

Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica

Brett E. Arenz^{a,*}, Benjamin W. Held^a, Joel A. Jurgens^a, Roberta L. Farrell^b,
Robert A. Blanchette^a

^aDepartment of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108-6030, USA

^bDepartment of Biological Sciences, University of Waikato, Hamilton, New Zealand

Received 8 September 2005; received in revised form 2 January 2006; accepted 17 January 2006

Available online 20 March 2006

Abstract

Microorganisms play a dominant role in Antarctic ecosystems, yet little is known about how fungal diversity differs at sites with considerable human activity as compared to those that are remote and relatively pristine. Ross Island, Antarctica is the site of three historic expedition huts left by early explorers to the South Pole, Robert F. Scott and Ernest Shackleton. The fungal diversity of these wooden structures and surrounding soils was investigated with traditional culturing methods as well as with molecular methodology including denaturing gradient gel electrophoresis (DGGE) using the internal transcribed spacer (ITS) regions of ribosomal DNA for identification. From historic wood and artifact samples and soils adjacent to the huts as well as soil samples obtained from the Lake Fryxell Basin, a remote Dry Valley location, and remote sites at Mt. Fleming and the Allan Hills, 71 fungal taxa were identified. The historic huts and associated artifacts have been colonized and degraded by fungi to various extents. The most frequently isolated fungal genera from the historic woods sampled include *Cadophora*, *Cladosporium* and *Geomyces*. Similar genera were found in soil samples collected near the huts. Sampling of soils from locations in the Transantarctic Mountains and Lake Fryxell Basin at considerable distances from the huts and with different soil conditions revealed *Cryptococcus* spp., *Epicoccum nigrum* and *Cladosporium cladosporioides* as the most common fungi present and *Cadophora* species less commonly isolated. DGGE revealed 28 taxa not detected by culturing including four taxa which possibly have not been previously described since they have less than 50% ITS sequence identity to any GenBank accessions. Fungi capable of causing degradation in the wood and artifacts associated with the expedition huts appear to be similar to those present in Antarctic soils, both near and at more remote locations. These species of fungi are likely indigenous to Antarctica and were apparently greatly influenced by the introduction of organic matter brought by early explorers. Considerable degradation has occurred in the wood and other materials by these fungi.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Antarctica; Fungi; Biodiversity; Historic huts; Ross island; Dry valleys; DGGE; Polar

1. Introduction

Antarctic explorers Robert F. Scott, Ernest Shackleton and their crews built three expedition huts on Ross Island, Antarctica in 1901–1911. These huts were used to house men and equipment for scientific investigations in the area as well as provide a base during attempts to explore the continent and reach the South Pole. Today, the structures and artifacts left at these sites have provided a remarkable

link to the past and the lives of these “Heroic Era” explorers. Despite the dry and cold climate of Antarctica, deterioration from both abiotic and biotic causes have occurred at these sites leading to concerns for the long term preservation of the historic structures (Blanchette et al., 2002; Held et al., 2003). In light of these concerns and its historical importance, Shackleton’s hut at Cape Royds (Fig. 1) was placed on the World Monuments Fund list of the 100 most endangered historic sites in the world.

Recent investigation has shown that the biotic forms of degradation are caused by fungi which produce a soft rot type of decay in woods that are in contact with the soil

*Corresponding author. Tel.: +1 612 625 6231; fax: +1 612 625 9728.

E-mail address: aren0058@umn.edu (B.E. Arenz).



Fig. 1. Hut at Cape Royds built by Ernest Shackleton in 1908 as a base for polar exploration is one of three huts on Ross Island where soil and wood samples were obtained.



Fig. 2. Crates in galley area inside Cape Evans Hut affected by surface fungal growth. Fungi from samples of interior and exterior woods and artifacts from the historic huts were used in this study to compare to fungi obtained from soils.

(Blanchette et al., 2004b). Surface fungal growth on wood (Fig. 2) and other artifacts inside the huts also have caused considerable degradation (Held et al., 2005). Previous investigations have shown that the soft rot attack is caused by species of *Cadophora* [some *Phialophora* species are now included in this genus (Harrington and McNew, 2003)] including *C. malorum*, *C. luteo-olivacea*, and *C. fastigiata* as well as several previously undescribed *Cadophora* species designated *C. species H*, *C. species E* and *C. species NH* (Blanchette et al., 2004b). During the austral summer, environmental conditions conducive to fungal growth include temperatures above 0 °C and relative humidity above 80% occur periodically in the huts (Held et al., 2005). Fungi reported from the inside of the Cape Evans hut include *Cladosporium cladosporioides*, *Hormonema dematioides*, *Penicillium echinulatum*, *P. expansum*, and *Geomyces* sp. (Held et al., 2005).

