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Lactic acid bacteria diversity and dynamics in colombian coffee fermentation

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ABSTRACT

Lactic acid bacteria (LAB) are recognized in coffee fermentation as key microorganisms in forming flavor and aroma precursors associated with high-quality beverages. In Colombia, although coffees with differential sensory characteristics are produced from one region to another, only some studies have been performed on the microbiology of coffee fermentation. This study aims to identify the LAB diversity and dynamics associated with coffee fermentation in the Sierra Nevada de Santa Marta (SNSM) through a temporal high-throughput sequencing approach, where the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Finally, LAB species were identified using the BLASTN algorithms of the NCBI GenBank. The coffee fermentation process that lasted 36 hours was dominated by the genera *Leuconostoc,* followed by *Lactobacillus* and *Weissella*. Of the 118 operational taxonomic units (OTUs) corresponding to LAB, it was possible to identify 50 bacterial species, among which 28 are reported for the first time in coffee fermentation. The species widely reported in coffee fermentation are *Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum* (basonym: *Lactobacillus plantarum*), *Levilactobacillus brevis* (basonym: *Lactobacillus brevis)*, and *Lactococcus lactis*. While the novel reports mainly correspond to species belonging to genera that were previously recognized as *Lactobacillus,* such as *Lactiplantibacillus, Paucilactobacillus, Secundilactobacillus, Liquorilactobacillus, Lacticaseibacillus, Schleiferilactobacillus, Loigolactobacillus, Ligilactobacillus, Lentilactobacillus, Limosilactobacillus,* and *Latilactobacillus.* These findings suggest that the indigenous LAB of the SNSM are responsible for generating metabolites that develop specific characteristics of coffee in the region, which is why coffee from the SNSM is protected by designation of origin. Isolates of the reported species should be considered for application as starter cultures.

Key words: LAB; *Lactobacillus*; *Leuconostoc*; sequencing; *Weissella*.

1 INTRODUCTION

Coffee is one of the most valuable agricultural commodities in the world and plays an important role in the economy of the producing countries (Infante et al., 2023; Kumar; Brooks, 2021). The global coffee demand is still growing, evidenced by the 2020/21 production of 101.2 million bags of *Coffea arabica*, with all producing regions expanding their output. South America's production increased the most at 5.6 percent, mainly in Brazil and Colombia, and it's expected to continue to increase (International Coffee Organization - ICO, 2021).

In Colombia, although the new generations of coffee farmers are trying other forms of processing, the coffee produced in the country is mainly processed using the wet method. In this method, the coffee beans are deposited in tanks with water after harvesting and de-pulping to give way to spontaneous fermentation. Finally, these are washed and dried under the sun (Huch; Franz, 2015).

During spontaneous fermentation, the metabolism of the indigenous microorganisms and the complex interaction within them significantly impact the composition of green coffee and, consequently, the flavor and aroma of the beverage that are developed during bean roasting. Bacteria and yeast are highly recognized due to their capacity of broke down mucilage molecules, mainly sugars, into simpler molecules such as alcohols, aldehydes, esters, ketones, and organic acids (Carvalho Ferreira et al., 2023; Elhalis; Cox; Zhao, 2023; Pereira et al., 2021).

Among the bacteria, the lactic acid bacteria (LAB) are an integral component of coffee processing in most producing countries. In addition to assisting in the process of removing the mucilage layer by the efficient use of pulp sugars and lactic acid formation (De Melo Pereira et al., 2020), they have been associated with the generation of compounds related to high coffee quality with distinct floral, fruity, and sweet notes (De Melo Pereira et al., 2016; 2020).

The LAB are comprised by the Lactobacillales order and are characterized by being non-spore-forming, Gram-positive, catalase-negative without cytochromes, nonaerobic or aerotolerant, acid-tolerant, and strictly fermentative bacteria with lactic acid as the major end-product during sugar fermentation (Tamang, 2014). Of these bacteria, *Leuconostoc* is the ubiquitous microbial genera reported worldwide (De Melo Pereira et al., 2020; Martins et al., 2020; Pothakos et al., 2020), even it has been reported as the most abundant through Colombian coffee fermentation (Cruz-O'Byrne; Piraneque-Gambasica; Aguirre-Forero, 2021; Peñuela-Martínez; Velasquez-Emiliani; Angel, 2023; Pino; Espinosa; Cabrera, 2023). However, other genera, such as *Weissella, Lactococcus,* and *Lactobacillus,* have also been reported in coffee-producing regions (De Melo Pereira et al., 2020).

