PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2688

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

# Fungal endophytic species *Fusarium annulatum* and *Fusarium solani* : Identification, molecular characterization, and study of plant growth promotion properties

**Soma Biswas & Suma Sarojini***

Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru 560029, India

*Email: suma@christuniversity.in

OPEN ACCESS

**ARTICLE HISTORY**

Received: 25 May 2023
Accepted: 19 September 2023

Available online
Version 1.0 : 28 December 2023

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

**Publisher's Note**: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing**: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting



**CITE THIS ARTICLE**

Biswas S, Sarojini S. Fungal endophytic species *Fusarium annulatum* and *Fusarium solani* : Identification, molecular characterization, and study of plant growth promotion properties. Plant Science Today (Early Access). https://doi.org/10.14719/pst.2688

## Abstract

Research on endophytic fungi has gained significant interest due to their potential to enhance plant growth directly by producing phytohormones, solubilizing macronutrients, fixing nitrogen, or indirectly inhibiting phytopathogens growth by producing ammonia, siderophore, hydrogen cyanide, or extracellular enzymes, thereby acting as biocontrol agents. The present study aimed to isolate fungal endophytes from *Alternanthera philoxeroides* and evaluate their plant growth promotion and antimicrobial activity. In total, nine fungal endophytic strains were isolated from different parts of *A. philoxeroides* such as leaves, roots, and stems. The results demonstrate that the strains MEFAphS1 and MEFAphR3 exhibited positive plant growth promotion properties, including phosphate solubilization, and IAA (Indoleacetic acid) production, and ammonia production. The IAA production was highest for MEFAphS1, with a concentration of 46.635±1.04 µg/mL, while MEFAphR3 displayed the highest ammonia production (0.903±0.01 µg/mL). The phosphate solubilization index (PSI) is the maximum for MEFAphS1 (1.5±0.10). MEFAphS1 also exhibited antibacterial activity against *Vibrio vulnificus, Streptococcus pneumoniae,* and *V. parahaemolyticus,* with the most substantial inhibition zone observed against *V. vulnificus* (28±1 mm). In contrast, MEFAphR3 showed an inhibition zone of 8±1.53 mm against *V. parahaemolyticus*. Molecular identification revealed the identity of the isolates MEFAphS1 and MEFAphR3 as *Fusarium solani* and *F. annulatum*. These results thus confirm the possible applications of the fungal endophytes as plant biofertilizers and bio-enhancers to increase crop productivity.

## Keywords

Plant growth promotion; fungal endophyte; antimicrobial; *Fusarium annulatum*; *F. solani*; *Alternanthera philoxeroides*

## Introduction

The world's population is expected to reach 9.687 billion by 2050, increasing demand for essential resources such as food and water (1). To meet the demand of the growing population, food production will need to rise by 70% by 2050 and potentially double or triple by 2100. At the same time, measures should be taken to reduce the environmental footprints of food production (2). Therefore, there is an urgent need to improve agricultural productivity sustainably to feed the rapidly growing population. The use of endophytesprovides an environment-friendly approach to enhance agricultural productivity sustainably.

Endophytes are microbes, such as fungi or bacteria that reside within the tissues of healthy plants without causing disease symptoms (3). Endophytes provide various benefits to plants including improved stress resilience, enhanced growth, nutrient absorption, and defence against diseases. The study of endophytes primarily focuses on two key areas: the exploration of valuable bioactive compounds produced by endophytes and their potential role as biocontrol agents (4, 5). Endophytic fungi have been observed to have a positive influence on plant growth and agricultural output. Various bioactive compounds have been synthesised by fungal endophytes, which play an essential role in plant-microbe interactions. Several of these metabolites exhibit pesticidal properties, making them potentially effective as biocontrol agents against a wide range of agricultural pathogens (6, 7). For example, endophytic fungi *Penicillium* spp. isolated from *Teucrium polium,* have demonstrated good antimicrobial activities and growth promotion traits like IAA production, ammonia production, and phosphate solubilisation (8). Endophytic fungi isolated from *Phaseolus vulgaris* demonstrated beneficial plant growth-promoting traits like IAA production, ammonia production, solubilizing phosphate, and fixing nitrogen and extracellular enzyme activities (9). The fungal endophyte *Fusarium solani,* isolated from an orchid, was reported to assist in plant growth and function as a biocontrol agent (10).

*Alternanthera philoxeroides* commonly known as Alligator weed, is a herbaceous amphibious weed belonging to the Amaranthaceae family, originating in South America. They are usually found in stagnant and slowly flowing water bodies, as well as in creeks, channels, riverbanks, and related areas that experience intermittent flooding (11). *A. philoxeroides* is one of the luxuriantly growing plants in Madiwala Lake, Bengaluru, which attracted our study of isolation and screening for potential endophytes. The degradation of these lakes primarily results from anthropogenic activities, including the release of treated or untreated municipal sewage, domestic effluents, and other harmful pollutants from industrial sources (12). The abundance of *A. philoxeroides* in the polluted Madiwala Lake indicates that these plants can thrive in harsh environments, likely due to the secretion of certain metabolites by their endophytes. Therefore, exploring their diverse functions could open up many doorways to their potential applications. A recent study reported the isolation of endophytic fungi from *A. philoxeroides* from Hulimavu Lake, Bangalore (13). This study aimed to investigate the isolation of potential fungal endophytes from different parts of *A. philoxeroides* with a focus on their abilities to promote plant growth and act as biocontrol agents.

