https://doi.org/10.1590/1678-4499.20220071

BASIC AREAS
Article

# Associative bacterial diversity of *pangolão*, a stress-resilient tropical grass

Michelle Justino Gomes Alves[1], Cybelle Souza Oliveira[1], Gisely Moreira Vitalino[1], Eric Xavier de Carvalho[2], José de Paula Oliveira[2], Giselle Gomes Monteiro Fracetto[1], Felipe José Cury Fracetto[1], Mario Andrade Lira Junior[1,*]

1. Universidade Federal Rural de Pernambuco – Agronomia – Recife (PE), Brazil.
2. Instituto Agronômico de Pernambuco – Recife (PE), Brazil.

**Received:** Apr. 5, 2022 | **Accepted:** Sep. 27, 2022

**Section Editor:** Hector Valenzuela

***Corresponding author:** mario.alirajr@ufrpe.br

**How to cite:** Alves, M. J. G., Oliveira, C. S., Vitalino, G. M., Carvalho, E. X., Oliveira, J. P., Fracetto, G. G. M., Fracetto, F. J. C. and Lira Junior, M. A. (2022). Associative bacterial diversity of *pangolão*, a stress-resilient tropical grass. Bragantia, 81, e4622. https://doi.org/10.1590/1678-4499.20220071

**ABSTRACT:** Some forage species, such as *pangolão* grass (*Digitaria eriantha* Steud. cv. Survenola), are resilient in tropical semiarid regions. A possible reason for this is the presence of endophytic and rhizospheric microorganisms. Thus, this study evaluated the diversity of associative bacteria in *pangolão* grass. Bacteria associated with the roots, culm, leaves, and rhizospheric soil were isolated and characterized in three municipalities of Pernambuco, Brazil. An initial phenotypic characterization was followed by a genotypic assessment by based repetitive extragenic palindromic-polymerase chain reaction (BOX-PCR) and partial sequencing of the 16S rRNA gene. We obtained 325 phenotypically-characterized isolates grouped into 243 strains with 100% similarity by BOX-PCR. The most diverse sampling environment was Araripina, and all factors affected bacterial diversity. There were 135 groups with 90% similarity, that were represented by a single strain each for sequencing. Among the sequenced strains, 118 showed 96.84–99.9% similarity with previously described strains, whereas 17 could not be identified. The following 18 genera were identified from three phyla, five classes, seven orders, and 13 families: *Achromobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Curtobacterium*, *Enterobacter*, *Herbaspirillum*, *Kosakonia*, *Ochrobactrum*, *Paenibacillus*, *Pantoea*, *Priestia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Shinella*, *Stenotrophomonas*, and *Variovorax*. The diversity of endophytic and rhizospheric bacteria may contribute to the resilience of *pangolão*, as various strains of these genera have been described as plant growth promoters. This is the first evaluation of *pangolão* bacterial diversity under tropical semiarid conditions. Since several of the genera include strains known to promote plant growth, we propose further research to evaluate this on crops.

**KEY WORDS:** endophytic bacteria, rhizospheric bacteria, environmental stress, semiarid, *Digitaria eriantha* Steud. cv. Survenola.

## INTRODUCTION

Brazilian livestock production is mainly based on pastures, either native or cultivated. Certain species located in these pastures, such as *Digitaria eriantha* Steud. cv. Survenola, known as *pangolão* grass, are tolerant to water deficit and low soil fertility (Navarro et al. 2005).

A possible coping mechanism for these conditions is the presence of endophytic microorganisms, that assist in plant growth and development and induce resistance to biotic and abiotic stresses, such as through phytostimulation, biofertilization, or biocontrol (Afzal et al. 2019).

Endophytic bacteria are found in the majority of plant species (Afzal et al. 2019), and many endophytes have been detected in association with a wide range of grasses with variable diversity (Lu et al. 2021). Unfortunately, no data were found in literature on endophytic bacteria from *Digitaria* species, and little information is available on these bacteria in tropical semiarid conditions.

Endophytic and rhizospheric bacterial diversity depends on several factors such as plant species, host plant tissue, and environmental conditions. High endophytic bacterial diversity has been found in wild rice roots (Chen et al. 2019), Brachiaria grasses (Mutai et al. 2017), and on the seashore paspalum adapted to warm saline environments in tropical areas (Liu et al. 2021), whereas high diversity was also found for both indole acetic acid-producing and phosphate-solubilizing bacteria in sugarcane (Teheran-Sierra et al. 2021).

This high diversity, coupled with the heterologous inoculation of bacteria isolated from one species into another, suggests the evaluation of the diversity of endophytic bacteria in stress-tolerant plants. For example, a strain of *Pseudomonas* sp. isolated from the roots of the desert-inhabiting legume *Alhagi sparsifolia* promoted drought resistance when inoculated into wheat (Zhang et al. 2020). Similarly, *Bacillus* isolated from *Cereus jamacaru*, a Brazilian native cactus, has been shown to induce drought resistance in several crops (Kavamura et al. 2013). Brazilian *Azospirillum* inoculants, which are entirely based on strains isolated from maize, are currently recommended for maize, wheat, rice, and Brachiaria, and used as co-inoculants for soybean, cowpea, and the common bean (Brasil 2011, Galindo et al. 2020).

Thus, this study aimed to isolate, characterize, select, and identify endophytic bacteria in different parts of *pangolão* grass and rhizospheric soil and verify the associated bacterial diversity.

## MATERIAL AND METHODS

Sampling was conducted in three municipalities of Pernambuco, Brazil, covering climates ranging from hot dry semiarid (Araripina) to hot tropical with winter rains (Gravatá and Nazaré da Mata, with lower average rainfall in the former), classified as BSh and As according to Köppen-Geiger's classification (CONDEPE/FIDEM, 2016) (Fig. 1). Gravatá and Nazaré da Mata *pangolão* pastures were on private properties and approximately 6 and 11 years old, with unknown fertilization practices. In Araripina, the samples were collected at the Experimental Station of Pernambuco Agronomic Institute and were at least 30 years old, with unknown fertilization during this time. After the first sampling in this field, part of the area was limed by the research station, allowing observation of the effects of liming on endophytic bacterial diversity.

At each sampling (Table 1), 20 plants were randomly collected from the field, grouped, and separated into leaves, culms, roots, and rhizospheric soil, followed by bagging and refrigeration until further analysis. All samplings were conducted in the rainy seasons for Gravatá and Nazaré da Mata, while Araripina was sampled during both the rainy and dry seasons. A single field was used for each location, except for Araripina, in which samples were collected in both limed and non-limed fields during the rainy season. In each site in Araripina, soil was collected, dried at 60 °C, sieved through a 2 mm mesh, and subjected to textural and chemical characterization based on Brazilian standard protocols (Teixeira et al. 2017).

