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ORIGINAL PAPER

Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica

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Abstract We surveyed the diversity and capability of producing bioactive compounds from a cultivable fungal community isolated from oligotrophic soil of continental Antarctica. A total of 115 fungal isolates were obtained and identified in 11 taxa of *Aspergillus*, *Debaryomyces*, *Cladosporium*, *Pseudogymnoascus*, *Penicillium* and *Hypocreales*. The fungal community showed low diversity and richness, and high dominance indices. The extracts of *Aspergillus sydowii*, *Penicillium allii-sativi*, *Penicillium brevicompactum*, *Penicillium chrysogenum* and *Penicillium rubens* possess antiviral, antibacterial, antifungal, antitumoral, herbicidal and antiprotozoal activities. Bioactive extracts were examined using ^{1}H NMR spectroscopy and detected the presence of secondary metabolites with chemical shifts. Our results show that the fungi present in cold-oligotrophic soil from Antarctica included few dominant species, which may have important implications for understanding eukaryotic survival in cold-arid oligotrophic soils. We hypothesize that detailed further investigations may provide a greater understanding of the evolution of Antarctic fungi and their relationships with other organisms described in that region. Additionally, different wild pristine bioactive fungal isolates found in continental Antarctic soil may represent a unique source to discover prototype molecules for use in drug and biopesticide discovery studies.

Keywords Antarctica · Drug discovery · Ecology · Fungi · Taxonomy

Introduction

Antarctica has an area of approximately 4 million km^2 and is reported to be the most pristine environment on the planet. However, only approximately 2 % of the total Antarctic area is ice free and comprises extensive mountains, nunataks and coastal outcrops (Azmi and Seppelt 1998). In general, Antarctic soils represent extreme habitats for the colonization and survival of life. Among the biota present in Antarctica,

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the microbial life is represented by archaea, bacteria and cyanobacteria. Fungi are the most highly diverse group in the different Antarctic ecosystems, including the extremophile soils. According to Fell et al. (2006), the soils of the continental region within Antarctica offer an interesting opportunity to investigate the regional-to-global environmental effects of microbial webs on community structures.

The kingdom Fungi represents an important element within the microbial communities in Antarctica. Various species of fungi have been isolated from different habitats including freshwater, the marine environment, rocks, plant tissues, lakes, marine sediments and different types of soil. The mycobiota of Antarctica have typically included yeasts and filamentous fungi consisting of species in the phyla *Chytridiomycota*, *Zygomycota*, *Glomeromycota*, *Ascomycota* and *Basidiomycota* (Ruisi et al. 2007). Additionally, fungi range in diversity across the latitudinal gradient in the sub-Antarctic islands, Antarctic Peninsula and continental Antarctic ecosystems. According to Rao et al. (2012), life in continental Antarctica is restricted to rare occurrences of some species of lichens, mosses, invertebrates and soil microbial communities. In continental Antarctica, fungi have been more often described in lichens symbioses; however, the occurrence of free-living fungi in soils remains poorly understood.

Fungal metabolic pathways are a rich source of bioactive compounds, including different classes of secondary molecules such as terpenoids, polyketides, alkaloids, polyacetylenes and others with different biological activities. Such molecules can be important prototype used to develop new drugs and pesticides. In recent years, the capability of different fungal communities across the Antarctic environments to produce compounds for use in biotechnological processes has been reported. However, few bioprospecting research programs have focused on the fungi present in unique and/or unexplored environments. Thus, the search for extremophile fungi in Antarctica may represent a useful strategy for finding new eukaryotic metabolic pathways and, consequently, new bioactive compounds (Santiago et al. 2012). For the reasons presented above, the aims of the present study were to characterize the diversity, richness, dominance and similarity of the fungal community present in the cold-arid oligotrophic soil of continental Antarctica region and to investigate the capability of these fungi to produce bioactive compounds with antimicrobial, antiviral, antitumoral, antiprotozoal, herbicidal and insecticidal activities.

Materials and methods

Study area

Thirteen soil samples were collected in the Ellsworth Mountains (Supplementary Fig. 1) in the summer of 2011 from ice-free areas of the Union Glacier region in the southern Heritage Range. Samples of soil at 0–8 cm depth were collected using sterile scoops, placed in Whirl–Pak (Nasco, Ft. Atkinson, WI) bags and kept at −20 °C until transported to the laboratory at the Federal University of Minas Gerais, where they were processed to isolate the fungi. The soil samples were studied by routine chemical analyses (pH, available *P*, exchangeable Ca^{2+}, Na^{+}, Mg^{2+}, K^{+} and Al^{3+}) according to international standard protocols established by Kuo (1996) and EMBRAPA (1997). Total organic carbon was determined according to Yoemans and Bremner (1988).

Fungal isolation

To isolate the fungal species from soil, 1 g of each sample was added to 9 ml of 0.85 % NaCl and 100 µL of a 10^{-1} dilution was inoculated onto YM (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 2 % glucose, 2 % agar), MEA (1.5 % malt extract, 1.5 % glucose, 0.1 % peptone, 2 % agar), DG18 (Oxoid, USA) and DRBC (Oxoid, USA) media containing 100 µg mL^{-1} of chloramphenicol (Sigma) and incubated at 5°, 10° and 15 ± 2 °C for 60 days. Fungal colony-forming units (CFUs) were counted, and subcultures were made of all of the morphologically distinct colonies from each sample. The subcultures were grouped into different morphotypes according to their cultural (colony color and texture, border type and radial growth rate) and micromorphological characteristics on YM agar. All fungal isolates were deposited in the Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais under code UFMGCB, which are available to be accessed by other scientists.

