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Bioprospection of Culturable Endophytic Fungi Associated with the Ornamental Plant *Pachystachys lutea*

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Abstract

Endophytes are fungi and bacteria that inhabit plant tissues without causing disease. Endophytes have characteristics that are important for the health of the plant and have been isolated from several plants of economic and medicinal interest but rarely from ornamental plants. The current study isolates and identifies endophytic fungi from the leaves of *Pachystachys lutea* and evaluates the antagonistic activity of these endophytes as well as cellulase production by the endophytes. Fungi were isolated by fragmentation from surface-disinfected leaves and were identified by the sequencing of the ITS gene and the genes coding for EF 1-α and β-tubulin followed by multilocus sequence analysis. Molecular taxonomic analysis revealed that 78% of the identified fungi belonged to the genus *Diaporthe*. We also identified strains belonging to the genera *Colletotrichum, Phyllosticta, Xylaria, Nemania,* and *Alternaria*. Most of the strains tested were able to inhibit the growth of pathogenic fungi, especially PL09 (*Diaporthe* sp.), which inhibited the growth of *Colletotrichum* sp., and PL03 (*Diaporthe* sp.), which inhibited the growth of *Fusarium oxysporum*. The production of cellulase ranged from 0.87 to 1.60 µmol/min. Foliar endophytic fungal isolates from *P. lutea* showed promising results for the in vitro control of plant pathogens and for cellulase production. This paper is the first report on culturable endophytic fungi isolated from the ornamental plant *P. lutea*.

Introduction

Society's concerns about the impact of agriculture on the environment have led to the use of biological control as one of the most discussed alternatives to reduce the intensive use of pesticides to control plant diseases, pests, and weeds. Furthermore, endophytic microorganisms have proved to be effective in blocking the growth of various groups of plant pathogens, similar to biological control agents [52].

By occupying inter- and intra-cellular spaces [13, 19], endophytes are capable of colonizing plant tissues without causing apparent damage [16]. Endophytic colonization may be beneficial for host plants in many ways, including promotion of plant growth, production of phytohormones, nitrogen fixation [16, 47], biological pest control [39], increased resistance of plants to stress conditions [2, 39], and inhibition or reduction of phytopathogen growth by mycoparasitism, antibiosis, production of metabolites, competition for nutrients, or resistance induction in plants [39].

Most plants have endophytic microorganisms, which include fungi and bacteria. In general, there are dominant species, which are fairly frequent in a particular host, and secondary species, which are rarer. Consequently, plants have a characteristic endophytic microbiota that is likely to be important for plant protection and maintenance. Since host specificity requires co-adaptation between the host plant and its fungal partner, a mutual influence arising from an ancient cohabitation and co-evolution may be suggested [34]. Factors shaping plant–endophyte interactions include

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transmission mode, infection pattern, plant age, environmental conditions, and genetic background [2].

In fact, these microorganisms have been widely studied due to the interactions between endophytes, plants, and other microorganisms, which requires a great variety of substances, including enzymes [11] and a wide range of bioactive secondary metabolites [32, 42]. Endophytes have developed special mechanisms to penetrate host tissues and to reside in close association with each other in the host tissues. Endophytes possess exoenzymes necessary for host colonization, while they grow well in the apoplastic washing fluid of the host [10]. To compete with pathogenic fungi, endophytes also release hydrolytic enzymes, such as proteases, glucanases, and chitinases, which are capable of degrading the cell walls of the fungal hyphae [16].

There have been reports of cellulase, amylase, phenoloxidase, pectinase, xylanase, tyrosinase, gelatinase, and lipase production by endophytic fungi isolated from various plants [11, 16]. Enzymes obtained from microorganisms are used in the detergent, starch, fuel, food, beverage, textile, paper, leather, and several other industries. Indeed, endophytes are a potential source of enzymes of interest for industrial use [11].

