Antimicrobial activity of microfungi from maritime Antarctic soil

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Abstract

The search for cold-adapted and cold-active fungi in extreme environments provides the potential for discovering new species and novel bioactive compounds. In this study, soil samples were collected from Deception Island, Wilhelmina Bay (north-west Antarctic Peninsula, Graham Land) and Yankee Bay (Greenwich Island), maritime Antarctica, for the isolation of soil fungi and determination of their antimicrobial activity. The soil-plate method, agar block, disc diffusion and broth micro-dilution assays were applied to characterize the thermal classes and antimicrobial activity of the isolated fungi. A total of 27 isolates of fungi were obtained from 14 soil samples, including 13 Ascomycota, 4 Zygomycota and 10 anamorphic fungi. Cold-active (psychrotolerant) fungi predominated over cold-adapted (psychrophilic) fungi. In the antimicrobial assay, 16 isolates showed substantial inhibitory activity against test bacterial pathogens. Ethyl acetate extracts of 10 competent isolates showed significant inhibition of bacterial pathogens. Antifungal activity was observed in the disc diffusion assay, but not in the agar block assay. Minimum inhibitory, bactericidal and fungicidal concentrations were determined using the broth micro-dilution method, with an average in the range of 0.78-25 mg ml^{-1} on the test microorganisms. Isolate WHB-sp. 7 showed the best broad spectrum antimicrobial activity, with the potential for biotechnological studies in antibiotic development.

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Key words: biodiversity, soil fungi, isolation, antibacterial, antifungal

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Introduction

Microorganisms are fundamental to all ecosystems and represent one of the the largest reservoirs of undescribed biodiversity. However, relatively little is known about their precise position and function in most ecosystems (Nichols et al. 2002). As well as providing novel biodiversity, they possess great potential for the discovery of new natural products. Raghukumar (2008) suggested that a focus on fungi from specialised ecological niches would be rewarding, as a basic understanding of their ecology would also help to reveal the novelty of an organism and its properties and abilities. The fungi of extreme environments, such as those of Antarctic terrestrial ecosystems, have the ability to withstand a range of stresses, including extremely low temperatures, desiccation, hypersaline (osmotic) stress and high levels of solar radiation (Bradner et al. 1999, Brett et al. 2006, Ruisi et al. 2007, Wynn-Williams 1996). Their survival and adaptation to such environments can increase their potential for the production of, for instance, characteristic bioactive compounds (Jensen et Fenical 2002), cold-active enzymes (Krishnan et al. 2011) and antifreeze proteins (Robinson 2001), providing clear opportunities in the fields of bioprospecting and biotechnology.

The influence of temperature on microbial growth characteristics has been a subject of interest for many years. Sinclair et Stokes (1963) defined 'psychrophiles' to be microorganisms which grow rapidly enough in culture at 0°C to form visible colonies. Later, Morita (1975) recognised that both psychrophilic and psychrotolerant organisms have the ability to grow at 0°C. The two can be differentiated as psychrophilic organisms have a maximum temperature for growth below 20°C, while psychrotolerant organisms have a maximum temperature for growth above 20°C. Mesophilic microbes have a minimum temperature for growth between 5 and 10°C and a maximum above 25°C (Robinson 2001). Lastly, thermophilic microbes have a minimum temperature for growth of 20°C and maximum of at least 50°C (Magan 2007), with an optimum often in the range 40-50°C.

Over the last decade, the application of molecular biological techniques has led to a rapid increase in the data available relating to Antarctic microbial diversity (Chong et al. 2015, Cowan et al. 2011, Vyverman et al. 2010). However, these data remain highly uneven in terms of spatial coverage (Chong et al. 2013, Chown et Convey 2007), and few functional studies have been carried out, not least as the majority of identified microbial diversity remains uncultured. The current study presents preliminary data on the diversity and antimicrobial activity of microfungi obtained from soil ecosystems in maritime Antarctica: Deception Island, Wilhelmina Bay (north-west Antarctic Peninsula, Graham Land) and Yankee Bay (Greenwich Island) (*see* Fig. 1).

