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Research

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Dry and Rainy Seasons Significantly Alter the Gut Microbiome Composition and Reveal a Key *Enterococcus* sp. (Lactobacillales: Enterococcaceae) Core Component in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Corn Strain From Northwestern Colombia

Marlon Felipe Higuita Palacio,[1] Olga I. Montoya,[1] Clara I. Saldamando,[2,] Erika García-Bonilla,[3] Howard Junca,[3,] Gloria E. Cadavid-Restrepo,[1] and Claudia Ximena Moreno-Herrera[1,4,]

[1]Grupo de Microbiodiversidad y Bioprospección, Facultad de Ciencias, Laboratorio de Biología Celular y Molecular, Universidad Nacional de Colombia Sede Medellín, Calle 59 A No. 63-20, 050003 Medellín, Colombia, [2]Grupo de Biotecnología Vegetal UNALMED-CIB, Facultad de Ciencias, Universidad Nacional de Colombia Sede Medellín, Calle 59A No. 63-20, Medellín, Colombia, [3]RG Microbial Ecology: Metabolism, Genomics & Evolution, Division of Ecogenomics & Holobionts, Microbiomas Foundation, LT11A, 250008 Chía, Colombia, and [4]Corresponding author, tel: (+57) 4309822, e-mail: cxmoreno@unal.edu.co

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Abstract

Spodoptera frugiperda is a polyphagous pest of several crops of economic importance. Nowadays, the insect is broadly distributed in America and, recently, in Africa, Asia, and Australia. The species has diverged into corn and rice strains. The role of the gut microbiota in insect physiology is relevant due to its participation in crucial functions. However, knowledge of seasonal variations that alter the gut microbiome in pests is limited. Gut microbiome composition between the dry and rainy seasons was analyzed with cultured and uncultured approaches in *S. frugiperda* corn strain larvae collected at Northwest Colombia, as seasonal microbiome changes might fluctuate due to environmental changes. On the basis of culture-dependent methods, results show well-defined microbiota with bacterial isolates belonging to *Enterococcus*, *Klebsiella* (Enterobacteriales: Enterobacteriaceae), *Enterobacter* (Enterobacterales: Enterobacteriaceae), and *Bacillus* (Bacillales: Bacillaceae) genera. The community composition displayed a low bacterial diversity across all samples. The core community detected with uncultured methods was composed of *Enterococcus*, *Erysipelatoclostridium* (Erysipelotrichales: Erysipelotrichaceae), *Rasltonia* (Burkholderiales: Burkholderiaceae), and *Rhizobium* (Hyphomicrobiales: Rhizobiaceae) genera, and Enterobacteriaceae family members. Significant differences in microbiome diversity were observed between the two seasons. The relative abundance of *Erysipelatoclostridium* was high in the dry season, while in the phylotype ZOR0006 (Erysipelotrichales: Erysipelotrichaceae) and *Tyzzerella* (Lachnospirales: Lachnospiraceae) genus, the relative abundance was high in the rainy season. The overall low gut bacterial diversity observed in the *S. frugiperda* corn strain suggests a strong presence of antagonist activity as a selection factor possibly arising from the host, the dominant bacterial types, or the material ingested. Targeting the stability and predominance of this core microbiome could be an additional alternative to pest control strategies, particularly in this moth.

Key words: corn fall armyworm, intestinal microbiome, bacterial diversity, next-generation sequencing

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a tropical insect considered an important pest in all Western Hemisphere (López-Edwards et al. 1999, Cano-Calle et al. 2015). The species has been reported in West and Central Africa in corn crops from contaminated material of corn from the eastern United States and the Greater Antilles (Nagoshi et al. 2017), and it has also been recently found in Asia and Australia (Food and Agriculture Organization of the United Nations [FAO] 2020, Nagoshi et al. 2020, Piggott et al. 2021). In Colombia, *S. frugiperda* is recognized as a primary pest of corn (*Zea mays*), and a secondary pest in sorghum (*Sorghum* spp.), cotton (*Gossypium hirsutum*), and pasture grasses (Garcia et al. 2002). Since 1986, *S. frugiperda* was molecularly classified into two

strains associated with its main host plants: corn and rice (Pashley and Martin 1987, Prowell et al. 2004). These two strains have also been widely reported in the United States (Nagoshi and Meagher 2004), México (Pecina-Quintero et al. 2015, Rosas-García et al. 2016), Argentina (Juárez et al. 2012, Murúa et al. 2015), and Brazil (Busato et al. 2004). Recently, the insect has also been reported in few countries of Africa (Goergen et al. 2016) and Asia (Nagoshi et al. 2020).

Given the migratory capability of species, this moth can move rapidly, especially its corn strain (Salinas-Hernandez and Saldamando-Benjumea 2011). In Colombia, this strain was genetically identified from corn, cotton, sorghum, sugar cane crops, and pasture grasses (Salinas-Hernandez and Saldamando-Benjumea 2011, Cano-Calle et al. 2015), demonstrating that the corn strain is more invasive than rice strain (Cano-Calle et al. 2015). Several diagnostic molecular markers have been used to differentiate both strains, where polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) of the mitochondrial gene cytochrome oxidase I (*COI*) and PCR of the tandem-repeated fragment *FR* (nuclear region For Rice) have been the most frequently used markers (Nagoshi and Meagher 2003, 2004; Cano-Calle et al. 2015). However, other markers, such as the mitochondrial gene Triosephosphate isomerase (*Tpi*), have also been successfully used to differentiate these strains (Nagoshi 2012). This gene was not used here as hybridization between both strains has been identified in previous investigations made in Colombia, with *FR* and *COI* markers. These two markers are located in different genomes facilitating the identification of hybrids between the corn and rice strains, particularly in cornfields (Cano-Calle et al. 2015).

Gut microbiota of some specialized niche feeders such as termites and aphids have been investigated and their enzymes achieve biochemical transformations of ecological, agricultural, and biotechnological importance (Chen and Purcell 1997, Warnecke et al. 2007). Therefore, analysis of gut microbiota is relevant for the improvement of the biological control applied to pests of economic importance such as *S. frugiperda* as the insect has spread from the Americas to Africa, Asia, and Australia very rapidly, demonstrating its ability to adapt to new habitats (Nagoshi et al. 2020, Piggott et al. 2021).

