African Journal of Biotechnology Vol. 11(64), pp. 12766-12772, 9 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB12.1403
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

The isolation and characterization of endophytic microorganisms from *Hyptis marrubioides* Epling roots

Luciana Cristina Vitorino[1]*, Fabiano Guimarães Silva[1], Marcos Antônio Soares[2], Edson Luiz Souchie[1] and William Cardoso Lima[2]

[1]Instituto Federal Goiano - Campus Rio Verde, Rodovia Sul Goiana, Km 01, Zona Rural, 75901-970, Rio Verde, GO, Brasil.
[2]Instituto de Biociências, Universidade Federal do Mato Grosso (UFMT), Av. Fernando Corrêa Costa, nº 2367, Bairro Boa Esperança, 78060-900, Cuiabá, MT, Brasil.

Accepted 25 July, 2012

Endophytic microorganisms asymptomatically colonize healthy plant tissues and may be related to the plant's resistance to attack by pathogens or even to the synthesis of secondary metabolites. The present study was aimed at isolating and characterizing endophytic strains from the root system of *Hyptis marrubioides*. Coarse and fine root fragments were collected for diaphanization and surface disinfection to isolate endophytes. After 10 days of incubation, we obtained the colonization rate (CR) of the fragments and the endophytic were purified and maintained in culture medium. The bacteria were partially characterized using Gram stain and a catalase test. Fungi were identified by distinguishing between reproductive structures using a microculture technique. While observing diaphanized root fragments, we found arbuscular mycorrhizal fungi (AMF) and dark septate endophytic (DSE) fungi in the fine and coarse roots of *H. marrubioides*. The endophytic CR was more significant in coarse root fragments. In both types of roots, the percentage of bacteria was higher than the percentage of fungi. Gram-positive and catalase-positive bacteria accounted for the majority of bacterial isolates, which were predominantly bacilli. Of all the fungal isolates, the majority had sporulating mycelium, which mainly consisted of fungi from the genus *Penicillium*, *Fusarium*, *Trichoderma* and *Papulaspora*.

Key words: Bacteria, fungus, Lamiaceae, root system.

INTRODUCTION

Endophytic microorganisms are defined by their ability to colonize plant tissues without causing symptoms or morphological changes (Strobel et al., 2004). Studies on the interaction between plants and endophytic microorganisms (Lacava et al., 2008; Dias et al., 2009) show that these organisms perform different and important ecological functions: they act to promote plant growth (Ahmad et al., 2008), protect the host against herbivory (Breen, 1994) and/or produce biologically active secondary metabolites, many of which are important for biotechnology (Cafêu et al., 2005). Few studies exist that describe the endophytic associations with the genus *Hyptis*. Notable among these is the study by Biradar and Reddy (2007) on the intensity of colonization by arbuscular mycorrhizal fungi (AMF) in roots of *Hyptis suaveolens* Poit and four other medicinal plants, as well as the influence of these fungi in the accumulation of biomass by plants inoculated. For this species, the efficiency index of mycorrhizal was approximately 44%. Also notable is the work of Selosse et al. (2009), who attempted to identify the presence of *Sebacinales* in *Hyptis verticillata* Jacq. However, the

*Corresponding author. E-mail: lu.vitorino@hotmail.com. Tel: (64) 3620 5600. Fax: (64) 3620 5616.

Abbreviations: AMF, Arbuscular mycorrhizal fungi; **CR,** colonization rate; **FAA,** formaldehyde glacial acetic acid and ethyl alcohol; **PDA,** potato dextrose agar; **CMA,** corn meal agar; **DSE,** dark septate endophytes.

