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Conservation

Culturable bacteria of the maize rhizosphere: conserving Mexican potential biotechnological resources

Bacterias cultivables de la rizósfera del maíz: conservando el potencial de los recursos biotecnológicos mexicanos

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Abstract

Rhizospheric microbiota diversity of crops in agroecosystems is understudied in Mexico and worldwide. The aim of the present work was to explore the diversity of culturable bacteria in maize fields. A bacterial collection consisting of 11,520 purified isolates was created from the rhizosphere of maize plants. Genomic DNA was obtained from each isolate and a region of 16S rDNA was sequenced. The 16S rDNA amplicon sequences were analyzed and grouped into Operational Taxonomic Units (OTUs), allowing the assemblage of 7,077 bacterial isolates into 185 non-singleton OTUs. OTUs belonged to 19 bacterial genera within Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes Phyla; with Firmicutes as the richest Phylum comprising 146 OTUs and 6 genera, and being *Bacillus* the richest genus. The soil core-community of 28 OTUs belonging to Firmicutes and 1 OTU from Proteobacteria was identified. The work discusses the role that the different bacterial populations identified within the maize rhizosphere may play, their potential use for biotechnological purposes, and the importance of conservation of microbiological resources using bacterial collections.

Keywords: Culturable microbiota; Maize; Bacterial populations; Microbial collections

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Resumen

La diversidad de la microbiota asociada a la rizósfera de cultivos en sistemas agrícolas ha sido pobremente estudiada en México y en todo el mundo. El objetivo de este trabajo fue identificar la diversidad de bacterias cultivables en campos de maíz. Se creó una colección de cepas de 11,520 aislados purificados a partir de la rizósfera de maíz. Se procedió a extraer el ADN genómico y secuenciar una región del 16S rADN de cada aislado. Las secuencias fueron analizadas y agrupadas en unidades operacionales taxonómicas (OTU). Esto permitió la agrupación de 7,077 cepas en 185 OTU pertenecientes a 19 géneros dentro de los phyla Firmicutes, Proteobacteria, Actinobacteria y Bacteroidetes; siendo Firmicutes el phylum más rico, incluyendo 146 OTU y 6 géneros, con *Bacillus* como el género con más especies. Se identificó la comunidad núcleo de los suelos conteniendo 28 OTU de Firmicutes y 1 OTU de Proteobacteria. Se discute el papel que juegan las diferentes poblaciones bacterianas identificadas en la rizósfera del maíz, su potencial para ser empleadas con propósitos biotecnológicos y la importancia de la conservación de los recursos microbianos empleando colecciones bacterianas.

Palabras clave: Microbiota cultivable; Maíz; Poblaciones bacterianas; Colecciones microbianas

Introduction

Plant roots provide a nutrient-rich environment for a large number of soil microorganisms. The rhizosphere, the zone in close proximity to the root surface, typically contains 10 to 100 times more microorganisms per gram than bulk soil (Haas et al., 2002). The organisms harbored by the rhizosphere can have a neutral, deleterious or beneficial effect on the plant. Different factors, such as land use, soil type, soil texture, pH, nitrogen availability and plant species affect the bacterial community structure (reviewed in Saleem et al., 2019). Furthermore, diversity and stability of plant-associated bacterial communities influence soil and plant quality, as well as ecosystem sustainability. Several studies have examined the effect of phytopathogens on the microbial diversity of plant-associated bacteria (Li et al., 2014; Mendes et al., 2011). These reports show that the presence of plant pathogens influences population dynamics in the rhizosphere, and that in some cases certain bacterial groups might affect disease development. Therefore, the analysis of the plant microbiota will allow identifying microorganisms with potential for plant growth promotion, phytopathogen control and bioactive compounds production for potential biotechnological applications (Douriet-Gámez et al., 2018; Figueroa-López et al., 2016; Ibarra-Galeana et al., 2017).

In Sinaloa, Mexico, particularly in Guasave Valley, natural vegetation is scarce, since most of the soil surface (~ 200,000 ha) is used for agriculture. There are very small conserved zones that show relictual vegetation of low deciduous forest (H. Ayuntamiento de Guasave, 1998). Agriculture in Sinaloa is highly technified. This is the highest maize producing state with around 0.5 million ha cultivated yearly under the scheme of intensive monoculture producing around 6 million metric tons of grain per year (SIAP, 2020). Soils submitted to monoculture for many years are highly susceptible to the loss of biological diversity (Liu et al., 2020; Trejo-Aguilar et al., 2013). Maize is affected by different biotic stresses including diverse bacterial and fungal pathogens that are currently treated with different agrochemicals and could be treated using biological control. This is the case of *Fusarium verticillioides*, a fungal pathogen causing stalk and ear rot of maize and being controlled by *Bacillus cereus* B25 (Lizárraga-Sánchez et al., 2015). A more complete understanding of the microbial ecology and diversity associated with the maize rhizosphere could help to improve plant health in field crops, reduce our dependence on chemical pesticides used in agriculture, and develop efficient biological control strategies. Therefore, it is important to characterize the microbial communities naturally associated with maize root systems, to identify potential growth promotion agents. To address this, studies have focused on characterizing rhizospheric maize bacterial communities using non-cultivation approaches and employing new sequencing technologies (Pereira et al., 2011). However, there is a drawback to metagenomic studies performed on DNA soil samples: dormancy allows bacteria to persist during unfavorable conditions, and surveys estimate that over 80% of the bacterial cells in the soil are dormant (Lennon & Jones, 2011). Moreover, the community of physiologically active bacteria within the soil is distinct from those that are dormant (Lennon & Jones, 2011). As a result, estimates of bacterial composition using standard DNA extractions from soil may not provide measurements that reflect the active players in the plant-microbe interaction, potentially obscuring field attempts to identify the agents of microbial control (Bulgarelli et al., 2013).

