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Bioprospecting of elite plant growth-promoting bacteria for the maize crop

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ABSTRACT. The use of plant growth-promoting bacteria (PGPB), which aims to replace chemical fertilizers and biological control, is a goal for achieving agriculture sustainability. In this scenario, our goal was to identify and evaluate the potential of bacteria isolated from maize roots to promote plant growth and be used as inoculants. We evaluated 173 bacterial strains isolated from the maize (*Zea mays* L.) rhizosphere for the properties of their PGPB *in vitro*. Twelve strains were positive for siderophores, indole acetic acid (IAA) production, biological nitrogen fixation (BNF), and phosphate solubilization. Sequence analysis of 16S rRNA identified these strains as belonging to the genera *Cellulosimicrobium*, *Stenotrophomonas*, *Enterobacter*, and *Bacillus*. The elite strains were evaluated under greenhouse conditions upon the inoculation of two maize hybrids, ATL100 and KWX628. The ability of the isolates to promote plant growth was dependent on the maize genotype; *Enterobacter* sp. LGMB208 showed the best ability to promote growth of hybrid ATL100, while *Enterobacter* sp. strains LGMB125, LGMB225, and LGMB274 and *Cellulosimicrobium* sp. strain LGMB239 showed the best ability to promote growth of hybrid KWX628. The results highlight the potential of bacterial genera little explored as maize PGPB but indicate the need to investigate their interactions with different plant genotypes.

Keywords: PGPB; maize hybrids; *Cellulosimicrobium*; *Enterobacter*; *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, with great economic importance in several countries (Yazdani, Bahamanyar, Pirdashti, & Esmaili, 2009). However, the species requires a high nutritional level to achieve economical yields, with nitrogen (N) and phosphorous (P) being the most limiting nutrients. The release of high-yield genotypes has increased the need for chemical fertilizers (Souza, Ambosini, & Passaglia, 2015). It is estimated that for an average yield of 7 - 9 t ha^{-1}, applications of 110 - 140 kg ha^{-1} N and 20 - 50 kg ha^{-1} P_2O_5 are required (Montañez & Sicardi, 2013), although the efficiency of fertilizer utilization by the plant rarely surpasses 50% efficiency (Halvorson, Peterson, & Reule, 2002). The poor efficiency of N use contributes to nitrate contamination of soil and groundwater, demanding alternatives to ensure competitive crop yields that are ecologically balanced (Majeed, Abbasi, Hameed, Imran, & Rahim, 2015). The use of microbial inoculants carrying plant growth-promoting bacteria (PGPB) is increasing (Hungria, Campo, Souza, & Pedrosa, 2010) but is still in the early stages of development (Souza et al., 2015).

PGPB can enhance plant growth and may offer protection against disease and abiotic stresses by different mechanisms (Kundan, Pant, Jadon, & Agrawal, 2015; Fukami, Ollero, Megías, & Hungria, 2017). These bacteria can act during plant growth by several mechanisms such as biological nitrogen fixation (BNF), phosphate solubilization and the production of siderophores and phytohormones. BNF activity is carried out by nitrogenase enzyme, the multiple subunits of which work in a concatenated fashion to provide nitrogen to the plant (Döbereiner, Day, & Dart, 1972). Some bacteria have a system to produce siderophores that complex with iron and provide iron to the plant, reducing free ions and offering protection against phytopathogens that colonize plants (Hungria et al., 2010). These authors also describe that a part of the P in Brazilian soil is in its organic form and is available through the activity of microorganisms. Lastly, bacteria can promote plant growth due to the production of phytohormones such as

auxins such as indole acetic acid (IAA). According to Chaiharn and Lumyong (2011), the IAA produced by rhizobacteria can stimulate root elongation, cell division and differentiation, and bacteria that are able to participate in these mechanisms can be beneficial for processes promoting plant growth.

The efficiency of PGPB is normally dependent on the plant genotype and the bacterial strain used (Yazdani et al., 2009); therefore, to improve the effectiveness of PGPB, it is necessary to select strains adapted to different culture conditions and plant genotypes (Majeed et al., 2015). In groups of grasses, some genera of PGPB such as *Serratia*, *Rhanella* and *Herbaspirillum* can be associated with improved plant development, but the main genera associated with maize yield are *Azospirillum, Pseudomonas* and *Azotobacter* (Szilagyi-Zecchin, Ikeda, & Mógor, 2017).

Our study aimed to identify and evaluate a collection of bacteria isolated from maize (Ikeda et al., 2013) in order to select strains to be used as inoculants. The bacteria were identified by phylogenetic analysis of the 16S rRNA sequence, and screening for plant growth-promoting ability was based on analyses of siderophore and IAA production, phosphate solubilization, and biological nitrogen fixation. The isolates that showed positive results for all variables analysed were selected for evaluation of plant growth-promotion under greenhouse conditions.

