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ORIGINAL ARTICLE

Epiphytic bacteria biodiversity in Brazilian Cerrado fruit and their cellulolytic activity potential

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Abstract The Cerrado is the second largest Brazilian biome, yet little is known about its wild fauna, flora and microbiota. This work aimed to identify epiphytic bacteria present in fruits native to three different regions of the Cerrado and to select cellulase-producing bacteria. Culture-dependent and culture-independent (PCR-DGGE) methods were used to characterize the microbiota from 32 native Cerrado fruits, and the selection of cellulase-producing bacteria was performed by a semi-quantitative test on carboxymethylcellulose agar medium. Analysis of the 16S rRNA gene sequences of 69 profile representatives showed that the isolates belonged to 29 bacterial genera (*Arthrobacter, Bacillus, Paenibacillus, Pseudomonas, Serratia, Staphylococcus, Streptomyces, Enterobacter*, *Microbacterium, Aerococcus, Bradyrhizobium, Methylobacterium, Erwinia, Pantoea, Acidithiobacillus, Ochrobactrum, Stenotrophomonas, Curtobacterium, Clostridium, Lactobacillus, Xanthomonas, Delftia, Klebsiella, Enterococcus, Burkholderia, Escherichia, Streptococcus, Citrobacter* and *Achromobacter*). Species in the genera *Methylobacterium, Stenotrophomonas, Clostridium, Pantoea* and *Enterobacter* were detected by both culture-dependent and culture-independent methods. The species *Lactobacillus fermentum, Acinetobacter* sp. and *Methylomonas methanica* were detected only by PCR-DGGE. Additionally, 30 % (178 isolates) of the bacteria tested were able to produce cellulase. The best producers belonged to the genera *Bacillus, Streptomyces, Paenibacillus, Enterobacter* and *Burkholderia*, indicating that this ecosystem could be an attractive source for the study of novel enzymes.

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Introduction

The Cerrado, which is a typical biome of the Brazilian tropical zone, is a savanna region that occupies approximately 2.0 million km^2, representing 23.1 % of the national territory. The Cerrado is considered the second-largest biome in Brazil and is surpassed in area only by the Amazon forest. The diversity of animals and plants is attributed to the variety of environments, which produce a great wealth of flowers and fruits, making the flora of the Cerrado the richest among the world's savannas (Walter 2006). The flora of the Cerrado has several fruit species with great potential for agricultural use, but these fruits are traditionally used by the local population. These fruits are exceptional mainly due to their pleasant and even exotic sensory traits, such as color, flavor and aroma. Generally, the fruits of the Cerrado are consumed raw or in the form of juices, liqueurs, ice creams, jellies and various jams (Silva et al. 2001; Almeida et al. 2008). Among the major fruit species of the savanna that are economically (for food) and scientifically important are the Marolo (*Annona crassiflora*), Pequi (*Caryocar brasiliense*), Ananás (*Ananas comosus*), Cagaita (*Eugenia dysenterica*), Araçá (*Psidium cattleyanum*), Gabiroba (*Campomanesia pubescens*), Lobeira (*Solanum lycocarpum*), Umbu (*Spondias tuberosa*) and Buriti (*Mauritia flexuosa*) (Soares et al. 2009). According to Silva et al. (2008a), some of these species may be potential sources of economic exploitation as research and technological development enable their use.

The surfaces of most plants are characterized by an associated epiphytic microbiota living in the phyllosphere (Kinkel et al. 2000). Plants provide a niche rich in nutrients for the growth and development of various groups of

microorganisms, especially bacteria. Mutualistic interactions between host plants and associated bacteria could have played a key role in the selection of a relatively specific fruit microbial community (Trivedi et al. 2011). The microbial biodiversity on fruits depends on regional environmental factors, such as the humidity, temperature and soil population, and on the physicochemical properties of each individual fruit species (Thomas and Soly 2009).

Only a few studies have reported the epiphytic microbiota on fruit from the Cerrado; microbial diversity has been studied for the Acerola, Pitanga, Umbu and Mangaba fruits (Trindade et al. 2002), for Pequi (Ferreira and Junqueira 2007) and for dried Cabacinha fruit (Amaral et al. 2001). The epiphytic microbiota is composed of bacteria, yeasts and filamentous fungi that may have pectinolytic, cellulolytic, proteolytic, and antimicrobial activities and may be pathogenic to humans (Trindade et al. 2002; Ferreira and Junqueira 2007).

Such microorganisms synthesize numerous industrially important compounds, such as vitamins, antibiotics, organic acids and enzymes. Of these metabolites, enzymes have been used in various industrial processes, mainly in food processing and waste treatment (Schmidell et al. 2001). Cellulases are enzymes that form a complex capable of acting on cellulosic materials and promoting their hydrolysis. These enzymes are highly specific biocatalysts that act synergistically to release sugars, including glucose, which is the main industrial interest due to the possibility of converting it into ethanol (Tolan 2002; Lynd et al. 2002). Some bacteria reported in the literature to have cellulolytic activity include *Acidothermus*, *Micromonospora, Streptomyces, Paenibacillus* and *Pseudomonas* (Talia et al. 2012), as well as *Bacillus pumilus* (Nagar et al. 2012), *Bacillus amyoliquefaciens* (Lee et al. 2008), *Bacillus subtilis* (Kim et al. 2009; Bano et al. 2013), *Marinobacter* (Shanmughapriya et al. 2010), *Vibrio* (Gao et al. 2010) and *Geobacillus* sp. (Assareh et al. 2012).

Little is known about the interactions that occur between typical fruits from the Cerrado and their epiphytic microbiota. The knowledge acquired during this study could enable an evaluation of the biotechnological potential of these microorganisms. The main purpose of this study was to investigate the diversity of epiphytic bacteria in the fruits of the Minas Gerais Cerrado using culture-dependent and culture-independent approaches and to evaluate the ability of these bacterial isolates to produce cellulase.

Materials and methods

Collection area and fruit sampling

Fruits were collected from preserved areas of the Cerrado in the state of Minas Gerais, Brazil, in regions near the cities of Arcos, Luminárias and Passos in January 2010 (rainy season). The fruits were collected at the following coordinates: Arcos, in the midwest region of Minas Gerais (20°17′29″S, 45°32′23″W), and Luminárias and Passos, in the southern region of Minas Gerais (21°31′26″S, 44°54′11″W and 20°43′08″S, 46°36′35″W, respectively). Thirty-two different species of fruits were collected, as described in Table 1 and Fig. 1. The amount of each fruit was sampled according to the availability in the area, season and size of fruit. Mature fruits were collected aseptically, placed in sterile plastic bags and transported under refrigeration (10 °C) to the Microbiology Laboratory of the Federal University of Lavras, Minas Gerais, Brazil. The fruits were stored in a freezer at −20 °C for further microbiological analysis.

Isolation of epiphytic bacteria

For microbial analyses, the fruit collected from each region and sample point was mixed to constitute a composite sample. Twenty grams from each fruit were added aseptically to flasks containing 180 mL peptone water (0.1 % (v/v) Himedia peptone, bacteriological) and were washed in a shaker at 140 rpm and 28 ° C for 30 min, after which 10-fold serial dilutions were prepared in peptone water. Bacterial counts were conducted on four different culture media. Nutrient agar medium (NA; 0.3 g/L meat extract, 0.5 g/L peptone and 1.5 g/L agar) and nutrient agar containing nystatin (0.4 g/L) were used as general media for the growth of mesophilic aerobic bacteria. Eosin–methylene blue agar (EMB; 1.0 g/L meat peptone, 0.5 g/L lactose, 0.2 g/L dipotassium phosphate, 0.04 g/L eosin, 0.0065 g/L methylene blue, 0.5 g/L sucrose and 1.5 g/L agar) was used for *Enterobacteriaceae*. Lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe agar (MRS; 1.0 g/L peptone, 1.0 g/L meat extract, 0.5 g/L yeast extract, 2.0 g/L glucose, 0.1 g/L Tween 80, 0.2 g/L dibasic potassium phosphate, 0.5 g/L sodium acetate, 0.2 g/L ammonium citrate, 0.005 g/L magnesium sulfate-7H_2O, 0.005 g/L manganese sulfate-7 H_2O and 1.5 g/L agar). Mesophilic aerobic bacteria and Enterobacteriacea were enumerated by the spread plate method, and LAB were enumerated by the pour plate method. The plates were incubated at 30 °C for 72 h. This temperature was chosen, because in the Cerrado the average temperature is 28–30 °C.

