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A metagenomic analysis reveals changes in the bacterial community structure due to infection by the rice yellow mottle virus

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This research aimed to explore the pathobiome to enhance the understanding of the roles played by disease-associated microbes. A comparative analysis of microbiomes in rice samples was conducted to shed light on the potential impact of the rice yellow mottle virus on microbiome composition. Forty samples were collected from rice fields of the Office du Niger in Mali. Bacterial DNA was extracted at the LaboREM-Biotech and the RT-PCR was conducted to confirm the presence of the virus. The next-generation sequencing of extracted bacterial DNA was employed to examine bacterial communities in both infected and uninfected plants. The Minnesota Supercomputing Institute, R-studio, Dada2 and Quiime were utilized to compare bacterial communities and identify bacteria present in healthy and infected rice plants. The results support the notion that plant infection by pathogenic viruses significantly influences microbial communities. The overall microbial diversity in infected and uninfected rice plants did not show a significant difference. Viral invasion led to alterations in microbiome members, potentially fostering colonization through mutualistic relationships or aiding in plant defense against pathogens during infection. The study highlighted the differential enrichment of bacteria from various families in plants, indicating a significant change in the rice bacterial community composition with infection.

Key words: Metagenomic analysis, microbiome, bacterial community, rice yellow mottle virus, viral infection, rice, Mali.

INTRODUCTION

Rice/Paddy (*Oryza sativa* L.) is a staple crop that forms the primary food source for over three billion people worldwide (Skamnioti and Gurr, 2009). Rice yellow mottle disease caused by the rice yellow mottle virus is a global

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problem that can lead to a 10-30% reduction in rice production each year, potentially feeding an additional 60 million people (Kirtphaiboon et al., 2021). Due to the pathogen's wide range of hosts and the evolution of new pathotypes, managing outbreaks is a challenging task (Valent, 2021; Devanna et al., 2022). Pathogenic microbes induce changes in plant phenotypes through tissue damage and the induction of plant defenses, which can alter plant immunity to colonization by microorganisms. Therefore, factors that influence the pathogen's impact on hosts will likely affect the colonization and growth of plant-associated microorganisms. Various host and environmental factors influence the structure and diversity of the plant microbiome (Compant et al., 2019; Dastogeer et al., 2020). The host's immunity level is one of the key factors shaping the plant microbiome community composition (Dastogeer et al., 2020).

Recent studies suggest that the plant microbiome can enhance the immune functions of the host plant (Vannier et al., 2019; Ma et al., 2021; Teixeira et al., 2021). It has been demonstrated that plants can selectively recruit microorganisms to have a positive impact on plant growth and health (Reinhold-Hurek et al., 2015; Sasse et al., 2018; Liu et al., 2021). Accumulating data suggests an ongoing battle between the host and its microbes to maintain microbiome homeostasis in the host (Paasch and He, 2021). In microbiome research, the concept of a "healthy microbiome" has garnered significant attention, although the definition of a "healthy microbiome" is not yet entirely clear (Bäckhed et al., 2012; Shanahan et al., 2021). Furthermore, by modifying the host's defense, a healthy microbiome maintains ecological stability in the host and prevents intruding microbial contaminants, such as those shielded against pathogen attacks. On the other hand, disease leads to a change in the microbiome termed "microbiome dysbiosis," a situation in which microbiome homeostasis is disrupted, and the organism becomes more vulnerable to potentially harmful microbial invaders.

Additionally, environmental sustainability calls for the innovation of natural biocontrol agents instead of chemical fungicides. Therefore, the rice-yellow mottle virus pathosystem has become a model system to study host-pathogen interactions. The microbiome can play a significant role in host defense and pathogen infection. This study aims to consider the pathobiome for a better understanding of the roles of disease-associated microbes.

MATERIALS AND METHODS

Rice samples (*Oryza sativa* L.) were collected from fields at two locations, namely, the Office du Niger (Niono, Séribala, Béwani, Moussa wéré, and Coumba wéré) and the Office riz Ségou (Markala) in Mali. Forty (40) complete rice plant samples (Cultivar Kogoni 911) were obtained from five sampling points in fields infected and non-infected by the rice yellow mottle virus. The sampling strategy involved randomly collecting rice samples from the four corners and the center of each part of the plots contaminated and uncontaminated by rice yellow mottle virus (horizontal sampling). In total, 20 samples were collected from infected plants and twenty (20) samples from non-infected plants. Each plant was individually packaged in a plastic bag and transported to the laboratory in a cooler containing dry ice to maintain a low temperature, minimizing potential disruptions to the microbial community. In the laboratory, the samples were subsequently stored at 4°C until processing, and processing was completed within 48 hours after collection.