Material and methods

A collection of 173 bacterial strains previously isolated from seven different maize genotypes (Ikeda et al., 2013) were used for *in vitro* evaluation of plant growth-promoting properties. All strains are deposited at the Culture Collection of the Laboratory of Genetics of Microorganism (www.labgem.ufpr.br) at the Federal University of Paraná, Curitiba, Paraná State, Brazil.

The ability to promote plant growth

Strains were first evaluated for phosphate solubilization and siderophore production abilities *in vitro*. Strains that were positive for both properties were selected for the evaluation of IAA production and BNF *in vitro*. Isolates positive for all analyses were then selected for the greenhouse experiment. Siderophore production was evaluated in semi-solid culture medium, as described by Schwyn and Neilands (1987), verifying the halo around the colonies. Phosphate solubilization was evaluated as described by Chagas Jr., Oliveira, Oliveira, and Willerding (2010) using glucose and yeast extract (GL) medium, verifying the translucent halo around the colonies. IAA production was evaluated according to Kuss, Kuss, Lovato, and Flores (2007) using DYGS medium (Rodrigues Neto, Malavolta Jr., & Victor, 1986). The results were obtained by spectrophotometric analysis at 530 nm and converted into units of $\mu g\ mL^{-1}$. BNF was evaluated according to Araújo et al. (2004) using JNFb semi-solid medium. The Kruskal-Wallis test ($p < 0.05$) was performed by the Assistat 7.7 program (Silva & Azevedo, 2016).

Phylogenetic analysis

Genomic DNA was extracted according to Sambrook, Fritsch, and Maniatis (1989). PCR to amplify the *16S rRNA* gene was performed using primers fD1 and rD1 following the conditions described by Menna et al. (2006). The PCR product was purified using the enzymes Exo1 and FastAP (ThermoScientific kit). DNA sequencing was performed using primers fD1, 362f, and 786f (Menna et al., 2006). Clean up of the sequencing product was performed using Sephadex™ G-50 medium® (GE Healthcare, Little Chalfont, UK) in a MultiScreen Column Loader® (Merck Millipore, Billerica, US). Amplicons were analysed in an ABI3500 Automatic Sequencer® (Applied Biosystems, Foster City, US). The sequences were inspected using the BioEdit program, version 7.2.5 (Hall, 1999) and aligned using ClustalW in MEGA software, version 6 (Tamura et al., 2013). Sequences were compared to sequences of reference and type strains retrieved from GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Maximum likelihood phylogenetic analysis was performed with Geneious software (Kearse et al., 2012). The GRT evolutionary model was determined using MEGA 6.0 (Tamura et al., 2013). The FigTree program, version 1.4.0 (Rambaut, 2012), was used to edit phylogenetic trees. Sequences obtained in this study were deposited in the GenBank database, and accession numbers were obtained.

Greenhouse evaluation

Twelve strains were selected for the evaluation of plant performance under greenhouse conditions. Bacteria were grown in LB medium (Sigma), adjusted to a concentration of 10^8 cells mL^{-1} and used at a volume of 0.5 mL to inoculate seeds of maize hybrids ATL100 and KWX628 provided by Semília Genética e Melhoramento Ltda. (Curitiba, Paraná State, Brazil). Seeds were superficially disinfected by immersion in

70% alcohol for 1 minute and 3% NaClO for 3 minutes, followed by three distilled water washes for 1 minute each. Plants were grown in Leonard jars and received sterile nutrient solutions (Broughton & Dilworth, 1971). Two experiments were conducted, with one for each maize hybrid, and each experiment comprised 15 treatments with four replicates: inoculation with 12 bacterial isolates, inoculation with the commercial strain *Azospirillum brasilense* Ab-V5 at 10^8 cells mL^{-1}, one negative control without N supplementation and one positive control with N supplementation. For the first solution, all jars contained 577.71 mg L^{-1} KNO_3 as a N supplement, and then the jars were refilled weekly with a sterile nutrient solution without N supplementation (except for the positive control). Plants were grown for 35 days at 28 ± 2ºC, and then biometric analysis was performed to measure the following: maize root length (cm), plant size (cm), root dry weight (g), number of leaves (per plant), leaf area (cm^2), leaf dry weight (g), N and P content (mg g^{-1}), stem diameter (mm) and plant height (cm). ANOVA and the Tukey test ($p < 0.05$) were performed by the Assistat 7.7 program (Silva & Azevedo, 2016).

