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Research Article

Bioprospecting Plant Growth-Promoting Bacteria Isolated from Maize (*Zea mays* L.) Roots

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Abstract

Maize (*Zea mays* L.) culture has a great importance in several countries, especially in Brazil the third-largest world producer. The increase in maize production has been achieved with a high use of fungicide; however, in view of a more sustainable agriculture plant growth promoting bacteria have been explored aiming for the replacement of chemical fertilizers and biological control. In this study, we investigated the bacterial community isolated from maize roots in order to evaluate their capacity of growth promotion as well as of inhibition of fungal species associated with maize leaf diseases. All isolates evaluated were positive for at least one of the parameters evaluated-growth promotion, enzymatic production or bio control. The best results were observed for *Enterobacter* sp. LGMB221 and *Bacillus* sp. LGMB242 that showed the high potential for growth promotion, acting in the early stage of maize seedlings development. *Bacillus* sp. LGMB152 showed the best enzymatic results, indicating that it might play a role against pathogens, a premise supported by the antagonist activity observed. The next steps involve evaluations under field conditions to confirm if these isolates have biotechnological potential as inoculants for the maize crop. In addition, we suggest that *Enterobacter* strains LGMB221 and LGMB235 and *Escherichia* strain LGMB159 might represent new species, indicating the high diversity of bacteria in maize rhizosphere that remains to be determined.

Keywords: 16S rRNA; Fungi antagonism; PGPB

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Introduction

Maize (*Zea mays* L.) is an important crop in the tropical climatic region and one of the three major food staple crops for the world's population. In Brazil, the third-largest world producer, the culture has great importance to the agribusiness [1]. The increase in grain production has been achieved mainly by genetic improvements, in association with fertilizers and agrochemicals application.

Especially in the past few years, the focus of many research groups is to find Plant Growth-Promoting Bacteria (PGPB) that will act in plant growth through one or more mechanisms, including biological nitrogen fixation, phosphate solubilization, production of hormones such as auxins, modifying root diameter [2,3].

Besides the use as PGPB, bacteria have attract attention for their potential to inhibit phytopathogens development, bean alternative to the application of fungicides, mitigating the environmental impacts and contributing to a more sustainable agriculture [4]. Studies performed with tomatoes (*Solanum lycopersicum*) [5], soybean (*Glycine max*) and cotton (*Gossypium arboretum*) [6] have shown that PGPB can act as antagonists to others pathogenic strains, been an effective biological controller, and in the same time increasing grain production. However, the selection of an effective PGPB consists of anex tensive and indispensable preliminary biochemical analysis *in vitro*. Despite the high amount of studies conducted in plant growth promoting, we decided to explore a previous culture collection of bacteria isolated from different maize genotypes including maize lineages and their respective hybrids [7], in order to evaluated if these isolates can act as plant growth promoting in a different hybrid from the one that they were isolated.

In this way, the aim of this study was to identify bacterial strains isolated from roots of different maize genotypes and to characterize their potential to be used as plant growth promoter and fungal biological controller by *in vitro* and *in vivo* evaluations.

Material and Methods

Strains and 16S rRNA gene sequencing

The bacteria used in this study were previously isolated from roots of different maize genotypes and were selected in view of the genetic diversity [7]. The bacteria were isolated from lineages LA, LB, LC, and LD and the hybrids FTH510, ATL100 and FX1453 (derived from the crosses LA × LB, LA × LC and LA × LD, respectively), provide by "Semilia Genética e Melhoramento Ltda" (Brazil).

The root samples were submerged in sterilized distilled water for one minute, immersed in 70% ethanol (v/v) for one minute, three minutes in sodium hypochlorite 3% (v/v), 30 seconds in 70% ethanol (v/v) and then washed three times in sterilized distilled water for one minute. After surface disinfesting, the samples were fragmented into 5 pieces of 8mm and aseptically transferred to plates containing one of the following solid culture media without nitrogen (N-free media): NFb, JNFb, LGI, or LGI-P [7]. The isolates are deposited at the Laboratory of Genetics of Microorganisms-LABGEM, Department of Genetics, Federal University of Parana, Curitiba, PR, Brazil.