In this work, special consideration was given to the discovery of bacterial isolates from corn fields in Sinaloa. These native isolates are already pre-adapted

to the prevailing edaphic and climatological conditions in this region. Additionally, co-existence for many years with the natural soil microbiota should provide these microorganisms with competitive advantages compared to recently introduced exotic species. This collection has been previously utilized for identifying native bacteria capable of exerting biocontrol against the fungal phytopathogen *F. verticillioides* (Figueroa-Lopez et al., 2014, 2016). Bacteria with the highest biocontrol potential have been tested in field trials with great success (Lizárraga-Sánchez et al., 2015). As a result of this work, we have identified *Bacillus cereus sensu lato* strain B25 as the best *F. verticillioides* antagonist. B25 displays a whole array of antagonistic mechanisms that can be used to control *F. verticillioides* growth (Douriet-Gámez et al., 2018), including B25 chitinases which expression is induced in response to *F. verticillioides* lysates and might be involved in direct control of *F. verticillioides* conidia germination (Figueroa-López et al., 2017; Morales-Ruiz et al., 2021). In addition, we have characterized in this collection phosphate-solubilizing bacteria with the potential for growth and phosphorus nutrition improvement in maize. We found 2 *Bacillus* species: *B. flexus* (B4) and *B. megaterium* (B5) as potential phosphate solubilizers associated to the maize rhizosphere (Ibarra-Galeana et al., 2017). This collection has been kept under scrutiny to find diverse isolates for different biotechnological purposes. For these reasons and because biotechnological use requires live organisms, our aim was to obtain culturable bacteria from the maize rhizosphere to examine the diversity of culturable bacterial populations. These will help us to identify bacterial strains with biotechnological potential and preserve them for further use.

Materials and methods

We sampled 5 locations in northern Sinaloa, Mexico, differing by planting date and by maize hybrid: *1)* Serrano (Salvador Alvarado municipality), *2)* Alhuey (Guasave municipality), *3)* 18 de Diciembre (Angostura municipality), *4)* Casa Blanca (Guasave municipality), and *5)* La Trinidad (Guasave municipality). In each location, 10 maize rhizosphere and bulk-soil samples were collected corresponding to 10 plants. Three to 4 kg of bulk soil were removed with a shovel from the stem base of each plant (0-30 cm in depth). The soil samples were transferred to the laboratory at room temperature and processed immediately. Soil particles adhering to the roots (rhizospheric soil) were collected. In both cases, rhizospheric and bulk soil samples from the 10 plants from each location were mixed thoroughly by using sub-samples of 1 kg for bulk-soil and 10 g for rhizospheric samples to prepare a composite sample by mixing thoroughly the 10 different samples corresponding to the 10 plants per location. Composite samples of bulk-soil were stored at room temperature (25 °C) for physicochemical characterization. Composite rhizosphere samples were used immediately to isolate microorganisms by serial dilutions.

In order to obtain bacterial isolates, 4 culture media were prepared in 100 mm-diameter Petri dishes to enrich for certain specific taxonomic groups: *1)* Luria Bertani (LB) medium was used to enrich for *Bacillus* isolates (Cavaglieri et al., 2005); *2)* Actinomycetes Isolation Agar (AIA) (Bressan & Fontes-Figueiredo, 2007); *3)* King B Agar (KBA) was used for *Pseudomonas* enrichment (Cavaglieri et al., 2004); and *4)* Man, Rogosa and Sharpe (MRS) medium was used for lactic acid bacteria (De Man et al., 1960). Colonies were collected from the serial dilutions plated on LB, KBA and MRS media after 24 hours growth and from AIA medium after 48-72 hours at 25 ºC.

To generate the maize rhizospheric culturable bacterial collection, 576 isolates were "picked" and arranged in 6 96-well plates from each specific culture medium. This yielded 2,304 isolates from each composite rhizospheric sample points; the complete collection therefore contained 11,520 isolates. Isolates were cryopreserved in triplicate at -70 °C in January 2009, using LB containing 15% glycerol (v/v) according to Pasarell and McGinnis (1992). Frozen stocks were made and 2 months later they were thawed and grown at 25 °C and 200 r/min in 2 mL 96-well plates containing 1.5 mL liquid medium, for either 24 hours (LB, KBA and MRS media) or 72 hours (AIA medium). Isolates were considered non-viable if no visible growth after culturing in the proper media was observed after thawing. Plates were centrifuged at 2,000 rpm for 10 min and the bacterial pellets from viable isolates were kept at -70 °C until processing for DNA extraction.