The coffee sector needs to guarantee a productive future and the sustainability of its processes, so it is increasingly necessary to generate new knowledge and innovative alternatives (ICO, 2021). This includes new fermentation techniques with the inoculation of microorganisms (starter cultures) to enhance the coffee quality, where a primary strategy for research is screening from the natural ecosystem (De Melo Pereira et al., 2020; Pereira et al., 2021). The study of microorganisms present in the fermentation processes of each producing place then becomes an essential step to knowing the dynamics and interactions of microbial communities and their diversity and, incidentally, understanding why there are distinctive flavors and aromas of each geographical region and niche.

In this sense, this study aims to identify the LAB present during the coffee fermentation process at the species level in the Sierra Nevada de Santa Marta (SNSM) in northern Colombia for the first time. The coffee from this zone is protected by designation of origin, and it's characterized by a clean and balanced cup profile with a medium-high and uniform body, medium acidity, chocolate flavor, and sweet and nutty notes in its fragrance and aroma (SIC, 2017). These results contribute to the knowledge gap on LAB diversity at the species level in coffee fermentation processes, generate new insights for understanding why SNSM coffee is differential, and lays the bases for future biotechnological applications for coffee innovation.

2 MATERIAL AND METHODS

2.1. Location and sampling

Coffee processing was performed through the wet method at a farm in the Sierra Nevada de Santa Marta in northern Colombia at 1619 m above sea level (11◦04′ 45.91"N - 74◦02′ 18.87"W), with environmental temperature between 14 – 19 °C. The fermentation process was conducted for 36 hours in cement tanks of approximately 10.4 m^3 of volume. Samples of 10g of Arabica coffee were collected randomly every 6 hours in sterile Falcon tubes and stored at – 80°C for further analysis. Additionally, pH was determined during fermentation with a Multiparameter edge pH Meter - HI2020 (HannaInstruments, Woonsocket, United States).

2.2. Total DNA extraction and Illumina high-throughput sequencing

For the total DNA extraction from samples, it was used a DNeasy® PowerLyzer® PowerSoil® Kit (Qiagen, Hilden, Germany) and the procedure suggested by the manufacturer was followed, which consists of successive steps: sample preparation, cell lysis, protein precipitation, PCR inhibitors remotion, DNA binding, DNA washing, and DNA elution (Qiagen, 2020). Each DNA obtained was quantified using a Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, California, United States) according to the supplied protocol, which is based on the fluorescence method (Invitrogen, 2022).

The DNA samples were normalized to a concentration of 10 ng/µL and, containing complementary adaptors (Caporaso et al., 2012), the bacterial 16S rRNA gene was amplified using the primers Bakt_341F and Bakt_805R (Herlemann et al., 2011) following the Illumina amplification program (Illumina, 2013). Sequencing was carried out on the Illumina MiSeq platform (Illumina, San Diego, CA, United States), generating 300 bp paired-end reads.

2.3. Sequences analysis

Reads data analysis was performed using MOTHUR v.1.39.5 (Schloss et al., 2009), and the sequences were aligned to the SILVA rRNA database v. 138 (Quast et al., 2012). During analysis, chimeric and non-bacterial lineages sequences were removed. OTUs were grouped considering a similarity threshold of 97%, and the phylogenetic classification was carried out with the RDP database, considering a bootstrap of 80 (Cole et al., 2014). Those belonging to the order Lactobacillales were filtered from the total bacteria genus identified. Finally, for LAB species identification, sequences were compared through the BLASTN algorithms of the NCBI GenBank and were assigned considering identification percentages ≥ 90%, E-value ≈ 0, and query cover = 100%.

3 RESULTS

3.1. Lactic acid bacteria diversity and dynamics

At the beginning of coffee fermentation in the SNSM, the lowest abundance of LAB is presented, which increases with a logarithmic behavior (R^2 = 0.92) until 18 hours at the same time that the pH decreases significantly following a linear behavior (R^2 = 0.98) from 5.4 to 4.3 (Figure 1A). The largest LAB population is observed at 24 hours and decreases again until the end of the fermentation process, while the pH remains without significant changes (4.1 ± 0.1).

