

Profiling of Gut Bacteria Composition of Field-collected and Laboratory-adapted Population of *Ostrinia furnacalis* under Antibiotic Exposure

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Abstract

All living organisms harbor gut bacteria that play dynamic roles in their lives. Among them, the Asian corn borer (ACB), *Ostrinia furnacalis*, stands out as a destructive pest in maize plantations. Due to the limited research, antibiotic-feeding insects may serve as excellent models for experimental studies of gut microbiota dynamics. In this study, we combine antibiotic-feeding treatment with metagenomic analysis of the 16S rRNA gene to descriptively examine the composition and predicted functions of gut bacteria in *O. furnacalis* larvae. The antibiotic-feeding assays showed no detectable effect of antibiotics on the development of *O. furnacalis*. However, high-throughput sequencing revealed shift in gut bacteria composition following antibiotic treatment. In the F₁ generation, antibiotic-treated larvae showed a reduction in *Enterococcus*, accompanied by a higher relative abundance of antibiotic-tolerance taxa; *Achromobacter* (24,08%), *Burkholderia-Caballeronia-Paraburkholderia* (24,47%), *Ochrobactrum* (19,70%), and *Paenibacillus* (18,33%). Whereas the control group remained dominated by *Enterococcus* (83,82%). In the F₂ generation, of both groups exhibited relatively similar bacterial profiles, sharing more than 50% of amplicon sequence variants (ASVs). Principal coordinate analysis (PCoA) indicated separation between field-collected and laboratory-adapted populations. Furthermore, the functional gene prediction based on KEGG orthologs suggested enrichment of the ABC transporter-associated pathway in antibiotic-treated samples, potentially related to transport processes and xenobiotic responses. Overall, this descriptive study provides baseline insight into gut microbiome variability in *O. furnacalis* under antibiotic exposure and highlights the need for future studies with biological replication to validate functional implications.

I. INTRODUCTION

Ostrinia furnacalis (Lepidoptera: Crambidae) is an important pest in maize production, and this insect is distributed across Asian countries, Australia, and Russia (Hu et al., 2024). In a tropical region, *O. furnacalis* could produce 9-12 generations in a year. The highest damage intensity has occurred during the tasseling and earing stages. According to He et al. (2003), the yield loss caused by *O. furnacalis* damaging maize was estimated to reach 6-9 million tons annually. During the vegetative stage, the whorl and leaf's parallel small holes were used for damage indications. During the generative stage, damage symptoms were a symmetrical hole in the stem surrounded by larva excrement. According to Subiadi et al. (2014), the vegetative stage (V10) had the highest yield loss proportion per larva per plant, resulting in a 4.94% grain weight reduction compared to the generative stage (R1) (4.56%) and (R2) (3.76%). Currently, insecticides are the primary method for controlling this pest population, while Bt crops have also been promoted as a control measure (G. Li et al., 2021). Therefore, sustainable and environmentally friendly management techniques are needed to control this pest successfully.

In addressing sustainable pest management, it is essential to consider the role of insect gut microbiomes, which support crucial functions for insect survival. The gut microbiota is crucial to insect life for processes like immunity, metabolism, reproduction, and adaptation to environmental changes (Frago et al., 2012). Various factors influence the insect gut microbial community, including diet, life stage, and habitat (Dongbiao et al., 2023). These varieties of factors could work dependently or independently. Although the gut of insects is home to a wide variety of microbial communities, bacteria are the most prevalent. In principle, insects have primary and secondary associated bacterial symbionts (Voirol et al., 2018). The primary, obligate insect-associated bacteria mainly help the host insect by supplying nutrients. The secondary, facultatively insect-associated bacteria may affect various aspects of the host insect's fitness, including enhancing the host's immune response to xenobiotic compounds, protecting against environmental changes, and influencing host plant selection (Douglas, 2015; Hammer & Bowers, 2015).

Many studies have been conducted on the gut microbial community of Lepidoptera species, revealing a lower range of bacterial symbionts in its gut. According to Prem Anand et al. (2009), the high alkaline pH in the lepidopteran gut and its rapid digestive processes influence the low variabilities of bacterial symbionts. For example, *Manduca sexta* has a low diversity of gut bacteria symbionts (Hammer et al., 2017). In *Spodoptera frugiperda* the most dominant gut bacterial community was Proteobacteria (Gichuhi et al., 2020). The recent work with the European Corn Borer (*Ostrinia nubilalis*) reveals the difference in microbial diversity between the lab-reared and field populations. The field population mainly consists of Proteobacteria and Bacteroidetes, while Firmicutes predominate in the lab-reared population (Belda et al., 2011). In the *Manduca sexta*, antibiotic suppression of gut bacterial activity did not significantly affect caterpillar weight gain, development, or survival (Hammer et al.,

2017). Furthermore, the bacterial symbiont could increase the resistance against imidacloprid insecticide in *Nilaparvata lugens* (Zhang et al., 2021).

Given the limited understanding of gut microbiome functions in *O. furnacalis*, it is critical to investigate the roles of the gut microbiome in the growth and development of *O. furnacalis*. Characterizing these microbial communities may uncover potential mutualistic interactions and provide a foundation for pest management strategies. Such knowledge could facilitate the development of microbial biocontrol approaches and improve the effectiveness of insecticides that indirectly disrupt gut-associated microbes. In this study, we investigated the composition and diversity of gut bacterial communities in *Ostrinia furnacalis* using 16S rRNA gene sequencing, comparing field-collected populations continuously exposed to an antibiotic mixture (ampicillin, chloramphenicol, rifampicin, and tetracycline) with laboratory-adapted populations.