Results

The ability to promote plant growth

Of the 173 bacterial strains evaluated, 70.5% (n = 122) and 56.5% (n = 98) were positive for siderophore production and phosphate solubilization, respectively, and 93 strains (53.7%) were positive for both traits and thus selected to verify BNF capacity and IAA production. In addition, 63.4% (n = 59) strains were positive for BNF, and 12.9% (n = 12) strains synthesized IAA. The 12 strains that showed positive results for all traits evaluated (LGMB125, LGMB149, LGMB202, LGMB208, LGMB225, LGMB228, LGMB229, LGMB239, LGMB274, LGMB319, LGMB322, and LGMB326) were submitted for other analyses.

Phylogenetic analysis

In the phylogenetic analysis of the *16S rRNA* gene of the 12 selected strains, LGMB229 showed higher similarity to *Cellulosimicrobium aquatile* and was also closely related to other *Cellulosimicrobium* species *C. funkei*, *C. Cellulans*, and *C. marinum,* which are in a different clade than other genera of the *Promicromonosporaceae* family (Figure 1). Strain LGMB149 shared high similarity with the type strain of *Stenotrophomonas maltophilia* (Figure 2).

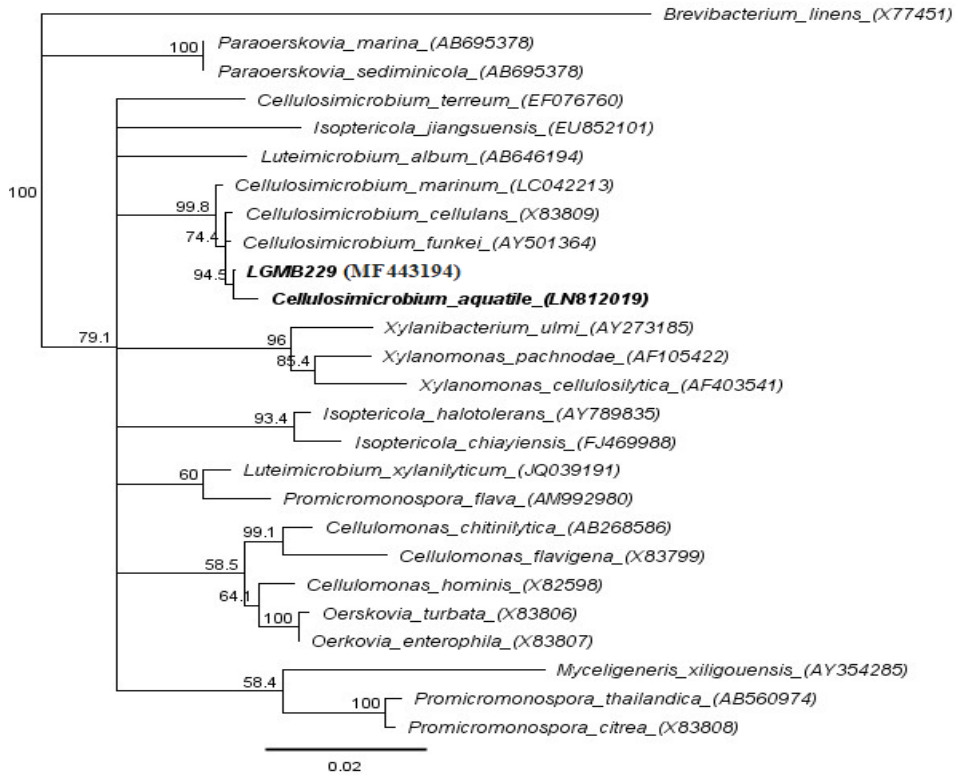

Figure 1. Maximum likelihood tree based on the *16S rRNA* gene (1057 bp) of LGMB229. The species *Brevibacterium linens* was used as an outgroup. Values on the node indicate bootstrap support. The bar indicates 2 substitutions per 1,000 nucleotides.

