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Endophytic microorganisms from leaves of *Spermacoce verticillata* (L.): Diversity and antimicrobial activity

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ABSTRACT

Endophytic microorganisms from the base and top leaves of *Spermacoce verticillata* were isolated and the antimicrobial potential was evaluated. A total of 56 strains were isolated in pure culture, 44 fungi and 12 actinobacteria. The isolation frequency was higher in the base leaves (12.5%), when compared to the top leaves (3.05%). Among all fungi and actionobacteria identified, the majority belonged to the genus *Guignardia* (25%) and *Microbispora* (41.66%), respectively. The antimicrobial screening was firstly evaluated by agar plug assay and showed that 28.57% of the isolates presented activity mainly against gram positive bacteria *Staphylococus aureus* (ATCC-6538) and *Bacillus subtilis* (UFPEDA-16). The microorganisms that presented the best activities were then selected, evaluated in different culture media broth, and tested by disk diffusion assay using their fermented broth. The microorganisms selected for this assay exhibited antimicrobial activity mainly for *Bacillus subtilis* (UFPEDA-16). Since many isolates showed inhibitory activity against pathogenic microorganisms, it is suggestive that endophytic microorganisms from *S. verticillata* could be an interesting source to explore for bioactive metabolites and new tools need to be employed to explore the real potential of these microorganisms.

INTRODUCTION

In the last decades, various studies have demonstrated that plants serve as reservoirs for innumerable microorganisms known as endophytes. By definition, these microorganisms live intra and/or intercellularly in the host plants, at least for one period of their life cycle, without causing apparent harm to them (Petrini, 1991; Bacon and White, 2000). Even with the existence of uncountable epiphytic and soil microorganims, diverse works have shown the potential of endophytes as a promising source of natural products for the discovery of a variety of different classes of bioactive molecules to beapplied in medicine, industry and agriculture (Schulz *et al.*, 2002; Gunatilaka, 2006; Joseph and Priya, 2011). Investigations have shown that the search for microorganisms of biotechnological interest based in the ethno-botanical pharmacology represents an alternative to discover new microorganisms and bioactive molecules (Li *et al.*, 2005; Qin et al. 2011; Zhao *et al.*, 2011). Based on these principles, the bioprospection of microorganisms isolate from new ecological niches constitutes an important strategy for obtaining more efficient and less toxic antibiotics, which may ultimately control pathogenic bacteria that are resistant to the diverse antibiotics commonly used in current time (Appelbaum and Jacobs, 2005; Butler and Buss, 2006; Peláez, 2006; Yoneyama and Katsumata, 2006; Rodríguez-Noriega *et al.*, 2010). The medicinal plant *Spermacoce verticillata* (L.) [*Borreria verticillata* (L.) G. F. W. Meyer], which belongs to the Rubiacea family, is native of South America where is popularly known as "vassourinha-de-botão", and is used in the treatment of various infections and inflammatory processes (Conserva and Ferreira, 2012). Studies have shown that the extract from this plant presents activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Ustue and Adamu, 2010). Therefore the present study sought to isolate, identify, and evaluate the potential antimicrobial activity of the endophytic microorganisms from leaves of *S. verticillata*.

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MATERIALS AND METHODS

Plant material

Five specimens of *S. verticillata* with approximately 43 cm in height and inflorescent, and with a healthy appearance were collected from the campus of the Federal University of Pernambuco (UFPE) (34º 56' 57" W; 8º 2' 53" S). Following the collection, the samples were conduced to the laboratory and processed in 24 h. A voucher specimen of the plant (Nº 42.234/UFPE) was deposited at the Herbarium Professor Geraldo Mariz – UFP, Department of Botany, UFPE, Recife, Brazil.

Isolation of microorganisms

Leaves of base (4 to 6 internodes), i.e. older leaves and top (8 to 10 internodes), i.e. younger leaves were individually collected. Following, the leaves were washed in running tap water and then surface-sterilized with 70% ethanol for 1 min, sodium hypochlorite (2.0-2.5% active Cl) for 4 min, and 70% ethanol for 30 s. The leaves were then washed three times with sterile distilled water (Araújo *et al.*, 2002).

