Extracellular enzymatic profiles and taxonomic identification of endophytic fungi isolated from four plant species

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ABSTRACT. Plants of medicinal and economic importance have been studied to investigate the presence of enzyme-producing endophytic fungi. The characterization of isolates with distinct enzyme production potential may identify suitable alternatives for specialized industry. At Universidade Estadual de Maringá Laboratory of Microbial Biotechnology, approximately 500 isolates of endophytic fungi have been studied over the last decade from various host plants, including medicinally and economically important species, such as *Luehea divaricata* (Martius et Zuccarini), *Trichilia elegans* A. Juss, *Sapindus saponaria* L., *Piper hispidum* Swartz, and *Saccharum* spp. However, only a fraction of these endophytes have been identified and evaluated

for their biotechnological application, having been initially grouped by morphological characteristics, with at least one representative of each morphogroup tested. In the current study, several fungal strains from four plants (*L. divaricata*, *T. elegans*, *S. saponaria*, and *Saccharum* spp) were identified by ribosomal DNA typing and evaluated semi-quantitatively for their enzymatic properties, including amylase, cellulase, pectinase, and protease activity. Phylogenetic analysis revealed the presence of four genera of endophytic fungi (*Diaporthe*, *Saccharicola*, *Bipolaris*, and *Phoma*) in the plants examined. According to enzymatic tests, 62% of the isolates exhibited amylase, approximately 93% cellulase, 50% pectinase, and 64% protease activity. Our results verified that the composition and abundance of endophytic fungi differed between the plants tested, and that these endophytes are a potential enzyme production resource of commercial and biotechnological value.

Key words: Endophytes; Fungi; Phylogenetic analysis; Enzymes

INTRODUCTION

Endophytic fungi exhibit complex interactions with their hosts. This fact has recently triggered several studies exploring their utilization as sources of novel bioactive natural products. Although the production of metabolites depends on several factors, fungi employ molecular pathways resulting in specific compounds. The products of these metabolic processes include important drugs such as penicillin and statins, and toxic substances such as aflatoxins (Keller et al., 2005).

Endophytic fungi synthesize a wide range of enzymes and natural products that demonstrate a variety of biological activities (Kusari et al., 2014). The bioactive secondary metabolites produced by such organisms include compounds that mimic metabolites synthesized by the host plant (Kusari et al., 2014). The production of novel compounds by endophytic fungi is of great pharmaceutical, industrial, and agricultural importance, since these metabolites may be unique in their structure and effective against several diseases (Mishra et al., 2014). For instance, Suryanarayanan et al. (2009) described several natural compounds with antifungal and antibacterial activity isolated from fungi.

Enzymes are potential biocatalysts for a large number of reactions. Microorganisms represent a viable alternative source of enzymes, as they may be cultured in large quantities within short time frames by fermentation, are biochemically diverse, and are amenable to genetic manipulation (Anbu et al., 2013).

Fungal endophytes produce several extracellular enzymes, such as pectinases, cellulases, lipases, amylases, laccases, and proteinases. Moreover, fungal enzymes play a key role in biodegradation and hydrolysis, mechanisms of significant importance in protection against invading pathogens, besides being crucial in obtaining nutrition from the host plant (Sunitha et al., 2013). Indeed, their principal functions comprise hydrolysis of food substances and defense against pathogens (Desire et al., 2014). Fungal enzymes are used in the beverage, food, confectionary, textile, and leather industries to simplify the processing of raw materials. In addition, such enzymes are often more stable than those derived from other sources (Raju et al., 2015).

The medicinal properties of certain plants may be related to the metabolites produced

by associated endophytic microorganisms. Endophytes isolated from such plants generate strongly fungicidal, bactericidal, and cytotoxic molecules, and produce enzymes used in various applications, including the degradation and biotransformation of organic compounds (Sudha et al., 2016).

