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Microbiome of the larvae of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) from maize plants

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Abstract

Fall armyworm (FAW), *Spodoptera frugiperda*, is an invasive insect pest with a diverse host range threatening food security in the African continent by causing severe economic damage to staple maize crop. FAW like all other insects are associated with various microbiota that influence their several characteristics and activities. The microbiota of insects plays important roles in their growth, development and environmental adaptation to their host plants or animals and not much is known about microbiota of FAW in Nigeria. In this study, the microbial community of FAW larvae from maize plants in Nigeria was investigated using Illumina PE250 NovaSeq of 16S rRNA V3-V4 gene region. A total of 1,160,133 sequence reads that ranged from 83,794 to 145,475 were obtained. They span through 2 kingdoms: bacteria (99.59%) and archaea (0.37%), 37 phyla, 59 classes, 78 orders, 145 families and 285 genera. The dominant taxonomic group of bacteria across the larval samples were Proteobacteria (58.32%), Firmicutes (35.87%) and Bacteroidetes (4.02%). There were no significant differences in bacteria species diversity and richness among the individual fall armyworm larva and samples from different geographical regions. The relative abundance of Bacteria phyla and classes also showed no significant differences among the larval samples. Significant differences were documented in bacteria orders Acidobacteriales and Aeromonadales, family Acidobacteriaceae_ (Subgroup_1) and genera Novosphingobium and Pedobacter. The results indicate that the gut of FAW larvae harbours a diverse set of bacteria and archaea biota. Understanding their ecological and functional relevance would provide novel insight on potential approaches for their exploitation in the integrated management of FAW in the tropics.

KEYWORDS

16S rRNA sequencing, archaea, fall armyworm, gut bacteria, invasive pest, maize host

1 | INTRODUCTION

Insects are the most successful group of animals in the universe in diversity and survival in almost all the ecological niches. The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an invasive insect and a highly polyphagous pest of the American origin (Cock, Besheh, Buddie, Cafa, & Crozier, 2017; Pogue, 2002; Todd & Poole, 1980). They attack several agricultural

crops of more than 180 plant species in 42 families (Ram, 2019), including maize (Casmuz et al., 2010; Silva et al., 2017). Maize and other economically important staple food crops in Africa are severely damaged by *S. frugiperda* larvae (Day et al., 2017) causing great economic losses and endangering food security in the region (CABI, 2018). FAW destroys young maize plants by attacking their growing points and burrows into cobs in older plants, thus affecting the yield quantity and quality severely (FAO, 2018). The larvae

generally stay inside the plant tissue and move into the soil to pupate and later emerge as adults. In maize, this pest has historically been controlled with synthetic insecticides, but their effectiveness is restricted because the larvae hide inside the maize whorl (Carvalho, Omoto, Field, Williamson, & Bass, 2013) and have developed resistance to several insecticides (Carvalho et al., 2013; Nascimento, Farias, Bernardi, Horikoshi, & Omoto, 2016). FAW has also developed field-evolved resistance to *Bacillus thuringiensis* (Bt) maize (Chandrasena et al., 2018; Farias et al., 2014; Huang et al., 2014; Omoto et al., 2016; Storer et al., 2010). *Bacillus thuringiensis* (Bt) maize is a transgenic crop that produces insecticidal proteins, which are currently the most successful biotechnological pest management application (Sanchis, 2011). FAW like other insects is known to live in close association with many microorganisms. They harbour various microbial communities that influence their interaction with other trophic levels and their environment (Acevedo et al., 2017). The insect host is affected by the actions and interactions of all the microbiota components such as bacteria, archaea, fungi, protozoa and viruses (Gurung, Wertheim, & Falcao Salles, 2019). Microorganisms are essential for plant-feeding insects whose diet is generally either low in nutrients, high in chemical defences or both. Insect-associated microorganisms provide several functions such as provision of nutrients, regulation of insect-immune responses, synthesis of pheromone and protection against parasites and predators (Douglas, 2015; Engel & Moran, 2013; Kikuchi et al., 2012). In insects, symbiotic bacteria play important roles in host physiology such as providing essential amino acids, prompting resistance against insecticides (Kikuchi et al., 2012), aid in digestion (Visotto, Oliveira, Ribon, Mares-Guia, & Guedes, 2009) and detoxify plant secondary metabolites such as terpenes and phenolics (Hammer & Bowers, 2015). Bacteria found in the regurgitated food can also influence plant responses to herbivore feeding by suppressing plant defences induction and increasing herbivore growth (Chung et al., 2013; Wang et al., 2016).