After the disinfection, the leaves were fragmented (± 0.25 cm^2) and transferred to Petri dishes containing the following culture media: potato dextrose agar – PDA supplemented with penicillin (75 µg/ml) and chloramphenicol (100 µg/ml) for isolation of fungi; starch casein agar – SCA and modified leaf agar medium – MLAM (100 g/L *S. verticillata* leaf extract; 15 g/L glucose, 15 g/L peptone, 15 g/L agar) containing nistatine (100 µg/ml) and cycloheximide (100 µg/ml) for the isolation of actinobacteria. The Petri dishes were then incubated at 28 ºC during 40 days. The efficiency of the disinfection was confirmed by inoculating the last washing water in Petri dishes with tryptone soy agar medium.

The microbial growth was observed daily and the colonies were purified and preserved in PDA or modified ISP2 – ISP2M (4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, 5 g/L starch, 15 g/L agar; pH 7.2) for posterior identification of fungi and actinobacteria, respectively. The frequency of isolation (*FI*) was calculated by observing the number of fragments with microbial colonies (*Ni*) in comparison to the total number of fragments (*Nt*) analyzed (*FI* = *Ni/Nt* x 100) (Araújo *et al.*, 2002).

Identification of microorganisms

The identification was carried at Mycology and Antibiotic Departments (UFPE), Recife, Brazil. For identification, the fungi were transferred to Petri dishes containing specific media: Czapek agar, 2% malt extract agar or PDA, and incubated at room temperature (28 ± 2ºC) for 30 days. After this period, macroscopic characters (colony diameter; color aspect and mycelial texture) and microscopic characters (somatic and reproductive microstructures) were evaluated (Dalmau, 1929).

The fungal identification was based on previous studies conducted by Ellis (1971), Domsch et al. (1993), Klich and Pitt (1994), De Hoog and Guarro (1995), Alexopoulos et al. (1996) and Frisvad and Samson (2004). The actinobacteria were cultivated in a variety of culture media such as ISP2M, SCA, and MLAM, and were further incubated at 28°C until 30 days. Afterwards, the identification of the genera level was performed by comparing their morphology of spore bearing hyphae with the entire spore chained structure (Shirling and Gottlieb, 1966). Futhermore, the type of cell wall was determined by thin layer chromatography using diaminopimelic acid as standard (Staneck and Roberts, 1974).

Screening and agar plug assay

Endophytic fungi and actinobacteria were selected for the study base on the antimicrobial activity submitted in the micelial agar plug assay (Ichikawa *et al.*, 1971).

This permitted a rapid and qualitative selection of the bioactive microorganisms. The fungi were grown on PDA at 28°C during 7 and/or 14 days, depending on fungal growth rate, and the actinobacteria on the media PDA supplemented 0.2% yeast extract, SCA, and ISP2M at 28°C for 7 days. Plugs of mycelium agar were cut with a flamed cork borer (6 mm diameter) and transferred to the surface of the medium previously spread with test microorganism.

For *Staphylococcus aureus* (ATCC-6538), *Bacillus subtilis* (UFPEDA-16), *Escherichia coli* (ATCC-25922), *Klebsiella pneumoniae* (ATCC-29665), *Pseudomonas aeruginosa* (ATCC-27853) the Müeller-Hinton agar – MHA was used. Both Sabouraud dextrose agar medium – SAB and MHA-MG supplemented with 2% glucose and methylene blue (0.5 µg/ml) were used for *Candida* spp. (Barry and Brown, 1996; Pfaller *et al.*, 2004). Petri dishes were incubated at 37°C for 24 h for the bacteria and at 30°C for 48 h for the fungi. The antimicrobial activity was confirmed by the visualization and measurement of inhibition zones.

Fermentation and disk diffusion assay

The bioactive microorganisms that showed the best activity in the agar plug assay were evaluated in different culture media broth. This provides alternative to select the best medium and incubation time for the production of the bioactive metabolites. For the preparation of the pre-inoculum, five plugs (6 mm in diameter) of growing culture were inoculated in 250 ml Erlenmeyer flasks containing 50 ml of the media potato dextrose broth – PDB, MEB and Czapek broth for fungi and ISP2M broth – ISP2MB, 2% malt extract broth – MEB and eurimycin production medium - MPE (20 g/L soya flour, 20 g/L glucose, 2 g/L $CaCO_3$, 5 g/L NaCl; pH 6.7) for actinobacteria.

