

## Chapter 2

# Why Study Endophytic Fungal Community Associated with Cacti Species?

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**Abstract** Dry environments have plants with special adaptations which enable them to live on nutrient-poor soils, in high temperatures, and with a scarcity of water. One of the most important features is the ability to establish symbiosis with different microorganisms. Studies on the diversity of endophytic fungi from dry environments are recent and scarce. The endophytic community (fungi and bacteria) of cacti has been studied in Australia, the United States, Brazil, and Mexico, revealing special features in their composition. Bioprospecting studies on these organisms are just as scarce as the ecological studies, but have shown promising results. In the United States and Brazil, endophytes isolated from cacti were selected for the production of enzymes, antimicrobials, and anticancer substances. The purpose of this chapter is to review the literature on this subject, highlight the benefits of endophytic fungi species for plants on dry environments (mainly the Cactaceae family), and to emphasize the usefulness of endophytes in biotechnological processes.

**Keywords** Brazilian fungi • Caatinga • Cactaceae • Diversity • Endophytes • Mycodiversity • Tropical dry forest

## 2.1 Introduction

Recently, Unterseher et al. (2012) defined the study of endophytic microorganisms as “endophytology”. A part of the microorganisms, mainly bacteria and fungi, live in plants, and they are called endophytes because they colonize the healthy tissues of the plant in some part of their life cycle without causing apparent damage (Petrini

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1991; Azevedo et al. 2000). The distinction between endophytic and pathogenic depends on the stage of the interaction of the microorganism with the host (Strobel et al. 2004). Therefore, application of these terms has a didactic meaning and still presents difficulties in determining the limits of this interaction (Azevedo et al. 2000). Definitions of endophytic fungi have been proposed over the years (Hyde and Soyong 2008; Azevedo and Araújo 2007; Mendes and Azevedo 2007). Similar to the concepts historically used, some authors have suggested that endophytic fungi studies can be called “endophytism” (Suryanarayanan 2013), and others denominate these fungi as “mycoendophytes” (Rai et al. 2014).

The Cactaceae family is originally from Mexico; it occurs on five continents (Hunt and Taylor 1990; Anderson 2001; Meiado et al. 2012, 2015), and it is divided into four subfamilies: *Maihuenioideae*, *Pereskioideae*, *Opuntioideae*, and *Cactoideae* (Taylor and Zappi 2008). The centers of diversity and distribution of cacti are located primarily in the southeastern part of the United States, Mexico, the Andes region, and the eastern part of Brazil (Taylor and Zappi 2004), except for the genus *Rhipsalis* Gaertn which is found from the Neotropics to Africa and southern Asia (Anderson 2001; Meiado et al. 2012). Brazil is considered the third largest center of family diversity on the American continent (Taylor, in Oldfield 1997), with a total of 37 genera of native Cactaceae, comprising about 30% of 120 species reported in the New World (Zappi et al. 2010). Using all publications available on endophytes from cacti, we wish to add more information to this subject, highlighting some of the benefits of endophytic fungi for plant species living in dry environments, and by suggesting new verification studies of the endophytic fungi community associated with cacti species.

## 2.2 Importance of Endophytic Fungi for Plants in Dry Environments

Dry habitats represent a promising research environment which, because of its environmental challenges, can be used to analyze and to characterize the structure of the symbiotic relationship between fungi and plants (Khidir et al. 2010). Only a few surveys have verified the endophytic fungi association with plants in dry environments; it has been observed in many semiarid fields that endophytic fungi community includes pigmented fungi (Suryanarayanan et al. 2005; Sun et al. 2012; Loro et al. 2012), suggesting that these fungi can help the plant to survive heat and drought, one of its key ecological functions (Khidir et al. 2010; Hubbard et al. 2014).

Pigmented endophytic fungi accumulate melanin in the wall of their cells, and this accumulation has been regarded as a mechanism to confer tolerance to environmental stresses, such as UV, microbial lysis, and defences against plant pathogenic microorganisms (Hyakumachi et al. 1987; Wang and Casadevall 1994; Jacobson et al. 1995; Kawamura et al. 1997; Redman et al. 2002; Hubbard et al. 2014). On the other hand, some experiments suggest that melanin accumulation in certain fungi species (e.g., *Alternaria alternata* and *Magnaporthe grisea*) is one character of pathogenicity (Kawamura et al. 1997) and that it also is involved with the virulence

factor of human and plant pathogenic fungi (Langfelder et al. 2003). Some surveys with melanized microorganisms and insects have shown that this pigment can protect against UV radiation (Mosse and Lyakh 1994; Geng et al. 2008). Reviewing studies on fungal melanin, Butler and Day (1998) proposed some benefits for fungi, such as protection against irradiation, enzymatic lysis, extreme temperatures, oxidizing agents, heavy metals binding, and fungicides.

