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Bacterial diversity in the rhizosphere of a transgenic *versus* a conventional maize (*Zea mays*)

Diversidad bacteriana en la rizosfera de un maíz (*Zea mays*) transgénico *versus* otro convencional

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Abstract. Genetically modified crops could cause negative effects on bacterial communities. In this study, we compared the bacterial community structure of two maize cultivars to determine whether the transgenic cultivar exerts a negative effect on bacterial communities inhabiting the rhizosphere. Cultivars included the genetically modified maize (*Zea mays*), with the *pat*-gene conferring resistance to the herbicide glufosinate (synonym: L-phosphinothricin), and the hybrid, conventional maize. Metagenomic DNA was extracted from the rhizosphere of plants grown in a greenhouse. Single-strand conformation polymorphism, based on polymerase chain reaction amplifying a partial subunit rRNA gene was used to characterize and generate genetic profiles that corresponded to the bacterial communities of the amplified products from the rhizosphere of the two maize cultivars. Genetic profiles of the rhizospheres consisted of distinguishable profiles, based on the chosen primer pairs. Similarity analyses of patterns found by binary matrix analyses showed no differences in the bacterial communities of the two cultivars. This analysis showed that the microbial population's structures of the conventional and genetically modified maize were very homogeneous. Genetic modification did not adversely affect the structural bacterial community in the rhizosphere of the transgenic maize cultivar.

Keywords: *Zea mays*; Glufosinate; Metagenomic DNA; Rhizosphere; Single-strand conformation polymorphism.

Resumen. Los cultivos genéticamente modificados pueden causar efectos negativos en las comunidades bacterianas. En este estudio, comparamos las estructuras de comunidades bacterianas de dos tipos de maíz: maíz genéticamente modificado (*Zea Mays*), transformado con el gen *pat* que le confiere resistencia al herbicida glufosinato, y un maíz híbrido convencional. El objetivo fue determinar si el cultivo transgénico ejerce un efecto en las comunidades bacterianas que habitan en la rizosfera. El ADN metagenómico fue extraído de la rizosfera de las plantas crecidas bajo condiciones de invernadero, utilizando suelo de regiones donde anualmente se cultiva el maíz. Se utilizó la técnica de Polimorfismo de Conformación de Cadena Sencilla (SSCP), basada en la reacción de la cadena de la polimerasa amplificando el gen 16S rRNA para caracterizar y generar los perfiles genéticos que correspondieran a las comunidades bacterianas de los productos amplificados de la rizosfera de los dos cultivos de maíz. Los perfiles genéticos de las rizosferas consistieron de perfiles distinguibles, basado en pares de primers seleccionados. El análisis de similitud de patrones encontrados por el análisis de matriz binaria demostró que no existen diferencias significativas en las comunidades bacterianas de ambos tipos de maíz. Este análisis indicó que las estructuras de las poblaciones microbianas del maíz convencional y genéticamente modificado son muy homogéneas. La modificación genética no afectó adversamente a la estructura de la comunidad bacteriana en la rizosfera del cultivo de maíz transgénico.

Palabras clave: *Zea mays*; Glufosinato; ADN Metagenómico; Rizosfera; SSCP.

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INTRODUCTION

Genetically modified or transgenic crops may improve agricultural productivity (Clive, 2013). The global transgenic crop surface area is growing continuously yearly despite the ongoing public debate around the use and commercialization of genetically modified crops. Approximately 175.2 million hectares of genetically modified crops were grown worldwide in 2013 (Clive, 2013). The maize has the greatest number of approved events or traits (introduced genes) followed by cotton, potato, canola and soybean (Clive, 2013). In Mexico, transgenic cotton and soybean were planted in about 0.1 million hectares. Also, experimental plots are being planted in Mexico with transgenic maize (Clive, 2013). However, an important concern is the introduction of transgenic crops into agricultural ecosystems because of their potential ecological affects on soil microbial communities, in particular rhizosphere microbes due to their intimate proximity (Fang et al., 2005; Mulder et al., 2006; Xue et al., 2011).

