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The Effect of Antibiotic Treatment on the Bacterial Community of the Brown Planthopper and Its Correlation with Rice Virulence

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Abstract: The prevention and control of planthoppers represent important issues for rice production. Current long-term control methods rely on pesticides, which raise concerns about environmental pollution. Recently, evidence has suggested that bacterial symbionts are important factors influencing the formation of Hemiptera insect biotypes and the selection of host plants for insects, which suggesting that targeting bacterial communities may be an effective alternative method for planthopper control. In this study, we perturbed the bacterial communities of the brown planthopper, *Nilaparvata lugens*, by feeding antibiotic-treated rice and used RNA-seq to examine the transcriptome of normal rice fed with perturbed BPHs by RNA-seq. Our results showed that the composition of the bacterial communities significantly changed after the perturbation, which was accompanied by changes in distinct biological processes of rice, especially the phenylpropanoid biosynthesis pathway, compared with the effect of the BPH feeding on rice without bacterial communities perturbation. Our work establishes a protocol for bacterial communities perturbation in BPH, demonstrating the link between bacterial community and the responses to BPH feeding and providing new insights into the interaction between BPH and rice.

Keywords: brown planthopper; bacterial community; rice; antibiotic treatment

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1. Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae), is a destructive phloem sap pest that costs heavy yield losses worldwide [1]. Outbreaks of BPH lead to "hopperburn" damage, resulting in an average direct yield loss of 1.19 million tons of rice fields in 2006–2015 [2]. There are typically two types of damage caused by BPH: direct and indirect damage. Direct damage is caused by BPH sucking on the leaf sheath and blades of the rice, which results in yellow leaves and decreases the rate of heading sprouting and seed setting. This kind of damage can also block the transportation of the photosynthesis product to the root, affecting the normal growth and physiological activities of rice [3]. When the damage becomes severe, the lower part of the rice turns black, and the rice wilts in pieces, resulting in a reduction in the harvest [1]. Indirect damage is caused by the spread of rice diseases by BPH. In some places, the diseases spread by BPH have caused even more severe losses than direct damage [4].

Due to the demand for rice, as one of the world's major food sources, there is an urgent need for pest control of BPH to reduce this loss of rice yield. Currently, the most widely used method is the frequent application of broad-spectrum insecticides; however, this leads to serious side effects, such as killing the natural enemies of BPH, threatening food safety and the environment because of the toxicity of the insecticide residue and being quickly overcome by BPH due to evolved resistances to insecticides [5]. Alternative

methods aiming at long-term control of BPHs include the use of a banker plant system [6] and transgenic plants [7,8]. The banker plant system tends to create an environment that attracts the natural predators of the pests to fight against the infestation. Although it is preventative and has little effect on the ecosystem, a banker plant requires extra cost and effort to manage. Using transgenic plants with resistant genes, on the other hand, is an efficient and cost-effective method. At present, 40 resistance genes, such as *Bph3*, *Bph6*, *Bph9*, *Bph14*, *Bph15*, *Bph18*, *Bph26*, *Bph29*, and *Bph32*, have been characterized in rice [9–18], which shows great promise for transgenic plants as an effective pest control method. However, BPH is still able to adapt and overcome the resistant rice after a few generations due to the rapid evolution of the new BPH biotype [19]. The biotypes of BPH show significant virulence differences among rice varieties. In general, four BPH biotypes are known for rice [20]. Biotype 1 can survive on and damage only those varieties that do not carry any resistance genes. Biotype 2 can survive on varieties carrying *Bph1* resistance gene and those susceptible to biotype 1. In addition, biotype 3 can survive on varieties having *bph2* resistance gene and those susceptible to biotype 1, respectively. This calls for further investigations into the molecular mechanisms of rice resistance and BPH adaptations.

The symbiotic association is a well-known phenomenon in insects. The symbionts play vital roles in growth, development, and reproduction [21]. The BPH harbors various microorganisms, including fungal symbionts and bacterial communities dispersed in the gut, hemolymph, fat body cells, eggs, and honeydew [22–24]. Recent studies showed that these microorganisms play essential roles in BPH survival and behavior. In addition, yeast-like symbionts (YLS) and *Arsenophonus nilaparvatae* in the fat body of BPH could supply complementary functions of steroid synthesis, nitrogen storage and recycling, essential amino acid synthesis, and vitamin synthesis [25–27]. Importantly, studies have shown that changes in YLS and bacterial communities might be one of the main reasons for the rapid adaptation of the BPH to resistant transgenic rice varieties [28–33]. Therefore, knowledge of the bacterial community is essential to fully understand the coevolution of BPH and rice, which will contribute to the advancement of long-term, eco-friendly, and cost-effective pest-control methods of BPH.

Recent studies showed that YLS and bacterial communities of BPH were changed during the selection and adaptation of BPH to different resistant rice varieties [19,29–31]. However, these studies seeking associations between the microorganisms and BPH virulence had only been described from two or three BPH colonies, each reared on a diverse host variety. In addition, this design did not consider the microorganism's variability between different BPH colonies and microorganisms' responses to host feeding [34].

In this study, we established a protocol for the perturbation of bacterial communities, and we used this perturbation model to investigate the possible changes in rice after being fed by BPHs with different bacterial communities.