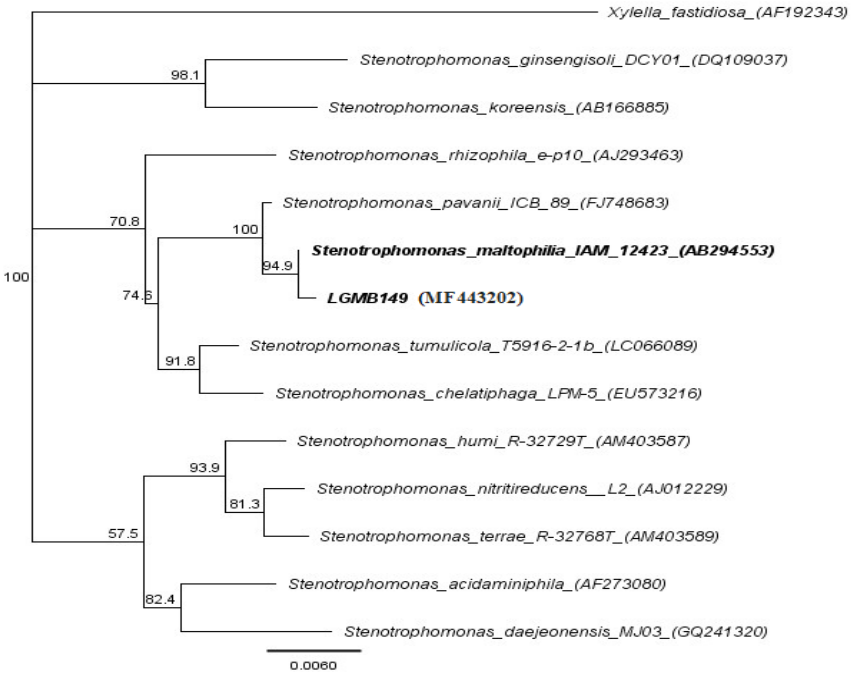

Figure 2. Maximum likelihood tree based on the *16S rRNA* gene (1378 bp) of LGMB149 and *Stenotrophomonas* species. The species *Xylella fastidiosa* was used as an outgroup. Values on the node indicate bootstrap support. The bar indicates 6 substitutions per 1,000 nucleotides.

Strains LGMB322, LGMB274, LGMB125, LGMB208, LGMB228, LGMB225, and LGMB239 were classified into the *Enterobacter* genus. Strains LGMB322 and LGMB225 were positioned on the same phylogenetic branch as *E. ludwigii*, while the remaining strains showed high similarity among themselves but were not clustered with any described species of *Enterobacter*; therefore, these strains might represent a new species (Figure 3). The remaining strains, LGMB202, LGMB319, and LGMB326, were classified as *Bacillus* sp. and were clustered with type strains of *B. siamensis, B. Vallismortis*, and *B. methylotrophicus* species (Figure 4).

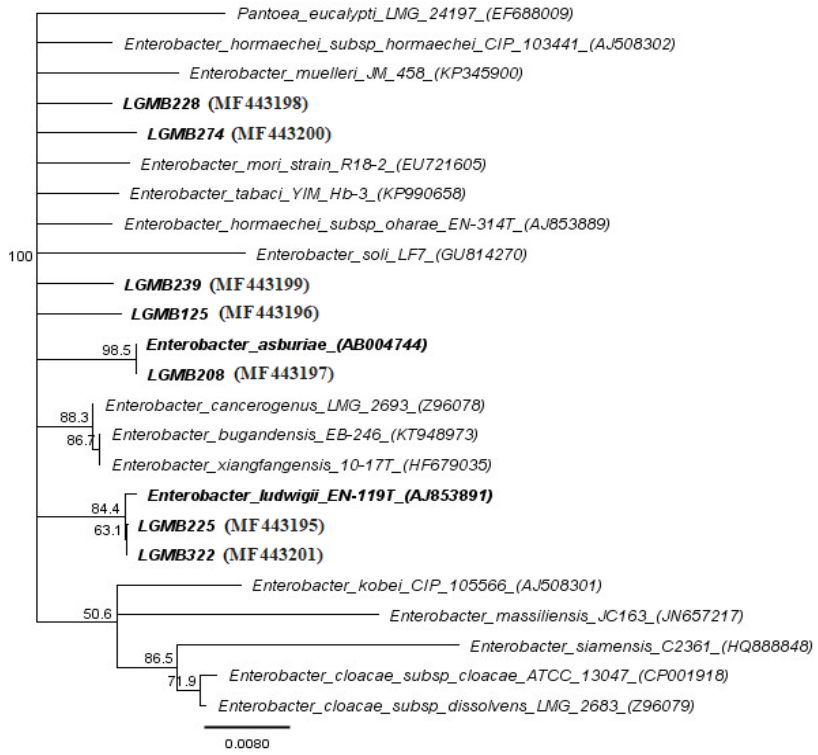

Figure 3. Maximum likelihood tree based on the *16S rRNA* gene (1087 bp) of LGMB125, LGMB225, LGMB228, LGMB239, LMGB274, and LGMB322 and *Enterobacter* species. *Pantoea eucalypti* was used as an outgroup. Values on the node indicate bootstrap support. The bar indicates 3 substitutions per 1,000 nucleotides.

