Comparison of the Bacterial Community and Characterization of Plant Growth-Promoting Rhizobacteria from Different Genotypes of *Chrysopogon zizanioides* (L.) Roberty (Vetiver) Rhizospheres

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Molecular approaches [PCR-denaturing gradient gel electrophoresis (DGGE)] were used to determine whether three different vetiver (*Chrysopogon zizanioides*) genotypes, commercially used in Brazil and considered economically important over the world, select specific bacterial populations to coexist in their rhizospheres. DGGE profiles revealed that the predominant rhizospheric bacterial community hardly varies regarding the vetiver genotype. Moreover, using traditional cultivation methods, bacterial strains were isolated from the different rhizospheres. Colonies presenting different morphologies (83) were selected for determining their potential for plant growth promotion. More than half of the strains tested (57.8%) were amplified by PCR using *nifH*-based primers, specific for the enzyme nitrogenase reductase. The production of siderophores was observed in 88% of the strains, while the production of antimicrobial substances was detected in only 14.5% of the isolates when *Micrococcus* sp. was used as the indicator strain. Production of indole-3-acetic acid and the solubilization of phosphate were observed in 55.4% and 59% of the isolates, respectively. In total, 44 strains (53%) presented at least three characteristics of plant growth promotion and were submitted to amplified ribosomal DNA restriction analysis. Twenty-four genetic groups were formed at 100% similarity and one representative of each group was selected for their identification by partial 16S rRNA gene sequencing. They were affiliated with the genera *Acinetobacter*, *Comamonas*, *Chryseobacterium*, *Klebsiella*, *Enterobacter*, *Pantoea*, *Dyella*, *Burkholderia*, or *Pseudomonas*. These strains can be considered of great importance as possible biofertilizers in vetiver.

***Keywords*: *Chrysopogon zizanioides*, *nifH* gene, plant growth-promoting rhizobacteria, phosphate solubilization, siderophore and auxin production, vetiver**

Vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] is a perennial, tropical grass of the family Poaceae with wide application over the world. Vetiver is very tolerant of some extreme soil and climate conditions, such as prolonged drought, flood, frost, salinity, acidity or alkalinity, agrochemicals, and extreme temperature from -22°C to 60°C (Dong *et al.*, 2003; Truong, 2003). It grows fast, presents a strong root system, and has a high survival rate. For these reasons, vetiver is being used for soil and water conservation in more than 100 countries (Dong *et al.*, 2003). In addition, vetiver also can be used as forage for livestock, ornaments, containers, industrial products (mainly pulp and paper), medicinal applications, and energy sources (Chomchalow and Chapman, 2003).

In tropical countries where vetiver thrives in natural condition, the use of vetiver root for essential oil extraction to produce perfume and other fragrant materials is well known (Chomchalow, 2001). The oil is a viscous light-brown oil with a rich green-woody earthy and nut-like fragrance, presenting a very complex chemical composition (mixture of sesquiterpene alcohols and hydrocarbons, Akhila and Rani, 2002). There are distinct differences in the quality of essential oil obtained from different geographic regions of the world, and vetiver genotypes producing oil with different notes and/or varying in the quantity of oil produced have been already described (Adams *et al.*, 2003; Lavania, 2003). Recently, it has been shown that axenic vetiver cultured *in vitro* produces only trace amounts of oil with a strikingly different composition compared with the oils from *in vivo* vetiver plants, suggesting a direct role of bacteria in the oil metabolism (Adams *et al.*, 2004; Champagnat *et al.*, 2006). Del Giudice *et al.* (2008) also suggested that bacteria may play a crucial role in the biotransformation of plant-derived terpenoids for the production of the final vetiver essential oil complex blend.

Besides the possibility of interfering in the essential oil production, bacteria found in the rhizosphere may contribute to the growth and health of vetiver. Different mechanisms of growth promotion by rhizobacteria have already been described for other plants (Glick, 1995; Gray and Smith, 2005), and the study of rhizobacteria which establish positive interactions with plant roots has increased because of

their potential use in sustainable agriculture (Défago *et al.*, 1994). Some examples of these mechanisms are: (i) increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; (ii) repression of soil-borne pathogens (by the production of HCN, siderophores, antibiotics, and/or competition for nutrients); and (iii) production of phytohormones such as indole-3-acetic acid (IAA). The bacteria presenting one or more of these characteristics are known as plant growth-promoting rhizobacteria (PGPR; Kloepper and Schroth, 1978).

