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Isolation and Characterization of an IAA-Producing *Aspergillus nigerfrom Avicennia marina*

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Abstract

Mangrove ecosystems harbor diverse endophytic fungi that contribute to plant health and stress tolerance. This study focused on the isolation, identification, and indole-3-acetic acid (IAA) production potential of an endophytic fungus from *Avicennia marina*. Surface-sterilized leaf, root, and branch tissues were cultured on PDA, and a distinct fungal isolate was obtained. Molecular identification using ITS1 and ITS4 primers followed by sequencing and BLAST analysis confirmed 99% similarity to *Aspergillus niger*. The isolate was cultured in tryptophan-enriched broth to assess its ability to synthesize IAA. Following extraction and colorimetric analysis using Salkowski reagent, the development of a pink color and absorbance at 530 nm confirmed IAA production. The results indicate that *A. niger* inhabits the internal tissues of A. marina and synthesizes IAA via a tryptophan-dependent pathway. These findings highlight the potential of this endophytic fungus as a plant growth-promoting organism that may support root development, nutrient uptake, and stress resilience. The study provides a basis for future applications of mangrove-derived endophytes in sustainable agriculture and ecological restoration.

Keywords. Mangrove; Endophytic Fungus; Molecular and Agriculture

Highlights

- *Aspergillus niger* was successfully isolated as an endophyte from *Avicennia marina* tissues
- ITS sequencing confirmed 99% similarity to *A.niger*
- The isolate produced detectable levels of IAA in tryptophan-supplemented media
- IAA production suggests potential for plant growth promotion and stress tolerance improvement

1. Introduction

Mangrove ecosystems sustain a rich diversity of microbial communities, supported by the unique structural adaptations of mangrove plants. In *Avicennia marina*, aerial roots known as pneumatophores facilitate oxygen uptake and improve air circulation and light penetration. Their porous structure also provides microhabitats that support various microorganisms, including endophytic fungi (D. H. Zainal Abidin *et al.,* 2021; L. T. M. Nguyen *et al.,* 2023).

Endophytic fungi inhabit internal plant tissues without causing visible symptoms and may form relationships that range from mutualistic to latent pathogenic, depending on environmental conditions. They contribute to plant health by producing inhibitory compounds, activating defense mechanisms, and enhancing tolerance to stresses such as salinity,

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heat, drought, and pollution through modulation of metabolic and antioxidant pathways (Z. Deng & L. Cao, 2017; M. Faiq *et al.,* 2025).

Fungi associated with mangrove plants have received growing attention due to the diversity of bioactive compounds they produce. These natural products exhibit a wide range of biological activities, including anticancer, anti-inflammatory, antimicrobial, antiparasitic, antiproliferative, antioxidant, and hypoglycemic properties (S. N. Bibi *et al.,* 2020; H. T. Khazaal *et al.,* 2023; Noviyanto *et al.,* 2023). The stressful and fluctuating conditions of mangrove environments encourage these fungi to develop distinct metabolic pathways that support competition and survival, resulting in specialized bioactive metabolites (P. Palanichamy *et al.,* 2018).

Microorganisms associated with plant roots also play a major role in plant development and stress management. Their interactions with plants contribute to increased biomass, reduced pathogen growth, and improved physiological performance (Oyserman *et al.,* 2018; Batista *et al.,* 2018). Root-associated microbial communities are shaped by plant-derived primary and secondary metabolites (Sasse *et al.,* 2018; Canarini *et al.,* 2019), while the microbes themselves support plant growth by releasing compounds that influence nutrient uptake, root elongation, and metabolic regulation (Tracanna *et al.,* 2021).

Among these beneficial microbes, species of *Aspergillus* filamentous ascomycetous fungi known for their industrial and biotechnological importance (Xin *et al.,* 2023) are recognized for their ability to promote plant growth. Several *Aspergillus* strains isolated from soil have shown strong interactions with plants and are used to enhance crop performance without environmental risk (Mohamed *et al.,* 2022). These fungi promote growth by secreting secondary metabolites that stimulate tissue development, increase biomass, and inhibit plant pathogens (El-Maraghy *et al.,* 2020). They also support vegetative growth by improving ion transport, producing enzymes such as amino cephalosporanic acid acylase, and activating plant immune responses (Kriaa *et al.,* 2015; Ismail *et al.,* 2019). In this study, endophytic fungi *(Aspergillus niger)* were isolated from *A. marina*, and identified using DNA barcoding. Its ability to produce indole-3-acetic acid (IAA) was evaluated to determine its potential as a plant growth-promoting fungus.