Fungal identification

The protocol for DNA extraction from filamentous fungi followed Rosa et al. (2009). The internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 (White et al. 1990). Amplification of the ITS region was performed as described by Rosa et al. (2009). Amplification of the β-tubulin (Glass and Donaldson 1995) and ribosomal polymerase B2 (Houbraken et al. 2012) genes were performed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Godinho et al. (2013). Yeasts were characterized via standard methods and the taxonomic keys according to Kurtzman et al. (2011). Yeast identities were confirmed by sequencing the D1-D2 variable domains of the large subunit ribosomal RNA gene using the primers NL1 and NL4 (Lachance et al. 1999) and the ITS region using the primers ITS1 and ITS4 (White et al. 1990).

Representative consensus sequences of fungal taxa were deposited into GenBank (Supplementary Table 1). To achieve

species-rank identification based on ITS, β-tubulin data and ribosomal polymerase B2, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al. 1997). The criteria suggested by Godinho et al. (2013) were used to interpret BLAST results from the GenBank database. The phylogenetic analysis were conducted using MEGA Version 5.0 (Tamura et al. 2011). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. To complete the molecular identification, the sequences of known-type fungal strains or reference sequences obtained from fungal species deposited in international culture collections found in GenBank were added to improve the accuracy of the phylogenetic analysis. The information about fungal classification generally follows Kirk et al. (2008), MycoBank (http://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org) databases.

Diversity, richness, dominance and distribution

To quantify species diversity, richness and dominance, we used Fisher's α, Margalef's and Simpson's indices, respectively. The similarities among fungal taxa from different sites were estimated using the Bray–Curtis measure. All diversity and similarity indices were performed using the computer program PAST, version 1.90 (Hammer et al. 2001).

Fungal cultivation and preparation of extracts for biological assays

All fungal isolates were cultivated using solid-state fermentation according to protocols established by Rosa et al. (2013). Briefly, a 5-mm diameter plug of fungus was place on 20 mL of YM medium at the center of 200 Petri dishes (90 mm diameter) and cultured for 15 days at 10 ± 2 °C. These fungal cultures were lyophilized for 72 h, cut in small pieces and transferred to 50-mL glass centrifuge tubes followed by the addition of 50 mL of dichloromethane (DCM, Fisher, USA). After 72 h at room temperature, the organic phase was filtered and the solvent removed under rotary evaporation at 40 °C. An aliquot of each dried extract was dissolved in DMSO (Merck) to prepare a 100 mg mL^{-1} stock solution, which was stored at −20 °C. Sterile YM medium was extracted using the same procedure. The sterile YM extract was used as the control in the screening procedure.

Assay for antimicrobial activity

Susceptibility testing against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 12600, *Pseudomonas aeruginosa* ATCC 10145, *Candida albicans* ATCC 18804, *Candida krusei* ATCC 6258 and *Cladosporium sphaerospermum* CCT 1740 was performed using a protocol established by Carvalho et al. (2012). All extracts (dissolved in DMSO) were diluted to a final concentration of 250 μg mL^{-1} for use in the antimicrobial assays. The results are expressed as the percent inhibition in relation to controls without drugs. All antimicrobial assays were performed in duplicate.

Assay with human cancer cell lines

The effect of crude extract on the survival and growth of the human cancer cell lines MCF-7 (breast) and TK-10 (renal) was determined using a colorimetric method developed at the National Cancer Institute, USA (Monks et al. 1991). All assays were run in triplicate wells and repeated at least once. All extracts (dissolved in DMSO) were diluted to a final concentration of 20 μg mL^{-1} for use in the cytotoxic assays. Etoposide (16 μg mL^{-1}) and cancer cell lines without fungal extracts were used in parallel as positive and negative controls, respectively. The results are expressed as percentage of growth inhibition in comparison to the control without drug.

Assay with *Leishmania amazonensis*

In vitro assays with promastigote forms of *Leishmania amazonensis* were performed according to protocols established by Teixeira et al. (2002). The results are expressed as percent inhibition in relation to controls without drugs. All extracts (dissolved in DMSO) were diluted to a final concentration of 20 μg mL^{-1} for use in the assay. 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 assays with intracellular amastigote forms of *Trypanosoma cruzi*

In vitro assays with amastigote forms of *T. cruzi* were performed according to protocols established by Romanha et al. (2010). Each extract diluted in DMSO was tested in duplicate at 20 μg mL^{-1}. Benznidazole at its IC_{50} (1 μg mL^{-1}) was used as positive control. The results were expressed as the percentage of growth inhibition.

Assays against *Dengue virus*

For antiviral activity assays against *Dengue virus 2*, monolayers of Baby Hamster Kidney 21 cells (BHK-21) grown in flat bottom, 96-well plates using Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 % fetal bovine serum (FBS), 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 0.25 μg mL^{-1} amphotericin B, were

exposed to different concentrations of fungal extracts for 72 h in the presence of *Dengue virus* 2. Fungal extracts were provided as 2 mg mL^{-1} in 10 % DMSO. Virus suspensions and extracts, at concentrations ranging from 100 to 0.78 mg mL^{-1} were simultaneously added to the plates, in two replicates. Controls for cells (uninfected untreated cells), virus (infected untreated cells) in the presence or not of DMSO were run in parallel during each experiment. The antiviral activity was evaluated by grading system of the peculiar cytopathic effect (CPE) caused by *Dengue virus* observed by optic microscopy (Tang et al. 2012) followed by colorimetric assay using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT/Sigma-USA) (Betancur-Galvis et al. 1999). The results are expressed as the percent of CPE inhibition in relation to controls without extracts. All antiviral assays were repeated at least three times.

Assay against fungal plant pathogens

The phytopathogens *Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Fusarium oxysporum* and *Botrytis cinerea* were used as target species for the antifungal assay according to protocols established by Wedge and Kuhajek (1998) and Wedge et al. (2009). The extracts were diluted in DCM and assayed at 180 μg $spot^{-1}$. Spots of the technical-grade fungicides benomyl, cyprodinil, azoxystrobin and captan (Chem Service, Inc. West Chester, Pennsylvania) at 2 μL (at 2 μM) diluted in ethanol were used as standard controls. All antifungal assays were performed in duplicate.