For bacteria identification, the genomic DNA was extracted by the phenol-chloroform method adapted from [8]. The isolates were re-identified based phylogeny analysis of full sequence of 16S rRNA gene. The amplification was performed using primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3') [9], as described by Menna et al., [10]. Sequencing reaction was performed with primers fD1, 362f (5'-CTCCTACGGGAGGCAGCAGTGGGG-3') and 786f (5'-CGAAAGCGTGGGGAGCAAACAGG-3'), DNA purification was performed using Sephadex™ G-50 DNA and DNA sequencing was performed on an automated DNA sequence Mega BACE™ 1000.

DNA sequences were compared with sequences available in the National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLAST tool [11]. Sequences from the type strains were obtained from Myco Bank (http://www.mycobank.org) and GenBank (http://www.ncbi.nlm.nih.gov/genbank). Alignments of DNA sequences were performed using the Bio Edit version 7.2.5 [12] and Clustal W [13] in MEGA v.6 [14]. Bayesian inference of the phylogeny was performed in MrBayes version 3.2.1 [15], with permutations allowed until a frequency of division ≤ 0.01 was reached. The General Time-Reversible (GTR) substitution model was used. Figure Tree version 1.4.2 was used to edit the phylogenetic trees that were constructed. Sequences obtained in this study were deposited in GenBank and accession numbers were obtained.

Plant growth promotion evaluation

Plant growth promotion was evaluated by the capacity of isolates to fix biological nitrogen, solubilize phosphate and produces iderophores, indole acetic acid and enzymes. In addition, we also evaluate the ability of strains to promote the growth of maize hypocotyl and root by seeds germination.

Analysis of siderophore production was carried out according to Schwyn and Neil [16] adapted by using solid DYGS medium (2g dextrose; 1.5g peptone; 2g yeast extract; 0.5g K_2HPO_4; 0.5g $MgSO_4$; 1.5g L-glutamic acid; 15g agar 15g, pH 7.0) adding CAS (60.5g of chromo azurol S into 50mL of distilled water plus 10mL of $FeCl_3.6H_2O$ 1mM in HCl 10mM) carefully mixed into 72.9mg of HDTMA (Hexadecyltrimethylamonium) dissolved in 40mL of distilled water. Phosphate solubilization was evaluated according to Chagas Junior et al., [17], using the culture medium GL (10g glucose; 2g yeast extract; 15g agar added to 0.25g/L of K_2HPO_4 solution and 1g/L$CaCl_2$, pH 6.5). Positive result was revealed by the halo formation around the colony.

Biological nitrogen fixation was evaluated as described by Araújo et al., [18], using the JNFb semi-solid medium (5g malic acid; 0.6g K_2HPO_4; 1.8g KH_2PO_4; 0.2g $MgSO_4.7H_2O$; 0.1g NaCl; 0.02g $CaCl_2.2H_2O$; 20mg yeast extract; 0.08mg $CuSO_4.5H_2O$; 2.4mg $ZnSO_4.7H_2O$; 2.8mg H_3BO_3; 2mg $Na_2MoO_4.2H_2O$; 2.35mg $MnSO_4.H_2O$; 65.6mg Na_2EDTA; 0.1mg biotin; 0.2mg pyridoxine; 4.5g KOH; 2mL bromothymol blue 0.5%; 2.2g agar, pH 6.8) [19]. The bacteria growth was revealed by the formation of a pellicle on the medium surface.

Indol Acetic Acid (IAA) production was evaluated using the methodology described by Kuss et al., [20], modified by using DYGS culture medium containing 10µL of tryptophan 10mg/mL. Salkowski solution ($FeCl_3.6H_2O$ 2% + H_2SO_4 37%) was added to reveal the results, absorbance values were measured by spectrophotometry at 530nm wavelength and final values were expressed in µg/mL. Correlation data for IAA production and seed germination was performed using Bio Estat 5.0 [21].