Studies related to the microbial communities associated with *S. frugiperda* gut are limited. In Argentina, two investigations were made on the gut metatranscriptome of *S. frugiperda* larvae collected from sorghum and corn (McCarthy et al. 2015, Rozadilla et al. 2020). In another study on larvae collected from cornfields in Brazil, the authors identified bacteria resistant to various insecticides, including strains belonging to the following genera/species: *Arthrobacter* sp. (Micrococcales: Micrococcaceae), *Enterobacter asburiae* (Pseudomonadales: Pseudomonadaceae), *Pseudomonas stutzeri*, and *Pseudomonas kilonensis* (Almeida et al. 2017). Recently, the presence of *S. frugiperda* was reported in Africa, where it has been evidenced the persistence of *Stenotrophomonas* (Xanthomonadales: Xanthomonadaceae), *Sphingobacterium* (Sph ingobacteriales: Sphingobacteriaceae), *Serratia* (Enterobacterales: Yersiniaceae), *Pseudomonas*, *Morganella* (Enterobacterales: Morganellaceae), *Delftia* (Burkholderiales: Comamonadaceae), and *Enterococcus* during insect metamorphosis and the predominance of Proteobacteria and Firmicutes phyla (Gichuhi et al. 2020).

Seasonal changes in gut microbiota have been widely observed in humans, as its composition depends on host genetic background, lifestyle, and environmental factors (Koliada et al. 2020); these changes were also observed in the gut of the horseshoe bat (*Rhinolophus ferrumequinum*) (Xiao et al. 2019), microbiota of different mulberry (*Morus* L.) cultivars (Ou et al. 2019), and mosquito microbiota (*Anopheles coluzzii*) (Krajacich et al. 2018), among others. However, this sort of study has never been made in *S. frugiperda*; for this reason, an analysis of the gut microbiome composition in the corn strain from larvae collected in cornfields at the Department of Antioquia (Colombia) was made to determine the predominant key members of its intestinal microbiota and the effect of seasonal differences and environmental temperature conditions on the gut microbiome. The results obtained here are relevant for the management of this pest as the microbiome plays a crucial role in insect physiology.

Materials and Methods

Ethics Approval

Sample collection was performed following the parameters of Colombian decree N° 1376. No specific permits were required for this study. *Spodoptera* larvae were collected from a private property and permission was received from the landowners before sampling.

Larvae Collection

Individuals of *S. frugiperda* larvae (third to the fourth instar) were obtained from a cornfield at the Estación Agraria 'Cotove' farm, Universidad Nacional de Colombia, located at the Santa Fe de Antioquia (6°31′54.0′N, 75°49′33.8′W) in Antioquia Department, Colombia. One hundred fifty larvae were collected in 2017 during March (dry season = season 1) and October (rainy season = season 2). After sampling, larvae were individually separated in small plastic containers under aerial conditions and with corn leaves and transported to the laboratory. All larvae were kept alive until processing.

Gut Dissection

To reduce vital functions, larvae were cooled at –20°C for 10 min. Later on, they were first washed with ethanol (70%) and then with phosphate-buffered saline (PBS) + Tween-20 solution (1%). Gravimetric data were taken from each processed larva. Dissections were performed using sterile forceps in PBS 1× buffer. The entire gastrointestinal tract was extracted, weighed, measured, and macerated in sterile PBS 1×. Each intestinal homogenate was preserved and processed at low temperatures. Half of the homogenate was used for conventional culture-based microbiological methods and the other half was frozen and used for the culture-independent molecular approaches (Yun et al. 2014, Vivero et al. 2016).

Strain Identification of *S. frugiperda*

To determine and confirm *S. frugiperda* host strains in the cornfields where the larvae were collected, genotyping was performed on larvae heads by using PCR-RFLP of the *COI* gene at the mitochondrial DNA and PCR for nuclear region FR (Cano-Calle et al. 2015). PCR amplification of genomic DNA was performed using JM76 forward (5′-GAGCTGAATTAGG(G/A)ACTCCAGG-3′) and JM77 reverse (5′-ATCACCTCC (A/T)CCTGCAGGATC-3′) primers. After the PCR reaction, digestion was performed using the restriction enzyme MspI. All samples were visualized on agarose gel at 2% (Vélez Arango et al. 2008).

For the FR region amplification, PCR reactions were performed using FR-a forward (5′-TTTTACACCGGTCACAACGA-3′) and FR-2 reverse (5′-GACATAGAAGAGCACGTTT-3′) primers. The rice strain pattern produces electrophoretic bands >500 bp, whereas the corn strain pattern produces no bands or one to three faint bands (Nagoshi and Meagher 2003, Vélez Arango et al. 2008, Cano-Calle et al. 2015).

Bacterial Isolation

The homogenates were serially diluted (up to 10^6) on Nutritional Agar (Merck, Germany) and MacConkey Agar (up to 10^6). The

plates were incubated aerobically at 37°C for 24 h (Vivero et al. 2016). After incubation, the total number of colony-forming units (CFU) per gram of each homogenate was determined. Representative morphotypes were isolated and characterized at the microscopic and macroscopic levels, and the isolates were conserved in 20% v/v glycerol at −80°C. Pure isolates were molecularly characterized by the Ribosomal Intergenic Spacer Analysis (RISA) and the sequence of the 16S rRNA gene (Vivero et al. 2016).

Molecular Analysis

DNA taken from the isolates was extracted using thermal lysis. PCR amplification of the internal transcribed spacer (RISA) was performed using the L1 and G1 primers and the mix reaction, and the program was used as previously reported (Jensen et al. 1993). The gels were stained with $AgNO_3$ (Amresco, Solon, Ohio) and analyzed using the GelCompar II program (Applied Maths Biosystems, Kortrijk, Belgium). ITS cluster analysis was performed using the Pearson coefficient and Simple Linkage cluster method. Seventy-five percent or more similarity between ITS standards was established as criteria for subsequent molecular identification.

To identify the bacterial isolates, the 16S rRNA gene was amplified with the universal primers Eubac 27 and 1492R (Moreno et al. 2002). Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced in both directions using the ABI PRISM 3700 Genetic Analyzer (Vivero et al. 2016).

PCR 16S rDNA–Temperature Gradient Gel Electrophoresis

The total genomic DNA was extracted and purified from homogenized tissue using 'FastDNA SPIN KIT' (Mp Biomedicals, Irvine, CA). The DNA integrity and concentration were analyzed as described previously (Montoya-Porras et al. 2018).