Herbicide assay

The herbicidal activities of fungal extracts were guided by *Lactuca sativa* (lettuce) and *Agrostis stolonifera* (bentgrass) bioassays according to Dayan et al. (2000). The fungal extracts (dissolved in acetone) were diluted to a final concentration of 1 mg mL^{-1}. Phytotoxicity was qualitatively evaluated by visually comparing the amount of germination of the seeds in each well with the untreated controls after 7 days. The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0–5, where 0 = no effect and 5 = no growth or no germination of the seeds. Each experiment was repeated in duplicate.

Mosquito larval bioassays

Larvae of *Aedes aegypti* L. used in these screening bioassays were hatched from the eggs obtained from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. The eggs were hatched and the larvae were maintained at a temperature of 27 ± 2 °C and 60 ± 10 % RH in a photoperiod regimen of 12:12 h (L:D). Bioassays were conducted using the system described by Pridgeon et al. (2009) to determine the larvicidal activity of these compounds against *Ae. aegypti*. All extracts (dissolved in ethanol) were diluted to a final concentration of 125, 62.5 and 31.25 ppm.

NMR spectroscopy

Bioactive fungal extracts were analyzed by NMR spectroscopy on a Varian INOVA 600 MHz spectrometer (Palo Alto, CA). ^{1}H NMR spectra were recorded in either $CDCl_3$ or MeOD. Samples were prepared at approximately 10 mg mL^{-1} and analysis recorded in 3 mm NMR tubes using a standard ^{1}H NMR pulse program.

Results

Fungal taxonomy

A total of 115 fungal isolates were recovered from eight soil samples collected in continental Antarctica. The fungal isolates were identified by molecular sequencing of the ITS, β-tubulin, ribosomal polymerase B2 and D1-D2 domains representing 11 taxa in the genera *Aspergillus*, *Debaryomyces*, *Cladosporium*, *Pseudogymnoascus*, *Penicillium* and one labeled as *Hypocreales* (Supplementary Table 1; Figs. 1, 2, 3). The taxa with the greatest densities were *Hypocreales* sp., *Penicillium brevicompactum*, and *Aspergillus sydowii*. In contrast, *Cladosporium* sp. 3, *Cladosporium* sp. 2 and *Debaryomyces hansenii* were rare components within the community. *Cladosporium* sp. 1, *Cladosporium* sp. 2, *Cladosporium* and sp. 3 (Figs. 1c, 2c), *Pseudogymnoascus* sp. (Fig. 1d) and *Hypocreales* sp. (Fig. 1a) presented low molecular identities and inconclusive information compared with known fungal sequences deposited in the GenBank database.

Fungal diversity

In general, the fungal community showed low diversity (Fisher's α = 1.42) and richness (Margalef = 1.25) and high dominance (Simpson = 0.8) indices; however, the diversity indices varied according to the different sites (Supplementary Table 2). Sites ELL 11, ELL 7 and ELL 13 showed the highest Fisher's α and Margalef indices, respectively, which also displayed moderate concentrations of TOC. At the same time, site ELL 8 showed the highest TOC concentration, but displayed moderate Fisher's and Margalef indices. The fungal diversity in the sites did not appear to be related to any other parameters that were measured.