Cellulases are the third most produced group of industrial enzymes worldwide due to their applications in cotton processing, paper recycling, and juice extraction and as enzymatic detergents and animal food additives [31]. Cellulases are classified into three groups: endoglucanases, which cleave the internal bonds of cellulosic fiber, generating oligosaccharides of different lengths and, therefore, new chain ends; exoglucanases, which are divided into cellobiohydrolases, which release cellobiose (glucose dimer) from the ends of cellulose, and glucanohydrolases, which are capable of directly releasing the glucose polymer; and β-glucosidase, which hydrolyses soluble oligosaccharides to glucose [22]. Endoglucanases are involved in plant colonization by endophytes [12].

Pachystachys lutea (Acanthaceae), popularly known as golden shrimp plant, is a subtropical shrub that is 90–120 cm tall and is commonly used as an ornamental plant [36]. This species is native to South America and was collected for the first time in the Amazon region; more precisely, this plant was first collected in the state of Acre, Brazil [56]. Studies of endophytes from ornamental plants are still rare and have mainly been conducted on Orchidaceae. In this family, endophytic fungi have been isolated from *Lepanthes* [5], *Bletilla ochracea* [51], and 54 other species; some of these endophytic fungi exhibited antimicrobial activity [54]. Fungi and endophytic bacteria have been isolated from Acanthaceae plants [33, 43].

These findings demonstrate the versatility of endophytic microorganisms isolated from different hosts as sources of information about host–plant interactions and biomolecule production. Therefore, the diverse endophytic fungi in *P. lutea* may be a rich source for the discovery of new, potentially bioactive compounds generated by these microorganisms. Current study isolates and identifies endophytic fungi in the leaves of *P. lutea*, and evaluates the antagonistic activity and cellulose production for them.

Materials and Methods

Isolation of Endophytic Fungi

P. lutea leaves were collected randomly from two specimens at the plant nursery of the Universidade Estadual de Maringá (23°24′S; 51°56′W). The rainfall during the month of collection was 151.3 mm, the average temperature was 24.1 °C, and the relative humidity was 63%. For the isolation of endophytes, the surfaces of 50 leaves were sterilized by immersion in 70% ethanol for 1 min, in 3% sodium hypochlorite for 4 min, and in 70% ethanol again for 30 s; then, the leaves were rinsed twice in autoclaved distilled water. The effectiveness of this method was verified by spreading 100 µL of the water from the final rinse on Petri dishes containing potato dextrose agar (PDA) medium (HiMedia®, Mumbai, India), pH 6.6, supplemented with tetracycline (Sigma, St. Louis, MO) (50 µg/mL in 50% ethanol) to prevent bacterial growth.

Then, 50 disinfected leaves were cut into small fragments measuring approximately 2 mm^2, which were then deposited (five fragments per plate, on a total of 100 plates) on plates containing PDA supplemented with tetracycline. The plates were incubated at 28 °C for 7 days. The colonization frequency (CF) (%) was determined as the ratio of the number of fragments colonized by fungi and the total number of fragments × 100. For fungal purification, the fungal isolates were transferred to PDA plates and grown for 7 days. Then, the fragments (5 mm^2) were crushed in 1 mL of an aqueous solution of 0.01% Tween 80, and a 100-µL aliquot of this solution was then spread on plates containing PDA and incubated for 24 h. Single colonies were immediately transferred to new plates with PDA and incubated for 7 days. If necessary, the process was repeated until pure colonies were obtained.

All strains were preserved according to Castellani's method [9] and deposited in the fungal collection of the Laboratory of Microbial Biotechnology (LBIOMIC) at the Universidade Estadual de Maringá, Brazil.

Multilocus Sequence Analysis (MLSA)

Genomic DNA extraction, amplification, and phylogenetic analysis were performed as described by Polonio et al. [38]. For MLSA, partial sequences of the region ITS1–5.8S–ITS2

and of the genes coding for elongation factor 1-α (EF1α) and β-tubulin (TUB) were used. Primers used for amplification and the PCR (polymerase chain reaction) conditions are presented in Supplementary Table 1.

