Fungal Diversity and Cellulytic Activity in the Historic Huts, Ross Island, Antarctica

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Abstract

The goal of this study was to undertake a microbial investigation of the Historic Huts areas on Ross Island, to gain knowledge of the fungal biodiversity and biochemical framework, focusing on the wood degrading potential of these fungi at both psychrophilic (cold) and mesophilic (moderate) temperatures. Eight hundred and forty nine samples were collected from three Heroic Era Historic Huts of Antarctica, from a variety of substrates but predominantly structural wood. The huts, *Discovery* Hut at Hut Point, *Terra Nova* Hut at Cape Evans and *Nimrod* Hut at Cape Royds, are located on Ross Island and were all assembled in the early 20th century by the Heroic Era explorers to house the expeditions, stores and animals. These wooden huts were abandoned when the expeditions left. The introduction of wood and other organic material to a pristine environment along with the creation of a microclimate within the harsh Antarctica environment created interesting sites for studying fungal diversity, wood decay and fungal cellulase enzymes in an extreme environment. Each hut can be classified as offering different conditions and circumstances for fungal propagules. Of the three huts, *Terra Nova* Hut is the only hut where there are visible fungal blooms within the hut and it, with *Discovery* Hut, had the greatest number of samples that contained fungi compared to *Nimrod* Hut which had the least. *Discovery* Hut, at less than 500 metres from the United States McMurdo Station, is the most visited by scientist and base staff and has been the most demonstrably affected by human impact of the three huts due to its closeness to the research stations on Ross Island

To ensure a full understanding of the fungal diversity of the Historic Hut sites, a variety of sampling techniques were used along with a variety of culture media. Two thousand and seventy six isolates consisting of 1177 filamentous fungi and 899 single celled microorganisms (yeast and bacteria) were isolated; all these cultures were frozen and now form the University of Waikato Antarctic Culture Collection. Five genera dominated the fungal isolates that were identified and these were *Cladosporium*, *Geomyces*, *Cadophora*, *Penicillium* and *Thelebolus*. The fungal diversity of these Historic Huts' communities is low but the members present are metabolically active, consistent with other microbial communities in the Antarctic. The Historic Huts and surroundings contain a diverse array of provision in the way of wood and supplies, which provide nutrient sources for

fungal growth. Endemic organisms present in the soil could have been enriched by using the introduced nutrient sources as primary and/or second metabolic substrates. In addition, fungi could have been introduced with the wooden huts and supplies when they were brought to Antarctica by the Heroic Era explorers, or introduced in the subsequent years with visitors and conservation work conducted at the sites. These introduced organisms, though, would have had to adapt to the change in climate and conditions posed by the Antarctic in order to survive and be subsequently isolated in this study.

A screen for carboxymethylcellulase (CMCase) activity was done on a selection of the fungal isolates as the first step to understand the cellulytic potential of the Antarctica fungal community inhabiting the huts. One hundred and six fungal isolates from a total of 404, that were screened were deemed to be CMCase positive, 27 fungal isolates were chosen for further study including quantifying the activity of extracellular endo-1,4-β-glucanase at psychrophilic and mesophilic incubation temperatures. All but one isolate could produce endo-1,4-β-glucanase activity at 4°C and many produced more endo-1,4-β-glucanase activity at 4°C than at 15°C.

Cadophora malorum 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporium* 805 were selected for in-depth study of growth characteristics including growth temperature preferences, growth on a variety of cellulose substrates, water activity, and carbon sources, the latter done by using a commercially available microtitre plate containing 95 carbon sources. All seven of the fungal isolates were classified as psychrotolerant and produced, when cultured at either 4°C or at 15°C, cellulase, protease, amylase, xylanase, and pectinase and mannanase enzyme activities. The range of water activity that the Antarctic *Penicillium roquefortii* isolates could grow at was distinctive when compared with food *Penicillium roquefortii* isolates. The utilisation of different carbon sources showed that like many studies of Antarctica organism they have a diverse range of enzymatic activity, but interestingly the activity does not differ greatly with incubation temperature with most carbon sources being used or not used at both incubation temperatures tested. Although it took longer for the fungi

to grow at the psychrophilic temperatures, the range of carbon sources they utilised was not reduced.

The protein composition of the extracellular supernatants was visualised using various electrophoretic and staining techniques. The cellulase activity of the protein bands was visualised by cellulose-containing zymograms, which illustrated that the cellulase complex in all fungi tested was multi-enzyme and differed between species, isolates and temperatures of culturing. The cellulase activity of *Cadophora malorum* 182 was enriched by purification techniques including ion exchange chromatography and native preparative electrophoresis. The protein complex was not purified to homogeneity, but enriched for a mixture of proteins and the mixture was described as having the following properties; a temperature range of β-1,4-glucan cellobiohydrolase activity from 20°C to 80°C with the optimum activity seen at 60°C, β-1,4-glucan cellobiohydrolase activity that is stable at 4, 25 and 40°C for at least 24 hrs, lost at 50 °C and 80°C within 24 hrs and 2 minutes respectively. Along with β-1,4-glucan cellobiohydrolase activity, the protein mixture contained Avicelase, CMCase, xylanase and mannanase activity.

The thesis research showed that there was limited fungal diversity in the Historic Huts and artefacts (a total of five dominant genera were identified) but the fungi are actively growing and producing viable spores in the cold of Antarctica and producing the necessary enzymes for degradation of wood. Although the metabolism and growth rate is slower at psychrophilic temperatures, the fungal isolates studied as part of this thesis research could still function enzymatically at cold temperatures and this includes the degradation of wood as evidenced by *in vitro* wood decay studies examined by scanning electron microscopy where two isolates of one species demonstrated the ability to degrade wood. The cellulase complex of the investigated fungal isolate was multi-enzymed and although the components were not purified to homogeneity, an enriched mixture of proteins had enzyme activity and stability in a broad temperature range, and activity to a variety of cellulosic substrates.

This thesis research adds to the knowledge of the fungal biodiversity in the Antarctic and increases the understanding of the biochemical framework, participating in relation to wood decay potential of these Antarctic fungal isolates.

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List of Abbreviations

UV	Ultraviolet
a_w	Water activity
Csp	Cold shock proteins
Cap	Cold acclimation proteins
PUFA	Polyunsaturated fatty acids
INP	Ice nucleation proteins
AFP	Antifreeze proteins
EPS	Exopolysaccarides
S_1	Outer secondary cell wall
S_2	Middle secondary cell wall
S_3	Inner secondary cell wall
VA	Vesicular arbuscular
AM	Arbuscular mycorrihizal
DP	Degree of polymerisation
UDP	Uridine-diphospate-glucose
CBD	Cellulose binding domain
SCAR	Scientific Committee on Antarctic Research
ASPA	Antarctic Special Protected areas
AHT	Antarctic Heritage Trust
DGGE	Denaturing gradient gel electrophoresis
CFU	Colony forming units
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CO_2	Carbon dioxide
rDNA	ribosomal DNA
ITS	Internal transcribed spacer
SD	Standard deviation
CMC	Carboxymethylcellulose
HEC	Hydroxyethylcellulose
VB	Vogel Bonner
YM	Yeast Malt
ME	Malt extract
PDA	Potato dextrose agar

CLPP	Community level physiological profiles
PAGE	Polyacrylamide gel electrophoresis
PAS	Periodic Acid Stain
MW	Molecular Weight
FPase	Filter paper activity
MOPS	4-Morpholine-propanesulfonic acid
SEM	Scanning Electron Microscope

Chapter 1 General Introduction to the PhD thesis

Research and Literature Review

1.1 Introduction to the PhD thesis research

During the Heroic Era of Antarctic exploration, Robert F. Scott assembled two huts on Ross Island in Antarctica, *Discovery* Hut at Hut Point in 1902 and *Terra Nova* Hut at Cape Evans in 1911 while Ernest Shackleton built one hut, *Nimrod* Hut, at Cape Royds in 1908. These three prefabricated wooden structures were erected to store supplies and house the expeditions for several years during exploration of the region. When those remaining expedition members left, the huts and supplies were used by members of other Antarctic expeditions until 1916, and subsequently abandoned until the late 1950s.

The presence of wood and other organic materials provided carbon and nitrogen sources for microorganisms that were either endemic to Antarctica or introduced with the timber or supplies and have adapted to the harsh conditions. It was hypothesised that microbial speciation was selected with the introduction of conditions such as the wood as a unique food source, posing opportunities for microbial evolution (Vincent, 2000). Many fungi have been isolated from Antarctica which are either supposedly endemic or introduced according to a review by Vishniac (1996). The determination of which fungi are endemic to Antarctica and which are introduced is made difficult for many reasons including introduction issues such as fungal spores being readily airborne, most plating techniques leading to the culturing of the more freeze thaw resistant spores rather than the unprotected hyphae, and the importance of correct identification of

species (Vishniac, 1996). Misidentification can lead to inaccurate biodiversity indices and rare species going unnoticed.

Much of the previous work on the fungi found in association with the Historic Huts focused on the long-term survival of organisms in the food supplies, human and the horse-associated materials, and thus due to this origin it was assumed that all organisms isolated were introduced with the materials (Meyer *et al.*, 1962; Meyer *et al.*, 1963; Nedwell *et al.*, 1994). Our recent investigations of fungi associated with Historic Hut structural timber in contact with the ground suggested that endemic species of Antarctic microbes colonised the wood and caused decay (Blanchette *et al.*, 2004). This doctoral thesis adds to the knowledge of Antarctic microorganisms with a multidisciplinary investigation of the diversity of fungi growing on the structural wood of the three Historic Huts of Ross Island and characterises some of the fungi, particularly with regard to the parameters of temperature and nutrients acceptable for primary and secondary metabolism.

The classical definitions, still used today to characterise organisms that live in the cold, are psychrophile and psychrotrophs. Both can grow at 0°C and a psychrophile is an organism having an optimal growth at about 15°C or lower, a maximal temperature for growth at about 20°C and a minimal temperature of growth at 0°C or below, while psychrotrophs have a maximal growth temperature above 20°C (Morita, 1975). With increasing industry interest in cold adapted enzymes, the cold, unique and isolated environment of the Antarctic has become a focus, of research. The search for psychrophilic fungi in Antarctica has for the most part led not to psychrophiles but to the discovery of many fungi that are psychrotrophs and specifically psychrotolerant strains of mesophiles adapted to

grow at temperatures as low as 1ºC (Kerry, 1990a; Abyzoz, 1993; Azmi and Seppelt, 1997). However psychrophilic fungi, have been isolated from Antarctica and are primarily from cryptoendolithic communities (Selbmann *et al.*, 2005), of the fellfield soils (Weinstein *et al*., 2000). Crytoendolithic microorganisms colonise spaces within rocks, and normally consist of a variety of microorganisms including cyanobacterial, algal, lichen, bacterial and fungal components (Hughes and Lawley, 2003). Psychrophilic yeasts have been isolated from Antarctica (Di Menna, 1960) and other psychrophilic fungi have been isolated from various parts of the world, including pink snow mould (Hoshino *et al.*, 1996) and various basidiomycetes (Inglis, 2000).

This doctoral thesis investigated the growth characteristics of a selection of psychrotrophic Antarctic fungal isolates along with their ability to utilise a variety of carbon sources, a variety of cellulose substrates, and their ability to grow at reduced water activity in order to determine their metabolism as a function of incubation temperature.

Research interest specifically in the Historic Huts, constructed of wooden timber, led to this thesis research focusing on wooden structures, cellulytic fungi and the cellulase enzymes produced by the fungi to breakdown the main component of wood. Wood is a relatively easy material to use for construction but because of its biological origin it is a complex construction material. Wood primarily consists of three organic polymers, cellulose, lignin and hemicellulose, which together make up the cell wall structure of wood fibres. Cellulose is a major component of wood comprising about 50% of an average wood fibre. The molecular structure of cellulose is a linear polymer of β-linked glucosyl units. Each glucose residue is

rotated 180º with respect to its neighbours along the main axis chain. Cellulose molecules are linear and form intra- and intermolecular hydrogen bonds. Cellulose is also hydrophobic, allowing for water molecules to be associated with the wood cells; and the tracheids are responsible for conduction of water and support while the parenchyma cells that constitute the wood rays store water and organic nutrients (Domec and Gartner, 2002).

Due to wood's affinity for moisture, it makes an ideal substrate for biological deterioration as it provides both nutrients and water that decay microorganisms require for proliferation. Many microorganisms are known to degrade cellulose in nature, producing the group of enzymes responsible for hydrolysis of cellulose, collectively known as cellulases. Fungi that have cellulase enzymes may be in the Ascomycota or Basidiomycota Classes (Eriksson *et al*., 1990). Cellulose degradation requires the action of three major classes of cellulase enzymes, as follows: Endo-1,4-β-glucanase (EC 3.2.1.4), which catalyses the hydrolysis of cellulose, randomly, by the hydrolysis of the β-(1-4)-glucosidic linkage; Exo-1, 4-β-glucanase, (EC 3.2.1.91), which catalyses hydrolysis and release of either glucose or cellobiose from the non-reducing end of the cellulose; and 1,4-β-glucosidase (EC 3.2.1.21), which hydrolyses cellobiose and water soluble cellodextrins to glucose. Some fungi can produce other oxidative enzymes, such as cellobiose dehydrogenase and cellobiose oxidase, which aid in catalysing the degradation of cellobiose. A great deal of information is known about microbial and enzymatic degradation of wood and wood components in temperate conditions (Blanchette *et al.*, 1990), but there is very little known from the Antarctic environment.

This doctoral thesis adds to the knowledge of cellulase breakdown by the characterisation of Antarctic fungal isolates, which were screened for cellulase activity, some of which were shown to produce endo-1,4-β-glucanase activity, the levels of which were quantified in extracellular supernatants of isolates grown at both cold and at temperate temperatures. Additionally, the extracellular supernatant was evaluated at cold and temperate temperatures when cultures were grown at 4°C and 15ºC for other relevant enzyme activities such as pectinase, amylase, xylanase and protease.

Psychrophilic enzymes have a high specific activity at low to moderate temperatures and are inactivated easily by moderate increases in temperature (Gerday *et al.*, 2000). The structure of psychrophilic enzymes is considered to be more flexible and less stable in order to increase energy efficiency and increase reaction rates (Gerday *et al.*, 2000).

This doctoral thesis describes the enrichment of the extracellular cellulases from Antarctic fungal isolate *Cadophora malorum* and the enzymes activity range with regard to temperature, stability and substrate.

1.2 Hypothesis, Aims and Objectives

1.2.1 Hypothesis

The hypothesis of this PhD thesis research was that the fungi isolated from the wood of the Antarctic Historic Huts would be capable of proliferation at cold temperatures, relative to the average Antarctic summer temperatures, with demonstrable enzymatic activity required for wood decay.

1.2.2 Aims

The aims of this PhD thesis research were focused on identifying the fungi and their enzymes relevant to degrading wood in the Antarctic, emphasising isolates from the century-old Historic Huts of the Heroic Period on Ross Island. The research aimed to gain an understanding of fungal diversity and the biochemical framework of the Antarctic isolates.

1.2.3 Objectives

Specific objectives of this thesis were as follows:

- Isolated filamentous fungi from a variety of Antarctic substrates of the Historic Huts in the Antarctic Specially Protected Areas (ASPA), using a selection of culture media, culturing techniques and sampling methods. All cultures preserved (as frozen isolates) to create an Antarctic Fungal Culture Collection.
- Determined fungal growth rates at various temperatures to identify psychrophilic or psychrotrophic preferences.
- Measured growth capabilities of a selection of fungi isolated from structural wood.
- Screened selected fungi isolated from a variety of substrates including structural wood from the Antarctic Historic Huts on Ross Island at different temperatures to determine fungal ability to produce cellulases.
- Identified fungal isolates to genus level using morphological characteristic and identified some of the fungi exhibiting cellulytic enzyme activity using molecular techniques.

- Characterised cellulytic activity using quantitative assays to determine levels of production (accumulated enzyme activity in the extracellular supernatant) and conditions under which this is optimal.
- Enriched and characterised cellulolytic enzymes from the species that most significantly produced cellulase enzymes under psychotrophic conditions.
- Showed growth and decay capabilities of selected of fungal isolates from structural wood at different temperatures.

1.3 Overview of thesis organisation

This thesis consists of eight chapters. In Chapter 1, the literature with regard to the Antarctica ecosystem, origin and survival of fungi, wood decay fungi, cellulose, cellulases and the history of the Antarctic Heroic Period of exploration are discussed. The literature relating to more specific aspects of the research objectives is provided at the beginning of the relevant chapters. Chapter 2 presents background and a summary of the research from the collaboration between The University of Waikato and The University of Minnesota including two publications of which this PhD research continues on from. Chapter 3 presents details of the collection of samples, isolation, and identification of fungi. Moisture content of the wood and the fungal spore distribution in the huts are described in this chapter. Chapter 4 describes the growth characteristics, including temperature preference, ability to grow on different cellulose sources, water activity and different carbon sources of a selection of fungal isolates described in Chapter 3. The screening, production and composition of cellulase by Antarctic fungal isolates described in Chapter 3 are detailed in Chapter 5 along with measurement

of the accumulated activity in the extracellular supernatant of other wood component degrading enzymes. Chapter 6 contains the protein enrichment of the cellulase complex from the extracellular supernatant of the Antarctic isolate *Cadophora malorum* 182 and biochemical characterisation of the proteins. A wood decay and colonisation study using Antarctic fungi is presented in Chapter 7. A general summary and overall discussion of the research findings presented in this thesis is provided in Chapter 8 as well as recommendations for future research. The 9 appendices include tables of the sample locations, presence/absences of fungi from the sample locations, results from spore traps, bait traps and culturing in Antarctica, moisture content of structural wood, presence/absence of fungi from the moisture content samples, the Antarctic Filamentous Fungal Culture Collection created by this thesis research, results of screening of 404 Antarctic fungal isolates for carboxymethylcellulase activity, identity of Antarctic fungi by BLAST search of DNA databases, graphs of cellulase production at various temperatures, possible identity of proteins by BLAST search of peptide sequence databases, publications of which the author of this doctoral thesis was a co author.

1.4 Literature Review

In this section, literature on the Antarctic environment, the microorganisms within it, and the strategies of these organisms to adapt to this unique environment are reviewed specifically relating to the ecosystems created by the Heroic Era Historic Huts. Literature on the physical conditions found in the Antarctic environment, along with the possible origins of microorganisms within this environment and how microorganisms adapt to cope with the environmental pressures placed on them is provided. An introduction to wood, including structure, composition,

decay and the microorganisms that cause this decay are addressed in the second part of this literature review. The history of the Antarctic Heroic Era Historic Huts, a description of their location and design, along with their care since the Heroic Era is presented in the third part of the literature review. Literature reviews relating to more specific aspects of the research objectives are provided at the beginning of the appropriate chapters.

1.4.1 Antarctic Ecosystem

The Antarctic Continent is indicated as the area south of latitude 60 degrees South (McGonigal and Woodworth, 2001) and emerged from the disintegration of the Gondwana super continent as a result of tectonic forces in the Cretaceous Period. The continent became more isolated through time (Figure 1.1), finally separating from Australia about 40 million years ago. It was around this time that the onset of the first major cooling in Antarctica occurred with the appearance of sea ice (Prothero, 1992). The average elevation of the continent is 2,160 metres (m) above sea level and at its thickest point the ice extends to a depth of 4,800 m.

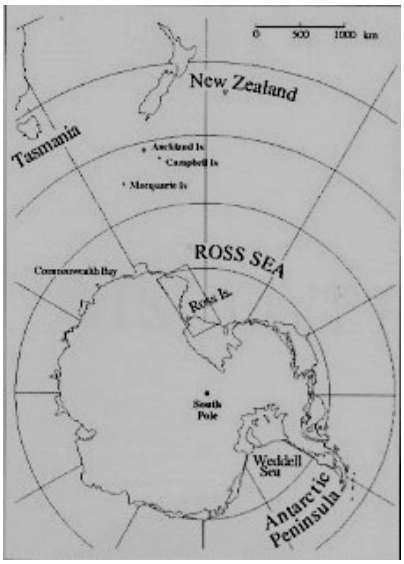

Figure 1.1: Map of Antarctica, Subantarctic Island (Macquarie, Campbell and Auckland Islands), New Zealand and Tasmania (AHT Conservation Plan *Discovery* Hut, Hut Point, 2004).

Antarctica is a rocky continent, the fifth largest on the Earth with an area of 12.38 million kilometre (km)^2 covered by an ice sheet of 11.97 million km^2, leaving only 3.45% of the continent ice free (Campbell and Claridge, 1987). The sea ice increases from 4 million km^2 during summer to 20 million km^2 in winter. This annual change is the greatest on Earth of ice creation and melting. The longest mountain range is the Trans-Antarctic Mountains with some of the peaks being 4000 m high, which extends across and divides the continent into two main geographical regions, East Antarctica and West Antarctica, with the later having an extending arm of the Antarctic Peninsula to Patagonia. The constant accumulation of snow on the ice sheet causes a steady flow of ice into the surrounding sea via the ice shelves and glacial ice streams.

The general climate of the continent is characterised by extreme cold, little precipitation and frequent strong winds from the south (Benninghoff and Bonner, 1985), which leads to the continent being classified as a polar desert. The lowest temperature recorded in Antarctica was -89.6°C at the Russian Base, Vostock on the inland icecap (*Antarctica Great Stories from the Frozen Continent*, 1985). The strongest wind gust of over 320 km/hr was recorded at Cape Denison (*Antarctica Great Stories from the Frozen Continent*, 1985).

Antarctic soils have developed under cold desert conditions (Ugolini, 1970). The general aridity and sub-zero temperatures have inhibited chemical weathering and biological processes of the soil and this is reflected in their unique properties specifically, a lack of organic material and high salt concentrations. Antarctic soils are mostly coarse-textured and often have a surface stone layer, and salt accumulation is frequent (Claridge and Campbell, 1985). Ornithogenic soils in the

vicinity of penguin colonies are enriched with nitrogen and phosphorus from decomposition of guano while other areas, like the Dry Valleys, are nutrient-limiting (Wynn-Williams, 1990). Youthful soils are soils that are still moist, contain less salt and are greyer in colour. As the Antarctic soils mature desert pavement, varnishing, frost crack, and polygon formations all appear. Salt accumulates, elemental and ionic composition, ratios, mineralogical character and soil structure all change (Cameron and Conrow, 1969). Also depth of ice cemented permafrost increases.

Ross Island is 4,000 km south of New Zealand, situated in the Ross Sea and is dominated by the active volcano Mount Erebus (3794 m elevation) (Figure 1.2). The Island is adjacent to the McMurdo and Ross Ice Shelves, the two shelves forming a vast floating mass of permanent ice the size of France. Ross Island is composed of basaltic volcanic rocks (Kyle, 1990) and is covered in snow and ice glaciers for most of the year. During summer, the soils adjacent to the shore line thaw to about 0.5 m depth and small melt ponds and streams form (Sheppard *et al.*, 1997).

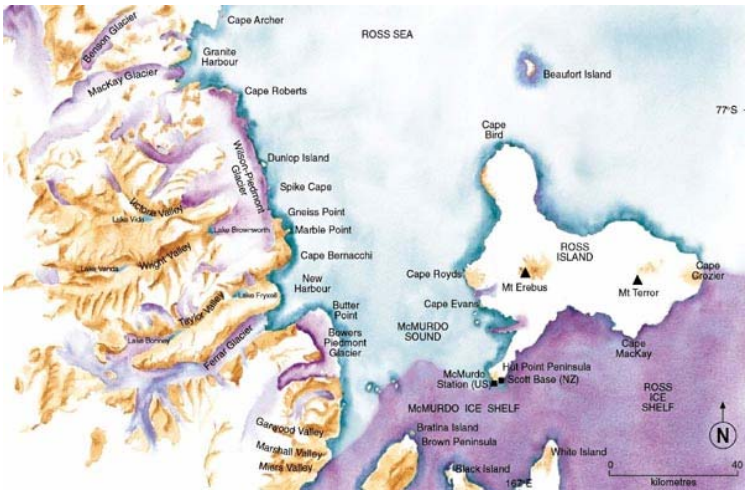

Figure 1.2: Map of Ross Island and the McMurdo Dry Valleys in southern Victoria Land, Antarctica (Waterhouse, 2001).

1.4.1.1 Moisture levels of Antarctica

The air over Antarctica is generally too cold to hold water vapour so there is very little precipitation but Antarctica does retain what moisture it receives, and about 75% of the world's fresh water is stored as ice on the continent. The extremely arid environment is due to the restricted availability of moisture under sub-zero temperatures (Campbell *et al*., 1987). Areas of Antarctica receive annual precipitation of snow and ice crystals equivalent to less than 0.5 centimetre (cm) of water. Precipitation is variable from year to year, ranging from 3 cm per year in the interior to 20-80cm on the continental margin (Benninghoff and Bonner, 1985). In contrast to the rest of the continent, the Antarctic Peninsula receives relatively high levels of precipitation; the northern end of the Peninsular receives the equivalent of 90 cm water per year (*Antarctica Great Stories from the Frozen Continent*, 1985) owing to its latitude and closeness to South America. The Dry Valleys, often referred to as the Ross Desert are one of the driest places on Earth, receiving less than 0.5cm of water per year which is slightly more than the Sahara Desert (*Antarctica Great Stories from the Frozen Continent*, 1985). The Trans-Antarctic Mountains create a precipitation shadow over the Dry Valleys and the katabatic winds effectively remove all moisture. All parts of the continent have persistent, moderately strong winds often producing blizzards. Liquid precipitation falls very rarely in the Ross Island area (Sheppard *et al*, 1997) so that any liquid water that flows and collects must be derived almost exclusively from the frozen state, either as snow, ice, or permafrost. In 1909, during the Heroic Era, Shackleton's party determined that the annual snowfall at Cape Royds was equal to about 9.5 inches (241.3 millimetre (mm)) of rain (Shackleton 1909).

Campbell *et al*. (1994) demonstrated that slight disturbance to soil cover in Antarctica can cause the release of water as a result of melting of permafrost. Removal of 50 cm from the active layer of soil; soil that is subject to annual or diurnal cycles of freezing and thawing and is above the permafrost can cause a loss of up to 1250 cubic metre (m^3) of water per hectare due to melting until a new equilibrium is reached. Soil moisture around Cape Royds is higher than that of soils from the Dry Valleys but like all Antarctica samples the moisture content rises from the surface to the depth of the cemented permafrost (Cameron and Conrow, 1969).

1.4.1.2 Temperatures of Antarctica

Antarctic temperatures have generally decreased since the break-up of Gondwana, according to Clarke and Crame (1989), but the trend has been punctuated by episodes of warming, either global or localised to the Antarctic (Clarke and Crame, 1989). The continent's high altitude and limited solar radiation means it has little chance to warm. The vast areas of ice reflect 40-90% of the incident solar radiation, thereby sustaining the lower temperature and causing a mass of cold air to accumulate on the polar plateau (McGonigal and Woodworth, 2001). This cold air flows out towards the sea as strong katabatic winds, which tend to isolate Antarctica from the northern continents. This isolation is accentuated by the cold waters of the Southern Ocean, which are cooled by the peripheral ice mass, the katabatic winds, and the fluctuating sea-ice. The net effect of this heat sink is to preserve a distinct easterly circulating circumpolar ocean of 35 million km^2. This cold ocean meets the warmer waters of the more northerly oceans at latitude 50ºS, in a relatively well defined front called the Antarctic Convergence. The isolating effect of the Antarctica Convergence is further accentuated by the large distance to the nearest major land mass which is 1000 km to South America

and over 4000 km to South Africa and to New Zealand. The warmest part of the continent is the Antarctic Peninsula with an average annual temperature of -5ºC. Coastal areas are generally warmer and have more cloud and precipitation than the central continent; they are also more strongly influenced by cyclonal activity (Weyant, 1966). Temperatures in the coastal regions generally range from -10ºC to -20ºC although this quickly drops to -40ºC a short distance from the coast. The average annual temperature of the central plateau is -50ºC (Campbell and Claridge, 1987) and variation in temperatures are in accordance with latitudinal and altitudinal parameters. The polar plateau receives little solar energy due to high reflectivity, whereas dark bare ground, for example in the Dry Valleys, coastal areas, and nunataks, can be heated up above freezing point relatively quickly (Campbell *et al*., 1987). A covering of snow insulates the soil from large temperature changes (Thompson *et al*., 1971) and the deeper the layer of snow, the less severe winter temperatures experienced at the soil surface (Davey *et al*., 1992). The sun is continuously above the horizon during summer leading to 24 hours (hr) of sunlight and is below the horizon for a considerable period around the winter solstice and for as long as six months at the South Pole.

1.4.2 Origins and Survival of Antarctic Microorganisms

1.4.2.1. Conditions affecting microbial life

With the onset of the first major cooling in Antarctica many of the original organisms became extinct as a result of the increasingly extreme climate (Clarke and Crame, 1989). The terrestrial fauna has an extremely low diversity with many soil communities being considered the simplest on earth (Clark *et al.* 2004). The coastal flora and fauna has risen from relatively recent immigration of species as almost this entire habitat was destroyed by recent glaciations (Convey, 2001).

The contrast in environmental variability in terrestrial versus marine systems has led to markedly different evolutionary pressures on organisms in these two ecosystems. Most of the southern marine areas are very stable in temperature with variations of <0.5ºC annually, whereas the terrestrial organisms can be exposed to daily temperature fluctuations of 20-40ºC and annual fluctuations as high as 40-80ºC in the upper surface of the soil due to solar heating during the summer (Convey, 1996). The diversity of the marine environment is assisted by the stability of the environment. The presence of primary producers makes it a rich nutrient source leading to greater species richness (Lizotte, 2001).

The terrestrial microbial biotas of the Antarctica inhabit a graded series of environments ranging from cold desert, through progressively warmer conditions of the continental coastal fringe and the maritime zone to sub-Antarctic. Antarctica is characterised by a combination of extreme environmental conditions such as low temperatures, strong winds, high ultraviolet (UV) exposure and low water availability (A_w) that constituent limiting factors for life settlement. These characteristics are given as an explanation for why the biology of Antarctica more than any other continent is dominated by microorganisms (Friedmann *et al*., 1993; Onofri, 1999). In extreme environments such as Antarctica, microhabitat and microclimates such as the cracks in rocks that endolithic communities inhabit are of particular importance in determining the distribution and abundance of microorganisms. Along with cold temperature, microorganisms must deal with desiccation, high salt concentration, cycles of freezing and thawing, periods of high and low solar radiation, high wind, low humidity, short duration of water availability, plus substratum instability (Cameron *et al*., 1968; Wynn-Williams, 1990) leading to restricted microbial populations in the essentially mineral soils.

Organic soils influenced by plants or animal life are of extremely limited occurrence on continental Antarctica and more common in maritime and sub Antarctic regions but microbial abundance is similar in Antarctic organic soils to that found in the equivalent soils elsewhere (Roser *et al.*, 1993, 1994).

The most pronounced biological activity probably occurs in surface soils warmed briefly by the summer sun and moistened by transient melt water. Antarctic soils support the simplest naturally occurring biotic communities anywhere in the world. These communities are simple in respect of the numbers of species present and the interactions that occur within the communities when compared with more complicated species rich communities found elsewhere (Block, 1984). Thus, they provide a unique opportunity to study whole communities and gain insights that might be applicable to more complex and less easily understood microbial communities. Temperature is the major factor in controlling the presence of liquid water, and in turn is a major factor in determining whether a substrate or an area is inhabited by microorganisms (Baross and Morita, 1978). Decomposition processes and microorganism activity in many Antarctic soils are largely inhibited by cold and aridity. Slow losses of organic matter are reported from ornithogenic soils by Campbell and Claridge (1966) and must be correspondingly greater in locally moist areas. Moisture availability in the form of liquid water is determined by frequency and length of exposure to solar radiation, air temperature, drying winds, exposure, slope and drainage of terrain and valley orientation (Cameron and Conrow, 1969).

Soil can affect survival and growth of organisms. Most soils of Antarctica contain an elevated level of salts, an unbalanced ionic composition and unfavourable

concentration of water soluble trace elements. High concentrations of salt limit the microbial population that can survive in these environmental niches (Cameron and Conrow, 1969). In contrast, high levels of soluble phosphorous and nitrogen encourages high productivity in microorganisms during the summer periods when moisture and temperature conditions are more favourable.

Using the Dry Valleys as a model, Cameron and Conrow (1969) indicated that the abundance, distribution and diversity of microbiota in soils, along with changes in moisture status, can be used as indications of the Dry Valley maturity. The likely ecological sequence taking place in the Dry Valleys is that moist and youthful soils possess a developing microflora that is enhanced due to favourable conditions but this population dies out or is reduced in abundance, distribution and diversity as the soil becomes more desiccated and reaches comparative maturity in the cold barren, windswept arid Antarctic desert areas. McKelvey Valley which was classified as the most mature soils in this study had the lowest microbial diversity compared with Wheelers Valley which was classified as the youngest soils in this study had the greatest microbial diversity.

Microorganisms exhibit three strategies for successful growth, reproduction and survival when faced with the harsh Antarctic environment and many species demonstrate different strategies in different niche dimensions (Vincent, 2000), as follows:

- Specialists that occupy narrow niches and have a competitive advantage relative to other colonising species e.g. having a high affinity nutrient transport system that allows growth at low substrate concentrations or fast

growth rates that allow them to withstand greater losses by predation and other removal processes.

- Generalists that grow suboptimally but survive because of their tolerance to environmental extremes.
- Generalists that occupy broad niches with periods of optimal and suboptimal growth and acclimation.

1.4.2.2 Introduced/Indigenous/Endemic Microorganisms

One of the major considerations that face microbiologists and mycologists studying Antarctic microorganisms is determining whether these organisms are indigenous, endemic or introduced to Antarctica. Antarctic microbial ecosystems provide useful models for general questions in evolutionary ecology, given the relative isolation of the region, the severe biological constraints imposed by the harsh environment, and the absence of higher plants and animals. Endemic organisms are defined as organisms that are only found in the place of interest, while indigenous is defined as originating from the place of interest, or present at the time of first human occupation, as opposed to exotic or introduced which are defined as bought into the place of interest by humans, or that have self introduced since the first human occupation. Areas such as Lake Vostok, located in the heart of Antarctica (78.5ºS, 106.8ºE), under the East Antarctic ice sheet, is a sealed environment due to the thick layer of ice that has accumulated over the top of the lake which has prevented introduction of new material. This lake provides a unique, natural culture collection of endemic microorganisms that have been isolated from the global gene pool over timescales (over 400,000 years) of evolutionary significance (Abyzov, 1993). Lake Vostak's ice sheet is providing insight into the introduction and evolution of microorganisms into the Antarctic environment and has acted as a collection depot for airborne propagules for over

400,000 years. Microbiological studies of cores drilled through the ice from 4.5 km above sea level to 1 km below sea level showed the presence of viable species including some unique to Antarctica (Abyzov, 1993). The diversity of organisms throughout the core has varied over time, indicating different introductions at different periods.

Most Antarctic environments, unlike Lake Vostok, however continue to receive microbial propagules from outside the Antarctic region as indicated by spore trap data of the air (Marshall, 1997), the microflora found in Antarctic snow and ice, the colonising taxa at geothermal sites on Mount Erebus, and the high frequency of apparently cosmopolitan species in most habitats (Kerry, 1990b; Azmi and Seppelt, 1998). Differences in environmental stability and selection pressure among environments are likely to influence the degree of adaptive radiation and microbial endemism. If microbial endemism (genotypes of microorganisms specific to geographical region (Vincent, 2000)) is possible, then Antarctica is one of the most likely regions for such organisms because of the isolated and extreme nature of the environment, reduced human influence, lack of transport into and around Antarctica, and few animals. The Antarctic environment is therefore an ideal ecosystem to examine the evolutionary processes that can give rise to microbial speciation.

There is evidence of endemic species in highly specialised niches on the continent such as the endolithic habitats and lake biomats in the McMurdo Dry Valleys of Antarctica. Selbmann *et al.* (2005) reported on the isolation of black, meristematic fungi, *Friedmanniomyces* sp. and *Cryomyces* sp., from cryptoendolithic lichen-dominated communities in Antarctic rock samples collected from Linnaeus

Terrace, McMurdo Dry Valleys. *Thelebolus globosus* Brumm. & de Hoog and *Thelebolus ellipsoideus* Brumm. & de Hoog are newly described fungi from the biomats of the McMurdo Dry Valleys and both are endemic to Antarctica according to de Hoog *et al*. (2005).

According to Vishniac (1996), to characterise a species as indigenous requires one or more of the following:

- the presentation of evidence of visible growth *in situ.*
- unique occurrence of the species with adaptation to the environmental conditions.
- occurrence of adapted species in excess of probable immigration numbers.

Uydess and Vishniac (1976) demonstrated in situ growth of bacteria in McMurdo Dry Valley soils indicating that some of the viable forms are indigenous. Vishniac and Hempling (1979) came to the same conclusion using the growth of the Antarctic yeast *Cryptococcus* spp., *Sporobolomyces* spp. and *Tilletiopsis* spp.. Atlas *et al*. (1978) reported that high levels of yeast isolated from Antarctica soils were due to the yeasts increased ability to survive after spore deposition from the air. Atlas *et al*. (1978) also stated the yeasts have an psychrophilic-psychrotrophic nature, which assists them to withstand the cold desiccation conditions of Antarctica as well as to grow during the diurnal freeze thaw cycles at the soil surface during the Antarctic summer. Their studies which involved sampling soils from 44 areas including the Dry Valleys and Tran Antarctic Mountains concluded that yeasts were more abundant in coastal soils where moisture contents were higher and in areas of high human impact and plant growth.

The invasion process for Antarctic microbes are more varied than for larger biota and includes atmospheric circulation, and vectoring by birds, fish, marine mammals and humans. Once established within the Antarctica environment, microorganisms can be redistributed by these same vectors. Arguably mosses may be the closest to microbes for introductions, Skotnicki *et al*. (1998) reported the finding of a single colony of *Bryum pseudotriquetrum* near Lake Fryxell that was identical to shoots from another turf some 40 km away at Cape Chocolate, indicating that mosses can be dispersed by the strong winds prevalent in the region, and that when environmental conditions are favourable enough for growth to occur, it is possible for moss propagules to colonise new areas. Spore trapping by Marshall (1997) on Signy Island showed that high concentrations of viable, locally derived propagules from plants infected with fungi were present as the Island is partially covered in vegetation.

Surprisingly, the volcanic areas of Antarctica contain very similar diversity of microorganisms as other volcanic areas around the world (Broady *et al*., 1987). In evolutionary biology, convergent evolution describes the process whereby organisms not closely related independently evolve similar traits as they both adapt to similar environments (Waterman, 1999). This similarity may be due to the unique soil and environmental conditions associated with volcanic environments such as low nitrogen and phosphorus levels, high levels of potentially toxic chemicals, high pH, and consistent temperature (Bargagli et al., 2004). Local cold propagules may find it to difficult to colonise this soil type due to the unique soil and environmental conditions.

The migratory nature of Antarctic birds, fish and mammals make them ideal vectors to introduce foreign microorganisms into Antarctica. *Geomyces pannorum* was shown to be dispersed around Signy Island on skua feathers (both on the skua and in molten feathers) and seal skins (Marshall, 1998). McRae *et al.* (1999) sampled Antarctic bird nests at Windmill Islands, and concluded that *Penicillium* sp. were more commonly isolated from areas inhabited by birds rather than areas where plants were present. They offered two explanations, one the increase in nutrients enhanced soil inhabiting *Penicillium* sp. growth or that *Penicillium* sp. were transported to Antarctica on the body and feathers of the birds. Siegfried (1981) estimated that bird materials contributed 0.4 tonnes (dry weight) ha $^{-1}$ year $^{-1}$ onto coastal lowlands. In areas of bird nesting this is estimated to be a lot more. Melick and Seppelt (1994) estimated that mosses and lichens contributed 2.4mg grm^{-1} sugars to the soil.

Climatic constraints and geological isolation are largely responsible for the present day low biodiversity and structural simplicity of the Antarctica ecosystem (Kennedy, 1995). Global warming has been suggested to be assisting in the survival of microorganisms in the Antarctic climate (Frenot *et al.*, 2005) and may also affect biodiversity in the future. The long term effects of global warming include reduction of the ice cover, leading to increased rock weathering, increased egression of water, melting of permafrost and increased soil activity. Antarctica is known for decreasing biodiversity the further south the latitude. As global warming takes affect, it is expected that species which are at present not found at the southern latitudes will begin to inhabit these latitudes. Many plants are constrained by the Antarctic environment and display dwarf, cushions and prostate growth forms but with global warming it is expected that they will take

on tall faliose, canopy and hummock-forming habits. As the temperature warms, if global warming is verified in the future, there will be many changes to the ecosystem functioning both negative and positive, plants will increase productivity but the nutrient limited soils of Antarctica will limit production. There will be an increase in decomposition rate due to increased microbial activity with the warmer climate to offset the nutrient limits but this will be affected by water levels and possible waterlogging of the soil could lead to reduced decomposition rates (Kennedy, 1995).

Global warming is in part a consequence of a reduction in the ozone layer in the atmosphere; ozone is the major component that adsorbs ultraviolet (UV). UV is known to be adsorbed by deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins and causes fatal damage to cells. Therefore it can be hypothesised that organisms which have the ability to prevent or repair UV damage will dominate the Antarctic ecosystems. An increase in atmospheric carbon dioxide (CO_2) is a consequence of global warming, increased CO_2 increases plant productivity but there must be other resources available to sustain long term increased productivity. Atmospheric green house gases concentration will not only affect the surface temperature but they will also affects the amount of precipitation. Cold air is not able to hold as much moisture as warm air so as the air warms the amount of water vapour in the air will increase. In Antarctica, it is expected that the water availability will increase leading to increasingly complex communities developing, changes in the ecosystem functioning and generally increased biological activity.

There are a large variety of cosmopolitan species being isolated and identified from habitats within Antarctica which also suggests airborne introduction. There is a growing pool of evidence that micoorganisms are arriving in Antarctica by atmospheric circulation. Much of the evidence is from the Antarctic Peninsula where pollen from South American plants has been found and spore trapping detected intermittent arrival of spores from other regions when wind conditions were appropriate (Marshall, 1997). Another indicator of atmospheric invasion is the biodiversity in the ice shelves, spores and cells from species of microbiota that have not been found as living cells within Antarctic ecosystems have been found trapped in layers in the ice shelf .

An increasing human presence in Antarctica has accelerated the potential arrival of foreign microoroganisms. The building of bases, increased logistics, importation of supplies, and disposal of rubbish increased the chances of introduction of new organisms. This includes the potential introduction of infectious diseases which can affect Antarctic mammals and birds. Once a microbial propagule arrives at a potential habitat in Antarctica, the environment is likely to exert a strong selection pressure. As Antarctica is open to such invasion from other regions, it is natural that microbiota will evolve within the environment to become new endemic species or via gene transfer to displace the already endemic organisms (Vincent, 2000). Two different views exist on the likelihood of endemic organisms. One is that everything is everywhere and that environmental conditions select what survives and proliferates; this view implicates that endemic organisms are rare (Staley and Gosink, 1999). The counter view is that the Antarctic region is more isolated than other parts of the world, that it is aerobiology differs from elsewhere, that local dispersal processes

favouring local species are more efficient than long range dispersal and that there has been environmental selection for specific adaptive strategies over a period of several million years leading to endemic Antarctic organisms (Vincent, 2000). Very little is known about either the levels of endemicity in the various microbial groups present in Antarctica or the presence or population trends of alien species. It was within the cryptoendolithic microbial communities, that the first endemic Antarctic fungal species *Friedmanniomyces endolithicus* anam.-gen. and *Friedmanniomyces endolithicus* sp. nov. were isolated (Onofri *et al.*, 2000).

Molecular analysis is allowing for more in depth investigation into origins and differences between Antarctic organisms and similar organisms in different regions. The comparison of Antarctic organisms DNA sequences with DNA sequences of other organisms contained in databases is leading to confirmation of the identity of Antarctic organisms and highlighting relativeness between Antarctic species and species from other regions. The more DNA sequences, from a variety of location and regions of the DNA strand analysed increases the accuracy of this type of analysis compared with the analysis of a small part of ribosomal DNA sequences.

Despite Antarctica's isolation, introduced microbes, fungi, plants and animals occur on most of the sub-Antarctic islands and some parts of the Antarctic continent. These have arrived over approximately the last two centuries, coinciding with human activity in the region. They have both direct and indirect impacts on the functioning of species in the limiting Antarctic ecosystems including substantial loss of local biodiversity and changes to ecosystem processes. Successful biological invasions are amongst the most significant threats

to biodiversity, posing both a significant threat to individual species and being responsible for major changes to ecosystem structure and functioning. Their extent and significance are likely to increase with global environmental change.

Biodiversity is another concern and the dangers of importation of microorganisms into Antarctica and the movement of these organisms between different parts of the continent was recognised (Wynn-Williams, 1996) but there have been few attempts to quantify or minimise the risk or assess the impact on native microbial floras. Azmi and Seppelt (1998) reported a total of 35 taxa of fungi from the Windmill Island of which 12 were restricted to soils in the vicinity of Casey Station suggesting their introduction were associated with human activities. Kerry (1990b) reported 20 taxa from Vestfold Hills and MacRobertson Land of which 10 were most common in sites affected by human activities and their presence was interpreted as a result of human activities. Human microorganisms discharged with raw sewage from Antarctic bases have been located in the surrounding marine environment and sea ice. Lisle *et al*., (2004) traced the distribution of *Clostridium perfringens* (an indicator organism for faecal contamination within an environment) from Antarctica's largest base, U.S. McMurdo station to just beyond 400 m from the base's sewage outfall noting that the concentration of bacteria decreased with sediment depth and distance from the outfall, but, nonetheless, *Clostridium perfringens*, was also found in the intestines of marine animals within the 400 m zone.

1.4.2.3 Cold temperature microorganisms

1.4.2.3.1 Cold temperature survival mechanisms

All living organisms possess the ability to respond to modifications in temperature and to cope with new thermal conditions (Thieringer et al. 1998). The response of organisms to stress will determine their ability to adapt and counter changes in the environment. A sudden reduction or increase in temperature will lead to a stress response, in many systems demonstrably followed production of shock proteins and continued exposure will lead to an adaptive response, as long as the drop or increase in temperature is not so large to cause death to the organism (Graumann and Marahiel, 1996). Many cold adaptive strategies have been reported in a variety of organisms including fish, bacteria, plants, algae and fungi. Living at cold temperatures, therefore, requires a multiplicity of crucial adaptations. Organisms that have adapted to the cold have been classified as psychrophiles or psychrotrophs. The definition of a psychrophile has been a topic of many publications. One of the most often quoted papers is Morita (1975) which defines psychrophiles as organisms having an optimal growth at about 15°C or lower, a maximal temperature for growth at about 20°C and a minimal temperature of growth at 0°C or below, while psychrotrophs differ from psychrophiles with maximal growth temperatures above 20°C. Cold adapted mesophiles are classified as psychrotrophs.

Sudden reductions in temperature cause the synthesis of cold shock proteins (Csps) and cold acclimation proteins (Caps) (Panoff *et al.*, 1998). Cold stress responses in organisms occur in two phases, a transient shock response and a continuous acclimation response. Csps are often the first response to a temperature change and are considered to be the beginning of cold-adaptation.

They are not restricted to psychrophiles and psychrotrophs and are also present in mesophiles and thermophiles when temperatures are reduced. The main function of Csps relates to the regulation of protein synthesis and messenger ribonucleic acid (mRNA) folding. The number of Csps synthesised increases with the severity of cold shock and analysis of genes reveals they have different characteristics (Georlette *et al.*, 2004). A major distinction between psychrophiles and their mesophilic and thermophilic homologies, is that psychrophiles synthesis house keeping proteins that are not affected by Csps; the number of Csps is high and proportional to the severity of the cold shock (Gounot and Russell, 1999). Often the synthesis of Csps is seen with a reduction of synthesis of so called "house keeping" proteins such as RNA polymerase, 6-phosphofructokinase, pyruvate kinase and phosphoglycerate kinase especially with mesophilic and thermophilic organisms. Not all Csps, though, are cold induced, and in *E.coli* only three (CspA, CspB and CspG) of the nine Csps are cold induced; CspD is induced at the onset of the stationary phase and during starvation and CspC and CspE are involved in cell division and the condensation of the chromosome at 37°C (Yamanaka, 1994). Csps function as RNA-chaperones in the regulation of translation. Binding of Csps to RNA is cooperative, non specific and prevents the formation of secondary structures thereby keeping RNA linear which is essential for efficient initiation of translation.

In addition to the Csps, psychrotrophic bacteria can express a second class of proteins, cold acclimation proteins (Caps), during balanced growth at low temperatures. These proteins are rarely seen in mesophilic organisms, and they play an important role in the physiology of cells during cold adaptation. These proteins are involved in maintaining metabolic functions at low temperature by

replacing cold-denaturated peptides. A low-temperature-specific proteolytic system has been described (Berger *et al.*, 1996), and Caps act as cold-specific proteases that eliminate denaturated proteins whose accumulation would be deleterious for the cells. Others are involved in maintaining membrane fluidity at low temperature or act either as antifreeze proteins or as anabolic enzymes involved in the synthesis of putative antifreeze substances. Caps are often seen later during continuous growth at cold temperatures and appear to be synthesised for a longer period of time (Phadtare *et al.*, 1999).

Lipid composition in the cell wall is central to survival in the cold as the membrane bilayer must remain in a fluid state to enable normal cellular processes including uptake of nutrients, transport of various compounds, electron and proton transport, photosynthesis, environmental sensing and recognition processes (Langworthy, 1982). Membranes are normally in a liquid crystalline form and will undergo a transition to a gel phase when the temperature drops. To compensate for the transition from liquid crystalline to gel, organisms change the degree of saturation of the hydrocarbon chains in the membrane phospholipids. Phospholipids with unsaturated fatty acids have lower melting points and a greater degree of flexibility than phospholipids with saturated fatty acids. The increase in unsaturated fatty acids in the membrane leads to an increase in membrane fluidity. Microorganisms regulate membrane fluidity in response to low growth temperatures by altering the lipid composition of the membrane to reduce the melting point of its constituent phospholipids (Melchoir, 1982). This reduction in phospholipids melting point is achieved by increasing the degree of fatty acid insaturation which may include the synthesis of polyunsaturated fatty acids (PUFA) (Russell, 1990). An increase in fatty acid insaturation with decreasing

growth temperature was observed for a number of Antarctic microorganisms (Finegold *et al.*, 1990; Fukunaga and Russell, 1990). Nichols *et al.* (2004) tested 38 strains of bacteria, isolated from Antarctica, for their ability to produce long chain PUFA. Five isolates produced long chain PUFA and seven isolates contained at least one other PUFA component. None of the bacterial strains contained as high levels of PUFA as found in alga and fish oil but the levels were higher than found in temperate marine bacteria.

Proteins embedded in the membrane, including key respiratory and transport proteins, function only when the membrane is in the fluid phase, and cease activity on the phase change to the solid state. Whether there is a progression of gradual change in membrane function as temperature decreases prior to the temperature of the phase change, or, whether there is constant full function followed by complete cessation of activity at the phase change temperature, is poorly understood. In virtually all natural environments, vital resources such as energy substrates, and the substrates necessary for growth (nitrogenous compounds, phosphate, etc.) are present at very low concentrations and are usually growth rate-limiting (Nedwell, 1999). Growth and survival depends upon the ability of a species to sequester these sparse resources in extreme competition with other species. If the uptake mechanism for a substrate depends on passive diffusion, then its rate of uptake is likely to be slow and influenced only by the concentration gradient across the membrane, although facilitated diffusion may increase the rate of passive uptake. Active uptake, though, depends upon the presence and activity of transporter proteins in the membrane which can accumulate substrates against a concentration gradient. Continued transporter activity depends upon the membrane maintaining a charged energetic state

required to power conformational changes which carry substrates across the membrane, and their activity can be affected by the fluidity of the membrane. The efficiency of active uptake by a microorganism from its external environment of any substrate at low concentration depends upon the 'affinity' of the organism for that substrate. Organisms are inhibited by lack of substrate at temperatures near their minimum for growth. Growth rates are increased either by a rise in temperature or by addition of substrates. There is an enhanced requirement for substrate near their lower temperature limit for growth of both mesophilic and psychrotrophic organisms. Wiebe *et al*. (1992) reported that four facultatively psychrophilic bacterial isolates when grown at 10°C and 15°C and above, substrate did not have an effect on growth rate but when grown at -1.5°C to 0°C the bacteria had an increased requirement for organic nutrients. Generation times were two to four times longer on low nutrient media than in high nutrient media.

When water is cooled under atmospheric pressure, it can keep a liquid state even below 0°C, which is known as the supercooled state. In the supercooled state, water naturally creates a number of embryo ice crystals known as ice nucleators that assemble water molecules onto their surfaces. The ice crystals become larger and contact with each other to construct a multi-crystalline-state of ice, leading to complete freezing of water. Ice nucleation proteins (INP) remove or inactivate ice nucleators and form on the outer membrane of some organisms (Kawahara, 2002). In contrast, antifreeze proteins (AFP) accumulate on the ice crystal surface to inhibit its further growth of the ice crystal (Sicheri and Yang, 1995). Antifreeze proteins have been reported in many organisms including fish, insects, algae, fungi and bacteria. They prevent the cell contents from freezing by binding to the ice crystals, causing curvature of the ice crystal and ultimately make it

thermodynamically unfavourable for the formation of further ice crystals and protect the cell membrane from cold-induced damage. The presence of antifreeze proteins in fungi is discussed in Section 1.4.2.3.3.

Many organisms that are exposed to the cold have an increase in trehalose or polyols in their cell fluid. Many extracellular enzymes from cryotolerant organisms are secreted with exopolymeric substance to prevent cold denaturation and assist with substrate requirements (Mancuso Nichols *et al.*, 2005).

Every phase of the enzyme catalytic cycle is sensitive to temperature. Generally, as the temperature drops, the speed of chemical reactions that enzymes catalyse also drops. Organisms can increase the speed of the reaction by either producing larger volumes of enzymes, possess enzymes characterised by temperature-independent reaction rates or synthesise cold efficient enzymes. Production of larger volumes of enzyme is rare as it is energetically expensive and difficult to maintain for a whole organism. Permanent adaptations are fixed in genomic DNA, which through the nucleotide sequences determines the amino acid sequences of proteins and their three dimensional structure. An organism cannot deliberately modify these parameters in order to adapt to unusual thermal conditions but it can to some extent modify the overall pattern of protein expressed through the thermal regulation of gene expression (Miyazaki et al., 2000).

1.4.2.3.2 Laboratory based production of psychrophiles

Olsen and Metcalf (1968) converted a mesophilic *Pseudomonas* isolate to a pseudo-psychrophilic *Pseudomonas* by UV mutation, resulting in the minimum temperature for growth lowered to 0°C with a maximum temperature for growth of 32°C, from the mesophilic maximum growth at 44°C there was a drop of 12°C

over the complete temperature range of growth was achieved. They also created psychrophilic tranductants with growth temperature minima and maxima characteristic of wild type psychrophilic pseudomonads from soil. Along with psychrophilic traits, they co-transducted trytophan dependence which confirmed that the growth temperature range was prescribed by a limited number of genetic loci or a closely linked constellation of characters. Although psychrophiles have temperature sensitive enzymes limiting growth much above 33°C it is unlikely that mutagenesis by ultraviolet light or transduction would change the total enzymatic makeup of the cell sufficiently to promote psychrophilicity if psychrophilicity was dependant on a whole array of temperature-sensitive enzymes. They concluded that psychrophilicity and perhaps the delineation of the growth temperature of mesophilic bacteria may reflect the temperature response of the product of a limited number of genetic loci whose primary function is the regulation of cell division in response to temperature fluctuations (Olsen and Metcalf, 1968).

1.4.2.3.3 Cold adaptation in fungi

Aspects of fungal life-history and morphology may be adaptations to cold tolerance. Abbreviated life cycles are found in fungi existing in harsh environments (Robinson, 2001). Physiological mechanisms conferring cold tolerance in fungi are complex; they include increases in intracellular trehalose and polyol concentrations, unsaturated membrane lipids, secretion of antifreeze proteins, enzyme active at low temperatures, acclimation of mycelium and spores, and melanin production in hyphae. Ecological mechanisms for survival include cold avoidance, sterile hyphae, short growth cycles, and fungal spores programmed to germinate annually in the spring and summer thus avoiding the cold months. A combination of these mechanisms may be necessary to survive

and no single mechanism appears to lead to tolerance of low temperatures by fungi (Robinson, 2001). All the components of a psychrophile or psychrotroph must be functional for a fungus to grow at low temperatures and cold adaptation must be an overall cellular phenomenon (Russell, 1990). If the changes in cell constituents are adaptations to cold temperatures there would be a seasonal pattern in these cell constituents. Montiel (2000) studied the concentrations of low molecular weight carbohydrate in winter, spring, and summer in a number of alga, mosses and lichens. Seasonal changes in total soluble carbohydrate concentrations were observed in all species with the concentration of total soluble carbohydrate highest in spring relative to winter. Montiel hypothesised that the relatively high concentration of carbohydrates functions as osmolytes in winter and as physiological buffering agents in spring.

Trehalose is an important storage compound in fungal vegetative cells and spores (Lewis and Smith, 1967) and is the most widely distributed disaccharide in fungi. Trehalose appears to be a general stress protectant in the cytosol and it is known to stabilise membranes during dehydration (Kandror *et al*., 1999). Trehalose is thought to prevent denaturation and precipitation of proteins, functions as a free radical scavenger and stabilises the cell membrane to maintain fluidity (Kandror *et al*., 1999). Accumulation of trehalose in fungal hyphae in response to low temperatures has been noted. *Humicola marvinii*, a psychrophilic fungi isolated from fellfield soil at Jane Col. Signy Island, Antarctica, grown at 5°C and at 15°C in liquid medium accumulated trehalose intracellulary to a greater extent at 5°C than at 15°C (Weinstein *et al.*, 2000). Another fungus from the same study *Mortierella elongata*, psychrophilic fungus, showed intracellular trehalose concentrations at 5°C, 75% higher than at 15°C (Weinstein *et al.*, 2000).

Glycol and mannitol increase in concentration to maintain turgor pressure against heat-mediated decreases in external water potential (Cooke and Whipps, 1993). Mannitol is thought to be important in protection against water stress and as a cryoprotectant. Weinstein *et al*. (1997) used an Antarctic isolate of *Humicola marvinii* to compare with *H. fuscoatra*, a mesophilc isolate, for the production of sugars. After 8 weeks of incubation at 15°C, the quantity of total sugars from the two isolates were not significantly different but when individual sugars and polyols were compared there were differences. *H. marvinii* produced high levels of mannitol (a cryoprotectant) compared with *H. fuscoatra* which produced more glucose and fructose (not known to be cryoprotectants) compared with *H. marvinii* which produced no fructose and very little glucose. Fungi have been reported to produce exopolysaccaride (EPS) in response to environmental stress including freeze and thaw. Selbmann *et al*. (2002) reported the production of EPS by *Phoma herbarum* CCFEE 5080, an Antarctic isolate. When the growth temperature for the organism was increased from 4°C and 16°C to 28°C, there was an increase in biomass but not in EPS production with a maximum EPS level of 12g/l at 4°C and at 16°C and 13.6g/l at 28°C but the time it took to produce maximum amounts of EPS was less at warmer temperatures, taking 192 hrs at 4°C, 120 hrs at 16°C and 96 hrs at 28°C. The ability of *Phoma herbarum* CCFEE 5080 to produce similar levels of EPS over a wide temperature range was possibly due to it being a cold tolerant mesophile. The cryoprotectant nature of EPS was shown by the repeated freezing and thawing of this fungus in the presence and absence of EPS. After four freeze thaw cycles without EPS, the fungal biomass was less, the duration of the lag phase was longer and the mycelia growth rate was slower. EPS were providing protection from the effects of repeated freeze thaw cycles. The production of ice crystals, variations in osmolarity and water

availability mean that freezing is well known to cause stress and consecutive freeze thaw cycles are more lethal for microorganisms than being in a continuous frozen environment (Vishniac, 1993). The structure and composition of membranes is likely to effect the temperature at which their properties change from an inactive gel phase to an active crystalline phase thus determining the ability of fungi to grow over specific temperature ranges. An increase in unsaturated lipid contents in the membrane with lower temperatures was reported by Weinstein *et al.* (2000). A psychrotrophic isolate of *Geomyces pannorum* showed altered lipid composition when grown at 5°C rather than 15°C, with increased unsaturated lipid content and overall unsaturation index. In the same study *Mortierella elongata* showed the presence of stearidonic acid, a fatty acid only reported in psychrotrophic zygomycetes and a lack of ergosterol.

Fungi require the maintenance of an aqueous environment for growth, to secrete enzymes and absorb carbon and nutrients. Extracellular and intracellular antifreeze proteins allow fungi to be active at below zero temperatures and they slow the formation of ice crystals. Antifreeze proteins (AFP) are essential for inhibiting the recrystallisation of ice and promote the survival of fungi through freeze and thaw cycles. In addition to preventing fungi freezing, the secretion of antifreeze proteins will prevent substrates from freezing allowing fungi to use them as nutrients. Antifreeze proteins and activity have been reported in snow moulds. Snider *et al.* (2000) reported on antifreeze activity from isolates of *Typhula incarnata*, *T. ishikariensis* and *T. phacorrhiza* in all fractions (growth medium, soluble hyphal fraction, and insoluble hyphal fraction). These fungi have peak growth temperatures at 10ºC. Interestingly, three other fungi (*Microdochium nivale*, *Sclerotinia borealis* and *S. homocarpa*) isolated from south western

Ontario with peak growth temperatures above 14ºC did not show antifreeze protein activity. The antifreeze activity in the growth medium of *T. phacorrhiza* isolate Tp94614 was shown to arise from protein molecules. The ice crystal structures associated with snow mould species showed different growth patterns from AFP found in fish, insects and plants indicating that the fungal AFP may bind to different planes of the ice crystal lattice.

The growth of psychrophiles and psychrotrophs at low temperatures has led to the search for enzymes with psychrophilic or cold active properties. Knowledge of these enzymes will lead to increased understanding on how these organisms thrive at cold temperatures and applications in the biotechnology industry including cold active proteases in cold water detergents.

From an ecological point, the low diversity of fungal species in soils of continental Antarctica is hypothesised to be offset by a wide variety of enzymes produced by each species (Fenice *et al.*, 1997). Enzyme activity has been found at low temperatures in soil fungi isolated from Antarctica. In a study by Weinstein *et al.* (1997), *Humicola marvinii*, a psychrophile isolated from fellfield soil in Antarctica, demonstrated capacity to solubilise inorganic phosphate and the production of extracellular proteinases at 15ºC while *H.fuscoatra*, isolated from the rhizosphere of *Gossypium* sp. in Nigeria, was not able to do so. Using plate screening techniques, (Fenice *et al.*, 1997) tested 33 strains of fungi isolated from different sites in Antarctica for their ability to produce 12 extracellular enzymes (polygalacturonase, pectinlyase, amylase, cellulase, chitinase, phosphatase, glucose oxidase, urease, proteinase, lipase, ribonuclease and deoxyribonuclease) at 25ºC, or at 20ºC for proteinase activity, or at the species optimum growth

temperature if not 25ºC. Lipase activity was present and in high quantities in almost all strains. Polygalacturonase, amylase, and phosphatase enzyme activities were common and found in 19, 18 and 21 of the 33 strains, respectively. Glucose oxidase, proteinase and deoxyribonuclease (DNAase) were absent or at low levels in all fungal isolates tested. The enzymes from many Antarctic isolates show thresholds of thermal inactivation at 28ºC and optimum temperatures of catalysis of 40-60ºC. These temperatures do not match the low temperature of psychrophilic growth. Cairns *et al.* (1995) concluded from a study of invertase from snow mould (*M. nivalis*) that the invertase demonstrated no cold active properties and resembled invertases from mesophiles. When growth data were considered, *M. nivalis* was metabolically a mesophilic organism. The same mesophilic phenomenon was exhibited by a chitinase of *Verticillium cfr lecanii* isolated from Antarctica with optimal growth of 25°C. The enzyme was active over a broad range of temperatures (5-60ºC), although at 5ºC its activity was 50% of that recorded at the optimum temperature which was 40ºC (Fenice *et al.*, 1998). Potentially, as few as one temperature sensitive limiting factor can confer a specific psychrophilic character which inhibits growth above the low optimum. This factor could be the loss of vital properties of intracellular proteins just above the upper limit of fungal growth (Hoshino *et al.*, 1997). Evidence does not implicate any enzyme as the sole determinate of psychrophilicity or psychrotrophy (Feller and Gerday, 1997).

Fungal acclimation could assist in cold tolerance. In laboratory studies using hyphae from *Fusarium oxysporum* f. sp. *lycopersici*, Robinson and Morris (1984) showed that transferring from 25ºC to 7ºC and holding for 2 hrs made the hyphae more tolerant to subsequent cooling to -2ºC than hyphae that had not been

prechilled. In the field, Addy *et al.* (1994) used blocks of temperate soils containing arbuscular mycorrhizal (AM) fungi which were either slowly cooled (2ºC d^{-1} until soils reached 5ºC) or held at room temperature (20ºC) before freezing at -12ºC. Infectivity of AM fungi was greater in soil that was slowly cooled before freezing. Additionally, *in vitro* studies showed that slowly cooled hyphae before freezing were still active while hyphae that had not been precooled before freezing activity were completely eliminated. Cells can be injured during freezing by endocellular ice crystal formation or dehydration which corresponds to hypothermal and osmotic stress (Mazur, 1984). Thammavongs *et al.* (2000) investigated the phenotypic adaptation of *Geotrichum candidum* to freeze thaw stress. Freeze resistance was greatest in the stationary phase of growth. Pre-incubation at chill temperatures lead to improved survival capacity to freeze thaw challenges. The longer the pre-incubation, the bigger the improvement in freeze thaw survival.

Flanagan and Scarborough (1974) wrote "the biomass of psychrophiles may vary seasonally from low values in spring to higher values in autumn," indicating that increased biomass before cold temperatures may increase the chances of a fungus surviving the winter period.

In many cold environments there is a predominance of fungi with dark melanised hyphae. Melanins are a common group of dark-brown to black pigments found among animals, plants and microorganisms and are thought to protect cells from UV damage owing to their strong adsorption in the UV region of the spectrum (Butler and Day, 1998). These pigments are not essential for growth and development, but rather they enhance the survival and competitive abilities of

species in certain environments (Bell and Wheeler, 1986). A high proportion of melanin-producing microorganisms have been associated with environmentally stressed areas such as deserts, alpine areas and the upper bioshere. Whether this is a survival strategies is still unsure. Evidence that presence of melanin is a survival strategy can be found in the fact that fungi with dark septate hyphae dominate the soil microbial communities of Antarctica (Smith and Reid, 1997). Melanin may protect dark septate hyphae from extreme temperatures and drought and so broaden the ecological niche of these fungi (Jumpponen and Trappe, 1998).

Fungal survival in cold environments may occur because of cold avoidance by seasonal dormancy rather than cold tolerance. One possible method of cold avoidance is for the fungus to re-establish annually in spring or summer from a cold-stable spore. This is more likely in maritime rather than continental Antarctica as positive air temperatures are experienced rarely and locally and at inland sites the mean daily temperature rarely rises above -10ºC (Convey and Block, 1996) although soil temperatures may be considerably higher. Vishniac (1996) wrote "It is generally thought that unprotected hyphomycetes hyphae do not survive freezing while spores often do". Limited research has been done on whether this is occurring in Antarctic fungi or whether spores can survive the cold periods to re-establish in warmer times. Greenland ice cores studied by Miteva and Brenchley (2005) showed that viable fungi could be recovered from a section of the core at 3,042.67 to 3,042.80m below the surface, but they were not studied. Willerslev *et al*. 1999 studied 2000-4000 yr old ice core samples from North Greenland using molecular techniques and of the 107 clones sequenced, 66 clones were assigned to fungal classes, Ascomycetes and Basidiomycetes.

Cold avoidance by Antarctic fungi could be by recolonisation in spring from spores or hyphal fragments dispersed from warmer climates. Long and short distance transport of fungal propagules is possible, although the number of propagules is low. Some fungi in Antarctica may survive by avoiding the extremely low temperatures during winter through annual germination from air spora during spring and summer. One possible flaw in this theory is that spore production in warmer climates often occurs at the end of the growing season, for instance autumn, which is a time when Antarctica is cooling down and conditions are not likely to be favourable for fungal spore germination. The production of spores is often a very efficient way for organisms to over winter and they may germinate from spore annually when conditions are favourable.

The number of spores produced by psychrophilic or psychrotrophic fungi could be another indicator of cold avoidance. By producing larger numbers of spores at lower temperatures, fungi increase their chances of at least the strain surviving through the colder temperatures. A study completed by Bertolini and Tian (1996) using a temperate isolate of *Penicillium hirsutum* showed that sporulation and germination were retarded at low temperatures and at -4ºC no germination occurred. Similar studies have not been reported with psychrophilic or psychrotrophic fungi.

1.4.2.4 Survival strategies to other extreme environments found in Antarctica in addition to temperature

The ability to adapt to the cold is not going to guarantee an organism's survival in Antarctica and there are many other factors that are going to affect its ability to survive, including low nutrients, high salt, lack of water, high UV levels. The soils of Antarctica are low nutrient environments which have scant and unpredictable rainfall and are poorly developed. These soils are low in organic matter and

available water and range from acidic to strongly alkaline on the surface. In habitats with low nutrients solid surfaces play an important role and have a strong effect on colonisation resulting in the formation of microbial films and layers. Oligotrophs are organisms that are capable of growth in a medium containing 1.2-16.8 mg of dissolved organic carbon per litre. The main characteristics of oligotrophic organisms include substrate uptake systems that are able to acquire nutrients from its surroundings; ideally, they have large surface area to volume ratio, high affinity uptake systems with broad substrate specificities and a resistance to environmental stress. Many microbes that have adapted to low nutrient environments produce appendages to enhance their surface area (Satyanarayana *et al.*, 2005).

Hypersaline evaporation ponds are present within Antarctica, several of which are stratified with respect to salinity. The freezing of ponds during the winter leads to pools containing high levels of saline water in the bottom. Also, within Antarctica, there are soils which have visible salt deposits on the surface which are less saline than the lakes but still saline enough to cause problems for organisms within the habitat. Halophilic organisms that tolerate high salt concentrations produce or accumulate compounds for osmoregulation including proline, oligosaccarides, glycine betaine, trehalose and *N*-acetylglutaminyglutamine amide.

Water activity (A_w) has been determined as a limiting factor for microbial growth. The environment in Antarctica is very dry due to a lack of precipitation, most of the available water is frozen, and the presence of salts within soils acts as a dessicant. Xenophilic organisms, growing in areas of low available water, produce

capsular material which increases the surface area over which or within which water could be absorbed and held over periods when water is not available allowing the organism to maintain themselves within a well hydrated environment. These capsules also allow the cells to aggregate and benefit from cross feeding and use dead cells for nutrients (Uydess and Vischniac, 1976).

During the summer, Antarctic organisms are exposed to high UV radiation. Microorganisms exposed to high levels of UV have four strategies to prevent damage; the first is to produce compounds that screen their cells from harm including flavonoids, sheath pigments and mycosporine like amino acids. A second strategy is escape from the UV by sinking deeper within the habitat. The third strategy is to produce quenching agents like carotenoids or enzyme systems such as superoxide dismutase that react with and neutralise the highly toxic reactive oxygen species produced by UV exposure. The final strategy is a variety of repair mechanisms to restore the cellular components after UV damage (Quesada and Vincent, 1997).

Cryptoendolithic fungi have developed special adaptations such as very slow growth rates, growing at very low temperatures and production of antibiotics to control algal growth (Onofri *et al.*, 2000). In a study by Onofri *et al.*, (2000) the production of extracellular enzymes pectinase, lipase, proteases and glycosidase was measured in three strains of cryptoendoendolithic fungi (*Pseudozyma* sp. CCFEE 5013, *Xylohypha* sp. CCFEE 5018 and a meristematic strain CCFEE 5001) over a temperature range of 0ºC to 27ºC. *Pseudozyma* sp. showed good proteinase and pectinase activity both at 10ºC and 20ºC and lipase activity at 10ºC, 20ºC and 27ºC while *Xylohypha* sp. showed protease activity only in the

range 10ºC to 27ºC. Strain CCFEE 5001, while growth was barely detectable, gave consistent glycosidase activity over all temperatures tested.

To maintain physiological processes at cold temperatures, psychrophilic organisms must compensate for reductions in molecular movement by either increasing the intracellular number of molecules, the efficiency of biochemical reactions/processes or by modifying the cellular environment. Psychrophilic organisms raise adenylate levels as temperature decreases. Metabolic rates measured by oxygen consumption appears to be elevated in some cold adapted organisms suggesting at least some level of metabolic compensation at low temperatures (Sommer and Pörtner, 1999). Elevated respiration rates can be explained by well documented increases in mitochondrial density at low temperatures (Johnston *et al.*, 1998). There is also evidence for compensation of enzymatic activity in some energy related pathways (Crockett and Sidell, 1990). These changes are likely to enhance the survival of cold-adapted organisms.

1.4.2.5 Cold adapted enzymes

Just as organisms have to adapt to their changing environment, cold adapted enzymes have features enabling them to function at low temperatures. Decreasing temperature has many effects on the aqueous system in which enzymes operate, including increased viscosity of water, reduced diffusion of solutes, reduced salt solubility, increased pH in biological buffers and an exponential drop in reaction rate due to the reduced thermal motion of the enzyme resulting from cold temperature (Marshall, 1997). The biggest challenge facing an organism living in a constantly cold environment is to maintain an adequate metabolic rate allowing optimum utilisation of nutrients, therefore, preventing the development of stress. The importance of this is illustrated by the fact that the rate of chemical reactions

is exponentially depending on temperature. A decrease in temperature by 10°C will cause a decrease in the reaction rate by a factor of 2 to 3 (Feller *et. al*., 1996). In the absence of any molecular adaptation, the rate of chemical reactions occurring in organisms living at temperatures close to 0°C would be depressed by a factor of approximately 10 when compared to growth at mesophilic temperatures. Psychrophilic enzymes have alterations to their molecular structure to create more plasticity of the structure leading to energy efficient accommodation of substrates (Gerday *et al*., 2000). They appear to be more sensitive to denaturing agents due to a weakening in intramolecular forces making them less stable but more flexible.

Possible structural changes to cold adapted enzymes include (Zecchinon *et al.*, 2001):

- A decrease in salt bridges, Hydrogen bonds and aromatic interactions, arginine content or proline residues in loops.
- A decrease in stabilisation of α helix dipoles.
- An increase in the clustering of glycine residues.
- Insertion or deletion of loops responsible for specific properties.
- Decrease in the density of charged residues at the surface of the protein.
- Increase in the density of hydrophobic residues exposed to solvents.

Proteins can contain one of these changes or many of these changes. The main physiological adaptation seen in psychrophilic enzymes is a higher specific activity at low temperature. They tend to have an apparent maximal activity shift. Once the temperature decreases, a lack of flexibility creates a selective pressure.

The selective pressure is directed toward the selection of an enzyme that shows good complementarity with the substrate at low energy cost.

Cold adapted enzymes have three basic features in common (Zecchinon *et al.*, 2001):

- Specific activity up to tenfold higher at low temperatures.
- An apparent maximum activity temperature shift towards low temperatures.
- A specific activity around 0°C which is never as high as that displayed by mesophilic counterparts at their environmental temperature showing that the adaptation to cold is not complete.

A cold adapted enzyme will be more flexible but on the negative side they are more heat labile and their stability will be in accordance with the low thermal energy of their natural environment. Two general strategies for increased flexibility of cold adapted enzymes have evolved, as follows:

- A general decrease in stability of the protein giving rise to an increase in overall plasticity of the molecular edifice which is probably required for the accommodation of macromolecular substrates. An example is cold adapted α amylase (Feller *et al.*, 1999).
- Evolution of the domain of the protein into a highly flexible unit while keeping another domain much more rigid, this in order to secure an appropriate affinity (K_m) of the protein for small substrates. An example is cold adapted phosphoglycerate kinase (Bentahir *et al.*, 2000).

Previous studies of the temperature adaptation of enzymes have focused on two properties, the Arrhenius Activation Energy, which is the barrier of the reaction

(Feller and Gerday, 1997) and the thermal stability of enzymes i.e. the ability of the enzyme to retain a functional conformation against increasing temperature. A combination of these properties has sometimes been presented as the temperature optima of the enzyme but this is not a biochemically valid parameter, being a result of assay duration as well as these properties. As Daniel *et al.* (2001) stressed in a graph of enzyme activity vs. temperature, the ascending limb results from the temperature coefficient of catalytic rate (i.e. the Arrhenius Activation Energy) until denaturation becomes significant then activity decreases from a combination of denaturation and thermal stability. Because the contribution of denaturation is dependent on assay duration as well as temperature the apparent optimum is shifted to lower temperatures for longer assays. To describe a true temperature optimum, a new model of thermal activation was described by Daniel *et al.* (2001) where enzymes can be in three states: active (N), inactive (I) and thermally denatured (D) i.e. N↔I→D. This means that temperature also affects the equilibrium position, between the inactive and active protein forms in addition to its other effects on activity. Therefore, even at zero in an enzyme activity vs. temperature plot when denaturation could have taken place, the enzyme will show a temperature optimum (Daniel *et al.*, 2001). This equilibrium model contrasts with the classical model which shows no optimum temperature of activity at zero time but an infinite increase in enzyme activity. Studies which have taken these factors into account show that some enzymes have real temperature optima. Thomas and Scopes (1998) showed that k_{cat} values for mesophilic and thermophilic 3-phosphoglycerate kinase reached a maximum and then decreased with higher temperatures before irreversible denaturation had any significance.

Laboratory based mutagenesis studies have shown that activity of an enzyme at low temperatures can be achieved without loss of thermal stability seen in nature. Zecchinon *et al.*, (2001) suggested that the decrease in stability of naturally cold adapted enzymes was due to a random genetic shift during divergent evolution from a mesophilic ancestor facilitated by the flexibility requirement rather than to a physical or chemical incompatibility between two properties. Feller and Gerday (1997) suggested that the thermal instability of cold adapted enzymes was negative selection; in cold adapted organisms, highly stable enzymes would be resistant to turnover by normal cellular degradation mechanisms and therefore, accumulate and ultimately be harmful to the organism. A fundamental requirement for all living organisms is the necessity to balance energy production with consumption. The rate of biological processes generally increases with rising temperatures in accordance with the Arrhenius principal, until a threshold is reached (Pörtner *et al.*, 1998).

1.4.3 Wood cell structure

Wood cell walls can be described as a composite of cellulose microfibrils embedded in an amorphous matrix similar to reinforced concrete but in reality there is a lot of matrix included in the cellulose microfibril and cellulose side chains associated with the matrix. Microfibrils have a structural role in the cell wall, imparting strength and contributing to its size and shape. Matrix material consist of hemicelluloses that are further associated with pectins and proteins in primary cell wall, and with lignin in secondary cell walls. Lignin is connected to hemicellulose by covalent lignin-carbohydrate bonds involving ester or ether linkage connections (Jeffries, 1990). Kerr and Goring (1975) proposed a model of a cell wall as a matrix of lignin and hemicellulose encrusting cellulose

microfibrils, distinguished into five cell wall layers (Figure 1.3). These five layers are the middle lamella, the primary wall and a three layer secondary wall. The individual cell-wall layers differ mainly in their fine structure, in orientation of the microfibrils and their chemical composition. In all plant material, neighbouring cell elements are connected by the middle lamella. The middle lamella consists of pectin and lignin which acts as a cementing substance to connect neighbouring cells to each other. The middle lamella impacts strength and stiffness to the cell wall.

The primary cell wall is often hard to distinguish from the middle lamella but is different in composition, and is a frame work of cellulose fibrils. The fibrils run scattered mainly transversely to the axis of the cell (Eriksson *et al.* 1990). In primary cell walls, xyloglucans form the interface between the microfibrils and the wall matrix.

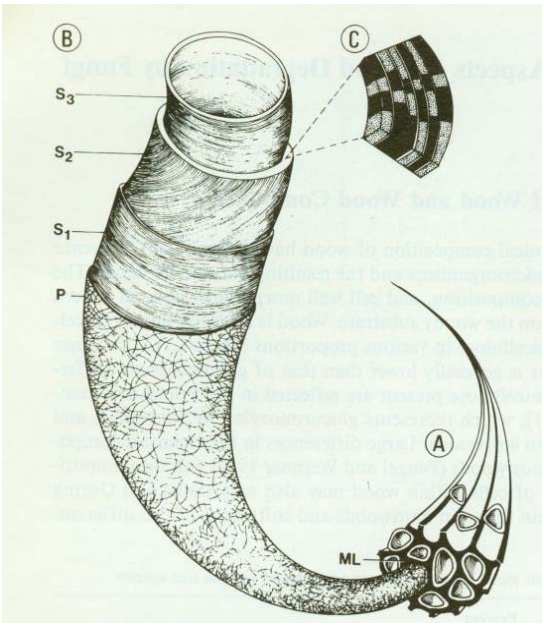

Figure 1.3: Diagram of the structure of cell wall layers in tracheids and ultrastructure arrangement of lignin and carbohydrates in the secondary wall (Eriksson *et al.*, 1990). (A) Traceids (B) Cell wall layers (C) Ultrastructure arrangement of lignin and carbohydrates in the secondary wall, redrawn from Kerr and Goring 1975; black: lignin-hemicellulose matrix; white hemicellulose; stippled: cellulose fibrils. ML: middle lamella; P: primary wall; S_1, S_2, S_3: layers of the secondary wall (Eriksson *et al.* 1990).

The secondary cell wall forms the largest part of the cell wall. Cellulose forms up to 94% of the defining chemical substance. It imparts high tensile strength to the cell. The secondary cell wall has a pronounced layer structure usually in the form of an outer (S_1), middle (S_2), and an inner (S_3) secondary wall (Figure 1.3). The thickness and arrangement of its cellulose fibrils differ in each layer (Figure 1.3). The S_1 cell wall lies next to the primary cell wall. Its cellulose fibrils exhibit a weak parallel arrangement, being orientated approximately transversely to the longitudinal axis of the cell. The S_2 cell wall forms the bulk of the cell wall. The cellulose microfibrils run parallel to each other in a shallow spiral in the direction of the cells longitudinal axis. Concentrically arranged individual lamellae composed of cellulose, lignin and hemicellulose follow one another, to form the S_2 cell wall. Zimmernann and Sell (1997) showed a different structure of the S_2 layer with the cellulose microfibrils displaying a radial orientation relative to the longitudinal axis of the cell and transverse to the middle lamella. In secondary cell walls the hemicellulose consists mainly of xylans, mannans, and glucomannans. The S_3 cell wall separates the cell wall from the lumen. The cellulose microfibrils are arranged in either parallel or slightly scattered and have a similar texture to primary cell wall material. It has less cellulose than the S_2 layer but exhibits a high degree of lignification.

1.4.4 Wood decay

Resistance to decay varies within a piece of timber or even from where the wood was taken from in a tree. The type of cell, chemical composition and cell wall morphology all affect the way enzymes attack the wood structure. Sapwood is most susceptible to decay, compared with heart wood, due to higher concentrations of resin acids and phenolics in the latter. Heartwood is the dark

region in the centre of a tree, where the wood cells which use to be sapwood have died and there is a build up of tannins, dyes and oils, while sapwood is the region surrounding the heartwood and consists of the live woody conducting cells which carry sap from the roots up to the leaves. Compression wood, which has thicker cell walls, higher concentration of lignin, a different chemical composition of lignin and hemicellulose and different orientation of cellulose micofibrils is more resistance to decay. Environmental conditions including moisture, oxygen, temperature, and pH also influence decay rates. If conditions are favourable for microbial growth then decay is more likely. Optimum moisture content for the two most common forms of decay fungi, white and brown rot are within a range of 40-80% based on oven dry weight of wood (Blanchette, 2000). Soft rot fungi have a wider range of favourable conditions while bacteria prefer saturated conditions.

1.4.4.1 Wood decay fungi

Tree species, possess differing attractiveness for fungal enzymes to breakdown either harvested or processed wood; this is manifested by the diverse patterns of wood decay observed. Beyond the purely visual changes, this has far reaching consequences for the mechanical properties of the fungus-infected wood, such as its strength or stiffness. The extent to which a microorganism can invade a substrate and the method it uses to do this will depend on its ability to degrade different wood cell types and wood cell wall constituents and also on its adaptability to the other conditions of the host and environment.

1.4.4.1.1 Brown rot

Brown rot is a wood decay caused exclusively by fungi of the Basidiomycete Class. This class consists of many families though the overwhelming majority of the brown rot fungi belong to the Polyporaceae family. Only 6% of all the known

wood decay fungi are now known to cause brown rot (Schwarze *et al.*, 2000). Cellulose and hemicellulose are degraded by brown rot fungi through extensive depolymerisation in the wood structure while lignin remains preserved in a slightly modified form (Green and Highley, 1997). Lignin is modified by brown rot fungi by the demethylation of phenolic and nonphenolic units (Kirk and Farrell, 1987) which appear to progress as fast as the attack on the polysaccharides. Cells are microscopically altered (Figure 1.4) but though their ultrastructure is only visibly changed at the late stages of decay, cell wall structure is often still easily distinguishable. Strength properties of wood are reduced quickly in the early stages of the decay process. Due to the preferential degradation of carbohydrates, the decayed wood acquires a brittle consistency, breaking up like cubes and finally crumbling into powder. The modified lignin remaining gives the decayed wood a characteristic consistency and brown colour. Due to its high levels of cellulose, the S_2 layer is the first site of brown rot decay. Brown rot decay is typically seen as numerous fine cracks and clefts occurring in the secondary wall extending from the S_2 to the S_1 layers (Figure 1.4). The cell walls become more porous with increasing decay.

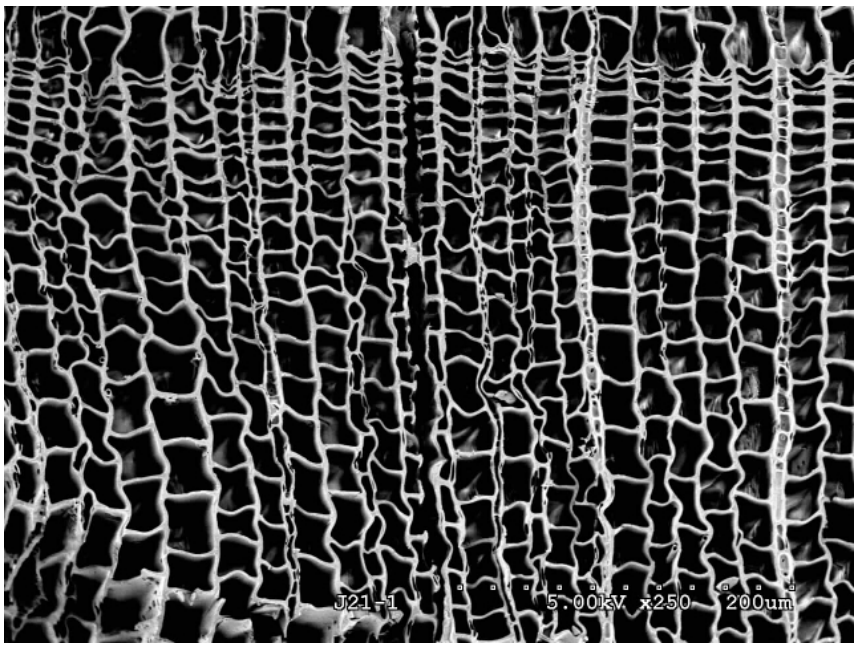

Figure 1.4: Micrograph of a transverse section of *Picea* sp.wood with brown rot showing the fungus has degraded some cells completely but not others. Cells have collapsed and have a "wavy' appearance (Source Joel Jurgens, unpublished micrograph).

Decay occurs away from the hypha which leads to the feature of very few visible hyphae but extensive decay. Movement of fungal hyphae from cell to cell is through pit membranes, or by direct penetration of the cell wall with the formations of apertures or boreholes.

1.4.4.1.2 White rot

The fungi causing white rot are represented in all the main groups of the Basidiomycetes and in some Ascomycetes (Sutherland and Crawford, 1981). The term white rot has been used to describe forms of wood decay in which the wood assumes a bleached appearance and where lignin, cellulose and hemicellulose are broken down. Two forms of white rot have been reported (Blanchette, 1984), selective delignification and simultaneous rot, which both result in chemically and morphologically different wood characteristics. One fungus can cause both forms of white rot. During selective delignification more lignin is broken down than hemicellulose and cellulose, this leads to white pocket rot, which is recognised as

light patches compared to preferential lignin degradation which leaves patches of pure cellulose. In the course of simultaneous rot the lignin, cellulose and hemicellulose are broken down at approximately the same rate. The hyphae grow in the lumen on the S_3 layer and the cell wall is broken down in the immediate vicinity of the hyphae which leads to the formation of erosion furrows. The hyphae sink into the cell wall. The parenchyma cells are colonised first and the hyphae penetrate from cell to cell via the pit membrane or by direct penetration through the cell wall (Figure 1.5). The cell wall of an infected cell is seen to be thin with the cell corner regions intact as they are more resistant to decay than other regions of the cell wall. Degradation is not uniform; one fibre may be totally decayed while the one beside may show no evidence of decay. If lignin within the middle lamella is degraded, a distinct decay pattern can be seen where the cells separate from each other and appear to peel off as individual cells.

Microscopically, a progression of erosion of the cell wall is seen with simultaneous rot, and occurs along with deterioration of the middle lamella due to selective delignification followed by progressive erosion of cellulose and hemicellulose (Figure 1.5). Lignin serves as a physical and chemical barrier to enzymatic degradation of wood polysaccharides (Kirk and Farrell, 1987). Removal of lignin, therefore, will expose cellulose and hemicellulose to degradation by cellulase and xylanase enzymes.

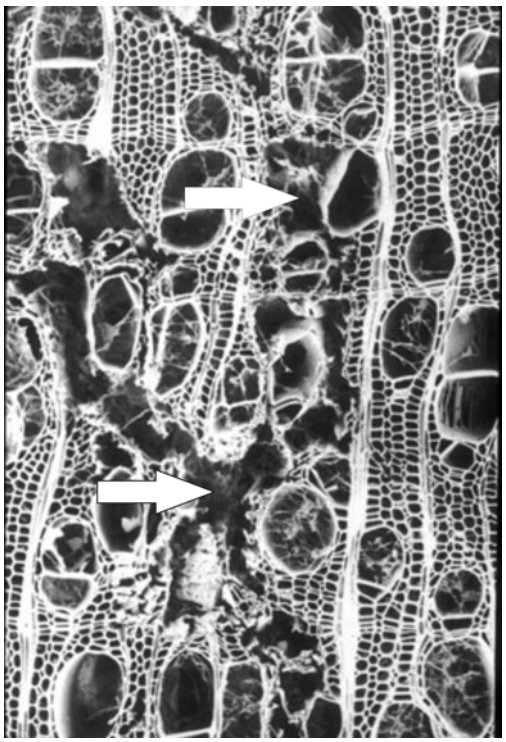

Figure 1.5: Micrograph of a transverse section of *Betula* sp. wood with white rot showing the fungus has degraded some cells completely but not others. White arrows indicate decay (Source Professor Robert A. Blanchette, unpublished micrograph).

White rot fungi are common parasites of heartwood in living trees and are aggressive decomposers of woody debris in the forest ecosystem (Blanchette, 1982). White rot Ascomycetes usually cause less loss of wood substance than Basidiomycetes and are restricted to angiosperms which contain syringyl lignin and can not degrade gymnosperm wood containing guaiacyl lignin (Kirk and Farrell, 1987).

1.4.4.1.3 Soft rot

Soft rot decay is characterised by a soft appearance and consistency of the wood. Ascomycetes and Deutromycetes are the main groups of fungi to cause soft rot. The characteristic feature of soft rot is the preferred growth of the hyphae within the secondary wall, which manifests itself in the form of typical cavities oriented longitudinally to the cell axis. Hyphae grow within the cell wall in the direction of the cellulose microfibril leading to the formation of cavities within the cell wall

which in cross section appear as small circular to oval holes in the secondary wall (Figure 1.6). Similar to white rot, destruction of the cell wall always takes place in the immediate vicinity of the hyphae.

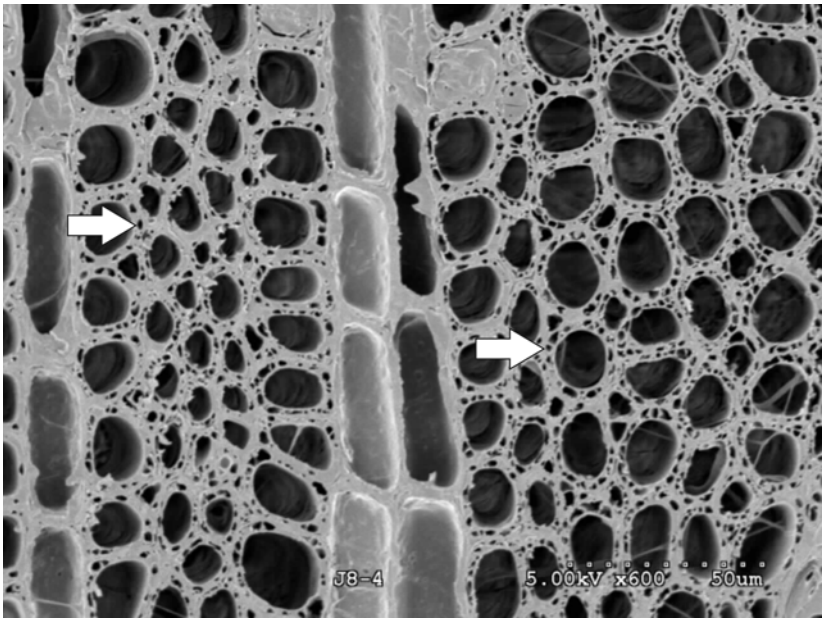

Figure 1.6: Micrograph of a transverse section of *Aquilaria* sp. wood with soft rot showing fungal hyphae within the cell wall and degradation cavities within the secondary cell walls. White arrows indicate soft rot cavities in the second cell wall (Source Joel Jurgens, unpublished micrograph).

Soft rot causes two different kinds of wood degradation generally distinguished as type1 and type 2. Some fungi are capable of causing both types of soft rot. Soft rot type 1 is characterised by the formation of a series of successive cavities with conically formed ends which follow the direction of the microfibril within the S_2 layer (Savory, 1954). Soft rot type 2 resembles a localised simultaneous rot, the degradation proceeding outwards from the lumen by the formation of small erosion furrows in the form of V-shaped notches (Corbett, 1965). The type of wood affects the rate and extent of soft rot; hardwoods seem to be more susceptible, due to the lignin within hardwood being syringyl-guaiacyl. In comparison, softwoods contain guaiacyl lignin, which is not easily degraded by soft rot fungi. The sequence of hyphal growth involves large diameter hyphae in

the lumen penetrating into the S_2 layer and forming fine hyphae which align their growth with the cellulose microfibrils and form L-bending or T-branching hypha. These hyphae grow within the S_2 layer and eventually return to the lumen and retain their wide diameter hyphae. Cavities arise from the enzymatic attack along the length of the fine hyphae (once it has stopped growing) as the hypha expands. New fine or proboscis hyphae form and emerge from the end of the cavity and form new cavities. This creates the image of a chain of cavities. Soft rot fungi are out competed by brown or white rot in favourable decay conditions however when conditions become less favourable (high or low moisture, high pH and high salt concentrations), soft rot fungi dominate.

1.4.4.2 Wood decay bacteria

Wood inhabiting bacteria are able to affect wood permeability, attack wood structure, work synergistically with other bacteria and fungi to predispose wood to fungal attack and have been reported to have an antagonistic effect on other wood inhabiting organisms (Eriksson *et al.* 1990). Bacteria and Actinomycetes are the most common wood inhibitors and are the initial colonisers in a succession of organisms that act synergistically with fungi to either breakdown wood or remove compounds that may be toxic to decay fungi. Bacteria are able to degrade wood both aerobically and anaerobically. Wood decay bacteria produce cellulolytic and pectinolytic enzymes which allow them to colonise the ray cells first and to be able to open up the crystalline structure of cellulose for future degradation. Bacteria form distinct decay patterns and are classified as erosion, cavitation, and tunnelling bacteria (Figure 1.7) (Singh and Butcher, 1991). Additionally, there are scavenger bacteria that utilise previously decayed material and primary bacteria that preferentially attack pit membranes (Blanchette, 1990). Erosion bacteria degrade secondary cell wall layers and deplete cellulose and hemicellulose from

the wood (Blanchette, 2000). Erosion bacteria can degrade wood under conditions that would inhibit both growth and activity of wood decay fungi (conditions of little or no oxygen) (Clausen, 1996). Tunnelling bacteria produce minute tunnels within the secondary cell wall. Tunnels have been reported that penetrate through the middle lamella and very little lignin loss has been reported (Daniel *et al.*, 1987). Cavitation bacteria form small diamond shaped or irregular cavities within the secondary wall that are orientated perpendicular to the long direction of the fibre (Singh and Butcher, 1991).

These cavities start in the cell wall near a pit chamber or directly within the secondary wall (Singh and Butcher, 1991). Wood inhabiting bacteria not only effect the wood cell wall but their actions open up wood structure for other microorganisms to utilise and upon their death provide important nitrogen resources for other bacteria and fungi that may inhabit the wood later in the decay sequence. Soil inhabiting bacteria also play a role in providing nitrogen, iron and sugar reserves for decay fungi to use. Many wood inhabiting bacteria are resistant to preservatives used to prevent wood decay in timber or have the ability to absorb or breakdown wood preservatives allowing decay by rot fungi. The ability of some bacteria to grow at higher temperatures than fungi also means they can cause decay in situation where decay fungi are inhibited (such as in chip piles where the temperature reaches 45-50°C (Clausen, 1996).

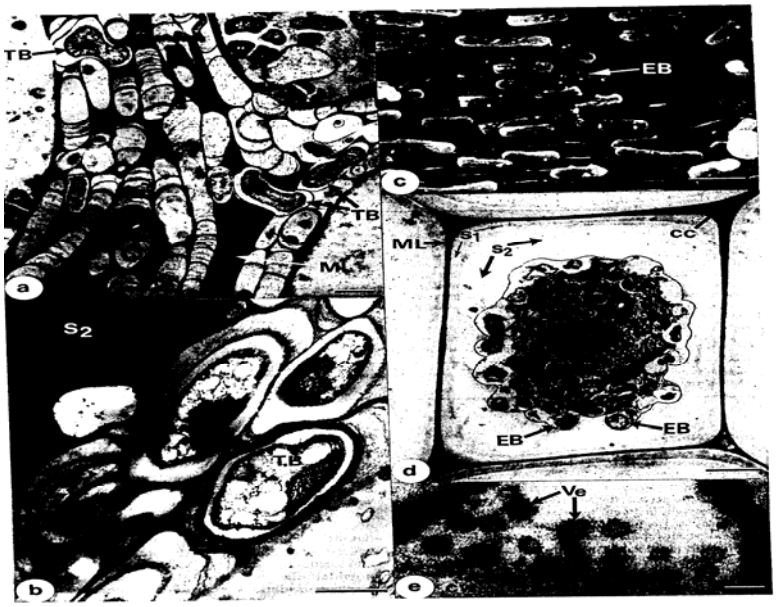

Figure 1.7: Transmission and scanning electron micrographs showing aspects of bacterial degradation of wood cell walls. (a) Tunnelling bacterial attack of *Homalium foetium*. (b) Tunnelling bacteria attack of *Pinus sylvestris* showing characteristic tunnel concentric secretions (arrowheads). (c,d) SEM and TEM micrographs showing complimentary features of erosion bacterial decay of *Betula verrucosa* fibre cell walls. (e) TEM micrographs showing the characteristic appearance of the membrane-bound vesicles produced by both tunnelling and erosion bacteria during decay of wood cell walls. Bars represent (a-d) 1.0μm; (e) 0.1μm; (Source Daniel, 1994).

1.4.4.3 Evidence of fossil fungi in Antarctica

Shackleton and his men reported finding fossil coniferous wood at latitude of 85ºS, on their trek to the South Pole in 1907. They (Shackleton, 1909) reported that "beacon sandstone formations which extend for at least 1100 miles from north to south in Antarctica contains coniferous wood associated with coal seams. It is probably of the Palaeozoic age". When the fossils in the beacon sandstone were described by Mr E.J. Goddard, in New South Wales, he concluded that the material was the xylem of a gymnospermous plant strongly resembling that of a coniferous plant (Shackleton, 1909). Additionally, they reported that peat deposits, formed of fungus, are now forming on the bottoms of some of the Antarctic glacial lakes near 77º and 78ºS. Balch (1912) reported that Frank

Debenham from Scott's second Antarctic expedition found coal in beacon sandstone at Granite Harbour.

Piozynski (1976) quoted a sentence written by Gardner in 1886 in reference to fossilised fungi "The fungi are destitute of chlorophyll and hence, or owing to their parasitic and saprophytic habits, any further development in them seems to have been arrested." Because of this lack of chlorophyll, fungi can be considered living witnesses of ancient environments.

The fossil record of ancient fungi is a valuable source of information about diversity, structure, evolution and activities. Fossil records have implicated fungi in processes including the following: the establishment of terrestrial plants; degradation of lignin in Devonian forests; parasitic relationship with plants and animals; and the development of ancient soils (Stubblefield and Taylor, 1988). Fossil evidence of decay has been hard to find, fungal mycelium among decaying debris has been documented in a variety of location which suggests wide spread decay activity. The earliest documented fungal rot comes from the Upper Devonian but there was probably at least some representative of the group earlier in the lower Palaeozoic (Stubblefield and Taylor, 1988). Branching septate hyphae with terminal and intercalary swellings were found throughout the secondary xylem of infected *Callixylon*. Tracheids attacked by fungi showed long, narrow grooves, oval cavities and severely degraded walls. The pattern of decay was consistent with present day white rots and hyphal morphology was consistent with either Ascomycetes or Basidiomycetes.

The research on fossil wood in Antarctica has lead to the discovery of decay patterns being seen in fossil wood from Fremouw Peak and Mount Augusta located in the Beardmore Glacier area. Araucarioxylon-type wood from the Triassic and specimen of *Vertebraria* from the Perimian demonstrated similar patterns of decay (Stubblefield and Taylor, 1986). They noted that two decay patterns were evident. The first one showed a thin cellulose framework indicating the removal of lignin from the cell wall and the second pattern showed separation of the primary and secondary cell wall and eventually the wall layers were removed completely. The fungal mycelia were branched, and septated with clamp junctions. The decay in the fossil wood was comparable in appearance to present day rot caused by white rot and white pocket rot Basidiomycetes.

Endochaetophora antarctica (White and Taylor, 1989) and *Mycocarpon asterineum* (Taylor and White, 1989) were isolated from Upper Beacon sediments (peat deposits) at Fremouw Peak (the lower-middle Triassic age). Both fungi were described as sporocarps. *Endochaetophora antarctica* was described as having hyphae, hyphal segments apparently representing arthrocondia and interwoven mycelia. They suggested that this fungus could be an ancestor to modern Ascomycotina. *Mycocarpon* did not correspond to any existent fungus and consequently is difficult to classify in modern taxa. Some features were considered to be advanced, others appeared to be more primitive, when compared with modern fungi. Their presence in peat, an environment of extensive degraded organic material, suggested that they may have been saprophytic, functioning as major decomposers. In modern ecosystems, endogonacecous fungi play a minor role in decomposition but are more known as important root symbionts, leading to the hypothesis that this symbiosis allowed plants to colonise the surface of the

earth. This led (Taylor and White, 1989) to hypothesise that these fungi evolved from shorelines and in swamps where organic material accumulated prior to evolution of land plants. In degrading organic matter, these terrestrial saprophytes may have produced the initial niches needed for the early vascular plants. The formation of fertile terrestrial habitats may have been an important prelude to the evolution of land plants. The relationship between early plants and saprophytes was one of interdependence with plants requiring nutrients liberated by saprophytes and they in turn obtained fixed carbon by degrading nonliving plant material.

Stubblefield *et al.* (1987) reported on the finding of silicified roots from Triassic Antarctica which showed evidence of extant vesicular-arbuscular (VA) mycorrhizae. Nonseptate fungal hyphae occurred within and between well preserved parenchymatous cells of the central cortex. The colonised roots were attributed to *Antarcticycas*, and fungal hyphae were the most abundant fungal structure. They concluded that the structure was organised like the arbuscle of modern day VA mycorrhizae and was found closely associated with a fungus that showed a pattern of colonisation, vesicles and clamydospores that was identical with those of extinct VA mychorrhizae. They believed that the Triassic roots were indeed vesicular-arbuscular mycorrhizae.

Along with fossil record evidence of fungi, there are the cryptoendilithic which are another source of fossil fungal material. The principal habitat for microbial life in Antarctica is a narrow zone under the sandstone rock surfaces (Friedmann, 1982). These narrow fissures, cracks and pores between mineral grains provide a comparatively milder microenvironment for the survival of cryptoendilithic and/or

chasmoendolithic cyanobacteria and lichens than rock surfaces (Golubic *et al.*, 1981). Wierzchos and Ascaso (2002) suggested that the Ross Desert, in the Mount Fleming region, was at the extreme limit of the physiological tolerance of inhabiting organisms and had lead to the extinction of microbial life. They used this location to look for the presence of fossilised microorganism. Using scanning electron microscopy with backscattered electron imaging and simultaneous use of X-ray energy dispersive spectroscopy to compare with live Antarctic crytoendoliths communities, evidence of fossil crytoendoliths communities were found, fossilised algae and hyphal cells were also seen.

1.4.5 Cellulose

Cellulose is the major polymeric component of plant material and is the most abundant polysaccharide on Earth (Bayer *et al.*, 1998). Cellulose consists of unbranched glucan polymer of linear chains of β-(1-4) linked D-glucose residues where every other glucose residue is rotated approximately 180 degrees (Figure 1.8). Cellobiose (a two glucose unit linked β-(1-4) bond) is the basic unit of cellulose, rather than glucose, as cellobiose is the major product from cellulose hydrolysis.

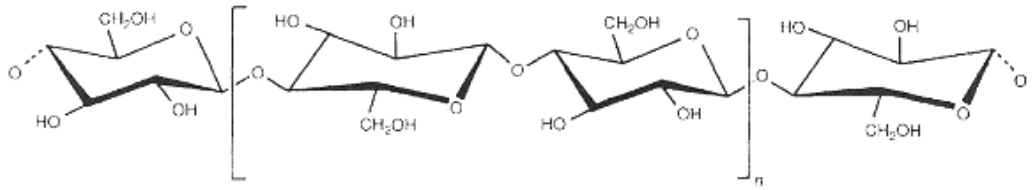

Figure 1.8: Diagram of the structural formula for the β-1,4-glucan polymer chain (cellulose). The repeating unit, cellobiose is indicated in brackets (Brown *et al.*, 1996).

The extended glucan chain polymer forms a flat ribbon-like structure that is further stiffened by Van der Waals forces, as well as intra- and intermolecular

hydrogen bonds between each glucosidic bond, leading to their parallel alignment to produce a regular crystalline arrangement of glucan chains. The glucan chain length in cotton and wood are reported to contain about 15,000 and more than 10,000 glucose residues, respectively, or Degree of Polymerisation (DP) units, which implies molecular lengths of 1-8μm (Kuga and Brown, 1991). Natural cellulose does not occur alone in free thread-like chains as found in commonly used synthetic cellulose substrates, it is usually present in bundles of parallel oriented chains, known as microfibrils. Microfibril size can vary from an elementary fibril which has approximately 36 chains to larger microfibrils of cellulosic algae which contain more than 1200 chains (Sugiyama *et al.*, 1985).

The most common crystalline form of native cellulose is cellulose I which is metastable and can be irreversibly converted into another crystalline state, cellulose II, the most stable allomorph known (Rånby, 1952). Cellulose I has parallel glucan chains and strong intramolecular hydrogen bonds. Cellulose II is seldom made in nature and it is generally the product of re-precipitation after swelling and dissolution of cellulose I with various solvents; it is commonly known as rayon. The glucan chains of cellulose II are anti-parallel probably as a result of chain folding (Kuga *et al.*, 1993). Other allomorphs of cellulose, cellulose III and IV, are known but seldom occur in nature (Sarko and Muggli, 1978). Cellulose microfibrils form amorphous regions (15% of the length), which appear to be located on the surface of the microfibril and crystalline regions (85% of the length), and appear to make up the core (Larsson *et al.*, 1997). Crystalline regions are considered to be more difficult to degrade than amorphous regions (Coughlan, 1985). The fraction of the total cellulose that is crystalline is considered an important parameter affecting the rate and extent of enzyme hydrolysis due to no pockets for the cellulase to bind. Many pre-treatments have

been tested to reduce crystallinity, and increase surface area. The aim for these pre-treatments is to increase cellulase enzyme hydrolysis by increasing adsorption of enzyme to create enzyme substrate complexes, a critical step in cellulose hydrolysis.

Cellulose is produced by bacteria as an extracellular polysaccharide, in algae, fungi and plants as a cell wall component for strength and protection (Eriksson *et al.*, 1990). In higher plants, cellulose synthesis occurs at rosette-like structures that consist of six hexagonally arranged subunits of the enzyme 1,4-β-D-glucan synthase, that are embedded in the plasma membrane (Brown *et al.*, 1996). Each rosette is thought to synthesise one microfibril. Cellulose synthases and related enzymes are family 2 glycosyltransferases (Brown *et al.*, 1996). Enzymes in this family catalyse glycosyl transfer reactions that involve inversion at the anomeric carbon leading to the formation of a β-linkage using α-linked uridine-diphosphate-glucose (UDP)-sugar donors (Saxena and Brown, 2000) A membrane anchored endo-1,4-β glucanase is also involved in the deposition of cellulose into the cell wall (Kimura and Kondo, 2002). Sucrose synthase is also present and is believed to degrade sucrose to fructose and UDP glucose (Kimura and Kondo, 2002), the substrate for cellulose synthesis. Cellulose is a major component of wood making up approximately 40-45% of dry weight in most wood species (Eriksson *et al.*, 1990). Within the plant cell wall, cellulose is usually found in association with hemicellulose and lignin.

1.4.5.1 Cellulose decomposition in general

The decomposition of plant litter and soil organic matter is an important process in all terrestrial ecosystems, and is also one of the major processes in the carbon cycle. Soil respiration is one of the most important contributors to the global

emissions of CO_2 to the atmosphere (Schlesinger and Andrews, 2000), particularly from forest ecosystems. Most terrestrial ecosystems act as carbon sinks in the global carbon cycle but they can also act as sources (Hill *et al.*, 2005), at least for some time. The decay of organic matter is critical to mineralization and nutrient cycling in ecosystems. Energy and nutrients obtained by plants eventually become incorporated into rock material that provides the resource base of complex food webs in soil. Because decomposition of organic materials is measured easily and serves as an integrator of the activities of organisms within a soil food web, decomposition serves as an indicator of soil condition. The rate of decomposition is a function of many characteristics and processes including chemical composition or quality of the organic material, temperature, moisture and composition of the decomposer community. Therefore, a significant difference in the rate of decomposition between sites signals either a change in decomposer community or condition of biotic and abiotic resources at a site. Plant litter is a mixture of labile (cellulose and hemicellulose) and recalcitrant (lignin) substrates that decompose at progressively slower rates. Cellulose and the lignin polymer are among the most abundant organic compounds on earth. Cellulose is the main carbohydrate constituent of plant cell walls and is decomposed by a wide variety of microorganisms. Lignin, an aromatic compound, coats cell walls and combines chemically with cellulose to form lignocellulose. In contrast with cellulose, lignin is one of the most resistant components in plant litter and only specialized organisms decompose it (Kirk and Farrell, 1987).

1.4.5.2 Cellulose decomposition in Antarctica

Cellulose decomposition was measured by Walton (1985) at South Georgia Island using both litter bags and standard cellulose substratum methods. The results of this study showed that the rate of decomposition in the *Polytrichum* mossbank

was 10mg $g^{-1}yr^{-1}$ compared to the decomposition rate in dried grass culms in a *Festuca* grassland, which was a magnitude greater at 140mg g^{-1} yr^{-1}. Analysis of the change in α-cellulose and holocellulose of the material in the litter bags showed that the percentage levels after 2.5 years were similar due to the level of cellulose in the grass being higher at the beginning of the experiment. The loss of grass material from the bags was due to cellulose breakdown while the lack of cellulose breakdown accounted for the low constant fraction loss in the moss beds. Cotton strips were used to study the decomposition of cellulose in three sites situated in Cumberland Bay, South Georgia and included a grassland, a moss bed and a peat mire. The mossbank once again showed the least ability to degrade cellulose. The grassland and mire demonstrated a similar rate of degradation at the surface, both had different profiles responses. The peat mire decomposition was at 120-150mm, with a rate at 260mm which was two times higher than at the same depth in the mossbank. The main decomposition occurred near the surface and the bottom (240-260mm) in the grassland and moss bank profiles. Peak cellulytic activity occurred in the latter part of the summer and although very slow over the winter the sites never showed nil breakdown. Decomposition was also noted in regions of the profile where the conditions were likely to be anaerobic. At the grassland site, the total tensile strength loss reached over 90% after two years compared to the mossbank which had only 55% breakdown. Walton (1985) reported on the PhD research of M.J. Smith which showed that cellulytic bacteria present at South Georgian sites were only very weakly active and that cellulose degradation was principally due to fungal activity. Hurst *et al*. (1983) studied litter and phylloplane fungi and determined that *Chrysosporium pannorum* and *Botrytis cinerea* both showed peak cellulase activity at 20°C while *Chaetophoma* sp. and *Cladosporium sphaerospermum* showed maximum activity at 25°C. All showed

significant activity at 1°C. Incubation of *P. flabellata* litter with *C. pannorum* showed an increase in percentage of dry weight loss when compared with sterile controls. The conclusion of this research was that the factors restricting decomposition on South Georgia Island were not principally temperature, bacteria and oxygen availability but the factors were related to plant form, substratum potential and dominance of fungi which is different than for the Arctic.

Yamamoto *et al.* (1991) reported on a preliminary study of cellulose decomposition by soil microorganisms carried out at Syowa Station and Langhovde Hut, Antarctica. The benchkote sheet method was used to test 14 sites in three locations: Yukidori Valley (an area of low human impact), Syowa Station and Langhovde Hut. Cellulose decomposition rates in the soil from Yukidori Valley and near a urinal barrel at Langhovde Hut ranged from a few percent while rates of 9-25% were measured from sites near a urinal drum at Syowa Station. The temperature and moisture content at the sites were lower than in temperate environments and the sandy soil which does not hold nutrients lead to lower numbers of cellulytic organisms. The two Antarctic stations showed different rates of decomposition which was put down to increased human impact in respect to increased supply of organic matter and nutrients at Syowa Station which was established over 20 years ago when the study was done, compared with Langhovde Hut which was established in 1986.

Greenfield (1992) commented on some unpublished work where the feathers, cellulose and moss stems held *in situ* in mesh bags at Cape Bird over nine years had shown <5% weight loss.

Heal *et al*. (1967) reported decomposition rates were slow in soils on Signy Island even though there was a large number of organisms. They concluded that this was due to the low temperature and the restricted period during which microbial activity can take place. Heal *et al.* (1967) reported that the number of microorganisms were similar in similar soils but varied greatly with different soil and vegetation types. This suggested that soil and vegetation type have a greater influence on numbers than does the climate. Fungi isolated from this study were the same species present in typical soil forms found in temperate zones. Heal *et al*. (1967) showed that the species they investigated were cold tolerant forms which probably showed further cold adaptation. They confirmed Holdgate's (1977) conclusion that ecological rather than distributional factors are of prime importance in determining the nature of the Antarctic vegetation probably also applies to microorganisms. All research to date shows that decomposition is possible but slower within the extreme Antarctic environment. Like the temperate environment the composition of the plant material the micoorganisms are exposed to is affecting the rate of decomposition.

1.4.6 Cellulase

1.4.6.1 General

Based on the abundance of cellulose, and the lack of its accumulation on Earth, one can suggest that cellulases are the most abundant carbohydratase in nature. Although cellulases are distributed throughout the biosphere, they are primarily manifested in microbial and fungal sources (Almin *et al*., 1975). It has been demonstrated that microorganisms are efficient degraders of cellulose, starch, and other carbohydrate components of plant hemicelluloses by the production of carbohydrases. These enzymes can be used for the conversion of biomass to

simple sugars and these sugars eventually form a myriad of compounds. These enzymes include cellulase, xylanase, and amylase among others. Microorganisms with such enzymes have been isolated in terrestrial as well as marine environments including bacteria, fungi and yeasts. While the cellulytic enzyme systems of different microorganisms are often distinct, the main features of cellulase degradation are the same.

Complete hydrolysis of cellulose requires cellulase, a multi enzyme system, successful adsorption of the enzymes onto the substrate and the removal of cellobiose which inhibits the action of some components of the cellulase complex. This system consists of endo-(1-4)-β-D- glucanases (EC 3.2.1.4), which act randomly along the cellulose chain and are more active on amorphous cellulose; exo-(1-4)-β-D- glucanases (EC 3.2.1.91) which release cellobiose units mainly from the chain ends and degrade preferentially crystalline cellulose (Carrard, 2000) and β-glucosidase (EC 3.2.1.21) acting on cellobiose to yield two glucose molecules (Mansfield *et al*., 1999). Purification and fractionation studies have revealed that all three major forms of cellulase occur in multiple forms and are likely all part of isozyme families. An example is that four to five endoglucanases and two cellobiohydralases (CBH) have been identified from *Trichoderma reesi* and *Trichoderma koningii.* Many of the cellulases are glycoproteins with large variations in polysaccarides contents (Tomme *et al.*, 1995). Most cellulases are composed of a catalytic domain joined to one or more ancillary domains or modules frequently by a recognised linker sequence (Gilkes *et al.*, 1991). Only a few microbial cellulases and the cellulase produced by higher plants have just a catalytic domain. The ancillary domains and modules include cellulose binding domains (CBD), duplicated segments, S-layer-like modules, and fibronectin-Type

III (Tomme *et al.*, 1995). The major function of the CBD is to deliver and associate its resident catalytic domain to the crystalline cellulose substrate. The binding is extremely stable although the enzyme may undergo lateral diffusion on the substrate surface (Jervis *et al.*, 1997). Some CBD appear to catalyse the disruption of the noncovalent interaction between the chains of the crystalline substrates (Din *et al.*, 1991) whereas others bind preferentially to non crystalline substrates (Johnson *et al.*, 1996). Cellulase enzymes are classified into families, according to the amino acid sequences similarities of their catalytic domains. This classification is based on hydrophobic cluster analysis. Cellulolytic enzymes are grouped into at least 15 of more than 80 known structural families of glycosyl hydrolases (Rabinovich *et al*., 2002). The linker regions of many cellulases are particularly sensitive to proteolysis, probably because they are exposed and the individual domains are released as discrete functional units (Gilkes *et al.*, 1988).

Sharrock (1988) defined synergism as a cooperative effect which causes a reaction catalysed by a combination of enzymes to proceed at a rate which exceeds the sum of the individual rates of reactions catalysed by the component enzymes acting in isolation. Most demonstrations of synergism involve a combination of endoglucanases and exoglucanases acting on crystalline cellulose but there have been reports of endo-endo (Mansfield *et al*., 1998) and exo-exo synergism (Hoshino *et al.*, 1987) when there are multiple forms of the enzyme present. The β-glucosidase component normally completes the synergistic interaction by hydrolysing cellobiose to glucose, thus relieving end product inhibition of exocellulobiohydrolase activity. An example of this synergism is the case of the two forms of Cellobiohydrolase, CBHI and CBHII in which CBHI prefers to hydrolyse cellulose Iα working with CBHII which prefers cellulose Iβ. The

dimorphism of cellulose I may also be related to the diversity of β-1,4-glucanase secreted by cellulytic micoorganism.

Fungal cellulases are different from bacterial systems; fungal enzymes can be produced as separate entities, each of them having specific actions. The most profound biochemical difference between fungal and bacterial systems is the end product produced during hydrolysis of cellulose. While fungal systems produce solely glucose during cellulose degradation, bacterial systems produce cellobiose, which is taken in to the cell and hydrolysed to glucose (Steenbakkers *et al*., 2003).

In the 1950's, Reese and Mandels (Mandels and Reese, 1964) began the pioneer work to understanding the role of cellulases in the degradation of cellulose. They also illustrated the importance of fungal cellulases in aggressive degradation of cellulose, specifically the cellulases produced by *Trichoderma reesei* Mandels and Reese (1999) introduced the C_1-C_x concept to explain how the cellulase system first opened up the cellulose matrix (C_1) while the more accessible substrate could then be hydrolysed by the C_x components. In 1979, Wood and McCrae showed that endoglucanases filled the roll of the proposed C_x but exoglucanases did not fill the role of a C_1 cellulase as they appeared to be able to breakdown cellulose from the non-reducing end and liberated cellibiose. The synergenistic C_1-C_x interaction model was modified to suggest that an endo-exo interaction results in more than just the additive interaction of the component cellulases.

The development of molecular biology, amino acid sequences, and crystallography has led to better understanding of the enzyme structure and has seen the re-classification of enzymes more on the structure of their cellulase

binding domain and catalytic domains rather the endo or exo action. Studies of cellulytic microorganisms was initially being motivated by the need to prevent degradation of cotton fabrics in the tropics (Mandels and Reese, 1964) but has refocused to the potential for producing fermentable sugar from cellulytic biomass (Saddler *et al*., 1992).

1.4.6.2. Bacterial cellulase

Cellulytic activity has been reported amongst gliding bacteria, Actinomycetes, gram-negative and gram-positive bacteria, both aerobic and anaerobic. The cellulytic system of bacteria is simpler than that of fungal origin because bacteria produce only endoglucanase and β-glucosidase. *Clostridium* spp. (Parsiegla *et al*., 1998; Murashima, 2002) are some of the most documented cellulase producers. Cellulosommes produced only by bacteria consist of discrete multifunctional, multienzyme complexes (Boisset *et al*., 1999). Cellulosommes are composed of large multidomain subunits called scaffoldin (Shoseyov *et al.*, 1992). This subunit contains a single CBD and multiple cohesion domains. The scaffoldin was found to be responsible both for cellulose binding via its resident CBD and for the integration of the enzyme subunits into cellulosomes through the intersubunit cohesion-dockerin integration (Béguin and Lemaire, 1996). Scaffoldin is tethered to the cell via a distinctive set of surface proteins. Cellulosome based enzymes have an additional docking domain and rely on the scaffoldin CBD for effective binding to the crystalline substrate when compared with noncellulosome enzymes.

1.4.6.3. Fungal cellulase

A wide diversity of fungi have the ability to synthesise cellulases. Oomycetes (*Leptomitus*, *Pythium*) and Ascomycetes (*Ceratocystis* and its anamorph *Verticicladiella*) synthesise cellulases to allow extension of their cellulose

containing mycelial walls. A diverse range of fungal species produce cellulase in response to plant interactions, be it endomycorrhizae formation or as plant pathogens. Some cellulolytic fungi that have evolved to fill specific ecological niches have become the prey of secondary consumers eg. *Termitomyces* spp. in the termite fungal gardens. The rumen of animals provides a unique niche for anaerobic cellulytic fungi. Anaerobic fungal cellulases have higher specific activities than aerobic versions but lesser amounts are produced. There are many other niches for cellulytic fungi that have not been explored. Two genera of fungi *Trichoderma* and *Aspergillus* have been studied extensively for cellulase production (Seiboth *et al*., 1997). *Trichoderma reesei* is one of the most prolific sources of cellulase enzymes due to its secretion of a family of different cellulolytic enzymes (Fujita, 2002). Several yeast genera have also been screened. These include *Candida*, *Debaromyces*, *Kluyveromyces* and *Pichia*. *Dedaromyces yamadae*, *K. marxianus*, and *C. chilensis* have shown the presence of thermoactive cellulase enzymes, a desired characteristic for industrial application (Saha, 1996).

Fungal cellulases appear to be generally comprised of distinct binding, hinge, and catalytic domains. Fungal endoglucanases and cellobiohydrolases are composed of a catalytic domain and a C-terminal CBD, joined by a linker rich in proline and hydroxy amino acid residues. Fungal β-1,4-glycanase have an N-terminal CBD; others have no CBD but contain C-terminal repeated sequences of undefined function (Black *et al.*, 1995).

1.4.6.4 Cellulase versus carbohydrate polymer hydrolytic enzymes

Along with hydrolytic enzymes, some organisms produce oxidative and phosphorolytic enzymes. It has been proposed that these enzymes are involved in

the initiation of cellulose degradation. These enzymes are believed to be the reason why aerobic cellulase systems produce more enhanced cellulose degradation than anaerobic cellulase systems. Oxidative enzymes involved in the cellulase system include cellobiose quinone oxidoreductase, lactonase, and cellobiose oxidase but they are synthesised in low amounts as compared to the carbohydrase enzymes measured in the laboratory conditions (Anders, 1994).

Cellobiose quinone oxidoreductase (EC 1.1.5.1) reduces quinones in the presence of cellobiose which is in turn oxidised to cellobiono-δ-lactone (Eriksson *et al.*, 1974). This enzyme is important in both cellulose and lignin degradation by preventing the repolymerisation of quinones and phenoxyradicals by the reduction with the concomitant oxidation of cellobiose (Eriksson *et al*., 1974).

Cellobionolactone is thought to induce cellulase components required for the degradation of cellulose. Bruchmann *et al.* (1987) showed that low levels of cellobionolactone promoted induction of the cellulase system but high levels inhibited the system.

Lactonase (EC 3.1.1.17) catalyses the hydrolysis of glucono-lactone and cellobiolactone. Along with having an inductive role in the cellulase system, lactonase also promotes cellulytic activity by removing lactones which have an inhibitory effect on the activity of β-glucosidase (Bruchmann *et al.*, 1987).

Cellobiose oxidase (EC 1.1.99.18) oxidises cellobiose and oligosaccharides with resultant disruption of the crystalline regions by the formation of uronic residues and hydrogen peroxides thus breaking hydrogen bonds between the cellulose

chains. This causes swelling of the cellulose and makes the crystalline parts more accessible (Anders, 1994). Cellobiose oxidase, along with glucose oxidase (EC 1.1.3.4), relieves end product inhibition of the endoglucanase and cellobiose.

Aerobic and anaerobic bacteria use phosphorylase enzymes for cellulose degradation. The substrates for these enzymes are cellobiose and higher cellodextrins and the products are glucose 1-phosphate and glucose. Cellobiose phosphorylase and cellodextrin phosphorylase perform the inorganic-phosphate-dependent phosphorolysis of β-glucosidic bonds yielding a D-glucose 1-phosphate and equimolar concentrations of D-glucose or cellodextrin with reduced chain length (Reichenbecher *et al.*, 1997).

Due to the complexity of cellulose in nature, most cellulytic organisms produce a varied consortium of enzymes to degrade cellulose. This consortium includes xylanases, mannanase, and lignin degrading enzymes along with cellulases. Hemicelluloses can be found within and between crystalline cellulose domains, this zone of cellulose-hemicellulose mix may require different enzymes to hydrolyse and could explain why cellulolytic micoorganisms synthesize a range of different cellulases with over lapping specificities and why some xylanases carry substrate binding domains with an affinity for cellulose (Tomme *et al.*, 1995).

1.4.7 Antarctic Heroic Era History

The history of each hut and its environments is detailed is this section, as well as the visitations and materials which remain on Ross Island that were relevant to the thesis research given potential of microbial introduction, disturbance, potential environmental change and substrate presence.

1.4.7.1 The Heroic Era

The Heroic Era began at the Sixth International Geographical Congress in London's Imperial Institute in 1895. On August 3, 1895, those present passed a resolution "That this Congress records its opinion that the exploration of the Antarctic Regions is the greatest piece of geographical exploration still to be undertaken. That in view of the additions to knowledge in almost every branch of science which would result from such a scientific exploration the Congress recommends that the scientific societies throughout the world should urge in whatever way seems to them most effective, that this work should be undertaken before the close of the century" (Lüdecke, 2003).

In addition to science, the geographical South Pole which lies at 90 degrees South, became the object of one of the last great races of discovery, the desire to be the first to reach the South Pole, which led to a number of expeditions being mounted and dozens of men risking their lives to conquer this last great frontier. This era is recorded as the Heroic Era (1895-1917). The explorers who were attempting to reach the Pole all built substantial "bases", especially for the time, from which to begin their trek. These bases will be discussed both in terms of their initial establishment, as well as subsequent party use.

1.4.7.1.1 Cape Adare

Carsten Borchgrevink led the first British Antarctic Expedition (BAE) in 1898 and landed at Cape Adare (named by Captain James Ross in 1841 after his friend Viscount Adare, MP for Glamorganshire (Harrowfield, 1995)) in February 1899. The BAE objectives included collecting scientific data and completing the first winter-over on the continent. They built two prefabricated *Pinus sylvesteris*

(Baltic pine) huts, a living hut and a store hut, which housed the 10 men, their supplies and equipment for the winter of 1899 (Figure 1.9).

Figure 1.9: Historical photograph of Borchgrevink's hut on Ridley beach, Cape Adare, February 1899 (Source Harrowfield, 1995).

The following summer they travelled south to Coulman Island and laid claim to the records of reaching the most southern point, being the first land based party to overwinter on the continent and the first to be equipped for exploration with specially designed and insulated buildings, dehydrated food, new forms of clothing and sledge dogs (Harrowfield, 1988). Borchgrevink's huts were visited in January 1902 by Scott's National Antarctic (*Discovery*) Expedition. The next expedition to winter over at Cape Adare was the Northern Party of Scott's second expedition the British Antarctic (*Terra Nova*) Expedition in 1911. They erected their own hut of *Pinus sylvesteris* (Baltic pine) about 20 metres north of Borchgrevink's living hut. The six men of the Northern party spent the winter at the hut and the next January trekked south to the Inexpressible Island. The men wintered over in a cave on the island, living on seals and penguins before trekking down the coast and crossing the sea ice to Hut Point on Ross Island. Since then,

the main shell of the Northern party hut essentially disintegrated, although the porch still stands (Harrowfield, 1995). Borchgrevink's huts, however, are still standing and today remain, together with their contents, in remarkably good condition, making Antarctica the only continent where the first dwellings erected by humans still exist.

1.4.7.1.2 Hut Point

Robert Falcon Scott's first expedition (1901-1904) had a scientific objective but the real goal was the South Pole (Lüdecke, 2003). On board *Discovery* were three huts; two small huts for scientific equipment and a large hut (11.3 metres^2 (m^2)) designed and prefabricated in Australia that was to be erected as a shore base. Scott chose Hut Point Peninsula (named by the *Discovery* shore crew when they had established their shore station there (Harrowfield, 1995)) on Ross Island to locate his first hut (Figure 1.10).

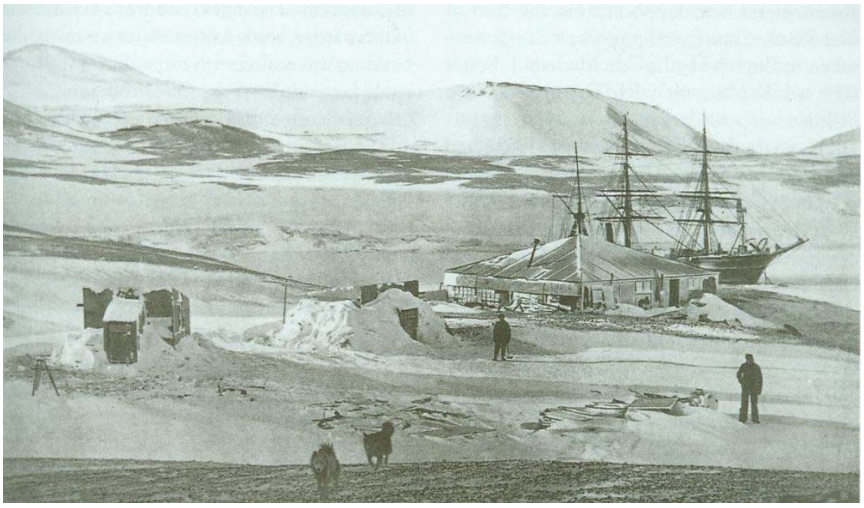

Figure 1.10: Historical photograph of the building of *Discovery* Hut in 1902. *Discovery* ship is in the background (Source Harrowfield, 1995).

The hut proved too hard to heat and was described as being more like a summer house. Consequently, it was never used as a base and the 47 expedition members lived on their ship, using the hut for scientific observations, drying equipment,

repairs and as an entertainment venue. During the first summer, much was accomplished including trips to Cape Crozier on the other side of Ross Island, the first ascent to the Polar Plateau and a record trip south by Scott, Shackleton and Wilson. A second winter was spent on board *Discovery* and in spring 1902 Scott led another sledging trip south, this time ascending to the Polar Plateau and exceeding the furthest point set the year before. In February 1904, the *Discovery* was freed from the ice and headed north. Being the furthest south of the Heroic Era huts, *Discovery* Hut was used by each of the subsequent expeditions to the Ross Sea. Scott's old hut was used as a staging post on depot laying journeys south and a safe haven when returning from expeditions south by Shackleton's (1907-1909) *Nimrod* Expedition and Scott used the hut as a staging post during his second and fateful last British Antarctic expedition (1910-1913). Seventeen men were trapped in *Discovery* Hut at the end of the sledging season in 1912. In the winter of 1912, Wilson, Bowers and Cherry-Garrard also used the hut on the way back from their epic mid-winter journey to Cape Crozier (as described in Cherry-Garrard's epic story entitled "*The Worst Journey in the World*"). Summer relief parties sheltered in *Discovery* Hut as they tried to reach Scott's ill-fated Polar team. Two years later, 1914, *Discovery* Hut was again visited by members of Shackleton's Ross Sea party who ventured to McMurdo Sound to lay depots for Shackleton who was attempting the first traverse of Antarctica from the Weddell Sea on the other side of the continent.

1.4.7.1.3 Cape Royds

Ernest Shackleton's British Antarctic Expedition departed from New Zealand in the *Nimrod* in January, 1908. Shackleton intended to establish his winter base in King Edward VII Land at the eastern end of the Ross Ice Shelf (Shackleton, 1909). However, the team found the Ice Shelf had been calved into the sea obliterating

their planned landing site and he was forced to head for McMurdo Sound. Thick sea ice prevented *Nimrod* from reaching Hut Point, so Shackleton settled on a site 32 kilometres north at Cape Royds (Shackleton, 1909) (named by the *Discovery* Expedition for its meteorologist Lieutenant Charles Royds RN. (Harrowfield, 1995)).

Shackleton's men constructed their base in a sheltered area below a ridge of volcanic rock near the Adelie penguin rookery at Pony Lake (Figure 1.11). Over the first summer season, David Edgeworth (the leader), Douglas Mawson, Alistair Mackay and a support party of three (McGonigal and Woodworth, 2001) made the first ascent of Mt. Erebus (3794m), the volcanic peak that dominates the Ross Island skyline (Shackleton, 1909).

Figure 1.11: Historical photo of the *Nimrod* Hut at Cape Royds, 1908. Mt Erebus in the background (Source Harrowfield 1995).

Over the winter the men published the first book in Antarctica, '*Aurora Australis*' and reams of paper as well as one copy of the book are still present in the hut. In the spring of 1908, their quest for the South Pole began using pony teams to drag supplies. When the ponies died, the men resorted to man-hauling their sledge via a new route over the Beardmore Glacier. After nearly two and a half months, they

reached a point just 156 km from the South Pole. Although the team could probably have made it to the Pole, with dwindling supplies and bad weather Shackleton knew they might not return safely and made the decision to turn back. The decision has subsequently been called the finest decision made in the history of Antarctic exploration (AHT Conservation Plan Shackleton's Hut, 2003). Shackleton and his men had beaten Scott's furthest south point by 598 km. The same summer another party of the expedition, Douglas Mawson, Alistair MacKay and Edgeworth David, reached the South Magnetic Pole for the first time after an epic 1,600 km trudge. The *Nimrod* collected the men in March 1909, the hut was locked up and they returned to the United Kingdom. After Shackleton's departure, the hut at Cape Royds was not used so extensively by subsequent expeditions but during Scott's second fateful expedition (1910-13) it was visited and lived in for short periods and on several occasions' members of Shackleton's Ross Sea Party foraged in the hut for supplies after they were stranded on Ross Island.

1.4.7.1.4 Cape Evans

The goal of Captain Scott's final expedition to the ice was to be science and geology, a matter of pride to be the first to claim the South Pole for Britain drove Scott to under take the expedition (King, 1999). Thick sea ice prevented the ship from reaching Hut Point and they landed instead some 25 kilometres further north on a narrow volcanic neck of land that Scott named Cape Evans (originally named the Skuary by the *Discovery* expedition and renamed on the *Terra Nova* expedition. Scott renamed it for his second in command Lieutenant Edward Evans RN (Harrowfield, 1995)). This largest of all huts in the Ross Sea Region was prefabricated in London, and was one of three taken south on the *Terra Nova* (Hoflehner, 2003) (Figure 1.12). The hut housed 25 men in the first year whose main job was to lay depots for the next summer's run for the Pole. Scott selected

his final team for the dash to the Pole besides himself; he chose Wilson, Oates, Evans and Bowers. The team reached the South Pole on 17 January 1912 but when they arrived they were met by the Norwegian flag flying that Amundsen's team had left some 35 days earlier. On January 13, 1913, the *Terra Nova* returned and the *Terra Nova* Hut at Cape Evans was closed.

Figure 1.12: Historical photograph of unloading supplies onto the beach at Cape Evans. The *Terra Nova* Hut in the background before the annex and stables were built (1911) (Harrowfield, 1995).

The final expedition of the Heroic Era was Shackleton's Imperial Trans-Antarctic Expedition (1914-17). Shackleton and men had planned to cross Antarctica from the Weddell Sea to Ross Island via the Pole. Shackleton's Ross Sea Party was charged with laying supply depots for Shackleton over two summers. The Party were stranded on Ross Island when their ship blew out to sea and was unable to return and they used the hut at Cape Evans as a base. Shackleton returned to rescue the Ross Sea Party in January 1917 and the Heroic Era drew to a close, particularly for the Ross Dependency.

1.4.7.2 Care of the huts since the Heroic Era

Following the end of the "Heroic Era" of exploration (1896-1917), the huts remained unvisited for a number of years until the first visitors came from the U.S. Operation High Jump in 1946-47 (Harrowfield, 1988). The historical importance of these sites was recognised and provisions of the Antarctic Treaty of 1959 provided protection for the buildings and artefacts. More recently, the Protocol on Environmental Protection to the Antarctic Treaty (1991) forbids the damaging or destruction of the historic sites and requires all governments to protect these objects of historic interest. In 1997 and 1998 at the Antarctic Treaty Consultative Meetings, the huts at Cape Adare, Hut Point, Cape Evans and Cape Royds received “protected area” designation. During the Trans-Antarctic Expedition 1955-58 (co-led by Sir Edmund Hillary), essential maintenance was carried at Shackleton’s hut located at Cape Royds and Scott’s second expedition hut located at Cape Evans.

In 1964, ice was excavated by four volunteers from the New Zealand Antarctic Society from *Discovery* Hut, at Hut Point. In 1969, the New Zealand Antarctic Division introduced a hut caretaker programme and for many years, two members of the New Zealand Antarctic Society went south to carry out essential maintenance on the Ross Island huts. A Strategy for the Preservation and Management of Historic Sites in the Ross Dependency, Antarctica was produced and subsequently these formed a Historic Sites Management Committee (Antarctic Heritage Trust, 1987). A five year Strategic Plan began for management of all the historic sites in the Ross Dependency. These efforts contributed to the preservation of the huts, and in April 1987, Antarctic Heritage Trust was formed to coordinate and manage the conservation efforts.

The Antarctic Heritage Trust (AHT) is an independent charitable trust based in Christchurch, New Zealand which was formed to care for the heritage of the Heroic Era located in the Ross Sea region of Antarctica on behalf of the international community. This heritage includes the four expedition bases associated with the Heroic Era explorers in the Ross Dependency.

In 2001, AHT recognised that a major conservation project was required to ensure this world heritage exists for future generations. In 2002, the Ross Sea Heritage Restoration Project was launched which involved the creation of conservation plans, detailed cost plans and work programmes for each of the four sites. These comprehensive plans provided an outline of the proposed conservation work at each of the sites and were complemented by Implementation Plans which detailed the physical works required for each hut. The Ross Sea Heritage Restoration Project commenced during the 2004/05 Antarctic summer season with the main focus on Shackleton's Hut at Cape Royds. Each Antarctic season for the next decade, experienced professionals travel south to the sites and undertake conservation work. The project is overseen by project managers with input from heritage specialists and consultants from around the world. The Trust's vision is twofold: first, to conserve the physical legacy of the huts and second, to create, through strategic partnerships, an educational outreach programme using modern technology so people around the world may experience this history and the project remotely.

1.4.7.3 Current Descriptions of Heroic Hut Sites

All three huts are located on the western lava beds and cinder cones of Mount Erebus volcano on Ross Island (Figure 1.13) in the Ross Dependency, Antarctica.

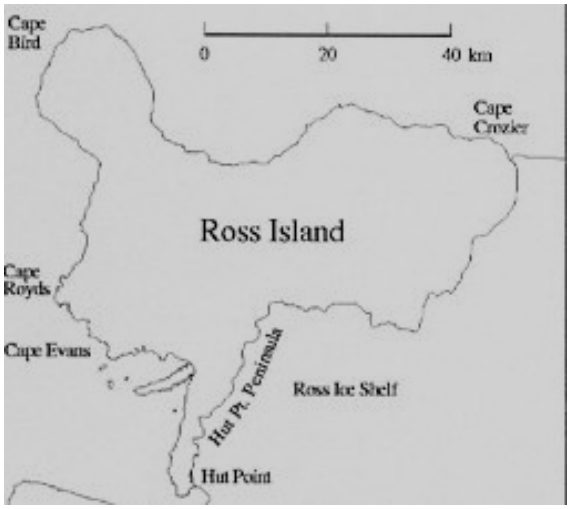

Figure 1.13: Map of Ross Island *Discovery* Hut is located at Hut Point, *Terra Nova* Hut at Cape Evans and *Nimrod* Hut at Cape Royds (Source AHT Conservation Plan *Discovery* Hut, Hut Point, 2004).

1.4.7.3.1 Hut Point: *Discovery* Hut - the British National Antarctic Expedition (1901-1904)

Discovery Hut (77º 50' 50"S, 166º 38' 30"E) was built at Hut Point Peninsula, an ice free area at the south-west extremity of Ross Island, approximately 32 km south of Cape Royds, and 25 km south of Cape Evans. The area is composed of loose scoria covered basalt bedrock. The site is on an area of land designated as Antarctic Specially Protected Area (ASAP) No. 158 by the Antarctic Treaty Consultative Meeting (1998) and endorsed by the Scientific Committee on Antarctic Research (SCAR). The hut is located at the end of Hut Point which juts out into McMurdo Sound in a south-westerly direction, forming the northern enclosure to Winter Quarters Bay, on the south side of a low saddle on the point. Towards the end of the point, there is a low hill due west of the hut. To the north east, the land rises steeply, while around the shore of Winter Quarters Bay, a well formed road leads to McMurdo Base, the largest United States base in Antarctica. Since 1958 the Hut Point site has had a large number of visitors both by vehicle and on foot because of it proximity to McMurdo Base and to New Zealand's Scott

Base, which is a further 3 km beyond McMurdo Base to the south east on the other side of Cape Armitage. There are no other structures presently (2006) in the area although, from the 1960's until the 1990's, there were industrial structures of McMurdo base to the north east of the hut.

Discovery Hut is a framed and panelled structure, prefabricated and made entirely of timber. It was designed in Melbourne and constructed by James Moore of Sydney, in 1900 (Pearson, 1992). A trial erection of the hut took place in Melbourne, in 1900, and the components coded (numbers, letters, and black painted bands can still be seen on the hut walls) before being loaded aboard the *Discovery* in 1901. The hut was erected at Hut Point in February 1902 and sited in a low saddle towards the end of Hut Point. The main door faces to the south west. Surveys of the hut undertaken in 2002 (AHT Conservation Plan *Discovery* Hut, Hut Point, 2004) show the building is 32 feet square (9.76 m^2) with a 5 foot (ft) 1 inch (in) (1.56 m) deep veranda on the north, east and south sides, and on the west side there is no veranda. The roof is a four sided pyramid rising to an apex. The floor walls and roof are all prefabricated panels fitted into a structural frame. The veranda has an apron which closes in the top third of the space between the posts. The original hut design included six skylights in the veranda roof to provide light into the area, the glass in these have been replaced but the skylights are as they were in the original plan (Figure 1.14).

Figure 1.14: Photograph of *Discovery Hut* in 1998. (Source Professor Robert A. Blanchette)

Two main species of wood have been identified from the exterior of the hut; *Pseudotsuga menziessii* (Douglas fir) was used for veranda posts and beams along with sidings and *Pinus sylvestris* (Scots pine) was used for sidings, roof and veranda (Held *et al.*, 2003). There is evidence that the exterior of the hut or parts of it were painted a terracotta colour (Harrowfield, 1995). The interior is divided into five areas, as follows: two porches, a store room, the main area (Figure 1.15) and a physical laboratory (Figure 1.16). The main area is divided by timber boxes, blankets, sacking and canvas to create a cooking area. Whether or not these current boxes and materials were the ones used in the Heroic Period is unknown.

Figure 1.15: Photograph inside the main area of *Discovery* Hut (Source Professor Robert A. Blanchette, 1998).

Two main species of wood have been identified from the interior of the hut *Pseudotsuga menziessii* (Douglas fir) was used for beams and floor repairs and *Pinus sylvestris* (Scots pine) was used for floor, walls and ceiling (Held *et al.*, 2003).

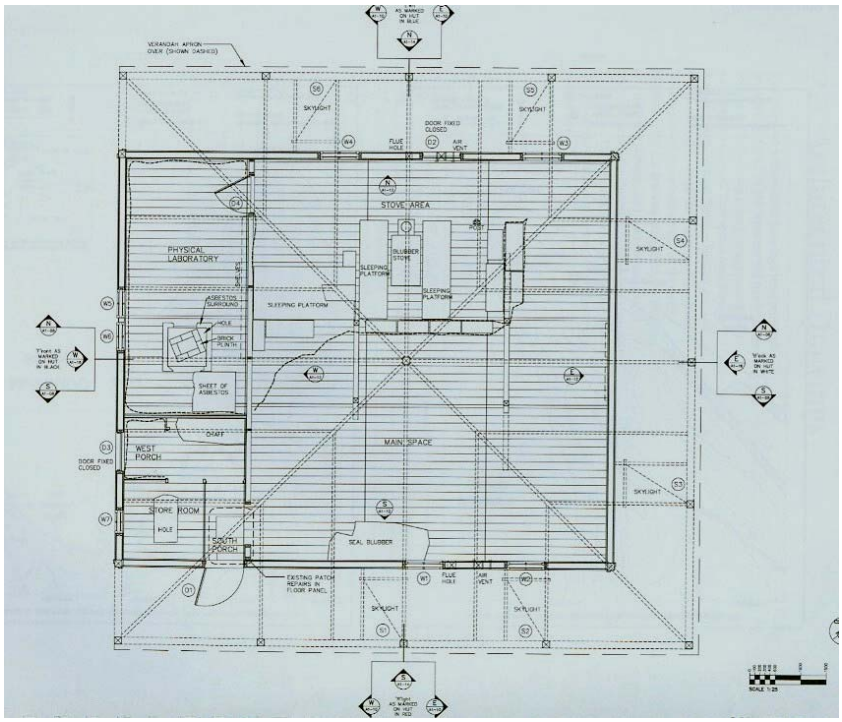

Figure 1.16: Map of *Discovery* Hut (Source AHT Conservation Plan *Discovery* Hut, Hut Point, 2004).

Discovery Hut has the smallest number of artefacts of the Historic Huts in the Ross Sea Region totalling some 350 items according to inventories taken during the summer work programmes from 1995-96 seasons to 2003. The scarcity of artefacts may be due to vandalism by visitors to the hut over approximately the last 50 years. The only artefacts outside the structure are found under the veranda and include a seal carcass, a winter awning, store boxes, tools and a pile of coal. Inside the hut the artefacts include mutton carcasses, chaff, dog and human provisions, utensils, kitchenware, personal effects, clothes, shoes, science equipment, primus stoves and candles. Besides all the artefacts bought to the site originally from outside of Antarctica, inside the hut are Antarctic derived artefacts of penguin carcasses and strips of seal blubber.

1.4.7.3.2 Cape Evans *Terra Nova* Hut - the British Antarctic Expedition (1910-1913)

Cape Evans is situated on the western side of Ross Island. The area is composed of loose scoria covered basalt bedrock. The site is an area of land designated as Antarctic Specially Protected Area (ASAP) No. 155 by the Antarctic Treaty Consultative Meeting (1998) and endorsed by the Scientific Committee on Antarctic Research (SCAR). A low ridge known as Wind Vain Hill shelters the site to the south, Mount Erebus dominates the backdrop of the hut to the east, to the north there is Home Beach, North Bay and the long cliffs of ice of the Barnes Glacier, while the distant view to the west is across McMurdo Sound to the Trans Antarctic Mountains. The hut is located on the North West side of Cape Evans and is built on a gentle sloping scoria beach facing Home Beach (Figure 1.17).

Figure 1.17: Photograph of *Terra Nova* Hut in January 2006.

Surrounding the hut are several other structures and a large number of stores and other items left by the expeditions. These include the remains of a stone hut, anchors from the *Aurora*, latrines, a meteorological screen, and an instrument shelter. A timber framed magnetic hut lined on the outside with asbestos is also present, it was used from 1911 until present day for magnetic observations and is now sheathed in a modern plywood structure due to the asbestos risk. On Wind Vain Hill, a cross is present, erected in 1916 for Mackintosh, Hayward and Spencer- Smith who died while part of Shackleton's Ross Sea party (1914-1917).

Terra Nova Hut at Cape Evans (78º 38' 10"S, 116º 25' 04"E) is the largest of the Historic Huts in the Ross Sea Region and was erected in January 1911. The hut is a rectangle shape with a gable roofed structure; the long axis orientated roughly north-east/south west. According to surveying done in 2002, the hut is 50 ft 7 in (15.415 m) long and 25 ft 7 in (7.795 m) wide. Inside, the side walls are 8 ft (2.45m) high and the end gables are 14 ft (4.3 m) high (AHT Conservation Plan, Scotts Hut Cape Evans, 2004). A stables area was added by Scott's expedition members within the first month at the site to the northern side of the hut which measured 50 ft 2in (15.3 m) by 11 ft 2 in (3.4m), and the western side had an area

called the annex which measured 36ft 8in (11.18m) by 9ft 4 in (2.86m) (Figure 1.18).

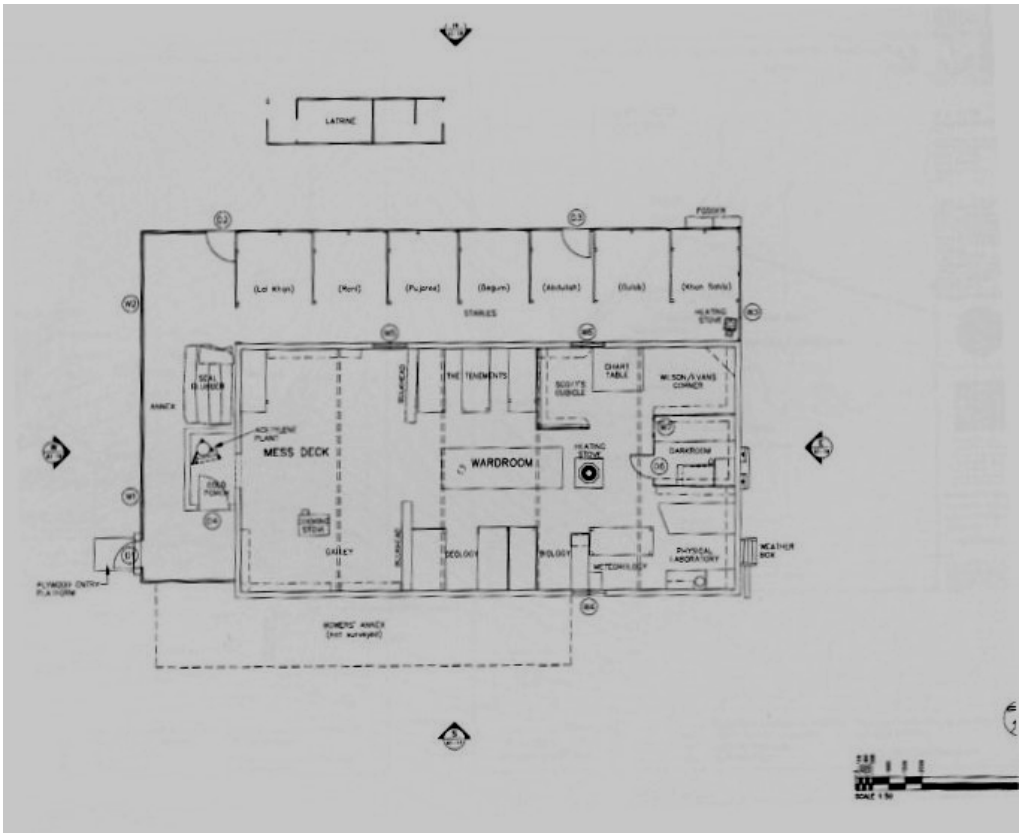

Figure 1.18: Map of *Terra Nova* Hut with stables and annex area shown (Source AHT Conservation Plan Scott's Hut, Cape Evans, 2004).

The *Terra Nova* Hut at Cape Evans is a timber structure, prefabricated in England. The building foundations are a series of wooden bearers placed directly on the ground. The wall lining consists of six layers. Inside the framing are two layers of tongue and groove boarding with Gibson seaweed quilted insulation, followed by another layer of tongue and groove then the weather boards. There are no structural walls in the interior of the hut (Figure 1.19). Three main species of wood have been identified from the exterior of the hut: *Pinus sylvestris* (Scots pine) used for North East (NE) and South East (SE) sidings, *Picea sp.* (Spruce) was used for the NE and stables siding and *Abies* sp. was used for North West

(NW) sidings and stable roof slats (Held *et al.*, 2003). New plywood and butyl clad were fixed directly to the original roof in 1991-1992, by conservators.

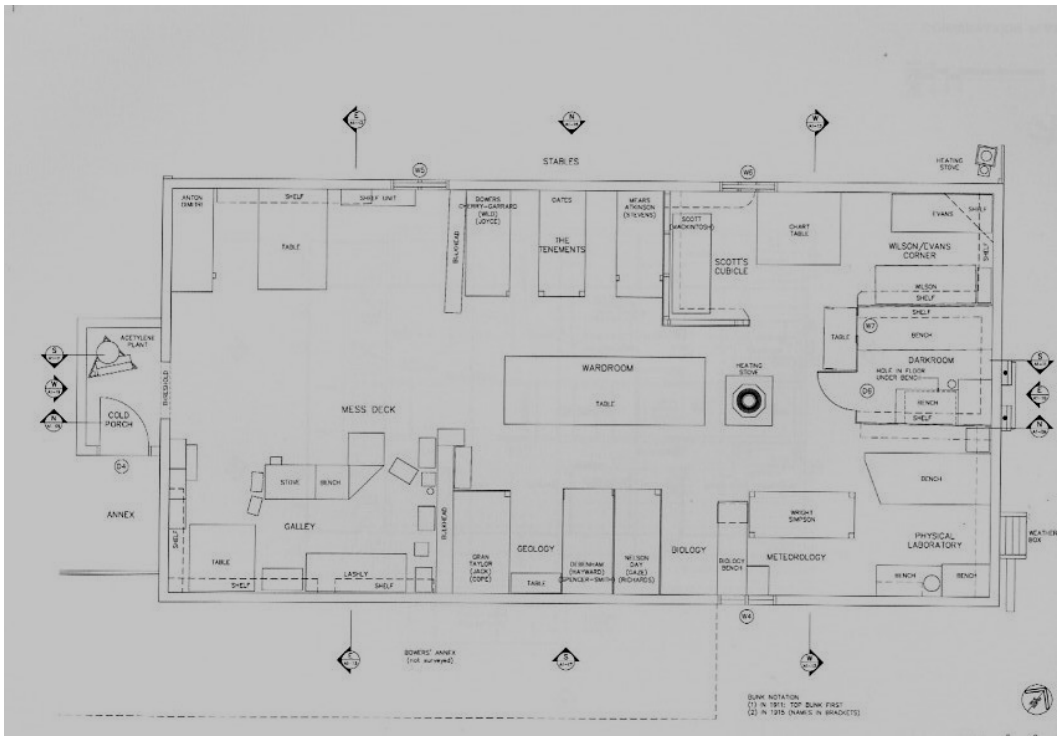

Figure 1.19: Map of the layout within the *Terra Nova* Hut (Source AHT Conservation Plan, Scott's Hut, Cape Evans, 2004).

The hut is entered through the door in the west wall of the annex; the original exterior door faces into the annex from a cold porch and from the cold porch to the main part of the hut (Figure 1.20).

Figure 1.20: Photograph of the annex area with the main door to the hut on the left (Source Professor Roberta Farrell, 2002).

The main part of the hut is one large space subdivided into two areas, the mess deck and the wardroom (Figure 1.21). These in turn are divided by stores boxes, and other left over materials to form a cubicle for Scott, a corner for Wilson/Evans, a darkroom, a physical laboratory, a geology area, the "tenements" (Quartermain, 1963) and a galley. The interior of the hut wood is finished in wood. The main species of wood have been identified from the interior of the hut *Pinus sylvestris* (Scots pine) was used for ceilings, floor and walls (Held *et al.*, 2003).

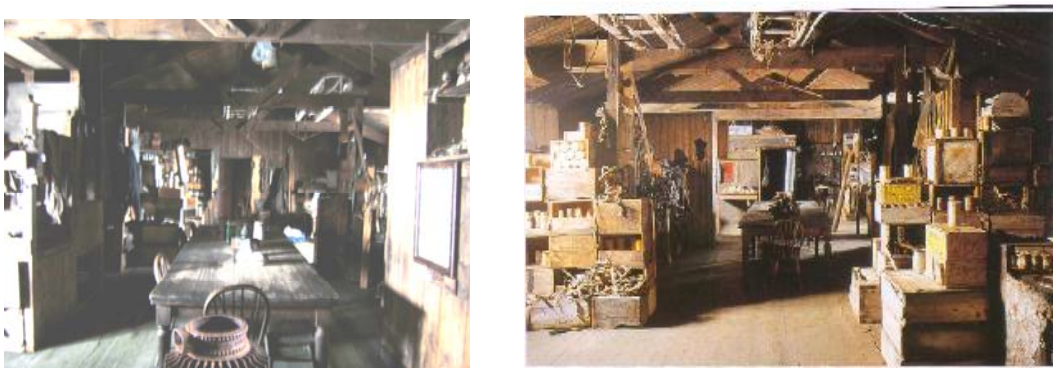

Figure 1.21: Photographs inside *Terra Nova* Hut. Left: looking from the darkroom to the main door, Scott's cubicle is to the right and the galley area is in the distant left. Right: looking the opposite direction with the darkroom in the distance and the galley area to the right. (2006, 1998)

Cape Evans has a total of 10,000 artefacts, both inside and outside the hut, within the ASPA. Outside, the collection of artefacts include fuel drums, remains of vehicles, metal, sledges and sledge parts, ladders, pipes, wire, nails, dog skeletons, human provisions, kitchenware, dog equipment, hay, and stores boxes (Figure 1.22).

Figure 1.22: Photograph of stores boxes outside at *Terra Nova* Hut. (2001)

Inside the hut, the artefacts include a bicycle, tools, fuel drums, skis, Allen acetylene plant, furniture, human provisions, bottle, rat traps, cutlery, kitchenware, stoves, baking ware, ropes, hooks, lamps, primuses, bedding material, clothing, horse gear, sleeping bags, shoes, books, hot water bottles, medicines, darkroom chemicals and equipment, laboratory equipment, car parts, stores boxes and hockey sticks. Besides all the artefacts brought to the site from outside of Antarctica, inside the hut are Antarctic derived artefacts of penguin eggs and strips of seal blubber (Figure 1.23).

Figure 1.23: Photograph of strips of seal blubber in Annex area at *Terra Nova* Hut (Source Lisa Robson, 2006).

1.4.7.3.3 Cape Royds *Nimrod* Hut - the British Antarctic Expedition (1907-1909)

Cape Royds is an ice free area at the western extremity of Ross Island. The ice free area is composed of till covered basalt bedrock. The historic area occupies the land designated as Antarctic Special Protected Area No 27. by the Antarctic Treaty Consultative Meeting (1998) and endorsed by the Scientific Committee on Antarctic Research (SCAR). It is located to the north east of Cape Royds adjacent to Backdoor Bay and is immediately to the east of the designated Site of Special Scientific Interest (SSSI0 No.1, an Adélie penguin rookery). Shackleton's British Antarctic Expedition 1907-1909 hut is the focal point of the area. As well as the hut, the area includes a meteorological screen which is situated on a low ridge to the south-west of the hut. The area also contains seven archaeological deposits including a rubbish dump, ship unloading sites, pony line, burn site and sheep skeleton. The *Nimrod* Hut (77°33'10.7"S, 166°10'6.5"E) is sited in an area where it is sheltered by low surrounding ridges and outcrops of volcanic kenyte. It is a conventionally framed prefabricated timber structure, rectangular in shape (Figure

1.24). The hut was sourced from a firm in East London which subsequently had its records destroyed in World War II bombing (R.Headland, personal communication). The hut, when surveyed in 2002 is 33 ft (10.1 m) long and 19ft 2in (5.855m) wide with a porch 6ft (1.845 m) long by 5ft 2in (1.59 m) wide. The long axis of the hut is orientated on a line east of north (AHT Conservation Plan Shackleton's Hut 2003). The foundations were formed by a series of timber piles set into the ground. The floor is tongue and groove. The porch and main door are at the west end of the hut. The walls are vertical tongue and groove.

Figure 1.24: Photograph of *Nimrod* Hut in 2003, Mount Erebus in the background (Source Professor Roberta Farrell).

Roofing felt and granular cork were used as insulation along with full stores boxes, which were stacked along the southern and eastern walls and volcanic soil poured in and round the boxes to fill gaps. The windows on the southern side are permanently shuttered, probably since Shackleton's time. Wire hold-down cables lay across the hut diagonally and are anchored in the ground by timber deadmen frozen into the permafrost. A stables area was built along the north side of the hut (Figure 1.25) and a garage/workshop was built next to the stables from fodder and stores boxes with a canvas roof. This area has been used by the Adélie penguin as

a nesting site and penguin feathers and guano has built up over the years. The north wall of the hut is covered with galvanised iron sheets to protect the hut from damage by the ponies which were housed along this wall. A stores hut was built out of stores boxes with hammocks used as a roof on the opposite side of the porch from Mawson's laboratory; this structure collapsed after the first blizzard in 1907. Mawson's laboratory was also built after the construction of the main hut and is separate from the main hut. An additional hut was built from cases near the south east corner of the hut (Figure 1.26).

Figure 1.25: Photograph of stables area at *Nimrod* Hut (Source Professor Roberta Farrell, 2002).

Two main species of wood have been identified from the exterior of the hut *Pinus sylvestris* (Scots pine) used for fascia boards and *Picea sp.* (Spruce) used for sidings (Held *et al*. 2003). New plywood and Butyl clad were fixed to the roof in 1990-1991 by AHT and canvas was placed over the top of the Butyl cladding in January 2006.

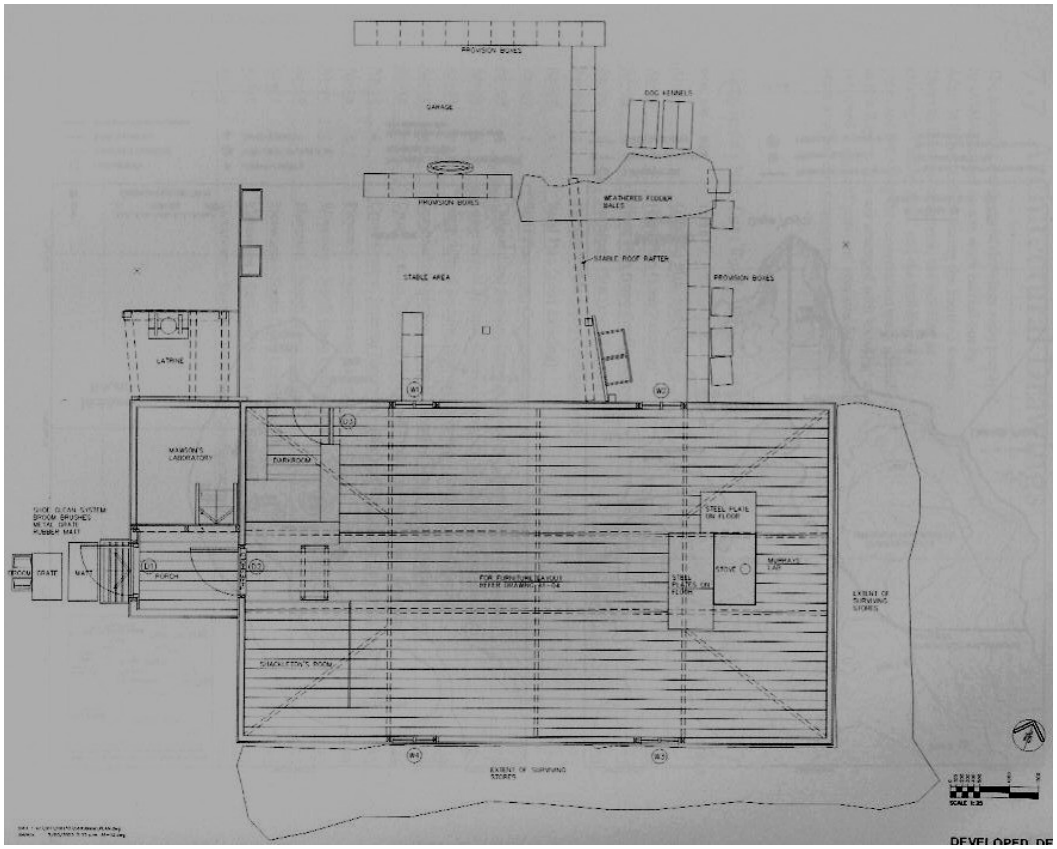

Figure 1.26: Map of *Nimrod* Hut showing stables area, and garage area (Source AHT Conservation Plan Shackleton's Hut 2003).

The hut is entered via the cold porch with Mawson's laboratory to the side. Inside the hut is one main area with two rooms off of it, one room was used by Shackleton as a bedroom, library and storage area for scientific equipment, the other room was used as a darkroom (Figure 1.27). The main area was divided by canvas into cubicles for the men with a galley area in the south east corner (Figure 1.28).

Figure 1.27: Photograph inside at *Nimrod* Hut looking towards main door canvas divide is in the right and door in the far right leads to the darkroom. (2004)

Two main species of wood have been identified from the interior of the hut, *Pinus sylvestris* (Scots pine) was used for ceiling beams and fascia boards and *Picea sp.* (Spruce) used for ceilings, floor and walls (Held et al 2003).

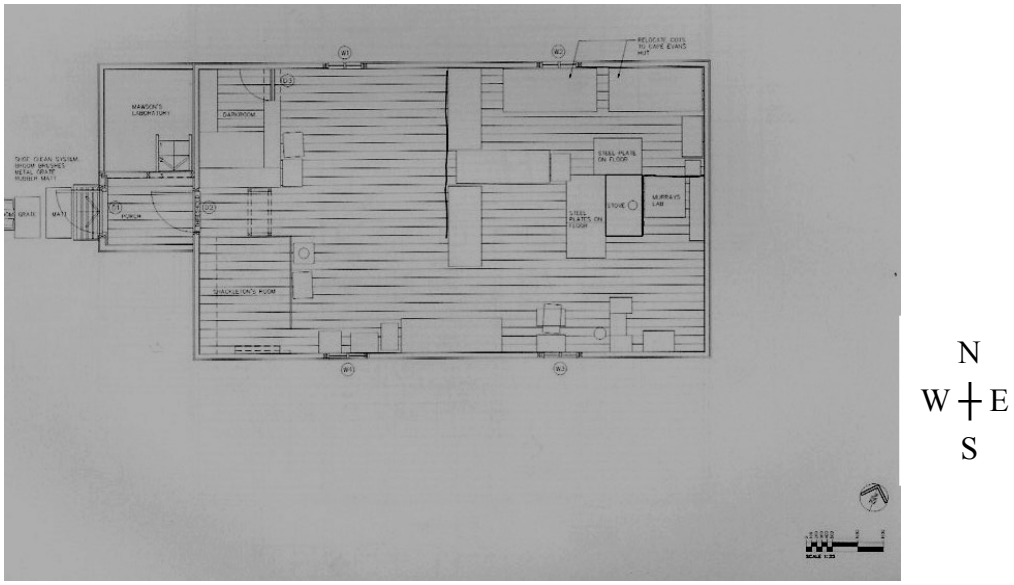

Figure 1.28: Map of inside the *Nimrod* Hut showing layout of artefacts inside (AHT Conservation Plan Shackleton's Hut 2003).

Cape Royds has a total of 3,000 artefacts both inside the hut and within the ASPA. Outside the collection of artefacts include: wheel and rim from the car,

fodder, dog kennels, human food provisions, iron sheets, wire ropes, horse gear, canvas, and scientific equipment and stores boxes. Inside the hut, the artefacts include tools, car lubricant, metal objects, candles, acetylene gas plant, furniture, human provisions, bottle, cutlery, kitchenware, “Mrs. Sam” stove, baking ware, bedding material, clothing, horse gear, dog gear, sleeping bags, boots/shoes, books, paper, medicines, darkroom chemicals and equipment, laboratory equipment, stores boxes and hurricane lamp.

Chapter 2 Background to PhD Research

2.1 History of Antarctic Science Events Relative to PhD Research

In January 1997, after a discussion at Scott Base between members of Antarctic Heritage Trust and Professor Roberta Farrell of the University of Waikato, a multidisciplinary project was conceived to study the deterioration of the Historic Huts of the Heroic Period on Ross Island. The disciplines used to address this study including biochemistry, mycology, microbiology, molecular biology and chemistry.

The first Antarctica New Zealand science event for this study, designated Event K024C, began in the 1998/1999 season and was a collaboration between Professor Roberta Farrell from the University of Waikato and Professor Robert Blanchette from the University of Minnesota. The aims of Event K024C were to evaluate the deterioration of the Huts and artefacts of the Heroic Period at Hut Point, Cape Evans and Cape Royds. The evaluation was both for microbiological damage, fungal and bacterial decay, and environmental, non-biological damage, including freeze/thaw, salt, and ultraviolet damages. The areas for evaluation were the interiors and exteriors of the huts, and surrounding areas, including outlying depots. The microorganisms, both fungal and bacterial, isolated from the Huts and artefacts were to be identified when readily possible and compared to Northern and Southern Hemisphere species; subjecture as to whether microorganism were indigenous to Antarctica or introduced with suggested possible modes of introduction either by the Heroic Explorers or subsequent visitors was also to be attempted.

In the 1999/2000 season, Antarctica New Zealand renamed Event K024C as Event K021, and the 1999/2000 season's research had the additional goals to isolate, identify and characterise the dominant microbes (bacterial and fungal) from a variety of Antarctic ecosystems including soil, structural timber, and artefactual materials such as hay and animal skins, in order to determine microbial diversity, and particularly to address biological indicators of climate change and microbial mechanisms for survival in extreme cold. The Event also had the extended laboratory goal to determine whether degradative enzymes were expressed by these microbes which can threaten the historic materials, and to assist definition of conservation management procedures. HOBO data loggers were installed in the huts to monitor temperature and humidity throughout the year.

Since 2000, Professor Blanchette received funding from U.S.A. National Science Foundation to study the deterioration of the wood and artefacts of the heroic period huts and the University of Minnesota contingent of K021 became NSF event number NSF BO 038.

From 2000 to 2004, Event K021 focused on three major aims:

1. To continue to identify the cause of biological and non-biological deterioration present in the Historic Huts and artefacts as well as wooden crates/packaging left behind at various sites in the Ross Dependency including the rock shelters at Granite Harbour and Cape Crozier. This aim sought to identify the cause of deterioration present in the Historic Huts by the following:
    - To determine the current condition of the deteriorated wood.

- To obtain a better understanding of the unique degradation processes responsible for the deterioration with particular attention to microbial and enzymatic decay.
- To investigate the historic fuel spills at Cape Evans.

2. To investigate the bio-diversity of the biological organisms in the Historic Hut areas, especially fungi, using molecular DNA probes as well as traditional morphologically-based taxonomic approaches. This aim addressed as follows:
    - To examine the origin of the organisms as to whether presumptively they were introduced to the continent during the Heroic period.
    - To determine whether the organisms were capable of mycotoxin production or other pathological conditions.
    - To test for persistence and viability of Antarctic organisms
3. To test conservationally acceptable materials for their long-term preservation on wood panels established at the Cape Evans, Cape Royds, and Hut Point sites in 1999.

In addition, the seasons from 2000-2004 had two additional aims which were in geographic areas distant from Ross Island but whose findings nonetheless added to those from Ross Island by having test sites distal to Ross Island for comparison.

1. To investigate bio-diversity in sites away from the historic sites this has included a late 1950's wooden crate that is located at New Harbour.
2. To study the Mt Fleming area for fungal biodiversity as this area is rich in preconifers fossils that show evidence of fungal decay.

The goals of Events K021 and NSF BO 038 for the 2005/2006 season were to address the ecosystem functioning of microorganisms isolated from the Antarctic Specially Protected Areas (ASPA) of the Ross Dependency that contain the Heroic Period Historic Huts, and from surrounding natural environments. The research focused on terrestrial fungi and *Bacillus* species. Both physiology of the whole microorganism as well as biochemistry of crucial enzymes were studied to understand the underlying mechanisms of cold adaptation, proliferation and life in extreme environments.

2.2 Published research findings

The Historic Huts and their contents of the Heroic Period in the Ross Dependency are a legacy of human exploration. After the first three seasons of research by the Universities of Waikato and Minnesota collaboration, it was concluded that the extreme polar environment had protected many of the wooden huts and artefacts of the Heroic Period of exploration from rapid decay but they were not free from deterioration. The results of this research have been used in the Ross Sea Heritage Restoration Project and three Conservation Plans citing this research have been published (AHT Conservation Plan Shackleton's Hut 2003; AHT Conservation Plan Scott's Hut, Cape Evans, 2004; AHT Conservation Plan *Discovery* Hut, Hut Point, 2004). These comprehensive plans provided an outline of the proposed conservation work at each of the sites and were complemented by Implementation Plans which detailed the physical works required for each Historic Hut.

A total of seven peer reviewed publications have resulted from the research of Events K021 and NSF BO 038. A list of all publication that the PhD author Shona M. Duncan has contributed is supplied in Appendix 9.

2.2.1 Discussion of Key Findings from the research of K021 and NSF BO 038

In Antarctica, wind erosion especially on the southern facing walls is a significant factor in non-biological degradation of the wood of the Historic Huts. Defibration of wood has been seen to be causing significant damage to the Historic Huts, most severely at *Terra Nova* Hut. Large concentrations of salt have been seen accumulating at evaporative surfaces. The origin of these salts is from snow accumulating on and around the hut by wind blown seawater and salts accumulating on the surface of the volcanic sands. The corrosive effect of these salts affects the middle lamella of the wood cells, which is mainly comprised of lignin and subsequently wood fibres detach. Often thought to be caused by freeze-thaw cycles or by salt crystal growth in the wood, this type of damage was identified as a chemical degradation of specific wood components. The cause of defibration is a chemical degradation of lignin, of key importance since lignin constitutes about 25% of wood composition. After the wood is defibrated, it is more susceptible to removal by the wind or washed off by melt water.