The sequences obtained corresponded to 8 families (Figure 1B), which include 14 identified and seven unidentified genera (Figure 1C). The most abundant order is Leuconostocaceae, represented mainly by *Leuconostoc* and *Weissella*, followed by Lactobacillaceae, with *Lactobacillus* as its main representative. It is also evident that the increase in the LAB population is led mainly by these three genera. Additionally, it can be observed that at the beginning, the populations are heterogeneous, and as time progresses, they become homogeneous while the pH decreases.

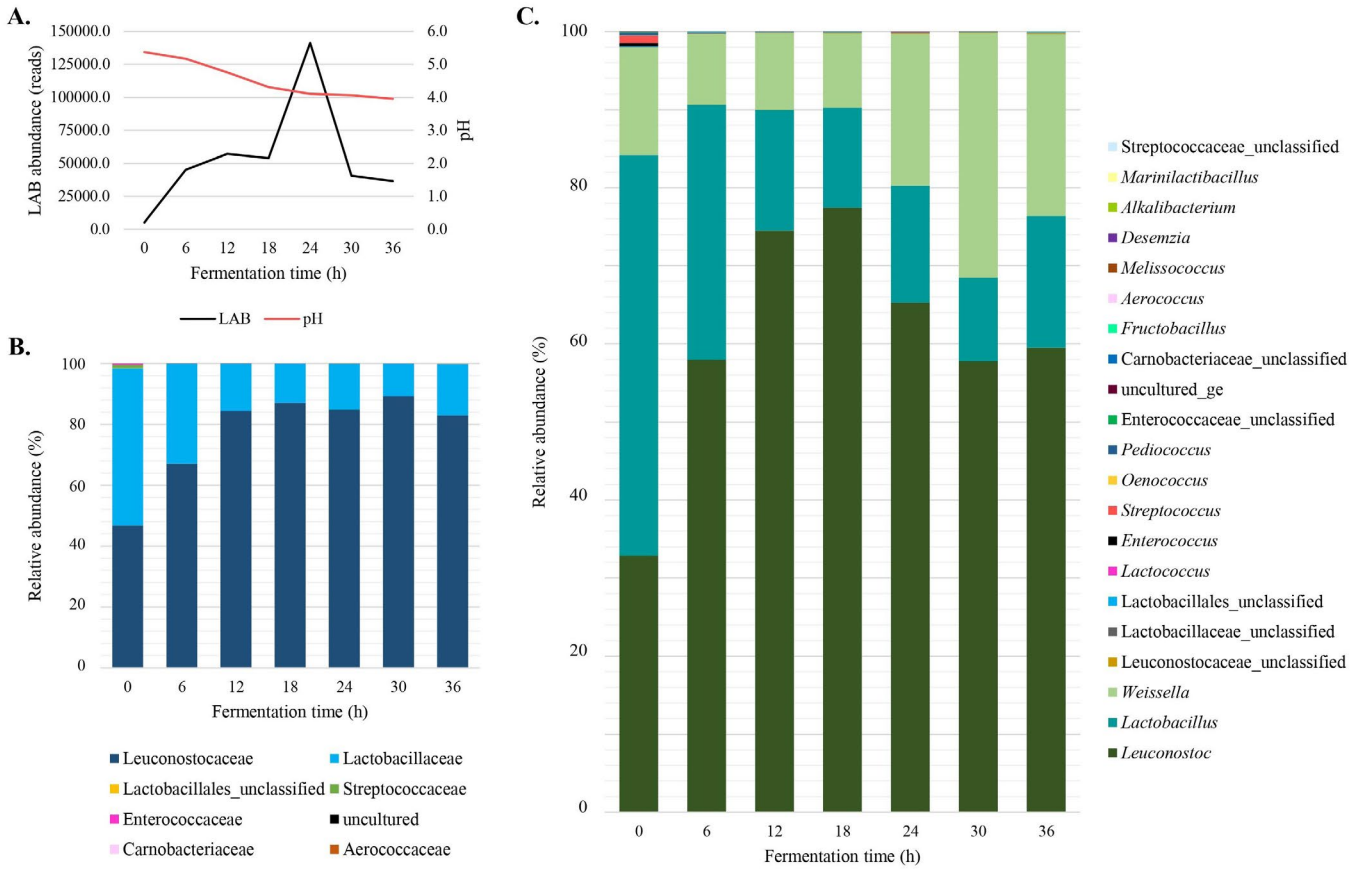

Figure 1: LAB abundance in coffee fermentation in the SNSM. A) Total LAB abundance; B) Relative abundance of LAB families; C) Relative abundance of LAB genus.