The DNA samples were used for 16S rDNA amplification between the V3 and V6 regions (566 bp) using 341F-GC domains and 907R primers (Muyzer et al. 1993). PCR reactions were performed as previously described by Gomez et al. (2011). PCR products were concentrated using a concentrator (Eppendorf 5301) and dissolved in ultrapure water. The PCR products (600 ng) were run on 6% (w/v) polyacrylamide–7 M urea gel in 1.25× TAE buffer with a temperature gradient of 66–69°C, and a ramp of 0.2°C/h for 15 h at 55 V in a DCode system (Bio-Rad, Hercules, CA). Furthermore, two reference markers of the 100 bp ladder (Thermo Fischer Scientific Inc., Carlsbad, CA, n.d.) and a pool of 16S rDNA fragments of two reference bacterial strains (*Escherichia coli* (Enterobacteriales: Proteobacteria) ATCC 8739 TM and *Bacillus cereus* ATCC 14579 TM) were used. Polyacrylamide gels were stained and analyzed using the GelCompar II software (Applied Maths Biosystems, Kortrijk, Belgium) (Rademaker and De Bruijn 1997). The cluster analysis was performed using the Pearson correlation and Complete Linkage method (Koeppel et al. 2008). Bands of interest were excised, and the DNA was eluted. The eluent DNA was used to perform the re-amplification with the 341F and 907R primers. The amplification products were purified and later sequenced. Additional analyses were performed for the isolate sequences re-using the methods described above.

Sequencing Analyses

The sequences that were obtained from the PCR of the 16 rDNA from the isolates and temperature gradient gel electrophoresis (TGGE) eluted bands were edited using the BioEdit program (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and CHIMERA CHECK software (http://www.bioinformatics-toolkit.org). We used a Basic alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the edited sequences and SeqMatch search tool from the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/seqmatch), downloaded reference and consensus sequences were aligned using Clustal W (Thompson et al. 1994) with MEGA7 (https://www.megasoftware.net) (Kumar et al. 2016) and the same program was used to construct a Neighbor-Joining (NJ) tree with bootstrapping. The sequences obtained here can be found in the NCBI GenBank databases with accession numbers SUB5452002 for isolates and SUB7508707 for TGGE eluted bands.

Microbiome Composition by 16S rRNA Gene Illumina Amplicon Sequencing

16S rRNA gene amplicons were obtained from the total DNA extractions and sequenced using the MiSeq Illumina platform. Alpha and beta diversity were estimated based on 97% similarity Operational Taxonomic Units (OTUS) using the QIIME 1.9.1 package (Kuczynski et al. 2011) using the pipeline as described by García-Bonilla et al. (2019). Variations in the relative abundance of OTUs in the respective seasonal conditions were tested for significance using the Kruskal–Wallis test. Phyloseq R 1.5.21 package was used for extracting graphic outputs (Pérez-Jaramillo et al. 2017).

According to national regulations, all available sequence information produced is available upon request in the GenBank database (MK786669–MK786690).

Results

Strain Identification

COI amplification of mitochondrial DNA and its subsequent digestion with the MspI enzyme allowed us to identify 27 individuals as the corn strain and one hybrid between the two strains (sample A205). This was confirmed using the amplification of the nuclear region FR (Supp Material 1 [online only]).

Bacterial Diversity Through Culture-Dependent Assays

Gut bacterial counts of *S. frugiperda* ranged from 6.4 log (CFU/g) (MacConkey Agar) to 7.4 log (CFU/g) (Nutritional Agar) in the first collection season, while CFU counts for the second season increased from 6 log (CFU/g) (McConkey Agar) to 27 log (CFU/g) (Nutritional Agar) (Supp Material 2 [online only]).

A total of 72 isolates were purified from both culture media, where differences were observed according to the parameters such as Gram staining and colony morphology after performing a macro- and microscopic characterization of each isolate. Fifty-seven percent of the isolates corresponded to Gram-positive bacteria, while 39% are Gram-negative bacteria, and only the remaining 4% were Gram-indeterminate bacteria.

RISA of Isolates

The dendrogram based on ITS isolated patterns analyzed with GelCompar II differentiated 12 clusters with a similarity percentage of 75% (Supp Material 3 [online only]). Representative isolates of each cluster were further selected for 16S rRNA gene sequencing.

Identification of Isolates With 16S rRNA Gene Sequences

Twenty-two total sequences obtained from RISA isolate clusters were phylogenetically analyzed using NJ tree. The percentages of

similarity between our isolates and reference sequences were between 98 and 100% (sequenced and cured fragments that were on average 1.000 bp). Isolates were related to the genera *Enterococcus*, *Klebsiella*, *Enterobacter*, and *Bacillus*, suggesting a predominance of Firmicutes phylum in the *S. frugiperda* gut corn strain (Table 1).

Bacterial Diversity Through Culture-Independent Assays

Microbial Community Analysis by PCR–TGGE

Twenty-eight intestinal samples (including 14 gut samples from the dependent culture methods) were subjected to total DNA extraction and further results were obtained using the TGGE technique, which separated 16S rRNA gene fragments of a size equivalent to 585 bp. These fragments showed differences in the banding patterns, suggesting some bacterial diversity (Fig. 1).

Phylogenetic Affiliations of Sequences Obtained From the TGGE Bands

Thirty-three bands with unique migration patterns and higher intensity were split, re-amplified, and sent to sequencing. From all sequences obtained, seven of them were successfully edited and correctly aligned with sequences related to the genus *Klebsiella* (NR_025635.1 *Klebsiella variicola*), *Enterococcus* (NR_113906.1 *Enterococcus mundtii*), and *Enterobacter* (NR_126208.1 *Enterobacter xiangfangensis*) (Fig. 2).