## Materials and Methods

### Chemicals and Reagents

Potato dextrose broth (PDB), nutrient broth (NB), sucrose, potassium iodide, mercuric chloride, tryptophan, indole acetic acid, bromocresol green, yeast extract, peptone, Salkowski's reagent, and Nessler's reagent were purchased from HiMedia (India). All reagents and chemicals used in this study were of analytical grade.

### Collection of Plant Sample

The *Alternanthera philoxeroides* sample was collected from Madiwala Lake, Bengaluru, India (Lat N $12^0$ 54' 3.492" Long E $77^0$ 37' 4.0044"), in clean plastic bags and processed within 24 h of collection (Fig. 1).

### Isolation of Fungal Endophytes

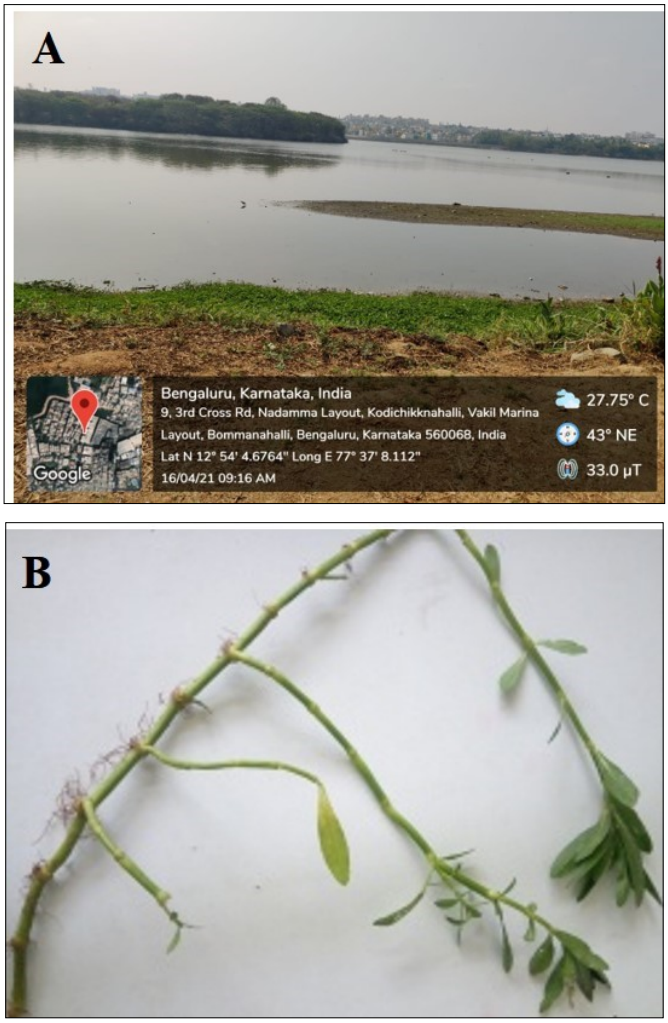


**Fig. 1**.(**A**) Geographical location showing the site for sample collection (**B**) Plant *Alternanthera philoxeroides* used for the study.

The plant sample was washed carefully to remove dirt particles by rinsing it repeatedly with tap water. For surface sterilisation, a modified method was used. Different parts of the plant (leaves, stem and root) were separated and immersed in 70% ethanol for a minute, followed by several rinses with sterile distilled water. For sterility check, 0.1 mL of the last rinse water was spread plated onto potato dextrose agar (PDA) medium. The sterilized plant parts were dried by blotting with sterile filter paper, cut into small 6 mm segments, and placed upside down into a PDA medium. The PDA medium was supplemented with 50 mg of chloramphenicol to inhibit the growth of bacteria. Once a single isolate was observed from the plant parts, it was transferred to PDA slants and stored at 4°C in a refrigerator for future use (14).

### *Molecular Identification of EndophyticFungi*

Fungal genomic DNA was extracted and used as a polymerase chain reaction (PCR) template with ITS1 and ITS4 primers. The quality of the PCR product was assessed on a 1.0% agarose gel, which showed a single band of approximately 600 bp. The PCR product was purified to eliminate any impurities. The forward and reverse DNA sequencing reactions of the PCR product were performed using ITS1 and ITS4 primers and the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer. By combining forward and reverse sequence data, a consensus sequence of the PCR product was generated using aligner software. The ITS region sequence was compared to the NCBI Genbank database using BLAST, and the top ten sequences with the highest identity scores were selected and aligned using ClustalW. A distance matrix and phylogenetic tree were then constructed using MEGA 10 (15, 16).

### *Plant Growth Promoting Traits*

#### IAA Production

The endophytic fungal strains were screened for production by inoculating the isolates in Czapek-dox broth with the composition of sucrose 30 g/L, $K_2HPO_4$ 1 g/L, $MgSO_4.7H_20$ 0.5 g/L, sodium nitrate 3 g/L, $FeSO_4.7H_2O$ 0.010 g/L, KCL 0.5 g/L (pH 7.3) supplemented with 0.1% (*w/v*) tryptophan and incubated at 25±2°C and 120 rpm in shaking incubator for 5 d. The supernatant was obtained by centrifuging the broth culture at 10,000 rpm for 10 min. Then, to 1 mL of supernatant, 2 mL of Salkowski reagent was added and incubated at room temperature for 30 min under dark conditions. The appearance of a pinkish-red color indicates IAA production. IAA production was measured quantitatively using a UV spectrophotometer at 530 nm. IAA standard was prepared at a 0-100 µg/mL concentration. All experiments were performed in triplicates (17, 18).

