Diversity Analysis of Endophytic Fungal Communities in *Oryza sativa* L. Using ITS Metabarcoding

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ABSTRACT

Background: Endophytic fungi are present in all parts of a plant without showing symptoms and aid the host in growth, nutrient uptake, and resistance against different stresses. In rice (*Oryza sativa* L.), being one of the major food crops of the world, analyzing these communities is important for sustainability in agriculture.

Objective: The study was aimed at studying the diversity, richness, and composition of fungal endophyte communities as present in the root, stem, and leaf of the rice plant using DNA barcoding techniques.

Method: The sterilized root, stem, and leaf samples were collected from the rice plants grown in the fields (n = 5 biological replicates per tissue). The fungal regions of ITS1 and ITS2 were PCR amplified and sequenced on the Illumina MiSeq platform.

Results: The worked yielded a total of 612,340 quality reads, producing 486 fungal ASVs from five phyla. Ascomycota (68.4%) and Basidiomycota (21.7%) are present in all tissues. The genera that were most common include Fusarium, Trichoderma, and Curvularia. The highest level of alpha diversity was exhibited by root tissues, with rapidly evolving debris, whereas the beta diversity showed a significant clustering specific to the tissues (PERMANOVA, $p < 0.05$).

Conclusion: The ITS metabarcoding method used helped establish the presence of an endophytic fungal community in the rice plants with the ability to promote the growth of plants and control diseases.

Keywords: Endophytic fungi; Rice (*Oryza sativa*); ITS sequencing; Mycobiome; Fungal diversity; Sustainable agriculture.

1. Introduction

Oryza sativa L., or rice, is a staple food in the diets of over half the earth's human inhabitants, hence the importance of understanding its microbiome. Endophytic fungi are organisms living within plants without causing any sicknesses, and they play a significant role in promoting plant health along with other microbes by improving nutrient assimilation, regulating phytohormones, and providing resistance against biotic and abiotic stresses. The importance of plant-microbe interactions is increasing as their contribution to sustainability is recognized.

The traditional culture-based techniques significantly underestimate fungi diversity; many endophytes are slow-growing, cannot be cultured in vitro, or cannot compete successfully with the other organisms [4]. Next generation sequencing (NGS), especially ITS metabarcoding, overcomes the limitations of the traditional techniques by sequencing the genetic material of the fungi [5, 6]. The ITS region is variable enough to be used as a specific barcode to identify the presence of fungi in samples [7].

Much research is being conducted on the rice microbiome, while diversity of endophytic fungi from different plant tissues remains poorly studied. In the following paper, we investigate endophytic fungi from rice root, stem, and leaf tissues using ITS metabarcoding and clarify diversity of the fungi in rice.

Materials and Methods

Sample Collection

Healthy rice plants (cv. IR64) were sampled at the tillering stage from an experimental field. Root, stem, and leaf tissues were collected separately, with five biological replicates per tissue type. Samples were surface-sterilized sequentially with 70% ethanol (1 min), 2% sodium hypochlorite (3 min), and sterile distilled water (three rinses) to eliminate epiphytic organisms, with sterilization efficacy confirmed by plating rinse water aliquots.

DNA Extraction

Genomic DNA was extracted using a CTAB-based protocol optimized for plant tissue. DNA integrity was verified via 1% agarose gel electrophoresis, and concentration/purity were assessed using a NanoDrop spectrophotometer (A260/280 ratio 1.8–2.0) and Qubit fluorometry.

ITS Metabarcoding

The ITS1 region was amplified using primers ITS1F/ITS2 with Illumina-compatible adapters. PCR products were purified, indexed, and pooled into sequencing libraries following the Illumina Nextera XT protocol, then sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end).

Bioinformatics Analysis

Raw reads underwent adapter trimming and quality filtering (Q ≥ 30) using Cutadapt and DADA2. Chimeric sequences were removed, and ASVs were inferred using the DADA2 pipeline. Taxonomic classification was performed against the UNITE fungal ITS reference database.

Diversity and Statistical Analysis

Alpha diversity (Shannon, Simpson, Chao1, evenness) and beta diversity (Bray–Curtis dissimilarity, PCoA) were calculated using QIIME2 and the vegan package in R. Rarefaction curves confirmed sequencing depth adequacy. Group differences were tested using ANOVA and PERMANOVA, with significance set at $p < 0.05$.

Results

Sequencing Output and Taxonomic Composition

Sequencing generated 612,340 quality-filtered reads clustering into 486 ASVs (Table 1). Ascomycota and Basidiomycota were the dominant phyla across all tissues, with *Fusarium*, *Trichoderma*, *Curvularia*, *Aspergillus*, and *Colletotrichum* as the most abundant genera (Table 3).

Table 1: Sample collection and sequencing statistics

Sample Type	Raw Reads	Filtered Reads	ASVs	Coverage (%)
Root	218,450	205,120	214	98.6
Stem	196,300	184,760	158	97.9
Leaf	231,900	222,460	114	96.4
Total	646,650	612,340	486	—

Diversity Patterns

Root tissues showed the highest alpha diversity (Shannon = 3.42, Chao1 = 231), followed by stem and leaf (Table 2). PCoA based on Bray–Curtis distances showed distinct tissue-specific clustering (PERMANOVA, $R^2 = 0.34$, $p = 0.001$), indicating compartmentalized fungal assembly.

