Different host plants alter structure, diversity, and function of gut bacterial communities in larval instars of Spodoptera frugiperda

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Article

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Abstract

Insect gut microbes have important roles in host feeding, digestion, immunity, and growth and development. The fall armyworm, *Spodoptera frugiperda*, is a major migratory agricultural pest worldwide, effects of host plants on its communities of gut bacteria remain to be investigated. Therefore, differences in gut bacterial communities were examined in *S. frugiperda* 5th and 6th instar larvae fed leaves of different host plants (corn, sorghum, highland barley, citrus). Metagenomic DNA was extracted from larval intestines, and abundance and diversity of gut bacteria were determined using 16S rDNA full-length amplification and sequencing. Highest richness and diversity of gut bacteria were in corn-fed 5th instar larvae; whereas in 6th instar larvae, richness and diversity were higher when larvae were fed other crops. Firmicutes and Proteobacteria were dominant phyla in gut bacterial communities of 5th and 6th instar larvae. According to LEfSe analysis, host plant had important effects on structure of gut bacterial communities in *S. frugiperda*. In an analysis using PICRUSt2 software, most predicted functional categories were associated with metabolism. Thus, the plant species attacked by *S. frugiperda* larvae affects their gut bacterial communities, and such changes are likely important in adaptation to a host plant.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major migratory agricultural pest of global concern. It originated in tropical and subtropical regions of America, where it is primarily distributed[1–3]. *S. frugiperda* invaded Ghana and Nigeria in Southwest Africa in 2016[4,5], was first found in India in 2018[6] and has since been recorded in Sri Lanka, Thailand, Myanmar, and other Asian countries[7], The armyworm invaded Yunnan, China, in December 2018 and then rapidly expanded to 1,538 counties in 26 provinces (cities, districts) across the country[8–10]. *S. frugiperda* is an omnivorous pest with strong migratory ability, high rate of reproduction, and short life cycle. It damages 353 species of plants in 76 families, including the major crops of corn, sorghum, sugarcane, barley, rice, pepper, wild oat, and potato[11]. Maize crops can suffer serious economic losses when attacked[12]. However, information on effective prevention and control strategies for *S. frugiperda* is lacking. *S. frugiperda* has developed resistance to a variety of insecticides, including diamide and neonicotinoids[13]. Because *S. frugiperda* is a newly invaded major agricultural pest in China, native natural enemy insects are being investigated to provide control[14]. Nevertheless, to prevent and control *S. frugiperda*, more effective green control methods are urgently needed.

Many microorganisms inhabit the insect gut, and those microbes are important in digestion of food, absorption of nutrients, and general metabolism[15,16]. The gut and associated microbes resist invasion and colonization by external pathogens[17,18], degrade harmful substances, and produce drug resistance[19]. Communities of gut bacteria can promote host absorption and utilization of food[20], and different foods can also affect composition and metabolic function of gut bacterial communities[21]. Although many plant tissues are low in nutrients, indigestible, or toxic, herbivorous insects are among the most numerous

and diverse groups of organisms[22,23]. Herbivorous insects have numerous morphological, behavioral, and physiological characteristics that enable them to overcome dietary barriers[24–26]. Some insects can adapt to new host plants, and in that process, changes occur in abundance and composition of gut enzymes that reduce toxicity of plant allelochemicals[27–29]. Therefore, composition and diversity of gut bacterial communities has been one of the recent hotspots of entomological research. Increasing understanding of the coevolution between insects and their gut bacteria can provide a theoretical basis for pest control[30–32].

The host plant is an important factor affecting insect gut bacteria. Feeding habits of insects affect composition and structure as well as diversity and function of gut bacterial communities[33]. Gut bacterial communities have been characterized in an increasing number of insects, including bees[34,35], fruit flies[36,37], beetles[38], termites[39], *Blattella germanica*[40], and the lepidopteran pests *Lymantria dispar*[41], *Plutella xylostella*[42], and *Grapholita molesta*[43]. In ground beetles, their food habits and habitats affect gut bacterial and fungal communities[38]. In cluster analysis of relative abundances of orthologous gene clusters, high similarities were observed among wood- and litter-feeding termites, but those groups had strong differences with humivorous species[39]. In *B. germanica*, gut bacteria are highly dynamic, and bacterial communities reassemble relatively rapidly and with different composition in a diet-specific manner (the highest diversity was associated with a no-protein diet)[40]. Flexibility of the gut bacteria is most likely because cockroaches are omnivorous with variable diets[40]. In a comparative analysis of *L. dispar* midgut bacterial diversity; the plant species consumed influenced composition of the gut bacterial community[41]. In *P. xylostella* larvae reared on artificial diet and different host plants, composition and diversity of gut bacterial communities vary significantly. Thus, host plants greatly influence composition and structure of gut bacterial communities in *P. xylostella*, which may be essential in long-term adaptation to host plants[42]. Similarly, gut bacterial communities of *G. molesta* are influenced by host diet and therefore may also be important in adaptation to hosts[43].

Although gut bacteria of *S. frugiperda* have been examined previously[44–47], how feeding on different host plants affects composition and functions of gut bacterial communities remain to be investigated. In this study, *S. frugiperda* larvae were reared on leaves of corn, sorghum, highland barley, or citrus. Then, 16S rDNA sequence amplification was used to compare effects of different host plants on structure, diversity, and functions of gut bacterial communities in *S. frugiperda*. The results will provide a foundation to generate new ideas for further study of effects of host plants on gut bacterial communities of *S. frugiperda*. In addition, new insights may lead to manipulation of gut bacterial communities for pest control of *S. frugiperda.*

Results

Sequence splicing, assembly analysis, and operational taxonomic unit distribution

Thirty-two *S. frugiperda* gut samples were examined. A total of 568,300 original reads and 748,360,295 bp of original bases were obtained(Table 1). After filtration, 15,802 high-quality average reads and 566 unique average reads were obtained(Figure S1). From 5th and 6th instar larvae raised on different host plants, 498 and 562 OTUs, respectively, were obtained from sequencing data (Table S1). Gut bacteria were classified into 9 phyla, 14 classes, 32 orders, 56 families, 93 genera, and 66 species. Differences in OTUs of gut bacteria in different larval instars fed different host plants are compared in a flower plot (Fig. 1). Although only seven OTUs of gut bacteria were shared among different *S. frugiperda* instars fed different host plants, they indicated there were similarities in composition of bacterial communities. In 5th instar larvae of *S. frugiperda*, the number of unique OTUs was 68 in those fed corn (ZmL1), 14 in those fed citrus (CrB1), 12 in those fed sorghum (SbL1), and two in those fed highland barley (HvL1). In 6th instar larvae, the number of unique OTUs was 54 in those fed sorghum (SbL2), 26 in those fed highland barley (HvL2), 15 in those fed corn (ZmL2), and two in those fed citrus (CrB2). Thus, composition of gut bacterial communities was different in *S. frugiperda*fed different host plants.

