Extremophiles (2012) 16:95–103
DOI 10.1007/s00792-011-0409-9

ORIGINAL PAPER

Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl.

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Received: 11 July 2011 / Accepted: 20 October 2011 / Published online: 10 November 2011

Abstract A total of 564 isolates of endophytic fungi were recovered from the plants *Deschampsia antarctica* and *Colobanthus quitensis* collected from Antarctica. The isolates were screened against parasites *Leishmania amazonensis* and *Trypanosoma cruzi* and against the human tumour cell lines. Of the 313 fungal isolates obtained from *D. antarctica* and 251 from *C. quitensis*, 25 displayed biological activity. Nineteen extracts displayed leishmanicidal activity, and six inhibited the growth of at least one tumour cell line. These fungi belong to 19 taxa of the genera *Alternaria*, *Antarctomyces*, *Cadophora*, *Davidiella*, *Helgardia*, *Herpotrichia*, *Microdochium*, *Oculimacula*, *Phaeosphaeria* and one unidentified fungus. Extracts of 12 fungal isolates inhibited the proliferation of *L. amazonesis* at a low IC_{50} of between 0.2 and 12.5 μg ml^{-1}. The fungus *Phaeosphaeria herpotrichoides* displayed only leishmanicidal activity with an IC_{50} of 0.2 μg ml^{-1}, which is equivalent to the inhibitory value of amphotericin B. The extract of *Microdochium phragmitis* displayed specific cytotoxic activity against the UACC-62 cell line with an IC_{50} value of 12.5 μg ml^{-1}. Our results indicate that the unique angiosperms living in Antarctica shelter an interesting bioactive fungal community that is able to produce antiprotozoal and antitumoral molecules. These molecules may be used to develop new leishmanicidal and anticancer drugs.

Keywords Antarctica · Bioprospecting · *Leishmania* · Secondary metabolites · Antitumoral activities

Communicated by M. da Costa.

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Introduction

Bioprospecting has been defined as the systematic search for organisms, compounds and genes, which might have a potential biotechnological benefit as well as lead to product development. During the recent past years, the relevance of microorganisms on bioprospecting for drug discovery has been increasing and, among microbial source, the bioactive molecules produced by fungi represent a chemical reservoir for discovering new compounds with antibiotic, antioxidant, immunomodulating, anticancer and antiparasitic compounds. Endophytic fungi commonly refer to a group of fungi that have an asymptomatic lifestyle inside living plant tissues (Petrini 1991). Several studies suggest that endophytic fungi are ubiquitous in nature and represent an important genetic resource for biotechnology. Endophytes have been recognised as potential sources of novel natural products for pharmaceutical, agricultural and industrial applications, especially due to their ability to produce secondary metabolites with different biological activities.

The vegetation of Antarctica is characterised by an extremely low number of species; there are only two native angiosperms, the grass *Deschampsia antarctica* Desv. (*Poaceae*) and *Colobanthus quitensis* (Kunth) Bartl. (*Caryophyllaceae*). The occurrence of these two angiosperms is limited to areas within maritime Antarctica that have the most favourable climatic conditions. These angiosperms were assayed to characterise their endophytic fungal communities (Rosa et al. 2009; Rosa et al. 2010a), which are interesting reservoirs of fungal species adapted to the extreme conditions in Antarctica and represented predominantly by the genera *Alternaria*, *Phaeosphaeria* and *Davidiella*. However, other endophytic taxa associated with both angiosperms displayed low ITS sequence identity to the sequences of the fungal species deposited in GenBank, suggesting that these fungi could be new or endemic species adapted to the extreme conditions of Antarctica.

Endophytic fungi have a complex relationship with their host plant, which to survive produce rich and complex bioactive molecules. According to Strobel et al. (2004), reasonable hypotheses should govern the plant selection strategy for the discovery of bioactive endophytic fungi; one of these includes plants from unique environmental settings, especially those with an unusual biology and possessing novel strategies for survival. The endemism and Antarctic survival of the endophytic fungi associated with *D. antarctica* and *C. quitensis* indicates that they may present different biochemical pathways able to generate new molecules for the development of new drugs. *Leishmania* are protozoan parasites that cause high morbidity and mortality levels and are recognised by the WHO as a major tropical public health problem (Asford 1997). There are currently no vaccines for leishmaniasis; although the drugs available for leishmaniasis treatment are toxic, expensive and sometimes ineffective, they are the only effective way to treat all forms of the disease (Croft and Coombs 2003). Chagas disease (American *Trypanosomiasis*) is caused by the haemoflagellate protozoan *Trypanosoma cruzi*. According to Reyes and Vallejo (2005), there is evidence that trypanocidal drug with nitrofuran and imidazole compounds can treat acute *T. cruzi* infection, but further studies are needed to develop new trypanocidal drugs. In addition, cancer has great medical, social and economic impacts around the world and some anticancer agents show serious cytotoxic effects not only to malignant cells, but also to normal tissues, including myelocytes and cells of the immune system (Anazetti et al. 2003).

