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Full Length Research Paper

Diversity of endophytic bacteria in the fruits of *Coffea canephora*

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Endophytic bacteria colonize the internal tissues of plants without causing infection or negative effects on their hosts. This study investigates the occurrence and diversity of culturable endophytic bacteria in the fruits of *Coffea canephora* at three developmental stages. Isolation and quantification were performed in R2A culture medium, and the diversity was established using molecular methods and analysis of fatty acid methyl esters (FAME). α- and γ-Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes were identified in the investigated community. *Kocuria turfanensis* and *Pantoea vagans* were identified as endophytes for the first time. Of the 18 species that were found, the following seven had not been previously described as endophytic in coffee fruits: *Bacillus thuringiensis*, *Bacillus licheniformis*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Enterobacter hormaechei*, *Chryseobacterium* sp., and *Ochrobactrum* sp. The diversity of endophytic bacteria varied during the three developmental stages that were investigated, and the diversity was greatest in fruits during the green stage, during which *Bacillus subtilis* predominated. The number of Gram-positive bacteria was larger than the number of Gram-negative bacteria during the two earliest developmental stages, whereas their numbers were similar during the ripe stage. The diversity was lowest during the ripe stage, and *Klebsiella oxytoca* was the predominant species at this stage, probably due to the higher caffeine and sugar contents in the fruits.

Key words: Coffee, bacterial community, sequencing, 16S rDNA, FAME.

INTRODUCTION

The economic and social importance of coffee can be assessed using data provided by the International Coffee Organization, which estimates that 75 to 125 million individuals worldwide are dependent on coffee-related activities. In Brazil, the coffee production chain generates more than eight million jobs (MAPA, 2012).

Coffee plants belong to the family Rubiaceae, which comprises approximately 650 genera and more than 13,000 species (Delprete and Jardim, 2012). The most widely cultivated are *Coffea arabica*, which produces superior-quality coffee beverages due to its organoleptic properties (Judd et al., 2008), and *Coffea canephora*, which produces neutral, weak-flavored, and very bitter coffee beverages (Ky et al., 2001). The raw material for the instant coffee industry is derived from *C. canephora,* and this species was used in 50 to 55% of the blends manufactured in Brazil in 2011, which represents a 20% increase compared to 2000 (ABIC, 2012). The Brazilian coffee research program is the largest in the world, and investments in this research have produced significant advances in breeding, plant nutrition, biotechnology, and other areas (MAPA, 2012). Recently, the map of the *C. arabica* genome was made available for functional genomics studies (Vieira et al., 2006). Although the International Coffee Genome Network - ICGN is currently mapping the genome of *C. canephora,* no studies investigating the colonization of its fruits by endophytes were

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found in a literature search

Most vascular plants host endophytic microorganisms including Gram-positive and Gram-negative α-, β-, and γ-proteobacteria, actinobacteria, firmicutes and bacteroidetes. Endophytic microorganis inhabit intra- or intercellularly without visibly harm the plant, competing by niche and can be detected by cultural or molecular methods (Rosenblueth and Martínez-Romero, 2006). The plant-microorganism interaction may occur in several parts of the plants such as the seeds, ovules, fruits, stems, leaves, roots, tubercles, buds, flowers, and inflorescences (Zhang et al., 2006). Endophytes can produce growth-promoting compounds and antimicrobial metabolites that increase the resistance of plants to disease, can maintain atmospheric nitrogen, and can perform other useful functions (Rosenblueth and Martinez-Romero, 2006).

The mucilaginous mesocarp of ripe coffee fruits comprises simple sugars, complex polysaccharides, proteins, lipids, minerals, and other compounds, which provide a culture medium that favors the establishment and growth of bacteria, fungi, and yeasts (Avallone et al., 2000). Confirmation of the presence of endophytes and the knowledge of the microbial diversity are crucial to an understanding of the interactions, functional metabolic pathways, products, and interconversion of possible microbial metabolites among the precursors that are associated with superior-quality coffee beverages. Therefore, the occurrence and characterization of endophytic populations in *C. canephora* fruits should be analyzed.

