# Fungal biodiversity in extreme environments and wood degradation potential

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by

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**Abstract**

This doctoral thesis reports results from a multidisciplinary investigation of fungi from extreme locations, focusing on one of the driest and thermally broad regions of the world, the Taklimakan Desert, with comparisons to polar region deserts. Additionally, the capability of select fungal isolates to decay lignocellulosic substrates and produce degradative related enzymes at various temperatures was demonstrated.

The Taklimakan Desert is located in the western portion of the People's Republic of China, a region of extremes dominated by both limited precipitation, less than 25 mm of rain annually and tremendous temperature variation. The organisms that inhabit this region are required to function in conditions that preclude most forms of life. Fungi are particularly interesting organisms for consideration of life in extreme environments since they absorb nutrients from their surroundings with diffusion taking place through the cell wall and plasma membrane requiring free water. The regions near the poles are another example of areas with extreme environmental conditions, with the north and south polar regions having similarities and differences to each other and to the Taklimakan Desert. All three regions experiences extreme cold but only the Taklimakan Desert has exceedingly warm temperatures. The Taklimakan Desert is diurnal and the polar regions have long periods of light and dark in summer and winter months, respectively. The annual precipitation in the specific polar sites is between 100-200 mm, 5-20 fold more than the Taklimakan Desert.

From soil, rock and wood collected in the Taklimakan Desert, 194 independent fungal isolates were generated and identified based on extracted DNA and analysis of the internal transcribed spacer (ITS) region of the rDNA. Dominant taxa were from closely related *Thielavia*, *Embellisia* and *Alternaria* genera. Total DNA extracted directly from environmental samples and subjected to molecular fingerprinting identified 51 consensus sequences almost entirely of taxa not represented by culturing, with the dominant taxa in the *Penicillium* and *Colletotrichum* genera.

The sequence data from the Taklimakan Desert cultured fungi were phylogenetically investigated by means of neighbor-joining analysis and compared to fungal sequences derived from various substrates collected at sites in Antarctica and the Arctic with wood as the common substrate from which isolates were obtained among all three locations. Based on comparisons of consensus sequences to the polar fungi and fungal databases, 72 isolates appear to represent novel taxa that may be endemic to the Taklimakan Desert and warrant further investigation.

Selected fungal isolates from the Taklimakan Desert, Arctic and Antarctic research were investigated to determine and compare their ability to degrade two types of lignocellulose substrates, *Pinus resinosa* and *Populus tremuloides*, the latter being a genera of tree identified in some locations of the Taklimakan Desert and the former as a model softwood example. Fungi from all regions were able to degrade these substrates to varying degrees though minimal weight loss was common. These isolates did not produce cellulase or lignin peroxidase concurrent to the temperatures

prevalent in the regions from which they were collected, posing interesting possibilities for their wood degradation pathways.

**Acknowledgements**

I would like to start by acknowledging the committee for this PhD thesis and most importantly my chief supervisor, Professor Roberta Farrell. You have given me the opportunity of a life time and the guidance, support and energy you have shown for this work and science in general is unparalleled. I would also like to thank Professor Steven Pointing and Dr. Chrissen Gemmill, for all of their valuable input into this thesis.

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During the period of this PhD thesis study many people have assisted in a variety of ways and if I failed to mention them, I sincerely apologize.

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All photographs and drawings were made by the author unless specifically noted.

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**List of Abbreviations**

| | |
|---|---|
| 1.5% MEA | Malt extract agar 1.5% |
| 0.5% MEA | Malt extract agar 0.5% |
| BSA | Basidiomycete semi-selective agar |
| BSA | Bovine serum albumin |
| C | Celsius |
| CA | California |
| CHNOS | Carbon, hydrogen, nitrogen, oxygen and sulfur |
| CMA | Corn meal agar |
| CMA+ | Corn meal agar + |
| CO | Colorado |
| DGGE | Denaturing gradient gel electrophoresis |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| E | East |
| EtOH | Ethanol |
| ETS | External transcribed spacer |
| g | Gram |
| IPC-MS | Inductively coupled plasma mass spectrometry |
| ITS | Internal transcribed spacer |
| Kg | Kilogram |
| l | Liter |
| LMEs | Lignin-modifying enzymes |
| LSU | Large subunit |
| m | Meter |
| M | Molar |
| MA | Massachusetts |
| ME | Maine |
| mg | Milligram |
| min | Minute |
| ml | Milliliter |
| mm | Millimeter |
| Ma | Million years |
| MODIS Spectroradiometer | Moderate Resolution Imaging |
| MYA | Malt yeast agar |
| N | North |
| NA | Nutrient agar |
| N/A | Not applicable |
| NaCl | Sodium chloride |
| NASA | National Aeronautic and Space Administration |
| PDA | Potato dextrose agar |
| PCR | Polymerase chain reaction |
| RNA | Ribonucleic acid |
| rRNA | Ribosomal RNA |

| | |
|---|---|
| Rpm | Revolutions per minute |
| SDA | Sabouraud dextrose agar |
| SDS | Sodium dodecyl sulfate |
| SLR | Single-lens reflex |
| SSU | Small subunit |
| TEMED | Tetramethylethylenediamine |
| U.S.A. | United States of America |
| US EPA | United States Environmental Protection Agency |
| UV | Ultraviolet radiation |
| WA | Water agar |
| WI | Wisconsin |
| µl | Microliter |
| µM | Micromolar |

# Chapter 1
# Introduction and literature review

## *1.1 Introduction to PhD thesis research*

This doctoral thesis is a multidisciplinary investigation of fungi isolated from extreme locations on Earth, focusing on one of the driest and thermally broad regions and comparing them to fungi isolated from other extreme environments. The thesis research encompassed the fields of mycology, molecular biology, wood science and biochemistry. The goals of the doctoral thesis were to identify the cultivable fungi from substrates that are present (soil, rock and wood) at a site in the hyper-arid Taklimakan Desert of western China, determine the fungal presence in this same desert by denaturing gradient gel electrophoresis (DGGE) molecular fingerprinting, use neighbor-joining analysis to establish the putative identity of fungi from the Taklimakan Desert and compare them to fungi isolated from deserts in polar regions. Additionally, the thesis had the goal to consider how fungi exist in their ecosystem and determine the impact of temperature on the capability of select cultivated isolates from this desert to decay lignocellulosic substrates and produce degradation related enzymes.

The collection site in the Taklimakan desert was selected based on minimal human impact and meteorological data; the location presented extreme variations in temperatures and very limited precipitation. Deserts are generally referred to as climatic regions that receive less than 250 mm of precipitation annually or where evaporation substantially exceeds precipitation for most of the year (Cressy 1960). The Taklimakan Desert's annual precipitation is less than 23 mm, placing it into the

most extreme precipitation related category of hyper-arid, defined as receiving less than 25 mm of precipitation annually (UNEP 1997). The temperatures near the collection site are also extraordinary with the lowest recorded, -21ºC and the highest at 43ºC, giving this location a minimum to maximum temperature range of 64ºC.

The conditions found in the Taklimakan Desert of limited precipitation and tremendous temperature variations significantly impact the ability of organisms to not only become established but survive. Most animals and vascular plants are only able to exist near rivers and oases, as they can not tolerate the conditions of severe desiccation and the thermally broad requirements this desert presents. However, there are microorganisms that can survive independently or in concert with other microorganisms in these harsh conditions and they include bacteria, eukaryotic algae and fungi. Perhaps the least studied group of microorganisms in these deserts consortiums are the fungi (Büdel 2005; States and Christensen 2001)

Although limited information has been presented about mechanisms for survival of fungi in deserts, for example the production of temperature related proteins and cryoprotectants to cope with temperature extremes (Deacon 2005; Robinson 2001), the production of extracellular polysaccharide layers to combat desiccation (Gorbushina et al. 2008) and the creation of relationships with other microorganisms to share resources (Gorbushina et al. 2005), even less is known about the biochemical strategies of nutrient acquisition of fungi in deserts.

Desert fungi are not limited to biomes such as the Taklimakan Desert, they can also be found in the deserts of polar regions (Newsham et al. 2009, Ludley and Robinson 2008). To complement the work completed in this PhD thesis on fungi from a site in the Taklimakan Desert and to enhance the understanding of not only the biodiversity but also biochemical strategies for survival of fungi in deserts, comparative studies were undertaken with fungi from deserts both in the Arctic and Antarctic. Although the sites in the Arctic and Antarctic receive slightly more precipitation with approximately 75 mm and 100-200 mm, respectively, they are well under the threshold that defines deserts. However, there are considerable differences in the average annual temperatures with the specific collection sites in Antarctica ranging from -15ºC to -30ºC, the site in the Arctic averaging -20 ºC, while the site in the Taklimakan Desert averages just under 12 ºC.

The substrates collected for fungal isolation varied between the Taklimakan Desert, Antarctica and the Arctic with wood obtained from all three. Soil, wood and various organic materials were collected from Antarctica, only wood was collected in the Arctic and soil, rock and wood were collected from the Taklimakan Desert. The common substrate throughout all sites is wood, with great differences in the origin of these samples. In the Arctic wood can originate from several sources such as, dwarf *Salix* sp. trees, wood brought in by past and present inhabitations, driftwood carried by ocean currents and deposited in tidal zones and the release of ancient wood from soils that were buried during catastrophic events many millions of years ago. In Antarctica the source of woody material is from early explorers that started to arrive on the continent approximately 100 years ago and from subsequent inhabitants. The

collection site in the Taklimakan Desert was completely void of vascular plants. However, there were 4 small samples collected within the 1 $km^2$ site. This type of material is exceedingly rare in the region and was likely brought to the location by the strong winds which frequently occur in the Taklimakan Desert (Kai et al. 2008). From the Arctic site, the wood originated from forests that once grew in this polar region and were buried millions of years ago by a catastrophic event. From Antarctica, the collected wood was from various structures and artifacts brought to the continent approximately 100 years ago.

It is important to note that the focus concerning the hyper-arid desert was based on a collection made at one site, at one time in the Taklimakan Desert and does not represent a full, compositional study of the entire desert. Nonetheless, 194 fungal isolates were cultured from the samples collected. Although the overall number of samples obtained in the Taklimakan Desert was relatively small at twenty, the quantity of microflora that was generated from the three substrates (soil, rock and wood) was significant.

The doctoral thesis was undertaken and administered by The University of Waikato in Hamilton, New Zealand under chief supervision of Professor Roberta L. Farrell. Research was in collaboration with co-supervisor Associate Professor Steven B. Pointing of The University of Hong Kong, who facilitated the field research and collections in the Taklimakan Desert in June 2007. The field work was followed by a period of research at the University of Hong Kong including cataloging and initial investigation of field samples immediately after their collection, followed by

importation of samples to New Zealand. The decay evaluation and electron microscopy were conducted at the University of Minnesota, U.S.A under the supervision of co-supervisor Professor Robert A. Blanchette because of the specfic facilities at University of Minnesota and Professor Blanchette's expertise in this field. Unfortunately, due to New Zealand's Ministry of Agriculture and Forestry requirements that indicate all fungal isolates must be identified prior to importation to New Zealand, the intended arrangement for returning to University of Waikato to complete the thesis research was changed in August 2008. All subsequent research with fungal isolates and DNA sequencing occurred at The University of Minnesota, with approval given by The University of Waikato's Higher Degree Committee, and supervision continued by Professors Farrell, Blanchette and Pointing. Antarctic research and isolated fungi from Antarctica undertaken by the PhD candidate and utilized in the PhD thesis research are from a collaborative investigation between University of Waikato's Professor Farrell and the research group of Professor Blanchette of The University of Minnesota, partially funded by the United States National Science Foundation Grant # 0229570. Canadian Arctic research was conducted by the PhD candidate under the supervision of Professor Blanchette at the University of Minnesota under Nunavut Research Institute Research License # 0100501 N-M and Parks Canada Permit # QQ-01-01.

### *1.2 Null-hypothesis, aims and objectives*

#### *1.2.1 Null-hypothesis*

*Null-hypothesis 1*: Fungal taxa from thermally distinct biomes do not occur in both hot and cold deserts.

*Null-hypothesis 2*: Fungi isolated from extreme desert conditions can not only colonize wood but have the ability to degrade this substrate

#### *1.2.2 Aims & Objectives*

The aims of this research were to investigate the biodiversity, evolutionary history and potential in utilizing lignocellulosic substrates of fungal constituents in lithic niches, wood and soils from a hot, hyper-arid desert region of Western China and compare them to fungi from cold deserts in polar regions.

There are three objectives to this PhD thesis research. The first objective was specific to the Western China desert region, the second was comparing those results to the polar regions and the third was considering lignocellulose as a nutrient; the specific objectives were as follows:

1.) a. Identify the cultivatable fungi from substrates present (soil, rock and wood) in a defined region of a hyper-arid desert in Western China.

b. Determine the fungal presence in a defined region of a hyper-arid desert in western China by denaturing gradient gel electrophoresis (DGGE) molecular fingerprinting.

2.) Use neighbor-joining analysis to establish putative identity of fungi from extreme deserts in Western China and from sites in the extreme environments of polar regions.

3.) Determine the capability of select cultivated isolates to decay lignocellulosic substrates and their ability to produce degradation related enzymes at various temperatures.

### *1.3 Literature review*

This literature review includes information on environments where samples were obtained as part of the PhD thesis research, a general overview of fungi, as well as background information on wood anatomy and properties and its degradation by microorganisms.

#### *1.3.1 Taklimakan Desert: general description, geography and vegetation*

The Taklimakan Desert is the largest, driest and warmest desert in the Peoples Republic of China and the second largest shifting sand desert in the world. It covers 337,000 $km^2$ (Sun and Liu 2006) (to put this in perspective, New Zealand's land mass covers 268,680 $km^2$) of which, 85% is shifting sand dunes that can reach heights of 100 to 200 m. The desert is located in the central portion of the Tarim Basin in the Uygur Autonomous Region of Xinjiang, in the northwestern portion of the country (Figure 1.1).

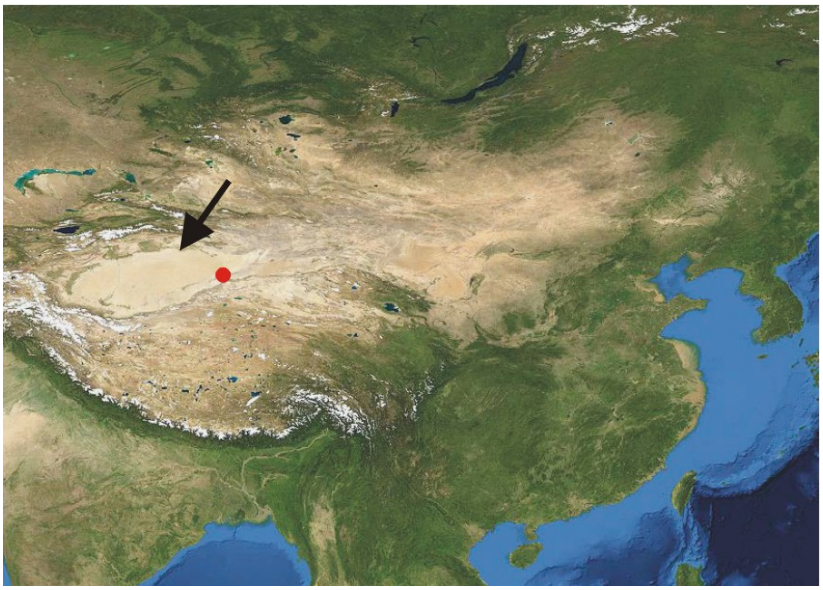

**Figure 1.1** Satellite image primarily of China and Mongolia with the arrow indicating the location of the Taklimakan Desert and circle identifying approximate collection site of soil, rock and wood samples (NASA).

The region is bound by the Kunlun Mountains to the south, and Pamir and Tian Shan Mountains to the west and north, with some of these surrounding mountains and plateaus reaching elevations of 4000-5000 m. The Taklimakan is one of the furthest points from any ocean and is located between 36.26º - 42.10ºN and 74.88º - 90.00ºE. Based on geologic investigations by Sun and Liu (2006), the age of this desert has been determined to be 5.3 Ma and was created by significant tectonic activity combined with marine regression modifying atmospheric circulation patterns causing the arid climate. The region has complex and diversified aeolian landforms with frequent sand dust storms created by the dramatic pressure gradients caused by air movement from the surrounding high elevation to the much lower basin (Figure 1.2).

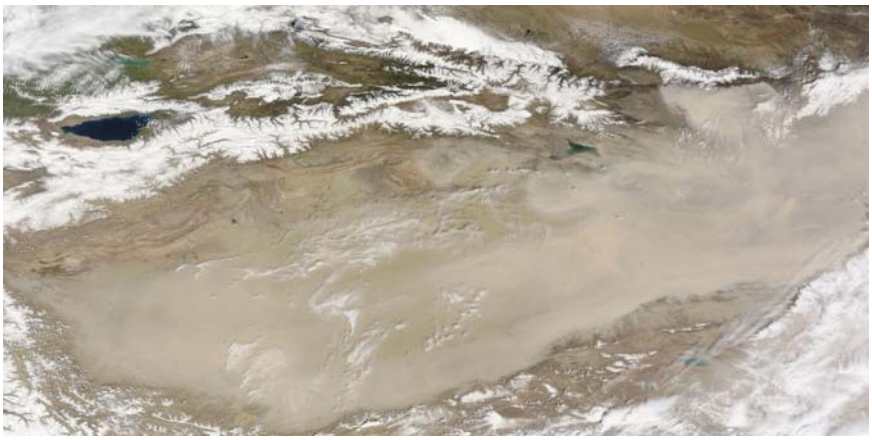

**Figure 1.2** Moderate Resolution Imaging Spectroradiometer (MODIS) captured by NASA's Terra satellite of a dust storm obscuring the floor of the Taklimakan Desert in China on April 17, 2009 (NASA).

The mineral dust originating from these sand storms can create dense dust layers from the ground up to 5.5 km (Kai et al. 2008) and have been found in Greenland snow (Bory et al. 2003) as well as the French Alps (Grousset et al. 2003). The fine nature of the Taklimakan dust results in it sustaining a very long transport time

playing an important role in the formation of cirrus clouds and therefore affecting the global radiation budget (Mikami et al. 2006) and additionally, is significant in supplying nutrients to the ocean (Duce and Tindale 1991).

Natural vegetation in the interior of the desert is very sparse; however, there are 27 species of plants (Zhou et al. 2006) primarily located around rivers and *Tamarix* dunes or cones. The river banks and oasis regions support the greatest diversity and largest species with woodlands of *Populus euphratica* and *P. pruinosa* dominating. The *Tamarix* cones which are located around the desert periphery are dominated by salt-tolerant vegetation such as *Tamarix ramosissima*, *Phragmites australis*, *Alhagi sparsifolia*, *Calligonium mongolicum*, *Nitraria sibirica* and *Scorzonera divaricata* (Qong 2002). A list of the common Taklimakan Desert plants can be found in Table 1.1.

**Table 1.1** Species of vascular plants and life form common to the Taklimakan Desert, Peoples Republic of China.

| **Species name** | **Life form** |
|---|---|
| *Alhagi sparsifolia* | Herb |
| *Calamagrostis pseudophragmites* | Reed |
| *Calligonum taklamakanensis* | Shrub |
| *Cistanche tubuloasa* | Grass |
| *Cynanchum kashgerisccum* | Grass |
| *Elaeagnus oxycarpa* | Tree |
| *Glycyrrhiza inflate* | Grass |
| *Halimodendron halodendron* | Shrub |
| *Heliotropium micranthum* | Grass |
| *Helogeton arachnoideus* | Grass |
| *Helogeton glomeratus* | Grass |
| *Hexinia polydichotoma* | Grass |
| *Inula salsoloides* | Grass |
| *Myricaria pulcherrima* | Shrub |
| *Nitraria sibirica* | Shrub |
| *Poacynum hendersonni* | Grass |
| *Phragmites australis* | Grass |
| *Phragmites communis* | Grass |
| *Populus euphratica* | Tree |
| *Populus pruinosa* | Tree |
| *Scorzonera divaricata* | Herb |
| *Tamarix ramosissima* | Shrub |
| *Tamarix hispid* | Shrub |
| *Tamarix hohenackeri* | Shrub |
| *Tamarix laxa* | Shrub |
| *Tamarix leptostachys* | Shrub |
| *Tamarix taklamakanensis* | Shrub |

(Dong et al. 2004; Qong et al. 2002; Thevs et al. 2008; Zhou et al. 2006)

Xinjiang is a region of temperature extremes with very hot summers and exceptionally cold winters. The highest temperature in China was recorded within the basin at 49.6°C and one of the lowest in the country was also recorded in the region at -51.5°C (Wei et al. 2005). The average annual temperature is 12.4°C with July being the hottest month having an average temperature of 28.2°C and January as the coldest month with an average temperature of -8.1°C. These averages don't express

the extremes well because diurnal temperature often fluctuate more than 20°C (Zhou et al. 2006). These extreme temperatures are further exemplified by surface readings in excess of 73°C (Xu et al. 2006), which create inversions that have also been shown to contribute significantly to dust storm outbreaks (Tsunematsu et al. 2005) (Figure 1.2). The region is also exceedingly dry with mean annual precipitation of less than 40 mm and some interior areas receiving less than 10 mm with annual evaporation exceeding 3000 mm or more than 70 times the annual precipitation (Wei et al. 2005, Han and Wang 2003), making it one of the driest places on earth. These extraordinary temperature variations (100°C, air and 124°C, surface) and minimal precipitation require the organisms that inhabit this region to function with extremely low and sporadic moisture availability and to have thermoflexibility that is unmatched in other terrestrial sites.

### *1.3.2 Polar regions*

The polar regions are the northern and southern ends of the Earth's axis surrounding the North and South Poles, respectively. These regions are primarily covered by polar ice caps, either on the Arctic Ocean in the north or in the continent of Antarctica in the south which is caused by heat radiation from the sun impacting these polar regions at an angle. The Earth's orbital plane is known as the ecliptic and thus the Earth's axial tilt is referred to as the obliquity of the ecliptic and the angle which the sun hits the ends of the Earth determines how much heat radiation is absorbed. When sunlight is intercepted by the earth at a lower angle (sun closer to the horizon), the energy of that light is spread over a larger area and is therefore weaker than if the sun

were higher overhead where the energy would be more concentrated on a smaller area, as occurs at the Equator (Figure 1.3).

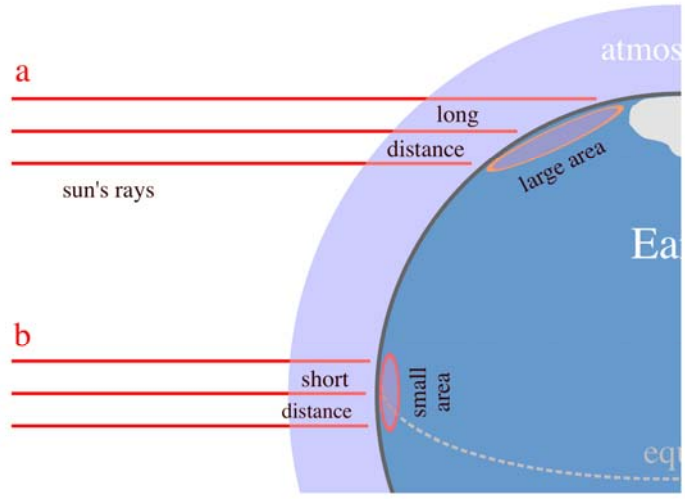


**Figure 1.3** Drawing representing the angle at which the Earth intercepts solar radiation from the sun in polar regions (a) and near the equator (b). Image from Wikipedia.

### *1.3.2.1 Antarctica*

The southern Polar Region is dominated by the continent of Antarctica which is located almost entirely south of the Antarctic Circle or below 66.5º South Latitude with only a small portion of the Antarctic Peninsula and Wilkes Land north of that line (Figure 1.4). The total area of Antarctica is 14 million sq km and only 280,000 sq km is not covered by ice (Clough and Draggan 2009). The average temperature over the entire continent is -49 °C and Antarctica contains 90% of Earth's ice and 70% of its freshwater (Clough and Draggan 2009). The total precipitation averaged over the entire continent, is approximately 166 mm (6.5 in) per year (Vaughan et al. 1999).

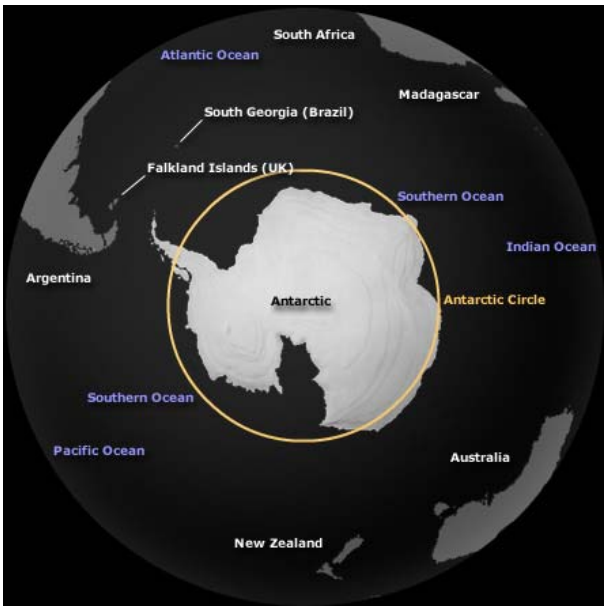


**Figure 1.4** Geographic representation of the continent of Antarctica (Webcam).

The Ross Dependency in Antarctica comprises all the islands and territories south of 60°S latitude between 160°E and 150°W longitude and is directly administered by New Zealand (Figure 1.5). It is an estimated 413,540 sq km and entails the permanent Ross Ice Shelf ice which covers 336,770 sq km. The area includes parts of the ice covered plateau, its landscape made up of glaciers, mountain ranges and deep crevasses. The Transantarctic Mountains extend across the continent, dividing the eastern and western ice sheets. The Ross Dependancy includes Ross Island with the active volcano, Mt Erebus rising to 3,794m. Ross Island is also the site of the New Zealand and American research stations, Scott Base and McMurdo Station, respectively (New Zealand Ministry of Foreign Affairs and Trade, 2009).

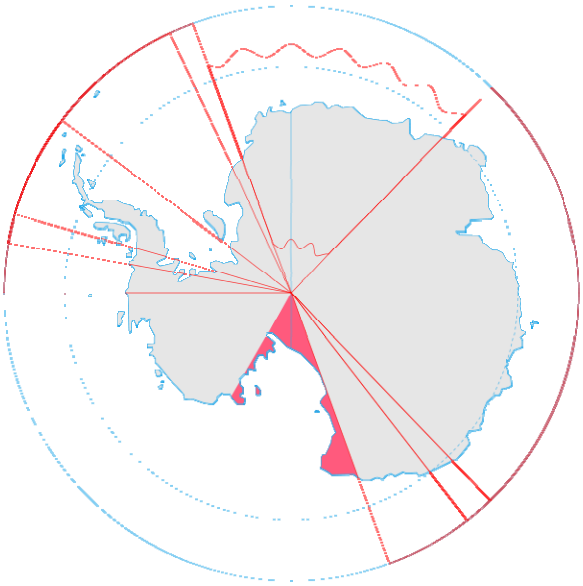

**Figure 1.5** Drawing of Antarctica with the Ross Dependency highlighted in red (Wikipedia).

The Dry Valleys region is a 4000 km$^2$ ice-free area of the Transantarctic Mountains from 77.81ºS to 77.84ºS and 160.8ºE to 164.8ºE, located between the Ross Sea and the Taylor Dome of the East Antarctic Ice Sheet (Summerfield et al. 1999). Mean annual temperatures for the valleys from seven meteorological stations during the period 1986 to 2000 ranged from -14.8ºC to -30.0ºC, depending on the site and period of measurement (Doran et al. 2002), with precipitation reaching a mean maximum of around 100 mm per year (Summerfield et al. 1999). Average annual temperature at McMurdo Station, Ross Island, Antarctica (77.88ºS, 166.73ºE) is -16.9º with the average monthly high temperature in January of -0.2ºC and the average monthly low temperature in August at -31.8ºC. The average annual precipitation at this site is 202.5 mm with the highest monthly average of 24.9 mm occurring during June. The significance of the samples collected will be described in full in Chapter 3 but the

collection sites were all in the Ross Sea Regions and specifically the Ross Dependency.

#### *1.3.2.2 Arctic*

The Arctic is a region in the northern hemisphere, north of the tree line, that covers approximately 7.2 x $10^6$ $KM^2$ (Tarnocai 2009) (Figure 1.6). Glaciers cover about 1.9 x $10^6$ $KM^2$ or 26% of the total area with 92% occurring in Greenland. This region is characterized by short, cold summers and extremely cold, long winters. The warmest temperatures occur during July with 7-10ºC in the southern portion of the Arctic and 3-5ºC in the north. Temperatures of -20 to -40ºC are found during the coldest month which is February. The total annual precipitation is approximately 60-160 mm and primarily occurs as snow (Tarnocai 2009). Based on data collected by the Meteorological Service of Canada from 1971 to 2000 at the nearest weather station to Eureka, Nunavut, Canada, 79°58.800N, 85°55.800W (collection site relevant to PhD thesis), had an average annual temperature of -19.7°C, with the highest average monthly temperature of 5.7°C occurring in July and the lowest of -38.4°C in February. The average annual precipitation over the 30-year period was 75.5 mm, with the greatest amount falling in August (14.9 mm) and the least in February (2.6 mm).

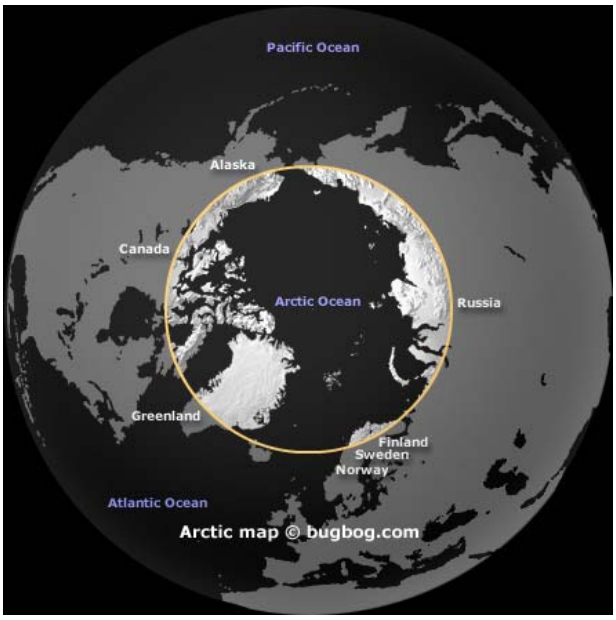

**Figure 1.6** Drawing representing the area within the Arctic Circle (Webcam).

### *1.3.3 Soils from desert regions*

#### *1.3.3.1 Hot deserts*

Soils from arid regions are distinguished from other biomes by commonly having low levels of organic matter and low biological activity. They are often slightly acidic to alkaline at the surface, are poorly developed, weather slowly and may have accumulations of calcium carbonates, soluble salts, gypsum or silica (Hendricks 1991). The texture of desert soils is usually coarse with rocks and gravel prevalent because they experience severe erosion from either wind or periodic rain events that disperse the finer particles elsewhere (Qi et al. 2002). The amount of soil carbon and nitrogen is generally believed to be in part regulated by precipitation, with decreasing C and N related to a decrease in precipitation (Amundson et al. 2001 and 2003.), suggesting that soils in desert biomes would be much lower in C and N than those in temperate and tropical regions. However, Ewing et al. (2007) found the inverse with relation to the hyperarid Atacama Desert in Chile, which has very similar conditions

to the Taklimakan Desert and had increasing N content in soils with a reduction in precipitation, stating that this was driven by the lack of biotic N cycling. The soil organic content at this site was 0.3 kg C $m^{-2}$ and organic N was 1.4 kg N $m^{-2}$.

#### *1.3.3.2 Cold deserts*

Antarctic soils are found in the ice free areas which are just 2% of the continent, of that, 90% are found near the continental coastline of the Antarctic Peninsula and the Ross Sea Region. The largest continuous ice free region is the McMurdo Dry Valleys with soils that are referred to as cold desert soils and classified as Anhyorthels (Bockheim, J.G. 2002). This soil type is defined by the moisture conditions associated with very cold, dry environments with the active layer reaching a depth of ~45 cm and below this depth the soils are dry enough that they are not ice-cemented (Barrett 2006). Soil pH is often slightly basic in the Dry Valleys and Ross Island with values ranging from 8.12-8.9 in the top centimeter and organic carbon and nitrogen values of 0.08-0.1% and 0.01-0.47%, respectively (Aislabie et al. 2008; Freckman et al. 1997).

The soils of Arctic Regions are dominated by cryogenic processes and can be either mineral or organic material but permafrost is within 1 m of the surface. These cryosolic soils can have a wide range of pH values (3.5-8.6) and in most cases this is dependent on the parent material. They are often low in nutrients, specifically nitrogen but both the mineral and organic soils contain large amounts of carbon (Tarnocai 2009).

### *1.3.4 Overview of Fungi*

Fungi are eukaryotic organisms that are members of the Kingdom Fungi and are primarily multicellular and heterotrophic. Unlike animals that ingest their food or plants that 'manufacture' their own food, most fungi produce networks of hyphae, which are essential in the absorption of nutrients from their surroundings. Fungi are able to do this by secreting enzymes or biomolecules into the substrates in which they are physically in contact with and these enzymes catalyze the reactions that dissemble the material into molecules that can be readily absorbed thus providing nutrients (Alexopoulos et al. 1996).

Reproduction by fungi is not as straight forward as it is for many organisms with some fungal species capable of only asexual reproduction, others are only known to reproduce sexually and most are capable of both forms of reproduction. Asexual reproduction does not involve the fusion of nuclei or meiosis, while sexual reproduction involves two nuclei joining followed by meiosis. When fungi reproduce asexually, new individuals initiate from fragments, fission, budding or mitotic spores originating from mother tissue. While sexual reproduction typically involves the union of two compatible nuclei, meiosis and the formation of a specialized spore that germinates into a new individual.

Fungi can generally be classified as being either saprophytes, parasites, symbionts and even predators, with several falling into multiple categories. Many fungi are saprophytes which live on or get their nourishment from dead organisms or decaying

organic matter. Saprophytes perform an important role in ecosystems by decomposing organic matter and are essential in nutrient cycling.

Fungi can also be parasitic on plants, animals and even other fungi. Within this group, there are three categories including necrotrophs, which kill host cells in advance of their hyphae, biotrophs, which are obligate parasites that obtain nutrients from living host cells and hemibiotrophs that initially require living host cells and eventually kill those host cells.

Some groups of fungi also have symbiotic relationships with organisms that are more mutualistic. One of the most important of these associations is between fungi and plants with as many as 90% of all plants species engaged in this mycorrhizal symbiosis, where fungal hyphae either grow on the root of a plant (ectomycorrhizae) or within the roots (endomycorrhiza). The fungi assist the plants by increasing their ability to absorb inorganic compounds and moisture and the fungi benefit by having access to carbohydrates produced by the plants (Smith and Read 2008). Another mutualistic relationship that fungi form is with algae and cyanobacteria to create lichens. Like the plants in the mycorrhizal association, the algae and cyanobacteria are the photobionts and produce energy from the sun that they share with the fungi, which provides minerals and water (Oksanen 2006). Lichens are incredibly resilient organisms that can grow on a wide variety of surfaces and inhabit any terrestrial biome.

There are also predatory fungi that actively trap tiny animals called nematodes and utilized them as a food source. These fungi typically live in soils and produce pheromones and toxins that attract nematodes (Barron 1992) and specialized structures such as rings, adhesive branches and nets that capture the animal and digest the body.

Isolating fungi from environmental samples such as soil is not an easy task as the organisms that inhabit this substrate can be exceedingly difficult to isolate into a pure culture. The reason for this is that there is a tremendous diversity of microorganisms (bacteria [actinobacteria], fungi, algae and protozoa) in soil samples and culturing just fungi, and more specifically an individual fungus, can be challenging because other microflora must be eliminated or their growth suppressed while still allowing the fungi of interest to grow. Using a broad array of culture media with various carbon sources, antibiotics, varying pH, as well as a range of isolation temperatures can assist in fungal isolation. Authors have found that isolating fungi from biological soil crusts in deserts is 'difficult' (States and Christensen 2001), while others have noted the great difficulty not only in isolating Basidiomycota from the Arctic and Antarctic soils but from soils in general (Ludley and Robinson 2008). Others have commented on the considerable difficulties associated with isolating fungi with a higher thermal preference from soils (Baumgardner 2009; Redman et al. 1999). It is estimated that only 5% of fungi are known and >1.5 million fungal species exist (Mueller 2004); part of the difficulty of studying these microorganisms beyond a molecular fingerprinting approach is the difficulty in obtaining pure isolates.

### *1.3.4.1 Nutrient acquisition by fungi*

To acquire the nutrients required for survival, fungi rely on other organisms to fix carbon. They are able to utilize most organic carbons as a source of nourishment, with some species having a more omnivorous existence allowing them to subsist on essentially any organic material and others requiring very specific sources of nutrition; mutualistic relationships exist between some fungi in order to maximize utilization of the carbon source. Regardless of the source of nourishment, fungi acquire nutrition in a similar manner primarily by means of extracellular enzymes, which are either excreted into the environment or are membrane bound. The extracellular enzymes are released from hyphae into a substrate which break down large, relatively insoluble compounds like carbohydrates, lipid and proteins into molecules that can be subsequently absorbed. The enzymes required for lignocellulosic metabolism will be discussed in detail in Section 1.38. Many fungi also excrete biomolecules such as organic acids which aid in ion displacement allowing these organisms to more readily absorb mineral ions which are important for growth.

For the diffusion of these soluble molecules through the cell wall and plasma membrane of the fungi to take place, free water must be present for metabolism. The role of water in metabolism and ultimately enzyme function can be seen to perform 4 possible roles (Dunn and Daniel 2004), as follows:

1.) Water may be essential for chemical reactions to take place; a hydrolysis reaction may be a more favorable reaction by assisting in the creation of greater entropy between the enzyme and substrate (Kornblatt and Kornblatt 1997).

2.) Water may be important in protein structure by forming hydrogen bridging bonds (Gronenborn and Clore 1997) and structure stabilization that appear important in native conformation of an enzyme (Meyer 1992).

3.) Some proteins also appear to only become active when there is direct contact with water and the protein surfaces, which indicates that a hydration threshold must be met.

4.) Water provides a medium for the diffusion of the enzyme to the substrate.

Relative to these four roles the fourth is extremely important for fungi using extracellular enzymes to facilitate nutrient acquisition as water provides a medium for degraded substrate material to reach the fungi. All fungi do not possess the same arsenal of enzymes and the type of substrate they can utilize as a food source is primarily determined by the enzymes they can produce. Recently, several fungi have been studied that appear to gain energy from ionizing radiation, not by enzymatic digestion. This process is similar to photosynthesis by plants, however, the biochemical pathway for this energy acquisition has not yet been determined and the amout of energy generated for the fungi appears to be relatively limited (Dadachova et al. 2008).

### *1.3.4.2 Diversity of fungal phyla*

The major taxonomic divisions or phyla of fungi are ever evolving based on molecular and mycological investigations. The most current classification utilizes recent molecular, multi gene phylogenetic studies and characteristics of their sexual and asexual reproductive structures, as well as spores to separate the groups. Figure 1.7 indicates the main phyla and briefly identifies characteristics that assist in separating them (Hibbett et al. 2007 and Alexopoulos et al. 1996).

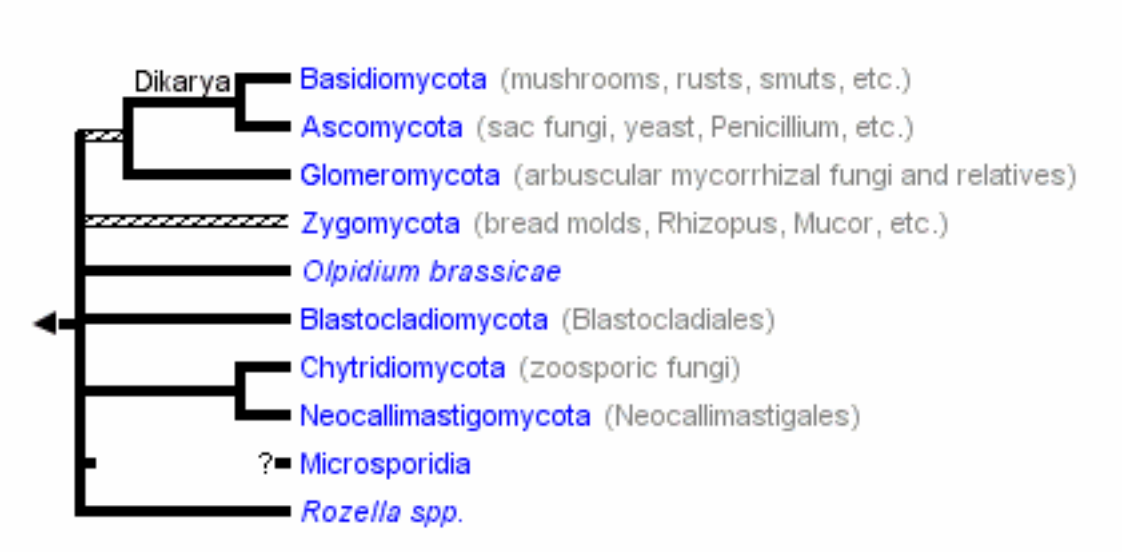


**Figure 1.7** Phylogenetic tree of Kingdom Fungi (Blackwell et al. 2009).

**Ascomycota:** The primary characteristic that distinguishes this Division/Phylum from others is the production of an ascus, or sac that holds ascospores when these fungi reproduce sexually (Figure 1.8). The phylum also consists of several 'imperfect' fungi that reproduce asexually via conidia and single celled yeast which are included in this group based on molecular similarities (Hibbett et al. 2007).

**Figure 1.8** Image of structures originating from sexual reproduction in Ascomycota, with an ascus holding ascospores (8) (© R. Vilgalys 1996).

**Basidiomycota:** The sexually reproducing members of this phyla produce very unique club shaped cells called basidia that produce basidiospores (Figure 1.9) and are the primary morphological characteristic separating them from other groups. Many members of this group also produce unique outgrowths on their hyphae called clamp connections. Like the Ascomycota, there are several asexually reproducing members producing conidia and unicellular yeasts that are included in this phylum based on molecular evidence.

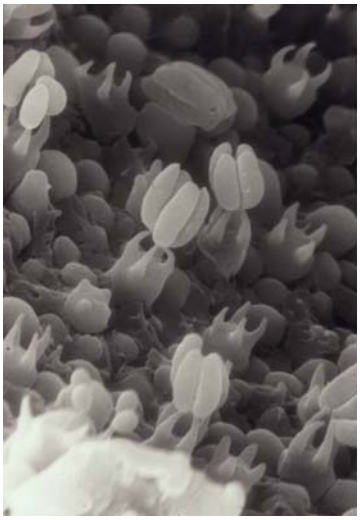

**Figure 1.9** Scanning electron micrograph of basidium bearing basidiospores (4) derived from sexual reproduction by fungi from the phylum Basidiomycota (R.A. Blanchette).

**Blastocladiomycota:** This is a recently designated group that has been split from the Chytridiomycota phyla based on molecular data, which is capable of both sexual and asexual reproduction and is characterized by a zoospore with a single flagellum (James et al. 2006).

**Chytridiomycota:** This group, like the Blastocladiomycota, with which they were previously grouped are characterized by a single flagellum originating from a zoospore.

**Glomeromycota:** Fungi in this group are considered obligate, mutualistic symbionts that form arbuscular mycorrhizal association within plants. They have not been found to reproduce sexually.

**Neocallimastigomycota:** This is another recently designated group, based on molecular evidence, found in anaerobic conditions in the gut of rumen and were initially classified with the Chytridiomycota. They are characterized by a lack of mitochondria and have only been found to reproduce asexually via zoospores with a single flagellum.

**Zygomycota:** The primary characteristics that define this phylum is the production of thick walled zygospores that form within zygosporangium after specialized hyphae called gametangia fuse during sexual reproduction. During asexual reproduction, Zygomycota produce sporangiospores on sporangia, which are very different from

the conidia formed by the Ascomycota and Basidiomycota as they are formed by cleavage of sporangial cytoplasm.

### *1.3.4.3 Internal transcribed spacer region*

Molecular techniques such as DNA sequencing have been very useful in assessing the identity and relatedness of fungi over the last several decades. Perhaps the most widely sequenced region of DNA for exploring the diversity of fungi is the internal transcribed spacer (ITS) by utilizing primers such as ITS1, ITS4, etc. The ITS refers to a segment of non-functional RNA that is between structural ribosomal RNAs (rRNA) on a common precursor transcript. This transcript is read from 5′ to 3′ and contains the 5′ external transcribed sequence (5′ ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and the 3′ ETS (Figure 1.10). It is extremely valuable in elucidating relationships between fungal genera and even species because it is easy to amplify even with small quantities of DNA, due to the high number of copies of ribosomal repeat in every fungal genome. Also, this region does not display a great deal of conservation which allows better resolution for identification because of the high degree of variation. It is also useful because ITS can be amplified reliably from most fungi using a single set of primers (Gardes and Brunes 1993).

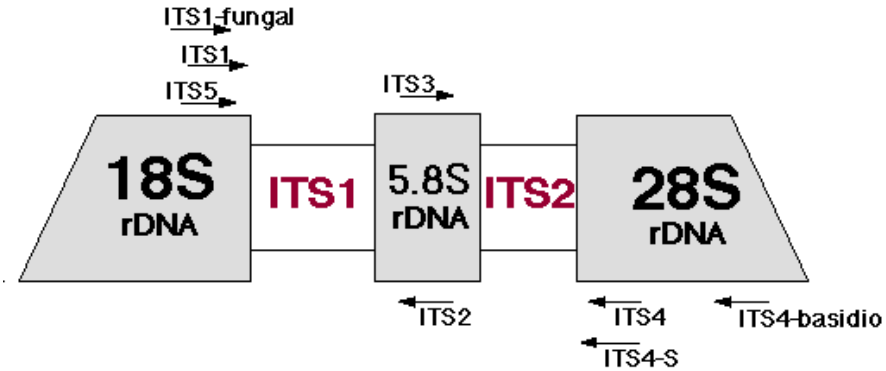


**Figure 1.10** ITS region of non-functional RNA that is between structural ribosomal RNAs (rRNA) on a common precursor transcript with primers and primer directions used to amplify this region. Drawing from Tom Bruns Laboratory, University of California at Berkeley (http://plantbio.berkeley.edu/~bruns/).

The ITS region sequences are also very useful because there are large public databases available for comparative purposes of this region such as GenBank (Benson et al. 2006). Although reference databases like GenBank have a tremendous amount of sequence data, this information is not always correct. In a recent study by Nilsson et al. (2006), the authors found that fungal DNA sequences from the International Nucleotide Sequence DataBase were incorrectly identified to species level 20% of the time and the majority lacked descriptive and up to date annotations. By employing additional primers that target genes or regions such as SSU (small subunit), LSU (large subunit), Beta-Tubulin or others, greater resolution and confidence can be generated in the final product. Also, the use of phylogenetic trees based on sequence data can be a useful tool in assigning putative identification to fungi and is reviewed in Section 1.3.4.3.

#### *1.3.4.4 Phylogenetic trees*

A phylogenetic tree is a graphical representation of the evolutionary relationships between taxon (Baum 2008). Phylogenetic analyses can be carried out at a number of taxonomic levels, from intra-population level through to deep branches and these analyses can be done using morphological and/or molecular data. These groups may be different species or genes from a common ancestor, which are delineated based on attributions such as traits, morphology, DNA sequences or amino acid sequences. A phylogenetic tree is a type of cladogram with branches of varying lengths that can correspond to the hypothetical or predicted time between the tested organisms or sequences. At the end of each branch or node is the most recent common ancestor of the descendent, often called a taxonomic unit or taxa. These cladograms are not

necessarily a complete and accurate depiction of the evolutionary history of organisms as there are a number of possible pathways that can produce patterns of relatedness, while the phylogenetic tree only illustrates the probability of relatedness, and that probability based on the specific attribution that was considered i.e. a specific gene sequence, a specific amino acid sequence. The patterns created by phylogenetic trees are strongly correlated with the amount and type of information utilized to generated them, with often a greater amount of information providing greater clarity in the evolutionary relationships. Although these problems exist, phylogenetic trees are still perhaps the most effective ways to establish and visualize relationships.

### *1.3.5 Extremophiles (extremotolorant organisms) and their environments*

Environmental conditions vary greatly throughout our planet, whether referring to air, land or water, and particularly the surface, subterranean or the depths of the sea. Despite these variations, microorganisms including Archaea, Bacteria and Eukarya, can be found inhabiting all of them. Microorganisms have specific requirements for survival and if any one of these requirements are not met or if they are greatly diminished or significantly elevated, their growth or existence will be in peril. However, there are a group of microorganisms that can not only cope with significant reductions or increases in vital requirements but they can actually flourish. These organisms are referred to as extremophiles or extremotolerent organisms because they exist in conditions that are considered extreme such that they are near the limits of cell functioning that limit enzyme activities or damage biomolecules (Rothschild and Mancinelli 2001). The organisms that live under adverse conditions are often

classified by the environmental extremes they tolerate, which are given in Table 1.2. Often, organisms are able to withstand not only a single condition that would be detrimental to most forms of life but they can endure multiple factors and these are given the title of polyextremophile. An example would be a microorganism that lives in exceedingly hot and dry conditions and would be referred to as a thermophilic xerophile. When these microorganisms from the three divisions (Archaea, Bacteria and Eukarya) are found growing in conditions that are considered extreme, they often form relationships with one another that allow the mutualistic organism to survive, where the individual would perish. By researching extremophiles, we expand our understanding of the vast biodiversity on earth and the mechanisms that allow them to survive the hostile conditions in which they live.

**Table 1.2** Extremophile classifications and associated environmental conditions.

| Extremophile organism class | Environmental condition |
|---|---|
| Acidophile | Optimum growth at pH level at or below pH 3 |
| Alkaliphile | Optimal growth at pH levels of 9 or above |
| Halophile | Requiring at least 2M concentrations of salt for growth |
| Xerophile | Growth in extremely dry, desiccating conditions |
| Radioresistant | Resistant to high levels of ionizing radiation, including ultraviolet and nuclear radiation |
| Piezophile (barophile) | Optimal growth at high hydrostatic pressure |
| Osmophile | Capable of growth in environments with high osmotic pressure i.e. high sugar concentration |
| Oligotroph | Capable of growth in nutritionally limited environments |
| Metalotolerant | Capable of tolerating high levels of dissolved heavy metals in solution |
| Lithoautotroph | Sole source of carbon is carbon dioxide |
| Hyperthermophile | Thrive at temperatures between 80–122°C |
| Thermophile | Thrives at relatively high temperatures, between 45 and 80°C |
| Psychrophile/Cryophile | Grows better at temperatures of 15°C or lower; not capable of growth above 20°C |
| Endolith | Lives inside rock, coral, animal shells, or in the pores between mineral grains of a rock |
| Chasmoendolith | Colonizes fissures and cracks in the rock |
| Cryptoendolith | Colonizes structural cavities within porous rocks |
| Euendolith | Penetrates actively into the interior of rocks forming tunnels that conform with the shape of its body |
| Hypolith | Lives inside rocks in cold deserts |
| Microaerophile | Survives under low oxygen conditions |
| Anaerobe | Survives in habitats lacking oxygen |

Adapted from Rothschild and Mancinelli 2001; Gadd et al. 2005

#### *1.3.5.1 Archaea extremophiles*

Archaea are thought to be among the oldest organisms on earth but they were not recognized as a separate domain from bacteria and Eukarya until Woese and Fox (1977) proposed their separation based on molecular investigations. Archaea are single celled organisms that lack a nucleus and other organelles within their cell and can only reproduce asexually. They were initially thought to be exclusively extremophiles, as they were only found living in adverse conditions. Additional sampling and analysis from non-extreme settings identified an abundance of these

organisms throughout our planet (DeLong 1998) and it is now considered they comprise as much as 20% of the earth's biomass (DeLong and Pace 2001). Within this domain, there are four primary extremophile groups that have been identified, as follows: acidophiles, alkaliphiles, halophiles and thermophiles, with some being polyextremophilic. The Archaea are truly amazing organisms with individuals able to live at pH 0 (Ciaramella et al. 2005), in salinity concentrations of 20-25% (Oren 2002), limited reduction in growth at 10 g arsenate per litre of water (Baker - Austin et al. 2007), grow at 122ºC (Takai et al. 2008) and even survive for short periods at 130ºC, which is the highest recorded temperature for any organism (Cowan 2004).

#### *1.3.5.2 Bacteria extremophiles*

Perhaps the most well studied division of extremophiles are the Bacteria, with *Thermus aquaticus* as the most well known of this group. This bacterium is a thermophilic organism originally isolated from a hot spring in Yellowstone National Park and was found to posses a thermotolerant enzyme called Taq DNA (Deoxyribonucleic acid) Polymerase which is used in PCR (polymerase chain reaction) DNA amplification, arguably one of the most important enzymes in molecular biology (Chien et al. 1976 and Saiki et al. 1988). Bacteria are single cell microorganisms that do not contain a nucleus and form most of the biomass on earth (Whitman 1998). Their great numbers are directly related to their ability to inhabit any biome on earth and even thrive under conditions that would halt cell function in other organisms. Within this division there is a unique group called cyanobacteria that obtain energy through photosynthesis. These photobionts are found in a wide variety of environments that are not considered extreme including marine and

terrestrial. However, they often flourish in hypersaline conditions (Oren 2000), exceedingly dry biomes (Pointing et al. 2007), hot springs (Steunou 2006) and very cold settings (Mondino et al. 2009 and Vincent 2000).

#### *1.3.5.3 Fungal extremophiles of hot and cold deserts*

As with Archaea and Bacteria, fungi are able to colonize and even thrive in adverse environmental condition (Amaral-Zettler et al. 2002) (The Kingdom of Fungi will be more generally discussed in Section 1.3.4). The term poikilotrophic or poikilophilic has been used to describe these organisms that can tolerate extreme environmental stress caused by fluctuating conditions (Gorbushina and Krumbein 1999). Depending on the condition or set of detrimental conditions present in a given environment, fungi have evolved numerous strategies to survive.