Endophytic fungal communities associated with plants living in extreme conditions, with their diversity of species and their diverse genetic and metabolic pathways, may be resources for intelligent screening for discovering new drugs to treat neglected diseases, as well as cancer. The aim of this study was to test the extracts of the endophytic fungi associated with these two plants against *Leishmania* (*Leishmania*) *amazonensis* and *Trypanosoma cruzi*, as well as against human tumour cell lines.

Methods

Isolation of the endophytic fungi

Endophytic fungal isolates were obtained from healthy specimens of *Deschampsia antarctica* Desv. (*Poaceae*) and *Colobanthus quitensis* (Kunth) Bartl. from the Admiralty Bay of King George Island in the South Shetland Islands, Antarctica during the austral summer between December 2006 and January 2007. The leaves were surface sterilised by successive dipping in ethanol 70% (1 min) and 2% sodium hypochlorite (3 min), followed by washing once with sterile distilled water (2 min) (Rosa et al. 2010a). The fragments were plated on Petri plates containing potato dextrose agar (PDA, Difco, USA) and chloramphenicol (100 μg ml^{-1}). The plates were incubated for up to 60 days at 15°C, and individual colonies were transferred to PDA and stored at 4°C. The endophytic fungal isolates associated from *C. quitensis* were obtained, as previously described by Rosa et al. (2010a). But briefly, fresh leaves of *C. quitensis* were collected from plants growing under natural conditions across a 25.5-km transect through Admiralty Bay, between January and February of 2008, during the austral summer. The leaves were subjected to surface sterilisation as described above. The fragments were plated on Petri plates containing PDA supplemented with chloramphenicol (100 μg ml^{-1}). The plates were incubated at 18°C for up to 60 days, and individual colonies were transferred to PDA and stored at 4°C. All fungal isolates used in this study were deposited in the Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais under UFMGCB codes.

Fungal cultures and extraction

Five millimetre-diameter plugs of each filamentous endophytic fungus were inoculated on the centre of Petri dishes (90 mm diameter, 20 ml PDA). The plates were incubated at 15 ± 2°C for 15 days. The culture materials from each Petri dish were dissected and transferred to 50-ml vials tubes containing 25 ml of ethanol. After 72 h at room temperature, the organic phase was decanted and the solvent was removed under vacuum centrifuge at 35°C. Endophytic yeasts were grown in 24-well plates containing GYMP medium (glucose 2%, yeast extract 0.5%, malt extract 1% and Na_2PO_4 0.2%) at 15 ± 2°C for 15 days. After incubation, 1 ml of ethanol was added to each well, and the content was macerated, incubated at 10°C for 48 h

and filtrated. The ethanol phase was transferred to 1.5-ml tubes and dried by evaporation in a vacuum centrifuge at 35°C. An aliquot of each dried extract was dissolved in dimethyl sulfoxide (DMSO, Merck, USA) to a 20 mg ml^{-1} stock solution, which was stored at −20°C.

Assay with *Leishmania amazonensis*

Promastigotes of *Leishmania (Leishmania) amazonensis* (strain IFLA/BR/196/PH-8) were obtained from lesions of infected hamsters. The parasites were grown at 26°C in pH 7.2 Schneider's medium and then stimulated to differentiate into amastigote-like forms by increasing the temperature (32°C) and lowering the pH (6.0) of the medium. After seven days under these conditions, 90% of the promastigotes were transformed into amastigote-like forms, which were then used in the bioassays. Amastigote density was adjusted to 1×10^8 parasites per ml, and 90 µl was added to each well of 96-well plates. Ten microlitres of extracts and control solutions were added to attain the desired concentrations. The plates were incubated at 32°C for 72 h, and then cell viability was determined using the MTT (5 mg ml^{-1}) assay (Teixeira et al. 2002). The results are expressed as percentage inhibition in relation to controls without drugs. Amphotericin B at 0.2 µg ml^{-1} (Fungison® Bristol-Myers Squibb B, Brazil) was used as a positive drug control. All assays were performed in triplicate.

