



***In silico* analysis of the biodiversity of endophytic fungi isolated from the Brazilian Cerrado**

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Abstract: Endophytic microorganisms are those that inhabit the interior of plant tissues and organs without causing damage to the plant, triggering a mutualistic interaction. These endophytes are known to produce compounds with various applications in the areas of biotechnology, pharmaceutical industry and agriculture; therefore, molecular methods are used to investigate the phylogeny of these organisms. The Brazilian Cerrado is the second largest biome in Brazil and is considered a hotspot, with a high diversity of endemic fauna and flora. The objective of this work was to analyze, through *in silico* analysis, the phylogeny of cultivable endophytic fungi isolated from plants found in the Brazilian Cerrado biome. A search was carried out for articles that worked with endophytes in the period between 2012 and 2022. The dendrogram was generated using the Neighbor-joining method based on the ITS1-5.8S-ITS2 conserved region obtained by GenBank codes of isolated endophytes. The genera *Xylaria*, *Diaporthe*, and *Colletotrichum* were isolated in more than three plants in the seven articles found. Most of the articles found related to the biodiversity of endophytic fungi from the Brazilian Cerrado aim at the bioprospecting of bioactive compounds, through culture-dependent methods; as such, a part of endophytic diversity may be lost due to the inability of certain endophytes to grow in artificial media. *In silico* analysis can assist in the investigation of phylogenetic relationships between endophytic fungi and has the potential to guide future work aimed at prospecting for bioactive compounds, phylogenetic identification, or biodiversity of this group of endophytes.

Keywords: *Ascomycota*; *endophytes*; *Dendrogram*; *ITS*.

Análise *in silico* da biodiversidade de fungos endofíticos isolados do Cerrado brasileiro

Resumo: Microrganismos endofíticos são aqueles que vivem no interior de tecidos e órgãos vegetais sem causar dano à planta, desencadeando uma interação mutualística. Esses endófitos são conhecidos por produzir compostos com diversas aplicações nas áreas de biotecnologia, indústria farmacêutica e agricultura; logo, métodos moleculares são utilizados para investigar a filogenia destes organismos. O Cerrado brasileiro é o segundo maior bioma do Brasil e é considerado um *hotspot*, com uma alta diversidade de fauna e flora endêmica. O objetivo deste trabalho foi analisar, por meio da análise *in silico*, a filogenia de fungos endofíticos cultiváveis isolados de plantas encontradas no bioma Cerrado brasileiro. Foi realizada uma busca por artigos que trabalharam com endófitos no período entre 2012 e 2022. O dendrograma foi gerado usando o método Neighbor-joining baseado na região conservada ITS1-5.8S-ITS2 obtida pelos códigos GenBank de endófitos isolados. Os gêneros *Xylaria*, *Diaporthe* e *Colletotrichum* foram isolados em mais de três plantas nos sete artigos encontrados. A maior parte dos artigos encontrados relacionados à biodiversidade de fungos endofíticos do Cerrado brasileiro visam à bioprospeção de compostos bioativos, através de métodos dependentes de cultura; como tal, uma parte da diversidade endofítica pode ser

perdida devido à incapacidade de certos endófitos crescerem em meios artificiais. A análise *in silico* pode auxiliar na investigação das relações filogenéticas entre fungos endofíticos e tem potencial para orientar trabalhos futuros voltados à prospecção de compostos bioativos, identificação filogenética ou biodiversidade deste grupo de endófitos.

Palavras-chave: *Ascomycota*; endófitos; *Dendrograma*; ITS.

Introduction

Endophytic microorganisms, mainly fungi and bacteria, are organisms that inhabit the interior of plant tissues and organs without causing any damage to their hosts. Endophytes can remain inside plants throughout their life cycle or during a phase of their development, occurring especially in the aerial parts of hosts without causing nodules or other external alterations. On the contrary, plant-endophyte interactions usually promote advantages to the host, such as modifications of plant physiology and protection against herbivores and phytopathogenic microorganisms (Azevedo et al. 2002, Peixoto et al. 2004, Verma et al. 2021).

As a result of the need to invade plant tissue to colonize it and to be able to compete against phytopathogens, endophytic fungi produce diverse molecules such as enzymes, steroids, flavonoids, alkaloids, xanthenes, phenolic compounds, among others, which have a variety of applications. The endophyte-host interaction is shown to be very intertwined, given the possible horizontal gene exchange. Some endophytes synthesize the same secondary metabolites produced by the host plant, such as *Taxomyces andreanae* isolated from *Taxus brevifolia* Nutt (Taxaceae), which synthesizes taxol, a molecule with high antitumor potential. Other plants, such as *Coffea arabica* L (Rubiaceae), *Mikania glomerata* Sprengel (Asteraceae), and *Sapindus saponaria* L (Sapindaceae), are consistently important sources for the isolation of endophytes, mainly those of medical interest (Ribeiro et al. 2017).

