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Diversity and antimicrobial activity of culturable endophytic fungi associated with the neotropical ethnomedicinal plants *Copaifera langsdorffii* and *Copaifera pubiflora*

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ABSTRACT

Medicinal plants represent a promising reservoir of diverse endophytic fungi, including taxa that are able to produce bioactive metabolites. In Brazil, the genus *Copaifera* includes species that are well known in folk medicine mainly due to their ability to produce oleoresin. In this study, we characterized the endophytic fungal communities associated with *Copaifera langsdorffii* and *Copaifera pubiflora* and investigated their ability to produce antimicrobial agents. We obtained 668 fungal isolates from the leaves, stems, and seeds of both plants, which were later classified into 64 taxa and 22 genera. *Diaporthe* sp. 6, *Xylariaceae* sp. 1, *Diaporthales* sp. 1, and *Diaporthales* sp. 2 were the most abundant taxa in *C. langsdorffii*, while *Phyllosticta* sp., *Diaporthe* sp. 7, *Diaporthales* sp. 3, and *Diaporthe miriciae* were the most abundant taxa in *C. pubiflora*. *Diaporthe* sp. 4, *Phyllosticta* sp., *Diaporthe* sp. 1, *Diaporthe* sp. 7, and *Neopestalotiopsis* sp. were the only taxa common between the two plants. Both plants were found to have high fungal diversity, especially *C. langsdorffii*. Six extracts displayed antibacterial, being *Alternaria* sp., *Diaporthe* sp. 1, *D. miriciae*, and *Diaporthe* sp. 14. Our results showed that different tissues of the ethnomedicinal plants *C. langsdorffii* and *C. pubiflora* are systematically colonized by rich and diverse endophytic fungal communities, and that some of the fungi are able to produce antimicrobial compounds, which may be explored in further studies as potential candidates for the development of new drugs.

1. Introduction

Recently, the search for natural bioactive compounds useful in medicine for application in treatment of various diseases, including those caused by drug resistant microorganisms, and in agriculture for development of pesticides that are more efficient and less toxic than the current pesticides has increased. According to Banerjee et al. (2015), natural products (NPs) are classical starting points for discovery of drugs, such as penicillin, lovastatin, silibinin, and paclitaxel. In agriculture, Yan et al. (2018) reported that NPs have been applied as fungicides, insecticides, and herbicides, which has substantially contributed to the increase in crop yield and quality worldwide, for example, spinosyn, avermectin, and phosphinothricin.

For decades, researchers have been searching for microorganisms that have the ability to produce NPs. These microorganisms are considered as vast reservoirs of bioactive secondary metabolites, which have applications in various fields. Among the diverse microbial communities found globally, organisms known as endophytes, which live asymptomatically inside plant tissues (Petrini 1991), represent an inexhaustible reservoir of novel bioactive metabolites with widespread applications and have attracted considerable interest as potential agents for the development of novel biomedical and agricultural products (Paramanantham et al., 2019). Among the endophytes, fungal species belonging to the phylum *Ascomycota*, *Basidiomycota*, and *Mucoromycota*, are well-represented.

According to Rodriguez et al. (2008), virtually all plants in a natural ecosystem seem to have some interactions with endophytic or some other associated fungi. According to Strobel and Daisy (2003), Strobel et al. (2004), and Yu et al. (2010), some reasonable strategies

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should be followed for plant selection to isolate endophytic fungal assemblages and among them, ethnomedicinal plants shelter interesting fungal taxa, which produce diverse bioactive compounds. The choice of medicinal plants for the isolation of endophytic fungi is one of the criteria adopted in recent research (Brissow et al., 2018; Carvalho et al., 2016, 2018; Carvalho et al., 2012a; Ferreira et al., 2020; Johann et al., 2012; Silva-Hughes et al., 2015; Wu et al., 2018). In this context, the plant genus *Copaifera* Linnaeus (Fabaceae), popularly known as "copaíba", "copaífera", and "pau-de-óleo" is widely used due to their ability to produce oleoresin, which is well known in folk medicine as a wound healing agent with anti-hemorrhoidal, purgative, anticarcinogenic, anti-inflammatory, antimicrobial, antiviral, local anesthetic, cytotoxic, insecticidal, or bactericidal properties (Arruda et al., 2019). In addition, oleoresin obtained from *Copaifera* of the Amazonian region of Brazil represents an important product in the Brazilian industry (Arruda et al., 2019). The medicinal copaíba species found in Brazil include *Copaifera langsdorffii* Desf. and *Copaifera pubiflora* Benth. *Copaifera langsdorffii* is one of the most abundant species and can be found in Brazil, Argentina, and Paraguay (Arruda et al., 2019). In contrast, *C. pubiflora* has wide distribution in northern South America, including Brazil, Colombia, Guyana, and Venezuela. As copaíba species have been used in folk medicine in different neotropical regions, the aim of this study was to characterize the endophytic fungal diversity associated with *C. langsdorffii* and *C. pubiflora* and investigate their ability to produce antimicrobial activities.

2. Methods and materials

2.1. Sample collection and isolation of fungal endophytes

Fifteen specimens of *Copaifera langsdorffii* were randomly collected in August 2011 at Serra of São José (21°05.105'S; 44° 12.140'W), a fragment of Tropical Rain Forest, Minas Gerais state, Brazil. Voucher specimen was deposited in the HUFSJ Herbarium of the Federal University of the São João del-Rei under the code 14283. Additionally, 15 specimens of *Copaifera pubiflora* were randomly collected in January 2012 in one Amazon forest area located at Serra da Prata Embrapa experimental field, Mucajaí, Roraima state, Brazil (2° 35'59.1"N; 60°56'35.7"W). Voucher specimen was deposited in the IAN, João Murça Pires Herbarium of the CPATU, EMBRAPA under the code IAN 187043.

Five healthy leaves, stems, and seeds samples of 15 individuals of each specimen were collected and stored in sterilized bags for less than 24 h at 10 °C during transfer to the laboratory. Initially the leaves and stems were washed and then cut into five 5-mm-long fragments using a flame-sterilised scalpel in a laminar flow hood to avoid air contamination of the tissues and all samples were processed separately. The fragments were surface disinfected by immersion in 2% Extran® (1 min), 70% ethanol (1 min) and 2% sodium hypochlorite (3 min), followed by a washing with sterile-distilled water (2 min) (Carvalho et al., 2012). For the isolation of endophytic fungi from the seeds, firstly the aril was removed and then each seed was split in half. Each fragment was surface disinfected by immersion in 2% Extran® (1 min), flamed at Bunsen burner, 70% ethanol (4 min), 2% sodium hypochlorite (5 min), and then wash with sterile distilled water (2 min). Afterwards, the fragments (leaves, stems and seeds) were inoculated onto potato dextrose agar (PDA; Difco, USA) supplemented with chloramphenicol (200 μg mL^{-1}) (Sigma Aldrich, USA). To test the effectiveness of the surface sterilization, 100 μL of the last water rinse was plated on PDA. All the plates were incubated for up to 60 days at 25 °C. The fungal colonies were purified on PDA media and deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais, Brazil under the code UFMGCB in cryotubes at −80 °C and in distillate-sterilized water (Castellani, 1967) at room temperature. The yeast colonies grown were picked from the plates and transfer to Yeast extract-Malt extract agar (YM; 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 2% agar) and pure cultures were obtained. Yeast cultures were preserved in GYMP broth (Glucose-yeast extract-malt extract-peptone broth; 2% glucose, 0.5% yeast extract, 1% malt extract, and 0.2% potassium phosphate dibasic) and 15% glycerol at −80 °C.

2.2. Fungal identification

Based on the characteristics of the culture, as colony color and texture, border type, and radial growth rate on culture media, the filamentous fungal isolates were grouped into different morphospecies (Fröhlichet al., 2000). The DNA was extracted following a protocol using chloroform: isoamyl alcohol according to Rosa et al. (2009). The isolates with similar morphological characteristics grouped were subjected to Polymerase Chain Reaction (PCR) fingerprinting using the microsatellite-primed PCR technique $(GTG)_5$ (Lieckefeldt et al., 1993). Based on the electrophoretic profile showed, an isolate among those with the same pattern of bands was selected for sequencing the internal transcribed region of the nuclear ribosomal RNA gene (ITS1-5.8S-ITS2), and β-tubulin gene (B-TUB) for the filamentous fungal. The internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 (White et al., 1990), and amplification of the ITS region was realized as described by Rosa et al. (2009). Amplification of the β-tubulin (Glass and Donaldson, 1995) was performed with the Bt2a/Bt2b primers according Gonçalves et al. (2015). The yeasts were grouped and identified according to the protocols established by Kurtzman et al. (2011). Yeast molecular identities were confirmed by sequencing the D1-D2 variable domains of the large-subunit rRNA gene using the primers NL1 and NL4 according to Lachance et al. (1999). Representative consensus sequences of fungal taxa were deposited into GenBank (Table 1). To achieve species-rank identification based on ITS, β-tubulin and D1-D2 data, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using Blast (Altschul et al., 1997). Fungal isolates with query coverage and identity $\geq$ 99% were considered to represent the same taxon, and taxa of filamentous fungi that displayed query coverage and identities $\leq$ 98% or an inconclusive taxonomic position were subjected to phylogenetic ITS and β-tubulin based analysis in comparison with sequences of the ex type species deposited in the GenBank database. The information about fungal classification generally followed the databases of dictionary Kirk et al. (2008), and websites MycoBank (http://www.mycobank.org/), and Index Fungorum (http://www.indexfungorum.org).