3.2. Identification of lactic acid bacteria species

From the bacterial genome sequencing, it was possible to obtain that 118 OTUs belong to the LAB population. When compared with the NCBI database to establish the species, 50 meet the established assignment criteria (Table 1), among which 28 have been found for the first time in coffee fermentation (Table 2). Of the species previously reported in more than two countries are *Leuconostoc mesenteroides, Leu. pseudomesenteroides, Leu. citreum, Leu. holzapfelii, Weissella cibaria, W. confusa, Lactiplantibacillus plantarum* (basonym: *Lactobacillus plantarum*), *Paucilactobacillus vaccinostercus* (basonym: *Lcb. vaccinostercus*), *Levilactobacillus brevis* (basonym: *Lcb. brevis*), *Pediococcus pentosaceus, Enterococcus faecium, E. casseliflavus,* and *Lactococcus lactis* (Table 2).

Additionally, this study presents novel reports of LAB genera and species in the coffee fermentation process that correspond mainly to genera from the family Lactobacillaceae, which includes the species *Lactobacillus taiwanensis,* and others from genera previously recognized as *Lactobacillus* such as *Lactiplantibacillus, Paucilactobacillus, Secundilactobacillus, Liquorilactobacillus, Lacticaseibacillus, Schleiferilactobacillus, Loigolactobacillus, Ligilactobacillus, Lentilactobacillus, Limosilactobacillus,* and *Latilactobacillus.* It was also possible to identify in coffee fermentation for the first time *Periweissella beninensis* (basonym: *Weissella beninensis*), *E. italicus, Bavariicoccus seileri, Lactococcus chungangensis, Lac. fujiensis,* and *Streptococcus salivarius.*

4 DISCUSSION

Coffee fermentation in the Sierra Nevada de Santa Marta is characterized by a high LAB diversity at the beginning of the process, which decreases over time due to the acidic conditions with the prevalence of communities that are more low pH tolerant. *Leuconostoc* and *Lactobacillus* were the most abundant genera during the entire fermentation process, in agreement with reports in other regions of Colombia, such as Quindio and Nariño (De Oliveira Junqueira et al., 2019; Peñuela-Martínez; Velasquez-Emiliani; Angel, 2023). *Leuconostoc* spp. are obligately heterofermentative LAB that metabolize glucose via the phosphoketolase pathway (Onyeaka; Nwabor, 2022). They require a pH at or above 4.8 to survive (Feiner, 2006), reason for which their abundance decreases again after 18 hours of fermentation. Species of this genus exhibit probiotic activities and the production of important flavor precursors in coffee and other products.

Table 1: LAB species present during the coffee fermentation process in the SNSM, according to the NCBI database.

