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Changes in Endophyte Communities across the Different Plant Compartments in Response to the Rice Blast Infection

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The rice blast disease, caused by the fungal pathogen, *Magnaporthe oryzae* (syn. *Pyricularia oryzae*), poses a significant threat to the global rice production. Understanding how this disease impacts the plant's microbial communities is crucial for gaining insights into host-pathogen interactions. In this study, we investigated the changes in communities of bacterial and fungal endophytes inhabiting different compartments in healthy and diseased plants. We found that both alpha and beta diversities of endophytic communities do not change significantly by the pathogen infection. Rather, the type of plant compartment appeared to be the main driver of endophytic community structures. Although the overall structure seemed to be consistent between healthy and diseased plants, our analysis of differentially abundant taxa revealed the specific bacterial and fungal operational taxonomic units that exhibited enrichment in the root and leaf compartments of infected plants. These findings suggest that endophyte communities are robust to the changes at the early stage of pathogen infection, and that some of endophytes enriched in infected plants might have roles in the defense against the pathogen.

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The world is facing significant challenges in ensuring food security for its growing population (Barrett, 2021; Mc Carthy et al., 2018). Crop production is constantly threatened by a variety of biotic and abiotic stressors, including drought, salinity, diseases, and pests, which collectively contribute to a loss of approximately 16% of global crop yields (Oerke, 2006). While extensive research has been conducted on the interactions between individual plants and pathogens, recent efforts have shifted towards understanding the complex interplay among plants, pathogens, and other microorganisms under diverse conditions (Bulgarelli et al., 2013; Santos and Olivares, 2021; Trivedi et al., 2022).

Plants, like humans and animals, host intricate microbial communities known as the "plant microbiome." There is a growing interest in unravelling the composition and functions of these microbiomes, with the aim of harnessing their potential for promoting host growth and disease resistance (Busby et al., 2017; Song et al., 2020; Zhang et al., 2021). Pathogenic microbes can alter plant phenotypes and influence the immunity of plants to colonization by other microorganisms. Several factors, including host immunity levels, environmental conditions, and exudation of compounds in the rhizosphere, shape the structure and diversity of plant microbiomes (Compant et al., 2008; Dastogeer et al., 2020). Recent studies have highlighted the ability of plant microbiomes to enhance host immune functions (Ma et al., 2021; Teixeira et al., 2021; Vannier et al., 2019).

Plant microbiomes are incredibly diverse, inhabiting various plant tissues and organs, such as seeds, roots, stems, leaves, flowers, and fruits (Berg et al., 2014; Philippot et al., 2013). Among these communities, endophytes,

which reside within plants without causing harm, have the potential to antagonize plant pathogens and influence host physiology and development (Mousa et al., 2015). Bacterial endophytes, in particular, contribute to host tolerance or resistance to biotic and abiotic stresses and have been explored for their use as biocontrol agents (Friesen et al., 2011; Mercado-Blanco and Lugtenberg, 2014; Rosenblueth and Martínez-Romero, 2006). Understanding plant-endophytic microbe interactions is crucial for developing sustainable agricultural practices with reduced reliance on pesticides and chemical fertilizers (Scholthof, 2001).

Rice (*Oryza sativa* L.) is a critical cereal crop feeding more than half of the global population (Khush, 2003). However, rice production is hampered by diseases such as bacterial leaf blight, blast, and sheath blight (Tian et al., 2007). Rice blast disease caused by *M. oryzae* poses a significant threat to rice production, causing substantial yield losses (Kirtphaiboon et al., 2021; Skamnioti and Gurr, 2009). Effective management of this disease is essential for global food security (Devanna et al., 2022; Valent, 2021). To address these challenges, sustainable agriculture requires eco-friendly approaches, including the use of beneficial microbes (Tian et al., 2007). Research has been conducted on the isolation of rice bacterial endophytes from different sources and for plant growth-promoting activities (Bertani et al., 2016; Elbeltagy et al., 2000; Hardoim et al., 2012; Mano and Morisaki, 2008; Walitang et al., 2017). In this study, we aimed to provide an overview of the diversity and distribution of bacterial and fungal endophytes from different plant compartments (root, stem, and leaf) of rice plants and the influence of the blast disease caused by *M. oryzae* on the structural composition of bacterial and fungal communities within the root, stem, and leaf compartments of rice plants.