A bulk soil sub-sample of 500 g was used for nutrient and physicochemical soil analyses (Supplementary material: Table S1). Texture was determined based on soil texture classification by particle size distributions (USDA), phosphate was analyzed according to Olsen et al. (1954), and organic matter was analyzed according to Walkley and Black (1934).

From the total 11,520 isolates, 95% (10,944) were viable, 10,080 isolates out of these were regrown and processed for DNA extraction, PCR amplification of 16S rDNA and sequencing. Bacterial DNA was extracted with the DNeasy® Blood & Tissue Kit (Qiagen; CA, USA). The following primers were used to amplify the complete 16S rDNA region (1,400 pb): F2C (5'- AGA GTT TGA

TCA TGG CTC -3') and C (5'- ACG GGC GGT GTG TAC -3') (Shi et al., 1997). The 25 µL PCR mixture contained 10 ng of DNA template, 1X reaction buffer, 10 pmol of each primer, 10 µM of each deoxynucleoside triphosphate (dNTP), and 1 U of Taq DNA polymerase (Invitrogen; Carlsbad, CA, USA). The PCR conditions included an initial denaturation step at 95 ºC (4 min); 32 cycles of denaturation at 95 ºC (1 min) followed by annealing at 60 ºC (30 sec) and extension at 72 ºC (1.5 min); and a final step at 72 ºC (5 min). PCR reactions were carried out in 96-well plates using a MyCycler thermal cycler (BioRad; CA, USA). Products were visualized by 1% agarose (w/v) gel electrophoresis in 0.5 X Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. PCR products were purified with a QIAquick PCR Purification kit (Qiagen; CA, USA) and quantitated using a Nanodrop 2000 UV-Vis spectrophotometer (Thermo Scientific). Approximately 400 ng of PCR product were used for the sequencing. The U1 primer (5' - CCA GCA GCC GCG GTA ATA CG - 3') (Lu et al., 2000) internal to the F2C/C amplicon was used for sequencing with an ABI 3730 XL automated sequencer at the National Laboratory of Genomics (LANGEBIO; Irapuato, Mexico). The resulting sequences ranged from 300 to 600 bp and contained a hypervariable region (domains V1-V3) (Sun et al., 2013).

Isolates were identified by sequence comparison against the GenBank databases and their genera identity were confirmed in the Ribosomal Database Project (RDP) (Cole et al., 2014). Ten thousand and eighty isolates out of 10,944 viable isolates were sequenced. Short (< 200 nt in length) and low-quality sequences were eliminated from the analyses. The resulting high-quality sequences were examined for the presence of chimeric sequences with the CHIMERA_CHECK software, available from the website (http://rdp.cme.msu.edu/); chimeric sequences were then discarded (yielding 7,077 sequences for the next analyses). A total of 7,077 sequences were grouped into operational taxonomic units (OTUs) (Supplementary material: Fig. S1) with the Cluster tool using the average neighbor algorithm from the Mothur program, v. 1.20.1 (http://www.mothur.org) using 0.03, 0.05 differences to construct rarefaction curves (Schloss et al., 2009). All singletons were removed to avoid any bias from minimally represented sequences. A total of 6,569 non-singleton sequences were grouped into OTUs defined by a 97% pairwise similarity threshold.

Sequences were aligned with the Clustal-W program (Thompson et al., 1994). A phylogenetic tree of OTUs representing all genera identified was constructed based on MEGA X (bootstrap = 1000), using the maximum likelihood method, and Tamura-Nei model.

Results

The bacterial strain collection, comprising 11,520 isolates, exhibited 95% survival efficiency 2 months after freezing and thawing, yielding 10,944 isolates. High quality 16S rDNA gene sequences were obtained from 10,080 isolates and after eliminating short, chimeric or low-quality sequences a total of 7,077 sequences with an average length ~ 300-600 nt were recovered for analysis and deposited in GenBank (with accession numbers JQ829081 through JQ836199).

A total of 6,569 non-singleton sequences were grouped in 185 OTUs (Table 1). Rarefaction analysis revealed that the species sampling effort curves calculated with 95% and 97% sequence identity have a positive slope with no evidence of approaching saturation (Supplementary material: Fig. S1).

Identification at the genus level using RDP and GenBank databases was successful for most isolates assigning putative taxonomic identities to each OTU in 19 genera (Table 1, Supplementary material: Table S2). These genera were grouped into 4 different bacteria phyla: Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria (Fig. 1).

Firmicutes and Proteobacteria displayed the greatest number of OTUs. Within Firmicutes, 146 OTUs were grouped into 6 genera, whereas Proteobacteria were represented by 34 OTUs grouped into 10 genera. Firmicutes was primarily represented by *Bacillus* and 5 other less abundant genera (Table 1). Notably, most of the putative Proteobacteria OTUs were grouped in the γ-Proteobacteria class. This class was represented mainly by the genera *Pseudomonas* and *Enterobacter* (15 and 4 OTUs, respectively) in addition to another 6 genera. The α- and β-Proteobacteria classes were solely represented by 1 OTU each (from the genera *Massilia* and *Rhizobium*, respectively) (Table 1).