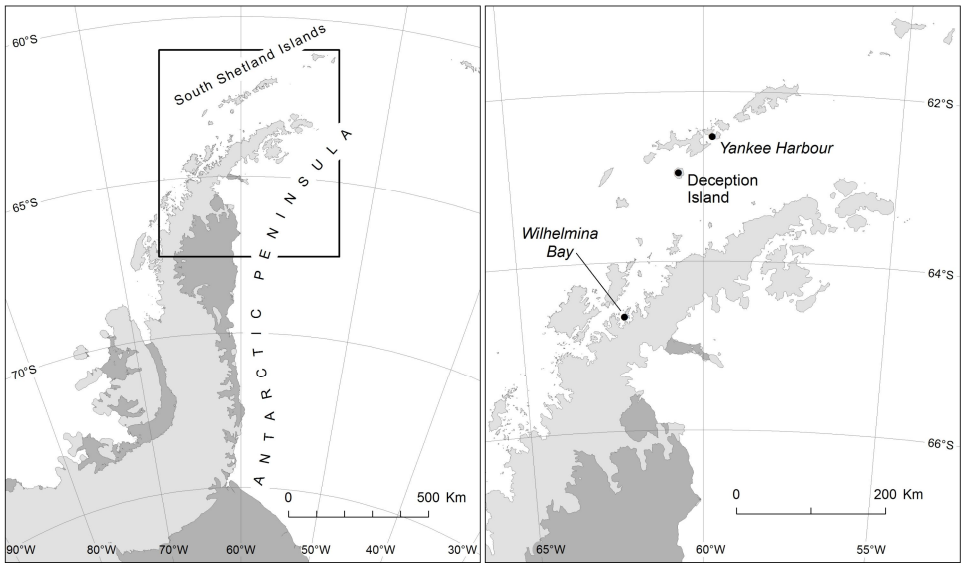

Fig. 1. Map of the Antarctic Peninsula region highlighting (inset) the three sampling locations at Deception Island, Wilhelmina Bay (north-west Antarctic Peninsula, Graham Land) and Yankee Bay (Greenwich Island).

Material and Methods

Study locations and soil sampling

Fourteen soil samples were collected during the austral summer in January 2008, during an expedition in collaboration with the Instituto Antarctico Chileno (INACH) and the Instituto Antarctico Ecuatoriano (INAE) (*see* Table 1). The samples were obtained from a depth of 10 cm using a sterile spatula and were stored immediately in sterile plastic bags. After collection, samples were rapidly returned to the research station, where they were refrigerated at 4°C, and were subsequently transported at this temperature to the National Antarctic Research Centre, Kuala Lumpur, Malaysia. Samples were refrigerated for about two weeks before they could be frozen at -20°C, and they were then kept frozen until further use in the analyses described below.

Isolation and identification of microfungi

A modified soil plate method (Warcup 1950) was applied to isolate fungi from the soil samples. Potato Dextrose agar (PDA) (supplemented with chloramphenicol) was chosen as an effective isolation medium (Azmi et Seppelt 1998). About 1 g of each soil sample was distributed into the sterile plate before the cooled PDA media was poured (5 replicates from each sample and for each incubated temperature). Inoculated plates were then incubated at 4 or 25°C for up to 6 weeks. In the case of soil samples obtained from geothermally active ground on Deception Island, an additional

incubation temperature of 50°C was used, in order to detect the presence of any thermophilic strains. However, no fungi were obtained in the latter incubation, and no further consideration is given here. Individual colonies were picked and sub-cultured onto fresh PDA plates.