The Bacterial Composition by 16 rRNA Gene Amplicon NGS

After filtering and cleaning the raw data for low-quality or chimeric sequences, the resulting dataset had read 250 nucleotides on average, consisting of 391.087 reads grouped into 7.093 OTUs with 97% similarity for all 11 samples analyzed. The median value in the clean datasets is 35.640 ± 4.338 reads per sample. Rarefaction analysis (Supp Material 4 [online only]) showed that the sequence coverage was more than 97% for all samples analyzed, signifying that the patterns of composition are representative of amplicon complexity and samples can be compared regarding alpha and beta diversity on specimens of *S. frugiperda* corn strain. Firmicutes and Proteobacteria were the most abundant phyla in all samples, where Firmicutes corresponded in most cases to 90% of the total bacterial community present, except for the A208 sample where phylum Proteobacteria accounts for 53% of the total community. *Enterococcus* was the most abundant genus in all samples (85–90%), except the sample A86 where *Erysipelatoclostridium* was the most abundant with almost 55% of the total community, communities grouped as 'rest of the community' corresponded to genera with less than 1% relative abundance within the total. A difference is observed for the collection season within the core community, where the genus *Erysipelatoclostridium* was more abundant in the dry season (season 1) of collection, decaying its abundance in the following season, whereas the ribotype from Firmicutes strain ZOR006 increased in the rainy season (season 2) as well as the genus *Tyzerella 3* (Figs. 3 and 4). Differences in the bacterial community composition were also observed in weighted and unweighted Unifrac distances showing a closer clustering of samples from the dry season. Although there is some similarity between the samples analyzed, samples from the rainy season are rather scattered across the dendrogram topology, indicating more variability in low-frequency OTUs (Supp Material 5 [online only]). Regarding *S. frugiperda* corn strain gut bacterial diversity, the alpha diversity indices were not significant since their box plot bars overlap, particularly Shannon and Simpson diversity indices. The values did not differ significantly between the diversity indices (*P* value: 0.44, Kruskal–Wallis test). The observed richness is greater in the dry collection season (season 1) (Fig. 4). The constrained analysis of principal coordinates (CAP) and principal coordinate analysis (PCoA) showed a well-defined clustering of bacterial communities, indicating that an estimated 30% of the differences observed was influenced due to seasonal conditions (dry vs rainy) (Fig. 5).

Table 1. Bacterial isolate IDs, GenBank accession number of the 16S rRNA gene sequences, and inferred phylogenetic affiliation of the closest relative reported in databases

Isolate ID	GenBank accession number	Phylogenetic affiliation
Sp59	MK786675	*Bacillus pumilus*
Sp29	MK786683	*Bacillus subtilis*
Sp1	MK786690	*Enterococcus* sp.
Sp8	MK786688	*Enterococcus* sp.
Sp22	MK786685	*Enterococcus* sp.
Sp28	MK786684	*Enterococcus* sp.
Sp33	MK786682	*Enterococcus* sp.
Sp53	MK786677	*Enterococcus* sp.
Sp67	MK786671	*Enterococcus* sp.
Sp72	MK786669	*Enterococcus* sp.
Sp35	MK786681	*Enterobacter cloacae*
Sp41	MK786679	*Enterobacter* sp.
Sp50	MK786678	*Enterobacter* sp.
Sp69	MK786670	*Enterobacter* sp.
Sp9	MK786687	*Enterobacter* sp.
Sp60	MK786674	*Enterobacter tabaci*
Sp11	MK786686	*Klebsiella pneumoniae*
Sp2	MK786689	*Klebsiella* sp.
Sp37	MK786680	*Klebsiella* sp.
Sp55	MK786676	*Klebsiella* sp.
Sp63	MK786672	*Klebsiella* sp.
Sp61	MK786673	*Klebsiella variicola*

Discussion

Nowadays, studies of intestinal microbiota associated with insects are a growing field of interest as new alternatives to find microorganisms and/or molecules for improving the biological control for pest management (Almeida et al. 2017, Orozco-Flores et al. 2017), especially for species that are difficult to control such as *S. frugiperda*. This moth represents an important corn pest worldwide (Nagoshi et al. 2020). The species has diverged into corn and rice strains that have genetically differentiated, and in Colombia, they have evolved reproductive isolation at pre- and postzygotic levels (Velasquez-Velez et al. 2011). These two strains are genetically differentiated with reduced gene flow in Colombia (Cano-Calle et al. 2015). In this country, they also differ in wing morphometrics (Cañas-Hoyos et al. 2014) and exhibit differences in resistance to insecticides and *Bacillus thuringiensis* endotoxins (Ríos-Díez and Saldamando-Benjumea 2011, Ríos-Díez et al. 2012). In this study, strain identification was performed on field-collected *S. frugiperda* corn strain specimens in Colombia (South America), and also a variation in the gut microbiome composition between the dry and rainy seasons was examined over the course of 1 yr in larvae collected from cornfields. Results obtained here corroborated the presence of the corn strain in 27 samples and a hybrid between both strains (sample 'A205')