Due to the variety of applications, these microorganisms have attracted the attention of the scientific community (Souza et al. 2004), and molecular methods are employed to expand the knowledge about endophytic diversity and taxonomy (Linnakoski et al. 2012). Ribosomal DNA (rDNA) is commonly used to estimate the diversity of fungal endophytes (Zervakis et al. 2004, Stefani & Berube 2006), in which amplification of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA by polymerase chain reaction (PCR), described by Mullis & Faloona in 1987, is combined with amplicon sequencing procedures and similarity analyses between sequences in studies and those deposited in databases such as GenBank (Rhoden et al. 2012, Orlandelli et al. 2012, Fryssouli et al. 2020).

The ITS1-5.8S-ITS2 region is widely used for molecular identification due to several advantages, such as satisfactory length and amplification, and universal primers (Zakaria & Aziz 2018). Although the conserved region is favorable for analyses at genus and species level, for higher taxonomic levels such as Family, Order and Class it remains poorly conserved, making greater similarity difficult (Hershkovitz & Lewis 1996).

The Cerrado, also known as Brazilian savanna, is characterized as the second largest biome in Brazil and is formed by seasonal forests and closed forests (*Cerradão*), savannas and open fields. Being a seasonal climate, it presents a dry period between the months of April to September and a rainy period between October and March (Ribeiro &

Walter 1998). The Brazilian savanna is considered a hotspot of world biodiversity, with diverse habitats and a high richness of vertebrates, including numerous endemic animals. Regarding flora, the Cerrado is considered the most diversified savanna in the world, with more than 7,000 plant species, consisting of tree, herbaceous, vine and shrub species, with almost 50% of the species being endemic (Klink & Machado 2005, Selari et al. 2021).

As a result of their relationships with their host plants, in addition to the influence of external factors present in the Cerrado, endophytic microorganisms isolated in this biome produce unique bioactive compounds. However, their biotechnological potential, ecology, and evolutionary and phylogenetic aspects remain poorly understood, requiring more studies focused on the Brazilian Cerrado and its endemic plants (Reis et al. 2022).

In silico study in biology consists of using computational tools to perform simulations of biological systems as testable predictions and how these systems behave (Palsson 2000, Ventura et al. 2006). Studies addressing the identification and biodiversity of microorganisms use sequences deposited in databases. The ITS region is formally used as the barcode for fungal identification (Bates et al. 2013), however, within this molecular identification process, errors and biases, such as primer incompatibility with certain fungal taxa, which subregion (ITS1 and ITS2) to use, and region length may occur, which can influence *in silico* studies (Bellemain et al. 2010, Tedersoo & Lindahl 2016).

The objective of this study was to perform an *in silico* analysis of the biodiversity and phylogeny of endophytic fungi isolated in the Brazilian Cerrado, directing future work that addresses the biodiversity, prospecting and phylogenetic relationships of endophytic fungi.

Material and Methods

To perform *in silico* analysis of the cultivable fungal community of the Brazilian Cerrado, the methodology of Rhoden et al. (2013) was used with modifications. The searches for articles were carried out in databases (Pubmed, Science Direct) and Google Scholar, with the keywords “endophytes”, “endophytic fungi”, “Brazilian savanna” and “Cerrado” (as well as the corresponding keywords in Portuguese), selecting works that used some part of the rDNA ITS1-5.8S-ITS2 conserved region for molecular identification, which presented the GenBank code, and were published between 2012 and September 2022.

No minimum similarity for identification of isolated endophytes was established. The sequences used for the analysis were obtained through NCBI (<https://www.ncbi.nlm.nih.gov/>) using GenBank codes provided in the articles. Aiming to phylogenetically evaluate only classified endophytic fungi, endophytes without taxonomic identification within NCBI were not considered for inclusion in the study.

The phylogenetic tree was elaborated using the software MEGA 11 (Tamura et al. 2021). The sequences obtained were aligned by

ClustalW2 (Thompson et al. 1994) and the phylogeny was performed by the Neighbor-joining method (Saitou & Nei 1987) with the bootstrap test of 500 repetitions.

Results

In total, seven articles were found which served as the basis for the elaboration of this study (Table 1). The search resulted in 57 genera of endophytic fungi, which were analyzed through the phylogenetic tree generated (Figure 1). Most of them belong to the phylum Ascomycota (Figure 2), representing 91% of the endophytes found (classes Sordariomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes, Pezizomycetes, and Saccharomycetes), and Basidiomycota was represented by 9% of the endophytes found (classes Agaricomycetes and Ustilaginomycetes).

For the analysis of the dendrogram, the orders of fungi were highlighted (Figure 3) and identified. The phylogenetic tree was divided into eight clades (1, 2, 3, 4, 5, 6, 7 and 8) to facilitate the analysis, discussing the main characteristics that emerged from the phylogenetic reconstruction of endophytes. Clade 1 is composed of fungi from the class Sordariomycetes, with representatives from the orders Xylariales, Amphisphaerales, Magnaporthales, Coniochaetales, and Hypocreales. Clade 2 was shown to be a diverse clade at the class and order levels, in which the orders Botryosphaerales and Capnodiales belong to the class Dothideomycetes. Thelebolales, Helotiales, Erysiphales, and Rhytismatales belong to the class Leotiomycetes. Magnaporthales belong to the class Sordariomycetes, and Eurotiales and Chaetothyrales to the class Eurotiomycetes.

