

# Exploratory study on interaction between bacteria and fungi from mulberry to intestine of *Bombyx mori*

**Chengxu Li**

Nanjing Normal University School of Life Sciences

**Shuo Xu**

Nanjing University of Chinese Medicine

**Chunjie Xiang**

Nanjing University of Chinese Medicine

**Shixia Xu**

Nanjing Normal University School of Life Sciences

**Qihai Zhou**

Nanjing Normal University School of Life Sciences

**junfeng zhang** (✉ [zhangjunfeng419@njucm.edu.cn](mailto:zhangjunfeng419@njucm.edu.cn))

Nanjing University of Chinese Medicine <https://orcid.org/0000-0001-5132-3975>

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## Research Article

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# Abstract

Many kinds of antibiotics in the environments deeply affect the microbial composition, but their effect on the interactions among the host microbiota is still poorly understood. This study used *Bombyx mori* as the model organism by feeding the antibiotics-treated mulberry leaf, preliminarily explored the effects of antibiotic exposure on the interaction of bacteria and fungi in the worm's intestine. Our results showed that the elimination of fungi significantly reduced the bacterial richness and diversity in the worm's intestine after exposure of anti-fungal amphotericin B, while the elimination of bacteria dramatically increased the richness and diversity of fungi after exposure of anti-bacterial ampicillin-streptomycin. The results revealed the intestinal bacteria and fungi of *Bombyx mori* were markedly shaped by the mulberry leaf flora. Anti-bacterial antibiotics enhanced the correlation between the host gut fungi and the diet-deriving fungi, while anti-fungal antibiotics weakened the correlation between the host gut bacteria and the diet-deriving bacteria. The data preliminarily established a simple model to explore the effect of antibiotics on the host microbiota interactions, and provided a way for further study on the environmental factors of intestinal bacteria-fungi interaction.

## Introduction

In recent years, antibiotics have been frequently found in the environments due to its extensive use (Liu et al. 2020), such as soil (Sun et al. 2017), lakes and rivers (Sta Ana et al. 2021). Antibiotics residues in the environment can alter the microbial structure (Bacanli et al. 2019) and cause bacteria to develop resistance (Wu et al. 2019). More worryingly, a review proposed that antibiotics residues in the environment could enter the human intestine through the food chain, and the original balance of microflora was disturbed not only in the environment but also in the host's gut (Zhu 2016). But until now, we still do not know how antibiotics affect the interaction of intestinal bacteria and fungi in the host intestine.

Microorganisms exist widely in nature with high richness and diversity, and their interactions are conducive to realize a variety of functions, which plays an important role in maintaining the stability of the ecosystem (Hao and Zhang 2016). In recent years, research on microbial interaction has attracted much attention. Study has found that the cooperative relationship between different bacteria in the human gut (Rakoff-Nahoum et al. 2016). Besides, some researchers investigated the rhizosphere microbial community of *Arabidopsis thaliana*, and found a negative correlation between bacteria and fungi (Durán et al. 2018), but others found the correlations between the core endophytic bacteria and fungi in *Oxytropis glacialis* were mainly synergistic (Xu et al. 2020). These inconsistent results may be explicated by the different hosts. The factors affecting microbial interaction and their underlying mechanism remain to be poorly understood.

Intestinal microorganism is the largest and most complex microecosystem, affecting a variety of host activities including digestion, immune response and pathogen defense (Biesebeke et al. 2004; Ke et al. 2017; Cifuentes et al. 2020), and has long been a hotspot in the field of microecology. Host genetics, dietary habits and environmental factors profoundly influence and shape the community structure of gut microbiome (Muegge et al. 2011; Benson et al. 2010). As regarding to the abiotic factors, temperature is the most crucial factor among environmental factors. Du et al. (2016) used *Bombyx mori* to investigate the influence of high temperature (34°C) on the intestinal microbial community, and observed the altered microbial community, the

most noteworthy point was that the dominant flora changed from *Sphingomonas* and *Pseudomonas aeruginosa* to *Clostridium* and *Lactococcus*. On the other hand, bacteria and fungi are the main biological factors and active participants in the formation of microecology. The relationship between them includes commensalism, amensalism, mutualism, and competition (Gao et al. 2018). Will the change of bacteria or fungi have an impact on the other party? How does this influence shape and alter the composition and function of microecological communities? Studies have shown that most of the intestinal flora of caterpillars comes from diet (Jones et al. 2019) and it can be speculated that food is the key factor affecting the host intestinal flora. There are great challenges in solving these problems because of the too complex interaction among diet, gut bacteria and fungi.

*Bombyx mori* is a kind of lepidopteran insect that feeds on mulberry leaves. Its diet is very simple, and is an ideal species to study the interaction between environment and host microecology (Zhejiang Agricultural University 1995). This study constructed a simple food chain, and mulberry leaves were pretreated with antibiotics to observe the changes in the relationship between intestinal flora of *Bombyx mori* and mulberry leaf flora caused by the loss of intestinal bacteria or fungi, which provided a theoretical basis for exploring the interaction of intestinal bacteria-fungi.