2. Materials and Methods

2.1. Acquisition of Biological Samples

The rice (*Oryza sativa* L.) used in this study was Taichung Native 1 (TN1), planted in a field in Guizhou province, China, and harvested in October every year. The BPH (biotype 1 colony) used in this study was kindly provided by Prof. Guangcun He (College of Life Sciences, Wuhan University). The BPH colony was collected at the Genetics Institute, Wuhan University in 2016.

2.2. Cultivation of Rice and Rearing of BPH

BPHs were reared on TN1 seedlings for maintaining the colony in School of Life Science, Xiamen University. The rice seeds were planted in basins of soil (obtained from a local rice field) for a month before being moved to a gauze net and incubated with BPH at 28–30 °C, L/D 16:8 (Figure S1).

2.3. Perturbation of Bacterial Communities of BPH

After husked, rice seeds were treated with 0.15% mercuric chloride for 20 min and washed six times with sterilized water. The TN1 seeds were then cultured on 1/2 MS culture

medium in test tubes (one seed per tube) at 25 °C, L/D 16:8 in an illumination incubator until the four-leaf stage. Rice seedlings were then placed in height 10 cm plastic cups (one seedling per cup) with 1/2 MS culture solution and 100 μg/mL antibiotic (rifampicin) for 48 h, before being fed on by BPH for 96 h (25 nymphs at the 5th instar per seedling) (Figure 1). The choice of the rifampicin-treatment time was based on our preliminary experiments, which showed that the rice seedlings could survive after 7 days of the 100 μg/mL rifampicin treatment, but for only about 3 days if the concentration of rifampicin increased to 200 μg/mL. With <100 μg/mL rifampicin treatment, the rice seedlings could survive for a longer time; however, the BPHs would still fall off the seedlings and die after 5 days, even though the rifampicin's concentration was <100 μg/mL. Therefore, to ensure sufficient time for the BPHs to feed on the rifampicin-treated rice and that BPHs absorbed a large amount of rifampicin. Consequently, we chose 48 h of rifampicin treatment for rice seedlings and then 96 h for BPHs feed on the rifampicin-treated rice seedlings.