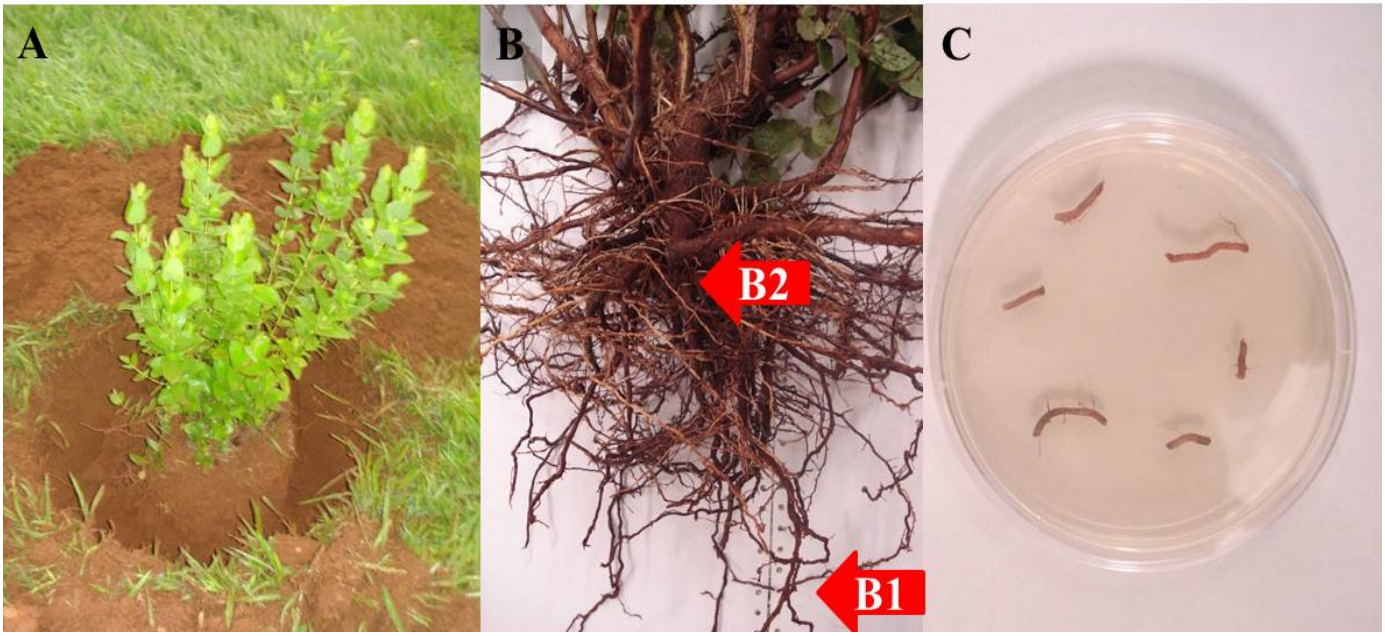

Figure 1. Photos showing (A) *Hyptis marrubioides* Epling removed from the soil, (B) two morphologically different classes of plant root from *H. marrubioides* Epling (B1 - coarse root; B2 - fine root) and (C) fragments of root inoculated in PDA medium for isolating endophytes.

authors failed to isolate any of these fungi from this specific plant species.

Species of the genus *Hyptis* have a characteristic aroma, which may have antifungal (De Oliveira et al., 2004), antibacterial (Souza et al., 2003), larvicidal (Costa et al., 2005) and antidepressant (Bueno et al., 2006) properties. The medicinal species *Hyptis marrubioides* Epling, which is found in the cerrado ecoregion (Rodrigues and Carvalho, 2001), has received attention because of its essential oil terpenoids, such as caryophyll-4(14),8(15)-dien-5β-ol, eudesma-4(15),7-dien-1β-ol, caryophyllene oxide and (β)-caryophyllene (Sales et al., 2007). Terpenes are used in traditional medicine for their antibacterial (Kang et al., 1992), anti-inflammatory (Shimizu, 1990) and even antitumor properties (Zheng et al., 1992). Since no data exist on endophytism in *H. marrubioides* Epling, this study was aimed at isolating and characterizing the endophytic strains of its root system.

MATERIALS AND METHODS

Obtaining plant material

The *H. marrubioides* Epling plant that was selected to assess the presence of endophytic microorganisms was a specimen originally cultivated *in vitro,* acclimatized and subsequently adapted to the external conditions of the greenhouse at the Plant Tissue Culture Laboratory, Goiano Federal Institute –Rio Verde Campus, the geographical coordinates of which are 17º 47' 53'' north latitude and 51º 55' 53'' south latitude, at an altitude of 743 m. The plant was entirely manually removed from the soil (Figure 1A) and placed in an insulated box, and the material was processed within 24 h.

Isolation of endophytic microorganisms

We were able to distinguish two classes of morphologically distinct roots from the *H. marrubioides* Epling root system: the first class consisted of coarse roots with a dark color and fine roots, which are lighter in color (Figure 1B). These two classes of roots were analyzed separately for the presence of endophytes. Root fragments of approximately 10 cm in length were collected and subjected to a disinfection pretreatment, based on the methods described by Petrini and Muller (1986). A number of approximately 1 cm fragments were fixed, kept in formaldehyde, glacial acetic acid and ethyl alcohol (FAA; 50% at a 5:5:90 ratio) for diaphanization and then stained to observe fungal structures. The remaining roots were immersed in water with a mild detergent, shaken at 150 rpm for 5 min and rinsed with distilled water. The material was disinfected under a laminar flow hood with 70% alcohol (v/v) for 1 min, 2.5% sodium hypochlorite (v/v) for 3 min and again with 70% alcohol (v/v) for 30 s. The root fragments were then rinsed three times with sterile distilled water and the excess moisture was removed using sterile filter paper. To control for the cleansing process, 500 µL of the water used in the final rinse of the samples was inoculated into nutrient broth and grown while shaking at 150 rpm overnight at room temperature.