The present study was done to obtain a more comprehensive list of fungi associated with degradation at the

huts, gain a better understanding of their relative abundance in soils adjacent to the huts and compare the fungal diversity present near the huts to those found in Antarctic soils at more distant, remote locations. In addition to traditional culturing methods for isolating fungi, denaturing gradient gel electrophoresis (DGGE) was utilized as a molecular method that allows rapid detection and identification of recalcitrant or cryptic species and/or species of low abundance. Samples were taken from wood, artifacts and soils at Discovery Hut, Cape Evans Hut and Cape Royds Hut as well as from soils at locations with historically very few human visitors including sites at the Allan Hills, Mt. Fleming and Lake Fryxell Basin, a location in the Antarctic Dry Valleys (Fig. 3). Soil samples from the area around McCraw Hut at New Harbor, Antarctica were also utilized. It should be noted that it is impossible to directly compare the diversity of fungi based on levels of human activity alone as there exists different soil conditions and a lack of introduced organic matter at the remote sites. Fungi were identified based on their internal transcribed spacer (ITS) sequences.

2. Materials and methods

Sampling was undertaken during the austral summers of 1999–2004 and carried out under permit guidelines of the Antarctic Conservation Act. Samples were obtained from wood, artifacts including straw, paper, flour, rope, burlap, butter, biscuits, and soils from Discovery Hut, Cape Evans Hut and Cape Royds Hut located on Ross Island, Antarctica. Soil samples were also obtained from Lake Fryxell Basin in the McMurdo Dry Valleys (samples were collected by Professor Diana Wall, Colorado State University) as well as the nearby ice free mountainous regions at Mt. Fleming and the Allan Hills. Exact site locations are listed in Table 1. Minuscule samples from structural wood and other artifacts were taken aseptically from inconspicuous locations and placed into sterile containers. Approximately 100–200 g of soil were also taken with a sterile scoop at each sampling site. Other samples from inside the huts were taken at locations where fungal growth was conspicuous using sterile swabs. All samples were placed in sterile bags or tubes and stored at below 0 °C until processed in the laboratory.

2.1. Culturing methodology

Fungi were isolated from samples by incubating a small sub-sample on media or streaking a swab sample across the surface of media. Soil samples were processed by diluting 1 g in 100 ml of sterile water. The particles were allowed to settle for 20 min and then 1 ml of the dilution was spread over each plate. Three types of media were used: malt extract agar (MEA) containing 1.5% Difco malt extract and 1.5% agar, an acidified MEA containing 2 ml of lactic acid added after autoclaving (AMEA) and a basidiomycete-select media (BSA) containing 1.5% malt extract,

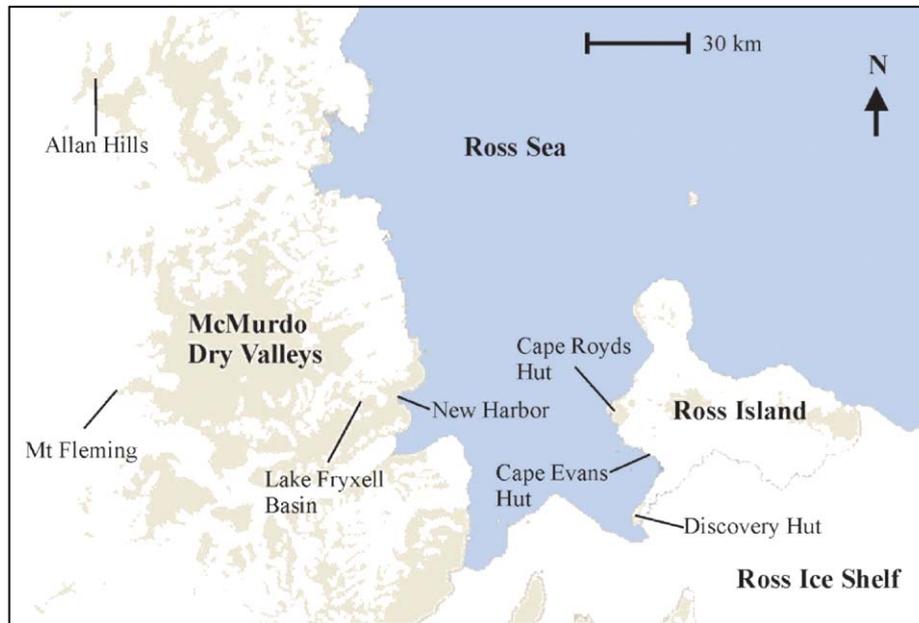


Fig. 3. Map of Ross Sea Region showing locations where samples were obtained for this study.

Table 1
Locations included in study and number of samples by type analyzed for fungal diversity

Location	Latitude	Longitude	Soil	Wood	Other ^a
<i>Ross Sea area</i>					
Cape Evans Hut	77°38'S	166°24'E	11	39	13
Cape Royds Hut	77°38'S	166°10'E	10	23	9
Discovery Hut	77°50'S	166°38'E	6	16	8
New Harbor	77°34'S	163°30'E	4		
<i>Dry Valley area</i>					
Lake Fryxell Basin	77°60'S	163°24'E	3		
<i>Mountain sites</i>					
Allan Hills	76°42'S	159°44'E	10		
Mt. Fleming	77°31'S	160°15'E	3		

^aOther artifacts included paper, cloth, straw and foodstuffs.