Materials and Methods

Experimental design. A rice variety, Nakdongbye, susceptible to an isolate of *M. oryzae*, KJ201 was used in this study. The sterilization protocol described by Bertani et al. (2016) was followed to prepare the seeds. The surface-sterilized seeds were germinated for seven days in 16/8 h light/darkness at 25°C and 70% relative humidity. In this experimental setup, seven-day-old seedlings were initially planted in nursery soil (Bunong, Gyeongju, Korea). Over the course of three weeks, we consistently applied a standard nitrogen solution to the plants at a rate of 40 mg/l on a weekly basis. This nitrogen solution comprised 75% NO_3^- and 25% NH_4^+. At the commencement of the fourth week, the plants were divided into two distinct groups: the control

group and the infected group. In the case of the infected groups, we followed the established protocol outlined by Berruyer et al. (2003) to administer a spore suspension of *M. oryzae* strain KJ201. Conversely, the control groups were sprayed with dH$_2$O. Disease progress was evaluated by counting lesions seven days post-inoculation (Ballini et al., 2013) using Adobe Photoshop software (Supplementary Fig. 1).

Sample preparation. In our experiment, we selected three plants from both the control (sprayed with dH$_2$O) and infected (7 days post-inoculation) groups, forming one sample. We maintained three replicates for each plant part. A single sample comprised 1 g of each plant part from three plants, considered a single sample. The roots, stems, and leaves of all the samples were separated, washed with running tap water, and rinsed three times with distilled water: RC, root control (healthy) sample; RI, root infected; SC, stem control; SI, stem infected; LC, leaf control; LI, leaf infected sample. To ensure the surface sterilization of the plant parts, we sequentially submerged the samples from each part in 70% ethanol for 5 min, 2.5% sodium hypochlorite for 2 min, and 70% ethanol for 1 min. After this, the samples were rinsed five times with sterile Millipore water (Elbeltagy et al., 2000). To confirm the effectiveness of sterilization, the final rinsing water was cultured on Luria-Bertani agar at 30°C for 24-48 h to detect bacterial growth, and potato dextrose agar at 25°C for 5 days to assess fungal presence (Schulz et al., 1998). The absence of microbial growth on triplicate media plates inoculated with 50 µl aliquots of the final rinse water, confirmed the efficacy of the surface sterilization technique. Subsequently, we stored a total of 18 samples at –80°C until DNA extraction.

DNA extraction, PCR amplification, and sequencing. The total genomic DNA of each sample was extracted using FastDNA Spin kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. PCR amplification of bacterial 16S rRNA genes (V3-V4 region) was performed using the universal primers 341F and 805R and for fungal 18S rRNA genes (ITS2 region) ITS3F and ITS4R were used. The PCR amplicons were purified with AgencourtAMPure Beads (Beckman Coulter Genomics, Inc., Brea, CA, USA) following the protocol described by Zheng et al. (2015). The paired-end sequencing was performed using the Illumina MiSeq platform at Macrogen (Seoul, Korea). PCR was performed in a 25 µl reaction volume containing 2.5 µl of template DNA, 12.5 µl of 2× KAPA HiFi HotStart Ready Mix (KAPA Biosystems, Boston, MA, USA), and 5 µl (1 µM) of each primer (Zheng

et al., 2015). PCR blockers pPNA and mPNA (Lundberg et al., 2013) were used to reduce contamination by plant host plastid and mitochondrial 16S amplicon.

Sequence analysis. The Quantitative Insights Into Microbial Ecology (QIIME 2 version 2020.8) pipeline was employed to process the sequencing data using EzMAP interface (Shanmugam et al., 2021). The sequences were quality-filtered and denoised using DADA2. Taxonomic classification of representative sequences was done using 'classifysklearn', a taxonomy classifier against SILVA_128 and UNITE database for bacteria and fungi, respectively. All non-chimeric sequences were clustered into 1,145 and 861 operational taxonomic units (OTUs) of bacteria and fungi, respectively, with a threshold of 97% similarity and 70% confidence level as default settings for OTU clustering. In our study, two analytical methods were utilized for deeper insights: DESeq2 was employed to analyse all OTUs, ensuring a comprehensive view that included low-abundance taxa. Conversely, the relative abundance analysis was conducted, applying thresholds of >0.5% for bacteria and >0.1% for fungi, respectively, focusing on more abundant taxa. This dual approach enhanced our understanding of microbial dynamics, as both prominent and low-abundance taxa were revealed, proving vital for a holistic view of the microbiome. Raw reads used in this study can be found in the NCBI Sequence Read Archive (SRA) under accession number: PRJNA1051002.

Statistical analysis and visualization. Statistical analyses were performed using R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was determined at $P < 0.05$, and wherever appropriate, the statistical significance was corrected for multiple hypothesis testing using the false discovery rate (FDR) method. Bacterial and fungal alpha diversity (Shannon diversity index and number of observed OTUs) were calculated from the rarefied OTU table using 'phyloseq' package. Wilcoxon rank-sum test was performed in R. Beta diversity (Bray-Curtis dissimilarity measures) was calculated with a normalized OTU table employing CSS method using 'metagenomeSeq' package. Relative abundance analysis was performed with normalized OTUs using 'phyloseq' package. Permutational multivariate analysis of variance (PERMANOVA) was conducted using the Adonis from the vegan package (v2.5-3) (Oksanen et al., 2018). The analysis of the microbial enrichment was performed using the DESeq2 package (Love et al., 2014) to identify the significantly enriched genera in pair-wise comparisons with an adjusted P-value (FDR adjusted $P < 0.01$).