Abiotic (e.g., soil physicochemical properties), and biotic factors (e.g., animals and grazers, plant phonology and species composition) are assumed to influence the structural and functional diversity of microbial communities in the rhizosphere (Gomes et al., 2001; Mansouri et al., 2002; Berg & Smalla, 2009). Furthermore, soil type has also been indicated as a major factor in determining the composition of rhizosphere microbial communities (Schmalenberger & Tebbe, 2002; Dohrmann & Tebbe, 2005; Fang et al., 2005; Berg & Smalla, 2009). Many studies have postulated that the composition of root exudates varies from plant to plant, and it affects the relative abundance and growth of microorganisms in the bulk soil and rhizosphere (Baudoin et al., 2003; Somers et al., 2004; Aira et al., 2010). Plant roots may exert strong effects on microbial communities on the rhizosphere through rhizodeposition of a specific root exudation and its composition (Bais et al., 2006; Nihorimbere et al., 2011). As a result, transgenic plants might change the soil environment and bacterial consortia qualitatively and quantitatively in the rhizosphere due to release of an altered composition of root exudates (Singh & Mukerji, 2006). In consequence, an altered composition of engineering roots leads to distinct microbial communities in the rhizosphere, and influence their functions (Dunfield & Germida, 2003; Hartmann et al., 2008). The release of proteins or an altered composition of root exudates from transgenic plants has been studied as a model system to evaluate the impact or effect of transgenic properties (Schmalenberger & Tebbe, 2003; Baumgarte & Tebbe, 2005; R. Miethling-Graff et al., 2010; Lottmann et al., 2010).

Moreover, it has been shown that each plants either species or cultivar can select their own specific bacterial community (Schmalenberger & Tebbe, 2002; Dohrmann & Tebbe, 2005; Buée et al., 2009). Therefore, plant genotypes might be more important than other factors in the selection of rhizobacterial communities, (e.g., soil origin, agricultural treatments: Miethling et al., 2000; Wieland et al., 2001, Schmalenberger & Tebbe, 2002; Miethling et al., 2003). However, glufosinate-resistant maize had no effect on the bacterial community composition in a field study (Schmalenberger & Tebbe, 2002). Hart et al. (2009) also showed that crop type (transgenic-glyphosate resistant corn or conventional corn) did not affect the denitrifying bacteria or fungal communities in the rhizosphere. Moreover, other studies in corn showed that no deleterious effects were caused on soil microbial communities after the release of *Bacillus thuringiensis* (Bt) (Cry endotoxin) into the soil. Other environmental factors such as plant genotype, age of plants and field heterogeneity were relatively more important (Saxena & Stotzky, 2001; Saxena et al., 2002; Blackwood & Buyer, 2004; Fang et al., 2005; Xue et al., 2011). Furthermore, Griffiths et al. (2006) concluded that although there are effects of the Bt trait or insecticides on soil microbial and faunal communities, they are relatively small compared with the main effect of soil type (field site) on all measured parameters, which may confound the effects of the natural variation between different maize lines.

Devare et al. (2004) employed a polyphasic approach, in which microbial biomass, activity, and T-RFLP analyses were combined to assess soil microbial ecology they cultivated the non-transgenic isoline CRW Bt corn, and the non-transgenic isoline treated with the pesticide tefluthrin. These authors concluded that CRW Bt corn and tefluthrin did not adversely affect neither the microbial biomass, and activity, nor the bacterial diversity or relative abundance. Moreover, Griffiths et al. (2007) conducted a greenhouse study to determine whether the variation in soil parameters under different, conventional maize cultivars exceeded differences between Bt (*Bacillus thuringiensis* protein, Cry1Ab) and non-Bt maize cultivars. Their results indicated that soil microbial community structure was affected by the plant growth stage but not by the Bt trait, and there was no measurable effect on soil microbial community structure by the Cry1Ab protein. Liu et al. (2008) compared seasonal effects of transgenic rice (express Cry1Ab protein, against lepidopteron pest) and the pesticide triazophos [3-(o,o-diethyl)-1-phenyl thiophosphoryl-1,2,4-triazol] on soil enzyme activities under field conditions. They found seasonal changes in rhizosphere soil microbial community composition throughout rice growth, indicating that the impact of the crop growth stage overweighed the application of triazophos and the *cry*1Ab gene transformation. Bt rice did not affect the rhizosphere soil microbial community composition over 2 years of rice cropping. Also, Kapur et al. (2010) performed a field experiment to determine the ecological consequences of cultivation of Bt cotton. They assessed the culturable and non-culturable microbial species in Bt cotton and non-Bt cotton soils. Their results indicated that cropping of Bt cotton did not adversely affect either the culturable or the non-culturable diversity of the microbial communities.