Sequences of the isolates were compared to the sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov) using BLASTn and limiting the alignment with type–strain sequences, and the isolates were identified based on percentage identity and sequence coverage. Based on the available phylogenetic data on the TreeBASE database, other species were also selected (Study S13943; S15707; S14141; S14146; S141147; SN1525; ID:14671; http://www.treebase.org). The sequences were then rescued and aligned with the online interface MAFFT [25] (http://mafft.cbrc.jp/alignment/server/). After alignment, multigene assembly of sequences was performed using SequenceMatrix [53] (http://gaurav.github.io/taxondna/).

For phylogenetic analysis based on the maximum likelihood and Bayesian inference, MrModelTest v. 2.3 [30] was used to choose the best evolutionary model. The phylogenetic tree was constructed using MrBayes v. 3.2.5 [44], taking into consideration the parameters generated by MrModelTest, with Markov chain Monte Carlo (MCMC), which lasted until the average standard deviation of the split frequencies was below 0.01 (100.000 generations). The Bayesian probability was demonstrated on the nodes between each individual. The tree was edited with FigTree v. 1.4.2 [41].

All sequences in the current study were deposited in GenBank and are available under the Accession Numbers listed in Supplementary Table 2.

In Vitro Antagonist Activity of Endophytic Fungi

The antagonistic activity of the endophytic fungi was evaluated against the pathogenic fungi *Fusarium oxysporum* (ATCC2163, André Tosello Foundation, Campinas, SP, Brazil) and *Colletotrichum* sp. (CNPUV378, Embrapa Grape and Wine, Bento Gonçalves, RS, Brazil) using a paired-culture technique described by Campanile et al. [8], with modifications described by Polonio et al. [37].

Competitive interactions between the endophytes and pathogens were analyzed in vitro on the scale described by Badalyan et al. [4], with modifications, based on four types of interactions: A, B, C, and D, with C and D being divided into subcategories. The interaction types are as follows: A = inhibition of mycelial growth with contact; B = inhibition from a distance; C = endophytic growth on the pathogen without initial inhibition; CA1 and CA2 = partial and complete endophytic growth, respectively, on the pathogen after initial inhibition with mycelial contact; CB1 and CB2 = partial and complete endophytic growth, respectively, on the pathogen after initial inhibition from a distance; D = pathogen growth on the endophyte without initial inhibition; DA1 and DA2 = partial and complete pathogen growth on the endophyte after initial inhibition with mycelial contact, respectively.

Detection of Cellulase Production

To select endophytes with cellulolytic activity in the extracellular medium, all isolates were inoculated in minimal medium (6 g/L $NaNO_3$, 5 g/L KCl, 1.5 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $ZnSO_4$, 0.01 g/L $FeSO_4$, and 15 g/L agar; pH 5.0) supplemented with 5 g/L yeast extract and 1% carboxymethylcellulose (CMC) (Sigma-Aldrich®, São Paulo, Brazil). Plates were incubated for 7 days at 28 °C and then visualized with 0.1% Congo red dye. Enzyme production was indicated by halo formation.

The fungi with the best results were studied by the cup–plate method as described by Souza et al. [48], with modifications.

Three mycelial discs were cultured in Mancini solution (2 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.9 g/L $Na_2HPO_4 \cdot 2H_2O$, and 1 g/L yeast extract in 1000 mL of distilled water; pH 5.0) with 0.5% CMC. The cultures were incubated for 7 days at 28 °C. Then, fungal mycelia were separated from the liquid media using sterilized gauze. The filtrate (enzyme crude extract) (50 µL) was inoculated into 6-mm-diameter holes removed from the central part of the culture media, CMC-agar (18 g/L agar, 10 g/L CMC, 0.1 M sodium acetate buffer; pH 5.0). A positive control experiment was performed using a commercially available enzyme (cellulase from *Aspergillus niger*, Sigma-Aldrich) at 1 mg/mL. The plates were incubated at 28 °C for 24 h and then stained with 0.1% Congo red dye. Tests were performed in triplicate. Enzyme production was indicated by halo formation; diameters were measured in mm. Mean rates were compared by the Scott–Knott test ($p < 0.05$) using the statistical program Sisvar 5.5 [14].

