Acta Botanica Brasilica, 2022, 36: e2020abb0426
doi: 10.1590/0102-33062020abb0426

Original article

Endophytic fungi from an overlooked plant species: A case study in *Kelissa brasiliensis* (Baker) Ravenna

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Received: September 20, 2020
Accepted: September 24, 2021

ABSTRACT

Studies involving endophytic fungi isolated from endemic plants and their antibacterial potential are largely unknown in the Pampa biome. In this study, we identified endophytic fungi isolated from *Kelissa brasiliensis* (Iridaceae), an endemic species of the Brazilian Pampa, and assessed their antibacterial activity. Endophytic fungi were first grouped based on macro-and micro-morphology, and one representative of each morphospecies was analyzed using sequences from the internal transcribed spacer (ITS) rDNA region. We then tested the fungal extracts against laboratory isolates of *Staphylococcus aureus* and *Escherichia coli* for antibacterial activity. A total of 30 endophytes were isolated from the tissues of *K. brasiliensis*, with the majority from the leaves. Endophytes were then grouped into seven morphospecies based on their morphological features and one representative from each was selected for phylogenetic analysis. The inference from the ITS rDNA sequences identified the endophytes of the seven selected morphospecies as belonging to six taxonomic groups: *Colletotrichum* (two), *Diaporthe* (one), *Epicoccum* (one), *Fusarium* (one), and *Pestalotiopsis* (one). The endophyte extracts revealed better results against *E. coli* than *S. aureus*, although the extracts from *Colletotrichum* and *Pestalotiopsis* sp. were statistically similar to the control antibiotic. Our study is a basis for endophytic fungi studies in Pampa.

Keywords: "bibi-pintadinha", Brazilian biome, *Colletotrichum*, endophytic fungi, *Pestalotiopsis*

Introduction

Kelissa is a monospecific genus endemic to the Pampa biome, and *Kelissa brasiliensis* (Iridaceae), popularly known in Brazil as "bibi-pintadinha," is an herbaceous bulbous species with a flowering period in the spring (September to November) and seed dispersal in December. This species is found in rocky environments in the southeastern depression, and in the center of the state of Rio Grande do Sul (Barroso 2006; Aguiar *et al.* 2009), and is considered vulnerable because of habitat reduction in its natural areas, mainly through agriculture (Barroso 2006).

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Kelissa brasiliensis is an endemic species that contributes to the environment of the Pampa biome, which contains approximately 3,000 plants (Pillar *et al.* 2009). However, due to the expansion of intense agricultural activities in the native fields, these species are threatened (De Oliveira *et al.* 2017). Pampa comprises more than 45 % of the Iridaceae species in Brazil (86 species in 14 genera of the total taxa described for the country), yet no studies involving the mycobiota associated with these species have been noted (Gil *et al.* 2015; Pastori *et al.* 2020). The reference to *K. brasiliensis* has only been identified in studies of plant propagation, chromosomal numbers, and floral lists (Barroso 2006).

Many plants have been studied as hosts of endophytic fungi in all other biomes in Brazil, given their excellent environmental service and biotechnological potential (Bezerra *et al.* 2019). Endophytic fungi are microorganisms that live in plant tissues without causing adverse effects to the plant. These fungi have been described in relation to hundreds of plants, and several studies have demonstrated their ability to produce a wide range of secondary metabolites with antibacterial, anticancer, and antifungal properties (Mishra *et al.* 2014; Rana *et al.* 2019). Saffron (*Crocus sativus*) is a unique species in the family Iridaceae with reports of endophytic fungi and biotechnological potential (Zheng *et al.* 2012; Raj *et al.* 2013; Wani *et al.* 2016; Chamkhi *et al.* 2018). In Brazil, the fungal endophyte community of Iridaceae species is still unknown.

Plants that live in poorly explored habitats similar to the Pampa biome have been used as sources for the discovery of new species of endophytic fungi, thereby providing increased data on the richness, diversity, and biological activity of this mycobiome (Hokama *et al.* 2016). Endemic species and plants at risk of extinction are among the most important hosts for revealing endophytes and their biotechnological potential (Omeje *et al.* 2017). Thus, the identification of endophytic fungi from the Iridaceae species, *K. brasiliensis*, which is endemic to the Pampa biome, presents a promising option for uncovering fungi with the potential to produce antibacterial agents for further biotechnological applications.

This study aimed to report the endophytic fungi richness of *K. brasiliensis*, an endemic plant of the Pampa Biome, and evaluate the antibacterial activity of these endophytes against clinical isolates.

