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ENDOPHYTIC FUNGI COMMUNITY IN *Eremanthus erythropappus* TREE FROM ANTHROPOGENIC AND NATURAL AREAS OF MINAS GERAIS

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HIGHLIGHTS

Natural habitat was the area with the highest number of endophytic fungi genera recovered from *E. erythropappus*

Some genera of endophytic fungi from *E. erythropappus* showed antagonism against phytopathogenic fungi

The most of the endophytic fungi isolated from *E. erythropappus* belong to the *Ascomycota phylum*

S. sclerotiorum was the most sensitive phytopathogen inhibited by endophytic fungi isolated from *E. erythropappus*

Keywords:
Antifungal activity
Biological control
Candeia
Cryptosporiopsis

ABSTRACT

It is know that many plants live in symbiosis with microorganisms that can be found on their interior, the endophytes. Environment and tissue type are modulating factors of this community, in which most of these microorganisms produce important antimicrobial molecules and they may be powerful biocontrol agents in agriculture. Thus, the aim of this study was to evaluate the community of *Endophytic fungi* from *Eremanthus erythropappus* in anthropogenic and natural areas (with human interaction, natural habitat and planned planting) of Minas Gerais State, Brazil, through cultivation-based approach and verify their antimicrobial activity against phytopathogenic fungal and pathogenic bacteria. The endophytic fungi isolated were identified by sequencing of the ITS region and subjected an in vitro antagonism test. The antagonisms that show antibiosis were submitted to tests on split plates to verify the volatile compound production. In the pairing testes, the endophytic fungi of the genera *Cryptosporiopsis, Diaporthe, Xylaria, Paraconiothyrium* and *Camarosporium* presented antibiosis against phytopathogenic fungi by releasing compounds in the medium. To our knowledge, this paper is the first report on the isolation of twelve genera fungi in *E. erythropappus* besides verifying their antagonist capacity, which opens the way for discovery of bioactive substances produced by endophytic fungi that inhibit pathogens.

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INTRODUCTION

Endophytic or endophytes are a group of highly diverse fungi that live inside plant tissues including leaves, petioles, stems, twigs, bark, root, fruit, flower and seeds for at least part of their life cycle without causing any immediate overt disease symptoms in their host (Hyde and Soytong, 2008). Once inside their host plant the relationship between them may range from latent phytopathogenesis to mutualistic symbiosis for a determinate period or the whole lifetime of the infected plant tissue (Rodriguez and Redman, 2008).

Endophytic fungi constitute a part of the unexplored fungal diversity and thus represent a new source for obtaining bioactive natural products with different potentialities not only in medical but extended to agricultural and industrial application (Omeje et al., 2017). In addition, these products exclusive of those to their host plants are important to increase the adaptability of both, such as the tolerances to biotic and abiotic stresses and they have proved to be effective in blocking the growth of various groups of plant pathogens, similar to biological control agents (Terhonen et al., 2016).

It is worth mentioning that, all plant species that exist in the earth is host to one or more endophytes (Dutta et al., 2014). The choice of plants for the isolating of endophytic fungi in search of potential bioactive compounds is an opportunities to found veritable sources of novel bioactive natural products. Biological biodiversity implies chemical biodiversity and tropical forests are excellent source of novel molecular structures and biologically active compounds (Redell and Gordon, 2000; Rajamanikyam et al., 2017; Sudha et al., 2016).

Genus Eremanthus (Candeia) belongs to the family Asteraceae and includes 22 species (Scolforo et al., 2012; Araújo et al. 2018). Among the species, *E. erythropappus* (DC.) is used for the production of fence posts and extraction of essential oil to produce alpha-bisabolol (Macleish, 1987; Oliveira et al. 2009). Most of the essential oil produced is exported and used by cosmetics and pharmaceutical industries in European countries (Barbieri and Borsotto, 2018). Alpha-bisabolol holds antiphlogistic, antifungal and dermatological properties and several derived products as creams, sunscreen, lotions, and medications, products for baby and adult skincare, among others have a high market demand (De Lucca et al., 2011, Araujo et al., 2018).