Due to the great diversity of Brazilian flora, the isolation and the characterization of these microorganisms, especially those associated with plants of medicinal and economic significance may be highly beneficial. The plants *Luehea divaricata*, *Trichilia elegans*, *Sapindus saponaria*, and *Saccharum* spp are used in traditional culture for the treatment of several diseases, and have also attracted commercial interest. Bernardi-Wenzel et al. (2010), Rhoden et al. (2012), García et al. (2012), and Leme et al. (2013) demonstrated the presence of endophytes in each of these plants, respectively. Although some endophytic fungi associated with these species have already been studied by our group, this represents only a fraction of the total number of fungal and bacterial isolates obtained from these plants belonging to our microbial laboratory collection, since endophytes were initially grouped by morphological characteristics, with representatives of each morphogroup being examined. Therefore, in the present study, with the aim of continuing the study of these previously isolated microorganisms, we sought to screen further endophytic fungi present in these plants to assess their biotechnological potential as enzyme producers. Furthermore, taxonomic identification of some of the endophytes in our fungal collection was carried out.

MATERIAL AND METHODS

Biological material

The endophytic fungi used in the current research were as follows: 8 isolates from *L. divaricata* Martius et Zuccarini (Bernardi-Wenzel et al., 2010); 10 isolates from *T. elegans* A. Juss (Rhoden et al., 2012); 11 isolates from *S. saponaria* L. (García et al., 2012); and 13 isolates from *Saccharum* spp (Leme et al., 2013), belonging to the collection of the Laboratory of Microbial Biotechnology of Universidade Estadual de Maringá, Maringá, PR, Brazil. The fungi were selected from the same morphogroup, considering colony morphology and coloration, mycelial form, and pigment production of each isolate.

Molecular identification of endophytic fungi

Genomic DNA was extracted following Raeder and Broda (1985) with modifications according to Pamphile and Azevedo (2002), except that beforehand, endophytes were grown for 7 days on plates containing potato dextrose broth medium at 28°C without agitation. The concentration and purity of genomic DNA were determined with a GENESYS 10S UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) by measuring the 260/280 nm optical density ratio. DNA integrity was analyzed by electrophoresis on 1% agarose gels, using a High DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as a molecular weight standard. The final DNA concentration was adjusted to 10 ng/mL.

Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS)1-5.8S-ITS2 ribosomal DNA (rDNA) region was performed using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCCGCTTATTGATATGC-3'; White et al., 1990), with an initial denaturation at 92°C for 4 min, followed by 35 cycles of denaturation at 92°C for 40 s, annealing at 52°C for 1 min 30 s, and extension at 72°C for 2

min, before a final extension at 72°C for 5 min.

PCR products were purified with a GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Amersham, UK) following the manufacturer protocol, and prepared for sequencing reactions using primer ITS4. Sequencing reactions were performed in a thermocycler as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 60°C for 1 min, prior to a final extension at 60°C for 5 min. Sequencing was performed in a MegaBACE 1000 sequencer (Amersham Biosciences), with injection and electrophoresis conditions of 1 kV for 90 s and 7 kV for 240 min, respectively.

The nucleotide sequences were analyzed, edited, and compared to homologous sequences deposited in GenBank (<http://www.ncbi.nlm.nih.gov>). For determination of genera and species, the BLASTN algorithm was used, based on the highest identity value obtained.

Phylogenetic analysis involved the construction of a dendrogram using the sequences obtained as above, together with those available in GenBank. Sequences were aligned using ClustalX and the dendrogram generated in MEGA5 (Tamura et al., 2011) by the neighbor-joining method with nucleotide p-distances, pairwise gap deletion, and 10,000 bootstrap (BP) replicates. All 16S rDNA gene sequences in the current study were deposited in GenBank under accession Nos. KX530728 through KX530754.