2.3. Relative abundance, diversity, richness, dominance, and distribution

The relative abundance of different fungal taxonomy was calculated to describe the fungal composition and diversity in different compartments of each plant species. The abundance of each taxon was calculated using the following formula: percentage of abundance of taxon A = number of isolates of taxon A x 100 / sum of isolates of all taxa. To quantify species diversity, richness and evenness, we used the following indices: (i) Fisher's α, (ii) Margalef's, and (iii) Simpson's, respectively, considering the total specimens number of each plant species. All of the results were obtained with 95% confidence, and bootstrap values were calculated from 1000 iterations. The rarefaction curve was calculated using the Mao Tao index. All diversity and similarity indices calculations were performed using PAST, version 1.90 (Hammer et al., 2001). Venn diagrams were prepared according to Bardou et al. (2014) to illustrate the comparison of fungal assemblages associated with the plants.

Table 1
Endophytic fungi obtained from *Copaifera langsdorffii* and *Copaifera pubiflora* identified by sequence comparison using BLASTn match and the NCBI GenBank database.

Host plant	[a]UFMGCB code	Tissue	Number of isolates	Top BLAST search results (GentrBank accession number)	Query cover (%)	Identrtity (%)	No of pb analyzed	Proposed taxa (GentrBank access codes)
Copaifera langsdorffii	7491	L, Se	12	*Alternaria alstroemeriae* (NR163686)[b]	100	100	448	*Alternaria* sp. (MT385147)[e]
	7581	Se	1	*Aspergillus lanosus* (MH860883)[b] *Aspergillus caelatus* (MK119757)[c]	87 88	95 91	462 457	*Aspergillus* sp. 1 (MT385148)[e] (MT375018)[f]
	7384	L	1	*Aspergillus fumigatus* (EF669791)[c]	98	100	440	*Aspergillus* sp. 2 (MT375019)[f]
	7521	S	1	*Chaetomium pseudoglobosum* (MH860267)[b] *Chaetomium unguicola* (KT214744)[c]	100 98	100 99	548 397	*Chaetomium* cf. *unguicola* (MT385149)[e] (MT375020)[f]
	7566	Se	3	*Cladosporium rugulovarians* (KT600459)[b]	100	100	429	*Cladosporium* sp. 1 (MT385150)[e]
	7568	Se	4	*Cladosporium subuliforme* (MH864124)[b]	100	100	465	*Cladosporium* sp. 2 (MT385151)[e]
	7207	L	2	*Colletotrichum cymbidiicola* (NR111694)[b] *Colletotrichum boninentrse* (HM585421)[c]	99 100	100 99	508 443	*Colletotrichum boninentrse* (MT385152)[e] (MT375021)[f]
	7219	L	4	*Colletotrichum phyllanthi* (NR111698)[b] *Colletotrichum karstii* (HM585428)[c]	99 100	100 100	503 379	*Colletotrichum karstii* (MT385153)[e] (MT375022)[f]
	7371	L, S	12	*Colletotrichum gigasporum* (NR145380)[b]	96	100	496	*Colletotrichum* sp.1 (MT385154)[e]
	7509	L	3	*Colletotrichum indonesientrse* (MH864562)[b] *Colletotrichum wanningentrse* (MG830286)[c]	100 100	100 98	491 452	*Colletotrichum* sp. 2 (MT385155)[e] (MT375023)[f]
	7510	L	1	*Colletotrichum sojae* (NR158358)[b]	98	100	492	*Colletotrichum* sp. 3 (MT385156)[e]
	7392	S	2	*Coniochaeta marina* (MK458764) [b]	97	100	493	*Coniochaeta* sp. (MT385157)[e]
	7327	L	2	*Curvularia soli* (NR152503)[b]	100	100	468	*Curvularia soli* (MT385158)[e]
	7243	L,S	28	*Chrysocrypta corymbiae* (NR120158)[b]	94	83	610	*Diaporthales* sp. 1 (MT385159)[e]
	7328	L, S	22	*Chrysofolia colombiana* (NR137993)[b] *Disculoides calophyllae* (KY979913)[c]	80 93	89 80	496 401	*Diaporthales* sp. 2 (MT385160)[e] (MT375024)[f]
	7457	S	2	*Diaporthe oxe* (NR111856)[b] *Diaporthe oxe* (KC344132)[c]	99 83	98 100	493 502	*Diaporthe* cf. *oxe* (MT385161)[e] (MT375025)[f]
	7216	L	5	*Diaporthe yunnanentrsis* (NR152472)[b] *Diaporthe masirevicii* (KJ197257)[c]	99 99	100 98	486 522	*Diaporthe* sp. 1 (MT385162)[e] (MT407175)[f]
	7296	S	3	*Diaporthe paranentrsis* (NR111857)[b] *Diaporthe myracrodruonis* (MK205291)[c]	100 99	98 94	419 403	*Diaporthe* sp. 2 (MT385163)[e] (MT407176)[f]
	7210	L, S	11	*Diaporthe foentriculina* (NR145303)[b] *Diaporthe inconspicua* (KC344091)[c]	100 84	98 100	486 491	*Diaporthe* sp. 3 (MT385164)[e] (MT407177)[f]
	7401	L, S	18	*Diaporthe velutina* (NR152470)[b] *Diaporthe velutina* (KX999223)[c]	100 99	99 97	485 480	*Diaporthe* sp. 4 (MT385165)[e] (MT407178)[f]
	7241	L, S	4	*Diaporthe maytentricola* (NR137826)[b] *Diaporthe hispaniae* (MG281296)[c]	99 100	97 95	492 507	*Diaporthe* sp. 5 (MT385166)[e] (MT407179)[f]
	7250	L, S	44	*Diaporthe baccae* (NR152458)[b] *Diaporthe inconspicua* (KC344091)[c]	100 82	94 100	389 481	*Diaporthe* sp. 6 (MT385167)[e] (MT407180)[f]
	7255	L, S	4	*Diaporthe sackstonii* (NR147537)[b] *Diaporthe yunnanentrsis* (KX999228)[c]	99 100	96 93	510 514	*Diaporthe* sp. 7 (MT385168)[e] (MT407181)[f]
	7198	L, S	15	*Diaporthe kochmanii* (NR111614)[b] *Diaporthe masirevicii* (KJ197257)[c]	98 100	100 98	491 520	*Diaporthe* sp. 8 (MT385169)[e] (MT407182)[f]
	7202	L, S	6	*Diaporthe sackstonii* (NR147537)[b] *Diaporthe serafiniae* (KJ197254)[c]	99 98	99 100	470 485	*Diaporthe* sp. 9 (MT385170)[e] (MT407190)[f]
	7460	L, S	13	*Diaporthe ocoteae* (NR147596)[b] *Diaporthe ocoteae* (KX228388)[c]	100 100	97 94	497 366	*Diaporthe* sp. 10 (MT385171)[e] (MT407183)[f]
	7254	S, Se	3	*Diplodia pseudoseriata* (MH863419)[b]	100	100	500	*Diplodia pseudoseriata* (MT385172)[e]
	7503	L	2	*Fusarium nepalentrse* (MH864615)[b]	99	100	459	*Fusarium* sp. 1 (MT385173)[e]
	7304	S	1	*Fusarium napiforme* (MH862670)[b]	99	100	461	*Fusarium* sp. 2 (MT385174)[e]
	7564	Se	1	*Hypoxylon griseobrunneum* (NR155184)[b] *Hypoxylon griseobrunneum* (KC977303)[c]	99 99	90 88	451 359	*Hypoxylon* sp. 1 (MT385175)[e] (MT407184)[f]
	7414	L	1	*Muscodor equiseti* (NR154155)[b]	100	100	535	*Muscodor equiseti* (MT385176)[e]
	7226	S	1	*Neopestalotiopsis dentrdrobii* (MK993572)[b] *Pestalotiopsis saprophyta* (JX399017)[c]	99 100	100 100	463 386	*Neopestalotiopsis* sp. (MT385177)[e] (MT407185)[f]

(*continued*)

Table 1 (*Continued*)

