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Beneficial microbes in coffee production and processing

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Abstract

Coffee, a globally traded agricultural commodity, faces significant challenges in sustainable production due to its high demand for chemical inputs. Recent research has highlighted the potential of microorganisms associated with coffee plants to enhance plant health, improve productivity, and contribute to sustainable agriculture. This review aims to synthesize current knowledge on the diversity, functionality, and application of coffee-associated microorganisms in both cultivation and postharvest processing. A comprehensive analysis of existing literature reveals that these microorganisms play crucial roles in plant growth promotion, bio control, and fermentation. Specific strains of bacteria and fungi have been identified as effective biofertilizers and bio pesticides, capable of enhancing nutrient uptake, suppressing pathogens, and improving coffee quality. The review also explores the potential of microbial starter cultures in controlled coffee fermentation, which can improve the sensory attributes of coffee. However, despite these promising developments, the practical application of these microorganisms under field conditions remains limited. Future research should prioritize in situ validation of microbial inoculants and integrate omics-based tools with culturomics approaches to isolate and deploy robust, functionally relevant strains. By harnessing the potential of the coffee microbiome, coffee production can become more sustainable, reducing dependency on chemical inputs and enhancing the quality, resilience, and environmental compatibility of coffee production systems. This review provides a comprehensive understanding of the coffee microbiome and its potential applications, offering insights into sustainable coffee production practices.

Keywords: Biofertilizers, bio pesticides, coffee microbiome and fermentation

1. Introduction

Coffee is a major plantation crop in southern India, cultivated over 292,000 hectares with an annual production of around 282,000 tonnes and export earnings of approximately \$200 million. The two main commercially grown species are *Coffea arabica* and *Coffea canephora* (robusta), raised in proportions of 70 and 30 % respectively (Davis *et al.*, 2006; Vieira *et al.*, 2006) ^[15, 83]. Despite being a monoculture, coffee is typically grown under the shade of diverse native trees, creating a forest-like environment that supports rich biodiversity (Venkatesha, 2010) ^[82]. The plant, a perennial from the Rubiaceae family, belongs to the *Coffea* genus, which includes about 100 species, though only *C. arabica*, *C. canephora*, and *C. liberica* are used for beverages. Arabica is native to Ethiopia's highlands (1,300-2,000 m), and Robusta is originating from tropical lowlands of Africa, together account for nearly all global coffee production. As the most traded tropical agricultural commodity and the second most consumed beverage after water. Coffee holds significant economic and ecological importance (Duongo *et al.*, 2020) ^[23]. Coffee (*Coffea* spp.) is one of the most important global commodities and a major source of income for millions involved in its cultivation, particularly in countries like India where it is traditionally grown under a two-tier canopy of diverse shade trees. This agro-forestry based system contributes to the sustainability of coffee farming by adding substantial organic matter to the soil through leaf litter and biomass from routine pruning. However, the increasing reliance on chemical fertilizers to enhance yield poses significant threats to soil health and long term productivity. In this context, sustainable soil fertility management has become crucial. Among various strategies, biofertilizers and microbial inoculants have emerged as promising alternatives due to their ability to enhance nutrient availability, stimulate beneficial microbial activity in the

rhizosphere, and improve soil structure while reducing dependence on synthetic inputs. Integrating such biologically based practices including biomass recycling, cover cropping, integrated nutrient management, and carbon-sequestering agro-forestry systems not only supports soil health and crop yield but also aligns with the broader goals of eco-friendly and economically viable coffee production (Nadaf *et al.*, 2024) ^[55].

The use of microbes in coffee farming and industry remains largely underutilized, despite their potential to reduce chemical inputs, enhance coffee quality, and improve farmer income through sustainable certifications (Mithöfer *et al.*, 2017) ^[52]. Additionally, integrating the plant microbiome into modern breeding strategies offers promising avenues for sustaining productivity (Nogales *et al.*, 2016; Orozco-Mosqueda *et al.*, 2018) ^[57, 58]. This review highlights (i) the role and potential of biofertilizers in sustainable coffee cultivation, particularly in enhancing soil fertility, plant health, and environmental resilience; (ii) summarizes current knowledge on the use of microorganisms for promoting plant growth and managing pests and diseases; and (iii) explores their possible applications in coffee processing.

2. Microorganisms associated with coffee

The microbiota associated with coffee is divided into two principal components. The first one is the indigenous microbiota composed of the microorganisms living in close association with the coffee plants in the soil at the vicinity of the roots (rhizosphere) at the surface (episphere), and inside the plant tissues (endosphere). The second component is related to the postharvest microbiota that encompasses all the microorganisms associated with the coffee cherries postharvest processing and its by-products including the fermentation, the drying steps, and the wastes.

2.1 The Indigenous Coffee Microbiota

The composition of plant-associated microbiota is influenced by a wide range of biotic and abiotic factors. These include the physical and chemical properties of the soil (Fierer, 2017; Tkacz *et al.*, 2020) ^[28, 74], the specific plant compartment (such as the rhizosphere, episphere, or endosphere), and the particular plant organ being studied (Compant *et al.*, 2019; Berg *et al.*, 2020; Tkacz *et al.*, 2020) ^[12, 8, 74]. Additionally, plant genotype has been shown to play a significant role in shaping the microbial community (Patel *et al.*, 2015; Mina *et al.*, 2020) ^[59, 51].