Clade 3 is composed mostly of Sordariomycetes fungi from the Diaporthales order, with emphasis on *Diaporthe*, which was the main genus due to the high number of isolated lineages mainly from *Vellozia gigantea*. The orders Sordariales (with *Chaetomium*, related with 59% similarity to *Colletotrichum* from Glomeroliales), Coniochaetales (with *Coniochaeta decumbens*, related with 100% similarity to *Fimetariella rabenhorstii* from Sordariomycetes), and Glomeroliales are divided within clade 3. Clade 4 is formed by only one fungus from Pezizales, the only order of the class Pezizomycetes.

Clade 5 contains the Pleosporales order. Representatives of several families were found: Didymosphaeriaceae (*Paraconiothyrium*, *Paraphaeosphaeria* and *Letendraea*), Massarinaceae (*Massarina*), Didymellaceae (*Didymella*, *Phoma* and *Epicoccum*), Trematosphaeriaceae, Tetrapsphaeriaceae, Phaeosphaeriaceae (*Phaeosphaeriopsis*), the base family of the order, Pleosporaceae (*Alternaria*, *Bipolaris*, *Curvularia*, *Preussia* and *Sporormiella*) and a fungus identified only at the suborder level Pleosporineae. Only two species are not contained in Pleosporales in this clade: Botryosphaerales (Dothideomycetes) and *Arthrobotrys* sp., with 99% similarity to *Bipolaris drechsleri*, belonging to the Orbiliales (Orbiliomycetes).

Clade 6, with 74% similarity, is characterized by an endophyte of the Dipodascaceae family (Saccharomycetales, Saccharomycetes) related to a group of basidiomycetes (Agaricomycetes), with 92% similarity, composed of *Trametes*, *Phaeophlebiopsis*, *Bjerkandera* (Polyporales) and *Filobasidium* (Filobasidiales). Clade 7 is formed only by *Pseudozyma* sp. (Ustilaginales; Ustilaginomycetes). Clade 8, at 100% similarity, is composed of *Yamadazyma riverae* (Saccharomycetales) and *Coniochaeta* sp. (Coniochaetales). This genus

also appears phylogenetically related to *Fimetariella* (Sordariomycetes) in the generated dendrogram.

Based on the ITS region sequences available, the genera *Colletotrichum*, *Diaporthe* and *Xylaria* were the most frequently found in the studies, each being isolated from at least four distinct host species (Figure 2). In total, 12 plant species were used (Figure 4), contained in nine families, with Myrtaceae (*Campomanesia xanthocarpa*, *Eugenia* aff. *bimarginata* and *Myrciaria floribunda*) being the most used for the studies. *Vellozia gigantea* (Velloziaceae) was the plant that presented the highest number of endophyte genera (26 genera), followed by *Stryphnodendron adstringens* (Fabaceae) (25) and *Baccharis trimera* (Asteraceae) (13). The other plants ranged from one to six isolated endophyte genera. *Alchornea castaneifolia*, *Myrciaria floribunda* and *Schinus terebinthifolia* (Anacardiaceae) were the plants that presented the lowest number of genera, with only one genus for each plant.

Discussion

Fungi are organisms that constitute one of the largest groups of eukaryotes, having a very large ecological importance due to the fact that they act in different niches, such as: decomposers, mobilizing and recycling different nutrients; as pathogens, affecting numerous plants and animals; and mutualists, offering essential support for plant maintenance in the form of mycorrhizae and endophytes (Gautam et al. 2022).

In general, plants have a very characteristic endophytic microbiota, which is thought to be very important for their health and maintenance (Azevedo 1999). Plant biodiversity in countries such as Brazil, with tropical and subtropical climates, is very large. Thus, it is estimated that most of the endophytic microorganisms found in these regions have not yet been classified, especially in the Brazilian Cerrado region, which has one of the largest biodiversities on the planet due to its unique edaphoclimatic characteristics (Damasco et al. 2018).

Within the area of endophytic biodiversity research, there are limitations in methodology that become significant obstacles. In relation to traditional methods, a part of endophytic diversity can be lost due to the inability of certain endophytes to grow in artificial media, or even be biased by the rapid growth of certain genera such as *Colletotrichum*, *Phomopsis*, *Phyllosticta* and *Xylaria*, thus requiring molecular techniques independent of culture. Dos Reis et al. (2023) reported a significant diversity of non-cultivable endophytes through molecular techniques in six Cerrado plant species. This largely explains the limited number of studies describing the cultivable biodiversity of fungi in plants from this biome.

Hershkovitz & Lewis (1996) addressed the applicability of the ITS region and reported its usefulness for phylogenetically evaluating organisms at the genus and species levels. For higher taxonomic levels (family, order, class), the ITS region may not be suitable due to the not very expressive sequence size. Based on this, it can be observed that some suprageneric taxa have endophytes more related to other taxa in the dendrogram than their original taxon.

