doi: 10.17221/523/2017-PSE

Unravelling the composition of soil belowground microbial community before sowing transgenic cotton

Lourdes VITAL, Jose A. NARVAEZ, Maria Antonia CRUZ, Eyra L. ORTIZ, Eric SANCHEZ, Alberto MENDOZA*

Instituto Politécnico Nacional, Reynosa, México
**Corresponding author: amendozah@ipn.mx*

ABSTRACT

Vital L., Narvaez J.A., Cruz M.A., Ortiz E.L., Sanchez E., Mendoza A. (2017): Unravelling the composition of soil belowground microbial community before sowing transgenic cotton. Plant Soil Environ., 63: 512–518.

Soils harbour enormously diverse bacterial communities that interact specifically with plants generating beneficial interactions between them. This study was the first approach to assess bacterial communities before sowing with three cotton genotypes, including both transgenic and conventional ones. The structure of bacterial communities was identified using the next generation sequencing analysis, ion torrent PGM (Personal Genome Machine™) sequencer technology, based on the V2–V3 16S rRNA gene region. Quantitative insights into microbial ecology pipeline were used to identify the structure and diversity of bacterial communities in bulk soil samples collected in the northeast of Mexico. Bulk soil textures and chemical properties, including most nutrients, were homogeneous in these bulk soil samples. Relative abundance analysis showed similar bacterial community structures. Dominant taxonomic phyla were Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, Gemmatimonadetes and Bacteroidetes, whereas the main families were Bacillaceae, Chitinophagaceae and Rhodospirillaceae with an abundance average of BS1 (bulk soil sample), BS2 and BS3 (24.85, 19.74 and 19.71%, respectively). Alpha diversity analysis showed a high diversity (Shannon and Simpson index) and a large value of the observed species found in bulk soils samples. These results allowed establishing the previous bacterial structural community in an unused soil before sowing it with a transgenic crop for the first time.

Keywords: rhizosphere; *Gossypium*; plant-microbe interactions; field

The composition of rhizosphere microorganisms in soils can vary greatly across space, plant species, soil type, root architecture and growth stage (Berg and Smalla 2009). Physicochemical and biological features have an important role in the soil for the establishment of plant-microbe interactions (Janssen 2006). As it was demonstrated, soil pH, structure, oxygen and nutrition levels in the rhizosphere differ from those in the bulk soil (Roesch et al. 2007). The soil is considered to harbour the most diverse bacterial communities on earth providing habitats for them (Roesch et al. 2007). Soil microbial communities play an important role in plant health, soil quality and ecosystem sustainability of agricultural systems (Rincon-Florez et al. 2013). Bacterial communities are important drivers for all biogeochemical cycles in terrestrial ecosystems and participate in most nutrient transformations in soil (Roesch et al. 2007, Rincon-Florez et al. 2013). There are biotic and abiotic factors that are assumed to influence the structural and functional diversity of bacterial communities in the rhizosphere soil, such as climate, season, herbicide application, management practices (Galazka et al. 2017a,b), integrated livestock-crop system (Acosta-Martínez

Supported by the National Council of Science and Technology (CONACYT-CVU/Becario: 421740), Grants No. SIP-20151063 and 20161362.

doi: 10.17221/523/2017-PSE

et al. 2010), soil quality (Galazka et al. 2017b), plant developmental stage and plant species (Berg and Smalla 2009). Transgenic plants can release proteins from the roots due to introduced genes, *Bt* protein being the most common (Schmalenberger and Tebbe 2002). To date, there are several strong controversies about the transgenic crops effects, not only in human and animal health but also in environmental features (Dunfield and Germida 2003). It has been showed that transgenic plants have a substantial influence on the structure of rhizobacterial communities (Dunfield and Germida 2003). However, in another study the bacterial communities were affected only by growth stage, herbicide application or soil type more than genetic modification (Schmalenberger and Tebbe 2002). Although molecular methods have been used to study the structure, diversity, and activity of soil bacterial communities (Rincon-Florez et al. 2013), few studies have employed the next generation sequencing (NGS) techniques to study the structure of bacterial communities in soils just before GMO (genetically modified organism) seeds sowing. The aim of the study was to characterize the ecological status of the diversity in soil microbial community before the introduction of transgenic crops to determine the effects of conventional or transgenic plants sown on these unused soils.