E. erythropappus is distributed in rocky fields of the interior plateau of the Center-West (Goiás and Brasília), Southeastern (states of Minas Gerais, Espírito Santo, Rio de Janeiro and São Paulo), in the middle of the secondary forest in coastal strips and in the Cerrado (Brazilian savanna) of Brazil (Macleish, 1987; Loeuille et al., 2012). The wood of *E. erythropappus* can be harvested from native areas or commercial plantations and the first work on modeling *E. erythropappus* was performed by Scolforo et al. (2004) in native fragments in the Aiuruoca region, Minas Gerais. Magalhães et al. (2008) showed for the first time the interaction of endophytic fungi with tree *E. erythropappus* from Park of Boqueirao, Ingaí – MG. It was observed that fungi belonging to *Xylaria* and *Phomopsis* genera were found in all organs sampled. The genera *Alternaria* and *Fusarium* demonstrated specificity in seed, *Nigrospora* and *Aspergillus* in leaf and *Dothiorella* in stem. This was the only work involving the isolation of endophytic fungi from tree *Eremanthus* and little is known about the biology and ecology of fungal endophytes that colonize this plant. Therefore, the aim of this study was to isolate and identify fungal endophytes survived inside the *E. erythropappus* from Aiuruoca and Bocaina de Minas region, Minas Gerais, Brazil, and to evaluate their ability for antimicrobial activity against some pathogenic bacteria and fungi.

MATERIAL AND METHODS

Plant material

Samples were collected from *E. erythropappus* from the Environmental Preservation Area of Serra da Mantiqueira, in three sites in the cities of Aiuruoca and Bocaina de Minas. At each site, the sampling was made from three trees from an area with human interaction (Area 1), other of natural habitat (Area 2) and planned planting (Area 3) (Figure 1).

The bark sample was performed at chest height with extraction of material 1 cm thick. Healthy leaves and seeds were also collected from the trees. All samples were transported to the Laboratório de Bioprospecção e Genética de Fungos Filamentosos (BIOGEN) at the Universidade Federal de Lavras (UFLA), Brazil, in plastic bag at 7°C inside a cool box.

Endophytic fungi isolation

Leaves and bark were washed under running tap water. Surface disinfection was done by treating the sample with sterile water (1 min), 70% ethanol (1 min), 2.5 % sodium hypochlorite (3 min), and sterile water three times, followed by drying on sterile filter paper. Approximately 100 μL of the last water wash was plated on PDA (Potato Dextrose Agar) and incubated at 25 °C,

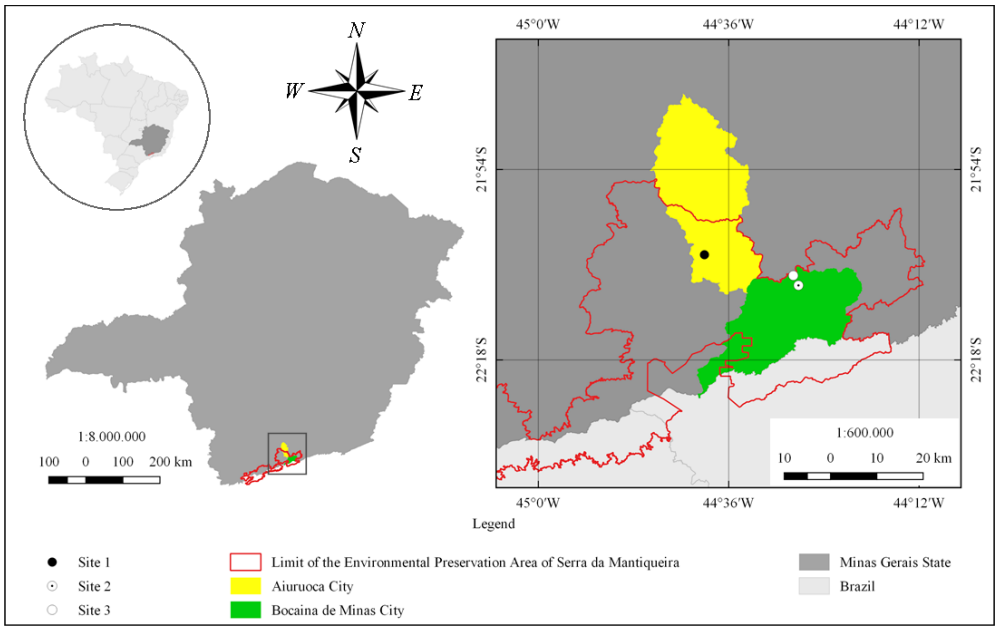

FIGURE 1 Map showing the sampling sites, created on the QGIS software version 2.18 utilizing the Geographic Coordinate Reference System and Datum SIRGAS 2000 from data of Instituto Brasileiro de Geografia e Estatística (IBGE)..