II. Methods

2.1. Insect

A field-collected population of larvae of *O. furnacalis* (200 larvae) was collected from a maize field in Magelang Regency, Central Java, Indonesia, in March 2024. The F_0 population was continuously fed with young maize cobs to maintain the composition of gut bacteria. The laboratory-adapted population was obtained from the Toxicology Room, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. This population has been maintained on an artificial diet following a well-established laboratory protocol since 2006 and is now in the F_{213} generation (Figure 1b). The artificial diet was made according to the procedure by (Trisyono, (2006) (unpublished); (Rahayu et al., 2018), consisting of an antibiotic (Tetracycline 500 mg/L). Each larva was placed into a plastic vial with a diameter of 4 cm and a height of 4,7 cm and allowed to grow in a place maintained at 25 °C temperature, 65% relative humidity, and a 12-h light-dark photoperiod. The adults were fed a 10% honey solution, and their offspring were used for the present study.

2.2. Antibiotic treatment

In this study, four antibiotics (ampicillin, chloramphenicol, tetracycline, and rifampicin) were used at specific concentrations to eliminate both gram-positive and gram-negative bacteria, as reported in previous studies (Xu et al., 2023). Based on these findings and preliminary experiments, a final solvent concentration of 500 µg/mL was selected, with an antibiotic mixture ratio of 1:1:1:1, resulting in a final concentration of 125 µg/mL for each antibiotic. The field-collected population was divided into two groups (Antibiotic and Control groups), each consisting of two treatments (Figure 1a). The antibiotic group was designated antibiotic-feeding and consisted of two generations of larvae (F_1 and F_2). In this group, first-instar larvae were fed with a natural diet dipped in the antibiotic solution until pupation. The control group was an untreated antibiotic group consisting of two generations of larvae (F_1 and F_2); the larvae were fed with a natural diet. Larva mortality was recorded at 3, 7, and 10 days after treatment (DAT). The larvae's weight was recorded on day 10, after which the treatment continued until pupation, when their weights were measured again. Furthermore, the larval and pupal weights and larval mortality were evaluated to assess any anomalies. The pupae were then transferred to rearing cages, where the adults were fed a 10% honey solution until the F_2 generation was produced. The F_2 generation was subjected to the same treatments as the F_1 generation, including both the natural diet and antibiotic feeding treatments. The laboratory- adapted population was maintained on an artificial diet following a well-established laboratory protocol (Figure 1b).

2.3. Gut Sample Preparation, DNA Extraction, and Library Construction

To examine the composition of gut bacteria without starving the larvae, five late-instar larvae were taken from each sample randomly: (1) Antibiotic group was sampled from each generation and named accordingly as With_AB_F1 and With_AB_F2; (2) Control group was sampled from each generation and named accordingly as No_AB_F1 and No_AB_F2; (3) Laboratory-adapted population larvae were sampled and name accordingly as Lab_Pop. Each sample consisted of five larvae pooled into a single composite sample with no biological replication. After being selected, the larvae sample was surface sterilized with 5% sodium hypochlorite and 70% ethanol for 90 sec and rinsed three times with phosphate-buffered saline (PBS) to remove surface-associated microorganisms. The dissection was performed under sterile conditions to isolate the whole gut tissues, which were preserved in absolute ethanol and stored at -80 °C to stabilize microbial DNA and minimize post-collection changes in microbiome

composition, as ethanol is reported to maintain DNA integrity and microbial community structure for sequencing analysis (Hildonen et al., 2019).

The composite of raw gut samples (5 gut/sample) was then transferred to the service provider GSI (Genetica Science Indonesia, Indonesia) for DNA extraction and sequencing. Total DNA from the larva gut was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300) following the manufacturer's protocol. The initial quantification and purity of the extracted DNA solution were determined using Nanodrop 2000 (Thermo Scientific). The PCR amplification was performed using target-specific primers (16S-V4), 515F (5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNGTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNGGACTACHVGGGTWTCTAAT-3'). The PCR product was visualized with Agarose Gel Electrophoresis and the concentration of the prepared library was measured using Qubit dsDNA High Sensitivity Assay Kits (Thermo Scientific). The final library products were then sequenced by Genetika Science (Jakarta, Indonesia) using the **Illumina** platform to generate paired-end raw reads.

2.4. Data analysis and statistics

Statistical analyses were conducted in RStudio. Data normality for larval survival, larval weight, and pupal weight was evaluated using the Shapiro–Wilk test. Following confirmation of normality, differences between control and antibiotic-treated groups were assessed using independent two-sample t-tests. Larval survival and larval weight were analyzed at the treatment level, while pupal weight was analyzed separately for males and females to account for sex-specific variation. All analyses were performed at a significance level of 0.05, and results are presented as mean $\pm$ standard error.

Library preparation was performed using the final PCR products. The final library was sequenced on **Illumina** platform to generate paired-end raw reads. Adapter and PCR primer sequences from the paired-end reads were removed using Cutadapt. DADA2 was used to correct sequencing errors and remove low-quality sequences and chimera errors. The resulting ASV data was used for taxonomic classification against **SILVA (silva_nr99_v138.1) (16S)**. Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3) (<https://www.R-project.org/>), Krona Tools (<https://github.com/marbl/Krona>), PICRUST2 (<https://github.com/picrust/picrust2>).