In vitro assay with amastigote intracellular forms of *Trypanosoma cruzi*

In vitro assay with amastigote forms of *T. cruzi* was performed according to protocols established by Buckner et al. (1996) with modifications. Briefly, parasites and culture procedures: *T. cruzi* (Tulahuen strain) expressing the *Escherichia coli* β-galactosidase gene were grown on monolayer of mouse L929 fibroblasts. Cultures to be assayed for β-galactosidase activity were grown in RPMI 1640 medium (pH 7.2–7.4) without phenol red (Gibco BRL) plus 10% foetal bovine serum and glutamine. Ninety-six well tissue culture microplates were seeded with L929 fibroblasts at 4.0×10^3 per well in 80 µl and incubated overnight. β-Galactosidase expressing trypomastigotes were then added at 4.0×10^4 per well in 20 µl. After 2 h, the medium with trypomastigotes that did not penetrate into the cells was discarded and replaced by 200 µl of fresh medium. After 48 h, the medium was discarded again and replaced by 180 µl of fresh medium and test extracts in 20 µl. Each extract was tested in triplicate. After 7 days of incubation, chlorophenol red β-D-galactopyranoside (CPRG) (100 mM final concentration) and Nonidet P-40 (0.1% final concentration) were added to the plates and incubated overnight at 37°C and the absorbance measured at 570 nm in an automated microplate reader. Benznidazole at its IC_{50} (1 µg ml^{-1}) was used as positive control. The results are expressed as percentage growth inhibition. All assays were performed in triplicate.

Assay with human cancer cell lines

The effect of crude extract on the survival and growth of the human cancer cell lines UACC-62 (melanoma), MCF-7 (breast) and TK-10 (renal) was determined using a colorimetric method developed at the National Cancer Institute, USA (Monks et al. 1991). Briefly, cells were inoculated in 96-well plates and allowed to stabilise for 24 h in a CO_2 incubator at 37°C. Solutions of extracts were added to attain the desired concentrations, and plates were incubated for 48 h under the same conditions. Trichloroacetic acid was added to each well to precipitate the proteins, which were quantified in a colorimetric assay using the dye sulphorodamine B. All assays were run in triplicate wells and repeated at least once. Etoposide at 16 µg ml^{-1} and cancer cell lines without fungal extracts were used in parallel as positive and negative controls, respectively. Results are expressed as percentage of growth inhibition in comparison to the control without drug.

Half maximal inhibitory concentration (IC_{50}) values

The bioactive crude extracts were submitted to half maximal inhibitory concentration (IC_{50}) determinations, which represent the measure of the effectiveness of a crude extract (or compounds) to inhibit the targets (in this study, protozoan or cancer cells) functions. This quantitative measure indicates how much of a particular crude extract is needed to inhibit a given biological process by half. The software GraphPad Prism version 4.03 was used to calculate the IC_{50} values using the non-linear curve fitting of two or more independent experimental datasets to a four parameter logistic dose–response model. No constraints were applied to the curve fitting calculations. All assays were performed in duplicate.

Molecular identification of the bioactive fungi

Molecular identification was performed on all bioactive endophytic fungi. Fungal DNA extraction was performed, as previously described by Rosa et al. (2009). The primers ITS1 and ITS4 were used to amplify the ITS regions between the SSU and LSU regions (ITS1-5.8S-ITS2) (White et al. 1990). Sequencing was performed using the methods described by Rosa et al. (2009). The sequences were analysed using SeqMan Π with Lasergene software (DNASTAR/Inc.), and a consensus sequence was obtained using the software Bioedit v. 7.0.5.3. To identify species by

rRNA gene sequencing, the consensus sequence was aligned with all sequences of related species retrieved from the GenBank database using the Fasta 2.0 program (Altschul et al. 1997). The consensus sequences were deposited into GenBank, and the accession numbers are shown in Table 2. The criteria used by Rosa et al. (2010a) was used interpret the sequences of the GenBank database: for sequence identities ≥98%, the genus and species were accepted; for sequence identities between 95 and 97%, only the genus was accepted; and, for sequence identities ≤95%, isolates were labelled as "unknown" fungi. The taxa were submitted to phylogenetic relationships to demonstrate the nearest relatives, which were estimated using MEGA Version 4.0 (Tamura et al. 2007). The maximum composite likelihood model was used to estimate evolutionary distance with bootstrap values calculated from 1,000 replicate runs.