Table 1
Effective reads data for subsequent analysis after quality control.

Saple_name	Raw_reads	Clean_reads	Base(nt)	AvgLen(nt)	Effetive(%)
HvL1.1	18434	16712	24823416	1485	90.66
HvL1.2	13733	12297	18259102	1484	89.54
HvL1.3	12142	10472	15560783	1485	86.25
HvL1.4	16263	14725	21871158	1485	90.54
SbL1.1	16291	14668	21773693	1484	90.04
SbL1.2	20232	18538	27167447	1465	91.63
SbL1.3	23156	19666	29188401	1484	84.93
SbL1.4	23467	21067	31264901	1484	89.77
ZmL1.1	17639	15895	23578489	1483	90.11
ZmL1.2	10148	9020	13191119	1462	88.88
ZmL1.3	12649	10831	15562182	1436	85.63
ZmL1.4	13328	12007	17818003	1483	90.09
CrB1.1	26445	23292	34155876	1466	88.08
CrB1.2	22652	20542	30485052	1484	90.69
CrB1.3	18589	16033	23804488	1484	86.25
CrB1.4	14176	12932	18909984	1462	91.22
HvL2.1	12065	11046	16295806	1475	91.55
HvL2.2	15120	13924	20641745	1482	92.09
HvL2.3	25269	21263	31586883	1485	84.15
HvL2.4	21157	19133	28414494	1485	90.43
SbL2.1	15491	13452	19895164	1478	86.84
SbL2.2	15980	14028	20809492	1483	87.78
SbL2.3	18850	16365	24214434	1479	86.82
SbL2.4	25471	22426	33299817	1484	88.05

Raw reads represent the number of original reads sequenced by PacBio. Clean reads are the number of high-quality reads obtained after quality control and splicing. Effective reads indicate the number of effective sequences with non-chimeras. AvgLen (nt) is the average sequence length of all samples. Effective (%) is the percentage of effective reads in raw reads.

Saple_name	Raw_reads	Clean_reads	Base(nt)	AvgLen(nt)	Effetive(%)
ZmL2.1	12346	11205	16617495	1483	90.76
ZmL2.2	19422	17006	25168430	1479	87.56
ZmL2.3	17704	16464	24431320	1483	93
ZmL2.4	13393	12090	17803176	1472	90.27
CrB2.1	25248	21817	32396921	1484	86.41
CrB2.2	13162	11940	17728360	1484	90.72
CrB2.3	16103	14615	21684846	1483	90.76
CrB2.4	22175	20180	29957818	1484	91
Total	568300	505651	748360295	47319	2852.5

Raw reads represent the number of original reads sequenced by PacBio. Clean reads are the number of high-quality reads obtained after quality control and splicing. Effective reads indicate the number of effective sequences with non-chimeras. AvgLen (nt) is the average sequence length of all samples. Effective (%) is the percentage of effective reads in raw reads.

Taxa annotation and relative abundance of operational taxonomic units

Relative abundance of gut bacteria was determined at different taxonomic levels. All samples typically included nine main phyla (Fig. 2a and Table S2). In 5th instar *S. frugiperda*, Firmicutes (78.48%) was the most abundant phylum among gut bacteria, followed by Proteobacteria (20.27%), with other phyla at much lower relative abundance, including Bacteroidetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Chloroflexi, and unidentified_Bacteria. In 6th instar larvae, Firmicutes (90.76%) was also the most abundant phylum, followed by Proteobacteria (7.57%). Phyla of bacteria were highly consistent between the two instars, with Firmicutes and Proteobacteria dominant phyla in both. There were no significant effects of host plants on phyla of gut bacteria ($P > 0.05$). Although the same phyla were dominant in guts of the two larval instars, their relative abundances were different.

Dominant genera in 5th instar *S. frugiperda* were primarily *Enterococcus* (78.26%) and *Ralstonia* (15.54%), with other genera at lower relative abundance, including *Pseudochrobactrum*, *Enterobacter*, *Klebsiella*, *Ochrobactrum*, *Alcaligenes*, *Myroides*, *Achromobacter*, and *Glutamicibacter* (Fig. 2b and Table S2). In 6th instar larvae, *Enterococcus* (90.54%) was also the dominant genus, but *Ralstonia* (0.43%) was less abundant. Whereas *Ralstonia* composed 43.33% of the gut community in 5th instar larvae fed citrus (CrB1), the genus composed only 0.03% in 6th instar larvae fed corn (ZmL2). The relative abundance of *Glutamicibacter* in 5th instar larvae fed corn (ZmL1) was 0.38%, which was significantly different from that on other host treatments, especially in 5th and 6th instar larvae fed (HvL1 and HvL2) and 5th instar larvae fed citrus (CrB1) ($p < 0.05$)(Fig. 2c). Thus, phyla and genera of gut bacteria in *S. frugiperda* reared on different hosts were the same, but relative abundances at each taxonomic level were different.

Diversity of gut bacteria in S. frugiperda larvae fed different host plants

Alpha diversity of bacterial communities in different treatments was analyzed (Fig. 3 and Table S3). The highest Chao index of gut bacteria was 108.34 in ZmL1, followed by 60.073 in SbL2. Thus, richness of gut bacterial communities was highest in larvae fed corn and sorghum. Fifth instar larvae fed corn (ZmL1) had the highest Shannon and Simpson diversity values (2.153 and 0.654, respectively). Shannon and Simpson indices between ZmL1 and CrB1 were significantly different ($P < 0.05$). Compared with 5th instar larvae fed corn, richness and diversity of gut bacteria decreased when larvae fed on leaves of other hosts. Compared with 5th instar larvae, diversity indices of gut bacterial communities in 6th instar larvae increased when fed citrus, sorghum, and highland barley. Thus, there were differences in gut bacterial communities between larval stages of *S. frugiperda*.

To better reflect the nonlinear structure of data on gut bacteria in *S. frugiperda* fed different hosts; nonmetric multidimensional calibration (NMDS) was performed on sequencing data based on Bray–Curtis distances (Fig. 4). The distance between gut bacteria in 5th instars fed corn and those in other host plant treatments was relatively large, indicating there were differences in gut bacteria among different treatments. Differences in gut bacterial communities in 5th and 6th instars fed different plants were analyzed (Fig. 5a and 5b). In 5th instars fed corn, the gut bacterial community was significantly enriched from genus to phylum to levels. According to linear discriminant analysis effect size (LEfSe), nine bacterial clades were consistently significantly enriched in ZmL1 samples (Fig. 5c). Each larval stage had a unique, significantly enriched set of bacteria at taxonomic levels ranging from phylum to species. For example, the genera *Pseudochrobactrum*, *Paenochrobactrum*, and *Ochrobactrum* were notably enriched in ZmL1, compared with other hosts, whereas *Enterobacter* was notably enriched in HvL1. *Providencia* was enriched in HvL2. Thus, different bacterial groups were enriched in different larval stages fed different host plants.