##### *1.3.5.3.1 Heat tolerant fungi (thermophiles)*

Fungi are not able to withstand the high temperatures that some Archaea and Bacteria can tolerate with a maximum temperature for survival of a fungal isolate from a thermal hot spring of 61.5ºC (Tansey and Brock 1973). Above 65ºC it appears that the hyphal cells become irreparably damaged (Magan 2007), however, some very resistant ascospores are able to withstand temperatures in excess of 80ºC (Baggerman and Samson 1988). The group of fungi that survive in this upper temperature limit are referred to as thermophiles and have minimum growth at 20ºC or above and maximum growth at 50ºC or above and include species such as *Scytalidium thermophilum* (Sanchez and Royse 2009) and *Aspergillus nidulans* (Magan 2007). In

a recent review of published thermotolerant fungal species Mouchacca (2007) found that the group is dominated by Ascomycetes, representing over 50%.

There appear to be several factors that allow some fungi to persist at these high temperatures, with one being the solubilization of lipids. Many thermophilic fungi have been shown to posses more lipids with saturated fatty acids in their membranes than mesophilic fungi (Satyanarayana1 2005), and this is also found in Archea and Bacteria. These saturated lipids have a higher melting point and assist in maintaining the integrity of cells at higher temperatures (Crisan 1973). Heat shock proteins also appear to play an important role in maintain cellular function at higher then optimal growth temperatures, which varies between fungi. Under heat induced stress these proteins, often called chaperones, assist in folding other proteins within the cell and destroy damaged proteins (Deacon 2005). Many fungi under a variety of different stress conditions, including elevated temperature, have the ability to synthesize the disaccharide trehalose which has been shown to provide several protective functions. This includes stabilizing intercellular enzymes and the prevention of denatured protein aggregation (Fillinger et al. 2001; Elbein et al. 2003; Al-Naama et al. 2009). It has also been found to stabilize cellular membranes during conditions of stress (Patist and Zoerb 2005).

#### *1.3.5.3.2 Cold tolerant fungi (psychrophiles & psychrotrophs)*

The group of fungi referred to as psychrophiles survive and even thrive at the lower end of the temperature spectrum and include genera such as *Mortierella* and *Mucor* spp. (Robinson 2001). These microorganisms have an optimum temperature for

growth of 15ºC, a maximum growth temperature of 20ºC and are able to grow at 0ºC. Psychrotrophic fungi are also able to grow at 0 ºC and can survive at temperatures above 20ºC. Both of these groups consist of a wide variety of fungi that have numerous strategies to combat the ill effects of low temperatures.

The physiological mechanisms that assist fungi in tolerating cold temperatures are not completely understood but several are known and they include; the synthesis of croprotectants such as arabitol, erythritol, glycerol, proline and trehalose, an increase in unsaturated membrane lipids, antifreeze proteins and enzymes that are active at low temperatures and the production of melanin in hyphae (Hoshino et al. 2009; Magan 2007; Robinson 2001).

There are other physiological mechanisms that trigger ecological and morphological characteristics in fungi when they are confronted with lower temperatures. These include sterile hyphae, cold avoidance, short growth cycles and fungal spores that will only germinate annually during warm periods. It does not appear that a single mechanism is responsible for the survival of fungi at low temperatures, rather a combination of mechanisms are required to tolerate and even thrive under this adverse condition (Robinson 2001; Rodrigues and Tiedje 2008).

#### *1.3.5.3.3 Rock associated fungi*

Fungi that are associated with rock are often found in habitats with extreme conditions and this association appears to be an adaptation of the microorganisms that is required to survive in low or high temperatures, extreme desiccation or high

ultraviolet radiation (UV) (Burford et al. 2003). There are several types of these fungi which are generally referred to as epi- or endoliths but others are called chasmoendolith, cryptoendolith, euendolith and hypolith depending on where they reside within rocks or the environmental conditions from where they are found (Table 1.2). There are species that grow independently on rocks such as *Friedmanniomyces endolithicus* growing as a cryptoendolith in the Dry Valleys of Antarctica (Onofri et al. 2004) or *Sarcinomyces petricola* growing on sun exposed marble in the Mediterranean Basin (Wollenzien et al. 1997) but many of the fungi that fall into this category grow as a consortium with other microorganisms (Gorbushina and Broughton 2009; Seymour et al. 2005).

These communities can consist of chemolithotrophic, chemoorganotrophic or photoautotrophic bacteria, eukaryotic algae or non-lichenized and lichenized fungi (Gorbushina et al. 2005). Perhaps the most ubiquitous example of one of these mutualistic symbiosic relationships is found in lichens, which consist of at least two organisms.

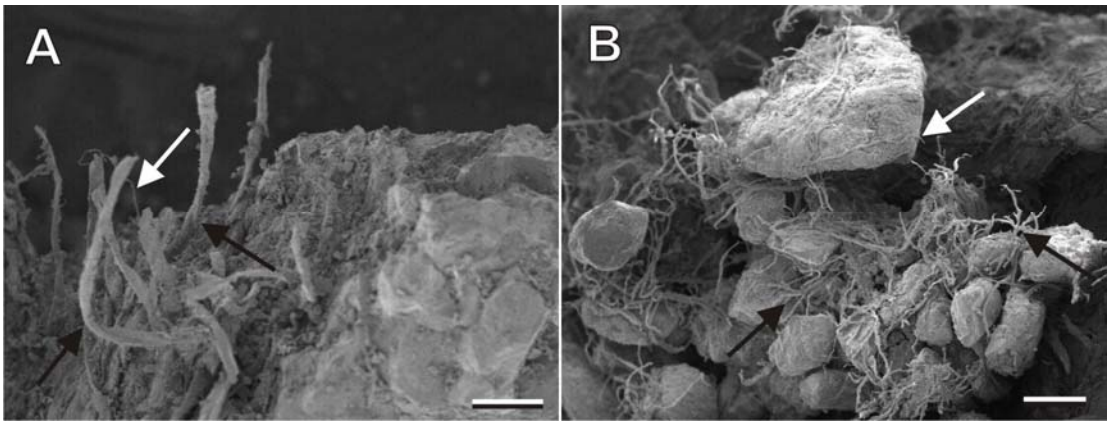


**Figure 1.11** Scanning electron micrographs of collections made in the Taklimakan Desert. (A) Filamentous cyanobacteria (black arrows) and fungal hyphae (white arrow) growing on rock surface (bar 100 μm). (B) Biological soil crust with soil particles attached by filamentous cyanobacteria (black arrows) and fungal hyphae (white arrow) (bar 100 μm). (J.A. Jurgens, unpublished)

The partnership is between a mycobiont, a fungus, and a photobiont which can be either a green alga or a cyanobacterium, with some lichens containing both. Similar communities can also be found in biological soil crusts which are associations between soil particles, cyanobacteria, algae, fungi, lichens and bryotrophs which live on top of or in the upper few millimeters of soil (Büdel 2005). Although these crusts can be found in a variety of biomes as pioneers, permanent soil crusts are most often found in regions that restrict vascular plants such as hot and cold deserts, polar, alpine and tundra settings (Galun and Garty 2001; Green and Broady 2001; Hansen 2001; Türk and Gärtner 2001; Ullmann and Büdel 2001). These symbiotic associations are thought to assist each organism in nutrient acquisition, reproduction and to avoid the loss of energy and nutrients under the harsh conditions from which they are found (Gorbushina 2007; Seymour et al. 2005).

### *1.3.6 Wood cell*

#### *1.3.6.1 Structure*

Wood is an organic material, produced as secondary xylem in the stems of trees and other plants. Wood is a conglomeration of cells, each cell composed of layers which initially consist of the primary wall and often additional layers are deposited to form the secondary wall. Wood varies depending upon the source plant material but the following is a general discussion of wood cell structure. The wood cell layers primarily consist of varying amounts of the organic polymers cellulose, hemicellulose and lignin. Between adjacent cells with primary walls is the middle lamella, which together forms the highly lignified compound middle lamella. Inside the primary wall develops the secondary wall, consisting of three distinct layers; S1, S2, and S3, which vary in thickness and arrangement of cellulose (Figure 1.12). The S1 is the outer most portion of the secondary wall and has the greatest amount of lignin with the microfibril orientation nearly perpendicular to the length of the cell. The largest layer in the secondary cell wall is the S2, composed primarily of cellulose fibrils oriented nearly perpendicular to those in the S1. The inner most layer is the S3 which has the greatest amount of hemicellulose compared to the others and a fibril arrangement similar to the S1. These various layers surrounding the inner cell cavity are called the lumen. The wood cell is composed of differing polymers, arranged in varying direction, which ultimately are responsible for the structural strength and stability of wood.

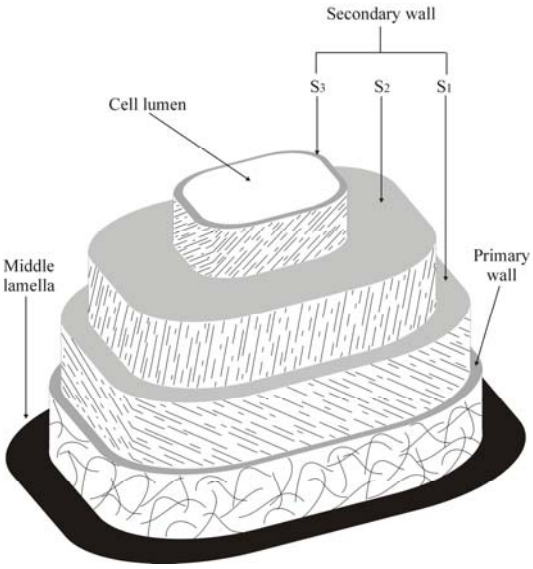


**Figure 1.12** Wood cell wall layers and orientation of microfibrils.

### *1.3.6.2 Wood cell wall chemical composition*

#### *1.3.6.2.1 Cellulose*

Cellulose is the most significant component of the wood cell wall in terms of its volume and is considered to be the most important raw material of plant origin as nearly half of the biomass produced by photosynthesis is made of this material (Eriksson et al. 1990). In the photosynthetic process, glucose is produced and the condensation of two of these molecules into a disaccharide called cellobiose becomes the basic structural units of cellulose. The two glucose molecules of cellobiose are linked through a β-(1-4)- glycosidic bond which leads to the glucose being rotated 180º and the cellulose polymer can consist of hundreds of glucose thus linked together (Figure 1.13). The cellulose polymer forms parallel chains which result in microfibrils that have intra- and intermolecular hydrogen bonds between each glucosidic bond. The microfibril can be a few to many cellulose chains. This

molecular arrangement of intra and inter bonds make the microfibrils incredibly strong and can be several thousand units in length. The microfibrils are a mix of very organized crystalline zones (60-70%) interspersed with noncrystalline (amorphous) regions (30-40%) bound tightly and are responsible for much of the wood cell wall tensile strength. Cellulose accounts for approximately 40-45% of wood cell walls (Zabel and Morrell 1992).

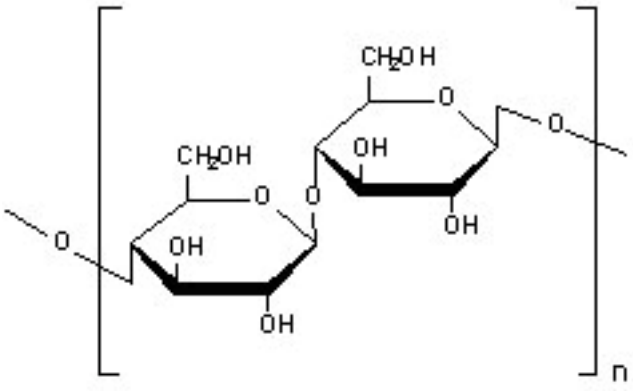


**Figure 1.13** Chemical structure of cellulose showing the bonds between glucose molecules (Alex Willis, Princeton University).

### *1.3.6.2.2 Hemicellulose*

Unlike cellulose, which is composed entirely of glucose, hemicellulose composition is a matrix of polysaccharides containing many different sugar monomers (Figure 1.14). These sugars include xylose, glucose, mannose, galactose, rhamnose and arabinose. The arrangement of these sugars in hemicellulose is also very different than the linear composition found in cellulose with the polymers of the various pentose and hexose units often having side chains and branches. These hemicellulose branches help bind the microfibrils to each other and to other matrix components,

particularly the pectins. This interlinked network of pectin and hemicellulose also help to bind adjacent cells to each other (Zabel and Morrell 1992). The type and amount of hemicellulose in the cell walls of hardwoods and softwoods are different. Softwoods typically have less hemicellulose than hardwoods with xylose as the most common constituent of hardwoods and mannose of conifers.

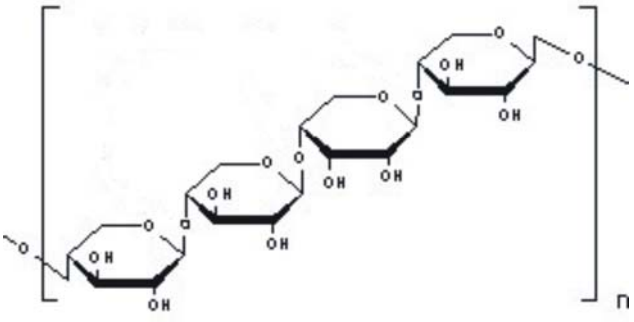

**Figure 1.14** Chemical structure of hemicellulose (Alex Willis, Princeton University).

### *1.3.6.2.3 Lignin*

Lignin is the second most abundant terrestrial biopolymer, second only to cellulose, accounting for approximately 30% of the organic carbon on earth and is the most abundant renewable aromatic polymer (Boerjan et al. 2003). Although the biosynthesis of lignin is complex and aspects of its biochemistry are still being studied, it is known that they are complex racemic aromatic heteropolymers primarily derived from three hydroxycinnamyl alcohol monomers (Figure 1.15). When these three monolignols are incorporated into the lignin polymer, *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units are produced which vary in their composition in hardwoods and softwoods. The lignin in hardwoods principally consists of guaiacyl and syringyl with only traces of *p*-hydroxyphenyl and the

softwoods are composed primarily of guaiacyl with minor amounts of *p*-hydroxyphenyl. Lignin plays a central role in the strength and stiffness of the wood cell wall and because of its hydrophobic nature it is important in the transport of water and solutes in the tree. It has also been shown to play a role in resistance to microorganisms and insects (Boerjan et al. 2003; Haygreen and Boyer 1989). The type of lignin is also of great importance in wood degradation by fungi, with the syringyl units having less resistance than guaiacyl. Greater concentrations of quaiacyl units are frequently found in the vessel elements of hardwoods and generally in softwoods, creating a more difficult substrate for most fungi to degrade (Blanchette et al. 1988; Eriksson et al. 1990). Lignin typically accounts for 20-30% of the wood cell wall composition, with softwoods often having a higher content than the hardwoods and a different composition.

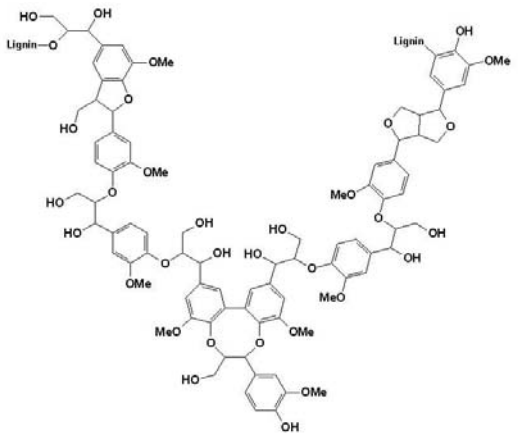


**Figure 1.15** Chemical structure of a small portion of the lignin polymer (Alex Willis, Princeton University).

#### *1.3.6.2.4 Additional components*

In addition to the three primary components, cellulose, hemicellulose and lignin, there are other carbohydrates and substances referred to as extractives found in the wood cell wall. The additional carbohydrates primarily consist of pectin, which is found in the middle lamella and in structures called bordered pits that are responsible for transport between cells, and starch, most often found in parenchyma cells and is responsible for storage of food reserves. Wood extractives are organic compounds that come in a wide range of classes, with the most important being the polyphenols, but others include tannins, oils fats and waxes. These substances are in part responsible for many of the physical properties of wood such as hardness and strength and can play a key role in resistance to microorganisms and insects (Zabel and Morrell 1992).

### *1.3.7 Fungal wood decay in temperate and tropical regions*

The decay of wood can occur in various biomes and under varying conditions with the most aggressive forms of degradation found in temperate and tropical terrestrial regions where conditions for fungal cell function are most conducive in facilitating growth and reproduction. Fungi that are responsible for the degradation of wood in these regions are often categorized into groups that have general names which represent either the color or texture of the substrate after decay has taken place.

#### *1.3.7.1 Brown rot decay in wood caused by fungi*

Wood that has been decayed by a brown rot fungus will generally have a brown appearance that is a result of extensive degradation of the cellulose and hemicellulose

and only minor alteration of the darkly colored lignin. Wood has considerable strength loss even at the early stages of brown rot, although cellular structure is often well defined. The process initiates with the fungi producing enzymes which catalyze a demethylation of lignin (Kirk 1971; Filley et al. 2002) that creates openings allowing small, diffusible, extracellular oxidants to access the cell causing an oxidative depolymerization of the cellulose and hemicellulose by means of an iron-peroxide reaction (Fenton reaction) (Jensen et al. 2001). Subsequent activity by the fungi produces hydrolytic enzymes that catalyze reactions on the hemicellulose, which releases the cellulose microfibrils and additional enzymes catalyze depolymerisation of both substrates. As decay progresses, the lignin rich framework of wood cells tend to have a wavy appearance, clearly exhibiting the significant strength loss caused by the fungi (Figure 1.16). In advanced stages, the wood cells become exceedingly brittle and will fracture into tiny cube-like fragments. Brown rot decay exclusively occurs in terrestrial systems with the greatest diversity and impact taking place in temperate and tropical biomes. However, there are examples of historically important wood being attacked by brown rot fungi in exceedingly dry regions, such as structural beams in Chacoan great houses (900-1200 AD) from a desert in the southwestern United States (Blanchette et al. 2004) and wood excavated from ancient tombs in Egypt (> 4000 years) (Blanchette et al. 1994).

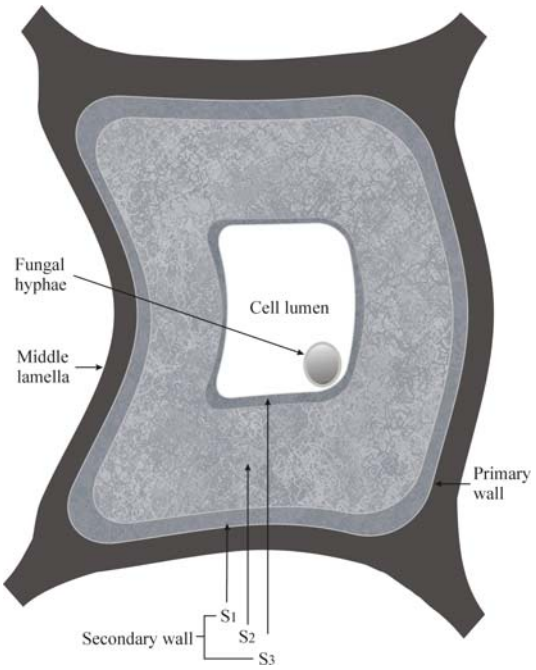


**Figure 1.16** Wood cell wall decayed by a brown rot fungus causing a depolymerization of the cellulose and hemicellulose, leaving a lignin rich, weakened substrate (drawing by J.A. Jurgens, In: Blanchette 2009).

### *1.3.7.2 White rot wood decay caused by fungi*

White rot decay fungi are a large group of fungi in the Basidiomycotina. They are capable of producing a broad array of oxidases, peroxidases, and hydrogen peroxide that initially depolymerize the darkly colored lignin, leaving the hemicellulose and cellulose, which give the remaining substrate a white appearance and the name of this decay category. The primary enzymes responsible for the degradation of the lignin are lignin peroxidase, manganese peroxidase, and laccase, however, several other enzymes also play a role (Cullen and Kersten 2004; Kirk and Farrell 1987). Although white rot fungi attack lignin, they are also able to metabolize all major cell wall components. The capability and extent with which the fungi do these activities is broad; for instance, some fungal white rot isolates have little activity on the cellulosic component and others have extensive activity on them. White rot decay fungi are

prolific wood degrading organisms in temperate and tropical terrestrial biomes and have not been identified as causing decay under extreme conditions.

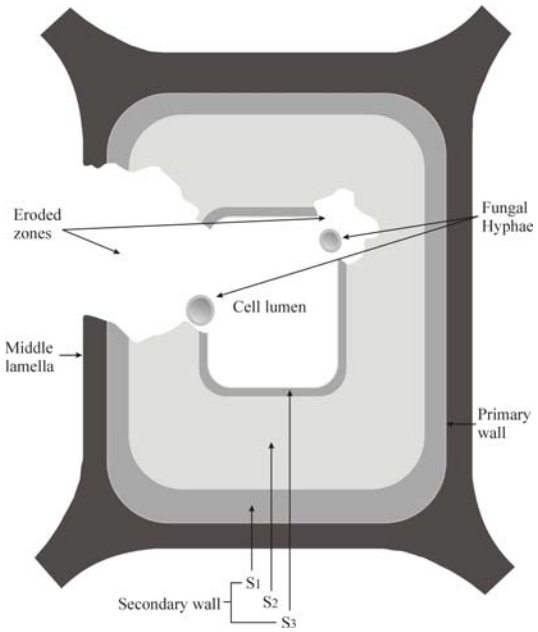


**Figure 1.17** White rot fungal decay of wood cell causing a simultaneous attack of all cell wall layers. (drawing by J.A. Jurgens, In: Blanchette 2009).

There are two forms of white rot decay which are differentiated based on the chemical and morphological characteristics of the degraded wood and the forms are caused by varying amounts, types and combinations of enzymes produced by fungi (Blanchette 1991). One type is a simultaneous attack of all major components within the cell wall causing an erosion and thinning of the wood cell (Figure 1.17). The second is a selective attack of the lignin components, leaving the cellulose and hemicellulose relatively unaltered. This type of decay can leave the cells separated from one another as the lignin rich middle lamella is degraded with only the very resistant cell corners remaining in advanced stages of decay (Figure 1.18) (Eriksson et al. 1990).

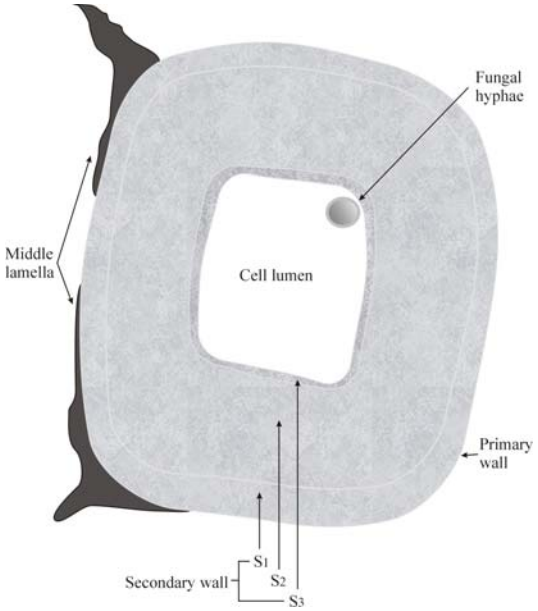


**Figure 1.18** A selective attack of the lignin rich middle lamella, leaving the remaining cellulose and hemicellulose in a wood cell wall is characteristic of one type of white rot decay by fungi (drawing by J.A. Jurgens, In: Blanchette 2009).

### *1.3.8 Wood degrading organisms in extreme environments*

All wood degrading microorganisms have general requirements for survival that include moisture, oxygen, nutrients, favorable temperature, suitable pH and a non-toxic substrate (Blanchette and Hoffman, 1994; Eaton and Hale, 1993). If any of these requirements are not provided, their development is greatly reduced or nonexistent. In most terrestrial conditions where these requirements are met, brown and white rot fungi are typically responsible for rapid degradation of wood (Blanchette and Hoffman, 1994). However, in adverse environments where the above listed requirements are in excess or greatly reduced, soft rot fungi and/or bacteria are the dominant organisms that attack wood, causing a relatively slow decay (Blanchette et al. 2004; Daniel and Nilsson 1997; Held et al. 2005; Singh and Butcher 1991).

#### *1.3.8.1 Fungal wood degradation in extreme environments*

##### *1.3.8.1.1 Soft rot decay*

Fungi that cause soft rot decay in wood belong to the *Ascomycota* and *Deuteromycota* phyla. They are not aggressive decay organisms and may not compete well with brown and white rot fungi under normal conditions but they are most often found in woods that have limited access to oxygen, or are found in environments of moisture extremes that will not support the basidiomycetous decay fungi. Fungal soft rot decay can be divided into two categories: type 1 or cavity formation and type 2 or erosion attack, both of which can be observed in hardwoods and softwoods. The type 1 form of decay is characterized by a series of angular or diamond shaped cavities in the S2 layer of the wood cell wall that follow the cellulose microfibril orientation when viewed longitudinally (Figure 1.19). When viewed transversely, these chains of cavities with conical ends are typically seen as round holes of varying sizes (Figure 1.20). The fungus enters the wood cell wall through the lumen by creating a small bore hole in the S3 layer and the hyphae create an L or T branch perpendicular to the bore hole which are aligned with the S2 microfibrils. The growth then stops temporarily until enzymes are released causing cavities to expand for a period and hyphal growth begins again with the production of proboscis hyphae, which may extend from one or both ends of the cavity (Hale and Eaton 1985b). This alternating growth and enzyme release creates a chain of cavities that are connected by angular ends (Hale and Eaton 1985a). Type 1 soft rot decay primarily affects the S2 and S1 layers, with minimal or no damage to the S3 and does not degrade the middle lamella (Eaton and Hale 1993; Khalili et al. 2001).

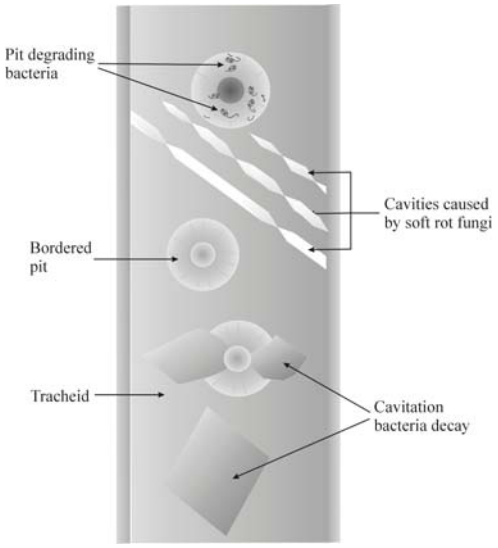


**Figure 1.19** Longitudinal view of a wood cell, with degradation caused by cavitation and pit degrading bacteria and soft rot fungi (Jurgens et al. 2003).

A complete erosion of the secondary wall and a slight modification of the middle lamella in advanced stages of degradation characterize the type 2 form of soft rot decay (Daniel and Nilsson, 1997). This decay reacts differently when eroding the secondary wall layers of hardwoods and softwoods (Eaton and Hale 1993), with the process in hardwoods often consisting of troughs of different sizes giving the degraded wood a striped appearance. The degradation occurs in all secondary cell wall layers, starting at the lumen and moving towards the middle lamellae. In softwoods, the fungus enters the wood cell through the S3 layer, but does not appear to degrade it. The decay progresses into the cellulose rich S2 layer and has only a limited effect on the S1 and no effect on the middle lamellae (Nilsson et al. 1989). In advanced stages of type 2 decay, only a framework of lignin rich middle lamellae will remain. Cellulose degradation is the primary focus of soft rot decay fungi, however, hemicellulose and lignin can also be degraded with the carbohydrates often being removed faster than the lignin in hardwoods and the lignin removed faster than the

hemicellulose and cellulose in softwoods (Eslyn et al. 1975; Haider and Trojanowski 1975 & 1980).

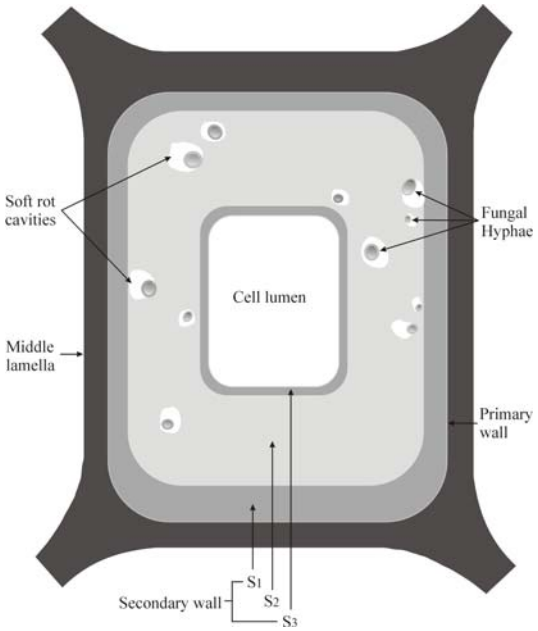


**Figure 1.20** Soft rot decay causing a selective attack of cellulose in the S2 layer of a wood cell wall (drawing by J.A. Jurgens, In: Blanchette 2009).

### *1.3.8.2 Bacterial wood degradation in extreme environments*

Bacteria require high moisture contents in the substrates they are degrading and are most common in waterlogged environments which tend to exclude most types of fungal decay, giving these Prokaryotes a competitive advantage. Some of the more commom genera identified in association with wood from these environments include *Cytophaga-Flavobacterium-Bacteroides* (CFB) *Pseudomonas*, *Cellvibrio* and *Brevundimonas* (Landy et al. 2008; Nilsson and Björdal 2008.). Wood degrading bacteria are often divided into three major groups, as follows: erosion, tunneling and cavitation bacteria (Jurgens et al. 2003). These descriptive names are based on the degradation appearance within the various wood cell wall layers the bacteria attack.

There are also pit degrading and scavenging bacteria which degrade wood or wood residues but they have not been studied extensively (Burnes et al. 2000; Singh and Butcher 1991). The pit degrading bacteria focus their attack on bordered pits and related structures and scavenging bacteria are considered secondary degraders that gain their required resources from residual material generated by primary degraders. These organisms may degrade substrates individually or they may work together to slowly degrade wood in marine environments.

#### *1.3.8.2.1 Erosion bacteria*

In marine environments one of the most dominant types of wood degradation appears to be caused by erosion bacteria (Singh and Kim 1997). This type of cell wall attack starts as conical troughs that initiate from the bacterium penetrating the S3 layer from the cell lumen and move toward the middle lamellae. The bacteria appear to preferentially degrade cell wall layers that have greater amounts of cellulose and hemicellulose and avoid regions of the cell with high lignin contents (Figure 1.21). The secondary cell wall, consisting of the S1, S2 and S3 layers, is the largest region of the cell wall and the layer most degraded. Although the bacteria penetrate localized areas of the S3, they do not fully degrade this layer. The S3 layer is often absent in areas of the wood cell wall where extensive degradation of the S2 layer has occurred and erosion troughs have coalesced into larger voids. It is not apparent, however, whether this is caused by direct bacterial degradation or a loss of structural support from the underlying S2 layer after it has been degraded (Daniel and Nilsson 1997). Within the secondary wall, erosion bacteria tend to follow the cellulose microfibril orientation; after advanced decay by these bacteria, the secondary wall is riddled with

holes but the middle lamella remains unaltered. Wood decay by bacteria requires some oxygen for degradation to progress but erosion bacteria apparently can tolerate near anaerobic conditions since they can be found in sediment covered, waterlogged woods (Daniel and Nilsson 1997).

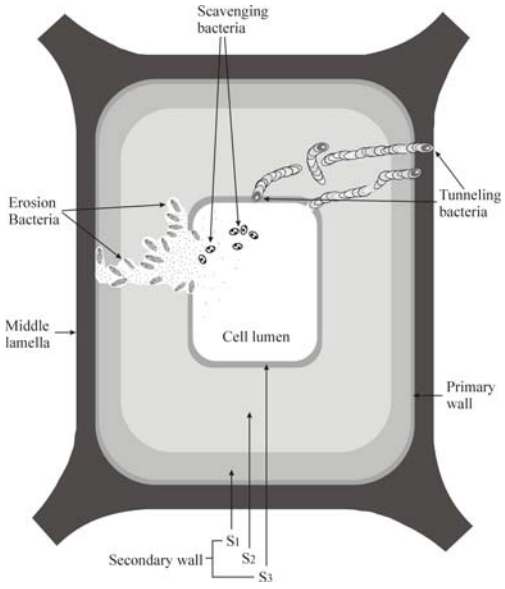


**Figure 1.21** Transverse view of a wood cell and cell wall layers with several types of degradation caused by wood degrading bacteria (Jurgens et al. 2003).

### *1.3.8.2.2 Tunneling bacteria*

Tunneling bacteria are aggressive organisms that not only have the ability to degrade the secondary wood cell wall but also the lignin and middle lamella region. They enter wood cells through the lumen and have also have been observed entering the secondary wall via the chambers of bordered pits when the lumen is full of extractives (Singh and Butcher

1991). Once the cell wall has been penetrated, they degrade all layers of the secondary wall and often do not follow the orientation of cellulose microfibrills in the

S2 layer. They produce small tunnels that are similar in diameter to the bacterium and as the decay progresses in the cell wall, concentric bands of residual materials from degradation and extracellular slime are left behind (Figure 1.21). Under favorable conditions the small tunnels eventually coalesce forming larger areas of degradation. One of the few areas of the wood cell that appears to be immune to degradation by tunneling bacteria is the corner regions of the middle lamellae (Singh and Butcher 1991). Although evidence of tunneling and erosion bacteria can often be found within the same cell wall, tunneling bacteria seem to require greater oxygen concentrations than erosion bacteria (Björdal 2000).

#### *1.3.8.2.3 Cavitation bacteria*

Similar to the erosion and tunneling bacteria, cavitation bacteria appear to enter the cell wall by boring a small hole in the S3 layer but do not degrade large areas of this layer. They focus on causing cavities in the S2 layer by removing the cellulose and hemicellulose and in advanced stages of decay the thin S3 appears to collapse because of reduced support. Studies have also shown that the cavities created by these bacteria are not limited to the S2 and can extend into the S1 layer (Singh and Butcher 1991). These cavities are often associated with bordered pits, which may implicate the pit chamber as another potential, however, they have also been observed in areas adjacent to the bordered pits suggesting direct penetration of the cell wall is also possible (Singh and Butcher 1991). The cavities are generally angular and often diamond shaped with the long axis running either parallel to or perpendicular to the long axis of the cell (Figure 1.19). This type of wood degrading bacteria does not appear to have the ability to degrade the lignin rich middle lamellae. The oxygen

requirement for cavitation bacteria is not yet known but they do not appear to be common in extremely oxygen depleted environments.

#### *1.3.8.2.4 Pit degrading bacteria*

Bordered pits of the wood cell wall appear to be only an entrance point for both cavitation and tunneling bacteria but there is a type of bacteria that seem to selectively degrade only the pit membrane and not the cell wall (Burnes et al. 2000; Singh 1997). These pit degrading bacteria preferentially degrade the nonlignified, pectin-rich region of the pit membrane including the margo and torus, which are primarily composed of cellulose (Figure 1.19). They accumulate in the pit chamber and attach to the microfibrils of the margo and completely destroy this structure, leaving the torus partially intact or even causing a complete dissolution of both structures.

#### *1.3.8.2.5 Scavenging bacteria and environmental influences*

The effects of scavenging bacteria and environmental influences often mask degradation patterns created by wood degrading bacteria and soft rot fungi (Blanchette 1995). Scavenging or secondary bacteria degrade the residual materials left after degradation by tunneling and erosion bacteria (Figure 1.21). They are not found in sound wood or areas were primary bacterial degradation is occurring and appear to rely on degraded cell wall components (Singh and Butcher 1991). The influence of environmental factors on bacterial degraded woods that have been exposed to marine environments for long periods often remove or alter residual material, creating large voids in the wood cell making positive identification of

primary degraders difficult. Scavenging bacteria appear to tolerate near anaerobic conditions, similar to that of erosion bacteria.

### *1.3.9 Wood degrading fungal enzymes*

#### *1.3.9.1 Lignocellulolytic enzymes*

Lignocellulolytic biomass consists of three primary components, cellulose, hemicellulose and lignin, with the enzymes responsible for their breakdown falling into three general categories, cellulases, hemicellulases and ligninases. These enzymes are essential in the carbon cycle on our planet and can be produced by a variety of organisms. However, the focus in this section will be on those produced by fungi.

#### *1.3.9.2 Cellulose degradation by cellulase complex*

To degrade cellulose is not an easy task as no individual enzyme is capable of complete hydrolysis of this substrate. A cellulase complex consisting of several enzymes acting sequentially and together as well as a radical based system based on redox chemistry are required to fully hydrolyse cellulose, and many of these enzymes have feedback inhibition from the catalyzed products of their reactions, thus a complicated biochemistry is observed for cellulolysis (Eriksson and Wood 1985).

These extracellular, hydrolytic enzymes initiate the reaction which breaks the long cellulose chain into shorter glucose polymers and eventually into glucose chains which can be readily absorbed by fungi (Figure 1.22). The set of hydrolytic enzymes

that are responsible for the degradation of crystalline cellulose are composed of the following enzymes (Baldrian & Valášková 2008):

- endo-1,4-$\beta$-glucanases (EC 3.2.1.4) which over the cellulose chain randomly attack and split $\beta(1\rightarrow4)$-glucosidic linkages.
- exo-1,4-$\beta$-glucanases (EC 3.2.1.91) that splits off either cellobiose or glucose from the non-reducing end of the cellulose.
- 1,4-$\beta$-Glucosidase (EC 3.2.1.21) hydrolysing cellobiose and other water-soluble cellodextrines to glucose .

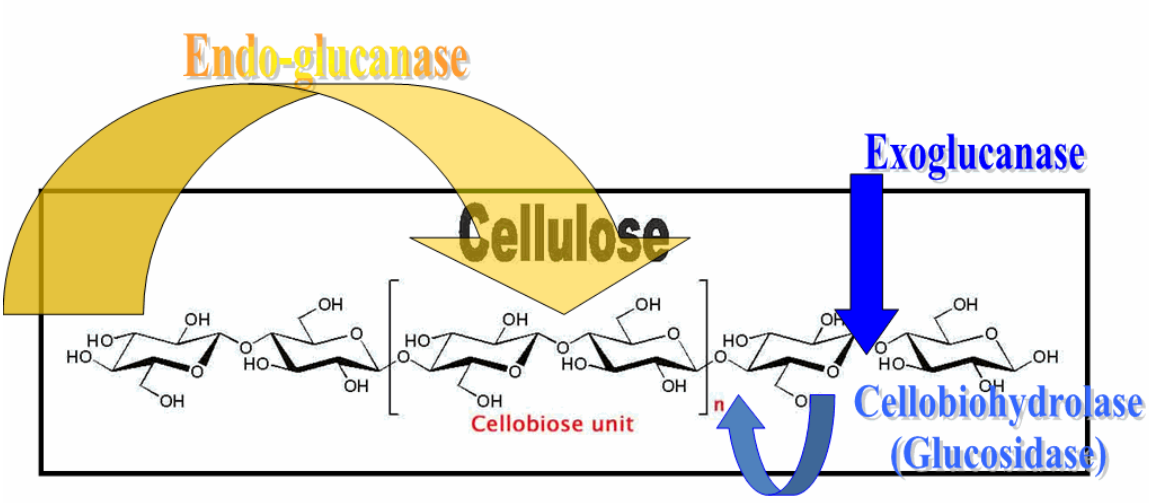


**Figure 1.22** Schematic of the cellulase complex responsible for cellulose degradation by fungi (R.L. Farrell, University of Waikato).

Additionally oxidative enzymes are required to fully degrade the cellulose polymers, which include Cellobiose: quinone oxidoreductase (cellobiose dehydrogenase) reduces quinones and phenoxy radicals in the presence of cellobiose, which is oxidised to cellobiono-$\delta$-lactone (Ander et al. 1990).

#### *1.3.9.3 Hemicellulose degradation by hemicellulase complex*

Hemicelluloses are the second most abundant component of lignocellulosic biomass, with xylan contributing to more than 70% of their structure (Dashtban et al. 2009). The heterogeneity of hemicellulose molecules makes their degradation difficult and requires a variety of enzymes, generally refered to as hemicellulases. These include; xylanases, β-xylosidase, endoglucanases, endomannanases, β-mannosidases β-mannanases, arabinofuranosidases, α-L-arabinanases and α -galactosidases (Karboune et al. 2009 & Saha 2003). When these enzymes work in concert, their synergistic action has been shown to be more efficient compared to their individual activity (Romero et al. 1999).

#### *1.3.9.4 Lignin degradation by lignin-modifying enzymes*

Lignin is the third most common polymer in lignocellulosic substrates and is considered the most recalcitrant component of this material to degrade (Sanchez 2009). The composistion of lignin generally includes three aromatic alcohols including coniferyl alcohol, sinapyl and p-coumaryl, which often link cellulose and hemicellulose, preventing penetration of enzymes to the interior of the lignocellulosic structure (Dashtban et al. 2009). The enzymes that are responsible for the degradation of lignin are refered to as lignin-modifying enzymes (LMEs) and are oxidative in their enzymatic mechanisms, not hydrolytic. There are two general groups of LMEs with the first including the peroxidases, such as lignin peroxidase, manganese peroxidase and versatile peroxidase, and many phenol-oxidases of the laccase type (Martinez 2005).

## Chapter 2

**Materials and methods relevant to Taklimakan Desert: sample collection, soil and wood analysis, fungal isolation and molecular analysis**

### *2.1 Sample Collection from Taklimakan Desert*

A total of 20 samples were collected near Ruoqiang in the Taklimakan Desert, Xinjiang Uyghur Autonomous Region of the People's Republic of China in June 2007. The map of the Taklimakan Desert in Figure 2.1 identifies the approximate sample collection location with a red dot at coordinates (38º 59' 3" N, 88º 05' 6"E).

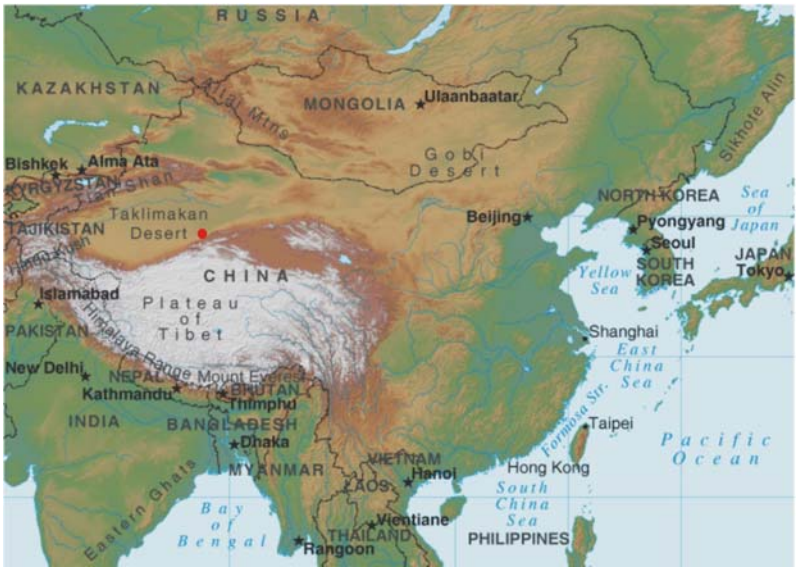


**Figure 2.1** Map of China with the red dot indicating the approximate location of Taklimakan Desert sample collection site.

Figure 2.2 shows a photograph of the actual site area. The samples were collected within a 1 square kilometer site. This site was selected because of proximity to the weather station at Ruoqiang, which has been identified to be the location with the least precipitation in China and one of the driest locations in the world based on meteorological data collected over 37 years (Hong Kong Observatory, The

Government of Hong Kong Special Administrative Region). The site was also selected because of its remote location and minimal human disturbance. All samples were collected by the PhD thesis candidate.

**Figure 2.2** Site where soil, rock and wood samples were collected in the Taklimakan Desert, China.

Samples of soil/sand, rock and wood (example shown in Figure 2.3) were aseptically collected and placed in sterile bags.

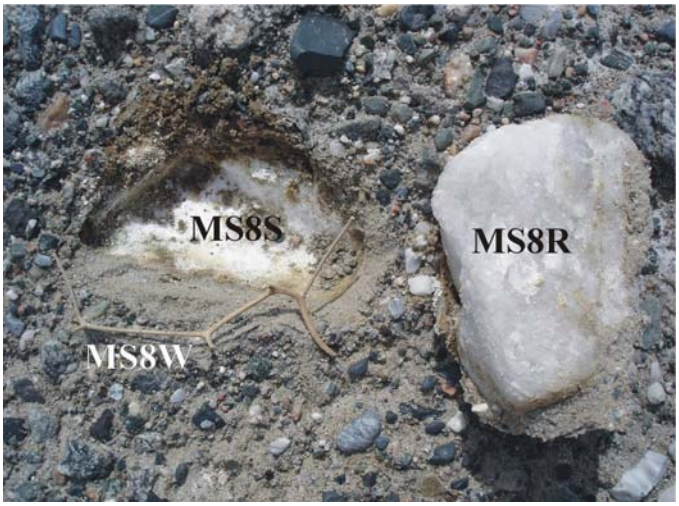


**Figure 2.3** Samples MS8S (soil), MS8R (rock) and MS8W (wood) *in situ* collected in the Taklimakan Desert.

All measures were taken to reduce the risk of cross contamination during the collection by using sterile gloves and instruments that were either wiped or sprayed with 70% EtOH between each collection. The samples were transported to the University of Waikato, New Zealand under Ministry of Agriculture and Forestry, New Zealand permit # 2007030892. These samples were stored at 5°C and after 1 month were taken to the University of Minnesota, U.S.A, under the United States Department of Agriculture, Animal and Plant Health Inspection Services, Plant Protection and Quarantine permit # P526P-08-0018. Transportation was done at ambient temperature and upon arrival at the final location; samples were stored at 5°C. During sampling in the desert, rocks were lifted from their original position and investigated to determine if there was a green or dark colored band in the region where they were in contact with the surface of the soil which indicated the potential presences of phototrophic algae or cyanobacteria. Rocks that exhibited this phenomenon were determined to potentially be colonized by microorganisms and were collected. These rocks were exceedingly rare and primarily white or translucent. Figure 2.4 shows a photograph *in situ* of a rock with a green area which had been in contact with the soil; this rock and soil were sampled and the sample numbers are respectively, MS2R and MS2S.

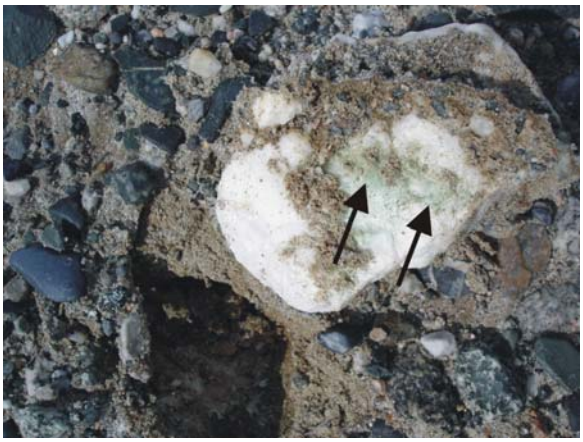

**Figure 2.4** Rock (MS2R) removed from its original position in the Taklimakan Desert with arrows identifying dark green zone, indicating potential colonization by microorganisms.

Approximately, 150-200 g of soil/sand was also collected with a sterile scoop directly under the rocks that were collected and if any organic material (i.e. wood) was within 10 cm of the same rock it was aseptically collected as well and placed into sterile bags. Samples of soil/sand, rock and wood that were not associated with one another were also collected in a similar manner. For the PhD thesis research a total of 7 rock, 9 sand/soil and 4 wood samples were collected and independently investigated (Table 2.1). This collection was completed in association with Associate Professor Steven Pointing and Dr. Maggie Lau of the University of Hong Kong who made additional collections during the same period and the results of a portion of their investigations will by included in a comparative study of some materials in this thesis and subsequently published. All remaining samples and cultures are archived at the University of Minnesota Department of Forest Pathology culture collection, under the supervision of Professor Robert Blanchette.

**Table 2.1** List of samples collected in one square kilometer site (38º 59' 3" N, 88º 05' 6"E) in the Taklimakan Desert for fungal isolation and associated investigation related to soil composition and isolation of environmental DNA.

| Sample number | Soil | Rock | Wood |
|---|---|---|---|
| MS2 | X[b] | X | |
| MS3 | X | | |
| MS4 | | X | |
| MS5 | X[a] | | |
| MS6 | X | | |
| MS8 | X[b] | X[b] | X |
| MS9 | X[a] | | |
| MS10 | X[a] | X | |
| MS11 | | X | |
| MS13 | | X | X |
| MS15 | X[a] | | X[b] |
| MS16 | X | X | |
| MSW | | | X |

[a]Samples used for soil testing.
[b]Samples used in DGGE investigation

### *2.2 Sample analysis of collections made in the Taklimakan Desert*

#### *2.2.1 Soil analysis*

Of the 9 soil samples taken, 4 were chosen (MS5S, MS9S, MS10S and MS15S) based on their association with the other collected rock or wood samples, or lack of association with these other substrates (Table 2.1), for soil analysis conducted by R.J. Hill Laboratories Limited, Hamilton, New Zealand. Table 2.2 is a summary of the methods used and the detection limits of the procedures.

**Table 2.2.** Parameters, methods and detection limits of soil analysis conducted on samples collected in the Taklimakan Desert, by R.J. Hill Laboratories Limited, Hamilton, New Zealand.

| **Parameters** | **Method used** | **Detection limit** |
|---|---|---|
| Dry and sieve sample | Air dry (35°C), sieved to pass 2 mm | N/A |
| Total recoverable digest | Nitric / hydrochloric acid digestion. US EPA 200.2 | N/A |
| pH | 1:2 v/v soil / water slurry, pH meter. APHA 4500-$H^+$ B $20^{th}$ ed. 1998 | 0.1 pH units |
| Dry matter | Dried at 103°C for 24 hours, gravimetric. | 0.1 g/100 g |
| Total recoverable calcium | Nitric / hydrochloric acid digestion, IPC-MS, US EPA 200.2 | 100 mg/kg dry weight |
| Total recoverable magnesium | Nitric / hydrochloric acid digestion, IPC-MS, US EPA 200.2 | 40 mg/kg dry weight |
| Total recoverable sodium | Nitric / hydrochloric acid digestion, IPC-MS, US EPA 200.2 | 40 mg/kg dry weight |
| Total recoverable phosphorus | Nitric / hydrochloric acid digestion, IPC-MS, US EPA 200.2 | 40 mg/kg dry weight |
| Total recoverable potassium | Nitric / hydrochloric acid digestion, IPC-MS, US EPA 200.2 | 100 mg/kg dry weight |
| Total organic carbon | 10% HCl, hotplate 2hrs, elementar combustion analyser. (Shimadzu 5000A TOC Analyser, Kyoto, Japan) | 0.05 g/100 g dry weight |
| Total carbon | Catalytic combustion (900°C, $0_2$), separation, thermal conductivity detector (Elementar, CHNOS elemental analyzer, vario MAX N/CN, Hanau, Germany). | 0.05 g/100 g dry weight |
| Total nitrogen | Catalytic combustion (900°C, $0_2$), separation, thermal conductivity detector (Elementar, CHNOS elemental analyzer, vario MAX N/CN, Hanau, Germany). | 0.05 g/100 g dry weight |

### *2.3 Microbial Isolation and morphological identification*

Soil/sand sample were sprinkled over 90 x 15 mm sterile, polystyrene Petri plates of each culture media described in section 2.3.1, distributed with approximately one sand grain per $mm^2$; for a total of 270 plates that were sealed with Parafilm. All rock samples were triple bagged in sterile Whirl Pak® bags (Nasco, Fort Atkinson, WI,

U.S.A.) in the laboratory, with the outside of each wiped with an alcohol pad and subsequently double bagged into 2 mm thick cotton sacks. The bagged rocks were placed on an anvil and struck with a hammer until they were pulverized. The resulting material was sprinkled over Petri plates of the different culture media (Section 2.3.1) as described above; for a total of 270 Parafilm sealed plates. Wood samples were cut with a sterile scalpel and 3 sections (approximately 1x1x3mm) of each placed onto each type of media; for a total of 120 plates sealed with Parafilm. All samples on each of the medium were inoculated without light, in triplicate with one of each having an incubation temperature of 2°C, 15°C and 25°C, for a total of 30 plates for each sample. Plates were monitored daily using a Nikon SMZ800 stereomicroscope (Nikon, Tokyo, Japan) for fungal growth. The plates were monitored daily for 28 days and hyphal tips were extracted with a sterile scalpel, transferred to 1.5% MEA and subsequent incubation at 22°C. All isolates are currently accessioned in duplicate at the University of Minnesota Forest Pathology culture collection.

### *2.3.1 Isolation Growth Media*

The following media, with indicated composition and method of preparation, were used for the isolation of fungi from all samples collected in the Taklimakan Desert. All samples listed in Table 2.1. were placed on all media types and monitored for up to 90 days. Unless otherwise indicated, all media were sterilized by autoclaving for 20 min at 121°C, and Petri plates were made immediately following, with approximately 15 ml media poured per 90 mm Petri plate. All chemical reagents were obtained from Sigma unless stated.

Basidiomycete semi-selective agar (BSA): BSA was made by adding 15 g malt extract, 15 g agar, 2 g yeast extract, 0.06 g benlate to 1 l deionized water. This was autoclaved for 20 minutes and cooled to 45°C before adding 0.01 g streptomycin sulfate and, according to a modification from Worrall (1991), 2 ml lactic acid.

Corn meal agar (CMA): CMA was made by adding 17 g corn meal agar to 1 l deionized water per the manufacturer's recommendation (Becton, Dickinson and Company, Sparks, MD, U.S.A.).

Corn meal agar + (CMA+): CMA was made by adding 17 g corn meal agar per manufacturer's recommendation (Becton, Dickinson and Company) to 1 l deionized water, autoclaved for 20 minutes and cooled to 45°C before adding 200 mg of chloramphenicol.

Malt extract agar 1.5% (1.5% MEA): This media was made by adding 1 l deionized water, 15 g malt extract, 15 g agar.

Malt extract agar 0.5% (0.5% MEA): This media was made by adding 1 l deionized water, 5 g malt extract, 15 g agar.

Malt yeast agar (MYA): The MYA media was made by adding 1 l deionized water, 15 g malt extract, 15 g agar, 2 g yeast.

Potato dextrose agar (PDA): PDA was made by adding 39 g potato agar per manufacturer's recommendation (Becton, Dickinson and Company) to 1 l deionized water.

Nutrient agar (NA): The nutrient agar media was made by adding 23 nutrient agar per manufacturer's recommendation (Becton, Dickinson and Company) to 1 l deionized water.

