Response to Edaphoclimatic Conditions and Crop Management of the Bacterial Microbiome of *Musa acuminata* Rhizosphere Profiled by 16S rRNA Gene Amplicon Sequencing

Francisco J. De la Torre-González,a Elisa Fernández-Castillo,a Dailen Azaharez-Llorente,b,d Jorge Lara,a Enrique Avendaño,a Alexis Castañeda,a Sergio Gómez,a José Gaxiola,c Ali Asaff-Torres,a,b Jorge Verdín,d

aInnovak Global, Chihuahua, Mexico
bCIAD-Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, Mexico
cGrupo Bananero “E”, Plantación Sonora, Teapa, Tabasco, Mexico
dCIATEJ-Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Zapopan, Jalisco, Mexico

Francisco J. De la Torre-González, Elisa Fernández-Castillo, Dailen Azaharez-Llorente, and Jorge Lara contributed equally to this work. Author order was determined randomly.

ABSTRACT  Bacterial rhizospheric microbiomes of *Musa acuminata* cultivated in farms close to the west and east Mexican coasts and with different climate, soils, and crop management practices were characterized by 16S rRNA gene amplicon sequencing. Results showed that rhizospheric microbiome composition changed along with seasonal weather but were mostly indifferent to soil type.

Banana (*Musa* spp.) is one of the most produced crops in the world (1). The rhizosphere and endosphere microbiomes of *Musa* spp. have been intensely investigated to address the major threat to banana production, *Fusarium* wilt (2–7). Nevertheless, the dynamic behavior of such microbiomes throughout seasons in different climates, soil types, and crop management practices have not been analyzed, despite the fact that they may provide valuable information to prevent diseases or increase productivity. Here, we report the 16S rRNA gene profiling of the *Musa acuminata* rhizosphere cultivated in two climate regimes, different soil types, and different types of crop management, which include the addition of biostimulants.

Bulk soil and roots of banana plants conventionally cultivated with or without microbial biostimulants (BioFit RTU and Mycoroot, Innovak, Mexico; 1 kg BioFit RTU + 1 kg Mycoroot/200 liters water/ha, three 4-month-spaced applications during the cropping year; 2 kg Mycoroot/200 liters water/ha, 2 weeks after each dual application) were sampled (random blocks) in three farms from southern Mexico close to the east and west coasts—plantation SB, Chiapas (14°54′25″N, 92°26′26″W; average temperature, 26.3°C; average annual rainfall, 2,158 mm), and plantations SO and RE, Tabasco (17°37′31″N, 92°57′05″W; average temperature, 26.9°C; average annual rainfall, 3,862 mm) (8, 9). The Chiapas samples were collected only during December 2017, while samples from Tabasco were collected from banana cultivated in three soil types, sandy loam, clay loam, and silty loam. All samples were triplicated, each one composed of seven plant roots (collected during the fluorescence emission of the mother plant) and seven 20-cm-deep soil columns for rhizosphere and bulk soil samples.