Assessment of biotechnologically relevant enzyme activity of endophytic isolates by cup-plate assay

Endophytic isolates were cultured on potato dextrose agar medium for 7 days before analysis of their amylase, cellulase, pectinase, and protease capacities. Three 5-mm mycelial discs per isolate were cultured in liquid medium (Manachini solution; 2 g/L KH_2PO_4 , 1 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.9 g/L $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 1 g/L yeast extract, and 1 L distilled water) supplemented with 0.5% substrate and a pH value adjusted to the corresponding enzyme, as follows: amylase (corn starch, pH 6.0); cellulase [carboxymethyl cellulose (CMC; Fluka, Gillingham, UK), pH 5.0]; pectinase [citrus pectin (Vetec, Duque de Caxias, Brazil), pH 2.5], and protease (gelatin, pH 6.9). Cultures were incubated at 28°C with shaking at 140 rpm for 96 or 120 h. Mycelia were then separated from the liquid medium with sterilized gauze. A disc 6 mm in diameter was removed from the center of the Petri dish of solid enzyme detection medium (cup plates) and the resulting hole filled with 50 μL filtered liquid medium. Starch agar (18 g/L agar, 10 g/L starch, and 0.1 M citrate-phosphate buffer, pH 5.0) was used for amylase; CMC agar (18 g/L agar, 10 g/L CMC, and 0.1 M Na^+ acetate buffer, pH 5.0) for cellulase; pectin agar (18 g/L agar, 10 g/L pectin, and 0.1 M Na^+ acetate buffer, pH 5.0) for pectinase; and gelatin milk agar (18 g/L agar, 10% gelatin solution, 10% skim milk solution, and 0.1 M citrate-phosphate buffer, pH 5.0) for protease. The following commercially available enzymes (Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls: porcine pancreatic α -amylase; *Aspergillus* sp cellulase; *Aspergillus niger* pectinase; and *Aspergillus oryzae* protease. The negative control consisted of liquid medium incubated without fungal inoculation.

The experiment was performed in triplicate, and plates were incubated at 28°C for between 24 and 168 h. Enzymatic activity was evaluated by the presence of a clear halo with an opalescent background, the size of which was measured in millimeters. With the exception of protease, they were visualized with indicators specific for each enzyme, as follows: iodine tincture (amylase); Congo red dye (cellulase); and 5 N HCl (pectinase).

Statistical analyses

All experiments were performed using a completely randomized design, and results were tested by analysis of variance and the *F*-test. Means were compared by the Scott-Knott test ($P < 0.05$) in the statistical program Sisvar 5.4 (Ferreira, 2011).

RESULTS

Molecular taxonomy based on rDNA and phylogenetic analysis of endophytic fungi

In the current study, taxonomic identification was achieved for 27 of the 42 endophytic fungi tested for enzyme function (Table 1).

Table 1. Identification of endophytic fungi isolated from *Luehea divaricata*, *Trichilia elegans*, *Sapindus saponaria*, and *Saccharum* spp based on sequencing of the ribosomal DNA internal transcribed spacer (ITS)1-5.8S-ITS2 region.

Host plant	Strain	BLAST (GenBank)				Taxonomic identification ³
		General alignment ¹	Identity	Type material alignment ²	Identity	
<i>Luehea divaricata</i>	G17	AY745986.1	98%	KJ197286.1	97%	<i>Diaporthe</i> sp
	G28	KM979780.1	99%	KJ197286.1	99%	<i>Diaporthe</i> sp
	G30	KP182396.1	98%	NR_120155.1	96%	<i>Diaporthe</i> sp
	G10	KM454881.1	100%	KF777180.1	99%	<i>Phoma</i> sp
	G13	KR093844.1	99%	NR_111850.1	99%	<i>Diaporthe</i> sp
<i>Trichilia elegans</i>	2-5	GQ461581.1	99%	KC343136.1	96%	<i>Diaporthe</i> sp
	3-17.1.11	GQ461582.1	99%	NR_111850.1	97%	<i>Diaporthe</i> sp
	3-2.2.1	KR093844.1	99%	KP004461.1	99%	<i>Diaporthe</i> sp
	2-68	KM999227.1	99%	KP004461.1	96%	<i>Diaporthe</i> sp
	2-1	KM979832.1	99%	KM453210.1	97%	<i>Diaporthe</i> sp
	2-58	KC145910.1	99%	KJ197287.1	96%	<i>Diaporthe</i> sp
<i>Sapindus saponaria</i>	Ss15	KP881432.1	97%	FJ427025.1	99%	<i>Phoma</i> sp
	Ss27	JQ753978.1	85%	KJ415528.1	85%	<i>Bipolaris</i> sp
	Ss69	KC981121.1	92%	KJ590728.1	91%	<i>Diaporthe</i> sp
	Ss01B	KC343053.1	98%	NR_111862.1	97%	<i>Diaporthe</i> sp
	Ss49	KM999227.1	99%	KP004461.1	97%	<i>Diaporthe</i> sp
<i>Saccharum</i> spp	C55	KP881432.1	97%	KF251258.1	87%	<i>Saccharicola</i> sp
	C58	GQ370379.1	98%	KF251258.1	94%	<i>Saccharicola</i> sp
	C40	GQ370379.1	99%	KF251258.1	96%	<i>Saccharicola</i> sp
	C61	GQ370379.1	97%	KF251258.1	96%	<i>Saccharicola</i> sp
	C20	GQ461582.1	99%	KC343136.1	96%	<i>Diaporthe</i> sp
	C34	GQ370379.1	99%	KF251258.1	95%	<i>Saccharicola</i> sp
	C35	GQ370379.1	99%	KF251258.1	95%	<i>Saccharicola</i> sp
	C36	GQ370379.1	99%	KF251258.1	95%	<i>Saccharicola</i> sp
	C42	GQ370379.1	100%	KF251258.1	96%	<i>Saccharicola</i> sp
	C24	GQ370379.1	99%	KF251258.1	95%	<i>Saccharicola</i> sp
	C56	GQ370379.1	99%	KF251258.1	95%	<i>Saccharicola</i> sp