Virus detection in samples by RT-PCR

Viral RNA was extracted from leaf samples using the Qiagen RNeasy Mini Kit. The concentration of each RNA was measured in ngµl^{-1} using the Biophotometer Eppendorf. Each total viral RNA sample was amplified by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) using two specific primer pairs, Prymv1 (Forward: TGCCAATACCTATCTCCACCA, and Reverse: TCACCTCTAGCGTTTGGTACG) and Prymv2 (Forward: CCCGCAGGACCATACTAACGA, and Reverse: GGGCTTCGTCACCTCTAGC), along with reagents from the Promega Access RT-PCR System Kit in a final reaction volume of 25 µl.

The amplification of the fragments occurred in a final reaction volume of 25 µl, following the program: Reverse transcription at 48°C for 45 min; Inactivation at 94°C for 30 sec; Denaturation at 94°C for 30 sec; Hybridization at 61°C for 1 min; Elongation at 68°C for 2 min; Final extension at 68°C for 7 min. Denaturation, hybridization, and elongation steps were repeated 45 times.

The RT-PCR products were separated on a 2% (w/v) low EEO Agarose D1 gel under the influence of an electric field in the migration tank at 80 volts for 1 h and 30 min. The gel was prepared using a 0.5X TBE (Tris, Borate, EDTA) solution and mixed with 30µl of 10% Ethidium Bromide (1mg/ML) for a 100mL tank. The products were visualized under ultraviolet (UV) light and then photographed using the Gel Documentation System E-BoX.

Analysis of bacterial genomic DNA

Roots, leaves, and grains were surface-sterilized by washing with 0.25% NaOCl for 1 min, followed by 70% EtOH for 40 sec, and subsequent rinsing with sterile water three times. The effectiveness of surface sterilization was assessed using the tissue imprint method (Greenfield et al., 2015). A cork borer was used to puncture and collect small tissues from within leaf symptomatic areas, referred to as the "symptomatic fraction," and from non-symptomatic tissues that showed no apparent disease symptoms, known as the "non-symptomatic fraction." Sterilized tissues were blot-dried on autoclaved absorbent paper, cut into small pieces, and stored at -80°C for further processing. All plant parts or debris were removed from bulk soil samples and the rhizosphere. After suspension in phosphate buffer, the soil was filtered through a sterile 100 µm cell sieve to remove any small plant parts and debris. Suspended soils were collected by centrifugation at 3,000 × g for 5 min, and the pellets were stored at -20°C until DNA extraction. Rhizosphere soils and plant parts from three out of the nine plants were combined to constitute a biological replicate.

DNA extraction and amplicon analysis

Total genomic DNA from plant tissues was extracted using the NucleoSpin R. Soil kit (Macherey-Nagel, Duren, Germany) with the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the