The common associations between insect guts and bacteria are facultative in nature and seem to perform context-reliant functions (Manson, Jones, & Felton, 2018). Caterpillars (larvae) of Lepidoptera have been suggested to naturally lack resident gut symbionts (Hammer, Janzen, Hallwachs, Jaffe, & Fierer, 2017). Associated bacteria have been studied in <0.1% of larvae of the recognized lepidopteran species using meta-taxonomics (Marchesi & Ravel, 2015), which revealed that current knowledge about the lepidopteran microbiome, and their roles is still very inadequate (Voirol, Frago, Kaltenpoth, Hilker, & Fatouros, 2018). Insect microbiomes could be determinant of the outcome of pest-natural enemies-host plant interactions (Ferrari, Vavre, & Lyon, 2011). Approaches that involve altering insect microbiomes are presently being evaluated for control and management of pests and vectors of plant diseases (Arora & Douglas, 2017; Beck & Vannette, 2017; Crotti et al., 2012; Perilla-Henao & Casteel, 2016). The resident microbiota have great potential to be used as improved control methods (Douglas, 2015). It is therefore of importance to gain insight into the composition and functional role of FAW gut microbiota. A study by Acevedo et al. (2017) on FAW gut bacteria using culture-dependent methods to isolate gut

bacteria from oral secretions of field-collected larvae revealed that the bacterial isolates regulated herbivore-induced defences. A recent study by Rozadilla, Cabrera, Virla, Greco, and McCarthy (2020) using meta-transcriptomic analysis revealed the presence of novel genes and other components in the gut microbiota of FAW larvae that were being expressed by the community. In this study, we used next-generation paired-end amplicon sequencing of 16S rRNA gene to characterize the microbiota of FAW larvae collected from maize plants in Nigeria. The diversity and potential functional roles of the identified microbiota were further discussed.

2 | MATERIALS AND METHODS

2.1 | Sample collection

The larvae of FAW *S. frugiperda* were randomly collected from maize plants at vegetative stage in the field from eleven different locations within three agroecological zones in Nigeria: namely, southeast, southwest and north (Table 1). Nigeria has a tropical climate with variable rainy and dry seasons depending on location. The south-eastern Nigeria is of the wet tropical type climate with mean annual temperature in the range of between 27 and 34°C. South-western temperature ranged between 21 and 34°C, while the northern Nigeria temperature can rise up to 44°C during the dry season.

Larvae were collected from the same maize variety known as OBA SUPPER 11, which matures in 90 days. Sampling was based on the availability of larvae in the field at the time of collection. Larval samples were collected from maize whorls during the rainy season (April to August 2019) and were preserved in 75% ethanol until use. The fifth-instar larvae used for this study were sorted from the pool of field samples in the laboratory using morphological characteristics of the *S. frugiperda* instars from the previous biology study of *S. frugiperda*.

2.2 | DNA extraction

Genomic DNA was extracted from 32 individual fifth-instar larvae sorted out from the samples using CTAB extraction buffer by grinding the insect tissue in liquid nitrogen with mortar and pestle. The ground sample was transferred to 2-ml tube followed by the addition of extraction buffer (pre-heated at 65°C) and DTT (1 mol/L). The mixture was vortexed and incubated for 20 min at 60°C (shaker, 900 rpm). One volume of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged at 10,000 g for 10 min. The upper phase was transferred to a new tube, and RNase A was added at 100 µg/ml final concentration and then incubated at 37°C for 1–2 hr. One volume of chloroform/isoamyl alcohol (24:1) was added, mixed and centrifuged for 10 min at 10,000 g. The supernatant was transferred into a new 1.5-ml tube, and 1/10 volume of NaAc (3M) and 1 volume of isopropanol (at room temp) were added and incubated for 10 min at room temperature and centrifuged for 30 min at 10,000 g at 4°C. The supernatant was discarded, and pellet was washed with 70% ethanol by centrifuging for

Sample 1D	Agroecological zones	Locations	Coordinate
B1-A	A	Abia (Umuahia)	Latitude 5.54°N; longitude 7.48°E
C1-B	B	Osun (isoka mopa)	Latitude, 7.20°N; longitude, 4.11°E
C2-C	B	Lagos (Akoka Yaba)	Latitude, 6.27°N; longitude, 3.23°E
D1-D	B	Osun (Ile-Ife)	Latitude, 7.49°N; longitude, 4.55°E
B2-E	C	Kogi (Anyigba)	Latitude, 7.29°N; longitude, 7.11°E
B3-F	C	Kano (Karaye)	Latitude, 11.78°N; longitude, 8.01°E
D6-G	C	Niger (New Bussa)	Latitude 9.53°N; longitude 4.31°E
D2-H	A	Cross River (Calabar)	Latitude, 4.95 N; longitude, 8.34 E
D3-I	A	Enugu (Enugu-Ezike)	Latitude, 6.94°N; longitude, 7.45°E
D5-J	B	Oyo (Akinyele-Ibadan)	Latitude, 7.29°N; longitude, 3.54°E
D4-K	B	Oyo (Idishin-Ibadan)	Latitude 7.41°N, longitude, 3.85°E

^aA = southeast; B = southwest; C = north.

