UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ

VANESSA BASSI PREGOLINI

ANÁLISE METATAXONÔMICA PARA INVESTIGAR A CONTRIBUIÇÃO DO MICROBIOMA AMBIENTAL NA EFICIÊNCIA DA FERMENTAÇÃO DE CAFÉ CONDUZIDA COM CULTURAS INICIADORAS

PONTA GROSSA

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Metataxonomic analysis to investigate the contribution of environmental microbioma in the efficiency of coffee fermentation conducted with starter cultures

Dissertação apresentada como requisito parcial à obtenção do título de Mestre em Biotecnologia do Programa de Pós-Graduação em Biotecnologia da Universidade Tecnológica Federal do Paraná.

Orientador: Prof. Dr Gilberto Vinicius de Melo Pereira.

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RESUMO

O café atualmente é uma das maiores commodities em valor de mercado no mundo, cultivada em mais de 70 países. Os frutos maduros do café abrigam diversos microrganismos, incluindo bactérias, leveduras e fungos filamentosos. Durante o processamento via úmida de pós-colheita de café, os frutos são despolpados e as grãos são submetidas ao processo de fermentação para a retirada da mucilagem aderida ao pergaminho. Essa fermentação é feita de forma não controlada e pode ser espontânea ou utilizando culturas iniciadoras. Este estudo teve como objetivo avaliar a influência da microbiota ambiental na atividade metabólica de culturas iniciadoras usadas para o controle do processo fermentativo em uma fazenda experimental situada na região do cerrado mineiro, Patrocínio, Minas Gerais. As seguintes amostras ambientais foram coletadas: solo, folhas, frutos inteiros, despolpados e água de lavagem. Em seguida, fermentação utilizando as culturas iniciadoras *Pediococcus acidilactici* LPBC161 e *Pichia fermentans* YC5.2 foi conduzida por 24 horas. Amostras da fração liquida da fermentação e grãos foram amostradas nos tempos de 0, 9, 18 e 24 horas. Os microbiomas de todas as amostras foram analisados por sequenciamento de nova geração através da plataforma Illumina. O metabolismo das culturas iniciadoras foram analisados através de técnicas cromatográficas nas amostras da fermentação. A microbiota ambiente foi dominada por Enterobacteriaceae e Saccharomycetales. Após o processo de fermentação, *Pichia* e a família Lactobacillaceae (que inclui *P. acidilactici*) representaram mais de 70% da comunidade microbiana total. A interação positiva entre as culturas iniciadoras resultou na formação de metabólitos primários (como etanol e ácido láctico) e importantes compostos impactantes de aroma (acetato de etila, acetato de isoamilo e isobutirato etílico). Através dos dados gerados, espera-se que estes resultados auxiliem produtores de café no Brasil a controlar melhor a etapa de fermentação e, consequentemente, trazer melhoria de qualidade do café produzido no país.

Palavras-chave: fermentação de café; ecologia microbiana; cultura iniciadora.

ABSTRACT

Coffee is currently one of the largest commodity in market value in the world, grown in more than 70 countries. The ripe fruits of coffee harbor several microorganisms, including bacteria, yeasts and filamentous fungi. During the wet processing of coffee postharvest, the fruits are pulped and the beans are submitted to the fermentation process for removal of the mucilage adhered to the parchment. This fermentation is done in an uncontrolled manner and can be spontaneous or by the use of starter cultures. This study aimed to evaluate the influence of the environmental microbiota on the metabolic activity of starter culture used to control the fermentation process in an experimental farm located in the cerrado region of Minas Gerais, Patrocínio. The following environmental samples were collected: soil, leaves, whole fruits, pulped and washing water. Then fermentation using the starter culture *Pediococcus acidilactici* LPBC161 and *Pichia fermentans* YC5.2 was conducted for 24 hours. Samples of the liquid fraction of fermentation and grains were sampled at 0, 9, 18 and 24 hours. The microbiomes of all samples were analyzed by new generation sequencing through the Illumina platform. The metabolism of the starter culture were analyzed by chromatographic techniques in the fermentation samples. The ambient microbiota was dominated by Enterobacteriaceae and Saccharomycetales. After the fermentation process, *Pichia* and the Lactobacillaceae family (which includes *P. acidilactici*) represented more than 70% of the total microbial community. The positive interaction between the starter cultures resulted in the formation of primary metabolites (such as ethanol and lactic acid) and important aroma impacting compounds (ethyl acetate, isoamyl acetate and ethyl isobutyrate). Through the data generated, it is expected that these results will help coffee producers in Brazil to better control the fermentation stage and, consequently, bring improvement in the quality of coffee produced in the country.

Keywords: coffee fermentation; microbial ecology; starter culture.

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SUMMARY

1 INTRODUCTION

With the increasing improvement in the processes of planting and pre-harvest management in the coffee markets, the need to develop technologies that improve the post-harvest processes arose. In this sense, biotechnology has important tools to add value to the product, developing or improving processes and increasing the quality of coffee.

From the perspective of processing, coffee fruits can be processed in different ways, according to post-harvest methods: dry processing (with the fruit intact) or wet method (pulping and fermentation). These processe is commonly complex, often not observing standardization causing defects and lack of consistency in the quality of the product, generating loss to producers (QUINTERO; MOLINA, 2015; PEREIRA *et al*., 2017)

Despite this, fermented coffee beans have gained prominence due to its excellent quality, aromas and differentiated flavors. Fermentation is a process carried out by microorganisms that has been studied and pointed out as responsible for producing compounds capable of influencing the quality of coffee after roasting. The effects of microorganisms present in post-harvest processing and their fermentation capacity in coffee have been studied in the last 10 years. Several studies have brought new perspectives in order to enhance the quality of coffee through fermentation inoculated by yeasts and lactic acid bacteria (most commonly reported microorganisms) (LEE *et al*., 2015; PEREIRA *et al*., 2014, 2016; ELHALIS *et al*., 2021).

Knowing the microbiota present in the environment and fruit of coffee processing allows to select beneficial microorganisms with specific characteristics and use them as starter crops to carry out coffee fermentation. Inoculation reduces fermentation time from 24h to 12h (PEREIRA *et al*., 2016). As coffee fermentation does not occur in a descontaminated way like other fermentations (wine and yogurt), inoculation of these microorganisms can act as control, as they help in reducing the growth of undesirable microorganisms. In addition, they can also contribute by producing compounds beneficial to the quality of coffee adding value to the final product.

Despite the large volume of coffee production, fermentation is still carried out in traditional uncontrolled processes where native microorganisms originating from grains, environment and processing equipment perform spontaneous fermentation

(SELVAMURUGAN; DORAISAMY; MAHESWARI, 2010). To transform fermentation into a more controlled process, it is crucial to develop early microbial cultures with adequate microbial and biochemical properties that can efficiently ferment and produce consistent high-quality coffee (ELHALIS *et al*., 2020). Due to this, the study of the microbiota involved in coffee fermentation, as well as the study of interactions with the starter cultures bring the need for the development and improvement of the fermentation process of coffee mucilage in order to promote a form of control and optimization of the wet method.

1.1 Objetives

- To evaluate the influence of the microbial communities from coffee farm processing and coffee fermentation.

1.2 Specific Objetives

- To develop a litetature review to better understand the functions and advantages of inoculating starter cultures in the coffee fermentation process.
- To identify microbioma present in soil, leaves, fruits and washing water -by high-throughput sequencing.
- To monitor and study metabolites produced in the fermentation process.
- To study the diversity and interaction of the starter microbiota with the local microbiota in the fermentation dynamics.

2 CHAPTER I - LITERATURE REVIEW

2.1 Coffee market

The global coffee market, which includes the production and export of coffee beans and beverage consumption, has greatly increased in recent years according to the International Coffee Organization (ICO, 2023). Coffee is one of the most popular beverages in the world (LEE *et al*., 2015) and plays an important role in various Brazilian scenarios, such as in the economic, political, social and environmental perspective. Brazil is the largest producer and exporter of coffee in the world, and the second largest consumer. Although there is a large number of coffee species (approximately 80), only the *Coffea arabica* e *Coffea canephora* have global economic importance (SARAIVA *et al*., 2009).

Coffee production is of great importance to the Brazilian economy. Approximately, Arabica coffees participated in exports with 64% and robust with 36% in the period from October 2020 to March 2021 (EMBRAPA, 2021). Six coffee producing states stand out in Brazil: Minas Gerais, São Paulo, Espírito Santo, Paraná, Bahia e Rondônia. With an estimated gross revenue of R$26 billion, the southeast region leads the ranking of the five coffee producing zones in 2021, equivalent to 87% of total revenue (EMBRAPA CAFÉ, 2021).

The coffee industry has a current concern to add value to its product, that is, to add inputs or services to coffee that value its price. Differing from traditional ones by several factors, such as mainly the quality of the beverage, origin of plantings, presence of bioaromas (added to the beverage by the action of the microbiota present in ferementation) and processing. Specialty coffees are products of the highest quality and their production and marketing are on the rise (SOUZA; SAES, 2018; MARCOMINI, 2008).

Therefore, new strategies aimed at consolidating the specialty coffee market in Brazil arise, such as changes in postharvest processes. Specialty coffees have changed international trade in recent years, from selling regular coffee to a special product (SITTIPOD *et al*., 2019), causing an increase in their popularity in global markets and driving the search for new postharvest technologies that help the production of specialty coffees (CÓRDOBA *et al*., 2021). The use of fermentation involving starter culture has been gaining space as a way to add value to the products developed thus adding new aromas to coffees (DEMITO, 2018; SAES *et al*., 2006)

Coffee processing is an agricultural practice that employs traditional production techniques, dedicated facilities and specialized equipment. The processing method is an important phase that may involve fermentation. Three processing methods are mainly used: dry, semi-dry (also called natural peeled) and wet method.

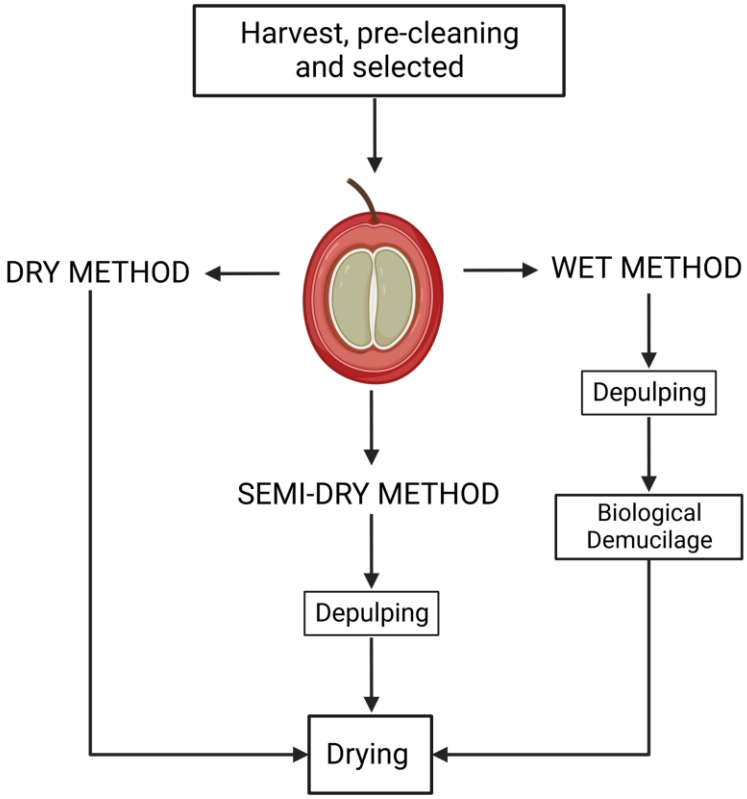
2.2 Postharvest processing

To produce coffee beans suitable for transport and roasting, it is necessary to separate the seeds from the outer layers (skin, pulp, mucilage and parchment), allowing the reduction of moisture from 65% to 12%. A crucial contributor to the quality of the coffee beverage is the series of post-harvest processes carried out to obtain dry beans suitable for roasting (PEREIRA *et al*., 2015).

The quality of coffee has recently become a great demand of coffee consumers and is directly related to its chemical compounds, especially those that constitute the flavor. This important sensory attribute of coffee is produced by several volatile compounds that are generated before and mainly during roasting. In addition, they are responsible for the generation of specialty coffees. However, chemical and volatile profiles vary and depend on environmental factors, altitude, coffee variety, storage conditions or processing methods and degree of roasting (EVANGELISTA *et al*., 2014b; SCHUMAN *et al*., 2014; ELHALIS *et al*. 2020)

In the international market, coffee is classified according to the post-harvest processing technology used to remove the outer layers adhered to the fruits (Figure 1): 'natural coffee', produced from the coffee beans processed on the farm by the simple method of drying the sun. Known as dry processing, it is a simpler process and requires less control during harvest. In this process all fruits are arranged in a terrace where they will be dried by the action of time or by mechanical dryers. However during drying the climatic conditions can affect the quality of the product by the action of fungi and bacteria that result in unwanted microbial growth. And 'washed coffee', produced from coffee beans that go through a number of relatively complex steps, including pulping, fermentation and sun drying or dryers, known as wet processing (ETHALIS *et al*., 2020; PEREIRA *et al*.,2019; SILVA, 2015; SARAIVA *et al*. 2010).

Figure 1 - Post-harvest processing technology



Source: The Author (2023)

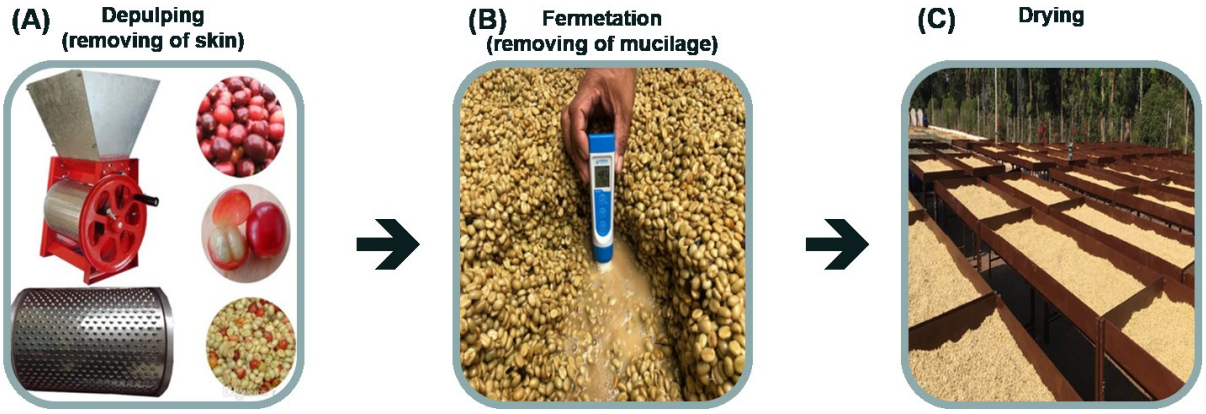
The dry method is the most used today in Brazil and in the world. (NADALETI *et al*., 2022). Despite this, the wet metling has been standing out due to the high quality of the final product. The production of microbial metabolites can reach the interior of the seed, thus leading to beneficial (organic acids of interest, esters, alcohols, sugars) or harmful effects (undesirable organic acids and toxins) on the quality of coffee beans (ELHALIS *et al*., 2021; SALEM *et al*., 2020; WANG *et al*., 2019, 2022). The use of starter cultures can control and improve wet processing, increasing the quality and valuo of the coffe beverage. In this work, the interaction between the environmental microbiota and the starter cultures during the wet coffee processing was studied, best addressed in the next topic.

2.2.1 Wet method

In the wet method (Figure 2) the coffee beans are first mechanically removed from the pulp, leaving the mucilage. Afterwards, the pulped beans are subjected to microbial degradation of the mucilage, characterized by submerging them underwater and subjecting them to fermentation for 24 - 72h. Which depends on the temperature conditions of the environment (SCHWAN; WHEALS, 2004; AVALLONE

et al., 2001; EVANGELISTA *et al*., 2015), it also involves a complex microbiological ecology that includes yeasts, bacteria and filamentous fungi (ELHALIS *et al*., 2020; EVANGELISTA *et al*., 2015 ; SILVA *et al*., 2008).

Figure 2 - Wet method: (A) Mecchanical depulping; (B) Removing of mucilageby fermentation; (C) Sun drying.



Source: The Author (2023)

This process arose when *Arabica coffee* began to be grown in tropical regions such as Colombia, Central America and Hawaii. In these countries, abundant rainfall and hot temperatures cause an immediate undesirable fermentation after harvest. The most practical way to avoid this harmful fermentation was to remove the external mesocarp tissue, rich in sugars, and subject the pulped fruits to an underwater tank process to achieve the desirable fermentation (BRANDO, 1999).

When the producer opts for the wet processing, it is possible to have in hand different patterns and nuances of aromas, as well as flavors, depending on the methodology adopted by the producer after harvest. However, the method itself does not guarantee quality, as a range of factors can interact between harvests until the end of coffee preparation (PEREIRA, 2017). Coffee from wet processing contains a higher acidity than other methods. Acidity is due to the reduction of pH by bacteria during wet fermentation. It is well accepted that the coffees resulting from the wet method produce roasted beans and coffee beverages that are characteristically different from those produced with the dry method. Wet processing coffees are known to have better quality, less body, higher acidity and more aroma than coffees produced by the traditional method (MAZZAFERA; PADILHA-PURCINO, 2004; PEREIRA *et al*., 2014).

2.3 Microbiota and coffee fermentation process

Through technology, controlled fermentation of coffee beans can increase the quality of coffee beverages. It can confer sensory notes of sweets, citrus and floral fruits, adding value and consistency to quality (LIN, 2010; EVANGELISTA *et al*., 2015; PEREIRA *et al*., 2014, 2015; SILVA, 2014). Mucilage contains high concentrations of pectins (alongside other polysaccharides such as cellulose), simple carbohydrates (mainly glucose and fructose), citric acid, malic acid, free amino acids and phenolic compounds, which can be used as nutrients or co-substrates by bacteria and yeasts (SILVA, 2014; WATERS *et al*., 2017). The physical and chemical properties of coffee mucilage vary according to species and variety. Studies have shown that the mucilage/water ratio increases with increasing altitude (CASTRO; MARRACCINI, 2006). Mucilage is composed of water (84.2%), protein (8.9%), reducing sugar (4.1%), pectatos (0.91%) and ash (0.7%) (BELITZ; GROSCH; SCHIEBERLE, 2009). Knowing these compositions is fundamental for understanding the fermentation of coffee.

