Environmental Microbiology (2006) **8**(7), 1212–1219 doi:10.1111/j.1462-2920.2006.01013.x

Endoglucanase-producing fungi isolated from Cape Evans historic expedition hut on Ross Island, Antarctica

Shona M. Duncan,[1] Roberta L. Farrell,[1]* Joanne M. Thwaites,[1] Benjamin W. Held,[2] Brett E. Arenz,[2] Joel A. Jurgens[2] and Robert A. Blanchette[2]

[1]*Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand.*

[2]*Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA.*

Summary

Early explorers of Antarctica's Heroic Era erected wooden buildings and brought large quantities of supplies to survive in Antarctica. The introduction of wood and other organic materials provided nutrient sources for fungi that were indigenous to Antarctica or were brought in with the materials and adapted to the harsh conditions. Seventy-two isolates of filamentous fungi were cultured on selective media from interior structural wood of the Cape Evans historic hut and 27 of these screened positive for the ability to degrade carboxymethyl cellulose (CMC). Four non-CMC-degrading isolates were added to a group of 14 CMC-degrading isolates for further study, and endo-1, 4-β-glucanase activity was demonstrated in the extracellular supernatant from all of these 18 isolates when grown at 4°C, and also when they were grown at 15°C. Isolates of *Penicillium roquefortii* and *Cadophora malorum* showed preference for growth at 15°C rather than 25°C or 4°C indicating psychrotrophic characteristics. These results demonstrate that cellulolytic filamentous fungi found in Antarctica are capable of growth at cold temperatures and possess the ability to produce extracellular endo-1, 4-β-glucanase when cultured at cold and temperate temperatures.

Introduction

In 1911, the *Terra Nova* hut was erected by the Robert F. Scott-led British Antarctic Expedition at Cape Evans on Ross Island, Antarctica. The wooden hut was prefabricated in England and was used in Antarctica to store supplies and house the expedition for several years during exploration of the region. When the expedition members left the continent, the hut and supplies were abandoned. Following Scott's Expedition, members of Shackleton's Ross Sea Party from the Imperial Trans-Antarctic Expedition 1914–1917 also occupied the hut through two winters. After this time, it was abandoned until the late 1950s when it has been visited periodically, increasingly by tourists in the past 5 years.

Many fungi isolated from Antarctica have been reported to be endemic or indigenous while others have been introduced (Vishniac, 1996). Much of the previous work on fungi found in association with the historic huts focused on the long-term survival of organisms in the food supplies and horse-associated materials (Meyer *et al.*, 1962; 1963; Nedwell *et al.*, 1994). Recent investigations of biological and non-biological causes of deterioration in the historic hut at Cape Evans produced evidence of decay fungi associated with exterior wood in contact with the ground, including several previously undescribed *Cadophora* species, suggesting at least some of these may be endemic species to Antarctica (Blanchette *et al.*, 2004). Along with providing a nutrient source for the fungi, the hut creates a microenvironment with conditions suitable for fungal growth during the austral summer; however, the fungi still have to survive and proliferate in the hut at average temperatures of –14.7°C, and maximum and minimum temperatures of 9.4°C and –35.1°C respectively (Held *et al.*, 2005).

Many microorganisms are known to degrade cellulose, a linear polymer of β-linked glucosyl units; the enzymes responsible for hydrolysis of cellulose are extracellular and collectively known as cellulases. Endo-1, 4-β-glucanase (EC 3.2.1.4) is a cellulase catalysing the hydrolysis of cellulose randomly by hydrolysis of the β $(1 \rightarrow 4)$-glucosidic linkage.

A psychrophile is defined as an organism capable of growth at or below 0°C but unable to grow above 20°C, whereas a psychrotolerant (also termed psychrotrophic) organism is capable of growth at around 0°C and can also grow above 20°C (Cavicchioli *et al.*, 2002). The search for psychrophilic filamentous fungi in Antarctica has so far been unsuccessful. Psychrotolerant strains of filamentous mesophilic fungi adapted to grow at temperatures as low as 1°C have been found (Kerry, 1990a; Abyzoz, 1993;

Received 2 November, 2005; accepted 30 January, 2006. *For correspondence. E-mail r.farrell@waikato.ac.nz; Tel. (+64) 7 8384704; Fax (+64) 7 8384976.

Table 1. Fungi from the Cape Evans hut demonstrating clearing of carboxymethyl cellulose, Index of Relative Enzyme activity determined for cultures grown at isolation temperature and at 4°C.

