Endophytic fungi from medicinal plant Bauhinia forficata: Diversity and biotechnological potential

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Abstract

Bauhinia forficata is native to South America and used with relative success in the folk medicine in Brazil. The diversity, antibacterial activity, and extracellular hydrolytic enzymes of endophytic fungi associated with this plant were studied. Plant samples, which included leaves, sepals, stems, and seeds, were used. Ninety-five endophytic fungal were isolated (18 from leaves, 22 from sepals, 46 from stems, and nine from seeds), comprising 28 species. The most frequently isolated species were Acremonium curvulum (9.5%), Aspergillus ochraceus (7.37%), Gibberella fujikuroi (10.53%), Myrothecium verrucaria (10.53%) and Trichoderma piluliferum (7.37%). Diversity and species richness were higher in stem tissues, and Sorensen’s index of similarity between the tissues was low. Eleven fungi showed antibacterial activity. Aspergillus ochraceus, Gibberella baccata, Penicillium commune, and P. glabrum were those with the greatest antibacterial activity against Staphylococcus aureus and/or Streptococcus pyogenes. Thirteen species showed proteolytic activity, particularly Phoma putaminum. Fourteen species were cellulase positive, particularly the Penicillium species and Myrmecridium schulzeri. All isolates tested were xylanase positive and 10 showed lipolytic activity, especially Penicillium glabrum. It is clear that the endophytic fungi from B. forficata have potential for the production of bioactive compounds and may be a source of new therapeutic agents for the effective treatment of diseases in humans, other animals, and plants. To our knowledge, this is the first study of endophytic fungi from different tissues of B. forficata and their biotechnological potential.

Key words: antibacterial agents, fungal endophytes, hydrolytic enzyme, symbiosis.

Introduction

Some microorganisms such as bacteria and fungi live as endophytes and inhabit the interiors of plants. According to Petrini (1991), they colonize healthy tissues of the aerial parts of the plant at some time in their life cycles without causing apparent damage, or producing visible external structures (Azevedo et al., 2000).

Several studies have demonstrated the ability of endophytic fungi to produce various compounds such as enzymes (Teske and Trentini, 1995; Bezerra et al., 2012b), antitumor substances (Chandra, 2012), antimicrobial substances (Souza et al., 2004; Siqueira et al., 2011; Pinheiro et al., 2013), and plant growth hormones (Hwang et al., 2011). The use of endophytic fungi in various industrial processes has aroused further study of these microorganisms, and led to the discovery of new compounds with industrial and pharmaceutical potential (Meng et al., 2011; Wang and Dai, 2011). The initial production of ‘Taxol’, an anticancer drug, for example, was from the medicinal plant Taxus brevifolia. A study of the endophytic fungi associated with T. brevifolia showed the ability of a new hyphomycete, Taxomyces andreanae Strobel, A. Stierle, D. Stierle & W.M. Hess, to produce Taxol in larger quantities than in traditional production (Stierle et al., 1993; Strobel et al., 1993). Besides knowing their biotechnological importance, the study of endophytic fungi has contributed to the
knowledge of diversity within this group, and new species have been reported with active extracellular metabolites (Siqueira et al., 2008, 2011).

Medicinal plants have been studied from the point of view of potential endophytic interactions, and have shown many benefits, such as production of antibiotics, secondary metabolites of pharmacological interest, biomarkers of vitality, and biological control agents against pests and diseases (Sun et al., 2008; Hilario et al., 2011; Bagchi and Banerjee, 2013; Pinheiro et al., 2013). Bauhinia forficata Link is widely used in folk medicine in Brazil. This plant has pharmacological and/or biological properties, and has been used in herbal preparations for the treatment of diseases such as diabetes, infections, and as a painkiller (Gupta, 1995; Teske and Trentini, 1995). It has also been reported that that Bauhinia species have in vitro antimicrobial activity against fungi (e.g. Aspergillus, Botrytis, Candida, Cladosporium, and Cryptococcus,) and bacteria (e.g. Salmonella, Staphylococcus, and Streptococcus) (Silva and Cechinel-Filho, 2002). Despite the biotechnological potential of Bauhinia, to our knowledge no-one has examined the endophytic fungal community of this plant or the biotechnological potential of these microorganisms.