1.5% agar, 0.2% yeast extract, 0.006% benlate, and with 0.2% lactic acid and 0.001% streptomycin sulphate added after autoclaving (Worrall, 1999). Cultures were incubated at 8 and 20 °C. After pure cultures were obtained via sub-sampling, genomic DNA was extracted from cultures with Qiagen DNeasy Plant Mini-kits using manufacturers instructions (Qiagen Sciences Inc., Germantown, MA). ITS sequences were amplified with the primers ITS1 and ITS4 (Gardes and Bruns, 1993). PCR amplification was performed with Amplitaq Gold PCR Master-mix and 1 µl template DNA using manufacturer's instructions (Applied Biosystems, Foster City, CA). A MJ Research PTC Mini-cycler (Watertown, MA) was used with the following profile: 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min followed by a final extension step of 72 °C for 5 min. PCR products of appropriate size were

verified by electrophoresing the amplicons on a 1% agarose gel with a SYBR green 1 (Molecular Probes, Eugene, OR) pre-stain and transilluminating with a Dark Reader DR45 (Clare Chemical Research, Denver, CO). Amplicons were purified using EXO-SAP (exonuclease-shrimp alkaline phosphatase) PCR product cleanup systems (USB Corporation, Cleveland, OH). Sequencing was performed for both primers using the ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems) and an ABI Prism 377 automated DNA sequencer. DNA sequence data were analyzed by Chromas software (Technelysium Ltd., Helensvale, Australia) and assembled into a consensus sequence based on the results of both primers. The sequences were compared to others in GenBank using BLASTn (Altschul et al., 1990) and the best match recorded.

2.2. Denaturing gradient gel electrophoresis (DGGE)

Samples from structural wood and other artifacts were ground to powder in liquid nitrogen in a sterile mortar and pestle. DNA from the pulverized samples was extracted with the Qiagen DNeasy Plant Mini-kit (Qiagen Sciences Inc.) using manufacturer's instructions. DNA from soil samples was extracted using the UltraClean Soil DNA Kit as per manufacturer's instructions (MO BIO Laboratories Inc., Carlsbad, CA). DNA was amplified by PCR with the primers ITS1F and ITS4, using the previously stated protocols. ITS1F is a fungal specific primer (Gardes and Bruns, 1993). The DNA products were then diluted 1/100 and re-amplified by PCR using the primers ITS3 (Gardes and Bruns, 1993) and ITS4*. The ITS4* primer in this case had a GC clamp (5' CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C 3') added to the

5' prime end of the amplicon to prevent total denaturation of the double stranded DNA fragment during DGGE. This nested PCR procedure is similar to that used by Anderson (2003), except for our use of the ITS2 region instead of the ITS1 region. The GC-clamped PCR amplicons were analyzed by a DGGE-2001 system (C.B.S. Scientific Company, Inc., Del Mar, CA). A variety of different denaturant concentration gradients were tested and it was found that a 30–60% gradient in the direction of electrophoresis produced the best band separation in the range of the gel. Vertical gradient 6.5% polyacrylamide gels were prepared with a GM-40 (C.B.S. Scientific Company, Inc.) gradient maker. The gels were run for 14 h in 1X TAE buffer at 60 °C and 70 V. After removal from the DGGE system, the gels were submerged in 50 ml of 1x TAE buffer plus 5 µl SYBR green for 10 min. The gels were visualized with a Dark Reader DR-45 (Clare Chemical Research, Dolores, CO). Bands were stabbed with 20 µl pipette tips and each individual stab placed in a microcentrifuge tube containing 20 µl of sterile distilled water for 20 min. Excised bands were re-amplified as described above and re-run through DGGE to ensure they appeared as single bands. In the event of multiple bands, they were again extracted and re-run until resolved into single bands or until three cycles of this procedure had been completed. Single bands were purified and sequenced as previously described with both primers ITS3 and ITS4 (without GC clamp).

3. Results

A total of 164 samples were analyzed by traditional culturing methods and 48 of these samples were also analyzed by DGGE. In total, from all samples (Table 1), 284 fungal ITS sequences were identified; including 184 from culturing and 100 from DGGE. These sequences were grouped into 71 distinct ITS sequence profiles (Table 2). BLAST identifications based on these sequences revealed a total of 39 different genera. The major groups identified include: filamentous ascomycetes (74%), basidiomycetous yeasts (21%), ascomycete yeasts (1%) and zygomycetes (1%). The most dominant genera observed as a percentage of total isolations were *Cadophora* (21%), *Geomyces* (14%), *Cladosporium* (13%), *Cryptococcus* (12%), *Rhodotorula* (3%), *Hormonema* (3%), *Exophiala* (2%). Thirty-two other genera made up the remainder (32%). The most frequently isolated genera from soil samples were *Cadophora* (20%), *Cryptococcus* (16%), *Geomyces* (11%), *Cladosporium* (7%) and the most frequently isolated from wood and artifact samples were *Cadophora* (21%), *Cladosporium* (18%), *Geomyces* (17%), *Cryptococcus* (8%), *Hormonema* (6%), *Rhodotorula* (3%), and *Fusarium* (3%). The above calculations were made under the conservative assumption that >95% ITS region sequence identity was enough to confidently group taxa into a genus (Landeweert et al., 2003). Four of the taxa had very poor best BLAST matches (<50% identity) and could not even

be tentatively identified. These are designated as AUNH1, AUNH2, AUNH3, and AUR1. These unknown types accounted for 3% of all sequences.

