Influence of Host Plants on the Diversity of Gut Microbiota Communities of Fall Armyworm *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

Juliana Amaka Ugwu (dr.amaka2013@gmail.com)
Forestry Research Institute of Nigeria https://orcid.org/0000-0003-1862-6864
Fred Asiegbu
Department of Forest sciences, University of Helsinki, P.O Box 27, FIN-00014 Helsinki, Finland.

Research Article

Keywords: host plants, microbial variability, fall armyworm, larvae, bacteria

Posted Date: June 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-657579/v1

Abstract

The gut bacteria of insects influence their host physiology positively, although their mechanism is not yet understood. This study characterized the microbiome of the gut of *Spodoptera frugiperda* larvae fed with nine different host plants; sugar cane (M1), maize (M2), onion (M3), cucumber (R1), tomato (R2), sweet potato (R3), cabbage L1), green amaranth (L2), and celocia (L3) by sequencing the theV3-V4 hypervariable region of the 16S rRNA gene using Illumina PE250 NovaSeq system. The results revealed that gut bacterial composition varied among larvae samples fed on different host plants. Three alpha diversity indices revealed highly significant differences on the gut bacterial diversity of S. *frugiperda* fed with different host plants.. Analysis of Molecular Variance (AMOVA) and Analysis of Similarity (ANOSIM) also revealed significant variations on the bacterial communities among the various host plants. Five bacteria phyla (Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria and Bacteroidetes) were prevalent across the larvae samples. Firmicutes (44.1%) was the most dominant phylum followed by Proteobacteria (28.5%). Linear discriminant analysis effect size (LEfSe) analyses showed that *S. frugiperda* larvae were enriched by diverse bacteria groups. Celocia (L3) and sweet potato (R3) were enriched in phylum Firmicutes by 15.1% and 14.2 % respectively while green amaranth (L2) and sugar cane (M1) were enriched in proteobacteria by 18.5% and 14.3% respectively. Genus *Enterococcus* was predominant and mostly enhanced by L3 with 21.7% incidence. Mann-Whitney' test revealed highly significant differences ($p<0.001$) on OTUs number among larvae samples. Our findings indicate that host plant is a major driver shaping insect gut microbiota.

Introduction

Insects inhabit a diverse set of niches, and are confronted with many different challenges like food sources, toxins, environmental dissipations, parasites and pathogens menace. They have developed strategies to cope with these challenges which often include creating symbiotic associations with microbes [1]. Native bacterial communities are usually concealed in insect guts Studies on the microbial communities associated with insect guts have recently been generating interest among researchers due to their economic and ecological importance [2]. Several host functions like development, defense against natural enemies, immune system response, energy utilization and food digestion, insect resistance, production of essential vitamins and gut physiology can be performed by microbes [3-4]. For instance, metabolic characteristics which are often absent in insects are often possessed by microorganisms and they can assist the insects to adapt to diverse host plants[5]. Herbivorous insects have developed strategies to overcome plant defenses through exploitation of microorganisms for their metabolic processes [6].

Bacteria in the insect gut can adapt to sudden changes in the insect diet or population structure [7].) The adaptive ability of the insects supports them in exploiting various types of food sources and which is the basis for the development of host-associated differentiation[2].The insect gut can be rich in microbial symbionts. The locations, functions and associations of these associates can vary considerably. Some insects possess special gut modifications or structures such as paunches, diverticula, and caeca to house symbionts while others lack morphological modifications [5]. Studies have shown that gut associated bacteria of lepidopteran insects could enhance plant growth and physiology by aiding nutrient acquisition and digestion [8]. Insects gut microbiota also metabolize toxic plant compounds [9], assist in the hydrolysis or synthesis of N-acyl amino acid conjugates which are elicitors of JA-mediated plant defenses [10]. They produce digestive proteases and protect the host from pathogens [11].

Fall army worm (FAW), *Spodoptera frugiperda* is a highly polyphagous lepidopteran insect in the family Noctuidae and a major agricultural pest causing huge economic loss in South America, the Caribbean, and lately in Africa [12 -13]. FAW has a very wide host range, it has been reported in about 80 plant species but they intermittently cause major economic damages to their major host plants such as maize, rice sorghum, wheat, alfalfa, cotton, turf, millet, soybean and grasses [14]. They are known to cause severe damage through feeding on the leaves /shoots of their major hosts resulting to leaf skeletonization. The leaves of the heavily infested maize usually look scruffy [15].

According to Silva et al. [16] fall armyworm develops faster and prefers maize and wheat but can also complete development on broadleaf crops like soybean and cotton. Presently, there is inferred evidence that plant-associated bacteria contribute significantly to bacterial diversity in herbivore guts [17-18]. However. it is not yet known if the variation is driven by replacement of bacteria from the environment or from the initial formation of gut bacteria.

The precise beneficial functions of gut microbes of fall army worm have not been determined. According to Acevedo et al. [19] some bacterial taxa have been observed to suppress plant defenses while other bacteria isolates can aggravate the harmful effects of plant defenses on caterpillars [20].

The variation in lepidopteran gut microbial communities is a major constraint in studying microbial function in plant interactions. [21]. Approaches that involve modifying insect microbiomes are being evaluated presently for control and management of pests and vectors of plant diseases [22].

A comprehensive knowledge of the bacterial communities of insect gut is vital for the full understanding of its hosts biology and ecology and will provide insight in the development of a novel strategies in pest management

Despite the economic importance of fall army worm, not much is known about how host plants influence their bacteria communities particularly in the tropics. In this study, we used bacterial 16S-rRNA sequencing to determine variations in gut communities of fall armyworm fed with nine different host plants *in vitro*.

Materials And Methods

Insect sampling and rearing

The fourth instar larvae of fall armyworm were collected from maize plants at Farmers field in Ibadan, Oyo state Nigeria during 2020 farming season and transferred to the Entomology and biology laboratory of the Federal College of Forestry Ibadan, Forestry Research institute of Nigeria (FRIN), Oyo state, Nigeria. Ibadan is in western part of Nigeria located within latitude 7 22' N and longitude 30 54' E of Greenwich Meridian Time (GMT) with annual rainfall of about 1300 to 1500mm and average relative humidity of about 80 to 85 % [23].

The larvae culture was maintained on potted maize plant under laboratory condition of ambient temperature 27 ± 0.5˚C, 80% relative humidity and 12:12 photoperiod until the adult emerged. The emerged adults were paired for mating in separate cages and fed with 10% sugar solution. They were supplied with different host plants in separate cages for oviposition. Nine host plants were used for rearing larvae used for this study (Table1). Each host plant had five biological replicates. The leaves used in feeding the larvae in the laboratory were sourced from farmers field and were rinsed with distilled water (dH_2O), dried on filter paper before being fed to *S. frugipeda* during the entire experimental period. The Fifth-instar larvae reared from the nine different host plants were

collected and preserved separately in 50 ml tubes in 95% ethanol and stored at 4 °C until used for DNA extraction at the Forest Pathology Laboratory, University of Helsinki, Finland (see below).

DNA Extraction and Sequencing

Genomic DNA was extracted from full bodies of each larvae samples by crushing directly with a sterilized mortar and pestle according to procedure by[24]De Cock et al (2019) in liquid Nitrogen using CTAB extraction buffer [25] at the Forest Pathology Lab (Department of Forest Sciences, University of Helsinki, Finland). The DNA extraction followed the same procedure as previously described [25]. The purity and concentration of DNA were monitored on 1% agarose gels and DNA was diluted to 1ng/μL using sterile water. 16S rRNA of distinct regions16S V3-V4 were amplified using specific primer with the barcode. Sequencing libraries were generated using NEBNext Ultra DNA Library. The quality of the library was assessed on Agilent Bioanalyzer 2100 system and the concentration was checked on Qubit@ 2.0 Fluorometer. The library was sequenced on Illumina platform to generate 250 bp paired-end reads.

