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Effect of *Digitaria eriantha* Endophytic Bacteria on Maize Growth in a Hydroponic System

Johny J. Mendonça [1], Michelle J. G. Alves [2], Gisely M. Vitalino [2], Maria C. S. Barreto [3], Eric X. Carvalho [4], José P. Oliveira [4], Maria L. R. B. Silva [4], Felipe J. C. Fracetto [2], Giselle G. M. Fracetto [2] and Mario A Lira Junior [2,*]

[1] Departamento de Solos, Universidade Federal do Rio Grande do Sul, Porto Alegre 90040-060, Brazil; mendonca.johny@yahoo.com.br
[2] Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Recife 52091-190, Brazil; michellejustinoalves@gmail.com (M.J.G.A.); gisellymore@gmail.com (G.M.V.); fracettofelipe@gmail.com (F.J.C.F.); giselle.fracetto@ufrpe.br (G.G.M.F.)
[3] Maná Alimentos Agroindústria Ltda, Araripina 56280-000, Brazil; mcsbarreto@gmail.com
[4] Instituto Agronômico de Pernambuco, Recife 50761-000, Brazil; eric.carvalho@ipa.br (E.X.C.); jose.paula@ipa.br (J.P.O.); luizabastos6@yahoo.com.br (M.L.R.B.S.)
* Correspondence: mario.alirajr@ufrpe.br

Abstract: Brazil is one of the largest grain producers worldwide, with yields heavily dependent on ecologically and financially expensive inputs. One possible approach to reduce these inputs is inoculation with plant-growth-promoting bacteria, whose large-scale use depends on a continual search for new genotypes for inoculant production. Several bacteria with potential for this have been isolated from plants that are more adapted to stressful environments. Thus, we aimed to evaluate the potential of pangolão grass (*Digitaria eriantha* cv. Suvernola) endophytic bacteria both in vitro and on maize growth. To this end, endophytic bacteria were isolated from pangolão grass of a tropical semiarid climate and a random subset of 80 strains was evaluated for biological nitrogen fixation, HCN, IAA and siderophore production and calcium phosphate solubilization, and later for maize growth promotion. All strains were positive for at least one of these in vitro growth promotion mechanisms and some strains increased maize plant height and root length, including some with better results than plants receiving commercial inoculants, confirming the potential of endophytic bacteria from stress-adapted plants. In vitro results had poor correlation with plant growth promotion, which indicates that the common practice of using these laboratory techniques as a pre-selection tool before a subset of strains is evaluated for plant growth promotion might result in the rejection of potentially interesting strains.

Keywords: corn; PGPB; inoculant; stress-adapted plants

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1. Introduction

Brazil is one of the major maize (*Zea mays*) producers and exporters, largely due to the intensive use of agrichemicals with high economic and ecological footprints [1,2]. An alternative is the inoculation with plant-growth-promoting bacteria (PGPB), already widely used in Brazil [3,4]. Generally, these inoculants allow for a reduction in fertilizer use and/or increase environmental stress resistance, particularly to drought, while maintaining or improving yield [5–8]. This is considered to be due to several mechanisms such as IAA, siderophore or HCN production, phosphate solubilization or biological nitrogen fixation [9–11].

The effect of PGPB inoculation under environmental stress suggests that plants adapted to these environments might be useful sources of these bacteria for crops [12–14]. This is already seen with the widespread use of maize-isolated *Azospirillum brasilense* strains for inoculants in maize, wheat, rice, Brachiaria grasses, soybean and common beans in Brazil [15]. At the same time, *Bacillus* and *Enterobacter* strains isolated from the rhizosphere

of drought-affected Bermuda grass (*Cynodon* spp.) reduced water stress in maize and wheat [14], while *Pseudomonas putida* and *P. brassicacearum* isolated from *Opuntia* sp. induced drought resistance in wheat [16] and root-endophytic *Kocuria arsenatis* ST-19 from the halophyte *Stipa tenacissima* reduced salt stress in tomato [17].

Another possible PGPB source is pangolão grass (*Digitaria eriantha* cv. Suvernola). This perennial species is well adapted to a low-fertility soil from a tropical semiarid climate in Northeast Brazil, and previous work from this group has found a high endophytic bacteria diversity and some strains with potential for maize growth promotion [18,19]. At the same time, while several of the putative growth promotion mechanisms are well identified, there is still a knowledge gap on how their laboratory determinations are linked to actual plant growth [20]. Since most of the literature on selecting strains for PGPB inoculants uses one or a combination of these growth promotion mechanisms evaluated in vitro as pre-selectors for plant assays [12,17,18,21], this knowledge gap might mean that researchers are not evaluating potentially promising bacterial strains.

Thus, this paper evaluates a randomly selected group of strains with regard to their maize growth promotion under controlled conditions and evaluates if the in vitro growth promotion characteristic values are well related to the actual growth promotion.

2. Materials and Methods

2.1. Sampling

Sampling was carried out in an area cultivated with pangolão grass in the experimental station of the Instituto Agronômico de Pernambuco—IPA, Araripina, Pernambuco, Brazil, with a semiarid hot climate Bsh, according to Köppen [22]. The soil was classified as Cutanic Acrisol (WRB 2006) and there was liming or fertilization for over 30 years [23].

The samplings were performed in December 2016 (dry season) and March 2017 (rainy season) to maximize environmental variability within the sampling area and increase potential biodiversity. Ten plants were collected in a transect in each sampling period, with the soil adhering to the roots, to form a compound sample and two of these samples were taken per environment (Table 1). A soil sample made of part of the soil from both samples was chemically characterized [24] (Table 2).

Table 1. Digitaria eriantha sampling from a tropical semiarid low-fertility site in Pernambuco, Brazil.

Date	Season	Liming	Coordinates
December	Dry	No	7°27′42.82″ S 40°25′12.71″ W
March	Rainy	No	7°27′48″25″ S 40°25′16.11″ W
		Yes	

Table 2. Soil chemical characterization in experimental plots in the 0–20 cm layer.