2. Materials and methods

2.1. Sample collection and prepartion

Collected samples were first washed under running water for 15 minutes to remove mud and surface debris, followed by thorough rinsing with sterile distilled water. Surface sterilization was carried out by immersing the materials in 75% ethanol for 1 minute, then in 5% sodium hypochlorite for 3 minutes for leaves and 5 minutes for roots and branches. This was followed by a brief treatment in 75% ethanol for 30 seconds and three final rinses with sterile distilled water to remove any residual disinfectant.

Leaves were aseptically cut into small sections measuring approximately 5 × 5 mm, while roots and branches were trimmed into 1 cm cross-sections and then split longitudinally to expose the internal tissues. All procedures were performed inside a biological safety cabinet to minimize contamination (Liu *et al.,* 2007).

2.2. Isolation of fungus

Three segments were placed in petri dishes containing PDA amended with chloramphenicol 500 mg/l (Al-Mahi I, *et al.,* 2003). The dishes were sealed with parafilm and incubated at 27°C for 3 -6 days. The incubation period for each fungus was recorded, and this was taken as the day the first visual growth was observed from the plating date and was considered as an incubation period of growth. Each distinct fungal colony was sub-cultured onto a fresh labeled PDA Petri plate twice to obtain pure isolates which were inoculated into 50 ml sterile Potato Dextrose Broth (TM Media, Rajasthan, India) (24 g in 1 L sterile seawater) and incubated for two weeks at room temperature. For the highest viability an aliquot of 1 ml fungal culture broth were labeled, and stored. (Mwamburi *et al.,* 2019).

Figure 1 Fungal broth (PDB)

2.3. Identification of fungus

The sample was subjected for DNA extraction using Phenol Chloroform method standardized by CAGL and the PCR was performed with ITS F& R primers. Sequencing was carried out for all the samples using ABI 3500 Genetic Analyzer. The sequences obtained from the samples were aligned and edited using MEGA software version 11. Similarity search was carried out using the aligned sequence against the sequences submitted in NCBI using NCBI-BLAST.

2.4. Determination of Indole-3-Acetic Acid (IAA)

2.4.1. IAA Screening Using Cell-Free Filtrate

Fungal isolates were inoculated into Potato Dextrose Broth (PDB) enriched with 0.5–1.0 g/L L-tryptophan to enhance IAA biosynthesis. The cultures were incubated at 28 °C for 7–10 days on a rotary shaker operating at 120–150 rpm.

2.4.2. Harvesting of Culture Supernatant

After the fermentation period, the culture broth was centrifuged at 10,000 rpm for 10 minutes, and the clear supernatant obtained was used for IAA extraction.

2.5. IAA Extraction Procedure

2.5.1. Acidification and Solvent Extraction

The pH of the culture supernatant was adjusted to 2.5–3.0 using 1 N HCl. Equal volumes of ethyl acetate were added to the acidified supernatant and mixed thoroughly for liquid–liquid extraction. This extraction step was repeated two to three times to improve IAA recovery. The ethyl acetate layers containing IAA were pooled.

2.5.2. Concentration of Extract

The combined ethyl acetate fractions were concentrated using a rotary evaporator under reduced pressure. The resulting residue was re-dissolved in a small volume of methanol or ethyl acetate for further analysis.

2.5.3. Colorimetric Estimation of IAA

IAA concentration was determined using the Salkowski reagent method. One milliliter of the extract was mixed with 2 mL of Salkowski reagent 0.5 M $FeCl_3$ in 35% perchloric acid and incubated in the dark for 30 minutes. The development of a pink color indicated the presence of IAA. Absorbance was measured at 530 nm using a UV–VIS spectrophotometer (UV-1800, CAT. No. 206-25400-38, Japan). Quantification was performed using a standard calibration curve prepared with known concentrations of pure IAA (Shahab et al. 2009).