MATERIAL AND METHODS

Description of the field trial and sample collection. The study was performed in an experiment, established in the spring 2015 in the north of Tamaulipas, México (25°96'40'N, 98°01'85'W). These bulk soil samples were categorized according to the type of cotton that would be planted next season: conventional 7A21 cotton (BSI); transgenic FM1740 B2RF cotton (BS2); and transgenic FM9250 GL cotton (BS3). Sampling soil was carried out in random, generating six sampling plots (~15 m^2) (two plots for each cotton cultivar) and in each plot three sampling spots were selected randomly. Bulk soil samples were placed in labelled bags, kept at 4°C, and transported to the laboratory. The bulk soil samples were combined in a composite sample. Samples were homogenized and passed through a 3 mm sieve. Soil analyses were carried out in the Experimental Field Rio Bravo at the National Research Institute Rio Bravo, Tamaulipas. The pH was measured in water solution (1:2); organic carbon (OC) was evaluated from inorganic carbon organic matter by the method of potassium dichromate (Walkely and Black); electrical conductivity (EC) was measured by using a potentiometer in a saturated paste; available inorganic nitrogen (NO_3^--N) by using the method of salicylic acid; available P was obtained by the method of Olsen; texture by the method of Bouyocos; available Fe by the method of DTPA (diethylenetriaminepentaacetic acid); K_{ex} was extracted by the ammonium acetate method; and Na, Mg, Ca and K (soluble cations) were measured by using an atomic absorption spectrophotometer in a saturated paste.

DNA extraction from soils and construction of the V2-V3-16S rDNA libraries. A total of 27 DNA extractions were extracted (three composite bulk soils per three replicates independently of DNA per three field plots) using the Power Soil DNA Isolation Kit (MOBIO, Carlsbad, USA). The quantity of purified DNA was measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and the quality was also determined by electrophoresis using the 1% agarose gel. For the nested polymerase chain reaction (PCR), the 16S rRNA gene was first amplified from the extracted community DNA by using primers 27F (GAGAGTTTGATCCTGGCTCAG) and 1495R (CTACGGCTACCTTGTTACGA). PCR conditions were; 5 min 95°C; 35 cycles of 60 s at 95°C, 60 s at 60°C and 90 s at 72°C; followed by 10 min at 72°C (Grifoni et al. 1995). Then, an aliquot (100 ng/µL) of the PCR products was used as a template for the V2–V3 region (252 bp) amplification. Conditions for this second PCR were; 5 min 95°C; 25 cycles of 30 s at 95°C, 30 s at 60°C and 45 s at 72°C, followed by 10 min at 72°C (Wuyts et al. 2002). The sequences of Forward primer and Reverse primer are given in Table 1 using the Ion Xpress barcodes (Life Technologies). All composite samples were pooled in equimolar concentrations and fractionated by electrophoresis in 3% agarose gel. Fragments of the 252 bp were purified from the gel by using the Wizard® SV Gel and PCR Clean-Up System (Promega®, Madison, USA). The DNA concentration of each library was quantified using the Qubit® 2.0 Fluorometer with Qubit™ dsDNA HS Assay Kit (Invitrogen™, Thermo Fisher Scientific, Wilmington, USA) according to the manufacturer's protocol. The amplicons were purified using the Agencourt AMPure XP

doi: 10.17221/523/2017-PSE

Table 1. Sequences of primers utilized in this study

Primer	Ion torrent linker primer	Golay barcode	Spacer	Linker-primer forward
V2-V3_344 F_BC8	CCATCTCATCCCTGCGTGTCTCCGAC TCAG	TTCCGATAAC	GAT	ACGGRAGGCAGCAG
V2-V3_344 F_BC9	CCATCTCATCCCTGCGTGTCTCCGAC TCAG	TGAGCGGAAC	GAT	ACGGRAGGCAGCAG
V2-V3_344 F_BC10	CCATCTCATCCCTGCGTGTCTCCGAC TCAG	CTGACCGAAC	GAT	ACGGRAGGCAGCAG
V2-V3_E534 R_trP1	CCTCTCTATGGGCAGTCGGTGAT	not applicable		ATTACCGCGGCTGCTGGC