Cluster analysis of relative abundance of predominant genera of bacteria

The cluster heat map in Fig. 6 shows annotation and abundance information for the top 35 genera based on relative abundance. The genera of gut bacteria in *S. frugiperda* fed corn (ZmL1, ZmL2), sorghum (SbL1, SbL2), barley (HvL1, HvL2), and citrus (CrB1, CrB2) were clustered in different branches. As shown in the horizontal direction, the abundance of each genus was different in different larval stages fed different hosts. For both 5th and 6th instar larvae of *S. frugiperda*, dominant genera of gut bacteria were also different when reared on different plants.

Prediction of functions of gut bacterial communities in S. frugiperda

To better understand the important functions of gut bacteria in *S. frugiperda*, relative abundances of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were predicted based on 16S rDNA gene sequences using PICRUSt2. Functions of gut bacteria primarily involved six types of metabolic pathways: metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases (Fig. 7a). Gut bacteria primarily functioned in metabolism-associated pathways, which accounted for 45.39 ± 1.07%. In analysis of the second functional layer of predicted genes (Fig. 7b, functions included membrane transport, signal transduction, carbohydrate

metabolism, amino acid metabolism, energy metabolism, cell motility, and xenobiotics biodegradation and metabolism, among other pathways.

Guts of 5th and 6th instar larvae of *S. frugiperda* reared on different plants were enriched in different functional proteins (Fig. 7c). For example, cold shock protein (K03704) and chitin-binding protein (K03933) were significantly enriched in 5th instar larvae reared on highland barley (HvL1). Gut microbiomes were enriched in several ABC transporter-related KOs(KEGG Orthogroups), including phosphate and amino acid transporters (K01999), permease protein (K02029, K01997, K01998), ATP-binding protein(K01996), periplasmic binding protein(K01999), hypothetical protein(K02030), peptide and nickel transporters (K02035). All of the predicted pathways perform the most important functions in the gut and therefore are important in overall growth and development of *S. frugiperda* larvae.

Discussion

Spodoptera frugiperda is an omnivorous pest that feeds on a wide range of crops, and analysis of metagenomic DNA of gut bacteria in *S. frugiperda* can provide the basis for pest control research. However, little is known about how host plants affect diversity of gut bacteria in *S. frugiperda*. It is essential to identify differences in gut bacteria of *S. frugiperda* feeding on different plants because of their potentially significant effects on larval growth and development. In this study, total number of OTUs was different in gut bacterial communities of *S. frugiperda* reared on leaves of different hosts, with numbers of OTUs in larvae fed corn and sorghum higher than those in larvae fed highland barley and citrus. Those results are most likely an indication that corn and sorghum are preferred oviposition host plants for *S. frugiperda.* Thus, diet strongly influenced the microbiome of *S. frugiperda*, as previously documented in other lepidopterans[48].

In this study, abundance and diversity of gut bacteria were analyzed in 5th and 6th instar larvae of *S. frugiperda* reared on leaves of corn, sorghum, highland barley, and citrus. In alpha diversity analysis, abundance and diversity of gut bacteria in HvL1, CrB1, and SbL1 showed an upward trend, compared with ZmL1. There were also differences in abundance and diversity of gut bacteria in larvae fed different plants, and diversity and abundance of gut bacteria in ZmL1 decreased. Different host plants can cause differences in insect gut microenvironments, which in turn lead to differences in gut microbial diversity [49]. The results of this study are consistent with those for other insects. Trano [50] found differences in dominant flora and their abundances in guts of *Thaumetopoea pityocampa* feeding on three different types of pine trees. In addition, Tinker and Ottesen[51] found that dietary substrate affects gut bacteria in *Periplaneta americana*, with changes in food leading to changes in the dietary matrix available for gut bacteria and ultimately changes in gut flora.

At the phylum level, composition of gut bacterial communities in 5th and 6th larvae was different among larvae fed different plants, but Firmicutes was the dominant phyla in both larval stages, followed by Proteobacteria. Dongbiao[52] found similar results with *S. frugiperda*, and gut bacterial communities of most samples were dominated by Firmicutes. The high abundance of Firmicutes on wild oats is due to

better absorption of different nutrients [52]. However, in larvae and adult *S. frugiperda* from different maize growing areas in Kenya, Firmicutes was only dominant in one Ngeria (Ngeria-l2) larva and two Kitale (Kitale-m2 and Kitale-m3) adult males[48]. At the genus level, *Enterococcus* was the dominant genus of gut bacteria in *S. frugiperda*. *Enterococcus* can degrade alkaloids and latexes and therefore has a stabilizing role in tolerant plants[53]. *Enterococcus* is also found in other lepidopterans[41–43]. As a result of long-term use, many insects are resistant to traditional chemical pesticides and baited sprays, which leads to increases in nontarget insect deaths and environmental pollution with a significant reduction in pest control quality performance[54]. However, *Enterococcus* and *Ralstonia* were mainly in CrB1 samples (fed on citrus). This difference might be due to differences in source and composition of the food[55]. *Ralstonia* originates from leaf surfaces, and *Ralstonia* bacteria are mostly plant pathogens that cause serious harm on a global scale[56].

To further study effects of host plants on diversity of gut bacterial communities, NMDS was used to examine beta diversity. Among samples from four different hosts, all samples of CrB1 were closely clustered together and separately from samples in other treatments, whereas there was overlap among samples in the other treatments. Therefore, components of CrB1 gut bacterial communities were clearly different from those in larvae fed other hosts. There were significant differences in structure of gut bacterial communities in larvae fed different hosts. The LEfSe analysis effectively detected differentially abundant bacterial taxa in gut microbiomes. A comparison with existing statistical methods and metagenomic analyses of environmental, gut microbiome, and synthetic data shows that LEfSe analysis consistently provides lower false positive rates and can effectively aid in explaining the biology underlying differences in microbial communities[57]. In general, the results in this study confirm that feeding on different host plants alters structure of gut bacterial communities in *S. frugiperda* larvae, similar to results for other lepidopterans[58].

