Extremophiles (2016) 20:425–435
DOI 10.1007/s00792-016-0833-y

ORIGINAL PAPER

Richness and bioactivity of culturable soil fungi from the Fildes Peninsula, Antarctica

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Received: 15 September 2015 / Accepted: 25 April 2016 / Published online: 3 May 2016

Abstract Since the discovery of penicillin, fungi have been an important source of bioactive natural products. However, as a specific resource, the bioactive potentiality and specificity of fungal metabolites from the Antarctic region have had little attention. In this paper, we investigated the diversity patterns and biological activities of cultivable fungi isolated from soil samples in Fildes Peninsula, King George Island, Antarctica. Fungal communities showed low abundance and diversity; a total of 150 cultivable fungi were isolated from eight soil samples. After being dereplicated by morphological characteristics and chemical fingerprints, 47 fungal isolates were identified by ITS-rDNA sequencing. We confirmed that these isolates belonged to at least 11 different genera and clustered into nine groups corresponding to taxonomic orders in the phylogenetic analysis. Using two different fermentation conditions, 94 crude extracts acquired from the abovementioned different metabolite characteristic isolates were screened by bioactivity assay and 18 isolates produced biologically active compounds. Compared with HPLC–DAD–UV fingerprint analysis of culture extracts and standard compounds, two bioactive components secalonic acid and chetracins were identified. Our research suggests that the abundance and diversity of Antarctic cultivable fungal communities exhibit unique ecological characteristics and potential producers of novel natural bioactive products.

Communicated by A. Oren.

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Keywords Fungal diversity · Metabolite characteristic · Cytotoxic · Antimicrobial · Antarctica

Introduction

Antarctica is characterized by its geographical isolation and extreme climate; most of the continent has experienced little to anthropogenic influence. Unique and harsh environments, such as low temperatures, low water availability, frequent freeze–thaw cycles, low annual precipitation, strong winds, high sublimation and evaporation, and high ultraviolet radiation, constitute significant limiting factors for plants and animals (Ruisi et al. 2007). Therefore, the biology of Antarctica, more than other continents, is dominated by microorganisms that are highly adapted to and can endure the extreme conditions (Friedmann 1993; Ruisi et al. 2007). Fungi are important microbial resources, the diversity and abundance of fungal communities have been investigated in various Antarctic niches, such as soils, (Bridge and Newsham 2009; Arenz and Blanchette 2011; Godinho et al. 2015), historic wood (Blanchette et al. 2010), lakes (Brunati et al. 2009; Gonçalves et al. 2012), sponges (Henríquez et al. 2014), macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014), bryophytes (Zhang et al. 2013a, b), and plants (Rosa et al. 2009, 2010; Santiago et al. 2012). As a result, species of Antarctic fungi are strikingly diverse and novel (Onofri et al. 2007; Ruisi et al. 2007).

Since the discovery of some bioactive natural products, such as penicillin and lovastatin, the powerful and potential biosynthetic activities of culture-dependent filamentous fungi have influenced human life. In view of the unique environment and severe competition, filamentous fungi that survive in Antarctica might be a potential resource for the

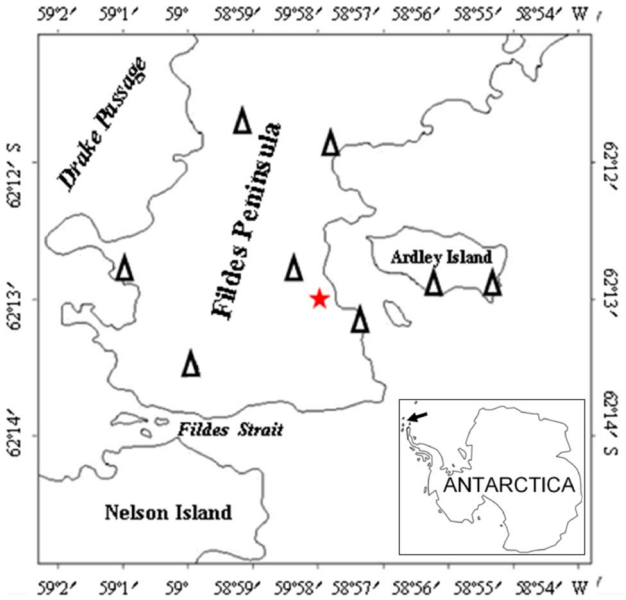

Fig. 1 *Map* of Fildes Peninsula at King George Island, Antarctica, showing locations (△) where the samples were obtained for this study. The Chinese Antarctic Great Wall Station (☆) was marked for *symbol*

discovery of bioactive metabolites (Liu et al. 2013). However, compared with the many studies on fungi in temperate regions, little is known about the bioactive potentiality and specificity of fungal metabolites from this remote site (Liu et al. 2013), even though Antarctic fungi have provided an abundant source of new natural products (Li et al. 2012; Wu et al. 2012, 2013; Figueroa et al. 2015; Zhou et al. 2015). Employing this effective strategy for discovering new bioactive metabolites, a lot of bioprospecting research focusing on fungi isolated in Antarctic regions has been carried out (Brunati et al. 2009; Santiago et al. 2012; Godinho et al. 2013, 2015; Furbino et al. 2014; Henríquez et al. 2014; Melo et al. 2014; Gonçalves et al. 2015).