as a sterilization control. Disinfected tissues were cut into 0.5-cm fragments and 5 fragments were plated on PDA plates containing cefotaxime (250 $\mu g \cdot mL^{-1}$) totaling 135 fragments of each tissue. For eliminating microorganisms from the surface, the seeds were rinsed in distilled water, soaked in sodium hypochlorite 5% (2 min), rinsed in ethanol 70% (2 min) and in autoclaved Milli-Q water three times. The seeds were dried on sterile filter paper for 15 minutes and five seeds were arranged on Petri dishes containing PDA/Cefotaxime medium. Following incubation at 25 °C, plates were checked regularly and fungal colonies emerging from the margins of sectioned tissues were subcultured onto PDA. Purified isolates were stored long-term in sterile microtubes containing sterile water, and kept at 4 °C. Initial grouping of fungal isolates into morphotypes, and their identification to the genus level were based on colony appearance, mycelium color, and structures of conidiomata, conidiophore, and conidia (size, color shape, ornamentation, etc.).

Molecular identification

Molecular identification of isolated fungi was made by sequencing the ITS (internal transcribed spacer) region from rDNA. The total DNA was extracted using *Wizard Genomic DNA Purification Kit* (PROMEGA) protocol. Amplifications of ITS was carried out in 30 μL reactions containing 15 μL Qiagen *Taq* PCR Master Mix kit, 12 μL of H_2O, 1 μL of each primer (10 pmol) ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), and 1 μL of genomic DNA at 10 ng.(μL)$^{-1}$. The reaction condition was 2 min at 95 °C, followed by 35 cycles of 30 sec of denaturation at 94 °C, 30 sec of primer annealing at 58 °C, 1 min of extension at 72 °C, with a final elongation of 7 min at 72 °C. Amplifications were performed in a Programmable Thermal Controller-100, (MJ Research, Inc) thermocycler. The PCR products were purified and sequenced at Macrogen (South Korea). Consensus sequences were assembled and edited using the software Sequencher 5.4 and compared against the GenBank database through BLAST searches using the Mega 6 software (Tamura et al., 2013). The closest hit sequences were checked for their authenticity and used as references for molecular identification of endophytic isolates.

Antibacterial activity of supernatant

The fungal supernatant was tested for antibacterial activity by the agar diffusion method described by Wikler (2006) with modifications. The bacteria provided by the Food Microbiology Laboratory (Departamento de Ciência dos Alimentos – DCA/UFLA) were *Escherichia coli* ATCC 3540, *Staphylococcus aureus* GL 5674, *Listeria monocytogenes* ATCC 19117 and *Salmonela enterica* Enteritidis S64. The bacteria were grown at 37 °C in 10 mL of TSB (Tryptone Soya Broth) overnight and transferred to 10 ml of saline solution until reaching a turbidity of 0.5 McFarland standard solution with a concentration of 10^8 CFU mL^{-1}. Then, 0.2 mL of the cultures were inoculated

on plates with TSA (Tryptone Soya Agar) medium. Paper disks with 5 μL of the endophytic fungi supernatant were placed over medium seeded with bacterial cultures. The plates with bacteria were incubated at 37 °C for 16 to 18 hours. After this period, the inhibition zone formations were observed. The negative control was 5 μL PDB (Potato Dextrose Broth) and the positive control was 5 μL of Chloramphenicol at concentration of 30 $\mu g \cdot mL^{-1}$.

Antifungal activity

The phytopathogenic fungi tested in the antagonism bioassays were *Fusarium solani*, *F. oxysporum*, *Sclerotinia sclerotiorum*, *Colletotrichum lindemuthianum* and *Phytophthora* sp. The fungi are deposited in the Coleção Micológica de Lavras (CML) at the Departamento de Fitopatologia at the Universidade Federal de Lavras, Brazil.

The endophytic and phytopathogenic fungi were cultivated for seven days at 25 °C on PDA medium. Mycelial disks (5 mm) of each endophyte were transferred to one side of a PDA plate. After seven days of incubation, 5 mm mycelial disks of plant pathogens were inoculated onto opposite sides of the plates containing the endophytes. Control plates contained only the pathogens inoculated. The bioassay was also performed on Petri dishes divided into two partitions to verify if those endophytic fungi that inhibited the growth of phytopathogenic fungi in the first test were producing bioactive volatile compounds instead of, or in addition to, compounds secreted in the culture medium (Strobel et al., 2001). Tests were performed in triplicate.

Furthermore, the antagonism interaction observed between endophytic fungi and plant fungi pathogens were separated into three classes: (I) Competition, inhibition by mycelial contact without growth over the phytopathogen; (II) Mycoparasitism, mycelial growth over phytopathogen colony; and (III) Antibiosis, diffusion of antimicrobial compound produced by the endophytic, forming an inhibition zone.