It is important to highlight that the majority of microorganisms associated with coffee plants have been documented in *Coffea arabica*, with evidence from around 170 scientific publications. In contrast, comparatively fewer studies have focused on other species such as *C. canephora*, *C. liberica*, and *C. congensis*, or did not specify the *Coffea* species at all. Given the complex and multifactorial nature of microbiota composition, we categorized the indigenous microbial communities of coffee plants based on their association with three primary plant compartments; the rhizosphere, episphere, and endosphere.

2.1.1 Microbial Communities in the Coffee Rhizosphere

Soil serves as a vast and underexplored reservoir of microbial diversity, much of which remains uncultivated and poorly understood (Mendes *et al.*, 2013) ^[50]. Among these soil microorganisms, plants play an active role in shaping the microbial community of their rhizosphere the narrow zone of soil surrounding the roots. Through the release of root exudates, plants can selectively enrich beneficial microbes from the soil (Hartmann *et al.*, 2008; Mendes *et al.*, 2013) ^[50, 36]. The growing

interest in rhizosphere microbiology is evident from the increasing use of the term “plant growth-promoting rhizobacteria” (PGPR), first introduced by Kloepper and Schroth in 1978. These rhizosphere-associated microbes have gained recognition for their ability to support plant health by enhancing nutrient uptake and improving tolerance to both biotic and abiotic stresses (Avis *et al.*, 2008; De Zelicourt *et al.*, 2013; Meena *et al.*, 2017) ^[5, 22, 48].

Studies conducted by Caldwell *et al.* (2015) ^[10], Lamelas *et al.* (2020) ^[45], and Rodríguez *et al.* (2020) ^[62] revealed that the bacterial diversity in the coffee rhizosphere is composed of eight phyla, 31 orders, 81 genera, and 42 species. The most frequently encountered and diversified phylum is Proteobacteria, with 45 genera and 22 species described. Within this phylum, *Pseudomonas* stands out as the most diversified and common genus. All studies that employed next-generation sequencing (NGS) to assess prokaryotic abundance and diversity in the coffee rhizosphere consistently reported the dominance of the Proteobacteria phylum and the *Pseudomonas* genus. The remaining bacterial diversity is distributed among the phyla Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Verrucomicrobia. In terms of fungal diversity, three phyla, eight orders, 16 genera, and 16 species were reported, with the phylum Ascomycota being the most represented. In contrast, other fungal phyla such as Basidiomycota and Mucoromycota are significantly underrepresented, with only two isolates reported from these groups (Tkacz *et al.*, 2020) ^[74].

2.1.2 Epiphytic Microbiome of Coffee Plants

In general, the plant epiphytic microbiota consists of a highly diverse group of microorganisms that are capable of attaching to and surviving on the surfaces of both aboveground and belowground plant tissues. This microhabitat presents unique environmental challenges particularly on leaf surfaces requiring specific adaptations for microbial survival and persistence. Moreover, the episphere often acts as a transitional or boundary zone, limiting microbial entry into internal plant tissues and thereby influencing their potential to become endophytes (Vandenkoornhuyse *et al.*, 2015; Duong *et al.*, 2020) ^[79, 23].

Two metabarcoding studies aimed to characterize the epiphytic communities on the surfaces of coffee leaves and cherries. However, both studies extracted DNA from crushed plant organs, making it impossible to distinguish epiphytic microorganisms from endophytes (James *et al.*, 2016; Veloso *et al.*, 2020) ^[37, 81]. The known diversity of coffee epiphytic microbiota includes seven phyla, 25 orders, 52 genera, and 42 species. Among these, the fungal kingdom is the most extensively studied, comprising three phyla, 15 orders, 34 genera, and 25 species. The most frequently reported fungal phylum is Ascomycota, followed by Basidiomycota and Mucoromycota.

2.1.3 Endophytic Microbial Communities in Coffee Plants

Endophytic microorganisms are defined by their ability to colonize the internal tissues of plants without causing any visible disease symptoms to their host. This endophytic lifestyle can vary, with some microorganisms residing within plant tissues for part of their life cycle, while others may remain internal for its entirety (Hardoim *et al.*, 2015) ^[35]. Notably, some endophytes can be transmitted vertically from parent to offspring, whereas others exhibit a range of colonization strategies (Frank *et al.*, 2017) ^[29]. Their ability to inhabit internal plant tissues is thought to protect them from environmental fluctuations in the soil and reduce competition with other microbes (Santoyo *et al.*, 2016)

[63]. Furthermore, endophytes are known to offer a wide array of benefits to plants, including growth promotion and enhanced stress resistance.