Results and discussion

Screening for biological activities and fungal identification

A total of 564 endophyte fungal isolates (313 isolates from *D. antarctica* and 251 from *C. quistensis*) were screened, and 25 isolates (4.43%) displayed at least one biological activity (Table 1). The extracts obtained from the endophytic yeasts did not show any biological activity, but the filamentous endophytic fungi did. Nineteen extracts displayed leishmanicidal activity and were able to inhibit the growth of *L. amazonensis* by more than 70%. Six fungal extracts displayed cytotoxic activity indicating greater than 60% inhibition. Of these six extracts, four were active against MCF-7 cells, one against TK-10 cells and one against UACC-62 cells. No extract displayed trypanocidal activity. All leishmanicidal extracts were obtained from endophytic fungi recovered from *D. antarctica*, while the majority (five) of the cytotoxic extracts were obtained from fungal endophytes isolated from *C. quitensis*. The phylogenetic comparison among the sequences of bioactive endophytes isolated from tissues of *D. antarctica* and *C. quitensis* and their nearest relatives fungal sequences deposited in GenBank are shown in Fig. 1.

Conidiogenesis is rare for the most endophytic fungi (Rosa et al. 2009; Vaz et al. 2009; Rosa et al. 2010a). For this reason, all bioactive fungal endophytes were identified by molecular analysis. Eighteen *Ascomycota* taxa were identified (Table 2), which belong to the genera *Alternaria*, *Antarctomyces*, *Cadophora*, *Davidiella*, *Helgardia*, *Herpotrichia*, *Microdochium*, *Oculimacula* and *Phaeosphaeria*.

Four endophytic fungi sequences had 88–96% nucleotide similarity with sequences of *Alternaria* species deposited in GenBank and were identified as *Alternaria* sp. 1, *Alternaria* sp. 2, *Alternaria* sp. 3 and *Alternaria* sp. 4. Recent studies have shown the occurrence of several *Alternaria* species as endophytes associated with tropical, temperate and polar plants (Morakotkarn et al. 2006; Wang et al. 2007; Rosa et al. 2009), which are able to produce different bioactive secondary metabolites with different bioactivities.

The fungus UFMGCB 2569 showed 96% identity with the sequence of uncultured fungus (EU516862) that was obtained from snow-covered soils in Austria, and 95 and 94% identity with the sequences of *Cadophora luteo-olivacea* (FJ486276) and *Cadophora malorum* (AB190402 and GU212434), respectively. The fungus UFMGCB 2569 was identified as *C. luteo-olivacea*, and had only a 2.6% nucleotide difference from *C. luteo-olivacea* (FJ486276). The *Cadophora* species have been isolated in soils as indigenous fungi in the Antarctica (Arenz et al. 2006); these were observed to attack historic wooden structures (Arenz and Blanchette 2009) and were in association with Antarctic moss (Tosi et al. 2002). According to Blanchette et al. (2010), some *Cadophora* species seem to have a circumpolar distribution in the Antarctic, as well as the Arctic, suggesting an adaptation to the extreme polar environment.

Seven endophytic fungi displayed nucleotide identities ranging from 90 to 94% with *Phaeosphaeria* species and were identified as *Phaeosphaeria* sp. 1–7. In addition, the taxon UFMGCB 2272 and 2672 displayed nucleotide identities of 98% to *Phaeosphaeria herpotrichoides* (FJ911873) that was previously recovered from seaweed thalli of *Adenocystis utricularis* in the Antarctica (Loque et al. 2010). In the Antarctica, *Phaeosphaeria* was identified as one of the most frequent endophytic genera associated with *D. antarctica* (Rosa et al. 2009), but occur also associated with mosses and lichens (Moller and Dreyfuss 1996) and in the soil (Connell et al. 2006). Drug discovery studies have demonstrated that the potential of *Phaeosphaeria* species to produce bioactive compounds is scarce. Zhang et al. (2008a, b) isolated the compound phaeosphenone, which has broad-spectrum antimicrobial activity, from *Phaeosphaeria* sp. Eleven compounds including naphthalenones, naphthoquinones, unsymmetrical naphthoquinone dimers and a chlorinated diphenyl ether have been isolated from *Phaeosphaeria* sp. from a tropical forest in Thailand and displayed antimycobacterial and tumoral activities (Pittayakhajonwut et al. 2008). However, to our knowledge, this is the first time that endophytic *Phaeosphaeria* species obtained from Antarctica displayed selectivity in leishmanicidal activity.