Representatives of the Ascomycota phylum were dominant in this work. This reinforces the competitive ability of representatives of this phylum to adapt to diverse conditions, such as those of the Brazilian Cerrado biome, prevailing in unique and specific ecological niches (Egidi et al. 2019).

Table 1. Selected articles with medicinal plants and their respective isolated endophytes.

Host plant	Family	Endophyte genera	Reference	Location
<i>Alchornea castaneifolia</i> (Willd.) A. Juss.	Euphorbiaceae	<i>Eutypella</i>	Vaz et al., 2012 L	09°20'526"S, 49°58'347"W, Tocantins
<i>Baccharis dracunculifolia</i> DC.	Asteraceae	<i>Cladosporium</i> , <i>Preussia</i>	Fernandes et al., 2018 L	19°52' S 43°58' W, Minas Gerais
<i>Baccharis trimera</i> (Less) DC.	Asteraceae	<i>Alternaria</i> , <i>Chaetomium</i> , <i>Diaporthe</i> , <i>Epicoccum</i> , <i>Guignardia</i> , <i>Nigrospora</i> , <i>Pestalotiopsis</i> , <i>Phomopsis</i> , <i>Phoma</i> , <i>Podospora</i> , <i>Preussia</i> , <i>Sporormiella</i> , <i>Xylaria</i> ,	Vieira et al., 2014 L	20°30'369"S; 43°37'837"W, Minas Gerais
<i>Campomanesia xanthocarpa</i> O.Berg	Myrtaceae	<i>Bjerkandera</i> , <i>Curvularia</i> , <i>Phomopsis</i>	Santos et al., 2020 L	23°35'09.1"S 47°31'08.9"W, São Paulo
<i>Eugenia aff. bimarginata</i> DC.	Myrtaceae	<i>Filobasidium</i> , <i>Letendreaea</i> , <i>Phaeosphaeriopsis</i> , <i>Pseudeurotium</i> , <i>Pseudozyma</i> , <i>Xylaria</i>	Vaz et al., 2012 L	10°36'043"S, 46°35'836"W, Tocantins
<i>Guazuma ulmifolia</i> Lam.	Malvaceae	<i>Bipolaris</i> , <i>Colletotrichum</i> , <i>Diaporthe</i>	Santos et al., 2020 L	23°35'08.6"S 47°31'08.1"W, São Paulo
<i>Lafoensia pacari</i> A.St – Hill.	Lythraceae	<i>Phaeophlebiopsis</i> , <i>Talaromyces</i>	Santos et al., 2020 L	23°35'08.2"S 47°31'06.7"W, São Paulo
<i>Myrciaria floribunda</i> (H. West ex Willd.) O.Berg.	Myrtaceae	<i>Trametes</i>	Vaz et al., 2012 L	09°20'526"S, 49°58'347"W, Tocantins
<i>Schinus terebinthifolia</i> Raddi	Anacardiaceae	<i>Diaporthe</i>	Tonial et al., 2017 –	–
<i>Siparuna guianensis</i> Aubl.	Siparunaceae	<i>Bjerkandera</i> , <i>Colletotrichum</i> , <i>Diaporthe</i>	Santos et al., 2020 L	23°35'06.2"S 47°31'25.4"W, São Paulo
<i>Stryphnodendron adstringens</i> (Mart.) Coville	Fabaceae	<i>Alternaria</i> , <i>Arthrotrichum</i> , <i>Aspergillus</i> , <i>Botryosphaeria</i> , <i>Cladosporium</i> , <i>Colletotrichum</i> , <i>Coniochaeta</i> , <i>Cytospora</i> , <i>Diaporthe</i> , <i>Fimetariella</i> , <i>Guignardia</i> , <i>Massarina</i> , <i>Muscodora</i> , <i>Neofusicoccum</i> , <i>Nigrospora</i> , <i>Paraconiothyrium</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phomopsis</i> , <i>Preussia</i> , <i>Pseudofusicoccum</i> , <i>Sordaria</i> , <i>Sporormiella</i> , <i>Trichoderma</i> , <i>Xylaria</i> ,	Carvalho et al., 2012 L and S	19°30'S, 43° 54'W 0°18'10.8"S e (21° 05'S, 44°12'W, Minas Gerais
<i>Vellozia gigantea</i> N.L. Menezes & Mello-Silva	Velloziaceae	<i>Bipolaris</i> , <i>Clonostachys</i> , <i>Coccomyces</i> , <i>Colletotrichum</i> , <i>Coniochaeta Crucellisporeopsis</i> , <i>Daldinia</i> , <i>Diaporthe</i> , <i>Didymella</i> , <i>Fusarium</i> , <i>Indurata</i> , <i>Myxotrichum</i> , <i>Neopestalotiopsis</i> , <i>Nigrospora</i> , <i>Pallidocercospora</i> , <i>Paraconiothyrium</i> , <i>Paraphaeosphaeria</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Pezicula</i> , <i>Phaeophleospora</i> , <i>Phyllosticta</i> , <i>Pseudocercospora</i> , <i>Trichoderma</i> , <i>Xylaria</i> , <i>Yamadazyma</i>	Ferreira et al., 2017 L and R	19° 14'874"S; 043°30'574"W, Minas Gerais

Plant organ used: L = leaves; S = stem; R = roots.