Results

Bacterial and fungal OTUs in plant root, stem, and leaf compartments.

A total of 4,214,060 bacterial and 4,134,835 fungal raw reads were sequenced from 18 samples. After quality filtering (Q = 30), DADA2 denoising, and chimera removal, OTUs with fewer than three reads and undesired taxa were excluded. All samples showed 99.78% (mean) in Good's coverage. The remaining OTUs were then classified at the genus level, yielding 90 unique bacterial OTUs and 53 unique fungal OTUs (Supplementary Table 1).

Analysis of overall bacterial endophyte compartments identified 90 OTUs. Root compartment, as expected, had the highest number of OTUs (89), followed by leaf (57) and stem (47) (Fig. 1A). A total of 41 OTUs were common
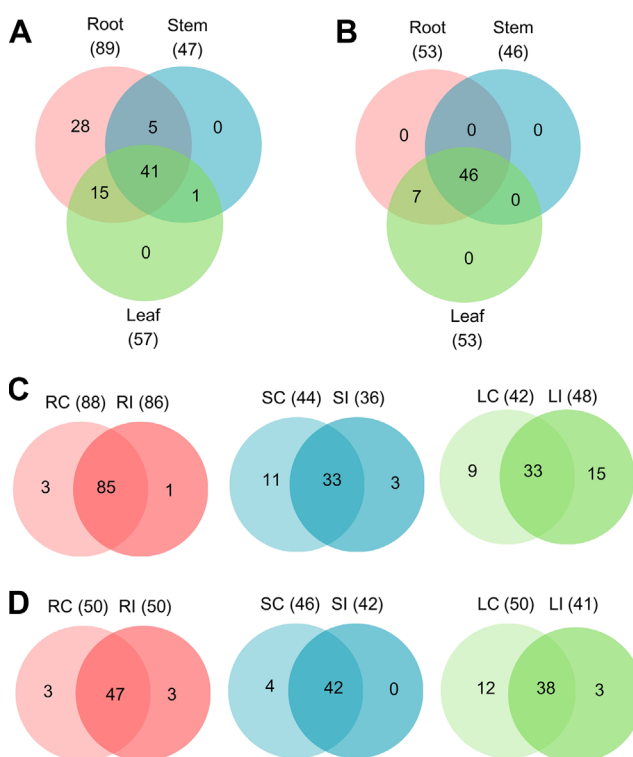

Fig. 1. Venn diagram showing distribution of operational taxonomic units (OTUs) at genus level in root, stem and leaf of rice plants. (A) Distribution of bacterial OTUs (shared and unique) (B) distribution of fungal OTUs (shared and unique) (C) distribution of bacterial OTUs (shared and unique) among control and infected compartments (D) distribution of fungal OTUs (shared and unique) among control and infected compartments. RC, root control (healthy) sample; RI, root infected; SC, stem control; SI, stem infected; LC, leaf control; LI, leaf infected sample.

to all the compartments, while 28 OTUs were exclusive to root compartment. There were no OTUs that are either present only in stem or leaf, suggesting the strong influence of root endophyte community on the formation of stem and leaf endophyte communities. Analysis of fungal endophytes yielded 53 OTUs in total, with 46 OTUs common in all the compartments, again suggesting the close relationships among the endophytic communities in different compartments (Fig. 1B). Surprisingly, no fungal OTUs were unique to the plant compartments.

Comparison of OTU sets between control and infected plants showed that most of OTUs are found in both conditions, regardless of plant compartments (Fig. 1C and D). Despite significant overlaps, two observations should be noted (1) that there are still OTUs unique to either control or infected samples, and (2) that proportions of unique taxa appear to be higher in leaf than in other compartments.