Figure 2.1: Photographs of wood defibration Left: Stable wall Terra Nova hut showing ice melting down the wall. Right: Closer photograph showing layers of wood cells washing off in the melting snow (Source Professor Robert A. Blanchette, 1998).

Soft rot was determined to be the main cause of biological degradation of the Historic Huts, and it was the only form of wood decay seen to be occurring in the

three Historic Huts in Antarctica (Blanchette et al, 2004). Unique soft rot wood decaying fungi were shown to be present in wood of the Historic Huts in contact with the ground, with a slow but progressive decay taking place. The decay pattern seen in the wood by Scanning Electron Microscopy (SEM) was characteristic of a soft rot fungus and was identified as *Cadophora* spp. These fungi are often out-competed by more common rot fungi in ideal decay conditions. *Cadophora* spp were isolated from through out the *Terra Nova* and *Nimrod* huts but was only isolated once at *Discovery* hut where no decay was observed in samples taken and subsequently observed by SEM.

Using molecular DNA analysis of the intervening transcribed region (ITS) of ribosomal DNA (rDNA) of the fungal isolates, *Cadophora malorum*, *C. luteo-olivacea*, *C. fastigata* and at least 3 new species were isolated and they appear to be soil colonizing organisms that are endemic to Antarctica. When *Cadophora malorum, C. luteo-olivacea* and *Cadophora* sp. strain E were reinoculated onto wood wafers of *Betula* and *Populus*, type 1 soft rot was seen after 12 months incubation at 24°C. Wood wafers of *Picea* and *Pinus* inoculated with the fungi showed no sign of decay after 12 months incubation but the wood was stained with dark hyphal growth.

In addition to the soft rot fungi, other moulds have been seen growing within all of the Historic Huts, and on foodstuffs and artefacts. Environmental monitoring, (Held *et al*., 2005) specifically temperature and relative humidity, provided extensive data on all the Huts. The Huts are offering favourable conditions for fungal growth (designated as temperature above 0°C and relative humidity >80%) for various periods of the year. At Cape Evans, conditions are favourable for

growth over the period 2000-2002 between 79 and 569 hours per year, the difference in the number of hours is due to the specific results of the dataloggers maintained at specific areas in the hut; obviously, micro-climates arise even within the hut. Areas where there is little air movement appear to be above 0°C and > 80% relative humidity for longer than areas with good airflow. At Cape Royds, the hours favourable for growth over the period 2000-2002 were less than Cape Evans, with the range being favourable for growth between 0-55 hours. At *Discovery* Hut conditions in the hut were never favourable for growth in the years 2001 and 2002 but in year 2000 the number of hours favourable for growth ranged from 8-11 hours. The *Terra Nova* hut has the most visible fungal growth of the three Historic Huts being monitored (Figure 2.2).

Figure 2.2: Photograph of mould growing in a stores box inside *Terra Nova* Hut. (2006)

Moisture accumulation after visitor entrance has also been investigated and the present guidelines for visitor numbers are not causing significant changes in relative humidity or temperature within the hut environment.

Investigations of deterioration and fungal biodiversity were conducted at sites containing wood away from the historic sites on Ross Island. One particular site is a wooden crate at New Harbour used in 1959 and 1960 by scientists as protection when transported across the sea ice and became a makeshift cookhouse and work area, after this it was abandoned (Held *et al*., 2006). The wood on the outside of the crate showed evidence of wind erosion, salt defibration and UV light degradation. A variety of fungi were isolated from the structure including *Cadophora fastigiata* from the wood of the crate; this species was not isolated from the Ross Island Historic Huts. A previously undescribed species of *Cadophora* (designated *Cadophora* C sp.NH) was also isolated from the New Harbour crate.

During the course of this research (Held *et al*., 2003) the wood species used to build the hut have been identified and a wide variety of different woods were used to construct the huts. The major wood genus used in *Discovery* and *Terra Nova* Hut was *Pinus* sp. and at *Nimrod* Hut the major wood genus was *Picea.*

Test panels consisting of blocks of wood treated with different silicon products and oil based paint were established at all three huts. After 2 years exposure to the Antarctic environment, the control blocks are showing signs of significant erosion.

From an assessment of environmental pollutants (Blanchette et al. 2004) at the historic sites, the following pollutants were noted:

- historic oil and motor spirit spills leading to petroleum contaminated soil
- a chemical spill in the Cape Evans Hut
- unlabelled bottles of chemicals, and laboratory equipment
- asbestos residues within the historic boundaries

- heavy metal contaminated soil due to lead based paint and deteriorating metal cans
- broken stores boxes and contents around the huts

A recommendation was made that all these pollutants require remediation to ensure they are not spread further into the Antarctic environment.

Arenz *et al*. (2006) investigated fungal biodiversity using traditional culturing methods as well as molecular methods denaturing gradient gel electrophoresis (DGGE) using the internal transcribed spacer (ITS) region of ribosomal DNA for identification. Samples included wood, artefacts, and soils from the three Historic Huts and soils from Lake Fryxell basin in the McMurdo Dry Valleys as well as Mount Fleming and Allan Hills. The dominant fungi from the Historic Hut wood and soils were *Cadophora*, *Cladosporium*, and *Geomyces* while the sites away from the huts contained *Cryptococcus* spp. *Epicoccum nigrum* and *Cladosporium cladosporiodes*. DGGE revealed 28 taxa not detected by culturing and 4 taxa which appeared to be not previously described.

As part of this thesis research, a study was completed on filamentous fungi from interior structural wood from 15 sites around the Cape Evans historic hut. They were screened for their ability to degrade carboxymethyl cellulose (CMC) and twenty seven were positive for cellulase activity. Endo-1,4-β-glucanase activity was demonstrated in the extracellular supernatant from isolates when grown at 4ºC, and also when they were grown at 15ºC. A temperature growth optima characterisation of five was reported and all showed the ability to grow at 4ºC (Duncan *et al*., 2006). Two isolates, *Penicillium roquefortii* 405 and *Cadophora malorum* 182, had higher growth rates, a larger fungal biomass accumulation per

hour in the log phase of growth at 15ºC rather than at 4ºC or at 25ºC, indicating psychrotrophic characteristics. Another *Penicillium roquefortii* isolate, 408, and *Cadophora malorum* 242 had a higher growth rate at 25ºC than at 15ºC or 4ºC. *Geomyces* sp. isolate 711 had similar rates of growth at all 3 temperatures. These results demonstrated that cellulolytic filamentous fungi found in Antarctica were capable of growth at cold temperatures and possessed the ability to produce extracellular endo-1,4-β-glucanase when cultured at cold and temperate temperatures.

The research presented in this PhD thesis includes the findings of the previous paragraph and continues on from the work presented in the following two papers. The findings of *Cadophora* sp., a soft-rot fungus isolated at the Historic Huts, led to the screening and investigation of the enzyme cellulase. These organisms break down the secondary cell wall an area of the wood cell structure rich in cellulose. The paper on the environment within the Historic Huts formed the direction of incubation temperature and experiment temperatures for many of the experiments completed during this thesis research.

2.2.2 Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L.,Harrington, T.C., Duncan, S.M., and Farrell, R.L. 2004. Wood Destroying Soft Rot Fungi in the Historic Expeditions Huts of Antarctica. *Applied Environmental Microbiology* 70: 1328-1335.

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Wood-Destroying Soft Rot Fungi in the Historic Expedition Huts of Antarctica

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Three expedition huts in the Ross Sea region of Antarctica, built between 1901 and 1911 by Robert F. Scott and Ernest Shackleton, sheltered and stored the supplies for up to 48 men for 3 years during their explorations and scientific investigation in the South Pole region. The huts, built with wood taken to Antarctica by the early explorers, have deteriorated over the past decades. Although Antarctica has one of the coldest and driest environments on earth, microbes have colonized the wood and limited decay has occurred. Some wood in contact with the ground contained distinct microscopic cavities within secondary cell walls caused by soft rot fungi. *Cadophora* spp. could be cultured from decayed wood and other woods sampled from the huts and artifacts and were commonly associated with the soft rot attack. By using internal transcribed spacer sequences of ribosomal DNA and morphological characteristics, several species of *Cadophora* were identified, including *C. malorum*, *C. luteo-olivacea*, and *C. fastigiata*. Several previously undescribed *Cadophora* spp. also were found. At the Cape Evans and Cape Royds huts, *Cadophora* spp. commonly were isolated from wood in contact with the ground but were not always associated with soft rot decay. Pure cultures of *Cadophora* used in laboratory decay studies caused dark staining of all woods tested and extensive soft rot in *Betula* and *Populus* wood. The presence of *Cadophora* species, but only limited decay, suggests there is no immediate threat to the structural integrity of the huts. These fungi, however, are widely found in wood from the historic huts and have the capacity to cause extensive soft rot if conditions that are more conducive to decay become common.

Three huts, *Discovery* hut built by Robert F. Scott and his crew in 1901, Cape Royds hut erected by Ernest Shackleton's *Nimrod* expedition in 1908, and Cape Evans hut built by Scott's *Terra Nova* expedition in 1911, were used for sheltering men and equipment for several years during scientific investigations and exploration of the South Pole region. The Cape Evans hut also was used by the Ross Sea party in 1914 to 1917, which was part of Shackleton's Imperial Trans-Antarctic expedition. These huts are now international heritage sites that are protected for their historic significance and cultural materials from the "Heroic Era" of exploration. Serious wood deterioration has become evident in the huts and artifacts during the past few decades, causing concern for the long-term preservation of these historic sites (2, 16, 17). It is a great misconception that the cold, dry polar climate protects organic material from decomposition (8, 19), and significant deterioration has occurred in the 90 to 100 years since the huts were built. Nonbiological deterioration of wood from the huts and artifacts caused by salt corrosion has resulted in significant damage (8). Microbial degradation of wood at these historic sites also may occur (6, 18), but nothing is known about the organisms responsible for the degradation, the frequency of occurrence, their distribution, or the extent of degradation that has occurred.

Wood deterioration in temperate and tropical forest ecosystems and in wood products has been widely studied, and many studies on microbial decay and the mechanisms of wood degradation have been published (11, 12, 27). Decay caused by many common white and brown rot fungi has been well characterized, but other types of decay, such as soft rot by fungi or bacterial degradation of wood, are not well understood (8). Soft rot is caused by fungi taxonomically classified in the phylum *Ascomycota*, including related asexual taxa, and the resulting decay usually is characterized by chains of cavities that form within the cell walls of wood. These biconical and cylindrical cavities form along the microfibrillar structure of the secondary wall and have a spiral orientation. The attack is localized to the secondary walls, and no degradation of the middle lamella occurs. Decay with microscopic evidence of such cavities is classified as type 1 soft rot (4). Another form of soft rot, type 2, also can occur. This type of attack does not form cavities within the cell wall but causes a progressive degradation of the secondary wall from the cell lumen to the middle lamella. In advanced stages of decay, the entire secondary wall may be completely degraded but the middle lamella between cells is not affected. The term soft rot is used because it was first identified from soft, decayed wood surfaces in contact with excessive moisture (14). Soft rot can occur not only when wood is wet but also in dry environments (5, 7, 13). Conditions that are exceedingly wet or dry apparently inhibit the growth of common and usually more aggressive wood-decaying basidiomycetes, but these adverse conditions do not limit colonization and decay by soft rot fungi. The extreme environmental conditions found in Antarctica have a strong impact on microbial growth and biodegradation. Cold temperatures, short austral summers, elevated salt concentrations,

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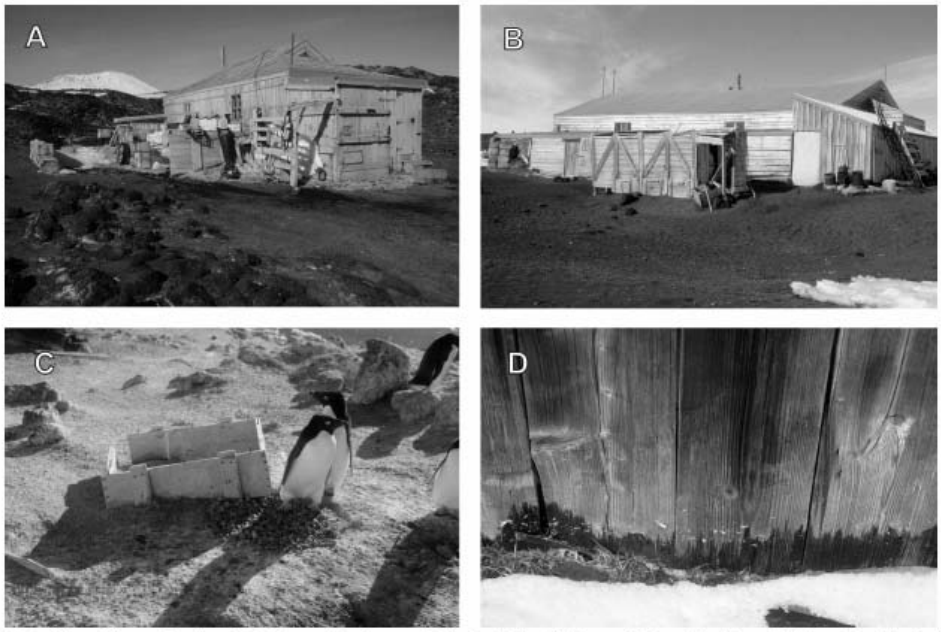

FIG. 1. Historic huts and artifacts left in Antarctica after expeditions by Robert F. Scott and Ernest Shackleton. (A) Cape Royds hut built in 1908, showing the hut structure and area used for the stables and storage adjacent to the hut. (B) Cape Evans hut built in 1911. The hut structure contains an annex and stable area enclosed within the walls of the hut. A row of historic latrines is in front of the hut. (C) Wooden storage box and Adele penguins nesting at Cape Royds. Many wooden storage boxes and other artifacts are located in the area around the huts. (D) Exterior wall boards from Cape Evans hut. Melt water from the ground is absorbed by the lower boards during the austral summer.

and high UV exposure as well as many other factors strongly influence the type of microorganisms that can survive at the site. The large quantities of wood from Europe used to build the expedition huts provide an unusual opportunity to study microbial decay processes occurring in this unique environment where wood did not previously exist. The origin of the decay microbes also is of interest, since no native higher plants with lignocellulose occur in the Ross Sea region. If wood-destroying fungi were brought into Antarctica with the wood used for the prefabricated huts, or subsequently by visitors, then these fungi would be similar to organisms found where the wood originated or would be common to other regions of the world.

This investigation was done to (i) evaluate wood decay present at the three historic huts in the Ross Sea region, (ii) identify fungi isolated from the decayed wood by using internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA), (iii) determine where these fungi are located in the historic huts, and (iv) evaluate their decay potential in laboratory studies. Little is known about deterioration of wood in polar regions, and our results provide new information on decay fungi that are present in the woods taken to Antarctica by the early explorers and elucidate the type and extent of degradation that has occurred over the past decades. In addition to advances in polar biology, these results should provide information crucial to conservators for preservation of these important historic sites.

MATERIALS AND METHODS

Collection of samples. Samples of wood were obtained from the huts and wooden artifacts within the historic boundaries of the *Discovery*, Cape Royds (Fig. 1A), and Cape Evans (Fig. 1B) huts on Ross Island, Antarctica. A wide range of woods, including pine, spruce, and birch, were used in the construction of the huts and for storage boxes and other items (18). Samples were obtained under Antarctic Conservation Act permit numbers 2001-015 and 2002-001. This work was done in cooperation with the Antarctic Heritage Trust, Antarctic New Zealand program K021, and the National Science Foundation (Washington, D.C.). Minute segments of wood exhibiting decay in contact with the ground and wood from various locations in the hut structures and from wooden objects outside of the huts were taken. Samples were placed in sterile plastic bags and brought to the laboratory for analysis. A portion of each sample was used for culturing microorganisms, and another was used for scanning electron microscopy. No excavations were made to obtain samples from the hut foundations below ground, and samples were taken only from accessible locations that did not disturb the historic site. When a sample was taken, only a small sliver of wood was removed from an inconspicuous location.

To compare fungi obtained from the historic huts with other fungi that may have colonized wood at another location in Antarctica, samples were obtained from a wooden structure taken to New Harbor, Antarctica, by New Zealand researcher John McCraw in November 1959. This wooden hut was used for shelter and storage and is located across the Ross Sea, approximately 64 km from

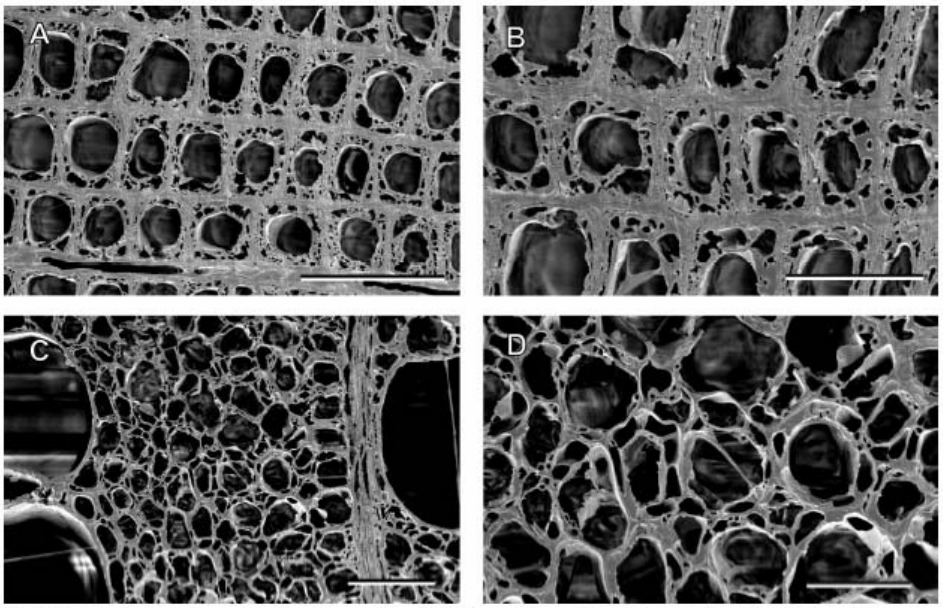

FIG. 2. Scanning electron micrographs of transverse sections of historic wood decayed by soft rot fungi. (A and B) Soft rot cavities in pine wood that was in contact with the ground from the exterior of Cape Evans hut. The secondary walls of tracheids contain numerous cavities of varying size. (C and D) Soft rot cavities in birch wood from a wooden storage box outside of the Cape Evans hut that was in contact with the ground. Advanced decay is present and large holes, formed by many secondary wall cavities that have coalesced together, are seen in fiber cell walls. Bar, 50 μm (A and C) or 25 μm (B and D).

the historic expedition huts. Samples were obtained by methods similar to those used for sample collection at the historic huts.

Sample analyses. Small wood segments were cut aseptically and placed on culture media to isolate the microorganisms present. Media used for isolations included 1.5% Difco malt extract agar (MEA), a basidiomycete-selective agar medium (24), and acidified MEA containing 2 ml of lactic acid added after autoclaving. Wood samples were prepared for scanning electron microscopy as previously described (9). Samples were frozen and cut in a cryostat freezing microtome, mounted on aluminum stubs, coated with gold, and examined with a Hitachi S3500N variable-pressure scanning electron microscope. Thin sections of wood also were cut and examined using light microscopy to detect soft rot cavities.

Identification of fungi based on rDNA sequences. Fungal cultures obtained from isolations were transferred and maintained on MEA. The two ITS regions ITS1 and ITS2 and the highly conserved 5.8S gene of the ribosomal repeat region were amplified and sequenced. The protocols for growing cultures, extracting DNA, PCR and automated sequencing, sequencing primers, and the cycling conditions were described previously (20). We sequenced both strands of all fragments to assure fidelity. BLAST searches were done with sequences from each of the fungi obtained from the wood samples, and similar sequences identified by the BLAST searches were used in phylogenetic analyses as previously described (15). The phylogenetic studies also utilized ITS sequences of related taxa from an earlier study (15), many of which were from cultures obtained from the Centraalbureau voor Schimmelcultures (CBS), including Antarctic strains of *Cadophora malorum* (CBS 257.89 [ITS sequence AY249058], CBS100584 [AY249062], CBS 100591 [AY249063], and CBS 377.77 [AY249064]); *C. luteo-olivacea* (CBS 141.41 [AY249066]); and *C. melinii* (CBS 268.33 [AY249072]). The nucleotide sequences were manually aligned, and the aligned DNA sequences were analyzed using PAUP version 4.0b10a (D. L. Swofford, Sinauer Associates, Sunderland, Mass.). After alignment, no gaps were greater than 3 bases, and gaps were treated as a "Newstate." Of 580 aligned characters, including gaps, 125 were eliminated because of ambiguous alignment, 287 were constant, and 47 were parsimony uninformative. *Tapesia cinerella* was the outgroup taxon in the ITS analysis, and1,000 bootstrap replications were run to determine confidence levels at branching points.

Laboratory decay studies. Wafers 10 by 10 by 2 mm were cut from sound wood of *Betula*, *Populus*, *Picea*, and *Pinus* samples, soaked in distilled water for 1 h, and autoclaved. Three sterile wood wafers were placed onto the surface of each actively growing fungal culture 8 to 10 days after inoculation on MEA. Duplicate plates of *C. malorum*, *C. luteo-olivacea*, and an undescribed *Cadophora* species designated as *Cadophora* sp. strain E were used for each wood type. Petri dishes were sealed with parafilm and incubated at 24°C. Thin sections were aseptically removed from the wafers and observed after 3- and 6-month incubations. Sections were examined using light microscopy for evidence of soft rot cavities within the secondary wall layers. Samples showing soft rot were prepared for scanning electron microscopy and photographed.

RESULTS

Many samples of wood in contact with the ground from the Cape Royds hut (Fig. 1B) had evidence of soft rot. These samples included wood from the stables area on the north side of the hut and wood from storage boxes and other wooden artifacts on the ground around the hut (Fig. 1C). Soft rot decay also was found at the Cape Evans hut in various wooden artifacts and miscellaneous pieces of historic wood located outside the hut (Fig. 1D). No soft rot was found in wood from the *Discovery* hut. Soft rot observed in the historic woods contained secondary cell wall cavities that were typical of a

TABLE 1. Isolate numbers, collection information, and DNA sequence accession numbers for representative isolates of *Cadophora* species from Antarctica

Species	Isolate number[a] and collection data	ITS rDNA[b]
C. malorum	2R20; Cape Royds hut, exterior wall board from northeast corner of hut just below ground	AY371503
	3R47; Cape Royds hut, miscellaneous piece of historic wood beneath ground	AY371505
	3E41-1; Cape Evans hut, exterior wall board from stable at ground line	AY371504
C. luteo-olivacea	E114; Cape Evans hut, exterior board from stable door at ground line	AY371507
	2E37; Cape Evans hut, wood from storage crate below ground	AY371508
	3E84; Cape Evans hut, board from latrine below ground	AY371509
	3E41-2; Cape Evans hut, exterior wall board from stable at ground line	AY371510
C. fastigiata	NH5-1; McCraw hut at New Harbor, exterior wood from east wall below ground	AY371511
Cadophora sp. strain E	4E71-1; Cape Evans hut, wood from bottom of fuel box located near hut	AY371506
Cadophora sp. strain H	H37; *Discovery* hut, interior wood from below floor in meat room	AY371512
Cadophora sp. strain NH	NH1-2; McCraw hut at New Harbor, exterior wood from southwest corner below ground	AY371513

[a] Isolate numbers are from collection of R. A. Blanchette, University of Minnesota.
[b] GenBank accession number.

type 1 form of soft rot. In transverse sections, the decay appeared as numerous holes of varying diameter within the secondary walls (Fig. 2). Some samples had only incipient stages of decay, with small cavities present in the cell walls of some cells, but others contained extensive soft rot and cell wall degradation. Soft rot was found in conifer woods (Fig. 2A and B) and in hardwoods (Fig. 2C and D). In decayed Venesta storage boxes made of birch plywood, very advanced stages of decay were found and cells were severely decayed. In woods with advanced soft rot, most of the secondary wall was removed and only remnants of the outer secondary wall and the middle lamella between cells remained (Fig. 2C and D). No evidence of brown or white rot by fungi or bacterial degradation of the wood was found in any of the woods examined. Salt defibration, a form of nonbiological deterioration with surface wood cells detaching due to a chemical attack on the middle lamella region, was common on exterior woods of the huts (8).

Phialophora-like fungi were the dominant microorganisms in isolations from wood with soft rot and other woods in contact with the ground. Based on the recent taxonomic revision of some of the *Phialophora*-like fungi (15), the isolated fungi belong to the genus *Cadophora*. Morphological characteristics of the fungi in culture and ITS sequences of rDNA identified the isolates as *C. malorum*, *C. luteo-olivacea*, *C. fastigiata*, and three undescribed *Cadophora* species designated strain E, H, and NH (Table 1; Fig. 3). Isolations made from other wood samples from the hut also yielded *Cadophora* spp. Eleven isolates of *C. malorum* and 6 of *C. luteo-olivacea* were obtained from Cape Royds hut, and 18 *C. malorum* isolates, 8 *C. luteo-olivacea* isolates, and 1 *Cadophora* sp. strain E isolate (isolate 4E71-1) were obtained from the Cape Evans hut. These species were not found in wood from the *Discovery* hut, but a different *Cadophora*, designated *Cadophora* sp. strain H (isolate H37), was obtained. Isolates of other fungi, such as species of *Geomyces*, *Penicillium*, and *Rhinocladiella*, also were recovered (<20% of the total isolates recovered) from wood in ground contact taken from the huts, but *Cadophora* species were the dominant fungi found. In the Cape Evans hut, the *Cadophora* spp. were widely distributed throughout the hut (Fig. 4). The wood from which *Cadophora* was isolated was often soft and sometimes discolored, but the wood did not always have distinct soft rot cavities when sections were examined with light or scanning electron microscopy.

Samples of wood from a structure erected at New Harbor, Antarctica, in 1959 also were obtained to compare the types of decay and fungi isolated from another location in Antarctica with that found at the historic huts on Ross Island. Wood from the hut at New Harbor in contact with the ground had extensive soft rot. Advanced stages of decay were observed using light and electron microscopy within the wood cell walls of many samples (micrographs not shown). Isolations from this wood yielded three different species of *Cadophora*: *C. malorum*, *C. fastigiata*, and *Cadophora* sp. strain NH (isolate NH1-2) (Table 1; Fig. 3).

Wood wafers of *Betula* and *Populus* inoculated in the laboratory with *C. malorum*, *C. luteo-olivacea* or *Cadophora* sp. strain E had a type 1 soft rot after 12 months of incubation (Fig. 5). Numerous soft rot cavities were present within secondary walls of wood fiber cells. These cavities often coalesced, resulting in large voids within the cell walls (Fig. 5C and D). In some cells, the entire S_2 layer of the secondary wall was degraded, leaving only the S_3 and middle lamella regions. Wafers of *Picea* and *Pinus* inoculated in the laboratory and examined after 12 months were stained with dark fungal growth, but no soft rot was found.

DISCUSSION

The only form of wood decay found at the historic huts on Ross Island in Antarctica was caused by soft rot fungi. Soft rot commonly occurs in wood exposed to extreme and adverse environmental conditions that inhibit other types of fungi from becoming established and causing wood decay. These conditions include waterlogged woods, wood treated with preservatives, and wood from relatively dry sites, such as buried ancient tombs (5, 10, 13, 23). This report is the first of wood decay from Antarctica, and its discovery suggests that the harsh and extreme environmental conditions found in Antarctica are favorable only for decay caused by soft rot fungi. Degradation apparently occurs when the ground surface thaws and melted water provides moisture for fungal growth. These fungi are active for a very short time each year during the austral sum-

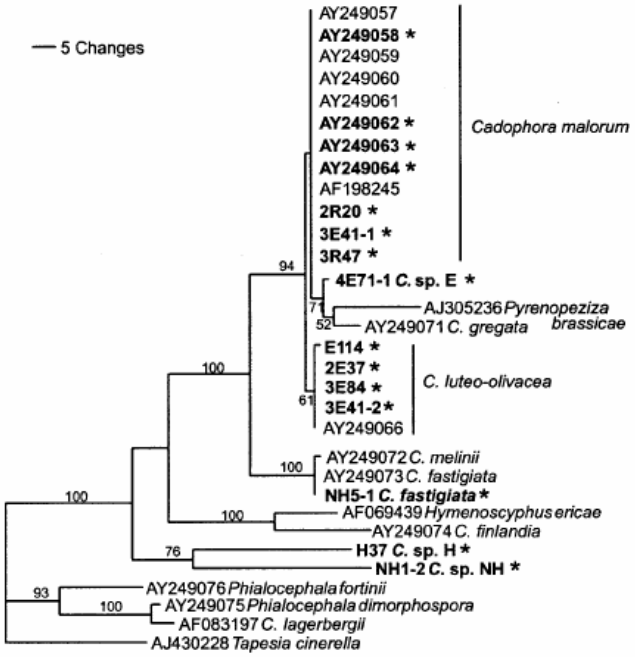

FIG. 3. One of 12 most parsimonious trees of *Cadophora* species and related discomycetes based on the ITS1, 5.8S, and ITS2 regions of rDNA. The tree is rooted to *T. cinerella*. Bootstrap values from 1,000 replications that were greater than 50% are indicated above or below the branches. Isolate numbers or accession numbers from Antarctic isolates are in bold and have asterisks. Consistency index = 0.7335; retention index = 0.8121; rescaled consistency index = 0.5957.

mer. If fungal growth occurs for a few days or weeks each summer, years may pass before appreciable soft rot is evident. In the 9 to 10 decades since the huts were built, enough time had passed for advanced decay to occur in some woods. The isolation of *Cadophora* spp. throughout the Cape Evans and Cape Royds huts indicates fungi capable of causing soft rot are well established. If conditions change within the huts (increase in temperature, increase in humidity, addition of nutrients, etc.) to become more conducive to decay, extensive soft rot could develop in many areas.

Although soft rot has occurred, it has not caused extensive damage to the historic structures. The oldest hut, *Discovery*, built in 1901, had no evidence of soft rot, and only one isolate of *Cadophora* was found at the site. *Discovery* hut was built with a wide veranda around three sides, and the ground around the hut is well drained. The veranda reduces the amount of moisture near the hut and shades the ground so that ground ice melts little if at all. These conditions apparently have helped to limit the duration of suitable conditions for fungal growth in wood in contact with the ground.

Soft rot fungi may grow very slowly and cause negligible decay in the absence of exogenous nutrients (25). The C/N ratio in wood is very high, and the lack of external sources of nitrogen can limit wood decay. Soft rot fungi have a remarkable ability to translocate nitrogen (13). For example, in an ancient wooden tomb of the legendary King Midas, soft rot fungi utilized the nitrogen from the King's body to allow decay to occur throughout the wooden tomb structure for hundreds of years (13). In the historic woods found in Antarctica, nutrients also are likely to influence the rate and extent of soft rot decay. The large number of soft rot fungi at Cape Royds and Cape Evans may be due to the abundance of nutrients there. A large penguin colony exists at Cape Royds, and penguins frequent the historic site. Penguin guano and feathers are always present around the hut. Another source of nutrients at these huts is from stores of food supplies at these sites. The deterioration of wooden storage crates and metal cans has resulted in them spilling their contents into the environment around the huts. Although no penguin colony exists at Cape Evans, penguins and skuas are common at the site. Cape Evans also has had nutrient input from historic sources, such as ponies and dogs used by the explorers and from latrines located adjacent to the hut. These sources, as well as modern deposits of skua and penguin guano, could provide the nutrients needed to facilitate soft rot fungal activity in the historic woods. In contrast, large quantities of food stores were not left around the

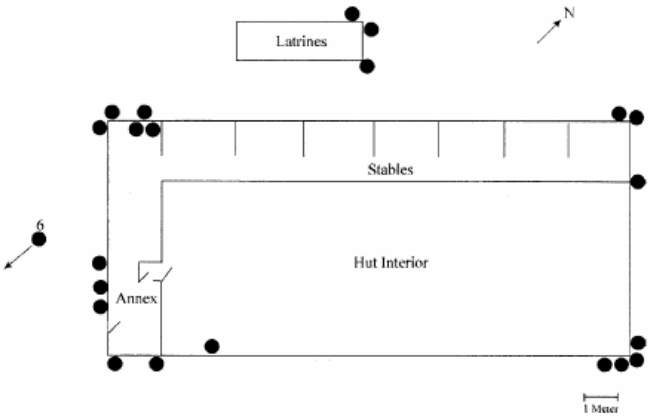

FIG. 4. Schematic drawing of Cape Evans hut showing locations (●) where *Cadophora* species were isolated. Six isolates were obtained from wooden artifacts around the hut, and the direction where these woods were located is indicated by an arrow. Although cultures of *Cadophora* were obtained from all of these locations, not all of the samples had soft rot.

Discovery hut, and there is no evidence of latrines next to the hut. The lack of penguins visiting this hut area, minimal levels of historic sources of nutrient deposits at this site, and reduced amounts of melt water adjacent to the hut due to its unique hut construction and good drainage at the site likely contributed to the near absence of soft rot fungi at *Discovery* hut.

In the wood wafer decay studies, no additional nutrients were added to the wood, yet the three species of *Cadophora* tested caused decay in *Betula* or *Populus* wood blocks. The extensive decay that occurred in the inoculated wood (Fig. 5) demonstrates the potential of *Cadophora* species to cause very serious degradation if conditions are favorable for decay. Soft rot was not found in the *Picea* or *Pinus* wood wafers. The presence of soft rot in conifer wood in contact with the ground at the Cape Evans and Cape Royds huts indicates that these fungi are able to attack these types of wood in the Antarctic environment. Laboratory conditions used for our analyses may not have been suitable for decay to occur in the conifer woods, since colonization occurred but no soft rot was evident. Increased incubation time and addition of nutrients also could have stimulated soft rot attack in the laboratory studies (25, 26). Additional studies are warranted to determine the optimal conditions for soft rot development in conifer wood and to determine the effect of other factors, such as exogenous nutrients, moisture, salts, and temperature, on fungal growth and degradation.

Recent phylogenetic analyses (15) showed that members of the genus *Cadophora* are anamorphs of *Helotiales* (discomycetes) and are distinct from the morphologically similar anamorph genus *Phialophora* in the *Chaetothyriales*. Morphologically, species of *Cadophora* are not easily differentiated from other species of *Cadophora* or from *Phialophora* species, but rDNA sequence analyses clearly separate these fungi. Since these fungi differ little in morphology, some misidentifications of *Phialophora*-like isolates from Antarctica probably have been made in the past. For example, two isolates (CBS 100584 [AY249062] and CBS 377.77 [AY249064]) from soil in Antarctica are listed as *C. fastigiata* but have the same ITS sequence as isolates of *C. malorum*. In addition, we found at least three unknown *Cadophora* species among our isolates, including the only isolate obtained from the *Discovery* hut, *Cadophora* sp. strain H. A second unknown species (NH 1-2) was obtained from McCraw's hut at New Harbor, and a third, *Cadophora* sp. strain E, was obtained from the Cape Evans hut. Additional sampling at the *Discovery* hut and at other locations in Antarctica is needed to obtain accurate information on the distribution of these species and their role as soft rot fungi or as decomposers of other organic materials in the Antarctic environment.

Cadophora species have been reported previously in Antarctica on mosses (3, 22) and in soils (21), including oil-contaminated soils (1). The occurrence of *Cadophora* spp. from Victoria Land to the Antarctic Shetland Islands demonstrates their wide distribution on the Antarctic continent. Results presented in this paper indicate these fungi are very common in the Ross Sea region in wood from the historic expedition huts. Their prevalence at the Cape Evans hut, the Cape Royds hut, and in the hut used by McCraw in 1959 suggests that *Cadophora* species are well adapted to the Antarctic environment and effectively colonize resources, such as wood. Extensive soft rot decay in wood at the McCraw hut after 44 years of exposure to the Antarctic environment indicates that some sites may be more conducive to soft rot decay than others. The hut at New Harbor is located near a stream channel that fills with melt water, and the gravel near the hut is wet for many weeks each summer. Although the expedition huts are 90 to 100 years old, soft rot is less extensive there than at the McCraw hut, indicating the conditions for decay at these locations on Ross Island may not be as conducive for decay. Nonetheless, *Cadophora* species have extensively colonized the historic

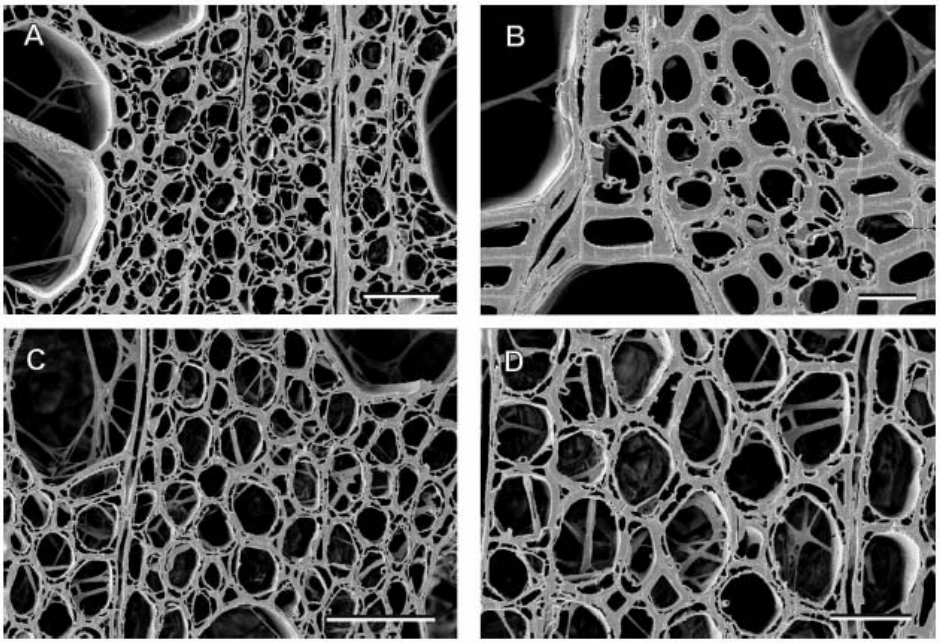

FIG. 5. Scanning electron micrographs of transverse sections from wood decayed in the laboratory with isolates of *Cadophora* from Antarctica. (A) Soft rot cavities in wood from *Betula* inoculated with *Cadophora* sp. strain E. Fibers between vessel elements are riddled with holes caused by the soft rot fungus. (B) Soft rot in birch wood inoculated with *C. malorum*. Cavities within the secondary walls of fibers are evident, with hyphae present in cell lumina and within the cavities created by the soft rot fungus. (C and D) Extensive soft rot in *Populus* wood inoculated with *C. luteo-olivacea*. Large cavities have formed in the fiber cell walls, and large numbers of hyphae are present. In many cells, the entire S_2 region of the secondary wall has been degraded. A residual S_3 layer adjacent to cell lumina and the middle lamella between cells are left. Wood cells with advanced soft rot have lost most of their original cell wall strength. Bar, 50 μm (A and C) or 25 μm (B and D).

woods at Cape Evans and Cape Royds, and since they are well established they could pose serious threats to the huts if conditions for decay were to become more favorable.

The soft rot fungi found in the historic huts probably were not brought to Antarctica by the early explorers. *Cadophora* species occur in temperate regions of the world, but they are not common wood decay fungi and are not frequently found in wood used for buildings. The great diversity of *Cadophora* species found in the historic woods, including several undescribed species, and their presence in soils and on dead moss thalli strongly suggest that these fungi are endemic to Antarctica. Additional investigations are needed to provide a more complete understanding of the biology of these microbes in Antarctica and to elucidate their role in the polar ecosystem. Studies of *Cadophora* and their ability to degrade wood also are needed as part of long-term conservation plans to preserve the huts and minimize conditions under which soft rot occurs.

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REFERENCES

1. **Aislabie, J., R. Fraser, S. Duncan, and R. L. Farrell.** 2001. Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biol. 24:308–313.
2. **Antarctic Heritage Trust.** 2003. Conservation report: Shackleton's hut, British Antarctic Expedition 1907–1909, Cape Royds, Ross Island, Antarctica. Antarctic Heritage Trust, Christchurch, New Zealand.
3. **Azmi, O. R., and R. D. Seppelt.** 1998. The broad-scale distribution of microfungi in the Windmill Islands, continental Antarctica. Polar Biol. 19:92–100.
4. **Blanchette, R. A.** 1998. A guide to wood deterioration caused by fungi and insects, p. 55–68. *In* K. Dardes and A. Rothe (ed.), The conservation of panel paintings. Getty Conservation Institute, Los Angeles, Calif.
5. **Blanchette, R. A.** 2000. A review of microbial deterioration found in archaeological wood from different environments. Int. Biodeterior. Biodegrad. 46: 189–204.
6. **Blanchette, R. A.** 2003. Deterioration in historic and archaeological woods from terrestrial sites, p. 328–347. *In* R. J. Koestler, V. R. Koestler, A. E. Charola, and F. E. Nieto-Fernandez (ed.), Art, biology, and conservation: biodeterioration of works of art. The Metropolitan Museum of Art, New York, N.Y.
7. **Blanchette, R. A., J. E. Haight, R. J. Koestler, P. B. Hatchfield, and D. Arnold.** 1994. Assessment of deterioration in archaeological wood from ancient Egypt. J. Am. Inst. Conserv. 33:55–70.
8. **Blanchette, R. A., B. W. Held, and R. L. Farrell.** 2002. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. Polar Rec. 38:313–322.

9. **Blanchette, R. A., and E. Simpson.** 1992. Soft rot decay and wood pseudomorphs in an ancient coffin (700 BC) from tumulus MM at Gordion Turkey. Int. Assoc. Wood Anatomists Bull. 13:201–213.
10. **Daniel, G. F., and T. Nilsson.** 1988. Studies on preservative tolerant *Phialophora* species. Int. Biodeterior. 24:327–335.
11. **Eaton, R. A., and M. D. C. Hale.** 1993. Wood: decay, pests and protection. Chapman and Hall, London, England.
12. **Eriksson, K. E., R. A. Blanchette, and P. Ander.** 1990. Microbial and enzymatic degradation of wood and wood components. Springer-Verlag, Heidelberg, Germany.
13. **Filley, T. R., R. A. Blanchette, E. Simpson, and M. Fogel.** 2001. Nitrogen cycling by unique wood decay fungi in the King Midas tomb, Gordion, Turkey. Proc. Natl. Acad. Sci. USA 98:13346–13350.
14. **Findlay, W. P. K.** 1984. Soft rot of timber—a review. J. Ind. Acad. Wood Sci. 15:1–11.
15. **Harrington, T. C., and D. L. McNew.** 2003. Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87:141–151.
16. **Harrowfield, D. L.** 1981. Sledging into history. Macmillan, Auckland, New Zealand.
17. **Harrowfield, D. L.** 1995. Icy heritage: historic sites of the Ross Sea region. Antarctic Heritage Trust, Christchurch, New Zealand.
18. **Held, B. W., R. A. Blanchette, J. A. Jurgens, R. L. Farrell, and S. Duncan.** 2003. Deterioration and conservation issues associated with Antarctica's historic huts, p. 370–389. *In* R. J. Koestler, V. R. Koestler, A. E. Charola, and F. E. Nieto-Fernandez (ed.), Art, biology, and conservation: biodeterioration of works of art. The Metropolitan Museum of Art, New York, N.Y.
19. **Hughes, J.** 2000. Ten myths about preservation of historic sites in Antarctica and some implications for Mawson's huts at Cape Denison. Polar Rec. 36:117–130.
20. **Paulin, A. E., T. C. Harrington, and D. McNew.** 2002. Phylogenetic placement of anamorphic species of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia 94:62–72.
21. **Sun, S., M. Huppert, and R. E. Cameron.** 1978. Identification of some fungi from soil and air of Antarctica. American Geophysical Union, Washington, D.C.
22. **Tosi, S., B. Casado, R. Gerdol, and G. Caretta.** 2002. Fungi isolated from Antarctic mosses. Polar Biol. 25:262–268.
23. **Wang, C. J. K.** 1990. Microfungi, p. 105–352. *In* C. J. K. Wang and R. A. Zabel (ed.), Identification manual for fungi from utility poles in the eastern United States. Allen Press, Lawrence, Kans.
24. **Worrall, J. J.** 1991. Media for selective isolation of hymenomycetes. Mycologia 83:296–302.
25. **Worrall, J. J., S. E. Anagnost, and C. J. K. Wang.** 1991. Conditions for soft rot of wood. Can. J. Microbiol. 37:869–874.
26. **Worrall, J. J., and C. J. K. Wang.** 1991. Importance and mobilization of nutrients in soft rot of wood. Can. J. Microbiol. 37:864–868.
27. **Zabel, R. A., and J. J. Morrell.** 1992. Wood microbiology. Academic Press, New York, N.Y.

2.2.3 Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., Blanchette, R.A. 2005. Environmental factors influencing microbial growth inside the Historic Expedition Huts of Ross Island, Antarctica. *International Biodegradation and Biodeterioration*, 55: 45-53.

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Environmental factors influencing microbial growth inside the historic expedition huts of Ross Island, Antarctica

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Abstract

Explorers to Antarctica during the Heroic Era of exploration built three wooden huts on Ross Island, Antarctica in 1902, 1908 and 1911. The structures were used as bases of operation while their occupants participated in scientific endeavors and strived to reach the South Pole. The huts, and the thousands of artifacts in and around them, have survived in the Antarctic environment for 9–10 decades, but deterioration has taken place. The successful preservation of these important historic structures and materials requires information on the agents causing deterioration and factors that influence microbial growth. Temperature and relative humidity (RH) were monitored in the expedition huts for several years. During the austral summer months of December and January it was common for temperatures to rise above 0°C and RH to exceed 80%. Extensive fungal growth was observed on wood and artifacts within the Cape Evans hut, and fungi isolated were identified as species of *Cladosporium*, *Penicillium*, *Cadophora*, *Geomyces* and *Hormonema*. The factors that influence RH within the huts and methods to control moisture and arrest microbial growth are discussed.

Keywords: Antarctica; Historic huts; Relative humidity; Temperature; Fungi

1. Introduction

During the Heroic Era of exploration, three huts were built on Ross Island, Antarctica. The huts served as bases for the expeditions of Ernest Shackleton and Robert F. Scott to explore the continent, carry out scientific investigations and to be the first to reach the South Pole. The structures housed men, supplies and food for several years during their endeavors. Robert Scott led the National Antarctic Expedition (1901–1904) and built *Discovery* hut in 1902 at Hut Point, which was also used by others in later expeditions. In 1908, Ernest Shackleton's British Antarctic Expedition built a relatively small hut adjacent to a penguin rookery at Cape Royds. Scott returned to Antarctica in 1911 leading the British Antarctic Expedition (1910–1913) and built another hut at Cape Evans in 1911. Thousands of artifacts were left in and around the huts as expedition members hastily left the Antarctic when relief ships arrived. Although the cold, dry Antarctic environment has aided preservation of the huts to a greater extent than if they were located in a more temperate climate, considerable deterioration has occurred over the past decades (Blanchette et al., 2002; Held et al., 2003). Fungi have been observed growing within the huts on wood, textiles and other artifacts. Fungi are dependent on moisture, and relative humidity above 80% is usually sufficient for mold growth to occur on wood (Pasanen et al., 1992). A preliminary study by Mason (1999) in the Cape Evans hut indicated that relative humidity is high within the historic structure and cultural properties cannot be effectively preserved in these conditions.

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Conditions that favor fungal growth appear similar to those occurring at other historic huts in Antarctica, such as Borchgrevink's hut at Cape Adare where molds have been found growing in the hut (Antarctic Heritage Trust, 2004) and Mawson's hut at Cape Denison, where RH above 95% is common making fungal growth a problem (Hughes, 1988). Increased visitation by tourists and their impact on raising relative humidity inside the huts have also been of concern (Hughes, 1992; Mason, 1999). Decay fungi that cause soft rot have been identified in exterior woods of the huts but little to nothing is known about the fungi growing within the huts on wood and other historic materials (Blanchette et al., 2004; Held et al., 2003).

This investigation was done to monitor relative humidity and temperature in Scott's hut at Hut Point (*Discovery* Hut), Shackleton's hut at Cape Royds and Scott's hut at Cape Evans and to determine what influence these environmental factors have on fungal growth within the huts and on artifacts. Fungi were isolated and identified from areas within the Cape Evans hut where extensive fungal growth was observed.

2. Materials and methods

Two Hobo® H8 Pro data loggers were placed in each of the historic huts at Cape Royds, Cape Evans and at Hut Point in December 1999 to record temperature and relative humidity. Three additional data loggers were placed in the huts at Cape Royds and Cape Evans and two additional data loggers within the hut at Hut Point in December 2000. One data logger was placed outside of Cape Evans hut in the historic meteorological station in 2000 but this data logger recorded only intermittent data due to its exposure to the extreme Antarctic environment. Locations of the data loggers at Cape Evans hut included (1) on a shelf near the entrance (galley/mess area) approximately 2.0 m from the floor, (2) near the center of the hut among food stores approximately 1.7 m from the floor, (3) under a bunk approximately 0.5 m from the floor, (4) in the darkroom near the ceiling approximately 2.2 m from the floor and (5) in the stables on a crate approximately 0.2 m from the ground. At Cape Royds hut they were located (1) on a shelf on the north wall approximately 2 m from the floor, (2) on the floor under a bunk directly below the logger on the north wall shelf, (3) on the floor among crates along the south wall adjacent to the galley, (4) 2.3 m from the floor on top of Shackleton's room near the entrance, (5) on a shelf in Shackleton's room 1.6 m from the floor. Hut point locations were (1) on the floor in the center of the hut behind crates, (2) along the east wall among boxes of stores approximately 0.2 m from the floor, (3) in the stove pipe hole in the false ceiling above the stove 2.0 m from the floor, (4) on a shelf in the physical laboratory approximately 1.6 m from the floor. All data loggers recorded temperature and relative humidity and had a sampling interval of one hour throughout the year. Data was downloaded from the loggers each year in the Austral summer during field visits to the huts. Boxcar® Pro 4.3 and Microsoft Excel software were used to analyze the data. The data collection period was from December 1999 to January 2003.

Samples of wood taken for fungal isolation (under Antarctic Conservation Act permits #2001-015, 2002-001 and 2004-019) were carefully removed from inconspicuous locations in the huts and placed in sterile sample bags. Sterile swabs were used to wipe the surface of wood where it was not possible to take a wood sample. Samples were kept frozen until isolations were made in the laboratory. Fungi were isolated by incubating small wood segments or wiping swabs on culture medium. Several types of culturing media were used for isolation of microorganisms including malt yeast agar (MYA) containing Difco malt extract 1.5%, yeast extract 0.2%, agar 1.5%, MYA with antibiotics added (chloramphenicol 0.2 g, streptomycin sulphate 0.1 g), MYA with antibiotics and cycloheximide (chloramphenicol 0.2 g, streptomycin sulphate 0.1 g, cycloheximide 0.4 g), a semi-selective media for basidiomycetes (malt extract 1.5%, yeast extract 0.2%, agar 1.8%, chloramphenicol 0.2 g, benlate 0.06 g, streptomycin sulphate 0.1g) (Worrall, 1991) and Vogel Bonner media, (glucose 25%, agar 2.0%, 20 ml VB concentrate containing 670 ml distilled water, K_2HPO_4· anhydrous 50%, $NaNH_4PO_4 \cdot 4PH_2O$ 17.5%, citric acid $\cdot H_2O$ 10%, $MGSO_4 \cdot 4H_2O$ 1%) (Vogel and Bonner, 1956). Plates were incubated at 4, 15 and 25 °C and pure cultures were transferred to separate plates for identification. Fungi were identified using taxonomic literature for these genera and analysis of rDNA internal transcribed spacer (ITS) sequences. DNA was extracted from cultures using Qiagen DNeasy Plant Mini-kits using manufacturer's instructions. The ITS sequences were amplified by PCR using primers ITS1 and ITS4 (Gardes and Bruns, 1993). PCR amplification was performed using Amplitaq Gold PCR Master-mix following manufacturer's instructions (Applied Biosystems) with 1 μl DNA. PCRs were performed in a MJ Research PTC Mini-cycler. PCR conditions were as follows: 94 °C for 5 minutes; 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. After visualization of amplicons on ethidium-bromide stained 1% agarose gels, the amplicons were purified using EXO-SAP (exonuclease-shrimp alkaline phosphatase) PCR product cleanup systems (USB Corporation). Sequencing reactions were performed using 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 assembled into contigs using Chromas software (Technelysium Ltd.) and the Emboss pairwise alignment algorithm (www.ebi.ac.uk/emboss/align/). The sequences were compared to those present in GenBank using BLAST and the best match was recorded.

3. Results

3.1. Cape Evans

Relative humidity within Scott's hut at Cape Evans was typically above 80% and sometimes above 90% in the austral summer months of December and January. Temperatures above 0 °C were common and reached as high as 9.4 °C within the hut during the monitoring period. Fig. 1 shows RH and temperature data over a three year period from a single data logger located in the center of the hut at approximately 1.7 m from the floor. Relative humidity averaged 74.6% for the 3 years with a maximum and minimum of 87.3% and 59%, respectively. The average temperature was −14.7 °C and the maximum and minimum were 9.4 and −35.1 °C, respectively, over 3 years. Data collected over a 4 week period during the austral summer, December 23, 2001–January 20, 2002, from a data logger placed in the galley near the inner hut entrance is shown in Fig. 2. The average RH and temperature for this period was 82.7% and 3.7 °C, respectively, and the maximum RH observed was 93.1% with a temperature range of −1.5 to 7.8 °C. The data logger placed in the stables area of the hut recorded relatively high RH over the same period of data collection. The average RH for that

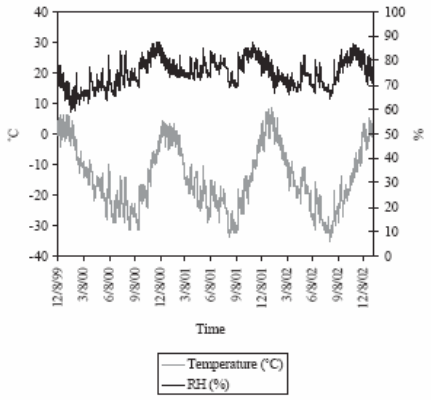

Fig. 1. Temperature and RH in Cape Evans hut over a three year period obtained from a data logger located in the middle of the hut, 1.7 m above the floor.

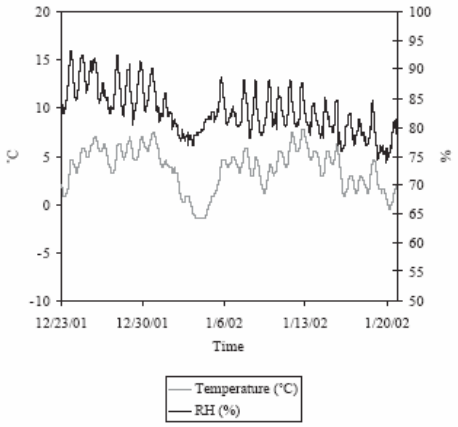

Fig. 2. Temperature and RH in Cape Evans hut over a four week period during the austral summer months of December and January.

period was 81% and the maximum and minimum was 93% and 64%, respectively, as compared to the average of 76.4% obtained from a data logger located near the center of the main hut.

Isolations were made from swabs taken from wood surfaces in the galley area of the hut where extensive mold growth was found. The fungi identified are listed in Table 1. The sequence from one particular fungus that was isolated infrequently did not match well with any presently in GenBank, and has remained unidentified. Mold growth was visually evident on a number of artifacts made of wood, leather and textiles in several areas. In the galley, extensive mold growth was observed in areas between wooden supply crates (Fig. 3), on the legs and underside of a table and on the lower portion of the south hut wall. The affected area also extends to an area of crates adjacent to bunk beds along the south wall. Moisture in this area appears to originate from frost that occurs on the wall in the galley (Fig. 4). This is likely due to the snow and ice that accumulates on the hut exterior (Fig. 5) causing the south wall to be colder than surrounding temperature in the hut. The high RH inside the hut and the temperature differential allows condensation to occur and frost builds up on the wall. As the temperatures rise in the hut, the melting of the frost and the presumably higher RH in this area provides sufficient moisture for fungi to grow prolifically.

To determine the duration of an environment inside the huts that may be conducive to mold growth, the number of hours per year in 2000 through 2002 in which the temperature was above 0 ° and RH was at or above 80% was calculated for each data logger and is displayed in Table 2. Although some differences were

Table 1
Fungi identified from wood located along the south wall in the galley area of Cape Evans hut

Isolate number	Location	Species identification
182	Wall at lower bunk	*Cadophora malorum*
711	Wall at lower bunk	*Geomyces* sp.
262	Wall at lower bunk	Unknown
487	Wall behind table, galley	*Cladosporium cladosporioides*
517	Wall behind table, galley	*Cadophora malorum*
660	Wall behind table, galley	*Cladosporium cladosporioides*
719	Wall behind bed frame, galley	*Cladosporium cladosporioides*
723	Wall near floor under bed frame, galley	*Penicillium echinulatum*
537	Near floor 1st box west of 1st bunk	*Penicillium expansum*
814	Near floor 1st box west of 1st bunk	*Cladosporium cladosporioides*
6E113-2	Wall above frost in galley	*Hormonema dematioides*
6E114-2	Wall above frost in galley	*Cadophora luteo-olivacea*
6E115-2	Wall above frost in galley	*Cadophora* sp. *E*

Fig. 3. Mold growth on food crates in the galley area of Cape Evans hut. The arrow on the right points to a large area of fungal growth and the arrow on the left points to scattered colonies of fungi.

found among the various positions for the data loggers, on average there were 287 h in 2001 and 247 h in 2002 that met these conditions inside Cape Evans hut.

3.2. Cape Royds

Shackleton's hut at Cape Royds is the smallest of the three huts studied. Fig. 6 shows data from a logger placed on the floor in the kitchen area over a three year period where RH ranged between 53.6% and 89.3% and

Fig. 4. Frost accumulation inside Cape Evans hut at the base of the south wall in the galley. When temperatures rise above 0 °C the frost melts producing sufficient moisture for mold growth in this area.

Fig. 5. Cape Evans hut in mid December showing a large amount of drifting snow that accumulates over the winter months.

averaged 71.6%. Temperatures were between −35.1 and 2.5 °C. Data from a logger placed on the floor under a bunk over a four week period during the austral summer from December 23, 2001 to January 20, 2002 showed the average temperature and RH was −0.1 °C and 77.4%, respectively (Fig. 7). The highest temperature and RH recorded for this period was 2.9 °C and 83.0%, respectively.

To determine if there are vertical temperature and RH differences occurring within the hut, two data loggers, one on the floor under a bunk and another approximately 2 m above it on a shelf were compared. Fig. 8 shows four weeks of data from these two loggers taken between December 15, 2000 and January 12, 2001. The environment on the floor has a slightly lower temperature averaging −2.9 °C as compared to the average shelf temperature of 0.2 °C. Relative humidity varied more significantly. The average RH on the floor was 84.2% compared to 63.6% on the shelf. It can also be noted that there were larger fluctuations in both temperature

Table 2
The number of hours per year in which temperature was greater than 0 °C and RH was greater than or equal to 80% in Cape Evans, Cape Royds and Discovery huts

Location	Year		
	2000	2001	2002
Cape Evans			
Floor, S wall, under bunk	79	269	433
Middle of hut (1.7 m)	268	157	83
Shelf, near entrance (2 m)		569	461
Darkroom, ceiling (2.2 m)		257	120
Stables, stores (0.2 m)		185	138
Cape Royds			
Floor, S wall	0	33	6
Shelf, N wall behind reams of paper (2 m)	13	0	0
Floor, N wall, under bunk		55	12
Behind acetylene generator (2.3 m)		3	0
Shelf, Shackleton's room (1.6 m)		0	5
Discovery hut			
Floor, center of the hut	8	0	0
Stove pipe hole, false ceiling, galley (2.0 m)	11	0	0
Shelf, physical laboratory (1.6 m)		0	0
Stores, east wall (0.2 m)		0	0

Values in parenthesis denote distance of data logger from floor.

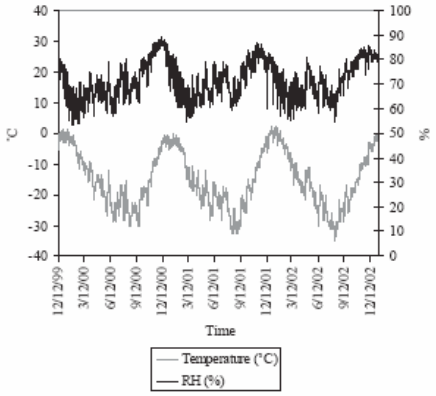

Fig. 6. Temperature and RH in Cape Royds hut over a three year period from a data logger located on the floor in the galley area.

and RH between the data loggers located on the shelf as compared to the data logger on the floor.

The hours per year at or above 0 °C and above 80% RH averaged from all five data loggers for the years 2001 and 2002 are shown in Table 2. This data shows that these conditions are more often occurring in lower areas of the hut than at higher positions. The number of hours with environmental conditions conducive for fungal growth is considerably less (30 h in 2001 and

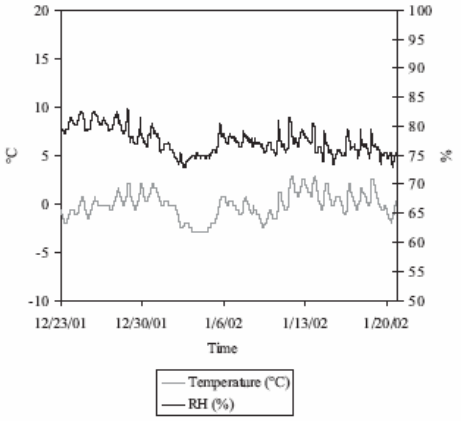

Fig. 7. Temperature and RH in Cape Royds hut over a four week period during the austral summer months of December and January.

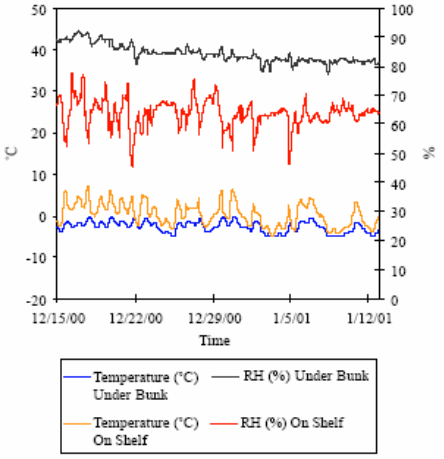

Fig. 8. A comparison of temperature and RH from two data loggers with different vertical positions in Cape Royds hut.

only 8 h in 2002) in this hut as compared to Cape Evans hut (Table 2). The temperature and RH data observed in Cape Royds hut shows a similar trend to the Cape Evans hut in which the number of hours at or above 0 °C and RH greater than 80% decreased between 2001 and 2002 at most data logger locations. This appears to indicate that the huts' environments were reacting similarly to exterior conditions. No extensive areas of active mold growth were observed in the hut but localized areas of dark fungal colonies exist on interior woods and artifacts.

3.3. Discovery hut

A representative view of temperature and RH data for Scott's hut at Hut Point (*Discovery* hut) from the data logger located on the floor in the center of the hut over a period of three years is presented in Fig. 9. The maximum and minimum RH for this time period was 91.3% and 49.2%, respectively, and averaged 73.5%. Temperatures ranged between −39.0 and 6.6 °C. Temperature and RH over a four week period from December 2001 through January 2002 recorded from the data logger on a shelf in the physical laboratory is shown in Fig. 10. The maximum and minimum RH was 76.4% and 62.3%, respectively, and averaged 68.3% for the four weeks. The maximum temperature reached was 8.2 °C and had an average temperature of 2.0 °C. During the three year period of data collection, only 19 h of temperature greater than 0 °C and RH above 80% were recorded in 2000 but no hours with these conditions occurred in 2001 and 2002 (Table 2). This is significantly lower than the hours at these conditions recorded for Cape Evans or Cape Royds huts. Extensive areas of fungal growth have not been observed in *Discovery* hut but several small, inactive colonies have been observed on various wall boards and some artifacts.

3.4. Visitor impacts

To determine if visitors entering the huts affect RH, visitor log books were used to determine when large groups of people (82 to 276 individuals) entered the hut during the 3 years of data collection. The selected dates for this evaluation were when groups of visitors entered

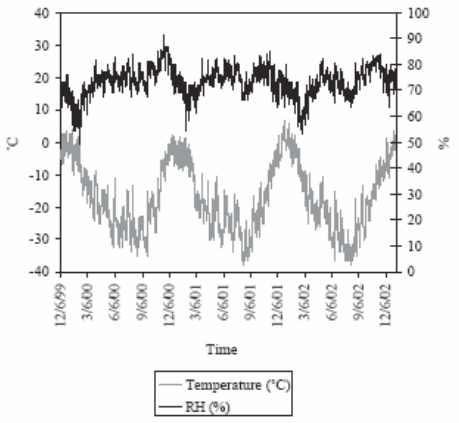

Fig. 9. Temperature and RH in *Discovery* hut over a three year period from a data logger located on the floor in the center of the hut.

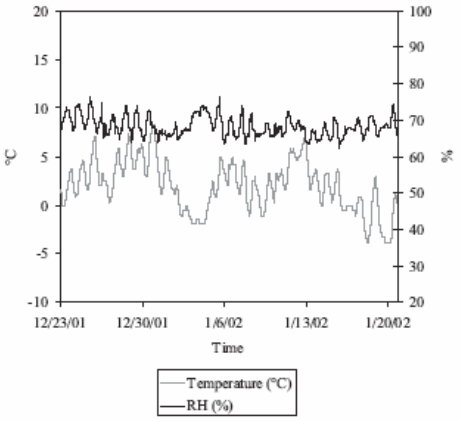

Fig. 10. Temperature and RH in *Discovery* hut over a four week period during the austral summer months of December 2001 to January 2002.

the huts, while having no visitors entering two days prior and two days after the events. Comparing temperature and absolute humidity on the day of the visit to days before and after these events show that moisture fluctuated with temperature and did not exhibit an increase during or after visitors entered the hut (data not shown). Several other visitor events were studied in each hut and the results were similar; no substantial elevation in RH was recorded even after large numbers of visitors entered the huts. To determine how exterior weather influences the interior conditions of the huts during times of visitation, the exterior and interior temperature and RH were studied over a five day period from February 11 to February 16, 2001 (Fig. 11). Twenty-eight individuals entered the hut on February 13, 2001. The average exterior RH and temperature during this period was 67.4% and −6.5 °C, respectively, as compared to the average RH of 74.8% and temperature of −4.7 °C for the interior. The data also shows no increase in RH from visitors entering the hut on February 13. It appears that although the exterior RH and temperature fluctuates more widely, the interior environment is very close to the average exterior environment.

4. Discussion

Environmental conditions are important factors that regulate microbial growth, and it is well-known that moisture is one of the main factors that influence microbial growth and decay in historic buildings located in temperate areas (Park, 1999). The results presented

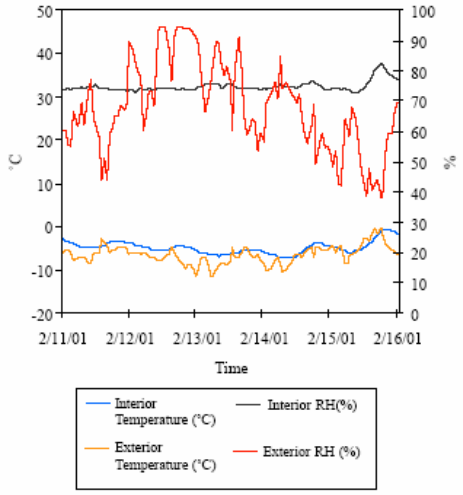

Fig. 11. Temperature and relative humidity of the exterior and interior environments at Cape Evans hut. Twenty-eight visitors entered the hut on 2/13/01. No visitors entered the hut 2 days before or after.

here suggest that RH has a significant effect on microbial growth in the Antarctic expedition huts of Ross Island. This is based on the presence of numerous, active fungal colonies and the high RH for extended periods in the Cape Evans hut, as compared to limited fungal growth and lower RH in the Cape Royds and *Discovery* huts. Although the huts have survived relatively well over the last 9–10 decades as compared to a more temperate or tropical location, they have been significantly impacted by Antarctica's unusual conditions (Blanchette, 2000; Blanchette et al., 2002; Held et al., 2003). An environment conducive for microbial growth has resulted in molds growing on wood, textiles and food stores in Cape Evans hut. Some evidence of mold also occurs in *Discovery* and Cape Royds huts but these areas are not widespread and appear to be localized. The monitoring of temperature and RH has provided important information about the huts internal environments and has demonstrated that despite their location in Antarctica, RH and temperature suitable for microbial growth occurs for an appreciable amount of time in all the structures and especially Cape Evans hut. Other historic structures in Antarctica also appear to have similar problems with mold growth such as Borchgrevink's hut at Cape Adare and Mawson's hut at Commonwealth Bay, Antarctica (Antarctic Heritage Trust, 2004; Hughes, 2000). Metal in the huts are also subjected to significant corrosion due to high RH and temperatures above 0 °C (Otineo-Alego et al., 2000). Food cans, metal equipment and artifacts in the huts have deteriorated significantly due to oxidation caused by these conditions.

4.1. Relative humidity and temperature

Relative humidity conducive for fungal growth ranges from 76% and 96% depending on temperature, length of time, substrate and fungal species (Coppock and Cookson, 1951; Block, 1953; Grant et al., 1989). As RH exceeds 80% conditions for fungal growth improve (Pasanen et al., 1992). The generally accepted range for RH of wooden cultural properties kept in museums to prevent fungal growth is between 47% and 55% (Thompson, 1978; Bachmann, 1992). Recently, revised environmental guidelines in Smithsonian Museum buildings have been established at 45% RH +/− 8% RH and 70 °F (21 °C) +/− 4 °F (2 °C) to insure no fungal growth occurs (Mecklenburg et al., 2004). Our results have shown relative humidity within the Ross Island historic huts is commonly over 80% during the austral summer months (Figs. 1, 6, and 9). This does not, always correlate with temperatures above 0 °C which is usually necessary for most types of mold growth to occur. However, fungi such as *Cladosporium* species have been observed to grow on some substrates when temperatures were −5 °C (Gill and Lowry, 1982). In our evaluations reported here, a conservative estimate of the number of hours during the year when temperatures were above 0 °C was used along with a RH of 80% or above as an indicator for adequate conditions for microbial growth. The data in Table 2 shows that Cape Evans hut has considerably more hours of high RH and temperature than the other huts. These differences in the temperature and RH observed between huts may be attributed to several factors. The huts are different in size, construction, insulative properties, aspect and location which all have an effect on the interior environment. Cape Evans hut commonly has snow that drifts around it from the SE side extending from the ground to the roofline. Snow often nearly encapsulates the structure except for areas on the east and west ends (Fig.5). The melting of this snow causes significant moisture problems in numerous locations around the hut. Cape Royds and *Discovery* huts have some accumulation of drifting snow but not to the depth and extent of Cape Evans hut. The authors have noted considerable snow ingress in the annex of Cape Evans hut as well as some areas in Cape Royds and *Discovery* huts. As the snow melts it can add moisture to the hut environment.

Prolific mold growth is occurring in the Cape Evans hut where microenvironments provide favorable conditions for fungal growth. This appears to be in places where there is also a lack of air movement. In the Cape Evans hut galley area, frost accumulates where the wall

and floor join and also along the wall boards about 0.5m from the floor (Fig. 4). Frost subsequently melts in the summer months forming free water on the surfaces of wood that provides ample moisture for fungal growth. The formation of free water also likely increases RH in this area creating a microenvironment conducive to mold growth. At present no significant decay is evident in the wood colonized by these fungi but degradation of paper, textiles and other substrates in the hut is likely occurring. *Cadophora* and *Cladosporium* species have the capacity to attack wood and other substrates and have been shown to cause a soft rot form of wood decay (Blanchette et al., 2004; Zabel et al., 1982). Continued growth of these fungi in the huts will undoubtedly result in significant damage over time.

Results obtained from data loggers in different huts show considerable differences when comparing the number of hours above 0°C and 80% RH. From the data in Table 2, it can be seen that in Cape Evans hut, the location near the entrance is warmer and more humid than other locations. This could be due to the data loggers close proximity to the door where it is likely influenced by the more humid air in the annex or it may be correlated to variations in conditions due to the difference in height from the floor. The data logger under the bunk also exhibits similar conditions, which may be due to decreased air flow in its position. A similar phenomenon is seen in the Cape Royds hut where loggers on the floor and under a bunk have more hours above 0°C and 80% RH than other loggers placed in different positions. Similar comparisons in *Discovery* hut were not made since there were only 18 total hours above 0°C and 80% RH over three years.

Visitors' impact on the interior environment of the huts has been a growing concern as hundreds of people enter the huts each year. Although the number of visitors to the huts is limited per season and at any one time by the Antarctic Treaty, the overall impact remains an important consideration for the conservation plans of the huts. Visitors originate from nearby U.S. McMurdo Station and New Zealand's Scott Base as well as from a number of cruise ships that visit the Ross Sea Region each year. The most probable impact visitors could have on the interior hut environment is increasing RH and/or adding moisture. This can occur by bringing snow in on footwear, increasing water vapor from breathing, or allowing air exchange with the outdoor environment. Temperature and RH collected on the days before, during and after large numbers of visitors entered the huts suggests that the current visitation does not cause a rise in RH during the visit or several days after. The amount of moisture in the air inside the huts appears to be more greatly influenced by frost build up, snow melt water, lack of air movement within some parts of the huts, and exterior environmental influences.

5. Recommendations

Relative humidity is sufficient in the huts, especially Cape Evans hut, to support fungal growth. To control this growth, moisture must be reduced. The most direct solution to the problem would be to dehumidify the interior hut air. However, this is extremely difficult given the remote location of the huts and lack of electricity. Localized areas where moisture and temperature are sufficient for mold growth suggest microenvironments of high RH exist in the huts, such as in the galley area of Cape Evans hut where *Cladosporium* and other fungi are growing profusely. The moisture source in these areas needs to be controlled. Possible methods may include periodically removing frost that accumulates on the lower wall or making structural changes and reducing snow on the exterior of the hut that will prevent frost from forming. Increasing air circulation could also help eliminate microclimates of high RH and limit mold growth where it is occurring. Ventilation should be considered to vent and remove warm moist air when it exists inside the huts. Regular inspections of artifacts and problem areas should take place so that mold does not grow unnoticed and cause irreversible harm. This is especially true in areas where air flow is limited and objects are in close proximity to one another. To remove existing fungal growth and the large number of spores that are currently present, hepa vacuuming followed by surface cleaning is warranted (Florian, 2002). Further monitoring of visitors to the huts and their possible effects will also be of value to insure that their numbers do not elevate RH in the future. Although our findings do not show visitors affecting the RH or temperature, continued diligence regarding snow removal from boots and clothing and monitoring the internal environment should be carried out.

The historic huts of Ross Island are valuable historic resources and conservation efforts are needed to protect them from further deterioration. Elevated relative humidity, temperature, and moisture are a continual threat to the huts and the artifacts in them. Addressing the current problems associated with the environmental conditions reported in this paper can be viewed as an integral part of future hut preservation efforts to ensure the protection of these iconic remnants from the Heroic Era of Antarctic exploration.

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References

Antarctic Heritage Trust, 2004. Conservation plan, the historic huts at Cape Adare. Antarctic Heritage Trust, Christchurch, New Zealand.

Bachmann, K., (Ed.), 1992. Conservation Concerns: a guide for collectors and curators. Smithsonian Institution Press, Washington D.C.

Blanchette, R.A., 2000. A review of microbial deterioration found in archaeological wood from different environments. International Biodeterioration and Biodegradation 46, 189–204.

Blanchette, R.A., Held, B.W., Farrell, R.L., 2002. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. Polar Record 38, 313–322.

Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., Farrell, R.L., 2004. Wood destroying soft-rot fungi in the historic expedition huts of Antarctica. Applied and Environmental Microbiology 70, 1328–1335.

Block, S.S., 1953. Humidity requirements for mold growth. Applied Microbiology 1, 287–293.

Coppock, J.B.M., Cookson, E.D., 1951. The effect of humidity on mould growth on constructional material. Journal of the Science of Food and Agriculture 2, 534–537.

Florian, M-L.E., 2002. Fungal facts. Solving fungal problems in heritage collections. Archetype Publications, London.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.

Gill, C.O., Lowry, P.D., 1982. Growth at sub-zero temperatures of black spot fungi from meat. Journal of Applied Bacteriology 52, 245–250.

Grant, C., Hunter, C.A., Flannigan, B., Bravery, A.F., 1989. The moisture requirements of moulds isolated from domestic dwellings. International Biodeterioration 25, 259–284.

Held, B.W., Blanchette, R.A., Jurgens, J.A., Duncan, S., Farrell, R.L., 2003. Deterioration and conservation issues associated with Antarctica's historic huts. In: Koestler, R.J., Koestler, V.R., Charola, A.E., Nieto-Fernandez, F.E. (Eds.), Art, Biology, and Conservation: Biodeterioration of Works of Art. The Metropolitan Museum of Art, New York and Yale University Press, New Haven, 370–389.

Hughes, J., 1988. The problems of preservation in a polar climate—the conservation of Sir Douglas Mawson's hut at Commonwealth Bay, Antarctica. Australian Institute for the Conservation of Cultural Material Bulletin 14, 1–32.

Hughes, J., 1992. Mawson's Antarctic huts and tourism: a case for on-site preservation. Polar Record 28, 37–42.

Hughes, J., 2000. Ten myths about the preservation of historic sites in Antarctica and some implications for Mawson's huts at Cape Denison. Polar Record 36, 117–130.

Mason, G., 1999. Modeling mass transfer inside Scott's hut Cape Evans, Antarctica. Thesis, University of Canterbury, Christchurch, New Zealand.

Mecklenburg, M.F., Tumosa, C.S., Pride, A., 2004. Preserving legacy buildings. HVAC retrofit: fitting the pieces together. A Supplement to ASHRAE Journal 46 (6), S18–S23.

Otineo-Alego, V., King, G., Hughes, J., Gillett, R., 2000. Rust from the Antarctic: a record of pollutants in wilderness environments. In: Proceedings for Corrosion and Prevention 2000. Australasian Corrosion Association, Auckland, pp. 34–41.

Park, C.S., 1999. Preservation Brief 39: holding the line: controlling unwanted moisture in historic buildings. U.S. Department of the Interior. Government Printing Office, Washington D.C.

Pasanen, A.L., Juutinen, T., Jantunen, M.J., Kalliokoski, P., 1992. Occurrence and moisture requirements of microbial growth in building materials. International Biodeterioration and Biodegradation 30, 273–283.

Thompson, G., 1978. The Museum Environment. Butterworths, London.

Vogel, H.J., Bonner, D.M., 1956. A convenient growth medium for *E. coli* and some other microorganisms. Journal of Biological Chemistry 218, 97–106.

Worrall, J., 1991. Media for selective isolation of *Hymenomycetes*. Mycologia 83, 296–302.

Zabel, R.A., Wang, C.J.K., Terracina, F., 1982. The fungal associates, detection and fumigant control of decay in treated southern pine poles. Electric Power Research Institute, Project 1471-1, Final Report.

Chapter 3 Mycology, Isolation and Identification of Microorganisms

3.1 Introduction

Generally the environment in Antarctica is unfavourable for growth of microorganisms as it is cold and low in water and nutrients. The introduction of wood to the Ross Island sites and other organic materials brought by the Heroic Period explorers provided unique nutrient sources either for indigenous Antarctic micoorganisms or for microorganisms brought in with the materials which persisted and adapted to the harsh conditions. Along with providing a nutrient source for microorganisms, the huts created a microenvironment with conditions suitable for fungal growth during the austral summer; however, the fungi still had to survive and proliferate in the huts at average temperatures of -14.7°C, and maximum and minimum temperatures of 9.4 and -35.1°C at the *Terra Nova* Hut, 6.6 and -39°C at *Discovery* Hut, and 2.5 and -35.1°C at the *Nimrod* Hut, respectively (Held *et al*., 2005).

The Historic Huts provide an environment which has had varying levels of human impact over a long period of time. The first occurrence of human impact at the study sites was at the site of *Discovery* Hut in 1901, ninety seven years before this PhD research began. Among the huts there is variation in ongoing human impact; *Discovery* Hut is by far the most visited of the three huts in this study, it is within walk distance of McMurdo Station, the American base and Scott Base, the New Zealand base on Ross Island. The *Terra Nova* and *Nimrod* Huts are both only accessible from the two bases by vehicle over the sea ice when it is safe or by helicopter at other times.

Chapter 3 describes the collection of samples from Antarctica, and isolation of fungi from structural wood and other organic materials from within and outside the three huts on Ross Island, Antarctica. A wide variety of sampling methods, isolation temperatures and growth media are discussed. Moisture content of the structural wood was used to investigate the condition of the wood for fungal growth. Quantitative air sampling was used to determine the viability of the fungi within the hut environments. The hypothesis, aims and objectives for this chapter

are presented first, followed by the literature review which discusses mycology in Antarctica, human impact, isolation of microfungi from organic material, air sampling for fungal spores and previous mycological work completed at the historic sites. The methods and materials, results and discussion on the findings of the fungal diversity from this unique environment are then described.

3.2 Hypothesis, Aims, and Objectives

This chapter focuses on the isolation and identification of the fungi found in the Historic Huts of Ross Island. The main hypothesis of this aspect of the thesis research was that viable fungi were present on the Historic Huts wood, artefacts and foodstuffs.

The main aim was to gain an understanding of the fungal diversity that was present in the Historic Huts as well as artefacts contained within and around it.

Two objectives were addressed, as follows:

- To isolate filamentous fungi from a variety of Antarctic substrates using a selection of culture media, culturing techniques and sampling methods.
- To preserve all organisms isolated both filamentous fungi and single celled organisms to create an Antarctic Fungal Culture Collection for future academic study and reference.

3.3 Literature Review

3.3.1 Mycology in Antarctica

The distribution of fungi in Antarctica is related to the distribution of hosts such as birds, invertebrate populations, human activity and vegetation, which consists of bryophyte and lichen communities. Mycological studies have been conducted in the sub-Antarctic region, the Antarctic Peninsula and Continental Antarctica including in the most ecologically stressed Antarctic regions, such as the Ross Desert and the ice-free regions of the McMurdo Dry Valleys. Onofri *et al.* (2004) reported from international literature that 1604 fungi or pseudofungi were recorded in continental Antarctica, belonging to 135 genera and 232 species and infraspecfic taxa. A summary of the reported phyla, and number of species, orders

and families are presented in Table 3.1. Water moulds (Kingdom Chromista) represents 0.6% while the remaining organisms (99.4%) are true fungi including yeasts.

Table 3.1: Summary of filamentous fungal phyla isolated from Antarctica, and number of species, orders and families within each phylum (Onofri *et al.* 2004).

Phylum	Number of species	Number of orders	Number of families
Chytridiomycota	7	3	3
Zygomycota	20	3	4
Ascomyota	33	10	18
Basidiomycota	1		

Most of the reported fungi were mitotic anamorphs; with 828 records, 725 belong to hyphomycetes (115 species belonging to 60 genera), and 103 to coelomycetes (3 species in the genus *Phoma*). Fifty four specific and intraspecific yeast taxa were recorded, including 10 genera and 13 species and varieties belonging to *Ascomycota* and 10 genera and 41 species and varieties to *Basidiomycota*.

Cameron (1971) suggested that *Penicillium* was the most common genus of fungi in the Antarctic. *Penicillium verrucosum* was the most common *Penicillium* isolate in a study by Heatwole *et al*. (1989) and was frequently recorded in other locations around Antarctica (Barker, 1977; Kerry, 1979; Fletcher *et al*., 1985; Kerry and Weste, 1985). McRae *et al.* (1999) isolated seven *Penicillium* species from a study of the occurrence of *Penicillium* spp. with plants and animals on Windmill Island, East Antarctica. *Chrysosporium pannorum* (*Geomyces* sp.), *Penicillium* spp. and *Cladosporium* spp. appear to be widespread (Fletcher *et al.*, 1985) and this prevalence is an indication of their importance in the Antarctic fungal flora. Their extensive distribution in natural ecosystems has been suggested to indicate that these fungi play a role in decomposition processes in these ecosystems (Fletcher *et al.*, 1985). *Cadophora* spp. (*Phialophora* spp.) have been reported previously in Antarctica (Amzi and Seppelt, 1998; Sun *et al*. 1998; Kerry, 1990b; Aislabie *et al*., 2001; Tosi *et al*., 2002) associated with organic material also indicating this organisms role in decomposition.

3.3.1.1 Human impact

The impact of humans has been studied at many sites including permanent stations, camp and study sites. The first record of human contamination was reported by Wicklow in 1968 when they isolated *Aspergillus fumigarus* (principal incitant of aspergillosis among penguins) from ornithogenic soils from the Cape Hallett penguin colony (Onofri and Tosi, 1992).

During the Dry Valley Drilling project, Cameron *et al.* (1977) reported on a site at McMurdo station, that was not only visibly changed but the indigenous population of bacteria, fungi and algae were completely eliminated and replaced by a few entirely different species of hydrocarbon-decomposing bacteria. At Lake Vida, a number of *Penicillium* spp. were found in samples before drilling, whereas after drilling there were substantial numbers of white moulds and some bacteria (Cameron *et al.,*1977). At Lake Fryxell, there were practically no microorganisms detected before drilling but a substantial number of *Penicillium* spp. were recovered after drilling and removal of the camp (Cameron *et al.*, 1974). At New Harbour, *Neurospora* sp., which was assumed to have been introduced via food stuffs from McMurdo Station or Scott Base, became the predominant contaminant in the Jamesway structure kitchen facility area and later became an obnoxious contaminant in the Eklund Biological Centre at McMurdo. One example of the survival of an introduced organism was a case reported by Cameron (1972). The opening of a can of food rations that was contaminated with a *Penicillium* sp., led to the subsequent dispersal of the organism around their remote campsite on Mount Howe; they were able to detect the organism in the next samples taken from this site.

In a study completed by Azmi and Seppelt (1998) investigating the distribution of microfungi in the Windmill Island region, they reported that there was a marked increase in fungal diversity in human disturbed sites. When contaminated sites were compared with pristine and high biota sites, higher numbers and types of fungi were isolated in the former along with a number of taxa that were not isolated from the pristine and high biota sites. *Penicillium* sp. dominated contaminated sites and eight additional taxa were also isolated from the contaminated sites.

Toyoda (1985) reported the isolation of *Cladosporium* sp. and *Penicillium* sp. from 68 sampling sites around Syowa Station, Antarctica while other genera were rarely isolated. They concluded that most of the fungal isolates were due to pollution by human beings. At Mawson and Davis Bases, Line (1988) concluded that the soils near the Bases contained a variety of fungi while pristine soils contained only *Verticillium* which was isolated occasionally. At the Italian base at *Terra Nova*, Mercantini *et al.* (1993) isolated keratinophilic fungi along with other fungi that were found in many Antarctic environments. Ten sites were sampled and *Chrysosporium* sp. was isolated from eight sites, making it the most commonly isolated fungal species in their research. The pathogenic fungi, *Microsporium gypseum* and *Trichophyton terrestre*, were isolated from two sites and one site, respectively, and both were attributed to human presence. The only site where there was no human, animal or bird presence (Mt. Melbourne) was negative for both keratinophilic fungi and for other forms of life. Mercantini *et al.* (1993) isolated fungi from five of the nine samples of soil taken from along the coast of the Ross Sea. Six dust samples were taken inside the Italian scientific base and fungi were isolated from all samples. *Trichophyton mentagrophytes* was isolated from the computer room which was attributed to a human carrier being on base at the time of sampling. This study was not repeated the next year so it is not known if the fungi isolated in this study were able to survive the winter. *Geomyces pannorum* var. *pannorum* was the most significant fungal component in the outside sites.

Kerry (1990b) sampled 129 sites at six locations at Vestfold Hills and MacRobertson Land, Antarctica to determine the influence of human activity on the fungal numbers and distribution. The samples were soil and plant communities both close and away from sites of human activity. Twenty fungal taxa were isolated. *Geomyces pannorum* and *Thelebolus microspora* were less common in oil contaminated soil but were in significant numbers in oil free soils near the oil contamination, indicating that the oil changed the natural microbial flora. All records of *Phialophora fastigiata* except one came from station areas, and particularly from the discarded wood and oil contaminated soil. This fungus is believed to have been introduced to this area from wooden packing boxes. The conclusions to be reached from the results of these studies were that most changes in the natural microbial flora were attributed to human activity.

During a study investigating fungal diversity at a variety of microsites in the Taylor Valley in The Dry Valleys, Antarctica, both in autumn and spring, Baublis *et al.* (1991) determined that more fungal species were isolated during spring than autumn (twenty two species in spring and nine species in autumn). *Chrysosporium pannorum* and *Phialophora largerbergii* were the only two species isolated in both seasons. The two microsites that contained human impact, soil near a base waste water barrel and chewing tobacco, contained a high density of fungal contamination.

The effect of the introduction of oil on the microbial hetetrophs in Antarctic soils was investigated by Aislabie *et al*. (2001). At three sites where oil was spilt, the soils were compared for numbers of carbon degraders, viable bacteria, yeast and fungi with uncontaminated soil from the same site. From the sites at Scott Base and Marble Point there was an increase in the number of culturable microorganisms (including fungal numbers) in oil contaminated soils but at the Wright Valley site there was no detectable increase in microbial numbers. For the culturable fungi the numbers in uncontaminated soils ranged from non-detectable to 430 per grm dry weight while in oil contaminated soils the number rose to a range from non-detectable at the Wright Valley to 8.5×10^4 per grm dry weight. A significant shift in the genera of culturable fungi was observed; *Chrysosporium* sp. were dominant in control soils, while *Phialophora* spp. were dominant in the oil contaminated soils at Scott Base and Marble Point.

3.3.1.2 Isolating fungi from organic material

Much of the Antarctic habitat is lacking in nutrients according to Wynn-Williams (1990). Fungi were found particularly to inhabit areas where plant and animal influence was seen and the nutrient levels were enhanced.

During a study of bacteria, fungi and other biota in the vicinity of Mirnyy Observatory, Meyer *et al.* (1967) analysed 60 different samples including air, soil, water, snow, and plant for microbial content. Moulds were isolated only from moss and lichen samples. Fungi, whether filamentous or yeasts, were isolated from all samples of plant material and all but one sample containing animal material from Mac.Robertson and Enderby Islands, Antarctica (Fletcher *et al.*, 1985). Fungi were also isolated though from soil showing no organic material. Few fungi were isolated from samples where the soil pH was above 8. From 4

samples taken, 1 contained fungus, *Penicillium cyclopium*. These results suggest that fungi are widespread on Mac.Robertson Island and Enderby Island. It was suggested that the fungi present in samples containing no visible organic matter were air spora and were inactive until being incubated on agar plates; in the natural ecosystem these air spora would be metabolising algae and small fragments of plant or animal debris.

Meyer *et al.* (1967) also supported the lack of species diversity seen in most locations sampled in Antarctica; this lack of diversity will be further discussed in Section 3.6. Material collected from mid-Victoria Land during 1987-1988 indicated that microfungi occurred most frequently in bird dung and soil and the levels were enhanced when mosses were present (Del Frate and Caretta, 1990; Caretta *et al.*, 1994).

Vishniac (1993) published a list of 82 fungal taxa representing macro- and microfungi recorded in Antarctica. Most of the filamentous fungi and yeasts listed were cosmopolitan species, but some appeared to be indigenous species. The occurrence of fungi in this extreme environment was interesting as is their normal association with mosses. *Phoma herbarum* was the most common species, and this supports the findings of McRae and Seppelt (1999); this species was observed to prefer moss as a nutritional substrate, according to Kerry (1990b). *Arthrobotrys superba*, *Conidiobolus* sp., *Verticillium psalliotae*, *V. lamellicola* and *Penicillium minioluteum* were new records for continental Antarctica. *Conidiobolus* sp. was the first record of a species of *Entomophthorales* in Antarctica.

Microfungi were isolated from 54 lichen, moss and plant samples collected at two sites on King George Island, Antarctica by Möller and Dreyfuss (1996). From a total of 409 isolates, 58 taxa, mainly Fungi Imperfecti and 63 different sterile mycelium (mycelium growth with no visible sexual or asexual structures to allow identification) were isolated.

Azmi and Seppelt (1998) investigated the distribution of microfungi in the Windmill Island region, sampling abiotic areas, biotically influenced areas and contaminated sites. Overall, there was found relatively low fungal biodiversity with 35 taxa representing 22 genera. From biota influenced sites, a greater number

and type of fungi were isolated. Within the variety of biota sampled, moss beds showed the highest degree of fungal colonisation; fungi associated with algae were very similar to the fungi associated with moss beds and lichens showed the poorest fungal diversity. There was also substrate specificity with some fungal species only being isolated from one substrate such as *Aspergillus* sp. and *Rhizopus stolonifer* were only isolated from algae.

McRae *et al.* (1999) investigated the association of *Penicillium* sp. with natural occurring plants and animals on Windmill Island, East Antarctica. From this study, seven *Penicillium* sp. were isolated and identified. They concluded that *Penicillium* sp. were more commonly isolated from areas influenced by birds than from areas influenced by plants. The one site where *Penicillium* sp. was not isolated, moss beds, was an area of high human impact.

Fungi were isolated from different moss samples in Victoria Land by Tosi *et al.* (2002). Twenty eight taxa belonging to 18 genera were identified. The most frequently isolated fungal species were *Cladosporium cladosporioides*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Geomyces pannorum* var. *pannorum*, *G. pannorum* var. *vinaceus*, *Mortierella antarctica*, *Cadophora malorum*, *Phoma herbarum* and *V. lecanii*. *Bryum pseudotriquetrum* was the moss richest in fungal species. However, the most frequently recorded species found associated with mosses in the study were isolated from sites very distant from Terra Nova Station, a contradictory finding to studies previously cited which stated fungal presence was greatest at sites with human impact but indicating that fungi isolated from mosses were host specific and cosmopolitan fungi introduced by humans were not able to colonise moss material. The conclusion was that the fungal communities in mosses were generally constituted by indigenous species, partly supported by the fact that most of the isolated species were psychrophilic.

In 1962-63, a comprehensive study of the Taylor and Wright Valleys in the McMurdo Dry Valleys was completed (Boyd *et al.*, 1966). During this study of soil, two different habitats were selected, one with visible signs of blue-green algae and moisture and one that was away from sources of moisture. From a total of 50 samples from four different sites in the Taylor Valley, the soil was found to be sterile and contained no microorganisms in 36 samples. Similar results were

recorded from the Wright Valley. There were also a number of areas within this study that had low counts of moulds and all these sites had high moisture contents as well as the presence of blue-green algae. The highest counts of moulds were from area 16 in the Wright Valley, where fungal counts ranged from 0-240 per gram of soil, samples from Marble Point where fungal counts ranged from 0-4300 per gram of soil and next to mummified seals, especially a seal carcass in area 7 where fungal counts ranged from 4-460 per gram of soil. The samples with high microorganism counts were all areas with relatively high moisture contents, human or animal impact.

Del Frate *et al.* (1990) reported on the isolation of fungi from soil, penguin, skua and petrel dung and bird feathers from Inexpressible Island to Cape King, in Victoria Land, Antarctica. Fungi occurred prevalently in bird dung and in soil especially when mosses were present. From 126 samples, 122 occurrences of fungi were recorded, representing 15 species of filamentous fungi and yeasts including the fungi *Acremonium strictum*, *Chrysosporium verrucosum*, *Cladosporium herbarum*, *Geomyces pannorum* var. *pannorum*, *Mortierella antarctica*, *Paecilomyces farinosus*, *Phialophora fastigiata*, *Phoma herbarum*, *Scolecobasidium salinum*, *Scytalidium thermophilium*, *Thelebolus microsporus*, *Thermomyces langinosus*, *Verticillium* sp. mycelia sterilia, the yeasts *Crytococcus albidus*, *Torulaspora delbrueckii*, white and pink yeasts.

Held *et al*. 2006 investigated a wooden crate at New Harbour, Ross Sea Region, Antarctica for the presence of fungi. Soft rot was found in wood in contact with the soil and four soft rot species *Cadophora malorum*, *Cadophora luteo-olivacea*, *Cadophora fastigiata* and an undescribed species of *Cadophora* were isolated. Other fungi isolated from the wood included *Cladosporium cladosporioides*, *Hormonema dematioides*, *Lecythophora hoffmanii* and *Penicillium mali*.

3.3.2 Mycological investigation at the Historic Huts on Ross Island

Much of the previous work prior to 1995 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 and Table 3.2 identifies the fungi reported from these studies. During investigation of microbiota of air and soil in Antarctica,

Meyer *et al.* (1962) found a food cache partly buried in the snow at Cape Evans and a sealed bottle of yeast contained within the cache was investigated for viable microorganisms. Meyer *et al.* (1963) studied human faeces and food stuffs from Cape Evans and Cape Royds. From the human faeces at both locations, aerobic and anaerobic bacteria were isolated but moulds were only isolated from the sample taken from Cape Royds. A tin of dry pearl barley was sampled from Cape Royds and a bottle of Heinz Tomato Catsup and hay were sampled at Cape Evans.

Nedwell *et al.* (1994) investigated the long term survival of microorganisms from Cape Evans and Cape Royds samples including dried peas, pearl barley, chaff/hay and pony dung patches. Although they isolated large numbers of bacteria, mostly spore forming *Bacillus* species, they only isolated a very small number of unidentified moulds. From soil samples taken at Cape Evans and horse dung samples from Cape Royds, Tubaki *et al.* (1965) isolated fungi from the soil. Thermophilic fungi were isolated from pony fodder at Cape Royds by Greenfield (1982). These fungi have a growth range of 25°C to 55°C (Greenfield, 1982).

Recent investigations of the Universities of Waikato and Minnesota collaboration 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 (Blanchette *et al.*, 2004). Farrell *et al.* (2004) and Held *et al.* (2005) reported on the environment within the Historic Huts and noted that it was favourable for fungal growth. Both reports also included a list of fungi which is reproduced in Table 3.3. By using traditional culturing methods, as well as molecular methodology including denaturing gradient gel electrophoresis (DGGE) to investigate fungal diversity of the Ross Island Historic Huts and surrounding soils, Arenz *et al.* (2006) determined that the most frequently isolated fungi from the Historic Hut woods sampled included species of *Cadophora*, *Cladosporium* and *Geomyces*. Similar species were found in soil samples collected near the huts (Arenz *et al.*, 2006). Table 3.3 presents a summary of the fungi that have been isolated from the Historic Huts along with location and material from which the fungi were isolated from from the Universities of Waikato and Minnesota collaboration.

Table 3.2: Filamentous micro-fungi isolated prior to 1995 from the Historic Huts on Ross Island, Antarctica.

Fungal species identified	**Location**	**Material fungi isolated from**	**Reference**
Absida corymbifera	Cape Evans	Sealed bottle of yeast	Meyer *et al.* (1962)
Rhizopus arrhizus	Cape Evans	Sealed bottle of yeast	Meyer *et al.* (1962)
Phoma sp.	Cape Royds	Faeces	Meyer *et al.* (1963)
Botrytis-like sp.	Cape Royds	Faeces	Meyer *et al.* (1963)
Unidentified dematiaceous mould	Cape Royds	Faeces	Meyer *et al.* (1963)
Mucor sp.	Cape Royds	Dry pearl barley	Meyer *et al.* (1963)
Two unidentified mould	Cape Evans	Heinz Tomato Catsup	Meyer *et al.* (1963)
Mucor sp.	Cape Evans	Hay	Meyer *et al.* (1963)
Penicillium sp.	Cape Evans	Hay	Meyer *et al.* (1963)
Undetermined dematiaceous mould	Cape Evans	Hay	Meyer *et al.* (1963)
Unidentified moulds	Cape Evans Cape Royds	Dried peas, pearl barley, chaff/hay and pony dung patches.	Nedwell *et al.* (1994)
Botrytricium piluliferum	Cape Royds	Horse dung	Tubaki *et al.* (1965)
Aspergillus restrictus	Cape Evans	Soil	Tubaki *et al.* (1965)
Chrysosporium verrucosum	Cape Evans	Soil	Tubaki *et al.* (1965)
Chrysosporium pannorum	Cape Evans	Soil	Tubaki *et al.* (1965)
Dendryphiella salina	Cape Evans	Soil	Tubaki *et al.* (1965)
Penicillium charlesii	Cape Evans	Soil	Tubaki *et al.* (1965)
Penicillium corylphilum	Cape Evans	Soil	Tubaki *et al.* (1965)
Penicillium crustosum	Cape Evans	Soil	Tubaki *et al.* (1965)
Paecilomyces sp.	Cape Royds	Pony fodder	Greenfield (1982)
Aspergillus sp.	Cape Royds	Pony fodder	Greenfield (1982)

Table 3.3: Filamentous microfungi isolted from the Historic Huts by the Universities of Waikato and Minnesota collaboration.

Fungal species identified	**Location**	**Material fungi isolated from**	**Reference**
Cadophora malorum	Cape Royds Cape Evans	Wood Wood, soil and other material	Blanchette *et al.* (2004) Arenz *et al.* (2006)
Cadophora luteo-olivacea	Cape Evans	Wood Soil, Wood and other material	Blanchette *et al.* (2004) Arenz *et al.* (2006)
Cadophora sp. strain E	Cape Evans	Wood	Blanchette *et al.* (2004)
Cadophora sp. strain H	Hut Point	Wood	Blanchette *et al.* (2004)
Geomyces sp.	Cape Evans	Wood	Held *et al.* (2005)
Cladosporium cladosporoides	Cape Evans Hut Point	Wood Soil and wood	Held *et al.* (2005) Arenz *et al.* (2006)
Penicillium echinulatum	Cape Evans	Wood	Held *et al.* (2005)
Penicillium expansium	Cape Evans	Wood	Held *et al.* (2005)
Horonema dematioides	Cape Evans	Wood Wood and other material	Held *et al.* (2005) Arenz *et al.* (2006)
Alternaria sp.	Cape Evans Cape Royds	Soil and wood	Arenz *et al.* (2006)
Ascobolus denudatus	Cape Evans	Other material	Arenz *et al.* (2006)
Cadophora fastigiata	Cape Evans Cape Royds	Soil and other material	Arenz *et al.* (2006)
Cadophora luteo-olivacea	Cape Royds Hut Point	Wood, soil and other material	Arenz *et al.* (2006)
Cadophora malorum	Hut Point	Wood, soil and other material	Arenz *et al.* (2006)
Cadophora sp. 4E71-1	Cape Evans	Wood, soil and other material	Arenz *et al.* (2006)
Cadophora sp. H37	Cape Royds	Wood	Arenz *et al.* (2006)
Cladosporium cladosporoides	Cape Royds Hut Point	Wood and soil	Arenz *et al.* (2006)
Cosmospora vilior	Cape Evans Cape Royds	Wood	Arenz *et al.* (2006)
Dactylella lobata	Cape Evans Cape Royds	Soil and Wood	Arenz *et al.* (2006)
Eurotium sp.	Cape Evans Cape Royds	Soil and Wood	Arenz *et al.* (2006)
Exophiala spiniferea	Cape Evans Hut Point	Soil and Wood	Arenz *et al.* (2006)
Geomyces sp. C239	Cape Evans Cape Royds Hut Point	Soil, wood and other material	Arenz *et al.* (2006)
Geomyces pannorum	Cape Evans Cape Royds Hut Point	Soil, wood and other material	Arenz *et al.* (2006)
Hormonema dematoides	Hut Point	Wood and other material	Arenz *et al.* (2006)
Monodictys castaneae	Cape Evans	Wood	Arenz *et al.* (2006)
Nectria sp.	Cape Royds	Wood	Arenz *et al.* (2006)
Paecilomyces inflatus version 1	Cape Royds	Wood and other material	Arenz *et al.* (2006)
Penicillium echinulatum	Cape Royds Hut Point	Soil wood and other material	Arenz *et al.* (2006)
Phoma sp.	Hut Point	Soil	Arenz *et al.* (2006)

Table 3.3: Filamentous microfungi isolted from the Historic Huts by the Universities of Waikato and Minnesota collaboration.

Fungal species identified	Location	Material fungi isolated from	Reference
Pseudodeurotium desertorum	Hut Point	Wood	Arenz *et al.* (2006)
Pseudodeurotium sp. olrium 176	Cape Evans Cape Royds	Other material	Arenz *et al.* (2006)
Sarea difformis	Hut Point	Wood	Arenz *et al.* (2006)
Thelobolus caninus	Cape Royds Hut Point	Soil and other materials	Arenz *et al.* (2006)
Thelobolus microspora	Cape Evans	Soil	Arenz *et al.* (2006)
Uncultured fungus isolate RFLP104	Cape Evans	Wood	Arenz *et al.* (2006)
Uncultured *Mortierellaceae*	Cape Royds	Soil	Arenz *et al.* (2006)

3.3.3 Air sampling

The pioneers of Antarctic microbiology investigated the microbiology of air, water, ice, snow, soil and animals in the early 1900's. The aerial microflora was negligible but they found a wide range of bacteria, actinomycetes and a pseudomycelial yeast. Ten years later McLean confirmed the near-sterility of Antarctic air at Commonwealth Bay but found viable bacteria and yeasts in falling snow, glacier ice and melt water. Many researches have hypothesised that some Antarctic microorganisms are blown in from distant continents on cyclonic or off shore winds, while others think that birds are the main transporters of microorganisms from foreign lands. The study of airborne fungal spores is a common practice worldwide mostly for allergen studies and plant pathology.

Recently in Antarctica, aerobiology has taken on greater importance as interest in introduction of alien organisms to the Antarctic environment has been recognised. According to Wynn-Williams (1991), the first study of airborne microorganisms in Antarctica was by Ekelöf in 1908. Many of the early studies were targeting bacteria although fungi were noted. The first approach was to allow propagules (gravitational settling) to settle onto exposed agar plates (Corte and Daglio, 1962). Volumetric samplers were used to trap constituents of the air spora but fungi were only found occasionally using this method (Meyer, 1962; Cameron *et al.*, 1972). Hirst and Gregory (1962) used a rotorad sampler (an instrument which uses sticky tape to capture the material) to investigate airborne spores at Halley Bay Brunt Ice Shelf, Antarctica and measured a mean spore concentration of 0.22 spores m^3 (cubic metre) (Marshall, 1997).

Cameron *et al.* (1974) began using air sampling in the summer of 1966-67 to investigate the distribution, abundance and variety of microorganisms present in the Antarctic environment. Information from these early studies was used as a base line when they (Cameron *et al.,* 1977) used aerobiology to determine the effect human activities were having on the Antarctic environment including the Dry Valley Drilling project, research bases, rubbish dumps and semi permanent structures, sampling both drilling sites and other inhabited areas. As expected, the abundance of bacteria and fungi were significantly higher inside the habitation and at obvious sites of contamination such as the McMurdo dump than in relatively unperturbed areas. There was an increase in numbers of aerial microorganisms after drilling from areas where there were detectable fungal numbers before drilling and areas with no detectable microorganisms before drilling showed no microorganisms after drilling. Cameron *et al.* (1973) presented data on the quantity of microbial spores in the air but they did not identify any of the micoorganisms. At Don Juan pond, aerial fungal spores were measured along with a study of aquatic and soil microbiology. Air samples were taken upwind and down wind from the campsite and non-indigenous soil microorganisms were isolated from the air including the following identified moulds: *Paecilomyces* sp., *Penicillium* spp. and *Monodictys austrina* (Cameron *et al.,* 1973).

Marshall (1997) used rotorod samplers to determine both quantitatively and qualitatively the year round seasonality of airborne fungal propagules at different sites on Signy Island. The results showed that the concentration was lower than those found in other regions of the world including the Arctic. The counts were higher during summer than winter; for example at Jane Col, a peak of 0.766 fungal spores per m^3 as recorded in early summer as compared to levels of no fungal spores in the winter months. *Chlamydospores* sp. were the most abundant at all the sites tested which is not consistent with the rest of the world where *Chlamydospores* sp. form a minor component of air samples and *Cladosporium* sp. the most numerous (Marshall, 1997). *Cladosporium* sp. conidia were the second most detected fungal spore in this Antarctic study. It was noted that the highest concentration of *Cladosporium* sp. spores were recorded when there had been an arrival of an air mass originating in South America. *Cladosporium sphaerospermum* was also detected and this was the first recording of this fungus on Signy Island.

Keratinophilic fungi have been studied extensively and it is believed that these organisms occur in areas of high plant and animal influence. In soils of the Ross Sea Region they have been found in areas frequented by birds, skuas and penguins, and areas of human activity. *Geomyces pannorum* is the most common keratinophilic species (Del Frate and Caretta, 1990). Keratinaceous material can be transported by the wind along with fungal material. Marshall (1998) reported results of the spread of keratinaceous material and the fungus *Geomyces pannorum* on Signy Island. Samples from the rotorad air sampler and Frisbee collector were cultured for fungal material; eight recoveries of *G. pannorum* were recorded from the three main sample sites and nine recoveries of *G. pannorum* from 53 rotorod collections at a minor sampling site. Microscopically, *G. pannorum* spores were undetectable and recovery from culturing was very low; 2.1% at main sample sites and 17% at the minor site. One interesting result was that the most recoveries of *G. pannorum* was during the period March to June, the onset of winter, when temperature fluctuates around 0ºC. This was attributed to the destruction of mycelia by freeze thaw cycles and release of spores from biomass mats.

Upton (1997) reported the use of settle plates to detect human commensals in the area around an Antarctic research station. They detected fungi at a level of 3 colony forming unit (CFU)/15 minutes exposure at one location from a sample of 13 sites around the accommodation block at Halley V station. They did detect bacteria propagules in all plates exposed inside the station and at one location outside the station.

The study of airborne microorganism is leading to a better understanding of the spread of organisms around Antarctica, their survival potential and threats they pose to the Antarctica environment. The use of air sampling techniques has also been successful in the assessment of human impact, and introduction of foreign organisms. The ability to detect low numbers of microorganisms in the air using air sampling machines is very useful for Antarctic research as the numbers are normally very low.

3.4 Materials and Methods

3.4.1 Events for sampling

As described in Section 2.1, Antarctic New Zealand Event K024C/K021 has been the field event for this multidisciplinary study. The PhD thesis author, Shona M. Duncan, participated in the Event six times in Antarctica; January 1998, December 1998, January 2001, January 2002, January 2004 and January 2006.

3.4.2 Samples locations and sampling method

3.4.2.1 Sample locations

Small samples of structural wood, organic material, soil, foodstuffs, swab samples, scraping samples of surfaces, air samples, floor sweeping, spore traps and bait traps were taken from the *Discovery* Historic Hut at Hut Point, *Terra Nova* Historic Hut at Cape Evans, and *Nimrod* Historic Hut at Cape Royds, Ross Island, Antarctica; (see specific details of the locations of the sampling in Table 3.4) in January 1998, December 1998, January 2001, January 2002, and January 2004. Field samples collected in Antarctica were placed into sterile containers, microbial isolations subsequently attempted in New Zealand, while other field samples were put directly on Petri dishes with specific growth media while in Antarctica. The isolation techniques are described in Section 3.4.3.

Table 3.4: Location of Historic Hut sites by GPS and Antarctic Specially protected area number.

Site	GPS	Antarctic Specially Protected Area (ASPA) number
Hut Point	77º 50' 50"S, 166º 38' 30"E	158
Cape Evans	78º 38' 10"S, 116º 25' 04"E	155
Cape Royds	77°33'10.7"S, 166°10'6.5"E	121

All samples were brought into New Zealand under the Ministry of Agriculture and Forestry Permit numbers 1998006429, 2000010576, 2001013830, 2002016832, 2003021075 and 2005027174. All sample vials, Petri dishes, and bait trap specimen containers, containing samples were kept cold (between 0-4°C) while in Antarctica and on return to New Zealand. Samples were then stored under sterile conditions at 4°C until isolations were made. All Petri dishes and bait trap specimen containers upon arrival back at the laboratory were incubated at 4°C, 15°C or 25°C depending on the experiment and year of sampling.

3.4.2.2. Wood samples

Minute (typically less than 1 cm long and 0.5 cm wide) segments of structural wood were aseptically collected from inconspicuous locations throughout the Historic Huts frame work. The samples were either removed from damaged areas where the wood fragments could be pulled off using sterile forceps or samples were removed from surfaces with a sterile scalpel and forceps. For samples of structural wood below ground level, a trowel was used to remove the soil and a minute sample was removed with a scalpel and forceps; the soil was replaced after the samples had been taken. Samples were taken from wooden artefacts from within the ASPA area and outside the protected area. These samples were taken from inconspicuous locations, damaged areas, or were pieces of wood that were lying on the ground around the sites.

3.4.2.3 Swab samples

Swab samples were taken from areas of visible fungal growth or areas where removal of a physical sample would leave a visible impression. The surface to be sampled was wiped over with a sterile, cotton swab saturated in autoclaved distilled water, which was kept in a sterile plastic tube. After wiping of the surface, the swab was returned to its sterile plastic tube.

3.4.2.4 Scraping samples

Scrapings were taken from surfaces with visual fungal growth or areas of accumulated material such as accumulated salt. Scrapings of structural wood and organic materials were aseptically collected using tweezers and scalpel from inconspicuous locations throughout the huts or a sterile 50ml Falcon tube was used to scrape the surface to be sampled; the rim of the tube was wiped with an alcohol wipe before the lid was replaced.

3.4.2.5 Organic material samples

Samples of organic materials were taken from various locations. Depending on the nature of the material, the samples were taken by either cutting the material away from the sample site using a sterile scalpel or if the material was loose, a sample was picked up with sterile forceps and placed in sterile vials.

3.4.2.6 Soil samples

Samples of soil were collected using a clean, ethanol sterilised trowel to uplift the sample, or to remove surface soil so a deeper sample could be taken with the trowel, and the collected soil was placed in a sterile bag.

3.4.2.7 Foodstuffs

Samples of foodstuffs were taken from open containers and were either picked up with sterile forceps or by cutting the sample using a sterile scalpel and placed into a sterile container.

3.4.2.8 Spore traps

Petri dishes containing growth medium (refer to Section 3.4.4) were placed in various locations around the huts, both interior and exterior. The lid of the Petri dish was removed and left off the plate for 30 minutes. During this time normal human activity continued. After the exposure time, the Petri dish was closed and sealed with parafilm.

3.4.2.9 Bait traps

A 70 ml polystyrene specimen bottle (with lid removed) containing 20 mls of growth medium agar was forced into the ground so the agar surface was in contact with the soil. The bottles were placed at various locations around the outside of the hut and left for 20 hrs. After 20 hrs, the specimen bottle was removed, recapped and sealed with parafilm.

3.4.2.10 Sampling and culturing on location

Swab, scraping, soil and food stuff samples were taken as outlined in Section 3.4.2.3, 3.4.2.4, 3.4.2.5, 3.4.2.6, and 3.4.2.7 but instead of placing in a sterile container, they were placed onto Petri dishes in growth medium. Petri dishes were sealed with parafilm.

3.4.2.11 Sweeping samples

The Antarctic Heritage Trust broom was used to sweep the floor of the huts, then sweepings were collected and a sample of the sweepings were placed in a sterile 50 ml Falcon tube.

3.4.3 Isolation of microorganisms from materials collected in Antarctica

3.4.3.1 Isolation of microorganisms from wood/solid material

The wood samples were surface sterilised by soaking for one minute in a 5% hypochlorite solution at room temperature, followed by two rinses in sterile, distilled water, then sliced on a sterile surface with a sterile scalpel and placed onto culture medium. Some samples such as wood scraping samples, soil samples or foodstuffs were shaken in order to make the sample more homogenous, by inverting sample container a number of times and then subsequently, a subsample was placed onto the culturing medium using sterile forceps.

For foodstuffs samples that were large or protected by a husk (e.g. corn, peas, beans), the samples were sliced with a sterile scalpel and then placed onto the culture media using sterile forceps.

Organic material samples were cut with a sterile scalpel and placed onto the culture medium.

3.4.3.2 Isolation of microorganisms from swab samples

Fungal isolations were accomplished by wiping swab samples over the surface of the agar medium starting with the medium containing the least amount of nutrient and finishing with the richest medium, in terms of carbon and nitrogen as given in Section 3.4.4.

3.4.4 Media and Reagents

The following media was used in the experiments related to this chapter's objectives:

Malt Yeast Extract Agar (YM) - 1.5% malt extract (Becton Dickinson and Company, USA), 0.2% yeast extract (Becton Dickinson and Company, USA), 1.8% agar (Becton Dickinson and Company, USA) in distilled water.

Malt Yeast Extract Broth (YM) - 1.5% malt extract, 0.2% yeast extract, in distilled water.

Media 4 - 1.5% malt extract, 0.2% yeast extract, 1.8% agar, 0.2 g/l (grams/litre) chloramphenicol (Applichem, Germany), 0.1 g/l streptomycin sulphate (Applichem, Darmstadt) in distilled water.

Media 6 - 1.5% malt extract, 0.2% yeast extract 1.8% agar, 0.2 g/l chloramphenicol, 0.1 g/l streptomycin sulphate, 0.4 g/l cycloheximide (Sigma, USA) in distilled water.

Media 7 - 1.5% malt extract, 0.2% yeast extract, 1.8% agar, 0.2 g/l chloramphenicol, 0.06 g/l benlate, 0.1 g/l streptomycin sulphate, 2 mls/l lactic acid (APS Ajax Finechem, Australia) in distilled water.

Vogel Bonner medium - 25% glucose (Scharlau, Spain), 2.0% agar, 20 ml VB concentrate (50% K_2HPO_4 (anhydrous) (APS Ajax Finechem, Australia), 17.5% $NaNH_4PO_4$.$4H_2O$ (APS Ajax Finechem, Australia), 10% citric acid.H_2O (APS Ajax Finechem, Australia), 1% $MgSO_4$.$7H_2O$(APS Ajax Finechem, Australia) in 670 mls distilled water.

Martin's medium – 0.1% K_2HPO_4 (anhydrous), 0.05% $MgSO_4$.$7H_2O$, 0.5% peptone (Becton Dickinson and Company, USA), 1% glucose, 50mg/l Rose Bengal (Serva Germany), 100ppm chloramphenicol, 1.5% agar in distilled water.

Malt extract agar- 1.5% malt extract, 2.0% agar in distilled water

Acidic malt extract agar – 3.0% malt extract, 0.3% peptone from soya bean (Difco Laboratories USA), 1.5% agar, 2mls/l lactic acid in distilled water.

NaCl agar- 1.5% malt extract, 0.2% yeast extract, 2.5% NaCl (APS Ajax Finechem, Australia), 1.8% agar in distilled water.

Potato Dextrose Agar (PDA) - 3.9% PDA powder (infusion from 200g Potato, 2.0% dextrose, 1.5% agar) (Sigma, USA) in distilled water.

Carboxymethylcellulose *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4 (BDH Laboratory Supplies, England), 15mls 1M KH_2PO_4 (BDH Laboratory Supplies, England), 6 mls 35% urea (APS Ajax Finechem, Australia), 3 mls 10% $CaCl_2$ (BDH Laboratory Supplies, England), 3 mls 10% $MgSO_4$.$7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl (APS Ajax Finechem, Australia), 0.51% $FeSO_4$ (APS Ajax Finechem, Australia), 0.186% $MnSO_4$.$4H_2O$ (BDH Laboratory Supplies, England), 0.166% $ZnCl_2$ (J.T. Baker USA), 0.2% $CoCl_2$ (BDH Laboratory Supplies, England), 2 mls Tween 80 (BDH Laboratory Supplies, England), 0.2% Carboxymethylcellulose (Sigma, USA) and 1.5% agarose (Seakem LE, Cambrex, USA) in distilled water.

Avicel *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Avicel (Serva, Germany) and 1.5% agarose in distilled water.

All media were sterilised by autoclaving for 20 minutes at 121°C. The antibiotic chloramphenicol was added prior to autoclaving but the antibiotics streptomycin sulphate and cycloheximide were filter sterilised through a 0.2 micron filter (Sartorius, Minsart) and added to the medium after it was autoclaved and cooled to 50°C. Lactic acid was autoclaved separately and added after the medium was cooled to 50°C and just before the medium was poured into 90mm Petri dishes.

Lactophenol cotton blue- 0.05% cotton blue (BDH Laboratory Supplies, England), 20% phenol (BDH Laboratory Supplies, England), 40% (v/v) glycerol (BDH Laboratory Supplies, England), 20% (v/v) lactic acid in distilled water

3.4.5 Incubation, single isolate production and storage of isolates

The Petri agar plates containing samples, spore traps, bait traps and samples plated in Antarctica were then incubated in an incubator (Contherm) at 4°C, 15°C or 25°C for up to six weeks. Microorganisms growing on the agar plates were transferred by subculturing from hyphal tips, colonies or spores to new agar plates until pure cultures were obtained. Pure cultures were given an acquisition number in the Antarctic Fungal Culture Collection at the University of Waikato, Hamilton, New Zealand, and stored -70°C using the following method: Universal bottles (total volume ~30 ml with a screw cap lid) containing 10 mls of YM broth were inoculated with the fungal isolate and incubated at isolation temperature (4°C, 15°C or 25°C) with shaking until visible growth was seen in the universal bottle. 800 microlitres (µL) of inoculum was placed into sterile Eppendorf tubes with 200µL of glycerol (sterilised by autoclaving for 20 minutes at 121°C), vortexed to distribute glycerol and then stored in -70°C ultra freezer (Sanyo Ultralow Freezer). Five replicates of each pure culture isolate were frozen.

3.4.5 Moisture content

Minute segments of structural wood were aseptically collected from inconspicuous locations throughout the inside of the hut. The samples were either removed from damaged areas where the wood fragments could be pulled off using sterile forceps, or, samples were removed from surfaces with a sterile scalpel and forceps. Samples were taken from the walls, locations near ceiling, middle of wall and down at floor level were sampled. Additional samples were taken from the ceiling beams, and the floor boards. Samples were placed in sterile vials for transport back to New Zealand. In the laboratory, samples were removed from their sterile vials, weighed and dried in an oven (Contherm) at 80°C to a constant weight. The moisture content was determined using the following equation:

Moisture content (%) = initial weight - oven dried weight / oven dried weight x100.

3.4.6 Fungal Identification: Classical mycology and molecular analysis

Pure cultures of fungi were grown on YM agar at the temperature of initial isolation 4°C, 15°C or 25°C; when confluent growth was visible, the plates were examined under a stereomicroscope to give an indication of general morphological structures. A small slice of fungal growth was removed from the YM agar plate and placed on a microscope slide with a drop of lactophenol cotton blue stain, and subsequently, a coverslip was placed over the sample and the slide was gently heated until the agar melted. Slides were viewed under a light microscope at magnifications up to 1000x. Structures were noted and compared with reported classical taxonomic morphological features (Barrnett and Hunter, 1972; Sun *et al.*, 1978) and classified into putative genera.

Thirty three fungal isolates were provided to members of the Forest Pathology Group at the University of Minnesota and identified using molecular techniques. DNA sequence analyses of the two internal transcribed spacer (ITS) regions of 18S ribosomal DNA, ITS1 and ITS2, were used to identify the isolates. 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 regions 1, 5.8S, and ITS region 2 were amplified using primers ITS1 and ITS4 (Gardes and Bruns, 1993). PCR amplification was done in a MJ Research PTC Mini-cycler

(Watertown, MA), with the following protocol: 94°C for 5 minutes; 35 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute followed by a final extension step of 72°C for 5 minutes. 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.

3.4.8 Air sampling

A total of ninety-six air samples were collected in Antarctica in January 2006 using a surface air sampler (Merck MAS-100 Eco®). This volumetric sampler, shown in Figure 3.1., aspirates air at a fixed speed for variable periods of time through a perforated plate containing 400-holes positioned over a Petri dish containing agar growth medium.

Figure 3.1: Photograph of Merck MAS-100 Eco® airsampler.

The sampler was operated at calibrated flow rates. Air samples were cultured on 90 mm Petri plates containing either YM agar, Carboxymethylcellulose *Trichoderma viride* medium A or Avicel *Trichoderma viride* medium A. After completion of air sampling, the plates were removed from the sampler, sealed with parafilm and kept cold (0-4°C) while both in Antarctica and travelling to Hamilton, New Zealand. Upon arrival at the University of Waikato laboratory, the plates were incubated at 2°C. Petri plates were examined every week for six weeks and the number of colonies on the agar plates was counted after three and

after six weeks. The number of colonies were adjusted using the positive hole conversion table provided by the manufacturer of the MAS-100; the conversion is based upon the principles that as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an empty hole decreases.

3.5 Results

3.5.1 Sample locations

Sampling of the three Historic Huts on Ross Island, *Discovery* Hut at Hut Point, *Terra Nova* Historic Hut at Cape Evans and *Nimrod* Historic Hut at Cape Royds, Ross Island, Antarctica, during the six field events in January 1998, December 1998, January 2001, January 2002 and January 2004, resulted in a total 849 samples being taken relevant to the PhD thesis research. These samples included wood, foodstuffs, organic material, soil, swabs, scrapings, sweeping samples, bait traps, spore traps, and culturing on location. The location and type of samples taken was dictated by the need to act in good conservation practise with regard to the iconic Historic Huts and not leave a visible mark once the sample was taken. Most of the structural wood samples were taken from areas where the walls had been damaged during the process of removing ice and therefore the sample removal was not evident. Locations from which samples were taken are described in Appendix 1 Tables A1.1, A1.9, A1.17, A1.20 and A1.23 from *Discovery* Hut. Tables A1.3, A1.11, A1.18, A1.21 and A1.24 from *Terra Nova* Hut. Tables A1.5, A1.13, A1.19, A1.22 and A1.25 from *Nimrod* Hut. Tables A1.7 and A1.15 from additional samples of historic material found outside the historic sites or Antarctic non-historic material.

One hundred and sixty samples were taken from *Discovery* Hut; 113 samples came from within the hut and 47 from outside the hut. Of the 160 samples, 112 were wood (69 from inside the hut, 42 from outside the hut and 1 from an artefact), 1 foodstuff, 2 organic materials, 2 from carcass, 4 chaff, 1 soil, 14 swabs, 4 scraping samples, 1 sweeping, and 19 spore traps.

Four hundred and one samples were from *Terra Nova* Hut at Cape Evans; 246 samples came from within the hut, 92 from within the annex/stables area and 63

from outside the hut. The annex/stable area was considered separate from the hut, as it does not have a floor and does not offer as much protection from the harsh Antarctic environment as the hut but does offer some protection. The stable area was also inhabited by ponies which created a different impact than the hut which was inhabited by humans. Of the 401 samples, 222 were wood (109 from inside the hut, 48 from outside the hut, 60 from annex/stable area, 1 from modern wood and 4 from artefacts), 1 foodstuff, 7 non-wood artefacts, 1 modern material, 1 straw, 2 soil samples, 105 swabs, 2 scraping, 3 sweeping samples, 3 bait traps, 16 cultures on site, and 38 spore traps.

Two hundred and seventy two samples came from the *Nimrod* Hut at Cape Royds, 156 from inside the hut, 20 from Mawson's laboratory and 96 samples from outside the hut. Samples from Mawson's laboratory were treated separate from samples inside and from outside the hut as it does not have a floor and is linked to the hut via the entrance porch. Of the 272 samples, 170 were from wood (89 from inside the hut, 70 from outside the hut, 9 from Mawson's laboratory and 2 from artefacts), 10 foodstuffs, 1 non-wood artefact, 1 soil sample, 2 modern material, 38 swabs, 3 sweeping samples, 4 bait traps, 17 cultures on site, and 26 spore traps.

Sixteen additional samples of historic material found outside the historic sites or Antarctic non-historic material were also sampled, 3 from inside a structure and 13 from outside and of these 16 samples, 11 were wood samples, and 5 were organic materials. These samples included wood taken from the Historic Huts and stored in a container at Scott Base, penguin guano and feathers, replacement wood that was stored at the Historic Huts and wood from the Historic Huts that was found away from the ASPA.

3.5.2 Overall percentage of samples from which microorganisms were isolated

Fungi were isolated from samples taken of structural wood, artefacts, and organic material whether physical samples or swabs, with or without obvious fungal contamination. From the eight hundred and forty nine samples taken, fungi were isolated from five hundred and sixty samples (61% of the samples). The percentages of samples containing fungi per hut were as follows:

76% of samples from *Discovery* Hut

69% of samples from *Terra Nova* Hut

39% of samples from *Nimrod* Hut

87% of samples of historic material found outside the historic sites or Antarctic non-historic material.

Presence/absence of fungi is described in Appendix 1, Tables A1.1, A1.9, A1.17, A1.20 and A1.23 from *Discovery* Hut. Tables A1.3, A1.11, A1.18, A1.21 and A1.24 from *Terra Nova* Hut. Tables A1.5, A1.13, A1.19, A1.22 and A1.25 from *Nimrod* Hut. Tables A1.7 and A1.15 from additional samples of historic material found outside the historic sites or Antarctic non-historic material.

3.5.3 Isolation of microorganisms from wood samples

Due to the number of samples taken at the three Historic Huts, along with the different locations within the huts varying, a percentage of the number of samples that contained fungi from the number of samples taken was calculated.

3.5.3.1 *Discovery* Hut

In *Discovery* Hut, there were slightly more fungi isolated from samples taken within the hut, 80%, rather than samples taken outside the hut, 62% as shown in Table 3.5. Within the hut, the percentages of fungi isolated were similar throughout regardless of sample type. Outside, the numbers of fungi isolated above the ground were less, 61% than below ground, 80%. The samples taken from hitching posts that were away from the hut but were left by the Heroic explorers contained the least number of fungi at 44% of samples taken had fungi isolated from them.

Table 3.5: Quantity of wood samples taken from *Discovery* Hut from which fungi were cultured.

Number of wood samples from inside the hut from which fungi were cultured (%)		**Number of wood samples from outside the hut from which fungi were cultured (%)**	
Floor	11 (79)	Above ground	14 (61)
Wall	33 (78)	Below ground	8 (80)
Ceiling	11 (85)	Post	4 (44)
Total	55 (80)	Total	26 (62)

Total number of structural wood samples investigated; 112.

3.5.3.2 *Terra Nova* Hut

In *Terra Nova* Hut, there were similar numbers of fungi isolated within the hut, 50% and outside the hut 48%, with the most samples containing fungi found in the annex/stables area, 71% as shown in Table 3.6. Figure 3.2 shows an example

of four wood samples taken from the *Terra Nova* Hut, two of which have fungi growing onto a VB agar plate from them and nothing growing from the other two wood samples.

Table 3.6: Quantity of wood samples taken from *Terra Nova* Hut from which fungi were cultured.

Number of wood samples from inside the hut from which fungi were cultured (%)		**Number of wood samples from outside the hut from which fungi were cultured (%)**		**Number of wood samples from annex/stables area from which fungi were cultured (%)**	
Floor	16 (55)	Above ground	12 (41)	Above ground	25 (86)
Wall	37 (52)	Below ground	14 (100)	Below ground	14 (100)
Ceiling	2 (22)	Latrine	0 (0)	Hut wall	10 (71)
Total	55 (50)	Total	23 (48)	Total	48 (71)

Total number of structural wood samples investigated; 222.

Within the hut, the percentages of fungi isolated were similar from floor and wall samples but the percentage of samples containing fungi was lower from the ceiling. Outside, the numbers of fungi isolated above the ground were less, 86% than below ground, where in the latter all samples contained fungi. None of the samples from the latrines contained fungi. The annex/stables area samples contained the most fungi with over 80% of all samples containing fungi. 71% of samples taken of the hut wall inside the annex area contained fungi compared with 41% and 52% from samples of wall samples taken from outside the hut and from inside the hut respectively.

Figure 3.2: Photograph of fungi growing from two out of the four wood samples taken from *Terra Nova* Hut.

3.5.3.3 *Nimrod* Hut

In the *Nimrod* Hut, there were significantly more fungi isolated outside the hut than inside, 62% versus 16% respectively with Mawson's laboratory samples showing a intermediate value between inside and outside at 33% of its samples containing fungi as shown in Table 3.7.

Table 3.7: Quantity of wood samples taken from *Nimrod* Hut from which fungi were cultured.

Number of wood samples from inside the hut from which fungi were cultured (%)		**Number of wood samples from outside the hut from which fungi were cultured (%)**		**Number of wood samples from Mawson's Laboratory area from which fungi were cultured. (%)**	
Floor	7 (35)	Above ground	14(27)	Above ground	3 (33)
Wall	7 (13)	Below ground	16 (89)		
Ceiling	0 (0)				
Total	14 (16)	Total	30 (62)	Total	48 (71)

Total number of structural wood samples investigated; 170.

Within the hut, the numbers of fungi isolated were highest, 35% from floor samples and no samples taken from the ceiling contained fungi. Outside, the numbers of fungi isolated above the ground 27% were significantly less than below ground at 89% positive.

3.5.4 Isolation of microorganisms from solid material samples

Tables 3.8-3.10 show the results of fungal isolation from the other samples, mainly artefacts, taken during the six sampling trips, from *Discovery* Hut (Table 3.8), *Terra Nova* Hut (Table 3.9) and *Nimrod* Hut (Table 3.10).

At *Discovery* Hut, fungi were isolated from all of the 10 samples of other material taken during the six field events.

Table 3.8: Quantity of other samples taken from *Discovery* Hut from which fungi were cultured.

Sample type	**Number of samples taken**	**% of samples that fungi were isolated from**
Wooden artefacts	1	100
Meat Carcass	2	100
Scrapping wall	2	100
Soil	1	100
Animal food/ Chaff	4	100

At *Terra Nova* Hut, fungi were isolated from 15 of the 19 samples of other materials which included wooden artefacts, food stuffs, scrapings of wall, soil, animal food/straw, non wooden artefacts and modern materials taken during the six field events. Fungi were not isolated from wood under a bunk inside the hut, soil from eastern end of hut, the non wooden artefact, sealing wax and modern mylar which is used to separate artefacts within the hut.

Table 3.9: Quantity of other samples taken from *Terra Nova* Hut from which fungi were cultured.

Sample type	Number of samples taken	% of samples that fungi were isolated from
Wooden artefacts	4	75
Food stuffs	1	100
Scrapping wall	2	100
Soil	2	50
Animal food/ Straw	1	100
Non wood artefacts	7	83
Modern materials	2	50

At *Nimrod* Hut, fungi were isolated from eight of the 14 samples taken during the six field events. Fungi were not isolated from flour and syrup leaking from a can taken from the east stores and food from the Backdoor Bay food cache. The one soil sample and the two samples of modern filler used for repairs to the hut did not contain fungi.

Table 3.10: Quantity of other samples taken from *Nimrod* Hut from which fungi were cultured.

Sample type	Number of samples taken	% of samples that fungi were isolated from
Food stuffs	10	70
Non wood artefact	1	100
Soil	1	0
Modern material	2	0

3.5.5 Isolation of organisms from swab samples

Swab samples were taken from locations where a physical sample could not be taken.

At *Discovery* Hut, fungi were isolated from 50% of the swab samples taken from hut wood and wooden artefacts. Fungi were isolated from all swab samples taken from non-wooden artefacts, of fungal mycelium as observed as "mould spots" on walls and artefacts (see Figure 3.3 for an example of mould spots) and modern material.

Figure 3.3: Photograph of mould spots on a box artefact (Source Professor Robert Blanchette, 2004).

At *Terra Nova* Hut, fungi were isolated from 82% of the swab samples taken from hut wood, and 69% of swabs of wooden artefacts. Fungi were isolated from all swabs taken from non-wooden artefacts, organic material (material of mixed origin often accumulated on the floor or walls), food and modern material samples. Fungi were isolated from 69% of swab samples taken of visible fungal mycelium as observed as "mould spots" on walls and artefacts. Section 3.6 discusses why the percentage was not higher for isolation of fungi from visible fungal mycelium.

At *Nimrod* Hut, fungi were isolated from 31% of hut structural wood samples, 60% of samples taken from Mawson's laboratory, and none of the swabs of wooden artefacts. The swabs taken of non-wooden artefacts contained fungi in 69% of the samples; the one swab of fungal mycelium which was present on the wall of the hut in the stores area produced a single fungal culture while the modern material contained no fungi.

3.5.6 Isolation of organisms from spore traps

3.5.6.1 *Discovery* Hut spore trap results

In 2002, YM agar (a general purpose rich nutrient medium) in an open Petri plate was used as "spore traps" at 7 locations. At *Discovery* Hut, the traps were set up inside and fungi were isolated from all spore traps. The fungi isolated from the spore traps, in 2002, were *Cladosporium* sp., and *Geomyces* sp.

In 2003, at *Discovery* Hut, three media, YM, Martin's and PDA, were used at 6 locations and fungi were isolated from all three media agar plates exposed inside and outside the hut at all locations. Figure 3.4 shows a typical YM agar spore trap plate after exposure for 30 minutes and incubation at 4°C. Fungi were also isolated from the site of the wood test panel frame (approximately 50 m from *Discovery* Hut) on the general purpose agar (YM and PDA) but not on the Martin's medium. The fungi isolated from the spore traps, in 2003, were *Cladosporium* sp., *Geomyces* sp. and unidentified fungi.

In 2004, at *Discovery* Hut, YM, NaCl and Media 7 agar plates were used at 6 locations 3 the same as 2003 and 3 new locations; fungi were isolated from all agar plates inside the hut except Media 7 which is selective for basidiomycete fungi.

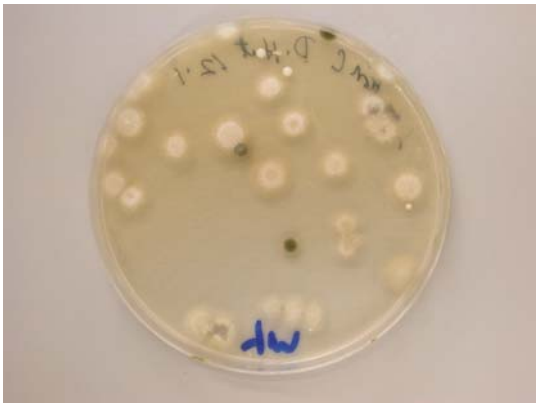

Figure 3.4: Photograph of spore trap plate showing *Cladosporium* sp. and *Geomyces* sp. collected from the air in the store room.

Outside the hut, fungi were isolated from all agar plates exposed near the seal carcass and on the YM plate near a post that was from the Heroic Era but was not attached to the hut. The fungi isolated from the spore traps, in 2004, were *Cladosporium* sp., and *Geomyces* sp.; no unidentified fungi, as had been isolated from spore traps in the previous year (2003), were found in 2004. Table of location, media and whether fungi were isolated from spore traps displayed in Appendix 2, Tables A2.1 to A2.3.

3.5.6.2 *Terra Nova* Hut spore trap results

In 2002, YM agar was used as spore traps at 10 locations at the *Terra Nova* Hut; the traps were set up inside the hut and in the annex area and fungi were isolated from all YM agar spore traps. The fungi isolated from the spore traps, in 2002, were *Cladosporium* sp., *Geomyces* sp., and two different unidentified white fungi. Figure 3.5 shows a typical YM agar spore trap plate after exposure for 30 minutes and incubation at 4°C.

In 2003, at the *Terra Nova* Hut YM, Martin's and PDA were used at 14 locations and fungi were isolated from all general purpose agar plates exposed inside the hut except on top of Evans bed. In the stable/annex area, fungi were isolated from the general purpose agar in the annex area and in stable 3 and outside. Fungi were also isolated from the site near the fuel drums, and in front of the hay but only on the general purpose agars (YM and PDA) and on spore traps at the wood test panel frame (approximately 250 m from *Terra Nova* Hut) but only on the general purpose agars (YM and PDA). The fungi isolated from the spore traps, in 2003, were *Cladosporium* sp., *Geomyces* sp., *Cadophora* sp. and several unidentified fungi.

In 2004, at *Terra Nova* Hut, YM, NaCl and Media 7 were used at 9 locations; fungi were isolated from two locations within the hut, the galley area and by the table containing the visitor's book. The fungi were only isolated on the general purpose medium. In the annex area, fungi were isolated from the YM agar in all locations except the annex area walkway to the stables. The fungi isolated from the spore traps, in 2004, were *Cladosporium* sp., *Geomyces* sp. and three unidentified fungi. Table of location, media and whether fungi were isolated is displayed in Appendix 2, Tables A2.4 to A2.6.

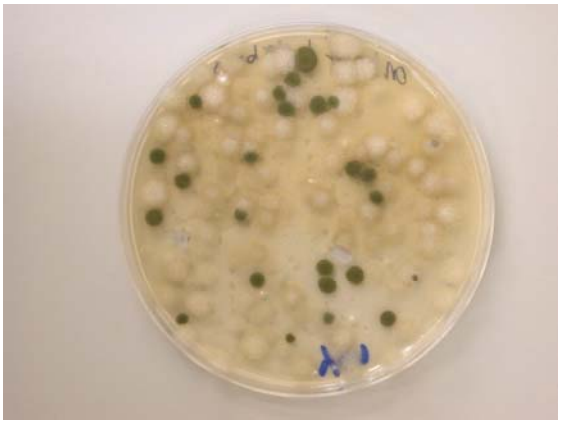

Figure 3.5: Photograph of spore trap plate showing *Cladosporium* sp. and *Geomyces* sp. collected from the air on top of the visitors table.

3.5.6.3 *Nimrod* Hut spore trap results

In 2002, YM agar was used as spore traps at 11 locations at the *Nimrod* Hut. The traps were set up inside the hut and in the Mawson's laboratory and fungi were isolated from four locations; the shelf above stores, under metal grill in entrance way, on the floor in the stores area and in Mawson's laboratory. The fungi isolated from the spore traps, in 2002, were *Cladosporium* sp., and *Geomyces* sp.

In 2003, at the *Nimrod* Hut YM, Martin's and PDA were used at 8 locations and fungi were isolated only from plates exposed in the middle of the hut and only on the general purpose agar. Figure 3.6 shows a typical YM agar spore trap plate after exposure for 30 minutes and incubation at 4°C. Outside, surprisingly, as compared to the other huts, no fungi were isolated from any of the locations with spore traps. The fungi isolated from the spore traps, in 2003, were *Cladosporium* sp., *Geomyces* sp., *Penicillium* sp. and several unidentified fungi.

In 2004, at *Nimrod* Hut YM, NaCl and Media 7 were used at 7 locations, 2 the same as 2003 and 5 new locations; fungi were isolated from two locations within the hut, a shelf in the galley area above the ham and under the window. The fungi isolated from the galley area were from Media 7 and the fungi from under the window were isolated on YM. The fungi isolated from the spore traps, in 2004, were *Cladosporium* sp., *Geomyces* sp. and two unidentified fungi. Table of location, media and whether fungi were isolated is present in Appendix 2 Tables A2.7 to A2.9.

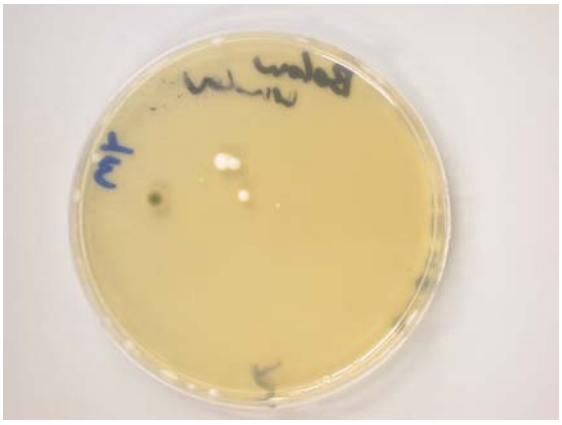

Figure 3.6: Photograph of spore trap plate showing *Cladosporium* sp. and an unidentified fungus collected from the air under the window.

3.5.7 Isolation of organisms from bait traps

Bait traps were established in 2004, at multiple locations in the *Terra Nova* Hut area, using YM and NaCl agar. Of three locations tested, two outside and one in the annex area, no fungi were isolated from the bait traps set between the latrines and the sea edge, fungi were isolated from the YM trap next to the door but not from the NaCl agar trap, and fungi were isolated from both media put in the annex area, under the spades which were hanging on the wall. Figure 3.7 shows typical growth within the bait trap and the microorganisms present when the bait trap was subcultured onto YM agar. The fungi isolated from the bait traps at *Terra Nova* Hut were unidentified. Table of location, media and whether fungi were isolated is presented in Appendix 2, Table A2.10.

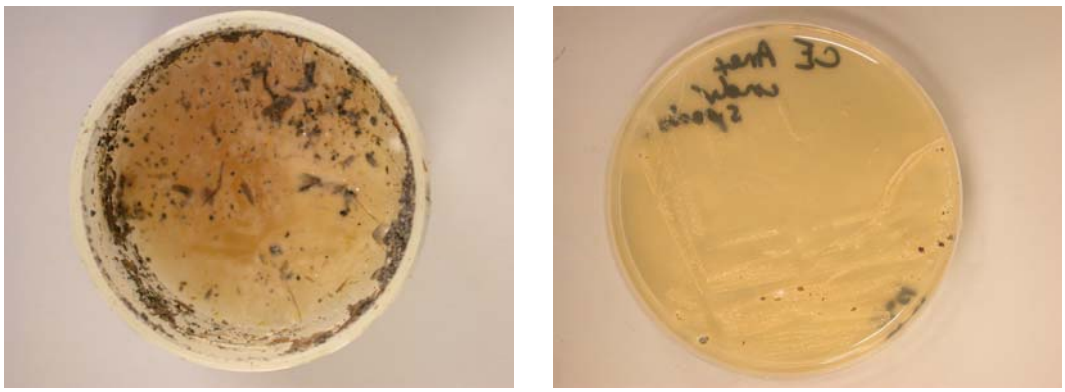

Figure 3.7: Photograph of bait traps. Left; bait trap after incubation in the laboratory at 4°C. Right; Petri plate showing isolation of organisms from bait trap.

Bait traps were established in 2004, at *Nimrod* Hut, using YM, Media 7, and NaCl agar. At *Nimrod* Hut, four locations outside were tested. No fungi were isolated from the bait traps by the meteorological station, and fungi were isolated from all three media set up in the latrine area and on YM and NaCl in the old dump, and on the YM media in front of the southern stores area. The fungi isolated from the six positive bait traps at *Nimrod* Hut were all *Geomyces* sp. and an unidentified fungus. Table of location, media and whether fungi were isolated is presented in Appendix 2, Table A2.10.

3.5.8 Isolation of organisms from culturing on location

Samples, swabs or wall scraping taken of material that appeared to have fungal contamination such as obvious fungal mould, (see Figure 3.8) were placed on agar plates.

Figure 3.8: Photograph of actively growing mould under the kitchen table in the galley area at *Terra Nova* Hut (2004).

In 2002, five sites were sampled and samples placed on YM agar and fungi were isolated from all five samples. From the swab of the dark mould below the window in *Terra Nova* Hut, a *Cladosporium* sp. and a white fungus were isolated; from in the galley area the isolate was a *Cladosporium* sp. (Figure 3.9

demonstrates the *Cladosporium* sp. from the galley area growing on YM agar) and from a second sample of the same contaminated galley area had a white fungus isolated along with the *Cladosporium* sp. From the dark room and the bunk in the laboratory area, a white fluffy filamentous fungal strain was isolated. In 2003, four swabs were plated onto YM agar in Antarctica and viable fungi grew from all four samples. These swabs were taken from the cup shelf and leg of galley table and a *Cladosporium* sp. was isolated from both. From the bunk next to the laboratory and from the dark room a white fluffy filamentous isolate along with a *Cladosporium* sp. were isolated. In 2004, eight samples were taken from the annex area, four from the stable containing butter boxes, and four from the alley way area. YM agar was used to isolate fungi from the butter box area, which included *Cladosporium* sp. and *Geomyces* sp. Media 7 was used for the alley way samples as they were all below ground but nothing was isolated from these sites. Table of location, media and whether fungi were isolated is presented in Appendix 2, Table A2.11 to A2.13.

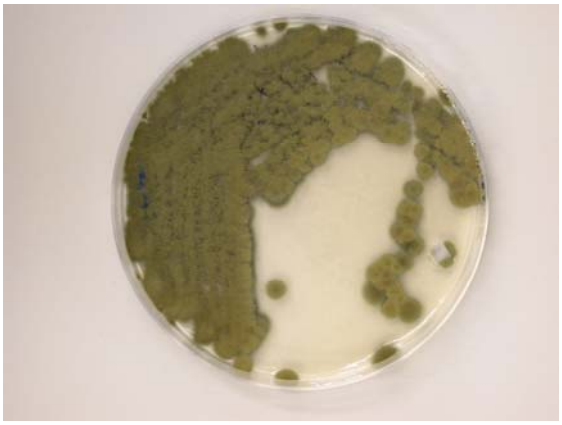

Figure 3.9: Photograph of agar plate from swabbing of wall of galley area at the *Terra Nova* Hut.

At the *Nimrod* Hut in 2002, the wall from Mawson's laboratory was scraped and the sample placed on YM agar in Antarctica and a *Geomyces* sp. was subsequently cultured and isolated. In 2003, sixteen samples were taken and plated onto YM agar, and fungi were isolated from four of the 16 samples; two samples taken from the wall next to the bacon and also from a sample of split peas which contained a *Cladosporium* sp. (Figure 3.10 demonstrates the *Cladosporium* sp. and yeast growing from the split pea sample on YM agar). The paper on the shelf next to the canvas contained two unidentified white filamentous fungi. Table of location, media and whether fungi were isolated is presented in Appendix 2, Table A2.14 and A2.15.

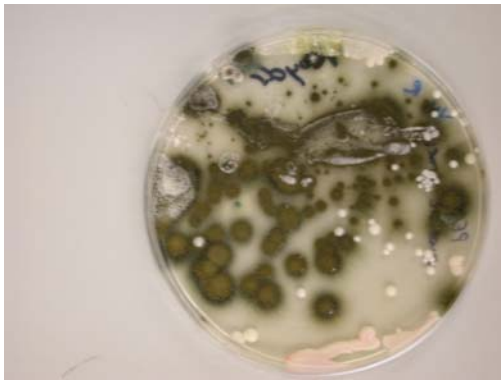

Figure 3.10: Photograph of microorganisms growing from split peas from the *Nimrod* Hut.

3.5.9 Fungal isolations from sweepings

Filamentous fungi were isolated from all sweeping samples (shown in Figure 3.11). A dark filamentous fungus was isolated from *Discovery* Hut, which was not identified. From *Terra Nova* Hut, dark fungi and two white fungi were isolated. The *Nimrod* Hut floor sweepings contained white fungi.

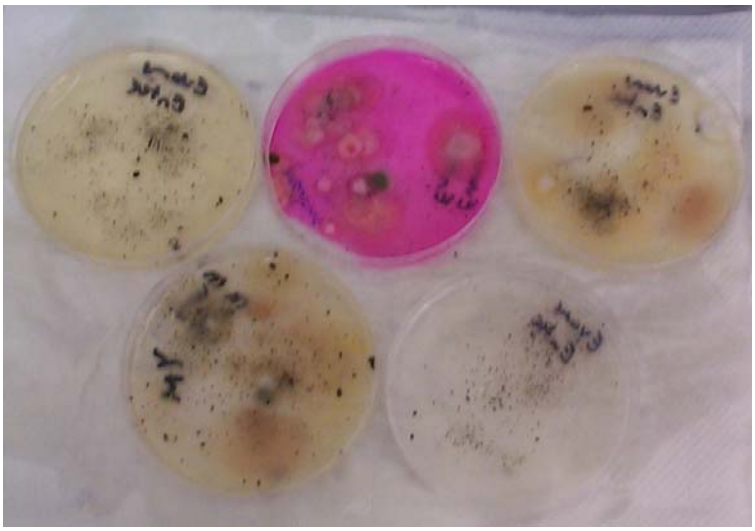

Figure 3.11: Photograph of microorganism growing from sweeping samples from entrance of the *Terra Nova* Hut.

3.5.10 Effect of media on number of samples that contained fungi

Over the six sampling Antarctic Events, seven different culture media were used to isolate fungi from physical samples and swabs. YM, a general purpose nutrient rich media, was used every year as follows: Media 7, selective for basidiomycetes; VB agar, selected for slow growing organisms by slowing the growth of faster growing fungi; Carboxymethylcellulose *Trichoderma viride* medium A, selective for cellulose degrading organisms as cellulose is the only carbon source in this medium; Acidic ME, selective for rot fungi; Media 4, selective for streptomycin resistant fungi and Media 6, selective for streptomycin

and cycloheximide resistant fungi. In January 1998, YM, Media 7, VB, Media 4 and Media 6 were used. In December 1998, YM, Media 7 and Media 4 were used to isolated microorganisms. In January 2001, YM, Media 7, Carboxymethylcellulose *Trichoderma viride* medium A and VB were used to isolate organisms. In January 2002, YM, Media 7, Carboxymethylcellulose *Trichoderma viride* medium A and VB were used to isolate microorganisms. In January 2004, YM and acidic ME agar were used to isolate fungi. Of the 719 samples taken, fungi were isolated from 308 samples on YM, 63 samples on Media 7, 155 samples on VB, 134 samples on Carboxymethylcellulose *Trichoderma viride* medium A, 56 samples on acidic ME agar, 73 samples on Media 4 and 28 samples on Media 6. Table 3.11 presents the locations, year samples were taken and number (percentage) of fungi isolated on the selection of media used in this PhD thesis research.

The significance of the efficiencies and types of fungi isolated on these various media will be discussed in Section 3.6. Location, media and whether fungi were isolated is given in Appendix 1 Tables A1.2, A1.10, A1.17, A1.20 and A1.23 from *Discovery* Hut. Tables A1.4, A1.12, A1.18, A1.21 and A1.24 from *Terra Nova* Hut. Tables A1.6, A1.14, A1.19, A1.22 and A1.25 from *Nimrod* Hut. Tables A1.8 and A1.16 additional samples of historic material found outside the historic sites or Antarctic non-historic material.

Table 3.11: Number (percentage) of fungi isolated on the selection of media used in this PhD thesis research.

Site	**Number of samples**	**YM (%)**	**7 (%)**	**VB (%)**	**CMC (%)**	**ME+ acid (%)**	**4 (%)**	**6 (%)**
Discovery Hut January 1998	17	13 (76)	3 (18)	6 (35)			8 (47)	4 (23)
Terra Nova Hut January 1998	56	25 (45)	2 (4)	18 (32)			18 (32)	18 (32)
Nimrod Hut January 1998	32	8 (25)	3 (9)	8 (25)			6 (19)	6 (19)
Other samples January 1998	15	3 (20)	0	3 (20)			5 (33)	0
Discovery Hut December1998	9	6 (67)	4 (44)				5 (55)	
Terra Nova Hut December1998	30	24 (80)	14 (47)				27 (90)	
Nimrod Hut December1998	14	4 (28)	2 (14)				3 (21)	
Other December1998	1	1 (100)	1 (100)				1 (100)	

Table 3.11: Number (percentage) of fungi isolated on the selection of media used in this PhD thesis research.

Site	Number of samples	YM (%)	7 (%)	VB (%)	CMC (%)	ME+ acid (%)	4 (%)	6 (%)
Discovery Hut January 2002	52	41 (79)	5 (10)	19 (36)	40 (77)			
Terra Nova Hut January 2002	147	66 (45)	20 (14)	72 (49)	61 (41)			
Nimrod Hut January 2002	66	17 (26)	3 (4)	14 (21)	18 (27)			
Discovery Hut January 2003	12	3 (25)	3 (25)	3 (25)	3 (25)			
Terra Nova Hut January 2003	28	14 (50)	2 (7)	5 (18)	6 (21)			
Nimrod Hut January 2003	37	4 (11)	1 (3)	7 (19)	6 (16)			
Discovery Hut January 2004	50	28 (56)				18 (36)		
Terra Nova Hut January 2004	80	40 (50)				29 (36)		
Nimrod Hut January 2004	73	11 (15)				9 (12)		
Total	719	308 (43)	63 (12)	155 (36)	134 (39)	56 (27)	73 (42)	28 (23)

3.5.11 Effect of temperature of incubation on number of samples from which fungi were isolated

From the samples taken in January 1998 and December 1998, a total of 173 were plated and cultured at three different temperatures, 4°C, 15°C and 25°C. Fungi were isolated at 4°C from 44% of the samples, 57% of the samples contained fungi capable of growth at 15°C and 68% of samples had fungi capable of growth at 25°C. From the 8 sites sampled during this comparison fewer fungi were isolated at 4°C than 15°C than 25°C at 5 sites. The percentage fungi isolated at 4°C from the 173 samples plated and cultured was statistically different from the percentage of fungi isolated at 15 °C ($p=0.015$) and from the percentage of fungi isolated at 25°C ($p=0.000$). The percentage of fungi isolated at 15 °C was statistically different from the percentage of fungi isolated at 25°C ($p=0.174$) using Fisher's exact tests.

Appendix 1, Tables A1.1 and A1.9 from *Discovery* Hut. Tables A1.3 and A1.11 from *Terra Nova* Hut. Tables A1.5 and A1.13 from *Nimrod* Hut. Tables A1.7 and A1.15 additional samples of historic material found outside the historic sites or Antarctic non-historic material.

Table 3.12: Number (percentage) of fungi that were isolated at 4, 15 and 25°C.

Sample site	Number of samples collected	4°C	15°C	25°C
Discovery Hut January 1998	17	9 (53)	10 (59)	15 (88)
Terra Nova Hut January 1998	56	20 (36)	27 (48)	42 (75)
Nimrod January 1998	32	10 (31)	14 (44)	17 (53)
Other sites January 1998	14	7 (50)	12 (86)	13 (93)
Discovery Hut December 1998	9	6 (67)	5 (55)	2 (22)
Terra Nova Hut December 1998	30	22 (73)	26 (87)	24 (80)
Nimrod December 1998	14	2 (14)	3 (21)	4 (28)
Other sites December 1998	1	1	1	1
Total	173	77 (44.5)	98 (56.6)	118 (68.2)

3.5.12 Moisture content of structural wood

Wood samples were collected in January 2003 and January 2004, from *Terra Nova* Hut and *Nimrod* Hut and in January 2004, from *Discovery* Hut, for determination of moisture content of the wood. At a number of sites around the hut wall, three samples were taken in a line from the near the ceiling (High), from the middle of the wall (Middle) and by the floor (Low). Additional random samples were also taken. Outside samples were taken above ground and below ground. Appendix 3 Tables A3.1 to A3.9 contains moisture content and location of samples.

At *Discovery* Hut, the moisture content ranged from 3 to 100% with a mean of 40%, SD± 32%. A breakdown of where samples were taken and the moisture contents in this location is presented in Table 3.13.

Table 3.13: Percent moisture in wood samples taken from *Discovery* Hut in 2004.

Location of sample	Range (%)	Mean (%)	Standard deviation (%)
Inside hut	12-95	43	29
Hut wall high	12-80	36	31
Hut wall middle	28-50	36	10
Hut wall low	12-81	48	31
Hut ceiling	75-95	87	11
Hut floor	20-22	21	2
Outside hut	3-101	35	30
Wood below ground	35-83	58	20
Wood above ground	3-101	25	30

At *Terra Nova* Hut, the moisture content ranged from 3 to 70% with a mean of 19%, SD± 18% in 2003, and 9 to 39% with a mean of 20%, SD± 7% in 2004. A breakdown of where samples were taken and the moisture contents in this location is presented in Table 3.14.

Table 3.14: Percent moisture in wood samples taken from *Terra Nova* Hut in 2003 and 2004.

Location of sample	Range (%)	Mean (%)	Standard deviation (%)
Inside hut 2003	3- 70	19	18
Hut wall high	8-14	12	3
Hut wall middle	3-16	11	6
Hut wall low	16-70	28	23
Hut ceiling	4-17	10	9
Hut floor	5-16	13	6
Inside hut 2004	9-39	20	7
Hut wall high	9-31	20	11
Hut wall middle	11-22	17	4
Hut wall low	3-70	19	18
Hut ceiling	3-98	18	26
Hut floor	13-25	19	6
Outside hut Wood above ground	8-52	28	19

At the *Nimrod* Hut, the moisture content ranged from 3 to 40% with a mean of 19%, SD± 10% in 2003 and 1 to 75% with a mean of 20%, SD± 18% in 2004. Outside samples were only taken in 2004 and were all above ground and the mean moisture content was 18% (range 11 to 30%, SD± 8%). A breakdown of where samples were taken and the moisture contents in this location is presented in Table 3.15.

Table 3.15: Percent moisture in wood samples taken from *Nimrod* Hut in 2003 and 2004.

Location of sample	Range (%)	Mean (%)	Standard deviation (%)
Inside hut 2003	3-40	19	10
Hut wall high	22-40	28	8
Hut wall middle	5-18	13	7
Hut wall low	5-21	13	7
Hut ceiling	0-33	19	17
Hut floor	12-33	20	10
Inside hut 2004	1-75	20	18
Hut wall high	4-11	9	4
Hut wall middle	23-75	45	27
Hut wall low	17-19	18	4
Hut ceiling	1-67	18	24
Hut floor	2-35	17	12
Outside Hut Wood above ground	11-30	18	8

The standard deviations for all groups of data were large, ranging from 3-39%, possibly due to the small sample size and length of time between sample collection and moisture content determination.

3.5.12.1 Culturing from samples taken for moisture content determination

In January 2004, sub samples of the samples taken for moisture content analysis (high, medium and low) taken from the wall on alleyway to kitchen area from *Discovery* Hut were cultured on YM agar and Acidic ME agar, all three samples

contained no fungi. A white unidentified fungus was isolated from the high and medium samples from the wall opposite the cooking area and no fungi were isolated from the low sample. Fungi were isolated from all three samples taken from the wall between door to main area and seal blubber, and they included *Cladosporium* sp., *Geomyces* sp. an unidentified dark fungus and an unidentified white fungus. Fungi were isolated from all three samples taken from the wall in the store room; they included *Cladosporium* sp., *Geomyces* sp., an unidentified dark fungus, *Thelebolus* sp. and an unidentified white fungus. Fungi were isolated from all three samples taken from the wall of the entrance way above the chaff pile; they included *Cladosporium* sp., *Geomyces* sp., and an unidentified white fungus. Also in January 2004, thirteen additional samples were taken outside the hut and fungi were isolated from six of them, three from the side of the hut that had the snow drift on it, two from wood from the store room/latrine side of the hut and one from the main door side of the hut. These samples contained *Geomyces* sp., *Penicillium* sp., *Thelebolus* sp., dark fungi, and white fungi. Three additional samples were taken inside the hut, from the wall above the stores, wall in pendulum room, wall above visitors table, fungi were isolated from all three samples. *Geomyces* sp., unidentified dark fungi and white fungi were isolated from these samples. Two of the three roof samples contained fungi, the roof of pendulum room and the roof of store room contained *Cladosporium* sp., an unidentified dark fungus and an unidentified white fungus. The roof samples from the cooking area contained no fungi. One of the two floor samples contained fungi, the samples from the middle of main area contained a white unidentified fungus and the samples next to sleeping platform contained no fungi. Table of location, moisture content and whether fungi were isolated is displayed in Appendix 3, Tables A3.1 and A3.2.

At the *Terra Nova* Hut, sub samples of the samples taken for moisture content analysis (high, medium and low) taken from the wall to left of main entrance above and below Anton/Dimitri bunk in January 2003, were also cultured on YM agar, media 7, VB and CMC Medium. From the three samples, *Cladosporium* sp., *Geomyces* sp. and an unidentified fungus were isolated from the low samples but no fungi were isolated from the high or medium sample. From the medium and low samples taken from the wall between the chart table and Evan's bed, *Geomyces* sp. and an unidentified fungus were isolated; no fungi were isolated

from the high sample. Fungi were isolated from all three samples taken from the wall between the biology bench and Nelson/Day's bunk, including *Penicillium* sp., *Cladosporium* sp. and an unidentified fungus. From the wall in the galley area above and below Lashly's bunk, *Cladosporium* sp. was isolated from the low sample and no fungi were isolated from the high or medium samples. From the fifth site, the store wall in the galley area, *Cladosporium* sp. was isolated from the middle samples and no fungi were isolated from the high and low samples. From the three floor samples taken, an unidentified white fungus was isolated from the floor next to the stove chimney in galley area, no fungi were isolated from the other two samples. From the three ceiling beam samples, an unidentified white fungus was isolated from the beam in the galley area; no fungi were isolated from the other two samples.

In January 2004, samples for moisture content were repeated at similar locations in the *Terra Nova* Hut, sub samples of the samples taken for moisture content analysis in January 2003 were also cultured on YM agar and acidic ME. From the two samples (medium and low) taken from the wall to left of main entrance above and below Anton/Dimitri's bunk *Cladosporium* sp. was isolated from both samples. From the medium sample taken from the wall between the chart table and Evan's bed, *Cladosporium* sp. was isolated; no fungi were isolated from the high or low sample. *Cladosporium* sp. was isolated only from the medium sample taken from the wall between the biology bench and Nelson/Day's bunk, and no fungi were isolated from the high or low samples. From the wall in the galley area above and below Lashly's bunk, *Cladosporium* sp. and an unidentified white fungus were isolated from the low sample and no fungi were isolated from the high or medium samples. From the one sample taken from the fifth site on the store wall in the galley area, *Cladosporium* sp. was isolated from the high sample. From the four floor samples taken, an unidentified white fungus was isolated from the floor to the left of main entrance below Anton/Dimitri's bunk; no fungi were isolated from the other three samples. From the four ceiling beam samples, an unidentified white fungus was isolated from the beam above Gran/Taylor's bunk and *Cladosporium* sp. from the beam over the Nelson/Day's bunk; no fungi were isolated from the other two samples. In January 2003, four additional samples were taken; two from the annex wall, and one from the darkroom and one from the frozen wood in the galley area. Fungi were isolated from all sites except the

darkroom. The annex wood contained *Geomyces* sp., *Thelebolus* sp. and unidentified white fungus, the frozen wood in the galley area contained *Cladosporium* sp. and an unidentified fungus. In 2004, a moisture content profile was taken from a new location, the entrance area, with an unidentified white fungus isolated. Six additional samples were taken from wood outside for moisture content analysis and from these six samples, fungi were isolated from three; two hut wall samples in stables area and one sample of the outside annex wall. The three samples contained *Cladosporium* sp., an unidentified white fungus, and an unidentified fungus. Ten additional samples were taken from within the hut, fungi were isolated from eight of them, the five samples that contained fungi were from areas where moisture content profile had been taken, one from the area below the window, stables side of hut, one from the tenements area and one from the floor in the entrance way. The fungi isolated from these samples included *Penicillium* sp., *Geomyces* sp., *Thelebolus* sp., unidentified white fungi, *Cladosporium* sp., and an unidentified fungus. Table of location, moisture content, and whether fungi were isolated is displayed in Appendix, Tables A3.3 to A3.5

At the *Nimrod* Hut sub samples of the samples taken for moisture content analysis in January 2003 were also cultured on YM agar, Media 7, VB and CMC Medium. No fungi were isolated from the four wall sites samples, the four floor samples or the four ceiling beam samples taken for moisture content sampling.

In January 2004, samples for moisture content were repeated at similar locations in the *Nimrod* Hut, sub samples of the samples taken for moisture content analysis in January 2004, were also cultured on YM agar and acidic ME. From the four wall site samples, the four floor samples and the four ceiling beam samples, *Cladosporium* sp. was isolated from a middle sample of the wall to the left of the stove and from low sample of the wall among the stores and an unidentified white fungus was isolated from the floor under the beds No fungi were isolated from any of the other sites. In January 2004, four additional samples were taken from the porch area, from Mawson's laboratory, from the darkroom and from Shackleton's room. No fungi were isolated from these samples. In 2004, 12 additional samples were taken from wood sampled outside for moisture content analysis and from these 12 samples, fungi were isolated from three; one hut wall sample in stables area below the ground, and two samples of hut wood from the

eastern wall among the store. The three positive samples contained *Penicillium* sp., *Thelebolus* sp., and an unidentified dark fungus. Sixteen additional samples were taken from within the hut, and fungi were isolated from none of them. Table of location, moisture content, and whether fungi were isolated is displayed in Appendix 3, Tables A3.6 to A3.8.

3.5.13 Repetitive sampling

3.5.13.1 Repeat sampling of particular areas at *Discovery* Hut which gave the same isolation results

Samples taken in 1998 of the chaff (Location 1 as given in Figure 3.11), both surface and inside the chaff, cultured a total of eight different fungi; *Cladosporium* sp., *Penicillium* sp. and *Geomyces* sp. were identified and five were unidentified. Also in 1998, samples in the interior and on the exterior surface of the mutton carcasses hanging in store room (Location 2, Figure 3.12) were taken. A *Penicillium* sp. was isolated from both samples. In 2002, swab samples were taken of the roof and wall of the latrine (Location 3, Figure 3.12). An unidentified white fungus with the same visual characteristics was isolated from both swabs. The wall behind the mutton carcasses in the store room (Location 4, Figure 3.12) was sampled twice in January 1998 and two unidentified fungi were isolated, one white fungus and one grey fungus. In December 1998, the wood under the blubber pile (Location 5, Figure 3.12), in the main area of the hut, was sampled. An unidentified dull green fungus with the same visual characteristics was isolated from both samples. The wall on the snow drift side of the hut (Location 6, Figure 3.12) was sampled twice, in 2003, and nothing was cultured from either sample.

3.5.13.2 Repeat sampling of particular areas at *Discovery* Hut which gave different isolation results

At three sites outside *Discovery* Hut, wood samples were taken at the same time from the same area above the ground and below the ground. At two sites, (Location 7 and 8, as given in Figure 3.12) both above the ground, there were no fungi isolated. At the third site, the sample hut wall NE corner (Location 9, Figure 3.12) just above the ground, an unidentified fungus was cultured and isolated. From the below ground samples, *Geomyces* sp., an unidentified dark fungus and *Thelebolus* sp. were isolated. In 2004, the wall of the cooking area (Location 10, Figure 3.12) was sampled three times; one as part of the moisture content study, a swab sample and an extra wood splinter sample was taken. Fungi were isolated

from the high and medium moisture content sample but not from the low moisture content sample, the swab or the extra wood sample. A white unidentified fungus was isolated from both the high and medium samples. The floor in the cooking area (Location 11, Figure 3.12) was sampled three times in 2002; one sample contained no fungi and two contained white unidentified fungi.

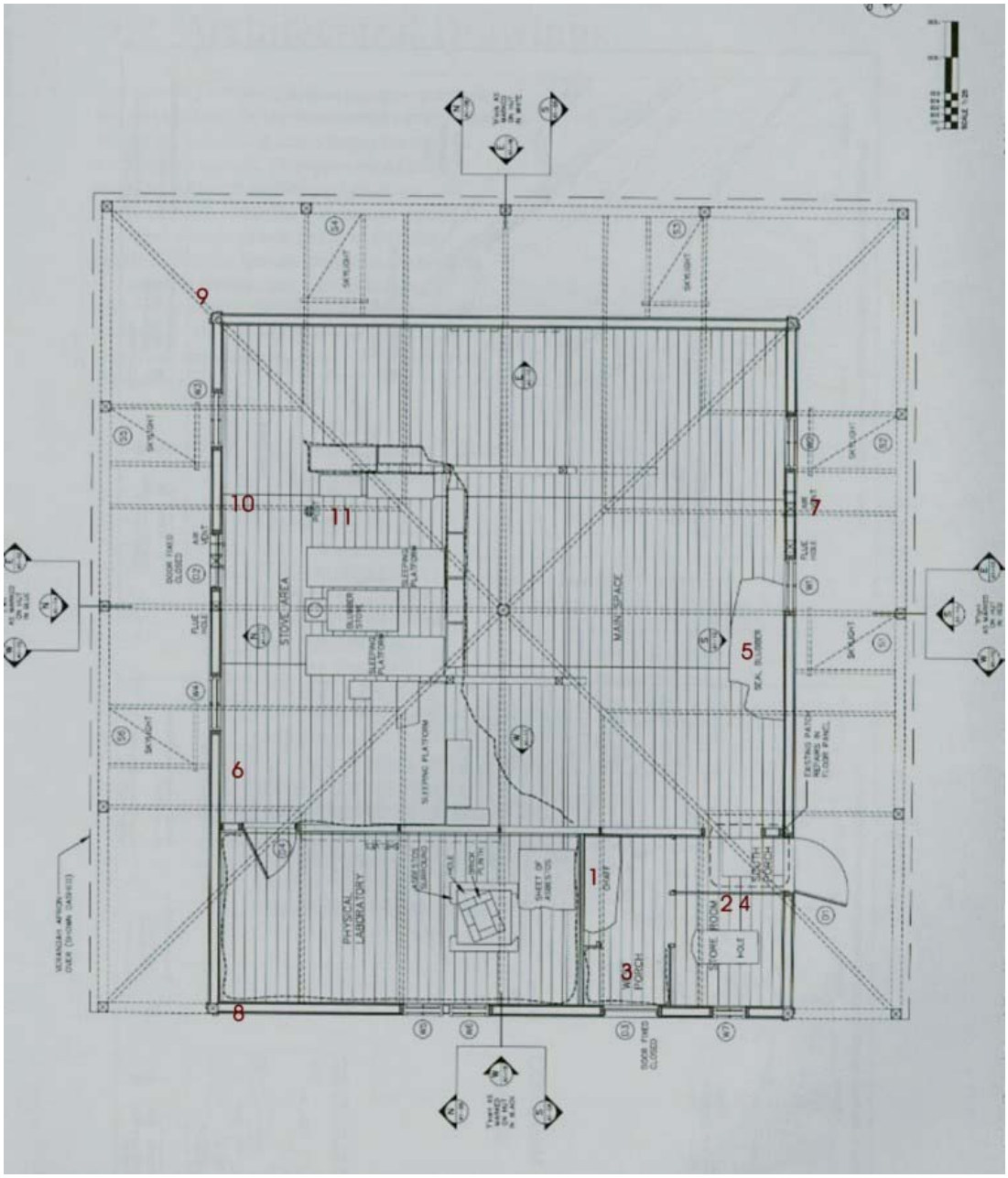

Figure 3.12: Map showing location of repetitive sampling sites at *Discovery* Hut (numbers indicate sample locations). (Source Conservation Plan *Discovery* Hut, Hut Point, 2004).

3.5.13.3 Repeat sampling of particular areas at *Terra Nova* Hut which gave the same isolation results

In 1998, wood under the Wright/Simpson bunk (Location 1, as given in Figure 3.13) was sampled twice and neither sample contained fungi. In 2002, the hut wall wood below the ground near the blubber pile (Location 2, Figure 3.13) was sampled twice, and *Penicillium* sp., *Cladosporium* sp., white fungi, and an unidentified fungus were isolated from both samples. The floor next to Scott's bed (Location 3, Figure 3.13) was sampled twice in 2004 and nothing was isolated from either sample. In 2002, the wall under the bunk in the 'tenements' area (Location 4, Figure 3.13) was sampled twice; the sampling was a swab sample and a wood sample and dark fungi with identical looking morphological features were isolated from both samples. In 2004, the floor in the main area next to the stove (Location 5, Figure 3.13), the floor between the laboratory bench (Location 6, Figure 3.13) and the bunks of Gaze/Richards (Location 7, Figure 3.13) were sampled twice and no fungi were isolated from any of the samples. The floor under the bunk of Anton/Dimitri (Location 8, Figure 3.13) was sampled twice in 2004 and white and dark fungi were isolated from both samples. The hut wall opposite stable 3 was sampled twice (Location 9, Figure 3.13); a dark fungus and a white fungus were isolated from both samples. The leg of the table in the galley area (Location 10, Figure 3.13) was swabbed three times in 2004, and *Cladosporium* sp. was isolated from all three samples. In 2004, a stores box next to the table in the galley area (Location 11, Figure 3.13) was swabbed twice and no fungi were isolated. In 2004, an unattached piece of wood just outside the hut (Location 12, Figure 3.13) was sampled twice and *Cladosporium* sp. were isolated from both samples. Two sites, with visible fungal growth below the window among the store on the stables side of the hut (Location 13 and 14, Figure 3.13), the wall in the galley area, were sampled twice and four times, in 2002, respectively. A white unidentified fungus was isolated from below the window among the stores on the stables' side of the hut and a dark fungus was isolated from all four samples taken from the galley wall. In 2002, the wooden railings in stable 7 (Location 15, Figure 3.13) were sampled twice and an unidentified white fungus was isolated from both samples. The wall next to the main door (Location 16, Figure 3.13) was sampled four times in 2002, and no fungi were isolated. In 2004, the wall next to the main door (Location 17, Figure 3.13) was sampled twice and a dark fungus, *Thelebolus* sp. and an unidentified fungus were isolated.