Few studies have indicated an influence in the composition and diversity of rhizosphere bacterial communities in agricultural soils during cropping of genetically modified crops (Dunfield & Germida, 2001; Castaldini et al., 2005; Lottmann et al., 2010). The effects of transgenic plants on the rhizosphere community have been observed, as in the case of transgenic canola (*Brassica napus*): the composition of rhizosphere bacteria of a transgenic cultivar could be distinguished from that on non-transgenic cultivars (Dunfield & Germida, 2001; Gyamfi et al., 2002; Dunfield & Germida, 2003). Dunfield & Germida (2003) conducted a field experiment to identify differences between the soil microbial community associated with growing genetically modified *versus* conventional canola. They concluded that the changes in the microbial community structure associated with genetically modified plants were temporary, and did not persist into the next field season. Also, Brusetti et al. (2005) found differences between the rhizosphere and bulk soil communities at different plant ages, as well as between transgenic Bt 176 and non-transgenic maize. These authors concluded that root exudates could determine the selection of different bacterial communities. Collectively, these studies seem to indicate that, generally, unintended modifications of rhizosphere-inhabiting communities are possible, but that the degree of variation will be influenced by the plant species and type of modification. However, effects detected to date have been minor in comparison with environmental factors such as agricultural practices, sampling date, soil type, field site, season and plant genotype (Schmalenberger & Tebbe, 2002; Dunfield & Germida, 2003; Griffiths et al., 2007; Lottmann et al., 2010). Also, maize plants (conventional and transgenic) collected 35 days after sowing established different rhizobacterial communities than those collected after 70 days grown in the same field (Schmalenberger & Tebbe, 2002).

In spite of the number of studies already done, further research is needed to clearly differentiate whether the genetic modification (e.g., transgenic maize, plant genotype) could affect the rhizobacterial communities. Moreover, it has been shown that plant age is more selective than field sites (Baumgarte & Tebbe, 2005). However, the effects of the transgenic property at a same growth stage are not clear. The objective of this study was to determine the effects of transgenic (herbicide resistant) *versus* non-engineered or conventional maize on their soil microbial community structures. Soil was recovered from the rhizosphere and analyzed by SSCP (Single Strand Polymorphism Conformation) of PCR-amplified 16S rRNA genes from the community DNA.

MATERIALS AND METHODS

Maize cultivars, soil sampling, and rhizosphere sampling. The transgenic maize (experimental line), an isogenic cultivar from the conventional maize, had the modified bacteria *pat*-gene for encoding for phosphinothricin-acetyltransferase which confers resistance to the herbicide glufosinate. The conventional maize variety used in this study was the hybrid 30P49. The agricultural soil was sandy clay, pH=8; electrical conductivity: 1.38 dS/m; organic matter: 6.75 g/kg; extractable potassium: 894 mg/kg; N-NO_3: 27.15 mg/kg; phosphorus: 10.2 mg/kg. The agricultural field selected to collect the soil samples was a site where conventional hybrid maize is cultivated (Tamaulipas, Mexico). Soil was collected from several sampling points in the field within 15 cm clearance strips and a 10 cm wide perimeter. Individual soil samples were pooled together to make 5 composite samples, followed by sieving (2 mm mesh) (Kapur et al. 2010).

The glufosinate-resistant and conventional maize plants were grown in 2.8 L pots filled with the sampling soil, and all planted pots were kept in a greenhouse (daylight approximately 12 h, average daily temperature ranged from 30 to 35 °C) (Brusetti et al., 2005). Pots were watered regularly with tapwater without fertilizer (Assigbetse et al., 2005). They were arranged in a randomized complete block design with 15 replicates per treatment. Sampling of the maize plants was performed after 30 days of plant growth (V6, vegetative stage). They were seedlings of uniform development. V6 is a phenological stage where nutrients are released by younger roots in the root hair zones, and consequently microbial activity is higher than at later stages (Semenov et al., 1999; Schmalenberger & Tebbe, 2002). Ten plants were randomly chosen from each treatment (Conventional or Transgenic maize plants); they were carefully uprooted to prevent root damage. Whole plants were placed in plastic bags and transported to the laboratory. At the laboratory, five composite samples (replicates) were obtained for each maize type by mixing two of any of the 10 sampled plants. Thereafter, a total of five samples was analysed for each treatment.