The aims of the present study were to: 1) investigate the endophytic mycobiota from leaves, sepals, stems and seeds of B. forficata; 2) determine the diversity and similarity of endophytic fungi from different tissues; 3) screen isolated endophytic fungi as potential agents against bacteria pathogenic to humans; and 4) detect the capacity of the endophytes to produce extracellular hydrolytic enzymes. To our knowledge, this is the first study of endophytic fungi from different tissues of B. forficata and their biotechnological potential.

Material and Methods

Isolation, identification, and frequency of endophytic fungi

The collection of leaves, stems, sepals, and seeds from healthy specimens of Bauhinia forficata was made randomly in the Didactic Garden of the Center of Biological Sciences, Federal University of Pernambuco, Recife, Brazil (8°3.047' S; 34°56.895' W), from September to October, 2008. Forty-five fragments were used from each plant tissue.

This botanical material was processed within 24 h, following Araújo et al. (2002) and Siqueira et al. (2011). After disinfection process, 6 mm disks were obtained from leaves and sepals, and 6 mm² fragments were obtained from stems and seeds. These were transferred to Petri dishes containing the potato dextrose agar (PDA) culture medium supplemented with chloramphenicol (100 mg L⁻¹). The dishes were incubated at 28 ± 2 °C for up to 30 days in alternated periods of dark and light. Fungal colonies were isolated, purified, and maintained in PDA for identification.

To check the efficacy of the surface sterilization, water samples (1 mL) from the last rinse were inoculated onto Petri dishes containing the same medium, using the same incubation conditions.

For identification of endophytic fungi, micro-cultivations were performed and the macro and micro morphological aspects of the somatic and reproductive structures were observed, using specific methodology and literature (Ellis, 1971; Sutton, 1980; Samson and Frisvad, 2004; Leslie and Summerell, 2006; Domsch et al., 2007).

The absolute frequency was calculated as the total number of endophytes isolated. For the relative frequency, the number of isolates in each species was divided by the total number of isolates (Larran et al., 2002).

Representative cultures of endophytic fungi isolated from B. forficata have been deposited into the URM Culture Collection of the Federal University of Pernambuco, Recife, Brazil (URM 5937, 5962, 5968, 5990, 5991, 5998-6001, 6011-6014, 6054-6059, 6229, 6234, and 6235).

Data analyses

The Simpson’s diversity index, Shannon-Wiener index, and Evenness were calculated using PAST 1.7 software (Hammer et al., 2001). To compare the frequency and richness of endophytic fungi on leaves, sepals, stems and seeds of B. forficata, the experimental design was completely randomized. Data were subjected to analysis of variance using the F test (ANOVA) (ASSISTAT Program version 7.7) and then the means were compared by Tukey test at 1% probability. Sorensen’s similarity coefficient was employed and a binary matrix was produced and used to calculate the similarity matrix (DICE coefficient, Sorensen) and to plot a UPGMA dendrogram using NTSYSpc 2.10.

Antibacterial activity of endophytic fungi

Human pathogenic bacteria were obtained from the Collection of Microorganisms, Department of Antibiotics (UPFEDA), Federal University of Pernambuco, Brazil. The endophytic fungi were subjected to an antibacterial assay using a solid medium (Ichikawa et al., 1971); this permitted a rapid and qualitative selection of the bioactive microorganisms. Each fungus was cultivated on the PDA surface in Petri dishes at 28 °C for 7 days. Six diameter disks were transferred to the surface of nutrient agar and/or brain heart infusion media previously spread with a test microorganism: Staphylococcus aureus Rosenbach (UPFEDA02), Streptococcus pyogenes Rosenbach (UPFEDA07), Mycobacterium smegmatis (Trevisan) Lehmann & Neumann (UPFEDA71), Bacillus subtilis (Ehrenberg) Cohn (UPFEDA86), Enterococcus faecalis (Andrewes & Horder) Schleifer & Klipper-Bülz (UPFEDA138), Salmonella typhi (Schroeter) Warren & Scott (UPFEDA478), Pseudomonas aeruginosa (Schroeter) Migula (UPFEDA735), Enterobacter
were isolated only from seeds. The colonization and frequency of endophytes was observed in stems and the lowest in seeds. The richness of endophytic fungi varied in different tissues of *B. forficata* \((F = 25.00; p < 0.01)\). Six, seven, and eight species were found in seeds, sepals, and leaves, respectively. The largest number (11) of species was found in the stems \((p < 0.01)\).