Plants that live in extreme environments present adaptations to these ecosystems. The ability of plants to tolerate drought can be a legacy of endophytic microorganisms (Rodríguez et al. 2008), and there is a possible involvement of genes in the expression of the capacity for tolerance, since these plants have been related to microorganisms for millions of years (Krings et al. 2007; Morsy et al. 2010). In addition, pigmented fungi can absorb more radiant energy than hyaline mycelium (Crabtree and Gessner 1982). Different studies in extreme regions, to which cacti are often subjected, have demonstrated a low diversity and a high colonization frequency of melanized fungi (Suryanarayanan et al. 2005; Bezerra et al. 2012b, 2013).

Other studies on viruses associated with endophytic fungi have demonstrated a new way in which the plants persist in a dry environment (Roossinck 2015). These viruses are called “mycovirus,” and their symbiosis with endophytes can guarantee ecological advantages to the host plant (Márquez et al. 2007). This benefit was demonstrated by Marquez et al. (2007) who studied the symbiosis between a virus, the endophyte *Curvularia protuberata*, and the grass *Dichanthelium lanuginosum*. These authors found that only when the virus was present in the association, the endophyte contributed to the plant’s survival in heat (>50 °C). Other endophytes, such as *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Phoma* have also been reported as being associated with different virus species (Bao and Roossinck 2013). These genera are commonly isolated from plants from dry environments, and their presence associated with viruses can guarantee the resistance of plants in extreme areas. A better understanding of this symbiosis (plant-fungus-virus) may contribute to future ecological and economic applications (e.g., sustainable agriculture) (Márquez et al. 2007; Roossinck 2011, 2012, 2015; Bao and Roossinck 2013).

Studies of the association of endophytic fungi with plants from semiarid regions were performed in the United States by Khidir et al. (2010), who studied the community of endophytic fungi associated with roots of grasses. Another study in Venezuela by Loro et al. (2012) analyzed the diversity of endophytic fungi from dominant grasses and sedges, and verified *Pleosporales* fungi as common endophytes. In the desert areas of China, Sun et al. (2012) explored the community of endophytic fungi in leaves and stems of eight plants species, and obtained *Alternaria* and *Fusarium* species as the most frequent endophytes isolated. In other Chinese regions with temperate, semiarid, and/or continental climates with an annual rainfall ranging from 50 to 381.8 mm and temperature of 6.7–11 °C, verification research of endophytic fungi community has been carried out (Unterseher et al. 2012; Jin et al. 2013). These studies have contributed to the understanding of the relationship of endophytic microorganisms and their benefits to the host, suggesting a beneficial contribution (e.g., resistance to abiotic factors) to plants growing in extreme regions.

Although there are still gaps in our understanding of the composition of the endophytic fungi communities, arid environments with a special community of plants are important in evaluating the role of endophytes in the global biological diversity, and to a broader evaluation of endophyte-plant interaction (Massimo et al. 2015).

### 2.3 Endophytic Fungi from Cacti

Despite the fact that arid and semiarid regions account for about 30% of the surface of the planet (Pell et al. 2007), few studies have investigated the association of endophytic fungi with plants in these areas. The association of these microorganisms with Cactaceae species is also incipient, but these cacti are among the most common plants in these habitats. Cacti belong to one of the most interesting families of arid, semiarid, and desert regions, due to the extensive set of special adaptations to water scarcity which allow them to be perennial (Rojas-Aréchiga and Vázquez-Yanes 2000). Figure 2.1 shows the studies of the endophytic fungi community from cacti species in different countries.