Morphological and biochemical characterization and grouping

Following incubation, the number of colony-forming units (CFU) was recorded, and the morphological characteristics (i.e., colony size, colony shape, edge morphology, color and brightness) of the different colony types were recorded. The square root of the total number of colonies for each morphotype was restreaked and purified. All isolates were initially screened for Gram staining, microscopic morphology

Table 1 Fruits species sampling in the Cerrado region of Fruit, Arcos and Luminárias (MG), used for the isolation of epiphytic bacteria

Passos	Scientific name	Popular name	Number of fruits
Passos region			
1	*Psychotria hoffmannseggiana*	Erva-de-rato	45
2	*Erythroxylum* sp.	Pimentinha-do-mato	52
3	*Erythroxylum suberosum*	Cabelo-de-negro	40
4	*Rudgea jasminoides*	Cafezinho-do-mato	48
5	*Miconia pepericarpa*	Pixirica	20
6	*Erythroxylum daphnites*	Muxiba	12
7	*Byrsonima crassifolia*	Murici-do-cerrado	7
8	*Psidium catleyaniu*	Araçá	5
9	*Schinus polygama*	Aroeirinha	35
10	*Picramnia parvifolia*	Pau-amargo	27
11	*Psittacanthus robustus*	Erva-de-passarinho	30
12	*Miconia cinerascens*	Jacatirão	14
13	*Erythroxylum campestre*	Fruta-de-pomba	12
14	*Davilla rugosa*	Bejuco-colorado	7
Arcos region			
15	*Hymenaea courbaril*	Jatobá	5
16	*Solanum aculeatissimum*	Arrebenta-boi	8
17	*Ananás comosus*	Ananás	7
18	*Annona crassiflora*	Marolo	10
19	*Psidium guajava*	Goiaba	7
20	*Caryocar brasiliense*	Pequi	15
21	*Annona geraensis*	Marolinho	1
22	*Lantana lilacina*	Midigrilo	3
Luminárias region			
23	*Kielmeyera speciosa*	Pau-santo	8
24	*Erythroxylum daphnites*	Muxiba	12
25	*Byrsonima crassifolia*	Murici-do-cerrado	7
26	*Miconia pseudonervosa*	Pixirica	20
27	*Leandra lacunosa*	Erva-de-jabuti	10
28	*Vitex montevidensis*	Azeitona-do-mato	7
29	*Myrsine coriacea*	Capororoca	30
30	*Miconia chamissois*	Pixirica	20
31	*Miconia cinerascens*	Jacatirão	12
32	*Miconia pepericarpa*	Pixirica	20

(shape, cell arrangements), catalase activity, motility and spore formation, as recommended in *Bergey's Manual of Determinative Bacteriology* (Brenner et al. 2005).

Purified isolates were stored at −80 °C in 20 % (w/w) glycerol; isolates originating from NA and EMB were stored in nutrient broth (Sigma, St. Louis, USA), and LAB were stored in MRS broth (Merck, Whitehouse Station, USA). The results of preliminary tests and the morphological and microscopic characterizations of several isolates were subjected to cluster analysis, and hierarchical clustering was performed using the Statistica 8.0 (Statsoft, Tulsa, UK, USA) program based on similarity matrices, which were generated by the agreement method (simple matching) using Euclidean distances and the unweighted pair group average algorithm (UPGMA). Among the isolates exhibiting the same morphological and biochemical characteristics (grouped with 100 % similarity), a representative from each group was selected and subjected to the molecular clustering technique, repetitive extragenic palindromic-polymerase chain reaction (Rep-PCR) and finally identified by 16S rRNA gene sequencing.

$(GTG)_5$-PCR genomic fingerprinting

Molecular fingerprinting-based grouping of the isolates was performed using repetitive extragenic palindromic (Rep)-PCR (GTG_5 primer), as described by Gevers et al. (2001). Total

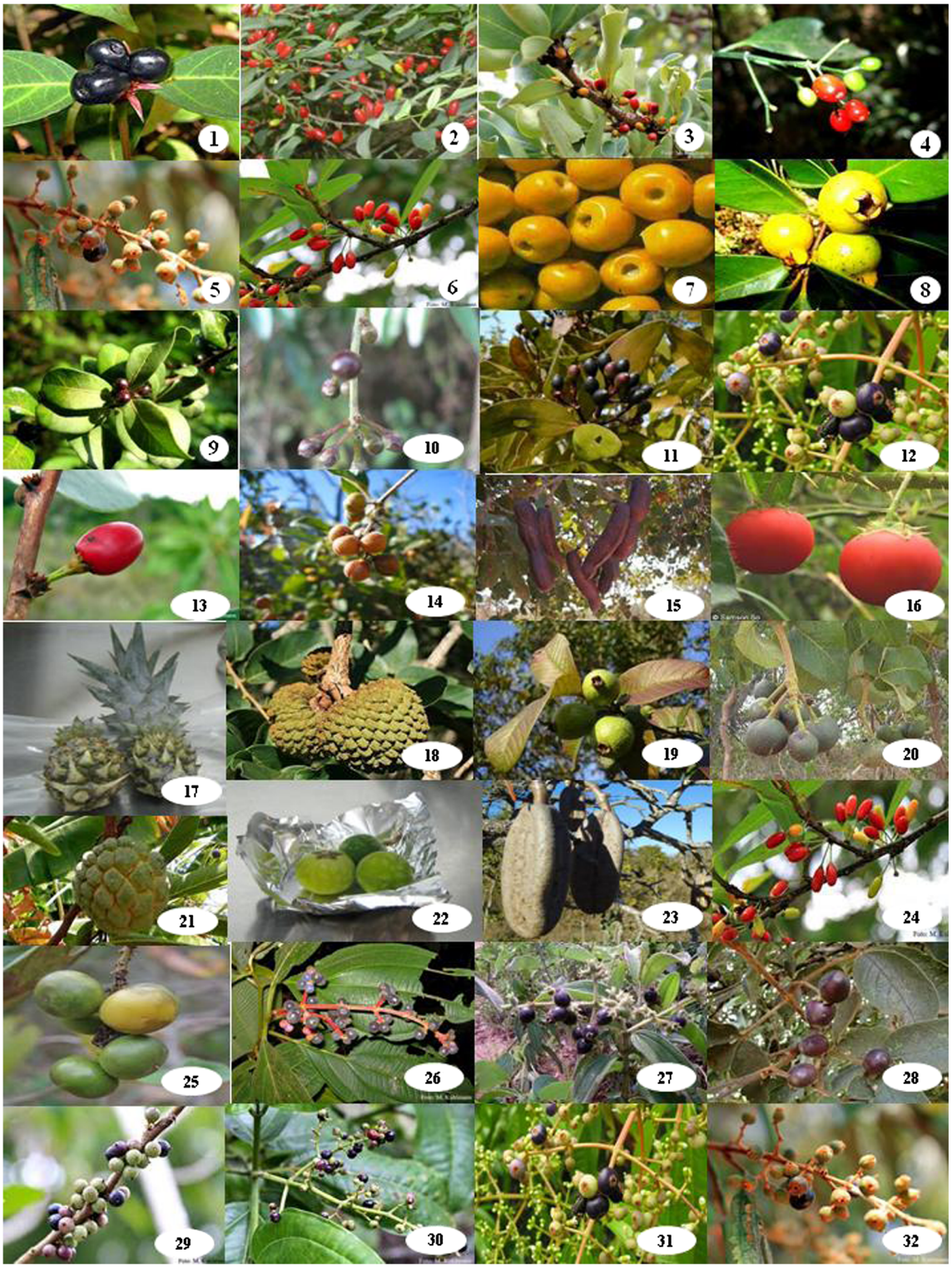

Fig. 1 Cerrado fruits collected in the regions of Passos, Arcos and Luminárias in Minas Gerais, Brazil, used for bacterial isolation according to Table 1