Diversity of endophyte communities. When we measured

the alpha diversity of endophytic communities using either species richness or Shannon index, we found that among all the samples, both bacterial and fungal endophytes in root show the highest alpha diversity, which is consistent with previous studies on endophytes (Fig. 2A and B) (Chen et al., 2021; Wang et al., 2016). For bacterial endophytes community diversities in stem and leaf appeared to be comparable, regardless of the infection. In contrast, fungal communities exhibited larger variations than bacterial counterparts. Such variation is especially pronounced in leaf tissue. Statistical analysis revealed significant differences in bacterial richness and Shannon diversity specifically within the root compartment ($P < 0.05$) (Fig. 2A). However, there were no significant differences observed in bacterial and fungal richness or Shannon diversity indices among the stem and leaf groups between the control and infected samples. Additionally, no significant difference was noted in fungal richness or Shannon diversity within the root samples ($P > 0.05$) (Fig. 2A and B).
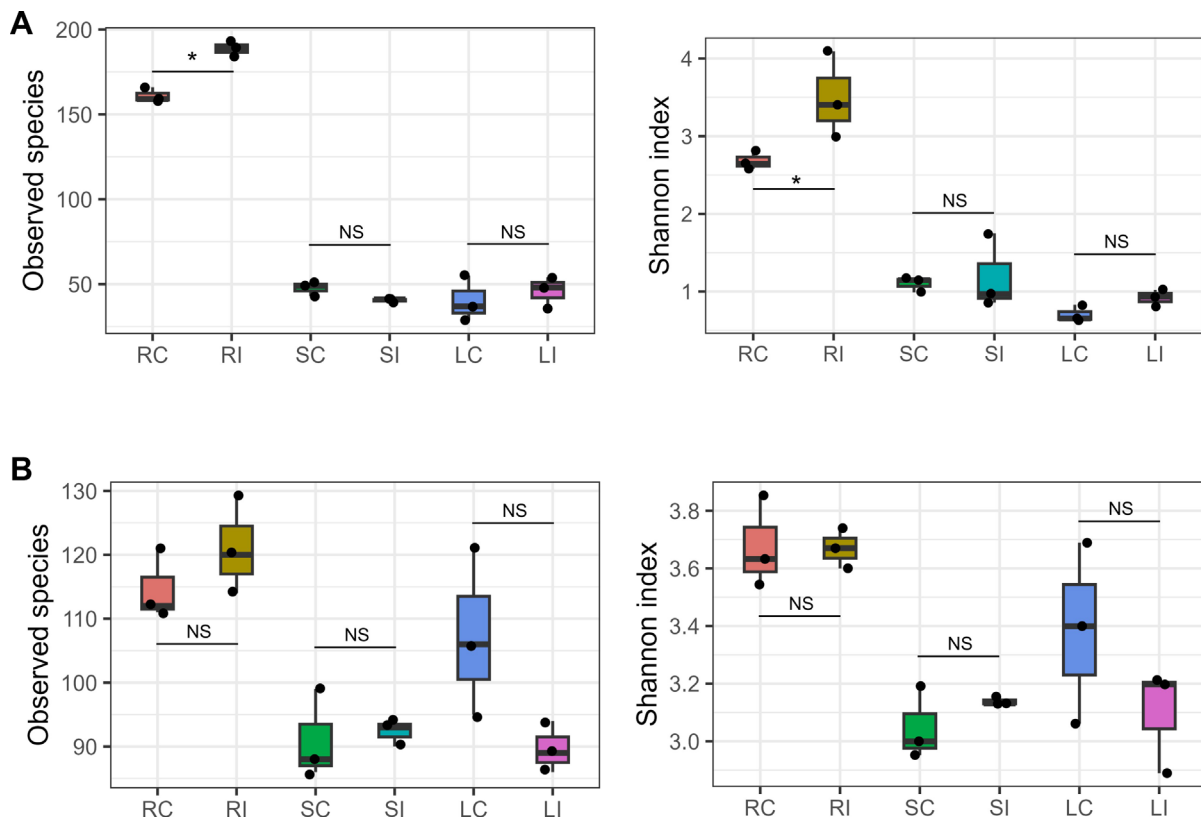

Fig. 2. Alpha diversity of the endophytic microbial communities in root, stem, and leaf of rice plants was assessed using rarefied operational taxonomic unit (OTU) file. (A) Boxplot of species richness and Shannon index of bacterial endophytic communities. Boxes indicate interquartile range (75% to 25% of the data, $n = 3$) and the median value is shown as a line in the box. (B) Boxplot of species richness and Shannon index of fungal endophytic communities. Boxes indicate interquartile range (75% to 25% of the data, $n = 3$) and the median value is shown as a line in the box. Asterisks indicate significant differences with P-values <0.05 (*); NS, not significant. RC, root control (healthy) sample; RI, root infected; SC, stem control; SI, stem infected; LC, leaf control; LI, leaf infected sample.

To compare the structures of endophyte communities across the plant compartments and treatment, principal coordinate analysis (PCoA) was performed using Bray-Curtis dissimilarity. The PCoA of endophyte communities revealed that the community structures are predominantly driven by the types of plant compartments in the rice plants ($P < 0.05$). In particular, the influence of the plant compartments seemed to be stronger for bacterial endophytes than fungal endophytes (Fig. 3A). In case of fungi, leaf endophyte community showed large variation in its structure (Fig. 3B). However, infection with the rice blast did not significantly impact the overall structures of bacterial and fungal endophytes across the compartments as indicated by PERMANOVA analysis ($P > 0.05$) (Supplementary Fig. 2A and B).