III. Results

3.1. Effect of antibiotic feeding on larva development

The antibiotic-feeding treatment was used to induce stress in the gut bacteria. The treatment showed no detectable effect on larval survival rate ($t = -1.87$, $df = 28$, $p = 0.072$). A similar pattern was observed for larval weight ($t = -0.25$, $df = 28$, $p = 0.803$) and for pupal weight in both males ($t = -1.58$, $df = 25.25$, $p = 0.127$) and females ($t = -1.51$, $df = 27.73$, $p = 0.143$). In other words, the growth performance of *O. furnacalis* larvae treated with antibiotics was comparable to that of the control group (Figure 2).

3.2. Diversity indices of bacterial ASV

Alpha diversity indices analyses revealed a distinct pattern in *O. furnacalis* gut bacteria communities across treatments (Figure 3). In the F_1 generation, With_AB_F1 exhibited lower observed ASVs but relatively higher Shannon and Simpson indices, indicating greater evenness despite reduced richness, whereas No_AB_F1 showed higher ASV richness but was dominated by fewer taxa. In the F_2 generation, With_AB_F2 and No_AB_F2 displayed moderate similarity. However, the control group (No_AB_F2) suggested stronger dominance by prevalent taxa. The laboratory-adapted population (Lab_Pop) closely mirrored With_AB_F2 in observed and Shannon indices, but indicated slightly higher dominance based on Simpson index. Consistent with these observations, the Venn diagram (Figure 4) showed ASV overlap of 25.9% (21/81) between With_AB_F1 and No_AB_F1, 50.5% (43/85) between With_AB_F2 and No_AB_F2, and 7.2% (13/179) across all groups.

The PCoA ordination based on the Bray-Curtis dissimilarity revealed distinct separation patterns in ASV composition across *O. furnacalis* gut communities (Figure 5), collectively explaining 76.22% variance (PCoA1 42%, PCoA2 34.2%). In the F_1 generation, With_AB_F1 and No_AB_F1 clustered distinctly along the PCoA2 axis, indicating divergent gut bacteria profiles of post-antibiotic

exposure. In the F2 generation, With_AB_F2 and No_AB_F2 showed closer proximity, suggesting partial compositional recovery or convergence, whereas Lab_Pop exhibited the most isolated pattern, reflecting established population stability.

3.3. Taxonomy Assignment

The high-throughput sequencing of *O. furnacalis* gut samples generated 100,000 sequence reads per pooled sample (5 guts/sample), retaining 99,969-99,994 valid sequences after quality control and chimera removal(average length 152-300 bp). These sequences clustered into 179 total ASVs spanning 5 phyla, 9 classes, 33 orders, 62 families, and 101 genera (Table 1). With_AB_F1 yielded 48 ASVs across 4 phyla and 33 genera, while With_AB_F2 showed 74 ASVs reaching 45 genera; No_AB_F1 and No_AB_F2 each produced 54 ASVs with 35 genera; Lab_Pop exhibited 74 ASVs with the broadest taxonomic resolution (5 phyla, 58 genera).

Table 1. Sequencing information of gut bacteria of *Ostrinia furnacalis* in the field-collected and laboratory-adapted population.

Sample Grouping	Sample	Original Sequence	Number of Valid Sequences	Sequence Length (bp)	ASVs Number	Number of Different Classification Orders				
						Phylum	Class	Order	Family	Genus
With AB	With_AB_F1	100000	99988	157-300	48	4	5	12	25	33
	With_AB_F2	100000	99993	155-251	74	4	7	17	34	45
No AB	No_AB_F1	100000	99988	160-300	54	4	5	14	27	35
	No_AB_F2	100000	99994	158-300	54	4	5	13	27	35
Lab	Lab_Pop	100000	99969	152-300	74	5	8	28	42	58

All *O. furnacalis* gut samples were dominated by four major bacterial phyla, which are Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidota; with Firmicutes and Proteobacteria representing the predominant groups (Figure 6a). In the F1 generation, Firmicutes exhibited high relative abundance in No_AB_F1(68%) and Lab_Pop (83%), whereas Proteobacteria predominated in With_AB_F1 (64.22%). This pattern shifted in the F2 generation, where Proteobacteria increased in both With_AB_F2 (66.74%) and No_AB_F2 (74.57%), accompanied by a decline in Firmicutes.

At the genus level (Figure 6b) *Enterococcus* dominated No_AB_F1 (83.82%), while With_AB_F1 showed a compositional shift toward *Achromobacter* (24.08%), *Burkholderia-Caballeronia-Paraburkholderia* (24.47%), *Ochrobactrum* (19.70%), *Paenibacillus* (18.33%), and other genera (11.62%). In the F₂ generation, a partial recovery was observed, with *Enterococcus* present in both With_AB_F2 (30.76%) and No_AB_F2 (24.35%). However, *Klebsiella* was dominant in No_AB_F2 (48.88%), while *Enterococcus* remained highly prevalent in Lab_Pop (92.43%).

