academicJournals

Vol. 7(31), pp. 4073-4083, 2 August, 2013
DOI: 10.5897/AJMR2013.5808
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antagonistic aptitude and antiproliferative properties on tumor cells of fungal endophytes from the Astroni Nature Reserve, Italy

Rosario Nicoletti[1]*, Anna De Filippis[2] and Elisabetta Buommino[2]

[1]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca CAT, Via Vitiello 108, Scafati 84018, Italy.
[2]Dipartimento di Medicina Sperimentale, Seconda Università di Napoli, Via De Crecchio 7, Napoli 80138, Italy.

Accepted 16 July, 2013

As a yet thoroughly explored component of biodiversity, endophytic fungi are stimulating a huge research activity worldwide concerning their occurrence, biocenotic role, and opportunities for exploitation in biotechnologies. This paper presents the results of an investigation on fungal endophytes from 28 species of trees and shrubs thriving at the Astroni Nature Reserve near Naples, Italy. One hundred and eight isolates were recovered, a number of which represent new records of endophytic occurrence in the corresponding host plants. In a bioassay-driven procedure for the selection of strains possibly producing antitumor compounds based on their antifungal properties *in vitro*, about 35% of the isolates induced fungitoxic effects, and 10% were mycoparasitic, with the species *Biscogniauxia mediterranea, Nemania serpens, Paraconiothyrium brasiliense* and *Phomopsis theicola* reported for the first time for such an aptitude. Inhibition of mycelial growth was confirmed for about 60% of the culture extracts prepared from these bioactive strains, and was mostly correlated to an antiproliferative activity in human tumor cell cultures. Particularly, five strains were selected to be further investigated for the purification and the characterization of putative cytostatic compounds.

Key words: Endophytic fungi, antagonism, mycoparasitism, culture extracts, antiproliferative activity.

INTRODUCTION

Fungal endophytes are a functional component of biodiversity of natural ecosystems. It is assumed that every plant harbours a community of dozens of endophytic species, many of which result to be unclassified or novel taxa associated to specific hosts (Arnold et al., 2000; Mueller and Schmit, 2007). Hence these microorganisms may well exceed in number the over 300,000 known plant species (Porras-Alfaro and Bayman, 2011), thereby representing a goldmine of undescribed biodiversity and a fertile ground for drug discovery (Staniek et al., 2008). In fact, their emerging implications in plant protection and growth promotion may be mediated by secondary metabolites (Gimenez et al., 2007; Wicklow and Poling, 2009), whose bioactivity is susceptible to be exploited in human medicine (Joseph and Priya, 2011). In the past 20 years more and more novel compounds have been characterized for their antibiotic, cytostatic, pro-apoptotic, or other pharmaceutical effects from endophytes from forest contexts worldwide, as it is also attested by a notable increase in

*Corresponding author. E-mail: rosario.nicoletti@entecra.it. Tel: +39.0818563631; Fax: +39.0818506208.

Abbreviations: PDA, Potato dextrose agar; **CDB,** Czapek-Dox broth; **DMEM,** Dulbecco's modified Eagle's medium; **MTT,** methyl thiazol tetrazolium.

the number of patents referring to this particular field (Porras-Alfaro and Bayman, 2011).

On the occasion of the International Year of Biodiversity in 2010, an investigation concerning fungal endophytes of forest trees aiming at the selection of strains able to produce cytostatic metabolites was started at the Astroni Nature Reserve near Naples, Italy, based on the evaluation of their antifungal properties along the lines of our previous experiences in the field (Nicoletti et al., 2008a,b). Astroni Nature Reserve, which is located in an ancient volcanic crater in the Campi Flegrei area and currently managed by the World Wide Fund for Nature (www.wwf.it/astroni.nt), represents a unique environment preserved throughout the ages within a developing urban context, and an interesting spot to be considered for investigating the microbial components of biodiversity.

MATERIALS AND METHODS

Isolations

Fungal strains were recovered from 5 cm cuttings taken from secondary branches of trees and shrubs showing no disease symptoms or signs. Surface sterilization was carried out by immersion in 96% ethanol for 1 min, followed by 5% sodium hypochlorite for 3 min, and washings in sterile distilled water. Subcortical tissues were then excised and placed on potato dextrose agar (PDA) amended with 200 ppm streptomycin sulphate in 9-cm diameter Petri dishes, which were incubated in the dark at 25°C. Emerging fungal colonies were transferred to fresh PDA dishes for completing the isolation procedure, and for storage of pure cultures in the mycological collection established at the CAT Research Unit.

Antifungal properties

Antifungal properties were evaluated against a strain (RT23) of *Rhizoctonia solani* AG-2-1/Nt in dual cultures on Czapek-Dox agar (CDA) in 9 cm Petri dishes, which were kept in the dark at 25°C. Observation of mycelial growth of the opposed colonies and the eventual inhibitory effects was carried out throughout two weeks. When colonies merged, the contact area was cut, mounted on slides, stained with lactophenol cotton blue, and inspected through the microscope at 100x to detect hyphal interactions. Contact points were then observed at higher magnifications (up to 600x).

Isolates inducing inhibitory effects on strain RT23 were cultured in 500 ml Czapek-Dox broth (CDB) in 1 l-Erlenmeyer flasks at 25°C; after two weeks in the dark, cultures were filtered at 0.45 µm, and the culture filtrate extracted three times with ethyl acetate. Culture extracts (CEs) were dried in a rotavapor at 45°C and weighed. After dissolving in 0.5 ml absolute ethanol, their fungitoxic properties were evaluated by adding 0.1 ml of the ethanolic solutions to 7.9 ml melted 2% water agar (WA) in 6 cm diameter Petri dishes, where strain RT23 was inoculated at the centre. Control plates were treated with 0.1 ml absolute ethanol only. Assays were performed in triplicate, and observation of mycelial growth (if any) was continued throughout 10 days.

Antiproliferative assay on HeLa cells

HeLa cells, deriving from human cervix epithelioid carcinoma, were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C, 5% CO_2. Effects on cell proliferation were evaluated in microplates (tissue culture grade, 96 wells, flat bottom) by means of a methyl thiazol tetrazolium (MTT) assay. To this purpose, 2 x 10^3 cells were plated in each well containing 100 µl DMEM, and treated with ethanolic solutions of the CEs, prepared as above, at the final concentrations of 100 and 150 µg ml^{-1}, with an amount of ethanol not exceeding 3% v/v which is known not to affect proliferation of HeLa cells (Buommino et al., 2004). Control cells were treated, or not, with 3 µl absolute ethanol. In two separate sets, the colorimetric assay was continued for 24 and 48 h respectively, after which 10 µl of the MTT labeling reagent [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; Roche Diagnostics] were added to each well, followed by 100 µl of a solubilization solution (10% SDS in 0.01 M HCl) 4 h later. Plates were incubated overnight. Spectrophotometric absorbance at 560 nm was measured through a microplate reader (BioRad). Each treatment was performed in triplicate, and effect is expressed as the mean of the three recorded values of optical density.

Identification of strains

The strains which were active in the biological assays were taxonomically identified by observations of their morphological and cultural features when possible, or sent to the Identification Service of the Fungal Biodiversity Centre (CBS, Utrecht, the Netherlands) for a classification based on DNA sequence homology.