Materials and methods

Study area and plant collection

Kelissa brasiliensis individuals were collected from a private area of the Pampa biome with native vegetation near the Reserve of Sanga da Bica (30°20'03" S, 54°19'18" W), in São Gabriel, Rio Grande do Sul, Brazil. Countryside vegetation and a temperate climate are predominant in this region, with an average temperature of 18 °C and annual precipitation ranging from 1,250 to 1,600 mm. The aerial parts of 12 healthy *K. brasiliensis* plants up to 7 cm tall and having one flower were randomly collected at three locations (30°20'50" S, 54°19'10" W; 30°20'48" S, 54°19'10" W; and 30°20'47" S, 54°19'09" W) (Fig. 1), along a pre-existing trail on the edge of the forest during the spring of 2017. Four specimens of *K. brasiliensis* were randomly collected

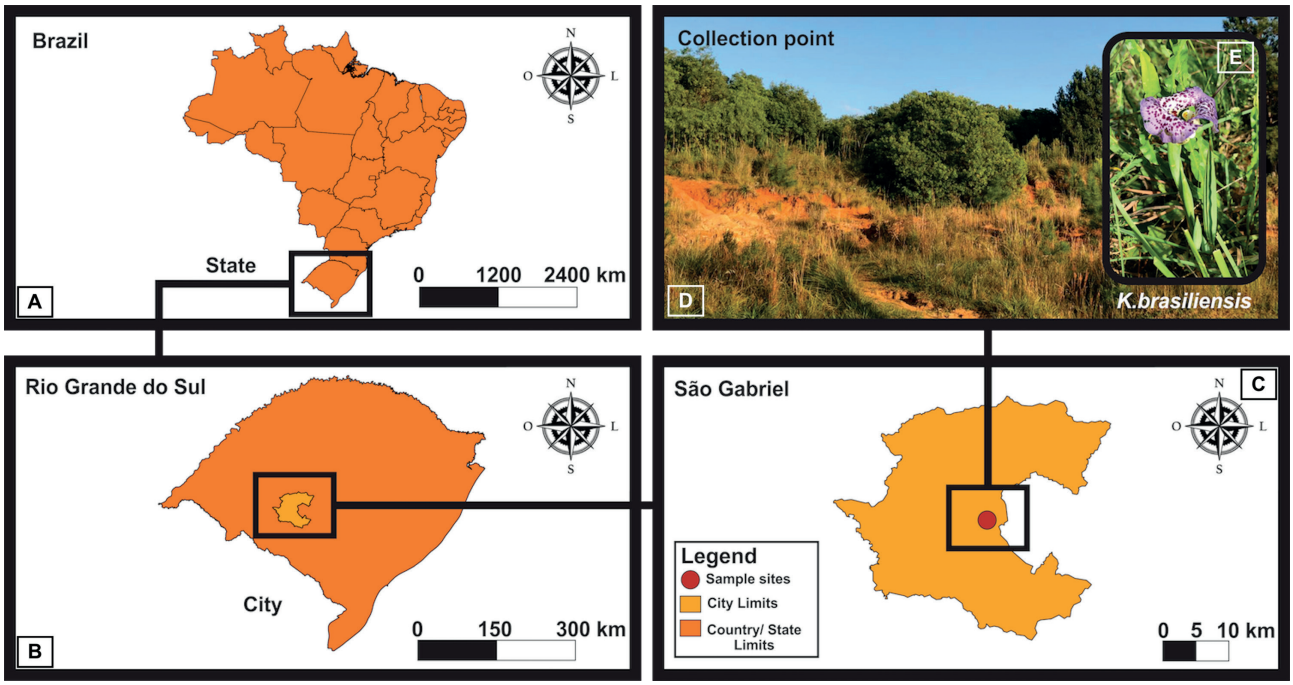

Figure 1. A-C. The geographical location of the Reserva Ecológica Sanga da Bica, São Gabriel, Rio Grande do Sul, Brazil. **D.** *Kelissa brasiliensis* (Baker) Ravenna. **E.** Collection point in the Reserva.

at each sampling point. The plants were stored individually in sterilized plastic bags, transported to the laboratory at ± 24 °C, and processed on the same day as collected.