In this study, we investigated the richness and bioactivity of cultivable filamentous fungi surrounding the Chinese Antarctic Great Wall Station (Fig. 1) on the Fildes Peninsula, King George Island, maritime Antarctica. The Fildes Peninsula is an ice-free area, with a polar marine climate, and the local terricolous vegetation is mainly made up of mosses and lichens. The terrestrial ecosystems of the Fildes Peninsula are varied, include rock fields, pristine soil and vegetation, penguin rookeries, seal haul-out areas, and human impacted sites (Smith 1984; Tin et al. 2009). Several studies are available on the diversity and abundance of filamentous fungi in this area (Ruisi et al. 2007; Rosa et al. 2009, 2010; Gonçalves et al. 2012; Zhang et al. 2013a, b); however, studies on diversity and bioactivity are scarce. In this study, we evaluated the cytotoxic and antimicrobial activities of cultivable filamentous fungi isolated in this region and demonstrate that Antarctic fungi are potential producers of natural bioactive products.

Materials and methods

Site, sampling, and isolation of cultivable fungi

The soil samples were collected from eight different points surrounding the Chinese Antarctic Great Wall Station (62°12′59″S, 58°57′52″W) located on the Fildes Peninsula, King George Island (Fig. 1). Cultivable fungi in soil samples were isolated by the spread-plate method. Fungi were incubated on Czapek yeast extract agar (CYA), potato dextrose agar (PDA), and yeast extract with supplements (YES) medium containing 150 mg/L chloramphenicol at 4, 15 and 25 °C, respectively, for 7–9 days. Fungal colonies with distinct morphologies were isolated from the Petri dishes, restreaked onto fresh dishes, and incubated for a further 48 h at the corresponding temperature. This restreaking process was repeated until pure morphotype colonies were obtained. The colonies were grouped into different morphotypes according to their appearance (colony color and texture, border type, and radial growth rate) on CYA medium.

DNA extraction, PCR amplification, and sequence analysis

DNA was extracted from the filamentous fungi following the protocol described by Zhang et al. (2008). The internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). Details of the PCR reactions can be found in Janso et al. (2005). The PCR products were purified and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). The sequence data in this study were deposited in the GenBank Database under the accession numbers JQ670942-JQ670943, JQ670947–JQ670954, JQ670959–JQ670966, JQ692138–JQ692142, JQ692144–JQ692147, JQ692149, JQ692152, JQ692154–JQ692165, JQ692168, JQ715699, JQ715705, and KJ700299–KJ700301. The sequences identified were compared with other sequences in the GenBank database (http://www.ncbi.nlm.nih.gov) by BLAST analysis, and aligned in ClustalW (Thompson et al. 1994). The criteria suggested by Godinho et al. (2013) were used to interpret the BLAST results from the GenBank database: for query coverage and sequence identities ≥98 %, the species was accepted; for query coverage and sequence identities between 95 and 97 %, only the genus was accepted, and for query coverage and sequence identities ≤95 %, isolates were labeled as "unknown" fungi. The phylogenetic

analysis was conducted using MEGA 5.0 (Tamura et al. 2011). The neighbor-joining method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs (Saitou and Nei 1987).

Fermentation and extract preparation

Each isolate was grown under two conditions: (1) static culture at 15 °C for 40 days and (2) shaken culture at 15 °C, 180 rpm for 15 days in 500 mL flask containing 100 mL liquid medium. Each test contained five replicates for each sample. The medium was composed of maltose (20 g/L), monosodium glutamate (10 g/L), glucose (10 g/L), mannitol (20 g/L), yeast extract paste (3 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), and tap water, pH was adjusted to 6.5.

The fermentation mixtures were processed as previously described by Wu et al. (2012, 2013). After cultivation, the fermentation mixture was extracted with an equal volume of ethyl acetate. The extracts were evaporated under reduced pressure and re-dissolved by methanol. Sterile medium was extracted using the same procedure. The sterile extract was used as the control in the screening procedure.

Metabolite fingerprint analysis

The Antarctic fungi extracts were analyzed in HPLC system (Waters Co.), which contained a model 600 pump, a model 996 diode array UV detector, and a ODS-AM C18 column (YMC, 4.6 × 250 mm, 5 μm). The gradient increased from 5 to 100 % methanol over 40 min and was retained at 100 % for 10 min.

Cytotoxic activity assay

Cytotoxic activities of the extractions were evaluated by the Sulforhodamine B (SRB) method (Skehan et al. 1990) using P388 lymphocytic leukemic cell line. Two hundred microliters of the cell suspensions were placed in 96-cell plates at a density of 2×10^5 cells mL^{-1}. Two microliters of the extraction solutions (10 μg/μL in MeOH) were added to each well (ultimate density = 0.1 μg/μL). Fluracil (ultimate density = 0.01 μg/μL) was used as the positive control. The culture was further incubated for 24 h at 37 °C in a humidified incubator at 5 % CO_2. Following drug exposure, the cells were fixed with 12 % trichloroacetic acid and the cell layer was stained with 0.4 % SRB. The results are expressed as percentage of growth inhibition in comparison to the control without drug.