Sabouraud dextrose agar (SDA): SDA was made by adding 1 l deionized water, 40 g dextrose, 15 g agar and 10 g polypeptone peptone, autoclaved for 20 minutes and modified from Sabouraud (1892), by cooling to 45°C before adding 0.1 g cycloheximide and 0.05 g chloramphenicol.

Water agar (WA): The WA media was made by adding 1 l deionized water to 15 g agar.

### *2.3.2 Morphological identification*

Fungal cultures were generally identified based on morphological characteristics after individual isolates were transferred to 1.5% MEA. The identifications were initially based on gross macroscopic features of the cultures such as colony color and mycelial habit, including whether the hyphae were arial or growth was within the medium. Microscopic examination followed with emphasis on hyphal and spore characteristics and the use of published materials to aid in the identification of the cultures (Barnett and Hunter 1998; Wang and Zabel 1990) and comparison to materials in the University of Minnesota Forest Pathology Laboratory type culture collection.

## *2.4 Molecular investigations*

### *2.4.1 Deoxyribonucleic acid extractions*

#### *2.4.1.1 Deoxyribonucleic extractions from pure fungal isolates*

Deoxyribonucleic acid extraction was performed on hyphal tip purified fungal isolates that were transferred from their original isolation media to 1.5% MEA and incubated at 22°C for a minimum of one week. A 2 x 2 $cm^2$ area of mycelia from these cultures were removed from the agar with a sterile scalpel. The fungal material

was placed in a sterile 1.5 milliliter (ml) microcentrifuge tube that contained 15-20, 3-mm sterile glass beads. Added to this was 500 microliters (µl) of lysis buffer (10% 1 M Tris pH 8.0, 10% 0.5 M Ethylenediamine tetracetic acid [EDTA], 10% of a 1% sodium dodecyl sulfate [SDS] solution and 70% sterile distilled $H_2O$) which was then vortexed for 1-2 minutes. The solution was transferred to another 1.5 ml microcentrifuge tube, 275 µl of 7 M ammonium acetate (pH 7.0) added and incubated for 5 minutes at 65 °C, followed by 5 minutes on ice. Five hundred µl of chloroform was added, vortexed for 1 minute, centrifuged at 15000 revolutions per minute (rpm) for 5 minutes and the supernatant was removed to another 1.5 ml microcentrifuge tube. This was precipitated with 1 ml of isopropanol, incubated for 5 minutes at room temperature and centrifuged for 7 minutes at 15000 rpm. The isopropanol was removed by being poured off, the remaining pellet washed with 500 µl of 70% ethanol (EtOH), centrifuged for 3 minutes, EtOH removed, centrifuged for 1 minute, removed remaining EtOH and allowed to dry. The pellet was rehydrated by adding 100 µl of sterile distilled $H_2O$ or 1x TE, briefly vortexed, incubated for 15 minutes at 37°C and stored at -20°C. This procedure was modified from Zhong and Steffenson (2001).

#### *2.4.1.2 Deoxyribonucleic acid extractions from environmental samples*

The DNA from environmental sample was extracted using the UltraClean™ Soil DNA Kit as per manufacturer's instructions (MO BIO Laboratories Inc., Carlsbad, CA, U.S.A.). Independent soil (MS2S), independent wood (MS15W) and concurrent soil and rock (MS8S and MS8R) (Table 2.1) were investigated in this study. Sample

MS15W was placed in a sterile mortar, ground to a fine powder in liquid nitrogen with a pestle and DNA extracted using the same kit.

### *2.4.2 Polymerase chain reaction amplification*

#### *2.4.2.1 Polymerase chain reaction of pure fungal isolates*

The polymerase chain reaction (PCR) for amplification of pure fungal isolates consisted of a mixture of the following: 25 µl AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Foster City, CA, U.S.A.), 9.5 µl PCR-grade water, 1µl of eucaryote specific ITS1 primer, 1 µl ITS4 primer (10µM) (Table 2.3) and one of the following amplification additives: 1 µl 360 GC Enhancer (Applied Biosystems), 1 µl dimethyl sulfoxide (DMSO), 0.4 µl bovine serum albumin (BSA) or 1 µl betaine anhydrous. A MJ Research PTC-200 Peltier Thermal Cycler (Watertown, MA, U.S.A) was used with the following profile: 95°C for 10 min (initial denaturation); 35 cycles of 95°C for 30 sec (denaturation), 50°C for 30 sec (annealing), 72°C for 1 min (extension), followed by a final extension of 72°C for 7 min.

**Table 2.3** Primer and direction, sequence and associated reference of primers used in polymerase chain reactions.

| Primer | Sequence 5′- 3′ | Reference |
|---|---|---|
| ITS1 (forward) | TCC GTA GGT GAA CCT GCG G | White et al. 1990 |
| ITS4 (reverse) | TCC TCC GCT TAT TGA TAT GC | White et al. 1990 |
| ITS1F (forward) | CTT GGT CAT TTA GAG GAA GTA A | Gardes and Bruns 1993 |
| ITS3 (forward) | GCA TCG ATG AAG AAC GCA GC | White et al. 1990 |
| ITS4(GC) (reverse) | CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C | Arenz et al. 2006 |

#### *2.4.2.2 Polymerase chain reaction amplification of environmental samples*

The DNA from the four environmental samples was amplified by PCR with the primers ITS1F, a fungal specific primer and ITS4 (Table 2.3) using the protocol detailed in Section 2.4.2.1. PCR products were diluted to 1/100 and re-amplified by PCR (Section 2.4.2.1) using the primers ITS3 and ITS4(GC) (Table 2.3), which had a GC clamp added to the 5′ end of the amplicon to prevent total denaturation of the double stranded DNA fragment during denaturing gradient gel electrophoresis (DGGE).

### *2.4.3 Electrophoreses of amplicons*

#### *2.4.3.1 Electrophoreses of amplicons from pure fungal isolates*

All DNA extractions from all samples were submitted to agarose gel electrophoresis to verify the presences of DNA by using the amplicons on a 1% TBE agarose gel. This was done by adding 7 µl of each PCR product to 1 µl SYBR® Green 1 nucleic acid gel stain (Cambrex Bio Science Rockland Inc., Rockland, ME, U.S.A.) and 2 µl of 6X concentration gel loading dye (New England BioLabs, Ipswich, MA, U.S.A.) to each well with 2 µl of 1kb DNA ladder (New England BioLab), 6 µl distilled water and 2 µl gel loading dye (New England BioLab) to the first well in each row for molecular weight standards. The gel was transilluminated with a DR-45M Dark Reader™ (Clare Chemical Research, Denver, CO, U.S.A.). The gels were then photographed with a Canon DS6041 digital SLR camera (Canon Inc. Tokyo, Japan) to estimate quantity and quality of DNA (i.e. to make certain that it was high molecular weight and not degraded DNA). Only high quality DNA samples which illuminated in the gel when placed on the Dark Reader™ were further analyzed, which

then included positive and negative controls for comparison. For DNA that did not provide favorable results, the procedure for extraction was repeated with the specific fungal isolate. This procedure was modified from Zhong and Steffenson (2001).

#### *2.4.3.2 Denaturing gradient gel electrophoresis of environmental samples*

The DGGE gel had a denaturant gradient concentration of 30-60% in the direction of electrophoresis. This was made by first mixing 250 ml stock solutions of both concentrations, which for the 30% final concentration consisted of 40.6 ml 40% acrylamide, 31.9 g urea, 30 ml deionized formamide and 179.4 ml 1X Tris-acetate-ethylenediaminetetraacetic acid (TAE) and the 60% final concentration consisting of 40.6 ml 40% acrylamide, 63.75 g urea, 60 ml deionized formamide and 149.4 ml 1X TAE. The gradient mixture gel was made by adding 4.3 ml of the 60% and 7.2 ml of the 30% to a 50 ml flask (A) and 8.6 ml of 60% and 2.9 ml to another flask (B), to each of these was added 80 µl of 10% ammonium persulfate and 5 µl TEMED. The gel was cast by allowing the contents of flask B to drain into the casting plate first and then flask A to fill the plate to the top (while mixing together) where a well comb was inserted and then allowed to polymerize for 1 hour. The gel was subsequently placed into a Denaturing Gradient Gel Electophoresis System, model DGGE-2001 (C.B.S. Scientific Company, Inc. Del Mar, CA, U.S.A.) and wells were loaded with 10 µl of the PCR product and 10 µl of 4x loading dye (New England BioLab). A well was also loaded with 5 µl 1kb DNA ladder (New England BioLab), 5 µl distilled water and 10 µl 4x loading dye (New England BioLab) for molecular weight standards. The gel was electrophoresed for 8 hours in 1X TAE buffer at 60°C and 100 V, modified from Arenz et al. 2006.

When removed from the DGGE system, the gel was immersed in a solution of 5 µl SYBR® Green diluted in 50 ml of 1x TAE buffer for 10 min and visualized as described in Section 2.4.3.1. Each band in the gel that was seen on the Dark Reader™ was stabbed with a 20 ml sterile pipette tip which was placed in a microcentrifuge tube containing 20 ml of sterile distilled water for 20 min. The excised bands were re-amplified as described in Section 2.4.2.1 with ITS3 replacing ITS1 (Table 2.3). These re-amplified bands were DNA sequenced and identified as explained in Sections 2.4.4 and 2.4.5. DNA sequenced material that appeared to have mixed results or multiple reads were analyzed again through the DGGE process as described above until a clean product with a single sequence was achieved.

### *2.4.4 Deoxyribonucleic acid sequencing*

DNA sequencing for the 194 pure isolates and from environmental samples were performed by submitting a mixture of 4.9 µl of PCR-grade water, 0.42 µl of 10 µM of the desired primer (Table 2.3) and 0.75 µl of PCR product from each DNA extraction to analysis by an ABI PRISM™ 3730xl DNA Analyzer automated DNA sequencer (Applied Biosystems) at the BioMedical Genomics Center, University of Minnesota, St Paul, Minnesota, USA. All sequences were replicated at least twice.

### *2.4.5 Analysis of deoxyribonucleic acid sequences*

The DNA sequences were analyzed by Chromas Pro version 1.42 Software (Technelysium Ltd., Helensvale, Australia) and consensus sequences constructed after both forward and reverse sequences were aligned. Each consensus sequence was

analyzed for missing and or weak base matches and the strongest peak from either the forward or reverse was include where there was discontinuity. These assembled sequences were compared to the nucleotide database in GenBank, using BLASTn (Altschul et al. 1990) with the best match and associated data recorded. This was done by entering the sequences into the database and recording the match with the highest identity percent and score.

### *2.4.6 Phylogeny*

Neighbor joining analysis was conducted on all consensus ITS sequences derived from fungal isolates generated from samples (soil/sand, rock and wood) collected in the Taklimakan Desert. Sequences were aligned in DS Gene (Accelrys Inc. San Diago, CA, U.S.A.) using CLUSTALW (Thompson et al. 1994) and trimmed. Phylogenetic trees, as discussed in Section 1.3.4.3, are shown with lines representing distance (scale at bottom of page) and bootstrap values with 1000 replicas, presented as a percentage. Top NCBI BLAST matches of sequences originating from either published or of reputable culture collections were included in the constructed phylogenetic trees to assist in determining evolutionary placement of cultures derived from Taklimakan Desert samples.

## Chapter 3

## Antarctic and Arctic fungal isolates: collaborative research and publications concerning the polar regions

### *3.1 Introduction to Antarctic and Arctic collaborative research*

Some of the most extreme environments in the world are in the polar regions. The north and south polar regions have similarities and differences and both experience extreme cold with large fluctuations in temperature due to the oblique sun angle and long periods of light and dark in summer and winter months, respectively. Since 1999, the University of Waikato and Minnesota have had an ongoing research collaboration in Antarctica studying fungal biodiversity and elucidating the mechanism of cold adaptation and proliferation of life in that extreme environment. In 2001 the University of Minnesota research team extended this work to the Canadian High Arctic. The PhD candidate Joel Jurgens, prior to beginning the PhD candidacy, collaborated in both polar regions as a research scientist employed by the University of Minnesota. During the PhD candidacy, the polar regions research collaboration continued and is part of the PhD research. In chapter 3, the fungal isolates from the polar regions isolated by Joel Jurgens are described. In addition, chapter 3 lists the publications completed during the PhD candidacy of which Joel A. Jurgens was the primary author or a contributing author. Specific emphasis relative to the PhD thesis is given to the two publications related to Arctic and Antarctic investigations resulting in fungal isolates specifically used in the thesis research for comparative purposes. A brief description of Joel Jurgens' contribution to the research and writing of all publications and a copy of those with available PDF versions are included.

### *3.2 Antarctic and Arctic fungal isolates used in PhD thesis research*

Sequences of similar fungal taxa generated from soils, wood and organic materials collected in the Dry Valleys and Ross Island, Antarctica from Arenz et al. (2006) and sequences from similar fungi originating from mummified wood collected on Ellesmere Island in the Canadian Arctic (Jurgens et al. 2009) were included to compare these fungi from Polar deserts (Table 3.4) to those isolated and subsequently sequenced from the Taklimakan Desert.

The fungal sequences from the Antarctic that were used to compare diversity between this region, the Arctic and the Taklimakan Desert were generated from samples of soils, woods and various organic materials collected from 3 sites in the McMurdo Dry Valleys and 3 historic sites on Ross Island, Antarctica (Arenz et al. 2006) (Table 3.1). They were investigated by either traditional culturing and subsequent analysis of the ITS region of the rDNA or by DGGE examination.

The Arctic fungi that were utilized for comparison to those of the Antarctic and Taklimakan Desert were from collections made in the west valley below the retreating ad Astra Ice cap, Ellesmere Island, Nunavut, Canada (81°N, 76°W) (Jurgens et al. 2009) (Table 3.1). The samples that the fungal isolates originated from were mummified woods that had been buried and recently exposed by water movement and frost heaving. These woods are thought to be from the Eocene period of 33-55 million years ago, when forests dominated the region and were likely buried as a result of a massive catastrophic event which assisted in their preservation.

**Table 3.1** List of top BLAST matches with accession numbers of fungi from Arenz et al. (2006) Antarctic study and Jurgens et al. (2009) Arctic study included in phylogenetic investigations to compare with sequenced fungi from The Taklimakan Desert.

| Top BLAST Match & Accession # | Substrate | Accession | Region |
|---|---|---|---|
| Uncultured Basidiomycete (AM999656) | Wood | FJ457769 | Arctic |
| *Exophiala* sp. (DQ317336) | Wood | FJ457766 | Arctic |
| *Phialocephala* sp. (EF485233.1) | Wood | FJ457767 | Arctic |
| *Acephala* sp. (EU434829) | Wood | FJ457768 | Arctic |
| *Acephala* sp. (EU434829) | Wood | FJ457770 | Arctic |
| Fungal sp. AB10 (FJ235943) (*Cadophora* sp.) | Wood | FJ457771 | Arctic |
| Fungal sp. AB29 (FJ235962) | Wood | FJ457772 | Arctic |
| Uncultured fungus clone N3 (EF434036) | Wood | FJ457773 | Arctic |
| cf. *Pyrenopeziza revincta* (AJ430226) | Wood | FJ457774 | Arctic |
| Uncultured fungus clone N3 (EF434036) | Wood | FJ457775 | Arctic |
| *Exophiala spinifera* (AY843179) | Soil & wood | DQ317336 | Antarctica |
| *Pezizomycetes* sp. 11217B (GQ153164) | Soil | DQ317369 | Antarctica |
| *Thelebolus caninus* (AY957550) | Soil | DQ317350 | Antarctica |
| *Geomyces pannorum* (AY873967) | Soil & wood | DQ317339 | Antarctica |
| *Pseudeurotium* sp.olrim 176 (AY787729) | Organic material | DQ317348 | Antarctica |
| *Cadophora malorum* (AY249064) | Soil, wood & organic material | DQ317328 | Antarctica |
| *Penicillium echinulatum* (AF033473) | Soil, wood & organic material | DQ317344 | Antarctica |
| *Fusarium oxysporum* (AY188919) | Wood | DQ317368 | Antarctica |
| *Nectria* sp. (AY805575) | Wood | DQ317342 | Antarctica |
| *Cladosporium cladosporioides* (AY213641) | Soil & wood | DQ317332 | Antarctica |
| *Phoma herbarum* (AY293791) | Soil | DQ317345 | Antarctica |
| *Epicoccum nigrum* (AF455455) | Soil | DQ317367 | Antarctica |
| *Alternaria* sp. (AB470836) | Soil & wood | DQ317386 | Antarctica |
| *Chaetomium funicola* (AJ279450) | Wood | DQ317331 | Antarctica |
| *Ulocladium chartarum* (AY625071) | Soil | DQ317352 | Antarctica |
| *Uncultured Mortierellaceae* (AJ879650) | Soil | DQ317354 | Antarctica |

### *3.3 Arctic and Antarctic publications authored or co-authored during PhD candidacy*

The following citations were completed by the PhD candidate during the PhD candidacy.

The PhD candidate participated in the following associated with each publication:

- Pre trip planning and preparation
- Site sample collection and investigation
- Laboratory studies involving fungal isolation, molecular identification and wood species identification
- Scientific discussions and contributions to manuscript writing and editing

Additionally, the candidate was the event field leader associated with the Pointing et al. 2009 manuscript.

Jurgens, J.A., Blanchette, R.A., and Filley, T.R. 2009. Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic. *Polar Biol*. 32:751-758

Arenz, B.E., Held, B.W., Jurgens, J.A., Farrell R.L. and Blanchette, R.A. 2006. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. *Soil Biology and Biochemistry* 38: 3057-3064

The following publications, except for the book chapters are included in Appendix 2 as PDF versions.

Blanchette, R.A., Held, B.W., Arenz, B.E., Jurgens, J.A., Baltes, N.J., Duncan, S.M. and Farrell, R.L. 2009. An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Book chapter. In press.

Pointing, S.B. Lacap, D. C., Chan, Y., Lau, M., Jurgens. J.A., Farrell, R.L. 2009. Highly specialized microbial biodiversity in hyper-arid Polor desert. *Proc. Natl. Acad. Sci.* (USA) 106: 19964-19969

Blanchette, R.A., Held, B.W. and Jurgens, J.A. 2008. Northumberland House, Fort Conger and the Peary Huts in the Canadian High Arctic: current condition and assessment of wood deterioration taking place. In: *Historical Polar Bases – Preservation and Management*. Barr, S. and Chaplin, P. (eds). ICOMOS Monuments and Sites No.XVII. International Polar Heritage Committee, Oslo, Norway. pp.96. ISBN 978-82-996891-2-0

Farrell, R.L., Duncan, S.M., Blanchette, R.A., Held, B.W., Jurgens, J.A. and Arenz, B.A. 2008. Scientific evaluation of deterioration of historic huts of Ross Island, Antarctica. In: *Historical Polar Bases – Preservation and Management*. Barr, S. and Chaplin, P. (eds). ICOMOS Monuments and Sites No.XVII. International Polar Heritage Committee, Oslo, Norway pp.96. ISBN 978-82-996891-2-0

Duncan, S.M., Minasaki, R., Farrell, R.L., Thaites, J.M., Held, B.W., Arenz, B.E., Jurgens, J.A. and Blanchette, R.A. 2008. Screening fungi isolated from historic Discovery Hut on Ross Island, Antarctica for cellulose degradation. *Antarctic Science* 20: 463-470

Duncan, S.M., Farrell, R.L., Thwaites, J.M., Held, B.W., Arenz, B.E., Jurgens J.A. and Blanchette, R.A. 2006. Endoglucanase-producing fungi isolated from Cape Evans historic expedition hut on Ross Island, Antarctica. *Environmental Microbiology* 8: 1212-1219

### *3.3.1 Antarctic and Arctic publications used in PhD thesis research*

The publications, Jurgens et al. 2009 and Arenz et al. 2006, are particularly relevant to the PhD thesis as they describe the specific polar regions fungi used in the thesis research, as shown in Table 3.1. The relevant contributions of the PhD candidate Jurgens, to the papers are given and PDF versions of the pubications.

Jurgens, J.A., Blanchette, R.A., and Filley, T.R. 2009. Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic. *Polar Biol*. 32:751-758

The PhD thesis author, Joel A. Jurgens was the senior author of this publication and contributed as follows:

- Pre Arctic trip planning and preparation
- Arctic site sample collection and investigation
- Laboratory studies involving fungal isolation and molecular identification and wood species identification
- Manuscript writing, preparation and submission

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ORIGINAL PAPER

# Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic

**Joel A. Jurgens · Robert A. Blanchette · Timothy R. Filley**

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**Abstract** Non-permineralized or mummified ancient wood found within proglacial soil near the ad Astra Ice Cap (81°N, 76°W), Ellesmere Island, Canada was investigated to ascertain the identification of the trees, current morphological and chemical characteristics of the woods and the fungi within them. These woods, identified as *Betula*, *Larix*, *Picea* and *Pinus*, were found with varying states of physical and chemical degradation. Modern microbial decomposition caused by soft rot fungi was evident and rDNA sequencing of fungi obtained from the samples revealed several species including *Cadophora* sp., *Exophiala* sp., *Phialocephala* sp., as well as others. Analytical $^{13}$C-labeled tetramethylammonium hydroxide thermochemolysis showed the lignin from the ancient wood was in a high degree of preservation with minor side chain alteration and little to no demethylation or ring hydroxylation. The exposure of these ancient woods to the young soils, where woody debris is not usually prevalent, provides carbon and nutrients into the polar environment that are captured and utilized by unique decay fungi at this Arctic site.

**Keywords** Non-permineralized wood · Ellesmere Island · Fungi · Wood decay · Biodeterioration · Lignin chemistry

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## Introduction

The Canadian High Arctic contains several deposits of non-permineralized, ancient woody materials in various states of preservation (Francis 1988; Basinger et al. 1988; Wheeler and Arnette 1994). Perhaps the most well studied of these deposits is located on Axel Heiberg Island near the Geodetic Hills. These woods have been primarily dated to the Eocene period (34–55 million years ago) based on geomorphology and vertebrate fossils found in the same strata layers. However, other sites such as the region near the ad Astra Ice Cap, Ellesmere Island, Nunavut Province, Canada (Fig. 1) also have mummified woods that have not been adequately studied. Unlike the woods of Axel Heiberg that have been dated using features in the surrounding sedimentary strata layers, the woods near the ad Astra Ice Cap have been displaced from their original location to proglacial soils (Fig. 2) and their age cannot be determined with accuracy making dating difficult. However, they are likely to be of a similar age ranging from the Paleocene–Eocene transition period of ~55 million years ago when dramatic global warming occurred (Zachos et al. 2001) and the Eocene–Oligocene boundary ~33 million years ago when a pronounced cooling initiated (Zanazzi et al. 2007), as this period of time provided temperatures adequate to support flora of this type in the Arctic. This age is further supported by the proximity to the dated material on Axel Heiberg and species composition similarities between the two sites (Jahren 2007). The current environment, based on data collected by the Meteorological Service of Canada from 1971 to 2000 at the nearest weather station to the collection site (Eureka, Nunavut, Canada, latitude 79°58.800′N; longitude 85°55.800′W), has an average annual temperature of −19.7°C, with the highest average monthly temperature of 5.7°C occurring in July and the lowest of −38.4°C in

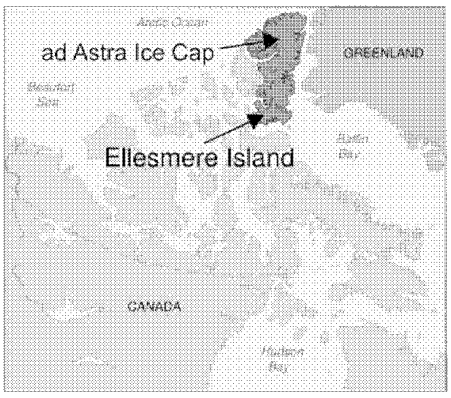


**Fig. 1** Map of the Canadian High Arctic Region indicating mummified wood collection location near the ad Astra Ice Cap on Ellesmere Island

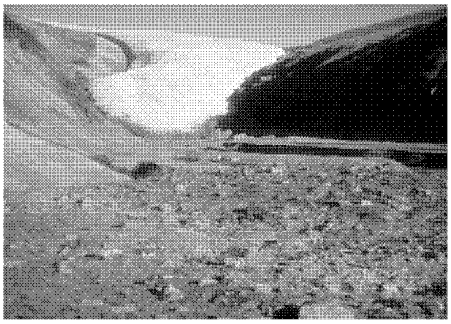

**Fig. 2** Valley at the base of the retreating ad Astra Ice Cap on Ellesmere Island, Nunavut, Canada where mummified wood was found

February. The average annual precipitation over the 30-year period was 75.5 mm, with the greatest amount falling in August (14.9 mm) and the least in February (2.6 mm). Investigations of these woods provide an excellent opportunity to gain a better understanding of the past landscape of this region, as well as an opportunity to study the microorganisms that utilize this labile food source, relative to glacial soils with sparse organic matter, in this harsh environment.

Much of the mummified wood that has been investigated in the Canadian High Arctic has been from the class gymnospermea, belonging primarily to *Metasequoia* (Visscher and Jangels 2003) or *Larix* (Jagels et al. 2001) genera. Additional evidence of members from Cupressaceae, Pinaceae and Taxodiaceae in the form of cones, leaves, needles, palynomorphs, twigs and wood have been identified and a review of these studies has been presented by Basinger (1991) and Jahren (2007). Although, similar findings of fruit, leaves, nuts and palynomorphs of dicot angiosperms have been found in these same assemblages with the gymnosperms, limited evidence of wood from the angiosperm group persist. Only one example was found in the Geodetic Hills of Axel Heiberg Island as an in situ stump and tentatively identified by Jagels et al. (2005) as belonging to either, the Lauraceae, Euphorbiaceae or Flacourtiaceae families.

Previous micromorphological and chemical investigations of mummified woods from different regions of the Canadian Arctic have shown that extensive alterations within these woods had taken place. In these studies, the residual wood had high but variable lignin content, thought to be controlled by the degree of vitrification (Kaelin et al. 2006) with the lignin from some samples being methoxyl group deficient, sidechain degraded and of a more condensed lignin state as compared to lignin in modern wood (Blanchette et al. 1991; Obst et al. 1991). Many of the woods from these investigations also had extensive carbohydrate degradation with complete loss of hemicellulose and very little crystalline cellulose remaining. Others, however, have been able to extract hemicellulose and cellulose from wood samples collected in this region (Jahren and Sternberg 2003; Jagels and Day 2004) indicating that some polysaccharides can survive in the residual cell wall layers. Ultrastructural studies of mummified wood samples removed from sedimentary layers at Axel Heiberg and other locations in the Arctic have shown no evidence of microbial degradation, paleo or modern (Blanchette et al. 1991). Although free of attack from fungi and bacteria, advanced stages of degradation were evident and deterioration appeared to be driven by nonbiological chemical hydrolysis mechanisms (Kaelin et al. 2006). The reasons for the lack of paleo biological degradation are uncertain but most likely involve a unique burial condition or conditions that limited microbial activity quickly. These conditions included rapid and deep burial (some reaching depths of 300 m) that restricted oxygen and possibly preserved an abundance of tannin-like compounds in some of the plant materials that may have helped to suppress microbial decomposition at early stages of burial (Schoenhut et al. 2004). Although microbial attack was inhibited, the wood appeared to be affected by a gradual hydrolysis and chemical attack (Obst et al. 1991). The loss of carbohydrates and conversion of lignin by abiotic processes, as well as the ultrastructural changes that were observed corresponded to the changes taking place during the formation of coalified wood (Blanchette et al. 1991).

This study was done to identify the type of ancient woods found at this high Arctic site and to ascertain their current morphological and chemical characteristics, as

well as to determine the fungal diversity within the woods after release from the glacier and exposure to the polar environment.

## Materials and methods

Mummified wood samples were initially found in the west valley below the retreating ad Astra Ice cap, Ellesmere Island, Nunavut, Canada (81°N, 76°W) by Parks Canada personnel from the Quttinirpaaq National Park (Figs. 2, 3). In cooperation with Parks Canada and under Park Permit # QQ-01-01 and Nunavut Research Institute Research License # 0100501 N-M, several sites near the ad Astra Ice Cap were visited and small sections of wood in contact with the ground were collected, placed in sterile bags and kept cool during transport to the University of Minnesota where they were investigated. Wood segments from the samples were cultured for microorganisms using three types of growth medium: 1.5% Difco malt extract agar (MEA), MEA with 2 ml of lactic acid added after autoclaving and a semi-selective media for Basidiomycetes that included 15 g of malt extract, 15 g of agar, 2 g of yeast, 0.06 g of benlate with 0.01 g of streptomycin sulfate and 2 ml of lactic acid added after autoclaving. All ingredients for each media type were added to 1 l of deionized water. Incubation was at 20–22°C since previous studies have shown fungi from Polar Regions are primarily psychrotrophs and grow well above 20°C (Robinson 2001). Plates were checked daily for fungal growth and pure cultures were transferred to individual plates. Fungi from pure cultures were identified using analysis of rDNA internal transcribed spacer sequences as previously described by Held et al. (2005).

Other segments from the same wood samples were used for micromorphologial observations and chemical analyses. Samples of wood were prepared for scanning electron microscopy (SEM) using previously described techniques (Blanchette and Simpson 1992) and images taken using a Hitachi S3500 N SEM. Hand sections of samples were also made and viewed under a Nikon Eclipse E600 with images captured using ACT1 software connected to a Nikon DXM 1200F digital camera.

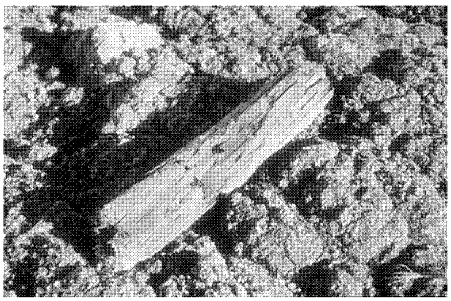

**Fig. 3** Mummified wood found near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada. The wood was separating along the annual rings and the surface in contact with the ground was soft and decayed

Lignin chemistry of the wood was assessed using $^{13}$C-labeled tetramethyl ammonium hydroxide (TMAH) thermal chemolysis according to methods outlined in Filley (2003) and Filley et al. (2006). This procedure decomposes lignin into methylated monomers for gas chromatographic analysis and permits the assessment of the number of free hydroxyls on the lignin structure, thereby assessing microbial or chemical demethylation and the contribution of tannin and phenol components to the lignin monomers (Filley et al. 2006). Briefly, 100–300 μg of dried and powdered sample were added to a platinum cup along with 3 μl of a 25% w/v solution of $^{13}$C-TMAH in water. The sample was then placed into an isothermally heated (350°C) Shimadzu Pyr4A pyrolyzer, interfaced to a GC17A gas chromatograph containing a Restek Rtx-5MS capillary column with helium flow. Quantification of fragmentation and structure was by mass spectrometry using a Shimadzu QP5050A mass spectrometer. Sixteen methylated phenols commonly released in the TMAH chemolysis/pyrolysis of natural organic matter comprising guaiacyl (G), syringyl (S) and cinnamyl (C) monomers (Table 1) were analyzed for their original aromatic methoxyl/hydroxyl content by determining

**Table 1** Methylated lignin phenols analyzed using TMAH thermochemolysis

| TMAH thermochemolysis products | |
|---|---|
| G4 | 3,4-Dimethoxybenzaldehyde |
| G5 | 3′,4′-Dimethoxyacetophenone |
| G6 | 3,4-Dimethoxybenzoic acid, methyl ester |
| S4 | 3,4,5-Trimethoxybenzaldehyde |
| G7 | *cis*-2-(3,4-Dimethoxyphenyl)-1-methoxyethylene |
| G8 | *trans*-1-Methoxy-2-(3,4-dimethoxyphenyl) ethylene |
| S5 | 3′,4′,5′-Trimethoxyacetophenone |
| P18 | *trans*-4-(4-Methoxyphenyl) acrylic acid, methyl ester |
| S6 | 3,4,5-Trimethoxybenzoic acid, methyl ester |
| G14 | 1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane (*erythro* or *threo*) |
| S7 | *cis*-2-(3,4,5-Trimethoxyphenyl)-1-methoxyethylene |
| G15 | 1-(3,4-Dimethoxyphenyl)-1,2,3-trimethoxypropane (*erythro* or *threo*) |
| S8 | *trans*-1-Methoxy-2-(3,4,5-dimethoxyphenyl) ethylene |
| G18 | *trans*-4-(3,4-Dimethoxyphenyl) acrylic acid, methyl ester |
| S14 | 1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane (*threo* or *erythro*) |
| S15 | 1-(3,4,5-Trimethoxyphenyl)-1,2,3-trimethoxypropane (*erythro* or *threo*) |

the number of $^{13}$C-labeled methyl groups added during the procedure using mass spectral methods modified from Filley et al. (2006). Commercially available standards and unlabeled TMAH thermochemolysis of the samples investigated herein were used to obtain the appropriate baseline ion fragment ratios to permit accurate calculation of the $^{13}$C-labeled methyl groups added. Carbon elemental analysis was performed using a Carlo Erba 1108 C/H/N analyzer. In addition to the Arctic wood samples, modern samples of birch, pine and spruce were assessed and used for comparison.

## Results

Wood examined on the ground or partially buried in soil at the ad Astra Ice Cap had obvious weathering and a bleached appearance. Some wood surfaces had defibrated zones and a delamination that was evident at the earlywood/latewood transition within the annual rings (Fig. 3). In addition to surface weathering and delamination at the edges of exposed wood pieces, some wood in contact with the ground appeared soft and decayed. Light microscope and SEM observations of anatomical characteristics allowed identification and readily displayed the exceptional preservation of these woods (Fig. 4a, b). The woods were identified as *Pinus* (subgenus *Strobus*) representing nearly 39% of the collection, *Picea* at just over 33%, *Betula* with nearly 14% and *Larix* consisting of 11% (Table 2). Several micromorphological characteristics were utilized to identify the wood samples to the generic level (Hoadley 1995; Schweingruber 1990). The gymnosperm samples were separated on the bases of the regular presence (*Pinus*) or absence (*Picea* and *Larix*) of resin canals, pitting in the rays and the type of walls and pitting in the ray tracheids. Ray or cross-field pitting separated the *Pinus* samples having window pits, from the *Larix* and *Picea* samples which had piceoid pitting. Further separation was based on features in the ray tracheids which included smooth walls in the *Pinus* subgenus *Strobus*, while *Picea* type I bordered pitting was present in the *Picea* samples and *Larix*-type in the *Larix* samples. The *Betula* samples were identified based on their diffuse-porous vessel arrangement and grouping, very small, alternate intervessel and ray-vessel pitting, as well as the presence of scalariform perforation plates.

To evaluate the modern biological degradation taking place in the various collections of wood, sections were examined with light and scanning electron microscopy.

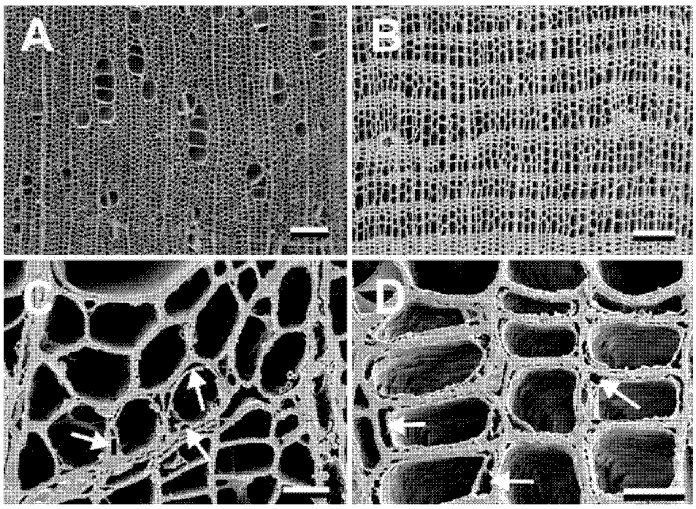


**Fig. 4** Scanning electron micrographs of mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada. **a** Section of birch (*Betula* sp.) wood from the inner part of a sample showing an excellent state of preservation with all cell wall layers intact and free of degradation (*Bar* 100 μm), **b** section of pine (*Pinus* subgenus *Strobes.*) (*Bar* 150 μm) wood from the inner region of a sample showing cell walls with no defect or alterations. **c** Soft rot attack in birch (*Betula* sp.) from the outer region of a wood sample. The degradation patterns of soft rot consist of cavities formed in the $S_2$ layer of the cell wall (*arrows*). Some cell walls are so extensively degraded that the entire secondary wall has been removed by the fungal attack (*Bar* 10 μm), and **d** soft rot attack from the surface of a pine (*Pinus* subgenus *Strobes.*) sample. Cavities in the $S_2$ layer of the cell wall (*arrows*) can be seen and fungal hyphae is present within the wood cells (*Bar* 25 μm)

**Table 2** Non-mineralized, Eocene aged wood identification from the ad Astra Ice Cap region, Ellesmere Island, Nunavut Province, Canada

| Sample identification | Number of samples |
|---|---|
| *Betula* sp. | 6 |
| *Larix* sp. | 4 |
| *Picea* sp. | 12 |
| *Pinus* subgenus *Strobus* | 14 |

Many wood sections had cells that appeared sound, primarily from the interior portion of the sample, and no evidence of biological attack was observed. However, some samples had fungal hyphae present and displayed distinct wood decay characteristics of attack by soft rot fungi. Cell walls contained chains of cavities typical of type 1 soft rot activity. These cavities formed in the $S_2$ layer of the secondary wall in cells of the angiosperm and gymnosperm woods. Scanning electron micrographs of transverse sections of the decayed regions in both wood types displayed extensive cavities (Fig. 4c, d) characteristic of recent decay and similar to soft rot decay described by Blanchette et al. (2004) and Eriksson et al. (1990) present in modern woods. No other forms of fungal or bacterial degradation were found.

Culturing of wood segments on the various growth media yielded several fungal cultures, however, no growth was obtained on the semi-selective media used to preferentially isolate Basidiomycetes but a Basidiomycete was isolated from malt extract agar. Fungi that were isolated in pure culture were identified by sequencing the ITS region of the rDNA. Blast search results of these sequences with GenBank accessions with a greater than 97% similarity revealed several fungi based on best Blast matches including *Acephala* sp., *Exophiala* sp., *Phialocephala* sp., Fungal sp. AB10 (*Cadophora* sp.), Fungal sp. AB29—isolated from Antarctic soil, cf. *Pyrenopeziza revincta* and Uncultured fungus clone N3—DNA from Alaskan soil (Table 3). A Basidiomycete with only an 88% match to an uncultured fungus was also isolated.

All wood samples showed excellent preservation of lignin with only minor modifications apparent with respect to the modern analogs analyzed for comparison (Table 4). Our results were consistent with previous chemical studies on mummified, non vitrified, wood from this region using TMAH thermochemolysis in that proxies for lignin side chain oxidation and presence of intact lignin monomers showed the wood to be remarkably "fresh" (Kaelin et al. 2006). For example, the G6/G4 ratios, which are frequently used as a proxy for the degree of side chain oxidation and increases with increasing microbial decay, are only mildly higher than the fresh counterparts with the exception of sample FW 2. The modern samples range from 0.31 to 0.61 and the mummified woods range from 0.45 to 0.67, while FW 2 had a value of 1.02 indicating a greater degree of biological degradation than the other samples. Additionally, the S6/S4, a proxy for syringyl based lignin decay, values for the angiosperm woods shows only minor elevation when compared to the control. An additional proxy for the lignin side chain alteration, $\Gamma_{(S,G)}$, which is defined as [G6/(G14 + G15)], showed that there is loss of extractable monomers with the intact glycerol side chain as this value for both the guaiacyl and syringyl analogs is substantially higher in the mummified than fresh woods (Table 3). It is thought that the increase in this ratio can be promoted by chemical condensation processes or loss of side chain hydroxyl groups.

**Table 3** Fungi cultured from mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada and identified based on BLASTn search of the ITS region of rDNA

| Sample | Best BLAST match (accession #) | Match (%) | Overlap[a] | Accession # |
|---|---|---|---|---|
| FW 1 | *Exophiala* sp. (DQ317336) | 100 | 468/468 | FJ457766 |
| FW 2 | *Phialocephala* sp. (EF485233.1) | 98 | 428/433 | FJ457767 |
| FW 3 | *Acephala* sp. (EU434829) | 99 | 436/437 | FJ457768 |
| FW 4 | Uncultured fungus (AM999656) (unknown Basidiomycete) | 88 | 466/529 | FJ457769 |
| FW 5 | *Acephala* sp. (EU434829) | 99 | 403/404 | FJ457770 |
| FW 13 | Fungal sp. AB10 (FJ235943) (*Cadophora* sp.)[b] | 99 | 439/440 | FJ457771 |
| FW 14 | Fungal sp. AB29 (FJ235962) (isolated from Antarctic soil)[c] | 99 | 421/423 | FJ457772 |
| FW 16 | Uncultured fungus clone N3 (EF434036) (DNA from Alaskan soil)[c] | 97 | 494/509 | FJ457773 |
| FW 17 | cf. *Pyrenopeziza revincta* (AJ430226) | 99 | 456/458 | FJ457774 |
| FW 18 | Uncultured fungus clone N3 (EF434036) (DNA from Alaskan soil)[c] | 97 | 496/510 | FJ457775 |

[a] Overlap of ITS alignment of best BLAST match in base pairs

[b] Based on authors unpublished phylogenetic neighbor joining analysis

[c] Provenance of fungal isolate or DNA

**Table 4** Tetramethylammonium hydroxide lignin concentration proxies from mummified wood collected near the ad Astra Ice Cap, Ellesmere Island, Nunavut, Canada

| Sample and wood identification | Guaiacyl Ac/Al (G6/G4) | Syringyl Ac/Al (S6/S4) | Gamma-G | Gamma-S | Demethylation[a] (monomers of full lignin side chain) | Demethylation[b] (low molecular weight monomer) |
|---|---|---|---|---|---|---|
| FW 1 (*Picea*) | 0.67 | | 1.05 | | 0.96 | 0.92 |
| FW 2 (*Larix*) | 1.02 | | 0.34 | | 0.97 | 0.93 |
| FW 3 (*Pinus*[c]) | 0.52 | | 0.60 | | 0.99 | 0.92 |
| FW 4 (*Pinus*[c]) | 0.45 | | 0.65 | | 0.99 | 0.94 |
| FW 5 (*Pinus*[c]) | 0.47 | | 0.54 | | 0.99 | 0.93 |
| FW 13 (*Picea*) | 0.57 | | 0.57 | | 0.98 | 0.92 |
| FW 14 (*Pinus*[c]) | 0.54 | | 0.50 | | 0.99 | 0.94 |
| FW 15 (*Betula*) | 0.57 | 0.74 | 0.46 | 1.91 | 0.95 | 0.93 |
| FW 16 (*Pinus*[c]) | 0.67 | | 0.94 | | 0.98 | 0.95 |
| FW 17 (*Pinus*[c]) | 0.60 | | 0.65 | | 0.99 | 0.94 |
| FW 18 (*Pinus*[c]) | 0.49 | | 0.53 | | 1.00 | 0.94 |
| AF 1 (*Betula*) | 0.43 | 0.65 | 0.23 | 0.66 | 0.97 | 0.92 |
| AF 2 (*Picea*) | 0.60 | | 0.70 | | 1.00 | 0.94 |
| *Betula*[b] | 0.61 | 0.56 | 0.11 | 0.18 | 0.98 | 0.88 |
| *Picea*[d] | 0.31 | | 0.13 | | 0.99 | 0.91 |
| *Pinus*[d] | 0.33 | | 0.14 | | 0.99 | 0.91 |

[a] Full lignin structure monomers (G14, G15, S14, S15)

[b] Lower molecular weight monomers (G4, G6, G7, G8, S4, S6, S7, S8)

[c] Subgenus *Strobus*

[d] Modern wood samples used for comparison

As both the abiotic hydrolysis reaction chemistry, the generally accepted slow process which transformed the mummified Arctic woods, and soft rot fungal decay may cause lignin demethylation, we investigated the degree of aromatic hydroxyl content on the thermochemolysis products. The data (Table 4) indicate that biotic or abiotic demethylation in the remaining lignin in the mummified woods was limited. The compounds indicative of the full lignin structure (G14, G15, S14, S15) have methoxyl contents indistinguishable from the modern analogs (Table 4). The lower molecular weight monomers (G4, G6, G7, G8, S4, S6, S7, S8) which are a combination of analytical breakdown products and environmental decay products show greater demethylation (or hydroxyl content) in the modern woods than the mummified counterparts. No relationship was evident between demethylation extent and the proxies for side chain oxidation.

## Discussion

In contrast to past research completed in the Arctic on mummified woods that found *Metasequoia* to be the dominant genera (Jahren 2007), our study revealed an absence of this type of wood at the site based on morphological characteristics of the samples collected. This, however, may simply be related to the limited number of samples collected (36) or perhaps gives some suggestion of the topography of the site when the burial event occurred. This assemblage of species including *Betula, Larix, Picea* and *Pinus* and the lack of *Metasequoia*, which is considered more of a swamp or floodplain species, indicate that the collection site was most likely a foothill of montane region (Richter and LePage 2005; Williams et al. 2003). As the original context of these samples has been disturbed by both glaciations and water movement it is also possible that the wood has been washed down from these higher elevation sites. The presence of *Betula* wood is very unique in this region as angiosperm wood appears to be exceedingly rare with only one published example and its identity was not determined but was tentatively suggested to belong to one of three families (Jagels et al. 2005).

Although Wheeler and Arnette (1994) noticed fungal hyphae in mummified *Picea* sp. wood collected in Alaska, this article is the first report of modern biological degradation and identification of the decay fungi associated with ancient mummified woods after their release into the Arctic environment. Many of the fungi isolated from the mummified woods that exhibited soft rot decay when examined microscopically have previously been shown to cause this same type of degradation in laboratory studies. *Phialophora finlandia*, recently renamed *Cadophora finlandia* based on a molecular taxonomic study (Harrington and McNew 2003) and *Phialocephala fortinii* have shown the ability to degrade various polysaccharides including cellulose, laminarin, starch and xylan (Caldwell et al. 2000). Also, Blanchette et al. (2004) and Held et al. (2006) conducted laboratory decay studies on *Betula* wood using *Cadophora* species isolated from Antarctica which revealed extensive type 1 soft rot after 16 weeks. In other laboratory studies, Worrall et al. (1997) found that isolates of *Phialocephala* sp. and *Phialophora* (*Cadophora*) sp. were capable of

causing seven to thirty times greater weight loss in birch (*Betula*) wood as compared to pine (*Pinus*) after 12 weeks. Since birch is very susceptible to soft rot attack it is not surprising that this mummified wood was affected more severely during the relatively short time of exposure. Fungi that cause soft rot appear to be the predominant decay fungi in Polar Regions. In other studies done in the Antarctic, as well as the Arctic, soft rot were the only type of decay fungi observed (Arenz et al. 2006; Blanchette et al. 2004; Blanchette et al. 2008). These fungi appear endemic to the area and well established in soils. They will colonize carbon and nutrient resources introduced into the environment and the ancient mummified woods, with their chemical signatures resembling modern woods, are readily attacked and degraded by these fungi.

There are few direct chemical studies on the nature of soft rot decay on lignin chemistry. Studies of both laboratory inoculations (Shary et al. 2007) and field sampled woods (Nelson et al. 1995; del Rio et al. 1998) indicate that some soft rot fungi are capable of lignin side chain oxidation to produce low molecular weight aromatic acids. Their chemistry is thought to most resemble the chemical action of white rot decay (Shary et al. 2007) and there is no direct evidence for extensive demethylation chemistry such as would be evident after brown rot decay.

Comparing the state of lignin chemistry of the mummified Arctic woods with the modern analogs demonstrates that the wood preservation mechanisms at this Arctic site did not result in any observable demethylation of the remaining lignin. In fact, the mummified woods exhibited slightly higher methoxyl contents for the low molecular weight lignin products than the modern analogs. The source of the higher phenol containing monomers in modern lignin can be attributed to non-core lignin associated with carbohydrates that are lost upon decay or in this case possibly hydrolysis (Filley et al. 2006). The lack of measurable demethylation in the mummified woods also suggests that there are no hydrolysable tannin decomposition products (Yang et al. 2005) and that the hydrolysis chemistry thought to be responsible for sugar decomposition, as well as changes to lignin chemistry (Kaelin et al. 2006) was incapable of methoxyl carbon removal. Alternatively, demethylated lignin fragments may have further reacted to produce material not released by this chemolytic procedure.

Both measures of side chain oxidation (Ac/Al and Gamma for both G and S monomers) in the mummified woods demonstrate a wide range of values, consistent with the findings of Kaelin et al. (2006) but show only mild oxidation as compared to the modern samples. Although birch samples FW 15 and AF 1 had only slightly higher levels of the syringyl lignin compared to the control, the dominant expression of soft rot was exhibited by the gamma-S values which were 3.5–10 times greater than the control sample (Table 3). The mummified gymnosperm samples with soft rot displayed a similar expression of these chemical proxies with most having significantly higher gamma-G values compared to the controls with slightly higher Guaiacyl levels. As the soft rot decay in the samples were observed to be primarily a surface phenomenon, it is possible that the chemical signature of this relatively limited decay was diluted with nondegraded interior lignin when the wood samples were ground.

This paper addresses the first documented occurrence of modern colonization and utilization of this ancient substrate by microorganisms. The effect of fungi on mummified woods exposed to the environment at other locations has not been investigated but results from this study suggest that degradation, although slow under Arctic environmental conditions may be occurring more widely than previously suspected. With the addition of this 'fresh', relatively easily metabolizable plant material, rates of stabilized soil organic matter should dramatically increase (Fontaine et al. 2007), and this Eocene wood may contribute to the creation of microbial and nutrient hot spots. The role of these fungi in the Arctic has received little attention and most isolates that were found with poor sequence similarity to Genbank accessions or isolates that match unclassified and unknown species may be new taxa and warrant additional investigation.

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- Pre Antarctic trip planning and preparation
- Antarctic site sample collection and investigation
- Laboratory studies involving fungal isolation
- Scientific discussions and contributions to manuscript writing and editing

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

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**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.


*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

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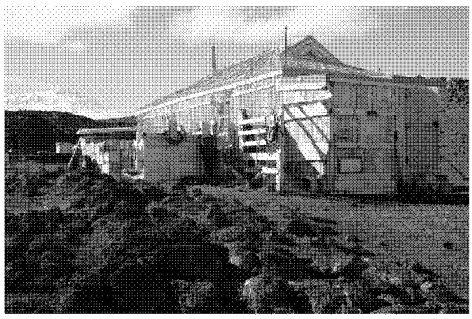

Fig. 1. Hut at Cape Royds built by Ernest Shackleton in 1908 as a base for polar exploration is one of three huts on Ross Island where soil and wood samples were obtained.

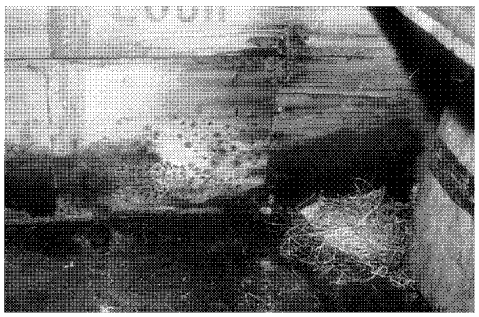

Fig. 2. Crates in galley area inside Cape Evans Hut affected by surface fungal growth. Fungi from samples of interior and exterior woods and artifacts from the historic huts were used in this study to compare to fungi obtained from soils.

(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. Miniscule 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,

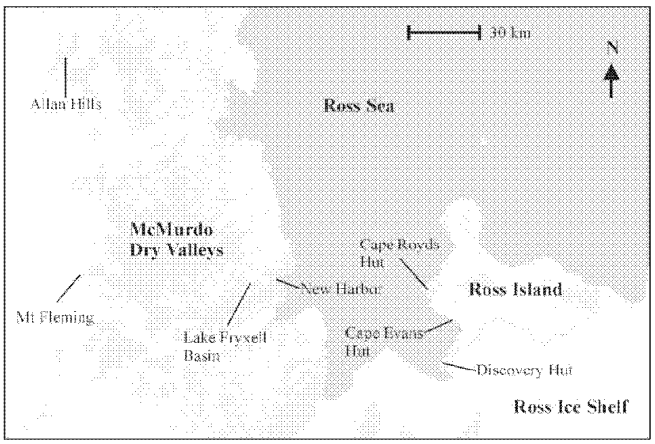


Fig. 3. Map of Ross Sea Region showing locations where samples were obtained for this study.