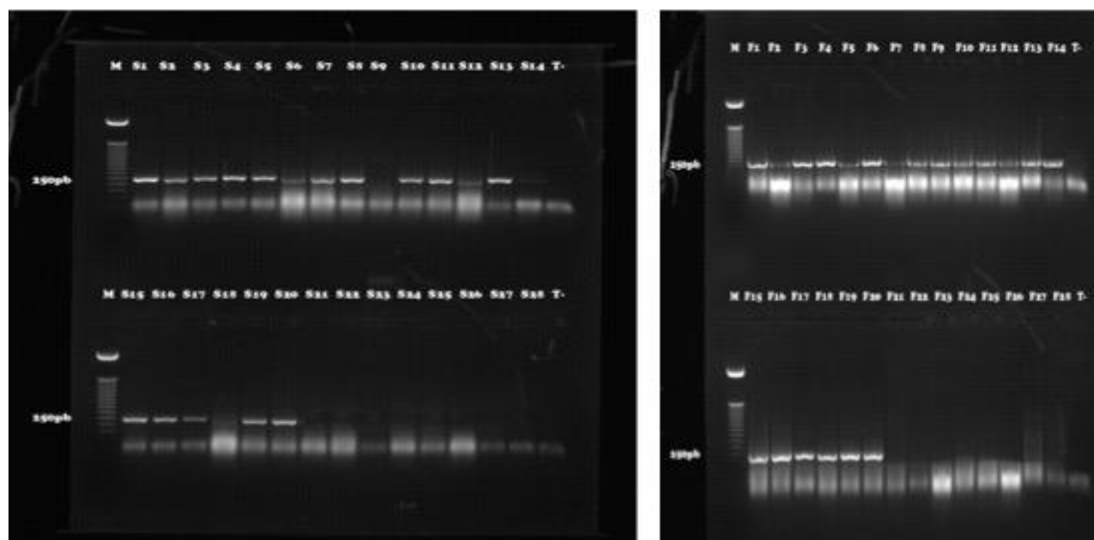

Figure 1. RT-PCR Profiles of Twenty-Eight (28) Plant Samples with the Prymv Primer.

protocols outlined in the manuals. DNA samples were eluted in 50 µL of nucleases-free water and used for bacterial analysis. DNA quantity and quality were measured using a Nanodrop 2000 diluted to 100 ngµl^{-1} and stored at -20°C.

Amplicon libraries were prepared using the Nextra XT index kit (Illumina Inc.) following the 16S metagenomic sequencing library preparation protocol (Part # 15044223 Rev. B). Primers for amplifying the V3-V4 hypervariable region of the bacterial 16S rRNA gene were designed and synthesized in the bioinformatics laboratory of the University of Minnesota's College of Pharmacy in the United States. The prokaryotic primers V3-Forward and V4-Reverse were composed of 5'CCTACGGGNBGCASCAG 3' and 5'GACTACNVGGGTATCTAATCC 3', respectively. The amplicon with Illumina adapters was sequenced using i5 and i7 primers that added multiplexing index sequences as well as common adapters required for cluster generation, following Illumina standard protocols (20). Amplicon libraries were purified with AMPure XP beads, checked on an Agilent High Sensitivity (HS) chip on a Bioanalyzer 2100, and quantified using a Qubit dsDNA HS Assay kit (Life Technologies) on a fluorometer.

Sequencing, data processing, and amplicon sequence variant identification

Next-generation sequencing of the samples was performed on the Illumina platform. Data generated for the two hyper-variable V3-V4 regions of the 16S rRNA gene were combined, and paired-end sequences were assembled using FLASH. Data analysis was conducted using the Quantitative Insight into Microbial Ecology (QIIME) software (Bolyen et al., 2019). Sequences shorter than 200 bp or containing ambiguous characters, quality scores (Phred) less than 25, non-matching barcode sequences, the presence of homopolymers (greater than 6 nucleotides), or any discrepancies with primer sequences were excluded from the analysis.

Operational taxonomic unit (OTU) clustering was performed at a 97% similarity level among sequences using the Uclust OTU picker version 1.2.22 (Edgar et al., 2011). Chimeric sequences within OTUs were detected using Chimera Slayer (Haas et al., 2011) and removed using the filter-fasta.py script. The representative sequence of each OTU was aligned with reference sequences in Greengenes (http://greengenes.lbl.gov/) using the PyNast program (CAPORASO et al., 2010). The minimum identity value for including a sequence in the alignment was set at 75%. A phylogenetic analysis was performed with FastTree using default parameters (Price et al., 2009). Taxonomic assignment of OTUs was carried out with an 80% confidence threshold using the Ribosomal Data Project's Bayesian naive ribosomal RNA classifier (http://rdp.cme.msu.edu/classifier/classifier.jsp) (Wang et al., 2007). A heat map was constructed using the CIMminer tool (http://discovery.nci.nih.gov) to visualize the most abundant genera (Scherf et al., 2000).

The Minnesota Supercomputing Institute (MSI), R-studio, Dada2, and QIIME were employed to compare bacterial profiles among samples and identify bacteria of interest present in soils and rice plants not infected by the virus but absent in infected ones (Fierer et al. 2010). After identification, these bacteria underwent a BLAST search against the NCBI database to define their respective functions. BLAST provides a score for each alignment and uses this score to give a statistical evaluation of the alignment's relevance (the probability that it occurred by chance). It was used to search the NCBI sequence database for segments that are locally homologous to the provided sequence (query sequence) and to calculate alignment scores.

