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Molecular Identification of Endophytic Fungi Isolated from Axonopus compressus (Lawn grass) around Tirunelveli district

Abstract: Endophytes are associated with grasses and produce positive and negative effects for host. It enhances the biometric parameters and increase the biomass value. Some endophytes produce active substances that exert a negative influence on grass fed life stock. The aim of the research was analysing the diversity of endophytic fungi on lawn grass. Fungal identification was carried out through morphological characteristic and molecular analysis of DNA sequence generated from ITS rRNA region. Totally ten species of endophytic fungi were isolated from 50 plant sample such as *Aspergillus niger, Aspergillus fumigatus*, *Alternaria alternata, Alternaria solani, Cladosporium cladosporioides Epicoccum nigrum, Fusarium chlamydosporum, Fusarium lateritium and Pythium acanthicum,* and. From the above-mentioned species the most prevalent genus was *Alternaria* and *Fusarium*

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Introduction

Endophytic fungi, which reside within plant tissues without causing immediate harm to their hosts, play a crucial role in plant health and ecology (Petrini 1992; Avezedo 2000). They make symbiotic relations with all plants and mediated plant resistance against biotic stress and abiotic stress. (Saikkonen et al., 1998; Kuldau et al., 2008; Gupta et al., 2021) These fungi are known for their ability to enhance plant resistance to various biotic and abiotic stresses, including pathogens, pests, and environmental extremes. In addition, endophytic fungi often produce a range of bioactive compounds with potential applications in agriculture, medicine, and industry. According to previous research in tropical region showed that endophytic fungi have detrimental effect on some insects from various taxonomic groups. For instance, endophytic fungi on grasses have been reported to inhibit the growth and development of the feeding insects. (Prajapati et al., 2021)

Axonopus compressus, commonly known as carpet grass or lawn grass, is a short-spreading grass that forms creeping stems with long stolon spread by above ground runners. Also is widely distributed species used in landscaping and erosion control due to its rapid growth and dense coverage. It forms dense turf which prevents soil erosion and land sliding. Despite its widespread use, there is limited research on the endophytic fungal communities associated with *Axonopus compressus*, particularly in the context of the Tirunelveli district's unique environmental conditions. Such grasses are found in all ecosystems around the world, and they play important ecological and economic roles.

Exploring the endophytic fungal diversity within *Axonopus compressus* from the Thirunelveli district can provide insights into the symbiotic relationships between these fungi and their host grass. Understanding this relationship is essential for several reasons:

- Ecological Health: Identifying endophytic fungi can reveal how they contribute to the health and resilience of *Axonopus compressus*, potentially offering insights into natural mechanisms for stress tolerance and pathogen resistance. According to various research done by Uenoet al., 2024; Perez, Gundel, Ghersa, & Omacini, 2013; Zabalgogeazcoa, 2012 fungi colonize the surface, intercellular

spaces, tissues and cells of grasses both in the phyllosphere and in the rhizosphere. Fungi can exert pathogenic, parasitic and mutualistic effects on grasses.

- Biotechnological Potential: Many endophytic fungi produce secondary metabolites with pharmacological and agricultural benefits. Identifying and characterizing these fungi could lead to the discovery of novel bioactive compounds. Systemic fungal endophytes enter several interactions with grasses, including antagonistic, asymptomatic and mixed interactions (Zabalgogeazcoa., 2012). Non-systemic endophytes can be phytopathogenic, entomopathogenic, mutualistic, saprotrophic and latent saprotrophic fungi, capable of producing phytohormones and allelochemical compounds (Cruz et al., 2020)

Environmental Adaptation: The specific fungal species present in *Axonopus compressus* could offer clues about the adaptability of the grass to local environmental conditions, including soil composition, moisture levels, and climatic factors.

Objectives of the Study

JThe primary objective of this study is to isolate and molecularly identify endophytic fungi from *Axonopus compressus* in the Tirunelveli district. The research aims to:

1. Isolate Endophytic Fungi: Extract and culture endophytic fungi from different parts of the grass to obtain a diverse fungal collection.
2. Characterize Morphological Features: Examine the morphological characteristics of the isolated fungi to provide preliminary identification.