The annex wood near the door to the latrine (Location 18, Figure 3.13) was sampled twice in 2003, fungi were isolated from both samples, a *Geomyces* sp. and *Thelebolus* sp. were isolated. A wallet found behind a bunk (Location 19, Figure 3.13) and was swabbed inside and out in December 1998 and a variety of unidentified white fungi were isolated. A ski on the wall of the annex (Location 20, Figure 3.13) was sampled twice, in 1998, and a cream and a dull green fungus were isolated from both samples.

3.5.13.4 Repeat sampling of particular areas at *Terra Nova* Hut which gave different isolation results

At the *Terra Nova* Hut, the door step to the hut (Location 2 as given in Figure 3.13) was sampled twice, in 2002, and one sample contained a white fungal isolate and the second sample contained a dark fungal isolate. A box next to the stove was sampled twice (Location 22, Figure 3.13) in 1998. From one sample, a *Cladosporium* sp. and from the other an unidentified white fungus was isolated. A selection of boots within the hut (Location 23, Figure 3.13) were sampled both inside and outside the boot, in January 1998 (7 samples); *Cladosporium* sp., *Penicillium* sp. and a white fungus were isolated in January 1998, and in December 1998 (2 samples), *Cladosporium* sp. and *Penicillium* sp. were isolated along with a *Geomyces* sp. and two unidentified white fungi. In 2002, at nine sites, samples were taken from the same location one above the ground and one below the ground. The sample taken of the doorstep above ground (Location 24, Figure 3.13) contained no fungi and the sample below ground contained *Thelebolus* sp. and a white fungus. The sample next to the door step (Location 25, Figure 3.13) above the ground contained a white fungus, *Thelebolus* sp. and an unidentified fungus, and below the ground an unknown fungus was isolated. The annex wall along the main door side (Location 26, Figure 3.13), above ground, contained no fungi, but below the ground a *Thelebolus* sp. and an unknown fungus were isolated. The wall next to the door from the annex to the latrine (Location 27, Figure 3.13), above the ground, contained a dark fungus and below the ground, the wood contained a dark fungus, *Thelebolus* sp., and a white fungus. The annex door (Location 28, Figure 3.13), above ground sample, contained an unidentified fungus and the below the ground sample contained a white fungus, and *Thelebolus* sp. The stable door (stable 4) (Location 29, Figure 3.13), above ground, contained a dark fungus, and below ground a dark fungus, a white fungus, and *Thelebolus* sp. From the wall next to the hay (Location 30, Figure 3.13),

above the ground, nothing was isolated but below the ground, a dark fungus and a white fungus were isolated. From the wall (Barnes Glacier side of the hut) (Location 31, Figure 3.13) above the ground, nothing was isolated and below the ground a white fungus was isolated. Wood from the corner of the hut contained no fungi above the ground and the two samples taken below the ground contained *Thelebolus* sp., dark fungus, and white fungi. Within the stables area two samples (Location 32 and 33, Figure 3.13) were taken of the timber rails that divide the stables into cubicles from above and below the ground; above the ground, fungi were isolated from between stables 4 and 5 but not from stable 7. Below the ground, fungi were isolated from both sites; a pale fungus, white fungus and an unidentified fungus. The entrance way floor (Location 34, Figure 3.13) and the door frame (Location 35, Figure 35.13) were each sampled twice, in 2002, and an unidentified white fungus was isolated from one of the two samples from each site. The wall in the entrance way (Location 36, Figure 3.13) was sampled twice, in 2002, with fungi being isolated from one of the samples which contained an unidentified fungus. In 2004, the entrance way wall (Location 37, Figure 3.13) was sampled five times and fungi were isolated from two of the samples both of which contained a white fungus and a dark fungus. The wall in the darkroom (Location 38, Figure 3.13) was sampled five times, in 2002 and three times, in 2004. From the five samples taken, in 2002, a *Cladosporium* sp. and a white fungus were isolated from one sample. In 2004, a *Cladosporium* sp. was isolated from one of the samples. In 2004, the annex wall next to the door into the hut (Location 39, Figure 3.13) was sampled four times one below the ground and three above the ground; a low (just above the ground) sample, a medium (halfway up wall) sample and a high (up by the ceiling) sample. A dark fungus was isolated from the below ground sample and a *Thelebolus* sp. was isolated from the low sample and no fungi were isolated from the other samples.

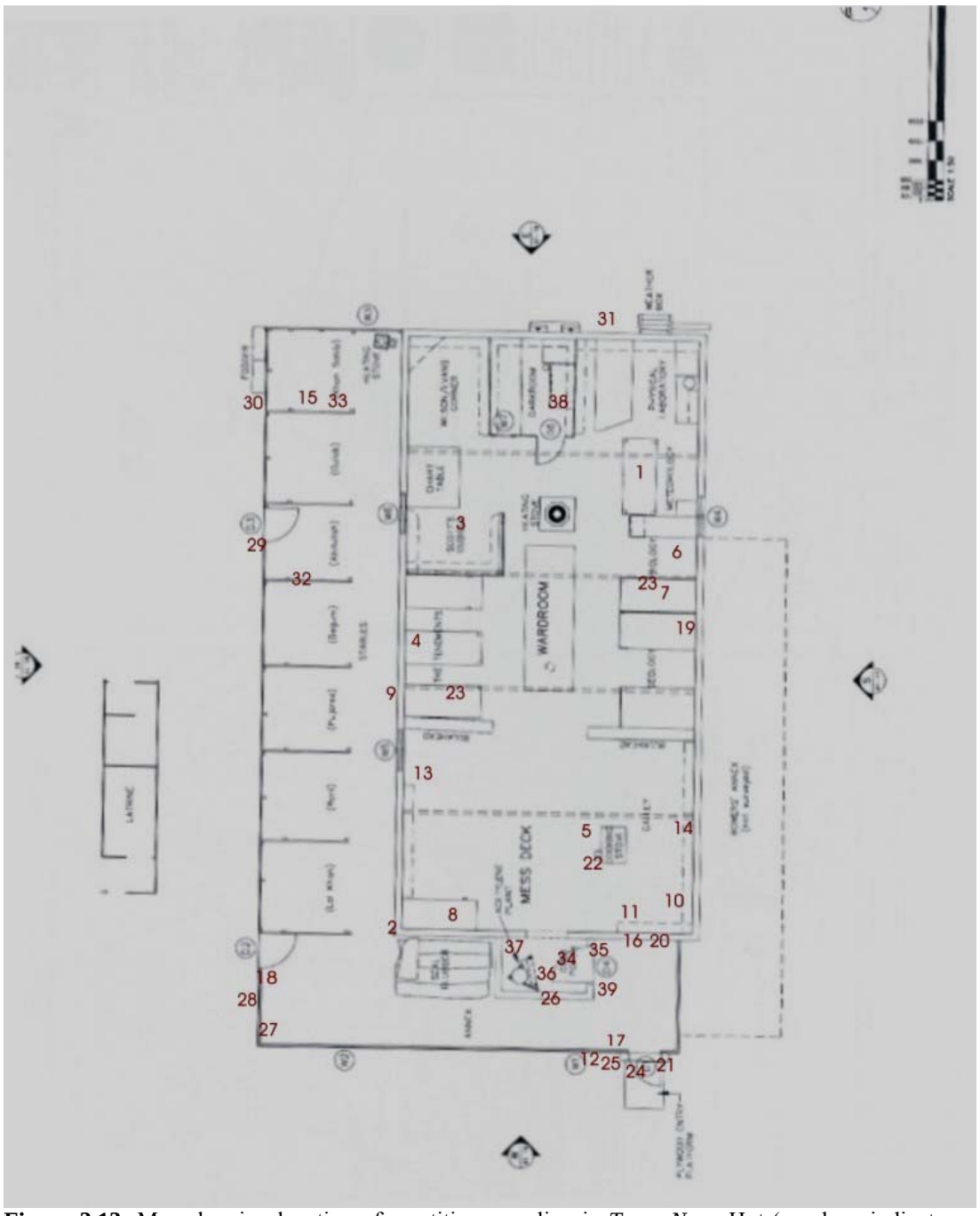

Figure 3.13: Map showing location of repetitive sampling in *Terra Nova* Hut (numbers indicate sample locations). (Source Conservation Plan, Scott's Hut Cape Evans, 2004).

3.5.13.5 Repeat sampling of particular areas at *Nimrod* Hut which gave same isolation results

At the *Nimrod* Hut, five locations (Location 1,2,3,4, and 5 as given in Figure 3.14) were sampled above and below ground. Fungi were not isolated from samples above ground but were isolated from three of the four samples taken below ground; the only location where fungi were not isolated was in the stables area. A boot liner and shoe were sampled (Location 6, Figure 3.14) inside and out, in January 1998, and *Penicillium* sp. and two different white fungi were isolated

from both samples. Two samples were taken of wood that was being stored as replacement wood (Location 7, Figure 3.14), in January 1998, and a variety of *Penicillium* sp. were isolated from both samples. The wall under the acetylene plant (Location 8, Figure 3.14) was sampled twice, in 1998, and no fungi were isolated. The wall below the window on the darkroom side of the canvas (Location 9, Figure 3.14) was sampled twice, in 2002, and no fungi were isolated from either sample. The wall in the darkroom (Location 10, Figure 3.14) was sampled twice, in 2002, and nothing was isolated from either sample. In 2004, two samples of outside wood were taken from the front of the porch area (Location 11, Figure 3.14) and nothing grew from either sample. Wood from the latrine area (Location 12, Figure 3.14) was taken twice, in 2004, and nothing was isolated from either sample. The ceiling beam next to the canvas divide (Location 13, Figure 3.14) was sampled twice, in 2004, and nothing grew from either sample. In 2002, the floor in front of the stores on the stables side of the hut (Location 14, Figure 3.14) was sampled by taking two wood samples; both contained no fungi. The wall outside Shackleton's room (Location 15, Figure 3.14) and the wall above the couch (Location 16, Figure 3.14) were sampled twice, in 2004, and fungi were not isolated from either location.

3.5.13.6 Repeat sampling of particular areas at *Nimrod* Hut which gave different isolation results

In December 1998, two samples of flour (Location 17 as given in Figure 3.14) were taken, one of which showed visible signs of fungi and one that appeared to be fungal free. The fungal free sample did not culture any fungi but the visibly contaminated sample contained a white fungus with black spores, and a dark fungus. In 2002, the floor in front of the stores on the stables side of the hut (Location 18, Figure 3.14) was sampled by taking a wood sample and a swab sample; the wood sample contained a *Geomyces* sp. and the swab sample contained no fungi. The wall below the window in the bed area of the hut (Location 19, Figure 3.14) was sampled by taking a wood sample and a swab sample. The wood sample contained no fungi but the swab sample contained a *Penicillium* sp. In 2004, the wall next to the biology laboratory (Location 20, Figure 3.14) was sampled three times and *Cladosporium* sp. were isolated from two of the three samples. The wall in Mawson's laboratory (Location 21, Figure 3.14) was sampled multiple times, in January and December 1998, and in 2004. In January and December 1998, fungi were isolated from three of the four samples; a

Penicillium sp. and an unidentified fungus. In 2004, none of the three samples produced any isolated fungi.

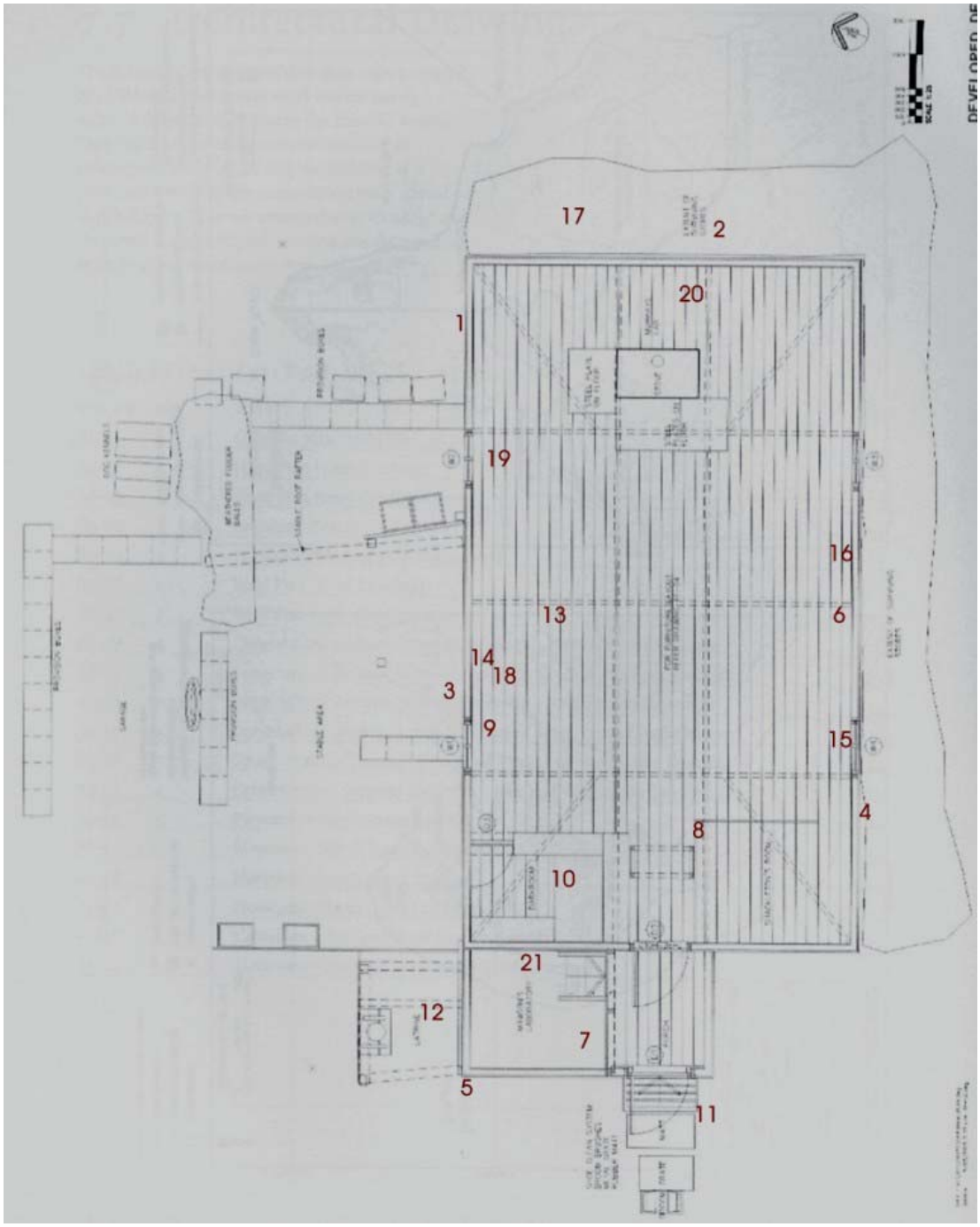

Figure 3.14: Map showing location of repetitive sampling in *Terra Nova* Hut (numbers indicate sample locations). (Source Conservation Plan, Shackleton's Hut 2003).

3.5.14 Results from sampling same sites sampled on different sampling trips

3.5.14.1 Results from sampling same sites at *Discovery* Hut sampled on different sampling trips

Inside *Discovery* Hut, seventeen sites were sampled at least twice on different sampling trips, from 11 sites fungi were isolated every time the site was sampled, 3 sites contained no fungi but on subsequent samplings fungi were isolated and from 2 sites fungi were isolated from the first sampling but not from subsequent samplings. At one site fungi were isolated from the first and third sampling but not the second. At one site on the ceiling in the store room identical fungi were contained in both samples. Five sites had less fungal species on the second or third sampling, 4 sites had the same number of fungal species (not always the same fungal species) on the second or third sampling, and 7 sites had more fungal species on the second and third sampling trips. Table 3.16 and 3.17 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.16: Location, years of sampling and fungi isolated from sampling same site on two different sampling trips inside *Discovery* Hut.

Location	Years sampled	Fungi isolated from samples taken on first sampling trip	Fungi isolated from samples taken on second sampling trip
Hole in store room floor (Figure 3.15)	1998, 2002	*Penicillium* sp., *Geomyces* sp., an unidentified dark fungus, a cream fungus	*Geomyces* sp., an unidentified white fungus
Ceiling of the store room (Figure 3.15)	2002, 2004	A dark fungus	A dark fungus
Store room wall (Figure 3.15)	2002, 2004	A white fungus	*Cladosporium* sp., *Geomyces* sp., an unidentified dark fungus, *Thelebolus* sp. and an unidentified white fungus
Wall behind the mutton carcasses in the store room (Figure 3.15)	1998, 2002	One white, one grey unidentified fungi,	No fungi were isolated
Mould on the walls and ceiling of the latrine	1998, 2002	*Penicillium* sp	An unidentified white fungus
Chaff (in the entranceway) (Figure 3.16)	January 1998, December 1998	Five unidentified fungi, *Cladosporium* sp. *Penicillium* sp., *Geomyces* sp.	Six unidentified fungi

Table 3.16: Location, years of sampling and fungi isolated from sampling same site on two different sampling trips inside *Discovery* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**
Pendulum room wall under the window	1998, 2002	*Penicillium* sp.	An unidentified white fungus
Dark wood on the ceiling main area	1998, 2002	*Penicillium* sp.	An unidentified white fungus, unidentified dark fungus
The wall between the door to the main area and the seal blubber	2002, 2004	A white unidentified fungal	*Cladosporium* sp., a *Geomyces* sp., an unidentified dark fungus, an unidentified white fungus
The wall above the AHT desk	2002, 2004	An unidentified white fungus, *Thelebolus* sp.	*Geomyces* sp.
The wall above the stores area	2003, 2004	No fungi were isolated	*Geomyces* sp., *Cladosporium* sp.
Wood under the blubber pile in the main area of the hut	1998,2002	An unidentified dull green fungus	An unidentified white fungus, *Thelebolus* sp., an unidentified fungus with white spikes.
The floor in the main area	2002, 2004	A white unidentified fungus	*Geomyces* sp

Table 3.17: Location, years of sampling and fungi isolated from sampling same site on three different sampling trips inside *Discovery* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**
Roof in the entrance way	January 1998, December 1998, 2002	An unidentified white fungus	No fungi were isolated	An unidentified white fungus, *Cladosporium* sp
Wall above the chaff (Figure 3.16)	1998, 2002, 2004	No fungi were isolated	An unidentified white fungus	*Cladosporium* sp., *Geomyces* sp., an unidentified white fungus
Pendulum room behind the door	1998, 2002 2004	No fungi were isolated	No fungi were isolated	A white unidentified fungus
Wall to the galley area	2002, 2004	A white fungus	No fungi were isolated	No fungi were isolated

Figure 3.15: Photograph of store room in *Discovery* Hut showing mutton carcasses and hole in floor. (Source Professor Robert Blanchette 1998).

Figure 3.16: Photograph of entrance to *Discovery* Hut showing chaff pile. (Source, Professor Roberta Farrell, 1998).

Outside *Discovery* Hut, 5 sites were sampled at least twice on different sampling trips, from 3 sites fungi were isolated every time the site was sampled, and from 2 sites fungi were isolated from the first sampling but not from subsequent samplings. No sites contained the identical fungi in both samples. Two sites had less fungal species on the second or third sampling, 2 sites had the same number of fungal species (not always the same fungal species) on the second or third sampling, and 1 site had more fungal species on the second and third sampling trips. Table 3.18 provides a summary of what sites outside *Discovery* Hut were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.18: Location, years of sampling and fungi isolated from sampling same site on two or three different sampling trips outside *Discovery* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**
Wood behind the seal carcass (Figure 3.17)	2002, 2003 2004	Unidentified white fungus	No fungi were isolated	No fungi were isolated
Veranda wood from above the coal pile (Figure 3.17)	2002, 2004	one white and one dark unidentified fungi	*Geomyces* sp. and an unidentified fungus	
Veranda post on the snow drift side of the hut	2002, 2004	One dark and one white unidentified fungi	*Geomyces* sp. and an unidentified fungus	
Buried board on the snow drift side of the hut	2002 ,2004	Unidentified dark fungus	*Geomyces* sp, *Penicillium* sp., *Thelebolus* sp., and an unidentified white fungus	
Wood from the corner of the hut	2002 ,2004	*Thelebolus* sp., dark fungi, white fungi	No fungi were isolated	

Figure 3.17: Photograph of under the veranda at *Discovery* Hut showing seal carcasses and coal (2006).

3.5.14.2 Results from sampling same sites at *Terra Nova* Hut sampled on different sampling trips

Inside *Terra Nova* Hut, 24 sites were sampled at least twice on different sampling trips; from 13 sites, fungi were isolated every time the site was sampled, 4 sites contained no fungi but on subsequent samplings fungi were isolated, from 5 sites fungi were isolated from the first sampling but not from subsequent samplings and from 2 sites no fungi were isolated on any sampling trip. One site the wall above and below the window between the laboratory bench and the bunk and contained the identical fungi at all samplings. Eight sites had less fungal species on the

second or third sampling, 8 sites had the same number of fungal species (not always the same fungal species) on the second or third sampling, and 8 sites had more fungal species on the second and third sampling trips. Table 3.19 and 3.20 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.19: Location, years of sampling and fungi isolated from sampling same site on two different sampling trips inside *Terra Nova* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**
Door step (Figure 3.21)	1998, 2002	*Geomyces* sp., an unidentified white fungus	A white fungus, a dark fungus
Wall in the entrance area	2002, 2004	An unidentified fungus	A white fungus and a dark fungus
Floor in the entrance way	2002,2004	An unidentified white fungus	A dark fungus and a white fungus
Wall galley area behind the table (Figure 3.18)	1998, 2004	*Cladosporium* sp., and *Cadophora malorum*	An unidentified fungus
Wall in the galley with a build up of frozen water. (Figure 3.18)	1998, 2003	*Cladosporium* sp., *Cadophora* sp.	*Cladosporium* sp. and an unidentified fungus
Wall under the bunks in the 'tenements area' (Figure 3.19)	2002, 2004	Dark fungus	No fungi were isolated
The wall between the store and window on the stable side of the hut	2002, 2004	White fungus	White fungus, an unidentified fungus
Floor in front of the stores area and next to the table with AHT sign-in book	2002, 2004	White fungus	No fungi were isolated
Floor in the main area next to the chimney was sampled in.	2003, 2004	An unidentified white fungus	No fungi were isolated
Floor between the laboratory bench and Nelson/Day bunk	2003, 2004	No fungi were isolated	No fungi were isolated
Roof beam above the galley area	2003, 2004	An unidentified fungi	No fungi were isolated
Roof beam above Gran/Taylors (Figure 3.20)	2003, 2004	No fungi were isolated	An unidentified fungi
Roof beam above the dark room (Figure 3.20)	2003, 2004	No fungi were isolated	No fungi were isolated
A box next to the stove.	January 1998, December 1998	No fungi were isolated	*Cladosporium* sp., an unidentified white fungus
Selection of boots within the hut	January 1998, December 1998	*Cladosporium* sp., *Penicillium* sp. and a white fungus	*Cladosporium* sp. and *Penicillium* sp. *Geomyces* sp. and two unidentified white fungi

Table 3.20: Location, years of sampling and fungi isolated from sampling same site on three or more different sampling trips inside *Terra Nova* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**	**Fungi isolated from samples taken on fourth sampling trip**
Galley wall next to the bed (Figure 3.18)	1998, 2002, 2003, 2004	*Penicillium* sp., *Cladosporium oxysporum*, *Cadophora malorum*, two unidentified white fungi	*Cladosporium* sp. and an unidentified fungus	*Cladosporium* sp.	*Cladosporium* sp.
The wall among stores galley area (Figure 3.18)	2002, 2003, 2004	No fungi were isolated	*Cladosporium* sp.	*Cladosporium* sp.	
Hut wall in the geology	1998, 2002, 2004	*Penicillium* sp. ,a white fungus	A white fungus, a dark fungus, an unidentified fungus	*Penicillium* sp. *Thelebolus* sp.	
Wall above and below the window between the laboratory bench and the bunk	2002, 2003, 2004	*Cladosporium oxysporum*	*Cladosporium oxysporum*	*Cladosporium oxysporum*	
The wall between the chart table and Scott's bed (Figure 3.19)	2002, 2003, 2004	White fungus	*Geomyces* sp., an unidentified fungus	*Cladosporium* sp.	
Wall above and below Anton/ Dimitri's bunk	2002, 2003, 2004	No fungi were isolated	*Cladosporium* sp.	*Cladosporium* spp.	
Wall above and below Anton/ Dimitri's bunk	2002, 2003, 2004	No fungi were isolated	*Cladosporium* sp.	*Cladosporium* spp.	
The floor next to Scott's bed and the chart table (Figure 3.19)	2002, 2003, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated	
Roof in the darkroom	1998, 2002, 2004	Fungus with white fine mycelium	Fungus with white fine mycelium	No fungi were isolated	
Wall in the darkroom. (Figure 3.21)	1998, 2002, 2004	White fungus, an unidentified, dull green, white fine mycelium fungi	White fungus, *Cladosporium* sp.	*Cladosporium* sp.	


Figure 3.18: Photographs inside *Terra Nova* Hut Left: Galley area showing supplies on the wall. Right; Galley wall showing build up of frozen water. (Source Professor Robert Blanchette, 2001).


Figure 3.19: Photographs inside *Terra Nova* Hut Left: Scott's cubicle showing chart table in right corner. Right; Tenements area. (Source Joel Jurgens, 2006).

Figure 3.20: Photograph inside *Terra Nova* Hut Main area showing main table and darkroom in the background. (Source Lisa Robson, 2006).


Figure 3.21: Photograph inside *Terra Nova* Hut Left; Inside the darkroom. Right; Annex area door to hut is on the left. (Source Lisa Robson, Professor Roberta Farrell, 2006, 2003).

Outside *Terra Nova* Hut, in the annex/stable area, 10 sites were sampled at least twice on different sampling trips; from 4 sites fungi were isolated every time the site was sampled, 4 sites contained no fungi but on subsequent samplings fungi were isolated, from 1 site fungi were isolated from the first sampling but not from subsequent samplings and from another 1 site no fungi were isolated on any sampling trip. None of the sites contained the identical fungi at all samplings. Two sites had less fungal species on the second or third sampling, 2 sites had the same number of fungal species (not always the same fungal species) on the second or third sampling, and 6 sites had more fungal species on the second and third sampling trips. Table 3.21 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.21: Location, years of sampling and fungi isolated from sampling same site on different sampling trips Annex/stables area of *Terra Nova* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**
Annex wall wood on the snow drift side of the hut (Figure 3.22)	2002, 2004	No fungi were isolated	No fungi were isolated
Wall next to the main door (Figure 3.22)	2002, 2004	No fungi were isolated	Dark fungus, *Thelebolus* sp., an unidentified fungus being isolated
Annex wall next to the door into the hut (Figure 3.21)	2002, 2004	No fungi were isolated	Dark fungus *Thelebolus* sp.
Hut wall stables area	2002, 2004	Unidentified dark fungi, white fungi, *Thelebolus* sp.	Unidentified dark fungi, white fungi
The hut wall in the annex area	2002,2004	*Thelebolus* sp.	*Thelebolus* sp., a dark fungus, a white fungus, an unidentified fungus
The doorstep into the annex	2002, 2004	*Thelebolus* sp., a white fungus	No fungi were isolated
Wood from near blubber pile	1998, 2002	No fungi were isolated	A white fungus, an unidentified fungus
Post in stable 3 stables area	1998, 2002	An unidentified white fungus	White fungus, *Thelebolus* sp.
Resin from the stables area	January 1998 December 1998	No fungi were isolated	Two white unidentified fungi, a cream fungus
Horse harness in the stable area	January 1998 December 1998	*Penicillium* sp., two white fungi	Three white fungi

Figure 3.22: Photograph of annex area in *Terra Nova* Hut looking from stable. (Source Professor Roberta Farrell, 2003).

3.5.14.3 Results from sampling same sites at *Nimrod* Hut sampled on different sampling trips

Outside *Nimrod* Hut, 5 sites were sampled at least twice on different sampling trips; from 3 sites fungi were isolated every time the site was sampled, from 1 site fungi were isolated from the first sampling but not from subsequent samplings and from 1 sites no fungi were isolated on any sampling trip. Two sites had less fungal species on the second or third sampling, 2 sites had the same number of fungal species (not always the same fungal species) on the second or third sampling, and 1 site had more fungal species on the second and third sampling trips. Tables 3.22 and 3.23 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.22: Location, years of sampling and fungi isolated from sampling same site on two different sampling trips outside *Nimrod* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**
Beans from the southern stores (Figure 3.23)	January 1998 December1998	Numerous unidentified fungi	Numerous unidentified fungi
Latrine area (Figure 2.23)	2002, 2004	No fungi were isolated	No fungi were isolated
Porch area (Figure 2.23)	2002, 2004	White fungi	*Geomyces* sp., *Penicillium* sp., *Cladosporium* sp., unidentified fungus

Table 3.23: Location, years of sampling and fungi isolated from sampling same site on three different sampling trips outside *Nimrod* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**
Stores area at the back of the hut (Figure 3.23)	2002, 2003, 2004	*Thelebolus* sp. and a dark fungus	No fungi were isolated	No fungi were isolated
Hut wall in the stables area (Figure 3.23)	2002, 2003, 2004	*Geomyces* sp., *Thelebolus* sp., a white fungus.	*Penicillium* sp., *Thelebolus* sp., dark fungi unidentified fungi.	*Thelebolus* sp., a dark fungus.

Figure 3.23: Photograph of stores around southern and eastern side of the *Nimrod* Hut. (Source, Professor Robert Blanchette 1998).

Figure 3.24: Photograph of outside *Nimrod* Hut. Left: Stables area. Right: Front of hut, latrine area is to the left.(Source Author, Professor Roberta Farrell 2006, 1998).

Inside *Nimrod* Hut, 18 sites were sampled at least twice on different sampling trips; fungi were isolated every time from none of the sites sampled, 2 sites contained no fungi but on subsequent samplings fungi were isolated, from 5 sites fungi were isolated from the first sampling but not from subsequent samplings and from 11 sites no fungi were isolated on any sampling trip. Five sites had less fungal species on the second or third sampling, 11 sites had the same number of fungal species (at *Nimrod* Hut all 11 sites had no fungi isolated from any of the

sampling events) on the second or third sampling, and 2 sites had more fungal species on the second and third sampling trips. Table 3.24 and 3.25 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.24: Location, years of sampling and fungi isolated from sampling same site on two or three different sampling trips inside *Nimrod* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**
Wall under the acetylene plant (Figure 3.26)	1998, 2002	No fungi were isolated	No fungi were isolated	
Wall behind the ham (Figure 3.26)	1998, 2003	No fungi were isolated	*Penicillium* sp.	
Wall above the couch (Figure 3.25)	2002, 2004	No fungi were isolated	No fungi were isolated	
Inside ceiling beam above Shackleton's room	2003, 2004	No fungi were isolated	No fungi were isolated	
Ceiling beam above biology laboratory	2003, 2004	No fungi were isolated	No fungi were isolated	
Ceiling beam above the canvas divide	2003 , 2004	No fungi were isolated	No fungi were isolated	
Darkroom (Figure 3.26)	1998, 2002, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated
Wall among the stores in the galley area (Figure 3.26)	1998, 2002, 2003	No fungi were isolated	No fungi were isolated	No fungi were isolated
Floor in front of the stores stable side of the hut	2002, 2003, 2004	Unidentified fungi	No fungi were isolated	No fungi were isolated
Wall behind the biology laboratory	2002, 2003, 2004	No fungi were isolated	No fungi were isolated	*Cladosporium* sp.
Wall below the windows on the stable side of the hut (Figure 3.26)	2002, 2003, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated

Table 3.24: Location, years of sampling and fungi isolated from sampling same site on two or three different sampling trips inside *Nimrod* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**
Floor in the corner under the bed	2002, 2003, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated
Floor in front of the doorway to Shackleton's room	2002, 2003, 2004	Unidentified fungi	No fungi were isolated	No fungi were isolated
Floor in front of the stores stable side of the hut (Figure 3.26)	2002, 2003, 2004	Unidentified fungi	No fungi were isolated	No fungi were isolated
Floor in front of the stove	2002, 2003, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated
Darkroom floor (Figure 3.26)	2002, 2003, 2004	Unidentified fungi	No fungi were isolated	No fungi were isolated
Shackleton's room	1998, 2002, 2004	No fungi were isolated	No fungi were isolated	No fungi were isolated

Table 3.25: Location, years of sampling and fungi isolated from sampling same site on four or more different sampling trips inside *Nimrod* Hut.

Location	**Years sampled**	**Fungi isolated from samples taken on first sampling trip**	**Fungi isolated from samples taken on second sampling trip**	**Fungi isolated from samples taken on third sampling trip**	**Fungi isolated from samples taken on fourth sampling trip**	**Fungi isolated from samples taken on fifth sampling trip**
Mawson's laboratory	January 1998, December 1998, 2002, 2003, 2004	*Penicillium* sp., an unidentified fungus	*Penicillium* sp., an unidentified fungus	White fungus	White fungus	No fungi were isolated
Wall outside Shackleton' s room	1998, 2002, 2003 2004	*Penicillium* sp., an unidentified green fungus	No fungi were isolated	No fungi were isolated	No fungi were isolated	

Figure 3.25: Photograph of wall above couch inside *Nimrod* Hut (Source Dr Jo Thwaites 2004).

Figure 3.26: Photograph inside *Nimrod* Hut. Left: Stores wall, hams are hanging in the centre of Figure. Right: Wall on stables side of hut door on left leads out of the hut (acetylene plant is above this doorway), door on right leads to darkroom (2001).

3.5.14.4 Results from sampling same above ground and below ground sites on different sampling trips

Wood from above and below the ground was sampled from all three huts on a variety of sampling trips. One site at *Discovery* Hut was sampled and both above ground samples contained no fungi while the below ground sample contained fungi in the first sampling but nothing was isolated from the second sampling. Table 3.26 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Four sites at *Terra Nova* Hut were sampled on multiple sampling trips, one site above ground contained no fungi at both sampling times, one site contained more fungi, the second sampling and one contained less fungi on the second sampling and one site was only sampled above ground once. Below the ground samples, from the four sites sampled, only one was sampled multiple times and less fungal species were isolated from the second sampling trip. Table 3.26 provides a

summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.26: Location, years of sampling and fungi isolated from sampling taken above and below the ground from same site on different sampling trips at *Discovery* Hut and *Terra Nova* Hut.

Hut	**Location**	**Year**	**Number of samples taken above ground**	**Fungi isolated**	**Number of samples taken below ground**	**Fungi isolated**
Discovery Hut	Wood from the corner of the hut	2002	1	No fungi were isolated	2	*Thelebolus* sp., dark fungi, white fungi
Discovery Hut	Wood from the corner of the hut	2004	1	No fungi were isolated	1	No fungi were isolated
Terra Nova Hut	Annex wall next to the door into the hut	2002	1	No fungi were isolated		Not sampled
Terra Nova Hut	Annex wall next to the door into the hut	2004	3	*Thelebolus* sp.	1	Dark fungus
Terra Nova Hut	The hut wall in the stables area	2002	5	Unidentified dark fungi, white fungi and *Thelebolus* sp.	2	Unidentified dark fungi, white fungi and *Thelebolus* sp.
Terra Nova Hut	The hut wall in the stables area	2004	2	unidentified dark fungi and white fungi	1	Unidentified dark fungi and white fungi
Terra Nova Hut	Hut wall in the annex area	2002		Not sampled	1	Unidentified dark fungi and white fungi
Terra Nova Hut	Hut wall in the annex area	2004	2	*Thelebolus* sp., a dark fungus, a white fungus and an unidentified fungus.		Not sampled
Terra Nova Hut	Doorstep into the annex	2002	2	No fungi were isolated	1	*Thelebolus* sp. and a white fungus
Terra Nova Hut	Doorstep into the annex	2004	1	No fungi were isolated		Not sampled

Four sites at the *Nimrod* Hut were sampled multiple times on different sampling trips. No fungi were isolated from the samples taken above the ground at any of the 4 sites sampled. Below the ground at one site, the hut wall behind the stables area, had the same fungal species isolated from three sampling trips. From one site no fungi were isolated from any of the sampling trips, one site had more fungal species in the second sampling trip and one site which was sampled three times had more fugal species on the second sampling and less on the third sampling. Table 3.27 provides a summary of what sites were repeat sampled, when and what fungi were isolated at each sampling.

Table 3.27: Location, years of sampling and fungi isolated from sampling taken above and below the ground from same site on different sampling trips at *Nimrod* Hut.

Hut	**Location**	**Year**	**Number of samples taken above ground**	**Fungi isolated**	**Number of samples taken below ground**	**Fungi isolated**
Nimrod Hut	The hut wall behind the stables	2002	1	No fungi were isolated	1	*Thelebolus* sp., white fungus
Nimrod Hut	The hut wall behind the stables	2003	1	No fungi were isolated	2	*Thelebolus* sp., white fungus
Nimrod Hut	The hut wall behind the stables	2004		Not sampled	2	*Thelebolus* sp., white fungus
Nimrod Hut	The hut wall in the stables area	2002	2	No fungi were isolated	2	*Geomyces* sp., *Thelebolus* sp., white fungus
Nimrod Hut	The hut wall in the stables area	2003	4	No fungi were isolated	4	*Penicillium* sp., *Thelebolus* sp., dark fungi, unidentified fungi.
Nimrod Hut	The hut wall in the stables area	2004	3	No fungi were isolated	3	*Thelebolus* sp., dark fungus
Nimrod Hut	The latrine area	200	1	No fungi were isolated	2	No fungi were isolated
Nimrod Hut	The latrine area	2004	1	No fungi were isolated	1	No fungi were isolated
Nimrod Hut	The porch area	2002	1	No fungi were isolated	3	White fungi
Nimrod Hut	The porch area	2004	2	No fungi were isolated	2	*Geomyces* sp., a *Penicillium* sp., *Cladosporium* sp.,unidentified fungus

3.5.15 Classical mycology and molecular identification of fungi

Classical mycological techniques were used to identify 643 fungal isolates (55% of the total number of filamentous fungi in The University of Waikato Fungal Culture Collection) to genera level. Of the 643 that were identified, 35% were assigned to the genus *Geomyces* sp., 28% were assigned to the genus *Cladosporium* sp., 23% were assigned to the genus *Penicillium* sp., 10% were assigned to the genus *Thelebolus* sp. and 3% were assigned to the genus *Cadophora* sp.. Many of the fungal isolates could not be identified due to not producing sexual structures. An example of the microscopic structure of the five most dominant genera *Penicillium* sp. (Figure 3.27), *Cladosporium* sp. (Figure 3.28), *Cadophora* sp. (Figure 3.29), *Geomyces* sp. (Figure 3.30), and *Thelebolus* sp. (Figure 3.31) are shown.

The structure for a *Penicillium* sp. is described as septated mycelium with erect sepatate condiphore which branches at the apex, penicillate, ending in phialides, conidia are 1 celled, mostly ovoid in shape and in basipetal chains.

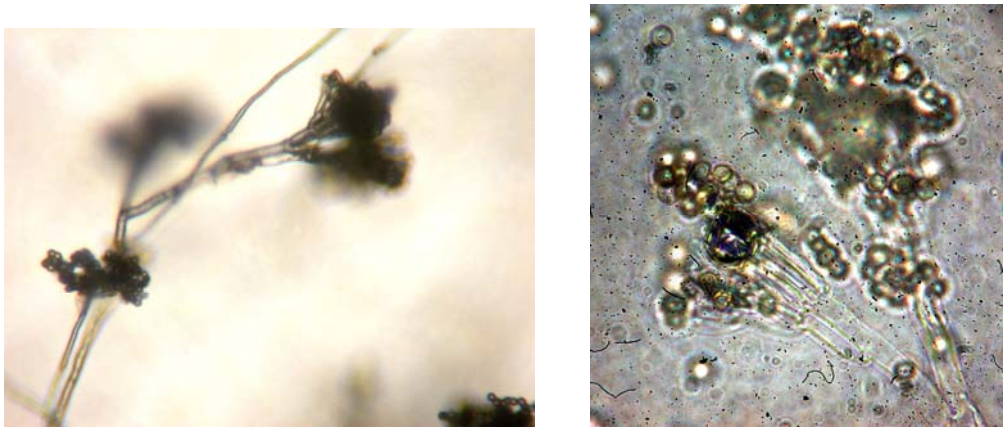

Figure 3.27: Photograph of the structures of *Penicillium* sp. 405 Left: the structure of *Penicillium* sp. 405 under 200 x magnifications. Right: the structure of *Penicillium* sp. 405 under 400x magnification.

The structure for a *Cladosporium* sp. is described as dark septated mycelium with erect dark sepatate condiphore, dark ovoid conidia are in acropetal chains.

Figure 3.28: Photograph of the structures of *Cladosporium* sp. 805 Left: the structure of *Cladosporium* sp. 805 under 200 x magnifications. Right: the structure of *Cladosporium* sp. 805 under 400x magnification.

The structure for a *Cadophora* sp. is described as dark mycelium with phialides clustered on a condiophore. Phialospores are produced endogenously and are single celled ovoid in shape.

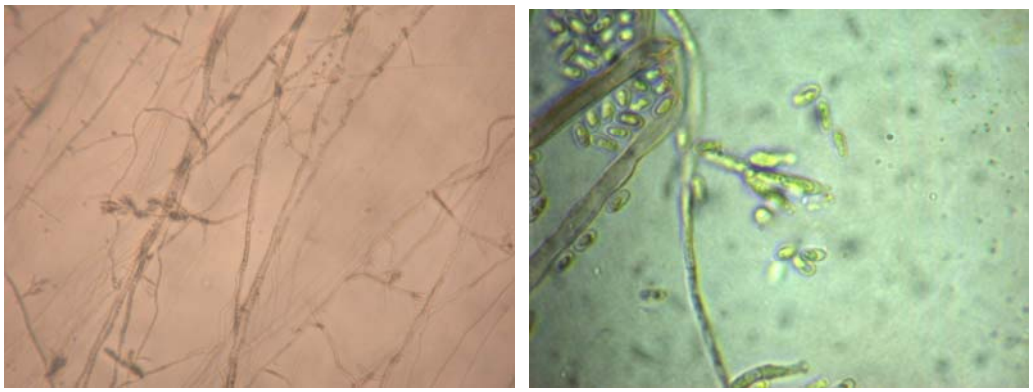

Figure 3.29: Photograph of the structures of *Cadophora* sp. 242 Left: the structure of *Cadophora* sp. 242 under 200 x magnifications. Right: the structure of *Cadophora* sp. 242 under 400x magnification.

The structure for a *Geomyces* sp. is described as mycelium with erect condiphore, with conidia on the tip which are pyriform in shape.

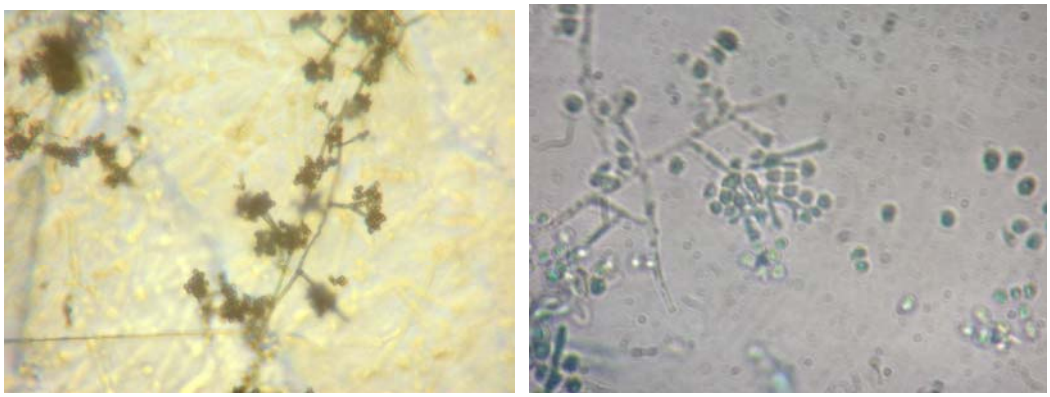

Figure 3.30: Photograph of the structures of *Geomyces* sp. 711 Left: the structure of *Geomyces* sp. 711 under 200 x magnifications. Right: The structure of *Geomyces* sp. 711 under 400x magnification.

The structure for a *Thelebolus* sp. is described as mycelium with ascomata containing asci. The ascospores are seen free around the slide.

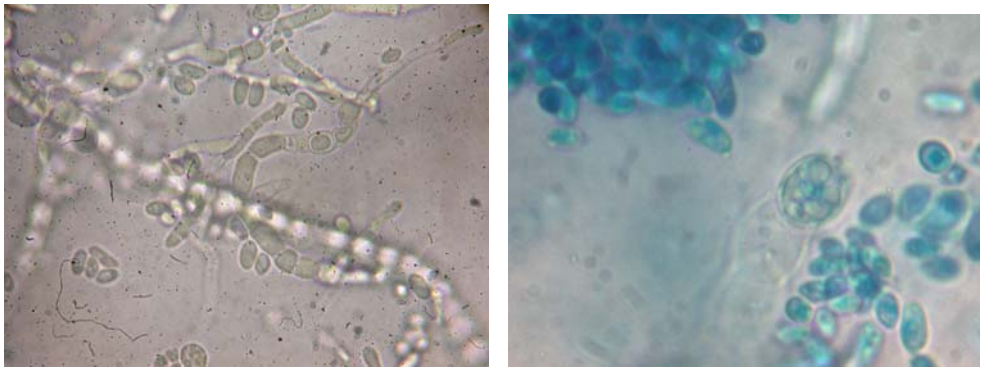

Figure 3.31: Photograph of the structures of *Thelebolus* sp. 4163 Left: The structure of *a Thelebolus* sp. 4163 under 200x magnification Right: The structure of *Thelebolus* sp. 4163 under 400x magnification.

Fungal isolates from structural wood relevant to the findings that were written up in the manuscript published in *Environmental Microbiology* as "Endoglucanase producing fungi isolated from Cape Evans Historic Expedition Hut on Ross Island, Antarctica" were initially identified by structural morphology and verified by DNA molecular methods (Duncan *et al*., 2006). In addition eighteen isolates which had been isolated from sampling at *Nimrod* and *Discovery* Huts were also identified by DNA molecular techniques.

Appendix 6 contains results of BLAST search of DNA sequences for potential matches with the DNA database.

The 33 fungal isolates identified by molecular techniques were as follows:

- Six *Cadophora malorum* (str166) came from two samples of the southern wall inside the *Terra Nova* Hut, from a Heroic Era unattached post outside at the *Discovery* Hut, and outside in the stables are at the *Nimrod* Hut.
- *Cadophora luteo-olivacea* strain A171 came from the stables area at the *Nimrod* Hut.
- *Penicillium* sp. came from a spore trap set up in the darkroom inside the *Nimrod* Hut.
- Three *Penicillium roquefortii* came from exterior wood from the *Terra Nova* Hut that had been removed and stored in a container at Scott Base and from the stables area at the *Nimrod* Hut.
- The *Penicillium solitum* strain FRR 1 came from the wall of the *Terra Nova* Hut near the floor and under a bunk.

- Two *Penicillium expansum* strain ATCC came from two samples inside the *Terra Nova* Hut, the roof of the dark room and the wall of the hut next to boxes.
- Seven *Cladosporium oxysporum* came from soil near a veranda post at *Discovery* Hut and from a variety of locations within the *Terra Nova* Hut, galley wall, wall under a bunk, roof of the darkroom and a spore trap set up in the 1st stable on top of the butter boxes.
- Six *Geomyces* sp. C239/10G came from soil near a veranda post at *Discovery* Hut, the southern wall and the door step inside the *Terra Nova* Hut, from a spore trap set up above the hams inside the *Nimrod* Hut and from a bait trap set up in the old dump at the *Nimrod* Hut.
- All 6 unidentified fungi were from the genus *Pseudodeurotium* but all had percent matches below 95%. They came from a spore trap set up in the main area at the *Discovery* Hut, the southern wall, the door step inside, a spore trap set up next to the blubber in the annex area in the *Terra Nova* Hut and the outside hut wall in the stables area at the *Nimrod* Hut.

One of the 33 fungal isolates was isolated at 25°C, 9 at 15°C and 23 at 4°C. Fourteen of the 33 fungi were isolated on YM agar, 1 on Media 7, 5 on VB agar, 7 on Media 4 and 3 on Media 6.

3.5.16 Air sampling

From six sites at Cape Hallett, eleven sites at Hut Point, twenty five sites at Cape Evans and nineteen sites at Cape Royds, air samples were taken onto YM or CMC Medium (CMC) or Avicel *Trichoderma viride* medium A (Avicel) using the Merck MAS-100 Eco®. The volume of air collected over the surface of the agar plate ranged from 50 to 500 litres at a rate of 100 litres per minute.

3.5.16.1 Air sampling Cape Hallett

Cape Hallett, is ASPA 106, containing a penguin colony, nesting skua, moss beds and lichen along with the abandoned Cape Hallett station a joint United States/New Zealand research station, (occupied from 1956 to 1973). Cape Hallett was also the location of the first LGP study site and for the period 2003 to 2005 there was a semi permanent New Zealand camp situated there. A total of twelve air samples were taken at Cape Hallett from six sites (Location of the six sites are shown in Figure 3.33), nine outside in the open environment and three inside the tents of the camp. The average of colony forming units per m^3 (CFU/m^3) outside

was 13 with a range of 0 to 40 on all media used. Samples taken outside onto YM agar had an average of 13 CFU/m^3, when sampled onto CMC or Avicel, the average was 11 CFU/m^3 and 17 CFU/m^3 respectively. Figure 3.32 shows the air sample collection over the moss bed at Cape Hallett. From the three samples taken within the polar haven tent and a polar tent, the average was 31 CFU/m^3, with a range from 10 to 75 (Table 3.28 shows the exact numbers of CFU/m^3 at each site and for each sample).

Outside the largest numbers of fungal propagules were recorded in the moss beds on the rocky scree slope behind the camp and inside, it was in the kitchen/dining room polar haven tent.

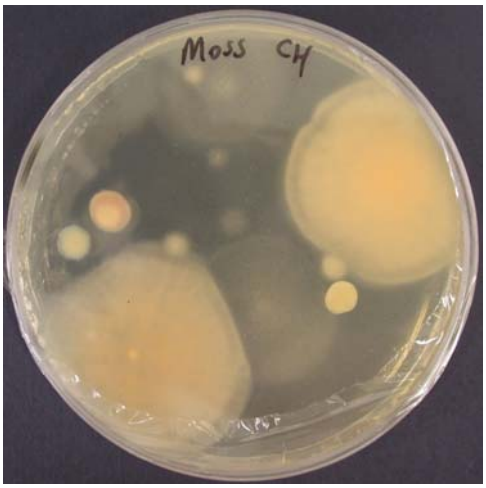

Figure 3.32: Photograph of agar plate from air sample taken from within the moss beds at Cape Hallett, media YM. The fungi are 2 *Thelebolus* sp., 4 *Geomyces* sp. and 2 white spreading fungi.

The fungi isolated from Cape Hallett outside in the environment were *Cladosporium* sp., *Geomyces* sp., *Thelebolus* sp., a white spreading fungus, and a white fungus. Inside the two tents the fungi were *Cladosporium* sp. and *Geomyces* sp.

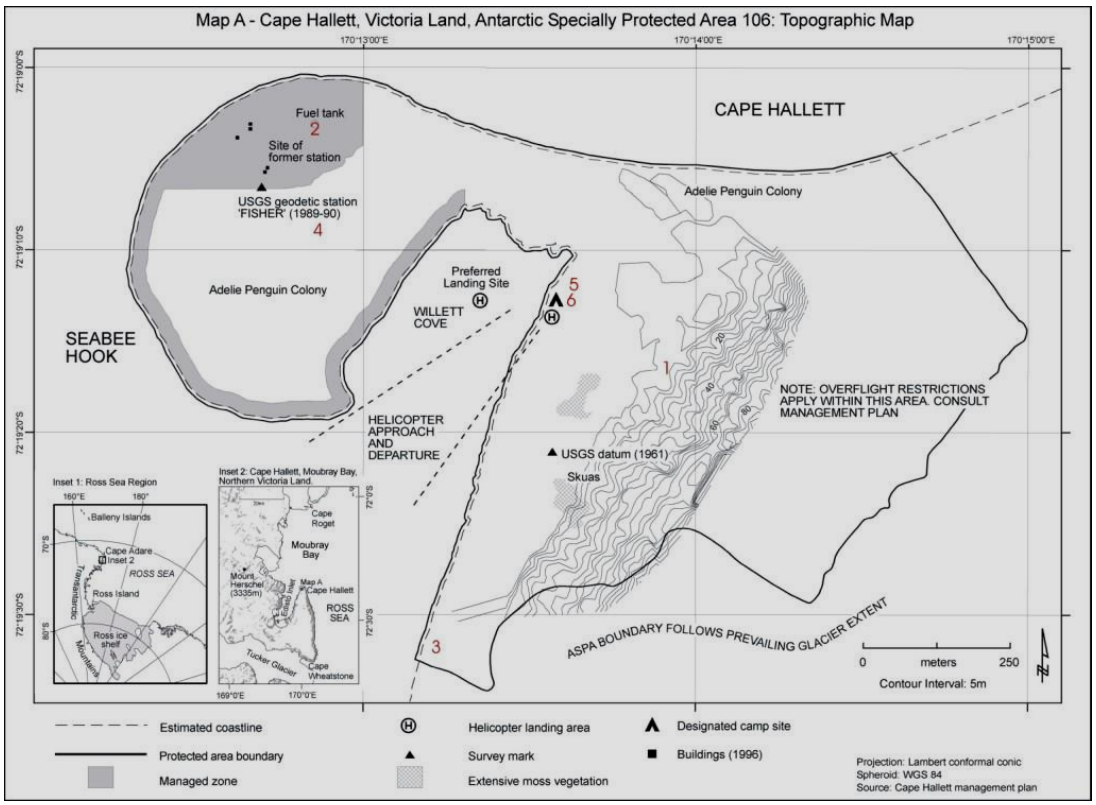

Figure 3.33: Map showing location of air sampling sites at Cape Hallett. (Source Cape Hallett Management Plan)

Table 3.28: Colony forming units per m^3 for air sample taken at Cape Hallett. Media used YM - Yeast Extract, CMC - Carboxymethylcellulose *Trichoderma viride* medium A and Avicel - Avicel *Trichoderma viride* medium A.

Location Number	Sample location	Volume of air sample	Media	Total colony forming units (CFU/m^3)	Fungal CFU/m^3
1	Within a moss bed	200	YM	60	40
1		200	Avicel	110	35
1		200	CMC	40	25
2	Remediation area	500	CMC	0	0
3	Away from campsite and other areas of visible life	500	YM	0	0
3		500	CMC	0	0
4	Within the penguin colony	200	YM	5	0
4		200	Avicel	5	0
4		200	CMC	890	20
5	Polar tent showing mould growth	200	YM	15	10
6	Haven tent used for kitchen and dining area	200	YM	10	10
6		200	CMC	80	75

3.5.16.2 Air sampling Hut Point

At Hut Point, from the eleven sites sampled (Location of the eleven sites are shown in Figures 3.35 and 3.36), a total of twenty samples were taken, six outside and 14 within *Discovery* Hut. The six samples from outside had an average of 4 CFU/m^3, with a range from 0 to 15. Inside *Discovery* Hut, the average was 1,930 CFU/m^3 for the fourteen samples taken, with the range from 0 to greater than 26,800 (Table 3.29 show the exact numbers of CFU/m^3 at each site and for each sample).

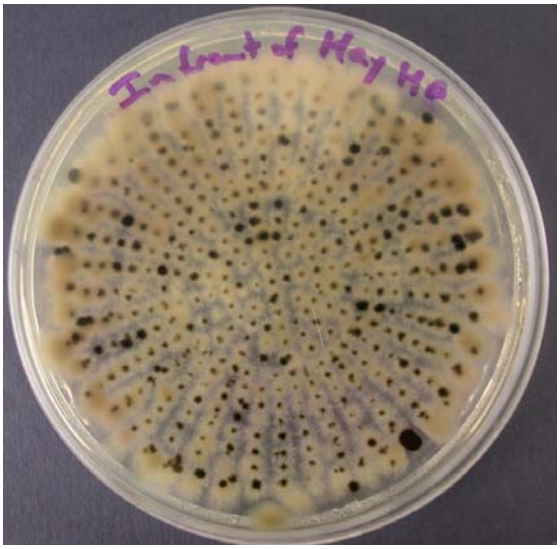

Figure 3.34: Photograph of agar plate from air sample taken from in front of the chaff inside *Discovery* Hut, media YM. . The fungi are *Cladosporium* sp. and *Geomyces* sp..

Samples taken onto YM agar had an average of 3350 CFU/m^3, while onto CMC and Avicel, the average were 73 CFU/m^3 and 133 CFU/m^3, respectively. Outside the largest number of fungal propagules was recorded next to the main door, while inside, the highest number of fungal propagules was recorded in front of the chaff in the entrance way (Figure 3.34). The fungi isolated from the air sampling outside Hut Point were *Cadophora* sp., *Cladosporium* sp., *Geomyces* sp. and unidentified white fungi. Inside *Discovery* Hut, the fungi isolated were *Cladosporium* sp. *Geomyces* sp., *Thelebolus* sp., and white fungi.

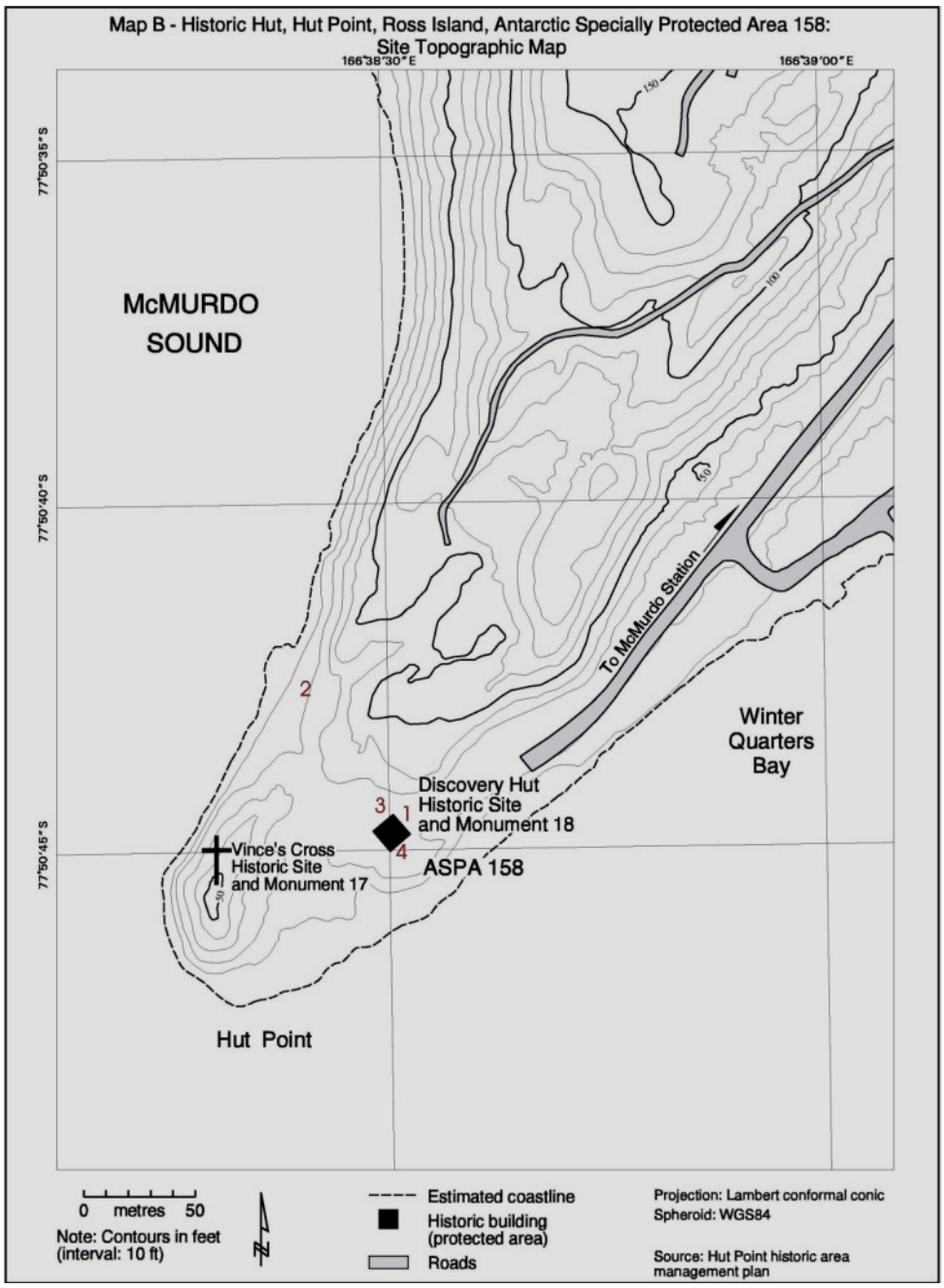

Figure 3.35: Map showing location of air sampling sites at Hut Point. (Source, Hut Point Historic Area Management Plan).

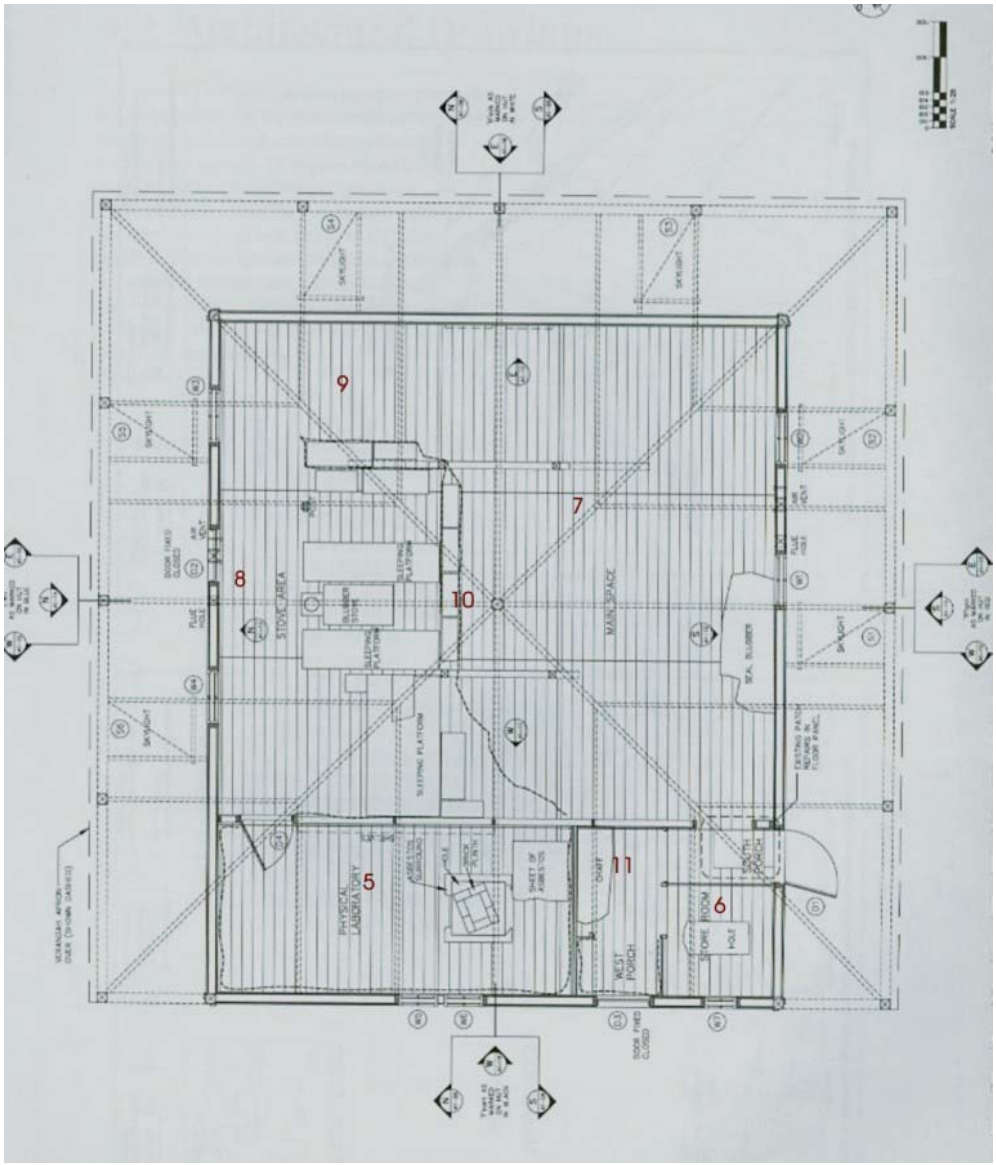

Figure 3.36: Map showing location of air sampling sites inside *Discovery* Hut. (Source, Conservation Plan *Discovery* Hut, Hut Point, 2004).

Table 3.29: Colony forming units per m^3 for air sample taken at Hut Point. Media used YM - Yeast Extract, CMC - Carboxymethylcellulose *Trichoderma viride* medium A and Avicel - Avicel *Trichoderma viride* medium A.

Location number	Sample location	Volume of air sample	Media	Total colony forming units (CFU/m^3)	Fungal CFU/m^3
1	On top of commemorative rock	200	YM	0	0
2	Away from hut, by biodiversity study site	200	YM	20	0
3	Outside on snow drift on side of hut	500	YM	156	0
4	Outside next to main door	200	YM	55	10
4		200	CMC	15	15
4		200	Avicel	0	0

Table 3.29: Colony forming units per m^3 for air sample taken at Hut Point. Media used YM - Yeast Extract, CMC - Carboxymethylcellulose *Trichoderma viride* medium A and Avicel - Avicel *Trichoderma viride* medium A.

Location number	Sample location	Volume of air sample	Media	Total colony forming units (CFU/m^3)	Fungal CFU/m^3
5	Inside in the pendulum room	100	YM	10	5
5		100	Avicel	0	0
5		100	CMC	10	10
6	Inside in meat room	100	YM	320	170
6		100	Avicel	0	0
6		100	CMC	420	170
7	Inside main room	100	YM	80	30
7		100	Avicel	40	40
7		100	CMC	40	40
7		500	YM	126	62
8	Inside near cooking area	100	YM	20	20
9	Inside in passageway to cooking area	100	YM	340	130
10	Inside among the stores in main area	100	YM	140	90
11	Inside in front of hay in entrance way	100	YM	>26,280	>26,280

3.5.16.3 Air sampling Cape Evans

At Cape Evans, from the 25 sites sampled from, a total of thirty three samples were taken, thirteen outside the hut and twenty inside the *Terra Nova* Hut (Location of the twenty five sites are shown in Figures 3.38-3.40). The average fungal CFU/m^3 from outside the hut was 7 with a range for the 13 samples of 0 to 60. When the outside samples were separated into two groups, away from the hut and around the hut, the average was 15 $CFU/m^3$and 4 CFU/m^3, respectively. The largest numbers of fungal propagules were recorded in the outside environment was in the samples taken from the wannigan and from in front of the hay bales. Inside the *Terra Nova* Hut, the average was 1680 CFU/m^3, with a range from 165 to greater than 13,140. The average when the collection was onto YM agar was 2,120 CFU/m^3 and when CMC or Avicel were used the average was 853 CFU/m^3 and 865 CFU/m^3 respectively. (Table 3.30 show the exact numbers of CFU/m^3 at each site and for each sample).

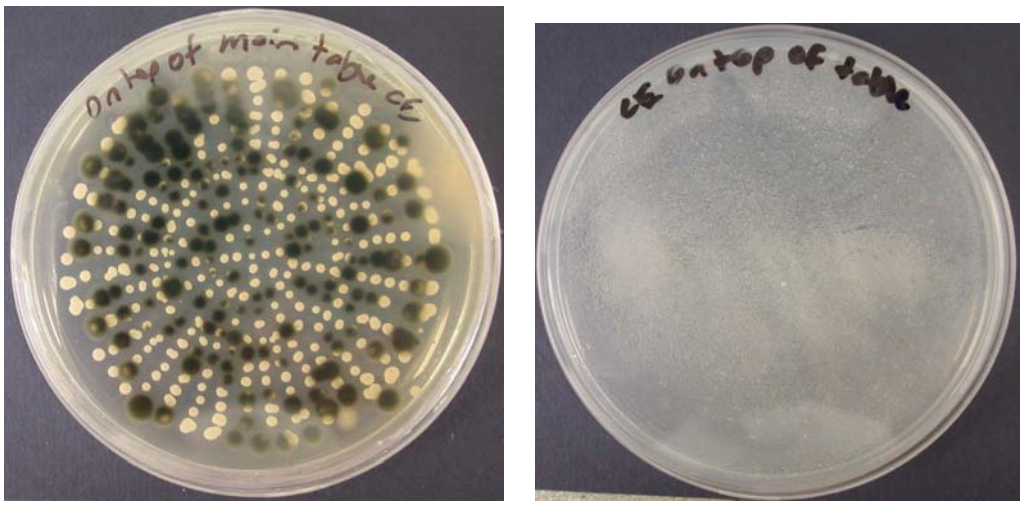

Figure 3.37: Photograph of agar plate from air sample taken on top of main table inside *Terra Nova* Hut, media YM on the left, Avicel on the right. . The fungi on media YM are *Cladosporium* sp. and *Geomyces* sp.. The fungi on the Avicel plate *Cladosporium* sp..

The highest numbers of fungal propagules inside the hut were recorded from the sample taken under the bunk opposite the laboratory area but significant numbers of fungal propagules were recorded from samples taken from the galley area. Figure 3.37 shows the levels detected from a sample taken in the middle of the hut with both YM agar and Avicel agar. Outside in the Cape Evans area, the fungi isolated were *Cladosporium* sp., *Geomyces* sp., *Thelebolus* sp. and white fungi. Inside the *Terra Nova* Hut the fungi isolated were *Cladosporium* sp., *Geomyces* sp. an unidentified white fungi, and pink fungi.

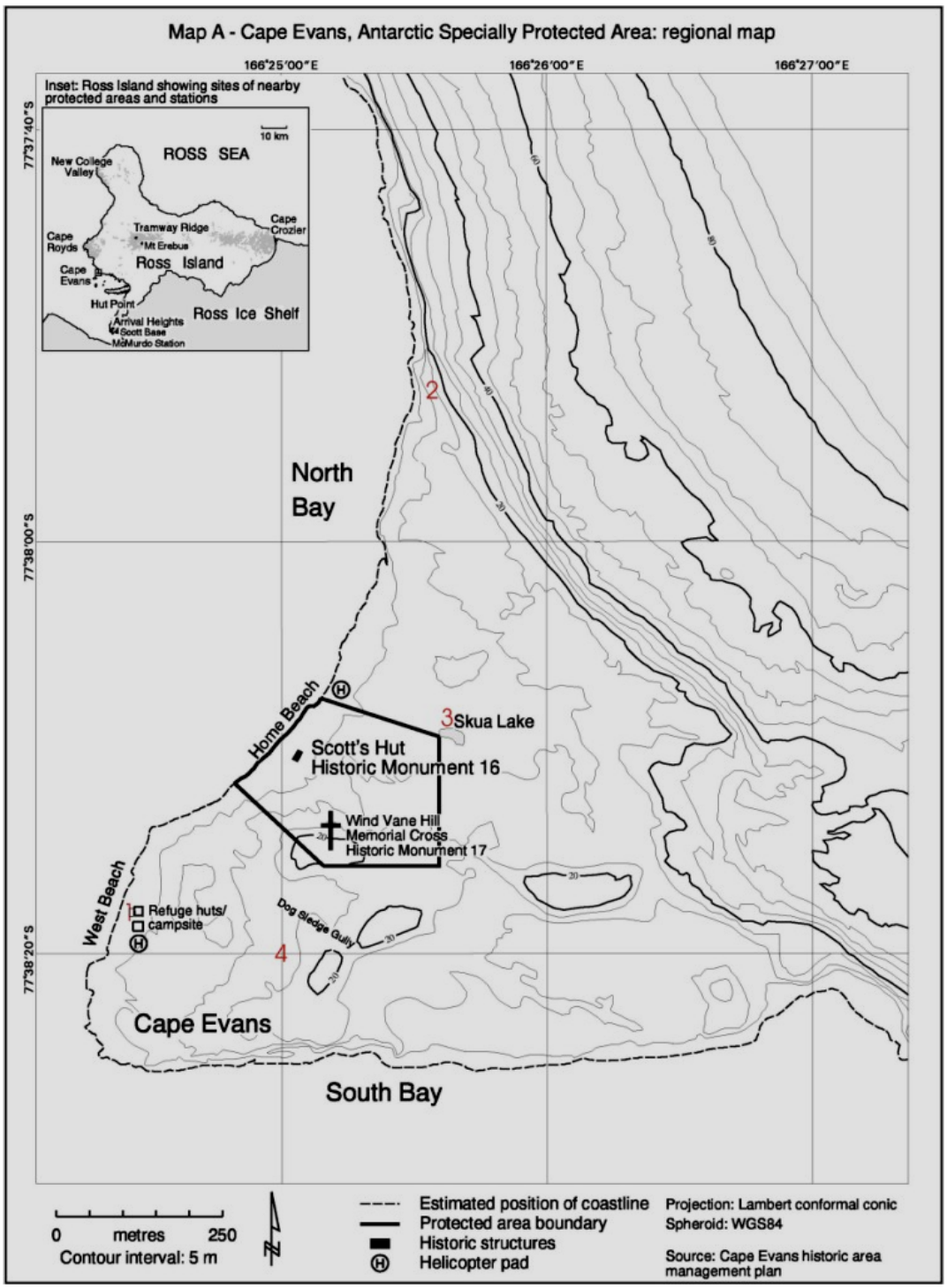

Figure 3.38: Map showing location of air sampling sites at Cape Evans. (Source Cape Evans Historic Area Management Plan).

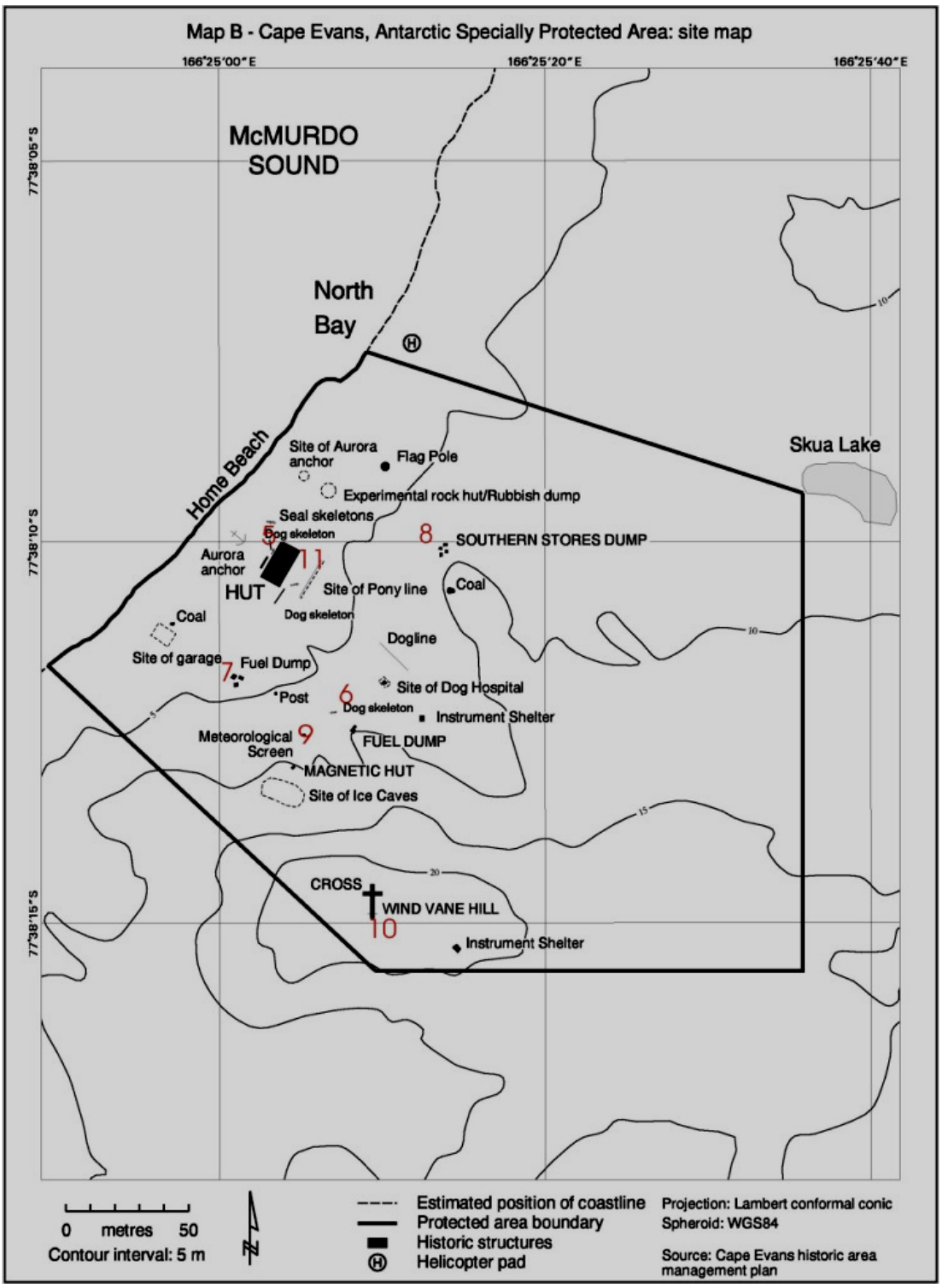

Figure 3.39: Map showing location of air sampling sites within ASPA 155. (Source Cape Evans Historic Area Management Plan).

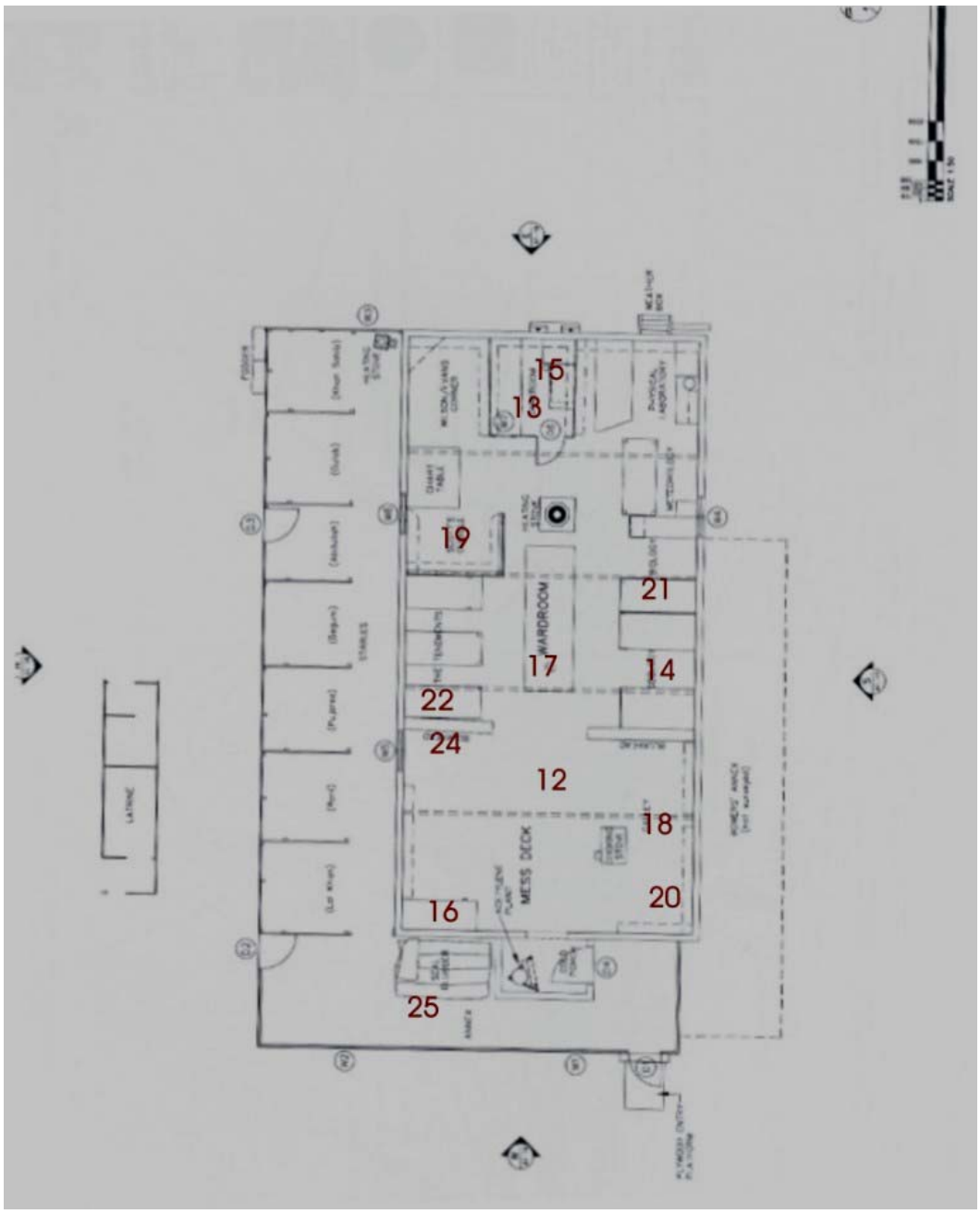

Figure 3.40: Map showing location of air sampling sites within *Terra Nova* Hut. (Source Conservation Plan Scott's Hut, Cape Evans, 2004).

Table 3.30: Colony forming units per m^3 for air sample taken at Cape Evans. Media used YM - Yeast Extract, CMC - Carboxymethylcellulose *Trichoderma viride* medium A and Avicel - Avicel *Trichoderma viride* medium A.

Location number	**Sample location**	**Volume of air sample**	**Media**	**Total colony forming units (CFU/m^3)**	**Fungal CFU/m^3**
1	In the wannigan	500	YM	134	60
2	The glacier end of home beach	500	YM	6	2
2		500	CMC	2	2
3	By skua lake	500	YM	0	0
4	By melt pond near biodiversity study site	500	YM	0	0
5	In front of the hay next to the stables	500	YM	202	14
6	Outside by a broken flour box and the dog skeleton	500	YM	10	8
7	Stores area 1 outside	500	YM	4	0
8	Stores area 2 outside	500	YM	26	4
9	In the meteorological screen outside	500	YM	0	0
10	By cross on wind vane hill	500	YM	12	2
10		500	CMC	0	0
11	Outside on top of stores box on the snow drift side of the hut	500	YM	14	0
12	Inside on floor of main room	500	YM	>5256	1448
12		500	CMC	1436	1030
13	Inside hut on top of darkroom	200	YM	1745	440
13		200	CMC	820	300
14	Inside bunk area next to galley	200	YM	3260	955
14		200	CMC	6045	165
15	Inside dark room	200	YM	2210	675
15		200	CMC	1585	240
16	On top of meteorological screen in corner of hut	200	YM	2165	1130
16		200	CMC	2040	1345
17	On top of the table in the middle of the main room	200	YM	>13,140	1030
17		200	Avicel	2865	865
18	Galley area	50	YM	5820	3100
18		50	CMC	4700	2040
19	Scott's bunk	200	YM	945	580
20	Under table in galley area	50	YM	1550	600
21	Under bunk lab area near mouldy boot	200	YM	>13,140	>13,140
22	Under bunk Birdie Bowers area near mouldy boot	200	YM	>13,140	1755
23	In store box stables side of hut	200	YM	2420	1105
24	In stores galley area	100	YM	2730	1610

Table 3.30: Colony forming units per m^3 for air sample taken at Cape Evans. Media used YM - Yeast Extract, CMC - Carboxymethylcellulose *Trichoderma viride* medium A and Avicel - Avicel *Trichoderma viride* medium A.

Location number	Sample location	Volume of air sample	Media	Total colony forming units (CFU/m^3)	Fungal CFU/m^3
25	Annex area next to blubber facing towards stables area	500	YM	54	8
25		500	Avicel	76	28

3.5.16.4 Air sampling Cape Royds

At Cape Royds, from a total of nineteen sites, a total of 28 samples were taken, 18 outside and 10 inside the *Nimrod* Hut. Outside the hut, the average was 74 CFU/m^3, with a range from 0 to 560 for the 18 samples taken (Location of the nineteen sites are shown in Figures 3.42-3.44). Outside, the highest numbers of fungal propagules were recorded from the stables area and latrine area. Figure 3.41 shows the level of spores trapped in a sample taken within the stables area on YM agar.

Figure 3.41: Photograph of agar plate from air sample taken from within the stable are at *Nimrod* Hut, media YM. The fungi are 1 *Cladosporium* sp. and 3 *Geomyces* sp..

Inside the *Nimrod* Hut, the average was 14 CFU/m^3 with a range from 2 to 32. The highest numbers of fungal propagules were recorded from a sample taken from the high shelf by the biology laboratory. Table 3.31 shows the exact numbers of CFU/m^3 at each site and for each sample. At Cape Royds, the fungi isolated outside were *Cladosporium* sp. *Geomyces* sp., large unidentified white fungi, small white fungi and white spreading fungi. Inside the *Nimrod* Hut, the

fungi isolated were *Cladosporium* sp., and unidentified white and large white fungi.

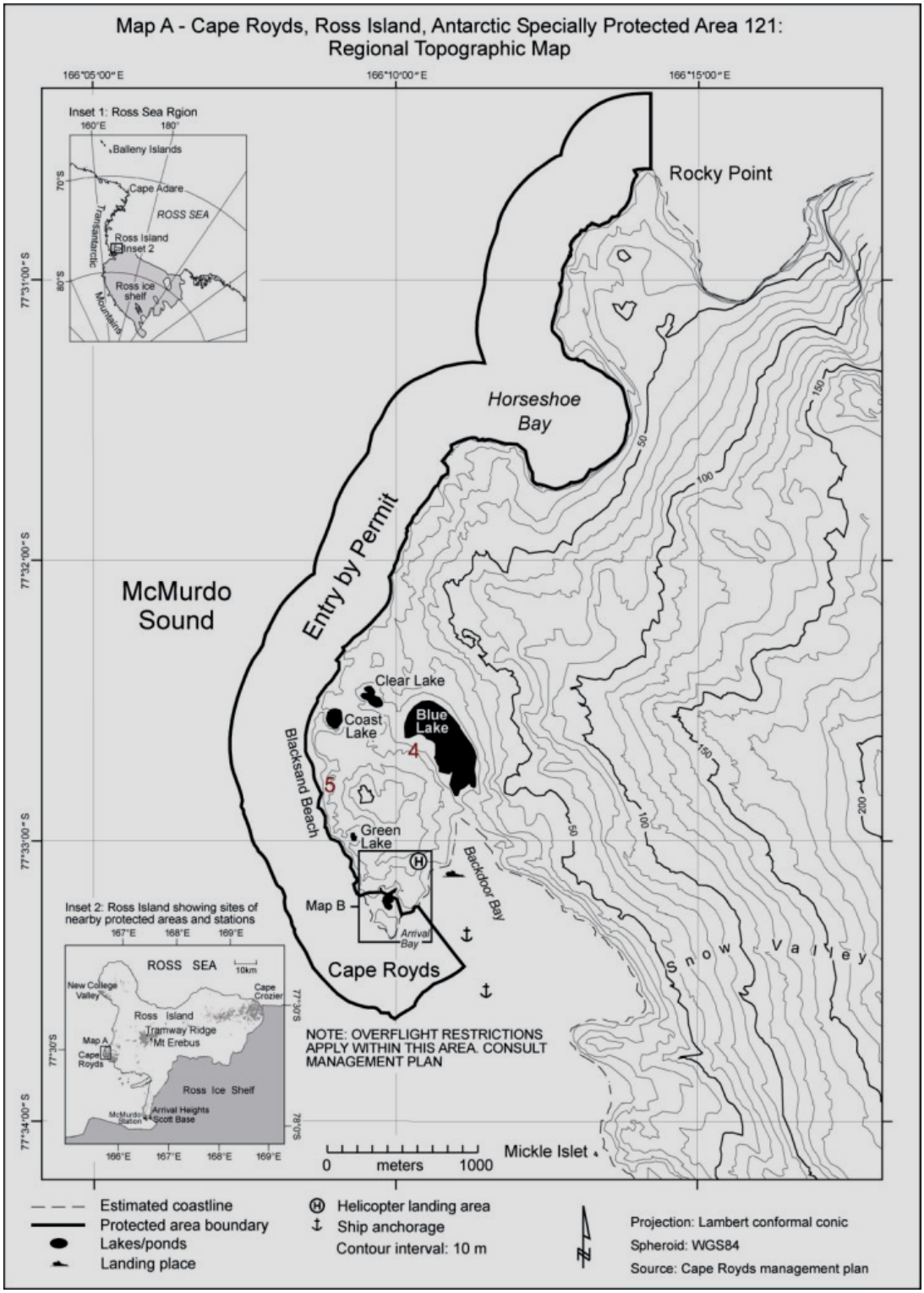

Figure 3.42: Map showing location of air sampling sites at Cape Royds. (Source Cape Royds Management Plan).

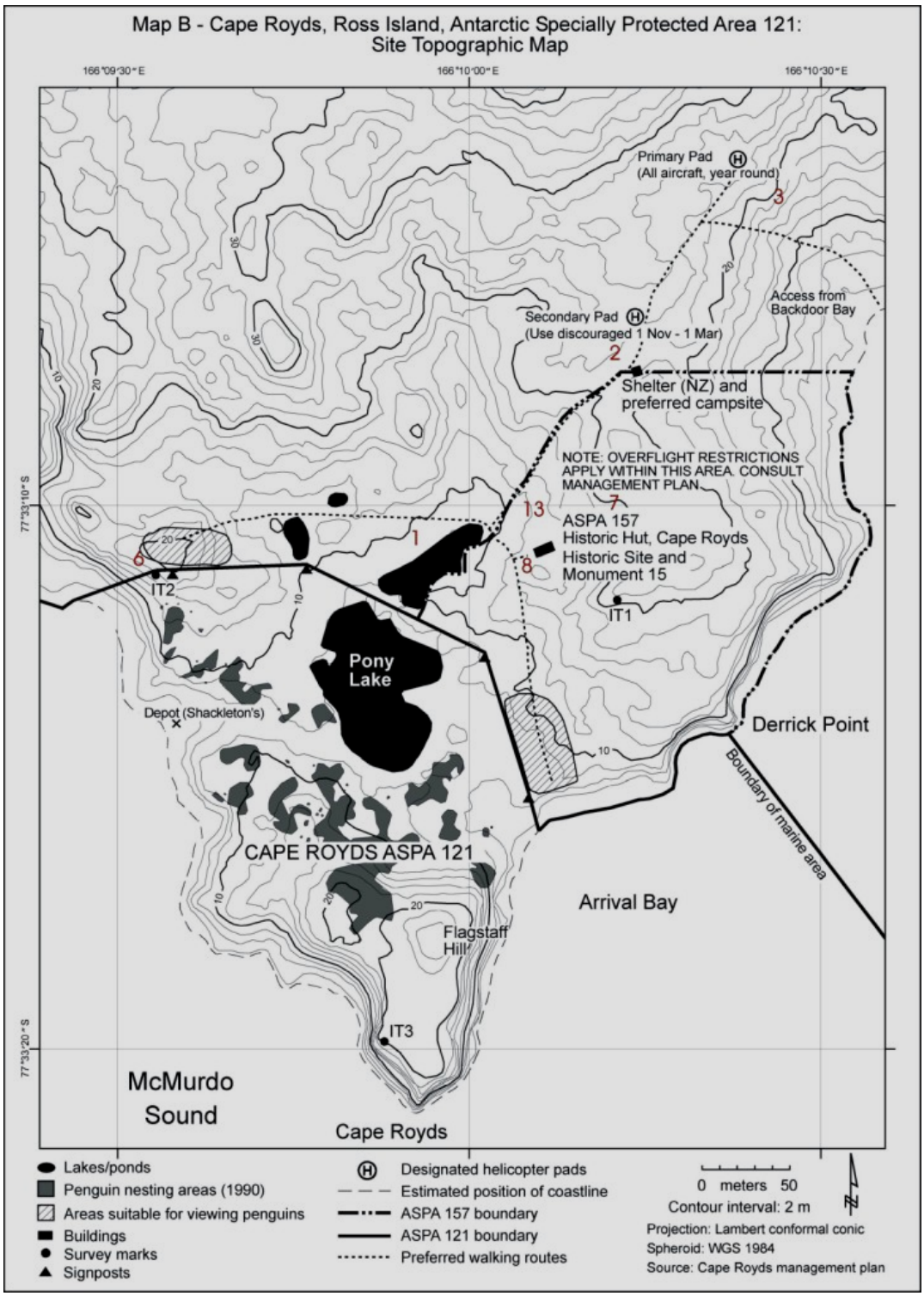

Figure 3.43: Map showing location of air sampling sites with the ASPA 122. (Source Cape Royds Management Plan).

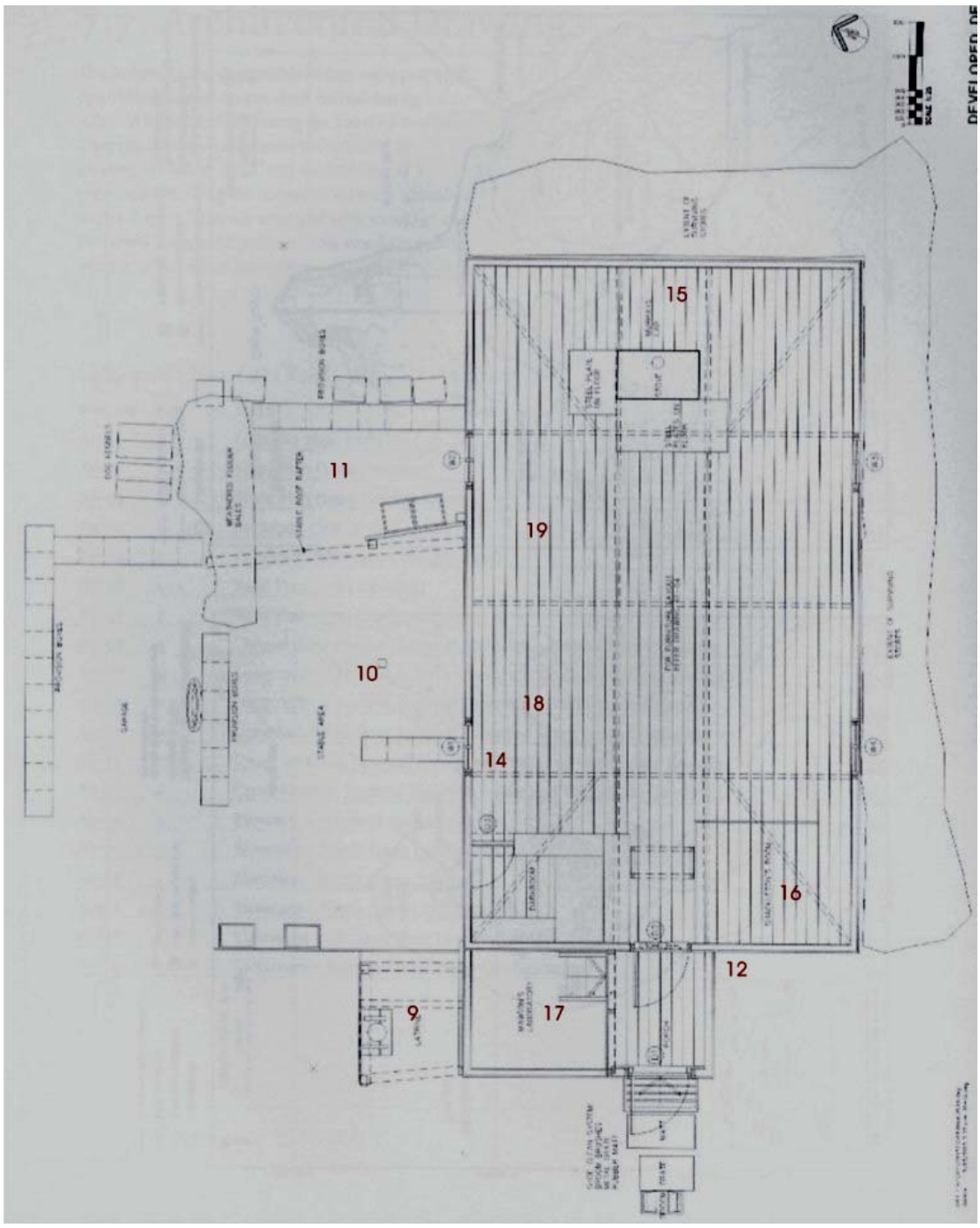

Figure 3.44: Map showing location of air sampling sites within *Nimrod* Hut. (Source Conservation Plan Shackleton's Hut 2003).

Table 3.31: Colony forming units per m^3 for air sample taken at Cape Royds. Media YM-Yeast Extract, CMC-Carboxymethylcellulose *Trichoderma viride* medium A and Avicel-Avicel *Trichoderma viride* medium A.

Location number	**Sample location**	**Volume of air sample**	**Media**	**Total colony forming units (CFU/m^3)**	**Fungal CFU/m^3**
1	Outside by Pony lake	500	YM	0	0
2	In the wannigan	500	YM	10	10
3	Next to the biodiversity study site	500	YM	0	0
4	Near melt pond away from hut	500	YM	0	0
5	On top of rise next to sea and away from the hut	500	YM	0	0
5		500	CMC	0	0
6	On rise above penguin colony	500	YM	0	0
6		500	CMC	0	0
7	Behind the hut by metal poles	500	YM	0	0
7		500	Avicel	20	20
8	On top of the pile of material that had been removed from around and under the hut	500	YM	86	56
9	Latrine area	500	YM	1656	204
10	1st Stable area	200	YM	610	560
10		200	Avicel	305	305
11	2nd stable area back of stables	200	YM	30	4
12	Under the hut by entrance	500	YM	74	66
12		500	Avicel	28	28
13	In dump site	500	YM	>5256	36
13		500	Avicel	10	10
14	Inside main area on high shelf above the window	500	YM	30	16
15	Inside on shelf in biology area above open box of biscuits	500	YM	60	32
16	On shelf inside Shackleton's room	500	YM	66	30
16		500	Avicel	6	6
17	Inside Mawson's laboratory	500	YM	30	8
17		500	Avicel	2	2
18	Main area between canvas divide and stove	500	YM	26	18
18		500	Avicel	8	8
19	Main area between canvas divide and door	500	YM	30	8
19		500	Avicel	14	14

3.6 Discussion

The main aim of this research was to gain an understanding of the fungal diversity present in the Historic Huts and materials contained within and around it. Two main objectives were addressed:

- To isolate filamentous fungi from a variety of Antarctic substrates using a selection of culture media, culturing techniques and sampling methods.
- To preserve the isolates and to create an Antarctic Fungal Culture Collection for future academic study and reference.

Eight hundred and forty nine samples were taken and fungi were isolated from five hundred and sixty of these samples. Fungi were successfully isolated from all types of substrates sampled and fungi were isolated by all sampling methods. The wood samples were all from the surface to 5 mm below of the material being sampled but due to the surface sterilising of the wood before culturing, the fungi isolated from these samples were all able to penetrate into the wood to a certain extent.

Over the period of this study, January 1998 to January 2006, the evidence of active fungal growth became more evident in the three Historic Huts, the most dramatic increase in visible fungi was in *Terra Nova* Hut at Cape Evans. This is consistent with the historic record Boyd *et al.* (1966) mentioned the presence of mould growing on leather and other objects in Ponting's darkroom in the Cape Evans Hut when they were in the field in 1963, but no culturing of these fungi was done so no comment on viability was made. The percentage of samples containing fungi was similar in the *Discovery* Hut and *Terra Nova* Hut but at *Nimrod* Hut the percentage was a lot less. The low percentage of samples containing fungi at the *Nimrod* could be due to many factors, including size of hut, construction differences, location, condition of artefacts, number of visitors and snow accumulation.

Held *et al*. (2005) reported that the number of hours when conditions were favourable for fungal growth, by the criteria of hours per year when temperatures were above 0°C and relative humidity was above 80%, *Nimrod* Hut in 2001 was

0-55 hrs and in 2002 the range was 0-12 hours with the most favourable place being the floor compared with *Terra Nova* Hut where conditions were favourable for 185-569 hrs in 2001 and 83-461 hours in 2002. Inside *Discovery* Hut, it is favourable for the least number of hours of the three huts but still has a high percentage of samples containing fungi. The range of hours represent the data as collected from dataloggers at the minimum and maximum number of hours, and reflects the microclimates that exist within each hut. As a possible explanation for the reasons for the huts vary with regard to temperature and relative humidity, *Nimrod* Hut is further away from the sea than the other two huts, is sheltered by surrounding ridges, the front of the hut is well above the ground while the other two huts are built directly on the ground. Both *Discovery* Hut and *Terra Nova* Hut have snow collect up along their walls over winter while *Nimrod* Hut has very little snow accumulation. At *Nimrod* Hut there is no visible accumulation of water within the hut, *Discovery* Hut has wind-blown snow inside after the winter and *Terra Nova* Hut has the southern wall with a build-up of ice.

From the samples of historic material found outside the historic sites or Antarctic non-historic material, the percentage from which fungi were isolated was higher due to many of the samples coming from the penguin colony which is a nutrient enhanced environment; also a number of the wood samples were from the Historic Huts but had been stored in a locked container at Scott Base therefore had human interference as a possible explanation.

At *Discovery* Hut there were more fungi isolated from wood inside the hut than outside the hut, this could possibly be due to human impact as this hut is the most visited of the three huts but offers the least favourable conditions for fungal growth. Fletcher *et al*. (1985) when studying the relationship between organic material and fungal colonisation in Antarctica noted that many areas containing no organic material also contained fungi concluded that these fungi were airspora which were not active until put onto agar plates and incubated in warmer conditions; this could also be an explanation of why there are high counts of fungi within *Discovery* Hut.

At *Terra Nova* Hut the percentage of wood samples containing fungi was similar both inside the hut and outside, but also at *Terra Nova* Hut the annex/stables area

samples did contain more fungi possibly due to the area having a gravel floor and having contained lots of enrichment sources like seal blubber, horses, and animal food.

At the *Nimrod* Hut, the percentage of samples containing fungi was highest outside, this could possible be due to the stables area being along the north side of the hut. This area originally housed the horses and has become a popular nesting site for the Adélie penguins from the nearby rookery. Also along the southern and eastern wall a row of boxes were placed to insulate the hut and over time these boxes have broken and the contents have spilt, once again enriching the environment.

Due to the need to act in good conservational practice, the use of swabs was found to be an acceptable alternative to taking physical samples. At *Discovery* Hut fungi were isolated from 72% wood physical samples and 100% of other samples and when swabs were used instead of physical samples, fungi were isolated from 50% of swabs taken from wood and 100% of swabs taken from other materials. At both *Terra Nova* and *Nimrod* Hut the percentage of samples from which fungi was isolated from swabs was greater than physical samples but the sampling was biased to swabs of sites of possibly active fungal growth.

Spore traps showed that the air in the Historic Huts contained viable fungal material either from sporulating actively growing fungi or from hyphal fragments. The air sampling confirmed the results of the spore traps but provided quantification of the number of fungal propagules within the hut, outside the hut and from the air away from the Historic Huts. Interestingly, the fungal species isolated by both methods were the same. Both methods showed less viable fungal numbers outside than inside.

The bait trap experiments showed that there were bacteria and fungi in the soils around the hut, and bait traps that were slightly away from the Historic attracted no fungi while the bait traps closer to the huts had isolated fungi. Sodium chloride medium was used in the bait traps and this was specifically done as the soil and wood in this area is very high in salt (Held et al., 2003) and it was hoped to enrich for salt tolerant organisms at both *Terra Nova* and *Nimrod* Hut the organisms

were the same as isolated from YM agar. To complete this study a wide range of locations needs to be screened and possibly an antifreeze reagent added to the media to prevent freezing of the agar.

The fungal blooms within the *Terra Nova* Hut that were sampled and placed on agar plates on location were all actively growing which confirmed the results of physical sampling, swabbing and the two air sampling methods. The samples taken from below ground in the annex area did not contain fungi but no fungal bloom was visible and the media used for these samples was a very selective media designed to isolate basidiomycetes. At *Nimrod* Hut the number of positive growth from visible fungal blooms was less. The fungal blooms in this hut are less visible and have a dryer more dormant look to them. Mawsons laboratory area where there are clear black mould spots was positive for fungal growth but not the organism expected, which was investigated and identified as a *Cladosporium* sp..

The huts are swept after visits by tourists, science events working in the huts and by base staff. The fact that these results show that these sweepings are rich in fungal material creates the question of whether this practice is adding to the problem by spreading this material back into the hut and possibly increasing spore spread into the air. The physical wood samples taken from the floor often contained no fungi but this could be explained that these fungi left by sweeping do not penetrate the top layer of the wood, (the samples had been surface sterilisation with hypochlorite) while the sweeping samples contained fungi and the spore traps/air samples showed that fungal material was dropping to the floor and accumulating on the floor.

Fungi were isolated on all media used in this study, from the general purpose agar YM to the more selective Media 4, 6 and 7. There was no dominant fungal species that grew on one agar type and not on others. The number of samples containing fungi that could grow on CMC indicated that many possessed the ability to degrade cellulose, which will be discussed in detail in Chapter 5.

The incubation temperature did affect the number of fungi isolated from samples as the total number of fungi isolated at 4°C was less than at both 15°C and 25°C. The number of fungi isolated at 15 °C was less than at 25 °C. From more

temperate environments the number of isolates cultured from plates incubated at 4°C would be expected to be lower than numbers from plates incubated at warmer temperatures. Isolation temperature can bias the frequency and diversity of fungi isolated but in this study the dominant organisms were isolated at all three temperatures used. It was not possible to determine if temperature of isolation affects the frequency of isolation of the less dominant fungi.

From a study completed by Carreiro and Koske (1992) they concluded that the temperature of isolation affected the species of fungi isolated. When they isolated from forest soil material at 0°C and 25°C, they found that *Zygomycetes* dominated at 0°C while at 25°C. *Deuteromycetes* and *Mortierella* were dominant. Most fungi isolated at 0°C were psychrotrophs capable of growing from 0°C to temperatures greater than 25°C. However, psychrophiles were also isolated. Isolations at 25°C were mostly mesophiles with growth minima between 5°C and 10°C and maxima above 25°C.

Due to the need to act in good conservational practise, it was not possible to take samples big enough to complete accurate moisture content calculation. There was no correlation between moisture content of the wood and presence of fungi.

When a site was sampled repeatedly on one sampling trip, there was variation in the diversity of fungi isolated between samples and whether fungi were isolated from the sample. From the sites sampled on a number of sampling trips, there was no pattern to the presence or absence of fungi. There were a few samples that on the first sampling contained no fungi which in subsequent samplings were found to contain fungi, a number of samples that contained fungi which were not reisolated in latter sampling and a few sample sites that never contained fungi. Many samples contained different fungi as a function of different samplings. In all examples of repetitive sampling, the variations in fungi could be due to changes in fungal diversity both with time, location of sample and difficulties in getting consistency in the procedure between the samplings themselves. These changes could be due to competition for nutrients, and or the natural progression of fungal species as each species creates favourable conditions for another species. Fungi could be restricted to small areas and the second sample could be out of this area from which an isolate was found in the first sample. A survival mechanism for

fungi is thought to be that they can become dormant in winter and then re establish when conditions are more favourable; this strategy favours spore forming fungi as spores are thought to be more resilient to unfavourable conditions. There is the possibility of new fungi being introduced between samplings leading to new fungi being isolated on subsequent samplings.

All the dominant genera (*Cadophora* sp., *Geomyces* sp., *Thelebolus* sp., *Cladopsorium* sp. and *Penicillium* sp.) in the study have been isolated in Antarctica from many locations and environments as discussed in Section 3.3.1, 3.3.1.1 and 3.3.1.2. *Cladopsorium oxysporum* has not been previously recorded from Antarctica but many *Cladosporium* sp. have been isolated. These fungi are not endemic to Antarctica as they have been isolated from locations other than Antarctica, and it is difficult to determine if these fungi are indigenous to Antarctica. The study completed by Blanchette *et al*. (2004) investigating the soft rot isolated from the Historic Huts suggested that *Cadophora* sp. found in the Historic Hut could be endemic due to the undescribed species found in hut wood and their presence in other material including soil and moss. Vishniac (1993) suggested that *Geomyces* sp. and *Thelebou*s sp. were indigenous due to both species being widespread in Antarctica. From this PhD research it is not possible to determine accurately the origins of the fungi isolated in this study, but it can be speculated that the *Cadophora* sp. are endemic, *Geomyces* sp. and *Thelebolus* sp. are indigenous and the *Cladopsorium* sp. and *Penicillium* sp. are introduced organisms.

It has been reported by Smith and Reid (1997) that many soil fungal communities in cold environments are dominated by fungi with dark hyphae, and in this study two of the five dominant fungal genera are dark. The colony colour of both *Cladosporium* sp. and *Cadophora* sp. are dark green/black, and many of the *Penicillium* sp. are also dark green/green in appearance. The *Geomyces* spp. were pale in early growth but darken on the underside to brown as the culture aged.

The number of fungal species that could be identified by mycological methods was limited by the number of isolates that did not produce recognisable structures, 643 isolates were identified by their structures.

The summary of the 33 fungi identified by molecular methods is as follows:
6 were identified as *Cadophora malorum* (str166)
1 as *Cadophora luteo-olivacea* strain A171
1 as *Penicillium* sp.
3 as *Penicillium roquefortii*
1 as Penicillium *solitum* strain FRR 1
2 as *Penicillium expansum* strain ATCC
7 as *Cladosporium oxysporum*
6 as *Geomyces* sp. C239/10G
6 remained unidentified as the match with fungal sequences within the database were not strong enough to confirm identity.

The presence of the three Historic Huts on Ross Island, Antarctica has altered the natural microbial biota in the area they were built. Schofield (1971) commented that the damage to and alteration of the land biota and environments of the Antarctic continent by early explorers was insignificant and of little consequence when compared with modern day. It was also noted that wood and other organic materials brought to Antarctica had been preserved virtually unchanged but that the microflora of the area has species with characteristics that indicated an origin away from Antarctica. The diversity of fungi within this environment is limited but what fungi are there are proliferating and it is proposed that the fungi are both endemic and introduced.

The University of Waikato Antarctic Fungal Culture Collection created by this thesis research contains a total of 2076 isolates, 1177 of which are filamentous fungi and 899 are single cell microorganisms either yeast or bacteria. Full catalogue of The University of Waikato Antarctic Fungal Culture Collection is presented in Appendix 4. Three hundred and nine fungi isolated from *Discovery* Hut, 622 fungi from *Terra Nova* Hut, 222 fungi from *Nimrod* Hut and 24 isolated from other locations were cultured during this PhD research and are now stored frozen in the Collection. Eight hundred and twenty seven cultures were isolated at 4ºC, 220 at 15ºC and 129 at 25ºC. Five hundred and thirty seven cultures were from YM agar, 54 from media 7, 75 from media 6, 205 from media 4, 149 from VB agar, 66 from CMC, 13 from Martin's medium, 52 from acidic malt extract agar, nine from PDA agar, and 17 from NaCl agar. Fungi from the frozen cultures

of this collection have been successfully recultured over the course of this research.

Fungal cultures isolated during this chapter of research have been quoted in the following publications:

Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., Blanchette, R.A. 2005.
Environmental factors influencing microbial growth inside the Historic Expedition Huts of Ross Island, Antarctica. *International Biodeterioration and Biodegradation*. 55**:** 45-53.

Farrell, R.L., Blanchette, R.A., Auger, M., Duncan, S.M., Held, B.W., Jurgens, J.E., Minasaki, R., *Scientific Evaluation of Deterioration in Historic Huts of Ross Island, Antarctica*. 2004.
In Polar Monuments and Sites Cultural Heritage Work in the Arctic and Antarctic Regions. ISBN: 82-996891-1-2, International Polar Heritage Committee, ICOMOS, Norway. pp.33-38.

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

Chapter 4 Growth characterisation of microorganisms

4.1 Introduction

The 1177 filamentous fungal cultures of the University of Waikato Antarctic Culture Collection, isolated from material collected from the Historic Huts on Ross Island, Antarctica and discussed in Chapter 3, provided a diverse range of microorganisms for the continuing investigation of what allows these fungi to survive in Antarctica and their potential relationship to mesophilic fungi. This chapter describes the investigation of seven Antarctic isolates; *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805.

The selected fungi were characterised according to growth rates as a function of temperature, growth on various cellulose carbon sources, differing water activity and ability to utilise 95 different carbon sources using BIOLOG microtitre plates. Additionally, the isolates were characterised by the temperature range in which they could grow. According to Ogunseitan (2004) organisms are classed into particular groups depending upon their growth range, as follows:

- Psychrophilic organisms have a growth range of minimum -10°C, maximum 20°C with an optimum growth temperature of 10-15°C.
- Psychrotrophic organisms have a growth range of minimum -10°C, maximum 45°C with an optimum growth temperature of 20-40°C.
- Mesophilic organisms have a growth range of minimum 5°C, maximum 52°C with an optimum growth temperature of 28-43°C.
- Thermophilic organisms have a growth range of minimum 30°C, maximum 70°C with an optimum growth temperature of 50-65°C.
- Hyperthermophilic organisms have a growth range of minimum 65°C, maximum 110°C with an optimum growth temperature of 80-105°C.

Fungi classified by growth range according to *Ainsworth and Bisby's Dictionary of the Fungi* (Hawksworth *et al.*, 1995) group slightly different from the general classification discussed above:

- Psychrophilic fungi grow below 10°C with an optimum growth temperature of below 20°C.
- Psychrotolerant fungi grow below 10°C with optimum growth temperature below 20°C.
- Mesophilic fungi grow between 10°C and 40°C with an optimal growth temperature of 20-35°C.
- Thermotolerant fungi maximum growth temperature 50°C and minimum growth temperature well below 20°C.
- Thermophilic fungi grow between 20°C and 50+°C with an optimal growth temperature of 40 to 50°C.

Due to the complex nature of wood, consisting of many specific components including polymers, cellulose, hemicellulose, lignin, pectin and starch, etc as well as extractives it is not possible to understand the growth of fungi on cellulose using wood as the substrate. The alternative is to either purify cellulose from wood, which is problematic in order to ensure there are no contaminates, or to use synthetic cellulose. In this study, three synthetic celluloses were used as cellulose substrates, carboxymethylcellulose, hydroxyethylcellulose and Avicel.

The fungal isolates were evaluated for their requirement of water for growth. The reduction of water activity in material is used in the food industry to prevent fungal spoilage and techniques have been developed to quantify how fungi cope with low water activity (A_w). According to Grant (2004) water activity is the effective water content expressed as its mole fraction. Antarctica is known to be an environment of low water availability due to much of the water being frozen and very little precipitation; therefore, given that the fungal isolates were isolated from Antarctica and can proliferate within the environment, it was hypothesised that they would be able to grow at lower A_w.

Biolog™ has been used for the identification of fungi by their substrate utilisation patterns and for accessing complete fungal communities by the

inoculation of plates with environmental samples. In this study it was used to detect differences for each of the chosen isolates in carbon utilisation at psychrophilic (cold) and mesophilic (moderate) temperatures.

This chapter begins firstly, with a review of published research on growth rate and enzyme activity of Antarctic isolates, including background on the methods used to investigate the ability of the selected fungi to grow at different A_w and to grow on a range of different carbon sources. The materials and methods, results and discussion of the growth characteristics at varying temperatures and water activity rate and biochemical variation are subsequently described.

4.2 Hypothesis, Aims, and Objectives

This chapter focuses on the microbiological framework of Antarctic fungal isolates including investigation of their growth characteristics and relationship to mesophilic organisms.

The main hypothesis of this aspect of the thesis research was that the fungi isolated from the wood of the Antarctic Historic Huts could proliferate at cold temperatures, relative to the average Antarctic summer temperatures with demonstrable enzymatic activity.

The main aim was to gain an understanding of the fungal biochemistry and growth characteristics of a selection of fungi that had been isolated from the Historic Huts themselves and materials contained within and around them.

Two objectives were addressed:

- Fungal growth rate measurements at various temperatures were conducted to determine whether organisms had psychrophilic or pychrotrophic preferences.
- Fungal growth rate measurements on different carbon sources, cellulose sources, and water availability were conducted to characterise primary and secondary metabolism of the isolates.

4.3 Literature review

4.3.1 Definition of psychrophiles and psychrotrophs

The definition of psychrophile has been a topic of many discussions but for the purpose of this thesis, psychrophiles are defined according to the definition of Morita (1975) as "organisms having an optimal growth at about 15°C or lower, a maximal temperature for growth at about 20°C and a minimal temperature of growth at 0°C or below, while psychrotrophs (psychrotolerant) have a maximal growth temperature above 20°C".

Polar Regions cover 14% of the earth's surface, and oceans cover 71%. In total, more than 90% of the earth's surface is 5°C or colder (Morita 1975). As genuinely psychrophilic microorganisms are restricted to permanently cold environments, ocean water is one of the best sources of these organisms as it has been cold for millions of years (Morita, 1975). Other permanently cold environments include the polar and alpine regions (Feller and Gerday, 1997). In contrast, psychrotrophic microorganisms are able to grow in a wide range of environments that undergo thermal fluctuations. Even in permanent cold environments most microorganisms are psychrotrophic rather than psychrophilic, this being due to a lack of nutrients restricting the metabolism of the psychrophiles (Helmke and Weyland, 2004). The habitats of psychrotolerant microorganism include the earth's atmosphere which can reach -40°C at high altitudes, snow, ice and caves in Polar Regions, freshwater lakes, mountainous rivers and streams (Baross and Morita, 1978). The guts of fish, frozen and chilled foods are other sources (Gounot, 1991).

Soils, especially in Polar Regions, are unstable cold environments. The temperature of soils can fluctuate greatly due to periodic heating by solar radiation and cooling due to no sun in the winter period, so bacteria isolated are predominantly psychrotolerant. However, microbial growth will only occur in soils where liquid water is present (Baross and Morita, 1978). Cameron and Merek (1971) completed experiments where water was added to four Antarctic Dry Valley soils and the plates were incubated at either 8 or 25°C for 4 weeks. After this time the number of bacteria was re counted and in three of the four samples the count had risen from less than 10^1 to around 10^6 per gram of soil. When obtaining fungal species from low temperature environments,

isolation temperature will bias the frequency of isolating psychrophiles. Psychrophiles have by definition growth temperature optima of 15°C so temperatures above this temperature will stress them possibly preventing growth. Additionally, psychrophiles are reported to be more likely to be isolated from a stable cold environment with very little change in temperature rather than psychrotrophs, which have a much wider growth range and are found in more temperature variable environments and are more tolerant to temperature increase and decrease. Carreiro and Koske (1992) concluded from a study that the temperature of isolation affected the species of fungi isolated. When they isolated from forest soil material at 0°C and 25°C, they found that *Zygomycetes* dominated at 0°C while at 25°C *Deuteromycetes* and *Mortierella* were dominant. Most fungi isolated at 0°C were psychrotrophs capable of growing from 0°C to temperatures greater than 25°C. However, psychrophiles were also isolated. Isolations at 25°C were mostly mesophiles with growth minima between 5°C and 10°C and maxima above 25°C.

The physiological mechanisms conferring cold tolerance are complex and there is not one single adaptation determining cold tolerance (the ability to resist or survive the cold), as all the cell components of a psychrophile or psychrotroph must be functional for the fungus to grow at low temperatures. The upper growth limit of cold tolerant fungi can result from the lack of activity of a single enzyme. Psychrophilic is the term used to classify an organism, prokaryotic or eukaryotic, which is living permanently at temperatures close to the freezing point of water in thermal equilibrium with the medium (Hoyoux *et al.*, 2004).

Psychrophiles have developed mechanisms of adaptation to temperature including a huge range of structural and physiological adjustments in order to cope with the deleterious effects of low temperatures such as increase in cell size, formation of double cell wall, production of cold shock proteins and cold adapted enzymes (Beales, 2004). Indeed they display metabolic fluxes at low temperatures that are more or less comparable to those exhibited by closely related mesophiles living in moderate temperatures (Zecchinon *et al.*, 2001).

Temperature is one of the most important environmental factors of life as it influences most biochemical reactions. Most psychrophilic

micoorganisms are not only adapted to low temperatures but also to other environmental constraints. One example is deep sea water wherein organisms are adapted to not just low temperatures but also to extremely high pressure, therefore they are both psychrophiles and piezophiles (Yayanos, 1995).

Life under low temperature conditions was identified as early as 1887 by Fortser who reported that microorganisms isolated from fish could grow well at 0°C (Morita, 1975). As early investigators were mostly interested in whether bacteria were able to grow at or close to 0°C, confusion rose over the definition of cold-adapted bacteria (Morita, 1975). Investigators found it difficult to tell if bacteria were growing at these low temperatures or just surviving. Most bacteria found to grow around 0°C were originally defined as being psychrophilic or cold loving. However, this definition failed to take into account cardinal growth temperatures, often the optimum temperature for growth i.e. that temperature at which cell division occurred at the highest rate, which was around 20-30°C. Most investigators were unaware that bacteria truly adapted to the cold would die at temperatures above 20°C and it is likely that their lack of success in the isolation of cold adapted bacteria was a result of using unsuitable source materials and handling techniques (Herbert, 1986). As a result of this, most psychrophilic bacteria discovered by early studies were later found to be psychrotolerant. It was not until 1964 that the first real psychrophilies, all isolated from the marine environment, were reported (Morita 1975). Since then, numerous organisms, in particular bacteria, yeasts, unicellular algae and fungi, have been found to successfully colonise low temperature environments in which they contribute to the ecosystem (Russell 1992; Gounot and Russell 1999). According to Herbert (1986) in order to isolate psychrophilic bacteria, the environment from which the source material was sampled must have been kept constantly below 20°C over its history. After sampling, the material must be kept cool, and all laboratory work carried out with precooled media, pipettes and other equipment.

Cooling to low temperatures reduces the rate at which chemical reactions occur, increases the viscosity of water, denatures proteins and increases the relative permittivity of water thus reducing attraction between ions of opposite charge and markedly affecting acidic and basic residues of proteins. There have been no reports of microbial growth below -12°C which is consistent with the

known physical state of aqueous solutions at subzero temperatures (Russell, 1990). Dilute aqueous solutions will generally supercool to -10°C and occasionally to -20°C and most cells remain unfrozen at -10°C to -15°C even though temperatures are 9 to 14 degrees below the freezing point of their cytoplasm and there is extracellular ice in the growth media (Russell, 1990). Nucleation of the supercooled cytoplasmic water does not occur above this temperature because small ice-nuclei are barred from entering the cell by the plasma membrane. Supercooled water has a higher vapour pressure than that of extracellular ice so water will move out of the cell thereby concentrating the intracellular milieu. At temperatures below -10°C to -15°C, the cell water begins to freeze thus further concentrating the intercellular salts up to 3 molar. The resulting ionic imbalance, altered pH and lowering of water activity have a toxic effect on the microorganisms which will either prevent them from functioning or possibly kill them due to environment within and outside the organism causing destruction of the cell and stoppage of the ability of the organism to metabolise. The lower growth temperature limit for psychrophiles is fixed not by the cellular properties of cellular macromolecules but by the physical properties of aqueous solvent systems inside and outside the cell.

4.3.2 Antarctic isolated fungal growth at low temperatures

Temperature affects the rate of fungal spore germination and subsequently the rate of growth of the resulting mycelium. It also affects fungal growth indirectly as freezing limits growth by making water unavailable. Freezing may result in tissue and membrane damage with subsequent loss of nutrients (Melick and Seppelt, 1994).

The ability of Antarctic isolated fungi to grow at low temperatures has been studied frequently. Fungi that were isolated from material collected from around the Showa base, Prince Olav Coast, in 1959-1960 were tested for their ability to grow at varying temperatures by Tubaki (1961). The four isolated species (*Blodgettia borneti*, *Chrysoporium pannorum*, *C. verrucosum* and *Cylindrium griseum*) could all grow at 5°C and did not grow at 30°C and all had an optimum growth temperature between 20-25°C. It was also noted that *Blodgettia borneti* and *Chrysoporium pannorum* grew while being stored in a fridge at 1°C for a month.

Zucconi *et al.* (1996) screened 35 strains of microfungi from Victoria Land for growth at temperatures ranging from 0°C to 45°C. The fungi were isolated from samples taken from a range of locations lying between 72°30'S to 77°52'S and consisted of hot soil samples from Mt. Melbourne, coastal sites rich in mosses, beaches close to the Italian research base, Lamplugh Island, Whitmer Peninsular and Gondwana Station. Of the 35 fungal strains tested, 31 were classified as psychrotolerant, one was classified as a thermotolerant mesophile, which was isolated from the hot soils of Mt Melbourne, and two strains were classified as psychrophiles, *Geomyces pannorum* var. *pannorum* no. 5 from Crater Cirque and a pink yeast no. 2 which were isolated from "Campo Icaro".

Azmi and Seppelt (1997) investigated the growth of six fungi (*Alternaria alternata*, *Chrysosporium pannorum*, *Nectria peziza*, *Thelebolus microspora*, "mycelium sterile" and *Phoma* cf. *herbarum*) isolated from soil in the Windmill Islands, continental Antarctica and found that all six showed poor growth at 0°C. The temperature optimum for these six fungi was 20°C and they concluded that all the fungi they studied were cold tolerant strains of mesophiles adapted to grow at temperatures as low as 0°C. Interestingly, acid media were preferred for growth. *Chrysoporium pannorum*, *Phoma* cf. *herbarum* and *Nectria peziza* all grew best at pH 3-4 while "mycelium sterile", *Alternaria alternata*, *Thelebolus microspora* all grew best at pH 5-6. Culture media had little effect on growth but, apart from *Thelebolus microspora*, all fungi grew poorly on nutrient agar. They also noted that it was possible that the fungi may have been introduced to the Windmill Islands by human activity due to all fungi isolated in this study being classified as cold tolerant mesophiles.

Dix and Webster (1995) in a review of fungal ecology reported cold tolerant fungi capable of growth at 0°C and psychrophilic fungi with low maximum and growth optimum. They noted that all the major subdivisions of Eumycota were represented in Antarctica, Fungi Imperfecti and Mycelia Sterilia dominate.
Additionally cold tolerant fungi are found associated with contamination and spoilage of food in cold storage. Winter crops are also susceptible to diseases caused by cold tolerant fungi including *Typhula* sp. and *Fusarium nivale* (Dix and Webster, 1995).

Hurst *et al.* (1983) investigated the growth rate of five fungal species (*Botrytis cinerea*, *Chaetophoma* sp., *Chrysosporium pannorum*, *Cladosporium sphaerospermum* and *Mucor hiemalis*) isolated from three sub-Antarctic phanerogams at King Edward Cove, Antarctica and all could grow at 1°C and all showed growth optima between 15°C and 25°C. Only *Mucor hiemalis* would grow at 30°C.

Latter and Heal (1971) showed that isolates of single fungal species may differ in their physiology depending on their climatic origin. Fungi were isolated from three sites, temperate (decaying petioles of *Pteridium aquilinum* from Roudsea Wood National Nature Reserve, Great Britain), cold temperate (soil and litter of *Jucus squarrosus* Moor House National Nature Reserve, Great Britain) and oceanic Antarctic (litter and peat under a *Polytrichum* mat at Signy Island, Antarctica). The growth rates of the fungi were measured over a temperature range of 1°C to 25°C. No single species was isolated from all three sites but the genus *Mortierella* was isolated at all sites and this fungal genus was the most tolerant to reducing temperature.

The predominance of psychrotrophy rather than psychrophilicty in Antarctica maybe occurs because while psychrotrophic fungi have the ability to grow around 0°C, temperatures of substrata at various times of the year are much higher than low air temperatures. For instance, Möller and Dreyfuss (1996) wrote "Although average air temperatures in the maritime Antarctica are around freezing point, local soil temperatures and microclimates may rise to 15°C through solar radiation". Möller and Dreyfuss (1996) reported 10% of the 106 tested microfungi isolated from lichen, moss and plant samples from two Antarctic sites on King George Island, South Shetlands appeared to be true psychrophiles, 46% were psychrotolerant and 44% were mesophiles. A higher proportion of psychrophiles were isolated from lichens, whereas mosses appeared to contain more mesophiles and plants more psychrotolerant fungi. The difference between psychrophile population percentages of lichen versus psychrotolerant population in plants was related back to origin of material. Plant endophytes have migrated to Antarctica with their plant hosts after the last glacial period while the fungi on lichens may have survived the ice age on nunataks; no rational was given for the high

percentage of mesophiles found in mosses. The mesophiles that were isolated from this study were deemed to be from cosmopolitan origin and that their survival was put down to warm surface temperatures.

Kerry (1990a) investigated the effect of temperature on the growth rate of fungi isolated from sub Antarctic Macquarie Island and Casey Station, Antarctica. The linear growth rate was measured on agar plates at temperatures ranging from 4°C to 35°C. Nine isolates of leaf colonising fungi and five isolates of litter colonising fungi from Macquarie Island and six isolates of leaf and soil colonisers from the Casey Station region were tested. All fungi grew at 4°C. Maximum growth rates were between 10°C and 20°C for 13 of the 15 isolates from Macquarie Island and all six of the Casey isolates. Maximum growth of all species was at temperatures above those normally prevailing in their natural environments. None of the isolates could be defined at psychrophilic but were classified as psychrotrophs.

The low proportion of psychrophilic fungi isolated from Antarctic environments may be because the isolations have not been performed in winter when low temperatures prevail, or have not been performed from substrata that experience solely low temperatures.

4.3.3 Enzyme activity in Antarctic fungi

Chitinolytic enzymes are present in more than 25% of Antarctic fungi (Onofri *et al.*, 2000). This activity is presumably due to the large amounts of chitin in ornithogenic soils reflecting the krill rich diets of penguins (Onofri, 1999). Fenice (1998) compared the chitinolytic activity of an Antarctic *Verticillium* cfr. *lecanii* with two *Trichoderma harzianum* strains. *Verticillium* cfr. *lecanii* produced the same level of chitinase activity at 25°C as the two *T. harzianum* strains but at 4°C the activity of the *Verticillium* was four times higher than the *Trichoderma* strains and the *Verticillium* enzyme was active over a broad range of temperature (5-60°C); at 5°C, its relative activity was still 50% of that recorded at 40°C. *Verticillium* cfr. *lecanii*, with its ability to produce high levels of chitinase at 4°C makes it of interest to the biotechnology industry including such applications as biocontrol of pathogenic fungi in cold storage and treatment of chitin-rich wastes at low temperatures.

Bradner *et al.* (1999a) reported on the hydrolytic activity of seven Antarctic fungal isolates. They were screened for xylanase, β-glucanase, proteinase, lipase, cellulase, mannanase, and chitinase activity at 10°C, 21°C, 28°C and 37°C using plate screening. All isolates effectively secreted proteinase and hemicellulase into the media at all temperatures. At low temperatures chitinase activity of Antarctic isolates, *Penicillium* sp. and two *Trichoderma* isolates showed activity at 10°C and 28º but not at the intermediate temperatures indicating that a different set of enzymes were synthesised at different temperatures. Lipase activity was highest at moderate temperatures for all isolates.

Finotti (1996) compared the metabolic differences between two Antarctic *Geomyces* spp. to gain understanding of the strategies used by these fungi to survive and adapt their energy balance at different temperatures. The two fungi used in the study had different temperature optima, one *Geomyces pannorum* var. *pannorum* had an optimum growth temperature of 25°C and the other *G. pannorum* var. *vinaceus* had an optimum growth temperature of 4°C. When incubated at their optimal growth temperature both showed different growth rates. *G. pannorum* var. *vinaceus* which had a growth optimum of 4°C, grew slower than *G. pannorum* var. *pannorum* taking an extra 40 days to reach a similar mycelium dry weight to *G. pannorum* var. *pannorum* at 25°C. The energy expenditure of *G. pannorum* var. *pannorum* was higher than for *G. pannorum* var. *vinaceus* when grown at the optimal growth temperatures of each fungus. When glucose and lipid consumption was compared, *G. pannorum* var. *vinaceus*, seemed to prefer the utilisation of lipids rather than glucose while *G. pannorum* var. *pannorum* preferred glucose. The preference for lipids by *G. pannorum* var. *vinaceus* was speculated to be due to the need to maintain membrane fluidity with a need to increase unsaturated fatty acids. Additionally, the increase of CO_2 production and O_2 consumption (the RQ ratio) was very slow in the 4°C optimal *G. pannorum* var. *vinaceus* compared with the 25°C optimal *G. pannorum* var. *pannorum*. The results showed that the two *G. pannorum* strains possess different metabolisms in respect to energy expenditure and with completely different modes of glucose and lipid utilisation.

Fungi with a broad enzymatic competence possess high eco-nutritional versatility, with an ability to survive in a vegetative state and to overcome environmental changes that might normally be harmful (Fenice *et al.*, 1997). High nutritional versatility could be a successful adaptation criterion in terrestrial Antarctica micro-habitats that are generally characterised by low competition (Zucconi, 1996). Fenice *et al.* (1997) plate screened 33 fungal isolates from Victoria Land for their ability to produce twelve extracellular enzymes. More than 50% of the fungi tested produced extracellular polygalacturonase on pectin and/or polypectate and pectin lyase on pectin. Eighteen of the 33 fungal isolates demonstrated amylase activity. Cellulase activity, albeit low, was detected in 12 isolates. Nine strains showed chitinase activity. For phosphatase and glucose oxidase production, 21 strains showed phosphatase activity and seven strains showed glucose oxidase activity. Urease activity was detected in ten stains, while proteinase activity was detected in seven strains using gelatine liquefaction as the test method. All the strains tested showed lipase activity on at least one of the four Tween detergent substrates tested. Ribonuclease activity was detected in 17 strains. Overall the strains investigated showed a diverse enzymatic pattern.

Hughes and Lawley (2003) isolated one fungus (there was another black fungus that could be seen in the community but could not be cultured) from an endolithic community within a gypsum crust from Two Step Cliffs, Alexander Island, Antarctic Peninsula. Using molecular techniques and morphological features the fungus was identified as *Verticillium* sp. The *Verticillium* sp. in this study was screened for the production of phosphatase, lipase, proteinase and glycosidase exo-enzymes and the *Verticillium* sp. was able to degrade a wide range of substrates including the recycling of phosphorus by phosphatase. When comparing their ability to produce exo-enzymes with that of *Verticllium lecanii* from the continental Antarctica as found in the literature (Fenice *et al.*, 1997), it appeared that *Verticllium* spp. from both locations produced a diverse range of enzymes and as in other fungal habitats in Antarctica, the endolithic community may be low in fungal biodiversity but high in physiological diversity. A broad range of enzymatic activity indicates high versatility in substrate utilisation, an ability to live in a vegetative rather than spore state and an enhanced ability to overcome environmental change which would normally be harmful to the

organism. It may not be adaptation to the low temperatures which influences survival of organisms in the harsh Antarctic environment but rather survival during freeze-thaw cycles and desiccation. According to Hughes and Lawley (2003), the endolithic community within this gypsum crust, from Two Step Cliffs, Alexander Island, can during the summer experience surface temperatures of 1-19°C, causing rapid desiccation of the crust, the addition of snow which melts on the warm rock surface leads to a cycle of freeze-thaw, desiccation/rewetting. While in winter the initial freezing event can reduce the temperature of the crust to <-10°C.

4.3.4. Carbon utilisation using BIOLOG microplate technology

The abilities of particular species to catabolise a selection of carbon substrates have been extensively used to identify and characterise bacteria and recently this approach has been extended for use in the fungal kingdom with increasing use and importance over recent years, both in the identification of fungi and the analysis of fungal communities. The analysis of utilisation of different carbon substrates has expanded to include the analysis of microbial communities on a functional basis, rather than just to identify species. The Biolog 96 well microtitre plate system provided a rapid, straight forward method of assessing fungal functional diversity. The Biolog FF microplate (designed for fungal identification) consisted of 96 200µL wells each containing one of 95 sole carbon sources–including carbohydrates, carboxylic acids, amino acids and other miscellaneous carbon sources plus one blank well and an indicator tetrazolium dye. The ability to metabolise the individual substrates was indicated by a colour change in the dye based on a redox reaction, which occurred as the substrate was oxidised and a change in turbidity indicated growth of the fungus. The resulting reaction patterns were used to identify the fungus and provided an extensive and detailed amount of information regarding the metabolic properties of each tested fungus. The reaction patterns can be submitted to the Biolog FF database which contains fingerprints of over 500 taxa of fungi from over 120 genera and together with traditional fungal identification methods the system can be used to successfully identify organisms to various taxonomic levels. Carbon utilisation patterns can also be used to successfully characterise a fungus to species or even sub-species levels as relevant metabolic, genetic, and physiological factors and systems required

specific-carbon source use, can vary greatly between fungal taxa.

The literature had diverse results cited for the ability of microorganisms to utilise a variety of substrates as a function of temperatures. Derry *et al.* (1999) studied the functional diversity of microorganisms in Arctic soils at three incubation temperatures (30°C, 10°C and 4°C) using sole carbon source utilisation. They concluded that temperature did affect substrate utilisation with lower temperatures showing greater richness and evenness when compared with the more mesophilic temperatures. They reported that this difference was due to 4°C and 10°C being temperatures that the soil and microorganisms were more likely to have been subjected and ability to function. Psychrophilic organisms have an optimum growth temperature of 15°C and an upper limit of 20°C. While at 25°C the mesophilic microbial community increased and masked the psychrophilic and psychrotolerant communities. Claussen *et al.* (2003) used Biolog microtitre plate technology to determine differences in community level physiological profiles (CLPPs) from two soils using five different C-substrate profile microtitre plates at five different temperature regimes. Their results showed that the CLPP's were relatively unaffected by the different temperature regimes.

4.3.5 Water activity

Water activity or water potential is the best estimate of thermodynamically available water. In the harsh environment of Antarctica, it is difficult to make field measurements so water content is used to measure water status. It is not the ideal measurement, as with plant material it takes into account water that is not available to all fungi. McRae and Seppelt (1999) investigated the effect of moss water content on fungal diversity at Windmill Islands, continental Antarctica. Within the study, the water content of the moss ranged from 0.5 to 312% dry weight while water content of the soil ranged from 1 to 665%. Their results demonstrated that there was no relationship between fungal diversity and either moss moisture content or soil moisture content. In all situations, the diversity of fungi was small. They concluded that temperature was the controller as temperature influences the amount of water present in the environment, the photosynthetic activity of the mosses, plant distribution, available nutrients and gas diffusion. The lowest recorded water activity (A_w) for growth as

reported by Grant (2004) was 0.61 for sugar and for salt 0.75. Pure water has a water activity of 1 with all other solutions having A_w values less than 1 (Grant, 2004).

4.3.6 Isolation in Antarctica of the main species investigated in this thesis

All the genera isolated during the course of this research had been reported previously in Antarctica. *Penicillium roquefortii* has only been reported once previously. *Cladosporium* spp. has been isolated may times but none identified as *Cladosporium oxysporum*.

4.3.6.1 Isolation of *Cadophora* spp. from Antarctica

Phialophora sp. has been isolated from many locations within Antarctica. According to Harrington and McNew (2003) "The anamorph genus *Phialophora Medlar* has been used for a wide range of species with simple, little-differentiated condiphores, flask-shaped to straight phialides, and hyaline to pigmented conidia." Based on a recent taxonomic revision of the *Phialophora*-like fungi (Harrington and McNew 2003) many fungi previously identified as *Phialophora* sp. have been renamed *Cadophora* sp. after ITS and 18S(LSU) rDNA analysis. The *Cadophora* species, substrates isolated from, location in Antarctica from where they were isolated are all presented in Table 4.1.

Table 4.1: Isolation of *Cadophora* spp. in Antarctica, substrate isolated from, and location of isolation.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Phialophora sp.	Soil, Oil contaminated soil	Ross Island Marble Point	Aislabie *et al.* (2001)
Phialophora sp.	Mummified seal	Cape Bird Canada Glacier	Greenfield (1981)
Phialophora sp.	Soil	Ross Island	Arenz *et al.* (2006)
Phialophora cf. *alba*	Moss, lichen and plant	King George Island	Möller and Dreyfuss. (1996)
Phialophora dermatitidis	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Phialophora dermatitidis	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Phialophora dermatitidis	Mummified seal	Cape Bird Canada Glacier	Greenfield (1981)
Phialophora dermatitidis	Air	Ross Island	Sun *et al.* (1978)
Phialophora fastigata	Skua feathers and soil Soil	Edmonsom Point Terra Nova Bay	Del Frate and Caretta (1990)
Phialophora fastigata	Mummified seal	Cape Bird Canada Glacier	Greenfield (1981)

Table 4.1: Isolation of *Cadophora* spp. in Antarctica, substrate isolated from, and location of isolation.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Phialophora fastigata	Soil, wood, oil contaminated soil	Davis Station Mawson Station	Kerry (1990b)
Phialophora fastigata	Air, soil beneath moss, algal remains	Terra Nova Italian base Gondwana Station	Onofri *et al.*(1994)
Phialophora fastigata	Soil	Deception Island	Sun *et al.* (1978)
Phialophora fastigata	Sea	Italian Base	Zucconi *et al.* (1996)
Phialophora gougerotii	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Phialophora gougerotii	Various	Victoria Land	Cameron *et al.* (1976)
Phialophora gougerotii	Mummified seal	Cape Bird Canada Glacier	Greenfield (1981)
Phialophora gougerotii	Soil	Ross Island	Sun *et al.* (1978)
Phialophora hyaline	Moss	King George Island	Möller and Dreyfuss. (1996)
Phialophora lagerbergii	Soil	Taylor Valley	Baublis *et al.* (1991)
Phialophora lagerbergii	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Phialophora lagerbergii	Various	Victoria Land	Cameron *et al.* (1976)
Phialophora lagerbergii	Soil	Ross Island	Sun *et al.* (1978)
Phialophora malorum	Soil Moss and Algae	Windmill Island	Azmi and Seppelt (1998)
Phialophora malorum	Moss, lichen and plant	King George Island	Möller and Dreyfuss. (1996)
Phialophora melinii	Moss	King George Island	Möller and Dreyfuss. (1996)
Phialophora verrucosa	Mummified seal	Cape Bird Canada Glacier	Greenfield (1981)
Cadophora malorum	Historic wood Soil, Other material	Ross Island New Harbour Mount Fleming	Blanchette *et al.* (2004) Held *et al.* (2005) Held *et al.* (2006) Arenz *et al.* (2006)
Cadophora malorum	Moss	Starr Nuatak, Kay Island, Harrow Peaks	Tosi *et al*. (2002)
Cadophora malorum	Vegetation	Edmonson Point	Tosi *et al*. (2005)
Cadophora luteo-olivacea	Historic wood Soil other material	Ross Island New Harbour Mount Fleming Lake Fryxell basin	Blanchette *et al.* (2004) Held *et al.* (2005) Held *et al.* (2006) Arenz *et al*. (2006)
Cadophora fastigata	Historic wood	Ross Island	Blanchette *et al.* (2004) Arenz *et al.* (2006)
Cadophora fastigata	Vegetation	Edmonson Point	Tosi *et al*. (2005)
Cadophora sp.	Historic wood	Ross Island	Blanchette *et al.* (2004) Held *et al.* (2005) Held *et al.* (2006) Arenz *et al.*(2006)
Cadophora sp.	Vegetation	Edmonson Point	Tosi *et al*. (2005)
Cadophora sp.	Plant material	Antarctic peninsular	Jumpponen *et al*. (2003)

4.3.6.2 Isolation of *Cladosporium* spp. from Antarctica

Cladosporium spp. are usually found associated with plants in Antarctica (Meyer *et al.* 1967) and soil in close proximity to plants. According to Vishniac (1993) *Cladosporium* spp. are common cosmopolitan species. The *Cladosporium* species, substrates isolated from, location in Antarctica from where they were isolated are all presented in Table 4.2.

Table 4.2: Isolation of *Cladosporium* spp. in Antarctica, substrate isolated from and location.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Cladosporium sp.	Soil	Windmill Island	Azmi and Seppelt (1998)
Cladosporium sp.	Soil	Ross Island	Greenfield *et al.* (1981)
Cladosporium sp.	Soil	Wilkes Island	Heatwole *et al.* (1989)
Cladosporium sp.	Soil beneath moss, moss, moss with lichen	Carezza Lake, Crater Cirque, Prior Island, Whitmer Peninsula	Onofri *et al.* (1994)
Cladosporium sp.	Soil	Newfoundland Coast	Mercantini *et al.* (1993)
Cladosporium sp.	Lichen	King George Island	Möller and Dreyfuss (1996)
Cladosporium cladosporoides	Soil under moss	Crater Cirque	Fenice *et al.* (1997)
Cladosporium cladosporoides	Soil	Ross Island	Greenfield *et al.* (1981)
Cladosporium cladosporoides	Plant material, soil	Signy Island	Pugh and Allsop (1982)
Cladosporium cladosporoides	Soil, Moss, Algae, Wood	Mawson Station	Kerry (1990b)
Cladosporium cladosporoides	Soil under moss	Crater Cirque	Zucconi *et al*. (1996)
Cladosporium cladosporoides	Historic wood	Ross Island	Held *et al.* (2005)
Cladosporium cladosporoides	Historic wood, Soil	Ross Island, New Harbour, Allan Hills	Arenz *et al.* (2006)
Cladosporium cladosporoides	Moss	Gondwana Lake, Prior Island, Bruce Point, Edmonson Point, Starr Nunatak, Kay Island, Kohler head, Harrows Peak	Tosi *et al*. (2002)
Cladosporium cladosporoides	Vegetation	Edmonson Point	Tosi *et al*. (2005)
Cladosporium herbarum	Moss, Soil under moss	Crater Cirque, Whitmer Peninsular	Fenice *et al.* (1997)
Cladosporium herbarum	Petrel dung and feathers, soil	Kay Island, Vegetation Island	Del Frate and Caretta (1990)
Cladosporium herbarum	Moss. Soil under moss.	Crater Cirque, Whitmer Peninsula.	Zucconi *et al*. (1996)
Cladosporium herbarum	Lichen	King George Island	Möller and Dreyfuss (1996)
Cladosporium herbarum	Moss	Kay Island, Kohler Head	Tosi *et al*. (2002)

Table 4.2: Isolation of *Cladosporium* spp. in Antarctica, substrate isolated from and location.

Fungus	Substrate from which isolated	Location	Reference
Cladosporium sphaerospermum	Air	Ross Island	Sun *et al.* (1978)
Cladosporium sphaerospermum	Moss	Prior Island,	Tosi *et al*. (2002)
Cladosporium resinae	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Cladosporium resinae	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Cladosporium resinae	Soil	Ross Island	Greenfield *et al.* (1981)
Cladosporium resinae	Soil	Mawson Base	Line (1988)
Cladosporium elatum	Microbial mat	Lake Hoare	Baublis *et al.* (1991)

4.3.6.3 Isolation of *Geomyces* spp. from Antarctica

Geomyces spp. have been reported as indigenous to Antarctica and have been isolated from the martime regions to the McMurdo Dry Valleys (Vishniac, 1993). *Geomyces* spp. are often the most commonly isolated fungi from bird influenced regions and are known to be keratinolytic organisms. The *Geomyces* species, which substrate isolated from, location in Antarctica from where they were isolated are presented in Table 4.3.

Table 4.3: Isolation of *Geomyces* spp. (*Chrysosporium*) in Antarctica, substrate isolated from and location.

Fungus	Substrate from which isolated	Location	Reference
Chrysosporium sp.	Soil	Ross Island Marble Point	Aislabie *et al.* (2001)
Chrysosporium sp.	Soil, Moss	Windmill Island	Azmi and Seppelt (1998)
Chrysosporium sp.	Soil	Mawson Base	Line (1988)
Chrysosporium sp.	Soil	Newfoundland Coast	Mercantini *et al.* (1989)
Chrysosporium sp.	Plant material Bird nests	Signy Island	Pugh and Allsopp (1982)
Chrysosporium carmichael	Dust	Terra Nova Bay	Mercantini *et al.* (1993)
Chrysosporium indicum	Soil	E.Frey Base	Caretta and Piontelli (1977)
Chrysosporium keratinophilum	Soil	Desolación Island	Caretta and Piontelli (1977)
Chrysosporium merdarium	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Chrysosporium merdarium	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Chrysosporium merdarium	Soil	Ross Island	Sun *et al.* (1978)
Chrysosporium pannorum	Soil	Windmill Island	Azmi and Seppelt (1997)
Chrysosporium pannorum	Soil, Moss, Algae, Lichens	Windmill Island	Azmi and Seppelt (1998)

Table 4.3: Isolation of *Geomyces* spp. (*Chrysosporium*) in Antarctica, substrate isolated from and location.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Chrysosporium pannorum	Microbial mat Mud , melt water, $CaCO_3$ precipitate	Lake Hoare Canada Glacier	Baublis *et al.* (1991)
Chrysosporium pannorum	Soil	McMurdo Dry Valleys	Cameron *et al*. (1977)
Chrysosporium pannorum	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Chrysosporium pannorum	Animal material	Mac.Robertson Land	Fletcher *et al.* (1985)
Chrysosporium pannorum	Soil	Ross Island	Greenfield (1981)
Chrysosporium pannorum	Soil	Windmill Island	Heatwole *et al.* (1989)
Chrysosporium pannorum	Plant material	Signy Island	Hurst *et al*. (1983)
Chrysosporium pannorum	Litter material	Macquarie Island	Kerry (1990a)
Chrysosporium pannorum	Soil	Terra Nova Bay Newfoundland Coast	Mertcantini *et al*. (1993)
Chrysosporium pannorum	Plant material, moss, soil, Bird nests	Signy Island	Pugh and Allsopp (1982)
Chrysosporium pannorum	Soil	Ross Island	Sun *et al*. (1978)
Chrysosporium pannorum	Soil	West Ongul Isalnd	Tubaki (1961)
Chrysosporium pannorum	Soil	Ross Island	Tubaki and Asano (1965)
Chrysosporium pannorum	Soil	Kay Island, Terra Nova Base, Cape king	Onofri *et al*. (1994)
Chrysosporium verrucosum	Penguin dung and feathers, Soil, Petrel feathers	Inexpressible Island, Kay Island, Edmonson Point , Vegetation Island, Terra Nova Base, Cape King	Del Frate and Caretta (1990)
Chrysosporium verrucosum	Moss	Signy Island	Hurst *et al*. (1983)
Chrysosporium verrucosum	Moss	Kay Island	Onofri *et al.* (1994)
Chrysosporium verrucosum	Soil	Ross Island	Tubaki and Asano (1965)
Chrysosporium verrucosum	Soil	McMurdo Dry Valleys	Cameron *et al*. (1977)
Chrysosporium verrucosum	Soil	West Ongul Isalnd	Tubaki (1961)
Geomyces sp.	Historic wood	Ross Island	Held *et al.* (2005)
Geomyces sp.	Soil, Moss, Algae, Lichens	Windmill Island	Azmi and Seppelt (1998)
Geomyces sp.	Plant material	Signy Island	Latter and Heal (1971)
Geomyces sp.	Soil under moss, moss, algal remains, soil and penguin dung, soil with algae and lichens, fresh moss, moss with lichen, soil lacking vegetation	Carezza Lake, Bakers Rock, Cape Russel, Edmonson Point, Gondwana Station. Inexpressible Island, Kay Island, Lamplung Island, Prior Island	Onofri *et al.* (1994)
Geomyces sp. C239	Soil, Historic wood, other material	Ross Island Lake Fryxell basin	Arenz *et al.* (2006)

Table 4.3: Isolation of *Geomyces* spp. (*Chrysosporium*) in Antarctica, substrate isolated from and location.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Geomyces sp. GFI	Soil	New Harbour	Arenz *et al.* (2006)
Geomyces pannorum	Soil, Historic wood, other material	Ross Island	Arenz *et al.* (2006)
Geomyces pannorum	Soil, contaminated soil, wood, rock, moss, algae,	Vestfold Hills, Mawson Station	Kerry (1990b)
Geomyces pannorum	Soil, feather, air	Signy Island	Marshall (1998)
Geomyces pannorum	Moss	Windmill Island	McRae and Seppelt (1999)
Geomyces pannorum	Moss, soil beneath moss	Crater Cirque, Edmonson Point, Kay Island	Onofri *et al.* (1994)
Geomyces pannorum	Soil	Signy Island	Weinstein *et al.* (2000)
Geomyces pannorum	Soil historic wood, other materials	Ross Island	Arenz *et al.* (2006)
Geomyces pannorum var. *pannorum*	Soil	Kay Island, Terra Nova base.	Del Frate and Caretta (1990)
Geomyces pannorum var. *pannorum*	Soil under moss, moss, Moss and soil under moss, Moss and sand under moss	Kay Island, Giardino, Carezza Lake, Campo Icaro, Crater Cirque	Fenice *et al.* (1997)
Geomyces pannorum var. *pannorum*	Soil	Terra Nova Bay Newfoundland Coast	Mercantini *et al.* (1993)
Geomyces pannorum var. *pannorum*	Lichen, moss	King George Island	Möller and Dreyfuss (1996)
Geomyces pannorum var. *pannorum*	Soil under moss, moss, Moss and soil under moss, Moss and sand under moss	Kay Island, Giardino, Carezza Lake, Campo, Icaro, Crater Cirque	Zucconi *et al.* (1996)
Geomyces pannorum var. *pannorum*	Moss	Cape Irizar, Starr Nunatak, Cape Reynolds, Cape Satsrugi, Kay Island, Kohler Head, Harrow Peaks,	Tosi *et al*. (2002)
Geomyces pannorum var. *vinaceus*	Soil	Terra Nova Bay Newfoundland Coast	Mercantini *et al.* (1993)
Geomyces pannorum var. *vinaceus*	Lichen, moss	King George Island	Möller and Dreyfuss (1996)
Geomyces pannorum var. *vinaceus*	Moss	Gondwana Lake, Bruce Point, Adelie Cove, Tinkers glacier, Snowy Point, Camp Irizar, Harrow Peaks,	Tosi *et al*. (2002)
Geomyces vulgare	Soil	Terra Nova Bay Newfoundland Coast	Mercantii *et al.* (1993)

4.3.6.3 Isolation of *Penicillium* spp. from Antarctica

Penicillium spp. have been reported as indigenous to Antarctica and have been isolated from the martime regions to the McMurdo Dry Valleys (Vishniac, 1993). *Penicillium* spp. are often the most commonly isolated fungi from bird influenced regions and is known to be a keratinolytic organisms (McRae and Seppelt, 1999). Cameron (1971) suggested that *Penicillium* spp. were the most common genus of fungi in the Antarctic. The *Penicillium* species, substrate from which isolated, location in Antarctica from where they were isolated are presented in Table 4.4.

Table 4.4 Isolation of *Penicillium* spp. in Antarctica, substrate isolated from and location.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Penicillium sp.	Soil, Moss, Algae	Windmill Island	Azmi and Seppelt (1998)
Penicillium sp.	Soil	Wright Valley	Boyd *et al.* (1966)
Penicillium sp.	Soil	Mt Melbourne	Broady *et al.* (1987)
Penicillium sp.	Air	Don Juan Pond	Cameron *et al.* (1972)
Penicillium sp.	Air	Lake Vanda, Lake Vida, Lake Fryxell	Cameron *et al*. (1974)
Penicillium sp.	Soil	Desolación Island , E.Frey Base, General O'Higgins Base	Caretta and Piontelli (1977)
Penicillium sp.	Plant material, Soil	Mac.Robertson Land Enderby Land	Fletcher *et al.* (1985)
Penicillium sp.	Soil	Ross Island	Greenfield (1982)
Penicillium sp.	Soil	Windmill Island	Heatwole *et al.* (1989)
Penicillium sp.	Moss	Signy Island	Hurst *et al.* (1983)
Penicillium sp.	Contaminated soil, moss, soil,wood	Vestfold Hills, Mawson Station	Kerry (1990b)
Penicillium sp.	Plant material	Signy Island	Latter and Heal (1971)
Penicillium sp.	Soil	Newfoundland Coast	Mercantini *et al.* (1989)
Penicillium sp.	Soil	Terra Nova Bay Newfoundland Coast	Mercantini *et al.* (1993)
Penicillium sp.	Hay	Ross Island	Meyer *et al.* (1963)
Penicillium sp.	Soil beneath moss, mummified seal	Carezza Lake, Inexpressible Island	Onofri *et al.* (1994)
Penicillium sp.	Moss	Mount Melbourne	Tosi *et al*. 2002
Penicillium adametzi	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Penicillium adametzi	Soil	Wright Valley	Cameron *et al.* (1976)
Penicillium adametzi	Soil	Ross Island	Greenfield (1981)
Penicillium adametzi	Soil	Wright Valley	Sun *et al.* (1978)
Penicillium adametzi	Soil	Ross Island	Tubaki and Asano (1965)
Penicillium brevicompactum	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium brevicompactum	Pant material and soil	Enderby land	Fletcher *et al.* (1985)
Penicillium brevicompactum	Plant litter	Macquarie Isalnd	Kerry (1990a)

Table 4.4 Isolation of *Penicillium* spp. in Antarctica, substrate isolated from and location.

Fungus	Substrate from which isolated	Location	Reference
Penicillium brevicompactum	Moss	King George Island	Möller and Dreyfuss (1996)
Penicillium brevicompactum	Moss	Mount Melbourne	Tosi *et al*. (2002)
Penicillium canescens	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Penicillium canescens			Cameron *et al.* (1976)
Penicillium canescens	Soil, Air	Victoria Valley, Ross Island	Sun *et al.* (1978)
Penicillium canescens	Soil	Ross Island	Tubaki and Asano (1965)
Penicillium charlesii	Soil	Ross Island	Tubaki and Asano (1965)
Penicillium chrysogenum	Soil, Moss, Algae	Windmill Island	Azmi and Seppelt (1998)
Penicillium chrysogenum	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Penicillium chrysogenum	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Penicillium chrysogenum	Soil	E.Frey Base	Caretta and Piontelli (1977)
Penicillium chrysogenum	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium chrysogenum	Soil	Ross Island	Greenfield (1981)
Penicillium chrysogenum	Soil, air	Ross Island, Heald Island, Wright Valley, Taylor Valley,	Sun et al (1978)
Penicillium citreo-viride	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Penicillium citreo-viride	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Penicillium citreo-viride	Air	Lake Bonney	Sun *et al.* (1978)
Penicillium commune	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium commune	Moss	Windmill island	McRae *et al*. (1999)
Penicillium corylophilum	Soil	McMurdo Dry Valleys	Cameron *et al.* (1977)
Penicillium corylophilum	Soil	McMurdo Dry Valleys	Cameron *et al.* (1976)
Penicillium corylophilum	Moss	Windmill Island	McRae and Seppelt (1999)
Penicillium corylophilum	Soil, Air	Wright Valley, Ross Island, Victoria Valley	Sun *et al.* (1978)
Penicillium corylophilum	Soil	Ross Island	Tubaki and Asano (1965)
Penicillium crustosum	Soil	Ross Island	Tubaki and Asano (1965)
Penicillium cyclopium	Animal material Soil	Mac.Robertson Land Enderby Land	Fletcher *et al.* (1985)
Penicillium cyclopium	Soil	Wilkes Island	Heatwole et al (1989)
Penicillium cyclopium	Plant Litter	Macquarie Island	Kerry (1990a)
Penicillium decumbes	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium echinulatum	Soil	Wilkes Island	Heatwole *et al.* (1989)
Penicillium echinulatum	Historic wood Soil, other material	Ross Island New Harbour	Held *et al.* (2005) Arenz *et al.* (2006)
Penicillium expansum	Soil, Moss, Algae	Windmill Island	Azmi and Seppelt (1998)
Penicillium expansum	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium expansum	Soil	Ross Island	Greenfield (1981)
Penicillium expansum	Historic wood	Ross Island	Held et al (2005)

Table 4.4 Isolation of *Penicillium* spp. in Antarctica, substrate isolated from and location.

Fungus	**Substrate from which isolated**	**Location**	**Reference**
Penicillium frequentans	Soil	Ross Island	Greenfield (1981)
Penicillium funiculosum	Soil	Ross Island	Greenfield (1981)
Penicillium glabrum	Moss	King George Island	Möller and Dreyfuss (1996)
Penicillium janthinellum	Air	Macquarie Island, Cabo Primavera, Ellsworth, Bahia Esperanza	Corte and Daglio (1962)
Penicillium janthinellum	Moss	King George Island	Möller and Dreyfuss (1996)
Penicillium jensenii	Soil	Taylor Valley	Baublis *et al.* (1991)
Penicillium jensenii	Leaf, soil	Casey Station	Kerry (1990a)
Penicillium lilacinum	Soil	Desolación Island, Puerto Williams, E.Frey Base, A Prat base, Gen.O'Higgins base	Carretta and Piontelli (1977)
Penicillium lilacinum	Soil	Ross Isalnd	Greenfield (1981)
Penicillium miniluteum	Moss	Gondwana lake, Mount Melbourne	Tosi *et al*. (2002)
Penicillium notatum	Mud, microbial mat	Canada Glacier	Baublis *et al.* (1991)
Penicillium oxalicum	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium palitans	Soil, moss	Windmill Island	Azmi and Seppelt (1998)
Penicillium ramigena	Air	Cabo Primavera, Macquarie Island	Corte and Daglio (1962)
Penicillium roquefortii	Air	Cabo Primavera	Corte and Daglio (1962)
Penicillium solitum	Moss	Windmill island	McRae and Seppelt (1999)
Penicillium soppi	Microbial mat	Lake Hoare	Baublis *et al.* (1991)
Penicillium cf. spinulosum	Plant material	Enderby Land	Fletcher *et al.* (1985)
Penicillium spinulosum	Soil, feather, air	Signy Island	Marshall (1998)
Penicillium verrucosa	Microbial mat	Lake Hoare	Baublis *et al.* (1991)
Penicillium waksmanii	Moss	Signy Island	Pugh and Allsopp (1982)

Overall, the fungi selected for indepth growth characteristic studies are found in a wide range of locations and in association with a variety of substrates.

This chapter demonstrates the growth characteristic, including growth temperature range, ability to utilise a variety of substrates for metabolism and growth in a range of water activity conditions for seven Antarctic isolates.

4.4 Materials and Methods

4.4.1 Media

The following media were used in the experiments related to this chapter's objectives:

Malt Yeast Extract Agar (YM) - 1.5% malt extract, 0.2% yeast extract, 1.8% agar in distilled water.

Malt Yeast Extract Broth (YM broth)-1.5% malt extract, 0.2% yeast extract, in distilled water.

Carboxymethylcellulose *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7$ H_2O, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Carboxymethylcellulose and 1.5% agarose in distilled water.

Avicel *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Avicel and 1.5% agarose in distilled water.

Hydroxyethylcellulose *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Hydroxyethylcellulose (Aldrich, USA) and 1.5% agarose in distilled water.

All media were sterilised by autoclaving for 20 minutes at 121°C, then dispensed into 90 mm Petri dishes.

4.4.2 Growth characteristics of fungi in liquid media

The following seven Antarctica fungal isolates were characterised for their growth rate in liquid medium: *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805. These strains were selected on the results from the cellulase screening study described in Section 5.5.1. These strains were isolated from the sites and samples as given in Table 4.5. The fungal isolates were grown on liquid medium to determine growth curves and growth rates at 4°C, 10°C, 15°C, 20°C and 25°C.

Table 4.5: Fungal isolate, and isolation hut, location and temperature.

Fungal isolate	**Hut**	**Location**	**Isolation temperature**
Cadophora malorum 182	Cape Evans	Swab damp spot against wall lower bunk on S side	15°C
Cadophora malorum 242	Cape Evans	Swab damp spot against wall lower bunk on S side	15°C
Penicillium roquefortii 405	Cape Evans	Exterior wood sample from 2nd boards removed from cape Evans and store in container at Scott base	15°C
Penicillium roquefortii 408	Cape Evans	Exterior wood sample from 2nd boards removed from cape Evans and store in container at Scott base	15°C
Geomyces sp. 711	Cape Evans	Swab damp spot against wall lower bunk on S side	4°C
Geomyces sp. 824	Hut Point	Soil NE corner post of veranda	4°C
Cladosporium oxysporum 805	Hut Point	Soil NE corner post of veranda	4°C

Each of the seven isolates were grown independently in three 250 ml flasks containing 50 ml of YM broth, inoculated with 5ml of inoculum which had been prepared as a 48 hr growth in YM Broth in a Universal bottle at experimental temperature. Flasks were shaken on a rotary shaker (Chiltern) with the rotary shaker revolving at 120 rpm and flasks were set at the different temperatures (4°C, 10°C, 15°C, 20°C and 25°C) of study. 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 at which time a steady dry weight was reached. For fungal growth, incubations at 4°C, mycelial dry weight was determined every 3 days for 31 days; for 10°C, 15°C , 20°C and 25°C, mycelial dry weight was determined every day for 10 days. Dry weight (mg/ml) was calculated and plotted against days of incubation to create growth curves. The growth rate was

determined by calculating the change in mycelial dry weight per hour during log phase of growth.

4.4.3 Sustained growth of Antarctic fungal isolates at 4°C

Two fungal isolates, *Cadophora malorum* 182, and *Geomyces* sp. 711, were grown repeatedly on liquid medium at 4°C to determine their ability to grow consistently at 4°C.

The method used for this growth experiment was as given in Section 4.4.2. Once cultures had reached the stationary phase of growth, after 11 days, 5mls of stationary phase liquid inoculum was transferred to 50 mls of fresh YM broth in a 250 ml flask. Flasks were incubated at 4°C and shaken on a rotary shaker set at 120 rpm. For fungal growth, mycelial dry weight was determined every 3 days, for an incubation period of 31 days. Growth curves were completed 3 times. All flasks, media, and transfers were maintained and done at 4°C.

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

The seven fungi as described in Section 4.4.2, *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805, were grown on solid media containing various cellulose carbon sources along with medium containing no cellulose and YM medium to compare the ability of the fungi to grow on different forms of cellulose at three temperatures, 4°C, 15°C, and 25°C.

Single isolates of fungi were first grown on the appropriate test medium and incubated at test temperatures prior to establishment of the experiment. Plugs (6mm) of actively growing fungi from the colony margins were placed at the centre of three 90mm plastic Petri dishes of the test medium. Plates consisted of Carboxymethyl cellulose *Trichoderma viride* medium A, Avicel *Trichoderma viride* medium A, and Hydroxyethylcellulose *Trichoderma viride* medium A (Mandels *et al.*, 1962). YM agar plates and *Trichoderma viride* medium plates with no cellulose carbon source were also used to serve as controls of a rich carbon and nitrogen medium and a cellulose-free medium, respectively.

Plates were incubated at 4°C, 15°C or 25°C until the stationary phase of growth 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.

4.4.5 Radial extension rate at varied water activity (A_w)

The seven fungi as described in Section 4.4.2 were grown on solid media with varying available water at 15°C.

Single isolates of fungi were first grown on YM agar. A conidial suspension was prepared by flooding the surface of the fungal culture with sterile saline solution containing Tween 80 (0.9% NaCl, 0.1% (v/v) Tween 80). Ten microlitres of the conidial suspension were placed at the centre of three 90mm plastic Petri dish of the test medium (Sautour *et al*., 2001). The test medium consisted of plates of YM agar with varying amounts of water replaced with glycerol to produce water activity (A_w) ranging from 1- 0.92 (Gervais, 1988) as given in Table 4.6.

Table 4.6: Volume of Glycerol required for decreasing water activity.

Required A_w	1	0.98	0.96	0.94	0.92
Glycerol (grms/Litre Distilled water)	0	102.02	203.94	306.22	409.26

To maintain water activity of the substrate at the exact value throughout the culturing period, the Petri dishes corresponding to one water activity value were placed in a jar in which relative humidity was controlled by a glycerol water solution of the same A_w as the agar plates. The glycerol water solution was changed every 3 days for the duration of the experiment. Plates were incubated at 15°C until stationary phase of growth 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 radial extension intrinsic growth rate was determined by calculating the change in colony diameter per day from inoculation to stationary phase.

4.4.7 Carbon Utilisation

The seven fungi as described in Section 4.4.2 were grown on a range of carbon substrates to determine their carbon utilisation patterns at 1°C and at 15°C.

Preparation and establishment of the Biolog FF microplate carbon utilisation system were conducted, following the steps outlined by Kubicek *et al.* (2003). Colonies of the fungi to be examined were prepared on YM agar until heavy confluent mycelial growth was present. The surface of each agar plate was swabbed with a sterile wetted cotton swab with the collected fungal matter transferred to a Universal bottle containing 16 mL of sterile phytagel solution (0.25% Phytagel (Gibco BRL, USA), 0.03% Tween 40). This was mixed by vortexing for 5 seconds. The solution was adjusted to 75% transmission at 590nm using a spectrophotometer (Thermospectronic Heλios γ). Subsequently, 100μL was aseptically pipetted into each of the 96 wells of the Biolog FF Microplate (Biolog, Hayward, CA). One plate of each isolate was incubated at 1°C and 15°C for 22 days and 12 days, respectively. Optical density was measured every 3 days using a microtitre plate spectrophotometer (Labsystemmultiskan RC) at wavelength 490 nm (to detect the reduction of Iodonitrotetrazolium violet to the red coloured formazan dye) and 750 nm (to reflect mycelium production). To quantify the production of formazan attributed to mitochondrial activity (an indiction that the fungal cells were metabolically active), a redox value was calculated by subtracting the 750 nm reading from the 490 nm reading. Absorbance readings were standardised by the subtraction of the water blank absorbance reading and the individual well absorbance was divided by the average of all 95 well absorbance readings. The complete lists of substrates tested in this experiment are given in Table 4.7.

Table 4.7: Carbon substrates found in BIOLOG FF microtitre plates, divided into chemical guilds (Preston-Mafham *et al.*,2002).

Chemical guild	**Substrate**	**Chemical formula**
Miscellaneous	Adenosine	$C_{10}H_{13}N_5O_4$
	Amygdalin	$C_{20}H_{27}NO_{11}$
	Adenosine-5'-monophosphate	$C_{10}H_{14}N_5O_7P$
	Bromo succinic acid	$C_4H_5O_4Br$
	D-Lactic acid methyl ester	$C_4H_8O_3$
	Glucose-1-phosphate	$C_6H_{13}O_9P$
	Glycerol	$C_3H_8O_3$
	Salicin	$C_{13}H_{18}O_7$
	Succinic acid mono-methyl ester	$C_5H_8O_4$
	Uridine	$C_9H_{12}N_2O_6$
Polymers	α-Cyclodextrin	$C_{36}H_{60}O_{30}$
	β-Cyclodextrin	$C_{42}H_{70}O_{35}$
	Dextrin	$C_6H_{10}O_5$
	Glycogen	$(C_6H_{10}O_5)n$
	Tween 80	

Table 4.7: Carbon substrates found in BIOLOG FF microtitre plates, divided into chemical guilds (Preston-Mafham *et al.*,2002).

Chemical guild	**Substrate**	**Chemical formula**
Amines/amides	2-Amino ethanol	C_2O_7NO
	D-Glucosamine	$C_6H_{13}NO_5$
	Glucuronamide	$C_6H_{11}NO_6$
	L-Alaninamide	$C_3H_8N_2O$
	Putrescine	$C_4H_{12}N_2$
	Succinamic acid	$C_4H_7NO_3$
Amino acids	γ-Amino butyric acid	$C_4H_9N0_2$
	Glycyl-l-glutamic acid	$C_7H_{12}N_2O_5$
	L-Alanine	$C_3H_7NO_2$
	L-Alanyl-glycine	$C_5H_{10}N_2O_3$
	L-Asparagine	$C_4H_8N_2O_3$
	L-Aspartic acid	$C_4H_7NO_4$
	L-Glutamic acid	$C_5H_9NO_4$
	L-Ornithine	$C_5H_{12}N_2O_2$
	L-Phenylalanine	$C_9H_{11}NO_2$
	L-Proline	$C_5H_9NO_2$
	L-Pyroglutamic acid	$C_5H_7NO_3$
	L-Serine	$C_3H_7NO_3$
	L-Threonine	$C_4H_9NO_3$
Carboxylic acids	β-Hydroxy butyric acid	$C_4H_8O_3$
	γ-Hydroxy butyric acid	$C_4H_8O_3$
	α-Keto glutaric acid	$C_5H_8O_5$
	2-Keto-d-gluconic acid	$C_6H_9O_7$
	D-Galacturonic acid	$C_6H_{10}O_7$
	D-Gluconic acid	$C_6H_{12}O_7$
	D-Glucuronic acid	$C_6H_{10}O_7$
	D-Malic acid	$C_4H_6O_5$
	D-Saccharic acid	$C_6H_{10}O_8$
	Fumaric acid	$C_4H_4O_4$
	L-Lactic acid	$C_3H_6O_3$
	L-Malic acid	$C_4H_6O_5$
	N-Acetyl-l-glutamic acid	$C_7H_{11}NO_5$
	p-Hydroxy phenylacetic acid	$C_8H_8O_3$
	Quinic acid	$C_7H_{12}O_6$
	Sebacic acid	$C_{10}H_{18}O_4$
	Succinic acid	$C_4H_6O_4$
Four-carbon carbohydrates	*i*-Erythritol	$C_4H_{10}O_4$
Five-carbon carbohydrates	Adonitol	$C_5H_{12}O_5$
	D-Arabinose	$C_5H_{10}O_5$
	D-Arabitol	$C_5H_{12}O_5$
	D-Ribose	$C_5H_{10}O_5$
	Xylitol	$C_5H_{12}O_5$
	D-Xylose	$C_5H_{10}O_5$
	L-Arabinose	$C_5H_{10}O_5$

Table 4.7: Carbon substrates found in BIOLOG FF microtitre plates, divided into chemical guilds (Preston-Mafham *et al.*,2002).

Chemical guild	Substrate	Chemical formula
Six-carbon carbohydrates	α-D-Glucose	$C_6H_{12}O_6$
	D-Fructose	$C_6H_{12}O_6$
	D-Galactose	$C_6H_{12}O_6$
	D-Mannitol	$C_6H_{14}O_6$
	D-Mannose	$C_6H_{12}O_6$
	D-Psicose	$C_6H_{12}O_6$
	D-Sorbitol	$C_6H_{14}O_6$
	D-Tagatose	$C_6H_{12}O_6$
	L-Fucose	$C_6H_{12}O_5$
	L-Rhamnose	$C_6H_{12}O_5$
	L-Sorbose	$C_6H_{12}O_6$
	m-Inositol	$C_6H_{12}O_6$
Seven-carbon carbohydrates	α-Methyl-D-galactoside	$C_7H_{14}O_6$
	β-Methyl-D-galactoside	$C_7H_{14}O_6$
	α-Methyl-D-glucoside	$C_7H_{14}O_6$
	β-Methyl-D-glucoside	$C_7H_{14}O_6$
	Sedoheptulosan	$C_7H_{12}O_6$
Eight-carbon carbohydrates	*N*-Acetyl-D-galactosamine	$C_8H_{15}NO_6$
	N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$
	N-Acetyl-D-mannosamine	$C_8H_{15}NO_6$
Twelve-carbon carbohydrates	α-D-Lactose	$C_{12}H_{22}O_{11}$
	Arbutin	$C_{12}H_6O_7$
	D-Cellobiose	$C_{12}H_{22}O_{11}$
	D-Melibiose	$C_{12}H_{22}O_{11}$
	D-Trehalose	$C_{12}H_{22}O_{11}$
	Gentiobiose	$C_{12}H_{22}O_{11}$
	Lactulose	$C_{12}H_{22}O_{11}$
	Maltitol	$C_{12}H_{24}O_{11}$
	Maltose	$C_{12}H_{22}O_{11}$
	Palatinose	$C_{12}H_{22}O_{11}$
	Sucrose	$C_{12}H_{22}O_{11}$
	Turanose	$C_{12}H_{22}O_{11}$
>Twelve-carbon carbohydrates	Maltotriose	$C_{18}H_{32}O_{16}$
	D-Melezitose	$C_{18}H_{36}O_{16}$
	D-Raffinose	$C_{18}H_{32}O_{16}$
	Stachyose	$C_{24}H_{42}O_{21}$

4.5 Results

4.5.1 Growth characteristics of fungi in liquid media

Cadophora malorum 182 grew over the complete range of temperatures tested (as seen in Figure 4.1). Growth at 4°C had a lag phase of 3 days, compared to 2 days for growth at 10 and 15°C, 1 day at 20°C and 0.5 of a day at 25°C. The exponential phase of growth was longer at 4°C (15 days) than at the other temperatures tested with the exponential phase of growth being 5 days at 10°C, 3 days at 15°C, 6 days at 20°C and 3.5 days at 25°C. The maximum biomass accumulated was at 10°C after 7 days and was 9.08mg/ml.