TABLE 1 The sample collection locations and their coordinates

5 min at 10,000 g at 4°C. The supernatant was discarded, and the pellet was dried in a sterile hood and resuspended in TE buffer. Samples were quantified with NanoDrop 2000C Spectrophotometer (Thermo Scientific) according to manufacturer's protocol.

2.3 | Illumina NovaSeq system

Samples were submitted to Novogene Bioinformatics Technology (UK) for paired-end sequencing of the 16S rRNA V3–V4 region on the Illumina NovaSeq system, where the primers CCTAYGGGRBGCASCAG and GGACTACNNGGGTATCTAAT were used for the generation of the V3–V4 region amplicon (Klindworth et al., 2013).

Amplicon was performed on a paired-end Illumina platform to generate 250-bp paired-end raw reads (raw PE) and then assembled and pre-treated to obtain clean tag.

The original data obtained from the high-throughput sequencing platforms were transformed to sequenced reads by base calling. Raw data were recorded in a FASTQ file, which contains sequenced reads and corresponding sequencing quality information (Cock, Fields, Goto, Heuer, & Rice, 2010).

2.4 | Sequencing data processing

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer

sequence and were merged using FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>), and the splicing sequences were called raw tags (Magoč & Salzberg, 2011).

Quality filtering on the raw tags was performed under specific filtering conditions to obtain the high-quality clean tags (Bokulich et al., 2013) according to the Qiime (V1.7.0) http://qiime.org/scripts/split_libraries_fastq.html (Caporaso et al., 2010)) quality-controlled process. The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (UCHIME Algorithm http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar, Haas, Clemente, Quince, & Knight, 2011) to detect chimera sequences, and then, the chimera sequences were removed according to Haas et al. (2011). The effective tags were finally obtained. All sequence reads were archived in the Sequence Read Archive under the BioProject: PRJNA631705.

2.5 | OTU cluster and taxonomic annotation

Sequence analysis was performed by UPARSE software (UPARSE v7.0.1001 <http://drive5.com/uparse/>) (Edgar, 2013) using all the effective tags. Sequences with ≥97% similarity were assigned to OTUs. Representative sequence for each OTU was screened for further annotation. For each representative sequence, Mothur software was performed against the SSUrRNA database of SILVA Database (<http://www.arb-silva.de/>) (Wang, Garrity, Tiedje, & Cole, 2007) for species annotation at each taxonomic rank (threshold: 0.8 ~ 1) (Quast et al., 2012) (kingdom, phylum, class, order, family, genus,

species). OTU abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences (D4-K), and after the normalization, it was not used further for detailed analysis because of low sequence depth. Subsequent analysis of alpha diversity and beta diversity was all performed based on this output-normalized data. The sequences from three sample replicates in each sample were pooled together; the sequences were analysed to estimate the overall bacterial diversity and richness in each sample. Species richness was estimated using a rarefaction curve and the richness estimators Chao (Chao, 1984) and ACE (Chao & Lee, 1992), whereas the Shannon–Weaver index provides more information about community composition and evenness by considering relative abundances (Kim et al., 2017). Rarefaction curve was created by randomly selecting certain amount of sequence data from the samples, and then counting the number of the species, they represent (i.e. the number of OTUs).

2.6 | Statistical analyses

Multiple sample analysis of molecular variance (AMOVA) R was used to test the significant differences in microbial diversity within the populations between individual larval samples from different locations. Permutational analysis of variance (PERMANOVA) pairwise comparison was conducted to compare sample groups from different geographical regions, while principal component analysis (PCA) was used to compare bacteria community structures across all samples based on the relative abundance of bacteria phyla or genus using SPSS version 25 software. A p value of $<.05$ was considered statistically significant.

Non-parametric independent-samples Kruskal–Wallis test was used to determine the minimum significant difference ($p < .05$) in relative abundance, species richness (Chao 1) and diversity index (Shannon) of bacteria between samples using IBM SPSS Statistics 25.