(Beckman Coulter, Inc®, USA) system. Then, each library was quantified and pooled together with the equimolar amounts (60 pmol/L). The emulsion polymerase chain reaction was carried out using the Ion OneTouch™200 Template v2 DL (Life Technologies®, Thermo Fisher Scientific, Wilmington, USA) according to the manufacturer's instructions. Libraries were sequenced at the Ion Torrent PGM (Life Technologies®). Template enrichment with Ion Sphere Particles (ISPs) was employed the Ion OneTouch™ 2 System (Life Technologies®). The sequencing was carried out using the Ion 314 Chip Kit v2 and the Ion Torrent PGM™ platform (Life Technologies®).

Metagenomic sequence analysis. Reads were filtered by the Ion Torrent PGM software according to Murugesan et al. (2015) based on their barcodes in bulk soil. All raw reads were trimmed to the removal of tags, primers and these were quality-filtered by using the Trimmomatic (quality score > 20, read length = 150–190 bp). Sequence analyses were processed by using the QIIME-1.9.0-amd64. vdi version (Caporaso et al. 2010). Open reference operational taxonomic units were determined at the 97% similarity using the USEARCH algorithm (Edgar 2010). Sequence alignments were done against the Greengenes core set (DeSantis et al. 2006).

Diversity, statistical and bioinformatics analysis. Alpha diversity was computed to estimate the observed species (operational taxonomic units – OTUs), species richness with the Chao1 estimator, species diversity with Shannon and Simpson. Data of the biochemical characteristics of soil were analysed by one-way ANOVA at $P < 0.05$ level of significance and the Tukey's post hoc analyses to compare three soils samples ($n = 3$) by using the Statistica package (Statistica v.8.0, Statsoft, Tulsa, USA). A heatmap was generated using the statistical analyses of metagenomic profiles (STAMP) software (Parks et al. 2014) for bacterial relative abundances. The associated dendrograms were obtained using the unweighted pair group method with arithmetic mean with a clustering threshold of 0.75. Resulting sequencing data sets were uploaded to the NCBI server (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/). SRP100208 sequence read archive submission was processed.

RESULTS AND DISCUSSION

The agricultural soil texture of bulk soil sample 1 (BS1), 2 (BS2) and 3 (BS3) appears to be a loam soil. The mean pH of all bulk soil samples was mildly alkaline, the mean organic matter content and electric conductivity were similar in all three bulk soil samples ($P < 0.05$). The ANOVA and Tukey's comparisons showed that different soils samples did not vary significantly in nutrients such as P, Fe, Cu, Zn and K (Table 2). The only significant differences between bulk soil samples were observed for removable K, NO_3^--N, Mn, Ca and Na values. Therefore, the soil texture and composition were uniform across all fields. This result is in agreement with Garza-Cano et al. (2005) who concluded that soils of this region are poor in nitrogen and phosphorus, and thus the bacterial communities are adapted to these conditions.

Also, it is known that there is a strong correlation between the parameters of soil quality and biodiversity indicators (Gałązka et al. 2017a). Bacterial communities structures were determined before sowing plants according to the season, plant species and growth, which are important factors affecting soil microbial communities (Berg and Smalla 2009, Gałązka et al. 2017a). Our results are in compliance with the findings of Schmalenberger