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

| Location | Latitude | Longitude | Soil | Wood | Other[a] |
|---|---|---|---|---|---|
| *Ross Sea area* | | | | | |
| 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 | | |
| *Dry Valley area* | | | | | |
| Lake Fryxell Basin | 77° 60′S | 163° 24′E | 3 | | |
| *Mountain sites* | | | | | |
| Allan Hills | 76° 42′S | 159° 44′E | 10 | | |
| Mt. Fleming | 77° 31′S | 160° 15′E | 3 | | |

[a]Other 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 subsampling, 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 Minicycler (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 submersed 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%), *Rhodoturula* (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 | | | | | | | | | |
| *Alternaria* sp. | 100 | 551/551 | C,D | E,R,AH, LF | 3 | 2 | | 5 | DQ317386 |
| *Antarctomyces psychrotrophicus* [AJ133431] | 97.6 | 321/329 | D | R | 1 | | | 1 | DQ317323 |
| *Ascobolus denudatus* [AY500528] | 88.1 | 267/303 | C | E | | | 1 | 1 | DQ317324 |
| *Ascobolus stercorarius* [AY372073] | 92 | 319/346 | D | NH | 1 | | | 1 | DQ317325 |
| *Ascomycete sp.* [AA1279460] | 92.9 | 353/380 | C | R | | 1 | 1 | 2 | DQ317343 |
| *Cadophora fastigiata* [AY805584] | 100 | 547 | C | E,R | | 1 | 1 | 2 | DQ317326 |
| *Cadophora luteo-olivacea* [AY249068] | 100 | 542 | C,D | E,H,R,NH,MF,LF | 12 | 3 | 1 | 16 | DQ317327 |
| *Cadophora malorum* [AY249064] | 100 | 521 | C,D | E,H,R,NH,MF | 11 | 13 | 3 | 27 | DQ317328 |
| *Cadophora* sp. 4E71-1 [AY371506] | 100 | 543 | C,D | E | 3 | 7 | 2 | 12 | DQ317329 |
| *Cadophora* sp. H37 [AY371512] | 100 | 461 | C | R | | 2 | | 2 | DQ317330 |
| *Chaetomium funicola* [AJ279450] | 94.7 | 392/414 | C | R | | 1 | | 1 | DQ317331 |
| *Cladosporium cladosporioides* [AY213641] | 99.1 | 538/543 | C,D | E,H,R,NH,AH | 9 | 28 | | 37 | DQ317332 |
| *Cosmospora vilior* [AY805574] | 99.4 | 471/474 | C | E,R | | 3 | | 3 | DQ317333 |
| *Dactylella lobata* [U51958] | 94.7 | 501/529 | C | E,R | 1 | 3 | | 4 | DQ317334 |
| *Epicoccum nigrum* [AF455455] | 100 | 342 | D | MF,LF,NH,AH | 6 | | | 6 | DQ317367 |
| *Eurotium* sp. [AF455536] | 99.6 | 554/556 | C | E,R | | 1 | 1 | 2 | DQ317335 |
| *Exophiala spinifera* [AY843179] | 100 | 528 | C,D | E,H | 3 | 3 | | 6 | DQ317337 |
| *Fusarium oxysporum* [AY188919] | 99.7 | 336/337 | D | E,R | | 4 | | 4 | DQ317368 |
| *Geomyces* sp. C239/10G [AY345347] | 99.8 | 560/561 | C,D | E,H,R,LF | 9 | 3 | 6 | 18 | DQ317337 |
| *Geomyces* sp. GFI 22 [AJ608988] | 94.4 | 470/498 | C | NH | 1 | | | 1 | DQ317338 |
| *Geomyces pannorum* [AY873967] | 99.8 | 565/566 | C,D | E,H,R | 5 | 10 | 7 | 22 | DQ317339 |
| *Geopyxis* sp. [AY465441] | 94.7 | 160/169 | D | E,NH | 1 | | 1 | 2 | DQ317369 |
| *Hormonema dematioides* [AF013228] | 99.1 | 579/584 | C | E,H | | 6 | 3 | 9 | DQ317340 |
| *Leptosphaerulina trifolii* [AY831558] | 100 | 341 | D | NH | 1 | | | 1 | DQ317370 |
| *Microdochium bolleyi* [AJ279454] | 100 | 355 | D | MF | 1 | | | 1 | DQ317371 |
| *Monodictys castaneae* [AJ238678] | 93.9 | 419/446 | C | E | | 1 | | 1 | DQ317341 |
| *Nectria* sp. olrim171 [AY805575] | 99.8 | 484/485 | C | R | | 1 | | 1 | DQ317342 |
| *Penicillium echinulatum* [AF033473] | 100 | 528 | C | R,H,NH | 2 | 2 | 1 | 5 | DQ317344 |
| *Phaeosphaeria* sp. | 94.8 | 165/174 | D | R | 1 | | | 1 | DQ317372 |
| *Phialophora* sp. RR 90-121 [AF083204] | 97.6 | 248/254 | D | E | 2 | | | 2 | DQ317373 |
| *Phoma herbarum* [AY293791] | 100 | 522 | C,D | R,MF | 3 | | | 3 | DQ317345 |
| *Phoma* sp. GS9N1a [AY465466] | 99.8 | 533/534 | C | H,NH | 2 | | | 2 | DQ317346 |
| *Pseudeurotium desertorum* [AY129288] | 95.1 | 481/506 | C | H | | 1 | | 1 | DQ317347 |
| *Pseudeurotium* sp.olrim 176 [AY787729] | 100 | 450/450 | C | E,R | | | 2 | 2 | DQ317348 |
| *Sarea difformis* [AY590786] | 99.4 | 488/491 | C | H | | 1 | | 1 | DQ317349 |
| *Thelebolus caninus* [AY957550] | 99.2 | 475/479 | C,D | R,H | 1 | | 1 | 2 | DQ317350 |
| *Thelebolus microsporus* [AY957552] | 99.2 | 477/481 | C | E | 1 | | | 1 | DQ317351 |
| *Ulocladium chartarum* [AY625071] | 100 | 548 | C | NH | 1 | | | 1 | DQ317352 |
| Uncultured fungus isolate RFLP104 [AF461665] | 94 | 518/551 | C | E | | 1 | | 1 | DQ317353 |
| Zygomycota | | | | | | | | | |
| *Uncultured Mortierellaceae* [AJ879650] | 96.3 | 498/517 | C,D | R | 2 | | | 2 | DQ317354 |
| Ascomycete yeasts | | | | | | | | | |
| *Candida parapsilosis* [AF455530] | 100 | 495 | C,D | R,LF | 2 | | | 2 | DQ317355 |
| *Debaryomyces hansenii* [AF210326] | 99.4 | 635/639 | C | R | | | 1 | 1 | DQ317356 |
| *Dipodascus australiensis* [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 |
| *Bulleromyces albus* [AF444664] | 99.6 | 283/284 | D | R | 1 | | | 1 | DQ317375 |
| *Cryptococcus albidosimilis* [AF137601] | 99.6 | 558/560 | C | E,R | 1 | | 1 | 2 | DQ317358 |
| *Cryptococcus antarcticus* [AB032670] | 100 | 588 | C | AH | 1 | | | 1 | DQ317359 |
| *Cryptococcus carnescens* [AB105438] | 99.4 | 501/504 | C,D | E,R | 1 | 1 | 2 | 4 | DQ317388 |
| *Cryptococcus foliicola* [AY557600] | 97.6 | 320/328 | D | H | 1 | | | 1 | DQ317376 |
| *Cryptococcus friedmannii* [AF145322] | 99.8 | 632/633 | C | R,AH | 3 | | | 3 | DQ317360 |
| *Cryptococcus hungaricus* [AF272664] | 97.5 | 306/314 | D | MF | 1 | | | 1 | DQ317377 |
| *Cryptococcus laurentii* [AJ421006] | 99.3 | 434/437 | C,D | E,H | 2 | | | 2 | DQ317361 |
| *Cryptococcus skinneri* [AF444305] | 91 | 315/346 | D | MF | 1 | | | 1 | DQ317378 |
| *Cryptococcus* sp. | 100 | 584 | C,D | E,R,AH | 4 | 2 | | 6 | DQ317387 |
| *Cryptococcus* sp. NRRL Y-17490 [AF444449] | 99.8 | 407/408 | C | R | 1 | | | 1 | DQ317379 |
| *Cryptococcus tephrensis* [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 |
|---|---|---|---|---|---|---|---|---|---|
| *Cryptococcus victoriae* [AY188380] | 99.8 | 488/489 | C,D | E,R,H | 2 | 4 | 1 | 7 | DQ317363 |
| *Cryptococcus vishniacii* [AB032691] | 100 | 557 | C | E,AH | 1 | | 1 | 2 | DQ317364 |
| *Cryptococcus wieringae* [AF444383] | 99.7 | 380/381 | D | AH | 3 | | | 3 | DQ317380 |
| *Dioszegia hungarica* [AF444467] | 99.4 | 316/318 | D | AH | 1 | | | 1 | DQ317389 |
| *Malassezia restricta* [AY387144] | 99.6 | 455/457 | D | R,MF,AH, LF | 5 | | | 5 | DQ317381 |
| *Mrakia* sp. [AY038826] | 100 | 420 | D | E | | | 1 | 1 | DQ317382 |
| *Rhodotorula mucilaginosa* [AF444541] | 99 | 400/404 | D | E | | | 1 | 1 | DQ317383 |
| *Rhodoturula laryngis* [AB078500] | 99 | 577/583 | C | E,R,H | 4 | 1 | 3 | 8 | DQ317365 |
| *Sporidiobolus salmonicolor* [AY015434] | 99.5 | 599/602 | C | H | | 2 | | 2 | DQ317366 |
| *Sporobolomyces symmetricus* [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 | |
| AUR1 | 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.

[a]Overlap of ITS alignment of best BLAST match in base pairs.

[b]Method of detection, (C) culturing, (D) DGGE.

[c]Sampling 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 |
| *Cadophora* (21.6%) | *Cadophora* (26.3%) | *Cryptococcus* (30.6%) |
| *Cladosporium* (18.3%) | *Geomyces* (14.1%) | *Epicoccum* (13.9%) |
| *Geomyces* (17%) | *Cryptococcus* (11.1%) | *Cladosporium* (11.1%) |
| *Cryptococcus* (8.5%) | *Epicoccum* (6.1%) | *Cadophora* (8.3%) |
| *Hormonema* (5.9%) | *Cladosporium* (5.1%) | *Malassezia* (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. victoriae* 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.

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## Chapter 4
## Fungal biodiversity of Taklimakan Desert

### *4.1 Site climate data*

#### *4.1.1 Temperature*

The Taklimakan Desert is located in the western region of the People's Republic of China and is considered hyper-arid based on Long-term climate data collected at several stations. The Taklimakan Desert is a region of meteorological extremes in both temperature and precipitation. While the basin that the desert is located within has average annual temperatures of 12.4°C, the closest weather station (Ruoqiang, China: 39.0 N, 88.2 E) to the sample collection site is slightly lower at 11.6°C (Table 4.1). During the warmest month of July, the average daily high temperature is also very similar with the desert as a whole reaching 28.2ºC and the station at Ruoqiang obtaining 27.4ºC. The average maximum temperature during this month is 35.6 ºC, with the maximum recorded from 1953 to 2008 of 42.9ºC.

**Table 4.1** Climatological information from Ruoqiang, China weather station: 39.0 N, 88.2 E, altitude: 888 m, which is the closest station to the sample collection site in the Taklimakan Desert.

| | **Jan** | **Feb** | **Mar** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** | **year** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Avg. rainfall (mm)** | 1 | 0.5 | 0.7 | 1.2 | 1.8 | 3.9 | 8.8 | 2.3 | 0.4 | 0.3 | 0.5 | 0.9 | **22.9** |
| **Avg. daily temp. (°C)** | -8 | -2.1 | 7 | 15.3 | 21 | 25.2 | 27.4 | 26.1 | 20.1 | 11.1 | 1.5 | -6.4 | **11.6** |
| **Avg. max. temp. (°C)** | -1 | 5.5 | 15.4 | 24 | 29.4 | 33.4 | 35.6 | 34.6 | 29.4 | 20.8 | 9.5 | 0.5 | **19.8** |
| **Avg. min. temp. (°C)** | -13.7 | -8.7 | -0.7 | 6.9 | 12.2 | 16.4 | 19.1 | 17.3 | 11.2 | 2.9 | -4.4 | -11.3 | **3.9** |

Data derived from 454 months of collections made between 1953 and 1990
World Meteorological Organization weather station ID: PC51777

The average low temperature during the coldest month of January at the Ruoqiang weather station is also very similar to that of the readings averaged over the entire basin varying by only 0.1ºC, with the station near the collection site averaging -8 ºC. The average low temperature over the above mentioned 55 year period of data collection at Ruoqiang was -13.7 ºC and the lowest recorded was -21 ºC, giving an overall minimum to maximum temperature range of 64ºC.

### *4.1.2 Precipitation*

The average annual recorded precipitation at the Ruoqiang weather station for the period of 1953 to 1990 is exceedingly low at 22.9 mm (Table 4.1) and it is the station with least amount of precipitation in China (Hong Kong Observatory, The Government of Hong Kong Special Administrative Region). The month receiving the greatest amount of moisture is the month after the sample collection took place, July, with an average rainfall value of 8.8 mm. This is also the month with the highest average temperatures (27.4ºC), which creates the greatest evaporation potential allowing little of the actual moisture that falls during this period to be utilized by the organisms that inhabit the region. During the months of September through November, the least amount of moisture falls with the cumulative average of 1.2 mm, and meteorological records have also shown periods of several consecutive months without any measurable precipitation (Hong Kong Observatory, The Government of Hong Kong Special Administrative Region).

### *4.2 Soil analysis*

The soil analysis of 4 samples (MS5S, MS9S, MS10S and MS15S) from the Taklimakan Desert determined that the pH of all samples was relatively alkaline, ranging from pH 8 to 8.3 (Table 4.2). This pH range is similar to what has been recorded in other deserts with similar meteorological conditions to the Taklimakan Desert such as the Atacama Desert in Northern Chile with a pH range of 7.3-7.6 (Okoro et al. 2009), Polar deserts in the Antarctic Dry Valleys at pH 8.73-9.29 (Barrett et al. 2004) and a site on Ellesmere Island in the Canadian Arctic with a pH of 7.3 (Tarnocai 2009). Although all of these examples are basic, the pH can vary greatly depending on parent material, vegetative cover and moisture (Sposito 2008). Interestingly, the Taklimakan Desert has a relatively high pH and is similar to the Antarctic Dry Valleys, potentially selecting for more alkalophilic organisms.

**Table 4.2** Soil analysis completed by R.J. Hill Laboratories Limited, Hamilton, New Zealand on selected samples collected in the Taklimakan Desert with parameters, methods and detection limits of analysis found in Table 2.2.

| | **MS5S** | **MS9S** | **MS10S** | **MS15S** |
|---|---|---|---|---|
| pH | 8 | 8.2 | 8.2 | 8.3 |
| Dry Matter (g/100g as rcvd) | 93.2 | 97.3 | 97.9 | 96.9 |
| Total Recoverable Calcium (mg/kg dry wt) | 48700 | 47400 | 37200 | 42400 |
| Total Recoverable Magnesium (mg/kg dry wt) | 14500 | 13700 | 11500 | 13900 |
| Total Recoverable Sodium (mg/kg dry wt) | 1140 | 1790 | 1810 | 2250 |
| Total Recoverable Potassium (mg/kg dry wt) | 1100 | 1200 | 1530 | 1640 |
| Total Organic Carbon (g/100g dry wt) | 0.09 | 0.11 | 0.08 | 0.11 |
| Total Carbon (g/100g dry wt) | 1.67 | 1.83 | 1.37 | 1.64 |
| Total Nitrogen (g/100g dry wt) | <0.05 | <0.05 | <0.05 | <0.05 |
| Total Recoverable Phosphorus (mg/kg dry wt) | 586 | 429 | 354 | 499 |

The Taklimakan Desert is characterized by a quartz pavement embedded in mineral soil. The absence of any higher plant cover severely restricts the input of organic material to this desert soil. This terrain is typical of hyper-arid deserts and was also

characteristic of the other desert study site in the Antarctic Dry Valleys, thus offering consistency in sampling substrate.

Concerning the soil analysis, the specific components were compared with other extreme environments, particularly those in polar regions. The total carbon levels measured for the 4 Taklimakan Desert samples were low, ranging from 1.37-1.83%, approximately 10 fold higher than what has previously been found in a study conducted in the Dry Valleys of Antarctic at 0.09-0.14% (Barrett et al. 2004). When the total organic carbon is compared between samples from this PhD thesis research collected in the Taklimakan Desert site, average ~ 0.01%, and two sites in the Atacama Desert, 0.02-0.03% (Okoro et al. 2009), and from the Antarctic studies, ~ 0.05% (Ewing et al. 2008) and 0.016-0.024% (Barrett et al. 2004), the Taklimakan Desert site has the least organic carbon. The levels of total carbon were similar to the Arctic site previously discussed at Ellesmere Island investigation with 2.3% total carbon (Tarnocai 2009).

### *4.3 Morphological and molecular identification of fungi*

Isolation and cultivation of fungal strains from desert soils, rocks and organic substrates generated 194 phylotypes; presumptive identification based on morphological structures as stated in Section 2.3.2 were conducted. These putative identifications are given in Table A1 Appendix 1. The isolation media and temperature, top BLAST match with associated accession number, percent identity and overall nucleotide overlap of the internal transcribed spacer (ITS) region are shown in Tables 4.3-4.5. Sequences were generated from amplification of the ITS

region of nuclear rRNA genes generated from the 20 samples consisting of 9 soils, 7 quartz and 4 woody substrates. Concerning classical mycological culturing of fungi from collected samples, the greatest number of fungi, 103 cultures, grew from the soil samples. The rock samples generated 60 fungal isolates and 31 fungal isolates were obtained from the wood sample. While many phylotypes displayed 98-100% ITS sequence homology to existing NCBI GenBank records, many displayed relatively low similarity to any known fungal phylotype (84-95%) and likely represent novel fungal diversity; to be further discussed in Section 4.4.

The number of fungi from each sample varied greatly with some samples, such as MS9S, generating only 1 isolate and others like MS10R from which 28 independent fungal isolates were made. Within each sample, after comparing the isolated DNA PCR amplification sequence, some isolates appeared to be the same based on GenBank top BLAST matches, while there appeared to be slight morphological difference in culture. For example, from sample MS15S, the top BLAST match of isolates 77, 115, 116, 117, 122, 123, 124 and 125 had a 93-94% similarity to *Thielavia* sp. B27 (EU620166). However, there were other samples, such as MS10S, MS11R, MS13R, MS16R and MS13W that did not have any repeat matches. Although most of the top BLAST matches did have an identity to at least the generic level, these identities are questionable as they have not been published or did not come from a reliable culture type collection. Others matched sequences that were simply identified as 'Fungal sp.', 'Ascomycete' or were from environmental studies and labeled 'Uncultured Fungus', indicating very little to nothing about potential relationships of the fungi sequenced in this study.

**Table 4.3** List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS2S*** | | | | | | |
| 1 | MYA | 25 | *Phoma betae* strain 17 | EU594572 | 99 | 497/502 |
| 3 | 1.5%MEA | 25 | *Phialocephala* sp. T64106 | DQ122928 | 100 | 523/524 |
| 6 | PDA | 25 | Uncultured endophytic fungus clone 59-11-07 | EF505090 | 100 | 478/478 |
| 7 | WA | 25 | Fungal endophyte isolate 9098 | EF420014 | 99 | 458/462 |
| 10 | MYA | 25 | Uncultured endophytic fungus clone 59-11-70 | EF505100 | 99 | 399/400 |
| 12 | WA | 25 | Uncultured fungus isolate CA10 | EF159163 | 96 | 482/501 |
| 13 | WA | 25 | Uncultured fungus isolate CA10 | EF159163 | 96 | 481/501 |
| 14 | CMA | 25 | Fungal sp. YNLF-26 | DQ426531 | 100 | 473/473 |
| 15 | CMA | 25 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | 100 | 471/471 |
| 20 | CMA+ | 25 | *Thielavia microspora* | AJ271577 | 94 | 431/458 |
| 23 | MYA | 25 | *Phoma macrostoma* | DQ093700 | 99 | 446/450 |
| 24 | 0.5%MEA | 25 | *Fusarium* sp. NRRL25622 | AF158306 | 99 | 482/483 |
| 25 | CMA | 25 | *Gibberella* sp. BF22 | AM901682 | 100 | 461/461 |
| 30 | 1.5%MEA | 25 | *Penicillium citreonigrum* strain NRRL 2046 | EF198647 | 100 | 475/475 |
| 88 | MYA | 15 | *Sordariomycete* sp. BC38 | DQ317345 | 98 | 468/475 |
| 90 | PDA | 15 | *Stachybotrys chlorohalonata* strain ATCC 9182 | AY185567 | 99 | 498/499 |
| 92 | NA | 15 | *Ulocladium* sp. CID68 | EF589899 | 99 | 501/502 |
| 95 | PDA | 15 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | 100 | 435/435 |
| 96 | CMA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 441/468 |
| 99 | WA | 15 | Fungal sp. GFI 145 | AJ608974 | 88 | 465/523 |
| 106 | WA | 15 | *Humicola fuscoatra* | AJ279444 | 93 | 440/470 |
| 108 | 1.5%MEA | 15 | *Aureobasidium pullulans* strain CBS 110377 | AY139391 | 98 | 474/480 |
| 109 | 1.5%MEA | 15 | *Lecythophora* sp. UBCtra1453C | AY219880 | 89 | 444/494 |

**Table 4.3** (Continued) List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS3S*** | | | | | | |
| 9 | MYA | 25 | Uncultured fungus | AJ875362 | 100 | 469/469 |
| 104 | NA | 15 | Uncultured fungus | AJ875362 | 100 | 469/469 |
| ***MS5S*** | | | | | | |
| 2 | 0.5%MEA | 25 | *Paecilomyces* major isolate GZDXIFR-H-57-2 | DQ243696 | 94 | 349/370 |
| 5 | CMA | 25 | *Alternaria tenuissima* | AJ867284 | 99 | 448/449 |
| 11 | CMA+ | 25 | *Alternaria alternata* | EF452443 | 100 | 322/322 |
| 16 | CMA | 25 | *Alternaria alternata* | AY787684 | 99 | 360/362 |
| 19 | CMA+ | 25 | Uncultured fungus clone 12(366-13) | EU437434 | 84 | 377/448 |
| 27 | 0.5%MEA | 25 | *Aporospora terricola* isolate 35/1.5 | DQ865097 | 97 | 463/475 |
| 28 | CMA+ | 25 | Uncultured fungus clone 12(366-13) | EU437434 | 84 | 434/511 |
| 33 | 1.5%MEA | 25 | *Retroconis fusiformis* strain CBS 330.81 | EU040239 | 91 | 414/453 |
| 41 | SDA | 25 | *Amaurascopsis reticulatus* | AJ271434 | 80 | 414/516 |
| 87 | CMA | 15 | *Ulocladium consortiale* | AY278837 | 99 | 480/483 |
| 101 | 1.5%MEA | 15 | *Alternaria tenuissima* | AJ867284 | 99 | 409/412 |
| 102 | CMA | 15 | *Alternaria alternata* | EF452443 | 99 | 507/508 |
| 103 | WA | 15 | *Alternaria alternata* | EF452443 | 100 | 478/478 |
| 142 | CMA+ | 25 | Uncultured fungus clone 12(366-13) | EU437434 | 85 | 435/510 |

**Table 4.3** (Continued) List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS6S*** | | | | | | |
| 4 | 1.5%MEA | 25 | *Retroconis fusiformis* strain CBS 330.81 | EU040239 | 91 | 458/503 |
| 8 | 1.5%MEA | 25 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 474/474 |
| 18 | 0.5%MEA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 495/499 |
| 21 | 0.5%MEA | 25 | *Sydowia polyspora* | AY781224 | 99 | 512/513 |
| 26 | 1.5%MEA | 25 | *Fusarium* sp. NRRL 43682 | EF453161 | 100 | 414/414 |
| 85 | PDA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 445/472 |
| 86 | MYA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 442/470 |
| 94 | CMA+ | 15 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | 100 | 484/484 |
| 97 | 0.5%MEA | 15 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 499/499 |
| 105 | CMA | 15 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 499/499 |
| 112 | 1.5%MEA | 15 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 477/477 |
| ***MS8S*** | | | | | | |
| 60 | 0.5%MEA | 2 | *Preussia fleischhakii* strain CBS 56563 | DQ468019 | 84 | 398/472 |
| 61 | PDA | 15 | *Stemphylium* sp. EGS48-089 | AY329186 | 100 | 487/487 |
| 62 | NA | 25 | *Thielavia* sp. B27 | EU620166 | 94 | 449/477 |
| 63 | 1.5%MEA | 2 | *Thielavia* sp. B27 | EU620166 | 94 | 449/476 |
| 64 | CMA | 25 | *Thielavia appendiculata* | AJ271584 | 89 | 448/498 |
| 65 | WA | 15 | *Thielavia appendiculata* | AJ271584 | 90 | 452/502 |
| 66 | 0.5%MEA | 25 | *Thielavia basicola* | AJ271591 | 88 | 454/511 |
| 69 | BSA | 15 | *Alternaria alternata* | EF452443 | 100 | 488/488 |
| 70 | 1.5%MEA | 15 | Ascomycete sp. nasa65 | DQ683978 | 95 | 451/471 |
| 71 | CMA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 524/527 |

**Table 4.3** (Continued) List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS8S*** | | | | | | |
| 72 | 1.5%MEA | 25 | *Chaetomium* sp. 73-19-O-Mexico | AY560520 | 90 | 409/454 |
| 73 | 1.5%MEA | 25 | Ascomycete sp. IZ-1109 | AM921746 | 84 | 393/467 |
| 74 | 0.5%MEA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 513/517 |
| 75 | 0.5%MEA | 25 | *Ulocladium consortiale* | AY278837 | 99 | 490/492 |
| 76 | CMA | 15 | *Stachybotrys chlorohalonata* strain ATCC 9182 | AY185567 | 100 | 497/497 |
| 114 | PDA | 15 | *Stemphylium* sp. EGS48-089 | AY329186 | 100 | 473/473 |
| ***MS9S*** | | | | | | |
| 67 | WA | 25 | *Alternaria alternata* | EF452443 | 100 | 493/493 |
| ***MS10S*** | | | | | | |
| 68 | 0.5%MEA | 2 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 499/499 |
| 184 | 0.5%MEA | 15 | *Sordariomycete* sp. 7670B | EU680539 | 100 | 476/476 |
| 211 | CMA | 25 | *Penicillium citreonigrum* strain NRRL 2046 | EF198647 | 99 | 457/458 |
| ***MS15S*** | | | | | | |
| 50 | 0.5%MEA | 25 | *Ulocladium botrytis* isolate CHHUB1 | AF267139 | 99 | 464/465 |
| 77 | 1.5%MEA | 15 | *Thielavia* sp. B27 | EU620166 | 93 | 436/464 |
| 115 | MYA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 436/463 |
| 116 | 1.5%MEA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 432/459 |
| 117 | PDA | 25 | *Thielavia* sp. B27 | EU620166 | 94 | 449/477 |
| 120 | SDA | 15 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | 100 | 467/467 |

**Table 4.3** (Continued) List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS15S*** | | | | | | |
| 121 | PDA | 25 | *Emericella quadrilineata* isolate NRRL 4992 | EF652493 | 99 | 333/334 |
| 122 | 1.5%MEA | 25 | *Thielavia* sp. B27 | EU620166 | 94 | 461/489 |
| 123 | PDA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 444/471 |
| 124 | NA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 462/489 |
| 125 | MYA | 25 | *Thielavia* sp. B27 | EU620166 | 93 | 370/394 |
| ***MS16S*** | | | | | | |
| 32 | 0.5%MEA | 25 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | 100 | 484/484 |
| 35 | WA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 496/498 |
| 36 | WA | 25 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 499/499 |
| 37 | PDA | 25 | *Alternaria alternata* | EF452443 | 99 | 482/483 |
| 38 | CMA | 25 | *Alternaria* sp. IBL 03136 | DQ682562 | 100 | 494/494 |
| 39 | CMA | 25 | *Alternaria alternata* | EF452443 | 100 | 482/482 |
| 40 | 1.5%MEA | 25 | *Paecilomyces* sp. MTCC 6328 | EF550986 | 94 | 421/447 |
| 42 | 0.5%MEA | 25 | *Chaetomium* sp. 73-19-O-Mexico | AY560520 | 89 | 425/475 |
| 43 | SDA | 25 | *Lecythophora* sp. UBCtra1453C | AY219880 | 91 | 427/467 |
| 44 | NA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 516/519 |
| 45 | CMA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 518/522 |
| 82 | CMA | 15 | *Thielavia* sp. B27 | EU620166 | 94 | 462/489 |
| 83 | MYA | 15 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 516/520 |
| 84 | WA | 15 | *Thielavia* sp. B27 | EU620166 | 100 | 487/487 |

**Table 4.3** (Continued) List of taxa isolated from soil (S) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS16S*** | | | | | | |
| 84 | WA | 15 | *Thielavia* sp. B27 | EU620166 | 100 | 487/487 |
| 91 | 0.5%MEA | 15 | *Thielavia subthermophila* | AJ271575 | 99 | 477/478 |
| 93 | WA | 15 | *Paecilomyces* sp. MTCC 6328 | EF550986 | 94 | 423/448 |
| 98 | MYA | 15 | *Chaetomium globosum* isolate aurim1231 | DQ093659 | 99 | 495/499 |
| 100 | PDA | 15 | *Alternaria alternata* | EF452443 | 100 | 485/485 |
| 107 | 0.5%MEA | 15 | Uncultured Chaetomiaceae clone A1_k_ | EU754950 | 98 | 486/495 |
| 110 | 1.5%MEA | 15 | *Thielavia basicola* | AJ271591 | 89 | 465/521 |
| 126 | PDA | 25 | *Thielavia appendiculata* | AJ271584 | 89 | 447/498 |
| 205 | PDA | 15 | *Stachybotrys* sp. BF15 | AM901678 | 100 | 497/497 |

**Table 4.4** List of taxa isolated from rock (R) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS2R*** | | | | | | |
| 131 | PDA | 25 | *Oxyporus corticola* strain R-3610 | EF011124 | 100 | 555/555 |
| 156 | PDA | 25 | Uncultured soil fungus clone 137-5 | DQ421195 | 88 | 502/566 |
| 166 | CMA+ | 25 | Fungal sp. YNLF-26 | DQ426531 | 99 | 459/460 |
| 170 | PDA | 25 | *Oxyporus corticola* strain R-3610 | EF011124 | 100 | 555/555 |
| 229 | MYA | 15 | *Sordariomycete* sp. pgp-hsf | DQ227290 | 99 | 520/521 |
| ***MS4R*** | | | | | | |
| 46 | 0.5%MEA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 508/511 |
| 47 | MYA | 25 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | 99 | 494/495 |
| 55 | SDA | 2 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | 99 | 492/493 |
| 58 | 1.5%MEA | 2 | *Phoma macrostoma* | AJ310557 | 98 | 456/461 |
| 59 | NA | 2 | *Alternaria alternata* | EF452443 | 100 | 494/494 |
| 78 | MYA | 15 | *Phoma macrostoma* 2-1-1-1 | AJ310557 | 98 | 454/459 |
| 79 | WA | 15 | Ascomycete sp. nasa65 | DQ683978 | 94 | 451/475 |
| 81 | MYA | 15 | *Phoma macrostoma* 2-1-1-1 | AJ310557 | 98 | 456/461 |
| 145 | 1.5%MEA | 25 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | 100 | 436/436 |
| 212 | MYA | 15 | *Phoma macrostoma* | AY618249 | 99 | 428/432 |
| 228 | 0.5%MEA | 15 | *Fusarium proliferatum* | FJ040179 | 100 | 476/476 |

**Table 4.4** (Continued) List of taxa isolated from rock (R) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS8R*** | | | | | | |
| 54 | NA | 2 | *Bjerkandera adusta* isolate wb340 | AF455468 | 99 | 543/547 |
| 56 | 1.5%MEA | 2 | *Macrophoma* sp. 244-465 | DQ100416 | 91 | 461/504 |
| 80 | CMA+ | 25 | Fungal sp. YNLF-26 | DQ426531 | 100 | 478/478 |
| 206 | 1.5%MEA | 2 | *Macrophoma* sp. 244-465 | DQ100416 | 93 | 413/441 |
| ***MS10R*** | | | | | | |
| 29 | MYA | 2 | *Thielavia appendiculata* | AJ271584 | 89 | 436/488 |
| 51 | SDA | 25 | *Lecythophora* sp. UBCtra1453C | AY219880 | 89 | 447/498 |
| 130 | MYA | 25 | Fungal endophyte isolate 9097 | EF420014 | 98 | 443/448 |
| 132 | PDA | 25 | *Thielavia coactilis* | AJ271585 | 95 | 448/469 |
| 133 | PDA | 25 | *Aporospora terricola* | DQ865100 | 96 | 415/432 |
| 134 | PDA | 25 | *Lecythophora* sp. UBCtra1453C | AY219880 | 89 | 432/484 |
| 137 | NA | 15 | *Alternaria* sp. IA202 | AY154681 | 99 | 514/516 |
| 138 | BSA | 15 | *Alternaria* sp. IA317 | AY154719 | 100 | 477/477 |
| 147 | BSA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 502/505 |
| 151 | PDA | 25 | Fungal endophyte isolate 9097 | EF420014 | 95 | 473/494 |
| 152 | MYA | 2 | *Chaetomium globosum* isolate aurim1231 | DQ093659 | 99 | 478/482 |
| 153 | CMA | 25 | *Sordariomycete* sp. BC38 | DQ317345 | 98 | 450/455 |
| 167 | WA | 25 | *Chaetomium globosum* strain UAMH 7142 | AY625061 | 88 | 367/417 |
| 168 | MYA | 2 | *Thielavia appendiculata* | AJ271584 | 89 | 450/501 |
| 169 | PDA | 2 | *Thielavia coactilis* | AJ271585 | 92 | 451/487 |
| 171 | PDA | 2 | Ascomycete sp. nasa65 | DQ683978 | 94 | 443/467 |

**Table 4.4** (Continued) List of taxa isolated from rock (R) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS10R*** | | | | | | |
| 172 | SDA | 2 | *Lecythophora* sp. UBCtra1453C | AY219880 | 94 | 369/393 |
| 173 | NA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 485/486 |
| 174 | 0.5%MEA | 2 | *Embellisia phragmospora* strain EGS 27-098 | FJ357314 | 99 | 517/521 |
| 175 | CMA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 489/490 |
| 176 | CMA+ | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 100 | 484/484 |
| 177 | CMA | 25 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | 100 | 456/456 |
| 178 | NA | 2 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | 100 | 477/477 |
| 179 | PDA | 2 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | 100 | 441/441 |
| 180 | 1.5%MEA | 2 | *Alternaria tenuis* isolate NW694 | EU520208 | 100 | 488/488 |
| 226 | NA | 15 | *Apodus deciduus* | AY681199 | 87 | 388/444 |
| 227 | 0.5%MEA | 15 | *Phanerochaete laevis* | AY219348 | 99 | 572/574 |
| 231 | NA | 15 | *Apodus deciduus* | AY681199 | 87 | 423/485 |
| ***MS11R*** | | | | | | |
| 31 | SDA | 25 | Fungal sp. ARIZ B487 | FJ613076 | 85 | 399/465 |
| 48 | CMA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 521/524 |
| 49 | 1.5%MEA | 25 | Uncultured soil fungus clone CS3M5c36P | EU480267 | 92 | 241/261 |
| 53 | SDA | 25 | *Thielavia tortuosa* | AJ271592 | 82 | 437/527 |
| 57 | 0.5%MEA | 2 | *Ulocladium* sp. CID68 | EF589899 | 99 | 493/496 |

**Table 4.4** (Continued) List of taxa isolated from rock (R) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS13R*** | | | | | | |
| 182 | MYA | 25 | Fungal sp. EXP0549F | DQ914710 | 94 | 446/472 |
| 183 | NA | 2 | *Phaeosphaeria volkartiana* isolate CBS 590.86 | AF439509 | 99 | 493/497 |
| ***MS16R*** | | | | | | |
| 129 | NA | 25 | *Alternaria alternata* isolate NW562cb | EU520077 | 100 | 388/388 |
| 148 | 1.5%MEA | 2 | *Cladosporium subinflatum* strain CPC 12041 | EF679389 | 100 | 466/466 |
| 160 | NA | 25 | *Alternaria alternata* | EF452443 | 100 | 463/463 |
| 207 | 1.5%MEA | 2 | *Cladosporium* sp. PZ-2006e | DQ780407 | 100 | 444/444 |
| 210 | CMA+ | 15 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 491/492 |

**Table 4.5** List of taxa isolated from wood (W) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MSW*** | | | | | | |
| 203 | PDA | 2 | *Embellisia phragmospora* strain EGS 27-098 | FJ357314 | 99 | 517/518 |
| 230 | SDA | 15 | *Thielavia intermedia* | AJ271588 | 83 | 402/483 |
| 232 | 0.5%MEA | 2 | *Thielavia microspora* | AJ271577 | 92 | 458/493 |
| ***MS8W*** | | | | | | |
| 187 | BSA | 15 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 485/486 |
| 188 | 0.5%MEA | 15 | Ascomycete sp. nasa65 | DQ683978 | 94 | 439/463 |
| 191 | PDA | 25 | *Ulocladium consortiale* | AY278837 | 99 | 476/477 |
| 192 | BSA | 25 | *Ulocladium consortiale* | AY278837 | 99 | 491/492 |
| 197 | MYA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 519/522 |
| 201 | CMA+ | 15 | Ascomycete sp. nasa65 | DQ683978 | 95 | 457/478 |
| 209 | BSA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 100 | 480/480 |
| 216 | 1.5%MEA | 15 | Ascomycete sp. nasa65 | DQ683978 | 95 | 421/439 |
| 217 | WA | 2 | Ascomycete sp. nasa65 | DQ683978 | 95 | 415/433 |
| 218 | MYA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 498/501 |
| 219 | PDA | 2 | *Ulocladium* sp. CID68 | EF589899 | 99 | 515/516 |
| 224 | SDA | 2 | Ascomycete sp. nasa65 | DQ683978 | 95 | 448/468 |

**Table 4.5** (Continued) List of taxa isolated from wood (W) collected in the Taklimakan Desert.

| Isolate | Media | Temp. | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|---|---|
| ***MS13W*** | | | | | | |
| 193 | 1.5%MEA | 25 | *Alternaria alternata* | EF452443 | 100 | 488/488 |
| 194 | PDA | 25 | *Thielavia hyalocarpa* | AJ271583 | 93 | 437/466 |
| 195 | SDA | 25 | *Ulocladium consortiale* | AY278837 | 99 | 491/494 |
| 196 | WA | 15 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 516/520 |
| 215 | 0.5%MEA | 2 | Ascomycete sp. nasa65 | DQ683978 | 95 | 429/449 |
| 225 | 1.5%MEA | 2 | *Cladosporium tenuissimum* isolate 029.4 | FJ228171 | 100 | 455/455 |
| ***MS15W*** | | | | | | |
| 198 | 0.5%MEA | 15 | Uncultured Pyronemataceae clone DGGE band BD6 | DQ317369 | 95 | 303/317 |
| 199 | 1.5%MEA | 25 | Uncultured Pyronemataceae clone DGGE band BD6 | DQ317369 | 95 | 310/324 |
| 200 | PDA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 491/492 |
| 208 | MYA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 513/516 |
| 213 | SDA | 2 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | 99 | 481/482 |
| 214 | CMA+ | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 497/501 |
| 220 | MYA | 2 | Fungal sp. AB58 | FJ235991 | 91 | 492/538 |
| 221 | 0.5%MEA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 499/500 |
| 222 | SDA | 2 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 484/486 |
| 223 | BSA | 25 | *Tetracladium setigerum* isolate NW313 | EU622246 | 99 | 495/498 |

### *4.4 Phylogenetic analysis*

#### *4.4.1 Basidiomycota*

The fungi isolated from samples collected in the Taklimakan Desert that were determined initially to belong to the Basidiomycota phyla based on alignment of all isolated sequences and neighbor joining analysis, were realigned and analyzed in the absence of individuals from other phyla, resulting in 4 distinct lineages with strong bootstrap support (Figure 4.1). These terminal clades all represent common wood decay fungi found throughout temperate and tropical regions. The *Oxyporus corticola*, *Bjerkandera adusta* and *Phanerochaete laevis* clades show extremely high levels of support and relatedness to the associated isolates from the Taklimakan Desert. Fungal isolate 156 from rock sample MS2R is distant from *Sistotrema sernanderi*, its nearest published NCBI BLAST match and the ITS sequence of this isolate had only an 88% match to the closest submitted sequence in this database. Fungal isolate 156 may, therefore, represent a new species based on DNA analysis of the ITS region and comparison to the NCBI data base although further study is required. Although all of the isolates are known as wood decay organisms, they were isolated from rock and were not isolated from the wood samples taken from the desert or the soils. There is a complete absence of higher plant cover at this location, so lignocellulose input is exceedingly rare. The only Basidiomycete representative sequence from Polar regions included in this tree was isolated from mummified wood in the Canadian High Arctic (Jurgens et al. 2009) and is quite distant from any known fungi (based on BLAST search) or Taklimakan Desert fungi isolated. No Basidiomycota from Antarctica were included, as all of the fungi from this phyla isolated in the comparative study (Arenz et al. 2006) were yeasts.

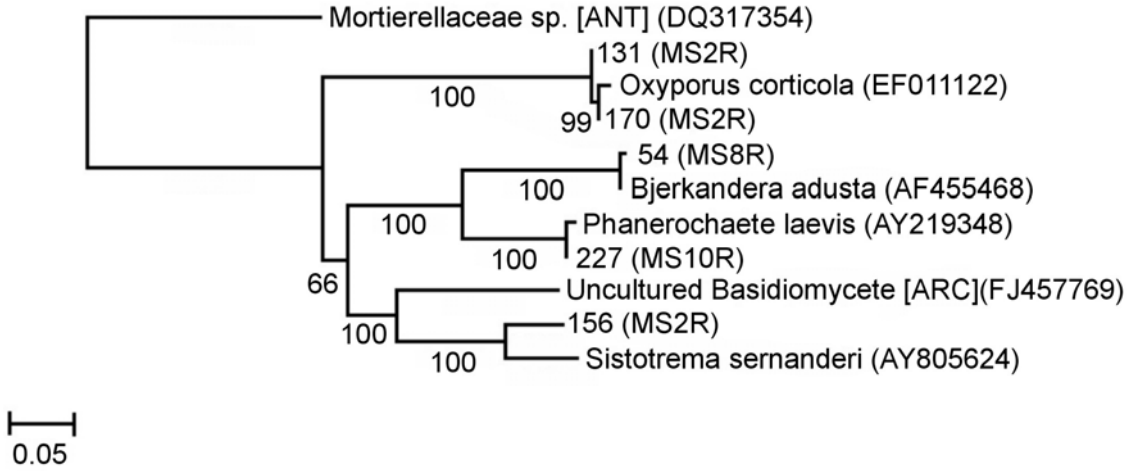


**Figure 4.1** A neighbor-joining phylogenetic tree based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between Basidiomycota isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The individual with [ARC] was isolated from a sample collected in the Arctic (Jurgens et al. 2009). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

### *4.4.2. Ascomycota*

The Ascomycota fungi isolated from the soil, rock and wood samples collected in the Taklimakan Desert for this PhD thesis research represent a diverse assemblage with members belonging to 13 orders based on ITS sequence data (Bisby et al. 2009; Hibbett et al. 2007) (Figure 4.2). The first group, which is represented in Figure 4.3 as clade A, is the most diverse with taxa from 5 orders. The greatest number of these are in the Helotiales, an order of the class Leotiomycetes, including the genera *Acephala, Cadophora, Geomyces,* and *Phialocephala.* Others from clade A include *Thelebolus* from the order Thelebolales, *Exophiala* from the order Chaetothyriales,

several from the family Pyronemataceae**,** order Pezizales and *Aureobasidium* from the Dothideales order. Clade B (Figure 4.4) includes taxa from the family Trichocomaceae, order Eurotiales and clade C (Figure 4.5) also includes members from a single order, the Hypocreales. In clade D (Figure 4.6), the order Microscales, with a representative of *Scedosporium* and several *Cladosporium* sp. from the order Capnodiales were found, however, the taxonomy of the latter group has received a great deal of revision recently (Schoch et al. 2007). As investigations into the evolutionary associations of the Capnadiales continues, with specific regions of genes or amino acids targeted, greater resolution and likely additional separations will be made. Clade E (Figure 4.7) had a distinct separation into two lineages, with *Lecythophora* from the order Chaetothyriales and *Apodus* from Sordariales. Clades F and G (Figures 4.8 and 4.9) were both from the order Sordariales with taxa from *Thielavia* and *Chaetomium.* Clades H, I, J, K and L (Figures 4.10, 4.11, 4.12, 4.13 and 4.14) had the greatest diversity within a single order consisting of *Phoma, Epicoccum, Alternaria, Embellisia, Ulocladium* and *Stemphylium,* all of which are in the Pleosporales. Clade M (Figure 4.15) has taxa that are not necessarily clearly assigned to specific orders but are likely from either Pleosporales or Sordariales because of the branching in this region of the tree.

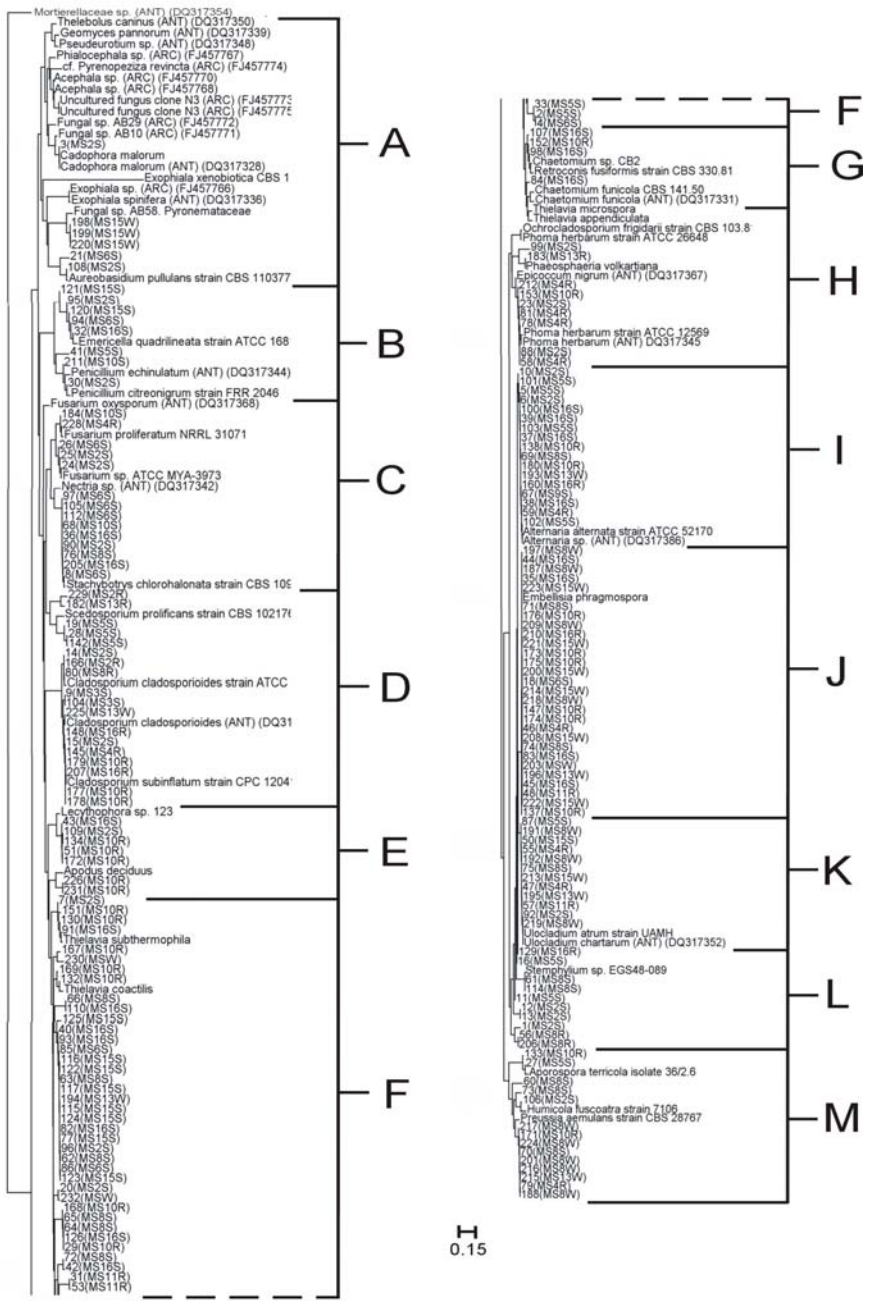


**Figure 4.2** A neighbor-joining phylogenetic tree based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between Ascomycota isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The individuals with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

**Clade A**

Clade A is perhaps the most diverse group primarily because of the inclusion of isolates from both the Arctic (Jurgens et al. 2009) and the Antarctic (Arenz et al. 2006), collectively called Polar fungi (Figure 4.3). When these Polar fungi are not viewed, the Taklimakan Desert isolates only account for 6 isolates in 3 subclades, the first of which is associated with a CBS *Aureobasidium pullulans* strain that is a strongly supported, an exact match to isolate #108 (MS2S) and more distant but well supported to isolate #21 (MS6S). *Aureobasidium pullulans* is one of the dominant fungi isolated from hypersaline environments, including dry and/or salty sites in Antarctica (Onofri et al. 2006) and is considered to be halophilic (Gunde-Cimerman et al. 2000; Buchalo et al. 1998).

Another, is a group of 3 morphologically and by DNA sequence identical isolates designated isolates #198, 199 and 220, which were all generated from the wood sample MS15W, which shows a high degree of support but some distance to their closest match from Antarctic soil (Fungal sp. AB58) from the Family *Pyronemataceae*. With only an identification to the family level and with this based solely on ITS sequences, it is difficult to know exactly what the evolutionary relationship is between these 3 isolates and the *Pyronemataceae* sequence. As discussed further in Chapter 6, this is an example where more DNA sequences and of various genes are required to determine more about the evolutionary relationship between isolates. *Pyronemataceae* is a very large family with approximately 500 known species (Kirk et al. 2001) with most from temperate to Arctic/alpine regions and a few taxa from the tropics (Perry et al. 2007). Members of this family have

primarily been considered saprotrophic, fungi which gain nutrition by a process of chemoautotrophic extra-cellular digestion involved in the processing of dead or decayed organic matter but most species have not been well studied. However, some have been found to be parasites of bryophytes (Benkert 1993) and more recently many are seen as ectomycorrhizal associates (Fujimura et al. 2005; Smith et al. 2006).

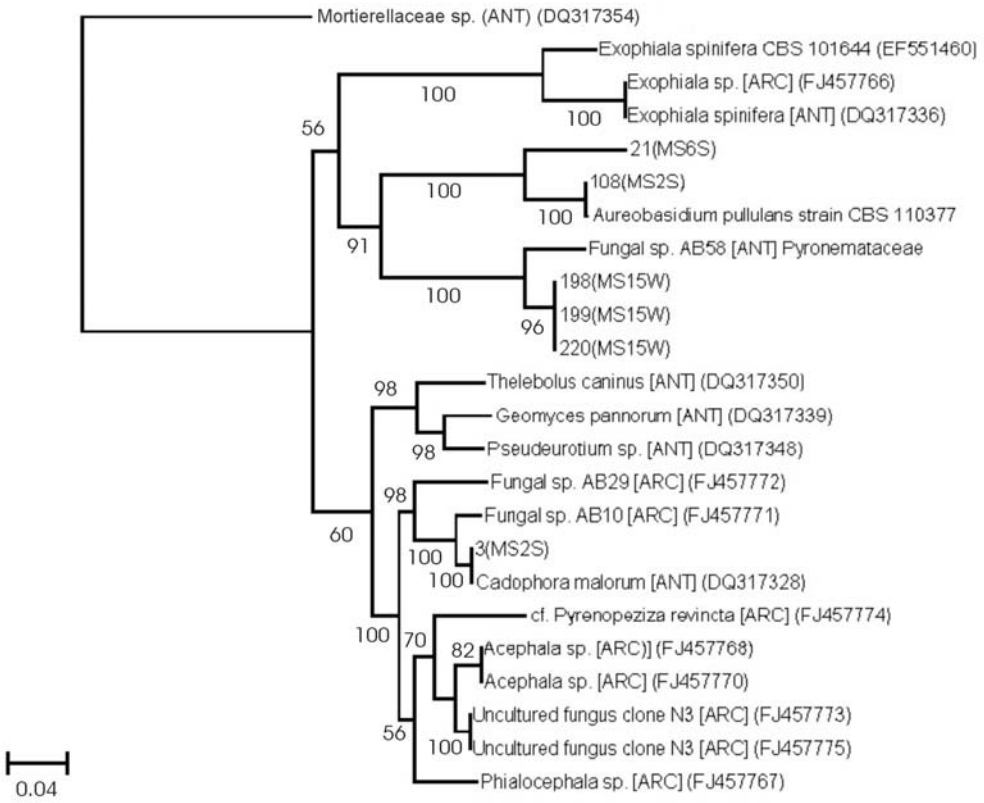


**Figure 4.3** A neighbor-joining phylogenetic tree of the A clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

The third subclade that has a Taklimakan isolate included is highly supported and matches the ITS sequence to the Antarctic isolate *Cadophora malorum* (DQ317328). The *Cadophora* genera is a common inhabitant of soils and wood in Polar regions (Blanchette et al. 2004; Arenz et al. 2006; Jurgens et al. 2009). Laboratory decay studies conducted by Blanchette et al. (2004) and Held et al. (2006) on *Betula* sp. using *Cadophora* species isolated from Antarctica revealed extensive type 1 soft rot after 16 weeks. Based on a recent molecular taxonomic study by Harrington and McNew (2003), many *Phialophora* sp. were renamed to the *Cadophora* group.

***Clade B***

This clade is from the Trichocomaceae family with culture collection reference sequences from both the *Penicillium* and *Emericella* genera (Figure 4.4). These are both very common fungal genera that can be isolated from countless substrates collected in various environments. *Penicillium* is a broad group with many members that has been found to tolerate adverse condition allowing it to fit into numerous extremophile categories such as psychotolerent (Duncan et al. 2006), thermophilic (Salar and Aneja 2007), halophilic (Cantrell et al. 2006; Trüper and Galinski 1986), osmophilic (Pitt and Hocking 1997), xerophilic (Dix and Webster 1995), acidophilic (López-Archilla 2004), alkalophilic (Kladwang et al. 2003), radioresistant (Dadachova and Casadevall 2008) and endolithic (Etienne and Dupont 2002). The *Penicillium* group exhibits 100% support throughout clade B with all desert fungi originating from soils. The *Emericella* branch also displayed strong bootstrap support with all of its members also residing from soils. Isolate #121 (MS15S) branched prior to the other groups and did not match any submitted accession to GenBank, which

indicates that it is unique when compared to other in this date base and requires further study.

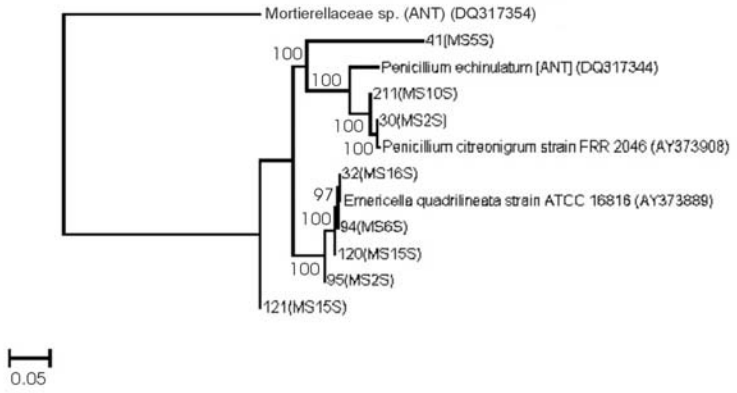


**Figure 4.4** A neighbor-joining phylogenetic tree of the B clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

### *Clade C*

Clade C consists of 14 isolates derived from Taklimakan Desert substrates, 13 originating from soils and 1 from rock (Figure 4.5). Two subclades separated with well supported branches terminating in the genera *Fusarium* and *Stachybotrys*. Within the *Fusarium* subclade, there was further separation into the highly supported branch of *Fusarium* sp. (FJ614644), which was closely aligned with the 3 soil isolates #24, 25 and 26 and *Fusarium proliferatum* (AF291061), which again displayed a high

degree of support and evolutionary similarity isolates #184 and 228. *Fusarium* is considered one of the most abundant and important soil inhabiting fungi. It is often referred to as ubiquitous and contains environmental, agricultural and human health importance (Wakelin et al. 2008).