Determination of Endoglucanase Activity

Endoglucanase activity was determined as described by Ghose [19], adapted for CMC analysis. A 0.5 mL aliquot of crude enzymatic extract was pipetted into test tubes containing 0.5 mL of CMC solution (1% w/v) in sodium citrate buffer (50 mM; pH 4.8). Then, 1 mL of DNS (3,5-dinitrosalicylic acid) was added into the tubes after 15 and 30 min of incubation at 40 °C.

To prepare the DNS solution, 4.0 g of sodium hydroxide (Panreac®, Barcelona, Spain) was dissolved in 50 mL of distilled water, to which 2.5 g of DNS reagent (Dinâmica®, Diadema, Brazil) was added, and the mixture was homogenized. Meanwhile, 75 g of sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) (Nuclear®, Diadema, Brazil) was dissolved in 125 mL of distilled water under constant agitation.

The solutions were mixed with heating until completely dissolved. After cooling, the volume of the mixture was adjusted to 250 mL, and the mixture was stored at room temperature and protected from light.

After homogenization of the contents of the tubes, the tubes were heated for 5 min and then cooled. Then, 3 mL of distilled water was added to each tube, and the contents of each tube were subsequently homogenized by vortexing for 15 s. The absorbance of the reaction was measured at a wavelength of 540 nm by a spectrophotometer [29].

A blank comprising crude enzymatic extract, CMC solution (1% w/v) and DNS, which were immediately mixed, was measured for each sample. The absorbance of the blank of each sample was subtracted from the measurement of the corresponding test sample. The absorbance values were compared to a standard glucose curve.

Each sample was assayed in triplicate. One unit of endoglucanase activity was defined as the amount of enzyme required to liberate 1 µmol/min/mL glucose. Average rates were compared by the Scott–Knott test ($p < 0.05$) using the statistical program Sisvar 5.5 [14].

Results

Isolation

The isolation rate was 26.6%. From the obtained isolates, 85 were randomly selected and grouped into 23 morpho-groups based on macroscopic characteristics, including morphology and characteristics observed when the isolates were grown on PDA culture media: sporulation, mycelial properties, mycelial coloration, coloration of the reverse side of the Petri dish, pigmentation of the culture medium, and average diameter of the colony. One of the fungi from each morpho-group was randomly selected for molecular identification based on DNA sequencing (Supplementary Fig. 1).

Multilocus Sequence Analyses

Multilocus sequence analyses (MLSAs) revealed that 78% of the isolates identified belonged to the genus *Diaporthe*. These isolates were categorized into four main groups (Supplementary Fig. 2).

The endophytic isolates PL01, PL64, and PL43, belonging to group A, were sub-clustered into sub-group 1A, exhibiting 100% Bayesian probability (BP) with *Diaporthe anacardii* CBS 720.97.

In sub-group 2B, with 100% BP, the isolates PL39, PL74, PL63, PL73, and PL66 were clustered with another three *Diaporthe infecunda* strains taking into account that only the ITS and EF1-α genes were sequenced for strains PL74, PL63, and PL66.

In sub-group 1C, with 87% BP, the isolates PL03 and PL09 were clustered with different species of *Diaporthe*; however, the identity of these isolates was confirmed to be *Diaporthe* only at the genus level.

Sub-group 2C, with isolates PL40, PL53, and PL47, was clustered with *Diaporthe schini* CBS 133181 and presented 100% BP. The strains were grouped based on the sequences of the ITS and EF1-α genes.

In group D, the isolates PL18, PL50, PL61, PL67, and PL71 were clustered with *Diaporthe* sp. 2 LGMF932 and *Diaporthe mayteni* CBS133185 with 100% BP. These isolates were classified taxonomically as *Diaporthe* sp.