A range of studies on the microbial community structures of soil and/or rhizospheres has been performed on the basis of DNA extracted directly from these habitats (Duineveld *et al.*, 2001; Smalla *et al.*, 2001; Mota *et al.*, 2008). The diversity of target genes, such as 16S rRNA, can be assessed by means of molecular fingerprinting approaches such as PCR-denaturing gradient gel electrophoresis (PCR-DGGE). However, traditional cultivation methods are still being extensively used to isolate bacteria naturally inhabiting this environment (Mota *et al.*, 2002; Albuquerque *et al.*, 2006). In this study, we aimed to analyze the genetic diversity of the bacterial community associated with the rhizospheres of different vetiver genotypes, after six months of plant cultivation, by PCR-DGGE fingerprinting. This approach will help to elucidate whether the different vetiver genotypes, commercially used in Brazil, select specific bacterial populations to coexist with them. Furthermore, we attempted to isolate bacterial strains presenting plant growth-promoting characteristics from the same rhizospheres.

Materials and Methods

Sampling site
The study area was located in Aracaju, Sergipe State, in the northeast of Brazil. Plants were collected from the experimental farm "Campus Rural-UFS". The soil of this area is characterized as a red-yellow argisoil. The experimental plot consisted of lines with spaces of 1 m between lines and 1 m between plants. Three genotypes (UFS-VET001, UFS-VET003, and UFS-VET004) of vetiver from the Active Germplasm Bank of the Federal University of Sergipe were planted in each line. The only difference that has been observed among the genotypes is the composition of the essential oil produced. For example, while valerianol is found only in the oil produced by UFS-VET001, α-cadinol is detected only in that produced by UFS-VET004. Vetiselinenol and epi-zizanone are not detected in UFS-VET003 and UFS-VET001 oils, respectively. Lines received 500 kg of NPK (6-24-12) per hectare, plus micronutrients (Hortosafra, Brazil) and 10,000 L/ha of cattle manure for soil fertilization. Five plants of each vetiver genotype were harvested six months after sowing and the roots shaken to remove the loosely attached soil. The adhering soil of the five plants was pooled and considered as the rhizosphere soil. Samples were kept at -20°C before DNA extraction.

Isolation and maintenance of bacterial strains from *C. zizanioides* (vetiver)
Rhizosphere soil (1 g) from each vetiver genotype was mixed with 9 ml of distilled water, and serial dilutions of the rhizosphere samples were plated onto TBNR agar (thiamine-biotin-nitrogen; Seldin *et al.*, 1984); all steps were performed under aerobic conditions. The plates were incubated for 5 days at 28°C in Gaspak jars filled with a gas mixture of 80% N_2, 10% CO_2, and 10% H_2. This gas mixture was introduced once in the jars only to facilitate the selection of putative nitrogen fixers. A complete anaerobic atmosphere was never achieved inside the jars, and the presence of residual oxygen was indicated by an anaerobic indicator (BR55; Oxoid, England) placed in the interior of the jars.

Colonies presenting different morphologic characteristics were selected for further purification under aerobic conditions. Bacterial cultures were stored at room temperature on trypticase soy broth (TSB) agar with 1% (w/v) $CaCO_3$.

DNA extraction from bacterial cultures and rhizosphere soil samples
Genomic DNAs were extracted from all bacterial strains by using the protocol described by Seldin and Dubnau (1985). The total microbial community DNA was extracted directly from the rhizosphere samples (0.5 g of each sample in duplicate) using FastPrep Spin kit for soil DNA (BIO 101 Systems, USA). DNA preparations were visualized after electrophoresis in a 0.8% agarose gel in 1× TBE buffer (Sambrook *et al.*, 1989) to assess their integrity and then stored at 4°C prior to PCR amplification.

PCR amplification of *nifH* gene
NifH gene fragments (360 bp) were amplified by PCR using the genomic DNAs from all bacterial isolates using the primers PolF; 5'-TGCGAYCCSAARGCBGACTC-3' and PolR; 5'-ATSGCCATCATYTCRCCGGA-3', following the same conditions as described by Poly *et al.* (2001).