## Materials And Methods

### Sample collection

The silkworm species were "Suchao 2", which were incubated in the laboratory at 37°C in the dark incubator. They were divided into 4 groups and fed from hatching to fifth instar larvae. Firstly, all the fresh mulberry leaves were rinsed by sterile deionized water. Secondly, the fresh mulberry leaves were soaked in the 10  $\mu\text{g mL}^{-1}$  amphotericin B solution (Sigma products, CAS1397-89-3), double antibiotics containing 400 U  $\text{mL}^{-1}$  penicillin and 400  $\mu\text{g mL}^{-1}$  streptomycin solution (TransGen products, FG1010-01) for 10 min, respectively. Leave mulberry leaves to dry naturally for feeding the worms according to the previous literature (Li et al. 2016). Finally, the control group was fed the fresh mulberry leaves, the amphotericin group was fed the amphotericin B treated leaves to delete the gut fungi, the antibiotic group was fed the double antibiotics treated leaves to delete the gut bacteria. Food should be kept adequate throughout the process.

### Total DNA extraction, PCR amplification and sequence determination

Total DNA were extracted from 6 mulberry leaves and ~ 300 mg feces produced by each *Bombyx mori*, and 1% agarose gel electrophoresis was used to control the quality of DNA. Using the extracted DNA as template and amplified by polymerase chain reaction (PCR). The V3-V4 region of the bacterial 16S rRNA genes was performed using forward primer 341F (5'-CCTAYGGGRBGCASCAG-3') and reverse primer 806R (5'-GGACTACNNGGGTATCTAAT-3'); the ITS1-ITS2 region of the fungal 18S rRNA genes was amplified using the forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and reverse primer ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Xu et al. 2021). The PCR was performed in a 20  $\mu\text{L}$  mixture containing 10 ng DNA extract, 0.8  $\mu\text{L}$  of each primer (5  $\mu\text{mol L}^{-1}$ ), 0.4  $\mu\text{L}$  FastPfu Polymerase, 4  $\mu\text{L}$  5×FastPfu Buffer, 2  $\mu\text{L}$  dNTPs (2.5  $\text{mmol L}^{-1}$ ), replenish to 20  $\mu\text{L}$  with ddH<sub>2</sub>O. All samples were amplified on an ABI GeneAmp 9700 (ABI, USA) using the following parameters: initial denaturation at 95°C for 5 min, 27 cycles of denaturation at

95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s, and an extension at 72°C for 10 min, then store at 10°C for use (Xu et al. 2019). After we purified and quantified the products of amplification, high-throughput sequencing was performed by Illumina company.

### Statistical analysis of data

The raw data was optimized by removing the low-quality sequences using the QIIME (version 1.17) software, and chimeric sequences were identified and deleted using the UCHIME algorithm (Patil et al. 2021). The refined sequences were clustered to the operational taxonomic units (OTUs) by 97% similarity using UPARSE (Version 7.1), and the OTUs was assigned according to the SILVA database with 70% confidence threshold (Pavlovska et al. 2021). Alpha and Beta diversity were analyzed based on the original OTUs abundance. After homogenization of OTUs, relative abundance, community composition, linear discriminant analysis of the effect size (LEfSe), linear discriminant analysis (LDA) and Venn analysis were carried out. In addition, SPSS software (Version 21) was used to conduct the non-parametric test and Spearman correlation analysis. Visualization of the microbial symbiotic was realized by Cytoscape software.

## Results

### Alpha diversity

When the silkworms grew to the fifth instar larvae by feeding the antibiotics-treated mulberry leaves, PCR amplification and agarose electrophoresis detection results showed that the negative amplification rate of 18S rRNA gene ITS1-ITS2 region of silk-worm feces was 85% (17/20) in the mulberry leaves treated with amphotericin. Six fecal samples with negative 18S rRNA gene ITS1-ITS2 PCR amplification were selected for V3-V4 region amplification of 16S rRNA, and gut bacteria were profiled by high-throughput sequencing. As regarding to the silkworms feeding by penicillin-streptomycin treated mulberry leaves, the negative rate of V3-V4 region of 16S rRNA gene in silkworm feces was 90% (18/20), and six fecal samples with negative 16S rRNA PCR amplification were selected for 18S rRNA gene ITS1-ITS2 region amplification and gut fungal high-throughput sequencing. At the same time, 6 mulberry leaves and 6 fecal samples of the control group were selected for both 16S rRNA gene V3-V4 region and 18S rRNA gene ITS1-ITS2 region amplification simultaneously. Ultimately, average  $37830 \pm 6702$  16S rRNA gene sequences and  $44136 \pm 9361$  18S ITS1-ITS2 gene sequences were obtained from each sample, and 607 bacterial OTUs and 533 fungal OTUs were obtained according to 97% similarity.