It is important to emphasize that over half of the studies on coffee endophytes have concentrated on arbuscular mycorrhizal fungi (AMF), a distinct group of endophytic symbiotic fungi classified within the Mucoromycotina phylum and the Glomeromycota subphylum (Spatafora *et al.*, 2016) [67]. These fungi are considered mutualistic obligate symbionts that colonize plant roots, while their external mycelium extends into the soil to absorb water and inorganic nutrients such as phosphorus, nitrogen, and other essential elements which are then transferred to the host plant in exchange for carbon compounds (Genre *et al.*, 2020) [30]. In addition to enhancing plant nutrition, AMF have been widely recognized for improving plant resilience to both biotic and abiotic stresses (Chen *et al.*, 2018; Begum *et al.*, 2019) [11, 7].

Prates Júnior *et al.* (2019) [60] reported the presence of a wide range of arbuscular mycorrhizal fungi (AMF) species in soils associated with coffee plants in Brazil. The identified species included *Glomus sp.*, *Gl. spinuliferum*, *Gl. fuegianum*, *Gl. glomerulatum*, *Gl. aggregatum*, *Acaulospora sp.*, *Ac. mellea*, *Ac. sieverdingii*, *Ac. tuberculata*, *Ac. scrobiculata*, *Ac. herrerae*, *Ac. foveata*, *Ac. nivalis*, *Ac. verna*, *Ac. morrowiae*, *Ac. denticulata*, *Scutellospora sp.*, *Sc. pernambucana*, *Archaeospora trappei*, *Ambispora leptoticha*, *Am. leptoticha*, *Gigaspora sp. 1*, *Rhizophagus fasciculatus*, *Dentiscutata biornata*, *De. cerradensis*, and *Paraglomus occultum*. The study compared plots under agro-ecological and conventional management, as well as forest fragments. Molecular analyses revealed a higher AMF diversity index in agro-ecological and agro-forestry systems compared to conventional systems, highlighting the significant influence of agricultural practices on AMF community structure.

Similarly, Vallejo-Torres *et al.* (2019) [78], working with the coffee variety Caturra in Peru, observed positive effects from applying an AMF consortium. These included increases in plant height, leaf area, root biomass, root colonization capacity, and extraradical mycelium density. The AMF species identified in this study included *Acaulospora rugosa*, *Ac. foveata*, *Ac. mellea*, *Ac. tuberculata*, *Ac. rhemii*, *Acaulospora sp.*, *Glomus sp. 1*, *Glomus sp. 2*, *Gl. geosporum*, *Gl. sinuosum*, and *Ambispora appendicula*. Another Peruvian study reported *Glomus brohultii*, *Gl. fuegianum*, *Funnelformis geosporum*, *Claroideoglomus claroideum*, *Glomus sp.*, *Scutellospora sp.*, *Sclerocystis sp.*, and *Acaulospora mellea* as the most common AMF species associated with coffee crops.

In Saudi Arabia, Mahdi *et al.* (2010) investigated the diversity of AMF colonizing coffee roots. Molecular and phylogenetic analyses revealed *Glomus* as the predominant genus, followed by *Claroideoglomus*, *Acaulospora*, and *Gigaspora*. DNA sequence data showed similarities with species such as *Claroideoglomus claroideum*, *Glomus microaggregatum*, *G. mosseae*, *Rhizophagus intraradices*, *Gigaspora rosea*, and *Acaulospora longula*. These findings offer a valuable foundation for developing targeted AMF-based bioinoculants to enhance coffee crop health and productivity.

2.2 Microbial Communities in Coffee Post harvest Stages

Microorganisms are known to play crucial roles during coffee postharvest processes, particularly in the degradation of the mucilage layer during fermentation (Agate and Bhat, 1966). Beyond this, they can significantly influence the organoleptic quality of the final product either positively (Elhalis *et al.*, 2020a) [25] or negatively (Ndayambaje *et al.*, 2019) [56] as well as

its safety, particularly in relation to the presence of mycotoxins (Urbano *et al.*, 2001) [75].

A review of existing studies reveals that the coffee postharvest microbiota comprises 18 phyla, 93 orders, 346 genera, and 446 species from both the bacterial and fungal kingdoms. Fungi, including yeasts, are the most extensively studied microorganisms in this context. Among yeasts, the most frequently cited genera include *Pichia* (28 citations), *Candida* (26), *Saccharomyces* (21), *Hanseniaspora* (16), *Torulaspora* (15), *Wickerhamomyces* (13), *Meyerozyma* (12), and *Debaryomyces* (10). Notably, metabarcoding studies have consistently reported *Pichia* and *Candida* as the dominant genera in terms of relative abundance during coffee fermentation (De Bruyn *et al.*, 2017; De Oliveira Junqueira *et al.*, 2019; Zhang *et al.*, 2019b; Elhalis *et al.*, 2020a, b) [16, 19, 86, 25, 26].