RESULTS

Samples showing rice yellow mottle virus presence after RT-PCR analysis

The results of the RT-PCR analysis aimed at confirming the presence or absence of the rice yellow mottle virus in the soil and rice samples collected from the Office du Niger rice fields. The analysis of the results indicates that twenty (20) out of the forty (40) processed rice samples are infected with the rice yellow mottle virus, as evidenced by their positivity with the Prymv2 primer pair, as shown in the RT-PCR results of the plant samples on different migration profiles, consisting of a single band of approximately 250 bp with Prymv2 (Figure 1).

Figure 2. Relative abundance of different phyla in the communities defined in samples from infected plants (FI1 to FI17) and Non-Infected Plants (FNI1 to FNI17).

Identification of bacteria present in plants

A total of 3,569,403 sequences were obtained from the rice samples, of which 2,298,023 were high-quality reads with an average length of approximately 250 base pairs. These sequences were used for subsequent analyses. A total of 1,866,676 different microorganisms were identified, with 956,233 species in infected plants and 952,713 species in non-infected plants. The number of microorganisms ranged from 50 to 102,407, depending on the condition of the rice plant samples (infected or non-infected). The relative abundance of different phyla in the communities defined in Samples from infected and non-infected plants is presented in Figure 2.

However, the results of the analysis of variance (ANOVA) did not show any significant differences between infected and non-infected plants at $P < 0.05$. In infected plant samples, the number of bacterial species ranged from 136 to 33,332, while non-infected plants exhibited a species richness ranging from 322 to 47,480 bacteria.

Forty-two bacterial phyla were detected in the analyzed plant samples (Figure 2). Approximately 8% of the reads were unclassified at the phylum level. Three phyla accounted for 82.6% of the total relative abundance in all samples. These predominant phyla were classified in the following order: Proteobacteria 58%, Firmicutes 19.4%, Bacteroides 5.2% (Figure 2). The relative abundance of each of these phyla varied depending on the soil samples' origin. Overall, the relative abundance was not significantly different. A total of 94 classes, 193 orders, 308 families, and 614 genera were identified. At the