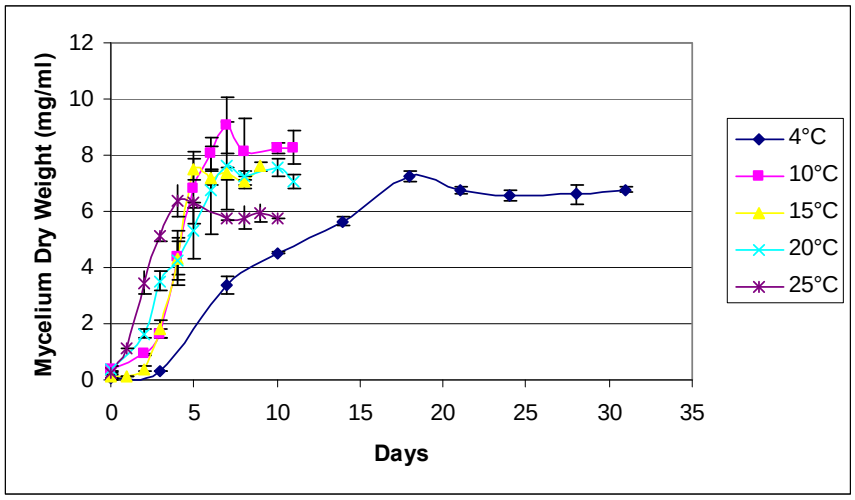

Figure 4.1: Graph of the growth curves for *Cadophora malorum* 182 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Cadophora malorum 242 grew over the complete range of temperatures tested (as seen in Figure 4.2). Growth at 4°C had a lag phase of 3 days, compared to 2 days for growth at 10, 15 days at 15°C, 1 day at 20°C and 0.5 of a day at 25°C. The exponential phase of growth was longer at 4°C (14 days) than at the other temperatures tested with the exponential phase of growth being 6 days at 10 and 15°C, 5 days at 20°C and 7 days at 25°C. The maximum biomass accumulated was at 4°C after 17 days and was 7.7 mg/ml.

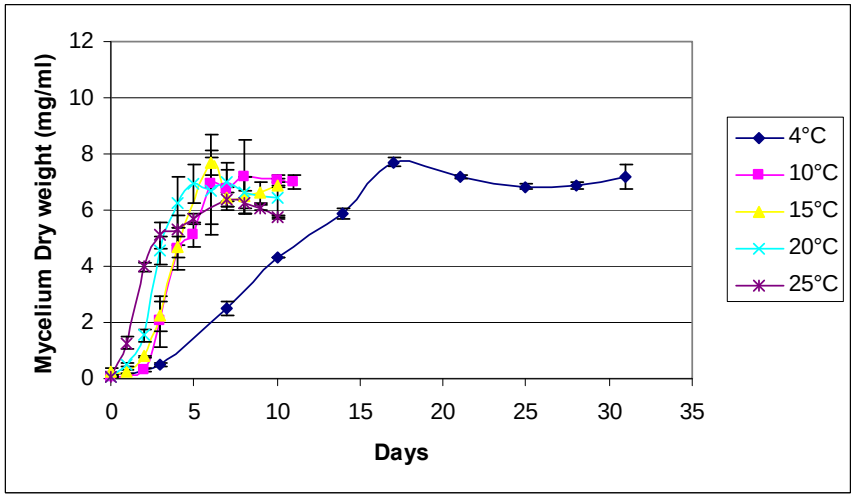

Figure 4.2: Graph of the growth curves for *Cadophora malorum* 242 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Penicillium roquefortii 405 grew over the complete range of temperatures tested (as seen in Figure 4.3). Growth at 4°C had a lag phase of 2 days, compared to 1 day for growth at 10, 15 and 20°C and 0.5 of a day at 25°C. The exponential phase of growth was longer at 4°C (9 days) than at the other temperatures

tested with the exponential phase of growth being 6 days at 10 and 20°C, 7 days at 15°C and 7.5 days at 25°C. The maximum biomass accumulated was at 20°C after 8 days and was 7.64 mg/ml.

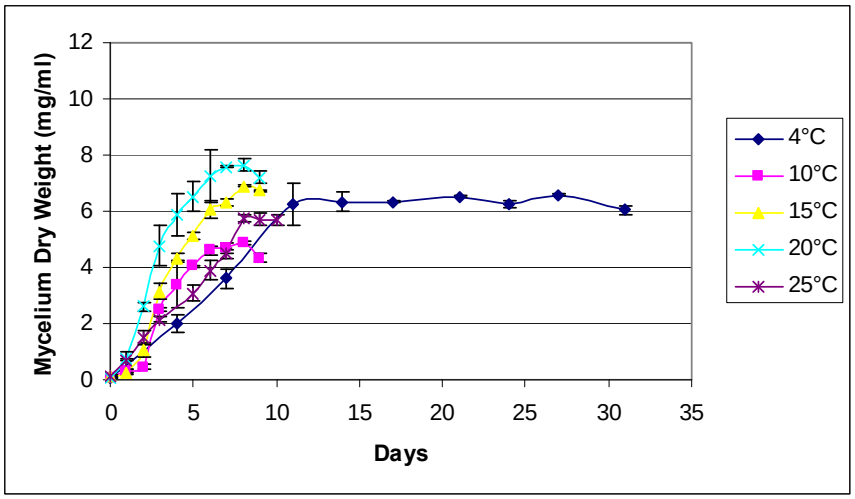

Figure 4.3: Graph of the growth curves for *Penicillium roquefortii* 405 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Penicillium roquefortii 408 grew over the complete range of temperatures tested (as seen in Figure 4.4). Growth at 4°C had a lag phase of 2 days, compared to 3 day for growth at 10, 1 day at 15 and 20°C and 0.5 of a day at 25°C. The exponential phase of growth was longer at 4°C (14 days) than at the other temperatures tested with the exponential phase of growth being 3 days at 10, 15 and 20°C, and 3.5 days at 25°C. The maximum biomass accumulated was at 25°C after 4 days and was 7.73 mg/ml.

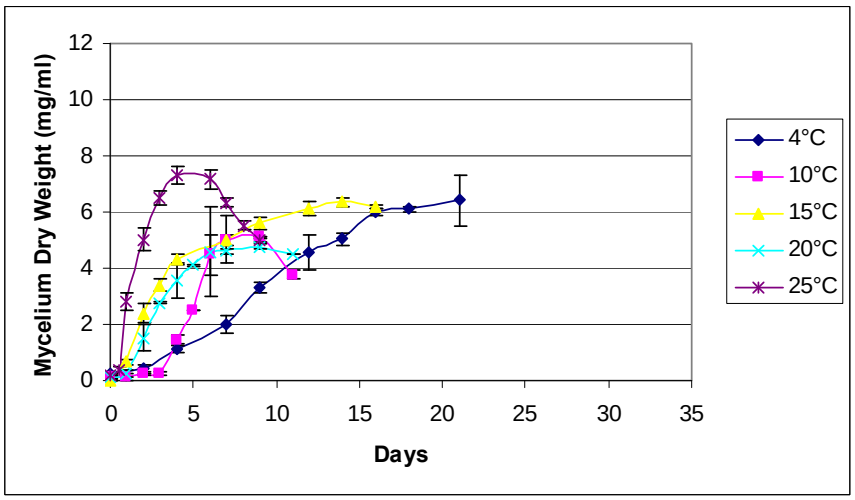

Figure 4.4: Graph of the growth curves for *Penicillium roquefortii* 408 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Geomyces sp. 711 grew over the complete range of temperatures tested (as seen in Figure 4.5). Growth at 4°C had a lag phase of 3 days, compared to 1 day for growth at 10, 15 and 20°C and 2 days at 25°C. The exponential phase of growth was longer at 4°C (21 days) than at the other temperatures tested with the exponential phase of growth being 9 days at 10, and 25°C, and 6 days at 15 and 20°C. The maximum biomass accumulated was at 4°C after 24 days and was 7.7 mg/ml.

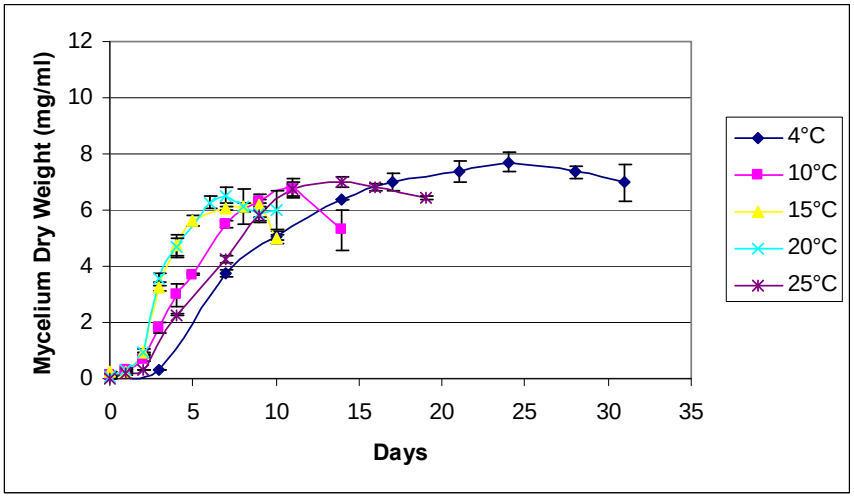

Figure 4.5: Graph of the growth curves for *Geomyces* sp. 711 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Geomyces sp. 824 grew over the complete range of temperatures tested (as seen in Figure 4.6). Growth at 4°C had a lag phase of 3 days, compared to 1 day for growth at 10°C and 20°C and 2 days at 15 and 25°C. The exponential phase of growth was longer at 4°C (21 days) than at the other temperatures tested with the exponential phase of growth being 7 days at 10°C, 5 days at 15 and 20°C and 6 days at 25°C. The maximum biomass accumulated was at 15°C after 8 days and was 7.84 mg/ml.

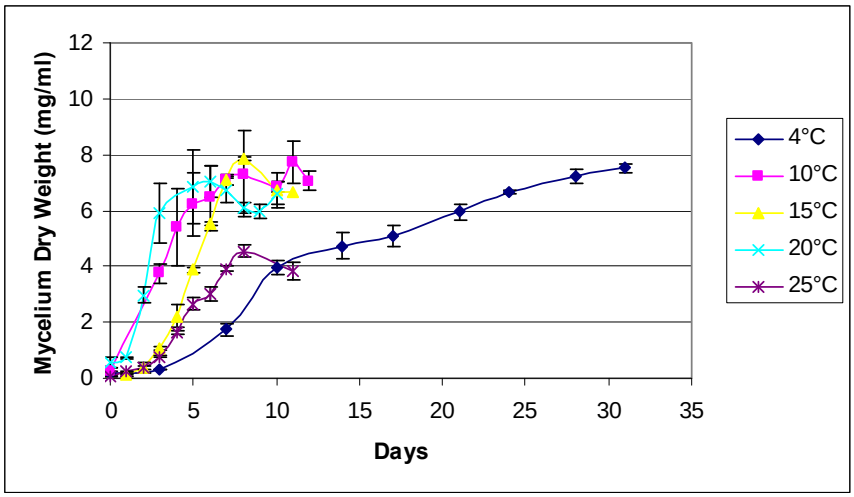

Figure 4.6: Graph of the growth curves for *Geomyces* sp. 824 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Cladosporium oxysporum 805 grew over the complete range of temperatures tested (as seen in Figure 4.7). Growth at 4°C had a lag phase of 5 days, compared to 3 day for growth at 10°C and 20°C, 1.5 days at 15°C and 1 day 25°C. The exponential phase of growth was longer at 4°C (14 days) than at the other temperatures tested with the exponential phase of growth being 8 days at 10 and 20°C, 8.5 days at 15°C and 7 days at 25°C. The maximum biomass accumulated was at 4°C after 19 days and was 10.22 mg/ml.

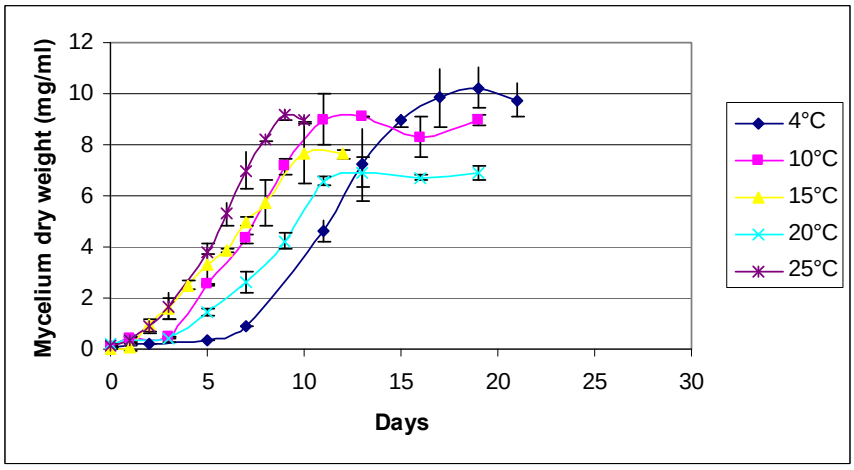

Figure 4.7: Graph of the growth curves for *Cladosporium oxysporum* 805 at 4, 10, 15, 20, and 25°C. Vertical bars represent the standard error.

Figure 4.8 shows the growth rate of the seven isolates as measured at 5 temperatures (4, 10, 15, 20 and 25°C). All four species showed the ability to grow at 4°C, *Penicillium roquefortii* 405 and *Cladosporium oxysporum* 805 both had the fastest growth rate (largest fungal biomass accumulation per hour in the log phase of growth) at 4°C. *Cadophora malorum* 182 had higher growth

rates and a larger fungal biomass accumulation per hour in the log phase of growth at 15°C than at the other 4 temperatures tested. *Cadophora malorum* 242 had near identical growth rates and fungal biomass accumulation per hour in the log phase of growth at 10, 15 and 20°C than at 4 and 25°C. *Penicillium roquefortii* 405 had higher growth rates and a larger fungal biomass accumulation per hour in the log phase of growth at 15°C than at the other 4 temperatures tested. *Penicillium roquefortii* 408 had a higher growth rates and a larger fungal biomass accumulation per hour in the log phase of growth at 25°C than at the other 4 temperatures tested but had the same growth rates and fungal biomass accumulation per hour in the log phase of growth at 10,15 and 25°C. *Geomyces* sp 711 had higher growth rates and a larger fungal biomass accumulation per hour in the log phase of growth at 15 and 20°C than at the other 3 temperatures tested. *Geomyces* sp. 824 had a slightly higher growth rate and a larger fungal biomass accumulation per hour in the log phase of growth at 15°C than at 25°C and both were higher than at the other 3 temperatures tested. *Cladosporium oxysporum* 805 had higher growth rates and a larger fungal biomass accumulation per hour in the log phase of growth at 25°C than at the other 4 temperatures tested.

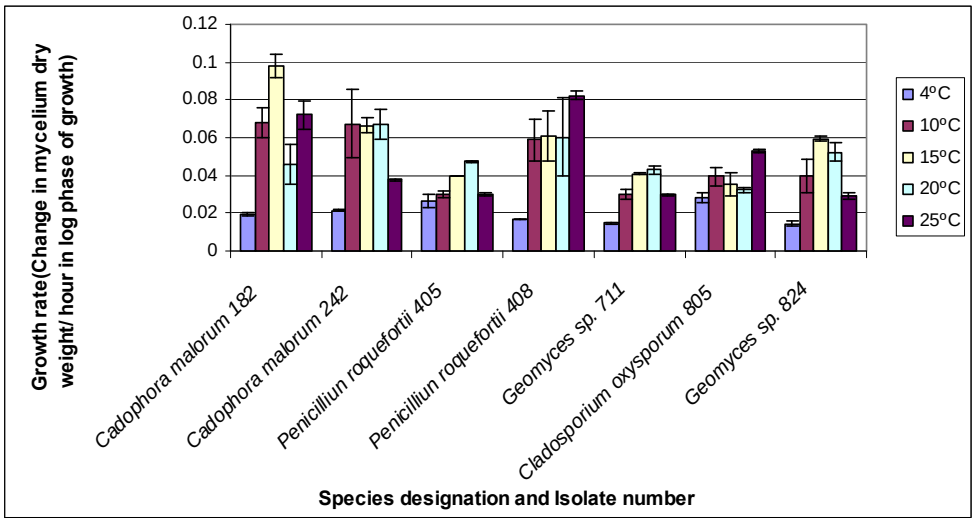

Figure 4.8: Graph of the growth rates of *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 in YM liquid broth. Vertical bars represent the standard error.

4.5.2 Sustained growth of Antarctic fungal isolates at 4°C

Sustained growth of fungal isolates *Cadophora malorum* 182, and *Geomyces* sp. 711 in YM Broth at 4°C was assessed.

Both *Cadophora malorum* 182 and *Geomyces* sp. 711 were repeatedly able to sustain growth at 4°C as shown in Figure 4.8 and 4.9. The slopes for *Cadophora malorum* 182 were as follows:

- 1st inoculation was 0.38
- 2nd inoculation 0.37
- 3rd inoculation 0.35

The slopes for *Geomyces* sp. 711 were as follows

- 1st inoculation 0.41
- 2nd inoculation 0.41
- 3rd inoculation 0.38

Interestingly the rate of growth, as determined by the slope of the curve during active growth, was nearly the same for all three inoculations for both isolates showing that the cultures were responding the same physiologically to each exposure at 4°C.

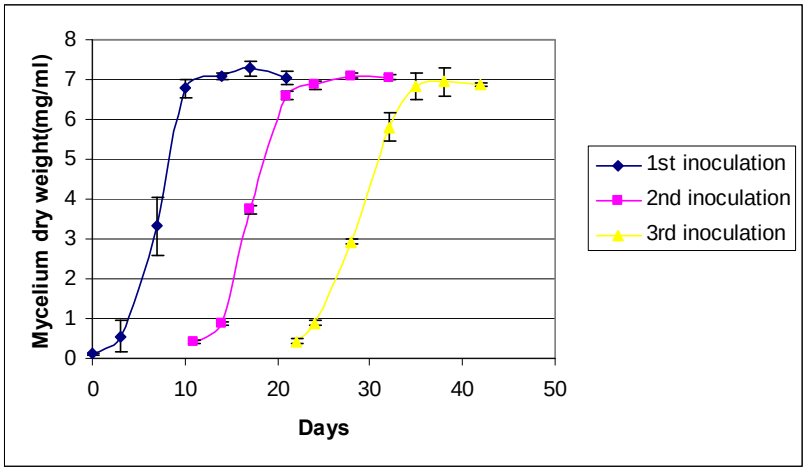

Figure 4.9: Graph of the sustained growth of *Cadophora malorum* 182 in YM broth at 4°C. Vertical bars represent the standard error.

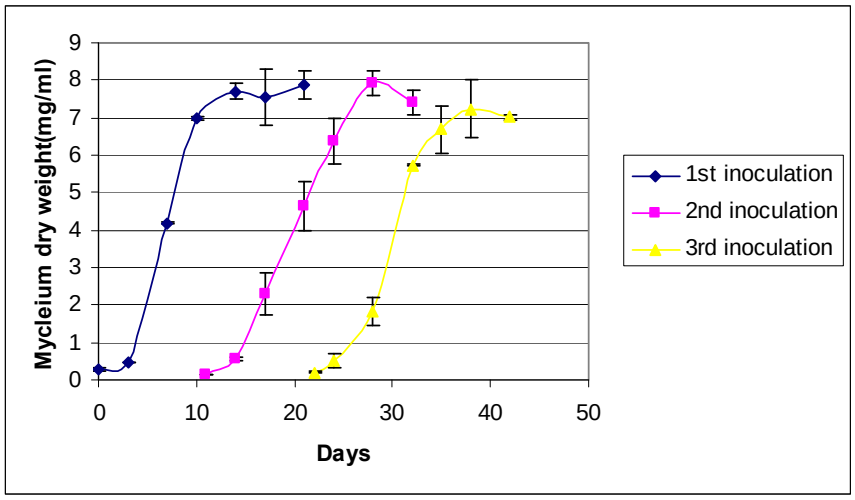

Figure 4.10: Graph of the sustained growth of *Geomyces* sp. 711 in YM broth at 4°C. Vertical bars represent the standard error.

4.5.3 Radial Hyphal extension rate on various media and selected cellulose carbon sources

The seven fungi described in Section 4.4.2, selected for growth on various media and cellulose carbon sources all demonstrated the ability to grow on all three carbon sources tested. *C. malorum* 182, *C. malorum* 242, *P. roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711 and *Geomyces* sp. 824 all grew faster at 25°C but all could still grow at 4°C on YM medium as seen in Figure 4.11. The exception was *Cladosporium oxysporum* 805, it had fastest growth rate at 15°C, but still grew at 25°C or 4°C on YM medium.

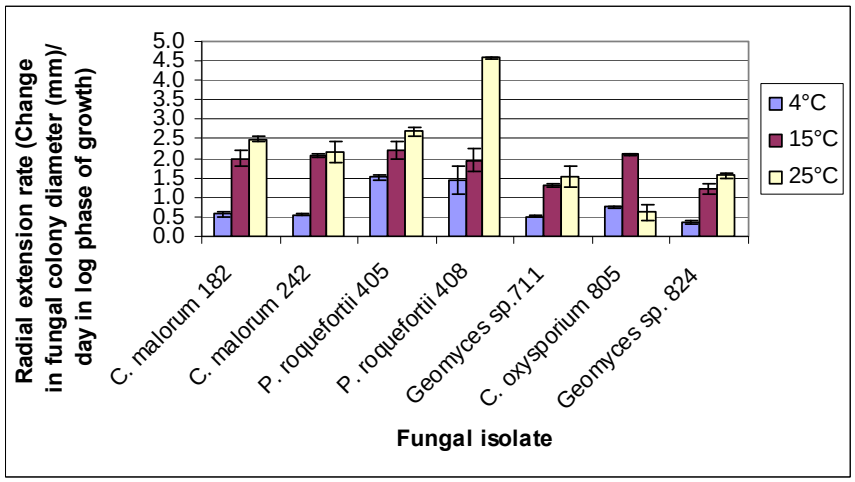

Figure 4.11: Graph of the radial extension rates of *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 on YM medium. Vertical bars represent the standard error.

Both *C. malorum* strains 182 and 242 grew on all sources of cellulose tested at 4°C, 15°C and 25°C but did not grow on the agar plates containing no

cellulose at 4°C, 15°C and 25°C (Figure 4.11 and Figure 4.12). The growth rate at 4°C was the same on all three sources of cellulose. The growth rate at 15°C and 25°C was slower when the two *C. malorum* strains were grown on hydroxyethylcellulose (HEC) compared with the growth rate on CMC and Avicel as seen in the Figure 4.12 and 4.13.

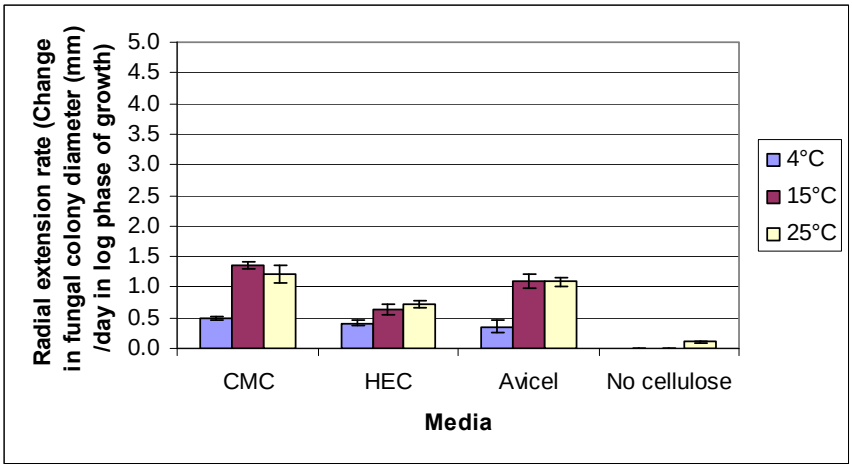

Figure 4.12: Graph of the radial extension rate of *Cadophora malorum* 182 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

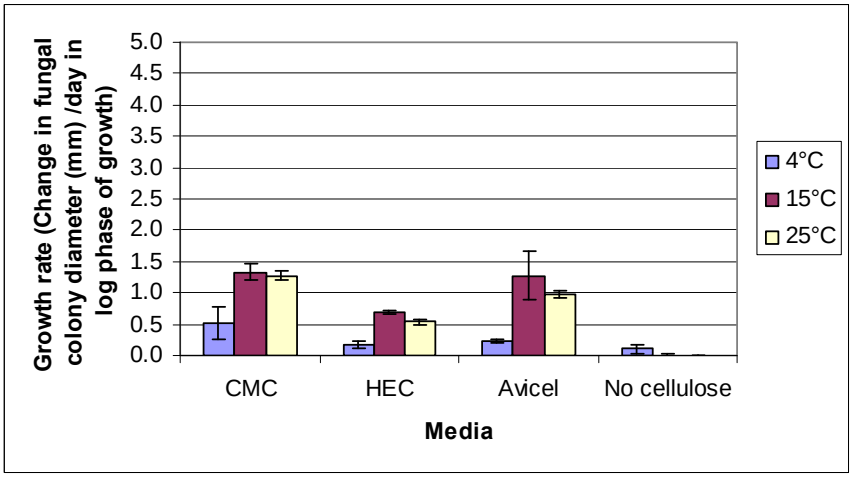

Figure 4.13: Graph of the radial extension rate of *Cadophora malorum* 242 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

Both *P. roquefortii* strains 405 and 408 grew on all sources of cellulose tested; the two *Penicillium* isolates also grew on the agar plates containing no cellulose at 4°C, 15°C and 25°C, but the growth was slower than on cellulose plates at 4°C, 15°C and 25°C (Figure 4.14 and Figure 4.15). The growth rate at 4°C was slower when *P. roquefortii* 405 was grown on HEC compared with the growth rate on the other two sources of cellulose. The growth rate was the same at 15°C on HEC and Avicel and was faster when *P. roquefortii* 405 was grown on medium containing CMC as a carbon source. The growth rate at 25°C was less on

HEC and the same on CMC and Avicel. *P. roquefortii* 408 grew the fastest of the seven fungi used in this experiment on all media containing cellulose. The growth rate at 4°C was slower when *P. roquefortii* 408 was grown on Avicel compared with the growth rate on the other two sources of cellulose. The growth rate was the same at 15°C on HEC and CMC and was faster when *P. roquefortii* 408 was grown on medium containing Avicel as a carbon source. The growth rate at 25°C was less on HEC and more when *P. roquefortii* 408 was grown on Avicel.

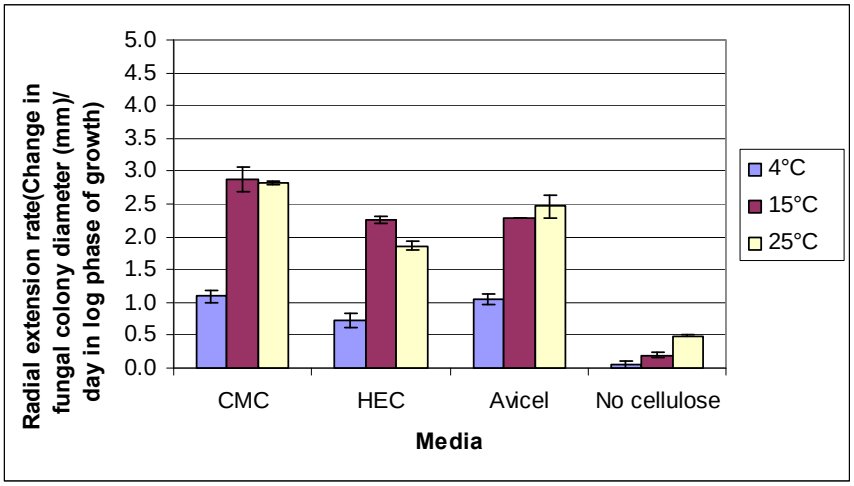

Figure 4.14: Graph of the radial extension rate of *Penicillium roquefortii* 405 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

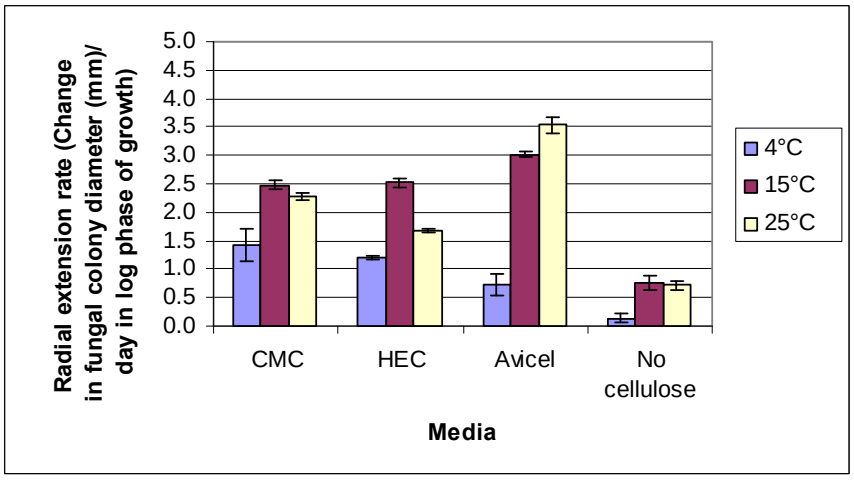

Figure 4.15: Graph of the radial extension rate of P*enicillium roquefortii* 408 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

Geomyces sp. 711 grew on all sources of cellulose tested including the agar plates containing no cellulose at 4°C, 15°C and 25°C (Figure 4.16). The growth rate at 4°C was the same on all three sources of cellulose as the growth rate was the same at 15°C on all three sources of cellulose, but it was slower when

Geomyces sp. was grown on the medium containing no cellulose as a carbon source. The growth rate at 25°C was less on Avicel than the other two carbon sources and less by about half on the medium containing no cellulose as a carbon source.

Geomyces sp. 824 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 (Figure 4.17). 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 source of cellulose but was slower when *Geomyces* sp. was grown on the medium containing no cellulose as a carbon source. The growth rate at 25°C was less on HEC and Avicel and less on the medium containing no cellulose as a carbon source (Figure 4.17).

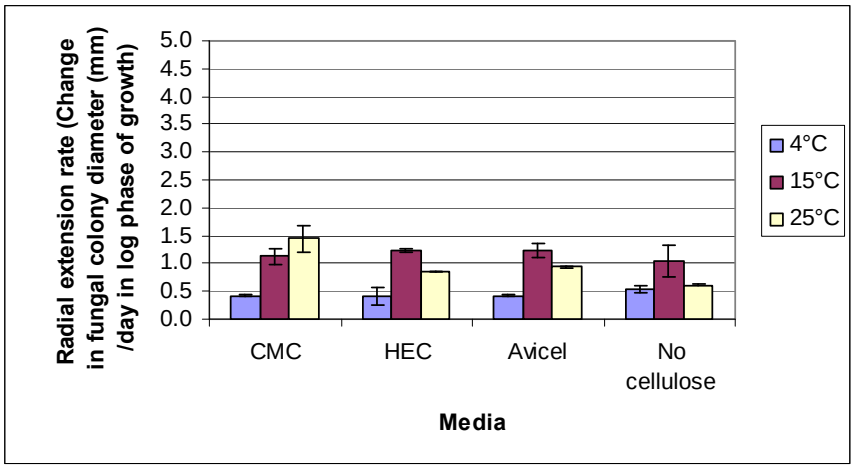

Figure 4.16: Graph of the radial extension rate of *Geomyces* sp. 711 on different cellulose carbon sources on at 4, 15 and 25°C. Vertical bars represent the standard error.

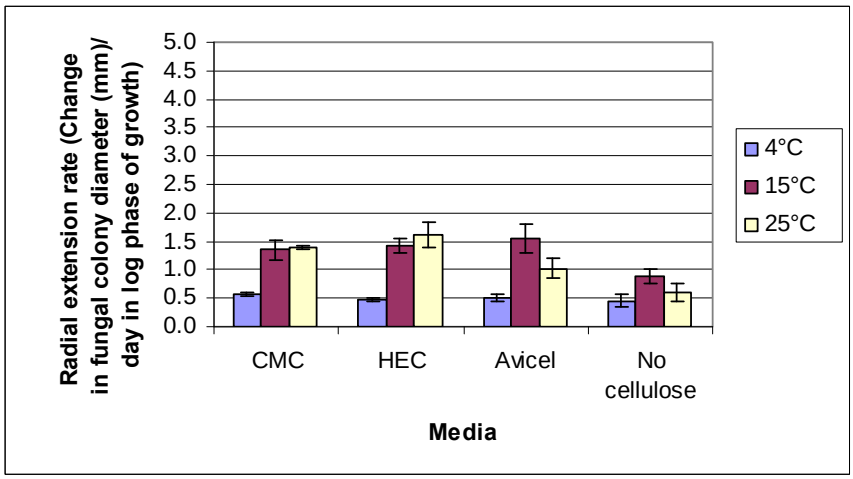

Figure 4.17: Graph of the radial extension rate of *Geomyces* sp. 824 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

C. oxysporum 805 grew on the all three cellulose sources at 4°C, 15°C and 25°C, with a similar growth rate at each of the temperatures, but did not grow on the medium containing no cellulose (Figure 4.18).

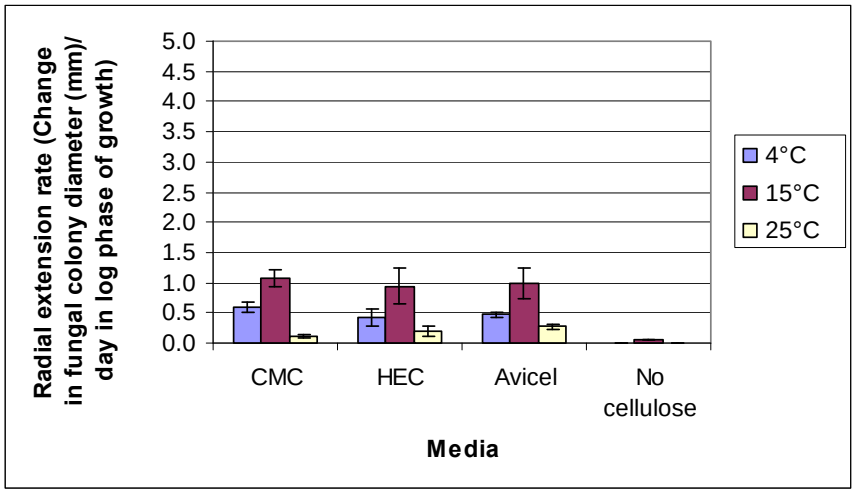

Figure 4.18: Graph of the radial extension rate of *Cladosporium oxysporum* 805 on different cellulose carbon sources at 4, 15 and 25°C. Vertical bars represent the standard error.

4.5.4 Radial extension rate at varied Water activity (A_w)

All seven fungi described in Section 4.4.2, selected for evaluating growth at varied water activity, grew over a range of water activity values from 1 to 0.90. For both strains of *C. malorum*, 182 and 242, the optimal water activity value for growth was found to be 1.0; below 0.92, no growth occurred. For both strains of *P. roquefortii*, 405 and 408, the optimal water activity value for growth was found to be 0.98 and growth occurred over the entire range of water activity values tested. For *Geomyces* sp. 711, the optimal water activity value for growth was found to be 1.0, and like the *C. malorum* isolates, below 0.92 no growth occurred. For *Geomyces* sp. 824, the optimal water activity value for growth was found to be 1.0; below 0.94 no growth occurred. For *C. oxysporum* 805, the optimal water activity value for growth was found to be 0.98 and growth occurred over the entire range of water activity values tested.

The two *P. roquefortii* strains had the fastest growth rates of the seven fungi tested and the two *Geomyces* sp. were the slowest growing fungi of the seven tested by 25% compared with the two *P. roquefortii*. The maximal radial extension rates were at the water activity value of 0.98 for *P. roquefortii* 405 and *P. roquefortii* 408, 22 and 29.2 μm/hr, respectively.

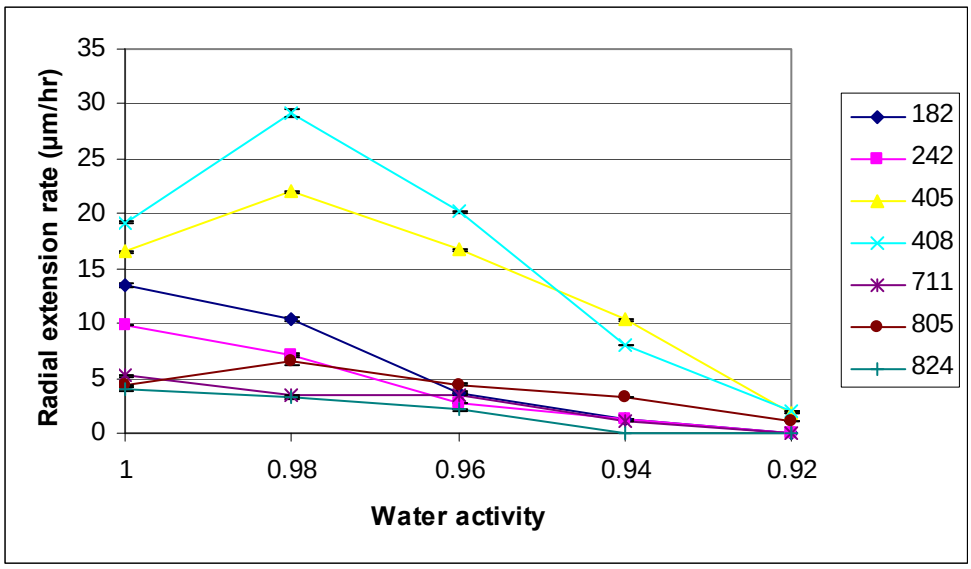

Figure 4.19: Graph of the radial extension rate of *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 on media of different substrate water activity. Vertical bars represent the standard error.

4.5.5 Carbon Utilisation

The carbon sources were divided into their substrate guilds as published by Preston-Mafham *et al.* (2002) as amines/amides, amino acids, carbohydrates (C4, C5, C6, C7, C8, C12 and >C12), carboxylic acids, polymers and miscellaneous as given in Table 4.7.

Fifty six carbon sources were used by all seven fungi tested at both temperatures. There was no carbon source that was not used by any of the fungi tested at either temperature. None of the fungi used glucuronamide at 15°C and only 2 used glucuronamide at 1°C. Only *C. malorum* 182 used adenosine-5- monophosphate at 15°C and at 1°C *C. malorum* 182 and *Geomyces* sp. 711 were the only two fungi to use adenosine-5-monophosphate. Adenosine was only used by *Geomyces* sp. 824 and *P. roquefortii* 405 at 15°C and at 1°C, *Geomyces* sp. 824 and *P. roquefortii* 405 along with *Geomyces* sp. 711 used adenosine. The two *Geomyces* sp. were the only fungal isolates to use uridine at 1°C and at 15°C, *Geomyces* sp. 824 and *P. roquefortii* 405 were the only two fungal isolates to utilise uridine. All fungi tested used glycyl-L-glutamic acid at 15°C while at 1°C only the two *Geomyces* sp. isolates used glycyl-L-glutamic acid. L-fucose was used by two isolates (*P. roquefortii* 408 and *C. oxysporum* 805) at 15°C and by five isolates at 1°C. Both *P. roquefortii* isolates used N-acetyl-D-galactosamine at

15°C but none of the other isolates did, at 1°C *P. roquefortii* 408 and *C. malorum* 242 were the only two isolates to use N-acetyl-D-galactosamine. Amygdalin was utilised by all fungal isolates at 1°C and only by the two *P. roquefortii* isolates, *C. malorum* 242 and *C. oxysporum* 805 at 15°C. The full lists of the isolates utilising carbon sources at designated temperatures is presented in Tables 4.8-4.19

Table 4.8: Growth of the seven isolates on amines/amides as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
2-Amino ethanol	-	+	-	+	+	+	+	+	+	+	+	-	+	+
D-Glucosamine	+	+	+	+	+	+	-	-	+	+	-	+	-	+
Glucuronamide	-	-	-	-	-	-	-	-	+	-	+	-	-	-
L-Alaninamide	-	+	+	+	+	+	-	-	+	+	+	+	-	-
Putrescine	-	-	-	-	+	+	+	+	+	-	+	+	-	-
Succinamic acid	-	+	+	+	+	+	-	-	+	+	+	-	+	+

Table 4.9: Growth of the seven isolates on amino acids as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
γ-Amino butyric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycyl-l-glutamic acid	-	+	-	+	-	+	-	+	+	+	+	+	-	+
L-Alanine	-	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Alanyl-glycine	+	+	+	+	+	+	+	-	+	+	+	+	+	+
L-Asparagine	+	+	+	+	+	+	-	-	+	+	+	+	+	+
L-Aspartic acid	+	+	+	+	+	+	-	-	+	+	+	+	+	+
L-Glutamic acid	-	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Ornithine	+	+	+	+	+	+	-	-	+	+	+	+	+	+
L-Phenylalanine	+	+	+	+	+	+	-	-	+	+	+	+	-	+
L-Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Pyroglutamic acid	+	+	-	+	+	+	-	-	+	+	+	+	+	+
L-Serine	+	+	+	+	+	+	-	+	+	+	+	+	+	+
L-Threonine	-	-	-	-	+	+	-	-	+	-	+	+	-	+

Table 4.10: Growth of the seven isolates on a four carbon carbohydrate as a carbon source.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
i-Erythritol	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.11: Growth of the seven isolates on five carbon carbohydrate as a carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
Adonitol	+	+	+	+	+	+	-	-	-	+	+	+	+	+
D-Arabinose	+	+	+	+	+	+	-	+	-	+	-	-	+	+
D-Arabitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	-	+	+	+	+	+

Table 4.12: Growth of the seven isolates on six carbon carbohydrate as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
α-D-Glucose	+	+	+	+	+	+	+	+	+	+	-	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	-	+	+	+	+	+	+	+	-	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	-	+	+	+	+	+
D-Psicose	-	-	+	+	+	+	-	+	+	-	-	-	+	+
D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Tagatose	-	-	+	+	+	+	-	+	+	-	+	+	+	+
L-Fucose	-	-	+	-	+	+	+	+	+	-	-	-	-	+
L-Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LSorbose	+	+	+	+	+	+	+	+	+	+	-	-	+	+
m-Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.13: Growth of the seven isolates on seven carbon carbohydrate as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
α-Methyl-D-galactoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β-Methyl-D-galactoside	-	-	+	+	+	+	+	+	-	-	+	+	+	+
α-Methyl-D-glucoside	+	+	+	+	+	+	+	+	+	+	-	+	-	+
β-Methyl-D-glucoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sedoheptulosan	+	+	-	-	-	-	-	-	+	+	+	+	-	-

Table 4.14: Growth of the seven isolates on eight carbon carbohydrate as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
N-Acetyl-D-galactosamine	-	-	+	+	+	+	-	+	-	-	-	-	-	-
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-Acetyl-D-mannosamine	+	-	+	+	+	-	-	+	-	-	-	-	+	-

Table 4.15: Growth of the seven isolates on twelve carbon carbohydrate carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
α-d-Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arbutin	+	+	+	+	+	+	+	-	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+	+	-	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gentiobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactulose	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Maltitol	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Palatinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turanose	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.16: Growth of the seven isolates on greater the twelve carbon carbohydrate as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
Maltotriose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Melezitose	+	+	+	+	+	+	-	+	+	+	+	-	+	+
D-Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stachyose	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.17: Growth of the seven isolates on carboxylic acids as a carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
β-Hydroxy butyric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
γ-Hydroxy butyric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	-
α-Keto glutaric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2-Keto-D-gluconic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galacturonic acid	+	+	+	+	+	+	-	-	+	+	-	-	+	+
D-Gluconic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucuronic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Malic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Saccharic acid	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Fumaric acid	+	+	+	+	+	+	-	+	+	+	+	+	+	+
l-Lactic acid	+	-	+	+	+	+	+	+	+	+	+	+	+	+
l-Malic acid	+	+	+	+	+	+	+	-	+	+	+	+	+	+
N-Acetyl-l-glutamic acid	+	+	+	+	+	+	-	+	+	+	+	+	+	+
p-Hydroxy phenylacetic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Quinic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebacic acid	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Succinic acid	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.18: Growth of the 7 isolates on polymer as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
α-Cyclodextrin	+	+	+	+	+	+	+	-	+	+	-	+	+	+
β-Cyclodextrin	+	+	+	+	+	+	-	-	+	+	-	+	+	+
Dextrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycogen	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+	-	+	+	+	+	+	+

Table 4.19: Growth of the 7 isolates on miscellaneous compounds as carbon sources.

Fungal isolate	182		242		405		408		711		824		805	
Temperature of incubation (°C)	1	15	1	15	1	15	1	15	1	15	1	15	1	15
Adenosine	-	-	-	-	+	+	-	-	+	-	+	+	-	-
Amygdalin	+	-	+	+	+	+	+	+	+	-	-	+	+	+
Adenosine-5'-monophosphate	-	-	-	-	-	+	-	-	+	-	+	+	-	-
Bromo succinic acid	+	+	+	+	+	+	-	+	+	+	+	-	+	+
d-Lactic acid methyl ester	-	-	+	-	+	+	-	-	+	-	+	+	-	-
Glucose-1-phosphate	-	-	+	+	+	+	+	-	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Succinic acid monomethyl ester	-	-	+	+	+	+	+	+	+	-	-	-	+	-
Uridine	-	-	-	-	-	-	-	-	+	-	+	+	-	-

4.5.5.1 *Cadophora malorum* 182 substrate utilisation

Cadophora malorum 182, cultured at 1°C, utilised 73 of the 96 carbon sources; when grown at 15°C, the fungus utilised 79 of the 96 carbon sources. When grown on amides/amines at 1°C, *Cadophora malorum* 182 utilised one of the six tested while at 15°C, four were utilised. When grown on amino acids as the carbon sources, *C. malorum* 182 at 1°C utilised nine of the thirteen tested and at 15°C twelve were utilised. The only four-carbon carbohydrate was utilised at both temperatures. All seven five-carbon carbohydrates were utilised at both temperatures. At 1°C, nine of the twelve six-carbon carbohydrates were utilised while eight were utilised at 15°C. Six of the seven-carbon carbohydrate were utilised at 1°C and at 15°C. At 1°C and 15°C, the eight-carbon carbohydrate, one was also not utilised. When grown on twelve and greater than twelve-carbon carbohydrates, all sixteen tested were utilised at both 1°C and 15°C. There were seventeen carboxylic acids tested and at 1°C, fourteen were utilised and at 15°C all seventeen were utilised. When polymers were used as the carbon source, all five tested were utilised at both temperatures. Ten miscellaneous carbon

compounds were tested and at both 1°C and 15°C, four were utilised.

4.5.5.2 *Cadophora malorum* 242 substrate utilisation

Cadophora malorum 242, grown at 1°C utilised 87 of the 96 carbon sources and when grown at 15°C, the fungus utilised 84 of the 96 carbon source. When grown on amides/amines at 1°C, *Cadophora malorum* 242 utilised four of the six tested, while at 15°C, three were utilised. When grown on amino acids as the carbon sources, *C. malorum* 242 at 1°C, utilised ten of the thirteen tested and at 15°C, twelve were utilised. The only four-carbon carbohydrate was utilised. All seven five-carbon carbohydrates were utilised at both temperatures. At 1°C, all of the twelve six-carbon carbohydrates were utilised while eleven were utilised at 15°C. All of the seven-carbon carbohydrate were utilised at 1°C and at 15°C one was not utilised. At 1°C, all three eight-carbon carbohydrates were utilised, while at 15°C, one was also not utilised. When grown on twelve and greater than twelve-carbon carbohydrates all sixteen tested were utilised at both 1°C and 15°C. There were seventeen carboxylic acids tested and at 1°C and at 15°C, all seventeen were utilised. When polymers were used as the carbon source, all five tested were utilised at both temperatures. Ten miscellaneous carbon compounds were tested and at 1°C seven were utilised and at 15°C, six were utilised.

4.5.5.3 *Penicillium roquefortii* 405 substrate utilisation

Penicillium roquefortii 405, grown at both 1°C and 15°C, utilised 91 of the 96 carbon sources. When grown on amides/amines at 1°C and at 15°C *Penicillium roquefortii* 405 utilised five of the six tested. When grown on amino acids as the carbon source, *P.roquefortii* 405 at 1°C utilised twelve of the thirteen tested and at 15°C all thirteen were utilised. The only four-carbon carbohydrate was utilised at both temperatures. All seven five-carbon carbohydrates were utilised at both temperatures. At 1°C and at 15°C, all of the twelve six-carbon carbohydrates were utilised. All of the seven-carbon carbohydrate were utilised at 1°C and at 15°C with the exception of one. At 1°C, all three eight-carbon carbohydrates were utilised, while at 15°C, two were utilised. When grown on twelve and greater than twelve-carbon carbohydrates all sixteen tested were utilised at both 1°C and at 15°C. There were seventeen carboxylic acids tested and at 1°C and at 15°C, all seventeen were utilised. When polymers were used as the carbon source,

all five tested were utilised at both temperatures. Ten miscellaneous carbon compounds were tested and at 1°C eight were utilised and at 15°C nine were utilised.

4.5.5.4 *Penicillium roquefortii* 408 substrate utilisation

Penicillium roquefortii 408, grown at 1°C, utilised 66 of the 96 carbon sources tested and for growth at 15°C, this fungus utilised 71 of the 96 carbon sources tested. When grown on amides/amines at 1°C and at 15°C, *Penicillium roquefortii* 408 utilised two of the six tested. When grown on amino acids as the carbon sources, *P.roquefortii* 408 at 1°C utilised five of the thirteen tested and at 15°C six of thirteen were utilised. The only four-carbon carbohydrate, tested, was utilised at both temperatures. Five of the seven, five-carbon carbohydrates were utilised at 1°C and at 15°C, six were utilised. At 1°C, ten of the twelve and at 15°C, all of the twelve six-carbon carbohydrates were utilised. All of the seven-carbon carbohydrate were utilised at 15°C and at 1°C, all except one were utilised. At 1°C, one of the three eight-carbon carbohydrates was utilised, while at 15°C, all of the eight-carbon carbohydrates were utilised. When grown on twelve-carbon carbohydrates, all twelve tested were utilised at 1°C and at 15°C, ten of the twelve were utilised. When grown on the greater than twelve-carbon carbohydrates at 1°C, three of the four were utilised and at 15°C, all four were utilised. There were seventeen carboxylic acids tested and at 1°C, thirteen were utilised and at 15°C, fifteen of the seventeen were utilised. When polymers were used as the carbon source, at 1°C four of the five tested were utilised and at 15°C only dextrin and glycogen were utilised. Ten miscellaneous carbon compounds were tested and at 1°C, four were utilised and at 15°C, five were utilised.

4.5.5.5 *Geomyces* sp. 711 substrate utilisation

Geomyces sp. 711, grown at 1°C, utilised 88 of the 96 carbon sources tested and when grown at 15°C, the fungus utilised 79 of the 96 carbon sources tested. When grown on amides/amines at 1°C, *Geomyces* sp. 711 utilised all of the six tested while at 15°C, four were utilised. When grown on amino acids as the carbon sources *Geomyces* sp. 711 at 1°C utilised all thirteen tested and at 15°C, twelve were utilised. The only four-carbon carbohydrate was utilised at both temperatures. At 1°C, four of the seven, five-carbon carbohydrate were utilised and at

15°C, all seven five-carbon carbohydrates were utilised. At 1°C, eleven of the twelve six-carbon carbohydrates were utilised while eight were utilised at 15°C. All of the seven-carbon carbohydrates were utilised at 1°C and at 15°C all except one was utilised. At 1°C and 15°C, only one eight-carbon carbohydrate was utilised. When grown on twelve and greater than twelve-carbon carbohydrates all sixteen tested were utilised at both 1°C and 15°C. There were seventeen carboxylic acids tested and at 1°C and at 15°C all seventeen were utilised. When polymers were used as the carbon source, all five tested were utilised at 15°C and at 1°C, only one was not utilised. Ten miscellaneous carbon compounds were tested and at 1°C, all ten were utilised and 15°C, four were utilised.

4.5.5.6 *Geomyces* sp. 824 carbon utilisation

Geomyces sp. 824, grown at both 1°C and 15°C, utilised 80 of the 96 carbon sources tested. When grown on amides/amines at 1°C, *Geomyces* sp. 824 utilised five of the six tested while at 15°C, three were utilised. When grown on amino acids as the carbon sources *Geomyces* sp. 824 at 1°C and at 15°C utilised all of the thirteen tested. The only four-carbon carbohydrate tested was utilised at both temperatures. Six of the seven five-carbon carbohydrates were utilised at both temperatures. At 1°C, eight and at 15°C, nine of the twelve six-carbon carbohydrates were utilised. At 1°C, four of the five seven-carbon carbohydrates were utilised and at 15°C, all five were utilised. At 1°C and 15°C the eight-carbon carbohydrate, only one was utilised. When grown on twelve and greater than twelve-carbon carbohydrates all sixteen tested with the exception of one twelve-carbon carbohydrate, were utilised at 1°C. At 15°C, ten of the twelve-carbon carbohydrates were utilised and three of the greater than twelve-carbon carbohydrates were utilised. There were seventeen carboxylic acids tested and at 1°C and at 15°C, sixteen of the seventeen were utilised. When polymers were used as the carbon source, all five tested were utilised at 15°C and at 1°C, three of the five polymers were utilised. Ten miscellaneous carbon compounds were tested and at both 1°C and 15°C eight were utilised.

4.5.5.7 *Cladosporium oxysporum* 805 carbon utilisation

Cladosporium oxysporum 805, grown at 1°C, utilised 79 of the 96 carbon sources tested were utilised and when grown at 15°C, this fungus utilised 84 of the 96 carbon sources tested. When grown on amides/amines at 1°C, *Cladosporium oxysporum* 805 utilised two of the six tested while at 15°C, three were utilised. When grown on amino acids as the carbon sources, *C. oxysporum* 805 at 1°C, utilised ten of the thirteen tested and at 15°C, thirteen were utilised. The only four-carbon carbohydrate was utilised at both temperatures. All seven five-carbon carbohydrates were utilised at both temperatures. At 1°C, eleven of the twelve six-carbon carbohydrates were utilised while all twelve were utilised at 15°C. At 1°C, three of the five seven-carbon carbohydrates were utilised and at 15°C four were utilised. At 1°C and 15°C, the eight-carbon carbohydrate, only one was not utilised. When grown on twelve and greater than twelve-carbon carbohydrates, all sixteen tested were utilised at both 1°C and 15°C. There were seventeen carboxylic acids tested and at 1°C, all seventeen were utilised and at 15°C, sixteen were utilised. When polymers were used as the carbon source, all five tested were utilised at both temperatures. Ten miscellaneous carbon compounds were tested and at 1°C, six were utilised and at 15°C, five were utilised.

4.5.5.8 Summary of carbon utilisation study

In order to compare each fungal isolate's ability to utilise a substrate guild, the bar graphs given in Figures 4.20 – 4.30 were made. When the isolates were grown on amine and amides (Figure 4.20), the two *Geomyces* sp. 711 and 824 and *Cadophora malorum* 242 used a greater number of amines and amides at 1°C than 15°C. Both *Penicillium roquefortii* 405 and 408 used the same number of amine and amides at both temperatures. *Cadophora malorum* 182 only used D-glucosamine at 1°C but used 4 amine and amides at 15°C.

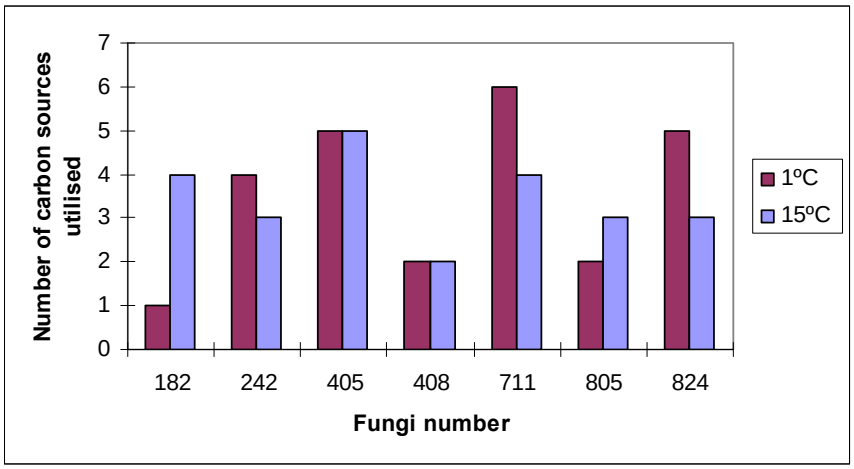

Figure 4.20: Graph of the number of amine and amide carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Six amine and amide carbon sources were tested.

When grown on amino acids (Figure 4.21), all the isolates except the two *Geomyces* sp. 711 and 824 used a larger number of amino acids at 15°C than at 1°C. *Penicillium roquefortii* 408 used the least number of amino acids at both temperatures.

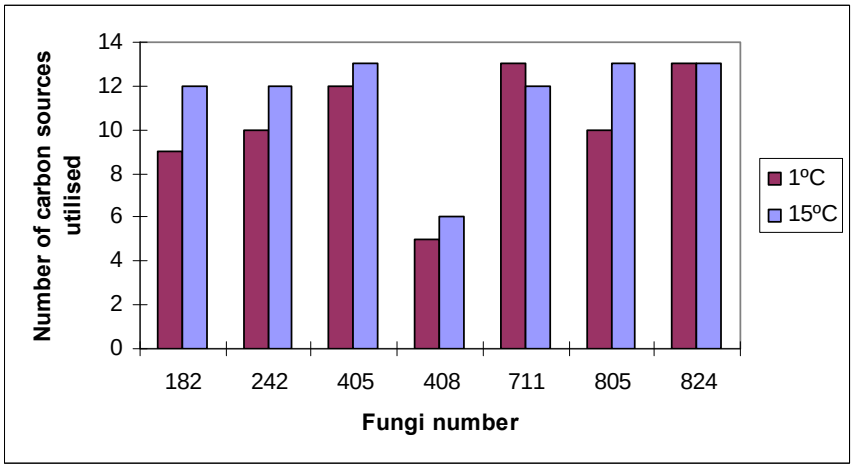

Figure 4.21: Graph of the number of amino acid carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Thirteen amino acid carbon sources were tested.

All fungal isolates used the one (i-erythritol) four-carbon carbohydrate tested at both temperatures.

All fungi except *Penicillium roquefortii* 408 and the two *Geomyces* sp. 711 and 824 used all seven five-carbon carbohydrates tested (Figure 4.22) at both temperatures. *Geomyces* sp. 824 used the same number of five-carbon

carbohydrates at both temperatures.

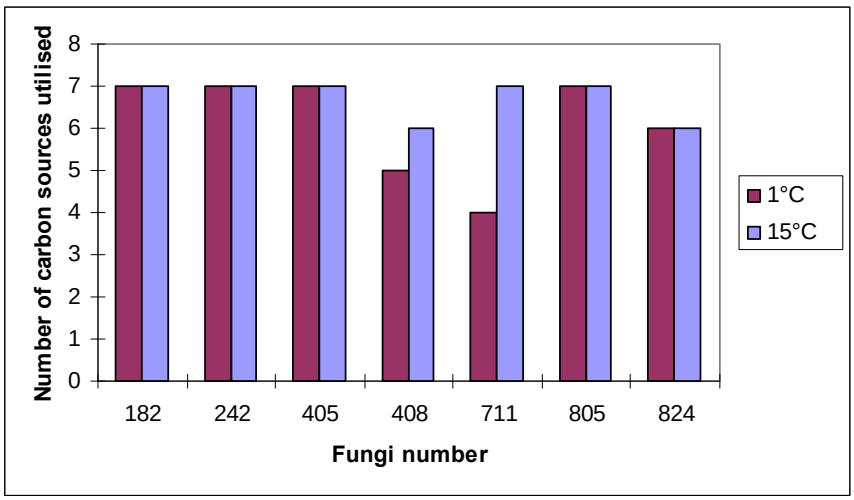

Figure 4.22: Graph of the number of five-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Seven five-carbon carbohydrates carbon sources were tested.

When grown on six-carbon carbohydrates, both *Cadophora malorum* 182 and 242, along with *Geomyces* sp. 711 used more six-carbon carbohydrates at 1°C than at 15°C. *Penicillium roquefortii* 405 used all 12 six-carbon carbohydrates at both temperatures while the other strain of *Penicillium roquefortii* 408 used less six-carbon carbohydrates at 1°C than at 15 °C (Figure 4.23).

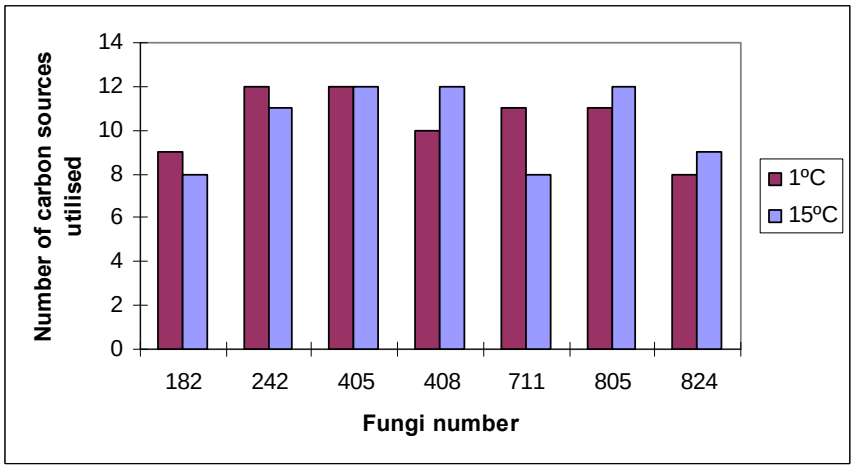

Figure 4.23: Graph of the number of six-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Twelve six-carbon carbohydrate carbon sources were tested.

Cadophora malorum 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, and *Geomyces* sp. 711, used all seven-carbon carbohydrates at 1°C and four seven-carbon carbohydrates at 15°C. *Penicillium roquefortii* 408 and

Geomyces sp. 824 used all seven-carbon carbohydrates at 15°C and four seven-carbon carbohydrates at 1°C. *Cladosporium oxysporum* 805 used the least number of seven-carbon carbohydrates at 1°C (Figure 4.24).

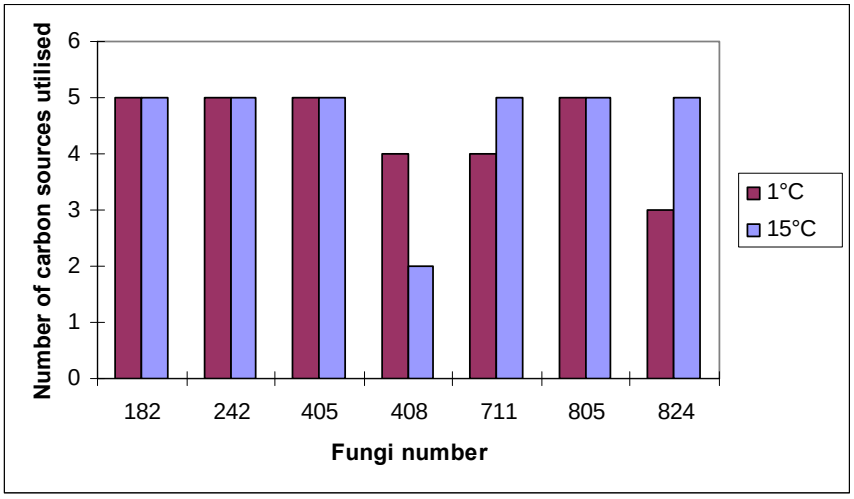

Figure 4.24: Graph of the number of seven-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Five seven-carbon carbohydrates were tested.

When comparing growth on eight-carbon carbohydrates *Cadophora malorum* 182 and 242 and *Penicillium roquefortii* 405 all utilised more eight-carbon carbohydrates at 1°C than at 15°C. *Penicillium roquefortii* 408 used more eight-carbon carbohydrates at 15°C than at 1°C. Both *Geomyces* sp. 711 and 824 used one (N-acetyl-D-glucososamine) of the eight-carbon carbohydrates at both temperatures (Figure 4.25).

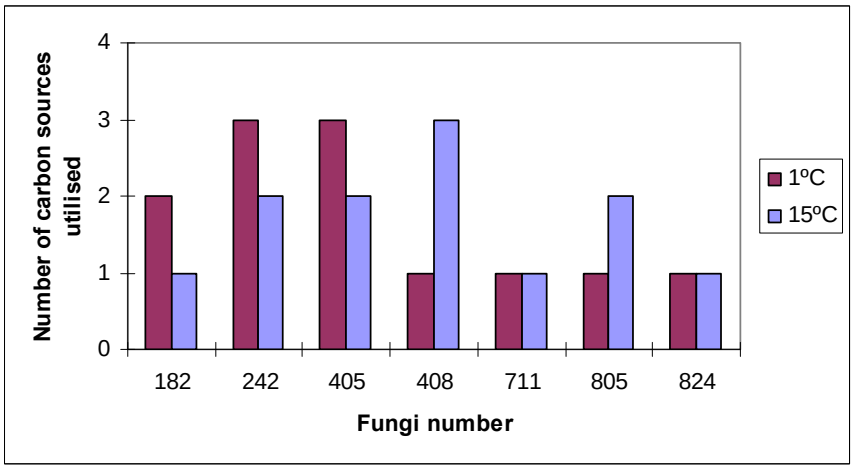

Figure 4.25: Graph of the number of eight-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15°C. Three eight-carbon carbohydrate carbon sources were tested.

All fungi except *Penicillium roquefortii* 405 and *Geomyces* sp. 824 used twelve-carbon carbohydrates at both temperatures. *Penicillium roquefortii* 405 used all twelve twelve-carbon carbohydrates at 1°C and ten at 15°C (Figure 4.26).

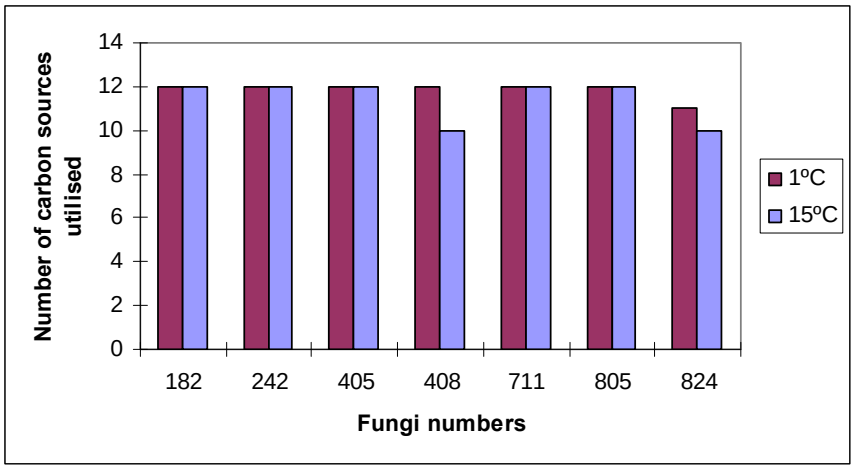

Figure 4.26: Graph of the number of twelve-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15 °C. Twelve twelve-carbon carbohydrate carbon sources were tested.

All fungi except *Penicillium roquefortii* 408, and *Geomyces* sp. 824 used all 4 carbohydrates with >twelve-carbon carbohydrate at both temperatures. *Penicillium roquefortii* 408 used all four at 15°C and three at 1°C while *Geomyces* sp. 824 used all 4 at 1°C and 3 at 15°C (Figure 4.27).

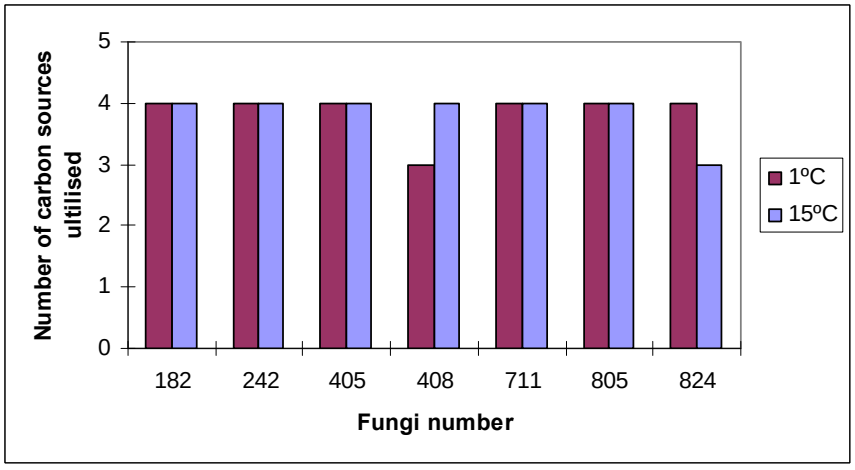

Figure 4.27: Graph of the number of greater than twelve-carbon carbohydrate carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15 °C. Four greater than twelve-carbon carbohydrates were tested.

Cadophora malorum 242, *Penicillium roquefortii* 405, *Geomyces* sp. 711, used all 17 carboxylic acids at both temperatures. *Cadophora malorum* 182 and

Penicillium roquefortii 408 both used more carboxylic acids at 15°C than 1°C; *Cadophora malorum* 182 used all 17 at 15°C. *Geomyces* sp. 824 used all 17 at 1°C and 16 at 15°C (Figure 4.28).

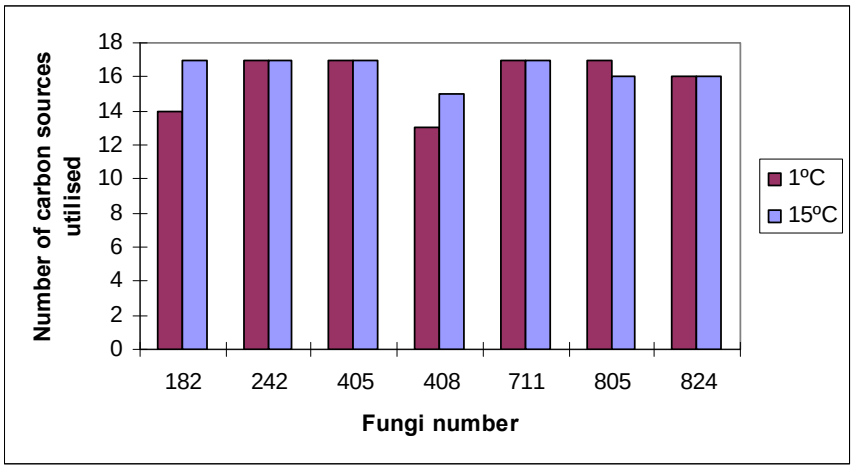

Figure 4.28: Graph of the number of carboxylic acid carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15 °C. Seventeen carboxylic acid carbon sources were tested.

Cadophora malorum 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, and *Cladosporium oxysporum* 805 utilised all five polymers at both temperatures. *Penicillium roquefortii* 408 utilised more polymers at 1°C than at 15°C while both *Geomyces* sp. 711 and 824 used more polymers at 15°C than at 1°C (Figure 4.29).

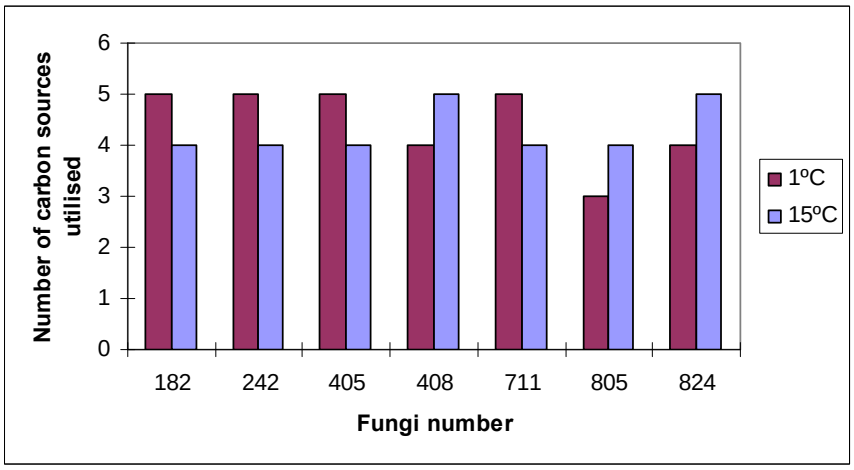

Figure 4.29: Graph of the number of polymer carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15 °C. Five polymer carbon sources were tested.

Both *Penicillium roquefortii* 405 and 408 utilised more miscellaneous carbon sources at 15°C than at 1°C while *Cadophora malorum* 242, *Geomyces* sp. 711 and *Cladosporium oxysporum* 805 utilised miscellaneous carbon

sources at 1°C than at 15°C. *Cadophora malorum* 182 and *Geomyces* sp. 824 utilised the same number of miscellaneous carbon sources at both temperatures. *Cadophora malorum* 182, four miscellaneous carbon sources out of the ten tested and *Geomyces* sp. 824 eight miscellaneous carbon sources out of the ten tested (Figure 4.30).

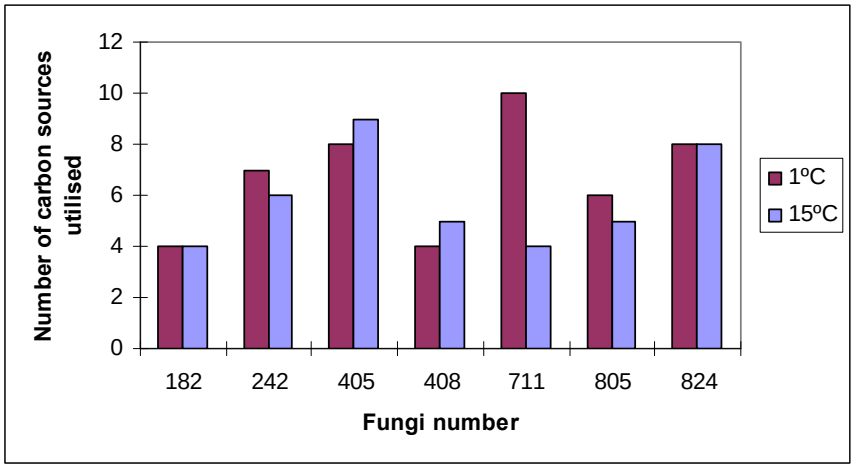

Figure 4.30: Graph of the number of miscellaneous carbon sources utilised by *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 at 1 and 15 °C. Ten miscellaneous carbon sources were tested.

4.6 Discussion

The main aim of this aspect of the research was to gain an understanding of the fungal biochemistry and growth characteristics of a selection of fungi that had been isolated from the Historic Huts themselves and materials contained within and around them.

Two objectives were addressed:

1. Fungal growth rates at various temperatures to determine whether psychrophilic or psychrotrophic preferences.
2. Fungal growth on different carbon sources, cellulose sources, and water availability were conducted to characterise primary and secondary metabolism of the isolates.

The 7 isolates studied in depth whose results were presented in this chapter, *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805, could all grow at 4°C, but all grew

slowest and with the longest lag phase at 4°C. It has been reported that the production of more fungal biomass at low temperatures is a survival mechanism (Robinson 2001) and in this study *Cadophora malorum* 242, *Geomyces* sp. 711, and *Cladosporium oxysporum* 805 all produced more fungal biomass when grown at 4°C than when grown at the other temperatures tested. Using Morita's (1975) definition of psychrophiles and psychrotrophs, none of these fungi could be characterised as psycrophiles and were all classified as psychrotrophs. Onofri *et al.* (2004) reported that the following fungal species were able to grow at the following temperatures: *Cladosporium cladosporiodes* growth range 0-32 °C, being able to grow at -3 °C and possibly as low as -10 °C; *Cladosporium herbarum* growing at -6°C; *Cadophora malorum* growing over the range of -3 to 45°C; and *Penicillium* sp. and *Geomyces pannorum* growing at -2°C. Zucconi *et al*. (1996) noted in their discussion that microorganisms that were better adapted to Antarctic terrestrial environments appeared to have wider growth rate curves than those that were less adapted to Antarctica terrestrial environments, which had narrower curves. They used the width of the growth curve at half of the maximum growth rate as an indicator of the adaptation of the species to thermal instability of the environment. Using this method on the results from the fungi tested in this research all the fungi had very broad growth ranges indicating that the seven fungi tested were all well adapted to the Antarctica environment.

Both *Cadophora malorum* 182 and *Geomyces* sp. 711 could sustain repeated growth at 4°C, indicating cold adaptation. Unadapted microorganisms would have failed to grow at the cold temperature but would demonstrate growth upon an increase in incubation temperature while mesophilic microorganisms would not grow due to cell death due to stress or would have required an acclimation period where cold shock and eventually cold acclimation proteins would be produced to allow the organism to adapt to the temperature before normal metabolism and growth could resume.

Growth optima on solid media were different than when the organisms were grown in liquid media, for all six of the seven isolates (Comparison can be seen in Table 4.20). This is common as different conditions, such as substrate availability, and exposure to environment are offered by the two different methods.

Cladosporium oxysporum was the only isolate with an optimum temperature for growth less on solid media than liquid media.

Table 4.20: Comparison of optimum growth temperature on liquid and solid media.

Isolate	Optimum growth temperature grown on liquid media °C	Optimum growth temperature grown on solid media °C
Cadophora malorum 182	15	25
Cadophora malorum 242	10-20	15-25
Penicillium roquefortii 405	20	25
Penicillium roquefortii 408	25	25
Geomyces sp. 711	20	25
Geomyces sp. 824	15	25
Cladosporium oxysporum 805	25	15

The results from growth on solid media showed that all isolates except *Cladosporium oxysporum* demonstrated fastest growth rates at 25° than 15°C but all could still grow at 4°C. *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 all had growth optima of 25°C or above. *Cladosporium oxysporum* 805 grew fastest on agar plates at 15°C and grew slightly faster at 4°C than at 25°C with growth optima of 15°C. The difference did not change the classification of the fungi with all being deemed to be psychrotrophs.

The predominance of psychrotrophy rather than psychrophilicity in Antarctica may be because psychrotrophic fungi have the ability to grow around 0°C while not being affected by the warmer temperatures of substrata at various times of the year when compared with the low air temperatures. For instance, Möller and Dreyfuss (1996) wrote, "Although average air temperatures in the maritime Antarctica are around freezing point, local soil temperatures and microclimates may rise to 15°C through solar radiation".

The following described studies were published using Antarctic fungal isolates related to those used in this PhD study. According to Tubaki (1961), *Chrysoporium pannorum* (*Geomyces pannorum*), *C. verrucosum* could all grow at 5°C and did not grow at 30°C and all had an optimum growth temperature between 20- 25°C. It was also noted that *Chrysoporium pannorum* grew

while being stored in a fridge at 1°C for a month. Zucconi *et al.* (1996) screened 35 strains of microfungi for growth at temperatures ranging from 0 to 45°C. Of the 35, 31 were classified as psychrotolerant, one was classified as a thermotolerant mesophile and two strains were classified as psychrophiles. These were *Geomyces pannorum* var. *pannorum* no. 5 from Crater Cirque and a pink yeast no. 2. Azmi and Seppelt (1997) investigated the growth of six fungi including *Chrysosporium pannorum*, and found that all six showed poor growth at 0°C. The temperature optimum for these six fungi was 20°C and they concluded that all the fungi they studied were cold tolerant strains of mesophiles adapted to grow at temperatures as low as 0°C. Hurst *et al.* (1983) investigated the growth rate of five fungal species including *Chrysosporium pannorum*, and *Cladosporium sphaerospermum* and all could grow at 1°C and all showed growth optima between 15 and 25°C. Kerry (1990a) investigated the effect of temperature on the growth rate of fungi isolated from the subantarctic. The linear growth rate was measured from 4°C to 35°C. Included in the study were two *Chrysosporium pannorum* and *Penicillium brevi-comactum*, *P. cyclopium*, and *P. jensenii*. All fungi grew at 4°C. Maximum growth rates for the five fungi were 20°C Maximum growth of all species was at temperatures above those normally prevailing in their natural environments.

When comparing the results of the published studies with the results of this PhD thesis research it is important to consider several features including the origin of the fungal isolate, that there can be variation in physiology due to degrees of adaptation, and the media used in the experiment can affect results. Latter and Heal (1971) showed that isolates of single fungal species may differ in their physiology depending on their climatic origin. Land *et al.* (1987) reported variation in growth range and rate between species of the same genus and the same strains of a genus especially with the dominant species in their study *Cladosporium* sp. and *Penicillium* sp. Of the ten *Cladosporium sphaerosperum* used in their study, four did not grow at 2°C. With those that did, the number of days before growth started ranged from 6 to 10 days. The results of this thesis are within the range of work done by previous researchers.

Cellulose is a secondary nutrient source for fungi. Secondary nutrients are nutrients that require enzymes to be produced that are not always produced - these enzymes are induced and are produced only when the organism is experiencing certain conditions such as carbon starvation. Cellulose is a secondary nutrient, requiring depolymersiation to release glucose which is a primary nutrient. Cellulose normally occurs as a mixture of two forms, 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 the study and presented in this chapter were synthetic. Avicel is relatively crystalline and less soluble, compared with carboxymethylcellulose and hydroxyethylcellulose which are both water soluble. All seven fungal isolates grew on all three cellulose carbon sources indicating these fungi can degrade different types of cellulose. Avicel being more crystalline would have been more difficult for the fungi to degrade but all fungi grew at least as well if not fastest on Avicel at least one temperature when compared to the other two more amorphous celluloses. Both *Penicillium roquefortii* isolates and both *Geomyces* sp. grew on the media containing no cellulose indicating that they were using other carbon sources within the media to grow and this carbon source most likely would be the setting agent agarose through all grew faster when cellulose was present. Both *Cadophora malorum* isolates and *Cladosporium oxysporum* 805 did not grow on media containing no cellulose indicating that they could not utilise any other carbon sources in the media and were only utilising cellulose when it was present. Section 5.6 will discuss the enzymes these fungi are using to breakdown cellulose and the cellulase activity when these cellulose substrates are used in an assay.

All the fungal isolates had an optimal growth rate at A_w 1.0 except the two *Peniciliium roquefortii* which had an optimal growth rate at A_w 0.98. The two *Penicillium roquefortii* isolates and *Cladosporium oxysporum* 805 grew to an A_w of 0.92 while the two *Cadophora malorum* isolates did not grow at an A_w of 0.92. *Geomyces* sp 711 grew at an A_w of 0.94 but not at A_w 0.92 and the other *Geomyces* sp 824 stopped growing at an A_w of 0.94. Onofri *et al.* (2004) reported that the following fungal species were xerophilic and could grow at the following A_w: *Geomyces pannorum* (0.89), *Cladosporium sphaerospermum* (0.89) and *Penicillium* sp (0.78- 0.79).

Much of previous water activity studies used food spoilage fungi. The only fungal species that growth optima and maxima have been determined from previous studies used in this PhD thesis research was *Penicllium roquefortii* used in cheese manufacture, which has a reported optimal water activity of 0.97 and grew over a range of 1 to 0.86 when grown at 25°C and pH 5.0, according to research completed by Gervais *et al.* (1988). Figures 4.31 and 4.32 compare the graphs for the cheese manufacture *P. roquefortii* and the two Antarctic *P.roquefortii* isolates. The two Antarctic isolates were grown at 15°C (isolates 405 and 408 have an optimum growth temperature of 25°C on solid media) so the radial extension rate is not as fast as the cheese isolate which was grown at 25°C. For the Antarctic isolates the experimental water activity went to an A_w of 0.92 while the study by Gervais *et al.* went to an A_w of 0.88 so it can only be speculated that the Antarctic organism would be able to grow at lower A_w. Therefore, it was concluded that the Antarctic isolates are distinctive from that described by Gervais *et al.* (1998).

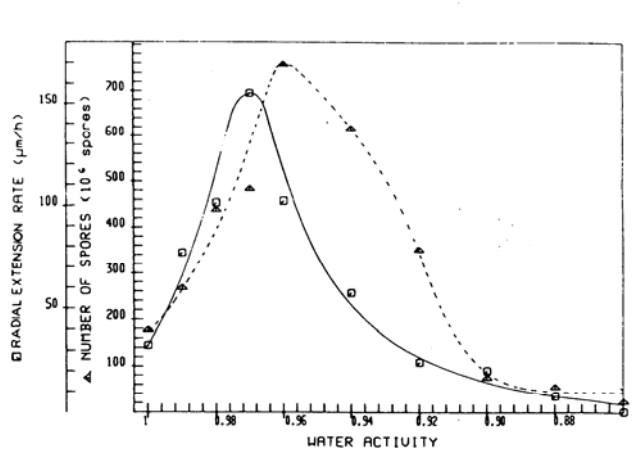

Figure 5. Influence of the substrate water activity on the (□) radial extension rate and on the (△) sporulation intensity of *P. roqueforti*.

Figure 4.31: Diagram of the influence of the substrate water activity on the radial extension rate and on the sporulation intensity of *P. roquefortii* (source Gervais et al (1988)).

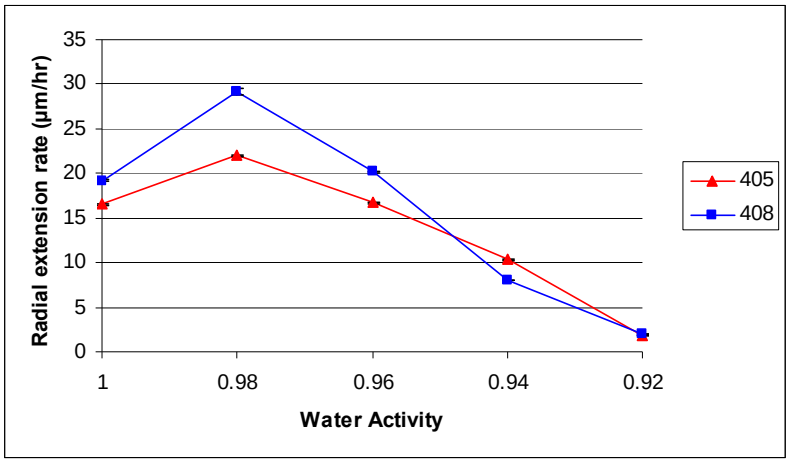

Figure 4.32: Graph of the influence of the substrate water activity on the radial extension rate for two Antarctic *P.roquefortii* isolates.

Gock *et al.* (2003) used *Penicillium roquefortii* from lake sediment, Antarctica in a study looking at water activity, temperature and pH effects on xerophilic fungi. The temperatures used in his study were 25, 30 and 37°C. At 25°C the minimum A_w for growth of *Penicillium roquefortii* was 0.82 and at 30°C the minimum was 0.86 and at 37°C *Penicillium roquefortii* did not grow.

This experiment was the first time Biolog FF microplates were used to study difference in carbon utilisation by one organism at different temperatures. Derry *et al.* (1999) studied the functional diversity of microorganisms in Arctic soils at three incubation temperatures (30°C, 10°C and 4°C) using sole carbon source utilisation. They concluded that temperature did affect substrate utilisation with lower temperatures showing greater richness and evenness when compared with the more mesophilic temperatures. Claussen *et al.* (2003) used Biolog microtitre plate technology to determine differences in community level physiological profiles (CLPPs) from two soils using five different C-substrate profile microtitre plates at five different temperature regimes. Their results showed that that the CLPP's were relatively unaffected by the different temperature regimes. The results of the carbon utilisation illustrated that there was no universal differences in carbon utilisation or not, when fungal isolates were grown at either 1°C or grown at 15°C but there are many changes in carbon utilisation when comparing each Antarctic fungal isolate when grown at either 1°C or 15°C. Glycyl-L-glutamic acid was used by all fungi at 15°C but only by the two *Geomyces* sp. at 1°C. There were a few carbon sources that were used by all fungal isolates at 15°C and by very few at 1°C. Glucuronamide was only used by the two *Geomyces* isolates at 1°C and by none of the other fungi at either temperature tested. Adenosine-5-monophosphate was not utilised by many of the isolates at either temperature. Adenosine was only used by the *Geomyces* and *Penicillium* sp.

In conclusion, this chapter reports on the study focusing on the response of Antarctic isolates to various temperatures, substrates and water activity. All the fungal isolates were classified as psychrotolerant. The two species tested could sustain growth at 4°C. All fungal isolates used in this study could grow on the three synthetic celluloses used as carbon substrates. Both *Penicillium roquefortii* and both *Geomyces* sp. could also grow on media containing no cellulose

as a carbon source. When compared with food isolated *Penicillium roquefortii*, the two Antarctic isolated *Penicillium roquefortii* showed different growth characteristics when grown on media of different A_w. When the fungal isolates were grown on 95 different carbon sources using Biolog FF microtitre plates, to determine the range of carbon source these isolates would use when incubated at 1°C and 15°C, there were no carbon sources used by all isolates at both temperatures and no carbon sources not used by all isolates at either temperatures. All isolates had a different spectrum of carbon source utilisation at each temperature tested. The biggest difference between growth at psychrophilic temperatures and mesophilic temperatures was that the growth rate was slower at the colder temperatures.

From the results from this chapter a better understanding of the microbiological framework of Antarctic fungi has been established. This knowledge aids in increasing the understanding of what makes these fungi different from mesophilic fungi and how they are growing and proliferating in the cold Antarctic environment.

Results from this chapter of research have been quoted in the following publication:

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.

Chapter 5 Cellulase

5.1 Introduction

The research of Antarctica New Zealand Event K021 and NSF BO-038 formed the basis of this PhD thesis research to investigate the ability of fungi isolated from the Historic Huts in Antarctica to produce the extracellular enzymes collectively called cellulases.

The secondary cell wall of wood cells make up the greatest proportion of cell wall thickness (Barnett and Bonham, 2004), are rich in cellulose microfibrils and the orientation of these microfibrils contributes to the properties of the woody plant and the timber that is produced by the wood. The publication by Blanchette *et al*., (2004) used scanning electron microscopy (SEM) and identified soft rot cavities in the secondary cell wall of wood cells. From rDNA sequencing of the fungal isolates from soft rotted wood, *Cadophora* sp were identified, and their decay capability was confirmed by inoculation in the laboratory of sterile wood wafers and subsequent examination by SEM (Blanchette *et al.*, 2004).

The fact that these fungi were causing decay in this cellulose rich region led to this PhD research focusing on the cellulase enzymatic capabilities of these fungi. Wood inhabiting fungi are faced with a mixed substrate of cellulose, hemicellulose and lignin along with pectin, phenolics and resin acids within the cell walls. Therefore, these microorganisms require either a wide variety of wood degrading enzymes activity to fully breakdown the wood or the ability to utilise one of the components of the cell wall in order for the decay observation to be manifested by SEM. Proteins in the sapwood provide a source of nitrogen for organisms that produces proteases (Gharibian *et al*., 1996).

This chapter reports on the screening of fungi isolated from Antarctica for cellulase activity, quantification of levels of endo-1,4-β-glucanase accumulating in the supernatant after culturing the isolates at varying temperatures, and the use of electrophoresis to demonstrate the complex of enzymes produced. The supernatant that contained cellulase was screened for accumulation of other wood cell degrading enzymes along with protease activity.

The hypothesis, aims and objectives of this chapter are firstly presented. Next, a literature review is provided at the beginning of this chapter and introduces the terminology of cellulase. Also, previous research on the screening of Antarctic fungi for cellulase production, cold adapted cellulases from Antarctic organism, the use of Congo red for cellulase screening, cellulase production by fungi investigated in this PhD study, wood degrading enzymes and protease are reviewed. This chapter provides methods and materials, results and a discussion on the production of cellulase and other wood degrading enzymes by Antarctic isolated fungi.

5.2 Hypothesis, Aims, Objectives

This chapter focuses on the fungi isolated from the wood of the Antarctic Historic Huts and their having enzymatic (cellulase) activity ranges reflecting activity in the unique environmental conditions.

The main aim of this aspect of the research was to gain an understanding of the cellulase activity of fungi present in the Historic Huts themself and materials contained within and around them.

Objectives were addressed as follows:

- At a variety of temperatures, selected fungi isolated from a variety of substrates including structural wood of the Antarctic Historic Huts on Ross Island were screened to determine ability to produce cellulytic activity.
- Cellulytic activity was characterised using quantitative assays to determine levels of production and conditions under which this was optimal.
- Examine the cellulase complex using electrophoresis techniques.
- Quantify levels of other wood cell degrading enzymes.
- Quantify protease activity.

5.3 Literature Review

5.3.1 Cellulase terminology

5.3.1.1 Cellulase complex

The term cellulase complex normally refers to the complete repertoire of enzymes involved in cellulase hydrolysis; a complete cellulase complex is required in the

complete hydrolysis of crystalline cellulose. Often the enzymes of the complex are in some physical association except β-glucosidase, which is usually not found physically associated with the exo or endo cellulases.

5.3.1.2 Endoglucanases

Endo-β-(1,4)-glucanases (or 1,4-β-D-glucan-4-glucanohydrolases, EC 3.2.1.4), commonly referred to as endoglucanases, are characterised by their random hydrolysis of β-(1,4)-glucosidic linkages, although the degree of randomness may vary amongst the several different endoglucanases which are normally produced by a single organism (Wood and McCrae, 1979). Acting on soluble cellulose derivatives, their random cleavage causes rapid decrease in chain length and hence changes in viscosity relative to the release of reducing end groups. When acting on cellodextrins, the rate of hydrolysis increases with the degree of polymerisation within the limits of substrate solubility, with cellobiose and cellotriose being the major final product (Reese, 1977). The fact that little monomers are produced reflects a degree of non-randomness in that the terminal linkages are less affected than the internal linkages. The anomeric linkage of the product is not inverted by the hydrolysis.

5.3.1.3 Exoglucanases

Exo-β-(1-4)-glucanase (or 1,4-β-D-glucan cellobiohydrolases, EC 3.2.1.91) cleave cellobiose units from the non-reducing ends of cellulose molecules. Some examples have been purified to the point where they produce no detectable reduction in carboxymethylcellulose (CMC) viscosity although some reducing sugar production from CMC was still possible (Wood, 1975). Presumably endwise hydrolysis would advance until a substituted glucose residue was encountered.

5.3.1.4 Glucosidases

Exo-β-(1,4)-glucosidase (or 1,4-β-D-Glucan glucohydrolases, EC 3.2.1.74) cleave glucose units successively from the non-reducing end of the glucan. They are distinguished from β-glucosidase by their preference for substrates of longer chain length and by the inversion of their products (Reese, 1968).

β-glucosidase (or β-D-glucoside glucohydrolase, EC 3.2.1.21) hydrolyse cellobiose and other very short chain β-1,4-oligoglucosides up to cellohexaose to form glucose (Reese, 1968). The term cellobiase is a misleading pseudonym since it suggests complete specificity for cellobiose. Most cellobiases are in fact active on a range of β-dimers of glucose (Lee and Fan, 1980). Unlike exoglucosidases,

the rate of hydrolysis of cellobiose decreases markedly as the degree of polymerisation of the substrate increases and the beta configuration is retained by the product.

5.3.1.5 Carboxymethylcellulase (CMCase)

The capacity to hydrolyse carboxymethylcellulose should not be considered synonymous with endoglucanase activity since other classes of enzyme can also be involved particularly in the release of reducing sugars (Wood, 1975).

5.3.1.7 Avicelase

Similarly the capacity to hydrolyse Avicel which is microcrystalline wood alpha-cellulose is not indicative of the action of any one class of enzyme alone (Cohen *et al*., 2005).

5.3.2 Screening of Antarctic fungi for cellulase activity

Several research groups have screened Antarctic isolated fungi for extracellular enzyme activity including cellulases and have published their results.

Of 33 fungal strains isolated from Victoria Land, plate screened at 25ºC and at their optimal growth temperature if 25ºC was not optimal, by Fenice *et al*. (1997), 12 strains showed low cellulase activity, which included *Alternaria* sp., 1 of 3 *Arthrobotrys ferox*, *Aspergillus versicolor*, 1 of 2 *Dendryphiella salina*, 1 of 5 *Geomyces pannorum*, *Phoma sorghina*, 2 of 3 *Phoma* sp., 2 of 3 *Verticillium* cfr. *lecanii*, 1 of 3 hyaline mycelia and 1 of 2 pink yeasts.

Bradner *et al*. (1999a) screened 7 Antarctic isolates for hydrolytic activity at 10ºC, 21ºC, 28ºC and 37ºC and screened for cellulase activity but did not report results.

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 along with other substrates at 20ºC. All except *Mucor hiemalis* grew on and were able to cause clearing of cellulose agar plates. They reported that *Botrytis*, *Chrysosporium pannorum*, *Chaetophoma*, *and Cladosporium sphaerospermum* all demonstrated cellulase activity at 1ºC.

5.3.3 Congo red cellulase screening

The interaction of the direct dye Congo red with polysaccharides containing contiguous (β-1,4) linked D-glucopyranosyl units and also a significant interaction with β-(1,3)-D-glucans and possibly some hemicellulosic galactomannans (Teather and Wood, 1982) forms the basis of the Congo red plate screening, a rapid and selective test. Congo red has been used to determine cellulose utilisation by bacteria in complex ecosystems including bovine rumen (Teather and Wood, 1982), and soil samples (Hendricks *et al*., 1995). This method does not allow for any quantitative comparison of enzyme activity, though, therefore variations in growth rate, variations in the rate of enzyme production and diffusion rate means that comparisons can not be made.

5.3.4 Cellulase from Antarctic microorganisms

Garsoux *et al*. 2004 purified from an Antarctic bacterium *Pseudoalteromonas haloplanktis* a cold adapted cellulase (CelG). The psychrophilic CelG enzyme had similar k_{cat} (0.18 s^{-1}) and k_{cat}/K_m (0.35mM^{-1} s^{-1}) values at 4°C to mesophilic Cel5 from *Erwinia chrysanthemi* at 30-35°C. Like many cold adapted proteins, CelG was heat labile with a half life at 45°C of 40 minutes while the mesophilic Cel5 was unaffected at this temperature; at the optimal temperature for activity (55°C) of Cel5, CelG is totally inactive. The protein structure of the psychrophilic CelG was compared with the structure of CelG from *Erwinia chrysanthemi* and no difference was found in the catalytic cleft but several changes were noted in the overall structure such as increased loop and backbone mobility, decrease in hydrophobic effect on folding, ionic interactions and folding compactness, weak electrostatic interactions, imbalance in surface charges, which could explain the heat-labile nature of the psychrophilic CelG.

5.3.5 Literature review of cellulases produced by fungi studied in this PhD thesis research.

5.3.5.1 *Cadophora malorum (Phialophora malorum)*

Berg (1978) studied the cellulase formation and location in the fungus *Phialophora malorum* isolated from peat in a subarctic bog on different carbon sources (the genus *Phialophora* was split into two genera in 2003 (Harrington and McNew, 2003) and this species has been reclassified as *Cadophora malorum*). The cellulase was found to be partly cell free and partly cell bound. The fungus

grew on cellulose and carboxymethyl cellulose but glucose and cellobiose repressed cellulase formation. The unicellular stage (non-mycelial form) did not appear to grow on carboxymethylcellulose or cellulose but mycelium was formed on these carbon sources. They concluded that *Phialophora malorum* grew well on salt media at pH 5.3 and 7.1 and temperatures 18-22ºC, with 24ºC as the upper limit for growth. The fungus had no special growth factor requirements. Carbon sources such as glucose, cellobiose, amylose, inulin, xylan, pectate, cellulose and carboxymethylcellulose (CMC) DS 0.4) could be utilised, but CMC (DS 0.8) did not support growth. (DS; Degree of substitution indicates the average number of etherified hydroxyl groups in a glucose unit) When grown on cellulose plates containing partially hydrolysed cellulose a clearing zone was seen after 2 weeks, but not when cotton linters chromatography powder was used as the carbon source. In shaking liquid culture the unicellular form of the fungus was dominant, in stationary liquid culture, mycelium developed and dominated. The stationary culture grew slower whereas a culture on cellobiose or reprecipitated cellulose grew out in 5-6 days and the pH increased slightly during cultivation. Their results suggested that cellulase was only produced from mycelium. Cellobiose and glucose caused repression of cellulase production.

3.5.3.2 *Penicillium roquefortii*

No literature could be found reporting cellulase activity of *Penicillium roquefortii* but cellulase has been reported in many *Penicillium* sp. including *Penicillium decumbens* (Mo *et al*., 2004; Chen and Jin, 2006), *Penicillium echinulatum* (Sehnem *et al*., 2006), *Penicillium janthinellum* (Wang and Gao, 2000**;** Oliveira *et al*., 2006), *Penicillium brasilianum* (Jørgensen *et al*, 2005; Jorgensen and Olsson, 2006), *Penicillium occitanis* (Chaabouni et al., 2005), *Penicillium pinophilum*, *P. persicinum* (Jorgensen *et al*. 2005) *Penicillium funiculosum* (van Wyk, 1999; van Wyk and Leogale, 2001), *Penicillium ulaiense* (Rajal *et al*., 2002), and *Penicillium chrysogenum* (Nuero and Reyes, 2002)

5.3.5.3 *Geomyces* sp.

Geomyces spp. have been considered to be involved in the decomposition of organic material especially keratinous waste and have been isolated from decomposing forest litter Kushwaha (2000) reported the production of 19 enzymes from 390 strains of *Chrysoporium* (*Geomyces*), cellulase activity was noted from the following two species *C. indicum*, and *C. keratinophilum.* Rice *et al*. (2006) demonstrated cellulase activity from ten *Geomyces pannorus* isolates

from bait traps set in a peat bog. *Chrysosporium lucknowense* is a cellulolytic fungus belonging to euascomycetes. It produces a wide range of enzymes that catalyse the biodegradation of cellulose and hemicelluloses. The major cellulase secreted by *C. lucknowense* is CBHI (Cel7A) belonging to family 7 of glycoside hydrolases (Gusakov et al. 2005).

Fenice *et al*. (1997), showed low cellulase activity, in 1 of 5 Antarctic isolates of *Geomyces pannorum* screened for cellulase activity. Hurst *et al*. (1983) reported cellulase activity by an Antarctic isolate *Chrysosporium pannorum* at both 1ºC and 20ºC.

5.3.5.4 *Cladosporium oxysporum*

There have been no publications of *Cladosporium oxysporum* producing cellulase but much has been published on various *Cladosporium* spp. producing cellulase including *Cladosporium herbarum* (Barbosa *et al*., 2001), *Cladosporium* sp. (Abrha and Gashe., 1992). Abrha and Gashe (1992) used a *Cladosporium* sp. they isolated locally and studied effect of different carbon and nitrogen sources on the production of cellulase. They found that this *Cladosporium* sp. could degrade all types of native and modified cellulose. The greatest production of cellulase was when the organism was grown on carboxymethylcellulose rather than Avicel, filter paper or cotton. They concluded that KNO_3 was the preferred nitrogen source and that the surfactant Tween 80 increased cellulase production by 1.5 to 4.5-fold. The cellulase complex produced by the *Cladosporium* sp. was active between pH 4 to 8, with optimal activity at pH 5. The highest cellulase activity was recorded at 60°C and was stable for 24hrs at this temperature. Hurst *et al*. (1983) reported cellulase activity by *Cladosporium sphaerospermum* at both 1ºC and 20ºC.

This literature provides ample evidence that the fungal genera studied during this PhD research are capable of producing cellulase and causing cellulose degradation.

5.3.6 Wood component and cell wall degrading enzymes

Extracellular enzymes which breakdown the carbohydrate polymers present in the plant cell wall are important to both pathogenic and saprophytic microorganisms to overcome host resistance and to utilise organic and inorganic materials. Enzymatic hydrolysis of most polysaccharides will release reducing sugars which are the focus of many assays for accessing the breakdown of these substrates.

Starch is located in the storage tissue of wood and is an easily available nutrient source as it is present unmasked by any encrusting material in the cell (Zabel and Morrell, 1992). Starch and glycogen are hydrolyzed by amylases, of which there are two general types: α- and β-amylase. α-amylase (EC 3.2.1.1) is found in nearly all plants, animals, and microorganisms. α-amylase (endoamylase) catalyzes the hydrolysis of internal α-1,4-glucan linkages in starch or glycogen containing three or more α-1,4-linked glucose units, yielding a mixture of maltose and glucose.
β-amylase (EC 3.2.1.2; exoamylase) occurs in resting seeds. β-amylase releases successive maltose units from the nonreducing end of a polysaccharide chain by hydrolysis of α-1,4-glucan linkage.

Pectins are a group of colloidal substances with a high proportion of anhydro-galacturonic acid and have a variable and complex composition (Highley, 1997). They are mainly located in the middle lamella and primary cell wall. D-galacturonic acid and its methyl ester are linked as poly-(α-galactopyranosyl)-uronic acid in the backbone of pectin. Blocks of galacturonic acid are interspaced by linked α-L-rhamnopyranosyl units. Nonrhamnose sugars like galactose, arabinose, glucose, mannose, and xylose also occur. Acetyl ester groups are supposedly linked to galacturonate residues in the backbone, whereas feruloyl ester groups are thought to be linked to the neutral sugar side chains, at least in sugar beet pectin (Highley, 1997). Pectinases (pectate lyase [PL], polygalacturonase [PG], and pectin methylesterase [PME]) have been studied in mostly in pathogens and in more detail than any other wall depolymerase (Highley, 1997). Pectin methylesterase (EC 3.1.1) catalyzes the hydrolysis of methyl ester groups of pectinic acids, converting pectinic acids to pectic acids (polygalacturonic acid). Pectate lyase (EC 4.2.2) breaks down pectin by a trans-elimination reaction. Polygalacturonase hydrolyse pectic acids to galacturonic acid (Highley, 1997).

The hemicelluloses constitutes approximately 20-30% of the cell wall, and are both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid (Highley, 1997). The xylose-based hemicelluloses are usually termed xylans. Xylan is the major component of hardwood hemicellulose. Xylans are hydrolysed by β-xylanase (EC 3.2.1.8) which act randomly on the xylan backbone to produce short oligosaccharides and by β-xylosidase (EC 3.2.1.37) which hydrolyse the oligosaccharides to D-xylose (Highley, 1997).

Mannan substrates consist of a varying composition but usually have a glucose or galactose component. The hemicellulose in softwood is half xylan and half galactomannan. The main enzyme in mannan depolymerisation is β-1,4, mannanase which hydrolyses the main chain of β-1,4-mannan to linear and branched oligosaccharides which are further cleaved by glycosidases and esterases including acetylmannanesterase, α-galactosidase, β-glucosidase and β-mannosidase (Dekker and Richards, 1976; Tenkanen *et al*., 1993)

5.3.7 Proteases

The breakdown of proteins in the sapwood of the tree or timber provides wood inhabiting fungi with a source of nitrogen (Gharibian *et al*., 1996). Proteases have been reported to increase the activity of endoglucanases and thus play an important role in the degradation of cellulose (Eriksson et al., 1990). They are thought to either activate the endoglucanases or destroy protein endogucanase inhibitors.

Proteases are regarded as degradative enzymes which are capable of cleaving proteins into small peptides and amino acids and whose role it is to digest nutrient protein or to participate in the turnover of cellular protein (North, 1982) It has been demonstrated that they play a role in a range of cellular processes. The ability of proteolytic enzymes to catalyse selective modification of proteins by limited cleavage means that some proteases have a regulatory function along with a role in intracellular protein turnover, digestion, protein translocation, sporulation, and germination. Proteases play an important role in pathogenesis, for example, in the penetration of the host organism, in countering host defense mechanisms, and in nutrition during infection (North, 1982).

Proteases from a number of sources, both microbial and non-microbial, are in widespread use in the food industry (baking, brewing, cheese manufacturing, meat tenderizing), in the tanning industry, and in the manufacture of biological detergents. Proteases may be classified in a number of ways. For example, on the basis of the pH range over which they are active (acid, neutral, or alkaline), on the basis of their ability to hydrolyze specific proteins (keratinase, elastase, collagenase, etc.), or on the basis of their similarity to well-characterized proteases such as pepsin, trypsin, chymotrypsin, or the mammalian cathepsins. The most satisfactory classification scheme is based on the catalytic mechanism (North, 1982). There are four different types of protease, and these can be distinguished from one another on the basis of their sensitivity to various inhibitors. The major groups are aspartic proteases (EC .4.23), metalloproteases (EC 3.4.24), serine proteases (EC 3.4.21) and cysteine proteases (EC 3.4.22) (North, 1982).

5.4 Materials and methods

5.4.1 Media and Reagents

The following media and reagents were used in the experiments related to this chapter's objectives:

Carboxymethylcellulose *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Carboxymethylcellulose and 1.5% agarose in distilled water.

Avicel *Trichoderma viride* medium A - 14 mls 10% $(NH4)_2$ SO_4, 15mls 1M KH_2PO_4, 6 mls 35% urea, 3 mls 10% $CaCl_2$, 3 mls 10% $MgSO_4.7H_2O$, 1 ml Trace elements solution (10 mls concentrated HCl, 0.51% $FeSO_4$, 0.186% $MnSO_4.4H_2O$, 0.166% $ZnCl_2$, 0.2% $CoCl_2$), 2 mls Tween 80, 0.2% Avicel and 1.5% agarose in distilled water.

Cellulose Broth- 1% Avicel, 1.5% soya bean flour (Sigma, USA), 1.5% K_2HPO_4, 0.5% $(NH_4)_2SO_4$, 0.006% $CaCl_2.2H_2O$ (BDH Laboratory Supplies, England), 0.006% $MgSO_4.7H_2O$, 0.02% (v/v) Tween 80 in distilled water.

Universal buffer- 2.473 grms boric acid (APS Ajax Finechem, Australia), 2.721 mls phosphoric acid (Merck, Germany), 2.299 mls acetic acid (APS Ajax Finechem, Australia), 1 ml 20% sodium azide (BDH Laboratory Supplies, England), and 1000 mls distilled water. 0.2M NaOH was added until pH 4.8 was obtained. $CaCl_2$ was added to a concentration of 7.5 mM.

Endo-1,4-β-glucanase substrate- 1% hydroxyethylcellulose in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate)

0.05M citrate buffer pH 4.8- Solution 1-0.05M citric acid (10.51grms/l) (APS Ajax Finechem, Australia), Solution 2-0.05M tri sodium citrate (14.71grms/l) (APS Ajax Finechem, Australia). Adjust pH to 4.8 by adding 667mls solution 1 to 1l of solution 2.

Avicelase substrate- 1% Avicel in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate).

Carboxymethylcellulase substrate- 1% carboxymethylcellulose in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate).

β-1,4-glucan cellobiohydrolase substrate- 1mM p-Nitrophenyl β-D cellobioside (Sigma, USA), in 50mM sodium acetate (APS Ajax Finechem, Australia) pH 4.8.

Dinitrosalicylic acid reagent- 1% 2-hydroxy-3,5,-dinitrobenzoic acid (Merck, Germany), 1.6% NaOH (added slowly), 30% Rochelle salts/Potassium sodium tartrate (APS Ajax Finechem, Australia), (added in small portions with continuous stirring and filter to remove particulate material) in distilled water.

Protease substrate solution- 0.2% Azocasein (Sigma, USA), in 50 mM 4-Morpholine-propanesulfonic acid (MOPS) (Boehringer Mannhiem, Germany) containing 5 mM $CaCl_2$ pH 7.0 at 40°C.

Protease stop reagent- 15% Trichloroacetic acid (APS Ajax Finechem, Australia) in distilled water.

Amylase substrate-1% soluble starch (Merck, Germany), in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate). Heat to dissolve.

Xylanase substrate-1% birchwood xylan (Sigma, USA), in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate). Microwave on high until fully dissolved.

Mannanase substrate- 1% locust bean gum (Sigma, USA), in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate). Homogenizing at 60°C, heat to boiling point with continuous stirring and allowed to cool, cover and slowly stir overnight at 4°C

Polygalacturonic acid pectinase substrate- 1% polygalacturonic acid (Sigma, USA), in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate).

Pectin pectinase substrate- 1% pectin (Sigma, USA), in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate). Heat to dissolve.

SDS Acrylamide separating gel- 12.5 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 7.5 ml 1.5M TrisCl (APS Ajax Finechem, Australia), pH 8.8, 0.3 ml 10% Sodium dodecylsuphate (BDH Laboratory Supplies, England), 7.5 ml 1% CMC or HEC, 2.1 ml distilled water (total volume 30mls). Add 150 µl 10% Ammonium persulphate (Plusone, Pharmacia Biotek, Uppsala, Sweden), and 10 µl TEMED (Plusone, Pharmacia Biotek, Uppsala, Sweden), after degassing and before pouring.

Acrylamide stacking gel- 0.67 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 1.25 ml 0.5 M TrisCl pH 6.8, 0.05 ml 10% sodium dodecylsulphate, 3 ml distilled water (total volume 5mls). Add 25 µl 10% Ammonium persulphate and 2.5 µl TEMED after degassing and before pouring.

SDS-Tris-Glycine electrophoresis buffer- 0.025M Tris, 0.192M glycine (APS Ajax Finechem, Australia), 0.1% Sodium dodecylsulphate pH 8.3 in distilled water.

Native acrylamide separating gel- 12.5 ml Biorad 40% acrylamide/Bis solution 29:1 3.3%C, 7.5 ml 1.5M TrisCl pH 8.8, 7.5 ml 1% CMC or HEC, 2.4 ml distilled water (total volume 30mls). Add 150 µl 10% Ammonium persulphate and 10 µl TEMED after degassing and before pouring.

Native acrylamide stacking gel- 0.67 ml Biorad 40% acrylamide/Bis solution 29:1 3.3%C, 1.25 ml 0.5 M TrisCl pH 6.8, 3.05 ml distilled water (total volume 5mls). Add 25 µl 10% Ammonium persulphate and 2.5 µl TEMED after degassing and before pouring.

Tris-Glycine electrophoresis buffer- 0.025M Tris, 0.192M glycine, pH 8.3 in distilled water.

Coomassie stain solution- 0.025% Coomassie Blue R-250 (BDH Laboratory Supplies, England), 40% (v/v) methanol (Scharlau, Spain), 7% (v/v) glacial acetic acid in distilled water.

Coomassie Destaining solution I- 40% (v/v) methanol, 7% (v/v) glacial acetic acid in distilled water.

Coomassie Destaining solution II- 5% (v/v) methanol, 7% (v/v) glacial acetic acid in distilled water.

Silver stain fixing solution- 40% (v/v) absolute ethanol (BDH Laboratory Supplies, England), 10% (v/v) acetic acid in distilled water.

Silver stain sensitizing solution- 30% (v/v) absolute ethanol, 6.8% (w/v) sodium acetate (anhydrous), 0.2% (w/v) sodium thiosulphate (pentahydrate) (BDH Laboratory Supplies, England), in distilled water. Within 1 hr of use add 0.125% (v/v) glutaraldehyde (BDH Laboratory Supplies, England).

Silver stain silver solution- 0.25% (w/v) silver nitrate (APS Ajax Finechem, Australia), in distilled water. Within 1hr of use add 0.015% (v/v) formaldehyde (Andrews, Takapuna, Auckland).

Silver stain developing solution-2.5% (w/v) sodium carbonate (anhydrous) (BDH Laboratory Supplies, England), in distilled water. Within 1 hr of use add 0.0074% (v/v) formaldehyde.

Silver stain stop solution- 1.5% (w/v) Ethylenediaminetetraacetic acid (disodium salt) (Na_2EDTA) (BDH Laboratory Supplies, England), in distilled water.

Silver stain preserving solution- 30% (v/v) absolute ethanol, 4%(v/v) glycerol (APS Ajax Finechem, Australia), in distilled water.

Periodic acid (PAS) staining reagent 1- 5% phosphotungstic acid (BDH Laboratory Supplies, England), in 2N HCl.

PAS staining reagent 2- 7% methanol, 14% acetic acid in distilled water.

PAS staining reagent 3- 1% periodic acid (BDH Laboratory Supplies, England), in 7% trichloroacetic acid.

PAS staining reagent 4- 0.5% sodium metabisulfite (BDH Laboratory Supplies, England), in 0.1N HCl.

Cellulose gel- 7.5 ml 1% CMC, 0.45grms agarose in 22.5mls Universal buffer pH 4.8.

5.4.2 Screening for cellulase activity using an agarose plate technique

Four hundred and four Antarctic fungal isolates as described in Chapter 3 isolated from a variety of sample substrates, particularly structural wood samples, were screened for their ability to degrade carboxymethylcellulose.

Single isolates of fungi were inoculated as a streak line down the middle of the cellulose/agarose 90 mm Petri plates which consisted of carboxymethylcellulose *Trichoderma viride* medium A (Mandels *et al*. 1962) and incubated at isolation temperature, 4°C, 15°C or 25°C. The incubation periods at the prescribed temperatures were as follows: after two days at 25°C, one week at 15°C, or six weeks at 4°C. Thereafter, the plates were flooded with 0.1% Congo red and

allowed to react for 30 minutes followed by destaining with 1M NaCl for 60 minutes according to the method described by Teather & Wood (1982). The width of fungal growth and the zone of clearing in the cellulose medium were measured at three locations along the streak line. The Index of Relative Enzyme Activity (which compared the width of the clearing zone with the width of fungal growth) was determined in order to designate which fungi were deemed as producing cellulase (Bradner *et al*. 1999a).

After the screening process mentioned above, 60 fungal isolates isolated at 15°C or 25°C were subsequently screened by the same method for their ability to degrade carboxymethylcellulose at 4°C.

Fungal isolates *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805, as described in Section 4.4.2, were also screened for their ability to degrade Avicel at 4°C using the same method as above but with Avicel *Trichoderma viride* medium A in place of carboxymethylcellulose *Trichoderma viride* medium A

5.4.3 Endoglucanase production

In order to grow fungi for studying endoglucanase activity, an adaptation of the methods of Bradner and colleagues (Bradner *et al*. 1999b) was used. Twenty seven fungal isolates were used to study endoglucanase activity based on the cellulose screening results.

Fungi were grown on YM agar for one week at 15°C or were grown for four weeks at 4°C, then harvested by scraping the fungal growth off the top of the agar plate and were rinsed with 2 ml of saline solution (0.9% NaCl, 0.01% Tween 80). The cells (hyphal fragments and spores) were added to 50 mls of cellulose broth in a 250 ml flask. Flasks were shaken on a Chiltern shaking platform rotating at 150 rpm and a two ml sample taken every day for 15 days for fungi grown at 15°C and every 3 days for 36 days for fungi grown at 4°C. The fungal cells were separated from the culture supernatant by centrifugation.

5.4.4 Measurement of Endo-1, 4-β-glucanase Catalytic Activity

Endo-1,4-β-glucanase catalytic activity was determined from the culture supernatant using the protocol described by Bailey *et al*. (1992). The enzyme supernatants (320 µl) were mixed with 480 µl of Endo-1,4-β-glucanase substrate solution and after ten minutes incubation at 50°C, the reaction was stopped with the addition of 1.2 ml dinitrosalicylic acid reagent and subsequent boiling in a water bath for five minutes. The absorbance was measured spectrophotometrically at 540 nm against a blank, which was the same volume as the sample but the enzyme supernatant for the blank was added when the samples were boiled. 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.

5.4.5 Total protein

During the course of this research total protein was measured by two methods.

- The Bradford method using a protein assay kit (Bio-Rad Laboratories, Richmond, CA, U.S.A.) according to the manufacturer's instructions, using bovine serum albumin as the standard.
- A binding copper ion- protein method using a 2-D Quant kit (Amersham Bioscience, Sweden) according to manufacturer's instructions, using bovine serum albumin as the standard.

5.4.6 Composition of extracellular cellulase complex using sodium dodecyl sulphate polyacrylamide electrophoresis (SDS PAGE)

Seven fungi as described in Section 4.4.2, *C. malorum* 182, *C. malorum* 242, *P. roquefortii* 405, *P. roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *C. oxysporum* 805, were studied regarding the composition of the cellulase complex accumulating in the supernatant using electrophoresis.

Migration or mobility of proteins through the continuous polyacrylamide gel matrix is dependant on size, with small proteins migrating faster than larger proteins. Using the SDS-PAGE method the electrophoretic mobility was dependant only on molecular weight as the net charge and shape of the individual proteins is negated by the presence of SDS. SDS denatures the protein to a linear polypeptide chain and binds on average one SDS molecule to every two amino

acid residues, this binding of negatively charged detergent overrides the intrinsic charge of individual proteins (Creighton, 1993).

Individual fungal isolates were grown in 1 litre of cellulose broth at 4°C and 15°C. Fungal biomass was removed by centrifugation and the extracellular supernatant (referred to as protein preparations in this chapter) was concentrated using ultrafiltration with a polyethersulphone membrane 10,000 MW cut-off. All protein preparations were prepared using a SDS-PAGE clean-up kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. The kit uses precipitation and co precipitation to quantitatively precipitate the sample proteins and removes interfering substances such as detergents, salts, lipids, phenolics and nucleic acids. Protein preparations were subjected to sodium dodecyl sulphate (SDS) polyacrylamide electrophoresis (PAGE) using the Hoefer SE 600 Ruby electrophoresis system. The samples, typically 70 µl, were boiled for 5 minutes, and loaded in duplicate onto the 12.5% acrylamide gel containing a final concentration of 0.1% hydroxyethylcellulose (to determine endo-1,4-β-glucanase activity) or 0.1% carboxymethylcellulose (to determine carboxymethylcellulase activity) gel, one duplicate per lane from lane 2-7 and the other duplicate sample per a lane from lanes 9-14. SDS-Tris-glycine buffer was used as the electrophoresis buffer. Electrophoresis was conducted at room temperature at 60 volts, 30 milliamps until the bromophenol blue dye front had run off the bottom of the gel, approximately 20 hours. Broad SDS-PAGE molecular mass protein markers (range 6,500-200,000 M_r) and pre-stained broad range molecular mass markers (ranges 6,900- 209,000 M_r) were electrophoresed on each gel. The gel was cut in half and one half of the gel was used to visualise the protein bands by Coomassie Blue protein staining as described in Section 5.4.7. The other half of the gel was washed with rocking in 2.5% Triton X 100 for 1 hour and subsequently rinsed in MilliQ water for 10 minutes. This rinsing was repeated 3 times, and the gel was incubated at 50°C in preheated Universal Buffer pH 4.8 for 40 minutes. After incubation, the gel was washed in MilliQ water for 5 minutes and this was repeated 3 times and then stained with 0.1% Congo red for 30 minutes. After staining, the gel was incubated in 1M NaCl until clearing zones were visible. To increase contrast, the gels were put into 5% (v/v) acetic acid, which converted the background from a red to a dark blue colour. This method was adapted from that used by Teather and Wood (1982).

5.4.7 Coomassie Blue protein staining method

The method used was as per the instructions in the protein electrophoresis technical manual produced by Amersham Biosciences (Sweden).

Gels were submerged in Coomassie Blue staining solution for 4hrs to overnight while shaking slowly on a laboratory shaker (Chiltern). The bulk of stain was removed by replacing Coomassie Blue stain solution with Coomassie Blue destaining solution I, slowly shaken for 30 minutes. The Coomassie Blue destaining solution I was replaced with Coomassie Blue destaining solution II, shaken slowly until gel background becomes clear. Coomassie Blue destaining solution II may require changing during this step.

5.4.8 Composition of extracellular Endo-1, 4-β-glucanase complex using Native electrophoresis

Native or non-denaturing gel electrophoresis is run in the absence of SDS. While in SDS-PAGE the electrophoretic mobility of proteins depends primarily on their molecular mass, in native PAGE the mobility depends on both the protein's charge and its hydrodynamic size. The charge driving the electrophoresis is governed by the intrinsic charge on the protein at the pH of the running buffer. Since the protein retains its folded conformation, its hydrodynamic size and mobility on the gel will also vary with the nature of this conformation (higher mobility for more compact conformations, lower for larger structures).

The protein preparations created in Section 5.4.3 were subjected to native electrophoresis. All enzyme preparations were prepared using a SDS-PAGE clean-up kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions with the following adaptations in step 14 when an equal volume of SDS-PAGE sample buffer with reductant was added, an equal volume of loading buffer was added (10% sucrose and 0.02% bromophenol blue), and in step 15 when the tube was placed in a boiling water bath for 3 minutes this step was omitted. Enzyme preparations were subjected to native polyacrylamide electrophoresis using the Hoefer SE 600 Ruby electrophoresis system. The samples, typically 50 µl, were loaded in duplicate onto the 12.5% acrylamide gel minus SDS containing a final concentration of 0.1% hydroxyethyl cellulose, one

duplicate per lane from lane 2-7 and the other duplicate sample per a lane from lanes 9-14. Tris-glycine buffer was used as the electrophoresis buffer. Electrophoresis was conducted at room temperature at 60 volts, 30 milliamps until the bromophenol blue dye front had run off the bottom of the gel, approximately 20 hours. High molecular weight (HMW) markers for Native electrophoresis (66000-669000M_r) were electrophoresed on each gel. The gel was cut in half and one half of the gel was used to visualise the protein bands by Silver protein staining as described in Section 5.4.9. The other half of the gel was washed with rocking in MilliQ water for 10 minutes, 3 times and afterwards incubated at 50ºC in preheated Universal Buffer pH 4.8 for 40 minutes. After incubation, the separation gel was washed in MilliQ water for 5 minutes and this was repeated 3 times and then stained with 0.1% Congo red for 30 minutes. After staining, the gel was incubated in 1M NaCl until clearing zones were visible. To increase contrast, the gels were put into 5% (v/v) acetic acid, which converted the background from a red to a dark blue colour. This method was adapted from that used by Teather and Wood (1982)

5.4.9 Silver protein staining method

Silver nitrate stains rely on the reaction of silver ions with protein under weakly acidic conditions, followed by the reduction of silver ion to metallic silver by formaldehyde under alkaline conditions. The proteins are first fixed in the gel, then glutaraldehyde is added to sensitise the protein. The silver nitrate solution is added which reacts with the protein, the silver ions are converted to metallic silver by the addition of formaldehyde in sodium bicarbonate which drops the pH to alkali. The reaction is stopped by the addition of EDTA.

The method used for visualising protein bands with silver stain was as per the instructions in the protein electrophoresis technical manual produced by Amersham Biosciences.

Staining times can differ according to gel thickness. All times in this method were for a 1.5mm gel. Gels were submerged in silver staining fixing solution for 30 min with gently shaking. The silver staining fixing solution was replaced with silver staining sensitising solution, gently shaken for 30 min. Washed with distilled water 3 times for 10 min. The gel was then gently shaken in silver staining silver

solution for 30 min before washing twice for 1 min in distilled water. Distilled water was replaced with silver staining developing solution and gently shaken for 5-10 minutes. Silver staining stop solution replaced the silver stain developing solution once protein bands appeared and before the background went dark. Gel was left in stop solution for 10 minutes with shaking. Gels were washed 3 times for 5 min in distilled water before being placed into silver stain preserving solution for 30 min.

5.4.10 Periodic acid stain (PAS) staining of glycoproteins

SDS-PAGE electrophoresis as described in Section 5.4.6 minus the cellulose substrates was performed on the enzyme preparation from Section 5.4.3. After electrophoresis the gel was cut in half and one half of the gel was used to visualise the protein bands by silver protein staining as described in Section 5.4.9. The other half of the gel was placed in a glass dish and submerged in PAS staining reagent 1, rocked gently for 90 minutes at room temperature, then soaked twice in 500mls of PAS staining reagent 2 for 1hr. The oligosaccharides were oxidised by soaking gel in PAS staining reagent 3 for 1hr, followed by soaking in PAS staining reagent 4 until gel fades from the initial amber colour (approximately 1hr). The gel was then stained in the dark and on ice with Schiffs reagent (BDH Laboratory Supplies, England) until pink bands appear (Kohn 1961).

5.4.11 Documentation of gels

Stained gels were photographed using a luminescent image analyzer (LAS 1000plus) (Fujifilm, Japan) used in the digitize exposure mode and with transparent light (DIA).

Exposure time for the protein gels was automatic but for the zymograms the exposure time was manually set at 1 sec. Images were edited using Image gauge ver. 3.12 (Fujifilm, Japan).

5.4.12 Composition of extracellular Endo-1, 4-β-glucanase complex using Isoelectrofocusing (IEF) electrophoresis

Differences in proteins isoelectric points are the basis of separations by isoelectric focusing (IEF). The pI is the pH at which a protein will not migrate in an electric field and is determined by the charged groups in the protein. Proteins can carry

positive, negative or zero charge depending on their local pH, and for every protein there is a specific pH at which its net charge is zero; this is its pI. When a protein is placed in a medium with a pH gradient and subjected to an electric field it will initially move towards the electrode with the opposite charge. During migration through the pH gradient the protein will pick up or lose protons. As it migrates the net charge and the mobility will decrease and the protein will slow down. Eventually the protein will arrive at the point in the pH gradient which is equal to its pI. Here it will be uncharged and hence stop migrating.

The enzyme preparations created in Section 5.4.3 were subjected to IEF electrophoresis using an Ettan IPGphor II isoelectric focusing system (Amersham Biosciences). All protein preparations were prepared using a 2D-clean-up kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. At step 14 the protein preparations were rehydrated in 350µl of Destreak reagent containing 0.5% IPG buffer (3-10). Rehydrated protein solutions were centrifuged. 18 cm Immobiline Dry strips pH 3-10 were rehydrated in the Immobiline reswelling tray by pipetting 350µl of rehydrated protein solution into the channel and placing the Dry strips on top gel side down, the channel was filled with Drystrip cover solution and left for 10-20hrs. Rehydrated Immobiline Dry strips were transferred to the Ettan IPGphor Cup loading manifold which was prefilled with Immobiline DryStrip cover fluid. Strips are placed under the fluid with the anodic end resting on the appropriate mark, strips were centred down the length of the channel. Wet (150µl distilled water) pre cut paper wicks were placed at each end of the Dry strip such that one end of the wick overlaps the end of the gel. The electrode assembly was placed on top of the wicks and locked into place by swivelling the cams into the closed position. The lid was shut and the pre set program was run. Broad range pI markers for IEF electrophoresis (3.5-9.3pI) were electrophoresed with each run. For 18 cm Immobiline Dry strips pH 3-10 the run is step 1, step and hold, 500V for 1hr, step 2, gradient, 1000V for 1 hr, step 3, 8000V for 2.3 hrs, step 4, step and hold, 8000V for 10 min. After electrophoresis the strips were removed from the manifold and washed with rocking in 2.5% Triton X 100 for 1 hour and subsequently rinsed in MilliQ water for 10 minutes. This rinsing was repeated 3 times before being laid onto a cellulose gel, incubated at 50°C for 60 minutes to allow the cellulose active bands to breakdown the cellulose in the gel. After

incubation, the cellulose gel was washed in MilliQ water for 5 minutes and this was repeated 3 times and then stained with 0.1% Congo red for 30 minutes. After staining, the gel was incubated in 1M NaCl until clearing zones were visible. To increase contrast, the gels were put into 5% (v/v) acetic acid, which converted the background from a red to a dark blue colour. This method was adapted from that used by Teather and Wood (1982). The Immobiline Dry strips were silver stained as described in Section 5.4.9.

5.4.13 Measurement of Cellulase Activity using different cellulose sources

Avicelase, carboxymethylcellulase, catalytic activity was determined of the culture supernatant created in Section 5.4.3 using the protocol described by Bailey *et al*. (1992). The extracellular supernatants (320 µl) were mixed with 480 µl of Avicelase, carboxymethylcellulase substrate solution respectively (for the Avicelase assay the substrate was stirred continuously during the dispensing of the 480 µl) and after ten minutes incubation at 15°C or 50°C, the reaction was stopped with the addition of 1.2 ml dinitrosalicylic acid reagent and subsequent boiling in a water bath for five minutes. The absorbance was measured spectrophotometrically (Thermospectronic Heλios γ) at 540 nm against a blank, which was the same volume as the sample but the enzyme supernatant for the blank was added when the samples were boiled. All assays were performed in duplicate. Activity was expressed as micromoles glucose released per minute and converted to specific activity by dividing by the total protein in the supernatant.

Filter paper catalytic activity was determined from the culture supernatant using an adaptation of the protocol described by Bailey *et al*. (1992). The enzyme supernatants (320 µl) were mixed with 480 µl of citrate buffer solution containing two 1cm by 1cm pieces of cellulose filter paper (Whatman No 1 filter paper) and after ten minutes incubation at 15°C or 50°C, the reaction was stopped with the addition of 1.2 ml dinitrosalicylic acid reagent and subsequent boiling in a water bath for five minutes. The absorbance was measured spectrophotometrically at 540 nm against a blank, which was the same volume as the sample but the enzyme supernatant for the blank was added when the samples were boiled. All assays were performed in duplicate. Activity was expressed as micromoles glucose released per minute and converted to specific activity by dividing by the total protein in the supernatant.

β-1,4-glucan cellobiohydrolase activity was determined from the culture supernatant using an adaptation of the protocol described by Magalhães *et al*. (2006). The enzyme supernatant (100µl) was incubated with 500µl of β-1,4-glucan cellobiohydrolase substrate solution and after ten minutes incubation at 15°C or 50°C, the reaction was stopped with the addition of 1 ml 1M sodium carbonate. The absorbance was measured spectrophotometrically at 410 nm against water blank. All assays were performed in duplicate. Activity was expressed as micromoles of nitrophenol released per minute and converted to specific activity by dividing by the total protein in the supernatant.

5.4.14 Measurement of activity of additional enzymes associated with wood component and cell wall degradation.

Amylase, xylanase, mananase, pectinase, catalytic activity was determined from the culture supernatant created in Section 5.4.3 using the protocol described by Bradner *et al*. (1999). The enzyme supernatants (320 µl) were mixed with 480 µl of substrate solution (amylase, xylanase, mannanase and two substrates for pectinase activity, polygalacturonic acid pectinase and pectin pectinase) and after ten minutes incubation at 15°C or 50°C, the reaction was stopped with the addition of 1.2 ml dinitrosalicylic acid reagent and subsequent boiling in a water bath for five minutes. The absorbance was measured spectrophotometrically at 540 nm against a blank, which was the same volume as the sample but the enzyme supernatant for the blank was added when the samples were boiled. All assays were performed in duplicate. Activity was expressed as micromoles glucose released per minute and converted to specific activity by dividing by the total protein in the supernatant.

5.4.15 Measurement of protease activity

Protease activity was determined from the culture supernatant created in Section 5.4.3 using a modification of the protocol described by Peek *et al*. (1993). The buffer was changed to 50mM 4-Morpholine-propanesulfonic acid (MOPS) pH 7.0, the volumes of enzyme supernatant and substrate were changed and the assay was run at 15 or 50°C. The enzyme supernatants (100 µl) were mixed with 500 µl of protease substrate solution and after ten minutes incubation at 50°C, the reaction was stopped with the addition of 0.5 mls protease stop reagent. After a further 15 minutes, the tubes were spun in a bench top mini centrifuge at 13,200 rpm

(Eppendorf Model 5415D) for 5 minutes. The absorbance was measured spectrophotometrically at 420 nm against a blank which was the same volume as the sample but the enzyme supernatant for the blank was added after the stop reagent had been added. All assays were performed in duplicate. Activity was expressed unit (ΔA_{420} of 1.0 per hour) and converted to specific activity by dividing by the total protein in the supernatant.

5.5 Results

5.5.1 Screening for cellulase activity using an agarose plate technique

Four hundred and four Antarctic fungal isolates from a variety of sample sites, particularly structural wood were screened for their ability to degrade carboxymethylcellulose. The full list of cellulase screening results is presented in Appendix 5. Table A5.1 fungi isolated at 4°C, Table A5.2 fungi isolated at 15°C, Table A5.3 fungi isolated at 25°C, Table A5.4 fungi isolated from December 1998 sampling trip and Table A5.5 fungi isolated from January 2001 sampling trip. The highest percentages (31%) of carboxymethylcellase (CMCase) positive organisms were isolated from the *Terra Nova* Hut. Of the fungi isolated from *Discovery* Hut, 25% were CMCase positive while 16% of fungi isolated from *Nimrod* Hut were CMCase positive. The percentage of CMCase positive organisms from *Terra Nova* Hut and *Discovery* Hut was statistically different from the percentage of CMCase positive organism isolated from *Nimrod* Hut. ($p=0.015$ and 0.019 respectively) The percentage of CMC positive fungi isolated from *Terra Nova* Hut was not statistically different from the percentage of CMCase positive fungi found in *Discovery* Hut ($p=0.135$) using Fisher's exact tests. Table 5.1 shows the number and percentage of CMC positive organisms from the four hundred and four Antarctic fungal isolates screened from the three Historic Huts and other locations. Figure 5.1 shows two typical positive results. The fungal growth can be seen as a red streak down the middle of the plate with the yellow/orange clear zone around the fungal growth. The stained undegraded cellulose is red making up the rest of the agar plate

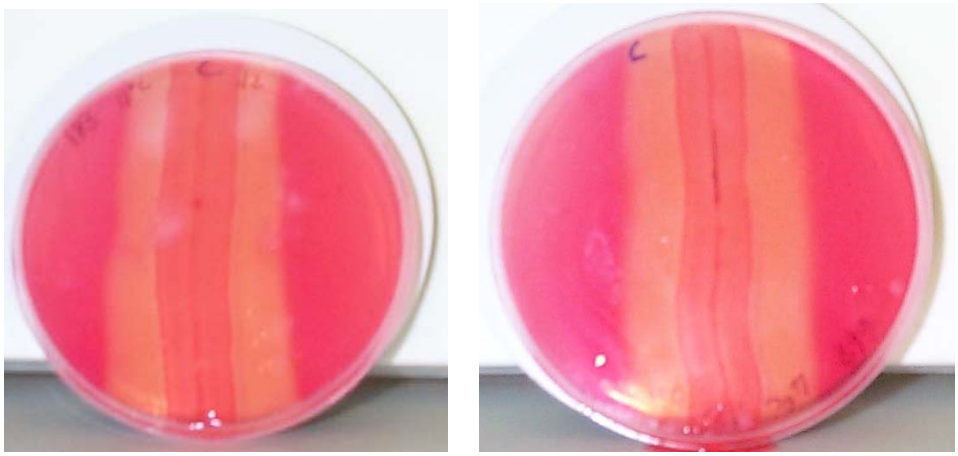

Figure 5.1: Photograph of two CMCase positive fungi growing on Carboxymethylcellulose *Trichoderma viride* medium A

Table 5.1: Number and percentage of CMCase positive organisms from the 404 screened.

	***Discovery* Hut**	***Terra Nova* Hut**	***Nimrod* Hut**	**Other locations**
Number of fungal isolates tested for CMC activity	80	235	61	28
Number of fungal isolates demonstrating clearing of CMC	19	73	10	4
% of total isolates that were CMC positive.	25	31	16	14

Of the fungal isolates isolated from wood substrates, 33% were able to degrade carboymethylcellulose (Table 5.2). Thirty one percent of fungi isolated from wood from outside the huts were carboxymethylcellulase positive while 37% of fungi isolated from wood inside the hut were positive. Twenty percent of fungi from wooden artefacts were carboxymethylcellulase positive.

Table 5.2: Number and percentage of CMCase positive organisms isolated from wood substrates within the 3 ASPA.

	Swab wood outside	**Wood outside**	**Swab wood inside hut**	**Wood inside**	**Swab inside walls**	**Wood door step**	**Swab roof inside**	**Swab wood shelf**	**Wood bench**	**Swab wood box**
Number of fungal isolates tested for CMC activity	23	15	21	7	68	3	8	2	1	22
Number of fungal isolates displaying clearing of CMC	10	2	7	3	27	0	3	1	0	4
% of total isolates that were CMC positive.	43	13	33	43	40	0	37	50	0	18

Of the fungal isolates from other substrates within the hut, 23% were carboxymethylcellulase positive (Table 5.3), Fifty five percent of fungi isolated from visible fungal mycelium within the hut were carboxymethylcellulase positive.

Table 5.3: Number and percentage of CMCase positive organisms isolated from other substrates within the Historic huts.

	Swab of leather boot	**Swab leather horse harness**	**Swab cloth**	**Straw**	**Straw plus extra material**	**Swab fungal hyphae**	**Sleep bag fibre**	**Swab book**	**Food plant origin**	**Food**
Number of fungal isolates tested for CMC activity	27	19	21	28	17	9	3	3	15	17
Number of fungal isolates displaying clearing of CMC	3	10	3	10	2	5	0	0	1	7
% of total isolates that were CMC positive.	11	53	14	36	12	55	0	0	7	41

Fungi isolated from substrates found outside the hut, 5 % of isolates from wood away from the historic sites were carboxymethylcellulase positive with one fungus out of nineteen demonstrating ability to degrade carboxymethylcellulose (Table 5.4).

Table 5.4: Number and percentage of CMCase positive organisms isolated from other substrates from outside the Historic Huts.

	Corn/soil	**Oil/Soil**	**Air**	**Penguin guano**	**Cape Adare**	**Wood away from Historic site**	**Resin**	**Swab of brooms**
Number of fungal isolates tested for CMC activity	4	9	7	4	6	19	4	17
Number of fungal isolates demonstrating clearing of CMC	0	0	1	0	2	1	0	4
% of total isolates that were CMC positive.	0	0	14	0	33	5	0	23

When the isolation temperatures of the fungi were taken into account, interestingly, very similar percentages of CMCase positive organisms were found at all three temperatures (Table 5.5) which were not statistically different.

Table 5.5: Number and percentage of CMCase positive organisms isolated at 4, 15 or 25°C.

Isolation Temperature	**4°C**	**15°C**	**25°C**
Number of fungal isolates tested for CMC activity	187	154	63
Number of fungal isolates demonstrating clearing of CMC	49	42	15
% of total isolates that were CMC positive.	26	27	25

The 60 fungal isolates isolated at 15°C or 25°C were screened for their ability to degrade carboxymethylcellulose at 4°C; the 60 consisted of 37 that were isolated at 15°C and 23 that were isolated at 25°C. Of the 37 isolated at 15°C, 7 could degrade CMC at both 15°C and 4°C, 7 degraded CMC at 15°C and not at 4°C, 6 degraded CMC at 4°C but not at 15°C and 17 did not degrade CMC at either temperature. Figure 5.2 shows the index of relative enzyme activity for the fungal isolates isolated 15°C when tested at 15°C and 4°C.

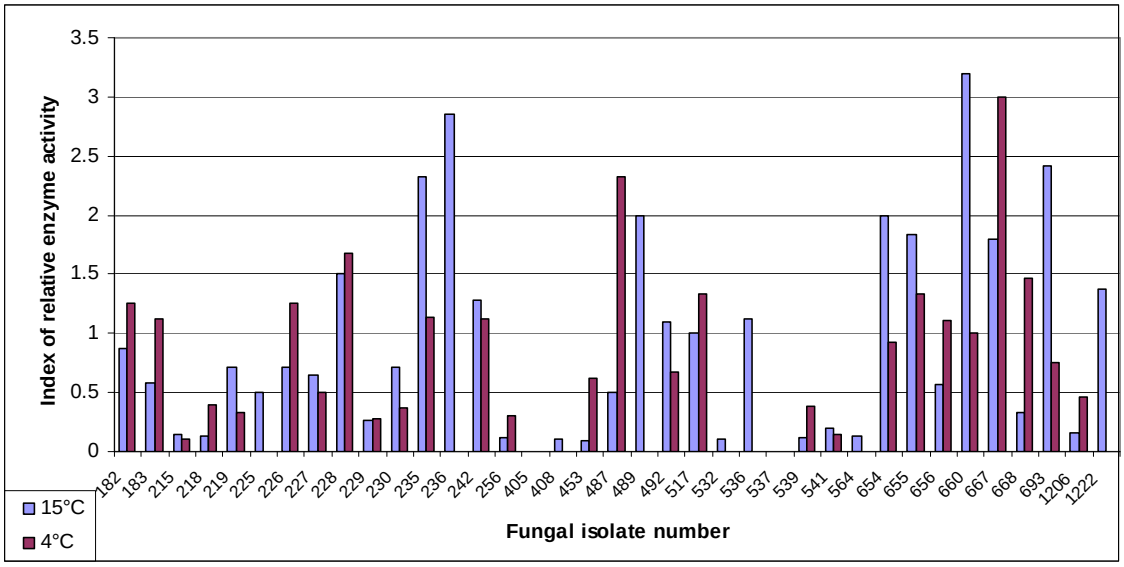

Figure 5.2: Graph of the comparison of the index of relative enzyme activity at 15°C and at 4°C for the 37 fungal isolates isolated at 15°C.

Of the 23 fungal isolates isolated at 25°C, 1 degraded CMC at both 25°C and 4°C, 7 degraded CMC at 25°C and not at 4°C, 3 degraded CMC at 4°C and not at 25°C and 12 did not degrade CMC at either temperature. Figure 5.3 shows the index of relative enzyme activity for the fungal isolates isolated 25°C when tested at 25°C and 4°C. Table A5.6 in Appendix 5 has complete results of this screening data.

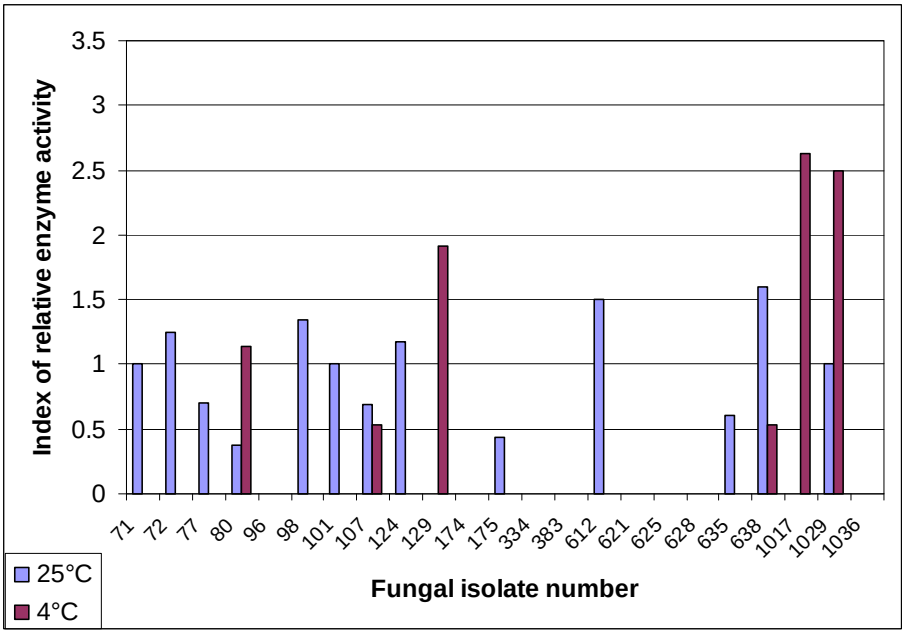

Figure 5.3: Graph of the comparison of the index of relative enzyme activity at 25°C and at 4°C for the 23 fungal isolates isolated at 25°C.

When the 7 fungi that were described in Section 4.4.2 were screened for their ability to degrade both carboxymethylcellulose and Avicel, only *Cadophora*

malorum 242 and *Penicillium roquefortii* 405 showed an ability to degrade both cellulose substrates; the amount of Avicel degraded was very small when compared with the degradation of carboxymethylcellulose. Using the criteria of the earlier experiments, none of the fungi could be classified as degrading Avicel as the factors were not greater than 1.

Table 5.6: Screening of *C. malorum* 182, *C. malorum* 242, *P. roquefortii* 405, *P. roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *C. oxysporum* 805 for CMCase and Avicelase activity

Fungal isolate	**Index of relative enzyme activity when grown on CMC**	**Index of relative enzyme activity when grown on Avicel**
Cadophora malorum 182	1.26±0.09	0
Cadophora malorum 242	2.33±0.47	0.85±0.20
Penicillium roquefortii 405	2.28±0.40	0.58±0.11
Penicillium roquefortii 408	1.71±0.24	0
Geomyces sp. 711	0.66±0.12	0
Geomyces sp. 824	1.14±0.20	0
Cladosporium oxysporum 805	1.34±0.13	0

± S.D.

5.5.2 Measurements of Endo-1, 4-β-glucanase Catalytic Activity

The levels of extracellular endo-1,4-β-glucanase activity produced by 7 fungi from *Discovery* Hut and 20 extracellular endo-1,4-β-glucanase producing fungi from *Terra Nova* Hut were measured. The graphs of levels of endo-1,4-β-glucanase activity accumulation over the growth periods 15 days when fungi were grown at 15°C and 35 days when grown at 4°C for all 27 fungi are presented in Appendix 7, Figures A7.1-A7.27. Figures 5.4 and 5.5 show the cellulase enzymes levels when they were at their peak which was after 10 days at 15°C and 28 days at 4°C. All of the fungi selected from *Discovery* Hut for this experiment demonstrated the production of endo-1,4-β-glucanase (endoglucanase) activity. Figure 5.2 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 from *Discovery* Hut were cultured at either 4°C or 15°C. As shown in Figure 5.4, 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. Levels of accumulated endo-1, 4-β-glucanase activity were measured at 4°C and 15°C, ranged from 62 specific activity units to 9.4 specific activity units for fungi grown at 4°C and 58 specific activity units to 5.6 specific activity units for cultures at 15°C. From the levels of accumulated endoglucanase activity, isolates 226, 228 and 816 produced more

endoglucanase activity at 15°C than 4°C. Isolate 227 produced more endoglucanase activity at 4°C than 15°C. Isolate 129 produced slightly more endoglucanase activity at 15°C than 4°C. The two identified *Cladosporium* strain 805 and *Geomyces* sp. 824 produced more endoglucanase activity at 4°C than 15°C.

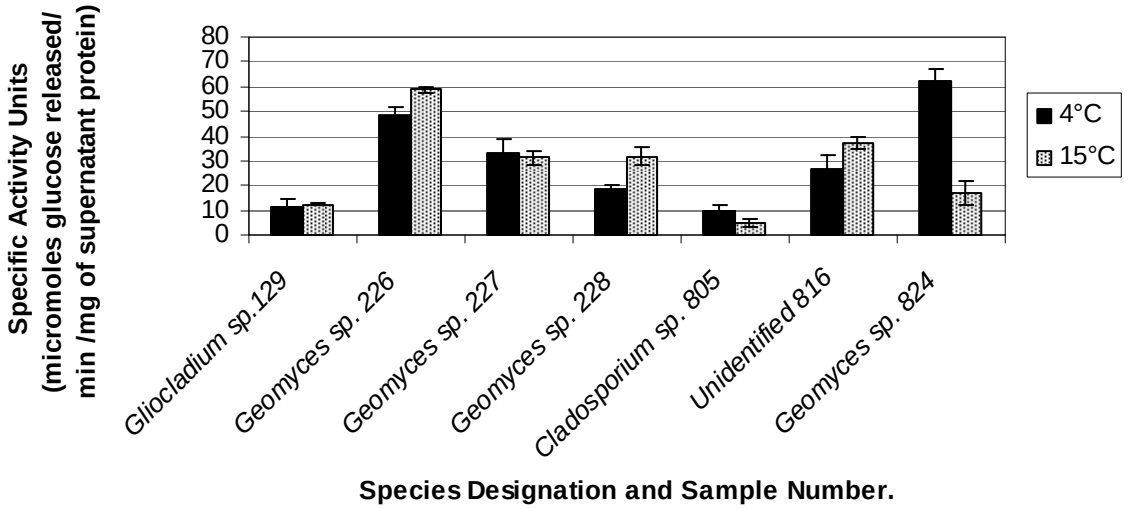

Figure 5.4: Graph of the comparison of specific activity units of endo-1, 4-β-glucanase (micromoles of glucose min^{-1} ml^{-1}/milligram of soluble protein in the supernatant) for 7 fungi isolated from *Discovery* Hut when grown at 4°C and 15°C. Vertical bars represent the standard error.

All of the fungi selected for this experiment from *Terra Nova* Hut demonstrated the production of endo-1,4-β-glucanase (endoglucanase) activity. Figure 5.5 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. As shown in Figure 5.5, of the 20 fungi tested, 8 produced more endo-1,4-β-glucanase activity at 4°C than at 15°C, one produced endo-1,4-β-glucanase activity at 4°C and not at 15°C, and one did not produce endo-1,4-β-glucanase activity at 4°C. Levels of accumulated endo-1,4-β-glucanase activity were measured at 4°C and 15°C ranged from 127 specific activity units to no activity for fungi grown at 4°C and 112 specific activity units to no activity for cultures at 15°C. From the levels of accumulated endo-1,4-β-glucanase activity of the 5 *Cadophora malorum,* isolate number 80 and isolate number 668, produced more endo-1,4-β-glucanase activity at 15°C than 4°C, both unidentified strains produced more endo-1,4-β-glucanase activity at 4°C, and 3 of the 5 *Cladosporium* sp, isolate numbers 660, 667, 719, produced more endo-1,4-β-glucanase activity

at 4°C than 15°C. Four *Penicillium* isolates 405, 408, 537, 1029 produced more endo-1,4-β-glucanase activity at 15°C than 4°C. *Penicillium sp.* isolate number 723 produced more endo-1,4-β-glucanase activity at 4°C than 15°C and two of the three *Geomyces* isolate numbers 711, 749 isolates produced more endo-1,4-β-glucanase activity at 4°C than 15°C.

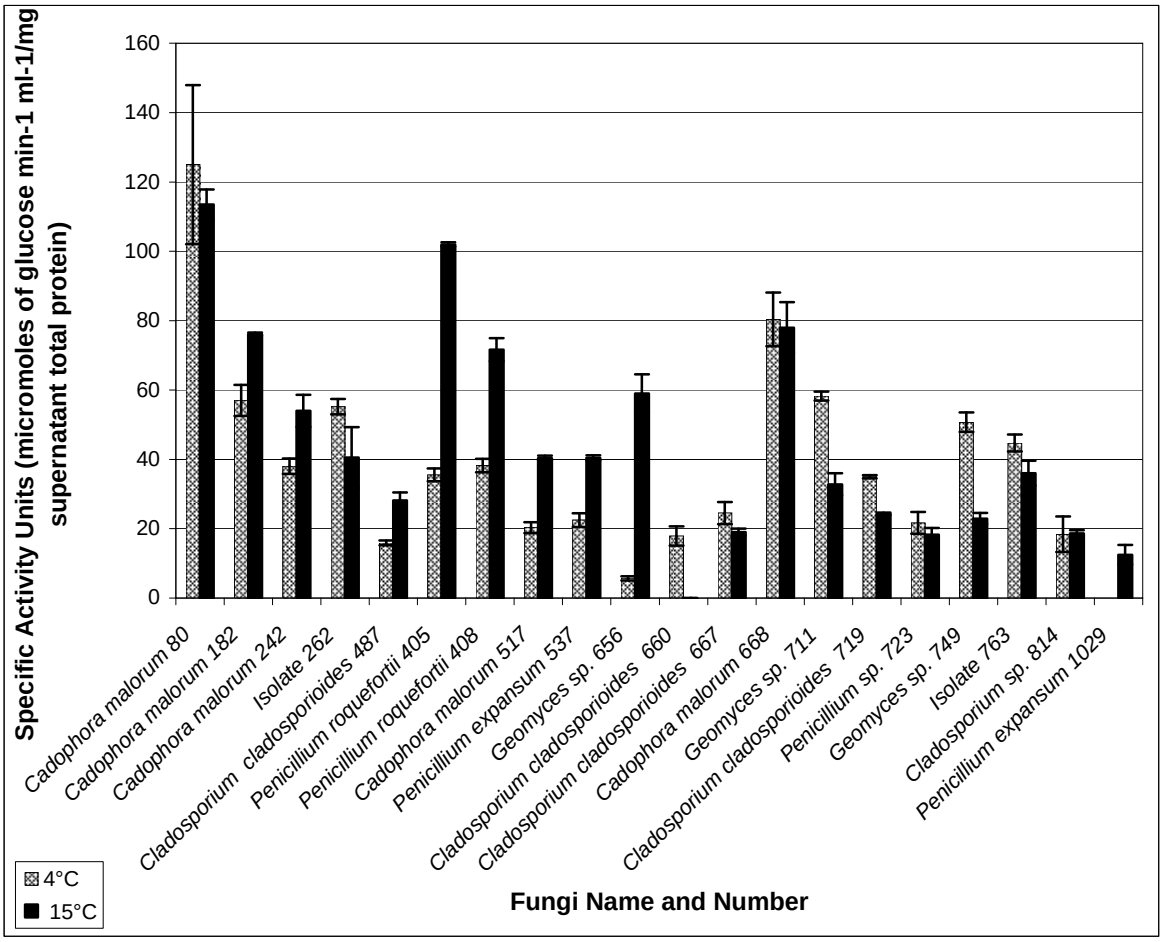

Figure 5.5: Graph of the comparison of specific activity units of endo-1, 4-β-glucanase (micromoles of glucose min^{-1} ml^{-1}/milligram of soluble protein in the supernatant) for 20 fungi isolated from *Terra Nova* Hut when grown at 4°C and 15°C. Vertical bars represent the standard error

5.5.3 Total protein

The levels of total protein were determined for the 27 fungi used in the study of endoglucanase activity (Table 5.7). The mean level of protein in the supernatant when grown at 4°C was 0.75mg /ml compared with 1.03mg/ml when grown at 15°C. 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 statistically different (*P*-value=0.03) with the isolates producing 27% less total protein (0.28mg/ml) in the supernatant when grown at 4°C than at 15°C.

Table 5.7: Protein levels in the enzyme supernatant for the 27 fungal isolates selected for endoglucanase activity when grown at 4 and 15°C.

Fungi number Incubation temperature	mg/ml Protein when grown at 4°C using Biorad protein assay kit.	mg/ml Protein when grown at 15°C using Biorad protein assay kit
80	0.32	0.52
129	0.58	2.08
182	0.52	0.78
226	0.58	1.28
227	0.8	1.28
228	0.79	0.66
242	0.6	0.74
262	0.5	1.16
487	0.61	0.42
405	0.79	1.00
408	0.78	0.88
517	1.88	1.34
537	1.8	1.14
656	0.47	1.0
660	0.54	0.44
667	0.96	0.84
668	0.46	0.5
711	1.04	2.56
719	0.56	0.78
723	0.94	0.82
749	0.73	1.88
763	0.7	0.86
805	0.34	1.4
814	0.5	1.4
816	0.74	0.78
824	0.94	0.76
1029	0.98	0.4

The level of protein in the supernatant was measured for the material used for the cellulase and other wood degrading enzymes experiments (Table 5.8). These supernatant samples had been concentrated by ultrafiltration. The mean level of protein in the supernatant when grown at 4°C was 0.98 mg /ml compared with 2.13 mg/ml when grown at 15°C. 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=0.104) due to the concentrating process being a random event.

Table 5.8: Protein levels in the enzyme supernatant for the 7 fungal isolates selected for cellulase and other wood degrading activity when grown at 4 and 15°C.

Fungi number Incubation temperature	mg/ml Protein when grown at 4°C using 2-D Quant kit	mg/ml Protein when grown at 15°C using 2-D Quant kit	mg/ml Protein when grown at 25°C using 2-D Quant kit
182	0.96	1.01	NT
242	0.93	2.92	NT
405	1.09	2.41	3.98
408	0.26	4.09	NT
711	2.99	3.42	NT
805	0.12	0.36	NT
824	0.52	0.73	NT

NT:not tested

5.5.4 Mycelium Dry weight

The fungal mycelium dry weight was determined as described in Section 4.4.2 for the 27 fungi used in the study of endoglucanase activity at time of harvest. The mean fungal mycelium dry weight when grown at 4°C was 18.2 mg /ml compared with 16.4 mg/ml when grown at 15°C. 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 = 0.022). This difference indicates cultures grown at 4°C required 11% more fungal biomass (1.8 mg dry weight) to achieve the same levels of endoglucanase activity as cultures grown at 15°C.

Table 5.9: Mycelium dry weight for the 27 fungal isolates selected for endoglucanase activity when grown at 4 and 15°C.

Fungi number	**Mycelium dry weight when grown at 4°C**	**Mycelium dry weight when grown at 15°C**
80	17.6	15.18
129	20.30	17.5
182	16.99	15
226	16.4	15.8
227	17.2	17.13
228	17	13.85
242	16.86	13.38
262	16.82	14
487	20.40	21.33
405	17.30	13.63
408	18.9	16.66
517	20.30	15.38
537	18.47	14.38
656	17.27	12.4
660	22.69	16.7
667	19.7	18.1
668	17.74	16.97

Table 5.9: Mycelium dry weight for the 27 fungal isolates selected for endoglucanase activity when grown at 4 and 15°C.

Fungi number	Mycelium dry weight when grown at 4°C	Mycelium dry weight when grown at 15°C
711	13.15	11.98
719	24.10	17.13
723	19.6	16.97
749	19.66	11.15
763	17.57	23.78
805	14.7	19.6
814	21.7	18.4
816	17.65	20.33
824	12.47	18.01
1029	14.60	13.77

5.5.5 Composition of extracellular cellulase complex using SDS PAGE electrophoresis

When fungal isolates *Cadophora malorum* 182, 242, *Penicillium roquefortii* 405, 408, and *Geomyces* sp.711 were grown at 15°C, they produced more extracellular protein than when grown at 4°C as evidenced by larger mean total protein values at 15°C, 2.13 mg/ml compared with 0.98 mg/ml at 4°C, and by more intense and greater number of bands on the Coomassie Blue stained SDS page gel as can be seen in Fig.5.6. The endo-1,4-β-glucanase zymogram activity gels (Fig. 5.7) showed multiple clearing bands. Fungi isolate numbers 242, 405, 408 and 711 showed differences in endo-1,4-β-glucanase complex composition when they were grown at 4°C, compared with growth at 15°C. *Cadophora malorum* 182, produced 1 wide bright clearing band, 3 finer paler bands at both 4 and 15°C; *Cadophora malorum* 242 produced 1 wide bright clearing band and 5 finer paler bands at both 4°C and 15°C. The clearing bands were brighter (indicating more clearing and suggestive of greater activity) at 15°C than at 4°C and an additional 2 fine bands were seen.

Penicillium roquefortii 405 when grown at 4°C produced three bands, all bright, and when grown at 15°C a total of seven bands were visualized. The bands that were not visualised at 4°C but were at 15°C were all larger proteins. *Penicillium roquefortii* 408 produced 1 bright clearing and 3 paler clearing bands when grown at 4°C compared with four bright clearing bands, two of the bands were wide and covered an area where there were 2 pale bands when grown at 4°C. The one band that was not visualised at 4°C but was seen at 15°C was just larger than one of the bright bands.

Geomyces sp.711 produced 5 clearing bands when grown at 4°C, 4 bright and one pale. When grown at 15°C 4 clearing bands were visualised. The one band that was seen at 4°C and not at 15°C was a smaller protein, according to apparent size as indicated by mobility in the gel. The cellulase enzyme composition was different as indicated by SDS-PAGE for all 5 organisms as they all showed different banding patterns.

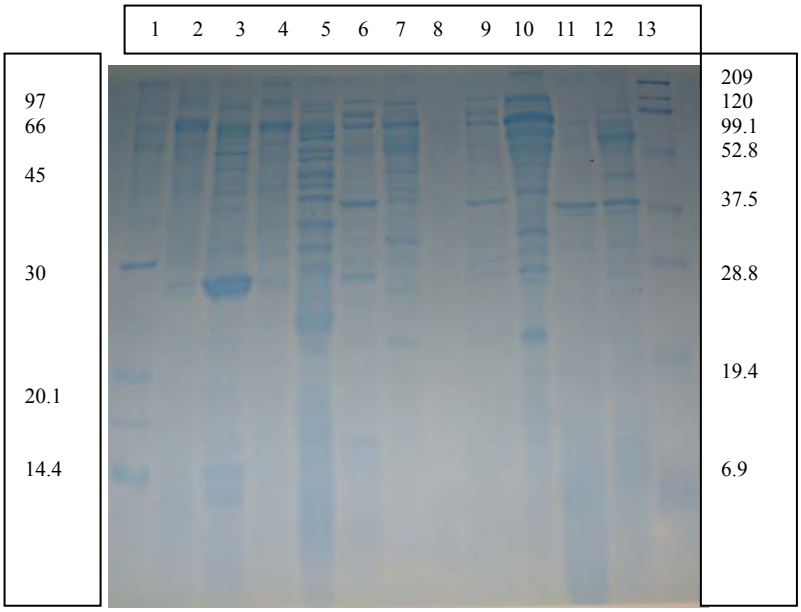

Figure 5.6: Photograph of coomassie blue stained SDS-PAGE of extracellular supernatants of five fungi at 4 and 15 °C for the hydroxyethyl cellulose zymogram (Figure 5.7).
Lane 1: LMW markers
Lane 2: 182 4°C
Lane 3: 182 15°C
Lane 4: 242 4°C
Lane 5: 242 15°C
Lane 6: 405 4°C
Lane 7: 405 15°C
Lane 8: Blank,
Lane 9: 408 4°C
Lane 10: 408 15°C
Lane11: 711 4°C
Lane 12: 711 15°C
Lane 13: Prestained broad range MW markers

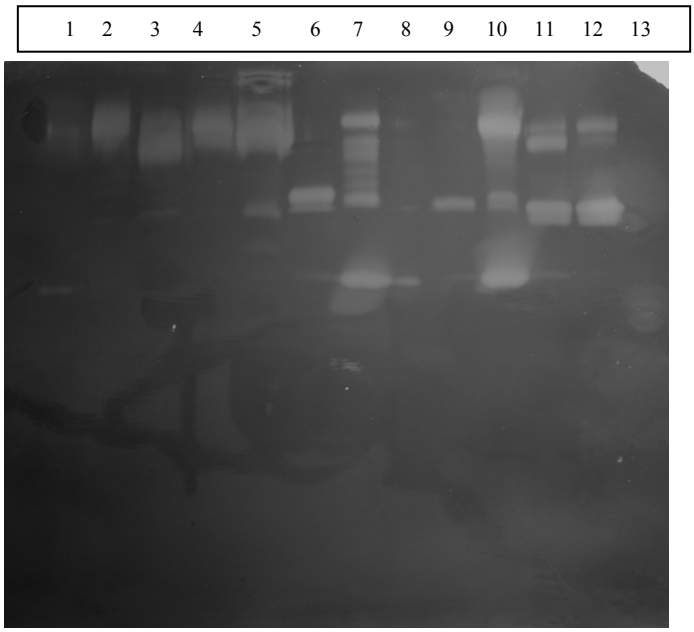

Fig. 5.7: Photograph of the Hydroxyethylcellulose zymogram of SDS-PAGE protein gel (Figure 5.6) showing protein bands that demonstrated endo-1, 4-β-glucanase activity.
Lane 1: LMW markers,
Lane 2: 182 4°C
Lane 3: 182 15°C
Lane 4: 242 4°C
Lane 5: 242 15°C
Lane 6: 405 4°C
Lane 7: 405 15°C
Lane 8: Blank
Lane 9: 408 4°C
Lane10: 408 15 °C
Lane 11: 711 4°C
Lane 12: 711 15°C
Lane 13: Prestained broad range MW markers

When the enzyme supernatants from *Cadophora malorum* 182, 242, *Penicillium roquefortii* 405, 408, and *Geomyces* sp.711 were grown at 4°C and 15°C were subjected to SDS-PAGE electrophoresis and a carboxymethylcellulose zymogram was used to visualise cellulose activity, the protein bands (Figure 5.8) were similar to the earlier SDS-PAGE electrophoresis (Figure 5.6). The carboxymethylcellulase (CMCase) zymogram activity gels (Figure. 5.9) showed multiple clearing bands. Fungi isolate numbers 242, 405, and 408 showed differences in CMCase complex composition when they were grown at 4°C, compared with growth at 15°C. *Cadophora malorum* 182 produced 3 finer paler clearing bands at both 4°C and 15°C, at 15°C one band was brighter than at 4°C. *Cadophora malorum* 242 produced 3 finer paler clearing bands at 4°C. At 15°C, 5 clearing bands were visualised an additional 2 fine bands were seen at the top of the gel along with an extra clearing band just above the smallest CMCase clearing band. *Penicillium roquefortii* 405, when grown at 4°C, produced seven pale

clearing bands. When grown at 15°C a total of seven bands were visualized but they were brighter than at 4°C. *Penicillium roquefortii* 408 produced 2 bright clearing bands when grown at 4°C compared with three bright clearing bands and 1 pale clearing band. The two bands that were not visualised at 4°C but were seen at 15°C were apparently by mobility larger proteins as was the pale band and one of the bright bands. *Geomyces* sp.711 produced 3 clearing bands in identical locations of mobility when grown at 4°C and at 15 °C. The cellulase enzyme composition was different for all 5 organisms as they all showed different banding patterns.

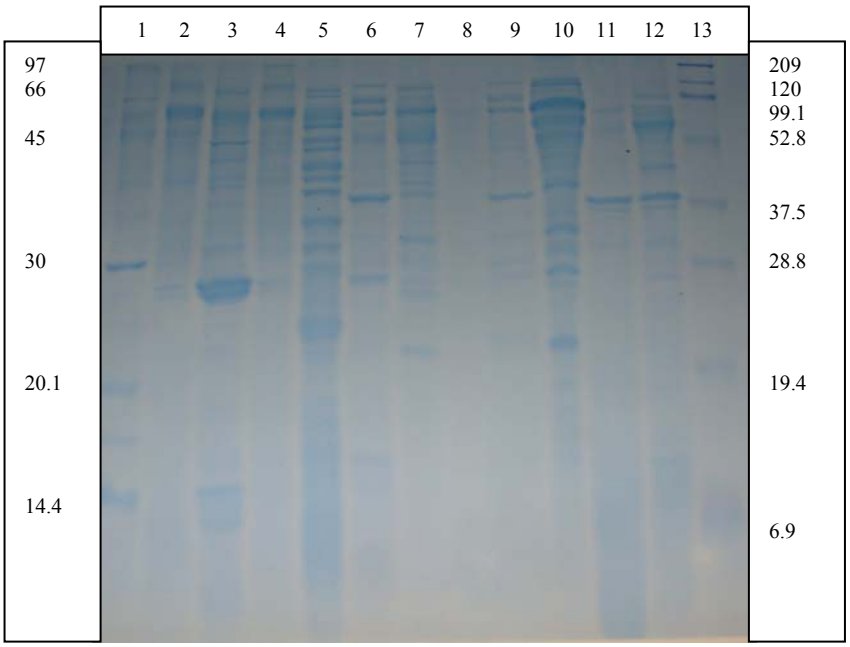

Figure 5.8: Photograph of the coomassie blue stained SDS-PAGE of extracellular supernatant of five fungi at 4 and 15°C for the carboxymethylcellulose zymogram (Figure 5.9).
Lane 1: LMW markers,
Lane 2: 182 4°C
Lane 3: 182 15°C
Lane 4: 242 4°C
Lane5: 242 15°C
Lane 6: 405 4°C
Lane 7: 405 15°C
Lane 8: Blank,
Lane 9: 408 4°C
Lane10: 408 15°C
Lane 11: 711 4°C
Lane 12: 711 15°C
Lane 13: Prestained broad range MW markers.

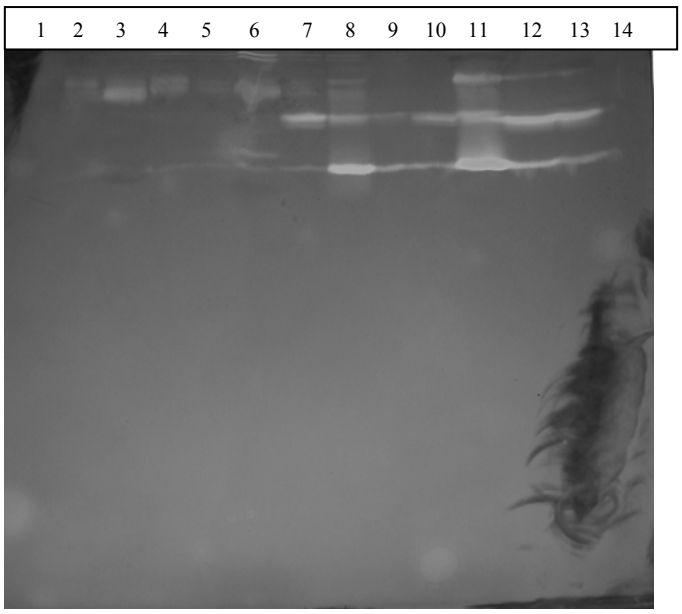

Figure 5.9: Photograph of the carboxymethylcellulose zymogram of SDS-PAGE gel (Figure 5.8) showing protein bands that demonstrated carboxymethylcellulase activity.
Lane 1: LMW markers
Lane 2: 182 4°C
Lane 3: 182 15°C
Lane 4: 242 4°C
Lane 5: Blank,
Lane 6: 242 15°C
Lane 7: 405 4°C
Lane 8: 405 15°C
Lane 9: Blank
Lane 10: 408 4°C
Lane 11: 408 15°C
Lane12: 711 4°C
Lane 13: 711 15°C
Lane 14: Prestained broad range MW markers

5.5.6 Composition of extracellular cellulase complex using Native electrophoresis

The enzyme supernatants from cultures of *Cadophora malorum* 182, 242, *Penicillium roquefortii* 405, 408, grown at 4°C and 15°C, were subjected to Native electrophoresis and hydroxyethylcellulose zymogram. The numbers of protein bands seen on the native gel (Figure 5.10) were less when compared with SDS-PAGE electrophoresis (Figures 5.6 and 5.8). The endo-1,4-β-glucanase zymogram activity gels (Figure 5.11) showed multiple clearing bands. Fungi isolate numbers 182, 405, and 408 showed differences in endo-1,4-β-glucanase complex composition when they were grown at 4°C, compared with growth at 15°C. *Cadophora malorum* 182, produced a wide clearing band at the top of the gel and 4 finer paler clearing bands at 4°C, and at 15°C only one band wide band

at the top of the gel was visualised. *Cadophora malorum* 242 produced 1 wide clearing band at the top of the gel at both 4°C and 15°C. *Penicillium roquefortii* 405 when grown at 4°C produced three clearing bands, and when grown at 15°C a total of three bands were visualized, the one at the top of the gel stretched from the top of the gel to the same point as the second clearing band on the zymogram of the culture grown at 4°C extracellular supernatant as subjected to electrophoresis, the band of the smallest cellulase positive is paler at 15°C than at 4°C and the band that was not visualised at 4°C is between the wide band and the smallest cellulase positive band. *Penicillium roquefortii* 408 produced 4 clearing bands when grown at 4 and 15°C. The first clearing band at 15°C is a lot wider than at 4°C. The cellulase enzyme composition was different for all 5 organisms as they all showed different banding patterns.