Microorganisms are believed to play at least two crucial functions in fermentation. The first is the breaking of the mucilage layer of coffee beans, the presence of mucilage may favor unwanted microbian growth (MASOU, 2006). The second function is the production of microbial metabolites that can migrate to the inside of the coffee beans and contribute to the flavor and aroma characteristics of coffee products (EVANGELISTA *et al*., 2015; PEREIRA *et al*., 2014, 2015).

During coffee fermentation, sugars are consumed by microorganisms, producing primary metabolites such as organic acids and alcohols, which leads to a decline in pH and increased temperature (LEE *et al*., 2015; PEREIRA *et al*., 2019; 2020; ELHALIS; COX; ZHAO, 2020). These stressful environmental conditions can affect the growth and metabolic activities of the microflora during coffee fermentation. With the molecular methods developed for the study of microorganisms and ecology, some of the limitations of ecological and taxonomic methodologies were overcome, which took to great advances in understanding the diversity of yeasts, bacteria and filamentous fungi in coffee fermentations (MASOUD *et al*., 2004; PEREIRA *et al*., 2015).

The microbial ecology of a coffee fermentation process can be highly variable. In general, the fermentation microbiota can be characterized by a mixture of yeasts and fermentative bacteria (Enterebacteria, lactic acid bacteria (LAB), acetic

acid bacteria (AAB)) (SILVA *et al*., 2000; AVALLONE *et al*., 2001; MASOUD *et al*., 2004). The bacterial aspect of fermentation is driven by lactic acid bacteria, some Enterobacteriaceae and Bacillus and in general, lactic acid bacteria have been reported as more numerous. Some of the mais dominant microorganisms in cofffe fermentations are *Leuconostoc* sp., *Pediococcus* sp., Enterobacteriaceae, *Pichia* sp., *Candida*, *Hanseniaspora*. The condition of low oxygen tension and high activity determine that oxidative and mesophilic species of Ochratoxin a (OTA) produce. (PEREIRA *et al*., 2017; CARVALHO NETO *et al*., 2017; CARVALHO NETO *et al*., 2018; PEREIRA *et al*., 2020; VALE *et al*., 2021).

Microbial activity during fermentation solubilizes the pulp material that surrounds the beans and produces a variety of final metabolic products (alcohol and organic acids). The characteristic flavor of coffee has its origin in the chemical composition of the grain due to the release of metabolites and its diffusion to the beans during fermentation (SILVA, 2014). For example, RIBEIRO *et al*. (2014, 2017) brings that organic acids mainly affect sweet taste and acidity. The bioactive compounds trigonelin and chlorogenic acids are precursors of volatile compounds that contribute to the flavor and aroma of roasted coffee and Lee *et al*. (2015) brings that the precursors of volatile alcohol produce rose-like and fruity flavors.

However, the fermentation step can also be detrimental to the quality of the coffee cup if not conducted properly, as undesirable microorganisms generate metabolites such as short-chain fatty acids (e.g., butyrate and propionate), contributing to undesirable flavors (BRANDO e BRANDO, 2014; AVALLONE *et al*., 2002; SILVA *et al*., 2000, 2008; LIN, 2010). Consequently, the chemical composition of green coffee beans, resulting from the wet processing of coffee cherries, is directly impacted by the various microorganisms that thrive on coffee cherry substrates (AVALLONE *et al*., 2002; PEREIRA *et al*., 2017).

2.3.1 Lactic acid bacterias (LAB)

According Pereira *et al*. (2020b) Lactic acid bacteria (LAB) are a microbial group of substantial economic importance, used extensively as a starter culture in the production of fermented foods. During coffee processing, lactic acid bacteria (LAB) from multiple ecosystems (water, native soil, air, and plant) find in the cherry pulp a rich environment for their development (PEREIRA *et al*., 2020a). The quality of coffee is also influenced by acid composition, which is generally recognized as a flavor precursor for coffee quality descriptors (BORÉM *et al*., 2016). Malic, succinic and

citric acids are part of the fruit composition and contribute to the acidity of the beverage (ELHALIS *et al*., 2020; EVANGELISTA *et al*., 2014). Lactic, acetic and succinic acids increase during fermentation. Lactic acid bacteria are recognized for producing lactic and acetic acid (AVALLONE *et al*., 2002).

The release of organic acids by LAB during coffee fermentation is essential for reducing pulp pH. Accelerates the fermentation process to aid in the breaking of pectin and prevents the growth of microorganisms such as enterobacteria and filamentous fungi that are not beneficial for fermentation (CARVALHO NETO *et al*., 2018a; CARVALHO NETO et al., 2018b; JUNQUEIRA *et al*., 2019; VALE *et al*., 2019). Acetic acid above 1 mg/mL can lead to an undesirable formation of onion flavor in green coffee beans. However, its concentration decreases during the roasting process and generates important flavor-active metabolites such as pyrirides and pyrorolls (SILVA *et al*., 2013; PEREIRA *et al*., 2019).

Some metabolites derived from LAB (e.g., aldehydes, ketonas and superior alcohols) may influence sensory attributes of coffee beverage with distinct floral, fruity and buttery perceptions (PEREIRA *et al*. 2019; WANG *et al*., 2019). The formation of higher alcohols by LAB is the result of amino acid catabolism through the Erlich pathway (PEREIRA *et al*. 2019). Keons may be the result of lipopolitical metabolism of LAB species. In the fermentation of cocoa beans, the detection of keones has been associated with desirable aromas of herbs and fruits. (CEVALLOS-CEVALLOS *et al*. 2018; ZHAO *et al*., 2014). In roasted coffee beans, keones are related to sweetness scores (ELHALIS *et al*. 2021). Chart 1 shows the relationship of LAB and its main metabolites produced in the coffee fermentation.

Chart 1 - Main metabolites produced by BALs related to coffee

Metabolite	**Sensorial description**	**Producing LAB**	**References**
Acetaldehyde	Fruity	*Leuconostoc mesenteroides; Leuconostoc citreu; Lactobacillus brevis*	Bruyn *et al*. 2017
Acetoin	Dairy; Milky	*Lactobacillus rhamnosus*	Wang *et al*., 2019
Benzaldehyde	Fruity	*Lactobacillus plantarum*	Pereira *et al*., 2019
Ethyl acetate	Fruity; Grape	*Leuconostoc lactis*; *Pediococcus sp*	Pereira *et al*., 2016

Phenylacetaldehyde, Phenylacetate and Phenylethanol	Floral; Fruity	*Lactobacillus plantarum*	Smit *et al*. 2005; Vermeulen *et al*. 2006; Pereira *et al*., 2019
Latic acid	Acid; Sour	All species	Carvalho Neto *et al*., 2018
2,3-butanedione	Buttery aroma	*Lactobacillus plantarum*	Pereira *et al*. 2019
3-metilbutanal, 3-metilbutanol and ácido 3-metilbutírico	Nutty; Leafy; Cocoa	*Lactococcus lactis*	Smit *et al*. 2005; Vermeulen *et al*. 2006

Source: The Author (2023)

Some studies also bring the implementation of bacteria in coffee fermentation, mainly lactic acid bacteria (LAB) (SOUZA *et al*., 2017; PEREIRA *et al*., 2016).

2.3.2 Yeasts

Yeasts are among the most frequently isolated and studied microorganisms from fermenting coffee beans. They are considered to be important to the fermentation performance and coffee flavor development. Consequently, yeast is the microbial group most widely studied in coffee fermentations, which metabolic function has been elucidated in recent studies (PEREIRA *et al*., 2014; EVANGELISTA *et al*., 2014). The most frequently occurring yeast species during coffee processing are *Pichia kluyveri*, *Pichia anomala*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*. (BRESSANI *et al*., 2021; EVANGELISTA *et al*., 2014).

In addition, play an important role in coffee fermentation, impacting the flavor and aroma profile (ELHALIS *et al*., 2020). These volatiles can diffuse into the coffee beans which may influence in its chemical composition (PEREIRA *et al*., 2015). High concentrations of flavor compounds, such as isoamyl acetate, ethyl acetate, were detected in fermentations conducted by *P. fermentans* (Chart 2) (ELHALIS, 2021). For exemple 2-hexanal, D-limonene, 1,2-epoxy-3-propyl acetate and isoamyl acetate has fruity flavor; 4-methoxy-benzenamine, nonanol has floral flavor (PEREIRA *et al*., 2019; PATRIGNANI *et al*., 2016; ELHALIS *et al*., 2020).

Chart 2- Main volatiles and their sensorial description.

Metabolite	Sensorial description	References
2-hexanal	Green; Fruity	Pereira *et al*., 2019; Patrignani *et al*., 2016
nonanol	Floral; Herbal	
D-limonene	Citrus; Fruity	
4-methoxy-benzenamine	Sweet scent; Floral;	

1,2-epoxy-3-propyl acetate	Herbaceous; Fruity	
Ethanol	Alcoholic	Elhalis *et al*., 2020
acetaldehyde	Pungent; Swet	
ethyl acetate	Anise; Pineapple	
isoamyl acetate	Fruity	
3-methyl-1-butanol	Banana; Pear	Elhalis *et al*., 2020
2-furanmethanol	Burnt; Sweet; Caramel aroma	

Source: The Author (2023)

Several studies have characterized spontaneous fermentation, from which the strains were isolated and used as starter cultures (EVANGELISTA *et al*., 2014a, b; MARTINEZ *et al*., 2017; BRESSANI *et al*., 2018). In addition, yeast can also inhibit the growth of mycotoxogenic fungi. Pereira *et al*. (2015) conducted a fermentation using as starter *P. fermentans* YC5.2 and concluded that it can be used as an alternative to ensure the hygiene control of fermentation process and to prevent a microbial contamination spoils the beans.

2.4 Starter cultures

The use of selected microorganisms in the food industry is quite common, especially in the production of wine, beer, cheese, bread, and yogurt. In practice, the main objective of using starter cultures is to enable the standardization of the fermentation process, increase safety parameters, and optimize processing time (PEREIRA, 2020).

There are three main properties that need to be met for the choice of the starter culture. First, she must be able to master the native microbiota. It is necessary to be tolerant and have capacity of adaptation within coffee environment conditions. It is desirable that it has a pectinolytic action for a degradation of mucilage. Finally, it has been reported that some yeasts and lactic acid bacteria can inhibit growth of filamentous fungi (PEREIRA *et al*., 2017)

During coffee fermentation, most microorganisms that participate in the process come from the environment, such as soil, air, plants and other sources (SILVA *et al*., 2008a; SILVA, 2015). The perspective of the application of starter cultures in coffee fermentation is to reduce fermentation times (pectinolytic activity) and drying, better process control, higher sensory quality. Some of these flavor attributes are: caramel, chocolate, woody, yellow fruits and almonds, and safety

attributes (inhibition of the growth of toxigenic fungi), improving the sensory quality and economic value of the product (SILVA *et al*., 2013; EVANGELISTA *et al*., 2014; PEREIRA *et al*., 2016)

Some studies inoculated yeasts and bacteria from the lactic acid alone or as coinoculated starter cultures. Pereira *et al*. (2015) instilled the yeast *Pichia fermentans* and obtained an improvement in the fermentation process, reducing fermentation time and increasing the sensory quality of the coffee beverage. Bravim *et al*. (2023) inoculated Bacillus licheniformis bactéria as a starter culture and concluded that there was production of desirable sensory notes. Chart 3 shows some studies that used these starter cultures in coffee fermentation and their response to this inoculation.

Chart 3 -Starter culture and their response to inoculation

	Starter Culture	Response to inoculation	Reference
Yeast	*Torulaspora delbrueckii* *Saccharomyces cerevisiae* *Candida parapsilosis*	The use of yeasts is an alternative for sensorial differentiation of coffee	Martins *et al*., 2019; Bressani *et al*., 2021
	Pichia fermentans	Was successfully implemented as a starter cultue. Increased the production of specific volatile aroma compounds	Pereira *et al*., 2015
	Candida parapsilosis *Pichia guilliermondii*	Resulted in a beverage with a distinctive flavor (caramel and fruity) and good sensory quality.	Evangelista *et al*., 2014
LAB	*Bacillus licheniformis*	Desirable sensory notes showed to be a potential alternative way to reduce the fermentation time	Bravim *et al*., 2023
	Lactobacillus plantarum		Pereira *et al*., 2016
Yeast and LAB	*Pichia fermentans* and *Pediococcus acidilactici*	Increased consumption of sugar in coffee pulp and the production of metabolites	Vale *et al*., 2019
	Saccharomyces cerevisiae, *Torulaspora delbrueckii*, *L. mesenteroides*, and *Lactobacillus plantarum*	Metabolism of each starter culture influenced the formation of the volatile and non-volatile compound and sensory profile	Cassimiro *et al*., 2022

Source: The Author (2023)

LAB and yeast are known to coexist and cooperate in various fermented foods and beverages, such as coffee, cocoa, kefir and wine (PEREIRA *et al*., 2015;

CHEN, 2021; MINNAAR *et al*., 2019). Yeasts contribute to the development of LAB through cellular autolysis; they provide essential amino acids and vitamins for their development. On the other hand, organic acids (such as lactic acid) produced by LAB decrease the pH, contributing to the growth of yeasts. These factors provide an unfavorable environment for the growth of pathogenic bacteria and filamentous fungi, providing food security (CANON *et al*., 2020).

Implementing coffee starter crops can be a very laborious process, but once successfully implemented, it can turn an inconsistent process into an economically valuable proposition. Recently, *Pediococcus acidilactici* LPBC161 was isolated from ripe coffee cherries and the genome was sequenced to provide information on lab adaptation and metabolism mechanisms in coffee processing (MUYNARSK *et al*. 2019). Vale *et al*. (2019) evaluated the use of *P. acidilactici* LPBC161 as starter and co-cultivated with *Pichia fermentans* YC5.2 during coffee bean fermentation on the farm. This process contributed to the acidification of coffee and improvement of the quality of the beverage (VALE *et al*. 2019). The fact that one group contributes to the growth of the other encourages us to use the co-cultivation between *Pediococcus* and *Pichia* yeast as a strategy to improve the microbiological and sensory quality of coffee. To explore this path, it is important to first understand how the development of the mixed starter culture is influenced by the native microbiota.

3 CHAPTER II INFLUENCE OF ENVIRONMENTAL MICROBIOTA ON THE ACTIVITY AND METABOLISM OF STARTER CULTURES USED IN COFFEE BEANS FERMENTATION

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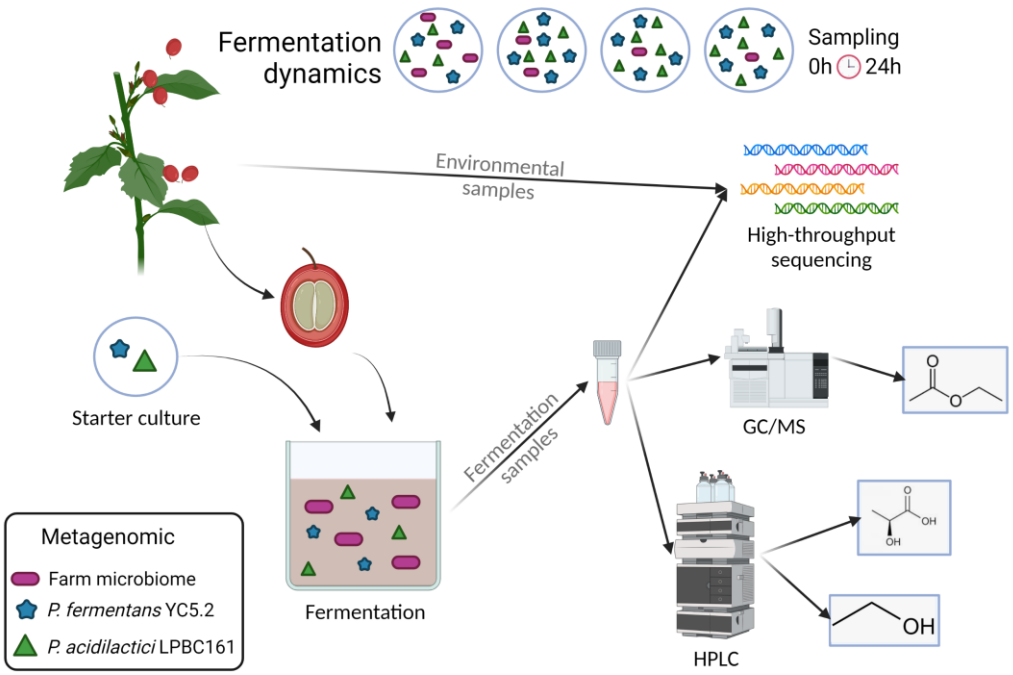
Figure 3 - Graphical Abstract



Source: The Authors (2021)

3.1 Abstract

Microbial activity is an integral part of agricultural ecosystems and can influence the quality of food commodities. During on-farm processing, coffee growers use a traditional method of fermentation to remove the cherry pulp surrounding the beans. Here, we investigated the influence of the coffee farm microbiome and the resulting fermentation process conducted with selected starter cultures (*Pichia fermentans* YC5.2 and *Pediococcus acidilactici* LPBC161). The microbiota of the coffee farm (coffee fruits and leaves, over-ripe fruits, cherries before de-pulping, depulped beans, and water used for de-pulping beans) was dominated by Enterobacteriaceae and Saccharomycetales, as determined by Illumina-based amplicon sequencing. In addition, 299 prokaryotes and 189 eukaryotes were identified. Following the fermentation process, *Pichia* and the family Lactobacillaceae (which includes *P. acidilactici*) represented more than 70% of the total microbial

community. The positive interaction between the starters resulted in the formation of primary metabolites (such as ethanol and lactic acid) and important aroma-impacting compounds (ethyl acetate, isoamyl acetate, and ethyl isobutyrate). The success competitiveness of the starters towards the wild microbiota indicated that coffee farm microbiota has little influence on starter culture-added coffee fermentation. However, hygiene requirements in the fermentation process should be indicated to prevent the high microbial loads present in coffee farm soil, leaves, fruits collected on the ground, and over-ripe fruits from having access to the fermentation tank and transferring undesirable aromas to coffee beans.