Metagenomic DNA extraction. Surrounding soil adhered two millimeters or less in diameter to roots (Hartmann et al., 2008) was separated from the bulk soil by gently shaking the root system (Barriuso et al., 2011). The term "rhizosphere" describes the narrow zone of soil that surrounds the roots (Philippot et al., 2012). Total genomic DNA was extracted from 0.25 g of rhizosphere soil samples using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to their protocol. Extracted DNA was stored at 4 °C. This kit was used because it requires less time per sample, produces less toxic waste, and recovers DNA of higher purity (Dohrmann & Tebbe, 2004).

Amplification of 16S rRNA gene from directly extracted gDNA. For bacterial community analysis, the 16S ribosomal RNA genes were amplified from the extracted community gDNA, at 50 ng/mL according to Baudoin et al. (2003) using primers Com1 (5'- CAG CAG CCG CGG TAA TAC-3') and Com2Ph (5'- CCG TCA ATT CCT

TTG AGT TT-3') (Schwieger & Tebbe, 1998), with one of the primers phosphorylated at the 5' end, resulting in PCR products corresponding to positions 519–926 of the 16S rRNA gene of *Escherichia coli* (Brosius et al., 1981). Specific primers were chosen to amplify part of the 16S rRNA gene; primers hybridizing to highly conserve regions within this gene were selected for estimates of structural diversity of the most dominant bacteria (Schmalenberger & Tebbe, 2003).

Consequently, selected phylogenetic groups of the bacterial community were amplified with taxon-specific primers followed by a second PCR (nested PCR) with the universal Com-primers (Com1 and Com2Ph). Primers used to amplify members of the α-proteobacteria were F203-α (5'-CCG CAT ACG CCC TAC GGG GGA AAG ATT TAT -3') and R1492-Ph (5'-TAC GG (G/T) TAC CTT GTT ACG ACT T -3') which was phosphorylated at the 5' end (Weisburg et al., 1991; Gomes et al., 2001). For the Actinobacteria, primers used were F243HGC-F (5'- GGA TGA GCC CGC GGC CTA-3') and R1387-Ph (5'-CGG TGT GTA CAA GG CCG GGA ACG-3') which was phosphorylated at the 5' end (Heuer et al., 1997). Annealing conditions in the PCR were 60 s at 63 °C for the Actinobacteria, and 60 s at 56 °C for the α-proteobacteria primers. The thermocycling conditions were those described by Dohrmann and Tebbe (2004). The amplification of the products was confirmed by 3% agarose gel electrophoresis. Briefly, an aliquot of the PCR products (1 μL of the PCR solution was diluted 100-fold) was added as template DNA for the second PCR for each specific group, which was conducted with Com primers, as described above, except that only 25 cycles were run. The PCR reaction mixture of 25 μL contained 0.5 μM of each primer (Alpha DNA, Montreal, QC). Each nucleotide consisted of a triphosphate at a concentration of 0.2 mM (Promega®), and 1.25 U of Taq polymerase (Go Taq, Promega, Madison, WI) with the corresponding 1× PCR buffer containing 1.25 mM $MgCl_2$. All reagents, including the Taq polymerase, were prepared as a master solution that was inserted using a pipette into the PCR tubes. Template DNA (50 ng) was added to a final volume of 25 μL for each PCR. The DNA was quantified by 1.5% agarose gel electrophoresis, using a marker of molecular weight DNA lambda (Promega-Markers® Lambda Ladders) and the Kodak MI Application, Molecular Imaging Software v.5.0.1.27 (Carestream Health, Rochester, NY). The thermocycling was conducted with 200 μL PCR tubes (Axigen®) in a Mastercycler, Eppendorf, Hamburg, Germany. All primers used in this study were synthesized by Alpha-DNA Montreal, QC.