The cultures were submitted to a rotary shaker at 180 rpm at room temperature (28 ± 2ºC) for 48 h. After the cultivation, an aliquot of 10 ml of each pre-inoculum was transferred to 500 ml Erlenmeyer flasks containing 90 ml of the respective media and subjected to the same conditions for 144 h. At every 24 h, the fermentation broth was centrifuged at 225 g for 15 min and 30 µl of the supernatant was utilized for the antimicrobial activity test using the disk diffusion method (Bauer *et al.*, 1996).

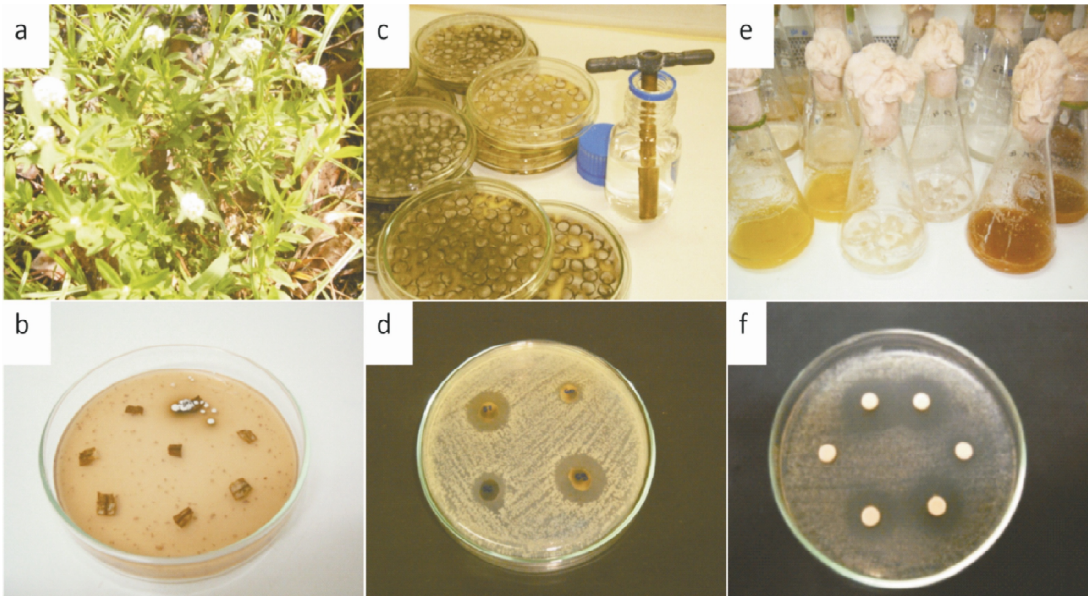

Fig. 1: Isolation of endophytic microorganisms from leaves of *S. verticillata* (**a** and **b**); agar plug screening assay (**c** and **d**) and fermentation and antimicrobial activity by disk diffusion assay (**e** and **f**).

RESULTS AND DISCUSSION

A total of 720 fragments (360 from the base and 360 from the top) were obtained from *S. verticillata* leaves (Figure I). Among these 240, fragments were used to isolate fungi and 480 fragments were used to isolate actinobacteria. In the present study, a total of 56 endophytes were isolated, 78.6% fungi and 21.4% actinobacteria (Table I). The frequency of fungi and actinobacteria was 7.56%. For the base leaves (older) the frequency was 12.5%, while for the top leaves (younger) the frequency was 3.05%. Similar findings were reported by many studies (Arnold *et al.*, 2000; Frohlich *et al.*, 2000; Bussaban *et al.*, 2001; Photita *et al.*, 2001; Chareprasert *et al.*, 2006). In such studies, older leaves were shown to present a higher occurrence of isolated endophytic microorganisms, when compared with younger leaves because of their longer exposure time in the environment what could provide an increase in the inoculum over them and consequently an increase in the endophytic microorganism frequency (Wilson and Carroll, 1994). Among the 44 endophytic fungi isolated, 38 strains belong to leaves from the base and 6 strains belong from the top, resulting in a total fungi frequency of 18.33%. However, this frequency may be considered low when compared to previous studies, for example the fungi frequency showed in the leaves from *Azadirachta indica* and *Lippia sidoides* with 42.5% and 50.41% respectively (Siqueira *et al.*, 2011; Tenguria and Khan, 2011).