Host plant	[a]UFMGCB code	Tissue	Number of isolates	Top BLAST search results (GentrBank accession number)	Query cover (%)	Identrtity (%)	No of pb analyzed	Proposed taxa (GentrBank access codes)
	7523	S	1	*Nigrospora hainanentrsis* (NR153480)[b]	100	100	437	*Nigrospora hainanentrsis* (MT385178)[e]
	7561	L, Se	10	*Pentricillium crustosum* (NR077153)[b]	100	100	496	*Pentricillium* sp. 1
				Pentricillium palitans (KJ834480)[c]	99	93	353	(MT385179)[e] (MT407186)[f]
	7232	S	1	*Pestalotiopsis kentryana* (NR147549)[b]	100	100	542	*Pestalotiopsis australasiae*
				Pestalotiopsis australasiae (KM199409)[c]	100	100	395	(MT385180)[e] (MT407187)[f]
	7502	L	10	*Phyllosticta fallopiae* (NR147316)[b]	100	100	479	*Phyllosticta* sp. (MT385181)[e]
	7569	L, Se	4	*Epicoccum italicum* (NR158264)[b]	98	99	466	*Pleosporales* sp. 1 (MT385182)[e]
	7222	S, Se	3	*Preussia persica* (NR137730)[b]	96	98	461	*Preussia* sp. 1 (MT385183)[e]
	7411	L, S	2	*Preussia flanaganii* (NR077168)[b]	100	90	494	*Preussia* sp. 2 (MT385184)[e]
	7330	L	3	*Beltrania pseudorhombica* (NR148074)[b]	100	99	527	*Sordariomycetes* sp.
				Lepteutypa fuckelii (MK523337)[c]	83	87	398	(MT385185)[e] (MT407188)[f]
	7306	S	1	*Trichoderma atroviride* (MH862505)[b]	100	100	580	*Trichoderma* sp. (MT385186)[e]
	7546	L, S, Se	32	*Amphirosellinia fushanentrsis* (NR 153514)[b]	100	93	489	*Xylariaceae* sp. 1 (MT385187)[e]
	7389	S	1	*Podosordaria muli* (NR158883)[b]	100	89	505	*Xylariaceae* sp. 2 (MT385188)[e]
	7473	S	1	*Xylaria bambusicola* (NR153200)[b]	100	95	465	*Xylariaceae* sp. 3 (MT385189)[e]
Copaifera pubiflora	7789	L	4	*Neopestalotiopsis brasilientrsis* (MG686469)[b]	100	99	464	*Amphisphaeriaceae* sp.
				Pestalotiopsis saprophyta (JX399017)[c]	78	100	443	(MT385190)[e] (MT418929)[f]
	7945	Se	2	*Aspergillus fumigatus* (NR121481)[b]	100	100	507	*Aspergillus fumigatus*
				Aspergillus fumigatus (EF669791)[c]	98	100	494	(MT385191)[e] (MT407189)[f]
	7937	S	1	*Aspergillus coremiiformis* (NR135393)[b]	98	89	508	*Aspergillus* sp. 3
				Aspergillus caelatus (MK119759)[c]	88	91	476	(MT385192)[e] (MT418930)[f]
	7855	L, S	4	*Botryosphaeria dothidea* (KF766151)[b]	100	99	409	*Botryosphaeria* sp. (MT385193)[e]
	7944	Se	1	*Chaetomium globosum* (MH858130)[b]	100	100	458	*Chaetomium* cf. *globosum*
				Chaetomium globosum (KT214742)[c]	88	100	385	(MT385194)[e] (MT418940)[f]
	7598	L	4	*Colletotrichum aeschynomentres* (NR120133)[b]	99	100	488	*Colletotrichum* sp. 4 [MT385195][e]
	7617	L, S	35	*Chrysofolia colombiana* (NR137993)[b]	94	87	438	*Diaporthales* sp. 3 (MT385196)[e]
	7719	L, S	26	*Diaporthe miriciae* (NR147535)[b]	100	99	424	*Diaporthe miriciae*
				Diaporthe miriciae (KJ197262)[c]	98	98	472	(KX588623)[e]
	7720	L, S	16	*Diaporthe passifloricola* (NR147595)[b]	100	99	454	*Diaporthe* sp. 1 (KX588624)[e]
				Diaporthe rosae (MG843878)[c]	81	100	408	(MT418939)[f]
	7873	S	1	*Diaporthe foentriculina* (NR145303)[b]	100	98	499	*Diaporthe* sp. 4 (MT385197)[e]
				Diaporthe hispaniae (MG281296)[c]	100	97	372	(MT418938)[f]
	7812	L, S	51	*Diaporthe yunnanentrsis* (NR152472)[b]	100	96	490	*Diaporthe* sp. 7 (MT385198)[e]
				Diaporthe yunnanentrsis (KX99928)[c]	99	92	460	(MT418937)[f]
	7722	L, S	14	*Phomopsis tuberivora* (NR160066)[b]	99	96	490	*Diaporthe* sp. 11
				Diaporthe myracrodruonis (MK205291)[c]	99	91	477	(MT385199)[e] (MT418936)[f]
	7609	S	2	*Diaporthe maytentri* (NR111852)[b]	100	91	499	*Diaporthe* sp. 12
				Diaporthe myracrodruonis (MK205291)[c]	96	95	350	(MT385200)[e] (MT418935)[f]
	7908	L, S	11	*Diaporthe bohemiae* (NR164425)[b]	100	92	489	*Diaporthe* sp. 13
				Diaporthe ocoteae (KX228388)[c]	100	91	371	(MT385201)[e] (MT418934)[f]
	7927	L	1	*Diaporthe aspalathi* (NR165951) [b]	99	96	466	*Diaporthe* sp. 14
				Diaporthe podocarpi-macrophyll (KX999207)[c]	100	86	398	(MT385202)[e] (MT418933)[f]
	7612	L, S	3	*Endomelanconiopsis entrdophytica* (MH863083)[b]	100	100	446	*Endomelanconiopsis* sp. (MT385203)[e]
	CM-Y412	Se	2	*Eremothecium coryli* (NR155097)[b]	97	97	627	*Eremothecium coryli*
				Eremothecium coryli (KY107671)[d]	100	99	541	(MT385212)[e] (MT386009)[g]
	7933	S	2	*Fusarium continuum* (NR159818)[b]	87	93	452	*Fusarium* sp. 3 (MT385204)[e]
	7613	L, S	3	*Hypoxylon pulicicidum* (JX183075)[b]	94	96	457	*Hypoxylon* sp. 2 (MT385205)[e]
	7794	L, S	5	*Neopestalotiopsis javaentrsis* (MH855207)[b]	100	100	494	*Neopestalotiopsis* sp.
				Neopestalotiopsis protearum (LT853251)[c]	98	100	355	(MT385206)[e] (MT418932)[f]

(*continued*)

Table 1 (*Continued*)

Host plant	[a]UFMGCB code	Tissue	Number of isolates	Top BLAST search results (GentrBank accession number)	Query cover (%)	Identrtity (%)	No of pb analyzed	Proposed taxa (GentrBank access codes)
	7623	L, S, Se	6	*Pentricillium citrinum* (MH856132)[b]	99	100	495	*Pentricillium* sp. 2 (MT385207)[e]
	7823	L, S	15	*Pestalotiopsis papuana* (NR147553)[b]	100	100	490	*Pestalotiopsis colombientrsis*
				Pestalotiopsis colombientrsis (KM199421)[c]	100	100	330	(MT385208)[e] (MT418931)[f]
	7687	L	66	*Phyllosticta fallopiae* (NR147316)[b]	100	100	553	*Phyllosticta* sp. (MT385209)[e]
	7825	S	3	*Pseudochaetosphaeronema martinelli* (NR132930)[b]	93	94	483	*Pleosporales* sp. 2 (MT385210)[e]
	7916	L, S, Se	17	*Amphirosellinia fushanentrsis* (NR153514)[b]	99	92	490	*Xylariaceae* sp. 4 (MT385211)[e]

[a] UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. Taxa subjected to sequentrcing and phylogentretic analysis based on the [b]ITS, [c]β-tubulin, [d]D1/D2. Sequentrces codes deposited in GentrBank: [e]ITS1-5.8S-ITS2, [f]β-tubulin, and [g]D1/D2. L: leaf, S: stem, Se: seed

2.4. Fungal and plant extracts

The production of the fungal extracts followed the protocols established by Santiago et al. (2012). For the filamentous fungi, five mm diameter plugs of each fungi isolate were inoculated into on 20 mL of PDA medium at the center of Petri dishes (90 mm diameter). For the yeasts, each isolate was streak at the same media culture. The plates were incubated at 25 ± 2 °C for 15 days. The fungal culture from each Petri dish were cut and transferred to 50 mL centrifuge tubes containing 35 mL of ethanol (PA, ≥ 99.8%, Vetec, Brazil). After 48 h at 10 °C the organic phase was filtered and the solvent was removed under a vacuum centrifuge at 35 °C. Concomitantly, the leaves and stems of each specimen of *C. langsdorffii* and *C. pubiflora* were macerated with 35 mL of ethanol and incubated at 10 °C for 15 days. The solvent was also removed under a vacuum centrifuge at 35 °C. An aliquot of each dried extract (fungal and tissue plant) was dissolved in dimethyl sulfoxide (DMSO; Merck, USA) to prepare a 100 mg mL^{-1} stock solution which was stored at −20 °C.

2.5. Antimicrobial assays

The extracts produced from all the endophytic fungi recovered and tissues plant were tested against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 12600, *Pseudomonas aeruginosa* ATCC 10145, *Candida albicans* ATCC 60193, *Candida krusei* ATCC 6258, and *Cladosporium sphaerospermum* CCT 1740. The experiments were established following the protocols described by Carvalho et al. (2012) using a modified version of the methods for bacteria (NCCLS M7 - A6, v. 23 n° 2, 2003), yeast (AFTS-EUCAST 7.1 Rodríguez-Tudela et al., 2003), and filamentous fungi (NCCLS M38 – A2, v. 22, n° 16, 2002) (Wedge and Kuhajek, 1998). The bacteria and yeast were grown at 37 °C in Miller-Hinton (Difco, USA) and Sabouraud (Himedia, India) media, respectively. After 24 h the microbial inocula were prepared by diluting the cell suspensions appropriately in Mueller-Hinton broth and RPMI1640 (supplemented with 2% glucose) media for bacteria and yeast, respectively. Fifty microliters of the inocula were added to each well in a 96-well plate and adjusted to 1-2 × 10^8 bacterial cells mL^{-1} and 1 × 10^6 yeast cells mL^{-1}. Twenty-five microliters of extract (previously dissolved in DMSO were diluted to a concentration of 1 mg mL^{-1}) and control solutions (culture medium, DMSO and positive control), as well as 25 μL of each medium, was added to attain the desired concentrations and the plates were incubated at 37 °C for 24 h. As an indicator of microorganism growth, 10 μl of 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT; Sigma, USA) (dissolved in sterile water at 5 mg mL^{-1}) was added to each well and incubated at 37 °C for 4 h. The results are expressed as per cent inhibition in relation to the controls without drugs. Chloramphenicol (32 μg mL^{-1}) and amphotericin B (2 μg mL^{-1}) (both from Sigma, USA) were used as positive drug controls for the bacterial and yeast assays, respectively.