Isolate No.	Isolation temperature (°C)	Index of relative enzyme activity[a]	
		Determined for isolates cultured at isolation temperature	Determined for isolates cultured at 4°C
98	25	1.34	NG
101	25	1.0	NG
107	25	1.62	0.53
124	25	1.17	NG
235	15	2.33	1.14
236	15	2.85	0
242	15	1.28	1.12
262	4	1.10	1.10
489	15	2.0	0
492	15	1.1	0.67
517	15	1.0	1.33
536	15	1.12	0
638	25	1.6	0.53
654	15	2.0	0.93
655	15	1.8	1.33
660	15	3.2	1.0
667	15	1.8	3.0
693	15	2.42	0.75
711	4	1.0	1.0
719	4	2.75	2.75
749	4	1.42	1.42
750	4	1.42	1.42
779	4	3.0	3.0
814	4	1.27	1.27
821	4	1.33	1.33
1029	25	1.0	2.5
1222	15	1.37	NG

a. Index of relative enzyme activity compares the width of the clearing zone of carboxymethylcellulose with the width of fungal growth.
NG, no growth of the fungal isolate at 4°C.

Azmi and Seppelt, 1997) as well as psychrophilic yeasts (DiMenna, 1960). Psychrophilic fungi, including pink snow mould *Microdochium nivale* (syn *Fusarium nivale*) (Hoshino *et al*., 1996), *Typhula ishikariensis* (Hoshino *et al*., 1998) and various low temperature basidiomycetes (Inglis *et al*., 2000), have been isolated from various parts of the world. The research findings of the present study demonstrate isolation of indigenous Antarctic psychrotolerant filamentous fungi, identified to four genera and seven taxa. Additionally, fungal isolates were shown to produce endo-1, 4-β-glucanase when cultured at 4°C, as well as at mesophilic temperatures of 15°C and 25°C.

Results

Isolation of fungi

Seventy-two filamentous fungi were isolated from swabs, wall scrapings or small slivers of wood taken from 15 sites around the interior of the Cape Evans hut, the samples were from floor, walls, ceiling, a shelf and wall boards, the latter removed from the historic hut by conservators and stored in a container at Scott Base. Of these 72 filamentous fungi, 27 isolates were from plates incubated at 4°C, 29 from plates incubated at 15°C and 16 from plates incubated at 25°C. The number of fungi isolated on each selective media were as follows: 24 isolates on YM agar; 18 isolates on Media 4 (for streptomycin-resistant fungi; Harrington, 1981); 18 isolates on Vogel Bonner (VB) agar (a minimal medium for selection of slow-growing fungi; Vogel and Bonner, 1956); 9 isolates on Media 6 (for cycloheximide-resistant fungi; Harrington, 1981); and 2 isolates on Media 7 (preference for basidiomycetous fungi).

Screening for cellulytic activity

All of the 72 Antarctic fungal isolates were screened for cellulytic activity by using the carboxymethyl cellulose (CMC) Congo red plate technique. Twenty-seven isolates, including eight initially isolated at 4°C, demonstrated clearing of CMC with an Index of Relative Enzyme Activity (Bradner *et al*., 1999a) of 1 or greater. Results are shown in Table 1 of activity at isolation temperature, and if the isolate was isolated at 15°C or 25°C, also activity when the isolate was cultured at 4°C.

Of the 72 original Antarctic fungal isolates, 18 fungal isolates were chosen for further study, and identified by morphology and molecular characterization to belong to four genera and seven taxa (Table 2). The 18 isolates were comprised as follows: 9 of the 27 that cleared CMC, 5 of the 72 that showed clearing of CMC at 4°C but not at their isolation temperature and 4 *Penicillium* sp. that

Table 2. Identification of fungi from the Cape Evans hut, sample location, isolation temperature and isolation media.

Isolate No.	Identity of fungus	Isolation temperature (°C)	Isolation media
80	*Cadophora malorum*	25	VB
182	*Cadophora malorum*	15	VB
242	*Cadophora malorum*	15	YM
405	*Penicillium roquefortii*	15	YM
408	*Penicillium roquefortii*	15	4
487	*Cladosporium cladosporioides*	15	4
517	*Cadophora malorum*	15	YM
537	*Penicillium expansum*	15	4
656	*Geomyces* sp.	15	6
660	*Cladosporium cladosporioides*	15	6
667	*Cladosporium* sp.	15	VB
668	*Cadophora malorum*	15	VB
711	*Geomyces* sp.	4	6
719	*Cladosporium cladosporioides*	4	VB
723	*Penicillium* sp.	4	VB
749	*Geomyces* sp.	4	VB
814	*Cladosporium* sp.	4	YM
1029	*Penicillium expansum*	25	4

Media: YM, YM agar; 4, Media 4; 6, Media 6; VB, Vogel Bonner medium.

showed no clearing of CMC at their isolation temperature or at 4°C. The isolation temperatures of the 18 selected isolates chosen were 4°C (5 isolates), 15°C (11 isolates) and 25°C (2 isolates). Five isolates came from YM agar, three from Media 4, four from Media 6 and six from VB agar. Table 2 shows identity, isolation temperature and isolation media of the 18 identified fungal isolates.