¹Accession numbers with highest identity values when compared to endophytic fungal isolate sequences using BLAST searches and the GenBank database; ²BLAST alignment limited to type materials available in GenBank;

³taxonomic identification based on the phylogenetic analysis.

Phylogenetic analysis revealed four main clades (A, B, C, and D; Figure 1), including sub-clades (A1, B2 and D1). Sub-clade A1, with 100% BP support, grouped isolates of the *Diaporthe* genus (including type strains). At least one fungal endophyte associated with each host plant featured in this clade: 2-5, 3-17.1.11, 2-58, 2-1, 2-68, and 3-2.2.1 isolated from *T. elegans*; Ss69, Ss01B, and Ss49 isolated from *S. saponaria*; G30, G13, G28, and G17 isolated from *L. divaricata*; and C20 associated with *Saccharum* spp (Figure 1).

Figure 1. Phylogenetic relationships between sequences of endophytic fungi isolated from *Luehea divaricata*, *Trichilia elegans*, *Sapindus saponaria*, and *Saccharum* spp and those of other fungi deposited in GenBank. Filled triangle = isolates from *Trichilia elegans*; open triangle = isolates from *Saccharum* spp; filled diamond = isolates from *Sapindus saponaria*; open diamond = isolates from *Luehea divaricata*; filled circle = type strains.

Figure 1. Phylogenetic relationships between sequences of endophytic fungi isolated from *Luehea divaricata*, *Trichilia elegans*, *Sapindus saponaria*, and *Saccharum* spp and those of other fungi deposited in GenBank. Filled triangle = isolates from *Trichilia elegans*; open triangle = isolates from *Saccharum* spp; filled diamond = isolates from *Sapindus saponaria*; open diamond = isolates from *Luehea divaricata*; filled circle = type strains.

Enzymatic activity

Evaluation of the enzymatic properties of endophytic fungal isolates obtained from the four plant species showed that 62% exhibited amylase, approximately 93% cellulase, 50% pectinase, and 64% protease activity.

According to our statistical analyses (Table 2), isolates G16 and G30 of *L. divaricata*, 2-58 of *T. elegans*, Ss73 of *S. saponaria*, and C58 and C16 of *Saccharum* spp showed significantly larger halos of enzymatic action (HEA) in relation to amylase activity, compared to other isolates of the same plants. Isolates Ss73 of *S. saponaria* and C58 and C16 of *Saccharum* spp produced HEAs of 11.8, 13.4, and 12.0 mm, respectively, larger than those resulting from the positive control enzyme.

Table 2. evaluation of the enzyme activities of endophytic fungi isolated from *Luehea divaricata*, *Trichilia elegans*, *Sapindus saponaria*, and *Saccharum* spp.