Isolation of endophytic fungi

Under aseptic conditions, the plants were divided into three parts (capsules, stems, and leaves). The central part of each fragment was cut into three subfragments measuring approximately 1 cm, which were subjected to superficial disinfestations (de Andrade *et al.* 2018). Briefly, the plant tissues were washed in 70 % alcohol for 1 min, followed by 2 % sodium hypochlorite for 3 min, and finally in distilled and autoclaved water for 2 min. The disinfected sub-fragments were inoculated in Petri dishes containing potato dextrose agar (PDA) culture medium supplemented with chloramphenicol (100 μg mL^{-1}) to restrict bacterial growth. The efficiency of the disinfestation protocol was tested by inoculating 1 mL of the washing water in Petri dishes containing PDA medium. Plates were incubated at 20 °C under a photoperiod (16 h light and 8 h dark) for up to 20 days. When the fungal endophytes were first observed from the seventh day, they were isolated in Petri dishes containing PDA and incubated under the same conditions described above.

Fungal endophyte identification

Fungal endophyte isolates were identified and grouped into morphological species according to the macro- (size of colonies after seven days growing in PDA culture medium, color, shape, and texture) and micro-morphological characteristics of their somatic and reproductive structures (*e.g.,* Booth 1971; Barnett & Hunter 1999; Guo *et al.* 2000). After grouping the initial morphological species, seven isolates were selected for molecular analysis.

Molecular identification of fungal isolates was performed using rDNA sequences of the internal transcribed spacer (ITS) region. Fungal isolates were inoculated on PDA and the DNA was extracted on day 7th day using the PureLink^{tm} Plant Kit. The ITS rDNA region was amplified using primers ITS1 and ITS4 (White *et al.* 1990), as described by Andrade *et al.* (2018) (95 °C for 5 min, 40 cycles of 94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s, with a final extension at 72 °C for 5 min). The resulting PCR amplifications were purified using the Wizard® SV Gel kit and PCR Clean-Up System, following the manufacturer's protocol. Sequencing was conducted using an ABI Prism 3500 Genetic Analyzer (ACTGene Company).

Sequences from the endophytes were analyzed and edited manually using BioEdit v.7.2.5 (Hall 1999), and the consensus sequence was determined using the SeqMan software by DNASTAR (Burland 2000). The BLASTn tool of the NCBI GenBank database was used to search for similar sequences to those of the seven endophytic fungi, and the resulting selections (Tab. S1 in supplementary material) were used to construct a matrix that was aligned using MEGA v. 7 (Kumar *et al.* 2016). Phylogenetic analysis based on maximum likelihood (ML) was conducted with the RAxML-HPC BlackBox v.8.2.8 (Kozlov *et al.* 2019) using the CIPRES Science Gateway (Miller *et al.* 2010). The best nucleotide model, GTR + I + G, with 1,000 bootstrap replicates were used in this analysis. ML bootstrap (ML-BS) values equal to or higher than 70 % are shown near the nodes. Sequences generated during this study were submitted to GenBank under the accession numbers MK778368–MK778374, and the ITS alignment was deposited in TreeBASE (study S24809). After identification, the isolated morphotypes were stored in distilled water (Castellani 1967) and deposited in the Herbário Bruno Edgar Irgang at the Universidade Federal do Pampa (codes: HBEI 0009–HBEI 0015).

Frequency and abundance of endophytic fungi

The frequency of plant tissue colonization (FC %) was estimated as suggested by Rajagopal & Suryanarayanan (2000) using the formula FC % = Ni/Nf × 100, where Ni = the number of endophytic isolates and Nf = the total number of fragments inoculated. To calculate the percentage abundance (PA %) of each genus, we applied the methodology used by Rosa *et al.* (2010). Thus, we considered the occurrence of each genus (Og) and the occurrence of all genera (Otg) (PA % = Og × 100/Otg). These values were used to determine the prevalence of different genera in the fungal endophyte community of *K. brasiliensis*.

The absolute (f) and relative (fr) frequencies were calculated according to the method described by Larran *et al.* (2002). The absolute frequency (f) was calculated according to the total number of fungi isolated from *K. brasiliensis*, and the relative frequency (fr) from the number of isolates of each morphotype divided by the total number of isolates.

Fungal fermentation and antibacterial activity

Fermentation and antibacterial activity tests were performed as described by Teixeira *et al.* (2011). The seven selected endophytes were cultured on PDA for 14 days at 25 °C, after which the 9 mm diameter culture disks were transferred to 500 mL Erlenmeyer flasks containing 200 mL Czapek-Dox liquid medium (0.5 g KCl, 1 g KH_2PO_4, 2 g $NaNO_3$, 30 g sucrose, 0.01 g $FeSO_4.H_2O$, and 0.5 g $MgSO_4$. 7 H_2O per 1,000 mL distilled water) previously sterilized at 120 °C for 20 min. Five discs of each culture were inoculated per flask and maintained on shaker tables at 180 rpm for 7 d at 25 °C. The cultures were then subjected to two successive vacuum filtrations using Whatman #1 paper and filtered using 0.22 μm Millipore membranes. The final extract was stored at 4 °C until antibacterial assays were performed.