Isolated fungi were categorized based on the incubation temperature at which they were isolated: mesophilic fungi grew better at 25°C than 4°C, psychrotolerant fungi grew equally well at 25°C and 4°C, and psychrophilic fungi grew better at 4°C than 25°C. All fungi were identified using both morphological characteristics (including colour and texture of the colony, fruiting body, spore size and pigmentation) (Barnett et Hunter 1972, Sun et al. 1978) and molecular biological approaches. Molecular analyses followed Krishnan et al. (2016), in which the intergenic transcribed spacer (ITS) region of the isolates was sequenced and aligned with sequences deposited in the GenBank database [WP1]. Sequences with the highest identities were selected. The threshold of 4% sequence difference in the ITS (ITS1, 5.8S and ITS2) region was designated as the threshold for species delimitation (Smith et al. 2007). This also takes into account PCR/sequencing error rates and the reported 1.5% difference in ITS sequences between isolates of the same species in community studies (Izzo et al. 2005). Fungal isolates were deposited in the fungal culture collection at the National Antarctic Research Centre (NARC), University of Malaya, Kuala Lumpur.

Location	Latitude	Longitude	No. of samples	Site description
Deception Island	62°58'42.2"S	60°42'71.5"W	1	Geothermally-influenced
	62°58'25.0"S	60°43'92.5"W	2	Geothermally-influenced
Wilhelmina Bay	64°33'495"S	62°11'279"W	1	Fellfield
	64°33'25.0"S	62°11'269"W	1	Fellfield
	64°33'47.4"S	62°11'23.0"W	2	Hillside, vegetated with mosses
	64°33'52.0"S	62°11'32.2"W	2	Hillside, vegetated with mosses
Yankee Bay	64°20'07.4"S	64°08'29.1"W	5	Rocky soil

Table 1. Sampling locations and corresponding number of samples obtained.

Bioactivity screening

Agar-block assay

In order to test for the presence of antimicrobial activity in the fungal isolates obtained, the following test microorganisms were used, selected as they are representative human pathogens: *Staphylococcus aureus* subsp. *aureus* Rosenbach MTCC 96 (ATCC 9144), *Bacillus subtilis* subsp. *subtilis* (Ehrenberg) Cohn (ATCC 6051), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 27853), *Escherichia coli* (Migula) Castellani & Chalmers MTCC 443 (ATCC 25922) and *Candida albicans*

(Robin) Berkhout MTCC 3017 (ATCC 90028). All were obtained from the Microbiology Department, University of Malaya. Test microorganisms were subcultured and incubated at 32°C for 24 h in preparation for the antimicrobial screening. On the day of the assay, test microorganisms were inoculated in a universal bottle containing Muller-Hinton broth and the density of the bacterial suspension was adjusted to the 0.5 McFarland standard. A sterile cotton swab was used to spread the bacterial suspension onto the surface of the agar plates. Luria Base agar (LBA) plates were inoculated with test bacteria, and Sabaraud Dextrose agar (SDA) plates were inoculated with test yeast. Agar blocks (6 mm diameter) were then immediately cut from the growing edge of the target fungal colony (of 1-2 weeks old on PDA medium) with a flame-sterilised No.1 corkborer and placed onto the surface of LBA and SDA plates inoculated with the pathogens as described above. Plates were then incubated at 37±1°C for 18-24 h (up to 48 h for the yeast pathogen). Fungal bioactivities were simply determined by the presence of a clear zone of inhibition around the blocks and, where present, the diameter of the inhibition zone was measured.

Disc diffusion assay

Three agar blocks (approximately 6 mm in diameter) of actively growing fungal colony (of 1-2 weeks old on PDA medium) from each test isolate were inoculated into 200 ml Potato Dextrose broth (PDB) in each of 3×500 ml Erlenmeyer flasks. The flasks were then incubated under stationary conditions at 25°C (to allow classification of mesophilic strains) or 4°C (psychrophilic strains) for up to 3-4 weeks. After the incubation period, cultures were centrifuged (to facilitate the filtration of mycelia) at 3000 rpm and 4°C for 10 min, followed by filtration of the supernatant using Whatman No.1 filter paper under vacuum. The filtrate was extracted twice with an equal volume (1:1) of ethyl acetate (EtOAc), with the aid of a separating funnel and vigorous shaking. EtOAc layers were combined and evaporated to dryness using a rotary evaporator adjusted to 240/25 mbar and 40°C. Crude extracts were stored at 4°C prior to use.