genomic DNA was extracted and analyzed by electrophoreses on 1 % agarose gel. PCR amplification was performed according to Pereira et al. (2011). Two microliters of DNA was added to 12.5 μL of Taq PCR Master Mix (Qiagen, São Paulo, Brazil), 8 μL of H_2O, 0.25 μL of bovine serum albumin (BSA), 0.25 μL of formamide and 2 μL of the primer GTG_5 (5′ - GTG GTG GTG GTG GTG - 3′) (Pereira et al. 2012). PCR was performed with the following cycling conditions: 5 min initial denaturation at 94 °C; 30 cycles of 95 °C for 30 s, 45 °C for 60 s and 60 °C for 5 min; and a final elongation at

60 °C for 16 min. Subsequently, the amplification products were separated on a 2.0 % (w/v) agarose gel at 70 V for 4 h and stained with SYBR Green (Invitrogen, Foster City, CA, USA). The Rep-PCR profiles were normalized, and cluster analysis was performed with the Bionumerics V6.5 software package (Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was calculated on the basis of Dice's coefficient of similarity using the unweighted pair group method with the arithmetic averages clustering algorithm (UPGMA).

Identification of isolates by 16S rDNA sequencing

Partial sequences of the 16S rDNA gene were used to identify representative bacteria from the analysis of the Rep-PCR gel. The primers were 27f (5′- AGAGTTTGATCCTGGCTCAG - 3′) and 1512r (5′ -ACGGCTACCTTGTTACGACT - 3′), and PCR amplification was performed as described by Wang et al. (2006). The PCR products were purified and sequenced using an ABI3730 XL automatic DNA sequence (Macrogen, Seoul, South Korea), and the sequences were compared with the GenBank database using the BLAST algorithm (National Center for Biotechnology Information, MD, USA).

DGGE analysis

Total DNA was extracted from the same microbial suspension used for plating (diluted 10 times). To obtain a pellet, the samples were centrifuged at 14,000 rpm for 3 min. Purification of total DNA was performed using the "DNA Purification from Tissues" kit (QIAamp DNA Mini Kit; Qiagen, Chatsworth, CA, USA) following the manufacturer's instructions. After extraction, an internal fragment of the V3 region of the 16S rDNA gene was amplified with the universal primers 338f gc (5′- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG - 3′; the GC clamp is underlined) and 518r (5′- ATTACCGCGGCTGCTGG - 3′) (Ovreas et al. 1997). All reactions were performed in a 25 μL volume containing 0.625 U Taq DNA polymerase (Promega, Milan, Italy), 2.5 mL of 10× buffer, 0.1 mM dNTP, 0.2 mM each primer, 1.5 mM $MgCl_2$ and 1 μL of extracted DNA. The amplification was performed according to Ramos et al. (2010). PCR products (5 μL plus 2 μL of loading dye) were checked by electrophoretic separation on a 1 % agarose gel at 70 V for 40 min.

The PCR products were analyzed by DGGE using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, Richmond, CA, USA). Polyacrylamide gels consisting of 8 % (v/v) polyacrylamide (Sigma-Aldrich) in 0.5× TAE buffer and a 20–50 % denaturing gradient (100 % denaturing polyacrylamide solution corresponds to 7 M urea and 40 % (v/v) formamide) were used. Electrophoresis was performed for 4 h at 120 V in 1× TAE buffer at a constant temperature of 60 °C using the DCode System (Bio-Rad). After electrophoresis, the gels were stained with SYBR Green I (Invitrogen) for 30 min and then visualized and digitally imaged using the Infinity VX2 System (Vilber Lourmat).

All different bands were excised from the acrylamide gels by inserting a pipette tip into the band, and the DNA in the band was left to elute overnight at 4 °C in 50 μL of Milli-Q water. Before sequencing, the excised bands (5 μL) of the eluited band were re-amplified using the same PCR conditions as described above, and the PCR products were purified using the QIAEX® III extraction kit (Qiagen) according to the manufacturer's protocol. The purified PCR products were sequenced by Macrogen using an ABI3730 XL automatic DNA sequence. The sequences were then compared to the GenBank database using the BLAST algorithm (National Center for Biotechnology Information).

In order to express differences of bacterial communities among fruits, the presence/absence of amplicons in the DGGE profiles was converted into a binary matrix, which was used for hierarchical clustering applying Jaccard similarity and the Ward algorithm, through Systat 8.0 software.

Screening for cellulose-degrading bacteria

The bacterial isolates were subjected to a semi-quantitative analysis according to the method proposed by Kasana et al. (2008) for the selection of cellulase-producing bacteria. The medium for the qualitative measurement of extracellular cellulase activity contained the following (per liter): 2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 2 g carboxymethylcellulose (CMC), 0.2 g peptone and 17 g agar. Bacterial cultures were standardized to a cell density of 8 log CFU/mL and then inoculated by microdrop onto CMC agar. After 48 h incubation at 28 °C, the plates were stained with iodine to visualize the halo of cellulolytic activity around each colony and to facilitate the selection of cellulose-degrading bacteria.

The enzyme index (EI) was determined from the ratio between the average diameter of the cellulose degradation halo and the diameter of the colony. The EI was calculated only for those isolates with degradation halo size >3 cm. The isolates with EI values greater than 4.0 were considered efficient cellulase-producing bacteria.

Results

Enumeration of bacterial groups

In the present study, 32 fruits obtained from 3 different regions of the Brazilian Cerrado were used. A total of 600 bacterial colonies were isolated from different Cerrado fruits. The mesophilic and *Enterobacteriaceae* media (NA and EMB, respectively) permitted the isolation of 85 % of the total

isolates, and the lactic acid bacteria medium (MRS) enabled the isolation of the remaining 15 %. In the Passos region, the average population of bacteria isolated on the NA medium ranged from 2.59 log CFU/g (Aroeirinha fruit) to 3.42 log CFU/g (Bejuco-colorado fruit) (Table 2). The MRS medium showed the lowest bacterial populations, with counts varying from <1 log CFU/g (Bejuco-colorado fruit) to 2.88 log CFU/g (Fruta-de-pomba fruit). The counts on the EMB medium varied from 1.42 log CFU/g in Pau-amargo fruit to 3.48 log CFU/g in Erva-de-rato fruit.