The bacterial component of the postharvest microbiota spans 14 phyla, 59 orders, 227 genera, and 176 species documented across 51 publications. The most commonly reported phyla include *Firmicutes* (38 citations), *Proteobacteria* (33), *Actinobacteria* (14), and *Bacteroidetes* (7). At the genus level, frequently cited bacteria include *Bacillus* (21), *Leuconostoc* (20), *Lactobacillus* (18), *Enterobacter* (18), *Pseudomonas* (17), *Klebsiella* (16), *Pantoea* (12), *Erwinia* (11), *Serratia* (11), *Lactococcus* (10), and *Acinetobacter* (10). These findings align with results from metabarcoding and metagenomic studies, which consistently identify lactic acid bacteria (LAB), particularly from the genus *Leuconostoc*, and acetic acid bacteria (AAB) as dominant during fermentation. Additional dominant bacterial genera, such as *Bacillus*, *Erwinia*, and *Pseudomonas*, are primarily associated with the *Firmicutes* and *Proteobacteria* phyla (De Bruyn *et al.*, 2017; De Carvalho Neto *et al.*, 2018; De Oliveira Junqueira *et al.*, 2019; Zhang *et al.*, 2019a,b; Elhalis *et al.*, 2020a,b) [16, 17, 19, 86, 87, 25, 26].

In total, 22 species of AAB have been identified during coffee postharvest stages, belonging to the genera *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, and *Kozakia*. Additionally, 23 LAB species have been reported, including members of *Bifidobacterium*, *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weissella*. These microorganisms collectively contribute to the biochemical transformations that occur during postharvest handling, affecting both the quality and safety of coffee.

3. Native microbes as biofertilizers in coffee cultivation

The term 'rhizosphere' was first introduced by the German agronomist and plant physiologist Lorenz Hiltner to describe the interface between plant roots and soil a zone characterized by intense microbial activity. The word is partly derived from the Greek term "rhiza," meaning root (Hartmann *et al.*, 2008) [36]. The microbial population in the rhizosphere differs significantly from that of the surrounding bulk soil, primarily due to the presence of root exudates that serve as a nutrient source for microbes (Burdman *et al.*, 2000) [9]. Research has demonstrated that many plant-associated microbes can positively influence plant growth, nutrient uptake, seed germination, and disease control (Gevers *et al.*, 2012) [31]. Therefore, the plant-microbe interaction plays a crucial role in enriching microbial communities and shaping their structure and activity. The rhizosphere is frequently targeted as a preferred niche for isolating plant growth-promoting (PGP) microorganisms with potential use as biofertilizers.

Plant growth-promoting microbes are broadly categorized into two types; plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi. PGPR were initially recognized

as bacteria capable of enhancing plant growth while simultaneously suppressing plant pathogens. Among the most extensively studied genera are *Alcaligenes*, *Pseudomonas*, *Azospirillum*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Enterobacter*, *Gluconacetobacter*, *Burkholderia*, *Arthrobacter*, *Rhizobium*, *Bradyrhizobium*, and *Serratia*. Numerous studies have shown that, when appropriately inoculated into soil or applied to seeds with suitable strains, formulations, and agronomic practices PGPR not only offer biocontrol against pathogens but also enhance nutrient uptake, stimulate root and shoot development, improve seed germination, and increase plant resilience to environmental stresses. Additionally, they play a crucial role in biological nitrogen fixation and phosphate solubilization. PGPR also influence the production of key phytohormones such as indole acetic acid (IAA) [76], gibberellins, cytokinins, and ethylene. Among, Plant growth promoting fungi like Arbuscular mycorrhizal fungi, and other beneficial fungi act as Biocontrol agent.

Inoculation of bacteria of the genus *Azospirillum* spp on coffee seeds helps their growth, thereby reducing the time of each of their c stages (germination, sprouting and "chapola"). An effective strain (CaUAP160) was found to be applied as biofertilizers for Garcinia variety. An effective strain (CaUAP290-38) was found to be applied as biofertilizers for cultivation of caturra coffee. The largest population of *Azospirillum* spp was found in the rhizosphere after 180 days (<https://patents.google.com>).

The synergistic interaction between Plant Growth-Promoting Rhizobacteria (PGPR) and Vesicular Arbuscular Mycorrhiza (VAM) has emerged as a promising strategy to enhance nutrient dynamics and plant vigor in coffee cultivation. According to findings by Ali *et al.* (2025) [2], the combined use of PGPR and VAM significantly improved both biomass production and macronutrient uptake particularly nitrogen (N), phosphorus (P), and potassium (K) in coffee plants. The study evaluated six treatment regimes, including a control (P0: without PGPR and VAM), and various combinations of PGPR (10, 15, and 20 g per plant) with VAM (5 or 10 g per plant). Notably, treatments P4 (PGPR 10 g/plant + VAM 10 g/plant) and P5 (PGPR 15 g/plant + VAM 10 g/plant) produced the most substantial improvements in wet and dry biomass accumulation. Beyond nutrient acquisition, these combinations enhanced the plants' tolerance to environmental stressors, underscoring their dual role in promoting growth and resilience. The results of Ali *et al.* (2025) [2] support the co-application of PGPR and VAM as an ecologically sound and effective approach for sustainable coffee production under varying agro-climatic conditions.