Antimicrobial substance assay
The overlay method described by Rosado and Seldin (1993) was used to detect antimicrobial activity. It was performed using TSB agar plates on which all isolates were inoculated as a 5 μl spot from an overnight culture. After incubation at 32°C for 48 h, the cells were killed by exposure to chloroform vapor for 15 min. The plates were then flooded with suspensions containing the indicator strain (*Micrococcus* sp.) as performed by von der Weid *et al.* (2005). Antimicrobial substance production was indicated by clear zones of inhibition around the bacterial colonies after incubation for 24 to 48 h at 32°C.

Phosphate solubilization
The test described in Rosado *et al.* (1998) was used to determine the ability of the isolates to solubilize phosphate (calcium phytate). Clear zones around the bacterial colonies after incubation for 5 days at 32°C indicated a positive test.

Siderophore production
All isolates were screened for the production of siderophores in King B agar supplemented with the complex chrome azurol S [CAS/iron(III)/hexadeciltrimethyl ammonium bromide] as described by Schwyn and Neilands (1987). Strains were inoculated as a 5 μl spot in plates and incubated at 32°C from 4 to 7 days. Bacterial growth and the formation of a

yellow halo around the colonies were recorded as a positive test for siderophore production.

Bacterial production of IAA

All strains were grown in 3 ml of TSB medium for 48 h at 32°C, and then 100 µl of these cultures were inoculated in triplicate in 3 ml King B medium and maintained at 27°C for 72 h in the dark in a rotary shaker (100 rpm), as described by Bent *et al*. (2001). Uninoculated tubes were used as controls. After that, the production of IAA in the culture supernatants was estimated qualitatively after adding 1 ml of Salkowski's reagent (1.875 g of FeCl₃·6H₂O, 100 ml H₂O, and 150 ml of H₂SO₄ at 96% of purity) to 1 ml of each supernatant (Tang and Bonner, 1947). Whenever a reddish color was visualized after the addition of the reagent, the test was considered positive.

Amplified ribosomal DNA (rDNA) restriction analysis (ARDRA)

The gene encoding 16S rRNA from selected isolates was amplified by PCR using the pair of universal primers pA and pH and the conditions described in Massol-Deya *et al*. (1995). Negative controls (without DNA) were run in all amplifications and PCR products were visualized by 0.8% agarose gel electrophoresis in TBE at 80 V for 4 h at room temperature, and stained with ethidium bromide. Samples (10 µl) of the 16S rRNA gene-amplified products were then digested with the endonucleases *Hin*fI, *Hae*III, and *Rsa*I (Invitrogen, Brazil) for 16 h, according to the manufacturer's protocols. Agarose (1.5%) gel electrophoresis of restricted DNA was performed at 80 V for 3 h at room temperature. The results of ARDRA were collected into a matrix indicating the presence or absence (scored as 1 or 0, respectively) of specific bands in the different restriction analyses. Simple matrixes were obtained by comparing pairs of strains using the simple matching (SM) coefficient, and the final dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA). For these analyses, the NTSYS software package (version 2.02, Exeter Software, USA) was used.

PCR amplifications of 16S rRNA coding genes for DGGE and for sequencing

Fragments of 16S rRNA genes were PCR-amplified using DNA from rhizosphere soils with primers U968F-GC; 5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G AAC GCG AAG AAC CTT AC-3' and L1401R; 5'-CGG TGT GTA CAA GAC CC-3' described by Nübel *et al*. (1996). When the DNAs used were from the representative strains of ARDRA groups the forward primer did not contain the GC clamp. The 50 µl reaction mix contained 1 µl of template DNA, 10 mM Tris-HCl (pH 8.3), 10 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTPs, 1.25 U of *Taq* DNA polymerase (Promega, USA), and 0.2 µM of each primer. The amplification conditions were: 1× (2 min, 94°C), 35× (1 min, 94°C; 1.5 min, 48°C; 1.5 min, 70°C), and a final 10 min extension at 70°C. Negative controls (without DNA) were run in all amplifications. The PCR products were visualized by agarose gel electrophoresis (1.4% gel) followed by staining with ethidium bromide. Amplicons

were stored at -20°C until DGGE analysis.

DGGEs were carried out using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad Laboratories, Germany). PCR products (approximately 150 ng) were applied directly onto 8% (w/v) polyacrylamide gels in 1×TAE buffer (40 mM Tris-acetate; pH 7.4, 20 mM sodium acetate, 1 mM disodium EDTA) containing a denaturing gradient of urea and formamide varying from 40 to 60%. The gels were run for 16 h at 60°C and 65 V. After electrophoresis, the gels were stained for 30 min with SYBR Green I (Invitrogen - Molecular Probes) and photographed under UV light by using a STORM apparatus (Amersham Pharmacia Biotech, Germany).