Table 2 Average population of bacteria in four culture media from Passos, Arcos and Luminárias regions

Fruit	Total mesophilic bacteria	Enterobacteriaceae	Lactic acid bacteria
Passos region			
1	3.35±0	3.48±0	<1
2	2.81±0	2.52±0.08	<1
3	2.89±0.08	<1	<1
4	2.84±0.04	2.62±0.09	<1
5	2.81±0.02	2.28±0.01	<1
6	3.24±0.09	2.46±0.06	<1
7	3.32±0.03	2.84±0.01	<1
8	2.87±0.05	1.47±0.07	<1
9	2.59±0.02	<1	<1
10	2.86±0.01	1.42±0.02	<1
11	3.27±0.01	2.84±0.02	<1
12	3.0±0	1.54±0.09	<1
13	2.72±0.07	2.19±0.01	2.88±0.08
14	3.42±0.06	2.96±0.02	<1
Arcos region			
15	3.4±0.05	2.96±0.01	2.62±0.01
16	2.56±0.06	3.45±0.02	<1
17	2.7±0	<1	<1
18	3.09±0.01	2.55±0.06	3.39±0.05
19	2.7±0.02	<1	<1
20	2.67±0.03	<1	<1
21	3.34±0.02	<1	<1
22	3.13±0.01	<1	<1
Luminárias region			
23	2.11±0.06	<1	<1
24	3.28±0.03	3.16±0.05	<1
25	3.13±0.05	1.10±0	<1
26	2.58±0.05	<1	<1
27	2.74±0.08	<1	<1
28	3.47±0.06	2.22±0.01	<1
29	2.76±0.03	1.39±0.07	<1
30	2.85±0.01	<1	<1
31	2.4±0.02	1.35±0.02	<1
32	3.43±0	<1	<1

±Standard Deviation. Total Mesophilic bacteria = Mesophilic bacteria grown in NA and in NA containing nystatin; <1 log CFU/g means that no colonies were found. For names of the fruits, see Table 1

Fruit from the Arcos region had higher bacterial populations than fruit from the other regions studied. Counts on the NA medium ranged from 2.56 log CFU/g (Arrebenta-boi) to 3.4 log CFU/g (Jatobá) (Table 2). The counts on the MRS medium ranged from <1 log CFU/g (Arrebenta-boi, Ananás, Goiaba, Pequi, Marolinho and Midigrilo fruits) to 3.39 log CFU/g (Marolo fruit). Counts on EMB varied between 2.55 log CFU/g in Marolo fruit and 3.45 log CFU/g in Arrebenta-boi fruit.

In the Luminárias region, the highest count on the NA medium, 3.47 log CFU/g, was associated with Azeitona-do-mato, whereas the lowest count, 2.11 log CFU/g, was associated with Pau Santo fruit. The LAB population was less than 1 log CFU/g in many fruits collected, as shown in Table 2. No LAB was detected in the fruits collected from the Luminárias region. The lowest count on EMB was 0.39 log CFU/g (Jacatirão fruit), and the highest count was 3.16 log CFU/g (Muxiba fruit) (Table 2).

The fruits Erva-de-rato (fruit 1), Murici (fruit 7), Erva-de-passarinho (fruit 11) and Bejuco-colorado (fruit 15), which belonged to the Passos region, had the highest population averages, with detected values above 3.0 log CFU/g (Table 2). In the Arcos region, the fruits with the highest counts were Jatobá (fruit 15), Arrebenta-boi (fruit 16) and Marolo (fruit 18). In the Luminárias region, the fruits Muxiba (fruit 24) and Azeitona-do-mato (fruit 28) showed the highest bacterial counts.

Same species fruits like Muxiba (fruits 6 and 24), Murici-do-cerrado (fruits 7 and 25) and Pixirica (fruits 5 and 32) were collected in Passos and Luminárias region. These species in both regions showed a higher population abundance of mesophilic bacteria (up to 30 % each) than Enterobacteriaceae and lactic acid bacteria (Table 2). However, the lactic bacteria group was lower for all the fruits, except for fruit 32 from the Luminárias region that also showed a low population of Enterobacteriaceae.

Erva de rato (Passos), Marolo (Arcos) and Azeitona do mato (Luminárias) fruits had the highest counts of Enterobacteriacea (3.48 log CFU/g), lactic acid bacteria (3.39 log CFU/g) and total mesophilic bacteria (3.47 log CFU/g), respectively (Table 2).

Rep-PCR-based fingerprinting and identification of isolates

Six hundred isolates were characterized as described in "Materials and methods", and were initially classified according to the fruit of origin. Isolates were selected from the groups obtained and were again subjected to group analysis corresponding to each of the sample regions (Passos, Arcos e Luminárias) (data not shown). After grouping based on

morphological and biochemical characteristics, isolates (at least one for each fruit) were selected and subjected to characterization by REP-PCR. Sixty-eight different band profiles were obtained, 31, 25, and 12 of which belonged to the Passos, Arcos, and Luminárias regions, respectively. At least one isolate from each fruit was chosen. This characterization yielded 31 different band profiles in the Passos region, 25 in the Arcos region and 12 in the Luminárias region

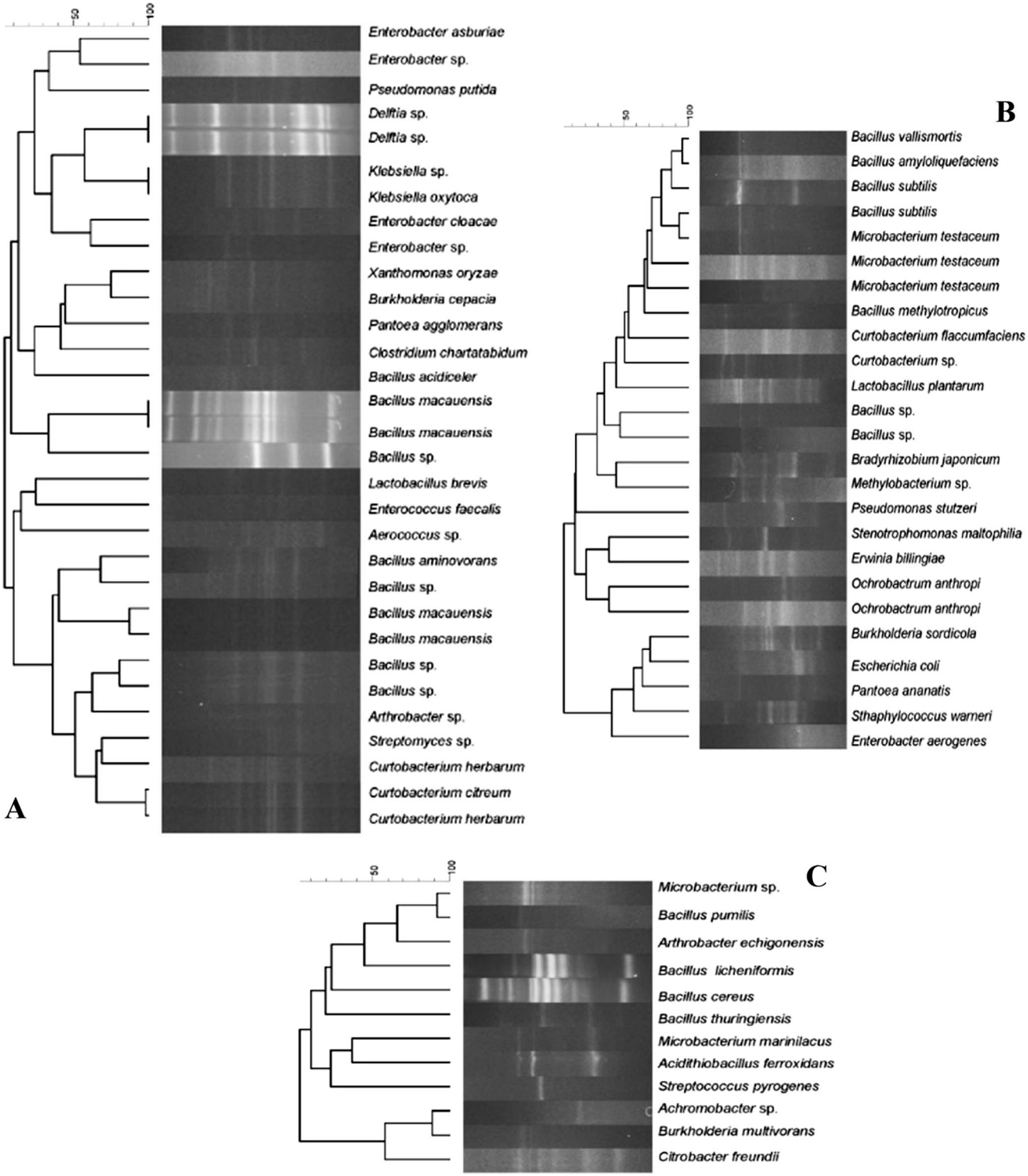

Fig. 2 Dendrogram obtained by cluster analysis of rep-PCR (GTG^5) fingerprints of bacteria isolated from cerrado fruits. The dendrogram is based on Dice's coefficient of similarity using the unweighted pair group method with the arithmetic averages clustering algorithm (UPGMA) – Region of Passos (*A*), Arcos (*B*) and Luminárias (*C*)