Antibacterial activity was evaluated using quantitative biological assays in triplicate with strains of *Escherichia coli* (TOP10 Invitrogen®) and a wild strain of *Staphylococcus aureus*. The *S. aureus* strain was isolated from milk products and identified using selective media, biochemical tests, microscopy, and gram staining. The strains can be accessed at the voucher UNI15 in the Microbiology Research Laboratory of the Federal University of Pampa, São Gabriel Campus, Brazil, for future studies and reproducibility tests.

The cup-plate diffusion technique (Rose & Miller 1939) was used to verify the potential of the endophyte extract against the two test microorganisms. The amoxicillin + clavulanate (AMC) (40 mg/mL) antibiotic was used as a positive control and the fermentation medium without fungus was used as a negative control. The tested microorganisms were pre-inoculated in Luria-Bertani (LB) liquid medium (1 g tryptone, 0.5 g yeast extract, and 1 g NaCl to 1,000 mL of distilled water) and incubated at 37 °C for 16 h in the dark. Bacteria were seeded (100 µL) on the surface of Muller Hinton medium (2.0 g meat extract, 17.5 g cassava acids, 1.5 g starch, and 15 g agar to 1,000 mL distilled water). Three 9 mm cup-plates were equidistantly drilled in each Petri dish, and 100 µL of the fungal extract was added at each point. Plates with extracts were incubated for 20 min in a laminar flow cabinet until the culture medium fully absorbed the extract. The plates were incubated at 37 °C for 24 h in the dark. The test was performed in triplicate and the antibacterial activity was evaluated by the formation and measurement of the inhibition halo. The results were considered using the mean values of the three replicates per plate.

The size of the inhibition halo values did not follow a normal distribution (Wickham 2016) and were statistically evaluated using Wilcoxon's nonparametric test (Bauer 1972; Hollander & Wolfe 1973) in the R software (R Development Core Team 2019). Values were compared using the p-value, where $p < 0.05$ indicated that the treatments differed statistically from the control.

Results

Endophytic fungi of Kelissa brasiliensis

In total, 30 endophytic fungi were isolated from 108 plant fragments, with a colonization frequency rate (FC) of 27.7 % (Tab. 1). Fifteen fungi were isolated from leaves (FC = 41.66 %), followed by ten from capsules (FC = 27.77 %) and five from stems (FC = 13.88 %) (Tab. 1). Thirty endophytes isolated were grouped into seven morphospecies according to their morphological characteristics (isolates F6KB, F8KB, F13KB, F14KB, F16KB, F17KB, and F18KB) and subsequently identified at the genus level through ITS rDNA region sequencing.

Phylogenetic analysis (Fig. 2) using the sequences obtained from the endophytes and those from the GenBank database showed that the endophytic fungi belonged to five families of the Sordariomycetes and Dothideomycetes (Ascomycota) classes represented by five genera. Three of the isolates (F6KB, F16KB, and F17KB) were phylogenetically grouped with sequences of *Colletotrichum* (Glomerellaceae), while F16KB and F17KB were grouped as the same species because of the formation of a separate cluster. The remaining isolates were grouped as follows: F18KB with *Pestalotiopsis* (Amphisphaeriaceae), F13KB with *Fusarium* (Nectriaceae), F14KB with *Diaporthe* (Diaporthaceae), and F08KB with *Epicoccum* (Didymelaceae). All genera clustered with significant support values (bootstrap value >95 %). Using the results from morphological grouping species along with phylogenetic analysis, *Colletotrichum* was the most representative genus (15 isolates, PA = 50 %), followed by *Pestalotiopsis* (PA = 33.33 %, ten isolates), and *Diaporthe* (PA = 10 %, three isolates). *Epicoccum* and *Fusarium* were represented by one isolate each.

Antibacterial activity of endophytic fungi

All extracts tested showed activity against *E. coli* and *S. aureus* with inhibition halos varying from 11–32.4 mm (Tab. 2). *Escherichia coli* exhibited the highest sensitivity to endophytic fungi extracts (halos of 18–27.3 mm), and the extract from *Colletotrichum* sp. 2 F17KB presented the greatest activity in the test (27.3 mm) when compared to the control (27.4 mm). The extracts were less efficient against *S. aureus* (halos of 11.1–32.4 mm) when compared to the control (38.2 mm). The most efficient extracts were obtained

Table 1. Endophytic fungi isolated from stem, leave and capsule of *Kelissa brasiliensis*, an endemic Iridaceae species from the Pampa biome in Brazil.