Antimicrobial activity assay

Antimicrobial activity of the extracts was estimated using the agar-diffusion method. The five microorganisms indicated were the bacteria *Escherichia coli*, *Mycobacterium phlei*, *Proteus mirabilis*, *Staphylococcus aureus*, and the fungus *Candida albicans*. We seeded 10^6 cells of the indicated microorganisms onto corresponding medium plates. Test extractions were dissolved in methanol at a concentration of 1 μg/μL. Chloromycetin and streptomycin (0.1 μg/μL) were used as positive controls for bacteria and amphotericin B (0.1 μg/μL) for yeast. Five microliters of the solutions were absorbed onto individual paper disks (6 mm diameter), which placed on the surface of the agar. The assay plates were incubated at 37 °C for 24 h, and the diameter of the growth inhibition zone was measured.

Results

Cultivation of filamentous fungi from Antarctic samples obtained a total of 150 isolates from eight soil samples (Table 1). The growth experiments showed that most of these isolate were psychrotrophic (data not shown; optimum growth temperature <25 °C). All of the isolates were dereplicated using morphological characteristics and metabolite HPLC–DAD–UV fingerprint analysis. A total of 47 isolates were retained and further analyzed using the ITS-rDNA sequences, which identified 30 individual taxa (Table 2). Individual sequences were compared with sequences in the GenBank database by BLAST analysis, which revealed that a portion of these taxa ($n = 26$, 86.7 %) were closely affiliated with known fungal isolates, but the other taxa ($n = 4$, 13.3 %) were obviously different from the sequences in NCBI database.

Thirty individual taxa were identified taxonomically based on ITS sequences that aligned with the type sequences from related species retrieved from the GenBank database (Tables 1, 2). The identified isolates were assigned to the genera *Acremonium*, *Antarctomyces*, *Aspergillus*, *Cladosporium*, *Pseudogymnoascus*, *Oidiodendron*, *Penicillium*, *Pyricularia*, *Rhizoscyphus*, *Thelebolus*, *Mortierella*, and order *Helotiales*. The diversities and distributions of fungal communities varied according to the different sites. Some sites displayed low richness and high dominance indices (Table 1). The most dominant genera in these samples belonged to *Cladosporium* (GW7), *Pseudogymnoascus* (GW8, GW25), and *Mortierella* (GW2, GW20). At the same time, the most diversiform fungal resources were discovered in sample GW31, in which 11 taxa of seven different filamentous fungi genera were obtained. Nine taxa from six genera and ten taxa from three genera were isolated from samples GW3 and GW8, respectively. *Aspergillus* and *Penicillium* species were frequently found in almost all of the samples, but were not predominant. More fungal communication richness and abundance was observed in clay soil than in either sand or loam soil (Table 1).

Table 1 Cultivable fungi isolated from different samples in Antarctica

Best BLAST match	Samples number [soil texture]								
	GW2 [sand]	GW3 [clay]	GW6 [loam]	GW7 [loam]	GW8 [clay]	GW20 [loam]	GW25 [loam]	GW31 [clay]	Total
Ascomycota									
Acremonium implicatum			3						3
Antarctomyces psychrotrophicus			1						1
Aspergillus aculeatus			2					1	3
A. flavus								4	4
A.terreus			1	1				1	3
A. versicolor			1	1					2
Cladosporium cladosporioides				5				7	12
C. sphaerospermum			1						1
Helotiales sp.		2							2
Oidiodendron truncatum							2		2
Penicillium chrysogenum		1			1	1		2	5
P. citrinum			4		5			3	12
P. crustosum					1				1
P. oxalicum								4	4
Pseudogymnoascus pannorum		1			4		1		6
P. vinaceus		7			1		5		13
P. sp. 1		2			8		1		11
P. sp. 2		3	2						5
P. sp. 3					9	1	1		11
P. sp. 4					4				4
P. sp. 5					7		3	5	15
Pyricularia sp.								3	3
Rhizoscyphus sp.		3							3
Thelebolus microsporus		2							2
Zygomycota									
Mortierella Antarctica						3			3
M. gamsii								4	4
M. sp. 1						4			4
M. sp. 2	2	1							3
M. sp. 3					3				3
Unidentified fungus 1								5	5
Total fungal isolates	2	22	15	7	43	9	13	39	150

According to the different chemical fingerprints, 94 extracts were acquired from the 47 isolates fermented by the shaken and stationary cultures. A total of 18 isolates produced biologically active compounds, among them, four isolates displayed intense bioactivity (Table 3). According to the cytotoxic screening, *Cladosporium sphaerospermum* GW7-7 and *Oidiodendron truncatum* GW25-13 showed 68.4 and 62.0 % inhibitation rate to P388 cell line, which were approximately equivalent to the value of fluracil. Eight isolates belong to the genera *Penicillium*, *Mortierella*, *Rhizoscyphus*, order *Helotiales*, and one unidentified taxa, and were exhibited intermediate cytotoxic activity. In the antimicrobial screening, the extracts from 14 isolates inhibited at least one of the indicated microorganisms. Among these, *Aspergillus flavus* GW31-9 and *O. truncatum* GW25-13 inhibited the fungus *C. albicans* and the latter also showed intense inhibition to *M. phlei*. *Aspergillus terreus* GW7-5 inhibited the Gram-negative bacteria *E. coli* and *P. mirabilis* more than any other isolate. Five isolates exhibited intermediate inhibition of the Gram-positive bacteria *S. aureus*. Additionally, through comparative HPLC–DAD–UV fingerprint analysis of culture extracts and standard compounds, we found that the highly active isolates *C. sphaerospermum* GW7-7 and *O. truncatum* GW25-13

Table 2 Antarctica culturable fungi differentiated with chemical characteristics in this study as identified by ITS sequences