Quantifying amounts of accumulated Endo-1, 4-β-glucanase at different temperatures

All of the *Cadophora*, *Cladosporium*, *Geomyces* and one of the *Penicillium* isolates were demonstrated to produce endo-1, 4-β-glucanase (endoglucanase) activity. Figure 1 shows the levels of accumulated endoglucanase activity, expressed as units (micromoles glucose released per minute per mg of protein in the supernatant) in the extracellular supernatant when fungal isolates were cultured at either 4°C or 15°C (initial growth experiments showed that maximal endoglucanase activity was determined on average for the isolates cultured at 4°C after 28 days and cultured at 15°C after 10 days; data not shown). Total protein levels in the supernatant after 28 days versus 10 days in culture were compared between 4°C and 15°C, respectively, and were not statistically different (*P*-value = 0.241). When total fungal biomass at time of harvest (determined when the maximum level of endoglucanase activity was obtained at 15°C and, for the cultures at 4°C, when a similar level of endoglucanase activity to the 15°C cultures was detected) was compared between fungi cultured at 4°C and at 15°C, there was a statistical difference (*P*-value = 0.00). This difference indicates cultures grown at 4°C required 20% more fungal biomass (3.25 mg dry weight) to achieve the same levels of endoglucanase activity as cultures grown at 15°C.

Levels of accumulated endoglucanase activity were measured at 4°C and 15°C, and were not statistically different (*P*-value = 0.190) when total protein levels in the supernatant were used to standardize the levels of accumulated endoglucanase. The levels of accumulated endoglucanase activity at 4°C and at 15°C were statistically different (*P*-value = 0.002) when total fungal biomass, at time of determining accumulated endoglucanase activity, was used to standardize the levels of accumulated endoglucanase.

As shown in Fig. 1, of the 18 fungi tested, eight produced more endoglucanase activity at 4°C than at 15°C, one produced endoglucanase activity at 4°C and not at 15°C, and one did not produce endoglucanase activity at 4°C. From the levels of accumulated endoglucanase activity of the *Cadophora malorum* isolates, 80 and 668 produced more endoglucanase activity at 15°C than at 4°C, and of the *Cladosporium* isolates, 660, 667 and 719 produced more endoglucanase activity at 4°C than at 15°C.

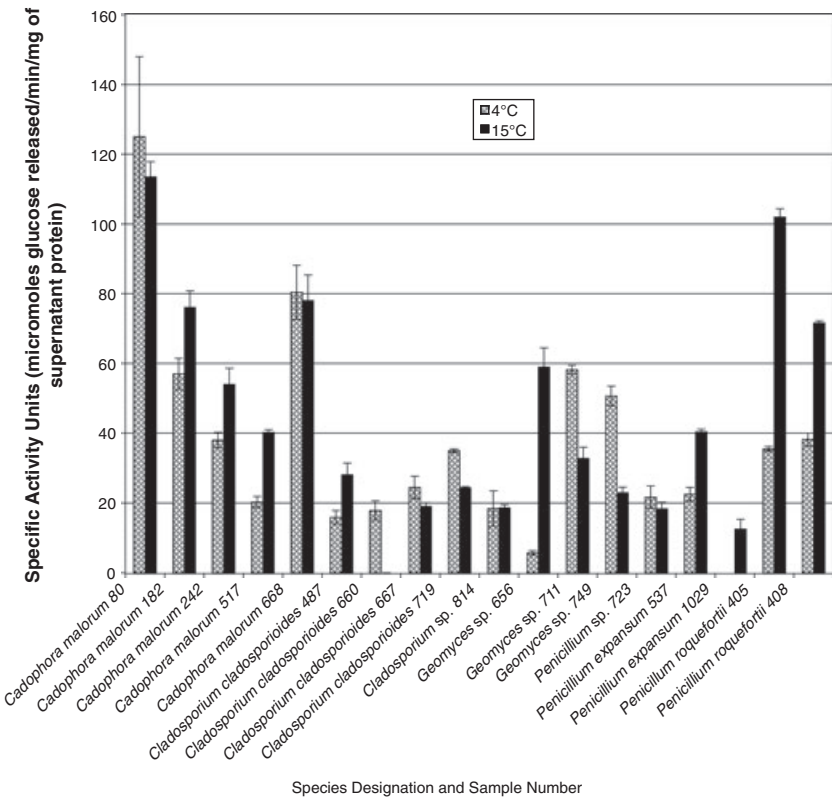

Fig. 1. Graph of specific activity units of cellulase (micromoles glucose released per minute per mg of supernatant protein) for the selected 18 fungi at 4°C and 15°C.