A slight modification of the method described by Bauer et al. (1966) was used. Three yeasts and five bacterial strains were selected as test microorganisms, namely *Candida albicans* (Robin) Berkhout (ATCC 90028), *Saccharomyces cerevisiae* Meyen ex E. C. Hansen (ATCC 18824), *Schizosaccharomyces pombe* Lindner (JCM 8274), *Staphylococcus aureus* Rosenbach 1884, *Bacillus subtilis* (Ehrenberg) Cohn (ATCC 6051), *Bacillus cereus* Frankland and Frankland (ATCC 11778), *Escherichia coli* (Migula) Castellani & Chalmers MTCC 443 (ATCC 25922) and *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 27853). First, 20 mg/ml working extract was prepared by dissolving the crude extract in dimethyl sulfoxide (DMSO). Then 10 µl of the test extract was absorbed on a sterile 6 mm paper disc and immediately placed onto the surface of LBA and SDA inoculated with test microorganisms as described above. Plates were incubated at 37±1°C for 18-24 h (up to 48 h for yeast pathogens). Any zone of inhibition obtained was again measured.

Disc diffusion is a qualitative assay, and therefore does not allow determination of the 'minimum inhibitory concentration' (MIC), or distinguishing between bacteriostatic and bactericidal effects (Ncube et al. 2008). Therefore, microtiter plate or broth microdilution approaches (Eloff 1998) were used to determine MICs against the test microorganisms with reproducible results.

Broth microdilution method

Based on the results from the disc diffusion assay, the minimum inhibitory concentrations (MIC) of extracts from *A. fumigatus* DCP-sp. 1, and unidentified isolates DCP-sp. 11, WHB-sp. 7 and DCP-sp. 7 (*i.e.* those with good or excellent bioactivity) against the test microorganisms were evaluated. DMSO, which showed no activity on the test microorganisms, was used as a negative control. Chloramphenicol, which exhibited the highest activity on the test microorganisms, was used as a positive control.

Minimum inhibitory concentrations (MIC) (Andrews 2001, Paudel et al. 2008) of fungal extracts were determined in U96 MicroWell plates (8×12 wells), with a range of concentrations (25.0 – 0.195 mg ml^{-1}) being prepared. An aliquot of 150 µl of PDB with the pathogen (after adjustment to turbidity of 0.5 McFarland standard units) was placed in the well labeled A-1, and further aliquots of 100 µl into each of the remaining wells labeled from A-2 to A-8. Next, a 50 µl aliquot containing 100 mg ml^{-1} concentration of the fungal extract was pipetted into well A-1 and mixed thoroughly (to obtain a final concentration of 25 mg ml^{-1}). Then, 100 µl from well A-1 was transferred to well A-2 and mixed thoroughly. This serial dilution process was repeated across the plate to well A-8. Plates were sealed and incubated at 35–37°C overnight, or again up to 48 h for tests involving yeast. Prior to assessing results, resazurin dye was added to all the wells, and a further 20 min. incubation completed to clarify the break-point (of completely inhibited growth) with color change.

MBC and MFC assays

Minimum bactericidal (MBC) or fungicidal (MFC) concentration was determined as the lowest concentration of the extract which completely killed the test microorganisms. MBC and MFC assays commenced at the last stage of the MIC assay. For these assays, after the overnight incubation step, and before the addition of dye, about 10 µl (loop-full) from each well of the serial dilutions was streaked onto a separate sterile plate of Muller-Hinton agar (MHA) and incubated overnight. The highest concentration of inoculum that produced a sterile plate was considered as the MBC/MFC.

Results

Fungal isolates

A total of 27 distinct fungal strains (based on their colony morphology) were isolated from the 14 soil samples (Table 2). Deception Island yielded most isolates (13), followed by Wilhelmina Bay (8) and Yankee Bay (6). Among the 27 strains, 13 were Ascomycota, 4 Zygomycota and 10 anamorphic fungi (Table 2). *Geomyces* sp. were dominant while other common Antarctic soil species were also isolated, including *Penicillium chrysogenum*, *Aspergillus fumigatus* and *Antarctomyces psychrotrophicus*. The 27 isolates obtained included 15 psychrophilic, 9 mesophilic and 1 psychrotolerant strains.