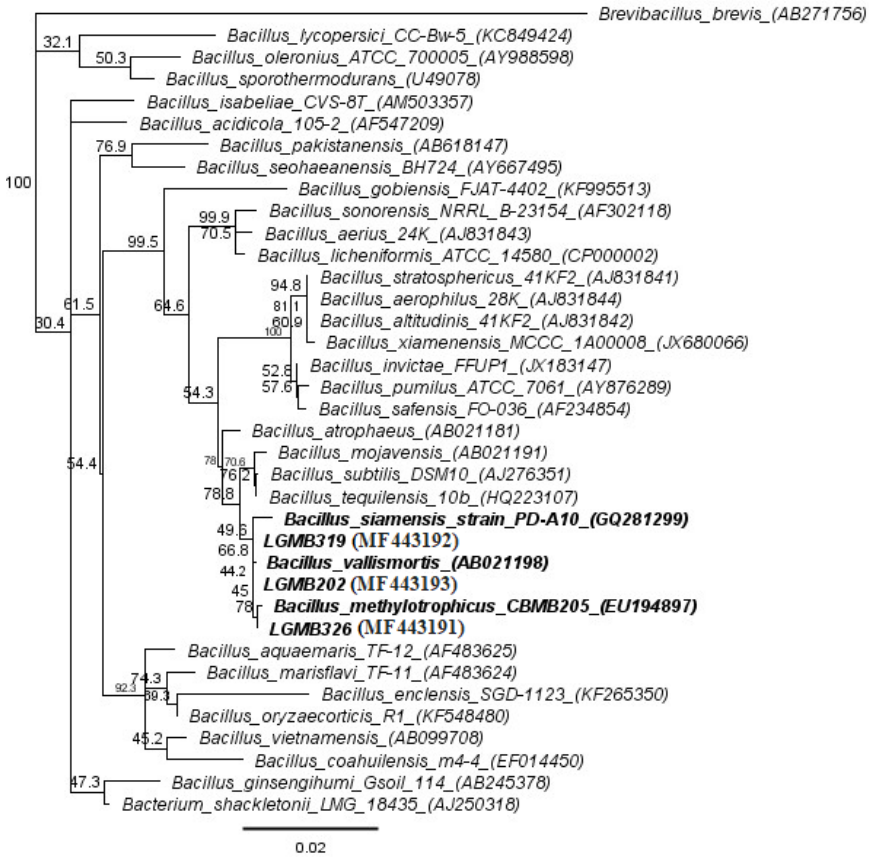

Figure 4. Maximum likelihood tree based on the 16S rRNA gene (1107 bp) of LGMB202, LGMB319, and LGMB326 and *Bacillus* type strains belonging to Clade 1. The species *Brevibacillus brevis* was used as an outgroup. Values on the node indicate bootstrap support. The bar indicates 2 substitutions per 1,000 nucleotides.

Greenhouse evaluation

The selected strains showed the capacity to promote maize growth under greenhouse conditions (Tables 1 and 2). For hybrid ATL100, all treatments improved plant growth in comparison to plant growth in the non-inoculated and non-N control (Table 1). Treatment of maize with strain LGMB208 showed promising results, with higher values for stem diameter, root mass and concentration of P in leaves observed in this treatment than in the treatment with *A. brasilense* strain Ab-V5, which is used in commercial inoculation of the maize crop in Brazil, and in the non-inoculated control supplemented with N. In addition, inoculation with strains LGMB228, LGMB229, and LGMB239 showed a larger stem diameter than the control treatments (Table 1). Compared to inoculation with strain Ab-V5 and treatment with N in the controls, inoculation with strain LGMB274 promoted an increase in plant size (for hybrid ATL100). Root diameter was increased through inoculation with strains LGMB319 and LGMB326, while inoculation with strains LGMB208, LGMB229, and LGMB326 increased root dry weight. Additionally, inoculation with LGMB125, LGMB149, LGMB208, and LGMB319 increased P concentration in leaves, resulting in a 13% to almost 17% increase when compared to P concentration in leaves following inoculation with strain Ab-V5 and a 4 to 8% increase in comparison to P concentration in leaves following N supplementation. All strains evaluated were able to increase the concentration of N in leaves in comparison to leaves of the non-inoculated non-N control but always had lower N content than leaves of the N control (Table 1). Compared to inoculation with the commercial strain Ab-V5, inoculation with LGMB149, LGMB208, LGMB322, and LGMB326 resulted in higher N concentration in leaves (Table 1).

For hybrid KWX628, inoculations with each of the selected strains from our study showed similar performances to inoculation with *A. brasilense* Ab-V5 regarding the number of leaves, leaf area, root weight, and leaf dry weight (Table 2). Inoculation with *Enterobacter* sp. LGMB239 resulted in the largest increase in stem diameter and root volume. Moreover, inoculation with *Bacillus* sp. LMGB225 also increased stem diameter, root length, and P concentration in leaves. Interestingly, compared to treatment with Ab-V5, treatment with strains *Enterobacter* sp. LGMB125 or LGMB274 resulted in an increase in N concentration in

leaves. Emphasis should be given to treatment with strain LGMB125, which increased the N concentration in leaves by approximately 25% compared to that measured in maize treated with Ab-V5 and to that in the N control (Table 2).