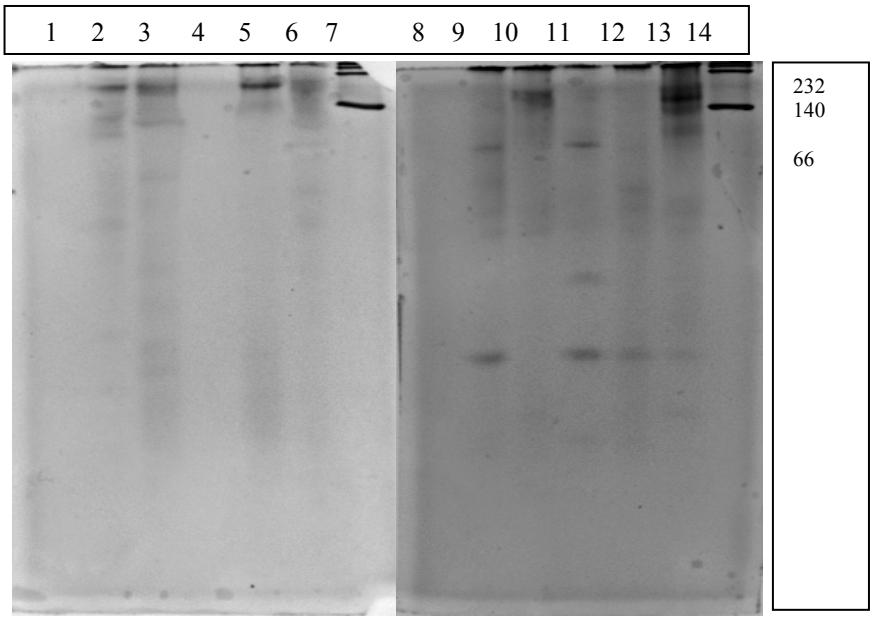

Figure 5.10: Photograph of the silver stained Native gel of the extracellular supernatant of five fungi at 4 and 15°C for the hydroxyethylcellulose zymogram (Figure 5.11).
Lane 1: Blank
Lane 2: 182 4°C
Lane 3: 182 15°C
Lane 4: Blank
Lane 5: 242 4°C
Lane 6: 242 15°C
Lane 7: HMW markers
Lane 8: Blank
Lane 9: 408 4°C
Lane 10: 405 15°C
Lane 11: 405 25°C
Lane 12: 408 4°C
Lane 13: 408 15°C
Lane 14: HMW markers

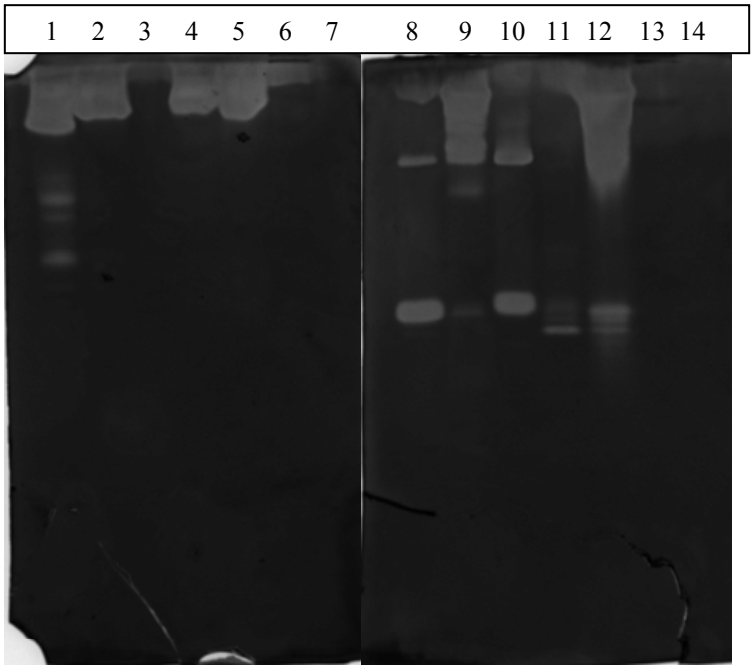

Figure 5.11: Photograph of the hydroxyethylcellulose zymogram of Native electrophoresis protein gel (Figure 5.10) showing protein bands that demonstrated endo-1, 4-β-glucanase activity.
Lane 1: 182 4°C
Lane 2: 182 15°C
Lane 3: Blank
Lane 4: 242 4°C
Lane 5: 242 15°C
Lane 6: Blank
Lane 7: HMW markers
Lane 8: 405 4°C
Lane 9: 405 15°C
Lane 10: 405 25°C
Lane 11: 408 4°C
Lane 12: 408 15°C
Lane 13: Blank
Lane 14: HMW markers

5.5.7 Composition of extracellular cellulase complex using Isoelectrofocusing (IEF) electrophoresis.

The enzyme supernatants from cultures of *Cadophora malorum* 182, 242, *Penicillium roquefortii* 408, grown at 4°C and 15°C and *Penicillium roquefortii* 405 grown at 4, 15 and 25°C were subjected to isoelectrofocusing electrophoresis and a hydroxyethylcellulose zymogram. The numbers of protein bands observed were more (Figure 5.12 and 5.13) than when using SDS-PAGE or Native electrophoresis due perhaps to the different separation criteria of protein pI rather than the molecular size and shape of the protein. The endo-1,4-β-glucanase zymogram activity gels (Figure. 5.12 and 5.13) showed multiple clearing bands with the major activity between pH 3.75 to 6.85. Fungi isolate numbers 242, 405, 408 and 711 showed differences in endo-1,4-β-glucanase complex composition

when they were grown at 4°C, compared with growth at 15°C. *Cadophora malorum* 182 produced 6 bright clearing bands in the pH range 5-5.85 and many paler bands in the pH range 5.85-6.55 at 4°C. At 15°C the clearing bands were paler but in the same pH range as at 4°C. *Cadophora malorum* 242 produced 7 clearing bands in the pH range 5 to 6.55 at 4°C and 15°C one large bright band was visualised in the pH range 4.55-6.55. *Penicillium roquefortii* 405 when grown at 4°C produced 1 bright clearing band at pH 4.55 and 6 fainter bands at the pH range 5.2-5.85; when grown at 15°C a total of three bright bands were visualized, one wide one at pH 5 and two finer ones in the pH range 5.5-5.85. Five faint bands were seen in the pH range 5.85-6.85. When grown at 25°C, the extracellular supernatant upon isoelectrofocusing electrophoresis showed there was one bright clearing band at pH 4.5 and a large number of paler clearing bands at pH range 6.55-6.85. *Penicillium roquefortii* 408 produced one bright clearing band at pH 4.5 and a group of paler band in the pH range 6.55 to 6.85 at both 4°C and 15°C. When *Geomyces* sp. 711 was grown at 4°C and the extracellular supernatant was subjected to IEF electrophoresis, two bright clearing bands were visualised one large band at pH 5.2 and a smaller one at pH 5.85, multiple faint bands were seen from pH range 4.5 to 5.2, while when grown at 15°C and the extracellular supernatant was subjected to IEF electrophoresis, one bright clearing band was seen at pH 4.55 and fainter bands from pH range 5.2 to 5.85 The cellulase enzyme composition was different for all 5 organisms as they all showed different banding patterns but many bands were seen in the majority of fungi and both temperatures.

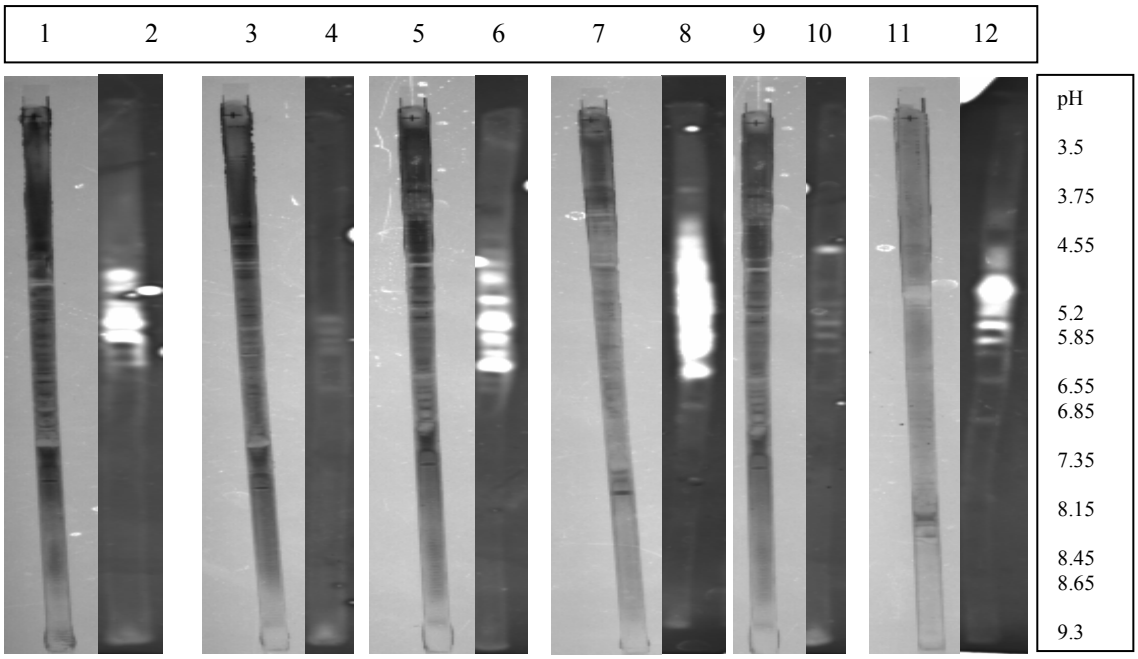

Figure 5.12: Photograph of IEF strips and hydroxyethylcellulose zymograms of extracellular supernatant of three fungi at 4 and 15°C.
Lane 1: IEF 182 4°C
Lane 2: Zymogram 182 4°C
Lane 3: IEF 182 15°C
Lane 4: Zymogram 182 15°C
Lane 5: IEF 242 4°C
Lane 6: Zymogram 242 4°C
Lane 7: IEF 242 15°C
Lane 8: Zymogram 242 15°C
Lane 9: IEF 405 4°C
Lane 10: Zymogram 405 4°C
Lane 11: IEF 405 15°C
Lane 12: Zymogram 405 15°C

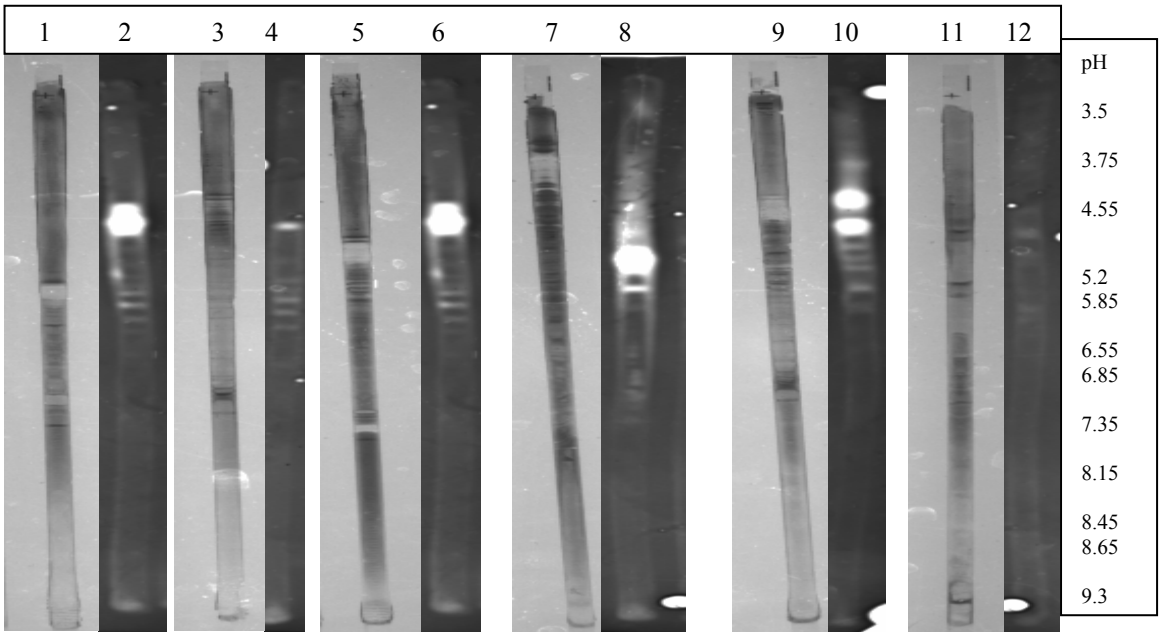

Figure 5.13: Photograph of IEF strips and hydroxyethylcellulose zymograms of extracellular supernatant of three fungi at 4, 15 and 25°C.
Lane 1: IEF 405 25°C
Lane 2: Zymogram 405 25°C
Lane 3: IEF 408 4°C
Lane 4: Zymogram 408 4°C
Lane 5: IEF 408 15°C
Lane 6: Zymogram 408 15°C
Lane 7: IEF 711 4°C
Lane 8: Zymogram 711 4°C
Lane 9: IEF 711 15°C
Lane 10: Zymogram 711 4°C
Lane 11: IEF standards
Lane 12: Zymogram IEF standards

5.5.8 PAS staining of glycoproteins

The enzyme supernatant from cultures *Cadophora malorum* 182, 242, *Penicillium roquefortii* 405, 408, and *Geomyces* 711 grown at 4°C and 15°C and additional at 25°C for *Penicillium roquefortii* 405 were subjected to SDS-PAGE electrophoresis and stained with PAS stain to determine the number of glycosylated protein bands in the enzyme supernatant. Figure 5.14 shows all the proteins in the extracellular supernatant while Figure 5.13 shows the bands that are glycosylated which appear as pink stained. All the culture supernatants contained glycosylated protein bands.

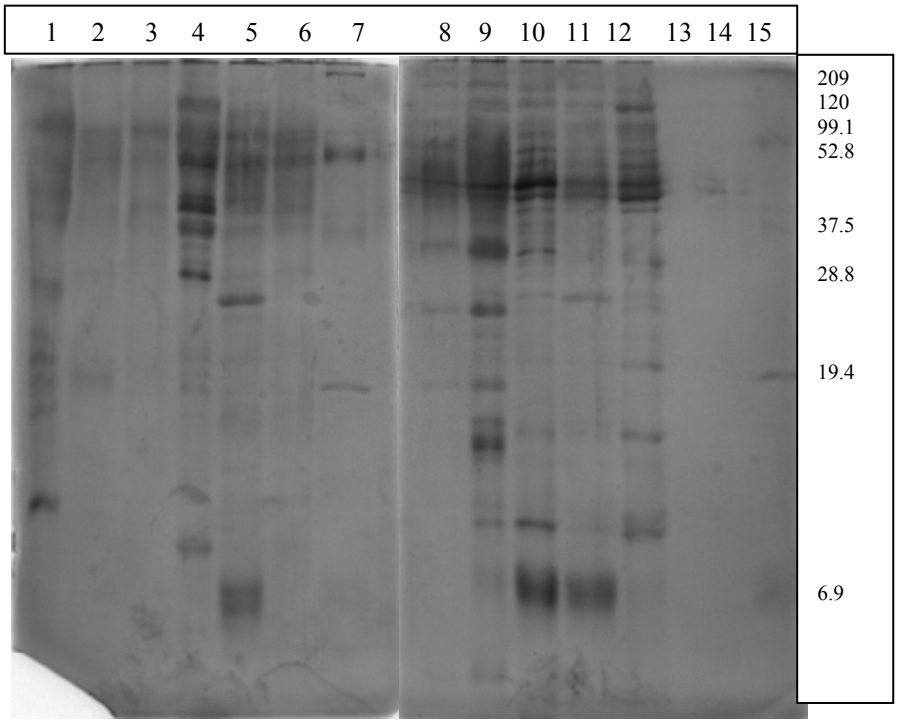

Figure 5.14: Photograph of the silver stained SDS-PAGE of extracellular supernatant of five fungi at 4 or 15 or 25°C.
Lane 1: 182 4°C
Lane 2: 182 15°C
Lane 3: 242 4°C
Lane 4: 242 15°C
Lane 4: 405 4°C
Lane 6: 405 15°C
Lane7: Broad range MW markers,
Lane 8: 405 25°C
Lane 9: 408 4°C
Lane 10: 408 15°C
Lane 11: 711 4°C
Lane 12: 711 15°C
Lane 13: Blank
Lane 14: Blank
Lane 15: Prestained broad range MW markers

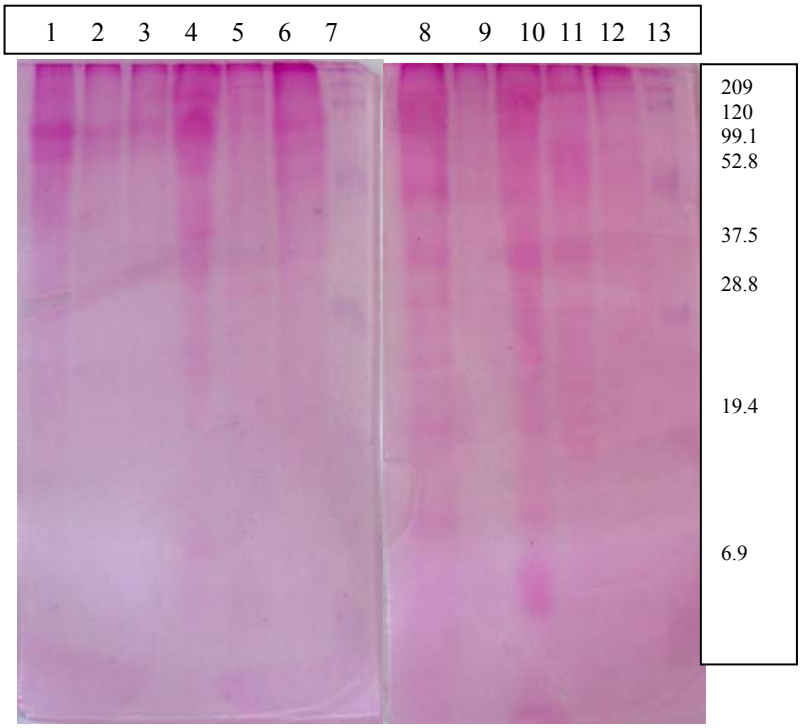

Figure 5.15: Photograph of the PAS staining of SDS-PAGE electrophoresis (Figure 5.14) of five fungi at 4 or 15 or 25°C. Bright pink bands are the glycosylated proteins.
Lane 1: 182 4°C
Lane 2: 182 15°C
Lane 3: 242 4°C
Lane 4: 242 15°C
Lane 5: 405 4°C
Lane 6: 405 15°C
Lane7: Broad range MW markers
Lane 8: 405 25°C
Lane 9: 408 4°C
Lane 10: 408 15°C
Lane 11: 711 4°C
Lane 12: 711 15°C
Lane 13: Prestained broad range MW markers

5.5.9 Measurement of Cellulase Activity using different cellulose substrates

The cellulase enzyme activities of the culture supernatant from seven Antarctic isolates described in Section 4.4.2 were measured with five different cellulose substrates. The greatest mean activity was filter paper activity when the assay was incubated at 50°C.

5.5.9.1 Filter paper activity.

The filter paper activity (FPase) was greatest when the assay was incubated at 50 °C rather than at 15°C for all isolates and cultures except for *Cadophora malorum* 182 grown at 15 °C, which had greater enzyme activity when the assay was run at 15°C. *Geomyces* sp. 824 accumulated the most FPase of all the fungi tested when grown at 4°C and the assay incubated at 50°C. FPase production ranged from 1.53 to 19.65 micromoles glucose released/minute/mg of protein in the supernatant

when fungal isolates were grown at 4°C and from 0.29 to 6.97 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.10).

5.5.9.2 Avicelase activity

The Avicelase enzyme activity was greatest when the assay was run at 50°C rather than at 15°C except for *Cadophora malorum* 182 grown at 15°C and *Penicillium roquefortii* 405 grown at 25°C which both had higher activity when the assay was incubated at 15°C. *Penicillium roquefortii* 408 had the greatest accumulation of Avicelase activity of all the fungi tested when grown at 15°C and the assay was run at 50°C. Avicelase production ranged from 0.69 to 53.3 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 4°C and from 0.24 to 12.6 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.10).

5.5.9.3 Endo-1, 4-β-glucanase activity

The endo-1,4-β-glucanase enzyme activity was greatest when the assay was run at 50 °C rather than at 15°C for all fungi tested. *Geomyces* sp 711 had the greatest accumulation of endo-1,4-β-glucanase activity of all the fungi tested when grown at 4°C and the assay was run at 50°C. Endo-1,4-β-glucanase production ranged from 0.56 to 14.04 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 4°C and from 1.51 to 23.57 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.10).

5.5.9.4 Carboxymethylcellulase activity

The Carboxymethylcellulase (CMCase) enzyme activity was greatest when the assay was run at 50°C rather than at 15ºC for all fungi except *Cadophora malorum* 182 grown at 15ºC which had greater activity when the assay was incubated at 15 ºC. *Cadophora malorum* 182 had the greatest accumulation of CMCase activity of all the fungi tested when grown at 4ºC and the assay was run at 50ºC. CMCase production ranged from 44.8 to 123.8 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 4°C and from 13.8 to 102.7 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.10).

5.5.9.5 β-1,4-glucan cellobiohydrolase activity

The β-1,4-glucan cellobiohydrolase enzyme activity was greatest when the assay was run at 50 ºC rather than at 15ºC for all fungi tested. *Penicillium roquefortii* 405 had the greatest accumulation of β-1,4-glucan cellobiohydrolase activity of all the fungi tested when grown at 4ºC and the assay was run at 50ºC. β-1,4-glucan cellobiohydrolase production ranged from 0.043 to 0.95 pmol p-nitrophenol released/minute/mg of protein in the supernatant when fungal isolates were grown at 4°C and from 0.016 to 0.396 pmol p-nitrophenol released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.10).

Table 5.10: Cellulase enzyme activities on different cellulose substrates at both 15°C and 50°C of the culture media from seven fungal isolates grown at 4, 15 and 25°C.

Cellulose	Filter paper		Avicel		Carboxymethyl cellulose		Hydroxyethyl cellulose		p-nitrophenyl β-D cellobiose	
Enzyme	Filter paper activity (Total cellulase activity)		Avicelase		CMCase		Endo-1,4-β-glucanase		β-1,4-glucan cellobiohydrolase	
	15°C	50°C	15°C	50°C	15°C	50°C	15°C	50°C	15°C	50°C
182 4°C	1.60	19.65	0.41	3.72	22.18	76.35	5.94	14.04	0.029	0.117
15°C	3.78	0.98	0.53	0.88	89.77	20.87	4.09	1.51	0.143	0.396
242 4°C	0.31	3.07	0.36	0.69	17.53	94.41	2.31	13.07	0.0156	0.043
15°C	0.30	3.29	0.07	0.24	3.39	26.33	1	3.35	0.0048	0.016
405 4°C	0.63	2.81	0.42	1.23	18.02	95.87	0.90	6.39	0.32	0.95
15°C	0.44	6.97	0.13	1.22	15.2	37.7	2.13	6.76	0.048	0.35
25°C	0.24	0.94	0.09	0.23	7.39	27.5	0.92	0.66	0.0017	0.015
408 4°C	1.69	3.81	0.22	2.11	17.68	123.8	2.27	12	0.1	0.92
15°C	0.043	0.68	0.36	12.6	2.75	13.8	0.32	23.57	0.0063	0.079
711 4°C	1.01	1.53	1.24	53.3	36.32	44.8	1.51	0.56	0.09	0.082
15°C	0.15	0.29	0.053	0.40	8.62	23.5	1.33	7.54	0.041	0.026
805 4°C	0.49	5.0	1.42	9.58	24.4	110.5	3.75	6.75	0.1	0.05
15°C	0.20	2.19	0.5	2.94	9.30	31.38	0.97	6.53	0.02	0.041
824 4°C	0.63	1.73	0.55	1.46	11.3	147.3	1.19	10.17	0.042	0.1
15°C	0.19	1.30	0.89	0.88	25.3	102.7	6.39	11.2	0.036	0.071

Filter paper, Hydroxyethyl cellulose, Carboxymethyl cellulose, Avicel are expressed as micromoles glucose released/minute/mg of protein in the supernatant
p-nitrophenyl β-D cellobiose is expressed as pmol p-nitrophenol released/minute/mg of protein in the supernatant.

5.5.10 Measurement of activity of additional enzymes associated with wood cell wall degradation.

All the fungi in this study produced other wood cell degrading enzymes. The greatest mean enzyme activity was for the mannanase enzyme when the assay was incubated at 50ºC.

5.5.10.1 Amylase activity in the culture supernatant

Amylase production ranged from 0.34 to 28.89 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 4°C and from 1.32 to 268.3 micromoles glucose released/minute/mg of protein in the supernatant when fungal isolates were grown at 15°C (Table 5.11). *Cladosporium oxysporum* 805 produced the greatest amylase activity of all fungi tested when grown at 4ºC. The amylase activity was greater when the assay was run at 50°C rather than at 15ºC. *Cadophora malorum* 182 and 242, *Penicillium roquefortii* 408 and *Cladosporium oxysporum* 805 all accumulated more amylase in the culture supernatant at 4ºC than at 15ºC. The amylase activity when the assay was run at 15ºC ranged from 3 to 68% of the levels seen when the assay was run at 50ºC.

5.5.10.2 Pectinase activity in the culture supernatant

Two substrates were used to determine pectinase activity. When polygalacturonic acid was used as a substrate the level of pectinase enzyme in the supernatant ranged from 0.11 to 195.4 micromoles glucose released/minute/mg of protein in the supernatant when grown at 4°C while grown at 15°C the range was 0.26 to 18.8 micromoles glucose released/minute/mg of protein in the supernatant (Table 5.11). Polygalacturonic acid was the only substrate where the activity at 15ºC was greater than at 50ºC for most of the fungal supernatants tested. *Cadophora malorum 182* produced the greatest amylase activity of all fungi tested when grown at 4ºC. More pectinase activity was measured in the supernatants of *Cadophora malorum* 182 at both incubation temperatures, *Cadophora malorum* 242 at 4ºC, *Penicillium roquefortii* 405 at 15ºC and 25ºC and *Penicillium roquefortii* 408 at 15ºC when the assay was conducted at 15ºC rather than 50ºC. When pectin was used as the substrate for the pectinase assay, the activity ranged from 0.36 to 51.27 micromoles glucose released/minute/mg of protein in the supernatant when grown at 4ºC compared to a range of 0.79 to 84.17 micromoles glucose released/minute/mg of protein in the supernatant when grown at 15ºC. *Cladosporium oxysporum* 805 produced the greatest amylase activity of all fungi

tested when grown at 4ºC. The levels of pectinase activity were larger when the assay was run at 50ºC than 15ºC for all fungi except *Cadophora malorum* 182 when grown at 15ºC, *Geomyces* sp. 711 at 4ºC.

5.5.10.3 Xylanase activity in the culture supernatant

When xylan was used as a substrate the level of xylanase enzyme in the supernatant ranged from 3.18 to 87.46 micromoles glucose released/minute/mg of protein in the supernatant when the culture was grown at 4°C while grown at 15°C the range was 2.84 to 209.6 micromoles glucose released/minute/mg of protein in the supernatant (Table 5.11). *Penicillium roquefortii* 408 produced the greatest xylanase activity of all fungi tested when grown at 4ºC.

5.5.10.4 Mannanase activity in the culture supernatant

When locust bean gum was used as a substrate the level of mannanase enzyme in the supernatant ranged from 4.33 to 96.67 micromoles glucose released/minute /mg of protein in the supernatant when grown at 4°C while grown at 15°C the range was 23 to 624 micromoles glucose released/minute/mg of protein in the supernatant (Table 5.11). *Penicillium roquefortii* 405 produced the greatest mannanase activity of all fungi tested when grown at 4ºC. The levels of mannanase activity were larger when the assay was run at 50ºC than 15ºC for all fungi except *Cadophora malorum* 182 when grown at 15ºC, *Cladosporium oxysporum* 805 at 4ºC.

Table 5.11: Amylase, pectinase, xylanase, and mananase activities at both 15 and 50°C of the culture media from seven fungal isolates grown at 4, 15 and 25°C.

Substrate	Starch		Polygalacturonic acid		Pectin		Larchwood xylan		Locust bean gum	
Enzyme	Amylase		Pectinase		Pectinase		Xylanase		Mannanase	
	15°C	50°C	15°C	50°C	15°C	50°C	15°C	50°C	15°C	50°C
182 4°C	11.12	104	195.4	2.01	40.76	75.1	29.48	78.85	17.81	99.37
15°C	2.41	1.32	15.56	0.65	18.06	4.59	87.46	33.0	63.36	23.00
242 4°C	2.53	31.3	2.06	1.31	11.67	30.3	11.2	2.84	21.68	91.6
15°C	0.49	2.49	0.31	0.35	6.73	14.7	12.9	21.5	19.86	48.28
405 4°C	3.55	34.3	0.64	3.14	17.49	31.9	20.9	101.1	4.33	30.0
15°C	5.10	16.93	123.6	1.51	0.36	19.25	31.40	63.5	32.0	61.8
25°C	0.46	2.81	6.34	0.84	4.92	8.04	13.9	27.98	5.07	624
408 4°C	6.0	47.3	57.3	12.57	51.27	230	31.92	209.6	7.57	90.77
15°C	0.34	12.56	0.54	0.28	1.83	23.9	3.18	30.39	1.20	33.3
711 4°C	3.41	5.01	0.40	1.2	1.53	0.79	9.23	21.4	6.51	85.3
15°C	7.66	11.7	0.11	0.26	3.15	16.28	13.97	49.59	5.47	73.68
805 4°C	59.1	268.3	7.33	18.8	30	84.17	18	120	96.67	167.3
15°C	28.89	188.6	0.75	5.44	8.44	24.22	19.78	9.55	31.1	24
824 4°C	3.81	17.86	0.67	3.67	6.53	8.36	4.57	8.15	5.06	33.8
15°C	5.89	52.19	0.253	2.13	5.38	7.93	6.81	6.16	7.51	81.51

Amylase, pectinase, xylanase, manannase are expressed as micromoles glucose released/minute/ mg of protein in the supernatant

5.5.11 Protease activity

All 7 fungi produced protease activity with azocasein as the substrate. *Cladosporium oxysporum* 805 produced the most protease of all the fungi when grown at 4°C and the assay was run at 50°C. When the assay was run at 50°C the protease activity was greater than when the assay was run at 15°C except in the case of *Penicillun roquefortii* 405 and 408 grown at 15°C. The drop in activity when the assay was run at 15°C rather than 50°C was within a range of 20% to 77% except for *Penicillun roquefortii* 405 and 408 grown at 15°C which both had higher activity when the assay was run at 15°C. *Penicillun roquefortii* 405 and 408, *Geomyces* 711 and 824 and *Cladopsorium oxysporum* 805 all produced more protease when grown at 4°C than at 15 or 25°C.

Table 5.12: Protease activities at both 15°C and 50°C of the culture media from seven fungal isolates grown at 4, 15 and 25°C.

Substrate	Azo casein	
Enzyme	Protease	
	15°C	50°C
182 4°C	0.48	2.42
15°C	4.09	6.77
242 4°C	1.68	3.32
15°C	2.09	6.03
405 4°C	1.94	3.04
15°C	0.76	0.08
25°C	0.66	1.84
408 4°C	8.38	10.9
15°C	0.15	0.14
711 4°C	3.17	5.99
15°C	2.05	6.01
805 4°C	54.3	129.3
15°C	13.72	30
824 4°C	18.73	51.38
15°C	12.3	21.92

Protease is expressed as AU=ΔA_{420} of 1.0 per hour/ mg of protein in the supernatant

5.6 Discussion

The main aim of this aspect of the thesis research was to gain an understanding of the cellulase activity of fungi present in the Historic Huts themselves and materials contained within and around them.

Objectives were addressed as follows:

- At a variety of temperatures, selected fungi isolated from a variety of substrates including structural wood of the Antarctic Historic Huts on Ross Island were screened to determine ability to produce cellulytic activity.

- Cellulytic activity was characterised using quantitative assays to determine levels of production and conditions under which this was optimal.
- The cellulase complex proteins were examined using electrophoresis techniques with activity zymograms.
- Levels of other extracellular cell wood degrading enzymes were quantified in culture supernatants of selected fungi.
- Levels of extracellular protease activity were quantified in culture supernatants of selected fungi.

The objective to screen large numbers of fungi for cellulase activity led to the use of a plate screening method. This method along with the calculation of an index of relative activity, gave a measure of the production of active carboxymethylcellulase, and as judged by this PhD thesis gave a reliable method for screening fungi for cellulase activity. Cellulase activity was reported in temperate isolates of *Cladosporium* (Abrha and Gashe., 1992; Barbosa *et al*., 2001), *Cadophora malorum* (Berg,1978), and many species of *Penicillium* (van Wyk, 1999; Wang and Gao, 2000**;** van Wyk and Leogale, 2001; Nuero and Reyes, 2002; Rajal *et al*., 2002; Jørgensen *et al*, 2003; Mo *et al*., 2004; Chaabouni et al., 2005; Jorgensen *et al*. 2005; Chen and Jin, 2006; Sehnem *et al*., 2006; Oliveira *et al*., 2006; Jorgensen and Olsson, 2006). There are limited reports of *Geomyces sp*. producing cellulase at temperate temperatures (Kushwaha,2000) but Fenice *et al*. (1997) reported low cellulase activity from 1 of 5 *Geomyces pannorum*, and no cellulase activity from 1 *Cladosporium cladosporioides* and 2 *Cladosporium herbarum* isolated in Antarctica. Hurst *et al*. (1983) reported on Antarctic isolates of *Chrysosporium pannorum* and *Cladosporium sphaerospermum* having an ability to degrade cellulose along with other substrates at 20ºC and both demonstrated cellulase activity at 1ºC.

Just like the percentage of samples from which fungi were isolated (as discussed in Chapter 3), the percentage of CMCase positive fungi screened showed that *Discovery* Hut and *Terra Nova* Hut contained similar percentages of CMCase positive fungi while *Nimrod* Hut contained a lower percentage of CMCase positive fungi. The percentage of CMCase positive fungi from pieces of wood substrate located inside the hut were higher than found outside but swabs of wood were reverse. This reversal of CMCase positive fungi isolated from swabs may

reflect that the swab samples only picked up a surface sample and that organisms producing CMCase activity are found more frequently from within the wood cells themselves. The highest percentage of CMCase positive fungi from non-wood samples were from those taken of visual fungal mycelium, indicating that areas of visible fungal growth are potential areas of decay. Very few of the fungi isolated from leather boots inside the huts could degrade CMC but 52.6% of the fungi isolated from the leather horse harnesses could, possible due to the different uses these leather items performed. The levels of CMC-degrading fungi isolated from outside the hut were low, possibly due to a lack of suitable substrate and harsher conditions. The temperature of isolation did not affect the ability of the isolated fungi to degrade cellulose.

When the 7 selected fungi were screened for their ability to degrade Avicel, only two *Cadophora malorum* 242 and *Penicillium roquefortii* 405 showed a clearing zone but these were not large enough to declare that these organisms were Avicel degraders. This does not mean that the fungi were not degrading the Avicel but they were not breaking down the (β-1-4) linked D-glucopyranosyl units to prevent the Congo red staining the Avicel. From the variety of cellulase enzyme assays conducted and the fact that the fungi could grow on the media containing Avicel indicated that they have potential to use Avicel as a carbon source.

All fungi tested for endoglucanase activity in liquid culture showed activity at either 4°C or 15°C or at both temperatures. 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; no direct correlation between the relationships of activity:temperature and growth:temperature 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 in a 15°C culture. When grown at 4°C, cultures produced less protein in the extracellular supernatant when compared with culturing at 15°C suggesting a lesser efficiency of growth at 4°C which may be a result of cold adaptation. There could also be different extracellular proteins produced when the fungal isolate was cultured at each of the two temperatures, supported by the SDS-PAGE results.

The various methods of electrophoresis used in this PhD thesis research demonstrated that the Antarctic isolates were producing multiple cellulose degrading enzymes. The cellulase enzyme composition appears to be different for all 5 isolates studied in depth in this PhD thesis research; this is not uncommon as the literature shows fungi have incomplete sets of cellulase enzymes and individual isolates may not be digesting the cellulose completely (Eriksson *et al*., 1990). Additionally there was observed a difference in cellulase composition in the supernatant of fungal isolates *Cadophora malorum* 242, *Penicillium roquefortii* 405 and *Penicillium roquefortii* 408 when grown at 4°C and at 15°C, according to the mobility of protein bands capable of clearing Congo red stained cellulose in SDS-PAGE zymograms. Whether the difference in mobility was due to different gene products or post translational modification of the same protein or a combination of these explanations would require purification of the protein. When different cellulose substrates were used the banding patterns were also different. The banding patterns were different between methods but this is to be expected as each separates by a different feature of the proteins and create gels with different numbers and locations of the protein bands. Production of multiple cellulase enzymes is common among fungi presumably due to the heterogeneous nature of cellulose and the structural changes that occur to cellulose during depolymerisation. As reported by Tomme *et al*. (1995), many fungi not only require all three enzymes (Endo-1,4-β-glucanase (EC 3.2.1.4), Exo-1,4-β-glucanase, (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21)) to breakdown cellulose but many have multiple versions of the same enzyme.

Fenice *et al*. (1997) reported enzyme activities from Antarctica fungal isolates. Due to differences in testing techniques it was only possible to compare the cellulase activity of genera that were common between their study and this thesis research. For the isolates from this PhD research the temperature of isolation was used as the incubation temperature for determining the level of cellulase activity. The wood isolates from this PhD research showed higher levels of cellulase activity (halo diameters ranging from less than 10 mm to greater than 25mm) compared with the isolates from soil or sand under mosses and mosses (many isolates did not produce cellulose and the largest halo diameter was less than 10mm) (Table 5.13). In the Fenice *et al*. 1997 study they used, + to indicated halo diameter of up to 10mm, ++ between 11 and 25mm and +++ more than 25mm.

For the purpose of comparison the results from this thesis have been converted to the same format as used by Fenice *et al*. (1997) in their study.

Table 5.13: Comparison of cellulase activity reported by Fenice *et al*. (1997) and cellulase activity measured during this PhD research.

Tested strain	Habitat	Study	Cellulase activity
Cladosporium cladosporiodes	Soil under moss	Fenice *et al*. (1997)	-
*C.herbarum*1	Moss	Fenice *et al*. (1997)	-
C.herbarum 2	Soil under moss	Fenice *et al*. (1997)	-
Cladosporium cladosporiodes 487	*Terra Nova* Hut Swab wall behind table	PhD thesis	+
Cladosporium cladosporiodes 660	*Terra Nova* Hut Swab wall behind table	PhD thesis	++
Cladosporium cladosporiodes 667	Exterior wood sample from 2nd boards removed from Cape Evans and store in container at Scott base	PhD thesis	++
Cladosporium cladosporiodes 719	*Terra Nova* Hut Wall scrapping underneath bed frame	PhD thesis	+++
Cladosporium oxysporum 805	*Discovery* Hut Soil NE corner post of veranda	PhD thesis	+++
Cladosporium sp. 814	*Terra Nova* Hut Swab piece of wood near floor 1st boxes W of 1st bunk on S wall	PhD thesis	+++
Geomyces pannorum v. pan. no.1	Soil under moss	Fenice *et al*. (1997)	-
Geomyces pannorum v. pan. no.2	Moss and soil under moss	Fenice *et al*. (1997)	-
Geomyces pannorum v. pan. no.3	Moss	Fenice *et al*. (1997)	+
Geomyces pannorum v. pan. no. 4	Moss and sand under moss	Fenice *et al*. (1997)	-
Geomyces pannorum v. pan. no.6	Moss and soil under moss	Fenice *et al*. (1997)	-
Geomyces sp 226	*Discovery* Hut Soil NE corner post of veranda	PhD thesis	+
Geomyces sp 227	*Discovery* Hut Soil NE corner post of veranda	PhD thesis	+++
Geomyces sp 228	*Discovery* Hut Soil NE corner post of veranda	PhD thesis	++
Geomyces sp 656	*Terra Nova* Hut Swab damp spot against wall lower bunk on S side	PhD thesis	++
Geomyces sp 711	*Terra Nova* Hut Swab damp spot against wall lower bunk on S side	PhD thesis	+++
Geomyces sp 749	*Terra Nova* Hut Swab doorstep	PhD thesis	+++
Geomyces sp 824	*Terra Nova* Hut Swab boot from under Taylor's bunk frozen white mycelium artefact number E/C/264.2	PhD thesis	+++

Symbols + halo diameter ≤ 10mm, ++ halo diameter between 11-25mm, +++ halo diameter ≥ 25mm.

Other enzymes, common to both studies were compared on a presence/absence scheme. Fenice *et al*. (1997) recorded pectinase activity (polygalaturonase, pectinlysase) in all three *Cladosporium* sp. and 3 of the 5 *Geomyces* sp., amylase activity was recorded for none of the *Cladosporium* isolates but all the *Geomyces*

isolates, and protease activity was reported for none of the similar isolates. In contrast in this PhD thesis research, pectinase, amylase and protease activity was reported for the one *Cladosporium* sp. and both *Geomyces* sp. tested, indicating that fungi isolated from wood potentially produced higher levels of wood degrading enzymes than fungi from soil and other organic material. The differences between the two results could be related to the use of different methods, substrates or could relate to the origin and the fungal isolates themselves.

The supernatant from the seven fungi investigated for cellulase activity on different cellulose substrates showed enzyme activity on all substrates. Many fungal cellulase are recognised as having activity to a variety of cellulose substrates (Jørgensen *et al*., 2005). The cellulase activity did not appear to be affected by how synthetic the substrate was; none of the substrates produced increased or decreased cellulase activity in all fungal isolates and there was variation between isolates of the same species. There are no trends of more cellulase being produced at 4°C than 15°C, or their being less activity when the assay is run at 15°C rather than 50°C. The same lack of trends was seen in the activity rates of other wood degrading enzymes studied. The enzyme activities of the five enzymes tested were not higher at either temperature of incubation, or assay temperature for all fungi involved in the study.

Bradner *et al*. (1999a) presented data on the production of protease, xylanase, β-mannanase and chitinase from 7 Antarctic isolates at 4 temperatures (10°C, 21°C, 28°C and 37°C). They concluded that protease and hemicellulases were the most efficiently secreted by all isolates at all temperatures. They found variation between isolates of the same species. *Penicillium* sp. was the only genus that was common to their work and this PhD thesis research and they found protease, xylanase, and β-mannanase activity at all temperatures tested which this PhD thesis research also reported.

Hemicellulase activity was determined by Bradner *et al*. (1999b) using six Antarctic fungal isolates including 3 *Penicillium* spp. (*Penicillium expansum*, *P.hirsutum* and *P.commune*). Peak activity for mannanase for the 3 *Penicillium* spp. was between 40-50°C and when the mannanase activity at 10°C was compared with activity at 40-50°C, the activity level remaining at 10°C was

between 10-60% (Table 5.14). In this PhD research the mannanase activity for the two *Penicillium roquefortii* isolates was measured at 15°C and 50°C. Taking the measurement at 50°C as peak activity the activity level remaining at 15°C was between 1-52 % (Table 5.14).

Table 5.14: Comparison of mannanase activity remaining after assaying at 10°C reported by Bradner *et al*. (1999b) and mannanse activity remaining after assaying at 15°C during this PhD research.

Fungal isolate	Temperature of culturing	% of activity at 10°C when compared with activity at temperature of peak activity.	% of activity at 15°C when compared with activity at 50°C	Reference
Penicillium expansum	25	10		Bradner *et al*. (1999b)
P.hirsutum	25	40		Bradner *et al*. (1999b)
P.commune	25	60		Bradner *et al*. (1999b)
Penicillium roquefortii 405	4		14	PhD thesis
Penicillium roquefortii 405	15		52	PhD thesis
Penicillium roquefortii 405	25		1	PhD thesis
Penicillium roquefortii 408	5		8	PhD thesis
Penicillium roquefortii 405	15		4	PhD thesis

The reduction in mannanase activity was greater in this PhD research but this may be due to different species being used, lower incubation temperature of the fungus. Differences in protein, for instance primary, secondary, and/or tertiary structural difference, and/or post translational modification differences which results in the enzyme being not so well cold adapted.

From Bradner *et al*. (1999b) study peak activity for xylanase for the 3 *Penicillium* spp. was between 50-60°C and when the xylanase activity at 10°C was compared with activity at 50-60°C, the activity level remaining at 10°C was between 10-25% (Table 5.15) . In this PhD research the mannanase activity for the two *Penicillium roquefortii* isolates was measured at 15°C and 50°C. Taking the measurement at 50°C as peak activity the activity level remaining at 15°C was between 10-49% (Table 5.15).

Table 5.15: Comparison of xylanase activity remaining after assaying at 10°C reported by Bradner *et al*. (1999b) and xylanase activity remaining after assaying at 15°C during this PhD research.

Fungal isolate	Temperature of culturing	% of activity at 10°C when compared with activity at temperature of peak activity.	% of activity at 15°C when compared with activity at 50°C	Reference
Penicillium expansum	25	10		Bradner *et al*. (1999b)
P.hirsutum	25	10		Bradner *et al*. (1999b)
P.commune	25	25/20		Bradner *et al*. (1999b)
Penicillium roquefortii 405	4		21	PhD thesis
Penicillium roquefortii 405	15		49	PhD thesis
Penicillium roquefortii 405	25		49	PhD thesis
Penicillium roquefortii 408	5		15	PhD thesis
Penicillium roquefortii 405	15		10	PhD thesis

The reduction in xylanase activity was less in this PhD research but this may be due to different species being used, lower incubation temperature of the fungus, differences in protein, for instance primary, secondary, and/or tertiary structural difference, and/or post translational modification differences which results in the enzyme being better cold adapted.

Proteases have been reported to increase the activity of endoglucanases and play an important role in the degradation of cellulose (Eriksson *et al*., 1990). They are thought to either activate the endoglucanases or destroy protein endogucanase inhibitors. In this thesis research, *Cladosporium oxysporum*, which had in the extracellular supernatant the greatest protease activity, did have the greatest endo-1,4-β-glucanase activity but this trend was not present in other fungi tested. Cold acclimation proteins are thought to be proteases. A low-temperature-specific proteolytic system has been described (Berger *et al.*, 1996), and Caps act as cold-specific proteases that eliminate denaturated proteins whose accumulation would be deleterious for the cells. Of the 7 isolates in this thesis research where protease activity was detected, five showed greater protease activity when the fungal isolate was grown at 4°C rather than 15°C indicating the presence of cold acclimation proteins.

In conclusion, this chapter reports on a study focusing on the production of cellulases by Antarctic isolates at mesophilic and psychrophilic temperatures. The screening confirmed that cellulytic fungi existed within the hut environments and

were present on both wooden and non wooden materials. When the endo-1,4-β-glucanase activity accumulating in the culture supernatant was quantified in the extracellular supernatant of fungi grown at 4 and 15°C, it was concluded that all fungi could produce endo-1,4-β-glucanase at 4°C and in many cases produced more enzyme activity at the lower temperature. The cellulase enzyme complex was a multi enzyme complex and was different both between species and between isolates. The complex was in many cases different depending on temperature of incubation, as determined by mobility in SDS-PAGE. The cellulase enzyme activity assays confirmed the variation in enzyme composition seen in the SDS-PAGE gels with the activity being variable on the five cellulose substrates tested and there were not set trends with each isolates culture supernatant appearing to contain different combinations of the enzymes.

The other wood degrading enzymes amylase, pectinase, xylanase and mannanase had demonstratable enzyme activity in the extracellular supernatants when fungal isolates were grown at 4ºC and 15ºC and additionally at 25ºC for *Penicillium roquefortii* 405. These enzymes showed similar variable activities between species and temperature and no conclusion could be drawn which covered all the fungal isolates tested. The presence of these enzymes in the culture supernatant indicates that the Antarctic fungal isolates are all capable of degrading other wood components along with cellulose.

Protease activity was generally higher when the fungal isolate was grown at 4°C rather than 15°C, indicating possible cold acclimation or adaptation (for instance, the ability in the cold to degrade proteins more efficiently so as to provide more readity carbon and nitrogen).

Results from this chapter of research have been quoted in the following publication:

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.

Chapter 6 Description of β-1,4-glucan cellobiohydrolase activity of *Cadophora malorum*

6.1 Introduction

With the increased interest in cellulases for industrial purposes, there has been increased focus on microorganisms that can produce cellulases, including the range of enzymes produced, their specificity and stability.

Extracellular enzymes can be screened relatively straightforwardly as they are secreted into the culture medium; the extracellular supernatant can be enriched for a group of proteins, or individual proteins purified by separating the extracellular medium from a fungal culture with a minimal amount of cell lysis. This prevents the target enzyme(s) from becoming mixed with intracellular proteins and thus reducing the number of enrichment/purification steps required.

Filamentous fungi are good producers of cellulase with *Trichoderma reesei* being the most studied. In general, the fungal cellulase system consists of three classes of enzymes; 1,4-β-D-glucan cellobiohydralases, endo-1,4-β-D-glucanases and 1,4-β-D-glucosidase. Most fungi produce multiple cellulases with similar enzyme activity. As discussed in Chapter 5, *Cadophora malorum* 182 produced a multi-protein cellulase complex, as evidenced by SDS-PAGE zymogram gel results.

This chapter begins firstly, with a review of published research on purification and characterisation of cellulases from fungi. The materials and methods, results and discussion of the partial purification of the cellulases of the Antarctic isolate *Cadophora malorum* 182. The temperature profile for the partially purified enzyme follows along with a description of enzyme stability and activity with regard to a variety of substrates.

6.2 Hypothesis, Aims, Objectives

This chapter focuses on the partial purification and characterisation of the cellulase complex from the Antarctic isolate *Cadophora malorum* 182. The main hypothesis of this aspect of the thesis research was that an Antarctic fungus

produces cellulase with enzymatic activity ranges reflecting the unique environmental conditions.

The main aim was to gain an understanding of the cellulase complex of a fungal isolate that originates from a cold environment, both the composition of the cellulase complex and the characteristics of the cellulases within the complex.

The objective addressed was as follows:

- Enrich or purify and characterise cellulolytic enzyme(s) from the fungal species that most significantly produced cellulase enzymes under psychotrophic conditions.

6.3 Literature Review

6.3.1 Cellulase purification

Complex cellulase systems present considerable purification problems due to the occurrence of several isoforms. Additional confusion has surrounded the number of cellulases due to some catalytic domains appearing in culture filtrates separated from the cellulose binding domain (Ståhlberg *et al*., 1988). Culture filtrates from fungal growth often contain, beside a number of cellulases and isoforms, hemicellulases, amylases, proteases and others, altogether dozens of proteins. Naturally, they can not be resolved in a single chromatographic run, since the chromatogram will contain overlapping peaks. The electrophoretic analysis of the fractions will prove co-elution of several proteins in most of the peaks. Purification steps including multiple chromatographic runs exploiting the unique properties of the proteins with varied run conditions and chromatographic media will be needed to purify the cellulase components, and other strategies may also need to be employed.

The use of affinity chromatography (van Tilbeurgh *et al*., 1984) is limited due to difficulties in elution of enzymes from the supports. Affinity chromatography separates proteins on the basis of a reversible interaction between the protein and a specific ligand attached to a chromatographic matrix.

Ion-exchange chromatographic methods have most often been applied (Goyal *et al*., 1991). Ellouz *et al*. (1987) suggested a multi-step separation method based on ion-exchange. Ion-exchange chromatography separates proteins with differences in charge. The charged protein attaches to the opposite charged chromatography media and by altering the conditions usually by increasing the salt concentration, the proteins will unbind and be eluted from the chromatography media. Depending on the strength of the binding proteins will elute at different concentration of salt allowing the separation of a variety of proteins. Yu *et al*. (1993), proposed chromatofocusing as a method to get better resolution of crude cellulases than ion-exchange chromatography, but overlapping peaks and co-elution still caused a problem in the evaluation. Chromotofocusing is a variant of ion-exchange chromatography. The proteins bind at high or low pH. The pH is then adjusted up or down, when the pI of the bound protein is reached the protein unbinds and is eluted.

Medve *et al*. (1998) described a purification technique based on ion-exchange chromatography using new materials with high resolution power. Fast protein liquid chromatography (FPLC) was used to separate and purify three major cellulases; cellobiohydrolase I, cellobiohydrolase II and endoglucanase I. The purification procedure consisted of three different ion-exchange chromatographic steps. Medve *et al*. (1988) experienced difficulties with "ghost" peaks in the chromatograms, as they created doubt about the purity of the preparations and destroyed the resolution between the "real" peaks. Tomaz and Queiroz (1999) described the successful use of hydrophobic interaction chromatography (HIC) to separate the cellulase complex of *Trichoderma reesei*. The hydrophobic interaction was used for the binding of nonpolar regions, on the surface of the proteins, to adsorbents with hydrophobic ligands. The adsorption increases with high salt concentration in the media and elution (and separation) was carried out by decreasing the salt concentration of the eluent.

6.3.2 Characterisation of cellulase activity

Characterisation of enzymes includes various electrophoresis techniques. Investigation of the effect of pH and temperature on activity and stability, degradation products, substrate specificity and amino terminal acid sequence (Ruiz-Arribas *et al*. 1995)

The most common methods for quantification of cellulases are based on measuring activity against substrates like filter paper, microcrystalline cellulose or carboxy methyl cellulose (CMC). These methods are very useful when the aim is to get overall information about the cellulase activity of the sample in question, but if the aim is to get specific information about the single enzymes of the cellulase complex they fail, since these substrates are not selective against the individual enzymes. Further complication is caused in the evaluation of the results by the synergism of the enzymes. Synthetic substrates, like methylumbelliferyl derivatives of cellooligosaccharides, are also used and reported to be more specific e.g., in combination with inhibitors they can be used to identify purified cellulases (van Tilbeurgh and Claeyssens, 1985).

6.4 Materials and methods

6.4.1 Media and Reagents

Cellulose Broth- 1% Avicel, 1.5% soya bean flour, 1.5% K_2HPO_4, 0.5% $(NH_4)_2SO_4$, 0.006% $CaCl_2.2H_2O$, 0.006% $MgSO_4$ .7H2O, 0.02% (v/v) Tween 80 in distilled water.

20 mM TEA/HCL pH 7.5- 2.66 mls Triethanolamine (APS Ajax Finechem, Australia) in 700 of distilled water, pH adjusted to 7.5 with concentrated HCl. Volume made up to 1 L with distilled water.

20 mM TEA/HCL pH 7.5 + 1M NaCl - 2.66mls Triethanol amine, 58.44 g NaCl in 700 ml of distilled water, pH adjusted to 7.5 with concentrated HCl. Volume made up to 1 L with distilled water.

10 mM Sodium acetate (NaAc) pH 4.6- 0.8203g Sodium Acetate in 700 mls distilled water, adjust pH with concentrated HCl. Volume made up to 1 L with distilled water.

1 M NaAc pH 4.6- 82.03g Sodium Acetate in 700 mls distilled water adjust pH with concentrated HCl. Volume made up to 1 L with distilled water.

Universal buffer- 2.473 grms boric acid, 2.721 mls phosphoric acid, 2.299 mls acetic acid, 1 ml 20% sodium azide, and 1000 mls distilled water. 0.2M NaOH was added until pH 4.8 was obtained. $CaCl_2$ was added to a concentration of 7.5 mM.

Endo-1,4-β-glucanase substrate- 1% hydroxyethylcellulose in 0.05 M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate).

0.05 M citrate buffer pH 4.8- Solution 1-0.05M citric acid (10.51grms/l), Solution 2-0.05 M tri sodium citrate (14.71grms/l). Adjust pH to 4.8 by adding 667 mls solution 1 to 1 L of solution 2.

Avicelase substrate- 1% Avicel in 0.05 M citrate buffer pH 4.8 (0.05 M citric acid, 0.05 M tri sodium citrate).

Carboxymethylcellulase substrate- 1% carboxymethylcellulose in 0.05 M citrate buffer pH 4.8 (0.05 M citric acid, 0.05 M tri sodium citrate).

β-1,4-glucan cellobiohydrolase substrate- 1mM p-Nitrophenyl β-D cellobioside in 50mM sodium acetate pH 4.8.

Dinitrosalicylic acid reagent- 1% 2-hydroxy-3,5,-dinitrobenzoic acid, 1.6% NaOH (added slowly), 30% Rochelle salts/Potassium sodium tartrate (added in small portions with continuous stirring and filter to remove particulate material) in distilled water.

Protease substrate solution- 0.2% Azocasein in 50 mM 4-Morpholine-propanesulfonic acid (MOPS) (Boehringer Mannhiem, Germany) contained 5 mM $CaCl_2$ pH 7.0 at 40°C.

Protease stop reagent- 15% Trichloroacetic acid in distilled water.

Amylase substrate-1% soluble starch in 0.05 M citrate buffer pH 4.8 (0.05M citric acid, 0.05 M tri sodium citrate). Heated to dissolve.

Xylanase substrate-1% birchwood xylan in 0.05 M citrate buffer pH 4.8 (0.05 M citric acid, 0.05M tri sodium citrate).

Mannanase substrate- 1% locust bean gum in 0.05M citrate buffer pH 4.8 (0.05 M citric acid, 0.05 M tri sodium citrate). Homogenizing at 60 °C, heat to boiling point with continuous stirring and allowed to cooled, covered and slowly stirred overnight at 4°C

Polygalacturonic acid pectinase substrate- 1% polygalacturonic acid in 0.05 M citrate buffer pH 4.8 (0.05M citric acid, 0.05M tri sodium citrate).

Pectin pectinase substrate- 1% pectin in 0.05M citrate buffer pH 4.8 (0.05M citric acid, 0.05 M tri sodium citrate). Heated to dissolve.

SDS Acrylamide separating gel- 12.5 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 7.5 ml 1.5 M TrisCl pH 8.8, 0.3 ml 10% Sodium dodecylsuphate, 7.5 ml 1% CMC or HEC, 2.1 ml distilled water (total volume 30mls). Added to 150 µl 10% Ammonium persulphate and 10 µl TEMED after degassing and before pouring.

Acrylamide stacking gel- 0.67 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 1.25 ml 0.5 M TrisCl pH 6.8, 0.05 ml 10% sodium dodecylsulphate, 3 ml distilled water (total volume 5mls). Add 25 µl 10% Ammonium persulphate and 2.5 µl TEMED after being degassed and before being poured.

SDS-Tris-Glycine electrophoresis buffer- 0.025 M Tris, 0.192 M glycine, 0.1% Sodium dodecylsulphate pH 8.3 in distilled water.

Native acrylamide separating gel- 12.5 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 7.5 ml 1.5 M TrisCl pH 8.8, 7.5 ml 1% CMC or HEC, 2.4 ml distilled water (total volume 30mls). Add 150 µl 10% Ammonium persulphate and 10 µl TEMED after degassing and before pouring.

Native acrylamide stacking gel- 0.67 ml Biorad 40% Acrylamide/Bis solution 29:1 3.3%C, 1.25 ml 0.5 M TrisCl pH 6.8, 3.05 ml distilled water (total volume 5mls). Add 25 µl 10% Ammonium persulphate and 2.5 µl TEMED after degassing and before pouring.

Tris-Glycine electrophoresis buffer- 0.025 M Tris, 0.192M glycine, pH 8.3 in distilled water.

Coomassie stain solution- 0.025% Coomassie Blue R-250, 40% (v/v) methanol, 7% (v/v) Glacial acetic acid in distilled water.

Coomassie destaining solution I- 40% (v/v) methanol, 7% (v/v) glacial acetic acid in distilled water.

Coomassie destaining solution II- 5% (v/v) methanol, 7 % (v/v) glacial acetic acid in distilled water.

Silver stain fixing solution- 40% (v/v) absolute ethanol, 10% (v/v) acetic acid in distilled water.

Silver stain sensitizing solution- 30% (v/v) absolute ethanol, 6.8% (w/v) sodium acetate (anhydrous), 0.2% (w/v) sodium thiosulphate (pentahydrate) in distilled water. Within 1 hr of use added 0.125% (v/v) glutaraldehyde.

Silver stain silver solution- 0.25% (w/v) silver nitrate in distilled water. Within 1hr of use added 0.015% (v/v) formaldehyde.

Silver stain developing solution-2.5% (w/v) sodium carbonate (anhydrous) in distilled water. Within 1 hr of use added 0.0074% (v/v) formaldehyde.

Silver stain stop solution- 1.5% (w/v) Ethylenediaminetetraacetic acid (disodium salt) (Na_2EDTA) in distilled water.

Silver stain preserving solution- 30% (v/v) absolute ethanol, 4%(v/v) glycerol in distilled water.

Periodic acid (PAS) staining reagent 1- 5% phosphotungstic acid in 2N HCl.

PAS staining reagent 2- 7% methanol, 14% acetic acid in distilled water.

PAS staining reagent 3- 1% periodic acid in 7% trichloroacetic acid.

PAS staining reagent 4- 0.5% sodium metabisulfite in 0.1N HCl.

6.4.2 Cultivation conditions

Cadophora malorum isolate 182 was grown in eight 2 L flasks containing 1 L of cellulose broth at 4°C for 28 days. Fungal biomass was removed by centrifugation and the extracellular supernatant was concentrated using ultrafiltration with a polyethersulphone membrane 10,000 MW.

6.4.3 Protein Purification conditions

The stratagem for protein purification was to use an AKTA (Amersham Bioscience, Sweden) Fast Protein Liquid chromatography performed at room temperature, a similar method to that described by Jørgensen *et al*. (2003). All chromatographic columns were from Amersham Biosciences, Sweden. Before separation, the concentrated extracellular supernatant was desalted and buffer exchanged to 20 mM TEA/HCL pH 7.5 using a Hi Prep 26/10 Desalting column. A single protein peak was observed, collected and pooled for further purification.

The protein fractions from the desalting step were pooled and aliquots loaded onto a Hiload 26/10 Q Sepharose Fast Flow column which was equilibrated in 20 mM TEA/HCL pH 7.5, the column was washed to remove unbound proteins, then a gradient using 20 mM TEA/HCL pH 7.5 + 1 M NaCl was used to remove the bound proteins. Five ml fractions were collected. The unbound proteins were frozen in a -20°C freezer. The bound proteins were collected from each run, pooled and concentrated using ultrafiltration with a polyethersulphone membrane 10,000 MW. The bound enzyme fraction was desalted and buffer exchanged to 10 mM NaAc pH 4.6 using a Hi Prep 26/10 Desalting column (Amersham, Sweden). The protein fractions were collected and pooled for further purification. The pooled protein fraction aliquots were loaded onto a Mono Q 10/100 GL column, which was equilibrated in 10 mM NaAc pH 4.6, the column was washed to

remove unbound proteins, then a gradient using 1 M NaAc pH 4.6 was used to remove the bound proteins. Two ml fractions were collected through the complete purification run.

6.4.4 Preparative Native electrophoresis

Fractions 11 or 13 (depending on run, but observed as a peak at the same relative position in the chromatograph) were pooled, concentrated using a 10 ml stirred cell system with a 10kMW cut off (PAL Life Sciences, USA) and subjected to native gel electrophoresis. The concentrated fractions were mixed with an equal volume of loading buffer and loaded onto a 12.5% acrylamide gel minus SDS. Tris glycine buffer was used as the electrophoresis buffer. Electrophoresis was conducted at room temperature at 250 volts, 50 milliamps for 3 hours then 100 volts, 30 milliamps until the bromophenol blue dye front had run off the bottom of the gel, approximately 15 hours. Once electrophoresis was completed, the edge of the gel was cut off and silver stained to determine location of the protein bands. The gel was cut with a scalpel at the appropriate locations of stained bands and the gel strips placed in a tube with 10mM sodium acetate buffer pH 4.6, and glass beads. The tubes were vortexed and chilled repeated times until the gel strip was well broken and subsequently frozen at -20°C in the freezer overnight before being centrifuged and the β-1,4-glucan cellobiohydrolase activity was determined using the method described in Section 5.4.13.

6.4.5 Electrophoresis of enriched material

SDS polyacylamide gel electrophoresis (SDS PAGE) was conducted as described in Section 5.4.6, and native electrophoresis and hydroxyethylcellulase zymogram as described in Section 5.4.8 were used to determine purity of the enzyme preparation. The time that the zymogram was incubated was reduced to 20 minutes. Gels were stained by Coomassie Blue protein staining method as described in Section 5.4.7, silver protein staining method as described in Section 5.4.9, and PAS staining of glycoproteins as described in Section 5.4.10

6.4.6 Identification of the protein bands

A second strip from the gels run in Section 6.4.4 was Coomassie Blue protein stained and the visualised bands were cut out of the gel and sent to The

Proteomics Facility at The Centre for Genomics and Proteomics at the University of Auckland for peptide mass finger printing and de novo sequencing.

6.4.7 Biochemical Characterisation

6.4.7.1 Temperature profile

The partial purified protein, as extracted from the native gel, was evaluated for β-1,4-glucan cellobiohydrolase activity at various temperatures by the method described in Section 5.4.13. The temperatures tested were 2, 4, 10, 15, 20, 30, 40, 50, 60, 70, 75 and 80°C. The pH of the substrate was adjusted for the different temperatures.

6.4.7.2 Enzyme stability

The temperature stability of the partially purified enzyme was measured at 4, 25, 40, 50 and 80°C. One ml of the enzyme was placed in an Eppendorf tube and placed in the preheated water bath set at appropriate temperature and 200 µl samples were taken at varying times depending on the temperature of the stability study; the β-1,4-glucan cellobiohydrolase assay was conducted as described in Section 5.4.13.

6.4.7.3 Cellulase activity using different cellulose sources.

Avicelase, filter paperase and carboxymethylcellulase catalytic activity was determined for the partially purified enzyme using the protocol described in Section 5.4.13. Endo-1,4-β-glucanase activity was determined using the protocol described in Section 5.4.4 and β-1,4-glucan cellobiohydrolase activity was determined as described in Section 5,4,13, with the only modification that the assay was run for 30 minutes rather than 10 minutes.

6.4.7.4 Measurement of activity of additional enzymes associated with wood cell wall degradation

Amylase, xylanase, mannanase and pectinase catalytic activity was determined for the partially purified protein using the protocols described in Section 5.4.14 with the only modification being that the assay was run for 30 minutes rather than 10 minutes.

6.4.7.5 Measurement of protease activity

Protease activity was determined for the partially purified protein using the protocol described in Section 5.4.15 with the only modification that the assay was run for 30 minutes rather than 10 minutes.

6.5 Results

6.5.1 Enrichment of cellulases from *Cadophora malorum* 182

The culture supernatant of *Cadophora malorum* 182 grown on Avicel at 4°C temperature was subjected to Ion Exchange Chromatography similar to the methods described by Jørgensen *et al*. (2003). From the first desalting step, fractions 3-6 demonstrated cellulase activity (Figure 6.1), and were pooled and loaded onto the next step of the enrichment process. Hiload 26/10 Q Sepharose Fast Flow column was used for the next step and a large peak of protein did not bind to the column and is seen in the chromatograph flow through (fractions 7-17 in Figure 6.2); the proteins represented by these fractions demonstrated endo-1, 4-β-glucanase activity, and were frozen for later work, as described in Section 6.4.3. From the gradient region of the chromatography, three broad unseparated peaks were observed (fractions 39-80 in Figure 6.2). Endo-1,4-β-glucanase was detected from these three broad peaks with the highest activity on a volume basis being from fractions 45-50. After being pooled, concentrated, and desalted, fractions 39-80 were subjected to additional chromatography using a Mono Q 10/100 GL column. Unbound proteins, fractions 2 to 4 (Figure 6.3), were pooled and frozen for future work. Endo-1,4-β-glucanase activity was present in these fractions and β-1,4-glucan cellobiohydrolase activity was not detected as shown in Table 6.1. From the gradient phase of the chromatography, six major peaks were detected (fractions 8-19 Figure 6.3). Endo-1,4-β-glucanase activity was detected in fractions 10-18, with greatest activity detected in fractions 11, 15 and 17 (Table 6.1). β-1,4-glucan cellobiohydrolase activity was detected in fraction 8, 10 to 16 (Table 6.1) with peak activity in fractions 10,14,15 (Table 6.1).

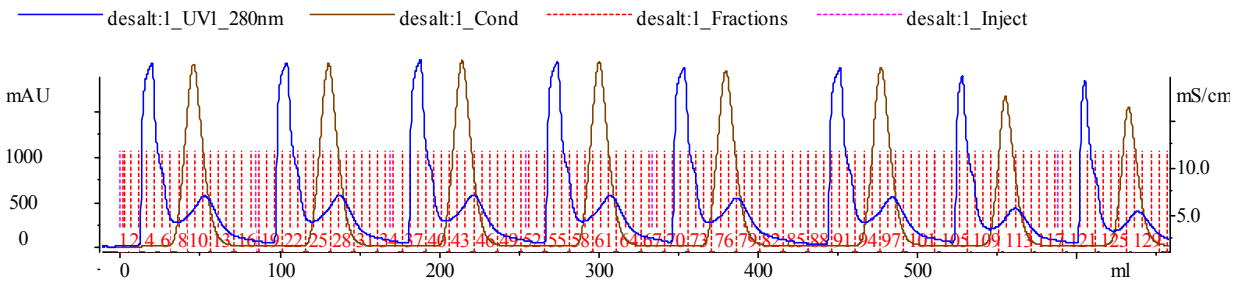

Figure 6.1: Chromatograph of the desalting process. Eight runs are shown on this chromatograph.

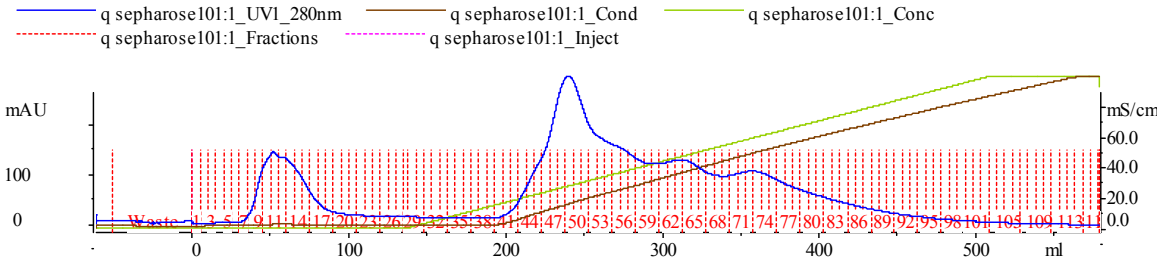

Figure 6.2: Chromatograph of typical Sepharose purification run.

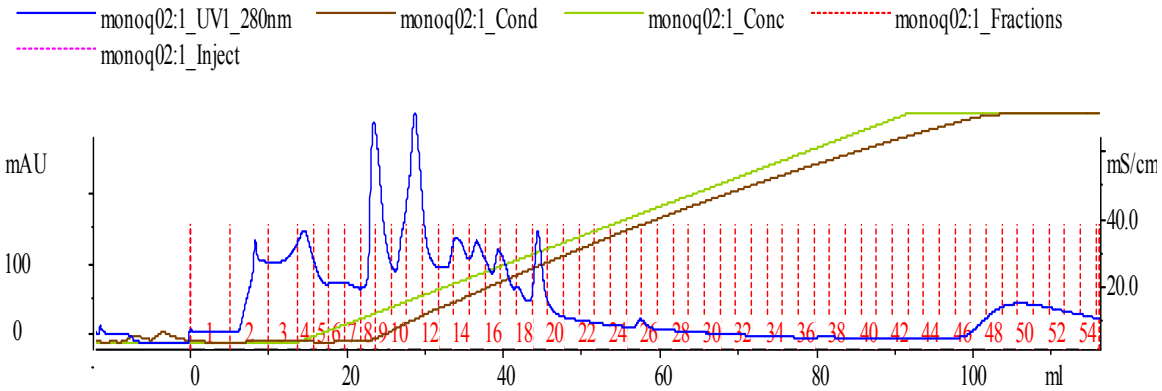

Figure 6.3: Chromatograph of the MonoQ purification run.

Table 6.1: MonoQ fractions, protein levels, Endo-1, 4-β-glucanase and β-1,4-glucan cellobiohydrolase activity.

MonoQ Fraction	Protein (ug/ml)	Endo-1, 4-β-glucanase activity (micromoles glucose released per minute)	β-1,4-glucan cellobiohydrolase activity (pmol p-nitrophenol released per minute per ml)
2	338	0.96	0
4	272	1.38	0
6	172	0	0
8		0	0.00033
9	808	0	0
10		1.57	>0.25
11	818	2.355	0.0117
12	354	1.07	0.0092
13	466	1.23	0.0067
14	242	0.787	>0.25
15	278	1.71	>0.25
16	474	1.55	0.0003
17	266	1.965	0
18	212	1.17	0
19		0	0

6.5.2 Preparative Native gel electrophoresis

Fractions 11 or 13 (depending upon which chromatogram, but at the same relative position) were subjected to native gel electrophoresis to separate proteins. Subsequently, stained proteins were cut from the gel and washed out of the gel. Figure 6.4 shows the stained native gel and location of the cuts which were made at 0 mm to remove the stacking band, 3 mm, 6 mm, 10 mm, 15 mm, 19 mm, 24 mm, 31 mm, 42 mm, 50 mm, 64 mm and 70 mm. β-1,4-glucan cellobiohydrolase

activity and protein concentration was determined for each slice after extraction from the gel. The highest activity was determined in the 3 mm slice (Table 6.2).

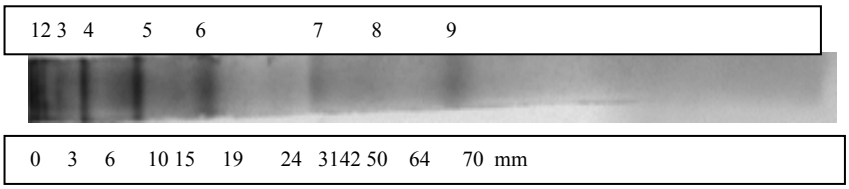

Figure 6.4: Photograph of native preparative electrophoresis strip, top scale show location of bands, bottom scale shown location of cuts.

Table 6.2: Native preparative gel slices, protein levels and β-1,4-glucan cellobiohydrolase activity.

Gel slice	Protein ug/ml	β-1,4-glucan cellobiohydrolase activity (pmol p-nitrophenol released per minute per ml)
0-3	21.4	0.014
3-6	57.4	0.0027
6-10	0	0.0016
10-15	0	0.0047
15-19	119	0
19-24	124.2	0.002
24-31	242.2	0
31-42	208.8	0.002
42-50	108.8	0
50-64	111.4	0.0026
64-70	193.4	0

From 75 mls of concentrated *Cadophora malorum* 182 culture media, via 3 purification steps, 15 mls of partially purified β-1,4-glucan cellobiohydrolase was produced as documented in Table 6.3. The starting material had a β-1,4-glucan cellobiohydrolase activity/mg total protein of 0.16 after desalting and the β-1,4-glucan cellobiohydrolase activity/mg total protein had dropped to 0.11 due to this step not being a purification step and the volume of sample rising to 100ml. After the first purification step, ion exchange chromatography using Q Sepharose, the total protein had dropped to 149 mg from the starting level of 281.7 mg and β-1,4-glucan cellobiohydrolase activity/mg total protein had risen to 0.25. Again the desalting step was used to remove salts and to buffer exchange to the buffer required for the next purification step there was no enrichment of β-1,4-glucan cellobiohydrolase activity. The next purification step, ion exchange chromatography using a MonoQ column, removed more protein with the total protein level at 9.9 mg, and β-1,4-glucan cellobiohydrolase activity/mg total protein was 0.42. The final step the native preparative electrophoresis caused a reduction in β-1,4-glucan cellobiohydrolase activity/mg total protein to 0.3.

Therefore, by the stratagem used, the β-1,4-glucan cellobiohydrolase activity was enriched in the final sample from the starting material by 1.88 fold.

Table 6.3: Enrichment details for partial purification of β-1,4-glucan cellobiohydrolase.

	Volume of material mls	Protein mg/ml	Total protein mg	β-1,4-glucan cellobiohydrolase activity (pmol p-nitrophenol released per minute per ml)	Total β-1,4-glucan cellobiohydrolase activity
Concentrated *Cadophora malorum* culture media (Starting material)	75	3.7	277	45	0.16
Q Sepharose starting material after desalting	110	2.4	264	30.8	0.11
Gradient peak from Sepharose purification and concentrating	31	5.0	155	37.2	0.25
MonoQ starting material after desalting	58	2.2	128	29.6	0.23
Conc Peak 2-12 from monoQ purification	2	5.0	10	4.2	0.42
Band 3 mm from native preparative electrophoresis	15	0.5	7.5	0.6	0.30

6.5.3 Electrophoresis of enriched material

SDS-PAGE electrophoresis was used to assess purity of the protein and as can be seen in Figure 6.5 the number of protein bands had not reduced significantly from the Ion exchange chromatograph step of the purification process. When comparing lane 1, the concentrated extracellular supernatant, with lane 6 fractions 2,3,4,6,8,9,11 and 12 from Mono Q chromatography. By the final MonoQ step, there are a number of protein bands that are darker (more intense) in lane 6 than lane 1 indicating greater levels of that protein in the sample, though the stain is only relative and not quantitative for protein. Lane 7 in Figure 6.8 shows the proteins present after the native preparative electrophoresis and the number of bands is significantly less with many of the abundant proteins, darker bands not being present.

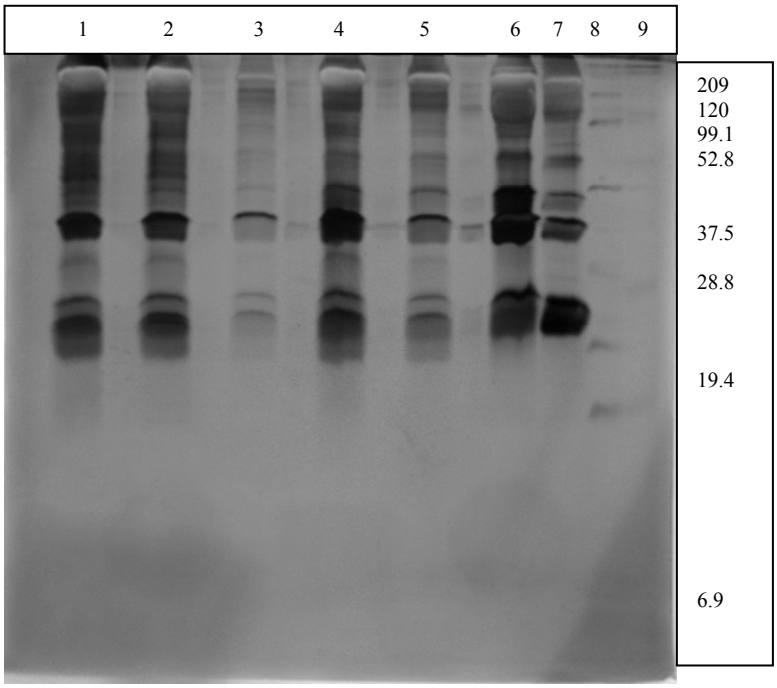

Figure 6.5: Photograph of SDS-page of *Cadophora malorum* extracellular supernatant and after the various purification steps.
Lane 1: 182 4°C concentrated extracellular supernatant, the starting material
Lane 2: Starting material desalted before Q Sepharose chromatography
Lane 3: Material from gradient section of Q Sepharose separation
Lane 4: Material in lane 3 concentrated
Lane 5: Mono Q initial loading sample
Lane 6: Fractions 2,3,4,6,8,9,11 and 12 from Mono Q chromatography
Lane 7: Fractions 5 and 7 from Mono Q chromatography
Lane 8: Broad range MW markers
Lane 9: Prestained broad range MW markers.

When the material from the purification steps were subjected to native electrophoresis and HEC zymograms, the number of bands in the native gel was less by the last step of the purification process as seen in Figure 6.6, and there was no visible cellulase activity in the concentrated *Cadophora malorum* 182 culture media in lane 1 but activity was seen as clearing bands in lanes 5, 6, 7. as seen in Figure 6.7. The reduction in the incubation time was presumed to be the cause of no cellulase clearing bands in lanes 1, 2, 3 and 4.

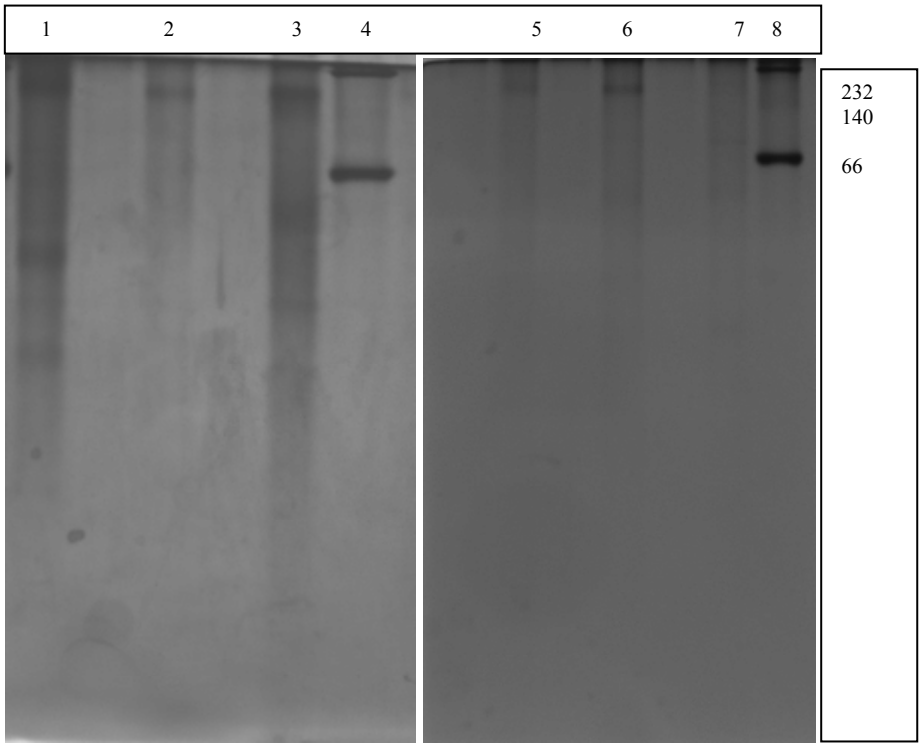

Figure 6.6: Photograph of native page of *Cadophora malorum* extracellular supernatant and after the various purification steps.
Lane 1: 182 4°C concentrated extracellular supernatant, the starting material
Lane 2: Starting material desalted before Q Sepharose chromatography
Lane 3: Material from gradient section of Q Sepharose separation
Lane 4: material in lane 3 concentrated
Lane 5: Mono Q initial loading sample
Lane 6: Fractions 2,3,4,6,8,9,11 and 12 from Mono Q chromatography
Fractions 2,3,4,6,8,9,11 and 12 from Mono Q step
Lane 7: HMW markers

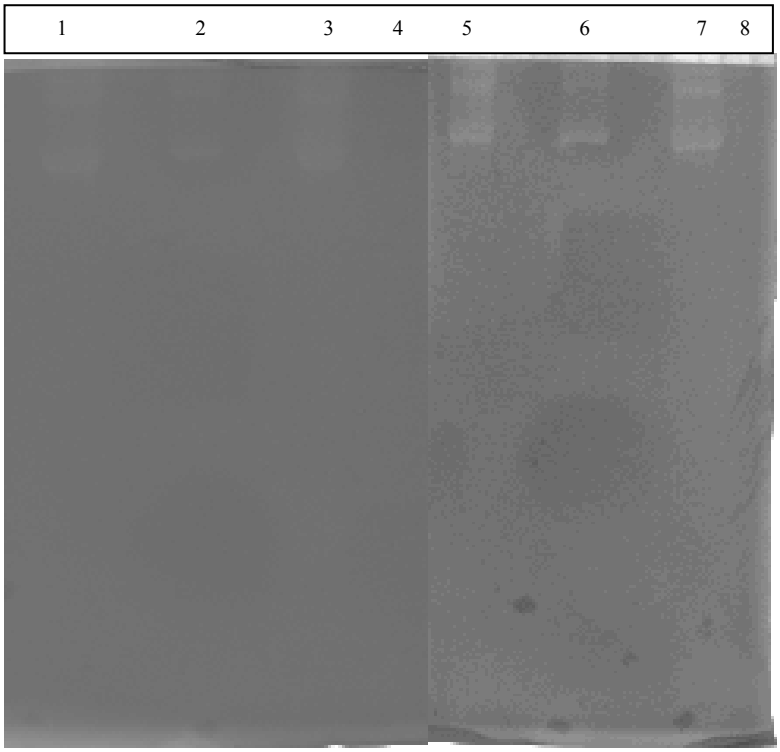

Figure 6.7: Photograph of hydroxyethylcellulose zymogram of *Cadophora malorum* extracellular supernatant and after the various purification steps.
Lane 1: 182 4°C concentrated extracellular supernatant, the starting material
Lane 2: Starting material desalted before Q Sepharose chromatography
Lane 3: Material from gradient section of Q Sepharose separation
Lane 4: Material in lane 3 concentrated
Lane 5: Mono Q initial loading sample
Lane 6: Fractions 2,3,4,6,8,9,11 and 12 from Mono Q chromatography
Lane 7: Fractions 5 and 7 from Mono Q chromatography
Lane 8: Prestained broad range MW markers

To determine if any proteins were glycosylated and how many were glycoproteins, a SDS-PAGE gel was PAS stained (Figure 6.8) and all lanes had a dark pink band at the top of the gel, lanes 1-5 contained an additional one main pink band and lane 6 and 7 showed two additional pink bands indicating two glycoproteins, one at the same position as lanes 1-5 and another that is slightly smaller as it is further into the gel.

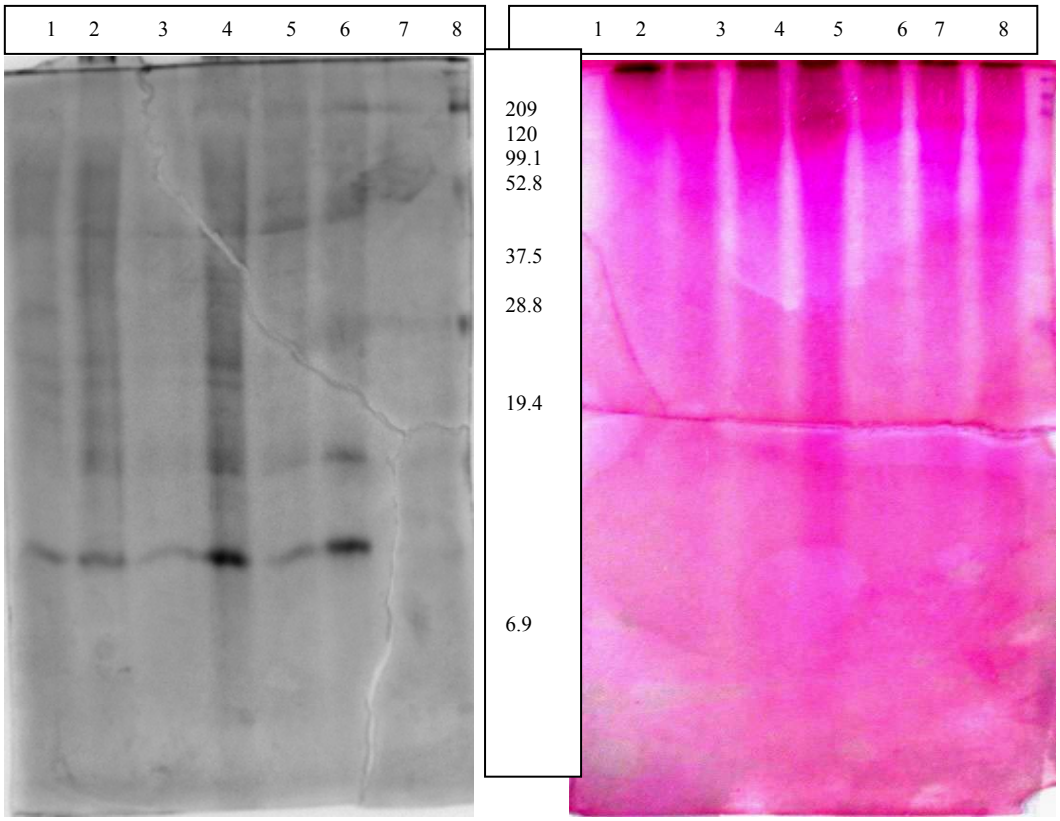

Figure 6.8: Photograph of left; SDS page of Cadophora malorum extracellular supernatant and after the various purification steps. Right; PAS Staining of SDS-PAGE electrophoresis on the left.
Lane 1: 182 4°C concentrated extracellular supernatant, the starting material
Lane 2: Starting material desalted before Q Sepharose chromatography
Lane 3: Material from gradient section of Q Sepharose separation
Lane 4: Material in lane 3 concentrated
Lane 5: Mono Q initial loading sample
Lane 6: Fractions 2,3,4,6,8,9,11 and 12 from Mono Q
Lane 7: Band 3mm from native preparative gel electrophoresis
Lane 8 Prestained broad range MW markers

6.5.4 Identification of the protein bands

From the most resolved peaks eluted from the Mono Q chromatography demonstrating endo-glucanase activity, preparative native gel and Coomassie blue staining showed nine protein bands, which were excised and sent for N terminal sequencing with the aim of identification; the preparative gel is seen in Figure 6.9.

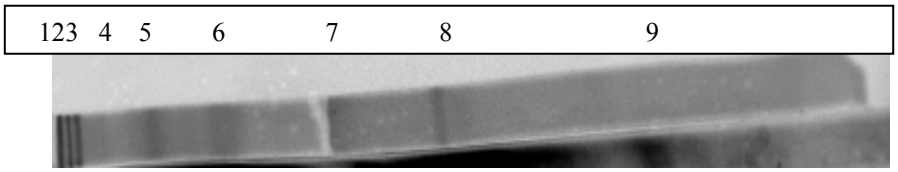

Figure 6.9: Coomassie blue stained gel of the preparative native electrophoresis. Scale along gel show bands 1=WF1, WF=2, 3=WF3, 4=WF4, 5=WF5, 6=WF6, 7=WF7, 8=WF8 and 9=WF9.

Though individually stained bands were excised, none of the bands were found to be pure (homogeneous) and the results from the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) required interpretation. The MASCOT search results of the protein databases showed there were very few protein sequences that matched sufficiently to the protein sequences generated from the MALDI-TOF sequences. Protein band WF9 was the only protein that the match was significant with a protein score above 64 but the number of mass values searched was low, and there were a number of missed cleavages and the protein molecular weight did not match which meant that the protein could not be unequivocally identified. Protein band WF1 contained proteins that were close to the required 65 protein score but there were also too many missed cleavages and the protein molecular weight also did not match, to be sure of the identification.

Protein bands WF1, WF2, WF3 and WF9 were submitted to Q-STAR XL Hybrid MS/MS system. WF1, WF2 and WF3 formed Band 3mm, which was used for the enzyme description, and WF9 as it was the protein band furthest away from the area of interest and should not contain any proteins in common with the area of interest. When the data from an LC-MS/MS run was searched using the Mascot software against the fungal protein sequences, there were significant matches for each protein band due to the fact that the bands were heterogenous and not representing a single protein.

A β-glucosidase 1 precursor was identified from WF2 and WF3. De Novo sequencing was used to identify the peaks that did not match any proteins in the database using the MASCOT software. These were the more dominant peptides in the samples and when the peptide sequences were BLAST searched using the ExPASy SIB BLAST Network service against submitted fungal protein sequences, a number of β-glucosidases were found to be present and in some cases were common in more that one band or more than one peptide sequence within a band identified the protein. The common proteins were identified as seen in Table 6.4. all protein matches are seen in Appendix 8. None of the proteins in WF9 were found in WF1, WF2 or WF3.

Table 6.4: Proteins identified in the Native Gel excised bands.

Potential identity of peptide sequence	**Band identified in Excised gel**
H antigen precursor [Ajellomyces capsulata (Histoplasma capsulatum)]	WF1
Hypothetical protein [FG06605.1] [Gibberella zeae (*Fusarium graminearum*)]	WF1
Beta-glucosidase-like protein [Magnaporthe griseea (Rice Blast fungus (*Pyricularia grisea*)]	WF1
Hypothetical protein NCU08755.1 [NCU08755.1] [*Neuropsora crassa*]	WF1,WF3
Predicted protein [SNOG_11881] [*Phaeosphaeria nodorum* SN15]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria*]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1,WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1,WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria sp*. S-93-48]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)*]*	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1, WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f. sp. triticae*]	WF1
Thermostable beta-glucosidase [bgl1] [*Thermoascus aurantacus*]	WF1 WF3
Beta-glucosidase [*Talaromyces emersonii*]	WFI, WF3
Hypothetical protein [SNOG_06393] [*Phaeosphaeria nodorum* SN15]	WF1
Hypothetical protein [AN7055.2] [*Emericella nidulans* (*Aspergillus nidulans*)]	WF1
Glycolate oxidase [AO090010000085] [*Aspergillus oryzae*]	WF1
Hypothetical protein [ATEG_09314] [*Aspergillus terreus* NIH2624]	WF2
Beta-glucosidase-related glycosidases AO090701000274] [*Aspergillus oryzae*]	WF2
Hypothetical protein [AN2217.2] [*Emericella nidulans* (*Aspergillus nidulans*)]	WF2
Beta-glucosidase [Afu5g07080] [*Aspergillus fumigatus* (*Sartorya fumigate*)]	WF2
Predicted protein [CHGG_02072] [*Chaetomium globosum* CBS 148.51]	WF2
Beta-glucosidase [*Talaromyces emersonii*]	WF3
Beta-glucosidase precursor (EC 3.2.1.21) [bgl1] [*Aspergillus niger*]	WF3
Beta-glucosidase precursor (EC 3.2.1.21) [bgln] [*Aspergillus niger*]	WF3
Beta-glucosidase (EC 3.2.1.21) [bgl1] [Aspergillus níger]	WF3
Beta-D-glucosidase [bglA] [*Aspergillus kawachi* (*Aspergillius awamori* var. *kawachi*)]	WF3
Beta-glucosidase 1 precursor (EC 3.2.1.21) (gentiobiase) (Cellobiase) (Beta-D-glucosidase glucohydrolase)	WF3
Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria f.sp. avenaria*]	WF3

6.5.5 Biochemical Description

The bands in the first 3 mm of the native gel (henceforth called Band 3mm) had the greatest β-1,4-glucan cellobiohydrolase activity, contained proteins identified as β-glucosidase and though heterogeneous, was used for description of biochemical properties, particularly to have an overall picture of the impact of temperature on β-1,4-glucan cellobiohydrolase to understand whether this protein may be relevant for activity in the context of the Antarctic environment. Band 3mm proteins were removed from the gel by cutting out the first 3mm and the

extracting into buffer and all the following activities were conducted with the supernatant from this excision.

6.5.5.1 Temperature profile

There was no β-1,4-glucan cellobiohydrolase activity observed when assayed at temperatures 0, 4, 10 or 15°C and there was activity at the assay temperature of 20°C, steadily increasing with increased temperature until the optimum temperature for activity was 60°C, as shown in Figure 6.10 and there was less activity at 70°C and very little activity when the assay was run at 80°C.

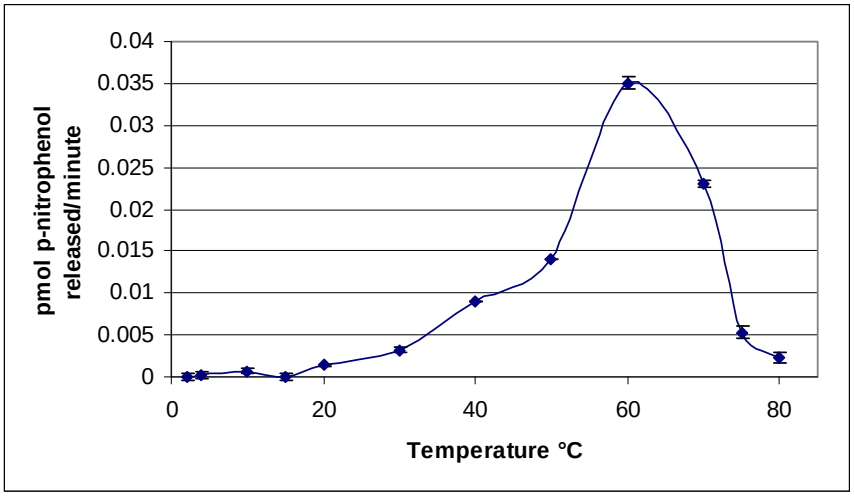

Figure 6.10: Graph of temperature profile for the partial purified protein Band 3mm. Vertical bars represent the standard error.

6.5.5.2 Enzyme stability

The partially purified protein Band 3 mm was stable at 4°C and at 25°C for over 24 hrs. At both 4°C and at 25°C, the β-1,4-glucan cellobiohydrolase enzyme activity rose over the first hour and then decreased for the incubation (until stop of experiment at 24 hours) but never dropped to the 0 time activity level (Figure 6.11 and 6.12). When the tube which had been incubated at 25°C was left on the laboratory bench for 2 hrs the level of enzyme activity was still 0.37 pmol p-nitrophenol released/minute.

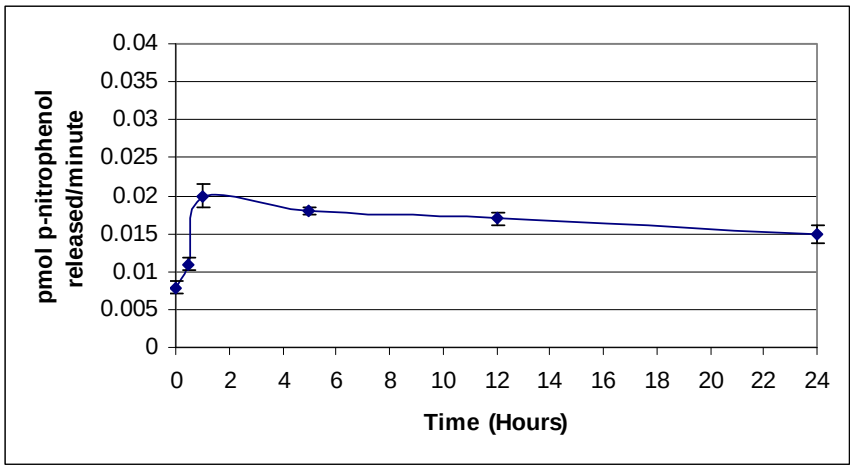

Figure 6.11: Graph of stability at 4°C of the partially purified protein. Vertical bars represent the standard error.

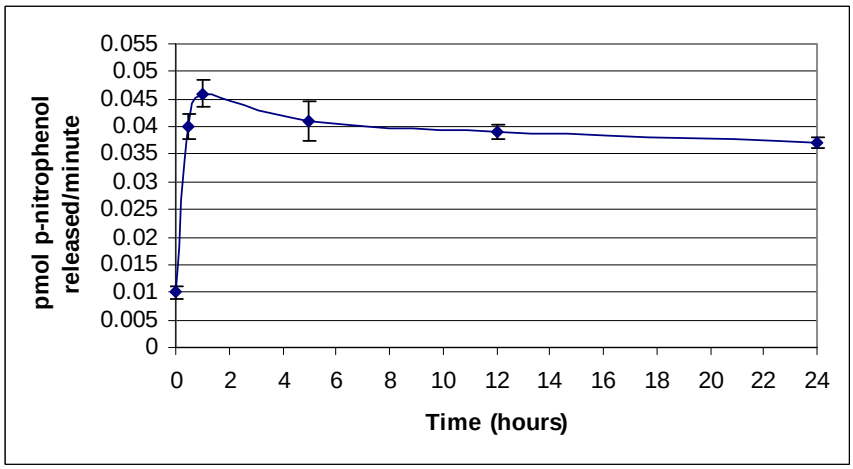

Figure 6.12: Graph of stability at 25°C of the partially purified protein Band 3 mm. Vertical bars represent the standard error.

At 40°C, the β-1,4-glucan cellobiohydrolase enzyme activity increased over the first hour and then dropped steadily to be back to the zero time enzyme activity (Figure 6.13) after 24hrs.

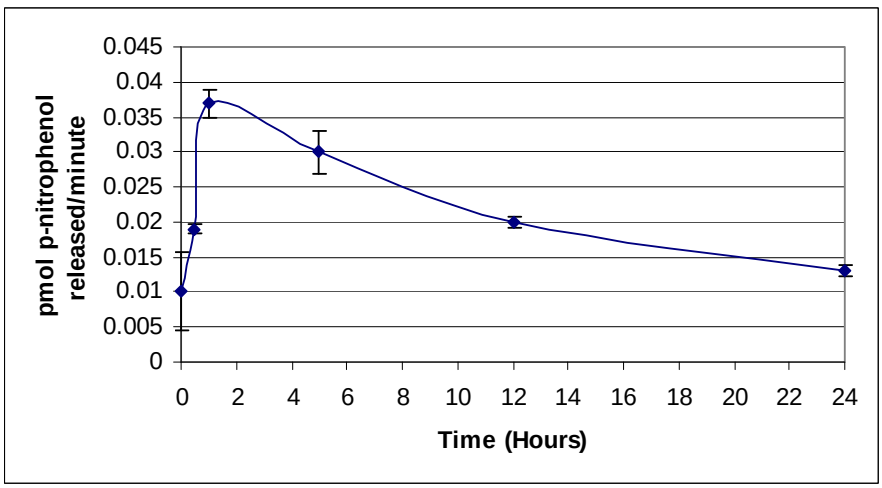

Figure 6.13: Graph of stability at 40°C of the partially purified protein Band 3 mm. Vertical bars represent the standard error.

At 50°C, the enzyme steadily lost activity and after 5hrs the activity was 50% of the activity at 0 time and by 24 hours the activity was at the detection limit of the assay (Figure 6.14).

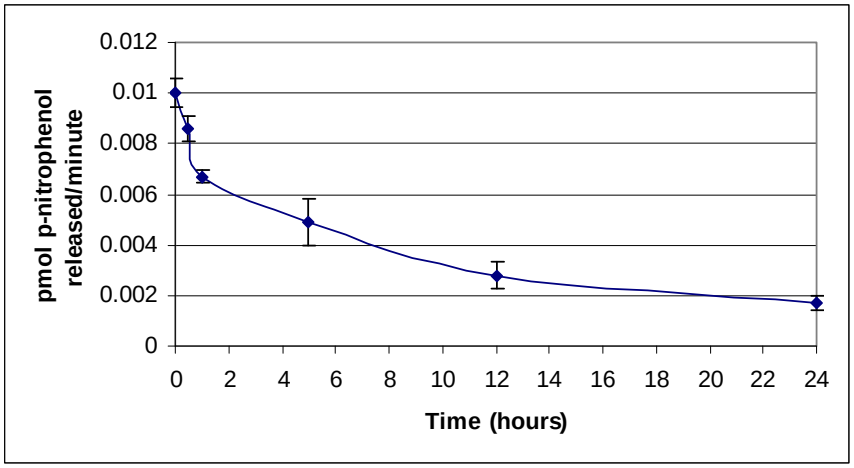

Figure 6.14: Graph of stability at 50°C of the partially purified protein Band 3 mm. Vertical bars represent the standard error.

At 80°C, the enzyme had lost 50% of its activity after less than 1 minute and the activity was below the detection limit of the assay after 2 minutes (Figure 6.15).

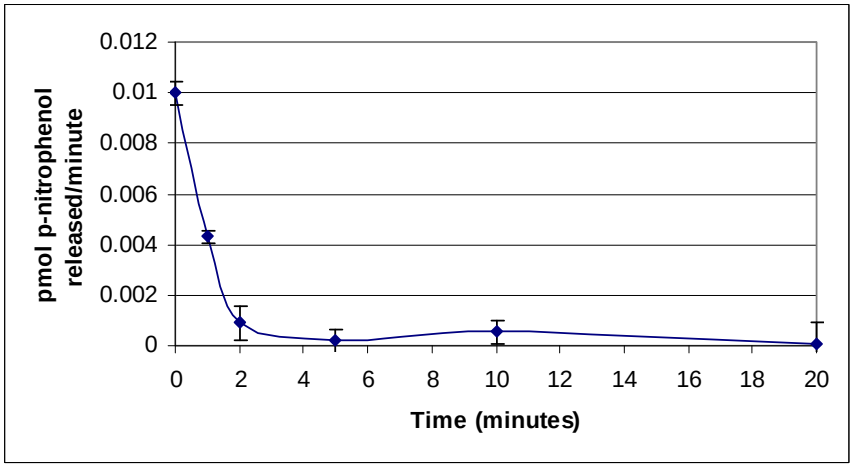

Figure 6.15: Graph of stability at 80°C of the partially purified protein Band 3 mm. Vertical bars represent the standard error.

6.5.5.3 Cellulase activity of Band 3mm partially purified protein using different cellulose sources.

No Endo-1,4-β-glucanase or filter paper catalytic activity was detected on hydroxyethylcellulose or cellulose filter paper, respectively, for partially purified Band 3 mm and the following activities were measured:

- Avicelase catalytic activity was 1.34 micromoles glucose released/minute/ mg of protein.

- Carboxymethlycellulase catalytic activity was 0.48 micromoles glucose released/minute/ mg of protein.
- β-1,4-glucan cellobiohydrolase activity was 0.028 pmol p-nitrophenol released/minute/mg of protein.

6.5.5.4 Measurement of activity of additional enzymes associated with wood cell wall degradation

Neither amylase nor pectinase catalytic activity was detected for Band 3mm partially purified protein. The following activities were measured:

- Mannanase activity was 0.36 micromoles glucose released/minute/ mg of protein.
- Xylanase activity was 0.86 micromoles glucose released/minute/ mg of protein.

6.5.5.5 Measurement of protease activity

Protease activity for Band 3mm partially purified protein was 0.48 AU (ΔA_{420} of 1.0 per hour/ mg of protein).

6.6 Discussion

Cellulases are notoriously difficult to fractionate and purify. In this PhD thesis the methods of Jørgensen *et al*. (2003) were used as a guideline for the purification strategy. Anion exchange was used to separate potential xylanases, which should not bind to the column matrix, from cellobiohydralases and endoglucanases which bound to the column but were eluted at different salt concentrations. After this initial purification the various peaks would be submitted to different purification regimes to remove any additional proteins that were present from the first step. In the purification process described in the thesis research, there were not distinct peaks (Figure 6.2) in the gradient section of the first purification step so all fractions were pooled and subjected to the next purification step which was another anion exchange step but at a lower pH and using acetate buffer. This provided more defined peaks but there was still overlap between the peaks (Figure 6.3). One fraction, (11 or 13, depending upon which chromatograph, but at the same relative position) was chosen for preparative native electrophoresis and the protein bands in the region of 0-3mm were removed and used for the activity description study. Overall the β-1,4-glucan cellobiohydrolase activity was increased about two fold and the protein levels

dropped from 3.7 mg/ml to 0.5 mg/ml. SDS-PAGE electrophoresis demonstrated that the protein had not been purified to homogeneity. Native gel electrophoresis with hydroxethylcellulase zymogram demonstrated that β-1,4-glucan cellobiohydrolase activity had been enriched for a mixture of proteins referred to as Band 3mm. Band 3mm was shown to contain three Schiff stain positive proteins, indicated three glycosylated proteins.

The bands seen on the preparative native gel were identified using peptide mass finger printing, LC-MS/MS and De Novo sequencing. All nine protein bands were multiple proteins, and the peptide matches were not to a high enough score to conclusively identify any of the proteins. A number of β-glucosidases were found to be present and in some cases were common in more that one band or more than one peptide sequence within a band identified the protein. The fact that the peptides did not match could indicate the proteins found within these bands are new, and are not like any other submitted sequences, supporting the uniqueness of the *Cadophora malorum* 182.

The properties of Band 3mm were described in relation to temperature profile, enzyme stability and activity with regard to a variety of substrates. β-1,4-glucan cellobiohydrolase activity was seen over the temperature range 20°C to 80°C with the optimum activity seen at 60°C. Band 3mm partially purified protein was stable at 4°C and at 25°C and demonstrated an unusual phenomenon of increased activity after incubation at both temperatures and at 40°C. At 40°C, the β-1,4-glucan cellobiohydrolase activity had started dropping after 1hr and after 24 hrs the activity level was back to the zero time levels. While at 50°C, the activity had dropped by 50% of the zero time activity after 5hrs and was close to the detectable limit of the assay by 24 hrs. At 80°C, β-1,4-glucan cellobiohydrolase activity in Band 3mm was below the detectable limit of the assay after 2 minutes. Band 3mm demonstrated Avicelase, carboxymethylcellulase and β-1,4-glucan cellobiohydrolase activity. Endo-1-4-β- glucanase and filter paper activity, which was present in the unpurified *Cadophora malorum* 182 culture supernatant, but was not present in the partially purified Band 3mm. Mannanase and xylanase activity was present in Band 3mm but there was no amylase or pectinase activity which was present in the unpurified *Cadophora malorum* 182 culture supernatant.

Band 3mm contained protease activity. Table 6.5 illustrates the level of enzyme activity in the culture supernatant before and after purification.

Table 6.5: Comparison of enzyme activity in *Cadophora malorum* 182 culture supernanant and after purification in Band 3mm.

Enzyme activity	Culture supernant of *Cadophora malorum* 182	Band 3mm
Filter paper activity	19.65	0
Avicelase	3.72	1.34
CMCase	76.35	0.48
Endo-1-4-β- glucanase	14.04	0
β-1,4-glucan cellobiohydrolase	0.117	0.028
Amylase	104	0
Pectinase (Polygalacturonic acid)	2.01	0
Pectinase (Pectin)	75.1	0
Xylanase	78.85	0.86
Mannanase	99.37	0.36
Proteinase	2.24	0.48

From these results a better understanding of the cellulase components of Antarctic fungal isolate *Cadophora malorum* 182 has been established. Perhaps most importantly, the thesis research has demonstrated many of the proteins accumulated extracellularly by the fungal isolate were glycosylated, demonstrated that even though the isolate was cultured at 4°C, the dominant β-1,4-glucan cellobiohydrolase activity was apparently optimal under the conditions of the assay at 60°C. This knowledge aids in increasing the understanding of these fungi and how they are surviving and proliferating in the cold Antarctic environment.

Chapter 7 Laboratory wood decay and colonisation study

7.1 Introduction

In natural environments, fungi grow differently from when they are grown on artificial media or on synthetic cellulose sources in a laboratory. This is due to differences between the *in vivo* versus *in vitro* environment such as differences in surface phenomena, moisture content, physical structure and chemical composition of the substrate and different nutrient environment. *In vitro* studied do not consider totally the fungal response to the wood product in the same conditions as found in nature, but does make it possible to conduct laboratory based experiments where fungi are exposed to wood but under more controlled conditions.

Wood inhabiting fungi play a major role in the decomposition of wood and the reduction of complex structures to simpler forms. The degradation of wood takes place in a number of clearly defined stages, each of which is the result of the activity of particular groups of microorganisms. The success of the process is in part governed by moisture content. Often the initial colonisers are bacteria which can predispose wood to other scavenger organisms such as primary moulds and sapstain fungi. The "true" wood decay fungi follow with white and brown rot fungi dominating with their characteristic decay patterns. Finally the cellulytic moulds complete the degradation (Levy and Eveleigh, 1987).

In living trees cracks in wood form pathways for wood inhabiting fungi to overcome the reaction zone, the region where the trees natural defence mechanisms accumulate in the hope of preventing infection (Schwarze *et al*. 2000) and for wood "in service" the largest fungal, group that degrade wood are Basidiomycetes

This chapter describes the results of a laboratory based fungal wood decay and colonisation study. Firstly, a review of the literature discusses research investigating decomposition in Antarctica and the decay potential of the fungi used in this study. The materials and methods, results and discussion on the decay

and colonisation potential of five Antarctica fungal isolates from structural wood of the Historic Huts are then described.

7.2 Hypothesis Aims and Objectives

This chapter focuses on the potential decay of wood by fungal isolates cultured from wood found in Antarctica. The primary hypothesis of this portion of thesis research was that wood inhabiting fungi isolated from the Historic Huts were would be able to colonise and degrade wood.

The main aim of this aspect of the thesis research was to gain an understanding of the potential for wood inhabiting fungi to decay wood at psychrophilic and mesophilic temperatures.

The objective addressed as follows:

- Show growth and decay capabilities of selected fungal isolates from structural wood at different temperatures.

7.3 Literature review

7.3.1 Decomposition in Antarctica

Pugh and Allsopp (1982) investigated the distribution of microfungi especially cellulytic and keratinophilic fungi on Signy Island, Antarctica. Samples were collected from a variety of habitats and substrates including herbs, mosses, soil, keratinous material, air and miscellaneous materials (seal skin, skua pellets, nest material and seaweeds). Fungi capable of growing on cellulose agar were isolated from vascular plants such as; *Colobanthus quitensis* (leaves, litter and roots) and *Deschampsia antarctica* (leaves and litter) and mosses *Polytrichum alpestre*, *Calliergon sarmentosum* and *Calliergon sarmentosum* (live green leaves for all three mosses and also dead brown leaves for *Calliergon sarmentosum*) and seaweed. Sterile mycelia were the most common isolated group according to the literature and *Chrysporium pannorum* was the most abundant fungal species isolated. *Chrysporium pannorum* was isolated more frequently on cellulose agar than malt agar and demonstrated cellulytic activity by clearing zones being visible underneath the colonies.

7.3.2 Decay potential of the fungi used in this study

Cadophora spp. are recognised soft rot fungi and can cause cavities in the secondary cell wall of wood. Blanchette *et al*. (2004) identified *Cadophora* sp. as causing decay in wood in contact with the ground at the Historic Hut sites in Antarctica. Soft rot is visualised as chains of cavities within the secondary cell wall of the wood cell. They are biconical and cylindrical voids which form along the microfibrillar structure of the secondary cell wall and have a spiral orientation. The soft rot attack is localised to the secondary cell wall with no evidence of decay in the middle lamella and the other layers of the cell wall. Soft rot attack is divided into two types, type 1 which creates cavities in the cell wall and type 2 which creates an erosion of the entire secondary cell wall directly below the fungal hyphae (Eriksson *et al.*, 1990). *Cadophora* sp. isolated from the Antarctic Historic Huts was shown to decay *Betula* and *Populus* wood blocks at 24°C after 6 months incubation by Blanchette *et al.* (2004).

Cladosporium spp. are very common soil and wood inhabiting fungi. They are commonly isolated from air samples from all regions of the world (Marshall, 1997). They are known to cause decay in fruit, vegetables and processed food (Singh *et al*., 1983; Sugar and Powers, 1986; Wang, 2003). *Cladosporium* spp. are often the first colonisers of leaf and decay litter (Rai, 1976) and are not recognised as major decay causers of wood but they are often isolated in the company of wood decay fungi and can cause discolouration of the wood.

Like *Cladosporium* spp., *Penicillium* spp. are recognised as causing decay in fruit, vegetables and processed food (Barkai-Golan, 1973; Sanderson, 1995; Amiri and Bompeix, 2005). *Pencillium* is a surface mould that has been attributed with causing colouration of wood. Ejechi (1997) reported the use of a *Penicillium* sp. to retard the decay of obeche *(Triplochiton scleroxylon)* wood in the field for 11 months (January-November) covering dry and wet seasons in a tropical environment. A study by Rice *et al.* (2006) showed that both *Penicillium* sp. and *Geomyces* sp. isolated by bait traps from peat bogs caused weight loss of *Sphagnam fuscum* leaves, and both are thought to be part of a consortium of fungi which are involved in the decomposition process in peat bogs. Like *Cladosporium* and *Penicillium*, *Geomyces* spp. are soil inhabiting fungi, and are thought to be involved in the decay and decomposition process. Both *Penicillum* spp. and

Geomyces spp. are not recognised as wood decay fungi but are often isolated from decayed wood in the presence of decay fungi.

7.4 Materials and methods

7.4.1 Media and Reagents

The following medium and reagents were used in the experiments related to this chapter's objectives:

Malt Yeast Extract Agar (YM) - 1.5% malt extract, 0.2% yeast extract, 1.8% agar in distilled water.

Formalin Acetic Acid (FAA) fixative- 50 ml absolute ethanol (95%), 5 ml glacial acetic acid, 10 ml formaldehyde (37-40%) and 35 ml distilled water.

7.4.2 Wood wafer preparation and inoculation with fungi

Picea sp. (spruce) wood wafers were inoculated with the following five fungi (one set of three wafers per temperature per fungal isolate): *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805, in order to determine the ability of these fungi to decay wood at psychrophilic and mesophilic temperatures.

The method of Blanchette *et al.* (2004) was used for the inoculated wafer experiment with new wood provided by conservation carpenters (Event K441) engaged by Antarctic Heritage Trust and collected from Cape Royds in January 2006. Spruce was sourced in the United Kingdom, shipped to New Zealand where it was kiln dried prior to being taken to Antarctica and used for repairs to the *Nimrod* Historic Hut by the conservation carpenters. The spruce was reimported back to New Zealand under MAF Permit Number 2005027174. Wafers measuring 15x15x2 mm were made at University of Waikato by cutting the wood with a sharp blade. The wafers were soaked in distilled water for 1hr and autoclaved at 121°C for 20 minutes. Cultures were allowed to grow on YM agar plates 2°C, 4°C, 10°C, 15°C, or 25°C and once actively growing fungi covered a significant portion of each plate (required 8-26 days to achieve growth requirement) three sterile wood wafers were added and the incubation continued at 2°C, 4°C, 10°C, 15°C, or 25°C. Three sterile wood wafers were placed on the surface of a non

inoculated YM agar plate as a control and were incubated in parallel with the fungal inoculated wood wafers. The Petri dishes containing wood wafer on the fungi were sealed with parafilm and plates were incubated at the same temperatures mentioned above. Thin tangential sections were aseptically removed with a razor blade from one of the wafers in each plate and observed after 6 and 13 weeks. Sections were examined using light microscopy Olympus BX40 (Japan) for evidence of fungal mycelium.

7.4.3 Scanning Electron Microscopy

After 13 weeks, one wafer from each fungal isolates, at each temperature set, was removed for scanning electron microscopy investigation. Wood wafers were placed in a universal bottle and FFA fixative added, and wafers were left in fixative until sectioned. Transverse sections of the spruce wafers were cut using a sledge microtome to create a clean surface to view with SEM. The samples were then mounted onto an aluminium stub with double-sided carbon tape and chromium-coated in a sputter coater (Emitech, Kent, England). The samples were examined with a Jeol (Japan) Field Emission Scanning Electron Microscope.

7.5 Results

7.5.1 Light microscopy

After 6 and 13 weeks all wood wafers examined showed evidence of fungal hyphae on the surface of the wood wafer but no hyphae could be seen in sections taken below the first few rows of wood cells.

7.5.2 Laboratory decay study

Wood that had not been inoculated with fungi showed by SEM wood cells that are uniform in shape and cell wall thickness as shown in Figure 7.1.

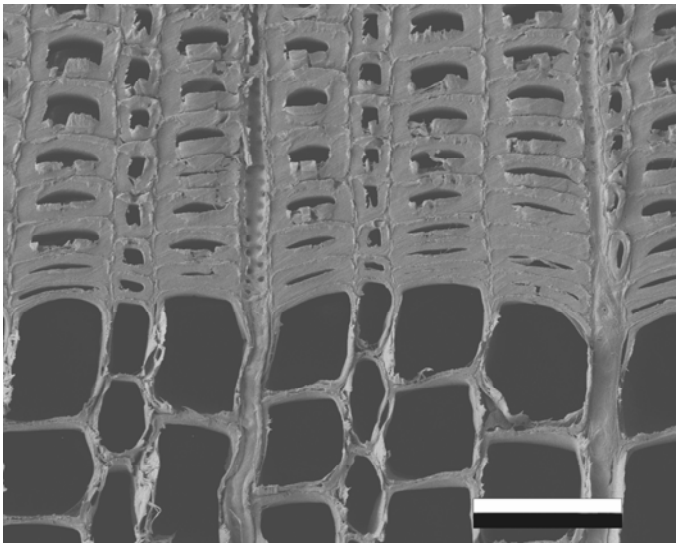

Figure 7.1: Micrograph of control wood *Picea* sp. not inoculated with fungi, latewood in top of figure, earlywood in bottom of figure. Magnification 500x, Micron bar 50µm.

Small cavities were seen in the secondary cell wall indicating the type 1 form of soft rot fungal activity in wafers inoculated with the two *Cadophora malorum* isolates, 182 and 242, and no soft rot decay was seen in wafers inoculated with *Penicillium roquefortii* 405, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805. Evidence of decay was seen in wafers inoculated with *Cadophora malorum* 182, incubated at 25°C and at 10°C as can be seen in Figure 7.2 and 7.3 where the decay cavities in the secondary cell wall are high lighted by the yellow arrow. Fungal hyphae (highlighted by the white arrows) can be seen in a number of cells as strands crossing the lumen and in the decay cavities. The number of hyphae visible in the 10°C micrograph is greater than at 25°C. This is due to greater hyphal growth on the wafer surface as seen in Figure 7.3 rather than further into the wafer as seen in the wafer incubated at 25°C (Figure 7.2). No evidence of decayed wood cells were visible in wafers incubated at 15°C, 4°C or 2°C.

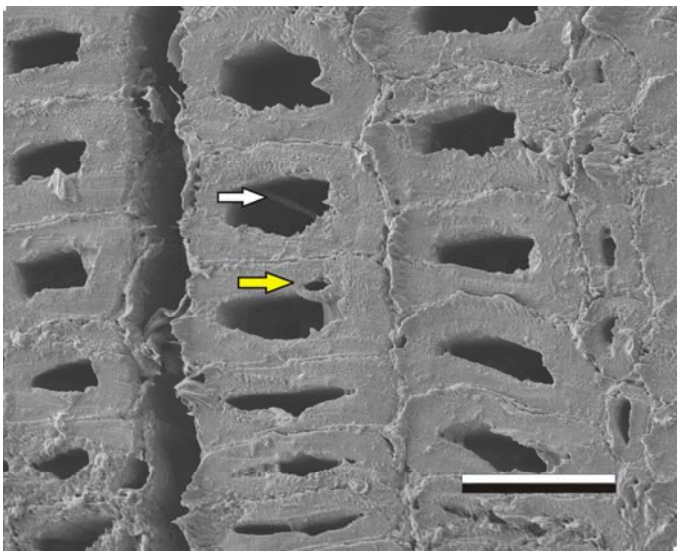

Figure 7.2: Micrograph of *Cadophora malorum* 182 growing on *Picea* sp. (spruce) wafers at 25°C. The yellow arrow indicates soft-rot cavities and the white arrow indicates fungal hyphae. Magnification 1300x, Micron bar 20µm.

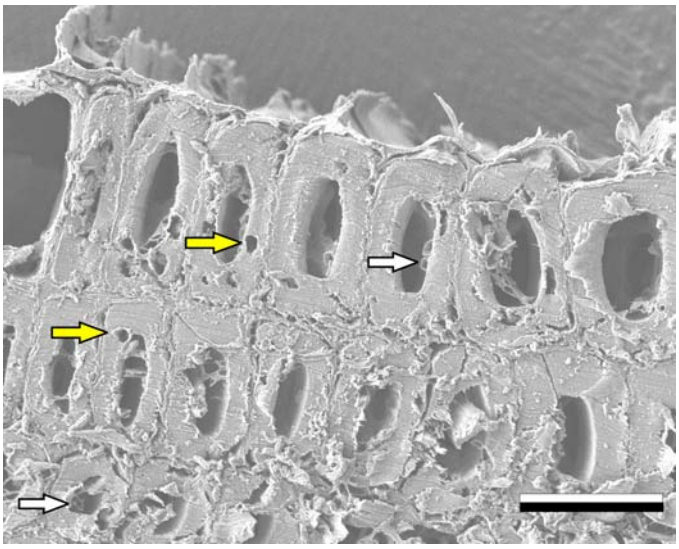

Figure 7.3: Micrograph of *Cadophora malorum* 182 growing on *Picea* sp. wafers at 10°C. The yellow arrows indicate soft-rot cavities and the white arrows indicate fungal hyphae. Magnification 1200x, Micron bar 20µm.

Evidence of early stages of decay was seen in wafers inoculated with *Cadophora malorum* 242 when incubated at 25°C but not at the other temperatures tested. Figure 7.4 shows typical soft-rot cavities formed in the secondary cell wall and are highlighted by the yellow arrow. Fungal hyphae (highlighted by the white arrows) can be seen in a number of cells as strands crossing the cell lumen. Much of the cell damage seen in this micrograph is due to mechanical damage caused by the microtome knife when preparing the section.

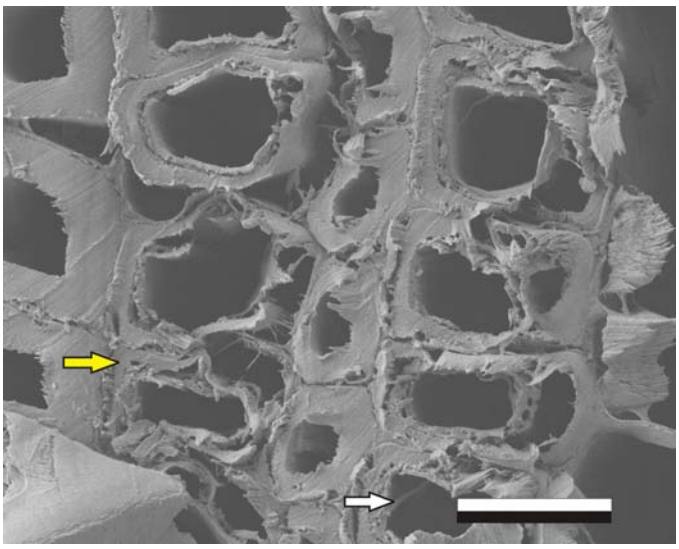

Figure 7.4: Micrograph of *Cadophora malorum* 242 growing on *Picea* sp. (spruce) wafers at 25°C. The yellow arrow indicates soft-rot cavities, and the white arrow indicates fungal hyphae. Magnification 1300x, Micron bar 20µm.

7.5.3 Colonisation of spruce wafers by Antarctica fungi

As all the fungi in the study were isolated from wood which had been surface sterilised (see Section 3.4.3.1), in addition to looking at the ability of the Antarctic fungi to decay the spruce wafers, the ability of the fungi to colonise the wood wafers was also investigated.

Cadophora malorum 182 colonised the wood at all temperatures tested and hyphae were seen in the early and latewood at all temperatures. Only visual quantification was conducted and from that it was apparent that colonisation of the latewood was not prolific as in the earlywood, also colonisation was greater on the outer portion of the wafer when compared to the inner.

Figures 7.5 to 7.9 show *Cadophora malorum* 182 fungal hyphae colonising the earlywood of spruce wafers at 2°C, 4°C, 10°C, 15°C and 25°C. The hyphae can be seen as strands crossing the cell lumen. Figures 7.2, 7.3, and 7.10 shows *Cadophora malorum* 182 fungal hyphae colonising the latewood of the spruce wafers at 2°C, 10°C and 25°C. In Figure 7.5 there are large numbers of hyphae due to the micrograph being taken close to the wafer edge where hyphae were observed macroscopically. Some of the hyphae appear to be surrounded by mucilaginous material but not as much as was seen when the fungus and wood wafer were incubated at 4°C.

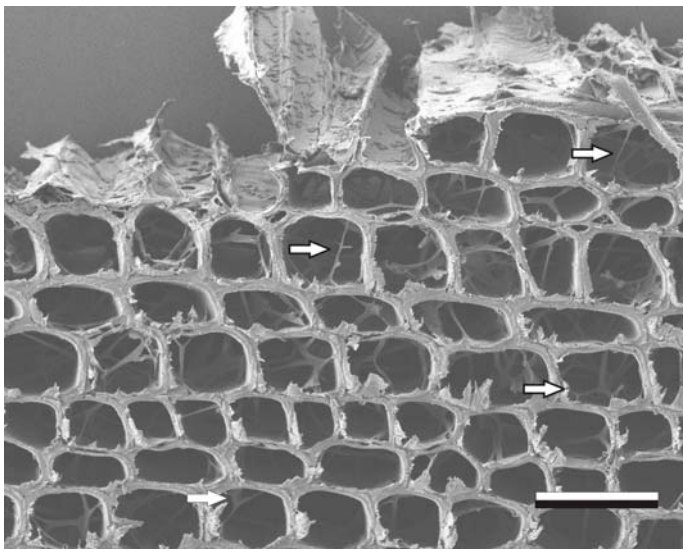

Figure 7.5: Micrograph of *Cadophora malorum* 182 colonising the earlywood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50 µm.

In Figure 7.6 *Cadophora malorum* 182 hyphae are surrounded by a mucilaginous matrix that was only seen when the fungi and wood wafers were incubated at temperatures below 4°C. The large damaged region visible in the lower section of the micrograph was done when the wood wafer was cut prior to inoculation with fungi; the fungal hyphae have managed to grow across this damage.

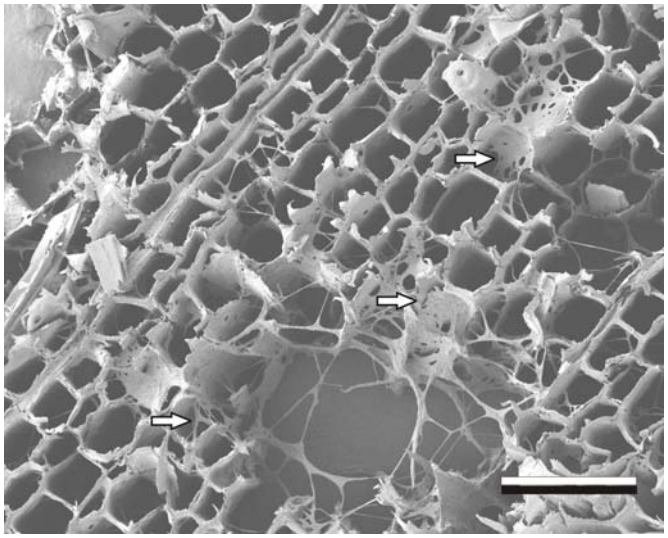

Figure 7.6: Micrograph of *Cadophora malorum* 182 colonising the earlywood of *Picea* sp. wafers at 4°C. The white arrows indicate fungal hyphae. Magnification 230x, Micron bar 100 µm.

The *Cadophora malorum* 182 fungal hyphae in Figure 7.7 starts along the surface and spreads into the wood cells via a damaged area on the surface of the wafer. No hyphae can be seen in the latewood on the right side of the micrograph.

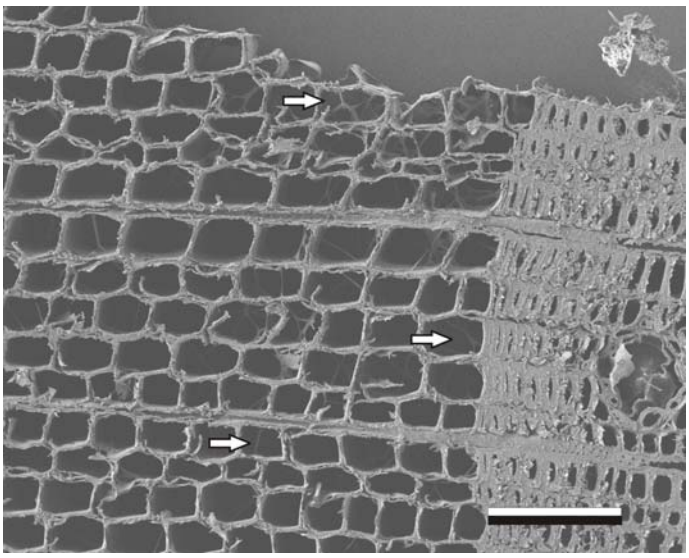

Figure 7.7: Micrograph of *Cadophora malorum* 182 colonising earlywood of *Picea* sp. wafers at 10°C. The white arrow indicates fungal hyphae. Magnification 220x, Micron bar 100µm.

When Cadophora malorum 182 was inoculated onto wood and incubated at 15°C, there was very little fungal hyphae seen both on the surface of the wood wafer and inside the wood cells as can be seen in Figure 7.8.

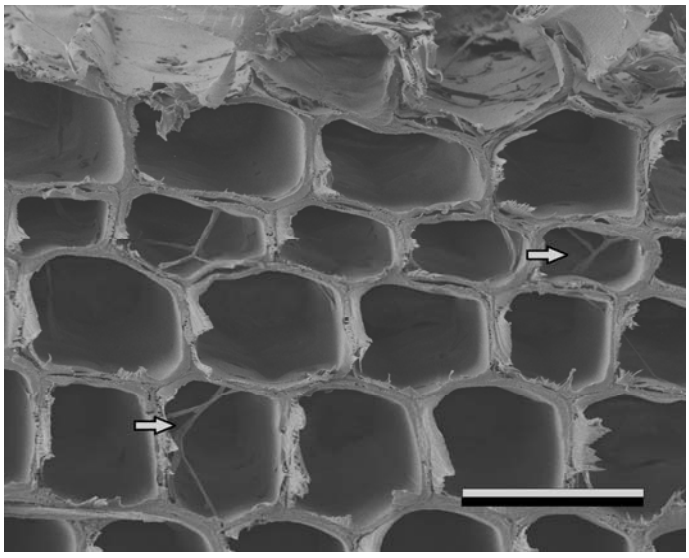

Figure 7.8: Micrograph of *Cadophora malorum* 182 colonising earlywood of *Picea* sp. wafers at 15°C. The white arrows indicate fungal hyphae. Magnification 600x, Micro bar 50µm.

When *Cadophora malorum* 182 was inoculated onto wood wafers and incubated at 25°C, fungal hyphae were seen both on the surface of the wood wafer and inside the wood cells as can be seen in Figure 7.9. The hyphae can be seen crossing from one side of the cell lumen to the other.

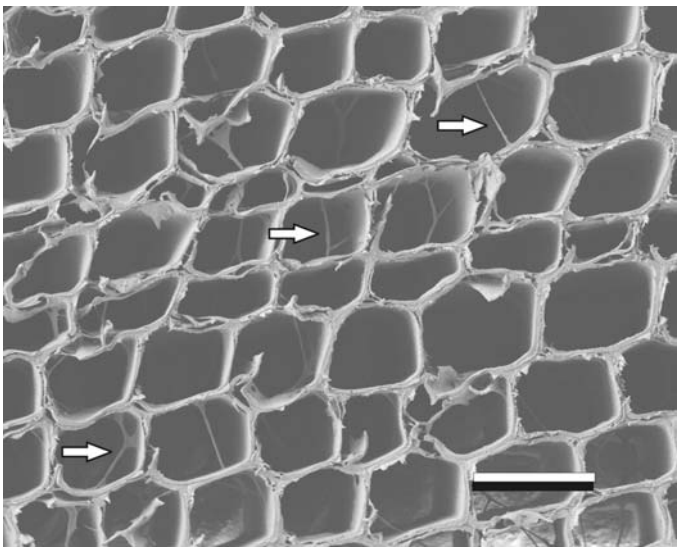

Figure 7.9: Micrograph of *Cadophora malorum* 182 colonising earlywood of *Picea* sp. wafers at 25°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50µm.

When *Cadophora malorum* 182 was inoculated onto wood and incubated at 2°C, fungal hyphae were seen in the latewood (Figure 7.10) as well as the earlywood (Figure 7.5). In Figure 7.10 fungal hyphae can be seen within the cells and their presence indicated that given time decay cavities could develop.

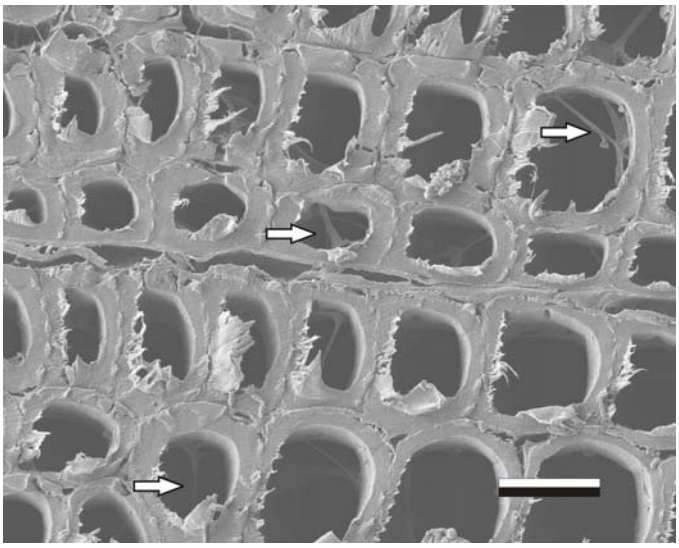

Figure 7.10: Micrograph of *Cadophora malorum* 182 colonising latewood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 800x, Micron bar 20µm.

Cadophora malorum 242 colonised the wood at all temperatures tested with fungal hyphae present in both the early and latewood. The colonisation of the latewood was not as prolific as the earlywood. Figures 7.11 to 7.14 shows *Cadophora malorum* 242 fungal hyphae colonising the earlywood of the spruce wafers at 2°C, 4°C, 10°C and 15°C respectively and Figures 7.4 and 7.15 shows *Cadophora malorum* 242 fungal hyphae colonising the latewood of the spruce

wafers at 10°C and 25°C. Extensive fungal colonisation can be seen in Figure 7.11 as well as mucilaginous material. Greater numbers of hyphae were found near the wafer surface when compared to the inner portion of samples

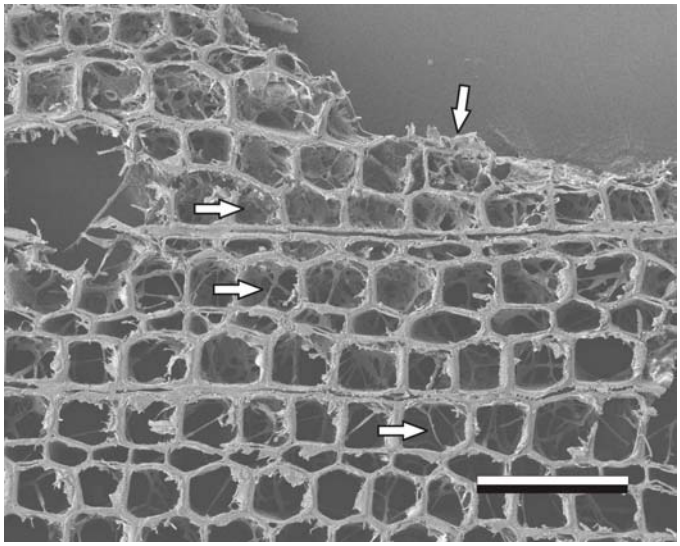

Figure 7.11: Micrograph of *Cadophora malorum* 242 colonising earlywood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 220x, Micron bar 100µm.

When *Cadophora malorum* 242 was incubated at 4°C, the hyphae were less visible but appeared to have penetrated further into the wood wafer when compared with other temperatures. Figure 7.12 shows fungal hyphae growing across several cell lumen and the cracks seen in this section are due to mechanical damage caused during the sectioning process and are not biological damage.

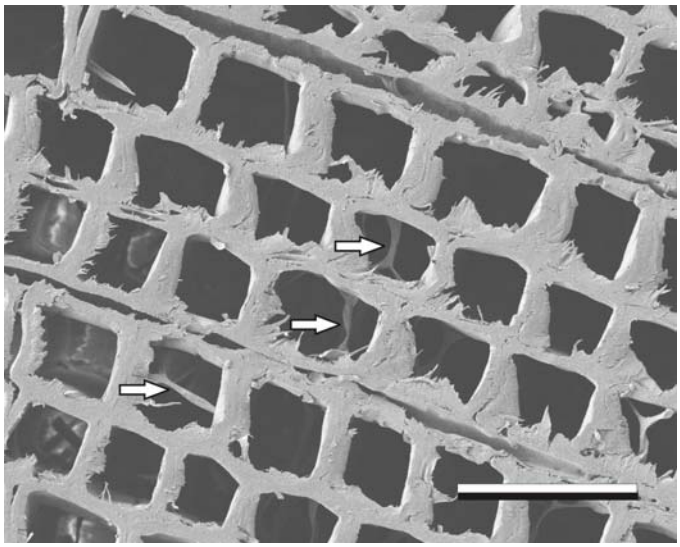

Figure 7.12: Micrograph of *Cadophora malorum* 242 colonising earlywood of *Picea* sp. wafers at 4°C. The white arrows indicate fungal hyphae. Magnification 600x, Micron bar 50µm.

Figure 7.13 shows fungal hyphae growing at 10°C and colonising wood cells.

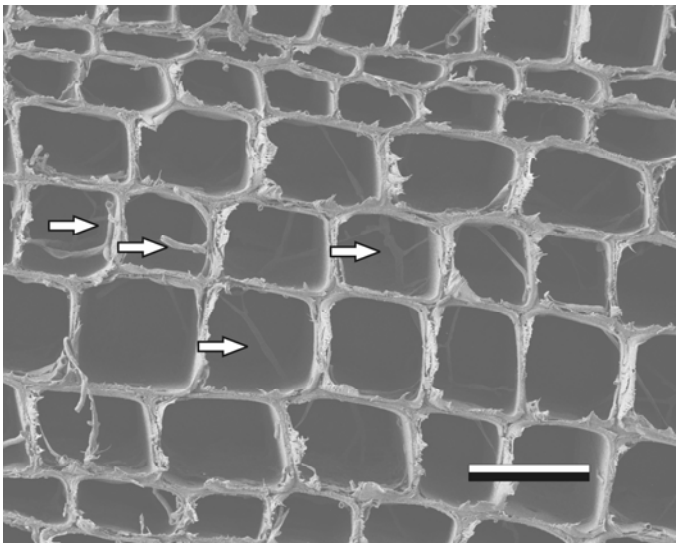

Figure 7.13: Micrograph of *Cadophora malorum* 242 colonising the earlywood of *Picea* sp. wafers at 10°C. The white arrows indicates fungal hyphae. The arrow on the far left indicate fungal hyphae growing along the wood cell wall. Magnification 500x, Micron bar 50µm.

Figure 7.14 is a micrograph with the image taken towards the middle of the wood wafer so the amount of fungal hyphae seen is less than what is observed toward the surface of the wood wafer.

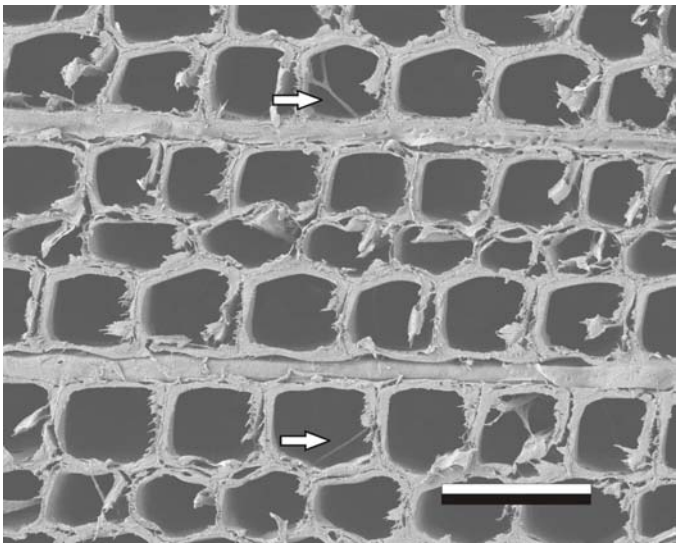

Figure 7.14: Micrograph of *Cadophora malorum* 242 colonising earlywood of *Picea* sp. wafers at 15°C. The white arrows indicate fungal hyphae. Magnification 500x, Micron bar 50µm.

Cadophora malorum 242 colonised the latewood as well as the earlywood. In Figure 7.15 hyphae can be seen growing both across the cell lumens and along the cell wall. No soft rot decay can be seen but the presence of fungal hyphae indicates that given time there is a potential for decay to occur.

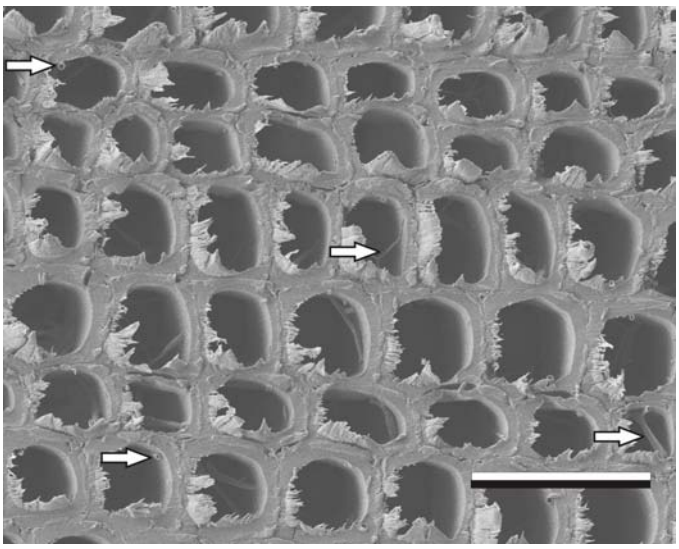

Figure 7.15: Micrograph of *Cadophora malorum* 242 colonising latewood of *Picea* sp. wafers at 10°C. The white arrows indicate fungal hyphae. Magnification 600x, Micron bar 50µm.

When the micrograph images in Figures 7.11 to 7.14 showing colonisation of earlywood were compared with Figures 7.4 and 7.15 colonisation of latewood the amount of colonisation seen visually in the micrographs of the earlywood region was similar except at 2°C (where the micrograph was taken at the surface of the wafer) to the amount of colonisation visualised in the latewood region of the wafers.

Penicillium roquefortii 405 colonised the wood but only on the surface and the first few layers of wood cells below the surface at all temperatures tested. Fungal hypha were seen in the earlywood of all samples but colonisation was more prolific at 2°C than at 25°C. There was no visible fungal mycelium in the latewood cells when viewed using the SEM. Figure 7.16 to 7.18 shows *Penicillium roquefortii* 405 fungal hyphae colonising the earlywood of the spruce wafers at 2°C, 10°C and 25°C respectively.

Figure 7.16 shows *Penicillium roquefortii* hyphae growing across the cell lumen at 2°C but are rather scarce and are surround by mucilaginous material as seen with both *Cadophora* isolates grown at the same temperature. The surface of the wood wafer has greater colonisation and can be seen in the bottom left of the micrograph.

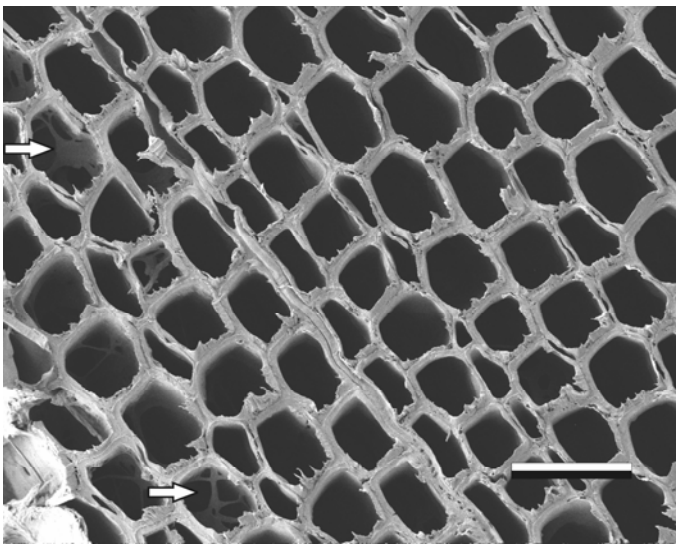

Figure 7.16: Micrograph of *Penicillium roquefortii* 405 colonising earlywood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 500x, Micron bar 50µm.

Figure 7.17 shows fungal hyphae growing throughout the wood cells. There were large masses of hyphae on the surface of the wood wafer that were smeared across the cut surface of this section.

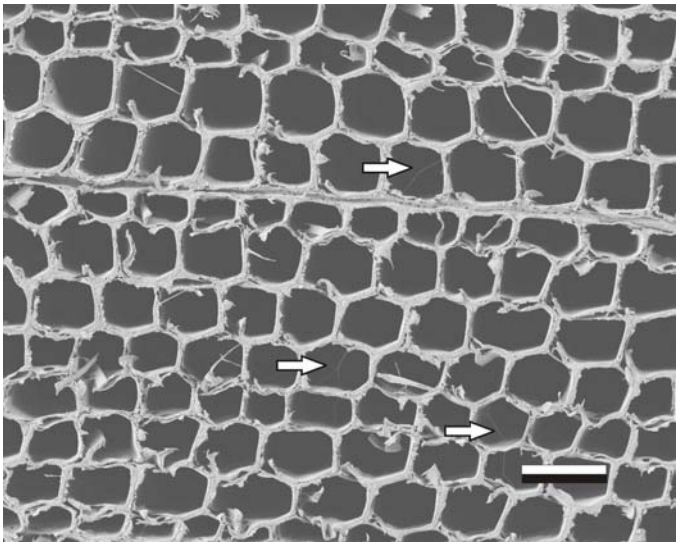

Figure 7.17: Micrograph of *Penicillium roquefortii* 405 colonising earlywood of *Picea* sp. wafers at 10°C. The white arrows indicate fungal hyphae. Magnification 300x, Micron bar 50µm.

Penicillium roquefortii mycelium and spores can be seen only growing on the surface of the wood wafer when incubated at 25°C in Figure 7.18. The hyphae do not appear to have penetrated into the wood cells below the surface of the wood wafer.

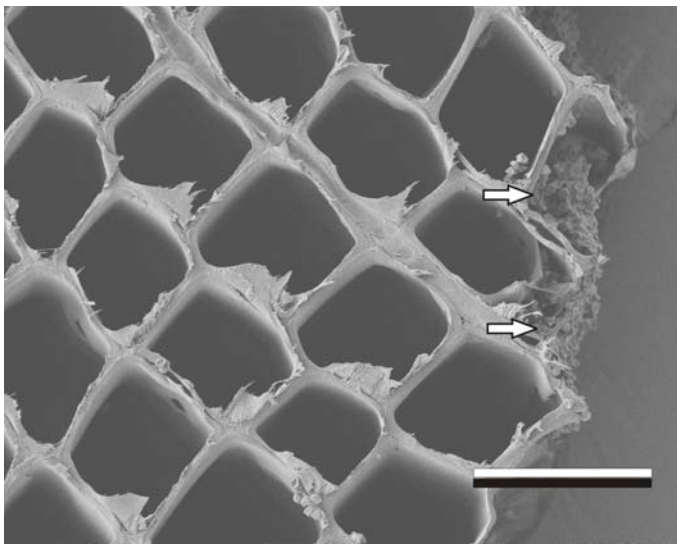

Figure 7.18: Micrograph of *Penicillium roquefortii* 405 grown on *Picea* sp. wafers at 25°C. The white arrows indicate fungal hyphae. Magnification 600x, Micron bar 50µm.

When the micrograph images in Figures 7.16 to 7.18 showing colonisation of earlywood were compared, the amount of colonisation and fungal hyphae seen visually increased as the temperature increased.

Cladosporium oxysporum 805 colonised only the wood cells on the top of the wafer and the first few layers of wood cells below the surface at all temperatures tested. Fungal hyphae were seen in the earlywood at all temperatures and colonisation of the earlywood was more prolific at 25°C than at 2°C. There was no colonisation observed of the latewood in any of the samples.

Figures 7.19 to 7.23 shows *Cladosporium oxysporum* 805 fungal hyphae colonising the earlywood of the spruce wafers at 2°C, 4°C, 10°C, 15°C and 25°C. Figure 7.19 shows primarily *Cladosporium oxysporum* hyphae growing on the surface of the wood wafer and with only limited hyphae growing in the third layer of wood cells.

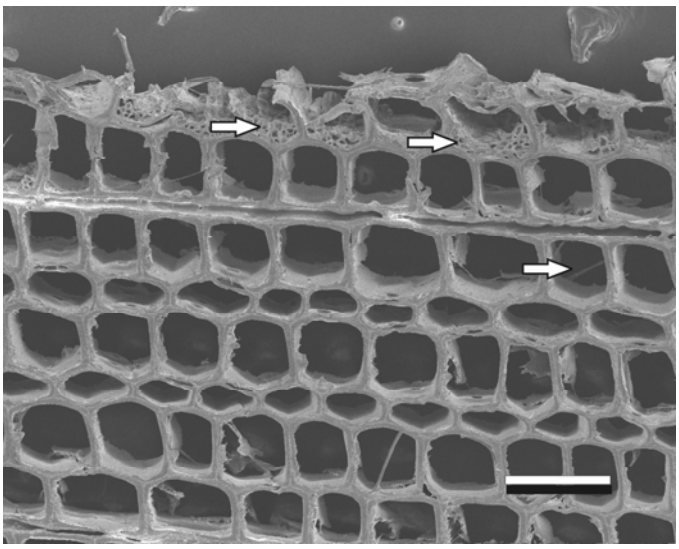

Figure 7.19: Micrograph of *Cladosporium oxysporum* 805 colonising earlywood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 350x, Micron bar 50µm.

Fungal hyphae can be seen on the surface of the wood wafer in Figure 7.20. This micrograph is an image at the end of the wood wafer and the fungus appears to have penetrated further into the wood wafer via a crack that has formed through to the latewood which is in the bottom right of the micrograph.

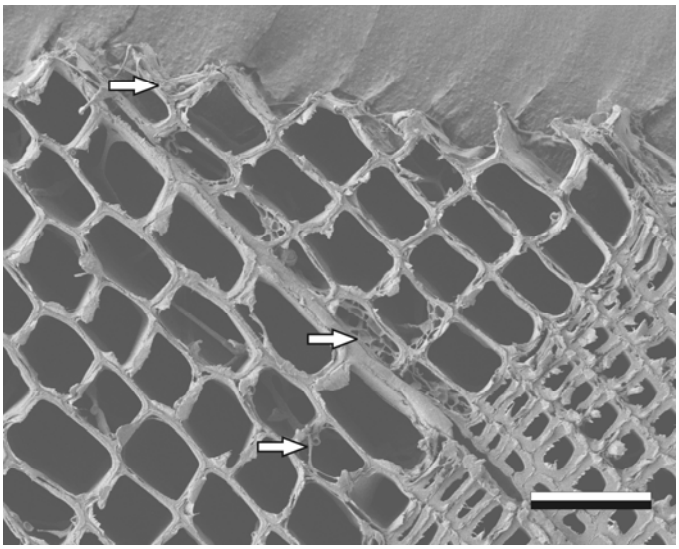

Figure 7.20: Micrograph of *Cladosporium oxysporum* 805 colonising earlywood of *Picea* sp. wafers at 4°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50µm.

Cadosporium oxysporum 805 fungal hyphae and spores can also be seen in Figure 7.21 be visualised in a region of structural damage caused by the sectioning process. Similar to other incubation temperatures of this fungus growth was limited to the surface and no evidence of decay was apparent.

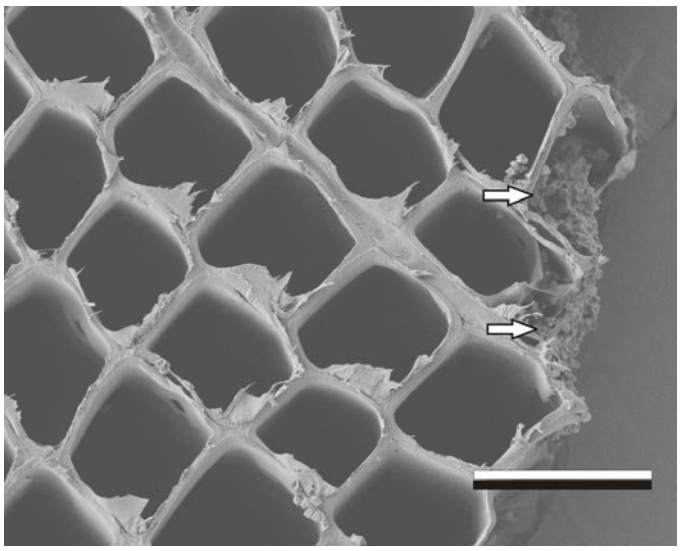

Figure 7.21: Micrograph of *Cladosporium oxysporum* 805 colonising earlywood of *Picea* sp. wafers at 10°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50µm.

Figure 7.22 shows a large area of fungal mycelium on the surface of the wood wafer, with no visible hyphae penetrating wood cells.

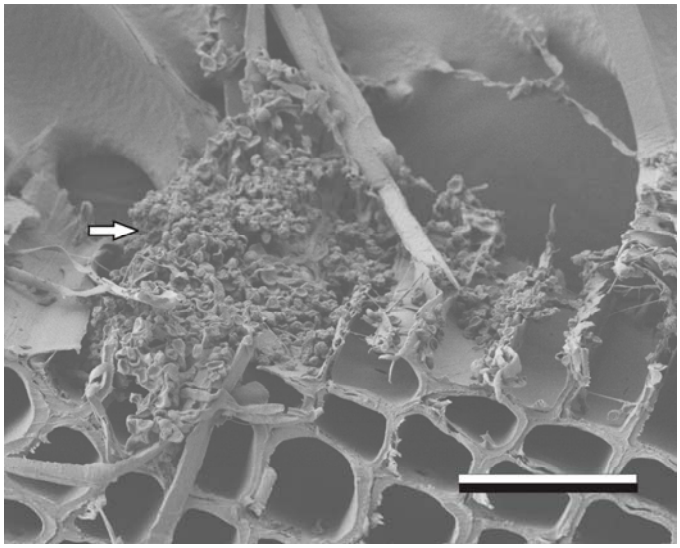

Figure 7.22: Micrograph of *Cladosporium oxysporum* 805 colonising the earlywood of *Picea* sp. wafers at 15°C. The white arrow indicates fungal hyphae. Magnification 600x, Micron bar 50µm.

At 25 °C the *Cladosporium oxysporum* hyphae were observed on the surface of the wood wafer, down to the fifth layer of cells and between the wood cells (Figure 7.23). Penetration of the wood wafer by the fungi appears to be due to the fungal hyphae growing into and through damaged areas caused during the wafer cutting where wood cells have separated from each other.

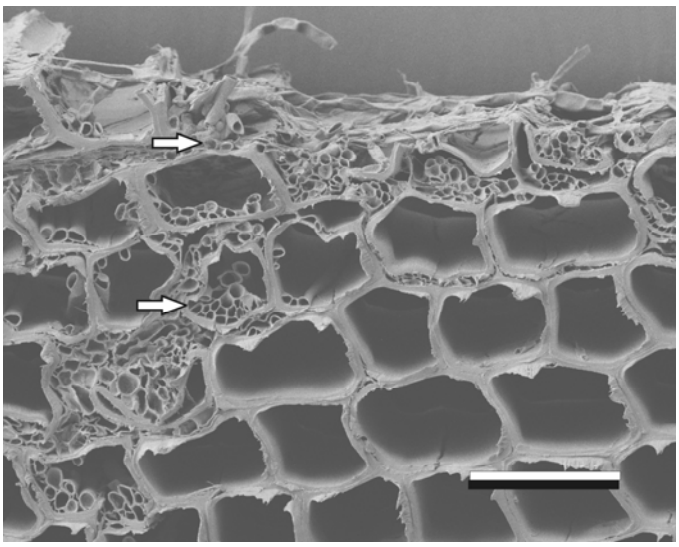

Figure 7.23: Micrograph of *Cladosporium oxysporum* 805 colonising earlywood of *Picea* sp. wafers at 25°C. The white arrows indicate fungal hyphae. Magnification 500x, Micron bar 50µm.

When the micrograph images in Figures 7.19 to 7.23 showing colonisation of earlywood were compared, the amount of colonisation and fungal hyphae seen visually in the micrographs increased with increasing temperature. In Figures 7.20, 7.21 and 7.23 where the wafer had been damaged the fungal hyphae had colonised further into the wafer at 25°C the hyphae had reached the fifth layer of cells.

Geomyces sp. 824 colonised the wood but only on the surface of the wafer and first few layers of wood cells below the surface. This was observed at all temperatures tested with most of the fungal hyphae observed in the earlywood. Colonisation of the earlywood was more prolific at 2°C than at 25°C. There was no colonisation observed of the latewood cell lumen in any of the samples.

Figures 7.24 to 7.26 shows *Geomyces* sp. 824 fungal hyphae colonising the earlywood of the spruce wafers at 2°C, 4°C and 10°C. Figure 7.27 shows *Geomyces* sp. 824 fungal hyphae lying on the surface of the latewood of the spruce wafers at 25°C.

At 2°C the fungal hyphae can be seen on the surface of the wood wafer, with a single hyphae seen in the sixth layer of wood cells. Most of the hyphae seen in Figure 7.24 were smeared across the section when the section was cut as they appear to be are lying across the top of the section.

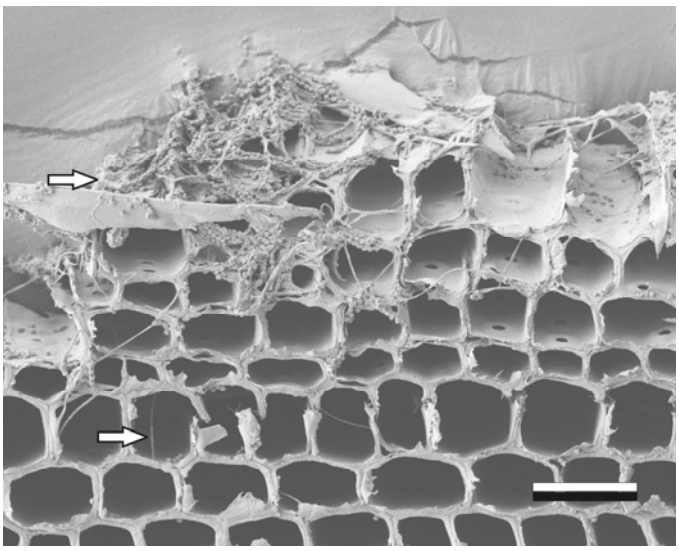

Figure 7.24: Micrograph of *Geomyces* sp. 824 colonising the earlywood of *Picea* sp. wafers at 2°C. The white arrows indicate fungal hyphae. Magnification 350x, Micron bar 50µm.

Figure 7.25 shows a few *Geomyces* sp. hyphae crossing the wood cell lumen after 13 weeks incubation at 4°C.

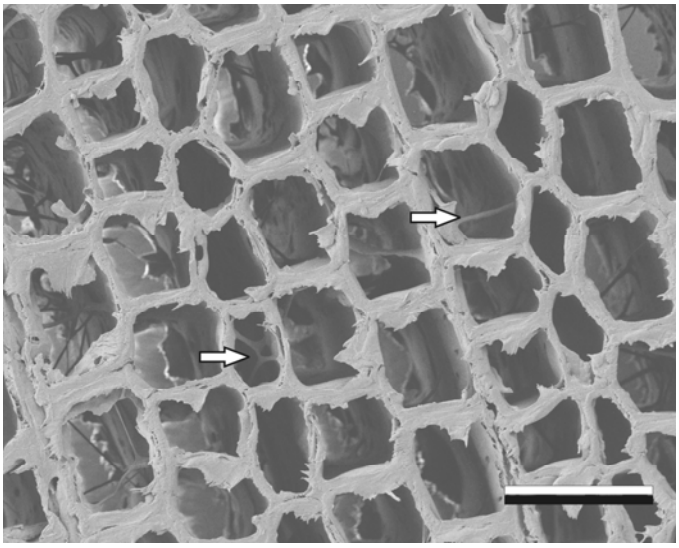

Figure 7.25: Micrograph of *Geomyces* sp. 824 colonising earlywood of *Picea* sp. wafers at 4°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50µm.

Fungal hyphae and spores on the surface of the wood wafer with no hyphae observed in the wood cells in Figure 7.26 when *Geomyces* sp. were incubated at 10°C.

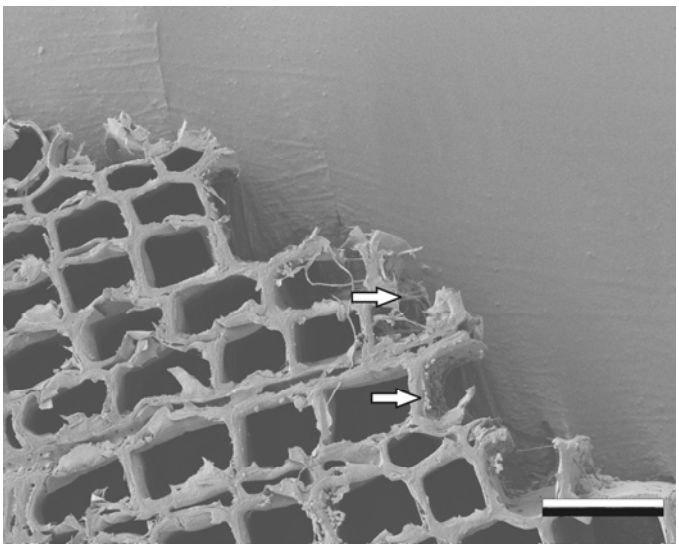

Figure 7.26: Micrograph of *Geomyces* sp. 824 colonising earlywood of *Picea* sp. wafers at 10°C. The white arrows indicate fungal hyphae. Magnification 400x, Micron bar 50µm

Geomyces sp. hyphae and spores were seen at 25°C on the surface of the wood wafer in a region of latewood but just as with the earlywood there was no penetration of the wood cells below the surface (Figure 7.27).

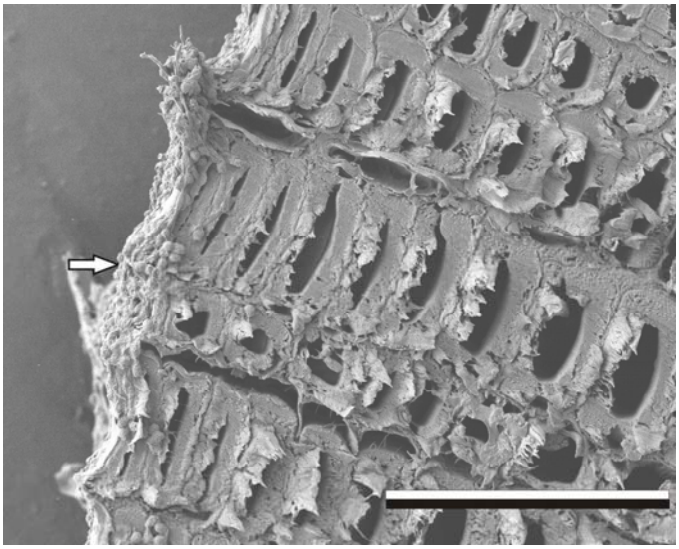

Figure 7.27: Micrograph of *Geomyces* sp. 824 lying on the surface of the latewood of *Picea* sp. wafers at 25°C. The white arrow indicates fungal hyphae. Magnification 400x, Micron bar 50µm.

When the micrograph images in Figures 7.24 to 7.26 showing colonisation of earlywood were compared, the amount of colonisation and fungal hyphae seen visually in the micrographs decreased with the temperature of incubation with the largest amount of fungal hyphae seen at 2°C.

7.6 Discussion

Fungal isolates *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 used in this decay and colonisation study were originally isolated from structural wood inside the *Terra Nova* Hut while isolate *Geomyces* sp. 824 was isolated from timber outside the *Discovery* Hut.

In this PhD thesis research, using wafers of spruce, both *Cadophora malorum* isolates 182 and 242 produced evidence of early stage soft-rot decay cavities at 25°C and at 10°C for *Cadophora malorum* 182. A small number of typical type 1 soft rot cavities were seen in the secondary cell wall, these results are consistant with the decay observed on *Betula* and *Populus* wood blocks at 24°C for 6 months by Blanchette *et al*. (2004). In this PhD thesis research fungal hyphae colonised the latewood but no decay cavities were seen at 2°C and 4°C. This colonisation of the latewood cells indicated that if the experiment was allowed to continue longer than 13 weeks there was the possibility that decay would be seen. None of the other 3 isolates tested showed evidence of any type of decay.

Fungal hyphae were seen only on the surface of the wood wafers using light microscopy due to many reasons such as the short incubation time of the experiment, sections were hand cut rather than cut with a microtome and sections not being stained. Using scanning electron microscopy to visualise the fungal hyphae, all the fungi tested colonised the wood to varying degrees. The two soft-rot causing, fungi *Cadophora malorum* 182 and 242, colonised well into the wood structure of both latewood and earlywood. The two *Cadophora malorum* isolates and *Penicillium roquefortii* isolate 405 appeared to produce an extracellular mucilaginous material when colonising wood at 2°C. Additionally, when incubated at 4°C, the *Cadophora malorum* 182 isolate also was shown to produce the extracellular mucilaginous material. The excretion of extracellular mucilaginous material was also noted when the *Cadophora malorum* isolates were grown at 4°C in liquid YM broth. This material was not further investigated as part of the PhD thesis research but will be discussed in Chapter 8 as part of future suggested research.

Though no visible evidence was seen by scanning electron microscopy it was concluded that the fungal hyphae were moving from cell to cell via the pits and ray cells. The colonisation appeared to be close to ray cells and the hyphae appeared to be crossing cells and appearing in the cells next to each other.

Penicillium roquefortii, *Geomyces* sp and *Cladopsporium oxysporum* are known as mould fungi and are likely to be primarily using the free sugars, cellulose and hemicellulose released from the damage done to the wood cells during cutting of wafers (Nofal and Kumaran, 1999). In this study *Penicillium roquefortii*, *Geomyces* sp and *Cladopsporium oxysporum* colonised the surface of the wood wafer and into the first and second layer of wood cells indicating limited ability to colonise wood after 13 weeks at the 5 test temperatures.

The results presented demonstrate that fungi isolated from the Historic Huts can colonise wood and one species *Cadophora malorum* can cause soft rot decay in spruce wood in only 13 weeks. The presence of fungal hyphae in the latewood of the spruce when this experiment was run at 2ºC suggests that soft rot decay might occur at psychrophilic temperatures if the experiment continued longer. Although the other fungal isolates in this study were mainly seen on the wood wafer surface, when the wood wafer was damaged they were able to colonise further into the wood structure via cracks in the structure.

Chapter 8 General Discussion and Conclusions

This PhD research evolved from collaborations between the University of Waikato and the University of Minnesota to investigate the biological and non-biological decay of the Antarctic Heroic Era Historic Huts.

The hypothesis of this thesis research was that the fungi isolated from the wood of the Antarctic Historic Huts were capable of proliferating and some were capable of wood degradation at cold temperatures, relative to the average Antarctic summer temperatures, with enzymatic activity ranges reflecting the activity in the Antarctic unique environment.

The aims of this PhD thesis research were focused on identifying the role of fungi and their enzymes in degrading wood in the Antarctic, emphasising the century-old Historic Huts of the Heroic Period on Ross Island. The research aimed to gain an understanding of fungal diversity and biochemical framework of the Antarctic isolates.

To investigate the fungal biodiversity, a total of 849 samples were taken from a variety of Antarctic substrates of the Historic Huts in the Antarctic Specially Protected Areas (ASPA). Fungal isolates were cultured from five broad substrates, using seven sampling methods and seven culture media were used. Isolation temperature did affect the numbers of fungi isolated from the samples as the total number of fungi isolated at 4°C was less than at both 15°C and 25°C. The number of fungi isolated at 15 °C was less than at 25 °C. The five dominant genera (*Cladosporium*, *Geomyces*, *Cadophor*a, *Penicillium* and *Thelebolus*) in this thesis research have all been isolated before in Antarctica and are dominant in other habitats. Arenz *et al*. (2006) showed by DGGE and culturing that the five most frequently identified fungal genera in wood and artefacts were *Cadophora*, *Cladosporium*, *Geomyces*, *Crytococcus* and *Hormonema*, while in soils the five most frequently identified fungal genera were *Cadophora*, *Geomyces*, *Crytococcus*, *Epicoccum* and *Cladosporium*. *Penicillium* and *Thelebolus* were cultured five and three time respectively from the study sites. Generally the

biodiversity was low at the Antarctic Historic Huts on Ross Island but the species that were present were physiologically active.

The thirty three fungi that were successfully identified by molecular techniques were from the five dominant species with no *Thelebolus* cultures sent for identification and six cultures remained unidentified due to weak matches in the database searched.

This PhD research provides an Antarctic Filamentous Fungal Culture Collection containing a total of 2076 isolates; 1177 filamentous fungi isolates, 899 single celled microorganisms and has provided a resource for future work on Antarctic microorganisms.

There were differences between the three huts; both *Discovery* and *Terra Nova* had similar percentages of samples from which fungi were isolated while *Nimrod* hut had a lower percentage of samples from which fungi were isolated. This difference could be due to many factors. *Terra Nova* Hut is the only hut where there are visible signs of fungal growth within the hut. The reason for the increased fungal growth in *Terra Nova* Hut maybe due to the work reported by Held *et al*. (2005) that the number of hours when conditions were favourable for fungal growth, by the criteria of hours per year when temperatures were above 0°C and relative humidity was above 80%, showed that at *Terra Nova* Hut in 2001 conditions were favourable for fungal growth for 185-569 hours and 83-461 hours in 2002. This was at least ten times higher than at Nimrod Hut which in 2001 was favourable for fungal growth for 0-55 hrs and in 2002 the range was 0-12 hours. At *Discovery* Hut conditions in the hut were never favourable for growth in the years 2001 and 2002 but in year 2000 the number of hours favourable for growth ranged from 8-11 hours. The results from spore trapping and air sampling confirmed that the huts contained viable fungal material from spores from actively growing fungi or hyphal material. The presence of viable fungal material within the hut reinforces recommendations of Held *et al*. (2005) that the environment within the hut needs to be controlled to be kept as unfavourable for fungal growth as possible.

The repetitive sampling showed that in many cases the fungal diversity in certain areas was changing on a yearly basis. These variations in fungal diversity could be attributed to changes in fungal numbers both with time and location of sample, and/or due to competition for nutrients, and/or the natural progression of fungal species as each species creates favourable conditions for another species. Fungi could be restricted to small areas and the second sample could be out of this area. A survival mechanism for fungi is thought that it can become dormant in winter and then re-establish when conditions are more favourable; this strategy favours spore forming fungi as spores are thought to be more resilient to unfavourable conditions. There is the possibility of new fungi being introduced between samplings leading to new fungi being isolated on subsequent samplings.

The origin of the fungal isolates cultured during this PhD research was not determined by this thesis work but it is recognised that there are possibly many different methods of introduction rather than being indigenous to Antarctica, such as wind, birds, human etc.

The seven fungi, *Cadophora malorum* 182, *Cadophora malorum* 242, *Penicillium roquefortii* 405, *Penicillium roquefortii* 408, *Geomyces* sp. 711, *Geomyces* sp. 824 and *Cladosporium oxysporum* 805 that were studied in depth were all classified as psychrotolerant rather than psychrophilic as all could grow at 4°C and could still proliferate at 20°C. Other investigators have found that the dominant fungal species in Antarctica are psychrotolerant (Tubaki, 1961; Zucconi *et al.*, 1996; Azmi and Seppelt, 1997; Hurst *et al*. 1983; Kerry 1990b). Using the method of Zucconi *et al*. (1996) for determining cold adaptation all seven were classified as cold adapted. Both *Cadophora malorum* 182 and *Geomyces* sp. 711 were shown to be cold adapted as both could sustain growth at 4°C and were not affected by a drop in temperature, and demonstrated the same growth rate in all three inoculations. All fungi could grow with limited water availability but not as low as reported by Onofri *et al*. (2004). When the range of water activity that the Antarctic *Penicillium roquefortii* isolates could grow was compared with food isolates of *Penicillium roquefortii*, the Antarctic isolates are distinctive from the food isolates described by Gervais *et al*. (1998). The utilisation of different carbon sources showed that like many studies of Antarctica organisms, they have a diverse range of enzymatic activity, but interestingly the activity does not differ

greatly with incubation temperature with most carbon sources being used or not used at both incubation temperatures tested. Although it took longer for the fungi to grow at the psychrophilic temperatures, this did not limit the range of carbon sources they utilised. The presence of wood degrading enzymes was investigated for differences in enzyme accumulation extracellularly at different temperatures.