The diversity of the endophytic community isolated from different tissues of *B. forficata* was compared using indices of \( \alpha \)-diversity. The Simpson’s dominance of endophytic fungi was higher in the seeds. Both Simpson and Shannon-Wiener’s diversity indices were higher in the stems. The species richness was also greater in the stems. There was little difference in species evenness among the tissues studied \((p < 0.01)\). Sorensen’s similarity index showed that although the similarity between stems and seeds may be considered low, only 25% similarity between these tissues was sufficient to make them cluster differently from the leaves and sepals \((p < 0.01)\). The diversity of the endophytic community isolated from different tissues of *B. forficata* was compared using indices of \( \alpha \)-diversity. The Simpson’s dominance of endophytic fungi was higher in the seeds. Both Simpson and Shannon-Wiener’s diversity indices were higher in the stems. The species richness was also greater in the stems. There was little difference in species evenness among the tissues studied \((p < 0.01)\). Sorensen’s similarity index showed that although the similarity between stems and seeds may be considered low, only 25% similarity between these tissues was sufficient to make them cluster differently from the leaves and sepals \((p < 0.01)\).

Antibacterial activity of endophytic fungi

Thirty-two endophytic fungi isolated from *B. forficata* were tested for antibacterial activity by disc diffusion assay against 10 clinical isolates of human pathogenic bacteria. Of 32 isolates, 11 (34.3%) showed antibacterial activity against one or more bacteria. Gram-positive bacteria were more sensitive than gram-negative. *Penicillium commune*, *Gibberella baccata*, *P. glabrum*, and *Aspergillus ochraceus* exhibited a high range of antibacterial activity, and inhibited the growth of four out of 10 pathogenic bacteria. *Khuska oryzae* only showed activity against *Salmonella typhi*; however, this fungus was the only one to inhibit this bacterium and it exhibited the maximum inhibition zone \((38 \text{ mm})\) among the fungi tested \((p < 0.01)\). The diversity of the endophytic community isolated from different tissues of *B. forficata* was compared using indices of \( \alpha \)-diversity. The Simpson’s dominance of endophytic fungi was higher in the seeds. Both Simpson and Shannon-Wiener’s diversity indices were higher in the stems. The species richness was also greater in the stems. There was little difference in species evenness among the tissues studied \((p < 0.01)\). Sorensen’s similarity index showed that although the similarity between stems and seeds may be considered low, only 25% similarity between these tissues was sufficient to make them cluster differently from the leaves and sepals \((p < 0.01)\).

Screening to detection the capacity to produce enzymes

The 19 endophytes randomly screened for enzyme production were representative of the taxa rare and more frequent. Table 4 shows the results of cultivating the endophytic fungi in specific solid media for the purposes of detecting their ability to produce proteases, cellulases, xylanases, and lipases.

Among the species analyzed, 14 (73%) showed cellulolytic activity. Five of these showed a ZA between 0.9 and 1.0 \((p < 0.01)\). *Penicillium commune*, *P. glabrum* \((p < 0.01)\), and *Aspergillus ochraceus* \((p < 0.01)\) showed activity against one or more bacteria. Gram-positive bacteria were more sensitive than gram-negative. *Penicillium commune*, *Gibberella baccata*, *P. glabrum*, and *Aspergillus ochraceus* exhibited a high range of antibacterial activity, and inhibited the growth of four out of 10 pathogenic bacteria. *Khuska oryzae* only showed activity against *Salmonella typhi*; however, this fungus was the only one to inhibit this bacterium and it exhibited the maximum inhibition zone \((38 \text{ mm})\) among the fungi tested \((p < 0.01)\).