Sequencing of PCR products and phylogenetic analysis

The PCR products from one representative of each ARDRA group were sequenced using an ABI Prism 3100 automatic sequencer. The 16S rRNA gene sequences were compared with the GenBank database using the BLAST-N facility (www. ncbi.nlm.nih.gov/blast) of the National Center for Biotechnology Information (NCBI). Sequences of closely related strains were recovered from the GenBank database and aligned to the sequences obtained in this study using the
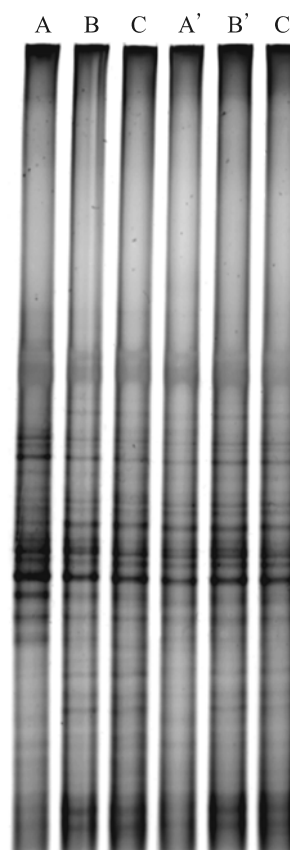

Fig. 1. DGGE fingerprints of 16S rRNA gene fragments amplified from rhizosphere soil DNA templates obtained from three vetiver genotypes: (lanes A, A') - UFS-VET003, (lanes B, B') - UFS-VET001 and (lanes C, C') - UFS-VET004

CLUSTAL X software (Thompson *et al.*, 1997). A phylogenetic tree was constructed based on partial 16S rRNA gene sequences by Neighbor-Joining (NJ) method, using MEGA 3.1 software (Kumar *et al.*, 2004).

Results

Analysis of bacterial DGGE fingerprints
DNA was recovered from all vetiver rhizosphere samples. Bacterial community fingerprints were generated for each sampling, and reproducible DGGE profiles of bacterial 16S rDNA were obtained between duplicate rhizosphere soil samples (Fig. 1). The DGGE profiles were found to be very similar among the three genotypes. No difference (presence or absence of bands) could be observed visually when the three patterns were compared (Fig. 1), indicating that the predominant bacterial community found in the rhizospheres does not vary regarding the vetiver genotype.

Isolation of bacterial strains from the rhizospheres of vetiver
Samples from the rhizosphere of the three different genotypes of vetiver (UFS-VET001, UFS-VET003, and UFS-VET004) gave rise to colonies with different morphologies

on TBNR agar medium, after incubation for 5 days. Among them, a total of 83 colonies (listed in Table 1) were chosen for purification and characterization procedures: 27 strains from UFS-VET001, 18 strains from UFS-VET003, and 38 strains from UFS-VET004. The different isolates were designated VET followed by the vetiver genotype number (001, 003, or 004) and by progressive numbers of isolation.

Plant growth promotion properties of bacterial strains isolated from vetiver rhizospheres
The potential for nitrogen fixation of all bacterial isolates was tested by PCR amplification using *nifH* gene (coding for the enzyme nitrogenase reductase)-based primers and the conditions described previously (Poly *et al.*, 2001). The expected PCR product of 360 bp was observed in 48 (57.8%) of the isolates: 11 from UFS-VET001, 7 from UFS-VET003, and 30 from UFS-VET004 (Table 1).

Solubilization of phosphate was observed in 49 (59%) of the isolates: 11 from UFS-VET001, 8 from UFS-VET003, and 30 from UFS-VET004 (Table 1). Only 12 (14.5%) of the isolates - 5 from UFS-VET001, 4 from UFS-VET003, and 3 from UFS-VET004 - were able to inhibit the bacterial indicator strain *Micrococcus* sp. in plate assays (Table 1). On the other hand, the majority of the isolates (73, correspon-

Table 1. Plant growth promotion characteristics presented by the bacterial isolates from the vetiver rhizospheres and their ARDRA groups