Seed germination was evaluated using the commercial hybrid maize SX2530 provided by Semilia Genética e Melhoramento Ltda, in order to evaluate the interaction of the isolated bacteria with a different hybrid from the one that they were isolated. Seeds were superficially disinfested by immersion in 70% ethanol (v/v) for one minute, three minutes in sodium hypochlorite 3% (v/v), 30 seconds in 70% ethanol (v/v) and then washed three times in sterilized distilled water for one minute. The experiment was performed using 48 seeds for each isolate. The bacteria were growth in LB culture medium. Microbiolization was done by adding the seeds to the culture medium containing 10^8cells/mL during two hours at 37°C. Microbiolized seeds were placed in germination paper humidified with distilled water and incubated in Biochemical Oxygen Demand (BOD) at 28°C for seven days [22]. Evaluations of length (cm) and volume (cm^3) of roots and hypocotyls were verified using Win-Rhizov.4.0 software (Regent Systems, Quebec, Canada). For statistical analysis, Kruskal-Wallis test ($p < 0.05$) was performed by Assistat 7.6 Beta [23].

Enzymatic profile

The production of extracellular enzymes such as amylase, pectinase, cellulase, chitinase, lipase, proteases and urease were investigated once they can act in Plant Growth Promotion (PGP) and indicate a biological controller potential. Amylase production was performed in MM9 medium (200mL of salt solution containing 12.8g $Na_2HPO_4.2H_2O$; 3g KH_2PO_4; 0.5g NaCl; 1g NH_4Cl added to 2mL $MgSO_4$ 1M; 10g glucose; 0.1mL $CaCl_2$ 1M; 15g agar; pH 7.0) containing 0.5% yeast extract and 1% soluble starch [24]. Result was revealed by iodine added to colonies grown. Pectinase was also evaluated in MM9 medium containing 1% of pectin [25]. Cellulase and chitinase tests were performed according to Renwick et al., [26]. Cellulase production was revealed by Congo Red added to amid mineral culture medium (0.02g $CaCO_3$; 0.01g $FeSO_4.7H_2O$; 1.71g KCl; 0.05g $MgSO_4.7H_2O$; 4.11g $Na_2HPO_4.12H_2O$; 15g agar; 0.5% carboxy methylcellulose, pH 5.0) and chitinase was evaluated in MM9 with 0.08% colloidal chitin. The assay for lipase production was carried out in solid culture sterase medium (10g peptone; 5g NaCl; 0.1g $CaCl_2.2H_2O$; 15g agar, pH 7.4) [27]. Protease production was evaluated in skimmed milk and agar medium (1.000mL skimmed milk heated at 55°C; 6g Tripticase-Soy-Agar; 20g agar, pH 7.0) [28]. Positive results were evaluated by the presence of halo around the colonies.

Urease production was evaluated in urease culture medium (0.5g Na_2HPO_4; 0.5g K_2HPO_4, 0.5g; 0.2g $MgSO_4.7H_2O$; 10mL NaCl 10%; 1g yeast extract; 2.5mL bromothymol blue 0.5%, pH 5,8) [29]. Positive result was revealed by the change of culture medium to blue color.

Fungi antagonism

The antifungal activity was evaluated using the dual culture method [30] and six fungi isolated from lesions on maize leaves: *Alternaria* sp. (LGMF1021) and *Diaporthe* sp. (LGMF1054) [31]; *Cercosporazeae-maydis* (LGMF1047) and *Bipolaris maydis* (LGMF1048) provided by the Biological Institute of São Paulo; *Fusarium verticillioides* (LGMF1046) and *Colletotrichum graminicola* (LGMF1044) provided by the culture collection of Phytopathogenic Fungi Prof. Maria Menezes. The bacteria and the phytopathogen fungi were previously cultured on PDA medium, pH 5.8 for seven days. One disc (6mm) from the phytopathogen was in one side of the petri dish, and in the opposite site a bacteria streak was inoculated, the experiment was performed in five replicas triplicate, and incubated at 28°C for seven days. To determine the Inhibition Percentage (IP), the diameters of colonies were measured, and the IP was calculated according to the following formula: IP = mycelial growth in the control-mycelial growth in the treated sample/mycelial growth in the control × 100. For statistical analysis, Kruskal-Wallis test ($p < 0.05$) was performed by Assistat 7.6 Beta [23].

Statistical Analysis

Seed germination was evaluated by length (cm) and volume (cm^3) of roots and hypocotyls comparing the treatments with the control without the inoculation of bacteria. Fungi antagonism was evaluated comparing the growth of fungus in the treatments and without the inoculation of bacteria. The data of both experiments were submitted to normality and homogeneity tests and once they did not satisfy the conditions for ANOVA, they were submitted to the non-parametric test using Kruskal-Wallis at 95% of significance ($p < 0.05$) performed on Assistat 7.6 Beta software [23].