Processing of Sequencing data

Paired-end reads was assigned to samples based on their unique barcode and shortened by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the QIIME (V1.7.0.) quality-controlled process [26]. The tags were compared with the reference database (Gold database) using UCHIME algorithm to detect chimera sequences and the chimera sequences were removed to obtain the effective Tags [27]. Sequence analysis was done using Uparse software and sequences with ≥97% similarity was assigned to the same OTUs. Species annotation was done for each representative sequence. The Green Gene Database was used to annotate taxonomic information (Multiple sequence alignment was conducted using the MUSCLE software to study relationships of different OTUs and differences in dominant species. OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. All sequence reads were archived in the Sequence Read Archive in NCBI under the BioProject: PRJNA734744

Data Analysis

Alpha diversity indices (Observed-species, Shannon, ACE and Chao1) were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3) Beta diversity on both weighted and unweighted unifrac were calculated with QIIME software (Version 1.7.0). Principal component analysis (PCA) and Principal Coordinate Analysis (PCoA) were performed in R to compare bacteria community structures across all larvae samples based on the relative abundance. Unweighted Pair-group Method with Arithmetic Means (UPGMA) clustering was done with QIIME software (Version 1.7.0) to interpret the distance on average linkage. Analysis of Molecular Variance (AMOVA) was calculated in mothur to determine differences in microbial community structure among groups. The ADONIS test was also performed in R 3.5.1 to analyze differences in the entire bacterial communities of M1, M2, M3, R1, R2, R3, L1, L2, L3, L1 samples. Linear discriminant analysis (LDA) effect size (LEfSe) was conducted using LEfSe software to detect significant abundant bacterial taxa among the host plant groups. The factorial Kruskal-Wallis sum-rank test ($\alpha = 0.05$) was done to identify significant differences in the bacterial communities between groups.

Results

Structural changes in the bacterial communities

The number of valid reads varied among different samples (Table 2). A total of 6,274,405

high quality reads with an average length of 422 bp were obtained. Rarefaction analysis indicated that the number of species increased rapidly before reaching a plateau (Fig. S1).

Bacterial diversity among all the larvae group fed with different host plants were highly significant as demonstrated by three parameters (observed species richness, ACE and Chao1 (Table 1, Fig.1, Fig.S2, Fig. S3). Shannon index showed no significant difference among all the larvae group from the different host plants, although significant differences existed between larvae samples fed with celocia (L3) and cucumber (R1) with p value = 0.0207 by Shannon wilcox test. Simpson index also showed no significant differences among all the larvae groups fed with the various host plants.

Principal component analysis (PCA) by clustering showed that the bacterial communities of all the larvae groups were not totally different although some groups like M1 and R1 groups were distinct from other larvae groups (Fig. S4). This indicates that host plants had a significant effect on the larvae of FAW gut bacterial communities. Principal coordinate analysis (PCoA) was calculated based on weighted and unweighted UniFrac, which considers both community membership and relatedness of community members. These distances were visualized by PCoA plots which displayed similarity between communities (Fig. 2). The unweighted pair group method with arithmetic means (UPGMA) clustering analyses also indicated that the entire bacterial communities of bacteria group were clearly distinct from each other (Fig.3). Analysis of molecular variance (AMOVA) revealed that the bacterial communities were significantly different among the various larvae groups (p=0.002).

Comparison of Bacterial communities of FAW larvae from different host plants

Nine phyla (Firmicutes. Proteobacteria. Cyanobacteria. Actinobacteria. Bacteroidetes Acidobacteria Patescibacteria Chloroflexi and Verrucomicrobia) were present in all the samples at varied proportions (Fig.4a). Firmicutes was the most abundant phylum and accounted for 44.1 % of the total bacteria with 15.1% and 14.2 % from larvae fed with celocia (L3) and sweet potato (R3) plants respectively (Table S1). Proteobacteria with 28.5% of the total bacteria followed in abundance and was dominated in the larvae group fed on green amaranth (L2) and sugar cane (M1) with 18.5% and 14.3% respectively. Cyanobacteria constituted 11.4% of the total phyla and was mostly enriched by larvae groups fed with cabbage (L1) and cucumber (R1) with 20.0% and 17.9% respectively. Actinobacteria recorded 9.4% bacteria phyla representation and was mostly enriched in M3 and R2 with 24.3% and 20.9% respectively. Phylum Bacteroidetes was only 5.4% and was mostly enriched by R1 and L2 with 36.2% and 22.8% respectively. Acidobacteria recorded 0.35% of the total bacteria phyla and was enriched by M1and R1 with 40.7%and 38.3% respectively. Chloroflexi constituted 0.09% of the total bacteria phyla in the gut of *S. frugiperda* and was mostly contributed by R1 and M1 with 40.8% and 35.4% respectively. Phylum Rokubacteria constituted only 0.05% of total bacteria and was found in three groups; M1, R1 and M2 with 47.9%, 34.1% and 17.9 % respectively (Table S1). Unweighted Hierarchical clustering tree of all gut bacterial groups based on taxon distribution revealed gemmatimodetes and Chloroflexi to be among the top ten bacteria phyla in the gut of *S. frugiperda* and was only recorded in three samples (R1, M1 and M2) (Fig.3).

Eight bacteria classes were prevalent across all the larvae groups; Gammaproteobacteria, Bacilli, Erysipelotrichia, Oxyphotobacteria, Actinobacteria, Bacteroidia, Alphaproteobacteria, and Clostridia. Bacilli was the most dominant class representing 28.8% of the total classes followed by Gammaproteobacteria with 23.2%. Class Bacilli was mostly enriched by L3 and M1 with 21.3% and 15.8% respectively while Gammaproteobacteria was mostly enriched by L2 and M1 with 20.4 and 14.9% respectively (Fig.4b). Erysipelotrichia was 13.9% and was mostly enriched by M3 and L2 groups with 19.5% and 19.5% representation respectively (Table S2). The number and relative abundance of bacterial phylum to species levels varied among the different groups fed with different host plants, although there were no significant differences among them (Table 3). However, M1 sample recorded highest number of bacteria order and families with 196 and 328 respectively while R1 recorded highest number of bacterial genus and species with 578 and 416 respectively. Order Lactobacillales was the most dominant order observed in all the samples, followed by Erysipelotrichales (Fig. S5). They constituted 28.4% and 13.9% of all the bacteria order sequenced respectively. The order Lactobacillales and Erysipelotrichales were mostly enhanced in L3 and M3 larvae samples with 21.6% and 19.5% respectively (Table S3).

The resulting heatmap shows the relative abundances of the top 35 OTUs at the family level facilitating the identification of species that vary in abundance in each sample (Fig.5). Enterococcaceae was the predominant bacterial family in all the larvae samples constituting 28.3 % of all the bacteria families recorded (Fig. S6). Erysipelotrichaceae with 13.9 % and Moraxellaceae (13.3%) followed Enterococcaceae in occurrence. The family Enterococcaceae was mostly enhanced by L3 and M1 with 21.59% and 15.8% respectively while Erysipelotrichaceae and Moraxellaceae were mostly enriched by M3(20.5%) and L2(30.3%) respectively (Table S4). The relative abundance and number of bacterial genera varied in different larvae samples. The predominant genus *Enterococcus* (28.2%) was mostly enhanced in L3 followed by M1 with 21.7% and 15.7% (Fig. S7). *Erysipelatoclostridium* (13.5%) and Acinetobacter (13.33%) followed *Enterococcus* in relative abundance among the top 10 bacteria genera recorded. *Erysipelatoclostridium* and *Acinetobacter* were mostly enriched in L2 sample with 20.0% and 30.3% respectively (Table S5). The genus *Klebsiella* was mostly supplied by M1 (58.7%) while *Corynebacterium_1* was dominant in M3 sample. Other minor bacteria genera apart from the top ten found in the different larvae samples was about 34.4%. Larvae samples fed with R1 was mostly dominated by genus *Weeksella* (64.0%).