OTU	Species	Query Cover	E value	% Identify	Accession
Otu0001	*Leuconostoc mesenteroides* *Leuconostoc pseudomesenteroides*	100%	0 0	98.92%	MT597709.1 MT597672.1
Otu0004	*Lactiplantibacillus plantarum*	100%	0	98.71%	AP019815.1
Otu0005	*Weissella cibaria* *Weissella confusa*	100%	0	98.71%	OQ430854.1 OQ359414.1
Otu0006	*Paucilactobacillus vaccinostercus* *Paucilactobacillus suebicus*	100%	0	99.14%	LN907853.1 LC071852.1
Otu0013	*Secundilactobacillus paracollinoides*	100%	0	99.14%	LC483558.1
Otu0014	*Liquorilactobacillus mali*	100%	0	99.57%	CP045035.1
Otu0017	*Lacticaseibacillus baoqingensis* *Lacticaseibacillus suibinensis* *Lacticaseibacillus camelliae*	100%	0	99.14%	MK110841.1 MK110835.1 MG383755.1
Otu0022	*Levilactobacillus brevis*	100%	0	99.14%	JX185497.1
Otu0027	*Lacticaseibacillus pantheris*	100%	0	98.28%	LC383826.1
Otu0032	*Liquorilactobacillus nagelii*	100%	0	98.28%	CP018180.1
Otu0039	*Lactiplantibacillus plajomi*	100%	0	98.49%	MG646914.1
Otu0040	*Lacticaseibacillus paracasei*	100%	0	99.78%	CP092498.1
Otu0054	*Schleiferilactobacillus harbinensis*	100%	0	99.14%	CP045180.1
Otu0055	*Leuconostoc fallax*	100%	0	99.35%	MF327682.1
Otu0057	*Loigolactobacillus coryniformis*	100%	0	99.57%	CP042392.1
Otu0069	*Ligilactobacillus murinus* *Ligilactobacillus animalis*	100%	0	99.14% 99.14%	MT585453.1 MT538999.1
Otu0070	*Lactococcus lactis*	100%	0	98.71%	KM257670.1
Otu0073	*Lactiplantibacillus songbeiensis*	100%	0	98.71%	NR_179291.1
Otu0077	*Lacticaseibacillus absianus* *Lacticaseibacillus parakribbianus* *Lacticaseibacillus kribbianus*	100%	0	99.14%	NR_181070.1 MZ951105.2 MZ951057.1
Otu0082	*Leuconostoc citreum* *Leuconostoc holzapfelii*	100%	0	99.57%	MT573003.1 MT573002.1
Otu0086	*Enterococcus italicus* *Enterococcus faecium* *Enterococcus hirae*	100%	0	98.93%	MT473506.1 MN994403.1 MN249567.1
Otu0088	*Lentilactobacillus farraginis* *Lentilactobacillus parafarraginis* *Lentilactobacillus diolivorans*	100%	0	99.14%	MT117902.1 MT117900.1 MK644534.1
Otu0116	*Oenococcus kitaharae*	100%	0	99.57%	OP985037.1
Otu0134	*Enterococcus casseliflavus*	100%	0	99.36%	CP119296.1
Otu0145	*Lactobacillus taiwanensis*	100%	0	98.71%	CP059276.1
Otu0146	*Levilactobacillus brevis*	100%	0	98.92%	OM982565.1
Otu0155	*Periweissella beninensis*	100%	0	99.35%	LC521979.1
Otu0177	*Pediococcus pentosaceus*	100%	0	99.35%	CP118739.1
Otu0193	*Limosilactobacillus reuteri*	100%	0	98.71%	KX688681.1
Otu0207	*Fructilactobacillus fructivorans*	100%	0	99.35%	CP045562.1
Otu0259	*Limosilactobacillus fermentum*	100%	0	99.14%	CP121468.1
Otu0291	*Latilactobacillus sakei*	100%	0	99.14%	CP113247.1
Otu0304	*Weissella confusa*	100%	0	98.07%	MZ853226.1

Continue...

Table 1: Continuation.

OTU	Species	Query Cover	E value	% Identify	Accession
Otu0344	*Lactiplantibacillus plantarum* *Lactiplantibacillus pentosus* *Lactiplantibacillus argentoratensis*	100%	0	98.06%	MK971761.1 ON142104.1 ON108472.1
Otu0356	*Lactococcus chungangensis*	100%	0	99.78%	KY393046.1
Otu0410	*Bavariicoccus seileri*	100%	0	99.35%	JQ680460.1
Otu0430	*Lacticaseibacillus baoqingensis* *Lacticaseibacillus suibinensis* *Lacticaseibacillus camelliae*	100%	0	98.28%	MK110841.1 MK110835.1 MG383755.1
Otu0438	*Lactococcus fujiensis*	100%	0	99.35%	LC519867.1
Otu0463	*Streptococcus salivarius*	100%	0	99.36%	MT585537.1
Otu0589	*Paucilactobacillus nenjiangensis*	100%	0	99.35%	CP043939.1

On the other hand, *Lactobacillus* spp. do not contain the enzyme catalase, and they can be both homofermentative, which ferment sugars predominantly into lactic acid without producing gas, or heterofermentative, which ferment glucose into lactic acid as well as other substances such as acetic acid and produce CO_2 (Feiner, 2006). As well as *Leuconostoc* and *Lactobacillus* have been widely reported in various types of fermentation worldwide (De Melo Pereira et al., 2020; Table 2).