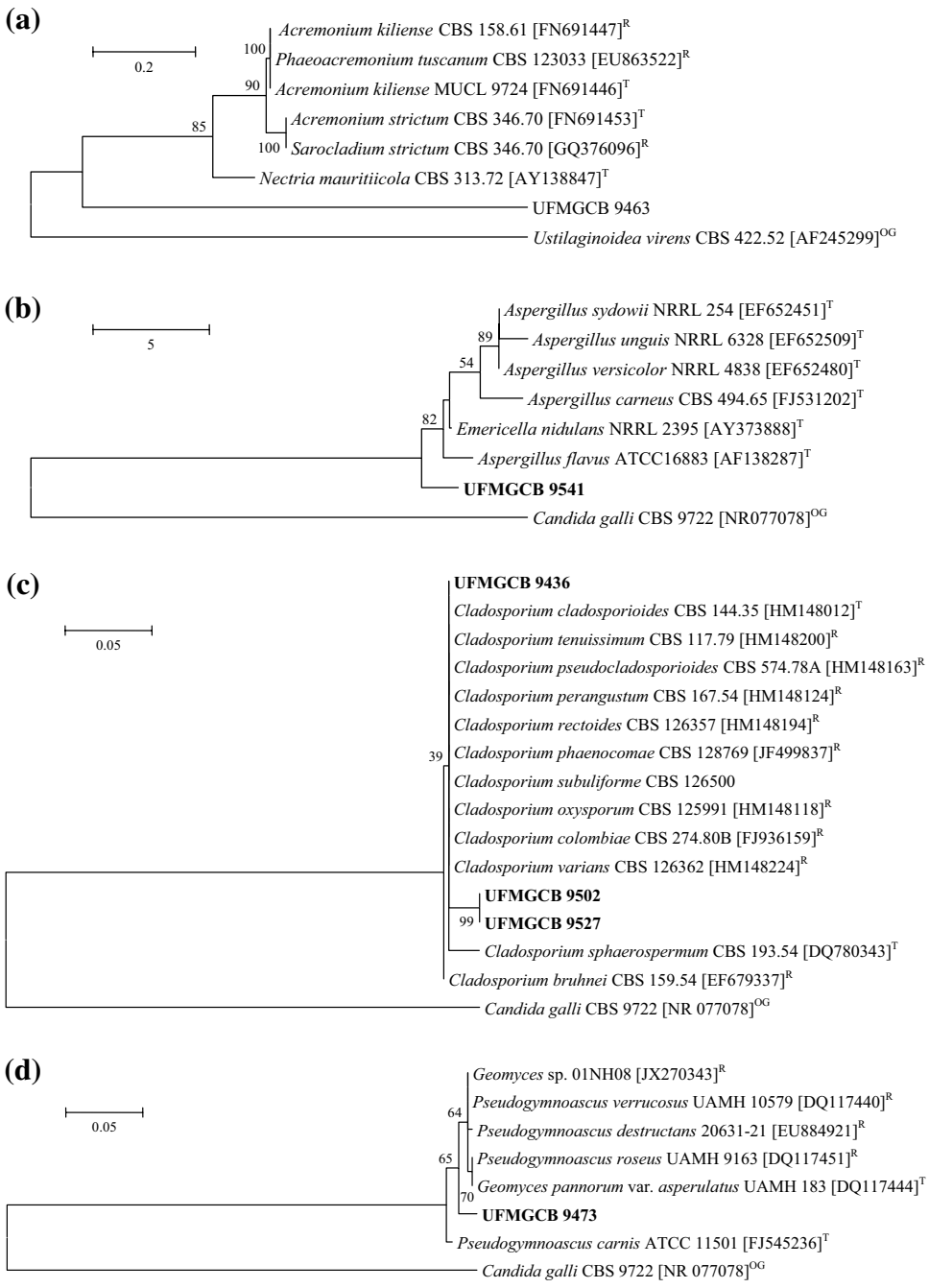
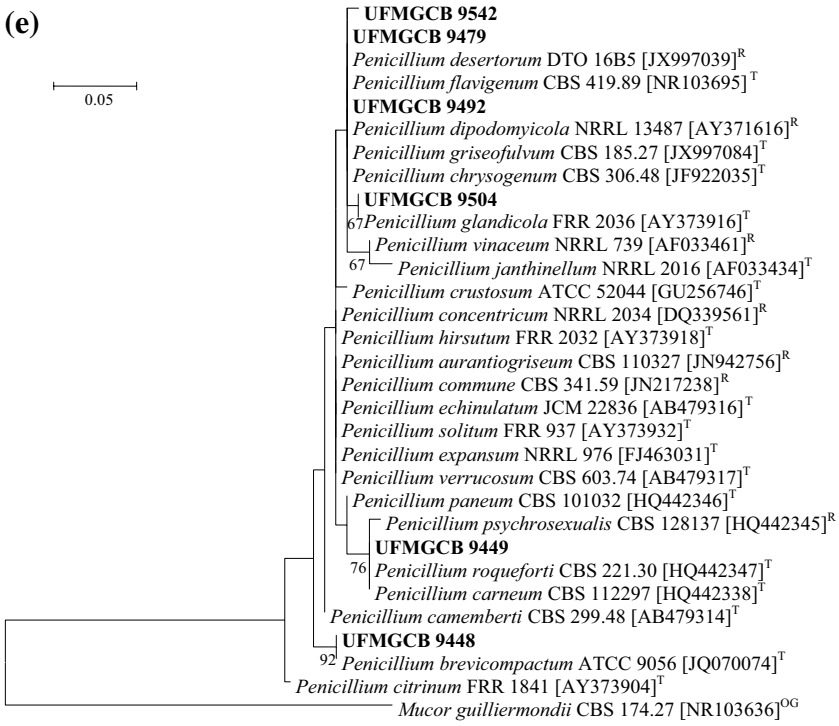


Fig. 1 Phylogenetic analysis of the sequences of fungi (*in bold*) isolated from continental Antarctic soil compared with type (**T**) or reference (**R**) sequences of the closest species following BLAST analysis, deposited in the GenBank database. The trees were constructed based on the ITS region sequences using the maximum composite likelihood method. *OG* reference sequence used as out-group

In addition to the low fungal diversity present in the cold-arid continental Antarctic soil, the Bray–Curtis similarity index (Supplementary Fig. 2) showed that the sites with the most similar species assemblages were ELL 12 and ELL 13, both with high concentrations of phosphorus.

The fungi isolated from the cold-arid soil of the Ellsworth Mountains in continental Antarctica showed a different temperature profile and type of culture media preference (Supplementary Table 3). The majority of the fungi grew at 10° and 15 °C. Only *Cladosporium* sp. 2 and *Cladosporium* sp. 3 grew at 5 °C and may exhibit a psychrophilic physiology. Additionally, *P. chrysogenum*, *P. rubens*, *P. allii-sativi*, *P. brevicompactum*, *Cladosporium* sp. 1, *Cladosporium* sp. 3, *Pseudogymnoascus* sp. and *D. hansenii* were able to grow on DG18 medium, which might indicate physiological capabilities to survive in xerophilic conditions. Additionally, *Cladosporium* sp. 3, which may be a new species, was isolated on DG18 medium at 5 °C and may represent a species adapted to the cold-arid conditions of continental Antarctica. However, further physiological studies are needed to characterize the abilities of these fungi to survive in extreme cold and arid conditions.