In the literature, only seven studies are known of endophytic fungi associated with cacti. The first survey was conducted by Fisher et al. (1994) in Australia, which studied the association of endophytic fungi with *Opuntia stricta* (Haw.) Haw. Eleven years after the first study, Suryanarayanan et al. (2005) studied 21 cacti species in



**Fig. 2.1** Geographical localization of the studies on endophytic fungi community from cacti species in different countries (The authors)

the desert areas in Arizona, and revealed a large diversity of endophytes belonging to phylum *Ascomycota*. In Brazil, the first study was by Bezerra et al. (2012a), who investigated the fungal endophytic composition of *Opuntia ficus-indica* (L.) Mill. These same authors also verified the endophytic fungal community associated with *Cereus jamacaru* DC. subsp. *jamacaru* in regions of the Brazilian tropical dry forest (Caatinga) (Bezerra et al. 2013). Figure 2.2 shows examples of Brazilian cacti species of which the endophytic fungi community has been studied.



**Fig. 2.2** Cacti species from Brazil in which the endophytic fungi community has been studied. (a) *Pilosocereus* sp. (b) *Tacinga* sp. (c) *Cereus* sp. (d) *Melocactus* sp. (e) *Opuntia* sp. (The authors) (Photographed by JDP Bezerra)



Another study in Missouri (the United States) was realized by Silva-Hughes et al. (2015); it verified the diversity and antifungal activity of endophytes of the medicinal cactus *Opuntia humifusa* (Raf.) Raf. with endophytes belonging to the genera *Alternaria*, *Aureobasidium*, and *Diaporthe* as the most frequent species. Also, in Brazil, Freire et al. (2015) demonstrated the influence of colonization of the insect *Dactylopius opuntiae* (Hemiptera: *Dactylopiidae*) on endophytic fungi community of the forage cactus *O. ficus-indica* growing on a family farming in the Caatinga ecosystem. Recently, in Mexico, Fonseca-García et al. (2016) verified the association of bacteria, archaea, and fungi with two native and sympatric cacti species *Myrtillocactus geometrizans* and *Opuntia robusta* and demonstrated a remarkable diversity of endophytes.

In addition to the endophytic fungi association, other studies have explored the community of endophytic bacteria and rhizoplane in cacti from the desert areas in Mexico, revealing the influence of these microorganisms on seed germination, and cacti permanence in their natural environment (Puente et al. 2004a, b, 2009a, b; Lopez et al. 2011, 2012). Table 2.1 summarizes the studies of endophytes (fungi and bacteria) associated with cacti species.

## 2.4 What Has the Study of Endophytic Fungi Associated with Cacti Revealed?

Although some authors indicate that the low diversity of endophytic fungi from plants in dry areas may be due to low rainfall and vegetation density (Arnold et al. 2000; Suryanarayanan et al. 2002, 2003, 2005; Massimo et al. 2015), these fungi can produce biomolecules of biotechnological interest (Wang and Dai 2011; Chandra 2012), and are contributing to the protection of their hosts against drought, salinity, herbivory, climate change, and diseases caused by pathogenic microorganisms (Redman et al. 2002; Hubbard et al. 2014).

The great importance of endophytes in biotechnological processes started from the production of the anticancer drug Taxol, by using endophytic fungi isolated from a medicinal plant in the United States (Stierle et al. 1993). After this discovery, different surveys were conducted to verify the potential of these microorganisms in medicine production, biofuels development, bioremediation, biological control of pests, and genetic engineering (Schulz et al. 2002; Strobel and Daisy 2003; Wang and Dai 2011; Chandra 2012).

Recently, studies of endophytic fungi in arid environments have drawn attention to their ecological and taxonomic benefits. However, the biotechnological potential of these fungi is still relatively small and requires bioprospecting research. In Brazil, Bezerra et al. (2012a) showed that endophytes isolated from the cactus *O. ficus-indica* growing in the Caatinga forest can be used in the production of pectinase, cellulase, xylanase, and protease enzymes. These mycologists demonstrated that the endophytes *Aspergillus japonicus* and *Penicillium glandicola* had activity to