Keywords: fermentation hygiene; coffee fermentation; *Pichia fermentans*; *Pediococcus acidilactici.*

3.2 Introduction

Coffee is a tropical crop grown in more than 50 countries, with the largest producers being Brazil, Vietnam, Colombia, Indonesia, Ethiopia, and Honduras (ICO, 2021). To produce coffee beans suitable for transportation and roasting, it is necessary to separate the seeds from the outer layers (skin, pulp, mucilage, and parchment). This process can be carried out by three different methods, namely dry, semi-dry, or wet processing, which reduces the moisture of the coffee beans from 65% to 12% (PEREIRA, 2017). In the dry processing, intact coffee fruits are exposed to the sun for approximately 30 days until they reach 12% moisture. Then the fruits are mechanically crushed to separate the beans from the outer layers. Wet processing is relatively more complex, as the fruits are mechanically de-pulped and placed in tanks containing large volumes of water for a natural fermentation to occur for 24 to 48 h. The fermentation process removes the mucilage layer adhered to the seeds, which are finally sun-dried to reach the desired moisture content. Finally, semi-dry processing presents stages of both dry and wet methods, where the coffee fruits are mechanically de-pulped and then sub-mitted to sun-drying (PEREIRA *et al*., 2019).

Recent studies using next-generation sequencing (NGS) have shown that more than 100 microbial genera are involved in coffee beans fermentation from Brazil, Ecuador, Colombia, Honduras, and Australia (CARVALHO NETO *et al*., 2017; BRUYN *et al*., 2017; JUNQUEIRA *et al*. , 2019; POTHAKOS *et al*., 2020; ELHALIS *et al*., 2020; ZHANG *et al*., 2019; VALE *et al*., 2021). However, temporal analyses of the fermentations revealed that the core microbiome is distinguished in three stages;

(i) Enterobacteriaceae, acetic acid bacteria (AAB), and lactic acid bacteria (LAB) are present in higher frequencies at the beginning of the fermentation, (ii) LAB (mainly *Leuconostoc*, *Lactococcus* or *Lactobacillus*) dominate the fermentation after 6 h and (iii) acid-tolerant LAB remains until the end of the process. Among the eukaryotic community, generally, yeasts belonging to the genus *Pichia* show a high prevalence throughout fermentation (JUNQUEIRA *et al*., 2019; POTHAKOS *et al*., 2020; CARVALHO NETO *et al*., 2018; CARVALHO NETO *et al*., 2020).

The success of coffee fermentation depends on the indigenous microbiota associated with coffee farm microbiome. Recently, the use of starter cultures has been indicted to replace this empirical process, making coffee fermentation more predictable and controlled (CARVALHO NETO *et al*., 2018; CARVALHO NETO *et al*., 2020). One limitation in the use of starter cultures is that fermentations are usually carried out in open tanks, which can favor contamination by the natural microbiota. Due to this lack of control, the starters must establish dominance over the high load of indigenous microorganisms. In recent years, several studies have shown that yeast and LAB species (e.g, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Pichia kluyveri*, *Pichia anomala*, *Pichia fermentans*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, and *Torulaspora delbrueckii*, *Candida railenensis*) were able to suppress the indigenous microbiota during coffee fermentation, as well as produce coffee beverages with desired characteristics (PEREIRA *et al*., 2014, 2016; ELHALIS *et al*., 2021; RIBEIRO *et al*., 2020; EVANGELISTA *et al*. 2014). However, the existence of a connection between coffee farm microbiome and the resulting starter culture added, coffee fermentation has not yet been investigated.

It was recently demonstrated that co-inoculation of *Pichia fermentans* YC5.2 and *Pediococcus acidilactici* LPBC161 increases the production of metabolites (lactic acid, ethanol, and ethyl acetate) during coffee fermentation, evidencing a positive interaction between these two microbial groups (VALE *et al*., 2019). In this sense, this study aimed to use a next-generation sequencing (NGS) to evaluate the influence of the microbial communities from coffee farm processing and coffee fermentation using *P. fermentans* YC5.2 and *P. acidilactici* LPBC161. In addition, the metabolites changes occurred during the fermentation process were studied by chromatographic techniques.

3.3 Material and methods

3.3.1 Cultivation of lactic acid bacteria and yeast

The starter cultures used in this study include the lactic acid bacterium *Pediococcus acidilactici* LPBC161 and the yeast *Pichia fermentans* YC5.2. These microbial cultures were previously isolated and selected from spontaneous coffee fermentations, as reported by Pereira *et al*. (2014) and Muynarsk (2019), and are deposited in the Microbial Cultures Collection of the Department of Bioprocess and Biotechnology Engineering of the Federal University of Paraná, UFPR, Curitiba, PR, Brazil. *P. acidilactici* LPBC161 and *P. fermentans* YC5.2 were reactivated in MRS (Merck Millipore, Burling-ton, MA) and YEPG broth (Himedia, Marg, India), respectively, at 30 °C for 24 h. To achieve a concentration of approximately 10^9 CFU/mL, 0.4 L of a culture of *P. acidilactici* LPBC161 was inoculated in Erlenmeyer containing 3.6 L of a culture medium composed of glucose (5g/L), yeast extract (5 g/L), ammonium citrate (5 g /L), ammonium phosphate (5 g/L), sodium acetate (2 g/L), manganese sulfate (0.05 g/L) and Tween 80 (0.1%) [20]. *P. fermentans* YC5.2 was also cultivated and inoculated in the same way, but a culture medium containing only glucose (5g/L), yeast extract (5 g/L) was used. Yeasts and bacteria were incubated at 30 °C for 24 h. After this period, the cells were centrifuged at 5,000 x g for 10 min and resuspended in 250 mL of sterile saline solution (0.9% NaCl) and stored at 4 °C until their proper use.

3.3.2 Area of study and sampling procedure

The experiments were conducted at Shalom farm situated in Patrocínio, Minas Gerais state, Brazil (18° 56' 38" S 46° 59' 34" O). The environmental samples were collected in triplicate and are composed of: (i) 100g of soil collected at 10 cm depth in an area of 1m2 in the treetops; (ii) 10 g of coffee leaves collected from the soil surface; (iii) 20 fruits collected from the soil surface; (iv) 10g of leaves collected from the branches 30 cm from the apical region; (v) 20 cherries collected from the coffee tree; (vi) 20 cherries before de-pulping; (vii) 20 over-ripe fruits collected from the coffee tree; (viii) 100 g de-pulped beans; ix) 50 mL water used for de-pulping beans. The samples were stored in falcon sterile tubes (50 mL) and transported to the Center of Agroindustrial Biotechnology of Paraná (CENBAPAR, Curitiba, Brazil) under refrigeration and maintained at −20 °C until further analysis.

Later, coffee cherries (*Coffea arabica* var. *Catuaí Amarelo*) were harvested manually and mechanically de-pulped. Approximately 100 kg of pulped coffee beans

were deposited in a 1 m^3 concrete tank containing about 50 L of water. Before inoculation, the cell viability of the starter cultures was determined by spread plate and the results were expressed in CFU/mL. Thus, the appropriate inoculum concentration of *P. fermentans* YC5.2 and *P. acidilactici* LPBC161 were adjusted to achieve an initial concentration of 10^7 CFU/mL and inoculated simultaneously in the fermentation tanks. About 100 g of coffee beans plus the liquid fraction of the coffee pulp were collected at 0, 8, 19, and 24 h. The liquid fraction was frozen in Falcon tubes sterilized at -20 °C for further analysis. Finally, the coffee beans sampled from each point were sun-dried to 12% moisture. Fermentation and sample collection were performed in triplicates.

3.3.3 Microbial community analysis by high-throughput sequencing

Before performing the total DNA extraction of the environmental samples, the microbial cells present in the samples were taken out. About 2.5 g of each sample was added to a tube containing 10 mL of saline solution (0.9% NaCl) and vigorously agitated by vortex (two treated for 2 min with an interval of 15 min). To eliminate coarse impurities, the solution was filtered through a 0.45 µm filter. Fermentation samples and water used for de-pulping beans were not submitted to this process. The total DNA extraction of the samples was performed by the Phenol-Chloroform method described by Carvalho Neto *et al*., (2018). Group-specific loci of both bacterial and fungal DNA were amplified through PCR. 20ng of DNA containing Illumina platform adapters (Caporaso *et al*., 2012), were used to amplify the hypervariable region V3/V4 of the bacterial 16S rRNA gene using the 515F-806R primers. The ITS fungal region was amplified by ITS1-ITS2 primers. Barcoded amplicons were generated by PCR under the following conditions: 95ºC for 3 min, and 18 cycles at 95°C for 30 s, 50 °C for 30 s, and 68°C for 60 s, followed by a final extension at 68°C for 10 min. Samples were sequenced in the MiSeq platform using the 500 V2 kits, following standard Illumina protocols. Bioinformatics analyses were performed according to Vale *et al*. (2021).

3.3.4 HPLC analysis of fermenting coffee pulp

The concentration of reducing sugars (glucose and fructose) and organic acids (lactic, citric, succinic, acetic, and propionic acids) in the liquid fraction of the coffee pulp mass in fermentation was determined by High-Performance Liquid Chromatography (HPLC). The samples were analyzed in an HPLC ® Agilent Technologies coupled to a refraction index (RID) and diode matrix (DAD) detector.

The separation of the compounds was obtained using a Hiplex-H column (300 × 7.7 mm) (Bio-Rad, Richmond, CA, USA) with an isocratic mobile phase composed of 4.0 mM H2SO4, with a flow rate of 0.5 mL min^{-1} for 30 min. The temperatures of the sample, column, and RID detector used during the entire race were 25, 70, and 50°C, respectively. The quantification of organic acids was performed in DAD at 210 nm while reducing sugars were determined in RID.

3.3.5 GC/MS analysis of coffee pulp and beans

The determination of volatile compounds presents in the fermentation liquid fraction and the chemical constitution of the coffee beans collected during the fermentation process were determined by Gas Chromatography Coupled to Mass Spectrophotometry (GC/MS). Briefly, the compounds were analyzed by Solid Phase Microextraction (SPME), using a DVB/CAR/PDMS Fibre (Supelco Co., Bellefonte, PA USA) and injected into GC/MS connected to an autosampler (GCMS2010 Plus, TQ8040, AO 5000; Shimadzu, Tokyo, Japan). The injection parameters used were according to the procedures described by Junqueira *et al*., (2019).

3.3.6 Statistical analysis

Statistical significance was calculated using a post-hoc comparison of means using Tukey's test. Analyses were performed using the Statistica program, version 10.0 (Statsoft Inc., Tulsa, OK, USA). The level of significance was established using a two-sided p-value < 0.05. A principal component analysis (PCoA) based on Weighted UniFrac Distances was constructed using the microbial relative prevalence data (at both family and genus levels). Analyses were performed using the Statistica program, version 10.0 (Statsoft Inc., Tulsa, OK, USA).

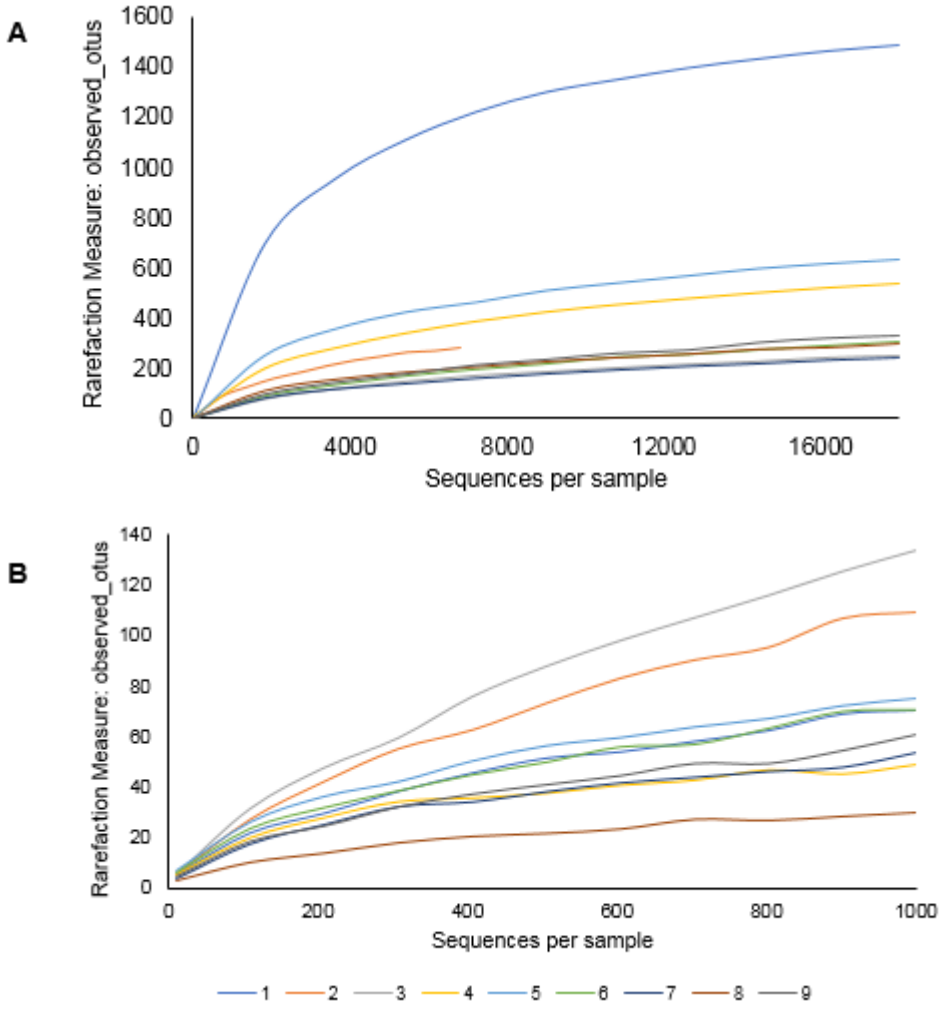
3.4 Results and discussion

3.4.1 Farm microbiome

To elucidate the influences of the environmental microbiota on the fermentation process, coffee fruits and leaves (collected from the coffee farm and on the ground), over-ripe fruits, cherries before de-pulping, de-pulped beans, and water used for de-pulping beans, were collected to analyze the bacterial and fungal communities. A total of 282.288 sequences from the hypervariable region V3-V4 of the 16S rRNA gene and 158.316 from the Internal Transcribed Spacer (ITS) rDNA gene were obtained from all samples. Sequences that presented identity above 97% were considered the same Operational Taxonomic Unit (OTUs). Thus, 299

prokaryotes and 189 eukaryotes were identified (Table S1). The rarefaction curves were satisfactory, suggesting that most bacterial and fungal communities were covered (Figure 4).

Figure 4 - Alpha rarefaction curves of observed OTUs (operational taxonomic units) from the environmental samples. (A) Bacterial analysis, (B) Fungal analysis. 1= Soil; 2= Leaves collected from the ground; 3=Fruits collected from the ground; 4= Leaves collected ffrom the coffee tree; 5 = Cherries collected from the coffee tree; 6 = Cherries before de-pulping; 7 = Over-ripe fruits; 8 = Depulped beans; 9 =Water used for de-pulping beans.