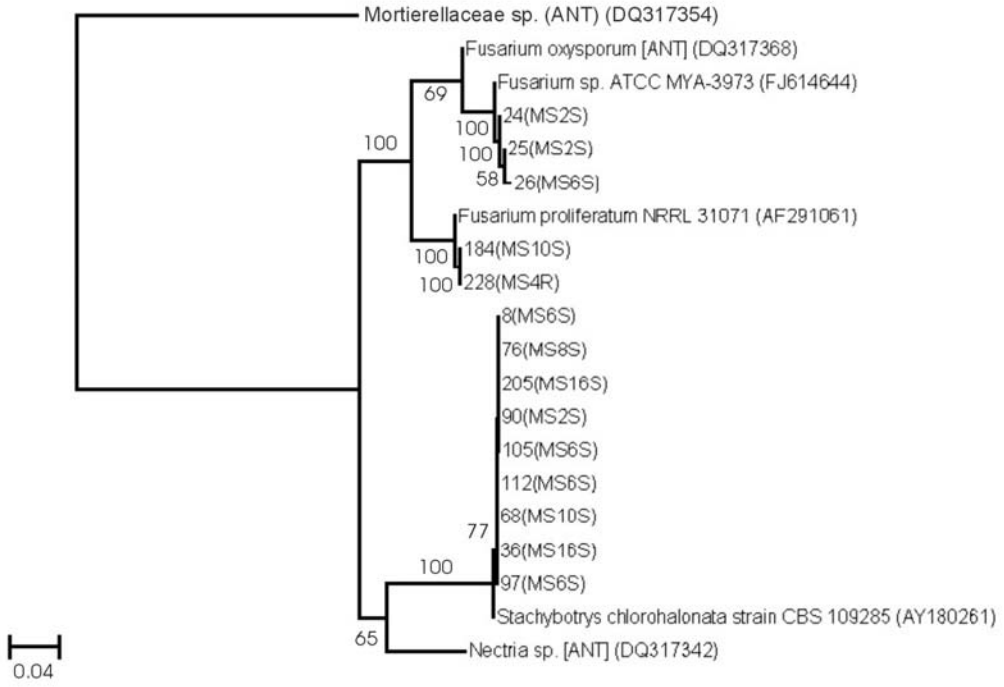


**Figure 4.5** Clade C neighbor-joining phylogenetic tree extracted from figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

The *Stachybotrys* group is highly supported with the known CBS strain and the 9 Taklimakan isolates having remarkable evolutionary similarity between all of the soil derived fungi. This genus has been isolated from diverse conditions including the

high saline waters of the Dead Sea (Buchalo et al. 2000). Anastasi et al. (2005) isolated *Stachybotrys* sp. from compost piles that reached a maximum temperature of 60ºC, while isolates have also been recovered from both Antarctica (Ruisi et al. 2007) and the Arctic (Gunde-Cimerman et al. 2003). The *Stachybotrys* group is also fairly common in 'damp buildings', where it has also been correlated to a serious human respiratory disease (Kuhn and Ghannoum 2003).

***Clade D***

Clade D primarily consists of members of the *Cladosporium* genera and displayed a great deal of similarity within the branch containing the known individuals *Cladosporium subinflatum and C. cladosporioides* with mixed bootstrap support (Figure 4.6). Several isolates from the Taklimakan Desert collection (#166, 14 and 80) were also very similar to the Antarctic sequence included in this tree from the isolate *Cladosporium cladosporioides* [ANT]. *Cladosporium* spp. are one of the most widely distributed and common fungi isolated from a broad range of substrates throughout the world. They are common endophytes (Brown et al. 1998), saprophytes on dead and dying plants, and often isolated from air, soil food, humans and several of substrates (Samsom et al. 2000; de Hoog et al. 2000).

The other clade surrounding *Scedosporium prolificans* had greater support but less affinity to this sequence originating from a Centraalbureau voor Schimmelcultures culture. The *Scedosporium* group is a serious human respiratory pathogen primarily affecting patients that have received heart and lung transplants and is highly resistant to common antifungal treatments (Tintelnot et al. 2009). The ability of some

members of this group to grow at the high temperatures found within the human body and resistance to antimicrobial agents may indicate why they were found in the harsh ecological conditions found in the Taklimakan Desert. The soil derived isolates from the Taklimakan (#19, 28, 142), although distant based on branching patterns making definitive identification difficult, have more similarity to the known CBS isolate than isolates #182 and 229 generated from the rock samples, which are quite distant and are not similar to others in GenBank.

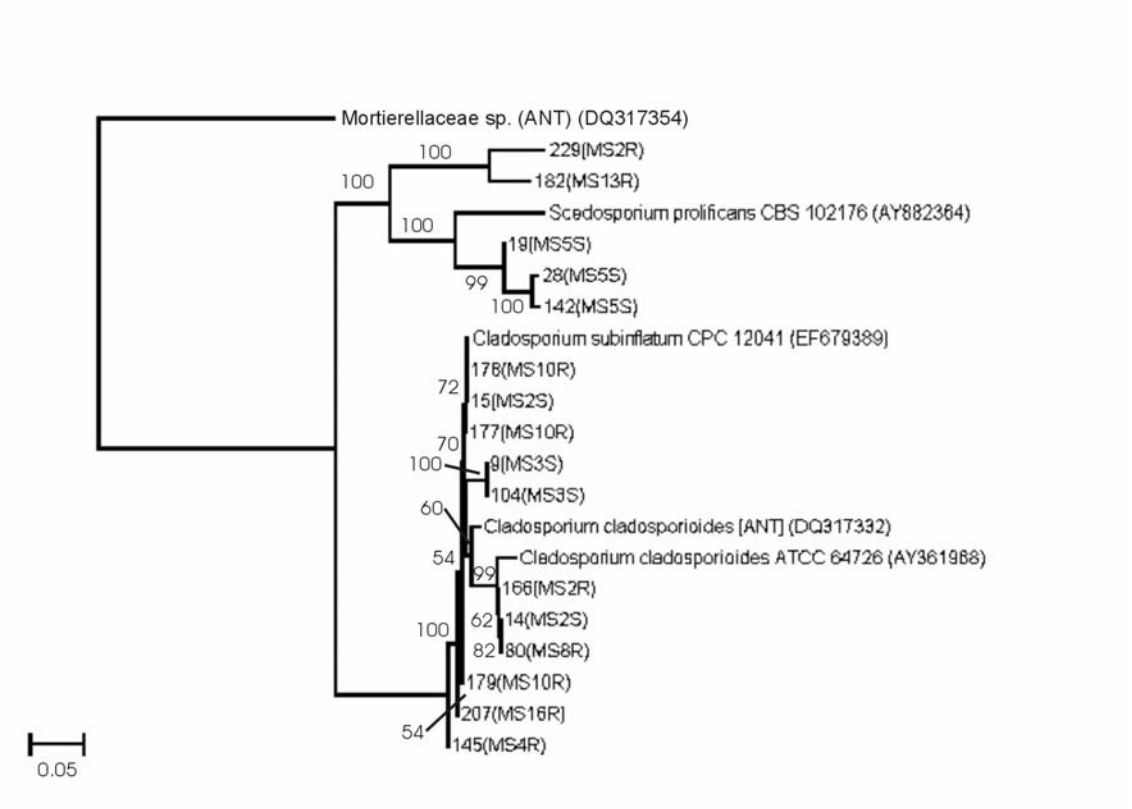


**Figure 4.6** A neighbor-joining phylogenetic tree of D clade extracted from Table 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

***Clade E***

Clade E divided into two subclades which were strongly supported (Figure 4.7) and consisted of the published sequences of a *Lecythophora* sp. (FJ228192) and *Apodus deciduus* (AY681199). Members of the *Lecythophora* genera are often identified as saprobes in soil, sewage water, and decaying wood and have also been found as a foliar endophyte of white spruce and as a root-associated fungus (Chang et al. 2005). They have also been characterized in more extremophilic settings such as extremely low pH (2.2) water with high concentrations of heavy metals (López-Archilla 2004), wooden structure in Antarctica (Held et al. 2006), rock from mountains in central Spain (Ruibal et al. 2008) and as human pathogens (Hoog et al. 2000). The genera *Apodus*, from the order Sordariales, which is evolutionarily closely related to *Thielavia* (clade F) and *Chaetomium* (clade G), has been cultured from dung (Cai et al. 2006).

Within the *Lecythophora* subclade, 5 closely related isolates generated from one rock and 2 soil samples were found, however, their evolutionary relatedness are separated by more than 10% and all require further characterization. The subclade with Taklimakan Desert isolates #226 (MS10R) and #231 (MS10R) are also distantly related to their closest BLAST sequence, *A. deciduus*, with only an 87% identity match and also warrant greater investigation.

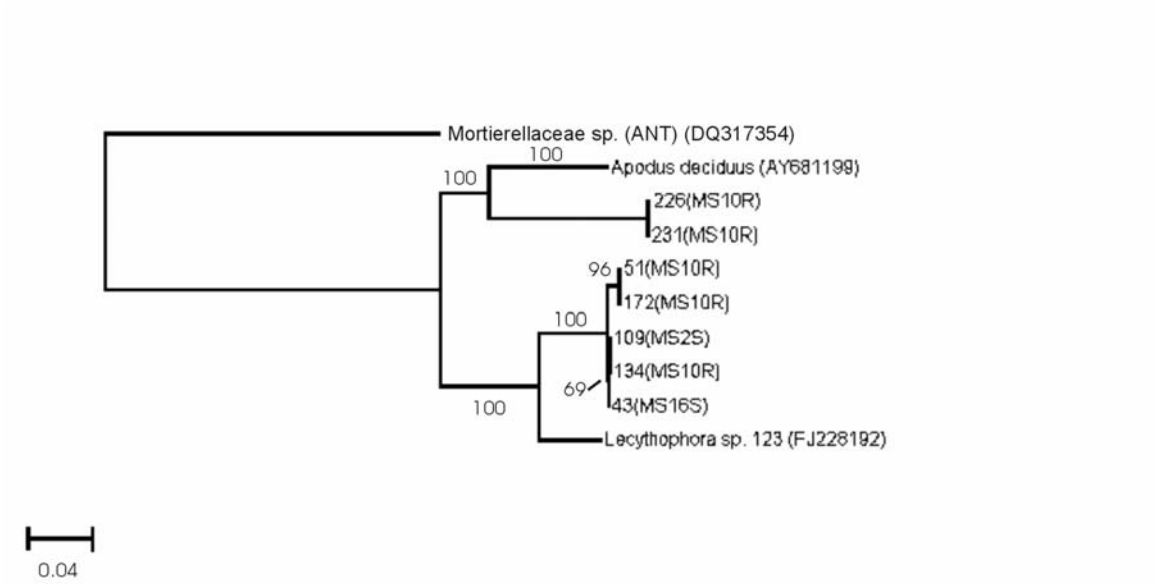


**Figure 4.7** A neighbor-joining phylogenetic tree of the E clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

***Clade F***

Clade F had the greatest number of representatives from the Taklimakan isolates with several branches showing close relatedness and mixed support to *Thielavia* sp. (Figure 4.8). The largest subclade within this group surrounded the CBS sequence of *Thielavia appendiculata* (AJ271584), representing 19 isolates, all derived from soils. Two other groups, comprising 5 soil and 2 rock derived samples, separated prior to the above mentioned group and all had < 94% identity matches to anything in the NCBI database but are clearly still within this genera because there are *Thielavia* sp. above and below them. A third group clustered with the known sequence of *Thielavia*

*subthermophila* (AJ271575) with individuals (#7, 91, 130 and 151) having strong support and these were generated from both soils and rock. There was also a small branch with 2 isolates [#20(MS2S) and #232(MSW)] that had a <95% BLAST match to *Thielavia microspora* and require more characterization to determine if they are new taxa. Another subclade had a sequence from #132 (MS10R) that was very similar and highly supported to *Thielavia coactilis* (AJ271585). There was also a group of 5 Taklimakan Desert isolates, #29, 64, 65, 126 and 168 from both soils and rock that branched near *T. coactilis* that requires more investigation to determine if they are the same species. The final group of #167 (MS10R) and #230 (MSW) branched prior to the main *Thielavia* clade and both had less than an 88% to any other submission in the NCBI database.

Members of the *Thielavia* genera are common soil inhabiting microorganisms but most often they are found in soils subjected to adverse environmental conditions. An example is *Thielavia antarctica,* a psychrotolerant species isolated from lichens collected on King George Island Antarctica (Stchigel et al. 2003). Other species have been isolated from the saline soils and water of the Dead Sea (Molitoris et al. 2000). In addition, thermotolerant species were found growing in camel dung on the Sinai Peninsula (Moustafa and Abdel-Azeem 2008) and 'heat tolerant species' were observed causing soft rot decay in compost piles (Tuomela et al. 2000).


**Figure 4.8** A neighbor-joining phylogenetic tree of the F clade extracted from Table 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

***Clade G***

This clade consists of isolates that display an affinity towards *Chaetomium*, which is evolutionarily closely related to the members of clade F, *Thielavia* sp. (Figure 4.9). Like *Thielavia,* the genus *Chaetomium* is found on a wide variety of substrates under diverse conditions. It is common in soils (Rodríguez et al. 2002), house dust (Udagawa et al. 1997), as a mycorrhizal associate (Violi et al. 2007) and can cause soft rot decay in wood treated with copper based preservatives (Bridžiuvienė and Levinskaitė 2007). The neighbor-joining tree of this clade is centered around the known CBS sequence of *C. funicola* (AJ279450) with weak support and relatively long branch length. The isolates from the Taklimakan Desert were generated from both soils and a rock sample, with #84 (MS16S) showing some relatedness to the Antarctic *C. funicola* (DQ317331). This clade overall has weak support and a high percentage of change based on branch length and requires further characterization.

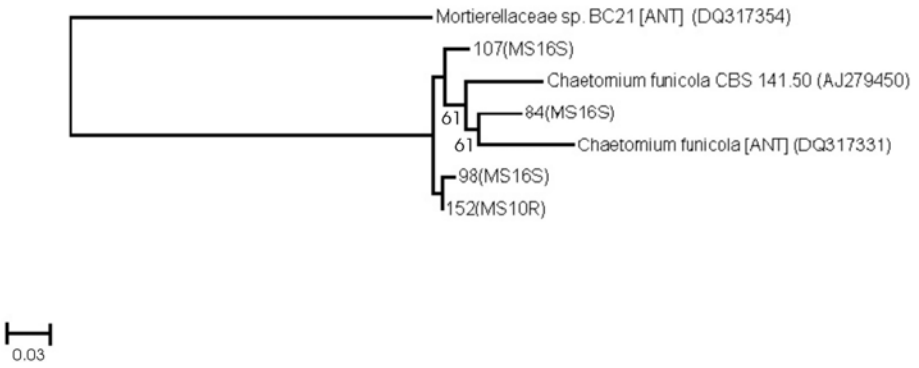


**Figure 4.9** A neighbor-joining phylogenetic tree of the G clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

### *Clade H*

The neighbor-joining analysis of clade H was dominated by Taklimakan Desert isolates that are very similar to *Phoma* sp. from published culture collection sequences (Figure 4.10). *Phoma* sp. has been called 'omnipresent' because of its wide distribution throughout various ecological niches (Aveskamp et al. 2009). It is primarily isolated from soils and a broad range of plant hosts including moncots and dicots in which it is either a pathogen, opportunist, saprobe or endophyte (Aveskamp et al. 2008). All branches had greater than 50% bootstrap support and the desert isolates originated from either soil or rock. Two Antarctic isolates fell into this clade, *Phoma herbarum* (DQ317345) and *Epicoccum nigrum* (DQ317367), which Arenal et

al. (2004) determined were synonymous based on morphology and phylogenetic sequence analysis of the ITS region

The other group that created a branch in clade H was related to *Phaeosphaeria* sp. and was highly supported. The *Phaeosphaeria* genus was originally grouped with *Leptosphaeria*, the anamorph of *Phoma*, until a recent investigation determined that phylogenetic analysis and morphological characteristic were varied enough to separate them into different but very similar groups (Camara et al 2002). The closest match to the known *Phaeosphaeria* sp. sequence was isolate #183 from MS13R, while #99 isolated from soil requires further characterization to determine how closely related it is to the others.

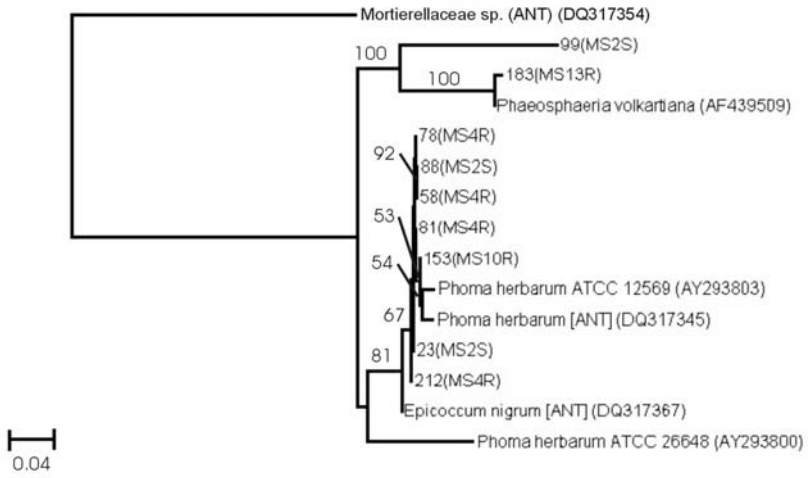


**Figure 4.10** A neighbor-joining phylogenetic tree of the H clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

***Clade I, J and K***

The clades exhibiting the greatest evolutionary relatedness are those of I, J, and K with representatives of known individuals including *Alternaria*, *Embellisia* and *Ulocladium*, respectively (Figures 4.11, 4.12 and 4.13). These groups not only cluster tightly during separate phylogenetic analysis but also when analyzed together (Figure 4.2). Many of the fungi from these groups are so similar morphologically that individuals had multiple generic names and definitive separation was not made until a phylogenetic study using rDNA from the nuclear internal transcribed-spacer region (ITS1/5.8S/ITS2), the mitochondrial small-subunit (mt SSU) and a portion of the glyceraldehyde-3-phosphate dehydrogenase (gpd) gene was used to determine their clustering (Pryor and Bigelow 2003). With the neighbor-joining analysis conducted in this PhD thesis research study, it was not possible to determine separation between most individuals from these groups by using the Zygomycete, *Mortierellaceae* sp. (DQ317354) as an outgroup as was done for the other trees because this did not create significant separation allowing delineation of taxa. To overcome this, the more related *Phoma herbarum* (AY293791) was used, however, the scale in these figures only represent a 1% change indicating how genetically similar they are.

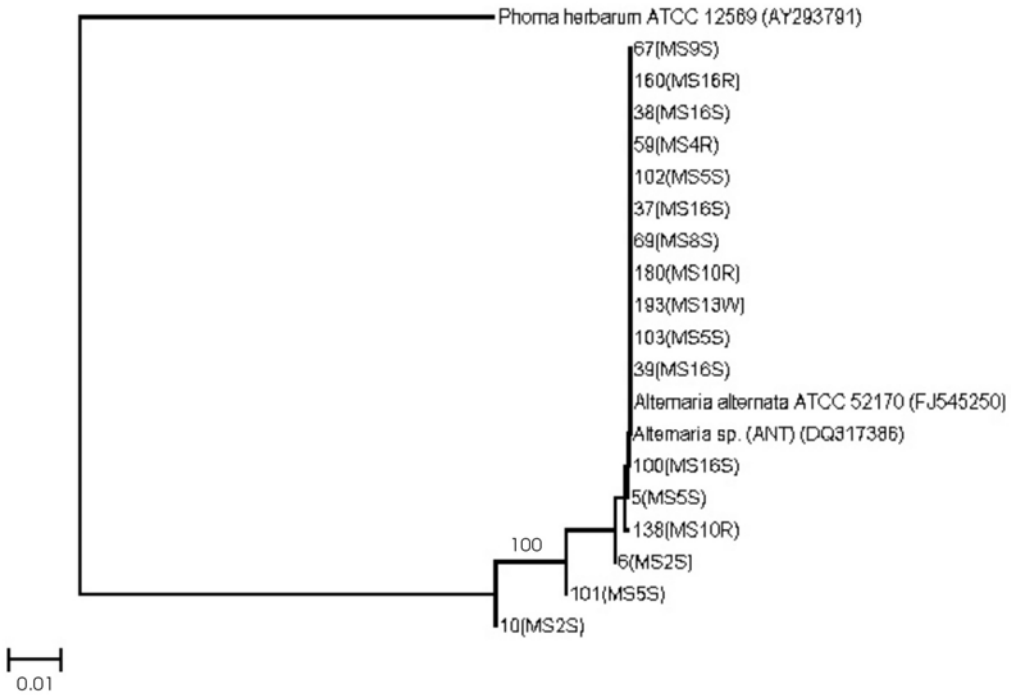


**Figure 4.11** A neighbor-joining phylogenetic tree of the I clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). *Phoma herbarum* (AY293791) is used as an outgroup.

Within clade I, there were 11 Taklimakan Desert isolates, #67, 160, 38, 59, 102, 37, 69, 180, 193, 103, 39 originating from soil, rock and wood matching *Alternaria alternata* ATCC (FJ545250) and the Antarctic isolate *Alternaria* sp. (DQ317386), and with #100 (MS16S), 5 (MS5S), 138 (MS10R) and 6 (MS2S) in very close

proximity. Only the isolates #101 (MS5S) and 10 (MS2S) deviated from the primary group, each by approximately 1%.

The '*Embellisia'* clade J was also closely knit with isolates from all substrates found in this clade, though there was a slight preference for isolates from the wood as 12 of the 28 were generated from this organic material. The entire clade has less than a 2% separation.

Clade K had 75% of the *Ulocladium* sp. Taklimakan Desert isolates on the same branch which had representatives from all substrates. Over 40% of the fungal sequences were derived from isolates originating from wood and there was less than a 0.5% change in all sequences including *Ulocladium chartarum* (DQ317352) from Antarctica and *Ulocladium atrum* (AY625072).

*Alternaria*, *Embellisia* and *Ulocladium* are all considered ubiquitous genera that can be found in nearly any environment and on any substrate as either a colony or spore. They also have a wide variety of roles, from saprobe, plant pathogen, soft rot decayer, and can be pathogenetic to humans (Halaby et al. 2001; Hoog et al. 2000; Hyde et al. 2007; Peever et al. 2002; Wang 1990).

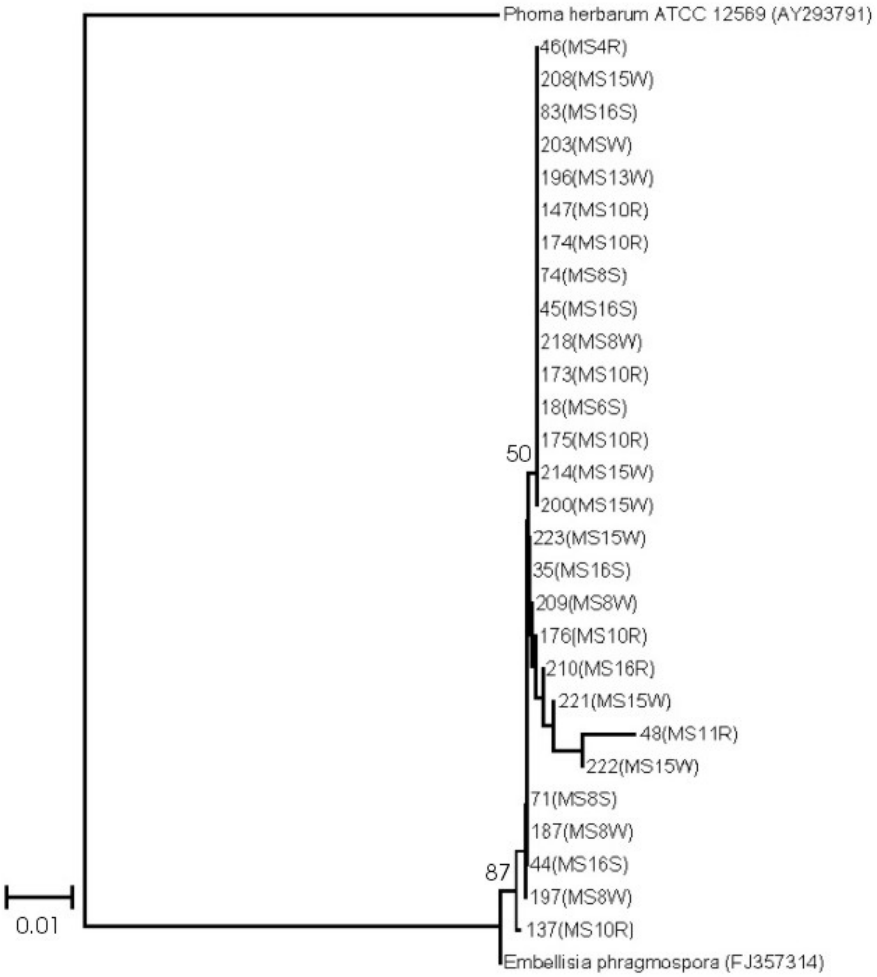


**Figure 4.12** A neighbor-joining phylogenetic tree of the J clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). *Phoma herbarum* (AY293791) is used as an outgroup.

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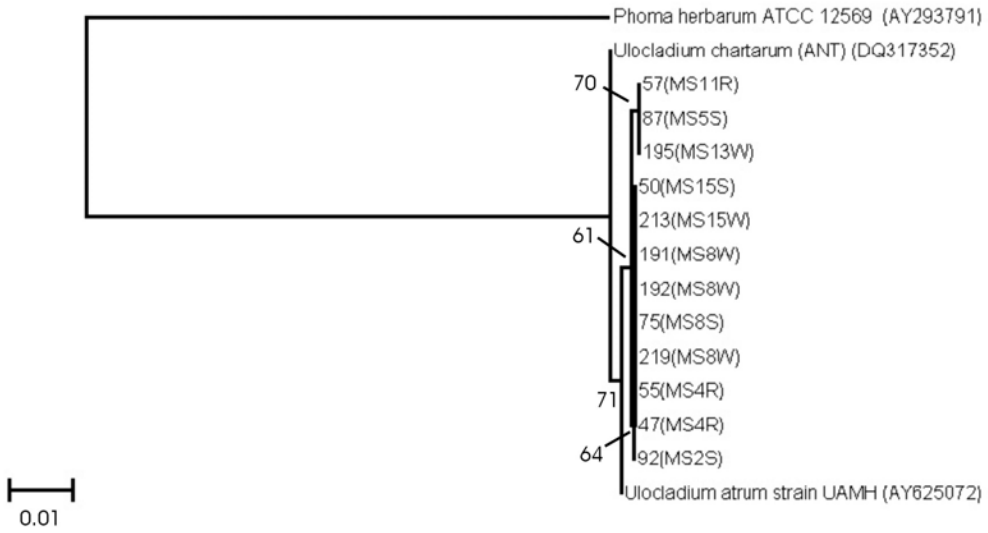


**Figure 4.13** A neighbor-joining phylogenetic tree of the K clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). *Phoma herbarum* (AY293791) is used as an outgroup.

***Clade L***

The neighbor-joining clade L had isolates similar to *Stemphylium* sp. (AY329186) (Figure 3.14) which has been referred to as a 'sister clade' to *Alternaria*, *Embellisia* and *Ulocladium* (Pryor and Bigelow 2003). This group however did not show the tight association present in I, J and K. It is a group that is again very diverse in its distribution with a similar ecology to the three above mentioned genera. Only isolates from soil and rock were found in this group which had high bootstrap support throughout but considerable separation from any submitted sequence in the NCBI

database, with the exception of #61 and 114 from (MS2S). Further characterization is required to gain a better understanding of these lineages.

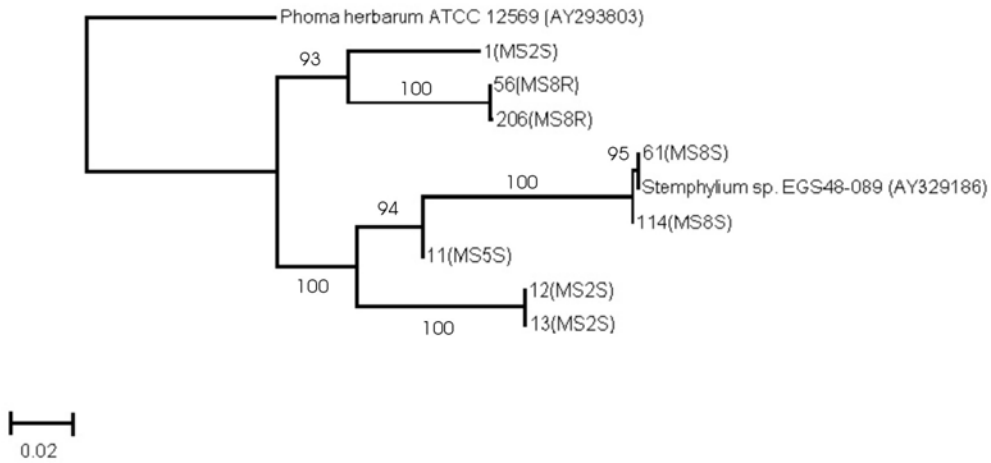


**Figure 4.14** A neighbor-joining phylogenetic tree of the L clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). *Phoma herbarum* (AY293791) is used as an outgroup.

### *Clade M*

Clade M separated into three primary subclades, the largest of which consisted of nine Taklimakan Desert isolates originating from 1 soil, 2 rock and 6 wood samples with the closest culture collection match to *Preussia aemulans* (DQ468017) from the Centraalbureau voor Schimmelcultures (Figure 3.15). This group showed relatively low bootstrap support and essentially no change between members. The distance from the known *P. aenulans* sequence is considerable but all isolates did have 94-

95% identity to their top BLAST match, a sequence of Ascomycete sp. nasa65 (DQ683978) which was isolated from soil in the hyperarid Atacama Desert of Chile (Conley et al. 2006). Besides showing similarity to the fungal isolate from the very comparable climatic conditions of the Atacama Desert, *Preussia* sp. can be found in soils, wood and plant debris (Cain 1961; Arenal et al. 2005).

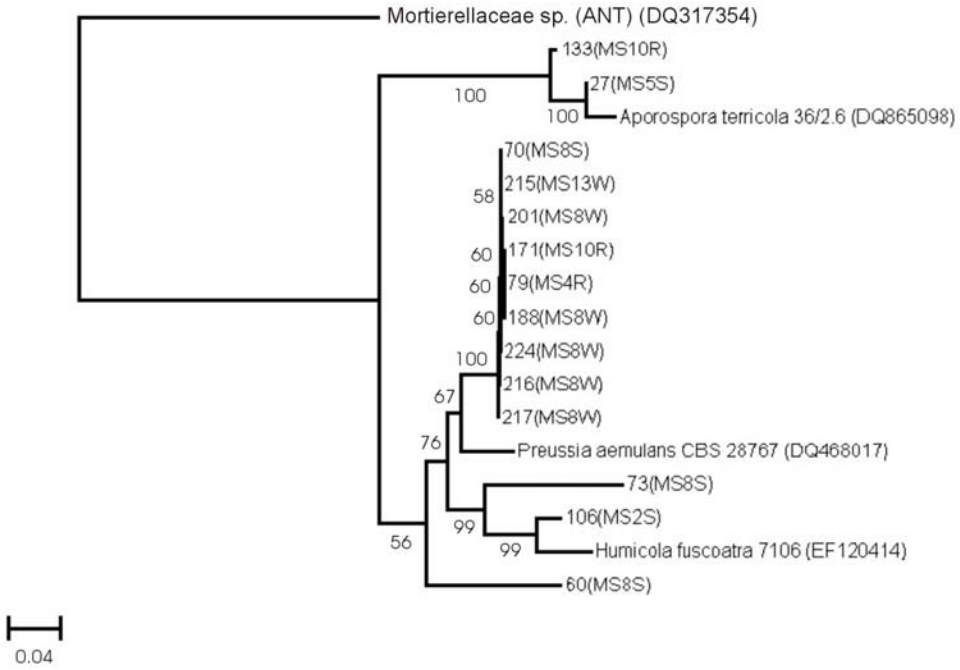


**Figure 4.15** A neighbor-joining phylogenetic tree of the M clade extracted from Figure 4.2 based on sequences of the internal transcribed spacer (ITS) region of nuclear rDNA showing the sequence similarities between isolates generated from samples collected in the Taklimakan Desert (#'s [MSXX]) and related fungi with GenBank accession numbers. Sequences were aligned by ClustalW in DS Gene software. Bootstrap values of more than 50% are shown and are based on 1000 replicates and branch lengths are proportional to base pair differences. The isolates with [ARC] were isolated from a sample collected in the Arctic (Jurgens et al. 2009) and [ANT] indicates isolates from samples collected in Antarctica (Arenz et al. 2006). A *Mortierellaceae* sp. (DQ317354), a Zygomycete is used as an outgroup.

A second subclade which had strong support but significant differences from the compared isolate of *Humicola fuscoatra* (EF120414) had two soil derived isolates,

#73 (MS8S) and 106 (MS2S). The *Humicola* genera contains a variety of thermophilic fungi, typically found in compost and dung (Mouchacca 1997), while Salar and Aneja (2006) found that *H. grisea* and *H. insolens* isolated from soils in Northern India grew at 55ºC but not below 20ºC.

Just prior to the *Preussia* and *Humicola* subclades, the Taklimakan Desert isolate of #60 (MS8S) separated with weak bootstrap support and a high degree of change. This isolate is interesting not just because of its distance from other sequences in the NCBI database with only an 84% match to *Preussia fleischhakii* strain CBS 56563 (DQ468019). Isolate #60, isolated from Taklimakan Desert soil was cultured on the relatively nutrient weak medium of 0.5% MEA at 2ºC and warrants further investigation.

The final subclade is delineated by the presence of the published sequence of *Aporospora terricola* (DQ865098), which is a taxon that has received limited study but was recently isolated from plant roots growing in sands with high salt concentrations in Southeastern Spain (Maciá-Vicente et al. 2008). The Taklimakan Desert isolates of #133 and 27 from rock and soil respectively, had very strong support for their separation and 96-97% match to the known sequence.

### *4.5 Diversity of fungi isolated from the Taklimakan Desert*

#### *4.5.1 Fungal diversity based on isolation substrate*

The filamentous fungi isolated from the collection site near Ruoqiang, China in the Taklimakan Desert were derived from three substrates, soil, rock and wood,

generating a total of 194 isolates. The greatest percentage of these were from soil totaling 53% (Figure 4.16), with rock samples contributing 31% and wood providing the remaining 16%. From the 9 soil samples, 103 individual isolates grew, averaging slightly more than 11 isolates per sample. The 7 rock samples generated 60 isolates, ~8.5 fungi per sample and 31 isolates were derived from the 4 wood samples averaging nearly 8 per sample.

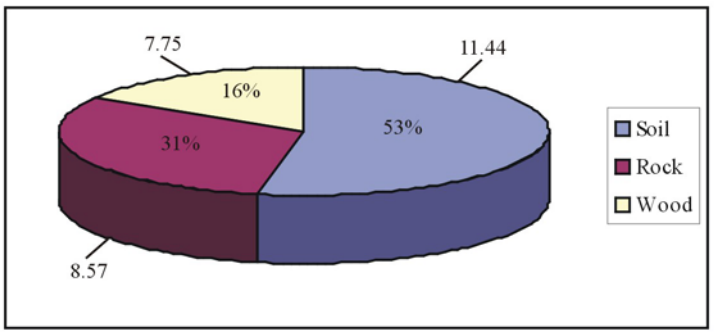


**Figure 4.16** Percentage of fungal isolates with relation to source substrate (soil, rock & wood) collected in the Taklimakan Desert and mean number of isolates from each substrate.

The number of fungal isolates generated from each sample are given (Figure 4.17, 4.18 and 4.19), soil, rock and wood, respectively. The greatest numbers of fungal isolates generated from soil samples were from MS2S (23 isolates) and MS16S (22 isolates) (Table 4.17). Rock samples were also collected in association with these soils, however, both MS2R and MS16R only generated 5 isolates each, which was less than the average number (Figure 4.16). Sample MS8S had the third greatest number of isolates (16 isolates) with the associated rock sample (MS8R) contributing one of the least number of isolates compared to all rock samples of only 4 (Figure 4.17) and the concurrent wood sample (MS8W) comprising the largest number of 'wood' isolates with 12 (Figure 4.18).

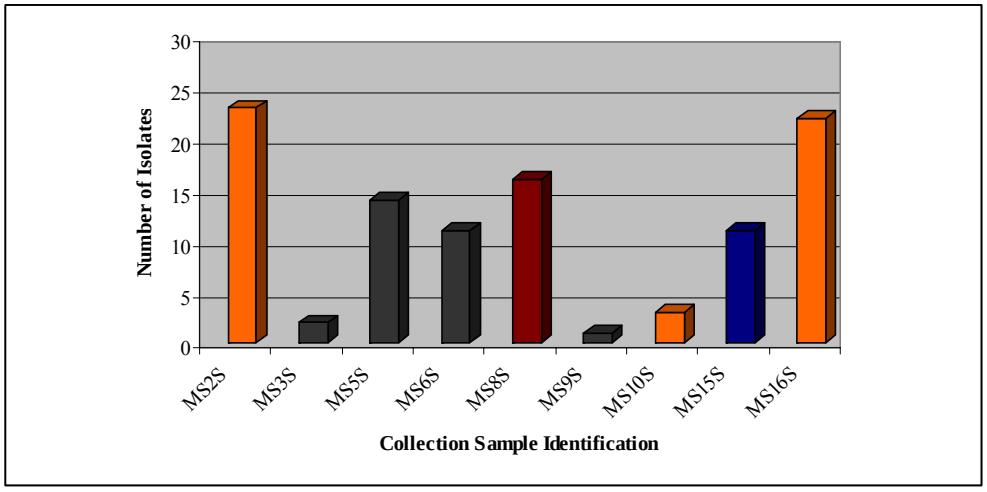


**Figure 4.17** Number of fungal isolates generated from soil samples collected in the Taklimakan Desert. Colored bars indicate samples of concurrent and independent collections based on substrate; orange bars: soil and rock, black bars: independent, red bar: soil, rock and wood, blue bar: soil and wood.

Sample MS10S only contributed 3 isolates to the total, while the rock sample MS10R collected directly above this soil had the greatest number, 28 isolates, from all substrates (Figures 4.17 & 4.18). The MS15 samples of both soil and wood generated nearly the same number of isolates with MS15S having 11 and MS15W having 10 isolates, nearly a third of all wood derived isolates.

The independently collected soil samples had a range of isolates generated from each, including 15 from MS5S, 11 from MS6S, 2 from MS3S and MS9S from which only 1 isolate was obtained. The independent rock collections contributed a total of 16 isolates with 11 from MS4R and 5 from MS11R. There was only a single independent wood sample collected, MSW, which added 3 fungal isolates.

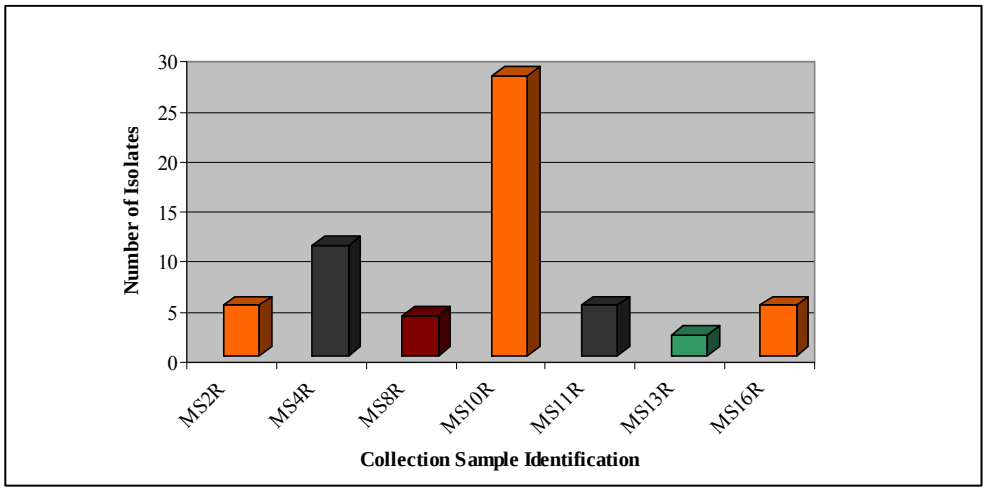


**Figure 4.18** Number of fungal isolates generated from rock samples collected in the Taklimakan Desert. Colored bars indicate samples of concurrent and independent collections based on substrate; orange bars: soil and rock, black bars: independent, red bar: soil, rock and wood, green bar: rock and wood.

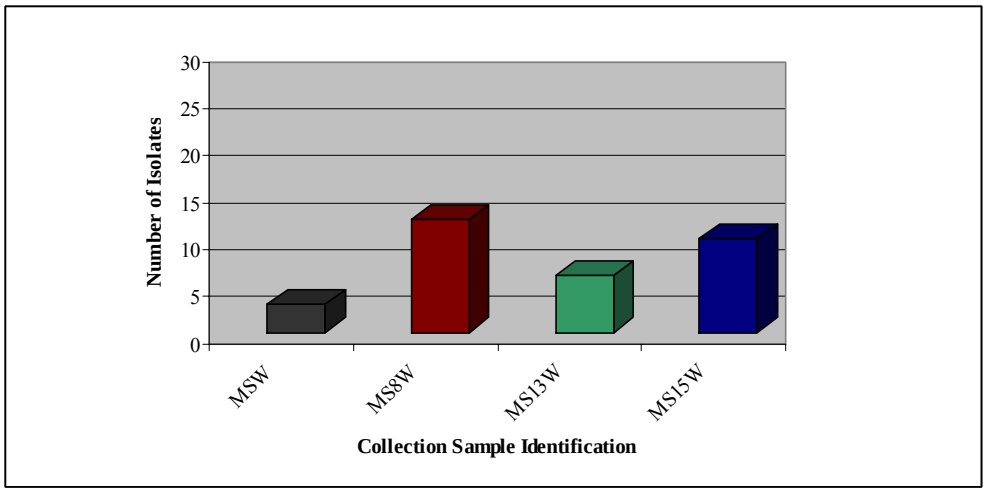


**Figure 4.19** Number of fungal isolates generated from wood samples collected in the Taklimakan Desert. Colored bars indicate samples of concurrent and independent collections based on substrate; black bars: independent, red bar: soil, rock and wood, green bar: rock and wood, blue bar: soil and wood.

To gain a better understanding of the independent fungal taxa associated with each of the 3 substrates collected in the Taklimakan Desert versus those taxa that occur together i.e. were concurrent in a specific substrate, fungi were placed into groups with their similar, known taxa from published reports and culture collections based on a <5% change / 95% similarity and/or if surrounding subclades were from the same genus (Figures 4.1 and 4.3-4.15). The greatest number of identifiable taxa based on these provisions were from the soil samples with 84 taxa, the rock samples had 39 taxa and wood with 27 taxa as depicted in a Venn Diagram (Figure 4.20).

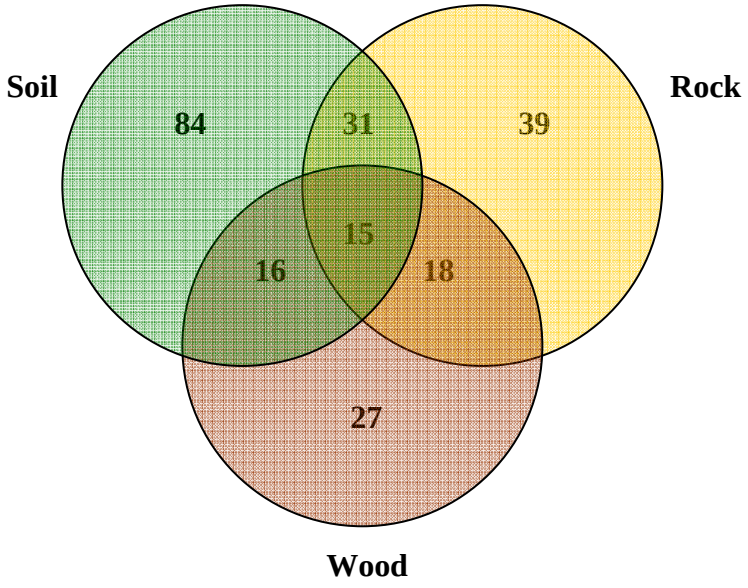


**Figure 4.20** Venn diagram of various substrates collected in the Taklimakan Desert and the number of overlapping fungal taxa from representative clades (based on neighbor-joining phylogenetic analysis) isolated from each.

Between the soil and rock there was nearly an 80% overlap from each substrate with 31 concurrent taxa. The rock and wood samples shared 18 taxa, while the soil and wood had only 16 taxa overlapping. There were 15 concurrent taxa when the 3 substrates were viewed together. These 15 common groups were very similar to the numbers of overlapping taxa between wood, and the soil and rock samples with 16

and 18 taxa, respectively, which shows that there is a significant correspondence between concurrent isolates from wood and the other 2 substrates.

### 4.5.2 Fungal diversity based on isolation media and temperature

The types of media used for isolating filamentous fungi from the various substrates can generally be divided into 3 categories based on the constituents added to each of the 10 media. The first group is the nutrient rich media, as follows: CMA, 1.5% MEA, MYA, NA and PDA; nutrient poor: 0.5% MEA, WA and BSA, CMA+ and SDA, the latter three include antibiotics. The greatest overall number of isolates generated across all substrates came from the nutrient rich group totaling 119. The nutrient poor and antibiotic media derived 43 and 32 isolates, respectively. Removing the bias based on the number of types of media in each category by determining the mean number of isolates per media group, the nutrient rich media's contributed nearly 24 per media type, nutrient poor with just over 21 and the antibiotic media with 10.

Soil generated fungal isolates on all types of media tested had the vast majority occurring on the nutrient rich media's of CMA, 1.5% MEA, MYA and PDA, however, the isolates from NA were limited (Figure 4.21). High numbers were recorded in both of the low nutrient media's as well, with 29% of the total from soils originating on either 0.5% MEA or WA. The antibiotic media contributed the least number of isolates derived from soils with a total of just 10% and only one isolate grew on the BSA.

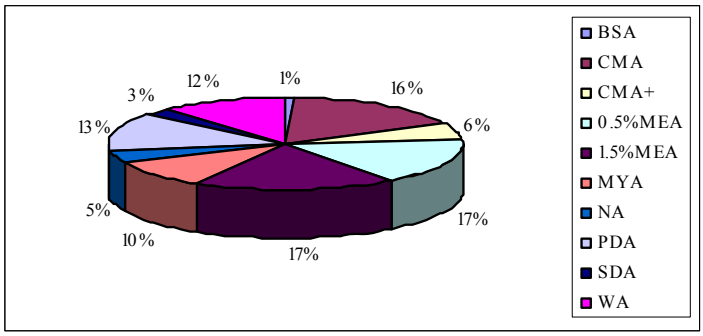


**Figure 4.21** Type of growth media associated with fungal isolations from soil collected in the Taklimakan Desert (total number of isolates: 103).

Overall, there were 31 fungi originating from wood and 60 from rock regardless of temperature or isolation nutrient medium. Fungi originating from the rock substrate also grew on all types of media with a definitive preference for the nutrient rich category, which had 71% of the total isolates (Figure 4.22). The nutrient poor media of 0.5% MEA and WA had very few fungi derived with only 8% and 3%, respectively. The 3 media with antibiotics present had a greater percentage of fungi originating from rock than soil but were still relatively low when compared to the total derived from rock regardless of media type with just 18%.

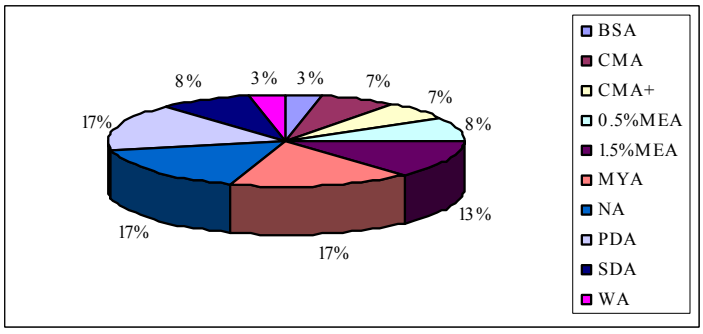


**Figure 4.22** Type of growth media associated with fungal isolations from rock collected in the Taklimakan Desert (total number of isolates: 60).

The fungi generated from wood samples were not as clear in their preference for isolation on one media category when compared to the others. Although there was a

high percentage of isolates found on the nutrient rich media as a whole (total 42%), fungi were not isolated on either CMA or NA (Table 4.23). The greatest number of isolated fungi from rock grew on the nutrient poor media of 0.5% MEA at 17% with the other member of this group, WA at 6%. The largest deviation from the previous two substrates with relation to isolations were the number of fungi generated from the antibiotic group, with 35% of the total coming from this category, 13% BSA, 6% CMA+ and 16% SDA.

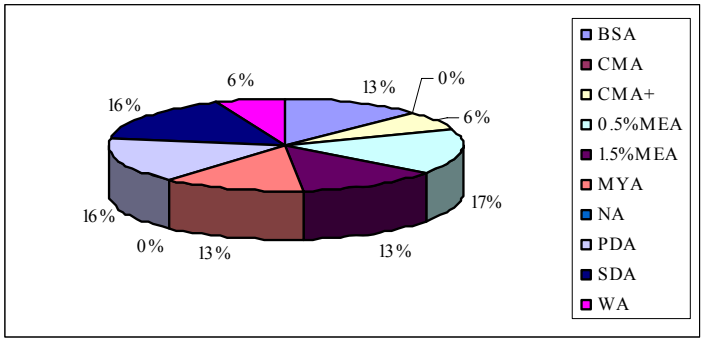


**Figure 4.23** Type of growth media associated with fungal isolations from wood collected in the Taklimakan Desert (total number of isolates: 31).

The differences in fungal isolations between substrates varied significantly when coupled with the isolation temperatures of 2ºC, 15ºC and 25ºC. Within the soils, there was a preference for successful isolations when culturing at the higher temperatures with 54% of the total number of fungi originating from this substrate isolated at 25 ºC and 28% of the total isolates across all substrates (Figure 4.24). The second highest number of isolates was also from the soils but at 15ºC (43 fungi), while the soils at 2ºC accounted for the least number of fungi, 4, when considering all isolates. The soil isolates accounted for 53% of the total fungi. When isolating from the rock samples, there was nearly an equal number of fungi generated from 25ºC (25 isolates) and at

2ºC (23 isolates), while culturing at 15ºC only produced 12 isolates. The fungi grown from wood had a similar distribution with relation to temperature as the rock samples. However, the number of cultures from 2ºC was slightly greater than those from 25ºC at 14 and 10, respectively, with 15ºC producing the second fewest across all substrates and temperatures, 7 isolates.

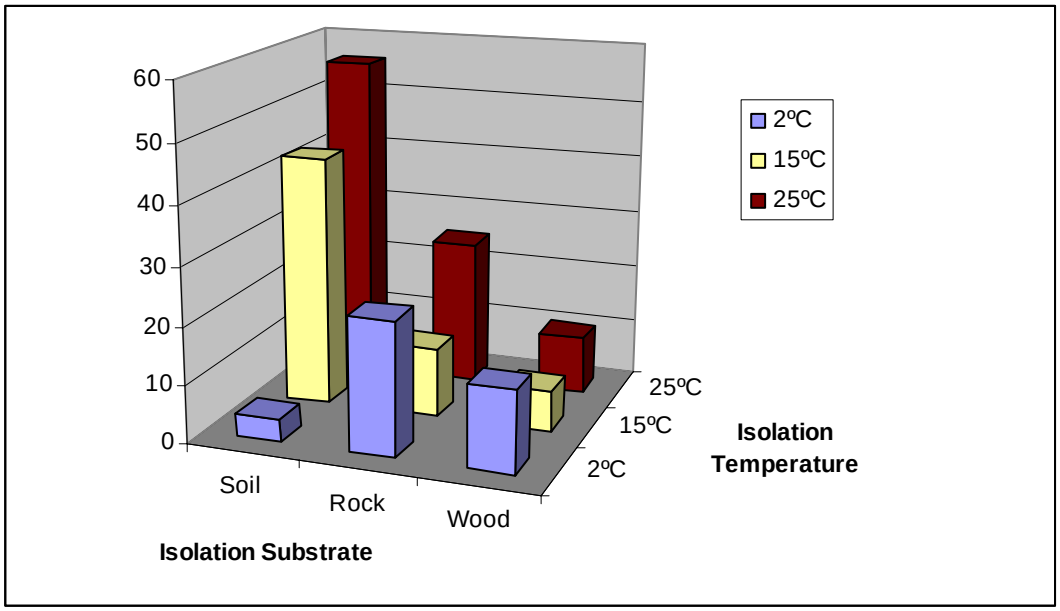


**Figure 4.24** Graph representing the number of fungi generated from soil, rock and wood samples collected in the Taklimakan Desert and isolated at 2ºC, 15ºC and 25ºC.

### 4.6 Uncultivated fungal diversity of the Taklimakan Desert

The DGGE investigations of fungi occurring on soils, rock and wood samples collected in the Taklimakan Desert produced 51 consensus ITS rDNA sequences (Tables 4.6-4.8). These were in addition to the DNA extractions from the pure cultures, DNA extractions from environmental samples, specifically 2 soil samples, MS2S and MS8S, 1 rock sample MS8R and 1 wood sample MS15W included in this study, which was conducted to determine the total fungal diversity present in each.