Table 2: Diversity indices of fungal communities

Tissue	Shannon Index	Simpson Index	Chao1	Evenness	Observed OTUs
Root	3.42	0.91	231	0.78	214
Stem	2.87	0.85	172	0.71	158
Leaf	2.31	0.79	126	0.65	114

Relative Abundance and Ecological Function

Dominant taxa displayed functionally relevant roles, ranging from biocontrol to latent pathogenicity (Table 3), suggesting a functionally diverse resident mycobiome.

Table 3: Relative abundance of dominant fungal taxa

Phylum	Genus	Relative Abundance (%)	Ecological Function
Ascomycota	*Fusarium*	24.6	Saprotroph/opportunistic pathogen
Ascomycota	*Trichoderma*	18.3	Biocontrol agent, growth promoter
Ascomycota	*Curvularia*	12.1	Stress-tolerance endophyte
Basidiomycota	*Rhizoctonia*	9.8	Latent pathogen
Ascomycota	*Colletotrichum*	8.4	Latent pathogen/endophyte

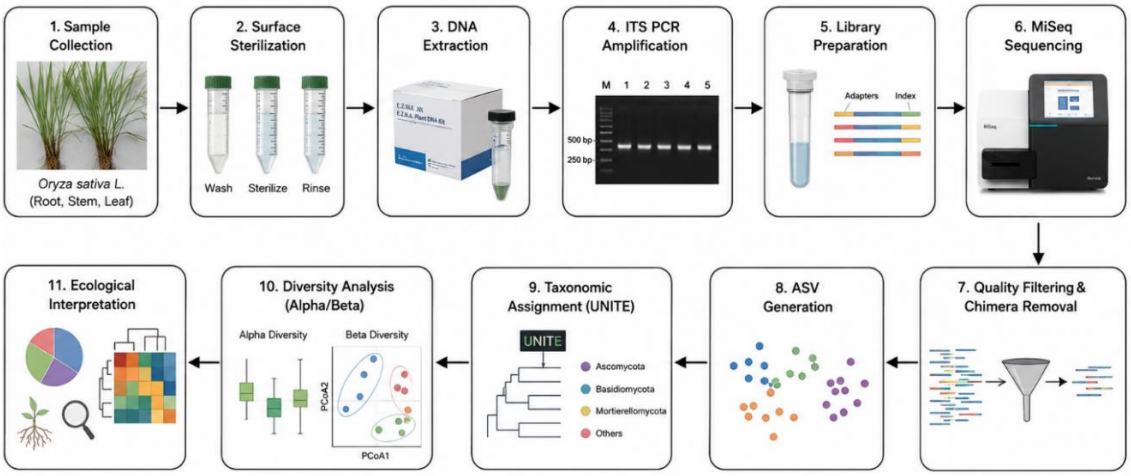

Fig 1: Workflow of ITS metabarcoding analysis of endophytic fungal communities in *Oryza sativa* L. Sample Collection → Surface Sterilization → DNA Extraction → ITS PCR Amplification → Library Preparation → MiSeq Sequencing → Quality Filtering & Chimera Removal → ASV Generation → Taxonomic Assignment (UNITE) → Diversity Analysis (Alpha/Beta) → Ecological Interpretation

Discussion

The predominance of Ascomycota and Basidiomycota aligns with previously reported patterns in graminaceous crops, reflecting their ecological versatility and adaptation to internal plant niches [8, 9]. The higher root diversity likely reflects direct rhizosphere-to-endosphere microbial recruitment, whereas aerial tissues host more selectively filtered communities [10]. Tissue-specific clustering observed via PCoA supports the concept of niche differentiation, wherein host physiology and microhabitat conditions shape distinct fungal assemblages [11].

Functionally, the co-occurrence of biocontrol agents such as *Trichoderma* alongside opportunistic taxa like *Fusarium* illustrates the dynamic equilibrium underlying rice endophytic health, where beneficial and latent pathogenic fungi coexist under regulated conditions [12]. Compared to culture-dependent surveys, ITS metabarcoding captured substantially greater richness, corroborating its superiority in resolving cryptic and slow-growing taxa [13].

These findings support the potential application of beneficial endophytes, particularly *Trichoderma* species, as biofertilizers and biocontrol agents in integrated rice management [14]. However, PCR amplification bias, primer specificity limitations, and reference database incompleteness remain inherent constraints of metabarcoding, potentially skewing relative abundance estimates [15]. Future work integrating metagenomics and metatranscriptomics would enable functional validation of taxa identified here, moving beyond taxonomic inventory toward mechanistic understanding of plant–fungal interactions.

Conclusion

This research confirms that ITS metabarcoding is capable of solving the taxonomic diversity and tissue-specific patterns of the endophyte fungi in rice. It showed that the mycobiome is represented primarily by Ascomycota, which contribute ecologically diverse functionalities. The identification of fungi containing useful taxa, such as Trichoderma, helps in formulating biofertilizers and biocontrol methods in rice cultivation. Further research is needed to transform the results into applicable technologies, and this calls for large-scale studies across several seasons and rice varieties.

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