The least diverse groups were the Actinobacteria and Bacteroidetes. In Actinobacteria we identified 31 and 4 sequences, belonging to the genera *Arthrobacter* (OTUs 114 and 248) and *Sinomonas* (OTU 174), respectively. In Bacteroidetes, only 6 sequences belonging to the genus *Sphingobacterium* were identified (OTU 243) (Table 1).

The core community was represented by 29 OTUs (highlighted in Table 1, Fig. 2) which were present in all 5 collection locations, 28 of these belong to Firmicutes with 26 from the genus *Bacillus* and only 1 OTU from *Lysinibacillus* and 1 from *Paenibacillus*. OTU 6 belonging to *Bacillus* was by far the most abundant one representing 23.69% of the total sequences. Only 1 OTU (36) belonged to γ-proteobacteria within the genus *Pseudomonas*.

Significant differences were detected between different collection locations in 5 (pH, P, K, Ca, Mg) of the 12-soil chemical or physical parameters evaluated in the bulk-soil samples [Supplementary material: Table S1]). We found significant pH changes between site I and the other sites. Site IV was significantly higher in P and Ca than the other sites. The sampling sites I and V were significantly lower than the remaining 3 points. Sites IV and V were higher on Mg than the remaining 3 points (Supplementary material: Table S1). Within these sites OTUs richness was: site I (117 OTUs) > site II (97) >, site V (83) > site IV (81) > site III (78 OTUs) (Table 1).

Discussion

The goal of our study was to analyze culturable bacterial communities from the maize rhizosphere. Although many purified bacterial isolates were sequenced and analyzed, our sampling effort was insufficient to reach a plateau in a rarefaction analysis of the 16S rDNA sequences; therefore, a complete diversity study of the bacteria present in the maize rhizosphere could not be performed. Among the phyla identified in our study Actinobacteria, Firmicutes and Proteobacteria were predominant; as reported before, the main groups of microorganisms that preferentially colonize maize plants belong to the phyla Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria (Li et al., 2014; Peiffer et al., 2013). These groups were identified in a microbiome study as the most changing taxa in the rhizospheres of sugar beet (Mendes et al., 2011), maize (Yang et al., 2017) and rice (Zhang et al., 2017) and some species of *Pseudomonas* (Proteobacteria) are associated with disease suppression of *Rhizoctonia solani* (Mendes et al., 2011). In agreement with our findings, Firmicutes and Proteobacteria have also been reported as the most abundant and diverse phyla in the maize rhizosphere of 20-day old maize seedlings (Pereira et al., 2011). In these reports, *Bacillus* and *Pseudomonas* were the major community components of the maize rhizosphere, using either culture-dependent or independent methods. This is consistent with our findings where Firmicutes was the most abundant bacterial phylum in the maize rhizosphere in this study. Additionally, the core community in this study was integrated mainly by the phylum Firmicutes and in less proportion by the phylum Proteobacteria corresponding well with previous studies that describe the presence of these phyla in the bacterial community of maize rhizosphere (Li et al., 2014; Omotayo et al., 2021; Peiffer et al., 2013). Both, Firmicutes and Proteobacteria have important members with diverse