Strain number	Closest BLAST match [GenBank accession number]	Query cover (%)	Identity (%)	No. of bp analyzed	Identification [GenBank accession number]
	Ascomycota				
GW6-4	*Acremonium implicatum* [HQ914932]	100	100	512	*Acremonium implicatum* [JQ692168]
GW6-2	*Antarctomyces psychrotrophicus* [FJ911878]	99	100	484	*Antarctomyces psychrotrophicus* [JQ692162]
GW6-1	*Aspergillus aculeatus* [HM236013]	100	100	498	*Aspergillus aculeatus* [JQ670953]
GW31-9	*Aspergillus flavus* [HQ288050]	100	100	519	*Aspergillus flavus* [JQ670961]
GW7-5	*Aspergillus terreus* [EF669603]	100	100	523	*Aspergillus terreus* [JQ670954]
GW6-15	*Aspergillus versicolor* [JQ301896]	100	100	497	*Aspergillus versicolor* [KJ700300]
GW31-1	*Cladosporium cladosporioides* [KF525807]	100	100	489	*Cladosporium cladosporioides* [JQ670943]
GW7-7	*Cladosporium sphaerospermum* [JX966573]	100	100	426	*Cladosporium sphaerospermum* [JQ670942]
GW31-6	Fungal endophyte isolate [EU687056]	99	90	344	Unidentified fungus [JQ692164]
GW8-6	*Geomyces pannorum* [HQ703417]	100	100	488	*Pseudogymnoascus pannorum* [JQ692144]
GW3-8	*Geomyces* sp. [DQ499474]	100	100	488	*Pseudogymnoascus* sp. 1 [JQ692139]
GW8-13	*Geomyces* sp. [DQ499474]	100	100	488	*Pseudogymnoascus* sp. 1 [JQ692147]
GW8-24	*Geomyces* sp. [DQ499474]	100	100	488	*Pseudogymnoascus* sp. 1 [JQ692149]
GW3-16	*Geomyces* sp. [KF986449]	100	99	488	*Pseudogymnoascus* sp. 2 [JQ692141]
GW6-6	*Geomyces* sp. [KF986449]	100	99	488	*Pseudogymnoascus* sp. 2 [JQ692142]
GW8-9	*Geomyces* sp. [KF986449]	100	100	488	*Pseudogymnoascus* sp. 3 [JQ692145]
GW8-40	*Geomyces* sp. [KF986449]	100	100	488	*Pseudogymnoascus* sp. 3 [JQ692154]
GW20-5	*Geomyces* sp. [KF986449]	100	100	488	*Pseudogymnoascus* sp. 3 [JQ692155]
GW25-5	*Geomyces* sp. [KF986449]	100	100	488	*Pseudogymnoascus* sp. 3 [JQ692158]
GW8-10	*Geomyces* sp. [HQ914925]	100	100	488	*Pseudogymnoascus* sp. 4 [JQ692146]
GW8-33	*Geomyces* sp. [DQ499474]	100	99	488	*Pseudogymnoascus* sp. 5 [JQ692152]
GW25-8	*Geomyces* sp. [DQ499474]	100	99	488	*Pseudogymnoascus* sp. 5 [JQ692160]
GW31-34	*Geomyces* sp. [DQ499474]	100	99	488	*Pseudogymnoascus* sp. 5 [JQ692161]
GW3-4	*Geomyces vinaceus* [KF986448]	99	100	488	*Pseudogymnoascus vinaceus* [JQ692138]
GW3-12	*Geomyces vinaceus* [KF986448]	99	100	488	*Pseudogymnoascus vinaceus* [JQ692140]
GW25-1	*Geomyces vinaceus* [KF986448]	99	100	488	*Pseudogymnoascus vinaceus* [JQ692156]
GW25-2	*Geomyces vinaceus* [KF986448]	99	100	488	*Pseudogymnoascus vinaceus* [JQ692157]
GW25-6	*Geomyces vinaceus* [KF986448]	99	100	488	*Pseudogymnoascus vinaceus* [JQ692159]
GW3-14	*Helotiales* sp. [JX852359]	99	97	427	*Helotiales* sp. [JQ715699]
GW25-13	*Oidiodendron truncatum* [FJ914713]	99	99	492	*Oidiodendron truncatum* [JQ692163]
GW3-11	*Penicillium chrysogenum* [KF986423]	100	100	512	*Penicillium chrysogenum* [JQ670960]
GW20-4	*Penicillium chrysogenum* [KF986423]	100	100	516	*Penicillium chrysogenum* [JQ670962]
GW31-7	*Penicillium citrinum* [KF986420]	100	100	482	*Penicillium citrinum* [JQ670963]
GW6-7	*Penicillium citrinum* [KF986420]	100	100	418	*Penicillium citrinum* [JQ670964]
GW6-14	*Penicillium citrinum* [KF986420]	100	100	442	*Penicillium citrinum* [JQ670965]
GW8-28	*Penicillium citrinum* [KF986420]	100	100	465	*Penicillium citrinum* [JQ670966]