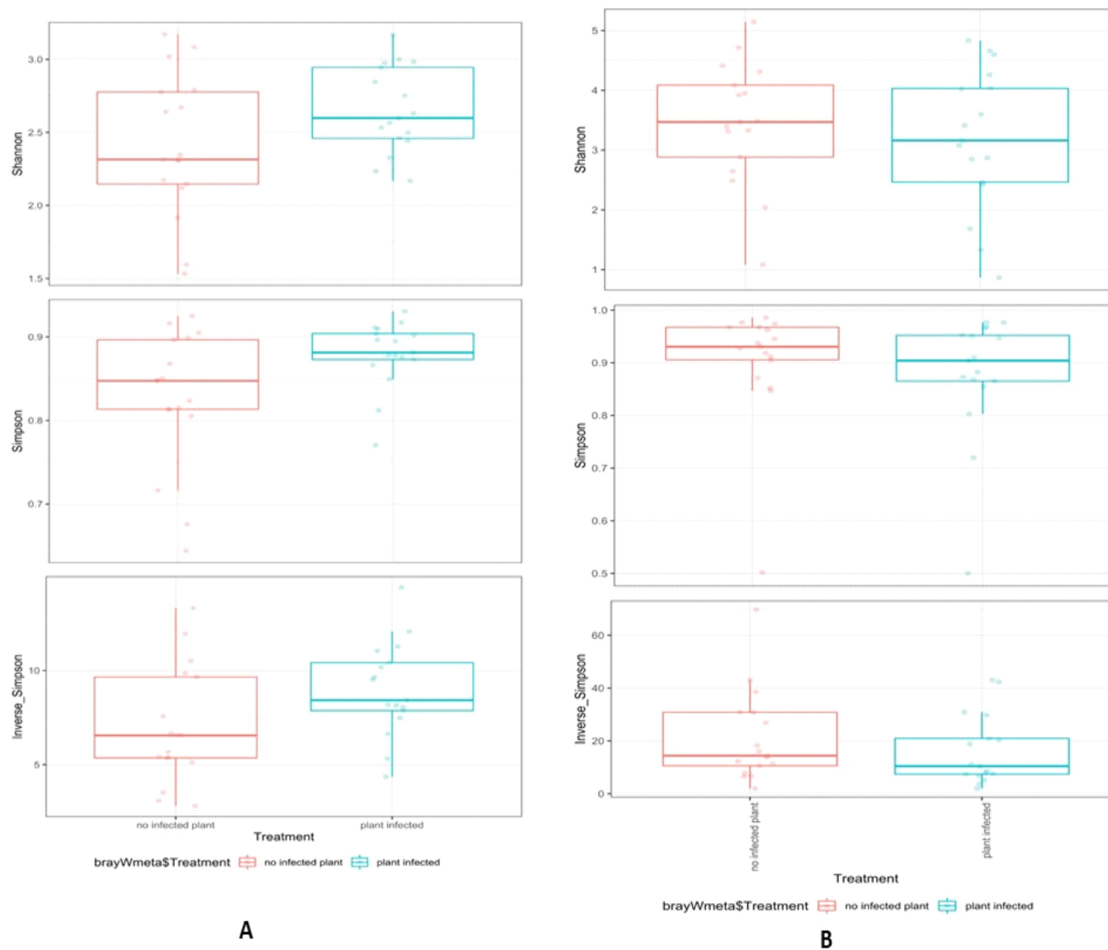

Figure 3. Results showing overall diversity (A) and specific diversity (B) of microbial species in virus-infected vs. non-infected rice plants.

genus level, Bacillus, Thiobacillus, Pedomicrobium, and Kaistobacter (formerly Sphingomonas), along with 47 other genera, were identified as the most abundant OTUs.

Microbial communities' diversity in healthy and virus-infected plants

This study analyzed and compared the alpha and beta diversity of the bacterial microbiome in infected and healthy rice plants from various locations in the Office du Niger area in Mali. As expected, both the overall microbial community (Figure 3A) and the specific microbial community (Figure 3B) in healthy plants were more diverse than those in plants infected with the rice yellow mottle virus. The estimated coverage based on OTU/Chao1 richness ranged from 56 to 71%.

PCoA revealed distinct bacterial assemblages in infected and healthy plants (Figures 4). Despite the fact that the number of identified microorganisms did not show a significant difference between the samples, PCoA clearly demonstrates that the overall microbial community of infected and healthy plants forms two relatively distinct groups (Figure 4A). In contrast to the overall microbial community, the specific microbial community of diseased and healthy plants overlaps, and thus, they do not form distinct groups (Figure 4B).

Alteration in the composition of the bacterial community in rice plants infected with the rice yellow mottle virus

Given the differences in composition and diversity of the microbiome community in virus-infected and healthy plants, the bacterial microbiome of healthy and diseased samples were compared. A significant difference was observed in the overall diversity of bacteria (Shannon and Chao1) between healthy and diseased samples. The