3 | RESULTS

3.1 | Illumina sequencing data

Paired-end sequencing of the V3–V4 region of the 16S rRNA gene yielded total raw sequence reads of 1,160,133 with average sequence lengths of 397–426 bp from the Illumina NovaSeq

sequencer. The sequences were assigned to the domains archaea and bacteria. All the reads had phred score greater than 30 ($>Q30$; error probability ≥ 0.001), and the average GC content in the range 51%–56% was observed in each sequence reads (Table S1). Application of quality filtering on the raw tags under specific filtering conditions according to the Qiime quality-controlled process resulted in 1,009,546 reads. Effective tags after removing the chimera sequence were 959,298 reads. The average base length of effective tags was 417–428 bp. The pre-processed reads from all samples were pooled and clustered into 1,420 OTUs based on their sequence similarity (similarity cut-off = 0.97), and 836 OTUs were identified from 959,298 after filtering to eliminate OTUs that could arise from sequencing errors and contamination. Successive analysis of alpha diversity and beta diversity was performed based on these normalized output data. A rarefaction curve was constructed for each sample to assess the sequencing depth and the species richness. The dilution curves of the final intergroup samples are presented in Figure 1.

3.2 | Bacteria community richness and diversity of *S. frugiperda* larvae

The larval samples from the different locations showed high microbial diversity and OTU species richness with small variation. The highest diversity was found in sample collected from Abia State (B1-A) with Shannon index of 3.9, and the highest species richness (Chao 1) was found in sample collected from Niger State (D6-G), while sample from Isoko Mopa-Osun State (C1-B) showed the lowest diversity and species richness (Table S2). There were no statistically significant differences in the microbial diversity and species richness of *S. frugiperda* from different agroecological regions (p value: .423) (Kruskal–Wallis test). However, species richness appeared to be higher in *S. frugiperda* from south-east region (A) and lowest in samples from south-west region (B) (Table 2).

3.3 | Bacteria community distribution and composition in *S. frugiperda* larvae identified by Illumina sequencing

The composition of bacteria present in the *S. frugiperda* larvae was grouped into each taxonomic category from phyla level to genus

TABLE 2 Sequencing analysis of 16S rRNA gene amplicons of *Spodoptera frugiperda* from different agroecological regions with diversity indices

Samples from different agroecological regions	No. of sequences	OTU coverage (%)	Observed OTU	Chao1	ACE	Shannon
A	1,108,416,667	74.9	3,863,333	4,441,477	4,584,143	2.7
B	12,129,375	83.9	3,275	3,139,185	3,230,148	1.4
C	114,138	85.8	330	341,529	3,467,923	2.2

^aA = south-east; B = south-west; C = north.

FIGURE 1 Rarefaction curves showing the observed number of operational taxonomic units (OTUs) at a genetic distance of 3% in the different larval samples [Colour figure can be viewed at wileyonlinelibrary.com]

level. The abundance of 10 major bacterial groups in each taxonomic category is presented in Table S3

A total of 37 bacterial phyla were detected in our samples. Among the phyla, Proteobacteria was the most dominant group followed by

Firmicutes and Bacteroidetes. Reads belonging to Euryarchaeota, Cyanobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Deferribacteres, Spirochaetes and other bacteria were found to be very few (>1%). These top ten bacteria phyla covered 99.7% of the

FIGURE 2 The distribution of sequences of bacterial 16S rRNA gene at the phylum level (top 10) of samples from different agroecological regions. (A = south-east; B = south-west; C = north) [Colour figure can be viewed at wileyonlinelibrary.com]

whole bacteria phyla, and other bacteria phyla were 0.3%. There were no significant differences ($p > .05$) in relative abundance of bacteria phyla among the individual larval samples (Figure S1). The relative abundance of bacteria phyla of sample groups from different agro-ecological zones (south-east, southwest and north) also showed no significant differences among the groups. However, the samples from southeast region were found to be dominated by higher number of bacteria phyla than samples from other regions. Samples from the north region were dominated by only three bacteria phyla namely Chloroflexi, Deferribacteres and Spirochaetes (Figure 2). The distribution of bacteria phyla of individual larvae from different locations is shown in Figure 3. The relative abundance of bacteria order was significantly different in two members: Acidobacteriales and Aeromandales with p values (.026 and .045), respectively. A total of 145 bacterial families were observed in the samples with only Acidobacteriaceae ($p = .025$) showing significant difference among the individual samples. At the genus level, 285 bacterial genera were present in our samples. The top 10 members of the group include *Klebsiella*, *Enterococcus*, *Acinetobacter*, *Enterobacter*, *Myroides*, *Sphingomonas*, *Comamonas*, *Sphingobacterium*, *Pseudomonas* and *Clostridium_sensu_stricto_5*. *Klebsiella* was the most dominant bacterial genus followed by *Enterococcus*, *Acinetobacter*, *Enterobacter* and *Myroides*. The proportion of *Pseudomonas* and *Clostridium_sensu_stricto_5* was <1% each, and these top members of the genera constituted 93.37% of the whole genera, while other genera was 6.67%. The lesser members of the bacteria genera among the top 20 were 12 including *Pseudomonas*, *Clostridium_sensu_stricto_5*, *Delftia*, unidentified_Chloroplast, *Chryseobacterium*, *Vagococcus*, *Paenibacillus*, *Erwinia*, *Rhizobium*, *Sporosarcina*, *Leucobacter* and *Faecalibaculum*, and their individual representation was >1% each. At genus level, the relative abundance of several genera showed no significant differences ($p > .05$) among the individual larva samples as well as in groups from different geographical regions. Only two members of the genera *Novosphingobium* and *Pedobacter* differed significantly between the individual larva samples with p values of .025 and .037, respectively (Figure S2). The relative abundance of bacteria genera among sample groups was present in similar pattern with the bacteria phyla (Figure 4).