doi: 10.17221/523/2017-PSE

Table 2. Chemical properties of soil samples

Property		BS1	BS2	BS3
OC	(%)	1.11 ± 0.17^{a}	0.53 ± 0.17^{a}	1.07 ± 0.17^{a}
pH	–	7.69 ± 0.12^{a}	7.95 ± 0.12^{a}	8.02 ± 0.12^{a}
EC	(dS/m)	1.44 ± 0.29^{a}	1.73 ± 0.23^{a}	1.22 ± 0.35^{a}
K		1946 ± 11.55^{a}	1666 ± 10.39^{b}	1326 ± 12.70^{c}
NO_3^--N		31.75 ± 1.44^{a}	31.92 ± 1.50^{a}	23.9 ± 1.73^{b}
P		28.8 ± 2.02^{a}	29.7 ± 1.73^{a}	26.7 ± 2.30^{a}
Fe		0.513 ± 0.03^{a}	0.586 ± 0.01^{a}	0.593 ± 0.00^{a}
Cu		0.895 ± 0.01^{a}	0.874 ± 0.02^{a}	0.891 ± 0.01^{a}
Zn	(mg/kg)	0.592 ± 0.02^{a}	0.650 ± 0.01^{a}	0.629 ± 0.01^{a}
Mn		41.977 ± 2.89^{a}	49.757 ± 0.58^{b}	37.601 ± 2.31^{a}
Ca^{2+}		95.59 ± 0.58^{b}	202.60 ± 0.87^{a}	56.91 ± 0.58^{c}
Mg^{2+}		12.15 ± 0.35^{a}	13.49 ± 0.07^{b}	12.88 ± 0.14^{ab}
Na^{+}		360.87 ± 0.27^{a}	360.41 ± 0.40^{a}	320.39 ± 0.29^{b}
K^{+}		10.17 ± 0.68^{a}	11.34 ± 0.23^{a}	10.17 ± 0.68^{a}

Data are shown as means and standard errors (n = 3); means followed by different letters are significantly different (Tukey's test; $P < 0.05$). S1, S2, S3 – bulk soil sample 1, 2 and 3; OC – organic carbon; EC – electrical conductivity; $^{+}$cations soluble in water

and Tebbe (2002) who proposed that soil quality have to be as homogeneous as possible for this type of studies involving GMOs. A total of 136 796 sequences from three samples were obtained from the NGS reactions and barcoding allowed us to analyse the samples (BS1 = 47 530; BS2 = 50 845; BS3 = 38 421). The bulk soil based on 16S rRNA gene profiling was characterized; the most abundant Phyla are: Proteobacteria (BS1 = 31.96%, BS2 = 38.62%, BS3 = 38.85%), Firmicutes (BS1 = 19.52%, BS2 = 11.39%, BS3 = 11.05%), Acidobacteria (BS1 = 12.64%, BS2 = 12.55%, BS3 = 12.98%), Actinobacteria (BS1 = 13.34%, BS2 = 10.33%, BS3 = 11.10%), Gemmatimonadetes (BS1 = 12.70%, BS2 = 16.46%, BS3 = 16.25%) and Bacteroidetes (BS1 = 4.94%, BS2 = 6.61%, BS3 = 5.43%) (Figure 1a). The relative abundances of the dominant phyla are similar to the results reported by Janssen (2006), where the majority of 16S rRNA gene soil clone libraries belong to the eight major bacteria phyla mentioned above. These results are consistent with previous studies that reported a large proportion of Firmicutes in soil (Acosta-Martínez et al. 2010). Phylum Proteobacteria show an extreme metabolic diversity and biological importance due to key roles

