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HALOTOLERANT AND HALOPHILIC FUNGI FROM COASTAL ENVIRONMENTS IN THE ARCTICS

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1. Introduction

Extreme environments on Earth have fascinated microbiologist since the discovery of extremophilic microorganisms, thriving in niches previously thought to be abiotic. The initial focus of attention was directed to unravelling the biodiversity of microorganisms inhabiting them. This may lead to the identification of new interesting extremophilic model organisms, enabling in-depth molecular studies (Aguilar, 1996).

This exciting field of extremophilic microbiology has so far been primarily dedicated to the study of prokaryotic microorganisms, due to the general belief that eukaryotes are unable to inhabit such environments. Contrary to these expectations, recent studies have revealed the presence of a diversity of eukaryotic microorganisms in extreme habitats. Amongst them fungi reaffirmed themselves as one of the ecologically most successful eukaryotic lineages, as they have been isolated from hypersaline waters (Gunde-Cimerman et al., 2000), at 10 km depth below the surface of the oceans (Nagahama et al., 2001), from extremely acidic mine waters (Hölker et al., 2004), and from the surface of rocks in arid and cold climates (Gorbushina et al., 1996; Sterflinger and Krumbein, 1997; Sterflinger et al., 1999). However, fungal diversity in many of these habitats is mostly unknown.

2. Halotolerant and Halophilic Fungi

So far, the best studied fungal extremophiles are halophilic and halotolerant fungi, inhabiting hypersaline waters of salt lakes and salterns around the world (Buchalo et al., 1998; Casamayor et al., 2002; Gunde-Cimerman et al., 2000; Petrovič et al., 2002). Through these studies it became evident that halophilic fungi use adaptive strategies different from the majority of halophilic prokaryotes. Although truly halophilic fungi do exist, the majority of fungi growing on hypersaline substrates displays a general xerophylic phenotype (Filtenborg et al., 2000; Northolt et al., 1995), reflected by their ability to grow at low water activity (a_w), unregarding the chemical nature of the solute

(Hocking, 1993; Pitt and Hocking, 1985a, 1985b). Fungal species are considered xerophilic if they grow well at water activities of 0.85 or less, corresponding to 17% NaCl or 50% glucose in the growth medium. According to the recent definition of halophily in fungi, those species which are isolated regularly with high frequency on selective saline media from environments at salinities above 10%, and are able to grow *in vitro* on media with at least 17% NaCl are considered halophilic (Gunde-Cimerman et al., 2004, 2005). Although halophilic fungi do not require salt for viability, they are, contrary to many halophilic prokaryotes, able to grow and adjust to the whole salinity range, from freshwater to solutions saturated with NaCl. They can adapt to this broad ecological amplitude by surviving periods of extreme environmental stress in a resting state, but when conditions change, they can use the available water immediately and respond with increased metabolic activity, growth and propagation. This adaptive halophilic behaviour, named poikilophilic halophily, enables continuous colonization of the hypersaline environments (Butinar et al., 2005a).

3. Fungi in Polar Regions

Cold polar regions are extreme environments, in which the majority of studies have been oriented towards psychrophilic bacteria, while the occurrence and diversity of psychrophilic fungi remained largely unknown.

So far fungi have been reported primarily in connection with sub-Arctic vegetation and soil in polar regions. Mainly basidiomycetous yeasts were isolated from berries, flowers, vegetation of the littoral zone, soils, forest trees, grasses (Babjeva and Reshetova, 1998) and Antarctic mosses (Tosil et al., 2002). Recently, fungi belonging to the Ascomycota and Basidiomycota, many of them new, were discovered in abundance below snow-covered tundra (Pennisi, 2003; Schadt et al., 2003). Although much of the water in tundra regions for most of the year is not biologically available, the peak in fungal activity was detected during winter, while in spring and summer bacteria prevailed (Hodkinson et al., 1999; Schadt et al., 2003).