**Antibacterial activity of endophytic fungi**

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zyme production (ZA = 0.83) while most of the fungi tested showed ZAs < 0.69 (very strong). Ten isolates (52%) were lipase positive. The fungi that stood out as good producers of this enzyme were: *Aspergillus ochraceus*, *A. chartarum*, *M. verrucaria*, *M. schulzeri*, and *P. glabrum*.

*Myrmecridium schulzeri* and *P. glabrum* were the best producers of all the fungi tested, and were classified as ‘very strong’ for cellulases, xylanases, and lipases, among the four enzymes tested.

**Discussion**

We found highest frequency of colonization by endophytic fungi in the stems (46 isolates), and the most frequent species were *Gibberella* (14.74%), *Myrothecium* (10.53%), and *Acremonium* (9.5%) (Table 1). In contrast, Siqueira *et al.* (2001) studied the species composition of endophytic fungi from *Lippia sidoides*, and found that colonization of leaves (50.41%) was higher than that of stems (35.40%); the most frequently isolated species were *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (12.3%) and *Alternaria alternata* (Fr.) Keissl. (7.08%). Analyzing the diversity of endophytic fungi from the leaves and stems of *Nyctanthes arbor-tristis*, Gond *et al.* (2012) isolated a larger number of fungi in the leaves (281 isolates) than in the stems (126 isolates). In Brazil, Mussi-Dias *et al.* (2012), investigating the composition of endophytic fungi in the leaves of 11 medicinal plants, reported the isolation of only 20 endophytes. In the same study, the researchers also analyzed leaves of *B. forficata* and recovered only two isolates identified as *Colletotrichum* sp. and *Nigrospora* sp., thus showing differing results from ours.

| Endophytic fungi               | Plant tissues | f  | fr (%) |
|-------------------------------|---------------|----|--------|
|                               | Leaves        | Sepals | Stems | Seeds |
| *Acremonium curvulum* W. Gams | 5             | 4    | 9      | 9.5   |
| *Ascotricha chartarum* Berk.  |               | 2    | 2      | 2.1   |
| *Aspergillus niger* Tiegh.    | 5             | 5    | 5.26   |
| *A. ochraceus* G. Wilh.       | 4             | 3    | 7      | 7.37  |
| *Cochliobolus australiensis* (Tsuda & Ueyama) Alcorn | 1 | 1    | 1.05  |
| *C. lunata* R.R. Nelson & F.A. Haasis | 1 | 5    | 6      | 6.32  |
| *Cladosporium oxysporum* Berk. & M.A. Curtis | 6 | 6    | 6.32   |
| *C. sphaerospermum* Penz.     | 5             | 5    | 5.27   |
| *Diplococcium spicatum* Grove |               | 1    | 1      | 1.05  |
| *Gibberella baccata* (Wallr.) Sacc. | 4 | 4    | 4.21   |
| *G. fujikuroi* (Sawada) Wollenw. | 5 | 5    | 10     | 10.53 |
| *Khusia oryzae* H.J. Huds.    | 3             | 3    | 3.15   |
| *Lasmenia balansae* Speg.     |               | 1    | 1      | 1.05  |
| *Myrmecridium schulzeri* (Sacc.) Arzanlou, W. Gams & Crous | 1 | 1    | 1.05  |
| *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar | 10 | 10   | 10.53  |
| *Nodulisporium* Preuss        | 1             | 1    | 1      | 1.05  |
| *Penicillium aurantiogriseum* Dierckx | 1 | 1    | 1.05  |
| *P. commune* Thom              | 2             | 2    | 2.1    |
| *P. corylophilum* Dierckx     |               | 1    | 1      | 1.05  |
| *P. glabrum* (Wehmer) Westling | 2 | 1    | 3      | 3.16  |
| *P. implicatum* Biourge       | 2             | 2    | 2      | 2.1   |
| *Phoma putaminum* Speg.       |               | 1    | 1      | 1.05  |
| *Phonopsis diachenii* Sacc.   | 2             | 2    | 2.1    |
| *Pithomyces atro-olivaceus* (Cooke & Harkn.) M.B. Ellis | 1 | 1    | 1.05  |
| *Spegazzinia tesserithra* (Berk. & M.A. Curtis) Sacc. | 1 | 1    | 1.05  |
| *Talaromyces faniculatus* (Thom) Samson, Yilmaz, Frisvad & Seifert | 2 | 2    | 2.1   |
| *Trichoderma piluliferum* J. Webster & Rifai | 7 | 7    | 7.37   |
| **Total**                     | 18            | 22   | 46     | 9      | 95 |
| **Species richness**          | 8             | 7    | 11     | 6      | 28 |
In our study some species were specific to plant tissues, for example, *A. chartarum* to seeds, *A. niger* to stems, *C. australiensis* to leaves, and *C. oxysporum* to sepals. Endophytic assemblages tend to be distributed in specific