From the endophytic fungi isolated, the *Mycelia sterilia* group had the highest occurrence (50%). *Mycelia sterilia* group is very common among the endophytic fungi as were shown also by biodiversity of endophytic fungi associated with Egyptian medicinal plants (Selim *et al.*, 2011). The Ascomycota fungi *Guignardia bidwelli* was found in a frequency of 25%. This finding was also observed in *Coffea arabica* leaves showing that the genus *Guignardia* occurs more often as endophytic of rather than epiphytic (Santamaría and Bayman, 2005).

The occurrence of this genus of endophyte has been observed in diverse tropical plants such as *Palicourea longiflora*, *Paullinia cupana* var. *sorbilis*, *Pueraria phaseoloides*, *Theobroma grandiflorum*, *Scleria pterota* and *Strychnos cogens* (Azevedo *et al.*, 2000; Souza *et al.*, 2004; Schulz and Boyle, 2005). Other fungi identified to the species level (20.45%) were members of Deuteromycota, including *Aspergillus stromatoide*, *Curvularia pallescens*, *Cladosporium cladosporioides*, *Penicillium aurantiogriseum*, and *Penicillium griseofulvum*. In addition, a member of Zygomycota, *Rhizomucor pusillus*, was also identified. Other fungi (4.55%) identified by the presence of clamp connections was member of the Basidiomycota, where the endophytes were shown in a low occurrence. The low occurrence of basidiomycetes also was observed in the leaves from *Melia azedarach* and *Samanea Saman* (Santos *et al.*, 2003; Chareprasert *et al.*, 2006).

Besides fungi, 12 endophytic actinobacteria were isolated, 7 strains recovered from base leaves and 5 strains from top leaves, with a total frequency of 2.5%. During the identification of these filamentous bacteria it was observed a higher occurrence of *Microbispora* (41.66%) followed by *Streptomyces* (25%), *Nocardia* (8.34%), and unidentified bacteria (25%) (Table I). A higher occurrence of *Microbispora* as endophyte was also observed in decaying leaves of *Lythrum* sp., *Kerria* sp., *Sasa* sp. and in leaves from *Zea mays* (Matsumoto *et al.*, 1998; Araújo *et al.*, 2000). All the isolated microorganisms were stored at the Microorganisms Collection of the Department of Antibiotics – UFPEDA and Department of Mycology, Fungi Cultures Collection, Mycotheca – URM, UFPE. Among the 56 microorganims isolated, 16 strains showed antimicrobial activity in the agar plug assay against *S. aureus* ATCC-6538, *B. subtilis*

UFPEDA-16, *E. coli* ATCC-25922, *K. pneumoniae* ATCC-29665 and *Candida* sp. URM-4224 (Figure I). These results highlight the great potential of these endophytes as antimicrobial metabolite producers, mainly against Gram positive bacteria (Table II). Studies have pointed out that actinobacteria and fungi are responsible for 45% and 38% of the antibiotics produced by microorganisms, respectively (Bérdy, 2005).

From the 16 strains that showed antimicrobial activity in the agar plug assay, 9 strains were selected for fermentation and disk diffusion assay (Figure I). Broth fermented was evaluated for 144 h at every 24 h against the same microorganisms that endophytes showed activity in the agar plug assay intending to determinate the time of maximum production of the bioactive metabolites by inhibition halo and the best broth medium at the conditions proposed (Table III). Fungi and actinobacteria showed a high activity against *B. subtilis* (UFPEDA-16) but only fungi showed activity for *S. aureus* (ATCC-6538). The best time of maximum production of the bioactive metabolites for all microorganisms was 120 h. The best medium was PDB for the fungi and ISP2M and MB for actinobacteria.The differences between the bioactivities showed by agar plug assay and the disk diffusion assay with the broth fermented could be directly correlated with the conditions of growth of the microorganism in solid and broth media, or the possibility of losing capacity of this biosynthesis when cultivated *in vitro* as reported by Owen and Hundley (2004) who showed that the production of bioactive compounds by endophytes is stimulated by the microorganism-plant interactions or by environmental factors. It behooves us to find the best ways to explore the most these microorganisms for the production of bioactive metabolites.