The analysis of correlation between IAA production and the seed germination were performed using the Pearson correlation test, considering hypocotyl and root growing, at Bio Estat 5.0 software [21].

Results

Bacteria identification

Among the 150 bacterial isolates from different maize genotypes, heterotic pairs and their respective commercial hybrids [7], eight strains-LGMB141, LGMB143, LGMB152, LGMB159, LGMB178, LGMB221, LGMB235, and LGMB242-were selected to evaluate their ability in promote plant growth. The isolates were identified by phylogenetic analysis of 16S rRNA gene and classified as belonging to the genera*Bacillus* (LGMB141, LGMB143, LGMB152, LGMB178, and LGMB242), *Escherichia* (LGMB159), and *Enterobacter* (LGMB221 and LGMB235). Four out of the eight *Bacillus* strains (LGMB141, LGMB143, LGMB152 and LGMB242), showed high similarity with five *Bacillus* species, while isolate LGMB178 was positioned in another clade with other *Bacillus* species (Figure 1 and S1). Isolate LGMB159 belong to the *Escherichia* genus differs from described species (Figure 2), in addition, strains LGMB221 and LGMB235 did not cluster with any described species in *Enterobacter* (Figure 3).

Characterization for plant growth promotion

Bacillus sp. LGMB141, LGMB143, LGMB152 and *Enterobacter* sp. LGMB221 and LGMB235 synthesized siderophores (Table 1). However, none of these isolates was able to solubilize phosphateor fix nitrogen. All isolates evaluated synthesized IAA, up to 25.75µg/Ml by *Bacillus* sp. LGMB143, followed by *Escherichia* sp. LGMB159 and *Enterobacter* sp. LGMB235, with 13.43µg/mL and 14.24µg/mL, respectively. In addition, Pearson correlation analysis showed that root length and volume are positively correlated to IAA production ($r = 0.49$ and $r = 0.44$, respectively) but negatively to hypocotyl ($r = -0.45$ for length and $r = -0.44$ for volume).

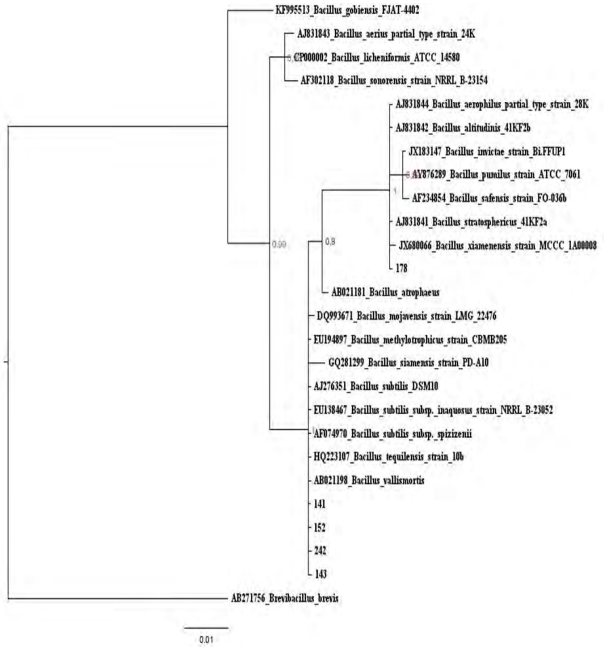

Figure 1: Baysian inferencetree based on the 16S rRNA gene of maize isolates and *Bacillus* type strains belonging to Clade 1. The species *Brevibacillusbrevis* was used as outgroup. Values on the node indicate bootstrap support. Bar indicates 10 substitutions per 1,000 nucleotides.

We also evaluated the capacity of the isolates in affecting maize seed development (Table 2). *Bacillus* sp. LGMB242 increased root volume by 25.7% and root length by 34.9%, in comparison with the control (without bacteria inoculation); the other strains did not show any improvement in the root length or volume. *Enterobacter* sp. LGMB221 increased hypocotyl length and volume by 21.8% and 33.2%, respectively, but no effect was observed by the other strains.