Table 2 continued

Strain number	Closest BLAST match [GenBank accession number]	Query cover (%)	Identity (%)	No. of bp analyzed	Identification [GenBank accession number]
GW8-17	*Penicillium crustosum* [EF634415]	100	100	504	*Penicillium crustosum* [KJ700301]
GW31-17	*Penicillium oxalicum* [KF986426]	100	100	517	*Penicillium oxalicum* [JQ670959]
GW31-8	*Pyricularia parasitica* [AY265340]	93	96	569	*Pyricularia* sp. [JQ692165]
GW3-18	*Rhizoscyphus* sp. [JX852328]	100	99	478	*Rhizoscyphus* sp. [JQ715705]
GW3-19	*Thelebolus microsporus* [JX852357]	100	100	484	*Thelebolus microsporus* [KJ700299]
	Zygomycota				
GW20-1	*Mortierella antarctica* [JX975843]	97	99	585	*Mortierella antarctica* [JQ670950]
GW20-2	*Mortierella fimbricystis* [KC008856]	93	99	567	*Mortierella* sp. 1 [JQ670951]
GW31-39	*Mortierella gamsii* [JX975968]	100	99	555	*Mortierella gamsii* [JQ670952]
GW2-1	*Mortierella parvispora* [JX976049]	100	95	555	*Mortierella* sp. 2 [JQ670947]
GW3-10	*Mortierella parvispora* [JX976049]	100	95	556	*Mortierella* sp. 2 [JQ670948]
GW8-1	*Mortierella sclerotiella* [JQ346223]	100	98	568	*Mortierella* sp. 3 [JQ670949]

Table 3 Bioactivities of the metabolites from active Antarctic culturable fungi fermented in two culture conditions (Static condition/Shaking condition)

Strain number	Cytotoxicity (%)[a]	Antimicrobial activity on: (mm)[b]				
		C. albicans	*E. coil*	*M. phlei*	*P. mirabilis*	*S. aureus*
Aspergillus aculeatus GW6-1	37.4/16.9	–/–	–/–	–/–	–/–	10.9/–
Aspergillus flavus GW31-9	27.9/23.1	**16.5/17.1**	12.2/–	–/–	11.7/10.3	–/–
Aspergillus terreus GW7-5	31.2/18.3	11.3/–	**17.2**/14.3	13.2/13.6	**15.3**/13.7	–/–
Cladosporium sphaerospermum GW7-7	11.2/**68.4**	–/–	–/–	–/–	–/–	–/12.9
Pseudogymnoascus sp. GW3-16	24.4/–	–/–	11.5/–	–/–	10.9/–	–/–
Pseudogymnoascus sp. GW8-9	18.4/–	–/–	11.4/–	13.6/–	11.1/–	–/–
Helotiales sp. GW3-14	49.0/–	–/–	–/–	–/–	–/–	–/–
Oidiodendron truncatum GW25-13	**62.0**/24.9	**15.9**/10.3	–/–	**16.7**/12.2	–/–	12.3/–
Penicillium chrysogenum GW3-11	36.5/43.7	12.4/13.1	–/–	–/–	–/–	10.9/11.2
Penicillium citrinum GW31-7	36.8/47.8	–/–	–/–	–/–	–/–	12.9/12.3
Penicillium citrinum GW8-28	33.8/32.6	13.2/13.9	–/–	–/–	–/–	–/–
Penicillium crustosum GW8-17	14.4/55.0	12.2/13.2	11.3/11.1	–/10.0	11.6/10.7	–/–
Penicillium oxalicum GW31-17	12.1/–	10.3/11.6	11.3/–	11.1/12.2	10.9/–	–/–
Pyricularia sp. GW31-8	–/–	–/–	13.6/12.2	–/–	12.4/13.1	–/–
Rhizoscyphus sp. GW3-18	19.6/42.5	–/–	–/–	–/–	–/–	–/–
Unidentified fungus GW31-6	57.6/–	12.2/–	11.2/–	12.2/–	11.9/–	–/–
Mortierella antarctica GW20-1	44.3/18.6	–/–	–/–	–/–	–/–	–/–
Mortierella sp. GW20-2	49.1/16.0	–/–	–/–	–/–	–/–	–/–
Amphotericin B	–	19.6	–	–	–	–
Chloromycetin	–	–	24.3	19.1	23.5	18.3
Fluracil	70.1	–	–	–	–	–
Streptomycin	–	–	18.4	20.2	19.9	12.1

[a] Cytotoxicity was estimated by the inhibiting rate (%) to P388 lymphocytic leukemic cell line. The inhibiting rate: >60 % is intense (bold), 40–60 % is media, 20–40 % is low, and <20 % is no activity, <10 % is not shown

[b] Antimicrobial activity was estimated by the inhibitory zone (mm) to five indicator microorganisms. The diameter of the inhibition zone: >15 mm is intense (bold), 10–15 mm is media, 6–10 mm is low/no activity and not shown. Indicator microorganisms: *C. albicans*, *Candida albicans*; *E. coil*, *Escherichia coli*; *M. phlei*, *Mycobacterium phlei*; *P. mirabilis*, *Proteus mirabilis*; *S. aureus*, *Staphylococcus aureus*. c. Antifungal Amphotericin B, antibacterial Chloromycetin and Streptomycin, and anticancer medication Fluracil were used as a *positive* control