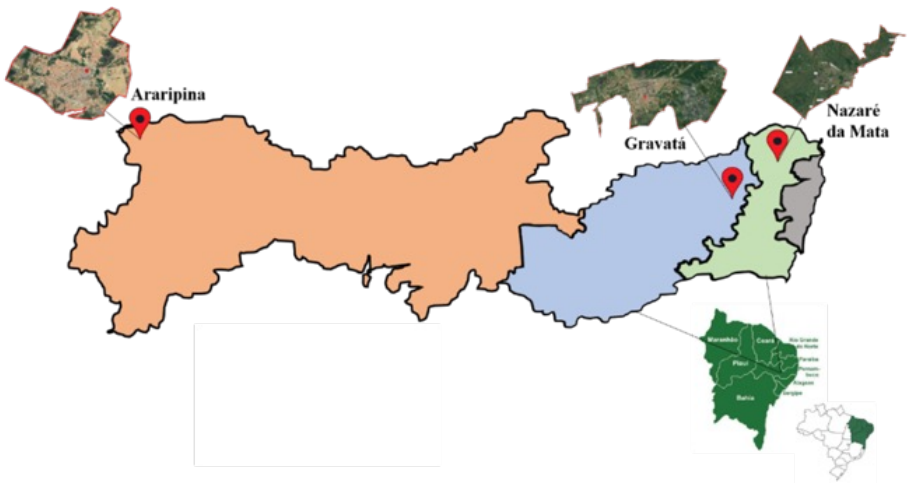


**Figure 1.** Sampling areas location in relation to Pernambuco state and the Brazilian northeast.

Leaves and colm were disinfected by washing in water, drying, swabbing in 70% ethanol, and rinsing in distilled autoclaved water. The roots were washed in water, cut into 10 cm pieces, immersed in 70% ethanol for 30 s, followed by 2.5% sodium hypochlorite, and washed five times with distilled autoclaved water. All plant samples were subsequently blended in autoclaved saline solution at a $10^{-1}$ dilution, followed by serial dilution from $10^{-3}$ to $10^{-7}$ in triplicate, also using autoclaved saline solution (Döbereiner et al. 1995).

At each dilution, samples were inoculated into penicillin flasks with semi-selective N-free semi-solid NFB culture media (Döbereiner et al. 1995), while in Araripina JNFB (Baldani et al. 1986) and JMV (Baldani et al. 1996) were also used. In all cases, the flasks were incubated at 28 °C for at least 48 h, after which samples from any dilution with visible growth were plated onto Petri dishes with yeast malt agar (YMA) media and bromothymol blue (Vincent 1970) and characterized by pH change (acidic, neutral, or alkaline), mucous production (absent or present), colony color (pink, yellow, cream or cream and yellow), opacity (opaque or translucent), form (circular or irregular), perimeter (full or irregular), and surface (smooth or irregular) (Silva et al. 2012).

**Table 1.** Sampling areas of *pangolão* grass (*Digitaria eriantha* cv. Survenola) in three municipalities of Pernambuco, Brazil.

| Municipality | Köppen-Geiger climate classification | Yearly average | | Sampling | Season | Liming | GPS coordinates | Field age (years) | pH | P | K | Ca | Mg | Na | Al | H+Al | Texture |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temperature (°C) | Rainfall (mm) | | | | | | | (mg·dm$^{-3}$) | ----------cmol$_c$·dm$^{-3}$---------- | | | | | | |
| Araripina | BSh | 24.4 | 924 | December 2016 | Dry | Without | 7°27'42"S 40°25'12"O | > 30 | 5.3 | 2 | 0.18 | 1.6 | 0.50 | 0.05 | 0.15 | 3.23 | Sandy loam |
| | | | | March 2017 | Rainy | With | 7°27'48"S 40°25'16"O | | 6.1 | 3 | 0.13 | 2.20 | 0.9 | 0.05 | 0.00 | 2.47 | Sandy loam |
| | | | | | | Without | | | 5.3 | 2 | 0.08 | 1.6 | 0.50 | 0.05 | 0.15 | 3.23 | Sandy loam |
| Gravatá | As | 23.8 | 972 | October 2017 | Rainy | Without | 8°8'6"S 35°22'51"O | ~ 6 | 5.7 | 3.5 | 0.22 | 0.85 | 0.8 | 0.06 | 0.12 | 3.42 | Sandy loam |
| Nazaré da Mata | As | 25.4 | 814 | November 2017 | Rainy | Without | 7°47'7"S 35°14'37"O | > 11 | 5.6 | 10 | 0.22 | 1.50 | 0.9 | 0.07 | 0.10 | 4.10 | Sandy loam |

All isolates were inoculated in 5 mL of trypticase soy broth (TSB) medium and shaken at 180 rpm for 72 h at 28 °C for DNA extraction (Lyra et al. 2019). Then, 2 mL of the bacterial suspension was centrifuged at 7,500 rpm for 3 min, the supernatant was discarded, and the pellet formed was used for DNA extraction using the MiniPrep Kit (Axygen), according to the manufacturer's recommendations. DNA integrity was analyzed by electrophoresis on a 0.8% agarose gel for 30 min in 0.5 × tris/borato/EDTA (TBE) buffer at 100 V, after being stained with SybrGold (Thermo Fisher Scientific). The 100 bp Plus DNA Ladder (Thermo Fisher Scientific) was used as a molecular standard, followed by DNA quantification using the NanoDrop 2000c (Thermo Fisher Scientific), standardization of the concentration to 20–30 ng·µL$^{-1}$, and storage at -20 °C.

A based repetitive extragenic palindromic-polymerase chain reaction (BOX-PCR) was performed using the BOX A1R (5'-CTACGGCAAGGCGACGCTGACG-3') primer (Versalovic et al. 1994). The amplification reaction was performed in a final volume of 10 µL containing 1 µL of template DNA (20–30 ng·µL$^{-1}$), 2 µM of primer, 0.3 mM of dNTPs, 1 µL of buffer

10 ×, 5 mM $MgCl_2$, 1.5 U Taq polymerase platinum, and Milli-Q water to complete the reaction. Amplification conditions were adjusted from Freitas et al. (2007) as follows: initial denaturation at 95 °C for 9 min, 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55 °C), extension (5 min at 72 °C), and a final extension cycle at 72 °C for 10 min. All reactions were performed using an Applied Biosystems 2720 thermocycler (Applied Biosystems). The amplified fragments were separated by electrophoresis, containing 0.5 × TBE buffer at 100 V, for 180 min on 1.2% agarose gels stained with SybrGold (Thermo Fisher Scientific).