The number and relative abundance of bacterial species also differed in larvae from different host plants ranging from 205 to 416. R1 sample with 416 number of bacterial species has higher number of bacteria species followed by M1 (415). The least bacterial species was recorded in L2 sample (Table 3). Uncultured_*Clostridium_sp* with 13.48 % of all the bacteria species was the most dominant species and was enriched in L2 and M3 with 20.0% and 19.2% respectively (Fig.S8). *Acinetobacter_calcoaceticus* and *Klebsiella_variicola* were mostly enhanced by L2 and M1 with 41,86% and 58,72% representation respectively. *Pectobacterium_carotovorum_subsp._carotovorum* and uncultured_ *Flavobacterium_sp* were mostly enhanced in M1 (50.2 %) and R1 (64.1%) respectively (Table S6). In total, 38724 OTUs were identified in all the larvae groups from different host plants. The number of OTUs significantly varied among the groups. The number of OTUs ranged among the larvae groups in the order of M1>R1>M2>R3>R2>L2>L1>L3>M3 (Table 3). M1 had the highest OTU numbers while M3 was the least among all the groups in the number of OTUs.

LEfSe analyses revealed that Phylum Cyanobacteria of class oxyphotobacteria, order chloroplast species *Sphingobacterium_multivorum* were enriched in larvae fed by L1 host plant (Fig. S9). Phylum Firmicutes of class Bacilli, order lactobacillales and family Enterococcaceae, genus Enterococcus and species *Acinetobacter_baylyi*

were enriched in L3 fed larvae. Phylum protobacteria of class Gammaproteobacteria, order Pseudomonadales and family Moraxellaceae, genus *Acinetobacter*, species *Acinetobacter_calcoaceticus* as well as order Cytophagales of family Spirosomaceae and *Leadbetterella* were enriched in L2 host plant as well as order Flavobacteriales of families Flavobacteriaceae and Weeksellaceae, order _Corynebacteriales of family Tsukamurellaceae, Micrococcaceae and order Rhodobacterales of family Rhodobacteraceae were enriched in R1 host samples

M1 host plants were also enriched in Class bacilli of the order Lactobacillales, the family Enterococcaceae and genus Enterococcus. M3 enhanced predominance of the class_Actinobacteria of the order Corynebacteriales within the family Corynebacteriaceae, genus Corynebacterium_ and species *Corynebacterium_variabile* (Fig. S10). A significant variation was found between the R1 and R3 groups. We found that 6 taxa were overrepresented and 10 were underrepresented, e.g. order Flavobacteriales (Fig. 11).

Discussion

In the present study, we analyzed the composition and relative abundance of the gut bacterial communities of the fifth instar larvae of *S. frugipeda* fed with nine different host plants under in vitro conditions. The results showed that the bacterial communities were substantially influenced by feeding of larvae on the nine different host plants. Earlier studies have suggested that life stage, environment and diet are the major factors that influence the formation of gut bacterial communities in insects [28-29,]. The results of this study are in line with findings from previous studies which showed that the bacterial community structure in many insect species are influenced by diets [30, 2]. The results from this study provided a further evidence that insect gut bacterial communities are influenced by diet which is dependent on their hosts.

High bacteria diversity with nine phyla (Proteobacteria, Firmicutes, Cyanobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Patescibacteria, Chloroflexi and Verrucomicrobia) in the gut of all the larvae samples were documented. Firmicutes was the predominant phylum among them followed by proteobacteria. This results corroborate with the findings of other researchers that bacteria phylum Firmicutes was the most abundant followed by Proteobacteria in the gut of late instar of *Spodoptera litoralis* [31] . Similarly, Gomes et al. [32] reported that Firmicutes was the predominant bacteria phylum in the gut of *S. frugiperda* larvae. However, two recent studies on the gut bacterial community of the fifth instar larvae of FAW revealed that Proteobacteria was most dominant phyla followed by Firmicutes [33,25]. Similar observation was made by several authors in other phytophagous insects, in particular lepidopterans [8,17,34] . Similarly, Lu et al. [2] reported that Proteobacteria and Enterobacteriaceae were the principal phylum and family respectively in the gut of fourth instar larvae of *Henosepilachna vigintioctopunctata* fed on two host plants *Solanum melongena* and *Solanum nigrum.* These variations could probably be attributed to the different host plants on which the larvae fed on, implying that host plants are major driving force to the community diversity in microbial composition of insect's gut.

This study also showed that the relative abundance of Firmicutes was significantly higher in larvae groups fed by celocia and sweet potato compared to other host plants. Studies have shown that Firmicutes function in energy absorption, and may cause diabetes and obesity in insects, mice and humans [31]). According to Chen et al.[31], studies on insects and other animals proved that increase in ability to extract energy from the diet are related to firmicutes increase. Thus, high proportion of Firmicutes observed in larvae fed on celocia and sweet potato suggests that the two plants have higher content of carbohydrates and polysaccharides than the other host plants. This may indicate that FAW larvae need more Firmicutes bacteria for the digestion hence the higher

number observed on their guts. Our results revealed that protobacteria were more dominant in green amaranth (L2) and sugar cane (MI) host plants. Proteobacteria can digest secondary metabolites (as terpenes, alkaloids, glycosides and phenolic compounds) of insect host plants and help to maintain the growth and development of insects and its absence leads to poor development in insects and other growth abnormalities[35].The higher proportion of Proteobacteria in larvae fed with green amaranth and sugarcane observed in our study suggest that those two host plants contain more secondary metabolites than other host plants, hence the higher proteobacteria to aid the metabolism. Proteobacteria have been reported to degrade insecticides [36]. The high number of proteobacteria may help the larvae tolerate plants that were treated with insecticides. Proteobacteria plays a diverse functional role and helps for adaptation to different hosts in nature[37-38].

Phylum Cyanobacteria was the third in the rank of bacteria phyla observed in this study constituting 11.4% of the total phyla and was mostly enriched in groups fed with cabbage (L1) and cucumber (R1). Oxyphotobacteria belong to the class of cyanobacteria observed in our larvae samples. These results are consistent with the study by Lu et al. [2] who reported that Oxyphotobacteria was found in small proportion on the fourth instar *H. vigintioctopunctata* fed with *solanum nigrum.* According to Soo et al. [39]phylum Cyanobacteria comprises of three classes namely; Oxyphotobacteria, Melainabacteria, and Sericytochromatia. A study by Utami et al. [40] revealed the presence of Melainabacteria in the gut of termite but this study detected Oxyphotobacteria in the gut of FAW larvae. Thus, this study has advanced the knowledge of Cyanobacteria group in the guts of insects. The information on any host associated variation in microbial structure and description of microbial communities of insects is vital for full understanding of insect ecology and development of innovative strategy for pest management. For instance, according to Crotti et al. [41] microbes can be used to improve Sterile Insect Technique (SIT) programs aimed at reducing the spread of insect borne pathogens and vectors and to protect beneficial insects. The baseline knowledge on the biology of the target species, and the characterization of microorganisms associated at the different life stages and its host need to be discovered[42] Furthermore, we identified a relatively high presence of Actinobacteria (9.4%) associated with *S. frugiperda* fed with different host plants This finding is consistent with the results of Strano et al. [34] who reported high incidence of Actinobacteria in the gut of *Thaumetopoea pityocampa* from conifer pine species. Actinobacteria has been previously reported in the guts of other insects like pyrrhocorid bugs where it acts as essential nutritional symbiont [43]. According to Kaltenpoth [44] Actinobacteria associated with insects provide protection against detrimental microorganisms. Remarkably, among Actinobacteria, reads associated with the genus Corynebacterium_1 were more abundant in *S, frugiperda* larvae fed with onion (M3) than other host plants. This result is of particular interest from an ecological point of view since it suggests a specific association between Corynebacterium_1 and *S. frugiperda* larvae feeding on onion plants. Ventura et al. [45] reported that Corynebacterium in the family of Corynebacteriaceae contains over 80 species and have been isolated from diverse samples like human clinical samples and animals, soil, marine environments, and dairy products. However, Corynebacterium have also been reported to occur in the gut of adult and larvae of *Anopheles gambiae* [46].