In the assay against the *C. sphaerospermum*, the conidia were harvested from 7- to 10-day-old cultures by flooding plates with 5 mL of 0.85% sterile saline and the resulting suspensions were filtered to remove the mycelial fragments. The conidia concentrations were determined photometrically from a standard curve of absorbance at 620 nm and the suspensions were adjusted with 0.85 % sterile saline to a concentration of 1.0 × 10^6 conidia mL^{-1} (Espinel-Ingroff and Kerkering, 1991; Wedge and Kuhajek, 1998). Fifty microliters of the conidia inocula was added to each well of a 96-well plate containing 25 μL of the extract or the control solution, as well as 25 μL of the medium RPMI1640. The plates were incubated at 25 °C for 48 h. Fungal growth was evaluated by measuring the absorbance of each well at 620 nm at 48 h and the mean absorbance values and standard errors were used to evaluate fungal growth inhibition. The fungicide benomyl (1.16 μg mL^{-1}) (Sigma, USA) was used as a positive standard in all assays. All antimicrobial assays were performed in duplicate and the final concentration of the crude extract was 250 μg mL^{-1}. The active crude extracts with antimicrobial activities ≥ 70% were selected to the minimum inhibitory concentration (MIC) determination. The culture medium, inoculum standardization, positive and negative controls, and evaluation of results were performed as described above in screening antimicrobial activity for the respective target microorganisms.

2.6. Statistical analysis

All the samples were tested in duplicate, in two independent experiments. Values represent the mean ± standard deviation (SD) of these experiments using the SoftMax Pro 5.3 (VersaMax microplate reader).

3. Results

3.1. Isolation and identification of endophytic fungi

A total of 900 fragments per plant were processed of which 668 fungi isolates were obtained. Of the total isolates, 308 isolates [172 (56%) from leaves, 110 (36%) from stems, and 26 (8%) from seeds] were recovered from *C. langsdorffii*, while the remaining 360 isolates [225 (63%) from leaves, 123 (34%) from stems, and 12 (3%) from seeds] were recovered from *C. pubiflora*. The isolates were classified into 64 taxa and 22 *Ascomycota* genera, which belonged to *Sordariomycetes* (70.3%), *Dothideomycetes* (18.7%), *Eurotiomycetes* (9.4%), and *Saccharomycetes* (1.6%). Among all the taxa identified, 15 (23.4%) occurred as singletons (occurrence = one isolate). Several taxa displayed low molecular taxonomic identities with sequences of fungi deposited in GenBank, and were identified at the family, class, or phylum levels and may represent new species, whereas 72 isolates could not be identified. The taxa *Diaporthe* sp. 6, *Xylariaceae* sp. 1, *Diaporthales* sp. 1, and *Diaporthales* sp. 2 were the most abundant in *C. langsdorffii* with 44 (14.6%), 32 (10.6%), 28 (9.3%), and 22 (7.3%) isolates, respectively. Among the fungi identified from *C. pubiflora*, the

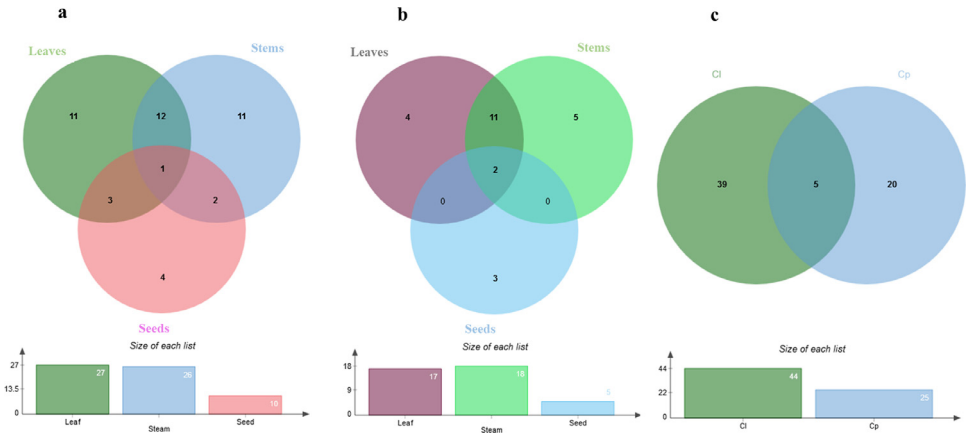

Fig. 1. Similarity of fungal assemblages associated with Neotropical ethnomedicinal plants *Copaifera langsdorffii* and *Copaifera pubiflora* represented by Venn diagrams. (**a**) Represents the total fungal distribution among leaves, stems, and seeds of *C. langsdorffii*. (**b**) Shows the fungal distribution among leaves, stems, and seeds of *Copaifera pubiflora*; (**c**) Shows the fungal distribution in both plants. Cl: *Copaifera langsdorffii* and Cp: *Copaifera pubiflora*.

most abundant taxa were *Phyllosticta* sp., *Diaporthe* sp. 7, *Diaporthales* sp. 3, and *Diaporthe miriciae* with 66 (22.3%), 51 (17.2%), 35 (11.8%), and 26 (8.8%) isolates, respectively. *Diaporthe* was the most abundant genera in both plant specimens with 125 (41.5%) and 122 (41.3%) isolates obtained from the leaves and stems of *C. langsdorffii* and *C. pubiflora*, respectively.

The distribution of fungal communities in the different plant tissues is shown in Fig. 1. In the isolates obtained from *C. langsdorffii*, only *Xylariaceae* sp. 1 was found to be common between all the three types of tissues examined, i.e., leaves, stems, and seeds. The majority of the fungal taxa were common between leaves and stem (*Diaporthe* sp. 6, *Diaporthales* sp. 1, *Diaporthales* sp. 2, *Diaporthe* sp. 4, *Diaporthe* sp. 8, *Diaporthe* sp. 10, *Colletotrichum* sp. 1, *Diaporthe* sp. 3, *Diaporthe* sp. 9, *Diaporthe* sp. 5, *Diaporthe* sp. 7, and *Preussia* sp. 2), while the taxa *Alternaria* sp., *Penicillium* sp. 1, and *Pleosporales* sp. 1 were common between leaves and seed (Fig. 1a). In the isolates obtained from *C. pubiflora*, *Xylariaceae* sp. 4 and *Penicillium* sp. 2 were found to be common between all three types of tissues (leaves, stems, and seeds), while 11 taxa were common between leaves and stems (*Diaporthe* sp. 7, *Diaporthales* sp. 3, *Diaporthe miriciae*, *Diaporthe* sp. 1, *Pestalotiopsis colombiensis*, *Diaporthe* sp. 11, *Diaporthe* sp. 13, *Neopestalotiopsis* sp., *Botryosphaeria* sp., *Endomelanconiopsis* sp., and *Hypoxylon* sp. 2) (Fig. 1b). Only the taxa *Diaporthe* sp. 4, *Phyllosticta* sp., *Diaporthe* sp. 1, *Diaporthe* sp. 7, and *Neopestalotiopsis* sp. were common between the two plants (Fig. 1c).

3.2. Diversity indices

In general, we observed a high diversity in the fungal communities associated with both the *Copaifera* species (Table 2); especially, the fungi associated with *C. langsdorffii* from fragment of Tropical Rain Forest displayed the highest diversity indices. The relative abundance of endophytic fungi associated of *C. langsdorffii* was calculated using 301 identified isolates and varied from 0.33 to 9.63% (Table 3). The abundance in the leaves was higher than stems and seeds (leaves > stems > seeds). However, some genera were obtained exclusively from one compartment of the plant, such as *Cladosporium* and *Hypoxylon* that occurred only from the seeds; *Chaetomium*, *Coniochaeta*, *Neopestalotiopsis*, *Pestalotiopsis*, and *Trichoderma* occurred only in the stems samples; and *Curvularia*, *Muscodor*, and *Phyllosticta* present only in the leaves. In relation to *C. pubiflora*, the relative abundance was calculated using the total of 295 identified isolates and varied from 0.33% to 22.37% (Table 3). The abundance in the leaves was also higher than stems and seeds (leaves > stems > seeds). *Chaetomium* was obtained from seeds of *C. pubiflora* and *Guignardia* obtained exclusively from the leaves.

Table 2
Diversity indices of fungal assemblages associated with neotropical ethnomedicinal plants *Copaifera langsdorffii* and *Copaifera pubiflora*

Diversity indices	*Copaifera langsdorffii*	*Copaifera pubiflora*
Number of taxa	44	25
Number of isolates	301	295
Fisher α	14.19	6.52
Margalef	7.53	4.22
Simpson	0.94	0.88

3.3. Antimicrobial activities

Among the 668 fungal extracts, the extracts obtained from six endophytes displayed antibacterial activity against at least one target, with inhibition ranging from 92% to 100% (Table 4). No fungal extract displayed activity against *P. aeruginosa*, *C. albicans*, *C. krusei*, and *C. sphaerospermum*. *Alternaria* sp. UFMGCB 7491 (recovered from *C. langsdorffii*), and *Diaporthe* sp. 14 UFMGCB 7927 (isolated from *C. pubiflora*) displayed a selective antibacterial activity against *E. coli* with inhibition rate of 100% and 99%, respectively. *Diaporthe miriciae* isolates UFMGCB 7646 and 7719, recovered from *C. pubiflora*, displayed a selective antibacterial activity against *S. aureus* with inhibition rate of 98% both. *Diaporthe miriciae* UFMGCB7701, and *Diaporthe* sp. 1 UFMGCB 7696, recovered from *C. pubiflora*, showed antibacterial activity against *E. coli* and *S. aureus* with inhibition rate ranging from 92% to 100%. In addition, 22 plant tissue extracts displayed antimicrobial activity against at least one target (Table 5).