Dendrograms of the sample areas and plant parts, individually and jointly, were constructed using Geljv2 (Heras et al. 2015) with the Jaccard coefficient and UPGMA algorithm. Groups with 100% similarity were considered as distinct strains and evaluated using the Shannon-Weaver's diversity (Shannon and Weaver 1949), Pielou's uniformity (Pielou 1959), Simpson's diversity and dominance (Simpson 1949) and Margalef's richness (Margalef 1956) indexes.

DNA from a representative strain of each BOX-PCR group with 90% similarity was amplified with 16S rRNA universal primers 27F (5'AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'TACGGTTAACCTT GTTACGACTT-3') (Weisburg et al. 1991). The amplification reaction with a final volume of 50 µL consisted of 2 µL DNA (20–30 $ng{\cdot}\mu L^{-1}$), 1.5 µL $MgCl_2$ (50 mM), 5 µL 10 × PCR buffer, 1 µL dNTPs (10 mM), 2 µL of each primer (10 µM), 0.6 µL of Taq DNA polymerase ($5\ U{\cdot}\mu L^{-1}$) and Milli-Q water to complete the reaction. The amplification reaction conditions were as follows: initial denaturation at 94 °C for 3 min, 30 cycles of denaturation (94 °C for 45 seconds), annealing (56 °C for 30 seconds), extension (72 °C for 2 min), and a final extension at 72 °C for 7 min. The amplified products were evaluated in 0.5 × TBE buffer at 100 V for 90 min on 1% agarose gel stained with SybrGold (Thermo Fisher Scientific) and visualized under ultraviolet light in an LPIX-HE photo documenter (Loccus, Brazil). The reactions were performed using a 2720 thermocycler (Applied Biosystems), followed by purification and sequencing by Macrogen (South Korea).

The sequences were compared to the type strains in the National Center for Biotechnology Information (NCBI) database. To determine the molecular identity, each strain was individually subjected to a similarity analysis using the MEGABLAST algorithm (highly similar sequences) or BLASTn algorithm (similar sequences). The same sequences were then analyzed for the percentage of molecular identity using the CLUSTAL W multiple progression method (Thompson et al. 1994) with the MEGA7.1 program (Kumar et al. 2018). The Juke-Cantor neighbor-join method was used to determine the similarity value and distance matrix and to build gene trees of the concatenated sequences for each isolate (Kumar et al. 2018). The significance of branching within trees was assessed by bootstrap analysis of 1,000 computer generated replicates. Sequences that did not show high similarity were not included in the phylogenetic tree.

## RESULTS AND DISCUSSION

Using the semi-specific culture media, 325 isolates were obtained, which restricted the endophyte population that could be isolated (Hernández-Pacheco et al. 2021).

Phenotypic characterization revealed that 80.3% of the isolates acidified, 3.7% alkalized, and 16% did not change the pH of the medium (Fig. 2 and Suppl. Table 1), which may be associated with the assimilation or production of organic acids and potentially aid in the solubilization of phosphates (Wang et al. 2020). Mucus production occurred with 72.3% of the isolates, which may indicate resistance to environmental stress (Khan and Singh 2021). Cream and yellow colony colors predominated with 45%, followed by cream (32%), yellow (21.2%), and pink (1.3%). As for the edge, shape, opacity, and surface, 88.9% were solid, 92.9% circular, 75.4% opaque, and 98.5% smooth, respectively (Fig. 2 and Suppl. Table 1).

Out of the 325 isolates, 315 amplified the BOX elements, forming bands from 228 to 1,736 bp, which identified 243 strains (Table 2), 78% of which were isolated at a single time, which indicates high diversity. This high diversity was also observed when plant parts or sampling areas were compared through the diversity indexes (Table 2), while dominance, equability, and high Margalef's richness indexes confirmed that the strains were equally abundant, which usually indicates greater stability and resistance to environmental stresses (Zhao et al. 2022). Considering the overall environments, Araripina had the most isolates and higher Shannon's (5.241), Simpson's (0.99) and Margalef's (7.502) indexes, followed by Nazaré da Mata and Gravatá. The lower diversity and higher dominance indexes found at Gravatá (Table 2) are likely due to this

stand being the youngest (6 years compared to 11 in Nazaré da Mata and > 30 in Araripina), which is comparable to that observed in *Lolium perenne* plants, which showed higher endophytic bacterial diversity in older plants than in younger ones (Tannenbaum et al. 2020). Another possible reason for the greater diversity found in Araripina is the drier environment, which could be more inducive to bacterial diversity (Wei et al. 2020).

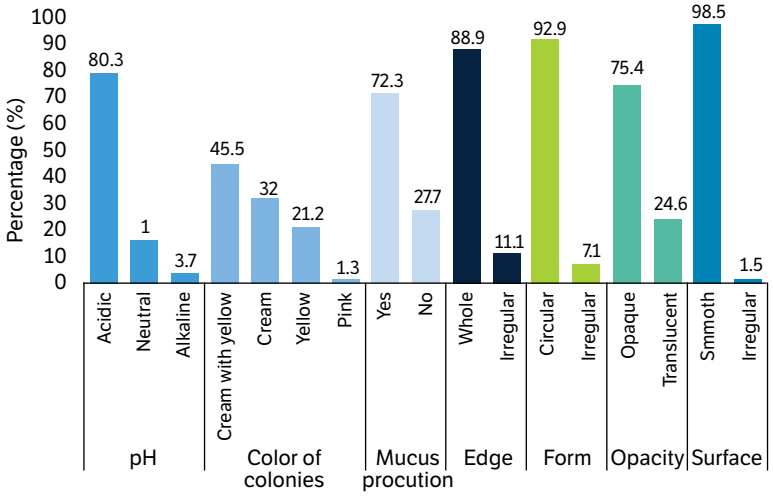


**Figure 2.** Percentage of bacterial isolates from *pangolão* grass (*Digitaria eriantha* cv. Suvernola) according to phenotypical characteristics.