Fungal isolate	Sampling site	Thermal class
Ascomycota		
Antarctomyces psychrotrophicus YKB-sp. 5*	Yankee Bay	Psychrophilic
Isolate DCP-sp. 9	Deception Island	Psychrotolerant
Isolate WHB-sp. 3	Wilhelmina Bay	Psychrophilic
Isolate YKB-sp. 1	Yankee Bay	Mesophilic
Aspergillus fumigatus DCP-sp. 1	Deception Island	Mesophilic
Geomyces sp. DCP-sp. 12	Deception Island	Psychrophilic
Geomyces sp. WHB-sp. 2*	Wilhelmina Bay	Psychrophilic
Geomyces sp. WHB-sp. 4*	Wilhelmina Bay	Psychrophilic
Geomyces sp. YKB-sp. 2*	Yankee Bay	Psychrotolerant
Geomyces sp. YKB-sp. 4*	Yankee Bay	Psychrophilic
Kabatiella zeae DCP-sp. 3*	Deception Island	Mesophilic
Kabatiella zeae DCP-sp. 5*	Deception Island	Mesophilic
Penicillium chrysogenum DCP-sp. 2*	Deception Island	Psychrophilic
Zygomycota		
Mucor sp. DCP-sp. 4	Deception Island	Psychrotolerant
Mucor sp. WHB-sp. 1	Wilhelmina Bay	Psychrophilic
Isolate DCP-sp. 6	Deception Island	Mesophilic
Zygomycete WHB-sp. 5	Wilhelmina Bay	Mesophilic
Anamorphic fungi		
DCP-sp. 10	Deception Island	Psychrophilic
DCP-sp. 11	Deception Island	Psychrophilic
DCP-sp. 14	Deception Island	Psychrophilic
DCP-sp. 7	Deception Island	Mesophilic
DCP-sp. 8	Deception Island	Mesophilic
WHB-sp. 6	Wilhelmina Bay	Psychrophilic
WHB-sp. 7	Wilhelmina Bay	Mesophilic
WHB-sp. 8	Wilhelmina Bay	Psychrophilic
YKB-sp. 3	Yankee Bay	Psychrophilic
YKB-sp. 6	Yankee Bay	Psychrophilic

Table 2. Collection sites and thermal classes of successfully isolated fungi from soils of Deception Island, Wilhelmina Bay and Yankee Bay, maritime Antarctica. *Identity determined through sequencing of ITS regions.

Agar-Block assay

18 fungal isolates exhibited antimicrobial activity against one or more Gram–negative and Gram–positive bacteria (Table 3). The diameter of the growth-inhibited zone varied among the isolates, ranging between 8-18 mm. The majority of isolates showed weak to intermediate activity. However, *Kabatiella zeae* DCP-sp. 5 and unidentified isolate DCP-sp. 14 exhibited excellent activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, with *K. zeae* also exhibiting good activity against *P. aeruginosa*. Also, the strains *Aspergillus fumigatus* DCP-sp. 1 and unidentified isolate DCP-sp. 7 showed good inhibition of *Bacillus subtilis* and *S. aureus*, respectively. None of the isolates demonstrated antifungal activity against the yeast pathogen.

Disc diffusion assay

Growth inhibitory zones (Fig. 2) were calculated from the mean of the five replicates (Table 4). DMSO, used as a negative control, demonstrated no antimicrobial activity against the eight test microorganisms. Chloramphenicol, used as a positive control, resulted in higher levels of inhibition across the test microorganisms compared with all 10 fungal isolates examined in this assay. Five extracts showed antimicrobial activity against the test microorganisms. The remaining five extracts did not show the activity in this assay that had been apparent in the agar block assay. Finally, isolate WHB-sp. 7 generated a large inhibition zone against *Saccharomyces cerevisiae*, while chloramphenicol did not.