**Table 4.6** List of taxa derived from DGGE investigation from soil (S) collected in the Taklimakan Desert, top BLAST match with associated accession number, percent identity and overall nucleotide overlap of the internal transcribed spacer (ITS) region.

| Sample | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|
| ***MS2S*** | | | | |
| 233 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 280/280 |
| 234 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 281/281 |
| 235 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 281/281 |
| 236 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 281/281 |
| 237 | *Wallemia muriae* strain EXF753 | AY302529 | 100 | 315/315 |
| ***MS8S*** | | | | |
| 244 | Iceman fungal clone T2709 | X88771 | 98 | 268/273 |
| 245 | Uncultured ascomycete clone Cw1 | EU520618 | 97 | 270/278 |
| 246 | *Colletotrichum circinans* | EU400140 | 100 | 325/325 |
| 248 | *Colletotrichum circinans* | EU400140 | 98 | 312/316 |
| 249 | *Colletotrichum circinans* | EU400140 | 100 | 324/324 |
| 251 | Uncultured *Epicoccum* ITS1 | FM165466 | 100 | 270/270 |
| 252 | Uncultured basidiomycete | AM901708 | 98 | 211/214 |
| 253 | Iceman fungal clone T2709 | X88771 | 98 | 263/268 |
| 254 | Iceman fungal clone T2709 | X88771 | 98 | 262/267 |
| 255 | *Colletotrichum circinans* | EU400140 | 100 | 326/326 |
| 256 | *Colletotrichum circinans* | EU400140 | 100 | 327/327 |
| 257 | Iceman fungal clone T2709 | X88771 | 94 | 249/263 |
| 258 | Iceman fungal clone T2709 | X88771 | 98 | 258/263 |
| 259 | *Colletotrichum circinans* | EU400140 | 100 | 330/330 |
| 260 | *Colletotrichum circinans* | EU400140 | 100 | 329/329 |

**Table 4.7** List of taxa derived from DGGE investigation from rock (R) collected in the Taklimakan Desert, top BLAST match with associated accession number, percent identity and overall nucleotide overlap of the internal transcribed spacer (ITS) region.

| Sample | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|
| ***MS8R*** | | | | |
| 239 | Iceman fungal clone T2709 | X88771 | 98 | 257/262 |
| 240 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 290/290 |
| 241 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 280/280 |
| 242 | *Penicillium* sp. QLF72 | FJ025202 | 98 | 264/268 |
| 243 | *Penicillium* sp. QLF72 | FJ025202 | 100 | 289/289 |
| 244 | Iceman fungal clone T2709 | X88771 | 98 | 268/273 |
| 245 | Uncultured ascomycete clone Cw1 | EU520618 | 97 | 270/278 |
| 246 | *Colletotrichum circinans* | EU400140 | 100 | 325/325 |
| 248 | *Colletotrichum circinans* | EU400140 | 98 | 312/316 |
| 249 | *Colletotrichum circinans* | EU400140 | 100 | 324/324 |
| 251 | Uncultured *Epicoccum* ITS1 | FM165466 | 100 | 270/270 |
| 252 | Uncultured basidiomycete | AM901708 | 98 | 211/214 |
| 253 | Iceman fungal clone T2709 | X88771 | 98 | 263/268 |
| 254 | Iceman fungal clone T2709 | X88771 | 98 | 262/267 |
| 255 | *Colletotrichum circinans* | EU400140 | 100 | 326/326 |
| 256 | *Colletotrichum circinans* | EU400140 | 100 | 327/327 |
| 257 | Iceman fungal clone T2709 | X88771 | 94 | 249/263 |
| 258 | Iceman fungal clone T2709 | X88771 | 98 | 258/263 |
| 259 | *Colletotrichum circinans* | EU400140 | 100 | 330/330 |
| 260 | *Colletotrichum circinans* | EU400140 | 100 | 329/329 |

**Table 4.8** List of taxa derived from DGGE investigation from wood (W) collected in the Taklimakan Desert, top BLAST match with associated accession number, percent identity and overall nucleotide overlap of the internal transcribed spacer (ITS) region.

| Sample | Top BLAST Match | Accession # | % ID | Overlap |
|---|---|---|---|---|
| ***MS15W*** | | | | |
| 262A | Iceman fungal clone T2709 | X88771 | 97 | 259/265 |
| 262B | *Geomyces* sp. BC7 | DQ317337 | 100 | 264/264 |
| 262C | *Mortierella* sp. 04M | AY842393 | 97 | 377/385 |
| 263A | Iceman fungal clone T2709 | X88771 | 97 | 263/269 |
| 263B | *Geomyces* sp. BC7 | DQ317337 | 99 | 268/270 |
| 264B | *Geomyces* sp. BC7 | DQ317337 | 100 | 276/276 |
| 264C | Iceman fungal clone T2709 | X88771 | 97 | 262/269 |
| 265 | Uncultured ascomycete | AM901770 | 99 | 272/273 |
| 266 | Ascomycete sp. HK-S279 | AM084528 | 96 | 221/229 |
| 267 | Uncultured ascomycete | AM901770 | 99 | 286/287 |
| 268 | Uncultured *Pyronemataceae* clone | DQ317369 | 95 | 276/290 |

The greatest number of consensus sequences derived from an individual sample was recovered from the rock sample MS8R with 20. However, based on the top BLAST matches of these consensus sequences only 6 separated taxa were found as several were repeated. The fungal DNA generated from this rock produced 7 bands in the DDGE gel that were found to be a 98-100% match to *Colletotrichum circinans* (EU400140), which is a plant pathogen and was from a collection of diseased *Phaseolus vulgaris*, the common bean (Chen et al. 2008). Another common repeat in this investigation with 6 matches was the Iceman fungal clone T2709 (X88771) from the grass clothing of a Neolithic mummy found on Austro-Italian Alps. Although the taxonomic placement of this sequence is not known, the best BLAST match to this clone was from historic wood collected on the Antarctic Peninsula (97%) by Arenz and Blanchette (2009), which clearly shows the ability of these fungi to tolerate

extreme conditions. There were also 4 repeats of sequences with a 98-100% match to *Penicillium* sp. QLF72 (FJ025202) which was isolated from soils collected in alpine grassland in eastern Qilian Mountains of China, just east of the Taklimakan collection site. The remaining sequences include a single match to an uncultured *Epicoccum* sp. (FM165466) (100%), Ascomycete (EU520618) (97%) and Basidiomycete (AM901708) (98%).

Sample MS8S had 15 total consensus sequences which account for the second greatest number derived from the DGGE investigation of these samples. The sequences were nearly identical to what was found in sample MS8R with 5 matching the Iceman fungal clone T2709 (X88771) (94-98%), 7 matching *Colletotrichum circinans* (EU400140) (98-100%) and 1 each of *Epicoccum* sp. (FM165466) (100%), Ascomycete (EU520618) (97%) and Basidiomycete (AM901708) (98%). The only sequence that was not found originating from the soil sample but was found associated with the rock was closely related to *Penicillium* sp. QLF72 (FJ025202). As sample MS8S was collected directly under MS8R, the concurrent identification of nearly the same fungal community could be expected. Figure 3.25 shows the concurrent verses independent ITS rDNA sequences derived from DGGE analysis of soil sample MS8R (rock) and MS8S (soil), with 15 from the soil sample and 20 from rock which had 4 *Penicillium* sp. (FJ025202) and 1 additional band that closely matched Iceman fungal clone T2709 (X88771) when compared to MS8S.

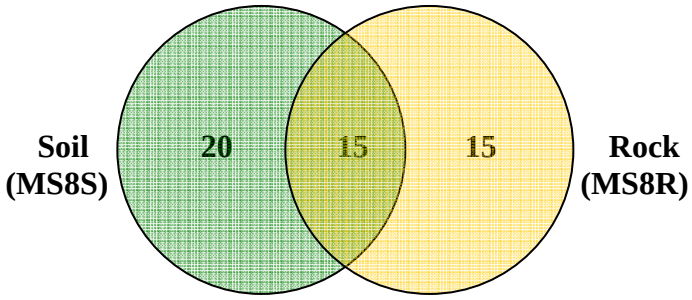


**Figure 4.25** Concurrent and independent fungal sequences from DGGE investigation from soil (MS8S) and rock (MS8R) samples collected in the same location from the Taklimakan Desert.

Sample MS2S from soil produced the least number of consensus sequences from this DGGE study with only 4. These were from only 2 taxa with *Penicillium* sp. QLF72 (FJ025202) accounting for 3 of the total, which is the same match associated with MS8R but the MS2S sequences had a 100% correspondence with this *Penicillium.* The other taxa that matched the Taklimakan sample sequence was *Wallemia muriae* strain EXF753 (AY302529) with 100% identity equivalence. The genus *Wallemia* represent one of the most xerophilic fungal groups and are very unique because they are Basidiomycetes, a phylum where this type of tolerance is exceedingly rare (Zalar et al. 2005). So rare, that based on their xerotolerance, morphology and ITS rDNA a new class was recently designated by Zalar et al. (2005), Wallemiomycetes covering the order Wallemiales.

The wood sample investigated by DGGE was MS15W with 11 consensus sequences. From this sample there were again repeats with 3 sequences similar to the Iceman fungal clone T2709 (X88771), all at 97% homology match. There were also 3 sequences that had a 99-100% match to *Geomyces* sp. BC7 (DQ317337), a sequence generated from fungal isolates and DGGE analysis generated from soils, wood and

organic materials found in the Ross Sea Region of Antarctica (Arenz et al. 2006). Members of the *Geomyces* genera are known to be psychrotolerant and have also readily been found in permafrost in the Arctic (Kochkina et al. 2007). There was also a 97% match to *Mortierella* sp. 04M (AY842393), which is another genus that includes psychrophilic species and is found in Antarctica (Gesheva 2009; Ruisi et al. 2007). *Mortierella* sp. have also been isolated from a river in Spain with a pH of <2.5 and exceedingly high levels of heavy metals including, 2280–3010 mg Fe, 94–172 mg Cu and 163–232 mg Zn (López-Archilla eta al. 2004). The uncultured Pyronemataceae clone (DQ317369) had a 95% similarity to one of the sequences from MS15W and this clone was generated from DGGE analysis of soils from Antarctica (Arenz et al. 2006). Another sequence had a 96% match to Ascomycete sp. HK-S279 (AM084528), which in turn is a 100% match to *Cladosporium colombiae* strain CBS 274.80B. There was also a sequence that was 99% similar to Uncultured ascomycete (AM901770), which gives very little detail of its identity but when this Uncultured ascomycete sequence was analyzed by a BLAST search, its top matches are the closely related *Alternaria* sp. and *Embellisia* sp. isolated from Antarctic and an Iranian desert, respectively.

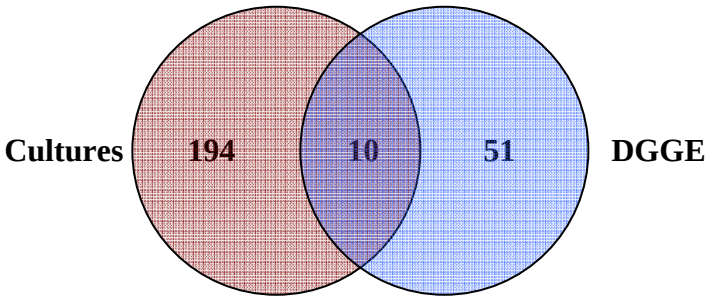


**Figure 4.26** Concurrent and independent fungal sequences from culturing and DGGE investigation of samples collected in the same location from the Taklimakan Desert.

Similar taxa based on sequence data were rare between cultured fungal isolates and those recovered from environmental samples with only 3 found by both techniques, for a total of 10 with repeats included (Fig. 4.26). One example is from wood sample MS15W, which generated sequences from both a culture and DGGE that both had a 95% affinity to their top BLAST match of an uncultured *Pyronemataceae* clone DQ317369. From MS8S a sequence was generated from DGGE analysis that had a top BLAST match of uncultured *Epicoccum* ITS1 (FM165466) (100%), which may be associated with culture sequences from MS2S, MS4R and MS10R that can be found in clade H (Fig. 4.10). This clade has branches that terminate in the closely related *Phoma* and *Epicoccum* genera (Aveskamp et al. 2008). MS2S and MS8R also generated sequences by DGGE (*Penicillium* sp. QLF72) (FJ0252020) that may be similar to culture sequences from both MS2S and MS10S which display similarity to *Penicillium* sp. under neighbor-joining phylogenetic analysis (Fig. 4.4). However, it is important to note that the incompatibility of direct comparisons between sequence data generated from cultures and environmental samples because of the differing sequence lengths from these two techniques would make any concrete statements of relatedness circumspect.

## Chapter 5
## Wood sample identification, fungal degradation of wood and fungal biochemistry

### *5.1 General introduction*

The primary focus of this chapter is wood. Wood is relevant to the PhD thesis as it was one of the sample types collected in the Taklimakan Desert, though rare, given that the 4 samples were the only woody materials observed in the one square kilometer collection site. Also, wood is the substrate from which many of the Antarctic and Arctic fungal sample were derived. Selected fungal isolates from the Taklimakan Desert, Antarctica and the Arctic were investigating as to their ability to degrade wood and produce wood degrading enzymes on select media.

### *5.2 Material and methods*

#### *5.2.1 Identification of wood samples*

The four wood samples, MS8W, MS13W, MS15W and MSW (Table 2.1) collected in the Taklimakan Desert were investigated to determine what species of plant the substrate originated from. This was completed by making hand sections of the material and viewing by means of a Nikon SMZ800 stereomicroscope, Nikon Eclipse E600 compound microscope (Nikon, Tokyo, Japan) or through scanning electron microscopy (Section 5.2.3). Under magnification these samples were identified based on a variety of morphological characteristics (Hoadley 1995; Schweingruber 1990).

### *5.2.2 Laboratory wood degradation testing*

#### *5.2.2.1 Fungal Isolates used in wood degradation testing*

Fungal isolates from the Taklimakan Desert samples that were determined to be dominant based on total isolations or were considered to be potential wood decay fungi based on molecular identification and literature review were included in a study to ascertain their ability to degrade lignocellulosic materials; the isolates are listed in Table 5.1.

**Table 5.1** Fungal isolate number, original sample identification and substrate or collection region, top BLAST match and accession # and putative identification based on phylogenetic, neighbor joining analysis of fungal isolates generated from collections in the Taklimakan Desert.

| Fungal isolate | Sample & substrate | Top BLAST Match | Accession # | Putative Identification[a] |
|---|---|---|---|---|
| 1 | MS2S (soil) | *Phoma betae* (Pb) | EU594572 | Unknown |
| 3 | MS2S (soil) | *Phialocephala* sp. (Ph) | DQ122928 | *Cadophora* sp. |
| 9 | MS3S (soil) | Uncultured fungus (Uf) | AJ875362 | *Cladosporium* sp. |
| 38 | MS16S (soil) | *Alternaria* sp. (Al) | DQ682562 | *Alternaria* sp. |
| 44 | MS16S (soil) | *Tetracladium setigerum* (Ts) | EU622246 | *Embellisia* sp. |
| 54 | MS8R (rock) | *Bjerkandera adusta* (Ba) | AF455468 | *Bjerkandera* sp. |
| 73 | MS8S (soil) | Ascomycete sp. (As) | AM921746 | Unknown |
| 84 | MS16S (soil) | *Thielavia* sp. (Th) | EU620166 | *Chaetomium sp.* |
| 98 | MS16S (soil) | *Chaetomium globosum* (Cg) | DQ093659 | Unknown |
| 109 | MS2S (soil) | *Lecythophora* sp. (Ls) | AY219880 | Unknown |
| 131 | MS2R (rock) | *Oxyporus corticola* (Oc) | EF011124 | *Oxyporus* sp. |
| 178 | MS10R (rock) | *Cladosporium subtilissimum*(Cs) | EF679390 | *Cladosporium* sp. |
| 191 | MS8W (wood) | *Ulocladium consortiale* (Uc) | AY278837 | *Ulocladium* sp. |
| 201 | MS8W (wood) | Ascomycete sp. nasa65 (An) | DQ683978 | Unknown |
| 203 | MSW (wood) | *Embellisia phragmospora* (Ep) | FJ357314 | *Embellisia* sp. |

[a]The putative identifications are based on Molecular Investigation of Fungi, as described in Section 4.3, and Phylogeny, as described in Section 4.4.

**Table 5.1** (continued) Fungal isolate number, original sample identification and substrate or collection region, top BLAST match and accession # and putative identification based on phylogenetic, neighbor joining analysis of fungal isolates generated from collections in the Taklimakan Desert.

| Fungal isolate | Collection region | Top BLAST Match | Accession # | Putative Identification[a] |
|---|---|---|---|---|
| | Arctic | Uncultured Basidiomycete (1) | FJ457769 | Unknown |
| | Arctic | *Exophiala* sp. (2) | FJ457766 | *Exophiala* sp. |
| | Arctic | *Phialocephala* sp. (3) | FJ457767 | *Phialocephala* sp. |
| | Arctic | Fungal sp. AB10 (4) | FJ457771 | *Cadophora* sp. |
| | Arctic | Uncultured fungus clone N3 (5) | FJ457773 | Unknown |
| | Antarctic | *Exophiala spinifera* (6) | DQ317336 | *Exophiala* sp. |
| | Antarctic | *Cadophora malorum* (7) | DQ317328 | *Cadophora* sp. |
| | Antarctic | *Cladosporium cladosporioides* (8) | DQ317332 | *Cladosporium* sp. |
| | Antarctic | *Phoma herbarum* (9) | DQ317345 | *Phoma* sp. |
| | Antarctic | *Alternaria* sp. (10) | DQ317386 | *Alternaria* sp. |
| | Antarctic | *Chaetomium funicola* (11) | DQ317331 | *Chaetomium* sp. |
| | Antarctic | *Ulocladium chartarum* (12) | EF679390 | *Ulocladium* sp. |

[a]The putative identifications are based on Molecular Investigation of Fungi, as described in Section 4.3, and Phylogeny, as described in Section 4.4.

#### *5.2.2.2 Wood species used in wood degradation testing*

The wood degradation study involved 2 wood species, *Pinus resinosa* and *Populus tremuloides* that were between 60 - 70 years old when harvested from the Nicolet National Forests, WI, U.S.A. Sections of each species were cut to 20 x 10 x 2 mm, hydrated with deionized water to a moisture content of 70-100%, autoclaved for 60 minutes and cooled to room temperature. Two sections of each wood species were placed in 3, 50 x 15 mm sterile plates of 1.5% MEA that had been inoculated with one of the selected fungal isolates (Table 5.1) and each wood section was elevated off the media by two 2 x 2 x 10 mm section of the same wood species on one side with the adjacent side in contact with the media; an example is shown in Figure 5.1. All plates were sealed with Parafilm to help reduce the occurrence of contamination by other microorganisms. Additionally, 10 plates of both wood types were included as controls and were not inoculated with fungi. The study continued for 20 weeks at 25ºC and then the wood sections were removed from the plates, dried at 50°C for 72 hours, weighed and percent weight loss was determined. These samples were also examined by scanning electron microscopy (Table 5.1), as described in Section 5.2.2. At the end of the study all plates were investigated to determine if contamination by other microorganisms had occurred by visual inspection of fungal morphological characteristics.

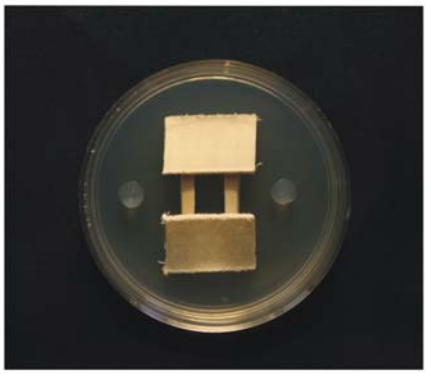

**Figure 5.1** Laboratory wood degradation study showing 2 *Populus tremuloides* sections, elevated on one side by additional wood sections of the same species and agar plugs with the inoculation fungus.

### *5.2.3 Scanning electron microscopy*

Selections of wood and lignocellulosic materials collected in the Taklimakan Desert (Section 2.1.) and wood samples of both species from the laboratory degradation testing plates (Section 5.2.2.2) were prepared for scanning electron microscopy by hydrating the samples in TBS™ Tissue Freezing medium™ (Triangle Biomedical Sciences, Durham, NC, U.S.A.) under vacuum, mounting on a brass stub at -20°C in a OM 2488 Minotome® microtome-crystat (International Equipment Company, Needham Heights, MA, U.S.A.) and cutting the section transversely on this device to create a clean face for viewing. After thawing and air drying for 48 hours, the specimens were placed on double sided, carbon adhesive tape which was adhered to aluminum stubs. Samples were then coated with gold in an EMS 76M Ernest Fullum sputter coater (Ernest F. Fullam, Inc., Schenectady, NY, U.S.A.) with a pressure setting of 250 microns Hg, and a current output of 15 microamps for 3 minutes. The aluminum stubs with the gold coated specimen were then placed in a Hitachi S3500

N (Hitachi, Tokyo, Japan) scanning electron microscope (SEM) and viewed transversely in low vacuum mode at 5 kilovolts (kV). Collected samples of rock were also placed on double sided, carbon adhesive tape that was adhered to an aluminum stub and viewed as mentioned above.

### *5.2.4 Cellulase assay*

Selected fungal isolates (Table 5.1) were screened for their cellulolytic activity by using a carboxymethyl cellulose (CMC) Congo red procedure assay. Individual 5 mm agar plugs of these fungi were placed on the center of plates 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). Three plates of each selected isolate were made and incubated at 2°C, 15°C or 25°C, respectively.

After 2 days at 25°C, 5 days at 15°C and 6 weeks at 2°C the plates were 'flooded' with ~15 ml of 0.1% Congo red and allowed to react for 30 minutes followed by destaining with 1 M NaCl for 60 min according to the method developed by Teather and Wood (1982). Two measurements perpendicular to each other of the zone of clearing (if present) were made in the cellulose media and the average was taken and the same system of measurement was completed for the fungal growth in these plates. By dividing this average area of clearing by the average area of fungal growth an index of relative enzyme activity was determined (Bradner et al. 1999).

### *5.2.5 Lignin peroxidase assay*

The selected fungi as given in Table 4.1 were investigated to determine their ability to produce lignin peroxidase and Mn-dependent peroxidase by utilizing an Azure B assay (Archibald 1992). To prepare this medium, 0.01% w/v of Azure B and 16 g of agar were added to 1000 ml of deionized water (Pointing 1999). This was autoclaved for 20 min at 121ºC and plates were poured as described in Section 2.3.1. Once the media had solidified, a 5 mm agar plug from an 1.5% MEA actively growing plate of the specific fungal isolate were transferred to the center of the Axure B plates and the assay was conducted in triplicate at three temperatures. The three Azure B plates of each isolate were incubated at 2°C, 15°C or 25°C, respectively, and monitored daily for 21 days for decolorization of the blue media, which would indicate the production of lignin peroxidase or Mn-dependent peroxidase. After the 21 days, all plates were incubated at 25°C to determine whether the individuals that did not produce these enzymes at lower temperatures were able to at this higher temperature.

## *5.3 Results and Discussion*

### *5.3.1 Identification of wood samples*

Four samples of wood were collected in the Taklimakan Desert and to determine the species of wood from the 4 samples, the wood cell arrangement, type and various other anatomical features were investigated using microscopy as described in Sections 5.2.1 and 5.2.3.

When sample MS8W was viewed transversely, the vessel arrangement was ring to

semi-ring-porous and vessels were primarily solitary (Figure 5.2 [A]). The rays were multiseriate and 7-16 cells wide. When viewed radially, the rays were heterocellular with the upper and lower most row upright. Also, the vessels had simple perforation plates and intervessel pitting was very small and numerous. Based on these anatomical features and the vegetation that is common in the riparian zones around the rivers and dunes (Table 1.1), sample MSW8 was determined to be *Tamarix* sp. This sample was only identified to the generic level as these species can not be distinguished from one using morphological criteria. During the microscopic investigation of this sample, fungal hyphae were found in several areas and white rot decay was evident with all cell wall layers affected by this degradation (Figure 5.2 [B]). These observations demonstrated a good justification for the lignocellulosic degradation study and the supposition that the fungi isolated from this site might produce wood degrading enzymes.

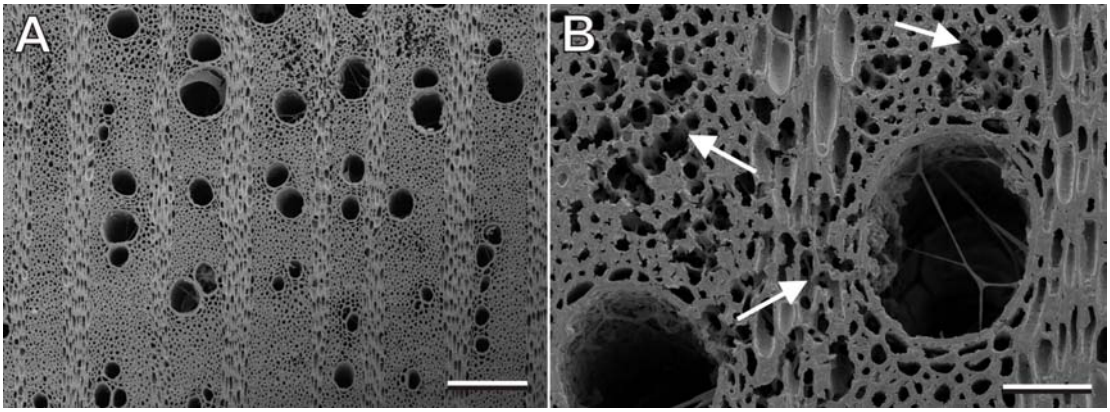


**Figure 5.2** Scanning electron micrograph of *Tamarix* sp. wood collected (MS8W) in the Taklimakan Desert. (**A)** Transverse view showing semi-ring porous vessel arrangement and larges multiseriate rays (Bar 200 μm). (**B**) White rot decay caused by fungi degrading all cell wall layers (arrows) (Bar 50 μm).

Sample MSW was determined to be *Populus* sp. based on the semi-ring porous vessel arrangement and uniserate rays when viewed transversely (Figure 5.3[A]). The radial plane exposed simple perforation plates and large, simple ray vessel pits. *Populus* is a common genus of tree in riparian zones, the interface between land and streams or rivers, in the Taklimakan Desert (Table 1.1) and although the collection site was many kilometers from a river, it is likely that the strong winds that are common in this desert carried this section of wood to this location. The two species of *Populus* from Taklimakan Desert region (*P. euphratica* and *P. pruinosa)* can not be differentiated from one another based on their wood anatomy. As with sample MS8W, MSW had fungal hyphae present in several areas but this sample did not display evidence of biological decay. However, in the outer portion of the sample, the fibers and vessels were separated from one another by an abiotic process called defibration which can be caused by either high UV or elevated levels of sodium salts (or a combination of both) chemically altering the lignin which cause the cell separation (Figure 5.3[B]). (Blanchette et al. 2002; Hon 1981).

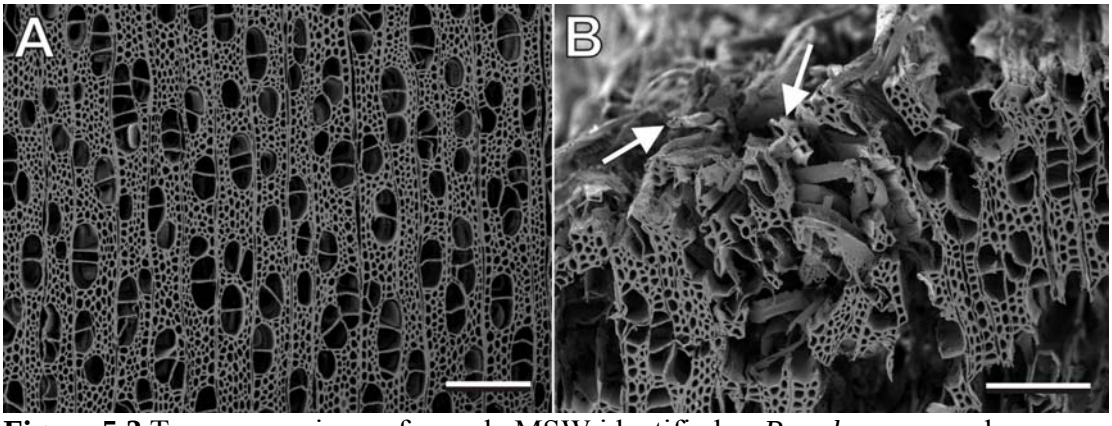


**Figure 5.3** Transverse views of sample MSW identified as *Populus* sp. wood collected in the Taklimakan Desert (**A**) showing diffuse porous, clustered vessel arrangement and uniseriate rays (Bar 200 μm). (**B**) Defibration of outer wood surface (arrows) caused by exposure to elevated UV or sodium (Bar 200 μm).

Samples MS13W and MS15W both appear to be branch or immature material as their cellular arrangement was disorganized and cell walls were very thin in areas indicating a more primary structure without secondary cell wall thickening (Figure 5.4 [A & C]). These features did not allow for positive identification of the samples. Both samples did have abundant fungal hyphae present (Figure 5.4 [D]).and sample MS13W had soft rot decay in selected cells affecting the $S_2$ cell wall layer (Figure 5.4 [B]).

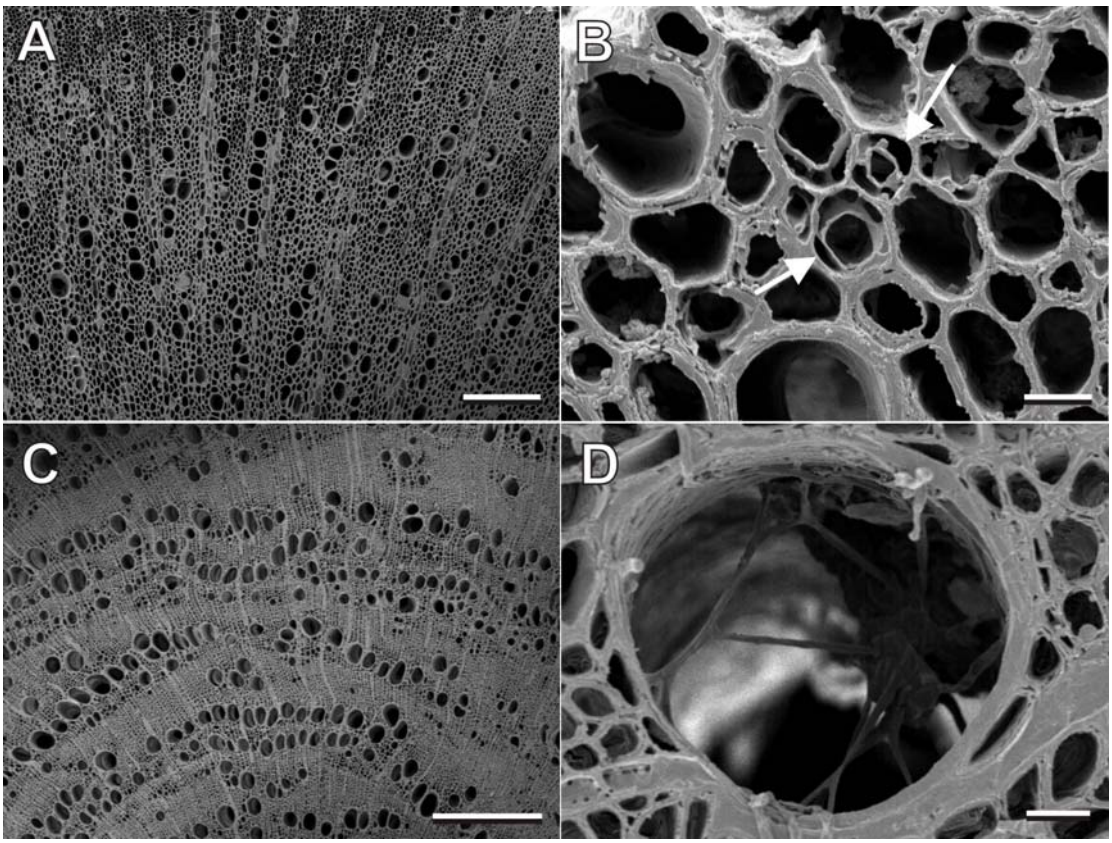


**Figure 5.4 (A)** Unknown wood species collected in the Taklimakan Desert (MS13W) with radial vessel organization and random ray arrangement (Bar 200 μm). (**B**) Sample MS13W exhibiting soft rot fungal decay attacking the $S_2$ cell wall layer (Bar 10 μm). (**C**) Sample MS15W displaying variation in vessel arrangement, growth ring boundries and ray patterns making positive identification unsuccessful (Bar 500 μm). (D) Extensive fungal hyphae in vessel of sample MS15W.

### *5.3.2 Laboratory wood degradation study*

#### *5.3.2.1 Weight loss*

The wood degradation weight loss study conducted on *Pinus* and *Populus* sp. inoculated with 15 selected isolates generated from soil, rock and wood samples collected in the Taklimakan Desert produced a weight reduction in nearly all samples tested (Table 5.2). The results are discussed based on samples which were observed to have the greatest weight losses, the least weight losses and no weight losses as indicated by Tables 5.2 and 5.3 and ANOVA analysis from Figure 5.5.

**Table 5.2** Fungal isolates generated from soil, rock and wood samples collected in the Taklimakan Desert, top BLAST match based on ITS consensus sequences (and abbreviated indicator) and percent mean wood mass loss (n=6) with standard deviations of *Pinus* sp. and *Populus* sp. utilized in a 20 week decay study.

| **Isolate** | **Top BLAST Match** | **Percent Mean Wood Mass Loss (n=6) (± S.D. of the Mean)** | |
|---|---|---|---|
| | | ***Pinus* sp.** | ***Populus* sp.** |
| 1 | *Phoma betae* (Pb) | 1.91 (± 0.78) | 3.2 (± 1.55) |
| 3 | *Phialocephala* sp. (Ph) | 2.79 (± 1.25) | 3.21 (± 1.77) |
| 9 | Uncultured fungus (Uf) | 2.19 (± 0.03) | 2.29 (± 0.96) |
| 38 | *Alternaria* sp. (Al) | 1.4 (± 0.68) | 1.24 (± 0.66) |
| 44 | *Tetracladium setigerum* (Ts) | 0.19 (± 0.5) | 1.01 (± 0.97) |
| 54 | *Bjerkandera adusta* (Ba) | 4.01 (± 1.51) | 7.85 (± 3.41) |
| 73 | Ascomycete sp. (As) | 0.36 (± 0.57) | 0.47 (± 0.62) |
| 84 | *Thielavia* sp. (Th) | 2.19 (± 1.43) | 2.22 (± 1.04) |
| 98 | *Chaetomium globosum* (Cg) | 3.15 (± 2.16) | 3.66 (± 2.15) |
| 109 | *Lecythophora* sp. (Ls) | 3.64 (± 0.58) | 3.24 (± 0.59) |
| 131 | *Oxyporus corticola* (Oc) | 7.13 (± 2.85) | 11.5 (± 4.37) |
| 178 | *Cladosporium subtilissimum*(Cs) | 3.2 (± 1.91) | 1.64 (± 1.13) |
| 191 | *Ulocladium consortiale* (Uc) | 2.64 (± 1.7) | 2.97 (± 2.15) |
| 201 | Ascomycete sp. nasa65 (An) | 1.23 (± 0.98) | 2.07 (± 0.77) |
| 203 | *Embellisia phragmospora* (Ep) | 1.58 (± 0.62) | 2.6 (± 0.71) |

The greatest losses were found in wood decayed by the Basidiomycetes, *Bjerkandera adusta* (isolate # 54) and *Oxyporus corticola* (isolate # 131). Both of these isolates produced more weight loss in the *Populus* sp. wood compared to the *Pinus* sp. with *Bjerkandera adusta* removing 7.85 (± 3.41) and *Oxyporus corticola* 11.5 (± 4.37) from the hardwood and 4.01 (± 1.51) and 7.13 (± 2.85), respectively, from the softwood. This may be due to the higher content of syringyl lignin units found in hardwoods which is more readily decayed than the more resistant quaiacyl units that are greater in softwoods (Blanchette et al. 1988; Eriksson et al. 1990). Both *Bjerkandera adusta* and *Oxyporus corticola* are white rot decay fungi and have been found during surveys of both southern China and northeastern China (Dai et al. 2003; 2006). The amount of decay in both wood types was lower than what has been found in a past study using *Bjerkandera adusta* where a 13.92-41.99% loss was found after 12 weeks in *Betula* sp. (Łakomy et al. 2005) and may indicate either unfavorable conditions in the current decay study or physiological differences in the isolates used. The only Basidiomycota from the comparative polar work was from the Arctic, Uncultured Basidiomycete (FJ457769), which had less than a 3% loss in both *Pinus* and *Populus* sp. wood (Table 5.3).

**Table 5.3** Fungal isolates from comparative Arctic and Antarctic investigations with top BLAST match based on ITS consensus sequences and percent mean wood mass loss (n=6) with standard deviations of *Pinus* sp. and *Populus* sp. utilized in a 20 week decay study.

| Region | Top BLAST Match | Percent Mean Wood Mass Loss (n=6) (± S.D. of the Mean) | |
|---|---|---|---|
| | | ***Pinus* sp.** | ***Populus* sp.** |
| Arctic | Uncultured Basidiomycete (1) | 2.12 (± 1.34) | 2.87 (± 0.75) |
| Arctic | *Exophiala* sp. (2) | 1.43 (± 0.92) | 2.61 (± 1.04) |
| Arctic | *Phialocephala* sp. (3) | 2.37 (± 0.41) | 3.11 (± 1.26) |
| Arctic | Fungal sp. AB10 (*Cadophora*) (4) | 2.72 (± 1.63) | 3.51 (± 0.93) |
| Arctic | Uncultured fungus clone N3 (5) | 3.47 (± 0.69) | 3.10 (± 0.88) |
| Antarctic | *Exophiala spinifera* (6) | 1.63 (± 0.51) | 1.22 (± 1.03) |
| Antarctic | *Cadophora malorum* (7) | 4.11 (± 2.68) | 5.54 (± 1.28) |
| Antarctic | *Cladosporium cladosporioides* (8) | 2.35 (± 1.08) | 2.95 (± 1.70) |
| Antarctic | *Phoma herbarum* (9) | 0.24 (± 0.37) | 0.78 (± 0.85) |
| Antarctic | *Alternaria* sp. (10) | 0.59 (± 0.27) | 0.81 (± 0.44) |
| Antarctic | *Chaetomium funicola* (11) | 3.10 (± 2.03) | 3.57 (± 1.82) |
| Antarctic | *Ulocladium chartarum* (12) | 2.68 (± 1.99) | 3.20 (± 1.61) |

There were other isolates tested that produced at least a 3% mean loss in at least one of the wood species and they included isolate # 1 (*Phoma betae*), # 3 (*Phialocephala* sp.), # 98 (*Chaetomium globosum*), # 109 (*Lecythophora* sp.), # 178 (*Cladosporium cf. subtilissimum*) and # 191 (*Ulocladium consortiale*) (Table 5.2). These are all soft rot wood decay fungi often found in adverse conditions such as water saturated wood in Polar regions or CCA treated timbers (Blanchette et al. 2004; Bridžiuvienė and Levinskaitė 2007; Bugos et al. 1988; Held et al. 2006; Jurgens et al. 2009; Kim et al. 2007). The amount of weight loss in each of the Taklimakan isolate tested wood samples was relatively low with a maximum of 3.66 (± 2.15) in *Populus* sp. wood, caused by isolate #98 (*Chaetomium globosum*) and may be caused by the laboratory

study conditions. Nutrient rich media, moisture content, temperature and even light can contribute to either a reduction or increase in growth or enzyme production in fungi with some, but not all soft rot fungi preferring conditions that are not as favorable as was created in this study (Eaton and Hale 1993; Schweingruber 2007). The type of wood used in weight loss studies utilizing soft rot decay fungi can also significantly influence decay with Eslyn and Highley (1976) finding 0 - 37.8% weight loss after 12 weeks in 15 wood species decayed by 6 soft rot fungi. The fungi from the polar studies which had similar weight loss are, *Cadophora malorum*, *Chaetomium funicola* and *Ulocladium chartarum* from the Antarctic and *Phialocephala* sp., Uncultured fungus clone N3 from the Arctic. These are all soft rot decay fungi and three, *Cadophora malorum, Phialocephala* sp. and Uncultured fungus clone N3 are in the '*Cadophora*' clade seen in Figure 4.3.

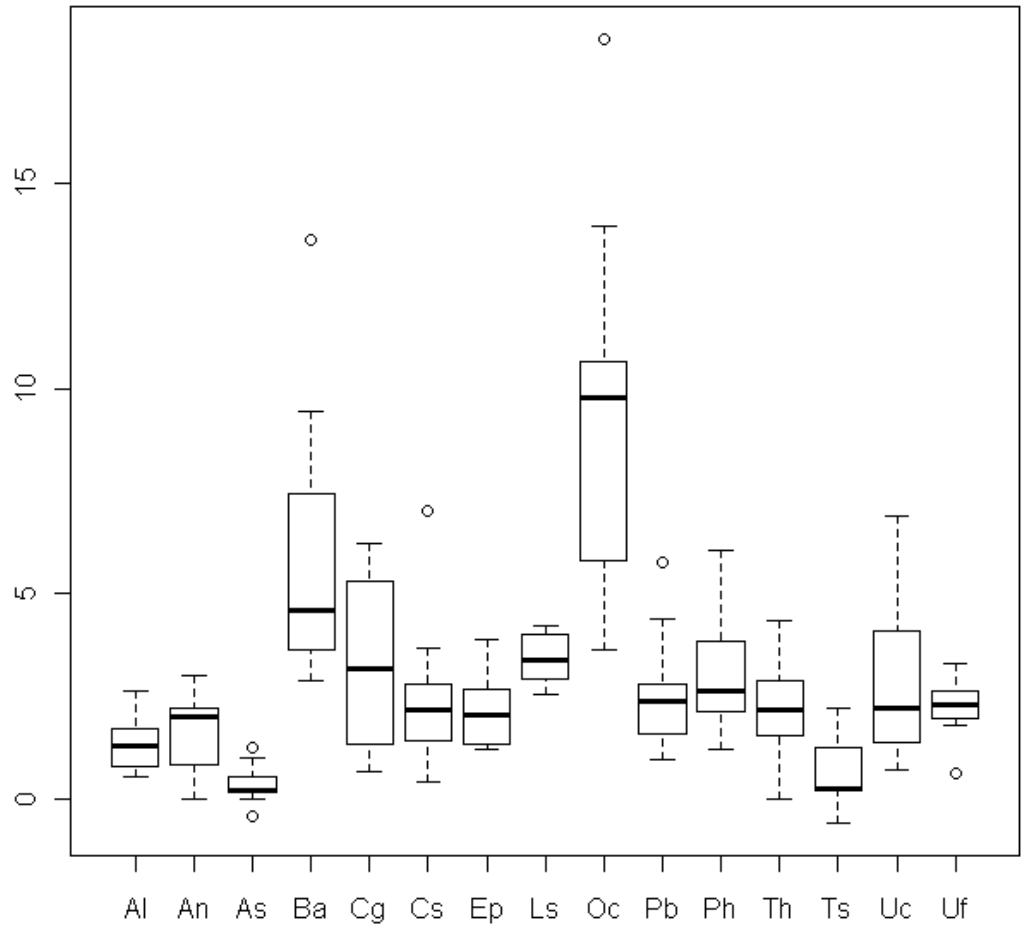


**Figure 5.5** ANOVA analysis of weight loss of *Pinus* and *Populus* sp. (n=12) wood decayed for 20 weeks by select fungi isolated from various substrates collected in the Taklimakan Desert. The horizontal line within the box represents the median, while the top of the box is the 75$^{th}$ percentile and the bottom is the 25$^{th}$ percentile. The whiskers represent 1.5 times the inter-quartile range and circles are outliers. P-value <0.001. (R Development Core Team 2009).

A third group was designated by having limited to nearly no weight loss with isolate #203 (*Embellisia phragmospora*) causing the greatest loss in this assemblage with 2.6% (± 0.71) in *Populus* sp. wood and # 44 (*Tetracladium setigerum*) causing the least loss of all fungi with 0.19 (± 0.5) in *Pinus* sp. (Table 5.2). Others in this group

include # 9 (Uncultured fungus), # 38 (*Alternaria* sp.), # 73 (Ascomycete sp.), #84 (*Thielavia* sp.) and # 201(Ascomycete sp. nasa65). The *Alternaria*, *Embellisia*, *Tetracladium* and *Thielavia* genera have all been shown to produce soft rot decay in wood in previous studies (Tuomela et al. 2000; Wang 1990) but their limited enzymatic activity may be related to the study conditions listed above or the inability of these specific isolates to degrade the substrates used in this investigation. All controls had weight loss of less than ±0.5.

#### *5.3.2.2 Scanning electron microscopy*

The *Pinus* sp. and *Betula* sp. wood blocks used in the 20 week decay study with fungal isolate # 131 *Oxyporus sp.* originating from a rock sample collected in the Taklimakan Desert exhibited the greatest amount of decay when viewed with the scanning electron microscope (Figure 5.6). This fungus also generated the most weight loss in the decay study with 7% loss in *Pinus* sp. and 11.5% reduction in *Betula* sp. wood. Figure 5.6 A and B show hyphal growth through the wood cell walls and the initial stages of lignin depolymerization causing wood cell separation. In Figure 5.6 C and D, a relatively large area at the latewood / earlywood transition exhibits extensive deterioration of all cell wall components with the exception of the highly resistant vessel walls. This region displays the simultaneous attack of all wood cell wall layers caused by various white rot decay fungi (Figure 1.15) and significantly compromises the wood strength even after this relatively short period of time.

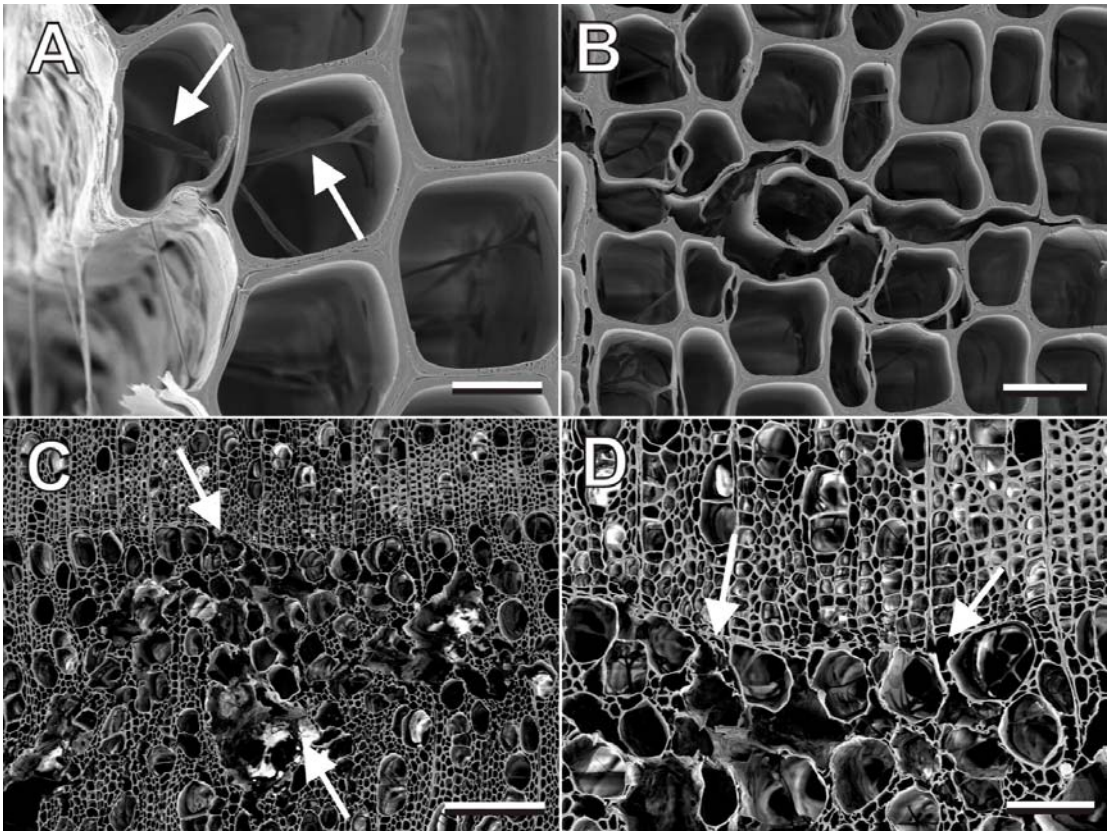


**Figure 5.6** (**A** and **B**) *Pinus* sp. wood with prolific hyphal growth and the initial stages of white rot decay caused by isolate #131 *Oxyporus* sp. generated from a rock collected in the Taklimakan Desert. Bar equals 25 µm in **A** and 200 µm in **B**. (**C** and **D**) Extensive white rot decay in *Betula* sp. wood after inoculation with isolate #131 *Oxyporus* sp. causing degradation of all cell wall layers except for the highly resistant vessels. Bar equals 250 µm in **C** and 100 µm in **D**.

In the laboratory wood degradation study weight loss section 5.3.2.1 the second greatest overall mean weight loss was seen in *Betula* sp. wood inoculated with isolate # 54, *Bjerkandera adusta*, which also had the second highest mean weight loss in *Pinus* wood. The weight loss in both of these wood types was lower than what has been seen in similar studies utilizing *Bjerkandera adusta* (Łakomy et al. 2005) but evidence of decay was found during SEM investigations. In the *Pinus* sp. wood inoculated with the fungus, prolific mycelial growth was seen primarily on the outer surface of the wood sample with limited decay evident (Figure 5.7, A and B).

However, white rot degradation was apparent in the *Betula* sp. wood with a depolymerization of the lignin in selected pockets throughout the samples that were viewed (Figure 5.7, C and D).

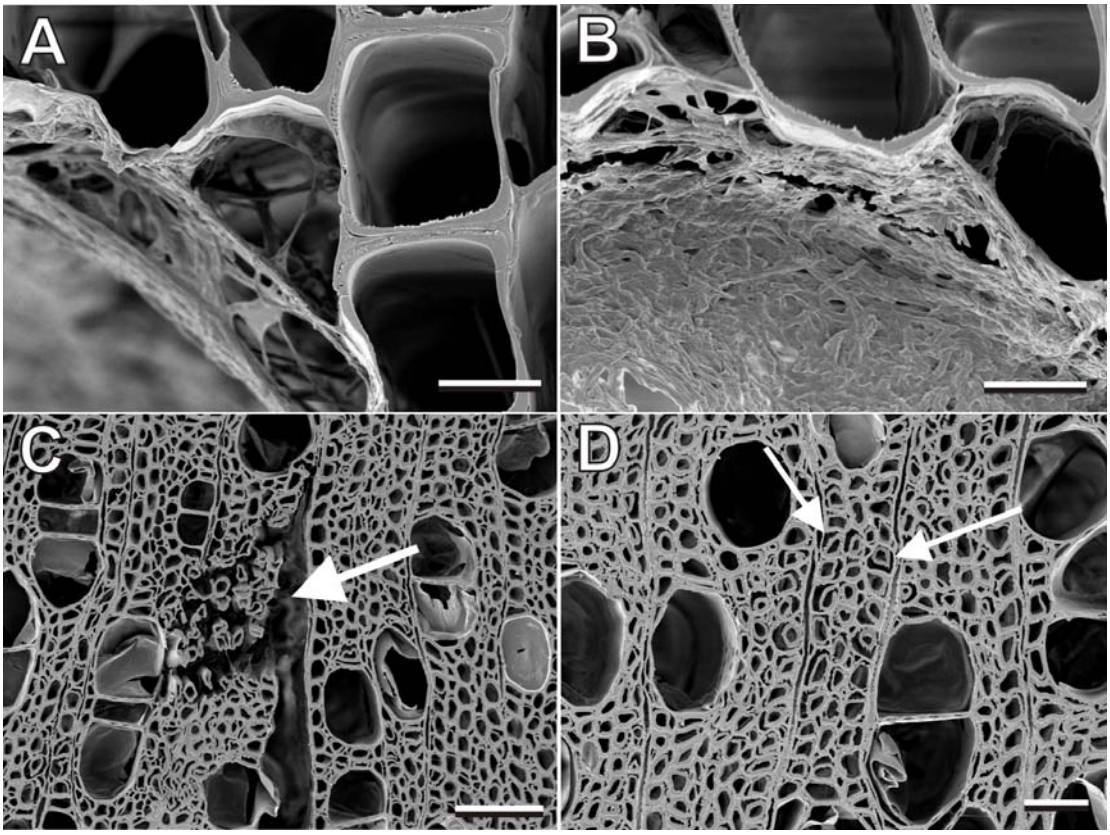


**Figure 5.7** (**A** and **B**) *Pinus* sp. wood inoculated with isolate #54, *Bjerkandera sp.* generated from a rock sample collected in the Taklimakan Desert, showing extensive hyphal growth on the wood surface (Bar 25 μm). (**C** and **D**) Selective delignification causing cell separation in *Betula* sp. wood with arrows indicate region of decay (Bar 100 μm).

In the *Betula* sp. wood decayed by various soft rot decay fungi generated from samples collected in the Taklimakan Desert including; # 1 (*Phoma betae*), # 3 (*Phialocephala* sp.), # 9 (Uncultured fungus), # 38 (*Alternaria* sp.), # 44 (*Tetracladium setigerum*), # 73 (Ascomycete sp.), # 84 (*Thielavia* sp.) # 98 (*Chaetomium globosum*), # 109, (*Lecythophora* sp.), # 178 (*Cladosporium cf.*

*subtilissimum*) and # 191 (*Ulocladium consortiale*), # 201 (Ascomycete sp. nasa65) and #203 (*Embellisia phragmospora*), only the incipient stages of type 2 soft rot decay were seen when viewed with the scanning electrom microscope (Figure 5.8). Although fungal growth was found throughout the samples the visual signs of decay were limited to only a few samples. Soft rot decay of wood is often an exceptionally slow process that has specific requirements that are not entirely understood and the conditions provided in this experiment may not have met the provisions needed to cause deterioration in the time allotted (Worrall et al. 1991; Worrall and Wang 1991).

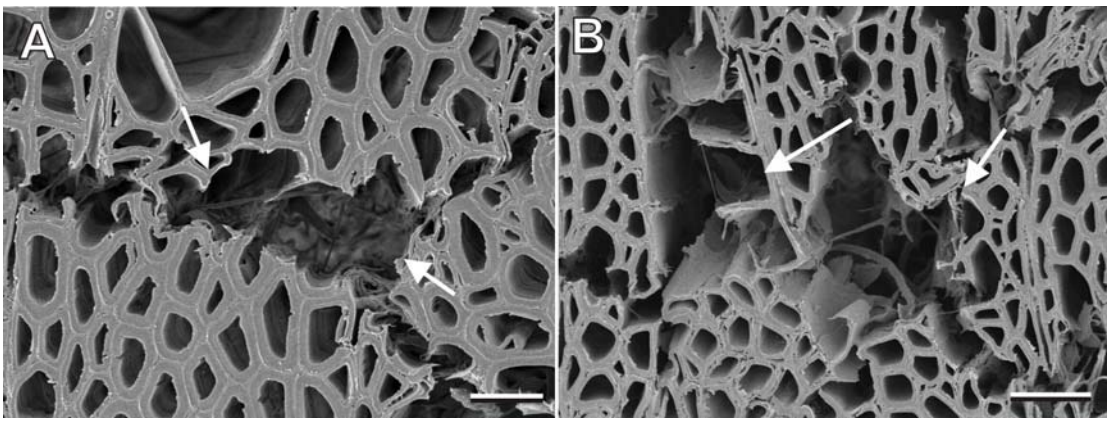


**Figure 5.8** Scanning electron micrographs of *Betula* sp. wood inoculated with isolated #109 Unknown, (*Lecythophora* sp.) (**A**) and # 98 Unknown, (*Chaetomium* sp.) (**B**), generated from samples collected in the Taklimakan Desert. Both samples show fungal hyphae and the incipient stages of type 2 soft rot decay (arrows). Bar equals 100 μm in A and 200 μm in **B**.