All bioactive extracts were subjected to MIC determination. The plant tissue extracts displayed MIC values ranging from 31.2 to >250 μg mL^{-1}. The extracts of *Alternaria* sp. UFMGCB 7491 and the tissue from which it was isolated, *C. langsdorffii* leaf extract (CL14L), exhibited activities against *E. coli* at 125 μg mL^{-1}. Extracts of three *Diaporthe* isolates displayed activity against *E. coli* ranging with MIC values from 62.5 to 250 μg mL^{-1}. Among these, the extracts of *D. miriciae* UFMGCB7701 and *Diaporthe* sp. 1 UFMGCB 7696 also displayed activities against *S. aureus* with MIC values ranging from 62.5 to >250 μg mL^{-1}.

4. Discussion

4.1. Fungal taxonomy and diversity

The tissues of the tropical plants have been considered an important natural habitat for endophytes and they comprise a significant

Table 3
Relative abundance of different fungal taxonomy associated with *Copaifera langsdorffii* and *Copaifera pubiflora*.

Host	Taxa	Leaves		Stems		Seeds	
		Number of isolates	Relative Abundance (%)	Number of isolates	Relative Abundance (%)	Number of isolates	Relative Abundance (%)
Copaifera langsdorffii	*Alternaria* sp.	11	3.65	0	0	1	0.33
	Aspergillus sp. 1	0	0	0	0	1	0.33
	Aspergillus sp. 2	1	0.33	0	0	0	0
	Chaetomium cf. *unguicola*	0	0	1	0.33	0	0
	Cladosporium sp. 1	0	0	0	0	3	0.99
	Cladosporium sp. 2	0	0	0	0	4	1.32
	Colletotrichum boninense	2	0.66	0	0	0	0
	Colletotrichum karstii	4	1.32	0	0	0	0
	Colletotrichum sp.1	8	2.65	4	1.32	0	0
	Colletotrichum sp. 2	3	0.99	0	0	0	0
	Colletotrichum sp. 3	1	0.33	0	0	0	0
	Coniochaeta sp.	0	0	2	0.66	0	0
	Curvularia soli	2	0.66	0	0	0	0
	Diaporthales sp. 1	25	8.30	3	0.99	0	0
	Diaporthales sp. 2	21	6.97	1	0.33	0	0
	Diaporthe cf. *oxe*	0	0	2	0.66	0	0
	Diaporthe sp. 1	5	1.66	0	0	0	0
	Diaporthe sp. 2	0	0	3	0.99	0	0
	Diaporthe sp. 3	7	2.32	4	1.32	0	0
	Diaporthe sp. 4	12	3.98	6	1.99	0	0
	Diaporthe sp. 5	3	0.99	1	0.33	0	0
	Diaporthe sp. 6	15	4.98	29	9.63	0	0
	Diaporthe sp. 7	1	0.33	3	0.99	0	0
	Diaporthe sp. 8	10	3.32	5	1.66	0	0
	Diaporthe sp. 9	4	1.32	2	0.66	0	0
	Diaporthe sp. 10	3	0.99	10	3.32	0	0
	Diplodia pseudoseriata	0	0	2	0.66	1	0.33
	Fusarium sp. 1	2	0.66	0	0	0	0
	Fusarium sp. 2	0	0	1	0.33	0	0
	Hypoxylon sp. 1	0	0	0	0	1	0.33
	Muscodor equiseti	1	0.33	0	0	0	0
	Neopestalotiopsis sp.	0	0	1	0.33	0	0
	Nigrospora hainanensis	0	0	1	0.33	0	0
	Penicillium sp. 1	1	0.33	0	0	9	2.99
	Pestalotiopsis australasiae	0	0	1	0.33	0	0
	Phyllosticta sp.	10	3.32	0	0	0	0
	Pleosporales sp. 1	1	0.33	0	0	3	0.99
	Preussia sp. 1	0	0	2	0.66	1	0.33
	Preussia sp. 2	1	0.33	1	0.33	0	0
	Sordariomycetes sp.	3	0.99	0	0	0	0
	Trichoderma sp.	0	0	1	0.33	0	0
	Xylariaceae sp. 1	12	3.98	19	6.31	1	0.33
	Xylariaceae sp. 2	0	0	1	0.33	0	0
	Xylariaceae sp. 3	0	0	1	0.33	0	0
Copaifera pubiflora	*Amphisphaeriaceae* sp.	4	1.35	0	0	0	0
	Aspergillus fumigatus	0	0	0	0	2	0.67
	Aspergillus sp. 3	0	0	1	0.33	0	0
	Botryosphaeria sp.	2	0.67	2	0.67	0	0
	Chaetomium cf. *globosum*	0	0	0	0	1	0.33
	Colletotrichum sp. 4	4	1.35	0	0	0	0
	Diaporthales sp. 3	31	10.50	0	0	4	1.35
	Diaporthe miriciae	11	3.72	15	5.08	0	0
	Diaporthe sp. 1	11	3.72	5	1.69	0	0
	Diaporthe sp. 4	0	0	1	0.33	0	0
	Diaporthe sp. 7	30	10.16	21	7.11	0	0
	Diaporthe sp. 11	5	1.69	9	3.05	0	0
	Diaporthe sp. 12	0	0	2	0.67	0	0
	Diaporthe sp. 13	7	2.37	4	1.35	0	0
	Diaporthe sp. 14	1	0.33	0	0	0	0
	Endomelanconiopsis sp.	1	0.33	2	0.67	0	0
	Eremothecium coryli	0	0	0	0	2	0.67
	Fusarium sp. 3	0	0	2	0.67	0	0
	Hypoxylon sp. 2	2	0.67	1	0.33	0	0
	Neopestalotiopsis sp.	2	0.67	3	1.01	0	0
	Penicillium sp. 2	1	0.33	3	1.01	2	0.67
	Pestalotiopsis colombiensis	8	2.71	7	2.37	0	0
	Phyllosticta sp.	66	22.37	0	0	0	0
	Pleosporales sp. 2	0	0	3	1.01	0	0
	Xylariaceae sp. 4	8	2.71	8	2.71	1	0.33

Table 4
Antimicrobial activities of the endophytic fungi extracts of *Copaifera langsdorffii* and *Copaifera pubiflora*.

Endophytic taxa	[a]UFMGCB code	Host	Antibacterial activity (%)			Antifungal activity (%)		
			[b]EC	[c]SA	[d]PA	[e]CA	[f]CK	[g]CIS
Alternaria sp.	7491	*Copaifera langsdorffii*	**100 ± 22** (125)	21 ± 66	0	0	0	0
Diaporthe miriciae	7646	*Copaifera pubiflora*	0	**98 ± 11** (250)	0	0	0	16 ± 3
Diaporthe sp. 1	7696		**100 ± 16** (125)	**92 ± 1** (250)	0	0	0	40 ± 36
D. miriciae	7701		**99 ± 3** (62.5)	**99 ± 2** (62.5)	0	20 ± 6	0	7 ± 0
D. miriciae	7719		0	**98 ± 1** (125)	0	0	0	40 ± 2
Diaporthe sp. 14	7927		**99 ± 3** (250)	0	0	29 ± 7	21 ± 3	0
Control drugs	Amph B		-	-	-	100	100	
	Chlo		100	93	62	-	-	-
	Ben		-	-	-	-	-	94

[a] UFMGCB = Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais. [b]*Escherichia coli* ATCC 11775
[c] *Staphylococcus aureus* ATCC 12600
[d] *Pseudomonas aeruginosa* ATCC 10145
[e] *Candida albicans* ATCC 60193
[f] *Candida krusei* ATCC 6258
[g] *Cladosporium sphaerospermum* CCT 1740. Concentrations: fungi extracts: 250 μg mL^{-1}, Amph B: amphotericin B (2 μg mL^{-1}), Chlo: chloramphenicol (32 μg mL^{-1}), Ben: benomyl (1.16 μg mL^{-1}). L: leaf, S: stem, "-"no tested. In bold, the most promising values of antifungal and antibacterial inhibition (≥70%) with the mean and coefficient of variation. The MIC values are in parentheses (μg mL^{-1}).

portion of the unknown fungal diversity. Among these tropical plants, those with medicinal properties have gained increasing attention due to their ability to host diverse fungal communities and taxa capable of producing various bioactive compounds. Of the total isolates, 397 fungi (59.4%) isolates were recovered from the leaves of *C. langsdorffii* and *C. pubiflora*. According to Stone et al. (2004), type of tissue and stage is one of the factors that may influence the efficiency of recovery and enumeration of endophytic microorganisms. The majority of the endophytic fungi associated with *C. langsdorffii* and *C. pubiflora* were represented by *Ascomycota*, which is the phylum dominant in different plant species (Arnold 2007). *Diaporthe*, *Xylariaceae*, *Phyllosticta*, and *Diaporthales* were the dominant fungal taxa recovered from *C. langsdorffii* and *C. pubiflora*.

The *Diaporthe*/*Phomopsis* genera represent a complex of anamorph/teleomorph fungi, which have already been isolated as saprobes, endophytes, and pathogens from different host plants (Ferreira et al., 2020). Associated with neotropical plants from South America, *Phomopsis* represents common endophytes genus (Carvalho

Table 5
Antimicrobial activities Copaifera langsdorffii and Copaifera pubiflora extracts.