Acidobacteria was relatively low in the guts of S. *frugiperda* larvae as observed in this study and was prevalent in larvae samples fed with three different host plants (M1, R1, and M2). Acidobacteria is known to be a principal bacteria phylum in tropical agricultural soils [47]. Several research using 16S rRNA gene sequencing and environmental shotgun metagenomic analyses have shown that the Acidobacteria denote a highly diverse phylum resident in a wide range of habitats around the globe[48]. Acidobacteria have been reported to play several roles such as carbohydrate metabolism [49], Nitrogen metabolism and production of Exopolysaccharide (EPS) [50] .Phylum gemmatimodetes and Chloroflexi were observed in small proportion in three larvae samples fed with

three different host plants. This is consistent with recent report by Leite-Mondin et al. [51] who detected very low abundance of Chloroflexi in the guts of *Trichoplusia ni* populations fed with exclusive diets.

We observed relatively high bacteria taxa associated within the gut of fall armyworm fed with different host plants ranging from 205- 416 species in 319 – 578 genera from 511- 1518 OTUs. Similarly, a recent study of the of Mexican bean beetle (*Epilachna varivestis)* regurgitant revealed a highly diverse microbiome with 1230 bacterial species in 577 genera [52] However, Jones et al. [53] reported less diversity and richness using culture dependent sequencing which detected about 100 OTUs in fall army larvae fed on maize and soybean. Bacteria associated with insects can confer their host with traits that allow them to exploit different host plant species in diverse ways, further defining the species host range [3] Shin et al., 2011).

Conclusions

Our study has confirmed that these diverse host plants impact differently on the structure and community diversity of the microbiome associated with larvae of fall armyworm. This study contributes in part to our understanding of the factors that influence gut microbiomes in fall armyworm larvae. It also offers insight into the composition of bacterial communities in FAW guts which are likely to be relevant for mediating plant–insect interactions. We showed that variation in host plants can affect the composition of bacterial gut communities of FAW larvae. Further research are needed to identify other factors that alter bacterial communities in each of these host plants, the mechanisms of the variations in insect microbiomes and the ecological implications of this variability in *S. frugiperda* larvae gut microbiota.

Declarations

Acknowledgements

We are grateful to Schlumberger Faculty for the Future Foundation (SFFTF) for research fellowship to JAU, University of Helsinki, Finland for the Laboratory Facilities and also Forestry Research Institute of Nigeria (FRIN) for support to JAU..

Funding: This work was supported by the Schlumberger Faculty for the Future- 2020 Postdoctoral Research Fellowship grant.

Conflict of interest

The authors declare no conflicts of interest.

Author contribution

JAU and FOA conceived and designed the experiments. JAU performed the experiments and wrote the manuscript. JAU analyzed the data and conducted statistical analyses. JAU and FOA contributed material. FOA and JAU edited, revised and approved the manuscript.

Data availability statement

The data that support the findings of this study are openly available in supplementary files as well as in Sequence Read Archive under the BioProject: PRJNA734744.

References

1. Douglas AE (2015) Multiorganismal Insects: Diversity and Function of Resident Microorganisms. Annu. Rev. Entomol. 60, 17–34. https://doi.org/10.1146/annurev-ento-010814-020822
2. Lü J, Guo W, Chen S, Guo M, Qiu B, Yang C, Lian T, Pan H (2019) Host plants influence the composition of the gut bacteria in Henosepilachna vigintioctopunctata. PLoS ONE 14, e0224213. https://doi.org/10.1371/journal.pone.0224213
3. Shin SC, Kim SH, You H, Kim B, Kim AC, Lee KA, Yoon JH, Ryu JH, LeeWJ (2011) Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling.Science 334:670–674. doi:10.1126/science.1212782
4. Engel P, Moran NA. (2013) The gut microbiota of insects–diversity in structure and function. FEMS Microbiol. Rev. 37(5): 699–735. https://doi.org/10.1111/1574-6976.12025 PMID: 23692388
5. .Dillon RJ.,Dillon VM (2004) The gut bacteria of insects: nonpathogenic interactions. Annu Rev Entomol 49(1): 71–9210.1146/annurev.ento.49.061802.123416
6. Frago E, Dicke M., Godfray HCJ, (2012) Insect symbionts as hidden players in insect–plant interactions. Trends Ecol Evol 27(12). 705–711. https://doi.org/10.1016/j.tree.2012.08.013 PMID: 22985943
7. Su L, Yang L, Huang S, Li Y, Su X, Wang F, Bo C, Wang ET, Song A, (2017) Variation in the Gut Microbiota of Termites (Tsaitermes ampliceps) Against Different Diets. Appl. Biochem. Biotechnol. 181, 32–47. https://doi.org/10.1007/s12010-016-2197-2
8. Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H, Qin B, Lin J, Wang Y, SongF, Li Y, Lin H, You M, (2017) Metagenomic Sequencing of Diamondback Moth Gut Microbiome Unveils Key Holobiont Adaptations for Herbivory. Front. Microbiol. 8, 663. https://doi.org/10.3389/fmicb.2017.00663
9. Mason C J, Lowe-Power T M, Rubert-Nason K F, Lindroth. R L, RaffaK F, (2016) Interactions between bacteria and aspen defense chemicals at the phyllosphere—Herbivore interface. J. Chem. Ecol. 42:193-201. https://doi.org/10.1007/s10886-016-0677
10. Ping L, Büchler R, Mithöfer A, Svatos A, Spiteller D, Dettner K, Gmeiner S, Piel,J, Schlott B, Boland W(2007) A novel Dps-type protein from insect gut bacteria catalyses hydrolysis and synthesis of N-acyl amino acids. Environ. Microbiol. 9:1572-1583. https://doi.org/10.1111/j.1462-2920.2007.01279.
11. .Shao Y, Chen B, Sun C, Ishida K, Hertweck C, Boland W, (2017) Symbiont-Derived Antimicrobials Contribute to the Control of the Lepidopteran Gut Microbiota. Cell Chemical Biology 24, 66–75. https://doi.org/10.1016/j.chembiol.2016.11.015
12. Goergen G, Kumar P L, Sankung S B, Togola A, Tamò M, (2016). First report of outbreaks of the fall armyworm Spodopterafrugiperda (J. E. Smith) (Lepidoptera. Noctuidae). a new alien invasive pest in west and central Africa. Plos One 11. e0165632)
13. Stokstad E, (2017) New crop pest takes Africa at lightning speed. Science 356, 473–474. https://doi.org/10.1126/science.356.6337.473
14. CABI, (2017) digest of an Evidence Note commissioned by the UK Department for International Development (*Fall Armyworm: Impacts and Implications for Africa*). The full report is available at www.invasive-species.org/fawevidencenote.
15. Odeyemi OO, Lawal BO, Owolade O F, Olasoji JO. EgbetokunOA,Oloyede- Kamiyo QO, Omodele T Anjorin FB (2020).Fall armyworm *Spodopterafrugiperda*(J. E. Smith) (Lepidoptera: Noctuidae): threat to maize