Season	Liming	pH	Ca	Mg	Al	Na	K	P	H+Al
		1:2.5	cmolc dm^{-3}					mg dm^{-3}	cmolc dm^{-3}
Dry	No	5.50	2.0	1.60	0.10	0.03	0.10	3.0	3.71
Rainy	No	5.1	0.55	0.50	0.45	0.06	0.07	3.0	4.86
Rainy	Yes	6.40	2.30	1.20	0.00	0.03	0.12	2.0	1.73

2.2. Isolation and Morphophysiological Characterization of Endophytic Bacteria

The plant samples were separated into leaves, culm and roots. The leaves and culms were washed under running water, dried on paper towels and washed in 70% alcohol [25]. The roots were washed under running water and cut into pieces of approximately 10 cm. The roots were disinfected with 70% alcohol for 30 s and washed with 2.5% sodium hypochlorite for 1 min and with autoclaved water five times.

A 1:9 weight/volume of each plant part and 0.85% sterile saline solution [26] was crushed for the 10^{-1} dilution, followed by ten-fold dilutions to 10^{-8} in the same solution. Aliquots of 0.1 mL of the diluted extracts were inoculated in triplicate in tubes containing 5 mL of the semi-solid nitrogen-free media NFB, JNFB and JMV [27,28].

The tubes were incubated at 35 °C for 72–96 h, and the most probable number of colony-forming units was determined [29] followed by phenotypic characterization of isolated colonies in YMA [30,31] and grouping at 100% by the paired groups algorithm with Jaccard Index, using the PAST 2.17 program [32]. The diversity indexes were calculated considering the phenotypical groups as taxonomic units. Eighty isolates were randomly chosen to maintain the group's representativeness and isolation conditions for later stages of the study.

2.3. Genetical Diversity

The isolates were grown in TSB culture medium and incubated at 30 °C at 180 RPM for 72 h, followed by DNA extraction, using the MiniPrep Kit (Axygen, Union City, CA, USA), according to the manufacturer's instructions. After extraction, DNA integrity was verified by electrophoresis on 0.8% agarose gel at 100 V for 30 min and DNA was quantified in a NanoDrop 2000c (Scientific Thermo, Waltham, MA, USA). Working DNA was standardized at 20–30 ng.μL^{-1} and stored at −20 °C.

The BOX element amplification used the oligonucleotide BOX-A1R (5′-CTACGGCAAGGCGACGCTGACG-3′). The amplification reaction with a final volume of 25 µL was as follows: 10% 10 X Buffer; 0.2 mM dNTPs; 2 mM $MgCl_2$; 0.3 U of Taq DNA platinum polymerase; and Template DNA (20–30 ng.μL^{-1}). The amplification conditions were initial denaturation at 95 °C for 9 min, 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55 °C) and extension (5 min at 72 °C) and one final extension cycle at 72 °C for 10 min [33].

The amplified fragments were separated by electrophoresis, containing 0.5 X TBE buffer at 100 V, for 360 min in 1.2% agarose gels, stained with SybrGold (Sigma-Aldrich, St. Louis, MO, USA) and photographed on a Loccus do Brasil LPIX-HE photocomputer.

Amplification was confirmed for 67 isolates, and a dendrogram was built using the Gelj v2 program using the Jaccard coefficient and UPGMA algorithm [34] at 70% similarity. Representatives of each BOX-PCR group were sequenced for the 16S rRNA gene, using primers 27F (5′-AGAGTTTGACCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [19]. The amplified products were evaluated on a 1% agarose gel and visualized under UV light. PCR products were sent to Macrogen (South Korea) for purification and sequencing and the sequences were compared to the NCBI database using the MEGABLAST algorithm. For sequences that did not show high similarity, the BLASTn algorithm was used. The same sequences were then analyzed comparatively with regard to the percentage of molecular identity, using the Clustal W multiple progression method [35] by the MEGA7.1 program [36].

Aligned sequences were used for phylogenetic analysis by the Neighbor-Joining method, using Kimura-2 parameters, applying a bootstrap with a minimum of 1000 replications, as described by Martins et al. [37]. Isolate numbers were evaluated by the chi-square test for a comparison between seasons, liming, plant part and medium used in the isolation.

2.4. In Vitro Growth Promotion Mechanism Evaluation

The isolates were tested for IAA production according to the method by Kuss et al. [38] modified by Barreto et al. [25], involving cultivation in TSB + 5 mM L-tryptophan, with Salkowski's reagent, storage for 30 min in the dark and readings at 520 nm using a spectrophotometer, with the readings compared to a standard curve.

Calcium phosphate solubilization was determined in NBRIP media after incubation for 15 days at 28 °C in the dark, with the solubilization index determined by the ratio between the solubilization and colony halos [39].

Siderophore production was evaluated by growth in TSB 1/10 for 72 h at 32 °C, followed by the change in color from blue to yellow in 15 min determined by the ratio of spectrophotometer readings at 630 nm for the sample and a blank [40].

Potential for biological nitrogen fixation was determined by the formation of growth pellicle in NFB semi-solid media and ascribed as positive or negative [26], while HCN production was also considered positive or negative by color change in filter paper impregnated with a 0.5% picric acid and 2% sodium carbonate solution in TSA medium [41].

2.5. Maize Growth Promotion Evaluation

Maize growth promotion was evaluated under nutrient solution conditions with Hoagland's nutrient solution [37] at ¼ strength and adjusted to 20% of N content in a greenhouse with cultivar AG 1051, recommended for corn-on-the cob and sillage production on a randomized block design with six replicates. The plants were grown in amber glass 330 mL bottles with two paper strips to support the plants and autoclaved.

The treatments included the 80 strains, an uninoculated control (NI) and a control inoculated with a commercial inoculant recommended for maize (AzzoFix™, with strains Ab-V5 and Ab-V6 of *Azospirillum brasilense*) (CI), with a third control with the same solution, but at full N content (N).

Maize seeds were superficially disinfected with 70% ethanol for 30 s and 2.5% sodium hypochlorite for 2 min, washed eight times in distilled, autoclaved water and germinated in sterile paper for four days at 25 °C, followed by transplant to the bottles and inoculation with 1 mL of bacterial broth with approximately 10^9 viable cells.ml^{-1}, as recommended by Brazilian legislation [15].

The plants were harvested 35 days after inoculation, and plant height (PH), leaf area (LA), culm diameter at root insertion (CD) and root length (RL) were measured. Plants were divided into root and shoot, dried at 65 °C for 72 h and shoot (SDM), root (RDM) and total (TDM) dry masses were determined.