**Table 2.** Number of isolates and strains and diversity indexes of *Digitaria eriantha* cv. Survenola bacteria from different sampling locations and isolation material, Pernambuco, Brazil.

| Isolation condition | Isolates | Strains | Dominance D | Simpson 1-D | Shannon H' | Margalef | Equitability J' |
|---|---|---|---|---|---|---|---|
| Total | 315 | 243 | 0.0049 | 0.9951 | 5.407 | 42.07 | 0.9845 |
| Araripina rainy season, without liming | 58 | 52 | 0.0208 | 0.9792 | 3.917 | 12.56 | 0.9913 |
| Araripina rainy season, with liming | 74 | 72 | 0.0142 | 0.9858 | 4.267 | 16.49 | 0.9976 |
| Araripina dry season | 97 | 90 | 0.0116 | 0.9884 | 4.481 | 19.54 | 0.9958 |
| Gravatá | 24 | 22 | 0.0486 | 0.9514 | 3.063 | 6.79 | 0.9908 |
| Nazaré da Mata | 61 | 60 | 0.0169 | 0.9831 | 4.088 | 14.35 | 0.9985 |
| Colm | 97 | 81 | 0.0146 | 0.9854 | 4.325 | 17.49 | 0.9841 |
| Leaves | 82 | 66 | 0.0181 | 0.9819 | 4.100 | 14.52 | 0.9822 |
| Roots | 61 | 59 | 0.0158 | 0.9842 | 3.976 | 14.11 | 0.9751 |
| Rhizospheric soil | 72 | 68 | 0.0154 | 0.9846 | 4.199 | 15.67 | 0.9953 |

Considering only Araripina, higher diversity was found in the dry season than that in the rainy season, with and without liming. While liming effects in the rainy season are reasonable, the higher diversity during the dry season is uncommon (Xu et al. 2018, Ou et al. 2019, Firrincieli et al. 2020), but it may relate to the use of multiple culture media, allowing for a broader range of cultivable bacteria (Döbereiner et al. 1995, Jia et al. 2022). Comparing the different plant parts and rhizospheric soil, diversity decreased on the culm, rhizospheric soil, leaves, and root, in that order (Table 2), although rhizospheric soil presented the highest uniformity. Since the difference between these sources is relatively small, and higher uniformity was

found in the rhizospheric soil, the results likely do not strongly deviate from the usual pattern for bacterial diversity found in these sources (Liu et al. 2017, Huang 2018, Zhang et al. 2020, Wang et al. 2022). Bacterial diversity varies between plant parts in a species dependent manner, with the most diverse part of maize being the culm, millet the roots, and both the culm and roots being more diverse for rice (Patel and Archana 2017). This also occurred on a cultivar basis, with drought resistant and tolerant millet genotypes presenting different bacterial diversity patterns (Manjunatha et al. 2019). Therefore, it is important to evaluate bacterial diversity in different plant parts, despite repeated isolation of the same strain.

Among the 135 groups with 90% similarity, 118 were identified at the genus level (96.84–99.9% similarity), while 13 (68–92.4% similarity) had insufficient similarity for taxonomic classification, and four strains showed no similarity with any genetic sequence in the GenBank database (Suppl. Table 2).

Based on the List of Prokaryotic names with Standing in Nomenclature (LPSN), three phyla, five classes, seven orders, 13 families, and 18 genera were identified (Table 3) (Parte et al. 2020). The main representative classes were γ-proteobacteria (*Enterobacter*, *Kosakonia*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas*), and α-proteobacteria (*Agrobacterium*, *Ochrobactrum*, *Rhizobium*, and *Shinella*), with 58 and 28% respectively, all of which found in the three collection sites and all plant parts. β-proteobacteria were found in smaller amounts, with 11 strains and four genera (*Achromobacter*, *Burkholderia*, *Herbaspirillum*, and *Variovorax*).

Proteobacteria domination found in *pangolão* grass has been previously described for widely divergent species, such as wheat (Robinson et al. 2016) and bamboo (Singh et al. 2021), which indicates a possible general pattern among endophytes.

**Table 3.** Distribution of bacterial strains isolated from *pangolão* grass (*Digitaria eriantha* cv. Survenola) in Pernambuco, Brazil.

| Phylum | Class | Order | Family | Genus | Strains |
|---|---|---|---|---|---|
| Proteobacteria | α - proteobacteria | | Rhizobiaceae | *Shinella* | 1 |
| | | | | *Agrobacterium* | 3 |
| | | | | *Rhizobium* | 26 |
| | | | Brucellaceae | *Ochrobactrum* | 3 |
| | β - proteobacteria | Burkholderiales | Oxalobacteraceae | *Herbaspirillum* | 1 |
| | | | Comamonadaceae | *Variovorax* | 4 |
| | | | | *Achromobacter* | 3 |
| | | | Burkholderiaceae | *Burkholderia* | 3 |
| | γ - proteobacteria | Enterobacterales | Enterobacteriaceae | *Enterobacter* | 10 |
| | | | | *Kosakonia* | 1 |
| | | | | *Pantoea* | 19 |
| | | | Yersiniaceae | *Serratia* | 1 |
| | | Pseudomonadales | Pseudomonadaceae | *Pseudomonas* | 15 |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* | 23 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | *Bacillus* | 1 |
| | | | Bacillaceae | *Priestia* | 1 |
| | | | Paenibacillaceae | *Paenibacillus* | 2 |
| Actinobacteria | Actinobacteria | Actinomycetales | Microbacteriaceae | *Curtobacterium* | 1 |
| | | Unclassified bacteria | | | 17 |

The genera found included several species described as plant growth promoters, such as *Stenotrophomonas* (Ramos et al. 2011), *Pseudomonas* (Josephine CM and Thomas 2021), *Enterobacter*, *Pantoea* (Lu et al. 2021), *Burkholderia*, *Herbaspirillum* (Van Deynze et al. 2018), *Rhizobium* (Hahn et al. 2016, Silva et al. 2020), *Bacillus*, and *Paenibacillus* (Govindasamy et al. 2010, Kavamura et al. 2013). A commercial product based on *Bacillus* strains has previously been licensed for use in maize to reduce drought effects, which was originally isolated from the native Brazilian cactus, *Cereus jamacaru* (Kavamura et al. 2013).

The most abundant genera were *Enterobacter*, *Pantoea*, *Pseudomonas*, *Rhizobium*, and *Stenotrophomonas*, although *Achromobacter*, *Bacillus*, *Burkholderia*, *Curtobacterium*, *Herbaspirillum*, *Kosakonia*, *Ochrobactrum*, *Paenibacillus*, *Serratia*, *Shinella*, and *Variovorax* were also present, and these genera have been observed in other grass endophytic communities such as Brachiaria and maize (Mutai et al. 2017, Mashiane et al. 2018).

Of the five most abundant genera, *Rhizobium*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas* were isolated from all locations (Fig. 3), whereas *Enterobacter* was not found in Nazaré da Mata, which might be a confirmation of the division of the endophytic community into systemic and transitory groups (Wani et al. 2015). These genera were isolated from all plant parts and the rhizospheric soil.