Previous studies on the occurrence of endophytic bacteria in coffee fruits or other parts of the plant and on the proteolytic activity of microorganisms have been conducted using techniques that require culturing in *C. arabica* (Sakiyama et al., 2001; Vega et al., 2005). There are no reports on the occurrence and diversity of endophytic bacteria in *C. canephora* fruits. Therefore, the present study investigated the occurrence and diversity of culturable bacteria in *C. canephora* fruits at three developmental stages using molecular methods, fatty acid methyl esters (FAMEs) analysis, and sequencing of the isolates' 16S rDNA.

MATERIALS AND METHODS

Sampling of the coffee fruits and isolation of endophytes

Samples of healthy *C. canephora* fruits in the green, green-yellow, and ripe developmental stages were collected at a farm in Viçosa County, state of Minas Gerais, Brazil, which is in Northern Zona da Mata at a latitude of 20º45'20" S, longitude of 45º52'40" W, and altitude of 658 m. Samples were collected from the middle third of coffee plants. Representative apparently healthy fruits were superficially decontaminated according to Sakiyama et al. (2001) with modifications. The fruits were pre-washed in running tap water, soaked in neutral detergent and rinsed in running distilled water. From this point, superficial decontamination of the fruits was performed aseptically. Briefly, the fruits were immersed twice in distilled water and once in 50 mM potassium phosphate buffer, pH 7.0. The fruits were then immersed in 70% (v/v) ethanol for 1 min, soaked for 5 min under vigorous agitation in 5% (v/v) sodium hypochlorite containing 0.05% (v/v) Tween 80 and then the fruits were immersed in 70% (v/v) ethanol for 1 min followed by immersion in 50 mM potassium phosphate buffer, pH 7.0, for 15 min. This superficial decontamination protocol was repeated once. The flaming step (Sakiyama et al., 2001) was omitted due to the delicate exocarp.

Two methods were used to establish the efficacy of the sterilization. The sterilized fruits were individually transferred to and incubated in R2A liquid culture medium (Reasoner and Gelrdreich, 1985) at 28°C for 72 h, and an aliquot of the sterilized water used in the final cleansing of the fruits was also inoculated into the culture medium and incubated under the same conditions.

Eight sterilized fruits in each developmental stage were used to establish the density of culturable endophytic bacteria per fruit (colony forming units [CFU] $fruit^{-1}$). The fruits were individually ground using a tissue homogenizer in tubes containing 10 mL of potassium phosphate buffer (0.05 M, pH 7.0). The suspension was filtered using sterilized gauze, and the filtered product was serially diluted in potassium phosphate buffer. Aliquots were streaked onto R2A solid medium and incubated at 28°C for up to 72 h.

Analysis of the fatty acids profile

Isolates of the endophytic bacteria were clustered according to the morphological characteristics of the colonies in R2A culture medium and were identified through cell total FAME analysis using the Sherlock® Microbial Identification System MIDI, Inc. coupled to gas chromatography (Peltroche-Llacsahuanga et al., 2000). For this purpose, cells from the axenic culture of each isolate were transferred to TSA culture medium (Trypticase Soy, 30 $g.L^{-1}$, agar 15 $g.L^{-1}$) and incubated at 30ºC for 24 or 48 h as needed.

The similarity index (SI) was used for identification, and isolates were considered unknown when the SI was lower than 0.2, positively identified at the level of genus when the SI was higher than 0.2, and positively identified at the level of species when the SI was higher than 0.5 (Buyer, 2003). The 140 endophytic isolates that were identified using FAME/MIDI were clustered using a Euclidean distance matrix analysis, and 77 isolates could not be identified.

DNA extraction, amplification, sequencing and analysis of the 16S rDNA sequences

Total DNA was extracted from the endophytic bacterial isolates that were not identified by FAME/MIDI using a DNA purification kit (Promega™, Madison, USA) and following the manufacturer's instructions.