Table 1. Growth parameters of maize hybrid ATL100 when inoculated with the 12 elite strains identified in this study, in comparison to those following inoculation with the commercial strain Ab-V5 of *Azospirillum brasilense* and those of the non-inoculated controls with or without N supplementation.

Treatment/ Strain identification	Number of leaves	Stem diameter (mm)	Plant size (cm)	Leaf area (cm^2)	Root length (cm)	Root volume (cm^3)	Root dry weight (g)	Leaf dry weight (g)	N concentration in leaves ($mg\ g^{-1}$)	P concentration in leaves ($mg\ g^{-1}$)
Enterobacter sp. LGMB125	8.0 a	7.5 ab	20.9 ab	630.1 a	29.4 a	24.2 ab	1.46 ab	2.37 a	21.51 abc	5.41a
Stenotrophomonas sp. LGMB149	8.2 a	7.7 ab	21.1 ab	658.6 a	28.4 a	23.8 ab	1.37 ab	2.73 a	22.01 ab	5.41 a
Bacillus sp. LGMB202	7.8 a	7.7 ab	20.7 ab	608.6 a	36.4 a	23.7 ab	1.48 ab	2.53 a	19.23 bcde	4.86 ab
Enterobacter sp. LGMB208	8.1 a	8.0 a	20.8 ab	542.1 ab	31.9 a	23.1 ab	1.66 a	2.82 a	22.27 ab	5.24 a
Enterobacter sp. LGMB225	8.0 a	7.6 ab	19.4 b	638.6 a	33.5 a	21.0 ab	1.38 ab	2.31 a	20.50 abcde	4.74 abb
Enterobacter sp. LGMB228	8.0 a	8.1 a	21.9 ab	759.5 a	29.6 a	19.4 ab	1.45 ab	2.93 a	19.43 abcde	4.26 bc
Cellulosimicrobium sp. LGMB229	7.7 a	8.2 a	22.1 ab	732.0 a	32.2 a	24.0 ab	1.72 a	3.00 a	17.83 de	4.31 bc
Enterobacter sp. LGMB239	8.1 a	8.1 a	22.4 ab	731.5 a	28.7 a	21.0 ab	1.49 ab	2.82 a	14.34 f	4.60 ab
Enterobacter sp. LGMB274	8.0 a	7.8 ab	23.0 a	692.1 a	27.0 a	23.2 ab	1.48 ab	2.70 a	17.35 ef	4.32 bc
Bacillus sp. LGMB319	7.8 a	7.7 ab	21.8 ab	667.0 a	32.2 a	27.9 a	1.49 ab	2.53 a	21.43 abcd	5.35 a
Enterobacter sp. LGMB322	8.0 a	7.6 ab	21.4 ab	687.2 a	27.0 a	25.4 ab	1.40 ab	2.72 a	22.40 ab	4.99 ab
Bacillus sp. LGMB326	7.7 a	7.8 ab	22.1 ab	702.5 a	30.1 a	28.4 a	1.71 a	2.81 a	22.38 ab	4.63 ab
Azospirillum brasilense Ab-V5	7.7 a	7.8 ab	21.1 ab	681.8 a	32.2 a	27.0 ab	1.55 ab	2.53 a	17.90 cde	4.63 ab
Nitrogen supplementation	7.7 a	6.5 ab	20.2 ab	647.6 a	29.9 a	23.2 ab	1.36 ab	2.27 a	23.22 a	5.01 ab
Nitrogen depletion	6.6 b	5.9 b	14.3 c	258.7 b	28.4 a	16.1 b	1.13 b	1.13 b	3.39 g	3.72 c

Note: Data represent the mean of four replicates, which, when followed by the same letter within each parameter, are not significantly different (Tukey test $p < 0.05$).

Table 2. Growth parameters of maize hybrid KWX628 when inoculated with the 12 elite strains identified in this study, in comparison to those following inoculation with the commercial strain Ab-V5 of *Azospirillum brasilense* and those of the non-inoculated controls with or without N supplementation.