RESULTS

An overall sample of 108 isolates of endophytic fungi was recovered from 28 species of trees and shrubs thriving at the Astroni Nature Reserve. As preliminarily screened for their antifungal properties in dual cultures on CDA against *R. solani*, 37 isolates exhibited some extent of inhibitory capacities (Figure 1). Considering their quite variable extent of growth on CDA, the inhibition of mycelial growth by the endophytic strains was recorded in terms of yes/no, regardless to the distance at which it was evident, and without any comparative intent. Except for a few cases, inhibition of mycelial growth was slowly overcome within the observation period, with the establishment of a contact between hyphae from the opposed colonies. Inspection through the microscope of the merging area disclosed evidence of mycoparasitic interactions established by 11 isolates, 6 of which had not previously induced an inhibitory effect (Table 1).

In the next stage of investigation, based on the evaluation of the antifungal properties of CEs, 15 isolates were found to be inactive. This may depend on different reasons, such as the failure by ethyl acetate to extract the alleged fungitoxic extrolites, their eventual structural unstableness, or the lack of a continuous production, which in contrast can be considered to occur in dual cultures where the strain is actively growing and the presence of *R. solani* may somehow stimulate its antibiotic potential. Consistent inhibitory effects were evident for the rest of the sample; particularly, CEs of 5 isolates completely inhibited strain RT23 throughout a week, while 17 isolates induced a retarded growth in

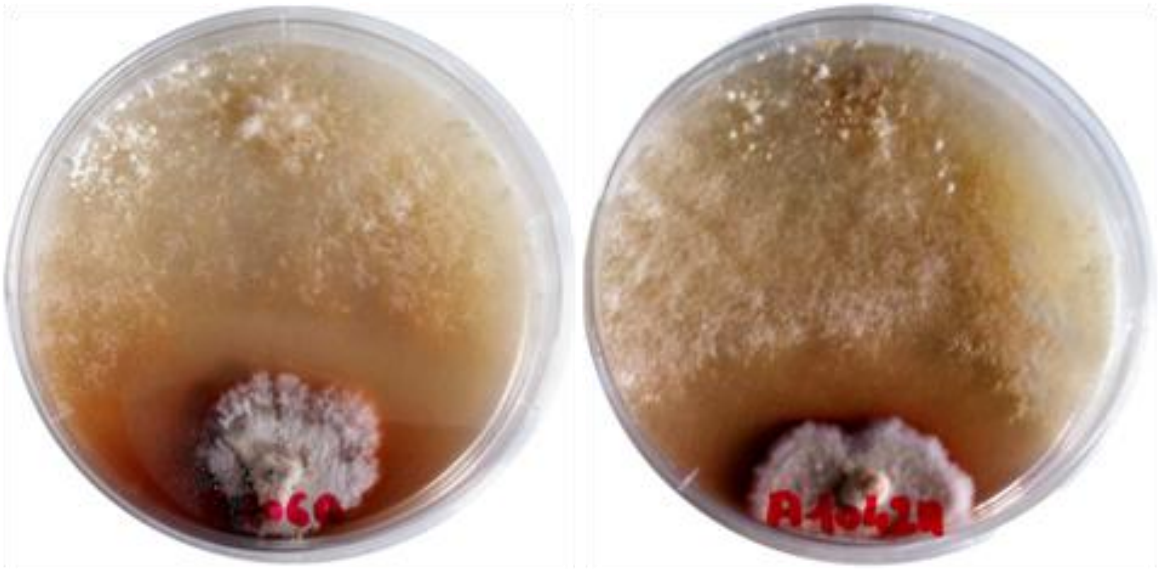

Figure 1. Inhibition of mycelial growth of strain RT23 in dual cultures with isolates A106A and A1042A.

Table 1. Strains of endophytic fungi which showed antagonistic properties against *R. solani* RT23.

Isolate	Species	Host plant	Inhibition by CEs	Mycoparasitism
A095A	*Biscogniauxia mediterranea*	*Carpinus betulus*	No	Yes
A095B	*B. mediterranea*	*C. betulus*	No	Yes
A1050A	*B. mediterranea*	*Quercus ilex*	No	Yes
A091D	*Botrytis cinerea*	*Q. ilex*	Yes (+)	No
A091E	*B. cinerea*	*Q. ilex*	Yes (+)	No
A1036B	*Cladosporium hillianum*	*Sambucus nigra*	Yes (++)	No
A1035B	*Colletotrichum* sp.	*Ulmus minor*	Yes	No
A1037A	*Embellisia indefessa*	*Fraxinus ornus*	Yes	No
A1021B	*Fusarium incarnatum-equiseti* s.c.	*Euonymus europaeus*	Yes (++)	No
A1022A	*Lecanicillium muscarium*	*Laurus nobilis*	No	Yes
A1026B	*L. muscarium*	*Myrtus communis*	No	Yes
A1041A	*Nemania serpens* var. *serpens*	*Ostrya carpinifolia*	Yes (+)	Yes
A1015D	*Neofusicoccum australe*	*M. communis*	Yes (+)	No
A1041C	*Paraconiothyrium brasiliense*	*O. carpinifolia*	Yes (++)	Yes
A1041G	*Penicillium chrysogenum*	*O. carpinifolia*	Yes (+)	No
A1104A	*P. chrysogenum*	*Eucalyptus globulus*	Yes (++)	No
A1102B	*Penicillium crustosum*	*Quercus robur*	Yes (+)	No
A1018A	*Penicillium echinulatum*	*C. betulus*	Yes (++)	No
A1017A	*Phoma* sp.	*Hedera helix*	Yes (+)	No
A1011C	*Phomopsis* sp.	*Q. ilex*	Yes	No
A1020A	*Phomopsis theicola*	*Acer campestris*	Yes (+)	Yes
A1103B	*Stemphylium* sp.	*Q. ilex*	Yes (+)	No
A1021A	*Trichoderma harzianum*	*E. europaeus*	No	Yes
A094A	unclassified *Sordariomycetes* sp.	*Quercus rubra*	Yes (+)	Yes
A1016A	unclassified *Sordariomycetes* sp.	*A. campestris*	Yes (+)	Yes
A106A	sterile mycelium 1	*Q. ilex*	Yes	No
A1041F	sterile mycelium 2	*O. carpinifolia*	Yes	No
A1042A	sterile mycelium 3	*Erica arborea*	Yes	No

++, Suppression of hyphal growth; +, less than 10 mm hyphal growth after one week.