Extract code	Vegetal specimen	Plant tissue	Antibacterial activity (%)			Antifungal activity (%)		
			[a]EC	[b]SA	[c]PA	[d]CA	[e]CK	[f]CIS
CL1L	Copaifera langsdorffii	L	**83 ± 0** (>250)	55 ± 37	0	0	16 ± 0	**89 ± 5** (>250)
CL14L		L	**70 ± 0** (125)	16 ± 2	0	23 ± 1	0	48 ± 1
CL15L		L	53 ± 0	**72 ± 5** (>250)	0	32 ± 0	13 ± 0	**87 ± 25** (>250)
CL2S		S	39 ± 0	36 ± 2	16 ± 0	4 ± 1	**89 ± 14** (250)	61 ± 9
CL15S		S	43 ± 0	55 ± 12	0	0	0	**75 ± 50** (>250)
CP4L	Copaifera pubiflora	L	**75 ± 0** (>250)	27 ± 22	0	16 ± 7	0	25 ± 6
CP5L		L	**82 ± 0** (>250)	21 ± 20	0	19 ± 7	0	37 ± 2
CP7L		L	**73 ± 0** (>250)	36 ± 2	0	0	0	30 ± 3
CP9L		L	**86 ± 0** (>250)	32 ± 10	0	24 ± 13	0	25 ± 1
CP10L		L	**84 ± 0** (>250)	57 ± 8	0	23 ± 9	0	32 ± 6
CP11L		L	**87 ± 0** (>250)	29 ± 0	0	21 ± 4	54 ± 44	35 ± 15
CP13L		L	**85 ± 0** (>250)	40 ± 24	0	19 ± 3	0	0
CP14L		L	**87 ± 0** (>250)	**78 ± 8** (>250)	0	31 ± 11	**91 ± 11** (>250)	**76 ± 11** (>250)
CP15L		L	**84 ± 0** (>250)	39 ± 22	0	27 ± 1	0	36 ± 4
CP1S		S	**76 ± 0** (>250)	14 ± 0	0	26 ± 6	**94 ± 4** (250)	**84 ± 12** (>250)
CP2S		S	**80 ± 0** (>250)	**84 ± 12** (250)	0	14 ± 7	47 ± 0	**78 ± 16** (125)
CP3S		S	**81 ± 2** (125)	61 ± 42	0	27 ± 5	63 ± 43	28 ± 6
CP4S		S	**82 ± 0** (>250)	49 ± 10	0	22 ± 3	0	28 ± 19
CP5S		S	**86 ± 0** (125)	**76 ± 1** (>250)	0	41 ± 16	0	47 ± 1
CP6S		S	**83 ± 0** (125)	63 ± 14	0	26 ± 3	**78 ± 15** (>250)	35 ± 8
CP7S		S	**83 ± 0** (>250)	58 ± 26	0	23 ± 8	**95 ± 10** (>250)	55 ± 39
CP8S		S	**81 ± 0** (31.2)	63 ± 10	0	32 ± 4	0	**81 ± 21** (>250)
Control drugs	Amph B		-	-	-	100	100	-
	Chlo		100	97	70	-	-	-
	Bem		-	-	-	-	-	94

[a] Escherichia coli ATCC 11775
[b] Staphylococcus aureus ATCC 12600
[c] Pseudomonas aeruginosa ATCC 10145
[d] Candida albicans ATCC 60193
[e] Candida krusei ATCC 6258
[f] Cladosporium sphaerospermum CCT 1740. Concentrations: extracts: 250 μg mL^{-1}, Amph B: amphotericin B (2 μg mL^{-1}), Chlo: chloramphenicol (32 μg mL^{-1}), Ben: benomyl (1.16 μg mL^{-1}). L: leaf, S: stem. In bold, the most promising values of antifungal and antibacterial inhibition (in percentage; ≥70%) with the mean and coefficient of variation. The MIC values are in parentheses (μg mL^{-1}).

et al., 2012; Ferreira et al., 2015, 2017a, 2020; Vieira et al., 2012). *Xylariaceae* is one of the largest and most diverse families of *Ascomycota* (Bitzer et al., 2008). Different *Xylariaceae* taxa have already been reported as endophytes in various plants, including tropical medicinal plants (Correia et al., 2018; Ferreira et al., 2015, 2020; Silva et al., 2010; Vieira et al., 2012, 2014).

Phyllosticta sp. (anamorph of *Guignardia*) was found to be the dominant taxa in the isolates obtained from the leaves of *C. pubiflora*. *Guignardia*/*Phyllosticta* have been previously reported as the dominant fungi in the leaves of *Dendrobium nobile* Lindl. (Orchidaceae) (Yuan et al., 2009), *Taxus chinensis* var. *mairei* (Lemée & H. Lév.) W.C. Cheng & L.K. Fu (Taxaceae) (Wu et al., 2013), and *Huperzia serrata* (Thunb.) Rothm. (Lycopodiaceae) (Xiong et al., 2015). According to Pandey et al. (2003) and Wu et al. (2013), *Guignardia* and *Phyllosticta* taxa are foliar endophytes or pathogens that are found in different plant hosts.

In addition, we recovered two yeast isolates identified as *Eremothecium coryli* (*Saccharomycetes*) from the seeds of *C. pubiflora*. In general, yeasts form a rare component of the endophytic communities (Vaz et al., 2009; Vieira et al., 2012; Ferreira et al., 2017a). *Eremothecium* genus was previously isolated from mustard seeds; it is known for its ability to produce riboflavin and has been reported as a dimorphic fungus (Gastmann et al., 2007). Furthermore, species of *Eremothecium* have already been reported as endophytic, plant pathogens, and recently as human pathogen (Multani et al., 2019; Schisler et al., 2010; Shipunov et al., 2008).

The composition of the endophytic fungi communities changed with the tissue. *Xylariaceae* sp. 1 was the only taxon that was recovered from leaves, stems, and seeds of *C. langsdorffii*. Yuan et al. (2009) recovered *Xylariaceae* from all tissues (leaves, stems, and roots) of *Dendrobium nobile*, a famous Chinese traditional medicinal plant. In addition, 12 taxa (179 isolates) were found to be common between leaves and stem tissues of *C. langsdorffii*, while 11 taxa (183 isolates) were found to be common between leaves and stems tissues of *C. pubiflora*. Some fungal endophytes seem to have affinity for different tissues based on their chemical and structural characteristics, which might be a reflection of their capacities to survive within a specific substrate (Wu et al., 2013).

4.2. Antimicrobial activities

Endophytic fungi of medicinal plants are currently being widely studied in the search for new, potentially useful secondary metabolites. The bioactive secondary metabolites produced by medicinal plants and endophytes have provided countless essential drugs for treatment of innumerable diseases, including those with bacterial origin (Glienke et al., 2012; Manganyi et al., 2018; Manganyi et al., 2019). Since the discovery that the diterpenoid taxol can be produced by the endophytic fungi *Taxomyces andreanae* (Strobel 2003), and, from their host, the medicinal plant *Taxus brevifolia* (Stierle et al., 1995), it was evidenced that the endophytic fungi might produce the same metabolites of their host plant (Carvalho et al., 2019). However, it is important to highlight that endophytic fungi are also producers of bioactive secondary metabolites that are different from those produced by their hosts and can be of interest for medicinal applications (Carvalho et al., 2019). Schulz et al. (2015) hypothesize that the role of the secondary metabolites produced by the endophytic fungi play *in situ* (*in planta*) can be to inhibit competitors. The authors suggested that in order to grow asymptomatically within their plant hosts, fungal endophytes would need to not only maintain a balanced antagonism with their plant host, but also with bacterial and fungal inhabitants of the host (Schulz et al., 2015).

Five of six active extracts were produced by fungi that were recovered from *C. pubiflora* and 17 of 22 plant tissue active extracts were also come from *C. pubiflora*. Fernández et al. (2018) reported that oleoresin produced by *C. pubiflora* displayed promising antibacterial activities against *S. aureus* and *Bacillus cereus*. However, the same authors related that the oleoresin produced by *C. langsdorffii* did not yield any promising antibacterial activity. In addition, regarding biofilm inhibition and eradication, the oleoresin from *C. pubiflora* exhibited MIC_{50} values of 100, 125, and 125 $\mu g\ mL^{-1}$ against *S. aureus*, *B. cereus*, and *Listeria monocytogenes*, respectively. Furtado et al. (2018) reported that the oleoresins and leaf extracts of different *Copaifera* species, including *C. pubiflora*, were neither cytotoxic *in vivo* nor genotoxic in both *in vitro* and *in vivo* assays under experimental conditions. For these reasons, *C. pubiflora* can be a good reservoir of bioactive endophytes that are able to produce antimicrobial compounds.

The isolates belonging to the taxa *Alternaria* and *Diaporthe* produced compounds with selective antibacterial activity. *Alternaria* species are commonly isolated as endophytes (Arnold 2008; Strobel and Daisy, 2003; Manganyi et al., 2018), and are potent producers of bioactive metabolites.