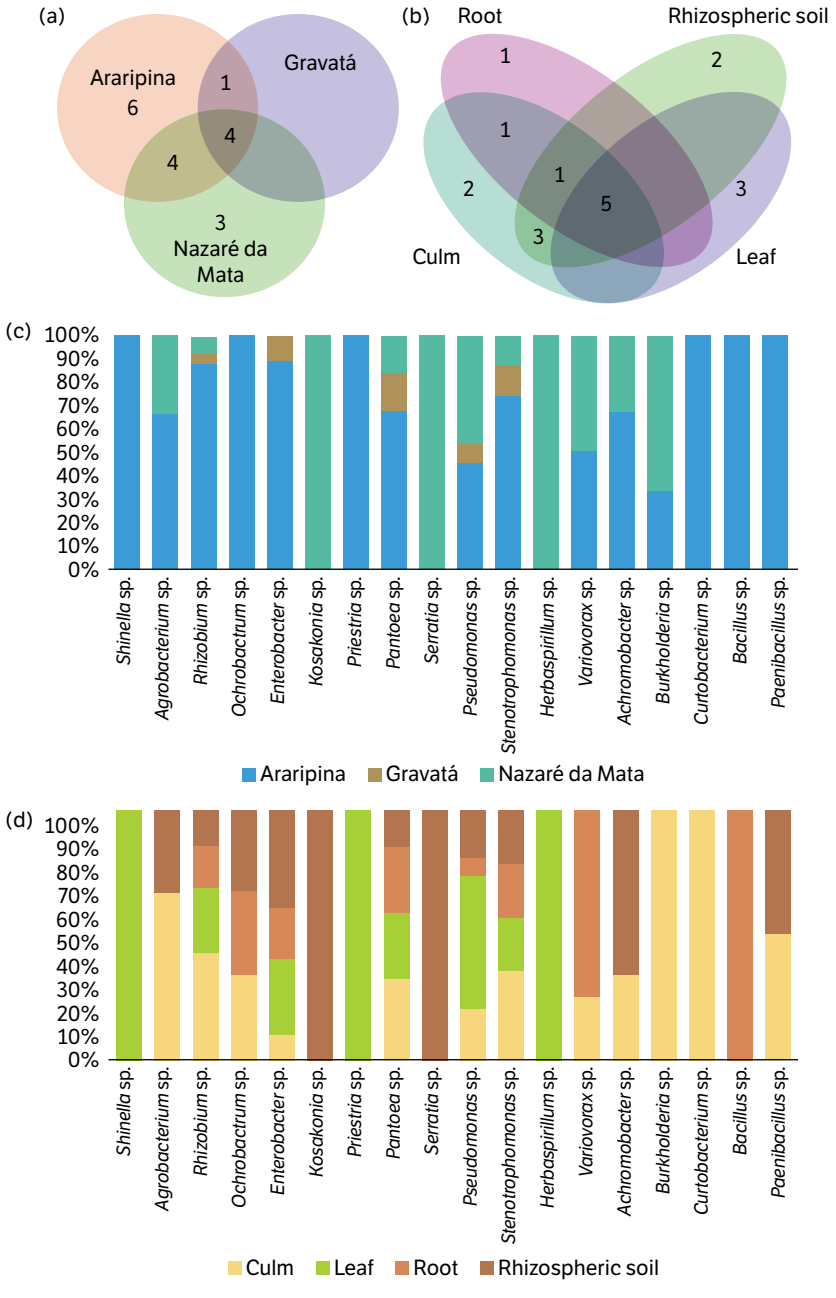


**Figure 3.** Number of bacterial genera from *pangolão* grass (*Digitaria eriantha* cv. Survenola) from Pernambuco, Brazil. Venn diagram represents the sharing of genera in (a) different locations and (B) plant parts. Proportion of isolates from each genus in (a) different locations and (b) plant parts. Proportion of isolates from each genus in (c) different locations and (d) plant parts.

The phylogenetic tree grouped the genera into six large polyphyletic groups (Fig. 4), with most strains (69%) in GI, GIII, and GVI, with no overlap with the outgroup based on *Escherichia coli*.

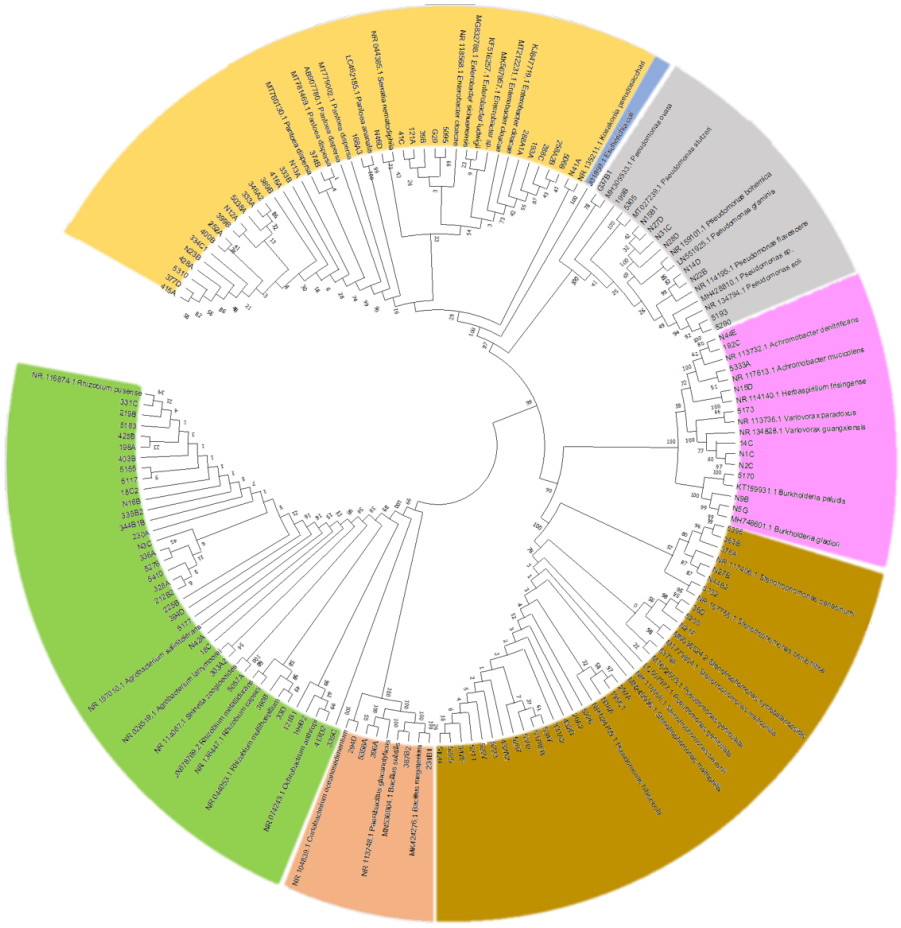

**Figure 4.** Neighbor-joining phylogenetic tree based on 16 S rRNA sequences from *pangolão* grass (*Digitaria eriantha* cv. Survenola) bacterial isolates from Pernambuco, Brazil.