3. Result

3.1. Isolation of Fungi

Endophytes were grown from mangrove leaves of *Avicennia marina* after a week, the isolated endophytes grown on PDA plates were grouped on the basis of morphological traits. One species showing distinct growth was selected for further screening process.

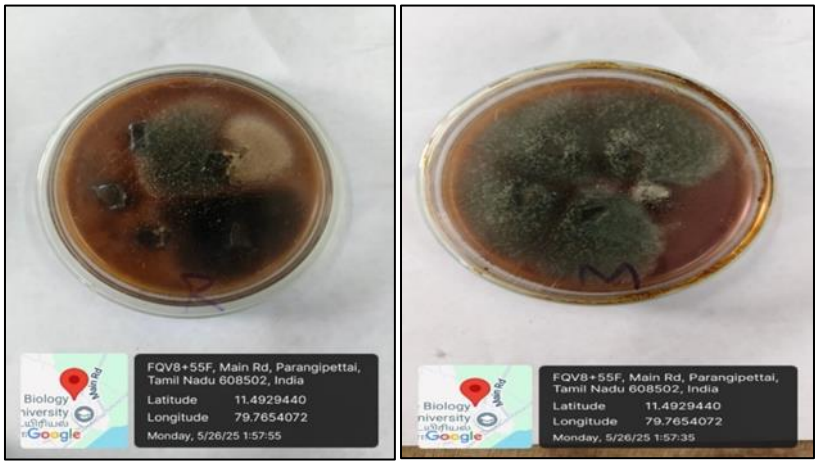

Figure 2& 3 Endophytes grown (PDA plates)

3.2. Molecular Identification of Endophytic Fungi

The *Avicennia officianalis* sample received was subjected to DNA extraction using the Phenol-Chloroform method standardized by CAGL, followed by PCR amplification using ITS1 (Forward: TCCGTAGGTGAACCTGCGG) and ITS4 (Reverse: TCCTCCGCTTATTGATATGC) primers. Sequencing was performed using an ABI 3500 Genetic Analyzer, and the resulting sequences were aligned and edited using MEGA version 11 software. A similarity search was carried out using NCBI-BLAST, which revealed that the sample (EF01) showed 99% similarity with *Aspergillus niger*. While this high similarity suggests the fungal isolate belongs to the Aspergillus genus, species-level confirmation requires further evidence, such as morphological studies and additional molecular markers. It is also recommended to sequence three to five isolates of the same species to ensure accurate identification before submitting sequences to NCBI. The edited FASTA sequence is provided for reference, along with the raw forward and reverse sequences.

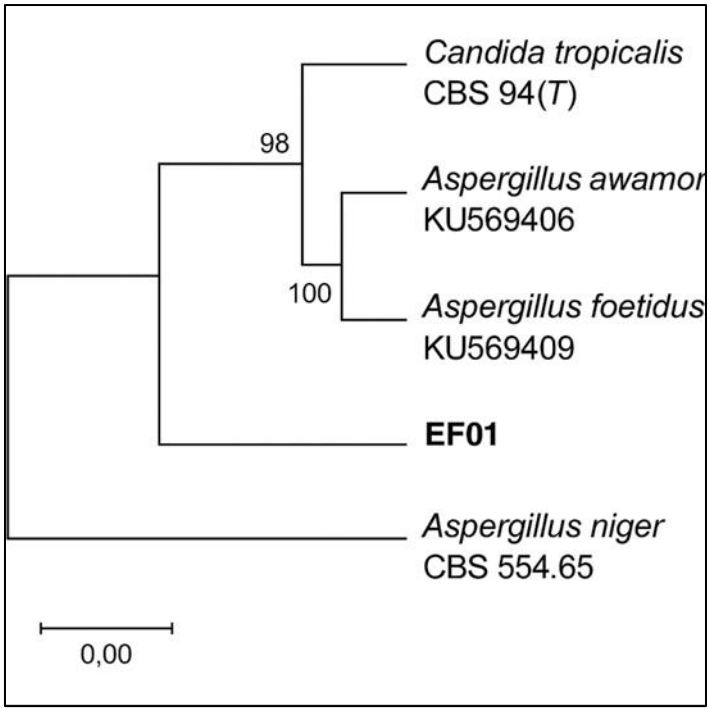

Figure 4 Phylogenetic tree

3.3. IAA Production from *A. niger*

After sub culturing, the endophytic fungi *Aspergilus niger* were inoculated in Czapek Dox broth with L-Tryptophan and grown for a week and then IAA was extracted from the fungal broth. Indole acetic acid determination and quantification IAA was determined in culture filtrates using Salkowski reagent. Salkowski solution gives a pink color in the presence of IAA which can be easily detected in the filtrates. The absorbance was measured at 530nm in UV-VIS spectrophotometer.