Treatment/ Strain identification	Number of leaves	Stem diameter (mm)	Plant size (cm)	Leaf area (cm^2)	Root length (cm)	Root volume (cm^3)	Root dry weight (g)	Leaf dry weight (g)	N concentration in leaves ($mg\ g^{-1}$)	P concentration in leaves ($mg\ g^{-1}$)
Enterobacter sp. LGMB125	6.7 ab	6.5 ab	17.6 ab	426.1 a	25.3 ab	13.6 abc	0.83 a	1.31 a	20.76 a	4.40 cd
Stenotrophomonas sp. LGMB149	7.2 a	6.8 ab	18.3 ab	474.4 a	27.2 ab	18.3 abc	1.04 a	1.51 a	18.32 abcd	4.57 bcd
Bacillus sp. LGMB202	7.2 a	6.8 ab	17.7 ab	489.1 a	20.8 ab	15.0 abc	1.01 a	1.40 a	16.99 abcd	4.60 abcd
Enterobacter sp. LGMB208	7.0 ab	6.6 ab	17.2 ab	441.7 a	23.8 ab	16.8 abc	0.97 a	1.23 a	16.65 abcd	4.48 bcd
Enterobacter sp. LGMB225	7.3 a	7.2 a	18.2 ab	577.7 a	28.8 a	16.7 abc	1.09 a	2.00 a	15.79 abcd	5.35 a
Enterobacter sp. LGMB228	7.2 a	6.0 ab	18.8 ab	407.4 a	20.8 ab	10.8 bc	0.77 a	1.14 a	16.13 abcd	4.79 abc
Cellulosimicrobium sp. LGMB229	7.0 ab	7.0 ab	18.7 ab	546.2 a	25.9 ab	20.3 ab	1.16 a	1.99 a	13.67 cd	5.17 ab
Enterobacter sp. LGMB239	7.7 a	7.8 a	19.8 a	674.4 a	26.9 ab	24.3 a	1.40 a	2.64 a	15.47 bcd	5.11 abc
Enterobacter sp. LGMB274	7.3 a	7.0 ab	18.9 ab	481.6 a	27.2 ab	17.3 abc	1.04 a	1.67 a	18.69 ab	4.02 d
Bacillus sp. LGMB319	7.3 a	7.0 ab	18.5 ab	489.7 a	24.0 ab	17.8 abc	1.03 a	1.63 a	15.44 bcd	4.87 abc
Enterobacter sp. LGMB322	7.5 a	6.9 ab	18.5 ab	576.6 a	22.4 ab	16.2 abc	0.96 a	1.93 a	18.48 abc	4.52 bcd
Bacillus sp. LGMB326	7.2 a	6.9 ab	18.7 ab	507.9 a	26.7 ab	16.1 abc	1.00 a	1.65 a	13.60 d	4.53 bcd
Azospirillum brasilense Ab-V5	7.5 a	6.7 ab	17.2 ab	486.3 a	20.2 b	15.9 abc	0.89 a	1.78 a	15.32 bcd	4.71 abcd
Nitrogen supplementation	7.3 a	6.5 ab	16.9 b	416.6 a	23.7 ab	12.6 abc	0.84 a	1.30 a	15.60 abcd	4.92 abc
Nitrogen depletion	6.1 b	5.1 b	11.8 c	153.6 b	23.0 ab	9.9 c	0.81 a	1.25 a	2.94 e	4.88 abc

Note: Data represent the mean of four replicates, which, when followed by the same letter within each parameter, are not significantly different (Tukey test $p < 0.05$).

Discussion

In vitro screening of the 173 bacterial strains isolated from maize roots for properties commonly associated with plant growth-promotion revealed that 70.5% of the bacterial strains produced siderophores and 56.5% solubilized phosphate; both parameters are considered important microbial features of PGPB (Majeed et al., 2015). These percentages are higher than those reported in studies of other species, *e.g.,* Chaiharn, Chunhaleuchanon, and Lumyong (2009) reported that 23% of isolates from rice roots (*Oryza sativa*) in Thailand were able to produce siderophores, while Reena, Aysha, Valli, Nirmala, and Vinothkumar (2013) reported that 36% of the isolates from tomato (*Solanum lycopersicum*) rhizosphere were able to produce siderophores. Siderophores are important for the availability of iron (Fe) and also help in antibiosis

against phytopathogenic microorganisms (Miethke & Marahiel, 2007). For P, the conversion of phosphate to available P has great importance under the P-limiting conditions (Ahemad & Kibret, 2014) commonly observed in Brazilian soils (Olibone & Rosolem, 2010). In addition, there is a global concern about the energy and costs involved in mining rock phosphate, which is neither eco-friendly, economically feasible or sustainable, and results in the emission of fluorine, disposal of gypsum and accumulation of cadmium (Cd) and other heavy metals in soils (Sharma, Sayyed, Trivedi, & Gobi, 2013).