Table 3 Identification of bacterial isolates, number of isolates by species, origin fruit (popular name), accession number in the corresponding GeneBank, percentage of similarity (% S) and estimated average population of bacterial isolates (log CFU/g) from Cerrado fruits belonging to regions Passos, Arcos and Luminárias, MG

Isolate	Number of isolates by species	Origin fruit (popular name)	Closest NCBI match	Accession number	S (%)	Population estimate log (CFU/g)
Passos						
UFLA BCEF 151	13 (43 %)	Erva-de-rato	*Bacillus* sp.	DQ451097.1	100	2.48
UFLA BCEF 154	17 (57 %)	Erva-de-rato	*Bacillus* sp.	DQ451097.1	100	2.60
UFLA BCEF 168	13 (35 %)	Pimentinha-do-mato	*Bacillus* sp.	DQ451097.1	100	2.00
UFLA BCEF 175	11 (30 %)	Pimentinha-do-mato	*Streptomyces* sp.	JN566179.1	100	2.30
UFLA BCEF 181	13 (35 %)	Pimentinha-do-mato	*Enterobacter* sp.	FJ686827.1	94	2.30
UFLA BCEF 194	17 (50 %)	Cabelo-de-negro	*Klebsiella* sp.	FJ823035.1	97	2.00
UFLA BCEF 197	17 (50 %)	Cabelo-de-negro	*Klebsiella oxytoca*	NR041749.1	97	2.90
UFLA BCEF 221	4 (100 %)	Cafezinho-do-mato	*Pantoea agglomerans*	JN162392.1	97	2.60
UFLA BCEF 285	7 (100 %)	Pixirica	*Bacillus* sp.	AY211147.1	96	2.30
UFLA BCEF 354	2 (100 %)	Jacatirão	*Lactobacillus brevis*	AB548884.1	100	2.00
UFLA BCEF 365	1 (1 %)	Muxiba	*Arthrobacter* sp.	JF901933.1	100	2.60
UFLA BCEF 368	1 (1 %)	Muxiba	*Bacillus macauensis*	NR042892.1	97	3.69
UFLA BCEF 369	17 (21 %)	Muxiba	*Bacillus macauensis*	NR042892.1	97	2.30
UFLA BCEF 370	17 (21 %)	Muxiba	*Delftia* sp.	DQ131821.1	97	2.70
UFLA BCEF 372	23 (28 %)	Muxiba	*Enterobacter* sp.	DQ536420.1	97	2.85
UFLA BCEF 385	23 (28 %)	Muxiba	*Clostridium chartatabidum*	NR029239.1	100	2.78
UFLA BCEF 449	23 (46 %)	Murici-do-cerrado	*Bacillus* sp.	FJ613547.1	96	2.78
UFLA BCEF 473	7 (14 %)	Murici-do-cerrado	*Enterobacter* sp.	DQ536420.1	97	3.75
UFLA BCEF 475	1 (2 %)	Murici-do-cerrado	*Aerococcus* sp.	AF076639.1	97	2.00
UFLA BCEF 477	19 (38 %)	Murici-do-cerrado	*Bacillus* sp.	DQ451097.1	100	2.60
UFLA BCEF 485	19 (100 %)	Araçá	*Enterobacter* sp.	CP003026.1	96	2.30
UFLA BCEF 532	13 (100 %)	Aroeirinha	*Curtobacterium* sp.	FN178369.1	96	2.00
UFLA BCEF 619	8 (100 %)	Pau-amargo	*Xanthomonas oryzae*	CP000967.1	100	3.04
UFLA BCEF 637	4 (14 %)	Erva-de-passarinho	*Burkholderia cepacia*	HQ607778.1	100	2.48
UFLA BCEF 678	19 (67 %)	Erva-de-passarinho	*Bacillus macauensis*	NR042892.1	100	2.30
UFLA BCEF 679	5 (19 %)	Erva-de-passarinho	*Bacillus macauensis*	NR042892.1	100	2.30
UFLA BCEF 872	9 (100 %)	Fruta-de-pomba	*Enterococcus faecalis*	FJ821315.1	100	2.78
UFLA BCEF 916	11 (35 %)	Bejuco-colorado	*Curtobacterium citreum*	FN178369.1	97	2.60
UFLA BCEF 922	9 (29 %)	Bejuco-colorado	*Curtobacterium* sp.	JF460761.1	96	4.67
UFLA BCEF 924	4 (12 %)	Bejuco-colorado	*Pseudomonas putida*	HM537229.1	100	3.30
UFLA BCEF 931	7 (24 %)	Bejuco-colorado	*Delftia* sp.	DQ131821.1	97	3.30
Arcos						
UFLA BCEF 1048	9 (32 %)	Arrebenta-boi	*Bradyrhizobium japonicum*	BA000040.2	100	2.30
UFLA BCEF 1050	12 (42 %)	Arrebenta-boi	*Curtobacterium* sp.	HQ324707.1	96	2.00
UFLA BCEF 1062	1 (4 %)	Arrebenta-boi	*Serratia* sp.	EU439032.1	96	2.60
UFLA BCEF 1064	3 (11 %)	Arrebenta-boi	*Erwinia billingiae*	FP236843.1	97	3.00
UFLA BCEF 1065	3 (11 %)	Arrebenta-boi	*Staphylococcus warneri*	HQ831387.1	99	3.00
UFLA BCEF 1084	12 (50 %)	Ananás	*Microbacterium testaceum*	AP012052.1	99	2.60
UFLA BCEF 1104	12 (50 %)	Ananás	*Burkholderia sordicola*	AF512826.1	97	3.26
UFLA BCEF 1110	11 (23 %)	Marolo	*Ochrobactrum* sp.	DQ295875.1	98	3.00
UFLA BCEF 1118	16 (33 %)	Marolo	*Bacillus subtilis*	HQ678662.1	97	2.60
UFLA BCEF 1135	12 (25 %)	Marolo	*Bacillus methylotrophicus*	JN648098.1	99	1.48
UFLA BCEF 1163	5 (10 %)	Marolo	*Stenotrophomonas maltophilia*	EU294137.1	100	2.26
UFLA BCEF 1180	3 (6 %)	Marolo	*Ochrobactrum anthropi*	AM490609.1	98	1.84
UFLA BCEF 1184	1 (3 %)	Marolo	*Pantoea* sp.	FJ605368.1	96	2.18

Table 3 (continued)