Endophytic fungi		Isolation sample			Genus abundance	Isolation frequency	
Isolates	Identification	Stem	Leaf	Capsule	(PA %)	f	Fr %
					50 %		
F6KB	*Colletotrichum* sp. 1	2	2			4	13.3
F16KB and F17KB	*Colletotrichum* sp. 2		11			11	36.6
					3.33 %		
F8KB	*Epicoccum* sp.	1				1	3.3
					3.33 %		
F13KB	*Fusarium* sp.	1				1	3.3
					10 %		
F14KB	*Diaporthe* sp.	1	2			3	10
					33.33 %		
F18KB	*Pestalotiopsis* sp.			10		10	33.3
Total		5	15	10	100 %	30	
Colonization rate (%)		13.88 %	41.6 %	27.77 %			

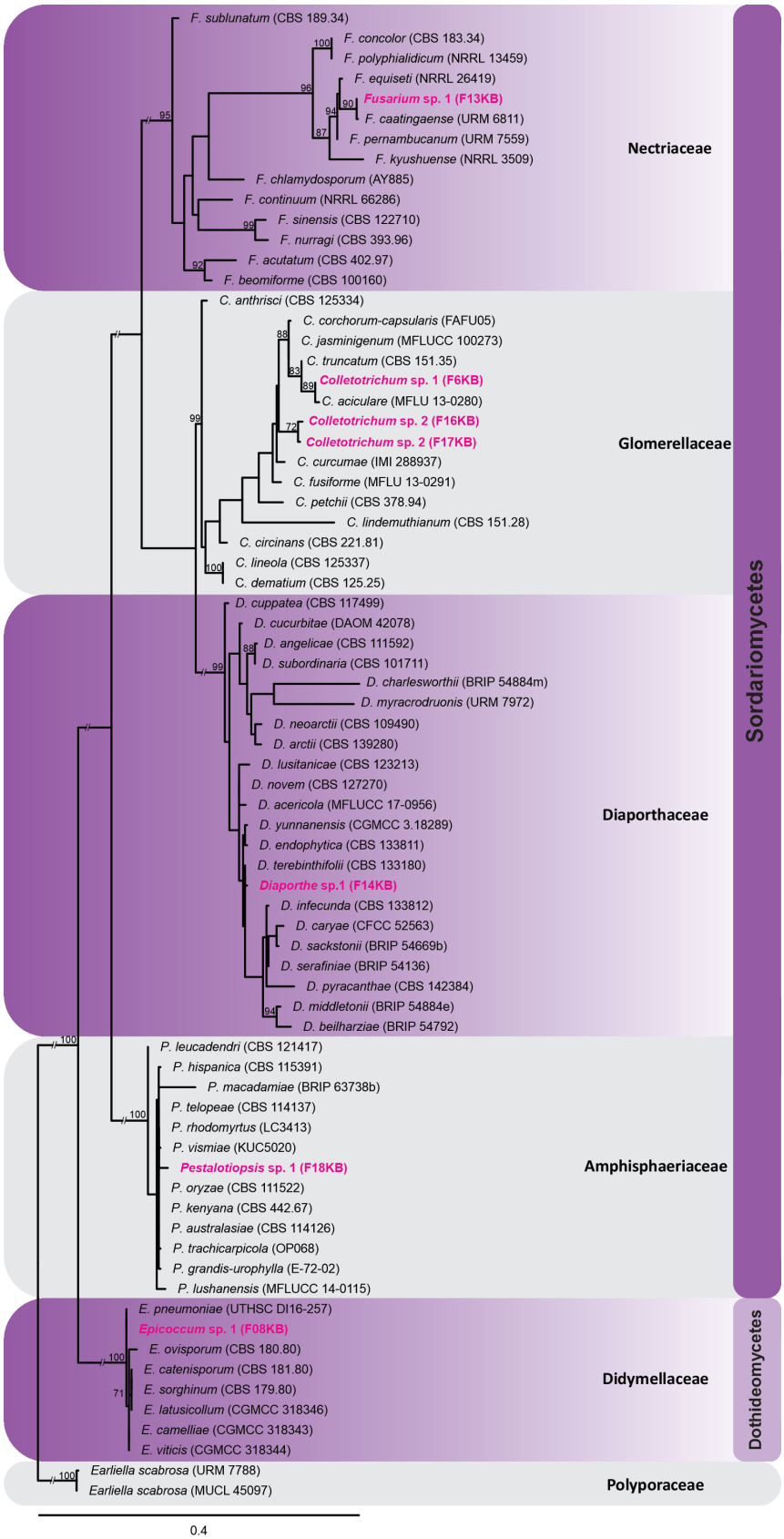

Figure 2. Maximum likelihood (ML) tree using ITS rDNA of endophytic fungi isolated from *K. brasiliensis*. Isolates obtained in our study are in bold and pink. ML bootstrap support values above 70 % are shown near nodes. The best nucleotide model GTR + I + G was used. The tree was rooted to *Earliella scabrosa* (URM 7788 and MUCL45097).

from *Colletotrichum* sp. 2 F17KB (32.4 mm), *Fusarium* sp. F13KB (31.1 mm), and *Pestalotiopsis* sp. F18KB (30.1 mm).

The extracts from *Colletotrichum* sp. 2 F17KB and *Pestalotiopsis* sp. F18KB showed the highest inhibition values against *E. coli* and *S. aureus* (Tab. 2), whereas that of *Epicoccum* sp. F8KB presented the lowest (18.6 and 11 mm) compared to the other tested extracts.

Statistical analysis indicated the highest sensitivity of *E. coli* to endophytic extracts ($p > 0.05$) when compared with the positive control (amoxicillin + clavulanate) (Tab. 2). In contrast, although some extracts had high inhibition values against *S. aureus*, they showed statistical differences from the positive control ($p < 0.05$) (Fig. 3).

Discussion