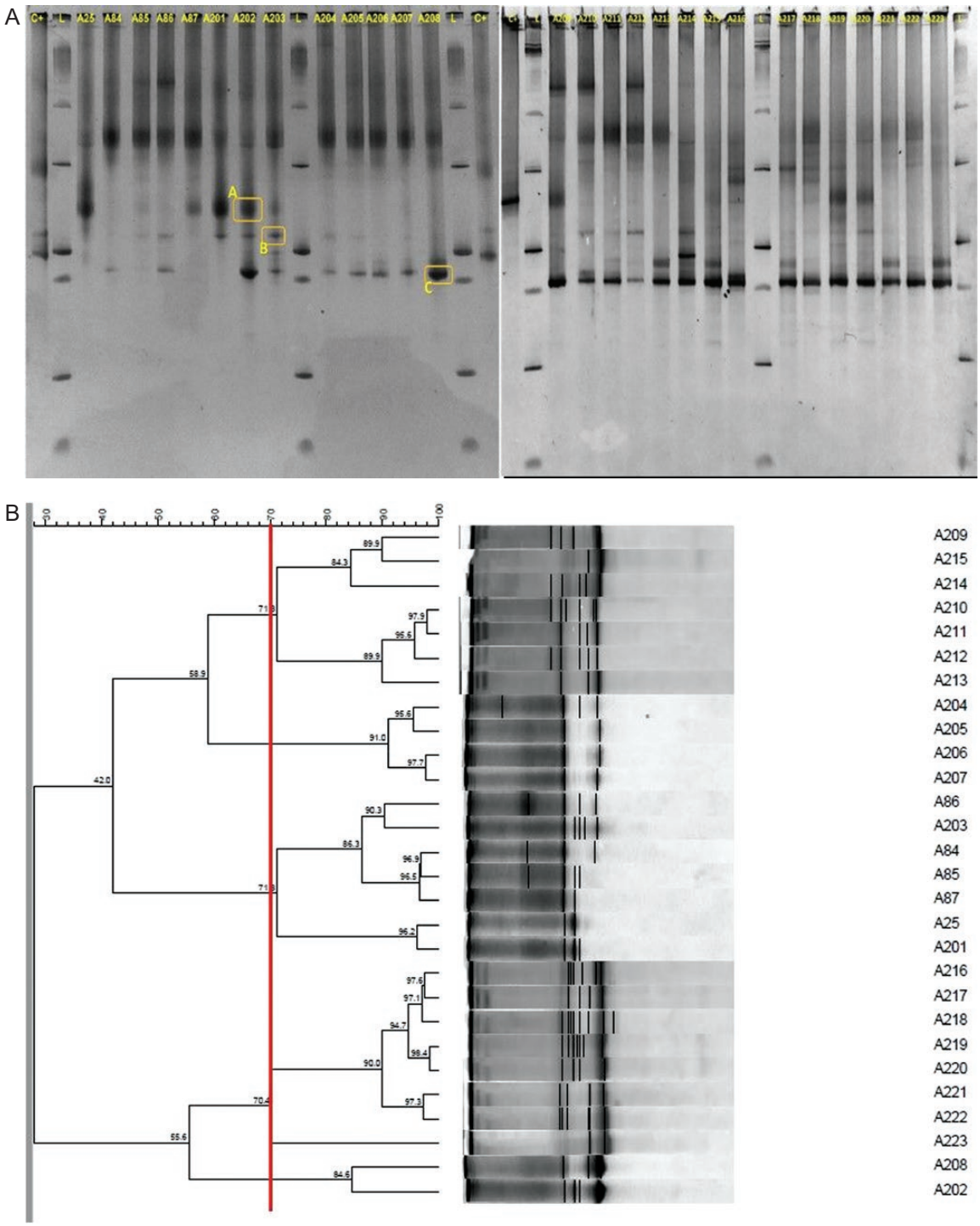

Fig. 1. TGGE fingerprints of 16S rRNA gene PCR amplification products. (A) TGGE fingerprints of 16S rRNA gene PCR amplification products between variable regions V3 and V6 of all total DNA samples obtained from the intestine of *S. frugiperda* larvae. L: 100 bp weight marker. C: TGGE reference banding pattern (*B. cereus* and *E. coli*). (B) Dendrogram (Pearson-Complete Linkage) obtained with GelCompar II software from the TGGE band patterns of the PCR fragments of the 16S rRNA genes retrieved from the total DNA of intestinal samples of *S. frugiperda* larvae.

(Cano-Calle et al. 2015), and previous studies have shown that the probability of the presence of this individual in corn crops is low, but due to its polyphagous nature, it can be found in a small proportion (Vélez Arango et al. 2008, Cano-Calle et al. 2015). Crosses between corn strain females with rice strain males are restricted in the laboratory. However, in nature, hybrids between rice strain females and corn strain males can be found in low proportions in the field (Velasquez-Velez et al. 2011). In Colombia, Cañas-Hoyos et al. (2017) demonstrated that both strains differ in the volatile composition produced by their females, and thus the production of hybrids in nature is limited due to the assortative mating between the strains.

The methods currently used for microbiota analysis include two approaches: culture-dependent and independent culture methodologies (Vivero et al. 2016). In the first case, there are advantages

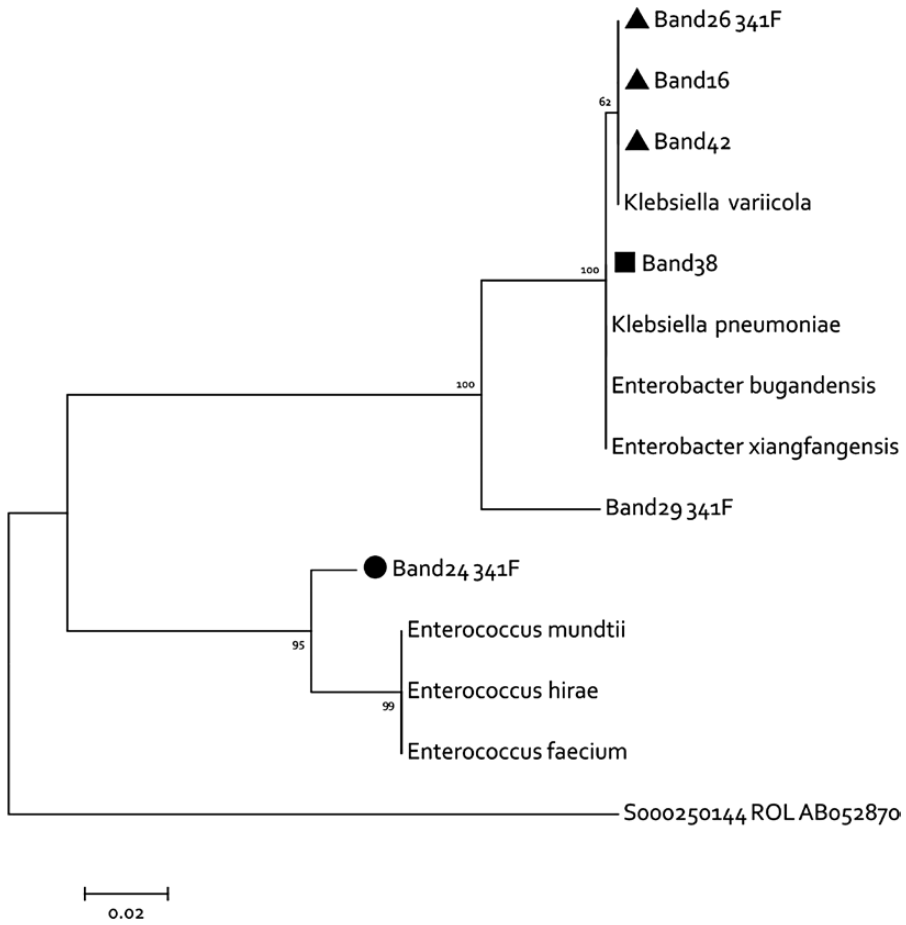

Fig. 2. Phylogenetic relationships of partial sequences of partial 16S rRNA gene sequences from TGGE bands. The phylogeny of the partial sequences of 16S rRNA genes corresponding to the bands obtained in TGGE patterns. The 16S rRNA gene sequence of the Bacterial Mollicutes Rice orange leaf phytoplasma (ROL) GenBank AB052870 was used as the outgroup. The phylogeny was inferred using the Maximum Likelihood method based on the Jukes–Cantor model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 14 nucleotide sequences. Labels correspond to Fig. 1A, Band A (circle), Band B (square), and Band C (triangle). All positions containing gaps and missing data were eliminated. There was a total of 343 positions in the final dataset.

over obtaining pure isolates because this allows the study of enzymes, peptides, and metabolites for various biotechnological purposes, although culturing approaches do not cover the total of the bacterial diversity that can be detected with culture-independent methods (Dillon and Dillon 2004, Tang et al. 2012). In this study, two culture media were selected to cover as many bacterial morphotypes as possible, and after their subsequent purification, 72 pure isolates were identified according to the macro- and microscopic characteristics of the cultures. Twenty-two isolates with a differential ITS band profile were selected to be taxonomically identified with 16S rRNA gene sequencing and showed a taxonomic association with the phyla Firmicutes and Proteobacteria, and the genera *Klebsiella*, *Enterobacter*, *Enterococcus*, and *Bacillus*; these results corroborate bacterial types reported from another Lepidoptera (Chen et al. 2016).