Alpha diversity analysis of the flora was conducted by the original OTUs distribution (**Table.1**). For bacteria, compared with the mulberry leaf group, the richness index (Ace, Chao, Observed OTUs) and diversity index (Shannon) of silkworm intestinal bacteria in the control group were significantly increased ( $P < 0.05$ ), but the intestinal bacterial richness and diversity index of silkworm which fed mulberry leaves treated with  $10 \mu\text{g mL}^{-1}$  amphotericin were observably decreased, indicating that the bacterial richness and diversity of silkworm were markedly reduced by the loss of intestinal fungi. For fungi, compared with the mulberry leaf group, the richness and diversity index of silkworm intestinal fungi in the control group were signally decreased ( $P < 0.05$ ), and there was no conspicuous change in the richness index of silkworm in-testinal fungi after feeding mulberry leaves treated with  $400 \text{ U mL}^{-1}$  penicillin and  $400 \mu\text{g mL}^{-1}$  streptomycin ( $P < 0.05$ ), but the fungal

diversity index was remarkably increased ( $P < 0.05$ ), indicating that antibiotic treatment could improve the diversity of intestinal fungi of silkworm.

**Table.1** Alpha diversity analysis of intestinal flora of *Bombyx mori*

		Ace	Chao	Shannon	Simpson	Observed OTUs
Bacteria	Control ( $n = 6$ )	178.2 ± 139.9	179.8 ± 139.5	2.533 ± 1.429	0.194 ± 0.101	168.2 ± 144.6
	Amphotericin ( $n = 6$ )	115.5 ± 10.9*	115.5 ± 12.4	1.845 ± 0.125*	0.256 ± 0.031*	105.4 ± 10.4*
	Mulberry Leaf ( $n = 6$ )	123.3 ± 18.8*	124.1 ± 21.0	1.740 ± 0.079*	0.277 ± 0.030*	105.4 ± 20.2*
	$H(P)$	1.434(0.488)	1.647(0.439)	6.313(0.043)	6.918(0.031)	0.261(0.878)
Fungi	Control ( $n = 6$ )	95.5 ± 18.9	83.5 ± 30.3	2.598 ± 0.950	0.211 ± 0.262	75.0 ± 30.5
	Ampicillin + Streptomycin ( $n = 6$ )	100.3 ± 57.8	97.5 ± 61.4	3.173 ± 0.629 $\Delta$	0.089 ± 0.050 $\Delta$	90.3 ± 56.3
	Mulberry Leaf ( $n = 6$ )	253.2 ± 53.6 $\Delta$	251.6 ± 55.1 $\Delta$	3.497 ± 0.254 $\Delta$	0.073 ± 0.031 $\Delta$	212.8 ± 39.9 $\Delta$
	$H(P)$	9.018(0.011)	9.029(0.011)	3.71(0.156)	2.946(0.229)	8.404(0.015)
* For bacteria, compared with control group, $P < 0.05$ . $\Delta$ For fungi, compared with control group, $P < 0.05$ .						

### Beta diversity analysis

Principal component analysis (PCA) is to simplify the original complex data by dimensionality reduction, so as to facilitate analysis. The similarity of sample composition was reflected as the distance between each point on the two-dimensional coordinate diagram (Deng and Zhang 2018). The results showed that the bacterial and fungal community structure of mulberry leaf were significantly different from silkworm gut. After feeding the mulberry leaves treated with amphotericin or antibiotics, the intestinal bacterial or fungal community structure of silkworm changed notably (Fig. 1). In terms of bacteria, the distance between the control group and the mulberry leaf group was far, suggesting that the similarity of bacterial community between mulberry leaf and silkworm gut was low. After feeding amphotericin-treated mulberry leaves, the bacterial community structure of silkworm gut was obviously changed, indicating that the loss of intestinal fungi observably affected the bacterial community structure (Fig. 1A). On the other hand, the similarity of fungal community between mulberry leaf and silkworm gut was not high also, and the intestinal fungal community structure of silkworm changed markedly after feeding mulberry leaves treated with antibiotics. Antibiotic group was close to the control group and located in the same quadrant, indicating that the fungal community structure of them was similar, and the loss of bacteria mainly resulted in the difference in PC1 direction between the two groups (Fig. 1B).

## Analysis of flora structure

The community structures of the bacteria and fungi were analyzed based on the homogenized OTUs distribution. A total of 20 known bacterial phyla were detected, among which the relative abundances of three dominant bacterial phyla (*Firmicutes*, *Cyanobacteria*, and *Proteobacteria*) accounted for 90–96%. The relative abundances of four known fungi phyla (*Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Mucoromycota*) ranked from 81–99%.