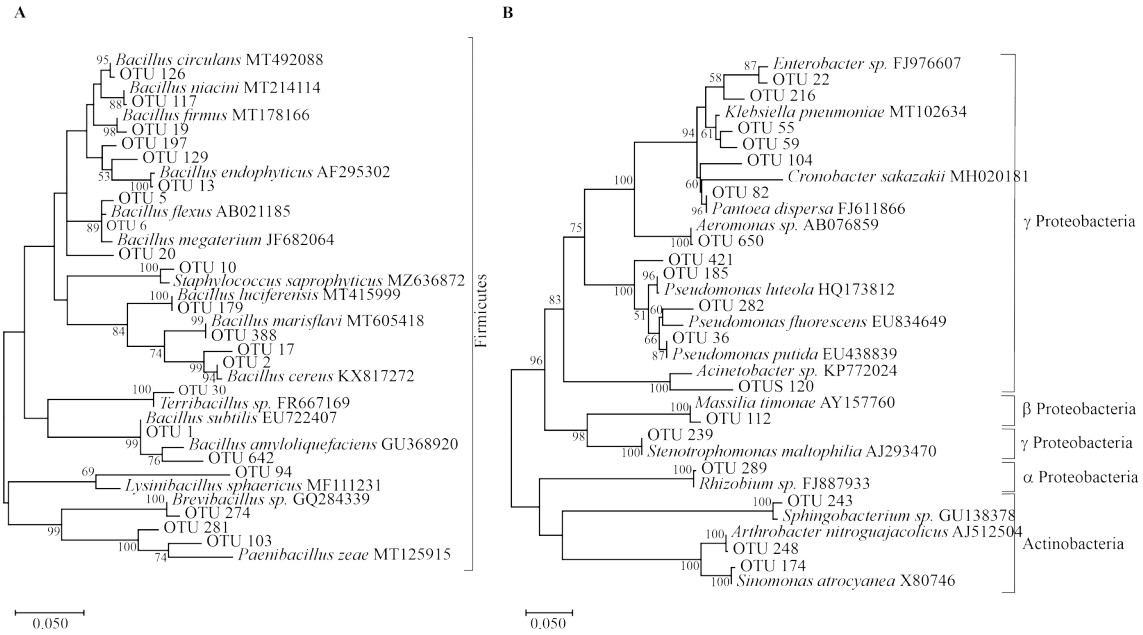

Figure 1. Maximum likelihood tree based on the partial 16S rDNA sequences (OTUs) from culturable bacterial isolates from maize rhizospheres and several sequences from NCBI. A, Firmicutes; B, Proteobacteria and Actinobacteria. Phylogenetic tree was constructed in MEGA X using maximum likelihood method and the Tamura-Nei model (bootstrap = 1,000), the highest log likelihood tree is shown. Only bootstrap support values higher than 50% are shown. The scale bar indicates the number of nucleotide substitutions per site.

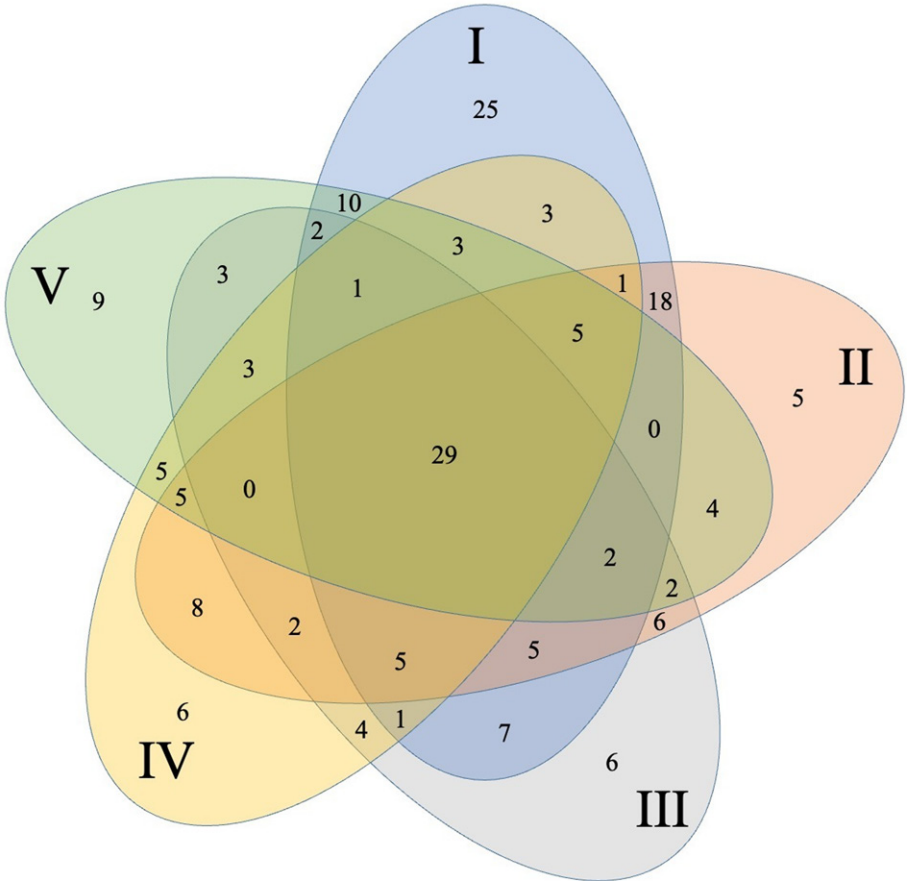

Figure 2. Venn's diagram for the OTUs richness within the sampled sites.

Table 1

Maize rhizosphere bacterial OTU distribution and colonies number in five collection sites in Sinaloa Mexico. Data were measured as total number of colonies sequenced per OTU (total sequences) and relative abundance as percentage of the total number of colonies sequenced. Highlighted in gray: OTUs that were present in all soil samples; N/D: not defined.