Several authors have suggested the crucial role of the intestinal microbiome in the development of insects, since it is related to aspects such as immunity, reproduction, digestion, nutrition, and production of metabolites such as pheromones, antimicrobial molecules, among others (Dillon and Dillon 2004). In Lepidoptera such as *Plodia interpunctella*, it has been demonstrated that the intestinal microbiota is important for the response of the insect to the biological control where 'Bt' endotoxins are used; additionally, it has been observed that the variation in the population dynamics of the intestinal microbiota can be associated with changes in gene expression related to the immune response of the host (Orozco-Flores et al. 2017). Other studies indicate the relationship between microbiota and insecticide resistance in *S. frugiperda*, where isolates of the genus *Enterococcus* sp. (Firmicutes), *Enterobacter* sp. (Proteobacteria), and *Serratia* sp. (Proteobacteria) have a major role in detoxification and modulation of host immune response (Almeida et al. 2017). In Colombia, previous studies have shown that *S. frugiperda* corn and rice strains differ in resistance to Bt endotoxins (Cry 1AC and Cr1AB) as the former is more resistant than the latter (Ríos-Díez et al. 2012). Also, both strains differ in resistance to insecticides as the former is more susceptible to λ-cyhalothrin and methomyl than the latter (Ríos-Díez and Saldamando-Benjumea 2011). Studies conducted by Almeida et al. (2017) have shown that *S. frugiperda* larvae gut bacteria can degrade insecticides. They also found a direct relationship between *S. frugiperda* immune response with the resident intestinal microbiota. For example, they observed the xenobiotic degradation potential of *Enterococcus* strains from the intestine of *S. frugiperda* and the resistance of various gut bacteria genera to pesticides such as λ-cyhalothrin, deltamethrin, ethyl chlorpyrifos, spinosad, and lufenuron, suggesting a possible influence of this type of bacteria on pesticide detoxification.

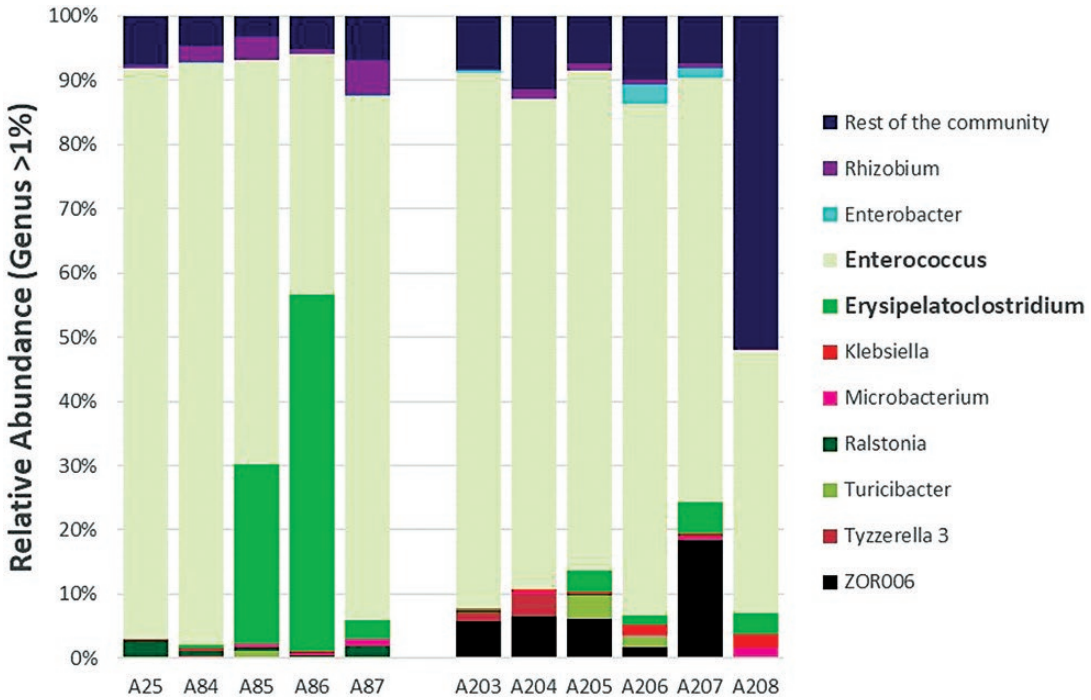

Fig. 3. Microbiome compositions of *S. frugiperda* larvae collected in two seasons. Relative abundance (%) of OTU-related bacterial genera found in 11 intestinal samples of wild specimens of *S. frugiperda* larvae as determined by Illumina sequencing analyses of hypervariable region V4 16S rRNA gene amplicons. A25–A87 = dry season, A203–A208 = rainy season.