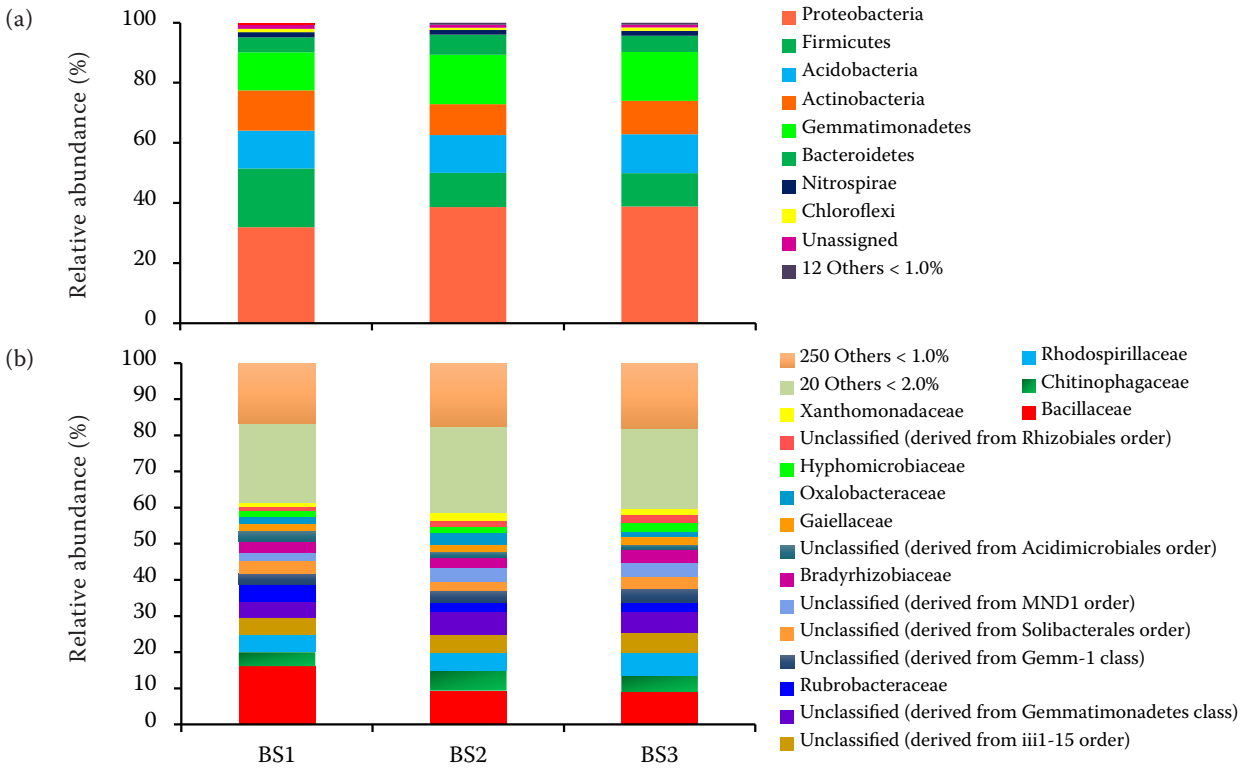

Figure 1. Taxonomic breakdown resulting from massive sequencing V2–V3 of the 16S rRNA of the bacterial communities of bulk soil samples. BS1, BS2, BS3 – bulk soil sample 1, 2 and 3. (a) All phyla and (b) all families

doi: 10.17221/523/2017-PSE

they play in the carbon, sulfur and nitrogen cycles (Kersters et al. 2006). Acidobacteria are among the most abundant bacterial phylum in a wide range of environments including soils (Janssen 2006) and members of this phylum may be important drivers of key processes in terrestrial ecosystems (Kielak et al. 2010). It has been suggested that Actinobacteria spp. in tropical soils represent a vast unexplored resource for the biotechnology of bioactive production (Suela Silva et al. 2013). Members of this phylum are reported to be an important component of soil communities playing a major role in organic matter turnover in soils and carbon cycling (Hodgson 2000). It has been shown that the relative abundance of Bacteroidetes was highest in soils with high carbon availability and organic matter decomposers (Fierer et al. 2007). The relative abundance of the major families was similar in all bulk soil samples where the Bacillaceae family (BS1 = 16.13%, BS2 = 9.37%, BS3 = 8.97%) was the most dominant in all bulk soil samples followed by the Chitinophagaceae (BS1 = 3.86%, BS2 = 5.43%, BS3 = 4.44%) and Rhodospirillaceae family (BS1 = 4.90%, BS2 = 4.94%, BS3 = 6.30%) (Figure 1b). This trend could emphasize the ability of these types of bacterial communities to survive very low substrate availability under lack of OC, NO_3^--N, phosphate and potassium. It is possible that these types of bacteria are able to survive extreme environmental conditions and appear to play an important role in sustaining soil processes. The total number of species observed was 6312 for BS1, 6772 for BS2 and 5540 for BS3 (Figure 2). Bulk soil samples show the largest expected number of species (Chao1 mean ± SE (standard error)), BS1 = 8215.696 ± 101.625, BS2 = 8591.013 ± 95.965, BS3 = 7660.995 ± 116.960 (Figure 2). Shannon's index shows that BS1 = 7.26, BS2 = 7.44 and BS3 = 7.23 are diverse. Simpson's index shows large values of dominance for BS1 = 0.9969, BS2 = 0.9978 and BS3 = 0.9972 and and some species dominate and are equally distributed in these soil environment (Figure 2). In addition, Kennedy (1999) and Acosta-Martínez et al. (2010) reported that diversity index is a single value that cannot fully represent the total makeup of a community, and thus, two communities may have the same diversity index value, but one may comprise low evenness and high richness, and the other may comprise high evenness and low richness. When comparing the bacterial communities across all samples at the genus level, the hierarchical clustering of bacterial profiles (vertical axis) shows that BS3 and BS2 samples were phylogenetically related with 100% similarity. BS1 was grouped in a separate branch of the tree and revealed that bacterial communities from S1 were at about 60% similar with BS2 and BS3. In our work, genus *Bacillus* sp. was more abundant in all bulk soil samples with a similar relative abundance (BS1 = 15.84%; BS2 = 9.17%; BS3 = 8.78%) followed by unclassified species derived from Gemmatimonadetes, iii-15, Rhodospirillaceae, Gemm-1, Solibacterales, Rubrobacteraceae, *MND1*, Flavisolibacter and Acidimicrobiales (Figure 3). The predominance of Firmicutes is attributed to their ability to encompass several spore-forming genera and these are able to survive under extreme conditions for a long time (Teixeira et al. 2010). *Bacillus* species are widely spread in soil environments and for many isolates they have numerous functions in