production in Nigeria. Nig. Agric. J. 51 (1): 101-108

16. .Silva DM. da- Bueno A, de -F, Andrade K, Stecca C dos S, Neves PMOJ, Oliveira MCN, de (2017). Biology and nutrition of Spodoptera frugiperda (Lepidoptera: Noctuidae) fed on different food sources. Sci. agric. (Piracicaba, Braz.) 74, 18–31. https://doi.org/10.1590/1678-992x-2015-0160
17. Chen B, Du ,K. Sun C, Vimalanathan A, Liang X Li Y, Wang B, Lu X, Li L, Shao Y(2018). Gut bacterial and fungal communities of the domesticated silkworm (Bombyx mori) and wild mulberry-feeding relatives. ISME J 12, 2252–2262. https://doi.org/10.1038/s41396-018-0174-1
18. Hannula SE, Zhu F, Heinen R Bezemer TM, (2019) Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. Nat. Commun. 10, 1254. https://doi.org/10.1038/s41467-019-09284-w
19. Acevedo FE, Peiffer M, Tan CW, Stanley BA, Stanley A, Wang J, Jones AG, Hoover K, Rosa C, Luthe D, Felton G (2017) Fall Armyworm-Associated Gut Bacteria Modulate Plant Defense Responses. MPMI 30, 127–137. https://doi.org/10.1094/MPMI-11-16-0240-R
20. Mason CJ, Ray S, Shikano I, Peiffer M, Jones AG, Luthe D, Hoover K, Felton GW, (2019)Plant defenses interact with insect enteric bacteria by initiating a leaky gut syndrome. Proc Natl Acad Sci USA 116, 15991–15996. https://doi.org/10.1073/pnas.1908748116
21. Mason C J. St. Clair A, PeifferM. GomezE, Jones A G, Felton G W, Hoover, K. (2020). Diet influences proliferation and stability of gut bacterial populations in herbivorous lepidopteran larvae. *PLOS ONE*, *15*(3), e0229848. https://doi.org/10.1371/journal.pone.0229848
22. Arora AK, Douglas AE(2017) Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. J. Insect. Phy.103, 10–17. https://doi.org/10.1016/j.jinsphys.2017.09.011
23. FRIN (2017). Forestry Research Institute of Nigeria Annual Bulletin, (74 pp). Ibadan: Nigeria.
24. De Cock M, Virgilio M, Vandamme P, Augustinos A, Bourtzis K, Willems A. De Meyer M, (2019) Impact of Sample Preservation and Manipulation on Insect Gut Microbiome Profiling. A Test Case With Fruit Flies (Diptera, Tephritidae). Front. Microbiol. 10, 2833. https://doi.org/10.3389/fmicb.2019.02833
25. Ugwu JA, Liu M, Sun H, Asiegbu FO, (2020) Microbiome of the larvae of Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) from maize plants. J Appl Entomol 144, 764–776. https://doi.org/10.1111/jen.12821
26. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley R.E, Lozupone CA, McDonald D, Muegge BD, PirrungM, Reeder J Sevinsky JR, Turnbaugh PJ, Walters WA. Widmann J, Yatsunenko T, Zaneveld J, Knight R. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 7, 335–336. https://doi.org/10.1038/nmeth.f.303
27. HaasBJ, Gevers D, Earl A M, Feldgarden M. Ward D V, Giannoukos G Ciulla D, Tabbaa D, Highlander S K, Sodergren E, Methé B, DeSantis T Z, Consortium T H M. Petrosino J F, Knight R, BirrenB W (2011)Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genom. Res.;21:494–504.
28. Kohl KD Varner J, Wilkening JL, Dearing MD, (2018) Gut microbial communities of American pikas (Ochotona princeps): Evidence for phylosymbiosis and adaptations to novel diets. J. Anim. Ecol. 87(2): 323–330. https://doi.org/10.1111/1365-2656.
29. KudoR, Masuya H, Endoh R, Kikuchi T, Ikeda H (2019) Gut bacterial and fungal communities in ground–dwelling beetles are associated with host food habit and habitat. ISME J 13: 676. https://doi.

org/10.1038/s41396-018-0298-3

30. Mikaelyan A, Dietrich C, Ko¨hler T, Poulsen M, Sillam-Dussès D, Brune A(2015)Diet is the primarydeterminant of bacterial community structure in the guts of higher termites. Mol. Ecol. 24(20): 5284–5295. https://doi.org/10.1111/mec.13376 PMID: 26348261
31. Chen B, Teh BS, Sun C, Hu, S,. Lu, X, Boland W, Shao Y, (2016) Biodiversity and Activity of the Gut Microbiota across the Life History of the Insect Herbivore Spodoptera littoralis. Sci. Rep. 6, 29505. https://doi.org/10.1038/srep29505
32. Gomes AFF, Omoto C, Cônsoli FL, (2020) Gut bacteria of field-collected larvae of Spodoptera frugiperda undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. J. Pest. Sci. 93, 833–851. https://doi.org/10.1007/s10340-020-01202-0
33. Gichuhi J, Sevgan S, Khamis F Van den Berg J, du Plessis H, EkesiS, Herren JK (2020) Diversity of fall armyworm, Spodoptera frugiperda and their gut bacterial community in Kenya. PeerJ 8, e8701. https://doi.org/10.7717/peerj.8701
34. Strano,CP, Malacrinò A Campolo O, Palmeri V, (2018)Influence of Host Plant on Thaumetopoea pityocampa Gut Bacterial Community. Microb. Ecol. 75, 487–494. https://doi.org/10.1007/s00248-017-1019-6
35. Dillon R, Charnley K, (2002) Mutualism between the desert locust Schistocerca gregaria and its gut microbiota. Res, in Microbiol.153, 503–509. https://doi.org/10.1016/S0923-2508(02)01361-X
36. Boone CK, Keefover-RingK, Mapes AC, Adams AS, BohlmannJ, Raffa KF (2013). Bacteria Associated with a Tree-Killing Insect Reduce Concentrations of Plant Defense Compounds. J. Chem. Ecol. 39, 1003–1006. https://doi.org/10.1007/s10886-013-0313-0
37. Bradley PH, Pollard KS (2017) *Proteobacteria* explain significant functional variability in the human gut microbiome. Microbiome. 5:36. https://doi.org/10.1186/s40168-017-0244-
38. Hauffe HC, Barelli C (2019) Conserve the germs: the gut microbiota and adaptive potential. Conserv Genet 20:19–27. https://doi.org/10.1007/s10592-019-01150-y
39. Soo RM, Hemp J, Parks DH, Fischer WW, Hugenholtz P,(2017) On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. Science 355: 1436–1440. https://doi.org/10.1126/ science.aa l3794 PMID: 28360330
40. Utami YD, Kuwahara H, Murakami T, Morikawa T, Sugaya K, Kihara K, Yuki M, Lo N, Deevong P, Hasin S, Boonriam W, Inoue T, Yamada A, Ohkuma M, Hongoh, Y, (2018) Phylogenetic Diversity and Single-Cell Genome Analysis of "*Melainabacteria*", a Non-Photosynthetic Cyanobacterial Group, in the Termite Gut. Microbes and environments 33, 50–57. https://doi.org/10.1264/jsme2.ME17137
41. Crotti E, Balloi A, Hamdi C, Sansonno L, Marzorati M, GonellaE., Favia G, CherifA, Bandi C, Alma A, Daffonchio D, (2012) Microbial symbionts: a resource for the management of insectrelated problems Microb. Biotechnol. 5:307–317. doi:10.1111/j. 1751-7915.2011.00312.x
42. Andongma AA, Wan L, Dong YC, li P, Desneux N, White JA, Niu CY(2015) Pyrosequencing reveals a shift in symbiotic bacteria populations across life stages of Bactrocera dorsalis. Sci. Rep. 5, 9470. https://doi.org/10.1038/srep09470
43. Salem H, KreutzerE, Sudakaran S, Kaltenpoth M. (2013) Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae) Environ. Microbiol. 15: 1956–1968. doi:10.1111/1462-2920.12001
44. Kaltenpoth M, (2009) Actinobacteria as mutualists: general healthcare for insects? Trends. Microbiol. 17:529–535. doi:10. 1016/j.tim.2009.09.006