Impact of *M. oryzae* infection on endophytic microbial communities in rice. Although there were no dramatic changes in the community structures of endophytes in response to the rice blast infection, there were still taxa showing variations in relative abundances or in presence/absence across the samples. To get more insight into the spatial (plant compartment) and disease-related (control vs. infection) patterns of endophytes, we combined phylogenetic analysis with the information on relative abundances of OTUs (Fig. 4A and B). For clarity and simplicity purposes, we included only the taxa, of which abundances are >0.5 and 0.1% for bacteria and fungi, respectively, across the samples. Such comparative analysis of the bacterial communities revealed a few taxa that are unique to root tissue and of which abundances are higher in the infected root than the control. These include OTU271 g_*Granulicella*, OTU471 g_*Nocardioides*, and OTU1267 g_*Rhodanobacter*. There were also taxa that were unique to either the control or the infected plants. For example, OTU901 o_Saccharimonadales, OTU1241 g_*Chujaibacter*, OTU606 f_Pirellulaceae, OTU540 o_Armatimonadales, OTU547 o_Armatimonadales, OTU693 f_Chitinophagaceae were observed only in the RI sample while OTU979 g_*Staphylococcus* and OTU1193 g_*Cronobacter* was solely found in the RC and SC samples respectively. These findings hint at the possibility of these bacteria being associated with plant health status.

Unlike bacterial endophytes displaying tissue-specific patterns, many fungal endophytes didn't seem to be restricted by the type of plant tissues (Fig. 4B). Nevertheless, there are some fungal taxa that are specific to or have higher abundances in particular plant compartments. For example, OTU1846 c_Pucciniomycetes was unique to the infected stem tissue, while OTU2430 g_*Botrytis* was found only in
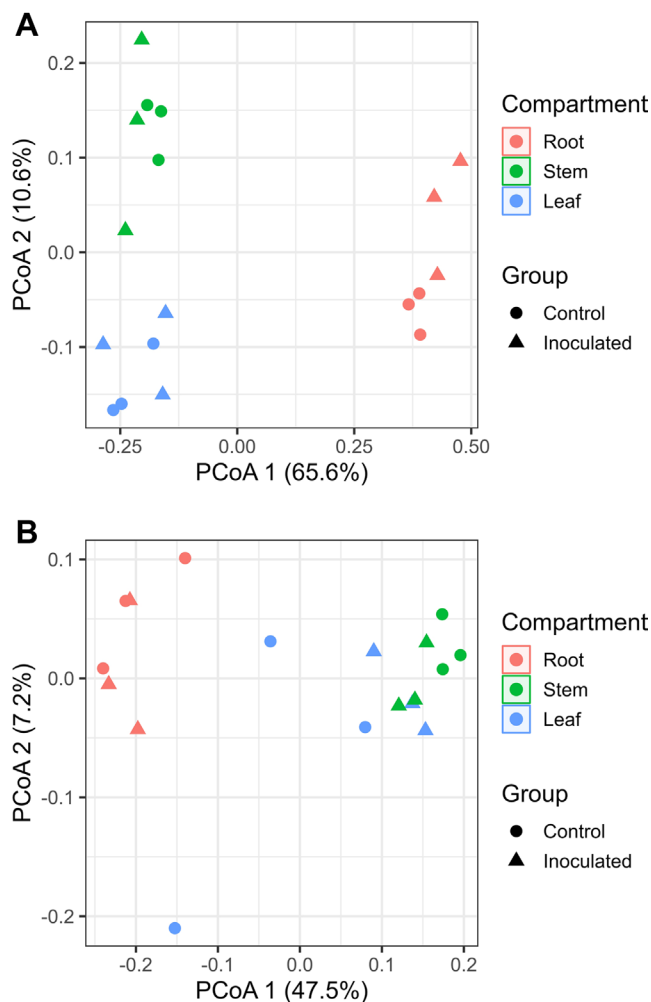

Fig. 3. Beta diversity of the endophytic microbial communities in root, stem, and leaf of rice plants. (A) Principal coordinate analysis (PCoA) plot using Bray-Curtis dissimilarity distance depicting endophytic bacterial community in root, stem, and leaf of rice plants ($n = 3$). Statistical analysis was conducted using permutational multivariate analysis of variance (PERMANOVA). PERMANOVA showed significant difference ($P = 0.001$) with R^2 value 0.72919. (B) PCoA plot using Bray-Curtis dissimilarity distance depicting endophytic fungal community in root, stem, and leaf of rice plants ($n = 3$). Statistical analysis was conducted using PERMANOVA. PERMANOVA showed significant difference ($P = 0.001$) with R^2 value 0.45624.