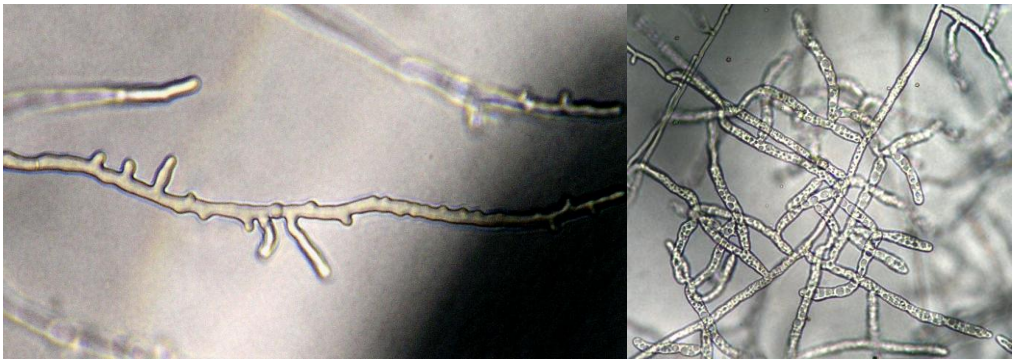

Figure 2. Proliferation of hyphal branching (left) and cell vacuolisation (right) induced by the culture extract of isolate A1041A.

comparison to the control. In most cases, this effect was associated with alterations of the hyphal structure consisting in the proliferation of hyphal branching and/or a diffuse vacuolisation of the cells (Figure 2), which were already documented to be induced by toxin-producing *Penicillium* strains on *R. solani* grown in the same conditions (Nicoletti et al., 2004). Isolates establishing a mycoparasitic interaction, and/or whose CEs induced an inhibitory effect, were taxonomically identified as reported in Table 1.

Some species are well known disease agents of forest trees which are reported to commonly colonize plant tissues endophytically, and to establish a pathogenic interaction in consequence of environmental stress, such as severe drought, fires, or insect defoliation. This is the case of *Biscogniauxia mediterranea* (De Not.) Kuntze (Vannini and Scarascia Mugnozza, 1991; Collado et al., 2001; Anselmi and Mazzaglia, 2005; Capretti and Battisti, 2007), *Nemania serpens* (Pers.) Gray (Chapela, 1989; Parfitt et al., 2010), and *Neofusicoccum australe* (Slippers, Crous and M.J. Wingf.) Crous, Slippers and A.J.L. Phillips (teleomorph *Botryosphaeria australis* Slippers, Crous and M.J. Wingf.) (Taylor et al., 2009; Sakalidis et al., 2011). Pathogenic behaviour also characterizes members in the *Fusarium incarnatum-Fusarium equiseti* species complex (FIESC), mostly on cropped plants; nevertheless our finding is within the context of a documented occurrence as tree endophytes of strains currently ascribed to this yet to be sorted taxonomic entity (Taylor et al., 1999; Li et al., 2008). *Botrytis cinerea* Pers. itself is mostly known as a plant pathogen exerting perthophytism, but its endophytic occurrence has been occasionally reported in a number of woody plants (Espinosa-Garcia and Langenheim, 1990; Linaldeddu et al., 2005), including *Quercus ilex* (Anselmi and Mazzaglia, 2005). Similar considerations pertain to three strains mentioned as *Colletotrichum* sp., *Stemphylium* sp., and *Phoma* sp., which could not be reliably identified at the species level; well known in plant pathology, these genera also include strains of undetermined species which have been reported for their endophytic habit (Schulz et al., 1993; Fisher et al., 1994; Yang et al., 1994; Lu et al., 2000; Hoffman et al., 2008).

The other identified species are mostly reported as saprophytes from disparate sources, but their more or less occasional recovery from forest plants seems to be more strictly referable to an endophytic habit. To this regard, a checklist of their cited hosts is provided in Table 2, from which it can be inferred that the findings at Astroni represent the first report of endophytism in the host plants indicated in Table 1 for the species *Paraconiothyrium brasiliense* Verkley, *Penicillium chrysogenum* Thom, *Penicillium crustosum* Thom, *Penicillium echinulatum* Raper and Thom ex Fassat., *Phomopsis theicola* Curzi and *Trichoderma harzianum* Rifai. Also, apart from a previous reference in a study where a strain was artificially inoculated on cucumber (Hirano et al., 2008), our findings may be regarded as the first documented report concerning a natural endophytic occurrence of *Lecanicillium muscarium* (Petch) Zare and W. Gams. However, it must be considered that this species has been typified since about a decade after its separation from the old taxon *Verticillium lecanii* (Zare and Gams, 2001), whose endophytic aptitude had already been ascertained (Vega, 2008).

Additional cases to be regarded as first reports of endophytic occurrence are represented by the findings of strains belonging to the very recently typified species *Cladosporium hillianum* Bensch, Crous and U. Braun (Bensch et al., 2010), and to *Embellisia indefessa* E.G. Simmons, considering that a conforming isolate previously recovered from within leaves of *Thlaspi caerulescens* (Coles et al., 1999) was later ascribed to a novel species, *Embellisia thlaspis* (David et al., 2000). As for *N. australe*, an endophytic strain of this widespread species was previously isolated from *Myrtus communis* at a Mediterranean maquis spot along the coastline of Punta Licosa, about 130 km south of Naples (Nicoletti, unpublished). Coupled with other findings on species in the Myrtaceae, such as *Agonis flexuosa, Syzygium*

Table 2. Checklist of cited hosts of some species of fungal endophytes recovered at Astroni.

Endophyte	Plant species	Geographic area	Reference
Paraconiothyrium brasiliense	*Aralia elata*	South Korea	Paul et al., 2007
	Alliaria petiolata	New Jersey (USA)	Damm et al., 2008
	Ginkgo biloba, Pinus tabulaeformis	China	Damm et al., 2008
	Picea glauca	South Quebec (Canada)	Damm et al., 2008
	Taxus chinensis	Qinba (China)	Liu et al., 2009
	Acer truncatum	Mount Donglin (China)	Liu et al., 2010a; Sun et al., 2011
	Cedrus deodara	Zijin mountain (China)	Liu et al., 2010b
	Phoradendron perrotetti, Tapirira guianensis	Brazil	de Abreu et al., 2010
	Pyrus communis	South Africa	Cloete et al., 2011
	Betula platyphylla, Ulmus macrocarpa	Mount Donglin (China)	Sun et al., 2012
	Cinnamomum camphora	Zijin mountain (China)	Han et al., 2012
	Holcoglossum sinicum	Yunnan (China)	Tan et al., 2012
Penicillium chrysogenum	*Avicennia marina*	Egypt	El-Morsy, 2000
	Catha edulis	Yemen	Mahmoud, 2000
	unidentified plant species	Peru	Singh et al., 2003
	Eichhornia crassipes	Damietta province (Egypt)	El-Morsy, 2004
	Cassia tora, Hemidesmus indicus	Malnad, Karnataka (India)	Krishnamurthy et al., 2008
	Cataranthus roseus	Varanasi, Mirazpur (India)	Kharwar et al., 2008
	Cistanche deserticola	China	Lin et al., 2008
	Hyosciamus muticus	Southern Egypt	El-Zayat et al., 2008
	Natural vegetation	Alicante province (Spain)	Macia-Vicente et al., 2008
	Austrocedrus chilensis, Prumnopitys andina	Las Trancas (Chile)	Hormazabal and Piontelli, 2009
	Canavalia cathartica	Nethravathi river (India)	Anita and Sridhar, 2009
	Oryza sativa	Karnataka (India) Malaysia	Naik et al., 2009 Zakaria et al., 2010
	Sesbania bispinosa	Nethravathi river (India)	Anita et al., 2009
	Fritillaria thunbergii	China	Tian et al., 2010
	Hevea brasiliensis	Madre de Dios (Peru)	Gazis and Chaverri, 2010
	Paris polyphylla	Wu-ding, Yunnan (China)	Xuan et al., 2010
	Adhatoda vasica	Karaikal (India)	Shukla and Mishra, 2012
	Vanda testacea, Bulbophyllum neilgherrense	Kaiga forest (India)	Sudheep and Sridhar, 2012
	Porteresia coarctata	Chorao Island (India)	Devi et al., 2012
	Salvadora oleoides	Haryana (India)	Dhankar et al., 2012
	Cannabis sativa	Mandi (India)	Gautam et al., 2013
Penicillium crustosum	*Coffea arabica*	Cacaohoatán (Mexico) Guatemala	Vega et al., 2006;
		Guatemala	Vega et al., 2008
		Chinchiná (Colombia)	Vega et al., 2010
	Cyperus malaccensis	Nethravathi estuary (India)	Karamchand et al., 2009
	Viguiera robusta	Brazil	Guimarães et al., 2010
	Persea americana	South Africa	Hakizimana et al., 2011

Table 2. Contd.