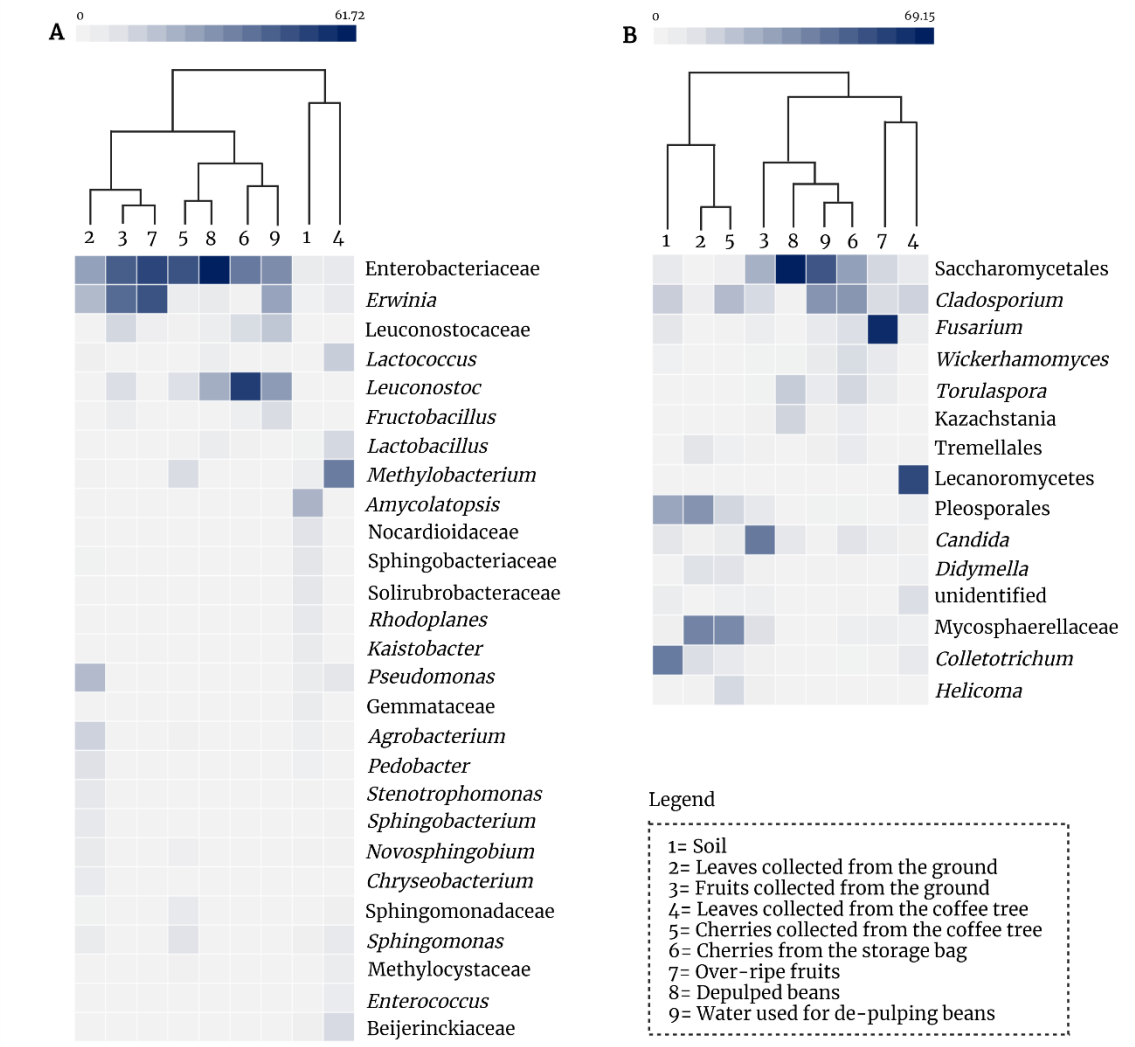
Source: The Authors (2021)

The presence and abundance of the major bacteria and fungi, defined as taxa with proportional abundance ≥2%, are reported in Figure 5. In general, Enterobacteriaceae and Saccharomycetales were the most abundant microbial groups. To facilitate visualization of the results, PCA analysis was constructed to group the coffee farm samples according to microbial abundance and diversity (Figure 6). Among the eukaryotes, the samples were divided into three clusters; (i) cherries before de-pulping, de-pulped beans, and water, characterized by the marked presence of the Saccharomycetales family; (ii) soil, leaves and fruits collected on the

ground, and fruits collected from the coffee tree, characterized by a low frequency of Saccharomycetales and dominance of specific groups such as Pleosporales, Mycosphaerellaceae, and Colletotrichum; and (iii) fruits collected on the ground, leaves collected from the coffee tree, and over-ripe fruits, characterized by the dominance of *Candida*, *Lecanoromycetes*, and *Fusarium*, respectively. These results suggest that the first grouping (mainly cherries before de-pulping and de-pulped beans) may favor the fermentative process due to the high incidence of Saccharomycetales. This order comprises about 1000 known species, including the yeasts *Pichia*, *Saccharomyces*, *Meyerozyma*, *Candida*, and *Hanseniaspora* that have been widely selected as starter cultures for coffee fermentation (ELHALIS *et al*. 2020; VALE *et al*., 2019; BRESSANI *et al*., 2020). Moreover, it is likely that most of the yeasts found in these samples are en-dophytic, since the coffee cherries collected from the coffee trees had a low incidence of Saccharomycetales (1.33%), while the de-pulped fruits contained a high frequency (69.15%) (Figure 5B). On the other hand, the marked presence of filamentous fungi, such as *Cladosporium*, *Colletotrichum*, *Fusarium*, and Mycosphaerellaceae, are undesirable in the fermentation process, and control measures should be adopted to reduce the contact of soil, leaves, fruits collect-ed from the ground, and over-ripe fruits with the fermentation tank. Interestingly, the genera Cladosporium and Fusarium were also observed at high frequency in soil from a Honduran coffee farm (VALE *et al*., 2021).

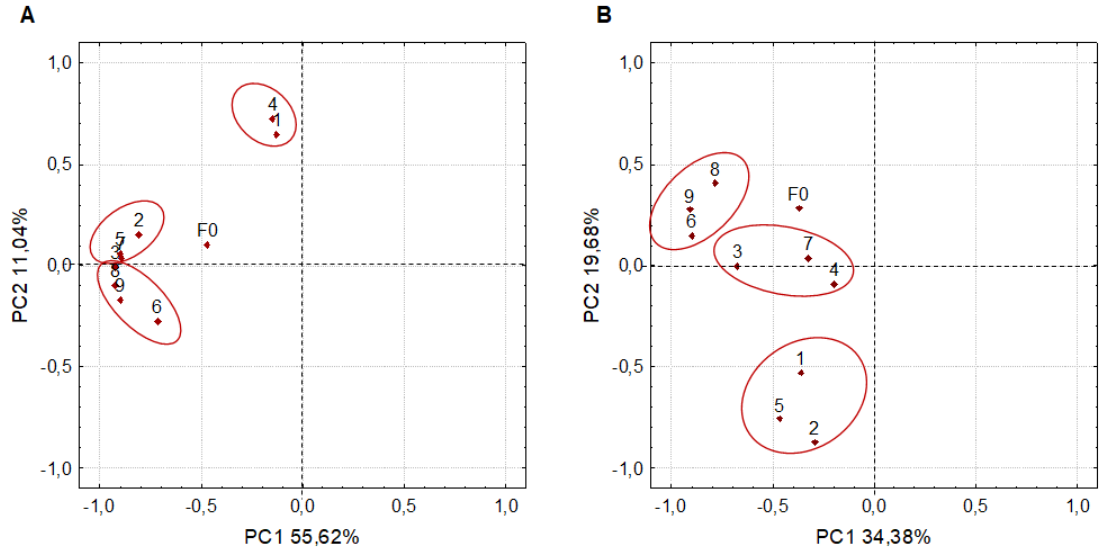
The bacterial group was also divided into three clusters; (i) leaves and fruits col-lected from the ground, cherries collected from the coffee tree and over-ripe fruits, by the high incidence of Enterobacteriaceae and Erwinia; (ii) cherries before de-pulping, depulped beans, and water, with high frequencies of Enterobacteriaceae and Leuco-nostoc; and (iii) soil and leaves collected from the coffee tree, with high dominated of Amycolatopsis and Methylobacterium, respectively, and low frequency of Enterobacteriaceae. *Lactococcus* and *Lactobacillus* were also identified in high populations in the coffee leaves (Figure 5A).

Figure 5 - Composition of bacteria (A) and fungi (B) from coffee farm samples. Only micro-organisms with prevalence superior to 2% are showed. The complete list of minor microbi-al groups is reported in the Supporting Information (Table 3).



Source: The Authors (2021)

Figure 6 - Principal component analysis, based on Weighted UniFrac Distances, according to microbial diversity and abundance. (A) Bacterial analysis, (B) Fungal analysis. 1= Soil; 2= Leaves collected from the ground; 3=Fruits collected from the ground; 4= Leaves collected from the coffee tree; 5= Cherries collected from the coffee tree; 6= Cherries before de-pulping; 7= Over-ripe fruits; 8= Depulped beans; 9= Water used for de-pulping beans; F0= Fermentation (0 h).



Source: The Authors (2021)

High incidence of Enterobacteriaceae has already been reported in grape and coffee plantations (ZHANG *et al*., 2019; VALE *et al*., 2021; NISIOTOU *et al*. 2011). In vineyards, this family is considered beneficial because they produce proteases, chitinases and glucanases, which make them excellent antagonistic microorganisms (PINTO *et al*., 2014). In addition, over 80% of the bacterial community of coffee fruits collected on the ground and over-ripe fruits was composed of Enterobacteriaceae and *Erwinia*, showing the ability of this group to secrete hydrolytic enzymes to support their growth on decaying organic matter (ZHOU; INGRAM, 2000). However, these microorganisms are not undesirable in coffee fermentation, as a correlation analysis performed by Zhang *et al*. (2019) showed that the main alcohols and esters produced from the course of the fermentation process were not associated with enterobacteria. In addition, Enterobacteriaceae are mainly associated formation of off-flavor metabolites, such as 3-isopropyl-2-methoxy-5-methylpyrazine, 2,3-butanediol, and butyric acid (GUEULE *et al*., 2015). *Leuconostoc* was observed in the coffee cherries before de-pulping (53.48%), de-pulped beans (20.24%), and in the water used for de-pulping beans (25.60%) (Figure 3A). After harvesting, coffee fruits are usually placed in storage bags for a few hours until they are processed. During this period, micro-cracks can be generated in the fruit skin and several amino acids and phosphorylated carbohydrates present in the coffee pulp

become accessible to the epiphytic microbiota of the cherries. The abundance of *Leuconostoc* in these samples may be associated with its ability to produce a wide range of saccharolytic enzymes, as well as having an elaborate carbohydrate uptake system (POTHAKOS *et al*., 2020). Shotgun metagenomic analysis of a coffee fermentation identified a gene that encodes hexose 6 phosphate: phosphate antiporter (uhpT). This transporter may favor *Leuconostoc* proliferation since phosphorylated hexoses can be transported into bacterial cells without ATP consumption (POTHAKOS *et al*., 2020).

The coffee farm soil showed a rich and complex microbial diversity, with 214 bacterial groups (Table 3). The high diversity found in the soil was also observed in other coffee-producing regions, such as China, Ecuador, Mexico, Brazil, and Honduras (ZHANG *et al*., 2019; VALE *et al*., 2021; ZHAO *et al*., 2018; CABRERA-RODRÍGUEZ *et al*., 2018; VELOSO *et al*. 2020). However, most of the bacterial groups that have been identified in coffee farm soils (e.g., Amycolatopsis, Bacillus, Bradyrhizobium, Pseudolabrys, Rhodoplanes, and Sphingomonas) are not associated with the fermentation process, so it should be avoided from having access to the fermentation tank.

Interestingly, coffee leaves showed a relatively high population of Lactococcus and Lactobacillus, which had not been reported so far. The ability of these strains to grow on leaves suggests that these microorganisms possess a metabolic versatility not yet explored, as LAB are auxotrophic for some amino acids and vitamins that are not bioavailable in coffee leaves (ZHANG *et al*., 2019; VALE *et al*., 2021; ZHAO *et al*., 2018; CABRERA-RODRÍGUEZ *et al*., 2018; VELOSO *et al*. 2020; PEREIRA *et al*., 2020). In addition, these LAB produce lactic acid as a primary fermentation product, so they are useful for coffee processing (PEREIRA *et al*., 2020).

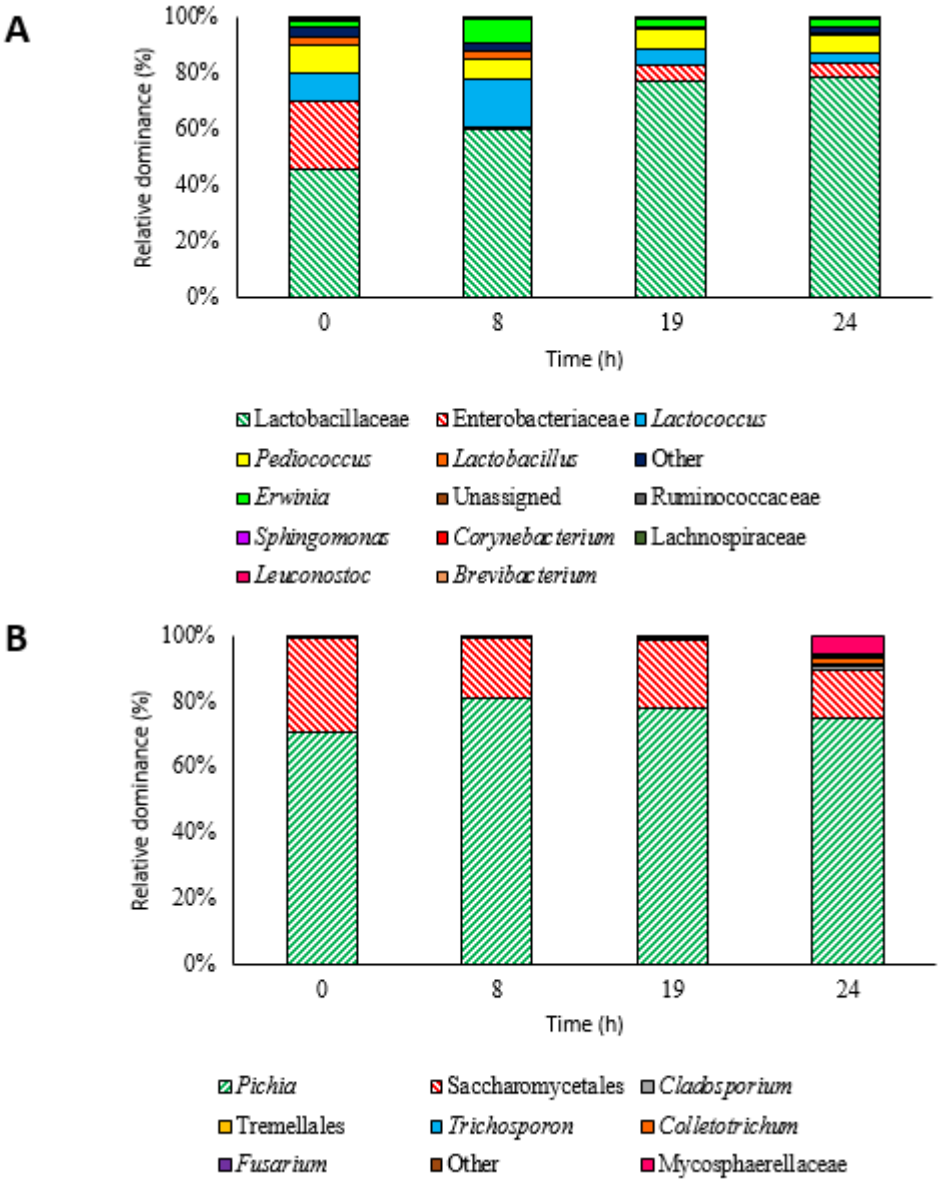
3.4.2 Microbiota dynamics during inoculated coffee fermentation

To improve the quality and complexity of fermented beverages, co-inoculation of LAB and yeast has been widely employed in the wine industry and more recently in coffee fermentations (VALE *et al*., 2021; SUN *et al*., 2021; BRESSANI, 2021). However, the starter cultures used in these processes must be able to suppress the growth of wild microbiota. The results reported in this study showed that co-inoculation favored the growth of the starter cultures (Figure 3-5). *Pichia* showed a population over 70% throughout the fermentative process. The

family Lactobacillaceae, which includes *Pediococcus*, was also dominant at the beginning of the fermentative process (45.13%) followed by Enterobacteriaceae (23.85%) and *Lactococcus* (10.11%). After 8 h, the OTU readings attributed to Lactobacillaceae increased to 59.38%, suppressing the growth of Enterobacteriaceae (0.45%) and other species. At the end of the process, the reading of Lactobacillaceae increased to 77.40% (Figure 7).

The predominance of *P. fermentans* YC5.2 over the wild microbiota is mainly associated with the metabolic versatility of this yeast in tolerating osmotic pressure (growth detected with up to 50% glucose and fructose), ability to grow over a wide pH range (pH 2.0 - 8.0), growth at temperatures ranging from 30 to 43°C, and tolerance to the main metabolites (ethanol, lactic acid, and acetic acid) produced in the course of the fermentation (Pereira *et al*., 2014). Recently, analysis of the *P. acidilactici* LPBC161 genome performed by Muynarsk *et al*. (2019) showed that its high fermentative capacity can be explained by the presence of several genes involved in the metabolism of sugars present in the coffee pulp, in addition to genes encoding proteins related to oxidative and alkaline stress. Vale *et al*. (2019) also demonstrated that there is a positive synergistic interaction between these two microbial The possible hypotheses to explain these inter-actions are (i) yeast autolysis releases nutrients, such as polysaccharides, riboflavin, and amino acids, favorable for bacterial growth, and (ii) acidification of the fermentation medium by LAB creates a favorable environment for yeast development.

Figure 7 - Prevalence and detection threshold of the highly persistent bacteria (A) and fungi (B) detected during coffee beans fermentation process conducted with selected starter cultures (Pichia fermentans YC5.2 and Pediococcus acidilactici LPBC161). The complete list of mi-nor microbial groups is reported in the Supporting Information (Table S2).



Source: The Authors (2021)

Although LAB and yeast are the main microbial group involved in coffee fermentations, the dominance of both these microbial groups may not be observed during spontaneous process. For instance, co-dominance of AAB (*Gluconobacter* and *Acetobacter*), LAB (*Leuconostoc* and *Lactococcus*), yeasts (*Hanseniaspora*, *Candida*, and *Pichia*), and the filamentous fungus *Fusarium* was recently observed in a spontaneous fermentation conducted in Honduras (VALE *et al*., 2021). The microbial dynamics of another spontaneous fermentation conducted in Australia also showed some peculiarities. Although LAB increased during the fermentation process,

it was noted that the population of Enterobacteriaceae and other subdominant microbial groups remained high after 36 h of fermentation (Elhalis *et al*., 2020). Thus, the use of starter cultures is seen as essential to ensure the growth of beneficial microorganisms (i.e., yeast and LAB) during coffee fermentation.

Interestingly, during the entire fermentation process, a total of 135 and 115 prokaryotic and eukaryotic groups were identified, respectively (Table S2). The fermentation started with only 86 prokaryotes and 45 eukaryotes. It was noted that some species (e.g., *Trichococcus*, *Terriglobus*, *Fusobacterium*, *Passalora*, and *Lecanosticta*) were not associated with the farm microbiome, suggesting that these microorganisms may have been introduced into the fermentation by human contact. In addition, several microbial genera (e.g., *Penicillium*, *Malassezia*, *Debaryomyces*, *Enterobacter*, *Pseudomonas*, and *Kaistobacter*), associated with farm microbiome, were detected only 8 h after the start of the fermentation process. Besides human contact, one of the contamination routes is likely to air since the coffee fermentations are carried out in open tanks. However, it is important to note that all these contaminants were present at frequencies ≤0.01%, showing that the starter cultures were able to suppress their growth. Thus, the use of starter cultures proves to be extremely important for maintaining desirable microbial groups during coffee beans fermentation.