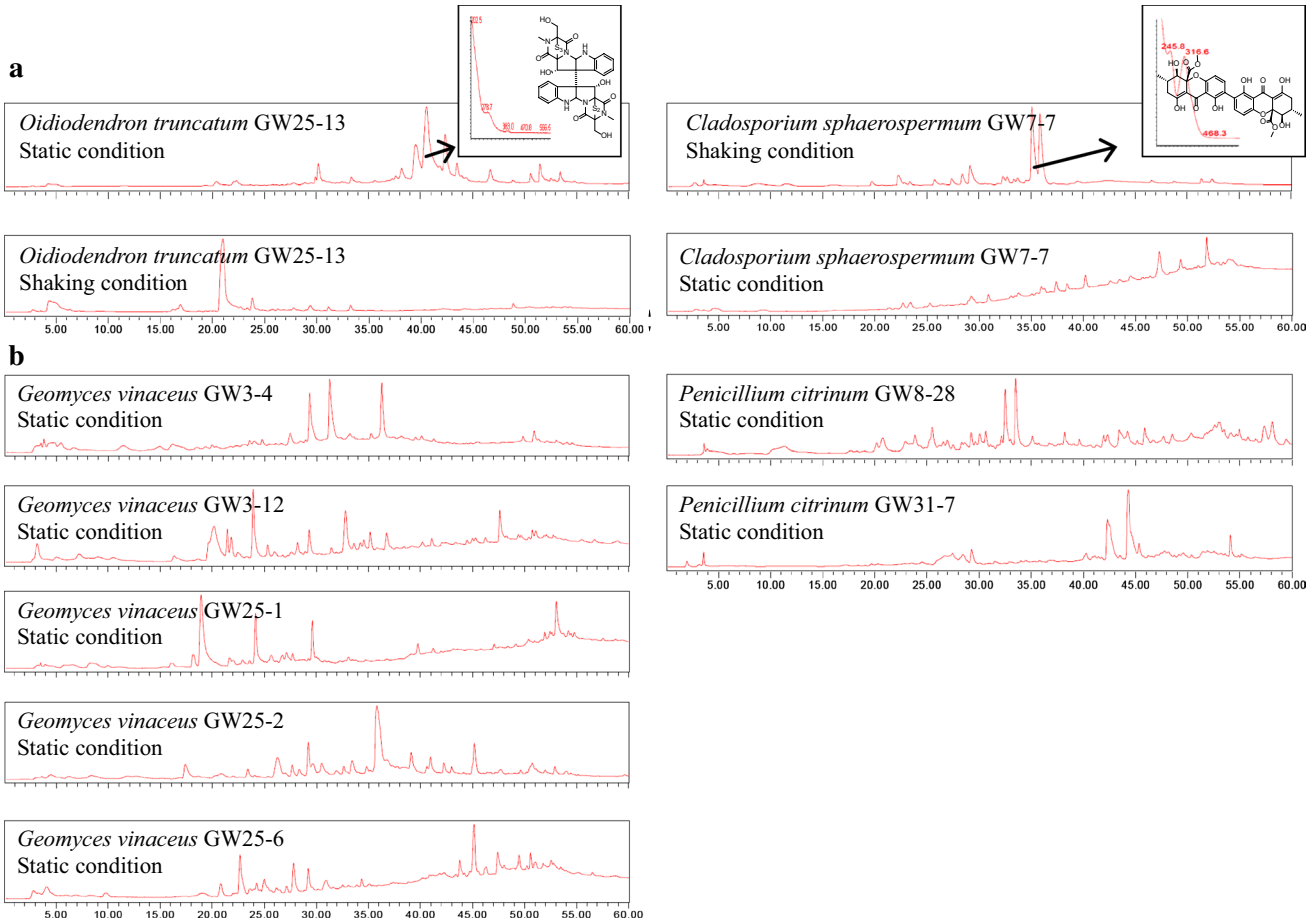

Fig. 2 Comparison of metabolite fingerprints using HPLC–DAD–UV (showed in 254 nm UV wavelength). **a** In different ferment condition of two bioactive strains, and shown the structure and UV absorption of the proposed active compounds in *black squares* (chetracins in *O. truncatum* and secalonic acid in *C. sphaerospermum*). **b** In same ferment condition of the similar strains

produced the bioactive components secalonic acid and chetracins, respectively (Fig. 2a).

Discussion

Richness of cultivable Antarctic fungi

Our results demonstrated the richness of culture-dependent filamentous fungi in King George Island and acquired some unidentified taxa from the region. There are a number of Antarctic expedition stations on King George Island, so the ecological system in this region has suffered anthropogenic influence. However, our results have shown that the fungal communities in the region are similar to the more unfrequented areas and many taxa considered common in aboriginal Antarctica were present.

The genera *Pseudogymnoascus* (typically and historically identified under the name *Geomyces*) and *Cladosporium* are widely distributed; they are the most frequently reported fungal genera in Antarctica (Arenz et al. 2006, 2011; Rosa et al. 2010; Gonçalves et al. 2012). According to Hayes (2012), the *Pseudogymnoascus* fungi are common in cold environments. Species in this genus tend to be psychrophilic and are ubiquitously distributed in the soils of arctic, alpine, and Antarctic regions. In Antarctica, members of the genus *Pseudogymnoascus* have been isolated from various samples collected in different habitats (Mercantini et al. 1989; Tosi et al. 2002; Arenz et al. 2006, 2011; Rosa et al. 2010; Godinho et al. 2013). As a prevalent genus in Antarctica, *Pseudogymnoascus* species generally have the ability to colonize and utilize different carbon sources and play a role in decomposition and nutrient cycling in this region (Arenz et al. 2006). It is thought that the genus *Pseudogymnoascus* may play a considerable role in the decomposition, and degradation of organic matter. In our results, *Pseudogymnoascus* was the most dominant genus in three samples GW3, GW8, and GW25, which contained more moisture and organics than other sand or loam samples, while *Pseudogymnoascus* species