The first group (GI) contained 32 strains in two subgroups. The first subgroup consisted of those similar to *Rhizobium* and *Agrobacterium* (25 strains, 98% similarity), *Shinella* (one strain, 100% similarity), *Rhizobium* (two strains, 58% similarity), *Ochrobactrum* (three strains, 99% similarity), while the second subgroup was not grouped with any genera. Five strains were included in GII, of which one was similar to *Curtobacterium*, two are similar to *Paenibacillus*, and the remaining two to *Bacillus* at 100% similarity.

GIII included 28 strains of *Stenotrophomonas*, and *Pseudomonas* split into two subgroups: one consisting of 22 strains with 70% similarity to these genera according to the bootstrap test to these genera, and the second one with the remaining six strains grouped with *Stenotrophomonas* at 57% similarity.

GIV contained the 11 *Burkholderia*, *Variovorax*, *Herbaspirillum*, and *Achromobacter* strains. Subgroup I matched three strains to *Burkholderia* at 100% similarity, while subgroup II matched four strains to *Variovorax* with 100% similarity, and the last four were grouped to *Achromobacter* and *Herbaspirillum* at 72% similarity.

GV related 11 strains to *Pseudomonas* (100% similarity). However, GVI connected 31 strains to *Enterobacter*, *Kosakonia*, *Serratia*, and *Pantoea*, with one strain in the subgroup showing 100% similarity to *Kosakonia*. In subgroup II, 10 strains were 54% similar to *Enterobacter*, one strain was 71% similar to *Serratia*, and 19 strains were similar to *Pantoea*.

This research is the first one to evaluate endophytic bacterial diversity on *pangolão* grass. These results indicate several factors, such as environmental conditions, plant parts, pasture age, season and liming, differently affect bacterial diversity. Since these bacteria were evaluated on a species well adapted to environmental stresses predicted to increase with global

climate change, several of the genera found are known to include species and strains known to promote plant growth and several other papers, indicating bacteria isolated from one plant species may promote plant growth on other species. We suggest further research evaluating these and similar strains as plant growth promoters for crops such as maize.

## CONCLUSION

Bacteria associated with *Digitaria eriantha* cv. Survenola are highly diverse, and this diversity varies according to environmental conditions, including plant compartment, pasture establishment time, season, and liming history. Proteobacteria were the most frequent bacteria associated with *pangolão* grass under all environmental conditions.

Although diversity was slightly higher in the culm, there were no major differences between plant parts and the rhizospheric soil, and diversity was higher in older pastures.

The diversity of endophytic and rhizospheric bacteria in *pangolão* grass may have promoted resilience, since many of the identified strains belong to genera known to promote plant growth. These strains should be further evaluated for growth promotion in this and other plant species.

Less studied drought tolerant grass genera, such as *Digitaria*, might be an interesting source of plant growth promoting bacteria for further studies, due to their endophytic bacteria diversity.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Alves, M. J. G., Oliveira, J. P. and Lira Junior, M. A. **Methodology:** Alves, M. J. G. and Fracetto, G. G. M. **Investigation:** Alves, M. J. G., Oliveira, C. S. and Vitalino, G. M. **Formal Analysis:** Alves, M. J. G. and Lira Junior, M. A. **Funding:** Carvalho, E. X., Oliveira, J. P. and Lira Junior, M. A. **Project Administration:** Carvalho, E. X. and Lira Junior, M. A. **Supervision:** Carvalho, E. X., Fracetto, G. G. M., Fracetto, F. J. C. and Lira Junior, M. A. **Writing – Original Draft:** Alves, M. J. G. **Writing – Review and Editing:** Alves, M. J. G., Carvalho, E. X., Oliveira, J. P., Fracetto, G. G. M., Fracetto, F. J. C. and Lira Junior, M. A.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

## FUNDING

Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco
https://doi.org/10.13039/501100006162
Grants Nos: BCT-0406-5.03/21, APQ-0453-5.01/15 and BPV-0008-5.01/19

Conselho Nacional de Desenvolvimento Científico e Tecnológico
https://doi.org/10.13039/501100003593
Grants Nos: 304107/2020-4, 401896/2013-7 and 483287/2013-0

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
https://doi.org/10.13039/501100002322
Finance Code: 001

## ACKNOWLEDGMENTS

To the Pernambuco Agronomic Institute for allowing the use of the research area, providing access to the soil biology laboratory and helping with all research activities.

## REFERENCES

Afzal, I., Shinwari, Z. K., Sikandar, S. and Shahzad, S. (2019). Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiological Research, 221, 36-49. https://doi.org/10.1016/j.micres.2019.02.001

Baldani, J. I., Baldani, V. L. D., Seldin, L. and Dobereiner, J. (1986). Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. International Journal of Systematic Bacteriology, 36, 86-93.

Baldani, V. L. D., Baldani, J. I. and Dobereiner, J. (1996). Meios de cultura específicos para o isolamento de bactérias endofíticas que fixam $N_2$ atmosférico. Comunicado técnico, 12. Seropédica: EMBRAPA-CNPAB.

[BRASIL] Ministério da Agricultura, Pecuária e Abastecimento. (2011). Instrução Normativa SDA nº 13, de 24 de março de 2011. Aprova as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos micro-organismos autorizados e recomendados para produção de inoculantes no Brasil, na forma dos Anexos I, II e III, desta Instrução Normativa. Diário Oficial da União, Brasília, Seção 1, p. 3.

Chen, Z., Liu, J., Yang, X., Liu, M., Wang, Y., Zhang, Z. and Zhu., D. (2019). Community composition and diversity of cultivable endophytic bacteria isolated from Dongxiang wild rice. Biodiversity Science, 27, 1320-1329. https://doi.org/10.17520/biods.2019219

[CONDEPE/FIDEM] Agência Estadual de Planejamento e Pesquisas de Pernambuco. (2016). Anuário estatístico de Pernambuco: Mapa 3.3 - Tipologia Climática. Available at: http://www.anuario.pe.gov.br/wp-content/uploads/downloads/2018/08/Mapa_3.3-Tipologia-clim%C3%A1tica.pdf. Accessed on: Jan. 7, 2021.

Döbereiner, J., Baldani, V. L. D. and Baldani, J. I. (1995). Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. Itaguaí: Embrapa-Agrobiologia.