For rhizospheric DNA extraction, excess soil on the roots was mechanically removed. Then, the roots were washed in 200 ml sterile phosphate-buffered saline (PBS)-Silwet Maxx (Arysta LifeScience, Mexico) (0.02% [vol/vol]) on sterile bottles and shaken at 250 rpm, 4°C for 20 min. Afterward, washed roots were taken off and submerged again in PBS-Silwet Maxx under sterile conditions, shaken at 250 rpm at 4°C for 20 min, and sonicated (VCX-
| Location          | Soil type   | Sample type  | Sampling date (mo/yr) | Sample name                                     | SRA accession no. for Rep 1 | Rep 2 | Rep 3 |
|-------------------|-------------|--------------|-----------------------|------------------------------------------------|-------------------------------|-------|-------|
| Chiapas SB        | Loamy       | Bulk soil    | 12/2017               | 16S bulk soil Chiapas Rep 1, 2, 3               | SRR12963519                   |       |       |
|                   |             | Rhizosphere-control | 12/2017               | 16S rhizosphere Chiapas Rep 2, 3               | SRR12963518                   |       |       |
|                   |             | Rhizosphere-biostimulant | 12/2017               | 16S rhizosphere Chiapas Biostimulant, Rep 1, 2, 3 | SRR13237956                   |       |       |
| Tabasco SO        | Sandy loam  | Bulk soil    | 2/2018                | 16S bulk soil sandy T1 Rep 1, 2, 3            | SRR12963529                   |       |       |
|                   |             | Rhizosphere-control | 2/2018                | 16S rhizosphere sandy T1 Rep 2, 3             | SRR12963521                   |       |       |
|                   |             | Rhizosphere-biostimulant | 2/2018                | 16S rhizosphere sandy T1 Biostimulant, rep 1, 2, 3 | SRR13237935                   |       |       |
|                   | Clay loam   | Bulk soil    | 6/2018                | 16S bulk soil sandy T2 Rep 1, 2, 3            | SRR12963559                   |       |       |
|                   |             | Rhizosphere-control | 6/2018                | 16S rhizosphere sandy T2 Rep 1, 2, 3          | SRR12963556                   |       |       |
|                   |             | Rhizosphere-biostimulant | 6/2018                | 16S rhizosphere sandy T2 Biostimulant, Rep 1, 2, 3 | SRR13237954                   |       |       |
|                   | Clay loam   | Bulk soil    | 12/2018               | 16S bulk soil sandy T3 Rep 1, 2, 3            | SRR12963543                   |       |       |
|                   |             | Rhizosphere-control | 12/2018               | 16S rhizosphere sandy T3 Rep 1, 2, 3          | SRR12963542                   |       |       |
|                   |             | Rhizosphere-biostimulant | 12/2018               | 16S rhizosphere sandy T3 Biostimulant, Rep 1, 2, 3 | SRR13237945                   |       |       |
| Tabasco SO        | Clay loam   | Bulk soil    | 2/2018                | 16S bulk soil clay T1 Rep 1, 2, 3             | SRR12963517                   |       |       |
|                   |             | Rhizosphere-control | 2/2018                | 16S rhizosphere clay T1 Rep 1, 2, 3           | SRR12963569                   |       |       |
|                   |             | Rhizosphere-biostimulant | 2/2018                | 16S rhizosphere clay T1-biostimulant, rep 1, 2 | SRR13237932                   |       |       |
|                   |             | Bulk soil    | 6/2018                | 16S bulk soil clay T2 Rep 1, 2, 3             | SRR12963553                   |       |       |
|                   |             | Rhizosphere-control | 6/2018                | 16S rhizosphere clay T2 Rep 1, 2, 3           | SRR12963552                   |       |       |
|                   |             | Rhizosphere-biostimulant | 6/2018                | 16S rhizosphere clay T2 Biostimulant, Rep 1, 2, 3 | SRR13237951                   |       |       |
|                   | Clay loam   | Bulk soil    | 12/2018               | 16S bulk soil clay T3 Rep 1, 2, 3             | SRR12963536                   |       |       |
|                   |             | Rhizosphere-control | 12/2018               | 16S rhizosphere clay T3 Rep 1, 2, 3           | SRR12963534                   |       |       |
|                   |             | Rhizosphere-biostimulant | 12/2018               | 16S rhizosphere clay T3 Biostimulant, rep 1, 2, 3 | SRR13237941                   |       |       |