healthy leaf tissue and OTU2596 g_*Setophoma* was found solely in stem control. OTU2088 g_*Blastobotrys* exhibited higher abundance in infected leaf. Meanwhile, fungal taxa such as OTU2208 g_*Fusarium* exhibited higher abundance in healthy root control, while, OTU2390 f_Cephalothecaceae and OTU2457 g_*Oidiodendron* exhibited higher abundances in the healthy root control and infected root tissue respectively. OTU1966 c_Agaricomycetes exhibited
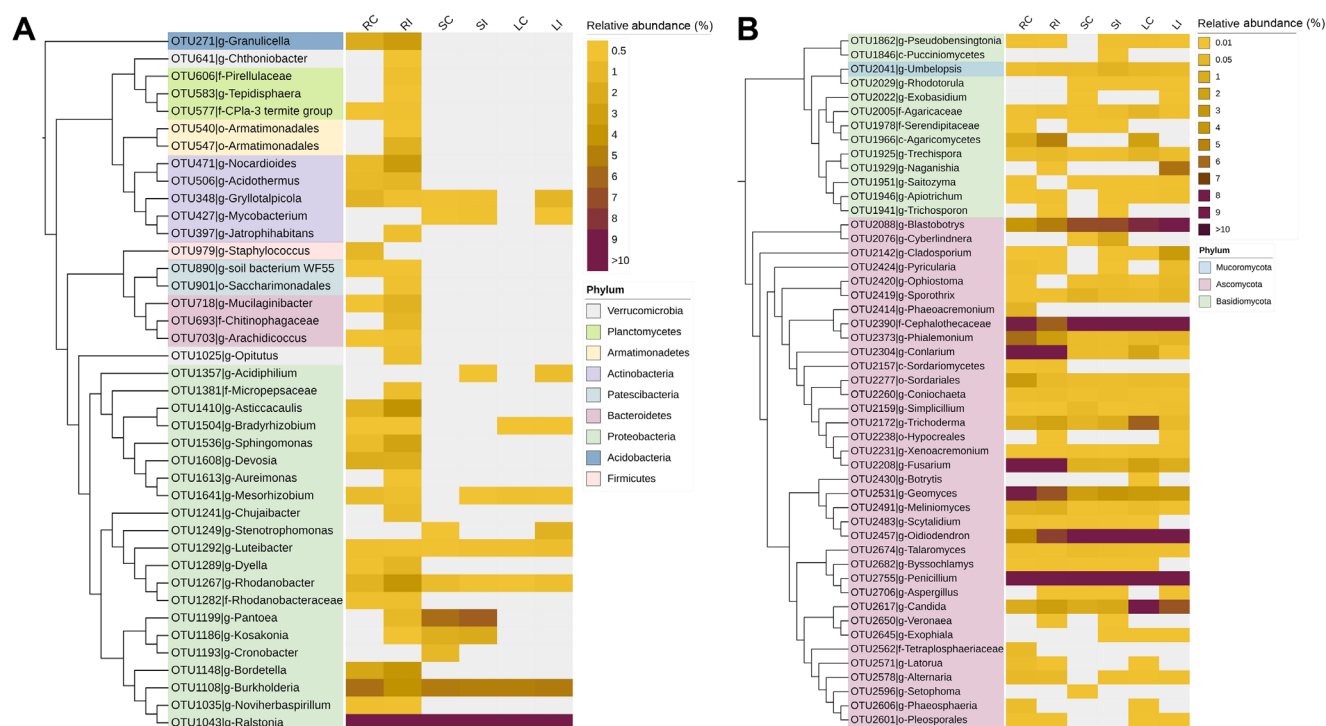
Fig. 4. Phylogenetic tree of bacterial (A) and fungal operational taxonomic units (OTUs) (B) with relative abundance information across the samples. Only the taxa having relative abundance greater than 0.5% and 0.1%, respectively, were used. Relative abundances of OTUs are shown as heatmap to the right of the phylogenetic tree. Figure was produced from the interactive Tree of Life (iTOL, http://itol.embl.de/). RC, root control (healthy) sample; RI, root infected; SC, stem control; SI, stem infected; LC, leaf control; LI, leaf infected sample.

higher abundance in infected root tissue as well as healthy leaf tissue. OTU2172 g_*Trichoderma* exhibited higher abundance in healthy leaf tissue. These results underscore the nature of endophytic microbial communities within different compartments when confronted with *M. oryzae*. The distinct microbial signatures associated with health and infected statuses may offer insights into the biological responses of rice to pathogen attack, with implications for disease management and plant health.

Identification of differentially abundant taxa. In order to identify differentially abundant taxa among samples, DESeq2 was employed to analyse all OTUs, ensuring a comprehensive view that included low-abundance taxa. This analysis revealed distinct bacterial taxa in root and leaf compartments in comparing control and infected plants. In roots, 14 OTUs displayed differential abundance. Specifically, 1 OTU was enriched in RC sample, while 13 were enriched in RI samples. Our analysis showed that OTU1580 g_*Methylovirgula* (Proteobacteria)was more enriched in the RC samples, whereas OTU429 g_*Mycobacterium*, OTU1241 and 1247 g_*Chujaibacter*, OTU1530