Six approximately 1 cm root fragments were distributed on the surface of Petri dishes containing PDA medium (infusion of 200 g potatoes, 20 g dextrose, 15 g agar and water to a final volume of 1000 ml) (Figure 1C). The plates were incubated in a bacteriological incubator at 30°C and observed daily for 10 days. Colonization was assessed daily for 10 days to determine the colonization rate (CR) (Petrini et al., 1992), using the formula:

$$\text{Colonization Rate} = \frac{\text{Number of fragments with endophytes}}{\text{Total number of fragments analyzed}} \times 100$$

Collection, purification and maintenance of endophytes

Individual bacterial colonies were purified using the streaking method; the fungi were purified by taking a fragment of the young mycelium that emerged from the edges of the root fragments. The isolates were stored on nutrient agar plates.

Characterization of endophytic isolates

The bacterial isolates were tested for their morphological and biochemical characteristics (catalase enzyme activity). A Gram stain (Humphries, 1974) was performed to determine the characteristics of the cell wall, cell shape and the arrangement of cells. The morphology of the endophytic bacterial strains was observed on slides under a microscope. For staining, 15 µL of a bacterial culture that was grown in nutrient broth overnight at room temperature with shaking at 150 rpm was heat-fixed onto a slide and then stained. To test the strain for catalase enzyme activity, 15 µL of the culture was placed on a slide and 15 µL H_2O_2 (30%) was added. The catalase-positive strains were characterized by the intense production of bubbles.

Furthermore, the fungal isolates were characterized using a micro-cultivation technique on slides after first being cultivated on corn meal agar (CMA) for seven days at 30°C. The slides were then stained with lactophenol. The structures were observed using a Leica DM500 photomicroscope with a Leica ICC 50 camera, which was adapted to use LAZ EZ software, version 1.8.0. The samples were then compared to other samples reported in the literature (Sutton, 1980; Carmichael et al., 1980; Alexopoulos et al., 1996).

Diaphanization of root material

To verify the existence of fungal structures inside the roots of *H. marrubioides* Epling, samples that had been previously fixed in FAA were diaphanized using the modified method described by Koskey and Gemma (1989). Briefly, these roots were immersed in KOH (2%), autoclaved and transferred to a new solution of KOH (2%) for 24 h. The samples were then transferred to an alkaline solution of ammonia (0.5%) and H_2O_2 (0.5%) in water for 60 min. Finally, the roots were stained with 0.05% trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol, water) (Phillips and Hayman, 1970). The fragments were mounted on slides and observed under a microscope. Finally, the fungal structures were classified according to Petrini (1986) and Peterson et al. (2004).

Experimental design

The experiment was conducted using a completely randomized design in which the treatments included two types of roots: coarse and fine. Seven plates were inoculated per treatment. Each plate, containing six pieces of root, was considered a sampling unit. Analysis of variance was used to analyze the data and the relative means for the rate of colonization were compared using the Tukey's test (5%). All analyses were performed with the help of the statistical software SISVAR (Ferreira, 2003).

RESULTS AND DISCUSSION

Microscopic analysis of *H. marrubioides* Epling roots showed that fungal structures were present in both types of roots that were studied. There were no significant morphological changes or symptoms of disease in the roots that were collected for this work. The observed structures allowed us to identify the fungi as a group of arbuscular mycorrhizal fungi (AMF) and as another endophytic group known as dark septate endophytes (DSE) (Figure 2). These groups were also found in all of the plants from the family Lamiaceae that were analyzed by Weishampel and Bedford (2006) while studying species from four wetlands in New York City. Moreover, while determining the presence of AMF and DSE in the recolonizing vegetation of a forest in Texas, Stevens et al. (2010) evaluated the species *Teucrium canadense* L. In the Lamiaceae; they found the presence of these two fungal groups, which occurred at an average frequency.