Molecular Identification: Utilize molecular techniques, including DNA sequencing of ribosomal RNA genes, to accurately identify the fungal species present.

Methodology

1. Sample Collection

- Source: Collect samples of *Axonopus compressus* from different locations around the Tirunelveli district. Ensure to collect both healthy and symptomatic parts of the grass to obtain a diverse range of endophytes.
- Processing: Totally of 50 plants were collected and transport the samples in sterile containers to the laboratory and processed them to avoid contamination.

2. Isolation of Endophytic Fungi

2.1. Surface Sterilization: In the laboratory, the collected plant samples were surface sterilized with disinfection (70% ethanol for 5 s, 1% sodium hypochlorite for 20 s, rinsed three times in sterile deionized water) to remove external contaminants. Rinse with sterile water.

2.2. Tissue Extraction: Cut the sterilized grass into small pieces (1 cm) and place them on a suitable fungal isolation medium, such as. From Each plant one leaf samples with length of 1 cm each were placed on Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA). The inoculated samples were incubated at 25°C for 21 days. The fungal growth index was estimated at 0.75 per leaf. Fungal colonies were transferred to fresh PDA, and fungal mycelia were sampled after 14 days for molecular analyses.

3. Morphological Identification

Observe and document the morphological characteristics of the fungal colonies (e.g., colour, texture, growth pattern). Use a microscope to examine the fungal structures, including hyphae, spores, and conidia, to assist in preliminary identification.

4. Molecular Identification

4.1. DNA Extraction: DNA isolation of the fungal isolates was done with the Cetyl trimethyl ammonium bromide (CTAB) method(Kenjar et al.,2021). After incubation 5gm of fungal mycelium was ground to a fine powder with sterile mortar & pestle and transfer to a plastic sterile tube. The pre-warmed isolation buffer (10 ml) was added to fine powder and mixed properly then it was incubated for 60 min at 65°C with occasional stirring. After incubation, tubes were left at room temperature for a few mins for cooling. Ten ml of chloroform: isoamyl alcohol (24: 1) was added to mixture and mixed properly. Centrifuge was done at 10,000 rpm for 20 mins at room temperature (24°C). After centrifugation, the supernatant was transferred to fresh tube. Ice- cold

isopropanol (0.6 volume) and 3M sodium acetate (0.1 volume) were added to the aqueous phase and incubated at -20°C for 30 mins. Reaction mixture was centrifuged at 10,000 rpm for 10 min at 4°C after incubation, and then the aqueous phase was discarded. DNA pellet was obtained and was washed with ethanol (75%) and was centrifuged at 10,000 rpm for 10 min at 4°C, the aqueous phase was discarded. The DNA pellet was dried and dissolved in 200 µl of distilled water.

4.2. PCR Amplification: Amplify fungal DNA regions using specific primers. Fungal DNA was multiplied using 18s rRNA gene primers. Internal Transcribed Spacer (ITS): ITS1 and ITS4 primers are often used for fungal identification. ITS1F (5'-TCCGTAGGTGAACCTGCGG) primer and ITS4R (5'-TCCTCCGCTTATTGATATGC) primer. The obtained sequence data were aligned by using the BLAST software (http://blast.ncbi.nlm.nih.gov) algorithm at NCBI.

- Sequencing: Sequence the PCR products to obtain the DNA sequence of the targeted regions.

5. Data Analysis

Sequence Analysis: Fragments of the ITS sequence were analysed in the MEGA6 program (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) with the Clustal W function. This helps in identifying the species or genus of the isolated fungi.

Phylogenetic Analysis: The obtained nucleotide matrix was used to determine the most appropriate evolutionary model and to develop a phylogenetic tree (maximum likelihood).This methodology provides a structured approach to identifying endophytic fungi at the molecular level, ensuring accurate and reliable results.