Shoot dry masses were used to determine growth relative to uninoculated control (ERC), to full N rate (ERN) and to commercial inoculant (ERIC) as below:

$$\mathrm{ERC}(\%) = \left(\frac{\text{SDM treatment}}{\text{average SDM uninoculated control}}\right) \times 100$$

$$\mathrm{ERN}(\%) = \left(\frac{\text{SDM treatment}}{\text{average SDM 100\% N rate control}}\right) \times 100$$

$$\mathrm{ERIC}(\%) = \left(\frac{\text{SDM treatment}}{\text{average SDM commercial inoculant control}}\right) \times 100$$

2.6. Data Analysis

In vitro growth promotion mechanisms were grouped with the Jaccard index and UPGMA algorithm using PAST 2.17c [42]. Maize experiment data were preprocessed to eliminate outliers and transformed by log10, enough to guarantee ANOVA requisites, and evaluated by ANOVA at 10% significance due to relatively high variability. When appropriate, Dunnett's test was applied separately for each control treatment. Plant variables with more differentiation from the controls were also evaluated by Tukey's test at 10% significance to better evaluate individual strains. In vitro and in vivo data were also used for correlation and principal component analysis, considering only the strain values.

3. Results

3.1. Endophytic Bacteria in Digitaria eriantha

Endophytic populations were found in the culm, leaf and root of pangola grass as well as in the rhizospheric soil in all evaluated conditions in all isolation media. The population density was higher in the culm during the dry season. NFB had the lowest population

estimate while JMV had the highest one, 6.7 × 10^2 and 1.2 × 10^4 cells gram^{-1}, respectively (Figure 1).

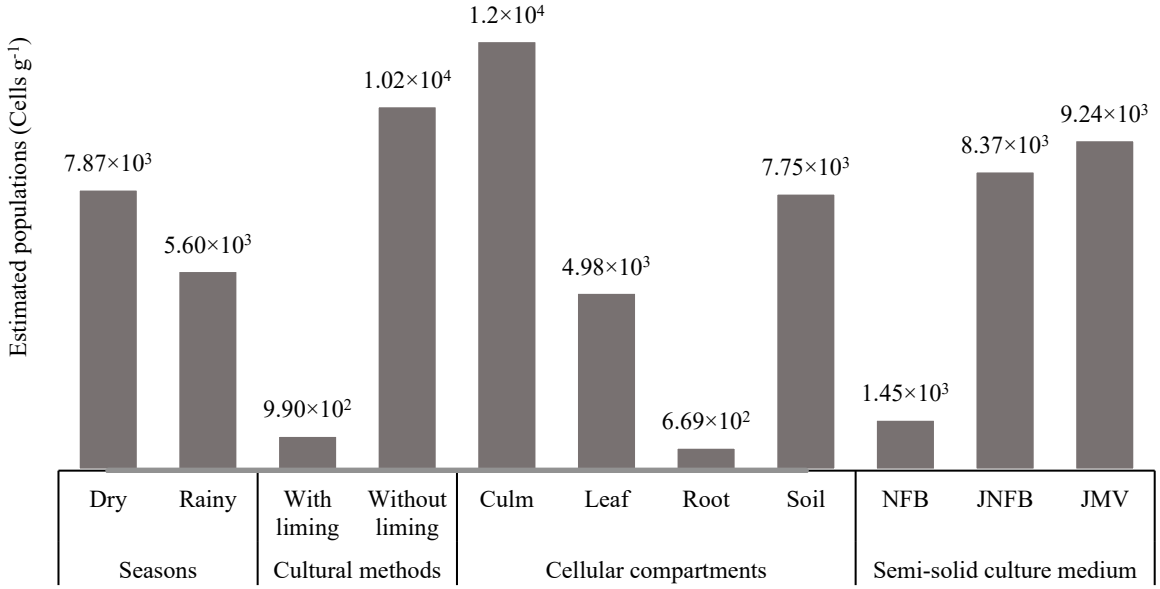

Figure 1. Endophytic bacterial populations isolated from *D. eriantha* at different sampling seasons (dry or rainy), with or without liming, from different compartments (endophytic from culm, leaf or root or rhizospheric) and using different culture media (NFB, JNFB or JMV).

Altogether, 316 isolates were obtained with higher proportions in the culm, in the rainy season and in the limed plot, using the JNFB based on the comparisons for each of these factors separately, although the difference between the dry and rainy seasons was not significant (Table 3).

Table 3. Number and percentage of endophytic or rhizospheric bacterial isolates from *D. eriantha* at different sampling seasons (dry or rainy), with or without liming, from different compartments (endophytic from culm, leaf or root or rhizospheric) and using different culture media (NFB, JNFB or JMV), compared through the χ^2 test.

Condition	Number of Isolates	%	Pr > qui^2
Dry	131	41.59	0.12057
Rainy	184	58.41	
With lime	102	55.74	0.00282
Without lime	81	44.26	
Culm	162	51.43	6.6 × 10^{-36}
Leaf	62	19.68	
Root	79	25.08	
Rhizospheric soil	12	3.81	
JMV	26	8.25	2.2 × 10^{-26}
JNFB	168	33.33	
NFB	121	38.41	

The 316 isolates formed 73 phenotypical groups at 100% similarity, with up to 55 isolates per group, which showed high phenotypic diversity of endophytic bacteria of *D. eriantha*. The Shannon diversity index (H) was 3.58 (Table 4), while the dominance index (D) was close to zero. The Simpson index (1-D) presented values close to 1, indicat-

ing high uniformity and richness, according to the equitability index (J) and richness (Margalef), respectively.

Table 4. Phenotypic diversity indexes of endophytic or rhizospheric bacterial isolates from *D. eriantha* at different sampling seasons (dry or rainy), with or without liming, from different compartments (endophytic from culm, leaf or root or rhizospheric) and using different culture media (NFB, JNFB or JMV).

Condition	Groups	Isolate	Dominance	Simpson	Shannon	Margalef	Equitability	Chao
Total	73	316	0.054	0.945	3.58	12.51	0.834	104.1
Dry	53	131	0.038	0.961	3.62	10.67	0.912	82.55
Rainy	49	185	0.086	0.913	3.12	9.19	0.801	68.46
Culm	52	163	0.063	0.936	3.37	10.01	0.855	73.23
Leaf	26	62	0.081	0.918	2.86	6.05	0.880	43.5
Root	34	79	0.061	0.938	3.13	7.55	0.889	55.11
Rhizospheric soil	10	12	0.125	0.875	2.21	3.62	0.959	46
With lime	35	104	0.097	0.902	2.96	7.32	0.833	48.6
Without lime	30	81	0.087	0.912	2.87	6.59	0.844	125
JMV	18	26	0.082	0.917	2.71	5.21	0.939	48.33
JNFB	53	168	0.047	0.952	3.49	10.15	0.879	78.09
NFB	44	122	0.072	0.927	3.23	8.95	0.855	65

3.2. Genotypic Diversity

Of the 80 representative isolates, 67 amplified with the BOX-A1 oligonucleotide at 70% similarity, with very diverse bands and cluster profiles (Figure 2) regardless of the isolation site or method.