(Continued on next page)
| Location       | Soil type | Sample type           | Sampling date (mo/yr) | Sample name                                      | SRA accession no. for Rep 1 | SRA accession no. for Rep 2 | SRA accession no. for Rep 3 |
|----------------|-----------|-----------------------|-----------------------|--------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Tabasco SO     | Silty loam| Bulk soil             | 2/2018                | 16S bulk soil tab silty T1 Rep 1, 2, 3           | SRR12963566                 | SRR12963565                 | SRR12963564                 |
|                |           | Rhizosphere-control   | 2/2018                | 16S rhizosphere tab silty T1 Rep 1, 2, 3         | SRR12963563                 | SRR12963561                 | SRR12963560                 |
|                |           | Rhizosphere-biostimulant | 2/2018             | 16S rhizosphere tab silty T1 Biostimulant, rep 1, 2 | SRR13237930                 | SRR13237929                 |                             |
|                |           | Bulk soil             | 6/2018                | 16S bulk soil tab silty T2 Rep 1                 | SRR12963546                 |                             |                             |
|                |           | Rhizosphere-control   | 6/2018                | 16S rhizosphere tab silty T2 Rep 1, 2, 3         | SRR12963545                 | SRR12963544                 |                             |
|                |           | Rhizosphere-biostimulant | 6/2018             | 16S rhizosphere tab silty T2 Biostimulant, rep 1, 2, 3 | SRR13237948                 | SRR13237947                 | SRR13237946                 |
|                |           | Bulk soil             | 12/2018               | 16S bulk soil tab silty T3 Rep 1, 2, 3           | SRR12963530                 | SRR12963528                 | SRR12963527                 |
|                |           | Rhizosphere-control   | 12/2018               | 16S rhizosphere tab silty T3 Rep 1, 2, 3         | SRR12963526                 | SRR12963525                 | SRR12963524                 |
|                |           | Rhizosphere-biostimulant | 12/2018             | 16S rhizosphere tab silty T3 Biostimulant, rep 1, 2, 3 | SRR13237938                 | SRR13237937                 | SRR13237936                 |
| Tabasco RE     | Silty loam| Bulk soil             | 2/2018                | 16S bulk soil tab RE T1 Rep 1, 2, 3             | SRR13234470                 | SRR13234469                 | SRR13234458                 |
|                |           | Rhizosphere-control   | 2/2018                | 16S rhizosphere tab RE T1 Rep 1, 2, 3           | SRR13234450                 | SRR13234449                 | SRR13234448                 |
|                |           | Rhizosphere-biostimulant | 2/2018             | 16S rhizosphere tab RE T1 Biostimulant, rep 1, 2, 3 | SRR13234447                 | SRR13234446                 | SRR13234445                 |
|                |           | Bulk soil             | 6/2018                | 16S bulk soil tab RE T2 Rep 1, 2, 3             | SRR13234444                 | SRR13234468                 | SRR13234467                 |
|                |           | Rhizosphere-control   | 6/2018                | 16S rhizosphere tab RE T2 Rep 1, 2, 3           | SRR13234466                 | SRR13234465                 | SRR13234464                 |
|                |           | Rhizosphere-biostimulant | 6/2018             | 16S rhizosphere tab RE T2 Biostimulant, rep 1, 2, 3 | SRR13234463                 | SRR13234462                 | SRR13234461                 |
|                |           | Bulk soil             | 12/2018               | 16S bulk soil tab RE T3 Rep 1, 2, 3             | SRR13234460                 | SRR13234459                 | SRR13234457                 |
|                |           | Rhizosphere-control   | 12/2018               | 16S rhizosphere tab RE T3 Rep 1, 2, 3           | SRR13234456                 | SRR13234455                 | SRR13234454                 |
|                |           | Rhizosphere-biostimulant | 12/2018             | 16S rhizosphere tab RE T3 Biostimulant, rep 1, 2, 3 | SRR13234453                 | SRR13234452                 | SRR13234451                 |

*Rep, replicate.*
Ultrasonic Processor; Fisher Scientific) at 70% frequency for 5 min. The rhizosphere fraction was recovered by centrifugation (3,857 × g, 4°C, 20 min) and kept at −80°C until metagenomic DNA extraction. Bulk soil and rhizosphere (250 mg) metagenomic DNA were extracted with a DNeasy PowerSoil kit (Qiagen, Germany) following the manufacturer's instructions, and after extraction, its integrity and concentration were assessed by agarose gel electrophoresis and UV spectroscopy.