**Table 2.1** (continued)

Cacti	Endophytic		Number of isolates	Identification	Country	Reference
	Fungi	Bacteria and/or archaeal				
<i>Pachycereus pringlei</i> (S. Watson) Britton and Rose <i>Stenocereus thurberi</i> (Engelm.) Buxb. <i>Opuntia cholla</i> F.A.C. Weber	–	<i>Acinetobacter</i> , <i>Bacillus</i> , <i>Citrobacter</i> , <i>Puentibacillus</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> , <i>Staphylococcus</i>	26	16S rRNA gene	Mexico	Puente et al. (2009a)
<i>Mammillaria fraileana</i> (Britton and Rose) Boed.	–	<i>Azotobacter</i> , <i>Enterobacter</i> , <i>Bacillus</i> , <i>Pseudomonas</i>	–	16S rRNA gene	Mexico	Lopez et al. (2011)
<i>Opuntia ficus-indica</i> (L.) Mill.	–	<i>Agrobacterium</i> , <i>Paracoccus</i> , <i>Sphingomonas</i> , <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Escherichia</i> , <i>Klebsiella</i> , <i>Kluyvera</i> , <i>Samonella</i> , <i>Stenotrophomonas</i> , <i>Bacillus</i> , <i>Chryseobacterium</i> , <i>Puentibacillus</i>	37	Physiology and biochemistry	Brazil	Costa and Melo (2012)
<i>Opuntia ficus-indica</i> (L.) Mill.	<i>Acremonium</i> , <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Monodictys</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Tetraploa</i> , <i>Xylaria</i>	–	44	Morphology	Brazil	Bezerra et al. (2012a)
<i>Opuntia</i> sp. (cultivars)	–	<i>Azospirillum</i> , <i>Herbaspirillum</i> , <i>Azotobacter</i> , <i>Azomonas</i> , <i>Gluconacetobacter</i>	22	16S rRNA gene	Brazil	Lyra et al. (2013)
<i>Cereus jamacaru</i> DC. subsp. <i>jamacaru</i>	<i>Acremonium</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Boeremia</i> , <i>Candida</i> , <i>Chrysomyilia</i> , <i>Cladosporium</i> , <i>Cochliobolus</i> , <i>Curvularia</i> , <i>Cytospora</i> , <i>Debaryomyces</i> , <i>Fusarium</i> , <i>Gibberella</i> , <i>Guignardia</i> , <i>Nigrospora</i> , <i>Penicillium</i> , <i>Pestalotiopsis</i> , <i>Phoma</i> , <i>Phomopsis</i> , <i>Pseudocochliobolus</i> , <i>Purpureocillium</i> , <i>Redaellia</i> (=Aspergillus), <i>Sarcocladium</i> , <i>Trichoderma</i> , <i>Rhodotorula</i> , <i>Sporobolomyces</i> , <i>Sterigmatomyces</i> , <i>Tritirachium</i> , <i>Cunninghamella</i> , <i>Synechalastrum</i>	–	560	Morphology, physiology and biochemistry	Brazil	Bezerra et al. (2013)
<i>Opuntia humifusa</i> (Raf.) Raf	<i>Aureobasidium</i> , <i>Alternaria</i> , <i>Disporthe</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Paraconiothyrium</i> , <i>Pestalotiopsis</i> , <i>Biscogniauxia</i> , <i>Cryptococcus</i> , <i>Epicoccum</i> , <i>Phoma</i>	–	108	ITS rDNA, <i>tef1</i> and $\beta$ -tubulin genes	USA	Silva-Hughes et al. (2015)



<i>O. ficus-indica</i> healthy and infested by <i>Dactylopius opuntiae</i>	<i>Acremonium</i> , <i>Aspergillus</i> , <i>Chrysomylla</i> , <i>Cladosporium</i> , <i>Cunninghamella</i> , <i>Curvularia</i> , <i>Fusarium</i> , <i>Mucor</i> , <i>Penicillium</i> , <i>Phialophora</i> , <i>Phoma</i> , <i>Rhizoctonia</i>	–	100	Morphology	Brazil	Freire et al. (2015)
<i>Myrtillocactus geometrizans</i> (Mart. ex Pfeiff.) Console and <i>Opuntia robusta</i> H.L. Wendl. ex Pfeiff.	<i>Agaricales</i> , <i>Ascomycota</i> , <i>Capnodiales</i> , <i>Chaetothyriales</i> , <i>Coniochaetales</i> , <i>Dorthideales</i> , <i>Eurotiales</i> , <i>Hymenochaetales</i> , <i>Hysteriales</i> , <i>Hypocreales</i> , <i>Helotiales</i> , <i>Leotiales</i> , <i>Pleosporales</i> , <i>Sordariales</i> , <i>Xylariales</i>	<i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Alphaproteobacteria</i> , <i>Bacilli</i> , <i>Betaproteobacteria</i> , <i>Chloracidobacteria</i> , <i>Deltaproteobacteria</i> , <i>Gammaproteobacteria</i> , <i>Gemmatimonadetes</i> , <i>Oscillatoriothymicidae</i> , <i>Spartobacteria</i> , <i>Solihacteres</i> , <i>Sphingobacteria</i> , <i>Thaumarchaeota</i>	–	Molecular techniques	Mexico	Fonseca-García et al. (2016)
<i>Tacinga inamoena</i> (K. Schum.) N. P. Taylor and Stuppy, <i>Pilosocereus gounellei</i> (F. A. C. Weber) Byles and G. D. Rowley, and <i>Melocactus zehntneri</i> (Britton and Rose) Luetzelb.	<i>Ascomycota</i> , <i>Basidiomycota</i> , <i>Mucoromycotina</i>	–	–	Polyphasic taxonomy	Brazil	JDP Bezerra et al. (2017) (personal communication)