#### Ammonia Production

The isolated strains were evaluated for their ability to produce ammonia using peptone water media consisting of sodium chloride 5 g/L, peptone 10 g/L (pH 7.2 ± 0.2) and incubated at 25 ± 2°C for 5 d. The broth cultures were centrifuged at 10000 rpm for 10 min to obtain the culture supernatant. Then, 1 mL of Nessler's reagent was added to 1 mL of the supernatant. The development of deep yellow-to-brown color was considered a positive indication of ammonia production, which was quantitatively measured using a UV spectrophotometer (8, 18).

#### Phosphate Solubilization

The phosphate solubilizing ability of the fungal isolates was examined qualitatively using a modified method. The isolates were inoculated onto Pikovskaya's agar medium and incubated at 25 ± 2°C for 7 d under dark conditions (19, 20). The development of a yellow halo zone around the colony indicates the phosphate solubilizing efficiency of the isolated fungal endophytes. Phosphate solubilization index (PSI) was also measured using the following equation (21).

Phosphate Solubilization Index = (colony diameter + halo zone diameter)/colony diameter

### *Preparation of Crude Fungal Extract*

To prepare crude fungal extracts, 4-5 discs of freshly grown fungal endophytes were dipped into the potato dextrose broth (PDB) and incubated for 10 d at 25 ± 2°C at 120 rpm in a shaking incubator. The fungal mycelia were filtered using Whatman filter paper after incubation. The filtrate was then used for antibacterial studies.

### *Tested Organisms Used for Antimicrobial Activity*

Five pathogenic bacterial strains, such as gram-positive: *Bacillus cereus*, *Streptococcus pneumoniae,* and gram-negative: *Escherichia coli*, *Vibrio parahaemolyticus*, and *V. vulnificus* were used for determining the antibacterial activity of the isolated fungal endophytes. The stock cultures were maintained at 4°C and inoculated on nutrient broth followed by incubation at 37°C for 24 h under shaking conditions.

### *Antimicrobial Assay*

Agar well diffusion assay was used to determine the antibacterial activity of the isolated fungal endophytes. Under sterile conditions, the freshly prepared bacterial inoculum (0.1 mL) was used for spread plating over the nutrient agar plates. Wells of 6 mm diameter were created on the plates using a sterile borer. The crude extract (50 µL) from each isolated fungal strain was poured into the wells using micropipette. Plates were then incubated at 37°C for 24 to 48 h. The presence of a zone of hydrolysis was an indicator of antibacterial activity (22).

### *Statistical Analysis*

Microsoft Excel 2016 was used for data processing. One-way analysis of variance (ANOVA) was used to examine the significance of differences ($p<0.05$) in the results using IBM SPSS statistics 21. All the tests were performed in triplicates (n=3) and expressed as mean ± standard deviation (SD).

## Results and discussion

### *Isolation and Molecular Characterization*

In our present study, nine endophytic fungi were isolated from different parts (stem, roots and leaf) of *Alternanthera philoxeroides* from Madiwala Lake, Bangalore. After sterility check, the absence of fungal growth on control plates has confirmed that the isolated microbes were indeed endophytes. Among all the isolates, MEFAphR3 and MEFAphS1 isolated from root and stem respectively were selected based on their screening for plant growth promotion and antimicrobial activity. The obtained endophytic fungal strains were cultured in sabouraud dextrose agar (SDA) plates and subcultured in SDA slants for further studies. The colony characteristics of the two fungal strains were white cottony mycelium with purple pigmentation for MEFAphR3 and red pigmentation for MEFAphS1. The initial identification of the endophytic fungus was carried out by examining its primary features. Subsequent identification of the isolates was conducted through staining with lactophenol cotton blue (as shown in Fig. 2) and molecular identification using ITS sequence analysis (as illustrated in

Fig. 3). The species MEFAphR1 was determined to be *Fusarium annulatum*, and MEFAphS1 was identified as *F. solani*. The sequence of MEFAphR1 was submitted to NCBI under accession number OM866184. It's worth noting that the isolate *F. solani* had been previously submitted and reported in one of our research works (unpublished data). To the best of our knowledge, this marks the first report of endophytic fungi belonging to the *Fusarium* genus from *A. philoxeroides*, showcasing both plant growth potential and antimicrobial activity.

of IAA, and ammonia production, and phosphate solubilization (Table 1). For IAA production, a change in color to pink upon the addition of Salkowski's reagent to the culture supernatant confirmed the presence of IAA (Fig. 4A). The IAA content in the isolates MEFAphS1 and MEFAphR3 was found to be 46.635±1.04 µg/mL and 4.925±0.46 µg/mL respectively, at 0.1% (*w/v*) tryptophan concentration. IAA serves various role in plant development, including stimulating abscission, promoting cell growth, inhibiting the growth of lateral shoots, participating in the formation of