Isolate		Halo of enzymatic action (mm) ^{1,2}			
		Amylase	Cellulase	Pectinase	Protease
<i>Luehea divaricata</i>	G16	10.2 ^d	12.2 ^e	-	12.2 ⁱ
	G30	10.2 ^d	16.2 ^c	8.0 ^b	18.6 ^c
	G28	9.4 ^f	15.0 ^d	8.0 ^b	13.5 ^b
	G17	9.2 ^g	16.0 ^c	-	20.0 ^a
	G10	9.1 ^g	14.9 ^d	-	-
	G13	8.6 ^h	13.6 ^c	-	15.0 ^g
	G21	8.3 ^h	10.4 ⁱ	8.0 ^b	15.8 ^c
	G14	-	15.3 ^d	-	8.0 ^j
<i>Trichilia elegans</i>	2-58	9.8 ^c	13.1 ^f	-	15.0 ^g
	2-1	9.5 ^f	15.7 ^c	-	10.0 ^k
	2-23	9.0 ^g	14.9 ^d	8.0 ^b	10.0 ^k
	3-2.2.1	8.0 ⁱ	10.3 ^j	-	15.3 ^f
	3-1.2.1	-	9.9 ^j	-	19.2 ^b
	2-68	-	11.8 ^b	8.0 ^b	20.0 ^a
	3-17.1.11	-	10.8 ⁱ	8.0 ^b	16.3 ^d
	2-5	-	10.6 ⁱ	8.0 ^b	-
	2-42	-	-	8.0 ^b	-
	2-43	-	12.4 ^e	8.0 ^b	13.4 ^b
<i>Sapindus saponaria</i>	Ss73	11.8 ^b	14.0 ^c	8.0 ^b	12.0 ^j
	Ss69	10.0 ^e	16.8 ^b	-	15.0 ^g
	Ss27	9.8 ^c	-	8.0 ^b	-
	Ss16	9.0 ^g	9.6 ^j	-	16.0 ^c
	Ss52	9.0 ^g	10.2 ^j	-	20.0 ^a
	Ss15	8.0 ⁱ	11.3 ^b	-	-
	Ss01B	-	12.1 ^b	8.0 ^b	20.0 ^a
	Ss31	-	12.4 ^e	-	20.0 ^a
	Ss34	-	11.8 ^b	8.0 ^b	13.4 ^b
	Ss49	-	10.0 ^j	-	-
	Ss50	-	10.7 ^j	-	12.3 ^j
<i>Saccharum</i> spp	C58	13.4 ^a	11.3 ^b	8.0 ^b	12.0 ^j
	C16	12.0 ^b	13.7 ^c	-	-
	C24	10.6 ^d	9.3 ^j	8.0 ^b	-
	C34	10.4 ^d	11.2 ^b	-	7.4 ^m
	C40	10.4 ^d	12.0 ^b	8.0 ^b	8.0 ^j
	C61	10.2 ^d	10.2 ^j	8.0 ^b	-
	C42	9.8 ^c	11.7 ^b	-	-
	C60	9.8 ^c	9.7 ^j	7.3 ^c	-
	C35	8.9 ^g	11.4 ^b	6.6 ^d	-
	C20	-	11.5 ^b	-	-
	C36	-	10.7 ^j	-	-
	C55	-	11.9 ^b	-	7.7 ^m
	C56	-	-	8.0 ^b	-
Positive controls		11.0 ^c	27.0 ^a	13.0 ^a	20.0 ^a

¹Means followed by the same letter in the same column do not differ according to the Scott-Knott test (<0.05). ²For values less than or equal to 6.0, no halo was considered to have formed.

Regarding cellulase activity, isolates G30 and G17 from *L. divaricata*, 2-1 from *T. elegans*, Ss69 from *S. saponaria*, and C16 from *Saccharum* spp generated significantly bigger HEAs (16.2, 16.0, 15.7, 16.8, and 13.7 mm, respectively; Table 2) than other isolates of the same plants. However, none produced an HEA larger than that of the positive control.