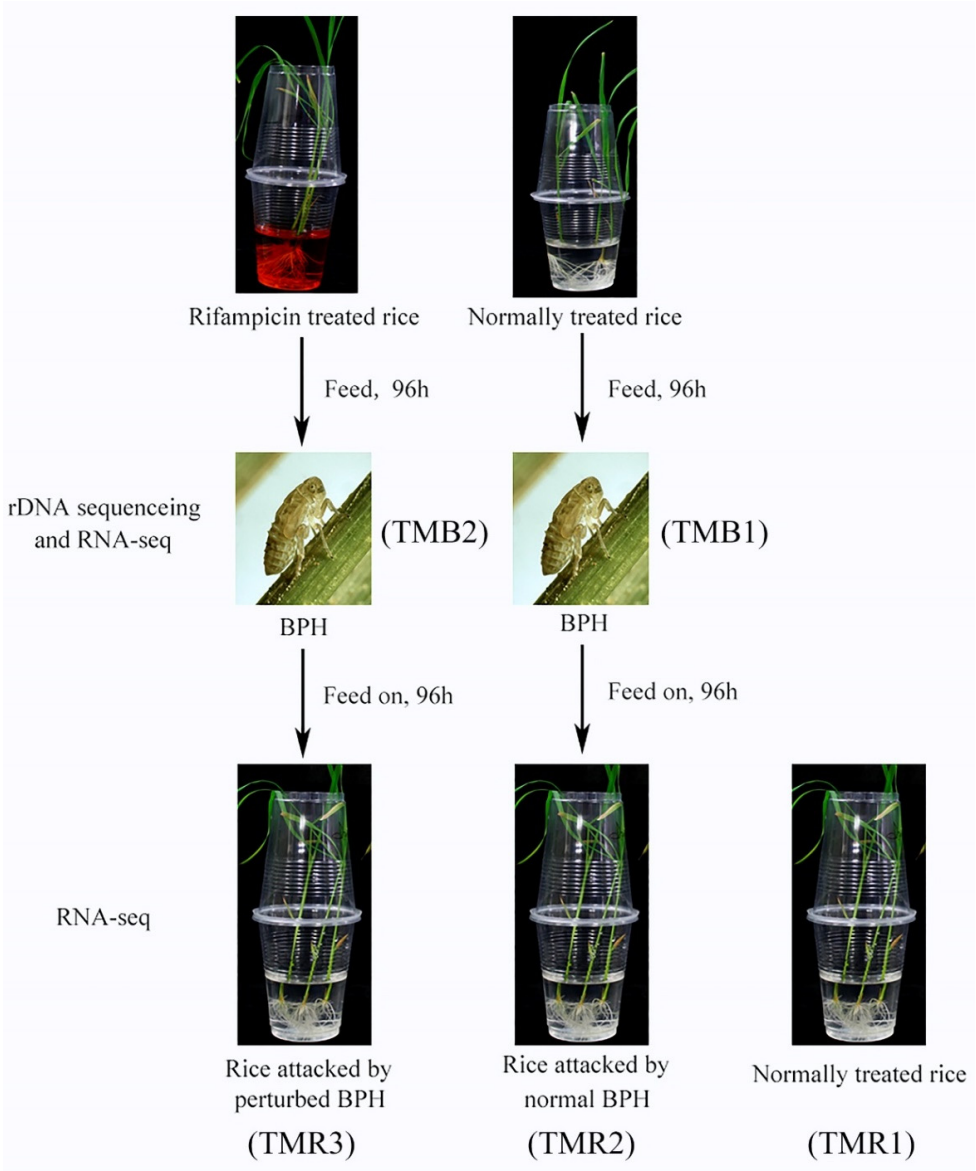

Figure 1. Perturbation of bacterial communities of BPH. Rice was treated with/without rifampicin before BPH feeding. The rifampicin will change the composition of the bacterial communities of BPH, which consequently affects BPH transcriptome. In turn, the change in BPH will also affect the rice being fed by BPH. To examine the effect of the changes of bacterial communities, we sequenced the 16S rRNA of the bacterial communities to confirm the perturbation result and used RNA-seq to assess the transcriptome of BPH and rice. In parentheses are the sample names of RNA-seq sequencing.

2.4. 16S rRNA Sequencing of Bacterial Communities of Treated/Untreated BPH

BPHs (20 nymphs for one pool, repeated three times) fed with rifampicin-untreated rice for 96 has a negative control (named BPHTN1) and BPHs (20 nymphs for one pool, repeated three times) fed with rifampicin-treated rice for 96 h (named BPHRIF) were collected, respectively. To exclude the potential noise caused by microorganisms on the surface of BPH, BPHs were treated with 5% NaClO for 2 min, then washed 5–6 times with sterilized water [29]. Then, 200 µL of the water from the last wash was transfered onto an LA plate at 37 °C overnight. This sterilization process was repeated until there were no colonies formed on the LA plate after incubation. We then extracted DNA of the whole bodies of BPH using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. The V3 + V4 region of the 16S rRNA was amplified by PCR using the following primers: 338F: 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′. The PCR reaction system included: 10 µM primers, 0.2 mM dNTPs, 0.25 µM MgCl2, 1 x PCR reaction buffer, and 3 units of Taq PCR polymerase. The PCR program was set to 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 50 s, then 72 °C for 10 min. The amplified DNA was sent to Biomarker Technology (Beijing, China, http://www.biomarker.com.cn/, accessed on 15 November 2021) for 16S rRNA high-throughput sequencing library preparation.

2.5. RNA-Seq of BPH Feeding on Rifampicin-Treated and Untreated Rice

BPHs (20 nymphs for one pool, repeated three times) were fed with rifampicin-untreated rice for 96 h as a negative control (named TMB1) and BPHs (20 nymphs for one pool, repeated three times) were fed with rifampicin-treated rice for 96 h named TMB2 before being collected in a 1.5 mL EP tube. According to the manufacturer's instructions, RNA was extracted using the Eastep® Super Total RNA Extraction Kit (Promega, Shanghai, China). The RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). The RNA-seq library construction and sequencing were performed by Novogene technology (Tianjin, China, http://www.novogene.com/, accessed on 15 November 2021).

2.6. RNA-Seq of Rice Fed on by Treated/Untreated BPH

Rice seedlings were cultured with 1/2 MS culture solution as a negative control (one seedling of each, named TMR1). Rice seedlings fed by rifampicin untreated BPH named TMR2; Rice seedlings fed by rifampicin treated BPH named TMR3, all the samples were repeated 3 times. The leaf sheaths of the rice seedlings fed by rifampin treated/untreated BPH were collected, and the RNA was extracted using the Eastep® Super Total RNA Extraction Kit and RNA-seq library construction, and sequencing was performed by Novogene technology.

2.7. Analysis of 16S rRNA Sequencing Data

Pair-end sequencing raw reads were trimmed and filtered, then assembled to tags. Paired-end reads were merged using FLASH (v 1.2.7) [35]. Quality filtering on the raw tags was performed to obtain the high-quality clean tags using Trimmomatic [36]. Chimera sequences were removed using UCHIME [37], and the effective tags were obtained finally. Sequences with 97% similarity were assigned to the same operational taxonomic units (OTUs). Representative sequences for each OTU were screened for further annotation. Annotation of the unique tags was performed using RDP classifier (v 2.2) based on 16S rRNA classification database SilvaS (Release 119) [38,39]. Downstream analysis, including the Rarefaction curve, Shannon curve, α-diversity, chao1, ACE, the Shannon index, and Simpson index, etc., were carried out by Mothur (v 1.34.0) [40]. In addition, Whittaker's index was used to analyze the β-diversity [41].

2.8. Analysis of BPH RNA-Seq Data

Reads were trimmed and filtered, then aligned to a genome reference of BPH (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/014/356/525/GCF_014356525.1_ASM1435652v1/, accessed on 15 November 2021) using Tophat2 [42]. Alternative splicing was predicted by Cufflinks [43]. New transcripts were annotated by aligning to COG, KEGG, Swiss and GO. FPKM was used to quantify the expression levels of genes, and differentially expressed genes were selected by Log2FC $\geq$1. The processed data can be obtained by accession number PRJNA763742.