Few studies exist on the biodiversity of fungi in Antarctic soils (Vishniac and Onofri, 2003). Such soils represent an interesting habitat for xerophilic fungi, since they exhibit extraordinary aridity, with a correspondingly low a_w, as well as a relatively high salt content (Vishniac and Onofri, 2003). The major soluble salts in Antarctic soils are sulfates, chlorides, nitrates of Na, K, Mg and Ca. Besides, microbial life is exposed to low temperatures, low nutrient availability, seasonally increased UV radiation, and geographic isolation (Onofri et al., 2004). In contrast to the mycobiota present in mesophilic soils, dominated primarily by diverse ascomycetous filamentous fungi (Domsch et al., 1980), diverse basidiomycetous yeasts prevail in Antarctic soils. The dominant yeast genera are *Candida*, *Cryptococcus* and *Leucosporidium* (Vishniac and Klinger, 1986; Vishniac and Onofri, 2003). In all cases their diversity was low and dominated by a few highly specialised and often endemic taxa (Abyzov, 1993; De Wit et al., 2003). The highest halotolerance for yeasts isolated from Antarctic soil was recorded for *Cr. albidus* and *Cr. himalayensis* (9% NaCl) (Onofri et al., 2004), although basidiomycetous yeasts in general show low salt tolerance and inability to grow on media with low a_w.

Viable yeast and fungi were isolated sporadically also from Siberian permafrost sediments, firmly fixed by ice. They were maintained in a frozen state for extended periods, but upon thawing they were nevertheless able to resume their metabolic activity (Takano, 2004). The most common yeast genera were again *Cryptococcus* with the most frequently encountered species being halotolerant *Cr. albidus*, followed by *Sporobolomyces*, *Rhodotorula* and *Cystofilobasidium.* They were found with the highest frequency of occurrence in the youngest layers, less than 10.000 years old, although they were also detected in three millions years old Pliocene samples. In all cases the share of the yeasts represented 25% of all aerobic heterotrophs, independent of the organic matter content (Dmitriev et al., 1997a, 1997b; Gerday et al., 1998; Rivkina et al., 2000).

The occurrence of fungi in polar aquatic habitats has even been less investigated. Yeasts and fungi were isolated from fresh water samples, benthic microbial mats and biofilms on pebbles beneath the ice of Antarctic lakes (Baublis et al., 1991; De Wit et al., 2003).

From polar offshore sea waters mainly basidiomycetous yeasts of the genera *Leucosporidium*, *Rhodosporidium* and *Sporobolomyces* were isolated (Jones, 1976). Sequences belonging to Eumycota were detected in up to 3000 m deep Antarctic polar front waters (Lopez-Garcia et al., 2001). There is no report on the isolation of fungi from sea ice, although their characteristic small subunit rRNA gene sequences were present in DNA extracted from diverse Antarctic and one Arctic sea ice sample (Brown and Bowman, 2001). Fungi were detected as well in the hypersaline Antarctic Lake Wanda (Kriss et al., 1976).

The presence of fungi was least investigated in polar glaciers. Filamentous fungi and yeasts were found in the microbial cryoconite holes that probably serve as biological refuges during extreme cold (Margesin et al., 2002; Reeve et al., 2002). Viable filamentous fungi and yeasts have been isolated from 10,000-13,000 years old Greenland ice (Ma et al., 1999a, 1999b), 12,000 years old Antarctic Vostok ice core sections (Christner et al., 2000, 2002), and even from Antarctic ice layers up to 38,600 years old (Abyzov, 1993). In all these cases the isolated fungi were filamentous and their numbers were low, while viable yeasts of the genera *Cryptococcus* and *Rhodotorula* have been found only in the upper, younger ice-sheet horizons and surface layers of ice and snow. The oldest yeasts were isolated from horizons 700-3,250 years old (Abyzov, 1993).

By PCR amplification of fragments of the eukaryotic 18S rRNA gene, a diversity of fungi was identified in 2000-4000 years old ice-core samples from North Greenland. They were not tested for viability (Price, 2000). All findings of fungi in glacier ice were interpreted as the result of coincidental Aeolian deposits of spores or mycelium into the ice during its geological history.

4. Potential Ecological Niches for Halophilic/Halotolerant Fungi in the Coastal Arctic Environment

Due to the adaptive fungal behaviour at low a_w, we have assumed that coastal Arctic environments, in particular diverse type of ice, could represent a potential ecological habitat for halotolerant and halophilic fungi. High concentrations of NaCl create both ionic and osmotic stress, while high concentrations of sugars and drought cause osmotic stress. There is great similarity between osmotic stress and matric water stress since in both cases the water activity is low. As water becomes ice it is not biologically available, and diverse types of ice can therefore be characterized as well as low a_w environments. Additionally, freezing leads to cellular dehydration due to reduced water absorption, while high salinity causes the same effects due to osmotic imbalances.