For the remaining endophytic isolates, sequence comparison against the NCBI database did not yield DNA sequences with close identities to the DNA sequences of those endophytes; this observation was true when considering all three genes (ITS, EF1α, and TUB) together as well as when only considering two of the three sequenced genes. Therefore, these endophytic strains were clustered into four groups of different genera based on the partial information obtained from the NCBI database (Supplementary Fig. 3).

In sub-group 1.1, the endophyte PL75 was clustered with different species of *Alternaria* (based on the analysis of ITS and EF1-α genes). Consequently, molecular taxonomical classification was confirmed only at the genus level as *Alternaria*.

In sub-group 1.2, the endophyte PL49 was clustered with five strains of *Phyllosticta capitalensis* (1908) [synonyms: *Phyllostictina pyriformis* (1955), *Guignardia mangiferae* (1968), and *Guignardia endophyllicola* (2001)] and confirmed as *P. capitalensis* by analysis of ITS and EF1-α genes.

In sub-group 2.2, the endophyte PL36 was clustered with three strains of *Xylaria berteroi* with 99% BP based on the analysis of the ITS and TUB genes. When these two genes were considered, the endophyte PL27 (sub-group 2.4) was clustered with *Nemania* sp. FL0031 with 100% BP, while strain PL45 (sub-group 3.2) was clustered with three strains of *Colletotrichum fructicola*. Taxonomy was confirmed at the species level.

In Vitro Antagonist Activity of Endophytic Fungi

The analysis of variance of antagonistic activities measured for the endophytic fungi showed statistically significant differences between the endophytic strains tested against phytopathogens. These data are shown in Table 1 along with the percentages of inhibition and types of competitive interactions.

The analysis of the antagonism of 20 endophytic fungi against *F. oxysporum* yielded four statistical groups. The best performance was observed for strain PL03 (*Diaporthe* sp.), which exhibited a 64.62% inhibition rate (Table 1; Supplementary Fig. 4). Three types of interactions were

Table 1 Antagonism of endophytic fungi from *P. lutea* against pathogenic fungi. Average plant pathogen area (*A*), percentage inhibition rate (IP%), and competitive interaction (CI)

Endophytes	Pathogenic fungi					
	F. oxysporum			*Colletotrichum* sp		
	A	IP%	CI	*A*	IP%	CI
PL01 (*Diaporthe anacardii*)	30.74[c]	28.06	CA1	39.99[b]	16.89	A
PL03 (*Diaporthe* sp.)	15.16[d]	64.62	A	22.78[d]	52.66	A
PL09 (*Diaporthe* sp.)	17.36[d]	59.37	A	19.47[d]	59.54	A
PL18 (*Diaporthe* sp.)	32.61[b]	23.68	CA1	38.76[b]	19.45	DA1
PL19 (NI)	25.20[c]	41.02	A	27.76[c]	42.31	A
PL27 (*Nemania* sp.)	41.76[a]	2.27	CA1	42.39[a]	11.91	A
PL35 (NI)	36.33[b]	14.98	DA1	42.42[a]	11.84	DA1
PL36 (*Xylaria berteroi*)	37.76[b]	11.63	A	45.18[a]	6.11	A
PL38 (NI)	43.42[a]	0,00	CA1	45.05[a]	6.38	A
PL39 (*Diaporthe infecunda*)	31.02[c]	27.40	A	36.29[b]	24.58	A
PL40 (*Diaporthe schini*)	21.35[d]	50.03	CA1	21.80[d]	54.70	CA1
PL43 (*Diaporthe anacardii*)	28.29[c]	33.79	CA1	38.18[b]	20.66	DA1
PL45 (*Colletotrichum fructicola*)	30.01[c]	29.77	CA1	39.65[b]	17.60	A
PL50 (*Diaporthe* sp.)	41.34[a]	3.25	A	36.73[b]	23.67	DA1
PL53 (*Diaporthe schini*)	20.02[d]	53.15	CA1	25.17[c]	47.69	CA1
PL56 (NI)	37.82[b]	11.49	DA1	38.33[b]	20.34	DA1
PL58 (NI)	37.42[b]	12.43	A	41.25[b]	14.28	DA1
PL64 (*Diaporthe anacardii*)	28.29[c]	33.79	CA1	39.02[b]	18.91	DA1
PL66 (*Diaporthe infecunda*)	24.73[c]	42.12	A	25.84[c]	46.3	A
PL75 (*Alternaria* sp.)	36.71[b]	14.09	DA1	41.08[b]	14.63	DA1
Control	42.73[a]	–	–	48.12[a]	–	–