The isolate UFMGCB 2630 displayed 94% nucleotide sequence divergence with *Helgardia* sp. (AM262430), the fungus UFMGCB 2567 displayed 93% sequence identity to different sequences of unidentified fungi and *Oculimacula*

Table 1 Fungal crude extracts which displayed biological activities equal to or above 60% when tested at 20 μg ml^{-1} against human tumour cells, amastigote forms of the *Leishmania amazonensis* and *Trypanosoma cruzi*

Endophytic taxa	UFMGCB code[a]	Inhibition of human tumoral cells			Antiprotozoal activity	
		MCF-7[b]	TK-10[c]	UACC-62[d]	Killing of LA	Killing of TC
Alternaria sp. 1	2301	6 ± 0.6	13 ± 0.8	0 ± 1.5	**72 ± 0.6**[e] **(20 ± 0.7)**[f]	0 ± 0
Alternaria sp. 2	2508	1 ± 0.7	11 ± 1.4	9 ± 2.4	**94 ± 0.6 (20 ± 0.6)**	9 ± 4.8
Alternaria sp. 3	2564	0 ± 0.7	19 ± 1.4	1 ± 2.6	**91 ± 0.6 (12.5 ± 0.1)**	4 ± 1.4
Alternaria sp. 4	2673	14 ± 0.6	29 ± 1.3	1 ± 2.6	**89 ± 0.3 (3.2 ± 0.1)**	0.6 ± 0.8
Cadophora luteo-olivacea	2569	0 ± 0.7	17 ± 1.4	5 ± 2.5	**94 ± 0.4 (20 ± 0.5)**	9 ± 3.3
Helgardia sp.	2630	7 ± 0.7	26 ± 1.3	4 ± 2.5	**90 ± 0.5 (3.4 ± 0.2)**	4 ± 5.7
Herpotrichia sp.	2682	2 ± 0.7	15 ± 1.4	5 ± 2.5	**94 ± 0.5 (3.5 ± 0.1)**	0 ± 0
Phaeosphaeria herpotrichoides	2272	0 ± 0.6	19 ± 0.9	0 ± 1.5	**70 ± 0.3 (20 ± 0.3)**	0 ± 0
Phaeosphaeria sp. 1	2515	0 ± 0.8	0 ± 1.5	0 ± 2.6	**94 ± 0.7 (20 ± 0.8)**	2.1 ± 3
Phaeosphaeria sp. 2	2518	0 ± 0.7	32 ± 1.3	6 ± 2.5	**94 ± 0.3 (20 ± 0.3)**	3 ± 5.6
Phaeosphaeria sp. 3	2528	5 ± 0.7	8 ± 1.4	2 ± 2.5	**91 ± 0.2 (1.9 ± 0.4)**	5 ± 3.4
Phaeosphaeria sp. 4	2560	0 ± 0.7	0 ± 1.5	3 ± 2.5	**94 ± 0.5 (7 ± 0.8)**	2 ± 3.7
Phaeosphaeria sp. 5	2649	0 ± 0.7	31 ± 1.3	1 ± 2.6	**89 ± 0.3 (5.5 ± 0.6)**	4 ± 6.1
Phaeosphaeria sp. 6	2650	9 ± 0.7	24 ± 1.3	0 ± 2.6	**82 ± 0.5 (5 ± 0.7)**	4 ± 0.9
Phaeosphaeria sp. 7	2667	0 ± 0.7	42 ± 1.2	6 ± 2.5	**93 ± 0.5 (20 ± 0.8)**	1.7 ± 2.5
Phaeosphaeria sp. 8	2669	0 ± 0.7	10 ± 1.4	3 ± 2.5	**74 ± 0.3 (0.4 ± 0.9)**	0 ± 0
Oculimacula sp.	2567	12 ± 0.6	24 ± 1.3	1 ± 2.5	**83 ± 0.4 (20 ± 0.4)**	3 ± 3.1
Endophytic fungus	2661	0 ± 0.8	**60 ± 1.2 (20 ± 0.8)**	3 ± 2.5	44 ± 0.7	6 ± 3
Antarctomyces psychrotrophicus	2666	0 ± 0.7	25 ± 1.3	6 ± 2.5	**84 ± 0.5 (9.3 ± 0.6)**	2.1 ± 3.0
Phaeosphaeria herpotrichoides	2672	3 ± 0.7	34 ± 1.3	0 ± 2.6	**86 ± 0.5 (0.2 ± 0.8)**	1.5 ± 2.1
Davidiella tassiana	2455	**82 ± 0.3 (20 ± 0.5)**	43 ± 0.8	11 ± 1.2	33 ± 0.5	8.5 ± 2.2
Microdochium sp.	2290	**62 ± 0.4 (17 ± 0.4)**	25 ± 1	27 ± 1.2	8 ± 0.5	8 ± 7.2
Microdochium phragmitis	2479	**77 ± 0.3 (8 ± 0.5)**	53 ± 0.8	18 ± 1	40 ± 0.5	4 ± 5
Microdochium phragmitis	2656	**61 ± 0.4 (12.5 ± 0.4)**	30 ± 0.9	4 ± 1.3	34 ± 0.5	3 ± 4.4
Endophytic fungus	2603	0 ± 0.7	20 ± 0.9	**75 ± 1.5 (20 ± 0.7)**	47 ± 0.5	2.6 ± 3.7
Control drugs	Etoposideo	44.5 ± 0.3	80 ± 1	83 ± 0.7	–	–
	Amphotericin B	–	–	–	71 ± 0.3	–
	Benznidazole	–	–	–	–	73 ± 9.7