Isolates[a] / ARDRA group[b]	PCR *nifH*	Phosphate	AMS	Siderophore	IAA
VET001.26, VET003.10	-	-	-	-	-
VET001.5	-	-	-	-	+
VET001.27, VET003.3	+	-	-	-	-
VET001.19, VET004.22	-	+	-	-	-
VET001.1, VET001.14, VET004.7, VET004.21	-	-	-	+	-
VET001.10	-	+	+	-	-
VET001.13	+	-	-	-	+
VET001.3, VET001.20	-	-	+	+	-
VET003.12	+	-	+	-	-
VET001.22, VET001.23, VET003.9, VET004.15	-	+	-	+	-
VET001.6, VET001.7, VET003.4, VET003.5, VET003.6, VET003.11, VET003.13, VET004.13, VET004.20	-	-	-	+	+
VET001.4, VET001.8, VET001.12, VET001.15, VET001.16, VET003.7, VET003.8, VET004.8, VET004.9, VET004.19	+	-	-	+	-
VET001.9 (9), VET004.32 (4)	+	-	-	+	+
VET001.11 (5), VET001.18 (6)	-	+	+	+	-
VET003.14 (12), VET004.14 (7), VET004.34 (8), VET004.37 (13), VET004.38 (10)	+	+	-	+	-
VET001.2 (1), VET001.21 (3), VET003.1 (18), VET003.15 (19), VET004.4 (20), VET004.16 (20)	-	+	-	+	+
VET003.17 (20), VET003.18 (20)	-	+	+	+	+
VET003.16 (17), VET004.23 (24)	+	+	+	+	-
VET001.17 (14), VET001.24 (15), VET001.25 (2), VET003.2 (11), VET004.1 (20), VET004.2 (21), VET004.3 (22), VET004.5 (20), VET004.6 (16), VET004.10 (20), VET004.11 (16), VET004.12 (16), VET004.17 (16), VET004.18 (20), VET004.24 (20), VET004.25 (23), VET004.26 (20), VET004.27 (20), VET004.29 (20), VET004.31 (16), VET004.33 (20), VET004.35 (16), VET004.36 (16)	+	+	-	+	+
VET004.28 (20), VET004.30 (16)	+	+	+	+	+

[a] The different isolates were designated VET followed by the vetiver genotype number (001, 003 or 004) and by progressive numbers of isolation.
[b] Numbers in parentheses correspond to the 16S rRNA gene-based genotypes formed (ARDRA group) within strains presenting at least three characteristics of plant growth promotion.

ding to 88% of the total strains studied) produced sidero-phores. Twenty-one siderophore producing strains were from UFS-VET001, 15 from UFS-VET003, and 37 from UFS-VET004 (Table 1). Production of IAA in the culture super-

natants was observed in 46 (55.4%) of the isolates: 10 from UFS-VET001, 10 from UFS-VET003, and 26 from UFS-VET004 (Table 1).