Penicillium echinulatum	*Cyperus malaccensis*	Nethravathi estuary (India)	Karamchand et al., 2009
Phomopsis theicola	*Maytenus ilicifolia, Spondias mombin*	Paranà (Brazil)	Rodrigues Gomes, 2008
	Cirsium arvense	New Zealand	Dodd et al., 2010
Trichoderma harzianum	*Zea mays*	Devon (England)	Fisher et al., 1992
	Eucalyptus nitens	Canberra (Australia)	Fisher et al., 1993
	Quercus pubescens	Chianti (Italy)	Ragazzi et al., 2003
	Quercus robur	Fagaré (Italy)	Ragazzi et al., 2003
	Theobroma galeri	North-west Ecuador	Evans et al., 2003
	Terminalia arjuna	South India	Tejesvi et al., 2005
	Coffea arabica, C. robusta	Sao Paulo state (Brazil)	Sette et al., 2006
	Cyperus malaccensis	Nethravathi river (India)	Karamchand et al., 2009
	Hevea brasiliensis	Madre de Dios (Peru)	Gazis and Chaverri, 2010

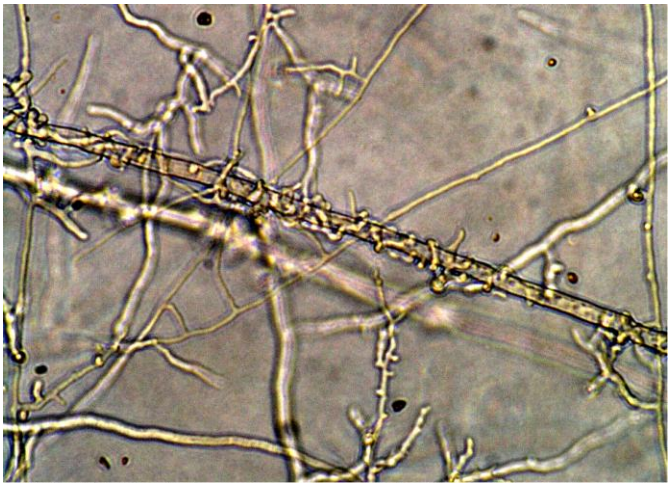

Figure 3. Mycoparasitic interactions established against strain RT23 by isolate A095A.

cordatum and *Eucalyptus* spp. (Barber et al., 2005; Pavlic et al., 2007; Taylor et al., 2009), our observations provide further evidence for a possible more general occurrence as an endophyte in plants belonging to this family. The species *P. theicola* (teleomorph *Diaporthe neotheicola* A.J.L. Phillips and J.M. Santos) was described long ago based on isolates from leaves and twigs of *Camellia sinensis* at the Botanical Gardens of the University of Pavia, Italy (Curzi, 1927); since then its occurrence has apparently been neglected until the years 2000s, when the application of biomolecular techniques for fungal identification has disclosed a more widespread distribution as both an endophyte and a pathogen (Mostert et al., 2001). A few bioactive isolates could not be ascribed to known taxonomic entities by reason of lack of sporulation on culture media, confirming that coming across unidentifiable fungi is a quite ordinary circumstance in endophyte assemblage studies (Promputtha et al., 2005; Wang et al., 2005). For example, no hit in any available database was obtained for isolate A1017A (*Phoma* sp.) as DNA sequencing was performed of genes coding for LSU, ITS, actin and the elongation factor 1-alpha (Meijer, pers. commun.). On the other hand, rDNA-ITS sequencing of isolates A094A and A1016A showed a 100% identity with sequences deposited in GenBank (GQ153076 and GQ153090) corresponding to a couple of unclassified endophytic strains belonging in the Sordariomycetes, recovered in Arizona respectively from *Cupressus arizonica* and *Juniperus deppeana* (Hoffman and Arnold, 2010; Hoffman, pers. commun.).

The species *Trichoderma harzianum* and *Lecanicillum muscarium* are known for their active involvement in plant protection against agents of biotic adversities. While the former is fundamentally a fungal antagonist (Vinale et al., 2008), the latter is primarily considered as an entomopathogen, but also reported for its ability to exert parasitism against plant pathogenic fungi (Goettel et al., 2008). The mycoparasitic behaviour of these two species was confirmed in our dual cultures, where the three available strains performed hyphal coiling and penetration by means of haustorium-like structures. More unexpectedly, the same hyphal reactions were observed in dual cultures with isolates of *P. brasiliense*, *P. theicola,* the unclassified Sordariomycetes species, *N. serpens* and *B. mediterranea* (Figure 3), introducing their possible opportunistic mycoparasitic aptitude. While it is already known in *Paraconiothyrium*, with the species *P. minitans* even employed for the preparation of biopesticides (Verkley et al., 2004; Whipps et al., 2008), to our knowledge there are no previous citations of mycoparasitism within *Biscogniauxia, Nemania* and *Phomopsis*.

CEs of 22 isolates which proved to be fungitoxic against strain RT23 were then assayed for their cytostatic activity on HeLa cells (Figure 4). Cell proliferation was strongly reduced by CEs of isolates A1017A, A1020A, A1021B, A1041C and A1104A at both the concentrations tested, with the latter inducing the most remarkable inhibition (73%). Increasing the dose by 50% (150 µg/ml) did not induce a corresponding decrease of cell proliferation, with the notable exception of isolate A1041G whose effect