3.4. Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology Analysis of Differentially Expressed Transcripts Between Field-collected Laboratory-adapted Populations Larvae

PICRUSt2 analysis paired with the KEGG Orthologs database was used to predict functional gene profiles from the gut bacterial community in *O. furnacalis*,focusing on the 20 most abundant orthologs across treatments (Figure 7). With_AB_F1 exhibited the highest abundances, shown by darker red color gradients, particularly for K03088 (RNA polymerase sigma-70 factor, ECF subfamily, Prokaryotic type) and K00059 (3-oxocyl-[acyl-carrier protein] reductase). Several other orthologs in With_AB_F1 exhibited strong correlations with ABC transporter family functions, which were comparatively lower in No_AB_F1. In the F₂ generation, With_AB_F2 and No_AB_F2 showed broadly similar ortholog abundance patterns across all categories, whereas Lab_Pop consistently showed the lowest predicted abundances.

IV. Discussions

As Lepidopteran pests, *Ostrinia furnacalis* larvae exhibit a simple gut structure and a rapid digestive system, which shape its gut bacteria communities. Previous research has examined the gut bacteria composition of the field-collected and lab-reared population of its sibling species, the European corn borer (*Ostrinia nubilalis*) (Belda et al., 2011). However, studies on *O. furnacalis*

remain limited, particularly on the gut bacteria composition. This exploratory analysis examines larval gut bacteria community dynamics across field-collected F1-F2 generations reared on a natural diet, with and without antibiotic exposure conditions, contrasted against long-established laboratory colonies continuously reared on tetracycline-supplemented artificial diet since 2006. The design utilized pooled larval guts (5 individuals/sample) to capture compositional trends associated with diet type and antibiotic exposure without biological replication. Antibiotic exposure showed no detectable fitness cost to larvae. However, the treatment elicited marked compositional changes in gut bacteria composition, with the drop in the Firmicutes abundance as the dominant phyla from 68% (No_AB_F1) to < 15%, while a major increase was observed in the Proteobacteria phyla of With_AB_F2 (66.74%) and No_AB_F2 (74.57%). These findings suggest that suppression of viable gut bacteria had no negative consequences on the fitness of *O. furnacalis* larvae. This observation aligns closely with the prior study in *Manduca sexta* where the suppression of gut bacteria by the influence of antibiotics had no significant effect on larval growth or survival (Hammer et al., 2017). Our findings were also aligned with previous research in lepidopteran insects, suggesting that antibiotic feeding could alter the gut microbial community (B. Chen et al., 2018; Y. Chen et al., 2021; G. Li et al., 2020; Martínez-Solís et al., 2020; Thakur et al., 2016). In F₁ generation, With_AB_F1 displayed reduced ASV richness coupled with elevated evenness based on the Shannon and Simpson indices (Figure 3). Furthermore, the PCoA of With_AB_F1 revealed separation from No_AB_F1, suggesting acute dysbiosis which affecting composition of the bacterial community. In more detail, the change in gut bacteria composition was shown by the collapse of *Enterococcus* dominance from 83.82% in No_AB_F1 to less than 1% in With_AB_F1 (Figure 6b). A decrease in Firmicutes was aligned with the significant reduction in the genus of *Enterococcus*, noted in fall armyworm (*Spodoptera frugiperda*) larvae fed an antibiotic-containing diet (Y. Chen et al., 2021; Fu et al., 2024). *Enterococcus* is a bacterial genus that inhabits the gut of insects fed a variety of crops. In lepidopteran species, *Enterococcus* is one of the most common bacteria in the digestive tract throughout the life cycle (Voirol et al., 2018). *Enterococcus* plays a significant role in the insect host's life as a core gut. In *O. nubilalis*, the bacterial genus *Enterococcus* is known to have a role in exhibiting cellulolytic metabolism, in which cellulose is the main component of its diet (J. Li et al., 2022). *Enterococcus*, which is present in gypsy moth larvae, can acidify the environment in which it lives, allowing it to settle in alkaline niches. This likely shields the gut from harmful substances that become active in an alkaline environment (Broderick et al., 2006). Furthermore, the decreased abundance of core bacteria in the F₁ generation of the antibiotic group induces dysbiosis. This condition creates an ecological opportunity for opportunistic proliferation of antibiotic-tolerant taxa, including *Achromobacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Ochrobactrum*, and *Paenibacillus* to thrive and persist in the gut.

In the F2 generation, gut bacterial communities showed signs of partial recovery. This result showed by 50% of ASVs overlap between With_AB_F2 and No_AB_F2 (Figure 4b). The PCoA plots also showed that the F2 groups were closer together (Figure 5). *Enterococcus* returned partially to 30.76% (With_AB_F2) and 24.35% (No_AB_F2), whereas *Klebsiella* became prominent at 48.88% (No_AB_F2). These patterns suggest that the core bacteria can rapidly adapt and recolonize despite continued antibiotic exposure (Upfold et al., 2023). However, the Lab_Pop sample stood apart in the PCoA, highlighting its unique microbial composition (Figure 5). This likely reflects long-term adaptation to tetracycline in the artificial diet. The artificial diet was first developed in 2006 and has been used since then. According to Belda et al. (2011), the microbial community of *O. nubilalis* became less diverse during the rearing period with an artificial diet containing antiseptics compared to the original starter population from the field.