Cota et al. (2008) and Johann et al. (2012) reported that altenusin, isolated from the endophytic fungus *Alternaria* sp. and from the leaves of the bioactive plant *Trixis vauthieri* DC (Asteraceae), was able to inhibit Trypanothione reductase with an IC_{50} value of 4.3 ± 0.3 μM and exhibited strong antifungal activity against *Paracoccidioides brasiliensis* with MIC values ranging between 1.9 and 31.2 $\mu g\ mL^{-1}$. In addition, Vieira et al. (2014) reported the extract of *Alternaria* sp. exhibited antifungal activity against *P. brasiliensis* with MIC value of 31.2 $\mu g\ mL^{-1}$. *Diaporthe*/*Phomopsis* complex is a known producer of different bioactive compounds (Carvalho et al., 2012; Ferreira et al., 2017b). Ferreira et al. (2017b) demonstrated that the extracts of *D. miriciae* isolated from *Vellozia gigantea* N. L. Menezes & Mello-Silva (Velloziaceae) showed antifungal activity against *C. albicans*, *C. glabrata*, *C. krusei*, antibacterial against *S. aureus* methicillin resistant, and antimalarial activity. The endophyte *D. miriciae* produced epoxycytochalasin H, which displayed high antimalarial activity against chloroquine-resistant *P. falciparum* with an IC_{50} approximately 3.5-fold lower than that with chloroquine (Ferreira et al., 2017b). Medeiros et al. (2018) reported that the crude extract of the endophytic *Diaporthe terebinthifolii*, isolated from the medicinal plant *Schinus terebinthifolius* Raddi (Anacardiaceae), demonstrated high antibacterial activity against *E. coli*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, methicillin-sensitive *S. aureus*, and methicillin-resistant *S. aureus*. In contrast, Carvalho et al. (2012) showed that the extract of two isolates of *Diaporthe* cf. *phaseolorum* obtained from *Stryphnodendron adstringens* did not displayed antibacterial activity but displayed antitumor activities.

5. Conclusion

Our results showed that different tissues of the ethnomedicinal plant species, *C. langsdorffii* and *C. pubiflora*, are systematically colonized by rich and diverse endophytic fungal communities. Among these fungal communities, extracts of fungi recovered from both plants, *C. langsdorffii* and *C. pubiflora*, are able to produce antimicrobial compounds, which might be explored in further studies as potential candidates for the development of new drugs.

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Conflicts of interest

The authors declare any conflict of interest.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipmanm, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25, 3389–3402.

Arnold, A.E., 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biol. Rev. 21, 51–66.

Arnold, A.E., 2008. Hidden within our botanical richness, a treasure trove of fungal endophytes. Plant Press 32, 13–15.

Arruda, C., Mejía, J.A.A., Ribeiro, V.P., Borges, C.H.G., Martins, C.H.G., Veneziani, R.C.S., Ambrósio, S.R., Bastos, J.K., 2019. Occurrence, chemical composition, biological activities and analytical methods on *Copaifera* genus—a review. Biomed. Pharmacother. 109, 1–20.

Banerjee, P., Erehman, J., Gohlke, B.O., Wilhelm, T., Preissner, R., Dunkel, M., 2015. Super Natural II-a database of natural products. Nucl. Acids Res. 43, 935–939.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C., 2014. Jvenn: an interactive Venn diagram viewer. BMC Bioinform. 15, 293.

Bitzer, J., Læssøe, T., Fournier, J., Kummer, V., Decock, C., Tichy, H.V., Piepenbring, M., Persoh, D., Stadler, M., 2008. Affinities of *Phylacia* and the daldinoid *Xylariaceae*, inferred from chemotypes of cultures and ribosomal DNA sequences. Mycol. Res. 112, 251–270.

Brissow, E.R., Silva, I.P., Siqueira, K.P., Senabio, J.A., Pimenta, L.P., Januário, A.H., Magalhães, L.G., Furtado, R.A., Tavares, D.C., Junior, P.A.S., Santos, J.L., Soares, M.A., 2018. 18-Des-hydroxy Cytochalasin: an antiparasitic compound of *Diaporthe phaseolorum*-92C, an endophytic fungus isolated from *Combretum lanceolatum* Pohl ex Eichler. Parasitol. Res. 116, 1823–1830.

Carvalho, C.R., Gonçalves, V.N., Pereira, C.B., Johann, S., Galliza, I.V., Alves, T.M.A., Rabello, A., Sobral, M.E.G., Zani, C.L., Rosa, C.A., Rosa, L.H., 2012. The diversity, antimicrobial and anticancer activity of endophytic fungi associated with the medicinal plant *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) from the Brazilian savannah. Symbiosis 57, 95–107.

Carvalho, C.R., Wedge, D.E., Cantrell, C.L., Hughes, A.F.S., Pan, Z., Moraes, R.M., Madoxx, V.L., Rosa, L.H., 2016. Molecular Phylogeny, Diversity and Bioprospecting of Endophytic Fungi Associated with wild Ethnomedicinal North American Plant *Echinacea purpurea* (Asteraceae). Chem. Biodivers. 1, 1–10.

Carvalho, C.R., Ferreira-D'Silva, A., Wedge, D.E., Cantrell, C.L., Rosa, L.H., 2018. Antifungal activities of cytochalasins produced by *Diaporthe miriciae*, an endophytic fungus associated with tropical medicinal plants. Can. J. Microbiol. 64, 835–843.

Carvalho, C.R., Ferreira, M.C., Amorim, S.S., Florindo, R.H.S., Assis, J.C.S., Zani, C.L., Rosa, L.H., 2019. Bioactive Compounds of Endophytic Fungi Associated with Medicinal Plants. In: Yadav, A.N., Mishra, S., Singh, S., Gupta, A. (Eds.), Recent Advancement in White Biotechnology Through Fungi. Springer, Cham, pp. 303–361.

Castellani, A., 1967. Maintenance and cultivation of common pathogenic fungi in distilled water. Am. J. Trop. Med. 42, 181–184.

Correia, A.M.L., Lira, S.P., Assis, M.A., Rodrigues, A., 2018. Fungal endophyte communities in *Begonia* species from the brazilian atlantic rainforest. Curr. Microbiol. 75, 441–449.

Cota, B.B., Rosa, L.H., Caligiorne, R.B., Rabello, A.L.T., Alves, T.M.A., Rosa, C.A., Zani, C.L., 2008. Altenusin, a biphenyl isolated from the endophytic fungus *Alternaria* sp., inhibits trypanothione reductase from *Trypanosoma cruzi*. FEMS Microbiol. Lett. 285, 177–182.

Espinel-Ingroff, A., Kerkering, T.M., 1991. Spectrophotometric method of inoculum preparation for the *in vitro* susceptibility testing of filamentous fungi. J. Clin. Microbiol. 29, 393–394.

Fernández, Y.A., Damasceno, J.L., Abrão, F., Silva, T.S., Cândido, A.L.P., Fregonezi, N.F., Resende, F.A., Ramos, S.B., Ambrosio, S.R., Veneziani, R.C.S., Bastos, J.K., Martins, C.H.G., 2018. Antibacterial, preservative, and mutagenic potential of *Copaifera* spp. oleoresins against causative agents of foodborne diseases. Foodborne Pathog. Dis. 15, 1–8.

Ferreira, M.C., Vieira, M.D.L.A., Zani, C.L., De Almeida Alves, T.M., Junior, P.A.S., Murta, S.M., Romanha, A.J., Gil, L.H.V.G., Carvalho, A.G.O., Zilli, J.E., Vital, M.J.S., Rosa, C.A., Rosa, L.H., 2015. Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). Biochem. Syst. Ecol. 59, 36–44.

Ferreira, M.C., Cantrell, C.L., Wedge, D.E., Gonçalves, V.N., Jacob, M.R., Khan, S., Rosa, C.R., Rosa, L.H., 2017a. Diversity of the endophytic fungi associated with the ancient and narrowly endemic neotropical plant *Vellozia gigantea* from the endangered Brazilian rupestrian grasslands. Biochem. Syst. Ecol. 71, 163–169.

Ferreira, M.C., Cantrell, C.L., Wedge, D.E., Gonçalves, V.N., Jacob, M.R., Khan, S., Rosa, C.A., Rosa, L.H., 2017b. Antimycobacterial and antimalarial activities of endophytic fungi associated with the ancient and narrowly endemic neotropical plant *Vellozia gigantea* from Brazil. Mem. Inst. Oswal. Cruz 112, 692–697.

Ferreira, M.C., De Assis, J.C.S., Rosa, L.H., 2020. Diversity of endophytic fungi associated with *Carapichea ipecacuanha* from a native fragment of the Atlantic Rain Forest. South Afr. J. Bot. 57, 1–5.

Fröhlich, J., Hyde, K.D., Petrini, O., 2000. Endophytic fungi associated with palms. Mycol. Res. 104, 1202–1212.

Furtado, R.A., de Oliveira, P.F., Senedese, J.M., Ozelin, S.D., de Souza, L.D.R., Leandro, L.F., de Oliveira, W.L., da Silva, J.J.M., Oliveira, L.C., Rogez, H., Ambrósio, S.R., Veneziani, R.C.S., Bastos, J.K., Tavares, D.C., 2018. Assessment of genotoxic activity of oleoresins and leaves extracts of six *Copaifera* species for prediction of potential human risks. J. Ethnopharmacol. 221, 119–125.

Gastmann, S., Dünkler, A., Walther, A., Klein, K., Wendland, J., 2007. A molecular toolbox for manipulating *Eremothecium coryli*. Microbiol. Res. 162, 299–307.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323–1330.

Glienke, C., Tonial, F., Gomes-Figueiredo, J., Savi, D., Vicente, V.A., Maia, B.H.S., Possiede, Y.M., 2012. Antimicrobial activity of endophytes from Brazilian medicinal plants. In: Bobbarala, V. (Ed.), Antibacterial Agents. 1st Edn InTech, Rijeka, pp. 239–254.