2.9. Analysis of Rice RNA-Seq Data

Reads were trimmed and filtered, then aligned to a rice genome reference of the indica variety 9311 (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/004/655/GCA_000004655.2_ASM465v1/, accessed on 15 November 2021) using Tophat2 [42]. Alternative splicing was predicted by Cufflinks [43]. New transcripts were annotated by aligning to COG, KEGG, Swiss and GO. FPKM was used to quantify the expression levels of genes, and differentially expressed genes were selected by Log2FC $\geq$ 1. The processed data can be obtained by accession number PRJNA763799.

3. Results

3.1. Change in Bacterial Communities after Feeding Antibiotic-Treated Rice

Because it is challenging to feed antibiotics directly to the BPH, we perturbed the bacterial communities by providing the BPH antibiotic-treated rice to explore the possible role of the bacterial communities of BPH (Figure 1). Rice was treated with/without rifampicin in the 1/2 MS culture solution for 48 h, then BPHs were fed on the rice sheath. During the feeding of BPH on rice, rifampicin was ingested by the BPHs, changing the composition of the bacterial communities. High-throughput sequencing methods were applied to investigate bacterial communities of BPH (by 16S rRNA sequencing). To further investigate the effects of rifampicin-treated/untreated BPH and rice response to rifampicin-treated/untreated BPH, high-throughput sequencing methods were applied for the investigation (by RNA-seq).

16S rRNA paired-end sequencing of the bacterial communities yielded 853,242 raw reads in total, and 694,774 clean tags after assembly and filtering. In total, we identified 146 OTUs, with 139 in perturbed BPHs and 134 in normal condition BPHs.

The Venn diagram presented in Figure 2A shows that there are 127 (87.0% of total) shared bacterial communities between BPH before and after treatment, although 19 (13% of total) bacterial communities are uniquely found in either of the two (Figure 2A). The alpha-diversity index shows that the biodiversity of bacterial communities increased after feeding BPH rifampicin-treated rice (Table S1). The annotation of each OTU in SilvaS (Release 119) generated the classifications of the OTUs on different levels. At the phylum level, the most abundant bacteria communities are Bacteroides, Proteobacteria, Actinobacteria, and Cyanobacteria, among which Proteobacteria is the most abundant one, followed by Bacteroides. Compared with BPHTN1, the rifampicin treatment led to a decrease in the relative abundance of Bacteroides, while there was an increase in Actinobacteria and Cyanobacteria (Figure 2B). At the genus level, in BPHTN1, the most relatively abundant genus was *Wolbachia*, followed by *Cardinium* and *Arsenophnus*, while in BPHRIF, *Arsenophnus* was the most abundant one, and *Pantoea* increased significantly, while *Wolbachia* and *Cardinium* decreased (Figure 2B). These results clearly show that our treatment of BPH with rifampicin-treated rice changed the bacterial communities of BPH.

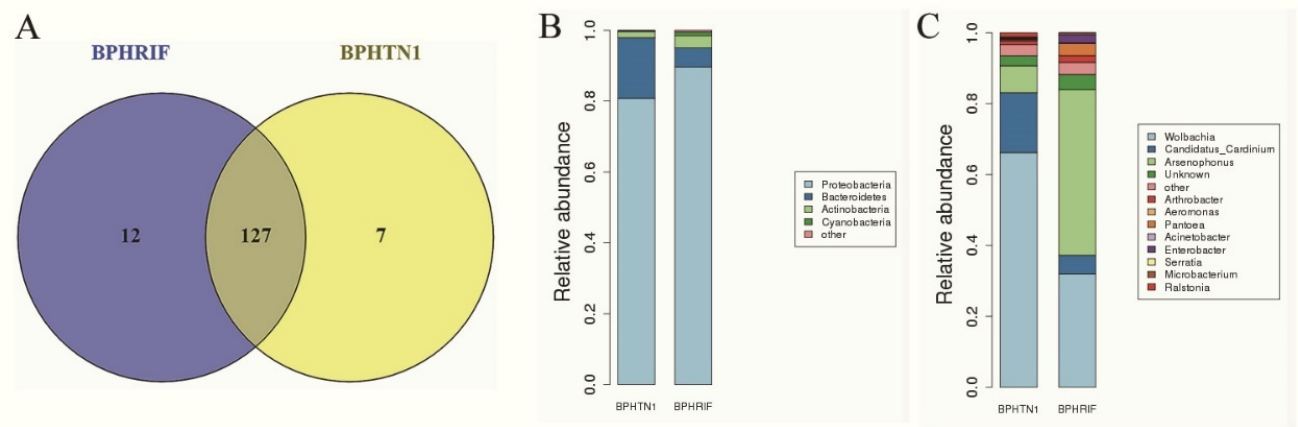

Figure 2. The changes of bacterial communities of BPH. (**A**) Venn diagram shows the similarities and differences of the bacterial communities of BPH feed on the rice treated with/without rifampicin. (**B**) Relative abundance of the bacterial communities on phylum level. (**C**) Relative abundance of the bacterial communities on genus level. Note: the abbreviation BPHRIF and BPHTN1 in (**A**–**C**) indicated the sample names of 16S rRNA sequencing; the ordinate indicates the relative abundance percentage; one color represents a species, the color block length represents the relative abundance ratio of the species; the part with abundance less than 1% is merged into Others, Unknown OTUs that are not taxonomically annotated.