Average phytopathogen area followed by the same letter does not differ by Skott-Knott test ($p < 0.05$)

NI not identified

reported: type CA1 (partial growth of the endophyte on the pathogen after initial inhibition with mycelial contact), 45%; type A (inhibition of mycelial growth with contact), 40%; and type DA1, 15% (Table 1).

The antagonistic activity of 20 endophytic fungi against *Colletotrichum* sp. was also evaluated. Four statistical groups were obtained for *Colletotrichum* sp., with the best inhibition rate obtained for endophyte PL09 (59.54%), identified as *Diaporthe* sp. Again, three types of interactions were reported: type A, 50%; type DA1, 40%; and type CA1, 10% (Table 1, Supplementary Fig. 4).

Enzyme Production

The statistical analysis of the results from the cup–plate test of cellulase production revealed significant differences between the control and the endophytes tested, with the formation of four separate groups (Table 2). The best results were reported for the endophyte PL01 (*Diaporthe anacardii*), with halos measuring 15.02 mm, followed by PL67 (*Diaporthe* sp.), with halos of 12.89 mm; PL36 (*Xylaria berteroi*); PL03 (*Diaporthe* sp.) and PL35, with halos ranging between 11.62 and 11.07 mm (Table 2, Supplementary Fig. 5).

Spectrophotometric analysis of cellulase production revealed results ranging between 0.87 and 1.60 µmol/min of endoglucanase with no significant difference (Table 2).

Table 2 Evaluation of cellulase production by endophytic fungi isolated from *P. lutea*

Endophytic fungi	Halos degradation	Endoglucanase (µmol/min)
PL01 (*Diaporthe anacardii*)	15.02[b]	1.60[a]
PL03 (*Diaporthe* sp.)	11.18[d]	1.06[a]
PL35 (NI)	11.07[d]	0.87[a]
PL36 (*Xylaria berteroi*)	11.62[d]	1.08[a]
PL67 (*Diaporthe* sp.)	12.89[c]	1.23[a]
Control	22.33[a]	–

Average halos degradation followed by the same letter does not differ by Skott-Knott test ($p < 0.05$)

NI not identified

Discussion

The colonization frequency of *P. lutea* leaves was 26.6%. The colonization may even be as high as 100% in plants from tropical environments and between 1 and 40% in plants from northern and Arctic ecosystems [34, 39, 42].

In this study, the endophytic genus *Diaporthe* was the one most abundant (78%) in the foliar tissue of *P. lutea*. Similarly, several authors showed that one or two species of endophytes appear in high abundance in different host. Bogner et al. [6] reported that *Fusarium oxysporum* and *F. solani* were the most frequently observed endophytes in tomato roots from Kenya. Pamphile and Azevedo [34] showed that the most prevalent endophyte in close association with maize seeds from different genotypes was *Fusarium verticillioides* (= *Fusarium moniliforme*). These results are also consistent with other reports in which the genus *Diaporthe* is commonly isolated from several plants, such as *Sapindus saponaria* [18], *Eichhornia azurea* [1], *Vitis labrusca* [13], and *Mikania glomerata* [37], and from agricultural crops with high economic value, such as cocoa [45], coffee [55], soybean [26], and common bean [21]. Rhoden et al. [42] observed an 84.3% abundance of *Diaporthe* sp. endophytic isolates in the leaves of *Trichilia elegans*. Ferreira et al. [15] analyzed the diversity of endophytic fungi associated with *Vellozia gigantean*, and *Diaporthe* was seen to be the most abundant genus, with 70 endophytic isolates recovered from the leaves and roots.