Statistical analysis

The fungal diversity of each area was calculated using the Fisher's Alpha test. In addition, from the contingency table we calculated the Pearson chi-square test. To perform all analyzes, the RStudio 3.4.4 program was used.

RESULTS

Endophytic fungi isolation

A total of 103 endophytic fungi were isolated from samples of *E. erythropappus*: 58 isolates were recovered from bark, 32 from seed and 13 from leaf (Table 1 and Figure 2b). All fungi were molecularly identified totaling 17 different genera (Table 2). *Cryptosporiopsis* sp. (21.3 %), *Diaporthe* sp. (15.5 %), *Xylaria* sp. (14.5 %) and *Camarosporium* sp. (13.5 %) were the endophytic fungi more abundant, accounting for 65 % of all isolates (Table 2). Moreover, except the endophyte *Xylaria* sp., all showed specificity for some *E. erythropappus* tissue.

TABLE 1 Total number of endophytic fungi isolated from *E. erythropappus* by area and tissue sampled and Chi-squared values obtained from comparisons of frequencies of endophytic recovered from tissue by area.

Areas	N° of isolates / tissue			Total	No. of genera	Alpha Fisher	Chi-square	df
	bark	seed	leaf					
Natural habitat	52	9	4	65	13	6.113		4
Human interaction	5	13	7	25	9	6.177	*	4
Planned planting	1	10	2	13	6	4.322		4
Total	58	32	13	103	28	-	-	-

*Show significant different al level of 0.05 with p-value = 9.248×10^{-9}.

Natural habitat showed the highest number of isolates and genera recovered, with 65 isolates and 13 genera of endophytic fungi recovered. In the area of human interaction 25 isolates and nine genera were obtained, while at planned planting 13 isolates and six genera were obtained (Figure 2a). In the areas of human interaction and planned planting, the tissue with the highest amount of endophytic fungi was the seed, with 13 (52 %) and 10 (76.9 %) fungal isolates, respectively, while in natural habitat the bark had the highest isolation, with 52 (80.0 %) of the endophytic fungi obtained (Figure 2b). Specifically, the leaf presented greater genera diversity in the area human interaction (5 genera), the bark in the area natural habitat (13 genera) and seed equally in the areas natural habitat e planed planting (4 genera) (Figure 2c).

Chi-squared value obtained from comparisons of frequencies of endophytic recovered from tissues of *E. erythropappus* in different areas showed a significant difference ($X^2 = 43.235$, $p < 0.05$). Therefore, the test showed that this frequency is significantly different among the areas sampled, in which natural habitat showed the highest frequency of recovered isolates in a greater variety of genera. Similarly, the Fisher's Alpha diversity test presents lower diversity for the planned plantation area, as expected, while the values found for natural habitat and human interaction areas suggest that the two areas present similar diversity, since the Fisher's Alpha test considers the number of genera found, which genera and their respective number of endophytic fungi recovered.

Except the endophyte *Acremonium* sp., all the fungi isolates from bark have occurred in the area 2, differently from isolates of leaf and seed tissues, both with greater

TABLE 2 Molecular identification of endophytic fungi recovered from *E. erythropappus* based on ITS rDNA analysis, number of isolates and occurrence areas by host tissue and relative frequency of isolation.

Molecular identification	Number of isolates [occurrence areas] by tissue type			Relative frequency (%)[a]
	Seed	Leaf	Bark	
Cryptosporiopsis sp.	nf	nf	22 [1/2]	21.36
Diaporthe sp.	16 [1/2]	nf	nf	15.54
Xylaria sp.	nf	7 [1/2/3]	8 [2]	14.56
Camarosporium sp.	nf	nf	14 [2/3]	13.59
Cladosporium cladosporioides	7 [1/2/3]	nf	1 [2]	7.77
Not identified	2 [1/2/3]	nf	4 [1/2]	5.83
Alternaria alternata	3 [3]	2 [1]	1 [2]	5.83
Periconia sp.	nf	1 [1]	1 [2]	1.94
Xylariaceae	nf	1 [2]	1 [2]	1.94
Paraconiothyrium sp.	1 [2]	nf	1 [2]	1.94
Peniophora sp.	1 [3]	nf	1 [2]	1.94
Epicoccum nigrum	1 [2]	nf	1 [2]	1.94
Pleosporales	nf	nf	1 [2]	0.97
Trametes villosa	nf	nf	1 [2]	0.97
Acremonium sp.	nf	nf	1 [1]	0.97
Anthostomella sp.	nf	1 [1]	nf	0.97
Muscodor sp.	nf	1 [1]	nf	0.97
Coprinellus radians	1 [3]	nf	nf	0.97
Total	32	13	58	100.00

[a]Relative frequency was calculated as the number of identified isolates of a specie divided by the total number of endophytic fungi isolates (32 + 13 + 58 = 103); bIsolation areas: human interaction [1], natural habitat [2] and planned planting [3]. nf = not found.

occurrence in the areas 1 and 3, respectively (Table 1). Most of endophytic fungal isolates (58 fungi, 56.3 %) were present in bark samples. *Camarosporium* sp., Pleosporales, *Trametes villosa*, *Acremonium* sp. and *Cryptosporiopsis* sp. showed specificity for this plant tissue.