3.4.3 Chemical analysis of the fermentation liquid fraction and coffee beans

The sugar consumption profile and metabolic formation showed the activity of the starter cultures used during the fermentation process. Glucose and fructose were consumed during fermentation and transformed mainly into lactic acid (associated with the metabolism of *P. acidilactici* LPBC161) and ethanol (associated with the metabolism of *P. fermentans* YC5.2) as shown in Table 4. It is possible to observe a decrease in sugars (glucose and fructose) during the 24 hours of fermentation (Table 1). Both glucose and fructose were partially consumed until the end of the process. During the fermentation process, sugars were used for microbial growth and a significant amount of ethanol and lactic acid were produced, causing a drop in pH (5.16 to 4.13). The reduction of pH levels below 4.5 is a method widely used by coffee producers to determine the end of fermentation of coffee beans during the processing of wet (CARVALHO NETO *et al*., 2018; DICKS; ENDO, 2009). In addition, in conjecture with organic acid production, ethanol generation inhibits the growth of undesirable microorganisms during the fermentation process.

Table 1 - Concentration of volatile compounds (area*10^5), organic acids, ethanol, and reducing sugars (g l−1) during coffee beans fermentation process conducted with selected starter cultures (*Pichia fermentans* YC5.2 and *Pediococcus acidilactici* LPBC161).

Compound	0 h	8 h	19 h	24 h
GC-MS (area)				
***Higher alcohols* (2)**				
1-Butanol, 3-methyl	0.27 ± 0.02^a	0.39 ± 0.02^b	0.63 ± 0.00^c	0.60 ± 0.00^c
2- Heptanol	0.17 ± 0.01^a	0.13 ± 0.00^a	ND	ND
***Ester* (4)**				
Ethyl acetate	4.10 ± 0.13^a	2.24 ± 0.78^b	3.05 ± 0.10^c	4.80 ± 0.02^a
Methyl acetate	ND	ND	3.80 ± 0.41^a	0.49 ± 0.04^b
Ethyl isobutyrate	ND	ND	0.63 ± 0.00	ND
Isoamyl acetate	ND	0.25 ± 0.00^a	0.37 ± 0.08 ^{a}	0.27 ± 0.00 ^{a}
***Aldehyde* (3)**				
Butanal, 3 methyl	0.86 ± 0.06^a	0.78 ± 0.05^{ab}	0.21 ± 0.00^b	0.74 ± 0.28^{ab}
Butanal, 2 methyl	0.63 ± 0.26^a	0.34 ± 0.00^a	0.13 ± 0.01^a	0.32 ± 0.00^a
Benzeneacetaldehyde	0.23 ± 0.02^a	0.33 ± 0.10^a	ND	ND
HPLC (g/L)				
Glucose	1.08 ± 0.11^a	0.75 ± 0.09^{ab}	0.52 ± 0.14^b	0.62 ± 0.21^b
Fructose	2.52 ± 0.22^a	1.78 ± 0.49^b	1.26 ± 0.13^b	1.47 ± 0.06^b
Lactic acid	ND	0.43 ± 0.04^a	1.24 ± 0.21^b	1.41 ± 0.12^b
Ethanol	ND	0.37 ± 0.12^a	0.50 ± 0.09^a	0.81 ± 0.08^b
pH	5.16	4.50	4.17	4.13

Source: The Authors (2021)

HPLC analysis of the liquid fermentation fraction revealed the high presence of lactic acid and ethanol. These compounds were associated with bal and yeast metab-olism, respectively. Lactic acid was the main organic acid produced, reaching a con-centration of 1.41 g/L at 24 h (Table 1). This primary metabolite is mainly produced by the central carbon metabolism of homofermentative LAB (DICKS; ENDO, 2009). The diffusion of ac-ids in the grains can influence the flavor and final quality of the beverage (PEREIRA *et al*., 2014; EVANGELISTA *et al*. 2013). Lactic acid is known to attribute desirable lactic sensory notes and contribute to the acidity and body of the final beverage (PEREIRA *et al*., 2016). Ethanol showed a constant increase throughout fermentation (0.8 g L-1), indicating a high metabolic activity of non-Saccharomyces yeasts (ELHALIS *et al*., 2020).

Among the volatiles, GC-MS analyses identified 9 compounds, 2 higher alcohols, 4 esters and 3 aldehydes (Table 1). Ethyl acetate was the most abundant com-pound formed during fermentation. *P. fermentans* YC5.2 was selected based

mainly on the production of this metabolite, which plays an important role in the development of aroma for coffee beans (PEREIRA *et al*., 2014). The diffusion and persistence of ethyl acetate in the grain contributes to the development of desirable fruit notes and nuances of grape/cherry in the coffee bevarege (PEREIRA *et al*., 2019). Other volatile compounds produced during fermentation, associated with the metabolism of early cultures, include isoamyl acetate and ethyl isobutyrate.

In addition to aromatic function, yeast used in this study as an starter culture has been reported in previous studies as producers of pectinitic enzymes. Thus, it assists in the degradation of pectin present in coffee pulp and mucilage, producing metabolites that spread within coffee beans, favoring the flavor formation of the final beverage (SILVA *et al*., 2021). Significantly, the roasted grains of fermentation with the inoculation of YC5.2 and LPBC161 brought these metabolites derived from yeast. Table 2 shows the compounds detected inside the coffee beans during the fermentation process. Twenty-five compounds were identified: 3 organic acids, 7 upper alcohols, 8 aldehydes, 1 terpene, 2 terpenes, 1 pyrazine, 1 ketone, and 2 hydrocarbons. Although the identified compounds fluctuated throughout the fermentation process, acetic acid, isorvaléric acid, butanal, 3-methyl, hexanal, benzeacetaldehyde, benzaldehyde and acetone were observed in high proportions. Interestingly, even though the main compound is identified in the liquid fraction of fermentation, ethyl acetate was only identified in coffee beans after 7 pm. In addition, isoamyl acetate and ethyl isobutyrate were not identified, which may also come from the initial metabolism.

The diffusion of volatile compounds to coffee beans is not fully understood. However, Silva *et al*. (2021) demonstrated that the compounds butanal, 2-fenontanol and isoamyl acetate presented different transfer rates from culture medium to coffee beans. The study suggested that the differences observed in the diffusion of compounds in grains may be associated with three processes (i) the parchment layer is a barrier, which "acts" as a filter; (ii) the compounds suffered metabolic reactions, decreasing their amount in coffee beans; (iii) there is an interaction between volatiles and yeast, significantly reducing the transfer of these compounds.

Table 2 - Concentration of volatile compounds (area*10^5) inside coffee beans collected during coffee fermentation process conducted with selected starter cultures (*Pichia fermentans* YC5.2 and *Pediococcus acidilactici* LPBC161).

(continue)

Compound (area)	0 h	8 h	19 h	24 h
***Organic acid* (3)**				
Acetic acid	5.35 ± 0.04^{a}	6.03 ± 0.86^{a}	9.99 ± 0.33^{b}	9.73 ± 0.84^{b}
Butanoic acid, 3-methyl	0.33 ± 0.03^{a}	0.44 ± 0.24^{ab}	0.58 ± 0.08 ^{ab}	0.85 ± 0.18 ^{b}
Isovaleric acid	0.36 ± 0.06^{a}	1.39 ± 0.18^{b}	0.54 ± 0.12^{ac}	0.77 ± 0.04^{c}
***Higher alcohols* (7)**				
Propanol, 2-methyl	0.16 ± 0.00^{a}	0.13 ± 0.00^{ab}	0.11 ± 0.00^{b}	0.11 ± 0.02^{b}
1-Octen-3-ol	0.41 ± 0.03^{a}	0.29 ± 0.05^{b}	0.22 ± 0.00^{b}	0.20 ± 0.03^{b}
2-Hexanol, 5 methyl	0.59 ± 0.10^{a}	0.59 ± 0.06^{a}	0.46 ± 0.05^{ab}	0.33 ± 0.01^{b}
1-Butanol, 3-methyl	-	0.60 ± 0.24^{a}	0.28 ± 0.00^{a}	0.59 ± 0.12^{a}
2-Heptanol, 3-methyl	-	-	-	0.13 ± 0.03
1-Butanol, 2-methyl	-	-	-	0.28 ± 0.00
Phenylethyl Alcohol	-	-	-	0.26 ± 0.03
***Aldehyde* (8)**				
Butanal, 3 methyl	3.33 ± 0.46^{ab}	4.39 ± 0.33^{b}	2.48 ± 0.18^{c}	1.50 ± 0.79^{c}
Butanal, 2 methyl	0.70 ± 0.07^{ab}	0.76 ± 0.07^{b}	0.26 ± 0.00^{ac}	0.39 ± 0.00^{c}
Hexanal	2.01 ± 0.32^{a}	3.49 ± 0.56^{bc}	3.97 ± 0.52^{c}	2.44 ± 0.27^{ab}
Heptanal	0.20 ± 0.02^{a}	0.07 ± 0.01^{b}	-	-
Benzeacetaldehyde	1.06 ± 0.06a	1.07 ±0.03^{a}	0.94 ± 0.05^{ab}	0.81 ± 0.02^{b}
Benzaldehyde	1.56 ± 0.06^{a}	1.23 ± 0.12^{b}	0.90 ± 0.03^{c}	0.89 ± 0.06^{c}
Pentanal	0.61 ± 0.06^{a}	0.58 ± 0.21^{a}	0.58 ± 0.04^{a}	0.49 ± 0.04^{a}
Methional	0.70 ± 0.00^{a}	0.79 ± 0.22^{a}	0.67 ± 0.12^{a}	0.30 ± 0.04^{b}
***Ester* (1)**				
Ethyl acetate	-	-	0.13 ± 0.01^{a}	0.25 ± 0.00^{b}
***Terpenes* (2)**				
Linalol	0.65 ± 0.00a	0.71 ± 0.04^{ab}	0.95 ± 0.00^{b}	0.61 ± 0.16^{a}
Limonene	-	-	0.17 ± 0.01	-
***Pyrazine* (1)**				
2-Isobuttyl-3-methoxypyrazine	0.23 ± 0.11	-	-	-
***Ketones* (1)**				
Acetoin	0.47 ± 0.05^{a}	3.49 ± 0.51^{bc}	4.15 ± 0.08^{c}	3.30 ± 0.09^{b}

Table 2 - Concentration of volatile compounds (area*10^5) inside coffee beans collected during coffee fermentation process conducted with selected starter cultures (*Pichia fermentans* YC5.2 and *Pediococcus acidilactici* LPBC161).

(conclusion)

Compound (area)	0 h	8 h	19 h	24 h
***Hydrocarbons* (2)**				
Toluene	0.23 ± 0.10[a]	0.22 ± 0.01[a]	-	-
Nonane, 3-methyl-5-propyl	0.27 ± 0.00	-	-	-
***Furanone* (1)**				
Furan, 2-pentyl	-	0.33 ± 0.07[a]	0.27 ± 0.06[a]	0.15 ± 0.03[a]

Source: The Authors (2021)

Finally, several other compounds identified in the roasted grains in the fermentation process were detected. These volatiles can be generated mainly during the course of fermentation by biochemical reactions within the bean or even by reactions that occur during the roasting process. It is known that the volatile fraction of coffee beans develops mainly in the form of alcohols, acids, esters and aldehydes (GONZALEZ-RIOS *et al*., 2007). Compounds of these classes are associated with flavor during coffee fermentation.

3.5 Conclusion

The coffee farm microbiome is composed of a rich microbiome diversity dominated by Saccharomycetales and Enterobacteriaceae. Coffee cherries before de-pulping and depulped beans harbour beneficial microorganisms (yeast and LAB) for the fermentation process. On the other hand, enterobacteria, filamentous fungi and other microbial groups presents in soil, leaves, fruits collected from the ground and over-ripe fruits may transfer unwanted aromas to coffee beans. Therefore, they should be prevented from having access to the fermentation tank. Thus, cleaning procedures should be performed to prevent the growth of these unwanted microbial groups.

The inoculation with high titers of selected yeast and LAB start culture modulates the overall fermentation over wild microbiota, with efficient sugar mucilage consumption and aroma compounds formation. Thus, the results of this study showed that the introduction of starter cultures is essential to control the coffee fermentation process in terms of both kinetics and quality of the resulting product.

4 GENERAL COCLUSIONS

The results of this study showed that *Pediococcus acidilactici* LPBC161 and *Pichia fermentans* YC5.2 were successfully implemented as starter cultures in coffee fermentation during wet processing. Their metabolic activities during fermentation process was shown to influence the final volatile fraction of roasted coffe beans. In addition, *Pediococcus acidilactici* LPBC161 and *Pichia fermentans* YC5.2 were able to control the development of undesirable microorganisms. These results suggest that mixed cultures can be used by combining the aromatic and functional microbial properties, improving the quality and value of the final product.

With the data generated in this project, a better understanding of the microbial interaction environment and starters used during coffee wet processing was obtained. This will enable a better control of the process and improvement in the quality of the coffe beans produced in Brazil. However, future studies should be conducted to confirm the best mixed cultures to be used and assess its ability to inhibit the growth of unwanted microbiota. As well as the interaction with different microbiotas of the Brazilian states.

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APPENDIX A - Complete list of minor microbial groups of fungi and bacteria in environmental samples

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations. (continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Fungi									
Saccharomycetales	3.10	0.04	21.44	3.29	1.33	27.33	8.95	69.15	51.71
Cladosporium	12.25	1.71	8.04	10.85	18.45	30.39	7.28	0.34	31.05
Fusarium	3.98	0.14	1.72	2.01	0.48	6.44	65.59	0.13	3.03
Wickerhamomyces	0.93	<0.01	0.57	0.15	0.07	7.46	3.00	0.58	2.88
Torulaspora	0.03	-	0.57	-	0.06	9.03	2.62	12.42	2.67
Kazachstania	0.03	-	0.77	-	0.07	2.32	0.05	10.13	1.09
Tremellales	0.06	4.36	0.67	0.51	1.18	2.67	0.38	0.26	1.00
Kodamaea	-	-	0.10	-	0.02	0.71	0.89	0.28	0.78
Lecanoromycetes	-	-	-	55.89	-	-	-	-	-
Other	2.57	8.94	6.03	2.31	6.27	0.94	0.29	0.18	0.66
Dothideomycetes	-	-	-	0.77	-	-	-	-	-
Saccharomyces	-	0.14	0.29	-	1.54	0.98	0.73	1.56	0.65
Pleosporales	25.15	31.28	3.44	1.49	9.18	0.59	0.18	0.11	0.64
Candida	4.06	0.75	39.81	1.03	2.58	4.99	2.22	3.67	0.53
Dothioraceae	0.03	0.11	-	1.75	0.38	0.08	0.58	0.09	0.45
Didymella	0.08	5.58	0.67	1.18	5.13	0.55	0.01	0.03	0.44
Issatchenkia	-	-	0.10	-	-	0.98	0.85	0.03	0.41
unidentified	2.23	0.05	1.53	6.84	1.26	0.55	0.06	0.02	0.25
Nectriaceae	0.28	<0.01	0.38	0.15	0.15	0.20	2.41	<0.01	0.24
Pleosporaceae	0.08	0.58	0.19	0.21	0.68	0.08	0.02	<0.01	0.21
Mycosphaerellaceae	1.04	36.72	5.93	1.23	34.74	1.61	1.97	0.10	0.15
Colletotrichum	39.77	6.65	0.77	3.19	3.06	0.31	0.43	0.05	0.13
Helicoma	-	0.44	1.05	-	8.52	-	0.16	<0.01	0.08
Citeromyce	-	-	-	-	-	-	0.02	-	0.08
Xylariales	-	-	-	-	<0.01	0.20	0.09	-	0.07
Meyerozyma	-	-	-	-	-	0.27	<0.01	0.38	0.07
Hanseniaspora	-	-	-	-	-	-	0.01	0.06	0.06
Aureobasidium	-	-	-	-	<0.01	-	0.04	0.02	0.05
Strelitziana	0.03	1.14	0.19	0.36	2.29	0.04	0.08	<0.01	0.05
Starmerella	-	-	-	-	-	-	-	-	0.05
Saturnispora	-	-	-	-	-	-	-	0.11	0.04
Hypocreales	0.23	-	0.48	0.41	-	0.24	0.08	<0.01	0.04
Chaetothyriales	0.08	0.04	0.38	0.15	0.97	0.08	0.04	<0.01	0.04