PICRUSt2 software was used to analyze functions of gut bacteria[59]. There were 35 predicted functions of gut bacteria in larvae feeding on different hosts, with most related to metabolic functions. In analysis of differences in KEGG metabolic pathways, guts of 5th and 6th instar larvae fed different hosts were obviously enriched with different functional proteins in most metabolic pathways. In ZmL1 and ZmL2 treatments (larvae fed corn), samples were enriched with different functional proteins, with genes associated with ABC transport function accounting for the largest proportion. Notably, the bacterial detoxification pump is based on ABC transporters in several main categories: the ABC superfamily[60], the major promoter superfamily[61], and the small multidrug resistance family[62]. Composition of gut bacterial communities in *S. frugiperda* and ability of members of those communities to metabolize insecticides differ depending on the diversity of chemicals used to treat the host[63]. Accumulation of detoxification and defense genes in the gut of *S. frugiperda* may be related to the diversity of food intake or the variable host environment. The specific factors of influence still need to be verified in further experiments.

This study showed that different host plants had important effects on structure and diversity of gut bacterial communities in *S. frugiperda*. Host-induced changes in structure and metabolic functions of gut

bacterial communities likely assist *S. frugiperda* larvae in adapting to differences in food. This work provides a good foundation for further exploration of interactions between gut bacteria and food in *S. frugiperda*. Further research on gut microbes may also provide new perspectives and directions for biological control of *S. frugiperda*.

Materials And Methods

Insect collection and laboratory feeding

S. frugiperda were collected from corn field in Base of Xindu, Sichuan Academy of Agricultural Sciences, China. The larvae were fed on artificial diet contained (g L^{-1}): soybean powder (225), wheat powder (125), yeast (40), casein (20), cholesterol (0.6) and agar (30) [64]. Insects were reared at our laboratory for three generations at 27 ± 1℃ with 70 ± 5% relative humidity, and the light:dark = 16:8 h photoperiod. Larvae were separately reared on leaves of corn, sorghum, highland barley, and citrus in the laboratory. Host plants included corn (Zea mays L. var. Chengdan 11, ZmL), sorghum (Sorghum bicolor L. Moench. Chuannuo 15, SbL), highland barley (Hordeum vulgare L. var. Kangqing 9, HvL) and citrus (Citrus reticulata Blanco. Chunjian, CrB). Plants were cultivated to the 3–4-true-leaf stage. Newly hatched larvae were reared to 5th and 6th instars on fresh young leaves of the four different host plants.

A total of 32 gut samples of 5th and 6th instar larvae of *S. frugiperda* fed different host plants were collected and profiled. Thus, the experiment had two treatment factors: host plant and larval stage. Host plants were corn (Zm), sorghum (Sb), highland barley (Hv), and citrus (Cr), and larval stages were 5th instar (L1 or B1) and 6th instar (L2 or B2). Therefore, there were eight treatment combinations: ZmL1, ZmL2, SbL1, SbL2, HvL1, HvL2, CrB1, CrB2.

Processing of S. frugiperda **larvae**

To ensure gut bacteria were at a relatively stable state, *S. frugiperda* larvae were transferred to new centrifuge tubes and starved for 24 h in a natural environment. After all materials were prepared, dissections were performed on an ultra-clean bench. First, beakers were prepared with sterile water and absolute ethanol. Larvae were removed from centrifuge tubes, soaked in absolute ethanol for 90 s, and then blotted on filter paper. Larvae were then washed three times with sterile water, blotted dry, and placed in petri dishes. Under a stereomicroscope, the head of a larva was held with pointed tweezers, and medical scissors were used to cut along the abdomen below the mouth. Ganglion, salivary glands, martensiian ducts, fat bodies and other organs were carefully removed. Then, the intestine was completely removed, placed in a sterile centrifuge tube, quickly frozen with liquid nitrogen, and stored at − 80°C.

DNA extraction and 16S rDNA sequencing

To extract total DNA from gut contents, a PowerSoil DNA Isolation Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) was used according to protocol provided by the manufacturer. Integrity of extracted DNA was

confirmed by agarose gel electrophoresis. Extracted DNA was quantified in a Qubit 2.0 (Invitrogen, Carlsbad, CA, USA), and 10 ng/ul was used for amplification and sequencing of the 16S rDNA gene from 32 samples. PCR full-length amplification was performed using the 16S primers F (5'AGAGTTTGATCCTGGCTCAG3') and R (5'GNTACCTTGTTACGACTT3') with Phusion® High-Fidelity PCR Master Mix(New England Biolabs Inc., Ipswich, MA, USA) under the following conditions: 94˚C 5 min; 35 cycles of 94˚C 30s, 56˚C 30s, 72˚C 30s; 72˚C 5 min. Followed by product purification, construction of a SMART bel library, and sequencing on PacBio[65]. Total DNA was sent to Beijing Novogene Bioinformatics Technology Co., Ltd., (Beijing, China) for sequencing.

Statistical analyses

PacBio offline data were exported to a bam file through PacBio's SMRT analysis software(version 7.0). After samples were distinguished according to barcodes, operational taxonomic unit (OTU) clustering and classification analysis were performed. Sequences that were less than 1340 bp or greater than 1640 bp were removed. Use Uparse software (Uparse version 7.0.1001 http://drive5.com/uparse) to cluster Clean Reads, The sequences were clustered into OTUs (Operational Taxonomic Units) with 97% identity, Species annotation analysis was performed using the Mothur method with the SSUrRNA database of SILVA (http://www.arb silva.de/). We used MUSCLE (version 3.8.31, http://www.drive5.com/muscle/) software to perform rapid multiple sequence alignment, and then obtained all OTUs representative sequences. The subsequent analysis of alpha diversity and beta diversity were based on the standardized data.

According to results of OTU clustering, abundances of OTUs were analyzed, and a petal map was prepared. QIIME software (version 1.9.1)was used to calculate alpha diversity indices, including Chao1, Simpson, and Shannon. ANOVA (One-way analysis of variance) followed by Tukey's tests were performed to test the difference between host plants. R software (version 2.15.3) was used to analyze the differences between groups in beta diversity index, and we used the R vegan package to obtain a NMDS. LEfSe (LDA Effect Size) analysis was used to test significance of differences in composition and structure of bacterial communities in samples from different treatments. Last, based on the KEGG database(https://www.kegg.jp/), PICRUSt2(https://github.com/picrust/picrust2) was used to predict metabolic functions of bacterial communities.

Declarations

Data availability

The data presented in this study are available in the Supplementary Materials. **Acknowledgments**

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Contributions

Conceived and designed experiments:Y.P.W and X.L. Performed the experiments: Y.P.W, C.Y.Y., C.H.L. Analyzed the data: Y.P.W., J.W.G., X.Y.C.and H.L.L. Contributed reagents/materials/analysis tools:Y.P.W., C.C.Z., Q.D.C., S.C. Wrote the paper: Y.P.W.

Statement

The experimental research on plants complied with “Forest Law of the People's Republic of China” and “Regulations of the People's Republic of China on Plant Quarantine”.