Weissella, the third most abundant genus in this study, also has communities of interest in coffee fermentation processes, reported in Colombia, Brazil, Ecuador, Africa, and Taiwan (Braga et al., 2023; De Bruyn et al., 2016; De Oliveira Junqueira et al., 2019; Evangelista et al., 2015; Hamdouche et al., 2016; Leong et al., 2014; Pereira et al., 2022; Pothakos et al., 2020). Their populations' dynamics demonstrate their high resistance to low pH since their abundance increases in the last hours of fermentation.

Of the identified species, *Leuconostoc mesenteroides, Lactiplantibacillus plantarum* (basonym: *Lactobacillus plantarum*), *Levilactobacillus brevis* (basonym: *Lcb. brevis*) and *Lactococcus lactis*, are the most reported in other coffee fermentation processes (Table 2). *Leu. mesenteroides* and *Lcp. plantarum* have been studied as starter cultures in coffee fermentation using the traditional wet method (Ribeiro et al., 2020) and the SIAF method (self-induced anaerobiosis) (Cassimiro et al., 2022).

The study carried out by Ribeiro et al. (2020) showed the highest percentages of aldehydes, ketones, lactones, phenols, pyrans, and pyrrole when *Leu. mesenteroides* was used as starter culture, while pyrazines and pyridines were higher in the case of *Lcp. plantarum.* At the same time, it was possible to identify the presence of 2-Furfurylthiol (FFT) in roasted coffee inoculated with *Leu. mesenteroides;* a compound known as the most important odorant reported in roasted coffee, with a high influence on the aroma of freshly roasted coffee (Cerny et al., 2021; Grosch et al., 2000; Mcgorrin, 2011). Concerning the inoculations with *Leu. mesenteroides* performed by Cassimiro et al. (2022) demonstrated the production of higher sweetness compared to spontaneous fermentation, as well as honey, chestnuts, milk chocolate and almonds notes. While the use of *Lcp. plantarum* produces milky/dairy notes. When using them as co-inoculums, obtaining beverages with caramel, milk chocolate, and honey notes was possible.

Lev. Brevis is recognized for the production of metabolites that influence sensory characteristics of the beverage, such as styrene (sweet, floral, almond), D-limonene (sweet, orange, citrus), phenylacetaldehyde (honey, chocolate), butyrolactone (milky, creamy with fruity peach-like afternote), 2-Phenethyl acetate (sweet, honey, floral, rosy with a slight fruity body), linalool (citrus, orange, lemon, floral), and 2-methyl-Butanoic acid (fruity, dirty, acidic with a dairy buttery) (De Carvalho Neto et al., 2018). Regarding *Lac. lactis,* (Wang et al., 2020) found that the coffee fermentation performed by this species led to roasted beans with a stronger nutty note in accordance with the N-heterocycle pathway viadicarbonyl production, showing potential for aroma modification in controlled fermentations.

Of the novel reports of LAB, it is possible to highlight the presence of *Periweissella beninensis* (basonym: *W. beninensis*), which was isolated for the first time in submerged cassava fermentations (Padonou et al., 2010) and has also been reported in Brazilian cocoa bean fermentation (Snauwaert et al., 2013). As well as those isolated from traditional Chinese pickle *Lactiplantibacillus songbeiensis* (Liu; Gu, 2019), *Paucilactobacillus nenjiangensis* (Gu et al., 2013), *Lacticaseibacillus baoqingensis* (Long; Gu, 2019), and *Lcc. suibinensis* (Long et al., 2020). From cheese such as Italian (*Enterococcus italicus*) (Fortina et al., 2004) and German red smear soft (*Bavariicoccus seileri*) (Schmidt et al., 2009), silage such as *Lactobacillus taiwanensis* and *Lentilactobacillus diolivorans* (Krooneman et al., 2002; Wang et al., 2009), *Secundilactobacillus paracollinoides* from brewery environments (Suzuki et al., 2004), and *Liquorilactobacillus nagelii* from fermented wine (Edwards et al., 2000).

Table 2: Identification of previous reports of the LAB species present during the coffee fermentation process in the SNSM.