About extracellular enzymes production, *Enterobacter* sp. LGMB235 produced lipase and urease, *Bacillus* sp. LGMB143 produced pectinase and LGMB141 protease. *Bacillus* sp. LGMB152 produced the four enzymes and cellulase either. Chitinase was produced by isolates LGMB221 and LGMB235 (both classified as *Enterobacter* sp.).

Antifungal activity

In the anti fungal analysis all isolates except for LGMB178, inhibited the growth of *Alternaria* sp. (LGMF1021) by more than 50%, isolates LGMB235 (*Enterobacter* sp.) and LGMB242 (*Bacillus* sp.) showed the highest inhibition of 66.0%. *Enterobacter* sp. LGMB221 and *Bacillus* sp. LGMB143 had a notable inhibition of *Colletotrichum graminicola* (LGMF1044) by 74.5 and 67.6%, respectively.

Against *Fusarium verticillioides* (LGMF1046), *Bacillus* sp. LGMB143 and LGMB152 showed the highest antifungal activity, inhibiting the phytopathogen development 60.0% and 58.8%, respectively. Against *Cercosporazeae-maydis* (LGMF1047) all isolates were able to inhibit the pathogen growth by more than 50%, and LGMB152 and LGMB221 showed the highest antifungal activity (Table S1). Few isolates were effective against *Bipolarismaydis* (LGMF1048) and *Diaporthe* sp. (LGMF1054) growth, with LGMB152 inhibiting the first by 74.3%, and LGMB143 and LGMB152 in habiting the latest by 62.2% (Table 3 and S1).

Discussion

Several studies have reported the benefits of PGPB inoculation. The plant-growth promotion effect is influenced by both biotic and a biotic factors, including the species of bacteria used and their capacity of producing enzymes, siderophores, IAA, among other components, such as secondary metabolites that can act inhibiting phytopathogens. In this context, we tested bacteria from roots of different maize genotypes, heteroticpairs, and their respective commercial hybrids to explore their capacity in growth promotion and fungal bio control.

Among the 150 bacterial isolates obtained in our previous study, eight were selected for PGPB ability as well as antagonism properties based on the variability observed in a BOX PCR analysis [7]. Strain LGMB159 was identified as *Escherichia* sp., but probably representing a new species and the same was observed for *Enterobacter* spp. strains (LGMB221 and LMGB235). Isolates belonging to Enterobacteriaceae family (*Enterobacter, Escherichia, Pantoea*) have been commonly described as plant-associated bacteria, and previously studies showed a high ability of these generat produce indoles, such as IAA that has an important aspect in plant growth promotion [32-34]. In addition, bacteria belonging to genus *Bacillus* are common associated with plant growth promotion due the production of different factors, such as, IAA, siderophores, HCN and ammonia [35-37]. These elements are crucial for agricultural crops, wild plants and microalgae [38-41].

Siderophores production act stimulating plant growth by multiple mechanisms, including the provision of iron to plants, production of phytohormones and organic acids that can act solubilizing phosphate. These mechanisms can act direct and indirectly as making nutrients available for plant absorption or depriving pathogenic organisms of essential elements to survival [34]. In our study, strains LGMB141, LGMB143, LGMB152, LGMB221, and LGMB235 showed siderophores production, that besides acting in iron assimilation by plants, can also act as an iron kidnapper from phytopathogens [37]. However, none of the isolates evaluated showed the ability to solubilize phosphate or fixing nitrogen.

All strains produced considerable amounts of IAA, with an emphasis to *Bacillus* sp. LGMB143; followed by *Escherichia* sp. LGMB159 and *Enterobacter* sp. LGMB235. The amount of IAA produced by these strains is substantially higher than in other studies with bacteria of the same genus [42,43]. However, the results are alike to that reported under similar environmental conditions from this region [44], which may suggest that these strains are able to produce IAA by the same biosynthetic pathways. Considering the effects on seed germination, *Bacillus* sp. LGMB242 increased root length and volume, while *Enterobacter* sp. LGMB221 increased hypocotyl length and volume. Harsh et al., [45], found that some microbial strains can produce bio stimulants such as auxins from precursors existing in plants roots, like IAA. However, in our study, strains that increased the root and hypocotyl development are not the strains with the highest IAA production *in vitro*. This data can be explained by once the addition of microbial auxin can change the optimal level of endogenous auxin and can cause plant growth inhibition [46].