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
Actinobacteria	*Arthrobacter*	OTU 114		23		3	2	28	0.43
	Arthrobacter	OTU 248	1	2				3	0.05
	Sinomonas	OTU 174		2		1	1	4	0.06
Bacteroidetes	*Sphingobacterium*	OTU 243		1	5			6	0.09
Firmicutes	*Bacillus*	OTU 1	127	200	122	121	39	609	9.27
	Bacillus	OTU 2	117	172	130	194	372	985	14.99
	Bacillus	OTU 3	1	1				2	0.03
	Bacillus	OTU 4	12	22	13	26	23	96	1.46
	Bacillus	OTU 5	67	33	33	34	24	191	2.91
	Bacillus	OTU 6	425	281	304	370	176	1,556	23.69

Table 1. Continued

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
	Bacillus	OTU 7	17	65	26	43	80	231	3.52
	Bacillus	OTU 8	14	15	7	5	3	44	0.67
	Bacillus	OTU 12	19	35	5	19	8	86	1.31
	Bacillus	OTU 13	2	30	19	3	16	70	1.07
	Bacillus	OTU 15	2	5	3	8	7	25	0.38
	Bacillus	OTU 16	7	4	3	3	3	20	0.3
	Bacillus	OTU 17	9	33	4	18	10	74	1.13
	Bacillus	OTU 18	11	23	2	11	6	53	0.81
	Bacillus	OTU 19	10	25	66	21	30	152	2.31
	Bacillus	OTU 20		1		1	1	3	0.05
	Bacillus	OTU 21	79	47	30	48	21	225	3.43
	Bacillus	OTU 23	4	3	1	1		9	0.14
	Bacillus	OTU 25		2			5	7	0.11
	Bacillus	OTU 26		1		3	6	10	0.15
	Bacillus	OTU 34	2					2	0.03
	Bacillus	OTU 43	4	1		5	2	12	0.18
	Bacillus	OTU 51	2		1			3	0.05
	Bacillus	OTU 52	2					2	0.03
	Bacillus	OTU 54	3					3	0.05
	Bacillus	OTU 60	1		1		1	3	0.05
	Bacillus	OTU 79	2					2	0.03
	Bacillus	OTU 84	2					2	0.03
	Bacillus	OTU 95	65	76	18	53	19	231	3.52
	Bacillus	OTU 98	1	1	2	3	2	9	0.14
	Bacillus	OTU 113	1	3	3	2	10	19	0.29
	Bacillus	OTU 117	41	11	11	15	13	91	1.39
	Bacillus	OTU 118	2				2	4	0.06
	Bacillus	OTU 121		1			3	4	0.06
	Bacillus	OTU 123		2	1			3	0.05
	Bacillus	OTU 124	1	1				2	0.03
	Bacillus	OTU 125	2	3	1			6	0.09
	Bacillus	OTU 126	1	3	2	1	1	8	0.12
	Bacillus	OTU 127	1	1				2	0.03
	Bacillus	OTU 128	25	12		14	1	52	0.79
	Bacillus	OTU 129		4				4	0.06
	Bacillus	OTU 130	7	7	5	5	3	27	0.41
	Bacillus	OTU 131	6	3	2	1	4	16	0.24
	Bacillus	OTU 134	4	8	2	5	2	21	0.32
	Bacillus	OTU 137	2	2	1	4		9	0.14

Table 1. Continued

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
	Bacillus	OTU 138		2				2	0.03
	Bacillus	OTU 141		2				2	0.03
	Bacillus	OTU 151			1		1	2	0.03
	Bacillus	OTU 156	1	1	1		1	4	0.06
	Bacillus	OTU 157			2	1	1	4	0.06
	Bacillus	OTU 159	2	2	5	5	4	18	0.27
	Bacillus	OTU 162		1	2		3	6	0.09
	Bacillus	OTU 163					2	2	0.03
	Bacillus	OTU 172		1			1	2	0.03
	Bacillus	OTU 175	1				1	2	0.03
	Bacillus	OTU 179	1			6	1	8	0.12
	Bacillus	OTU 183	1				1	2	0.03
	Bacillus	OTU 190	1				1	2	0.03
	Bacillus	OTU 195				2		2	0.03
	Bacillus	OTU 196	1			3		4	0.06
	Bacillus	OTU 197	1			2		3	0.05
	Bacillus	OTU 202				2		2	0.03
	Bacillus	OTU 208	1	1				2	0.03
	Bacillus	OTU 212	1	1		3		5	0.08
	Bacillus	OTU 220		1		1		2	0.03
	Bacillus	OTU 224		1		1		2	0.03
	Bacillus	OTU 228			1		1	2	0.03
	Bacillus	OTU 231	1	3	1			5	0.08
	Bacillus	OTU 251		2				2	0.03
	Bacillus	OTU 252		1		1		2	0.03
	Bacillus	OTU 253		2	1		2	5	0.08
	Bacillus	OTU 254	1	1				2	0.03
	Bacillus	OTU 259	1	2				3	0.05
	Bacillus	OTU 260	2	1				3	0.05
	Bacillus	OTU 261	1	1				2	0.03
	Bacillus	OTU 262	1	1				2	0.03
	Bacillus	OTU 266	2	2	1	1	1	7	0.11
	Bacillus	OTU 267	11	5		11	8	35	0.53
	Bacillus	OTU 268		1		1		2	0.03
	Bacillus	OTU 269	3	1				4	0.06
	Bacillus	OTU 270	8	3	2	7	3	23	0.35
	Bacillus	OTU 272		2		1	1	4	0.06
	Bacillus	OTU 273	1	5	5		3	14	0.21
	Bacillus	OTU 275	1	1				2	0.03