3.4 | Domain Archaea

The domain archaea were represented by four phyla, three classes, four orders, seven families and seven genera (Table 3). Euryarchaeota (72.22%) was the most dominant phylum followed by Bathyarchaeota (11.11%). At the class level, Methanomicrobia (57.14%) was the most dominant order followed by Thermoplasmata (14.29%). Methanomicrobiales (42.85) was the dominant order, while *Methanosaeta* and *Methanolinea* with both 25% were leading at the genus level.

3.5 | Comparisons of bacteria biota of *S. frugiperda* from previous studies and our study

The major dominant bacteria genera reported from various authors on the gut of *S. frugiperda* is presented in Table 4.

4 | DISCUSSION

This study revealed that the microbiome of the fall armyworm *S. frugiperda* larvae were dominated by Proteobacteria followed by Firmicutes at the phylum level. This observation is in line with the earlier reports on the gut bacteria biota of other lepidopteran insects such as in European Corn Borer *Ostrinia nubilalis* (Belda et al., 2011), Diamondback Moth, *Plutella xylostella* (L.) (Ramya, Venkatesan, Srinivasa Murthy, Jalali, & Verghese, 2016; Xia et al., 2013, 2017), the African maize stem borer *Busseola fusca* (F.) (Snyman, Gupta, Bezuidenhout, Claassens, & van den Berg, 2016), the Spruce Budworm (*Choristoneura fumiferana*) (Landry, Comeau, Derome, Cusson, & Levesque, 2015), Pine processionary moth, *Thaumetopoea pytiocampa* (Denis & Schiff.) (Strano, Malacrino, Campolo, & Palmeri, 2018), Silkworm *Bombyx mori* (Chen et al., 2018) and *S. frugiperda* (Gichuhi et al., 2020).

A recent review that compared microbiota studies of 30 lepidopteran species found that the most dominant phylum across

FIGURE 3 Principal component analysis (PCA) of bacterial communities in *Spodoptera frugiperda* individual larval samples from different locations based on the relative abundance of bacterial genera. Symbol '●' represents bacterial phylum

FIGURE 4 The distribution of fractional sequences of bacterial 16S rRNA gene at the genus level (top 20) from different agroecological regions (A = south-east; B = south-west; C = north) [Colour figure can be viewed at wileyonlinelibrary.com]

species was Proteobacteria within the family Enterobacteriaceae (Voirol et al., 2018). However, recent studies using high-throughput sequencing (NGS) of 16S rDNA to analyse the gut microbiota of late-instar field-captured *H. armigera* larvae revealed that Actinobacteria was the dominant phylum followed by Proteobacteria and Firmicutes (Ranjith, ManiChellappan, Harish, Girija, & Nazeem, 2016) and *S. frugiperda* (Rozadilla et al., 2020). Another NGS study of the late-instar of the gut microbiota in *S. littoralis* found that Firmicutes was the most abundant phylum, followed by Proteobacteria (Chen et al., 2016; Shao, Arias-Cordero, Guo, Bartram, & Boland, 2014).

Four bacteria genera, *Enterococcus*, *Pseudomonas*, *Delftia* and *Serratia*, observed in this study have previously been isolated from *S. frugiperda* (Almeida, Moraes, Trigo, Omoto, & Cônsoli, 2017; Acevedo et al., 2017; Gichuhi et al., 2020). Additionally, *Staphylococcus*, *Microbacterium* and *Arthrobacter* recorded were previously isolated from *S. frugiperda* in Brazil (Almeida et al., 2017). Other isolates documented in this study such as *Enterobacter* and *Klebsiella* have been previously identified in oral secretions of *S. frugiperda* in Pennsylvania, USA (Acevedo et al., 2017).