Firrincieli, A., Khorasani, M., Frank, A. C. and Doty, S. L. (2020). Influences of climate on phyllosphere endophytic bacterial communities of wild poplar. Frontiers in Plant Science, 11, 203. https://doi.org/10.3389%2Ffpls.2020.00203

Freitas, A. D. S., Vieira, C. L., Santos, C. E. R. S., Stamford, N. P. and Lyra, M. D. C. C. P. (2007). Characterization of isolated rhizobia of pachyrhyzus erosus cultivated in saline soil of the state of Pernambuco, Brazil. Bragantia, 66, 497-504. https://doi.org/10.1590/S0006-87052007000300017

Galindo, F. S., Teixeira Filho, M. C. M., Silva, E. C., Buzetti, S., Fernandes, G. C. and Rodrigues, W. L. (2020). Technical and economic viability of cowpea co-inoculated with *Azospirillum brasilense* and *Bradyrhizobium* spp. and nitrogen doses. Revista Brasileira de Engenharia Agrícola e Ambiental, 24, 305-312. https://doi.org/10.1590/1807-1929/agriambi.v24n5p304-311

Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V. and Annapurna, K. (2010). *Bacillus* and *Paenibacillus* spp.: Potential PGPR for Sustainable Agriculture. In: D. K. Maheshwari (Ed.). Plant growth and health promotion bacteria (p. 333-364). Berlin: Springer.

Hahn, L., Sá, E. L. S. D., Osório Filho, B. D., Machado, R. G., Damasceno, R. G. and Giongo, A. (2016). Rhizobial inoculation, alone or coinoculated with *Azospirillum brasilense*, promotes growth of wetland rice. Revista Brasileira de Ciência do Solo, 40, e0160006. https://doi.org/10.1590/18069657rbcs20160006

Heras, J., Domínguez, C., Mata, E., Pascual, V., Lozano, C., Torres, C. and Zarazaga, M. (2015). GelJ – a tool for analyzing DNA fingerprint gel images. BMC Bioinformatics, 16, 270. https://doi.org/10.1186/s12859-015-0703-0

Hernández-Pacheco, C. E., Orozco-Mosqueda, M. D. C., Flores, A., Valencia-Cantero, E. and Santoyo, G. (2021). Tissue-specific diversity of bacterial endophytes in Mexican husk tomato plants (*Physalis ixocarpa* Brot. ex Horm.), and screening for their multiple plant growth-promoting activities. Current Research in Microbial Sciences, 2, 100028. https://doi.org/10.1016/j.crmicr.2021.100028

Huang, Y.-H. (2018). Comparison of rhizosphere and endophytic microbial communities of Chinese leek through high-throughput 16S rRNA gene Illumina sequencing. Journal of Integrative Agriculture, 17, 359-367. https://doi.org/10.1016/S2095-3119(17)61731-3

Jia, H., Xi, Z., Ma, J., Li, Y., Hao, C,; Lu, M., Zhang, Z.-Z. and Deng, W.-W. (2022). Endophytic bacteria from the leaves of two types of albino tea plants, indicating the plant growth promoting properties. Plant Growth Regulation, 96, 331-343. https://doi.org/10.1007/s10725-021-00779-5

Josephine CM, R. and Thomas, J. (2021). Rhizosphere competent *Pseudomonas indoloxydans* (F3-47) as a plant growth promoter and enhancer of *Zea mays* L. under greenhouse and field trials. Current Trends in Biotechnology and Pharmacy, 15, 411-418. https://doi.org/10.5530/ctbp.2021.3s.34

Kavamura, V. N., Santos, S. N., Silva, J. L., Parma, M. M., Avila, L. A., Visconti, A., Zucchi, T. D. and Taketani, R. G., Andreote, F. D. and Melo, I. S. (2013). Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiology Research, 168, 183-191. https://doi.org/10.1016/j.micres.2012.12.002

Khan, A. and Singh, A. V. (2021). Multifarious effect of ACC deaminase and EPS producing *Pseudomonas* sp. and *Serratia marcescens* to augment drought stress tolerance and nutrient status of wheat. World Journal of Microbiology and Biotechnology, 37, 198. https://doi.org/10.1007/s11274-021-03166-4

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution, 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Liu, H., Carvalhais, L. C., Crawford, M., Singh, E., Dennis, P. G., Pieterse, C. M. J. and Schenk, P. M. (2017). Inner plant values: Diversity, colonization and benefits from endophytic bacteria. Frontiers in Microbiology, 8, 2552. https://doi.org/10.3389%2Ffmicb.2017.02552

Liu, T., Zhai, C., Zhang, J. and Coulter, J. A. (2021). Genetic diversity and promotion plant growth of culturable endophytic diazotrophs associated with seashore paspalum cultivars. Journal of Crop and Horticultural Science, 49, 243-257. https://doi.org/10.1080/01140671.2021.1893193

Lu, L., Chang, M., Han, X., Wang, Q., Wang, J., Yang, H., Guan, Q. and Dai, S. (2021). Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. Journal of Applied Microbiology, 131, 1919-1931. https://doi.org/10.1111/jam.15082

Lyra, M. C. C. P., Freitas, A. D. S., Silva, M. L. R. B. D., Bezerra, R. V., Silva, V. S. G. D., Silva, A. F., Mergulhão, A. C. E. S., Dantas, E. F. and Santos, C. E. R. S. (2019). Diversity of rhizobia isolated from nodules of indigenous tree legumes from the Brazilian dry forest. Acta Agronomica, 68, 47-55. https://doi.org/10.15446/acag.v68n1.61243

Manjunatha, B. S., Paul, S., Aggarwal, C,; Bandeppa, S., Govindasamy, V., Dukare, A. S., Rathi, M. S., Satyavathi, C. T. and Annapurna, K. (2019). Diversity and tissue preference of osmotolerant bacterial endophytes associated with pearl millet genotypes having differential drought susceptibilities. Microbial Ecology, 77, 676-688. https://doi.org/10.1007/s00248-018-1257-2

Margalef, R. (1956). Información y diversidad específica en las comunidades de organismos. Investigación Pesquera, 3, 99-106.

Mashiane, A. R., Adeleke, R. A., Bezuidenhout, C. C. and Chirima, G. J. (2018). Community composition and functions of endophytic bacteria of Bt maize. South African Journal of Science, 114, 88-97. https://doi.org/10.17159/sajs.2018/20170018

Mutai, C., Njuguna, J. and Ghimire, S. (2017). Brachiaria Grasses (*Brachiaria* spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. Microbiologyopen, 6, e00497. https://doi.org/10.1002/mbo3.497

Navarro, D. L., Rodríguez, P. I. E., González, C. S. and Torres, D. R. (2005). Umfolozi o pangola peluda: un pasto que comienza a ser cultivado. INIA Divulga, 29-32.