Isolate	Number of isolates by species	Origin fruit (popular name)	Closest NCBI match	Accession number	S (%)	Population estimate log (CFU/g)
UFLA BCEF 1192	7 (47 %)	Jatobá	*Enterobacter aerogenes*	FJ380123.1	97	2.07
UFLA BCEF 1199	8 (53 %)	Jatobá	*Bacillus vallismortis*	JF912890.1	99	1.60
UFLA BCEF 1211	8 (16 %)	Goiaba	*Bacillus* sp.	HM233971.1	99	2.08
UFLA BCEF 1215	13 (21 %)	Goiaba	*Methylobacterium* sp.	EU912441.1	96	1.48
UFLA BCEF 1250	13 (21 %)	Goiaba	*Bacillus* sp.	HM233971.1	99	1.30
UFLA BCEF 1251	13 (21 %)	Goiaba	*Escherichia coli*	GU329913.1	97	1.30
UFLA BCEF 1252	13 (21 %)	Goiaba	*Bacillus amyloliquefaciens*	JF460751.1	97	2.30
UFLA BCEF 1266	1 (33 %)	Pequi	*Pseudomonas stutzeri*	GQ480478.1	100	1.90
UFLA BCEF 1280	2 (77 %)	Pequi	*Lactobacillus plantarum*	GQ468312.1	97	2.00
UFLA BCEF 1320	13 (65 %)	Marolinho	*Microbacterium* sp.	JN084138.1	96	3.60
UFLA BCEF 1322	7 (35 %)	Marolinho	*Microbacterium testaceum*	AB478972.1	97	2.18
UFLA BCEF 1335	3 (100 %)	Midigrilo	*Curtobacterium* sp.	AB695338.1	96	1.70
Luminárias						
UFLA BCEF 1399	1 (100 %)	Pau-santo	*Achromobacter* sp.	HQ256543.1	94	2.60
UFLA BCEF 1473	5 (100 %)	Muxiba	*Microbacterium marinilacus*	AB286020.1	97	2.11
UFLA BCEF 1489	11 (100 %)	Murici-do-cerrado	*Burkholderia* sp.	AP009385.1	96	1.90
UFLA BCEF 1538	3 (100 %)	Erva-de-jabuti	*Microbacterium sp.*	GQ280061.1	96	1.84
UFLA BCEF 1568	4 (40 %)	Azeitona-do-mato	*Bacillus* sp.	CP000813.1	92	1.30
UFLA BCEF 1590	1 (10 %)	Azeitona-do-mato	*Arthrobacter echigonensis*	GU326383.1	98	4.00
UFLA BCEF 1591	5 (50 %)	Azeitona-do-mato	*Bacillus thuringiensis*	CP002508.1	99	3.00
UFLA BCEF 1603	1 (100 %)	Caporoca	*Bacillus* sp.	JF815044.1	94	2.00
UFLA BCEF 1652	1 (12.5 %)	Pixirica	*Bacillus* sp.	JN133843.1	94	2.00
UFLA BCEF 1666	6 (75 %)	Pixirica	*Acidithiobacillus ferrooxidans*	CP001219.1	100	2.00
UFLA BCEF 1691	6 (100 %)	Jacatirão	*Streptococcus pyogenes*	AE009949.1	97	1.00
UFLA BCEF1739	1 (12.5 %)	Pixirica	*Citrobacter freundii*	EF450115.1	97	2.78

(Fig. 2). As shown in Fig. 2, the Rep-PCR technique was able to roughly separate the Gram-positive and Gram-negative bacteria into two groups. Exceptions were *Clostridium chartatabidum* for Muxiba fruit from region Passos (A) and *Staphylococcus warneri* for Arrebenta-boi fruit from Arcos (B).

The results of the polyphasic identification of all 68 isolates from the fruits of the three Cerrado regions studied are depicted in Table 3 and Fig. 2. The identified isolates belonged to 29 different genera. Only *Bacillus* and *Burkholderia* genera were common to all three regions. *Delftia, Klebsiella, Xanthomonas, Clostridium, Enterococcus, Aerococcus* and *Streptomyces* were exclusively encountered in the fruits collected in the Passos region. Bacterial community of fruits from the Arcos region was characterized by *Staphylococcus, Escherichia*, *Ochrobactrum, Erwinia, Stenotrophomonas, Methylobacterium* and *Bradyrhizobium. Acidithiobacillus*, *Streptococcus, Citrobacter* and *Achromobacter* were found only in fruits from the Luminaria region.

Bacillus showed the highest species biodiversity. With regard to fruits from the Passos region, *Bacillus* was found in six different fruits (Table 3), with *B. macauensis* the most frequently occurring species (Muxiba and Erva-de-passarinho). The Muxiba fruit showed the highest diversity of genera (*Arthrobacter* sp., *Bacillus* sp., *Delftia, Enterobacter* sp., *Chartatabidum* and *Clostridium*). In the Arcos region, four different *Bacillus* species were identified: *B. subtilis* and *B. methylotrophicus* (Marolo fruit), *B. vallismortis* (Jatobá fruit) and *B. amyloliquefaciens* (guava fruit). Another genus that had representatives in two different fruits (pineapple and Marolinho) from the Arcos region was *Microbacterium*. The Arrebenta-boi had the highest diversity of genera (*Bradyrhizobium, Curtobacterium, Serratia, Erwinia* and *Staphylococcus*). Three different fruits (Azeitona-do-mato, Caporoca e Pixirica) from Luminárias had representatives of the genus *Bacillus*, and *B. thuringiensis* species was identified in the Azeitona-do-mato fruit.

Bacteria detected by PCR-DGGE

As shown in Table 4 and Fig. 3, the fruits from the Passos, Arcos and Luminárias regions displayed different bacterial profiles. However, the fruits from the Arcos region showed

similar profiles, except for fruits 18, 21 and 22. Differences among fruits from the same region were evaluated through dendrograms (Fig. 3b–d). Among the regions, fruits from Passos showed the highest bacterial diversity. The fruits 10, 11, 12 and 14 from the Passos region showed similar band profiles. The same was also observed for fruits 1, 2, 3, and 4, which were clustered into one unique group. Two groups of fruits were found in the Arcos region; the first, including the fruits 15, 16, 17, 19 and 20, showed no difference in microbial composition. The second group comprised of fruits 18, 21 and 22 had a different profile (Fig. 3c).

To determine the composition of the epiphytic bacterial populations in the Cerrado fruit samples, the bands in the PCR-DGGE gel were excised, and the DNA was recovered and sequenced. We were not able to identify the minor bands l and q because they could not be amplified after excision from the gel due to their low abundance.

In the Passos region, the fruits Muxiba (fruit 6), Araça (fruit 8) and Fruta-de-pomba (fruit 13) showed a DGGE profile distinct from that (Fig. 3) of the other fruits of this region; these three fruits showed the highest number of bands. In the Passos region fruits, the species *Methylobacterium gregans*, *Stenotrophomonas* sp. and *Methylobacterium* sp. were identified by a culture-independent approach. *Stenotrophomonas* sp. (band e) was detected in the Muxiba (fruit 6) and Jacatirão (fruit 12) fruits.

The profiles of the bacterial communities present in the samples from Arcos were more homogeneous, with the exception of the species *Methylomonas methanica* being identified only in Marolinho (fruit 21) and one band unidentified in Midigrilo (fruit 22) (Fig. 3a, c). The bacterial species identified in the fruits of this region were *Lactobacillus fermentum* and *M. methanica*. *L. fermentum* was identified by PCR-DGGE in the fruits of Jatobá (fruit 15), Arrebenta-boi (fruit 16), Ananás (fruit 17), Goiaba (fruit 19) and Pequi (fruit 20).

Fruits from the Luminárias region showed the lowest number of bands of the fruits analyzed (Fig. 3a, d). The fruits Pau-santo (fruit 23), Muxiba (fruit 24) and Murici-do-cerrado (fruit 25) presented profiles with similar bands, which were identified as the *Acinetobacter* sp. The fruits Pixirica (fruit 30), Jacatirão (fruit 31) and Pixirica (fruit 32) also presented similar band profiles that were identified as the *Enterobacter aerogenes* (m) and uncultivable α-Proteobacterium (n). *Clostridium* sp. (band o) and *Pantoea* sp. (band p) were detected in two different fruits from the Luminárias region as Pixirica (fruit 26) and Azeitona-do-mato (fruit 28) fruits.

Screening for cellulose-degrading bacteria

Six hundred isolates from fruits of the Brazilian Cerrado were tested for their ability to produce cellulase. Among them, 178 isolates (30 %) were capable of forming a halo of cellulose degradation around bacterial colonies. Nine isolates were selected because their cellulase activity, in terms of Enzymatic Index, was higher than 4 (Table 5).