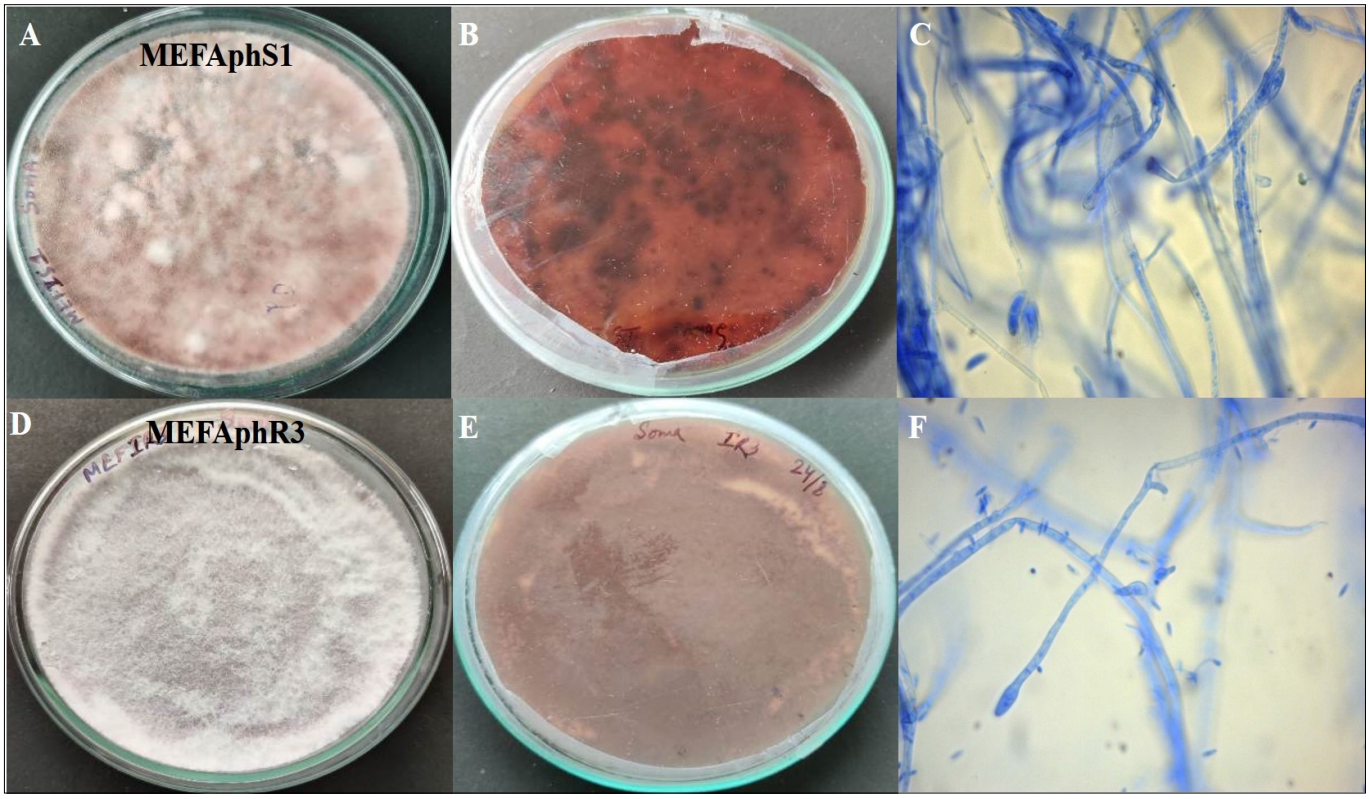


**Fig. 2**. Fungal plate culture grown on SDA media (**A**, **B**) front view and rear view of MEFAphS1 (**D**, **E**) front view and rear view of MEFAphR3 and (**C**, **F**) lactophenol cotton blue staining depicting fungal spores and mycelium of MEFAphS1 (*Fusarium solani*) and MEFAphR3 (*F. annulatum*).