At genus level, 8 dominant bacterial genera were found: *Bacillus*, *Chloroplast-norank*, *Lactococcus*, *Carnobacterium*, *Mitochondria-norank*, *Streptococcus*, *Exiguobacterium*, and *Enterococcus*. Compared with the mulberry leaf group, the relative abundance of silkworm gut bacteria in the control group was apparently different. After feeding the mulberry leaves treated with 10  $\mu\text{g mL}^{-1}$  amphotericin, the relative abundance of silkworm gut bacteria had no evident change (Fig. 2A), indicating that the loss of fungi had little effect on it. The dominant fungal genera were *Cladosporium*, *Nigrospora*, *Tausonia*, *Aureobasidium*, *Penicillium*, et al. Compared with the mulberry leaf group, the relative abundance of silkworm gut fungi in the control group was significantly different too. After feeding the mulberry leaves treated with 400 U  $\text{mL}^{-1}$  penicillin and 400  $\mu\text{g mL}^{-1}$  streptomycin, the relative abundance of each fungal genus obviously changed (Fig. 2B), indicating that the antibiotic treatment had a great effect on the relative abundance of intestinal fungi genera of silkworm.

Among the 154 bacterial genera, Venn analysis results presented 56 shared bacterial genera (Fig. 2C), such as *Bacillus* (15.92%), *chloroplast-norank* (15.70%), *Carnobacterium* (15.53%) and *Lactococcus* (15.26%). Among the 221 fungal genera, here observed 38 shared fungal genera (Fig. 2D), including *Trichosporon*, *Trichomerium*, *Didymella* and *Ceratocystis*.

## LDA discriminant analysis of flora

LDA was used to compare two or more LDA groups and to identify the potential markers (Ma et al. 2019). The histogram below showed the LDA scores of prominent genera in each group at the OTU level (Fig. 3). Seven bacterial genera, a bacterial taxonomic group and 18 fungal genera with significantly high abundance were found in the mulberry leaf group; 12 bacterial genera and 4 fungal genera were found in the control group, which have extremely high abundance. Multiple bacterial genera were also enriched in the amphotericin group, such as *Aerococcus*, belonging to the order *Lactobacillales*, phylum *Firmicutes*. In the antibiotic group, markedly abundant fungal genera included *Aspergillus*, belonging to the order *Eurotiales*, phylum *Ascomycota*. To sum up, we think amphotericin or antibiotic treatment can affect the composition of bacterial or fungal genera in the intestine of silkworm.

## Analysis of symbiotic relationship of flora

Among the shared 56 bacterial genera (Fig. 2C) and 38 fungal genera (Fig. 2D), bacteria and fungi genera with average relative abundance greater than 0.1% were considered as the core flora, and 37 bacterial genera and 21 fungal genera were arranged to conduct symbiotic network. In general, the changing tendency of the bacteria was homologous between the mulberry leaf (Fig. 4A) and the control group (Fig. 4B). After the silkworms were exposed to amphotericin B, the tendency of the bacteria in silkworm intestine did not change significantly, while the tendency of the fungi was notably changed after the exposure to penicillin and

streptomycin (Fig. 4C). Based on the flora of mulberry leaf, the status of the dominant bacteria in the silkworm gut was more closed related to the mulberry leaf bacteria than that of the dominant fungi, however, the bacterial intercept became lower (Fig. 4D) while the fungal intercept became higher after the exposure of antibiotics (Fig. 4E). The results suggested that intestinal bacteria and fungi may come from mulberry leaf, the deletion of gut fungi weaken the correlation between gut bacteria and diet-deriving bacteria, but the deletion of bacteria strengthen the correlation between gut bacteria and diet-deriving fungi. In a word, exposure of dietary antibiotics exerted different influence on the host's gut flora.

Finally, based on core genera, the symbiotic networks were constructed (Fig. 5). In the mulberry leaves, multiple bacteria with higher abundance showed significantly positive or negative correlation in aggregation, such as the first 8 bacteria genera with higher relative abundance (*Bacillus*, *Lactococcus*, *Carnobacterium*, *Streptococcus*, *Exiguobacterium*, *Enterococcus*, *Pseudomonas*, and *Paenibacillus*). These 8 bacterial genera were negatively correlated with 5 fungal genera (*Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, and *Moesziomyces*) which have higher relative abundance (Fig. 5A). In the gut of silkworm, the above 8 bacterial genera were positively correlated with 9 genera such as *Geobacillus*, and the correlation coefficient was slightly reduced (Fig. 5B). The results indicated that the bacterial symbiosis changed signally in different habitats (mulberry leaf and silkworm gut), but the fungal symbiosis had no obvious rule. In the gut of silkworms which fed mulberry leaves treating with amphotericin B, the bacteria with high abundance showed apparently positive correlation, such as the population of positively correlated bacteria expanded dramatically, including 17 genera (*Bacillus*, *Lactococcus*, *Carnobacterium*, *Streptococcus*, *Exiguobacterium*, *Enterococcus*, *Pseudomonas*, *Paenibacillus*, *Geobacillus*, *Acinetobacter*, *Brochothrix*, *Leuconostoc*, *Fusobacterium*, *Cronobacter*, *Enhydrobacter*, *Enterobacter*, and *Lysinibacillus*), and the total relative abundance reached more than 47% (Fig. 5C). In the gut of silkworms which fed mulberry leaves treating with antibiotics (penicillin and streptomycin), the number of pairs of fungi with significantly positive correlation increased from 2 to 7 (such as *Golubevia* and *Periconia*), while the number of pairs of fungi with dramatically negative correlation increased from 3 to 5, suggesting that the loss of bacteria in the gut of silkworm resulted in the abnormal proliferation of fungi (Fig. 5D).