Brazilian Cerrado Fungi: *In Silico* Biodiversity

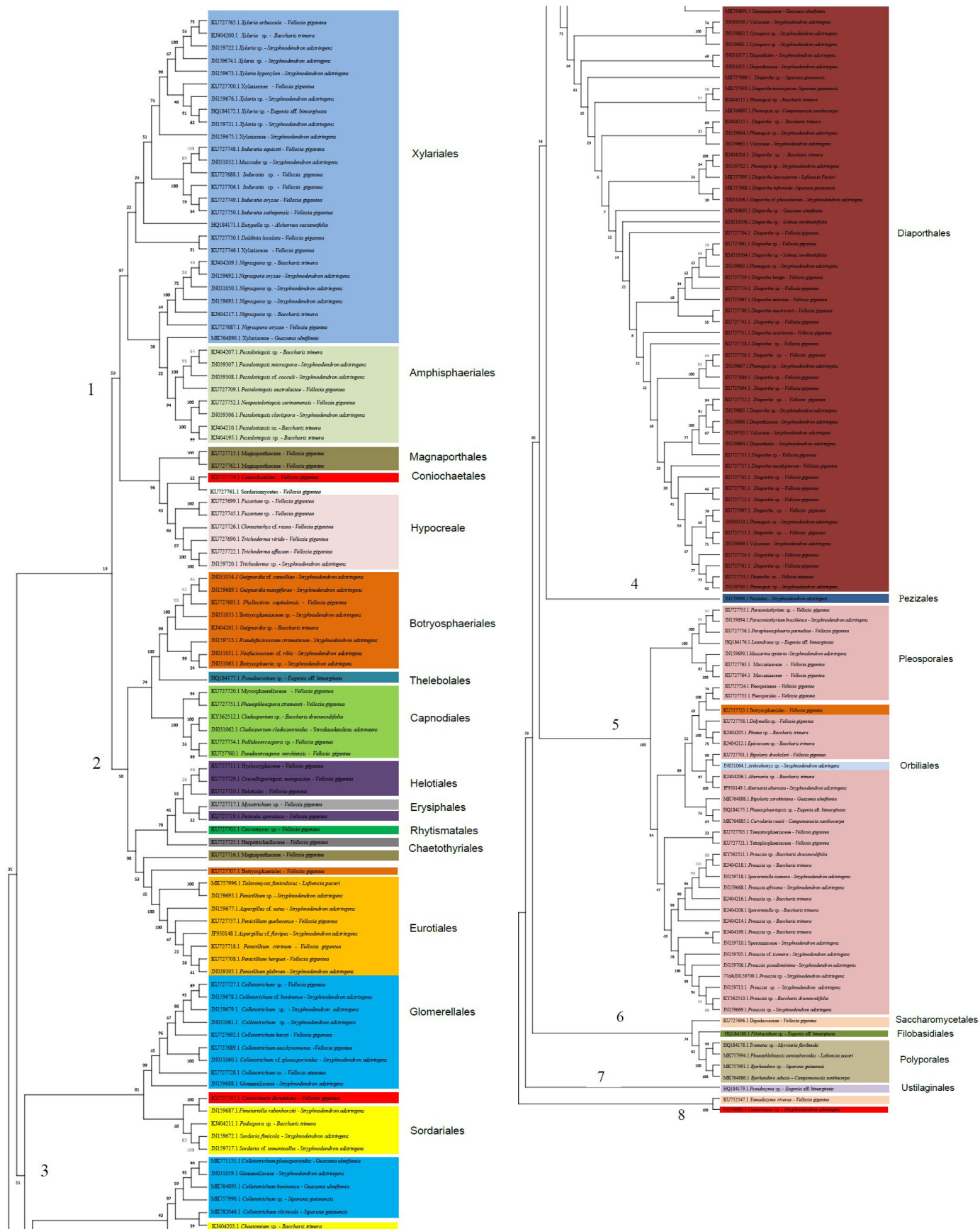


Figure 1. Phylogenetic analysis. The phylogenetic tree was elaborated using the MEGA 11 software (Tamura et al. 2021), with sequences aligned by ClustalW (Thompson et al. 1994) and phylogeny performed by the neighbor-joining method and with the bootstrap test of 500 repetitions.