In our study, structures such as arbuscular, vesicular and intensely stained blue hyphae marked the presence of AMF (Figure 2E and F). Some of these vesicles occupied almost the entire volume of the cell, whereas other vesicles were slightly less developed. We observed the presence of a Paris-type arbuscular mycorrhizal association, characterized by the intense involvement of hyphae in the root cortex (Figure 2D). Arum-type associations were not seen in the stained fragments. Smith and Smith (1997) suggested a preference for Paris-type associations in plants from the cerrado ecoregion under natural conditions. However, in studies of AMF in Lamiaceae, such as the one reported by Zubek and Blaszkowski (2009), a tendency for Arum-type associations has been identified. They analyzed 31 medicinal species and found AMF in 30 of them, 23 of which were of the Arum-type. Of these species analyzed, seven were Lamiaceae, while only one was a Paris-type association. The benefits of associating with AMF have been shown by the efficiency with which some species enhance the vegetative growth and nutritional status of plants (Nunes et al., 2010). The edaphic and climatic conditions of cerrado *stricto sensu* limit the growth and establishment of plant species. Herbs such as *H. marrubioides* do not have deep root systems and therefore do not reach the water table. Consequently, these plant species need to overcome the water stress that is imposed by the first 2 m of soil depth. The formation of mycorrhizae allows for a greater volume of soil to be explored (Siqueira, 2002), which may be a decisive factor for the establishment and growth of these herbs as it increases the availability of nutrients and water that are key to generating and transporting photo-assimilates (Martins et al., 1999).

The presence of DSE was confirmed by the intraradicular identification of myelinated microsclerotial hyphae (Figure 2B, G, H and I). Microsclerotia were detected inside the root cortex, where some occupied the entire volume of the cortical cells. Other small microsclerotial structures coexisted with the dark septate hyphae, suggesting that they correspond to different developmental stages of DSE or different DSE species. In some plants, DSE are more frequent than AMF (Yuan et al., 2010). Upon analyzing the roots of seven medicinal Lamiaceae; Zubek and Blaszkowski (2009) found that