Fig. 1 continued



Bioprospection

Under the growth conditions we used, 17 fungal isolates produced compounds with biological activities against the targets screened (Supplementary Table 4) and (Table 1). Among them, the extracts of different isolates of *Aspergillus sydowii*, *Penicillium allii-sativi*, *P. brevicompactum*, *P. chrysogenum* and *P. rubens* showed antibacterial, antifungal, antitumoral, antiprotozoal and herbicidal activities, which have equal or greater activity compared with the control drugs. Extracts from *P. allii-sativi*, *P. brevicompactum*, *P. chrysogenum*, in particular, the *P. brevicompactum* (strain UFMGCB 9480) showed a broad high antiviral activity against *Dengue virus* 2 and antiprotozoal activity against *T. cruzi,* the agent of the Chagas disease (both neglected tropical diseases), strong antifungal activity against the fungal plant pathogen *Colletotrichum gloesporioides*, as well as herbicidal activity. In contrast, the extracts of *P. brevicompactum* (strain UFMGCB 9478) and *P. chrysogenum* (strain UFMGCB 9438) had selective activities against *C. gloeosporioides*. No activity was observed for any extracts against *Aedes aegypti* larvae at the highest concentration of 125 ppm. Bioactive extracts were examined using ^{1}H NMR analysis to inspect for the presence of secondary metabolites with interesting chemical shifts (Fig. 4). In general, all of the extracts contained the presence of fatty acid functional groups, and most extracts contained signals indicative of the presence of triglycerides. More importantly, downfield ^{1}H NMR signals for nearly all of the extracts indicated the presence of highly functionalized secondary metabolites due to the presence of protons in the aromatic and olefinic regions.

Discussion

Fungal taxonomy, distribution and diversity

In the present study, the soil samples were collected in the Heritage Range that forms the southern part of the Ellsworth Mountain system. The fungi isolated from the cold-arid oligotrophic soil of Heritage Range belongs to the classes *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, *Saccharomycetes* and *Sordariomycetes*. According to Ruisi et al. (2007), *Ascomycota* in anamorphic stages is the most common fungal phylum found in Antarctica; these fungi seem to have given up sexual reproduction as a simplified means to conclude their life cycles in a shorter time without high metabolic costs. Most of the known Antarctic fungi are cosmopolitan, cold-tolerant species that have been selected by, or have adapted to, the prevailing low temperatures (Möller and Dreyfuss 1996).

In our study, *Hypocreales* sp. (which might represent a new fungal species), *P. brevicompactum* and *A. sydowii* showed the highest densities and were the dominant species in the fungal community; the genus *Penicillium* also occurs predominantly in the cold-arid soil sampled. According to McRae et al. (1999), the genus *Penicillium* is cosmopolitan

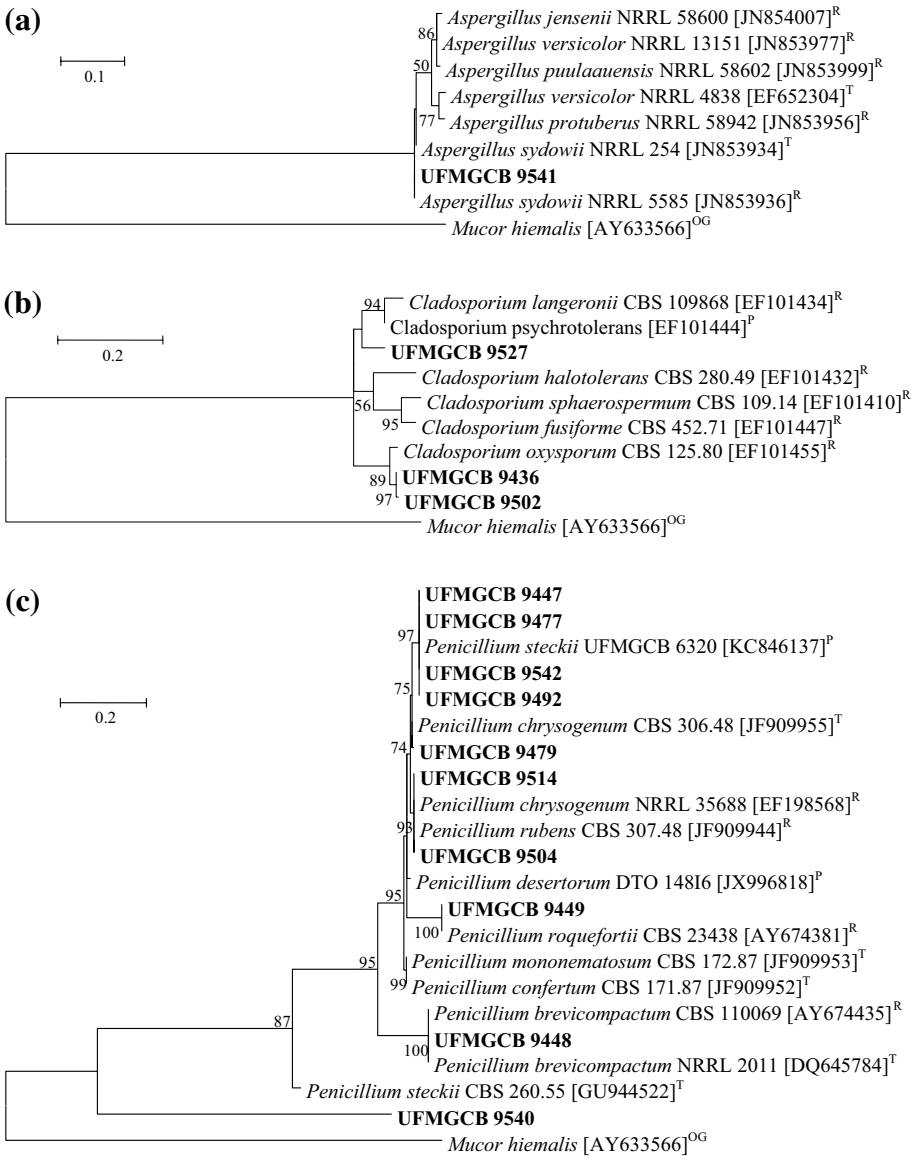


Fig. 2 Phylogenetic analysis of the β-tubulin sequences of the UFMGCB fungi (*in bold*) compared with type (**T**) or reference (**R**) sequences of the closest species following BLAST analysis, deposited in the GenBank database. The trees were constructed based on the β-tubulin gene sequences using the maximum composite likelihood method. *OG* reference sequence used as out-group