Antibacterial activity of supernatant

The tests that evaluate the potential inhibition of supernatants of endophytic fungi against pathogenic bacteria showed no inhibition of *E. coli*, *S. aureus*, *L. monocytogenes* and *S. enteritidis*.

Antifungal activity

Endophytic fungi were also tested against five plant pathogens. Figure 3 shows the interaction classes observed between endophytic and phytopathogenic fungi. The antagonisms that show an inhibition halo were tested for volatile compounds production and exhibited negative results indicating that the inhibitory compounds are released into the culture medium.

Class I, the most frequent accounting for 72.24% of the results, showed inhibition of pathogenic fungi by competition for space and/or nutrients. In Class II, *Trametes villosa* did not overgrow only the colony of *Fusarium oxysporum*, but also others phytopathogens. The results of antibiosis antagonism (Class III), that includes all antagonism tests that resulted in the inhibition halo, are shown in Table 3. In total, 94 endophytic fungi isolates with antagonistic action against phytopathogenic fungi were observed. *S. sclerotiorum* was the most sensitive phytopathogen with inhibition ranging from 58.7 to 93.7 % (five genera of endophytic were antagonists to *S. sclerotiorum*). However, greater diversity of antagonists affected the *C. lindemuthianum* (10 genera of antagonists) while only two endophytic fungi reduced the growth of *Phytophthora* sp. (4.9 to 26 % of inhibition).

DISCUSSION

Natural habitat showed the highest number of isolates and genera, this result is associated to the diversity of vegetation that, consequently, implies in the greater diversity of the endophytic microbial community,

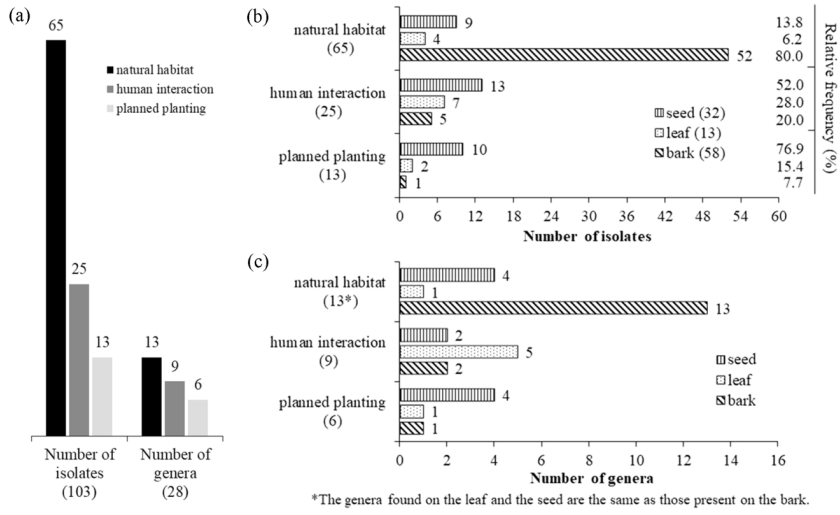

FIGURE 2 (a) Total number of isolates and genera of fungal endophytes recovered from *E. erythropappus* in function of the different areas studied. Number and relative frequency of (b) isolates and number of (c) endophytic fungi genera from different tissues in function of three areas studied: human interaction, natural habitat and planned planting.