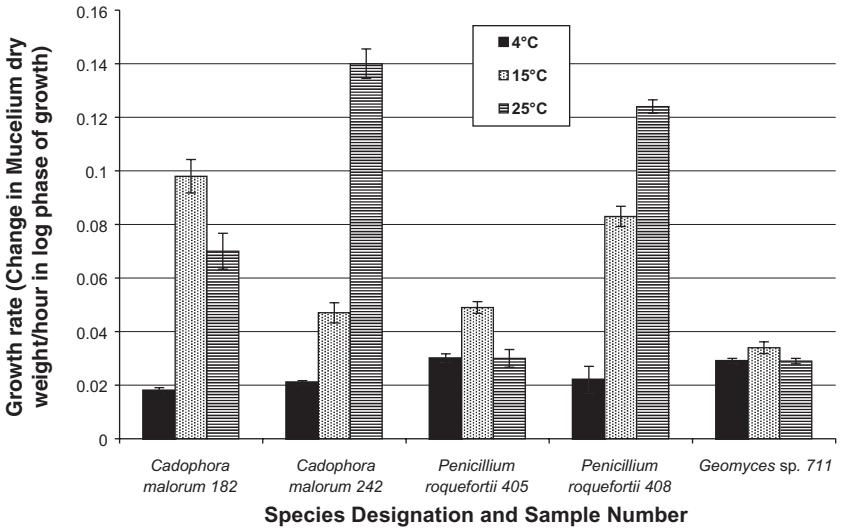

Fig. 2. Graph of growth rate of the five selected fungi at 4°C, 15°C and 25°C.

Penicillium isolate 723 had more endoglucanase activity at 4°C than at 15°C but *Penicillium* isolates 405, 408, 537 and 1029 produced more endoglucanase activity at 15°C than at 4°C. *Geomyces* isolates 711 and 749 produced more endoglucanase activity at 4°C than at 15°C.

Growth temperature characteristics

Five of the 18 fungal isolates were selected for temperature growth optima characterization. Figure 2 shows the growth rates of these isolates as measured at three temperatures. All five species showed the ability to grow at 4°C. *Penicillium roquefortii* 405 and *C. malorum* 182 had higher growth rates, a larger fungal biomass accumulation per hour in the log phase of growth, at 15°C than at 4°C or 25°C. Another *P. roquefortii* isolate, 408, and *C. malorum* 242 had a higher growth rate at 25°C than at 15°C or 4°C. *Geomyces* sp. isolate 711 had similar rates of growth at all three temperatures.

Discussion

The historic huts have provided a unique environment and metabolic substrates for Antarctic fungi in this otherwise pristine region. Although we can not be sure of their origin, the 72 filamentous fungal isolates described in this study adapted to their ecological niche in order to survive, as was demonstrated by the ability to culture them. Their origins have the potential to be from a very diverse range, including fungi endemic or indigenous to Antarctica or fungi introduced by human activity. Human introductions may have taken place a number of different ways including by the historic era explorers on their supplies or animals brought with them from the Northern Hemisphere, or acquired during their stops in Southern Hemisphere ports, or by the many scientists and tourists who have visited the huts since the late 1950s. Vincent (2000) has hypothesized that increased human disturbance leads to larger microbial speciation. Human impacts have led to selection of certain fungal species which either were previously already there, and are able to utilize the new nutrient sources introduced by humans to a greater extent than others, or that new species have been brought in with the humans and materials, leading to a diversity profile that is different from adjacent pristine environments. The findings of the work described the capability of fungi isolated from the Antarctic historic huts to produce endoglucanase in culture, and specifically when cultured at cold temperatures including 4°C. The fungi that were growing on the interior structural woods of the historic hut at Cape Evans were capable of cellulose breakdown activity at 4°C and 15°C, thus these enzymes are functional in the Antarctic ecosystem and over time, these organisms undoubtedly will have significant impact on the wood of the hut structure and artefacts. It is likely that both indigenous and introduced fungi were isolated from the interior structural woods of the historic hut at Cape Evans, Ross Island, Antarctica, and their growth optima demonstrated that the fungi not only survive in the Antarctica environment but are capable of proliferating. Therefore, we feel these findings support the Vincent hypothesis and that larger microbial speciation caused by adaptation has been demonstrated in the historic hut. Fungi that were endemic to Antarctica would not have previously encountered wood as a substrate and have adapted to survive on it and use it as a source of carbon and energy, as they produce the extracellular enzyme activity required to degrade wood; 'hitchhiking' fungi on the wood timbers would have had to survive and adapt to the harsh and cold Antarctic environment.