In this study, *Klebsiella* (Proteobacteria) was the most dominant bacteria genera followed by *Enterococcus* in the gut of *S. frugiperda*.

These results support the recent report by Acevedo et al. (2017) who found *Klebsiella* and *Enterococcus* in *S. frugiperda* larvae but not as the predominant members of the bacteria genera. *Klebsiella* sp. was also reported to be prominent in the DNA data set of adult *S. littoralis* (Chen et al., 2016). However, Gichuhi et al. (2020) did not report the presence of *Klebsiella* in *S. frugiperda* rather recorded high proportion of *Enterococcus* in two larval samples from Nigeria in Kenya. Other authors also documented high relative abundances of *Enterococcus* and unclassified Enterobacteriaceae in the United States (Jones, Mason, Felton, & Hoover, 2019). Members of these genera are widespread in nature and have been previously isolated from guts of other insects such as Cotton Leaf worm, *Spodoptera littoralis* (Shao et al., 2014) and larvae of Sugarcane borer *D. saccharalis* (Dantur, Enrique, Welin, & Castagnaro, 2015). *Enterococcus* and *Klebsiella* were also shown to be abundantly present in the gut of grasshoppers (Stoops et al., 2016).

Interestingly, *Serratia*, *Bacillus*, *Pseudomonas* and *Clostridium* species, which have been reported to have insecticidal (entomopathogenic) properties (Castagnola & Stock, 2014), were also documented. These findings are in agreement with the recent report by Gichuhi et al. (2020) on the occurrence of the same bacteria groups in *S. frugiperda* in their study.

TABLE 3 The abundance of taxonomic categories from phyla to order level of *Spodoptera frugiperda* as occurred in the larvae of *Spodoptera frugiperda* (in % of each category, given in parenthesis)

Phylum	Class	Order	Family	Genus
Euryarchaeota (72.22)	Methanomicrobria (57.14)	Methanomicrobiales (42.85)	Methanosetaeaceae (21.42)	Methanoseta (25)
Bathyarchaeota (11.11)	Thermoplasmata (14.29)	Methanosarcinales (35.71)	Methanoregulaceae (21.42)	Methanolinea (25)
Diapherotrites (5.56)	Methanobacteria (21.43)	Thermoplasmatales (14.29)	Methanobacteriaceae (21.42)	Methanomassiliicoccus (8.33)
WSA2 (5.56)	Unidentified_Diapherotrites (7.14)	Unidentified_Diapherotrites (7.14)	Methanosarcinaceae (14.28)	Methanomethylovorans (8.33)
MEG (5.56)			Unidentified_Diapherotrites (7.14)	Methanobacterium (16.67)
			Unidentified_Thermoplasmatales (7.14)	Candidatus Iainarchaeum (8.88)
			TMEG (7.14)	Methanosarcina (8.33)

The presence of *Arthrobacter woluwensis* (Actinobacteria) in *S. frugiperda* larvae is in line with earlier reports on other lepidopteran insects. *Arthrobacter ilicis* (Actinobacteria) was found in the larval gut of *Helicoverpa armigera* in India (Ranjith et al., 2016). Similarly, analysis of 16S rDNA of gut bacterial community in oriental army worm, *Mythimina seprata*, revealed the presence of the *Arthrobacter species* (He, Nan, Zhang, & Li, 2013). Occurrences of these bacteria in the gut of insects may aid in eliciting the binding of synthetic insecticide molecules (organophosphorus and carbamates groups) to the enzyme and prevent activity of the insecticides on the target organisms (Ranjith et al., 2016).

The gut bacteria *Clostridia* sp. and *Microbacterium* identified in *S. frugiperda* are associated with the breakdown of plant cell wall components such as cellulose and xylan. Similarly, *Acidobacterium* sp., *Clostridia* sp., *Microbacterium* sp., *Flavobacter johnsoni* and *Thermobia* sp. found in *H. armigera* gut have been reported to be involved in digestion of cellulose in the gut of other insects (Castaneda & Mallo, 2013; Huang, Sheng, & Zhang, 2012; Ranjith et al., 2016). Also, *Pseudomonas* sp. and *Clostridia* sp. detected in our study have been reported to be responsible for degradation lignocelluloses in wood-feeding beetles and termites (Lynd, Weimer, van Zyl, & Pretorius, 2002; Rizzi et al., 2013). The *Candidatus* sp. detected in the gut of *S. frugiperda* is capable of providing essential amino acids to insects flourishing on nutrient-deficient diets (Moran & Mira, 2001; Nikoh, Hosokawa, Oshima, Hattori, & Fukatsu, 2011). Thus, the associations were considered beneficial to the *S. frugiperda* in the improvement of the nutrient status of their diet. In this study, we identified *Burkholderia* bacteria, which are capable of detoxifying pesticides as well as *Enterococcus* sp. and *Rhodococcus* sp. that functions in detoxifying plant toxins. Similarly, Ranjith et al. (2016) reported that *Burkholderia*, *Enterococcus* sp. and *Rhodococcus* sp. were detected in the gut of *H. armigera*.