**Table 2 - Diversity indices for endophytic fungi isolated from Bauhinia forficata.**

| Indices             | Leaves  | Sepals  | Stems  | Seeds  |
|---------------------|---------|---------|--------|--------|
| Simpson’s dominance | 0.1914  | 0.1901  | 0.1257 | 0.2099 |
| Simpson’s diversity | 0.8086  | 0.8099  | 0.8743 | 0.7901 |
| Species richness    | 8       | 7       | 11     | 6      |
| Shannon-Wiener      | 1.842   | 1.772   | 2.206  | 1.677  |
| Evenness (E)        | 0.7888  | 0.8401  | 0.8252 | 0.8916 |

**Figure 1 - Sorensen’s similarity coefficient for the endophytic fungi isolated from Bauhinia forficata.**

**Table 3 - Antibacterial activity (halo size in mm) of endophytic fungi isolated from Bauhinia forficata.**

| Endophytic fungi          | Pathogenic bacteria (UFPEDA)* |
|--------------------------|-------------------------------|
|                          | 02   | 07   | 71   | 86   | 138  | 478  | 735  | 739  | 740  | 224  |
| **Sepals**               |      |      |      |      |      |      |      |      |      |      |
| Gibberella baccata       | 22   | 21   | 22   | 17   |      |      |      |      |      |      |
| Khuska oryzae            |      |      |      |      |      |      |      |      |      |      |
| Penicillium commune      | 24   | 21   | 21   | 17   |      |      |      |      |      |      |
| **Stems**                |      |      |      |      |      |      |      |      |      |      |
| Aspergillus niger        | 11   | 15   |      |      | 15   |      |      |      |      |      |
| A. ochraceus             |      |      |      |      |      |      |      |      |      |      |
| Cochliobolus lunatus     | 28   |      |      |      |      |      |      |      |      |      |
| Penicillium glabrum      | 24   | 37   | 21   | 21   |      |      |      |      |      |      |
| **Seeds**                |      |      |      |      |      |      |      |      |      |      |
| Ascotricha chartarum     |      |      |      |      |      |      |      |      |      |      |
| Aspergillus ochraceus    | 16   | 14   | 13   | 15   |      |      |      |      |      |      |
| P. coryophilum           | 15   |      |      |      |      |      |      |      |      |      |
| P. glabrum               |      |      |      |      |      |      |      |      |      | 11    |

*Pathogenic bacteria: *S. aureus* (UFPEDA02), *S. pyogenes* (UFPEDA07), *M. smegmatis* (UFPEDA71), *B. subtilis* (UFPEDA86), *E. faecalis* (UFPEDA138), *S. typhi* (UFPEDA478), *P. aeruginosa* (UFPEDA735), *E. aerogenes* (UFPEDA739), *P. vulgaris* (UFPEDA740), and *E. coli* (UFPEDA224).
hosts and specific tissues (Siqueira et al., 2011). Xing et al. (2010) observed tissue specificity for endophytic fungi in the roots, stems, and leaves of Panax quinquefolium. Indeed, Petri et al. (1992) concluded that different plant tissues and organs may represent distinct microhabitats. Moreover, the host-plant age, associated vegetation, elevation, and exposure can all affect endophytic assemblages and frequency of colonization (Kusari et al., 2013).