Results

The molecular identification of endophytic fungi isolated from *Axonopus compressus* in the Tirunelveli district yielded a diverse range of fungal species. The distribution of the isolates and their frequencies are summarized as follows:

Figure-1 Colony Images and Microscopic images of Isolated endophytic fungus

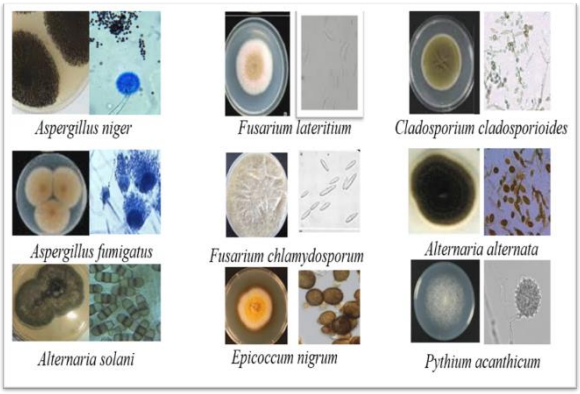
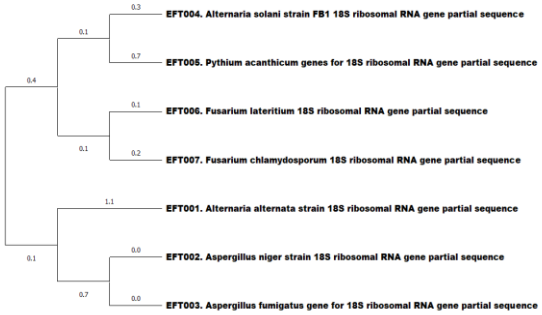


Table-1 Distribution of the isolates and their frequencies

S. No	Endophytic fungi	Number of isolates (n)	Frequency (%)
1	*Alternaria alternata*	9	42
2	*Alternaria solani*	5	23
3	*Epicoccum nigrum*	1	4
4	*Fusarium chlamydosporum*	3	14
5	*Fusarium lateritium*	1	23
6	*Aspergillus niger*	1	4
7	*Aspergillus fumigatus*	1	4
8	*Pythium acanthicum*	1	4
9	*Cladosporium cladosporioides*	1	4
10.	*Fusarium lateritium*	5	23
	Total number of specimens (S)	**21**	
	Total number of taxa (T)	**10**	

Figure-2 Phylogenetic analysis of isolated endophytic fungi



Evolutionary analysis by Maximum Likelihood Method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei., 1993) The tree with the highest log likelihood (-5794.32) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. This analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1754 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018)

Discussion

The study identified a total of 10 fungal taxa from *Axonopus compressus*, with a total of 21 isolates. The fungal community was predominantly composed of *Alternaria alternata*, which was the most frequently isolated species, representing 42% of the total isolates. This high frequency suggests that *A. alternata* may have a prominent role in the endophytic community of this grass species.

Alternaria alternata and *Fusarium lateritium* were the most abundant, each constituting 23% of the total isolates. This indicates that these species might be particularly well-suited to the endophytic niche within *Axonopus compressus*. The high prevalence of *Alternaria* species could also reflect its adaptability and potential mutualistic benefits with the host plant.

Several fungi were represented by only a single isolate, including *Epicoccum nigrum, Fusarium lateritium, Aspergillus niger, Aspergillus fumigatus, Pythium acanthicum and Cladosporium cladosporioides*. These species, though less common, contribute to the overall fungal diversity and may offer unique ecological or biochemical properties. Alternaria alternata

The diversity of endophytic fungi isolated from *Axonopus compressus* highlights the complexity of plant-microbe interactions. Many of these fungi, particularly those with high frequency, could have potential applications in agriculture, such as biological control agents or biofertilizers. Additionally, the bioactive compounds produced by these fungi could be explored for pharmaceutical applications.

The current study provides a snapshot of the endophytic fungal diversity in a specific region. To gain a more comprehensive understanding, future research should consider broader geographic sampling and include a variety of plant species. Additionally, exploring the functional roles and metabolic profiles of these fungi could provide valuable insights into their ecological and practical significance.

Conclusion

This study successfully identified a diverse range of endophytic fungi associated with *Axonopus compressus* in the Tirunelveli district, with *Alternaria alternata* and Fusarium lateritium being the most prevalent. The findings underscore the ecological complexity of endophytic communities and highlight potential areas for further research into the roles and applications of these fungi. Understanding these interactions can contribute to better management practices in agriculture and uncover new opportunities for utilizing fungal biodiversity in various fields.

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