The genus *Diaporthe* comprises close to 800 species and is distributed worldwide, with a great variety of hosts. Species of this genus may be phytopathogens, saprophytes, or endophytic symbionts [20, 46]. Some species of *Diaporthe* may be either pathogenic or harmless endophytes, depending on the type of host and the health of the host [20].

Other species found in the leaves of *P. lutea* were *Alternaria* sp., *Phyllosticta capitalensis, Xylaria berteroi, Nemania* sp., and *Colletotrichum fructicola*. The genera found in the current study may be associated with the isolation approach used, which generally excludes the detection of non-culturable species, as highlighted by Bogner et al. [6]. Consequently, the number of genera observed in *P. lutea* leaves in the current study may represent only a fraction of the total fungal diversity present. A major limitation of crop-dependent studies for unraveling the diversity of endophytes is the prevalence of fast-growing ubiquitous species. On the other hand, rare species with minor competitive strength and more specialized requirements may remain undiscovered [2]. It should be noted that the time between plant collection and endophyte isolation, the culture medium selected, the size of the plant fragment, and the growth conditions could also affect infection frequency, diversity, and species composition [3].

The endophytes that inhabit leaves are under a different set of selective pressures than those inhabiting other parts of the plant. Leaves have a short life span compared to stems and roots, but leaves are more biochemically dynamic, more sensitive to changes in environmental conditions, and more prone to damage caused by herbivores. The implications of these differences have not yet been explored, and they can be used to elucidate general patterns of the evolution of the life histories and specificity between the host and the endophyte [3]. Therefore, different environmental pressures can select endophytes with different physiological abilities, such as enzyme production and antagonistic activity against phytopathogens of economically important plant cultivars.

The current study demonstrated the ability of several endophytic fungi of *P. lutea* to reduce the mycelial growth of two phytopathogenic fungi. The endophytic fungi tested were efficient against *F. oxysporum* and *Colletotrichum* sp. Diseases caused by *F. oxysporum*, especially fusarium wilt, and crown and root rot in tomato plants (*Solanum lycopersicum* L., formerly, *Lycopersicon esculentum* Mill.), have been and continue to be among the most intensively studied plant diseases. These pathogens cause extensive damage to this important vegetable crop in fields and in greenhouses and continue to be the major limiting factors in tomato production [28]. *Colletotrichum* spp., an ascomycete, is a pathogenic fungus that causes anthracnose, a highly destructive plant disease, in a wide range of plants, including vegetables, fruits, legumes, and perennial trees, worldwide, with severe economic repercussions [23].

Endophytic fungi with the highest antagonistic activity against *F. oxysporum* and *Colletotrichum* sp. in the current study belong to the genus *Diaporthe*. This genus is known as a prolific source for the production of natural products, many of which possess antifungal activity. Tanneye et al. [50] characterized three dihydropyrones, phomopsolides A, B, and C, and a stable alpha-pyrone obtained from *Diaporthe maritima* with potent in vitro antifungal and antibiotic activities. Specian et al. [49] investigated secondary metabolites from endophytic *D. helianthi* by column chromatography and ^{1}H and ^{13}C nuclear magnetic resonance and identified the phenolic compound 2(-4 hydroxyphenyl)-ethanol (Tyrosol). These bioactive compounds, including Tyrosol, have antimicrobial effects against human and phytopathogenic bacteria. Several studies have demonstrated the ability of *Diaporthe* to inhibit other fungi and bacteria. The antifungal activity of *D. citri*, isolated from *Mikania glomerata*, was verified by in vitro tests against *Fusarium solani* and *Didymella bryoniae* [37]. *Diaporthe phaseolorum* isolated from *Espeletia* sp. demonstrated significant activity against the plant pathogen *Phytophthora infestans*; this organism contains a gene encoding an amylase, which was differentially expressed during the interaction and is possibly involved in this antagonism [40]. Compounds obtained from *Diaporthe*

sp. have also demonstrated potent antifungal and antibacterial activity against gram-positive and gram-negative bacteria [27, 37, 50].