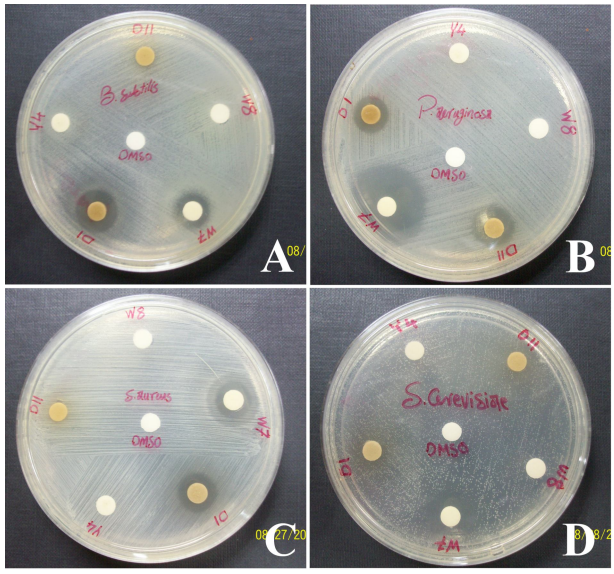

Fig. 2 . Photographs of antibacterial activity of fungal extracts demonstrated through disc diffusion. **A&C:** only W7 and D1 extracts showed intermediate to good activity against *Staphylococcus aureus* and *Bacillus subtilis*. **B:** *Pseudomonas aeruginosa* showed very high susceptibility to W7 extract, good to D11, intermediate to D1. **D:** W7 was the only extract that exhibited very good antifungal activity against *Saccharomyces cerevisiae.*

Fungal isolate	Antibacterial activity				Antifungal activity
	E.c	P.a	B.s	S.a	C.a
Antarctomyces psychrotrophicus YKB-sp. 5	+	NA	NA	NA	NA
DCP-sp. 9	NA	NA	NA	NA	NA
WHB-sp. 3	+	NA	NA	NA	NA
YKB-sp. 1	NA	NA	NA	NA	NA
Aspergillus fumigatus DCP-sp. 1	NA	NA	+++	NA	NA
Geomyces sp. DCP-sp. 12	NA	NA	NA	NA	NA
Geomyces sp. WHB-sp. 2	NA	NA	NA	NA	NA
Geomyces sp. WHB-sp. 4	+	NA	+	+	NA
Geomyces sp. YKB-sp. 2	NA	NA	NA	NA	NA
Geomyces sp. YKB-sp. 4	+	NA	+	+	NA
DCP-sp. 10	NA	NA	NA	NA	NA
DCP-sp. 11	+	++	+	+	NA
DCP-sp. 14	+	++++	+	NA	NA
DCP-sp. 7	NA	NA	+	+++	NA
DCP-sp. 8	NA	NA	NA	NA	NA
WHB-sp. 6	+	NA	NA	NA	NA
WHB-sp. 7	NA	NA	+	++	NA
WHB-sp. 8	NA	NA	+	+	NA
YKB-sp. 3	NA	NA	NA	NA	NA
YKB-sp. 6	+	NA	NA	NA	NA
Kabatiella zeae DCP-sp. 3	+	NA	NA	NA	NA
Kabatiella zeae DCP-sp. 5	+	+++	+	++++	NA
Mucor sp. DCP-sp. 4	NA	NA	NA	+	NA
Mucor sp. WHB-sp. 1	NA	NA	NA	NA	NA
Penicillium chrysogenum DCP-sp. 2	NA	NA	+	++	NA
DCP-sp. 6	+	NA	NA	NA	NA
WHB-sp. 5	+	NA	NA	NA	NA

Table 3. Results from preliminary screening of fungal bioactivity on test microorganisms in the agar-block assay. NA: No activity, (+): 7-10mm, (++): 11-12mm, (+++):13-15mm, (++++): >15mm, E.c: *Escherichia coli*, P.a: *Pseudomonas aeruginosa*, B.s: *Bacillus subtilis*, S.a: *Staphylococcus aureus*, C.a: *Candida albicans*.