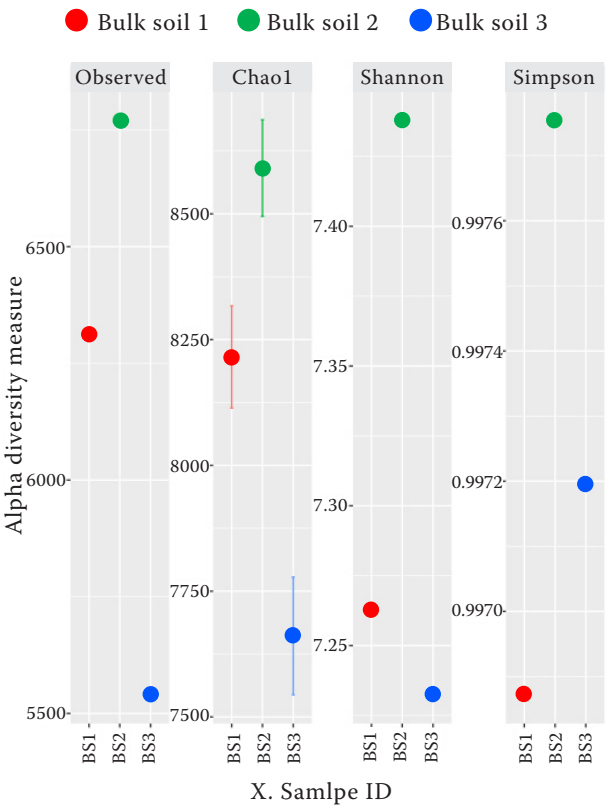

Figure 2. Taxonomic diversity features of bulk soil based on 16S rRNA plots. Alpha diversity measurements for bulk soil samples (BS1, BS2, BS3 – bulk soil sample 1, 2 and 3). Estimated number of the observed species, Shannon index, Simpson index and Chao1 for each sample