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Figures

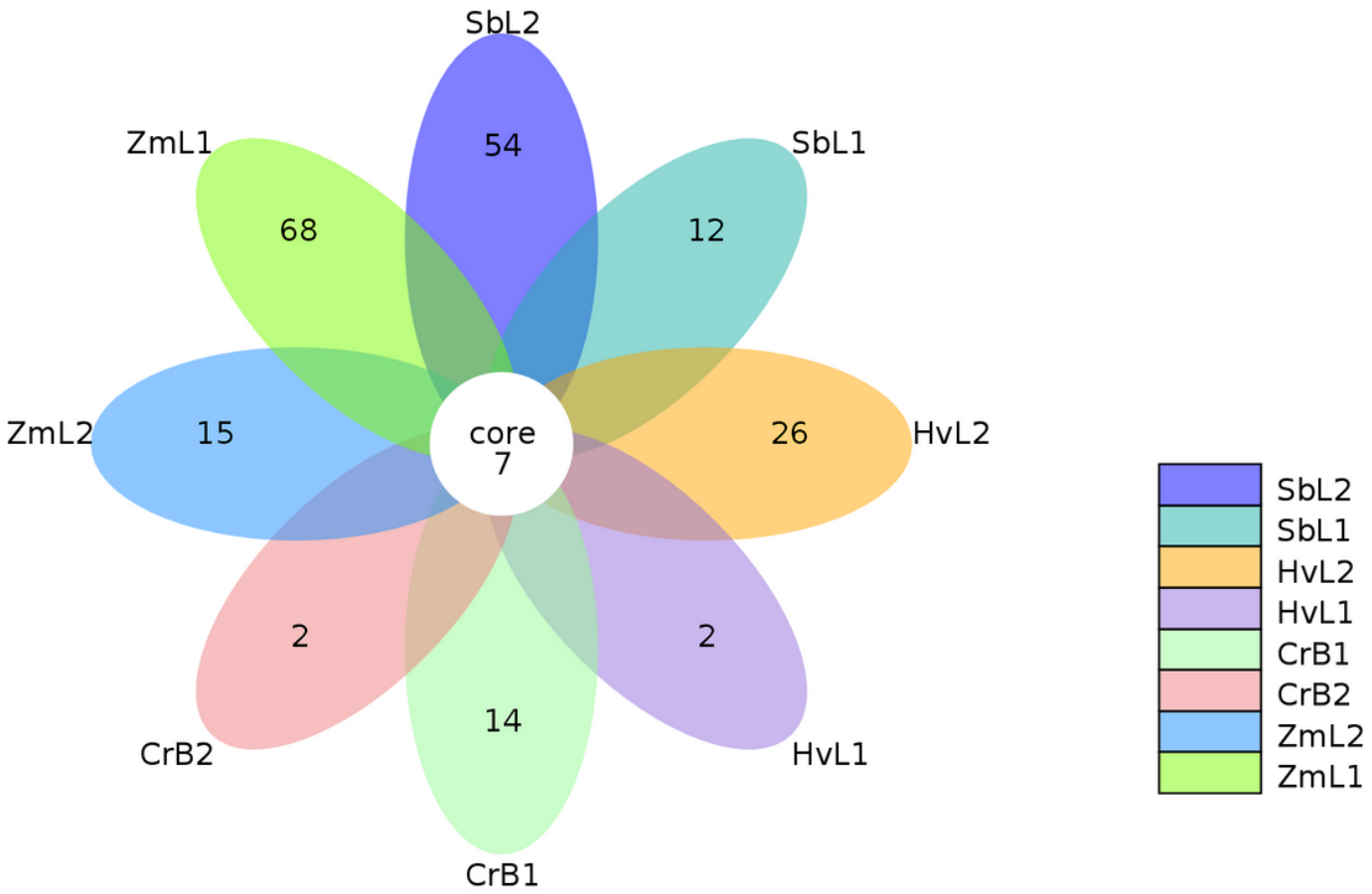

Figure 1

Flower plot of bacterial operational taxonomic units (OTUs) in guts of *S. frugiperda* larvae fed leaves of different host plants.

Each petal in the flower represents a treatment, and the core number in the overlapped parts of the petals represents the number of OTUs shared among treatments. The numbers at petal edges represent the number of unique OTUs in a treatment. Treatments: letters represent different host plants (Zm, corn; Sb,

sorghum; Hv, highland barley; Cr, citrus), and numbers represent different larval instars (L1 or B1, 5th instar; L2 or B2, 6th instar).