We observed similarities as well as variations in our studies from the reports of previous work on microbiome of *S. frugiperda* from different countries. From the five studies, so far reported in different countries (Brazil, USA, Kenya, Argentina and Nigeria) *Enterococcus* was persistent in gut of the *S. frugiperda* larvae. The genus *Pseudomonas* was recorded in four countries (Brazil, USA, Kenya and Nigeria), while *Enterobacter* was persistent in studies at three different countries (USA, Argentina and Nigeria). Similarly, *Chryseobacterium*, *Comamonas* and *Sphingobacterium* were recorded in three countries (USA, Kenya and Nigeria). The composition of these bacteria genera found in the gut of *S. frugiperda* larvae varied in samples from different countries. These variations indicate that environment could be a driving force in the composition of bacteria as seen from the previous report on the bacteria diversity in the gut of *S. frugiperda*. Andersen, Chapman, and Artz (2013) also reported that environmental conditions may be a stronger driver of microbial community composition. The variation in the bacteria composition in the gut of *S. frugiperda* larvae as seen from the previous studies could also be attributed to different maize genotypes, geographical locations and different developmental stage of larvae. However, the observed insignificant differences in the relative abundance and

TABLE 4 Previous report on dominant bacteria genera of *Spodoptera frugiperda* in different countries

Acevedo et al. (2017) (USA)	Almeida et al. (2017) (Brazil)	Jones et al. (2019) (USA)	Gichuhi et al. (2020) (Kenya)	Rozadilla et al. (2020) Argentina	Current study (Nigeria)
<i>Enterobacter</i>	<i>Arthrobacter</i>	<i>Acinetobacter</i>	<i>Achromobacter</i>	<i>Enterobacter</i>	<i>Acinetobacter</i>
<i>Klebsiella</i>	<i>Delftia</i>	<i>Aquabacterium</i>	<i>Acinetobacter</i>	<i>Enterococcus</i>	<i>Chryseobacterium</i>
<i>Pantoea</i>	<i>Enterococcus</i>	<i>Arthrobacter</i>	<i>Aeromonas</i>	<i>Janibacter</i>	<i>Clostridium_sensu_stricto_5</i>
<i>Raoultella</i>	<i>Leclercia</i>	<i>Bradyrhizobium</i>	<i>Carnobacterium</i>	<i>Lysobacter</i>	<i>Comamonas</i>
<i>Serratia</i>	<i>Microbacterium</i>	<i>Chryseobacterium</i>	<i>Chryseobacterium</i>	<i>Pediococcus</i>	<i>Delftia</i>
	<i>Pseudomonas</i>	<i>Comamonas</i>	<i>Citrobacter</i>	<i>Rubrobacter</i>	<i>Enterobacter</i>
	<i>Staphylococcus</i>	<i>Enterococcus</i>	<i>Comamonas</i>	<i>Vibrio</i>	<i>Enterococcus</i>
		<i>Flavobacterium</i>	<i>Cutibacterium</i>	<i>Xanthomonadaceae</i>	<i>Erwinia</i>
		<i>Leuconostoc</i>	<i>Delftia</i>	<i>Xylanophilus</i>	<i>Faecalibaculum</i>
		<i>Luteibacter</i>	<i>Enterococcus</i>		<i>Klebsiella</i>
		<i>Methylobacterium</i>	<i>Lysinibacillus</i>		<i>Leucobacter</i>
		<i>Ochrobactrum</i>	<i>Morganella</i>		<i>Paenibacillus</i>
		<i>Pseudomonas</i>	<i>Ochrobactrum</i>		<i>Pseudomonas</i>
		<i>Ralstonia</i>	<i>Pseudomonas</i>		<i>Bradyrhizobium</i>
		<i>Rhizobium</i>	<i>Serratia</i>		<i>Rhizobium</i>
		<i>Sphingobacterium</i>	<i>Sphingobacterium</i>		<i>Sphingobacterium</i>
		<i>Sphingomonas</i>	<i>Stenotrophomonas</i>		<i>Sphingomonas</i>
		Unclassified Enterobacteriaceae			<i>Sporosarcina</i>
		Unclassified Enterobacteriaceae			Unidentified Chloroplast
					<i>Vagococcus</i>

Note: The most commonly represented bacteria genera in different studies are highlighted with the same colour across the rows.

microbial diversity of samples from different agroecological regions in this study could be attributed to the unvarying developmental stage of the larvae used, the same maize host genotype and larvae enclosure within the maize whorls at the point of sampling/ collection that may limit the effects of the environments.