The highest values found for the enzymatic index were 5.7 and 5.15 for strains of *B. subtilis* UFLA BCEF 1130 and *Paenibacillus illinoisensis* UFLA BCEF 1197, respectively (Table 5).

Discussion

To date, little is known about the epiphytic microbiota of fruit native to the Cerrado in the state of Minas Gerais. This study identified the epiphytic bacteria of 32 different fruits native to the Brazilian Cerrado. Differences in climate (temperature), fluctuations in the seasons (rainy and dry), the time of fruit ripening and geographic location may influence the epiphytic microbial population of plants and fruits (Chand-Goyal and Spotts 1996; Thomas and Soly 2009). The average annual rainfall in the Cerrado is 1,500 mm, and temperatures are generally mild throughout the year, ranging between 22 and 30 °C (Klink and Machado 2005). In the regions where samples were collected for this study, the precipitation was above 200 mm and the temperature was approximately 28 ° C,

Table 4 Identification of bands excised from DGGE after amplification of 16S rDNA using the bacterial universal primers 338f and 518r extracted from 32 fruit samples from regions of Passos, Arcos and Luminárias (Table 1)

Band	Species	Source	% Similarity
a	Uncultivable bacterium	FJ406571.1	90
b	Uncultivable soil bacterium	GQ351443.1	74
c	*Methylobacterium gregans*	HM803941.1	90
d	Uncultivable bacterium	FJ403001.1	78
e	*Stenotrophomonas* sp.	FJ609992.1	90
f	*Methylobacterium* sp.	JN590732.1	92
g	Uncultivable bacterium	FN421647.1	68
h	Uncultivable bacterium	JF681620.1	85
i	Uncultivable bacterium	EF990686	99
j	Uncultivable bacterium	GU764869.1	99
k	*Lactobacillus fermentum*	JQ083644.1	99
l	Unidentified	–	–
m	*Enterobacter aerogenes*	CP002824.1	100
n	Uncultivable *α-Proteobacterium*	FJ938135.1	97
o	*Clostridium* sp.	FJ609997.1	98
p	*Pantoea* sp.	FJ611805.1	85
q	Unidentified	–	–
r	*Acinetobacter* sp.	CR543861.1	80
s	*Methylomonas methanica*	CP002738.1	100

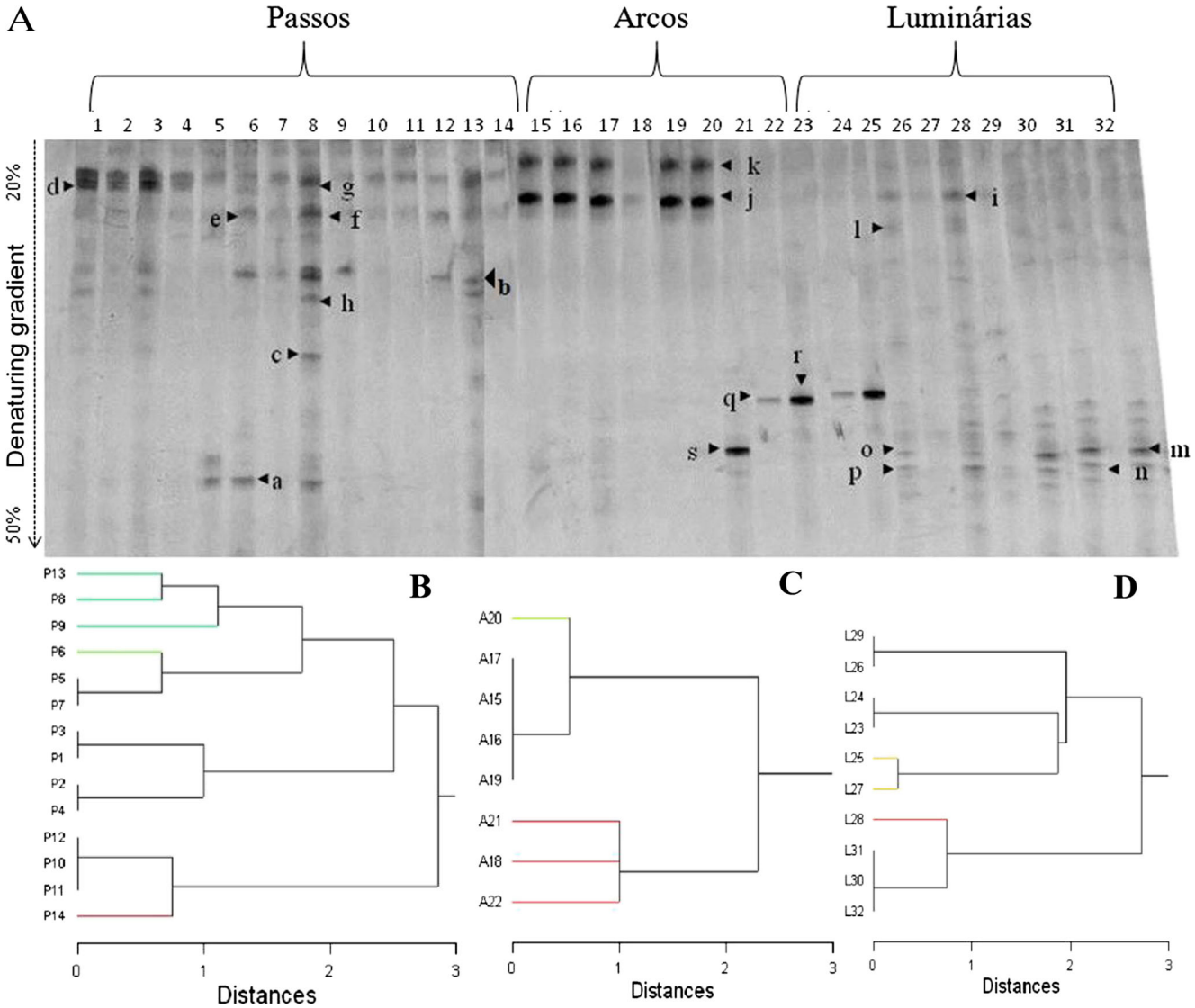

Fig. 3 **a**. Denaturing Gradient Gel Electrophoresis profiles of bacterial V3 regions of the 16S rRNA gene amplified from fruits of the region Passos, Arcos and Luminárias. The *numbers* refer to the different fruit samples collected: *1–15* (erva-de-rato, pimentinha-do-mato, cabelo-de-negro, cafezinho-do-mato, pixirica, muxiba, murici-do-cerrado, araçá, aroeirinha, pau-amargo, erva-de-passarinho, jacatirão, fruta-de-pomba and bejuco-colorado, respectively); *16–23* fruits of Arcos (jatobá, arrebenta-boi, ananás, marolo, goiaba, pequi, marolinho and midigrilo, respectively); *24–32* fruits of Luminárias (pau-santo, muxiba, murici-do-cerrado, pixirica, erva-de-jabuti, azeitona-do-mato, capororoca, pixirica, jacatirão and pixirica, respectively). **b** dendogram from fruits of the region Passos; **c** dendogram from fruits of the region Arcos and dendogram from fruits of the region Luminárias

which may have influenced the prevalence of mesophilic bacteria among the isolated species.

Fruits native to the Cerrado have high levels of sugars, proteins, minerals, fatty acids (Silva et al. 2001), B vitamins and carotenoids (Agostini-Costa and Vieira 2000). In this context, the media culture used (NA, EMB and MRS) were efficient to isolate different groups of bacteria from Cerrado fruits like Enterobacteriacea, lactic acid bacteria and mesophilic bacteria. Studies quantifying aerobic mesophilic bacteria and "enterobacteria" showed high CFU counts, with counts higher than 5 log CFU/g, in fruits such as orange, mango and apple, among others (Badosa et al. 2008; Seow et al. 2012). Pereira et al. (2011) reported a strawberry-associated LAB population of approximately 3.05 log CFU/g, similar to the results obtained in this work.