## Discussion

There is a continuous interaction between microorganisms in the human intestinal tract, an extremely complex ecosystem (Hevia et al. 2015). In the foreseeable future, the interaction among the intestinal microorganisms and host will still be a hotspot (Erin et al. 2021). Thus, the silkworm with the simplest diet was collected as a model to overcome the complexity of diet-driving intestinal microbial interaction in the mammals besides human. The present study used 16S rRNA and 18S rRNA ITS1-ITS2 gene amplicon sequencing to explore the community structure and interaction of bacteria and fungi in mulberry leaf and silkworm intestine. The preliminary results showed that the loss of bacteria had a significant impact on the fungi and vice versa. And the exposure of antibiotics to inhibit or kill gut microbes could significantly increase the correlation between silkworm's gut microbiota and the corresponding microbiota from its food (mulberry leaf), as well as markedly affected the interaction between bacteria and fungi in the intestine.

To further study the difference of microbiota in diverse habitats, LDA based on the genus level was designed to reveal the key biomarker microbiota. The abundances of many bacteria and fungi genera were distinctly

increased in the mulberry leaf, while 12 bacterial genera and 4 fungal genera were markedly increased in the gut of silkworm, including 2 dominant bacterial genera (*Bacillus* and *Enterobacter*) identified by Yuan et al. (2006). We can speculate that the intestinal microbial system of silkworm changed during the process of feeding mulberry leaves. Additionally, the abundances of several bacterial and fungal genera were significantly increased after the exposure of the antibiotics, and the antibiotics could break the balance between bacteria and fungi, indicating that exposure of the antibiotics cause the massive proliferation of opportunistic pathogens such as *Aerococcus* and *Aspergillus*.

The hypothesis that the diet-driving shape of gut microbiota has been gradually confirmed by mounting evidence (Claesson et al. 2012; Johnson et al. 2019), and diet-driving alteration of the gut microbiota have been suspected to be responsible for the increasing prevalence of chronic diseases such as obesity and inflammatory bowel disease (David et al. 2014). For example, our latest epidemical literatures showed that the risk of gastric cancer was significantly related to the bacteria but not the fungi in case of matching the lifestyle, the phenomena was observed both in the oral and fecal samples, which suggested that lifestyle played a key role in driving the alteration of gut microbiota, especially the fungi (Wu et al. 2020; Xu et al. 2021). An animal investigation also showed that the gut mycobiome of healthy mice was shaped by the environment and correlated with metabolic outcomes in response to diet (Mims et al. 2021). As well as known, the interactions between microorganisms includes parasitism, commensalism and mutualism, many evidences have confirmed that the mutualism of bacteria-fungi plays a major role in promoting and maintaining the plant's health and performance (Guo and Narisawa 2018), suggesting that the symbiotic state of bacteria-fungi in the environment exert important impact on host health and disease. Our data showed that the bacterial and fungal abundances in mulberry leaf were significantly different from that in the silkworm intestine. Interestingly, exposure of antibiotics obviously shrunk the differences of microbiota in the silkworm's gut and its food, especially the positively correlated fungi were observed between silkworm intestine and mulberry leaf after the exposure of antibacterial penicillin-streptomycin, suggesting that antibiotic exposure could promote the direct correlation between host's gut microbiota and the feeding diet.

Since the industrial revolution, many kinds of antimicrobial agents are broadly used for bacteriostasis or sterilization as a natural or artificial synthetic chemical substance, and play an essential role in medical treatment and significantly promote the human life span (Wang 2014). However, the abuse of antimicrobial agents especially the antibiotic directly or indirectly causes a series of emerging human health problems (Huang 2021). Gudda et al. (2020) conducted a risk assessment of antibiotic contamination of vegetables on human intestinal microbiota, pointing out that the presence of dietary antibiotics may put stress on the intestinal microbiota, disturb the balance of gut microbiota, and finally promote the abnormal proliferation of the opportunistic and pathogenic bacteria. In our study, the diversity, abundances, and symbiosis network of bacteria or fungi in the gut of silkworm were all significantly disturbed after the exposure of antibiotics.

Up to now, there are still few studies on dietary antibiotics, this study used silkworm as a model to reveal the preliminary rule of interaction between the exposure of dietary antibiotics and the intestinal bacteria-fungi. This rule is that the exposure of dietary antibiotics significantly promotes the direct correlation between the food-derived microbiota and the host gut microbiota especially the fungi, which bring the important clue for us to pay more attention on the food antibiotic residues and to regulate the social behavior of antibiotic application.

## Declarations

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## Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Author Contributions

Chengxu Li performed the data analysis and wrote the manuscript. Shuo Xu and Chunjie Xiang conducted the experiment and collected samples. Shixia Xu suggested the constructive comments of the study. Qihai Zhou designed the study and performed the data analysis. Junfeng Zhang designed and supervised the study, reviewed the draft. All authors read and approved the final manuscript.