From studies carried out on pristine soils of Antarctica, very few fungal species are present but *Cadophora*, *Penicillium*, *Geomyces* and others have been found (Kerry, 1990b). Therefore, all of the fungal species isolated in this study have been previously reported in Antarctica but previously they were not characterized biochemically as being functional in the ecosystem from which they were isolated. *Cadophora malorum* (syn *Phialophora malorum*; Harrington and McNew, 2003) was isolated from seal-influenced soil samples from Peterson Island (one of the Windmill Islands) (Azmi and Seppelt, 1997) and on moss (Tosi *et al*., 2002). *Cadophora* spp. were isolated from wood in contact with the ground in the historic expedition huts of Ross Island as well as at New Harbor which is across the Ross Sea from Ross Island (Blanchette *et al*., 2004). *Penicillium expansum* and *P. roquefortii* were noted from Antarctic air samples (Corte and Daglio, 1962). There were reports of *Cladosporium cladosporioides* from many locations around Antarctica but specifically only on human and animal foodstuffs in *Discovery* Hut, Ross Island (Minasaki *et al*., 2001). *Geomyces* spp. have been isolated from areas with both little biotic influence and seal-influenced soil samples from Peterson Island, of the Windmill Islands (Azmi and Seppelt, 1997).

None of the fungi isolated in this study could be defined as psychrophilic. All species grew at 4°C but also to varying levels at 25°C. During austral summers, relative humidity and temperature in the Cape Evans hut provide a unique microenvironment with adequate conditions for fungal growth according to Held and colleagues (2005) but the average temperature for a 4-week period in the summer is only 3.7°C, hence the ecological relevance of our studies conducted at 4°C.

Penicillium roquefortii isolate 405 and *C. malorum* isolate 182 both showed higher growth rates at 15°C than at 25°C and *Geomyces* sp. isolate 711, which grew at a similar rate at all three temperatures, should be classified as psychrotrophic. *Penicillium roquefortii* isolate 408 and *C. malorum* isolate 242 both showed higher growth rates at 25°C than at 15°C and 4°C and should be classified as cold-tolerant mesophiles.

Although cellulase activity has been reported in temperate isolates of *Cladosporium* (Abrha and Gashe, 1992), *C. malorum* (Berg, 1978) and many species of *Penicillium* (Jorgensen *et al*., 2002), there have been no reports of *Geomyces* sp. producing cellulase. Additionally, there is no information published on cellulase activity of any of these organisms at psychrophilic temperatures. Of the 18 fungi identified from this Antarctic historic hut, all produced detectable levels of endoglucanase activity, at either 4°C or at 15°C and 14 showed cellulytic activity (using CMC as the cellulose source) at their isolation temperatures of 4°C, 15°C or 25°C (data not shown). Sixteen fungi produced endoglucanase at both 4°C and 15°C. Within species there were variations in levels of accumulated endoglucanase activity with some isolates producing more endoglucanase at 4°C than at 15°C while others were the opposite; therefore, no direct correlation between activity:temperature and growth:temperature relationship could be assigned. Statistical analysis showed that more biomass was required in a 4°C culture to produce the same amount of endoglucanase activity as a 15°C culture; we have yet no explanation for this but suggest it may be a result of cold adaptation, including a lesser efficiency of growth at 4°C, perhaps as a result of stress, or different extracellular enzymes produced between the two temperatures.

Experimental procedures

Sample collection

Small samples of structural wood, swab samples, or scrapings of wood surfaces were taken from the *Terra Nova* historic hut (78°38′10″S, 116°25′04″E) at Cape Evans, Ross Island, Antarctica, in January 1999 and December 1999. Three samples were taken from historic hut wood that were removed from the hut by conservators before 1998 and stored in a locked, unheated shipping container at Scott Base, Antarctica. Minute segments of structural wood were aseptically collected from inconspicuous locations throughout the hut. Swab samples were taken from the hut by wiping a sterile, autoclaved distilled water saturated cotton swab over the surface of the wood or by taking scrapings from surfaces with visual fungal growth. All samples were taken under the Ministry of Agriculture and Fishery Permit No. 1999006429 and 2000010576. Samples were placed in sterile vials and kept cold while in Antarctica and on return to New Zealand. Samples were then stored under sterile conditions at 4°C until isolations were made.

Isolation of fungi

The wood samples were surface sterilized by soaking for 1 min in a 5% hypochlorite solution, followed by two rinses in sterile, distilled water, then sliced and cultured on a variety of enriched and semi-selective media prepared as agar plates for isolating fungi. The different media included: YM agar (yeast extract 0.2%, malt extract 1.5%, agar 1.8%); Media 4 (yeast extract 0.2%, malt extract 1.5%, agar 1.8%, chloramphenicol 0.2 g l^{-1}, streptomycin sulfate 0.1 g l^{-1}) for isolation of streptomycin-resistant fungi (Harrington, 1981); Media 6 (yeast extract 0.2%, malt extract 1.5%, agar 1.8%, chloramphenicol 0.2 g l^{-1}, streptomycin sulfate 0.1 g l^{-1}, cycloheximide 0.4 g l^{-1}) for isolation of cycloheximide-resistant fungi (Harrington, 1981); Media 7 (yeast extract 0.2%, malt extract 1.5%, agar 1.8%, chloramphenicol 0.2 g l^{-1}, benlate 0.06 g l^{-1}, streptomycin sulfate 0.1 g l^{-1}, lactic acid 2 ml) for selection of basidiomycetous fungi; and VB medium (glucose 25%, agar 2.0%, 20 ml of VB concentrate containing 670 ml of distilled water, K_2HPO_4·anhydrous 50%, $NaNH_4PO_4 \cdot 4H_2O$ 17.5%, citric acid·H_2O 10%, $MGSO_4 \cdot 7H_2O$ 1%) a minimal medium for the selection of slow-growing fungi