The application of rhizobacteria, specifically *Bacillus nitrificans* and *Bacillus velezensis*, has been shown to significantly enhance the growth, production, and phytochemical content of Balinese robusta coffee plants. A study conducted by Suriani, *et al.*, (2025) [70] utilizing a randomized group approach with four treatments (control, *B. nitrificans*, *B. velezensis*, and a consortium of both) demonstrated that the consortium treatment yielded the most pronounced effects, outperforming single treatments and the control group. Both rhizobacteria strains exhibited plant growth-promoting properties, including IAA production, nitrogen fixation, and phosphorus solubilization, and effectively colonized coffee roots. These findings suggest that *B. nitrificans* and *B. velezensis* can function as effective biofertilizers and bio stimulants, enhancing coffee plant growth, yield, and phytochemical content. The use of these rhizobacteria in sustainable agriculture practices holds promise for improving coffee production while reducing reliance on synthetic

fertilizers.

It has been recognized that endophytic fungi have a considerable capacity to improve the nutrient up-taking ability of plants from soil both macro-elements (e.g., calcium, magnesium, nitrogen, phosphorus, potassium) and micro-elements (e.g., boron, zinc). One of the pioneering studies in this area, conducted by Jimenez-Salgado *et al.* (1997) [39], identified *Acetobacter diazotrophicus* a nitrogen (N)-fixing bacterium—associated particularly with the root systems of coffee plants. The study suggested that an increase in populations of endophytic nitrogen-fixing bacteria can significantly enhance biological nitrogen fixation. The bacteria demonstrated a strong ability to colonize plant tissues extensively, thereby contributing positively to plant nitrogen nutrition.

In addition to their presence within root tissues, *A. diazotrophicus* was also found to inhabit the coffee rhizosphere. This dual presence may be explained by the natural deposition of fallen coffee leaves and berries on the soil surface. These plant residues are decomposed by the soil microbiota, enriching the rhizosphere with organic matter and carbon sources—particularly sugars which in turn support the proliferation and activity of nitrogen-fixing bacteria like *A. diazotrophicus*. This interaction suggests a probable mutualistic symbiosis between coffee plants and nitrogen-fixing bacteria, although direct experimental evidence for this relationship remains limited. Further supporting this, Pratiwi *et al.* (2020) [61] isolated several additional nitrogen-fixing bacterial species from coffee roots, identifying *Rahnella aquatilis* and *Pseudomonas tolaasii* as particularly effective nitrogen fixers. These bacteria are capable of converting atmospheric nitrogen into ammonium, which is readily absorbed and utilized by coffee plants to support growth and development (Jha *et al.*, 2012) [38]. Moreover, Pratiwi *et al.* (2020) [61] also assessed the phosphate (P) solubilizing capabilities of various bacterial isolates. Among these, *Rahnella aquatilis* and *Kluyvera intermedia* exhibited the highest and most significant phosphate-solubilizing activity. Their findings align with those of Muleta *et al.* (2013) [53], who reported several phosphate-solubilizing rhizobacterial species associated with coffee roots. In a related study, Teshome *et al.* (2017) further confirmed the role of phosphate-solubilizing bacteria in coffee systems. Their isolations identified *Pseudomonas* spp. (14.5%) as the most dominant phosphate solubilizers, followed by *Rhodococcus* spp. (9.1%), *Stenotrophomonas* spp. (7.3%), *Citrobacter* spp. (3.6%), *Gordonia* spp., and *Bacillus* spp. (3.6%). These results reinforce the potential application of rhizosphere and endophytic bacteria in sustainable coffee production through their roles in nitrogen fixation and phosphorus solubilization.

Phosphate-solubilizing bacteria (PSB) play a pivotal role in enhancing phosphorus availability in soils, particularly in phosphorus-deficient cropping systems such as coffee plantations. In a study conducted in Kavre, Nepal, Kunwar *et al.* (2018) [42] isolated 26 distinct PSB strains from the rhizosphere of *Coffea arabica* seedlings using Pikovskaya's agar medium, where phosphate solubilization was indicated by clear halo formation. Of these, eight isolates with solubilization indices greater than 1.60 were selected for detailed evaluation. Morphological and biochemical characterization revealed that the majority were Gram-negative rods, closely resembling members of the genus *Pseudomonas*. The phosphate-solubilizing potential of these isolates was tested using tricalcium phosphate (TCP) and Morocco rock phosphate (RP) in both agar and broth-based media. Notably, *Pseudomonas* sp. PSB 10 demonstrated the highest solubilization in Pikovskaya's broth (407.5 ± 2.50

µg/ml) and NBRIP-RP broth (263.3 ± 2.92 µg/ml), while *Pseudomonas* sp. PSB 11 exhibited superior performance in NBRIP broth (529.17 ± 6.14 µg/ml). Furthermore, *Pseudomonas* sp. PSB 20 recorded the highest solubilization index ($SI = 3.71 \pm 0.19$) on NBRIP-RP agar, suggesting strong efficacy in mobilizing poorly soluble phosphate sources. Seed germination bioassays using *Coffea arabica* seeds inoculated with PSB strains (PSB 1, PSB 10, PSB 11, and PSB 20) revealed significant improvements over control treatments. Among these, PSB 20 showed the most pronounced effects, significantly increasing shoot length, shoot biomass, and chlorophyll content in seedling trials. In greenhouse conditions, PSB 20 also enhanced both shoot and root biomass beyond that achieved with conventional phosphate fertilizers. These findings by Kunwar *et al.* (2018) [42] emphasize the potential of indigenous rhizospheric *Pseudomonas* spp. particularly isolate PSB 20 as effective biofertilizer candidates for sustainable *Coffea arabica* cultivation. Their multifunctional traits, including phosphate solubilization and plant growth promotion, support their application in low-input and organic coffee farming systems.