45. Ventura M, Canchaya C, Fitzgerald GF, Gupta RS, van Sinderen D (2007) Genomics as a means to understand bacterial phylogeny and ecological adaptation: the case of bifidobacteria. Antonie van Leeuwenhoek 91, 351–372. https://doi.org/10.1007/s10482-006-9122-6
46. Minard G, Mavingui P, Moro CV,(2013) Diversity and function of bacterial microbiota in the mosquito holobiont. Parasit Vectors. 6:146. https:// doi.org/10.1186/1756-3305-6-146.
47. .De Chaves MG, Silva GGZ, Rossetto R, Edwards R.A, Tsai SM, Navarrete AA(2019) Acidobacteria Subgroups and Their Metabolic Potential for Carbon Degradation in Sugarcane Soil Amended With Vinasse and Nitrogen Fertilizers. Front. Microbiol. 10, 1680. https://doi.org/10.3389/fmicb.2019.01680
48. Kielak .AM, Barreto CC, Kowalchuk GA, van Veen JA, Kuramae EE,(2016) The Ecology of Acidobacteria: Moving beyond Genes and Genomes. Front.Microbiol.;7:744. DOI: 10.3389/fmicb.2016.00744.
49. .Rawat SR, Männistö MK, Starovoytov V, Goodwin L, Nolan M, Hauser L, Land,M, Davenport KW, Woyke T, HäggblomMM (2012) Complete genome sequence of Terriglobus saanensis type strain SP1PR4T, an Acidobacteria from tundra soil. Stand. Genomic Sci. 7, 59–69. https://doi.org/10.4056/sigs.3036810
50. Whang,K.S, Lee JC, Lee H-R, Han S-I, Chung SH, (2014) Terriglobus tenax sp. nov., an exopolysaccharide-producing acidobacterium isolated from rhizosphere soil of a medicinal plant. International Journal of Systematic and Evolutionary Microbiology 64, 431–437. https://doi.org/10.1099/ijs.0.053769-0
51. Leite-Mondin, M DiLegge MJ, Manter DK, WeirTL, Silva-Filho MC, Vivanco JM. (2021)The gut microbiota composition of Trichoplusia ni is altered by diet and may influence its polyphagous behavior. Sci. Rep. 11, 5786. https://doi.org/10.1038/s41598-021-85057-0
52. Gedling C R, Smith CM, LeMoine C M R Cassone BJ, (2018) The Mexican bean beetle (Epilachna varivestis) regurgitome and insights into beetle-borne virus specificity. Plos One 13, e0192003.
53. Jones AG, Mason CJ, Felton GW, Hoover K, (2019) Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. Sci Rep 9, 2792. https://doi.org/10.1038/s41598-019-39163-9

Tables

Table 1. Host plants species used for rearing FAW larvae

Sample designation/code	Plant's common name	Scientific name	Family	Part of plant used in feeding the larvae
M1(A)	Sugar cane	*Saccharum officinarum*	Poaceae	leaves
M2 (C)	Maize	*Zea mays*	Gramineae	leaves
M3 (H)	Onion	*Allium cepa*	Alliaceae	leaves
R1(B)	Cucumber	*Cucumis sativus*	Cucurbitaceae	fruits
R2 (F)	Tomato	*Solanum lycopersicum*	Solanaceae	fruits
R3 (G)	Sweet potato	*Ipomoea batatas*	Convolvulaceae	roots
L1 (D)	Cabbage	*Brassica oleracea*	Brassicaceae	leaves
L2(E)	Green amaranth	*(Amaranthus viridis*	Amaranthaceae	leaves
L3 (I)	Celocia	*Celosia argentea*	Amaranthaceae	leaves

Table 2. Alpha diversity indices of the gut bacterial community of FAW larvae fed with nine different host plants

Sample designation /Code	Number of valid read valid read	Observed species richness	Ace	Chao1	Shannon	Simpson
M1(A)	139,753±10.1	1314.40±413.4	1575.06±451.9	1472.67±432.5	4.07±1.4	0.78±0.1
M2(C)	141,52±4.8	983.80±442.5	1164.66±532.1	1112.99±482.2	4.34±0.6	0.80±0.1
M3(H)	133,266±4.1	457.20±45.8	529.11±52.1	508.79±57.4	3.84±1.0	0.80±0.1
R1(B)	140,933±6.3	1321.80±536.8	1562.04±659	1587.68±798.7	5.34±1.6	0.87±0.1
R2(F)	139,339±4.3	606.40±115.0	723.71±104.9	703.84±103.9	3.84±1.1	0.74±0.2
R3(G)	139,982±5.3	624.20±30.9	725.79±41.7	717.93±35.9	4.18±0.6	0.79±0.1
L1(D)	141,393±5.8	527.00±41.2	625.31±29.9	607.21±36.1	3.78±0.7	0.77±0.1
L2(E)	139,581±7.3	544.00±66.2	633.48±98.5	624.19±112.2	3.97±1.1	0.75±0.2
L3(I)	137,081±4.3	471.60±141.5	557.79±159.1	540.28±153.4	3..46±0.7	0.78±0.1
P	0.793	<0.001	<0.001	<0.001	0.250	0.873
F	0.573	7.994	8.348	6.873	1.353	0.464

Table 3. Number of gut bacteria taxonomic categories in different host plants

Sample designation/code	Phylum	class	order	family	genus	species	OTUs
M1(A)	30	91	196	328	568	415	1518.0±420.2
M2(C)	34	87	187	310	541	364	1117.20±509.0
M3(H)	19	32	85	168	347	219	511,8±47.6
R1(B)	30	91	182	311	578	416	1444.4±533.4
R2(F)	20	31	89	171	354	212	695.8±111.4
R3(G)	16	27	75	156	349	237	701.6±30.8
L1(D)	14	25	75	158	348	218	597.6±37.6
L2(E)	17	29	69	141	319	205	618.6±81.3
M3(H)	19	32	85	168	347	219	511.8±47.6
L3(I)	19	33	77	150	214	198	539.8±157.4

Figures

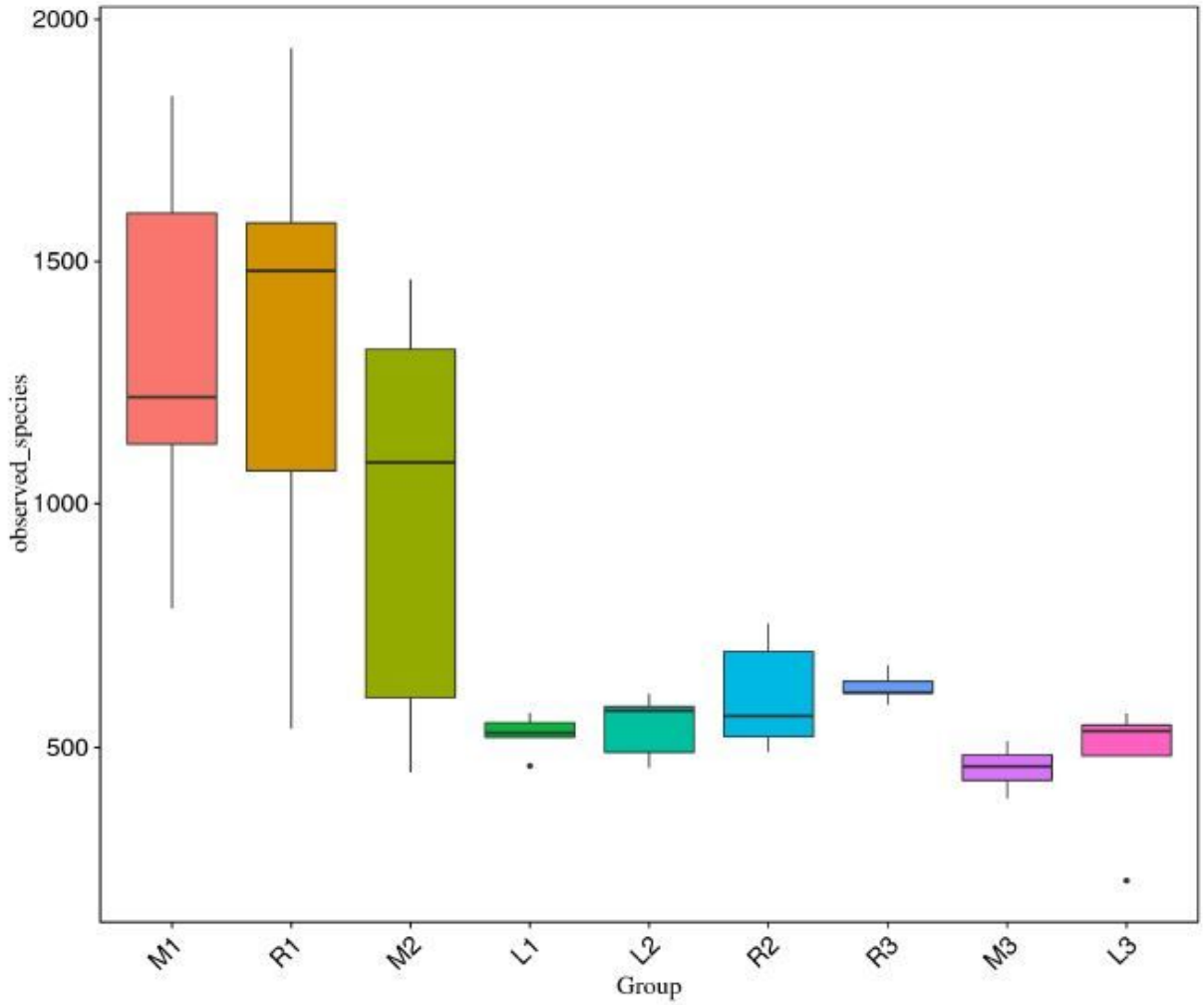

Figure 1

The observed bacteria species richness of FAW larvae group fed with different host plants .Group: L1, cabbage; L2, green amaranth; L3, celocia; M1, sugarcane; M2, maize; M3, onion; R1, cucumber; R2, tomato; R3, sweet potato