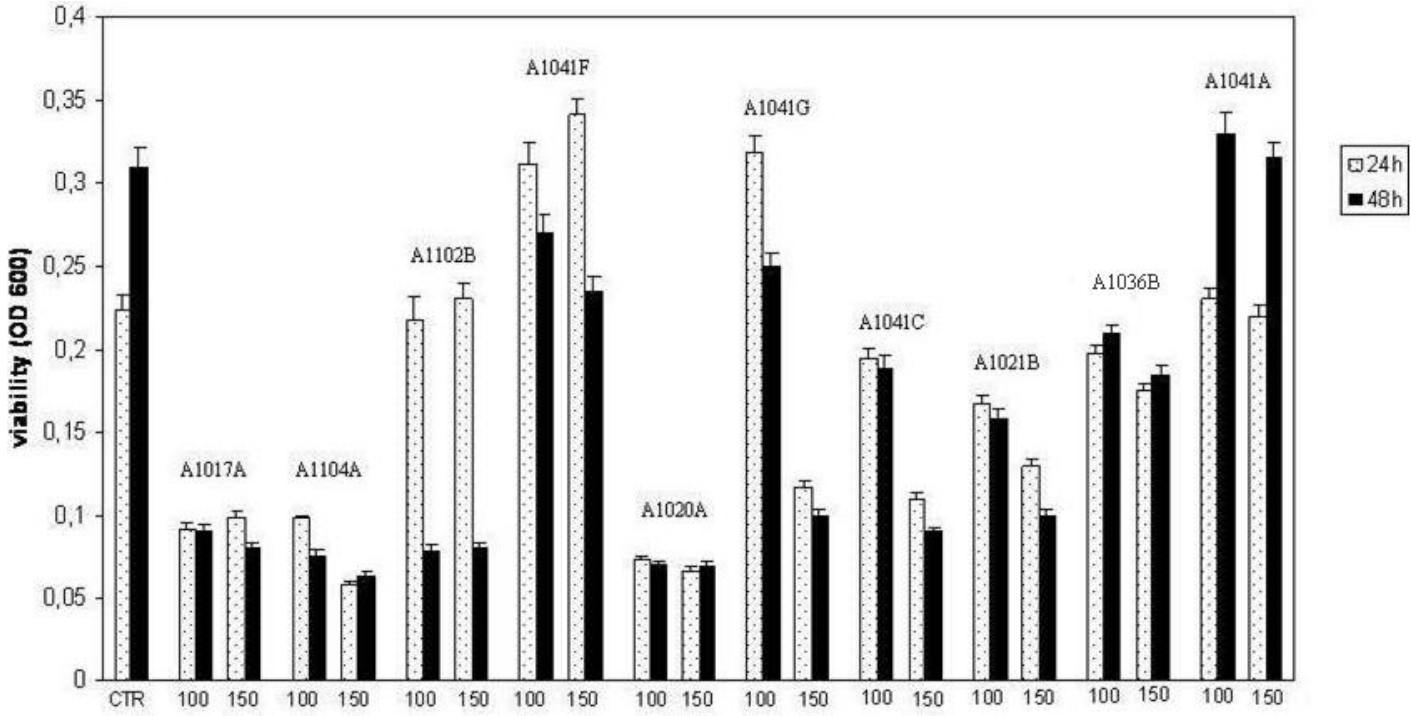

Figure 4. Antiproliferative activity on HeLa cells of CEs of selected endophytic isolates, as evaluated at the doses of 100 and 150 µg/ml at 24 and 48h. Control (CTR) refers to cells treated with 3 µl absolute ethanol.

was more than doubled at the higher dose. On the other hand, an extended incubation time (48 h) just slightly increased the inhibitory effect for all isolates but A1102B, whose antiproliferative properties were only consistent at 48 h at both concentrations. In the case of isolate A1041F, and of isolate A1041G at the lower dose, a stimulatory effect on cell proliferation was evident at the first remark which reversed to inhibitory at 48 h. Finally, a weak cytostatic effect as visualized for A1036B was recorded for the rest of the isolates, with the exception of A1041A which did not appreciably differ from the control.

DISCUSSION

In recent years, quite fruitful results have been obtained in prospecting fungal diversity for the bioassay-oriented search of new antitumor drugs (Cheng et al., 2009; Suryanarayanan et al., 2009; Kharwar et al., 2011), which may be based on a correlation with their fungitoxic activity (Huang et al., 2001; Li et al., 2005). As a whole, our investigation confirmed that this effect represents a useful indicator to be considered. In fact, although not being systematic and based on a methodology eventually implying the loss of a number of interesting strains which may not be able to grow on CDB or to produce bioactive extrolites at effective concentrations in this culture medium, our preliminary screening has lead to the selection of a few isolates to be further characterized with reference to one or more extrolites which could account for the observed bioactivity. Particularly, our attention will focus on isolates A1104A (*P. chrysogenum*), A1021B (FIESC), A1041C (*P. brasiliense*), A1020A (*P. theicola*), and A1017A (*Phoma* sp.). While *P. chrysogenum* and some species currently included in the FIESC have been quite widely investigated for their ability to produce mycotoxins and other bioactive compounds (Logrieco et al., 2002; Frisvad et al., 2004, Nicoletti et al. 2008c), there is a paucity of data concerning *P. brasiliense* and *P. theicola.* So far, the latter has only been mentioned for an inhibitory activity against the agent of citrus black spot (*Guignardia citricarpa*) (Rodrigues Gomes, 2008), although the production of fungitoxic compounds related to antagonism has been reported from several *Phomopsis* spp. (Horn et al., 1994; Corrado and Rodrigues, 2004; Silva et al., 2005). Some clues of fungitoxic effects by strains of *P. brasiliense* can be inferred from the literature (Paul et al., 2007; Liu et al., 2010a), and the opportunity to more thoroughly investigate this species as a source of bioactive compounds is corroborated by the recent finding of an endophytic strain from Chinese yew (*Taxus chinensis*) producing the renowned antitumor pharmaceutical taxol (Liu et al., 2009). Finally, a series of fungitoxic and cytostatic extrolites has been also reported from strains of *Phoma* spp. (El-Kady and Eman Mostafa, 1995; Weber et al., 2004).

At the same time, in our study novel indications have

been gathered on endophyte diversity, both in terms of their distribution and hosts, and with reference to their functions and ecological role. Particularly, the above-mentioned finding of two unclassified strains disclosing an identity with other endophytes previously recovered from botanically unrelated hosts at the very distant geographical location of Mount Lemmon, Arizona, represents an evidence of a likely widespread diffusion of a currently anonymous species.

Likewise, the recovery of the species *C. hillianum*, whose recent description is only based on two isolates from bulrush (*Typha orientalis*) collected in New Zealand (Bensch et al., 2010), is destined to modify its current status as an endemism. Actually, the above circumstances should be regarded as an encouragement for a more systematic collection of data and their report in the literature and/or in accessible online databases, in order to prevent the loss of novel information on fungal diversity resulting from either exhaustive investigations or incidental isolations.

More interestingly, we found 11 isolates to be able to exert mycoparasitism against *R. solani.* Although this fungus is not an epigeous pathogen of forest plants, a number of its mycoparasites, including the above-mentioned *L. muscarium* and *T. harzianum*, are also aggressive against other basidiomycetes inciting forest plant diseases, such as rusts and wood decay (Evans et al., 2003; Verma et al., 2007; Goettel et al., 2008). More in general, it is a feature of many mycoparasites to present a wide host range including taxonomically diverse plant pathogens (Whipps, 2001; Verma et al., 2007; Vinale et al., 2008). The occurrence of endophytic mycoparasites, as well as *T. harzianum*, has been reported from *Theobroma* spp. and proposed to be exploited for novel strategies in cacao protection (Evans et al., 2003; Bailey et al., 2008). Considering that about 10% of the isolates in our sample are mycoparasitic, this aptitude seems to be more than just occasional within endophyte assemblages; if confirmed *in vivo* against more relevant disease agents of forest trees, it could introduce a novel insight for evaluating the biocenotic interactions and the outcome on plant health by these microorganisms.