The laboratory decay study on *Pinus* sp. wood utilizing the 13 soft rot decay fungi produced only a 3.64% weight loss caused by isolate # 109, *Lecythophora* sp. which was the greatest amount by any soft rotter on this type of wood and the least was caused by isolate # 44, *Tetracladium setigerum* with 0.19%. When these 13 samples were viewed by stereo, compound and electron microscopy no evidence of decay was found, however prolific fungal growth was apparent on all samples including those

inoculated with cultures # 3 (*Cadophora* sp) and 9 (*Cladosporium* sp.) (Figure 5.9). This may in part be related to the greater resistance found in *Pinus* sp. degraded by soft rot decay fungi when compared to *Betula* sp. wood. This was seen in a study conducted by Held et al. (2005) where *Cadophora fastigiata* was able to produce 20–27% weight loss in *Betula* sp. wood but only 1–3% reduction in *Pinus* sp.

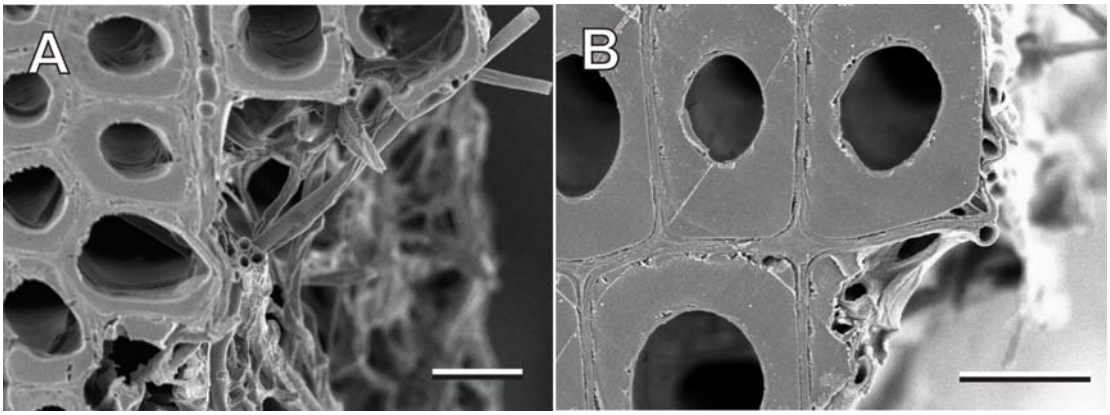


**Figure 5.9** Scanning electron micrographs of fungal hyphae from isolate #3, *Cadophora* sp. (**A**) and # 9, *Cladosporium* sp. (**B**) originating from substartes collected in the Taklimakan Desert, growing on *Pinus* sp. wood. Bar equals 25 μm in **A** and 20 μm in **B**.

### *5.3.3 Cellulase assay*

To determine the relative cellulase activity of twelve of the fungal isolates generated from the samples collected in the Taklimakan Desert, a carboxymethyl cellulose (CMC) Congo red assay, as described in Section 5.2.3, was utilized. The results of the assay are given in Figure 5.10 and show that all but 2 of the 12 isolates, # 3 *Cadophora* sp. and # 178 *Cladosporium* sp., were capable of producing cellulase when the isolates were cultured on CMC agar plates with culturing at either 2ºC, 15ºC or 25ºC. Isolates #3 and 178 did not produce cellulase by the criteria of the assay at any of the temperatures.

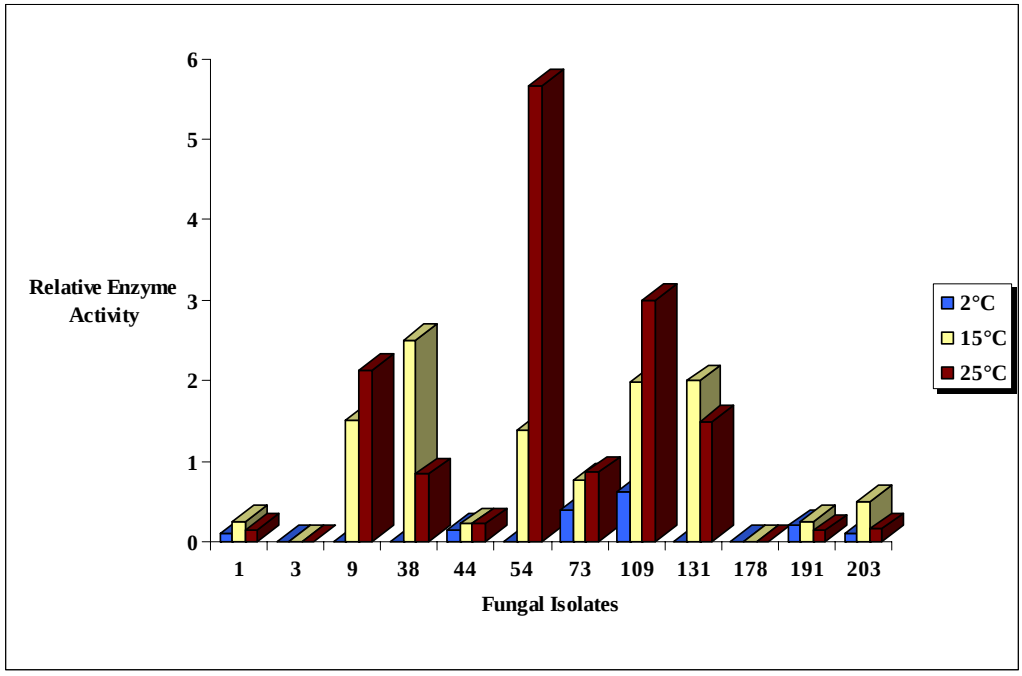


**Figure 5.10** Comparison of the relative enzyme production determined by a carboxymethyl cellulose (CMC) Congo red assay of selected fungal isolates generated from samples collected in the Taklimakan Desert and grown at 2ºC, 15ºC and 25ºC.

The isolates # 1 (Unknown), 9 (*Cladosporium* sp.), 38 (*Alternaria* sp.), 44 (*Embellisia* sp.), 54 (*Bjerkandera* sp.), 73 (Unknown), 109 (Unknown), 131 (*Oxyporus* sp.), 191 (*Ulocladium* sp.) and 203 (*Embellisia* sp.) all had cellulase activity at 25 ºC. The most pronounced activity observed at all three temperatures was seen in isolate # 54 (*Bjerkandera* sp.), when the assay was conducted on a culture at 25ºC; this activity was nearly twice as much as what was recorded in isolate # 109 (Unknown) when assayed at the same temperature, which displayed the second highest activity. As mentioned in Section 5.3.2.1 (Weight loss), *Bjerkandera* are white rot decay fungi which primarily decay lignin components of wood but are capable of degrading all cell wall materials. Other studies have similar results, for example Oses et al. (2006) found a 'strong' reaction, (the author's term for highest

ranking), from a *Bjerkandera* sp. isolated from *Drimys winteri* wood when conducting a cellulase (Cellulose-azure) assay on agar based media. The cellulase activity of this isolate was reduced by nearly 80% at 15ºC and there was no activity at 2ºC.

The relative cellulase activity of the polar isolates at 25ºC was very different from the complementary study completed with the Taklimakan isolates (Figure 5.11). All of the isolates; Uncultured Basidiomycete (1), *Exophiala* sp. (2), *Phialocephala* sp. (3), Fungal sp. AB10 (*Cadophora*) (4), Uncultured fungus clone N3 (5), *Exophiala spinifera* (6), *Cadophora malorum* (7), *Cladosporium cladosporioides* (8), *Phoma herbarum* (9), *Alternaria* sp. (10), *Chaetomium funicola* (11) and *Ulocladium chartarum* (12), exhibited cellulose activity in this assay. Although none of the polar isolates had similar activity to that of the Basidiomycota from the Taklimakan, the Antarctic *Cadophora malorum* (7) did have the greatest reaction in this group with a value of nearly 3. The activity in the polar isolates was interesting as all produced cellulase at 25ºC, with only 2 isolates giving higher values at 15ºC, *Phialocephala* sp. (3) and *Ulocladium chartarum* (12) and the least or no reaction was seen at 2ºC.

Of the 10 isolates that had cellulase enzyme activity at 25ºC, they all also showed activity at 15ºC. Five of these isolates, # 1 (Unknown), 38 (*Alternaria* sp.), 131 (*Oxyporus* sp.), 191 (*Ulocladium* sp.) and 203 (*Embellisia* sp.) , # 1 (Unknown), 38 (*Alternaria* sp.), 131 (*Oxyporus* sp.), 191 (*Ulocladium* sp.) and 203 (*Embellisia* sp.), displayed greater activity at 15ºC when compared to 25ºC and Isolate # 38 exhibited three times more activity at 15ºC than at 25ºC. Only isolates that had cellulase

activity at 15ºC and 25ºC demonstrated activity at 2ºC and all were less than what was seen at the higher temperatures. Isolates # 9 (*Cladosporium* sp.), 38 (*Alternaria* sp.), 54 (*Bjerkandera* sp.) had activity at the higher two temperature but none at 2ºC and isolate # 44 (*Embellisia* sp.), had lower activity over all but nearly the same across all temperatures.

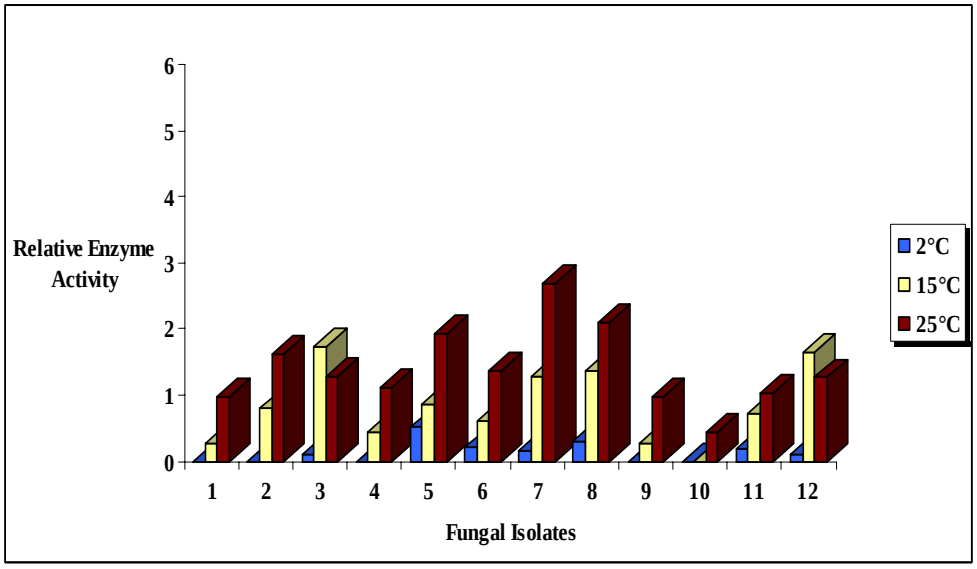


**Figure 5.11** Comparison of the relative enzyme production determined by a carboxymethyl cellulose (CMC) Congo red assay of selected fungal isolates generated from samples collected in the Arctic and Antarctic at 2ºC, 15ºC and 25ºC.

### *5.3.4 Lignin peroxidase assay*

The lignin peroxidase assay utilizing Azure B, with 15 fungal isolates generated from substrates collected in the Taklimakan Desert and tested at 3 temperature regimes showed enzyme activity in nearly half of the fungi tested (Table 5.4). Of the isolates that showed clearing of the media stain, the greatest number was at 25ºC and included; # 1 (Unknown), 3 (*Cadophora* sp.), 9 (*Cladosporium* sp.), 38 (*Alternaria* sp.), 44 (*Embellisia* sp.), 178 (*Cladosporium* sp.), 191(*Ulocladium* sp.) and 203(*Embellisia* sp). At 15ºC these same fungi also showed clearing, with the

exception of #3 and 38 and at 2ºC isolate # 1, 9, 44 and 203 displayed enzyme activity by clearing the media. All of these isolates are soft rot decay fungi (Blanchette et al. 2004; Held et al. 2006; Tuomela et al 2000; Wang 1990) that primarily degrade the cellulytic components of lignocellulolytic materials but also have the capacity to degrade lignin (Eslyn et al. 1975; Haider and Trojanowski 1975 & 1980).

**Table 5.4** Selected fungal isolates, associated substrate and identifier generated from collections made in the Taklimakan Desert used in an Azure B assay for detecting lignin peroxidase and Mn dependant peroxidase utilization at 2°C, 15°C and 25°C.

| Fungal isolate | Sample & substrate | Temperature | | |
|---|---|---|---|---|
| | | 2°C | 15°C | 25°C |
| 1 | MS2S (soil) | + | + | + |
| 3 | MS2S (soil) | - | - | + |
| 9 | MS3S (soil) | + | + | + |
| 38 | MS16S (soil) | - | - | + |
| 44 | MS16S (soil) | + | + | + |
| 54 | MS8R (rock) | - | - | - |
| 73 | MS8S (soil) | - | - | - |
| 84 | MS16S (soil) | - | - | - |
| 98 | MS16S (soil) | - | - | - |
| 109 | MS2S (soil) | - | - | - |
| 131 | MS2R (rock) | - | - | - |
| 178 | MS10R (rock) | - | - | + |
| 191 | MS8W (wood) | - | + | + |
| 201 | MS8W (wood) | - | - | - |
| 203 | MSW (wood) | + | + | + |

Interestingly, the 2 white rot decay fungi # 54 (*Bjerkandera* sp.) and # 131 (*Oxyporus* sp.) did not show evidence of lignin peroxidase and Mn dependant peroxidase

production in this assay. Although most white rotters are capable of degrading all primary cell wall materials including cellulose, hemicellulose and lignin, their primary focus is most often the lignolytic components and there was no indication of activity in these 2 isolates similar to what was found in the above mentioned soft rot decay fungi. The *Bjerkandera* sp., # 54 isolate did however produce a darker purple halo around the inoculation plug at 15 and 25ºC but this was not consistent with the activity seen in the other isolates that unambiguously displayed clearing of the media and thus was not considered a positive result because of this discontinuity. Heinfling et al. (1998) found similar results when testing lignin peroxidase activity of *Bjerkandera adusta* on 3 different media dye assays and was only able to obtain significantly positive results with the addition of veratryl alcohol.

At 25ºC the polar isolates that were similar to the Taklimakan Desert isolates based on consensus sequence data also caused clearing of the lignin peroxidase assay media; these included, Uncultured Basidiomycete (1), *Phialocephala* sp. (3), Fungal sp. AB10 (*Cadophora*) (4) from the Arctic and *Cadophora malorum* (7), *Cladosporium cladosporioides* (8), *Phoma herbarum* (9), *Alternaria* sp. (10), *Chaetomium funicola* (11) from the Antarctic (Table 5.5). These reactions all correspond well with the similar species from the Taklimakan Desert with the exception of the *Ulocladium chartarum* (12) from Antarctica which did not produces lignin peroxidase at 25ºC, while # 191 *Ulocladium consortiale* from the Taklimakan Desert did. At 15ºC all of the Arctic isolates that reacted at 25ºC, failed to react at this lower temperature. However, all of the Antarctic isolates that cleared the media at

25ºC had the same result at 15ºC and the only culture that produced lignin peroxidase at 2ºC was *Cladosporium cladosporioides* (8) from the Antarctic.

**Table 5.5** Fungal isolates from comparative Arctic and Antarctic investigations with top BLAST match based on ITS consensus sequences, associated accession number and used in an Azure B assay for detecting lignin peroxidase and Mn dependant peroxidase utilization at 2°C, 15°C and 25°C.

| **Region** | **Top BLAST Match** | **Accession** | **Temperature** | | |
|---|---|---|---|---|---|
| | | | **2°C** | **15°C** | **25°C** |
| Arctic | Uncultured Basidiomycete (1) | FJ457769 | - | - | + |
| Arctic | *Exophiala* sp. (2) | FJ457766 | - | - | - |
| Arctic | *Phialocephala* sp. (3) | FJ457767 | - | - | + |
| Arctic | Fungal sp. AB10 (*Cadophora*) (4) | FJ457771 | - | - | + |
| Arctic | Uncultured fungus clone N3 (5) | FJ457773 | - | - | - |
| Antarctic | *Exophiala spinifera* (6) | DQ317336 | - | - | - |
| Antarctic | *Cadophora malorum* (7) | DQ317328 | - | + | + |
| Antarctic | *Cladosporium cladosporioides* (8) | DQ317332 | + | + | + |
| Antarctic | *Phoma herbarum* (9) | DQ317345 | - | + | + |
| Antarctic | *Alternaria* sp. (10) | DQ317386 | - | - | + |
| Antarctic | *Chaetomium funicola* (11) | DQ317331 | - | + | + |
| Antarctic | *Ulocladium chartarum* (12) | EF679390 | - | - | - |

After the 21 day monitoring period all plates were incubated at 25ºC to determine if the lignin peroxidase and Mn dependant peroxidase production was temperature dependant or if other factors contributed to the recorded reactions during this 3 week period. After 1 week of growth at 25ºC all 8 isolates from the Taklimakan Desert (# 1, 3, 9, 38, 44, 178, 191 and 203) and from the polar regions (1, 3, 4, 7, 9, 10, 11) that originally displayed activity at 25ºC but not at 2ºC and 15ºC had clearing of the media and those that did not have clearing during the first 21 days had the same results after this additional week. This test only identified the lignin peroxidase production in these fungi and did not consider other lignin degrading enzymes such as laccases.

## Chapter 6
## Conclusions and future research

The aims of the doctoral thesis research were to investigate the biodiversity, evolutionary history and potential in utilizing lignocellulosic substrates, of fungal constituents in lithic niches, soils and vascular plant materials from a hot, hyper-arid desert region of Western China and compare them to fungi from cold deserts in polar regions.

There were three objectives to the research; the first objective was specific to the Western China desert region, the second was comparing those results to the polar regions and the third was considering lignocellulose as a nutrient. The specific objectives and their addressment were as follows:

***Objective 1a:*** Identify the cultivatable fungi from substrates present (soil, rock and wood) in a defined region of a hyper-arid desert in western China.

***Conclusions:***

Culturing fungi from the Taklimakan Desert substrates of soil, rock and wood and putatively identifying the fungi based on the ITS region of rDNA generated 194 phylotypes. Concerning classical mycological culturing of fungi from collected samples, the greatest number of fungi, 103 cultures, grew from the soil samples. The rock samples generated 60 fungal isolates and 31 fungal isolates were obtained from the wood sample.

***Objective 1b:*** Determine the fungal presence in a defined region of a hyper-arid desert in western China by denaturing gradient gel electrophoresis (DGGE) molecular fingerprinting.

***Conclusions:***

The DGGE investigations of fungi occurring on soils, rock and wood samples collected in the Taklimakan Desert produced 51 consensus ITS rDNA sequences, with *Colletotrichum* sp. (EU400140) and *Penicillium* sp. (FJ025202) as the dominant taxa. In this study a rare, based on current research, Basidiomycota that was not cultured, *Wallemia muriae*, was also identified. Filamentous fungi from this Phylum are very rare in deserts and this species represents on of the most xerophilic fungal taxa known (Polona et al. 2005).

***Objective 2:*** Use neighbor-joining analysis to establish putative identity of fungi from extreme deserts in western China and from the extreme environments of polar regions.

**Conclusions:**

Based on comparisons of consensus sequences to the polar fungi and fungal databases, 72 cultured isolates appear to represent novel taxa, which may be endemic to the Taklimakan Desert but further characterization of these isolates is required.

***Objective 3:*** Determine the capability of select cultivated isolates to decay lignocellulosic substrates and their ability to produce degradation related enzymes at various temperatures.

**Conclusions:**

Fungi from these three regions were able to degrade the *Pinus* and *Populus* sp. wood to varying degrees though minimal weight loss was common and the isolates did not produce cellulase or lignin peroxidase at temperatures prevalent in the regions from which they were collected.

There were two *Null-hypotheses* associated with this investigation.

*Null-hypothesis* 1 was; fungal taxa from thermally distinct biomes do not occur in both hot and cold deserts. The results of this study showed that null hypothesis 1 was confirmed when the dominant taxa from the Taklimakan Desert and the comparative studies in the Arctic and Antarctic are considered. However, recent results published during the PhD thesis period, one book chapter now in press to which the PhD candidate contributed (Blanchette et al. 2009), indicates that null hypothesis 1 was not proven. The dominant taxa isolated from the Taklimakan Desert were *Thielavia* sp. While not found in the thesis comparative publications (Jurgens et al. 2009; Arenz et al. 2006) from the Arctic and Antarctic, they have been isolated in both of these polar regions, as reported by Frisvad (2008); Stchigel et al. (2003) and were recently found to be one of the dominant taxa at a site on Ross Island, Antarctica, associated with historic organic materials and soils (Blanchette 2009). The authors found that *Thielavia* sp. contributed 17% of the total isolates cultured from this site, representing the second most dominant taxa.

The presence of filamentous Basidiomycota in the Taklimakan Desert are unique when compared to the complementary investigations in polar regions by Jurgens et al. (2009) and Arenz et al. (2006), as well as research conducted by others in both polar and hot deserts (Büdel 2005; Conley et al. 2006; Ludley and Robinson 2008). Many of the other fungi that were isolated and putatively identified based on top BLAST matches and neighbor-joining analysis are ubiquitous taxa that can readily be found in various biomes throughout the world. Studies have shown that the dust and fungal spores from the Taklimakan Desert can be carried great distances by the strong winds generated in this desert and deposited at locations like Greenland and the French Alps (Bory et al. 2003; Grousset et al. 2003; Kai et al. 2008) and it is likely that this same type of deposition occurs within the Taklimakan Desert but further study is required to confirm this.

The second *null-hypothesis* stated fungi isolated from extreme desert conditions can not only colonize wood but have the ability to degrade this substrate. The results of this investigation indicated that this null-hypothesis was confirmed. All of the fungi from both, the Taklimakan Desert, Arctic and Antarctic reduced the weight of the two substrates utilized in the lignocellulic decay study, however, the amount of degradation was minimal. The fungi utilized in the degradation study did not produce the amount of weight loss seen in other decay studies with similar taxa (Held et al. 2005; Wikström 2005; Worrall et al. 1991; Worrall et al. 1997) and physical signs of degradation in the wood cell walls were limited. This may by a symptom of the fungi not functioning well under the conditions that were created in this investigation or it may indicate a unique degradation system. The Taklimakan Desert isolates may not

have the same 'robustness' of gene expressions, genes, enzyme production or stability common to fungi isolated from other locations (Held et al. 2005; Wikström 2005; Worrall et al. 1991; Worrall et al. 1997). It is also possible that the isolates are selectively attacking materials such as starches, sugars and extractives within the substrates and not degrading the primary cell wall components. Addition study of this, including analysis of all wood components prior to and after decay has taken place is justified to assist in determining the degradation pathway of these fungi.

Although few Basidiomycota were isolated and sequenced in this investigation, all of the cultures in this phylum were derived from rocks collected in the Taklimakan Desert. As seen in the comparative collections conducted by Jurgens et al. (2009) and Arenz et al. (2006) in the Arctic and Antarctic, respectively, Basidiomycota fungi are exceedingly rare in these polar deserts. In the Arctic, Jurgens et al. (2009) isolated only 1 culture from this Phylum, with the closest NCBI BLAST match of Uncultured Basidiomycete (AM999656) and with the neighbor-joining analysis in Figure 4.1 no definitive association with known fungi could be established for this culture. In Arenz et al. (2006) the collections in Antarctic deserts did not generate any filamentous Basidiomycota, with only yeast such as *Cryptococcus* sp. and *Rhodotorula* sp. identified.

The fact that 5 Basidiomycota were isolated only from three separate rock samples collected in the Taklimakan Desert is curious for several reasons:

First, as noted above by Jurgens et al. (2009), Arenz et al. (2006) and in a review by Ludley and Robinson (2008) of Basidiomycota in Arctic and Antarctic ecosystems, the presence of filamentous fungi from this Phylum on the Antarctic continent and much of the Arctic are extremely uncommon.

Second, in hot deserts such as the Atacama in Northern Chile, Conley et al. (2006) did not isolate any Basidiomycota and Büdel (2005) in a review of two deserts in the United States and the Sahara of Northern Africa only identified 1 fungus from this Phylum, *Podaxis pistillaris*, when isolating from rocks and soil crusts.

Third, all of the Basidiomycota isolated from rocks in this study are commonly considered wood degrading white rot fungi including *Bjerkandera* sp., *Oxyporus* sp. and *Phanerochaete* sp. but they were not isolated from soils or their preferred substrate, wood.

Fourth, the Basidiomycota from the Taklimakan Desert were all isolated on the high nutrient media of NA and PDA, yet they were derived from rock which is nearly devoid of nutrients.

These points raise questions such as could the fact that these Basidiomycota were isolated only from rock indicate that they are simply subsisting on a substrate that is present, which may indicate an ecological and physiological shift to take advantage of what is in their environment for survival? Also, as these fungi were only isolated from rock and not from what is considered the 'preferred' substrate, wood, and they

were not isolated from soils that were collected in direct contact with the rocks, could this indicate that they may require a relationship with a consortium of other microorganisms to subsist in this harsh environment? The isolation of these Basidiomycota could be based on spores or hyphal fragments brought into this site by the strong winds in the Taklimakan Desert but why have they only been isolated from rocks and not the other substrates, notably the wood? These points and the questions they raise warrant additional study and future research to determine why these fungi are in the Taklimakan Desert and what their roles are.

The sequenced strains produced many unexpected fungi such as those closely related to endophytes, human pathogens and wood-decay fungi. This creates questions such as are they present due to dispersal of propagules, are they resident inoculum awaiting stochastic nutrient events, or are they more physiologically plastic than previously thought? These questions are important and if answered will increase our knowledge of the physiology, ecological significance, enzymatic potential and human health issues associated with these unique fungi.

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**Appendix 1 Supplementary tables of top BLAST match and putative identification of Taklimakan Isolates**

**Table A1** List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 1 | *Phoma betae* strain 17 | EU594572 | Unknown |
| 2 | *Paecilomyces* major isolate GZDXIFR-H-57-2 | DQ243696 | *Thielavia* sp. |
| 3 | *Phialocephala* sp. T64106 | DQ122928 | *Cadophora malorum* |
| 4 | *Retroconis fusiformis* strain CBS 330.81 | EU040239 | *Thielavia* sp. |
| 5 | *Alternaria tenuissima* | AJ867284 | *Alternaria alternata* |
| 6 | Uncultured endophytic fungus clone 59-11-07 | EF505090 | *Alternaria alternata* |
| 7 | Fungal endophyte isolate 9098 | EF420014 | *Thielavia subthermophila* |
| 8 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 9 | Uncultured fungus | AJ875362 | *Cladosporium* sp. |
| 10 | Uncultured endophytic fungus clone 59-11-70 | EF505100 | Unknown |
| 11 | *Alternaria alternata* | EF452443 | Unknown |
| 12 | Uncultured fungus isolate CA10 | EF159163 | Unknown |
| 13 | Uncultured fungus isolate CA10 | EF159163 | Unknown |
| 14 | Fungal sp. YNLF-26 | DQ426531 | *Cladosporium* sp. |
| 15 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | *Cladosporium subtilissimum* |
| 16 | *Alternaria alternata* | AY787684 | *Alternaria* sp. |
| 18 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 19 | Uncultured fungus clone 12(366-13) | EU437434 | Unknown |
| 20 | *Thielavia microspora* | AJ271577 | Unknown |
| 21 | *Sydowia polyspora* | AY781224 | Unknown |
| 23 | *Phoma macrostoma* | DQ093700 | *Phoma* sp. |
| 24 | *Fusarium* sp. NRRL25622 | AF158306 | *Fusarium* sp. |
| 25 | *Gibberella* sp. BF22 | AM901682 | *Fusarium* sp. |
| 26 | *Fusarium* sp. NRRL 43682 | EF453161 | *Fusarium* sp. |

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**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 27 | *Aporospora terricola* isolate 35/1.5 | DQ865097 | *Aporospora* sp. |
| 28 | Uncultured fungus clone 12(366-13) | EU437434 | Unknown |
| 29 | *Thielavia appendiculata* | AJ271584 | *Thielavia* sp. |
| 30 | *Penicillium citreonigrum* strain NRRL 2046 | EF198647 | *Penicillium citreonigrum* |
| 31 | Fungal sp. ARIZ B487 | FJ613076 | *Thielavia* sp. |
| 32 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | *Emericella quadrilineata* |
| 33 | *Retroconis fusiformis* strain CBS 330.81 | EU040239 | *Thielavia* sp. |
| 35 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 36 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 37 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 38 | *Alternaria* sp. IBL 03136 | DQ682562 | *Alternaria alternata* |
| 39 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 40 | *Paecilomyces* sp. MTCC 6328 | EF550986 | *Thielavia* sp. |
| 41 | *Amaurascopsis reticulatus* | AJ271434 | Unknown |
| 42 | *Chaetomium* sp. 73-19-O-Mexico | AY560520 | *Thielavia* sp. |
| 43 | *Lecythophora* sp. UBCtra1453C | AY219880 | Unknown |
| 44 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 45 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 46 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 47 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | *Ulocladium* sp. |
| 48 | *Tetracladium setigerum* isolate NW313 | EU622246 | Unknown |
| 49 | Uncultured soil fungus clone CS3M5c36P | EU480267 | Unknown |
| 50 | *Ulocladium botrytis* isolate CHHUB1 | AF267139 | *Ulocladium* sp. |
| 51 | *Lecythophora* sp. UBCtra1453C | AY219880 | Unknown |
| 53 | *Thielavia tortuosa* | AJ271592 | *Thielavia* sp. |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 54 | *Bjerkandera adusta* isolate wb340 | AF455468 | *Bjerkandera adusta* |
| 55 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | *Ulocladium* sp. |
| 56 | *Macrophoma* sp. 244-465 | DQ100416 | Unknown |
| 57 | *Ulocladium* sp. CID68 | EF589899 | *Ulocladium* sp. |
| 58 | *Phoma macrostoma* | AJ310557 | *Phoma* sp. |
| 59 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 60 | *Preussia fleischhakii* strain CBS 56563 | DQ468019 | Unknown |
| 61 | *Stemphylium* sp. EGS48-089 | AY329186 | *Stemphylium* sp. |
| 62 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 63 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 64 | *Thielavia appendiculata* | AJ271584 | *Thielavia* sp. |
| 65 | *Thielavia appendiculata* | AJ271584 | *Thielavia* sp. |
| 66 | *Thielavia basicola* | AJ271591 | *Thielavia* sp. |
| 67 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 68 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 69 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 70 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 71 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 72 | *Chaetomium* sp. 73-19-O-Mexico | AY560520 | *Thielavia* sp. |
| 73 | Ascomycete sp. IZ-1109 | AM921746 | Unknown |
| 74 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 75 | *Ulocladium consortiale* | AY278837 | *Ulocladium* sp. |
| 76 | *Stachybotrys chlorohalonata* strain ATCC 9182 | AY185567 | *Stachybotrys chlorohalonata* |
| 77 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 78 | *Phoma macrostoma* 2-1-1-1 | AJ310557 | *Phoma* sp. |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 79 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 80 | Fungal sp. YNLF-26 | DQ426531 | *Cladosporium* sp. |
| 81 | *Phoma macrostoma* 2-1-1-1 | AJ310557 | *Phoma* sp. |
| 82 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 83 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 84 | *Thielavia* sp. B27 | EU620166 | *Chaetomium* sp. |
| 85 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 86 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 87 | *Ulocladium consortiale* | AY278837 | *Ulocladium* sp. |
| 88 | *Sordariomycete* sp. BC38 | DQ317345 | *Phoma* sp. |
| 90 | *Stachybotrys chlorohalonata* strain ATCC 9182 | AY185567 | *Stachybotrys chlorohalonata* |
| 91 | *Thielavia subthermophila* | AJ271575 | *Thielavia subthermophila* |
| 92 | *Ulocladium* sp. CID68 | EF589899 | *Ulocladium* sp. |
| 93 | *Paecilomyces* sp. MTCC 6328 | EF550986 | *Thielavia* sp. |
| 94 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | *Emericella quadrilineata* |
| 95 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | *Emericella sp.* |
| 96 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 97 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 98 | *Chaetomium globosum* isolate aurim1231 | DQ093659 | Unknown |
| 99 | Fungal sp. GFI 145 | AJ608974 | Unknown |
| 100 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 101 | *Alternaria tenuissima* | AJ867284 | *Alternaria* sp. |
| 102 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 103 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 104 | Uncultured fungus | AJ875362 | *Cladosporium* sp. |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 105 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 106 | *Humicola fuscoatra* | AJ279444 | Unknown |
| 107 | Uncultured Chaetomiaceae clone A1_k_ | EU754950 | Unknown |
| 108 | *Aureobasidium pullulans* strain CBS 110377 | AY139391 | *Aureobasidium pullulans* |
| 109 | *Lecythophora* sp. UBCtra1453C | AY219880 | Unknown |
| 110 | *Thielavia basicola* | AJ271591 | *Thielavia* sp. |
| 112 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 114 | *Stemphylium* sp. EGS48-089 | AY329186 | *Stemphylium* sp. |
| 115 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 116 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 117 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 120 | *Emericella quadrilineata* isolate NRRL 4904 | EF652484 | *Emericella quadrilineata* |
| 121 | *Emericella quadrilineata* isolate NRRL 4992 | EF652493 | *Emericella sp.* |
| 122 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 123 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 124 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 125 | *Thielavia* sp. B27 | EU620166 | *Thielavia* sp. |
| 126 | *Thielavia appendiculata* | AJ271584 | *Thielavia* sp. |
| 129 | *Alternaria alternata* isolate NW562cb | EU520077 | *Alternaria* sp. |
| 130 | Fungal endophyte isolate 9097 | EF420014 | *Thielavia subthermophila* |
| 131 | *Oxyporus corticola* strain R-3610 | EF011124 | *Oxyporus corticola* |
| 132 | *Thielavia coactilis* | AJ271585 | *Thielavia* sp. |
| 133 | *Aporospora terricola* | DQ865100 | Unknown |
| 134 | *Lecythophora* sp. UBCtra1453C | AY219880 | Unknown |
| 137 | *Alternaria* sp. IA202 | AY154681 | *Embellisia* sp. |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 138 | *Alternaria* sp. IA317 | AY154719 | *Alternaria alternata* |
| 142 | Uncultured fungus clone 12(366-13) | EU437434 | Unknown |
| 145 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | *Cladosporium* sp. |
| 147 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 148 | *Cladosporium subinflatum* strain CPC 12041 | EF679389 | *Cladosporium sp.* |
| 151 | Fungal endophyte isolate 9097 | EF420014 | *Thielavia* sp. |
| 152 | *Chaetomium globosum* isolate aurim1231 | DQ093659 | Unknown |
| 153 | *Sordariomycete* sp. BC38 | DQ317345 | *Phoma* sp. |
| 156 | Uncultured soil fungus clone 137-5 | DQ421195 | Unknown (Basidiomycete) |
| 160 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 166 | Fungal sp. YNLF-26 | DQ426531 | *Cladosporium* sp. |
| 167 | *Chaetomium globosum* strain UAMH 7142 | AY625061 | Unknown |
| 168 | *Thielavia appendiculata* | AJ271584 | *Thielavia* sp. |
| 169 | *Thielavia coactilis* | AJ271585 | Unknown |
| 170 | *Oxyporus corticola* strain R-3610 | EF011124 | *Oxyporus corticola* |
| 171 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 172 | *Lecythophora* sp. UBCtra1453C | AY219880 | Unknown |
| 173 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 174 | *Embellisia phragmospora* strain EGS 27-098 | FJ357314 | *Embellisia* sp. |
| 175 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 176 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 177 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | *Cladosporium cf. subtilissimum* |
| 178 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | *Cladosporium cf. subtilissimum* |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 179 | *Cladosporium cf. subtilissimum* CBS 172.52 | EF679390 | *Cladosporium sp.* |
| 180 | *Alternaria tenuis* isolate NW694 | EU520208 | *Alternaria alternata* |
| 182 | Fungal sp. EXP0549F | DQ914710 | Unknown |
| 183 | *Phaeosphaeria volkartiana* isolate CBS 590.86 | AF439509 | *Phaeosphaeria* sp. |
| 184 | *Sordariomycete* sp. 7670B | EU680539 | *Fusarium* sp. |
| 187 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 188 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 191 | *Ulocladium consortiale* | AY278837 | *Ulocladium* sp. |
| 192 | *Ulocladium consortiale* | AY278837 | *Ulocladium* sp. |
| 193 | *Alternaria alternata* | EF452443 | *Alternaria alternata* |
| 194 | *Thielavia hyalocarpa* | AJ271583 | *Thielavia* sp. |
| 195 | *Ulocladium consortiale* | AY278837 | *Ulocladium* sp. |
| 196 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 197 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 198 | Uncultured Pyronemataceae clone DGGE band BD6 | DQ317369 | Unknown |
| 199 | Uncultured Pyronemataceae clone DGGE band BD6 | DQ317369 | Unknown |
| 200 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 201 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 203 | *Embellisia phragmospora* strain EGS 27-098 | FJ357314 | *Embellisia* sp. |
| 205 | *Stachybotrys* sp. BF15 | AM901678 | *Stachybotrys chlorohalonata* |
| 206 | *Macrophoma* sp. 244-465 | DQ100416 | Unknown |
| 207 | *Cladosporium* sp. PZ-2006e | DQ780407 | *Cladosporium* sp. |
| 208 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 209 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 210 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |

**Table A1** (Continued) List of fungal taxa isolated from soil, rock or wood collected in the Taklimakan Desert, based on top BLAST match and putative identification from phylogenetic neighbor joining analysis.

| Isolate | Top BLAST Match | Accession # | Putative Identification |
|---|---|---|---|
| 211 | *Penicillium citreonigrum* strain NRRL 2046 | EF198647 | *Penicillium citreonigrum* |
| 212 | *Phoma macrostoma* | AY618249 | *Phoma* sp. |
| 213 | *Ulocladium* sp. CID68 haplotype ulo068 | EF589899 | *Ulocladium* sp. |
| 214 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 215 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 216 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 217 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 218 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 219 | *Ulocladium* sp. CID68 | EF589899 | *Ulocladium* sp. |
| 220 | Fungal sp. AB58 | FJ235991 | Unknown |
| 221 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 222 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 223 | *Tetracladium setigerum* isolate NW313 | EU622246 | *Embellisia* sp. |
| 224 | Ascomycete sp. nasa65 | DQ683978 | Unknown |
| 225 | *Cladosporium tenuissimum* isolate 029.4 | FJ228171 | *Cladosporium* sp. |
| 226 | *Apodus deciduus* | AY681199 | Unknown |
| 227 | *Phanerochaete laevis* | AY219348 | *Phanerochaete laevis* |
| 228 | *Fusarium proliferatum* | FJ040179 | *Fusarium* sp. |
| 229 | *Sordariomycete* sp. pgp-hsf | DQ227290 | Unknown |
| 230 | *Thielavia intermedia* | AJ271588 | Unknown |
| 231 | *Apodus deciduus* | AY681199 | Unknown |
| 232 | *Thielavia microspora* | AJ271577 | Unknown |

**<u>Appendix 2</u> Supplementary publications of Joel A. Jurgens completed between 2006 - 2009 relevant to the PhD topic**

PNAS

# Highly specialized microbial diversity in hyper-arid polar desert

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**The McMurdo Dry Valleys in Antarctica are a cold hyperarid polar desert that present extreme challenges to life. Here, we report a culture-independent survey of multidomain microbial biodiversity in McKelvey Valley, a pristine example of the coldest desert on Earth. We demonstrate that life has adapted to form highly-specialized communities in distinct lithic niches occurring concomitantly within this terrain. Endoliths and chasmoliths in sandstone displayed greatest diversity, whereas soil was relatively depauperate and lacked a significant photoautotrophic component, apart from isolated islands of hypolithic cyanobacterial colonization on quartz rocks in soil contact. Communities supported previously unreported polar bacteria and fungi, but archaea were absent from all niches. Lithic community structure did not vary significantly on a landscape scale and stochastic moisture input due to snowmelt resulted in increases in colonization frequency without significantly affecting diversity. The findings show that biodiversity near the cold-arid limit for life is more complex than previously appreciated, but communities lack variability probably due to the high selective pressures of this extreme environment.**

Antarctica | biodiversity | endolith | extremophile | hypolith

The McMurdo Dry Valleys located in southern Victoria Land, Antarctica are a unique biome comprising the largest ice-free region on the Antarctic continent. They are designated by international treaty as an Antarctic Special Managed Area (1) to reflect their environmental significance. Classified as a hyperarid desert, the Dry Valleys are among the most threatened environments from climate change due to their polar location (2), and environmental stress is more pronounced in the higher inland valleys (3). They are characterized by extreme cold and dry conditions, resulting in sublimation of snowfall with minimal localized liquid-water input to soils (4–6). The Dry Valleys floor comprises dry permafrost (7) that commonly supports a polygonal surface terrain characterized by a desert pavement of sandstone, quartz, and granitic rocks embedded in mineral soils. Higher plants and animals are absent (8), although microbial colonization has been recorded for some Dry Valleys locations. This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 20).

Endolithic colonization of pore spaces within rocks has been recorded in isolated high Dry Valleys sites (9, 10). Colonization as chasmoliths in cracks and fissures was reported as relatively infrequent (9, 10). In lower valleys experiencing less environmental stress, hypolithic communities have been recorded beneath quartz rocks in soil contact (11, 12) in addition to soil microbial communities (13–17). To date, a comprehensive multidomain diversity assessment comparing these refuge communities has been lacking, and so, community structure remains unresolved, and uncertainty exists as to whether distinct taxa colonize niches near the cold arid limit for life or if they represent an extension of autochthonous soil or aquatic communities from less extreme locations.

An understanding of biodiversity in such communities advances our knowledge of microbial ecology under extreme stress and also informs potential effects of environmental change to endangered polar landscapes. Here, we present findings from a polyphasic molecular study targeting all domains of life to fully characterize microbial diversity in four distinct microbial niches occurring concomitantly in the dry permafrost of the high inland McKelvey Valley. We identify highly-specialized communities that supported previously unreported polar bacteria and fungi, and demonstrate that landscape-scales and stochastic moisture input had little impact on community structure.

## Results

Chasmoliths and endoliths occurred exclusively in above-ground sandstone with a mean frequency of 1 and 3%, respectively ($n$ = 100). Hypoliths occurred exclusively on quartz in soil-contact with a mean frequency of 4.9% ($n$ = 1,260) in typical polygons. A single snowmelt-influenced polygon supported a near 5-fold higher frequency of colonization (22%), and there was no significant difference in available quartz substrate between "dry" and snowmelt-influenced polygons. Microscopy of colonized rock surfaces revealed *Chroococcidiopsis*-like cyanobacterial morphotypes dominated sandstone substrates, whereas oscillatorian cyanobacterial morphotypes dominated quartz. Copious extracellular polymeric substance was present around colonized areas.

We used real-time quantitative (q)PCR to estimate the absolute and relative abundance of recoverable phylotypes for archaea, bacteria, and eukarya as a proxy for relative biomass (Table 1). Whereas each lithic niche supported both eukaryal and bacterial phylotypes, soil supported bacterial phylotypes only. Presence of a soil-based inhibitor to eukaryal PCR amplification was discounted after successful recovery of amplicons from artificially "spiked" soil samples. Eukaryal phylotypes in lithic niches accounted for a relatively low abundance (<5%) of total recoverable phylotypes. The hypolithon supported greatest overall abundance of recoverable phylotypes, with values for surrounding soil several orders of magnitude lower.

Variation in multidomain community structure among all colonized rocks and soil samples was assessed using terminal restriction fragment length polymorphism (t-RFLP) (Fig. 1). The relative contribution of each domain-specific t-RFLP profile to overall community diversity/abundance in any given sample was calculated based on relative abundance obtained from qPCR data. Differences between soil and rock substrates were significant (ANOSIM, Global R = 0.719, $P < 0.001$). Multiple rank correlations (BEST analysis) of abiotic (Table S1 and S2) and community diversity/abundance data (t-RFLP) revealed that the combination of factors most important in influencing community diversity in soils were soluble salts, K and C ($\rho_W$ 0.274). Combinations including sodium

Author contributions: S.B.P. and R.L.F. designed research; S.B.P., Y.C., D.C.L., M.C.Y.L., and J.A.J. performed research; S.B.P., Y.C., D.C.L., M.C.Y.L., and R.L.F. analyzed data; and S.B.P. and R.L.F. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. FJ490210-490344 and FJ895042-FJ895089).

See Commentary on page 19749.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0908274106/DCSupplemental.

**Table 1. Diversity statistics and recovery of microbial phyla from soils, sandstone chasmoliths, sandstone endoliths, and quartz hypoliths in McKelvey Valley, McMurdo Dry Valleys, Antarctica**

| | Soil | Hypolith | Chasmolith | Endolith |
|---|---|---|---|---|
| | | Diversity indices | | |
| | | Alpha diversity | | |
| Shannon's index | 3.3 | 2.3 | 2.8 | 2.8 |
| Simpson Diversity Index | 1 | 0.8 | 0.9 | 0.9 |
| Pielou's Evenness | 0.9 | 0.7 | 0.7 | 0.7 |
| | | Omega diversity | | |
| $F_{ST}$ bacteria | 0.299 | 0.305 | 0.301 | 0.301 |
| Fr eukarya | — | 0.105 | 0.083 | 0.083 |
| AvTD | 77 | 68 | 84 | 83 |
| VarTD | 255 | 1293 | 425 | 393 |
| | | qPCR (SSU rRNA copy no.) | | |
| Archaea | 0 | 0 | 0 | 0 |
| Bacteria | $0.06 \times 10^6$ | $55 \times 10^6$ | $0.8 \times 10^6$ | $2.4 \times 10^6$ |
| Eukarya | 0 | $6.4 \times 10^4$ | $1.5 \times 10^4$ | $0.3 \times 10^4$ |
| | | Clone libraries | | |
| | | Bacteria | | |
| No. clones | 180 | 169 | 144 | 172 |
| No. RFLP-defined phylotypes | 72 | 24 | 41 | 43 |
| No. O.T.U. (97% cutoff) | 48 | 11 | 25 | 27 |
| Chao 1 richness | 44.2 | 8.8 | 23.4 | 25.4 |
| Coverage, % | 87 | 95 | 91 | 95 |
| Average similarity to known phylotypes using BLAST | 94 | 97 | 95 | 96 |
| | | Eukarya | | |
| No. clones | — | 58 | 55 | 55 |
| No. RFLP-defined phylotypes | — | 10 | 10 | 8 |
| No. O.T.U. (97% cutoff) | — | 1 | 4 | 5 |
| Chao 1 richness | — | 1.8 | 2.1 | 4.1 |
| Coverage, % | — | 100 | 98 | 98 |
| Average similarity to known phylotypes using BLAST | — | 99 | 99 | 98 |
| | | Phylum abundance, % | | |
| Cyanobacteria | 0 | 95 | 64 | 56 |
| [Chroococcidiopsis sp.] | — | [0] | [57] | [51] |
| [Nostocales] | — | [0] | [1] | [5] |
| [Oscillatoriales] | — | [95] | [0] | [0] |
| [Unidentified cyanobacterium] | — | [0] | [6] | [0] |
| Acidobacteria | 21 | 2 | 2 | 5 |
| Actinobacteria | 33 | 2 | 5 | 4 |
| Bacteriodetes | 2 | 0 | 7 | 16 |
| Chloroflexi | 3 | 0 | 0 | 0 |
| Deinococcus-Thermus | 6 | 0 | 0 | 2 |
| Gemmatimonadetes | 8 | 0 | 0 | 0 |
| Planctomycetes | <1 | 0 | 0 | 2 |
| Alpha proteobacteria | 0 | 1 | 4 | 4 |
| Beta proteobacteria | 4 | 0 | 0 | 0 |
| Gamma proteobacteria | 0 | 0 | 8 | 4 |
| Unidentified bacteria | 13 | 0 | 8 | 2 |
| Ascomycota | 0 | 0 | <1 | <1 |
| Basidiomycota | 0 | 0 | <1 | <1 |
| Chlorophyta | 0 | <1 | 2 | 4 |

and moisture content resulted in significant but weaker correlations. For rock substrates, the soluble salts, organic carbon, and moisture content were below detectable limits, and the most important variables determining community structure among quartz and sandstone were Cl ($\rho w$ 0.794) and Cl, K, and porosity ($\rho w$ 0.688).

We were able to assign putative identification to a relatively high percentage (92%) of t-RFLP peaks. These data revealed some general trends among substrates. Chasmoliths and endoliths were dominated by *Chroococcidiopsis* phylotypes, whereas *Leptolyngbya*-like phylotypes dominated hypoliths. Cyanobacterial signals were absent from all but three soil samples, and comprised a very low fraction (<10%) of overall t-RFLP signal. Soil t-RFLP profiles indicated communities dominated by Acidobacteria, Alphaproteobacteria and Actinobacteria. Chasmoliths and endoliths supported fungal and algal phylotypes, whereas hypoliths supported only algal eukaryotes. No eukaryal signals were recorded for any soils.

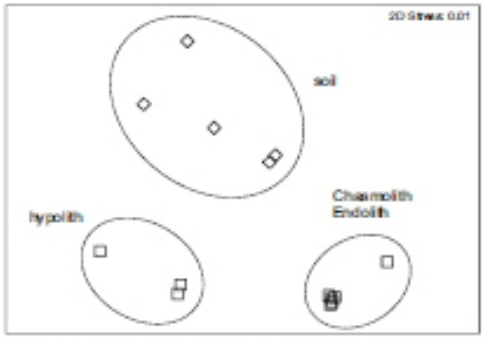


**Fig. 1.** Nonmetric multidimensional scaling plot of Bray Curtis similarities for bacterial and eukaryal rRNA gene phylotypes recovered from soils, sandstone chasmoliths and endoliths, and quartz hypoliths in McKelvey Valley. Dashed line represents statistically significant groupings (ANOSIM, Global R = 0.719, $P < 0.1$, $n = 14$).

To further characterize community structure, we constructed clone libraries based on near full-length 16S and 18S rRNA gene sequences to determine phylogenetic identity for all recoverable phylotypes. Estimates of sampling effort revealed high coverage for bacterial and eukaryal libraries (Table 1). All operational taxonomic units (O.T.U.) were assigned a phylogenetic identity based on near full-length rRNA sequence (Fig. 2; Fig. S1–S3).

Archaea-specific primers failed to generate archaeal sequences, despite attempts with varying PCR stringency and alternative sets of archaea-specific PCR primers. Therefore, it was concluded that there were no recoverable archaeal phylotypes in our Dry Valleys samples.

Interpolation of clone library sequence data with qPCR data allowed an estimate of relative abundance for all phylotypes across all domains in the community (Table 1). The lithic substrates were all dominated by cyanobacteria; for chasmoliths and endoliths, these cyanobacteria comprised diazotrophic *Chroococcidiopsis* and Nostocales phylotypes, whereas for hypoliths, they were exclusively Oscillatoriales (Fig. 2). An additional source of bacterial phototrophy was indicated by *Chloroflexi* in soil and endolithon. Other bacterial phylotypes were phylogenetically diverse, and overall, bacterial diversity spanned 14 phyla (Fig. S1), although in lithic niches, they comprised relatively few of the total recoverable phylotypes. The only ubiquitous phyla in the Dry Valleys terrain were the acidobacteria and actinobacteria, although cyanobacteria were ubiquitous among lithic niches.

The eukaryal phylotypes resolved phylogenetically within the Chlorophyta (Algae), Ascomycota (Fungi), and Basidiomycota (Fungi). Whereas chlorophytes were indicated for all lithic niches, the fungi were present only in endolithic and chasmolithic communities. A relatively restricted phylogenetic diversity was indicated for the chlorophytes with two closely related groups within the Trebouxiophyceae and a single *Bracteococcus* phylotype (Fig. S2). Among the fungi, four genera were indicated by phylotypes affiliated within the Dothideomycetes (Ascomycota), Sordariomycetes (Ascomycota), and Cystobasidiomycetes (Basidiomycota) (Fig. S3). We recovered a single endolithic fungal phylotype (FJ490293) that displayed low phylogenetic affinity with any known taxon. This taxon accounted for ~10% of recoverable eukaryal phylotypes, and

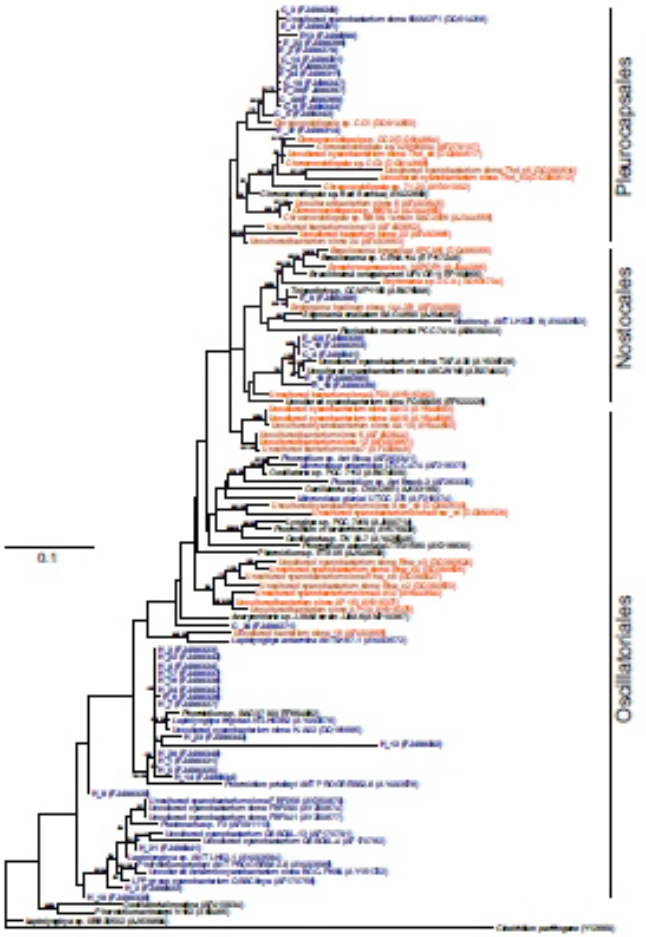


**Fig. 2.** Phylogenetic relationships among cyanobacterial 16S rRNA phylotypes recovered from sandstone chasmoliths and endoliths and quartz hypoliths in McKelvey Valley. Phylotypes recovered during this study are shown in bold type. Sequence code prefix denotes location. H, hypolith; C, chasmolith; E, endolith. Blue font denotes Antarctic and glacial phylotypes, orange font denotes phylotypes from nonpolar deserts, black font denotes nondesert phylotypes. Tree topologies are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1,000 replications (second number). (Scale bar, 0.1 nt changes per position.)

so may represent a previously uncharacterized lichen mycobiont or free-living fungus. Similarly unidentified bacteria with little phylogenetic affiliation to any known phylum accounted for 2–13% of phylotypes and were most common in soil.