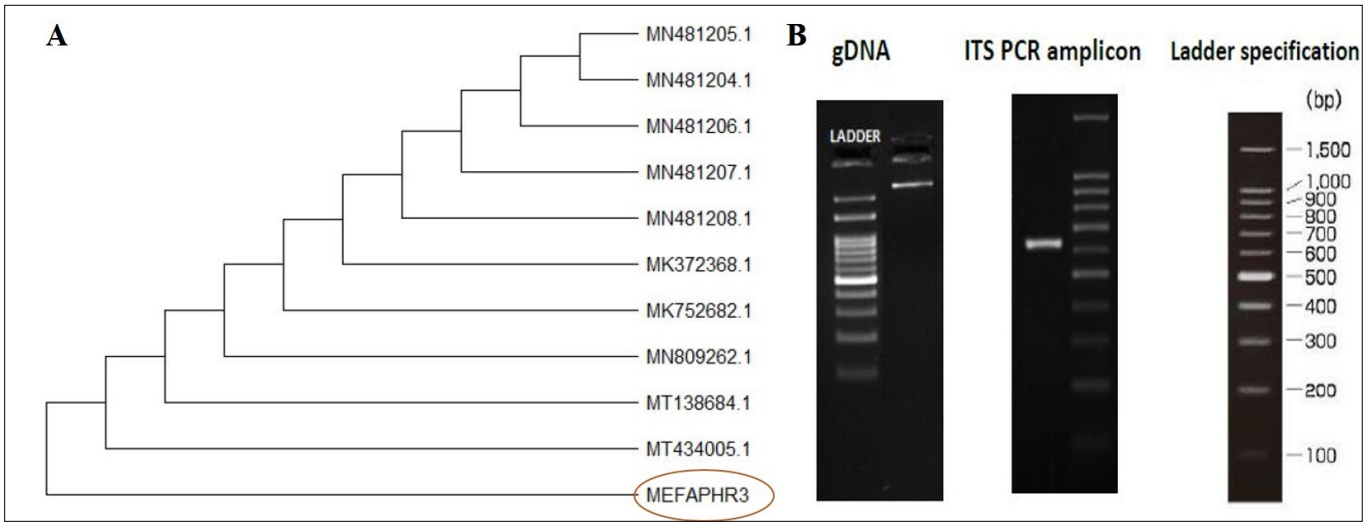


**Fig. 3**. (**A**) The phylogenetic analysis of the isolate *Fusarium annulatum* MEFAphR3 obtained in the study along with sequences from NCBI using MEGA 5 with neighbor-joining method (**B**) species identification of the isolated fungal endophyte *F. annulatum* MEFAphR3: genomic DNA isolation (**left**), PCR amplification of the ITS region (**middle**) and DNA ladder specifications (**right**).

### *Determination of Plant Growth-Promoting Attributes*

Qualitative and quantitative tests were performed to determine the plant growth-promoting traits of the isolated fungal endophytes. These tests included the evaluation

phloem and xylem tissues, and influences the elongation and growth of roots. The production of IAA plays a crucial role in regulating growth-promoting characteristics (23). Many researchers have previously supported that the

**Table 1.** Plant growth promotion capability of the isolated fungal endophytes from *Alternanthera philoxeroides.*

| Fungal endophytes | Isolate identified as | Plant growth promoting traits | | |
|---|---|---|---|---|
| | | Ammonia production (µg/mL) | IAA production (µg/mL) | Phosphate solubilisation Index (PSI) |
| MEFAphS1 | *Fusarium solani* | 0.743±0.07 | 46.635±1.04 | 1.5±0.10 |
| MEFAphR3 | *Fusarium annulatum* | 0.903±0.01 | 4.925±0.46 | 1.37±0.08 |

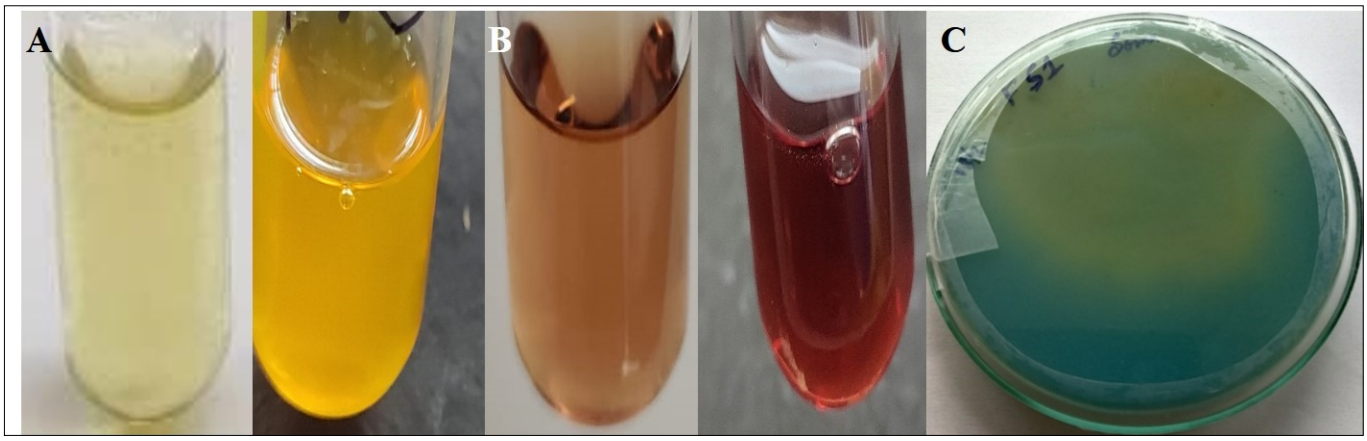


**Fig. 4**. Screening for plant growth promotion by fungal endophyte isolated from *Alternanthera philoxeroides* (**A**) Ammonia production by *Fusarium annulatum* MEFAphR3 (Control, treated sample) (**B**) IAA production by *F. solani* MEFAphS1 (Control, treated sample) and (**C**) Phosphate solubilization (in Pikovskaya's media supplemented with 500 µL of 0.5% bromocresol green) by *F. solani* MEFAphS1.

biosynthetic pathway involving tryptophan is more favourable for auxin production compared to conditions where tryptophan is absent (24, 25).

The isolation of the endophytic fungi, *Fusarium* spp. and *Alternaria* spp., was earlier reported from the leaves of *Solanum nigrum.* These fungi exhibited IAA production with the values of 54 and 30 µg/mL respectively (26). Similarly, 12 endophytic fungi were isolated from various parts of the wild orchid *Vanda cristata*, all of which demonstrated plant growth-promoting abilities. The isolate *F. solani* (PVR1) was tested positive for IAA production and phosphate solubilization and showed a weak intensity of ammonia production. The concentration of IAA was recorded as 30 µg/mL, 48 µg/mL, and 87 µg/mL at different tryptophan concentrations of 1 mg/L, 2 mg/L, and 5 mg/L, respectively. The phosphate concentration for *F. solani* (PVR1) was 0.427 µg/mL (10).