Single strand conformation polymorphism (SSCP) analysis. The SSCP was generated for each sample to assess whether the structural diversity of the bacterial communities associated with transgenic maize were different from those associated with non-transgenic maize cultivars. The PCR products were re-suspended in 8 mL of loading buffer (formamide, EDTA, bromophenol blue, and xylene cyanole); the samples of DNA were denatured for 5 min at 95 °C, then immediately cooled on ice and loaded into the pockets of a non-denaturing, temperature controlled, vertical polyacrylamide gel for SSCP electrophoresis for 7 h at 10 °C and 50 V in a Mini-Protean 3-cell apparatus (Bio Rad). The gel was composed of 0.5 × MDE solutions (Lonza Rockland, Rockland, ME) in 0.5 × TBE buffer, 7.3 cm long, 8 cm wide, and 0.75 mm thick. After electrophoresis, the DNA was visualized with a silver staining kit (Bio-Rad Laboratories, Hercules, CA). Statistical analyses of the SSCP profiles were conducted from gels that had been loaded with the respective rhizosphere samples in a randomized order (Schwieger & Tebbe, 1998; Dohrmann & Tebbe, 2004). The polyacrylamide gels were run under the same conditions, each gel carrying samples of all replicate rhizospheres of the two types of maize.

Digital image analysis of SSCP profiles. SSCP profiles were analyzed by R software (R Foundation for Statistical Computing, Vienna, Austria). Calculation of the similarity matrix was based on a binary matrix. The clustering method used the UPGMA procedure for clustering profiles, based on their similarity (Baumgarte & Tebbe, 2005) to compare the similarity of 16S rRNA gene from SSCP profiles. We analyzed 4 replicates for each treatment for cluster analysis, except sample GM5 (see Figures 2 and 3).

RESULTS AND DISCUSSION

The plant rhizosphere is a dynamic environment in which many factors may affect the structure and species composition of the microbial communities that colonize the roots (Berg & Smalla, 2009). The yield of PCR-amplified DNA obtained from rhizosphere samples was from 65 to 380 ng of DNA/g of rhizospheric soil. It has been previously shown that rhizosphere communities vary between plant species and even between cultivars (Germida & Siciliano, 2001). SSCP profiles looking for dominant bacteria were composed of 5 main bands (Fig. 1).

SSCP targeting *Alpha-proteobacteria* was composed by a similar number of bands, but profiles for the *Actinobacteria* group showed only a few bands (Figs. 2 and 3). These results indicated a low species richness in this type of soil (semi-arid and alkaline). It has been shown that different soil textures affected more the microbial populations than transgenic varieties (Baumgarte & Tebbe, 2005; Fang et al., 2005; Barriuso & Mellado, 2012). Because of this, our study was conducted in the same type of soil. This prevented differences on soil characteristics that may be reflected in the bacterial communities or abundance.

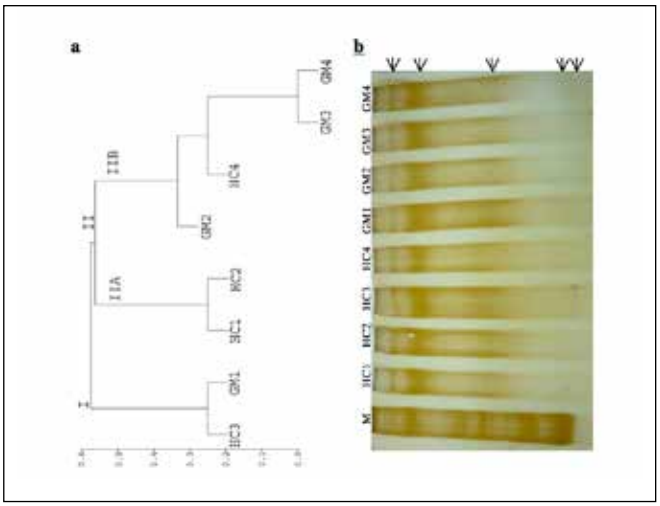

Fig. 1. (a) SSCP genetic profile from bacteria domain, from conventional hybrid (HC1-4) and genetically modified (GM5-8) maize using Marker *Xanthomonas* sp. strain. (b) UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis.
Fig. 1. (a) Perfil SSCP genético del dominio bacteria, las muestras de la rizosfera de maíz HC (carriles 1-4) y del GM (carriles 5-8) (b) Análisis de Clúster UPGMA (del inglés "Unweighted Pair Group Method using Arithmetic averages").