Comparing the soils from around the huts in the Ross Sea area to those at the Dry Valley and mountain sites showed that *Cadophora* and *Geomyces* were the two most commonly isolated genera in the Ross Island and New Harbor soils, whereas in the Dry Valley area and mountain soil samples the most common fungi belonged to the genera *Cryptococcus* and *Epicoccum* (Table 3). Some *Cadophora* species were identified from all of the sites except the Allan Hills. Identifications from samples taken from the Lake Fryxell Basin, Allen Hills and Mt. Fleming sites had equal numbers of filamentous fungi (50%) and yeasts (50%). Samples taken from the Ross Island and New Harbor locations produced a higher proportion of filamentous micro-fungi (76%) than yeasts (24%). Sixty one percent of taxa that were identified from historic wood or other artifact samples were also found in soil samples. Twenty-eight taxa, including the four unknown types, were detected and identified by DGGE and not by culturing methods. Conversely, 25 taxa were detected by traditional culturing methods and not by DGGE.

4. Discussion

The fungi present in the historic wood and artifact samples were similar to those in soils located near the huts as well as the soils in the more remote locations but to a lesser degree. However, some species causing degradation in the huts, such as *C. malorum*, *C. luteo-olivacea*, *C. cladosporioides*, and *Geomyces* sp., were also found in the very remote soils sampled (Lake Fryxell Basin, Mt. Fleming and Allan Hills sites). The presence of previously unreported species of *Cadophora* in Antarctica and the prevalence of these fungi at many locations in Antarctica suggests that they are indigenous (Blanchette et al., 2004a, b).

Analyses of Antarctic soils from other areas of high human impact have revealed species similar to those that we report. Line (1988) has identified the presence of *Cladosporium* spp., *G. pannorum*, and *Phialophora fastigiata* (syn. = *C. fastigiata*, Harrington and McNew, 2003) in soils and other substrates near Mawson Station in MacRobertson Land and Davis Station near the Vestfold Hills. *Phialophora* sp. (syn. = *Cadophora*) were found to replace *Chrysosporium* as the dominant species in oil contaminated sites in the McMurdo Sound region (Aislabie et al., 2001). In our study we analyzed soil samples around a historic fuel depot at the Cape Evans hut that had petroleum hydrocarbon contamination (Blanchette et al., 2004a). *Cadophora* spp. were found in five out of eight of these petroleum contaminated soil samples.

Cadophora spp. have also been reported associated with Antarctic mosses (Tosi et al., 2002), a mummified seal carcass (Greenfield, 1981), skua feathers and soil (Del Frate and Caretta, 1990) from the Ross Sea area. The

Table 2

Taxa identified from samples using culturing or denaturing gradient gel electrophoresis from soil, wood and other samples obtained from several locations in the Ross Sea region of Antarctica