The shift in gut bacteria is linked to microorganisms' adaptability to changes in feed ingredients and the gut microenvironment. The reduction of *Enterococcus* as a core bacterium due to antibiotic exposure may increase susceptibility to pathogens or toxic compounds. In conjunction, some of the antibiotic-tolerant taxa were reported in higher abundance, which are *Achromobacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Ochrobactrum*, *Paenibacillus*, and *Acinetobacter* (Figure 5b). These bacteria are reported to have a role in detoxifying xenobiotic compounds. *Achromobacter* has been reported to break down and use xenobiotic substances to survive, as well as develop resistance to insecticides in its insect host (Jing et al., 2020; Wang et al., 2023). The exact roles of *Ochrobactrum* are not fully understood in the insect gut. However, according to Yadav et al. (2022), this bacterium can defend insects by producing a variety of enzymes. As Lee et al. (2017) reported, *Acinetobacter* has several antibiotic resistance pathways, such as enzymatic degradation, target alterations, efflux pumps, and permeability abnormalities.

In agreement, PICRUSt2 analysis predicted functional gene profiles from 16S rRNA data across *O. furnacalis*, with the highest activity observed in With_AB_F1. Among the key orthologs, K03088 (RNA polymerase sigma-70 factor, ECF subfamily, Prokaryotic type) showed the highest abundance, which is reported to regulate gene expression in response to specific

environmental cues, particularly under extracellular stress conditions. They often manage stress responses and adapt to changing conditions (L. Li et al., 2019). Additionally, several enriched KEGG orthologs were associated with the ABC transporter family, which showed relatively high abundance. ABC transporters are transmembrane proteins involved in the transport of lipids, inorganic ions, and especially xenobiotic degradation processes (Broehan et al., 2013). Genes related to this transporter have been reported to increase under stress conditions, such as insecticide exposure (Tang et al., 2022). These predicted functional genes declined in F₂ generation, coinciding with bacterial recovery, and were lowest in Lab_Pop following prolonged antibiotic exposure. However, PICRUST2 provides only descriptive predictions based on taxonomy, not direct functional evidence from metagenomics. Overall, these results highlight the potential for environmental antibiotic exposure to influence gut microbiome dynamics in *O. furnacalis*, providing a basis for future studies investigating the ecological and functional consequences of microbiome alteration in this important pest species.

V. Conclusion

This pilot study established descriptive patterns of antibiotic-induced gut bacteria dynamics in a field-collected and laboratory-adapted population of *O. furnacalis*. Antibiotic exposure triggered dysbiosis without fitness costs, followed by generational recovery, and distinct laboratory-adapted population composition. Field-collected population showed greater predicted xenobiotic metabolism potential than lab lines via PICRUST2 analysis. These foundational trends highlight microbiota responses in the tropical maize pest. Further investigation is necessary to fully understand the role of gut microbiota in this species. Additionally, more detailed studies on microbiota-pesticide interactions are essential for a comprehensive understanding and IPM applications.

Declarations

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Author Contributions

H. N. H. led the conceptualization, formal analysis, and writing of the original draft, and contributed equally to the review and editing of the manuscript. N. P. R. A. K. contributed to supervision, supported the conceptualization and original draft preparation, and participated equally in manuscript review and editing. Y. K. and W. contributed equally to the review and editing of the manuscript. Y. A. T. led the supervision, supported the conceptualization and original draft preparation, and contributed equally to the review and editing.

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Data Availability

All the sequences have been deposited in GenBank (accession numbers: Lab Control RNA-seq of insect gut, SRR31035788; Antibiotic RNA-seq of insect gut F1, SRR31035792; Antibiotic RNA-seq of insect gut F2, SRR31035791; No Antibiotic RNA-seq of insect gut F1, SRR31035790; No Antibiotic RNA-seq of insect gut F2, SRR31035789).

Competing interest: The authors declare no competing interest.

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Figures

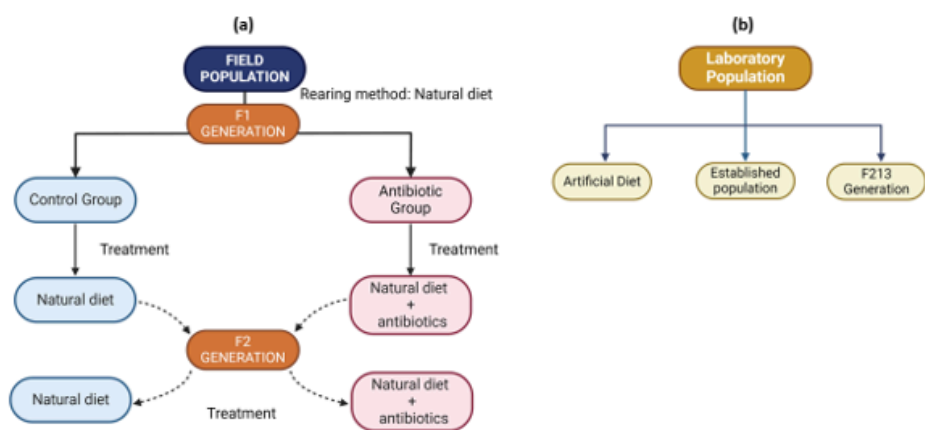


Figure 1

Experimental design of antibiotic treatment on field-collected and laboratory-adapted populations across generations, comparing natural and artificial diets. a) Field population b) Laboratory population.