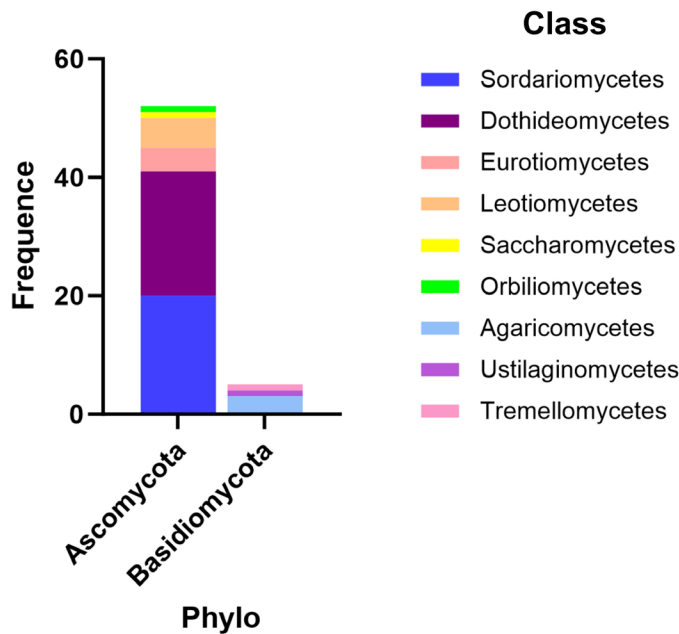


Figure 2. Class-level frequency of the endophytes in the Phyla Ascomycota and Basidiomycota.

Dos Reis et al. (2023), when analyzing the endophytic fungal diversity of leaves from six forest tree species of the Brazilian Cerrado through a culture-dependent method, also found the Sordariomycetes class to be predominant (80%) among the isolates, followed by Dothideomycetes (19.2%).

The prevalent order found in this work, Xylariales, is described as presenting mainly terrestrial saprophytes, with dark-colored perithecia and developed stroma. This is the main order of clade 1, having representatives of its type family, Xylariaceae (*Xylaria*, *Muscodora* and *Induratia*), related at 26% similarity to Diatrypaceae (*Eutypella*). These two families are reported in the scientific literature as related groups (Glawe & Rogers 1986, Zhang et al. 2006).

Diaporthe was the main genus found in this study. This result is similar to the works of Reis et al. (2023) and Noriler et al. (2018) in research on the fungal community of Cerrado plants. This genus is quite diverse and has pathogenic and non-pathogenic representatives, producers or not of bioactive secondary metabolites (Reis et al. 2022).

The Pezizales order highlighted in clade 4 has representatives unevenly distributed around the world and is considered one of the basal taxa of Ascomycota (Pfister & Healy 2021). The Pleosporales order, from clade 5, according to Hyde et al. (2013), comprises the most representative of Dothideomycetes, with more than 40 families, and are saprophytic fungi of plant matter in terrestrial and aquatic environments, as well as phytopathogens.

Basidiomycetes were found with lower incidence, which is possibly related to the culture-dependent approach observed in this study, since most basidiomycetes do not grow in the conventional media used in laboratory (Reis et al. 2023). However, clade 6 was represented by a basidiomycete of the Polyporales order, which are considered important lignin recyclers, directly impacting the carbon cycle and presenting various arrangements of the hymenophore and varied types of basidiocarps (Binder et al. 2013).

Pseudozyma sp. from clade 7 is a yeast-like fungus with studies on the antagonistic potential against other yeasts of scientific interest (Avis & Bélanger 2002). In clade 8, *Yamadazyma riverae* has asexual species related to *Candida* spp. Its genus was initially proposed for *Pichia* species, but in 2010, the taxon had genetic basis to support the genus proposal (Lopes et al. 2015).

Rhoden et al. (2013) performed an *in silico* phylogenetic analysis of fungi isolated from various plant families in Brazil, using sequences deposited in the GenBank database by 12 authors between 2005 and 2012, to obtain the alignment to determine genetic distance. Among the 12 studies analyzed, 73 plant species belonging to 13 families and various genera were obtained, including *Hypocrea*, *Trichoderma*, *Gibberella*, *Coniophora*, *Epicoccum*, *Sclerostagonospora*, *Bensingtonia*, *Rhodotorula*, *Candida*, *Trichoderma*, *Fusarium*, *Clonostachys*, *Bionectria*, *Glomerella*, *Arthrinium*, *Epicoccum*, *Phaeosphaeria*, *Cladosporium*, *Mycosphaerella*, *Aspergillus*, *Talaromyces*, *Penicillium*, *Guignardi*, *Phomopsis*, *Diaporthe*, *Gongronella*, *Colletotrichum*, among others. The result was very close to that presented in this study, where 57 genera of endophytic fungi were found. It is worth noting that only studies that isolated fungi from plants in the Cerrado biome were analyzed in this work. Therefore, it is believed that the fungi isolated from this region represent a large part of the cultivable endophytic fungal genera found in Brazil.