Of the 93 isolates positive for both siderophore production and phosphate solubilization, 63.4% were positive for the BNF trait. N is considered to be the most essential nutrient for plant growth because it impacts protein content (Lana, Dartora, Marini, & Hann, 2012). This property, in addition to the ability of these isolates to solubilize phosphate and produce siderophores, demonstrates their high potential to provide nutrients to the maize crop (Ahemad & Kibret, 2014; Souza et al., 2015; Sharma, Kulkarni, & Jha, 2016). Twelve isolates were able to produce considerable amounts of IAA (7.5 to 39.1 μg mL^{-1}). IAA production is frequently observed in bacteria able to enhance plant growth (Fukami et al., 2017) through the enhancement of lateral root initiation, cell enlargement and an increase in root area (Zahid, Abbasi, Hameed, & Rahim, 2015). The screening of bacterial strains that show multifactorial traits may result in higher effectiveness in plant growth-promotion and an increase in the probability of success with different plant genotypes and edaphoclimatic conditions (Deepa, Dastager, & Pandey, 2010; Majeed et al., 2015).

Strain LGMB229 was related to *Cellulosimicrobium aquatile*, a species recently isolated from the freshwater Panagal reservoir at Nalgonda, India (Sultanpuram, Mothe, Chintalapati, & Chintalapati, 2015) but not reported from other sources. For the closely related species *C. cellulans* and *C. funkei,* growth promotion has been reported in *Phaseolus vulgaris* grown in chromium (Cr)-contaminated soil (Karthik et al., 2016). *C. funkei* was also able to improve the growth of *Brassica juncea* and showed antagonistic activity against plant pathogens associated with this species (Singh, Kumar, & Agrawal, 2014). *C. cellulans* has been previously isolated from the semi-arid region in Brazil and reported to promote maize growth (Kavamura et al., 2013). Strain LGMB149 is related to *Stenotrophomonas maltophilia*, a species commonly associated with growth promotion of several plants that also shows antagonistic activity against several plant pathogens (Islam, Akanda, Prova, Islam, & Hossain, 2016; Kumar & Audipudi, 2015; Li et al., 2016; Alavi et al., 2013). Interestingly, *S. matophilia* has also been reported to be a N_2-fixing symbiont of *P. vulgaris* (Cardoso, Hungria, & Andrade, 2012).

The largest number of selected bacterial strains belong to the *Enterobacter* genus; strains LGMB225 and LGMB322 were similar to *E. ludwigii,* however strains LGMB125, LGMB208, LGMB228, and LGMB235 have not shown high similarity with any described species. *E. ludwigii* was primarily described as a clinical pathogen (Hoffmann et al., 2005) and has been characterized as an effective PGPB with the ability to promote N_2 fixation, phosphate solubilization and IAA production (Shoebitz et al., 2009; Singh, 2013), features usually observed in *Enterobacter* species (Lin et al., 2012; Chen et al., 2016), and has also been reported to be a symbiont of *P. vulgaris* (Cardoso et al., 2012). The production of high amounts of IAA *in vitro* did not result in high radicular development under greenhouse conditions, suggesting that multiple mechanisms are involved in this process.

The remaining strains LGMB202, LGMB319, and LGMB236, showed high similarity with strains from the *Bacillus* genus. *Bacillus* encompasses a large number of species, several of which are classified as PGPB because of their use of different mechanisms such as siderophore production, which is also associated with the antagonism of several phytopathogens (Nautiyal et al., 2013; Souza et al., 2015; Armada, Probanza, Roldanc, & Azcona, 2016). In addition, this genus shows a positive effect on competitive interactions with bacteria and fungi (Beneduzi, Ambrosini, & Passaglia, 2012).

Most of the selected strains improved maize growth under greenhouse conditions, and the increase in N in maize leaves can be attributed to BNF activity. Although inoculation of cereals with PGPB may only partially replace the chemical fertilizers, the use of our strains in combination with decreased amounts of fertilizers may represent a useful alternative for farmers, decreasing the cost as well as the environmental impact (Fukami, Nogueira, Araujo, & Hungria, 2016). A clear effect due to the interaction of different plant genotypes with bacterial strains was observed. For hybrid ATL100, inoculation with strains *Enterobacter* sp. LGMB208 and *Bacillus* sp. LGMB319 showed increased plant growth, while for hybrid KWX628, the largest increase in plant growth was achieved through inoculation with *Enterobacter* sp. strains LGMB125, LGMB239, and LGMB274 and *Bacillus* sp. LGMB225.

Conclusion

Twelve elite bacterial strains were identified that exhibited siderophore production, phosphate solubilization, indole acetic acid production and biological nitrogen fixation properties that were evaluated *in vitro*. Their performance as elite strains for the inoculation of maize plants was confirmed under greenhouse conditions, but interaction with plant genotypes was observed. The elite strains were identified as belonging to the genera *Cellulosimicrobium*, *Stenotrophomonas*, *Enterobacter*, and *Bacillus*.

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