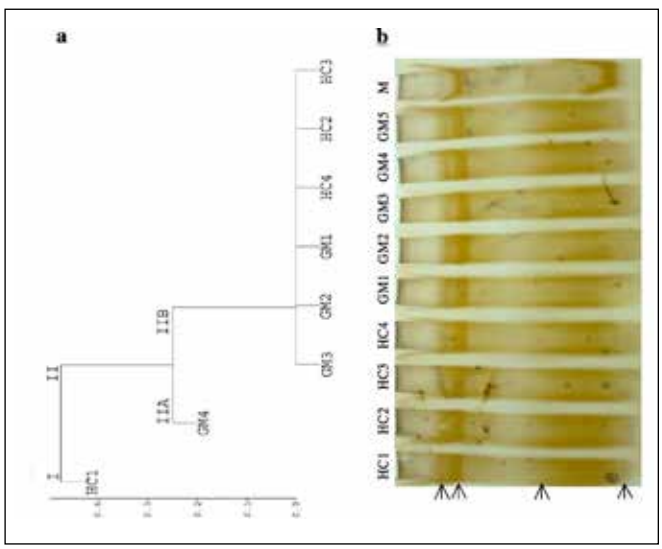

Fig. 2. (a) SSCP genetic profile from group *Alpha-proteobacteria,* from conventional hybrid (HC1-4) and genetically modified (GM5-9) maize using Marker *Xanthomonas* sp. strain. (b) UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis. Exceeding number of samples could not be analyzed on the same gel.
Fig. 2. (a) Perfil SSCP genético del grupo bacteriano específico *Alfaproteobacteria*, las muestras de la rizosfera de maíz híbrido convencional (carriles 1-4) y del GM (carriles 5-9) y utilizando como Marcador (M) la cepa *Xanthomonas* sp. (b) Análisis de Clúster UPGMA (del inglés "Unweighted Pair Group Method using Arithmetic averages"). El número de muestras exceden y no se pudieron analizar en el mismo gel.

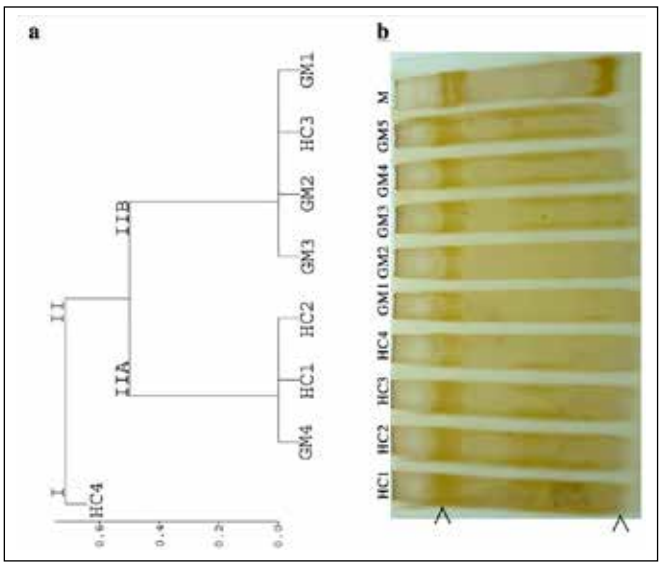

Fig. 3. (a) SSCP genetic profile from group *Actinobacteria*, from conventional hybrid (HC1-4) and genetically modified (GM5-9) maize using Marker *Arthrobacter* sp. strain. (b) UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis. Exceeding number of samples could not be analyzed on the same gel.
Fig. 3. (a) Perfil SSCP genético del grupo bacteriano específico *Actinobacteria*, las muestras de la rizosfera de maíz híbrido convencional (carriles 1-4) y del GM (carriles 5-9) utilizando como marcador (M) a la cepa *Arthrobacter* sp. (b) Análisis de Clúster UPGMA (del inglés "Unweighted Pair Group Method using Arithmetic averages"). El número de muestras exceden y no se pudieron analizar en el mismo gel.

Cluster analysis of bacterial communities formed two groups (I and II). Group I presented a 42.5% similarity to Group II. Group II was further divided in two subgroups (IIA and IIB), where Group IIA had a similarity of 46% to Group IIB. These subclusters contained most of the bacterial communities from the rhizosphere of both transgenic and conventional maize. Group I contained profiles of conventional and transgenic maize. Using the universal bacterial domain, there were no differences in the structure of the bacterial communities (Fig. 1a). Previous studies have suggested that microbial community composition in maize is independent of the study cultivar (Schmalenberger & Tebbe, 2002; Dohrmann et al., 2013), on soil properties (Baumgarte & Tebbe, 2005), genotypes (Aira et al., 2010), and growth stages (Gomes et al., 2001; Li et al., 2014). Therefore, these results differ from those reported by Aira et al. (2010) who mention that plant genotype modifies the structure of maize rhizosphere microbial communities.