Best BLAST match	%Id	Overlap ^a	MoD ^b	Locations ^c	Soil	Wood	Other	Total	Accession
Ascomycetes, filamentous									
<i>Alternaria</i> sp.	100	551/551	C,D	E,R,AH, LF	3	2		5	DQ317386
<i>Antarctomyces psychrotrophicus</i> [AJ133431]	97.6	321/329	D	R	1			1	DQ317323
<i>Ascobolus denudatus</i> [AY500528]	88.1	267/303	C	E			1	1	DQ317324
<i>Ascobolus stercorarius</i> [AY372073]	92	319/346	D	NH	1			1	DQ317325
<i>Ascomycete</i> sp. [AA1279460]	92.9	353/380	C	R		1	1	2	DQ317343
<i>Cadophora fastigiata</i> [AY805584]	100	547	C	E,R		1	1	2	DQ317326
<i>Cadophora luteo-olivacea</i> [AY249068]	100	542	C,D	E,H,R,NH,MF,LF	12	3	1	16	DQ317327
<i>Cadophora malorum</i> [AY249064]	100	521	C,D	E,H,R,NH,MF	11	13	3	27	DQ317328
<i>Cadophora</i> sp. 4E71-1 [AY371506]	100	543	C,D	E	3	7	2	12	DQ317329
<i>Cadophora</i> sp. H37 [AY371512]	100	461	C	R		2		2	DQ317330
<i>Chaetomium funicola</i> [AJ279450]	94.7	392/414	C	R		1		1	DQ317331
<i>Cladosporium cladosporioides</i> [AY213641]	99.1	538/543	C,D	E,H,R,NH,AH	9	28		37	DQ317332
<i>Cosmospora vilior</i> [AY805574]	99.4	471/474	C	E,R		3		3	DQ317333
<i>Dactylella lobata</i> [U51958]	94.7	501/529	C	E,R	1	3		4	DQ317334
<i>Epicoccum nigrum</i> [AF455455]	100	342	D	MF,LF,NH,AH	6			6	DQ317367
<i>Eurotium</i> sp. [AF455536]	99.6	554/556	C	E,R		1	1	2	DQ317335
<i>Exophiala spinifera</i> [AY843179]	100	528	C,D	E,H	3	3		6	DQ317337
<i>Fusarium oxysporum</i> [AY188919]	99.7	336/337	D	E,R		4		4	DQ317368
<i>Geomyces</i> sp. C239/10G [AY345347]	99.8	560/561	C,D	E,H,R,LF	9	3	6	18	DQ317337
<i>Geomyces</i> sp. GFI 22 [AJ608988]	94.4	470/498	C	NH	1			1	DQ317338
<i>Geomyces pannorum</i> [AY873967]	99.8	565/566	C,D	E,H,R	5	10	7	22	DQ317339
<i>Geopyxis</i> sp. [AY465441]	94.7	160/169	D	E,NH	1		1	2	DQ317369
<i>Hormonema dematioides</i> [AF013228]	99.1	579/584	C	E,H		6	3	9	DQ317340
<i>Leptosphaerulina trifolii</i> [AY831558]	100	341	D	NH	1			1	DQ317370
<i>Microdochium bolleyi</i> [AJ279454]	100	355	D	MF	1			1	DQ317371
<i>Monodictys castaneae</i> [AJ238678]	93.9	419/446	C	E		1		1	DQ317341
<i>Nectria</i> sp. olrim171 [AY805575]	99.8	484/485	C	R		1		1	DQ317342
<i>Penicillium echinulatum</i> [AF033473]	100	528	C	R,H,NH	2	2	1	5	DQ317344
<i>Phaeosphaeria</i> sp.	94.8	165/174	D	R	1			1	DQ317372
<i>Phialophora</i> sp. RR 90-121 [AF083204]	97.6	248/254	D	E	2			2	DQ317373
<i>Phoma herbarum</i> [AY293791]	100	522	C,D	R,MF	3			3	DQ317345
<i>Phoma</i> sp. GS9N1a [AY465466]	99.8	533/534	C	H,NH	2			2	DQ317346
<i>Pseudeurotium desertorum</i> [AY129288]	95.1	481/506	C	H		1		1	DQ317347
<i>Pseudeurotium</i> sp.olrim 176 [AY787729]	100	450/450	C	E,R			2	2	DQ317348
<i>Sarea difformis</i> [AY590786]	99.4	488/491	C	H		1		1	DQ317349
<i>Thelebolus caninus</i> [AY957550]	99.2	475/479	C,D	R,H	1		1	2	DQ317350
<i>Thelebolus microsporus</i> [AY957552]	99.2	477/481	C	E	1			1	DQ317351
<i>Ulocladium chartarum</i> [AY625071]	100	548	C	NH	1			1	DQ317352
Uncultured fungus isolate RFLP104 [AF461665]	94	518/551	C	E		1		1	DQ317353
Zygomycota									
Uncultured <i>Mortierellaceae</i> [AJ879650]	96.3	498/517	C,D	R	2			2	DQ317354
Ascomycete yeasts									
<i>Candida parapsilosis</i> [AF455530]	100	495	C,D	R,LF	2			2	DQ317355
<i>Debaryomyces hansenii</i> [AF210326]	99.4	635/639	C	R			1	1	DQ317356
<i>Dipodascus australiensis</i> [AF157596]	99	243/244	D	E			1	1	DQ317374
Basidiomycete yeasts									
Antarctic yeast [AY033643]	99.7	575/577	C	E,H	2	1		3	DQ317357
<i>Bulleromyces albus</i> [AF444664]	99.6	283/284	D	R	1			1	DQ317375
<i>Cryptococcus albidosimilis</i> [AF137601]	99.6	558/560	C	E,R	1		1	2	DQ317358
<i>Cryptococcus antarcticus</i> [AB032670]	100	588	C	AH	1			1	DQ317359
<i>Cryptococcus carnescens</i> [AB105438]	99.4	501/504	C,D	E,R	1	1	2	4	DQ317388
<i>Cryptococcus foliicola</i> [AY557600]	97.6	320/328	D	H	1			1	DQ317376
<i>Cryptococcus friedmannii</i> [AF145322]	99.8	632/633	C	R,AH	3			3	DQ317360
<i>Cryptococcus hungaricus</i> [AF272664]	97.5	306/314	D	MF	1			1	DQ317377
<i>Cryptococcus laurentii</i> [AJ421006]	99.3	434/437	C,D	E,H	2			2	DQ317361
<i>Cryptococcus skinneri</i> [AF444305]	91	315/346	D	MF	1			1	DQ317378
<i>Cryptococcus</i> sp.	100	584	C,D	E,R,AH	4	2		6	DQ317387
<i>Cryptococcus</i> sp. NRRL Y-17490 [AF444449]	99.8	407/408	C	R	1			1	DQ317379
<i>Cryptococcus tephrensii</i> [DQ000318]	98.8	324/328	D	E			1	1	DQ317362

Table 2 (continued)