3.2. Change in BP Transcriptome after Feeding Rifampicin-Treated Rice

Next, we examined the effect of the bacterial communities of BPH changes on BPH and rice. RNA from BPHs fed rifampicin-treated/untreated rice was extracted and sequenced, producing 39.79 Gb of data via Illumina Hiseq3000 paired-end sequencing. In total, 42.99–47.54 million raw reads were obtained from each sample (Table 1). Prior to mapping, low-quality and adapter reads were filtered, and 42.05–46.68 million clean reads from each sample were retained (Table 1). All samples had Q30 values greater than 93.67%, and the GC content ranged from 43.55% to 44.56% (Table 1).

Table 1. Statistical summary for all RNA-seq samples.

Samples	Raw Reads	Clean Reads	GC Content	% ≥ Q30
TMB1-1	42,986,218	42,049,682	44.56	93.91
TMB1-2	44,165,642	43,209,258	44.54	93.76
TMB1-3	47,541,582	46,680,550	43.72	93.73
TMB2-1	45,485,624	44,656,850	43.55	93.67
TMB2-2	44,521,806	43,586,560	44.12	93.79
TMB2-3	46,011,104	45,052,222	44.25	93.77
TMR1-1	45,073,386	44,379,244	54.97	93.56
TMR1-2	45,142,932	44,365,444	54.39	93.26
TMR1-3	45,295,128	44,536,280	53.79	93.21
TMR2-1	45,913,532	44,847,806	52.56	93.00
TMR2-2	82,922,232	81,738,152	46.86	93.02
TMR2-3	80,85,2316	79,583,178	49.48	93.75
TMR3-1	81,61,2046	80,658,880	48.45	93.50
TMR3-2	81,958,948	80,806,938	47.85	93.22
TMR3-3	86,133,980	85,114,310	49.46	93.70

Differential expression genes (DEGs) analysis showed 530 significantly changed genes, among which 183 genes (34.5%) were up-regulated and 347 genes (65.5%) were down-regulated. GO (gene ontology) analysis of these DEGs were enriched in the following biological processes: the oxidation-reduction process, protein targeting to mitochondrion and the de novo IMP biosynthetic process, and the molecular function of structural constituents of ribosomes. The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis showed that these DEGs were enriched in oxidative phosphorylation and protein

processing in the endoplasmic reticulum (Figure 3A), which are consistent with the results of GO analysis. To confirm the expression changes in the oxidative phosphorylation pathway, we selected three annotated genes (*LOC111044521*, *LOC111058946*, and *LOC120352526*) and performed quantitative real-time PCR (qRT-PCR) in BPH samples (TMB1 and TMB2). The qRT-PCR results (Figure 3B) showed up-regulation of TMB2 compared with TMB1, which is consistent with the transcriptome results.

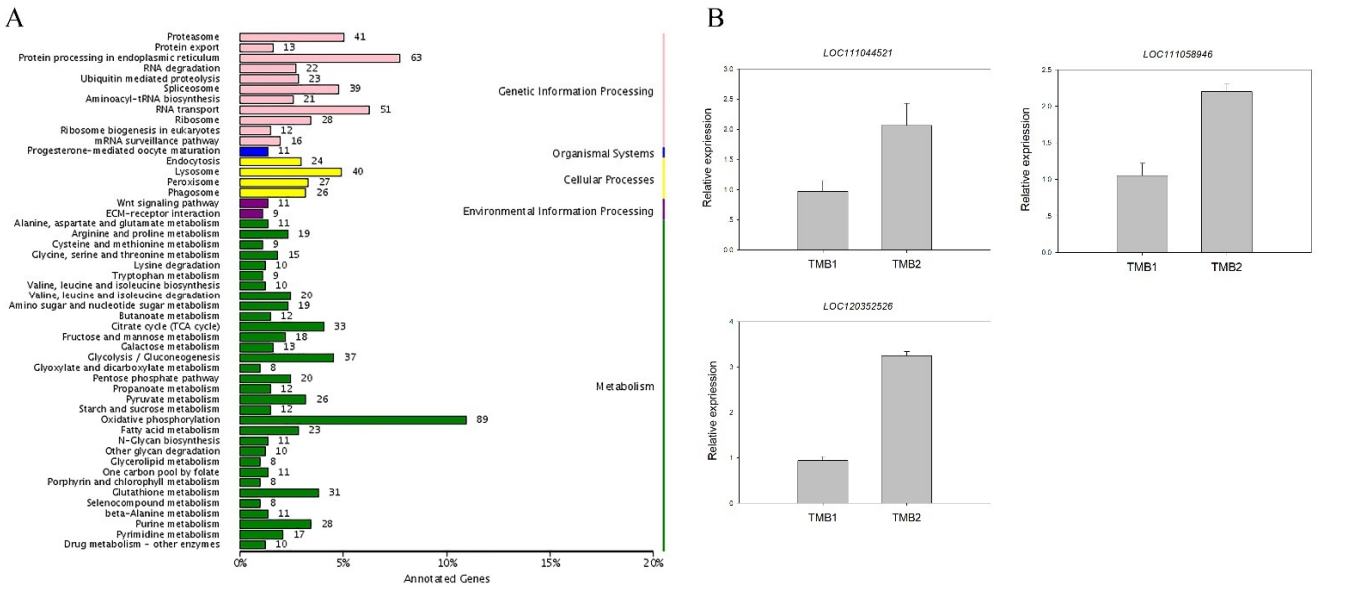

Figure 3. The changes of transcriptome of BPH with/without rifampicin treatment. (**A**) KEGG pathway enrichment analysis of the DEGs comparing BPHs feeding on the rice with/without rifampicin treatment. The abscissa indicates the ratio of the number of genes annotated to the pathway and the number of genes on the annotation, the ordinate indicates the name of the KEGG metabolic pathway. (**B**) Quantitative real-time PCR validation of the expression changes in the three genes enriched in the oxidative phosphorylation pathway. The error bars were S.D.