LA, *Leishmania amazonensis*; TC, *Trypanossoma cruzi*

[a] UFMGCB = Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais

[b] MCF-7 = mammary

[c] UACC-62 = melanoma

[d] TK-10 = kidney

[e] The promising values (mean and standard error) in percentage of biological inhibition

[f] The promising values (mean and standard error) in inhibition at 50% (IC_{50}) in μg ml^{-1} of fungal extracts are given in bold

species and was identified as *Oculimacula* sp. The genera *Helgardia* and *Oculimacula* are an anamorph/teleomorph complex. In the Antarctica, Upson et al. (2009) obtained *Helgardia* and *Oculimacula* taxa as dark septate endophytes (DSE) associated with the roots of *C. quitensis*. The fungus UFMGCB 2682 showed only 1% sequence difference from that of *Herpotrichia juniperi* (FJ839639) and was, therefore, identified as *H. juniperi*. The genus *Herpotrichia* includes species described as endophytes associated with plants living in cold ecosystems. This is the first study that shows the leishmanicidal activity of the *Helgardia*/*Oculimacula* complex and the *Herpotrichia* taxa living in the Antarctic ecosystems.

The fungus UFMGCB 2666 shared 96% divergent nucleotides with *Antarctomyces psychrotrophicus* (AM489755). The genus *Antarctomyces* is represented only by *A. psychrotrophicus*; it was isolated from Antarctic soil (Stchigel et al. 2001) and from the thalli of the seaweed *A. utricularis*