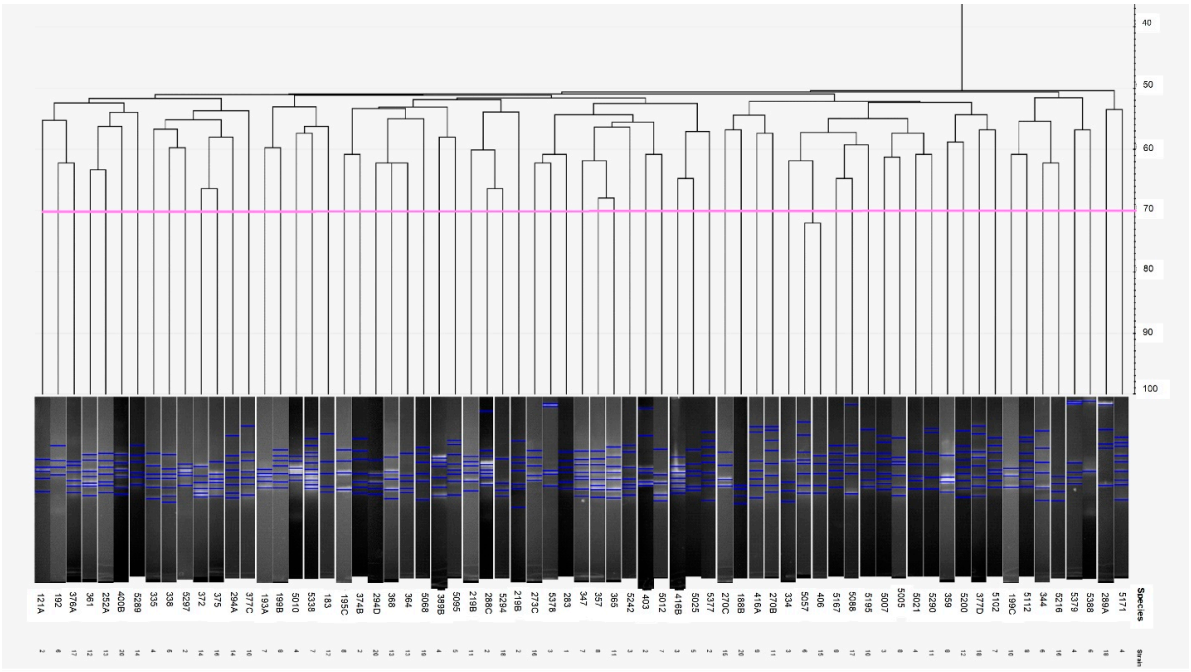

Figure 2. BOX-PCR dendrogram for endophytic or rhizospheric bacterial isolates from *D. eriantha*. The horizontal line indicates the 70% similarity line for the groupings.

Approximately 79% of the isolates had an identity greater than 96% using the MEGABLAST algorithm (Supplementary Table S1), while fourteen isolates had an identity less than 96%, and thirteen isolates were not found to be highly similar to any entry in the GenBank. For the isolates that did present a low percentage of identity, the BLASTn algorithm was used, and the isolates showed an identity ca. 67–95% to *Enterobacter*, *Pseudomonas*, *Rhizobium*, *Shinella*, *Shingomonas*, *Stenotrophomonas*, *Bacillus*, *Staphylococcus* and two isolates of bacteria with no resemblance to those cataloged on GenBank.

Four phyla, five classes, ten orders, one suborder, fifteen families and twenty different genera of bacteria were identified (Table 5). The γ-proteobacteria class had representatives of the *Enterobacter*, *Erwinia*, *Pseudomonas*, *Pantoea* and *Stenotrophomonas* genera. As for α-proteobacteria, the genera *Massilia*, *Beijerinckia*, *Rhizobium* and *Shinella* were identified (Table 5).

Table 5. Taxonomic classification of endophytic and rhizospheric bacteria from *D. eriantha* under a tropical semiarid condition.

Phylum	Class	Order	Family	Genera	Total	%
Actinobacteria	Actinomycetales	Actinomycetales	Microbacteriaceae	*Curtobacterium*	1	1.49
			Micrococcaceae	*Kocuria*	1	1.49
			Nocardioidaceae	*Nocardioides*	2	2.99
		Micrococcales	Microbacteriaceae	*Microbacterium*	2	2.99
Proteobacteria	α-Proteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	1	1.49
		Rhizobiales	Rhizobiaceae	*Rhizobium*	8	11.94
				Shinella	4	5.97
				Agrobacterium	6	8.95
	γ-Proteobacteria	Enterobacteriales	Enterobacteriaceae	*Enterobacter*	6	8.95
				Erwinia	1	1.49
				Pantoea	8	11.94
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	6	8.95
		Lysobacterales	Lysobacteraceae	*Stenotrophomonas*	4	5.97
	β-Proteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes*	1	1.49
			Burkholderiaceae	*Burkholderia*	1	1.49

The culm, root and leaf had more genera than the rhizospheric soil (Figure 3). The most abundant genera were *Pantoea* and *Rhizobium* with eight isolates each, followed by *Agrobacterium*, *Enterobacter* and *Pseudomonas* with six representatives and *Stenotrophomonas* and *Shinella* with four, while the environmental conditions with most genera were dry season, no liming and when NFB was used for isolation.

3.3. In Vitro Plant-Growth-Promoting Mechanisms

All isolates produced IAA, most (53%) more than 50 $g \cdot L^{-1}$ (Supplementary Table S2), and siderophores, while 70, 34 and 14% were positive for BNF, phosphate solubilization and HCN production, respectively (Figure 4). Seven groups were formed based on in vitro plant-growth-promoting characteristics (Figure 5). Isolates 376A (*Stenotrophomonas*) and 361B (*Agrobacterium*) formed a group with high IAA production and average-to-high phosphate solubilization and were positive for BNF, HCN and siderophore production, while the largest group had strains negative for phosphate solubilization and HCN production.