Family	Genus	Species	Coffee fermentation
Leuconostocaceae	*Leuconostoc*	*Leu. mesenteroides*	Quindio – Colombia (Peñuela-Martínez; Moreno-Riascos; Medina-Rivera, 2023); Brazil (Braga et al., 2023; Pereira et al., 2022; Ribeiro et al., 2018; Vilela et al., 2010); Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020; Zhang et al., 2019); Australia (Elhalis; Cox; Zhao, 2020); China (Feng et al., 2016); Cameroon (Hamdouche et al., 2016); Thailand (Nasanit; Satayawut, 2015); Mexico (Avallone et al., 2001)
		Leu. pseudomesenteroides	Brazil (Braga et al., 2023); Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020; Zhang et al., 2019); Taiwan (Leong et al., 2014)
		Leu. fallax	Ecuador (Pothakos et al., 2020; Zhang et al., 2019)
		Leu. citreum	Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020); Taiwan (Leong et al., 2014)
		Leu. holzapfelii	Ecuador (De Bruyn et al., 2016); Ethiopia (De Bruyne et al., 2007)
	Weissella	*W. cibaria*	Brazil (Pereira et al., 2022); Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020)
		W. confusa	Brazil (Braga et al., 2023); Ecuador (De Bruyn et al., 2016); Taiwan (Leong et al., 2014)
	Periweissella	*Per. beninensis* (basonym: *W. beninensis*)	**No reported**
	Oenococcus	*O. kitaharae*	Ecuador (Pothakos et al., 2020)
Lactobacillaceae	*Lactobacillus*	*Lcb. taiwanensis*	**No reported**
	Lactiplantibacillus	*Lcp. plantarum* (basonym: *Lcb. plantarum*)	Quindio – Colombia (Peñuela-Martínez, Moreno-Riascos and Medina-Rivera, 2023); Brazil (Braga et al., 2023; Pereira et al., 2022; Ribeiro et al., 2018; Vilela et al., 2010); Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020; Zhang et al., 2019); Taiwan (Leong et al., 2014); Thailand (Nasanit; Satayawut, 2015)
		Lcp. plajomi (basonym: *Lcb. plajomi*)	**No reported**
		Lcp. songbeiensis (basonym: *Lcb. songbeiensis*)	**No reported**
		Lcp. pentosus (basonym: *Lcb. pentosus*)	Quindio – Colombia (Peñuela-Martínez, Moreno-Riascos and Medina-Rivera, 2023)
		Lcp. argentoratensis (basonym: *Lcb. argentoratensis*)	**No reported**
	Paucilactobacillus	*Pau. vaccinostercus* (basonym: *Lcb. vaccinostercus*)	Brazil (Braga et al., 2023); Ecuador (Pothakos et al., 2020; Zhang et al., 2019)
		Pau. suebicus (basonym: *Lcb. suebicus*)	Ecuador (Pothakos et al., 2020)
		Pau. nenjiangensis (basonym: *Lcb. nenjiangensis*)	**No reported**
	Secundilactobacillus	*Sec. paracollinoides* (basonym: *Lcb. paracollinoides*)	**No reported**

Continue...

Table 2: Continuation.