The Pampa is the only Brazilian biome not mentioned in a survey of studies on endophytic fungi (Bezerra *et al.* 2019; Savi *et al.* 2019). This is the first known study of endophytic fungi from plants of the Pampa biome, revealing the fungal endophyte community associated with *K. brasiliensis* and its antibacterial activity.

The richness of endophytic fungi associated with *K. brasiliensis* is similar to that observed in other two studies of Iridaceae species (Raj *et al.* 2013; Wani *et al.* 2016). We isolated 30 fungal endophytes from 108 fragments and found a colonization rate of 27.7 %. Studies with saffron (*Crocus sativus*, Iridaceae) showed similar colonization rates of endophytic fungi of 21 % (Raj *et al.* 2013) and 29.7 % (Wani *et al.* 2016). The colonization rate in our study was also similar to the isolation rates of several other Brazilian plants, such as two species of medicinal herbs, *Mentha piperita* (40.8 %) and *M. canadensis* (14.6 %) (Herrmann *et al.* 2019); a shrub species of the family Apocynaceae, *Calotropis procera* (32.1 %) (Nascimento *et al.* 2015); and the medicinal plant *Myracroduon urundeuva* (10.43 % in the Caatinga forest and 39.58 % in an upland forest) (Pádua *et al.* 2019).

In our study, leaves showed the highest colonization rate (41.66 %), followed by capsules (27.77 %), and stem (13.88 %). In studies with Iridaceae plants, the most significant number of endophytic isolates were found in corms (Raj *et al.* 2013; Wani *et al.* 2016) although leaves were not used for endophyte isolation. Similar to our study, leaves were the most colonized tissues (colonization rate