## Data Availability Statement

The datasets presented in this study has been deposited in Entrez (<https://www.ncbi.nlm.nih.gov/sra/PRJNA769808>), the Submission ID is SUB10504772.

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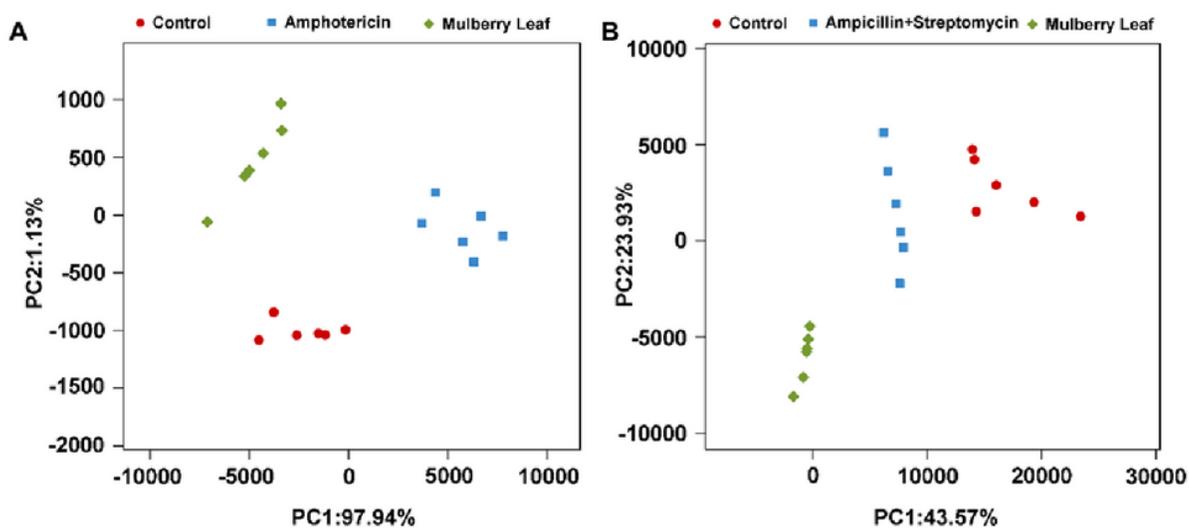
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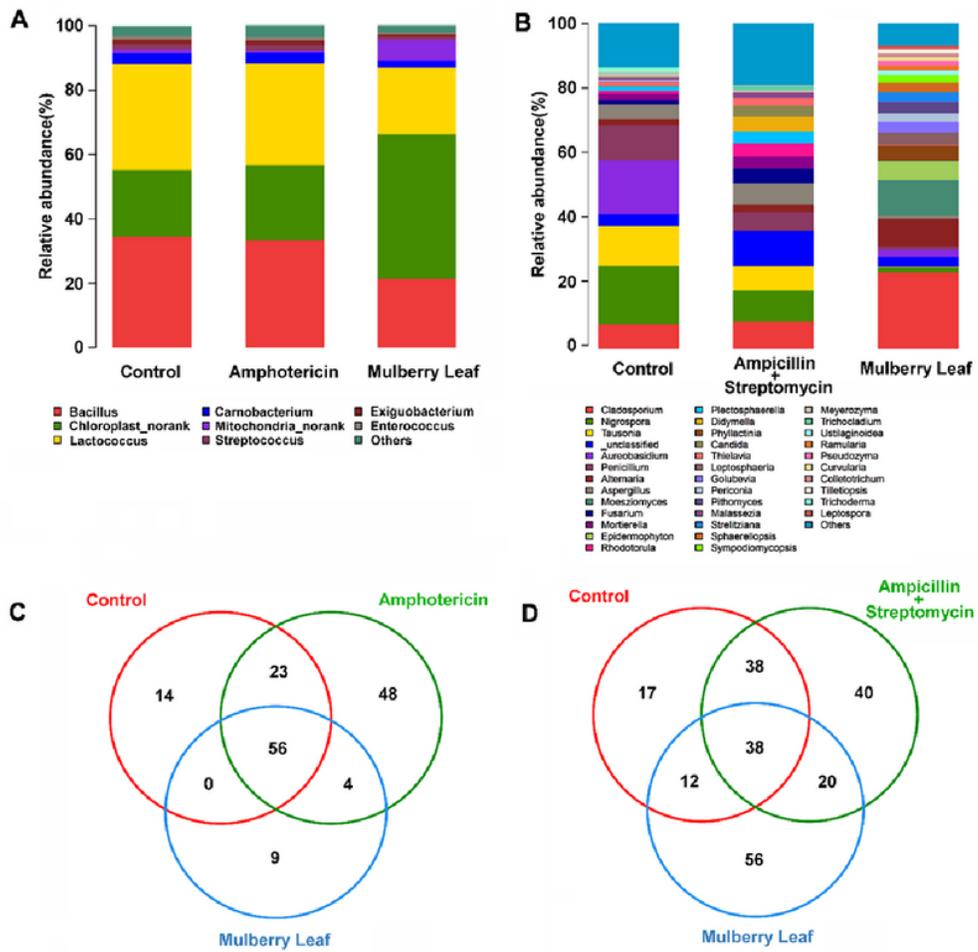
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## Figures