Gonçalves, V.N., Cantrell, C.L., Wedge, D.E., Ferreira, M.C., Soares, M.A., Jacob, M.R., Oliveira, F.S., Galante, D., Rodrigues, F., Alves, T.M.A., Zani, C.L., Junior, P.A.S., Murta, S., Romanha, A.J., Barbosa, E.C., Kroon, E.G., Oliveira, J.G., Gomez-Silva, B., Galetovic, A., Rosa, C.A., Rosa, L.H., 2015. Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. Environ. Microbiol. 18, 232–245.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1–9.

Johann, S., Rosa, L.H., Rosa, C.A., Perez, P., Cisalpino, P.S., Zani, C.L., Cota, B.B., 2012. Antifungal activity of altenusin isolated from the endophytic fungus *Alternaria* sp. against the pathogenic fungus *Paracoccidioides brasiliensis*. Rev. Iberoam. Micol. 29, 205–209.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. Dictionary of the Fungi. CAB International, Wallingford.

Kurtzman, C.P., Fell, J.W., Boekhout, T., 2011. The Yeasts: A Taxonomic Study. Elsevier Science, Amsterdam. fifth ed.

Lachance, M.A., Bowles, J.M., Starmer, W.T., Barker, J.S.F., 1999. *Kodamaea kakaduensis* and *Candida tolerans*, two new yeast species from Australian Hibiscus flowers. Can. J. Microbiol. 45, 172–177.

Lieckfeldt, E., Meyer, W., Börner, T., 1993. Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. J. Basic Microbiol. 33, 413–426.

Manganyi, M.C., Regnier, T., Kumar, A., Bezuidenhout, C.C., Ateba, C.N., 2018. Biodiversity and antibacterial screening of endophytic fungi isolated from *Pelargonium sidoides*. South Afr. J. Bot. 116, 192–199.

Manganyi, M.C., Regnier, T., Tchatchouang, C.K., Bezuidenhout, C.C., Ateba, C.N., 2019. Antibacterial activity of endophytic fungi isolated from *Sceletium tortuosum* L. (Kougoed). Ann. Microbiol. 69, 659–663.

Medeiros, A.G., Savi, D.C., Mitra, P., Shaaban, K.A., Jha, A.K., Thorson, J.S., Rohr, J., Glienke, C., 2018. Bioprospecting of *Diaporthe terebinthifolii* LGMF907 for antimicrobial compounds. Folia Microbiol. (Praha) 63, 499–505.

Multani, A., Rustagi, A., Epstein, D.J., Gomez, C.A., Budvytiene, I., Banaei, N., Brown, J.M., Liu, A.Y., 2019. *Eremothecium coryli* bloodstream infection in a patient with acute myeloid leukemia: first case report of human infection. Diagn. Microbiol. Infect. Dis. 95, 77–79.

National Committee for Clinical Laboratory Standards, 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. NCCLS document M38-A, Pennsylvania, USA.

National Committee for Clinical Laboratory Standards, 2003. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard. NCCLS document M7-A6, Pennsylvania, USA. 6 ed.

Pandey, A.K., Reddy, M.S., Suryanarayanan, T.S., 2003. ITS-RFLP and ITS sequence analysis of a foliar endophytic *Phyllosticta* from different tropical trees. Mycol. Res. 107, 439–444.

Paramanantham, P., Pattnaik, S., Siddhardha, B., 2019. Natural products from endophytic fungi: synthesis and applications. In: Singh, B. (Ed.), Advances in Endophytic Fungal Research. Springer, Cham, pp. 83–103.

Petrini, O., 1991. Fungal endophytes of tree leaves. In: Andrews, J.H., Hirano, S.S. (Eds.), Microbial Ecology of Leaves. Springer, New York, pp. 179–197.

Rodriguez, R.J., Henson, J., Volkenburgh, E.V., Hoy, M., Wright, L., Beckwith, F., Kim, Y-O., Redman, R.S., 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME J. 2, 404–416.

Rodríguez-Tudela, J.L., Barchiesi, F., Bille, J., Chryssanthou, E., Cenca-Estrella, M., Denning, D., Donnelly, J.P., Dupont, B., Fegeler, W., Moore, C., Richardson, M., Verweij, P.E., 2003. Method for determination of minimal inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Eucast Discussion Document 7.1. Clin. Microbiol. Infect. 9, 1–8.

Rosa, L.H., Vaz, A.B.M., Caligiorne, R.L.B., Campolina, S.A., Rosa, C.A., 2009. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (Poaceae). Polar Biol. 32, 161–167.

Santiago, I.F., Alves, T.M.A., Rabello, A., Sales, P.A.J.R., Romanha, A.J., Zani, C.L., Rosa, C.A., Rosa, L.H., 2012. Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. Extremophiles 16, 95–103.

Schulz, B., Haas, S., Junker, C., Andrée, N., Schobert, M., 2015. Fungal endophytes are involved in multiple balanced antagonisms. Curr. Sci. 109, 39–45.

Silva, G.H., Oliveira, C.M., Teles, H.L., Bolzani, V.S., Araujo, A.R., Pfenning, L.H., Young, M.C.M., Costa-Neto, C.M., Haddad, R., Eberlin, M.N, 2010. Citocalasinas produzidas por *Xylaria* sp., um fungo endofítico de Piper aduncum (Piperaceae)#. Quim. Nova 33, 2038–2041.

Silva-Hughes, A.F., Wedge, D.E., Cantrell, C.L., Carvalho, C.R., Pan, Z., Moraes, R.M., Madoxx, V.L., Rosa, L.H., 2015. Diversity and antifungal activity of the endophytic fungi associated with the native medicinal cactus *Opuntia humifusa* (Cactaceae) from the United States. Microbiol. Res. 175, 67–77.

Stierle, A., Strobel, G., Stierle, D., Grothaus, P., Bignami, G., 1995. The 25 search for a taxol-producing microorganism among the endophytic fungi of the pacific yew, *Taxus brevifolia*. J. Nat. Prod. 58, 1315–1324.

Stone, J.K., Polishook, J.D., White, J.F., 2004. Endophytic fungi. In: Muller, G., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of Fungi. Inventory and Monitoring Methods. Elsevier Academic Press, pp. 241–270.

Strobel, G., 2003. Endophytes as sources of bioactive products. Microb. Infect. 5, 535–544.
Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67, 491–502.
Strobel, G., Daisy, B., Castillo, U., Harper, J., 2004. Natural products from endophytic microorganisms. J. Nat. Prod. 67, 257–268.
Shipunov, A., Newcombe, G., Raghavendra, A.K.H., Anderson, C.L., 2008. Hidden diversity of endophytic fungi in an invasive plant. Am. J. Bot. 95, 1096–1108.
Schisler, D.A., Janisiewicz, W.J., Boekhout, T., Kurtzman, C.P., 2010. Agriculturally important yeasts: biological control of field and postharvest diseases using yeast antagonists, and yeasts as pathogens of plants. In: Kurtzman, C.P., Fell, J.W., Boekhout, T. (Eds.), The Yeasts: A Taxonomic Study. 5th edn Elsevier Science, pp. 45–52.
Vaz, A.B.M., Mota, R.C., Bomfim, M.R.Q., Vieira, M.L.A., Zani, C.L., Rosa, C.A., Rosa, L.H., 2009. Antimicrobial activity of endophytic fungi associated with *Orchidaceae* in Brazil. Can. J. Microbiol. 55, 1381–1391.
Vieira, M.L.A., Hughes, A.F.S., Gil, V.B., Vaz, A.B.M., Alves, T.M.A., Zani, C.L., Rosa, C.A., Rosa, L.H., 2012. Diversity and antimicrobial activities of the fungal endophyte community associated with the traditional Brazilian medicinal plant *Solanum cernuum* Vell. (Solanaceae). Can. J. Microbiol. 58, 54–66.
Vieira, M.L.A., Johann, S., Hughes, F.M., Rosa, C.A., Rosa, L.H., 2014. The diversity and antimicrobial activity of endophytic fungi associated with medicinal plant *Baccharis trimera* (Asteraceae) from the Brazilian savannah. Can. J. Microbiol. 60, 847–856 .
Wedge, D.E., Kuhajek, J.M., 1998. A microbioassay for fungicide discovery. SAAS Bulletin. Biochem. Biotechnol. 11, 1–7.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.
Wu, L., Han, T., Li, W., Jia, M., Xue, L., Rahman, K., Qin, L., 2013. Geographic and Tissue Influences on Endophytic Fungal Communities of *Taxus chinensis* var. *mairei* in China. Curr. Microbiol. 66, 40–48.
Wu, Y.Y., Zhang, T.Y., Zhang, M.Y., Cheng, J., Zhang, Y.X., 2018. An endophytic fungi of *Ginkgo biloba* L. produces antimicrobial metabolites as potential inhibitors of FtsZ of *Staphylococcus aureus*. Fitoterapia 128, 265–271.
Yan, Y., Liu, Q., Jacobsen, S.E., Tang, Y., 2018. The impact and prospect of natural product discovery in agriculture. EMBO Rep. 19, e46824.
Yu, H., Zhang, L., Li, L, Zheng, C., Guo, L., Li, W., Sun, P., Qin, L., 2010. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol. Res. 165, 437–449.
Yuan, Z.L., Chen, Y.C., Yang, Y., 2009. Diverse non-mycorrhizal fungal endophytes inhabiting an epiphytic, medicinal orchid (*Dendrobium nobile*): estimation and characterization. World J. Microbiol. Biotechnol. 25, 295–303.
Xiong, Z.Q., Yang, Y.Y., Liu, Q.X., Sun, C.C., Jin, Y., Wang, Y., 2015. Endophytes in the plant *Huperzia serrata*: fungal diversity and discovery of a new pentapeptide. Arch. Microbiol. 197, 411–418.