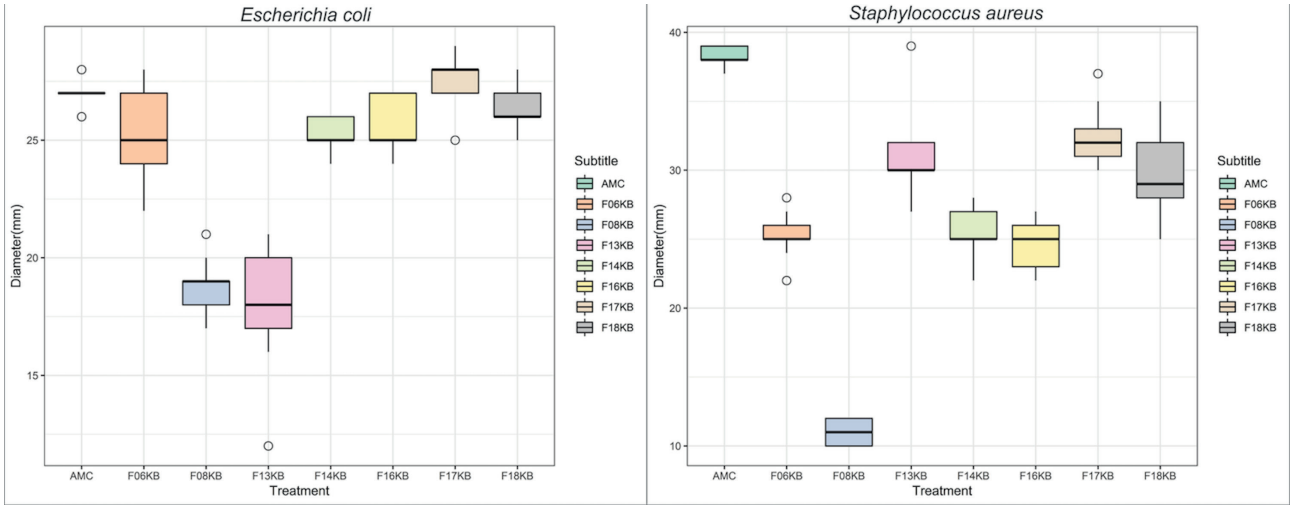

Figure 3. Boxplot whit mean values of the inhibition halos (mm) of endophytic fungi extracts against two bacteria using the Wilcoxon test.

Table 2. Inhibition halos of extracts produced by endophytic fungi isolated from stem, leave and capsule of *Kelissa brasiliensis*, an endemic Iridaceae species from the Pampa biome in Brazil.

Endophytic fungi		*E. coli*		*S. aureus*	
Isolates	Identification	Inhibition halo (mm)	p-value	Inhibition halo (mm)	p-value
F6 KB	*Colletotrichum* sp. 1	25.0	0.12014*	25.2	0.00032
F16 KB	*Colletotrichum* sp. 2	25.6	0.02243	24.5	0.00034
F17 KB		27.3	0.15000*	32.4	0.00040
F8 KB	*Epicoccum* sp.	18.6	0.00030	11.0	0.00030
F13 KB	*Fusarium* sp.	18	0.00031	31.1	0.00034
F14 KB	*Diaporthe* sp.	25.2	0.00067	25.4	0.00033
F18 KB	*Pestalotiopsis* sp.	26.4	0.22114*	30.1	0.00034
AMC (Amoxicillin + Clavulanate)		27.4	-	38.2	-

* Values with statistical significance.

of 50.41 %) of *Lippia sidoides*, a wild medicinal bush found in northeastern Brazil, whereas stems revealed a lesser colonization rate (35.40 %) (Siqueira *et al*. 2011). In another study with *Acmella ciliate*, a small Asteraceae species, Ortiz-Ojeda *et al*. (2020) reported that the leaves were the most colonized plant tissue (40 isolates), followed by the stems (16 isolates).

The 30 endophytes isolated were represented by seven morphospecies and six taxonomic groups, with the *Colletotrichum* (15 isolates) genus being the most abundant (PA 50 %). In contrast, saffron (Iridaceae) showed the greatest abundance with the genus *Rhizoctonia* (27.7 %, 13 isolates) among the six genera observed, followed by *Fusarium* (25.5 %, 12 isolates) (Raj *et al*. 2013). In a similar study with saffron, Wani *et al*. (2016) reported 294 isolates belonging to 19 genera, with *Phialophora* (15 %, 44 isolates) and *Cadophora* (12.9 %, 18 isolates) as the most common. Our results, along with these unique studies of fungal endophytes from Iridaceae species, show that the fungal communities may vary among plant tissues, species of the same family, and biomes.

The endophytic fungal genera found in our study have previously been listed as endophytes in different parts of plants in other biomes (Vieira *et al*. 2014; Hamzah *et al*. 2018; Larran *et al*. 2018; Pádua *et al*. 2019). *Colletotrichum* species are one of the most common endophytes, and are the most abundant in the study of the endophytic community associated with *Carapichea ipecacuanha* (Rubiaceae), a medicinal plant in Brazil, Colombia, and Central America (Ferreira *et al*. 2020), as well as in *Begonia fishcheri* and *B. olsoniae* (Begoniaceae) in the Brazilian Atlantic rainforest (Correia *et al*. 2018).

Endophytic *Pestalotiopsis* isolates were found only in capsules of *K. brasiliensis*, whereas isolates of *Colletotrichum* were obtained only from the fragments of leaves and stems, confirming specificity. According to Petrini *et al*. (1992), different organs and plant tissues comprise specific microenvironments in which the endophytic communities may have a certain distribution in the tissues (*e.g.*, Ganley & Newcomb 2006; Xing *et al*. 2010; Bezerra *et al*. 2015).