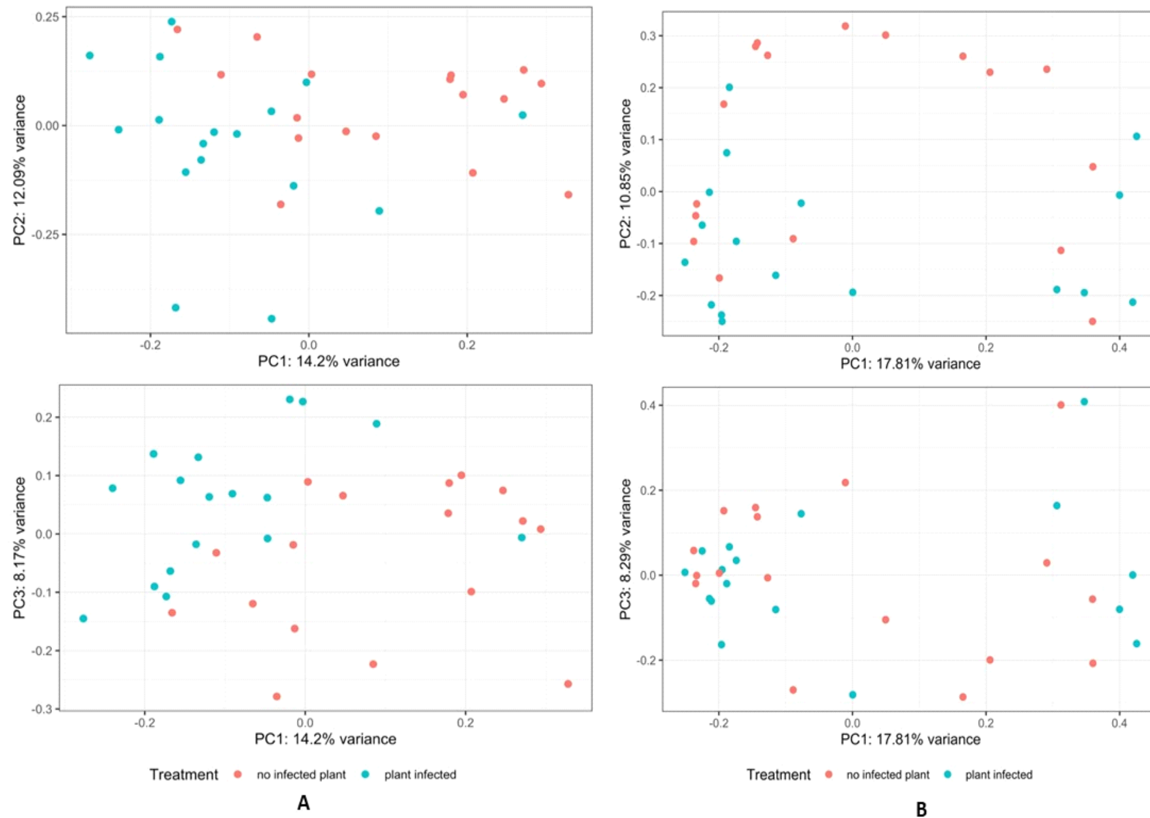

Figure 4. Weighted principal coordinates analysis of the overall microbial community (A) and specific community (B) of virus-infected and healthy plants.

comparison of bacterial communities in healthy and diseased plants revealed a significant difference. Several bacterial genera present in healthy plants are entirely absent in virus-infected plants (Figure 5).

The most abundant phylum in non-infected leaves was Proteobacteria, followed by Firmicutes, Planctomycetes, Actinobacteria, and Bacteroidetes (Table 1). Some families were only found in healthy plants. These families include Trueperaceae, Moraxellaceae, Burkholderiaceae, Hyphomicrobiaceae, JG30-KF-CM45, Sphingomonadaceae, Rubinisphaeraceae, and Paenibacillaceae (Figure 3). The number of bacteria per Phylum present in infected and non-infected leaves and the percent variation caused by the virus infection are presented in Table 1.

Infection of these plants by the yellow leaf virus resulted in a 53% reduction in Proteobacteria, a 64% reduction in Planctomycetes, and a 94% reduction in Chloroflexi (Table 1). Conversely, the presence of the virus in plants led to an 88.65% increase in Firmicutes and an 84.47% increase in Bacteroidetes compared to the content in healthy plants (Table 1). Furthermore, several abundant bacterial families in healthy samples were completely absent from virus-infected plant samples. These families include Trueperaceae, Moraxellaceae, Burkholderiaceae, Hyphomicrobiaceae, JG30-KF-CM45, Sphingomonadaceae, Rubinisphaeraceae, and Paenibacillaceae. At the genus level, Trupera, Acinetobacter, Hydrogenophaga, Filomicrobium, Castellaniella, Sphingobium, Planctomicrobium, and Ammoniphilus, which were abundantly present in healthy plant samples, were entirely absent from virus-infected plant samples (Figure 5).