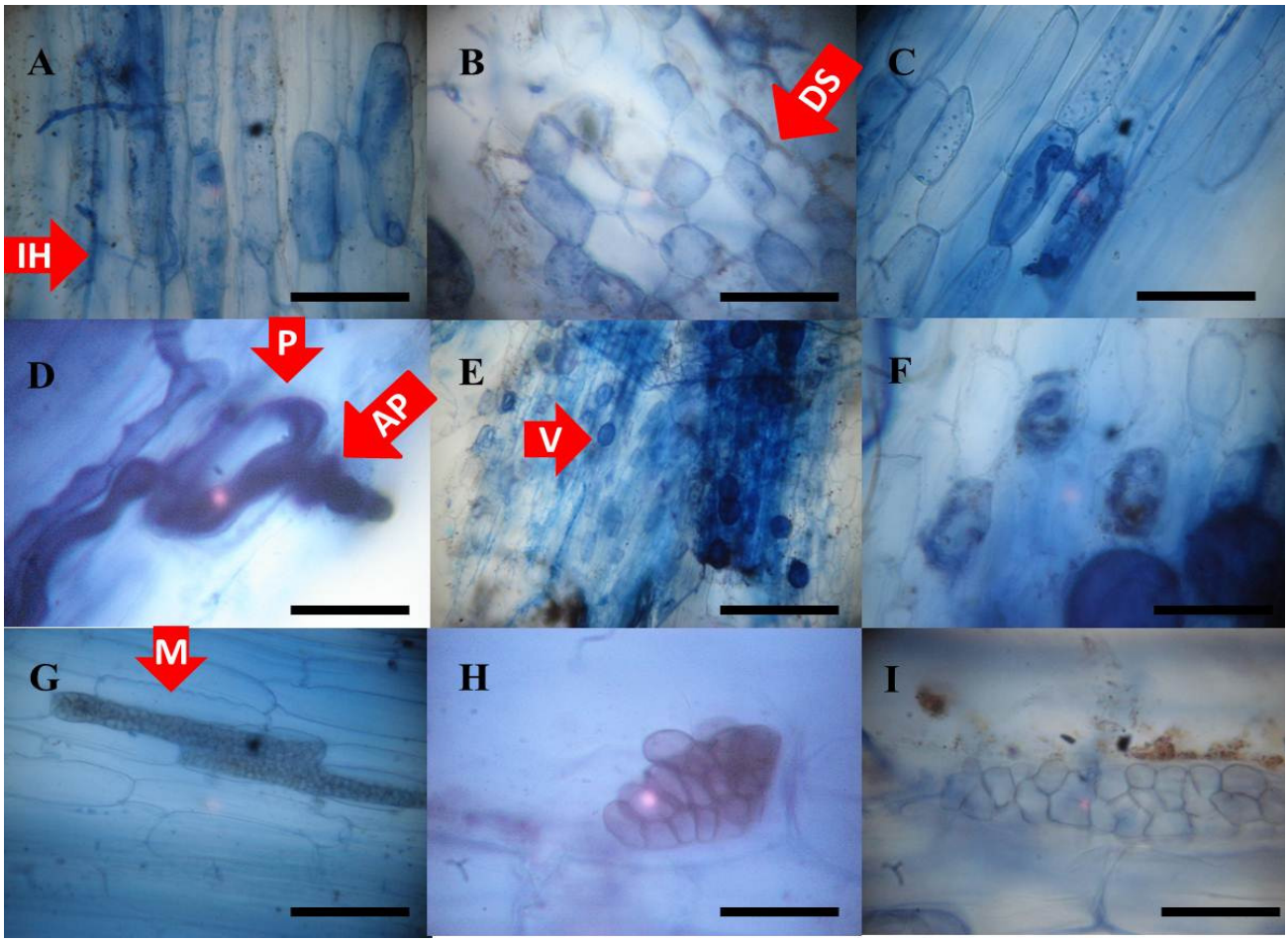

Figure 2. Extensive colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in *Hyptis marrubioides* Epling roots. (A) Blue endophytic hyphae colonizing the cortex intracellularly; (IH) internal hyphae. (B) Co-occurrence of myelinated and blue endophytic hyphae; DS - Dark septate hyphae. (C) Blue hyphae occupying the cell cytoplasm. (D) Paris-type (P) and appressorium-type (AP) arbuscular mycorrhizal association. (E) V - AMF vesicles. (F) Increased magnification of AMF vesicles. (G) M - microsclerotia internally occupying cortical cells; (H and I) increased magnification of microsclerotia. Scale bars: A, B, E and G = 50 µM; C, D, F, H and I = 20 µM.

these fungi colonized all of the analyzed plants with a colonization frequency of 66.9% in *Lavandula angustifolia* Mill. Moreover, the absence of turbidity in the nutrient broth that was inoculated with the rinse water shows the effectiveness of the process of cleaning the surface of the samples, indicating that the microorganisms that grew on the isolation medium were, in fact, of endophytic origin.

The analysis of variance for the colonization rate showed a difference between the two types of roots analyzed; however, there was not a significant difference between the treatment repetitions. This demonstrates that colonization progressed in a similar manner for all of the plates that were analyzed. However, the results were more significant for the coarse root fragments; 42 fragments were analyzed for each type of root, of which 95.2% of the coarse root fragments and 35.6% of the fine root fragments resulted in colonization. This difference may be related to the age of the tissue; the coarse roots may be older roots and therefore have a higher colonization rate. This relationship is demonstrated in other studies, including the study by Espinosa-Garcia and Langenheim (1990), which is consistent with the hypothesis that endophytes are predominantly transmitted horizontally in plants. Older plant tissues would have had more time to accumulate the endophytes from the environment, as opposed to tissues being initially colonized (Taylor et al., 1999). In both types of roots analyzed, the contribution of bacteria to the total colonization was higher than that of the fungi. These results were achieved using the same BDA culture medium, which is a selective medium for fungi. It is possible that the absence of antibacterial in the medium influenced the appearance of large numbers of bacteria compared to the small number of fungi. Monesso et al. (2008) while working with *Viguiera robusta* Gardner, an endemic species to the cerrado, observed an inhibition of

the growth of foliar endophytic bacteria in BDA using terramycin (50 mg ml^{-1}). However, Pimentel et al. (2010) did not use antibiotics in BDA when isolating endophytes associated with *Pinus taeda* L. needles and still managed to isolate 17 genera of fungi.

Significant changes in populations of autochthonous or even introduced endophytes have been reported (Roesch et al., 2007). These variations are attributed to the type of host plant, the age of the plant, the type of tissue, the sampling period and the environment (Zinniel et al., 2002). In this study, we found 42 isolates of endophytic bacteria, 27 of which were from coarse root fragments and 15 of which were from fine root fragments. Of the isolated bacteria, majority (69%) were Gram-positive, corresponding to 63% of coarse root isolates and 80% of fine roots isolates. With regard to catalase enzyme production, 55% of the isolates were catalase positive, including 51.8% of the coarse root isolates and 60% of the fine root isolates. The majority of the bacterial isolates were bacillus-shaped (57%), representing 44.4% of the coarse root isolates and 73.3% of the fine root isolates. We also isolated nine strains of endophytic fungi, seven of which had differentiated reproductive structures on PDA and CMA media and two of which were considered sterile mycelium because they did not produce reproductive structures on these media. The colonization rate was higher for the coarse root fragments where we found six fungal isolates all of which had differentiated reproductive structures, and lower for the fine root fragments where we recorded three isolates, only one of which had differentiated reproductive structures. These numbers were, however, low compared to other studies that isolated endophytes. In a study of *Taxus chinensis*; 115 fungi were isolated from bark segments and these were grouped into 23 genera, of which *Diaporthe*, *Phomopsis*, *Acremonium* and *Pezicula* were the dominant genera; 13 representative species were able to produce taxol (Liu et al., 2009).