A recent investigation by Suharjono and Yuliatin (2022) [69] into the bacterial communities of the coffee rhizosphere in Malang, East Java, provided valuable insights into microbial diversity and their functional potential in supporting coffee plant growth. Soil samples were collected from *Coffea canephora* (Robusta) and *Coffea arabica*, and analyzed using both culture-independent and culture-dependent approaches. Genomic DNA was extracted using the Fast DNA Spin Kit and sequenced via the Illumina platform, revealing *Proteobacteria* as the dominant phylum, with *Bradyrhizobium elkanii* emerging as the most abundant species in both coffee types, showing no significant difference in relative abundance. Concurrently, culturable bacterial isolates were obtained using serial dilution methods on Tryptic Soy Agar, Pikovskaya Agar, and Nitrogen-Free Bromothymol Blue Agar to target IAA-producing, phosphate-solubilizing, and nitrogen-fixing bacteria, respectively. The selected isolates were identified through 16S rDNA sequencing. Notably, isolate S1.6.3.2 (*Bacillus subtilis* DSM 10) exhibited the highest IAA production at 104.46 µg/mL; isolate W3.5 (*Pseudomonas putida* S18) showed the greatest phosphate-solubilizing activity at 4.5 µg/mL; and isolate W1.2 (*Bacillus methylotrophicus* SY2) demonstrated significant ammonia-fixing capability at 21.54 µg/mL. These isolates exhibit strong potential as biofertilizers.

Candidates, owing to their ability to enhance soil fertility and promote coffee plant growth, thereby contributing to more sustainable and productive coffee agroecosystems.

3. Microorganisms as biocontrol agents against plant diseases and pests in coffee

The microbiota associated with coffee plants have emerged as valuable resources for the development of biologically based disease and pest management strategies. Numerous bacterial and fungal taxa isolated from the rhizosphere, phyllosphere, and endosphere of *Coffea* species have demonstrated antagonistic activities against key phytopathogens and nematodes affecting coffee cultivation. Prominent bacterial genera include *Pseudomonas*, *Bacillus*, *Burkholderia*, and *Streptomyces*, while fungal genera such as *Trichoderma* and *Verticillium* have also shown promise in biocontrol applications.

3.1 Mechanisms of Action

Several microbial isolates exhibit biocontrol activity through the production of hydrolytic enzymes, secondary metabolites, and

volatile compounds. Enzymes such as chitinases, proteases, and lipases contribute to the degradation of fungal cell walls, while metabolites like hydrogen cyanide (HCN), siderophores, and various antibiotics have been implicated in the suppression of pathogen growth (Muleta *et al.*, 2009; Kejela *et al.*, 2016; Duong *et al.*, 2021) [54, 40, 24].

3.2 In Vitro Antagonism

The antagonistic potential of coffee-associated microorganisms has been widely demonstrated through dual culture and agar diffusion assays. These assays revealed strong inhibitory effects against major coffee pathogens, including *Hemileia vastatrix* (the causal agent of coffee leaf rust, CLR), *Gibberella xylarioides* (responsible for coffee wilt disease, CWD), as well as species of *Colletotrichum* and *Fusarium* (Silva *et al.*, 2012; Haddad *et al.*, 2013) [66, 32].

3.3 In Plant Validation

Greenhouse and growth chamber experiments have corroborated in vitro results, showing that microbial inoculants can significantly reduce disease severity. For instance, pre- or co-inoculation of *Bacillus subtilis* and *Pseudomonas fluorescens* was effective in managing CLR symptoms (Shiomi *et al.*, 2006; Silva *et al.*, 2012; Haddad *et al.*, 2014) [66, 65, 33]. Similarly, antagonistic strains of *Streptomyces* and *Burkholderia* were shown to suppress CWD in controlled conditions.

3.4 Nematode Suppression

Beyond fungal pathogens, coffee-associated microorganisms have shown activity against plant-parasitic nematodes. Endophytic bacteria such as *Paenibacillus* spp. and arbuscular mycorrhizal fungi (AMF) have demonstrated efficacy against *Meloidogyne incognita* (root-knot nematode) and *Pratylenchus coffeae* (root lesion nematode), reducing galling and improving plant tolerance (Vaast *et al.*, 1997a; Mekete *et al.*, 2009; Asyiah *et al.*, 2018) [77, 49, 3].