were consistently absent from the samples that lacked decayed organic matter. The genus *Cladosporium* was another prevalent group in our study. This oligotrophic genus is cosmopolitan, but is also found in Polar Regions and other cold environments. *Cladosporium* species are associated with plants and mosses (Meyer et al. 1967; Tosi et al. 2002) and are isolated in rocks and soils in Antarctica (Onofri et al. 2000; Arenz et al. 2006; Brunati et al. 2009). According to Arenz et al. (2006), *Cladosporium* was one of the dominant genera in soil of McMurdo Dry Valleys, Antarctica. Gonçalves et al. (2012) obtained three *Cladosporium* species from water sampled from a lake on the Antarctic Peninsula.

Like *Pseudogymnoascus* and *Cladosporium*, the genera *Aspergillus* and *Penicillium* are the most frequently isolated filamentous soil fungi belonging to cosmopolitan, globally distributed species in previous studies on soils sampled from Antarctica (Adams et al. 2006; Arenz et al. 2006, 2011; Onofri et al. 2007). It is difficult to determinate if these fungi are endemic to Antarctica, because of their global distribution and cold-tolerance.

Because they are psychrophilic, some species, e.g., *Antarctomyces psychrotrophicus* and *Thelebolus microsporus*, are considered cold-environment-specific genera (Stchigel et al. 2001; de Hoog et al. 2005). Some other isolates, such as GW3-18 and GW31-8 belonging to the genera *Rhizoscyphus* and *Pyricularia*, were not grown when temperatures exceeded 25 °C in the initial cultivation. However, with serial subcultivation these isolates acquired the ability to grow in moderate temperatures. According to Zhang et al. (2013b), *Rhizoscyphus* species isolated from three bryophyte species in the Fildes Region was mesophilic with an optimum temperature above 20 °C. *Pyricularia* is a widespread fungal genus that includes some plant pathogen species, which cause rice blast disease; however, there are no reports of the genus in Antarctica. Therefore, some species of these genera may be endemic and have survived by adapting to the extreme conditions.

The genus *Mortierella*, belonging to the phylum *Zygomycota*, frequently occurs in different aqueous samples from Antarctica, such as wet soil (Adams et al. 2006; Arenz et al. 2006, 2011; Bridge and Newsham 2009), lake water (Gonçalves et al. 2012), bryophytes (Tosi et al. 2002; Zhang et al. 2013a), and macroalgae (Godinho et al. 2013; Furbino et al. 2014). In our study, the genus was only isolated from one group in dry sand with a low water ratio. This may be the result of the humid climate in the insular region. Our results suggest that the *Mortierella* genus is prevalent in this region, which is in agreement with previous reports (Tosi et al. 2002; Adams et al. 2006; Arenz et al. 2006, 2011; Bridge and Newsham 2009; Gonçalves et al. 2012, 2015; Godinho et al. 2013; Zhang et al. 2013a; Furbino et al. 2014).

In this study, most of the isolates were successfully classified at either the species or genus level based on the ITS sequences of standard isolates deposited in the GenBank database according to the BLAST results, except for the isolates GW31-6 and GW3-14. The unidentified fungus GW31-6 showed low similarity (90 %) with the endophyte fungus (EU687056) sequence obtained from tropical forest grasses and was described as an unidentified fungus. The fungus GW3-14 showed 97 % identity with the *Helotiales* sp. (JX852359) sequence isolated from bryophytes as culturable endophytic fungi in Antarctica (Zhang et al. 2013b) and was identified at order level as *Helotiales* sp. These fungi could represent novel fungal species. However, more morphological and taxonomic studies are necessary to correctly identify these fungal taxa.

In our results, fungal community richness and abundance differed among samples, even though the collection sites are in the same geographic location (Table 1, Fig. 1). Fungal communities in clay soil were more diverse and abundant than those from either dry or infertile soil. This result is similar to those of Connell et al. (2006) and (Arenz and Blanchette 2011) who reported that cultivable fungal distribution and abundance patterns in Antarctic soil was positively correlated with soil nutrients and moisture. In general, compositions of different Antarctic samples determine the distinctive and characteristic microbial distributions.

Bioactivity of cultivable Antarctic fungi

In recent decades, Antarctic fungi have gradually become an important resource for bioactive compounds (Liu et al. 2013). However, systematic bioactive investigations have yet to be caught the attention. In this study, we screened the cytotoxic and antimicrobial activities of fungal isolates, of which 10 exhibited cytotoxic activity and 14 exhibited antimicrobial activity.

The *C. sphaerospermum* GW7-7 and *O. truncatum* GW25-13 extracts showed the highest cytotoxic activity against P388 cell line in our screening tests. Comparing the retention time and absorption spectrum of standard compounds by HPLC–DAD–UV, we determined that the GW7-7 and GW25-13 extracts contained secalonic acid and chetracin as a main component, respectively, which may play a key cytotoxic role as the major bioactive component. Secalonic acid, a common toxic metabolite in filamentous fungi, exhibits cytotoxic activity against various cancer lines (Kurobane et al. 1987; Zhang et al. 2009). Recently, Li et al. (2012) obtained the chetracins B and C, belonging to the epipolythiodioxopiperazines (ETPs), from *O. truncatum* isolated from King George Island, Antarctica, which exhibited potent cytotoxicity against five human cancer lines in the nanomolar range. Additionally, *O.*

truncatum GW25-13 exhibited intense antifungal and antimycobacterium activity. According to Elliott et al. (2007), the ETP metabolites not only possess strong cytotoxicity, but also show potential antimicrobial activity.