Additionally, among the fungal isolates from the coarse root, one was classified as belonging to the genus *Penicillium* (Ascomycota, Eurotiomycetes and Eurotiales), a very common genus of endophyte that is widely studied for the production of bioactive substances. One endophyte from this genus isolated from mangrove plants, was characterized by Shao et al. (2010) as a producer of penicillin, a cytotoxic alkaloid. This type of fungi has been found to be associated with several plant species, including *Melia azedarach* L. (Marinho et al., 2009), *Murraya paniculata* (L.) Jack (Rutaceae) (Marinho et al., 2007) and *Aegiceras corniculatum* (L.) Blanco, and the endophytic strain of *Penicillium* obtained from this plant synthesized cytotoxic polyketide (Lin et al., 2008). *Fusarium* (Ascomycota, Sordariomycetes and Hypocreales) is another genus that we identified in the coarse root isolates in which we observed the presence of reproductive hyphae carrying conidiophores, arising from the edges of the slides of the microculture. This genre is commonly reported as endophytic. *Fusarium* was the most common endophyte that was isolated from two *Annonas* and is related to growth promotion in these plants (Silva, 2006). In the study by Herrera (2010), *Fusarium* was considered to be an important root fungal association (RFA), due to its wide distribution and close relationship to North American grasses.

In the present work, the genus *Trichoderma* (Ascomycota, Sordariomycetes and Hypocreales) was also isolated from coarse root fragments. The microscopic observations of this fungus also showed the presence of reproductive hyphae carrying conidiophores. Endophytic associations with this fungus have already been reported in several studies. The ample colonization of plant species by *Trichoderma* has been explained by the fact that this fungus has biocontrol activity of plant pathogens (Ownley et al., 2010) and also because it induces systemic resistance, which was arguably, shown by Perazzolli et al. (2011). Among the coarse root isolates, we also found the genus *Papulaspora* (Ascomycota). This fungus is identified by its dark brown hyphae and by its resistance structures known as sclerotia. This genus has drawn attention because of the antimicrobial capacity of its culture extracts (Ramos et al., 2010). However, extracts of endophytic *Papulaspora*, as demonstrated in studies by Gallo et al. (2010), have cytotoxic activity and are also a source of 14 secondary metabolites, three of which have recently been described for the first time.

Generally, the present study isolated root endophytes from *H. marrubioides* Epling. In future studies, we hope to isolate endophytes from other organs of this plant to assess the systemic occurrence of the strains found in this work.

Conclusions

The following were concluded thus:

(i) The staining of the roots of *H. marrubioides* Epling revealed the presence of AMFs and DSEs in two types of roots analyzed.
(ii) The rate of colonization was higher in the coarse root fragments.
(iii) The contribution of bacteria to the total colonization was higher in the two types of roots analyzed.
(iv) Gram-positive and catalase-positive bacteria accounted for the majority of bacterial isolates, which were predominantly bacilli. Of all the fungal isolates, the majority had sporulating mycelium.

ACKNOWLEDGEMENTS

The authors thank the Research Support Foundation of the Goias State (FAPEG), CAPES, and PIBIC programs for their financial support.

REFERENCES

Ahmad F, Ahmad I, Khan MS (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. 163:173-181.

Alexopoulos CJ, Mims CW, Blackwell M (1996). Introductory Mycology, IV edition.John Wiley & Sons, New York p. 868.

Biradar R, Reddy CN (2007). Response of five medicinal plants to vesicular arbuscular mycorrhizal inoculations in unsterile soil.Mycorrh. News 19:25-29.

Breen JP (1994). *Acremonium* endophyte interactions with enhanced plant resistance to insects. Annu. Rev. Entomol. 39:401-423.

Bueno AX, Moreira ATS, Silva FT, Estevam CS, Marchioro M (2006). Effects of the aqueous extract from *Hyptis pectinata* leaves on rodent central nervous system. Rev. Bras. Farmacogn. 16:317-323.

Cafêu MC, Silva GH, Teles HL, Bolzan VS, Araújo AR (2005). Substâncias antifúngicas de *Xylaria*sp., um endofítico isolado de *Palicoureamarcgravii* (Rubiaceae). Quim. Nova. 28:991-995.