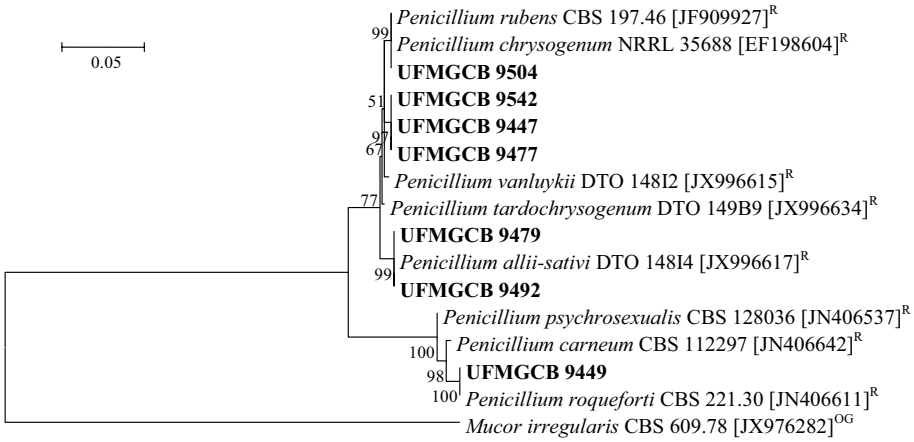


Fig. 3 Phylogenetic analysis of the ribosomal polymerase B2 gene sequences of the *Penicillium* species (UFMGCB) *in bold* compared with reference (**R**) sequences of the closest species following BLAST analysis, deposited in the GenBank database. The tree was constructed based on the polymerase B2 gene sequences using the maximum composite likelihood method. *OG* reference sequence used as out-group

Table 1 Phytopathogenic antifungal, herbicidal and activities of the extracts of fungal species from cold-arid oligotrophic soil of the continental Antarctica

		Targets to detect pesticides compounds						
		Phytopathogenic fungi[a]					Plants	
Fungal species	UFMGCB	*C. gloesporioides*	*C. fragariae*	*C. acutatum*	*F. oxysporum*	*B. cinerea*	*L. sativa*	*A. stolonifera*
Aspergillus sydowii	9541	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
Penicillium allii-sativi	9451	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
P. allii-sativi	9458	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**3**	**3**
P. allii-sativi	9508	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0	0
P. allii-sativi	9509	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**2**
P. allii-sativi	9524	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**3**	**2**
Penicillium brevicompactum	9446	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
P. brevicompactum	9448	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
P. brevicompactum	9478	**26 ± 2.7**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**3**
P. brevicompactum	9480	**25.8 ± 3**	**6 ± 0.5**	**4 ± 0.3**	0 ± 0	0 ± 0	**2**	**4**
P. brevicompactum	9538	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**1**
Penicillium chrysogenum	9466	0 ± 0	**7 ± 0**	0 ± 0	0 ± 0	0 ± 0	**3**	**3**
P. chrysogenum	9470	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**2**
P. chrysogenum	9445	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
P. chrysogenum	9534	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**1**
P. chrysogenum	9438	**24.3 ± 2.2**	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	**4**
Penicillium rubens	9496	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**1**	0
Control drugs	Benomyl	**20.5 ± 0.7**	**19.5 ± 0.7**	**14.5 ± 2.1**	–	**24 ± 1.4**	–	–
	Cypro	**22.5 ± 0.7**	**20 ± 0**	**11.5 ± 0.7**	–	–	–	–
	Cap	**19.5 ± 0.7**	**17.5 ± 0.7**	**15 ± 1.4**	**15.5 ± 0.7**	**24 ± 1.4**	–	–
	Azo	**29.5 ± 0.7**	–	**17 ± 1.4**	–	–	–	–

The bioactive extracts are in bold

[a] Mean inhibitory clear zones and standard errors were used to determine the level of antifungal activity against each fungal species; *UFMGCB* Culture of Microorganisms and Cells of the Universidade Federal de Minas Gerais. Technical-grade agrochemical fungicides, *Ben* benomyl, *Cypro* cyprodinil, *Cap* captan, *Azo* azoxystrobin with different modes of action were used at 2 µL (at 2 µM) as internal standards. – not assayed. Fungal targets: *C. gloesporioides*, *Colletotrichum gloesporioidesi*; *C. fragariae*, *Colletotrichum fragariae*; *C. acutatum*, *Colletotrichum acutatum*; *F. oxysporum*, *Fusarium oxysporum albicans*; *B. cinerea*, *Botrytis cinerea*. The qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0–5, where 0 = no effect and 5 = no growth or no germination of the seeds. Plant targets: *L sativa*, *Lactuca sativa* (lettuce); *A. stolonifera*, *Agrostis stolonifera* (bentgrass). Against the phytopathogens fungi, the extracts were assayed at 180 µg spot^{-1}. For the herbicidal activity, the extracts were assayed and 1 mg mL^{-1}

and found virtually everywhere from the tropics to the poles; however, its occurrence, diversity and frequency may be limited in some regions of the world (Pitt 1979). *Penicillium* has been described as the most common fungal genus in Antarctic ecosystems (McRae et al. 1999), as it is found in lakes (Gonçalves et al. 2012), soils (Azmi and Seppelt 1998), historic woodlands (Arenz et al. 2006), ice (D'Elia et al. 2009), macroalgal thalli (Loque et al. 2010) and marine sediments (Gonçalves et al. 2013). According to Corry (1987), most of the filamentous fungi found in areas distant from the marine coast are *Penicillium* species that are known to produce spores able to tolerate dry conditions. *Penicillium brevicompactum* is one of the most xerophilic penicillia and is resistant to ultraviolet radiation (Gehrke et al. 1995); it is known as an in vitro cellulose decomposer, and its ecological role may be mycophily and/or mycoparasitism (Scott et al. 2008). Fletcher et al. (1985) demonstrated that *P. brevicompactum* was one of the taxa that was most often isolated from the soil of Enderby Land, Antarctica. *Aspergillus* is a cosmopolitan genus commonly isolated from soil and plant debris. In Antarctica, *Aspergillus* species were isolated from ornithogenic soil (Wicklow 1968). *Aspergillus sydowii* has a worldwide distribution and occurs in many substrates in indoor and outdoor environments, including soil (Klich 2002). In Antarctica, *A. sydowii* was isolated from the soil of the Ross Sea Region and Antarctic Peninsula (Arenz and Blanchette 2011).