Table 1. Continued

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
	Bacillus	OTU 288		1		1		2	0.03
	Bacillus	OTU 321	2					2	0.03
	Bacillus	OTU 324	1		1			2	0.03
	Bacillus	OTU 325	2					2	0.03
	Bacillus	OTU 331	2					2	0.03
	Bacillus	OTU 336	1		1			2	0.03
	Bacillus	OTU 365	2					2	0.03
	Bacillus	OTU 372	1				1	2	0.03
	Bacillus	OTU 381					2	2	0.03
	Bacillus	OTU 388			9	17	5	31	0.47
	Bacillus	OTU 390				1	1	2	0.03
	Bacillus	OTU 398	1				1	2	0.03
	Bacillus	OTU 401				1	1	2	0.03
	Bacillus	OTU 409					3	3	0.05
	Bacillus	OTU 411				1	1	2	0.03
	Bacillus	OTU 412				4		4	0.06
	Bacillus	OTU 416			1	2		3	0.05
	Bacillus	OTU 423		1		1		2	0.03
	Bacillus	OTU 433				2		2	0.03
	Bacillus	OTU 451	3				2	5	0.08
	Bacillus	OTU 454	1	1	2			4	0.06
	Bacillus	OTU 456	2			1	1	4	0.06
	Bacillus	OTU 459	2	1	2	1		6	0.09
	Bacillus	OTU 464		1	1			2	0.03
	Bacillus	OTU 469	1		1			2	0.03
	Bacillus	OTU 489		1	1			2	0.03
	Bacillus	OTU 490	1		1			2	0.03
	Bacillus	OTU 494	1	1				2	0.03
	Bacillus	OTU 495	2					2	0.03
	Bacillus	OTU 505	2					2	0.03
	Bacillus	OTU 510	1	1				2	0.03
	Bacillus	OTU 521	1		1	2	1	5	0.08
	Bacillus	OTU 523	4		1			5	0.08
	Bacillus	OTU 541			1		2	3	0.05
	Bacillus	OTU 567	1		2	1		4	0.06
	Bacillus	OTU 568				2		2	0.03
	Bacillus	OTU 575				2		2	0.03
	Bacillus	OTU 576		1		1		2	0.03
	Bacillus	OTU 577	2					2	0.03

Table 1. Continued

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
	Bacillus	OTU 584	1	1				2	0.03
	Bacillus	OTU 592			1	1		2	0.03
	Bacillus	OTU 593			1	1		2	0.03
	Bacillus	OTU 596		1	1	1		3	0.05
	Bacillus	OTU 607			2			2	0.03
	Bacillus	OTU 625		1	1			2	0.03
	Bacillus	OTU 642			2			2	0.03
	Bacillus	OTU 662			2			2	0.03
	Brevibacillus	OTU 274		1		2		3	0.05
	Lysinibacillus	OTU 94	29	4	14	56	157	260	3.96
	Lysinibacillus	OTU 375	1				11	12	0.18
	Lysinibacillus	OTU 385	1			3	2	6	0.09
	Lysinibacillus	OTU 392					2	2	0.03
	Lysinibacillus	OTU 393					2	2	0.03
	Paenibacillus	OTU 103	1	2	5	1	3	12	0.18
	Paenibacillus	OTU 281		2	1	1		4	0.06
	Paenibacillus	OTU 387				3	1	4	0.06
	Paenibacillus	OTU 394	1	3		4	2	10	0.15
	Paenibacillus	OTU 422	1	1	8	2		12	0.18
	Paenibacillus	OTU 453	2					2	0.03
	Staphylococcus	OTU 10	1	4		2	1	8	0.12
	Terribacillus	OTU 30	3	6	3	2		14	0.21
	Terribacillus	OTU 441	1			1		2	0.03
	N/D	OTU 221	1	1				2	0.03
α-Proteobacteria	*Rhizobium*	OTU 289		3	1			4	0.06
β-Proteobacteria	*Massilia*	OTU 112					2	2	0.03
γ-Proteobacteria	*Acinetobacter*	OTU 120	1		5		8	14	0.21
	Acinetobacter	OTU 164					2	2	0.03
	Acinetobacter	OTU 167					2	2	0.03
	Acinetobacter	OTU 169	15				2	17	0.26
	Aeromonas	OTU 650			18			18	0.27
	Cronobacter	OTU 104	2					2	0.03
	Enterobacter	OTU 22	340	20	39			399	6.07
	Enterobacter	OTU 216	2	1				3	0.05
	Enterobacter	OTU 335	2					2	0.03
	Enterobacter	OTU 368	3					3	0.05
	Klebsiella	OTU 55	4					4	0.06
	Klebsiella	OTU 59	2					2	0.03
	Klebsiella	OTU 313	4					4	0.06