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Capnodiales	0.03	0.02	0.10	-	0.03	-	<0.01	-	0.
Lachancea	-	-	-	-	-	0.08	0.01	0.04	0.02
Alternaria	0.06	0.02	-	0.31	-	-	0.01	<0.01	0.02
Mortierella	0.08	<0.01	0.19	0.31	-	0.08	0.06	<0.01	0.02
Aspergillus	-	-	-	0.41	-	0.04	0.02	0.10	0.01
Dothiorella	-	-	-	0.05	-	-	-	<0.01	0.01
Cyphellophora	-	-	0.10	-	0.67	-	0.04	<0.01	0.01
Debaryomyces	0.03	-	0.10	-	-	0.04	<0.01	<0.01	0.01
Saccharomycetaceae	-	-	-	-	-	-	-	0.03	0.01
Beauveria	0.03	-	0.10	0.15	-	-	-	-	<0.01
Penicillium	0.06	-	-	0.05	-	0.12	0.03	-	<0.01
Pseudorobillarda	-	-	-	-	-	-	-	-	<0.01
Hannaella	-	0.59	-	-	-	-	-	-	<0.01
Nigrospora	0.03	-	-	1.44	<0.01	-	<0.01	-	<0.01
Trichocomaceae	0.03	<0.01	-	0.05	-	-	0.03	-	<0.01
Cystofilobasidium	-	-	-	-	-	-	-	<0.01	<0.01
Naganishia	-	-	-	-	-	-	-	<0.01	<0.01
Zygosaccharomyces	-	-	-	-	-	-	-	-	<0.01
Capnodiaceae	-	-	-	0.05	-	0.12	-	-	<0.01
Conocybe	-	-	-	-	-	-	-	-	<0.01
Mucor	-	-	-	-	-	-	-	-	<0.01
Trichosporon	0.03	-	0.10	-	-	0.08	<0.01	-	<0.01
Chaetomium	0.14	<0.01	0.10	0.36	-	-	0.01	-	<0.01
Bipolaris	0.03	-	-	0.05	-	-	-	-	<0.01
Clohesyomyces	0.03	-	-	0.10	-	-	<0.01	<0.01	<0.01
Helotiales	0.03	-	0.96	-	-	-	0.09	<0.01	<0.01
Sporobolomyces	-	-	-	-	-	-	<0.01	-	<0.01
Talaromyces	0.03	-	0.10	0.10	0.03	0.04	0.12	-	<0.01
Chaetomiaceae	0.03	-	-	0.15	-	-	<0.01	-	<0.01
Meira	-	-	-	-	0.06	-	0.05	-	<0.01
Rhodotorula	-	-	-	-	-	-	-	-	<0.01
Sordariales	0.03	-	0.29	0.15	-	0.04	0.01	<0.01	<0.01
Trichoderma	0.03	-	0.57	0.05	0.07	0.04	0.06	-	<0.01
Bacidia	-	-	-	-	-	-	-	-	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Bionectriaceae	0.03	<0.01	-	-	-	-	<0.01	-	<0.01
Glomeraceae	-	0.01	-	0.15	<0.01	-	-	-	<0.01
Hirsutella	-	-	-	-	-	-	-	-	<0.01
Parmeliaceae	-	-	-	-	-	-	-	-	<0.01
Pleomassariaceae	0.03	-	-	-	-	-	-	-	<0.01
Septobasidium	-	-	-	-	0.12	-	-	-	<0.01
Chaetosphaeriaceae	0.06	0.01	-	-	<0.01	-	0.01	-	<0.01
Cryphonectriaceae	-	-	-	-	-	-	0.14	-	<0.01
Cryptococcus	-	-	-	-	-	-	-	<0.01	<0.01
Dioszegia	-	-	-	-	-	-	-	-	<0.01
Lecanorales	-	-	-	-	-	-	-	-	<0.01
Magnaporthaceae	-	-	-	-	-	0.04	<0.01	-	<0.01
Malassezia	-	-	-	-	0.03	-	<0.01	-	<0.01
Pichia	-	-	-	-	-	-	-	<0.01	<0.01
Stagonosporopsis	-	-	-	-	-	-	-	-	<0.01
Acarosporina	-	-	-	-	-	0.04	0.02	-	<0.01
Aplosporella	-	-	-	-	-	-	-	-	<0.01
Cantharellales	-	-	-	0.05	0.06	-	-	-	<0.01
Curvularia	0.06	-	-	0.05	-	-	<0.01	-	<0.01
Entoloma	-	-	-	0.05	-	-	-	-	<0.01
Lasiosphaeriaceae	-	<0.01	-	0.10	-	-	<0.01	-	<0.01
Microdochium	0.06	-	0.10	-	-	-	0.03	-	<0.01
Myrothecium	-	-	-	-	-	-	-	-	<0.01
Phyllozyma	-	-	-	0.15	-	-	-	-	<0.01
Rachicladosporium	-	-	-	-	-	-	-	-	<0.01
Venturiales	-	-	-	0.05	-	-	-	-	<0.01
Agaricaceae	-	-	0.10	-	-	-	-	-	<0.01
Articulospora	-	-	-	-	-	-	-	-	<0.01
Buellia	-	-	-	-	-	-	-	-	<0.01
Bullera	-	-	-	-	-	-	-	-	<0.01
Chaetosphaeria	-	-	-	0.05	-	-	-	-	<0.01
Chloridium	-	-	-	-	-	-	-	-	<0.01
Choanephoraceae	-	-	-	-	-	-	-	-	<0.01
Claroideoglomus	-	-	-	0.05	-	-	-	-	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Clavicipitaceae	-	-	-	-	-	-	-	-	<0.01
Coniochaetaceae	-	-	-	-	0.02	-	-	-	<0.01
Coniochaetales	-	-	-	-	-	-	-	-	<0.01
Debaryomycetaceae	-	-	-	-	-	-	-	-	<0.01
Diaporthe	-	-	-	-	-	-	0.01	-	<0.01
Dictyosporium	-	-	-	-	-	-	-	-	<0.01
Elsinoaceae	-	-	-	-	-	-	-	<0.01	<0.01
Exophiala	-	<0.01	-	-	-	0.04	-	-	<0.01
Gongronella	0.03	<0.01	-	-	-	-	-	-	<0.01
Helotiaceae	-	-	-	-	-	-	-	-	<0.01
Hyaloscyphaceae	-	-	-	-	<0.01	-	<0.01	-	<0.01
Kwoniella	-	-	-	-	-	-	-	-	<0.01
Lecanicillium	-	-	-	-	-	-	-	-	<0.01
Lepiota	-	-	-	-	-	-	-	-	<0.01
Metacordyceps	-	-	-	-	-	-	-	-	<0.01
Metarhizium	-	<0.01	0.10	-	-	0.04	-	-	<0.01
Nakazawaea	-	-	-	-	-	-	-	-	<0.01
Phaeosphaeria	-	<0.01	-	-	-	-	-	-	<0.01
Phaeosphaeriaceae	-	-	-	-	0.07	-	-	-	<0.01
Physciaceae	-	-	-	-	-	-	-	-	<0.01
Pseudeurotiaceae	-	-	-	-	-	-	0.02	<0.01	<0.01
Pseudophialophora	-	-	-	-	-	-	-	-	<0.01
Rhytidhysteron	-	-	-	-	-	-	-	-	<0.01
Septobasidiaceae	-	-	-	-	0.05	-	-	-	<0.01
Sporormiaceae	0.03	-	-	0.05	-	-	-	-	<0.01
Tubeufiaceae	-	-	-	0.05	-	-	<0.01	-	<0.01
Westerdykella	-	<0.01	-	-	-	-	-	-	<0.01
Sporidiobolales	-	-	-	-	-	-	0.02	-	-
Subulicystidium	-	-	-	-	-	-	0.01	<0.01	-
Lindgomycetaceae	-	-	0.10	-	-	-	0.01	-	-
Myrmecridium	-	-	-	-	-	-	0.01	-	-
Paraglomerales	-	-	-	-	-	-	0.01	-	-
Eurotiales	-	-	-	-	-	-	<0.01	0.02	-
Archaeosporaceae	-	-	-	-	-	-	<0.01	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Ophiostoma	-	-	-	-	-	-	<0.01	-	-
Rhizophlyctis	0.06	-	-	-	-	-	<0.01	-	-
Apiosporaceae	-	-	-	-	-	-	<0.01	-	-
Atractiellales	-	<0.01	0.10	0.05	-	-	<0.01	<0.01	-
Glomerales	-	-		0.05	-	-	-	-	-
Auricularia	-	-	-	-	-	-	<0.01	-	-
Auriculariales	-	-	-	-	-	-	<0.01	-	-
Cordycipitaceae	-	-	-	-	-	-	0.02	-	-
Diatrypaceae	-	-	-	-	-	0.08	<0.01	-	-
Glomus	-	-	-	-	-	-	<0.01	-	-
Hypholoma	-	-	-	-	-	-	<0.01	-	-
Ophiocordycipitaceae	-	-	-	-	-	-	<0.01	-	-
Paraglomus	-	-	0.10	-	-	-	<0.01	-	-
Periconia	-	-	-	-	-	-	<0.01	<0.01	-
Scedosporium	-	-	-	-	-	-	<0.01	-	-
Strophariaceae	-	-	-	-	-	-	<0.01	-	-
Trechispora	-	-	-	-	-	-	<0.01	-	-
Umbelopsis	-	-	-	-	-	-	<0.01	-	-
Purpureocillium	2.74	0.26	0.29	0.05	0.05	0.04	-	<0.01	-
Ceriporia	-	-	-	-	-	-	-	<0.01	-
Ochroconis	-	-	-	-	-	-	-	<0.01	-
Cercospora	-	-	-	-	0.06	-	-	<0.01	-
Cyberlindnera	-	-	-	-	-	-	-	<0.01	-
Microascaceae	-	-	-	-	-	-	-	<0.01	-
Phaeosphaeria	-	-	-	0.05	-	-	-	-	-
Thermomyces	-	-	-	-	-	-	-	<0.01	-
Microstromatales	-	-	-	-	0.11	-	-	-	-
Tetraplosphaeria	-	-	-	-	0.06	-	-	-	-
Symmetrospora	-	-	-	-	0.04	-	-	-	-
Annulohypoxylon	-	-	-	-	<0.01	-	-	-	-
Ganodermataceae	0.03	-	0.19	-	<0.01	-	-	-	-
Multiseptospora	-	-	-	-	<0.01	-	-	-	-
Phanerochaetaceae	-	0.30	-	-	-	-	-	-	-
Ceratobasidiaceae	-	<0.01	-	-	-	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Entolomataceae	-	<0.01	0.10	-	-	-	-	-	-
Herpotrichiellaceae	-	<0.01	0.10	0.15	-	-	-	-	-
Sordariaceae	-	<0.01	-	-	-	-	-	-	-
Preussia	-	-	-	-	-	0.04	-	-	-
Diversispora	0.08	-	0.10	0.05	-	-	-	-	-
Glomerellaceae	0.03	-	-	-	-	-	-	-	-
Pestalotiopsis	0.03	-	0.10	-	-	-	-	-	-
Verticillium	0.03	-	-	-	-	-	-	-	-
Pseudocercospora	-	-	-	0.41	-	-	-	-	-
Sordariomycetes	-	-	-	0.41	-	-	-	-	-
Conlarium	-	-	0.19	0.10	-	-	-	-	-
Hypotrachyna	-	-	-	0.05	-	-	-	-	-
Agaricomycetes	-	-	-	0.05	-	-	-	-	-
Monographella	-	-	-	0.05	-	-	-	-	-
Orbiliaceae	-	-	-	0.05	-	-	-	-	-
Scytalidium	-	-	-	0.05	-	-	-	-	-
Castanediella	-	-	0.19	-	-	-	-	-	-
Clitopilus	-	-	0.10	-	-	-	-	-	-
Ganoderma	-	-	0.10	-	-	-	-	-	-
Lycoperdon	-	-	0.10	-	-	-	-	-	-
Psathyrellaceae	-	-	0.10	-	-	-	-	-	-
Bacteria									
Enterobacteriaceae	2.30	24.05	43.77	2.77	47.00	35.58	50.70	61.72	30.02
Erwinia	0.80	16.61	39.97	2.88	1.91	0.37	47.01	2.54	23.24
Leuconostocaceae	-	0.01	7.94	0.18	0.73	6.68	1.21	1.56	12.85
Lactococcus	0.23	0.89	0.01	10.83	0.19	0.10	-	1.56	0.35
Leuconostoc	0.23	0.03	5.88	-	5.46	53.49	0.15	20.24	25.61
Fructobacillus	-	<0.01	1.53	-	0.05	1.28	0.22	0.11	6.46
Acetobacteraceae	0.43	0.07	<0.01	0.12	0.81	0.03	0.02	-	0.01
Methylobacterium	1.74	0.41	0.04	34.79	6.57	0.14	0.06	0.23	0.03
Amycolatopsis	18.33	-	<0.01	-	-	-	-	-	-
Yaniella	<0.01	0.02	0.01	0.24	0.03	0.02	<0.01	<0.01	<0.01
Acetobacter	-	-	0.02	-	-	0.06	0.01	0.84	0.06
Gluconobacter	-	-	0.05	-	0.02	0.02	<0.01	0.05	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Lactobacillaceae	-	-	<0.01	0.06	-	0.02	<0.01	0.67	0.05
Lactobacillus	0.52	0.07	0.06	7.65	0.32	0.66	0.05	1.84	0.23
Weissella	-	-	0.04	-	-	0.18	-	-	0.21
Pantoea	0.02	0.20	0.15	0.06	0.02	-	0.17	0.01	0.12
Staphylococcus	0.53	0.09	0.06	1.71	0.68	0.11	0.04	0.12	0.02
Enterococcus	0.03	0.03	<0.01	2.06	-	-	-	<0.01	0.02
Pseudomonas	2.15	16.47	0.02	3.59	0.24	0.03	-	0.05	0.03
Methylocystaceae	-	-	-	2.00	0.69	0.01	-	-	-
Ruminococcaceae	1.45	-	0.01	0.29	1.83	<0.01	<0.01	0.02	<0.01
Kaistobacter	2.60	0.01	<0.01	-	0.25	-	0.01	-	<0.01
Beijerinckiaceae	0.93	-	<0.01	7.12	0.13	<0.01	-	-	-
Clostridium	-	-	-	-	0.02	-	<0.01	0.03	-
Gemmataceae	2.12	-	<0.01	-	-	-	-	-	<0.01
Erythrobacteraceae	1.83	0.03	<0.01	-	-	-	-	-	<0.01
Sphingomonadaceae	-	0.59	0.03	0.88	2.75	0.06	0.02	-	-
Sphingomonas	0.93	2.23	0.02	2.71	4.42	0.11	<0.01	0.08	0.05
Bacillus	1.08	0.04	-	0.53	0.20	-	-	0.05	<0.01
Alloiococcus	0.17	-	-	-	0.22	-	-	0.06	-
Pseudomonadaceae	-	0.25	-	1.65	1.67	0.05	-	0.57	<0.01
Novosphingobium	0.30	2.64	0.02	0.29	1.27	0.08	0.01	-	<0.01
Gemmata	1.14	-	-	-	0.04	<0.01	-	0.05	-
Sphingobacterium	-	3.17	0.02	0.29	-	-	-	-	<0.01
Solirubrobacteraceae	3.64	-		0.12	-	<0.01	-	-	<0.01
Comamonadaceae	1.37	1.05	<0.01	0.06	0.56	0.02	0.01	0.14	0.02
Chryseobacterium	0.25	2.42	-	0.12	0.12	<0.01	-	-	0.01
Luteibacter	0.08	0.02	-	-	0.32	0.01	-	-	<0.01
Micrococcaceae	1.37	<0.01	-	0.24	0.10	<0.01	-	-	-
Oxalobacteraceae	0.43	1.60	-	0.18	0.10	-	-	-	<0.01
Bradyrhizobium	0.72	0.01	-	1.71	-	-	-	-	-
Nocardioidaceae	4.50	-	-	0.18	0.10	-	0.01	-	-
Agrobacterium	1.39	9.42	0.01	0.47	0.60	0.05	-	0.05	0.01
Chitinophagaceae	1.20	0.01	<0.01	0.47	0.49	<0.01	-	-	-
Gaiellaceae	1.78	-	-	0.29	-	-	-	-	-
Rhodospirillaceae	1.27	<0.01	-	-	-	-	-	-	-
Pedobacter	1.10	5.02	-	0.06	-	0.01	<0.01	-	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Actinomycetospora	0.49	0.02	-	1.06	1.13	<0.01	-	-	-
Rhodoplanes	3.04	<0.01	-	-	-	-	-	-	-
Phormidium	1.37	0.02	-	-	-	-	-	-	-
Stenotrophomonas	0.43	3.46	0.01	-	-	0.02	-	0.05	<0.01
Paenibacillus	0.43	-	<0.01	-	1.30	0.01	0.02	1.13	-
Prevotella	-	-	-	-	1.19	-	-	0.64	<0.01
Rikenellaceae	0.09	-	-	0.06	1.13	<0.01	-	0.04	-
Microbacteriaceae	0.08	1.15	-	0.82	-	0.01	-	-	<0.01
Aurantimonadaceae	0.24	0.11	-	0.41	1.06	0.02	0.01	0.09	0.01
Hymenobacter	0.22	0.04	-	0.71	1.93	-	<0.01	-	-
Paenibacillaceae	0.03	1.03	-	0.12	-	-	-	-	<0.01
Sphingobacteriaceae	4.02	0.55	-	-	0.09	0.03	-	-	0.05
Dolo_23	1.78	-	-	-	-	-	-	-	<0.01
Pseudanabaenaceae	1.45	-	-	-	-	-	-	-	-
Geodermatophilaceae	0.87	<0.01	-	0.12	0.03	-	-	-	-
Candidatus Nitrososphaera	0.87	-	-	-	-	-	-	-	-
Modestobacter	0.75	-	-	-	-	-	-	-	-
Geodermatophilus	0.71	-	-	-	0.71	-	-	-	-
Patulibacteraceae	0.71	0.01	-	-	-	-	-	-	-
Pseudonocardia	0.71	-	-	-	-	-	-	-	-
Nocardioides	0.70	<0.01	-	-	<0.01	-	-	-	-
Conexibacteraceae	0.67	-	-	-	-	-	-	-	-
DA101	0.63	-	-	-	-	-	-	-	-
Bdellovibrio	0.55	0.03	-	0.29	-	-	-	-	<0.01
Micromonosporaceae	0.52	-	-	-	0.19	-	-	-	-
Burkholderia	0.47	0.03	0.04	-	0.28	<0.01	-	-	-
Xanthomonadaceae	0.44	0.11	<0.01	-	-	0.05	-	0.04	-
Intrasporangiaceae	0.44	-	<0.01	-	-	-	-	-	-