Best BLAST match	%Id	Overlap ^a	MoD ^b	Locations ^c	Soil	Wood	Other	Total	Accession
<i>Cryptococcus victoriae</i> [AY188380]	99.8	488/489	C,D	E,R,H	2	4	1	7	DQ317363
<i>Cryptococcus vishniacii</i> [AB032691]	100	557	C	E,AH	1		1	2	DQ317364
<i>Cryptococcus wieringae</i> [AF444383]	99.7	380/381	D	AH	3			3	DQ317380
<i>Dioszegia hungarica</i> [AF444467]	99.4	316/318	D	AH	1			1	DQ317389
<i>Malassezia restricta</i> [AY387144]	99.6	455/457	D	R,MF,AH, LF	5			5	DQ317381
<i>Mrakia</i> sp. [AY038826]	100	420	D	E			1	1	DQ317382
<i>Rhodotorula mucilaginosa</i> [AF444541]	99	400/404	D	E			1	1	DQ317383
<i>Rhodotorula laryngis</i> [AB078500]	99	577/583	C	E,R,H	4	1	3	8	DQ317365
<i>Sporidiobolus salmonicolor</i> [AY015434]	99.5	599/602	C	H		2		2	DQ317366
<i>Sporobolomyces symmetricus</i> [AY364836]	100	390	D	E		1		1	DQ317384
Uncultured basidiomycete yeast [AJ581040]	98.9	264/267	D	AH	1			1	DQ317385
Unclassified									
AUNH1	29.4	123/418	D	NH	1			1	
AUNH2	30.7	121/394	D	NH,LF	3			3	
AUNH3	41.9	142/339	D	NH	1			1	
AURI	44.2	180/407	D	R	4			4	

Identification was made using BLASTn searches of the ITS region of rDNA and % identity to best BLAST match is given.

^aOverlap of ITS alignment of best BLAST match in base pairs.

^bMethod of detection, (C) culturing, (D) DGGE.

^cSampling locations: Ross Sea sites include Cape Evans Hut (E), Cape Royds Hut (R), Discovery Hut (D), New Harbor (NH). Dry Valley and mountain sites include Lake Fryxell Basin (LF), Allan Hills (AH), Mt. Fleming (MF).

Table 3

The five most frequently identified fungal genera based on BLAST results from the Ross Sea region or Dry Valley and Mountain sites

Ross Sea region		Dry Valley and mountain sites
Wood and artifacts	Soils	Soils
<i>Cadophora</i> (21.6%)	<i>Cadophora</i> (26.3%)	<i>Cryptococcus</i> (30.6%)
<i>Cladosporium</i> (18.3%)	<i>Geomyces</i> (14.1%)	<i>Epicoccum</i> (13.9%)
<i>Geomyces</i> (17%)	<i>Cryptococcus</i> (11.1%)	<i>Cladosporium</i> (11.1%)
<i>Cryptococcus</i> (8.5%)	<i>Epicoccum</i> (6.1%)	<i>Cadophora</i> (8.3%)
<i>Hormonema</i> (5.9%)	<i>Cladosporium</i> (5.1%)	<i>Malassezia</i> (8.3%)

presence of these fungi in areas away from high human impact and on material not introduced by humans as well as the high ITS region genetic diversity of Antarctic specimens, [three named species and three unnamed (Blanchette et al., 2002)] suggest these are native saprophytes. Its prevalence in areas of higher human activity and on introduced substrates such as wood, straw, and carbon enriched oil in soils from spills, indicates it has a high degree of saprophytic aggressiveness and colonizes new nutrient sources more rapidly than other soil saprotrophs.

Dry Valley and Ross Island soils are highly mineral in composition. Ross Island soils are largely composed of black volcanic scoria and have relatively higher amounts of organic matter deposition, especially in areas near penguin rookeries and skua nests, in the form of guano and feathers (Cowan and Ah Tow, 2004). These maritime ornithogenic soils have been reported to be sites of high microbiological activity, especially from bacteria (Tatur, 2002). The hut at Cape Royds is located very close to a large active Adelie

penguin rookery and the soils near the hut are influenced by direct ornithogenic inputs as well as wind dispersed inputs. Feathers are rich in keratin and have been suggested as possible substrates for keratinophilic fungi such as *G. pannorum* (Marshall, 1998). From our results, *Geomyces* spp. apparently have the ability to colonize and utilize other carbon sources since they were also found in samples of wood, straw, fur, biscuits, flour, and paper. At present, little is known about the ability of *Geomyces* to cause decay of wood or other organic materials. Its widespread occurrence, however, strongly suggests that it has a role in decomposition and nutrient cycling in Antarctica.

Dry Valley soils have reduced microbial diversity apparently due to low moisture availability and organic inputs as well as other harsh environmental stresses at the location (Cowan and Ah Tow, 2004). A previously published report indicated soil moisture at the McMurdo Sound coastal area, which encompasses our Ross Sea area sampling locations, averaged 5% whereas soils of inland Dry Valley sites averaged closer to 1% (Campbell et al., 1997). Areas of localized moisture can occur near sporadic meltwater streams. Previous investigators have found the fungal diversity of Dry Valley soils to have a higher abundance of yeasts (Vishniac, 1996). Our findings corroborate this with an equal number of identifications of yeasts and filamentous fungi in the Dry Valley and Transantarctic Mountain samples as compared to the filamentous fungi dominated soils from the Ross Sea area. It should also be noted that in the Dry Valley samples the only fungi detected by culturing methods were yeasts such as *Cryptococcus antarcticus*, *C. friedmannii*, *C. vishniacii*, and *Candida parapsilosis*. The use of DGGE detected six

additional species of yeasts and eight species of filamentous fungi not found using traditional culturing methods. This discrepancy may be due to either the more sensitive nature of DGGE to detect fungi in low amounts or the DNA was from non-viable propagules present in the samples. The study presented here shows how DGGE and traditional culturing can be used together to provide more accurate information on fungal diversity in Antarctica.