Table . 1: Distribution of endophytes isolated of old and young leaves of *Spermacoce verticillata*.

Endophytes	**Isolates n°**	**Isolates n° from leaves of *S. verticillata***	
		Base	**Top**
Fungi			
Aspergillus stromatoides Raper & Fennell	1	-	1
Curvularia pallescens Boedijn	1	1	-
Cladosporium cladosporioides (Fresen.) G.A. de Vries	1	1	-
Guignardia bidwellii (Elli) Viala & Ravaz	11	11	-
Penicillium aurantiogriseum Dierckx	1	-	1
Penicillium griseofulvum Dierckx	1	-	1
Rhinocladiella cellaris (Pers.) M.B. Ellis	1	1	-
Rhinocladiella mansoni (Castell.) Schol-Schwarz	1	1	-
Rhizomucor pusillus (Lindt) Schipper	2	-	2
Basidiomycota	2	2	-
Mycelia sterilia	22	21	1
Subtotal	**44**	**38**	**6**
Actinobacteria			
Microbispora sp.	5	1	4
Streptomyces sp.	3	2	1
Nocardia sp.	1	1	-
Unidentified	3	3	-
Subtotal	**12**	**7**	**5**
Total	**56**	**45**	**11**

– : not detected

Table. 2: Screening of endophytic microorganisms for antimicrobial activity by agar plug assay.

Cod.	**Endophyte**	**Culture Media**	**Microorganisms tests**				
			S. aureus **ATCC-6538**	***B. subtilis*** **UFPEDA-16**	***E. coli*** **ATCC-25922**	***K. pneumoniae*** **ATCC-29665**	***Candida*** **sp. URM-4224**
FAA1	*Microbispora* sp.	ISP2M	+	++	-	-	++
FAA3	*Streptomyces* sp.	ISP2M	+	-	-	-	-
FBA4	*Streptomyces* sp.	ISP2M	+	++	-	-	+
FBA7	*Streptomyces* sp.	ISP2M	+	++	-	-	+
FBA8	*Microbispora* sp.	ISP2M	+	++	-	-	+
FAA10	*Microbispora* sp.	ISP2M	+	++	-	-	-
FAA11	*Microbispora* sp.	ISP2M	++	++	-	-	++
FB45	*Mycelia sterilia*	PDB	++	++	-	-	-
FB44	*Mycelia sterilia*	PDB	+	+	+	-	-
FB56	*Mycelia sterilia*	PDB	++	+	-	-	-
FB58	*Mycelia sterilia*	PDB	++	++	+	-	-
FB59	*Mycelia sterilia*	PDB	++	++	++	+	-
FB60	*Mycelia sterilia*	PDB	++	+	-	-	-
FB68	*Mycelia sterilia*	PDB	+	-	-	-	-
FA80	*Penicillium griseofulvum*	PDB	++	++	-	-	-
FA81	*Penicilium aurantiogriseum*	PDB	++	++	-	-	-
FAA1	*Microbispora* sp.	ISP2M	+	++	-	-	++

Activities were classified according to the diameter of the clear zones around the point of application of the sample:
–: no antimicrobial activity
+: the inhibition zone is less than 15 mm
++: the inhibition zone is more than 15 mm

Table. 3: Evaluation of antimicrobial activity by disk diffusion assay by measuring the size of inhibition zones.

N°	Endophyte	Culture Media	Time (h)	Microorganisms tests (inhibition zone mm)				
				S. aureus **ATCC-6538**	***B. subtilis*** **UFPEDA-16**	***E. coli*** **ATCC-25922**	***K. pneumoniae*** **ATCC-29665**	***Candida*** **sp. URM-4224**
FAA1	*Microbispora* sp.	MEB	120	-	13	nt	nt	-
FBA8	*Microbispora* sp.	ISP2MB	96	-	19	nt	nt	nt
FAA10	*Microbispora* sp.	ISP2MB	120	-	14	nt	nt	nt
FAA11	*Microbispora* sp.	MEB	120	-	14	nt	nt	-
FB58	*Mycelia sterilia*	PDB	96	17	15	-	nt	nt
FB59	*Mycelia sterilia*	PDB	120	16	15	11	-	nt
FA80	*Penicillium griseofulvum*	PDB	120	22	17	nt	nt	nt
FA81	*Penicilium aurantiogriseum*	PDB	120	20	17	nt	nt	nt

–: no antimicrobial activity
nt: not tested

CONCLUSIONS

The findings of the present study showed that the endophytic fungi and actinobacteria obtained from *S. verticillata* leaves have a great potential for the synthesis of bioactive compounds. Future studies will be developed as an attempt to isolate the substances that may be responsible for its antimicrobial activity.