The same genus could be found in different in vitro plant-growth-promoting characteristic phenotypical groups, while a single group had different genera.

3.4. Maize Growth Promotion

While some strains were significantly different from either NI or CI (Supplementary Table S3 synthesized in Figure 6), the same did not occur in comparison to N. While 22 strains were significantly different from NI for at least one of the characteristics, isolates 195C (IAA and siderophore producer) and 5242 (positive for BNF, and an IAA and siderophore producer) were significantly higher for plant height, root length, leaf area and total dry mass.

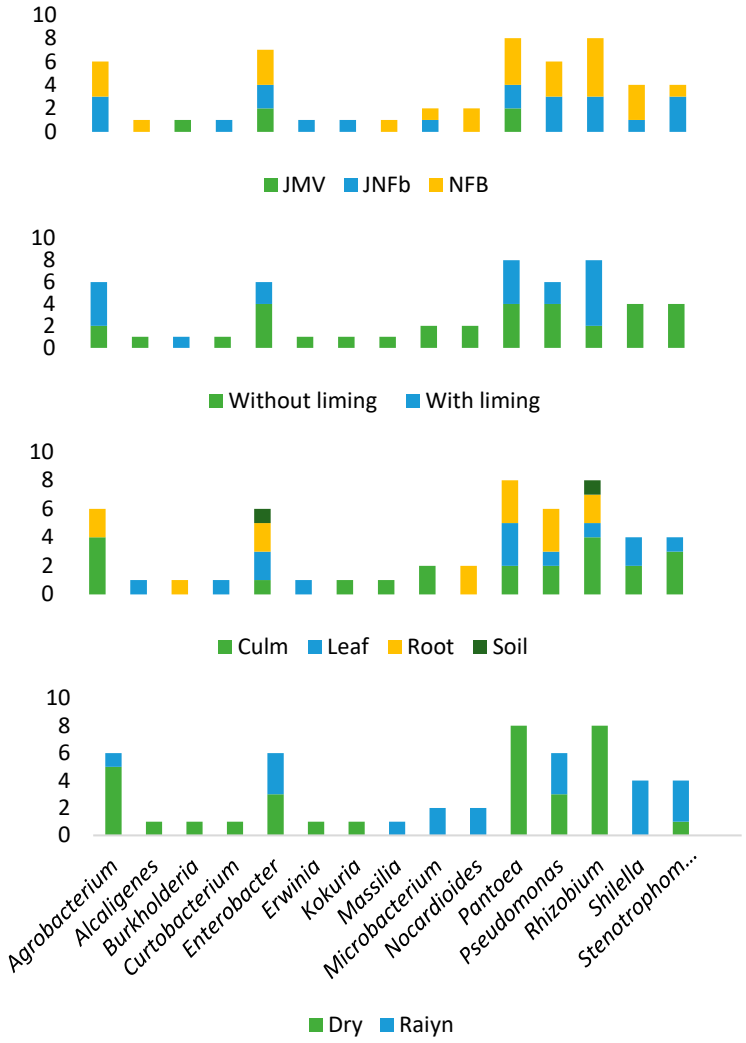

Figure 3. Genera of endophytic or rhizospheric bacterial isolates from *D. eriantha* at different sampling seasons (dry or rainy), with or without liming, from different compartments (endophytic from culm, leaf or root or rhizospheric) and using different culture media (NFB, JNFB or JMV).

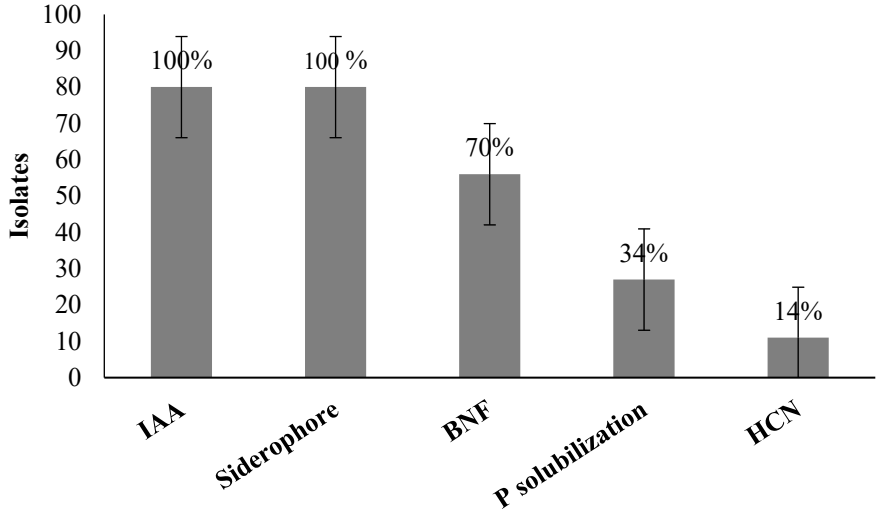

Figure 4. Proportion of endophytic or rhizospheric bacterial isolates from *D. eriantha* presenting different growth promotion characteristics (IAA, siderophore and HCN production, potential biological nitrogen fixation and P solubilization) under in vitro evaluation.