Bacteria domain cluster patterns shared five bands, and some of them were dominant bands in the acrylamide gel (Fig. 1b). This was attributed to members of specific groups, such as profiles of α-*Proteobacteria* (Fig. 2) and *Actinobacteria* (Fig. 3).

The profiles obtained by using the specific primers for α-*Proteobacteria* and *Actinobacteria* were present in lower

numbers than the nonspecific profiles (universal bacterial community primers). This result suggests that the two groups are less represented or less diverse. Further, our results suggest that the dominant members of the bacteria communities were similar and ubiquitous in both types of maize, and there were no important changes at this level in the bacterial structure in either type of maize, as postulated by Baumgarte & Tebbe (2005). From the structural diversity of the α-*Proteobacteria* SSCP profiles, we found four bands that were shared (Fig. 2). Cluster analysis showed that Groups I and II had about 50% similarity. Subgroups IIA and IIB were similar (75%) in the transgenic and conventional maize rhizospheres. Subgroup IIB contained most of the replicates of the α-*Proteobacteria* community in the transgenic and conventional maize rhizospheres. The remaining replicates were contained in Group I (in conventional maize), and only in one replicate of the transgenic maize in Subgroup IIA (Fig. 2). This was most likely due to artifact technical manipulation.

The *Actinobacteria* profiles contained one strong band that was shared by all replicates. These bands could be due to a specific Actinobacteria group highly represented at the rhizosphere of both types of maize. The similarities of the SSCP patterns were low for Groups I and II (28%). Group II contained almost all bacteria communities, and when separated into Subgroups IIA and IIB, similarity between the subgroups was even 50%. Thus, these results indicated that these groups were different, and that the plant can select for species-specific richness (Hartmann et al., 2004). However, both groups shared similar species richness derived from conventional and genetically modified maize. Also, our results indicate that it does not exist a drastic shift in the bacterial populations that inhabited the rhizosphere from both types of maize. Most replicates in Group II from these profiles were similar in transgenic and conventional maize, as found for the α-*Proteobacteria* cluster analysis. The remaining samples of conventional maize rhizospheres were found in Group I (Fig. 3). As it was mentioned before, even for *Actinobacteria,* differences between transgenic and non-transgenic maize were not present.

Differences in the specific profiles of the cultivars, particularly those of the transgenic maize, could not be detected. No differences in bacteria community profiles were found between transgenic and conventional maize rhizospheres, confirming our greenhouse results.

Earlier reports have indicated changes on microbial communities with the use of genetically modified crops (Dunfield & Germida, 2003; Brusetti et al., 2005). However, the SSCP analysis showed that the conformation of the rhizosphere microbial structure did not significantly differ between the conventional and genetically engineered maize with the *pat* gene. Our results are consistent with those reported by Schmalenberger & Tebbe (2002) and Dohrmann et al. (2013) based on the rRNA gene profiling technique and pyrosequencing under the same field and climate conditions. Schmalenberger & Tebbe (2002) found patterns of dominant bacteria in maize rhizosphere. Dohrmann et al. (2013) indicated that the rhizobacterial community of a GM maize did not respond drastically to the presence of proteins in the root tissue. The individual genetic profiles were very similar and reduced, because few bands were observed even if we used the domain bacteria α-*Proteobacteria* and *Actinobacteria*. A few minor variations on microbial community structures could have occurred because of the similar environmental factors. The results presented in this study contribute to the idea that the extent to which the plant influences community composition and structure in the rhizosphere may be different depending not only on the plant species but also on small modifications in their genotype (transgenic plants). This has been shown by other reports (Dunfield & Germida, 2003).

CONCLUSIONS

The present study of bacterial richness in the rhizosphere indicated that herbicide-resistant, transgenic maize did not cause adverse effects or changes on the structure of the microbial community. The structure of the bacterial communities was stable in the *Bacteria* domain, *Alpha-Proteobacteria* and *Actinobacteria* groups from transgenic maize with respect to conventional maize using SSCP analysis. Only a few variations were observed but no drastic changes. This study contributed to resolve some questions about the safe option of using transgenic crops, in this case, herbicide-resistant transgenic maize.

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