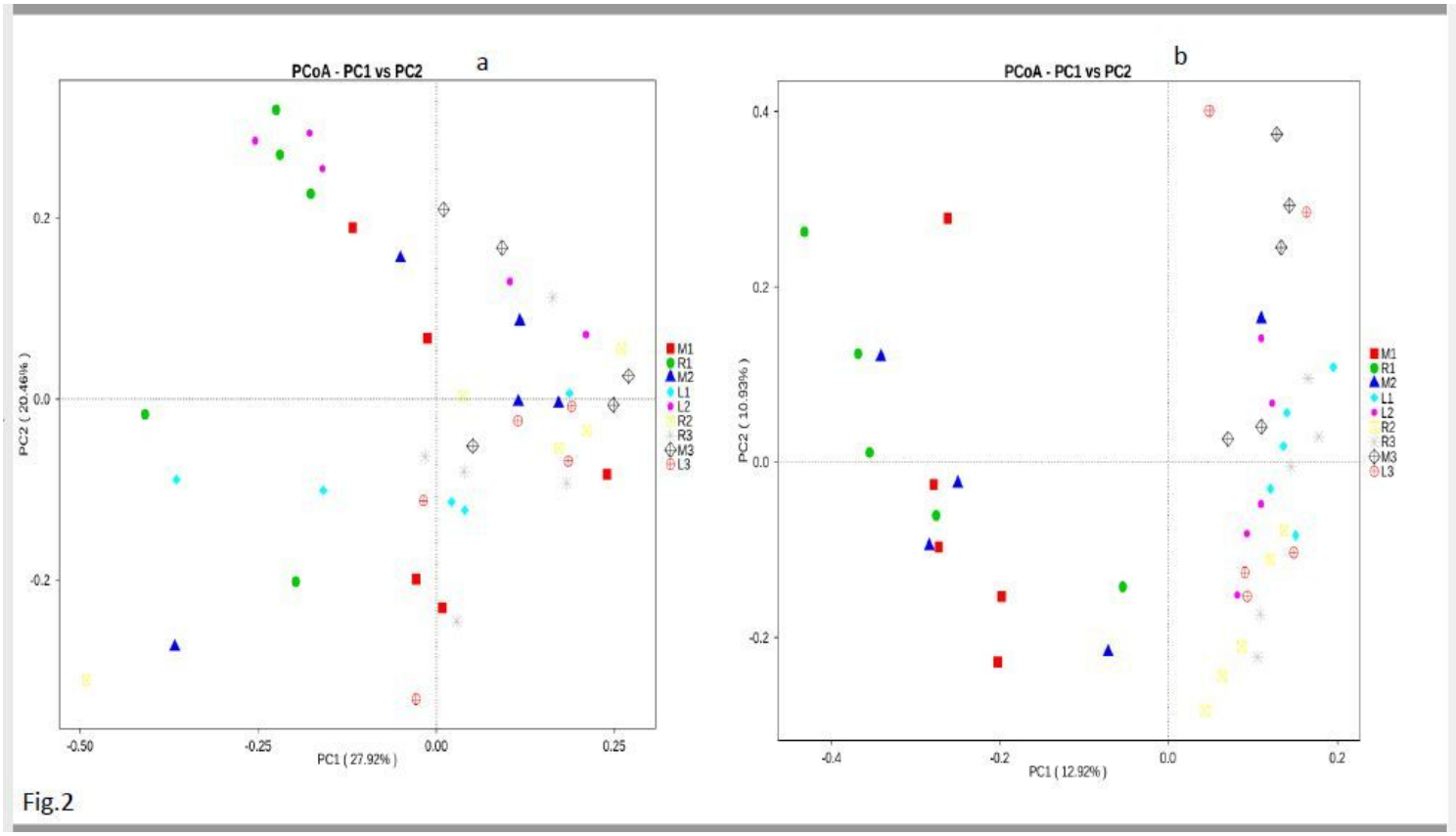

Fig.2

Figure 2

Principal coordinate analysis performed on the entire gut bacterial community in all the larvae groups. a, weighted; b, unweighted unweighted , L1, cabbage; L2, green amaranth; L3, celocia; M1, sugarcane; M2, maize; M3, onion; R1, cucumber; R2, tomato; R3, sweet potato

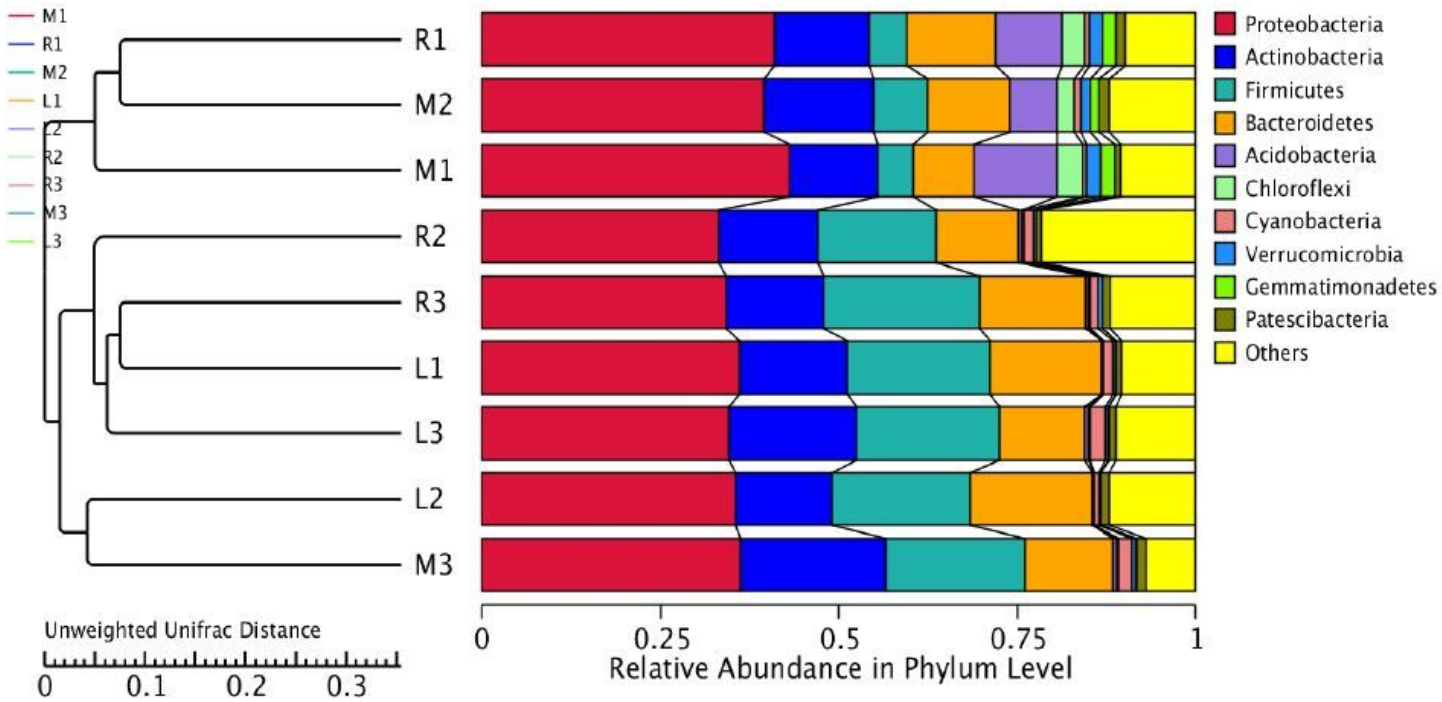

Figure 3

UPGMA Unweighted Hierarchical clustering tree of all gut bacterial groups based on taxon distribution Group: L1, cabbage; L2, green amaranth; L3, celocia; M1, sugarcane; M2, maize; M3, onion; R1, cucumber; R2, tomato; R3, sweet potato