Although many species of fungi are commonly described as endophytes, others can be found occasionally colonizing the host tissue and are isolated only once or twice in several samples (Siqueira et al., 2011; Pinheiro et al., 2013). The literature reports Alternaria, Cladosporium, Colletotrichum, Guignardia, Phoma, Phomopsis, Phyllosticta, and Xylaria as endophytes of various plant tissues (Costa et al., 2012; Bezerra et al., 2012a, 2012b, 2013). We found that A. chartarum, C. australiensis, D. spicatum, L. balansae, P. corylophilum, P. glabrum, P. atro-olivaceus, M. schulzeri, S. tessartihra, and T. piluliferum are reported as the first occurrence of these endophytes in Brazil.

Species of Aspergillus, Gibberella, and Penicillium that we isolated have been questioned as true endophytic fungi. However, several studies with various species of plants, including medicinal plants, have reported the isolation of species within these genera as endophytes (Bezerra et al., 2012a, 2013; Kusari et al., 2013).

The diversity of endophytic fungi was highest in the stems (Table 2). Kumar and Hyde (2004), analyzing endophytic fungi associated with Tripterygium wilfordii, reported the highest Shannon diversity index in the twig xylem followed by the leaves. In our study, species richness was higher in the stems (11 species) and the Sorensen similarity index was about 25% between the tissues of stems and seeds (Table 2). In contrast, Gond et al. (2012), studying medicinal plants of India, observed higher species richness of endophytic fungi in leaves than in stems, and a greater similarity in species distribution of fungi in the leaves. Studying the diversity and dynamics of fungal endophytes in leaves, stem and roots of Chinese medicinal plant Stellera chamaejasme, Jin et al. (2013) verified that Simpson’s dominance (D) and Evenness (E) were greater in the roots (D = 0.99 and E = 0.34) than in the other studied tissues. Researching the endophytic fungal communities from Madhuca indica at different locations in India, Verma et al. (2013) observed a greater diversity in stem (D = 0.18) and in leaves (E = 0.6). These authors suggest that the highest frequency of colonization in stem may be due to spore abundance of a few dominant endophytes in stem tissue.

Endophytic fungi have shown potential in the synthesis of a wide range of biologically active metabolites (Aly et al., 2011), and are a diverse field of study and a valuable resource with enormous potential. New approaches must be designed efficiently and chemical study of this diversity needs to be intensified to discover and develop new medicines (Schulz et al., 2002). We screened the antibacterial activity of endophytic metabolites against clinical isolates of human pathogenic bacteria. A total of 34.3% of the isolates showed activity. Among these, Penicillium commune, P. glabrum, Gibberella baccata, and Aspergillus ochraceus stand out as the most effective against all gram-positive strains. Similarly, Shim et al. (2006) demonstrated that Penicillium griseofulvum Dierckx produces secondary metabolites, such as mycophenolic acid, active against this group of bacteria. Cui et al. (2011) studied the antimicrobial and antitumor activity of endophytic fungi isolated from the medicinal plant Aquilaria sinensis (Thymelaeaceae), and found that species within Fusarium [Gibberella] also demonstrated activity against S. aureus and B. subtilis.

Among the three fungi that showed activity against gram-negative bacteria, two belong to Aspergillus. This genus has been reported as a good inhibitor of microorganisms by Souza et al. (2004), who studied the antimicrobial activity of endophytic fungi isolated from toxic plants in the Brazilian Amazon, and by Sadananda et al. (2011), who tested endophytes from Tabebuia argentea against fungi and bacteria pathogenic to humans.

Although Myrothecium verrucaria was frequently isolated (10.41%) from B. forficata, it showed no antibacterial capacity. Some research has shown other capacities of M. verrucaria, such as potential to be a biological control agent of invasive plants from the inoculation of spores (Clarke et al., 2007) and to be included in bioherbicide formulations (Hoagland et al., 2007). However, some isolates of this species may produce mammalian toxins (Anderson and Hallett, 2004). Furthermore, this species has been reported to produce enzymes (Halliwell, 1961).