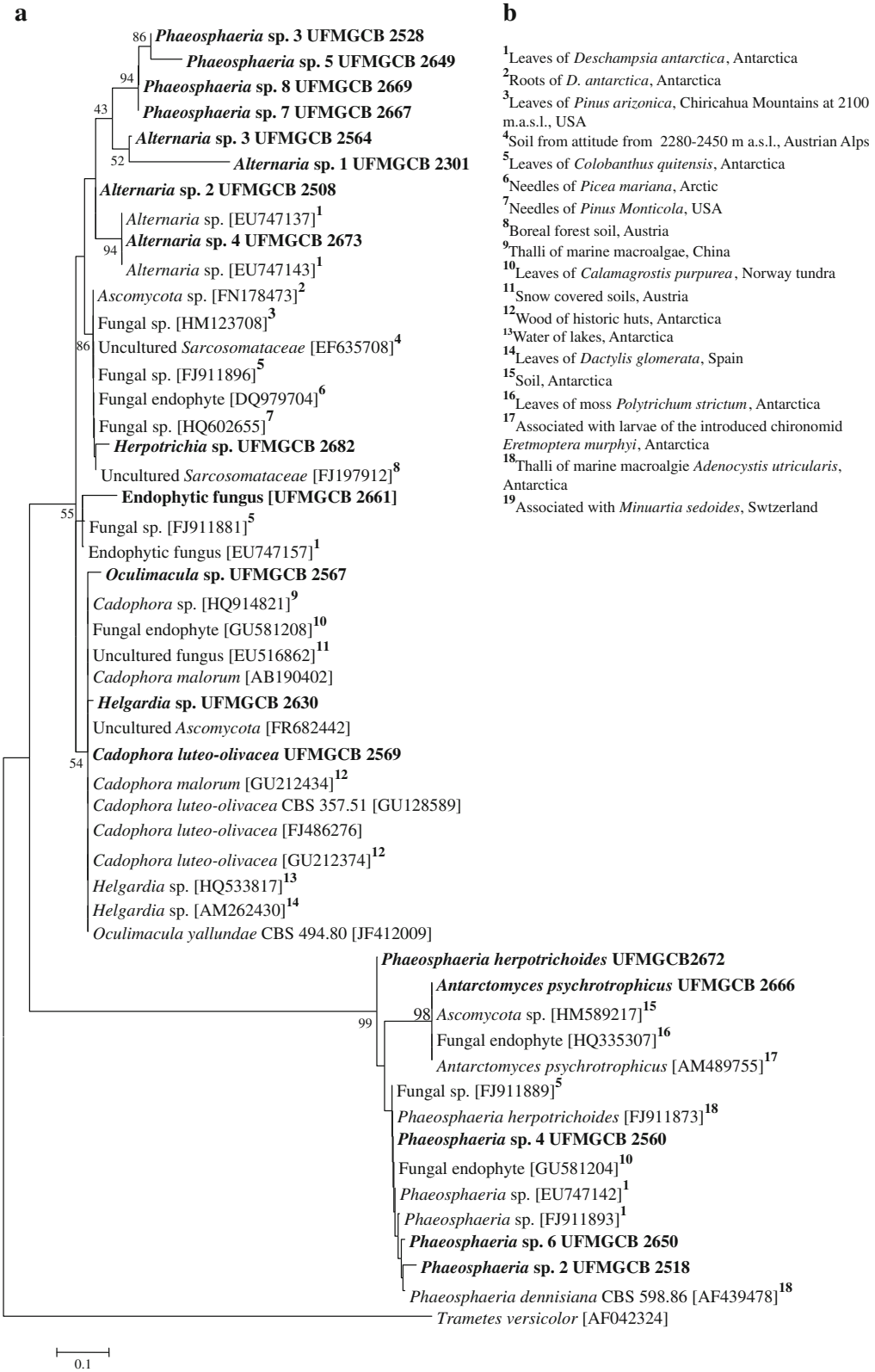

Fig. 1 **a** Phylogenetic tree showing the nearest relatives of bioactive endophytic fungi (in *bold*). The tree was constructed based on the rRNA gene sequences (ITS1-5.8S-ITS2) using the maximum composite likelihood model. The tree was rooted with *Trametes versicolor* [AF042324] as outgroup. **b** Substrates and origins of nearest relatives' fungal sequences

Table 2 Top BLAST match to the ITS1-5.8S-ITS2 sequence of a representative isolate from each endophytic fungus, with substratum and origin of sequence, and identification with GenBank accession number

UFMGCB[a] code	Identity (%)	Top BLAST search results [GenBank accession number]	Substratum and origin	Proposed species or taxonomic group [GenBank accession number]
2301	90	*Alternaria* sp. [EU747137]	Leaf of *Deschampsia antarctica*, Antarctica	*Alternaria* sp. 1 [JN247811]
2508	95	*Alternaria* sp. [EU747153]	Leaf of *D. antarctica*, Antarctica	*Alternaria* sp. 2 [JN247809]
2564	88	*Alternaria* sp. [EU747137]	Leaf of *D. antarctica*, Antarctica	*Alternaria* sp. 3 [JN247806]
2673	96	*Alternaria* sp. [EU747143]	Leaf of *D. antarctica*, Antarctica	*Alternaria* sp. 4 [JN2478101]
2666	96	*Ascomycota* sp.	Soil, Antarctica	*Antarctomyces psychrotrophicus* [JN247807]
2569	96	Uncultured fungus [EU516862]	Soil covered by snow, Austria	*Cadophora luteo-olivacea* [JF800174]
2661	92	Fungal sp. [FJ911881]	Leaf of *Colobanthus quitensis*, Antarctica	Endophytic fungus [JN247815]
2630	94	*Helgardia* sp. [AM262430]	Leaf of *Dactylis glomerata*, Spain	*Helgardia* sp. [JN247812]
2682	94	Fungal sp. [HQ602655]	Needle of *Pinus Monticola*, USA	*Herpotrichia* sp. [JN247813]
2567	93	Endophytic fungus [GU581208]	Leaf of *Calamagrostis purpurea*, Norway	*Oculimacula* sp. [JN247814]
2272	98	*Phaeosphaeria herpotrichoides* [FJ911873]	Thalli of *Adenocystis utricularis*, Antarctica	*P. herpotricoides* [JN247823]
2672		*Phaeosphaeria herpotrichoides* [FJ911873]	Thalli of *A. utricularis*, Antarctica	*P. herpotricoides* [JN247808]
2518	92	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 1 [JN247816]
2528	92	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 2 [JN247817]
2560	98	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 3 [JN247818]
2649	91	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 4 [JN247819]
2650	89	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 5 [JN247820]
2667	96	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 6 [JN247821]
2669	95	*Phaeosphaeria* sp. [EU747142]	Leaf of *D. antarctica*, Antarctica	*Phaeosphaeria* sp. 7 [JN247822]