DISCUSSION

The study presents a critical assessment of bacterial communities sampled from rice plants infected and non-infected by the rice yellow mottle virus to provide a comprehensive view of the rice-associated microbiome. Bacterial communities associated with rice have been studied using culture-dependent and culture-independent methods (Edwards et al., 2015; Bertani et al., 2016; Kanasugi et al., 2020; Kim and Lee, 2020; Sinong et al., 2020). The study profiled the 16S and ITS2 regions to reveal bacterial community composition. The results support the concept that infection of plants by pathogenic viruses plays a crucial role in shaping microbial

SequenceID	Infected_Sum	more_noninfect	nonInfected_su	domain	phylum	class	order	family	genus	species
TACGGAGGGT	0	TRUE	814	Bacteria	Deinococcus-Th	Deinococci	Deinococcales	Trueperaceae	Truepera	NA
TACAGAGGGT	0	TRUE	637	Bacteria	Proteobacteria	Gammaproteob	Pseudomonada	Moraxellaceae	Acinetobacter	baumannii/septicus/ursingii
TACGTAGGGT	0	TRUE	407	Bacteria	Proteobacteria	Gammaproteob	Betaproteobact	Burkholderiacea	Hydrogenophag	flava
TACGTAGGGT	0	TRUE	407	Bacteria	Proteobacteria	Gammaproteob	Betaproteobact	Burkholderiacea	NA	NA
TACGAAGGGG	0	TRUE	278	Bacteria	Proteobacteria	Alphaproteobac	Rhizobiales	Hyphomicrobiac	Filomicrobium	NA
TACGTAGGGG	0	TRUE	269	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobia	JG30-KF-CM45	NA	NA
TACGGAGGGT	0	TRUE	209	Bacteria	Proteobacteria	Gammaproteob	NA	NA	NA	NA
TACGTAGGGT	0	TRUE	179	Bacteria	Proteobacteria	Gammaproteob	Betaproteobact	Burkholderiacea	Castellaniella	caeni/daejeonensis/defragrans/denitrificans/ginsengisoli
TACGTAGGGG	0	TRUE	142	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobia	JG30-KF-CM45	NA	NA
TACAGAGGGT	0	TRUE	141	NA	NA	NA	NA	NA	NA	NA
TACGGAGGGG	0	TRUE	138	Bacteria	Proteobacteria	Alphaproteobac	Sphingomonada	Sphingomonada	NA	NA
TACGAACTGC	0	TRUE	129	Bacteria	Planctomycetes	Planctomycetac	Planctomycetale	Rubinisphaerac	Planctomicrobiu	NA
TACGTAGGGG	0	TRUE	122	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillacea	Ammoniphilus	NA
TACGGAGGGA	0	TRUE	110	Bacteria	Proteobacteria	Alphaproteobac	Sphingomonada	Sphingomonada	Sphingobium	NA
TACGTAGGGT	0	TRUE	105	Bacteria	Proteobacteria	Gammaproteob	Betaproteobact	Burkholderiacea	NA	beijingensis/chironomi/denitrificans/ebreus/konkukae/nitroreducens/oryzae/pentelensis
TACGTAGGGG	0	TRUE	70	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobia	JG30-KF-CM45	NA	NA

Figure 5. Bacteria present only in non-infected plants.

Table 1. Number of bacteria per Phylum present in infected and non-infected leaves and the variation (%) caused by the virus infection.

Variable	Number of bacteria per Phylum		
	Infected leaves	Uninfected leaves	Variation (%)
Proteobacteria	12205	40574	53.75
Chloroflexi	98	3566	94.65
Firmicutes	128884	7740	-88.70
Planctomycetes	1546	7044	64.01
Actinobacteria	1395	4445	52.22
Bacteroidetes	55897	4705	-84.47
Deinococcus	20	211	82.68

communities. The study demonstrated that overall microbial diversity in infected and non-infected rice plants did not differ significantly. However, it was observed that the number of bacteria in infected plants differed from that in non-infected plants. Interestingly, in the analyses, it was observed a significant increase in the number of certain groups of bacteria in samples from diseased plants compared to samples from healthy rice plants. Although several previous studies have reported distinct and more diverse plant bacterial communities in different crops (Praeg et al., 2019; Hinsu et al., 2021), a distinct separation of the bacterial community in diseased and non-diseased rice plants was not found. This may be due to the fact that most bacterial genera were negatively correlated with the rice yellow mottle virus in the microbiome of diseased plants. When bacterial communities in healthy and diseased plants were compared, changes were observed in the correlation pattern between the rice yellow mottle virus and other microorganisms (Table 1). For example, Firmicutes and Bacteroidetes significantly increased in infected plants, while bacteria from other phyla significantly decreased in the same plants, indicating a change in microbiome interactions due to infection with the rice yellow mottle virus. The observations indicate that pathogen invasion alters members of the microbiome, which could either contribute to colonization through mutualistic relationships or act in defense of the plant against the pathogen during the infection process. In other words, microbial members present in asymptomatic tissues may play a role in limiting pathogen invasion into the tissues, which would otherwise lead to disease symptoms.