The only taxon that was isolated with significant frequency (nine times) from wood or other artifact samples but not found in soil samples was *H. dematioides*. However, this species has been previously reported in Antarctic aerial samples (Chalmers et al., 1996) and in soil samples (Baublis et al., 1991; Kerry, 1990) and is more commonly referred to by its synonym *Aureobasidium pullulans*. There were a number of taxa isolated only once or twice from historic wood and artifacts but not from soils. Many of these taxa had best BLAST matches to species that have not been previously reported from Antarctica: *Ascobolus denudatus*, *Exophiala spinifera*, *Monodictys castaneae*, *Paecilomyces inflatus*, *Pseudeurotium desertorum*, *Sarea difformis*, *C. tephrensis*, and *Sporobolomyces symmetricus*. At present there can only be speculation as to their possible indigenous nature, but it is likely that at least some of these species were introduced by humans in the last century. This is also true for taxa identified infrequently in soil samples: *A. stercorarius*, *Leptosphaerulina trifolii*, *Microdochium bolleyi*, *Ulocladium chartarum*, *C. parapsilosis*, *Bulleromyces albus*, *C. foliicola*, and *C. hungaricus*.

Determining which fungi may be indigenous to Antarctica is difficult and previous investigators have considered criteria of whether the organism is actively growing and metabolizing or simply existing in a dormant state (often as spores). Vishniac (1996) proposed that to establish a fungal species as an Antarctic indigene one must show “visible growth in situ” or “unique occurrence (i.e. new species)”. While the artificial nature of the huts in this environment must be acknowledged, the findings of this study provide more evidence that fungi in Antarctic soils are able to colonize substrates introduced by humans. The abundance and broad distribution of the fungi found, especially *Cadophora* and *Geomyces* species, point towards their likely indigenous nature and important role in nutrient cycling in Antarctica.

Species of fungi that appear to be not only indigenous to Antarctica, but also endemic (not found outside Antarctica) were identified. *Antarctomyces psychrotrophicus* was first identified and described by Stchigel et al. (2001) from King George Island in the South Shetland Islands. *C. victoricae* from Southern Victoria Land (Montes et al., 1999) and *C. vishniacii* (Vishniac and Hempfling, 1979) from Ross Desert soils also had good BLAST matches with two taxa reported in this study. The three unnamed species of *Cadophora* (Blanchette et al., 2004b) as well as the four unclassified types should also be evaluated as possible endemic Antarctic species. This is in contrast to *C.*

cladosporioides which like other *Cladosporium* spp. is cosmopolitan and has a high abundance in air samples in many areas of the world (Marshall, 1997).

This work has provided a more complete knowledge of fungi associated with the historic woods and artifacts on Ross Island as well as in soils of the Ross Sea Region. New evidence for the possible indigenous nature of certain fungal species has been presented. Eleven taxa have also been identified that have less than 95% identity on best BLAST matches, indicating that their phylogenetic relationships are not clear and these species are possibly different from those previously reported. The four unclassified types that had best BLAST matches of less than 50% identity indicate there are large genetic differences reflecting they may possibly belong to new groups of fungi that are undescribed or not represented in GenBank. More research needs to be done on the phylogenetic relationships of these taxa, their presence in other locations of Antarctica and what their ecological roles may be. The results of this research also emphasize the importance of using molecular methods of detection in addition to traditional culturing methods in surveys of biodiversity to obtain a more precise analysis of the fungi present.

Acknowledgements

The authors would like to thank Shona Duncan and Joanne Thwaites of the University of Waikato, New Zealand for assistance in field work and Professor Diana Wall of Colorado State University for providing soil samples from the Lake Fryxell Basin and for organizing this special issue of Soil Biology and Biochemistry. We also thank Dr. Jason Smith Department of Plant Pathology, University of Minnesota for his help with DGGE methodology and manuscript review. We would also like to thank Nigel Watson and the Antarctic Heritage Trust for their cooperation and support, the personnel at Scott Base and McMurdo Station for their assistance in carrying out this research in Antarctica and Antarctica New Zealand for their support. This research is based upon work supported by the National Science Foundation. This project serves as partial fulfillment of the requirements for Master of Science degree at the University of Minnesota.

References

- Aislabie, J., Fraser, R., Duncan, S., Farrell, R.L., 2001. Effects of oil spills on microbial heterotrophs in Antarctic soils. *Polar Biology* 24, 308–313.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410.
- Anderson, I.C., Campbell, C.D., Prosser, J.I., 2003. Diversity of fungi in organic soils under a moorland—Scots pine (*Pinus sylvestris* L.) gradient. *Environmental Microbiology* 5, 1121–1132.
- Baublis, J.A., Wharton Jr, R.A., Volz, P.A., 1991. Diversity of micro-fungi in an Antarctic dry valley. *Journal of Basic Microbiology* 31, 3–12.