The screening of fungal isolates for cellulase activity determined that 26% of the fungi screened were capable of producing cellulases. Cellulase activity has been reported in temperate isolates of *Cladosporium* (Abrha and Gashe, 1992), *C. malorum* (Berg, 1978), *Geomyces* sp. Kushwaha (2000) and many species of *Penicillium* (Jorgensen *et al*., 2005). However, there is no information published on the quantitative levels of cellulase activity of any of these organisms at psychrophilic temperatures. Using agar plates containing cellulose Fenice *et al*. (1997) reported low cellulase activity from 1 of 5 *Geomyces pannorum*, and no cellulase activity from 1 *Cladosporium cladosporioides* and 2 *Cladosporium herbarum* isolated in Antarctica, while Hurst *et al*. (1983) reported Antarctic isolates of *Chrysosporium pannorum* and *Cladosporium sphaerospermum* having an ability to degrade cellulose along with other substrates at 20ºC and both demonstrated cellulase activity at 1ºC using plate screening methods. Of the 27 fungi identified from two of the Antarctic Historic Hut in this study, (7 from *Discovery* Hut and 20 from *Terra Nova* Hut) all produced detectable levels of β-endoglucanase activity, at either 4°C or at 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; no direct correlation between the relationships of activity:temperature and growth:temperature 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 in a 15°C culture. When grown at 4°C, cultures produced less protein in the extracellular supernatant when compared with culturing at 15°C suggesting a lesser efficiency of growth at 4°C which may be a result of cold adaptation. There could also be different extracellular proteins produced when the fungal isolate was cultured at each of the two temperatures, supported by the SDS-PAGE results. The variety of electrophoretic techniques used all showed that the cellulase found in these Antarctic isolates constitutes a complex of multiple enzymes, different at the two temperatures tested and

different for each species along with variations between isolates of the same species.

A single cellulase enzyme was not purified to homogeneity as part of the PhD research. The properties of a mixture of proteins with enriched cellulase activity were described with relation to temperature profiles, enzyme stability and activity toward a variety of substrates. Various chromatographic and electrophoretic methods were used to partially purify a group of proteins which had enhanced β-1,4-glucan cellobiohydrolase enzyme activity; these proteins were large as they were found in the first 3mm of an SDS-PAGE gel and were identified as Band 3mm for the description process. Band 3mm was comprised of three visible protein bands on a native preparative gel. There was a large number of possible proteins in these bands as attempts to identify using MS/MS gave multiple possible protein identities and many peptide sequences which were unable to be identified due to no similar sequence in the searched databases. None of the peptide matches were strong enough to give a definite identification to any of the proteins but selection of proteins including β-glucanases were consistently suggested as possible matches. The use of 2-D electrophoresis would assist in separating these multiple protein bands to single protein bands. Band 3mm displayed β-1,4-glucan cellobiohydrolase activity over the temperature range 20°C to 80°C with the optimum activity demonstrated at 60°C. Band 3mm was stable at 4°C and at 25°C for at least 24 hrs and was stable at 40°C for 1 hr before activity started to drop. At 4°C, 25°C, and 40°C, Band 3mm β-1,4-glucan cellobiohydrolase showed an unexplained phenomena of increased activity when incubated at each of the temperatures. When incubated at 50°C and 80°C, enzyme activity was below the detectable limit of the assay after 24 hrs and 2 minutes, respectively. During the purification process endo-1,4-β glucanase and FPase activity, present in the extracellular supernatant, was removed but Band 3mm still had Avicelase, CMCase and β-1,4-glucan cellobiohydrolase enzyme activity. Proteins producing amylase and pectin activity were not present in Band 3mm (and had been in the total extracellular supernatant) but the partially purified protein still retained xylanase and mannanase activity. After the purification process the protease activity was still present.

At least one species, *Cadophora* sp., isolated in Antarctica demonstrated wood degradation at psychrophilic temperatures after 13 weeks incubation. Also, fungal

hyphae were seen in the latewood of wood wafers at 2°C suggesting that given time they might cause wood decay at psychrophilic temperatures. The other three species tested in this thesis research showed no decay though after 13 weeks incubation all could colonise wood to varying degrees.

In conclusion, this PhD thesis reports the following:

- Fungal diversity Historic Huts of the Heroic Period on Ross Island is low but the fungi that are present are physiologically active and are proliferating.
- Fungal isolates used for the in-depth studies were all
    - Psychrotolerant.
    - Metabolised a wide variety of carbon sources at psychrophilic temperatures, and at mesophilic conditions with the only difference being that the metabolism rate was slower at the colder temperature.
    - Grew in media with reduced water activity.
- Fungi associated with wood are capable to degrade a variety of cellulose carbon sources at both mesophilic temperatures and psychrophilic temperatures.
- Cellulase enzyme complexes of the fungi were all
    - Multi-enzymed
    - Different at the two temperatures (4°C and 15°C) tested
    - Different for different species
    - Different between isolates of the same species
- The culture supernatant contained other wood degrading enzymatic activity besides just cellulase.
- Band 3mm, a partially purified protein from *Cadophora malorum* 182 that was not purified to homogeneity, had the following properties:
    - β-1,4-glucan cellobiohydrolase activity over the temperature range 20°C to 80°C with optimum activity seen at 60°C.
    - Stable β-1,4-glucan cellobiohydrolase activity at 4, 25 and 40°C for at least 24 hrs.
    - Loss of β-1,4-glucan cellobiohydrolase activity at 50°C within 24 hrs.

- Loss of β-1,4-glucan cellobiohydrolase activity at 80°C with in 2 minutes.
- Along with β-1,4-glucan cellobiohydrolase activity, Band 3mm contained enzymatic activities of the following enzymes:
  - Avicelase
  - CMCase
  - xylanase
  - mannanase
  - protease

Results from this thesis were published in a peer reviewed journal. This paper focused on the fungi isolated from *Terra Nova* Hut, and the β-endoglucanase activity these fungi were producing at mesophilic and psychrophilic temperatures.

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In summary, the following objectives were identified as possible milestones for future Antarctic fungal research:

- Investigate the production of extracellular mucilaginous material by some of the fungal isolates when grown at psychrophilic temperatures.
- Continue the effort to complete the purification and identification of the cellulase complex from *Cadophora malorum.*
- Determine the temperature equilibrium T_{eq} for the purified cellulases.
- Investigate the cause of the activity rise when the enzyme is incubated at temperatures where activity is low if the β-1,4-glucan cellobiohydrolase activity assay is run at this temperature.
- Explore the other wood degrading enzymes that the Antarctica fungal isolates are producing.

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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 4ºC, and also when they were grown at 15ºC. Isolates of *Penicillium roquefortii* and *Cadophora malorum* showed preference for growth at 15ºC rather than 25ºC or 4ºC indicating psychrotrophic characteristics. These results demonstrate that cellulolytic filamentous fungi found in Antarctica are capable of growth at cold temperatures and possess the ability to produce extracellular endo-1, 4-β-glucanase when cultured at cold and temperate temperatures.

Introduction

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

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

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

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

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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	2.85	0
242	15	1.28	1.12
262	4	1.10	1.10
489	15	2.0	0
492	15	1.1	0.67
517	15	1.0	1.33
536	15	1.12	0
638	25	1.6	0.53
654	15	2.0	0.93
655	15	1.8	1.33
660	15	3.2	1.0
667	15	1.8	3.0
693	15	2.42	0.75
711	4	1.0	1.0
719	4	2.75	2.75
749	4	1.42	1.42
750	4	1.42	1.42
779	4	3.0	3.0
814	4	1.27	1.27
821	4	1.33	1.33
1029	25	1.0	2.5
1222	15	1.37	NG

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

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

Results

Isolation of fungi

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

Screening for cellulytic activity

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

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

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

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

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

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

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

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

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

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

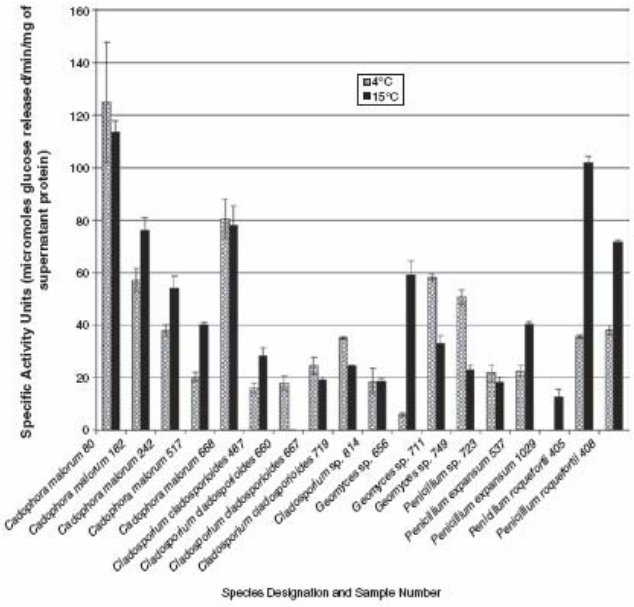

Fig. 1. Graph of specific activity units of cellulase (micromoles glucose released per minute per mg of supernatant protein) for the selected 18 fungi at 4°C and 15°C.

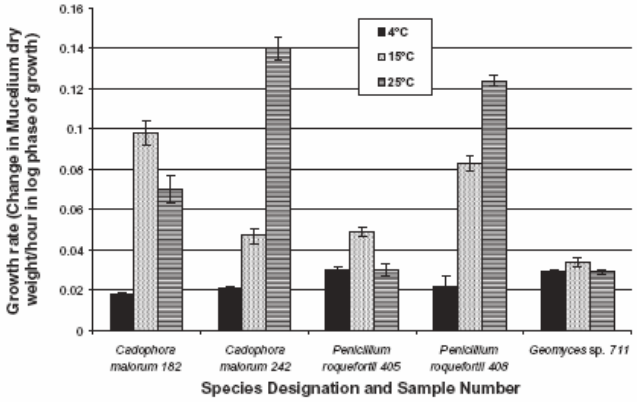

Fig. 2. Graph of growth rate of the five selected fungi at 4°C, 15°C and 25°C.

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

Growth temperature characteristics

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

Discussion

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

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

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

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

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

Experimental procedures

Sample collection

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

Isolation of fungi

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

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

Molecular characterizations, particularly DNA sequence analyses of the two internal transcribed spacer (ITS) regions of ribosomal DNA, ITS1 and ITS2, were used to confirm identity. Fungal material was scraped from pure cultures and DNA extracted using Qiagen DNeasy plant mini-kits, following manufacturer's instructions (Qiagen Sciences, Germantown, MA). The rDNA ITS regions 1, 5.8S, and ITS region 2 were amplified using primers ITS1 and ITS4 (Gardes and Bruns, 1993). Polymerase chain reaction amplification was performed in a MJ Research PTC Mini-cycler (Watertown, MA), with the following protocol: 94°C for 5 min; 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min followed by a final extension step of 72°C for 5 min.

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

Growth characteristics of fungi

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

Detection and analysis of cellulytic activity

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

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

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

Statistical analysis

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

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References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

References

Abrha, B., Gashe, B.A. 1992. Cellulase production and activity in a species of *Cladosporium*. *World Journal of Microbiology and Biotechnology* 8: 164-166.

Abyzoz, S.S. 1993. *Microorganisms in the Antarctic Ice.* Wiley-Liss, New York. pp. 265-295.

Addy, H.D., Schaffer, G. F., Miller, M. H., Peterson R. L. 1994. Survival of the external mycelium of a VAM fungus in frozen soil over winter. *Mycorrhiza* 5:1-5.

Aislabie, J., Fraser, R., Duncan, S., Farrell, R. L. 2001. Effects of Oil spills on microbial heterotrophs in Antarctic soils. *Polar Biology* 24: 308-313.

Almin, K.E., Eriksson, K-E., Pettersson, B. 1975. Extracellular enzyme system utilized by the fungus *Sporotrichum pulverulentum* (*Chrysporium lignorum*) for the breakdown of cellulose II Activities of five endo-1,4-glucanases towards carboxymethylcellulose. *European Journal of Biochemistry* 51: 207-211.

Amiri, A., Bompeix, G. 2005. Diversity and population dynamics of *Penicillium* spp. on apples in pre- and post harvest environments: consequences for decay development. *Plant Pathology* 54: 74-81.

Anders, P. 1994. The cellobiose-oxidizing enzyme CBQ and CbO as related to lignin and cellulose degradation- a review. *FEMS Microbiology Reviews* 13: 297-312.

Antarctic Heritage Trust. 1987. http://www.heritageantarctica.org/index.cfm/Who/Background/

Antarctic Heritage Trust. 2003. *Conservation Plan Shackleton's Hut. British Antarctic Expedition 1907-1909 Cape Royds, Ross Island, Antarctica.* Antarctic Heritage Trust. Christchurch.

Antarctic Heritage Trust. 2004. *Conservation Plan Scott's Hut, Cape Evans.* Antarctic Heritage Trust. Christchurch.

Antarctic Heritage Trust. 2004. *Conservation Plan Discovery Hut, Hut Point.* Antarctic Heritage Trust. Christchurch.

Arenz, B.E., Held, B.W., Jurgens, J.A., Farrell, R.L., 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.

Atlas, R.M., DiMenna, M. E., Cameron, R. E. 1978. Ecological Investigations of Yeasts in Antarctic Soils. *Antarctic Research Service* 30: 27-34.

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

Azmi, O.R., Seppelt, R.D. 1998. The broad-scale distribution of microfungi in the Windmill Islands region, continental Antarctica. *Polar Biology* 19: 92-100.

Bailey, M. J., Biely, P., Poutanen, K. 1992. Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology* 23: 257-270.

Baker, C.J., Whalen, C.H., Bateman, D.F. 1977. Xylanase from *Trichoderma pseudkongingii*: purification, characterisation and effects on isolates plant cell walls. *Physiological Biochemistry* 67: 1250-1258.

Balch, E.S. 1912. Scott's second Antarctic expedition. *Bulletin of the American Geographical Society* 44: 270-277.

Barbosa, M-A. G., Rehn, K-G, Menezes, M., Mariano, R.L.R. 2001. Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzymatic characterization. *Brazilian Journal of Microbiology* 32: 98-104.

Bargagli, R., Skotnicki, M. L., Marri, L., Pepi, M., Mackenzie, A., Agnorelli, C. 2004. New record of moss and thermophilic bacteria species and physico-chemical properties of geothermal soils on the northwest slope of Mt. Melbourne (Antarctica). *Polar Biology* 27: 423-431.

Barkai-Golan, R. 1973. Species of *Pencillium* causing decay of stored fruit and vegetable in Israel. *Mycopathologia* 54:141-145.

Barker, R.J. 1977. A biological reconnaissance of the Bunger Hills. *ANARE Technical Memo* 67: 29-30.

Barnett, J.R., Bonham, V.A. 2004. Cellulose microfibril angle in the cell wall of wood fibres. *Biological Reviews* 79: 461-472.

Baross, J.A., Morita, R.Y. 1978. Microbial life at low temperatures: Ecological aspects. In Kushner, D.J. (ed.). *Microbial Life in Extreme Environments*. Academic Press, London. pp. 9-71.

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

Baublis, J.A., Wharton, R. A., Volz, P. A. 1991. Diversity of micro-fungi in an Antarctic Dry Valley. *Journal of Basic Microbiology* 31: 3-12.

Bayer, E.A., Chanzy, H., Lamed, R., Shoham, Y. 1998. Cellulose, cellulase and cellulosomes. *Current Opinion in Structural Biology*. 8: 548-557.

Beales, N. 2004 Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comprehensive Reviews in Food Science and Food Safety* 3: 1–20.

Béguin, P, Lemaire, M. 1996. The cellulosome: an exocellular, multiprotein complex specialized in cellulose degradation. *Critical Reviews in Biochemistry and Molecular Biology* 31: 201-236.

Bell, A.A., Wheeler, M.H. 1986. Biosynthesis and functions of fungal melanins. *Annual Reviews Phytopathology* 24: 411-51.

Benninghoff, W.S., Bonner, W.N. 1985. *Man's Impact on the Antarctic Environment: A procedure for evaluating impacts and logistic activities*. Scientific Committee on Antarctic Research. Cambridge, England.

Bentahir, M., Feller, G., Aittaleb, M., Lamotte-Brasseur, J., Himri, T., Chessa, J-P., Gerday, C. 2000. Structural, kinetic, and calorimetric characterization of the cold-active phosphoglycerate kinase from the Antarctic *Pseudomonas* sp. TACII18. *The Journal of Biological Chemistry* 275: 11147-11153.

Berg, B. 1978. Cellulose Degradation and Cellulase Formation by *Phialophora malorum*. *Archives Microbiology* 118: 61-65.

Berger,F., Morellet, N., Menu, F., Potier, P. 1996. Cold shock and cold acclimation proteins in the psychrotrophic bacterium *Arthrobacter globiformis* SI55. *Journal of Bacteriology* 178: 2999-3007.

Bertolini, P., Tian, S.P. 1996. Low temperature biology and pathogenicity of Penicillium hirsutum on garlic in storage. *Postharvest Biology and Technology* 7: 83-89.

Black, G. W., Rixon, J.E., Clarke, J.H., Hazlewood, G. P., Ferreira, L.M., Bolam, D.N., Gilbert, H .J. 1997. Cellulose binding domains and linker sequences potentiate the activity of hemicellulases against complex substrates. *Journal of Biotechnology* 57: 59-69.

Blanchette, R.A. 1982. Progressive stages, of discoloration and decay associated with the canker-rot fungus *Inotontus obliquus* in birch. *Phytopathology* 72: 1272-1277.

Blanchette, R.A. 1984. Selective delignification of eastern hemlock by *Gandoderma tsugae*. *Phytopathology* 74: 153-160.

Blanchette, R.A., Nilsson, T., Daniel G., Abad, A.1990. Biological Degradation of Wood. In: Rowell, R.M., Barbour, R.J. (ed.). *Archaeological Wood: Properties, Chemistry, and Preservation, Advances in Chemistry Series*, vol. 225. American Chemical Society, Washington, DC. pp. 141-174.

Blanchette, R.A. 2000. A review of microbial deterioration found in archaeological wood from different environments. *International Biodeterioration and Biodegradation* 46: 189-204.

Blanchette, R.A., Held, B.W., Jurgens, J.A., Aislabie, J., Duncan, S.M., Farrell, R.L. 2004. Environmental pollutants in Antarctica from the Robert F. Scott and Ernest H. Shackleton expeditions during the 'Heroic Era' of exploration. *Polar Record* 40: 143-151.

Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., Farrell, R.L. 2004. Wood-Destroying Soft Rot Fungi in the Historic Expedition Huts of Antarctica. *Applied and Enviromental Microbiology* 70: 1328-1335.

Block, W. 1984.Terrestrial microbiology, invertebrates and ecosystems In Laws R.M. (ed) *Antarctica Ecology* Vol. 1. Academic Press, London. pp 163-236.

Boisset, C., Chanzy, H., Henrissat, B., Lamed, R., Shoham, Y., Bayer, E.A. 1999. Digestion of crystalline cellulose substrates by the *Clostridium thermocellum* cellulosome: structural and morphological aspects. *The Biochemical Journal* 340: 829-835.

Boyd, W.L., Staley, J.T., Boyd, J.W. 1966. Ecology of soil microorganisms of Antarctica. *Antarctic Research Series* 8: 125-159.

Bradner, J.R., Gillings, M., Nevalainen, K.M.H. 1999a. Qualitative assessment of hydrolytic activities in Antarctic microfungi grown at different temperatures on solid media. *World Journal of Microbiology and Biotechnology* 15: 131-132.

Bradner, J.R., Sidhu, R.K., Gillings, M., Nevalainen, K.M.H. 1999b. Hemicellulase activity of Antarctic microfungi. *Journal of Applied Microbiology* 87: 366-370.

Broady, P., Given, D., Greenfield, L., Thompson, K. 1987. The biota and environment of fumaroles on Mt Melbourne, Victoria Land. *Polar Biology* 7: 97-113.

Brown, R.M. Jr, Saxena, I.M., Kudlika, K. 1996. Cellulose biosynthesis in higher plants. *Trends in Plant Science* 1: 149-156.

Bruchmann, E-E., Schach, H., Graf, H. 1987. Role and properties of lactonase in a cellulase system. *Biotechnology and Applied Biochemistry* 9: 146-159.

Butler, M.J., Day, A.W. 1998. Fungal melanins: a review. *Canadian Journal of Microbiology* 44: 1115-1136.

Cairns, A.J., Howarth, C.J., Pollock, C.J. 1995. Characterisation of acid invertase from the snow mould *Monographella nivalis*: A mesophilic enzyme from a psychrophilic fungus. *New Phytologist* 130: 391-400.

Cameron, R.E., King, J., David, C.N. 1968. Soil toxicity in Antarctic Dry Valleys. *Antarctic Journal of the United States* 9: 116-119.

Cameron, R.E., Conrow, H.P. 1969. Soil moisture, relative humidity, and microbial abundance in Dry Valleys of Southern Victoria Land. *Antarctic Journal of United States* 4: 23-28.

Cameron, R.E. 1971. Antarctic Soil Microbial and Ecological Investigations. In Quam P.O., Porter H.D. (ed.). *Research in the Antarctic*. AAAS Publication Washington DC pp. 137-189.

Cameron, R.E., Melick, E.L. 1971. Growth of bacteria in soils from the Antarctic Dry Valleys. *Technical Report 32-1522*. Jet Propulsion Laboratory, Pasadena California.

Cameron, R.E. 1972. Microbial and ecological investigations in Victoria Dry Valley, Southern Victoria Land, Antarctica. *Antarctic Terrestrial Biology* 195-260.

Cameron, R.E., Morelli, F.A., Honour, R.C. 1973. Aerobiological monitoring of dry valley drilling sites. *Antarctic Journal* 211-214.

Cameron, R.E., Morelli, F.A., Donlan, R., Guilfoyle, J., Markley, B., Smith R. 1974. DVDP environmental monitoring. *Antarctic Journal* 141-144.

Cameron, R.E., Honour, R.C., Morelli, F.A. 1976. Antarctic Microbiology-preparation for Mars life detection, quarantine, and back conatmination. In Heinrich M.R., (ed.). *Extreme Environments*. Academic Press Inc., New York. pp.57-82.

Cameron, R.E., Honour, R.C., Morelli, F.A. 1977. Environmental impact studied of Antarctic sites. In llano, G.A (ed.) *Adaptation within Antarctic Ecosystems: Proceedings of the Third SCAR Symposium on Antarctic Biology*. Smithsonian Institution, Washington DC pp. 1157-1176.

Campbell, I.B., Claridge, G.G.C. 1966 A sequence of soils from a penguin rookery, Inexpressible Island, Antarctica. *New Zealand Journal of Science* 9: 361-372.

Campbell, I.B., Claridge, G.G.C. 1987. *Antarctica: Soils, weathering processes and environment*. Elsevier, Amsterdam.

Campbell, I.B., Claridge, G.G.C. Balks, M.R., Campbell, D.I. 1987 Moisture content in soils of the McMurdo Sound and Dry Valley region of Antarctica. In Lyons, W.B., Howard-Williams, C. Hawes, I. (ed.). *Ecosystem processes in Antarctic ice-free landscapes.* Balkema , Rotterdam. pp. 61-76.

Campbell, I.B., Claridge, G.G.C., Balks, M.R. 1994. The effect of human activities on moisture content of soils and underlying permafrost from the McMurdo Sound region, Antarctica. *Antarctic Science* 6: 307-314.

Carrard, G., Koivula, A., Söderlund, H., and Béguin, P. 2000. Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose. *Proceedings of the National Academy of Sciences of the United States of America* 97: 10342-10347.

Carreiro, M. M., Koske, R.E. 1992. Effect of temperature on decomposition and development of microfungal communities in leaf litter microcosms. *Canadian Journal of Botany* 70: 2177-2183.

Carretta, G., Piontelli, E. 1977. *Microsporum magellanicum* and *Cunninghamella antarctica*, new species isolated from Australia and Antarctic soil of Chile. *Sabouraudia* 15: 1-10.

Carretta, G., Del Frate, G., Margiarotti, A.M. 1994. A record of *Arthrobotrys tortor* Jarowaja and *Engyodontium album* (Limber) de Hoog from Antarctica. *Boletín micológico* 9: 9-13.

Chaabouni, S.E., Mechichi, T., Limam, F, Marzouki, N. 2005. Purification and Characterization of Two Low Molecular Weight Endoglucanases Produced by *Penicillium occitanis* Mutant Pol 6. *Applied Biochemistry and Biotechnology* 125: 99-112.

Cherry-Garrard, A. 1937. *The Worst Journey in the World*. Chatto and Windus, London.

Chen, H., Jin, S. 2006. Effect of ethanol and yeast on cellulase activity and hydrolysis of crystalline cellulose. *Enzyme and Microbial Technology* 39: 1430-1432.

Claridge, G.G.C., Campbell, I.B. 1985. Physical geography - soils. In Bonner, W.N., Walton, D.W.H. (ed.). *Key environments, Antarctica.* Pergamon Press, Oxford. pp. 62-70.

Clark, M.S., Clarke, A., Cockell,C.S., Convey, P., Detrich III, W., Fraser, K.P.P., Johnston, I.A., Methe, B.A., Murray, A.E., Peck, L.S., Römisch, K., Rogers, A.D. 2004. Antarctic genomics. *Comparative and Functional Genomics* 5: 230-238.

Clarke, A., Crame, J.A. 1989. The origin of the Southern Ocean marine fauna. In. Crame, J.A. (ed.). *Origins and evolution of the Antarctic biota.* The Geological Society, London. pp. 253-268.

Clausen, C.A. 1996. Bacterial Association with decaying wood: a review. *International Biodeterioration and Biodegradation*. 37: 101-107.

Claussen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., Hart, S.C. 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. *FEMS Microbiology Ecology* 1506: 1-10.

Cohen, R., Suzuki, M.R., Hammel, K.E. 2005. Processive Endoglucanase Active in Crystalline Cellulose Hydrolysis by the Brown Rot Basidiomycete *Gloeophyllum trabeum. Applied and Environmental Microbiology*. 71: 2412-2417.

Convey, P., Block, W. 1996. Antarctic dipterans: ecology, physiology and distrubution. *European Journal of Entomology* 93: 1-13.

Convey, P. 2001. Antarctic Ecosystems. In Levin, S.A. (ed.). *Encyclopaedia of Biodiversity* Vol. 1. Academic Press, San Diego. pp. 171-184.

Cooke, R.C., Whipps, J.M. 1993. *Ecophysiology of fungi*. Blackwell Scientific Publications, Oxford, U.K..

Corbett, N.H. 1965. Micro-morphological studies on the degradation of liginified cell walls by Ascomycetes and Fungi Imperfectii. *Journal Institute of Wood Science* 4: 18-29.

Corte, A., Daglio, C. A. N. 1964. A mycological study of the Antarctic air. In Carrick, M., Holdgate, W., Prevost, J. (ed.). *Biologie antarctique*. Hermann, Paris, France.

Coughlan, M.P. 1985. The properties of fungal and bacterial cellulases with comment on their production and application. *Biotechnology and General Engineering* 3: 39-109.

Creighton, T.E. 1993. *Proteins: structures and molecular properties*. 2nd edition. W.H. Freeman and Company, New York.

Crockett, E. L., Sidell, B. D. 1990. Some pathways of energy metabolism are cold adapted in Antarctic fishes. *Physiological Zoology* 63: 472–488.

Daniel, G., Nilsson, T., Singh, A.P. 1987. Degradation of lignocellulosic by unique tunnel-forming bacteria. *Canadian Journal of Microbiology* 33: 943-948.

Daniel, G. 1994. Use of electron microscopy for aiding our understanding of wood biodegradation. *FEMS Microbiology Reviews* 13: 199-233.

Daniel, R.M., Danson, M.J., Eisenthal, R. 2001. The temperature optima of enzymes: a new perspective on an old phenomenon. *TRENDS in Biochemical Science* 26: 223-225.

Davey, M.C., Pickup, J., Block, W. 1992. Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarctic Science* 4: 383-388.

de Hoog, G.S., Göttlich, E., Platas, G., Genilloud, O., Leotta, G., van Brummelen, J. 2005. Evolution and ecology of the genus *Thelebolus* in Antarctica. *Studies in Mycology* 51: 33–76.

Dekker, R.F.H., Richards, G.N. 1976. Hemicellulases: their occurrence, purification, properties and mode of action. *Advances in Carbohydrate Chemistry and Biochemistry* 32: 277-352.

Del Frate, G., Caretta, G. 1990. Fungi Isolated from Antarctic Material. *Polar Biology* 11: 1-7.

Derry, A.M., Staddon, W.J., Kevan, P.G., Trevors, J.T. 1999. Functional diversity and community structure of micro-organisms in three arctic soils as determined by sole-carbon-source-utilization. *Biodiversity and Conservation* 8: 205-221.

Di Mena, M.E., 1960. Yeasts from Antarctica. *Journal of General Microbiology* 23: 295-300.

Din, N., Gilkes, N.R., Tekant, B., Miller Jr., R.C., Anthony, R., Warren, J., Kilburn, D.G. 1991. Non-Hydrolytic Disruption of cellulose Fibres by the Binding Domain of a bacterial cellulase. *Biotechnology* 9: 1096-1099.

Dix, N.J., Webster, J. 1995. *Fungal Ecology.* Chapman and Hall, London.

Domec, J-C., Gartner,B.L. 2002 How do water transport and water storage differ in coniferous earlywood and latewood? *Journal of Experimental Botany* 53: 2369-2379.

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

Ejechi, B.O. 1997. Biological Control of Wood Decay in an OpenTropical Environment with *Penicillium* sp. and *Trichoderma viride*. *International Biodeterioration & Biodegradation* 39: 295-299.

Ellouz, S., Durand, H., Tiraby, G. 1987. Analytical separation of *Trichoderma reesei* cellulases by ion-exchange fast protein liquid chromatography. *Journal of Chromatography* 396: 307-317.

Eriksson, K.E., Pettersson, B., Westermark, U. 1974. Oxidation: an important enzyme reaction in fungal degradation of cellulose. *FEBS Letters* 49: 282-285.

Eriksson, K.E., Blanchette, R. A., Ander, P. 1990. *Microbial and Enzymatic Degradation of Wood and Wood Components.* Springer-Verlag, Berlin.

Farrell, R.L., Blanchette, R.A., Auger, M., Duncan, S.M., Held, B.W., Jurgens, J.E., Minasaki, R. 2004. *Scientific Evaluation of Deterioration in Historic Huts of Ross Island, Antarctica*. In Polar Monuments and Sites Cultural Heritage Work in the Arctic and Antarctic Regions. ISBN: 82-996891-1-2, International Polar Heritage Committee, ICOMOS, Norway. pp. 33-38.

Feller, G., Narinx, E., Arpigny, J.L., Aittaleb, M., Baise, E., Genicot, S., Gerday, C. 1996. Enzymes for psychrophilc organisms. *FEMS Microbiology Reviews*. 18: 198-202.

Feller, G., Gerday, C. 1997. Psychrophilic enzymes: molecular basis of cold adaptation. *Cellular and Molecular Life Sciences* 53: 830-841.

Feller G, d'Amico, D, Gerday, C.1999. Thermodynamic stability of cold-active alpha-amylase from the Antarctic bacterium *Alteromonase haloplanctis*. *Biochemistry* 38: 4613-4619.

Fenice, M., Selbmann, L., Zucconi, L., Onofri, S. 1997. Production of extracellular enzymes by Antarctic fungal strains. *Polar Biology* 17: 275-280.

Fenice, M., Selbmann, L., Di Giambattista, R., Federici, F. 1998. Chitinolytic activity at Low temperature of an Antarctic strain (A3) of *Verticillium lecanii*. *Research Microbiology* 149: 289-300.

Finegold, L., Singer, MA, Federie, T.W., Vestal, J.R. 1990. Composition and thermal properties of membrane lipids in cryptoendolithic lichen microbiota from Antarctica. *App1ied and EnvironmentalMicrobiology* 56: 1191-1194.

Finotti, E, Paolino, C., Lancia, B., Mercantini, R. 1996. Metabolic Differences Between Two Antarctic Strains of *Geomyces pannorum*. *Current Microbiology* **32**: 7-10.

Flanagan, P.W., Scarborough, A.M.1974. Physiological groups of decomposer fungi on tundra plant remains. In Holding, A.J., Heal, O.W., MacLean, S.F.J., Flanagan, P.W. (ed.). *Soil organisms and decomposition in tundra.* Tundra Biome Steering Committee, Stockholm. pp. 159–181.

Fletcher, L.D., Kerry, E.J., Weste, G.M. (1985) Microfungi of Mac.Robertson and Enderby Lands, Antarctica. *Polar Biology* 4: 81-88.

Frenot, Y., Chown, S.L., Whinam, J., Selkirk, P.M., Convey, P., Skotnicki, M., Bergstrom, D.M. 2005. Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* 80: 45-72.

Friedmann, E.I. 1982. Endolithic microorganisms in the Antarctic cold desert. *Science New Series* 215: 1045-1053.

Friedmann, E.I., Kappen, L., Meyer, M.A., Nienow, J.A. 1993. Long term productivity in the cryptoendolithic microbial community of the Ross Desert. *Microbial Ecology* 25: 57-69.

Fujita, Y., Takahashi, S., Ueda, M., Tanaka, A., Okada, H., Morikawa, Y., Kawaguchi, T., Arai, M., Fukuda, H. Kondo, A. 2002. Direct and efficient production of ethanol from cellulosic material with a yeast strain displaying cellulolytic enzymes. *Applied and Environmental Microbiology* 68: 5136-5141.

Fukunaga, N., Russell, N.J. 1990. Membrane lipid composition and glucose uptake in two psychrotolerant bacteria from Antarctica. *Journal of General Microbiology* 136: 1669-1673.

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

Garsoux, G., Lamotte, J., Gerday, C., Feller, G. 2004. Kinetic and structual optimization to catalysis at low temperature in a psychrophilic cellulase from the Antarctic bacterium *Pseudoalteromonas haloplanktis*. *Biochemical Journal.* 384: 247-253.

Georlette, D., Blaise, V., Collins, T., D'Amico, S., Gratia, E., Hoyoux, A., Marx, J-C., Sonan, G., Feller, G., Gerday, C. 2004. Some like it cold: biocatalysis at low temperatures. *FEMS Microbiology Reviews* 28: 25-42.

Gerday, C.M.A., Bentahir, M., Chessa, J-P., Claverie, P., Collins, T., D'Amico, S., Dumont, J., Garsoux, G., Georlette, D., Hoyoux, A., Lonhienne, T., Meuwis, M-A., Feller, G. 2000. Cold-adapted enzymes: from fundamentals to biotechnology. *TRENDS in Biotechnology* 18: 103-107.

Gervais, P., Molin, P., Grajek, W., Bensoussan, M. 1988. Influence of the water activity of a solid substrate on the growth rate and sporogenesis of filamentous fungi. *Biotechnology and Bioengineering* 31: 457-463.

Gharibian, S., Hoffert, C., Abraham, L. D., Breuil, C. 1996. Localizing an *Ophiostoma piceae* proteinase in sapwood of four tree species using polyclonal antibodies. *New Phytologist* 133: 673-679.

Gilkes, N.R., Warren, R.A., Miller, Jr., R.C., Kilburn, D.G. 1988. Precise excision of the cellulose binding domains from two *Cellulomonas fimi* cellulase by a homologous protease and the effect on catalysis. *The Journal of Biological Chemistry* 263: 10401-10407.

Gilkes, N. R, Henrissat, B, Kilburn, D.G., Miller Jr, R.C, Warren, R.A. 1991. Domains in microbial beta-1, 4-glycanases: sequence conservation, function, and enzyme families. *Microbiology and Molecular Biology Review* 55: 303-315.

Gock, M.A., Hocking, A.D., Pitt, J.I., Poulos, P.G. 2003. Influence of temperature, water activity and pH on growth of some xerophilic fungi. *International Journal of Food Microbiology* 2481: 11-19.

Golubic, S., Friedmann, I., Schneider, J. 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology* 51: 475-478.

Gounot, A.M. 1991. Bacterial life at low temperature: physiological aspects and biotechnological implications. *Journal of Applied Bacteriology* 71: 386-397.

Gounot, A.M., Russell, N.J. 1999. Physiology of cold-adapted microorganisms. In Margesin, R, Schinner, F., (ed.). *Cold-Adapted Organisms*. Springer Verlag, New York. pp. 33–51.

Goyal, A., Ghosh, B., Eveleigh, D. 1991. Characterisation of Fungal Cellulase. *Bioresource Technology* 36: 37-50.

Grant, W.D. 2004. Life at low water activity. *Philosophical Transactions of the Royal Society of London. Series B.* 359**:** 1249-1267.

Graumann, P., Marahiel, M.A. 1998. A superfamily of proteins that contain the cold-shock domain. *Trends in Biochemical Science* 23: 286-290.

Graumann, P., Marahiel, M.A. 1999 Some like it cold: response of microorganisms to cold shock. *Archives in Microbiology* 166: 293-300.

Green, F., Highley, T. L. 1997. Mechanism of brown-rot decay: Paradigm or paradox. *International Biodeterioration and Biodegradation* 39: 113–124.

Greenfield, L. 1981. Soil microbiology studies. *Antarctic expedition No19* Christchurch, University of Canterbury. pp.4-22.

Greenfield, L.G., 1982. Thermophilic microbes in Shackleton's pony fodder, Cape Royds. *NewZealand Antarctic Record* 4: 21.

Greenfield, L.G. 1992. Precipitation nitrogen at maritime Signy Island and continental Cape Bird, Antarctica. *Polar Biology* 11: 649-653.

Grounot, A.M., Russell, N.J. 1999. Physiology of cold-adapted microorganisms In Margesin, R., Schinner, F. (ed.). *Cold-Adapted Organisms. Ecology, Physiology, Enzymology and Molecular Biology*, Springer-Verlag, Berlin-Heidelberg. pp. 33-55.

Gusakov, A.V., Sinitsyn, A. P., Salanovich, T. N., Bukhtojarov, F. E., Markov, A. V., Ustinov, B. B., van Zeijl, C., Punt, P., Burlingame, R. 2005. Purification, cloning and characterisation of two forms of thermostable and highly active cellobiohydrolase I (Cel7A) produced by the industrial strain of *Chrysosporium lucknowense*. *Enzyme and Microbial Technology* 36: 57-69.

Harlow, W.M. 1970. *Inside wood masterpiece of nature*. The American Forestry Association. Washington.

Harrington, T.C., Mc New, D.L., 2003. Phylogenetic analysis places Thephialphora-like anamorph genus *Cadophora* in the Helotiales. *Mycotaxon* 87: 141-151.

Harrowfield, D.L. 1988. Historic sites in the Ross Dependency, Antarctica. *Polar Record* 24: 277-284.

Harrowfield, D.L. 1995. *Icy Heritage: Historic Sites of the Ross Sea Region*. Christchurch, Antarctic Heritage Trust.

Hawkesworth, D.L., Kirk, P.M., Sutton, B.C., Pegler D.N. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*. CAB International, Wallingford, UK. pp 455.

Heal, O.W., Bailey, A.D., Latter, P.M. 1967. Bacteria, fungi and protozoa in Signy Island soils compared with those from temperate moorlands. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, A Discussion of the Terrestrial Antarctica Ecosystem*. 252: 191-197.

Heatwole, H., Saenger, P., Spain, A., Kerry, E., Donelan, J. 1989. Biotic and chemical characteristics of some soils from Wilkes Land, Antarctica. *Antarctic Science* 1: 225-234.

Held, B.W., Blanchette, R.A., Jurgens, J.A., Duncan, S., Farrell, R.L. 2003. *Deterioration and conservation issues associated with Antarctica's historic huts*, In Koestler, R.J., Charola, A.E., Nieto-Fernadez, F.E. (ed.). Art, Biology and Conservation of Works of Art. Metropolitan Museum of Art, New York. pp. 370-389.

Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., Blanchette, R.A. 2005. Environmental factors influencing microbial growth inside the Historic Expedition Huts of Ross Island, Antarctica. *International Biodegradation and Biodeterioration* 55: 45-53.

Held, B.W., Jurgens, J.A., Duncan, S. M., Farrell, R.L., Blanchette, R.A. 2006. Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica. *Polar Biology* 29: 526-531.

Helmke, E., Weyland, H. 2004. Psychrophilic versus psychrotolerant bacteria -occurance and significance in polar and temperate marine habitats. *Cellular and Molecular Biology* 50: 553-561.

Hendricks, C.W., Doyle, J.D., Hugley, B. 1995. A new solid medium for enumerating cellulose-utilizing bacteria in soil. *Applied and Environmental Microbiology* 61: 2016-2019.

Herbert, R.A. 1986. The ecology and physiology of psychrophilic microorganisms In Herbert, R.A., Codd, G.A., (ed.). *Microbes in Extreme Environments.* The Society of General Microbiology, Academic Press, London. pp. 1-24.

Highley, T.L. 1997. Carbohydrolase Assays. In Dashek, W.V. (ed) *Methods in plant biochemistry and molecular biology*. Boca Raton, FL. CRC Press pp 309-321.

Hill, M.J., Roxburgh, S. H., Carter, J.O., McKeon, G.M. 2005. Vegetation state change and consequent carbon dynamics in savanna woodlands of Australia in response to grazing, drought and fire: a scenario approach using 113 years of synthetic annual fire and grassland growth. *Australian Journal of Botany* 53: 715-739.

Hirst, J. M., Gregory, P. H. 1962. Interim report on fungal investigations at Halley Bay, 1961, and mycological examination of snow melt water from Halley Bay, 1960–61. *British Antarctic Survey Archives*, Cambridge, United Kingdom.

Hoflehner, J., Hofleher, K., Harrowfield, D.L. 2003 *Frozen History-the Legacy of Scott and Shackleton*. J. Hoflehner, Wels-Austria.

Holdgate, M.W., 1964. Terrestrial ecology in the maritime Antarctica. In Carrick, R., Holdgate, M.W., Prévost, J. (ed.). *Biologie Antarcticique*. Hermann, Paris. pp. 181-194.

Hoshino, T., Ohgiya, S., Shimanuki, T., Ishizaki, K. 1996. Production of low temperature active Lipase from the pink snow mold *Microdochium nivale* (syn *Fusarium nivale*). *Biotechnology Letters* 18: 509-510.

Hoshino, E., Shiroishi, M., Amano,Y., Nomura, M., Kanda T. 1997. Synergistic actions of exo-typer cellulase in the hydrolysis of cellulose with different crystallinities. *Journal of Fermentation and Bioengineering* 84: 300-306.

Hoshino,T., Tronsmo, A.M., Matsumoto, N., Sakamoto,T., Ohgiya, S., Ishizaki, K. 1997. Purification and characterization of a lipolytic enzyme active at low temperature from Norwegian *Typhula ishikariensis* group III strain *European Journal of Plant Pathology* 103: 357–361.

Hoyoux, A., Blasise, V., Collins, T., D'Amico, S., Gratia, E., Huston, A.L., Marx, J-C., Sonan, G., Zeng, Y., Feller, G., Gerday, C. 2004. Extreme catalysts from low-tempertaure environmnets. *Journal of Bioscience and Bioengineering* 98: 317-320.

Hughes, K.A., Lawley, B. 2003. A novel Antarctic microbial endolithic community within gypsum crusts. *Environmental Microbiology* 5: 555-565.

Hurst, J.L., Pugh, G.J.F., Walton, D.W.H. 1983 Fungal succession and substrate utilization on the leaves of three south georgia phanerogams. *British Antarctica Survey Bulletin* 58: 89-100.

Inglis, G.D., Popp, A.P., Selinger, L.B., Kawchuk, L.M., Gaudet, D.A., McAllister, T.A. 2000. Production of cellulase and xylanase by low-temperature basidiomycetes. *Canadian Journal of Microbiology* 46: 860-865.

Jeffries, T.W. 1990. Biodegradation of lignin-carbohydrate complexes. *Biodegradation* 1: 163-167.

Jervis, E.J., Hayes, C.A., Kilburns, D.G. 1997. Surface diffusion of cellulase and their isolated binding domains an cellulose. *The Journal of Biological Chemistry* 272: 24016-24023.

Johnston, I.A., Calvo, J., Guderley, H., Fernandez, D., Palmer, L. 1998. Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. *Journal of Experimental Biology* 201:1-12.

Johnson, P., Joshi, M., Tomme, P., Kilburn, D., McIntosh, L. 1996. Structure of the N-terminal cellulose-binding domain of *Cellulomonas fimi* Cen C determined by nuclear magnetic resonance spectroscopy. *Biochemisry 35*: 14381-14394.

Jørgensen, H., Mørkeberg, A., Krogh, K.B.R., Olsson, L. 2005. Production of cellulases and hemicellulases by three *Penicillium* species: effect of substrate and evaluation of cellulase adsorption by capillary electrophoresis. *Enzyme and Microbial Technology* 36: 42-48.

Jørgensen, H.,Eriksson, T., Börjesson, J., Tjerneld, F., Olsson, L. 2003. Purification of five celulases and one xylanase from *Penicillium brasilianum* IBT 20888. *Enzyme and Microbial Technology* 32: 851-861.

Jørgensen, H., Olsson, L. 2006. Production of cellulases by *Penicillium brasilianum* IBT 20888—Effect of substrate on hydrolytic performance. *Enzyme and Microbial Technology* 38: 381-390.

Jumpponen, A., Trappe, J.M. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonising fungi. *New Phytologist* 140: 295-310.

Jumpponen, A., Newsham, K.K., Neises, D.J. 2003. Filamentous ascomycetes inhabiting the rhizoid environment of the liverwort *Cephaloziella varians* in Antarctica are assessed by direct PCR and cloning. *Mycologia* 95: 457-466.

Kandror, O., Deleon, A., Goldberg, A.L. 2002. Trehalose synthesis is induced upon exposure of Escherichia coli to cold and is essential for viability at low tempertures. *Proceedings of the National Academy of Sciences of the United States of America* 99: 9727-9732.

Kawahara, H. 2002. The structure and function of ice crystal-controlling proteins from bacteria. *Journal of Bioscience and Bioengineering* 94: 492-496.

Kennedy, A.D. 1995. Antarctic Terrestrial Ecosystem Response to Global Enviromental Change. *Annual Review of Ecology Systems* 26: 683-704.

Kerr, A.J., Goring, D.A.I. 1975. The ultrastructural arrangemnet of the wood cell. *Cellular Chemistry Technology* 9: 563-573.

Kerry,E. 1979. Microbiological studies of soil from Sabrina Island, Antarctica. In Lewis D. (ed.). *Voyage to the ice. The Antarctic expedition of Solo*. Wm Collins, London. pp.112-114.

Kerry, E.J., Weste, G.M. 1985. Succession in the microflora of leaves and litter of three plant species at sub-Antarctic Macquarie Island. In Siegfried, W.R., Condy, P.R., Laws, R.M. (ed.). *Antarctica nutrient cycles and food webs*. Springer, Berlin Heidelberg. pp. 597-605.

Kerry, E. 1990a. Effect of Temperature on Growth Rates of Fungi from Subantarctic Macquarie Island and Casey, Antarctica. *Polar Biology* 10: 293-299.

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

Kimura, S., Kondo, T. 2002. Recent progress in cellulose biosynthesis. *Journal of Plant Research* 115: 297-302.

King P. 1999 *Scott's Last Journey*. Harper Collins, New York.

Kirk, T.K., Farrell, R.L. 1987. Enzymatic "Combustion": The microbial degradation of Lignin. *Annual Review of Microbiology* 41: 465-505.

Kohn, J. 1961. A lipoprotein staining method for zone electrophoresis. *Nature* 28: 312-313.

Kubicek, C.P., Bissett, J., Druzhinina, I., Kullnig-Gradinger, C., Szakacs, G. 2003. Genetic and metabolic diversity of *Trichoderma*: a case study on South-East Asian isolates. *Fungal Genetics and Biology* 38: 310-319.

Kuga, S., Brown, R.M. Jr 1991. Physical structure of cellulose microfibrils: implications for biogenesis. In Haigler, C.H., Weiner, P.J. (ed.). Biosynthesis and Biodegradation of Cellulose. Marcel Dekker. pp.125-142.

Kuga, S., Takagi, S., Brown, R.M. Jr 1993. Native-folded chain cellulose II. *Polymer* 34: 3293-3297.

Kushwaha, R.K.S. 2000. The genus *Chrysosporium*, its physiology and biotechnological potential. In Kushwaha R.K.S., Guarro, J. (ed.). *Biology of Dermatophytes and other Keratinophilic Fungi*. Revista Iberoamericana de Micología, Bilbao. pp. 66-76.

Kyle, P.R. 1990. Erebus volcanic province. Summary. In LeMasurier, W.E. Thomson, J.W. (ed) *Volcanoes of Antarctic Plate and the Southern Oceans*. pp. 81-88.

Land, C.J., Banhidi, Z.G., Albertsson, A-C. 1987. Cold tolerant (psychrotrophic) moulds and blue stain fungi from softwood in Sweden. Growth rates in relation to pH and temperature. *Nordic Journal of Botany- Section of mycology* 7: 97-106.

Langworthy, T.A. 1982. Lipids of bacteria living in extreme environments. *Current Topics in Membranes and Transport* 17: 45-70.

Larsson, P.T., Wickholm, K., Iversen, T. 1997 A CP/MAS ^{13}C NMR investigation of molecular ordering in celluloses. *Carbohydrate Research* 302: 19-25.

Latter, P.M., Heal, O.W. 1971. A preliminary study of the growth of fungi and bacteria from temperate and Antarctic soils in relation to temperature. *Soil Biology Biochemistry*. 3: 365-379.

Lee, Y-H, Fan, L.T., Fan, L-S. 1980. Kinetics of hydrolysis of insoluble cellulose by cellulose. *Advanced Biochemical Engineering* 17: 131-168.

Lewis, D.H., Smith, D.C. 1967. Sugar alcohols (polyols) in fungi and green plants I. Distrubution, physiology and metabolism. *New Phytologist* 66: 143-184.

Levy, J.F., Eveleigh, D.E. 1987. The natural history of the degradation of wood. *Philosophical Transactions of the Royal Society of London. Series A, Mathermatical and Physical Sciences.* 321: 423-433.

Line, M.A. 1988. Micobial flora of soma soils of Mawsons Base and the Vestfold Hills, Antarctica. *Polar Biology* 8: 421-427.

Lisle, J.T., Smith, J. J., Edwards, D.D., McFeters, G. A. 2004. Occurrence of Microbial Indicators and *Clostridium perfringens* in Wastewater, Water Column Samples, Sediments, Drinking Water, and Weddell Seal Feces Collected at McMurdo Station, Antarctica. *Applied and Environmental Microbiology* 70: 7269–7276.

Lizotte, M.P. 2001. The contribution of sea ice algae to Antarctic marine primary production. *American Zoologist* 41: 57-73.

Lüdecke, C. 2003. Scientific collaboration in Antarctica (1901-04): a challenge in times of political rivalry. *Polar Record* 39: 35-48.

Magalhães, P.O., Ferraz, A., Milagres, A.F.M. 2006. Enzymatic properties of two β-glucosidases from *Ceriporiopsis subvermispora* produced in biopulping conditions. *Journal of Applied Microbiology* 101: 480-486.

Mancuso Nichols, C., Garon Lardiere, S., Bowman, J.P., Nichols, P.D., Gibson, J.A.E., Guézennec, J. 2005. Chemical characterisation of exopolysaccharides from Antarctic marine bacteria. Microbial Ecology 49**:** 578-589.

Mandels, M., Parrish, F.W., Reese, E.T. 1962. Sophorose as an inducer of cellulase in Trichoderma viride. *Journal of Bacteriology* 83: 400-408.

Mandels, M., Reese, E.T., 1964. Fungal cellulases and the microbial decomposition of cellulosic fabric. *Developments in Industrial Microbiology* 5:5–20.

Mandels, M., Reese, E.T. 1999. Fungal Cellulase and the Microbial Decomposition of Cellulosic Fabric. *Journal of Industrial Microbiology and Biotechnology* 22: 225-240.

Mansfield, S.D., Saddler, J.N., Gübitz G.M. 1998. Characterization of endoglucanases from the brown rot fungi *Gloeophyllum sepiarium* and *Gloeophyllum trabeum*. *Enzyme and Microbial Technology* 23: 133–140.

Mansfield, S.D., Mooney, C., Saddler, J.N. 1999. Substrate and Enzyme Characterisation that limit Cellulose Hydrolysis. *Biotechnology Progress* 15: 804-816.

Marshall, C.J. 1997. Cold adapted enzymes. *Trends in Biotechnology* 15: 359-364.

Marshall, W.A. 1997. Seasonality in Antarctic Airborne Fungal Spores. *Applied and Enviromental Microbiology* 63: 2240-2245.

Marshall, W.A. 1998. Aerial transport of keratinaceous substrate and distrubution of the fungus *Geomyces pannorum* in Antarctic soils. *Microbial Ecology* 36: 212-219.

Mazur, P. 1984. Freezing of living cells: mechanisms and implications. *American Journal of Physiology: Cell Physiology* 16: 125–142.

McGonigal, D., Woodworth, L. 2001. *Antarctica: The complete story*. Auckland, Random House New Zealand.

McRae, C.F., Alisa, D., Hocking, Seppelt, R.D. 1999. *Penicillium* species from terrestrial habitats in the Windmill Islands, East Antarctica, including a new species, *Penicillium antarcticum*. *Polar Biology* 21**:** 97-111.

McRae, C.F., Seppelt, R.D. 1999 Filamentous fungi of the Windmill Islands, continental Antarctica. Effect of water content in moss turves on fungal diversity. *Polar Biology* 22**:** 389-394.

Medve, J., Lee, D., Tjerneld, F. 1998. Ion-exchange chromotographic purification and quantitative analysis of Trichoderma reesei cellulase cellobiohydrolase I, II and endoglucanase II by fast protein liquid chromotography. *Journal of Chromotography A* 808**:** 153-165.

Melick, M.D., Seppelt, R.D. 1994. The effect of hydration on carbohydrate levels, pigment content and freezing point of *Umbilicaria decussata* at a continental Antarctic locality. *Cryptogamic Botany* 4**:** 212-217.

Melchior, D.L. 1982. Lipid phase transitions and regulation of membrane fluidity in prokaryotes. *Current Topics in Membranes and Transport* 17**:** 263-307.

Mercantini, R., Marsella, R., Moretto, D., Finotti, E. 1993 Keratinophilic fungi isolated from the Antarctic environment. *Mycopathologia* 122: 169-175.

Mercantini, R., Marsella, R., Cervellati, M.C. 1989 Keratinophilic fungi isolated from Antarctic soil. *Mycopathologia* 106: 47-52.

Meyer, G.H. 1962. Microbiology populations of Antarctic air, soil snow and melt pools. *Polar Record* 11: 317-318.

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

Meyer, G.H., Morrow, M.B., Wyss, O. 1963. Viable organisms from faeces and foodstuffs from early Antarctic expeditions. *Canadian Journal of Microbiology* 9: 163-167.

Meyer, G.H., Morrow, M.B., Wyss, O. 1967. Bacteria, fungi and other biota in the vicinity of Mirnyy Observatory. *Antarctic Journal* 248-255.

Miteva, V.I., Brenchley, J.E. 2005. Detection and isolation of ultrasmall microorganisms from 120,000-year-old Greenland glacier ice core. *Applied and Environmental Microbiology* 71: 7806-7818.

Miyazaki, K., Wintrode, P.L., Grayling, R.A., Rubingh, D.N., Arnold, F.H. 2000. Directed Evolution Study of Temperature Adaptation in a Psychrophilic Enzyme. *Journal of Molecular Biology* 297: 1015-1026.

Mo, H., Zhang, X., Li, Z. 2004. Control of gas phase for enhanced cellulase production by *Penicillium decumbens* in solid-state culture. *Process Biochemistry* 39:1293-1297.

Möller, C., Dreyfuss, M.M. 1996. Microfungi from Antarctica lichens, mosses, and vascular plants. *Mycologia* 88: 922-933.

Montiel, P.O. 2000. Soluble carbohydrates (trehalose in particular) and cryoprotection in polar biota. *Cryoletters* 21: 83-90.

Morita, R.Y. 1975. Psychrophilic Bacteria. *Bacteriological Reviews* 39: 144-167.

Murashima, K., Kosugi, A. Doi, R.H. 2002. Thermostabilization of cellulosomal endoglucanase EngB from *Clostridium cellulovorans* by *in vitro* DNA recombination with non-cellulosomal endoglucanase EngD. *Molecular Microbiology* 45: 617-626.

Nedwell, D.B. 1999. Effect of low temperature on microbial growth: lowered affinity for for substrates limits growth at low temperature. *FEMS Microbiology Ecology* 30: 101-111.

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

Nichols, D.S., Miller, M.R., Davies, N.W., Goodchild, A., Raftery,M., Cavicchioli, R. 2004. Cold adaptation in the Antarctic archaeon Methanococcoides burtonii involves membrane lipid unsaturation. *Journal of Bacteriology* 186: 8508-8515.

Nofal, M., Kumaran, K. 1999. Durability assessment of wood-frame construction using the concept of damage functions. Eds. M.Lacasse and D. J. Vanier *Durability of Building Materials and Components* 1: 766-779.

North, M.J. 1982. Comparative biochemistry of the proteinases of Eucaryotic microorganisms. *Microbiological Reviews* 46: 308-340.

Nuero, O.M., Reyes, F. 2002. Enzymes for animal feeding from *Penicillium chrysogenum* mycelial wastes from penicillin manufacture, *Letters in Applied Microbiology* 34: 413 - 416.

Ogunseitan, O. 2004. *Microbial Diversity: Form and Function in Prokaryotes*. Blackwell, London.

Oliveira, L.A., Porto, A.L.F. Tambourgi, E.B. 2006. Production of xylanase and protease by *Penicillium janthinellum* CRC 87M-115 from different agricultural wastes. *Bioresource Technology* 97: 862-867.

Olsen, R.H., Metcalf, E.S. 1968. Conversion of mesophilic to psychrophilic bacteria. *Science* 162: 1288-1289.

Onofri, S., Tosi, S. 1992. *Arthrobotrys ferox* sp. a springtail-capturing hyphomycete from contiental Antarctica. *Mycotoxon* 42: 445-451.

Onofri, S., Tosi, S., Persiani, A.M., Maggis, O., Riess, S., Zucconi, L. 1994. *Mycological researches in Victoria Land, terrestrial ecosytems*. In Battaglia, B., Bisol, P.M., Varotto, V. (ed.). Scienza e cultura Proceedings of the 2nd meeting on Antarctic Biology, Padova, 26-28 Febuaray 1992. Edizioni Universitarie Patavine, Italy. pp.19-32.

Onofri, S. 1999. Antarctic microfungi. In. Seckbach. J. (ed.). *Enigmatic microorganisms and life in extreme environments*. Kluwer, Dordrecht, pp. 323-336.

Onofri, S.M.F., Cicalini, A.R., Tosi, S., Magrino, A., Pagano, S., Selbmann, L., Zucconi, L., Vishniac, H., Ocampo-Friedmann, R., Friedmann, E.I. 2000. Ecology and biology of microfungi from Antarctic rocks and soils. *Italian Journal of Zoology* Supplement 1: 163-167.

Onofri, S., Selbmann, L., Zucconi, L., Pagano, S. 2004. Antarctic microfungi as model exobiology. *Planetary and Space Science* 52: 229-237.

Panoff, J.-M., Thammavongs, B., Gueguen, M., Boutibonnes, P. 1998. Cold Response in Mesophilic Bacteria. *Cryobiology* 36: 75-83.

Parsiegla, G., Juy, M., Reverbel-Leroy, C., Tardif, C. Belaïch, J-P., Driguez, H. Haser, R. 1998.The crystal structure of the processive endocellulase CelF of *Clostridium cellulolyticum* in complex with a thiooligosaccharide inhibitor at 2.0 Å resolution. *The EMBO Journal* 17: 5551–5562.

Pearson, M. 1992. Expedition huts in Antarctica: 1899-1917. *Polar Record* 28: 261-276.

Peek, K., Veitch, D.P., Prescott, M., Daniel, R. M., MacIver, B., Bergquist, P.L. 1993. Some characteristics of a proteinase from a thermophilic *Bacillus* sp. expressed in *Escherichia coli*: comparison with the native enzyme and its processing in E. coli and in vitro. Applied and Environmental Microbiology 59: 1168-1175.

Phadtare, S., Alsina, J., Inouye, M. 1999. Cold-shock response and cold-shock proteins. *Current Opinion in Microbiology* 2: 175-180.

Piozynski, K.A. 1976. Fossil fungi. *Annual Reviews* 237-246.

Pörtner, H.O., Hardewig, I., Sartoris F.J,van Dijk P.L.M. 1998. Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? In: Pörtner HO, Playle R (ed.). *Cold ocean physiology*. Cambridge University Press, Cambridge. pp. 88-120.

Preston-Mafham, J., Broddy, L., Randerson, P.F. 2002. Analysis of micobial community functional diversity using sole-carbon-source utilization profiles- a critique. *FEMS Microbiology Ecology* 42: 1-14.

Prothero, D.R. The Late Eocene-Oligocene Extinctions. *Annual Review Earth Planetary Science* 22: 145-165.

Protocol on Environmental Protection to the Antarctic Treaty. 1991.

Pugh, G.J.F., Allsopp, D. 1982. Microfungi on Signy Island, South Orkney Islands. *British Antarctic Survey Bulletin* 57: 55-67.

Quartermain, L. 1963. *Two Huts in the Antarctic*. R.E. Owen, Wellington.

Quensada, A., Vincent, W.F. 1997. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *European Journal of Phycology* 32: 335-342.

Rabinovich, M.L., Melnick, M.S., Bolobova, A.V. 2002. The structure and mechanism of action of cellulolytic enzymes. *Biochemistry (Moscow)* 67: 850-871.

Rai, B. 1976. Study of fungi on decaying leaves of *Saccharum munja* by differential washing techniques. *Plant and Soil* 44: 233-236.

Rajal, V.B.,Carrillo, L., Cuevas, C.M. 2002. Studies on exocellular hydrolases from a new *Penicillium ulaiense* strain. *World Journal of Microbiology and Biotechnology* 18: 713-714.

Rånby, B.G. 1952. The mercerization of cellulose. III. A phase transition study using electron diffraction. *Acta Chemica Scandinavica* 6: 128-135.

Readers Digest 1985. *Antarctica Great Stories from the Frozen Continent*. NSW, Reader's Digest Service Pty Limited.

Reese, E.T., Maguire, A,H., Parrish, F.W. 1968. Glucosidases and exo-glucanases. *Canadian Journal of Biochemistry* 46: 25–34.

Reese, E.T. 1977. Degradation of polymeric carbohydrates by microbial enzymes. In Loewus, F.A., Runeckles, V.C. (ed.). *Recent advances in phytochemistry*. Plenum Publishing Corporation, New York. pp. 311-367.

Reichenbecher, M., Lottspeich, F., Bronnrnmeier, K. 1997. Purification and properties of a cellobiose phosphorylase (CepA) and cellodextrin phosporylase (CepB) from the cellulolytic thermophile *Clostridium stercorarium*. *European Journal of Biochemistry* 247: 262-267.

Rice, A. V., Tsuneda, A., Currah, R. S. 2006. *In vitro* decomposition of Sphagnum by some microfungi resembles white-rot of wood. *FEMS Microbiology Ecolology* 56: 372–382.

Robinson, P.M., Morris, G.M. 1984. Tolerance of hyphae of *Fusarium oxysporum* f. sp. *lycopersici* to low temperature. *Transactions of the British Mycological Society* 83: 569-573.

Robinson, C.H. 2001. Cold Adaption in Arctic and Antarctic fungi. *New Phytologist* 151: 341-353.

Roser, D.J., Seppelt, R.D., Ashbolt, N. 1993. Microbiology of orthinogenic soils from the Windmill Island, Budd Coast, continental Antarctica: microbial biomass distribution. *Soil Biology and Biochemistry* 25:165-175.

Roser, D.J., Seppelt, R.D., Nordstrom, O. 1994. Soluble carbohydrate and organic content of soils and associated microbiota from the Windmill Islands, Budd Coast, Antarctica. *Antarctic Science* 6: 53-59.

Ruiz-Arribas, A., Fernandez-Abalos, J.M., Sanchez, P., Garda, A.L., Santamaria, R.I. 1995. Overproduction, purification, and biochemical characterization of a xylanase (Xys1) from *Streptomyces halstedii* JM8. *Applied and Environmental Microbiology*. 61: 2414-2419.

Russell, N.J. 1990. Cold adaptation of microorganisms. *Philosophical Transactions of the Royal Society of London B* 326: 595-608.

Russell, N.J. 1992. Physiology and molecular biology of psychrophilic microorganisms. In Herbert, R.A., Sharp, R.J.(ed.). *Molecular Biology and Biotechnology of Extremophiles*. Chapman and Hall Inc, New York. pp. 203-224.

Saddler, J.N., Brownell, H.H., Clermont, L.P.,Levitin, N. 1982. Enzymatic hydrolysis of cellulose and various pretreated wood fractions. *Biotechnology and Bioengineering* 24: 1389–1402.

Saha, B., Bothast, R. 1996. Production, purification and characterization of a highly glucose-tolerant novel ß-glucosidase from *Candida peltata*. Applied and Environmental Microbiology. 62: 3165-3170.

Sanderson, P.G., Spotts, R.A. 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium*. *Phytopathology* 85: 103-110.

Sarko, A., Muggli, R. 1974. Packing analysis of carbohydrates and polysaccharides. III. *Valonia* cellulose and cellulose II. *Macromolecules* 7: 486–494.

Satyanarayana, T, Raghukumar, C, Shivaji, S. 2005. Extremophilic microbes: Diversity and perspectives. *Current Science* 89: 78–90.

Sautour, M., Rouget, A., Dantigny, P., Dives, C., Bensoussan, M. 2001. Prediction of conidial germination of *Penicillium chrysogenum* as influenced by temperature, water activity and pH. *Letters in Applied Microbiology* 32: 131-134.

Savoy, J.G. 1954. Breakdown of timber by Ascomycetes and Fungi Imperfectii. *Annuals of Applied Biology* 41: 336-347.

Saxena, I.M., Brown, R.M. 2000. Cellulose synthases and related enzymes. *Current Opinion in Plant Biology* 3: 523-531.

Schlesinger, W.H., Andrews, J.A. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48: 7–20.

Schofield, E. 1971. Preserving the scientific value of cold desert ecosystems: Past and present practises and a rationale for the future. In Parker, B.C. (ed.). *Proceedings of the colloquium on conservation problems in Antarctica*. pp. 193-225.

Schwarze, F.W.M.R., Engels, J., Mattheck, C. 2000. *Fungal strategies of wood decay in trees*. Springer-Verlag, Berlin.

Sehnem, N.T. de Bittencourt, L.R., Camassola, M., Dillon, A.J.P. 2006. Cellulase production by *Penicillium echinulatum* on lactose. *Applied Microbiology and Biotechnology* 72:163-167.

Seiboth, B., Hakola, S., Mach, R.L., Suominen, P.L., Kubicek,C.P. 1997. Role of four major cellulases in triggering of cellulase gene expression by cellulose in Trichoderma reesei. J*ournal of Bacteriology* 179: 5318-5320.

Selbmann, L., Onofri, S., Fenice, M., Federici, F., Petruccioli, M. 2002. Production and structural characterization of the exopolysaccharide of Antarctic fungus *Phoma herbarum* CCFEE 5080. *Research in Microbiology* 153: 585-592.

Selbmann, L., de Hoog, G.S., Mazzaglia, A., Friedmann, E.I., Onofri, S. 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology* 51: 1–32.

Shackleton, E. H. 1909. Some Results of the British Antarctic Expedition, 1907-9 *The Geographical Journal* 34: 481-500.

Sharrock, K.R., 1988. Cellualse assay methods: a review. *Journal of Biochemical and Biophysical Methods* 17: 81-106.

Sheppard, D.S., Deely, J.M., Edgerley, W.H.L. 1997. Heavy metal content of meltwaters from the Ross Dependency, Antarctica. *New Zealand Journal of Marine and Frshwater Research* 31: 313-325.

Shoseyov, O., Takagi, M., Goldstein, M.A., Doi, R.H. 1992. Primary sequence analysis of *Clostridium cellulovorans* cellulose binding protein A. *Proceedings of the National Academy of Sciences of the United States of America* 89: 3483-3487.

Sicheri, F., Yang, D. S. 1995. Ice-binding structure and mechanism of an antifreeze protein from winter flounder. *Nature* 375: 427–431.

Siegfried, W.R. 1981. The role of birds in ecological processes affecting the functioning of the terrestrial ecosystem at sub-Antarctic Marion Island. *Comité National Francais des Recherches Antarctiques* 51: 493-499.

Singh, S., Thakur, D.P., Singh, J.P. 1983. Post harvest decay of ripe tomato fruits caused by Cladosporium oxypsorum *Indian Phytopathology* 36: 723-724.

Singh, A.P., Butcher, J.A. 1991. Bacterial degradation of wood cells: A review of degardation patterns. *Journal of Institute of Wood Sciences* 12: 143-157.

Skotnicki, M.L., Selkirk, P.M., Ninham, J.A. 1998. RAPD analysis of genetic variation and dispersal of the moss Bryum pseudotriquetrum from Southern Victoria Land, Antarctica *Polar Biology* 20: 121-126.

Smith, S.E., Reid, D.J. 1997. *Mycorrhizal symbiosis* 2nd edn. Academic Press Ltd, London, UK.

Snider, C.S., Hsiang, T., Zhao, G., Griffith, M. 2000. Role of ice nucleation and antifreeze activities in pathosenesis and grwoth of snow molds. *Phytopathology* 90: 354-361.

Sommer, A., Portner, H.O., 1999. Exposure of *Arenicola marina* (L.) to extreme temperatures: adaptive flexibility of a boreal and a subpolar population. *Marine Ecolology Programme Service* 181: 215–226.

Ståhlberg, J., Johansson, G., Pettersson, G. 1988. A binding-site-deficient, catalytically active, core protein of endoglucanase III from the culture filtrate of Trichoderma reesi. *European Journal of Biochemistry* 173: 179-183.

Staley, J.T., Gosink, J.J. 1999. Poles Apart: Biodiversity and Biogeography of sea ice bacteria. *Annual Review of Microbiology*. 53: 189-215.

Steenbakkers, P.J.M., Harhangi, H.R., Bosscher, M.W. van der Hooft, M.M.C. Keltjens, J.T., van der Drift, C., Vogels, G.D., op den Camp, H.J.M. 2003. beta-Glucosidase in cellulosome of the anaerobic fungus Piromyces sp. strain E2 is a family 3 glycoside hydrolase**.** *Biochemistry Journal* 370: 963–970.

Stubblefield, S.P., Taylor, T. N. 1986. Wood decay in silicified gymnosperms from Antarctica. *Botanical Gazette* 147: 116-125.

Stubblefield, S.P., Taylor, T. N., Trappe, J. M. 1987. Vesicular-Arbuscular Mycorrhizae From The Triassic of Antarctica. *American Journal of Botany* 12: 1904-1911.

Stubblefield, S.P., Taylor, T. N. 1988. Tansley Review No 12 Recent advances in palaemycology. *New Phytologist* 108: 3-25.

Sugar, D., Powers, K. 1986. Interaction among fungi causing postharvest decay of pear. *Plant Diseases* 70: 1132-1134.

Sugiyama. J., Harada, H., Fujiyoshi, Y., Uyeda, N. 1985. Lattice images from ultrathin sections of cellulose microfibrils in the cell wall of Valonia macrophysa Kutz, *Planta* 166: 161-168.

Sun, S.H., Huppert, M., Cameron, R. E. 1978. Identification of Some Fungi from Soil and Air of Antarctica. *Antarctic Research Service* 30: 1-26.

Sutherland, J.B., Crawford, D.L. 1981. Lignin and glucan degradation by species of the *Xylariaceae Transactions of the British Mycological Society* 76: 335–337.

Taylor, T.N., White Jr., J. F. 1989. Fossil Fungi (endogonaceae) from the Triassic of Antarctica. *American Journal of Botany* 76: 389-396.

Teather, R. M., Wood, P. J. 1982. Use of Congo Red-Polysaccharide Interaction in Enumeration and Characterisation of Cellulytic Bacteria from the Bovine Rumen. *Applied and Environmental Microbiology*. 43: 777-780.

Tenkanen, M., Puls, J., Rättö, M., Viikari, L. 1993. Enzymatic deacetylation of galactoglucomannans. Applied and Microbiological biotechnology 39: 159-165.

Thammavongs, B., J-M. Panoff, M. Gueguen. 2000. Phenotypic adaption to freeze-thaw stress of the yeast-like fungus *Geotrichium candidum*. *International Journal of Food Microbiology* 60: 99-105.

Thieringer, H.A., Jones, P.G. Inouye, M. 1998. Cold shock and adaptation. *BioEssays* 20: 49–57.

Thomas, T.M., Scopes, R.K. 1998. The effect of temperture on the kinetics and stability of mesophilic and thermophilic 3-phosphoglycerate kinase. *Biochemical Journal* 330: 1087-1095.

Thompson, D.C., Bromley, A.M., Craig, R.M.F. 1971. Ground temperatures in an Antarctic Dry Valley. *New Zealand Journal of Geology and Geophysics*. 14: 477-483.

Tomaz, C.A., Queiroz, J.A. 1999. Studies on the chromotographic frcationation of Trichoderma reesei cellulases by hydrophoboic interaction. *Journal of Chromotography A* 865: 123-128.

Tomme, P., R. A.J. Warren, N.R. Gilkes. 1995. Cellulose Hydrolysis by Bacteria and Fungi. *Advances in Microbial Physiology* 37: 1-81.

Tosi, S., Casado, R., Gerdol, R.,Caretta, G. 2002. Fungi isolated from Antarctic Mosses. *Polar Biology* 25: 262-268.

Tosi, S., Onofri, S., Brusoni, M., Zucconi, L., Vishniac, H. 2005. Response of Antarctic soil fungal assemblages to experimental warming and reduction of UV radiation. *Polar Biology* 28: 470-482.

Toyoda, S., Enokida, N., Matsumae, A., Aiso M. 1985. Microbiological investigation of the human pollution at Syowa Station in Antarctica. Special reference to the specimen collected by the 23rd Japanese Antarctic Research Expedition. II. *Journal of Antibacterial and Antifungal Agents*. 13: 541-546.

Toyoda ,S., Matsumae, A., Ghoda, A., Aiso, M. 1986. Total number of bacteria and fungi as an index for human pollution at Soyowa Station in Antarctica. I. Special reference to the specimen collected by the 18th Japanese Antarctic Research Expedition. Japan Journal of Bacteriology. 41: 527-534.

Tubaki, K., 1961. On some fungi isolated from Antarctic materials. *Biological results of Japanese Antarctic Research Expedition* 14: 3-9.

Tubaki, K., Asano, I. 1965. Additional species of fungi isolated from the Antarctica materials. JARE 1956-62. *Scientific Report Ser.E.* 27:1- 12.

Ugolini, F.C. 1970. Antarctic soils and their ecology. In Holdgate M.W. (ed). *Antarctic Ecology* Vol 2. Academic Press, London. pp. 673-692.

Upton, M., Pennington, T.H., Haston, W., Forbes, K.J., 1997. Detection of human commensals in the area around an Antarctic research station. *Antarctic Science* 9: 156-161.

Uydess, I.L., Vishniac, W.V. 1976, Electron microscopy of Antarctica soil bacteria. In Heinrich M.R. (ed.). *Extreme Environments*. Academic Press, New York. pp. 29-56.

van Tilbeurgh, H., Claeyssens, M. 1985 Detection and diferrentiation of cellulase components using low molecular mass fluorogenic substartes. *FEBS Letters* 187: 283-288.

van Wyk J.P.H. 1999. Saccharification of paper products by cellulase from *Penicillium funiculosum* and *Trichoderma reesei*. *Biomass and Bioenergy* 16: 239-242.

van Wyk, J.P.H., Leogale, P.B. 2001. Saccharification of wastepaper mixtures with cellulase from Penicillium funiculosum. *Biotechnology Letters* 23: 1849-1852.

Vincent, W.F. 2000. Evolutionary Origins of Antarctic microbiota: invasion, selection and endemism. *Antarctic Science* 12: 374-385.

Vishniac, H.S. 1993. The microbiology of Antarctic soils. In Friedmann, E.I. (ed.). *Antarctic Microbiology*. Wiley-Liss, New York. pp. 297-341.

Vishniac, H.S. 1996. Biodiversity of yeasts and filamentous fungi in terrestrial Antarctic ecosystems. *Biodiversity and Conservation* 5: 1365-1378.

Vishniac, H.S., Hempling, W.P. 1979. *Crytococcus vishniacii* sp. nov., an Antarctic yeast. *International Journal of Systematic Bacteriology* 29: 153-158.

Walton, D.W.H. 1985 cellulose decomposition and its relationship to nutrient cycling at South Georgia. In Siegfried, W.R., Condy, P.R., Laws, R.M. (ed.). *Antarctic Nutrient Cycles and Food Webs*. Springer-Verlag, Berlin, Heidelberg. pp.192-199.

Wang, C.Y. 2003. Maintaining postharvest quality of raspberries with natural volatile compounds**.** *International Journal of Food Science & Technology* 38: 869-875.

Wang, J.L., Gao, P.J. 2000. In vitro expression of *Penicillium janthinellum* cellobiohydrolase I gene in a coupled transcription-translation system. *Journal of Biotechnology* 81: 205-209.

Waterhouse, E.J. 2001. *Ross sea Region: A State of the Environment Report for the Ross Sea Region of Antarctica*. New Zealand Antarctic Institute, Christchurch.

Waterman, T.H. 1999. The Evolutionary Challenges of Extreme Environments (Part 1). *Journal of Experimental Zoology* 285: 326–359.

Weinstein, R.N., Palm, M., Johnstone, K., Wynn-Williams, D.D. 1997. Ecological and physiological characterisation of *Humicola marvinii*, a new psychrophilic fungus from fellfield soils in the maritime Antarctica. *Mycologia* 89: 706-711.

Weinstein, R.N., Montiel, P. O., Johnstone, K. 2000. Influence of growth temperature on lipid and soluble carbohydrate synthesis by fungi isolated from fell field soil in the Maritime Antarctic. *Mycologia* 92: 222-229.

Weyant, W.S. 1966 The Antarctic atmosphere: climatology of the surface environment. In Weyant, W.S. (ed.). *Antarctic Map Folio Series*. American Geography Society, New York. pp.1-13.

White, Jr.J.F, Taylor, T.N. 1988. Triassic Fungus from Antarctica with Possible Ascomycetous Affinities. *American Journal of Botany* 75: 1495-1500.

Wiebe, W.J., Sheldon, J.R., Pomeroy, L.R. 1992. Bacterial growth in the cold: Evidence for an enhanced substrate requirement. *Applied and Environmental Microbiology* 58: 359-364.

Wierzchos, J., Ascaso, C. 2002. Micobial fossil record of rocks from the Ross Desert, Antarctica: implications in the search for past life on Mars. *International Journal of Astrobiology* 1: 51-59.

Willerslev, E., Hansen, A.J., Christensen, B., Steffensen, J.P., Arctander, P. 1999. Diversity of holocene life forms in fossil glacier ice. Procedings of the national academy of Science 96: 8017-8021.

Wood, T.M. 1975. Properties and mode of action of cellulase. *Biotechnology and Bioengineering Symposium* 5:111-137.

Wood, T.M., McCrae, S.I. 1979. Synergism between enzymes involved in the solubilization of native cellulose. *Advanced Chemical Ser* 181: 181-209.

Wynn-Williams, D.D. 1990. Ecological aspects of Antarctic microbiology. *Advances in Microbial Ecology*. 11: 71-146.

Wynn-Williams, D.D. 1991. Aerobiology and colonization in Antarctica- the BIOTAS Programme. *Gana* 30: 380-393.

Wynn-Williams, D.D. 1996. Antarctic microbial diversity: the basis of the polar ecosystem process. *Biodiversity and Conservation* 5: 1271-1293.

Yamanaka. K. 1994. Cold shock response in *Escherichia coli*. *Journal of Molecular Microbiology and Biotechnology*. 2:193-202.

Yamamoto, H., Shuji Ohtani, Kadzunori Tatsuyama and Masaru Akiyama. 1991. Preliminary report on cellulolytic activity in the Antarctic region (Extended Abstract). *Proceedings of the NIPR Symposium on Polar Biology* 4: 179-182.

Yayanos. A.A. 1995 Microbiology to 10,500 metres in the deep sea**.** *Annual Reviews Microbiology* 49: 777-805.

Yu, A.H.C, Lee D., Saddler J. N. 1993. A quantitative approach to the study of the adsorption/desorption of cellulase components in a crude cellulase mixture. *Biotechnology Techniques* 7: 713-718.

Zabel R.A., Morrell, J.J. 1992. *Wood Microbiology: decay and its prevention*. Academic Press, California.

Zecchinon, L., Claverie, P., Collins, T., D'Amico, S., Delille, D., Feller, G., Georlette, D., Gratia, E., Hoyoux, A., Meuwis, M-A., Sonan, G., Gerday, C. 2001. Did pyschrophilic enzymes really win the challenge? *Extremophiles* 5: 313-321.

Zimmerman T, Sell J. 1997. The fine structure of the cell wall on transverse-fracture surfaces of longitudinally tension-loaded hardwoods. *Research and Works Reports, EMPA Department of Wood* 115: 32

Zucconi, L., Pagano, S., Fenice, M., Selbmann, L., Tosi, S., Onofri, S. 1996. growth temperature preference of fungal strains from Victoria Land, Antarctica. *Polar Biology* 16: 53-61.

Appendix 1 Sample locations and presence/absence of fungi

Sample numbers, locations of the 849 samples taken as part of this PhD thesis research are given in Appendix 1.
For January 1998, and December 1998 isolations were done at 4, 15 and 25°C. Whether fungi were isolated from a sample is indicated by a + if fungi were isolated or- if no fungi were isolated.
Different media were used each year. Whether fungi were isolated from a sample is indicated by a + if fungi were isolated or- if no fungi were isolated.

Discovery Hut January 1998

Table A1.1: Sample identification, location at *Discovery* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; January 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
H22	Soil NE corner post of veranda	+	+	+
H32	Straw behind pick axe surface	+	+	+
H33	Straw same as H32 but interior 1cm	+	+	+
H34	Mutton carcass Exterior wall meat and fat	-	-	+
H35	Straw in hole in meat room floor	+	+	+
H36	Mutton carcass meat Internal	-	-	+
H39	Swab Mould on surface of roof latrine room	+	+	+
H41	Roof in porch above straw Dark wood	+	+	+
H43	Swab Wall behind mutton carcass	+	+	-
H44	Scrapped wood wall behind carcass	-	-	+
H46	Salt deposit upper window next to pendulum	-	-	+
H47	Compacted rubbish floor of pendulum room	+	+	+
H51	Swab behind the door in the pendulum room	-	-	-
H52	Ice/muck top of wood inside pendulum area	+	+	+
H59	Oats bag in pendulum room Number H C/4.2	-	+	+
H65	Swab ceiling main area	-	-	+
H66	Dark wood ceiling main are by door	-	-	+

Table A1.2: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut January 1998.

Sample ID	Media 4	Media 6	YM	Media 7	V B Agar
H22	+	+	+	+	+
H32	+	-	+	-	+
H33	+	+	+	-	+
H34	-	-	+	-	-
H35	+	+	+	-	+
H36	-	-	+	-	-
H39	-	-	-	-	-
H41	+	-	+	-	-
H43	+	-	+	-	-
H44	-	-	+	-	-
H46	-	-	+	-	-
H47	+	-	+	+	-
H51	-	-	-	-	-
H52	+	+	+	+	+
H59	-	-	+	-	+
H65	-	+	-	-	-
H66	-	+	-	-	-

Terra Nova Hut January 1998

Table A1.3: Sample identification, location at *Terra Nova* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; January 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
E2	Wood Underneath box immediately left of front door on floor writing on box 2 S Oxtail soup	+	+	+
E4	Swab Underneath box immediately left of front door on floor	+	+	+
E10	Swab Lyle golden syrup (molasses) S W wall oozing out	-	+	+
E11	Swab doorstep	+	+	+
E15	Swab wall behind table	+	+	+
E19	Swab on S wall near floor underneath bed frame	+	+	+
E22	Wall scraping underneath bed frame	+	+	+
E23	Swab box W of 1st bunk S wall	-	-	+
E24	Swab Box E of stove Black mottle surface	-	-	-
E27	Swab under candles on box W of 1st bunk S wall	+	-	+
E28	Swab Can of real turtle soup on boxes W of first bunk on S wall Artefact number E/BF/ 425.1-2	+	+	-
E30	Swab piece of wood near floor 1st boxes W of 1st bunk on S wall	+	+	+
E33	Swab wall between 1st and 2nd bunk S side	-	-	+
E35	Swab damp spot against wall lower bunk on S side	+	+	+
E38	Swab boot under 2nd bunk on s side	+	+	+
E43	Swab under mitten 2nd bunk	-	-	-
E44	Swab inside neck collar 2nd bunk	-	-	+
E45	Swab mitt N end 1st bunk (mycelium colonies)	-	+	-
E46	Swab table cloth between 1st and 2nd bunk	+	+	+
E53	Swab Wood underneath bunk 4	-	-	-
E53	Wood underneath bunk 4	-	-	-
E55	Leather strap attached 3 feet from floor just E of window on S	-	-	+
E68	Wood Door step into darkroom	-	-	+
E72	Swab board under bench in from corner (swab of mycelium)	-	+	+
E73	Swab left of darkroom Entrance under bench	-	+	+
E76	Wood fibre bottom of shelf above table to the left of darkroom Entrance	-	+	+
E79	Fibres canvas stool under table near Scott's bed	+	-	-
E83	Swab boots at the south end of Scott's bed	-	+	+
E84	Swab wall next to bunk closest to food boxes	-	-	+
E88	Swab Flax fibre on shelves on North side under first window	-	-	+
E93	Swab bottom box containing wholemeal flour scattered black blotches	-	+	+
E95	Swab under shelf	-	+	-
E102	Swab Under table on W wall left of Entrance	-	-	+
E103	Swab Wall under table west of Entrance	-	+	+
E104	Swab Box on floor E of table on west wall of Entrance	-	-	-
E106	Swab on wall just S of entrance darkroom	-	-	-

Table A1.3: Sample identification, location at *Terra Nova* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; January 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
E109	Swab box from top of door into darkroom	-	-	+
E113	Swab blubber	+	-	+
E116	Soil around blubber	-	-	+
E127	Swab horse harnesses	+	+	+
E129	Swab green post in stall 3	-	-	+
E131	Hard resinous material on top of hay in stall 4	-	-	+
E132	Swab Cloth bag that resin was in stall 4	+	+	+
E134	Feather stall 4	+	+	+
E139	Wood corner hut near blubber	-	-	-
E141	Scraping white mycelium on s wall by blubber	-	-	+
E144	Swab\outside W wall of porch	-	-	+
E145	Swab boot from under Taylor's bunk frozen white mycelium artefact number E/C/264.2	-	-	+
E146	Swab same boot as E145 but from upper to sole junction	+	+	+
E147	Same boot asE145 but at toe of boot	+	+	+
E148	Same boot as E145 but on sole by nail green	+	+	-
E149	Swab Boot frozen textile upper part white matted mycelium from single colony	-	-	+
E151	Swab enclosed area on the way to the stables green surface mould Artefact numbers E/B/656.2 E/B/664	-	+	+
E153	Swab lower third cupboard Right side just before entry	-	-	+
E195	Soil sample along E end	-	-	-
M	Mylar	-	-	-

Table A1.4: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 1998.

Sample ID	**Media 4**	**Media 6**	**YM**	**Media 7**	**V B Agar**
E2	+	+	+	-	+
E4	+	+	+	-	+
E10	+	-	-	-	-
E11	-	+	+	-	+
E15	+	+	+	-	+
E19	+	+	+	-	+
E22	+	+	+	-	+
E23	-	+	+	-	+
E24	-	-	-	-	-
E27	-	-	+	-	-
E28	-	-	+	-	+
E30	+	+	+	+	+
E33	-	+	-	-	-
E35	+	+	+	-	+
E38	+	+	+	-	+
E43	-	-	-	-	-
E44	-	-	-	-	-
E45	+	-	-	-	-
E46	+	-	+	-	+
E53	-	-	-	-	-
E53	-	-	-	-	-
E55	-	-	-	-	-
E68	-	-	+	-	-

Table A1.4: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 1998.

Sample ID	Media 4	Media 6	YM	Media 7	V B Agar
E72	-	+	-	-	-
E73	-	-	-	-	-
E76	-	-	+	-	-
E79	-	-	-	-	-
E83	-	-	-	-	-
E84	-	-	+	-	-
E88	-	-	-	-	-
E93	-	-	+	-	-
E95	-	-	-	-	-
E102	-	-	-	-	-
E103	+	-	-	-	-
E104	-	-	-	-	-
E106	-	-	-	-	-
E109	-	-	-	-	-
E113	-	-	-	-	-
E116	-	-	-	-	-
E127	+	+	+	-	+
E129	-	-	-	-	-
E131	-	-	-	-	-
E132	+	+	+	-	+
E134	+	+	+	-	+
E139	-	-	-	-	-
E141	-	-	-	-	-
E144	-	-	-	-	-
E145	-	-	-	-	-
E146	+	+	+	-	+
E147	+	+	+	+	+
E148	+	+	+	-	+
E149	-	-	+	-	-
E151	-	-	-	-	-
E153	-	-	+	-	-
E195	-	-	-	-	-
M	-	-	-	-	-

Nimrod Hut January 1998

Table A1.5: Sample identification, location at *Nimrod* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; January 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
R15	Corn back left corner among soil	+	+	+
R22	Oil soil under crate N side	+	+	+
R54	Beans S side (good ones)	-	+	+
R59	Lentils (good condition)	+	+	+
R84	Swab canvas under bed	-	+	+
R85	Swab British Antarctic Expedition 1907 box on east wall	-	-	-
R88	Swab wall under Shackleton's bed n side	-	-	-
R90	Swab boot under bed against N wall	-	-	+
R92	Swab boot between beds on n wall	+	+	+
R97	Swab box under sacking partition under stove	-	-	-
R98	Swab oven cloth hanging behind stove	-	-	+
R100	Swab lab right by door	+	+	-
R101	Swab lab wall between lab and Hut	-	+	-
R102	Swab lab in shelf	-	-	-
R103	Swab Entrance way just before acetylene plant	-	-	-
R104	Swab entrance way left next to saw	-	-	-
R105	Swab wall between canvas and bunk	-	-	-
R106	Swab floor just along from Shackleton's bunk	-	+	+
R107	Swab Entrance way just before acetylene plant next to sample R103	-	-	-
R110	Swab wall behind boot on E wall	-	-	-
R111	Swab table behind stove	-	-	-
R114	Swab behind ham	-	-	-
R116	Swab behind boxes on S wall	+	+	+
R118	Swab from oily looking box on S wall	-	-	-
R133	Swab East wall Mawson's lab	-	+	+
R152	Swab sleeping bag S bunk	-	-	-
R153	Swab mould on Bottle W window on N side	-	-	+
R154	Swab boot liner on top of bench on s side	+	-	+
R155	Same as R154 but inside	+	+	+
R156	Swab shoe under bench s side	-	-	+
R157	Same as R156 but inside	+	+	+
R158	Swab horse harness on W wall	+	+	+

Table A1.6: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut January 1998.

Sample ID	Media 4	Media 6	YM	Media 7	V B Agar
R15	+	+	-	-	-
R22	+	+	+	-	+
R54	-	-	-	+	+
R59	+	+	+	+	+
R84	-	-	-	-	-
R85	-	-	-	-	-
R88	-	-	-	-	-
R90	-	-	-	-	-
R92	-	-	+	-	-
R97	-	-	-	-	-
R98	-	-	-	-	-
R100	-	-	-	-	-
R101	-	-	-	-	-
R102	-	-	-	-	-
R103	-	-	-	-	-
R104	-	-	-	-	-
R105	-	-	-	-	-
R106	-	-	-	-	+
R107	-	-	-	-	-
R110	-	-	-	-	-
R111	-	-	-	-	-
R114	-	-	-	-	-
R116	+	+	+	-	+
R118	-	-	-	-	-
R133	-	-	+	-	+
R152	-	-	-	-	-
R153	-	-	+	-	-
R154	-	-	-	-	-
R155	-	-	-	-	-
R156	-	-	-	-	-
R157	+	+	+	-	+
R158	+	+	+	+	+

Historic material found outside the historic sites or Antarctic non- Historic material, January 1998

Table A1.7: Sample identification, location at other location on Ross Island and presence of fungi when samples were cultured at 4, 15 or 25ºC; January 1998.

Sample ID	Sample location	4 ºC	15ºC	25ºC
W6	Interior wood sample from 1st boards removed from Cape Evans and store in container at Scott base	+	+	+
W7	Exterior wood sample from 2nd boards removed from cape Evans and store in container at Scott base	+	+	+
W8	Interior wood sample from 2nd boards removed from Cape Evans and store in container at Scott base	+	+	+
PS1	Penguin guano	-	+	+
PS2	Penguin guano	-	+	+
PS3	Penguin guano	+	+	+
PS4	Penguin guano	+	+	+
W1	Wood sample covered with penguin guano	-	+	+
W2	Wood sample covered with penguin guano	-	+	+
W3	External sample of wood found outside2	-	-	-
W4	Internal sample of same wood as in W3	-	-	+
TF	Penguin tail feather	-	+	+
R136	Swab of new wood S wall of Mawson's lab unattached	+	+	+
R137	Swab of new wood S wall of Mawson's lab unattached	+	+	+

Table A1.8: Sample identification, media used to culture fungi and presence of fungi; other locations January 1998.

Sample ID	Media 4	Media 6	YM	Media 7	V B Agar
W5	-	-	-	-	-
W6	-	-	-	-	-
W7	+	-	-	-	-
W8	-	-	+	-	-
PS1	-	-	-	-	-
PS2	-	-	-	-	-
PS3	-	-	-	-	-
PS4	+	-	-	-	-
W1	-	-	-	-	-
W2	-	-	-	-	-
W3	-	-	-	-	-
W4	-	-	-	-	-
TF	+	-	-	-	+
R136	+	-	+	-	+
R137	+	-	+	-	+

Discovery Hut December 1998

Table A1.9: Sample identification, location at *Discovery* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; December 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
2H45	Swab Bottom of bag on bed W of stove	-	+	-
2H50	Swab Old broom	+	+	-
2H51	Swab Roof in entrance way	-	-	-
2H52	Swab wall above chaff	-	-	-
2H53	Swab Straw broom	+	+	+
2H54	Swab under blubber in main area of hut	+	-	-
2H55	Swab AHT broom	+	+	-
2H56	Wood under blubber in main area of hut	+	-	-
2H57	Straw entrance way	+	+	+

Table A1.10: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut December 1998.

Sample ID	**Media 4**	**YM**	**Media 7**
2H45	-	+	-
2H50	+	+	+
2H51	-	-	-
2H52	-	-	-
2H53	+	+	+
2H54	-	+	+
2H55	+	+	-
2H56	+	-	-
2H57	+	+	+

Cape Evans December 1998

Table A1.11: Sample identification, location at *Terra Nova* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; December 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
2E39	Wood outside white box S side store	+	+	+
2E53	Straw outside NE corner	+	+	+
2E72	Swab handle of main door	-	+	-
2E78	Swab of mould biscuit stables area	+	+	+
2E82	Resin Gum stable 4	+	+	+
2E87	Butter Stall 1	+	+	+
2E89	Swab leather harness stall2	+	+	+
2E90	Swab stores box stall1	+	+	+
2E95	Swab ceiling above blubber	+	+	-
2E96	Swab ski on wall in annex	+	+	+
2E98	Swab roof in annex	-	+	+
2E99	Swab near blubber	+	+	+
2E100	Swab mouldy ski	+	+	-
2E102	Swab box E of stove	+	-	-
2E104	Swab fungal mycelium by Nelson's bunk	-	+	+
2E107	Swab wall between bunks south side of hut	-	-	+
2E108	Swab box next to bunk	-	-	-
2E111	Reindeer fibres sleeping bag bottom bunk	+	+	+
2E112	Swab book in darkroom	+	-	+
2E113	Swab roof of darkroom	-	+	+
2E114	Swab table in darkroom	+	+	+
2E115	Swab fungal mycelium dark room	+	+	+
2E119	Swab boot under Bowers bunk	+	+	-
2E121	Swab Scott's pillow	+	+	+
2E122	Swab book in galley area	-	+	+
2E123	Swab box next to stove galley area	-	+	+
2E124	Swab box next to bed in kitchen	+	+	+
2E134	Swab Inside of wallet found by AHT	+	+	+
2E135	Swab outside of wallet found by AHT	+	+	+
2E136	Swab AHT broom	+	+	+

Table A1.12: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut December 1998.

Sample ID	Media 4	YM	Media 7
2E39	+	+	+
2E53	+	+	+
2E72	+	-	-
2E78	+	+	+
2E82	+	+	+
2E87	+	+	+
2E89	+	+	-
2E90	+	+	-
2E95	+	+	-
2E96	+	+	-
2E98	+	+	-
2E99	+	+	-
2E100	-	+	+
2E102	+	+	-
2E104	+	+	+
2E107	-	-	-
2E108	-	-	-
2E111	+	+	+
2E112	+	+	-
2E113	+	-	-
2E114	+	+	-
2E115	+	+	+
2E119	+	+	-
2E121	+	+	+
2E122	+	-	+
2E123	+	-	-
2E124	+	+	-
2E134	+	+	+
2E135	+	+	+
2E136	+	+	+

Cape Royds December 1998

Table A1.13: Sample identification, location at *Nimrod* Hut and presence of fungi when samples were cultured at 4, 15 or 25ºC; December 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
2R23	Flour E side wall	-	-	-
2R27	Celery E side stores	-	+	+
2R30	Goop E side stores	-	-	-
2R38	Beans E side stores	+	+	+
2R40	Soil leaking can	-	-	-
2R63	Swab wall Mawson's lab	-	-	+
2R64	Swab wall Mawson's lab	-	-	-
2R67	Swab dark room wall	-	-	-
2R68	Fibres from darkroom wall	-	-	-
2R85	Swab hand broom	-	-	-
2R86	Swab AHT broom	-	-	-
2R87	Swab Scrubbing brush	-	-	-
2R88	Flour Mouldy collected by AHT	+	+	+
2R89	Food backdoor bay depot	-	-	

Table A1.14: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut December 1998.

Sample ID	**Media 4**	**YM**	**Media 7**
2R23	-	-	-
2R27	+	+	-
2R30	-	-	-
2R38	+	+	+
2R40	-	-	-
2R63	-	+	-
2R64	-	-	-
2R67	-	-	-
2R68	-	-	-
2R85	-	-	-
2R86	-	-	-
2R87	-	-	-
2R88	+	+	+
2R89	-	-	-

Historic material found outside the historic sites or Antarctic non- Historic material, December 1998

Table A1.15: Sample identification, location at other locations around Ross Island and presence of fungi when samples were cultured at 4, 15 or 25ºC; December 1998.

		Growth		
Sample ID	**Sample location**	**4 ºC**	**15ºC**	**25ºC**
2R90	Wood outside hut area	+	+	+

Table A1.16: Sample identification, media used to culture fungi and presence of fungi; other locations December 1998.

Sample ID	**Media 4**	**YM**	**Media 7**
2R90	+	+	+

Discovery Hut January 2001

Table A1.17: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4HP1	Inside Middle of floor pendulum room	-	-	+	-
4HP2	Inside Wood wall below window pendulum room	+	-	+	-
4HP3	Inside Wood ceiling above window pendulum room	-	-	-	-
4HP4	Inside Wood board behind door pendulum room	+	-	+	+
4HP5	Inside 1st post in cooking area	+	-	+	-
4HP6	Inside Floor in galley area	+	-	-	+
4HP7	Inside Floor in front of stove galley area near sample 4HP5	-	-	-	-
4HP8	Inside Wall from alley way between galley area and main area	+	-	+	+
4HP9	Inside Roof alley way between galley area and main area	+	-	+	-
4HP10	Inside Floor of mutton room between hole and door way covered with hay	+	-	+	+
4HP11	Inside Mouldy blubber hole in mutton room floor	+	-	+	+
4HP12	Inside Wood mutton room floor	+	-	+	+
4HP13	Inside Wood roof of mutton room far side of hole	-	-	+	+
4HP14	Inside Mutton room wall by window	+	+	+	+
4HP15	Outside veranda opposite main door above coal	+	+	+	+
4HP16	Outside hut wood plaque end of hut deep below ground	+	-	+	+
4HP17	Outside veranda wood post snow drift side of hut	+	+	+	+
4HP18	Outside wood hitching post Vince's cross side of hut	+	-	+	+
4HP19	Outside hut doorstep Vince's cross side of hut next to pendulum room window	+	+	+	+
4HP20	Outside board by door Vince's cross side of hut between mutton room and pendulum room windows	+	-	-	+
4HP21	Outside snow drift side of hut below Galley area window ground level	+	-	+	-
4HP22	Outside beside seal carcass below ground	+	-	-	+
4HP23	Outside sample of main door step lower side	+	-	+	+
4HP24	Outside Main door side of hut between main door and seal carcass very weathered board	+	-	+	+
4HP25	Outside Verhanda post wood opposite main door below ground	+	+	+	+
4HP26	Inside wall beside seal blubber	+	-	+	+
4HP27	Inside wall above hay bail	+	-	+	+
4HP28	Inside roof latrine area	+	-	+	+
4HP29	Inside wall behind mutton carcass	-	-	-	-
4HP30	Inside wall in latrine area left side	-	-	+	+
4HP31	Inside floor in front of hay bail	+	-	+	+
4HP32	Inside wall right side from main door	+	-	+	-

Table A1.17: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4HP33	Inside roof corner of inside door to main area	+	-	+	+
4HP34	Inside wall left side as you enter hut	+	-	+	+
4HP35	Inside floor doorway to main area	+	-	+	+
4HP36	Inside main area wall right when you enter	+	-	-	+
4HP37	Inside post middle of hut	+	-	+	+
4HP38	Inside main post main area close to artefacts that form the wall between main area and galley area	-	-	+	+
4HP39	Inside 2nd post in main area	+	-	+	+
4HP40	Inside wall above visitor sign in area	+	-	+	+
4HP41	Inside Wall near roof walkway between main area (closer to main area)and galley area	-	-	-	+
4HP42	Inside wall above seal blubber	-	-	-	-
4HP43	Inside ceiling above seal blubber	+	-	+	+
4HP44	Inside Floor middle of main area	+	-	+	+
4HP45	Inside floor opposite window on main door side of hut	+	-	+	+
4HP46	Inside black roof wood in main area close to door to entrance area	+	-	+	+
4HP47	Inside wall between window and seal carcass main door side of hut	+	-	-	+
4HP48	Inside wall snow side of hut between window and corner	+	-	+	-
4HP49	Inside Floor next to sleeping platform	+	-	+	-
4HP50	Inside wall between galley area and pendulum room next to bench	-	-	-	+
4HP51	Inside wall next to mutton carcass main door side of hut	-	-	-	+
4HP52	Inside roof main area of hut	+	-	+	+

Terra Nova Hut January 2001.

Table A1.18: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CE1	Outside door step to hut	+	+	+	+
4CE2	Outside below ground below door step	+	-	+	+
4CE3	Outside below ground 2.5 metres from main door	+	+	+	+
4CE4	Outside below ground corner of main door and stable side of hut	+	+	+	+
4CE5	Outside below ground Annex wall stable side of hut	+	+	+	+
4CE6	Outside below ground Annex wall by door	+	+	+	+
4CE7	Outside below ground Stables area by door	+	+	+	+
4CE8	Outside below ground Stable wall by hay bails	+	-	+	+
4CE9	Outside below ground Barnes glacier side of hut 3.5 metres along hut wall	+	-	-	-
4CE10	Outside below ground Corner of snowdrift and Barnes glacier sides of hut	+	-	+	+
4CE11	Outside below ground Corner of snow drift and Barne's Glacier sides of hut	+	-	+	+
4CE12	Outside below ground Latrine area Barnes glacier side	+	-	+	+
4CE13	Outside below ground Corner of latrine Barnes glacier and sea side	+	-	+	+
4CE14	Outside below ground 1st Latrine sea side	+	+	+	+
4CE15	Outside below ground Latrine corner of stables and main door side	+	-	+	+
4CE16	Outside Snow drift side of hut main door end corner	-	-	-	-
4CE17	Outside Snow drift side close to snow drift	-	-	-	-
4CE18	Outside Snow drift side between samples 4CE16 and 4CE17	-	-	-	-
4CE19	Outside Snow drift side of hut annex wall	-	-	-	-
4CE20	Outside Main door side of hut by door	-	-	-	-
4CE20b		-	-	-	-
4CE21	Outside Main door side of hut 6 metres from main door	-	-	+	-
4CE22	Outside Main door side of hut 4 metres from main door	+	-	+	+
4CE23	Outside corner of main door and stable side of hut	-	-	-	-
4CE24	Outside Sables side of hut 3.5 metres from corner	+	+	+	-
4CE25	Outside Stables side of hut between the two stable doors	-	-	-	-
4CE26	Outside Stables side of hut between stable door and hay bails	+	+	+	-
4CE27	Outside Stables side of hut above hay bails	-	-	-	-
4CE28	Outside Stable wall Barnes glacier side of hut	-	-	-	-
4CE29	Outside Snow drift side of hut Barnes glacier end	-	-	-	-
4CE30	Outside Stables side of hut 6.5 metres from main door corner	+	-	+	-
4CE31	Outside Latrine main door side	-	-	-	-

Table A1.18: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CE32	Outside Inside 1st latrine	-	-	-	-
4CE33	Inside Annex area 1 metre from main door towards stables	+	-	-	+
4CE34	Inside annex area hut wall below tools right side as you go to stables area.	-	-	-	-
4CE35	Inside annex area above seal blubber	-	-	+	+
4CE35	Inside annex area above seal blubber	+	-	-	-
4CE36	Inside stables area Stable roof 1 metre from annex area	-	-	+	-
4CE37	Inside stables area hut wall 2 metre from annex area	+	-	+	+
4CE38	Inside stables area hut wall 6.5 metre from annex area	+	+	+	+
4CE39	Inside stables area hut wall 13 metre from annex area	-	-	+	-
4CE40	Inside stables area Roof in stable 7 far end of stables	-	-	-	-
4CE41	Inside stables area Stable 2 divide between stable2/3	-	+	+	+
4CE42	Inside stables area Stable3 divide between stable ¾	-	-	-	-
4CE43	Inside stables area Stable5 divide between stable 4/5	+	-	+	+
4CE44	Inside stables area Stable 6 wall	+	-	-	+
4CE45	Inside stables area Stable 7 divide between stable 7/6	+	-	+	+
4CE46	Inside stables area Stable7 wall between stable 7 and annex	-	-	-	+
4CE47	Inside stables area Roof sample Stable 7	+	+	+	+
4CE48	Inside annex area wall between annex and stable 7	+	-	-	-
4CE49	Inside annex area main door side 8 metres from main door	-	-	-	-
4CE50	Inside annex area main door side 5 metres from main door	+	+	+	-
4CE51	Inside annex area Door frame to hut from annex	-	-	+	-
4CE52	Inside annex area hut wall beside door to hut next to tools	-	-	-	-
4CE53	Inside annex area Annex wall snow drift side of hut	-	-	-	-
4CE54	Inside annex area right next to main door to outside snow drift side.	-	-	-	-
4CE55	Inside annex area below door step	+	-	+	+
4CE56	Inside annex area below ground main door side 1.5 metres from main door	+	-	+	+
4CE57	Inside annex area below ground wall between annex and stable 7	+	+	+	+
4CE58	Inside stables area below ground Stable 4 divide between stable 4/5	+	+	+	+
4CE59	Inside stables area below ground Stable 1 stable wall Barnes glacier side of hut	+	-	+	+
4CE60	Inside stables area below ground hut wall beside stove far end of stables	+	-	+	+

Table A1.18: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CE61	Inside stables area below ground hut wall stables area 4 metres from annex end	+	-	+	-
4CE62	Inside stables area below ground door way to stables area next to seal blubber	+	-	+	+
4CE63	Inside stables area below ground door way to stables area next to seal blubber	+	-	-	+
4CE64	Inside annex area below ground hut wall below tools right side as you go to stables area.	+	-	+	+
4CE65	Inside annex area below ground under doorstep of door to hut from annex	+	-	+	+
4CE66	Inside annex area below ground Annex wall snow drift side of hut	+	-	+	+
4CE67	Inside annex area below ground right next to main door to outside snow drift side.	-	-	-	+
4CE68	Inside annex area below ground below door step	+	-	+	+
4CE69	Outside Latrine wall sea side	-	-	-	-
4CE70	Outside Latrine wall Barne's glacier side	-	-	-	-
4CE71	Outside Latrine wall stables side Barnes glacier end	-	-	-	-
4CE72	Outside Latrine roof stables side	-	-	-	-
4CE73	Outside Latrine wall stables side main door end	-	-	-	-
4CE73b	Inside Floor sample just inside entrance way	-	-	+	-
4CE74	Inside floor sample Entrance area under platform	-	-	-	-
4CE75	Inside hut wall entrance area left side as you enter	+	-	-	+
4CE76	Inside Door from annex to entrance way	-	-	-	-
4CE77	Inside hut wall between entrance door and hut door	-	-	+	-
4CE78	Inside Entrance way door step	+	-	+	-
4CE79	Inside door step door from entrance way to inside hut	+	-	+	+
4CE80	Inside floor main area by chimney	+	+	+	+
4CE81	Inside floor Between Ponting's dark room and main table	-	-	-	-
4CE82	Inside floor Under chart table Scott's area	+	-	+	-
4CE83	Inside floor Between bunks and main table stables side of hut	-	-	-	-
4CE84	Inside floor Stables side of hut between stores and visitors sign in area	-	-	+	+
4CE85	Inside floor Under form stables side of hut	+	+	+	+
4CE86	Inside Wall sample Galley stores area	-	-	-	-
4CE87	Inside Under bed Galley area	+	+	+	+
4CE88	Inside under bunk next to Galley area snow drift side of hut	-	-	+	-
4CE89a	Inside under table between 1st set of bunks snow drift side of hut	-	-	-	+
4CE90	Inside Wall sample between bunk and Lab bench	-	-	-	-

Table A1.18: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CE90b	Inside 2nd Wall sample between bunk and Lab bench	+	-	+	+
4CE91	Inside Chemistry table.	-	-	-	-
4CE92	Inside Window surround lab area snow drift side of hut	-	-	-	-
4CE93	Inside wall sample lab area snowdrift side of hut	-	-	-	+
4CE94	Inside wall sample lab area Barnes Glacier side of hut	-	-	-	-
4CE95	Inside dark room door	-	-	-	-
4CE96	Inside Above Evan's bed Scott's area	-	-	-	-
4CE97	Inside Wall stables side of hut between Evan's bed and chart table	+	-	-	-
4CE98	Inside Wall stables side of hut between chart table and Scott's bed	-	+	-	-
4CE99	Inside Wall above Scott's bed	-	-	-	-
4CE100	Inside Wall between Scott's bed and Main hut area	+	-	-	+
4CE101	Inside Wall between Scott's bed and Bunks on Stables side of hut	+	+	+	-
4CE102	Inside hut wall stables side of hut under middle bunk	+	-	-	-
4CE103	Inside wall stables side of hut under window between sores and sign in table	+	-	-	-
4CE104	Inside Wall stables side of hut next to table and form	-	-	+	+
4CE105	Inside Main door side of hut under platform with leather on top behind form	+	-	+	+
4CE106	Inside wall next to platform with leather on	-	-	-	-
4CE107	Inside wall between platform and main door to hut	-	-	-	-
4CE108	Inside dark room Floor	-	-	-	-
4CE109	Inside dark room wall between dark room and lab near floor	-	-	-	-
4CE110	Inside dark room hut wall	-	-	-	-
4CE111	Inside dark room roof	-	-	-	-
4CE112	Inside dark room wall door side of dark room	-	-	+	-
4CE113	Inside Annex area roof	-	-	+	-
4CE114	Inside Stables area door step of bay with Steve	+	-	+	+
4CE115	Inside board on annex wall with tools	+	-	+	+
4CE116	Outside wood from stables area under butyl roof covering	+	-	+	-
4CE117	Inside Roof beam over main table	-	-	-	+
4CE118	Outside wax sample	-	-	-	-
4CE16a	Swab of dark fungus on wall below window on store side of hut.	+	-	+	+
4CE18a	Swab of fungus on 2nd boxes in Galley area	-	-	+	-
4CE18b	Swab of 2nd fungus on 2nd boxes in Galley area	-	-	+	-
4CE19a	Swab of white fungus in Dark room	+	-	+	-

Table A1.18: Sample identification, media used to culture fungi and presence of fungi *Terra Nova* Hut January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CE19b	Swab of white fungus bunk in bunk area near laboratory.	-	-	-	-
4CE16a	Swab of dark fungus on wall below window on store side of hut.	+	-	+	+
4CE16b	Swab of dark fungus on wall below window on store side of hut.	-	-	-	-
4CE17a	Swab of dark fungus on boxes in Galley area.	-	-	+	-
4CE17b	Swab of dark fungus on boxes in Galley area.	+	-	+	+
4CE125	Swab of	-	-	-	-
4CE126	Swab of	-	-	-	-
4CE127	Swab of Stores to right as you enter main hut door	-	-	-	-
4CE128	Swab of Box with Lanterns, books and shoes on	-	-	-	-
4CE121	Swab of Bunk next to row of boxes in Galley area snowdrift side of hut	-	-	-	-
4CE122	Swab of dark room in front of door	+	-	+	+
4CE123	Swab of Box under lab bench	-	-	-	-
4CE124	Swab of sunlight soap shelf above sample 4CE18 and 4CE19	-	-	-	-
4CE75a	Swab of hut wall entrance area left side as you enter	-	-	-	-
4CE89	Swab of under table between 1st set of bunks snow drift side of hut	+	-	+	+
4CE102a	Swab of hut wall stables side of hut under middle bunk	-	-	+	-
4CE109a	Swab of dark room Wall between dark room and lab near floor	-	-	+	-
4CE112a	Swab of dark room wall door side of dark room	-	-	-	-
4CE112b	Swab of dark room wall door side of dark room	+	-	-	+
4CE119	Swab of Shelf below window Stables side of hut near table	-	-	-	+
4CE120	Swab of cup shelf Galley area	-	-	-	+

Nimrod Hut January 2001

Table A1.19: Sample identification, media used to culture fungi and presence of fungi; *Nimrod Hut* January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CR1	Outside Below ground corner of stable area and back stores area	+	-	+	+
4CR2	Outside Behind stores back of hut	+	-	+	+
4CR3	Outside Below ground Corner of porch front of hut	+	-	+	+
4CR4	Outside Below ground by the door step latrine side	+	-	+	+
4CR5	Outside Below ground corner of Mawson's lab and latrine	+	-	+	+
4CR6	Outside Below ground between sample 4-5	+	-	-	+
4CR7	Outside Below ground corner of latrine	+	-	+	+
4CR8	Outside Below ground post on the way to stable area	+	-	+	+
4CR9	Outside Below ground stable area hut side	+	-	+	+
4CR10	Outside Below ground post in the middle of the stable area	+	-	+	+
4CR11	Outside above sample 1	-	-	-	-
4CR12	Outside above the stores back of hut	-	+	-	-
4CR13	Outside above stores penguin colony side of hut towards back of hut	-	-	-	-
4CR14	Outside above stores penguin colony side of hut towards front of hut	-	-	+	+
4CR15	Outside board attached to hut porch penguin colony side of hut	-	-	-	-
4CR16	Outside porch penguin colony side of hut	+	-	-	-
4CR17	Outside doorstep right side	-	-	-	-
4CR18	Outside board down main door	-	-	-	-
4CR19	Outside wall front of hut	-	-	-	-
4CR20	Outside wall between hut and latrine	-	-	-	-
4CR21	Outside post on the way to the stable	-	-	-	-
4CR23	Outside stable area hut wall close to latrine	-	-	-	-
4CR24	Outside stable area 1st window	-	-	-	-
4CR25	Inside floor next to wall in front of stores on the stable side of hut	-	-	-	-
4CR26	Inside floor in front of stores on stable side of hut	-	-	-	+
4CR27	Inside floor in front of Shackleton's room	-	-	+	+
4CR28	Inside floor in front of stove	-	-	-	-
4CR29	Inside floor between bed and couch	-	+	-	-
4CR30	Inside floor under acetylene plant				
4CR31	Inside floor in porch	+	-	+	-
4CR32	Inside floor darkroom	+	-	-	+
4CR33	Inside wall below store shelf stables side of hut	-	-	-	-
4CR34	Inside wall dribble down wall close to sample 33	-	-	-	-
4CR35	Inside wall below window bed are of hut stable side of hut	-	-	-	-
4CR36	Inside wall black dribble close to sample 35	-	-	-	-
4CR37	Inside porch area wall Mawson's lab side	-	-	-	-
4CR38	Inside porch area wall Mawson's lab side close to door to lab	-	-	-	-

Table A1.19: Sample identification, media used to culture fungi and presence of fungi; *Nimrod Hut* January 2001.

Sample ID	Sample location description	YM	Media 7	VB	CMC
4CR39	Inside porch area wall Mawson's lab side of hut close to sample 38	-	-	+	-
4CR40	Inside wall from behind the 1st ham	-	-	-	+
4CR41	Inside wall from behind the 2nd ham	-	-	-	-
4CR42	Inside wall Mawson's lab above door	-	-	-	-
4CR43	Inside door inside into the hut	-	-	-	-
4CR44	Inside porch area door frame	-	-	-	-
4CR45	Inside Mawson's lab door step	+	-	-	-
4CR46	Inside Mawson's lab door step support below the cork	+	-	+	+
4CR47	Inside Mawson's lab breaking up doorstep wood	-	-	-	-
4CR48	Inside Mawson's lab wall below stores	+	-	-	-
4CR49	Inside cork from floor	+	-	-	+
4CR50	Inside dark room wall stable side of hut	-	-	-	-
4CR51	Inside roof dark room	-	-	-	-
4CR52	Inside Dark room just below door step dust spot	-	-	-	-
4CR53	Inside wall Shackleton's room wall entrance side of hut	-	-	-	-
4CR54	Inside roof Shackleton's room	-	-	-	-
4CR55	Inside wall just outside Shackleton's room	-	-	-	-
4CR56	Inside wall stores side of hut among stores	-	-	-	-
4CR57	Inside wall under acetylene plant	-	-	-	-
4CR58	Inside wall stores side of hut above couch	-	-	-	-
4CR59	Inside wall back wall of hut next to biologist lab	-	-	-	-
4CR60	Outside stable area top of 2nd window	-	-	-	-
4CR61	Outside stable area wall next to 2nd window	-		-	-
4CR62	Outside stable area wall between 1st and 2nd window	-	-	-	-
4CR63	Outside hut wall behind stables area	-	-	-	-
4CR64	Outside post in the middle of the stable area	-	-	-	-
4CR65	Outside stable area board from post in middle of stable area to hut	-	-	-	-
4CR26a	Inside floor in front of stores on stable side of hut	-	-	-	-
4CR33a	Inside wall below store shelf stables side of hut	-	+	-	+

Discovery Hut January 2003

Table A1.20: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut 2003.

Sample ID	Sample location description	YM	Media 7	VB	CMC
5HP1	Outside, wood from post between hut and McMurdo bay above ground.	-	-	-	-
5HP2	Outside, wood from post between hut and McMurdo bay below ground.	+	+	+	+
5HP3	Outside, wood from post between hut and Road above ground	-	-	-	-
5HP4	Outside, wood from post between hut and Road below ground	+	+	+	+
5HP5	Outside, wood from behind *Discovery* awning	-	-	-	-
5HP6	Outside, wood from second post between hut and road.	-	-	-	-
5HP7	Outside, wood from corner near seal carcass	-	-	-	-
5HP8	Outside, wood from corner of verhanda up by metal brace.	-	-	-	-
5HP9	Outside, wood from damp spot under window to mutton room.	+	+	+	+
5HP10	Outside wood from door side of hut mutton room end of wall.	-	-	-	-
5HP11	Inside, wood from behind stores wall.	-	-	-	-
5HP12	Inside wood from behind door in pendulum room.	-	-	-	-

Terra Nova Hut January 2003.

Table A1.21: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut 2003.

Sample ID	Sample location description	YM	Media 7	VB	CMC
5CEMC1 H	Inside, wood from wall to left of main door.	-	-	-	-
5CEMC1 M	Inside, wood from wall to left of main door.	-	-	-	-
5CEMC1 L	Inside, wood from wall to left of main door.	-	+	+	+
5CEMC2 H	Inside, wood from wall on stables side of hut by table in Scott's area.	-	-	-	-
5CEMC2 M	Inside, wood from wall on stables side of hut by table in Scott's area.	-	-	-	-
5CEMC2 L	Inside, wood from wall on stables side of hut by table in Scott's area.	-	+	-	-
5CEMC3 H	Inside, wood from wall on snow drift side of hut between lab bench and bunk.	-	+	-	-
5CE MC3 M	Inside, wood from wall on snow drift side of hut between lab bench and bunk.	-	+	-	-
5CE MC3 L	Inside, wood from wall on snow drift side of hut between lab bench and bunk.	-	+	-	-
5CE MC4 H	Inside, wood from Galley area right of main door.	-	-	-	-
5CE MC4 M	Inside, wood from Galley area right of main door.	-	-	-	-
5CE MC4 L	Inside, wood from galley area right of main door.	-	+	-	+
5CE MC5 H	Inside, wood stores area near galley	-	-	-	-
5CE MC5 M	Inside, wood stores area near galley	-	+	-	+
5CE MC5 L	Inside, wood stores area near galley	-	-	-	-
5CE MC F1	Inside, wood from floor near stove main area	-	+	-	-
5CE MC F2	Inside, wood from floor on snow drift side of hut between lab bench and bunk.	-	-	-	-
5CE MC F3	Inside, wood from floor Scott's area between Scott's bed and table.	-	-	-	-
5CE MC B1	Inside, wood from beam above sample MC F1	-	+	-	-
5CE MC B2	Inside, wood from beam snow drift side of hut between bunks next to galley area.	-	-	-	-
5CE MC B3	Inside wood from beam above Pontings darkroom.	-	-	-	-
5CE TNSD	Outside, wood from roof above snow drift.	-	+	-	-
5CE WNB	Outside, wood wall Barnes Glacier side of hut	-	-	-	-
5CE DSH	Outside, wood from stable door step near Hay bails.	-	+	+	+
5CE FL	Inside, wood from frozen line in galley area	+	+	+	+
5CE AW1	Wood from Annex area near door to latrines	+	+	+	-
5CE AW2	Wood from Annex area near door to latrines	-	+	+	+
5CE Dark room	Inside wood from ceiling in Dark room.	-	-	-	-

Nimrod Hut January 2003.

Table A1.22: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut 2003.

Sample ID	Sample location description	YM	Media 7	VB	CMC
5CRMC1 H	Inside, wood from wall near Shackleton,s room	-	-	-	-
5CR MC1 M	Inside, wood from wall near Shackleton,s room	-	-	-	-
5CR MC1 L	Inside, wood from wall near Shackleton,s room	-	-	-	-
5CR MC2 H	Inside, wood from wall stables side of hut next to window.	-	-	-	-
5CR MC2 M	Inside, wood from wall stables side of hut next to window.	-	-	-	-
5CR MC2 L	Inside, wood from wall stables side of hut next to window.	+	+	+	-
5CR MC3 H	Inside, wood from back wall of hut left side of stove.	-	-	-	-
5CR MC3 M	Inside, wood from back wall of hut left side of stove.	-	-	-	-
5CR MC3 L	Inside, wood from back wall of hut left side of stove.	-	-	-	-
5CR MC4 H	Inside, wood from wall in stores area between shelves.	-	-	-	-
5CR MC4 M	Inside, wood from wall in stores area between shelves.	-	-	-	-
5CR MC4 L	Inside, wood from wall in stores area between shelves.	-	-	-	-
5CR 1	Outside, wood from behind stores nearest door	-	-	-	-
5CR 2	Outside, wood from behind stores ¼ of the way along the wall starting at main door end.	-	-	-	-
5CR3	Outside, wood from behind stores 1/2 of the way along the wall starting at main door end.	-	-	-	-
5CR4	Outside, wood from behind stores 1/2 of the way along the back wall.	-	-	+	+
5CR5	Outside, wood from behind stores on the corner between back wall and main stores wall.	-	-	-	-
5CR6	Outside, wood from behind stores on the corner between back wall and stables wall.	-	+	+	-
5CR7	Outside, wood from behind stores on the corner between back wall and stables wall.	-	-	+	+
5CR8	Outside, wood from wall between corner of back wall and stables wall.	-	-	-	+
5CR9	Outside, wood from stores boxes of back wall of stable area.	-	-	+	+
5CR10	Outside, wood from hut wall in stables area under penguin feathers.	-	+	+	+
5CR11	Outside, wood from hut wall in stables area under window under penguin feathers.	-	-	-	-
5CR12	Outside, wood from hut wall in stables area in corner to latrine.	-	+	+	+
5CROil Box	Outside wood from oil boxes in garage area	-	-	-	-

Table A1.23: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut 2003.

Sample ID	Sample location description	YM	Media 7	VB	CMC
5CR MCR1	Inside, wood from beam above Shackleton's room.	-	-	-	-
5CR MCR2	Inside, wood from beam in stores area.	-	-	-	-
5CR MCR3	Inside, wood from beam in bed area left of stove	-	-	-	-
5CR MCR4	Inside, wood from beam in main area to left of main entry door.	-	-	-	-
5CR MC5	Inside, wood from roof of Shackleton's room	-	-	-	-
5CR MC6	Inside, wood from roof of darkroom	-	-	-	-
5CR MC7	Inside, wood from wall of porch area	-	-	-	-
5CR MCMa	Inside, wood from wall in Mawson's lab	-	-	-	-
5CR MCF1	Inside, wood from floor middle of main area	-	-	-	-
5CR MCF2	Inside, wood from floor in bead area	-	-	-	-
5CR MCF3	Inside, wood from floor just outside Shackleton's room	-	-	-	-
5CR MCF4	Inside, wood from floor just before stores area	-	-	-	-

Discovery Hut January 2004

Table A1.24: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6HPMC1H	Wall opposite cooking area	+	-
6HPMC1M	Wall opposite cooking area	-	+
6HPMC1L	Wall opposite cooking area	-	-
6HPMC2H	Wall on alleyway to galley area	-	-
6HPMC2M	Wall on alleyway to galley area	-	-
6HPMC2L	Wall on alleyway to galley area	-	-
6HPMC3H	Outside wall between door to hut and seal blubber	+	+
6HPMC3M	Outside wall between door to hut and seal blubber	+	-
6HPMC3L	Outside wall between door to hut and seal blubber	+	+
6HPMC4H	Outside wall mutton room	+	-
6HPMC4M	Outside wall mutton room	+	-
6HPMC4L	Outside wall mutton room	+	+
6HPMCR1	Roof pendulum room	+	-
6HPMCR2	Roof same area as HPMC1	-	-
6HPMCR3	Roof mutton room above mutton carcass	+	+
6HPMCF1	Floor middle of main area	+	+
6HPMCF2	Floor next to sleeping platform	-	-
6HPMC5H	Entrance way above straw	+	-
6HPMC5M	Entrance way above straw	-	-
6HPMC5L	Entrance way above straw	+	+
6HPI1	Outside wall main area above stores	+	-
6HPI2	Internal wall entrance way right side	+	-
6HPI3	Outside wall in pendulum room	+	+
6HPI4	Floor between stove and artefact wall to main area	-	-
6HP1	Below ground between main door and seal carcass	+	+
6HP2	Pole sample above Sample 6HP1	-	-
6HP3	Below ground corner post behind seal carcass	-	-
6HP4	Above ground Baton	+	+
6HP5	Wall ice drift side of hut	-	-
6HP6	Ice drift side of hut Veranda pole below ground	-	-
6HP7	Wet wood middle veranda post snow drift side	+	+
6HP8	Wall along from wet patch on snow drift side of hut	+	-
6HP9	Board just above ground Vince's Cross side of hut	+	-
6HP10	Board below ground Vince's Cross side of hut	+	+
6HP11	Below sample 6HP11 Board below ground corner of Vince's cross side of hut and main door	+	+
6HP12	Board just below ground corner of Vince's cross side of hut and main door	-	-
6HP13	Wood from window frame	+	-

Table A1.24: Sample identification, media used to culture fungi and presence of fungi; *Discovery* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6HP14	Board on main door side of hut above ground	-	-
6HP15	Wood from above main door wet wood	+	+
6HP16	Wood from outside veranda opposite the main door	+	+
6HP17	Board from inside veranda main door side of hut	-	-
6HP18	Defiberated wood below ground snow drift side of hut	+	+
6HP18V	Inside Veranda wood snowdrift side of hut	-	-
6HP19	Wood from roof Vince's cross side of hut.	+	+
6HP20	Inside veranda wood opposite seal carcass	-	-
6HPSWAB1	Swab wall behind mutton carcass in mutton room	+	+
6HPSWAB2	Swab wall snow drift side of hut between door to pendulum room and cooking area	-	-
6HPSWAB3	Swab shelf in pendulum room	-	-
6HPClad1	Outside wall opposite cooking area	-	-
6HPClado2	Outside wall on alleyway to galley area	-	-

Terra Nova Hut January 2004

Table A1.25: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CEMC1M	Wall left of main entrance behind supplies and table	+	+
6CEMC1L	Wall left of main entrance behind supplies and table	+	+
6CEMC2H	Wall Scott's area between chart table and Evans bed	-	-
6CEMC2M	Wall Scott's area between chart table and Evans bed	+	-
6CEMC2L	Wall Scott's area between chart table and Evans bed	-	-
6CEMC3H	Wall between lab bench and bunks	-	-
6CEMC3M	Wall between lab bench and bunks	+	+
6CEMC3L	Wall between lab bench and bunks	-	-
6CEMC4H	Galley area behind bed frame	-	-
6CEMC4M	Galley area behind bed frame	-	-
6CEMC4L	Wall Frozen sample	+	+
6CEMC5H	Wall above stores in galley area	-	+
6CEMC EntH	Wall in Entrance area	+	-
6CEMC EntM	Wall in Entrance area	-	-
6CEMC EntL	Wall in Entrance area	-	-
6CEMCB1	Roof beam in galley area	-	-
6CEMCB2	Roof beam after galley area towards darkroom	+	-

Table A1.25: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CEMCB3	Roof beam next closest to darkroom	+	+
6CEMCB4	Roof beam just outside darkroom	-	-
6CEMCF1	Floor next to the chimney in galley area	-	-
6CEMCF2	Floor same area as CEMC3	-	-
6CEMCF3	Floor between table and Scott's bed	-	-
6CEMCF4	Floor under bench near CEMC1	+	+
6CE1	Outside next to main door	+	+
6CE2	Batten above ground by main door	+	+
6CE3	Annex area alleyway to stables area below spades	+	+
6CE4	Annex area post on outside wall alley way to stables area	+	+
6CE5	Annex area by door to latrines	+	+
6CE6	Board annex area next to inside door to hut below ground	+	+
6CE7	Board annex area next to inside door to hut 2 boards up	+	-
6CE8	Board annex area next to inside door to hut 3m up loose wood	-	-
6CE9	Board annex area next to inside door to hut left side 1.5m up	-	-
6CE10	Annex area alleyway to stables area below spades 1.5 m up	-	-
6CE11	Hut wall stables stable 1	+	+
6CE12	Hut wall stables stable 3 1m up	-	-
6CE13	Hut wall stables stable 5 2 m up	+	+
6CE14	Hut wall stables stable 7 0.5 m up	+	+
6CE15	Hut wall stables stable 5 1m up	+	-
6CE16	Outside Snow drift side of hut wall 2m up	-	-
6CE17	Inside entrance right side 20 cm up	-	-
6CE18	Wall sample in same area as CEMC1	+	-
6CE19	Swab of wall area under sleeping platform left side	+	-
6CE20	Swab under sleeping platform left side	+	+
6CE21	Wall below widow on stables side of hut among stores	+	+
6CE22	Wall bunks area stables side of hut	-	-
6CE23	Inside between Scott's bed and chart table 1m up	-	-
6CE24	Inside between Scott's bed and chart table 0.3m up	-	-
6CE25	Darkroom swab of white fungus on wall 1.5m up	-	-
6CE26	Laboratory wall between benches 0.2m up	-	-
6CE27	Laboratory area snow drift side 0.5 m up	-	-
6CE28	Wall same site as CEMC3	-	-

Table A1.25: Sample identification, media used to culture fungi and presence of fungi; *Terra Nova* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CE29	Wall Entrance way CEMCEnt	+	-
6CE30	Wall snow drift side of hut under table from between bunks	+	+
6CE31	Floor between bunks on galley side of hut	+	+
6CE32	Floor laboratory area	+	+
6CE33	Wall Galley area above bunk	+	+
6CE34	Floor Galley area	+	-
6CE35	Floor main area next to chimney	-	-
6CE36	Floor between bunks and laboratory bench	-	-
6CE37	Floor main area in front of stores on stable side of hut	-	-
6CE38	Same site as CEMCF4	+	+
6CE39	Floor Entrance way.	+	+
6CE40	Door step to outside annex side	-	-
6CE41	Batten above stables	-	-
6CE42	Sample of sealer from wall above stable	+	-
6CE43	Annex wall above stables near hut wall	+	+
6CE44	Barnes glacier end of hut wall by roof	-	-
6CE45	Corner hut Barnes glacier/ snow drift end of hut by roof	-	-
6CE46	Snow drift side of hut Barnes glacier end of hut by roof	-	-
6CE47	Floor by chart table	-	-
6CESwab1	Table galley area inside front leg	-	-
6CESwab2	Table Galley area inside back leg	+	+
6CESwab3	Mould from box next to galley table	-	-
6CESwab4	White fungi in dark room	-	-
6CESwab5	White fungi on wall next to Galley table	+	+
6CESwab6	Dark wood from boxes next to Galley table	-	-
6CESwab7	White fungi from wall in dark Room	+	-
6CESwab8	Unattached boards just outside main door to hut	+	+
6CEScap9	Table in galley area	+	-
6CEScap10	Unattached Boards just outside main door to hut	+	+

Nimrod Hut January 2004

Table A1.26: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CR1	Wood outside corner of hut between latrine and main door below ground	+	+
6CR2	Wood by door step below ground	+	+
6CR3	Wood by door step stores side of hut below ground	+	+
6CR4	Wood back corner of the hut between back stores area and stables area below ground	+	+
6CR5	Wood between sample CR4 and stables area below ground	+	+
6CR6	Wood below ground stables area	+	+
6CR7	Wood below ground stables area near latrine	-	-
6CR8	Wood below ground latrine area	-	-
6CR9	Sealant on Main door side of hut	-	-
6CR10	Side beam next to sample 6CR9	-	-
6CR11	Wood next to sample 6CR10	-	-
6CR12	Wood Stores side of Porch area	-	-
6CR13	Wood Stores side of hut Facing Pony lake	-	-
6CR14	Wood stores side of hut behind stores 2 meters along wall	-	-
6CR15	Wood stores side of hut behind stores 3 meters along wall	-	-
6CR16	Wood stores side of hut behind stores 9 meters along wall	+	+
6CR17	Wood stores side of hut on corner with front of hut	-	-
6CR18	Wood back stores area behind stores 3 meters from side stores wall	+	+
6CR19	Wood back stores area behind stores 4 meters from side stores wall	-	-
6CR20	Wood back corner of the hut between back stores area and stables area near roof	-	-
6CR21	Wood between sample CR4 and stables area above ground	-	+
6CR22	Wood above ground stables area 7 meters from latrine	-	-
6CR23	Insulating material above ground stables area 6 meters from latrine	-	-
6CR24	Wood above ground stables area 5 meters from latrine	-	-
6CR25	Wood above ground stables area 2 meters from latrine	-	-
6CR26	Wood above ground stables area next to latrine	-	-
6CR27	Wood above ground stables area 1 metre from latrine	-	-
6CR28	Wood above ground latrine area	-	-
6CR29	Wood above ground latrine area near entrance	-	-
6CR30	Inside Porch area right side 2 metres from outside door	-	-
6CR31	Inside Porch area right side	-	-
6CR32	Inside Porch area left side	-	-

Table A1.26: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CR33	Inside Porch area left side near door to Mawson's lab	-	-
6CR34	Inside Swab Steps down into Mawson's lab	-	-
6CR35	Steps down into Mawson's lab	-	-
6CR36	Inside hut wall Mawson's lab 1.5 metres from door	-	-
6CR37	Inside hut wall Mawson's lab 2.5 metres from door	-	-
6CR38	Inside hut wall Mawson's lab near door	-	-
6CR39	Inside Wood inside door to hut	-	-
6CR40	Inside hut wall next to Shackleton's room Half way up wall	-	-
6CR41M	Inside hut wall next to Shackleton's room near roof	-	-
6CR42R	Inside hut wall 1metre from Shackleton's room ceiling beam	-	-
6CR43	Inside hut wall 1metre from Shackleton's room ceiling	-	-
6CR44	Inside hut wall next to Shackleton's room near roof Down by floor	-	-
6CR45M	Inside Wall stores side of hut next to couch half way up wall	-	-
6CR46H	Inside Wall stores side of hut next to couch near roof	-	-
6CR47	Inside Wall Backdoor By side of hut near floor among stores	+	-
6CR48M	Inside Wall Backdoor Bay side of hut bed area next to biologist lab Middle of wall	-	-
6CR49H	Inside Wall Backdoor Bay side of hut bed area next to biologist lab Near roof	+	-
6CR50C	Inside Wall backdoor bay side of hut bed area next to biologist lab ceiling	-	-
6CR51R	Inside Wall stables side of hut under bed in corner ceiling beam	-	-
6CR52L	Inside Wall stables side of hut under bed in corner	-	-
6CR53	Inside Wall stables side of hut above bed next to canvas curtain	-	-
6CR54	Inside Wall stables side of hut dark room side of canvas Near floor	-	-
6CR55	Inside Wall stables side of hut dark room side of canvas halfway up wall	-	-
6CR56H	Inside Wall stables side of hut dark room side of canvas near roof	-	-
6CR57R	Inside Ceiling stables side of hut dark room side of canvas near wall	-	-
6CR58	Inside Ceiling beam stables side of hut dark room side of canvas	-	-
6CR59	Inside sledge resting on ceiling beams	-	-
6CR60F	Inside Floor stores side of hut dark room side of canvas	-	-
6CR61F	Inside Floor stables side of hut dark room side of canvas	-	-

Table A1.26: Sample identification, media used to culture fungi and presence of fungi; *Nimrod* Hut 2004.

Sample ID	Sample location description	YM	MEA+ acid
6CR62F	Inside Floor stables side of hut under bed in corner	-	-
6CR63F	Inside Floor store area just behind stove	-	-
6CR64R	Inside Roof Shackleton's room	-	-
6CR65F	Inside Floor Dark room	-	-
6CR66	Inside Wall from darkroom	-	-
6CR67	Inside Wood from window stables side of hut	-	-
6CR68	Inside entrance way Shackleton's side of hut		
6CR69	Inside Door step out of hut	-	-
6CR70	Outside Door step out of hut	-	-
6CRSwab1	Wall in stores area next to bacon	+	-
Swab2	Paper on shelf stables side of hut	-	-
Swab3	Mawson's lab Moirs plum jar	-	-

Appendix 2 Results of Spore Traps, Bait traps, and culturing on location

Results of Spore traps, bait traps and culturing on site for the sampling trips January 2002 to January 2004 are given in Appendix 2.
Whether fungi were isolated from a sample is indicated by a + if fungi were isolated or- if no fungi were isolated.

Discovery Hut January 2002

Table A2.1: Location of spore traps, media used for culturing, fungi cultured; *Discovery* Hut January 2002

Location	Media	Fungi Isolated
Floor of mutton room	YM	+
Floor in front of chaff	YM	+
Floor entrance way to hut	YM	+
Floor main area of hut	YM	+
On top of artefacts main area of hut	YM	+
On bench next to stove	YM	+
On middle shelf in pendulum room	YM	+

Discovery Hut January 2003

Table A2.2: Location of spore traps, media used for culturing, fungi cultured; *Discovery* Hut January 2003.

Location	Media	Fungi Isolated
Outside	YM	-
Outside	Martins	-
Outside	PDA	-
Outside by wood block frame	YM	+
Outside by wood block frame	Martins	-
Outside by wood block frame	PDA	+
Mutton room	YM	+
Main area	YM	+
Main area	Martins	+
Main area	PDA	+
Galley area	YM	+
Galley area	Martins	+
Galley area	PDA	+
In artefacts between galley and pendulum room	YM	+

Discovery Hut January 2004

Table A2.3: Location of spore traps, media used for culturing, fungi cultured; *Discovery* Hut January 2004.

Location	Media	Fungi Isolated
Outside by hitching post	YM	+
Outside by hitching post	NaCl	-
Outside by hitching post	Media 7	-
Outside by seal carcass	YM	+
Outside by seal carcass	NaCl	+
Outside by seal carcass	Media 7	+
Inside by visitor sign in table	YM	+
Inside by visitor sign in table	NaCl	+
Inside by visitor sign in table	Media 7	-
Pendulum room under shelf with penguin skin on	YM	+
Mutton room	YM	+
Mutton room	NaCl	+
Mutton room	Media 7	-
Main area in front of seal blubber	YM	+

Terra Nova Hut January 2002

Table A2.4: Location of spore traps, media used for culturing, fungi cultured; *Terra Nova* Hut January 2002

Location	Media	Fungi Isolated
On floor in dark room	YM	+
On bed in galley area	YM	+
Top bunk lab area	YM	+
Floor stables side of hut between table and stores	YM	+
Under acetylene plant in entrance way to hut	YM	+
Under bunks Tenements area	YM	+
Chair in Scott's area	YM	+
On top of high shelf in laboratory area	YM	+
On top of main table	YM	+
Floor in main area of hut	YM	+
Next to mat by main door	YM	+
By stove in stables area	YM	+
Annex area next to shoe cleaning grate	YM	+
Stables area	YM	+
Annex area on shelves	YM	+

Terra Nova Hut January 2003

Table A2.5: Location of spore traps, media used for culturing, fungi cultured; *Terra Nova* Hut January 2003.

Location	Media	Fungi Isolated
Meteorology station	YM	-
Meteorology station	Martins	-
Meteorology station	PDA	-
Near fuel drums	YM	+
Near fuel drums	Martins	-
Near fuel drums	PDA	-
In front of hay	YM	+
In front of hay	Martins	-
In front of hay	PDA	+
Outside snow drift side of hut	YM	-

Table A2.5: Location of spore traps, media used for culturing, fungi cultured; *Terra Nova* Hut January 2003.

Location	Media	Fungi Isolated
Outside snow drift side of hut	Martins	-
Outside snow drift side of hut	PDA	-
Cape Evans Wood block frame	YM	-
Cape Evans Wood block frame	Martins	-
Cape Evans Wood block frame	PDA	-
End of annex	YM	+
End of annex	Martins	-
End of annex	PDA	-
Stable 3	YM	+
Stable 3	Martins	-
Stable 3	PDA	+
Darkroom	YM	-
Darkroom	Martins	-
Darkroom	PDA	+
On top of Evan's bed	YM	-
On top of Evan's bed	Martins	-
On top of Evan's bed	PDA	-
Floor middle of room	YM	+
Floor middle of room	Martins	-
Floor middle of room	PDA	+
Galley area	YM	+
Galley area	Martins	-
Galley area	PDA	+
On top of Bower's bunk	YM	+
On top of Bower's bunk	Martins	-
On top of Bower's bunk	PDA	+
On table Hayward's bunk area	YM	+
On table Hayward's bunk area	Martins	-
On table Hayward's bunk area	PDA	+
On top of lab bench	YM	+
On top of lab bench	Martins	-
On top of lab bench	PDA	-

Terra Nova Hut January 2004

Table A2.6: Location of spore traps, media used for culturing, fungi cultured; *Terra Nova* Hut January 2004.

Location	Media	Fungi Isolated
Annex area on top of butter boxes	YM	+
Annex area on top of butter boxes	NaCl	+
Annex area on top of butter boxes	Media 7	-
Annex area beside the blubber pile	YM	+
Annex area alley way to stables area	YM	-
On top of Oates bunk	YM	-
On top of Oates bunk	NaCl	-
On top of Oates bunk	Media 7	-
On floor next to stove chimney	YM	-
On floor next to stove chimney	NaCl	-
On floor next to stove chimney	Media 7	-
Between Evan and Wilson's bed	YM	-
Between Evan and Wilson's bed	NaCl	-
Between Evan and Wilson's bed	Media 7	-

Table A2.6: Location of spore traps, media used for culturing, fungi cultured; *Terra Nova* Hut January 2004

Location	Media	Fungi Isolated
On chair beside visitor sign in book	YM	+
On chair beside visitor sign in book	NaCl	-
On chair beside visitor sign in book	Media 7	-
On chair in galley area	YM	+
On chair in galley area	NaCl	-
On chair in galley area	Media 7	-
Under Wright and Simpson's bunk	YM	-
Under Wright and Simpson's bunk	NaCl	-
Under Wright and Simpson's bunk	Media 7	-

Nimrod Hut January 2002

Table A2.7: Location of spore traps, media used for culturing, fungi cultured; *Nimrod* Hut January 2002

Location	Media	Fungi Isolated
Shelf above stores southern side of hut	YM	-
Shelf above stores northern side of hut	YM	+
Bench in darkroom	YM	-
Among food stuffs Shackletons's room	YM	-
Under door mat in porch	YM	+
Onto of stores boxes	YM	-
On floor in stores area	YM	+
On floor between beds	YM	-
Under couch among shoes	YM	-
On top of stove	YM	-
On top of AHT gear in Mawson's laboratory	YM	+

Nimrod January 2003

Table A2.8: Location of spore traps, media used for culturing, fungi cultured; *Nimrod* Hut January 2003.

Location	Media	Fungi Isolated
Meteorological Station	YM	-
Meteorological Station	Martins	-
Meteorological Station	PDA	-
In front of southern stores area	YM	-
In front of southern stores area	Martins	-
In front of southern stores area	PDA	-
In stables area	YM	-
In stables area	Martins	-
In stables area	PDA	-
Cape Royds wood block frame	YM	-
Cape Royds wood block frame	Martins	-
Cape Royds wood block frame	PDA	-
Outside wannigan	YM	-
Outside wannigan	PDA	-
On floor main area of hut	YM	+
On floor main area of hut	Martins	-
On floor main area of hut	PDA	+
Shelf in front of window	YM	-
Shelf in front of window	Martins	-
Shelf in front of window	PDA	-
High shelf in stores area	YM	-
High shelf in stores area	Martins	-
High shelf in stores area	PDA	-

Nimrod Hut January 2004

Table A2.9: Location of spore traps, media used for culturing, fungi cultured; *Nimrod* Hut January 2004.

Location	Media	Fungi Isolated
Under beds	YM	-
Under beds	NaCl	-
Under beds	Media 7	-
Under stove	YM	-
Under stove	NaCl	-
Under stove	Media 7	-
On top of couch	YM	-
On top of couch	NaCl	-
On top of couch	Media 7	-
Door way to hut	YM	-
Door way to hut	NaCl	-
Door way to hut	Media 7	-
Shelf above hams in galley area	YM	-
Shelf above hams in galley area	NaCl	-
Shelf above hams in galley area	Media 7	+
Below window	YM	+
Below window	NaCl	-
Below window	Media 7	-
Darkroom	YM	-
Darkroom	NaCl	-
Darkroom	Media 7	-

Results from Bait traps from the *Terra Nova* and *Nimrod* Huts in January 2004

Table A2.10: Hut name, location of bait traps, media used for culturing, fungi cultured; *Terra Nova* and *Nimrod* Hut January 2004.

Hut	Location	Media	Fungi Isolated
Terra Nova	Between latrines and sea ice	YM	-
Terra Nova	Between latrines and sea ice	NaCl	-
Terra Nova	By the main door	YM	+
Terra Nova	By the main door	NaCl	-
Terra Nova	In annex area under spades	YM	+
Terra Nova	In annex area under spades	NaCl	+
Nimrod	Latrine area	YM	+
Nimrod	Latrine area	NaCl	+
Nimrod	Latrine area	Media 7	+
Nimrod	Old dump	YM	+
Nimrod	Old dump	NaCl	+
Nimrod	Next to weather station	YM	-
Nimrod	Next to weather station	NaCl	-
Nimrod	Next to weather station	Media 7	-
Nimrod	In front of southern side stores	YM	+

Sampling and culturing on location at *Terra Nova* Hut 2002, 2003 and 2004

Terra Nova Hut 2002

Table A2.11: Sampling technique, Location of sample, media used for culturing, fungi cultured; *Nimrod* Hut January 2002.

Sample type	Location	Media	Fungi Isolated
Swab	Dark fungus below window	YM	+
Swab	Fungus in kitchen	YM	+
Swab	Fungus in kitchen	YM	+
Swab	Fungus in dark room	YM	+
Swab	Fungus above bunk laboratory area	YM	+

Terra Nova Hut 2003

Table A2.12: Sampling technique, Location of sample, media used for culturing, fungi cultured; *Nimrod* Hut 2003.

Sample type	Location	Media	Fungi Isolated
Swab	Darkroom	YM	+
Swab	Bunk next to laboratory	YM	+
Swab	Leg of table in kitchen	YM	+
Swab	Cup shelf	YM	+

Terra Nova Hut 2004

Table A2.13: Sampling technique, Location of sample, media used for culturing, fungi cultured; *Nimrod* Hut 2004.

Sample type	Location	Media	Fungi Isolated
Swab	Butter box mould second box second level	YM	+
Swab	Support box by butter boxes	YM	+
Swab	Box fourth box first level	YM	+
Swab	Box fifth box of butter second level	YM	+
Swab	6CE3 Annex area alleyway to stables area below spades	Media 7	-
Swab	6CE4 Annex area post on outside wall alley way to stables area	Media 7	-
Swab	6CE5 Annex area by door to latrines	Media 7	-
Swab	6CE6 Board annex area next to inside door to hut below ground	Media 7	-

Sampling and culturing on location at *Nimrod* Hut 2002 and 2003

Nimrod Hut 2002

Table A2.14: Sampling technique, Location of sample, media used for culturing, fungi cultured; *Nimrod* Hut 2002.

Sample type	Location	Media	Fungi Isolated
Scraping	Wall in Mawson's laboratory	YM	+

Nimrod Hut January 2003

Table A2.15: Sampling technique, Location of sample, media used for culturing, fungi cultured; *Nimrod* Hut 2003.

Sample type	Location	Media	Fungi Isolated
Swab	Door to main area	YM	-
Swab	Wall near floor between window and canvas	YM	-
Swab	Paper on shelf northern side of hut	YM	+
Swab	Wall next to biology area	YM	-
Swab	Wall stores area next to bacon	YM	+
Scraping	Wall next to bacon	YM	+
Swab	Wall in porch next to tools	YM	-
Swab	Bacon	YM	-
Swab	Ham	YM	-
Swab	Wall Mawson's laboratory	YM	-
Sample	Peas outside southern stores	YM	+
Sample	Sawdust around salt bottles	YM	-
Sample	Flour outside Eastern stores	YM	-
Sample	Goop outside eastern stores wall	YM	-
Sample	Fibres from sleeping bag	YM	-
Swab	Black spot on floor	YM	-

Appendix 3 Moisture content of structural wood and presence/absence of fungi from Moisture content samples

Results of moisture content and presence/absence of fungal growth from wood samples taken at the three Historic Huts from sampling trips 2003 and 2004 are given in Appendix 3.

Moisture Content and fungal growth inside *Discovery* Hut 2004

Table A3.1: Sampling number, Location of sample, moisture content, fungi cultured; inside *Discovery* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
HPMC1	Wall High	16.8	+
	Wall Medium	36.8	+
	Wall Low	21.7	-
HPMC2	Wall High	0.0	-
	Wall Medium	28.6	-
	Wall Low	11.8	-
HPMC3	Wall High	0.0	+
	Wall Medium	300.0	+
	Wall Low	80.9	+
HPMC4	Wall High	11.8	+
	Wall Medium	27.6	+
	Wall Low	75.0	+
HPMCR1	Ceiling	95.2	+
HPMCR2	Ceiling	75.0	-
HPMCR3	Ceiling	92.2	+
HPMCF1	Floor	22.4	+
HPMCF2	Floor	19.7	+
HPMC5	Wall High	80.0	+
	Wall Medium	50.0	-
	Wall Low	48.9	+
HI1	Wall	15.7	+
HI2	Wall	21.6	+
HI3	Wall	21.6	+

Table A3.2: Sampling number, Location of sample, moisture content, fungi cultured; outside *Discovery* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
H1	Below ground	51.1	+
H3	Below ground	35.2	-
H5	Above ground	31.3	-
H7	Above ground	100.8	+
H8	Above ground	13.4	+
H9	Above ground	16.2	+
H10	Above ground	23.4	+
H12	Below ground	61.5	-
H14	Above ground	2.6	-
H17	Above ground	14.2	-
H18	Below ground	83.4	+
H18V	Above ground	9.7	-
H20	Above ground	10.6	-

Moisture Content and fungal growth *Terra Nova* Hut 2003 and 2004

Table A3.3: Sampling number, Location of sample, moisture content, fungi cultured; inside *Terra Nova* Hut 2003.

Sample ID	Location	Moisture content (%)	Fungal growth
CEMC1	Wall Middle	14.3	-
	Wall Low	16.7	+
CEMC2	Wall High	0	-
	Wall Middle	3.2	-
	Wall Low	16.1	+
CEMC3	Wall High	8.3	+
	Wall Middle	11.5	+
	Wall Low	19.2	+
CEMC4	Wall Middle	70	+
	Wall Low	12.1	-
CEMC5	Wall High	15.8	+
	Wall Middle	17.4	-
	Wall Low	16.7	+
CEMCB1	Ceiling	0	-
CEMCB3	Ceiling	3.8	-
CEMCB4	Ceiling	16.4	+
CEMCF1	Floor	5.5	-
CEMCF2	Floor	16	-
E43	Above ground	70	+
FL	Above ground	22.5	+
AW1	Above ground	18.5	+
AW2	Above ground	22.5	-

Table A3.4: Sampling number, Location of sample, moisture content, fungi cultured; inside *Terra Nova* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
CEMC1	Wall High	10.9	+
	Wall Middle	24.5	+
	Wall Low	-13.3	-
CEMC2	Wall High	17.6	+
	Wall Middle	15.4	-
	Wall Low	19.6	-
CEMC3	Wall High	13.8	+
	Wall Middle	18.8	-
	Wall Low	115.4	-
CEMC4	Wall High	20.0	+
	Wall Middle	200.0	-
CEMC5	Wall Middle	30.8	+
	Wall Low	9.5	-
CEMC Ent	Wall High	21.6	-
	Wall Middle	17.5	-
	Wall Low	30.0	+
CEMCB1	Ceiling	16.1	+
CEMCB2	Ceiling	11.5	-
CEMCB3	Ceiling	14.6	-
CEMCB4	Ceiling	14.0	-
CEMCF1	Floor	24.8	-
CEMCF2	Floor	12.7	+
CEMCF3	Floor	24.3	-
CEMCF4	Floor	51.9	+
E18	Wall	28.2	-
E21	Wall	24.6	-
E22	Wall	22.3	+
E28	Wall	39.1	+
E29	Wall	25.0	+
E31	Floor	116.7	+
E33	Wall	11.4	+
E34	Floor	20.1	+
E38	Floor	23.8	-
E39	Floor	19.1	-

Table A3.5: Sampling number, Location of sample, moisture content, fungi cultured; outside *Terra Nova* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
E8	Above ground	39.0	+
E11	Above ground	42.3	+
E15	Above ground	22.0	+
E40	Above ground	8.0	+
E41	Above ground	7.7	-

Moisture Content and fungal growth outside *Nimrod* Hut 2003 and 2004

Table A3.6: Sampling number, Location of sample, moisture content, fungi cultured; inside *Nimrod* Hut 2003.

Sample ID	Location	Moisture content (%)	Fungal growth
5CRMC1 H	Wall High	26.7	-
5CR MC1 M	Wall Middle	17.6	-
5CR MC1 L	Wall Low	21.2	-
5CR MC2 H	Wall High	25	-
5CR MC2 M	Wall Middle	17.6	+
5CR MC2 L	Wall Low	16.7	-
5CR MC3 H	Wall High	21.8	-
5CR MC3 M	Wall Middle	0	-
5CR MC3 L	Wall Low	10	-
5CR MC4 H	Wall High	40	-
5CR MC4 M	Wall Middle	5.3	-
5CR MC4 L	Wall Low	5	-
5CR MCR1	Ceiling	33.3	-
5CR MCR2	Ceiling	33.3	-
5CR MCR3	Ceiling	0	-
5CR MCR4	Ceiling	11.1	-
5CR MC5	Wall	16.7	-
5CR MC6	Wall	11.5	-
5CR MC7	Wall	13	-
5CR MCMa	Wall	14.9	-
5CR MCF1	Floor	11.7	-
5CR MCF2	Floor	22.8	-
5CR MCF3	Floor	14.3	-
5CR MCF4	Floor	33.3	-

Table A3.7: Sampling number, Location of sample, moisture content, fungi cultured; inside *Nimrod* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
R30	Wall	9.6	-
R31	Wall	19.0	-
R32	Wall	14.3	-
R35	Floor	20.5	-
R36	Wall	21.7	-
R38	Wall	20.4	-
R41	M	37.5	-
R42	R	66.7	-
R45	M	22.6	-
R46	H	3.8	-
R48	M	75.0	-
R49	H	11.2	-
R50	C	4.2	-
R51	R	12.5	-
R52	L	18.1	-
R53	W	27.3	-
R56	H	11.3	-
R57	R	11.1	-

Table A3.7: Sampling number, Location of sample, moisture content, fungi cultured; inside *Nimrod* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
R59	R	14.2	-
R60	F	15.5	-
R61	F	14.1	-
R62	F	2.0	-
R63	F	34.8	-
R64	R	1.2	+
R65	F	-4.1	-
R66	W	25.0	-
R67	W	0.0	-
DS inside	F	2.6	-
DS outside	F	15.9	-
CRMC2M	M	17.6	-
CRMC2L	L	16.7	-
CRMC4M	M	5.3	-
CRMC4L	L	5	-
CRMCR1	R	33.3	-
CRMCR2	R	33.3	-
CRMCR3	R	0	-
CRMCR4	R	11.1	-
CRMCF3	R	14.3	-
CRMCF4	R	33.3	-

Table A3.8: Sampling number, Location of sample, moisture content, fungi cultured; outside *Nimrod* Hut 2004.

Sample ID	Location	Moisture content (%)	Fungal growth
R6	Below ground	98.2	+
R8	Below ground	132.0	-
R18	Above ground	28.1	+
R11	Above ground	-5.6	-
R14	Above ground	17.5	-
R19	Above ground	2.6	-
R21	Above ground	11.4	-
R22	Above ground	3.6	-
R26	Above ground	2.9	-
R27	Above ground	3.2	-
R28	Above ground	8.5	-
R29	Above ground	14.6	-

Appendix 4

Antarctic Filamentous Fungal Culture Collection.

Location: –70°C Freezer lobby of PC2 laboratory C208

Curator: Professor Roberta Farrell

Culture number	Sample number	Media	Description	Incubation Temperature	Identification
1.			Yeast		
2.	Air	6	White Rough Raised Fungi	4,15	
3.	Air	6	White Rough Raised Fungi	15	
4.	Air	6	White Rough Raised Fungi	4,15	
5.	Air	6	White Rough Raised Fungi	4,15	
6.	Air	6	White Rough Raised Fungi	4	
7.	Air	MEA	Black/green Rough Fungi	4	
8.	Air	MEA	Black/green Rough Fungi	4	
9.	Air	MEA	Black/green Rough Fungi	4	
10.	Air	MEA	Black/green Rough Fungi	4	
11.			Bacteria		
12.			Yeast		
13.			Bacteria		
14.			Yeast		
15.			Yeast		
16.			Yeast		
17.			Bacteria		
18.			Yeast		
19.			Bacteria		
20.			Yeast		
21.			Yeast		
22.			Not frozen		
23.			Not frozen		
24.			Bacteria		
25.			Yeast		
26.			Yeast		
27.	Air	MEA	Black/brown fungi	4	
28.	Air	MEA	Dark Fungi	4	
29.			Bacteria		
30.			Not frozen		
31.	Air	MEA	White top Pale Orange base Fungi	4	
32.	Air	MEA	White top Yellow base Fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
33.	Air	MEA	White top Red/Orange base Fungi	4	
34.	Air	MEA	White top Red/Orange base Fungi	4	
35.	Air	MEA	White top Red/Orange base Fungi	4	
36.	Air	MEA	White top Red/Orange base Fungi	4	
37.	Air	MEA	White rough top White /cream base Fungi	4,15	
38.	Air	MEA	White rough top White /cream base Fungi	4,15	
39.	Air	MEA	White rough top White /cream base Fungi	4,15	
40.	Air	MEA	White rough top White /cream base Fungi	4,15	
41.			Bacteria		
42.			Bacteria		
43.	Air	MEA	Black/ Green rough Fungi	4,15	
44.	Air	MEA	Black/ Green rough Fungi	4,15	
45.			Yeast		
46.			Yeast		
47.			Bacteria		
48.			Bacteria		
49.	Air	MEA	White flat Fungi	4,15	
50.	Air	MEA	White flat Fungi	4,15	
51.	Air	MEA	White flat Fungi	4,15	
52.			Bacteria		
53.			Bacteria		
54.			Bacteria		
55.			Bacteria		
56.			Bacteria		
57.			bacteria		
58.	H32	MEA	Penicillium like	25	*Penicillium* sp.
59.			Not frozen		
60.			Not frozen		
61.	H46	MEA	Penicillium like	25	*Penicillium* sp.
62.			Not frozen		
63.			Not frozen		
64.	H46	MEA	Penicillium like	25	*Penicillium* sp.
65.	H59	MEA	Penicillium like	25	*Penicillium* sp.
66.			Not frozen		
67.			Not frozen		
68.			Not frozen		
69.			Not frozen		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
70.			Not frozen		
71.	H44	MEA	Penicillium like	25	*Penicillium* sp.
72.	H41	MEA	Penicillium like	25	*Penicillium* sp.
73.	H34	MEA	Penicillium like	25	*Penicillium* sp.
74.	H66	MEA	Penicillium like	25	*Penicillium* sp.
75.	E23	VB	Rhizopus like	25	*Rhizopus* sp.
76.			Not frozen		
77.	E33	6	Penicillium like	25	*Penicillium* sp.
78.			Bacteria		
79.			Bacteria		
80.	E35	VB	Black small fungi	25	*Cadophora malorum*
81.			Not frozen		
82.			Bacteria		
83.			Not frozen		
84.			Not frozen		
85.			Not frozen		
86.	E38	MEA	Penicillium like	25	*Penicillium* sp.
87.			Bacteria		
88.			Bacteria		
89.			Not frozen		
90.			Not frozen		
91.	E66	6	Penicillium like	25	*Penicillium* sp.
92.			Bacteria		
93.			Bacteria		
94.			Bacteria		
95.			Bacteria		
96.	E19	4	Penicillium like	25	*Penicillium* sp.
97.	E4	6	Penicillium like	25	*Penicillium* sp.
98.	E19	6	Penicillium like	25	*Penicillium* sp.
99.	E4	MEA	Penicillium like	25	*Penicillium* sp.
100.			Yeast		
101.	E19	MEA	Penicillium like	25	*Penicillium* sp.
102.			Bacteria		
103.			Yeast		
104.			Not frozen		
105.			Bacteria		
106.			Bacteria		
107.	E19	VB	Penicillium like	25	*Penicillium* sp.
108.			Not frozen		
109.			Bacteria		
110.	E153	MEA	Trichoderma like	25	*Trichoderma* sp
111.			Bacteria		
112.	E149	MEA	Trichoderma like	25	*Trichoderma* sp
113.			Bacteria		
114.			Bacteria		
115.			Bacteria		
116.	E147	MEA	Black/green fungi	15	
117.	E147	4	Black/green fungi	15	
118.	E147	VB	White fungi	15	
119.	E147	6	White fungi	15	
120.	H22	MEA	Penicillium like	25	*Penicillium* sp.
121.			Not frozen		
122.					