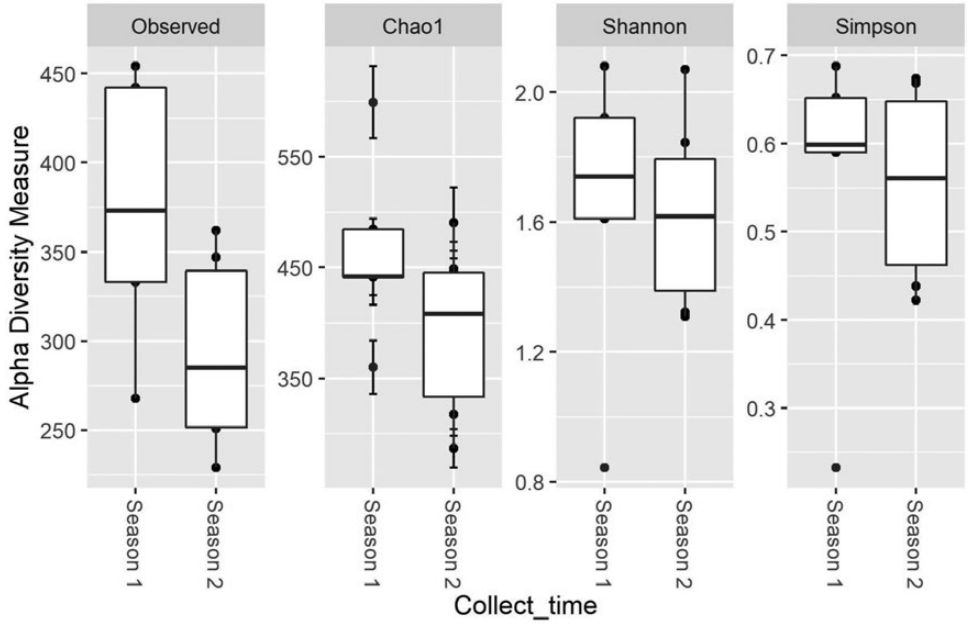

Fig. 4. Alpha diversity indices of the 11 intestinal samples of the *S. frugiperda* group according to the season of collection (season 1 = dry, season 2 = rainy).

As in this work, temporal stability of the gut microbiome was studied in two collecting seasons in the corn strain of *S. frugiperda*, due to dietary perturbations that contribute to shaping the composition of the microbiota, we observed a high degree of temporal stability in members of the corn strain microbiome similar to the metatranscriptomic study conducted by Rozadilla et al. (2020) in *S. frugiperda* from Argentina. These authors found that most microbiota were indigenous (83%), including Actinobacteria (*Rubrobacter* (Rubrobacterales: Rubrobacteraceae) *Xylaniohilus* (Aphelenchida: Aphelenchoidoidea), *Janibacter* sp. (Micrococcales: Intrasporangiaceae), *Streptomyces* sp. (Streptomycetales: Streptomycetaceae)), Proteobacteria (*Enterobacter cloacae*, *Xantomonadacae*, *Vibrio* (Vibrionales: Vibrionaceae), *Lysobacter* (Xanthomonadales: Xanthomonadaceae)), and Firmicutes (*Enteroc. mundtii*, *Pediococcus pentosaceus* (Lactobacillales: Lactobacillaceae)), and a

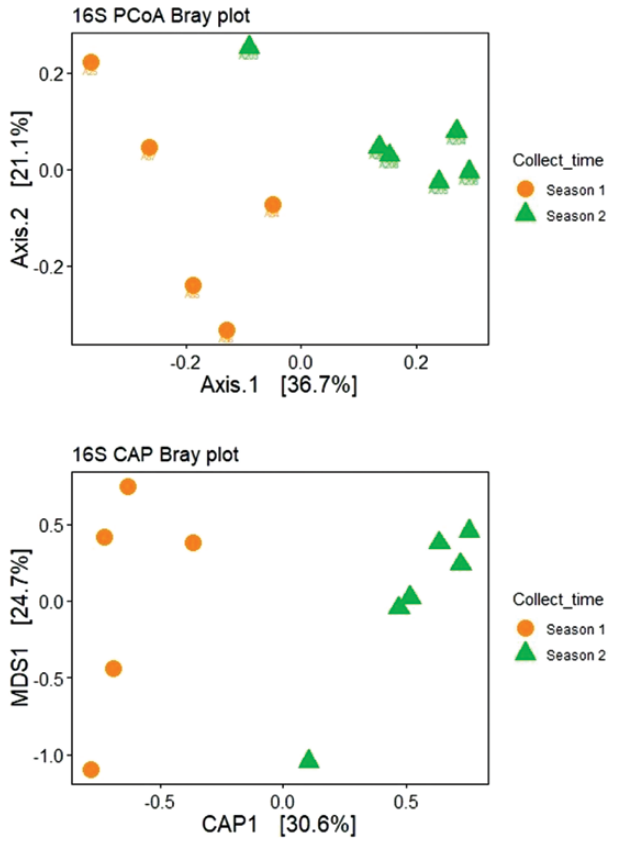

Fig. 5. PcoA and CAP obtained from the normalized data based on the Bray–Curtis similarity analysis for the 11 intestinal samples of *S. frugiperda* larvae 16S rRNA microbiome composition data (season 1 = dry, season 2 = rainy).

few were transient (17%). In contrast, Rozadilla et al. (2020) observed significant shifts in bacterial abundance and diversity between the two collecting seasons (dry and rainy), suggesting the importance of seasonal changes in *S. frugiperda* corn strain microbiota from Colombia. Within the results of this work, a correlation was found between taxonomic dependent and independent culture information since the partial sequences of the TGGE bands yielded the same information regarding the bacterial genera reported by the dependent culture analysis. However, analysis of massive sequencing of the 16S rRNA gene amplicons revealed that the most abundant genus was *Enterococcus*. This genus predominance has been widely reported in other Lepidoptera such as *Spodoptera littoralis*, where bacteria belonging to the *Enterococcus* genus can maintain themselves after metamorphosis (Chen et al. 2016). Our study found a marked difference within the bacterial communities concerning the collection season (dry or rainy), where the genus *Erysipelatoclostridium* drastically reduced, from dry to rainy season, with an increase in rainfall (≥134 mm) and a drop of ~2°C in the average temperature of the sampling site, while *Tyzerella 3* and the ZOR006 phylotype increased in abundance from rainy to dry season. According to alpha diversity indices, microbiome abundance was greater in the dry season (season 1) compared to the rainy season (season 2). These results coincide with the outcome obtained in *An. coluzzii* from Mali (Krajacich et al. 2018) as during the Sahelian dry season, this insect presented high microbial diversity, including the genera *Anaplasma* (Rickettsiales: Ehrlichiaceae), *Bacillus*, *Microvirga* (Hyphomicrobiales: Methylobacteriaceae), and *Intestinibacter* (Eubacteriales: Clostridiaceae), while in the riparian dry season, this mosquito presented a reduction of *Ralstonia* reads and an increase in *Janthinobacterium* (Burkholder iales: Oxalobacteraceae), and *Sphingomonas* (Sphingomonadales: Sphingomonadaceae) reads. In the case of cricket *Gryllus veletis*, no seasonal changes were observed during the analyses of temperature changes made in the laboratory, suggesting that microbiome changes are in concert with insect physiological changes (Ferguson et al. 2018).