Two isolates identified as *Mortierella* spp. showed modest cytotoxic activity. As one of the dominant groups in Antarctica, *Mortierella* isolates have seldom been reported in previous bioprospecting research of Antarctic fungi; thus bioactive metabolites derived from the genus *Mortierella* have not been widely known. Melo et al. (2014) found that the extract of an endophytic *Mortierella alpina* strain isolated from the Antarctic moss *Schistidium antarctici* displayed strong antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, and obtained two potential antibacterial diketopiperazines. It seemed that this genus may be a potential resource for bioactive metabolites.

Five isolates of *Penicillium* species showed modest bioactivity. The genus *Penicillium* is a significant source of secondary metabolites with various bioactivities (Frisvad et al. 2004). *Penicillium* species isolated from Antarctic region are usually more bioactive than other groups. Brunati et al. (2009) reported that a strain of *Penicillium chrysogenum* produced two main bioactive compounds, rugulosin and skyrin. Two novel skeleton compounds, penilactones A and B were isolated from an Antarctic deep sea derived fungus *Penicillium crustosum* (Wu et al. 2012). In recent bioactive screening of fungal strains isolated from various Antarctic habitats, extracts from *Penicillium* species were found to possess antiviral, antibacterial, antifungal, antitumoral, herbicidal, and antiprotozoal activities (Brunati et al. 2009; Godinho et al. 2013, 2015; Furbino et al. 2014; Gonçalves et al. 2015). According to Godinho et al. (2015), similar to *Penicillium*, *Aspergillus* species also produce many bioactive compounds, but few species found in Antarctica have been chemically investigated. Brunati et al. (2009) found that the extracts of two diverse species of *Aspergillus* isolated from Antarctic lake benthic mats exhibited relatively potent antimicrobial activity. Godinho et al. (2015) discovered a strain of *Aspergillus sydowii*, isolated from continental Antarctica oligotrophic soil, which extracts had broad bioactive spectrum. As the representative species producing the bioactive compounds aflatoxin and lavastatin, *A. flavus* GW31-9 and *A. terreus* GW7-5 isolated in this study were selectively inhibitory to the fungus *C. albicans* and the Gram-negative bacteria *E. coli* and *P. mirabilis*.

All of the *Pseudogymnoascus* species showed low antifungal activity (data not show), only two of which showed medium antimicrobial activity. Li et al. (2008) obtained the geomycins A–C from *Pseudogymnoascus* sp. isolated from Antarctic soil samples, which exhibited antifungal activity against *Aspergillus fumigatus* and antibacterial activity against *E. coli*, *S. aureus*, and *Streptococcus pneumoniae*, respectively. In our research, some *Pseudogymnoascus* species also revealed conspicuous intragenus and intraspecies metabolite diversity, the metabolite fingerprints revealed a significant difference despite the high similarity (≥99 %) among the ITS-rDNA sequences. While the isolates GW8-28 and GW31-7 identified as the same species, *P. citrinum* showed a specific antimicrobial spectrum and exhibited relatively high inhibitory action against different indicators because of their different characteristic metabolite fingerprints (Fig. 2b). This phenomenon is widespread in filamentous fungi; therefore, isolating and screening Antarctic fungi to find new bioactive metabolites is of great importance.

Additionally, we found that the extracts of some potential novel species displayed unpredicted bioactivities. *Helotiales* sp. GW3-14 and *Rhizoscyphus* sp. GW3-18 showed medium cytotoxicity, *Pyricularia* sp. GW31-8 showed selective inhibitory activity against *E. coil* and *P. mirabilis*, and the unidentified fungus GW31-6 produced an extract with a broad antimicrobial spectrum. The extracts from these novel isolates might contain some undiscovered bioactive molecules, and we intend to research these further.

Conclusion

The edaphon is an important source of bioactive compounds. In recent years, more attention has been paid to extreme environments edaphon. In this study, we investigated the richness and bioactivity of cultivable fungi derived from various soil samples from King George Island, Antarctica. Similar to previous research (Ruisi et al. 2007; Rosa et al. 2009, 2010; Gonçalves et al. 2012; Zhang et al. 2013a, b), we found fungal communities in this region with both dominant and rare fungal species that belong to endemic, cold-adapted, and cosmopolitan taxa, which displayed interesting and unique ecological characteristics. Furthermore, we found some highly bioactive isolates that we intend to research further; these unexploited isolates may be a new and unique resource. Our research provides even more evidence that Antarctic fungi have evolved genetic and metabolic mechanisms to adapt to their extreme living environment. This means that both the 'novel' and some 'common' fungi can produce uncommon metabolites (Santiago et al. 2012). Although extensive research on the metabolites of relative isolates is still necessary, the evidence suggests that Antarctic fungi are an important potential pharmaceutical resource pool.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 41376147), the

NSFC-Shandong Joint Fund (U1406402), and the Chinese Polar Environment Comprehensive Investigation and Assessment Programs (CHINARE2015-01-06-06).

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