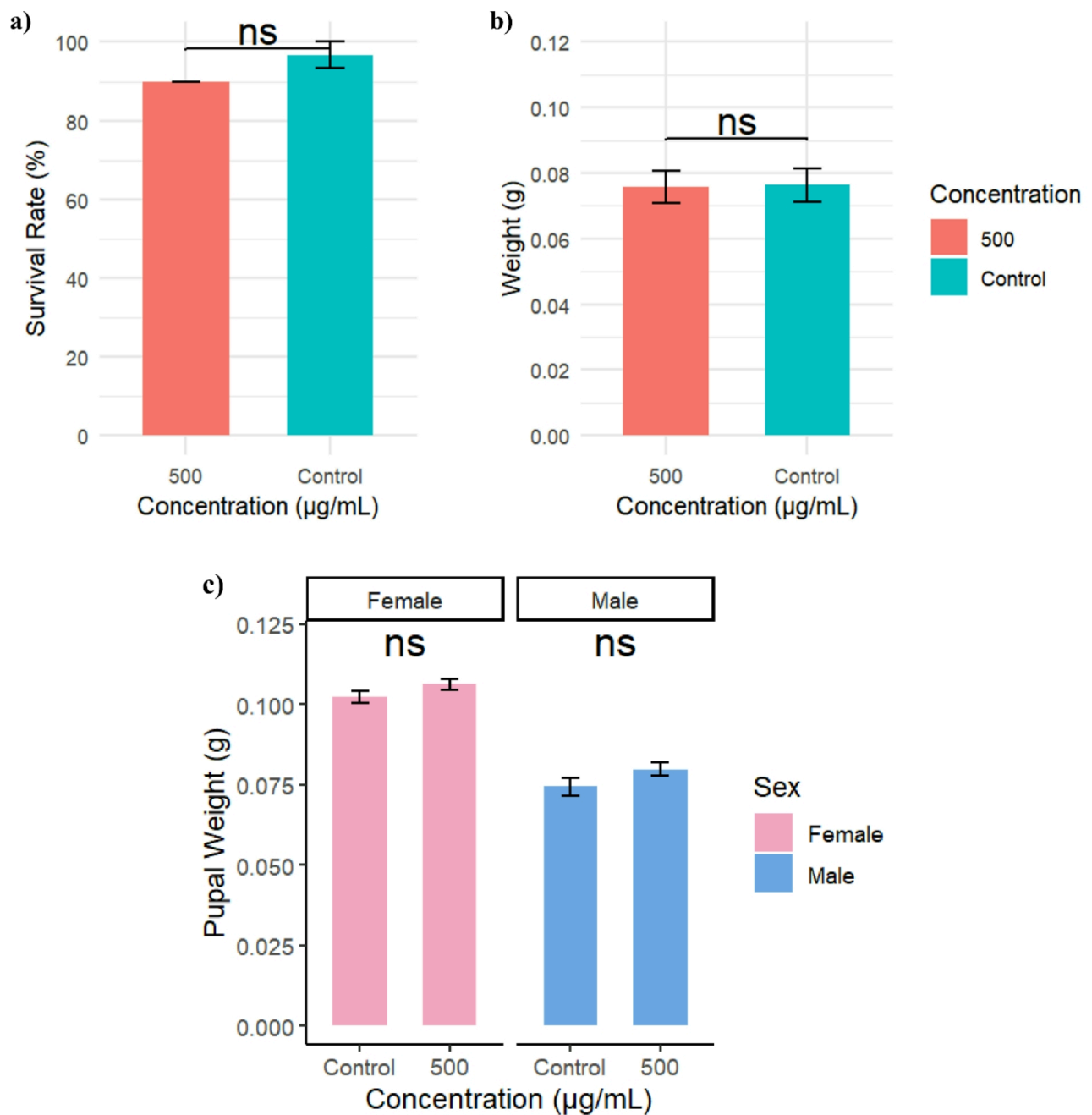


Figure 2

Effect of antibiotic feeding on the development of field-collected *Ostrinia furnacalis* in the F1 generation. (a) Larval survival (mortality), (b) fifth-instar larval weight, and (c) pupal weight (male and female shown separately). Bars represent mean $\pm$ standard error. No significant differences were observed between control and antibiotic-treated groups in any measured parameter (t-test, $p > 0.05$).

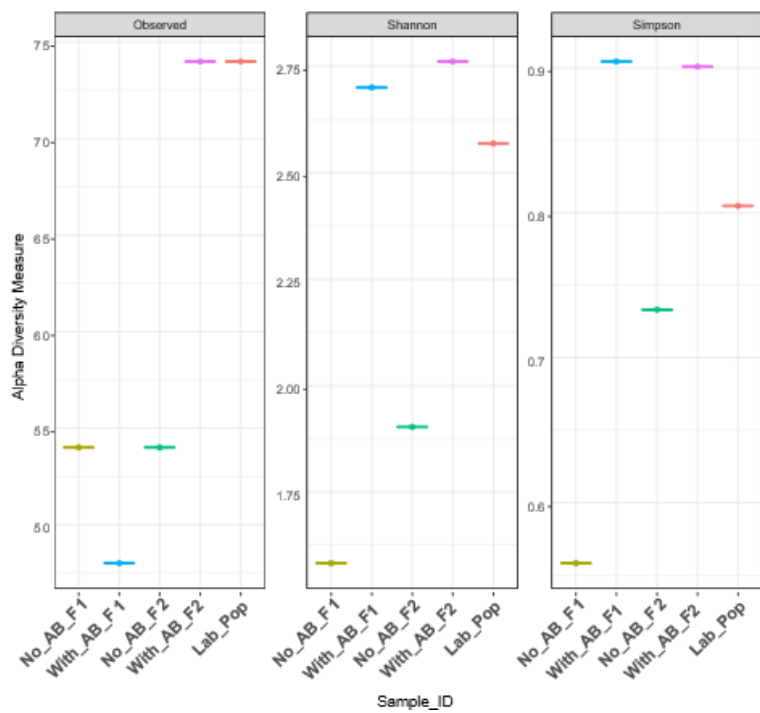


Figure 3

Alpha diversity analyses of the bacteria ASVs in the field-collected and laboratory-adapted population of *Ostrinia furnacalis*.