The universal eubacterial primers 27f/1392r (Heuer et al., 1997; Blackwood et al., 2005) were used to amplify the 16S rDNA. The reaction contained approximately 20 ng of DNA, buffer (Promega), 2.25 mM of $MgCl_2$, 210 mM of each primer, 250 µM of deoxynucleotides (dNTPs), and 0.02 U of Taq DNA polymerase (Promega). Amplification was performed using a thermocycler (Mastercycler Gradient, Eppendorf-Germany) set to perform an initial denaturation at 94ºC for 4 min followed by 35 denaturation cycles at 94ºC for 30 s, annealing at 60ºC for 1 min, and elongation at 72ºC for 1.5 min, with a final elongation step of 7 min at 72ºC. The amplicons were assessed using electrophoresis in a 1.2% (p/v) agarose gel that was stained with 0.5 $µg.mL^{-1}$ of ethidium bromide and visualized using a Stratagene Eagle Eye II Trans Illuminator and Imaging System™ (Stratagene, Cedar Creek, TX, USA). The fragments were sequenced using the above mentioned primers and Sanger's sequencing method. The sequences reported in this study have been submitted to GenBank database under the GenBank ID

numbers JX865440 to JX865456.

All of the sequences were compared against the nucleotide collection stored in the GenBank using the BLAST algorithm. The sequences exhibiting high identity values were imported into MEGA 4 software and aligned with other 16S rDNA fragments using ClustalW (Larkin et al. 2007). The sequence alignments were manually trimmed, and the phylogenetic trees were calculated based on the sequence alignments using the maximum parsimony method (Tamura et al. 2011). The robustness of the resulting trees and the levels of statistical significance of the internal nodes were calculated using bootstrap analysis with 1,000 replicates, and values above 50% were reported. Endophytic sequences that clustered with bootstrap values above 97% were considered identical.

The diversity of isolates in the library was investigated using Shannon's diversity indexes calculated using PAST 1.4 software (Hammer et al., 2001) and rarefaction analysis (Heck et al., 1975) using Analytic Rarefaction 1.3 (aRarefactWin) software (www.uga.edu/~strata/software/anRareReadme.html).

RESULTS AND DISCUSSION

The occurrence of interactions between plant and endophytic bacteria has previously been demonstrated in cultivable plants of economic importance such as rice, potato, wheat, sugarcane, maize, and others (Rosenblueth and Martínez-Romero, 2006). However, the literature contains no reports describing endophytes in *C. canephora* fruits, despite the economic and social importance of this plant in Brazil. Therefore, demonstration of the occurrence of such interactions and identification of the bacteria that become established in the fruits of *C. canephora* represent an indispensable starting point for future studies, such as those addressing the functionality of such an interaction.

From all isolates recovered from coffee fruits, 63 were identified by FAME/MIDI, most of which belonged to the Fimicutes phylum, within *Bacillus* genus: 48 isolates were classified as *B. subtilis,* with similarity index (SI) ranging from 0.510 to 0.930, six isolates as *B. megaterium*, SI from 0.556 to 0.678, two isolate as *B. thuringiensis,* SI of 0.538, two as *B. licheniformis,* SI of 0.665*,* and two as *B. cereus*, with SI ranging from 0,675 to 0.820. One γ-Proteobacteria, corresponding to three isolates, *Shigella sonnei*, was identified with SI ranging from 0,593 to 0.637. All similarity indexes were higher than 0.5 confirming the reliability of isolates identification (Buyer, 2003). Previous study reported the presence of *B. subtilis* and *B. megaterium* in the fruits and *B. cereus* and in the leaves of *C. arabica* (Vega et al., 2005). *B. cereus* is endophytic and is efficient at controlling coffee rust, which is the most important disease that affects coffee plants. Coffee rust is caused by *Hemileia vastatrix* and can result in losses of up to 40% in yield. *B. cereus* produces chitinases that are active against plant pathogens (Sadfi et al., 2001), as do strains of *B. thuringiensis* and *B. licheniformis* that have been isolated from the rhizosphere of *C. arabica* (Muleta et al., 2009). The present study is the first to identify *B. thuringiensis* and *B. licheniformis* as endophytes in coffee fruits.