The ammonia production by our isolates was confirmed as positive by the addition of Nesslers's reagent, resulting in the formation of a deep yellow-to-brown color (Fig. 4B). The ammonia production by the isolate MEFAphS1 and MEFAphR3 was found to be 0.743±0.07 µg/mL and 0.903±0.01 µg/mL, respectively. Ammonia production has an indirect effect on the growth of plants. They fulfil the requirement of nitrogen by the host plant and also inhibit the growth of phytopathogens, acting as biocontrol agents (27). The endophytic fungus *Fusarium* spp. showed medium to week ammonia production while *Agaricus* spp. and *Mycoleptodiscus* spp. showed the maximum intensity of ammonia production in the medium (10). Isolates MEFAphS1 and MEFAphR3 were also found to be positive for phosphate solubilization, as indicated by the development of a yellow halo zone around the colony, when grown in Pikovskaya's media containing bromocresol green indicator (Fig. 4C). The phosphate solubilizing index (PSI) for MEFAphS1 and MEFAphR3 were found to be 1.5±0.10 and 1.37±0.08, respectively. In another study, 15 endophytic fungi were isolated from the wheat plant out of which 11 strains solubilized phosphate exhibited PSI ranging from 2.08±0.03 to 5.16±0.36 and also showed visible halo zones around the colonies on Pikovskaya's agar medium. They also reported that 34% of their isolated endophytic fungus exhibited ammonia production (28). Another study showed that the fungal endophyte *F. solani* isolated from *Lotus tenuis* showed the highest phosphate solubilization activity (29). In our current findings, the plant growth-promoting traits IAA production and phosphate solubilization directly helps in plant growth. In contrast, ammonia production indirectly promotes plant growth by suppressing the proliferation of plant pathogens. The symbiotic association between the plant *A. philoxeroides* and endophytic fungus is crucial for their survival and growth under polluted conditions.

### *Antimicrobial Activity*

The selected endophytic fungi, MEFAphS1 and MEFAphR3, were further studied to investigate there *in vitro* inhibitory activity against *Escherichia coli, Bacillus cereus, Streptococcus pneumoniae, Vibrio parahaemolyticus,* and *V. vulnificus.* The isolate MEFAphS1 showed antibacterial activity against *V. vulnificus, S. pneumoniae,* and *V. parahaemolyticus,* with the highest zone of inhibition (28±1 mm) against *V. vulnificus* and the lowest against *S. pneumoniae* (10±2 mm). The isolate MEFAphR3, on the other hand, displayed antibacterial activity only against *V. parahaemolyticus,* with an inhibition zone of 8±1.53 mm (Fig. 5). Given that, *A. philoxeroides* is an invasive plant, the endophytic fungal population within the plant could probably confer the host for their ability to tolerate both abiotic and biotic stresses by producing various secondary metabolites. In a similar study, the endophytic fungus isolated from *Opuntia dillenii*, an invasive plant growing in the harsh South-Eastern region of Sri Lanka, showed the pres-

ence of the most-active fungal endophyte, *Fusarium* spp. The antibacterial compound equisetin of the *Fusarium* spp. showed antibacterial activity against *B. subtilis* and *Staphylococcus aureus* (30). In another study, 29 endophytic fungi were isolated from *Withania somnifera.* Most of them were *Fusarium* spp. and showed antagonistic activity against bacterial pathogens like *B. subtilis*, *E. coli*, and *S. aureus* (31). The endophytic fungus *Fusarium spp.* isolated from meniran leaves (*Phyllanthus niruri*), has shown antibacterial activity against *S. aureus, Streptococcus mutans, B. subtilis, E. coli, Pseudomonas aeruginosa,* and *Salmonella typhi* (32). Western Ghats, a biodiversity hotspot harbours a lot of pigment producing endophytic fungi which can be utilised in multiple industries (33). Earlier studies on endophytes in polluted lakes in Bangalore had revealed the presence of many bacteria and fungi with plant growth promoting and enzyme production capabilities (34-36).

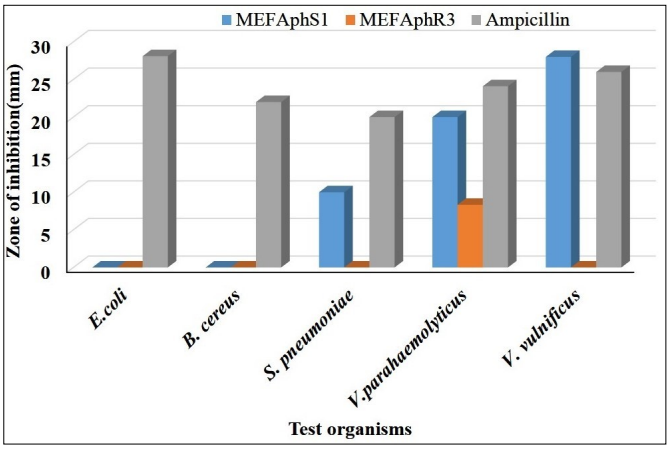


**Fig. 5**. Antibacterial activity of the selected fungal endophytes *Fusarium solani* MEFAphS1 and *F. annulatum* MEFAphR3 against test organisms *E. coli, B. cereus, S. pneumoniae, V. parahaemolyticus,* and *V. vulnificus*.