(Vogel and Banner, 1956). Fungal isolations were accomplished by wiping swab samples over the surface of the media or by aseptically placing wood scraping samples onto the culturing media mentioned above. The plates were then incubated at 4°C, 15°C or 25°C for up to 6 weeks. Organisms growing on the agar plates were transferred by subculturing from hyphal tips, colonies or spores to new agar plates. Fungi were identified on the basis of morphological and physiological characteristics into putative species using classical taxonomic morphological features (Barrnett and Hunter, 1972; Sun *et al*., 1978).

Molecular characterizations, particularly DNA sequence analyses of the two internal transcribed spacer (ITS) regions of ribosomal DNA, ITS1 and ITS2, were used to confirm identity. Fungal material was scraped from pure cultures and DNA extracted using Qiagen DNeasy plant mini-kits, following manufacturer's instructions (Qiagen Sciences, Germantown, MA). The rDNA ITS regions 1, 5.8S, and ITS region 2 were amplified using primers ITS1 and ITS4 (Gardes and Bruns, 1993). Polymerase chain reaction amplification was performed in a MJ Research PTC Mini-cycler (Watertown, MA), with the following protocol: 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.

Sequencing reactions were performed at the Advanced Genetic Analysis Centre (AGAC) at the University of Minnesota. Separate sequences were run with both the ITS1 and ITS4 primers, and combined to form a consensus sequence. This sequence was compared with those in GenBank using BLASTN to find the best match.

Growth characteristics of fungi

Isolates of each fungus were grown independently in three 250 ml flasks containing 50 ml of YM broth (yeast extract 0.2%, malt extract 1.5%) (Farrell *et al*., 1998; Schirp *et al*., 2003). At various time intervals, the dry weight of fungal biomass was determined by removing mycelia through filtration and drying at 65°C for 3 days. For fungal growth incubations at 4°C, mycelial dry weight was determined every 3 days for 31 days; for 25°C and 15°C this was performed every day for 10 days. The growth rate was determined by calculating the change in mycelium dry weight per hour during log phase of growth.

Detection and analysis of cellulytic activity

Fungi were screened for cellulase activity using an agarose plate technique as follows: plates consisted of *Trichoderma viride* medium A [14 ml of $(NH_4)_2SO_4$ 10%, 15 ml of KH_2PO_4 1 M, 6 ml of urea 35%, 3 ml of $CaCl_2$ 10%, 3 ml of $MgSO_4{\cdot}7H_2O$ 10%, 1 ml of Trace elements solution (10 ml of concentrated HCl, $FeSO_4$ 0.51%, $MnSO_4{\cdot}4H_2O$ 0.186%, $ZnCl_2$ 0.166%, $CoCl_2$ 0.2%), 2 ml of Tween 80, carboxymethylcellulose 0.2%, agarose 1.5%)] (Mandels *et al*., 1962). Single isolates of fungi were inoculated in a line down the middle of the cellulose/agarose plate and incubated at isolation temperature, 4°C, 15°C or 25°C, the fungi isolated at 15°C or 25°C were also screened at 4°C. After 2 days at 25°C, 1 week at 15°C or 6 weeks at 4°C the plates were flooded with 0.1% Congo red and allowed to react for 30 min followed by destaining with 1 M NaCl for 60 min according to the method developed by Teather and Wood (1982). The width of fungal growth and the zone of clearing in the cellulose medium were measured. The Index of Relative Enzyme Activity (which compared the width of the clearing zone with the width of fungal growth) determined which fungi were classified as producing cellulase (Bradner *et al*., 1999a).

In order to grow fungi for studying endoglucanase activity, an adaptation of the methods of Bradner and colleagues (1999b) was used. Endoglucanase-producing fungi were grown on YM agar for 1 week at 15°C or 4 weeks at 4°C, then harvested and rinsed with 2 ml of saline solution (0.9% NaCl, 0.01% Tween 80). The cells were added to 50 ml of cellulose broth [Avicel 1%, Soya bean flour 1.5%, K_2HPO_4 1.5% $(NH_4)_2SO_4$ 0.5%, $CaCl_2{\cdot}2H_2O$ 0.006%, $MgSO_4{\cdot}7H_2O$ 0.006%, Tween 80 0.02% (v/v)] in a 250 ml flask. Flasks were shaken at 150 r.p.m. for 10 days at 15°C grown samples and 28 days for the samples grown at 4°C. Growth and enzyme activity were measured from culture supernatant at 4°C and 15°C.