Within the complex frame of plant microbiomes, the facultative pathogenic behaviour of species such as *B. mediterranea, N. serpens* and *P. theicola* could find a counterpart in a possible detrimental effect on the spread of other more noxious fungi, thereby justifying the establishment of a compatible interaction with their host, as in the case of our isolates which were recovered from asymptomatic branches. Actually, the increasing concern for the threat of fungal pathogens, not only on plants but also on animals and humans within a temporal context of alteration of the natural ecosystems (Fisher et al., 2012), calls for a more thorough knowledge and the exploitation of any factor able to maintain an equilibrium even within such an apparently lesser biocenotic context.

ACKNOWLEDGEMENTS

The contribution of Martin Meijer and other specialists of fungal taxonomy at the CBS Fungal Biodiversity Centre (Utrecht, the Netherlands) is gratefully acknowledged.

REFERENCES

Anita DD, Sridhar KR (2009). Assemblage and diversity of fungi associated with mangrove wild legume *Canavalia cathartica*. Trop. Subtrop. Agroecosyst. 10:225-235.

Anita DD, Sridhar KR, Bhat R (2009). Diversity of fungi associated with mangrove legume *Sesbania bispinosa* (Jacq.) W. Wight (Fabaceae). Livestock Res. Rural Develop. 21 (5): article #67. http://www.lrrd.org/lrrd21/5/anit21067.htm, accessed on 20 September 2012.

Anselmi N, Mazzaglia A (2005). Correlation between the incidence of endophytic pathogenic fungi and oak decline in *Quercus ilex* L. after fire damages. IOBC/WPRS Bull. 28(8):93-99.

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA (2000). Are tropical fungal endophytes hyperdiverse? Ecol. Lett. 3:267-274.

Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, Vinyard BT, Holmes KA (2008). Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Biol. Control 46:24-35.

Barber PA, Burgess TJ, Hardy GESJ, Slippers B, Keane PJ, Wingfield MJ (2005). *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. Mycol. Res. 109:1347-1363.

Bensch K, Groenewald, Dijksterhuis J, Starink-Willemse M, Andersen B, Summerell BA, Shin HD, Dugan FM, Schroers H-J, Braun U, Crous PW (2010). Species and ecological diversity within the *Cladosporium cladosporioide*s complex (*Davidiellaceae*, *Capnodiales*). Stud. Mycol. 67:1-94.

Buommino E, Nicoletti R, Gaeta GM, Orlando M, Ciavatta ML, Baroni A, Tufano MA (2004). 3-*O*-Methylfunicone, a secondary metabolite produced by *Penicillium pinophilum*, induces growth arrest and apoptosis in HeLa cells. Cell Prolifer. 37:413-426.

Capretti P, Battisti A (2007). Water stress and insect defoliation promote the colonization of *Quercus cerris* by the fungus *Biscogniauxia mediterranea.* For. Pathol. 37:129-135.

Chapela IH (1989). Fungi in healthy stems and branches of American beech and aspen: a comparative study. New Phytol. 113:65-75.

Cheng Z, Pan J, Tang W, Chen Q, Lin Y (2009). Biodiversity and biotechnological potential of mangrove-associated fungi. J. For. Res. 20:63-72.

Cloete M, Fourie PH, Damm U, Crous PW, Mostert L (2011). Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. Phytopathol. Mediterr. 50:176-190.

Coles KE, David JC, Fisher PJ, Lappin-Scott HM, Macnair MR (1999). Solubilisation of zinc compounds by fungi associated with the hyperaccumulator *Thlaspi caerulescens*. Bot. J. Scot. 51:237-247.

Collado J, Platas G, Pelaez F (2001). Identification of an endophytic *Nodulisporium* sp. from *Quercus ilex* in central Spain as the anamorph of *Biscogniauxia mediterranea* by rDNA sequence analysis and effect of different ecological factors on distribution of the fungus. Mycologia 93:875-886.

Corrado M, Rodrigues KF (2004). Antimicrobial evaluation of fungal extracts produced by endophytic strains of *Phomopsis* sp. J. Basic Microbiol. 44:157-160.

Curzi M (1927). *De novis theae micromycetibus pathogenis*. Atti dell'Istituto Botanico dell'Università e Laboratorio Crittogamico, Pavia, Ser. 3(3):59-72.

Damm U, Verkley GJM, Crous PW, Fourie PH, Haegi A, Riccioni L (2008). Novel *Paraconiothyrium* species on stone fruit trees and other woody hosts. Persoonia 20:9-17.