## Conclusion

The current research has highlighted the capacity of fungal endophytes isolated from *Alternanthera philoxeroides* to produce substances that enhance plant growth. Specifically, the isolates *Fusarium solani* and *F. annulatum* demonstrated growth-promoting activities, including phosphate solubilisation, and ammonia, and IAA production. Additionally, *Fusarium* spp. exhibited significant antibacterial activity. The plant growth-promoting traits and antimicrobial activity exhibited by these *Fusarium* spp. suggest their potential use as both plant growth enhancers and biocontrol agents. Consequently, incorporating these strains into the agricultural industry has the potential to reduce reliance on synthetic fertilizers and promote sustainable farming practices, which warrants further scientific investigation. Subsequent studies, particularly those involving field experiments, are imperative for validating their effects on crop plants under natural growth conditions.

## Acknowledgements

The authors would like to acknowledge the Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru, for providing all the necessary infrastructural facilities and resources to conduct this work.

## Authors contributions

SS and SB carried out the conceptualization and design of the study. SB conducted experiments, analysed the data, and drafted the manuscript. SS critically revised the manuscript, supervised and gave the final approval.

## Compliance with ethical standards

**Conflict of interest**: The authors report no conflict of interests.

**Ethical issues**: None.

## References

1. United Nations Department of Economic and Social Affairs, Population Division. World Population Prospects; 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3
2. Poveda J. Insect frass in the development of sustainable agriculture- A review. AgronSust Develop. 2021;41:1-10. https://doi.org/10.1007/s13593-020-00656-x
3. Wilson D. Endophyte: The evolution of a term and clarification of its use and definition. Oikos. 1995;73:274-76. https://doi.org/10.2307/3545919
4. Khare E, Mishra J, Arora NK. Multifaceted interactions between endophytes and plant: Developments and prospects. Front Microbiol. 2018;9:2732. https://doi.org/10.3389/fmicb.2018.02732
5. Yao YQ, Lan F, Qiao YM, Wei JG, Huang RS, Li LB. Endophytic fungi harbored in the root of *Sophoratonkinensis*gapnep: Diversity and biocontrol potential against phytopathogens. Microbiology Open. 2017;6:e00437. https://doi.org/10.1002/mbo3.437
6. Dubey A, Saiyam D, Kumar A, Hashem A, Allah EFA, Khan ML. Bacterial root endophytes: Characterization of their competence and plant growth promotion in soybean (*Glycine max* (L.) Merr.) under drought stress. Int J Environ Res Public Health. 2021;18:931. https://doi.org/10.3390/ijerph18030931
7. Fontana D, de Paula S, Torres A, de Souza V, Pascholati S, Schmidt D, Neto DD. Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens. 2021;10:570. https://doi.org/10.3390/pathogens10050570
8. Hassan SE. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucriumpolium* L. J Adv Res. 2017;8:687-95. https://doi.org/10.1016/j.jare.2017.09.001
9. Ismail M, Amin M, Eid A, Hassan S, Mahgoub H, Lashin I, Abdelwahab A, Azab E, Gobouri A, Elkelish A *et al*. Comparative study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for *Phaseolus vulgaris* L. Cells. 2021;10:1059. https://doi.org/10.3390/cells10051059
10. Chand K, Shah S, Sharma J, Paudel MR, Pant B. Isolation, characterization and plant growth-promoting activities of endophytic fungi from a wild orchid *Vanda cristata.* Plant Signal Behav. 2020;15(5):1744294. https://doi.org/10.1080/15592324.2020.1744294
11. Xiao-Yun PA, Yu-Peng GE, Alejandro SO, Wen-Ju ZH, Bo LI, Jia-Kuan CH. Invasive *Alternanthera philoxeroides*: Biology, ecology and management. Journal of Systematics and Evolution. 2007;45(6):884. https://doi.org/10.1360/aps06134
12. Gorain B, Parama VR, Paul S. Heavy metals contamination in Madiwala and Lalbagh lakes of Bengaluru, Karnataka: Effect of idol immersion activities. Int J Curr Microbiol App Sci. 2018;7(10):2254-63. https://doi.org/10.20546/ijcmas.2018.710.260

13. Jayaram S, Sarojini S. Bioprospecting of fungal endophytes in Hulimavulake for their repertoire of bioactive compounds. ECS Transactions. 2022;107(1):10471. https://doi.org/10.1149/10701.10471ecst

14. Khalil AMA, Hassan SE, Alsharif SM, Eid AM, Ewais EE, Azab E, Gobouri AA, Elkelish A, Fouda A. Isolation and characterization of fungal endophytes isolated from medicinal plant *Ephedra pachyclada* as plant growth-promoting. Biomolecules. 2021;11 (2):140. https://doi.org/10.3390/biom11020140

15. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol. Evol. 1980;16(2):111-20. https://doi.org/10.1007/BF01731581

16. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 2018;35(6):1547-49. https://doi.org/10.1093/molbev/msy096

17. Munir E, Yurnaliza Y, Lutfia A, Hartanto A. Antifungal activity and IAA production by endophytic fungi isolated from *Elettaria*sp. IOP Conference Series: Earth and Environmental Science. 2021;782(4):042037. https://doi.org/10.1088/1755-1315/782/4/042037

18. Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Khan N. *In vitro* production of IAA by endophytic fungus *Aspergillusawamori* and its growth promoting activities in *Zea mays*. Symbiosis. 2019;77(3):225-35. https://doi.org/10.1007/s13199-018-0583-y

19. Jasim B, John Jimtha C, Jyothis M, Radhakrishnan EK. Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. Plant Growth Regul. 2013;71:1-11. https://doi.org/10.1007/s10725-013-9802-y

20. Soni SK, Singh R, Ngpoore NK, Niranjan A, Singh P, Mishra A, Tiwari S. Isolation and characterization of endophytic fungi having plant growth promotion traits that biosynthesizes bacosides and withanolides under *in vitro* conditions. Braz J Microbiol. 2021;52(4):1791-805. https://doi.org/10.1007/s42770-021-00586-0

21. Joe MM, Deivaraj S, Benson A, Henry AJ, Narendrakumar G. Soil extract calcium phosphate media for screening of phosphate-solubilizing bacteria. Agri Nat Resour. 2018;52(3):305-08. https://doi.org/10.1016/j.anres.2018.09.014

22. Kalyanasundaram I, Nagamuthu J, Muthukumaraswamy S. Antimicrobial activity of endophytic fungi isolated and identified from salt marsh plant in Vellar Estuary. Journal of Microbiology and Antimicrobials. 2015;7(2):13-20. https://doi.org/10.5897/JMA2014.0334

23. Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C. Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci. 2013;4:356. https://doi.org/10.3389/fpls.2013.00356

24. Fouda AH, Hassan SED, Eid AM, Ewais EED. Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepiassinaica* (Bioss.). Ann Agric Sci. 2015;60:95-104. https://doi.org/10.1016/j.aoas.2015.04.001

25. Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M, Lee IJ. Endophytic fungi from Frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid. PLoS One. 2016;11(6):e0158207. https://doi.org/10.1371/journal.pone.0158207

26. Khan AR, Ullah I, Waqas M, Shahzad R, Hong SJ, Park GS, Jung BK, Lee IJ, Shin JH. Plant growth-promoting potential of endophytic fungi isolated from *Solanumnigrum* leaves. World J MicrobiolBiotechnol. 2015;31(9):1461-66. https://doi.org/10.1007/s11274-015-1888-0

27. Mbai FN, Magiri EN, Matiru VN, Nganga J, Nyambati VCS. Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. American International Journal of Contemporary Research. 2013;3(4):25-40.

28. Ripa FA, Cao WD, Tong S, Sun JG. Assessment of plant growth promoting and abiotic stress tolerance properties of wheat endophytic fungi. Biomed Res Int. 2019;2019:6105865. https://doi.org/10.1155/2019/6105865

29. Nieva AS, Vilas JM, Gárriz A, Maiale SJ, Menéndez AB, Erban A, Kopka J, Ruiz OA. The fungal endophyte *Fusarium solani* provokes differential effects on the fitness of two Lotus species. Plant Physiol Biochem. 2019;144:100-09. https://doi.org/10.1016/j.plaphy.2019.09.022

30. Ratnaweera PB, de Silva ED, Williams DE, Andersen RJ. Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium* sp. BMC Complement Altern Med. 2015;15:220. https://doi.org/10.1186/s12906-015-0722-4

31. Salini TS, Divakaran D, Shabanamol S, Rebello S, Jisha MS. Antimicrobial and immunomodulatory potential of endophytic fungus *Fusarium solani* isolated from *Withania somnifera*. World J Pharm Res. 2014;3(10):879-90.

32. Yuniati Y, Rollando R. Isolation of antibacterial compounds from endophyte fungal of *Fusarium* sp. in *Phyllanthus niruri* Linn. leaves. Journal of Pharmaceutical Sciences and Research. 2018;10(2):260-64.

33. Sivakumar B, Rao NR, Poornamath BP, Jayaram S, Sarojini S. Multifarious pigment producing fungi of Western Ghats and their potential. Plant Science Today. 2022; 9(3):733-47. https://doi.org/10.14719/pst.1759

34. Philip I, Sarojini S, Biswas S, Jayaram S. Exploring the Potential of Bacillus velezensis, an Endophytic Bacteria Isolated from *Alternanthera philoxeroides* for Plant Growth Promotion and Bioremediation Properties. Journal of Pure & amp; Applied Microbiology. 2023; 17(3).https://doi.org/10.22207/JPAM.17.3.40

35. Philip I, Sarojini S, Biswas S, Jayaram S. Insights into the plant growth promotion properties of bacterial endophytes isolated from *Alternanthera philoxeroides* and *Eichhornia crassipes* from Bellandur lake, India. South African Journal of Botany. 2023; 162:588-603.https://doi.org/10.1016/j.sajb.2023.09.028

36. Jayaram S, Biswas S, Philip I, Umesh M, Sarojini S. Differential Laccase Production among Diverse Fungal Endophytes in Aquatic Plants of Hulimavu Lake in Bangalore, India. Journal of Pure & amp; Applied Microbiology. 2023;17(1). https://doi.org/10.22207/JPAM.17.1.19