- Blanchette, R.A., Held, B.W., Farrell, R.L., 2002. Defibrination of wood in the expedition huts of Antarctica: and unusual deterioration process occurring in the polar environment. *Polar Record* 38, 313–322.
- Blanchette, R.A., Held, B.W., Jurgens, J.A., Aislabie, J., Duncan, S., Farrell, R.L., 2004a. Environmental pollutants from the Scott and Shackleton expeditions during the 'Heroic Age' of Antarctic exploration. *Polar Record* 40 (213), 143–151.
- Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., Farrell, R.L., 2004b. Wood destroying soft-rot fungi in the historic expedition huts of Antarctica. *Applied and Environmental Microbiology* 70, 1328–1335.
- Campbell, R.B., Claridge, G.G.C., Balks, M.R., Campbell, D.I., 1997. Moisture content in soils of the McMurdo Sound and the Dry Valley region of Antarctica. In: Lyons, W.B., Howard-Williams, C., Hawes, I. (Eds.), *Ecosystem process in Antarctic ice-free landscapes*. Proceedings of an international workshop on polar desert ecosystems. Christchurch, New Zealand. A.A. Balkema, Rotterdam, pp. 61–77.
- Chalmers, M.O., Harper, M.A., Marshall, W.A., 1996. An Illustrated Catalogue of Airborne Microbiota from the Maritime Antarctic. British Antarctic Survey, Cambridge 175pp.
- Cowan, D.A., Ah Tow, L., 2004. Endangered Antarctic environments. *Annual Review of Microbiology* 58, 649–690.
- Del Frate, G., Caretta, G., 1990. Fungi isolated from Antarctic material. *Polar Biology* 11, 1–7.
- Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113–118.
- Greenfield, L., 1981. Soil microbiological studies. In: Greenfield, L., Wilson, G. (Eds.), *Antarctic Expedition No. 19*. University of Canterbury, Christchurch, New Zealand, pp. 4–22.
- Harrington, T.C., McNew, D.L., 2003. Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. *Mycotaxon* 87, 141–151.
- Held, B.W., Blanchette, R.A., Jurgens, J.A., Duncan, S., Farrell, R.L., 2003. Deterioration and conservation issues associated with Antarctica's historic huts. In: Koestler, R.J., Koestler, V.R., Charloa, A.E., Nieto-Fernandez, F.E. (Eds.), *Art, Biology, and Conservation: Biodeterioration of Works of Art*. The Metropolitan Museum of Art, New York and Yale University Press, New Haven, pp. 370–389.
- Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., Blanchette, R.A., 2005. Environmental factors influencing microbial growth inside the historic huts of Ross Island, Antarctica. *International Biodeterioration & Biodegradation* 55, 45–53.
- Kerry, E., 1990. Microorganisms colonizing plants and soil subjected to different degrees of human activity, including petroleum contamination, in the Vestfold Hills and MacRobertson Land, Antarctica. *Polar Biology* 10, 423–430.
- Landeweert, R., Leeflang, P., Kuyper, T.W., Hoffland, E., Rosling, A., Wernars, K., Smit, E., 2003. Molecular identification of ectomycorrhizal mycelium in soil. *Applied and Environmental Microbiology* 69, 327–333.
- Line, M.A., 1988. Microbial flora of some soils of Mawson Base and the Vestfold Hills, Antarctica. *Polar Biology* 8, 421–427.
- Marshall, W.A., 1997. Seasonality in Antarctic airborne fungal spores. *Applied and Environmental Microbiology* 63, 2240–2245.
- Marshall, W.A., 1998. Aerial transport of keratinaceous substrate and distribution of the fungus *Geomyces pannorum* in Antarctic soils. *Microbial Ecology* 36, 212–219.
- Montes, M.J., Belloch, C., Galiana, M., Garcia, M.D., Andrés, C., Ferrer, S., Torres-Rodriguez, J.M., Guinea, J., 1999. Polyphasic taxonomy of a novel yeast isolated from antarctic environment; description of *Cryptococcus victoriae* sp. nov. *Systematic and Applied Microbiology* 22, 97–105.
- Stchigel, A.M., Cano, J., MacCormack, W., Guarro, J., 2001. *Antarctomyces psychrotrophicus* gen. et sp. nov., a new ascomycete from Antarctica. *Mycological Research* 105, 377–382.
- Tatur, A., 2002. Ornithogenic ecosystems in the maritime Antarctic formation, development and disintegration. In: Beyer, L., Bölter, M. (Eds.), *Geocology of Antarctic Ice-free Coastal Landscapes*. Springer, Berlin, pp. 161–184.
- Tosi, S., Casado, B., Gerdol, R., Caretta, G., 2002. Fungi isolated from Antarctic mosses. *Polar Biology* 25, 262–268.
- Vishniac, H.S., 1996. Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems. *Biodiversity and Conservation* 5, 1365–1378.
- Vishniac, H.S., Hempfling, W.P., 1979. *Cryptococcus vishniacii* sp. nov., an Antarctic yeast. *International Journal of Systematic Bacteriology* 29, 153–158.
- Worrall, J., 1999. Media for selective isolation of *Hymenomyces*. *Mycologia* 83, 296–302.