All of the isolates that were identified using 16S rDNA sequencing exhibited 97 to 100% identity with sequences deposited at the National Center for Biotechnology Information (NCBI), except for the sequence from LEM 17, which exhibited 77% sequence identity with a sequence in NCBI (Table 1). A 16S rDNA sequence identity above 97% is generally considered to be the absolute boundary for species circumscription in phylogenetic studies (Al-Batayneh et al., 2011), although some authors have observed that values between 98.5% and 99% are more consistent (Stackebrandt and Ebers, 2006). The reliability of the alignment was confirmed using the E-values, which indicate the probability of obtaining an alignment with an equal or higher score using a random sequence of the same size and base composition (Kerfeld and Scott, 2011). LEM 17 was interpreted similarly to Velásquez et al. (2008), that this bacterial species has not been yet described or that its data have not yet been entered into the database.

The phylogenetic trees confirmed the diversity of the endophytic bacteria in *C. canephora* fruits, which includes isolates corresponding to Firmicutes, Bacteroidetes, Actinobacteria, and α- and γ-Proteobacteria (Figures 1, 2, 3, 4). The maximum parsimony method is the most widely used method for phylogenetic reconstructions (Park et al., 2010) because it allows robust analysis when sequences are closely related (Mauro et al., 2010).

All of the isolates belonging to the phylum Firmicutes clustered with significant to moderate bootstrap values. Values above 95 are rated as significant, values between 70 and 94 are rated as moderate, and values below 70 are rated as weak (Schneider, 2007). The bootstrap values corresponding to LEM 58 and LEM 95 were moderate and are given phylogenetic support of their being linked together, at a value of 99. This high value indicates that these isolates belong to the genus *Bacillus* (Figure 1). The genus *Bacillus* is one of the most common endophytic bacterial genera and prevails in soil, rhizosphere, and root endophytic communities (Hallman et al., 1997).

LEM 134 clustered with *Bacillus firmus,* LEM 25 with *Paenibacillus lautus,* and LEM 80 with *B. pumilus*. All of these isolates clustered with significant support (Figure 1). The bacteria from the genus *Bacillus* colonize several plants and may promote their growth through the production of gibberellins and auxins, which is a remarkable characteristic of *B. pumilus* in addition to atmospheric nitrogen fixation, phosphate solubilization (Forchetti et al., 2007), and activity as biological control agents (Mekete et al., 2009). These bacteria produce pectinolytic enzymes, primarily polygalacturonase and pectin lyase. An increased fermentation rate in cocoa tree and a better-quality final product have been attributed to the high pectinolytic ability of *Bacillus* sp. (Ouattara et al., 2008).

The phylogenetic relationships of six Actinobacteria*,* α-Proteobacteria, and Bacteroidetes isolates (Figure 2) exhibited high cluster bootstrap values. The results from

Table 1. Results of the comparison between the 16S rDNA sequences of the culturable endophytic bacterial isolates from *Coffea canephora* fruits and the sequences from the nucleotides collection recorded at the National Center for Biotechnology Information (NCBI).

Number of Isolate	Isolate identification (accession number)	Database sequence (accession number)	E-value	% Identity
Actinobacteria, α-Proteobacteria and Bacteroidetes				
5	LEM 166 (JX865440)	*Microbacterium* sp. NII-1012 (HM036663.1)	0	99
2	LEM 171 (JX865441)	*Kocuria turfanensis* strain GJM817 (HM209734.1)	0	100
2	LEM 38 (JX865442)	*Ochrobactrum* sp. MJ25 (GQ250447.1)	0	100
1	LEM 170 (JX865443)	*Agrobacterium tumefaciens* (FJ581441.1)	0	100
1	LEM 168 (JX865444)	*Janibacter melonis* strain MA1B-GFJ (FJ811878.1)	0	100
1	LEM 165 (JX865445)	*Chryseobacterium* sp. MH28 (EU182856.1)	0	100
Firmicutes				
2	LEM 80 (JX865446)	*Bacillus pumilus* strain DYJL55 (HQ317196.1)	0	100
12	LEM 58 (JX865447)	*Bacillus subtilis* strain CH1 (FR773878.1)	0	100
3	LEM 97 (JX865448)	*Bacillus amyloliquefaciens* strain EII-5 (FJ613553.1)	0	99
2	LEM 25 (JX865449)	*Paenibacillus* sp. J16-10 (AM162327.1)	0	99
1	LEM 134 (JX865450)	*Bacillus firmus* (HQ285922.1)	0	100
γ-Proteobacteria				
28	LEM 01 (JX865451)	*Klebsiella oxytoca* strain NFSt 18 (GQ496665.1)	0	99
6	LEM 05 (JX865452)	*Enterobacter hormaechei* strain DYM-6 (EF428236.2)	0	99
1	LEM 44 (JX865453)	*Escherichia coli* strain JCM12 (GQ202138.1)	0	100
8	LEM 17 (JX865454)	*Citrobacter freundii* strain F1 16S (FJ608234.1)	$1{,}00e^{-76}$	77
1	LEM 67 (JX865455)	*Pantoea vagans* C9-1 (CP002206.1)	0	97
1	LEM 145 (JX865456)	*Pantoea eucrina* (HQ455824.1)	0	100