3.3. Change in the Transcriptome of Rice after Being Fed by Perturbed BPH

We predicted that the changes in the bacterial communities and the BPHs after being fed with rifampicin-treated rice would ultimately induce different responses in rice sheathes fed by the BPH. To distinguish the effects of the bacterial communities change from the effects of feeding, we sequenced the mRNA of rice sheathes fed by rifampicin-treated BPH, untreated BPH, and rice sheathes that had not been fed. After trimming and quality control, the remaining clean data resulted in 87.91 Gb. In total, 45.07–86.13 million raw reads were obtained from each sample (Table 1). Prior to mapping, low-quality and adapter reads were filtered, and 44.36–85.11 million clean reads from each sample were retained (Table 1). All samples had Q30 values greater than 93.00%, and the GC content ranged from 46.86% to 54.97% (Table 1).

DEGs analysis was performed by comparing the FPKM of each gene in different samples. The genes with Log2FC $\geq$1 in two different samples were considered as DEGs, which yielded 8494, 12,489, and 4599 DEGs from the comparisons between sample TMR1 (normally grown rice sheath) vs. TMR2 (sheath of rice fed on by normally grown BPHs), TMR1 vs. TMR3 (sheath of rice fed on by rifampicin-treated BPHs), and TMR2 vs. TMR3, respectively. This indicated that, although BPHs fed on rice sheathes caused significant responses, the perturbation of the bacterial communities of BPH also affected the responses of the rice. Among these DEGs, 3835, 5698, and 2468 genes were up-regulated, and 4659, 7151, and 2131 genes were down-regulated, respectively. These results suggest that the mechanism of rice response to BPHs differed based on the presence of different combinations of the bacterial communities. GO analysis showed distinct enriched GO terms of TMR2 vs. TMR3 from TMR1 vs. TMR2 and TMR1 vs. TMR3. These indicated that

the changes in the bacterial communities caused different rice responses in the biological processes (Table 2).

Table 2. Significantly enriched GO terms of the DEGs generated from different pair-wise comparisons.

GO Category	TMR1 vs. TMR2	TMR1 vs. TMR3	TMR2 vs. TMR3
Biological Process	methylerythritol 4-phosphate pathway pentose-phosphate shunt photosystem II assembly	methylerythritol 4-phosphate pathway pentose-phosphate shunt photosystem II assembly	oxidation-reduction process tRNA methylation lipid metabolic process regulation of transcription, DNA-templated basipetal auxin transport (1->3)-β-D-glucan biosynthetic process
Molecular Function	chlorophyll binding	chlorophyll binding	chromatin binding peroxidase activity ATP binding

Next, we investigated the distribution of the DEGs of TMR2 vs. TMR3 in the KEGG pathways. Among the pathways with the top percentage of DEGs, the phenylpropanoid biosynthesis changed most significantly in ranking, from the third (TMR1 vs. TMR2) and the sixth (TMR1 vs. TMR3) to the first (TMR2 vs. TMR3) (Figure 4A and Figure S2). Since the total number of DEGs generated by comparing TMR2 vs. TMR3 was much less than TMR1 vs. TMR3 and TMR1 vs. TMR2, the enrichment of the DEGs in phenylpropanoid biosynthesis suggested that this pathway might be the pathway in rice that is most affected by the change in the bacterial communities s of BPH.