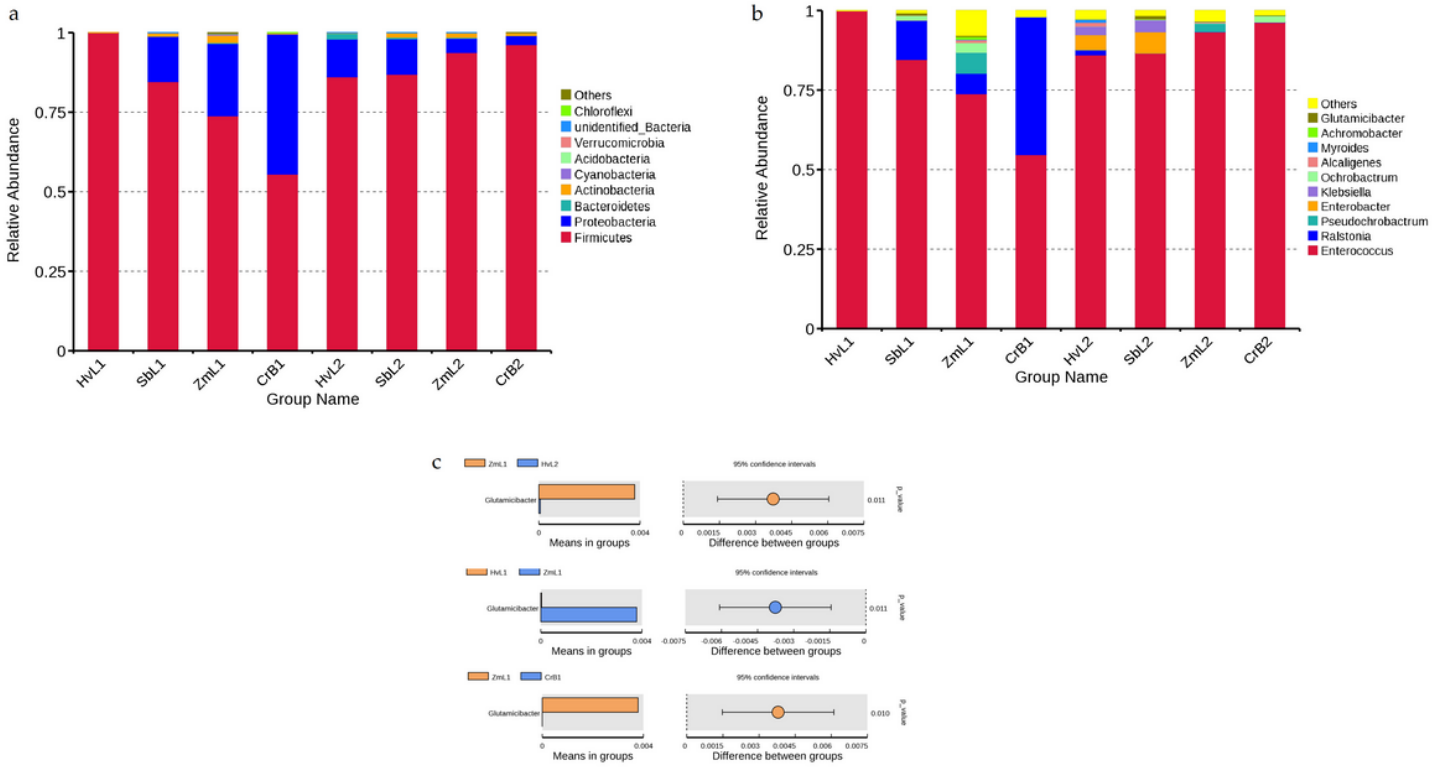

Figure 2

Relative abundance of the most predominant taxa of gut bacteria in *S. frugiperda* larvae fed leaves of different host plants. (a) Phyla and (b) genera. (c) T-test analysis of species differences between groups.

Others represents the sum of the relative abundances of all phyla (genera) other than the phyla (genera) in the figure. Each bar in the figure represents the mean value of species with significant differences in abundance between groups. It is the p-value for the between-group significance test for the corresponding differing species.

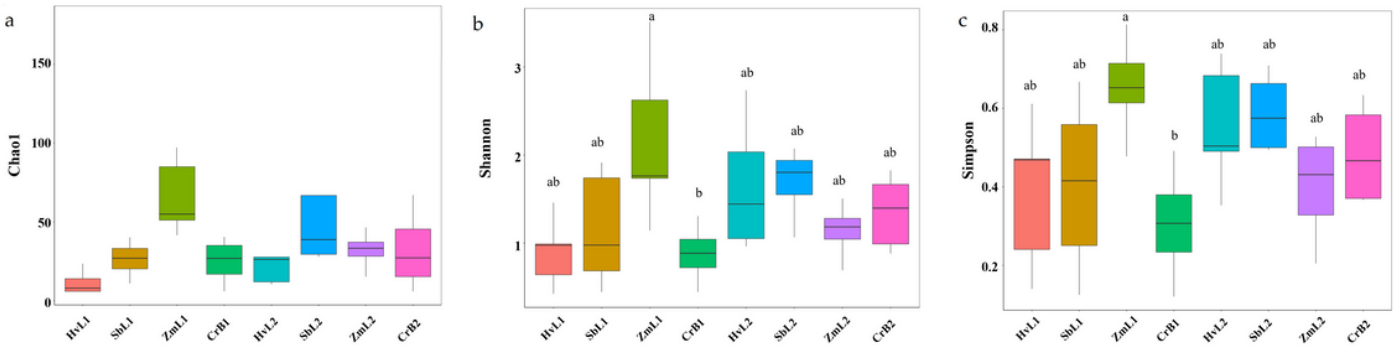

Figure 3

Box plots of (a) Chao1, (b) Shannon, and (c) Simpson alpha diversity indices of gut bacterial communities in *S. frugiperda* larvae fed leaves of different host plants.

The a, b indicate the significant differences in relative abundance in the same column in the mean values. Different letters above boxes indicate significant differences among treatments (one-way ANOVA, Tukey's post-hoc test) in the mean values.

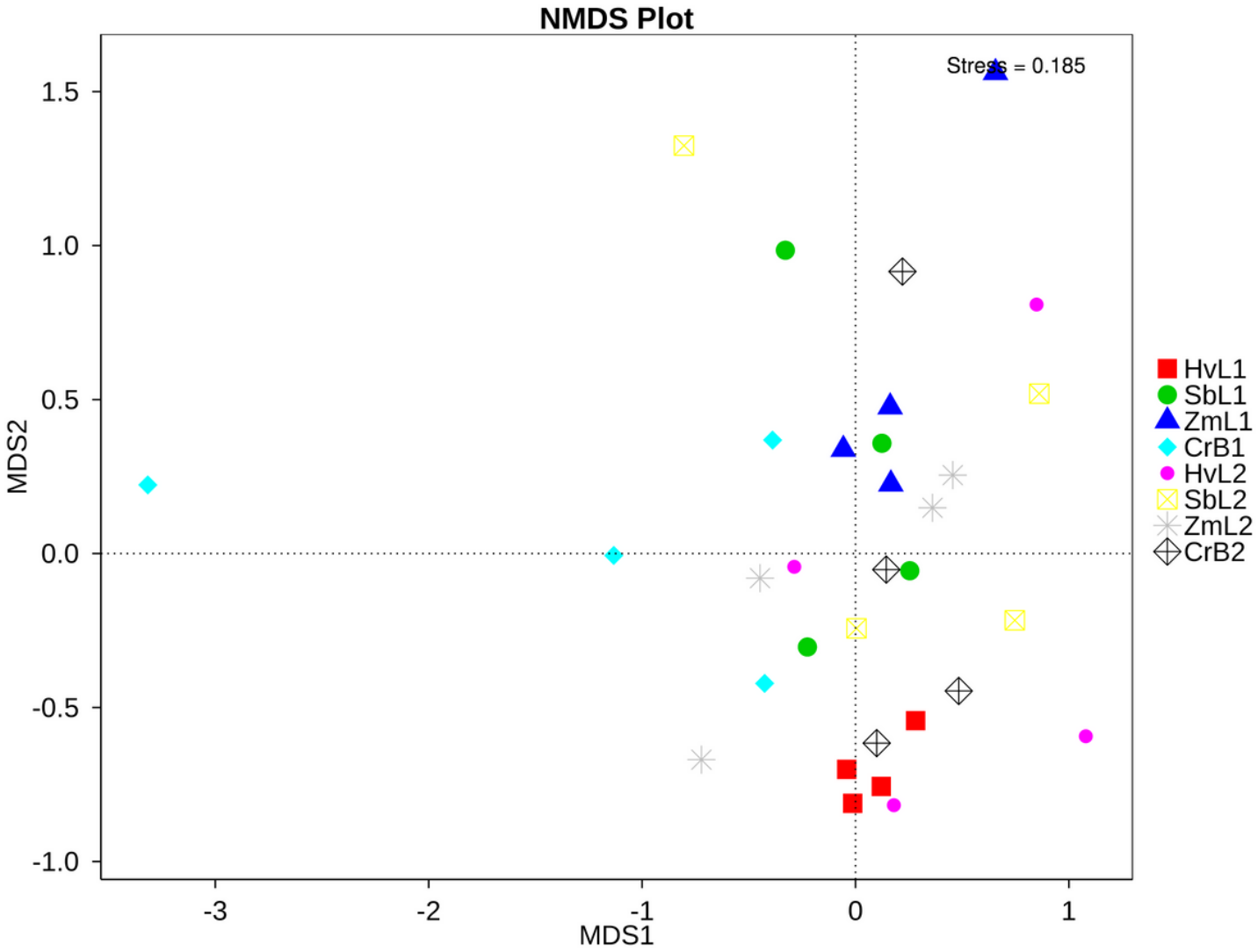

Figure 4

Nonmetric multi-dimensional scaling (NMDS) analysis of gut bacterial communities in *Spodoptera frugiperda* larvae fed leaves of different host plants.