4.1. SEA ICE

Sea ice is dominated by strong gradients in temperature, salinity, space, and light. Frozen seawater forms a semisolid matrix, permeated by a network of channels and pores, filled with brine formed from expelled salts as the ice crystals freeze together. The salinity of sea ice brines within channels and cracks of the sea ice (formed when salt is ejected during freezing) can rise as high as 200‰, while the salinity can drop to below 10‰ as the sea ice melts (Brown and Bowman, 2001). Sea ice is also an extreme environment regarding temperatures, which can range from -1°C to as low as -50°C in winter (Thomas and Dieckmann, 2002). Brine inclusions, in general ranging from several μm to cm in size, become increasingly disconnected at lower temperatures, although they remain liquid down to -35°C. Microorganisms inhabiting sea ice have to combine freezing tolerance with tolerance to dehydration caused by the lack of free water at low temperatures and/or high salinities. Changing water activity is therefore the dominant factor in the sea ice external chemistry (Deming, 2002; Junge et al., 2002; Krembs et al., 2002).

The evaporating ponds on the surface of sea ice, formed when spring surface melting occurs, form an additional potential habitat for halotolerant fungi. These melt-water ponds with temperature near 0°C are characterized by increased salinity, and they are occasionally nutrient rich due to ornithogenic contributions.

4.2. GLACIAL ICE

Atmospheric circulation over polar regions provides air-mass exchange with lower latitudes. As a result, microorganisms from air-borne terrestrial dust may become embedded in ice formed from snow. This microbial diversity is thus represented by taxa that are probably endemic to the polar regions as well as exotic species from temperate and tropical regions. These can originate from ocean mist, wind-borne pollen and soil particles, infected plant surfaces, and many other sources. They may have been transported and deposited by the action of waves, wind, rain, snow, animals, or by other means (Abyzov, 1993; Ma et al., 1999a, 1999b). Glacial ice thus provides a unique global source of microorganisms, enabling the study of both contemporary and ancient microbial diversity.

Glacial ice is known as an extremely stable, frigid and static environment. Microorganisms or their spores are protected from UV irradiation, and because they become desiccated, DNA damage is minimized. Viable microorganisms, randomly entrapped in ice even for thousands of years, are destined to be released during glacial melts or after the calving of icebergs into the ocean (Ma et al., 1999a, 1999b). The few existing studies of fungi in glacier ice have provided evidence on the viability and diversity of the randomly entrapped mycobiota.

Recent investigations have shown that glaciers are much more dynamic than previously assumed on the micro scale as well as on the geomorphological level. Ice in temperate glaciers is permeated by a continuous network of aqueous veins, formed at the linear junctions of three ice crystals. They are formed due to sea salts deposited as aerosols, that are essentially insoluble in ice crystals. These liquid veins can have high ionic strength, and due to the percolation of salts from the top of the glacier to its bottom, salts can be accumulated to relatively high concentrations in the bottom parts of polythermal glaciers (Price, 2000). Besides, due to quick seismic shifts (Ekström et al., 2003; Fahnestock, 2003) and cryokarst phenomena in connection with massive surface ablations, liquid water can temporarily appear as ponds or streamlets on the surface of the glacier and as caves or interglacial lakes, artesian fountains and moulins within the glaciers (Christner et al., 2000). These supraglacial waters can also reach the glacier bed and mix with groundwater and basal meltwater generated by frictional and geothermal melting of ice at the glacier base. These liquid waters interact with rocks and sediments, and hence contain high solute and suspended sediment concentrations. When frozen onto the basal glacier ice, they can be transported to the glacier margins, where it can be aseptically sampled. Until recently subglacial environments were thought to be abiotic, but lately viable bacteria have been found (Foght et al., 2004; Gaidos et al., 2004; Lanoil, 2004; Priscu et al., 1998; Siegert et al., 2001; Skidmore et al., 2000). In all these cases, there were no reports on the presence of fungi.