The genera *Colletotrichum*, *Diaporthe* and *Xylaria* were isolated from four different plant species (Figure 2), affirming their wide occurrence and ecological importance as endophytic organisms. *Colletotrichum* is well described in the scientific literature for presenting species that are usually intracellular or endophytic hemibiotrophic phytopathogens (Silva et al. 2020), in addition to serving as a genetic model for studies investigating the fungus-plant relationship (Perfect et al. 1999). *Diaporthe* is described as a genus with significant biotechnological value due to the prospecting of bioactive molecules with diverse activities with applications in the areas of biotechnology, agriculture and pharmacology, in addition to its species being found in a wide variety of biomes (Chepkirui & Stadler 2017, Sebastianes et al. 2017, Noriler et al. 2019). The genus *Xylaria*, in turn, is known to have wood saprophytic and endophytic species with wide global distribution and varied production of secondary metabolites with diverse activities (Macías-Rubalcava & Sánchez-Fernández 2017, Song et al. 2014).

In this study, certain genera were closely related in the dendrogram. Some researchers classify the genera *Preussia* and *Sporomiella* (Pleoporales) as synonyms (Chang et al. 2009), and in Diaporthales, *Phomopsis* is anamorph of *Diaporthe* (Diogo 2010). Caldart et al. (2016) describe that the bootstrap test is widely used to evaluate the reliability of the generated tree nodes and that a considerable percentage to support its credibility begins at 70%.

For molecular identification of fungi, the ITS region of rDNA or other regions are usually used (Raja et al. 2017). The sequence of the ribosomal genes of Polymerase II and the genes of β -tubulin, translation elongation factor 1- α , Actin, Calmodulin, Glyceraldehyde-3-phosphate dehydrogenase and Histone H3 can be used for identification (Gomes et al. 2013, Ferreira 2017). The articles found in this study used only the ITS region for endophyte identification, with the exception of Toniai et al. (2017), who performed identification based on multiple sequences of the ITS region and the Calmodulin, translation elongation factor 1- α ,

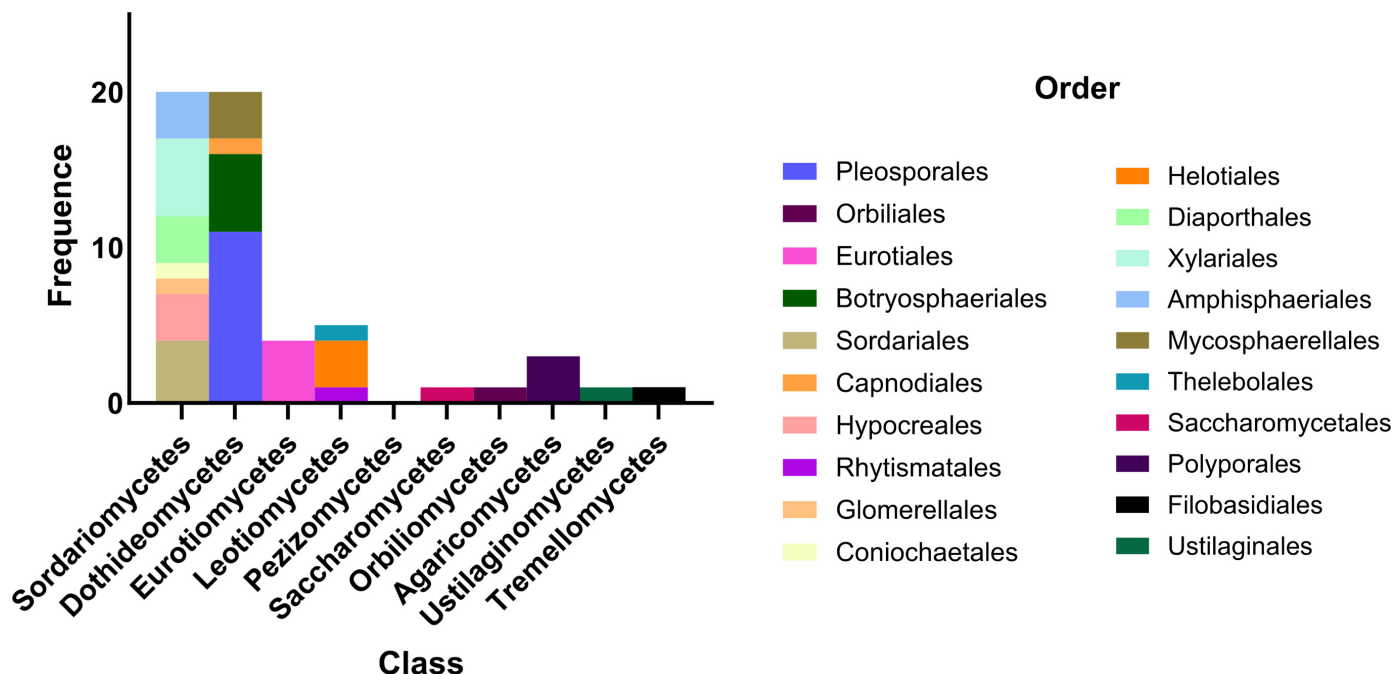


Figure 3. Order-level frequency of endophytes in the corresponding classes.