LEM, Laboratório de Ecologia Microbiana.

sequencing show that four of the six bacteria, LEM 166, LEM 167, LEM 169, and LEM 179, were indicated as *Microbacterium* with 99% identity; these bacteria were isolated from ripe *C. canephora* fruits (Table 1). Among these isolates, only LEM 166 was used in the phylogenetic reconstruction, and it clustered with *Microbacterium flavascens* (Figure 2). The genus *Microbacterium* is endophytic in tomato roots (Marquez-Santacruz et al., 2010) and in maize grains (Zinniel et al., 2002). The isolates LEM 171 and 178 in the present study are Actinobacteria that exhibit high identities with *Kocuria turfanensis* (Table 1) and are phylogenetically related to *Kocuria* sp. (Figure 2), which are endophytic in *C. arabica* leaves (Vega et al., 2005). Although *K.*

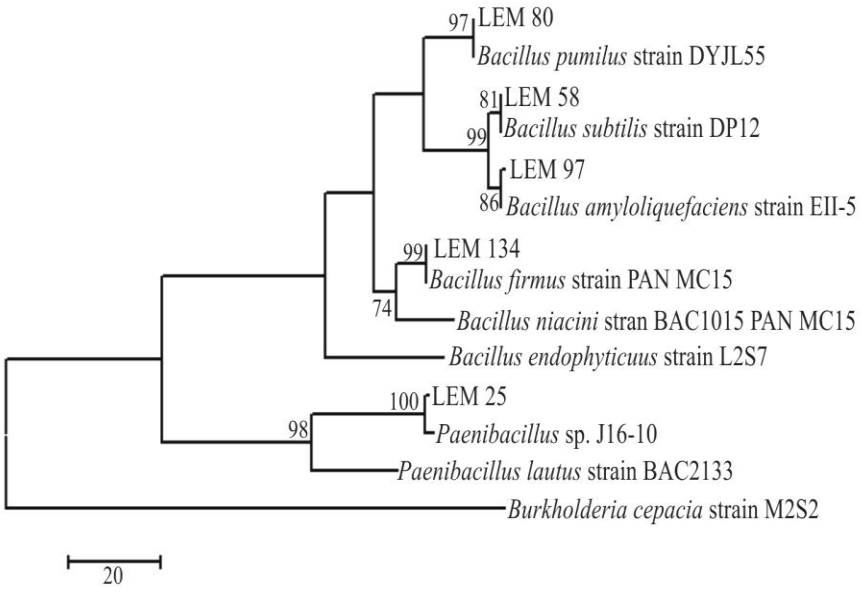

Figure 1. Phylogenetic relationships of endophytic bacterial isolates from the fruits of *Coffee canephora* that belong to the phylum Firmicutes.