Figure S1: Maximum likelihood tree based on the 16S rRNA gene of maize isolates and *Bacillus* type strains, in red is the isolates that belonging to Clade 1. The species *Brevibacillusbrevis* was used as out group. Values on the node indicate bootstrap support. Bar indicates 2 substitutions per 1,000 nucleotides.

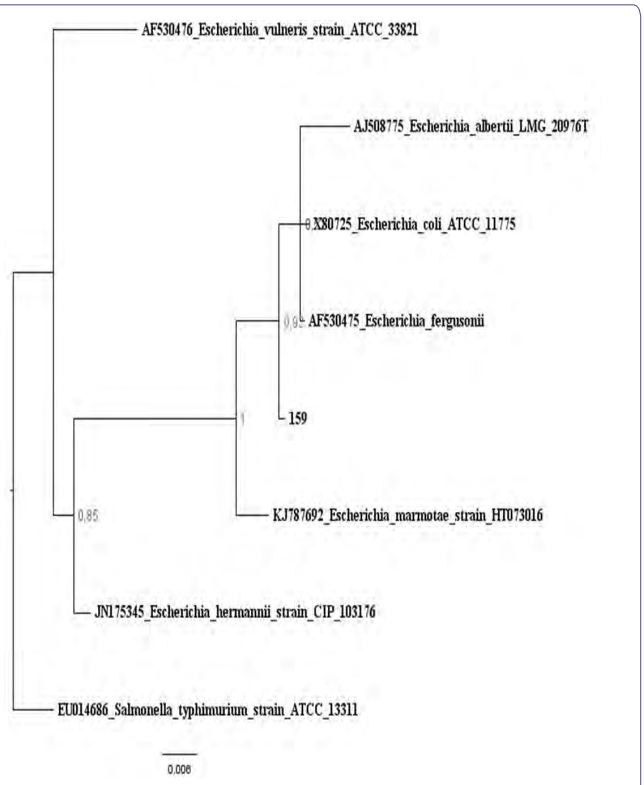

Figure 2: Baysian Inference tree based on 16S rRNA gene of maize isolates and Escherichia species. *Salmonella thyphimurium* was used as outgroup. Values on the node indicate bootstrap support. Bar indicates 6 substitutions per 1,000 nucleotides.

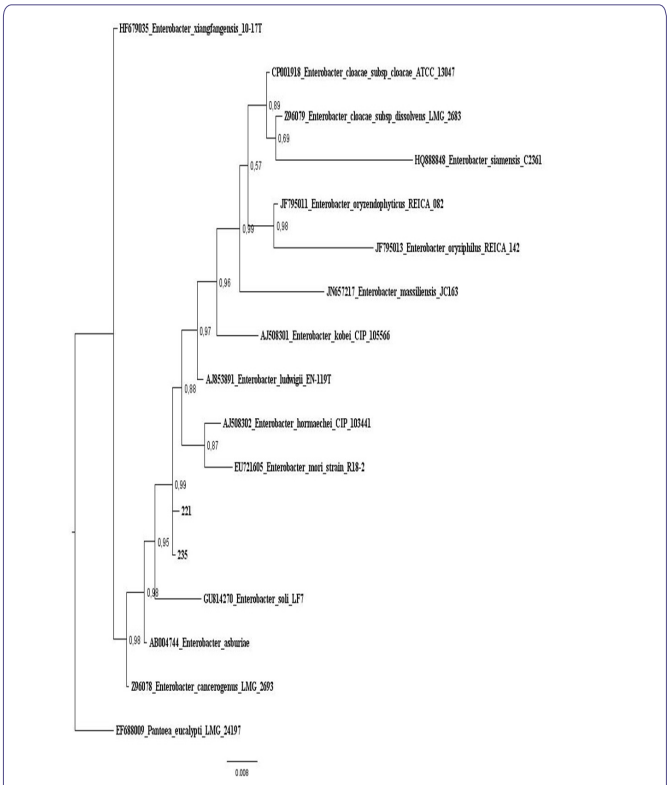

Figure 3: Baysian Inference tree based on 16S rRNA gene of maize isolates and *Enterobacter* species. *Pantoea eucalypti* was used as outgroup. Values on the node indicate bootstrap support. Bar indicates 8 substitutions per 1,000 nucleotides.