g_*Sphingomonas*, OTU693 f_Chitinophagaceae, OTU720 g_*Mucilaginibacter* (Bacteroidetes), OTU602 o_Planctomycetales, OTU606 f_Pirellulaceae (Planctomycetes), and OTU540 and 547 o_Armatimonadales (Armatimonadetes), OTU938 o_Candidatusadlerbacteria, OTU1210 f_Enterobacteriaceae, and OTU1264 f_Rhodanobacteraceae showed significant enrichment in the RI samples. In the stem compartment, only OTU1210 f_Enterobacteriaceae demonstrated differential enrichment in SI sample. In leaf compartment, OTU1249 g_*Stenotrophomonas* was differentially abundant in LI sample (|log2 fold change| > 2 and <–2, FDR<0.01). However, none of the bacterial OTUs showed notable enrichment in the LC samples (|log2 fold change| > 2 and <–2, FDR < 0.01) (Supplementary Table 2).

Among the fungal endophytes, OTU2200 g_*Fusarium* was significantly more abundant in the RC, but no OTU was significantly enriched in RI sample. To gain a comprehensive understanding of the origin and dynamics of root endophytes, we conducted a thorough analysis of bacterial and fungal communities in both bulk soil and the rhizosphere at genus level (Supplementary Fig. 3A and B).

Fusarium, a notable component of the soil microbiome, was observed in substantial quantities in both environments (Supplementary Fig. 3B). This dual observation of *Fusarium*'s presence in both bulk soil and rhizosphere highlights its ubiquity within the soil ecosystem and its potential impact on the dynamics of root endophytes. In stem samples, five fungal OTUs (OTU2229 o_Hypocreales, OTU2321 g_*Conlarium*, OTU2406 p_Ascomycota, OTU2670 g_*Talaromyces*, and OTU2719g_*Penicillium*) were enriched in the SC samples, while six fungal OTUs (OTU2108 p_Ascomycota, OTU2276 o_Sordariales, OTU2304 g_*Conlarium*, OTU2510 p_Ascomycota, OTU2723 and OTU2779 g_*Penicillium*) were significantly enriched in the SI samples. In leaf samples, eight OTUs (OTU1964 and OTU1966 c_Agaricomycetes, OTU2179 g_*Trichoderma*, OTU2304 g_*Conlarium*, OTU2312 and OTU2324 c_Sordariomycetes, OTU2524 c_Leotiomycetes, OTU2670 g_*Talaromyces*) were enriched in LC samples, whereas four OTUs (OTU2121 p_Ascomycota, OTU2211 g_*Fusarium*, OTU2424 g_*Pyricularia*, OTU2458 g_*Oidiodendron*) were significantly more abundant in the LI samples (|log2 fold change| > 2 and <−2, FDR < 0.01) (Supplementary Table 3).

To summarize, our analysis has revealed notable changes in microbial communities in response to *M. oryzae* infection across different plant tissues. These changes have been particularly evident in the abundance of bacterial and fungal OTUs in root and leaf samples, while stem samples have exhibited less pronounced differences.

Discussion

In this study, we sought to present a comprehensive examination of the diversity and distribution patterns of bacterial and fungal endophytes inhabiting various plant compartments (including roots, stems, and leaves) of rice plants. Our analysis highlights that the structural composition of bacterial and fungal endophytes exhibited notable dissimilarities across different plant tissues. Additionally, we investigated how the structural composition of these communities was affected by the presence of blast disease. Contrary to previous studies (Jakuschkin et al., 2016; Mannaa and Seo, 2021; Musonerimana et al., 2020), we did not observe statistically significant shifts in the structural composition of these microbial communities between control and infected groups across different plant compartments.

One potential explanation for the contradiction between our results and previous studies could be the limited number of replicates employed in our study. This limitation may have impacted our statistical power, making it difficult to detect subtle changes in community structure. Despite this limitation, it is essential to recognize that our findings do not negate the potential influence of infection on the plant microbiome; rather, they suggest that our study may not have had the necessary statistical robustness to detect such effects conclusively.

Interestingly, our results revealed specific OTU enrichments in infected samples, suggesting localized microbial responses to the pathogen challenge rather than broad-scale community restructuring. This nuanced observation aligns with the work of Duan et al. (2021), who reported that *M. oryzae* infection triggers systemic defense mechanisms in rice, leading to the accumulation of defensive metabolites that enhance plant immunity. Drawing from Duan et al.'s insights, the lack of pronounced shifts in our microbial community analysis might not directly result from pathogen colonization but could reflect the rice plant's systemic response to infection. This notion is supported by the enriched OTUs (Supplementary Tables 2 and 3), which might be associated with the plant's defensive reaction rather than the pathogen's direct effects.