Enzyme assays were used to determined levels of endo-1, 4-β-glucanase activity (Bailey *et al*., 1992). The amount of enzyme was determined after 10 and 28 days culturing at the designated temperature. To determine the endoglucanase activity, the quantities of reagents used were as follows: substrate 480 µl [hydroxyethyl cellulose 1% in 0.05 M citrate buffer pH 4.8 (0.05 M citric acid, 0.05 M sodium citrate, pH adjusted to 4.8 by adding citric acid solution to sodium citrate solution)], enzyme supernatant 320 µl, mixed and incubated for 10 min at 50°C. The reaction was stopped with the addition of 1.2 ml of dinitrosalicylic acid (2-hydroxy-3,5,-dinitrobenzoic acid 1%, NaOH 1.6% (added slowly), Rochelle salts 30% (added in small portions with continuous stirring and filter to remove particulate material) and incubation in a boiling water bath for 5 min. All samples were measured against a blank which was the same volume as the sample but the enzyme supernatant was added at the boiling stage. All assays were performed in triplicate and absorbance was measured at 540 nm. Activity was expressed as micromoles glucose released per min and converted to specific activity by dividing by supernatant total protein. Supernatant total protein levels were determined by the Bradford method using a Bio-Rad Laboratories (Richmond, CA, USA) protein assay kit according to the manufacturer's instructions, using Bovine Serum Albumin as the standard.

Statistical analysis

Statistical analysis was performed using the program MINITAB Version 14 (Minitab, State College, PA). Differences with respect to total protein levels in the cellulose broth supernatant, total fungal biomass at time of determining accumulated endoglucanase activity and levels of accumulated endoglucanase activity at 4°C and 15°C were investigated using a paired *t*-test with confidence interval.

Acknowledgements

We thank David Harrowfield for helpful comments and insights on the historic huts, Nigel Watson and conservators

of the Antarctic Heritage Trust for their support and cooperation during this study, support personnel of Scott Base for their assistance in carrying out this research in Antarctica and Antarctica New Zealand for logistic support. This research is based on work supported by the Vice Chancellor's Fund of The University of Waikato and by the National Science Foundation Grant 0229570. We also sincerely thank Margaret E. DiMenna from AgResearch, Ruakura, Hamilton, for assistance with identifying the fungi by classical taxonomic features and mycological references and Lisa Robson from the University of Waikato for laboratory assistance.

References

Abrha, B., and Gashe, B.A. (1992) Cellulase production and activity in a species of *Cladosporium*. *World J Microbiol Biotechnol* **8:** 164–166.

Abyzoz, S.S. (1993) Microorganisms in the Antarctic Ice. In *Antarctic Microbiology.* Friedman, E.I. (ed.). New York, USA: Wiley-Lis, pp. 265–295.

Azmi, O.R., and Seppelt, R.D. (1997) Fungi of the Windmill Islands, continental Antarctica. Effect of temperature, pH and culture media on the growth of selected microfungi. *Polar Biol* **18:** 128–134.

Bailey, M.J., Biely, P., and Poutanen, K. (1992) Interlaboratory testing of method for assay of xylanase activity. *J Biotechnol* **23:** 257–270.

Barrnett, H.L., and Hunter, B.B. (1972) *Illustrated Genera of Imperfect Fungi*. Minneapolis, MN, USA: Burgess.

Berg, B. (1978) Cellulose degradation and cellulase formation by *Phialophora malorum*. *Arch Microbiol* **118:** 61–65.

Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., and Farrell, R.L. (2004) Wood-destroying soft rot fungi in the historic expedition huts of Antarctica. *Appl Environ Microbiol* **70:** 1328–1335.

Bradner, J.R., Gillings, M., and Nevalainen, K.M.H. (1999a) Qualitative assessment of hydrolytic activities in Antarctic microfungi grown at different temperatures on solid media. *World J Microbiol Biotechnol* **15:** 131–132.

Bradner, J.R., Sidhu, R.K., Gillings, M., and Nevalainen, K.M.H. (1999b) Hemicellulase activity of Antarctic microfungi. *J Appl Microbiol* **87:** 366–370.

Cavicchioli, R.K., Siddiqui, S., Andrews, D., and Sowers, K.R. (2002) Low-temperature extremophiles and their applications. *Curr Opin Biotechnol* **13:** 1–9.

Corte, A., and Daglio, C.A.N. (1962) A mycological study of the Antarctic air. In *Symposium on Arctic Biology.* Carrick, R., Holdgate, M.W., and Prevost J. (eds). Paris, France: Hermann, pp. 115–120.

DiMenna, M.E. (1960) Yeasts from Antarctica. *J Gen Microbiol* **23:** 295–300.