Fruits are important microhabitats for wild microrganism in nature, mainly characterized by low pH, high concentration of mono- and di-saccharides and visitation of insects (Trindade et al. 2002). The presence of bacteria in fruits is a mutualistic relationship because there is availability of nutrients for microorganims, and plants, in turn, benefit from the bacterial associations by growth enhancement, stress reduction, or protection from pathogens (Trivedi et al. 2011).

The native fruits of the Brazilian Cerrado were characterized by culture-dependent methods (Table 3). Members of the *Bacillus* group were identified, and five out of nine strains

Table 5 Cellulase activity, expressed as enzyme index, of selected bacteria isolated from Cerrado fruits

Region/fruit	Strain	Enzyme index
Passos – Pimenta do mato	*Streptomyces* sp. UFLA BCEF175	3.9
Arcos - Marolo	*Bacillus subtilis* UFLA BCEF1124	2.75
	B. subtilis UFLA BCEF1130	5.7
	B. methylotrophicus UFLA BCEF1135	3.0
	Enterobacter sp. UFLA BCEF1181	1.8
Arcos - Goiaba	*B. amyloliquefaciens* UFLA BCEF1252	2.4
Arcos - Pequi	*Paenibacillus illinoisensis* UFLA BCEF1197	5.15
Luminárias – Murici-do-Cerrado	*Burkholderia multivorans* UFLA BCEF1489	2.85
Luminárias – Azeitona-do- Mato	*Bacillus pumilus* UFLA BCEF1568	1.95

Enzyme index = Ø halo/Ø colony

selected for cellulase activity belonged to this genus (Table 5). *Bacillus* species are known sources for the production of a variety of industrially important polysaccharide-hydrolyzing enzymes, such as amylases, cellulase, and pectinase (Horikoshi 1999; Shirai et al. 2001).

Species of the genera *Enterobacter*, *Escherichia*, *Klebsiella*, *Citrobacter*, *Pantoea*, *Erwinia* and *Serratia* were identified by culture-dependent methods in several of the studied fruits. These microorganisms belong to Enterobacteriaceae, and some species are commonly found in soil, water and animals and among the endophytic microbiota of fruits and plants (Pereira et al. 2012; Trivedi et al. 2011). Similarly, in a major study conducted to investigate the microbiological quality of apples in the European Union (EU), strains of *Citrobacter, Enterobacter, Klebsiella* and *Escherichia* were isolated from apples in Spain (Abadias et al. 2006). Some species belonging to the genera mentioned above are pathogenic to humans, so it is necessary to practise good hygiene and to sanitize fruits before consumption. Lactic acid bacteria, especially species belonging to the genera *Lactobacillus* and *Streptococcus*, were identified in this work by culture-dependent and independent approaches/methods. These bacteria are prominent in the spoilage of some fruits and fruit products because they are tolerant to low pH (Fleet 2003). On the other hand, Lactic acid bacteria produce a variety of antimicrobial substances and create unfavorable conditions for the growth of pathogens and toxigenic or spoilage organisms (Abriouel et al. 2006; Trias et al. 2008).

The presence of LAB in fruits has been reported in strawberry (Pereira et al. 2011), masau fruit (a fruit typical of South Africa) (Nyanga et al. 2007), apple (Trias et al. 2008), banana (Thomas and Soly 2009), pineapple (Di Cagno et al. 2010), grape (Nisiotou et al. 2011) and coffee (Silva et al. 2008b; Vilela et al. 2010).

In this study, a greater diversity of epiphytic bacteria was obtained by the culture-dependent method than by the culture-independent method. In addition, the culture-dependent method was able to identify a greater diversity of bacteria (28 different genera) than the independent method (8 different genera).

Bacteria of the genera *Clostridium*, *Pantoea* and *Methylobacterium* were identified by culture-dependent and culture-independent approaches. The fruits from the Luminárias region showed a lower diversity of bacteria by both methods (culture-dependent and culture-independent) than fruits of the Passos region but a greater diversity than fruits of the Arcos region.

The culture-dependent approach identified a greater diversity of species from genera such as *Bacillus*, *Streptomyces*, *Enterobacter*, *Klebsiella*, *Delftia*, *Aerococcus*, *Curtobacterium*, *Xanthomonas* and *Burkholderia*.

Species of the genera *Serratia, Staphylococcus, Streptomyces, Aerococcus, Bradyrhizobium, Erwinia, Acidithiobacillus, Ochrobactrum, Klebsiella, Enterococcus, Escherichia, Streptococcus, Citrobacter* and *Achromobacter* (Fig. 2; Table 3) were identified only by isolation techniques; this result may be due to these species likely having small population sizes (<3 log CFU/g) that are not detectable in the DGGE profiles of the microbial community (Muyzer et al. 1993).

However, the PCR-DGGE analysis allowed the detection of the species *Lactobacillus fermentum*, *Acinetobacter* sp. and *Methylomonas methanica*, which were not isolated by the culture-dependent method (Fig. 3). The differences between the culture-dependent and culture-independent methods for the different fruits analyzed may be due to the low populations or even cell death of some species or to the extraction method used (Pereira et al. 2011).

A semi-quantitative evaluation of cellulase production by the isolates from Cerrado fruits indicated that some isolates were able to detectably degrade cellulose. This result shows that these bacteria produce a complex mixture of cellulase enzymes. These enzymes, which collectively exhibit specificity for the glycosidic β-1,4 bond, are needed for the complete solubilization of cellulose and require synergy in their activities (Bon et al. 2008). The potential of cellulases has been reported in various industrial processes, including food,

textiles, laundry, pulp, paper and agriculture, and in the expansion of research and development (Trivedi et al. 2010; Nagar et al. 2012).

Among the bacteria studied, strains of *B. subtilis* (UFLA BCEF 1130) and *P. illinoisensis* (UFLA BCEF 1197) showed the best results during selection for the production of cellulase and had the highest measured enzyme index values (Table 5). This result shows that these strains can be used in future studies to optimize cellulase production, since these strains are able to degrade the cellulose fiber without chemical agents or another microorganism present. Complete cellulolytic systems are produced by different genera and species of bacteria and fungi (Zhang et al. 2006). Cellulolytic bacteria include aerobic species, such as *Actinomycetes*, facultative anaerobes, such as *Bacillus*, and anaerobes, such as *Clostridium* (Bon et al. 2008). *Paenibacillus* species, which were previously classified as *Bacillus*, have also been reported to produce enzymes with cellulolytic and xylanolytic activities. Cellulases with activity on both soluble and crystalline cellulose have been isolated from strains of *Paenibacillus* and *Bacillus* (Yeasmin et al. 2011).

Besides the two strains discussed above, one strain identified as *Streptomyces* sp. showed interesting cellulase activity. *Streptomyces* sp. are actinobacteria and can be isolated from air, soil, water, fungi, plants and humans, and many species produce extracellular enzymes for the degradation of macromolecules, such as lignin, cellulose, chitin and, for the partial degradation of starch. *Streptomyces* species are heterotrophic feeders and can utilize both simple and complex molecules as nutrients. Although proteases and amylases are mainly fungal and bacterial products, the possibility of using *Streptomyces* for enzyme production has been investigated (Yang and Wang 1999). *Streptomyces* species secrete extracellular enzymes, and approximately three-quarters of *Streptomyces* species may produce antibiotics (Gupta et al. 1995).

The biodiversity of the Cerrado is very broad and is strongly associated with the composition of the vegetation and with ecosystem variation (Machado et al. 2004). Most importantly, this study reported the diversity of epiphytic bacteria associated to fruits native to the Cerrado. Some bacteria isolated during this study may be further characterized, in order to exploit their biotechnological potential.

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