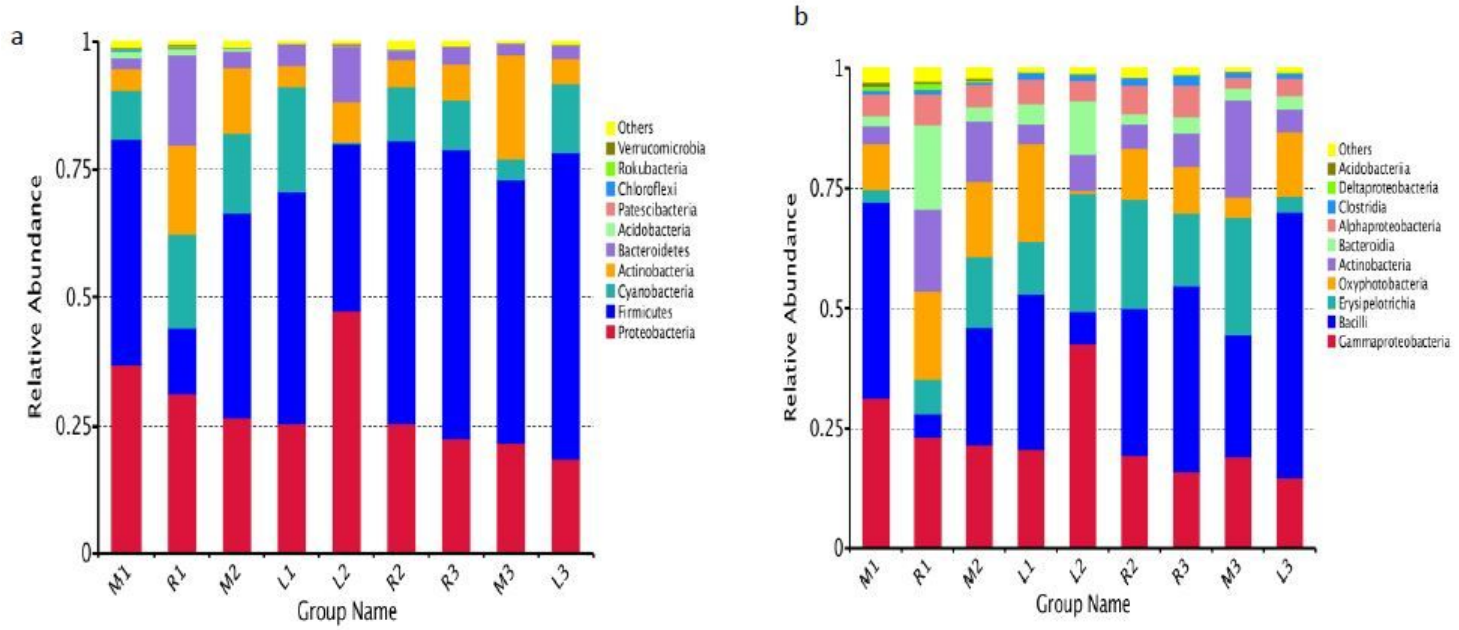

Figure 4

Relative abundance of gut bacterial phyla and class (top ten) in FAW larvae fed with nine host plants . a, Phylum, b, Class, Group: L1, cabbage; L2, green amaranth; L3, celocia; M1, sugarcane; M2, maize; M3, onion; R1, cucumber; R2, tomato; R3, sweet potato

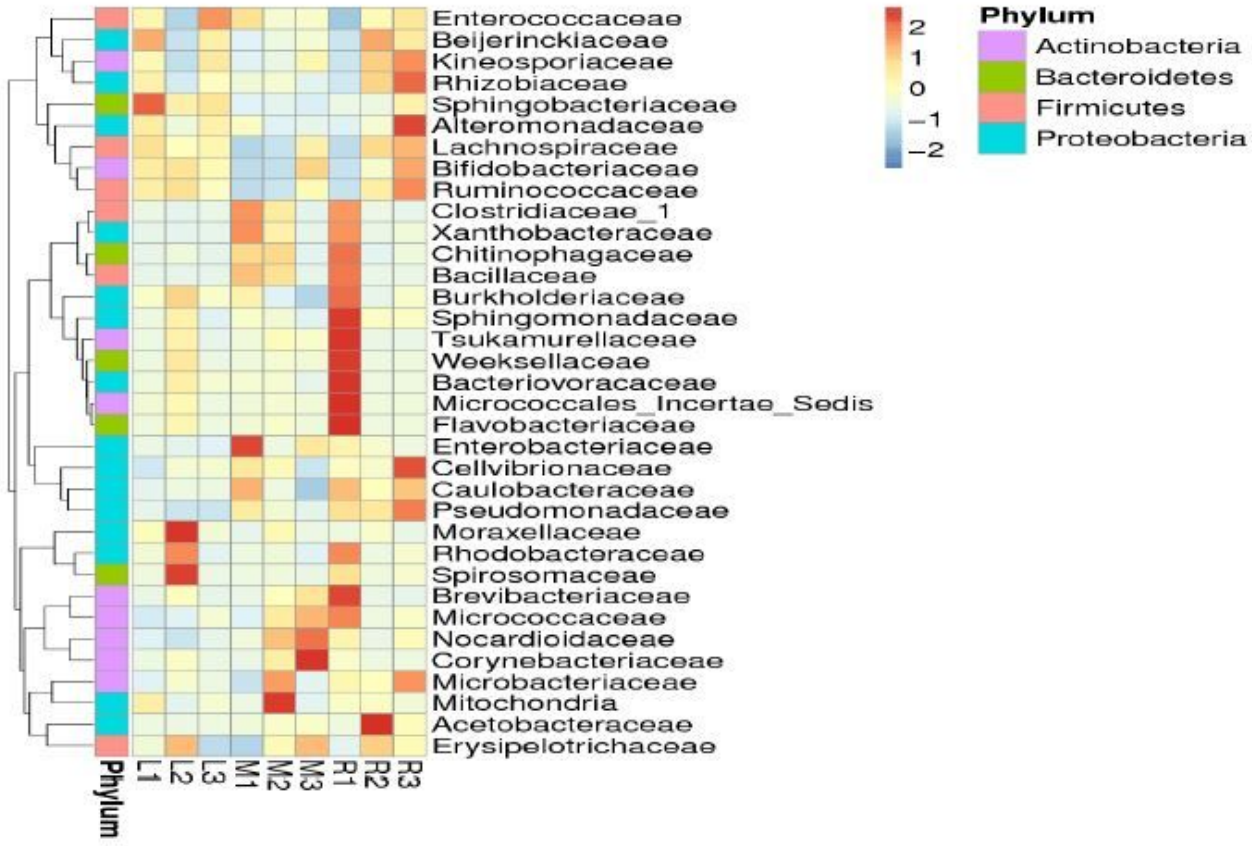

Figure 5

Heatmap showing the relative abundance and distribution across the different host plants used for rearing Spodoptera frugipeda larvae. The colour code indicates relative abundance, ranging from blue (low abundance) to yellow (moderate abundance) to red (high abundance). Group: L1, cabbage; L2, green amaranth; L3, celocia; M1, sugarcane; M2, maize; M3, onion; R1, cucumber; R2, tomato; R3, sweet potato

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryfilesInfluenceofhostplants.doc