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Research article

Biological control potential of endophytic fungus *Talaromyces wortmannii* FP-25 against multiple fungal diseases of corn and banana on Mindanao Island, the Philippines

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Abstract

Importance of the work: Antagonistic fungi offer a sustainable, eco-friendly biocontrol approach for disease management, providing an effective alternative to conventional chemical pesticides.
Objectives: To evaluate and propose a biological control agent from an endophytic fungal strain against three fungal pathogens: *Curvularia lunata*, *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) and *Stenocarpella maydis*.
Materials and Methods: The selected fungal endophyte was characterized based on morpho-cultural assessment on artificial media and ultrastructural examination using scanning electron microscopy. Molecular identification used the internal transcribed spacer (ITS) region sequencing and phylogenetic analyses. Antagonistic potential against selected fungal phytopathogens was evaluated using dual-culture assays to determine its efficacy as a prospective biological control agent.
Results: The endophytic fungus was morpho-culturally characterized and molecularly identified based on ITS region sequencing, revealing 100% sequence identity with *Talaromyces wortmannii*. The dual-culture assays demonstrated potential antagonistic activity of *T. wortmannii* FP-25 against *C. lunata*, Foc TR4 and *S. maydis*. Radial growth inhibition increased significantly from day 6 to day 12, with linear mixed-effects modeling confirming significant treatment × incubation interactions. Based on other studies, the antagonistic mechanisms may involve mycoparasitism, secretion of cell wall-degrading enzymes and production of antifungal secondary metabolites. Distinct temporal suppression patterns were observed among the evaluated phytopathogens.
Main finding: The endophytic fungus *T. wortmannii* FP-25 acted as an effective potential biocontrol agent, suppressing pathogen inocula via competition and potential antibiosis. Its activity against multiple economically important fungal phytopathogens and its time-dependent antagonism support its potential application in integrated disease management and represents the first report of biocontrol activity of *T. wortmannii* FP-25 against *C. lunata*, Foc TR4 and *S. maydis* in the Philippines

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Introduction

Corn and banana production are constrained severely by fungal diseases that significantly reduce yield, compromise grain and fruit quality and ultimately result in substantial economic losses for growers (Pangga et al., 2023). Among the most economically important fungal diseases of corn are *Curvularia* leaf spot and *Stenocarpella* ear rot. *Curvularia* leaf spot, caused by *Curvularia lunata*, induces extensive leaf necrosis, reduces photosynthetic efficiency and consequently leads to measurable yield reductions (Steckel, 2003; Mousa et al., 2015; Abate et al., 2017; Fauriah et al., 2023). *Stenocarpella* ear rot, caused by *Stenocarpella maydis*, results in kernel deterioration, mycotoxin contamination and considerable post-harvest losses (Luna et al., 2017). In banana-growing countries, banana production remains under severe threat from *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) because this soil-borne, vascular pathogen invades the xylem, disrupts water transport and ultimately causes wilting and plant death (Dita et al., 2018). The persistence of Foc TR4 in soil (Stover, 1962; Ekwomadu and Mwanza, 2023) and its capacity to spread across plantations have resulted in widespread epidemics and long-term economic instability in banana-growing regions. Conventional management of these diseases relies largely on chemical fungicides and resistant varieties; however, excessive chemical use has raised concerns regarding environmental contamination, pathogen resistance and negative effects on beneficial microorganisms (Ahmad et al., 2024). Consequently, sustainable alternatives have gained increasing attention, such as biological control using endophytic fungi. Endophytic fungi colonize internal plant tissues without causing visible disease symptoms and can enhance host resistance by suppressing pathogens, producing antifungal metabolites and inducing systemic defense responses (Fontana et al., 2021; Ling et al., 2024). Recently, it has been demonstrated that endophytic fungi play a crucial role in reducing disease severity by competing with pathogens for space and nutrients through their production of bioactive secondary metabolites (Manathunga et al., 2024) and have been shown to influence resistance against the species *Fusarium* and other foliar and ear-infecting pathogens (Lanubile et al., 2017).

Recently, the genus *Talaromyces* has attracted attention for its ecological diversity and its capacity to produce antimicrobial compounds. For example, several *Talaromyces* species have been reported as endophytes with antagonistic activity against important phytopathogens, including a wide range of phytopathogenic fungi (Grabka et al., 2022; Yang et al., 2024; Pereira et al., 2026) and have been shown to contribute to plant health by inhibiting phytopathogen growth and enhancing host defense mechanisms through metabolite production (Pereira et al., 2026). *Talaromyces wortmannii* is a relatively understudied species within this genus. Although it has been investigated mainly for its taxonomic characteristics and secondary metabolites (Bara et al., 2013; Lei et al., 2022), its potential role as a fungal biocontrol agent in crop systems remains largely unexplored. The current authors studied the antagonistic potential of *T. wortmannii* FP-25 as a fungal biocontrol agent against the important fungal phytopathogens, *C. lunata*, *S. maydis* and Foc TR4 (unpublished data). Understanding its activity against multiple economically important fungal phytopathogens may contribute to the development of environment-friendly and sustainable strategies for integrated disease management in corn and banana production systems.

Materials and Methods

Isolation and cultivation of fungal endophytes

Corm and pseudostem tissues from *Fusarium*-infected banana plants were sampled intentionally to isolate localized biocontrol candidates. This targeted strategy aimed to recover ecologically adapted endophytes with established tolerance to pathogen-induced stress and high *in situ* competitive capability within the primary host. Approximately 150–200 g of tissue per sample were wrapped in sterile tissue paper and stored in separate, sterile polythene bags. Isolation of fungal endophytes was conducted immediately after collection, following the general protocols of Damodaran et al. (2020), Pujade-Renaud et al. (2019), Khalil et al. (2021) and Correa-Delgado et al. (2024), with minor modifications. The collected corm and pseudostem tissues were cut into small segments (3 mm × 3 mm) and surface-sterilized by briefly immersing them and agitating for 30 s in a 100 mL solution containing 5% sodium hypochlorite. Then, the tissues were rinsed three times with sterile distilled water to eliminate residual disinfectant. Sterilized segments were placed aseptically at equal distances onto potato dextrose agar (PDA; Scharlau; Spain) supplemented with 150 parts per million (ppm) rifampicin to suppress bacterial contamination. The plates were incubated at 28 ± 2°C for 7 d. Subsequently, emerging fungal colonies were subcultured onto fresh PDA (Scharlau; Spain) plates to obtain pure isolates of potential fungal antagonists.

Isolation, morphological identification and cultivation of test fungal phytopathogens

Corn leaves were collected exhibiting characteristic symptoms of *C. lunata* leaf spot had small tan-to-brown lesions with yellow halos, with the ears infected by *S. maydis* ear rot, showing as white-to-gray fungal growth originating from the base and occasionally accompanied by black specks on the kernels. The samples were obtained from Cabanglasan, Bukidnon (8°03’36.0”N, 125°18’36.0”E), a major corn-producing area on Mindanao Island, the Philippines. In addition, banana samples showing infection by Foc TR4, specifically from corm and pseudostem tissues, were obtained from Alabel, Sarangani Province, the Philippines (6°8’24”N, 125°15’0”E). Each was placed in a sterile polythene bag, properly labeled and transported in a cooled container to the Plant Pathology Laboratory, Crop Protection Development, Syngenta Philippines Inc., Alabel, Sarangani Province, the Philippines. Fungal pathogens were isolated immediately upon arrival, following the general procedures described by Garcia-Aroca et al. (2018) and Wu et al. (2019), with slight modifications. Infected corn leaves and kernels, as well as diseased banana corm and pseudostem tissues, were washed separately using sterile distilled water and cut into approximately 5 mm² sections. These were surface-sterilized based on immersion in 100 mL of 5% sodium hypochlorite solution for 2 min,

followed by three rinses with sterile distilled water and blot-drying using sterile paper towels. The sterilized tissue segments were transferred aseptically onto PDA (Scharlau; Spain) supplemented with 150 ppm rifampicin to inhibit bacterial contamination. The plates were incubated in complete darkness at 28 ± 2°C for 7 d. At 7 d, actively growing mycelia these cultures were subcultured onto fresh PDA (Scharlau; Spain) plates to obtain pure isolates. Next, these purified cultures were maintained at 28 ± 2°C for 14 d prior to their use in the dual culture assays. Morphological identification under light microscopy was performed based on colony characteristics and diagnostic microscopic features, including hyphal morphology and conidial characteristics. *Stenocarpella maydis* was characterized based on dark pycnidia producing predominantly one-septate, cylindrical-to-ellipsoid conidia (Xia et al., 2011). In contrast, Foc TR4 was identified by the presence of hyaline, septate hyphae; falcate, multicellular macroconidia, oval to ellipsoidal microconidia and thick-walled terminal or intercalary chlamydospores (Taping et al., 2023), whereas *C. lunata* was distinguished by brown, septate hyphae, geniculate conidiophores and curved, typically three-septate conidia with an enlarged, darkly pigmented central cell (Santos et al., 2018).

Morphological and cultural characterization of fungal endophyte

The purified fungal endophyte isolates were grown in PDA (Scharlau; Spain) plates and incubated at 28 ± 2°C. Fungal growth was observed daily for 14 d and various colony growth-morphology parameters were recorded such as shape, texture, surface and color (Stolk and Samson, 1972). Samples were prepared for scanning electron microscopy (SEM) by mounting them onto aluminum stubs using double-sided carbon tape. The mounted specimens were sputter-coated with platinum using an ion sputter coater (Hitachi E-1045; Japan) to improve electrical conductivity and image quality. Microscopic observations were conducted using a Hitachi SU3800 scanning electron microscope (Japan) operating under high-vacuum conditions. Images were acquired using a secondary electron detector at an accelerating voltage of 3 kV, with a working distance of 5.1–6.1 mm. Digital micrographs were captured at multiple magnification levels (2,000×, 2,500×, 3,000×, 10,000×, and 20,000×) to examine surface morphology and structural details.

Molecular identification of the fungal endophyte

DNA barcoding

Genomic DNA was isolated from actively growing fungal mycelia using a standard fungal extraction protocol (Taping et al., 2023). The internal transcribed spacer (ITS) region was amplified using the universal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′), based on the methods described by White et al. (1990) and Taping et al. (2023), generating amplicons with an expected size of approximately 550–700 bp. Polymerase chain reaction (PCR) amplification was carried out in a total reaction volume of 25 µL, consisting of genomic DNA purified using the optimized Apical Scientific protocol (Lee et al., 2026), 0.5 pmol of each primer, 200 µM of each deoxynucleotide triphosphate, 0.5 U of thermostable DNA polymerase, the supplied PCR buffer and nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 98°C for 2 min, followed by 25 cycles of denaturation at 98°C for 15 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, with a final extension step at 72°C for 10 min. The amplified PCR products were purified using a standard PCR clean-up procedure (Lee et al., 2026). The quality and integrity of the amplicons were evaluated using the Labnet ENDURO™ Gel XL Electrophoresis System (USA). Briefly, 4 µL of each PCR product was loaded onto a 0.8% agarose gel containing 2× loading dye. The gel was stained with ethidium bromide for 5 min and DNA bands were visualized using the Azure 200 Gel Imaging Workstation (USA). Then, the purified amplicons were subjected to bidirectional sequencing using the universal primers M13F (-20) and M13R-pUC (-26) with the Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit (USA).

Sequence and phylogenetic analysis

Chromatogram data were edited and assembled using the BioEdit software (Hall, 1999). Primer sequences and low-quality paired-end regions were trimmed prior to analysis. The forward and reverse reads were aligned to generate a consensus sequence for each isolate. The resulting ITS sequences of the fungal endophytes were subjected to similarity searches using the Basic Local Alignment Search Tool (BLAST) to identify closely related sequences (Altschul et al., 1990). Identification of the fungal biocontrol isolates was based on comparisons with nucleotide sequences in the GenBank database, with the top 10 matches selected by maximum score and percentage identity. Phylogenetic analyses were performed using the MEGA11 software (Tamura et al., 2021). Evolutionary relationships among the isolates were inferred using the maximum-likelihood method (Saitou and Nei, 1987). A consensus phylogenetic tree was constructed from 1,000 bootstrap replicates to assess the reliability of the inferred clades (Kimura, 1980; Felsenstein, 1985).

Antagonism test of fungal endophyte

The fungal biocontrol agent in pure culture was evaluated for its antagonistic activity against corn and banana fungal phytopathogens using a dual culture technique (Cheng et al., 2019; Johnson et al., 2023). Mycelial plugs, 5 mm in diameter, were excised using a sterile cork borer from cultures aged 14 d of *C. lunata*, *S. maydis* and Foc TR4. These plugs were placed at the edge of separate 90 mm Petri plates, while 50 µL (approximately 1×10^8 spores) of the fungal biocontrol agent suspension were applied on the opposite side as a single 3 cm vertical streak using a sterile wire loop (Tian et al., 2021). Each dual culture setup was prepared in four replicates and pathogen-only plates served as controls. The plates were incubated at 28 ± 2°C for 6–12 d. A 6 d window was selected to capture optimal inhibition metrics immediately prior to colony confluence and crowding, as observed in the growth habits of the tested pathogens. Conversely, the 12 d assessment prevented total plate coverage in the controls, ensuring an unbiased comparative analysis. Antagonistic interactions were assessed using the Bell et al. (1982) five-stage rating system, where Stage 1 indicates complete overgrowth of the pathogen by the antagonist, Stage 2 indicates the antagonist overgrowing at least two-thirds of the pathogen, Stage 3 indicates an equal colonization by both, Stage 4 indicates the pathogen overgrowing at least two-thirds of the antagonist

and Stage 5 represents complete pathogen dominance. A mean stage score of ≤2 (rounded to the nearest whole number) indicated antagonistic activity. The pathogen inhibition percentage was calculated using Equation 1 (Skidmore and Dickinson, 1976):

$$\text{Inhibition} = (R1 - R2) / R1 \times 100 \quad (1)$$

where R_1 is the radial growth of the fungal phytopathogens in the control plate and R_2 is the radial growth of the fungal phytopathogens towards the antagonistic fungi, both measured in millimeters. The antagonistic efficacy of the fungal endophyte was evaluated according to Soytong (1988) and categorized based on the percentage of pathogen inhibition (very high = >75%, high = 61–75%, moderate = 51–60% and low = ≤50% antagonistic activity).

Statistical analyses

Radial growth data were analyzed using a linear mixed-effects model to account for treatment effects and repeated measurements over time. Treatments (single-culture fungal phytopathogens and their corresponding dual cultures with the fungal biocontrol agent and incubation days (day 6 and day 12) were treated as fixed effects, including their interaction. Incubation day was modeled as a categorical factor to enable direct comparison of treatment effects across sampling times. Models were fitted using restricted maximum likelihood and the significance of fixed effects was assessed using Type III analysis of variance with Satterthwaite's approximation for degrees of freedom (Satterthwaite, 1946). Model assumptions were evaluated using residual diagnostics. Estimated marginal means were computed for each treatment within day and pairwise comparisons were performed using Tukey-adjusted tests. All analyses were conducted in R, a language and environment for statistical computing and graphics (version 4.5.2; R Core Team, 2025) at a significance level of $\alpha = 0.05$.

Results

Morphological and cultural characterization of fungal endophyte as biocontrol agent

Three distinct endophytic fungal isolates (FP-25, FP-26, FP-27) were recovered from five processed samples of *Fusarium*-infected banana corm and pseudostem tissues. Initially, all isolates were screened for antagonistic activity against Foc TR4 using an *in vitro* dual-culture assay. Among the three isolates, FP-25 produced the greatest inhibition of Foc TR4 mycelial growth and consistently demonstrated the strongest antagonistic activity during the preliminary screening. Based on this superior inhibitory performance, FP-25 was selected for subsequent characterization against multiple phytopathogens. Morpho-culturally, the isolate FP-25 formed colonies with a predominantly floccose-to-cottony texture, characterized by dense sporulation, radial growth, a slightly elevated center and a gently depressed margin. Early mycelial growth appeared white to yellow, gradually shifting to bluish-green after 4 d as sporulation began. By 14 d, mature colonies exhibited a greyish-green coloration (Fig. 1A). The SEM revealed the characteristic penicillus structure (Fig. 1B), with phialides displaying a typical acerose shape that tapered into elongated necks and arranged metulae in verticils (Fig. 1C). Conidia were predominantly fusiform, ellipsoidal, pointed and ranged from smooth to finely spiny, forming long basipetal chains (Figs. 1D–1E). Based on these morphological, cultural and microscopic features, the isolate was assigned tentatively to the genus *Talaromyces*.

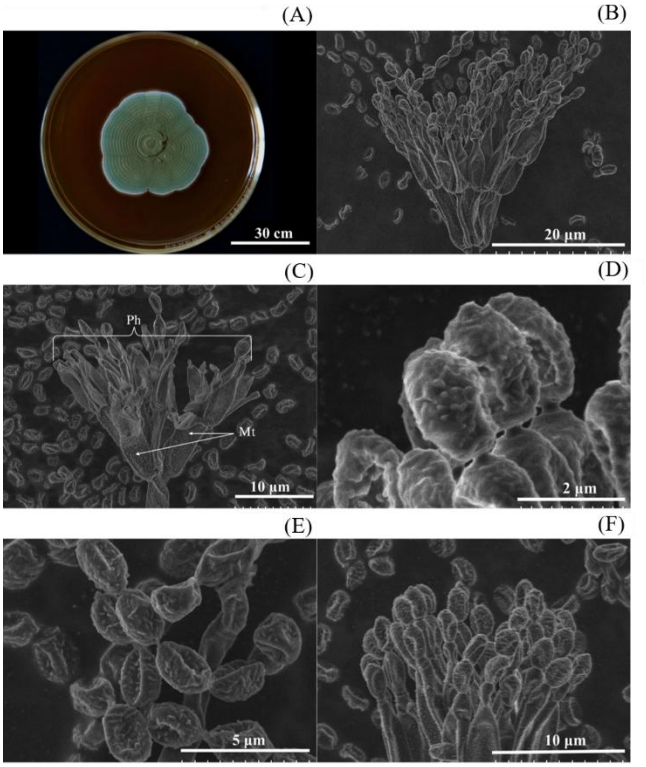


Fig. 1 Morphological and cultural characteristic of *Talaromyces* FP-25: (A) colony on potato dextrose agar medium at 28 ± 2°C after 14 d of incubation; (B) penicillus; (C) phialides (Ph) and metulae (Mt); (D–E) conidia; (F) conidia attached to phialides

Molecular identification and phylogenetic analysis

Identification of isolate FP-25 was confirmed based on molecular analysis. The PCR amplification of the target ITS regions yielded amplicons of the expected size, verified based on gel electrophoresis. Subsequent BLASTn analysis of the sequences against the GenBank database revealed that the fungal endophyte, isolate FP-25, shared 100% identity with *T. wortmannii*. The phylogenetic tree (Fig. 2) had a hierarchical branching pattern characteristic of an ITS-based phylogenetic analysis, with nodal support metrics at key divergence points. Phylogenetic analysis placed the target isolate firmly within the *Talaromyces* section *Islandici* complex (Yilmaz et al., 2014). The maximum likelihood consensus tree resolved the ingroup into two primary lineages, with a basal bootstrap support of 59%. The first lineage grouped *T. variabilis* LN286 (GenBank accession: MW646442.1) and *T. variabilis* LN675 (GenBank accession: MW646457.1) together, with a bootstrap value of 72%. The second lineage clustered the target isolate, *T. wortmannii* FP-25, directly with

the reference strain *T. wortmannii* CBS 316.63 (GenBank accession: MH858298.1), with 65% bootstrap support, while *T. variabilis* ZHKUCC 23-1032 (GenBank accession: PP029409.1) occupied an adjacent position within this clade. Overall, the molecular data placed isolate FP-25 within the immediate evolutionary boundaries of *T. wortmannii*.

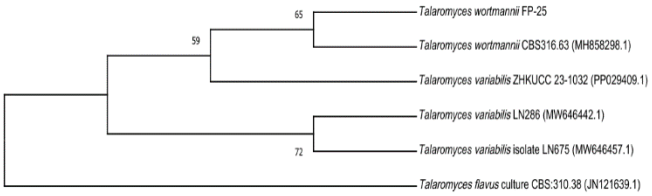


Fig. 2 Phylogenetic tree constructed using maximum likelihood (Kimura-2 parameter) based on internal transcribed spacer sequences, depicting evolutionary relationship of isolate FP-25 with closely related *Talaromyces* species, where *Talaromyces flavus* culture CBS:310.38 (GenBank accession: JN121639.1) was used as outgroup, bootstrap support values indicated at nodes reflect local topology instability due to high sequence identity among synonymous taxa and *Talaromyces variabilis* is a heterotypic synonym of *Talaromyces wortmannii.*

Antagonism of Talaromyces wortmannii FP-25 against fungal phytopathogens

The dual culture assay demonstrated a potent two-stage antagonistic interaction between *T. wortmannii* FP-25 and the target fungal phytopathogens. Across all tested phytopathogenic fungi, co-culturing with *T. wortmannii* FP-25 resulted in a substantial reduction in radial growth compared with single-culture treatments. Growth suppression was evident on day 6 and became more pronounced by day 12, indicating a time-dependent biocontrol effect. The magnitude of inhibition varied among the fungal phytopathogens, with consistent suppression observed for *C. lunata* (Fig. 3A), Foc TR4 (Fig. 3B) and *S. maydis* (Fig. 3C). This type of antagonism exhibits the highest degree according to Bell et al. (1982), where the antagonist *T. wortmannii* FP-25 almost completely overgrew the pathogen and dominated the surface of the medium from day 12 and at day 15 (Fig. 4).

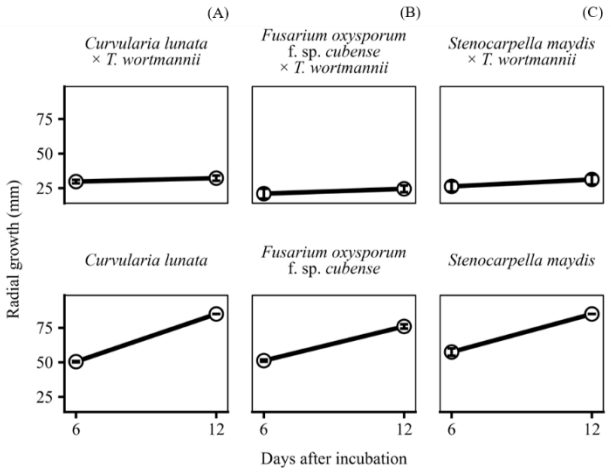


Fig. 3 Radial growth dynamics of fungal phytopathogens in dual culture with *Talaromyces wortmannii* FP-25 (top row) and in monoculture (bottom row) at 6 and 12 d after incubation. (A) *Curvularia lunata*, (B) *Fusarium oxysporum* f. sp. *cubense*, and (C) *Stenocarpella maydis*. Points represent mean radial growth, vertical bars represent SE, and shaded bands indicate mean ± SD. Within each incubation day, treatments did not differ significantly based on Tukey-adjusted pairwise comparisons of estimated marginal means at $\alpha = 0.05$.

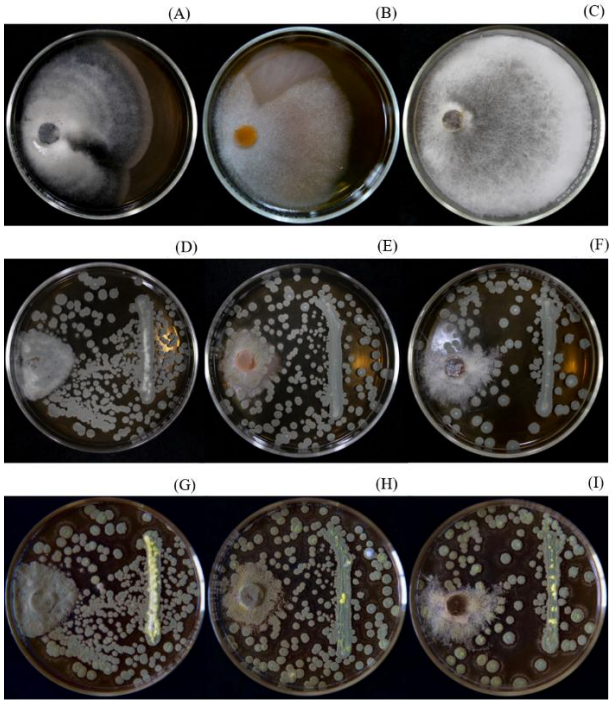


Fig. 4 Antagonistic activity of *Talaromyces wortmannii* FP-25 against fungal phytopathogens. Monocultures after 12 d incubation: (A) *Curvularia lunata*; (B) *Fusarium oxysporum* f. sp. *cubense* TR4; (C) *Stenocarpella maydis*. Dual cultures with *T. wortmannii* FP-25 after 12 d incubation: (D) *C. lunata*; (E) Foc TR4; (F) *S. maydis.* Dual culture after 15 d incubation: (G) *C. lunata*; (H) Foc TR4; and (I) *S. maydis*.

Linear mixed-effects modeling confirmed a significant effect of treatment (F value = 110.935, $p < 0.001$), incubation day (F value = 671.512, $p < 0.001$) and their interaction (F value = 85.181, $p < 0.001$), indicating that the effect of *T. wortmannii* FP-25 on radial growth differed between day 6 and day 12 (Table 1). The significant interaction between treatment and incubation day demonstrated that the antagonistic effect of *T. wortmannii* FP-25 on phytopathogenic fungi was time-dependent with differential levels of sensitivity. Across all tested fungal phytopathogens, dual culture with *T. wortmannii* FP-25 resulted in reduced radial growth relative to the single-culture controls, with inhibition generally becoming more pronounced by day 12. Across all the tested fungal phytopathogens, the percentage growth inhibition values increased from day 6 to day 12, mirroring the progressive decline in radial growth over time (Fig. 5). Based on the classification by Soytong (1988), this temporal shift resulted in several treatments moving from low or moderate antagonistic classes at day 6 into high or very high classes by day 12, indicating that early assessments alone may underestimate true antagonistic potential.

Table 1 Type III analysis of variance results from linear mixed-effects model assessing effects of treatment, incubation day and their interaction on radial fungal growth.

Source of variation	SS	MS	Num df	Den df	F	*p* value
Treatment	2,630.848	526.169	5	18	110.935	0.001
Incubation Day	3,185.019	3185.019	1	18	671.512	0.001
Treatment x Day	2,020.104	404.021	5	18	85.181	0.001

SS = sum of squares; MS = mean square; Num df = numerator degrees of freedom; Den df = denominator degrees of freedom; F = F statistic.

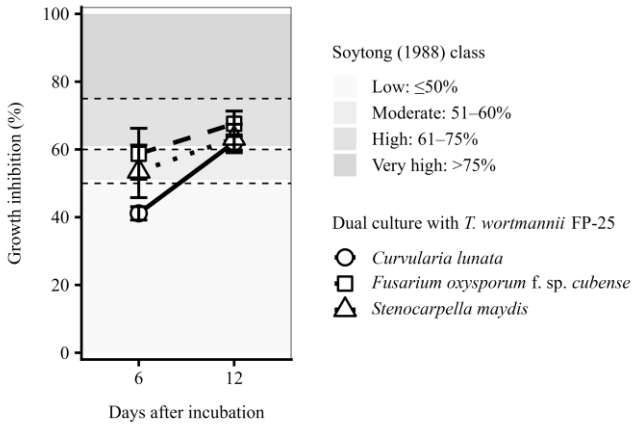


Fig. 5 Mean percentage growth inhibition of three fungal phytopathogens in dual culture with *Talaromyces wortmannii* FP-25 at 6 d and 12 d after incubation. Points represent mean and vertical bars represent SE. Gray background bands indicate the antagonistic activity classes of Soytong (1988).

Discussion

This study demonstrated the biocontrol potential of the endophytic fungus *T. wortmannii* FP-25 against three economically important fungal pathogens (*C. lunata*, *Foc* TR4, *S. maydis)*. The current authors believe this represents the first report in the Philippines of the biocontrol activity of *T. wortmannii* FP-25 against these pathogens. The results expand the currently recognized spectrum of antagonism by *T. wortmannii* and provide a basis for further investigation of its potential as a biological control agent under greenhouse and field conditions. Based on the current results, variation in inhibitory dynamics among the tested fungal phytopathogens reflected differences in susceptibility and competitive resilience. Specifically, *Curvularia lunata* had strong growth suppression by *T. wortmannii* FP-25 early in the incubation period, which had intensified by day 12, indicating high susceptibility to antagonism by *T. wortmannii* FP-25. In contrast, Foc TR4 showed limited inhibition by *T. wortmannin* FP-25 at day 6 but marked suppression at day 12, reflecting a delayed response that may have been associated with its rapid early growth and competitive resilience. *Stenocarpella maydis* showed consistent growth reduction at both assessment times, suggesting sustained antagonistic pressure from *T. wortmannii* FP-25 throughout incubation. The antagonistic behavior of *T. wortmannii* FP-25, based on the percentage growth inhibition, closely mirrored the statistically significant suppression of radial growth detected based on the linear mixed-effects modeling. The strong treatment, incubation day and treatment × day effects observed in the radial growth analysis indicated that pathogen suppression was time-dependent and varied among pathogens, a pattern consistently captured and further clarified by the inhibition classes of Soytong (1988). *Curvularia lunata* had low percentage growth inhibition by *T. wortmannii* FP-25 at day 6, with all replicate values falling within the low antagonistic activity class (≤50%) on the scale defined by Soytong (1988). By day 12, inhibition had increased markedly, with values shifting into high (62.06%) antagonistic classes. This clear temporal transition indicated that the antagonistic effects against *C. lunata* intensified over time rather than being immediately expressed. *Fusarium oxysporum* f. sp. *cubense* TR4 displayed moderate (58.71%) percent growth inhibition by *T. wortmannii* FP-25 as early as day 6, indicating an early onset of antagonistic activity. These inhibition levels increased at day 12, with values in the high (67.57%) antagonistic class. In *S. maydis*, the percentage growth inhibition by *T. wortmannii* FP-25 varied considerably at day 6, with moderate (53.52%) antagonistic classes. By day 12, inhibition values converged into the moderate to high (63.24%) antagonistic classes, indicating a more consistent antagonistic response over time. Collectively, these findings indicated that *T. wortmannii* FP-25 functioned not merely as a space competitor but also as a potential active mycoparasitic and antibiosis-driven antagonist (Halo et al., 2019; Abbas et al., 2024), capable of reducing pathogen inoculum load and displacing phytopathogens. This pattern suggested that *T. wortmannii* FP-25 may establish competitive dominance over time, a potential hallmark of mycoparasitism. By comparison, other studies have reported varying levels of inhibition against important phytopathogens in dual-culture assays using a well-established fungal biocontrol, such as *Trichoderma* species. For example, Catambacan and Cumagun (2021) reported a high inhibitory effect of *Trichoderma asperellum* TDC075, with 87.09% mycelial growth inhibition against Foc TR4. In another study, *T. asperellum* strains TRC900 and T34 exhibited approximately 40% inhibition of Foc TR4 after 1 wk under *in vitro* conditions (Sarma et al., 2025). For *C. lunata*, *T. harzianum* has been reported to achieve 76.28% mycelial growth inhibition (Oliveira et al., 2022), while Addangadi et al. (2017) reported growth inhibition levels of 84.44% and 69.44% for *T. viride* and *T. harzianum*, respectively, against *C. lunata* causing maize leaf spot. These reported values provide a comparative framework for interpreting the antagonistic activity observed in the current study. Furthermore, several *Talaromyces* species have been reported as effective antagonists against a broad range of fungal phytopathogens. For example, *T. flavus* and

T. purpureogenus have demonstrated strong inhibitory activity against *Verticillium dahliae* (Naraghi et al., 2010; Li et al., 2025). Antagonism against foliar pathogens has also been documented, including *T. pinophilus* against *Botrytis cinerea* (Abdel-Rahim and Abo-Elyousr, 2018) and *T. muroii* against *A. alternata* (Zhang et al., 2024). In addition, several *Talaromyces* species were reported to be effective against soil-borne and vascular pathogens, such as *T. islandicus* against *Fusarium* spp. (Zapata et al., 2024) and *T. apiculatus* against *Ganoderma boninense* (Goh et al., 2020). Notably, *T. helicus* var. *major*, *T. indigoticus* and *T. rotundus* have shown multiple antagonistic activities against *Colletotrichum capsici*, Foc TR4 and *Phytophthora palmivora* (Manoch and Dethoup, 2021). The antagonistic activity of *T. wortmannii* FP-25 is hypothesized to involve multiple mechanisms (Fig. 6), including mycoparasitism and the production of antifungal secondary metabolites, as reported in *Talaromyces* species above; however, these mechanisms were not directly examined in the current study.

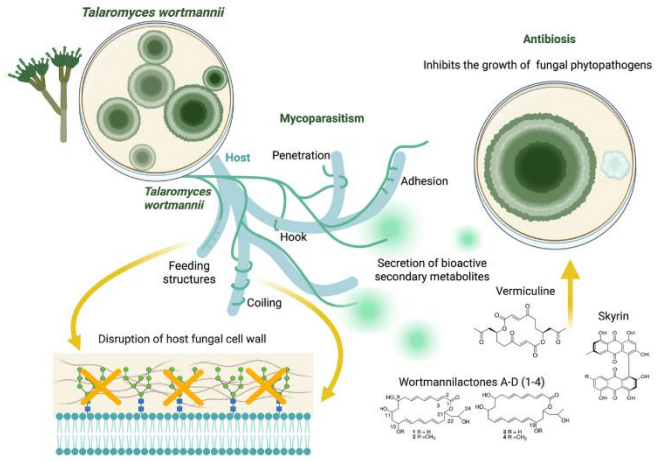


Fig. 6 *Talaromyces wortmannii* FP-25 inhibits the growth of fungal phytopathogens based on other studies, involving direct mycoparasitism and antibiosis through the production of bioactive secondary metabolites. Created in BioRender. Paslon Jr, F. (2026) https://BioRender.com/9s43wlh

Although the phylogenetic tree successfully assigned isolate FP-25 to *T. wortmannii*, the internal nodes separating the reference sequences yielded fluctuating, moderate bootstrap support values of 59%, 65% and 72%. Rather than reflecting true interspecific evolutionary divergence, this localized node instability is a recognized algorithmic artifact arising from database nomenclature and restricted phylogenetically informative sites within the single ITS locus. Notably, *T. variabilis* has been treated as a synonym of *T. wortmannii* following comprehensive taxonomic revisions integrating morphological characters and multilocus phylogenetic analyses (Samson et al., 2011; Yilmaz et al., 2014; Yilmaz et al., 2016; Houbraken et al., 2020). These studies concluded that these taxonomic names represented a single, monophyletic lineage when examined through the lens of Genealogical Concordance Phylogenetic Species Recognition (GCPSR). Therefore, the placement of FP-25 within the *T. variabilis* and *T. wortmannii* species complex is entirely consistent with the current taxonomic concept of *T. wortmannii sensu lato*. In the current study, the name *T. variabilis* was retained strictly to reflect the sequence annotations available in GenBank and to maintain consistency with the reference sequences used in the phylogenetic analysis. While simulation and empirical frameworks indicated that a ≥70% bootstrap value conservatively corresponded to a high historical probability for resolving distinct evolutionary lineages (Hillis and Bull, 1993; Soltis and Soltis, 2003), this operational threshold has been applied specifically to the demarcation of separate biological species. Because *T. variabilis* and *T. wortmannii* are established taxonomic synonyms representing a single species boundary, the fluctuating internal bootstrap scores (59% and 65%) simply reflected the random shuffling of near-identical sequences during pseudo-replication. Therefore, this local instability does not undermine the reliability of the clade, as a whole. Because isolate FP-25 groups definitively within this unified biological species complex, the molecular phylogenetic evidence strongly supports the identification of the target isolate as *T. wortmannii*. Furthermore, the tree serves as a useful reference for researchers navigating the transition from pre-2015 literature (in which *T. variabilis* predominated in certain geographic or ecological contexts) to the post-2015 unified nomenclature (Zhang et al., 2025). Accordingly, the name *T. wortmannii* was adopted throughout the current study and the ITS sequence of isolate FP-25 has been deposited in the NCBI GenBank database under the accession number PX930976 (https://www.ncbi.nlm.nih.gov/nuccore/PX930976). However, reliance on the ITS region alone has inherent limitations for fungal identification. Although ITS is recognized as the primary fungal DNA barcode, often, it is insufficient for accurate species-level discrimination because many closely related taxa have highly similar or identical ITS sequences, resulting in reduced taxonomic resolution (Schoch et al., 2012). Consequently, the use of additional genetic markers, such as β-tubulin (BenA), calmodulin (CaM), translation elongation factor 1-α (TEF1-α) and RNA polymerase II subunits (RPB1 and RPB2), is recommended frequently to improve species delimitation and phylogenetic inference (Nilsson et al., 2014).

Conclusion

In this study, strain FP-25, with high antagonistic activity, was identified as a fungal endophyte of a Foc TR4-infected banana plant. It was morphologically and molecularly identified as *T. wortmannii*. Laboratory experiments revealed that *T. wortmannii* FP-25 exhibited inhibitory activity against Foc TR4, *C. lunata* and *S. maydis* by reducing mycelial growth over time, indicating antagonistic potential against the tested fungal phytopathogens and representing the first report of biocontrol activity of *T. wortmannii* against *C. lunata*, Foc TR4 and *S. maydis* in the Philippines. However, this study was undertaken only under controlled *in vitro* conditions and its effectiveness under natural field conditions remains unknown. Therefore, the results indicate promising *in vitro* antagonistic potential that warrants further evaluation under greenhouse and field trials.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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