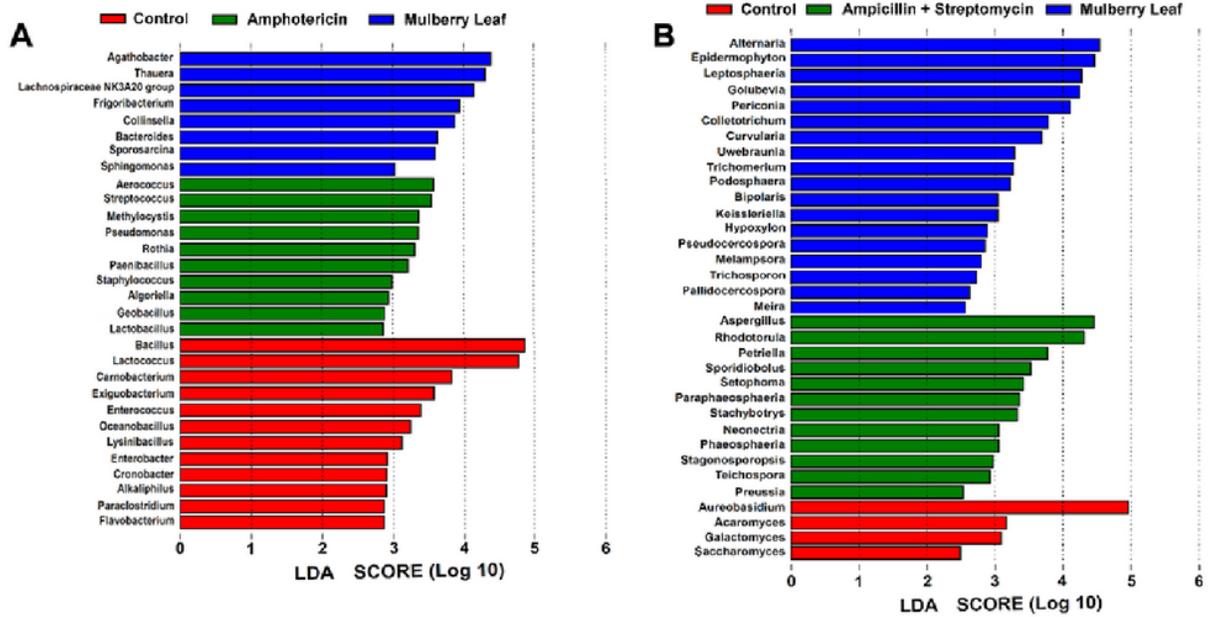
**Figure 1**

Effects of antibiotic treatment on intestinal flora of *Bombyx mori*. The bacteria were obviously different after the exposure of antifungal amphotericin B (A), and the fungi were also significantly different after the exposure of antibacterial ampicillin and streptomycin (B).