Penicillium chrysogenum and *P. rubens* are very common panglobal species found in indoor environments, deserts, dried foods, salterns and cheese (Frisvad and Samson 2004). In Antarctica, *P. chrysogenum* was detected in different samples, including soil with plant populations,

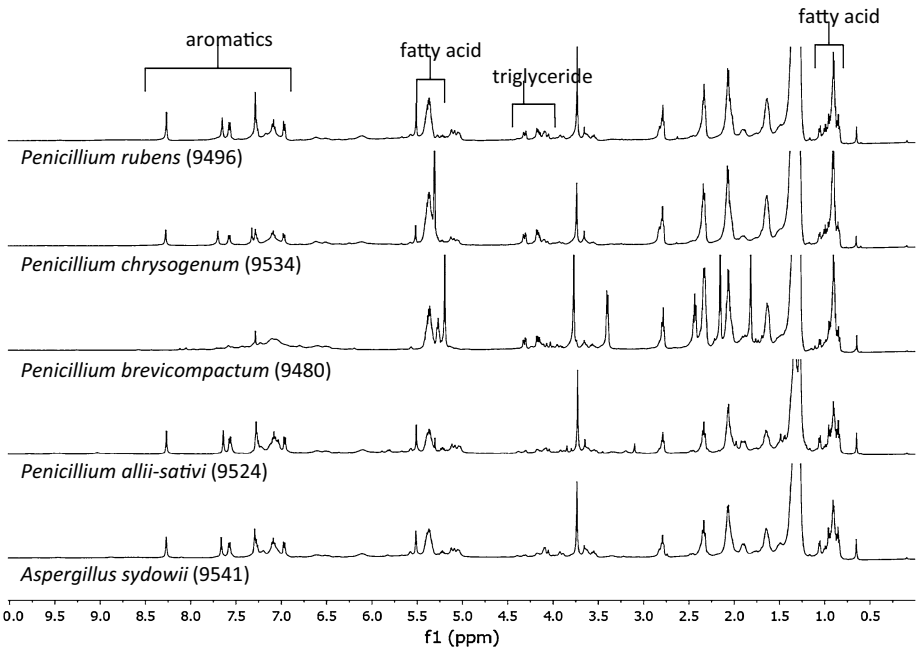


Fig. 4 ^{1}H NMR spectrum (600 MHz in $CDCl_3$) of representative crude extracts from each species of fungi. *Labels* indicate regions typically indicative of identifying protons for particular types of compounds

in ornithogenic soils (McRae et al. 1999) and was associated with macroalgae (Godinho et al. 2013). Zucconi et al. (2012) isolated *P. chrysogenum* as a dominant species present in permafrost in Antarctica. *Penicillium allii-sativi* was recently described as a new species isolated from garlic, soil, salterns, sorghum malt and mixed pig feed (Houbraken et al. 2012), and it has a broad distribution. This is the first report of *P. allii-sativi* in Antarctica.

Fletcher et al. (1985) suggested that the genus *Penicillium* has an extensive distribution in Antarctica, indicating that some species may play a role in decomposition processes in these ecosystems. The studies of Block (1994) and Rudolph and Benninghoff (1977) indicate that *Penicillium* species are major decomposers and represent an important element of the terrestrial nutrient cycle in the harsh Antarctic environment where a limited range of other microbial taxa can tolerate the extreme climate. McRae et al. (1999) suggested that *Penicillium* populations isolated from Antarctica may be useful as biological indicators of anthropogenic contamination because they are common contaminants of human foods, fibers, fuels and building materials.

Debaromyces hansenii, an ascomycetous yeast and a known cryotolerant and halotolerant species associated with food spoilage (Kurtzman et al. 2011), was isolated from soil of the Ross Sea Region and Antarctic Peninsula (Arenz and Blanchette 2011). According to Rao et al. (2012), the yeasts found in Antarctica may emerge as a physiological group well adapted to Antarctic soils, although a systematic landscape-scale survey is needed to test this hypothesis.

According to Bensch et al. (2010), *Cladosporium* is one of the largest genera of dematiaceous hyphomycetes with a worldwide distribution, which includes saprobic and parasitic species. However, some *Cladosporium* species are known only from specific hosts or have a restricted geographical distribution. *Cladosporium* has usually been associated with plants in Antarctica (Meyer et al. 1967), but was found as one of the dominant genera in the soil of Dry Valleys in Antarctica (Arenz et al. 2006).

Pseudogymnoascus species, typically and historically identified under the name *Geomyces*, have been frequently recorded in Antarctica with a ubiquitous distribution and from a number of substrates, including in the soils of cold regions (Mercantini et al. 1989), mosses (Tosi et al. 2002), leaves of *Colobanthus quitensis* (Rosa et al. 2010), thalli of macroalgae (Loque et al. 2010; Minnis and Lindner 2013; Furbino et al. 2014), freshwater lakes (Gonçalves et al. 2012) and the soil of the Ross Sea Region and Antarctic Peninsula (Arenz and Blanchette 2011). According to Lorch et al. (2013) and Minnis and Lindner (2013), the diversity of *Geomyces* and allies, including *Pseudogymnoascus* as revealed by DNA sequence data, is greater than previously recognized on the basis of traditional taxonomic methods.