Balneimonas	0.42	-	-	-	-	-	-	-	<0.01
Streptococcus	0.41	0.01	<0.01	0.65	0.41	-	-	0.09	-
Bradyrhizobiaceae	0.40	<0.01	-	0.35	0.10	0.01	<0.01	-	<0.01
EB1017	0.40	-	-	-	-	-	-	-	-
Segetibacter	0.37	-	-	-	-	-	-	-	-
Coxiellaceae	0.37	-	-	-	-	-	-	-	-
Sporichthyaceae	0.35	-	-	0.18	-	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Ralstonia	0.34	-	-	-	-	-	-	0.02	-
Aeromicrobium	0.34	0.03	-	-	-	<0.01	-	-	-
mb2424	0.34	-	-	-	<0.01	-	-	-	-
Rubellimicrobium	0.33	-	-	-	-	-	-	-	-
Sphingobium	0.32	0.26	0.01	0.65	0.54	<0.01	-	-	<0.01
Faecalibacterium	0.31	-	-	0.12	0.38	<0.01	<0.01	0.05	-
Syntrophobacteraceae	0.31	-	-	-	-	-	-	-	-
Ellin5301	0.29	-	-	-	-	-	-	-	-
Bacteroides	0.28	<0.01	0.02	-	0.10	<0.01	-	<0.01	-
Planctomyces	0.28	-	-	-	-	-	-	-	-
Ellin506	0.28	-	-	-	0.08	0.02	-	-	-
[Ruminococcus]	0.27	0.01	<0.01	0.65	0.62	<0.01	<0.01	<0.01	<0.01
Clostridiaceae	0.26	-	-	-	0.40	<0.01	<0.01	-	-
Mycobacterium	0.25	-	-	-	0.03	-	-	-	-
RB40	0.25	-	-	-	-	-	-	-	-
Afifella	0.25	-	-	-	-	-	-	-	-
C111	0.24	-	-	-	-	-	-	-	-
Flavisolibacter	0.23	-	-	-	-	-	-	-	<0.01
Ellin6075	0.23	-	<0.01	-	0.08	-	-	-	<0.01
Hyphomicrobiaceae	0.23	-	-	-	-	-	0.01	-	-
Phycicoccus	0.22	-	-	-	-	-	-	-	-
Virgisporangium	0.22	-	-	-	-	-	-	-	-
Methylobacteriaceae	0.21	0.01	-	0.71	0.30	-	0.02	-	<0.01
Rubrobacter	0.21	-	-	-	-	-	-	-	-
[Kouleothrixaceae]	0.20	-	-	-	-	-	-	-	-
AK1AB1_02E	0.20	-	-	-	-	-	-	-	-
Enhydrobacter	0.20	-	-	-	0.22	-	-	-	-
Lysobacter	0.20	-	-	-	-	-	-	-	<0.01
Fimbriimonas	0.19	-	-	-	0.23	-	-	-	-
Haliangiaceae	0.19	-	-	-	-	<0.01	-	-	-
Chthonomonas	0.16	-	-	-	-	-	-	-	-
Bacillaceae	0.15	0.02	0.02	0.47	0.27	0.05	0.05	0.01	<0.01
Legionella	0.15	-	-	-	-	-	-	-	-
Blautia	0.15	0.02	-	-	0.50	<0.01	-	-	-
Isosphaeraceae	0.14	-	-	-	-	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Leptolyngbya	0.14	-	-	-	-	-	-	-	-
Pseudonocardiaceae	0.13	-	-	-	-	-	-	-	-
Pedomicrobium	0.13	-	-	-	0.14	-	-	-	-
Hylemonella	0.12	0.23	-	-	0.06	<0.01	0.02	-	<0.01
Chthoniobacter	0.12	-	-	-	-	-	-	-	-
CM44	0.12	-	-	-	-	-	-	-	-
Devosia	0.12	0.01	-	0.06	0.15	<0.01	-	0.03	<0.01
Oscillospira	0.12	-	<0.01	-	0.62	<0.01	<0.01	0.02	-
[Entotheonellaceae]	0.12	-	-	-	-	-	-	-	-
Corynebacterium	0.12	0.03	0.01	0.18	0.22	0.03	-	0.01	<0.01
Faecalibacterium	0.31	-	-	0.12	0.38	<0.01	<0.01	0.05	-
Syntrophobacteraceae	0.31	-	-	-	-	-	-	-	-
Ellin5301	0.29	-	-	-	-	-	-	-	-
Bacteroides	0.28	<0.01	0.02	-	0.10	<0.01	-	<0.01	-
Planctomyces	0.28	-	-	-	-	-	-	-	-
Ellin506	0.28	-	-	-	0.08	0.02	-	-	-
[Ruminococcus]	0.27	0.01	<0.01	0.65	0.62	<0.01	<0.01	<0.01	<0.01
Clostridiaceae	0.26	-	-	-	0.40	<0.01	<0.01	-	-
Mycobacterium	0.25	-	-	-	0.03	-	-	-	-
RB40	0.25	-	-	-	-	-	-	-	-
Afifella	0.25	-	-	-	-	-	-	-	-
C111	0.24	-	-	-	-	-	-	-	-
Flavisolibacter	0.23	-	-	-	-	-	-	-	<0.01
Ellin6075	0.23	-	<0.01	-	0.08	-	-	-	<0.01
Hyphomicrobiaceae	0.23	-	-	-	-	-	0.01	-	-
Phycicoccus	0.22	-	-	-	-	-	-	-	-
Virgisporangium	0.22	-	-	-	-	-	-	-	-
Methylobacteriaceae	0.21	0.01	-	0.71	0.30	-	0.02	-	<0.01
Rubrobacter	0.21	-	-	-	-	-	-	-	-
[Kouleothrixaceae]	0.20	-	-	-	-	-	-	-	-
AK1AB1_02E	0.20	-	-	-	-	-	-	-	-
Enhydrobacter	0.20	-	-	-	0.22	-	-	-	-
Lysobacter	0.20	-	-	-	-	-	-	-	<0.01
Fimbriimonas	0.19	-	-	-	0.23	-	-	-	-
Haliangiaceae	0.19	-	-	-	-	<0.01	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Chthonomonas	0.16	-	-	-	-	-	-	-	-
Bacillaceae	0.15	0.02	0.02	0.47	0.27	0.05	0.05	0.01	<0.01
Legionella	0.15	-	-	-	-	-	-	-	-
Blautia	0.15	0.02	-	-	0.50	<0.01	-	-	-
Isosphaeraceae	0.14	-	-	-	-	-	-	-	-
Leptolyngbya	0.14	-	-	-	-	-	-	-	-
Pseudonocardiaceae	0.13	-	-	-	-	-	-	-	-
Pedomicrobium	0.13	-	-	-	0.14	-	-	-	-
Hylemonella	0.12	0.23	-	-	0.06	<0.01	0.02	-	<0.01
Chthoniobacter	0.12	-	-	-	-	-	-	-	-
CM44	0.12	-	-	-	-	-	-	-	-
Devosia	0.12	0.01	-	0.06	0.15	<0.01	-	0.03	<0.01
Oscillospira	0.12	-	<0.01	-	0.62	<0.01	<0.01	0.02	-
[Entotheonellaceae]	0.12	-	-	-	-	-	-	-	-
Corynebacterium	0.12	0.03	0.01	0.18	0.22	0.03	-	0.01	<0.01
5B-12	0.11	-	-	-	-	-	-	-	-
Ellin517	0.11	-	-	-	-	-	-	-	-
Microcoleus	0.11	-	-	-	-	-	-	-	-
Ruminococcus	0.11	-	-	-	0.15	<0.01	-	-	-
Caulobacteraceae	0.11	0.46	-	-	-	-	-	0.02	<0.01
Catellatospora	0.11	-	-	-	-	-	-	-	-
Acinetobacter	0.10	-	-	0.12	0.09	<0.01	-	0.12	<0.01
Adhaeribacter	0.10	-	-	-	-	-	-	-	-
Candidatus Koribacter	0.10	-	-	-	-	-	-	-	-
FFCH4570	0.10	-	-	-	-	-	-	-	-
OM27	0.10	-	-	-	-	-	-	-	-
Spirosoma	0.10	0.03	-	-	0.42	-	-	-	<0.01
Truepera	0.10	-	-	-	-	-	-	-	-
A17	0.09	-	-	-	-	-	-	-	-
Brachybacterium	0.09	0.03	0.02	0.35	0.05	0.02	<0.01	<0.01	<0.01
Nitrospira	0.09	-	-	-	-	-	-	-	-
Pseudoxanthomonas	0.09	<0.01	-	-	-	-	-	-	-
Sediminibacterium	0.09	-	-	-	-	<0.01	-	-	<0.01
Sporichthya	0.09	-	-	-	-	-	-	-	-
Terracoccus	0.09	-	-	-	-	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Dermacoccus	0.09	-	-	-	0.10	-	-	-	-
Brevibacterium	0.09	0.04	0.02	0.24	0.02	0.02	<0.01	<0.01	0.01
Koribacteraceae	0.09	-	-	-	-	-	-	-	-
Nostocaceae	0.09	-	-	-	0.13	-	-	-	-
Salinibacterium	0.09	0.02	-	0.06	-	-	-	-	-
Arthrobacter	0.08	-	-	-	<0.01	-	-	-	-
Bacteriovoracaceae	0.08	-	-	-	-	-	-	-	-
FFCH7168	0.08	-	-	-	-	-	-	-	-
Hyphomicrobium	0.08	-	-	-	-	-	-	-	-
Polaromonas	0.08	<0.01	-	-	0.05	-	<0.01	-	-
Massilia	0.08	0.03	-	0.06	-	-	-	0.02	-
Methylopila	0.08	-	-	-	-	-	-	-	-
Mycoplasma	0.08	-	-	-	-	-	-	-	-
Rubricoccus	0.08	-	-	-	-	-	-	-	-
Sinobacteraceae	0.08	-	-	-	-	-	-	-	-
Chloronema	0.07	-	-	-	-	-	-	-	-
Neisseriaceae	0.07	-	-	-	0.09	-	-	-	-
Phenylobacterium	0.07	-	-	-	-	-	-	-	<0.01
Pirellula	0.07	-	-	-	-	-	-	-	-
Streptomyces	0.07	-	-	-	-	-	-	-	-
5B-12	0.11	-	-	-	-	-	-	-	-
Ellin517	0.11	-	-	-	-	-	-	-	-
Microcoleus	0.11	-	-	-	-	-	-	-	-
Ruminococcus	0.11	-	-	-	0.15	<0.01	-	-	-
Caulobacteraceae	0.11	0.46	-	-	-	-	-	0.02	<0.01
Catellatospora	0.11	-	-	-	-	-	-	-	-
Acinetobacter	0.10	-	-	0.12	0.09	<0.01	-	0.12	<0.01
Adhaeribacter	0.10	-	-	-	-	-	-	-	-
Candidatus Koribacter	0.10	-	-	-	-	-	-	-	-
FFCH4570	0.10	-	-	-	-	-	-	-	-
OM27	0.10	-	-	-	-	-	-	-	-
Spirosoma	0.10	0.03	-	-	0.42	-	-	-	<0.01
Truepera	0.10	-	-	-	-	-	-	-	-
A17	0.09	-	-	-	-	-	-	-	-
Brachybacterium	0.09	0.03	0.02	0.35	0.05	0.02	<0.01	<0.01	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Nitrospira	0.09	-	-	-	-	-	-	-	-
Pseudoxanthomonas	0.09	<0.01	-	-	-	-	-	-	-
Sediminibacterium	0.09	-	-	-	-	<0.01	-	-	<0.01
Sporichthya	0.09	-	-	-	-	-	-	-	-
Terracoccus	0.09	-	-	-	-	-	-	-	-
Dermacoccus	0.09	-	-	-	0.10	-	-	-	-
Brevibacterium	0.09	0.04	0.02	0.24	0.02	0.02	<0.01	<0.01	0.01
Koribacteraceae	0.09	-	-	-	-	-	-	-	-
Nostocaceae	0.09	-	-	-	0.13	-	-	-	-
Salinibacterium	0.09	0.02	-	0.06	-	-	-	-	-
Arthrobacter	0.08	-	-	-	<0.01	-	-	-	-
Bacteriovoracaceae	0.08	-	-	-	-	-	-	-	-
FFCH7168	0.08	-	-	-	-	-	-	-	-
Hyphomicrobium	0.08	-	-	-	-	-	-	-	-
Polaromonas	0.08	<0.01	-	-	0.05	-	<0.01	-	-
Massilia	0.08	0.03	-	0.06	-	-	-	0.02	-
Methylopila	0.08	-	-	-	-	-	-	-	-
Mycoplasma	0.08	-	-	-	-	-	-	-	-
Rubricoccus	0.08	-	-	-	-	-	-	-	-
Sinobacteraceae	0.08	-	-	-	-	-	-	-	-
Chloronema	0.07	-	-	-	-	-	-	-	-
Neisseriaceae	0.07	-	-	-	0.09	-	-	-	-
Phenylobacterium	0.07	-	-	-	-	-	-	-	<0.01
Pirellula	0.07	-	-	-	-	-	-	-	-
Streptomyces	0.07	-	-	-	-	-	-	-	-
Thiobacillus	0.07	-	-	-	-	-	-	-	-
Armatimonadaceae	0.06	-	-	0.18	0.01	<0.01	-	-	-
Leptotrichia	0.06	-	-	-	-	-	-	-	-
Moraxellaceae	0.06	-	-	-	-	-	-	-	-
Rhizobiaceae	0.06	0.02	-	-	0.16	0.02	-	-	<0.01
Phyllobacteriaceae	0.06	0.19	<0.01	-	-	<0.01	-	-	-
[Chthoniobacteraceae]	0.06	-	-	-	-	-	0.02	-	-
A4b	0.06	-	-	-	-	-	-	-	-
Xenococcaceae	0.06	-	-	-	-	-	-	-	-
Kocuria	0.06	-	-	-	0.03	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
oc28	0.06	-	-	-	-	-	-	-	-
Saccharopolyspora	0.06	<0.01	-	-	-	-	-	-	-
Kineococcus	0.05	-	-	0.29	0.01	-	-	-	<0.01
PRR-10	0.05	-	-	-	-	-	-	-	-
Rhodobacter	0.05	0.44	-	0.53	-	-	-	-	-
Turicibacter	0.05	-	-	-	-	-	-	-	-
Actinopolymorpha	0.05	-	-	-	-	-	-	-	-
AKIW874	0.05	-	-	-	-	-	-	-	-
Butyricimonas	0.05	-	-	-	-	-	-	-	<0.01
Dorea	0.05	-	-	-	0.24	<0.01	-	0.03	-
Ktedonobacteraceae	0.05	-	-	-	-	-	-	-	-
Luteimonas	0.05	0.12	0.01	-	-	0.02	-	-	-
Streptosporangium	0.05	-	-	-	-	-	-	-	-
Jeotgalicoccus	0.04	<0.01	0.02	0.24	0.09	0.01	<0.01	<0.01	<0.01
Luteolibacter	0.04	-	-	-	-	-	-	-	-
Cytophagaceae	0.04	0.10	-	-	-	-	-	-	-
Ellin515	0.04	-	-	0.24	-	-	-	-	-
[Barnesiellaceae]	0.03	-	-	-	-	<0.01	-	-	-
Curtobacterium	0.03	0.11	-	0.47	0.02	-	-	-	<0.01
Lentibacillus	0.03	0.02	0.01	0.35	0.04	0.02	-	<0.01	<0.01
Chthonomonadaceae	0.03	-	-	-	-	-	-	-	-
Chlamydomonadaceae	0.03	-	-	-	-	-	-	-	-
Mycoplana	0.03	0.02	-	-	-	0.01	-	-	-
Myxococcaceae	0.03	-	-	-	-	-	-	-	-
Nitrosovibrio	0.03	-	-	-	-	-	-	-	-
Steroidobacter	0.03	-	-	-	-	-	-	-	-
Candidatus Solibacter	0.03	-	-	-	-	-	-	-	-
Hyphomonadaceae	0.03	-	-	-	-	-	-	-	-
Lachnospiraceae	0.03	<0.01	-	-	0.79	-	-	0.09	<0.01
Flavobacterium	0.02	0.36	-	-	-	-	-	-	-
Iamia	0.02	-	-	-	-	-	-	-	-
Labrys	0.02	-	-	-	0.02	<0.01	-	-	-
0319-6A21	0.02	-	-	-	-	-	-	0.03	-
Aerococcaceae	0.01	<0.01	-	-	<0.01	<0.01	-	<0.01	<0.01
Janthinobacterium	0.01	0.16	-	-	-	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Peptostreptococcaceae	0.01	-	-	-	-	<0.01	-	0.02	-
Ramlibacter	0.01	-	-	-	-	-	-	-	-
Caldilineaceae	<0.01	-	-	-	-	-	-	-	-
cc_115	<0.01	-	-	-	0.07	<0.01	-	-	-
Delftia	<0.01	-	-	-	-	-	-	-	-
Inquilinus	<0.01	-	-	-	-	-	-	-	-
Kineosporiaceae	<0.01	-	-	-	0.04	<0.01	-	-	-
OM60	<0.01	-	-	-	-	-	-	-	-
Oscillochloris	<0.01	-	-	-	-	-	-	-	-
Pirellulaceae	<0.01	-	-	-	-	-	-	-	-
Veillonella	<0.01	-	-	-	-	-	-	-	-
Ruaniaceae	<0.01	-	-	-	-	-	-	-	-
SMB53	<0.01	-	-	-	<0.01	-	-	-	-
Cellulomonas	-	0.93	-	0.06	-	-	-	-	-
Pigmentiphaga	-	0.67	-	0.12	-	<0.01	-	-	-
Sanguibacter	-	0.66	-	-	<0.01	-	-	-	-
Alcaligenaceae	-	0.41	-	-	-	<0.01	-	-	<0.01
Rhodobacteraceae	-	0.12	-	0.18	-	-	-	-	-
Saccharibacillus	-	0.09	-	-	-	-	-	-	-
Flavobacteriaceae	-	0.05	-	-	-	-	-	-	-
Trabulsiella	-	0.03	-	-	-	-	-	-	-
Achromobacter	-	0.02	-	-	-	-	-	-	-
Salana	-	0.02	-	-	-	-	-	-	-
Serratia	-	0.02	<0.01	0.12	-	-	-	0.06	0.06
Rhodococcus	-	0.02	-	0.06	0.39	-	-	-	-
Acidovorax	-	0.01	-	-	-	-	-	-	-
Dyadobacter	-	0.01	-	-	-	-	-	-	-
Enterobacter	-	0.01	<0.01	-	-	-	-	-	<0.01
Vibrio	-	<0.01	-	-	-	-	-	-	-
Cellulomonadaceae	-	<0.01	-	-	-	-	-	-	-
Facklamia	-	<0.01	-	-	-	-	-	-	<0.01
Virgibacillus	-	<0.01	-	-	<0.01	-	-	-	-
Christensenellaceae	-	-	<0.01	-	0.05	-	-	<0.01	-
Coprococcus	-	-	-	0.24	0.24	-	-	-	-
Roseomonas	-	-	-	0.18	-	-	-	-	<0.01