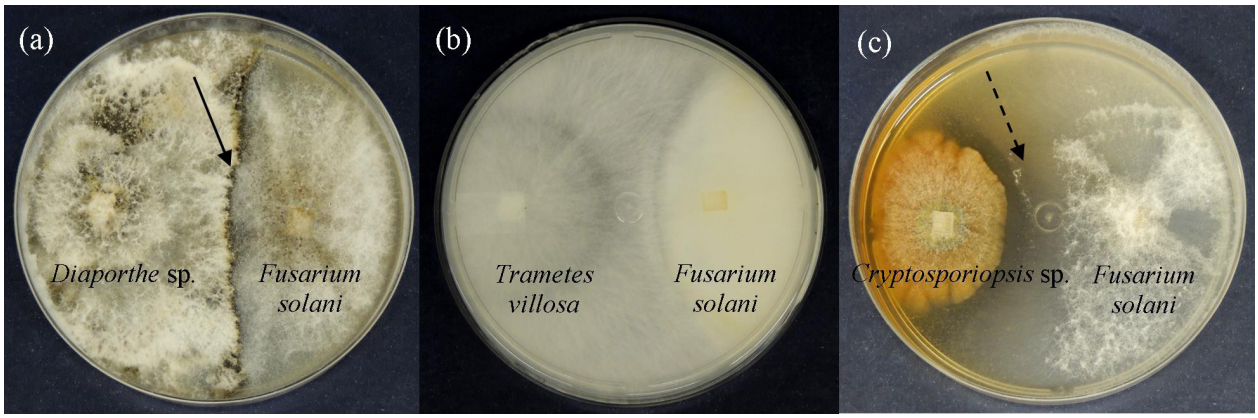

FIGURE 3 Endophytic fungi are on the left and the phytopathogenic fungi are on the right side. (a) Class I – Competition; (b) Class II – Mycoparasitism and (c) Class III – Antibiosis, inhibition zone formed. Continuous arrows show deadlock with mycelial contact and dashed arrows the zones of inhibition.

as well as increases the incidence of infections (Arnold, 2007; Arnold and Lutzoni, 2007). Except the endophyte *Acremonium* sp., all the fungi isolates from bark have occurred in the area 2. This result demonstrates the environment-tissue relationship influencing the endemic endophytic community of *E. erythropappus*.

According to the result of a planned planting area with lower diversity of endophytic fungi when compared to the diversity of other areas and can be explained by the homogeneity of the vegetation (Pádua et al., 2019). Meanwhile, the areas of natural habitat and human interaction showed close diversity value, suggesting that the heterogeneity of the vegetation of both environments result in the diversification of the endophytic fungi found (Padua et al., 2019).

However more relevant than the difference in diversity is the difference in the constitution of the microbial community (Rampelotto et al., 2013). The values of alpha Fisher can be considered relatively high when compared with other similar studies (Pawlowska et al., 2014; Bononi et al., 2018, Padua et al., 2019). Besides, some authors have demonstrated that fungal endophyte community may be influenced by diverse biotic and abiotic factors, such as the type of plant tissues; heterogeneous profile of microhabitats; and different substrates, climate and vegetation changes (Nascimento et al., 2015, Koide et al., 2017).

It is known that the largest amount of secondary metabolites, for example alpha-bisabolol, is present in the stem, which suggests that the community living in the stem is favored by the protective action of these metabolites (De Lucca et al., 2011). Moreover, endophytic fungi exhibit tissue specificity because of their adaptation to different physiological conditions in plants (Dutta et al., 2014). However, we also found endophytes generalists such as *Alternaria alternata* with occurrence in all tissues sampled.

Magalhães et al. (2008) reported the isolation of 159 endophytic fungi from *E. erythropappus* distributed in eight genera. However, our study identified fifteen genera, twelve of which were not described before: *Acremonium*, *Anthostomella*, *Camarosporium*, *Coprinellus*, *Cryptosporiopsis*, *Diaporthe*, *Epicoccum*, *Muscodor*, *Paraconiothyrium*, *Peniophora*, *Periconia* and *Trametes*. This fact probably occurred because the sampling were carried out in

TABLE 3 Endophytic fungi and number of isolates that showed antagonism by antibiosis against phytopathogenic fungi

Antagonist endophytic fungi	Number of isolates	Phytopathogenic fungi that have undergone antagonism and inhibition variation (%)[a]				
		Colletotrichum lindemuthianum	*Fusarium solani*	*Fusarium oxysporum*	*Sclerotinia sclerotiorum*	*Phytophthora* sp.
Cryptosporiopsis sp.	22	15 (31.8-54.6)	16 (52.3-67.9)	19 (56.9-72.4)	1 (58.7)	1 (26)
Diaporthe sp.	16	7 (20.5-81.8)	ne	ne	3 (62.7-93.7)	1 (4.9)
Xylaria sp.	15	2 (18.2-68.2)	3 (55.1-64.2)	ne	1 (69.8)	ne
Camarosporium sp.	14	4 (18.2-36.6)	4 (50.5-58.7)	ne	ne	ne
Cladosporium sp.	8	3 (22.7-34.1)	ne	ne	ne	ne
Not Identified	6	4 (31.1-70.5)	3 (58.7-64.2)	2 (66.4)	2 (61.1-87.3)	ne
Alternaria sp.	6	1 (54.6)	ne	1 (71.6)	1 (72.2)	ne
Epicoccum nigrum	2	2 (56.8-65.9)	2 (61.5-71.6)	2 (70.7-73.3)	ne	ne
Paraconiothyrium sp.	2	ne	1 (49.5)	1 (45.7)	ne	ne
Acremonium sp.	1	1 (10)	ne	ne	ne	ne
Anthostomella sp.	1	1 (9.1)	1 (55.1)	1 (62.1)	ne	ne
Pleosporales	1	ne	1 (56.7)	ne	ne	ne
Total (min-max)[b]	94	40 (9.1-81.8)	31 (49.5-67.9)	26 (45.7-73.3)	8 (58.7-93.7)	2 (4.9-26)