Table 1. Continued

Phyla/Class	Genera	OTU	Site I	Site II	Site III	Site IV	Site V	Total sequences	%
	Pantoea	OTU 82	68				1	69	1.05
	Pantoea	OTU 314	2					2	0.03
	Pseudomonas	OTU 36	24	20	14	24	5	87	1.32
	Pseudomonas	OTU 37	3					3	0.05
	Pseudomonas	OTU 39	2					2	0.03
	Pseudomonas	OTU 46	2					2	0.03
	Pseudomonas	OTU 50	2					2	0.03
	Pseudomonas	OTU 56	1	1				2	0.03
	Pseudomonas	OTU 75	2					2	0.03
	Pseudomonas	OTU 168		1			1	2	0.03
	Pseudomonas	OTU 185					4	4	0.06
	Pseudomonas	OTU 282		2				2	0.03
	Pseudomonas	OTU 383			121	5	9	135	2.06
	Pseudomonas	OTU 419				1	1	2	0.03
	Pseudomonas	OTU 421			1	1		2	0.03
	Pseudomonas	OTU 600			3			3	0.05
	Pseudomonas	OTU 661			2			2	0.03
	Stenotrophomonas	OTU 239	3	21	16			40	0.61
	Stenotrophomonas	OTU 240	1		1			2	0.03

activities such as carbon, sulfur, nitrogen cycling essential for nutrient cycling and are used in phytoremediation, biocontrol of phytopathogens and as plant biofertilizers (Omotayo et al., 2021).

Members of these phyla have been well-described as either plant growth-promoting rhizobacteria (PGPR) or as biocontrol agents (Nagórska et al., 2007). We have previously demonstrated the biotechnological potential of several isolates obtained in the present study. For example, *Bacillus cereus sensu lato* strain B25 (OTU 2 from this work) increased grain yield following seed bacterization, as compared to a non-inoculated control (Lizárraga-Sánchez et al., 2015). *In vitro* testing of *Bacillus* spp. in maize roots and kernels inhibited growth of the phytopathogen *Fusarium verticillioides* and the production of fumonisin B1, a mycotoxin produced by this fungus (Cavaglieri et al., 2004). B25 has been identified as *Bacillus cereus* in this study (Fig. 1, OTU 2) and by phylogenomic analysis (Douriet-Gámez et al., 2018). B25 exerts biocontrol of *F. verticillioides in vitro*, in greenhouse (Figueroa-López et al., 2016) and field experiments. This bacterium also inhibits total fumonisin production in grain (Lizárraga-Sánchez et al., 2015). OTU 2 is highly represented (14.99%) in the culturable bacterial population (985 out of 6,569 16S rDNA sequences) and belongs to the core community of OTUs being present in all sites sampled.

Bacillus megaterium isolated from the maize rhizosphere has been reported to promote growth and development of *Phaseolus vulgaris* and *Arabidopsis thaliana* (López-Bucio et al., 2007), as well as *B. flexus* and *B. megaterium* (both belonging to OTU 6 from this work) have demonstrated ability for P-solubilization in maize in pot experiments (Ibarra-Galeana et al., 2017). OTU 6 also belongs to the core community of OTUs and is by far the most abundant OTU representing 23.69%, or 1,556 sequences from the culturable bacterial population. *Bacillus thuringiensis* is used as a biocontrol agent of diverse phytopathogens (Lucon et al., 2010).

In this study, the second most abundant phylum in the maize rhizosphere was Proteobacteria, in which the most predominant genera were *Enterobacter* and *Pseudomonas*. In a previous study, *E. cloacae* was reported to display an endophytic distribution within maize stem and leaf tissues, and exhibited an antagonistic effect against *F. verticillioides*

(Hinton & Bacon, 1995). In this work, this genus was represented by 407 sequences and it was distributed across 3 locations (I, II, and III). *Pseudomonas* species have been previously described as biocontrol agents (Mendes et al., 2011). Costa et al. (2006) associated the functional and structural diversity of *Pseudomonas* by matching dominant ribotypes (DGGE) of *Pseudomonas* spp. in the maize rhizosphere with PCR-DGGE fingerprints of bacterial isolates that display an antagonistic potential against the phytopathogenic bacteria *Ralstonia solanacearum*. Two abundant OTUs have been previously identified as *P. putida* (OTU 36; *Ps*3) and *P. fluorescens* (OTU 383: *Ps*42) (Figueroa-López et al., 2016). OTU 36 belongs to the core community of OTUs.

Maize root exudates, such as sugars, organic acids, aromatics, and enzymes interact with soil traits, such as pH, water potential, texture, and nutrient availability, as well as existing microbial populations to promote plant growth and development (Peiffer et al., 2013).

The slight changes on soil physicochemical parameters found in our sampling points may account for differences in microbiota composition between sites (Etesami et al., 2017; Wang et al., 2019).

Although our study provides some insight into the culturable bacterial communities from the maize rhizosphere, a more thorough investigation including culture-independent methods is necessary to resolve which bacterial communities are associated with the maize rhizosphere in agroecosystems.

Since 2009, the viability (95%) of the bacterial isolates from the collection keeps being monitored periodically and the last time the collection viability has been measured in 2019, the viability remains at 89.6%. To the best of our knowledge, this constitutes the largest culturable maize rhizospheric bacterial collection in Mexico. This study also emphasizes the importance of microbial biodiversity conservation through the creation of bacterial collections designed to explore future industrial needs.

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