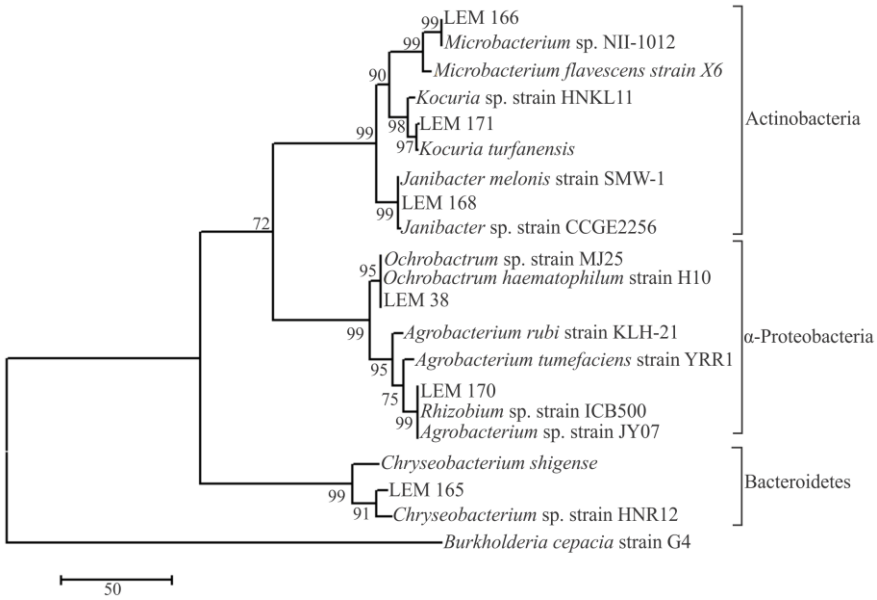

Figure 2. The phylogenetic relationships of endophytic bacterial isolates from the fruits of *Coffea canephora* corresponding to Actinobacteria, α-Proteobacteria and Bacteroidetes.

turfanensis has previously been identified in mammalian skin, soil, rhizoplane, water, and sea sediments (Kim et al., 2004), endophytic colonization of plants has not been previously reported for this bacterium.

LEM 168, which has 100% identity with the Actinobacteria *Janibacter melonis* (Table 1), clustered with *Janibacter* sp. and *J. melonis* (Figure 2). This bacterium spoils oriental melon (*Cucumis melo*) and causes significant economic losses in South Korea (Yoon et al., 2004). This observation demonstrates that one plant's endophytes may be pathogenic to other plants (Saikkonen et al. 2004). The 16S rDNA sequence of LEM 170 exhibited 100% of identity with that from *Agrobacterium tumefaciens,* actually known as *Rhizobium*

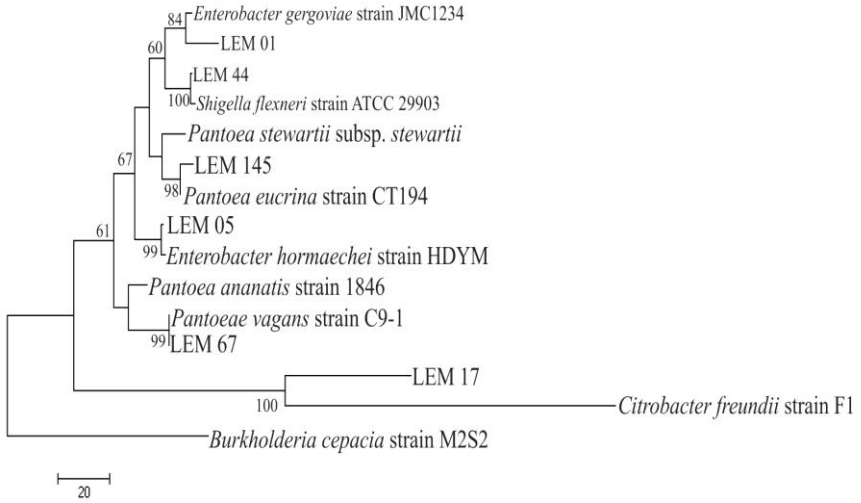

Figure 3. Phylogenetic relationships of the culturable endophytic bacterial isolates from *Coffea canephora* fruits that correspond to γ-Proteobacteria.