Each point in the figure represents a sample; distance between points represents degree of difference, and samples in the same treatment are the same color. When Stress is less than 0.2, the NMDS accurately reflects degree of difference between samples.

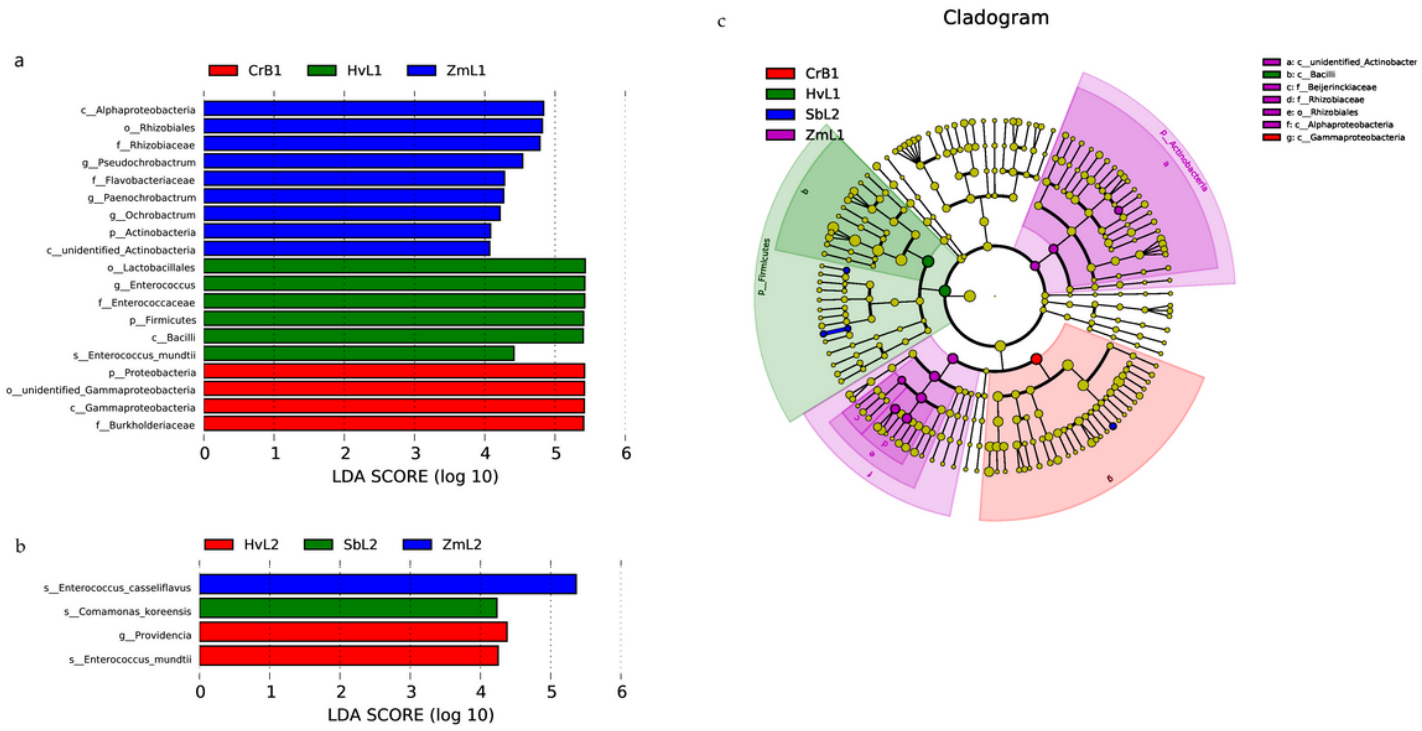

Figure 5

Linear discriminant analysis (LDA) of gut bacterial taxa in *Spodoptera frugiperda* larvae fed leaves of different host plants. Taxa with LDA score (log 10) greater than four in (a) 5th and (b) 6th instar larvae. (c) LEfSe (LDA Effect Size) analysis showing significant differences in bacterial taxa at the level of phylum, class, order, family, and genus, from inside to outside.

Small circles at different classification levels represent classifications at a particular level, and their diameters represent relative abundances. Nodes of different colors represent bacteria that were significantly enriched with the corresponding host. Significantly different biomarkers follow the group for coloring. Small yellow nodes indicate bacterial taxa that were not significantly different in guts of larvae fed different hosts.