doi: 10.17221/523/2017-PSE

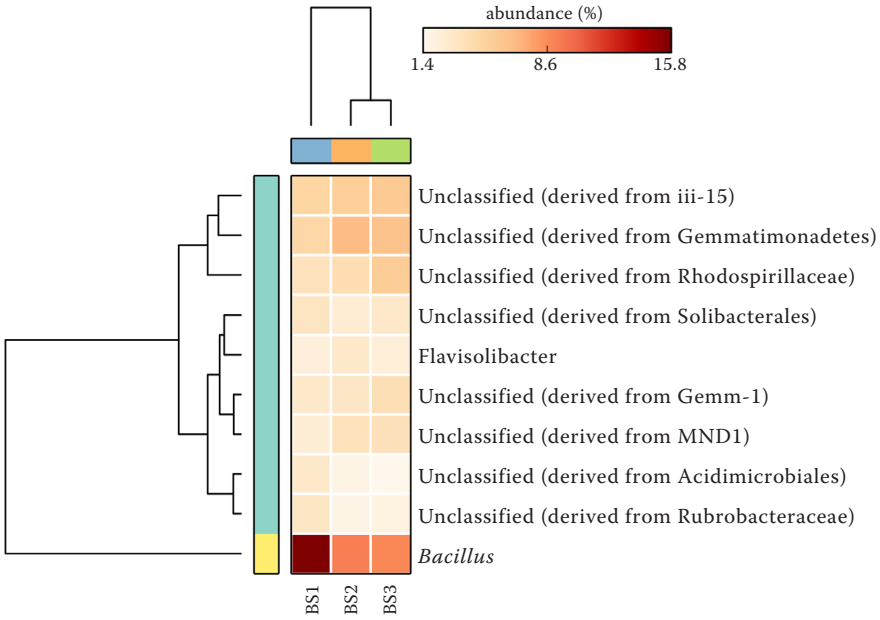

Figure 3. Heatmap showing the most abundant OTUs (operational taxonomic units) and hierarchical clustering of bacterial relative abundance. The figure shows in a colour representation how sampling soils 1 (BS1), 2 (BS2) and 3 (BS3) are clustered in the vertical axis and genera in the horizontal axis according to their abundance. The intensity of relative abundance ranges from 1.4 to 15.8 in and colour intensity ranges from orange (low abundance) to red (high abundance)

soils (Hong et al. 2009). The bacterial community structure and function may be also changed under different management practices, seasons and plant genotype (Dunfield and Germida 2003, Galazka et al. 2017a).

In conclusion, the massive sequencing analyses revealed that composition and dynamics of bacterial community in bulk soil samples were dominated by six phyla: Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, Gemmatimonadetes, and Bacteroidetes. The more abundant genus was *Bacillus* sp., which may play major roles in soil physicochemical changes and has essential functions associated to crops, considering some *Bacillus* sp. as plant growth promotion rhizobacteria as well as the Acidobacteria group that has the ability to use nitrite as an nitrogen source, responding to soil macro and micronutrients. Some other unclassified bacteria were detected where their function remains unknown such as Gemmatimonadetes, iii-15, Rhodospirillaceae, Gemm-1, Solibacterales, Rubrobacteraceae, MND1, Flavisolibacter, Acidimicrobiales, Gaiellaceae, Rhizobiales, Chitinophagaceae and Gemm-5. Members of the order Rhizobiales and family Rhodospirillaceae are of agricultural and ecological importance. The semiarid bulk soils share similar species richness, abundance and diversity. Such data are valuable and this study represents a broad initial background before planting and the first overview of the bacterial communities of a GMOs free soil located in the northeast of Mexico to discern the possible impact of transgenic cotton on the bacterial community associated to its rhizosphere.

Acknowledgement

We thank M. Sc. Manuel Moreno Legorreta for his statistical corrections and Susana Fernandez for her technical support.

REFERENCES

Acosta-Martínez V., Dowd S.E., Sun Y., Wester D., Allen V. (2010): Pyrosequencing analysis for characterization of soil bacterial populations as affected by an integrated livestock-cotton production system. Applied Soil Ecology, 45: 13–25.

Berg G., Smalla K. (2009): Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiological Ecology, 68: 1–13.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koening J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. (2010): QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7: 335–336.

DeSantis T.Z., Hugenholtz P., Larsen N., Rojas M., Brodie E.L., Keller K., Huber T., Dalevi D., Hu P., Andersen G.L. (2006):

doi: 10.17221/523/2017-PSE

Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology, 72: 5069–5072.

Dunfield K.E., Germida J.J. (2003): Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified canola (*Brassica napus*). Applied and Environmental Microbiology, 69: 7310–7318.

Edgar R.C. (2010): Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26: 2460–2461.

Fierer N., Bradford M.A., Jackson R.B. (2007): Toward an ecological classification of soil bacteria. Ecology, 88: 1354–1364.