Culture number	Sample number	Media	Description	Incubation Temp	Identification
123.			Bacteria		
124.	E19	4	Penicillium like	25	*Penicillium* sp.
125.			Not frozen		
126.			Not frozen		
127.	H66	6	Penicillium like	25	*Penicillium* sp.
128.	H65	6	Penicillium like	25	*Penicillium* sp.
129.	H22	6	White fungi	25	
130.			Bacteria		
131.	H33	MEA	Dark fungi	25	
132.			Bacteria		
133.	H47	4	Penicillium like	25	*Penicillium* sp.
134.			Bacteria		
135.			Yeast		
136.			Not frozen		
137.			Bacteria		
138.			Bacteria		
139.			Bacteria		
140.			Bacteria		
141.			Bacteria		
142.			Bacteria		
143.			Not frozen		
144.			Not frozen		
145.			Not frozen		
146.			Bacteria		
147.			Not frozen		
148.			Bacteria		
149.	E22	MEA	Penicillium like	25	*Penicillium* sp.
150.	E44	4	Small white fungi	25	
151.			Bacteria		
152.	E10	4	Penicillium like	25	*Penicillium* sp.
153.			Bacteria		
154.			Bacteria		
155.			Not frozen		
156.			Not frozen		
157.			Bacteria		
158.			Yeast/bacteria		
159.	E4	MEA	Penicillium like	25	*Penicillium* sp.
160.			Bacteria		
161.			Bacteria		
162.			Yeast		
163.			Not frozen		
164.			Yeast		
165.			Bacteria		
166.	E46	4	Penicillium like	25	*Penicillium* sp.
167.			Bacteria		
168.	E46	MEA	Penicillium like	25	*Penicillium* sp.
169.			Bacteria		
170.			Bacteria		
171.	E46	VB	White fungi with black dots	25	
172.			Bacteria		
173.			Bacteria		
174.	E35	MEA	Green Fungi	25	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
175.	E30	VB	White fungi	25	
176.			Bacteria		
177.			Bacteria		
178.			Bacteria		
179.			Yeast		
180.			Yeast		
181.	E38	6	White fungi with yellow base	15	
182.	E35	VB	Green/ black fungi	15	*Cadophora malorum*
183.	E35	VB	White furry fungi	15	
184.			Bacteria		
185.	E38	VB	Bright white fungi	15	
186.			Bacteria		
187.			Bacteria		
188.			Bacteria		
189.	H32	MEA	Green/ black fungi	15	
190.			Bacteria		
191.			Not frozen		
192.			Not frozen		
193.			Bacteria		
194.			Not frozen		
195.	H52	VB	White/green top fungi	15	
196.	H33	VB	Yellow base fungi	15	
197.	H33	VB	White fluffy fungi	15	
198.	H47	MEA	Black Fungi	15	
199.	H35	MEA	Cream dull fungi turning black	15	
200.	H33	MEA	Tan fungi	15	
201.			Not frozen		
202.	H52	MEA	Green/ black penicillium like	15	*Cladosporium* sp.
203.	H35	4	Tan dull Fungi	15	
204.	H33	4	Fungi	15	
205.	H33	4	Fungi same 204	15	
206.			Not frozen		
207.			Not frozen		
208.			Not frozen		
209.	H52	4	Green/ black penicillium like	15	*Cladosporium* sp.
210.			Not frozen		
211.			Yeast		
212.			Not frozen		
213.			Not frozen		
214.	E4	VB	Green/ black penicillium like	15	*Cladosporium* sp.
215.	E19	VB	Penicillium like	15	*Penicillium* sp.
216.			Not frozen		
217.	E4	MEA	Penicillium like	15	*Penicillium* sp.
218.	E19	MEA	Green/ black penicillium like	15	*Cladosporium* sp.
219.	E19	MEA	Penicillium like	15	*Penicillium* sp.
220.			Not frozen		
221.			Not frozen		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
222.	E4	6	White fungi	15	
223.			Yeast/bacteria		
224.			Not frozen		
225.	H22	VB	White fluffy fungi	15	
226.	H22	6	White fluffy fungi	15	
227.	H22	4	White fluffy fungi	15	
228.	H22	MEA	White fluffy fungi	15	
229.	H41	MEA	Tan dull Fungi	15	
230.	H41	4	Small white rough fungi	15	
231.			Bacteria		
232.			Bacteria		
233.			Bacteria		
234.			Bacteria		
235.	E76	MEA	Green penicillium like	15	*Penicillium* sp.
236.	E35	4	Green penicillium like	15	*Penicillium* sp.
237.			Not frozen		
238.	E38	4	Green penicillium like	15	*Penicillium* sp.
239.	E38	4	Bright white fungi	15	
240.			Bacteria		
241.			Not frozen		
242.	E35	MEA	Green fungi	15	*Cadophora malorum*
243.			Not frozen		
244.	E38	MEA	Green/ black penicillium like	15	*Cladosporium* sp.
245.			Bacteria		
246.	E38	MEA	Bright white fungi	15	
247.	E38	MEA	Blue/green penicillium like	15	*Penicillium* sp.
248.	E73	4	Pale green spreading fungi	15	
249.			Bacteria		
250.			Yeast		
251.	E19	VB	Green/ black penicillium like	15	*Cladosporium* sp.
252.			Not frozen		
253.			Not frozen		
254.	E4	4	Green/ black penicillium like	15	*Penicillium* sp.
255.			Not frozen		
256.	E19	4	Blue/green penicillium like	15	*Penicillium* sp.
257.			Not frozen		
258.			Not frozen		
259.			Not frozen		
260.	E147	4	White/green penicillium like	4	*Penicillium* sp.
261.	E147	MEA	White/green penicillium like	4	*Penicillium* sp.
262.	E35	7	White Fungi	4	*Pseudeurotium desertorum*
263.	E4	MEA	White fungi	4	
264.			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
265.			Yeast		
266.			Not frozen		
267.			Yeast		
268.			Yeast		
269.			Yeast		
270.			Yeast		
271.	E35	MEA	Tiny dark fungi	4	
272.			Yeast		
273.	E38	MEA	Dark fungi	4	
274.	E4	4	White furry fungi	4	
275.			Yeast		
276.	H32	MEA	Dark fungi	4	
277.			Bacteria		
278.	H32	4	White rough fungi	4	
279.			Yeast		
280.	H35	VB	Dark fungi	4	
281.	H35	MEA	Dark rough fungi	4	
282.			Yeast		
283.	H52	MEA	Dark rough fungi	4	
284.			Yeast		
285.	H35	4	Cream rough fungi	4	
286.			Yeast		
287.	H52	4	Cream rough fungi	4	
288.			Bacteria		
289.			Bacteria		
290.			Bacteria		
291.			Bacteria		
292.	W7	4	Fungi black dot	25	
293.			Not frozen		
294.			Bacteria		
295.			Bacteria		
296.			Bacteria		
297.			Bacteria		
298.			Bacteria		
299.	TF	4	Penicillium like	25	*Penicillium* sp.
300.			Yeast		
301.			Bacteria		
302.			Not frozen		
303.			Bacteria		
304.			Bacteria		
305.			Bacteria		
306.			Bacteria		
307.			Bacteria		
308.			Bacteria		
309.			Not frozen		
310.			Not frozen		
311.	R153	MEA	Penicillium like	25	*Penicillium* sp.
312.			Bacteria		
313.			Not frozen		
314.	R158	6	Penicillium like	25	*Penicillium* sp.
315.			Bacteria		
316.	R158	VB	Penicillium like	25	*Penicillium* sp.
317.	R158	4	Penicillium like	25	*Penicillium* sp.
318.	R158	7	Penicillium like	25	*Penicillium* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
319.			Yeast		
320.	R158	MEA	Penicillium like	25	*Penicillium* sp.
321.	R136	4	Penicillium like	25	*Penicillium* sp.
322.			Yeast		
323.			Yeast		
324.	R136	VB	Penicillium like	25	*Penicillium* sp.
325.			Bacteria		
326.			Bacteria		
327.			Bacteria		
328.			Bacteria		
329.	R136	MEA	Penicillium like	25	*Penicillium* sp.
330.			Bacteria		
331.			Bacteria		
332.			Yeast		
333.	R157	6	White rough fungi	25	
334.	R137	4	Penicillium like	25	*Penicillium* sp.
335.			Not frozen		
336.			Not frozen		
337.			Yeast		
338.	R137	VB	Penicillium like	25	*Penicillium* sp.
339.			Bacteria		
340.			Yeast		
341.			Bacteria		
342.			Bacteria		
343.			Bacteria		
344.			Bacteria		
345.			Bacteria		
346.			Bacteria		
347.			Bacteria		
348.			Bacteria		
349.			Bacteria		
350.			Bacteria		
351.			Bacteria		
352.			Yeast		
353.			Bacteria		
354.	R22	VB	Penicillium like	25	*Penicillium* sp.
355.			Bacteria		
356.	R22	4	Penicillium like	25	*Penicillium* sp.
357.			Bacteria		
358.			Bacteria		
359.	R22	MEA	Fungi Yellow pigment in agar	25	
360.			Yeast		
361.			Yeast		
362.			Yeast		
363.	R15	4	Black fungi	25	
364.	R59	4	Penicillium like	25	*Penicillium* sp.
365.			Not frozen		
366.			Yeast		
367.			Yeast		
368.			Bacteria		
369.			Bacteria		
370.			Bacteria		
371.			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
372.			Bacteria		
373.	R15	MEA	Fungi	25	
374.	R59	MEA	Penicillium like	25	*Penicillium* sp.
375.			Bacteria		
376.			Bacteria		
377.			Bacteria		
378.			Bacteria		
379.			Not frozen		
380.			Bacteria		
381.			Bacteria		
382.	E2	4	Penicillium like	25	*Penicillium* sp.
383.			Not frozen		
384.			Bacteria		
385.			Bacteria		
386.	E147	4	Penicillium like	25	*Penicillium* sp.
387.			Bacteria		
388.			Yeast		
389.			Not frozen		
390.			Not frozen		
391.			Not frozen		
392.			Bacteria		
393.			Bacteria		
394.			Bacteria		
395.			Bacteria		
396.			Bacteria		
397.			Bacteria		
398.	R59	MEA	Penicillium like	15	*Penicillium* sp.
399.	R116	MEA	Green rough fungi	15	
400.	R116	VB	Green rough fungi	15	
401.			Bacteria		
402.			Yeast		
403.	R116	4	Green rough fungi	15	
404.			Not frozen		
405.	W7	MEA	Penicillium like	15	*Penicillium roquefortii*
406.			Yeast		
407.			Yeast		
408.	W7	4	Penicillium like	15	*Penicillium roquefortii*
409.			Yeast		
410.			Bacteria		
411.			Bacteria		
412.			Bacteria		
413.			Bacteria		
414.			Bacteria		
415.			Bacteria		
416.			Not frozen		
417.			Not frozen		
418.			Bacteria		
419.			Bacteria		
420.			Bacteria		
421.			Bacteria		
422.			Bacteria		
423.			Bacteria		
424.			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
425.			Bacteria		
426.					
427.			Yeast		
428.			Yeast		
429.	R22	VB	Fungi	15	
430.			Bacteria		
431.			Bacteria		
432.	R22	4	Fungi	15	
433.			Not frozen		
434.			Not frozen		
435.			Not frozen		
436.	R22	6	Bright white fungi	15	
437.	R22	MEA	Fungi	15	
438.			Yeast		
439.			Not frozen		
440.			Yeast		
441.			Not frozen		
442.			Not frozen		
443.	R15	4	Penicillium like	15	*Penicillium* sp.
444.			Bacteria		
445.			Not frozen		
446.			Not frozen		
447.	R59	4	Penicillium like	15	*Penicillium* sp.
448.			Bacteria		
449.	R59	VB	Penicillium like	15	*Penicillium* sp.
450.			Bacteria		
451.			Bacteria		
452.	R15	6	Fungi	15	
453.			Not frozen		
454.	R133	VB	Penicillium like	15	*Penicillium* sp.
455.	R158	VB	Penicillium like	15	*Penicillium* sp.
456.	R158	4	Penicillium like	15	*Penicillium* sp.
457.	R158	7	Penicillium like	15	*Penicillium* sp.
458.			Yeast		
459.			Not frozen		
460.	R158	MEA	Penicillium like	15	*Penicillium* sp.
461.	R136	4	Penicillium like	15	*Penicillium* sp.
462.			Not frozen		
463.	R136	MEA	Penicillium like	15	*Penicillium* sp.
464.			Yeast		
465.			Bacteria		
466.			Yeast		
467.	R136	VB	Penicillium like	15	*Penicillium* sp.
468.			Bacteria		
469.			Bacteria		
470.			Bacteria		
471.			Bacteria		
472.	R157	6	White fungi red extracellular pigment	15	
473.	R137	4	Penicillium like	15	*Penicillium* sp.
474.			Not frozen		
475.	R157	4	White fungi	15	
476.			Not frozen		
477.			Bacteria		
478.			Not frozen		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
479.			Bacteria		
480.			Bacteria		
481.			Bacteria		
482.			Yeast		
483.			Bacteria		
484.			Bacteria		
485.			Yeast		
486.			Not frozen		
487.			Not frozen		
488.	E15	4	Penicillium like		*Cladosporium cladosporum*
489.			Yeast/bacteria		
490.	E15	VB	Penicillium like	15	*Penicillium* sp.
491.			Bacteria		
492.			Bacteria		
493.	E11	6	White fungi	15	
494.			Not frozen		
495.			Not frozen		
496.	E146	6	White fungi	15	
497.	E46	4	Penicillium like	15	*Penicillium* sp.
498.	E146	4	Black/green fungi	15	
499.	E46	MEA	Penicillium like	15	*Penicillium* sp.
500.	E146	MEA	Black/green fungi	15	
501.	E46	VB	Penicillium like	15	*Penicillium* sp.
502.			Not frozen		
503.	E127	4	White fungi	15	
504.	E148	4	Black/green fungi	15	
505.			Bacteria		
506.	E148	MEA	Black/green fungi	15	
507.			Not frozen		
508.	E148	VB	Dark green Penicillium like	15	*Cladosporium* sp.
509.			Bacteria		
510.			Bacteria		
511.	E127	6	White fungi	15	
512.			Bacteria		
513.			Yeast		
514.			Yeast		
515.			Bacteria		
516.			Not frozen		
517.			Not frozen		
518.	E15	MEA	Black/green fungi	15	*Cadophora malorum*
519.	E134	6	White fungi	15	
520.	E134	MEA	White fungi	15	
521.			Bacteria		
522.			Bacteria		
523.	E134	4	White fungi	15	
524.			Bacteria		
525.			Not frozen		
526.			Bacteria		
527.	E132	4	Cream/green fungi	15	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
528.	E132	6	Green/white fungi	15	
529.			Not frozen		
530.			Yeast		
531.	E132	MEA	Cream/green fungi	15	
532.	E2	VB	Green/white fungi	15	
533.	E22	VB	Dark green Penicillium like	15	*Cladosporium* sp.
534.			Bacteria		
535.			Bacteria		
536.	E2	4	Dark green Penicillium like	15	*Cladosporium* sp.
537.	E22	4	Dark green Penicillium like	15	*Cladosporium* sp.
538.	E30	4	Fibrous fungi	15	*Penicillium expansum* (strain ATCC)
539.	E2	MEA	Green fungi	15	
540.	E22	MEA	Green fungi	15	
541.			Not frozen		
542.	E30	MEA	Green fungi	15	
543.			Not frozen		
544.	R22	4	Bright white fungi	15	
545.			Yeast		
546.			Yeast		
547.			Bacteria		
548.			Not frozen		
549.			Not frozen		
550.			Not frozen		
551.			Not frozen		
552.			Not frozen		
553.	R22	VB	Rhizopus like	25	
554.			Not frozen		
555.			Not frozen		
556.			Bacteria		
557.			Bacteria		
558.			Not frozen		
559.			Not frozen		
560.			Not frozen		
561.			Not frozen		
562.			Not frozen		
563.			Bacteria		
564.			Not frozen		
565.			Bacteria		
566.			Not frozen		
567.			Bacteria		
568.	W2	4	Green/black Penicillium like	25	*Cladosporium* sp.
569.	PS1	6	Penicillium like	25	*Penicillium* sp.
570.	PS1	6	Penicillium like	25	*Penicillium* sp.
571.			Not frozen		
572.	PS1	4	Trichoderma like	25	*Trichoderma* sp
573.			Not frozen		
574.			Bacteria		
575.	R156	6	Penicillium like	25	*Penicillium* sp.
576.	PS1	MEA	Fungi	25	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
577.			Bacteria		
578.			Bacteria		
579.			Not frozen		
580.			Not frozen		
581.			Not frozen		
582.			Not frozen		
583.	R22	6	White fungi	25	
584.			Not frozen		
585.	H35	MEA	Penicillium like	25	*Penicillium* sp.
586.			Not frozen		
587.			Bacteria		
588.	E55	MEA	Rhizopus like	25	
589.			Not frozen		
590.			Not frozen		
591.			Not frozen		
592.			Not frozen		
593.			Not frozen		
594.			Bacteria		
595.			Not frozen		
596.			Bacteria		
597.			Bacteria		
598.			Not frozen		
599.			Not frozen		
600.			Not frozen		
601.	E146	6	White fungi	25	
602.			Bacteria		
603.	E146	6	White fungi	25	
604.			Bacteria		
605.			Bacteria		
606.	E11	6	Apricot fungi	25	*Thelebolaceae* sp.
607.			Bacteria		
608.			Not frozen		
609.			Yeast/bacteria		
610.			Bacteria		
611.			Bacteria		
612.	H32	6	Penicillium like	25	*Penicillium* sp.
613.	H22	4	Penicillium like	25	*Penicillium* sp.
614.			Bacteria		
615.			Bacteria		
616.	H36	MEA	Penicillium like	25	*Penicillium* sp.
617.			Bacteria		
618.	H41	7	White fungi	25	
619.	H34	7	Penicillium like	25	*Penicillium* sp.
620.			Bacteria		
621.	E103	4	Penicillium like	25	*Penicillium* sp.
622.	E102	VB	Cream fungi	25	
623.	E127	MEA	Penicillium like	25	*Penicillium* sp.
624.			Not frozen		
625.	E72	6	Penicillium like	25	*Penicillium* sp.
626.	E76	MEA	Penicillium like	25	*Penicillium* sp.
627.			Not frozen		
628.	E113	MEA	White fungi	25	
629.	E129	MEA	White fungi	25	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
630.			Not frozen		
631.			Bacteria		
632.			Not frozen		
633.			Not frozen		
634.			Not frozen		
635.			Not frozen		
636.	E30	VB	Penicillium like	25	*Penicillium* sp.
637.	E23	VB	Rhizopus like	25	
638.			Not frozen		
639.	E22	VB	White fungi	25	
640.			Bacteria		
641.			Not frozen		
642.			Not frozen		
643.			Not frozen		
644.			Not frozen		
645.	PS4	4	Trichoderma like	15	*Trichoderma* sp
646.			Bacteria		
647.	H47	4	Dark green Penicillium like	15	*Cladosporium* sp.
648.	R59	6	White fungi	15	
649.	E2	6	White fungi	15	
650.	E22	6	Dark green Penicillium like	15	*Cladosporium* sp.
651.	E30	6	White fungi	15	
652.	H35	6	White fungi	15	
653.	H52	6	White/green fungi	15	
654.	H33	6	White fungi	15	
655.	E19	6	Dark green fungi	15	
656.	E35	6	Dark green Penicillium like	15	*Cladosporium* sp.
657.	E35	6	White fungi	15	*Geomyces* sp.
658.			Bacteria		
659.			Bacteria		
660.	E148	6	Dark green Penicillium like	15	
661.	E15	6	Dark green Penicillium like	15	*Cladosporium cladosporum*
662.	R116	6	Dark green Penicillium like	15	*Cladosporium* sp.
663.			Bacteria		
664.			Not frozen		
665.			Bacteria		
666.			Bacteria		
667.			Bacteria		
668.	W7	VB	Black/green fungi	15	*Cladosporium cladosporum*
669.	W8	VB	Black fungi	15	*Cadophora malorum*
670.	TF	VB	Fungi	15	
671.	E95	VB	Dark Penicillium like	15	*Cladosporium* sp.
672.	H59	VB	Green/black fungi	15	
673.			Bacteria		
674.			Yeast		
675.			Yeast		
676.			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
677.			Bacteria		
678.					
679.			Bacteria		
680.			Yeast		
681.			Yeast		
682.			Yeast		
683.	R15	MEA	Dark spreading fungi	15	
684.			Bacteria		
685.			Bacteria		
686.			Bacteria		
687.			Not frozen		
688.			Bacteria		
689.	E72	MEA	Penicillium like	15	*Penicillium* sp.
690.			Bacteria		
691.			Bacteria		
692.	E28	MEA	Cream fungi	15	
693.	E28	MEA	Tan fungi	15	
694.			Bacteria		
695.	E19	6	Dark fungi	15	
696.			Bacteria		
697.			Yeast		
698.			Yeast		
699.			Yeast		
700.			Yeast		
701.			Yeast		
702.	R116	7	Cream fungi	4	
703.			Yeast		
704.			Bacteria		
705.			Yeast		
706.			Bacteria		
707.	H32	6	Cream/clear fungi	4	
708.	H32	6	Cream spreading fungi	4	
709.	H22	6	White fungi	4	
710.	R157	6	White fungi	4	
711.	R22	6	White fungi	4	
712.	E35	6	Large cream fungi	4	
713.	E35	6	Small dark fungi	4	*Geomyces* sp.
714.	E38	6	White fungi	4	
715.	E147	6	White fungi	4	
716.	H52	VB	Black spreading (280)	4	
717.	H33	VB	Black spreading (280)	4	
718.	H32	VB	Black spreading (280)	4	
719.	E2	VB	Dark green Penicillium like	4	*Cladosporium* sp.
720.			Yeast		
721.	E22	VB	Dark green Penicillium like	4	*Cladosporium cladosporum*
722.	E30	VB	Dark green Penicillium like	4	*Cladosporium* sp.
723.			Bacteria		
724.	E19	VB	Dark green Penicillium like	4	*Cladosporium* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
725.	E19	VB	Blue/green Penicillium like	4	*Penicillium* sp.
726.			Yeast/bacteria		
727.	E147	VB	Green/white fungi	4	
728.	R22	VB	White fungi	4	
729.			Yeast		
730.	H22	VB	White fungi	4	
731.			Bacteria		
732.			Bacteria		
733.	R116	VB	Green Penicillium like	4	*Penicillium* sp.
734.			Bacteria		
735.			Bacteria		
736.			Bacteria		
737.			Bacteria		
738.			Bacteria		
739.	E35	VB	Green fungi	4	
740.	E38	VB	Green fungi	4	
741.	E38	VB	Dark fungi	4	
742.			Bacteria		
743.			Not frozen		
744.			Not frozen		
745.			Not frozen		
746.	E46	VB	Pale green fungi	4	
747.	E146	VB	Dark green/white fungi	4	
748.			Bacteria		
749.			Bacteria		
750.			Yeast		
751.	E11	VB	White fungi	4	*Geomyces* sp.
752.	E15	VB	Dark green fungi	4	
753.			Bacteria		
754.			Bacteria		
755.			Bacteria		
756.	R59	VB	White fungi	4	
757.			Not frozen		
758.	E148	VB	Green fungi	4	
759.			Not frozen		
760.	E46	4	Penicillium like	4	*Penicillium* sp.
761.	E146	4	Green fungi	4	
762.	E146	4	White fungi	4	
763.			Not frozen		
764.			Yeast		
765.	E11	4	White fungi	4	*Pseudeurotium desertorum* (str CBS 986)
766.	E15	4	Green Penicillium like	4	*Penicillium* sp.
767.	E132	4	Grey fungi	4	
768.	E134	4	Bright white fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
769.	E134	4	Dull white fungi	4	
770.	H52	4	Dark Penicillium like	4	*Cladosporium* sp.
771.			Bacteria		
772.	H33	4	Black spreading fungi	4	
773.	H41	4	White fungi	4	
774.	E35	4	Dark Penicillium like	4	*Cladosporium* sp.
775.	E38	4	Pale green fungi	4	
776.	E38	4	Dark Penicillium like	4	*Cladosporium* sp.
777.	E19	4	Dark Penicillium like	4	*Cladosporium* sp.
778.	E2	4	Dark Penicillium like	4	*Cladosporium* sp.
779.	E2	4	Dull fungi	4	
780.	E2	4	White fungi	4	
781.	E22	4	Dark Penicillium like	4	*Cladosporium oxysporum*
782.	E30	4	Dark Penicillium like	4	*Cladosporium* sp.
783.			Bacteria		
784.	R22	4	White fungi	4	
785.	E127	4	White fungi	4	
786.	E148	4	Dark Penicillium like	4	*Cladosporium* sp.
787.			Bacteria		
788.			Bacteria		
789.	R59	4	Yellow fungi	4	
790.	W5	4	Dull fungi	4	
791.			Yeast		
792.			Yeast		
793.	W7	4	Dull fungi	4	
794.			Yeast		
795.			Yeast/bacteria		
796.			Yeast		
797.	R136	4	Penicillium like	4	*Penicillium* sp.
798.			Yeast		
799.			Yeast		
800.	R116	4	Penicillium like	4	*Penicillium* sp.
801.			Yeast		
802.	R137	4	Penicillium like	4	*Penicillium* sp.
803.	R157	4	Bright white fungi	4	
804.			Yeast		
805.	R157	4	Dull fungi	4	
806.	H22	4	White fungi	4	
807.	H22	4	Dark fungi	4	*Cladosporium oxysporum*
808.	H22	4	Dark green fungi	4	
809.	E35	MEA	Dark green Penicillium like	4	*Cladosporium* sp.
810.			Bacteria		
811.	E38	MEA	White powdery fungi	4	
812.	E134	MEA	White fungi	4	
813.			Not frozen		
814.	E22	MEA	Green Penicillium like	4	*Penicillium* sp.
815.			Bacteria		
816.	E30	MEA	Dark green Penicillium like	4	*Cladosporium* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
817.			Bacteria		
818.	H43	MEA	White fungi	4	
819.			Bacteria		
820.			Bacteria		
821.	H52	MEA	Bright white fungi	4	
822.			Not frozen		
823.	E19	MEA	Green Penicillium like	4	*Penicillium* sp.
824.	E19	MEA	Dark fungi	4	
825.	H41	MEA	White fungi	4	
826.	H22	MEA	White fungi	4	*Geomyces* sp.
827.			Yeast		
828.	H22	MEA	Dull green fungi	4	
829.			Bacteria		
830.	R59	MEA	Penicillium like	4	*Penicillium* sp.
831.	E132	MEA	Dull green fungi	4	
832.			Yeast		
833.			Yeast		
834.			Yeast		
835.	R22	MEA	White fungi	4	
836.			Yeast		
837.			Yeast		
838.			Yeast		
839.	W7	MEA	Spreading fungi	4	
840.			Yeast		
841.			Bacteria		
842.			Yeast		
843.			Bacteria		
844.			Not frozen		
845.			Bacteria		
846.	R116	MEA	Green Penicillium like	4	*Penicillium* sp.
847.	E127	MEA	White fungi	4	
848.			Bacteria		
849.	E148	MEA	Dark Penicillium like	4	*Cladosporium* sp.
850.	E27	MEA	Spreading fungi	4	
851.	E46	MEA	Penicillium like	4	
852.	E146	MEA	Dark Penicillium like	4	*Cladosporium* sp.
853.			Bacteria		
854.			Bacteria		
855.			Yeast		
856.	E28	MEA	Small dark fungi	4	
857.	R92	MEA	Spreading fungi	4	
858.			Yeast		
859.			Yeast		
860.			Yeast		
861.			Yeast		
862.			Yeast		
863.			Yeast		
864.			Yeast		
865.	E127	6	Dark fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
866.	E11	6	White fungi	4	
867.	E146	6	White fungi	4	
868.			Bacteria		
869.	R15	6	White fungi	4	
870.	E2	6	White fungi	4	
871.			Bacteria		
872.			Not frozen		
873.	E15	MEA	Green Penicillium like	4	*Penicillium* sp.
874.			Not frozen		
875.			Bacteria		
876.			Bacteria		
877.			Bacteria		
878.			Bacteria		
879.			Yeast		
880.	E134	6	Fungi	4	
881.			Bacteria		
882.			Bacteria		
883.	E154	VB	Dark spreading fungi	4	
884.			Yeast		
885.	H35	6	Tan fungi	4	
886.	H35	6	White fungi	4	
887.	H52	6	Fungi	4	
888.	H33	6	Fungi	4	
889.	H35	6	Penicillium like?	4	*Penicillium* sp.
890.	H35	6	Tan fungi	4	
891.	H47	6	Fungi	4	
892.	R116	6	Dark Penicillium like	4	*Cladosporium* sp.
893.	R136	6	Penicillium like	4	*Penicillium* sp.
894.			Bacteria		
895.	R158	6	Penicillium like	4	*Penicillium* sp.
896.	E132	6	Penicillium like	4	*Penicillium* sp.
897.			Yeast		
898.			Yeast		
899.	PS4	4	Penicillium like	4	*Penicillium* sp.
900.	H43	4	Grey fungi	4	
901.	H22	7	Tan fungi	4	
902.			Yeast		
903.			Yeast		
904.			Yeast		
905.	W5	7	White fungi	4	
906.			Yeast		
907.			Bacteria		
908.			Bacteria		
909.			Not frozen		
910.			Not frozen		
911.			Not frozen		
912.			Not frozen		
913.			Yeast/bacteria		
914.			Yeast		
1001	2E114	4	Cream fluffy top fungi	25	
1002	2E121	4	Cream flat top fungi	25	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1003	2E122	4	Cream fluffy top fungi	25	
1004	2E53	7	Cream fluffy top fungi	25	
1005			Not frozen		
1006	2E78	M	Slow growing white flat fungi, earthy odour	25	
1007	2E87	4	White top Orange base fungi	25	
1008	2E135	4	White top Orange /Tan base fungi	25	
1009	2E134	4	White top Orange / Tan base fungi	25	
1010	2E99	M	White powdery top fungi	25	
1011	2E136	M	White powdery fungi	25	
1013	2E111	7	White powdery fungi	25	
1014	2E39	7	White powdery fungi Cream base white top	25	
1015	2E99	M	White powdery fungi	25	
1016			Not frozen		
1017	2R63	M	Black/dark green fungi	25	
1018			Yeast		
1019			Yeast		
1020			Yeast		
1021	2E122	4	Large white fungi fluffiness in ring zones	25	
1022	2E136	4	Large white fungi fills whole plate Yellow/orange droplets on top centre	25	
1023	2E104	M	White spreading fungi	25	
1024	2E114	M	Yellow dry looking fungi	25	
1025	2R90	M	Fine white fungi	25	
1026			Not frozen		
1027	2E78	4	Green top Yellow/green base fungi	25	*Penicillium* sp.
1028	2R88	4	Green top Yellow/green base fungi	25	*Penicillium* sp.
1029	2E113	4	Green top Yellow base fungi	25	*Penicillium expansum* (strain ATCC)
1030	2H57		Small pink top brown/red base fungi	25	
1031	2E53	4	Yellow fluffy fungi	25	
1032			Not frozen		
1033			Not frozen		
1034	2E89	M	Small white flat fungi	25	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1035			Not frozen		
1036	2E107	M	White fine fungi	25	
1037			Bacteria		
1038			Bacteria		
1039	2E104	7	Small white fungi	25	
1040	2E82	4	Flat dull cream fungi.	25	
1041			Not frozen		
1042			Not frozen		
1043	2E78	4	Small powdery top fungi cream top cream bottom	25	
1044			Not frozen		
1045			Not frozen		
1046			Bacteria		
1047			Bacteria		
1048			Bacteria		
1049			Bacteria		
1050			Bacteria		
1051			Bacteria		
1052			Bacteria		
1053			Bacteria		
1054			Bacteria		
1055			Bacteria		
1056			Not frozen		
1057			Bacteria		
1058			Bacteria		
1059			Not frozen		
1060	2E72	4	Dull cream fungi	15	
1061	2E90	4	White fluffy fungi thick and strong	15	
1062	2E90	4	Dark green top and black base fungi	15	*Cladosporium* sp.
1063			Yeast		
1064	2E96	4	Dull cream fungi	15	
1065			Not frozen		
1066			Not frozen		
1067	2E87	4	Cream dull looking fungi with white powdery top.	15	
1068	2E82	4	White top yellow/tan base fungi	15	
1069	2E89	4	White/tan powdery top with yellow base	15	
1070			Yeast		
1071	2E111	4	Green/grey powdery top black base	15	
1072	2E111	4	White powdery top yellow/tan base	15	
1073			Yeast		
1074	2E122	7	White fluffy fungi	15	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1075	2E96	M	Cream fibrous fungi	15	
1076	2E136	M	Black fibrous fungi	15	
1077	2E134	4	White furry top fungi with yellow/tan base	15	
1078	2E135	4	White powdery fungi yellow base	15	
1079	2E134	M	Cream/green fine fungi	15	
1080	2E135	M	White fine fungi	15	
1081	2E114	M	White tree like fungi	15	
1082	2E78	M	White tree like fungi	15	
1083	2E121	M	White fine fungi sectored	15	
1084	2E53	M	Fine white fungi	15	
1085	2E111	M	Fine white fungi	15	
1086	2E104	4	White fungi fine white aerial hyphae	15	
1087	2E112	M	Fine hyphae fungi	15	
1088	2E87	M	Fine fungi yellow powdery top	15	
1089			Not frozen		
1090			Yeast		
1091			Yeast		
1092	2E123	4	Pale green powdery top fungi with dark green base	15	*Penicillium* sp.
1093	2E115	4	White powdery top fungi with yellow/tan base	15	*Penicillium* sp.
1094	2E115	4	Fungi	15	
1095	2H53	4	White powdery top fungi with yellow/tan base	15	*Geomyces* sp.
1096	2H53	4	Dull cream fungi	15	
1097	2H50	4	Dull cream fungi	15	
1098	2H50	4	White powdery top fungi with yellow/tan base	15	*Geomyces* sp.
1099	2H53	M	Dark green/black fibrous fungi	15	
1100	2H55	4	Pink powdery top fungi with tan base	15	
1101	2H57	4	Pink powdery top fungi with yellow base	15	
1102	2H57	4	Dull cream fungi	15	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1103	2H45	M	Fine hyphae fungi with fine green powdery spores on top.	15	
1104	Ad4	Y	White fluffy top fungi with yellow base.	15	
1105	Ad4	4	White fluffy top fungi with yellow base	15	
1106	Ad4	M	White circular sectored fungi	15	
1107	2H57	4	Dull green top fungi with green base	15	
1108	2E115	4	Dull green top fungi with green base	15	
1109	2E114	4	White powdery top fungi with yellow/tan base	15	
1110			Not frozen		
1111	2E90	M	Green fibrous fungi	15	
1112	2E78	4	Cream dull fungi	15	
1113	2E136	M	Fine hyphae fungi with fine green powdery top	15	
1114	2E121	4	White dull fungi	15	
1115	2E121	4	Pink/white powdery top fungi yellow/tan base	15	
1116	2E121	4	White fluffy top fungi with yellow/tan base	15	
1117	2E119	4	Dark green powdery top fungi with dark base	15	*Cladosporium* sp.
1118	2E100	M	Dark green fine hyphae fungi	15	
1119			Yeast		
1120			Yeast		
1121	2R88	4	Large fluffy fungi with black dot spores	15	
1122	2R88	M	Fine fibrous fungi	15	
1123	2R88	7	Large fluffy fungi with black dot spores	15	
1124	2E87	M	Fine cream hyphae fungi	4	
1125	2E115	M	Fine cream hyphae fungi	4	
1126	2E78	4	Cream fibrous top yellow base	4	
1127	2E78	4	White powdery top of dull fungi cream base	4	
1128	2E115	M	Fine hyphae fine spores on top	4	
1129	2E115	M	Fine hyphae Fine aerial hyphae	4	
1130	2E135	M	Fine cream hyphae fungi	4	
1131	2E134	M	Fine cream hyphae fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1132	2E90	M	Dark fibrous fungi	4	
1133	2E89	M	Fine mycelium	4	
1134	2E53	M	Fine hyphae fine powdery spores on top	4	
1135	2E90	4	Dark green/avocado top black base fungi	4	*Cladosporium* sp.
1136	2E89	4	White fibrous top fungi cream base	4	
1137	2E134	4	White fibrous top fungi dark tan base	4	
1138	2E135	4	White powdery top fungi with yellow/tan base	4	
1139	2E96	4	Green base fungi	4	
1140	2E121	4	Green base white powdery fungi	4	
1141	2E87	4	White powdery top Yellow/tan base	4	
1142	2E53	4	White powdery top cream base	4	
1143	2R88	M	Fine mycelium with fine aerial mycelium	4	
1144	2R88	7	Fine hyphae black dots of spores on top	4	
1145	2R88	4	Fine hyphae black dots of spores on top	4	
1146	2H57	4	Cream powdery fungi	4	
1147	2H57	M	Fine white hyphae fine spores on top	4	
1148	2H50	M	Fibrous fungi	4	
1149	2H53	M	Fine white hyphae fine spores on top	4	
1150			Yeast		
1151	2H53	4	Dull cream fungi	4	
1152	2H54	4	Dull cream fungi	4	
1153	2H50	4	Dull cream fungi	4	
1154	2E87	M	Fine mycelium fungi with green spores	25	
1155	2E135	M	Fine mycelium fungi with green spores	25	
1156			Not frozen		
1157			Bacteria		
1158			Not frozen		
1159			Yeast		
1160			Bacteria		
1161			Not frozen		
1162	2E135	7	Brown/tan fungi	25	
1163	2E134	7	Brown tan dry worm like fungi	25	
1164	2E99	M	White fluffy fungi	25	
1165			Yeast		
1166			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1167	2E82	7	Fine mycelium with green spores	25	
1168	2E136	M	Cream fibrous	25	
1169	2R27	4	Fine mycelium with green spores	25	
1170			Bacteria		
1171			Not frozen		
1172			Not frozen		
1173			Yeast		
1174	2R88	4	Dry raised dark base cream top fungi	25	
1175	2R90	4	Cream spore fungi with dark spots on base	25	
1176	2H57	4	Dark fibrous fungi	25	
1177			Not frozen		
1178			Bacteria		
1179			Bacteria		
1180			Bacteria		
1181			Bacteria		
1182			Bacteria		
1183			Bacteria		
1184			Bacteria		
1185			Bacteria		
1186			Bacteria		
1187			Bacteria		
1188			Bacteria		
1189			Bacteria		
1190			Bacteria		
1191			Bacteria		
1192			Bacteria		
1193	2E87	7	Fine hyphae fungi	15	
1194	2E121	7	Fine hyphae fungi	15	
1195	2E115	7	Fine hyphae fungi	15	
1196	2E135	7	Tan base fungi with cream/pale tan wrinkly top	15	
1197	2E134	7	Tan base fungi with cream/pale tan wrinkly top	15	
1198	2E100	7	Fine white hyphae	15	
1199			Yeast/Bacteria		
1200	2H57	7	Cream balls on top of each other fungi	15	
1201			Bacteria		
1202			Yeast		
1203			Bacteria		
1204	2E115	M	Fine hyphae turning yellow with fine aerial hyphae	15	
1205	2E39	M	Fine green black hyphae	15	
1206	2E95	M	Fine white hyphae	15	
1207			Bacteria		
1208			Not frozen		
1209			Not frozen		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1210	2H55	M	Fine white mycelium with fine aerial hyphae	15	
1211	2R90	M	Dark fibrous fungi	15	
1212			Not frozen		
1213	2H57	M	Fine mycelium with fine green spores on top	15	
1214	E253	M	Fine white to dark tan fibrous fungi	15	
1215	E253	M	Fine tan fibrous fungi	15	
1216			Yeast		
1217	2E122	4	Tan/brown base fungi with white mycelium spore mat on top	15	*Geomyces* sp.
1218	2E99	4	Fine white mycelium	15	
1219	2E114	4	Yellow/tan base fungi with pink fine spores on top	15	
1220	2E114	4	White fine fibrous fungi	15	
1221	2E78	4	Dark base fungi with dark green top	15	*Cladosporium* sp.
1222	2E113	4	Cream/white thick mycelium	15	
1223	2E53	4	Fine white mycelium	15	
1224	2E53	4	Cream/green dull fungi	15	
1225	2E121	4	Fine white mycelium with some aerial hyphae	15	
1226	2R38	4	Cream/pink base circular growth with white spores older growth has black dots on top	15	
1227	2R38	4	Cream fungi	15	
1228	2R38	4	Pale avocado base fungi with dark green base	15	
1229			Yeast		
1230	2E39	4	Cream dull fungi	4	
1231	2E121	4	Yellow/tan base fungi with white powdery top	4	
1232	2E121	4	Pale green base fungi with green/grey top	4	
1233			Not frozen		
1234	2E114	4	Yellow/tan base white powdery top fungi	4	*Penicillium* sp.
1235	2E114	4	Yellow base white powdery top fungi	4	
1236	2E115	4	Cream dull fungi	4	
1237	2E102	4	Fine white hyphae with aerial mycelium	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1238	2E111	4	Yellow/tan base fungi with white powdery top	4	*Penicillium* sp.
1239			Yeast		
1240	2E87	4	Dull fungi	4	
1241	2E82	4	Yellow base powdery top fungi	4	*Penicillium* sp.
1242	2E102	M	Cream fine mycelium with fine aerial mycelium	4	
1243			Yeast		
1244	2E99	M	White slow growing fine fungi	4	
1245			Not frozen		
1246	2E89	M	White slow growing fine fungi	4	
1247	2E96	M	Darkening branched fungi	4	
1248	2E39	M	Cream/white branching fungi	4	
1249	2E82	M	Cream base dull top fungi	4	
1250	2E87	M	Darkening branch like fungi	4	
1251	2E87	M	White fine mycelium fungi	4	
1252	2E124	M	Dark green top fungi with black base	4	*Cladosporium* sp.
1253	2E114	M	Cream fine mycelium with fine aerial mycelium	4	
1254	2E119	M	Cream/tan fine fungi	4	
1255	2E121	M	Darkening branch like fungi	4	
1256	2E134	7	Cream/tan base fungi with dull top	4	
1257	2E135	7	Cream/tan base fungi with dull top	4	
1258			Yeast		
1259			Not frozen		
1260			Not frozen		
1261			Not frozen		
1262			Yeast		
1263			Yeast		
1264			Yeast		
1265			Yeast		
1266			Yeast		
1267			Yeast		
1268			Yeast		
1269	2R90	4	Dark base cream top fungi	4	
1270			Yeast		
1271	2R90	4	Dark base avocado top fungi	4	*Cadophora* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
1272	2R90	4	Cream/tan base fungi with white fibrous top	4	
1273	2R38	M	White fine mycelium fungi with green spores on top	4	
1274	2R90	M	White fine mycelium fungi	4	
1275	2R90	M	Green /dark dull fungi	4	*Cadophora* sp.
1276	2H57	7	Slow growing cream dull fungi	4	
1277	2H55	M	Dull green branched forms tree like structure	4	
1278	2H56	M	Dull green fungi	4	*Cadophora* sp.
1279	2H53	4	Cream/tan base fungi with white fibrous	4	
1280	Ad 4	M	Cream/white dull fungi	4	
281	Ad 4	Y	Cream tan base fungi with white powdery top	4	
1282	Ad 4	4	Cream tan base fungi with white powdery top	4	
1283			Not frozen		
3001	H1.1	YM	Dark fungi	4	*Cladosporium* sp.
3002	R2.1	YM	Dark Fungi	4	*Cladosporium* sp.
3003	R12.3	YM	White/Green	4	
3004	H1.4	YM	White/Green	4	
3005			Unknown		
3006	R17.16	YM	Dark Fungi	4	*Cladosporium* sp.
3007	E7.3	YM	White/Green	4	
3008			Unknown		
3009			Unknown		
3010			Unknown		
3011	E10.4	YM	White with green spores	4	
3012	E19.0	YM	Spreading white fungi with spores	4	
3013	E10.1	YM	Spreading white fungi with spores	4	
3014	E6.1	YM	White with green spores	4	
3015			Unknown		
3016			Bacteria		
3017			Bacteria		
3018			Yeast		
3019	R6.2	YM	White fungi	4	*Geomyces* sp.
3020			Yeast		
3021			Yeast		
3022	E9.1	YM	Spreading white fungi with spores	4	
3023	EW2	YM	White fungi	4	*Geomyces* sp.
3024	EL1	YM	Dark fungi	4	*Cladosporium* sp.
3025	EL2	YM	White spreading fungi	4	
3026	RE2	YM	White fungi	4	*Geomyces* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3027	EL3	4	White fungi	4	*Geomyces* sp.
3028	HP1	4	Dark fungi	4	*Cladosporium* sp.
3029	RE1	4	White fungi	4	*Geomyces* sp.
3030	RE2	4	White fungi	4	*Geomyces* sp.
3031	RP2	4	White fungi	4	*Geomyces* sp.
3032	EW2	Martins	White fungi	4	*Geomyces* sp.
3033	EW3	Martins	White fungi	4	*Geomyces* sp.
3034	EE1	Martins	White fungi	4	*Geomyces* sp.
3035	HP1	Martins	Dark fungi	4	*Cladosporium* sp.
3036	RE1	Martins	White fungi	4	*Geomyces* sp.
3037	RE2	Martins	White fungi	4	*Geomyces* sp.
3038	RP1	Martins	Fluffy white fungi	4	
3039	RE3	Martins	White fungi	4	*Geomyces* sp.
3040	EW1	YM	Dark fungi	4	*Cladosporium* sp.
3041	HP1	4	Dark fungi	4	*Cladosporium* sp.
3042	EW3	YM	White/green fungi	4	*Penicillium* sp.
3043					
3044			Yeast/bacteria		
3045			Yeast/bacteria		
3046			Yeast/bacteria		
3047			Yeast/bacteria		
3048			Yeast/bacteria		
3049			Yeast/bacteria		
3050			Yeast/bacteria		
3051			Yeast/bacteria		
3052			Yeast/bacteria		
3053			Yeast/bacteria		
3054			Yeast/bacteria		
3055			Yeast		
3056			Yeast/bacteria		
3057			Yeast/bacteria		
3058			Yeast		
3059			Yeast		
3060			Yeast		
3061			Yeast		
3062	4HP4	YM	White fungi	4	*Geomyces* sp.
3063	4HP5	YM	White fungi	4	*Geomyces* sp.
3064	4HP6	YM	White fungi	4	*Geomyces* sp.
3065	4HP8	YM	White fungi	4	*Geomyces* sp.
3066	4HP9	YM	White fungi	4	*Geomyces* sp.
3067	4HP10	YM	White fungi	4	*Geomyces* sp.
3068	4HP10	YM	Dark fungi	4	*Cladosporium* sp.
3069	4HP11	YM	White fungi	4	*Geomyces* sp.
3070	4HP12	YM	White fungi	4	*Geomyces* sp.
3071	4HP14	YM	White fungi	4	*Geomyces* sp.
3072	4HP15	YM	White fungi	4	*Geomyces* sp.
3073	4HP15	YM	Dark fungi	4	*Cladosporium* sp.
3074	4HP16	YM	White fungi	4	*Geomyces* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3075			Yeast/bacteria		
3076	4HP16	YM	White fungi	4	*Geomyces* sp.
3077	4HP17	YM	White fungi	4	*Geomyces* sp.
3078	4HP17	YM	White fungi	4	*Geomyces* sp.
3079	4HP17	YM	Dark fungi	4	*Cladosporium* sp.
3080	4HP18	YM	White fungi	4	*Geomyces* sp.
3081	4HP19	YM	White fungi	4	*Geomyces* sp.
3082			Yeast/bacteria		
3083	4HP19	YM	White fungi	4	*Geomyces* sp.
3084	4HP20	YM	White fungi	4	*Geomyces* sp.
3085	4HP21	YM	Dark fungi	4	*Cladosporium* sp.
3086			Yeast/bacteria		
3087	4HP22	YM	White fungi	4	*Geomyces* sp.
3088			Yeast/bacteria		
3089	4HP23	YM	White fungi	4	*Geomyces* sp.
3090	4HP24	YM	White fungi	4	*Geomyces* sp.
3091	4HP25	YM	Apricot fungi	4	*Thelebolaceae* sp.
3092	4HP25	YM	White fungi	4	*Geomyces* sp.
3093	4HP25	YM	White fungi	4	*Geomyces* sp.
3094	4HP26	YM	White fungi	4	*Geomyces* sp.
3095	4HP26	YM	Apricot fungi	4	*Thelebolaceae* sp.
3096			Bacteria		
3097	4HP28	YM	White fungi	4	*Geomyces* sp.
3098	4HP31	YM	White fungi	4	*Geomyces* sp.
3099	4HP32	YM	White fungi	4	*Geomyces* sp.
3100			Yeast/bacteria		
3101	4HP33	YM	White fungi	4	*Geomyces* sp.
3102	4HP24	YM	White fungi	4	*Geomyces* sp.
3103	4HP31	YM	White fungi	4	*Geomyces* sp.
3104	4HP34	YM	White fungi	4	*Geomyces* sp.
3105	4HP35	YM	White fungi	4	*Geomyces* sp.
3106	4HP36	YM	White fungi	4	*Geomyces* sp.
3107	4HP37	YM	White fungi	4	*Geomyces* sp.
3108	4HP39	YM	White fungi	4	*Geomyces* sp.
3109	4HP40	YM	Apricot fungi	4	*Thelebolaceae* sp.
3110	4HP43	YM	White fungi	4	*Geomyces* sp.
3111	4HP43	YM	Dark fungi	4	*Cladosporium* sp.
3112			Yeast/bacteria		
3113	4HP44	YM	White fungi	4	*Geomyces* sp.
3114	4HP45	YM	White fungi	4	*Geomyces* sp.
3115	4HP46	YM	White fungi	4	*Geomyces* sp.
3116	4HP46	YM	Dark fungi	4	*Cladosporium* sp.
3117	4HP47	YM	Dark fungi	4	*Cladosporium* sp.
3118	4HP48	YM	White fungi	4	*Geomyces* sp.
3119	4HP49	YM	White fungi	4	*Geomyces* sp.
3120	4HP52	YM	White fungi	4	*Geomyces* sp.
3121	4HP31	YM	Green fungi	4	*Penicillium* sp.
3122			Yeast/bacteria		
3123			Bacteria		
3124	4HP17	7	Small white fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3125	4HP44	7	Small white fungi	4	
3126	4HP25	7	Small white fungi	4	
3127	4HP19	7	Small white fungi	4	
3128	4HP21	7	Small white fungi	4	
3129	4HP49	YM	White fungi	4	*Geomyces* sp.
3130	4HP43	YM	White fungi	4	*Geomyces* sp.
3131	4HP40	YM	White fungi	4	*Geomyces* sp.
3132	4HP23	YM	Fungi	4	
3133	4HP15	YM	White fungi	4	*Geomyces* sp.
3134			Bacteria		
3135	4HP23	YM	Brown fungi	4	
3136	4HP26	CC	White fungi	4	*Geomyces* sp.
3137	4HP22	CC	White fungi	4	*Geomyces* sp.
3138	4HP23	CC	White fungi	4	*Geomyces* sp.
3139	4HP13	CC	Dark fungi	4	*Cladosporium* sp.
3140	4HP30	CC	White fungi	4	*Geomyces* sp.
3141	4HP18	CC	White fungi	4	*Geomyces* sp.
3142	4HP16	CC	White fungi spikey	4	
3143	4HP47	CC	White fungi	4	*Geomyces* sp.
3144	4HP52	CC	White fungi	4	*Geomyces* sp.
3145	4HP41	CC	White fungi	4	*Geomyces* sp.
3146	4HP38	CC	White fungi	4	*Geomyces* sp.
3147	4HP34	CC	Dark fungi	4	*Cladosporium* sp.
3148	4HP43	CC	White fungi	4	*Geomyces* sp.
3149	4HP13	VB	White fungi	4	*Geomyces* sp.
3150	4HP26	VB	White fungi spikey	4	
3151	4HP1	VB	White fungi	4	*Geomyces* sp.
3152	4HP2	VB	White fungi	4	*Geomyces* sp.
3153	4HP4	VB	White fungi	4	*Geomyces* sp.
3154	4HP46	VB	White fungi	4	*Geomyces* sp.
3155	4HP38	VB	Dark fungi	4	*Cladosporium* sp.
3156	4HP40	VB	White fungi	4	*Geomyces* sp.
3157	4HP21	VB	White fungi	4	*Geomyces* sp.
3158	4HP8	VB	Dark fungi	4	*Cladosporium* sp.
3159	4HP9	VB	Dark fungi	4	*Cladosporium* sp.
3160	4HP27	VB	White fungi	4	*Geomyces* sp.
3161			Yeast/bacteria		
3162			Yeast/bacteria		
3163			Yeast/bacteria		
3164			Yeast/bacteria		
3165			Yeast/bacteria		
3166			Yeast/bacteria		
3167			Yeast/bacteria		
3168			Yeast/bacteria		
3169	4CE14	7	Fungi	4	
3170			Yeast/bacteria		
3171			Yeast/bacteria		
3172			Yeast/bacteria		
3173	4CE24	7	Dark fungi	4	*Cadophora* sp.
3174	4CE26	7	Dark fungi	4	*Cladosporium* sp.
3175			Yeast/bacteria		
3176			Yeast/bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3177			Yeast/bacteria		
3178			Yeast/bacteria		
3179			Yeast/bacteria		
3180			Yeast/bacteria		
3181	4CE50	7	Dark fungi	4	*Cladosporium* sp.
3182			Yeast/bacteria		
3183	4CE57	7	Pale fungi	4	
3184	4CE58	7	Pale fungi	4	
3185			Yeast/bacteria		
3186			Yeast/bacteria		
3187	4CE80	7	Small white fungi	4	
3188			Yeast/bacteria		
3189	4CE85	7	Small brown fungi	4	
3190	4CE87	7	Fungi	4	
3191			Yeast/bacteria		
3192			Yeast/bacteria		
3193			Dark fungi		*Cladosporium* sp.
3194	4CE1	7	Dark fungi	4	*Cladosporium* sp.
3195			Yeast/bacteria		
3196			Yeast/bacteria		
3197	4CE3	7	Fungi	4	
3198			Yeast/bacteria		
3199			Yeast/bacteria		
3200			Yeast/bacteria		
3201			Yeast/bacteria		
3202	4CE1	YM	White fluffy fungi	4	
3203	4CE2	YM	White fungi	4	*Geomyces* sp.
3204	4CE3	YM	Apricot fungi	4	*Thelebolaceae* sp.
3205	4CE4	YM	Apricot fungi	4	*Thelebolaceae* sp.
3206			Bacteria		
3207	4CE5	YM	Dark fungi	4	*Cladosporium* sp.
3208					
3209	4CE5	YM	Apricot fungi	4	*Thelebolaceae* sp.
3210			Yeast/bacteria		
3211	4CE6	YM	White fungi	4	
3212	4CE6	YM	Apricot fungi	4	*Thelebolaceae* sp.
3213			Yeast/bacteria		
3214			Yeast/bacteria		
3215	4CE7	YM	Dark fungi	4	*Cladosporium* sp.
3216	4CE7	YM	White fungi	4	*Geomyces* sp.
3217			Yeast/bacteria		
3218	4CE8	YM	Dark fungi	4	*Cladosporium* sp.
3219	4CE8	YM	Dark fungi	4	*Cladosporium* sp.
3220	4CE8	YM	White fungi	4	*Geomyces* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3221	4CE9	YM	Dark fungi	4	*Cladosporium* sp.
3222	4CE9	YM	White fungi	4	*Geomyces* sp.
3223			Yeast/bacteria		
3224	4CE10	YM	Apricot fungi	4	*Thelebolaceae* sp.
3225	4CE11	YM	Apricot fungi	4	*Thelebolaceae* sp.
3226	4CE11	YM	Dark fungi	4	*Cladosporium* sp.
3227			Yeast/bacteria		
3228	4CE11	YM	White fungi	4	*Geomyces* sp.
3229			Yeast/bacteria		
3230	4CE12	YM	White fungi	4	*Geomyces* sp.
3231	4CE13	YM	Apricot fungi	4	*Thelebolaceae* sp.
3232	4CE14	YM	White fungi	4	*Geomyces* sp.
3233	4CE15	YM	Apricot fungi	4	*Thelebolaceae* sp.
3234	4CE15	YM	White fungi	4	*Geomyces* sp.
3235	4CE16a	YM	Fungi	4	
3236	4CE17b	YM	Dark fungi	4	*Cladosporium* sp.
3237	4CE19a	YM	White fluffy fungi	4	
3238			Yeast/bacteria		
3239			Yeast/bacteria		
3240	4CE23	YM	Dark fungi	4	*Cladosporium* sp.
3241			Yeast/bacteria		
3242	4CE24	YM	Dark fungi	4	*Cladosporium* sp.
3243	4CE26	YM	Dark fungi	4	*Cladosporium* sp.
3244			Yeast/bacteria		
3245			Yeast/bacteria		
3246	4CE30	YM	Apricot fungi	4	*Thelebolaceae* sp.
3247	4CE33	YM	White fungi	4	*Geomyces* sp.
3248	4CE35	YM	White fungi	4	*Geomyces* sp.
3249	4CE37	YM	Dark fungi	4	*Cladosporium* sp.
3250	4CE38	YM	White fungi	4	*Geomyces* sp.
3251	4CE38	YM	Apricot fungi	4	*Thelebolaceae* sp.
3252	4CE41	YM	White fungi	4	*Geomyces* sp.
3253	4CE43	YM	White fungi	4	*Geomyces* sp.
3254	4CE44	YM	White fungi	4	*Geomyces* sp.
3255	4CE45	YM	White fungi	4	
3256	4CE47	YM	Apricot fungi	4	*Thelebolaceae* sp.
3257	4CE48	YM	White hyphae with dark structures fungi	4	
3258			Yeast/bacteria		
3259	4CE56	YM	White fungi	4	*Geomyces* sp.
3260	4CE56	YM	Apricot fungi	4	*Thelebolaceae* sp.
3261	4CE56	YM	White fungi	4	*Geomyces* sp.
3262	4CE55	YM	White fungi	4	*Geomyces* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3263	4CE55	YM	Apricot fungi	4	*Thelebolaceae* sp.
3264	4CE57	YM	White fungi	4	*Geomyces* sp.
3265	4CE57	YM	White fungi	4	*Geomyces* sp.
3266			Yeast/bacteria		
3267	4CE58	YM	Fungi	4	
3268	4CE59	YM	Apricot fungi	4	*Thelebolaceae* sp.
3269			Yeast/bacteria		
3270	4CE60	YM	Apricot fungi	4	*Thelebolaceae* sp.
3271	4CE60	YM	White fungi	4	*Geomyces* sp.
3272	4CE63	YM	White fungi	4	
3273			Yeast/bacteria		
3274			Yeast/bacteria		
3275	4CE66	YM	Apricot fungi	4	*Thelebolaceae* sp.
3276	4CE63	YM	Green fungi	4	*Penicillium* sp.
3277	4CE65	YM	White fungi	4	
3278	4CE64	YM	Apricot fungi	4	*Thelebolaceae* sp.
3279			Yeast/bacteria		
3280	4CE68	YM	Apricot fungi	4	*Thelebolaceae* sp.
3281	4CE68	YM	White fungi	4	*Geomyces* sp.
3282	4CE78	YM	Dark fungi	4	*Cladosporium* sp.
3283	4CE79	YM	White fungi	4	
3284	4CE80	YM	White fungi	4	
3285			Yeast/bacteria		
3286	4CE87	YM	Dark fungi	4	*Cladosporium* sp.
3287	4CE89 swab	YM	White fluffy fungi	4	
3288	4CE90b	YM	Fungi	4	
3289	4CE97	YM	White fungi	4	
3290	4CE100	YM	Dark fungi	4	*Cladosporium* sp.
3291	4CE100	YM	White fungi	4	
3292	4CE101	YM	White fluffy fungi	4	
3293	4CE102	YM	Dark fungi	4	*Cladosporium* sp.
3294	4CE105	YM	Green fungi	4	*Penicillium* sp.
3295	4CE105	YM	Dark fungi	4	*Cladosporium* sp.
3296	4CE112b	YM	White fungi	4	
3297	4CE114	YM	Funny fungi	4	
3298			Yeast/bacteria		
3299	4CE114	YM	Dark fungi	4	*Cladosporium* sp.
3300	4CE115	YM	Apricot fungi	4	*Thelebolaceae* sp.
3301			Yeast		
3302	4CE103	YM	White fungi	4	*Geomyces* sp.
3303	4CE75	YM	White fungi	4	
3304			Yeast/bacteria		
3305			Yeast		
3306			Yeast/fungi		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3307	4CE98	7	Fungi	4	
3308	4CE101	7	Small fungi	4	
3309			Yeast/bacteria		
3310			Yeast		
3311			Yeast		
3312			Yeast/bacteria		
3313	4CE4	7	Fungi	4	
3314	4CE122	YM	Fungi	4	
3315	4CE1	VB	White fungi	4	*Geomyces* sp.
3316	4CE2	VB	White fungi	4	*Geomyces* sp.
3317	4CE3	VB	White fungi	4	*Geomyces* sp.
3318	4CE5	VB	White fungi	4	*Geomyces* sp.
3319	4CE12	VB	Apricot fungi	4	*Thelebolaceae* sp.
3320	4CE14	VB	White fungi	4	*Geomyces* sp.
3321	4CE14	VB	White fungi	4	*Geomyces* sp.
3322	4CE16a	VB	White fungi	4	*Geomyces* sp.
3323	4CE17b	VB	Dark fungi	4	*Cladosporium* sp.
3324	4CE21	VB	Dark fungi	4	*Cladosporium* sp.
3325	4CE22	VB	Dark fungi	4	*Cladosporium* sp.
3326	4CE23	VB	Dark fungi	4	*Cladosporium* sp.
3327	4CE26	VB	Dark fungi	4	*Cladosporium* sp.
3328	4CE35	VB	Unknown fungi	4	
3329	4CE36	VB	Unknown fungi	4	
3330	4CE38	VB	White fungi	4	
3331	4CE37	VB	Apricot fungi	4	*Thelebolaceae* sp.
3332	4CE38	VB	White fungi	4	*Geomyces* sp.
3333	4CE39	VB	Unknown fungi	4	
3334	4CE43	VB	Dark fungi	4	*Cladosporium* sp.
3335	4CE45	VB	Unknown fungi	4	
3336	4CE41	VB	Dark fungi	4	*Cladosporium* sp.
3337	4CE51	VB	White fluffy fungi	4	
3338	4CE50	VB	Dark fungi	4	*Cladosporium* sp.
3339	4CE50	VB	Apricot fungi	4	*Thelebolaceae* sp.
3340	4CE58	VB	White fungi	4	*Geomyces* sp.
3341	4CE59	VB	Dark fungi	4	*Cladosporium* sp.
3342	4CE59	VB	White fungi	4	*Geomyces* sp.
3343	4CE60	VB	White fungi	4	
3344	4CE61	VB	Dark fungi	4	*Cladosporium* sp.
3345	4CE62	VB	Unknown fungi	4	
3346	4CE66	VB	Apricot fungi	4	*Thelebolaceae* sp.
3347	4CE73b	VB	White fluffy fungi	4	
3348	4CE77	VB	White fluffy fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3349	4CE77	VB	Apricot fungi	4	*Thelebolaceae* sp.
3350	4CE80	VB	Unknown fungi	4	
3351	4CE84	VB	White fluffy fungi	4	
3352	4CE85	VB	White fungi	4	*Geomyces* sp.
3353	4CE85	VB	Dark fungi	4	*Cladosporium* sp.
3354	4CE88	VB	Dark fungi	4	*Cladosporium* sp.
3355	4CE10	VB	White fungi	4	
3356	4CE89	VB	Dark fungi	4	*Cladosporium* sp.
3357	4CE90b	VB	Unknown fungi	4	
3358	4CE101	VB	Unknown fungi	4	
3359	4CE104	VB	Unknown fungi	4	
3360	4CE109	VB	Dark fungi	4	*Cladosporium* sp.
3361	4CE115	VB	Unknown fungi	4	
3362	4CE113	VB	Unknown fungi	4	
3363	4CE116	VB	Unknown fungi	4	
3364	4CE114	VB	Unknown fungi	4	
3365	4CE1	CC	Apricot fungi	4	*Thelebolaceae* sp.
3366	4CE2	CC	Apricot fungi	4	*Thelebolaceae* sp.
3367	4CE7	CC	Apricot fungi	4	*Thelebolaceae* sp.
3368	4CE16a	CC	White fluffy fungi	4	
3369	4CE16a	CC	White fungi	4	*Geomyces* sp.
3370	4CE17	CC	Apricot fungi	4	*Thelebolaceae* sp.
3371	4CE44	CC	White fungi	4	*Geomyces* sp.
3372	4CE41	CC	White fungi	4	
3373	4CE45	CC	White fungi	4	*Geomyces* sp.
3374	4CE22	CC	Apricot fungi	4	*Thelebolaceae* sp.
3375	4CE47	CC	Unknown fungi	4	
3376	4CE37	CC	Unknown fungi	4	
3377	4CE52	CC	Unknown fungi	4	
3378	4CE56	CC	Unknown fungi	4	
3379	4CE58	CC	Unknown fungi	4	
3380	4CE60	CC	White fungi	4	*Geomyces* sp.
3381	4CE63	CC	Dark fungi	4	*Cladosporium* sp.
3382	4CE65	CC	Unknown fungi	4	
3383	4CE75	CC	Unknown fungi	4	
3384	4CE79	CC	Apricot fungi	4	*Thelebolaceae* sp.
3385	4CE80	CC	Apricot fungi	4	*Thelebolaceae* sp.
3386	4CE85	CC	Unknown fungi	4	
3387	4CE87	CC	Unknown fungi	4	
3388	4CE90b	CC	Unknown fungi	4	
3389	4CE93	CC	White fungi	4	
3390	4CE100	CC	White fluffy fungi	4	
3391	4CE105	CC	White fluffy fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3392	4CE114	CC	Apricot fungi	4	*Thelebolaceae* sp.
3393			Yeast		
3394	4CE117	CC	White fluffy fungi	4	
3395			Yeast/bacteria		
3396			Yeast/bacteria		
3397			Yeast/bacteria		
3398			Yeast/bacteria		
3399			Yeast/bacteria		
3400			Yeast/bacteria		
3401			Yeast		
3402			Yeast		
3403			Yeast		
3404			Yeast		
3405			Yeast		
3406			Yeast		
3407			Yeast		
3408			Yeast		
3409			Yeast		
3410			Yeast		
3411			Yeast		
3412	4CR29	7	Penicillium	4	*Penicillium* sp.
3413	4CR33a	7	Penicillium	4	*Penicillium* sp.
3414			Yeast		
3415			Yeast		
3416			Yeast		
3417	4CR1	YM	Apricot fungi	4	*Thelebolaceae* sp.
3418	4CR1	YM	White fungi	4	
3419	4CR2	YM	Cladosporium like	4	*Cladosporium* sp.
3420			Yeast		
3421	4CR4	YM	Apricot fungi	4	*Thelebolaceae* sp.
3422	4CR4	YM	Dark fungi	4	*Cladosporium* sp.
3423	4CR3	YM	Penicillium	4	*Penicillium* sp.
3424	4CR5	YM	Unknown fungi	4	
3425			Yeast		
3426	4CR6	YM	White fluffy fungi	4	
3427			Bacteria		
3428	4CR7	YM	Unknown fungi	4	
3429	4CR8	YM	Apricot fungi	4	*Thelebolaceae* sp.
3430			Bacteria		
3431			Bacteria		
3432	4CR9	YM	White fungi	4	
3433	4CR10	YM	Geomyces like	4	*Geomyces* sp.
3434	4CR16	YM	Geomyces like	4	*Geomyces* sp.
3435			Yeast/bacteria		
3436	4CR26	YM	Geomyces like	4	*Geomyces* sp.
3437	4CR31	YM	White fungi	4	
3438	4CR32	YM	Geomyces like	4	*Geomyces* sp.
3439	4CR45	YM	Geomyces like	4	*Geomyces* sp.
3440	4CR46	YM	White fungi	4	*Geomyces* sp.
3441	4CR48	YM	White fungi	4	*Geomyces* sp.
3442	4CR49	YM	White fungi	4	
3443			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
3444			Yeast		
3445			Yeast		
3446	4CR2	CC	White fungi	4	
3447	4CR4	CC	White fungi	4	
3448	4CR5	CC	White fungi	4	
3449	4CR6	CC	White fungi	4	
3450	4CR7	CC	White fungi	4	*Geomyces* sp.
3451	4CR9	CC	Geomyces like	4	*Geomyces* sp.
3452	4CR10	CC	Geomyces like	4	*Geomyces* sp.
3453	4CR10	CC	Apricot fungi	4	*Thelebolaceae* sp.
3454	4CR14	CC	Geomyces like	4	*Geomyces* sp.
3455	4CR26a	CC	Penicillium	4	*Penicillium* sp.
3456	4CR27	CC	Geomyces like	4	*Geomyces* sp.
3457	4CR32	CC	Geomyces like	4	*Geomyces* sp.
3458	4CR40	CC	Penicillium	4	*Penicillium* sp.
3459	4CR2	VB	Penicillium	4	*Penicillium* sp.
3460	4CR3	VB	Penicillium	4	*Penicillium* sp.
3461	4CR14	VB	Penicillium	4	*Penicillium* sp.
3462	4CR39	VB	Penicillium	4	*Penicillium* sp.
3463	4CR49	CC	Unknown fungi	4	
3464			Bacteria		
3465			Yeast/bacteria		
3466			Yeast		
4001	5HP Mutton room	YM	Dark fungi	4	*Cladosporium* sp.
4002	5HP Mutton room	YM	Unknown fungi	4	
4003			Bacteria		
4004	5HP Main Area	Martins	Dark fungi	4	*Cladosporium* sp.
4005	5HP Main Area	Martins	Unknown fungi	4	
4006	5HP Main Area	YM	White fungi	4	*Geomyces* sp.
4007			Yeast		
4008	5HP Kitchen area	YM	Dark fungi	4	*Cladosporium* sp.
4009			Yeast		
4010	5HP Frame	PDA	Cream fungi	4	
4011	5HP Frame	YM	Green fungi	4	*Penicillium* sp.
4012	5CR Weather station	YM	Unknown fungi	4	
4013	5CR Weather station	PDA	Unknown fungi	4	
4014	5CR Outside Stores area	YM	Unknown fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4015	5CR Outside Stable area	YM	Unknown fungi	4	
4016					
4017	5CR Floor	PDA	Green fungi Penicillium like	4	*Penicillium* sp.
4018	5CR Floor	PDA	Green fungi Penicillium like	4	*Penicillium* sp.
4019			Bacteria		
4020			Bacteria		
4021			Bacteria		
4022			Bacteria		
4023			Bacteria		
4024	5CR High shelf stores area	PDA	Unknown fungi	4	
4025			Bacteria		
4026			Yeast		
4027			Bacteria		
4028			Bacteria		
4029			Bacteria		
4030			Bacteria		
4031			Bacteria		
4032	5CR Paper bed side	YM	Unknown fungi	4	
4033	5CR Paper bedside	YM	Unknown fungi	4	
4034			Bacteria		
4035			Bacteria		
4036	5CR Scraping of wall stores area next to bacon	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4037			Bacteria		
4038			Bacteria		
4039			Bacteria		
4040			Yeast		
4041			Yeast		
4042			Yeast		
4043	5CR Outside Peas	YM	Dark fungi	4	*Cladosporium* sp.
4044			Yeast		
4045			Yeast		
4046			Bacteria		
4047	5CR Outside Flour	YM	Unknown fungi	4	
4048	5CR Outside Flour	YM	Green fungi Penicillium like	4	*Penicillium* sp.
4049			Bacteria		
4050			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4051			Bacteria		
4052			Bacteria		
4053			Bacteria		
4054			Yeast		
4055			Yeast		
4056			Bacteria		
4057	5CR Sleeping bag	PDA	Unknown fungi	4	
4058			Yeast		
4059			Bacteria		
4060			Bacteria		
4061			Bacteria		
4062			Bacteria		
4063	5CR Outside Stable area	PDA	Unknown fungi	4	
4064	5CR Outside Stable area	YM	Unknown fungi	4	
4065	5CR Outside Stores area	YM	Unknown fungi	4	
4066	5CE Stable3	YM	Dark fungi Cadophora like	4	*Cadophora* sp.
4067			Bacteria		
4068			Bacteria		
4069			Bacteria		
4070			Bacteria		
4071			Bacteria		
4072			Bacteria		
4073			Bacteria		
4074			Bacteria		
4075			Bacteria		
4076	5CE oil drums	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4077			Yeast		
4078			Bacteria		
4079			Bacteria		
4080			Bacteria		
4081	5CE End of annex	YM	Unknown fungi		
4082	5CE End of annex	YM	Unknown fungi		
4083			Bacteria		
4084			Bacteria		
4085			Bacteria		
4086			Yeast		
4087	5CE Pontings dark room	YM	Green fungi Trichoderma like	4	*Trichoderma* sp.
4088	5CE Pontings dark room	PDA	Dark fungi		*Cladosporium* sp.
4089			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4090			Bacteria		
4091	5CE Evans bed	YM	Dark fungi Cadophora like	4	*Cadophora* sp.
4092			Bacteria		
4093			Bacteria		
4094	5CE Main area middle of floor	PDA	Unknown fungi		
4095			Bacteria		
4096			Yeast		
4097	5CE Kitchen area	Martins	Unknown fungi		
4098			Bacteria		
4099			Bacteria		
4100	5CE Bower's bunk	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4101			Bacteria		
4102			Bacteria		
4103			Bacteria		
4104	5CE Scraping Pointing's lab wall	YM	Unknown fungi	4	
4105			Bacteria		
4106			Bacteria		
4107			Bacteria		
4108	5CE Bunk next to lab bench	YM	Green fungi Trichoderma like	4	*Trichoderma* sp.
4109			Bacteria		
4110			Bacteria		
4111			Bacteria		
4112	5CE back leg of kitchen table	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4113			Bacteria		
4114			Bacteria		
4115			Yeast		
4116			Bacteria		
4117			Yeast		
4118			Bacteria		
4119	5CE Cape Evan's frame	Martins	Unknown fungi	4	
4120	5CE Cape Evan's frame	Martins	Unknown fungi	4	
4121			Bacteria		
4122			Bacteria		
4123	5CR12	VB	Apricot fungi	4	*Pseudodeuroti um bakeri* (strain CBS 878.71)
4124			Bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4125	5CR10	YM	Apricot fungi	4	*Thelebolaceae* sp.
4126			Bacteria		
4127			Bacteria		
4128			Bacteria		
4129			Yeast		
4130	5CR12	VB	Unknown fungi	4	
4131	5CR10	VB	Dark fungi	4	*Cadophora* sp.
4132	5CR10	VB	Apricot fungi	4	*Thelebolaceae* sp.
4133			Bacteria		
4134			Bacteria		
4135			Bacteria		
4136	5CR12	YM	Green fungi Penicillium like	4	*Penicillium* sp.
4137	5CR12	CC	Unknown fungi	4	
4138			Bacteria		
4139			Yeast		
4140			Pink yeast		
4141			Pink yeast		
4142	5CR12	VB	Unknown fungi	4	
4143			Bacteria		
4144			Bacteria		
4145			Bacteria		
4146			Bacteria		
4147			Bacteria		
4148	5CR8	YM	Dark fungi	4	*Cadophora luteo-olivacea*
4149	5CR7	YM	White fungi	4	*Pseudotium desertorum*
4150			Bacteria		
4151			Bacteria		
4152			Bacteria		
4153			Bacteria		
4154			Bacteria		
4155			Bacteria		
4156			Bacteria		
4157			Bacteria		
4158			Bacteria		
4159	5CR8	VB	Unknown fungi	4	
4160	5CR7	CC	Unknown fungi	4	
4161	5CR8	CC	Unknown fungi	4	
4162	5CR9	CC	Unknown fungi	4	
4163	5CEAW2	VB	Apricot fungi	4	*Thelebolaceae* sp.
4164	5CEAW2	VB	White fungi Geomyces like	4	*Geomyces* sp.
4165	5CEAW1	VB	Unknown fungi	4	
4166			Bacteria		
4167			Bacteria		
4168			Bacteria		
4169	5CEFL	VB	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4170			Bacteria		
4171	5CR9	VB	Unknown fungi	4	
4172			Bacteria		
4173	5CR6	YM	Spreading fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4174	5CR4	CC	Unknown fungi	4	
4175	5CR4	YM	Green fungi Penicillium like	4	*Penicillium* sp.
4176			Yeast		
4177			Pink yeast		
4178			Yeast		
4179			Bacteria		
4180	5CR4	VB	Green fungi Penicillium like	4	*Penicillium* sp.
4181	5CEDSNH	YM	Apricot fungi	4	*Thelebolaceae* sp.
4182			Bacteria		
4183			Bacteria		
4184			Bacteria		
4185	5CEMCF1	YM	White fungi	4	*Geomyces* sp.
4186	5CEMC5M	CC	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4187	5CEMC4L	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4188	5CEMC3L	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4189	5CEFL	YM	Unknown fungi		
4190	5CEFL	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4191			Bacteria		
4192			Bacteria		
4193			Bacteria		
4194			Bacteria		
4195			Bacteria		
4196	5CEAW1	YM	Apricot fungi	4	*Thelebolaceae* sp.
4197	5CEAW2	YM	Apricot fungi	4	*Thelebolaceae* sp.
4198			Bacteria		
4199			Bacteria		
4200	5HP4	YM	White fungi	4	*Geomyces* sp.
4201			Yeast		
4202			Yeast		
4203			Yeast		
4204			Yeast		
4205	5CEDSNH	YM	Unknown fungi	4	
4206			Bacteria		
4207			Bacteria		
4208			Bacteria		
4209			Bacteria		
4210	5HP9	YM	Unknown fungi	4	
4211			Bacteria		
4212	5CETNSD	YM	Green fungi Penicillium like	4	*Penicillium* sp.
4213			Yeast		
4214	5CEMC2L	VB	Unknown fungi	4	
4215			Bacteria		
4216			Yeast		
4217			Yeast		
4218	5CR2	YM	Unknown fungi	4	

Culture number	Sample number	Media	Description	Incubation Temp	Identification
4219			Yeast		
4220	5CR2	7	Unknown fungi	4	
4221	5HP2	VB	White fungi Geomyces like	4	*Geomyces* sp.
4222	5HP2	CC	Unknown fungi	4	
4223			Yeast		
4224	5HP2	YM	White fungi Geomyces like	4	*Geomyces* sp.
4225	5CEMC1 L	VB	White fungi Geomyces like	4	*Geomyces* sp.
4226	5CEMC1 L	YM	White fungi Geomyces like	4	*Geomyces* sp.
4227	5CEMC1 L	YM	Unknown fungi	4	
4228			Bacteria		
4229	5CEMC2 M	YM	White fungi Geomyces like	4	*Geomyces* sp.
4230			Yeast		
4231			Yeast		
4232	5HP9	CC	Unknown fungi	4	
4233	5HP4	CC	Unknown fungi	4	
4234	5HP4	YM	Dark fungi	4	*Cadophora malorum*
4235	5HP4	VB	White fungi Geomyces like	4	*Geomyces* sp.
4236			Bacteria		
4237	5CEMC2	YM	Unknown fungi	4	
4238	5CEMC2	CC	Unknown fungi	4	
4239			Bacteria		
4240	5HP4	VB	White fungi Geomyces like	4	*Geomyces* sp.
4241	5CEMC3 M	YM	Green fungi Penicillium like	4	*Penicillium* sp.
4242	5CEAW1	YM	White fungi Geomyces like	4	*Geomyces* sp.
4243	5HP9	YM	Unknown fungi	4	
4244	5CEMC1	YM	Dark fungi Cladosporium like	4	*Cladosporium* sp.
4245	5CR6	VB	Unknown fungi	4	
4246	5CEMC2 L	YM	Unknown fungi	4	
4247	5CR8	YM	Dark fungi	4	*Cadophora malorum*
4248	5CR6	VB	Unknown fungi	4	
4249	5CR9	YM	Dark fungi	4	*Cadophora malorum*
4250					
5001			Mixed culture		
5002			Mixed culture		
5003			Mixed culture		
5004			Mixed culture		
5005			Mixed culture		
5006			Mixed culture		
5007			Mixed culture		
5008			Mixed culture		
5009			Mixed culture		
5010			Mixed culture		
5011			Mixed culture		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5012			Mixed culture		
5013			Mixed culture		
5014	6CEBT Outside between latrine and sea	NaCl	Unknown fungi	4	
5015	6CRBT Old dump	NaCl	Unknown fungi	4	*Geomyces* sp. (str C239/10G)
5016	6CEBT Outside between latrine and sea	YM	Unknown fungi	4	
5017	6CRBT Latrine	YM	Unknown fungi	4	
5018	6CEST Beside blubber alleyway to stables	YM	Dark fungi	4	*Cladosporium* sp.
5019	6CEST Beside blubber alleyway to stables	YM	White fungi	4	*Pseudeurotium desertorum*
5020			Yeast		
5021	6CEST 4th box 1st level black green	YM	Dark fungi	4	*Cladosporium* sp.
5022	6CEST 4th box 1st level black green	YM	White fungi	4	*Geomyces* sp.
5023			Yeast		
5024	6CEST 5th box 2nd level butter box	YM	White fungi	4	*Geomyces* sp.
5025			Yeast		
5026			Yeast		
5027	6CEST 5th box 2nd level butter box	YM	Dark fungi	4	*Cladosporium* sp.
5028	6CEST Butter box mould 2nd box 2nd level	YM	Dark fungi	4	*Cladosporium oxysporum*
5029	6CEST Chair in kitchen area	YM	White fungi	4	*Geomyces* sp.
5030			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5031	6CEST Chair in kitchen area	NaCl	White fungi	4	*Geomyces* sp.
5032	6CEST Support box by butter box	YM	White fungi	4	*Geomyces* sp.
5033			Yeast		
5034			Yeast		
5035	6CEST Ontop of butter boxes	YM	Dark fungi	4	*Cladosporium* sp.
5036	6CEST Ontop of butter boxes	YM	White fungi	4	*Geomyces* sp.
5037			Yeast		
5038	6CEST Ontop of butter boxes	NaCl	White fungi	4	*Geomyces* sp.
5039	6CEST Ontop of buter boxes	YM	Dark fungi	4	*Cladosporium* sp.
5040			Yeast		
5041	6CEST Main area by chimney	YM	White fungi	4	*Geomyces* sp.
5042			Yeast		
5043			Yeast		
5044			Yeast		
5045			Yeast		
5046	6HPST LocationA	YM	White fungi	4	*Pseudeurotorum desertorum*
5047			Yeast		
5048			Yeast		
5049	6HPST Location A	NaCl	Dark fungi	4	*Cladosporium* sp.
5050	6HPST Location A	NaCl	White fungi	4	*Geomyces* sp.
5051			Yeast		
5052	6HPST Location B	YM	White fungi	4	*Geomyces* sp.
5053			Yeast		
5054	6HPST Location C	YM	Dark fungi	4	*Cladosporium* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5055	6HPST Location C	YM	White fungi	4	*Geomyces* sp.
5056			Yeast		
5057	6HPST Location C	NaCl	Dark fungi	4	*Cladosporium* sp.
5058	6HPST Location C	NaCl	White fungi	4	*Geomyces* sp.
5059	6HPST Location C	NaCl	White fungi	4	*Geomyces* sp.
5060	6HPST Location D	YM	White fungi	4	*Geomyces* sp.
5061	6HPST Outside by seal	YM	White fungi	4	*Geomyces* sp.
5062			Yeast		
5063			Yeast		
5064	6HPSTOutside by seal	NaCl	White fungi	4	*Geomyces* sp.
5065					
5066	6HPST Outside by hitching post	YM	White fungi	4	*Geomyces* sp.
5067			Yeast		
5068			Yeast		
5069			Yeast		
5070			Yeast		
5071			Yeast		
5072	6CRST Under stove	NaCl	White fungi	4	*Geomyces* sp.
5073			Yeast		
5074			Yeast		
5075			Yeast		
5076			Yeast		
5077			Yeast		
5078			Yeast		
5079	6CRST Below window	YM	Dark fungi	4	*Cladosporium* sp.
5080			Yeast		
5081	6CRST Darkroom	YM	Unknown fungi	4	
5082			Yeast		
5083	6CRST Darkroom	NaCl	Green fungi	4	*Penicillium* sp.
5084			Yeast		
5085			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5086			Yeast		
5087			Yeast		
5088			Yeast		
5089			Yeast		
5090			Yeast		
5091			Yeast		
5092			Yeast		
5093	6CEST On chair beside sign in table	NaCl	White fungi	4	*Geomyces* sp.
5094			Yeast		
5095			Yeast		
5096	6CEST 5th box of butter 2nd level	YM	White fungi	4	*Geomyces* sp.
5097	6CEST On top of butter boxes	YM	White fungi	4	*Geomyces* sp.
5098	6CEST Main area by Chimney	NaCl	White fungi	4	*Geomyces* sp.
5099	6CEST On chair in Kitchen	NaCl	Dark fungi	4	*Cladosporium* sp.
5100			Yeast		
5101			Yeast		
5102			Yeast/Bacteria		
5103			Yeast/Bacteria		
5104	6CRST Doorway	YM	White fungi	4	*Geomyces* sp.
5105	6CRST Doorway	YM	White fungi	4	*Geomyces* sp.
5106			Yeast		
5107			Bacteria		
5108			Bacteria		
5109			Yeast/Bacteria		
5110	6CRST Above hams	NaCl	White fungi		*Geomyces* sp.
5111			Yeast		
5112			Yeast/Bacteria		
5113			Mixed culture		
5114			Mixed culture		
5115			Mixed culture		
5116			Mixed culture		
5117			Mixed culture		
5118			Mixed culture		
5119			Mixed culture		
5120			Mixed culture		
5121			Mixed culture		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5122			Mixed culture		
5123			Mixed culture		
5124			Mixed culture		
5125			Mixed culture		
5126			Mixed culture		
5127			Mixed culture		
5128	6CRBT Old dump	YM	Green fungi	4	*Penicillium* sp.
5129			Mixed culture		
5130	6CRBT Old dump	YM	Green fungi	4	*Penicillium* sp.
5131	6CRBT Old dump	YM	White fungi	4	*Geomyces* sp.
5132	6CRST Dark room	YM	White fungi	4	*Geomyces* sp.
5133			Yeast		
5134			Yeast		
5135	6CEST E4	7	White fungi	4	*Geomyces* sp.
5136	6CEChair in Kitchen area	YM	White fungi	4	*Geomyces* sp.
5137			Bacteria		
5138			Yeast		
5139	6CEST Main area by chimney	NaCl	White fungi	4	*Geomyces* sp.
5140			Yeast		
5141	6HPST Location B	YM	White fungi	4	*Geomyces* sp.
5142	6HPST Outside by Hitching post	YM	White fungi	4	*Geomyces* sp.
5143	6HPST Outside by seal	YM	White fungi	4	*Geomyces* sp.
5144	6HPMC1 H	YM	White Fungi	4	*Geomyces* sp.
5145	6HPMC1 M	MEA	White fungi	4	*Geomyces* sp.
5146	6HPMC3 L	MEA	Dark fungi	4	*Cladosporium* sp.
5147	6HPMC3 H	YM	White Fungi	4	*Geomyces* sp.
5148	6HPMC3 H	MEA	Dark fungi	4	*Cladosporium* sp.
5149	6HPMC3 M	YM	White fungi	4	*Geomyces* sp.
5150			Yeast/bacteria		
5151			Yeast/bacteria		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5152	6HPMC4 L	MEA	Dark fungi	4	*Cladosporium* sp.
5153	6HPMC4 M	YM	Dark fungi	4	*Cladosporium* sp.
5154	6HPMC4 H	YM	White Fungi	4	*Geomyces* sp.
5155	6HPMC4 L	YM	Apricot fungi	4	*Thelebolaceae* sp.
5156			Yeast/bacteria		
5157			Yeast/bacteria		
5158	6HPMC5 L	MEA	White Fungi	4	*Geomyces* sp.
5159	6HPMC5 L	YM	Dark fungi	4	*Cladosporium* sp.
5160			Yeast/bacteria		
5161			Yeast/bacteria		
5162			Yeast/bacteria		
5163	6HPMCR 3	YM	Dark fungi	4	*Cladosporium* sp.
5164	6HPMCR 1	YM	White fungi	4	*Geomyces* sp.
5165			Yeast/bacteria		
5166			Yeast/bacteria		
5167	6HPMCF 1	YM	White Fungi	4	*Geomyces* sp.
5168	6HP1	YM	Apricot fungi	4	*Thelebolaceae* sp.
5169			Yeast/bacteria		
5170			Yeast		
5171	6HP1	MEA	White Fungi	4	*Geomyces* sp.
5172			Yeast/bacteria		
5173			Yeast/bacteria		
5174	6HP4	MEA	Dark fungi	4	*Cladosporium* sp.
5175			Yeast/bacteria		
5176			Yeast/bacteria		
5177	6HP7	MEA	Dark fungi	4	*Cladosporium* sp.
5178			Yeast/bacteria		
5179	6HP7	YM	Apricot fungi	4	*Thelebolaceae* sp.
5180			Yeast/bacteria		
5181	6HP8	YM	White fungi	4	*Geomyces* sp.
5182	6HP9	YM	Apricot fungi	4	*Thelebolaceae* sp.
5183			Yeast/bacteria		
5184	6HP10	YM	White fungi Geomyces like	4	*Geomyces* sp.
5185	6HP10	YM	Apricot fungi	4	*Thelebolaceae* sp.
			Yeast/bacteria		
5187	6HP10	MEA	Dark fungi	4	*Cladosporium* sp.
5188	6HP11	YM	White fungi Geomyces like	4	*Geomyces* sp.
5189	6HP11	YM	Apricot fungi	4	*Thelebolaceae* sp.
5190			Yeast/bacteria		
5191	6HP11	MEA	Dark fungi	4	*Cladosporium* sp.
5192	6HP11	MEA	Yeast/Bacteria		
5193	6HP13	YM	White fungi	4	*Geomyces* sp.
5194	6HP13	YM	White fungi Geomyces like	4	*Geomyces* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5195	6HP13	YM	Yeast/bacteria		
5196	6HP15	YM	White fungi	4	*Geomyces* sp.
5197	6HP15	MEA	Dark fungi	4	*Cladosporium* sp.
5198	6HP15	YM	Yeast/bacteria		
5199	6HP15	MEA	Yeast/bacteria		
5200	6HP18	MEA	Green fungi	4	*Penicillium* sp.
5201	6HP18	YM	White fungi Geomyces like	4	*Geomyces* sp.
5202	6HP18	YM	Apricot fungi	4	*Thelebolaceae* sp.
5203	6HP18	YM	Yeast/bacteria		
5204	6HP18	YM	White fungi	4	*Geomyces* sp.
5205	6HP19	MEA	Yeast/bacteria		
5206	6HP19	MEA	Apricot fungi	4	*Thelebolaceae* sp.
5207			Yeast		
5208	6HP19	YM	White fungi	4	*Geomyces* sp.
5209	6HPHI1	YM	White fungi Geomyces like	4	*Geomyces* sp.
5210	6HPHI1	YM	Dark fungi	4	*Cladosporium* sp.
5211	6HPHI2	YM	White fungi Geomyces like	4	*Geomyces* sp.
5212	6HPHI3	YM	White fungi	4	*Geomyces* sp.
5213	6HPHI3	YM	Yeast/bacteria		
5214	6HP Swab1	YM	Dark fungi	4	*Cladosporium* sp.
5215	6HP Swab1	MEA	Dark fungi	4	*Cladosporium* sp.
5216	6HP16	YM	Yeast/bacteria		
5217	6HP16	YM	Dark fungi	4	*Cladosporium* sp.
5218	6HP16	MEA	Apricot fungi	4	*Thelebolaceae* sp.
5219	6HP16	MEA	Yeast/bacteria		
5220	6HP16	MEA	Green fungi	4	*Penicillium* sp.
5221	6HP16	MEA	Yeast		
5222	6CE1	YM	Yeast/bacteria		
5223	6CE1	YM	Unknown fungi	4	
5224	6CE1	MEA	Dark fungi	4	*Cladosporium* sp.
5225	6CE1	MEA	Yeast/bacteria		
5226	6CE2	YM	Apricot fungi	4	*Thelebolaceae* sp.
5227	6CE2	YM	Yeast/bacteria		
5228	6CE2	MEA	Dark fungi	4	*Cladosporium* sp.
5229	6CE2	MEA	Yeast/bacteria		
5230	6CE3	YM	Apricot fungi	4	*Thelebolaceae* sp.
5231	6CE3	MEA	Dark fungi	4	*Cladosporium* sp.
5232	6CE3	MEA	White fungi	4	*Geomyces* sp.
5233	6CE4	YM	White fungi	4	*Geomyces* sp.
5234	6CE4	YM	Yeast/bacteria		
5235	6CE4	MEA	Unknown fungi	4	
5236	6CE4	MEA	Yeast/bacteria		
5237	6CE5	YM	Yeast/bacteria		
5238	6CE5	YM	Apricot fungi	4	*Thelebolaceae* sp.
5239			Yeast/bacteria		
5240	6CE6	YM	Dark fungi	4	*Cladosporium* sp.
5241			Yeast/bacteria		
5242			Yeast/bacteria		
5243	6CE7	YM	Apricot fungi	4	*Thelebolaceae* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5244	6CE11	MEA	Dark fungi	4	*Cladosporium* sp.
5245	6CE13	YM	Dark fungi	4	*Cladosporium* sp.
5246	6CE14	YM	Dark fungi	4	*Cladosporium* sp.
5247	6CE14	MEA	White fungi	4	*Geomyces* sp.
5248	6CE15	YM	White fungi	4	*Geomyces* sp.
5249	6CE18	YM	Dark fungi	4	*Cladosporium* sp.
5250	6CE19	YM	Dark fungi	4	*Cladosporium* sp.
5251	6CE20	MEA	Dark fungi	4	*Cladosporium* sp.
5252	6CE21	YM	White fungi	4	*Geomyces* sp.
5253	6CE21	YM	White fungi	4	*Geomyces* sp.
5254			Yeast		
5255	6CE21	MEA	Unknown fungi	4	
5256			Yeast		
5257	6CE29	YM	Dark fungi	4	*Cladosporium* sp.
5258	6CE30	YM	Dark fungi	4	*Cladosporium* sp.
5259	6CE30	YM	Yeast		
5260	6CE30	MEA	Green Fungi	4	*Penicillium* sp.
5261	6CE31	YM	Dark fungi	4	*Cladosporium* sp.
5262			Yeast		
5263	6CE31	MEA	White fungi	4	*Geomyces* sp.
5264			Yeast		
5265	6CE32	MEA	Dark fungi	4	*Cladosporium* sp.
5266	6CE33	MEA	Dark fungi	4	*Cladosporium* sp.
5267	6CE34	YM	Apricot fungi	4	*Thelebolaceae* sp.
5268	6CE34	YM	Dark fungi	4	*Cladosporium* sp.
5269			Yeast		
5270			Yeast		
5271	6CE38	MEA	Dark fungi	4	*Cladosporium* sp.
5272	6CE39	MEA	Dark fungi	4	*Cladosporium* sp.
5273			Yeast		
5274	6CE39	YM	White fungi	4	*Geomyces* sp.
5275	6CE42	YM	Dark fungi	4	*Cladosporium* sp.
5276			Yeast		
5277	6CE43	MEA	Unknown fungi	4	
5278			Yeast		
5279	6CE Swab2	YM	Dark fungi	4	*Cladosporium* sp.
5280	6CE Swab5	YM	Unknown fungi	4	
5281			Yeast		
5282			Yeast		
5283	6CE Swab5	MEA	Unknown fungi	4	
5284	6CE Swab7	YM	Dark fungi	4	*Cladosporium* sp.
5285	6CE Swab8	YM	Dark fungi	4	*Cladosporium* sp.
5286	6CE Scap9	YM	Dark fungi	4	*Cladosporium* sp.
5287	6CE Scrap10	YM	Apricot fungi	4	*Thelebolaceae* sp.
5288			Yeast		
5289	6CE Scrap10	MEA	Dark fungi	4	*Cladosporium* sp.

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5290			Yeast		
5291	6CEMC1 L	MEA	White fungi	4	*Geomyces* sp.
5292	6CEMC1 L	YM	Dark fungi	4	*Cladosporium* sp.
5293			Yeast		
5294			Yeast		
5295	6CEMC1 M	YM	Dark fungi	4	*Cladosporium* sp.
5296	6CEMC5 H	MEA	Dark fungi	4	*Cladosporium* sp.
5297	6CEMC2 M	YM	Dark fungi	4	*Cladosporium* sp.
5298	6CEMC3 M	MEA	Dark Fungi	4	*Cladosporium* sp.
5299	6CEMC4 L	YM	Dark Fungi	4	*Cladosporium* sp.
5300			Yeast		
5301			Yeast		
5302	6CEMCE ntH	YM	White fungi	4	*Geomyces* sp.
5303	6CEMCF 4	MEA	White fungi	4	*Geomyces* sp.
5304	6CEMCF 4	YM	White fungi	4	*Geomyces* sp.
5305	6CEMCB 2	YM	White fungi	4	*Geomyces* sp.
5306	6CEMCB 3	YM	Dark Fungi	4	*Cladosporium* sp.
5307	6CEB3	YM	White fungi	4	*Geomyces* sp.
5308			Yeast		
5309	6HPMCR 3	YM	White fungi	4	*Geomyces* sp.
5310	6HP9	YM	White fungi	4	*Geomyces* sp.
5311	6HP16	YM	Dark fungi	4	*Cladosporium* sp.
5312	6HP16	YM	White fungi	4	*Geomyces* sp.
5313	6HP11	YM	Unknown fungi	4	
5314			Yeast/bacteria		
5315	6HPMCF 1	MEA	Unknown fungi	4	
5316			Yeast/bacteria		
5317			Bacteria		
5318			Yeast		
5319	6CE20	MEA	Unknown fungi	4	
5320	6CR1	YM	Geomyces like	4	*Geomyces* sp.
5321	6CR1	YM	Unknown fungi	4	
5322			Yeast/bacteria		
5323	6CR1	MEA	Fungi/yeast	4	
5324	6CR2	YM	Unknown fungi	4	
5325			Yeast/bacteria		
5326	6CR2	MEA	Penicillium	4	*Penicillium* sp.
5327	6CR2	MEA	Cladosporium like	4	*Cladosporium* sp.
5328	6CR3	YM	Apricot fungi	4	*Thelebolaceae* sp.
5329			Yeast		
5330			Yeast		

Culture number	Sample number	Media	Description	Incubation Temp	Identification
5331	6CR4	YM	White fungi	4	*Geomyces* sp.
5332	6CR4	MEA	Penicillium	4	*Penicillium* sp.
5333	6CR5	YM	White fungi	4	*Geomyces* sp.
5334			Pink yeast		
5335			Yeast/bacteria		
5336	6CR5	MEA	Unknown fungi	4	
5337	6CR6	YM	Apricot fungi	4	*Thelebolaceae* sp.
5338			Yeast		
5339	6CR6	MEA	Dark fungi	4	*Cladosporium* sp.
5340			Yeast/bacteria		
5341			Yeast/bacteria		
5342			Yeast/bacteria		
5343			Yeast/bacteria		
5344			Yeast/bacteria		
5345			Yeast/bacteria		
5346			Yeast		
5347			Yeast		
5348			Yeast		
5349			Yeast/bacteria	4	
5350			Yeast/bacteria	4	
5351	6CR18	YM	Apricot fungi	4	*Thelebolaceae* sp.
5352			Yeast/bacteria		
5353	6CR18	MEA	Yeast/Fungi	4	
5354			Yeast		
5355			Yeast/bacteria		
5356	6CR21	MEA	Penicillium	4	*Penicillium* sp.
5357			Yeast		
5358			Yeast		
5359			Yeast		
5360			Yeast		
5361	6CR47	YM	Cladosporium like	4	*Cladosporium* sp.
5362	6CR49	YM	Cladosporium like	4	*Cladosporium* sp.
5363	6CR61	YM	White fungi	4	*Geomyces* sp.
5364	6CRSwab 1	YM	Cladosporium like	4	*Cladosporium* sp.
5365	6CR5	MEA	Penicillium	4	*Penicillium* sp.
5366	6CR3	MEA	Cadophora like	4	*Cadophora* sp.
5367	6CR15	MEA	Cadophora like	4	*Cadophora* sp.

Appendix 5
Results of CMCase screening using an agarose plate technique

Results from screen Antarctic fungal isolates for CMCase activity are given in Appendix 5.

Table A5.1: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated at 4°C.

Isolate number	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
2	4	5	0.25
6	6	7	0.17
9	4	6	0.5
32	4	8	1.0
36	10	19	0.9
39	4	6	0.5
50	7	No	0
261	6	10	0.67
263	10	13	0.3
271	6	9	0.5
278	7	No	0
281	6	8	0.33
283	6	8	0.33
285	6	10	0.67
287	5	8	0.6
707	6	9	0.5
709	5	No	0
711	5	No	0
713	4	9	1.25
714	9	11	0.22
715	6	11	0.83
716	6	11	0.83
719	5	No	0
721	6	8	0.33
722	11	13	0.18
725	3	No	0
728	4	No	0
738	9	12	0.33
739	5	7	0.4
743	6	10	0.67
744	9	11	0.22
745	8	10	0.25
749	7	No	0
750	10	15	0.5
754	4	7	0.75
756	5	9	0.8
758	7	No	0
759	2	5	1.5
765	12	15	0.2
766	4	No	0
767	11	13	0.18
770	4	No	0
771	5	8	0.6
773	7	12	0.71

Table A5.1: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated at 4°C.

Isolate number	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
774	4	No	0
778	10	12	0.2
788	5	7	0.4
791	2	No	0
795	6	9	0.5
800	10	12	0.2
801	6	9	0.5
805	4	6	0.5
806	7	10	0.43
807	5	9	0.8
809	3	No	0
810	5	7	0.4
816	8	12	0.5
819	13	17	0.38
820	7	11	0.7
822	6	14	1.33
824	5	6	0.2
826	9	12	0.33
828	5	8	0.6
829	8	12	0.5
833	4	6	0.5
837	3	5	0.67
844	7	9	0.28
847	7	No	0
850	4	6	0.5
854	7	9	0.28
855	35	No	0
863	4	7	0.75
864	12	19	0.58
865	4	5	0.25
867	7	9	0.28
868	6	10	0.67
878	5	No	0
881	7	No	0
883	4	8	1
884	4	6	0.5
889	4	6	0.
890	11	14	5
894	5	No	0
898	5	7	0.4
899	2	No	0
903	7	9	0.28

Table A5.2: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated at 15°C.

Isolate number	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
117	10	17	0.7
194	12	14	0.16
195	8	12	0.5
196	7	11	0.71
198	6	10	0.67
199	15	19	0.27
200	10	15	0.5
203	4	9	0.25
204	7	11	0.45
205	7	9	0.28
208	6	8	0.33
216	7	10	0.42
219	11	17	0.54
227	4	No	0
228	6	10	0.67
229	14	19	0.36
246	6	8	0.33
247	12	15	0.25
248	7	12	0.71
254	10	15	0.5
256	7	15	0.71
405	4	No	0
431	19	No	0
436	24	No	0
443	30	No	0
448	10	17	0.7
453	7	16	1.28
455	6	9	0.5
462	15	18	0.2
466	10	No	0
492	2	No	0
493	18	20	0.11
459	8	13	0.625
502	3	5	0.66
503	9	10	0.11
519	18	20	0.11
526	4	6	0.5
527	4	6	0.5
531	6	9	0.5
537	22	No	0
538	7	10	0.43
539	7	10	0.43
541	11	20	0.82
543	4	6	0.5
581	6	11	0.83
644	14	No	0
648	9	12	0.33
649	4	6	0.5
650	10	12	0.2
651	9	15	0.67
652	6	No	0

Table A5.2: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated at 15°C.

Isolate number	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
653	7	9	0.28
654	6	9	0.5
656	12	No	0
659	10	20	1
661	5	11	1.2
665	5	9	0.8
668	5	10	1
669	4	No	0
670	10	15	0.5
671	6	14	1.33
681	12	16	0.33
687	8	No	0
690	7	12	0.71
691	9	13	0.44
693	7	15	1.14

Table A5.3: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated at 25°C.

Isolate number	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
74	8	No	0
150	15	No	0
175	7	No	0
373	4	6	0.5
543	5	No	0
552	18	No	0
564	6	No	0
575	6	No	0
581	9	11	0.18
587	10	No	0
611	5	No	0
615	4	No	0
627	4	6	0.5
625	10	No	0
626	4	7	0.75
636	18	No	0
638	10	12	0.2
644	9	11	0.18

Table A5.4: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated from December 1998 sampling trip at isolation temperature.

Isolate Number	Temperature for growth	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
1001	25	5	No	0
1002	25	20	No	0
1003	25	2	No	0
1004	25	4	15	2.75
1007	25	10	26	1.60
1008	25	7	27	2.86
1009	25	12	24	1.00
1010	25	30	37	0.23
1011	25	15	25	0.67
1012	25	4	No	0
1013	25	24	No	0
1014	25	14	No	0
1015	25	27	34	0.26
1017	25	4	7	0.75
1021	25	3	No	0
1022	25	11	20	0.82
1027	25	9	11	0.22
1031	25	10	21	1.10
1032	25	7	12	0.71
1060	15	8	14	0.75
1060	15	10	12	0.20
1064	15	9	22	1.44
1067	15	7	14	1.00
1068	15	7	11	0.57
1074	15	14	20	0.43
1076	15	10	17	0.70
1077	15	9	15	0.67
1078	15	10	20	1.00
1079	15	5	12	1.40
1080	15	15	34	1.27
1081	15	7	17	1.43
1082	15	8	9	1.2
1083	15	8	10	0.25
1085	15	7	13	0.86
1086	15	52	No	0
1087	15	3	No	0
1088	15	11	22	1.00
1092	15	3	No	0
1093	15	8	17	1.12
1094	15	8	18	1.25
1095	15	4	8	1.0
1096	15	9	15	0.67
1098	15	7	12	0.71
1099	15	12	19	0.58
1100	15	15	20	0.33
1101	15	9	12	0.33
1103	15	5	No	0
1104	15	8	15	0.87
1105	15	7	14	1.00
1106	15	2	No	0

Table A5.4: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated from December 1998 sampling trip at isolation temperature.

Isolate Number	Temperature for growth	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
1107	15	7	16	1.28
1108	15	13	25	0.93
1109	15	10	13	0.30
1111	15	15	19	0.27
1113	15	18	21	0.17
1114	15	9	16	0.78
1115	15	11	15	0.36
1116	15	6	17	1.83
1117	15	7	11	0.57
1118	15	15	17	0.13
1121	15	60	No	0
1122	15	30	No	0
1123	15	40	No	0
1124	4	12	No	0
1126	4	6	No	0
1128	4	6	18	2.00
1129	4	31	No	0
1130	4	2	8	2.00
1131	4	3	11	2.67
1132	4	2	No	0
1133	4	7	9	0.28
1134	4	9	18	1.00
1135	4	5	10	1.00
1136	4	10	15	0.50
1137	4	7	17	1.43
1139	4	8	19	1.37
1140	4	5	12	1.40
1141	4	6	9	0.50
1142	4	8	18	1.25
1144	4	40	No	0
1145	4	20	No	0
1146	4	7	No	0
1147	4	7	15	1.14
1148	4	7	14	1.00
1149	4	9	11	0.22
1151	4	9	18	1.00
1152	4	9	12	0.33
1153	4	6	No	0
1162	25	16	28	0.75
1164	25	32	34	0.06
1167	25	25	34	0.36
1169	25	7	13	0.86
1174	25	12	26	1.17
1176	25	15	No	0
1178	15	6	17	1.83
1193	15	7	16	1.29
1195	15	3	No	0
1194	15	10	13	0.30
1198	15	7	18	1.57
1200	15	8	16	1.00

Table A5.4: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated from December 1998 sampling trip at isolation temperature.

Isolate Number	Temperature for growth	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
1204	15	8	19	1.37
1205	15	12	18	0.50
1206	15	10	22	1.20
1210	15	8	No	0
1214	15	5	14	1.80
1215	15	8	17	1.12
1217	15	11	No	0
1218	15	7	15	1.14
1219	15	8	18	1.25
1221	15	5	16	2.20
1222	15	5	9	0.80
1223	15	11	No	0
1224	15	12	15	0.25
1225	15	6	7	0.17
1226	15	7	No	0
1227	15	5	No	0
1228	15	15	24	0.60
1230	4	7	14	1.00
1231	4	10	20	1.00
1232	4	6	No	0
1233	4	3	No	0
1234	4	4	10	1.50
1236	4	8	21	1.62
1238	4	11	No	0
1242	4	2	No	0
1244	4	2	No	0
1245	4	2	No	0
1246	4	4	8	1.00
1248	4	5	10	1.00
1249	4	8	No	0
1250	4	7	19	1.71
1252	4	7	36	4.14
1254	4	7	10	0.43
1255	4	10	19	0.90
1256	4	6	No	0
1257	4	10	No	0
1269	4	3	No	0
1271	4	9	11	0.22
1272	4	8	11	0.37
1276	4	8	18	1.25
1277	4	9	12	0.33
1278	4	12	18	0.50
1279	4	10	No	0
1280	4	4	No	0
1281	4	6	10	0.67
1283	25	7	21	2.00

Table A5.5: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates isolated from January 2001 sampling trip at isolation temperature.

Isolate Number	Temperature for growth	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
4248	4	45	47	0.04
4224	4	15	32	1.13
4233	4	13	21	0.61
4222	4	14	25	0.78
4169	4	8	14	0.75
4187	4	4	10	1.5
4185	4	10	27	1.7
4232	4	13	28	1.15
4197	4	27	37	0.37
4189	4	8	14	0.75
4181	4	2	14	6.0
4186	4	20	25	0.25
4138	4	13	20	0.54
4174	4	31	42	0.35
4137	4	7	15	1.14
4238	4	11	26	1.36
4214	4	9	22	1.44
4125	4	7	11	0.57
4136	4	9	18	1.0
4137	4	7	11	0.45
4142	4	8	15	0.87
4245	4	19	24	0.26
4248	4	16	No	0
4249	4	6	14	1.33
4175	4	6	25	3.12
4180	4	8	30	2.75
4171	4	6	14	1.33
4123	4	13	34	1.61
4162	4	29	34	0.17
4161	4	45	56	0.24
4148	4	14	24	0.71

Table A5.6: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates at isolation temperature and at 4°C.

Isolate Number	Isolation Temperature (°C)	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
71	25	7	14	1.00	NG		
72	25	8	18	1.25	NG		
77	25	7	12	0.71	NG		
80	25	8	11	0.375	21	45	1.14
96	25	6	No	0	NG		
98	25	6	14	1.34	NG		
101	25	7	14	1.00	NG		
107	25	16	27	0.69	17	26	0.53
124	25	6	13	1.17	NG		
129	25	8	No	0	12	35	1.91
174	25	6	No	0	NG		
175	25	7	10	0.43	NG		
182	15	8	15	0.87	19	43	1.26
183	15	12	19	0.58	17	36	1.12
215	15	14	16	0.14	27	30	0.11
218	15	15	17	0.13	20	28	0.40
219	15	14	24	0.71	15	20	0.33
225	15	12	18	0.50	18	No	0
226	15	7	12	0.71	24	54	1.25
227	15	20	33	0.65	22	33	0.50
228	15	8	20	1.50	16	43	1.68
229	15	11	14	0.27	36	46	0.28
230	15	7	12	0.71	19	26	0.37
235	15	6	20	2.33	14	30	1.14
236	15	7	27	2.85	9	No	0
242	15	7	16	1.28	17	36	1.12
256	15	16	18	0.12	20	26	0.30
262	4				29	61	1.10
271	4				15	25	0.67
292	4				50	56	0.12
334	25	11	No	0	70	No	0
383	25	10	No	0	NG		
405	15	8	No	0	43	No	0
408	15	18	20	0.11	70	No	0
453	15	22	24	0.09	24	39	0.62
487	15	4	6	0.50	12	40	2.33
489	15	7	21	2.00	18	No	0
492	15	10	21	1.10	24	40	0.67
517	15	10	20	1.00	18	42	1.33
532	15	19	21	0.10	66	No	0
536	15	8	17	1.12	19	No	0
537	15	46	No	0	90	No	0
539	15	17	19	0.12	29	40	0.38
541	15	15	18	0.20	35	40	0.14
564	15	30	34	0.13	NG		
612	25	6	15	1.50	NG		
625	25	7	No	0	NG		
635	25	20	32	0.60	NG		
638	25	10	26	1.60	15	23	0.53

Table A5.6: Isolate number, measurement of growth and CMCase clearing, Relative Index of Enzyme Activity for isolates at isolation temperature and at 4°C.

Isolate Number	Isolation Temperature (°C)	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity	Colony growth (mm)	Colony Growth + clear zone (mm)	Index of relative enzyme activity
654	15	7	21	2.00	15	29	0.93
655	15	6	17	1.83	18	42	1.33
656	15	7	11	0.57	26	55	1.11
660	15	5	21	3.20	25	50	1.00
667	15	5	14	1.80	10	40	3.00
668	15	9	12	0.33	15	37	1.47
693	15	7	24	2.42	16	28	0.75
707	4				24	32	0.33
711	4				20	40	1.00
719	4				12	45	2.75
720	4				15	25	0.67
722	4				17	30	0.76
723	4				20	24	0.20
728	4				20	34	0.70
737	4				25	40	0.60
749	4				20	37	0.85
750	4				19	46	1.42
763	4				35	No	0
764	4				14	No	0
771	4				35	45	0.28
772	4				47	51	0.08
775	4				38	57	0.50
779	4				12	48	3.00
780	4				15	17	0.13
804	4				31	38	0.22
805	4				15	42	1.80
806	4				32	51	0.59
807	4				15	17	0.13
812	4				16	27	0.69
814	4				15	34	1.27
816	4				36	No	0
821	4				12	28	1.33
822	4				22	No	0
823	4				35	45	0.28
824	4				23	50	1.17
826	4				40	49	0.22
864	4				21	41	0.95
871	4				11	No	0
1017	25				11	40	2.63
1029	25	5	10	1.00	14	49	2.50
1206	15	19	22	0.16	28	41	0.46
1222	15	8	19	1.37	NG		
1278	4				36	46	0.28

Appendix 6
Results from BAST search of DNA Sequences

Table A6.1 Isolate number, Best Identity via BLAST search, Accession number, Score match and match length for wood related fungal isolates.

Fungal isolate number	Best BLAST match	Accession number	Score	% Match	Length Match
182	*Cadophora malorum* (str166)	>gi|30144887|gb|AY249064.1|	1003	99	516/521
242	*Cadophora malorum* (str166)	>gi|30144887|gb|AY249064.1|	906	99	465/468
262	*Pseudeurotium desertorum*	>gi|32394764|gb|AY129288.1|	779	94	475/501
405	*Penicillium roquefortii*	gi|34809409|gb|AY373929.1|	1045	99	547/552
408	*Penicillium roquefortii*	gi|34809409|gb|AY373929.1|	1015	99	514/515
487	*Cladosporium oxysporum*	>gi|13539213|emb|AJ300332.1|COX300332	971	98	515/521
517	*Cadophora malorum* (str166)	>gi|30144887|gb|AY249064.1|	981	98	507/513
660	*Cladosporium oxysporum*	>gi|13539213|emb|AJ300332.1|COX300332	991	99	516/521
656	*Geomyces* sp. C239/10G	gi|33622362|gb|AY345347.1|	959	97	524/540
711	*Geomyces* sp. C239/10G	>gi|33622362|gb|AY345347.1|	936	97	501/512
719	*Cladosporium oxysporum*	>gi|13539213|emb|AJ300332.1|COX300332	989	99	501/502
723	*Penicillium solitum* strain FRR	gi|34809412|gb|AY373932.1|	1092	99	553/554
749	*Geomyces* sp. C239/10G	gi|33622362|gb|AY345347.1|	1015	98	531/540
537	*Penicillium expansum* strain ATCC	gi|34809392|gb|AY373912.1|	1088	100	549/549
763	*Pseudeurotium desertorum* CBS 986	>gi|32394764|gb|AY129288.1|	775	94	473/499
779	*Cladosporium oxysporum*	>gi|13539213|emb|AJ300332.1|COX300332	989	99	515/520
805	*Cladosporium oxysporum*	>gi|13539213|emb|AJ300332.1|COX300332	981	99	500/502
814	*Cladosporium oxysporum*	gi|13539213|emb|AJ300332.1|COX300332	987	99	510/513
824	*Geomyces* sp. C239/10G	>gi|33622362|gb|AY345347.1|	821	94	467/492

Table A6.1 Isolate number, Best Identity via BLAST search, Accession number, Score match and match length for wood related fungal isolates.

Fungal isolate number	Best BLAST match	Accession number	Score	% Match	Length Match
1029	*Penicillium expansum* strain ATCC	>gi|34809392|gb|AY373912.1|	1102	99	558/559
4123	*Pseudeurotium bakeri* CBS 878.71	>gi|32394763|gb|AY129287.1|	728	93	469/501
4148	*Cadophora luteo-olivacea* strain A171	>gi|30144892|gb|AY249069.1|	985	99	501/503
4149	*Pseudeurotium desertorum*	>gi|32394764|gb|AY129288.1|	787	95	476/501
4173	*Penicillium roquefortii*	>gi|34809409|gb|AY373929.1|	1029	98	540/547
4234	*Cadophora malorum* strain A166	>gi|30144887|gb|AY249064.1|	1003	98	512/515
4247	*Cadophora malorum* strain A166	>gi|30144887|gb|AY249064.1|	1011	99	517/518
4249	*Cadophora malorum* strain A166	>gi|30144887|gb|AY249064.1|	1011	99	517/518
5110	*Geomyces* sp. C239/10G	>gi|33622362|gb|AY345347.1|	906	96	507/525
5015	*Geomyces* sp. C239/10G	gi|33622362|gb|AY345347.1|	989	96	507/527
5019	*Pseudeurotium desertorum*	>gi|32394764|gb|AY129288.1|	769	94	472/499
5028	*Cladosporium oxysporium*	gi|13539213|emb|AJ300332.1|COX300332	977	100	493/493
5046	*Pseudeurotium desertorum*	gi|32394764|gb|AY129288.1|	757	94	470/499
5083	*Penicillium* sp.	gi|34809412|gb|AY373932.1|	1017	99	517/519

Appendix 7

Endo-1, 4-β-glucanase accumulation over time at 4 and 15 °C

Graphs of Endo-1, 4-β-glucanase accumulation over time at 4 and 15 °C are given in Appendix 5.

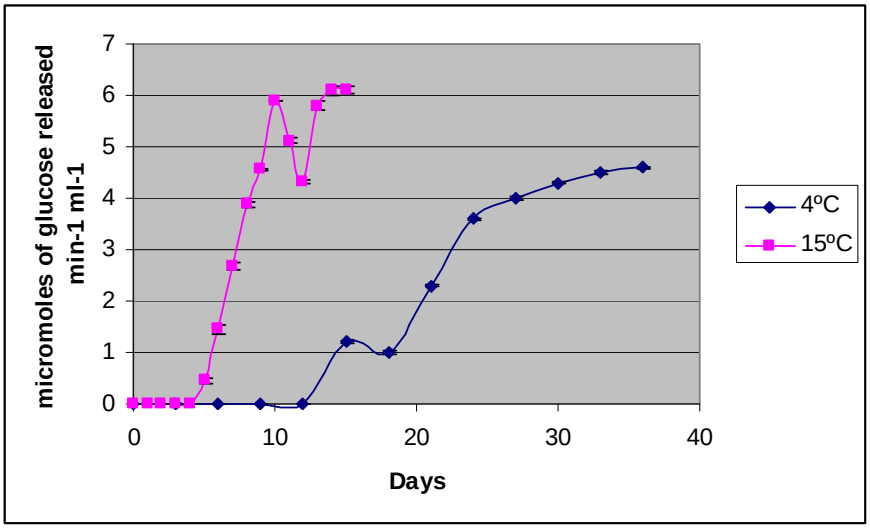

Figure A7.1: Graph of endo-1, 4-β-glucanase accumulation *Cadophora malorum* 80 at 4 and 15ºC. Vertical bars represent the standard error.

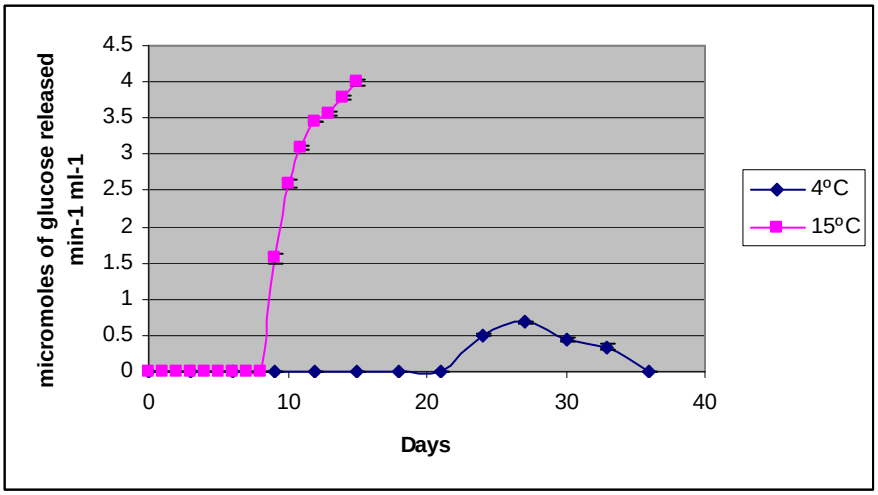

Figure A7.2: Graph of endo-1, 4-β-glucanase accumulation *Gliocladium* sp. 129 at 4 and 15ºC. Vertical bars represent the standard error.

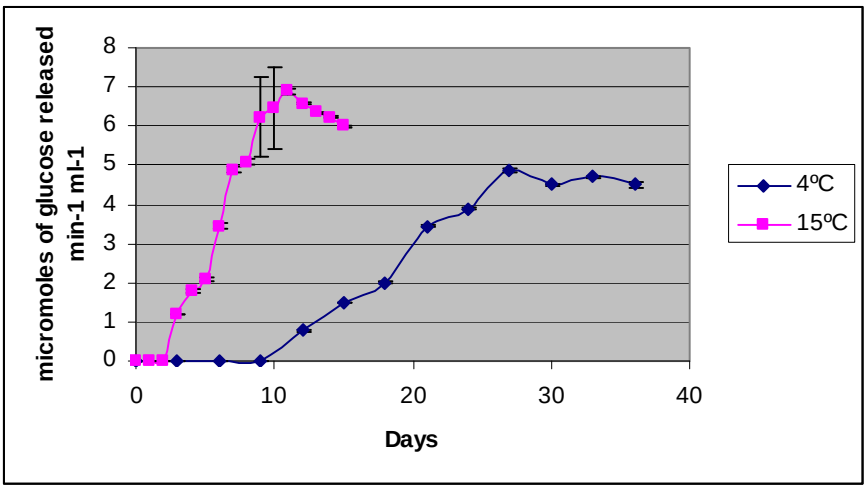

Figure A7.3: Graph of endo-1, 4-β-glucanase accumulation *Cadophora malorum* 182 at 4 and 15ºC. Vertical bars represent the standard error.

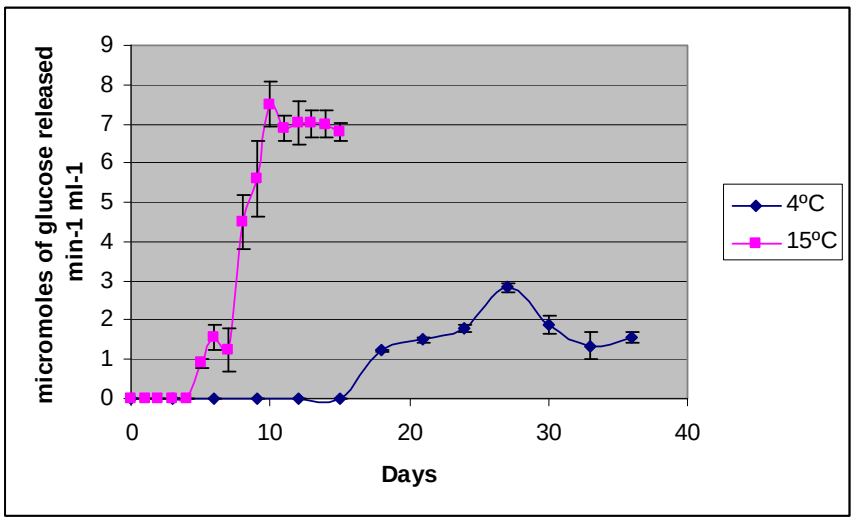

Figure A7.4: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 226 at 4 and 15ºC. Vertical bars represent the standard error.

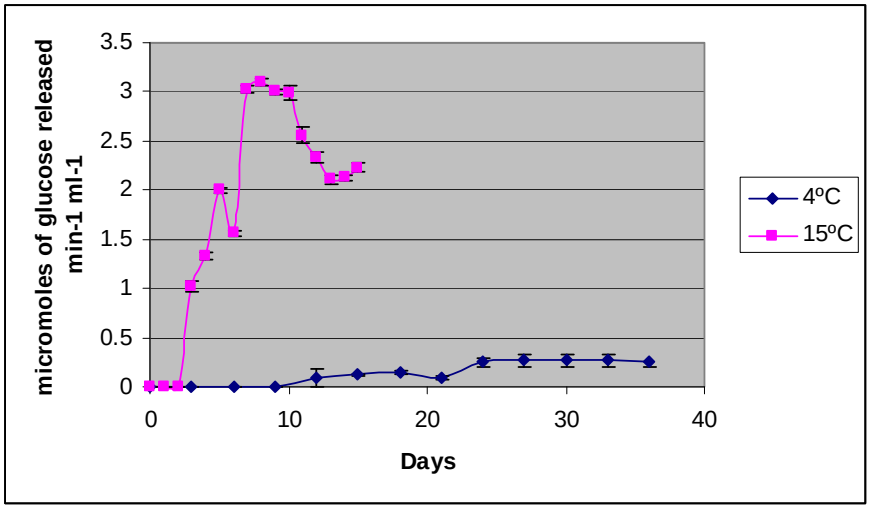

Figure A7.5: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 227 at 4 and 15ºC. Vertical bars represent the standard error.

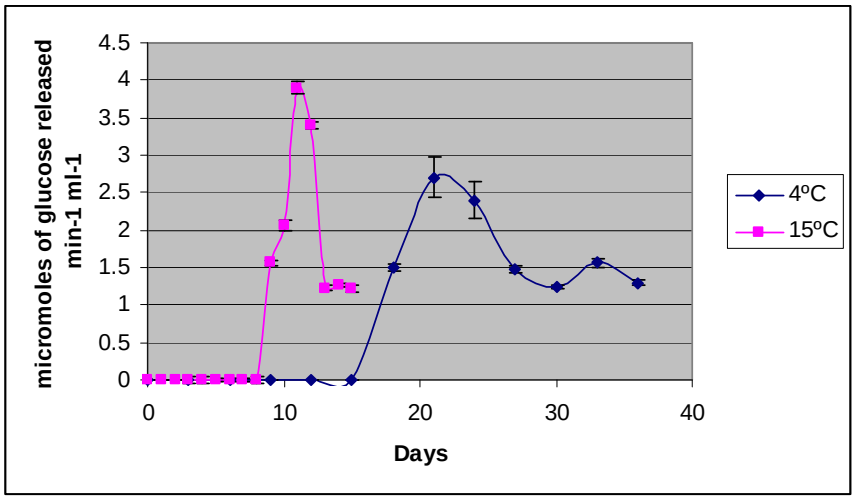

Figure A7.6: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 228 at 4 and 15ºC. Vertical bars represent the standard error.

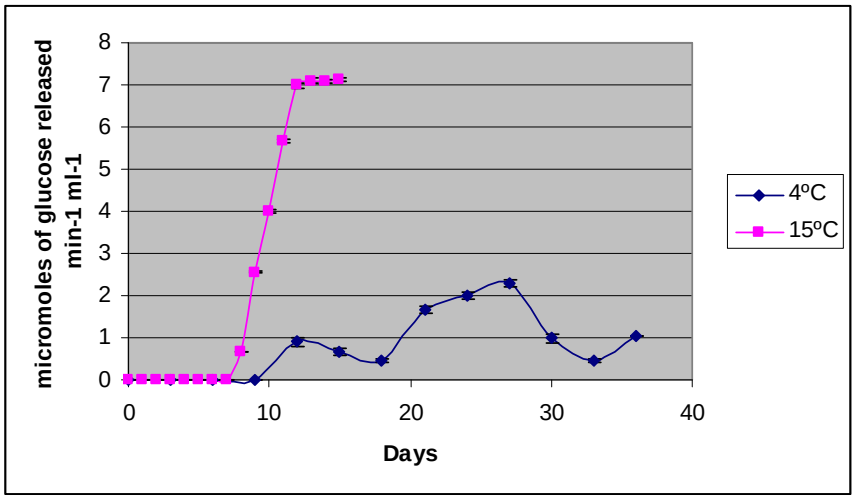

Figure A7.7: Graph of endo-1, 4-β-glucanase accumulation *Cadophora malorum* 242 at 4 and 15ºC. Vertical bars represent the standard error.

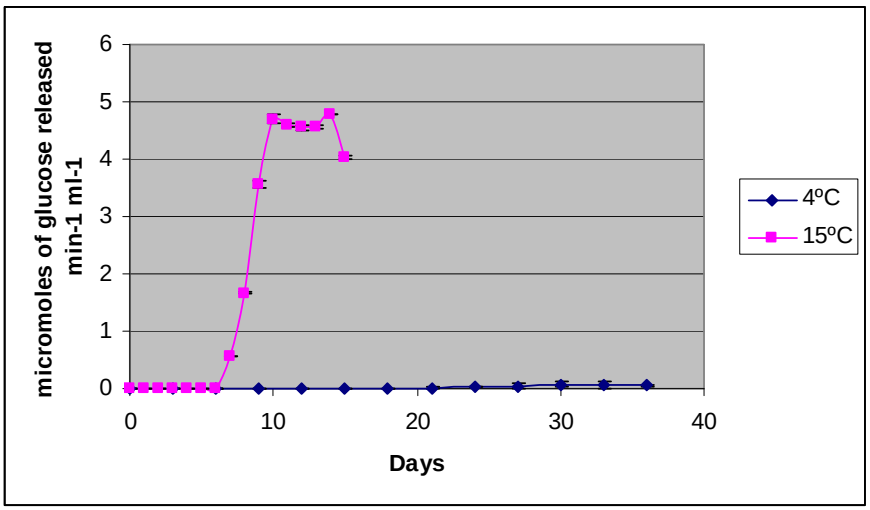

Figure A7.8: Graph of endo-1, 4-β-glucanase accumulation Isolate 262 at 4 and 15ºC. Vertical bars represent the standard error.

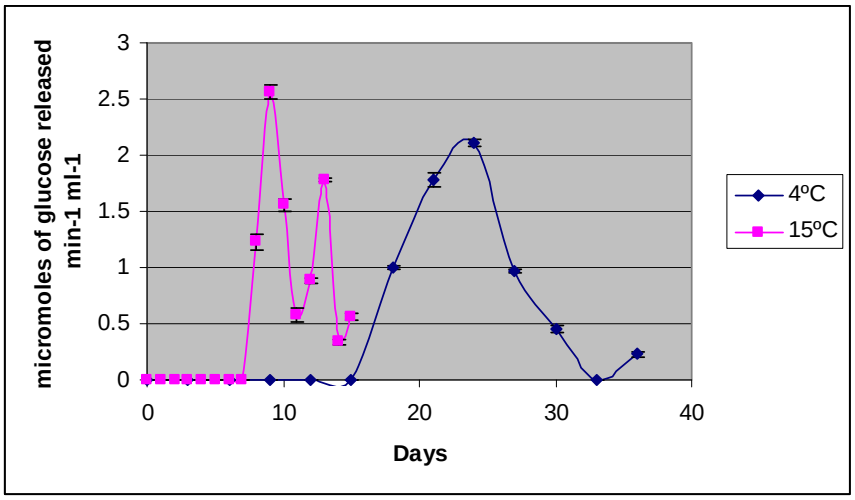

Figure A7.9: Graph of endo-1, 4-β-glucanase accumulation *Cladosporium cladosporoides* 487 at.4 and 15ºC. Vertical bars represent the standard error.

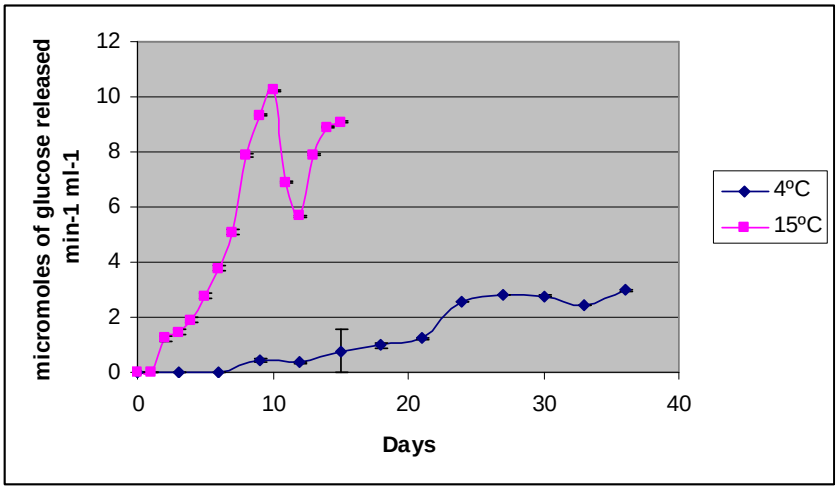

Figure A7.10: Graph of endo-1, 4-β-glucanase accumulation *Penicillium roquefortii* 405 at 4 and 15ºC. Vertical bars represent the standard error.

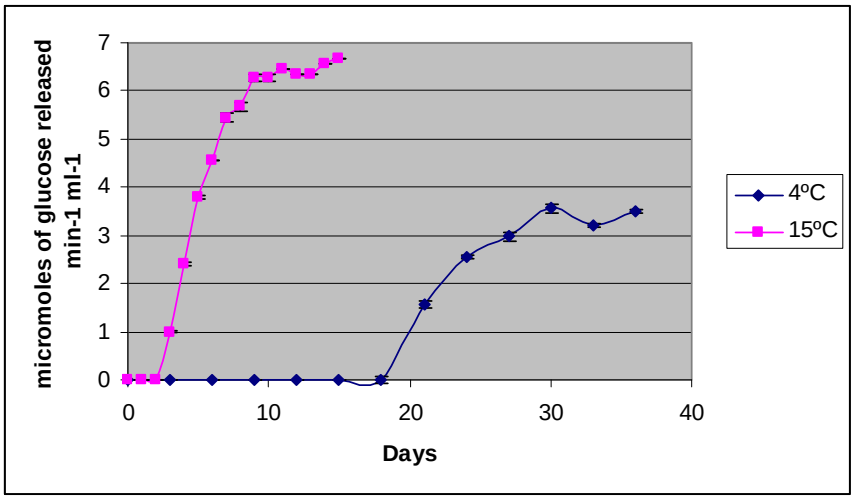

Figure A7.11: Graph of endo-1, 4-β-glucanase accumulation *Penicillium roquefortii* 408 at 4 and 15ºC. Vertical bars represent the standard error.

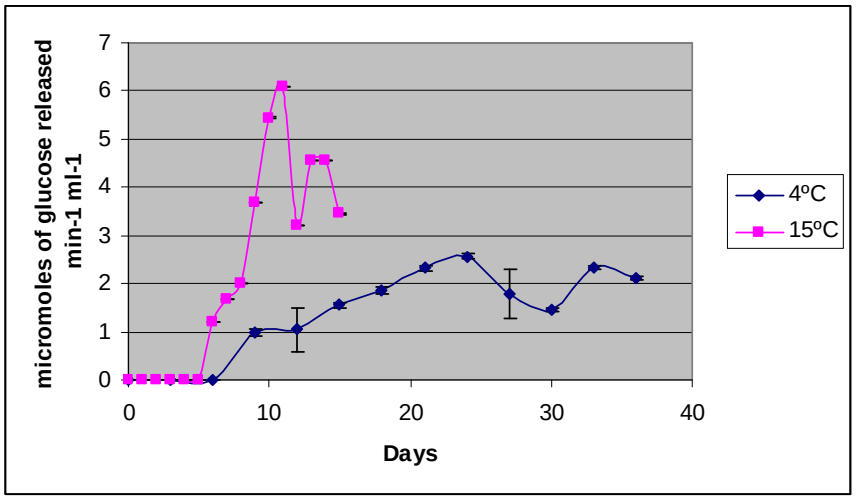

Figure A7.12: Graph of endo-1, 4-β-glucanase accumulation *Cadophora malorum* 517 at 4 and 15ºC. Vertical bars represent the standard error.

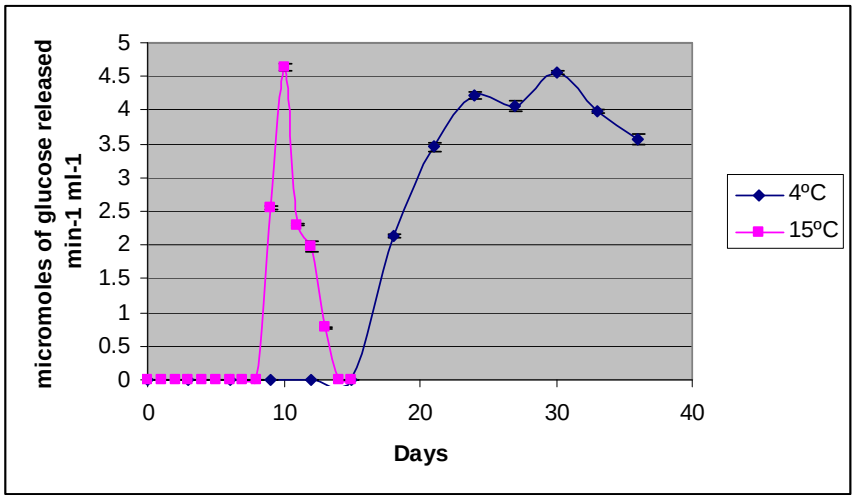

Figure A7.13: Graph of endo-1, 4-β-glucanase accumulation *Penicillium expansum* 537 at 4 and 15ºC. Vertical bars represent the standard error.

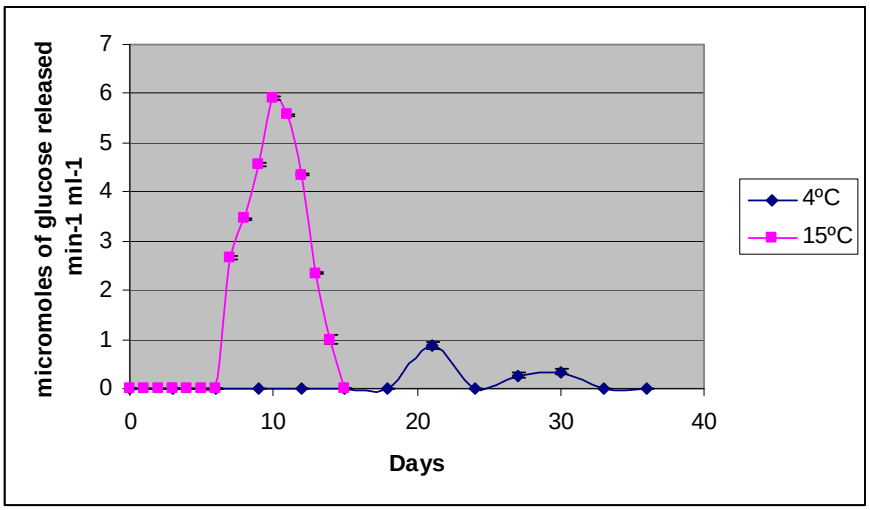

Figure A7.14: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 656 at 4 and 15ºC. Vertical bars represent the standard error.

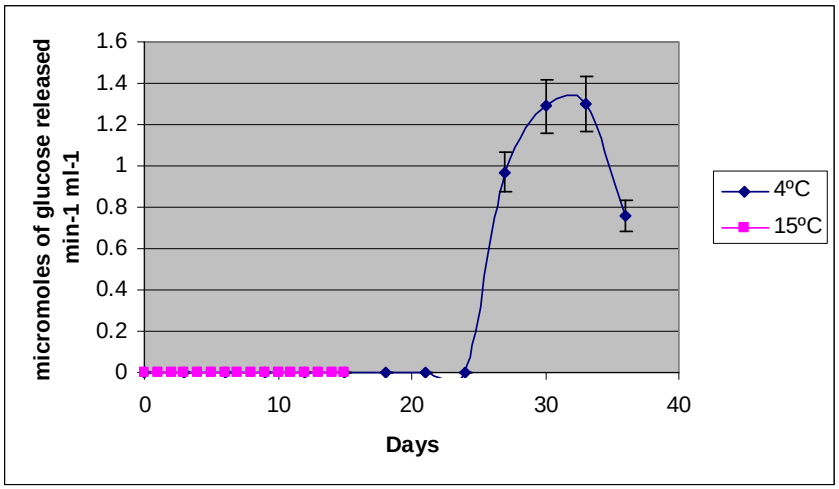

Figure A7.15: Graph of endo-1, 4-β-glucanase accumulation *Cladosporium cladosporoides* 660 *Geomyces* sp. 226 at 4 and 15ºC. Vertical bars represent the standard error.

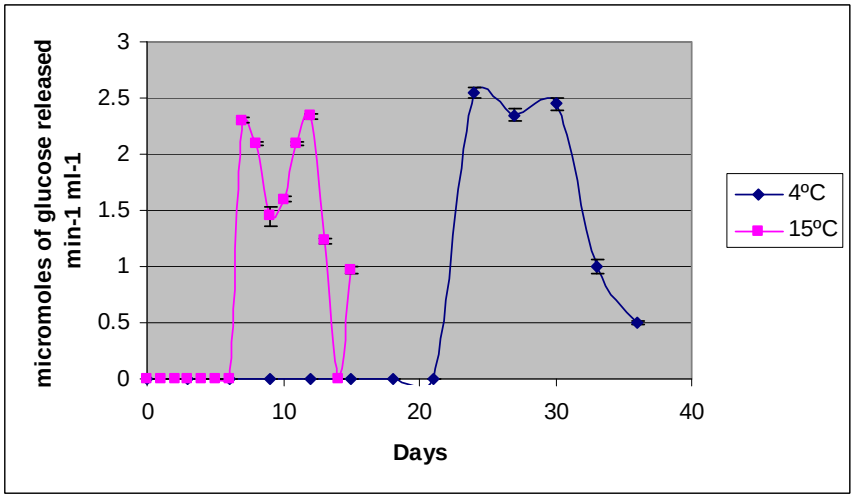

Figure A7.16: Graph of endo-1, 4-β-glucanase accumulation *Cladosporium cladosporoides* 667 at 4 and 15ºC. Vertical bars represent the standard error.

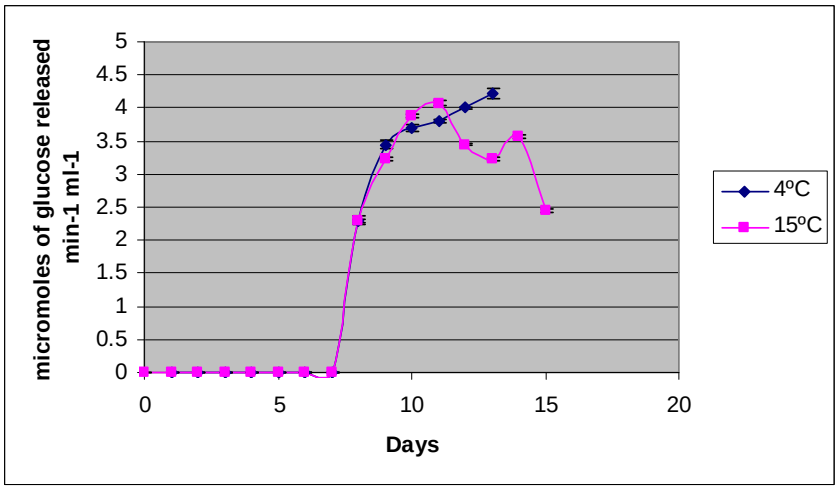

Figure A7.17: Graph of endo-1, 4-β-glucanase accumulation *Cadophora malorum* 668 at 4 and 15ºC. Vertical bars represent the standard error.

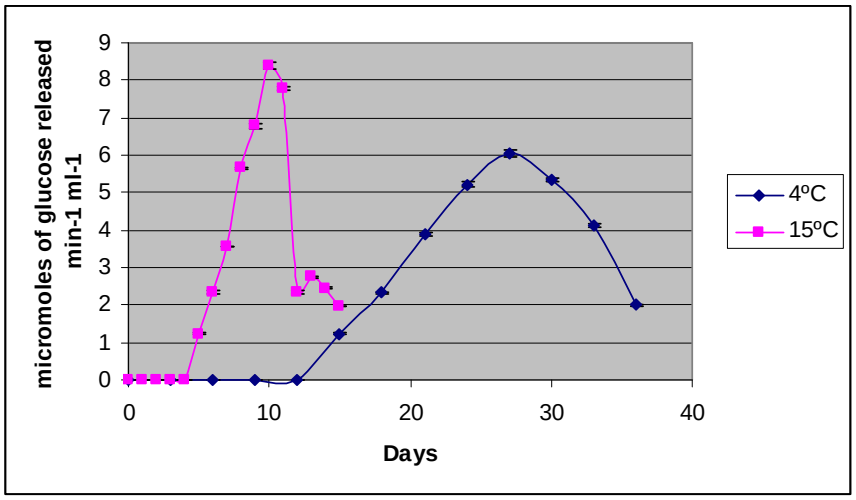

Figure A7.18: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 711 at 4 and 15ºC. Vertical bars represent the standard error.

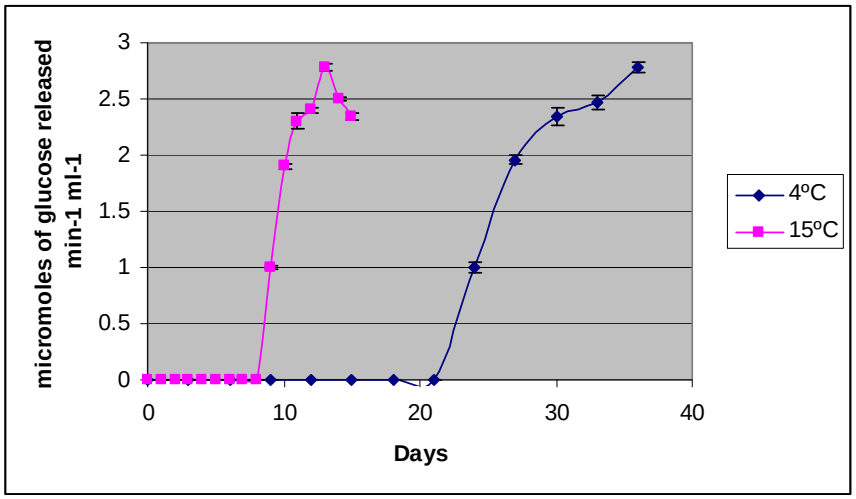

Figure A7.19: Graph of endo-1, 4-β-glucanase accumulation *Cladosporium cladosporoides* 719 at 4 and 15ºC. Vertical bars represent the standard error.

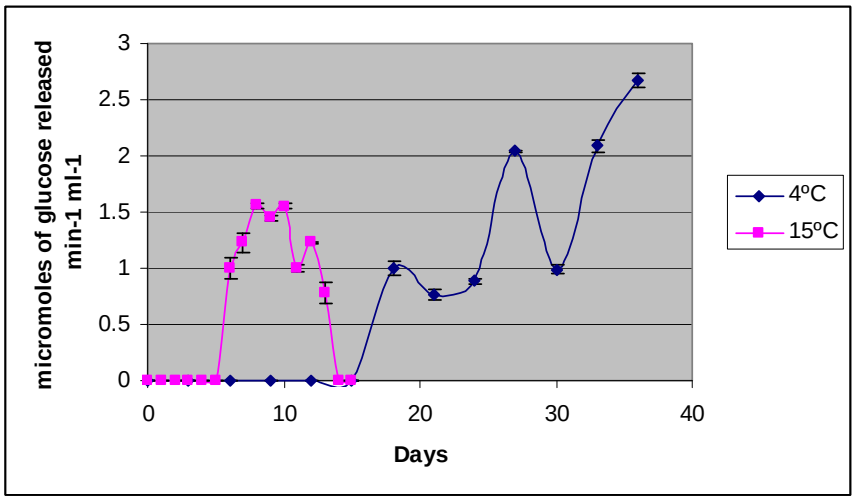

Figure A7.20: Graph of endo-1, 4-β-glucanase accumulation *Penicillium* sp. 723 at 4 and 15ºC. Vertical bars represent the standard error.

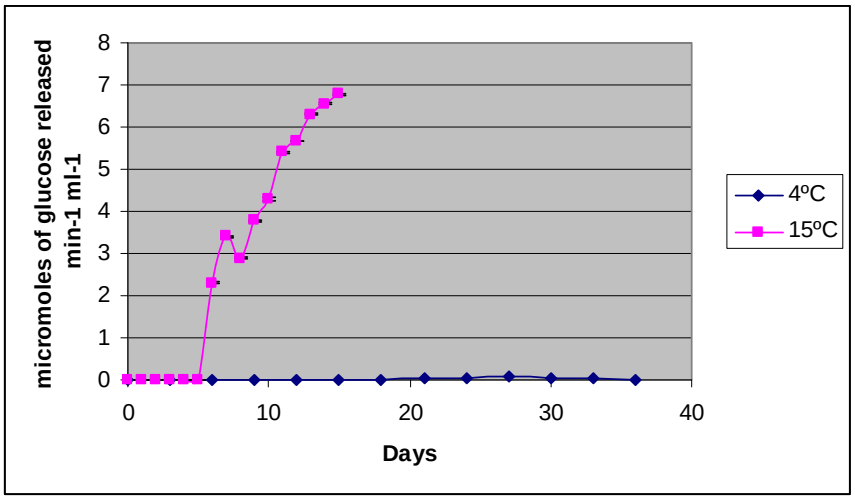

Figure A7.21: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 749 at 4 and 15ºC. Vertical bars represent the standard error.

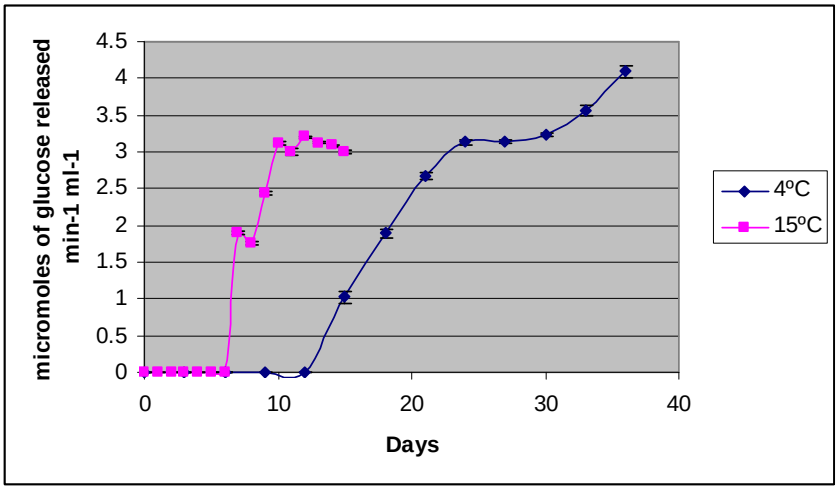

Figure A7.22: Graph of endo-1, 4-β-glucanase accumulation Isolate 763 at 4 and 15ºC. Vertical bars represent the standard error.

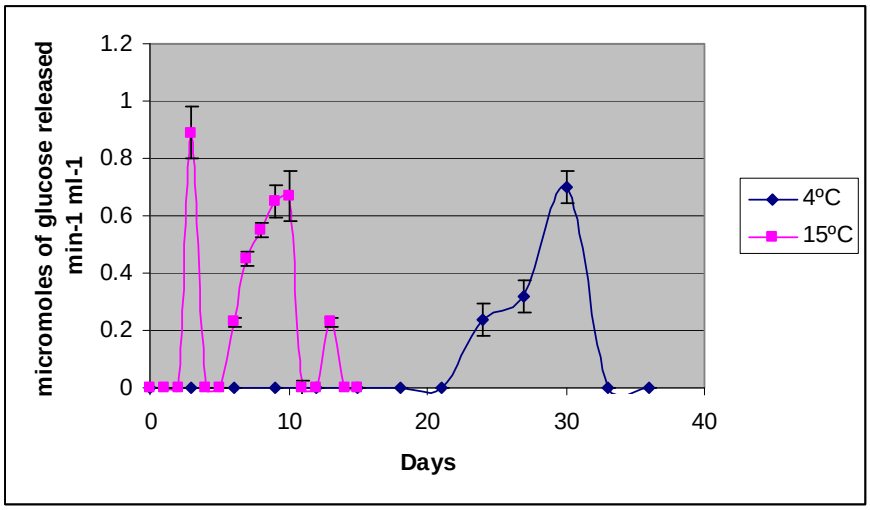

Figure A7.23: Graph of endo-1, 4-β-glucanase accumulation *Cladosporium* sp. 805 at 4 and 15ºC. Vertical bars represent the standard error.

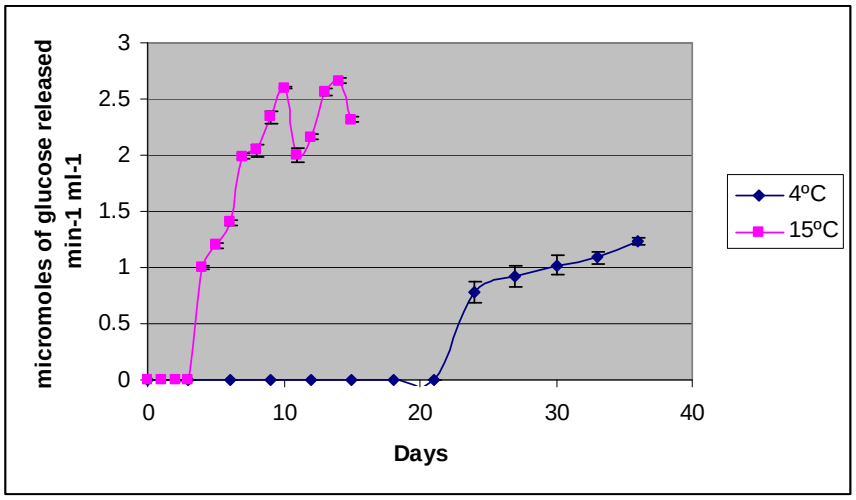

Figure A7.24: Graph of endo-1, 4-β-glucanase accumulation Cladosporium sp. 812 at 4 and 15ºC. Vertical bars represent the standard error.

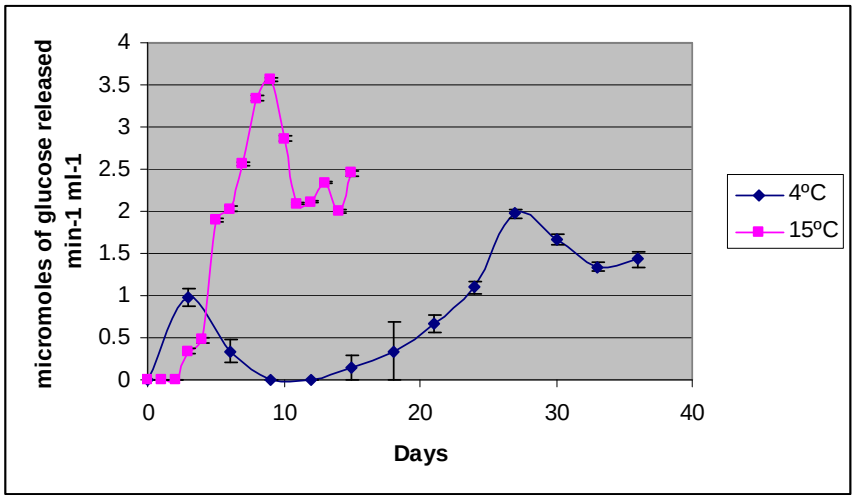

Figure A7.25: Graph of endo-1, 4-β-glucanase accumulation Unidentified 816 at 4 and 15ºC. Vertical bars represent the standard error.

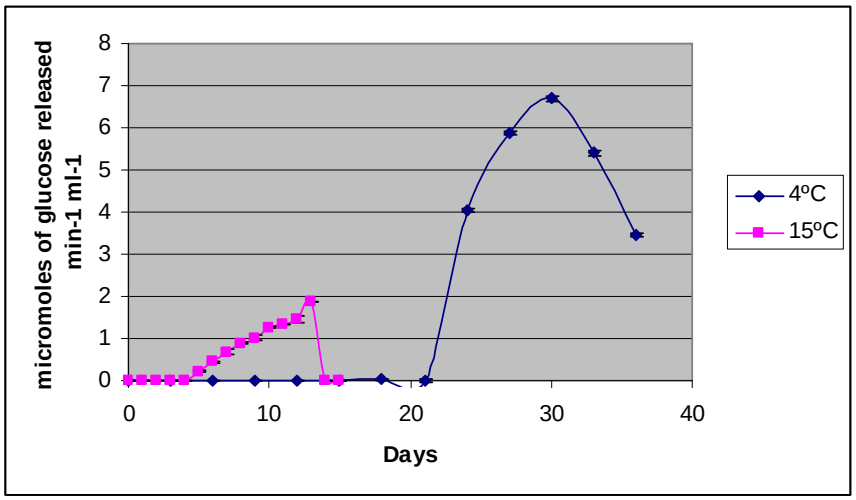

Figure A7.26: Graph of endo-1, 4-β-glucanase accumulation *Geomyces* sp. 824. at 4 and 15ºC. Vertical bars represent the standard error.

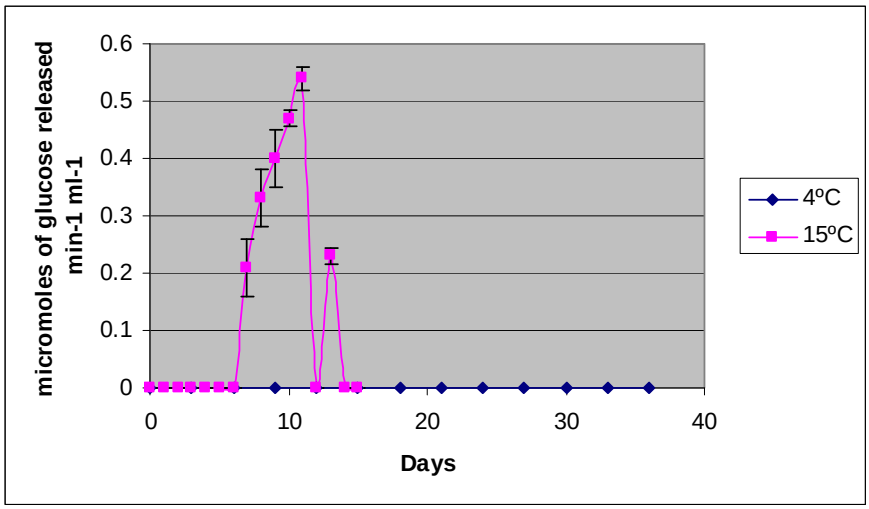

Figure A7.27: Graph of endo-1, 4-β-glucanase accumulation *Penicillium expasium* 1029 at 4 and 15ºC. Vertical bars represent the standard error.

Appendix 8

Results from protein sequencing and identification.

Table A8.1 Protein bands, and potential identity of peptide sequence for the less abundant proteins but the best matches with in the database.

Band identified in excised gel	**Potential identity of protein using LC-MS/MS with the best sequence matches from the MASCOT protein database search.**
WF 1	gi|1857716 catalase [*Aspergillus fumigatus*] gi|111068784 hypothetical protein SNOG_03173 [*Phaeosphaeria nodorum* SN15] gi|46114420 hypothetical protein FG03052.1 [*Gibberella zeae* PH-1]
WF2	gi|83771876 unnamed protein product [*Aspergillus oryzae*] gi|115437930 conserved hypothetical protein [*Aspergillus terreus* NIH2624] gi|111055949 hypothetical protein SNOG_15694 [*Phaeosphaeria nodorum* SN15] gi|50285689 unnamed protein product [*Candida glabrata*] gi|115389440 beta-glucosidase 1 precursor [*Aspergillus terreus* NIH2624] gi|46114420 hypothetical protein FG03052.1 [*Gibberella zeae* PH-1]
WF3	gi|50285689 unnamed protein product [*Candida glabrata*] gi|115389440 beta-glucosidase 1 precursor [*Aspergillus terreus* NIH2624]
WF9	No significant hits

Table A8.2: Protein band WF1, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF1	VGTVQLGPVLAGPGR	tr!Q4PEG0_USTMA Hypothetical protein [UM01503.1] [tr!Q0ZUL0_THEAU Thermostable beta-glucosidase [bgl1] [*Thermoascus aurantiacus*] tr!Q8TGI8_TALEM Beta-glucosidase [*Talaromyces emersonii*]
WF1	YLYDGLK ELSDAANLGVGDLK	tr!Q0UPC1_PHANO Hypothetical protein [SNOG_06393] [*Phaeosphaeria nodorum* SN15] tr!Q2TXM6_ASPOR Glycolate oxidase [AO090010000085] [*Aspergillus oeyzea*] tr!Q2KES4_MAGGR Hypothetical protein [MGG_ch7g962] [*Magnaporthe grisea* 70-15] tr!Q5AXC5_EMENI Hypothetical protein [AN7055.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q2UBB7_ASPOR Predicted protein [AO090102000034] [*Aspergillus oryzae*] sp!P40355!YJ31_YEAST Hypothetical 108.4 kDa protein in CBF1-NTA1 intergenic region [YJR061W] *[Saccharomyces cerevisiae* (Baker's yeast)] sp!Q12248!DAD1_YEAST DASH complex subunit DAD1 (Outer kinetochore protein DAD1) (DUO1 and DAM1-interacting protein 1) [DAD1] [*Saccharomyces cerevisiae* (Baker's yeast)] sp!P27895!CIN8_YEAST Kinesin-like protein CIN8 (Chromosome instability protein 8) [CIN8] [*Saccharomyces cerevisiae* (Baker's yeast)] tr!Q6BU39_DEBHA Similar to sp|Q12496 *Saccharomyces cerevisiae* YOL098c [DEHA0C14872g] [*Debaryomyces hansenii* (Yeast) (*Torulaspora hansenii*)]

Table A8.2: Protein band WF1, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF1	TPFTGEG TTR EGVSETL GPLSGLR LMVLDD GEFNK DFVSQLT LLEK YNANVA GEEGLAR FSMYANC YGTEGVA K	tr!Q0UPC1_PHANO Hypothetical protein [SNOG_06393] [*Phaeosphaeria nodorum* SN15] tr!Q5EMW3_MAGGR Beta-glucosidase-like protein [*Magnaporthe griseea* (Rice Blast fungus (Pyricularia grisea)] tr!Q5AXC5_EMENI Hypothetical protein [AN7055.2] [*Emericella nidulans* (*Asperfillus nidulans*)] tr!Q4I853_GIBZE Hypothetical protein [FG06605.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q00025_AJECA H antigen precursor [*Ajellomyces capsulata* (*Histoplasma capsulaum*)] tr!Q4I3W9_GIBZE Hypothetical protein [FG08089.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q2TXM6_ASPOR Glycolate oxidase [AO090010000085] [*Aspergillus oryzae*] tr!Q7RWP2_NEUCR Hypothetical protein NCU08755.1 [NCU08755.1] [*Neuropsora crassa*] tr!Q9Y857_KLULA Cytochrome b2 precursor (EC 1.1.2.3) [cyb2] [*Kluyveromyces lactis* (Yeast) (*Candida sphaerica*) tr!Q6CSA3_KLULA Similarity [KLLA0D02640g] [*Kluyveromyces lactis* (Yeast) (*Candida sphaerica*)
WF1	ASAGDAG LLACAK LAETTVD SAGDGDR LPSMGND LR DFVSQLT LLEK	tr!Q00025_AJECA H antigen precursor [*Ajellomyces capsulata* (*Histoplasma capsulatum*)] tr!Q5EMW3_MAGGR Beta-glucosidase-like protein [*Magnaporthe griseea* (Rice Blast fungus (*Pyricularia grisea*)] tr!Q4I853_GIBZE Hypothetical protein [FG06605.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q2H8N3_CHAGB Hypothetical protein [CHGG_03421] [*Chaetomium globosum* CBS 148.51] tr!Q1E0M5_COCIM Hypothetical protein [CIMG_03888] [*Coccidioides immitis* RS] tr!Q9UVC8_COCIM Beta-glucosidase precursor (EC 3.2.1.21) [bgl2] [*Coccidioides immitis*] tr!Q7RWP2_NEUCR Hypothetical protein NCU08755.1 [NCU08755.1] [*Neuropsora crassa*] tr!Q0U8N3_PHANO Predicted protein [SNOG_11881] [*Phaeosphaeria nodorum* SN15] tr!Q9P879_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria*] tr!Q68KY2_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f. sp. *triticae*] tr!Q68KY1_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria*] tr!Q68KY0_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria sp.* S-93-48] tr!Q68KX9_PHANO Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)]

Table A8.2: Protein band WF1, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF1	ASAGDAGLLA CAK LAETTVDSAG DGDR LPSMGNDLR DFVSQLTLLEK	tr!Q68KX7_PHANO Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (Septoria nodorum)] tr!Q68KX6_PHANO Beta-glucosidase [bgl1] [Phaeosphaeria nodorum (*Septoria nodorum*)] tr!Q68HW6_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f. sp. *triticae*] tr!Q68HW5_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f. sp. *triticae*] tr!Q68HW4_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f. sp. *triticae*] tr!Q68HW1_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f. sp. *triticae*] tr!Q4HUU0_GIBZE Hypothetical protein [FG11268.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q2HAQ4_CHAGB Predicted protein [CHGG_02700] [*Chaetomium globosum* CBS 148.51] tr!Q2GUQ9_CHAGB Predicted protein [CHGG_08295] [*Chaetomium globosum* CBS 148.51] tr!Q0ZUL0_THEAU Thermostable beta-glucosidase [bgl1] [*Thermoascus ayrantiacus*] tr!Q6FQ79_CANGA Similarity [CAGL0I08437g] [*Candida glabrata* (Yeast (*Torulopsis glabrata*)] tr!Q59QW5_CANAL Potential transcriptional regulator of filamentous growth (Transcription regulatory factor FLO8p) [CaO19.1093] [*Candida albicans* (Yeast)] tr!Q0CUC1_ASPTE Hypothetical protein [ATEG_02713] [*Aspergillus terreus* NIH2624] tr!Q8SUP0_ENCCU Hypothetical protein ECU08_1170 [ECU08_1170] [*Encephalitozoon cuniculi*] tr!Q7Z9M5_TRIRE Cel3b [cel3b] [*Trichoderma reesei* (*Hypocrea jecorina*)] tr!Q6CXI0_KLULA Similarities with sp|P47114 *Saccharomyces cerevisiae* YJR054w [KLLA0A08063g] [Kluyveromyces lactis (Yeast) (*Candida sphaerica*)] tr!Q5KIF3_CRYNE Expressed protein [CND03170] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q55TY6_CRYNE Hypothetical protein [CNBD3180] [*Cryptococcus neoformans* var. *neoformans* B-3501A]

Table A8.3: Protein band WF2, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF2	FTVLVGSSSEDLR	tr!Q0CAH0_ASPTE Hypothetical protein [ATEG_09314] [*Aspergillus terreus* NIH2624] tr!Q2U8V9_ASPOR Beta-glucosidase-related glycosidases [AO090701000274] [*Aspergillus oryzae*] tr!Q5BB63_EMENI Hypothetical protein [AN2217.2] [Emericella nidulans (*Aspergillus nidulans*)] tr!Q4WU38_ASPFU Beta-glucosidase [Afu5g07080] [*Aspergillus fumigatus* (*Sartorya fumigate*)] tr!Q8J261_TALEM Avenacinase [aven] [*Talaromyces emersonii*] tr!Q4I2E9_GIBZE Hypothetical protein [FG08609.1] [*Gibberella zeae* (*Fusarium graminearum*)]
WF2	WSNDGASACSSAGDVAAK	tr!Q2HCI2_CHAGB Predicted protein [CHGG_02072] [*Chaetomium globosum* CBS 148.51]
WF2	WAEVLAVTDRLSVSMPR	tr!Q7S3V1_NEUCR Predicted protein [NCU04916.1] [*Neurospora crassa*] tr!Q5KC19_CRYNE Alpha-1,2-mannosidase, putative [CNI00600] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q55NN3_CRYNE Hypothetical protein [CNBH0560] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q0U575_PHANO Hypothetical protein [SNOG_13089] [*Phaeosphaeria nodorum* SN15]
WF2	DDLASVVVNPRGVTPLDGLK	tr!Q0CE57_ASPTE Hypothetical protein [ATEG_08027] [*Aspergillus terreus* NIH2624] tr!Q2H3E0_CHAGB Hypothetical protein [CHGG_03706] [*Chaetomium globosum* CBS 148.51] tr!Q5BB63_EMENI Hypothetical protein [AN2217.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q4WU38_ASPFU Beta-glucosidase [Afu5g07080] [*Aspergillus fumigatus* (*Sartorya fumigate*)] tr!Q2U8V9_ASPOR Beta-glucosidase-related glycosidases AO090701000274] [*Aspergillus oryzae*] tr!Q0CAH0_ASPTE Hypothetical protein [ATEG_09314] [*Aspergillus terreus* NIH2624] tr!Q59TL5_CANAL Hypothetical protein [CaO19.1320] [*Candida albicans* (Yeast)]
WF2	LGAYYEAPESR AVAGDNAQVNGVAGAAANPR EVDLGLWNTR	tr!Q5KJL7_CRYNE Expressed protein [CNC06090] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q55WM8_CRYNE Hypothetical protein [CNBC1110] [*Cryptococcus neoformans* var. *Neoformans* B-3501A]
WF2	VTNLVPGANK	No hits

Table A8.4: Protein band WF3, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF3	VTVDLLYGR	No hits
WF3	DPYAMQSTL LDKVGDVQ LGPVAGAAP R	tr!Q0ZUL0_THEAU Thermostable beta-glucosidase [bgl1] [*Thermoascus aurantacus*] tr!Q8TGI8_TALEM Beta-glucosidase [*Talaromyces emersonii*] tr!Q5B9F2_EMENI Hypothetical protein [AN2828.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q0U8N3_PHANO Predicted protein [SNOG_11881] [*Phaeosphaeria nodorum* SN15] tr!Q68KX7_PHANO Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)] tr!Q68KX6_PHANO Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)] tr!Q7SBY8_NEUCR Predicted protein [NCU08420.1] [*Neurospora crassa*] tr!Q4WVB5_ASPFU Hypothetical protein [Afu5g11510] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!Q9P8F4_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgl1] [*Aspergillus niger*] tr!Q0PHW1_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgln] [*Aspergillus niger*] tr!Q30BH9_ASPNG Beta-glucosidase (EC 3.2.1.21) [bgl1] [*Aspergillus niger*] tr!P87076_ASPKA Beta-D-glucosidase [bglA] [*Aspergillus kawachi* (*Aspergillius awamori* var. k*awachi*)] sp!P48825!BGL1_ASPAC Beta-glucosidase 1 precursor (EC 3.2.1.21) (Gentiobiase) (Cellobiase) (Beta-D-glucoside glucohydrolase) [*Aspergillus aculeatus*] tr!Q9P879_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria*] tr!Q68KY2_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *triticae*] tr!Q68KY1_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *triticae*] tr!Q68KY0_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria* sp. S-93-48] tr!Q68KX9_PHANO Beta-glucosidase [bgl1] [*Phaeosphaeria nodorum* (*Septoria nodorum*)] tr!Q68HW6_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *triticae*] tr!Q68HW5_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *triticae*] tr!Q68HW4_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *avenaria*] tr!Q68HW1_9PLEO Beta-glucosidase [bgl1] [*Phaeosphaeria avenaria* f.sp. *avenaria*] tr!Q5B5S8_EMENI Hypothetical protein [AN4102.2] [*Emericella nidulans* (*Asperigillius nidulans*) tr!Q58IJ4_9EURO Extracellular beta-glucosidase [bgl1] [*Aspergillus avenaceus*] tr!Q4WGT3_ASPFU Beta-D-glucoside glucohydrolase [Afu7g06140] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!Q2UUD6_ASPOR Beta-glucosidase-related glycosidases [AO090009000356] [*Aspergillus oryzae*] tr!Q0CTD7_ASPTE Beta-glucosidase 1 [ATEG_03047] [*Aspergillus terreus* NIH2624] tr!Q0CFW5_ASPTE Hypothetical protein [ATEG_07419] [*Aspergillus terreus* NIH2624]

Table A8.4: Protein band WF1, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences.**
WF3	MVMSDLK VDLLASFLS LAREGTGQS TATQLPMV DSLR WGMCMQDS PLRK	tr!Q6C9C3_YARLI Similarity [YALI0D12342g] [*Yarrowia lipolytica* (*Candida lipolytica*)] tr!Q7S5P2_NEUCR Predicted protein [NCU05801.1] [*Neurospora crassa*] sp!Q12389!DBP10_YEAST ATP-dependent RNA helicase DBP10 (EC 3.6.1.-) (DEAD box protein 10) [DBP10] [*Saccharomyces cerevisiae* (Baker's yeast)] sp!Q4HZ42!DBP10_GIBZE ATP-dependent RNA helicase DBP10 (EC 3.6.1.-) [DBP10] [*Gibberellazeae* (*Fusarium graminearum*)] tr!Q4P5A8_USTMA Hypothetical protein [UM04705.1] [*Ustilago maydis* (Smut fungus)] sp!Q6CIR0!DBP10_KLULA ATP-dependent RNA helicase DBP10 (EC 3.6.1.-) [DBP10] [*Kluyveromyces lactis* (Yeast) (*Candida sphaerica*)] sp!Q6FNA2!DBP10_CANGA ATP-dependent RNA helicase DBP10 (EC 3.6.1.-) [DBP10] [*Candida glabrata* (Yeast) (*Torulopsis glabrata*)] sp!P48825!BGL1_ASPAC Beta-glucosidase 1 precursor (EC 3.2.1.21) (Gentiobiase) (Cellobiase) (Beta-D-glucoside glucohydrolase) [*Aspergillus aculeatus*] tr!Q9P8F4_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgl1] [*Aspergillus niger*] tr!Q0PHW1_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgln] [*Aspergillus niger*] tr!Q8TGI8_TALEM Beta-glucosidase [*Talaromyces emersonii*] tr!Q4WZB7_ASPFU Hypothetical protein [Afu2g17510] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!P87076_ASPKA Beta-D-glucosidase [bglA] [Aspergillus kawachi (*Aspergillus awamori* var. *kawachi*)] tr!Q0UB15_PHANO Predicted protein [SNOG_11049] [*Phaeosphaeria nodorum* SN15] sp!Q8NJM2!DBP10_ASPFU ATP-dependent RNA helicase dbp10 (EC 3.6.1.-) [dbp10] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!Q5B7Y1_EMENI Hypothetical protein [AN3349.2] [*Emericella nidulans* (*Aspergillus nnidulans*)] tr!Q7RWP2_NEUCR Hypothetical protein NCU08755.1 [NCU08755.1] [*Neurospora crassa*] tr!Q5AZ11_EMENI Hypothetical protein [AN6469.2] [Emericella nidulans (*Aspergillus nidulans*)] tr!Q4PBU0_USTMA Predicted protein [UM02423.1] [*Ustilago maydis* (Smut fungus)] tr!Q0CIF1_ASPTE Predicted protein [ATEG_06533] [*Aspergillus terreus* NIH2624]
WF3	TSAGDAGVL ACAK	tr!Q0ZUL0_THEAU Thermostable beta-glucosidase [bgl1] [*Thermoascus aurantiacus*] tr!Q8TGI8_TALEM Beta-glucosidase [*Talaromyces emersonii*]

Table A8.4: Protein band WF3, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences.**
WF3	LGAYYEAPESR EVSGALGSDST VLLK HDYVSEDWGK	tr!Q4IG28_GIBZE Hypothetical protein [FG03830.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q4ICV5_GIBZE Hypothetical protein [FG04953.1] [*Gibberella zeae* (*Fusarium graminearum*)] sp!P46958!IDS2_YEAST IME2-dependent signaling protein [IDS2]][*Saccharomyces cerevisiae* (Baker's yeast)] tr!Q873J5_NEUCR Probable ATP dependent RNA helicase (Hypothetical protein NCU03681.1) [68B2.060] [*Neurospora crassa*] sp!P48825!BGL1_ASPAC Beta-glucosidase 1 precursor (EC 3.2.1.21) (Gentiobiase) (Cellobiase) (Beta-D-glucoside glucohydrolase) [*Aspergillus aculeatus*] tr!Q0PHW1_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgln] [*Aspergillus niger*] tr!Q9P8F4_ASPNG Beta-glucosidase precursor (EC 3.2.1.21) [bgl1] [*Aspergillus niger*] tr!Q4P4L9_USTMA Hypothetical protein [UM04944.1] [*Ustilago maydis* (Smut fungus)] tr!Q30BH9_ASPNG Beta-glucosidase (EC 3.2.1.21) [bgl1] [*Aspergillus niger*] tr!P87076_ASPKA Beta-D-glucosidase [bglA] [*Aspergillus kawachi* (*Aspergillius awamori* var. k*awachi*)] tr!Q2HCI2_CHAGB Predicted protein [CHGG_02072] [*Chaetomium globosum* CBS 148.51]

Table A8.5: Protein bands WF9, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences.**
WF9	TESAPMTGGVD R QLLPGASSSHR MYYDGENTVR QPPALPFHSNVS MPAGYK QLDGAHSKPGS QDGMDGNSK	tr!Q86Z93_GIBMO Kinesin [KLP7] [*Gibberella moniliformis* (*Fusarium verticillioides*)] tr!Q2HCZ9_CHAGB Hypothetical protein [CHGG_01905] [*Chaetomium globosum* CBS 148.51] sp!P36011!STUA_EMENI Cell pattern formation-associated protein stuA [stuA] [*Emericella nidulans* (Aspergillus nidulans)] tr!Q0UUI9_PHANO Hypothetical protein [SNOG_04575] [*Phaeosphaeria nodorum* SN15] tr!Q755Z6_ASHGO AER371Cp [AER371C] [*Ashbya gossypii* (Yeast) (*Eremothecium gossypii*)] tr!Q5B0U4_EMENI STUA_EMENI CELL PATTERN FORMATION-ASSOCIATED PROTEIN [AN5836.2 [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q2GX19_CHAGB Predicted protein [CHGG_07485] [*Chaetomium globosum* CBS 148.51] tr!Q4P538_USTMA Hypothetical protein [UM04775.1] [*Ustilago maydis* (Smut fungus)] tr!Q0UQT3_PHANO Predicted protein [SNOG_05881] [*Phaeosphaeria nodorum* SN15] tr!Q0CXW3_ASPTE Hypothetical protein [ATEG_01471] [*Aspergillus terreus* NIH2624]

Table A8.5: Protein band WF9, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences.**
WF9	TESAPMTGGVDR QLLPGASSSHR MYYDGENTVR QPPALPFHSNVS MPAGYK QLDGAHSKPGS QDGMDGNSK	tr!Q0V0Q3_PHANO Hypothetical protein [SNOG_02411] [*Phaeosphaeria nodorum* SN15] tr!Q5B0E2_EMENI Hypothetical protein [AN5988.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q2H9N9_CHAGB Hypothetical protein [CHGG_03065] [*Chaetomium globosum* CBS 148.51] tr!Q1ZYK3_ASPNG Hypothetical protein [An12g05200] [*Aspergillus niger*] sp!Q0U6X2!MAK5_PHANO ATP-dependent RNA helicase MAK5 (EC 3.6.1.-) [MAK5] [*Phaeosphaeria nodorum* (*Septoria nodorum*)] tr!Q9P6X0_NEUCR Hypothetical protein 68B2.060 (Hypothetical protein NCU03681.1) [68B2.060] [*Neurospora crassa*] tr!Q0ULL5_PHANO Hypothetical protein [SNOG_07349] [*Phaeosphaeria nodorum* SN15] tr!Q4IJN1_GIBZE Hypothetical protein [FG02577.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q0TYV8_PHANO Hypothetical protein [SNOG_15385] [*Phaeosphaeria nodorum* SN15] sp!P33400!PACC_YEAST pH-response transcription factor pacC/RIM101 (pH-response regulator protein RIM101) (Regulator of IME2 protein 1) [RIM101][*Saccharomyces cerevisiae* (Baker's yeast)] tr!Q5KK10_CRYNE Hypothetical protein [CNC04670] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q5A7G7_CANAL Hypothetical protein [CaO19.5070] [*Candida albicans* (Yeast)] tr!Q5A792_CANAL Hypothetical protein [CaO19.12536] [*Candida albicans* (Yeast)] tr!Q55W86_CRYNE Hypothetical protein [CNBC2510] [*Cryptococcus neoformans* var. *neoformans* B-3501A] tr!Q4INA4_GIBZE Hypothetical protein [FG01304.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q4I691_GIBZE Hypothetical protein [FG07267.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q0TXW1_PHANO Predicted protein [SNOG_15595] [*Phaeosphaeria nodorum* SN15] tr!Q0CHA9_ASPTE Hypothetical protein [ATEG_06933] [*Aspergillus terreus* NIH2624] tr!Q92251_NEUCR Ascospore maturation 1 protein (Hypothetical protein NCU01414.1) (Asm-1) [Asm-1] [*Neurospora crassa*] tr!Q0U086_PHANO Hypothetical protein [SNOG_14941] [*Phaeosphaeria nodorum* SN15] tr!Q5BED9_EMENI Hypothetical protein [AN1091.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q4WH38_ASPFU Hypothetical protein [Afu7g08240] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!Q4I2C4_GIBZE AREA_GIBFU Nitrogen regulatory protein areA [FG08634.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q2HHL1_CHAGB Hypothetical protein [CHGG_00293] [*Chaetomium globosum* CBS 148.51] tr!Q0UFV6_PHANO Hypothetical protein [SNOG_09358] [*Phaeosphaeria nodorum* SN15]

Table A8.5: Protein band WF9, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	Sequence	Potential identity of the peptide sequences.
WF9	TGTSDVSVA TVDAEK WLDGLGR MPCPFVSDN SGK ATDPGLTYD AYK	tr!Q86Z93_GIBMO Kinesin [KLP7] [*Gibberella moniliformis* (*Fusarium verticillioides*)] tr!Q2HCZ9_CHAGB Hypothetical protein [CHGG_01905] [*Chaetomium globosum* CBS 148.51] sp!P36011!STUA_EMENI Cell pattern formation-associated protein stuA [stuA] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q0UUI9_PHANO Hypothetical protein [SNOG_04575] [*Phaeosphaeria nodorum* SN15] tr!Q755Z6_ASHGO AER371Cp [AER371C] [*Ashbya gossypii* (Yeast) (*Eremothecium gossypii*)] tr!Q5B0U4_EMENI STUA_EMENI CELL PATTERN FORMATION-ASSOCIATED PROTEIN [AN5836.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q2GX19_CHAGB Predicted protein [CHGG_07485] [*Chaetomium globosum* CBS 148.51] tr!Q4P538_USTMA Hypothetical protein [UM04775.1] [*Ustilago maydis* (Smut fungus)] tr!Q0UQT3_PHANO Predicted protein [SNOG_05881] [*Phaeosphaeria nodorum* SN15] tr!Q0CXW3_ASPTE Hypothetical protein [ATEG_01471] [*Aspergillus terreus* NIH2624] tr!Q0V0Q3_PHANO Hypothetical protein [SNOG_02411] [*Phaeosphaeria nodorum* SN15] tr!Q5B0E2_EMENI Hypothetical protein [AN5988.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q2H9N9_CHAGB Hypothetical protein [CHGG_03065] [*Chaetomium globosum* CBS 148.51] tr!Q1ZYK3_ASPNG Hypothetical protein [An12g05200] [*Aspergillus niger*] sp!Q0U6X2!MAK5_PHANO ATP-dependent RNA helicase MAK5 (EC 3.6.1.-) [MAK5] [*Phaeosphaeria nodorum* (*Septoria nodorum*)] tr!Q9P6X0_NEUCR Hypothetical protein 68B2.060 (Hypothetical protein NCU03681.1)[68B2.060] [*Neurospora crassa*] tr!Q0ULL5_PHANO Hypothetical protein [SNOG_07349] [*Phaeosphaeria nodorum* SN15] tr!Q4IJN1_GIBZE Hypothetical protein [FG02577.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q0TYV8_PHANO Hypothetical protein [SNOG_15385] [*Phaeosphaeria nodorum* SN15] sp!P33400!PACC_YEAST pH-response transcription factor pacC/RIM101 (pH-response regulator protein RIM101) (Regulator of IME2 protein 1) [RIM101] [*Saccharomyces cerevisiae* (Baker's yeast)] tr!Q5KK10_CRYNE Hypothetical protein [CNC04670] [*Cryptococcus neoformans* (*Filobasidiella neoformans*)] tr!Q5A7G7_CANAL Hypothetical protein [CaO19.5070] [*Candida albicans* (Yeast)] tr!Q5A792_CANAL Hypothetical protein [CaO19.12536] [*Candida albicans* (Yeast)] tr!Q55W86_CRYNE Hypothetical protein [CNBC2510] [*Cryptococcus neoformans* var. *neoformans* B-3501A] tr!Q4INA4_GIBZE Hypothetical protein [FG01304.1] [*Gibberella zeae* (*Fusarium graminearum*)]

Table A8.5: Protein band WF9, peptide sequences of dominant peptides and potential identity of peptide sequence.

Band identified in excised gel	**Sequence**	**Potential identity of the peptide sequences from the BLAST protein database search.**
WF9	TGTSDVSVATV DAEK WLDGLGR MPCPFVSDNSG K ATDPGLTYDAY K	tr!Q4I691_GIBZE Hypothetical protein [FG07267.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q0TXW1_PHANO Predicted protein [SNOG_15595] [*Phaeosphaeria nodorum* SN15] tr!Q0CHA9_ASPTE Hypothetical protein [ATEG_06933] [*Aspergillus terreus* NIH2624] tr!Q92251_NEUCR Ascospore maturation 1 protein (Hypothetical protein NCU01414.1) (Asm-1) [Asm-1] [*Neurospora crassa*] tr!Q0U086_PHANO Hypothetical protein [SNOG_14941] [*Phaeosphaeria nodorum* SN15] tr!Q5BED9_EMENI Hypothetical protein [AN1091.2] [*Emericella nidulans* (*Aspergillus nidulans*)] tr!Q4WH38_ASPFU Hypothetical protein [Afu7g08240] [*Aspergillus fumigatus* (*Sartorya fumigata*)] tr!Q4I2C4_GIBZE AREA_GIBFU Nitrogen regulatory protein areA [FG08634.1] [*Gibberella zeae* (*Fusarium graminearum*)] tr!Q2HHL1_CHAGB Hypothetical protein [CHGG_00293] [*Chaetomium globosum* CBS 148.51] tr!Q0UFV6_PHANO Hypothetical protein [SNOG_09358] [*Phaeosphaeria nodorum* SN15]
	LTAGPVAAGSK	No hits

Appendix 9

Publications that the PhD author Shona M. Duncan has made a contribution.

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

Held, B.W., Jurgens, J.A., Duncan, S. M., Farrell, R.L., Blanchette, R.A. 2006 Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica. *Polar Biology* 29: 526-531.

Held, B.W., Jurgens, J.A., Arenz, B.E., Duncan, S.M., Farrell, R.L., Blanchette, R.A. 2005. Environmental factors influencing microbial growth inside the Historic Expedition Huts of Ross Island, Antarctica. *International Biodegradation and Biodeterioration* 55: 45-53.