Carmichael JW, Kendrick B, Conners IL, Sigler L (1980). Genera of Hyphomycetes. University of Alberta Press, Edmonton p. 386.

Costa JGM, Rodrigues FFG, Angélico EC, Silva MR, Mota ML, Santos NKA, Cardoso ALH, Lemos TLG (2005). Estudo químico-biológico dos óleos essenciais de *Hyptismartiusii, Lippiasidoides*e *Syzigiumaromaticum*frente às larvas do *Aedes aegypti*. Rev. Bras. Farmacogn 15:304-309.

De Oliveira CMA, Silva MDR, Kato L, Da Silva CC, Ferreira HD, Souza LKH (2004). Chemical composition and antifungal activity of the essential oil of *Hyptis ovalifolia* Benth. (Lamiaceae). J. Braz. Chem. Soc. 15:756-759.

Dias ACF, Costa FEC, Andreote FD, Lacava PT, Teixeira MA, Assumpção LC, Araújo WL, Azevedo JL, Melo IS (2009). Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion.World J. Microbiol. Biotechnol. 25:189-195.

Espinosa-Garcia FJ, Langenheim JH (1990). The leaf fungal endophytic community of a coastal redwood population diversity and spatial patterns. New Phytol. 116:89-97.

Ferreira DF (2003). Sisvar: versão 4.2. Lavras: UFLA.

Gallo MBC, Cavalcanti BC, Barros FWA, Moraes MO, Costa-Lotufo LV, Pessoa C, Bastos JK,Pupo MT (2010). Chemical Constituents of *Papulas poraimmersa*, an Endophyte from *Smallanthus sonchifolius* (Asteraceae), and Their Cytotoxic Activity. Chem. Biodiver. 7:2941-2950.

Herrera J (2010). Shifting fungal endophyte communities colonize *Boutelou agracilis*: effect of host tissue and geographical distribution. Mycologia 102:1012-1026.

Humphries J (1974). Bacteriology. London: J. Murray p. 92.

Kang R, Helms R, Stout MJ, Jaber H, Nakatsu T (1992). Vietnamese culinary herbs in the United States. J. Agric. Food Chem. 40:2328-2332.

Koskey RE, Gemma JN (1989). A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92:486-505.

Lacava PT, Silva-Stenico ME, Araújo WL,Simionato AVC, Carrilho E, Tsai SM, Azevedo JL (2008). Detection of siderophores in endophytic bacteria *Methylobacterium* pp. associated with *Xylella fastidiosa* subsp. *pauca*. Pesq. Agropec. Bras. 43:521-528.

Lin Z, Zhu T, Fang Y, Gu Q, Zhu W (2008). Polyketides from *Penicillium* sp. JP-1, an endophytic fungus associated with the mangrove plant *Aegiceras corniculatum*. Phytochem. 69:1273-1278.

Liu K, Ding X, Deng B, Chen W (2009). Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*. J. Ind. Microbiol. Biotechnol. 36:1171-1177.

Marinho AMR, Marinho PSB, Rodrigues-Filho E (2007). Constituintes químicos de *Penicillim*sp. umendofítico de *Murrayapaniculata* (Rutaceae). Rev. Ciênc. Exatas Nat. 9:189-200.

Marinho AMR, Marinho PSB, Rodrigues-Filho E (2009). Esteroides produzidos por *Penicilliumherquei,* um fungo endofítico isolado dos frutos de *Meliaazedarach*(Meliaceae). Quim. Nova 32:1710-1712.

Martins CR, Miranda JCC, Miranda LN (1999). Contribuição de fungos micorrízicosarbusculares nativos no estabelecimento de *Aristidasetifolia*Kunth em áreas degradadas do Cerrado. Pesq. Agropec. Bras 34:665-674.

Monesso LS,Kawano CY, Ribeiro PH, Nomizo A, Goldman GH,Pupo MT (2008). Chaetoglobosinas produzidas por *Chaetomiumglobosum*, fungo endofítico associado a*Viguiera robusta*Gardn. (Asteraceae). Quim. Nova 31:1680-1685.

Nunes JLS, Souza PVD, Marodin GAB, Fachinello JC (2010). Effect of arbuscular mycorrhizal fungi and indolebutyric acid interaction on vegetative growth of 'Aldrighi' peach rootstock seedlings. Ciênc. Agrotec. 34:80-86.

Ownley BH, Gwinn KD, Veeega FE (2010). Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. Biol. Control 55:113-128.