Of the enzymes tested, the activity of pectinase was the lowest, with isolates C60 and C35 from *Saccharum* spp. demonstrating particularly reduced activity (HEAs of 7.3 and 6.6 mm, respectively; Table 2). None of the isolates from any of the four plants produced pectinase with an HEA higher than that of the positive control.

In tests for protease activity, isolates G17 of *L. divaricata*, 2-68 of *T. elegans*, and Ss52, Ss01B, and Ss31 of *S. saponaria* demonstrated HEAs significantly larger than those of the other fungi examined (all 20.0 mm; Table 2), and of a similar size to that observed with the positive control.

DISCUSSION

Several studies have reported the isolation of large numbers of endophytes from plants of medicinal and great economic importance. As in the current analysis, fungi of the genus *Diaporthe* were identified in investigations by Bernardi-Wenzel et al. (2010), Rhoden et al. (2012), García et al. (2012), and Leme et al. (2013). Rhoden et al. (2012) found members of the genus *Diaporthe* (*Phomopsis*) to be the most frequently observed endophytic species.

Diaporthe species (and their *Phomopsis* asexual states) have broad host ranges and are widely distributed, occurring as plant pathogens, endophytes, or saprobes, but also as pathogens of humans and other mammals. *Diaporthe* has been described as one of the most common genera of endophytic fungi in several plant hosts. Organisms in this genus have also been acknowledged as producers of interesting enzymes and secondary metabolites with antibiotic and anticancer activity (Gomes et al., 2013).

Leme et al. (2013) studied endophytic fungal communities isolated from sugarcane (*Saccharum* spp) leaves, identifying five genera, including *Saccharicola* (Massarinaceae). This genus was defined by Eriksson and Hawksworth (2003) to accommodate *Leptosphaeria bicolor* and *Leptosphaeria taiwanensis*, the *Stagonospora*-like asexual morphs of which were described by these authors.

Fungi of the genus *Saccharicola* were also identified in a study by Almeida et al. (2015) as part of an endophytic fungal community associated with the aquatic macrophytes *Eichhornia azurea* (Kunth) and *Eichhornia crassipes* (Mart.) of the Pontederiaceae family.

Endophytes of the genus *Bipolaris* were previously isolated from *S. saponaria* as *Cochliobolus*, their sexual morph (García et al., 2012). This genus was also recorded in studies by Bernardi-Wenzel et al. (2010), isolated from *L. divaricata*, and Orlandelli et al. (2012), from *Piper hispidum*. In the latter study, the authors evaluated the colonization of *P. hispidum* by foliar endophytic fungi and the diversity of such species in this medicinal plant, identifying 21 isolates belonging to 11 genera, with *Bipolaris* being the most frequently observed genus among the identified endophytes.

Furthermore, *Bipolaris* fungi have been isolated from medicinal and aquatic plants (Sakayaroj et al., 2010; Almeida et al., 2015). Members of this genus are the most widely distributed phytopathogens, and include species commonly associated with leaf spot, leaf blight, melting out, root rot, foot rot, and other disease symptoms that principally affect high-value crops of the Poaceae family (Manamgoda et al., 2014).

Various strains of the genus *Phoma* have been isolated from several medicinal plants, such as *L. divaricata* (Bernardi-Wenzel et al., 2010), *S. saponaria* (García et al., 2012), *Arisaema erubescens* (Wang et al., 2012), *P. hispidum* (Orlandelli et al., 2012), *Tinospora cordifolia*, *Calotropis procera* (Kedar et al., 2014), and *Curcuma longa* (Gupta et al., 2016). In the current study, *Phoma* isolates were only identified in samples of *L. divaricata* and *S. saponaria*. *Phoma* species are geographically widespread and comprise a large fungal group occupying diverse ecological niches. Although harmless saprobic examples abound, some *Phoma* species are well-known phytopathogens of economically important plants (Rai et al., 2014).

The use of endophytic fungi in various industrial processes has triggered an increasing number of studies concerning these microorganisms, and consequently, the discovery of novel compounds with enormous potential in a variety of applications (Meng et al., 2011; Duza and Mastan, 2013).