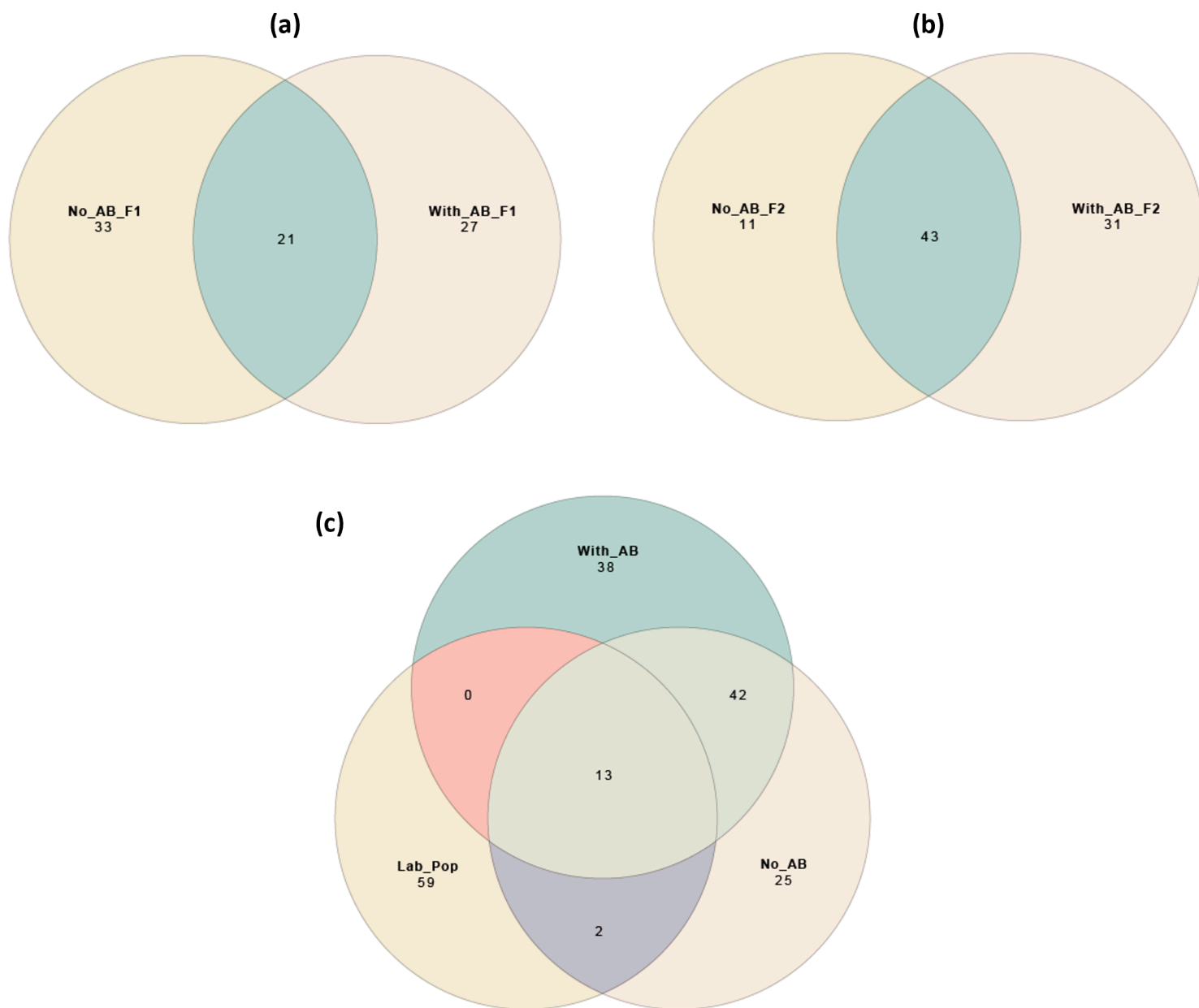


Figure 4

The Venn diagram of ASVs of the field-collected and laboratory-adapted population of *Ostrinia furnacalis*. a) The overlap of ASVs between the F₁ generation of antibiotic and control groups b) The overlap of ASVs between the F₂ generation of antibiotic and control groups c) The overlap of ASVs between the antibiotic, control, and laboratory groups. The overlapping circle shows the shared ASVs between treatments.