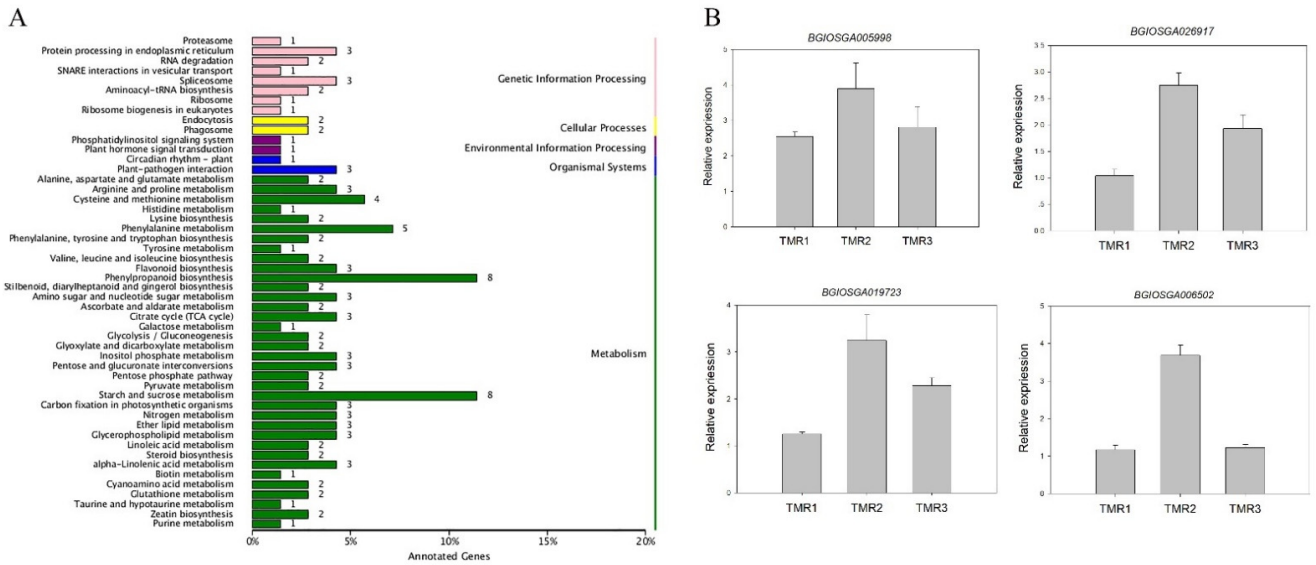

Figure 4. The changes of rice transcriptome fed by BPHs with/without rifampicin treatment. (**A**) KEGG pathway enrichment analysis of the DEGs comparing the rice fed by BPH with/without rifampicin treatment. (**B**) Quantitative real-time PCR validation of the expression changes in the four genes enriched in the phenylpropanoid biosynthesis pathway. The error bars were S.D.

To confirm the expression changes in the phenylpropanoid biosynthesis pathway, we selected four annotated genes (*BGIOSGA005998*, *BGIOSGA006502*, *BGIOSGA019723* and *BGIOSGA026917*) and performed quantitative real-time PCR (qRT-PCR) in all three rice samples (TMR1, TMR2, and TMR3), primers for real-time amplification were shown in Table S2. The qRT-PCR results (Figure 4B) clearly showed down-regulation of TMR3 compared with

TMR2, which is consistent with the transcriptome results. Since the four genes are crucial in downstream phenylpropanoid biosynthesis (Figure S3), the down-regulation of these four genes might have sizeable effects on the biosynthesis of downstream metabolites, such as lignin and flavonoids.

4. Discussion

As suggested in many studies, the microorganisms of BPH might change in the process of the adaptation of planthopper to altered environments and hosts (for example, insecticides and genetically modified rice with resistance genes) [19,28–30]. However, little was known about the potential effects of diverse microorganisms of BPH on its host. In this study, we delineated an interacting insect-microorganisms-plant system in which the rice transcriptome was influenced by the perturbed bacterial communities of BPH, and we identified gene expression changes in phenylpropanoids biosynthesis in rice after fed by BPH with different bacterial communities composition. To elucidate only the influences of the different microorganisms on the same genetic background of BPH and rice, we perturbed the bacterial communities of BPH by feeding with antibiotic-treated rice. The protocol did alter microorganisms' diversity and composition in BPHs, as confirmed by 16S rRNA sequencing.

The species of the bacterial communities of BPH differed in different tissues, life stages, and biotype populations [30,44]. Our results show that the Bacteroides, Proteobacteria, Actinobacteria, and Cyanobacteria were the dominant phylum in BPH. These four phyla are particularly strongly represented in other insects [45]. Furthermore, Bacteroides, Proteobacteria, and Actinobacteria were the most predominant phyla identical to the microorganisms previously studied in the small brown planthopper (SBPH), *Laodelphax striatellus*, another pest that sucks the phloem sap of rice plants [46].

Recent studies have shown that antibiotic usage causes alterations BPH's microorganisms. Significant differences in microbial communities were found in the gut and the fat body in BPHs treated with toyocamycin, tebuconazole, and zhongshengmycin. A decrease in the total number of YLS in the fat body and elevated mortality was observed, and *Serratia* significantly increased in the gut and fat body [47,48]. In our results, after rifamycin treatment, *Wolbachia* and *Cardinium* decreased, *Arsenophnus* and *Pantoea* increased significantly, which may be because *Arsenophnus* and *Pantoea* were able to occupy the space left by *Wolbachia* and *Cardinium* quickly. Previous studies showed that *Wolbachia* decreased in other insects after antibiotic treatment. In the bedbug *Cimex lectularius*, elimination of the *Wolbachia* resulted in retarded growth and sterility. These deficiencies were rescued by oral supplementation of B vitamins [49]. Similarly, after tetracycline treatment, the relative abundance of *Wolbachia* was reduced; however, *Asaia* was a significant increase in many tissues of SBPH [46]. So far, the effect of antibiotics on the bacterial communities in the gut, fat body, and other tissues of BPH has not been fully elucidated. During the puncture and oviposition process of insect-feeding plants, many microorganisms transmitted into plants, including phytoplasma, pathogenic bacteria, and viruses [50–52]. There are many microorganisms, including *Asaia*, *Cardinium*, and *Rickettsia*, in the salivary glands of some Hemiptera insects [53–55]. The specific role of these microorganisms in the interaction between Hemiptera insects and plants needs to be further clarified.