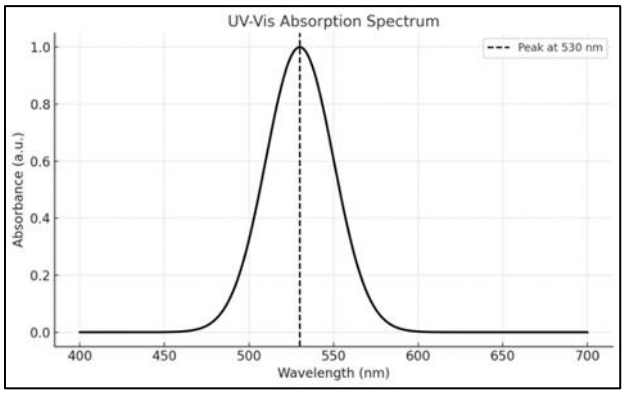

Figure 5 UV-Vis Absorption Spectrum

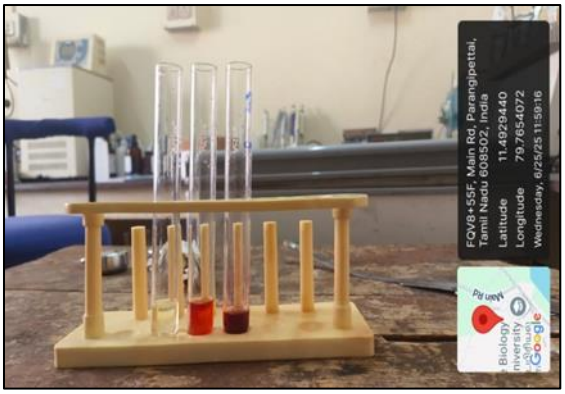

Figure 6 Indole acetic acid determination

4. Discussion

The present study confirms that *Aspergillus niger* is an endophytic fungus associated with the internal tissues of *Avicennia marina*. Successful isolation after surface sterilization indicates that the fungus naturally inhabits the host rather than existing as a surface contaminant. ITS sequencing showed 99% similarity with *A. niger*, supporting its identification and matching earlier reports of *Aspergillus* species being common endophytes in mangrove plants.

The isolate showed clear ability to produce indole-3-acetic acid (IAA), especially in tryptophan-enriched media. The pink coloration in the Salkowski assay and absorbance at 530 nm confirm IAA production. This agrees with earlier findings that many *Aspergillus* species use tryptophan-dependent pathways for IAA synthesis. Production of IAA by endophytic fungi plays an important role in plant growth, as it can enhance root elongation, nutrient uptake, and overall plant development.

Given the stressful conditions in mangrove habitats, IAA-producing endophytes such as *A. niger* may contribute to host adaptation by supporting root growth and improving physiological performance. The results indicate that this isolate may serve as a potential plant growth-promoting fungus and could be further explored for applications in agriculture and mangrove restoration.

5. Conclusions

This study demonstrates that *Aspergillus niger* is a naturally occurring endophytic fungus in *Avicennia marina* and can be reliably identified through ITS-based DNA barcoding. The isolate showed the capacity to synthesize indole-3-acetic acid (IAA), particularly in the presence of L-tryptophan, confirming its role as a potential plant growth-promoting fungus. The ability of the isolate to produce IAA highlights its significance in enhancing root development, nutrient uptake, and overall plant vigor. These findings suggest that *A.niger* from mangrove environments may be a promising candidate for future applications in sustainable agriculture and plant health improvement. Further studies on its biochemical pathways and plant interaction mechanisms will help establish its broader biotechnological potential.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Author Contributions

The first draft of the manuscript was written by Gowarthanan Ranganathan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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