Farrell, R.L., Kay, S., Hadar, E., Hadar, Y., Blanchette, R.A., and Harrington, T.C. (1998) Survey of sapstaining organisms in New Zealand and albino antisapstain fungi. In *Biology and Prevention of Sapstain, No. 7273.* Whistler, Canada: Forest Products Society Publication, pp. 57–62.

Gardes, M., and Bruns, T.D. (1993) ITS primers with enhanced specificity of basidiomycetes: application to the identification of mycorrhizae and rusts. *Mol Ecol* **2:** 113–118.

Harrington, T.C. (1981) Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia* **73:** 1123–1129.

Harrington, T.C., and McNew, D.L. (2003) Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. *Mycotaxon* **87:** 141–151.

Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., and Blanchette, R.A. (2005) Environmental factors influencing microbial growth inside the historic expedition huts of Ross Island, Antarctica. *Int Biodeter Biodegr* **55:** 45–53.

Hoshino, T., Ohgiya, S., Shimanuki, T., and Ishizaki, K. (1996) Production of low temperature active lipase from the pink snow mold *Microdochium nivale* (syn *Fusarium nivale*). *Biotechnol Lett* **18:** 509–510.

Hoshino, T., Tronsmo, A.M., Matsumoto, N., Araki, T., Georges, F., Goda, T., *et al.* (1998) Freezing resistance among isolates of a psychrophilic fungus, *Typhula ishikariensis*, from Norway. *Proceedings NIPR Symposium Polar Biol* **11:** 112–118.

Inglis, G.D., Popp, A.P., Selinger, L.B., Kawchuk, L.M., Gaudet, D.A., and McAllister, T.A. (2000) Production of cellulase and xylanase by low-temperature basidiomycetes. *Can J Microbiol* **46:** 860–865.

Jorgensen, H., Eriksson, T., Borjesson, J., Tjerneld, F., and Olsson, L. (2002) Purification and characterisation of five cellulases and one xylanase from *Penicillium brasilianum* IBT 20888. *Enzyme Microbiol Technol* **32:** 851–861.

Kerry, E. (1990a) Effect of temperature on growth rates of fungi from Subantarctic Macquarie Island and Casey, Antarctica. *Polar Biol* **10:** 293–299.

Kerry, E. (1990b) Microorganisms colonizing plants and soil subjected to different degrees of human activity, including petroleum contamination, in the Vestfold Hills and MacRobertson Land, Antarctica. *Polar Biol* **10:** 423–430.

Mandels, M.L., Parrish, F.W., and Reese, E.T. (1962) Sophorose as an inducer of cellulose in *Trichoderma viride*. *J Bacteriol* **83:** 400–408.

Meyer, G.H., Morrow, M.B., and Wyss, O. (1962) Viable micro-organisms in a fifty-year old yeast preparation in Antarctica. *Nature* **196:** 598.

Meyer, G.H., Morrow, M.B., and Wyss, O. (1963) Viable organisms from faeces and foodstuffs from early Antarctic expeditions. *Can J Microbiol* **9:** 163–167.

Minasaki, R., Farrell, R.L., Duncan, S., Held, B.W., Jurgens, J.A., and Blanchette, R.A. (2001) Mycological biodiversity associated with historic huts and artefacts of the heroic period in the Ross Sea region. In *Antarctica Biology in a Global Context SCAR International Biology Symposium*. Amsterdam, the Netherlands: Vrije Universiteit, p. S6P19.

Nedwell, D.B., Russell, N.J., and Cresswell-Maynard, T. (1994) Long-term survival of microorganisms in frozen material from early Antarctic base camps at McMurdo Sound. *Antarct Sci* **6:** 67–68.

Schirp, A., Farrell, R.L., and Kreber, B. (2003) Effects of New Zealand sapstaining fungi on structural integrity of unseasoned radiata pine. *Holz als Rohund Werkstoff* **61:** 369–376.

Sun, S.H., Huppert, M., and Cameron, R.E. (1978) Identification of some fungi from soil and air of Antarctica. *Antarctic Res Serv* **30:** 1–26.

Teather, R.M., and Wood, P.J. (1982) Use of Congo red–polysaccharide interaction in enumeration and characterisation of cellulytic bacteria from the bovine rumen. *Appl Environ Microbiol* **43:** 777–780.

Tosi, S., Casado, B., Gerdol, R., and Caretta, G. (2002) Fungi isolated from Antarctic Mosses. *Polar Biol* **25:** 262–268.

Vincent, W.F. (2000) Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarct Sci* **12:** 374–385.

Vishniac, H.S. (1996) Biodiversity of yeasts and filamentous fungi in terrestrial Antarctic ecosystems. *Biodivers Conserv* **5:** 1365–1378.

Vogel, H.J., and Bonner, D.M. (1956) A convenient growth medium for *E. coli* and some other microorganisms. *J Biol Chem* **218:** 97–106.