The study by Acevedo et al. (2017) in the United States revealed five major bacteria genera using culture-dependent methods, while in Brazil the gut of *S. frugiperda* revealed eight major bacterial genera that are resistant to different insecticides based on 16S rDNA sequence similarities obtained from heuristic search against sequences in NCBI and EzTaxon-e databases (Almeida et al., 2017). Another study in the United States and Puerto Rico reported 20 major bacteria genera to be associated with the gut of *S. frugiperda* larvae using bacterial 16S rRNA sequencing (Jones et al., 2019). Similarly, a study in Kenya using NGS reported 19 major bacteria genera in the gut of *S. frugiperda* (Gichuhi et al., 2020). The fifth report on *S. frugiperda* Argentina using high-throughput pyrosequencing of 16S rDNA reported 10 major bacteria genera with other non-bacterial group (Rozadilla et al. (2020). In the present study, 20 major bacterial genera were documented. The variations in microbial diversity of *S. frugiperda* as shown in previous studies could also be attributed to

the different molecular tools used by various authors. Prior studies have shown that molecular methods offer powerful tools for detecting bacterial and fungal structure and diversity in ecological samples and appraising the response of microbes to ecological changes (Allison & Martiny, 2008; Weedon et al., 2012).

This study revealed that Archaea are present in *S. frugiperda* larvae. This finding corroborates the recent report by Rozadilla et al. (2020) who also detected the presence of archaea in the gut of *S. frugiperda* larvae using high-throughput pyrosequencing of 16S rDNA, and it represented a high proportion of the metabolically active gut microbiota in *S. frugiperda*. Archaea have been revealed through metagenomic studies to be very diverse and prolific, and they play vital roles in nitrogen cycling in soils (Bates et al., 2011; Edwards et al., 2015; Siles, Öhlinger, Cajthaml, Kistler, & Margesin, 2018; Secil, Sevim, Demirbag, & Demir, 2012). There is insufficient information on the roles they play in their host (Gurung et al., 2019) although it has been suggested that they could affect plants (Taffner et al., 2018). In this study, Euryarchaeota was the most dominant archaea phylum followed by Bathyarchaeota in the gut of *S. frugiperda*. On the contrary, Rozadilla et al. (2020) reported only uncultured Crenarchaeota in the gut of *S. frugiperda* in their own study. However, phylum Euryarchaeota

has been reported in the hindguts of beetles, cockroaches, termites and millipedes (Brune, 2009; Egert et al., 2005; Hara, Shinzato, Seo, Oshima, & Yamagishi, 2002; Shinzato, Matsumoto, Yamaoka, Oshima, & Yamagishi, 1999; Šustr, Chroňáková, Semanová, Tajovský, & Šimek, 2014; Tinker & Ottesen, 2016). Similarly, shotgun metagenomic analysis of the gut of diamondback moth *Plutella xylostella* larvae revealed Euryarchaeota DNA in very low proportions (Xia et al., 2017).

In conclusion, the bacterial communities in *S. frugiperda* larval samples collected randomly from different locations in Nigeria were unravelled with aid of NGS sequencing. Significant differences and resemblances across samples and in relation to other studies on this species were observed. Bacteria were the dominant microbiota in the gut of *S. frugiperda*, while archaea were relatively a minor component of the microbiota community. Among the bacterial community, the most dominant group was Proteobacteria. Notable was the presence of insecticidal toxin-producing bacteria (entomopathogenic) in the gut of *S. frugiperda*. Geographical location did not influence the larval microbiome of *S. frugiperda* in this study. Characterizing the microbial diversity of this pest species in Nigeria is a significant step towards the exploration of novel, cost-effective biological-based management strategies. Further studies to analyse more samples from different environment and food sources to determine the significance of the taxa found in this study and their functional roles are ongoing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

JAU and FOA conceived the research. JAU and ML conducted the experiments. JAU and FOA contributed material. JAU and HS analysed data and conducted statistical analyses. JAU wrote the manuscript. JAU secured funding. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Tables S1–S3.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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