[a]Inhibition percentage was calculated by equation ([dmc – dma / dmc] *100), in which dmc = average diameter of the phytopathogen colony alone and dma = average diameter of the phytopathogen colony paired with the antagonist; [b]min = minimum and max = maximum percentage inhibition observed. ne = no effect.

different places, since we collected in the Mata Atlântica while Magalhães et al. (2008) collected in the Cerrado, two different biomes. Thus, those twelve different genera are reported for the first time in *E. erythropappus*.

Among the endophytic fungi isolates, some are described in the literature for presenting interesting biotechnological features. The genus *Xylaria* sp. is known for the production of secondary compounds that inhibit tumor cells and various microorganisms such as bacteria, protozoa, yeasts and filamentous fungi (Chen et al, 2011; Jang et al, 2007; Tansuwan et al., 2007; Jiménez-Romero et al, 2008), in addition to possessing an anti-inflammatory effect (Ko et al, 2011).

The genus *Muscodor,* also found in our study, is known as a producer of mixtures of volatile organic compounds, which inhibit growth of a wide variety of pathogenic fungi and bacteria, as well as some nematode and arthropod species (Strobel et al, 2001). While most of the isolates are Ascomycota, the species *Coprinellus radians* and *Trametes villosa* and the genera *Peniophora* spp. are from the phylum Basidiomycota. *T. villosa* and *Peniophora* spp. have been reported as producing important enzymes such as laccase (Niku-Paavola et al., 2004), enzyme used in industrial applications, including bioremediation, clarification of wine, ethanol production analysis and biosensors construction (Sigoillot et al., 2004).

Moreover, other endophytic genera found are known as producers of compounds and enzymes important in the pharmaceutical and agronomic industry. Among them, *Acremonium* sp., a producer of cephalosporin C (Hu et al., 2015); *Alternaria* sp., producer of mycotoxins in cereals and fruits (López, et al., 2016); *Cladosporium* sp., which produces antimicrobial compounds (Ding et al., 2008); *Coprinellus radians* that releases enzymes with peroxidase action (Aranda et al., 2009); *Cryptosporiopsis* includes species that produce antibiotics and herbicides (Schulz et al., 2002); *Diaporthe* sp. produces antibiotic (Lin et al. 2005) and anticancer compounds (Kumaran and Hur, 2009); *Periconia* sp. produces alkaloids (Verma, 2011) and *Paraconiothyrium* sp. releases Brefeldin A with antifungal, antiviral and anticancer properties (Khan et al, 2012). *Paraconiothyrium minitans* is a known mycoparasite capable of controlling plant diseases caused by fungal pathogens, including *S. sclerotiorum* (Whipps et al., 2008). Almeida et al. (2014) report the isolation of graminin B, compound with antibiotic activity obtained from fermentation broths of species *P. hawaiensis*.

No isolated endophytic fungus presented antibacterial activity. Previous studies reported that the extract of endophytic fungi belonging to genera identified in our study inhibited *E. coli, S. aureus* and *S. enteritidis* (Vieira et al., 2012; Xing et al., 2011; Li et al., 2015; Sorres et al., 2015; Yue et al., 2015; Hu et al., 2015; Kurzatkowski and Gębska-kuczerowska, 2015). However, those endophytic fungi were not isolated from *E. erythropappus*. Furthermore, extracts used in the previous studies probably present higher bioactive compound concentrations than those present in the supernatant we used.

The interaction classes competition, mycoparasitism and antibiosis were observed between endophytic and phytopathogenic fungi. According to Pal and Gardener (2006), promising results for inhibiting pathogens in the field are mycoparasitism and antibiosis. However, competition may represent an important mechanism of action in the endophytic interaction of the plant microbiome.