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REFERENCES

Alexopoulos C. J., Mims C. W. Blackwell M. Introductory mycology. 4ª Ed. John Wiley & Sons, New York (1996); 869 p.

Appelbaum P. C., Jacobs M. R. Recently approved and investigational antibiotics for treatment of severe infections caused by Gram-positive bacteria. Curr Opin Microbiol. 2005; 8: 510-517.

Araújo J. M., Silva A. C., Azevedo J. L. Isolation of endophytic actinomycetes from roots and leaves of maize (*Zea mays* L.). Braz Arch Biol Technol. 2000; 43: 447-451.

Araújo W. L., Lima S. O. A., Azevedo J. L., Marcon J., Sobral J.K., Lavaca P.T. Manual: Isolamento de Microrganismos Endofíticos. CALQ, Piracicaba (2002); 86 p.

Arnold A.E., Maynard Z, Gilbert G.S., Coley P.D., Kursar T.A. Are tropical fungal endophytes hyperdiverse? Ecol Lett. 2000; 3: 267-274.

Azevedo J. L., Maccheroni W., Pereira J. O., Araújo W. L. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J Biotechnol. 2000; 3: 40-65.

Bacon C. W., White Jr. J. F. Microbial endophytes. Marcel Dekker, New York (2000); 487 p.

Barry A. L., Brown S. D. Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. J. Clin. Microbiol. 1996; 34:2154-2157.

Bauer A. M., Kirby W. M. M., Sherris J. C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 43: 493-496.

Bérdy J. Bioactive microbial metabolites. J Antibiot (Tokyo). 2005; 58: 1-26.

Bussaban B., Lumyong S., McKenzie E. H. C., Hyde K. D. Endophytic fungi from *Amomum siamense*. Can J Microbiol. 2001; 47: 943-948.

Butler M. S., Buss A. D. Natural products – The future scaffolds for novel antibiotics?. Biochem Pharmacol. 2006; 71: 919-929.

Chareprasert S., Piapukiew J., Thienhirun S., Whalley A. J. S., Sihanonth P. Endophytic fungi of teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr.. World J Microbiol Biotechnol. 2006; 22: 481-486.

Conserva L. M., Ferreira J. C. *Borreria* and *Spermacoce* species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. Phcog Rev. 2012; 6: 46-55.

Dalmau L. M. Remarques sur la technique mycologique. Ann Parasitol. 1929; 7: 536-545.

De Hoog G. S., Guarro J. Atlas of clinical fungi. Centraalbureau voor Schimmelcultures, Baarn (1995); 720 p.

Domsch K. H., Gams W., Anderson, T. H. Compendium of soil fungi. Editora IHW Verlag V. I, San Francisco (1993); 859 p.

Ellis, M. B Dematiaceous hyphomycetes. Commonwealth mycological institute. Kew, Surrey, England (1971); 608 p.

Frisvad J. C., Samson R. A. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol. 2004; 49: 1-174.

Frohlich J., Hyde K.D., Petrini O. Endophytic fungi associated with palms. Mycol Res. 2000; 104: 1202-1212.

Gunatilaka A. A. L. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod. 2006; 69: 509-526.

Ichikawa T., Date M., Ishikura T., Ozaki A. Improvement of Kasugamycin - producing strain by the agar piece method and the prototroph method. Folia Microbiol. 1971; 16: 218-224.

Joseph B., Priya R. M. Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am. J. Biochem. Biotechnol. 2011; 1: 291-309.

Klich M. A., Pitt J. I. A laboratory guide to common *Aspergillus* species and their teleomorphs. CSIRO-Division of Food Processing, North Wales (1994); 116 p.