An important aspect of the present study was the low diversity represented by few fungal genera found in the soil of the Heritage Range. This contrasts with the study of Fell et al. (2006), which found a complex community of symbionts, saprobes, predators and parasitic/pathogenic fungi in the Dry Valleys soils of Antarctica, a region considered to be among the world's most extreme environments. However, the physic-chemical characteristics of the Heritage Range seem to be more limiting to life compared with the Dry Valleys, especially regarding the amount of organic compounds and pH.

Hypocreales sp. UFMGCB 9463, the three *Cladosporium* species and *Pseudogymnoascus* sp. which showed low molecular identities with the sequences of the nearest species deposited in GenBank, have changes that represent endemic taxa. These fungi could represent unknown fungi or a known group of fungi whose sequences have not been submitted to GenBank, and additional phylogenetic and morpho-physiological taxonomic studies are necessary to confirm whether these fungi represent new fungal taxa. Future taxonomic studies will describe the potential new fungal species, in particular, the taxon *Pseudogymnoascus* sp. UFMGCB 9473, which showed genetic similarity with species able to kill bats in Europe and North America.

Bioprospection

According to Santiago et al. (2012), the ability of Antarctic fungi to survive in extreme conditions suggests that they may display unusual biochemical pathways that allow them to generate specific or new molecules that could be used as prototypes to develop new drugs. Our bioprospecting results show that the extracts from *Penicillium* and *Aspergillus* species present in the cold-arid oligotrophic soil of Antarctica are able to produce strong antiviral, antibacterial, antifungal, antitumoral, antiprotozoal, and herbicidal compounds.

Aspergillus and *Penicillium* species are well-known producers of many bioactive compounds, but few species found in Antarctica have been chemically investigated. *Aspergillus sydowii* isolated from the deep sea produced 4-dihydroxy-3,5,6-trimethylbenzaldehyde, a strong cytotoxic agent (Li et al. 2007). From extracts of *A. sydowii*, Ren et al. (2010) isolated cytotoxic hetero-spirocyclic γ-lactams, and Trisuwan et al. (2011) obtained a hydrogenated xanthon with antioxidant activity. *Penicillium allii-sativi* produces several secondary metabolites, including penicillins (Houbraken et al. 2012). *Penicillium brevicompactum* produces mycophenolic acid (MPA), one of the oldest known antibiotics (Danheiser et al. 1986). *Penicillium rubens* produces the polyketide compound chloctanspirone A that inhibits human leukemia HL-60 and a lung cancer cell line (Ireland et al. 2003).

In Antarctica, Brunati et al. (2009) found *Penicillium* species living in Antarctic lakes, and these species were able to produce cytotoxic and antimicrobial compounds. Among them, the extract of *P. chrysogenum* displayed selective antibacterial activity against *S. aureus*, *Enterococcus faecium* and *E. coli*, due to the presence of rugulosin and skyrin, two bioactive bis-anthraquinones. The extract of *P. chrysogenum* recovered from the endemic Antarctic macroalga *Palmaria decipiens* yielded extracts with high and selective antifungal and/or trypanocidal activities (Godinho et al. 2013).

We also observed discrepancies among the bioactivities of different isolates of the same fungal species (*P. allii-sativi*, *P. brevicompactum* and *P. chrysogenum*). This result is not uncommon, as intra-specific genetic differences have already been observed for different fungal strains of the same species. In fact, distinct secondary metabolites can be produced by conspecific isolates in other fungi (Möller et al. 1996). This result indicates that the low inter-specific diversity of the fungal community present in the oligotrophic soil of continental Antarctica may be offset with a high intra-specific diversity of the different strains of the same species. Thus, if secondary metabolic diversity is of interest, it is important to keep different samples/isolates of the same species of extremophiles fungi in the culture collections. ^{1}H NMR signals indicating the presence of highly functionalized secondary metabolites are promising. These results together with the previous reports on interesting compounds having been isolated from these species, support studies aimed at the isolation and identification of the active compounds from the most promising extracts disclosed in this research.

Conclusions

Despite the published studies about fungi from Antarctica, little information exists about their ecological roles (Bridge and Spooner 2012). According to Fell et al. (2006), the micro-eukaryotic webs in continental Antarctica may offer a unique opportunity to investigate the regional-to-global environmental effects on the community structures of microbial webs. Our results show that the fungal community present in the cold-arid oligotrophic soils of the Antarctic continent includes a few dominant species. These may represent cold-adapted taxa with biochemical capabilities for surviving in the extreme conditions of the isolated and undisturbed habitat in continental Antarctica. These dominant taxa may have important implications for understanding eukaryotic survival in cold-arid oligotrophic soils. We hypothesize that detailed investigations of fungal occurrence and frequency dynamics over time may provide a more comprehensive understanding about the evolution and relationships among the indigenous fungal species with other organisms, including prokaryotes, autotrophic organisms, nematodes and alien species described for the region. The characterization and monitoring of these unique fungal communities associated with changes in the physic-chemical characteristics of the cold-arid oligotrophic soils over time might serve as a model to study the effect of climate changes in continental Antarctica. In addition, the fungal isolates of the community demonstrated be source of bioactive compounds that may confer advantages during competition with dominant species and help the species survive in the pristine environmental conditions. These bioactive

fungi may represent a potential source of prototype molecules to use in drug and agrochemical discovery studies. Additionally, among the *Penicillium* species obtained from continental Antarctic soil, one was reported as producer of the beta-lactam antibiotic penicillin. Different wild, pristine bioactive isolates of *P. allii-sativi*, *P. brevicompactum*, *P. chrysogenum* and *P. rubens* found in continental Antarctic soil may represent a unique source of new prototype molecules for drug and agrochemical discovery studies.

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