Our statistical analyses included approaches designed to assess both alpha diversity (number of taxa) and omega diversity (phylogenetic diversity) (Table 1). On the basis of Shannon and Simpson, estimates soils appeared most biodiverse; however, this estimate is misleading, because soils supported a relatively large number of closely related acidobacterial and actinobacterial phylotypes. Estimates of evenness indicated that soil communities were markedly more even in terms of taxon contribution to overall abundance than any lithic community. We exploited the sequence-based nature of our data to estimate phylogenetic diversity in the different communities. The $F_{ST}$ statistic was used to show that bacterial assemblages in soil, hypoliths, chasmoliths, and endoliths were significantly different from each other ($F_{ST} = 0.302$, $P < 0.00001$; AMOVA). Eukaryal assemblages in sandstone and quartz were also significantly different ($F_{ST} = 0.088$, $P < 0.00001$; AMOVA), although chasmolith and endolith eukaryal assemblages were not significantly different. Overall, community was significantly different at the phylogenetic level between each niche. Taxonomic distinctness (TD) estimates were used to illustrate the phylogenetic basis of community composition. Average (Av) TD reflects the frequency of phylogenetically defined species, and here, was used to show that soil and hypoliths were significantly less diverse than

chasmoliths and endoliths. Variation (Var) in TD reflects the level of phylogenetic species differentiation within a community, and clearly showed that soils were depauperate compared with lithic niches.

To address the question of how variable lithic communities are on a landscape scale in the Dry Valleys, we selected hypolithic colonization as an indicator, because this colonization occurred most frequently at all locations and there was no significant difference in colonization frequency for typical polygons within or between locations ($P > 0.05$). Because bacterial phylotypes accounted for >98% of recoverable phylotypes, we assessed variation in bacterial community t-RFLP profiles for the hypervariable 16S-23S ITS region ($n = 61$). No significant difference in community structure within or between locations could be delineated based on this highly variable marker (Fig. S4). The inclusion of samples in a polygon atypically experiencing moisture sufficiency (and significantly greater colonization frequency) due to localized snowmelt revealed that, although 14% of rocks displayed apparent differences in diversity/abundance profiles, this pattern was not significantly different from overall community structure on a landscape scale (Fig. S4).

## Discussion

Our study represents a complete assessment of terrestrial microbial biodiversity across surface niches at the cold-arid limit for life, and provides insights into ecosystem complexity under extreme stress. These highly-specialized communities face unpredictable effects from climate change. They may be out-competed by invasive species should warming occur, or encounter catastrophic ecosystem shift (18) should aridity increase with further cooling. Given that current global climate-warming trends are most pronounced in polar regions (19) but that increased localized cooling in the Dry Valleys region may also be occurring (5), it is timely to document this endangered biome.

We identified four distinct microbial communities as chasmoliths, endoliths, hypoliths, and in bulk soil that occurred concomitantly in polygonal terrain of McKelvey Valley. Lithic communities were dominated by different cyanobacteria, and overall, diversity spanned 16 phyla. This level of diversity suggests a greater ecosystem complexity in the high inland Dry Valleys than previously appreciated. Our data counters the view based on soil studies that cyanobacteria are restricted to wetter, more productive polar locations (16, 20); rather, we demonstrate that, under severe xeric stress, soil becomes too extreme, and the last refuge for life is in lithic niches where distinct communities develop.

The soil biota of McKelvey Valley was dominated by known radiation and desiccation tolerant taxa such as *Deinococcus* and *Rubrobacter*, and such adaptation has also been observed for moisture-impacted Dry Valley soils (13, 16). Nonpolar hyperarid soils in the Atacama Desert were instead dominated by *Frankia*-like actinobacteria at depths of 20–200 mm (21). The relatively low number of microorganisms and taxonomic evenness of the community, plus absence of primary producers, indicate that soil "communities" in McKelvey Valley may possibly represent a transient soil-bourne inoculum rather than a stable community. Conversely, the specific occurrence of Chloroflexi in soils, a phylum known to be important in tundra soils (22), suggests a true soil community may indeed exist. We did not investigate whether the deeper subsurface permafrost supported microorganisms.

The absence of *Chroococcidiopsis* in molecular and morphological examinations from hypolithon and most soils is surprising given that this cyanobacterium was the dominant taxon in sandstone substrates in our study and has been recorded as the main component of hypolithic communities in nonpolar deserts (23–25). A study focusing on cyanobacteria in soils of Beacon Valley also failed to generate amplicons using cyanobacteria-specific PCR primers, whereas they were commonly retrieved for soils of lower valleys supporting lakes with a presumed aquatic origin for phylotypes (11). The absence is unlikely to be a result of UV or desiccation stress, because it is one of the most radiation- and desiccation-tolerant organisms known (26). Rather, we suggest, in light of the fact that soils were also the only substrate that did not support algae, that this inland soil presents an environment that somehow precludes significant photoautotrophic colonization. This finding has major implications for productivity in such inland valleys, because it makes the isolated "islands" of lithic colonization the only significant source of primary production. The reasons for this absence are unclear. We did not identify levels of any soil abiotic variable likely to inhibit photosynthesis per se. We suggest that, in addition to increased exposure to incident light, highly xeric conditions, and lack of thermal buffering in soils, the relative instability of the soil substrate may also hinder colonization given the slow colonization rates for Antarctic microorganisms (27). Therefore, an upward revision of standing biomass and productivity in the Dry Valleys is warranted, because previous estimates have been based largely on soil and aquatic biota (2).

It has been previously shown that Antarctic lakes are a significant source of inoculum in lower lake-bearing valleys, with soils, hypoliths, and aquatic niches supporting the same cyanobacteria (11). There may also be an aquatic origin for oscillatorian hypoliths in our study, because they are common aquatic Antarctic cyanobacteria, although there are no lakes in the immediate vicinity of the study site (the closest, Lake Vashka, is >10 km distant). This finding suggests that lake dispersal reaches deep inland to McKelvey Valley, so why are they not also colonists of soil and sandstone niches? This absence may be explained by viewing these cyanobacteria as opportunists. They were absent from soil and sandstone, yet occurred on quartz in soil where entrapment of wind-dispersed lake-derived organic matter containing cyanobacterial and algal inoculum can be envisaged, and the microenvironment is known to be more favorable than surrounding soil (24, 28). Therefore, relatively rapid colonization under favorable conditions could occur, whereas for sandstone ingress on an exposed substrate and the microclimate may be too challenging (29).

The lichens recorded in our study displayed little similarity in terms of community diversity with sandstone endoliths from Beacon sandstone recovered 24 years ago (30). Both mycobiont and phycobiont phylotypes in our study affiliated with different genera as did cyanobacteria. This identification may indicate the existence of multiple lichen associations in the Dry Valleys; or more speculatively, our study may indicate Antarctic lichens reflect climatic warming trends and are becoming less unique, because the morphology and cyanobacterial composition of our lichens more closely resembled those recorded for alpine regions of Europe (31).

Nitrogen fixation has been demonstrated by *Chroococcidiopsis*-dominated communities from alpine endoliths of gypsum (32), whereas others conclude that polar and other endoliths largely used abiotic combined nitrogen sources (27). Data also indicated phylotypes indicating diazotrophic cyanobacterial, alpha proteobacterial and actinobacterial taxa. This can be viewed as a useful adaptation in this nitrogen-poor Dry Valleys location. All niches in McKelvey Valley supported a putative heterotrophic bacterial component presumably supported by microbial carbon and nitrogen input.

The lack of significant community variation among samples for a given substrate and between locations reflects the high selective pressure in these high inland sites where environmental stresses are exacerbated compared with lower valleys (6). It also illustrates that our study may be broadly applicable to the inland Dry Valleys in general due to reduced heterogeneity. Across all substrates, salinity-related factors were influential to community structure. It has been suggested that salinity may act as a stressor in certain dry valleys (33), although in our study, soils did not display inhibitory levels of soluble salts. Our multivariate analysis points to a complex interaction of salinity, carbon, moisture, and other variables in this ecosystem.

We used multiple approaches to attempt recovery of archaeal signatures from soils, quartz and sandstone, but all proved unsuccessful. It has been suggested that archaea are unable to tolerate the environmental stress in extreme xeric environments (34), and this inability may explain their absence. Other Dry Valleys studies have not generally focused on archaea, although they were also concluded to be absent from an endolith recovered from the Asgard Range (30). Given the high degree of environmental heterogeneity observed in Antarctic soils (2), their occurrence may yet be recorded elsewhere in the Dry Valleys biome where less moisture stress is experienced.

It has been estimated that polar endoliths are exceptionally persistent over geological time periods (35). Endoliths supported the greatest diversity of phylotypes, shared the greatest number of phylotypes with other niches, and they are very long-lived. Therefore, we propose that they act as a reservoir for terrestrial microbiota. This notion is supported by recent isotopic evidence indicating that some organic matter in Dry Valleys soils remote from sources of liquid water has an endolithic origin (36). Weathering of sandstone is accelerated by endolithic colonization (10), and therefore, can be envisaged to disperse endolithic taxa, although in a relatively slow manner. The relatively rare occurrence of endoliths may restrict the volume of inoculum released, but this dispersal may nonetheless represent an important source over time in high inland valleys where hydroterrestrial taxa may also be dispersed but are unable to proliferate. The occurrence of algae from the usually lichenized endolithic Trebouxiophyceae in McKelvey Valley hypolithon without a mycobiont may further indicate local dispersal of endolithic taxa.

Recovery of cyanobacterial phylotypes, including first records for these substrates, allowed comparison with several other deserts worldwide due to ubiquity of this phylum in desert lithic niches. A clear separation among phylogenetic lineages from Antarctic and nonpolar desert locations within the Pleurocapsales, Nostocales, and Oscillatoriales was evident. This pattern may indicate isolated and regionally seeded cyanobacterial populations occur. This idea supports the view that global occurrence among terrestrial soil bacteria is determined primarily by localized factors (37, 38), although the Antarctic is exposed to globally dispersed aerosols that likely disseminate microbial propagules in a near-ubiquitous manner (39). We suggest that extreme xeric low nutrient environments such as deserts minimize the possible interference of abiotic variables in biogeographic studies, and thus, lithic communities offer a useful model for further testing hypotheses related to biogeography in microbial ecology.

We recovered a limited number of bacterial and fungal phylotypes with low phylogenetic affiliation to any known taxon from other deserts or substrates worldwide. This observation indicates novel diversity at a high taxonomic level in inland Dry Valleys despite the environmental stresses exceeding maritime and lake-influenced Antarctic locations where similar claims for fungi (17) and cyanobacteria (11) have been made. Some records also point to a physiological plasticity among Antarctic taxa, for example, we identified basidiomycetous yeasts in endolithic niches yet these taxa also occurred as colonists of archaeological wood in the Antarctic (17).

We have characterized highly-specialized communities inhabiting multiple lithic and soil niches in the inland region of the most extreme cold desert on Earth. This diversity is in contrast to apparently more homogenous communities in the lower, wetter Dry Valleys. The occurrence of location-specific cyanobacterial lineages may be a result of lithic niches acting as local reservoirs for dispersal of microbial biomass. The specialized communities and possible endemism for certain phyla, together with climate-change related threats, emphasize the conservation value of the inland Dry Valleys ecosystem. Ironically, the frequency of hypolithic colonization may have potential as a bioindicator of climate change given that a comparison of colonization frequency in this study with maritime polar (40) and nonpolar deserts (24, 41) suggests that landscape-scale patterns may be closely related to climatic variables. Additional relevance lies with environmental similarities between polar regions on Earth and Mars during recent history and the implications for habitability of Mars (42).

## Materials and Methods

**Field Sampling.** The high inland McKelvey Valley (central valley coordinates 77°26′ S, 161°33′ E) was surveyed during Antarctica New Zealand event K02118 in January 2008. Frost polygons of 40 $m^2$ average area were used as in situ quadrats. All sandstone and quartz substrate was surveyed, and frequency of colonization for chasmoliths, endoliths, and hypoliths was recorded. Epilithic lichens were not observed. Soil samples were taken after removing the topmost 2.5 cm of loose soil to minimize transient particles in sampling and be consistent with the average depth of hypolithic colonization. A total of 10 randomly-selected and nonadjacent polygons were surveyed (five on a south-facing slope used in rRNA and ITS studies, plus five additional polygons from a north-facing slope for ITS studies). A single polygon from the southern slope that supported multiple chasmolithic, edolithic, and hypolithic colonization was selected for diversity comparison between niches. Soil and rock samples were sampled aseptically and stored in sterilized plastic containers with no headspace at −80 °C until processed.

**Abiotic Variables.** A suite of 18 abiotic variables, including moisture content, porosity, pH, soluble salts, total organic carbon, total nitrogen, and metals, were measured for each substrate. Ambient temperatures remained below freezing throughout the sampling period in January 2008 although solar heating of ground created isolated patches of snowmelt. Long-term climate data are available at the following link: www.scar.org. Moisture content and total organic content in soils and rocks were measured gravimetrically after heating to 100 and 450 °C, respectively. Rock porosity was measured by vacuum displacement. Soluble salts and pH were measured by potentiometric determination. Total carbon and nitrogen were determined using a thermal conductivity detector at 900 °C. All elemental tests were conducted after air-drying and nitric/hydrochloric acid digestion using ICP-MS according to the Environmental Protection Agency 200.2 for soils or using the EDX elemental scanning function during scanning electron microscopy of rock surfaces.

**Recovery of Environmental DNA and Target Loci.** Recovery of environmental DNA used a protocol optimized for lithic microorganisms (24). PCR amplification of rRNA genes was carried out using domain-specific forward primers for bacteria (43), eukarya (44), and archaea (22), and universal reverse primers (43, 45). Alternative sets of archaea-specific primers were also tested (46). The ITS region was amplified using rRNA gene-specific primers from flanking regions (47, 48).

**Real-Time Quantitative PCR.** PCR amplification was quantified in real-time (Prism 7000; Applied Biosystems) by flourometric monitoring with SYBR Green 1 dye (Invitrogen). All standard curves were constructed using plasmids from cloned rRNA genes (Qiagen) separately for archaea, bacteria, and eukarya.

**Terminal RFLP.** Restriction digests (MspI for 16S/18S rRNA, HaeIII for ITS) of FAM-labeled PCR amplicons were subjected to fragment analysis by capillary electrophoresis (3730 Genetic Analyzer; Applied Biosystems). The software Peri and R were used to identify true peaks and bin fragments of similar size (49). A virtual digest using HaeIII and MspI was carried out on the sequences retrieved from the bacterial and eukaryal clone libraries. This analysis allowed the assignment of phylogenetic identity to individual peaks.

**Clone Library Construction and Sequencing.** Samples were selected for clone library construction (PCR Cloning$^{plus}$ kit; Qiagen) based on those with t-RFLP profiles most similar to other samples for a given substrate. Transformants were screened using RFLP (MspI, HaeIII, and CfoI) before automated sequencing (3730 Genetic Analyzer; Applied Biosystems). Phylotypes were delineated on the basis of 97% sequence similarity using the freeware DOTUR (50). All sequences generated by this study have been deposited in the National Center for Biotechnology Information GenBank database under accession numbers FJ490210-490344 and FJ895042-FJ895089. Screening for possible chimeric sequences was made using Chimera.Check (http://rdp.cme.msu.edu). Approximate phylogentic affiliations were then determined by BLAST searches of the National Center for Biotechnology Information GenBank database (http://www.ncbi.nlm.nih.gov). Estimates of clone library sampling effort were made using the freeware EstimateS (51). Sampling effort was assessed by calculation of Coverage and Rarefaction curves, estimates of library richness were made using the nonparametric estimation ACE and Chao 1.

**Phylogenetic Analyses.** Multiple alignments were created with reference to selected GenBank sequences using BioEdit v7.0.9.0 (52). The alignments were tested against prescript models of evolution using the softwares PAUP* 4.0b10 (53) and Modeltest v3.0 (54). The criteria described by the most appropriate evolutionary model were input for maximum likelihood analysis using Genetic Algorithm for Rapid Likelihood Inference (GARLI) Version 0.96 Beta (55). Robustness of furcated branches was supported by both bootstrap values (1,000 replicates) determined using PAUP* 4.0b10 and Bayesian posterior probabilities (56) calculated using Bayes v3.0b4 (57). Values (in percentage) were shown on all branch nodes supported by >50% of the trees.

**Statistical Analyses.** Alpha diversity indices (Shannon's Index, Simpsons Diversity Index, and Pielou's Evenness) were calculated using untransformed data. Phylogenetic data were used to calculate the $\Phi_{ST}$ statistic, AvTD and VarTD. Quantification of the degree of phylogenetic differentiation between communities was expressed by the $\Phi_{ST}$ statistic (58) using the software Arlequin v3.0 (59). TD indices were calculated to reflect phylogenetic diversity within populations (60). Differences stated as significant were tested using one-way and two-way ANOVA, AMOVA, or analysis of similarity (ANOSIM). Multivariate analysis of diversity data were performed on square-root transformed diversity data, and on nontransformed normalized data for environmental variables. Nonmetric multidimensional scaling ordinations (NMDS) were used to visualize Bray Curtis Similarities (diversity data) and Euclidean Distances (environmental data). In BEST analyses, the BIO-ENV procedure was used to maximize the rank correlation between biotic and environmental data; thereby, establishing a ranking ($\rho w$) for the effects of environmental variables on diversity. All analyses were performed using Primerv6.1.6 (61). All results stated as significant have a confidence level of $P < 0.05$ unless stated otherwise.

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# Correction

**MICROBIOLOGY**

Correction for "Highly specialized microbial diversity in hyper-arid polar desert," by Stephen B. Pointing, Yuki Chan, Donnabella C. Lacap, Maggie C. Y. Lau, Joel A. Jurgens, and Roberta L. Farrell, which appeared in issue 47, November 24, 2009, of *Proc Natl Acad Sci USA* (106:19964–19969; first published October 22, 2009; 10.1073/pnas.0908274106).

The authors note that, on page 19964, left column, first paragraph, last sentence, "This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 20)" should instead read as "This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 64)." This error does not affect the conclusions of the article.

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CORRECTION

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# Screening fungi isolated from historic *Discovery* Hut on Ross Island, Antarctica for cellulose degradation

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**Abstract:** To survive in Antarctica, early explorers of Antarctica's Heroic Age erected wooden buildings and brought in large quantities of supplies. The introduction of wood and other organic materials may have provided new nutrient sources for fungi that were indigenous to Antarctica or were brought in with the materials. From 30 samples taken from *Discovery* Hut, 156 filamentous fungi were isolated on selective media. Of these, 108 were screened for hydrolytic activity on carboxymethyl cellulose, of which 29 demonstrated activities. Endo-1, 4-β-glucanase activity was confirmed in the extracellular supernatant from seven isolates when grown at 4°C, and also when they were grown at 15°C. *Cladosporium oxysporum* and *Geomyces* sp. were shown to grow on a variety of synthetic cellulose substrates and to use cellulose as a nutrient source at temperate and cold temperatures. The research findings from the present study demonstrate that Antarctic filamentous fungi isolated from a variety of substrates (wood, straw, and food stuffs) are capable of cellulose degradation and can grow well at low temperatures.

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**Key words:** cellulolytic, endo-1, 4-β-glucanase, microfungi, psychrotolerant

## Introduction

In 1902, *Discovery* Hut was erected at Hut Point, Ross Island, Antarctica, by the National Antarctic Expedition led by Robert F. Scott. This wooden building was the first structure to be built on Ross Island and was to serve as a shelter, a workshop and to store supplies. Due to the design of the hut it was too cold to house the men. When the expedition members left the continent, the hut and supplies were abandoned. The hut was also used extensively by four other expeditions in the Heroic Age, both as a key stepping stone to the southern latitudes and as shelter for those returning from the south. After use by Shackleton's 1914–1917 Imperial Trans-Antarctic expedition, the hut was abandoned until the late 1940s and visited only periodically until 1957 when restoration work began. In the past 20 years, the hut and the surrounding area has attracted increasing numbers of tourists from cruise ships and scientists from the nearby research facilities, Scott Base and McMurdo Station. Of the three historic huts, on Ross Island, *Discovery* Hut has been the most affected by human impact due both to being close to the research facilities and to the increasing numbers of visitors.

There are many reports of fungi isolated from Antarctic soils, some described as endemic or indigenous to the continent while many were reported to be introduced (Vishniac 1996). Onofri *et al.* (2004) reported that in Antarctica 0.6% of the known fungal species are water moulds (kingdom *Chromista*) and 99.4% are composed of true fungi including yeasts and filamentous fungi from the phyla *Chytidiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota*. Onofri *et al.* stated that most Antarctic filamentous fungi are cold tolerant mesophiles or psychrotrophs rather than psychrophiles. Psychrotrophic fungi (organisms capable of growth at around 0°C as well as grow above 20°C (Cavicchioli *et al.* 2002)) have been previously isolated from Antarctica and have been defined as strains of filamentous mesophilic fungi adapted to grow at temperatures of 1°C (Kerry 1990a, Abyzoz 1993, Azmi & Seppelt 1997).

Much of the previous work on fungi found in association with the Ross Island 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). Recently Held *et al.* (2005) reported on the presence of large fungal blooms within Scott's *Terra Nova* Hut at Cape Evans. Investigations of biological and non-biological causes of deterioration in the historic huts of Antarctica produced evidence of decay fungi associated with exterior wood in contact with the ground, including several previously undescribed *Cadophora* species, (based on the recent taxonomic revision of some of the *Phialophora*-like fungi (Harrington & McNew 2003)). Blanchette *et al.* (2004) suggested, on the basis of their molecular evidence, that some of these fungi are

indigenous species to Antarctica. Along with providing a nutrient source for the fungi, the hut creates a microenvironment with conditions suitable for fungal growth during the summer. Fungi found in *Discovery* Hut appeared well adapted to cold temperatures, and environmental monitoring within the hut indicated temperatures ranging from a maximum of 6.6°C to a minimum of -39°C (Held *et al.* 2005).

Many micro-organisms 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), one of the components of the cellulase enzyme complex catalyses randomly the hydrolysis of the β 1,4-glucosidic linkage.

Cellulose decomposition in the Antarctic region has been studied by Smith (1981) and Walton (1985) at South Georgia, Pugh & Allsopp (1982) at Signy Island and Yamamoto *et al.* (1991) at Syowa station and Langhovde hut, Antarctica. All concluded that cellulolytic fungi were present in the Antarctic region and that cellulose decomposition was occurring but at a slower rate when compared with more temperate environments. Antarctic fungi have been evaluated for extracellular enzyme activity including cellulases by Hurst *et al.* (1983), seven of eight fungi isolated from this study (*Acremonium terricola*, *Botrytis cinerea*, 2 *Chaetophoma* sp., *Chrysosporium pannorum*, *Cladosporium sphaerospermum*, and *Fusarium lateritium*) were grown on and were able to cause clearing of cellulose agar plates at 20°C. In addition they reported cellulase activity at 1°C for *B. cinerea*, *C. pannorum*, *Chaetophoma*, and *C. sphaerospermum*. Fenice *et al.* (1997) reported rather low cellulase activity in 12 of 33 fungal strains tested and Bradner *et al.* (1999) reported testing for cellulase activity along with other hemicellulolytic activity but did not present results for cellulolytic activity.

This investigation focused on fungi isolated from cellulose containing samples (wood, straw and food stuffs) from *Discovery* Hut. It was proposed that isolated fungi would be able to grow at temperatures typical of the summer months and that they would be able to degrade cellulose. The isolated fungi were screened for the ability to degrade carboxymethyl cellulose, production of the related enzyme endo-1, 4-β-glucanase (EC 3.2.1.4), and their ability to use a variety of synthetic celluloses as a nutrient source to determine their ability to degrade cellulose.

## Materials and methods

### *Sample collection*

In January 1999, December 1999 and December 2000 samples of wood, artefacts and organic material were collected from *Discovery* Hut, Hut Point, Ross Island, Antarctica (77°50'50"S, 166°38'30"E). Minute segments of structural wood, foodstuffs (straw, meat, molasses, biscuits and oats) and organic materials were aseptically collected using tweezers and scalpels from inconspicuous locations throughout the hut. Swab samples were taken, using a sterile cotton swab saturated with sterile distilled water from the more conspicuous areas. Scrapings were taken from surfaces with visible fungal growth or an accumulation of organic material was seen. All samples were taken under the New Zealand Ministry of Agriculture and Fishery permit numbers 1999006429 and 2000010576. Samples were placed in sterile vials and kept between 0–5°C while in Antarctica and during transit to New Zealand. Samples were then stored under sterile conditions at 4°C until isolations were made.

### *Isolation of fungi*

Fungi from the samples taken in January 1999 and December 1999 were isolated on the following media:

YM agar (yeast extract 0.2%, malt extract 1.5%, agar 1.8%),

Medium 4 (yeast extract 0.2%, malt extract 1.5%, agar 1.8%, chloramphenicol 0.2 g $l^{-1}$, streptomycin sulphate 0.1 g $l^{-1}$) for isolation of streptomycin resistant fungi (Harrington 1981),

Medium 6 (yeast extract 0.2%, malt extract 1.5%, agar 1.8%, chloramphenicol 0.2 g $l^{-1}$, streptomycin sulphate 0.1 g $l^{-1}$, cycloheximide 0.4 g $l^{-1}$) for isolation of cycloheximide resistant fungi (Harrington 1981),

Medium 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 sulphate 0.1 g $l^{-1}$, lactic acid 2 ml) for selection of Basidiomycetes, and

Vogel Bonner minimal medium (-25% glucose, 2.0% agar, 20 ml VB concentrate/litre (50% $K_2HPO_4$ (anhydrous), 17.5% $NaNH_4PO_4 \cdot 4H_2O$, 10% citric acid $\cdot H_2O$, 1% $MgSO_4 \cdot 7H_2O$ in 670 mls distilled water) for the selection of slow growing fungi (Vogel & Bonner 1956).

For fungal isolations wood samples were surface sterilized by soaking for one minute in a 5% hypochlorite solution, followed by two rinses in sterile, distilled water, sliced and cultured on a variety of enriched and semi-selective media prepared as agar plates. Organic material samples were cut with a sterile scalpel and placed onto culture media; swab samples were wiped over the surface of the media; wood scraping samples were aseptically placed onto the media. The agar plates were then incubated at 4°C, 15°C or 25°C for up to six weeks. Organisms growing on the agar plates were transferred by subculturing from hyphal tips, colonies or spores to fresh YM agar plates.

Fungi from the samples taken in December 2000 were isolated by the following method: a small amount of each sample (~1 g) was transferred into YM broth (yeast extract

**Table I.** Fungi isolated from samples taken from *Discovery* Hut and cultured at 4°C, 15°C and 25° on YM agar, Media 6, Media 4, Media 7, and Vogel Bonner medium.

| Sample location | Sample type | No. of fungi isolated | 4°C | 15°C | 25°C | YM | Media 6 | Media 4 | Media 7 | VB |
|---|---|---|---|---|---|---|---|---|---|---|
| Outside NE corner post of veranda | wood | 16 | + | + | + | + | + | + | + | + |
| Entrance way | straw | 9 | + | + | + | + | NT | + | + | NT |
| Behind pick axe, exterior sample | straw | 11 | + | + | + | + | + | + | - | + |
| Behind pick axe interior (1 cm) sample | straw | 12 | + | + | + | + | + | + | -; | + |
| Ceiling in porch above straw | wood | 7 | + | + | + | + | - | + | + | - |
| Wall above straw | swab | 0 | - | - | - | - | NT | - | - | NT |
| Mutton carcass exterior | food | 2 | - | - | + | + | - | - | + | - |
| Mutton carcass meat internal | food | 1 | - | - | + | + | - | - | - | - |
| Wall behind mutton carcass | swab | 2 | + | + | - | + | - | + | - | - |
| Wood from wall behind carcass | other | 1 | - | - | + | + | - | - | - | - |
| Floor hole in meat room | straw | 14 | + | + | + | + | + | + | + | - |
| Mould on latrine room ceiling | swab | 0 | - | - | - | - | - | - | - | - |
| Salt deposit upper window next to pendulum | wood | 2 | - | - | + | + | - | - | - | - |
| Wall behind door pendulum room | swab | 0 | - | - | - | - | - | - | - | - |
| Material from top of the pendulum area wood | other | 11 | + | + | + | + | + | + | - | + |
| Compacted muck pendulum room floor | other | 6 | + | + | + | + | + | + | + | - |
| Oats from bag, artefact number HC/4.2 | food | 4 | - | + | + | + | - | + | - | + |
| Bottom of bag on bed west of stove | swab | 1 | - | + | - | - | NT | - | - | NT |
| Old broom | swab | 4 | + | + | - | + | NT | + | + | NT |
| Straw broom in entrance | swab | 9 | + | + | + | + | NT | + | + | NT |
| From under blubber in hut | swab | 1 | + | - | - | - | NT | + | + | NT |
| From under blubber in hut | wood | 2 | + | - | - | + | NT | - | - | NT |
| Ceiling in entrance | swab | 0 | - | - | - | - | NT | - | - | NT |
| Straw underneath blubber | straw | 10 | NT | + | + | + | NT | + | - | NT |
| Straw across from door way | straw | 8 | NT | + | + | + | NT | - | - | NT |
| Fungal colony exterior western wall | scrapping | 7 | NT | + | - | + | NT | - | - | NT |
| Fungal colony on floor | scrapping | 3 | NT | + | - | + | NT | - | - | NT |
| Molasses on floor near the stove | food | 4 | NT | + | + | + | NT | - | + | NT |
| Mutton | food | 4 | NT | + | + | + | NT | - | + | NT |
| Biscuit from box in main room | food | 5 | NT | + | + | + | NT | - | + | NT |
| Total number of fungi isolated | | 156 | | | | | | | | |

Note + = fungi isolated from the sample, - = no fungi isolated from the sample, NT means not tested.
Footnote: YM agar (YM), general purpose agar; Media 4, for isolation of streptomycin resistant fungi; Media 6, for isolation of cycloheximide resistant fungi; Media 7 for selection of Basidiomycetes; and Vogel Bonner (VB) minimal medium for the selection of slow growing fungi.

0.2%, malt extract 1.5%). These were incubated with horizontal shaking movement at three temperatures; 4°C, 15°C, and 25°C for one week before being plated out onto YM agar, Medium 7, and Medium 4. For four weeks, all plates were observed daily to sub-culture filamentous fungi. Sub-cultures were isolated on the same media as their parental plates. All isolates were sub-cultured until colonies of uniform physical appearance were obtained.

### *Identification of fungi*

Fungi were identified into putative species using classical taxonomy based on morphology (Barrnett & Hunter 1972, Sun *et al.* 1978).

Molecular characterizations, particularly DNA sequence analyses of the two internal transcribed spacer regions of ribosomal DNA, ITS1 and ITS2, were used to confirm identity of the two species used for the radial hyphal extension rate on various media and selected cellulose carbon sources experiments. Fungal material was scraped from pure cultures and DNA extracted using Qiagen DNeasy plant mini-kits, following manufacturer's instructions (Qiagen Sciences Inc, Germantown, MA). The rDNA internal transcribed spacer (ITS) regions 1, 5.8S, and ITS region 2 were amplified using primers ITS1 and ITS4 (Gardes & Bruns 1993). PCR amplification was done 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 to those in GenBank using BLASTn to find the best match.

### *Detection of carboxymethyl cellulose activity and analysis of endoglucanase activity*

Fungi were screened for cellulase activity using a congo red agarose plate technique (Duncan *et al.* 2006), using plates

consisting of *Trichoderma viride* medium A (Mandels *et al.* 1962) (14 ml $(NH4)_2$ $SO_4$ 10%, 15 ml $KH_2PO_4$ 1 M, 6 ml urea 35%, 3 ml $CaCl_2$ 10%, 3 ml $MgSO_4 \cdot 7$ $H_2O$ 10%, 1 ml trace elements solution (10 ml concentrated HCl, $FeSO_4$ 0.51%, $MnSO_4 \cdot 4H_2O$ 0.186%, $ZnCl_2$ 0.166%, $CoCl_2$ 0.2%), 2 ml Tween 80, carboxymethyl cellulose 0.2%, agarose 1.5%). The Index of Relative Enzyme Activity (RA) (which compares the width of the clearing zone with the width of fungal growth) determined which fungi were producing cellulase (Bradner *et al.* 1999). For the screening it was determined that a RA value 1 or greater was significant carboxymethyl cellulase activity.

Endoglucanase activity was determined using methods described by Duncan *et al.* 2006. Fungi were grown on YM agar for one week at 15°C or four 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%, $CaCl_2$ $2H_2O$ 0.006%, $MgSO_4$ $7H_2O$ 0.006%, Tween 80 0.02% (v/v)) in a 250 ml flask. Flasks were shaken at 150 rpm and after 10 days for fungi grown at 15°C and 28 days for fungi grown at 4°C, the fungal cells were separated from the culture supernatant by centrifugation. Endo-1, 4-β-glucanase enzyme activity was determined from the culture supernatant (Bailey *et al.* 1992). The enzyme supernatants (320 µl) were mixed with 480 µl of substrate solution containing 1% hydroxyethyl cellulose in 0.05 M citrate buffer pH 4.8 (0.05 M citric acid, 0.05 M sodium citrate). After 10 min incubation at 50°C (demonstrated to be the temperature for optimal enzyme activity under these conditions (data not shown)), the reaction was stopped with the addition of 1.2 ml 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 subsequent boiling in a water bath for 5 min. The absorbance was measured spectrophotometrically at 540 nm 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. Activity was expressed as micromoles glucose released per minute and converted to specific activity by dividing by the total protein in the supernatant. The total protein measurements of the supernatant were carried out by the Bradford method using a protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin as the standard.

*Radial hyphal extension rate on various media and selected cellulose carbon sources*

Fungi were tested for their ability to grow on selected cellulose carbon sources using an agarose plate technique.

**Table II.** Screening fungi from the *Discovery* Hut for carboxymethyl cellulase activity: Index of Relative Enzyme activity at isolation temperature and at 4°C.

| Isolate # | Sample type | Isolation temperature (°C) | Index of relative enzyme activity Performed at isolation temperature | Performed at 4°C |
|---|---|---|---|---|
| 71 | food | 25 | 1.0 | NG |
| 72 | wood | 25 | 1.25 | NG |
| 129* | wood | 25 | 0 | 1.91 |
| 225 | wood | 15 | 0.5 | NG |
| 226* | wood | 15 | 0.71 | 1.25 |
| 227* | wood | 15 | 0.65 | 0.5 |
| 228* | wood | 15 | 1.5 | 1.68 |
| 229 | wood | 15 | 0.27 | 0.28 |
| 230 | wood | 15 | 0.71 | 0.37 |
| 612 | wood | 25 | 1.5 | NG |
| 707 | wood | 4 | 0.33 | 0.33 |
| 728 | wood | 4 | 0.2 | 0.2 |
| 771 | wood | 4 | 0.28 | 0.28 |
| 804 | wood | 4 | 0.22 | 0.22 |
| 805* | wood | 4 | 0 | 0 |
| 806 | wood | 4 | 0.59 | 0.59 |
| 816* | wood | 4 | 0 | 0 |
| 823 | wood | 4 | 0.28 | 0.28 |
| 824* | wood | 4 | 1.17 | 1.17 |
| 826 | wood | 4 | 0.22 | 0.22 |
| 1278 | wood | 4 | 0.28 | 0.28 |
| 2011 | straw | 15 | 0 | NG |
| 2013 | straw | 15 | 3.21 | 0.89 |
| 2020 | straw | 15 | 2.24 | 1.93 |
| 2026 | food | 15 | 4.0 | NG |
| 2028 | fungi | 15 | 0 | 0 |
| 2032 | fungi | 15 | 0.73 | 0 |
| 2033 | fungi | 15 | 1.28 | 0 |
| 2042 | fungi | 15 | 5.0 | 0 |
| 2044 | fungi | 15 | 1.53 | 1.62 |
| 2045 | straw | 15 | 1.09 | 0 |
| 2046 | straw | 15 | 3.0 | NG |
| 2054 | straw | 15 | 0 | 0 |
| 2099 | fungi | 15 | 11.0 | 2.25 |
| 2103 | fungi | 15 | 0 | 0 |
| 2111 | fungi | 15 | 0 | 1.13 |
| 2113 | food | 15 | 0 | NG |
| 2114 | food | 15 | 3.21 | 5.75 |
| 2117 | food | 15 | 2.5 | NG |

* Isolates used in further studies. NG = No growth

All the cellulose sources were synthetic; Avicel is non-soluble and relatively crystalline while both carboxymethyl cellulose and hydroxyethyl cellulose are both soluble. Plates consisted of *Trichoderma viride* medium A (Mandels *et al.* 1962). *Trichoderma viride* medium A plates with no cellulose carbon source and YM agar plates were also used as controls. Single isolates of fungi were first grown on the appropriate test medium and incubated at test temperatures prior to establishment of the experiment. For this a 6 mm plug of actively growing colony margins were placed at the centre of a 90 mm plastic Petri dish of the test medium. Plates were incubated at 4°C, 15°C or 25°C until the stationary phase of growth

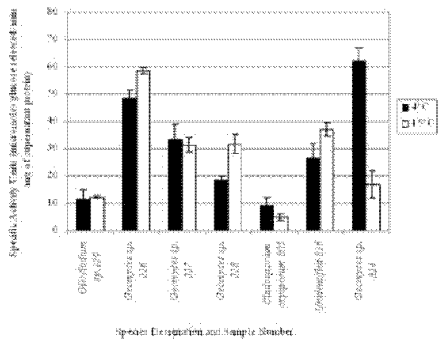

**Fig. 1.** Comparison of specific activity units of endoglucanase (micromoles of glucose $min^{-1}$ $ml^{-1}$/milligram of soluble protein in the supernatant) for the selected seven fungi at 4°C and 15°C

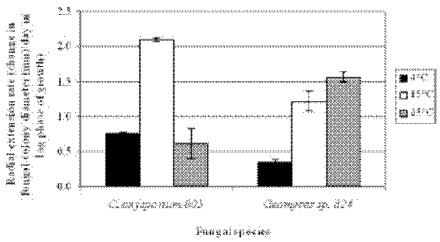

**Fig. 2.** Growth rates of *Cladosporium oxysporum* strain 805 and *Geomyces* sp. strain 824 on YM agar at 4°C, 15°C and 25°C.

was reached. Two diameter measurements were made daily of the fungal colony at right angles to each other until the diameter measurement failed to increase. The intrinsic growth rate was determined by calculating the change in colony diameter per day during the log phase of growth.

## Results

### *Isolation of fungi*

The 30 sites within *Discovery* Hut provided 156 filamentous fungi from swabs, wall scrapings, organic material or wood (Table I). Of these, 44 isolates were from agar plates incubated at 4°C, 73 from plates incubated at 15°C and 39 from plates incubated at 25°C. The number of fungi isolated on each selective medium was as follows: 76 isolates on YM agar; 40 isolates on Medium 4; 18 isolates on Medium 6; 14 isolates on VB agar; and eight isolates on Medium 7. Fungi were isolated from all physical samples and swabs of artefacts except for four swab samples of wood surfaces.

### *Identification of fungi*

Using classical taxonomy based on morphology six of the seven fungi used for further experiments were identified. Isolate numbers 226, 227, 228 and 824 were identified as *Geomyces* sp. Isolate 805 was identified as *Cladosporium* sp. Isolate 129 was identified as as *Gliocladium* sp. Isolate 816 could not be identified due to a lack of identifiable structures. Two fungal isolates, 805 and 824, were identified by morphological and molecular characterization as *Cladosporium oxysporum* (99% similarity, 500/502 overlap and Genbank accession number AJ300332) and a *Geomyces* sp. C239/10G (94% similarity, 467/492 overlap and Genbank accession number AY345347), respectively.

### *Screening for cellulolytic activity*

Of the 108 Antarctic isolates screened for cellulolytic activity on carboxymethyl cellulose (CMC) 29 isolates, including six initially isolated at 4°C, 20 isolated at 15°C and four isolated at 25°C, demonstrated clearing of CMC with an Index of Relative Enzyme Activity of 1 or greater. To screen Antarctic fungi isolated at temperate temperatures for CMC activity at cold temperatures (4°C), 28 fungi initially isolated at 15°C or 25°C (15 isolates were CMC positive, 13 were CMC negative) were also screened for cellulase activity at 4°C. Of the 15 fungi that showed CMCase activity at their isolation temperature, five also showed CMCase activity at 4°C, six did not grow at 4°C and four showed no CMCase activity at 4°C. Of the 13 fungi that showed no CMCase activity at their isolation temperature, three showed CMCase activity at 4°C, three did not grow at 4°C and seven showed no CMCase activty at 4°C. Data is shown in Table II 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 156 original Antarctic fungi, seven isolates were chosen for further study, and identified by morphological characteristics to belong to three genera, *Cladosporium*, *Geomyces*, and *Gliocladium* and one isolate remained unidentified as no sexual structures could be seen. The isolation temperatures of the seven selected isolates chosen were 4°C (three isolates), 15°C (three isolates) and 25°C (one isolate). Three isolates came from YM agar, two from Media 4, and two from Medium 6.

The seven isolates had the following characteristics: one showed clearing of carboxymethyl cellulose at isolation temperature, three showed clearing of carboxymethyl cellulose at 4°C but not at their isolation temperature and

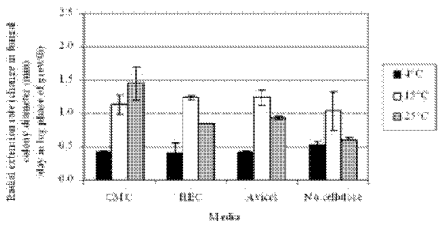

**Fig. 3.** Growth rates of *Geomyces* sp. strain 824 on three cellulose sources and media containing no cellulose as a carbon source.

three isolates showed no clearing of carboxymethyl cellulose at isolation temperature or at 4°C (Table II).

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

All of the fungi selected for this experiment showed endo-1, 4-β-glucanase activity. Figure 1 shows the levels of accumulated endoglucanase activity, expressed as specific activity units (micromoles glucose released per minute per mg of protein in the extracellular 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 occurred for the isolates cultured at 4°C after 28 days and cultured at 15°C after 10 days (data not shown)). Of the seven fungi tested, two produced more endoglucanase activity at 4°C than at 15°C, two produced similar endoglucanase activity at 4°C and 15°C, and three produced more endoglucanase activity at 15°C than at 4°C (Fig. 1). *Geomyces* sp. strains 226 and 228 produced more endoglucanase activity at 15°C than 4°C (58.6 and 31.8 specific activity units at 15°C compared with 48.8 and 18.6 specific activity units at 4°C respectively), but *Geomyces* sp. strains 227 and 824 produced more endoglucanase activity at 4°C than 15°C (33.5 and 63.7 specific activity units at 4°C compared with 31.5 and 17.1 specific activity units at 15°C respectively). *Cladosporium oxysporum* 805 produced more endoglucanase activity at 4°C than 15°C (9.4 specific activity units at 4°C compared with 5 specific activity units at 15°C). *Gliocladium* sp. strain 129 produced slightly more endoglucanase activity at 15°C than 4°C (12.5 specific activity units at 15°C compared with 11.7 specific activity units at 4°C). An unidentified strain 816 produced more endoglucanase activity at 15°C than 4°C (37.1 specific activity units at 15°C compared with 26.6 specific activity units at 4°C).

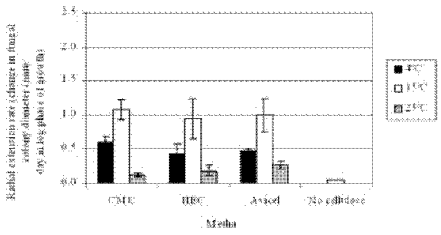

**Fig. 4.** Growth rates of the *Cladosporium oxysporum* strain 805 on three cellulose sources and media containing no cellulose as a carbon source.

*Radial hyphal extension rate*

The two fungi selected for this experiment demonstrated the ability to grow on YM medium at the three temperatures tested. The *Geomyces* sp. 824 grew fastest on YM medium at 25°C but could still grow at 4°C (radial extension rate at 25°C was 1.57 mm per day compared with 1.22 mm per hour at 15°C and 0.35 mm per day at 4°C) while the *C. oxysporum* 805 isolate showed fastest growth at 15°C compared with 25°C or 4°C on YM medium (radial extension rate at 25°C was 0.61 mm per day compared with 2.1 mm per day at 15°C and 0.76 mm per day at 4°C) (Fig. 2).

The two fungi were able to grow on all three carbon sources. The *Geomyces* sp. grew on all sources of cellulose tested and also grew on the agar plates containing no cellulose at 4°C, 15°C and 25°C (Fig. 3). The growth rate at 4°C was the same on all three sources of cellulose. The growth rate was the same at 15°C on all three sources of cellulose but was slower when *Geomyces* sp. was grown on medium containing no cellulose as a carbon source. The growth rate at 25°C was less on hydroxyethyl cellulose and Avicell and less on the medium containing no cellulose as a carbon source (Fig. 3). In contrast *C. oxysporum* grew on all three cellulose sources at 4°C, 15°C and 25°C, with a similar growth rate at each of the temperatures, but no growth on the medium containing no cellulose (Fig. 4).

## Discussion

The historic huts of Ross Island provide a unique environment and metabolic substrates for fungi in this relatively pristine region. Although we cannot be sure of their origin, it is apparent that the fungal isolates described in this study are well adapted to the environmental conditions of Antarctica. Vincent (2000) hypothesized that increased invasion from micro-organisms from elsewhere in the world into Antarctica leads to larger microbial speciation. Human impacts in Antarctica have led to selection of fungal species which were either native, and able to utilize the new nutrient sources introduced by humans, or were new non-native species brought in with the humans and materials. This has resulted in a diversity

profile that is different to the adjacent unimpacted environment. This research, in addition to the research reported by Duncan *et al.* (2006) and Held *et al.* (2005), suggests that other factors, including environmental influences, are impacting on fungal numbers and diversity. Fungal material is being introduced by the human visits but it is the ability to adapt to the environment that ultimately leads to an increase in fungal biomass and species diversity within the historic huts. It seems likely that both indigenous and introduced fungi were isolated from *Discovery* Hut when the fungi isolated from this study were compared with fungi identified in a more in-depth study on fungal diversity (Arenz *et al.* 2006) and their growth optima demonstrated that the fungi are not only surviving in the Antarctica environment but are capable of proliferating. Therefore, we feel these findings support Vincent's (Vincent 2000) hypothesis that larger microbial speciation caused by adaptation has been demonstrated in this research focused on the interior of the historic hut.

Of the two fungi identified by molecular techniques, *Geomyces* spp. have been isolated from pristine areas (Kerry 1990b) and areas with both little biotic influence and seal-influenced soil samples such as Peterson Island, off the Windmill Islands (Azmi & Seppelt 1997). There are no references to *Cladosporium oxysporum* being isolated in Antarctica but Mercantini *et al.* (1993) have reported isolation of *Cladosporium* sp. from areas of high biotic influence along the Newfoundland Coast, Ross Sea Region. Neither of the fungi used in this study could be defined as psychrophilic. Both species grew at 4°C but also at 25°C. *Cladosporium oxysporum* strain 805 showed higher growth rates at 15°C than at 25°C, and should be classified as a psychrotroph. *Geomyces* sp. strain 824 showed higher growth rates at 25°C than at 15°C and 4°C and should be classified as a cold tolerant mesophile. During summer temperatures averaging 2°C, and as high as 8.2°C, were reported by Held *et al.* (2005) in *Discovery* Hut. Compared with the other two historic huts on Ross Island, the number of hours that provided adequate conditions for fungal growth ($> 0$°C and $> 80$% RH) at *Discovery* Hut was substantially lower than found at the Cape Royds and Cape Evans huts (Held *et al.* 2005). This may explain the low amount of visible fungal growth and the presence of relatively small inactive colonies on the wood structure and artifacts compared to the other huts.

Many researchers have screened Antarctic fungi for extracellular enzyme activity. Of 33 fungal strains isolated from Victoria Land, by Fenice *et al.* (1997), (isolates screened at 25°C, and at their optimal growth temperature if 25°C was not optimal), 36.4% of the isolates demonstrated cellulase activity with halo diameters $\leq 10$ mm. When the results of this study of *Discovery* Hut fungi are expressed by the methods used by Fenice *et al.*, (which did not take into account the fungal colony size) the percentage of cellulase producing organisms is 61% with 18 isolates producing halos of diameters $\leq 10$ mm, 39 isolates producing halos measuring 11–25 mm and 20 isolates producing halos $> 25$ mm. When comparing cellulase production it is important to consider the substrate and habitat that the fungi were isolated from. The substrates in this research are structural wood, foodstuffs and organic material and the temperature inside the hut ranged from maximum of 6.6°C to a minimum of -39°C (Held *et al.* 2005) while the fungal isolates from Fenice *et al.* (1997) came from a habitat consisting of soil, moss, soil under moss, or moss and soil/sand under the moss. The air temperatures at time of collection were 0°C to -9°C, while the temperature of the substrata were always above 0°C and as high as 27°C. Fenice *et al.* did not describe the species of moss that the fungi were isolated from, but Walton (1985) noted that decomposition rates in *Polytrichum alpestre* as "low decomposition of plants due to high holocellulose and crude fibre, very low nutrient content and low microbial populations". Hurst *et al.* (1983) cultured fungi from leaf discs from three sub-Antarctic phanerogams and airspora beneath a grass canopy on the sub-Antarctic island of South Georgia. Eight fungi isolated from this study (*Acremonium terricola*, *Botrytis cinerea*, 2 *Chaetophoma* sp., *Chrysosporium pannorum*, *Cladosporium sphaerospermum*, *Fusarium lateritium* and *Mucor hiemalis*) were screened for their ability to degrade cellulose (by growth rate and clearing zones around fungal colonies) along with other substrates at 20°C. All except *M. hiemalis* grew on and were able to cause clearing of cellulose agar plates. They reported cellulase activity at 1°C for *Botrytis* at 70% of its maximum, *C. pannorum* at 60%, *Chaetophoma* at 50%, and *C. sphaerospermum* at 40%.

Cellulose is a secondary nutrient source for fungi. Cellulose is a mixture of crystalline cellulose, which is more resistant to microbial degradation, and amorphous cellulose, which is readily broken down to glucose (Eriksson *et al.* 1990). All cellulose substrates used in this study were synthetic. *Cladosporium oxysporum* strain 805 and *Geomyces* sp. strain 824 grew on all three cellulose carbon sources indicating these fungi can use many different types of cellulose as nutrient sources. The presence of these fungi within the historic huts, and their ability to degrade cellulose demonstrates that it is important to create conditions in the hut which are not conducive to fungal growth so that continued degradation of the historic textiles, paper, wood and other cellulosic substrates in the hut is reduced.

Quantitative cellulase activity at psychrophilic temperatures has been reported by the authors, (Duncan *et al.* 2006) in Antarctic fungi isolated from the Cape Evans historic hut. This previous study reported on 18 fungi identified from an Antarctic historic hut, all producing detectable levels of endoglucanase activity, at either 4°C or 15°C. The findings of the *Discovery* Hut work adds that fungi isolated from a variety of substrates within *Discovery* Hut were capable of cellulose breakdown activity at 4°C and 15°C and produce

carboxymethyl cellulase and endo-1, 4-β-glucanase activity in culture. Thus, these enzymes are likely to be functional in the Antarctic ecosystem and the organisms may have significant impact on the wood of the hut structure and cellulosic artefacts.

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

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## 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 → 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;

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**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 | 2.65 | 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.

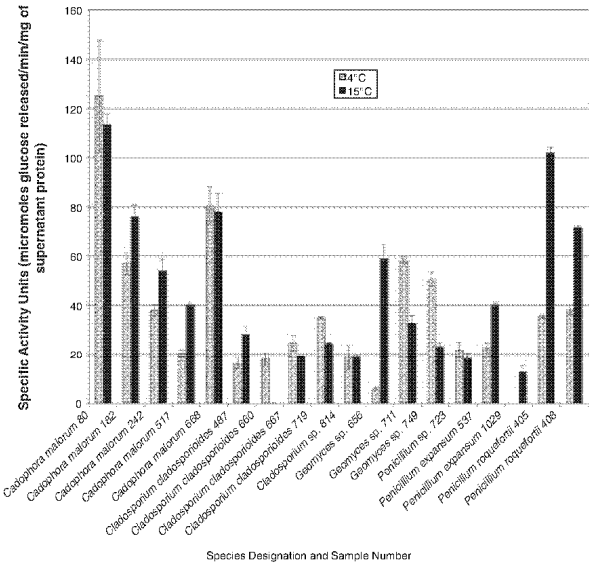


**Fig. 1.** Graph of specific activity units of cellulase (micromoles glucose released per minute per mg of supernatant protein) for the selected 18 fungi at 4°C and 15°C.

 

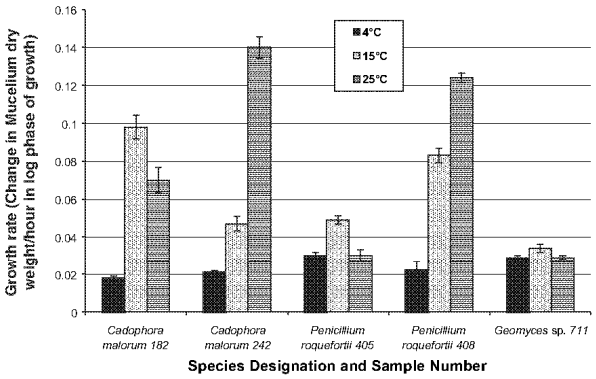


**Fig. 2.** Graph of growth rate of the five selected fungi at 4°C, 15°C and 25°C.

*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.

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