Gałązka A., Gawryjołek K., Grządziel J., Frąc M., Księżak J. (2017a): Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant, Soil and Environment, 63: 264–270.

Gałązka A., Gawryjołek K., Grządziel J., Księżak J. (2017b): Effect of different agricultural management practices on soil biological parameters including glomalin fraction. Plant, Soil and Environment, 63: 300–306.

Garza-Cano I., Pecina-Quintero V., Díaz-Franco A., Williams-Alanís H., Ramírez-De León A. (2005): Sorghum cultivated with biofertilizers, fitohormones, and inorganic phosphorus. Terra Latinoamericana, 23: 581–586.

Grifoni A., Bazzicalupo M., Di Serio C., Fancelli S., Fani R. (1995): Identification of *Azospirillum* strains by restriction fragment length polymorphism of the 16S rDNA and of the histidine operon. FEMS Microbiology Letter, 127: 85–91.

Hodgson D.A. (2000): Primary metabolism and its control in streptomycetes: A most unusual group of bacteria. Advances in Microbial Physiology, 42: 47–238.

Hong H.A., To E., Fakhry S., Baccigalupi L., Ricca E., Cutting S.M. (2009): Defining the natural habitat of *Bacillus* spore-formers. Research in Microbiology, 160: 375–379.

Janssen P.H. (2006): Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology, 72: 1719–1728.

Kennedy A.C. (1999): Microbial diversity in agroecosystem quality. In: Collins W.W., Qualset C.O. (eds.): Biodiversity in Agroecosystems. Boca Raton, CRC Press, 1–17.

Kersters K., De Vos P., Gillis M., Swings J., Vandamme P., Stackebrandt E. (2006): Introduction to the Proteobacteria. In: Dwarkin M., Falkow S., Rosenberg E., Schleifer K.H., Stackebrandt E. (eds): The Prokaryotes. 3rd Edition. Volume 5. New York, Springer, 3–37.

Kielak A.M., van Veen J.A., Kowalchuk G.A. (2010): Comparative analysis of acidobacterial genomic fragments from terrestrial and aquatic metagenomic libraries, with emphasis on acidobacteria subdivision 6. Applied and Environmental Microbiology, 76: 6769–6777.

Murugesan S., Ulloa-Martínez M., Martínez-Rojano H., Galván-Rodríguez F.M., Miranda-Brito C., Romano M.C., Piña-Escobedo A., Pizano-Zárate M.L., Hoyo-Vadillo C., García-Mena J. (2015): Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. European Journal of Clinical Microbiology and Infectious Diseases, 34: 1337–1346.

Parks D.H., Tyson G.W., Hugenholtz P., Beiko R.G. (2014): STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics, 30: 3123–3124.

Rincon-Florez V.A., Carvalhais L.C., Schenk P.M. (2013): Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity, 5: 581–612.

Roesch L.F., Fulthorpe R.R., Riva A., Casella G., Hadwin A.K., Kent A.D., Daroub S.H., Camargo F.A., Farmerie W.G., Triplett E.W. (2007): Pyrosequencing enumerates and contrasts soil microbial diversity. The International Society for Microbial Ecology Journal, 1: 283–290.

Schmalenberger A., Tebbe C.C. (2002): Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transgenic cultivar Bosphore. FEMS Microbiology Ecology, 40: 29–37.

Suela Silva M., Naves Sales A., Magalhães-Guedes K.T., Ribeiro Dias D., Freitas Schwan R. (2013): Brazilian Cerrado Soil Actinobacteria Ecology. Hindawi Publishing Corporation. BioMed Research International. Available at: http://dx.doi.org/10.1155/2013/503805

Teixeira L.C., Peixoto R.S., Cury J.C., Sul W.J., Pellizari V.H., Tiedje J., Rosado A.S. (2010): Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. The International Society for Microbial Ecology Journal, 4: 989–1001.

Wuyts J., Van de Peer Y., Winkelmans T., De Wachter R. (2002): The European database on small subunit ribosomal RNA. Nucleic Acids Research, 30: 183–185.

Received on August 18, 2017
Accepted on November 1, 2017
Published online on November 14, 2017