Fungal extract	Antibacterial activity					Antifungal activity		
	E.c	P.a	B.c	B.s	S.a	C.a	S.c	S.p
DCP-sp. 1	12	13	14	15	14	NA	NA	NA
DCP-sp. 11	8	15	NA	NA	NA	NA	NA	NA
Geomyces sp. YKB-sp. 4	NA	NA	NA	NA	NA	NA	NA	NA
WHB-sp. 7	12	28	12	13	13	NA	17	NA
WHB-sp. 8	NA	NA	NA	NA	NA	NA	NA	NA
Penicillium chrysogenum DCP-sp. 2	8	11	NA	NA	10	NA	NA	NA
DCP-sp. 7	8	13	8	8	12	NA	NA	NA
DCP-sp. 14	NA	NA	NA	NA	NA	NA	NA	NA
Geomyces sp. WHB-sp. 4	NA	NA	NA	NA	NA	NA	NA	NA
DCP-sp. 5	NA	NA	NA	NA	NA	NA	NA	NA
Chl.	30	16	30	24	32	NA	NA	NA
DMSO	NA	NA	NA	NA	NA	NA	NA	NA

Table 4. Biological activity of fungal extracts, chloramphenicol and dimethyl sulfoxide on test microorganisms, measured in millimeters, through the disc diffusion assay. DMSO: Dimethyl sulfoxide, Chl.: Chloramphenicol, E.c: *Escherichia coli*, P.a: *Pseudomonas aeruginosa*, B.c: *Bacillus cereus*, B.s: *Bacillus subtilis*, S.a: *Staphylococcus aureus*, C.a: *Candida albicans*, S.c: *Saccharomyces cerevisiae*, S.p: *Schizosaccharomyces pombe*, NA: No activity, (8-10mm): weak activity, (11-12mm): intermediate, (13-15): good, (>15): very good activity.

Fungal extract	*Test microorganism*											
	E. coli (mg/ml)		*B. subtilis* (mg/ml)		*B. cereus* (mg/ml)		*P. aeruginosa* (mg/ml)		*S. aureus* (mg/ml)		*S. cerevisiae* (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Aspergillus fumigatus DCP-sp. 1	3.13	3.13	1.56	1.56	3.13	6.25	6.25	6.25	1.56	1.56	-	-
DCP-sp. 11	12.5	12.5	6.25	6.25	-	-	6.25	12.5	-	-	-	-
DCP-sp. 7	-	-	-	-	3.13	6.25	-	-	0.78	25	-	-
WHB-sp. 7	12.5	12.5	1.56	12.5	0.78	3.13	1.56	1.56	0.78	12.5	6.25	6.25

Table 5. MICs and MBCs of fungal extracts against test microorganisms through the broth microdilution assay. MIC: Minimum inhibitory concentration. MBC: Minimum bactericidal concentration. MFC: Minimum fungicidal concentration.

Broth microdilution method

The extract from unidentified isolate WHB-sp. 7 recorded the lowest inhibitory concentration amongst the tested extracts. MBC and MFC values are shown in Table 5. The extract from *Aspergillus fumigatus* DCP-sp. 1 (D1) recorded the lowest MBC against *E. coli*, *S. aureus* and *B. subtilis*. That of isolate WHB-sp. 7 (W7) gave the lowest MBC against *B. cereus* and *P. aeruginosa*, and shared the lowest concentration with that of isolate DCP-sp. 7 (D7) against *B. subtilis* and *S. aureus*. That of isolate DCP-sp. 11 (D11) recorded relatively high MICs against the test pathogens.

Discussion

Biodiversity

Over 1,000 species of fungi have been reported from Antarctica to date (Bridge et Hughes 2010), with 13 species being reported specifically from Deception Island (Bridge et al. 2008). In this study, 27 apparently distinct fungal taxa were obtained (Table 2) from 3 locations, including 13 from Deception Island, 8 from Wilhelmina Bay and 6 from Yankee Bay. *Geomyces* sp. were common, a result that is consistent with previous studies (Krishnan et al. 2011, 2016). The relatively low number of isolates obtained in the current study may reflect methodological limitations, in particular the use of a single growth medium (PDA) which may favour faster growing fungi, and the extended period of refrigerated storage required prior to sample freezing.