Adapted from Bezerra et al. (2012b)

produce pectinolytic enzymes, and the endophytic fungus identified as *Xylaria* sp. was the best for producing cellulase enzymes in solid medium. According to these authors, all endophytes from *O. ficus-indica* were capable to produce xylanolytic enzymes, but they were very weak. Endophytes belonging to *Acremonium*, *Phoma*, and *Tetraploa* were the best to produce proteolytic enzymes.

Some cacti studied on the composition of endophytic microdiversity are used to feed humans and ruminants, and in traditional medicine (Paula and Ribeiro 2004; Andrade et al. 2006). In the United States, Silva-Hughes et al. (2015) described the diversity and the antifungal activity of endophytic fungi from the medicinal cactus *O. humifusa*. These authors demonstrated that these endophytes can be a source of bioactive molecules that inhibit or control pathogenic microorganisms. Similar results were obtained by Pires et al. (2015), who studied the antimicrobial capacity of endophytes isolated from cacti *C. jamacaru* subsp. *jamacaru*, *O. ficus-indica*, and *Pilosocereus gounellei* against pathogenic bacteria in humans. These mycologists found that 35% of the endophytes exhibited antagonism against pathogenic bacteria, especially for the endophytic fungi isolated from *C. jamacaru* subsp. *jamacaru*.

A review by Chandra (2012) on the use of endophytes in biotechnological processes demonstrated that endophytic fungi are new sources for substances with anti-cancer potential. The L-asparaginase antitumor substance produced by fungi has received attention in recent years because it has less collateral effects than L-asparaginase produced by bacteria (Kumar et al. 2013). The wide use of this anti-leukemic substance reinforces the verification of the potential of endophytes in the synthesis of anticancer enzymes (Audipudi et al. 2014; Chow and Ting 2015). Surveys using the endophytic fungal community from cacti species for producing L-asparaginase have been conducted with endophytes from *C. jamacaru* subsp. *jamacaru* (Santos et al. 2015a). The researchers used 44 endophytes; 30 were active on solid medium and 19 produced L-asparaginase in liquid medium. *Aspergillus*, *Fusarium*, and *Penicillium* species were the best to synthesize L-asparaginase, and they are indicated to production process of this enzyme. This first study demonstrated that endophytes from cacti have a great potential for biotechnological production of antileukemic enzymes and that they are suitable for further studies.

Endophytes associated with plants in arid ecosystems have revealed a great phylogenetic diversity in association with hosts (Massiano et al. 2015) and allowed for the discovery of new taxa or new fungi habitats (Siqueira et al. 2008; Oliveira et al. 2014; Bezerra et al. 2015; Nascimento et al. 2015; Santos et al. 2015b; Knapp et al. 2015; Bezerra et al. 2016). In addition to the ecological properties, studies have shown that these microorganisms have a great potential for use in plant resistance processes because of global temperature changes (especially those plants of economic importance), production of pharmacological metabolites, and they can contribute directly to the preservation of natural ecosystems. The data obtained from the use of this mycobiome may also provide information for the creation of environmental preservation areas, since the natural environments around the world are under anthropic pressure, including the protected areas in Brazil (Bernard et al. 2014). Endophytes associated with cacti and other plants from dry environments are

a microdiversity still little known. This endophyte diversity can bring great benefits to the taxonomy, ecology, and biotechnology, contributing to new endophytic fungal community studies with cacti from different ecosystems.

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