3.4 Field Applications

Although most studies have been conducted under controlled conditions, a few field trials have demonstrated the practical potential of microbial biocontrol. In one such study, application of *Verticillium lecanii* resulted in delayed CLR symptom development, though its overall efficacy was limited (Vélez and Rosillo, 1995) [80]. Daivasikamani and Rajanaika (2009) [14]. Evaluated biocontrol agents over a two-year period, reporting disease incidence reductions of 36% with *Bacillus subtilis* and 28% with *Pseudomonas fluorescens*. Despite these reductions being lower than those achieved with chemical fungicides, the results support the potential of biocontrol agents as complementary tools in integrated pest management strategies.

The application of plant growth-promoting bacteria (PGPB) offers a sustainable solution for enhancing coffee health and productivity. Several bacterial strains isolated from the phyllosphere, endosphere, and rhizosphere exhibited multiple beneficial traits, including disease suppression, phytohormone production, and growth stimulation in coffee seedlings. In particular, *Pseudomonas gozinkensis* (MN1F), *Paraburkholderia caribensis* (BEca88), and *Enterobacter cloacae* (RBca5) demonstrated consistent effectiveness in reducing the severity of coffee leaf rust, brown eye spot, and brown leaf blight, while also improving seed germination and seedling vigor. In a recent study, De Souza *et al.* (2025) [21] reported that these strains produced key metabolites such as indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and

antifungal compounds, and may also contribute to induced systemic resistance. These results highlight the potential of native PGPB strains as biotechnological tools for integrated disease management and growth enhancement in coffee

production systems. Their consistent performance under controlled conditions supports their further development into commercial bioinoculants for sustainable agriculture.

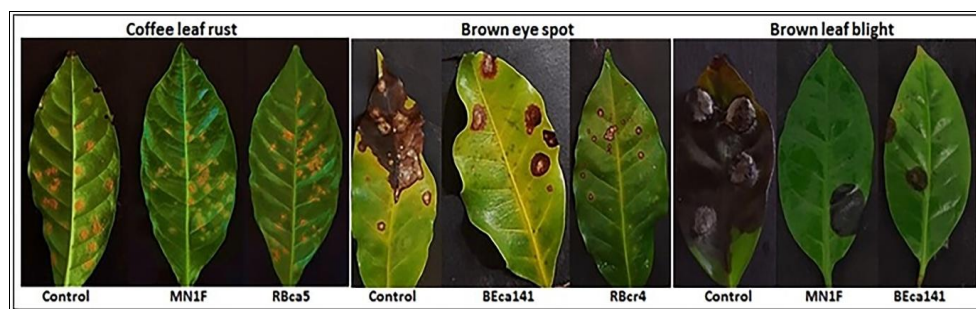


Fig 1: Biological control of diseases by the strains inoculated on coffee seedling and their effect. (De Souza *et al.* 2025) ^[21]

4. Role of microbes in post-harvest processing of coffee

In recent years, increasing attention has been directed toward the role of microorganisms in enhancing coffee quality during postharvest processing. Following the harvest of ripe coffee cherries, the beans undergo a series of processing steps to yield green (raw) coffee beans. Three principal methods wet, dry, and semidry processing are widely employed for this purpose. Microorganisms, particularly yeasts and bacteria, play a critical role during the fermentation stage by breaking down the mucilage layer through the secretion of pectinolytic enzymes, organic acids, and alcohols. The development of defined microbial starter cultures has become a focus of research, aiming to identify strains with desirable characteristics such as efficient mucilage degradation, stress tolerance, antifungal activity, and the potential to enhance the sensory attributes of the final product. As a result, the use of selected microbial inoculants during fermentation is gaining prominence for improving the flavour, aroma, and overall quality of green coffee beans (Haile and Kang, 2019) ^[34].

4.1 Functional Microorganisms and Enzymes in Coffee Bean Fermentation

Numerous studies, including the comprehensive review by Haile and Kang (2019) ^[34], have identified a diverse range of microbial species involved in coffee fermentation, with over 50 species of yeasts and bacteria reported to be active during the process. These microorganisms contribute significantly to the biochemical transformations that occur during fermentation, including the production of ethanol and various organic acids such as lactic, butyric, acetic, and higher carboxylic acids through the metabolism of pectin-rich substrates. In particular, cellulolytic species of *Bacillus* are known to secrete a variety of extracellular enzymes that facilitate the degradation of structural polysaccharides such as cellulose and pectin found in the skin, pulp, and mucilage of coffee cherries.

According to Haile and Kang (2019) ^[34], among the microbial enzymes involved in pectin degradation, three are considered particularly important: pectin lyase, polygalacturonase, and pectin methyl esterase. Pectin lyase catalyses the cleavage of pectin via a β -elimination mechanism, resulting in the formation of unsaturated galacturonic acid derivatives. Polygalacturonase, the primary enzyme involved in pectin hydrolysis during coffee fermentation, breaks down the α -1, 4 glycosidic linkages in polygalacturonic acid. Pectin methyl esterase initiates de-esterification by removing methoxyl groups from pectin, thereby generating pectic acid and methanol. These enzymatic activities

collectively facilitate the efficient removal of mucilage, thereby influencing the physical properties and quality of the final coffee product.