Khuskia oryzae [Nigrospora oryzae] produced the greatest inhibition zone (38 mm) against S. typhi, a causal agent of typhoid fever in humans. These results corroborate with recent studies that have shown the endophyte K. oryzae to be the main inhibitor of S. paratyphi (Gond et al., 2012); however, the halo formed by this species (22 mm) in our work was larger. The antibiotic chloramphenicol is considered the drug for the treatment of typhoid fever (Pinheiro et al., 2013) and according to the standard table of the Clinical and Laboratory Standards Institute (CLSI) a halo above 18 mm demonstrates that bacteria is sensitive to that antibiotic. Accordingly, K. oryzae was more efficient than the standard antibiotic and indicates the potential of the fungus for production of antibacterial compounds.

Enzymes are also among the wide variety of primary or secondary metabolites that can be produced by microorganisms, especially fungi (Stamford et al., 1998). In our work, species of Penicillium and Myrmecridium were among the best producers of cellulase. Similarly, Ruegger and Tauk-Tornisielo (2004) highlighted P. glabrum as an excellent producer of this enzyme. Myrmecridium
*schulzeri* was found in leaf litter species from the Caatinga ecosystem in Brazil (Cruz and Gusmão, 2009), showing cellulase production ability even in extreme environments.

The proteolytic activity displayed by isolates was mostly considered low, and 31% of the fungi tested failed to produce this enzyme in culture. These results are confirmed by Silva et al. (2006), where 82% of the fungi studied showed no ability to degrade medium containing casein as the protein source. The only fungus with strong production of this enzyme, *Phoma putaminum*, is recognized for its potential as a herbicide, its ability to produce protease, and its production of putaminoxin, a substance with high phytotoxic effects (Evidente et al., 1995).

In contrast, we found that all isolates tested were xylanase positive. This was also observed by Rugegger and Tauk-Tornisielo (2002); in their study, all isolates (e.g. *Aspergillus*, *Cladosporium*, *Penicillium*, and *Trichoderma* species) produced this enzyme, probably because they had been isolated from plant tissue, which has xylan in its cell walls. In our work, *Acremonium curvulum* was positive for xylanase only. However, Braz et al. (2009), studying different strains of *A. curvulum*, found that enzyme activity varied with the substrate used, emphasizing the view that the halo can be changed by the composition of the culture medium. This may explain the results we obtained.

Lipase production by microorganisms has no known function, but is thought to be related to the location and possibility of expansion of pathogenic fungal infection (Rivera-Orduña et al., 2011). In our study, the fungi that were obviously good producers of this enzyme were *A. chartarum*, *A. ochraceus*, *M. schulzeri*, *M. verrucaria*, and *P. glabrum*. Different results were gained by Silva et al. (2006), where *Aspergillus* and *Penicillium* isolated from *Annona* spp. did not produce lipase; demonstrating that the enzyme production capacity varies among species and that it is therefore necessary to select isolates.

Our results demonstrate that different tissues of the medicinal plant *B. forficata* harbor distinct endophytic fungi communities. The wide richness of endophytes in different tissues of *B. forficata* seems to have an important ecological role in healthy plant tissues. This can also be suggested from the different endophytic species reported as the first occurrence as endophyte to Brazil, confirming that *B. forficata* is a reservoir of diversity of fungal endophytes. The bioactive compounds produced by these fungi can be a source of new therapeutic agents for the effective treatment of diseases in humans, other animals, and plants. In addition, endophytes can be used in the production of enzymes of industrial and environmental interest. The species *A. ochraceus*, *F. lateritium*, *P. commune*, and *P. glabrum* are indicated for use in future experiments regarding antibacterial activity, and *M. schulzeri* and *P. glabrum* for enzymes production. Future studies of endophytic fungi from *B. forficata* are necessary to gain knowledge of its potential in the production of antimicrobial compounds and extracellular hydrolytic enzymes.

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