Blanchette, R.A., Held, B.W., Jurgens, J.A., Aislabie, J., Duncan, S.M., Farrell, R.L. 2004. Environmental pollutants in Antarctica from the Robert F. Scott and Ernest H. Shackleton expeditions during the 'Heroic Era' of exploration. *Polar Record* 40: 143-151.

Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., and Farrell, R.L. 2004. Wood Destroying Soft Rot Fungi in the Historic Expeditions Huts of Antarctica. *Applied Environmental and Microbiology* 70: 1328-1335.

Blanchette, R.A, B.W. Held, R.L. Farrell, S. Duncan. (2000). Wood deterioration in the historic huts of Antarctica. *Phytopathology* 90(6): 7.

Aislabie, J., Fraser, R., Duncan, S., Farrell, R.L. (2001). Effects of oil spills on microbial heterotrophs in Antarctic soils. *Polar Biology* 24: 308-313.

Book Chapters

Farrell, R.L., Blanchette, R.A., Auger, M., Duncan, S.M., Held, B.W., Jurgens, J.E., Minasaki, R. 2004. *Scientific Evaluation of Deterioration in Historic Huts of Ross Island, Antarctica*. In Polar Monuments and Sites Cultural Heritage Work in the Arctic and Antarctic Regions. ISBN: 82-996891-1-2, International Polar heritage Committee, ICOMOS, Norway. pp. 33-38

Held, B.W., Blanchette, R.A., Jurgens, J.A., Duncan, S., Farrell, R.L. 2003. *Deterioration and conservation issues associated with Antarctica's historic huts*, In Art, Biology and Conservation of Works of Art, Ed. Koestler, R.J., Charola, A.E., Nieto-Fernadez, F.E., Metropolitan Museum of Art, New York., pp. 370-389.

Farrell, R.L, Duncan, S.M. *Uniqueness of Antarctica and potential for commercial success*. 2005. In *Antarctic Bioprospecting*, Ed. Hemmings A.D., and Rogan-Finnemore M. Gateway Antarctica Special Publication Series Number 0501, ISBN: 0-476-01647-9,University of Canterbury, Christchurch, pp. 10-40

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- Pre Antarctic field event planning and preparation.
- Interview of Professor Emeritus John McCraw concerning construction, shipment, and use of crate.
- In Antarctica planning, preparation, sample collection, and visual observations.
- Scientific discussions and manuscript proof reading.

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ORIGINAL PAPER

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Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica

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Abstract Scientists working at New Harbor, Antarctica in November 1959 used a wooden crate as a makeshift workspace and kitchen. The structure has been used intermittently over the subsequent decades and still remains at the site with various materials left in and around it. The wooden structure was assessed for deterioration and samples collected to determine the diversity of fungi at the site after 43 years in the Antarctic environment. Results from these investigations are compared to the results from research on the historic huts of Ross Island, approximately 70 km east of New Harbor that were built 48–58 years earlier. Our analysis shows the wood of the New Harbor structure is extremely weathered and soft rot decay was detected in the wood in contact with the ground. Fungal cultures isolated from wood of the structure were identified using sequences of the internal transcribed spacer region of the rDNA. Several species of *Cadophora* were identified including *C. malorum*, *C. luteo-olivacea*, *C. fastigiata* and a previously undescribed species designated *C.* sp. NH. Laboratory decay experiments using two *Cadophora* species isolated from New Harbor demonstrated extensive decay and loss of biomass in hardwood wafers after 16 weeks. Other fungi isolated from the wood included species of *Cladosporium*, *Hormonema*, *Penicillium* and *Lecythophora*. Wind erosion has also severely affected the structure's exterior wood causing deep furrowing between earlywood and latewood cells. In general, the deterioration and fungi found at the site were similar to those found at the historic expedition huts on Ross Island, however, one species obtained is unique to the New Harbor site. This research expands our knowledge of the microbes colonizing wood brought into the polar environment and provides additional information on deterioration and decomposition processes occurring in Antarctica.

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Introduction

In November 1959, two scientists, J.D. McGraw and G.C. Claridge, used a large wooden crate (1×1×2.5 m) built in New Zealand to transport field gear in Antarctica. A Massey Ferguson tractor was used to pull the crate to New Harbor, Antarctica approximately 70 km west of Ross Island, with the two scientists inside the crate during the journey. The crate provided protection for the scientists from ice fragments and snow that came off of the tracks of the tractor. Once they arrived at New Harbor, the crate was converted into a makeshift cook house and work area. The structure was abandoned the following season and although evidence exists that others have used the structure during past years, no further detailed information is available about its subsequent use. The structure is presently situated approximately 300 m from the sea and less than 1 m from a seasonal stream (Fig. 1a, b). Visual observations indicated that exterior woods of the structure have suffered significant wind erosion leaving an eroded, uneven surface. In a preliminary study, fungi were isolated from wood and several species of *Cadophora* were found (Blanchette et al. 2004).

Although the deterioration of wood in more temperate climates has been widely studied and understood (Eaton and Hale 1993; Eriksson et al. 1990; Zabel and Morrell 1992), degradative processes of wood in the polar environment are not as well known. Deterioration of the historic huts of Ross Island has been researched and documented and involves the degradation of wood

Fig. 1 The 1959 structure at New Harbor and wind erosion of exterior woods. a View of the structure at New Harbor. The small stream in the foreground wets the soil under the structure. b A close view of the structure and the remaining materials left at the site. Hazardous materials have recently been removed from the site. c Advanced wind erosion of the exterior woods. The wind erodes the earlywood cells more easily than the more resistant, highly lignified late wood cells resulting in deep grooves in the wood

caused by non-biological (wind, ultraviolet light and salt) and biological (soft rot decay) effects (Blanchette et al. 2002; Blanchette et al. 2004; Held et al. 2003). The soft rot fungi isolated are of particular interest since some species identified have the capacity to cause extensive wood decay as well as being indigenous to Antarctica. These fungi likely have a broader role for organic matter degradation and nutrient recycling in Antarctica. Since the wooden structure at New Harbor has been in the Antarctic environment for only about four and a half decades, compared to the historic expedition huts that have been in Antarctica for nine to ten decades, it is an excellent study site providing a different time interval and location for assessing microbial diversity and deterioration in a polar environment. The objectives of this study were to: (1) assess and document the deterioration that has occurred in the wooden structure at New Harbor, Antarctica, (2) determine the fungal diversity associated with the wood and (3) compare these findings to those obtained for other introduced wood to Antarctica, specifically, the historic Ross Island expedition huts.

Materials and methods

Samples from the structure were taken in the austral summer of 2002 under Antarctic Conservation Act Permit number 2002-001. Minute samples of wood were taken several centimeters below ground near the base as well as other locations above ground from the structure for microbiological isolations and were placed in sterile bags. Samples were kept cool during transport and frozen upon arrival to the laboratory at the University of Minnesota. In the laboratory, microorganisms were isolated from wood samples by placing them on two types of culturing media, followed by an incubation period. The media used were 1.5% Difco malt extract agar (MEA) and acidified MEA with 2 ml of lactic acid added after autoclaving. Plates were incubated at 20 and 8°C for 4–8 days after which pure cultures were transferred to individual plates. Fungi were identified using taxonomic literature for these genera and analyses of rDNA internal transcribed spacer sequences were carried out as described by Held et al. (2005).

The laboratory decay study to test fungal species isolated from the New Harbor structure for their ability to decay wood was conducted in the laboratory over a 16-week period. Thin wafers of a hardwood (birch, measuring 1.5×1.5×0.2 cm) and a softwood (pine, measuring 1×1.5×0.2 cm) were used that were cut from sound wood blocks. Wafers were then dried and weighed to determine their dry weight. Two different nutrient solutions were infiltrated into wafers by autoclaving them in a corresponding solution. The two solutions used were: (1) a reduced nutrient solution, RNS, containing 1.5 g NH_4NO_3, 2.5 g KH_2PO_4, 2 g K_2HPO_4, 1 g $MgSO_4{\cdot}7H_2O$, 2.5 g glucose and 0.1 g thiamine per liter and (2) 2A (double Abrams) containing 6 g NH_4NO_3, 4 g KH_2PO_4, 5 g K_2HPO_4, 4 g $MgSO_4{\cdot}7H_2O$, 2.5 g glucose and 0.1 g thiamine per liter (Abrams 1948; Duncan 1965; Zabel et al. 1991; Worrall et al. 1991). Petri plates with media made with the same RNS and 2A nutrients and agar were inoculated with *Cadophora* sp. NH and *C. fastigiata* and allowed to grow for several weeks. After autoclaving, excess nutrient solution was decanted from the wafers and four wafers were placed directly onto growing cultures on plates made with the corresponding RNS or 2A media. Three plates were used for each fungus and nutrient solution.

Non-inoculated wafers with nutrients were used as a control. Parafilm was placed around the plates and the plates were incubated at 21°C for 16 weeks. After the incubation period, wafers were removed from the plates and excess mycelium was carefully removed. Wafers were then oven-dried and biomass loss determined [(dry wt. before inoculation - dry wt. after inoculation)/dry wt. before inoculation]. Wood samples were prepared for scanning electron microscopy (SEM) using techniques described previously by Blanchette and Simpson (1992) and was carried out using a Hitachi S3500N SEM.

Results

Wood samples from the structure at New Harbor were identified as several different species of *Pinus* and several unattached boards as *Nothofagus* sp. The exterior woods were still intact but severe weathering was observed. The most serious deterioration was due to wind erosion. This has left the wood surface uneven where the earlywood regions eroded more than the latewood regions causing a grooved appearance (Fig. 1c). Small fragments of gravel were frequently found wedged in these grooves. Small zones of defibrated wood just above the ground were found, but this form of non-biological deterioration was not widespread. A graying or whitening of exterior woods also was apparent on some areas of exterior woods. The interior of the structure appeared sound and was free of visible mold growth and deterioration.

Wood in contact with the ground was wet and often appeared to be discolored. In addition to the exterior boards of the structure, several boards and wood fragments were found lying on the ground near the hut. SEM of small samples taken from wood in ground contact indicated that soft rot decay was affecting the wood (Fig. 2). Large holes were present in secondary walls of the *Pinus* and *Nothofagus* woods typical of type 1 soft rot (Blanchette et al. 1990; Eaton and Hale 1993). Soft rot was found in several samples of wood that were in soil contact near the base of the structure. Fungal isolations from wood produced four species of *Cadophora* including *C. malorum*, *C. luteo-olivacea*, *C. fastigiata* and an undescribed species designated *C.* sp. NH (Table 1). Other fungi isolated from wood included *Cladosporium cladosporioides*, *Hormonema dematioides*, *Lecythophora hoffmanii* and *Penicillium mali*.

Microscopic examination of sections from birch wood wafers inoculated in the laboratory with *C. fastigiata* revealed extensive type 1 soft rot (Fig. 2). Although the most advanced decay was limited to the outer portions of the wafers, biomass losses with the 2R and RNS media showed a 27 and 20.3% average weight loss, respectively (Fig. 3). For the pine wood, weight losses were 1.2 and 3.1% for wafers inoculated with *C. fastigiata* on 2R and RNS media, respectively, and no decay was observed. *Cadophora* sp. NH grew slower on MEA than other *Cadophora* species and did not colonize the birch and pine wafers well. In these wood wafers a negligible weight loss of 0.6–3.2% was observed.

Discussion

Previous studies have shown that the cold, dry Antarctic environment does not completely inhibit deterioration processes and significant damage can occur over time

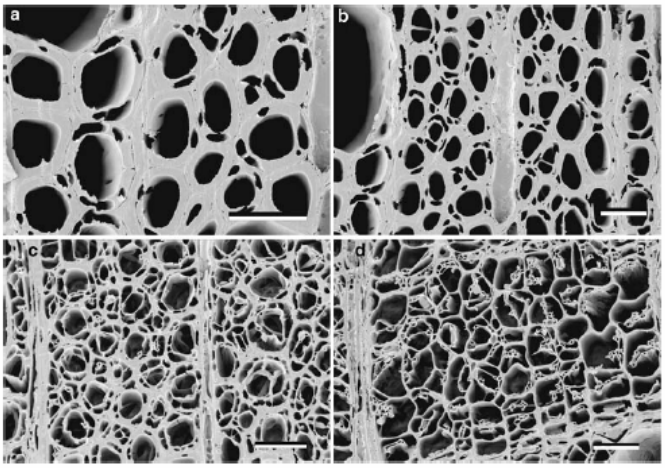

Fig. 2 Scanning electron micrographs showing soft rot cavities in the cell walls of wood. **a** and **b** Soft rot cavities in *Nothofagus* buried in soil at the base of the structure. Cavities coalesce to form large voids in the secondary cell walls of the wood. **c** and **d** Extensive soft rot decay in birch wafers inoculated with *Cadophora fastigiata* in the laboratory after 16 weeks. The secondary cell walls are almost completely degraded, leaving only the middle lamella which causes severe strength loss in the wood

Table 1 Fungal species isolated from various wood samples from the 1959 structure at New Harbor, Antarctica

Species	Collection data and isolate number[a]
Cadophora malorum	Wood in contact with soil; NH8-1; NH15-3
Cadophora luteo-olivacea	Wood, E sideboard below soil; NH6-1
Cadophora fastigiata	East sideboard, below soil; NH5-1
Cadophora sp. NH	Wood, below soil, SW corner; NH1-2, NH9-1
Cladosporium cladosporioides	East sideboard, below soil; NH6-3
Hormonema dematioides	Southwest corner sideboard, below soil; NH7-3
Lecythophora hoffmanii	Wood, below soil, SW corner; NH1-1
Penicillium mali	Wood, below soil, SW corner; NH1-3

[a]Cultures with two isolate numbers were found in two separate locations

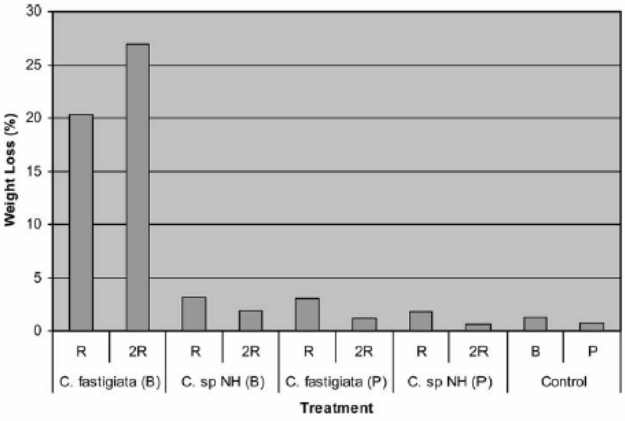

Fig. 3 Comparisons of percent weight losses of wood wafers, birch (*B*) and pine (*P*) when infiltrated with reduced nutrient (*R*) and double Abrams (*2A*) solution followed by inoculation with *Cadophora fastigiata* and *C.* sp. NH

(Blanchette et al. 2002; Blanchette et al. 2004; Held et al. 2003; Hughes 2000). The structure at New Harbor provided a good opportunity to study wood that has been in the Antarctic environment for a shorter period of time than the historic huts on Ross Island (43 years instead of 90). After only 43 years, severe damage from non-biological and biological agents has taken place.

Three types of non-biological deterioration are affecting the structure: wind abrasion, salt defibration and UV light degradation. Wind abrasion, which is commonly found in wood exposed to the Antarctic environment (Blanchette et al. 2002; Held et al. 2003; Harrowfield 1996; Hughes 2000), is affecting the wood at New Harbor in a similar way as the historic expedition huts in other locations (Fig. 1c). Exposure to powerful winds and particulate matter are the main factors responsible and currently no acceptable treatment exists to protect wood that is subjected to this type of deterioration. The second type of non-biological deterioration is in the form of defibration of the exterior wood caused by high concentrations of salts. This type of damage has been documented in the historic huts on Ross Island and in other environments where wood is in contact with high salt concentrations. It consists of a chemical degradation of the middle lamella that results in the separation of wood fibers (Blanchette et al. 2002). The structure at New Harbor is located near a seasonal stream that provides continuous moisture to the wood in ground contact. As moisture is absorbed by the wood it is evaporated above the ground and high concentrations of salt precipitate. Although the extent of exterior wood defibration is not severe, some damage is occurring to the structure. The third form of deterioration is from UV light causing a graying or whitening of wood surfaces, most easily seen on exterior, horizontal cross member woods of the structure. This type of deterioration involves the degradation of lignin in the wood surface caused by UV light absorption (Hon 1981; Gellerstedt and Petterson 1977). Although this damage is limited on the structure at New Harbor more significant effects can be seen on the huts on Ross Island which can be attributed to Antarctica's intense UV light levels (Booth et al. 1994).

Wood decay caused by soft rot fungi is a newly described phenomenon occurring in Antarctica (Blanchette et al. 2004; Held et al. 2003). Advanced stages of wood decay have been reported in wood in ground contact from Shackleton's 1909 hut and in various woods near Scott's Cape Evans hut on Ross Island (Blanchette et al. 2004). Species of *Cadophora*

have been found associated with this decay. Different environmental conditions between the two sites may be responsible for more decay present in some of the New Harbor samples despite being exposed for a shorter time period. Since the soil around the wooden structure at New Harbor is continuously wet during the austral summer, conditions for soft rot fungi are likely to be more conducive for decay. The fungal species causing the decay also may be more aggressive than those found affecting the woods on Ross Island. *C. fastigiata*, which this study shows to be capable of causing advanced stages of decay in laboratory studies, has not been isolated from wood associated with the Ross Sea huts. Cultures of *C. malorum*, *C. luteo-olivacea*, *C. fastigiata*, *H. dematioides* and *C. cladosporioides* have been isolated from samples of wood collected from the New Harbor structure (Table 1). Similar fungi, isolated from other materials have been previously shown to cause soft rot (Blanchette et al. 2004; Morrell and Smith 1988; Morrell and Zabel 1985; Zabel et al. 1982). The frequency that *Cadophora* species were isolated from the wood at New Harbor and the extensive decay that species of this genus produced in laboratory decay experiments (Fig. 2) indicates it is likely the major cause of the decay observed. Results from the laboratory wood decay studies also showed *C. fastigiata* caused considerable weight loss in birch but not pine wood (Fig. 3). In a previous investigation using a different isolate of *C. fastigiata*, Morrell and Zabel (1985) showed both type 1 and 2 soft rot in southern yellow pine and beech. Nilsson (1973) also showed that a strain of this fungus produced soft rot cavities in hardwood, pine and spruce. Additional research is needed on the physiology of these species to fully understand what governs their capacity to cause decay. It is well known that nutrients influence soft rot activity (Duncan 1965; Worrall and Wang 1991; Worrall et al. 1991) and increased concentrations of some nutrients are needed to cause soft rot unlike other forms of wood decay. The presence of nitrogen and other sources of nutrients from food stores left at the historic huts as well as penguin guano may influence the extent and rate of degradation in Antarctica.

Environmental conditions within the historic huts of Ross Island have been monitored using data loggers and high relative humidity and temperatures above 0°C have been reported (Held et al. 2005). The high relative humidity and excess moisture in areas of the huts provided conditions that allowed extensive mold growth to occur, especially in areas of restricted air movement. Unlike the historic huts, environmental monitoring at the New Harbor structure has not been carried out. Since surface molds were not observed on the interior of the structure it is a good indication that relative humidity is not high enough in this structure to support surface mold growth.

Apart from the Ross Sea huts, *Cadophora* species have been previously reported in Antarctica on a range of different substrates and locations. They have been found in soils in the Vestfold Hills and near Davis Station (Kerry 1990), in contaminated soils in the Ross Sea Region (Aislabie et al. 2001), and isolated from feathers and mosses from Victoria Land (Azmi and Seppelt 1998; Frate and Caretta 1990; Tosi et al. 2002). These reports indicate a wide polar distribution of the genera and suggest a role for this fungus in organic matter decomposition and nutrient cycling. A previously undescribed species of *Cadophora*, designated *C.* sp. NH isolated from the New Harbor site, has not been reported at any other location in Antarctica or the world. This species did not cause soft rot in the laboratory wood decay study and did not grow well on the pine and birch wood wafers, unlike *C.* sp. E an undescribed species isolated from Cape Evans which caused soft rot in birch and thought to be endemic to Antarctica (Blanchette et al. 2004). Additional studies that vary the substrate, nutrients and environmental conditions are needed to determine if *C.* sp. NH has the capacity to cause wood decay, and to elucidate its role in nutrient recycling in the Antarctic environment.

These studies have provided new information on the microbial diversity found on wood introduced into the Antarctic environment, the fungi causing wood decay and other factors responsible for deterioration taking place. The occurrence and role of the *Cadophora* species isolated from different locations in Antarctica demonstrate their widespread distribution and diversity. The previously undescribed species, *C.* sp. NH, discovered at this site suggests this fungus may be endemic to Antarctica (Blanchette et al. 2004). New studies are warranted to better understand this fascinating group of microbes and their biology and ecology in the polar environment.

Results presented here provide an assessment of deterioration and fungal diversity associated with the 43-year-old wooden structure at New Harbor. Environmental staff from both the US and New Zealand Antarctic programs have documented and inventoried items in and around the structure, and items that posed environmental concerns have been removed. At the present time, the structure's historic value and whether action will be taken for its conservation are still being determined. The scientific value of the structure, if left in place, appears to be significant since it provides an excellent study site to monitor microbial diversity and evaluate deterioration processes in the Antarctic environment. The presence of an undescribed fungal species suggests it may have unique microflora and further investigations are warranted.

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References

Abrams E (1948) Microbiological deterioration of organic materials. Its prevention and methods of test. U.S. Natl. Bur. Stan. Circ. No. 188

Aislabie J, Fraser R, Duncan S, Farrell RL (2001) Effects of oil spills in microbial heterotrophs in Antarctic soils. Polar Biol 24:308–313

Azmi OR, Seppelt RD (1998) Broad scale distribution of microfungi in the Windmill Islands, continental Antarctica. Polar Biol 19:92–100

Blanchette RA, Held BW Farrell, RL (2002) Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. Polar Rec 38:313–322

Blanchette RA, Simpson E (1992) Soft rot decay and wood pseudomorphs in an ancient coffin (700 BC) from tumulus MM at Gordion, Turkey. Int Assoc Wood Anatomists Bull 13:201–213

Blanchette RA, Nilsson T, Daniel G, Abad A (1990) Biological degradation of wood. In: Rowell RM, Barbour RJ (eds) Archaeological wood: properties, chemistry, and preservation. American Chemical Society, Washington. Adv Chem Ser 225:141–174

Blanchette RA, Held BW, Jurgens JA, McNew DL, Harrington TC, Duncan SM, Farrell RL (2004) Wood-destroying soft rot fungi in the historic expedition huts of Antarctica. Appl Environ Microbiol 70:1328–1335

Booth CR, Lucas T, Morrow JH, Weiler CS, Penhale PA (1994) The United States National Science Foundation's polar network for monitoring ultraviolet radiation. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects, AGU Antarctic research series, vol. 62. American Geophysical Union, Washington, pp 17–37

Duncan CG (1965) Determining resistance to soft-rot fungi. U.S. For. Prod Lab Res Pap No FPL-48

Eaton RA, Hale MDC (1993) Wood: decay pests and protection. Chapman & Hall, London

Eriksson KE, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin Heidelberg New York

Frate GD, Caretta G (1990) Fungi isolated from Antarctic material. Polar Biol 11:1–7

Gellerstedt G, Petterson EL (1977) Light-induced oxidation of lignin Part 2 The oxidative degradation of aromatic rings. Sven Papperstindn 80:15–21

Harrowfield DL (1996) The role of wind in the destruction of an historic hut at Cape Adare in Antarctica. Polar Rec 32:3–18

Held BW, Blanchette RA, Jurgens JA, Farrell RL, Duncan SM (2003) Deterioration and conservation issues associated with Antarctica's historic huts. In: Koestler RJ, Koestler VR, Charola AE, Nieto-Fernandez FE (eds) Art, biology, and conservation: biodeterioration of works of art. The Metropolitan Museum of Art, New York, pp 370–389

Held BW, Jurgens JA, Arenz BE, Duncan SM, Farrell RL, Blanchette RA (2005) Environmental factors influencing microbial growth inside the historic expedition huts of Ross Island, Antarctica. Int Biodeterior Biodegradation 55:45–53

Hon DN-S (1981) Photochemical degradation of lignocellulosic materials. In: Grassie N (eds) Developments in polymer degradation. Appl Sci Publ, London, pp 229–281

Hughes J (2000) Ten myths about preservation of historic sites in Antarctica and some implications for Mawson's huts at Cape Denison. Polar Rec 36:117–130

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

Morrell JJ, Smith SM (1988) Fungi colonizing redwood in cooling towers: identities and effects on wood properties. Wood Fiber Sci 20:243–249

Morrell JJ, Zabel RA (1985) Wood strength and weight loss caused by soft-rot fungi isolated from treated southern pine utility poles. Wood Fiber Sci 17:132–143

Nilsson T (1973) Studies on degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica Nr 104, Stockholm, Sweden, p 40

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

Worrall JJ, Wang CJK (1991) Importance and mobilization of nutrients in soft rot of wood. Can J Microbiol 37:864–868

Worrall JJ, Anagnost SE, Wang CJK (1991) Conditions for soft rot of wood. Can J Microbiol 37:869–874

Zabel RA, Morrell JJ (1992) Wood microbiology. Academic, New York

Zabel RA, Wang CJK, Terracina F (1982) The fungal associates, detection and fumigant control of decay in treated southern pine poles. Project 1471-1, Final Report, Electric Power Research Institute, p 93

Zabel RA, Wang CJK, Anagnost S (1991) Soft-rot capabilities of the major microfungi isolated from Douglas-fir poles in the Northeast. Wood Fiber Sci 23:220–237

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- In Antarctica planning, preparation, sample collection, and visual observations.
- Scientific discussions and manuscript proof reading.

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Environmental pollutants from the Scott and Shackleton expeditions during the 'Heroic Age' of Antarctic exploration

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ABSTRACT. Early explorers to Antarctica built wooden huts and brought huge quantities of supplies and equipment to support their geographical and scientific studies for several years. When the expeditions ended and relief ships arrived, a rapid exodus frequently allowed only essential items to be taken north. The huts and thousands of items were left behind. Fuel depots with unused containers of petroleum products, asbestos materials, and diverse chemicals were also left at the huts. This investigation found high concentrations of polyaromatic hydrocarbons in soils under and around the historic fuel depots, including anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, fluorene, and pyrene, as well as benzo[a]anthracene, benzo[a]pyrene, and fluoranthene, which are recognized carcinogens. Asbestos materials within the huts have been identified and extensive amounts of fragmented asbestos were found littering the ground around the Cape Evans hut. These materials are continually abraded and fragmented as tourists walk over them and the coarse scoria breaks and grinds down the materials. A chemical spill, within the Cape Evans hut, apparently from caustic substances from one of the scientific experiments, has caused an unusual deterioration and defibration on affected woods. Although these areas are important historic sites protected by international treaties, the hazardous waste materials left by the early explorers should be removed and remedial action taken to restore the site to as pristine a condition as possible. Recommendations are discussed for international efforts to study and clean up these areas, where the earliest environmental pollution in Antarctica was produced.

Contents

Introduction

A little more than 100 years ago, the members of the British National Antarctic Expedition, led by Robert Falcon Scott, built a large wooden building, later to become known as the *Discovery* hut, at Hut Point on Ross Island. This hut was to serve as a shelter, workshop, and supply store for the expedition members for three years during their exploration and scientific investigation of the area (Scott 1905). In 1908 the members of the British Antarctic Expedition, led by Ernest Shackleton, built another hut on Ross Island at Cape Royds; this would house a shore party of 15 men (Shackleton 1909, 1919). The members of Scott's ill-fated *Terra Nova* expedition (1910–13) erected a large prefabricated hut at Cape Evans to provide accommodation and also built a smaller structure that was framed in wood and lined with asbestos sheeting for taking magnetic observations (Harrowfield 1995; Pearson 1992; Scott 1913). Although it can be argued that geographical goals were primary to all three expeditions, they also had important scientific objectives. Each of the expeditions had one or more biologist, geologist, meteorologist, and physicist to carry out scientific programs. Along with an enormous amount of stores and equipment needed for survival in Antarctica during several years of exploration, there were some unusual items such as motorized vehicles and tractors. This new mode of transport for the early twentieth century was to be used for hauling supplies across the ice, but they were not overly successful. Large volumes of petroleum products brought for the vehicles were not used and remained in storage containers near the huts (Dougherty 1985). A considerable amount of asbestos was also transported to Antarctica for use as insulation around scientific equipment to prevent interference during magnetic observations and for other purposes. Knowledge of potential health hazards and environmental pollution that could result from these compounds being indiscriminately released into the environment did not exist at the time. In addition to crude petroleum products and asbestos, a wide array of chemicals was also brought to Antarctica along with battery acid, paint, and other substances containing heavy metals.

When the relief ships arrived to transport the shore parties back, initially, to New Zealand, there was a fast exodus to avoid the possibility of the ship getting frozen in the pack ice. Many non-essential materials were left at the huts, including unused food stores,

scientific and technical equipment, and personal items. Unused tanks of petroleum, materials containing asbestos, chemicals for experimentation, and thousands of other items were left behind. Today, the historic huts and most of the artifacts remain, providing a remarkable view into the lives of the explorers (Harrowfield 1995, 1981; Quartermain 1963). The international community has recognized the immense historical significance of the huts and artifacts for many years. Provisions of the Antarctic Treaty require all governments to adopt all adequate measures to protect the buildings and objects of historic interest from damage or destruction. The Protocol on Environmental Protection to the Antarctic Treaty of 1991 forbids the damaging, removal, or destruction of the historic sites, and, more recently, the huts have been designated as 'protected areas' by the Antarctic Treaty Consultative Meetings held in 1997 and 1998. During the past several years conservators from the Antarctic Heritage Trust have carried out work to protect and restore the huts. The investigations reported here were carried out in collaboration with the Antarctic Heritage Trust to identify hazardous materials and areas contaminated by potentially toxic pollutants at the historic sites in the Ross Sea region.

The dry, cold Antarctic environment has helped to preserve the huts and artifacts, but during the past 9–10 decades considerable deterioration has occurred (Blanchette and others 2002). Non-biological as well as biological deterioration has taken a toll on the wooden structures, metal objects, textiles, food stores, and other materials. During investigations to assess the abiotic deterioration and unique microbial decay occurring at the historic sites, hazardous pollutants originating from the early explorers were found to be contaminating the Antarctic environment at several locations. Since these sites are some of the most frequently visited areas for tourists in the Ross Sea region, exposure of the pollutants to humans should be a concern as well as the potential effects on terrestrial animal populations, aquatic life, and the Antarctic environment in general. This paper documents areas of environmental pollution that were found and identifies the specific types of pollutants present at each site. Results from these investigations suggest that despite the historic origin of the materials, the toxic substances should be removed to return the Antarctic environment to as pristine a condition as possible.

Sampling methods and analyses

Samples of soil, wood, and materials suspected of being asbestos were obtained from within the historic boundaries of Hut Point, Cape Evans, and Cape Royds on Ross Island, Antarctica. Samples were obtained during the austral summers of 2001 and 2002 under Antarctic Conservation Act permit numbers 2001-015 and 2002-001, and in cooperation with the Antarctic Heritage Trust, Antarctic New Zealand program K021, and the National Science Foundation.

Soil samples were collected from two fuel depot sites at Cape Evans near the historic hut. One site, the 'lower fuel depot,' is located south of the hut. The second site is located at a higher elevation to the southeast of the hut and is referred to in this paper as the 'upper fuel depot.' Both depots have wooden crates enclosing metal tanks that stored the petroleum products. Samples were taken immediately adjacent to the containers and at one-meter intervals from the ground surface and at various depths (5–15 cm) down to the ice-cemented layer. Samples were placed in non-reactive containers and frozen until analyzed. Levels of total petroleum hydrocarbons were determined by extracting the samples in methylene chloride, then analyzing the extracts by capillary gas chromatography with a flame ionization detector, as outlined in EPA Method 8015 (US Environmental Protection Agency 1992). Samples contaminated with hydrocarbons were also analyzed by using accelerated solvent extraction EPA Method 3545, followed by capillary gas chromatography with mass spectrometry and selected ion monitoring quantification EPA Method 3540 and 3630 (US Environmental Protection Agency 1992).

Elemental analyses were completed on samples of historic woods using multi-elemental inductively coupled plasma atomic emission spectroscopy. Wood samples from the historic huts and artifacts were carefully collected by selecting minute segments of wood from inconspicuous areas that appeared to have deteriorated or were suspected of heavy-metal contamination. Samples were kept in sterile containers and frozen until used. Methods used to prepare and analyze the samples were previously described (Blanchette and others 1994; Munter and Grand 1981). Soil samples obtained for elemental analyses were also placed in sterile containers and frozen until used. Previously described methods for sample preparation (Munter and Grand 1981) and inductively coupled plasma atomic emission analyses were used for these samples (US Environmental Protection Agency 1992).

Materials suspected of containing asbestos were sampled and examined microscopically. Locations were documented within the huts or within the historic boundaries where asbestos was found. At the Cape Evans site, large amounts of fragmented asbestos material were found littering the area around the hut. To obtain additional information on how much asbestos was present at the site, a survey was done at three snow-free areas adjacent to the hut. Three different surveyors counted asbestos materials greater than 1 cm^2 that were found on the ground surface; each area was surveyed twice by each examiner. This assessment provided an estimate of the amount of asbestos on the ground but made no attempt to determine asbestos materials below ground or those covered by ice and snow.

Environmental pollutants at the historic huts

The buildings and artifacts left in Antarctica by the early explorers provide a wealth of important historical information that deserves to be protected and preserved

long into the future. However, materials now considered to be environmental pollutants were also left behind, and these have a negative impact on the once pristine Antarctic environment. Aromatic hydrocarbons, asbestos, lead, and other potentially toxic materials contaminate the historic sites. Although these materials are part of the historic record of Antarctic exploration, their potentially harmful effects on the environment necessitate remedial action by the international community.

Petroleum-contaminated soils

Soil samples taken adjacent to historic petroleum containers and at 1- and 2-m intervals in two fuel depots located at Cape Evans hut (Figs 1 and 2) show that petroleum products have spilled or leaked from containers and have contaminated the area. At the lower fuel depot, high levels of total petroleum hydrocarbons were present adjacent to the containers from the soil surface to a depth of 15 cm, where the ice-cemented zone was located (Table 1). The contamination was also found at the soil surface and at a 5-cm depth located 1 m from the containers. A similar set of samples taken from the upper fuel depot immediately adjacent to the fuel boxes and at 1- and 2-m intervals showed that total petroleum hydrocarbons for C_7–C_9, and C_{10}–C_{14} were below the detectable limit. However, at the 5–10 cm depth soil sample, taken adjacent to the fuel container and just above the ice-cemented layer, 600 mg kg^{-1} of C_{15}–C_{36} compounds were present. All other soil samples taken away from the fuel containers at this depot had levels of hydrocarbons below the detectable limit. For both fuel depot sites, no attempt was made during this sampling to dig below the ice-cemented layer nor to move any of the containers since this would have resulted in excessive disturbance of the site. There appear to be few low molecular weight hydrocarbons present in these samples from the various locations tested (Table 1). The residual total petroleum hydrocarbons present at the site were unresolved complex material. Over the past decades these low molecular weight compounds may have been evaporated, leached, or transported out of the area. Since this is one of the oldest petroleum spills in Antarctica and the compounds have been in the soil for more than nine decades, some degradation by soil microbes could also have taken place. The high molecular weight compounds present in the contaminated soils at the lower fuel depot include high concentrations of phenanthrene, pyrene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (Table 2). Concentrations at this site are exceedingly high as compared to other petroleum spills in soils from around Scott Base, McMurdo Station, and the former Vanda Station (Aislabie and others 1999; Mazzera and others 1999). While naphthalene and or methylnaphthalenes predominated in samples from Scott Base and McMurdo Station in these previous investigations, they are absent

Fig. 1. Fuel depot at Cape Evans near the historic hut. High concentrations of polyaromatic hydrocarbons are in soils below and around the fuel containers.

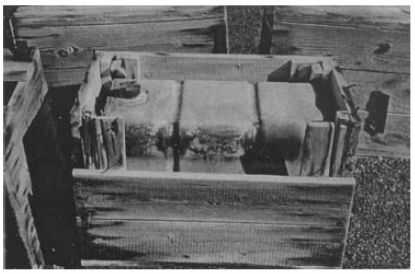

Fig. 2. Petroleum container at fuel depot showing a metal tank that contained the petroleum products enclosed in a wooded crate.

Table 1. Total petroleum hydrocarbons detected in soil from the lower fuel depot area at Cape Evans hut, Ross Island, Antarctica. Amount present is in mg kg^{-1} dry weight. * = soil samples taken adjacent to fuel container where soil appeared darkly stained and at 1 m (sample 2) and 2 m (sample 3) intervals out from the container. †= soil samples taken at ground surface–5 cm, 5–10 cm, and 10–15 cm. The lowest sample was taken from just above the ice-cemented soil. For sample 3, soil was taken only from a 0–5 cm depth. BLD = below level of detection.

Total petroleum hydrocarbons	Sample 1*			Sample 2			Sample 3
	0–5 cm†	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm
C_7–C_9	BLD	BLD	BLD	BLD	BLD	BLD	BLD
C_{10}–C_{14}	80	44	48	9	15	BLD	BLD
C_{15}–C_{36}	36,100	21,200	25,200	9330	13800	760	BLD

Table 2. Polycyclic aromatic hydrocarbons (PAH) identified in soil from the lower fuel depot area at Cape Evans hut, Ross Island, Antarctica. Amount present is in μg kg^{-1} dry weight. * = soil samples taken adjacent to fuel container and at 1 m (sample 2) and 2 m (sample 3) intervals out from the container. † = soil samples taken at the ground surface and at 5 and 15 cm below surface.

	Sample 1*			Sample 2			Sample 3
PAH	0–5 cm†	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm
Chrysene	2950	1780	2120	942	1230	52	6
Pyrene	2170	1610	1770	664	954	42	11
Phenanthrene	1700	2460	2430	723	791	78	11
Benzo[a]anthracene	1230	890	1040	453	633	24	6
Fluoranthene	770	610	710	208	208	18	14
Benzo[a]pyrene	370	260	280	83	79	6	3
Benzo[k]fluoranthene	370	260	240	130	118	10	3
Benzo[b]fluoranthene	330	220	340	87	108	6	10
Anthracene	180	150	160	46	63	5	2
Fluorene	120	250	230	51	73	4	1

in contaminated soils from Cape Evans. In addition, other compounds present in other Antarctic petroleum spills, such as acenaphthene and acenaphthylene, are not present. This may be attributed to the short time since spillage at Scott Base and McMurdo Station and because these lower molecular weight compounds are more susceptible to removal processes such as volatilization, leaching, or biodegradation. Several of the aromatic compounds found, including benzo[a]pyrene, benzo[a]anthracene and fluoranthene, are recognized carcinogens. Levels for most of the polyaromatic hydrocarbons detected in these samples were well above the acceptable levels established by the Dutch clean-up standards (Ministry for Housing, Spatial Planning and the Environment 1994), which range from 15 to 45 μg kg^{-1} dry weight. Although there are no guidelines established that indicate remediation is required for hydrocarbon-contaminated sites in the Antarctic, the levels of PAHs in the soils are above Dutch standards and should be of concern. The relevance of these standards in an Antarctic environment is not known but guidelines set for other parts of the world should be used as a standard in the polar regions until more precise guidelines are available.

Some of the wooden crates that contain petroleum cans at Cape Evans have painted labels indicating they contained 'motor spirits.' A previous analyses of the 'motor spirits' used by the *Terra Nova* expedition found approximately 22% n-alkanes, 37% iso-alkanes, 15% cyclo-alkanes, and 7% aromatics (Dougherty 1985). The petroleum contaminants within the soils reported in this paper indicate a much higher concentration of diverse aromatic compounds than are present in the sample of 'motor spirits' tested. This suggests that the fuel depot contains more varied materials such as lubricating engine oil and also possibly grease. The darkly stained surface soil in the depot area also suggests that other petroleum products were spilled at the site.

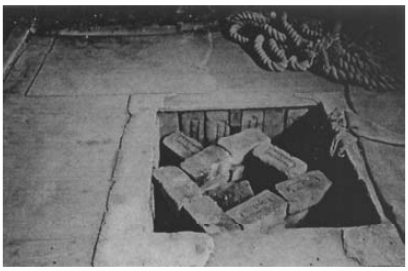

Fig. 3. Asbestos materials on floor around the remains of the pendulum apparatus used by Louis Bernacchi in the *Discovery* hut.

Asbestos

Materials that appeared to contain asbestos were sampled and analyzed microscopically to determine if asbestos was present. Materials containing 15–25% asbestos were found at the *Discovery* hut and the Cape Evans hut. At the *Discovery* hut, asbestos is present around the base of the pendulum apparatus used by Louis Bernacchi (Fig. 3) and on the interior west wall near the meat storage room, where slats of asbestos were used to hold a felt-like material on the walls. At Cape Evans, the entire magnetic observation hut, built on a hill southeast of the main hut, is lined with asbestos board. A large wooden containment building was erected over this structure many years ago and is only opened intermittently for inspection by conservators from the Antarctic Heritage Trust. A large amount of asbestos also litters the ground around the hut at Cape Evans. A survey of three areas near the hut showed that 54, 59, and 187 pieces of asbestos greater than 1 cm^2 were on the ground surface in snow-free areas adjacent to the hut (Fig. 4). No attempt was made in this survey to ascertain the amount of asbestos that was present below

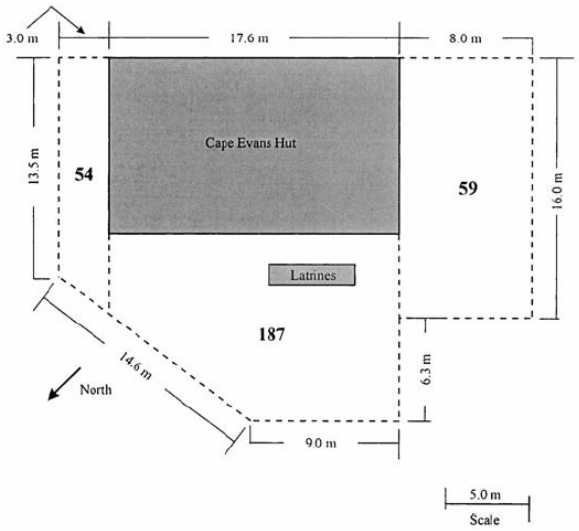

Fig. 4. Diagram of the area around Cape Evans hut with amounts of asbestos fragments greater than 1 cm^2 observed on the ground surface. Numbers in bold represent the mean number of asbestos fragments determined within three snow-free areas around the hut.

the soil surface or in areas covered by snow and ice. These fragments of asbestos appear to be continually fractured and broken into minute pieces as people visit the site and walk over the material. The coarse scoria at the site also grinds and abrades the asbestos materials as they are crushed and tramped upon. The asbestos can be found over a wide area around the hut (Fig. 5) and undoubtedly extends much farther under snow- and ice-covered areas not surveyed. The number of visitors to these historic sites has steadily increased in past years and a great deal of human activity takes place around the huts during the austral summer. The presence of large amounts of fragmenting asbestos where tourist activity takes place warrants action. In addition, as asbestos is abraded into minute particles it is distributed out into the Antarctic environment. At the Cape Evans hut, asbestos located at the shoreline is easily dispersed by winds into the sea and land around the hut. Although the effects of asbestos on Antarctic biota are not known, the continued release of this hazardous material into the environment should be of great concern.

Fig. 5. Snow-free area round Cape Evans hut that contains several hundred pieces of asbestos on the surface of the ground.

Heavy metals and other chemicals

Elemental analyses of selected wood and soil samples were completed to assess the presence of lead and other heavy metals at the sites (Tables 3 and 4). High concentrations of lead were found at specific locations at all three huts. At the *Discovery* hut, elevated lead concentrations were found in the soil immediately adjacent to the west wall and veranda post at the south side of the hut (Table 4). The exterior wallboards were painted with a terra cotta colored paint when erected and although little evidence of the paint is left on the wood the residual degraded paint appears to be present in the ground immediately next to the hut. Elevated concentrations of

Table 3. Elemental analyses of historic woods from the expedition huts of the Ross Sea Region. Elements in ppm.

Sample	Cu	Cr	Fe	Pb	Zn
***Discovery* Hut**					
South exterior wallboard	8	3	4454	38	40
Paint on interior wall	15	2	2577	1060	3452
Base of veranda post	47	6	2590	1271	39
Cape Evans hut					
Buried wood	39	8	3316	148	300
South exterior wallboard	19	4	431	6	100
Interior chemical spill					
Deteriorated shelf	1042	2194	16,980	964	36
Deteriorated table top	405	1802	9596	1801	134
Cape Royds hut					
Exterior fascia board					
South side	2	1	150	419	612
West side	5	2	724	8572	6637
North side	11	5	1595	10,988	22,894
Interior porch ceiling board	16	10	649	115	160
Deteriorated wooden crate	13	10	18,460	1088	84
Wooden crate on ground	17	1	2126	891	74
Exterior wallboard near ground on west side	14	3	2356	56	59

lead are not found at all locations around the hut and also are not found in samples taken a short distance away from the structure (Table 4: soil samples taken from the south side of the *Discovery* hut and from 3 m away from the hut). A sample of terra cotta paint located on an interior wall of the hut was analyzed and results indicated that the paint currently contained 1060 ppm lead (Table 3). Salt deterioration and extensive defibration of the exterior woods has been reported causing the surface fibers to detach (Blanchette and others 2002). Wind blasting particles of ice and grit also contribute to the degradation of the exterior wood surfaces. These weathering processes have apparently removed the lead paint coating that was on the wood and in some areas deposited the residual material in the ground below the wallboards. Since some sampling sites did not contain elevated lead concentrations (Table 3: south exterior wallboard sample at *Discovery* hut), the degraded paint and surface wood fibers may have been dispersed by strong winds or surface soils disturbed during previous work at the hut. Extremely high concentrations of lead and zinc were found on the north fascia board of the Cape Royds hut and elevated concentrations in the wallboards near the roof from the west and south sides of the hut (Table 3). Apparently a lead/zinc flashing material was used on the roof when the hut was built (but is no longer visible on the roof) and these concentrations most likely originate from these materials. Strips of lead-containing material have been observed by the authors around the Cape Evans hut; these presumably were part of the original roofing materials. Samples of soils and wood from storage crates that held canned goods and other food supplies also have elevated lead. These can be found in the refuse area near the Cape Royds hut, in storage goods

Table 4. Elemental analyses of soil samples from areas adjacent to the historic huts on Ross Island. Elements in ppm.

Sample	Cu	Cr	Fe	Mg	Na	Pb	Zn
***Discovery* Hut**							
South side of hut	15	11	17,308	15,086	11,323	18	50
East side near veranda post	23	32	27,917	18,551	13,253	298	64
West side of hut	28	35	24,550	17,279	12,888	1222	65
3 meters west from hut	20	41	34,315	20,669	12,522	21	71
Cape Evans hut							
North corner of hut	14	110	15,408	2708	15,071	7	125
Northwest side of hut	150	16	79,273	2096	9582	476	1083
Cape Royds hut							
North side of hut	12	3	37,418	2920	13,180	196	220
Refuse area near hut	252	11	33,106	4490	20,846	1621	159
East side of hut	7	1	101,256	3141	11,051	224	68

Fig. 6. Deteriorating cans and wooden crates of food stores left at the Cape Royds hut.

Fig. 7. Chemical spill inside the Cape Evans hut causing a deterioration of the wood. High concentrations of chromium and lead are associated with this caustic chemical spill.

Fig. 8. Bottles of chemicals on shelf in the Cape Evans hut. Most labels that identify the chemicals have deteriorated and cannot be read, but one labelled 'poison' remains.

Fig. 9. Many chemicals are still present in the Cape Evans hut, associated with the scientific apparatus left behind by the early explorers.

outside of the hut, and around the Cape Evans hut (Fig. 6). Many of the soils near these materials had concentrations of up to 1620 ppm lead (Table 4). In samples taken from soils without storage crates or deteriorated canned goods nearby (Table 4: sample taken from the north corner of Cape Evans hut), concentrations were only 7 ppm.

A very unusual type of wood deterioration was found within the Cape Evans hut affecting the shelves, wall, and table at the east end of the hut used by the expedition crew as a science laboratory (Fig. 7). Affected wood has been extensively defibrated, changing the normal structure of the wood to masses of brown fibrous material. Elemental analyses of this deteriorated wood showed high levels of copper, chromium, iron, and lead, suggesting that a caustic chemical spill had occurred. The chemicals were absorbed into the wood and have caused a destructive dissolution of the wood cell structure. This type of deterioration appears similar to advanced stages of attack observed by salts and other chemicals to wood (Blanchette and others 1991, 2002) where the lignified intercellular regions of the woody cell walls have been degraded. The deteriorated wood is also similar to degraded wood found in the historic laboratory of Thomas Edison, which apparently was affected by a similar type of chemical spill (Blanchette and others 1991). The chemicals caused a slow degradation of wood, and the process was allowed to continue unchecked because the laboratory is a protected historic building. In the Antarctic, the chemicals absorbed by the wood have had many decades to corrode and alter the cellular structure resulting in the current condition of the wood.

There are many chemicals in various unlabelled bottles, containers, and glass tubes or other scientific apparatus left within Cape Evans hut that should be evaluated to ascertain their identity (Figs 8 and 9). The high relative humidity found within the huts (authors, unpublished data) has promoted mold growth on paper, textiles, and even wood. These organisms have contributed to the poor condition of many of the labels on the chemical bottles and containers, and most have deteriorated. In the past, conservators from the Antarctic Heritage Trust have catalogued labelled chemical bottles in the historic huts, but many unknown substances remain. Chemical spills may occur by freeze–thawing of liquids and subsequent glass breakage, by inadvertent accidents

from curious tourists visiting the hut, or even accidental spills during conservation and research activities within the hut.

Recommendations for remedial actions

The early explorers inadvertently left toxic and hazardous materials in Antarctica that contaminate the historic sites and can have an adverse effect on the environment, wildlife, and human health. Remediation is needed and action by the international community is warranted to remove these pollutants from Antarctica. The following recommendations are provided as a forum for discussion on appropriate ways to remove these historic but hazardous materials from further contact with the Antarctic environment.

1. Petroleum spills. Large concentrations of polyaromatic compounds, many of which are known carcinogens, have spilled into the soils at the historic fuel depot areas. At the lower depot, the contaminants are present immediately below and around the containers and the contamination extends up to 1 m away. In the upper fuel depot, the contaminants appear restricted to the area immediately under and adjacent to the containers. The Protocol for Environmental Protection to the Antarctic Treaty (1991), in Annex III, Article 1, indicates that past and present waste-disposal sites on land and abandoned work sites of Antarctic activities shall be cleaned up by the generator of such wastes and the user of such sites. However, this does not include removal of any structure designated as a historic site or monument or the removal of any waste material in circumstances where the removal would result in greater adverse environmental impact than leaving waste material in its existing location. It appears appropriate that all containers should be emptied of their petroleum materials so that continued release of polyaromatic compounds into the environment does not occur. Since the levels of contamination in the soil exceeds current standards used to determine if clean-up is required at sites where petroleum spills have taken place, one method of control would be to remove the contaminated soil under and around the fuel boxes and transport it out of Antarctica for remedial treatment. The historic site, however, contains numerous artifacts on the ground and in the top layers of the gravel, and excavation and removal of the contaminated soil would need to be done under the supervision and guidance of an archaeologist. Disturbance of the historic site with its rich cultural heritage is of great concern, and drastic measures, such as the removal of large quantities of soil, need careful consideration. Additional studies are needed to determine if the level of contamination within the historic boundaries is confined to the site and if it poses a threat to the surrounding Antarctic environment. Since this is the oldest known petroleum spill in Antarctica, the site also has potential scientific value to serve as an experimental area for studying and isolating native micro-organisms that grow in the contaminated soils and may be able to degrade and detoxify these substances. These organisms could have potential for bioremediation of petroleum spills not only at Cape Evans but for use throughout the polar regions. The historic petroleum spills at Cape Evans will need careful consideration to determine the best action to take.

Motor tractors and vehicles were used by both Shackleton and Scott, and petroleum products apparently were used at all of the hut locations. Although this investigation documents the spills at the Cape Evans hut, additional but limited sampling did not detect petroleum compounds in soils around the *Discovery* hut or the Cape Royds hut. More extensive sampling is needed at these locations as well as other areas around the Cape Evans hut to determine if there are any other areas where petroleum contamination exist.

2. Materials containing asbestos that litter the area around Cape Evans should be removed. Several hundred pieces larger than 1 cm^2 are on the surface of the ground in the snow-free areas around the hut. These materials continue to be fragmented and abraded as visitors to the site walk around the hut. It is not known how much additional asbestos exists under snow and ice, but as it melts the exposed asbestos should be removed. The larger pieces of asbestos can be easily collected, properly contained, and shipped out of the Antarctic for disposal. The smaller sized materials (less than 1 cm^2) and materials below ground, however, will require significant effort to collect. The structure that contains the hut used for magnetic observations appears to be functioning. This structure has significant historic value and should remain at the site. Although the current wooden structure covering the hut functions very well to contain the asbestos-lined hut, Antarctica's severe weather is taking its toll on the wood (Blanchette and others 2002). Wind and salt will continually erode the plywood used in the building causing it to delaminate and deteriorate. This structure must be maintained and upgraded in the near future. A more stable and permanent containment building will be needed to insure that the large amount of asbestos in this historic hut remains confined to the building. A structure that can withstand severe storms (and possibly allow visitors to view the magnetic hut through Plexiglass or other similar material) is needed to prevent the possibility of an extremely large amount of asbestos material being broadcast into the environment should the containment structure be destroyed by katabatic winds or other extreme weather conditions.

The asbestos in the *Discovery* hut that is holding insulation materials to the wall is not in an area that could easily be abraded or fragmented by visitors but could be removed to avoid potential future problems. The asbestos around the pendulum apparatus, however, is adhered to the floor and is subject to disruption and shredding as visitors walk around this area. Removal of the asbestos should be considered or a consolidant and protective covering used to prevent further erosion of the material. Limiting visitor

access to the area with asbestos would also prevent any further disruption of the material and release of asbestos particles in the hut.

3. The presence of heavy metals around the hut from lead-deteriorated paint is localized to a few areas immediately adjacent to the *Discovery* hut. A greater concern is the heavy metals that appear associated with the deteriorated canned goods around the Cape Royds and Cape Evans huts. As more of the canned goods stored at the huts continue to deteriorate, additional metal ions will be released into the soil. The movement and distribution of these heavy metals into the Antarctic environment should be studied to determine if any potential hazards could develop. The lead/zinc flashing materials that are at the site should be removed to avoid future weathering and release of additional metal ions into the soil. The historic woods that currently have increased levels of lead pose no problems, but locations should be noted when future restoration work is done. High concentrations of chromium and lead exist in woods affected by the chemical spill inside the Cape Evans hut. Access to this area should also be restricted to avoid the fragile defibrated wood being disturbed and possibly dispersed within the hut. Since the caustic substance is still in the wood and deterioration is likely to be continuing, these areas need special conservation efforts to remove the toxic substances from the wood and consolidate the affected woods.

4. A review of all scientific apparatus and chemicals in the hut should be completed and toxic or hazardous materials identified. In the event of a future accidental chemical spill, materials to contain and clean up the chemicals are needed within the huts. Test tubes, flasks, and other scientific apparatus containing battery acids, caustic alkaline materials, or any hazardous material should be removed or secured to avoid any possibility of spillage and damage to the historic hut and artifacts.

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References

Aislabie, J., M.R. Balks, N. Astori, G. Stevenson, and R. Symons. 1999. Polyaromactic hydrocarbons in fuel-oil contaminated soils, Antarctica. *Chemosphere* 39: 2201–2207.

Blanchette, R.A., K.R. Cease, A.R. Abad, R.J. Koestler, E. Simpson, and G.K. Sams. 1991. An evaluation of different forms of deterioration found in archaeological wood. *International Biodeterioration* 28: 3–22.

Blanchette, R.A., J.E. Haight, R.J. Koestler, P.B. Hatchfield, and D. Arnold. 1994. Assessment of deterioration in archaeological wood from ancient Egypt. *Journal of the American Institute of Conservation* 33: 55–70.

Blanchette, R.A., B.W. Held, and R.L. Farrell. 2002. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. *Polar Record* 38 (207): 313–322.

Dougherty, G.J. 1985. Analysis of motor spirit from Captain Scott's Antarctic expedition 1910. *New Zealand Antarctic Record* 6: 43–44.

Harrowfield, D.L. 1981. *Sledging into history*. Auckland: Macmillan.

Harrowfield, D.L. 1995. *Icy heritage: historic sites of the Ross Sea region*. Christchurch: Antarctic Heritage Trust.

Mazzera, D., T. Hayes, D. Lowenthal, and B. Zielinska. 1999. Quantification of polycyclic aromatic hydrocarbons in soil at McMurdo Station, Antarctica.*The Science of the Total Environment* 229: 65–71.

Ministry for Housing, Spatial Planning and the Environment. 1994. *Environmental quality objectives in the Netherlands: risk assessment and environmental quality. Division Directorate for Chemicals, External Safety and Radiation Protection, P465*. Amsterdam: Ministry for Housing, Spatial Planning and the Environment.

Munter, R.C., and R.A. Grande. 1981. Plant tissue and soil extract analyses by ICP-AES. In: Barnes, R.M. (editor). *Developments in atomic plasma spectrochemical analyses*. Philadelphia: Heydon and Son: 653–673.

Pearson, M. 1992. Expedition huts in Antarctica: 1899–1917. *Polar Record* 28 (167): 261–276.

Quartermain, L.B. 1963. *Two huts in the Antarctic*. Wellington: R.E. Owen.

Scott, R.F. 1905. *The voyage of the 'Discovery'*. 2 vols. London: Smith Elder & Co.

Scott, R.F. 1913. *Scott's last expedition*. 2 vols. London: Smith Elder & Co.

Shackleton, E.H. 1909. *The heart of the Antarctic*. 2 vols. London: William Heinemann.

Shackleton, E.H. 1919. *South*. London: William Heinemann.

US Environmental Protection Agency. 1992. *Test methods of evaluating solid waste, physical/chemical methods, SW-846*. Third edition. Cincinnati, OH: Environmental Monitoring and Support Laboratory, Office of Research and Development.

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ORIGINAL PAPER

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Effects of oil spills on microbial heterotrophs in Antarctic soils

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Abstract Oil spillage on the moist coastal soils of the Ross Sea region of Antarctica can impact on populations of microbial heterotrophs in these soils, as determined by viable plate counts and a most probable number technique. Elevated numbers of culturable hydrocarbon degraders, bacteria and fungi were detected in surface and subsurface soils from oil-contaminated sites, compared with nearby control sites. Culturable yeasts were not detected in soil from coastal control sites, yet reached $>10^5$ organisms g^{-1} dry weight in contaminated soils. The presence of hydrocarbons in soils resulted in a shift in the genera of culturable filamentous fungi. *Chrysosporium* dominated control soils, yet *Phialophora* was more abundant in oil-contaminated soils. Hydrocarbon degraders are most likely bacteria; however, fungi could play a role in degradation of hydrocarbons or their metabolites. Depleted levels of nitrate detected in some contaminated soils and decreased pH may be the result of growth of hydrocarbon degraders. Numbers and diversity of culturable microbes from Antarctic soil varied depending on whether a pristine site or a human-impacted (in this case, by fuel spills) site is studied.

Introduction

The soils of the ice-free areas of Antarctica are formed under the most extreme conditions found on earth (Beyer et al. 1999). In the Ross Sea region, and along the Transantarctic Mountains, conditions are so extreme that higher plants are almost completely absent and the soils are microbially dominated ecosystems. Soils of the coastal fringe of East Antarctica and the Antarctic Peninsula are slightly warmer and moister; soil water contents are greater and the soils support a wider range of soil organisms and plant species. Because of the extremely slow rate at which soil processes operate, Antarctic soils are considered particularly susceptible to human-induced damage (Campbell et al. 1998). Petroleum hydrocarbons are the most likely source of pollution in the Antarctic (Cripps and Priddle 1991) and with tourism numbers to the continent on the increase, the potential for fuel spills also increases. While a number of papers describe the effects of fuel spill on marine ecosystems (Kennicutt et al. 1991; Cripps and Shears 1997), very little is known about the impact of hydrocarbons on land, despite the siting of most scientific stations on land.

Most reported fuel spills on Antarctic soils have occurred near scientific stations where storage and refuelling of aircraft and vehicles can result in such spills (Cripps and Priddle 1991). Oil contamination of soil was also a consequence of the Dry Valley Drilling Project (Parker and Howard 1977). Generally, the areas contaminated by terrestrial fuel spills are localised (Kennicutt et al. 1992) and the total area of contaminated soil in the Ross Sea region has been estimated at less than 2 ha (I.B. Campbell, unpublished work). Chemical characterisation of the hydrocarbon contaminants has identified *n*-alkanes from C9–C15 and polycyclic aromatic hydrocarbons (PAH), including those that occur on the USEPA Priority Pollutant List, though generally naphthalene and methyl naphthalenes are the predominant PAH detected (Aislabie et al. 1998, 1999).

As it is prohibitively expensive to transport contaminated soil from Antarctica to lower latitudes, decisions must be made about which oil-contaminated sites should be remediated and which should be left. These decisions need to be based on sound scientific information on both the fate of fuel spills, and their effects on terrestrial ecosystems. In this paper we report the effects of fuel spills on microbial populations in Antarctic soils, and in

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particular the effects on heterotrophic bacteria and fungi. To do this, we have compared the microbial properties of soils, both oil-contaminated and controls, taken from three locations in the Ross Sea region.

Materials and methods

Soil collection

Soil samples for analysis were collected from Scott Base on Ross Island (Lithic Haplorthel) (S77°50′53.9″; E166°45′40.7″), Marble Point (Typic and Glacic Haplorthel) (S77°25′9.9″, E163 40′55.1″) and the Wright Valley (Typic Anhyorthel) (S77 31′05.7″; E161°51′57.3″) (Balks et al., in press). Soils in the Scott Base area are impacted as a result of the establishment and habitation of the base for 40 years continuously; a hydrocarbon-contaminated site was sampled near a former storage area for drums of mixed oils. At Marble Point, the soil sampled was situated near the old Marble Point camp, which was inhabited from 1957 to about 1963 (Broadbent 1994). Oil stains were visible on the surface of the soil and it is assumed that they have been there for over 30 years. The soil samples selected for analysis from the Wright Valley came from a spill site near Bull Pass that occurred during seismic bore-hole drilling activities in 1985. The sampled site was downhill from the bore hole, and it is likely petroleum flowed to the sampling site along the interface between the soil and the relatively impermeable underlying bedrock. At each of the three sites the extent of the contamination appears to be limited to less than 100 m^2. Pits were dug at each site, and soil samples collected from a range of depths. The depth of the pits depended on the presence of an ice-cemented surface at Scott Base or Marble Point, or bedrock at Bull Pass. In addition, pits were dug and soil samples collected from control sites at least 30 m from the contaminated areas. Soil samples for microbial and chemical analysis were taken using an ethanol-swabbed trowel. Samples for all analyses, except for total petroleum hydrocarbon (TPH) analyses, were placed in sterile whirlpak bags, frozen at −20 °C, and transported to New Zealand for analysis. For TPH analyses, approximately 50 g soil was placed in gas-tight metal cans, and stored frozen until analysis in New Zealand.

Chemical analyses

Water content, pH, ammonia nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N), and total and available phosphorus were measured following Blakemore et al. (1987). Total carbon and total nitrogen were determined using a Leco FP-2000 analyser. To reduce the loss of hydrocarbons on drying, total carbon levels were determined using wet samples. Levels of total petroleum hydrocarbons were determined by extracting the samples in methylene chloride, and then analysing the extracts by capillary gas chromatography with a flame ionisation detector, essentially as outlined in EPA Method 8015 (US EPA 1987).

Microbial analyses

Total microbial counts were determined using epifluorescence microscopy with the stain diphenylamidino indophenol (DAPI) (Bottomley 1994). For enumeration of culturable heterotrophic bacteria and hydrocarbon degraders, soil (10 g wet weight) was shaken for 1 h at 4°C in 90 ml 0.1% (w/v) sterile sodium pyrophosphate containing 30 g glass beads (3 mm) and then diluted in phosphate-buffered saline as required. Numbers of culturable heterotrophic bacteria were then determined by plating soil dilutions onto R2A (Difco) agar plates and incubating at 16°C for 4 weeks. Relative differences in numbers of hydrocarbon-degrading microbes in the soil dilutions were determined by a most probable number technique, using 10 ml Bushnell Haas Medium (BH) (Difco) amended with 50 µl JP8 jet fuel as sole carbon and energy source. The five-tube method was used, and all tubes were incubated at 16°C for at least 6 weeks. Poisoned controls were prepared by adding 0.2 ml cHCl to each tube. To determine whether growth had occurred in the tubes, they were compared with the controls, and those that were both turbid and showed disruption to the film of oil on the surface of the medium were scored as positive.

To isolate and enumerate fungi (both filamentous fungi and yeasts), approximately 0.1 g soil, or soil dilutions in phosphate-buffered saline as described above, were inoculated onto yeast malt extract agar (Farrell et al. 1998). All plates were incubated at 16°C for at least 6 weeks. Representative colonies of filamentous fungi were purified, and their taxonomy derived both from their microscopic characteristics when stained with lactophenol blue, and by comparison with known Antarctic fungi (Sun et al. 1978) and "Illustrated genera of imperfect fungi" (Barnett and Hunter 1972). Presumptive yeast colonies were examined microscopically to confirm that they were unicellular fungal cultures.

Results

Chemical analyses

Chemical characteristics of the soils are shown in Table 1. Total petroleum hydrocarbon levels were elevated in oil-contaminated sites. The highest level of TPH was 29,100 mg kg^{-1} in surface soil from Marble Point. At the Marble Point site, hydrocarbon contamination was limited to surface soils and hydrocarbons were not detected below 27 cm, whereas at the Wright Valley site, high levels were detected in subsurface soils, yet were below detection in surface soil. TPH were not detected in soil from control sites.

The water content of all samples was low, but generally increased with depth. The lowest values were recorded in the inland soil from the Wright Valley, where the climate is more arid than the moister coastal sites of Scott Base and Marble Point. Lower water contents in surface soils are thought be due to drying by evaporation. The pH of the soils ranged from neutral (7.1) in soil from the Wright Valley to highly alkaline (9.6) in coastal soil from Marble Point; this range is typical of Antarctic soils. The pH levels of the surface oil-contaminated soils from Marble Point and Scott Base were slightly lower than the corresponding control sites. Total carbon content ranged from very low (0.02%) for the control site in the Wright Valley, to medium (5.3%) in the oil-contaminated soil from Marble Point. Enhanced levels of carbon at oiled sites can be attributed to hydrocarbon contamination. Total nitrogen content of all soil samples was very low. High levels of nitrate in soil from the Wright Valley are derived from small amounts of atmospheric nitrate in precipitation which have accumulated in these arid soils over very long periods of time (Claridge and Campbell 1977). Higher levels of nitrate were detected in control soil from Marble Point, compared with Scott Base. This may be an indication of a slightly greater age of the Marble Point soils, or perhaps of aridity and consequent lower biological activity (G.G.C. Claridge,

Table 1 Chemical properties of oil-contaminated and control soils from the Ross Sea region

Location	Depth (cm)	TPH (mg/kg dry wt)	Water content (%)	pH	Total C (%)	Total N (%)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	Total P (mg/kg)	Available P (mg/kg)
Scott Base										
Control	0–1	<30	1.8	8.9	0.10	0.006	3.3	1.3	1945	10
	1–7	<30	5.3	9.1	0.12	0.004	2.1	0.6	2157	13
	7–15	<30	3.7	8.9	0.09	0.003	1.5	0.3	2127	10
	15–30	<30	6.2	8.3	0.06	0.003	2.4	0	1806	12
Oil-contaminated	0–2	20300	1.5	7.8	4.72	0.010	2.4	0.2	1934	13
	2–10	21200	2.2	8.2	4.36	0.008	2.3	0.1	1699	12
Marble Point										
Control	0–3	<20	2.4	9.6	0.28	0.017	3.4	2.2	699	2
	3–15	<20	5.3	9.0	0.11	0.007	2.3	5.9	685	2
	15–32	<20	5.9	7.9	0.50	0.005	1.8	7.4	670	2
	32–45	<20	6.5	7.5	0.18	0.003	2.3	8.1	653	2
	45–70	<20	7.6	7.7	0.41	0.004	2.3	11.1	640	2
Oil-contaminated	0–3	29100	1.9	8.3	5.33	0.018	3.5	0.5	647	3
	3–12	18300	6.4	9.2	3.36	0.011	2.2	0.5	665	3
	12–27	200	11.4	9.5	0.20	0.004	3.2	0.5	608	10
	27–40	<20	11.8	9.3	0.14	0.002	2.3	0.5	734	6
	40–55	<20	11.5	9.2	0.21	0.002	2.4	0.5	775	6
	55–75	<20	12.5	9.2	0.20	0.002	2.4	0.5	513	3
Wright Valley										
Control	0–2	<20	0.2	7.6	0.03	0.008	1.5	0.0	221	0.6
	2–5	<30	1.0	7.7	0.02	0.005	1.2	2.9	227	2.7
	5–15	<30	1.9	7.3	0.02	0.052	0.3	129.0	265	2.8
	15–48	<30	2.0	7.1	0.02	0.037	0.2	103.8	271	4.1
	48–75	<30	3.0	7.2	0.02	0.034	0.0	82.1	250	2.6
	75–106	<30	4.5	7.4	0.03	0.031	1.1	86.5	228	1.4
	106–125	<30	4.5	7.5	0.03	0.031	0.1	76.8	233	1.3
Oil-contaminated	0–2	<30	0.4	7.4	0.02	0.01	1.2	24.5	340	0.4
	2–8	260	1.4	7.4	0.13	0.031	1.0	74.2	186	0.8
	8–16	1260	4.0	7.4	0.11	0.045	0.1	124.8	156	0.6
	16–35	960	4.8	7.3	0.07	0.045	0.2	116.6	210	0.6
	35–50	2470	4.3	7.3	0.04	0.024	0.2	52.0	209	0.7
	50–60	9000	9.3	7.6	0.20	0.02	0.2	41.2	261	0.7
	60–65	9500	14.2	7.6	0.34	0.019	0.2	40.2	303	0.7

personal communication). Levels of nitrate in oil-contaminated soils from Scott Base and Marble Point were depleted compared with control soils. Total P contents were low in the Wright Valley soils, medium in those from Marble Point and very high in soils from Scott Base. The levels of available P, however, were very low in soils from the Wright Valley and Marble Point, and low in soil from Scott Base.

Microbial analyses

Microbial characteristics of the soils are shown in Table 2. Total microbial counts ranged from 4×10^8 g^{-1} dried weight of soil to below countable levels. Highest numbers were detected in surface soils from Scott Base and Marble Point, with higher numbers in oil-contaminated soils from Marble Point compared with the control site. Total microbial counts of soils from the Wright Valley were below countable levels; however, bacteria were observed in surface soil samples from the control site. In general, numbers of microbes decreased down the soil profile. Microbes observed were predominantly bacteria; small cocci were observed in soil from control sites and larger rod-shaped bacteria in oil-contaminated samples. Occasionally, yeast cells were observed.

Numbers of culturable hydrocarbon degraders were elevated by up to 7 orders of magnitude in oil-contaminated soils from Scott Base and Marble Point compared with control sites. Generally, numbers decreased down the soil profile. Low levels were detected in control soils from Scott Base but not Marble Point. No hydrocarbon degraders were detected in soil from the Wright Valley site.

Culturable heterotrophic bacteria were detected in soil from Scott Base and Marble Point at all soil depths analysed; higher numbers (typically 2 orders of magnitude) were detected in oil-contaminated soils than control sites. Generally, numbers decreased down the soil profile. While bacteria were cultured from surface soils (0–8 cm depth) in the Wright Valley samples, lower down the soil profile bacteria were cultured only occasionally.

Culturable yeasts were detected in oil-contaminated soils from Scott Base and Marble Point but not in control soils, and numbers decreased with soil depth. At these sites the numbers of culturable yeasts reached 10^4–10^6 g^{-1} dried weight of surface soils. Similar levels were reported in contaminated soils from the former

Table 2 Microbial properties of oil-contaminated and control soils from the Ross Sea region (*ND* not detected)

Location	Depth (cm)	Total counts (g^{-1} dried weight)	MPN of hydrocarbon degraders (g^{-1} dried weight)	Number of culturable bacteria (g^{-1} dried weight)	Number of culturable yeasts (g^{-1} dried weight)	Number of culturable filamentous fungi[a] (g^{-1} dried weight)	Representative genera of filamentous fungi in order of decreasing appearance on plates[b]
Scott Base							
Control	0–1	1.2×10^8	33	3.4×10^6	ND	40	*Chrysosporium*, *Geotrichum*, *Phoma*, *Phialophora*, *Aureobasidium*
	1–7	1.0×10^8	230	4.1×10^5	ND	430	*Chrysosporium*, *Geotrichum*
	7–15	3.4×10^7	13	4.7×10^4	ND	36	*Chrysosporium*, *Geotrichum*
	15–30	5.7×10^6	13	2.5×10^3	ND	<10	Basidiomycete
Oil-contaminated drum storage	0–2	7.3×10^7	1.2×10^8	6.1×10^7	2.2×10^6	8.5×10^4	*Phialophora*
	2–10	6.6×10^7	1.2×10^8	1.4×10^7	2.8×10^4	4.4×10^4	*Phialophora*, *Phoma*, *Chrysosporium*
Marble Point							
Control	0–3	4.5×10^7	<10	3.7×10^5	ND	211	*Chrysosporium*, *Phoma*, Basidiomycete, *Alternaria*
	3–15	3.5×10^6	<10	8.6×10^4	ND	<10	*Chrysosporium*
	15–32	1.8×10^6	<10	2.4×10^4	ND	ND	
	32–45	$<10^6$	<10	9.3×102	ND	ND	
	45–70	ND	<10	<100	ND	<10	*Penicillium*, *Aureobasidium*
Oil-contaminated	0–3	4.0×10^8	1.1×10^7	5.3×10^7	1.4×10^5	2.3×10^4	*Phialophora*, *Aureobasidium*
	3–12	8.4×10^7	1.8×10^6	4.2×10^7	1.4×10^5	3.5×10^4	*Phialophora*, *Aureobasidium*
	12–27	9.5×10^7	8.8×10^4	6.6×10^6	1.7×10^3	677	*Phialophora*, *Penicillium*
	27–40	8.7×10^6	5.5×10^5	7.5×10^6	113	57	*Phialophora*
	40–55	3.8×10^6	1.6×10^4	3.2×10^6	508	<10	*Phialophora*
	55–75	2.4×10^6	3.7×10^5	3.1×10^6	1540	800	*Phialophora*, *Aureobasidium*
Wright Valley							
	0–2	$<10^6$	ND	5.6×10^3	ND	ND	
	2–5	$<10^6$	ND	8.4×10^3	ND	<10	Basidiomycete, *Aureobasidium*
	5–15	ND	ND	<100	<10	<10	*Geotrichum*
	15–48	ND	ND	<100	<10	ND	
	48–75	ND	ND	ND	ND	<10	Basidiomycete
	75–106	ND	ND	<100	ND	ND	
	106–125	ND	ND	<100	23	ND	
Oil-contaminated	0–2	ND	ND	<100	<10	<10	*Aureobasidium*
	2–8	ND	ND	1.4×10^2	<10	<10	*Sepedonium*, *Aureobasidium*
	8–16	ND	ND	ND	ND	ND	
	16–35	ND	ND	ND	ND	ND	
	35–50	ND	ND	<100	ND	<10	Unidentified
	50–60	ND	ND	ND	ND	<10	*Alternaria*
	60–65	ND	ND	<100	ND	ND	

[a] Determined on yeast malt extract agar
[b] Isolated on yeast malt extract agar or malt agar

McMurdo Dump site on Ross Island (Atlas et al. 1978). In the Wright Valley soil, low numbers of yeasts were detected in some samples.

Culturable filamentous fungi were detected in soil from Scott Base, Marble Point and the Wright Valley, though much lower numbers were cultured from soil from the Wright Valley. Filamentous fungi numbers tended to decrease with depth. Oil-contaminated soils from Scott Base and Marble Point had higher numbers of filamentous fungi than control soils. In control soils

from Scott Base and Marble Point, filamentous fungal isolates were predominantly *Chrysosporium* and *Geotrichum* species, whereas in oil-contaminated soils *Phialophora* was dominant.

Attempts to measure soil microbial biomass C using the fumigation-extraction technique (Vance et al. 1987) were unsuccessful due to low levels of microbial biomass (data not shown).

Discussion

Fuel spills on soil result in higher levels of soil carbon, which may either serve as substrates for microbial growth or prove toxic to microbial growth and activity (Bossert and Bartha 1984). In oil-contaminated soils from Scott Base and Marble Point elevated numbers of hydrocarbon degraders, and culturable heterotrophic bacteria and fungi, indicate that hydrocarbons spilled on Antarctic soils can serve as substrates for microbial growth. Elevated numbers of culturable microbes were detected in surface soils and lower down in the soil profile in moist coastal soils from Scott Base and Marble Point. No effects on culturable heterotrophs were observed in soils from the Wright Valley. However, at this site microbes were detected in surface soils rather than at depth where hydrocarbons accumulated. We have previously detected enhanced numbers of hydrocarbon degraders and heterotrophic bacteria in oil-contaminated surface soil samples from around Scott Base, Marble Point and near Lake Vanda in the Wright Valley (Aislabie et al. 1998; Balks et al. 1998). The low numbers of microbes detected in soils from the Wright Valley were not surprising as there have been reports of "sterile" soils from the Dry Valleys (reviewed by Vishniac 1993). Unlike numbers of culturable microbes, total microbial counts did not provide conclusive evidence of an increase in microbial abundance in response to oil spills. While higher total counts were detected in oil-contaminated surface soils from Marble Point, this was not observed for soils from Scott Base.

Unlike previous studies in Arctic tundra soils, increases in microbial populations in subsurface soils at Marble Point do not appear to have parallelled the downward migration of the oil (Sexstone and Atlas 1977). In the oil-contaminated location, elevated levels of microbes were detected at 55–75 cm, whereas total petroleum hydrocarbons were not detected below 27 cm. Of those bacteria able to be cultured, significant numbers are probably hydrocarbon degraders. The ratio of hydrocarbon degraders to culturable heterotrophic bacteria ranged from 1 in Scott Base soils to less than 0.2 in soils from Marble Point. This difference between sites probably reflects the time since oil spillage. While oil was spilled at Marble Point more than 30 years ago, spills at the Scott Base site could be less than a year old. The detection of elevated levels of hydrocarbon degraders, coupled with a decrease in soil nitrate levels and a decrease in soil pH in oil-contaminated soils from Scott Base and Marble Point, provide indirect evidence of the in situ growth and activity of indigenous hydrocarbon degraders at these sites.

Hydrocarbon degradative activity in Antarctic soils has been attributed to bacteria, due to their predominance in oil-contaminated soils (Kerry 1990). Hydrocarbon-degrading bacteria isolated from soils from the Ross Sea region have been identified as *Pseudomonas*, *Sphingomonas* or *Rhodococcus* species (Aislabie et al. 2000; Bej et al. 2000). The bacteria were cold tolerant rather than psychrophilic and were in most cases similar to degradative bacteria from cold climates. Significant enhancement in numbers of fungi in oil-contaminated soils indicate that they may also play a part in the degradation of hydrocarbons or their metabolites. Fungi are known to metabolise hydrocarbons (Atlas and Cerniglia 1995). In the soils examined in this study, a significant shift in the genera of culturable fungi was observed. While *Chrysosporium* dominated in control soils, *Phialophora* was dominant in hydrocarbon-contaminated soils from Scott Base and Marble Point. A similar observation was made by Kerry (1990) when examining soils from around Davis Station in East Antarctica. *Phialophora* spp. have recently been reported to degrade the hydrocarbon pyrene (Ravelet et al. 2000). Preliminary investigations of the in situ bacterial diversity of soils from Scott Base, both oil-contaminated and control, using molecular techniques, indicate a significant decrease in bacterial diversity in response to hydrocarbon contamination (D.J. Saul, unpublished work). It is possible that microbes capable of utilising hydrocarbons and/or their metabolites as a carbon source may dominate the oil-contaminated sites at Scott Base.

This study describes the effects of oil spills on culturable heterotrophic microbes in moist coastal soils of the Ross Sea region. The effects on microbes in dry inland soils are unclear from this study; at the Wright Valley spill site hydrocarbons accumulated in subsurface soils where microbes are either unculturable or non-existent. Further, attempts to extract DNA from these inland soils for in situ microbial diversity assessment have thus far been unsuccessful (D.J. Saul, unpublished work). The effects of hydrocarbon spills on other groups of microbes require investigation. While mineralisation of organically bound nitrogen was inhibited by the presence of McKee crude oil (Kohnleckner 1985), the effects of oil spills on other soil processes such as nitrogen fixation are unknown. The effect on Antarctic soil microfauna has also not been addressed. Attempts to determine the effects of hydrocarbons on nematodes in these soils were hampered by their low numbers (C.W. Yeates, personal communication).

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References

Aislabie J, McLeod M, Fraser R (1998) Potential of biodegradation of hydrocarbons in soil from the Ross Dependency, Antarctica. Appl Microbiol Biotechnol 49: 210–214

Aislabie J, Balks M, Astori N, Stevenson G, Symons R (1999) Polycyclic aromatic hydrocarbons in fuel-oil contaminated soils, Antarctica. Chemosphere 39: 2201–2207

Aislabie J, Foght J, Saul D (2000) Aromatic-hydrocarbon degrading bacteria isolated from soil near Scott Base, Antarctica. Polar Biol 23: 183–188

Atlas RM, Cerniglia CE (1995) Bioremediation of petroleum pollutants. BioScience 45: 332–338

Atlas RM, Di Menna ME, Cameron RE (1978) Ecological investigations of yeasts in Antarctic soils. Antarct Res Ser 30: 27–34

Balks MR, Aislabie J, Foght JM (1998) Preliminary assessment of the constraints to biodegradation of fuel spills in Antarctic soils. Proceedings of the World Congress of Soil Science, Montpellier, France 20–28 August 1998

Balks MR, Kimble J, Paetzold R, Aislabie J, Campbell I (in press) Effects of hydrocarbon contaminants on the temperature and moisture regimes of Cryosols of the Ross Sea region, Antarctica. Proceedings of the Second International Conference on Contaminants in Freezing Soil, Cambridge, 2–% July 2000

Barnett HL, Hunter BB (1972) Illustrated genera of imperfect fungi, 3rd edn. Burgess, Minneapolis, Minn

Bej AK, Saul DJ, Aislabie J (2000) Cold tolerance of alkane-degrading bacteria isolated from soil near Scott Base, Antarctica. Polar Biol 23: 100–105

Beyer L, Bockheim JG, Campbell IB, Claridge GGC (1999) Review. Genesis, properties and sensitivity of Antarctic Cryosols. ANS 11: 387–398

Blakemore LC, Searle PL, Daly BK (1987) Methods for chemical analysis of soils. NZ Soil Bureau Sci Rep 80

Bossert I, Bartha R (1984) The fate of fuel spills in soil ecosystems. In: Atlas RM (ed) Petroleum microbiology. Macmillan, New York, pp 435–473

Bottomley PJ (1994) Light microscopic methods for studying soil microorganisms. Methods of soil analysis, part 2. Microbiological and biochemical properties. Soil Science Society of America Book Series, no. 5, Madison, Wis

Broadbent ND (1994) An archaeological survey of Marble Point, Antarctica. Antarct J US 19: 3–6

Campbell IB, Claridge GGC, Campbell DI, Balks MR (1998) The soil environment of the McMurdo Dry Valleys, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. American Geophysical Union, Washington, DC, pp 297–322

Claridge IB, Campbell IB (1977) The salts in Antarctic soils, their distribution and relationship to soil processes. Soil Sci 123: 377–384

Cripps GC, Priddle J (1991) Hydrocarbons in the Antarctic environment. ANS 3: 233–250

Cripps GC, Shears J (1997) The fate in the marine environment of a minor diesel fuel spill from an Antarctic Research Station. Environ Monit Assess 46: 221–232

Farrell RL, Kay SJ, Hadar E, Hadar Y, Blanchette RA, Harrington TC (1998) Survey of sapstaining organisms in New Zealand and albina antisapstain fungi. Biology and prevention of sapstain. Forest Products Society Publication no. 7273. Whistler, Canada, pp 57–62

Kennicutt MC II, Sweet ST, Fraser WR, Stockton WL, Culver M (1991) Grounding of the Bahia Paraiso at Arthur Harbor, Antarctica. 1. Distribution and fate of oil related hydrocarbons. Environ Sci Technol 25: 509–518

Kennicutt MC II, McDonald TJ, Denoux GJ, McDonald SJ (1992) Hydrocarbon contamination on the Antarctic Peninsula. I. Arthur Harbor – subtidal sediments. Mar Pollut Bull 24: 499–506

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

Kohnleckner JC (1985) An investigation of the fate and effects of a paraffin based crude oil in an Antarctic terrestrial ecosystem. NZ Antarct Rec 6: 40–46

Parker C, Howard RV (1977) The first environmental impact monitoring and assessment in Antarctica. The Dry Valley Drilling Project. Biol Conserv 12: 163–177

Ravelet C, Krivobok S, Sage L, Steiman R (2000) Biodegradation of pyrene by sediment bacteria. Chemosphere 40: 557–563

Sexstone AJ, Atlas RM (1977) Mobility and biodegradability of crude oil in Arctic tundra soils. Dev Ind Microbiol 18: 673–684

Sun SH, Huppert M, Cameron RE (1978) Identification of some fungi from soil and air of Antarctica. Antarct Res Ser 30: 1–26

US EPA (1987) Test methods for evaluating solid waste: physical/chemical methods, 3rd edn. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19: 703–707

Vishniac HS (1993) Microbiology of Antarctic soils. In: Friedmann EI (ed) Antarctic microbiology. Wiley, New York, pp 297–341

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- Pre Antarctic field event planning and preparation.
- In Antarctica planning, preparation, sample collection, and visual observations.
- Specific research results of the PhD thesis author that were pre-publication were included in the Chapter including the findings that fungi were being isolated and positively screened for cellulase activity
- Scientific discussions and manuscript proof reading.

SCIENTIFIC EVALUATION OF DETERIORATION IN HISTORIC HUTS OF ROSS ISLAND, ANTARCTICA

Roberta L. Farrell, Robert A. Blanchette, Margaret Auger, Shona M. Duncan,
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Introduction

The British National Antarctic Expedition (1901-04) led by Robert F. Scott built a large wooden building at Hut Point on Ross Island, Antarctica, commonly referred to as *Discovery* Hut, to shelter and store supplies for 48 men for 3 years during their explorations of the South Polar Region. The British Antarctic Expedition led by Ernest Shackleton followed in 1907 and another hut was built on Ross Island at Cape Royds to house a shore party of 15 men. Scott returned in 1910 on the ill-fated *Terra Nova* British Antarctic Expedition. This 25-person expedition erected a large prefabricated hut at Cape Evans to provide accommodation and also built a smaller structure that was framed in wood and lined with asbestos sheeting for taking magnetic observations. *Discovery* Hut was used extensively by the latter expeditions in the Heroic Era as a key stepping stone to the southern latitudes and a shelter for those who returned from the south. Although all three expeditions had primary goals to discover new land and be first at the South Pole, they also had important scientific objectives. Each of the expeditions had one or more biologist, geologist, meteorologist and physicist to carry out the scientific programs. When the expeditions ended and relief ships arrived, a rapid exodus allowed only essential items to be returned to England. The huts and thousands of items were left behind, including food stores and fuel depots with unused containers of petroleum products, asbestos materials, and diverse chemicals.

A joint scientific collaboration began in 1997 between The University of Waikato in New Zealand and the University of Minnesota in the United States of America to evaluate the deterioration of the Ross Island historic huts and artifacts and their environs. The key to the collaboration has been to use state-of-the-art multi-disciplinary scientific methodology. Specifically, for the first time in the Antarctic, microbiology, wood chemistry, biochemistry, and molecular biology have been applied to the study of the deterioration, while the scientists have also worked with Antarctic Heritage Trust and conservation architects who are developing conservation plans for the Ross Dependency Historic areas. The four major goals of the collaboration are as follows:

1. Identify cause of non-biological & biological deterioration present in Historic Huts & artifacts.
2. Characterise environmental pollutants in the historic areas left behind from the 'Heroic Era' of exploration.
3. Test conservationally-acceptable materials for long-term preservation.
4. Investigate biodiversity in the Historic Hut areas, especially fungi and bacteria.

The collaboration has also determined the wood species used for the construction of the huts and various wooden artifacts found at the historic sites.[1] Identifications were made by taking small sections of wood from the structures and associated artifacts and examining them with light microscopy for anatomical characteristics, according to standard protocols. [2] This information is essential for conservation efforts if any of the deteriorated woods need to be replaced.

The Ross Island historic huts and surrounding areas attract many tourists as well as scientists and visitors from nearby McMurdo and Scott Bases and are therefore the most affected by decades of human activities of any Antarctic historic areas. Standards that guide research and conservation work conducted at the historic sites include those derived from the *Antarctic Treaty* (1959), and the Protocol on Environmental Protection to the *Antarctic Treaty*, known as the Madrid Protocol (adopted 1991) which provides, in Annex V, for the preservation and protection of historic sites as Specially Protected Areas or Specially Managed Areas. The Antarctica (Environmental Protection) Act of 1994 (the Antarctica Act) is the New Zealand legislation implementing The Protocol and under which all activities concerning the Ross Island historic huts pertain.
The Antarctic Heritage Trust (AHT) is a charitable trust formed in New Zealand in 1987 to conserve the historic

sites of the Ross Sea region of Antarctica. The joint scientific collaborative research results, an overview of which is reported in this paper, are directly contributing to the fulfilment of the work of AHT by demonstrating scientifically the state of the huts and environs.

Non-biological deterioration

Non-biological degradation processes can severely affect the physical and chemical structure of wood[3]. Morphological examination of minute wood samples, including light microscopy, scanning electron microscopy, and transmission electron microscopy are used by the Universities of Minnesota and Waikato collaboration to characterize decay patterns present. Chemical analyses for lignin, carbohydrates, extractives, etc. are difficult to carry out using very small samples, but minute samples are used with histological stains for light microscopy and electron dense stains for electron microscopy to identify and monitor the removal of cell wall components in deteriorated samples. Elemental analysis of wood is carried out by using multi-elemental inductively coupled plasma atomic emission spectroscopy.[3 a,b,c] These techniques have been very successful in other studies using small samples of archaeological woods to ascertain the type of degradation present and reveal important information on the current condition of the wood, extent of cell wall degradation and zones with the greatest structural losses[3 a].

Ultraviolet (UV) light, iron corrosion products, salts and other caustic compounds cause a deterioration that progresses from wood surfaces to inner regions of the wood. UV light may cause a selective attack of lignin and hemicellulose resulting in a defibration of the wood. Over time a gradual loss of the outer wood cells takes place and the surface gradually erodes away. Salt accumulations in wood cause chemical erosion of the lignified middle lamella and alterations to cellulose within the secondary walls. This chemical attack has only recently been described and the conditions for its occurrence elucidated.[4] Damage may occur quickly where large concentrations of salt are in contact with moist wood or very slowly as low concentrations of salt accumulate in wood after evaporation. Figure 1a illustrates cell damage of a sample of wood from the exterior wall of the Cape Royds Hut showing extensive disruption of wood tracheids. Cells have separated at the middle lamella region and appear defibrated. The secondary walls of latewood regions are intact, but the wood has little structural integrity left. The diffuse nature of the damage throughout the wood, lack of fungal mycelia and selective attack on the lignified middle lamellae suggest that deterioration of the surface layers of the wood is the result of salt attack. Although the exact process of salt deterioration in wood is not fully understood, it is apparent that the high salt concentrations cause a chemical reaction to take place in which the hemicellulose and lignin in the middle lamella is degraded.[4] This is exhibited in affected woods by a defibration of surface fibers, giving the wood surface a fuzzy appearance that can be seen in Figure 1b. There are many locations at all three huts that are affected by salt deterioration. All of these locations involve moisture absorption from melt water in pools on the ground, or by the melting of snow from the roofs directly on the huts or artifacts.[4]

Fig. 1a Scanning electron micrograph of a transverse section of wood showing a loss of middle lamella and a separation of cells.

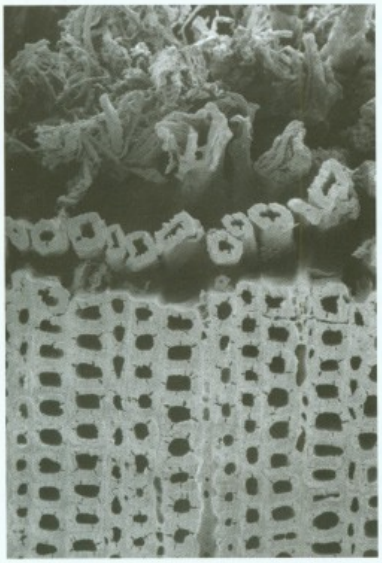
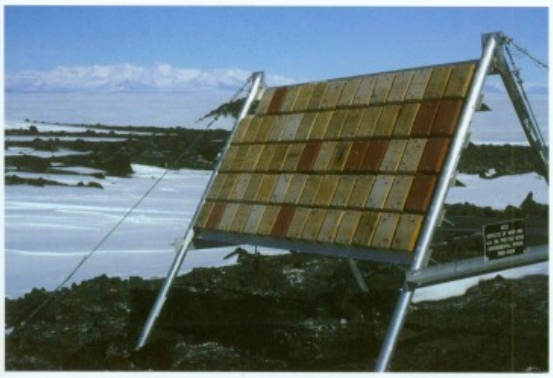


Fig. 1b Non-biological deterioration from Cape Evans hut. Chemical deterioration causing a defibration of wood. As snow melts, large amounts of the wood fibers are removed and the historic wood is gradually destroyed.



Test panel erected in January 2000 at Cape Evans. Similar test panels also erected at Cape Royds and Hut Point.

Wind erosion can be identified in many locations of the huts and associated artifacts.[5] High velocity winds originating from the South Pole carry airborne ice and scoria particles that cause a sand blasting effect on the exposed wood. Therefore most of the significantly eroded areas are those that face south. By using digital videography over the past six years the collaboration has documented that the exterior wood is not eroded uniformly by wind. Windborne particles erode the highly lignified, thick-walled latewood cells at a slower rate than the thin-walled earlywood cells, leaving affected wood with an uneven, furrowed appearance.

Three test panels, one at each hut location, were established to test possible treatments for use on the huts. Blocks of wood were treated, fixed to the panels, and maintained on the panels or removed for examination and study in laboratories. New treatments and wood combinations can be subsequently fixed to the panel. For initial consideration, in Austral Summer 2000, blocks of pine and spruce were treated with four silicon-based treatments and an oil based paint similar to the type that was originally used on *Discovery* Hut, and fixed to the panels. These wood blocks have been assessed and significant erosion has been shown to take place after only two years of exposure. These treated wood blocks are being monitored for the next 5-8 years.

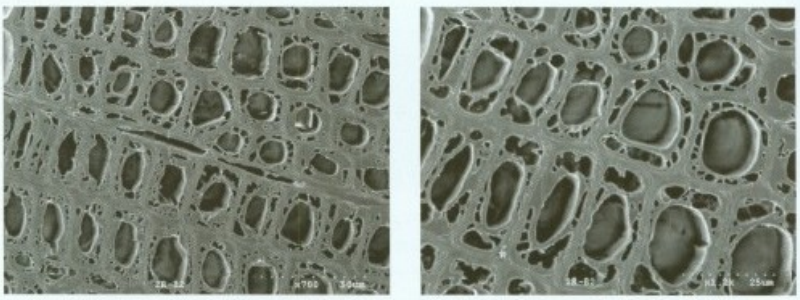
Biological deterioration and biodiversity in the Historic Huts

Biological degradation of wood and other organic matter is common in the huts. Actively growing fungi have been observed and isolated from walls, floors, ceilings and beams, clothing, leather, wood, foodstuffs and other artifacts within the huts. Previous investigations of mycoflora in the historic areas on Ross Island, Antarctica focused on long-term survival of microorganisms. Meyer, et al. (1962)[6] demonstrated the viability of filamentous micro-fungi from a sealed bottle of yeast from Cape Evans hut. They also isolated *Mucor* sp. from tinned barley from Shackleton's hut at Cape Royds and *Penicillium* sp., and unidentified dematiaceous fungi from hay at Cape Evans.[7]

The Universities of Waikato and Minnesota joint collaboration has isolated and identified wood decaying microorganisms present in the Historic Huts and environs, and addressed the general biodiversity of microorganisms present.[8] Sample collection was initiated by identifying affected wood, artifacts, soil, ice, debris etc. and placing small segments of these in sterile vials on site. Permits have been granted by the Ministry of Agriculture and Forestry, New Zealand to bring samples out of Antarctica. These samples are cultured in the Universities' laboratories on a wide variety of growth media, at various temperatures, typically in the range of 0 to 25 degrees Centigrade, for isolation of fungi and/or bacteria, or studied by molecular techniques. Pure cultures of fungi have been obtained and identified using various taxonomic keys from the mycological literature and/or molecular probes; examples of some of these from Cape Evans historic site are given in Table 1.[9] Cellulases, enzymes that catalyse the degradation of cellulose in fibers, such as wood and/or cotton textiles, have been isolated from several of these organisms and are now being characterised as to their role in the decay of wood at temperatures experienced within the huts. Environmental data loggers were placed by the joint collaboration at various locations and at varying heights with six dataloggers in each hut. The data is currently being processed to give both macro and microclimate information and has revealed that during the austral summer, temperatures rise above freezing and relative humidity within the structures is often well over 80% providing conditions conducive to microorganism growth and enzymatic activity.[1]

Filamentous micro-fungal diversity was also identified from the historic materials at *Discovery* Hut at Hut Point.[10] There were 22 taxa and 14 genera recorded from this study. Many cosmopolitan genera were isolated from the samples collected inside *Discovery* Hut. *Penicillium* species were most frequently isolated. Isolations made from straw samples contained a variety of other fungi. Visible fungal colonies on the wall and floor also contained many filamentous micro-fungi which were not found in the other samples.

An unusual wood destroying fungus is causing decay in the historic woods that are in contact with the ground.[11] Micromorphological examinations indicate just one type of decay, a soft-rot, is present in all of the



Scanning electron micrographs of transverse sections of historic wood from Cape Royds hut showing attack of the secondary wall layers by soft-rot fungi. The fungus penetrates the secondary cell wall and causes large cavities that coalesce causing significant damage.

deteriorated woods. The fungus grows into the wood cells, forming elongated cavities within the secondary wall layers. Soft rot fungi were isolated from all three of the historic huts in the Ross Sea region, but were found most prevalent in wood from Shackleton's Cape Royds hut. Pure cultures obtained from the historic woods were identified by morphological characteristics and phylogenetic analysis. Investigations of microbes in Antarctic soils and moss[12/13] revealed that *C. malorum* and other *Phialophora*-like fungi are present at many different locations, suggesting these fungi are endemic to Antarctica. Although it has been millions of years since trees grew on the Antarctic continent, fungi there have retained their degradative enzymes to attack wood when it is in contact with soil. A previous report has shown the presence of *Phialophora*-like species in preservative treated wood and indicated their tolerance for high concentrations of copper, arsenic and other toxic compounds.[14] A better understanding of what allows these organisms to live where others are inhibited is needed[15], and more information on the biochemistry of degradation is needed. New knowledge of these polar fungi is needed if we are going to find effective controls that can be used to preserve the huts long into the future. These microbes living at earth's most extreme environments will undoubtedly be a formidable challenge to control. Little is known about the mechanisms in which filamentous fungi can survive numerous freeze-thaw cycles[16] and how introduced organisms can adapt in the Antarctic climate regime.[17] Successful conservation of the huts requires an understanding of these mechanisms and the biology and ecology of these decay organisms so degradation processes can be controlled.

Another remnant of the Heroic Era on Ross Island is the extensive stores of seeds outside the huts, particularly at Cape Evans and Cape Royds. Concerns have been raised about the impact on the antarctic environment and fauna of both the seeds and micro-organisms proliferating on them – fungi and bacteria are actively growing on the seeds and have been identified. Alas for historic interests the seeds have not been able to be germinated.

General environment of the Historic Huts and environs

The environment of the Historic Huts is also being scientifically studied.[1] There are many chemicals in various unlabelled bottles, containers and in glass tubes or other scientific apparatus left within the huts that should be evaluated to ascertain their identity. The high relative humidity found within the huts has promoted mold growth on paper, textiles and even wood. These organisms have contributed to the poor condition of many of the labels. Chemical spills may also still occur by freeze-thawing of liquids and subsequent glass breakage or by inadvertent accidents from curious tourists who visit the huts, or even conservation and research activities within the huts. An historic chemical spill within the Cape Evans hut, apparently from caustic substances from one of the scientific experiments, has caused an unusual deterioration and defibration on affected woods. This deteriorated wood is similar to degraded wood found in the historic laboratory of Thomas Edison that was

affected by a similar type of chemical spill[18]. The chemicals caused a slow degradation of wood and the process continued unchecked since the laboratory is a protected historic building. In the Antarctic, the chemicals absorbed by the wood have had many decades to corrode and alter the cellular structure, resulting in the current condition of the wood.

Decaying stores around the hut are degrading the environment, and under current environmental protocols for Antarctica intervention to prevent further pollution is imperative. Fuel depots with unused containers of petroleum products, asbestos materials, and diverse chemicals were also left at the huts. The joint collaboration found high concentrations of polyaromatic hydrocarbons in soils under and around the historic fuel depots. Asbestos materials within the huts have been identified and extensive amounts of fragmented asbestos were found littering the ground around the Cape Evans hut. Within a relatively small area immediately adjacent to the hut, several hundred fragments of asbestos-containing materials are located on the ground surface. These materials are continually abraded and fragmented as tourists walk over them and the coarse scoria breaks and grinds down the materials. Wood and soil samples containing lead and other heavy metals have also been identified at the huts. Although these areas are important historic sites protected by international treaties, the hazardous waste materials left by the early explorers should be removed and remedial action has been proposed to restore the site to as pristine a condition as possible.

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References

(1) Held, B.W., Blanchette, R.A., Jurgens, J.A., Duncan, S., and Farrell, R.L., 2003: Deterioration and Conservation Issues Associated with Antarctica's Historic Huts. In: Koestler, R.J., Koestler, V.R., Charola, A.E., and Nieto-Fernandez, F.E., (Eds.), Art, Biology, and Conservation: Biodeterioration of Works of Art. The Metropolitan Museum of Art, New York, 370-389.

(2) Panshin, A.J., Zeeuw, C., 1970: Textbook of Wood Technology. Vol. 1. McGraw-Hill, New York.

[3 a] Blanchette, R.A., Haight, J. E., Koestler, R. J., Hatchfield, P. B. Arnold, D., 1994: Assessment of deterioration in archaeological wood from ancient Egypt. Journal of the American Institute of Conservation 33, 55-70.

[3 b] Munter, R. C., Grande, R. A. 1981: in *Developments in atomic plasma spectrochemical analyses*, ed. Barnes, R. M. (Heydon & Son, Philadelphia), pp. 653-673.

[3 c] U. S. Environmental Protection Agency 1992: *Test methods of evaluating solid waste, physical/chemical methods, SW-846*, 3rd edition (Environmental Monitoring and Support Laboratory, Office of Research and Development, Cinncinnati, Method 3051).

(4) Blanchette, R.A., Held, B.W., Farrell, R.L. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. 2002: Polar Record 38 (207): 313-322.

(5) Held, B.W., Blanchette, R.A., Duncan, S., Farrell, R.L. 1999: An Assessment of deterioration in the historic huts of Antarctica. International Biodeterioration and Biodegradation *44*:163.

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

(7) Meyer, G. H., Morrow, M. B., Wyss, O. 1963: Viable organisms from faeces and foodstuffs from early Antarctic expeditions. Canadian Journal of Microbiology 9. 163-167.

(8) Blanchette, R.A., Held, B.W., Farrell, R.L., Duncan, S. 2000: Wood deterioration in the historic huts of Antarctica. Phytopathology 90(6):7.

(9) Farrell, R.L., Duncan, S.M., 2003: Bioprospecting in Antarctica, Proceedings Gateway to Antarctica Workshop, Christchurch, NZ.

[10] Minasaki, R., Duncan, S., Farrell, R.L., Blanchette, R.A., Held, B.W., Jurgens, J.A., Watson, N. 2001: Mycological Biodiversity Associated with Historic Huts & Artefacts of the Heroic Period in Ross Sea Region. Scientific Committee on Antarctic Research. VIII SCAR International Biology Symposium. Antarctic Biology in a Global Context. Handbook. S6P19.

[11] Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., Farrell, R.L., 2003: Wood Destroying Soft-rot Fungi in the Historic Expeditions Huts of Antarctica. Applied and Environmental Microbiology, submitted.

[12] Aislabie, J., Fraser, R., Duncan, S., Farrell, R.L. 2001: Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biology, 24:308-313.

[13] Tosi, S., B. Casado, R. Gerdol, and G. Caretta. 2002: Fungi isolated from Antarctic mosses. Polar Biology 25:262-268.

[14] Daniel G. F. & Nilsson, T. 1988: Studies on preservative tolerant Phialophora species. International Biodeterioration. Int. Biodeterioration 24, 327.

[15] Vishniac, H. S. 1996: Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems. Biodiversity and Conservation 5. 1365-1378.

[16] Vincent, W. F. 2000: Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarctic Science 12. 374-385.

[17] Blanchette, R.A., Held, B.W., Jurgens, J.A., Aislabie, J., Duncan, S.M., Farrell, R.L. 2003: Environmental pollutants in Antarctica from the Robert F. Scott and Ernest H. Shackleton expeditions during the 'Heroic Era' of exploration. Polar Record, In press.

[18] Blanchette, R. A., Cease, K. R., Abad, A. R., Koestler, R. J., Simpson, E. & Sams, G. K. 1991: An evaluation of different forms of deterioration found in archaeological wood. *International Biodeterioration* 28, 3-22.

Table 1. **Isolation of fungi from Cape Evans hut and artifacts**

Fungal Genus Species	**Origin of wood sample**
Cladosporium sphaerospermum	Wall behind table, Wall under bed Wood near floor on south wall NE corner post
Geomyces pannorum	NE corner post Damp spot on interior wall south side Table cloth
Myceliophthora	Damp spot south wall Inside Door step Wall behind Mutton
Penicillium chrysogenum	Floor at base of south wall
Penicillium spinulosum	Dark Room Wall
Penicillium verrucosum	Wall near floor south side
Cadophora malorum	Damp spot south wall
	Wall behind table Interior wood

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PhD thesis author Shona M. Duncan was a co-author of this publication and contributed as follows:

- Pre Antarctic field event planning and preparation.
- Organised construction of wood test panels sourced and prepared wood blocks.
- In Antarctica planning, preparation, sample collection, and visual observations.
- Scientific discussions and manuscript proof reading.

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DETERIORATION AND CONSERVATION ISSUES ASSOCIATED WITH ANTARCTICA'S HISTORIC HUTS

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ABSTRACT During Antarctica's heroic era, explorers used Ross Island as a starting point for expeditions to the South Pole and other regions of Antarctica. Two huts built by Robert F. Scott in 1902 and 1911, and one built by Sir Ernest Shackleton in 1908, were used to escape the extreme Antarctic environment during their explorations. Large quantities of supplies brought to sustain the expeditions for several years were stored in and around the huts. Once the expeditions were completed, the huts and unused supplies were abandoned. Antarctica's dry, cold environment has provided some protection to the huts and artifacts, but significant deterioration has occurred over the past 9 to 10 decades and serious issues concerning preservation must be addressed if the huts are to be successfully preserved.

Biological degradation of wood is common in the huts. A soft-rot type of decay was found to be attacking wood in contact with the ground at several locations. The fungal species responsible for this decay are being characterized and studied. Actively growing fungi have been observed and isolated from clothing, leather, wood, foodstuffs, and other artifacts within the huts. Environmental data loggers placed at various locations throughout the three huts have revealed that during the austral summer, temperatures rise above freezing and relative humidity within the structures is often well over 80%, providing conditions conducive to mold growth.

The impact of visitors on relative humidity inside the huts was also studied. Nonbiological deterioration is evident in the huts, resulting in a defibration of wood surfaces. This unusual corrosive type of chemical degradation is caused by salts that affect the lignified regions of the woody cell wall, resulting in cell separation. Snowmelt carrying dissolved salts is absorbed by the wood and evaporated at the surface. Elemental analyses have shown that very high concentrations of salts are present in affected woods. Wind abrasion has also caused significant erosion of the huts' exterior wood.

Wood treatments are being studied in field tests at three locations on Ross Island, Antarctica, to determine their effectiveness as possible protectants for the exterior of the huts. Results from this work are providing important insights into how wood deteriorates in the polar environment and are creating a foundation of information to initiate conservation plans to preserve these historic sites for future generations.

INTRODUCTION In January of 1902 Robert F. Scott's Discovery expedition built the first of three huts in the Ross Sea Region of Antarctica. [FIG. 1] Modeled after an Australian outback structure, the 9.3 × 9.1-m hut was prefabricated and built at Hut Point on Ross Island to be used as living quarters and to store supplies. Explorations of the region were made, and scientific information was collected during the expedition. Sir Ernest Shackleton, who served with Scott during the Discovery expedition, mounted his own "British Antarctic Expedition" and returned to Antarctica with hopes of being the first to the South Pole. Shortly after arriving on Ross Island in early 1908 he built a hut 13 km north of Hut Point at Cape Royds. [FIG. 2] Situated adjacent to a large Adele penguin rookery, it is the smallest of the three huts studied and measures 7 × 5.8 m. The hut was used for several years during the expedition and was vital for survival while the expedition members readied themselves for the long journey to the pole. Having fallen 97 miles short of reaching the pole, Shackleton returned to England, leaving the hut and all materials associated with the expedition at the site. Scott returned to Antarctica in 1910 on the ill-fated Terra Nova expedition. He built a large hut, 15.5 × 7.5 m, at Cape Evans. [FIG. 3] This hut was the most spacious of the three huts on Ross Island and had a stable attached for the ponies that were brought from England for hauling supplies.

Since each of the expeditions left quickly when the relief ships arrived, the huts and thousands of artifacts were left behind. The dry cold conditions of the Antarctic environment have provided some protection from decay for the huts, but after

[1] Robert F. Scott's 1902 Discovery expedition hut in the Ross Sea region of Antarctica. [2] Sir Ernest Shackleton's 1908 hut 13 km north of Hut Point at Cape Royds, Ross Island. [3] Scott's 1910 Cape Evans hut from his Terra Nova expedition.

basidiomycete select agar (Worrall, 1991), and a 1.5% Difco acidified malt agar. Wood samples were also prepared for scanning electron microscopy using previously described methods (Blanchette et al., 2002).

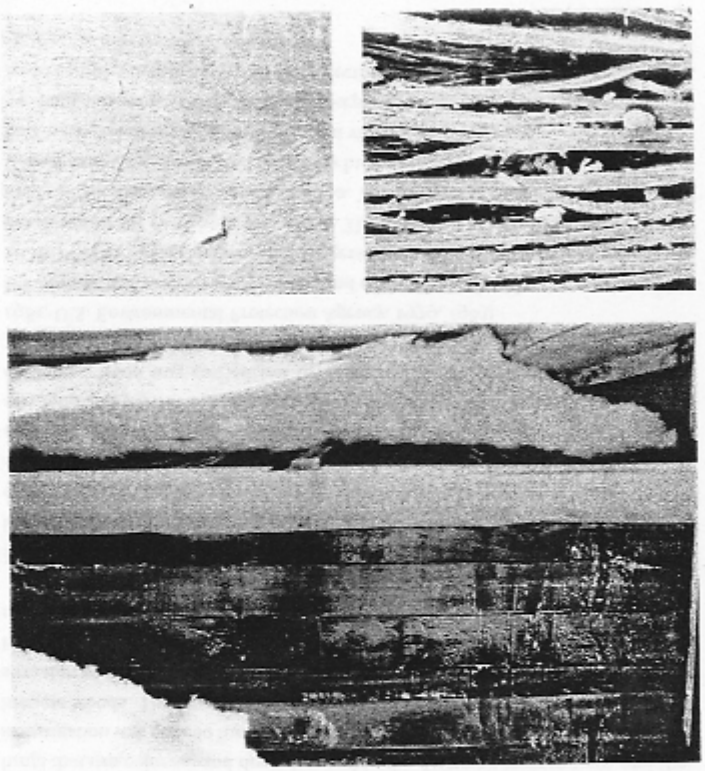
Wood identifications were made by taking small sections from wood and examining them with light microscopy for anatomical characteristics (Hoadley, 1990; Panshin and Zeeuw, 1970).

RESULTS

Wood Identification A large variety of different woods were used to construct the huts. [TABLE 1] The major woods used for Cape Evans and Discovery huts were *Pinus sp.* with characteristics of a hard pine. The species is a hard pine, likely *P. sylvestris*, as the wood originated from Europe. This wood was used for the floors, walls, and ceilings of the huts. Cape Royds hut is constructed primarily out of *Picea*. All plywood supply crates were identified as *Betula sp.* at all locations. Douglas fir is another important species and was used for the beams and corner posts on the veranda of Discovery hut. Boards that were replaced during conservation work many years ago on a section of the veranda siding are also Douglas fir.

Salt Deterioration Salt deterioration can be commonly found affecting the huts and associated artifacts. Affected wood has a fuzzy appearance when dry due to the separation of surface fibers. [FIGS. 4 AND 5] Under wet conditions affected surfaces of wood have the appearance of having been pulped. A severe defibration is taking place on the exterior siding of the stables on Cape Evans hut. [FIG. 6] Snow with elevated amounts of salts accumulates on the roof of the stables and melts as temperatures rise in the austral summer, causing melt water with significant amounts of sodium and chloride ions to drip onto the side wallboards. [TABLE 3] This salt solution cascades down the siding and is absorbed by the wood. The salts accumulate in the wood and deterioration occurs. The surface wood fibers become defibrated and detach from one another. Boards replaced by conservators on the hut as recently as 1991, from above the stables area, are already showing significant defibration when observed in 2002.

Cape Royds hut and Discovery hut are also affected by salt deterioration. At Cape Royds, the damage is especially severe along the north side in the stables area. Some of the most severe damage found on the interiors of the huts is in the porch of Cape Royds hut, in which the ceiling and wall boards are extensively bleached and defibrated. Table 2 shows concentrations of sodium ions of this wood exceed 50,000 ppm. Many wooden supply boxes located on the south and east sides of the



[4, 5] Salt-deteriorated wood showing a fuzzy appearance when dry due to the separation of surface fibers.
[6] Exterior siding of the stables of the Cape Evans hut showing severe defibration.

(TABLE 1) Wood identifications made from the Ross Island historic huts and associated artifacts

WOOD ID	LOCATIONS	
	INTERIOR	EXTERIOR
CAPE EVANS HUT		
Abies	Scott's table, supply boxes	NW siding, stables roof slats
Betula	Supply boxes (plywood)	Supply boxes (plywood)
Picea	Main table, darkroom, supply boxes	NE siding, stables siding, latrine, cross, sledge NW corner
Pinus (sylvestris)	Ceiling, floor, walls	NE, SE siding, fuel boxes, latrine
Pseudotsuga menziesii	Not found	Replaced siding on hut above stables
CAPE ROYDS HUT		
Abies	Supply box in kitchen	Doghouse
Betula	Supply boxes (plywood)	Supply boxes (plywood)
Picea	Ceiling, floor, walls, supply boxes	Siding, fence in stables, supply boxes
Pinus (sylvestris)	Ceiling beams, facia	Facia, meteorological station
Ulmus	Not found	Supply box in garage
DISCOVERY HUT		
Picea	Supply boxes, wall repair	Not found
Pinus (sylvestris)	Floor, walls, ceiling	Siding, roof, veranda
Pseudotsuga menziesii	Beams, floor repair	Beams, veranda corner posts, corner siding, replaced siding N side veranda

9–10 decades serious deterioration has occurred, and these problems must be addressed if the huts are to be preserved for the future.

Nonbiological types of deterioration are the most common forms of deterioration associated with the huts and artifacts. One very prevalent type of attack is due to high salt concentrations that corrode the wood surfaces. This chemical attack has only recently been described and the conditions for its occurrence elucidated (Blanchette et al., 2002). Wind erosion is another very serious form of nonbiological deterioration that occurs commonly on exterior woods, especially those with a southerly exposure. Biological deterioration can also be commonly found in association with the wood of the huts and artifacts that is in contact with the ground. A soft-rot type of decay has been found attacking woods in the stables area at the Cape Royds hut and in wooden artifacts at various locations at the historic site. The interiors of the huts have been affected by surface molds. Little is known about the fungi that can colonize and degrade wood in the Antarctic environment, and this investigation was done to further investigate their role in wood deterioration in the historic woods. This study also determined the wood species used for the construction of the huts and various wooden artifacts found at the historic sites. This information is essential for conservation efforts if any of the deteriorated woods need to be replaced.

MATERIALS AND METHODS Minute wood samples were taken from inconspicuous locations on the huts and other artifacts for fungal isolations, salt deterioration evaluations, elemental analysis, and wood identification, and they were stored in sterile containers until they were later studied in the laboratory. This work was done in cooperation with the Antarctic Heritage Trust and under Antarctic Conservation Act permit numbers 2001–015 and 2002–001. Elemental analysis of defibrated wood was carried out by using multi-elemental inductively coupled plasma atomic emission spectroscopy (Blanchette et al., 1994; Munter and Grande, 1981; U.S. Environmental Protection Agency, 1979, 1983).

Interior and exterior temperature and relative humidity data were taken using HOBO® H8 Pro data loggers. Data loggers were calibrated to take readings of temperature and RH every hour year round. The data loggers were downloaded during field visits to the huts in 2001 and 2002. The number of data loggers used in the Cape Evans, Cape Royds, and Discovery huts are 6, 5, and 4, respectively. Data loggers were placed throughout the huts at varying heights.

Fungal isolations were made by aseptically removing small segments from wood samples and placing them in selective media: 1.5% Difco malt extract agar,

hut are also damaged. Supply boxes made from plywood are not only defibrated but have also become delaminated. Wood sampled from beneath the veranda at Discovery hut had the highest concentration of salts from all woods sampled, with sodium concentrations of 246,321 ppm. [TABLE 2]

The huts themselves are not the only woods being affected by salt deterioration. Artifacts made from wood and exposed to salts and moisture are also affected. Wooden crates, doghouses, sledges, and even nonwooden objects such as rattan pony snowshoes, are being deteriorated. Salts also cause significant corrosion of metals at the historic huts.

Wind Erosion Wind erosion can be identified in many locations of the huts and associated artifacts. High-velocity winds originating from the South Pole carry airborne particles that cause a sand-blasting effect on the exposed wood. Therefore most of the significantly eroded areas are those that face south. Wood is not eroded uniformly by wind. [FIG. 7] Windborne particles erode the highly lignified, thick-walled latewood cells at a slower rate than they do the thin-walled earlywood cells, leaving affected wood with an uneven, furrowed appearance.

Three test panels, one at each hut location, have been set up to test possible treatments for use on the huts. Blocks of pine and spruce were treated with four silicon-based treatments and an oil-based paint similar to the type that was originally used on Discovery hut. Wood blocks have been assessed and more time is needed to complete the study. Significant erosion is taking place on the control blocks after only two years of exposure.

Biological Deterioration Minute samples from many areas suspected of being decayed were collected and examined. A unique form of decay has been found degrading wood in contact with the ground. It is described as a soft-rot type of decay causing cavities to form in the cell walls. Isolations have indicated that the decay is caused by *Phialophora*-like fungi. Figure 8 shows extensive cavities caused by the soft-rot fungi located in the secondary walls of the wood cells. [FIG. 8] Affected wood was from the stables area at Cape Royds hut. This area has significant amounts of penguin guano due to penguins gathering in this location for short periods of time. Pure cultures of the decay fungi have been obtained and are being characterized and studied further. Cultures similar to those causing soft rot have been isolated from many other woods at all three hut locations.

In addition to decay fungi, extensive mold growth has been found in Cape Evans hut. [FIG. 9] The fungal growth is most common on wood near or on the floor and

[TABLE 2] Elemental analysis (ppm) of wood from the Ross Island historic huts. Adapted from Blanchette et al., 2002.

LOCATION	MATERIAL	P	K	CA	MG	MN	AL	FE	NA
Cape Evans	Exterior defibrated wood	132	4355	6379	8331	49	558	431	98.650
Cape Royds	Porch defibrated ceiling	132	2828	7451	6091	71	647	649	52.970
Discovery Hut	Exterior defibrated wood	547	7070	4250	10.728	78	3285	2602	246.321
Sound modern wood	Construction board	102	527	403	133	51	5	10	7

[TABLE 3] Chloride and sodium concentrations in melt water from the Cape Evans stables roof. Elements in ppm. Adapted from Blanchette et al., 2002.

SAMPLE	CL	NA
Roof melt water	12.283	5.736
Old snow on roof	626	395
New snow on roof	34	23

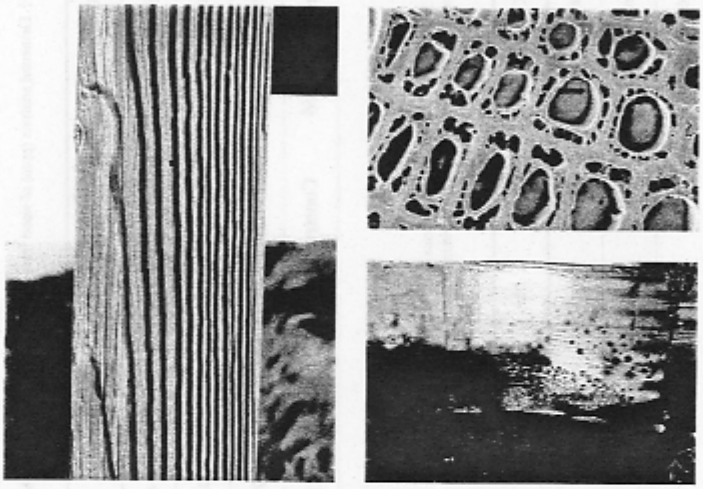
often in areas with limited air circulation. Frost accumulating at the junction of the wall and floor several feet away is probably contributing increased moisture to the area as temperatures rise and the ice melts, enabling the mold growth to occur. Cultures of the mold have been obtained and identified as *Cladosporium sphaerospermum* and *C. cladosporioides*.

Environmental Monitoring Temperature and relative humidity have been collected for over a two-year period. A graph showing temperature and RH for two years in Discovery hut is shown in figure 10. (FIG. 10) The RH over time is quite elevated, although the temperature rises above freezing only for a short period during the austral summer months. Relative humidity in the huts is elevated and is often above 80%. Figure 11 shows data for a one-month period from December to January 2002 inside Cape Evans hut, where the average RH is 84% and the maximum relative humidity observed was 93%. (FIG. 11) The average temperature for the same period is 1.4°C and reaches a maximum of 7°C. These conditions are conducive to mold growth within the hut.

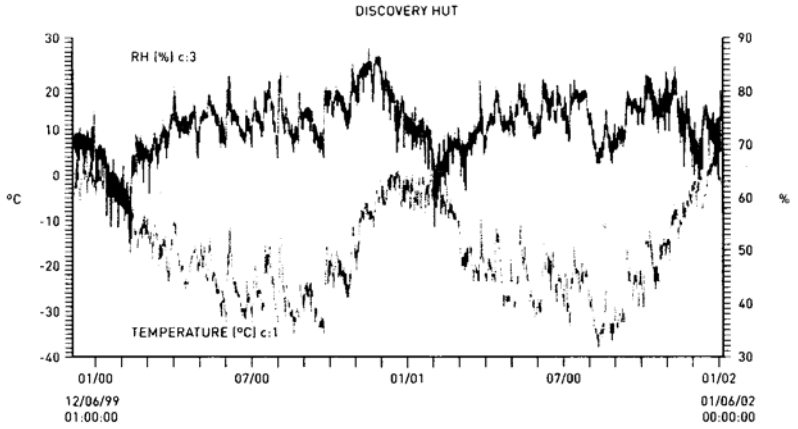
Hundreds of visitors from cruise ships or scientists carrying out research in the area enter the huts every season. The impact on the interior environment of the hut from visitors has not been previously determined. Using visitor data, several days were selected when large groups entered Cape Royds; the goal was to determine their possible effect on relative humidity. On Jan. 8, 2001, 66 visitors entered the hut. (FIG. 12) Four visitors entered the hut three days prior to Jan. 8, and three visitors entered the hut three days after the large group. Forty-five visitors entered the hut on Feb. 14, 2001, and there were no other visitors in the days prior to or after Feb. 14. (FIG. 13) Both events show no apparent impact on RH from the visitors entering the hut. Instead, the RH appears more reflective of the temperature, with increases in RH following increases in temperature.

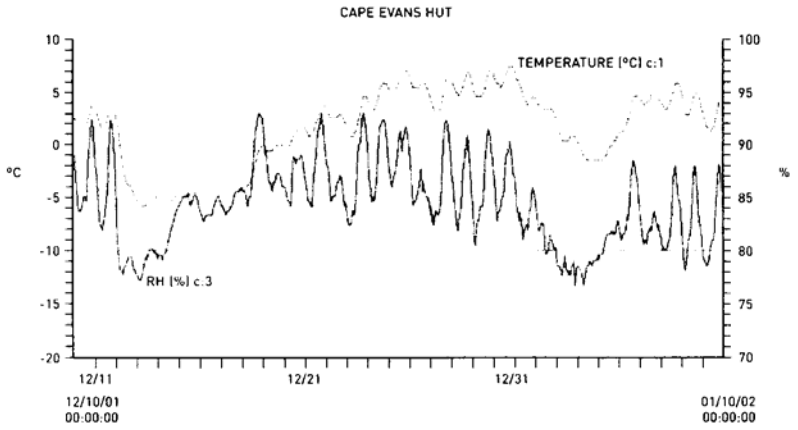
DISCUSSION

Wood Identification Woods used in the construction of the huts and wooden artifacts have been identified. (TABLE 1) For successful conservation of the historic huts the identification of the wood will help any restoration efforts to provide the appropriate wood species that was originally used. During this investigation it was found that some woods recently used in restoration work were not the same type used in the original structure. Harrowfield (1981) reports the general assumption that Discovery hut was made of Douglas fir (*Pseudotsuga menziesii*), while our investigations found Douglas fir was used for some main timbers and corner posts of

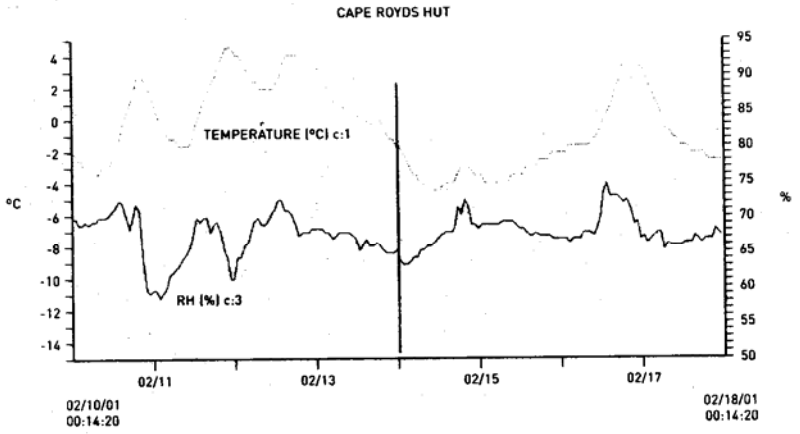


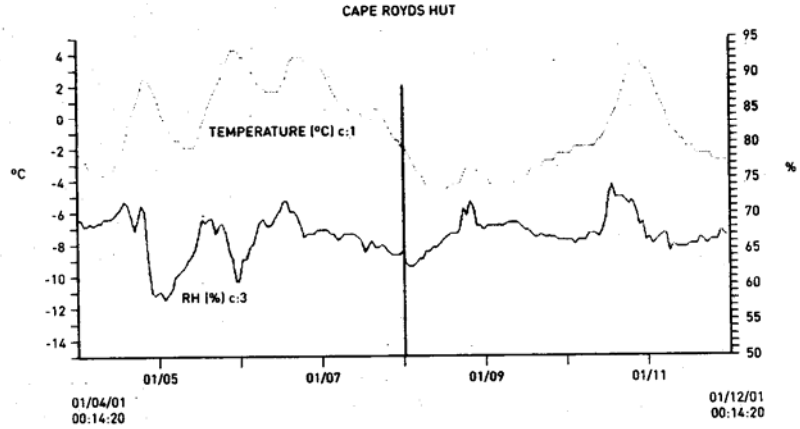
[7] Wood eroded by wind shows an uneven erosion. [8] Extensive cavities caused by the soft-rot fungi located in the secondary walls of the wood cells. [9] Extensive mold growth has been found in Cape Evans hut, most commonly on wood near or on the floor and often in areas with limited air circulation.





[10] Graph of relative humidity and temperature in Discovery hut over a 2-year period. [11] Graph showing relative humidity and temperature in Cape Evans hut over a one-month period indicating high relative humidity and temperatures above 0°C. The horizontal line indicates 80% relative humidity.





[12] Graph of relative humidity and temperature in Cape Royds hut. The vertical line indicates a day in which 66 visitors entered the hut. [13] Graph of relative humidity and temperature in Cape Royds hut. The vertical line indicates one day in which 45 visitors entered the hut.

with historic woods. Drainage can be improved in some areas adjacent to the huts where water tends to accumulate when snow melts. Also, melt water with high concentrations of dissolved salts drips onto the sides of some areas of the huts. Extending the drip line of the roof several inches out over the wall could avoid melt water coming in contact with the wood. Additional research is needed to fully understand this complex process of wood defibration from different types of salts in the polar environment as well as to find better methods to protect wood from the damage that salt causes.

Wind Erosion Wind erosion is a serious problem for the historic huts and artifacts, which are exposed to strong southerly winds. Research on wind movements around the historic hut at Cape Adare suggests that wind barriers may be effective at protecting the historic woods (Harrowfield, 1996). Wood protectants are also being tested as possible treatments to slow the process of wind erosion (author's unpublished data). The treatments will be evaluated at the end of the five-year study period. Further research is needed to identify coatings that may prove effective against nonbiological effects and are acceptable for use on the historic woods.

Biological Deterioration Soft rot is the only type of wood decay found at the historic sites investigated. The same species of soft rot that was isolated from decayed wood at Cape Royds has also been isolated from many other samples taken from wood in ground contact at Cape Evans and Discovery hut. It is interesting that these fungi seem to be common in the Antarctic environment but rarely in other parts of the world. There is limited knowledge about the biology and physiology of these unusual fungi, and their role in the Antarctic ecosystem is unknown (Vincent, 1998; Vishniac, 1992). The location in which soft rot occurs appears to be associated with an area where penguin guano accumulates. The increased nitrogen and elevated pH may influence the growth and decay rates of these fungi. This also may be a reason why soft rot is prevalent in wood from Cape Royds but the same fungi are not causing wood decay at other sites. Isolations of similar species have been made from other locations in Antarctica, indicating these fungi are likely to be endemic to the continent (Vincent, 1988; Sun et al., 1978).

The soft-rot fungi have only several weeks each year during which temperatures are high enough (above 0°C) to attack the wood. It is most likely that the decay would be much more extensive if temperatures were not limiting fungal activity. *Phialophora* species occurring in temperate areas have also been known to tolerate toxic substances such as copper, chrome, and arsenic in preservative-treated tim-

the veranda, and pine (*Pinus sp.* similar to *P. sylvestris*) was used for exterior siding, interior walls, floor, and ceiling. The misconception that the wood was Douglas fir was likely the reason for conservators using Douglas fir in 1964 to replace lost wall boards from the north side of the veranda. The original boards of the veranda still present on the hut are *Pinus sp.* Cape Evans hut has also undergone restoration and in 1991 some of the deteriorated siding on the northwest- or sea-side of the hut was replaced. Douglas fir was also used for this wood but the original woods remaining on the hut indicate the exterior boards were made of pine. Nearly all of the exterior wallboards sampled from Cape Evans are pine. At Cape Royds the hut has been thought to be made from fir (Harrowfield, 1981), but our investigations have found the wood is spruce (*Picea sp.* and most likely Norway spruce). Pony snowshoes previously thought to be bamboo (Harrowfield, 1981) are actually rattan. The identification of most of the major woods used in the huts is now confirmed [TABLE 1] and conservators can use this information for selecting the appropriate woods for any future restoration work.

Salt Deterioration Wood exposed to high salt concentrations can be severely deteriorated. In more temperate regions, a defibration of surface fibers has been identified on preservative-treated marine pilings, salt and fertilizer warehouses, and chemical plants (Johnson et al., 1992; Wilcox et al., 1991). In a study by Parameswaran (1981), the changes occurring in wood used from a potash fertilizer storehouse were similar to the alkali pulping process that takes place during papermaking. While the process appears to be similar, salt defibration associated with the historic huts occurs at a much slower rate. On Ross Island, salts commonly migrate to the surface of the soil, forming thin crusts (Campbell and Claridge, 1987). These salts are then blown and accumulate in snowdrifts. Salts from the Ross Sea also contribute aerosols of salt solutions that occur in snow and ice around the historic huts.

Although the exact process of salt deterioration in wood is not fully understood, it is apparent that the high salt concentrations cause a chemical reaction to take place in which the hemicellulose and lignin in the middle lamella are degraded (Blanchette et al., 2002). This is exhibited in affected woods by a defibration of surface fibers, giving the wood surface a fuzzy appearance. There are many locations at all three huts that are affected by salt deterioration. All of these locations involve moisture absorption from melt water in pools on the ground, or by the melting of snow from the roofs directly on the huts or artifacts. To best protect the huts against this problem, excess moisture accumulation must be kept from being in contact

bers (Daniel and Nilsson, 1988; Wang et al., 1990). Further research could provide information into factors responsible for their tolerance and give insight into potential methods of control of these decay fungi.

Environmental Monitoring Relative humidity within the huts is elevated for much of the year. Of greatest concern is the time during the austral summer in which both RH is high and temperatures are above freezing. A reduction of RH during these periods may limit the growth of surface molds. Also, air circulation in the huts is currently poor. Increasing circulation in problem areas where mold growth occurs may be beneficial. It does appear that visitors to the huts have no significant effect on RH inside the huts. [FIGS. 12 AND 13] Temperature and RH data will continue to be collected for several years to confirm these results.

CONCLUSIONS The research presented here has provided valuable information on the deteriorating effects influencing the Ross Island historic huts. The species of wood that the huts and associated wooden artifacts are made of have been identified, and the types of nonbiological and biological deterioration commonly associated with the huts have been identified.

The major forms of nonbiological deterioration are salt defibration and wind erosion. Salt solutions are repeatedly absorbed by the historic woods, which precipitate water at evaporative surfaces, causing high concentrations of salts to build up. Extensive defibration results, caused by a degradation of the middle lamella region of wood cells, which becomes weakened and deteriorated, causing cells to separate. Wind erosion over the past 9–10 decades has caused significant damage. Most affected is wood that is unprotected from the strong southerly winds, which carry windborne particles that slowly erode the wood surface. Earlywood is preferentially eroded at a faster rate than latewood, apparently due to lower amounts of lignin and thinner cell walls in the earlywood zone.

Biological organisms are also affecting the huts. An unusual type of soft rot has been found attacking wood in contact with the soil at Cape Royds hut. *Phialophora*-like fungi isolated from the decayed woods are being characterized and studied for their decay capacity. These fungi have also been commonly isolated from all of the historic structures and many of the wooden artifacts located outside the huts. Active mold growth caused by *Cladosporium sp.* has also been found growing on wood in the Cape Evans hut. Mold growth appears to be exacerbated by conditions of high relative humidity and temperatures above 0°c during the austral summer months. Environmental monitoring of temperature and relative humidity has been

initiated and will continue over the next several years. At present, the relative humidity is elevated above 80% for much of the summer months, and visits by large numbers of people (from cruise ships) do not appear to have a significant effect on the internal environment of the huts. The results provided from these investigations should serve as a resource for conservators involved in the preservation of these historic sites, allowing the huts to survive long into the future.

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REFERENCES

BLANCHETTE, R.A., HELD, B.W., and FARRELL, R.L., 2002. Defibration of wood in the expedition huts of Antarctica: an unusual deterioration process occurring in the polar environment. *Polar Record*, 38, 313–322.

BLANCHETTE, R.A., HAIGHT, J.E., KOESTLER, R.J., HATCHFIELD, P.B., and ARNOLD, D., 1994. Assessment of deterioration in archaeological wood from ancient Egypt. *Journal of the American Institute of Conservation*, 33, 55–70.

CAMPBELL, I.B., and CLARIDGE, G.G.C., 1987. *Antarctica: soils weathering processes and environment*. New York, Elsevier. 368 pp.

DANIEL, G.F., NILSSON, T., 1988. Studies on preservative tolerant Phialophora species. *International Biodeterioration*, 24, 327–335.

HARROWFIELD, D.L., 1981. *Sledging into history*. Macmillan, Auckland, 118 pp.

HARROWFIELD, D.L., 1996. The role of wind in the destruction of an historic hut at Cape Adare in Antarctica. *Polar Record*, 32, 3–18.

HOADLEY, R.B., 1990. *Identifying Wood*. Taunton Press, New Town, CT, 233 pp.

JOHNSON, B.R., IBACH, R.E., and BAKER, A.J., 1992. Effect of salt water evaporation on tracheid separation from wood surfaces. *Forest Products Journal*, 42, 57–59.

MUNTER, R.C., and GRANDE, R.A., 1981. Plant tissue and soil extract analyses by ICP-AES. In: Barnes, R.M., (Ed.), *Developments in Atomic Plasma Spectrochemical Analyses*, Heydon and Sons, Philadelphia, pp. 653–673.

PANSHIN, A.J., and ZEEUW, C., 1970. *Textbook of Wood Technology*, 3rd edition, McGraw Hill, New York.

PARAMESWARAN, N., 1981. Micromorphology of spruce timber after long-term service in a potash store house. *Holz als Roh und Werkstoff*, 39, 149–156.

SUN, S.H., HUPPERT, M., and CAMERON, R.E., 1978. Identification of some fungi from soil and air of Antarctica. *Terrestrial Biology* 3. Antarctic Research Series v.30, 26 pp.

U.S. ENVIRONMENTAL PROTECTION AGENCY, 1979. *Methods for chemical analysis of water and wastes*. Cincinnati, OH: Environmental Monitoring and Support Laboratory, Office of Research and Development (Method 325.2; EPA-600 4–79–020).

U.S. ENVIRONMENTAL PROTECTION AGENCY, 1983. *Methods for chemical analysis of water wastes*. Cincinnati, OH: Environmental Monitoring and Support Laboratory, Office of Research and Development (Method 200.7; EPA-600 4–79–020).

VINCENT, W.F., 1988. *Microbial ecosystems of Antarctica*. Cambridge University Press, Cambridge. 304 pp.

VISHNIAC, H.S., 1992. Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems. *Biodiversity Conservation*, 5, 1365–1378.

WANG, C.J., WANG, K., and ZABEL, R.A., 1990. *Identification manual for fungi from utility poles in the eastern United States*. American Type Culture Collection, Rockville, Maryland, 356 pp.

WILCOX, W., BOTSAI, E., and KUBLER, H., 1991. *Wood as a building material: A guide for designers and builders*. John Wiley & Sons, New York, 215 pp.

WORRALL, J.J., 1991. Media for selective isolation of hymenomycetes. *Mycologia*, 83, 296–302.

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- Assisted with literature reviewing.
- Assisted with writing chapter.
- Scientific discussions and manuscript proof.

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UNIQUENESS OF ANTARCTICA AND POTENTIAL FOR COMMERCIAL SUCCESS

ROBERTA L. FARRELL[*] & SHONA M. DUNCAN[*]

Bioprospecting is a somewhat new term but it is not a new activity and has been practiced for centuries. Advancements in isolation and manipulation of genetic material, particularly Deoxyribonucleic Acid (DNA), in the past twenty years has certainly broadened and intensified bioprospecting, particularly in certain environments. Bioprospecting in Antarctic activities and related policies have been discussed by the Scientific Committee on Antarctic Research (SCAR), the Committee for Environmental Protection (CEP) and at Antarctic Treaty Consultative Meetings (ACTMs). This chapter addresses bioprospecting from the scientific perspective, and is divided into three sections:

- Definition of Bioprospecting and the 'Unique' Antarctic Environment
- Overview of Bioprospecting Research Worldwide and Development in New Zealand
- Potential for Commercial Success

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The reader is recommended to several good reviews that have appeared recently, either specifically dealing with bioprospecting, or generally discussing the types of research that may lead to bioprospecting.[1]

DEFINITION OF BIOPROSPECTING AND THE 'UNIQUE' ANTARCTIC ENVIRONMENT

Bioprospecting is a subset of biotechnology, the application of biological sciences to industry and/or the environment. This application implies commercialisation and in regards to Antarctic research, this aspect of commercialisation has drawn the attention of scientists and policymakers alike as to whether bioprospecting is in conflict with the Antarctic Treaty. Commercialisation does not necessarily mean that scientific

[1] Onofri, S.L.S., L. Zucconi, S.Pagano. (2004) "Antarctic microfungi as models for exobiology." Planetary and Space Science 52: 229-237;
Nichols, D.S., Sanderson, K., Buia, A., van de Kamp, J., Holloway, P., Bowman, J.P., Smith, M., Nichols, C., Nichols, P.D., and McMeekin, T.A. (2002). Bioprospecting and Biotechnology in Antarctica" in Jabour-Green, J. and Haward, M. (Eds). The Antarctic: Past, Present and Future. Antarctic CRC Research Report #28. Hobart, pp 85-103;
Cavicchioli, R. K., Siddiqui, S., Andrews, D. and K.R. Sowers (2002). "Low-temperature extremophiles and their applications." Current Opinion in Biotechnology 13: 1-9;
Pazgier, M., M. Turkiewicz, H. Kalinowska and S. Bielecki (2003). "The unique cold-adapted extracellular subtilase from psychrophilic yeast Leucosporidium antarcticum." Journal of Molecular Catalysis B: Enzymatic 21: 39-42;
Deming, J. W. (2002). "Psychrophiles and polar regions." Current Opinion in Microbiology 5: 301-309.

results will not be disseminated – in fact, patent applications often appear in press faster than international journal publications. Also, biological cultures that may arise from commercial bioprospecting activities could be accessible to those wanting to fundamentally study the organisms. This chapter will not address the potential commercialisation conflicts but will focus on bioprospecting as a scientific activity.

Various definitions of bioprospecting exist. Dr. Dean Peterson, Science Strategy Manager of Antarctica New Zealand, defined bioprospecting as "The search for commercially valuable biochemical and genetic resources in plants, animals and microorganisms. These resources may be used in food production, pest control, the development of new pharmaceuticals and other biotechnological applications." The US National Park Service gives a broader definition of bioprospecting as "Scientific research that looks for a useful application, process or product in nature". The World Foundation for Environment and Development (WFED), who in 1997 provided the pivotal assistance to Yellowstone National Park that led to the first bioprospecting agreement between a US National Park and a private company, states "Bioprospecting refers to the search for valuable genetic or chemical compounds in nature." Inherent in all of these

definitions is the activity of looking or searching, which follows the definition of prospecting from The Oxford Dictionary, "Go on exploring expedition, institute search, (esp. for gold)". Bioprospecting is a scientific exploring activity to search for something useful from nature. Accepting these definitions, therefore, fundamental scientific research is not classified as bioprospecting, though the findings of bioprospecting may contribute to fundamental science.

The perception exists because of the term 'prospecting' that there is exploitation and potential for harm to the environment in which the bioprospecting took place. If bioprospecting occurred in a non-sustainable fashion, such that removal of biological material endangered its continued presence in Antarctica, then the negative connotation attached to the linkage of the term to prospecting would be accurate. However, the vast majority of bioprospecting occurring worldwide, and particularly in Antarctica, is done by taking small samples, each sample can be a swab or weigh less than one gram. The biological information is then 'harvested' in a sustainable fashion. This sustainable bioprospecting has been addressed worldwide, and is included in the WFED Bioprospecting and Biodiversity Benefit-Sharing Program ("Bioprospecting Program"), which has promoted a new conservation paradigm known as conservation-based

bioprospecting, specifically in the US National Park System and other conservation areas.

Most would agree there are key common stages of the bioprospecting activity regardless of specific final product. Jabour-Green and Nicol[2] considered there are four phases of bioprospecting for Antarctic biological resources. Though ours slightly differ from theirs, specifically with our consideration that screening for activity is part of research and development, we too consider there are 4 stages for successful bioprospecting:

1. Discovery – involving collection, screening, and describing.
2. Research and Development – involving isolation and purification of the useful biological and/or chemical, potential modification to improve efficacy, and testing.
3. Manufacturing – involving production, and concomitant activities such as testing for shelf life stability, potential toxicity testing, and any other testing required for the final stage, marketing.
4. Marketing – including registration requirements and sales.

[2] Jabour-Green, J. and D. Nicol. (2003). Bioprospecting in Areas Outside National Jurisdiction: Antarctica and the Southern Ocean. Melbourne Journal of International Law, 4: 76-111.

The discovery stage is that which garners the most interest, particularly in Antarctica, as the other three stages occur outside the region. Generally, stages 2, 3 and 4 require the most significant effort in terms of time and resources, and these stages will be considered later in this chapter. Potential for intellectual property protection is not only appropriate at the bioprospecting stage 1 but also at all the subsequent stages, whenever a novel idea is shown to have utility.

Who are the Bioprospectors? Bioprospectors are scientists who are either searching for something useful, as identified by a commercial group or a government requesting a certain 'activity'/biochemical/substitute chemical, or, just as often, a scientist on a strictly academic project suddenly noticing a valuable use for what started as curiosity-driven research. It is important to realise that the fruition is the same whether the outset was implicit commercial bioprospecting or the outcome of fundamental research, and the two should not be distinguished if resulting in utility. Bioprospecting scientists are from universities, research institutes or commercial firms.

What is there to bioprospect? The scope of Bioprospecting can be plants, animals, or microorganisms, in the terrestrial, freshwater or marine environments. Much of the literature is focused on microorganisms as a source of bioprospecting but Antarctic programmes also include

significant effort in animal and plant bioprospecting, particularly from the marine environment. Bioprospecting efforts are often linked with efforts to understand the Biodiversity of an area, meaning the diversity of life in an area. Novel biodiversity is thought to give rise to novel molecules which in turn may give rise to commercial potential. Bioprospecting can be done using the whole organism, parts of an organism (collectively called the 'biomass'), or prospecting genes without organisms. Examples of uses of the whole organism or 'biomass' for commercial application are as *in vivo* Biocatalysts, for instance in the brewing and baking industries, or in the production of antibiotics. Bioprospecting can also search for *in vitro* Biocatalysts, i.e. proteins, enzymes and bioactives, used in foods, detergents, pharmaceuticals, as well as thousands of other applications.

After finding an appropriate environment for the targeted bioprospecting, the environment is sampled, which may be accomplished by swabbing the environment with a sterile cotton swab, or taking a small sample of lichen or soil, even as little as one gram of material removed can be sufficient. Extracting molecules from plant or animal biomass, or culturing microorganisms from the sample, is accomplished by classical chemistry or microbiological techniques. Advanced culturing techniques are being applied to Antarctic

bioprospecting by the use of selective bioreactors. Wery et. al.[3] used a packed-column bioreactor inoculated with soil from Antarctica in order to isolate seventy-five heterotrophs which were protease producers, the bioreactor having been continuously fed with a casein-containing medium; this work continued to identify new proteases for commercialisation. Fundamental research findings were also produced in this study as some of the Antarctic strains identified in the bioreactor represent candidates for new species of *Chryseobacterium* and *Massilia*. Without the funding for the bioprospecting for proteases, would these new Antarctic species have ever been found?

Bioprospecting either as biomass or the genes without biomass provides the material to be screened and selected for the next stage of research, in many cases a targeted biological activity. The search for the targeted activity can be done by bioprospecting genes without necessarily harvesting the biomass, culturing the biomass, or even sighting the organism from which the genes are derived. Prospecting genes without organisms, "uncultured biomass", has been estimated to offer a 100-fold or more increase in genetic resource and

[3] Wery, N., Gerike, U., Sharman, A., Chaudhuri, J.B., Hough, D.W., and M. J. Danson (2003) "Use of a Packed-Column Bioreactor for Isolation of Diverse Protease Producing Bacteria from Antarctic Soil." Applied and Environ. Microbial. 69: 1457-1464.

bioprospecting by molecular techniques and expression libraries can access this resource.

The last decade of the twentieth century has seen the development of a plethora of molecular techniques to support this bioprospecting without culturing microorganisms or harvesting an entire organism. They require only a minute amount of deoxyribonucleic acid (DNA) isolated from the sample in order to study the entire genetic resource. Torsvik et. al.[4] estimated that in one gram of garden soil, DNA analysis detected the equivalent of 4,000 bacterial genomes while in one sample of marine sediment 12,000 bacterial genomes were estimated to be present. It has also been shown that whole phyla or the equivalent of phyla of bacteria and archaea have been detected but have yet to be cultured. "Because we have not grown these microbes in most cases we have no idea of their function or their potential importance. So far we have only cultured about 5,000 bacterial species out of a speculated 100,000+ species but some scientists have contested that there could be millions of species".[5]

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[4] Torsvik V., R. Sorheim and J. Goksoyr. (1996). "Total bacterial diversity in soil and sediment communities - a review." Journal of Industrial Microbiology 17:170-178.

[5] Bowman, J.P., (2001). "Antarctica a Global 'Hot Spot': Biodiversity and Biotechnology". The Australian Academy of Technological Sciences and Engineering, Academy Symposium Looking South - Managing Technology, Opportunities and the Global Environment, November 2001.

DNA is extracted from the sample by procedures analogous to those used in forensic science[6]. This extraction of DNA can occur in the field, as is presently being initiated by some science Events in the Antarctic or the extraction can be done using the Antarctic sample after returning from Antarctica back in the laboratory. The DNA is then broken into smaller pieces, either by physical forces, shearing, or by use of restriction digestion enzymes which act to cut DNA in designated places. The pieces of DNA are then joined (ligated) into a DNA vector which serves as a carrier for the bioprospected DNA pieces to be placed in an expression system, a small scale living production factory. DNA of itself does not confer biological activity; it is only the blueprint for such activity when it is expressed to its corresponding protein/enzyme/bioactive molecule in an expression system. The expression system for the initial screening of bioprospected DNA is most often a bacterium and *Escherichia coli (E. coli)* is commonly used. The ligated bioprospected DNA pieces in the DNA vector are taken up by E.coli by the transformation process. The transformed bacteria are put onto selection plates, and each bacterial colony which grows on the

[6] Moss, D., Harbison, S.-A. Saul, D.J. (2003). "An easily automated closed-tube forensic DNA extraction procedure using a thermostable proteinase." Int J Legal Med, 117: 340- 349.

selection plate expresses a piece of bioprospected DNA – this expression means the bioprospected DNA is transcribed and translated to the corresponding protein/enzyme/bioactive molecule encoded originally by the DNA. The transformed colonies are screened by tests or assays to find the desired activity, or by re-isolation and sequencing of the DNA. The screens for activity can be done by various methods and a simple screen is accomplished using a plate assay which shows the desired activity directly as a result of expression of the activity in the transformed bacterial colony. Types of activities assessed by plate assays include the following:

1. Anti-microbials

   Demonstrating killing of an overlay of target organisms by the expressed bioprospected piece of DNA in the transformed bacteria.

2. Proteinase

   Clears a milk-powder overlay by the expressed bioprospected piece of DNA in the transformed bacteria.

3. Hydrolases (cellulases, xylanases, mannanases)

   Clears a dye-linked substrate overlay by the expressed bioprospected piece of DNA in the transformed bacteria.

To bioprospect a molecule with a desired parameter, it is often advantageous to match the environment with the parameters. For instance, if one desires a heat-stable (thermophilic) molecule, it is appropriate to consider first bioprospecting a geothermal area, such as on top of Mount Erebus-an active volcano; or to find a salt tolerant (halophilic) molecule to go to an area where concentrated salts are present. It is the extreme habitats of Antarctica that make it a desirable place for bioprospecting opportunities, hopefully resulting in the discovery of unique molecules (Table 1.1).

HABITAT	DEFINING CONDITION	BIOPROSPECTING OPPORTUNITIES
Seawater, maritime lakes sea-ice	Low temperature	Cold active enzymes/biocatalysts; bioremediation; anti-freezes; polyunsaturated fatty acids, novel pigments
Southern Ocean seawater	Low nutrient concentration	High affinity catalysts & ligands
Hypersaline lakes	High salinity	Halotolerant enzymes; novel metabolites; novel pigments
Marine/lake sediment	Anaerobic/low temperature	Anaerobic biotransformations; Novel bioactives
Soil, lithic habitats	Cold, dry	Novel bioactives

Table 1.1: Antarctic and Southern Ocean ecosystems corresponding conditions and opportunities. From *Antarctica a Global 'Hot Spot': Biodiversity and Biotechnology* Bowman (2001).

With collection of samples in Antarctica, what are the chances that unique molecules will be discovered? Given a multimillion year timeline, and the obvious opportunities available to a micrometre-sized organism for dispersal, will such uniqueness depend upon a unique environment rather than geographic separation? Or upon microbial versatility? If so, how unique does this environment need to be – is there no chance of finding the same organism in Baffin land, Lapland, or Kamchatka? Is there a possibility that uniqueness rests on geographic separation? The answers to these questions are

likely to be messy - some locally unique organisms can certainly be found elsewhere from the so-called unique environment; see, for example, Hudson[7] on *Thermus* distribution. But since only a few percent of microorganisms have ever been identified by any means, let alone cultivated, estimates range from only 1-10% of probable species being described in culture[8] there is currently virtually no certainty of labelling an organism as unique to a single geographic source with any degree of scientific validity. Of course some of this depends upon what you mean by unique. If there are Antarctic-unique microbes, it is very difficult to prove conclusively. Blanchette et. al.[9] have proposed three Antarctic-unique fungi of the Genus *Cadophora* based on extensive studies of an unusual soft rot decay in the foundations of the Scott and Shackleton historic huts on Ross Island, and by specific DNA sequence information as compared to soft rot fungal species from around the world. Their claim of uniqueness was both functionality as well as DNA sequence information.

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[7] Hudson, J.A., Morgan, H.W., and R.M. Daniel. (1986). "A numerical classification of some Thermus isolates. " Journal of General Microbiology 132: 531-540.

[8] Bull, A.T., Goodfellow, M., and Slater, J.H. (1992). Biodiversity as a Source of Innovation in Biotechnology. Ann Rev. Microbiol. 42: 219-252;
Bull, A.T., Ward, A.C., Goodfellow, M., (2000). Search and Discovery Strategies for Biotechnnology. Microbiology and Molecular Biology Reviews. 46: 573-606.

[9] Blanchette, R.A., Held, B.W., Jurgens, J.A., McNew, D.L., Harrington, T.C., Duncan, S.M., and Farrell, R.L. (2004). Wood Destroying Soft Rot Fungi in the Historic Expeditions Huts of Antarctica. Appl. Environ. Microbiol, 70: 1328-1335.

OVERVIEW OF BIOPROSPECTING RESEARCH WORLDWIDE AND DEVELOPMENT IN NEW ZEALAND

Worldwide the search for possible novel biologics is already occurring with great speed and success, but in the future, it is expected to accelerate. A recent United Nations University Institute of Advanced Studies (UNU/IAS) report expressed concerns that "the search to unlock the secrets of these life form's success could be a repeat of the 19th century's gold rush, a free-for-all to find and patent new cancer treatments, antibiotics and industrial products".[10]

We highlight a few examples of international bioprospecting successes. So far, the discovery of antifreeze proteins (AFP) is one of the leading success stories of Antarctic biology. In the late 1960s Dr. Arthur DeVries showed that freezing resistance in Antarctic fish was due to blood serum glycoproteins that lowered their freezing temperature below that of the subzero sea surrounding them.[11] The ensuing years witnessed a great deal of work on AFPs in a number of phylogenetically diverse fish species, much of it by DeVries

[10] Lohan, D. and S. Johnston. (2003). "The International Regime for Bioprospecting Existing Pocilices and Emerging Issues for Antarctica" United Nations University Institute for Advanced Studies Report, Tokyo, Japan.

[11] DeVries, A. L. & Wohlschlag, D. E. (1969). Freezing resistance in some Antarctic fishes. Science 163, 1073–1075;
DeVries, A. L. (1971). Glycoproteins as biological antifreeze agents in Antarctic fishes. Science 172, 1152–1155.

and his colleagues[12]. The scope of organisms known to use AFP to survive the environment is ever increasing. AFP generally possess one or more of the following properties: thermal hysteresis, inhibition of ice recrystallisation, control of ice crystal shape and interaction with ice nucleators. AFP have been patented for many applications including benefits in the preservation and improved viability of cell suspensions, tissues and whole organs, and in the treatment of disease conditions (US Patent Number 5,358,931); to prepare novel ice-controlling molecules (US Patent Number 6,303,388); and with plant AFPs to limit the growth of ice crystals in frozen confectionary products (US Patent Number 6,096,867). There are proposed many more commercial uses for AFPs from wide ranging sources, such as use of antifreeze peptides from α- and γ-*Proteobacteria.*[13]

Much international research has led to patents and large pharmaceutical company interest. *Candida antarctica* lipase is referenced in many patents. In US Patent Number 6,074,863,

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[12] Scott, G. K., Fletcher, G. L., & Davies, P. L. (1986). Fish antifreeze proteins: recent evolution. Can. J. Fish. Aquat. Sci. 43, 1028–1034;
Davies PL, Hew CL, Fletcher GL. 1988. Fish antifreeze proteins: physiology and evolutionary biology. Can. J. Zool. 66:2611-17;
Cheng, C. C. & DeVries, A. L. di Prisco, G., ed. (1991) in Life Under Extreme Conditions (Springer, Berlin).

[13] Gilbert, J.A., Hill, P.J., Dodd, C.E.R. & Laybourn-Parry, J. (2003) Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology, 150: 171-180.

filed in 1994, the lipase was modified to increase specific activity and the invention sited uses of the modified lipase as a detergent enzyme; as a digestive enzyme; in ester hydrolysis, ester synthesis or interesterification; or the use of the lipase variant to avoid pitch trouble arising, e.g., in processes for preparing mechanical pulp and in paper-making processes using mechanical pulp. *Candida antarctica* lipase was selected because it has naturally a higher thermostability as noted in Patent WO8802775. The *Candida antarctica* modified lipase variant has been further developed and commercialised by Novo Nordisk.

Another example of Antarctic successful international bioprospecting is a medical invention (European Patent 1,402,898) involving the use of a glycoprotein Antarticine-NF3 produced by *Pseudoalteromonas antarctica*, a gram-negative bacteria. Antacticine-NF3 selectively improves cellular growth and improves scar formation in wounds.

New Zealand's interest in bioprospecting in Antarctica is increasing though only a few programmes are advanced beyond early stages. Environmental Risk Management Authority (ERMA) applications, required to bring material into New Zealand from Antarctica for the purpose of looking for new organisms, were only approved for two institutes as of April 2004. Both of these approvals were for the importation of

materials from Antarctica with the potential to be used for bioprospecting purpose. Additionally, to date, only a small number of patents have been lodged in New Zealand based on Antarctic bioprospecting[14] (WO02/04447, 2002).

Antarctica New Zealand has created the opportunity for New Zealand scientists to begin the discovery process of bioprospecting with the Science Strategy which includes Theme 3, Life in Extreme Environments: "Understanding of Antarctic biological processes such as freezing resistance will also provide a vital stimulus to diverse biotechnology industries." Also Theme 3A: Bio-diversity and Environmental Change: "Research into cold-tolerant bacteria, cryo-preservation and production of novel chemicals by diverse Antarctic organisms is potential value to fishing, agriculture, and aquaculture, medical and pharmaceutical industries". This direction has continued in its new focus document *Science Strategy 2003-2008*. In this strategic document, Theme 2 Southern Ocean Research considers the biodiversity and ecosystems of the Ross Sea Region. Due to the Antarctic Circumpolar Current, a unique environment has been created and the Southern Ocean is the most uncontaminated ocean in

[14] Daniel, R.M. and D. J. Saul (2002). Improvements in and relating to purification of solutions used for diagnostic techniques, NZ Patent Application No. 511681; Daniel, R.M. and D. J. Saul (2002). Improved procedure for diagnostic techniques, NZ Patent Application No. 511680.

the world. The taxonomic biodiversity of the benthic community along the inshore Victoria Land is rich but the interaction and primary production of this biodiversity is still untapped. Theme 3 deals with Terrestrial Biodiversity, both diversity and functioning of ecosystems. The strategy states "Understanding of Antarctic biological processes such as freezing, or desiccation resistance will also provide a vital stimulus to diverse biotechnology industry."

Additionally, the Latitudinal Gradient Project (LGP), coordinated by Antarctica New Zealand, allows for increased interest in bioprospecting potential. In the LGP, many scientists work together to look at all aspects of five locations along the Victoria Land coastline. The project is an international collaboration and encompasses many disciplines from geology, glaciology, to biodiversity of plants, animals and microorganisms both terrestrial and marine. LGP will clearly contribute to fundamental knowledge of the areas, but a clear-cut policy of bioprospecting has not been instituted for those participating in the project.

This increased focus in Antarctic biodiversity will lead to both greater fundamental understanding of the uniqueness of Antarctic organisms and their ecosystem functioning and without doubt resultant bioprospecting. The greatest concern may be how well this understanding and the biological and

genetic resources gained from bioprospecting are shared and disseminated. Biological and genetic resources are routinely shared by scientists for fundamental research from international culture collections, such as maintained in The Netherlands, USA, and Australia. Continued access of these culture collections for fundamental science studies will prevent the gold rush concerns that some have expressed, such as the opinion of Sam Johnston from the UNU/AIS as quoted in BBC News saying that "The search for extremophiles threatens the hallmarks of Antarctic scientific research, its transparency and cooperation." The importance of large culture collections was noted by McMeekin et. al.[15] in a submission to the Australian House of Representatives Standing Committee on Primary Industries and Regional Services Inquiry into the Regional Development of bioprospecting Industries stating that "Culture collections are important repositories of microbial biodiversity and are essential for the long term availability of authentic strains and their genes. They are also a key source of taxonomic expertise in the long term preservation of strains and organisms for biotechnology". We suggest the continued good science practice of making available the biological and genetic

[15] McMeekin, TA., D.S. Nichols and K.Sanderson. (2001). "Bioprospecting in Tasmania-A case study". A submission to the House of Representaves Standing Committee on Primary Industries and Regional Services Inquiry into the Regional Development of bioprospecting Industries.

resources of culture collections not only maintains the necessary collaborations of international science endeavours but also reduces the impact on the Antarctic environment of groups sampling for the same resource.

We highlight two published projects to give as examples of New Zealand successful bioprospecting and science. The first one looks at the bioactives produced by an Antarctic organism, their identification, unique properties, chemical composition and finally synthetic production. The second one looks at the isolation of an Antarctic organism, screening for specific enzyme activity, purification of the enzyme, gene cloning, pilot scale production and application studies.

University of Canterbury's Professor Murray Munro in collaboration with Professor John Blunt isolated a series of new bioactive metabolites called variolins from the Antarctic sponge *Kirkpatrickia varialosa.*[16] Dr. Jonathan Morris, also at Canterbury, devised an elegant synthetic route to the variolins thereby by-passing the need to recollect from under the ice and opening the way for the production of analogues. Synthesised derivatives were patented by the Spanish pharmaceutical

[16] Perry, N.B., L. Ettouati, M. Litaudon, J. W. Blunt, and M. H. G. Munro, S. Parkin and H. Hope (1994). Alkaloids from the Antarctic sponge Kirkpatrickia varialosa. : Part 1: Variolin b, a new antitumour and antiviral compound. Tetrahedron, Volume 50: 3987-3992.

company PharmaMar and >200 analogues have now been prepared based on the parent variolin skeleton. These analogues have been assessed in vitro and a selection is now in *in vivo* trials for anti-cancer properties.

Antarctica does not just offer an environment that is likely to produce organisms that are cold adapted. Mt Erebus offers an environment that is at the other end of extreme environments. Areas of Mt. Erebus are at temperatures of >95ºC. It was from this area that Professors Roy Daniel and Hugh Morgan of the Waikato Thermophile Unit isolated a thermophilic *Bacillus* (strain EA1) on their first expedition to Mt. Erebus in 1980 as part of a fundamental research study of the worldwide distribution and characteristics of thermophilic bacteria. As a result of further studies this organism was found to produce a protease enzyme, EA1. Though discovered in the early 1990s, EA1 was quite recently selected for an applications study, and the protease is now patented for a forensic analysis, as it is useful for extracting DNA[17]. With forensic samples it is important to reduce the chance of contamination from extraneous DNA and the sample size is often very small. Other methods of DNA extraction require multiple opening of tubes for addition and removal of

[17] Moss, D., Harbison, S.-A. Saul, D.J. (2003). "An easily automated closed-tube forensic DNA extraction procedure using a thermostable proteinase." Int J Legal Med, 117: 340- 349.

chemicals for the breaking of the cell wall and extraction of DNA from the samples. Proteinases are commonly used to break the protein rich cell wall but unless they are denatured they will interfere with the following DNA amplification steps. An earlier proteinase, also isolated by the Waikato Thermophile Unit, from the bacterium *Thermus* sp. Rt41a (the bacterium in this case found in Rotorua, NZ, and the enzyme marketed as PreTaq, Pacific Enzymes Ltd) worked on the same principals as EA1 but was more difficult to fully inactivate by heat. The EA1 results showed that proteinase-based systems offered better or at least as good quality DNA as more traditional methods and EA1 offered better options for automation of the process. Overall, EA1 proteinase offers a reliable, simplified and in some case superior procedure for small DNA recovered samples. Could another proteinase not isolated from Antarctica convey the same properties as EA1? Maybe or maybe not, but in the screen for the most efficacious enzyme by the Waikato Thermophile Unit and Dr David Saul of University of Auckland, it was EA1 that was most successful for the forensic applications of interest. This research and its accompanying patent application were discussed by Lohan and Johnston, 2003[18] as "this example illustrates how difficult it is

[18] Lohan, D. and S. Johnston. (2003). "The International Regime for Bioprospecting Existing Polices and Emerging Issues for Antarctica" United Nations University

to distinguish commercial activity from science research." We suggest that there is a continuum between fundamental and applied research, both of which lead to commercial activity, thus it is not necessary nor appropriate to distinguish them.

POTENTIAL FOR COMMERCIAL SUCCESS RESULTING FROM ANTARCTIC BIOPROSPECTING

Bioprospecting projects require many years lead time prior to commercialisation in order to establish the expression libraries, culture collections, chemical testing etc required for the stages of bioprospecting, as discussed in the first section. The initial bioprospecting activity is, as stated earlier, just the start of being able to realise a commercial outcome. The bioprospected molecule, whether a bioactive molecule or protein/enzyme, can be used as 'prospected' from an organism, or it can be manipulated to alter its properties. "Many of the world's most successful and valuable pharmaceuticals have been derived directly, or indirectly, from natural product sources eg acetylsalicylic acid (aspirin) from willow bark and penicillin from the fungus *Penicillium*" (Nichols et. al. 2002)[19]. The

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Institute for Advanced Studies Report, Tokyo, Japan.

[19] Nichols, D.S., Sanderson, K., Buia, A., van de Kamp, J., Holloway, P., Bowman, J.P., Smith, M., Nichols, C., Nichols, P.D., and McMeekin, T.A. (2002). "Bioprospecting and Biotechnology in Antarctica" in Jabour-Green, J. and Haward, M. (Eds). The Antarctic: Past, Present and Future. Antarctic CRC Research Report #28. Hobart, pp 85-103.

natural product can be commercialised as discovered or in an altered state from what was originally found in nature. With enzymes this can be done on the protein molecule itself or by recombinant DNA technology, either of which can be collectively called 'enzyme engineering'. Manufacturing is then required for the bioprospected molecule, either done by traditional chemical syntheses, or high technology syntheses, or in the case of a biological, such as protein/enzyme or secondary metabolite, in a dedicated expression system. The expression system is most often used for production in some type of fermentation process. With the targeted molecule produced there may be further engineering design modification required, including immobilisation of the molecule or derivatisation. Finally, there is further up-scaling to appropriate commercial manufacturing, with regulatory testing required on the final product to be marketed. In the commercialisation of a product from bioprospecting the most time consuming and costly areas are subsequent to the bioprospecting and initial laboratory stages, and they are product development, clinical trials, registration and marketing. According to Bio-IT World, 2002 in the late 1970s, based on a study by the Tufts Center for the Study of Drug Development, the average cost of developing a drug from target compound through animal models and clinical trials to market was a modest US$54 million. Ten years ago,

that figure jumped to US$231 million. Estimates from the late 1990s put the cost at around US$500 million. Two years ago the Tufts group released its third drug-cost report, garnering widespread publicity and no small amount of controversy. Using data on a random selection of home-grown drugs from 10 pharmaceutical companies, the report calculated the current cost of drug discovery at a staggering US$802 million — a 2.5-fold increase (in inflation adjusted terms) over the past decade. (Had costs risen merely at the pace of inflation, the current figure would be just US$318 million.) Much of this increase was attributed to the rising costs of clinical trials — recruiting thousands of patients, better safety screening — which increased at an annual rate of 12 percent, five times more than for pre-clinical R&D — and greater emphasis on treating chronic diseases. A new Tufts study, led by the center's director for economic analysis, Joseph DiMasi, includes estimates of the cost of drug candidates that fail during various stages of the discovery process.

Only five out of every 5,000 potential new drugs tested on animals reach clinical trials, and only one out of 5,000 ultimately wins approval by the FDA.

Therefore, in terms of Bioprospecting in Antarctica and science, it is important for all to realise that first, the investment in Bioprospecting can not be under-estimated in order to try

and achieve commercial success, and second, the chances for success are slim.

Just to do research and development in order to study biodiversity that may or may not lead to a bioprospecting goal requires extensive research effort and long lead time. For example, a collaborative project between the University of Waikato and the University of Minnesota evaluating deterioration of Historic Huts (Heroic Era 1895-1917) and the bio-diversity of Terrestrial Microorganisms has produced a fungal culture collection now totalling 2500 isolates but this has taken 7 years of research (Farrell et. al. 2004)[20]. Of these 2500 isolates, so far only 150 isolates have been screened for the hydrolytic enzyme cellulase, with several of these isolates now showing potentially unique cellulase activity at cold temperatures. It is this requirement of initially doing appropriate research and development to understand a field that makes it difficult to determine when commercial breakthroughs will be realised by the New Zealand Antarctic programmes.

Professor Murray Munro has suggested an equation to predict the commercial success, or "Hit" of a Bioprospecting activity. The equation is such that the probability of a "Hit" is

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[20] Farrell, R.L., Blanchette, R.A., Auger, M., Duncan, S.M., Held, B.W., Jurgens, J.E., Minasaki, R., Scientific Evaluation of Deterioration in Historic Huts of Ross Island, Antarctica. In *Polar Monuments And Sites Cultural Heritage Work In The Arctic And Antarctic Regions*. ISBN:82-996891-1-2. International Polar Heritage Committee, ICOMOS, Norway, 2004.

equal to the number of Samples taken multiplied by the relative Biodiversity of the area sampled multiplied by the number of assays conducted.

"Hits" = Samples x "Biodiversity" x Assays

We agree with Professor Munro's equation, which suggests most importantly to increase the probability of success it is important to launch a significant and major research effort. Munro's comments were directed at the time to bioprospecting in the marine environment and were based on figures supplied by Dr Peter Murphy on cytotoxicity screening by the National Cancer Institute of the USA on natural product extracts from a variety of sources. The Animal Kingdom in a specific environmental habitat, such as Marine Benthic, or Terrestrial Moist area, can be estimated depending upon the number of Phylum and Sub-Phylum considered being present, as given in the bar graph shown in Figure 1.1(below). The probability of the "Hit is calculated as "Biodiversity" is based on the diversity per area, as given in Figure 1.2. Nichols et. al. suggest the same overall opinion, but their paper suggests microorganisms representing the largest reservoir of undescribed biodiversity, and hence possesses the greatest potential for the discovery of new natural products. They estimate "the Earth currently

supports between 3 and 30 million species of organisms. Of these approximately 1.4 million have been described by science"[21].

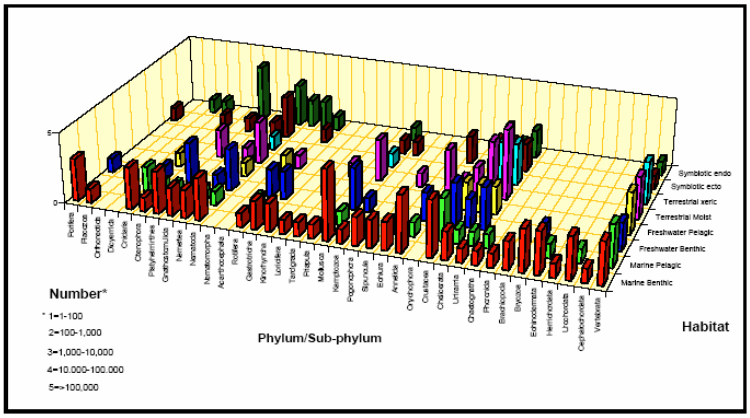

Figure 1.1 The Animal Kingdom by Phylum.

[21] Nichols, D.S., Sanderson, K., Buia, A., van de Kamp, J., Holloway, P., Bowman, J.P., Smith, M., Nichols, C., Nichols, P.D., and McMeekin, T.A. (2002). "Bioprospecting and Biotechnology in Antarctica" in Jabour-Green, J. and Haward, M. (Eds). The Antarctic: Past, Present and Future. Antarctic CRC Research Report #28. Hobart, pp 85-103.

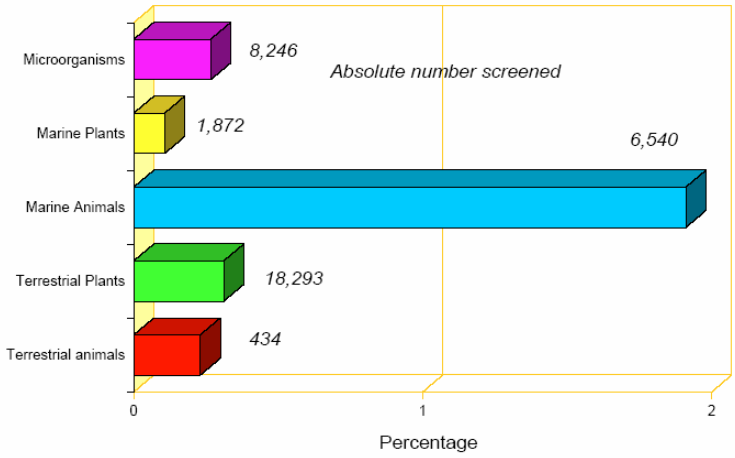

Figure 1.2 Probability of a "Hit".

Without totally being focused on where the greatest biodiversity lies on neither the Earth, nor the calculated final value for probability of success of bioprospecting, "Hits" require biodiversity and scientists working both diligently and smartly.

In summary, bioprospecting is occurring in Antarctica, and for more than ten years patents have been filed from the efforts. This activity is taking place worldwide, and in many cases with great benefit of international collaborative teams, and is increasing. Use of the latest biotechnologies will

facilitate bioprospecting, particularly by minimising its impact on the Antarctic environment, specifically techniques using unculturable. We believe coordinated funding for basic research is crucial to deliver eventual research outcomes necessary for commercialisation. Lastly, issues of access to research findings, and culture collections, as well as ownership and benefits if standardized across all the nations participating in Antarctic research would be helpful and maintain adherence to the key concepts of the Antarctic Treaty.

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