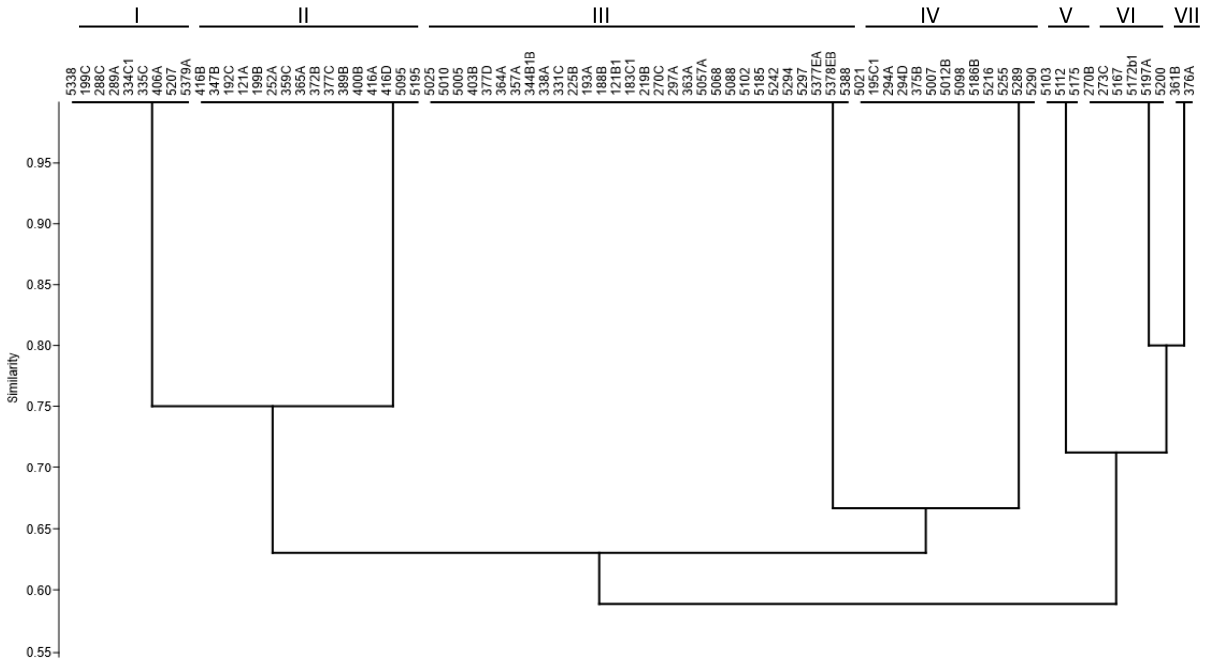

Figure 5. Dendrogram of endophytic and rhizospheric bacterial isolates from *D. eriantha* grouping based simultaneously on IAA, siderophores and HCN production, BNF and phosphate solubilization.

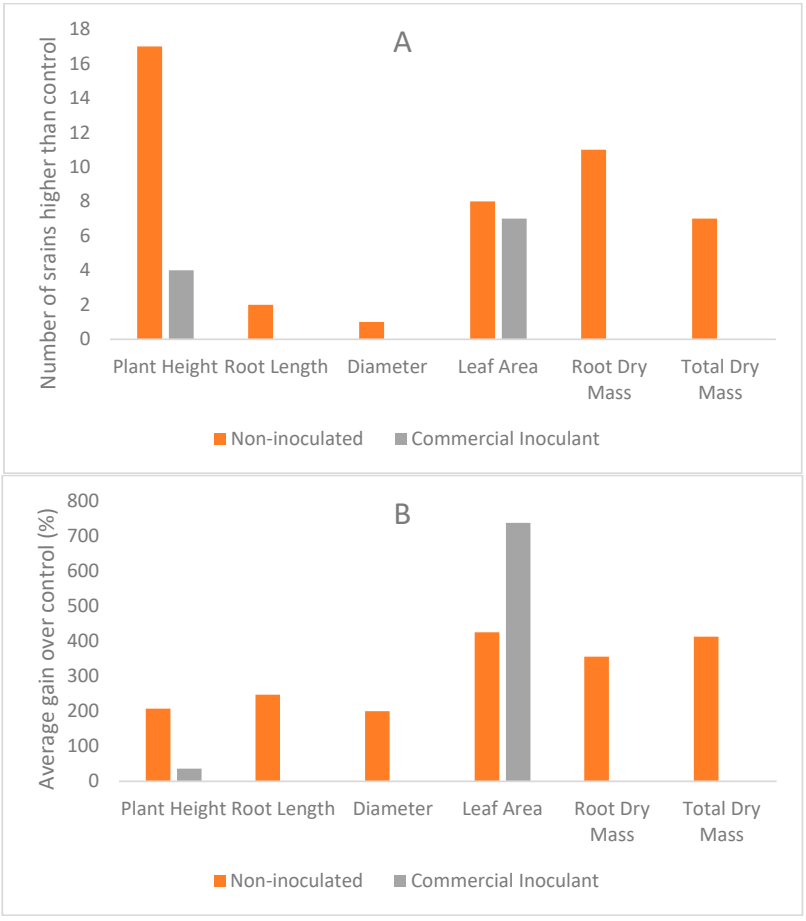

Figure 6. Number (**A**) of rhizospheric and endophytic strains from *D. eriantha* with significantly higher plant height, root length, culm diameter, leaf area root and total dry mass than plants non-inoculated or inoculated with a commercial inoculant and average percentage of gain (**B**).

The strains were grouped according to the results of the Tukey test into three groups (Supplementary Table S4 synthesized in Figure 7), and the group with the higher averages (strains classed as A in Supplementary Table S4) had taller plants with larger leaf areas than the commercial inoculant or N.