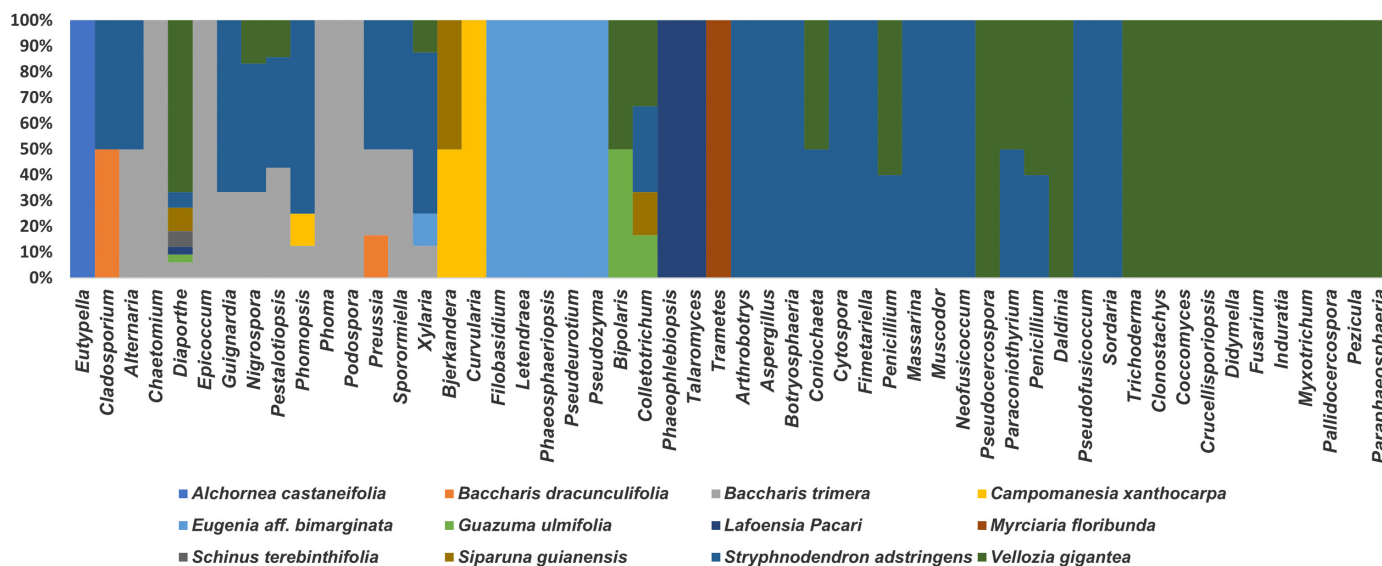


Figure 4. Frequency percentage of the diversity of endophytic fungi genera isolated from each plant species.

Histone H3 and β -tubulin genes, and Ferreira et al. (2017) used the ITS region, β -tubulin, Polymerase II and D1 and D2 regions.

Most of the results found related to the biodiversity of endophytic fungi from the Brazilian Cerrado aim at the bioprospecting of bioactive compounds through culture-dependent methods. Although the isolation methodology of this type of work cannot precisely evaluate the real diversity of fungi, efforts to obtain and evaluate the diversity of molecules of biotechnological interest from these organisms drive

studies in the area, providing data for the ecology, biodiversity and phylogeny of these organisms.

Certain metabolites can be produced from the unique interaction between endophyte-plant, due to the ability of endophytic fungi to produce secondary metabolites naturally produced by the plant (Nguyen et al. 2023). Therefore, the endophytes found in this study may express different compounds based on the host of the Cerrado flora. Therefore, the preservation of this biome is necessary due to its

biodiversity resulting from a spatial variety, that is, the diversity of ecosystems such as gallery forests, rupestrian grasslands, *cerradão*, among others (Machado et al. 2004). Consequently, the biodiversity of endophytic fungi related to endemic plants of the Cerrado and their biotechnological value are extremely relevant for the prospecting of new bioactive compounds.

Conclusion

The dendrogram presented was generated based on sequences obtained in the conserved ITS region, by the Neighbor-joining method, of various endophytic fungi isolated from the Brazilian Cerrado. Through it, it was possible to evaluate the applicability of *in silico* studies for the study of endophytic fungal biodiversity and their phylogenetic relationships. The developed cladogram included several orders of fungi, bringing the phylogeny of these groups based on the ITS region. This study has the potential to guide future work about the biodiversity of endophytic fungi and the prospecting of bioactive compounds from fungi and medicinal plants from the Brazilian Cerrado.

In summary, Brazil has a high biotechnological value preserved in the biodiversity of endophytic fungi. However, this potential is not yet fully known and defined. Knowledge of the diversity of endophytic fungi associated with plants from the Brazilian Cerrado can bring new perspectives on the ecology, phylogeny and biotechnological capacity of these organisms. Therefore, a more precise and detailed identification of isolated endophyte species is valuable and can be aided by *in silico* methods.

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Conflicts of Interest

The authors declare that there is no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

Data supporting the reported results are openly available in Figshare at: <https://doi.org/10.6084/m9.figshare.24246517>

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