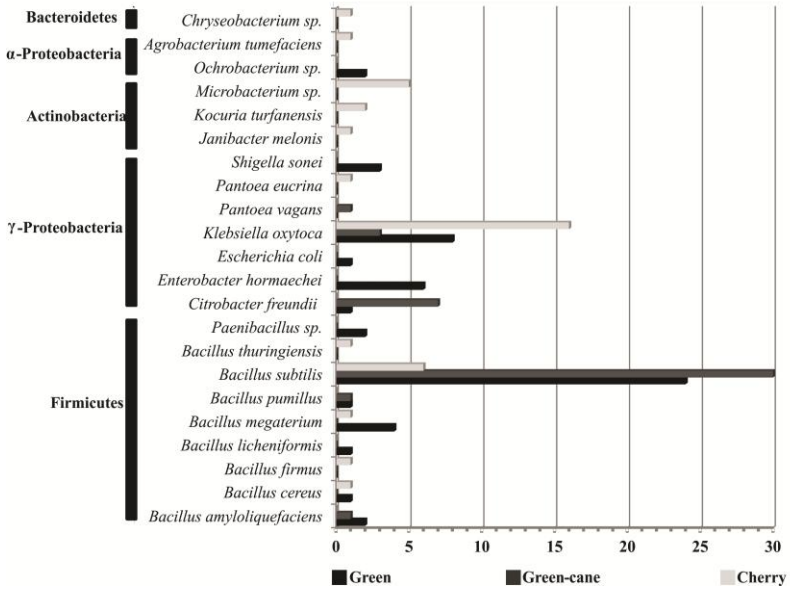

Figure 4. Eubacterial clusters and clones that were detected in *Coffea canephora* fruits.

radiobacter (Table 1). This bacterial species has previously been isolated from the rhizosphere (Muleta et al., 2009) and roots of *C. arabica* (Mekete et al., 2009), but the present studyis the first report of this bacteria being endophytic in coffee plant fruits. This bacterial species is genotypically related to *Agrobacterium* and *Rhizobium* (Figure 2). Bacteria from these two genera endophytically inhabit plant tissues and exhibit similar chemotaxonomic profiles. Therefore, some authors have suggested that they be united in a single genus, *Rhizobium* (Young et al., 2001).

LEM 38 clustered with *Ochrobactrum* sp. and *Ochrobactrum haematophilum* and formed a clade that is phylogenetically near an *Agrobacterium* sp. (Figure 2). *Ochrobactrum* is endophytic in soybean seeds, where it antagonizes the following three phytopathogenic fungi: *Fusarium oxysporum, Fusarium semitectum,* and *Cercospora kikuchii* (Assumpção et al., 2009). The single isolate that was identified as belonging to the phylum Bacteroidetes was LEM 165, which corresponded to

Table 2. Richness and diversity of endophytic bacteria isolated from *Coffee canephora* fruits during three developmental stages.

Variable	Green	Green-yellow	Ripe
Clones	57	45	38
Richness	13	6	11
Shannon	1.942	0.995	1.866

Chryseobacterium sp. (Table 1). Bacteria belonging to this genus reside in habitats including soil, fresh water, and sewage, as well as in association with plants and other hosts (Cho et al., 2010). The phylogenetic relationship between LEM 165 and *Chryseobacterium* sp., which is in the same clade as *Chryseobacterium shigense,* is supported by a high bootstrap value (Figure 2).

With regard to γ-Proteobacteria, comparison of the 16S rDNA sequences demonstrated that six of the isolates from *C. canephora* fruits, LEM 67, LEM 145, LEM 01, LEM 44, LEM 05, and LEM 17, are species from the following species of Enterobacteriaceae: *Klebsiella oxytoca*, *Enterobacter hormaechei*, *Pantoea eucrina*, *Pantoea vagans*, *Escherichia coli*, and *Citrobacter freundii* (Table 1). Except for *Citrobacter freundii*, each of these isolates exhibited a high sequence identity with a sequence available from NCBI. Upon phylogenetic analysis of the isolates, the high bootstrap values of the clades (Figure 3) confirmed that all of these bacteria belong to the family Enterobacteriaceae, which increases the reliability of the determined identities (Table 1). LEM 01 exhibits 98% identity with the *K. oxytoca* sequence (Table 1) and clustered with *Enterobacter gergoviae* in a phylogenetic analysis (Figure 3). *Klebsiella* sp. and *Enterobacter* sp. occupy a similar ecologic niche and are prone to lateral gene transfer (Dauga et al., 2002).