For insects, insecticide or antibiotic exposure can be considered joint environmental stress. The oxidative phosphorylation system is the primary ATP source in eukaryotic cells. Studies have shown that glutathione S-transferases (GSTs) confer resistance via direct metabolism and indirectly by protecting oxidative stress induced by imidacloprid exposure [56]. A previous study characterized genes responding to imidacloprid in the green peach aphid *Myzus persicae*. The result indicated that the response patterns of aphids to imidacloprid are complex, as demonstrated by changes in the expression of genes involved in oxidative phosphorylation, cuticle structure, and metabolic processes [57]. Another study indicated that energy metabolism pathways were significantly enriched in the BPH treated TN1 rice with water stress, suggesting that water stress enhanced the

energy metabolism of the BPH [58]. In our results, KEGG pathway analysis indicated that DEGs of rifampicin-treated/untreated BPH were enriched in the oxidative phosphorylation process. Oxidative phosphorylation genes up-regulation means that rifampicin treatment enhances the energy metabolism of the BPH. To improve the activity of detoxification enzymes in their bodies, BPH will consume more energy, which will lead to the oxidative phosphorylation metabolism pathway under antibiotic stress.

It would be expected that most of the responses of rice to BPH feeding may be mainly due to the piercing of the mouthpart and the saliva component [59,60]. Therefore, the changes in the transcriptome caused by the variations in the microorganisms might be trivial compared with the total changes. However, by comparing the transcriptome of the rice fed by BPH with/without perturbation of bacterial communities, we were able to detect subtle changes in the transcriptome and confirmed the down-regulation of the phenylpropanoids biosynthesis pathway in TN1 rice feedings by rifamycin-treated BPH.

The phenylpropanoid pathway is indispensable to plants because of its role in lignin biosynthesis and the production of many other vital compounds, such as flavonoids, coumarins, and lignans [61]. Previously published evidence has suggested that the metabolites downstream of phenylpropanoid biosyntheses, lignin, flavonoids, and salicylic acid (SA), might contribute to rice resistance to BPH. PAL is a key enzyme in the phenylpropanoid pathway. BPH infestation induced the expression of *PAL* genes [9]. Further research showed that BPH feeding induces the expression of an R2R3 MYB transcription factor, *OsMYB30*, which up-regulates the expression of *OsPALs* genes, leading to increased biosynthesis and accumulation of SA and lignin. As a result, the plants gained increased resistance to BPH [62]. Silencing of the *OsSLR1* gene enhanced constitutive levels of lignin and the rice resistance to BPH [63]. The white-backed planthopper (WBPH), *Sogatella furcifera*, is a serious phloem sap pest. Overexpression *OsF3H* gene accumulated significant amounts of the flavonols, kaempferol, quercetin, the anthocyanins delphinidin, and cyanidin in response to the stress induced by WBPH [64]. A recent study showed that a rice variety from Taiwan, Qingliu, displays a rarely observed dual resistance to the leaffolder (chewing insect) and the BPH. Although the responses of Qingliu to both insect pests appear vastly dissimilar, the phenylpropanoid pathway could be a convergent upstream pathway [65]. This study showed that the phenylpropanoid pathway is valuable for generating broad-spectrum resistance in rice cultivars.

Overall, our results linked the upstream pathway of lignin and SA, phenylpropanoid biosynthesis, with the rice response to different bacterial communities of BPH. However, the exact function of the bacterial communities of BPH remains unclear. Thus, we plan to conduct further studies on the processes of microorganisms. In addition, information on the effects of antibiotics on the diversity of microorganisms of BPH will be helpful in the integrated interactions between BPH and rice. Finally, our results provide evidence of plant gene expression changes in response to different bacterial communities, which may guide future exploration of pest control methods via bacterial communities of BPH.

5. Conclusions

We established a protocol for the perturbation of bacterial communities. Coupling this perturbation protocol with high-throughput 16S rRNA sequencing, we showed that feeding BPH with rifampicin-treated rice increased the abundance of *Arsenophnus* and *Pantoea* while significantly decreasing the abundance of *Wolbachia* and *Cardinium*. Transcriptomics analysis showed that oxidative phosphorylation and protein processing in the endoplasmic reticulum were significantly up-regulated in BPH fed with rifampicin-treated rice and that the phenylpropanoid biosynthesis pathway in the rice sheath was significantly affected by the changes of the bacterial communities of BPHs. Our results displayed coordinated changes in the bacterial communities, BPH, and rice, providing evidence of the significance of the bacterial communities of BPH and rice defense responses.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112327/s1, Figure S1: Cultivation of the rice and the BPH colony. (a) The rice

planted in basins. The soil in the basin was obtained from local rice field. (b) A gauze net was used to contain the BPHs and the rice. Figure S2: The KEGG pathway enrichment analysis of the differentially expressed genes comparing TMR1 with TMR2 (a) and TMR1 with TMR3 (b), respectively. Figure S3: The KEGG map of phenylpropanoid biosynthesis pathway. The genes in red are the four differentially expressed genes validated by qRT-PCR. Table S1: α-diversity index of BPH population. Table S2: List of the primers of rice DEGs in this study.

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Data Availability Statement: The RNA-seq sequencing data presented in this study are openly available, processed data can be obtained by accession number PRJNA763742 and PRJNA763799.

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