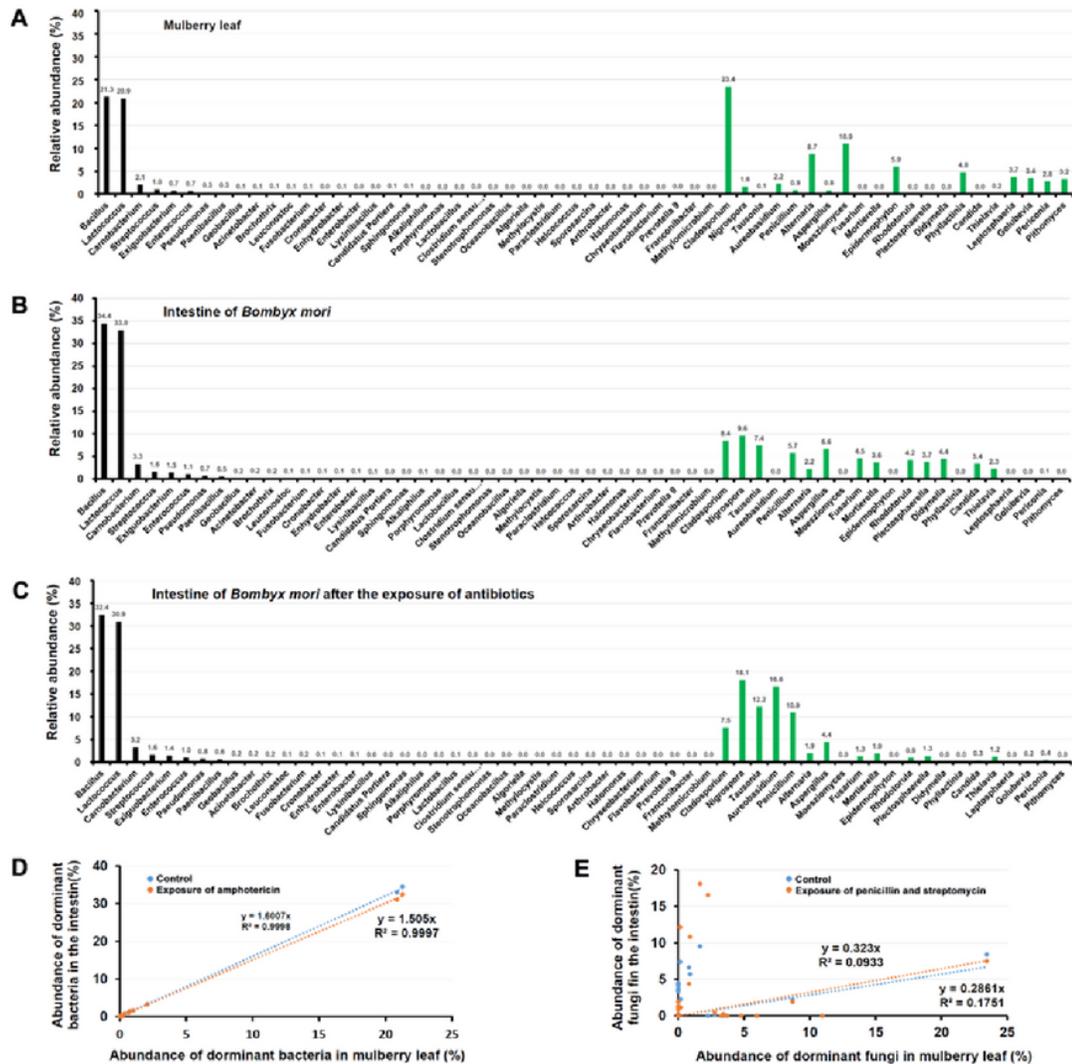
**Figure 2**

Genus-level analysis of the microbial community structure Here presented the genus-level structure of the bacteria treated by antifungal Amphotericin (A), and genus-level structure of the fungi treated by antibacterial Ampicillin and Streptomycin (B). The Venn Diagram showed the shared bacterial genera (C) and fungal genera (D).



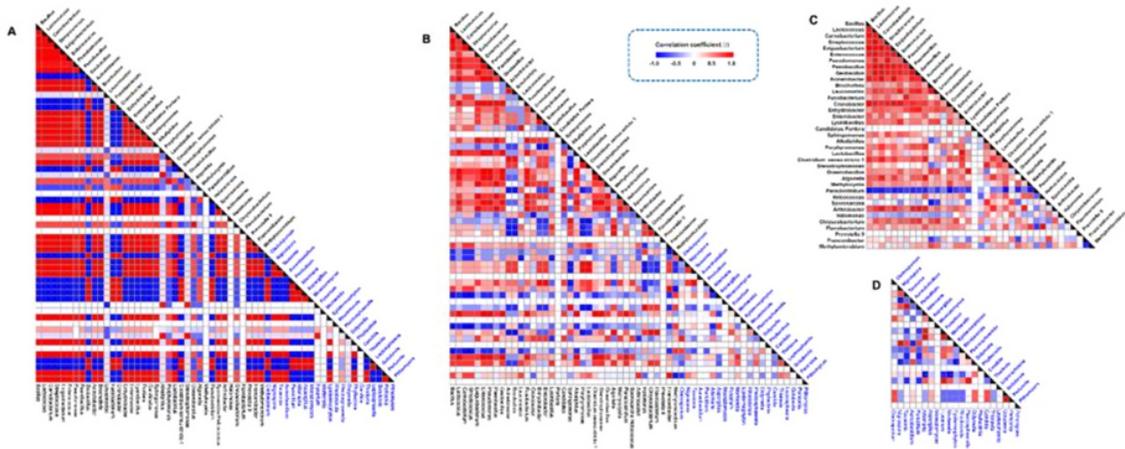
**Figure 3**

Enrichment of the potential marker flora related to the antibiotic treatment LDA resulted 30 enriched bacterial genera (A) and the 34 fungal genera (B) among the three groups.



**Figure 4**

Altered tendency of the core bacteria and fungi in silkworm gut Thirty-seven bacterial genera and 21 fungal genera were presented in the mulberry leaf (A) and the silkworm’s intestine (B). After exposure of antibiotics, the tendency of remaining bacteria (black) and fungi (green) in the silkworm intestine was distinct (C). Based on the flora of mulberry leaf, the bacterial intercept became lower after the exposure of amphotericin B (D), while the fungal intercept became higher after the exposure of ampicillin and streptomycin (E).



**Figure 5**

Analysis of symbiotic relationship of flora in different habitats (A) The symbiotic network in the mulberry leaves. (B) The symbiotic network in the silkworm feces of control group. (C) The symbiotic network in the silkworm feces of amphotericin group. (D) The symbiotic network in the silkworm feces of antibiotic group. Red represents significantly positive correlations; blue represents significantly negative correlation. \* The absolute value of correlation coefficient is greater than 0.85. The black font is bacteria and the blue font is fungi.