The isolate LEM 44 clustered with *Shigella flexneri* (Figure 3), which belongs to a bacterial genus that has been found in soils (Sabat et al., 2000) but not in interactions with plants. LEM 05 is another γ-Proteobacteria, and it exhibited high identity (Table 1) and a high bootstrap value (Figure 3) with *E. hormaechei*. This species has previously been described as endophytic in maize roots, where it decreases the rate of infection by *Fusarium verticillioides* (Pereira et al., 2010). The high identity (Table 1) and bootstrap (Figure 3) values exhibited by LEM 67 with *P. vagans* confirm its identification. Bacteria from the genus *Pantoea* are frequently associate with plants and colonize the rhizosphere, seeds, and other plant parts (Verma et al., 2001), and an interaction with coffee plants and their seeds has also been described (Vega et al., 2005).

The relationships among the data regarding the number of clones and analyses of the endophytes isolated from *C. canephora* fruits in the three developmental stages using the fatty acids profile and

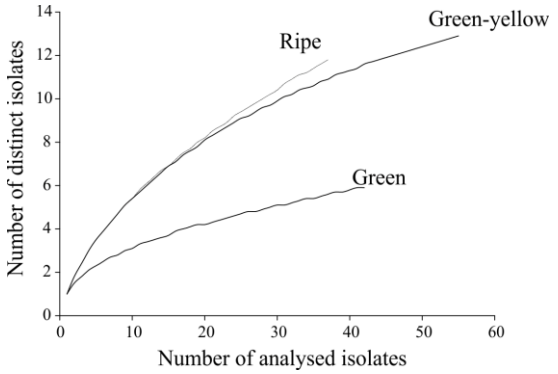

Figure 5. Rarefaction curves indicating endophytic bacterial diversity in coffee fruits isolates during three developmental stages: green, green-yellow and ripe stages.

16S rDNA sequencing were visualized using a library containing 22 representatives of Firmicutes, Bacteroidetes, Actinobacteria, and α- and γ-Proteobacteria (Figure 4). The predominant genus was *Bacillus*, *B. subtilis* was the predominant species during the green and green-yellow developmental stages, and *K. oxytoca* prevails in the ripe stage (Figure 4). Plants are able to select beneficial microorganisms that specifically colonize their rhizosphere (Nihorimbere et al., 2011), and *C. canephora* plants, together with the environmental factors at the sites where they are grown, may select the endophytes that associate with their fruits.

The greatest diversity of endophytic bacteria in fruits was observed during the green stage, followed by the ripe stage, and, finally, the green-yellow developmental stage (Table 2). During ripening, the concentration of caffeine increases in the coffee grains (Almeida et al., 2007), which may be one of the factors that contributes to the differences in the endophyte diversity in the three developmental stages. The growth of Gram-positive bacteria is inhibited in the presence of caffeine, which promotes cellular lysis by inhibiting functions such as protein synthesis (Dash and Gummadi, 2008). This effect is not observed in *K. oxytoca*, and the predominance of this species in ripe fruits might be attributed to possible effects of the caffeine concentration at this developmental stage and to the ability of this bacterial species to metabolize the cellobiose, cellotriose, xylobiose, xylotriose, saccharose, and monomeric sugars that are present in the lignocellulosic biomass (Grange et al., 2010). The predominance of *B. subtilis* during the green and green-yellow developmental stages may arise due to the characteristic secretion of depolymerizing enzymes by this bacterial species (Zhang and Zhang, 2010).

The rarefaction curve of the isolates obtained from the fruits during the green stage reached a plateau, while during the green-yellow and ripe stages, the decline in the sequence detection rates that are revealed by the curves demonstrates that a large portion of the diversity that was present in the grains was detected (Figure 5).

Conclusions

The occurrence and diversity of culturable bacteria in the fruits of *C. canephora* during three developmental stages were demonstrated using identification by FAME and sequencing of the 16S rDNA from the isolates. The bacterial diversity was greatest in fruits in the green stage. The isolated endophytic bacteria were phylogenetically diverse and corresponded to Firmicutes, Bacteroidetes, Actinobacteria, α-Proteobacteria, and γ-Proteobacteria. *Kocuria turfanensis* and *Pantoea vagans* were identified for the first time as endophytic bacteria, and *Bacillus thuringiensis*, *Bacillus licheniformis*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Enterobacter hormaechei*, *Chryseobacterium* sp. and *Ochrobactrum* sp. were identified as endophytic in coffee fruits.

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