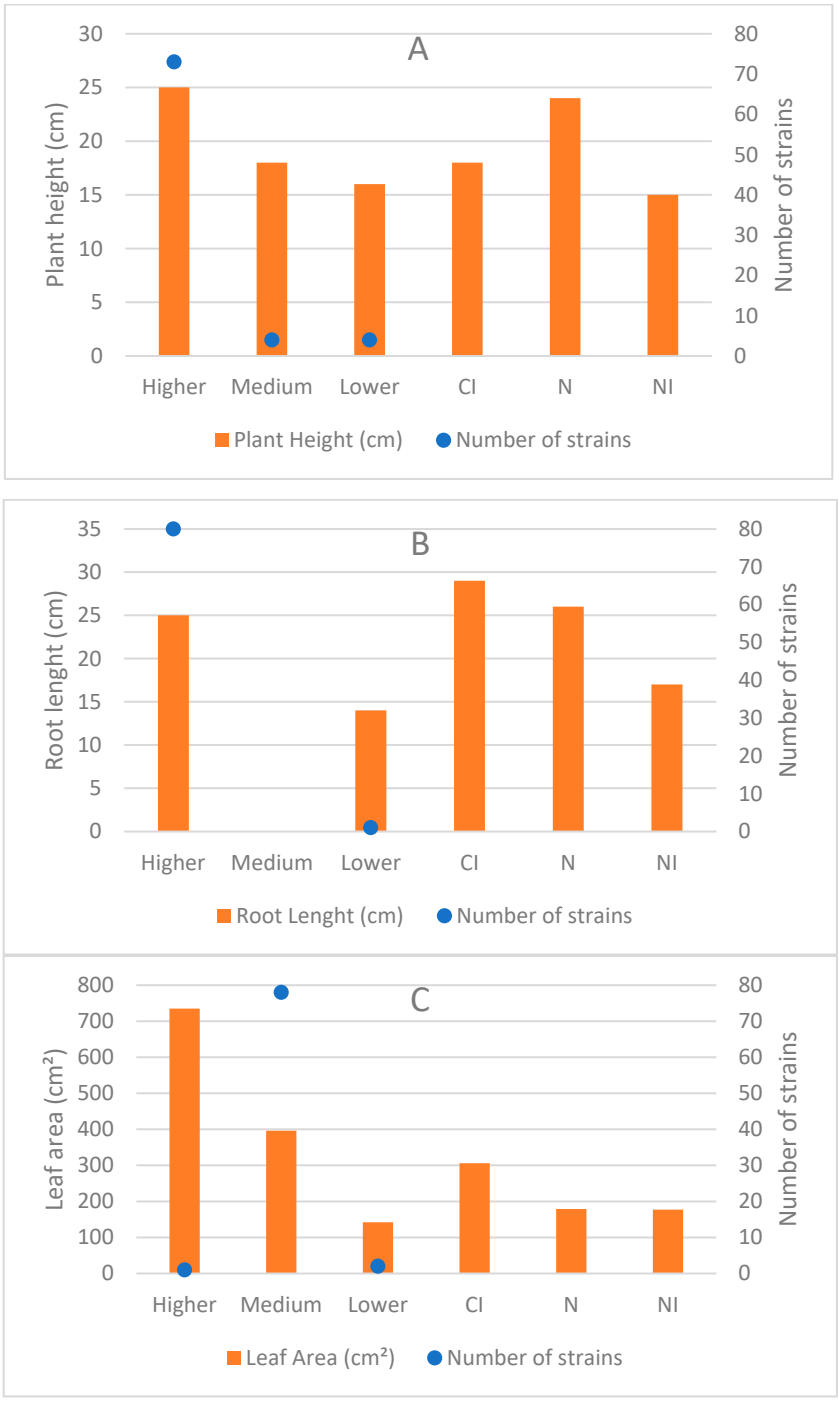

Figure 7. Average and strain number of different Tukey test groupings of rhizospheric and endophytic *D. eriantha* bacterial strains and control treatments (CI—commercial inoculant, N—full N rate, NI—non-inoculated). (**A**) Plant height, (**B**) root length, (**C**) leaf area.

Although phosphate solubilization had a significant correlation with plant height, culm diameter and root and total dry masses (Table 6), the correlation coefficients were too low (−0.19 to −0.28) to allow an important explanatory power, while the other correlations

were even lower and non-significant. A similar issue was found for the explanatory power through PCA, since five principal components (from a total of eleven) explained 70% of the total variation (Figure 8).

Table 6. Pearson correlations and their probability between in vivo effects on corn plant height (PH), root length (RL), neck diameter (D), leaf area (LA), shoot (SDM), root (RDM) and total (TDM) dry masses and in vitro plant-growth-promoting characteristics including IAA and siderophore production (IAA and Sid), phosphate solubilization (PS), presence or absence of growth in N-free media (BNF) and HCN production (HCN). Correlations in bold are significant at the 10% probability level.

		PH	RL	D	LA	SDM	RDM	TDM
IAA	R	0.03	−0.10	−0.07	≈0.00	0.10	−0.11	−0.14
	Prob	0.7820	0.3859	0.5207	0.9972	0.3837	0.3458	0.2257
Sid	R	0.04	0.11	−0.04	−0.12	−0.07	−0.05	−0.05
	Prob	0.7562	0.3153	0.7230	0.2991	0.5212	0.6831	0.6772
PS	R	**−0.21**	−0.17	**−0.20**	−0.15	−0.18	**−0.28**	**−0.19**
	Prob	**0.0575**	0.1433	**0.0706**	0.1965	0.1125	**0.0125**	**0.0898**
BNF	R	−0.12	0.14	0.07	−0.13	0.06	−0.02	−0.04
	Prob	0.2836	0.2046	0.5224	0.2580	0.5662	0.8574	0.7234
HCN	R	−0.15	0.06	−0.08	0.06	0.09	−0.08	−0.05
	Prob	0.1751	0.6009	0.4821	0.6019	0.4433	0.4742	0.6719

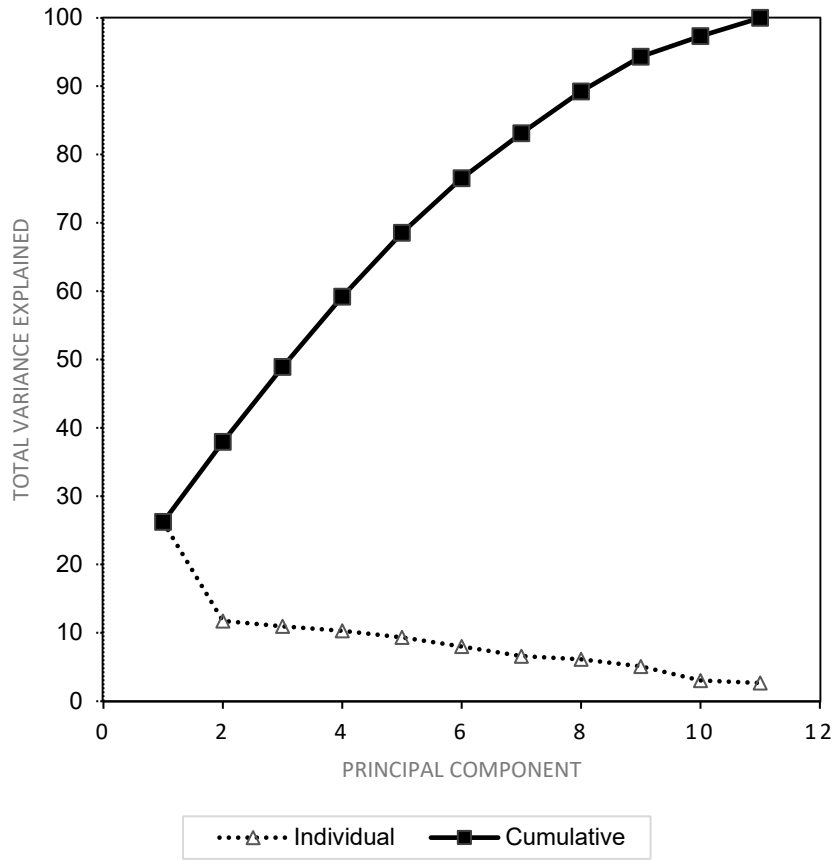

Figure 8. Principal component analysis for both in vitro and in vivo growth promotion characteristics.