Fungi of the phylum Basidiomycota, such as *T. villosa*, class II, are not commonly reported as endophytic and mycoparasite at the same time. Thus, a more complete study of *T. villosa* would be interesting in order to discover their biocontrol potential. In addition, studies with Basidiomycetes have increased considerably because of its ability to produce biotechnological compounds used in pharmacology and agriculture (De silva et al., 2013).

Some of the endophytic genera isolated from *E. erythropappus* are not commonly reported in biological control studies, such as *Cryptosporiopsis,* which inhibited the growth of *F. solani*, *F. oxysporum*, *C. lindemuthianum*, *S. sclerotiorum* and *Phytophthora* sp, especially an endophytic isolate of *Cryptosporiopsis* sp. that presented antibiosis against all phytopathogens, except *S. sclerotiorum*. Some species have been found as pathogen and endophyte and can produce secondary metabolites with antibacterial, antifungal and herbicidal activity (Schulz et al., 2002; Strobel et al., 1999). Among these metabolites, Strobel et al. (1999) described cryptocandin A from *Cryptosporiopsis* cf. *quercina*, which inhibits *Trichophyton* spp., *S. sclerotiorum*, *Candida albicans*, *Histoplasma capsulatum* and *Botrytis cinerea*. Li et al. (2000) also reported the production of cryptocin by this fungus, with antifungal activity against plant pathogens, such as *Pyricularia oryzae*. Thus, fungi of this genus may be candidates for more detailed studies on biological control.

Moreover, in our study two strains of *E. nigrum* showed antimycotic activity against *F. solani, F. oxysporum* and *C. lindemuthianum*. In previous studies *E. nigrum* was reported as having efficient control of brown rot in peach and nectarine postharvest (Larena et al., 2005; Mari et al., 2007), whereas *Diaporthe* sp. also presented inhibition of *S. sclerotiorum, C. lindemuthianum* and *Phytophthora* sp.

Interestingly, *Diaporthe* is a teleomorph of *Phomopsis*, which was reported as a producer of enzymes and secondary metabolites (Kobayashi et al. 2003; Dai et al. 2005). *Diaporthe* sp. was also reported as a producer of phytotoxic and mycoherbicide compounds (Andolfi et al., 2015).

In our study, the endophytic genus *Xylaria* sp. inhibit *F. solani*, *S. sclerotiorum* and *C. lindemuthianum*. Previous work with compounds produced by *Xylaria* species demonstrated that the secondary metabolites released by this fungus have significant antifungal activity against pathogens such as *F. solani*, *Alternaria solani*, *B. cinerea*, *Gibberella saubinetti*, *Phytium ultimun*, *Magnaporte grisea*, *Aspergillus niger*, *Alternaria panax*, *F. oxysporum*, *Phytophthora capsici*, *Alternaria mali*, *A. porri*, *Rhizoctonia solani*, *Fulvia fulva* and *Cylindrocarpon destructans* (Zhang et al. 2014; Baraban et al. 2013; Jang et al., 2007). Such reports suggest that the compounds produced by the genus *Xylaria* should be further studied to optimize their biocontrol activity also demonstrated herein.

Some endophytes of *E. erythropappus* were identified as belonging to the genus *Alternaria*. Kumar et al. (2011) also found *Alternaria* species as endophytic from *Tylophora indica*. They reported that *A. tenuissima* and *Alternaria* sp. showed activity against both *S. sclerotiorum* and *F. oxysporum*, which corroborates our results, in addition to *C. lindemuthianum*.

This study is one of the few reporting antifungal activity of some genera isolated from *E. erythropappus* such as *Camarosporium*, *Anthostomella*, *Acremonium* and *Paraconiothyrium* with potential for phytopathogenic fungus biocontrol opening the possibility of discovering new bioactive natural products with different potentialities in medical, agricultural and industrial application.

CONCLUSIONS

This work described, for the first time, the isolation of the endophytic fungi *Acremonium*, *Anthostomella*, *Camarosporium*, *Coprinellus*, *Cryptosporiopsis*, *Diaporthe*, *Epicoccum*, *Paraconiothyrium* and *Trametes* from *E. erythropappus* with occurrence in all tissue sampled as bark, seed and leaf and some isolates tissue-specific. The supernatant of endophytic fungi isolates did not inhibit the growth of the pathogenic bacteria. However, some isolates, such as *Cryptosporiopsis* sp. showed high inhibition capacity of the majority of the phytopathogens evaluated. Furthermore, this work is the first describing the antimicrobial activity of endophytic fungi present in *E. erythropappus* showing three classes of interaction against phytopathogenic fungi: competition; mycoparasitism and antibiosis. New studies should be performed to characterize the bioactive metabolites of the endophytic fungi isolated from *E. erythropappus*.

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