It was observed that samples infected with the rice yellow mottle virus exhibited different bacterial community structures compared to healthy plants. This study indicated that bacteria from several families were differentially enriched in healthy and diseased plants. In particular, members of the Trueperaceae, Moraxellaceae, and Burkholderiaceae families were significantly more abundant in the endosphere of healthy plants and absent in diseased plants (Figure 5). Members of the Trueperaceae, Moraxellaceae, and Burkholderiaceae

families are known for their ability to produce various bioactive compounds and play a crucial role in agriculture due to their potential for biological control against phytopathogens, including RYMV. The role of plant-associated microbiota in protecting against pathogenic viruses is well-documented. While it was not empirically examined, the differential bacterial assemblage may be linked to differential exudation of compounds through the endosphere due to the presence of the virus in rice plants, leading to the selection of specific microbial groups, thereby regulating the composition of their communities. The "cry for help" hypothesis suggests that plants recruit microbial partners to maximize their survival and growth when affected by external stress and is likely a conserved survival strategy across the plant kingdom (Liu et al., 2019, 2020; Gao et al., 2021).

However, the pathobiome of infected plants showed a clear separation from the microbiome of healthy plants. The concept of the pathobiome has been defined as the set of microbes interacting with a given pathogenic species and their influence on pathogenesis (Vayssier-Taussat et al., 2014; Jakuschkin et al., 2016). Characterizing the components of the pathobiome is an important consideration for understanding pathogenesis, persistence, transmission, and evolution of pathogens (Vayssier-Taussat et al., 2014). It has been demonstrated that changes in the composition, richness, and abundance of the microbiome occur due to pathogenic infection in plants (Jakuschkin et al., 2016; Musonerimana et al., 2020; Mannaa and Seo, 2021). The results from this study also revealed that the composition of the bacterial community in rice leaves changed considerably with viral infection.

In diseased plants, the relative abundances of Paenibacillus, Enterobacter, and several other bacteria often considered beneficial plant microbes showed a significant decrease in their abundance compared to healthy samples. These decreases imply that these taxa might be excluded due to a compromised local immune system or outcompeted by more efficient colonizers. The change in microbiome composition in diseased plants could be due to the degradation of plant tissues (necrotic/decomposed tissues) by the pathogen, leading to colonization by different microbiomes or a pathogen teaming up with commensals (Lundberg et al., 2012; Venturi and da Silva, 2012; Tláskal et al., 2016). On the other hand, the relative abundances of several bacteria, such as those belonging to Bacteroides, in leaf samples showed a marked increase compared to non-diseased samples. This suggests they might be involved in pathogenesis and maintain mutualistic relationships with the pathogen, or perhaps they are opportunistic and could take advantage of different ecological niches created by the invasion of the pathogen (Lundberg et al., 2012; Hu et al., 2020). Overall, it was deduced that foliar infection by the pathogenic rice yellow mottle virus causes a shift in rhizosphere bacteria. The rice yellow mottle virus in diseased plants is associated with interactions with various bacteria whose roles in the disease process need to be clarified. Bacteria that interact positively potentially benefit from invading pathogens, which could lead to the migration of many additional bacterial genera into diseased plants, eventually causing a viral epidemic. These results provide potential insights and a theoretical basis for isolating biological control agents against the rice yellow mottle virus in future work.

Conclusion

Based on the overall study, the study infers that plants infection by the pathogenic rice yellow mottle virus causes a shift in bacteria community from the rhizosphere. The rice yellow mottle virus in diseased plants is associated with interactions with various bacteria, including Bacillus, Enterobacter, and several other bacteria. The role of these bacteria in the disease processes needs to be clarified. Bacteria with positive interactions potentially benefit from invading pathogens, which could lead to the migration of many additional bacteria into diseased plants, eventually causing a viral epidemy. These results provide potential insights and a theoretical basis for isolating biological control agents against the rice yellow mottle virus in future research work.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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