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.

(continue)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
Larkinella	-	-	-	0.18	-	<0.01	-	-	-
Natronobacillus	-	-	-	0.12	-	-	-	-	-
Cystobacterineae	-	-	-	0.12	0.07	-	-	-	-
Enterococcaceae	-	-	-	0.12	-	-	-	<0.01	-
Methylosinus	-	-	-	0.12	-	-	-	-	-
Anaerobacillus	-	-	-	0.06	-	-	-	-	-
Erysipelotrichaceae	-	-	-	0.06	-	-	-	-	-
Planococcaceae	-	-	-	0.06	-	-	-	<0.01	-
Parabacteroides	-	-	-	0.06	-	-	<0.01	-	<0.01
Rhodobaca	-	-	-	0.06	-	<0.01	-	-	<0.01
Staphylococcaceae	-	-	-	0.06	-	-	-	-	-
Treponema	-	-	-	-	0.33	-	-	-	-
Veillonellaceae	-	-	-	-	0.22	-	-	0.02	-
Megasphaera	-	-	-	-	0.21	-	-	-	-
Ruminobacter	-	-	-	-	0.18	-	-	-	-
Polyangiaceae	-	-	-	-	0.17	-	-	-	-
Nocardiopsaceae	-	-	-	-	0.15	<0.01	-	<0.01	<0.01
[Fimbriimonadaceae]	-	-	-	-	0.11	-	-	-	-
0.104126706	-	-	-	-	0.10	-	-	-	-
Catenibacterium	-	-	-	-	0.08	-	-	-	-
S24-7	-	-	-	-	0.06	-	-	-	-
Gemellaceae	-	-	-	-	0.05	-	-	-	-
Brevibacillus	-	-	-	-	0.05	-	-	-	-
Bilophila	-	-	-	-	0.05	-	-	-	-
[Prevotella]	-	-	-	-	0.04	-	-	-	-
Rudanella	-	-	-	-	0.04	-	-	-	-
Micrococcus	-	-	-	-	0.04	-	-	-	<0.01
Succinivibrio	-	-	-	-	0.04	<0.01	-	-	-
p-75-a5	-	-	-	-	0.03	-	-	-	-
Frankiaceae	-	-	-	-	0.03	<0.01	-	-	-
Carnobacterium	-	-	-	-	0.03	-	-	-	-
RFN20	-	-	-	-	0.03	-	-	-	-
Phascolarctobacterium	-	-	-	-	0.03	-	-	-	-
Alcanivorax	-	-	-	-	0.02	-	-	-	-
Candidatus Arthromitus	-	-	-	-	0.02	-	-	-	-

Table 3 - Relative abundance (%) of fungi and bacteria detected in environmental samples collected from Brazil coffee plantations.
(conclusion)

Microorganism	Soil	Ground leaf	Fruit of the ground	Tree leaf	Tree cherry	Cherries before de-pulping	Over-ripe fruits	Depulped fruit	Water
CF231	-	-	-	-	0.02	-	-	-	-
Caloramator	-	-	-	-	0.02	-	-	-	-
Sutterella	-	-	-	-	<0.01	-	-	-	-
Klebsiella	-	-	-	-	<0.01	<0.01	-	<0.01	0.02
Salinicoccus	-	-	-	-	<0.01	<0.01	0.01	-	<0.01
Xiphinematobacter	-	-	-	-	-	<0.01	-	-	-
Nannocystis	-	-	-	-	-	<0.01	-	-	-
Aeromonadaceae	-	-	-	-	-	<0.01	-	-	-
Aneurinibacillus	-	-	-	-	-	<0.01	-	-	-
Aerococcus	-	-	-	-	-	<0.01	-	-	<0.01
Sporosarcina	-	-	-	-	-	<0.01	-	-	-
Saccharomonospora	-	-	-	-	-	<0.01	-	<0.01	<0.01
Sporolactobacillaceae	-	-	-	-	-	-	-	0.01	-
ACK-M1	-	-	-	-	-	-	-	-	0.02
Synechococcus	-	-	-	-	-	-	-	-	0.01
Providencia	-	-	-	-	-	-	-	-	<0.01
Cyclobacteriaceae	-	-	-	-	-	-	-	-	<0.01

Source: The Author (2021)

APPENDIX B - Complete list of minor microbial groups of fungi and bacteria during the inoculated fermentation process

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(continue)

Microorganisms	Fermentation time (h)			
	0	**8**	**19**	24
Fungi				
Pichia	70.37	80.88	77.92	74.21
Saccharomycetales	28.83	18.31	20.27	14.54
Cladosporium	0.23	0.24	0.29	1.59
Tremellales	0.13	<0.01	<0.01	<0.01
Trichosporon	0.09	0.03	0.03	0.30
Colletotrichum	0.06	0.06	0.11	1.94
Fusarium	0.05	0.07	0.16	0.72
Wickerhamomyces	0.03	0.02	0.02	0.02
Other	0.03	0.02	0.14	0.32
Torulaspora	0.02	0.02	0.03	0.03
Kodamaea	0.02	-	<0.01	<0.01
Mycosphaerellaceae	0.02	0.14	0.47	5.49
Dothioraceae	0.02	0.03	0.02	0.09
Pleosporales	0.01	0.02	0.09	0.02
Unidentified	<0.01	0.02	0.03	<0.01
Strelitziana	<0.01	<0.01	<0.01	0.08
Cutaneotrichosporon	<0.01	-	-	-
Issatchenkia	<0.01	<0.01	<0.01	<0.01
Helotiales	<0.01	-	<0.01	<0.01
Cystofilobasidium	<0.01	<0.01	-	-
Didymella	<0.01	<0.01	0.05	<0.01
Hannaella	<0.01	-	-	-
Aureobasidium	<0.01	<0.01	-	<0.01
Chaetothyriales	<0.01	<0.01	0.02	<0.01
Montagnulaceae	<0.01	<0.01	-	-
Helicoma	<0.01	<0.01	<0.01	0.16
Purpureocillium	<0.01	<0.01	<0.01	<0.01
Saccharomyces	<0.01	<0.01	0.07	<0.01
Hanseniaspora	<0.01	-	-	<0.01
Lycoperdon	<0.01	-	-	-
Trichoderma	<0.01	-	<0.01	<0.01
Aspergillus	<0.01	<0.01	0.01	-
Chaetomium	<0.01	-	<0.01	<0.01
Dermateaceae	<0.01	-	-	-
Ganodermataceae	<0.01	-	-	-
Hypocreaceae	<0.01	-	-	-
Hypocreales	<0.01	-	<0.01	0.01
Kazachstania	<0.01	-	<0.01	0.02
Metacordyceps	<0.01	-	-	-
Nectriaceae	<0.01	<0.01	<0.01	0.05
Onygenales	<0.01	-	-	-
Rhizophydiales	<0.01	-	-	-
Rhizopogon	<0.01	-	-	-
Rhizopus	<0.01	-	-	-

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(continue)

Microorganisms	Fermentation time (h)			
	0	**8**	**19**	24
Saccharomycetaceae	<0.01	<0.01	<0.01	-
Talaromyces	<0.01	<0.01	<0.01	0.06
Trechispora	-	0.02	-	-
Phaeococcomyces	-	<0.01	-	-
Exophiala	-	<0.01	<0.01	-
Penicillium	-	<0.01	-	<0.01
Diaporthaceae	-	<0.01	-	-
Malassezia	-	<0.01	-	-
Pseudophialophora	-	<0.01	-	-
Chaetomiaceae	-	<0.01	<0.01	-
Schizoparmaceae	-	<0.01	-	-
Gongronella	-	<0.01	0.02	<0.01
Thermomyces	-	<0.01	-	-
Schizoporaceae	-	<0.01	-	-
Clohesyomyces	-	<0.01	-	<0.01
Capnodiales	-	<0.01	-	<0.01
Mortierella	-	-	0.02	0.01
Xylaria	-	<0.01	-	-
Cyphellophora	-	<0.01	<0.01	-
Alternaria	-	-	0.02	<0.01
Scytalidium	-	<0.01	-	-
Ceratobasidiaceae	-	-	<0.01	-
Atractiellales	-	-	<0.01	<0.01
Lasiosphaeriaceae	-	-	<0.01	-
Dictyonema	-	-	<0.01	-
Debaryomyces	-	-	<0.01	0.16
Conlarium	-	-	<0.01	-
Chaetosphaeriaceae	-	-	<0.01	<0.01
Clitopilus	-	-	<0.01	-
Ostropales	-	-	<0.01	0.12
Myrmecridium	-	-	<0.01	-
Pleosporaceae	-	-	<0.01	<0.01
Xylariales	-	-	<0.01	<0.01
Cercospora	-	-	<0.01	-
Archaeosporaceae	-	-	<0.01	<0.01
Coniochaetales	-	-	<0.01	-
Dothiorella	-	-	<0.01	<0.01
Entolomataceae	-	-	<0.01	-
Magnaporthaceae	-	-	<0.01	<0.01
Conocybe	-	-	<0.01	-
Galerella	-	-	<0.01	-
Microdochium	-	-	<0.01	<0.01
Junewangia	-	-	<0.01	-
Lindgomycetaceae	-	-	<0.01	<0.01
Trichocomaceae	-	-	<0.01	<0.01
Sordariales	-	-	<0.01	<0.01
Oidiodendron	-	-	<0.01	-

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(continue)

Microorganisms		Fermentation time (h)			
		0	8	19	24
	Westerdykella	-	-	<0.01	-
	Meira	-	-	<0.01	-
	Glomeraceae	-	-	<0.01	<0.01
	Cercospora	-	-	-	<0.01
	Rhizophlyctis	-	-	-	<0.01
	Saccharomycetaceae	-	-	-	<0.01
	Auriculariales	-	-	-	<0.01
	Phaeosphaeria	-	-	-	<0.01
	Diaporthe	-	-	-	<0.01
	Edenia	-	-	-	<0.01
	Curvularia	-	-	-	<0.01
	Lecanosticta	-	-	-	<0.01
	Glomus	-	-	-	<0.01
	Lentitheciaceae	-	-	-	<0.01
	Microstromatales	-	-	-	<0.01
	Myrothecium	-	-	-	<0.01
	Passalora	-	-	-	<0.01
	Pestalotiopsis	-	-	-	<0.01
	Tetraplosphaeria	-	-	-	<0.01
	Thelephoraceae	-	-	-	<0.01
	Sporidiobolales	-	-	-	<0.01
	Tubeufiaceae	-	-	-	<0.01
	Meyerozyma	-	-	-	<0.01
	Bionectriaceae	-	-	0.02	<0.01
Bacteria					
	Lactobacillaceae	45.13	59.38	77.04	77.40
	Enterobacteriaceae	23.85	0.45	5.67	5.17
	Lactococcus	10.11	17.05	5.37	3.38
	Pediococcus	9.42	6.69	6.98	6.28
	Lactobacillus	3.37	2.88	0.75	0.78
	Other	3.29	2.53	0.33	1.75
	Erwinia	1.97	8.55	2.81	3.20
	Unassigned	0.71	0.54	0.53	0.18
	Ruminococcaceae	0.38	0.16	0.06	0.07
	Sphingomonas	0.16	0.02	<0.01	<0.01
	Corynebacterium	0.14	0.03	<0.01	0.14
	Lachnospiraceae	0.11	0.07	0.03	0.02
	Leuconostoc	0.07	0.22	0.05	0.20
	Faecalibacterium	0.06	0.04	0.01	<0.01
	Oxalobacteraceae	0.06	-	<0.01	<0.01
	Facklamia	0.05	0.02	<0.01	0.05
	Methylobacterium	0.05	0.04	<0.01	0.04
	Prevotella	0.05	0.11	<0.01	0.01
	Proteus	0.05	0.04	0.07	0.04
	Oscillospira	0.05	0.05	<0.01	0.02
	Ruminococcus	0.04	0.06	0.06	0.03
	Acetobacteraceae	0.04	-	-	-

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(continue)

Microorganisms	Fermentation time (h)			
	0	8	19	24
Bosea	0.04	-	-	-
Methylobacteriaceae	0.04	0.03	<0.01	<0.01
Prevotella	0.03	0.04	<0.01	<0.01
Agrobacterium	0.03	0.01	<0.01	-
Spirosoma	0.03	-	-	-
Erysipelotrichaceae	0.03	<0.01	<0.01	<0.01
Ruminococcus	0.03	<0.01	<0.01	0.01
Staphylococcus	0.03	-	-	0.01
Dietzia	0.02	<0.01	-	0.04
Phyllobacteriaceae	0.02	-	-	-
Brachybacterium	0.02	0.02	<0.01	0.06
Clostridium	0.02	-	<0.01	<0.01
Ochrobactrum	0.02	<0.01	<0.01	<0.01
Acinetobacter	0.02	-	<0.01	-
Brevibacterium	0.02	0.03	<0.01	0.10
Dorea	0.02	-	<0.01	-
Ectothiorhodospiraceae	0.02	-	-	-
Fructobacillus	0.02	0.04	0.02	0.04
Luteibacter	0.02	-	-	-
Rhodospirillaceae	0.02	-	-	-
Salinicoccus	0.02	-	-	-
Bacillaceae	0.01	-	-	-
Bradyrhizobiaceae	0.03	-	-	<0.01
Clostridiaceae	0.04	0.02	<0.01	<0.01
Coprococcus	0.01	<0.01	-	<0.01
Micrococcaceae	0.01	-	-	-
p-75-a5	0.01	<0.01	<0.01	<0.01
Phascolarctobacterium	0.01	0.01	<0.01	-
Succinivibrio	0.01	0.05	<0.01	-
Turicibacter	0.01	-	<0.01	<0.01
Williamsia	0.01	-	-	-
Planococcaceae	0.01	<0.01	-	<0.01
Eubacterium	<0.01	-	-	-
Acetobacter	<0.01	-	<0.01	-
Achromobacter	<0.01	-	-	-
Aerococcus	<0.01	-	-	-
Aurantimonadaceae	<0.01	-	-	-
Balneimonas	<0.01	-	-	-
CF231	<0.01	-	-	-
Chryseobacterium	<0.01	-	-	-
Comamonadaceae	<0.01	-	-	-
Ellin6075	<0.01	0.01	-	-
Gordonia	<0.01	-	-	-
Hymenobacter	<0.01	-	-	-
Jeotgalicoccus	<0.01	-	-	0.06
Leuconostocaceae	<0.01	0.08	0.06	0.19
Microbacterium	<0.01	-	-	-

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(continue)

Microorganisms	Fermentation time (h)			
	0	8	19	24
Mycobacterium	<0.01	-	-	-
Novosphingobium	<0.01	-	-	-
Piscirickettsiaceae	<0.01	-	-	-
Pseudoclavibacter	<0.01	-	-	<0.01
Rathayibacter	<0.01	-	-	-
Serratia	<0.01	0.02	<0.01	<0.01
Sphingobacterium	<0.01	0.07	<0.01	0.13
Synechococcus	<0.01	-	-	-
Campylobacter	<0.01	<0.01	<0.01	<0.01
Desulfobacteraceae	<0.01	<0.01	-	-
Paracoccus	<0.01	<0.01	-	<0.01
Pedobacter	<0.01	<0.01	-	-
Peptostreptococcaceae	<0.01	-	<0.01	-
Roseburia	<0.01	-	<0.01	<0.01
Sphingomonadaceae	<0.01	-	-	<0.01
Stenotrophomonas	<0.01	<0.01	<0.01	<0.01
Weissella	<0.01	-	<0.01	<0.01
Streptococcus	-	0.22	-	0.02
Brevibacillus	-	0.11	-	-
Anaerovibrio	-	0.04	<0.01	<0.01
Enterobacter	-	0.03	<0.01	<0.01
Luteimonas	-	0.03	-	0.07
Pseudomonas	-	0.03	<0.01	0.01
S24-7	-	0.03	<0.01	-
Burkholderia	-	0.02	<0.01	0.05
Enterococcus	-	0.02	<0.01	0.12
Methylocystaceae	-	0.02	<0.01	<0.01
Terriglobus	-	0.02	-	-
Enterococcaceae	-	0.01	<0.01	-
Megasphaera	-	0.01	-	-
Pantoea	-	0.01	-	-
Pelosinus	-	0.01	-	-
Bulleidia	-	<0.01	-	<0.01
Citrobacter	-	<0.01	-	<0.01
Fusobacterium	-	<0.01	<0.01	-
Selenomonas	-	<0.01	<0.01	<0.01
Xenococcaceae	-	<0.01	-	<0.01
RF16	-	-	<0.01	-
Bdellovibrio	-	-	<0.01	<0.01
Mycoplana	-	-	<0.01	-
Rhizobiaceae	-	-	<0.01	-
Trabulsiella	-	-	<0.01	-
Dysgonomonas	-	-	<0.01	<0.01
Lysobacter	-	-	<0.01	0.02
Sphingobacteriaceae	-	-	<0.01	0.05
Delftia	-	-	-	0.01
Oligella	-	-	-	0.01

Table 4 - The relative abundance (%) of fungi and bacteria during the inoculated fermentation process

(conclusion)

Microorganisms	Fermentation time (h)			
	0	8	19	24
Sporosarcina	-	-	-	0.01
Aerococcaceae	-	-	-	<0.01
Alcaligenaceae	-	-	-	<0.01
Candidatus Arthromitus	-	-	-	<0.01
Candidatus Koribacter	-	-	-	<0.01
Coriobacteriaceae	-	-	-	<0.01
Kaistobacter	-	-	-	<0.01
Neisseriaceae	-	-	-	<0.01
Blautia	-	-	-	<0.01
Brucellaceae	-	-	-	<0.01
Chitinophagaceae	-	-	-	<0.01
Flavobacteriaceae	-	-	-	<0.01
Isosphaeraceae	-	-	-	<0.01
Rikenellaceae	-	-	-	<0.01
Sinobacteraceae	-	-	-	<0.01
Trichococcus	-	-	-	<0.01
vadinHB04	-	-	-	<0.01
Dermabacteraceae	-	-	-	<0.01
Christensenellaceae	-	-	-	<0.01

Source: The Author (2021)