In addition to the great diversity of primary and secondary metabolites produced by endophytic fungi, a range of enzymes are also generated by these microorganisms (Bezerra et al., 2015). D'Souza and Hiremath (2015) performed qualitative amylase, cellulase, and pectinase assays of endophytic fungi isolated from 21 plants used in traditional medicine from forests of the Western Ghats, Goa, India. The authors verified that production of cellulase was common (45.07%), followed by that of amylase (36.62%), and pectinase (18.31%). In a similar study, Patil et al. (2015) qualitatively and quantitatively screened endophytic fungi extracted from seven medicinal plants for the production of extracellular enzymes, including amylase, protease, cellulase, and lipase. Their qualitative assay showed that of these enzymes, protease activity was most frequently observed, followed by cellulase, amylase, and lipase activity.

With a wide variety of industrial applications, such as in the food, fermentation, textile, paper, detergent, pharmaceutical, and sugar industries, amylases are important enzymes that may be employed in polysaccharide hydrolysis for the processing of starch. Amylases are derived from several sources, including plants, animals, and microorganisms. Enzymes from microbial sources are generally more suited to industrial demands, being cheaper to produce and more predictable, controllable, and reliable. *A. niger* is used for the commercial synthesis of amylase because of its high production capacity (de Souza and de Oliveira Magalhães, 2010; Jain et al., 2012).

In terms of production, cellulases are the third most industrially significant enzymes worldwide, because of their utility in applications and products such as cotton processing, paper recycling, juice extraction, enzymatic detergents, and animal food additives. Filamentous fungi are widely used in industry for the generation of cellulases, especially those of the genera *Aspergillus*, *Trichoderma*, *Humicola*, *Penicillium*, *Fusarium*, and *Phanerochaete* (Onofre et al., 2013).

Endophytic and pathogenic fungi induce the production of pectinases in the presence of pectic substances. Microbial pectinases are important enzymes in phytopathologic processes, plant-microbe symbiosis, and decomposition of dead plant material. Degradation of host tissue by phytopathogens generally begins with the production of pectinolytic enzymes, the major enzymes involved in plant pathogenesis. Endophytes that degrade pectic substances are likely to be latent pathogens (Choi et al., 2005; Sunitha et al., 2013).

The cellulase and pectinase activity of endophytic fungi isolated from oil-bearing seeds and medicinal plants has been described by Venkatesagowda et al. (2012) and Sunitha et al. (2013). Extracellular hydrolytic enzymes produced by fungal endophytes, particularly cellulase and pectinase, provide host-derived nutrients and resistance against pathogenic microbial infection.

Proteases represent two-thirds of global industrial enzyme requirements. They have a host of applications, notably in industrial bioremediation processes and the pharmaceutical and food industries, among others (Vardhini and Irfath, 2013; Orlandelli et al., 2015). Proteases are used in clinical contexts, especially in therapeutic use, for example, in the treatment of diabetes (Patil et al., 2015). These enzymes could be found in different organisms, including animals, plants, and microorganisms, particularly fungi (Vardhini and Irfath, 2013; Orlandelli et al., 2015).

According to Choi et al. (2005), the production of extracellular enzymes by endophytes may be involved in the host plant's resistance strategy against phytopathogenic microorganisms, or in the improvement of the plant's nutrition.

The numerous species of foliar endophytic fungi that inhabit plants of medicinal and economic importance represent a significant component of environmental microbial diversity. Moreover, they serve as a source of chemical compounds of medicinal and industrial consequence, including in their role as enzyme producers. Our results show that the composition and abundance of endophytic fungi differed among the studied plants, and that these fungi demonstrate potential as sources of enzymes of great commercial and biotechnological value.

Furthermore, enzymatic profiling may be used as an initial screen to select fungi capable of producing such proteins, and to provide preliminary information concerning the range of enzymes involved in the endophyte-host interaction. However, the technique applied in the present work did not yield quantitative results. With the aim of using these fungi in industrial applications as enzyme producers or biotransformation agents, future quantitative assays are required, including tests on agro-industrial wastes.

Conflicts of interest

The authors declare no conflict of interest.

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