In Brazil, *Pestalotiopsis* species have been registered in some geographical regions as saprophytes, phytopathogens, and endophytes (Kruschewsky *et al*. 2014). For example, *Pestalotiopsis oryzae* has not yet been reported in Brazil, but it has been isolated from *Telopea* sp. (Proteaceae), *Paris polyphilla* (Mellathiaceae), *Dysosma versipellis* (Berberidaceae), and rice (*Oryza sativa*) (Poaceae), which together with soybeans, are the largest monocultures in the Pampa region (Sentelhas *et al*. 2015). The genus *Epicoccum*, is a cosmopolitan plant pathogen mainly found on grasses in tropical regions (De Oliveira *et al*. 2018). Some species, including *Paspalum guenoarum* (Poaceae), have previously been reported in the Pampa territory of Brazil (Gasparetto *et al*. 2017; Liu *et al*. 2018; Zeng *et al*. 2018).

All extracts of the endophytic fungi tested had antibacterial potential against *S. aureus* and *E. coli*, six of which showed high inhibition values. The potential of endophytic fungi may be related to the habitat of the host, and new hosts may be a promising source of antibacterial metabolites (Strobel & Daisy 2003; Schulz & Boyle 2005). Raj *et al*. (2013) observed similar results with the strong potential of saffron endophytes to produce bioactive compounds against *E. coli*. In a similar study, the potential of secondary metabolites produced by endophytic fungi from saffron showed antibacterial activity, highlighting the *Rhizopus* ethyl acetate extract, which could inhibit all the bacteria tested (Chamkhi *et al*. 2018). In a survey of endophytic fungi from six plant species of the Cyperaceae family in Sri Lanka, Ratnaweera *et al*. (2018) observed that 91.6 % of the endophytes had metabolites with antibacterial properties, of which 33 % were able to inhibit the growth of *E. coli*.

Studies of endophytic *Colletotrichum*, *Fusarium*, *Epicoccum*, and *Pestalotiopsis* isolates have revealed antibacterial action against *S. aureus* and *E. coli* (Xing *et al*. 2011; Radić & Štrukelj 2012). In our study, the extracts of *Colletotrichum* sp. 2 F17KB and *Pestalotiopsis* sp. F18KB presented the highest growth inhibition values for both microorganisms tested. Endophytic *Colletotrichum* and *Pestalotiopsis* isolates had antibacterial activity against gram-positive and gram-negative bacteria, with extracts showing high inhibition values (95.4 to 100 mm) (Maria *et al*. 2005; Ferreira *et al*. 2015).

Similar to saprophytes and plant pathogens, endophytic microorganisms can exhibit varying degrees of host specificity, ranging from permanent associations with a single plant species to relationships involving a wide range of hosts (Petrini 1996). The host specificity exhibited by endophytic microbes can be complex, and although many strict associations seem to be formed in nature, this can be manipulated somewhat when developing novel endophyte–host associations. Cannon and Simmons (2002) described frequent isolation of identical endophytic strains from plant tissues of several species, which suggests that the extent of host preference/specificity in leaf endophytes is small; therefore, molecular research is being conducted on two key genera (*Pestalotiopsis* and *Colletotrichum*), the same common groups found in our study. The analysis of these factors is beyond the scope of this study but the loss of diversity, especially of endemic species, can result in negative consequences such as the loss of potentially associated organisms (Mommer *et al*. 2018; Chen *et al*. 2019; Li *et al*. 2020). Plant diversity is an essential factor for microbial biomass, and both the diversity and richness of fungi and bacteria are positively affected by the plants found in a given location. When losses in plant diversity are found, microbial biomass immediately decreases (Chen *et al*. 2019).

Our findings in this first study show that Iridaceae endemic to the Pampa biome has endophytic fungi with antibacterial potential, thus highlighting the importance of further studies with these fungal isolates. Endophytic fungi were more abundant in the leaves of the plant, and *Colletotrichum* and *Pestalotiopsis* were the most abundant genera and presented the best inhibition values in antibacterial tests. This survey is fundamental for the study of endophytic fungi in the Pampa biome, providing important information for the development of future studies of endophytic fungal communities in this Brazilian environment.

Acknowledgements

Special thanks to the researchers Rutilene Roll and Andrés Cañedo of the Laboratório de Pesquisa em Microbiologia of UNIPAMPA for the strains of microorganisms used in the experiments of antimicrobial activity given to the first author. This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Finance Code 001) and the Conselho Nacional de Desenvolvimento Científico (grant number 446234/2015-0). All authors thank the Universidade Federal do Pampa for their support in the development of this work.

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