Family	Genus	Species	Coffee fermentation
Lactobacillaceae	*Liquorilactobacillus*	*Liq. mali* (basonym: *Lcb. mali*)	Ecuador (De Bruyn et al., 2016)
		Liq. nagelii (basonym: *Lcb. nagelii*)	**No reported**
	Lacticaseibacillus	*Lcc. baoqingensis* (basonym: *Lcb. baoqingensis*)	**No reported**
		Lcc. suibinensis (basonym: *Lcb. suibinensis*)	**No reported**
		Lcc. camelliae (basonym: *Lcb. camelliae*)	**No reported**
		Lcc. pantheris (basonym: *Lcb. pantheris*)	Brazil (Braga et al., 2023)
		Lcc. paracasei (basonym: *Lcb. paracasei*)	**No reported**
		Lcc. absianus (basonym: *Lcb. absianus*)	**No reported**
		Lcc. parakribbianus (basonym: *Lcb. parakribbianus*)	**No reported**
		Lcc. kribbianus (basonym: *Lcb. kribbianus*)	**No reported**
	Levilactobacillus	*Lev. brevis* (basonym: *Lcb. brevis*)	Brazil (Braga et al., 2023; De Oliveira Junqueira et al., 2022; Pereira et al., 2022; Vilela et al., 2010); Ecuador (Pothakos et al., 2020; Zhang et al., 2019); Indonesia - Civet coffee (Muzaifa et al., 2019); Thailand (Nasanit; Satayawut, 2015); Mexico (Avallone et al., 2001)
	Schleiferilactobacillus	*Sch. harbinensis* (basonym: *Lcb. harbinensis*)	**No reported**
	Loigolactobacillus	*Loi. coryniformis* (basonym: *Lcb. coryniformis*)	**No reported**
	Ligilactobacillus	*Lig. murinus* (basonym: *Lcb. murinus*)	**No reported**
		Lig. animalis (basonym: *Lcb. animalis*)	**No reported**
	Lentilactobacillus	*Len. farraginis* (basonym: *Lcb. farraginis*)	**No reported**
		Len. parafarraginis (basonym: *Lcb. parafarraginis*)	Ecuador (Pothakos et al., 2020)
		Len. diolivorans (basonym: *Lcb. diolivorans*)	**No reported**
	Limosilactobacillus	*Lim. reuteri* (basonym: *Lcb. reuteri*)	**No reported**
		Lim. fermentum (basonym: *Lcb. fermentumi*)	Cameroon (Hamdouche et al., 2016)
	Fructilactobacillus	*F. fructivorans* (basonym: *Lcb. fructivorans*)	Indonesia - Civet coffee (Muzaifa et al., 2019)
	Latilactobacillus	*Lat. sakei* (basonym: *Lcb. sakei*)	**No reported**
	Pediococcus	*Ped. pentosaceus*	Brazil (Pereira et al., 2022); Ecuador (Zhang et al., 2019); Indonesia - Civet coffee (Muzaifa et al., 2019)
Enterococcaceae	*Enterococcus*	*E. italicus*	**No reported**
		E. faecium	Ecuador (Pothakos et al., 2020); Brazil (Ribeiro et al., 2018)
		E. hirae	Brazil (Ribeiro et al., 2018)
		E. casseliflavus	Brazil (Ribeiro et al., 2018); Thailand (Nasanit; Satayawut, 2015)
	Bavariicoccus	*B. seileri*	**No reported**

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Family	Genus	Species	Coffee fermentation
Streptococcaceae	*Lactococcus*	*Lac. lactis*	Australia (Elhalis; Cox; Zhao, 2020); Ecuador (De Bruyn et al., 2016; Pothakos et al., 2020; Zhang et al., 2019); Indonesia - Civet coffee (Muzaifa et al., 2019); Cameroon (Hamdouche et al., 2016); Thailand (Nasanit; Satayawut, 2015); Taiwan (Leong et al., 2014); Brazil (Vilela et al., 2010)
		Lac. chungangensis	**No reported**
		Lac. fujiensis	**No reported**
	Streptococcus	*Str. salivarius*	**No reported**

Leu = Leuconostoc; W = Weissella; Per = Periweissella; Lcb = Lactobacillus; Lcp = Lactiplantibacillus; Pau = Paucilactobacillus; Sec = Secundilactobacillus; Liq = Liquorilactobacillus; Lcc = Lacticaseibacillus; Lev = Levilactobacillus; Sch = Schleiferilactobacillus; Loi = Loigolactobacillus; Lig = Ligilactobacillus; Len = Lentilactobacillus; Lim = Limosilactobacillus; F = Fructilactobacillus; Lat = Latilactobacillus; Ped = Pediococcus; E = Enterococcus; B = Bavariicoccus; Lac = Lactococcus; Str = Streptococcus.

5 CONCLUSIONS

Coffee fermentation from the Sierra Nevada de Santa Marta in Colombia is characterized by harboring a high diversity of lactic acid bacteria, where the most abundant belong to the genera *Leuconostoc, Lactobacillus,* and *Weissella*. The highest abundance of the LAB occurred after 18 hours of fermentation, demonstrating the ability of this type of bacteria to tolerate acid conditions; however, as the fermentation progresses with the decrease in pH, the diversity decreases, prevailing those communities best adapted to these conditions.

The application of BLASTN algorithms to the sequences obtained allowed the identification of species and the establishment of new and novel reports on the coffee fermentation processes. Thus, this study becomes a new starting point to evaluate the possibility of carrying out new research on biotechnological applications with indigenous species of the region that optimize coffee production and potentiate the sensory characteristics of the beverage in a territory that already has a distinctive coffee that allows it to have protection by designation of origin.

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7 AUTHORS' CONTRIBUTION

RCO performed the experiment, conducted the data analysis, and wrote the manuscript, NPG supervised the research, reviewed, and approved the final version of the work. SAF co-supervised the research, reviewed, and edited the manuscript.

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