There has been increasing interest in endophytic microorganisms as biological control agents because they are capable of inhibiting plant pathogens via several mechanisms of direct or indirect inhibition. Endophytic organisms produce substances (antibiotics and enzymes) that directly inhibit pathogens or induce systemic resistance in the host; merely occupying space and mobilizing plant nutrients prevent infection by pathogens [17], with the added benefit of decreasing the levels of xenophobic chemical products that may damage the environment [24, 43]. The current study further highlights the well-documented role of endophytes as biocontrol agents and reinforces the observations from other studies that use the same methodologies employed in this study. Orlandelli et al. [32] evaluated the antifungal activity of endophytic fungi isolated from *Piper hispidum* against plant pathogens *Alternaria alternata, Colletotrichum* sp., *Phyllosticta citricarpa*, and *Moniliophthora perniciosa* and observed that, in some cases, the endophytes were more effective than commercial fungicides. This result highlights the capacity of these microorganisms to act as biological control agents.

One of the mechanisms by which endophytes compete with pathogenic fungi is by releasing hydrolytic enzymes. Panka et al. [35] confirmed that colonized plants invoke a faster defensive reaction against pathogens than non-colonized plants, thus increasing plant resistance against infection. Furthermore, microorganisms produce enzymes that facilitate their penetration into plants. Corrêa et al. [11] highlight endophytes as a new source of many interesting industrial enzymes, such as lipases, phytases, amylases, proteases, and cellulases. Moreover, the ability of endophytes to degrade the complex structure of lignocellulose makes them potentially useful in the exploitation of lignocellulosic biomass to produce ethanol and other value-added products.

Cellulase is involved in plant colonization by endophytes, and there is great interest in cellulase for industrial application [11, 12]. Cellulose is the most abundant renewable source of carbon on the earth's crust; however, in nature, few microorganisms are able to degrade cellulose by cellulase production; filamentous fungi are the most efficient degraders of cellulose [22].

The most studied models of cellulase production are *Trichoderma reesei, Aspergillus niger, Aspergillus nidulans*, and *Neurospora crassa*. Endoglucanase production by *T. reesei* may vary between 0.2 and 137 µmol/min/mg, depending on test conditions and substrate used. Endoglucanase production by *A. niger* and *A. nidulans* varies between 1.0 and 59 µmol/min/mg and 65.66 µmol/min/mg, respectively [22]. Endoglucanase production by endophytes of *P. lutea* may be compared to endoglucanase production by *T. reesei* and *A. niger*, taking into account differences in test conditions and substrates. Laboratory production of cellulase may be optimized to increase the efficiency of *P. lutea* endophytes.

The current analysis reported the relationship between antifungal and enzymatic activities. The strains PL01 (*Diaporthe anacardii*), PL03 (*Diaporthe* sp.), PL36 (*Xylaria berteroi*), and PL35 (unidentified) showed significant antifungal activity against at least one of the pathogenic fungi tested, in addition to producing cellulase. These observations are not unexpected since the same fungal endophyte has often exhibited a positive behavior in different in vitro tests [32, 37]. The species *Xylaria berteroi* has been isolated as an endophyte from grapevines (*Vitis labrusca* L.) and has shown significant activity against *F. oxysporum* [7].

Ornamental plants have rarely been studied for their endophytic biodiversity. The current analysis shows, for the first time, that endophytic fungi from *P. lutea* are promising agents for the biological control of diseases of agronomic interest and are useful for cellulase production.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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