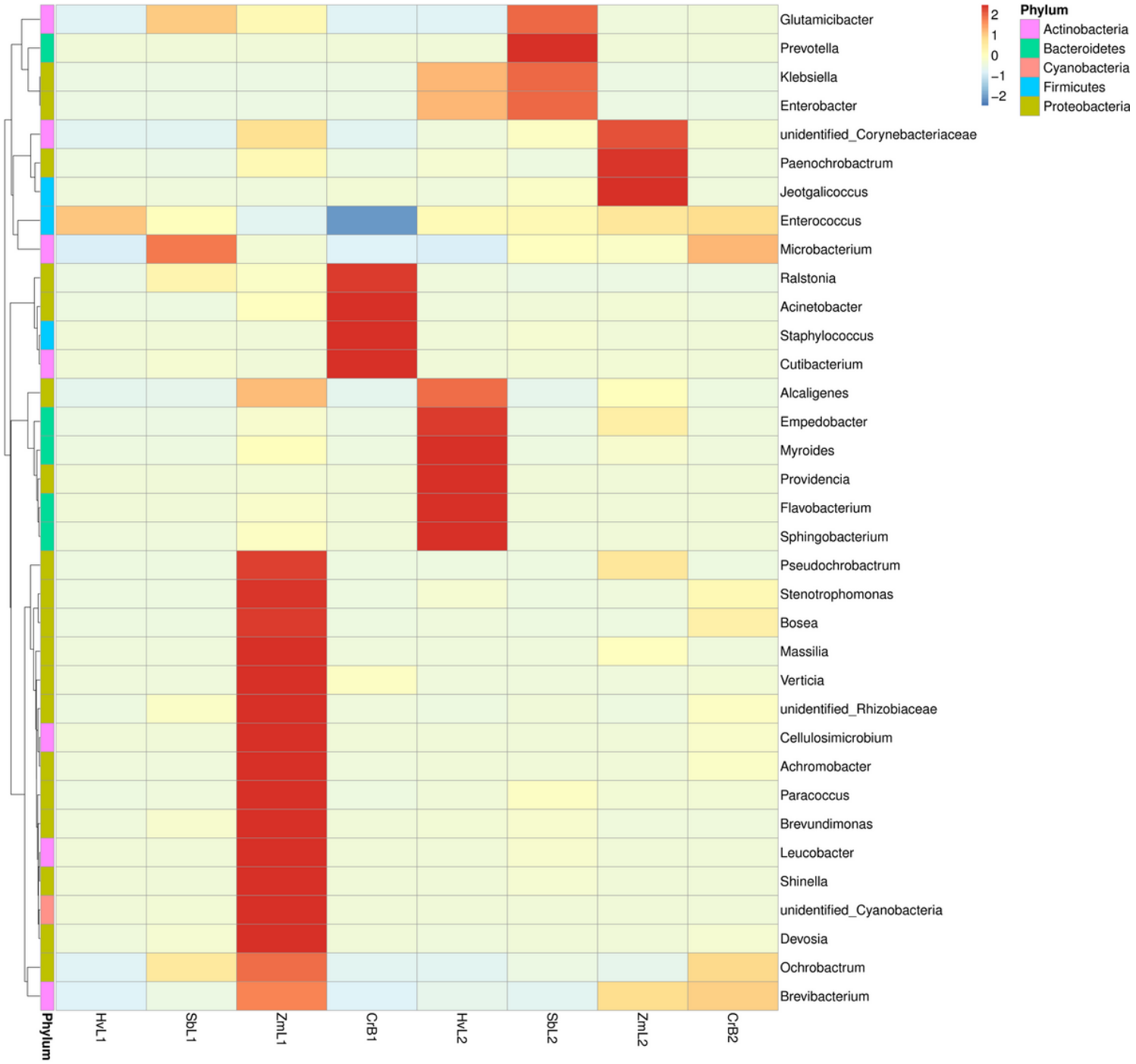

Figure 6

Heat map of relative abundances of the top thirty-five predominant genera of gut bacteria separated by phylum in *Spodoptera frugiperda* larvae fed leaves of different host plants. Treatment names are on the *x*-axis, and genus annotation is on the *y*-axis. The clustering tree for genera is on the left, and heat map values are Z-values obtained after relative abundances of each genus were standardized.

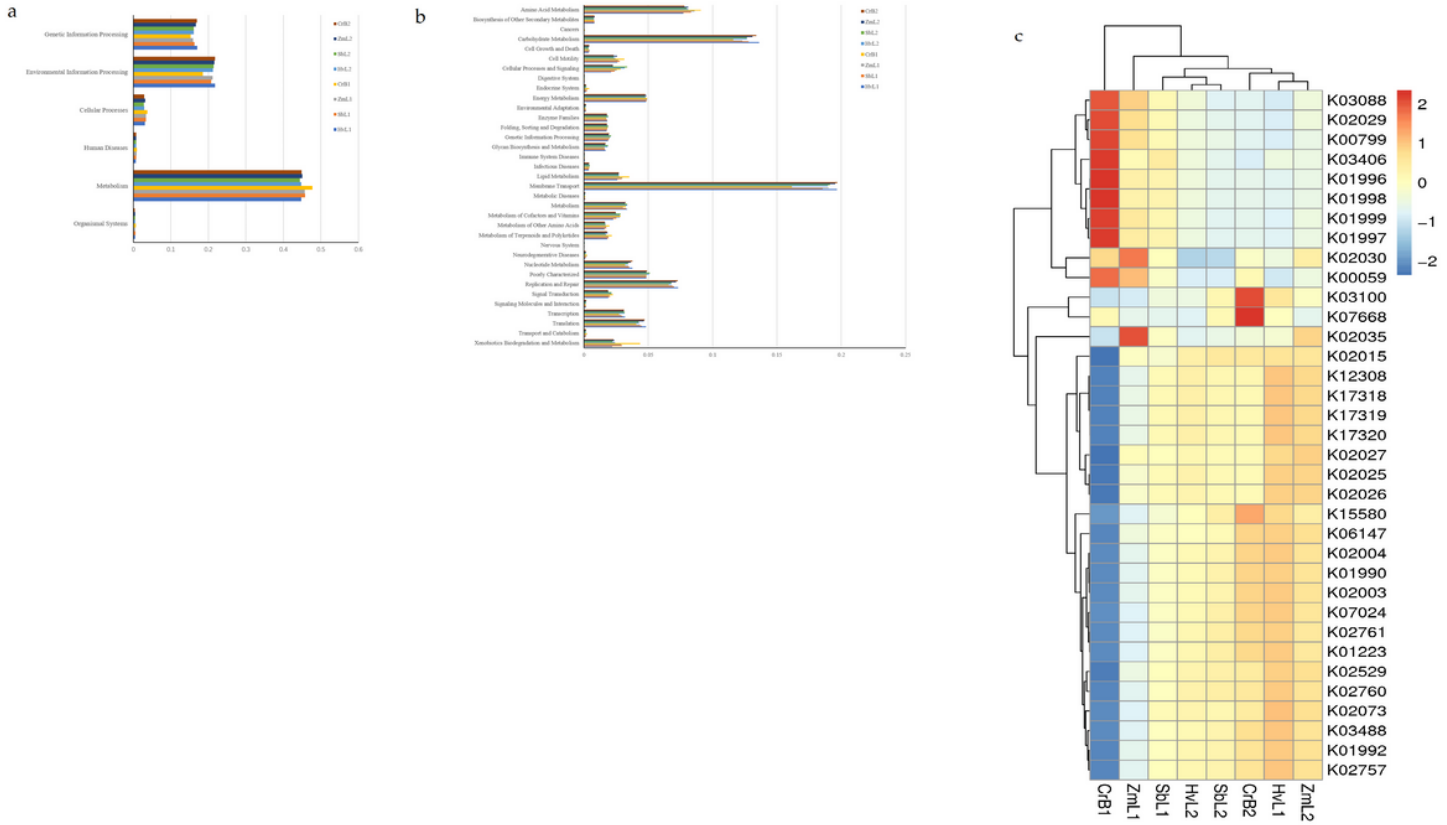

Figure 7

Annotations of KEGG predicted functions of gut bacterial communities in *Spodoptera frugiperda* larvae fed leaves of different host plants. (a) Level 1 and (b) level 2. (c) PICRUSt2 prediction of proteins based on functions in the KEGG database.

Supplementary Files

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

- Supplementarytablesandfigures.docx