Our analysis using DESeq2 revealed noteworthy enrichment of specific OTUs within the root and leaf compartments of the infected group (Supplementary Tables 2 and 3). This enrichment suggests that certain microbial taxa exhibited a preferential response to the infection within specific plant compartments. This nuanced change in the microbial community structure within these compartments hints at the intricate nature of the plant-pathogen-microbe interactions, where infection-induced shifts may not always be globally significant but can be discerned at a finer taxonomic resolution. In the root compartment, most of the bacterial OTUs showing significant differences in abundance distribution were RI-enriched, with only exception of OTU1580 g_*Methylovirgula*. *Methylovirgula*, a nitrogen-fixing bacterium (Dedysh et al., 2016), enrichment in root control plants suggests that it plays a beneficial role in nitrogen fixation, contributing to the overall health and nutrient balance of the plants in a non-stressed environment. On the other hand, the enrichment of OTU1530 g_*Sphingomonas* in RI samples is noteworthy due to its known associations with indole-3-acetic acid (IAA) and gibberellin production (Khan et al., 2014). While gibberellins are traditionally associated with plant growth, they also play a role in signaling pathways (Richards et al., 2001), affecting defense mechanisms involving jasmonic acid and salicylic acid (Pieterse et al., 2012). This suggests that *Sphingomonas*, with its potential to produce gibberellins, might influence both plant growth and defense responses. In the leaf compartment, our analysis identified significant enrichment

of OTU1249 g_*Stenotrophomonas* in response to pathogen infection. This aligns with the well-documented benefits of *Stenotrophomonas* species in promoting plant growth and defense. *Stenotrophomonas* spp., are known for producing plant growth hormone IAA (Suckstorff and Berg, 2003), nitrogen fixation (Liba et al., 2006; Park et al., 2005), and sulphur oxidation (Banerjee and Yesmin, 2002), which benefit plant health. These bacteria are commonly associated with plants, residing in the rhizosphere and internal plant tissues, including roots and stems (Berg et al., 1996; Juhnke and des Jardin, 1989). They also exhibit antifungal activity and produce volatile compounds that hinder plant pathogens (Kai et al., 2007; Stotzky and Schenck, 1976; Wheatley, 2002). One remarkable illustration of their role in plant defense is exemplified by *Stenotrophomonas rhizophila*-JLS11, which has been identified for its active involvement in the suppression of rice blast (Wei et al., 2020); a common and detrimental rice pathogen. These findings underscore the potential significance of *Stenotrophomonas* species in enhancing plant resilience and health during pathogenic challenges. Expanding our analysis to fungi, the enrichment of OTU2179 g_*Trichoderma* in LC samples is particularly notable. *Trichoderma*, widely recognized for its effectiveness in the biological control of plant diseases, as demonstrated by Tyśkiewicz et al. (2022), holds a prominent global position in both research and practical disease management initiatives. These endophytic fungi, known as *Trichoderma*, inhabit leaf tissues, roots, and sapwood, imparting a range of benefits to their host (Cummings et al., 2016). *Trichoderma*, as identified by Nguyen et al. (2016), serves as a significant microbial antagonist against *M. oryzae*. It employs various mechanisms to provide biocontrol benefits, including competition with pathogens for ecological compartments and nutrients, secretion of bioactive compounds with antagonistic properties, and stimulation of the plant's innate defense responses to resist pathogen attacks (Vurukonda et al., 2018). OTU2200 g_*Fusarium* was notably enriched in the RC samples, indicating its significant presence within the root microbiome under normal conditions. *Fusarium*, a diverse group of fungi, plays a pivotal role as a pathogen with a broad host range, affecting numerous plants and cereals vital for both human and animal nutrition. It specifically targets various plant parts, including grains, seedlings, heads, roots, and stems, resulting in a spectrum of diseases, reduced commercial yields, and a decline in crop quality (Lamprecht et al., 2011). The shift in *Fusarium* abundance, with enrichment in RC samples and depletion in root infected samples, aligns with the 'cry for help' hypothesis (Bakker et al., 2018; Liu et al., 2019, 2020), suggesting that rice plants actively manipulate their root microbiome in response to external stress, such as pathogen infection. This dynamic response, characterized by changes in *Fusarium* abundance, exemplifies a survival strategy conserved across the plant kingdom, as proposed by the 'cry for help' hypothesis.

Our comprehensive examination of samples from roots, stems, and leaves provides a holistic perspective on how endophytes react to the presence of *M. oryzae* in rice plants. These discoveries yield valuable insights into the dynamics of the endophytic microbiome and its significance for anticipating and addressing the disease. The results highlight the intricate nature of plant-microbe interactions, revealing the diverse responses of specific bacterial and fungal taxa to *M. oryzae* in various plant tissues. Moreover, the observed enrichment patterns in root and leaf compartment following infection imply that distinct microbial interactions and defense mechanisms are at play in each tissue. To further our understanding of plant health and microbial responses to infection, future research should focus on uncovering the roles and functions of these enriched microbial taxa within each compartment.

Conflicts of Interest

Junhyun Jeon, a contributing editor of the Plant Pathology Journal, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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