4.2 Starter Cultures for Coffee Fermentation

A starter culture refers to a defined microbiological preparation used to initiate and accelerate the fermentation process. In the food fermentation industry, starter cultures are well established in the production of cheese, yogurt, beer, and wine (Steinkraus, 2004; Schwan *et al.*, 2014) ^[68, 27]. Similarly, the application of starter cultures in controlled coffee fermentation has shown potential to enhance coffee quality and provide greater economic benefits to producers. As highlighted by Corsetti *et al.* (2012) ^[13], microorganisms selected for starter cultures should exhibit key characteristics such as being non-pathogenic, non-toxigenic, and well-adapted to the raw substrate and processing conditions. Additionally, they should contribute to desirable sensory attributes, extend product shelf life, reduce processing time, and inhibit the growth of food-borne pathogens.

Despite the promising potential, research on the use of defined starter cultures in coffee fermentation remains limited. The first notable study by Agate and Bhat (1996) ^[1] successfully introduced a starter culture composed of a mixture of three *Saccharomyces* species (*S. marxianus*, *S. bayanus*, and *S. cerevisiae* var. *ellipsoideus*), which enhanced fermentation efficiency by accelerating mucilage degradation.

Further work by Avallone *et al.* (2002) ^[4] evaluated the microbial and physicochemical characteristics of coffee fermentation using pectinolytic microorganisms isolated from spontaneous fermentations, including *Lactobacillus brevis* L166, *Erwinia herbicola* C26, *Bacillus subtilis* C12, and *Kluyveromyces fragilis* K211. However, the study reported that these inoculants did not significantly enhance polysaccharide degradation or improve the organoleptic qualities of the final beverage. The authors suggested that employing lactic acid bacteria may better replicate natural fermentation processes and deliver improved outcomes.

More recently, the use of aromatic yeast strains has been explored to enhance flavour development during dry, semidry, and wet processing methods (Evangelista *et al.*, 2014) ^[27]. Although coffee fermentation typically involves a diverse indigenous microbiota, most native microorganisms are presumed to lack specific traits necessary for enhancing the sensory quality of the final product. As such, only a select few species have been identified and utilized based on their positive impact on coffee flavour and aroma.

4.3 Yeast and Fungal Fermentation for Enhancing Green Coffee Bean Quality

A study by Kwak *et al.* (2018) ^[43] investigated the effects of yeast fermentation on the antioxidant activity and consumer acceptability of green coffee beans. The results showed that a 24-hour yeast fermentation significantly increased antioxidant activity, total polyphenol, and flavonoid contents. Although consumer acceptance of the fermented coffee was generally lower than that of the non-fermented control, one particular fermentation using *Saccharomyces* species received approximately 39.4% preference over the control, indicating potential for sensory improvement through targeted yeast inoculation.

Rhizopus oligosporus, a filamentous fungus commonly used as a starter in the production of fermented foods such as tempeh, also holds potential in coffee processing. During fermentation, *R. oligosporus* secretes a variety of extracellular enzymes, including proteases (Lee *et al.*, 2016) ^[46] and polysaccharide-degrading enzymes such as xylanase, polygalacturonase, cellulase, arabinase, β -D-glucosidase, and α -D-galactosidase (De Reu *et al.*, 1997) ^[20]. In a study by Lee *et al.* (2016) ^[46], solid-state fermentation of green coffee beans with *R. oligosporus* altered both volatile and non-volatile compound profiles. The metabolism of aroma precursors during fermentation resulted in the breakdown of some volatiles and the generation of others, which contributed to improved aroma precursor profiles. These precursors are crucial for the development of key odorant compounds such as pyrazines, thiols, furanones, and guaiacol, which are responsible for the characteristic coffee aroma after roasting. Given coffee's widespread global demand, even marginal improvements in its quality can significantly enhance marketability. The use of green coffee beans rather than parchment coffee as a substrate for fermentation presents an opportunity for coffee-processing industries to directly implement microbial fermentation strategies. However, further research is needed to isolate and characterize microorganisms that meaningfully enhance sensory attributes. Future studies should also explore the commercial potential of microbial fermentation for modulating the aroma and flavour of green coffee beans.

5. Conclusion

The exploration of coffee-associated microorganisms has revealed numerous beneficial roles across both cultivation and postharvest stages. These microbes contribute significantly to plant health by enhancing nutrient uptake, promoting growth, and suppressing pathogens, while also playing a central role in fermentation by influencing the sensory quality of the final product through enzymatic and metabolic activities. Advances in microbial characterization through both culture-dependent methods and high-throughput sequencing have enriched our understanding of microbial diversity and functionality in coffee ecosystems. Despite these promising developments, the practical application of these microorganisms under field conditions remains limited. Future research should prioritize *in situ* validation of microbial inoculants, especially considering the long growth cycle and environmental variability associated with coffee cultivation. Additionally, integrating omics-based tools with culturomics approaches will be essential for isolating and deploying robust, functionally relevant strains. Harnessing the full potential of the coffee microbiome offers a sustainable path forward, reducing dependency on chemical inputs and enhancing the quality, resilience, and environmental compatibility of coffee production systems.

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