Additionally, our results showed that both CAP and PCoA confirmed a change in the bacterial community between collecting seasons (season 1 = dry, season 2 = rainy) (approximately 30%). This result was due to an increase in precipitation and a decrease in the temperature of the sampling site where the larvae were collected. These last results are important since studies on the microbiome of insects have revealed that various insect life-history traits are correlated with their complex bacterial ecosystem and its host environmental changes (Krajacich et al. 2018). For example, in *An. coluzzii*, the authors found that changes in the microbiome composition from different dry environments in Mali were due to the differences between the habitats where larvae were collected rather than the physiological states of the mosquito. This result might be explained because microbes are ectotherms, and the microbiome of ectothermic animals will be exposed to the same temperature changes as their hosts (Ferguson et al. 2018). These fluctuations can potentially challenge individual microbe species, modify community interactions, and alter the functional host–symbiont relationship (Ferguson et al. 2018). For instance, Ferguson et al. (2018) simulated season changes in the laboratory to study the microbiota composition in *G. veletis* and they found that the abundance of *Pseudomonas* spp. decreased in cold temperatures, whereas *Wolbachia* sp. (Rickettsiales: Ehrlichiaceae) abundance increased under these temperatures. These results are relevant in the biological control of vectors of human diseases and also in pests since *Wolbachia* sp. represents one of the most relevant endosymbionts used for insects' management (Vivero et al. 2016).

In the year 2020 was found Actinobacteria (37.41%), uncultured bacteria (19.80%), Proteobacteria (1.68%), and Firmicutes (0.23%) where the phylum Actinobacteria was represented by *Rubrobacter xylanophilus* (Rubrobacterales: Rubrobacteraceae) (most abundant), *Janibacter* (Micrococcales: Intrasporangiaceae) (less abundant), and *Streptomyces* (less abundant) (Rozadilla et al. 2020). Also, they found that most of the uncultured bacteria corresponded to uncultured ammonia-oxidizing bacteria, phylum Proteobacteria was represented by *E. cloacae*, *Xanthomonadaceae*, *Lysobacter*, and *Vibrio*, which accounted for <2% of the gut microbiota, and phylum Firmicutes was represented by *Enteroc. mundtii* and *Pe. pentosaceus*. In this work, we found *Bacillus pumilus*, *B. subtilis*, various *Enterococcus* sp., *E. cloacae*, various *Enterobacter* sp., *Enterobacter tabaco*, *Klebsiella pneumoniae*, various *Klebsiella* sp., and *K. variicola*. The two studies would be different for (Rozadilla et al. 2020) studied the metatranscriptome (based on RNA analysis) of the species, whereas this work was made on the microbiome (based on DNA analysis).

Studies of other Lepidoptera have also shown a marked presence of Firmicutes and Proteobacteria phyla within their gut bacterial communities, for example, the intestinal microbiota related to *S. littoralis* has a predominance of phylum Firmicutes and more specifically to the genus *Enterococcus* (Chen et al. 2016). Another Lepidoptera, *Busseola fusca* (corn stalk borer), showed the predominance of the genera *Bacillus*, *Enterococcus*, and *Klebsiella* in its intestinal microbiota (Snyman et al. 2016); similarly, *Helicoverpa*

armigera showed gut bacterial communities associated with these two phyla, and the predominance of bacteria belonging to the Actinobacteria phylum (Ranjith et al. 2016, Snyman et al. 2016). Differences in the intestinal microbiota composition might be due to differences in host plants consumed by these pests; for example, in *S. littoralis* and *Bu. fusca*, both pests feed on plants of the family of Poaceae, such as corn, whereas *H. armigera* mainly feeds on tomato (*Solanum lycopersicum*). On the basis of our results, the central bacterial 'core' is consistent with that of other Lepidoptera, and according to their behavior within the crops, it can be suggested that, in general, the bacterial diversity of the Lepidoptera is low, the composition of the microbiota does not depend on the food consumed by the insect, but this food does have a direct relationship with the abundance of the bacterial communities associated with the intestine of the larvae of *S. frugiperda*, and that environmental characteristics such as temperature and precipitation might also influence the abundance of these communities.

The development of control strategies on Lepidoptera is urgently required to reduce the economic impact on crops of agricultural interest (Ranjith et al. 2016). In the case of the genus *Spodoptera*, the management of the species *S. littoralis*, *S. frugiperda*, *S. litura*, and *S. exigua* has been reported through mass capture, interruption of mating, and methods of attraction and death (Guerrero et al. 2014). Alternatively, biological control where the life cycle of larvae can be interfered with by manipulating their bacterial communities could be a feasible strategy that would mitigate the ecological impact of conventional biocide chemical pest control. The alteration of intestinal homeostasis seems to be a promising strategy for the biological control of a pest. It has been reported that the alteration in terms of the abundance of some intestinal isolates, such as the case of *Enteroc. mundtii*, leaves the insect without one of its natural defense mechanisms against pathogens, directly affecting its life cycle (Shao et al. 2017). It is, therefore, necessary to define experimental strategies for biological control of *S. frugiperda* strain corn in Colombia from the reported genera and perform comparative analyzes with the microbiota of *S. frugiperda* rice strain, which is also of great economic importance in this country.

In conclusion, our study found an overall low bacterial diversity in the intestine of *S. frugiperda*. However, the most important result observed in the *S. frugiperda* corn strain was the seasonal variation of gut microbiota due to temperature variations and rainfall. These results were obtained from a larval collection where these two variables had a direct effect on the relative abundance of bacterial communities present in the intestine of *S. frugiperda* and particularly in the relative abundance of *Erysipelatoclostridium*, phylotype ZOR0006 (*Erysipelotrichaceae*), and *Tyzerella* sp. as *Erysipelatoclostridium* was more abundant in the dry collecting season, while the phylotype ZOR0006 (*Erysipelotrichaceae*) and *Tyzerella* sp. were more abundant in the rainy season. In general, our study enriches our knowledge on the microbiome associated with *S. frugiperda* and will provide clues to develop potential biocontrol techniques against this corn pest, particularly it provides evidence of the relevance of seasonal changes in microbiota composition in insects for their biological control.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Data Availability

According to national regulations, all available sequence information produced is available upon request in the GenBank database (MK786669–MK786690).

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