16S V3-V4 rRNA was amplified with 337F/805R primers (25 PCR cycles) and indexed with a Nextera XT index kit v2 (Illumina) (8 PCR cycles) using Phusion (Thermo Fisher) DNA polymerase (10). 16S rRNA amplicon libraries were paired-end sequenced on an Illumina MiSeq platform. A total of 25,675,772 raw reads were obtained for 16S libraries (Table 1). Sequencing reads were analyzed with CLC Genomics Workbench 9.0 and CLC Microbial Genomics module 1.3 (Qiagen, Denmark). Raw reads were overlapped into single longer reads and fixed-length trimmed; chimeras and reads showing <100 abundance were removed. To identify operational taxonomic units (OTUs), filtered reads were clustered against the SILVA 16S database 138.1 (11) using 97% identity as clustering criteria. A total of 79,182 OTUs were predicted for 16S libraries.

Rhizosphere microbiomes were different from those of their surrounding bulk soil but derived from them. Bulk soil and rhizosphere microbiome composition changed along with seasonal weather and, in some cases, also after biostimulant application; however, soil type did not show any influence.

Data availability. The sequences obtained in this study were made public in the Sequence Read Archive (SRA) (accession numbers are listed in Table 1) via the National Center for Biotechnology Information (NCBI) under the accession number PRJNA673638.

ACKNOWLEDGMENT

This work was supported by Innovak Global, Chihuahua, Mexico.

REFERENCES

1. FOA. 2020. Banana statistical compendium 2019. FAO, Rome. http://www.fao.org/3/cb0466en/cb0466en.pdf.
2. Rossmann B, Müller H, Smalla K, Mpiira S, Tumuhairwe JB, Staver C, Berg G. 2012. Banana-associated microbial communities in Uganda are highly diverse but dominated by Enterobacteriaceae. Appl Environ Microbiol 78:4933–4941. https://doi.org/10.1128/AEM.00772-12.
3. Suhaimi NSM, Goh S-Y, Ajam N, Othman RY, Chan K-G, Thong KL. 2017. Diversity of microbiota associated with symptomatic and non-symptomatic bacterial wilt-diseased banana plants determined using 16S rRNA metagenome sequencing. World J Microbiol Biotechnol 33:168. https://doi.org/10.1007/s11274-017-2336-0.
4. Fu L, Ou Y, Shen Z, Wang B, Li R, Shen Q. 2019. Stable microbial community and specific beneficial taxa associated. J Microbiol Biotechnol 29:1624–1628. https://doi.org/10.4014/jmb.1904.04061.
5. Shen Z, Wang B, Zhu J, Hu H, Tao C, Ou Y, Deng X, Ling N, Li R, Shen Q. 2019. Lime and ammonium carbonate fumigation coupled with bio-organic fertilizer application steered banana rhizosphere to assemble a unique microbiome against Panama disease. Microb Biotechnol 12:515–527. https://doi.org/10.1111/1751-7915.13391.
6. Kaushal M, Swennen R, Mahuku G. 2020. Unlocking the microbiome communities of banana (Musa spp.) under disease stressed (Fusarium wilt) and non-stressed conditions. Microorganisms 8:443. https://doi.org/10.3390/microorganisms8030443.
7. Kaushal M, Mahuku G, Swennen R. 2020. Metagenomic insights of the root colonizing microbiome associated with symptomatic and non-symptomatic bananas in Fusarium wilt infected fields. Plants 9:263. https://doi.org/10.3390/plants9020263.
8. Díaz PG, Ruiz CJA, Medina GG, Cano GMA, Serrano AV. 2006. Estadísticas climatológicas básicas para el estado de Tabasco (periodo 1961–2003). INIFAP-CIRGOC, Veracruz, Mexico.
9. Serrano D-PA, López-Luna G, Cano-García A, Baez-González MA, Garrido-Ramírez ER. 2006. Estadísticas climatológicas básicas para el estado de Chiapas (periodo 1961–2003). INIFAP-SAGARPA: Libro Técnico No. 1, Ocozocautla, Chiapas, Mexico.
10. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41:e1. https://doi.org/10.1093/nar/gks808.
11. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. Nucleic Acids Res 41:DS90–DS96. https://doi.org/10.1093/nar/gks1219.