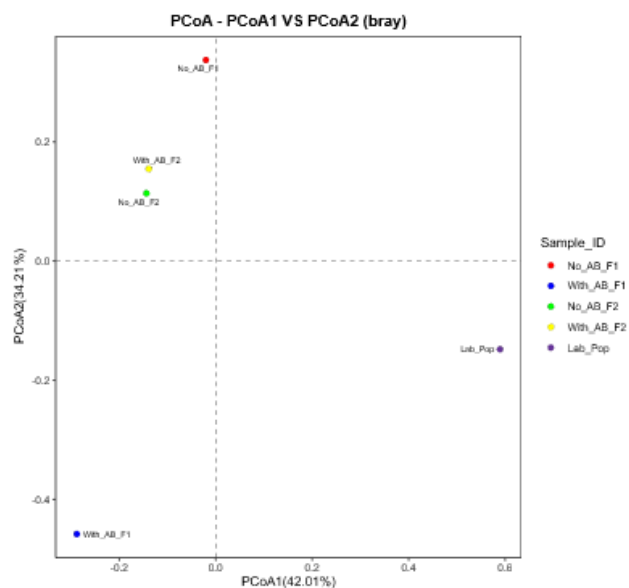


Figure 5

Analysis of Beta diversity with PCoA ordination based on Bray-Curtis distance for the bacterial community in the field-collected and laboratory-adapted population of *Ostrinia furnacalis*. The slight separation indicates high similarity, and the great separation indicates a high distinction in the composition of the gut bacteria.

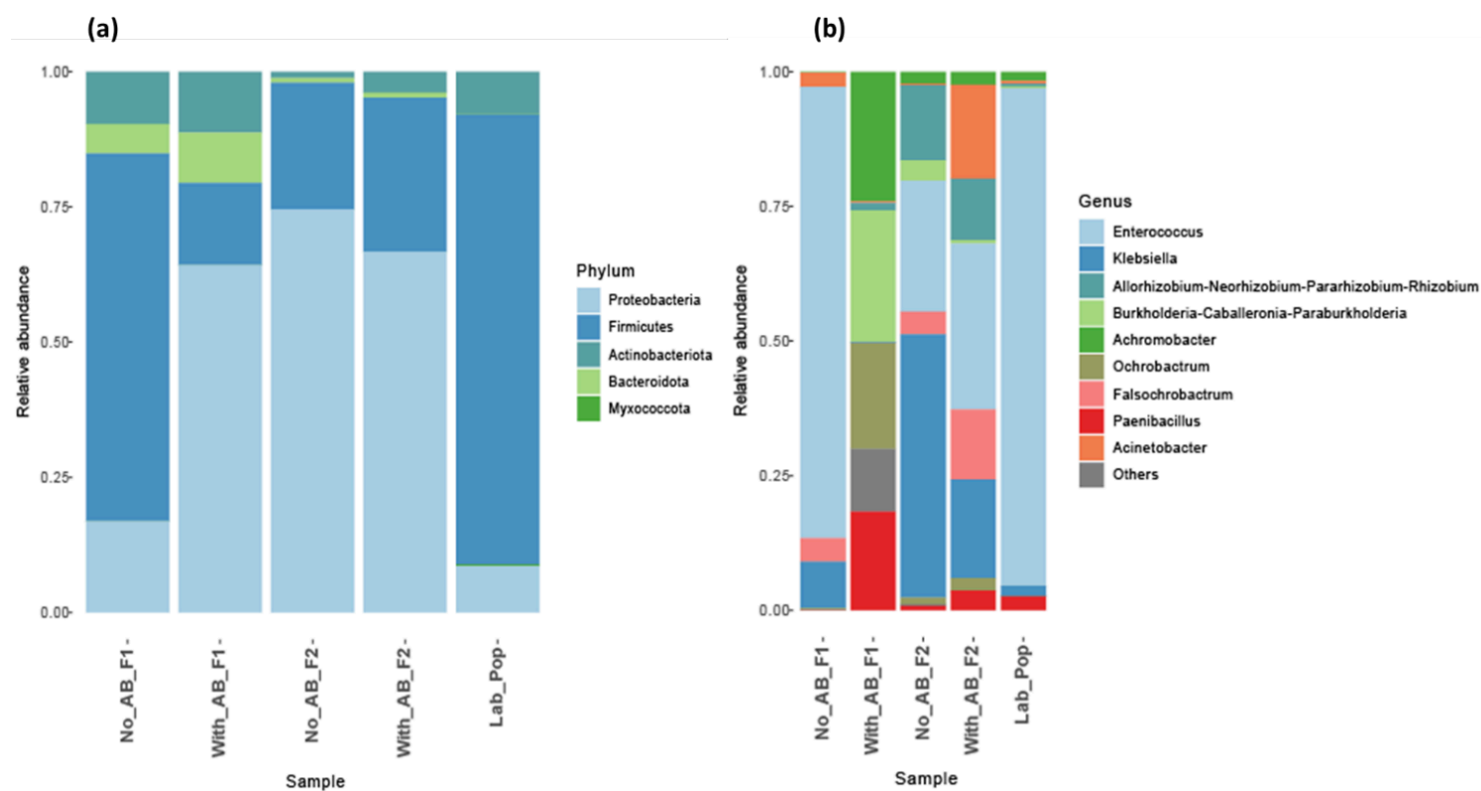


Figure 6

Taxonomic assignment of bacterial ASVs in the field-collected and laboratory-adapted population of *Ostrinia furnacalis*. Top 10 abundance of a) Phylum level; b) Genus level.



Figure 7

The hierarchical cluster heatmap shows the differential of the top 20 most abundant KEGG Orthologs in the antibiotic treatment group of *Ostrinia furnacalis* samples. The darker color gradient indicated more abundance.