4. Discussion

Digitaria eriantha harbors high endophytic bacteria diversity, several genera of which contain species with different plant growth promotion traits. Crop-associated microbiota directly influences plant productivity and health and is fundamental in suppressing pathogens, acquiring nutrients and tolerating environmental stress [43].

Pangola grass has a high population density of endophytic bacteria, especially in the culm, as also reported in wheat (*Triticum* spp.) [44] and in andropogon (*Andropogon gayanus* Kunth) and Buffel (*Cenchrus ciliari* L.) grasses in tropical semiarid non-degraded soils [45]. Although a higher proportion of endophytes in the culm than in the root is not expected or commonly reported, this might be due to reduced stress on the culm compared to the root [46]. At the same time, the relatively undegraded condition of the pasture [23] might allow relatively higher soil organic matter contents than degraded pastures, decreasing the competition for nutrients among microbial species [47]. While some studies have reported a larger endophytic bacteria population in grasses in the rainy season [48–50], likely due to a break in the dormancy of microorganisms previously inactive or non-functional [51], and our population estimates were higher for the dry season, this higher estimate did not result in more isolates, although the isolation effort was the same in both cases, and might be an artifact from the population estimative method. Most bacterial isolates were equally abundant, particularly in the rainy season, which might be either directly linked to water availability or indirectly through increased exudate production from more intensive plant growth [52].

Even considering the 70% similarity level, there was high genotypic diversity, as also observed in endophytic bacteria from maize [53] and sugarcane [54]. The isolates that amplified for the 16S rRNA showed similarity with *Curtobacterium*, *Alcaligenes*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kocuria*, *Massilia*, *Microbacterium*, *Nocardioides*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Shinella*, *Agrobacterium* and *Stenotrptophomonas*.

Some of this genera, such as *Enterobacter*, include strains that promote plant growth and mitigate the effect of drought on maize and wheat with phytohormone and siderophore production [14], while some *Pseudomonas*, *Microbaterium* and *Burkholderia* include excellent phosphate solubilizers, producers of siderophores and indolacetic acid [55–58]. On the other hand, the inoculation of *Pantoea* sp. in maize seedlings can increase leaf area, culm length and shoot dry biomass under water stress, even when isolated from cactus [21], perhaps due to high exopolysaccharide production [59].

The high IAA production levels found were similar to those from wheat endophytes [48], although higher than the most common range of up to 130 $mg{\cdot}L^{-1}$ [49–51]. At the same time, while we could not find the relation between HCN production and phosphate solubilization previously found [60], one *Stenotrophomonas* strain was positive for both, while other research also found strains from this genus producing IAA and solubilizing phosphate [9,48]. Some *Rhizobium* and *Enterobacter* had high IAA production, up to medium phosphate solubilization and were BNF- and HCN-positive, as frequently found for strains from these genera [48,61–68].

A point to consider, though, is the lack of apparent linkage between in vitro growth promotion characteristics and taxonomical characteristics, since while a single genus could have representative strains in different phenotypical groups, the same group would have strains from different genera, which invalidates the use of taxonomical identification as a pre-selector for possible growth promotion.

While most research aiming to find possible plant growth promoters pre-selects strains based on one or a combination of in vitro putative plant growth mechanisms [4,6,7,9,10,13,18], we still found possibly effective strains which did not present these mechanisms under the standard laboratory methods. The low correlation between these measures and plant effects questions this practice. For example, while the literature frequently indicates IAA production as one of the major plant-growth-stimulating mechanisms [69–71], our results indicate only a non-significant and very low correlation between IAA production in vitro and any of the plant variables (Table 6, p values between 0.23 and 1.00, and r values between −0.14 and 0.10), even though the highest IAA producer was also among those with better maize response. At the same time, other researchers also found this lack of linkage between IAA and plant response [21,72], perhaps due to plant response depending on its endogenous IAA levels [73]. While the literature also indicates that siderophore production and phosphate solubilization in vitro are usually linked to increased plant

growth [9,62,69,74–78], we did not find any indication of these being connected, with very low non-significant correlations between in vitro siderophore production and plant growth variables (p values ranging from 030 to 0.76, with r values ranging from −0.12 to 0.11; Table 6).

Considering multiple in vitro characteristics simultaneously, we still could not find a clear link between the in vitro and plant results, since the strains with the best plant results were not among those with the highest in vitro results, although the strains with better plant responses included more than one in vitro mechanism, indicating this might be a valid pre-selector [50,79].

Although our choice of a hydroponic system, which provides higher nutrient and water availability, might be partially responsible for the reduced correlations, we still observed gains from several strains, even when compared to a commercially produced inoculant, so this is not likely to be the only reason for the lack of correlation.

5. Conclusions

Pangola grass showed a great endophytic bacteria diversity, coexisting in different plant tissue compartments, with the greatest diversity in the culm. Several of the genera found are frequently considered to be plant growth promoters, which validates the use of stress-resistant plants as endophytic bacteria sources for plant evaluation.

While both in vitro and in vivo growth promotion was widespread, we could not find a link between them on a random sample of 80 strains, which indicates the in vitro evaluation is not a strong indicator of plant growth promotion.

Further research on developing accurate predictors of plant growth promotion under laboratory conditions is likely to be a promising area for future research, since these could reduce the number of strains submitted to the labor- and resource-intensive selection of plants, while at the same time reducing the chance of not evaluating the most suitable strains.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14122769/s1, Supplementary Table S1—Identity of endophytic bacteria from Digitaria eriantha based on 16S rRNA identity; Supplementary Table S2—Pangola grass endophytic bacteria and their in vitro plant growing characteristics; Supplementary Table S3—Effects of inoculation with endophytic bacteria on corn plant height (PH), root length (RL), culm diameter (D), leaf area (LA), shoot (SDM), root (RDM) and total (TDM) dry masses when compared with uninoculated (NI), inoculated with commercial inoculant (CI) and full N level (N) controls. * indicates the isolate result was different and higher than the respective control according to Dunnett's test at 10% significance; Supplementary Table S4—Effects of inoculation with endophytic bacteria on corn plant height (PH), root length (RL) and leaf area (LA).

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