Our incubation data indicate that psychrophilic taxa form a greater proportion of the fungi isolated than do mesophilic or psychrotolerant taxa. Tosi et al. (2002) also reported that a majority of isolated species from maritime Antarctica were psychrophilic. In contrast, a range of studies have suggested that Antarctic microfungi are typically psychrotolerant rather than psychrophilic (Robinson 2001, Frate et Caretta 1990, Onofri et al. 2004).

Qualitative bioactivity screening

An increasing number of recent studies have started to examine the antimicrobial activities of Antarctic fungal isolates. Li et al. (2008), Melo et al. (2014) and Gonçalves et al. (2015) have examined either diversity or the specific antimicrobial activity of microfungi from the terrestrial environment, with Brunati et al. (2009) and Henriquez et al. (2014) reporting similar studies in freshwater lakes and the marine environment, respectively. The fungal isolates obtained in the current study possessed a variety of broad spectrum antibacterial potential. During initial screening, 18 of 27 fungal isolates exhibited antibacterial activity against Gram–positive and Gram–negative bacteria (Table 3). Nedialkova et Naidenova (2005) also used this method to screen actinomycete strains from Antarctica, similarly reporting 60% of strains to possess antibacterial activity. Of these most were active against Gram–positive rather than Gram–negative bacteria. However, unlike the current study, these authors reported very weak antifungal activity using the agar-block method. In total 5 fungal species which demonstrated antibacterial activities in the agar-block assay did not

exhibit this activity when their extracts were tested in the disc diffusion assay. This could possibly be due to the extraction of the secondary metabolites prior to the disc diffusion assay, while certain compounds could be non-extractable due having polar properties. It is also possible that these compounds produced by Antarctic fungi could be heat sensitive and thereby be degraded when channeled through solvent evaporation temperatures. A similar obstacle was also reported by Moncheva et al. (2002) and Nedialkova et Naidenova (2005).

Compared with a recent study of antimicrobial activity in Antarctic Actinobacteria (Nedialkova et Naidenova (2005), extracts from the soil fungi obtained in the current study possessed higher activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Saccharomyces cerevisiae*, while having a relatively lower activity against *Bacillus subtilis* and *Staphylococcus aureus*. Overall, extracts from isolate isolate WHB-sp. 7 showed the best broad-spectrum antimicrobial activities, providing a potential target for biotechnological studies into antibiotic development.

Quantitative bioactivity screening

Certain fungal strains such as *Aspergillus fumigatus* DCP-sp. 1, isolate WHB-sp. 7 and isolate DCP-sp. 11 exhibited bactericidal activities even towards Gram–negative bacteria, although with different levels of activity. However, these levels were significantly lower than achieved by other organisms in similar environments. Paudel et al. (2008) also reported the MIC values of extracts from 5 Antarctic lichens against *B. subtilis* to be between 0.037 and 0.954 mg ml^{-1}, and against *S. aureus* to be between 0.069 and ≥1 mg ml^{-1}. Isolate WHB-sp. 7 was the only fungus exhibiting fungicidal activity, and also showed strong bactericidal activity on all test bacteria. In a study by Cabello et al. (2001), *Arthrinium arundinis* extract showed antifungal activity against yeast pathogens with no associated antibacterial activity, even at concentrations up to 64 μg ml^{-1} of the extract. They recorded MICs between 2-8 μg ml^{-1} against *Candida* sp. and 1 mg ml^{-1} against *Aspergillus fumigatus*. The MIC and MFC values of isolate WHB-sp. 7 were significantly higher.

Conclusion

While this study is necessarily preliminary in nature, it clearly demonstrates that a high proportion of Antarctic soil microfungi possess detectable antimicrobial activity, with some doing so at levels comparable to studies that have identified biotechnological potential. More detailed studies, using a greater range of isolation media and temperatures, would be of great value in building on the potential identified in the current study.

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