Identification was made using BLASTn searches of the ITS1-5.8S-ITS2 of the rRNA

[a] UFMGCB = Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais

(Loque et al. 2010). According to Arenz and Blanchette (2011), *A. psychrotrophicus* is endemic to the Antarctica; our results suggest that this species is able to live as endophytes of *D. antarctica* in the Antarctic ecosystem. The fungus UFMGCB 2661 had 92% sequence identity with that of the fungal sp. (FJ911881), which was recovered as an endophyte from the leaves of *D. antarctica* (Rosa et al. 2009), and 91% identity with different sequences of uncultured taxa. This isolate was, therefore, characterised as an unidentified endophytic fungus.

Determination of IC_{50} values

All bioactive fungal extracts were assayed to determine their IC_{50} values. The fungal extracts with leishmanicidal activities inhibited the proliferation of the *Leishmania* parasite with IC_{50} values between 0.2 and 20 μg ml^{-1} (Table 1). *Phaeosphaeria herpotrichoides* UFMGCB 2672 displayed the best IC_{50} value (0.2 μg ml^{-1}) in the leishmanicidal tests with a value equivalent to that of the control drug amphotericin B. In addition, *Phaeosphaeria* sp. UFMGCB 2669, *Oculimacula* sp. UFMGCB 2567, *Phaeosphaeria* sp. UFMGCB 2528, *Helgardia* sp. UFMGCB 2630 and *Herpotrichia* sp. UFMGCB 2682 displayed low IC_{50} values ranging from 0.4 to 3.5 μg ml^{-1} against the amastigote forms of *L. amazonensis*. Endophyte fungi have been found to be promising sources of leishmanicidal compounds. Rosa et al. (2010b) recovered endophytic fungi of the genera *Alternaria*, *Arthrinium*, *Cochliobolus*, *Fusarium*, *Gibberella* and *Penicillium* from tropical plants, which displayed leishmanicidal activity and IC_{50} values ranging from 4.6 to 24.4 μg ml^{-1}. In addition, the endophytic fungus *Cochliobolus* sp. UFMGCB 555 was able to produce the leishmanicidal compounds cochlioquinone A and isocochlioquin, which displayed IC_{50} of 1.7 and 4.1 μM, respectively (Campos et al. 2008). However, to our knowledge, this is the first work in which endophytic fungi obtained from plants from the Antarctica displayed leishmanicidal activity.

Six of the extracts tested displayed specific cytotoxic activity, four of which inhibited only the mammary MCF-7 cells with IC_{50} values ranging from 8 to 20 μg ml^{-1}. Among the six extracts displaying cytotoxic activity, the extracts of *Microdochium phragmitis* UFMGCB 2479 (IC_{50} of 8 μg ml^{-1}) and UFMGCB 2656 (IC_{50} of 12.5 μg ml^{-1}) displayed the best IC_{50} values to the MCF-7 tumour cell line compared with the control drug etoposide (16 μg ml^{-1}). *Microdochium* species have been described as endophytes (Márquez et al. 2007) and as DSE in the roots of the native prairie tallgrass (Mandyam et al. 2010); some species of *Microdochium* display strong antimicrobial and antifungal activities (Zhang et al. 2008a, b). These endophytic fungal taxa may be promising candidates for further studies of their leishmanicidal and cytotoxic compounds. This study shows that endophytic mycota associated with *D. antarctica* and *C. quitensis* are sources of leishmanicidal and anticancer molecules and may represent potential therapeutic treatments for leishmaniasis, a deadly tropical disease, as well as for cancer.

Acknowledgments This study had financial and logistic support from the Brazilian Antarctic Program, Marine of Brazil and Clube Alpino Paulista. This work is part of the API activity 403 and contributes to Microbiological and Ecological Responses to Global Environmental Changes in Polar Regions and INCT Criosfera. The authors would also like to express our great appreciation to Isis Galliza, Daniela N.B. Maia, Luciana F. Soares, and Fernanda L.M. Francisco for performing biological activities. It was financially supported by the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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