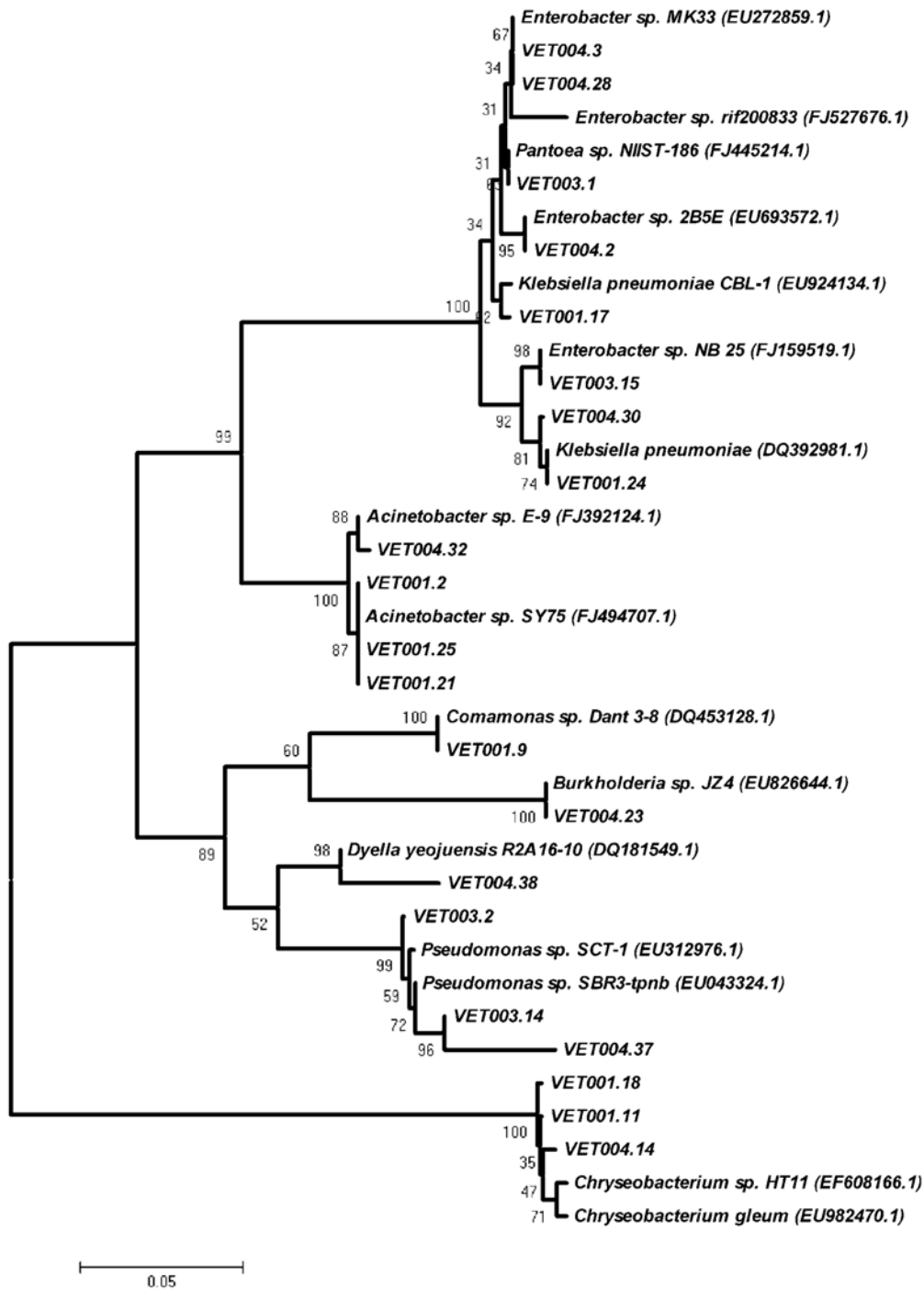Of the 83 strains, 44 presented three or more characteri-

Fig. 2. Phylogenetic tree of partial 16S rRNA gene sequences (433 bp) showing the relationship between 21 vetiver isolates with different related species found by Blast-n searches. The tree was constructed based on neighbor-joining method. Bootstrap analyses were performed with 1,000 repetitions and results are represented at the branch points. The GenBank accession number of each species is enclosed in parentheses.

stics of plant growth promotion. Only two strains (VET001.26 and VET003.10) gave negative results in all the five tests carried out in the present study, and two other strains (VET004.28 and VET004.30) were amplified by *nifH*-based primers and were able to produce IAA and siderophores, to solubilize phosphate and to inhibit the growth of the indicator strains used (*Micrococcus* sp.) at the same time (Table 1).

ARDRA and taxonomic identification of the isolates

The DNA samples of the 44 strains presenting at least three characteristics of plant growth promotion (Table 1) were amplified with universal primers (pA and pH) for the 16S rRNA gene sequence. As expected, all strains produced a single band of about 1,500 bp, and the PCR products were digested with *Hin*fI, *Hae*III, and *Rsa*I. A similarity analysis was then performed based on UPGMA by using 47 markers, which correspond to all different bands observed after the digestion with these three endonucleases (data not shown). Twenty-four different 16S rRNA gene-based genotypes were observed at 100% similarity (Table 1). Twenty-two groups were formed by only one strain while two groups (16 and 20) by 8 and 14 strains, respectively. At about 55% similarity, two groups were observed: one formed by five strains of the genotype UFS-VET001 and three of UFS-VET004, and the other formed by representatives of the three genotypes. Therefore, no particular correlation between the origin of the isolates and the ARDRA groups could be established.

To help the phylogenetic placement of the strains described in this study, one representative isolate from each ARDRA group was investigated with partial (433 bp) 16S rRNA gene sequencing. Sequences obtained clustered in three different taxonomic groups: *Gammaproteobacteria* (75% of the strains), *Betaproteobacteria* (10% of the strains), and *Flavobacteria* (15% of the strains). They were affiliated with the genera *Acinetobacter*, *Comamonas*, *Chryseobacterium*, *Klebsiella*, *Enterobacter*, *Pantoea*, *Dyella*, *Burkholderia*, or *Pseudomonas* with identity values varying from 96 to 100% (Fig. 2).