David JC, Coles K, Fisher J, Moss ST (2000). A new species of

Embellisia from soil with high levels of heavy metals. Mycoscience 41:533-537.
de Abreu LM, Almeida AR, Salgado M, Pfenning LH (2010). Fungal endophytes associated with the mistletoe *Phoradendron perrottettii* and its host tree *Tapirira guianensis*. Mycol. Progr. 9:559-566.
Devi P, Rodriguez C, Naik CG, D'Souza L (2012). Isolation and characterization of antibacterial compound from a mangrove endophyte fungus, *Penicillium chrysogenum* MTCC5108. Indian J. Microbiol. 52:617-623.
Dhankar S, Kumar S, Sandeep D, Yadav JP (2012). Antioxidant activity of fungal endophytes isolated from *Salvadora oleoides* Decne. Int. J. Pharm. Pharm. Sci. 4:380-385.
Dodd S, Ganley R, Bellgard S, Than D (2010). Endophytes associated with *Cirsium arvense* – a step toward understanding their role in the success/failure of *Sclerotinia sclerotiorum* as a bioherbicide. Proceedings XVII Australasian Weeds Conference, Christchurch, New Zealand. pp. 235-238.
El-Kady IA, Eman Mostafa M (1995). Production of cytochalasins C, D and E from Dematiaceous Hyphomycetes. Folia Microbiol. 40:301-303.
El-Morsy EM (2000). Fungi isolated from the endorhizosphere of halophytic plants from the Red Sea coast of Egypt. In Hyde et al. (eds) Aquatic mycology across the millennium, Fungal Diver. 5:43-54.
El-Morsy EM (2004). Evaluation of microfungi for the biological control of water hyacinth in Egypt. Fungal Diver. 16:35-51.
El-Zayat SA, Nassar MSM, El-Hissy FT, Abdel-Motaal FF, Ito S (2008). Mycoflora associated with *Hyoscyamus muticus* growing under an extremely arid desert environment (Aswan region, Egypt). J. Basic Microbiol. 48:82-92.
Espinosa-Garcia FJ, Langenheim JH (1990). The endophytic fungal community in leaves of a coastal redwood population diversity and spatial patterns. New Phytol. 116:89-97.
Evans HC, Holmes KA, Thomas SE (2003). Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycol. Progr. 2:149-160.
Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186-194.
Fisher PJ, Petrini O, Lappin Scott HM (1992). The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). New Phytol. 122:299-305.
Fisher PJ, Petrini O, Petrini LE, Sutton BC (1994). Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorca and Switzerland. New Phytol. 127:133-137.
Fisher PJ, Petrini O, Sutton BC (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. Sydowia 45:338-345.
Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud. Mycol. 49:201-242.
Gautam AK, Kant M, Thakur Y (2013). Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. Arch. Phytopathol. Plant Prot. 46:627-635.
Gazis R, Chaverri P (2010). Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. Fungal Ecol. 3:240-254.
Gimenez C, Cabrera R, Reina M, Gonzalez-Coloma A (2007). Fungal endophytes and their role in plant protection. Curr. Org. Chem. 11:707-720.
Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008). Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J. Invertebr. Pathol. 98:256-261.
Guimarães DO, Borges WS, Vieira NJ, de Oliveira LF, da Silva CHTP, Lopes NP, Dias LG, Durán-Patrón R, Collado IG, Pupo MT (2010). Diketopiperazines produced by endophytic fungi found in association with two Asteraceae species. Phytochemistry 71:1423-1429.
Hakizimana JD, Gryzenhout M, Coutinho TA, van den Berg N (2011). Endophytic diversity in *Persea americana* (avocado) trees and their ability to display biocontrol activity against *Phytophthora cinnamomi.* Proceedings VII World Avocado Congress, Cairns, Australia.
Han M, Liu T, Cai X, Chen K, Liu C, Kemp B, Xue Y, Gu Y (2012). A new endophytic *Paraconiothyrium brasiliens* LT161 shows potential in producing antifungal metabolites against phytopathogens. Afr. J. Microbiol. Res. 6:7572-7578.
Hirano E, Koike M, Aiuchi D, Tani M (2008). Pre-inoculation of cucumber roots with *Verticillium lecanii* (*Lecanicillium muscarium*) induces resistance to powdery mildew. Res. Bull. Obihiro Univ. 29:82-94.
Hoffman AM, Mayer SG, Strobel GA, Hess WM, Sovocool GW, Grange AH, Harper JK, Arif AM, Grant DM, Kelley-Swift EG (2008). Purification, identification and activity of phomodione, a furandione from an endophytic *Phoma* species. Phytochem. 69:1049-1056.
Hoffman MT, Arnold AE (2010). Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. Appl. Environ. Microbiol. 76:4063-4075.
Hormazabal E, Piontelli E (2009). Endophytic fungi from Chilean native gymnosperms:antimicrobial activity against human and phytopathogenic fungi. World J. Microbiol. Biotechnol. 25:813-819.
Horn WS, Schwartz RE, Simmonds MSJ, Blaney WM (1994). Isolation and characterization of phomodiol, a new antifungal from *Phomopsis.* Tetrahedron Lett. 35:6037-6040.
http://www.avocadosource.com/wac7/Section_01/HakizimanaJD2011.pdf, accessed on 03 June 2012.
Huang Y, Wang J, Li G, Zheng Z, Su W (2001). Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis.* FEMS Immunol. Med. Microbiol. 31:163-167.
Joseph B, Priya RM (2011). Bioactive compounds from endophytes and their potential in pharmaceutical effect; a review. Am. J. Biochem. Mol. Biol. 1:291-309.
Karamchand KS, Sridhar KR, Bhat R (2009). Diversity of fungi associated with estuarine sedge *Cyperus malaccensis* Lam. J. Agric. Technol. 5:111-127.
Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D (2011). Anticancer compounds derived from fungal endophytes:their importance and future challenges. Nat. Prod. Rep. 28:1208-1228.
Kharwar RN, Verma VC, Strobel G, Ezra D (2008). The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don. Curr. Sci. 95:228-233.
Krishnamurthy YL, Naik SB, Jayaram S (2008). Fungal communities in herbaceous medicinal plants from the Malnad region, southern India. Microbes Environ. 23:24-28.
Li H, Qing C, Zhang Y, Zhao Z (2005). Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World J. Microbiol Biotechnol. 21:1515-1519.
Li L-Y, Ding Y, Groth I, Menzel K-D, Peschel G, Voigt K, Deng Z-W, Sattler I, Lin W-H (2008). Pyrrole and indole alkaloids from an endophytic *Fusarium incarnatum* (HKI00504) isolated from the mangrove plant *Aegiceras corniculatum*. J. Asian Nat. Prod. Res. 10:775-780.
Lin Z, Wen J, Zhu T, Fang Y, Gu Q, Zhu W (2008). Chrysogenamide A from an endophytic fungus associated with *Cistanche deserticola* and its neuroprotective effect on SH-SY5Y cells. J. Antibiot. 61:81-85.
Linaldeddu BT, Franceschini A, Pulina MA (2005). Epidemiological aspects of *Biscogniauxia mediterranea* in declining cork oak forest in Sardinia (Italy). IOBC/WPRS Bull. 28 (8):75-81.
Liu C, Liu T, Yuan F, Gu Y (2010a). Isolating endophytic fungi from evergreen plants and determining their antifungal activities. Afr. J. Microbiol. Res. 4:2243-2248.
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.
Liu L, Gao H, Chen X, Cai X, Yang L, Guo L, Yao X, Che Y (2010b). Brasilamides A-D:sesquiterpenoids from the plant endophytic fungus *Paraconiothyrium brasiliense*. Eur. J. Org. Chem. 2010:3302-3306.
Logrieco A, Mulè G, Moretti A, Bottalico A (2002). Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. Eur. J. Plant Pathol. 108:597-609.
Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000). New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua.* Plant Sci. 151:67-73.
Maciá-Vicente JG, Jansson HB, Abdullah SK, Descals E, Salinas J, Lopez-Llorca LV (2008). Fungal root endophytes from natural vege-