Strain Code	GenBank Accession Number	Identification Based on 16S rRNA Phylogeny	IAA (µg/mL)	BNF	Siderophore	Phosphate Solubilization	Amylase	Cellulase	Lipase	Pectinase	Protease	Chitinase	Urease
LGMB 141	KY848228	Bacillus sp.	2.16	-	+	-	-	-	-	-	+	-	-
LGMB 143	KY848229	Bacillus sp.	25.75	-	+	-	-	-	-	+	-	-	-
LGMB 152	KY848230	Bacillus sp.	2.54	-	+	-	-	+	+	+	+	-	+
LGMB 159	KY848231	Escherichia sp.	13.43	-	-	-	-	-	-	-	-	-	-
LGMB 178	KY848232	Bacillus sp.	7.29	-	-	-	-	-	-	-	-	-	-
LGMB 221	KY848233	Enterobacter sp.	7.29	-	+	-	-	-	-	-	-	+	-
LGMB 235	KY848234	Enterobacter sp.	14.24	-	+	-	-	-	+	-	-	+	+
LGMB 242	KY848235	Bacillus sp.	7.59	-	-	-	-	-	-	-	-	-	-

Table 1: Strain identification, Gen Bank accession code, identification based on phylogeny analysis, quantitative results for production of Indol Acetic Acid (IAA) and qualitative results for Biological Nitrogen Fixation activity (BNF), phosphate solubilization, siderophores and enzymes production.

Note: + represent positive results; - represent negative results.

Inoculated Bacteria	Root Length (mm)	Root Volume (mm^3)	Hypocotyl Length (mm)	Hypocotyl Volume (mm^3)
Control without bacteria	372.13^{CD}	1.94^{BCD}	718.95^{CD}	23.51^{CD}
Bacillus sp. LGMB143	42.16^{A}	0.48^{A}	$563.36B^{CD}$	15.18^{ABCD}
Bacillus sp. LGMB152	370.14^{BCD}	2.11^{CD}	299.68^{ABC}	8.35^{ABC}
Escherichia sp. LGMB159	145.73^{ABC}	1.26^{ABC}	364.63^{ABCD}	12.88^{ABCD}
Bacillus sp. LGMB178	136.01^{AB}	0.91^{AB}	237.30^{A}	2.13^{A}
Enterobacter sp. LGMB221	261.80^{ABCD}	1.69^{ABCD}	492.49^{ABCD}	15.99^{BCD}
Enterobacter sp. LGMB235	225.64^{ABCD}	1.35^{ABCD}	875.89^{D}	31.32^{D}
Bacillus sp. LGMB242	296.50^{ABCD}	1.86^{ABCD}	262.30^{AB}	7.29^{AB}
Bacillus sp. LGMB141	467.92^{D}	2.61^{D}	350.45^{ABCD}	11.43^{ABCD}

Table 2: Statistical results for bacteria influence in length (mm) and volume (mm^3) of root and hypocotyl maize, using the hybrid SX2530 seeds.

Straincode	ID16S RNA	LGMF1021	LGMF1044	LGMF1046	LGMF1047	LGMF1048	LGMF1054
		Alternaria sp.	*Colletotrichumgraminicola*	*Fusarium verticillioides*	*Cercosporazeae-maydis*	*Bipolarismaydis*	*Diaporthe* sp.
LGMB141	*Bacillus* sp.	1.57^{A}	3.40^{C}	2.67^{B}	2.47^{B}	3.63^{B}	3.53^{B}
LGMB143	*Bacillus* sp.	0.57^{A}	1.67^{ABC}	1.27^{AB}	0.60^{AB}	3.50^{B}	3.10^{AB}
LGMB152	*Bacillus* sp.	0.63^{A}	1.10^{AB}	1.07^{A}	0.83^{AB}	2.23^{AB}	1.33^{A}
LGMB159	*Escherichi*a sp.	0.57^{A}	1.77^{ABC}	1.10^{A}	0.53^{A}	0.93^{A}	1.33^{A}
LGMB178	*Bacillus* sp.	0.57^{A}	1.50^{ABC}	1.80^{AB}	1.17^{AB}	2.57^{AB}	2.57^{AB}
LGMB221	*Enterobacter* sp.	1.27^{A}	1.60^{ABC}	2.00^{AB}	1.27^{AB}	2.37^{AB}	2.07^{AB}
LGMB235	*Enterobacter* sp.	0.60^{A}	0.87^{A}	2.23^{AB}	0.57^{A}	1.80^{AB}	2.40^{AB}
LGMB242	*Bacillus* sp.	0.53^{A}	2.03^{BC}	2.17^{AB}	1.23^{AB}	2.57^{AB}	3.33^{AB}

Table S1: Inhibition of paired cultures antagonism test from bacteria against fungi associated with lesions on maize leaves.