Li H., Qing C., Zhang Y., Zhao Z. Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World J Microbiol Biotechnol. 2005; 21: 1515-151.

Matsumoto A., Takahashi Y., Mochizumi M., Iwai Y., Omura S. Characterization of actinomycetes isolated from fallen leaves. Actinomycetology. 1998; 12: 46-48.

Owen N. L., Hundley N. Endophytes – the chemical synthesizers inside plants. Sci Prog. 2004; 87: 79-99.

Peláez F. The historical delivery of antibiotics from microbial natural products – can history repeat?. Biochem Pharmacol. 2006; 71: 1981-1990.

Petrini O. Fungal endophytes of tree leave. In: Andrews, J. H. & Hirano, S. S. (Eds) Microbial ecology of leaves. Springer-Verlag, New York (1991); pp. 179-197.

Pfaller M. A., Boyken L., Messer S. A., Hollis R. J., Diekema D. J. Stability of Müeller-Hinton agar supplemented with glucose and

methylene blue for disk diffusion testing of fluconazole and voriconazole. J Clin Microbiol. 2004; 42: 1288-1289.

Photita W., Lumyong S., Lumyong P., Hyde K. D. Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycol Res. 2001; 105: 1508-1513.

Qin S., Xing K., Jiang J. H., Xu L. H., Li W. J. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl. Microbiol. Biotechnol. 2011; 89: 457-473.

Rodríguez-Noriega E., Seas C., Guzmán-Blanco M., Mejía C., Alvarez C., Bavestrello L., Zurita J., Labarca J., Luna C. M., Salles M. J., Gotuzzo E. Evolution of methicillin-resistant *Staphylococcus aureus* clones in Latin America. Int J Infect Diseases. 2010; 14: e560-e566.

Santamaría J., Bayman P. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). Microb Ecol. 2005; 50: 1-8.

Santos R. M. G., Rodrigues-Fo E., Rocha W. C., Teixeira M. F. S. Endophytic fungi from *Melia azedarach*. World J Microbiol Biotechnol. 2003; 19: 767-770.

Schulz B., Boyle C. The endophytic continuum. Mycol Res. 2005; 109: 661-686.

Schulz B., Boyle C., Draeger S., Römmert A. K. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res. 2002; 106: 996-1004.

Selim K. A., El-Beih A. A., AbdEl-Rahman T. M., El-Diwany A. I. Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants. Mycosphere. 2011; 2: 669-678.

Shirling E. B., Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol. 1966; 16: 313-340.

Siqueira V. M., Conti R., Araujo J. M., Souza-Motta C. M. Endophytic fungi from the medicinal plant *Lippia sidoides* Cham. and their antimicrobial activity. Symbiosis. 2011; 53: 89-95.

Souza A. Q. L., Souza A. D. L., Astolfi Filho S., Belém-Pinheiro M. L., Sarquis M. I. M., Pereira J. O. Atividade antimicrobiana de fungos endofíticos isolados de plantas tóxicas da Amazônia: *Palicourea longiflora* (aubl.) rich e *Strychnos cogens* bentham. Acta Amaz. 2004; 34: 185-195.

Staneck J. L., Roberts G. D. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. J. Appl Microbiol. 1974; 28: 226-231.

Tenguria R. K., Khan F. N. Distribution of endophytic fungi in leaves of *Azadirachta indica* A. JUSS. (Neem) of Panchmarhi biosphere reserve. Curr Bot. 2011; 2: 27-29.

Ustue O. A., Adamu H. M. Phytochemical screening of *Borreria verticillata* leaves. J Agric Biotech Ecology. 2010; 3: 108-117.

Wilson D., Carroll G. C. Infection studies of *Discula quercina*, an endophyte of Quercus garryana. Mycologia.1994; 86: 635-647.

Yoneyama H., Katsumata R. Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci Biotechnol Biochem. 2006; 70: 1060-1075.

Zhao K., Penttinen P., Guan T., Xiao J., Chen Q., Xu J., Lindstrom K., Zhang L. L., Zhang X. P., Strobel G. A. The diversity and antimicrobial activity of endophytic actinomycetes isolated from medicinal plants in Panxi Plateau, China. Curr Microbiol. 2011; 62: 182-190.

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