tation in Mediterranean environments with special reference to *Fusarium* spp. FEMS Microbiol. Ecol. 64:90-105.
Mahmoud A-LE (2000). Mycotoxin-producing potential of fungi associated with qat *(Catha edulis)* leaves in Yemen. Folia Microbiol. 45:452-456.
Mostert L, Crous PW, Kang J-C, Phillips AJL (2001). Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa:morphological, cultural, molecular and pathological characterization. Mycologia 93:146-167.
Mueller GM, Schmit JP (2007). Fungal biodiversity:what do we know? What can we predict? Biodiver. Conserv. 16:1-5.
Naik BS, Shashikala J, Krishnamurthy YL (2009). Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. Microbiol. Res. 164:290-296.
Nicoletti R, Buommino E, De Filippis A, Carella A, Brancato V, Tufano MA (2008a). Antiproliferative and pro-apoptotic effects of culture extracts of novel endophytic fungi. Abstract Book PSE Symposium on Natural Products in Cancer Therapy, Napoli, Italy. p. 31.
Nicoletti R, Buommino E, De Filippis A, Lopez-Gresa MP, Manzo E, Carella A, Petrazzuolo M, Tufano MA (2008b). Bioprospecting for antagonistic *Penicillium* strains as a resource of new antitumor compounds. World J. Microbiol. Biotechnol. 24:189-195.
Nicoletti R, Ciavatta ML, Buommino E, Tufano MA (2008c). Antitumor extrolites produced by *Penicillium* species. Int. J. Biomed. Pharm. Sci. 2:1-23.
Nicoletti R, De Stefano M, De Stefano S, Trincone A, Marziano F (2004). Antagonism against *Rhizoctonia solani* and fungitoxic metabolite production by some *Penicillium* isolates. Mycopathologia 158:465-474.
Parfitt D, Hunt J, Dockrell D, Rogers HJ, Boddy L (2010). Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecol. 3:338-346.
Paul NC, Kim WK, Woo SK, Park MS, Yu SH (2007). The fungal endophytes in roots of *Aralia* species and their antifungal activity. Plant Pathol. J. 23:287-294.
Pavlic D, Slippers B, Coutinho TA, Wingfield MJ (2007). Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. Plant Pathol. 56:624-636.
Porras-Alfaro A, Bayman P (2011). Hidden fungi, emergent properties:endophytes and microbiomes. Ann. Rev. Phytopathol. 49:291-315.
Promputtha I, Jeewon R, Lumyong S, McKenzie EHC, Hyde KD (2005). Ribosomal DNA fingerprinting in the identification of non-sporulating endophytes from *Magnolia liliifera* (Magnoliaceae). Fungal Diver. 20:167-186.
Ragazzi A, Moricca S, Capretti P, Dellavalle I, Turco E (2003). Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. For. Pathol. 33:31-38.
Rodrigues Gomes R (2008). *Phomopsis* spp. endófitos de plantas medicinais:diversidade genética e antagonismo ao fungo *Guignardia citricarpa*. PhD Dissertation, Universidad Federal do Paraná, Curitiba, Brazil.
Sakalidis ML, Hardy GEStJ, Burgess TI (2011). Class III endophytes, clandestine movement amongst hosts and habitats and their potential for disease; a focus on *Neofusicoccum australe*. Australasian Plant Pathol. 40:510-521.
Schulz B, Wanke U, Draeger S, Aust H-J (1993). Endophytes from herbaceous plants and shrubs:effectiveness of surface sterilization methods. Mycol. Res. 97:1447-1450.
Sette LD, Passarini MRZ, Delarmelina C, Salati F, Duarte MCT (2006). Molecular characterization and antimicrobial activity of endophytic fungi from coffee plants. World J. Microbiol. Biotechnol. 22:1185-1195.
Shukla M, Mishra MK (2012). Mycoflora associated with five commonly used medicinal plants of Karaikal (U.T. of Puducherry). Int. J. Sci. Res. Publ. 2(12):1-4.
Silva GH, Teles HL, Trevisan HC, Bolzani VdaS, Young MCM, Pfenning LH, Eberlin MN, Haddad R, Costa-Neto CM, Araújo AR (2005). New bioactive metabolites produced by *Phomopsis cassiae*, an endophytic fungus in *Cassia spectabilis*. J. Braz. Chem. Soc. 16:1463-1466.
Singh SB, Zink DL, Guan Z, Collado J, Pelaez F, Felock PJ, Hazuda DJ (2003). Isolation, structure, and HIV-1 integrase inhibitory activity of xanthoviridicatin E and F, two novel fungal metabolites produced by *Penicillium chrysogenum*. Helv. Chim. Acta 86:3380-3385.
Staniek A, Woerdenbag HJ, Kayser O (2008). Endophytes:exploiting biodiversity for the improvement of natural product–based drug discovery. J. Plant Interact. 3:75-93.
Sudheep NM, Sridhar KR (2012). Non-mycorrhizal fungal endophytes in two orchids of Kaiga forest (Western Ghats), India. J. For. Res. 23:453-460.
Sun X, Ding Q, Hyde KD, Guo LD (2012). Community structure and preference of endophytic fungi of three woody plants in a mixed forest. Fungal Ecol. 5:624-632.
Sun X, Guo L-D, Hyde KD (2011). Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Diver. 47:85-95.
Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS (2009). Fungal endophytes and bioprospecting. Fungal Biol. Rev. 23:9-19.
Tan X-M, Chen X-M, Wang C-L, Jin X-H, Cui J-L, Chen J, Guo S-X, Zhao L-F (2012). Isolation and identification of endophytic fungi in roots of nine *Holcoglossum* plants (Orchidaceae) collected from Yunnan, Guangxi, and Hainan provinces of China. Curr. Microbiol. 64:140-147.
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.
Taylor K, Barber PA, Hardy GEStJ, Burgess TI (2009). *Botryosphaeriaceae* from tuart (*Eucalyptus gomphocephala*) woodland, including descriptions of four new species. Mycol. Res. 113:337-353.
Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Shetty HS (2005). Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W. and A. (Combretaceae). World J. Microbiol. Biotechnol. 21:1535-1540.
Tian F, He R-F, Li M-Y, Wang J, Ye B-P (2010). The endophytic fungus strain FTJZZJ09 isolated from the bulbs of *Fritillaria thunbergii* and its antibacterial metabolites. Microbiology/Weishengwuxue Tongbao 37:1475-1480.
Vannini A, Scarascia Mugnozza G (1991). Water stress:a predisposing factor in the pathogenesis of *Hypoxylon mediterraneum* on *Quercus cerris*. Eur. J. For. Pathol. 21:193-201.
Vega FE (2008). Insect pathology and fungal endophytes. J. Invertebr. Pathol. 98:277-279.
Vega FE, Posada F, Aime MC, Peterson SW, Rehner SA (2008). Fungal endophytes in green coffee seeds. Mycosystema 27:75-84.
Vega FE, Posada F, Peterson SW, Gianfagna TJ, Chaves F (2006). *Penicillium* species endophytic in coffee plants and ochratoxin A production. Mycologia 98:31-42.
Vega FE, Simpkins A, Aime MC, Posada F, Peterson SW, Rehner SA, Infante F, Castillo A, Arnold AE (2010). Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and Puerto Rico. Fungal Ecol. 3:122-138.
Verkley GJM, da Silva M, Wicklow DT, Crous PW (2004). *Paraconiothyrium*, a new genus to accommodate the mycoparasite *Coniothyrium minitans*, anamorphs of *Paraphaeosphaeria*, and four new species. Stud. Mycol. 50:323-335.
Verma M, Brar SK, Tyagi RD, Surampalli RY, Valéro JR (2007). Antagonistic fungi, *Trichoderma* spp.:panoply of biological control. Biochem. Eng. J. 37:1-20.
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008). *Trichoderma*-plant-pathogen interactions. Soil Biol. Biochem. 40:1-10.
Wang Y, Guo LD, Hyde KD (2005). Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. Fungal Diver. 20:235-260.
Weber RWS, Stenger E, Meffert A, Hahn M (2004). Brefeldin A production by *Phoma medicaginis* in dead pre-colonized plant tissue:a strategy for habitat conquest? Mycol. Res. 108:662-671.
Whipps JM (2001). Microbial interactions and biocontrol in the

rhizosphere. J. Experim. Bot. 52:487-511.
Whipps JM, Sreenivasaprasad S, Muthumeenakshi S, Rogers CW, Challen MP (2008). Use of *Coniothyrium minitans* as a biocontrol agent and some molecular aspects of sclerotial mycoparasitism. Eur. J. Plant Pathol. 121:323-330.
Wicklow DT, Poling SM (2009). Antimicrobial activity of pyrrocidines from *Acremonium zeae* against endophytes and pathogens of maize. Phytopathology 99:109-115.
Xuan Q, Bao FK, Pan HM, Liu AH (2010). Isolating fungal endophyte from *Paris polyphylla* Smith var. *yunnanensis* and identifying their antibacterial ability. Afr. J. Microbiol. Res. 4:1001-1004.
Yang X, Strobel G, Stierle A, Hess WM, Lee J, Clardy J (1994). A fungal endophyte-tree relationship:*Phoma* sp. in *Taxus wallachiana.* Plant Sci. 102:1-9.
Zakaria L, Yaakop AS, Salleh B, Zakaria M (2010). Endophytic fungi from paddy. Tropic. Life Sci. Res. 21:101-107.
Zare R, Gams W (2001). A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73:1-50.