Ou, T., Xu, W.-F., Wang, F., Strobel, G., Zhou, Z.-Y., Xiang, Z.-H., Liu, J. and Xie, J. A. (2019). Microbiome study reveals seasonal variation in endophytic bacteria among different mulberry cultivars. Computational and Structural Biotechnology Journal, 17, 1091-1100. https://doi.org/10.1016/j.csbj.2019.07.018

Parte, A. C., Sardà Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C. and Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International Journal of Systematic and Evolutionary Microbiology, 70, 5607-5612. https://doi.org/10.1099/ijsem.0.004332

Patel, J. K. and Archana, G. (2017). Diverse culturable diazotrophic endophytic bacteria from *Poaceae* plants show cross-colonization and plant growth promotion in wheat. Plant and Soil, 417, 99-116. https://doi.org/10.1007/s11104-017-3244-7

Pielou, E. C. (1959). The use of point-to-plant distances in the study of the pattern of plant populations. Journal of Ecology, 47, 607-613. https://doi.org/10.2307/2257293

Ramos, P. L., Van Trappen, S., Thompson, F. L., Rocha, R. C. S., Barbosa, H. R., De Vos, P. and Moreira-Filho, C. A. (2011). Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 61, 926-931. https://doi.org/10.1099/ijs.0.019372-0

Robinson, R. J., Fraaije, B. A., Clark, I. M., Jackson, R. W., Hirsch, P. R. and Mauchline, T. H. (2016). Endophytic bacterial community composition in wheat (*Triticum aestivum*) is determined by plant tissue type, developmental stage and soil nutrient availability. Plant and Soil, 405, 381-396. https://doi.org/10.1007/s11104-015-2495-4

Shannon, C. E. and Weaver, W. (1949). The mathematical theory of communication. Urbana: University of Illinois Press.

Silva, F. B., Winck, B., Borges, C. S., Santos, F. L., Bataiolli, R. D., Backes, T., Bassani, V. L., Borin, J. B. M., Frazzon, A. P. G. and Sá, E. L. S. (2020). Native rhizobia from southern Brazilian grassland promote the growth of grasses. Rhizosphere, 16, 100240. https://doi.org/10.1016/j.rhisph.2020.100240

Silva, M. D. O., Freire, F. J., Lira Junior, M. A., Kuklinsky-Sobral, J., Costa, D. P. D. and Lira-Cadete, L. (2012). Isolamento e prospecção de bactérias endofíticas e epifíticas na cana-de-açúcar em áreas com e sem cupinicida. Revista Brasileira de Ciência do Solo, 36, 1113-1122. https://doi.org/10.1590/S0100-06832012000400006

Simpson, E. H. (1949). Measurement of diversity. Nature, 163, 688. https://doi.org/10.1038/163688a0

Singh, L., Ruprela, N., Dafale, N. and Thul, S. T. (2021). Variation in endophytic bacterial communities associated with the rhizomes of tropical bamboos. Journal of Sustainable Forestry, 40, 111-123. https://doi.org/10.1080/10549811.2020.1745655

Tannenbaum, I., Kaur, J., Mann, R., Sawbridge, T., Rodoni, B. and Spangenberg, G. (2020). Profiling the *Lolium perenne* microbiome: from seed to seed. Phytobiomes Journal, 4, 281-289. https://doi.org/10.1094/PBIOMES-03-20-0026-R

Teheran-Sierra, L. G., Funnicelli, M. I. G., Carvalho, L. L., Ferro, M. I. T., Soares, M. A. and Pinheiro, D. G. (2021). Bacterial communities associated with sugarcane under different agricultural management exhibit a diversity of plant growth-promoting traits and evidence of synergistic effect. Microbiology Research, 247, 126729. https://doi.org/10.1016/j.micres.2021.126729

Teixeira, P. C., Donagemma, G. K., Fontana, A. and Teixeira, W. G. (2017). Manual de Métodos de Análise de Solo. Brasília: EMBRAPA.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22, 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Van Deynze, A., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., Schwartz, K. D.; Berry, A. M., Bhatnagar, S., Jospin, G., Darling, A., Jeannotte, R., Lopez, J., Weimer, B. C., Eisen, J. A., Shapiro, H.-Y., Ane, J.-M. and Bennett,

A. B. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLoS Biology, 16, e2006352. https://doi.org/10.1371/journal.pbio.2006352

Versalovic, J., Schneider, M., De Bruijn, F. J. and Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, 5, 25-40.

Vincent, J. M. (1970). A manual for the practical study of root-nodule bacteria. Oxford: Blackwell.

Wang, C., Ma, H., Feng, Z., Yan, Z., Song, B., Wang, J., Zheng, Y., Hao, W., Zhang, W., Yao, M. and Wang, Y. (2022). Integrated organic and inorganic fertilization and reduced irrigation altered prokaryotic microbial community and diversity in different compartments of wheat root zone contributing to improved nitrogen uptake and wheat yield. Science of the Total Environment, 842, 156952. https://doi.org/10.1016/j.scitotenv.2022.156952

Wang, X., Xie, H., Ku, Y., Yang, X., Chen, Y., Yang, N., Mei, X. and Cao, C. (2020). Chemotaxis of ***Bacillus cereus*** YL6 and its colonization of Chinese cabbage seedlings. Plant and Soil, 447, 413-430. https://doi.org/10.1007/s11104-019-04344-y

Wani, Z. A., Ashraf, N., Mohiuddin, T. and Riyaz-Ul-Hassan, S. (2015). Plant-endophyte symbiosis, an ecological perspective. Applied Microbiology and Biotechnology, 99, 2955-2965. https://doi.org/10.1007/s00253-015-6487-3

Wei, Z., Friman, V. P., Pommier, T., Geisen, S., Jousset, A. and Shen, Q. (2020). Rhizosphere immunity: Targeting the underground for sustainable plant health management. Frontiers of Agricultural Science and Engineering, 7, 317-328. https://doi.org/10.15302/J-FASE-2020346

Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173, 697-703. https://doi.org/10.1128/jb.173.2.697-703.1991

Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K. K., Kim, Y.-M., Zink, E. M., Engbrecht, K. M., Wang, Y., Gao, C., Degraaf, S., Madera, M. A., Sievert, J. A., Hollingsworth, J., Birdseye, D., Scheller, H. V., Hutmacher, R., Dahlberg, J., Jansson, C., Taylor, J. W., Lemaux, P. G. and Coleman-Derr, D. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proceedings of the National Academy of Sciences, 115, E4284-E4293. https://doi.org/10.1073/pnas.1717308115

Zhang, L., Zhang, W., Li, Q., Cui, R., Wang, Z., Wang, Y., Zhang, Y.-Z., Ding, W. and Shen, X. (2020). Deciphering the root endosphere microbiome of the desert plant ***Alhagi sparsifolia*** for drought resistance-promoting bacteria. Applied and Environmental Microbiology, 86, e02863-19. https://doi.org/10.1128/aem.02863-19

Zhao, Y., Li, T., Shao, P., Sun, J., Xu, W. and Zhang, Z. (2022). Variation in bacterial community structure in rhizosphere and bulk soils of different halophytes in the yellow River Delta. Frontiers in Ecology and Evolution, 9, 816918. https://doi.org/10.3389/fevo.2021.816918