Perazzolli M, Roatti B, Bozza E, Pertot I (2011).*Trichoderma harzianum* T39 induces resistance against downy mildew by priming for defense without costs for grapevine. Biol. Control 58:74-82.

Peterson RL, Massicotte HB, Melville LH (2004). Mycorrhizas: Anatomy and Cel Biology. Natl. Res. Counc. Canada p. 173.

Petrini O, Muller E (1986). Haupt und nebenfruchtformeneuropaischerpilze. Mycologia Helv. 1:501-627.

Petrini O, Stone J, Carroll FE (1992). Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. Can. J. Bot. 60:789-796.

Petrini O (1986).Taxonomy of endophytic fungi of aerial plant tissues. In: NJ Fokkema, LVan Den Heuvel(editors). Microbiology of the phyllosphere. Cambridge: University Press, pp. 87-175.

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-160.

Pimentel IC, Figura G, Auer CG (2010). Fungos endofíticos associados a acículas de *Pinus taeda*. Summa Phytopathol. 36:85-88.

Ramos HP, Braun GH, Pupo MT, Said S (2010). Antimicrobial activity from endophytic fungi *Arthrinium* state of *Apiospora montagnei* Sacc. and *Papulaspora immerse*. Braz. Arch. Biol. Technol. 53:629-632.

Rodrigues VEG, Carvalho DA (2001). Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do alto Rio Grande – Minas Gerais. Ciênc. Agrotec. 25:102-123.

Roesch LFW, Passaglia LMP, Bento FM,Triplett EW, Camargo FAO (2007). Diversidade de bactérias diazotróficasendofíticas associadas a plantas de milho. Rev. Bras. Ciênc 31:1367-1380.

Sales JF, Pinto JEBP, Botrel PP, Oliveira CBA, Ferri PH, Paula JR, Seraphin JC (2007). Composition and chemical variability in the essential oil of *Hyptis marrubioides* Epl. J. Essential Oils Res. 19:552-556.

Shao C, Wang C, Gu Y, Wei M, Pan J, Deng D, She Z, Lin Y (2010). Penicinoline, a new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system from an endophytic fungus *Penicillium* sp. Bioorg. Med. Chem. Lett. 20:3284-3286.

Selosse MA, Dubois MP, Alvarez N (2009). Do Sebacinales commonly associate with plant roots as endophytes? Mycol. Res. 113:1062-1069.

Shimizu M (1990). Quantity estimation of some contaminants in commonly used medicinal plants. Chem. Pharm. Bull. 38:2283-2287.

Silva RLO, Luz JS, Silveira EB, Cavalcante UMT (2006). Fungos endofíticos em *Annona*spp.: isolamento, caracterização enzimática e promoção do crescimento em mudas de pinha (*Annonasquamosa* L.). Acta Bot. 20:649-655.

Siqueira JO, Lambais MR, Stumer SL (2002). Fungos micorrízicosarbusculares. Biot. Ci. Desenv. 4:12-21.

Smith FA, Smith SE (1997). Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol. 137:373-388.

Souza LKH, Oliveira CMA, Ferri PH, Oliveira JG, Souza AH, Fernandes ODL, Silva MDR (2003). Antimicrobial activity of *Hyptis ovalifolia* towards dermatophytes. Mem. I Oswaldo Cruz 98:963-965.

Stevens KJ, Wellner MR, Acevedo MF (2010). Dark septate endophyte and arbuscular mycorrhizal status of vegetation colonizing a bottomland hardwood forest after a 100 year flood. Aquat. Bot. 92:105-111.

Strobel G, Daisy B, Castillo U, Harper J (2004). Natural products from endophytic microorganisms. J. Nat. Prod. 67:257-268.

Sutton BC (1980). Coelomycetes - Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England p. 1900.

Taylor JE, Hyde KD, Jones EBG (1999). Endophytic fungi associated

with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. New Phytol. 142:335-346.
Weishampel PA, Bedford B (2006). Wetland dicots and monocots differ in colonization by arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza 16:495-502.
Yuan ZL, Zhang CL, Lin FC, Kubicek CP (2010). Identity, diversity, and molecular phylogeny of the endophytic microbiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. Appl. Environ. Microbiol. 76:1642-1652.
Zheng GQ, Kenney PM, Lam LKT (1992). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. Nat. Prod. 55:999-1003.
Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaven AK (2002). Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants. Appl. Environ. Microbiol. 68:2198-2208.
Zubek S, Blaszkowski J (2009). Medicinal plants as hosts of arbuscular mycorrhizal fungi and dark septate endophytes. Phytochem. Rev. 8:571-580.