Note: Data followed by the same letter are not statistically different. Kruskal-Wallis test $p < 0.05$. Analysis were performed independently for each treatment.

Isolated Strain	Phytopathogeninhibition in %					
	***Alternaria* sp.**	***Colletotrichum graminicola***	***Fusarium verticillioides***	***Cercosporazeae-maydis***	***Bipolaris maydis***	***Diaporthe* sp.**
Bacillus sp. LGMB141	63.91	50.98	52.56	75.71	3.58	12.18
Bacillus sp. LGMB143	59.66	67.65	60.05	66.26	38.48	62.23
Bacillus sp. LGMB152	63.91	48.04	58.8	78.41	74.29	62.23
Escherichia sp. LGMB159	63.91	55.88	32.58	52.77	29.29	27.29
Bacillus sp. LGMB178	19.32	52.94	25.09	48.72	34.8	41.45
Enterobacter sp. LGMB221	61.78	74.51	16.35	77.06	50.41	32.01
Enterobacter sp. LGMB235	66.03	40.2	18.85	50.07	29.29	5.57
Bacillus sp. LGMB242	66.03	53.92	23.85	64.91	48.58	33.9

Table 3: Percentage of inhibition of phytopathogens by the isolated bacteria in dual culture.

Production of extracellular enzymes by microorganisms plays an important role in plant pathogens control, in addition to other biotechnological applications [47]. About the enzymes evaluated in our study, just two strains (*Bacillus* sp. LGMB178 and *Escherichia* sp. LGM159) showed no extracellular enzyme production, but one strain (*Bacillus* sp. LGMB152) was able to produce six out of seven enzymes analyzed. Several reports have been shown that plant rhizospheric strains exhibit high enzymatic activity, in addition to other antifungal metabolites [48], and the hydrolytic enzymes produced by these strains can degrade the structural matrix of fungal cell walls and therefore act as antifungal factors [49].

LGMB152 play a role on enzyme activity against maize pathogens, and when evaluated the antifungal activity, once more, this

strain showed promising results with high activity against five out of the six pathogens evaluated (*Alternaria* sp., *Fusarium verticilioides*, *Cercosporazeae-maydis*, *Bipolaris maydis* and *Diaporthe* sp.) and moderate activity against *Colletotrichum graminicola*. This latest, is an important fungus of maize crops [33,50,51]. However, strain LGMB235, which produced enzymes lipase, chitinase and urease, showed considerable activity only against *Alternaria* sp. and *Cercosporazeae-maydis*, suggesting that there was not a direct correlation between the production of extracellular enzymes and phytopathogens inhibition, but enzyme production activity can help to protect plant against pathogenic fungi. Therefore, bioprospecting of PGPB aiming at their use as bio control, has high importance to reduce the use of fertilizers and pesticides favoring a sustainable agriculture.

Conclusion

In this study, we explored the bacterial community isolated from roots of maize lineages and hybrids in order to evaluate their capacity for growth promotion as well as of inhibition of major maize phyto pathogens. All isolates evaluated were positive for at least one of the parameters evaluated-growth promotion, enzymatic production or bio control. *Enterobacter* sp. LGMB221 and *Bacillus* sp. LGMB242 showed the highest potential for growth promotion. *Bacillus* sp. LGMB152 produced the largest number of evaluated enzymes, acting as an antagonist for different fungal associated with maize diseases. The next steps involve the evaluations under field conditions, to confirm if these isolates have biotechnological potential as inoculants for the maize crop. Beside the PGPB potential, we suggest that *Enterobacter* strains LGMB221 and LGMB235 and *Escherichia* strain LGMB159 might represent new species.

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