Discussion

The bacterial communities of the rhizospheres of three vetiver genotypes were investigated using DGGE analysis of the PCR-amplified 16S rRNA gene. This approach provides an overview of the profiles of the bacterial communities that may encompass a range of fundamental processes associated with ecosystem functioning, such as organic matter decomposition, nutrient cycling, and plant growth stimulation (Brimecombe *et al.*, 2001; Duineveld *et al.*, 2001; Smalla *et al.*, 2001; Mota *et al.*, 2008). The DGGE fingerprints obtained for the bacterial communities analyzed here hardly varied, regardless of different vetiver genotypes (Fig. 1). This observation supports the idea that the rhizospheres harbor bacterial populations determined by the soil conditions where these vetiver genotypes are cultivated. Previous studies have also indicated that soil type was found to be the determining factor on the rhizosphere microflora (da Silva *et al.*, 2003; Garbeva *et al.*, 2004). Moreover, the difference observed in the composition of the essential oils produced by the different

genotypes does not seem to influence the bacterial community found in their rhizospheres. Indeed, Del Giudice *et al.* (2008) demonstrated by light and transmission electron microscopy that bacteria in close association with the essential oil are located in the cortical parenchymatous essential oil-producing cells and in the lysigen lacunae of vetiver plants.

The study of bacterial population structure in rhizosphere has attracted great interest because of the positive response of many plants to inoculation with suitable plant growth-promoting bacterial strains. In the present study, using a culture-dependent approach, 83 strains were isolated from the rhizosphere soil of the three genotypes of vetiver in a nitrogen-poor medium (TBNR agar, Seldin *et al.*, 1984). This medium was used to select potential nitrogen-fixing strains, one of the positive effects resulting from interactions between microorganisms and plants. After PCR amplification using *nifH*-based primers, about 60% of the strains showed to harbor this nitrogenase structural gene (Table 1). However, the expression of the enzyme by the different bacterial strains isolated has not been tested yet. The remaining strains were considered only efficient nitrogen scavengers, as suggested by Seldin *et al.* (1983). Other characteristics of plant growth promotion were also determined, including the production of antimicrobial substances. The low number of strains able to inhibit the indicator strain used (*Micrococcus* sp.) can be explained by either the wrong choice of this indicator or by the inexistence of phytopathogens which usually commit vetiver plants. *Micrococcus* sp. has been extensively used as an indicator strain to test the production of antimicrobial substances by different bacterial strains (von der Weid *et al.*, 2005; Tupinambá *et al.*, 2008). On the other hand, the majority of the isolates (88%) produced siderophores. Production of IAA and solubilization of phosphate was observed in 55.4% and 59% of the isolates, respectively (Table 1). Strains of *Paenibacillus polymyxa* (Rosado *et al.*, 1998; Mota *et al.*, 2008) and *Acinetobacter calcoaceticus* (Kang *et al.*, 2009) have been previously described as producers of extracellular auxin and also as phosphate solubilizers.

ARDRA was used in this study to assist in distinguishing among taxonomic groups. This technique has been shown to be a useful tool for screening environmental bacterial isolates and/or clone libraries (Sjöling and Cowan, 2003). Representatives of the different ARDRA groups were used for partial 16S rRNA gene sequencing. After identification by Blast search tool of the NCBI GenBank database, the majority of the sequences were related to *Gammaproteobacteria*. Gram-negative bacteria belonging to *Acinetobacter*, *Comamonas*, *Cryseobacterium*, *Klebsiella*, *Enterobacter*, *Pantoea*, *Dyella*, *Burkholderia*, and *Pseudomonas* were isolated from the rhizospheres of vetiver (Fig. 2). Strains of many of these genera have been already described as plant growth-promoting rhizobacteria (De Los Santos *et al.*, 2001; Kang *et al.*, 2009; Sulbarán *et al.*, 2009; among others).

On the basis of the results of this investigation, different PGPR isolated from the rhizosphere of vetiver usually found in the northeast of Brazil became available for further studies *in vivo* to determine the strains that efficiently promote the vetiver growth in a tropical soil. The strains presented here may offer a potential for use as biofertilizers in vetiver.

Acknowledgements

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