5. Isolation of Fungi from Arctic Coastal Environments

A study involving isolation of halotolerant and halophilic fungi from an Arctic coastal environment was performed in Kongsfjorden, one of the largest fjords found at the western coast of Spitsbergen, Svalbard, located at 79°N, 12°E. It runs from ESE to WNW, joins with Krosfjorden at the mouth and continues into the Greenland Sea. The fjord has a narrow, long shape, is 26 km long and 8 km wide, with several small islands, a steep coastal line, and a deep (140 m) water body. The tide is diurnal with a range of ca. 2 m. Fjord water is in contact with the overlying atmosphere through its surface all year. The water in the fjord is rather immobile, but is occasionally stirred by the wind. It is warmer and less salty than open sea at the same latitude, with an annual mean temperature around -5°C and spring/summer temperatures which can be as high as 3.8°C. The salinity decreases as the season progresses. The fjord is filled with Atlantic deep water at 34.95 psu at the end of the winter. Fresh water is added in summer due to the ablation of glaciers, and this reduces the mean salinity to a minimum of 34.00 psu (Ito and Koduh, 1997). The fjord remains mainly unfrozen despite its location at high latitude, as only the coastal part of the fjord develops a stable sea ice cover in most years. The majority of the drainage basin is covered by glaciers, and most of these reach the sea. Therefore many small pieces of glacier ice are found on the surface of the fjord during most of the year (Ito and Koduh, 1997).

Isolation conditions were designed to accommodate xerotolerant/halotolerant fungi by using media with high concentrations of salt or sugar, and thus low a_w, such as previously used for the isolation of halotolerant/xerotolerant fungi from temperate hypersaline environments (Gunde-Cimerman et al., 2000). These media should give a selective advantage to cultivable microorganisms adapted to ice, thereby possibly enabling the isolation of higher fungal colony forming units (CFU) numbers than previously reported (Gunde-Cimerman et al., 2000, 2003).

Melanized yeast-like fungi were identified by their morphology, their physiology, and by sequencing of ITS rRNA to the species level (Zalar et al., 1999). Isolates of filamentous fungi were identified to the species level by morphology, physiology, and in most cases also by secondary metabolite profiles using HPLC-DAD (Smedsgaard, 1997; Sonjak et al., 2005). The identification methods for non-melanized yeasts followed those described by Yarrow (1998), and the strains were examined as well by sequence analyses in the D1/D2 region at the 5' end of the large subunit rRNA.

All isolates are maintained in a genetically stable way in the Culture Collection of the National Institute of Chemistry (MZKI) (Slovenia) and in the EXF Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia). Most penicillia are preserved as well in the fungal collection (IBT) at the Centre for Microbial Biotechnology (BioCentrum-DTU), Denmark, while yeast-like strains are preserved in CBS, Utrecht, The Netherlands.

5.1. PHYSICO-CHEMICAL CHARACTERISTICS

The pH of the water from which isolates were obtained varied between 7.1 and 7.4 in all samples. The highest cation concentrations were determined for sea water and the lowest for glacial ice, where they ranged from 5 to 340 mg sodium kg^{-1}, from 20 to 310 mg potassium kg^{-1} and 70 to 550 mg magnesium kg^{-1}, respectively. The highest phosphorus content was determined in glacier-ice samples and the lowest in the sea water (<1.00) (Gunde-Cimerman et al., 2003).

5.2. FREQUENCY OF OCCURRENCE

In Kongsfjorden sea water the highest CFU of fungi were determined on a general-purpose medium, with a_w 1.0, at 10°C incubation temperature. The CFU was as high as $3x10^3$ CFU l^{-1}, while it was considerably lower on medium with a_w lowered to 0.946 (Fig. 1). Almost all detected fungi were non-melanized yeasts and diverse penicillia, with few melanized yeasts. The frequency of occurrence was considerably higher than reported in polar offshore waters, usually in the range of 1-12 CFU l^{-1} and only occasionally up to$2x10^2$ CFU l^{-1} (Jones, 1976).

It was assumed that higher fungal CFU numbers than previously reported will be obtained from diverse types of ice on media with lowered water activity, due to the prevention of osmotic imbalances. In accordance, the total number of fungal CFU isolated from sea-ice on medium with a_w 1.0 was only up to $2x10^2$ CFU l^{-1}, but when using medium with a_w lowered to 0.946, it increased up to $7x10^3$ l^{-1} for sea ice and up to $1x10^4$ l^{-1} for molten glacier ice (Fig. 1) (Gunde-Cimerman et al., 2003).

In both types of ice non melanized yeasts predominated, although in samples of glacier ice with gypsum inclusions, melanized yeast-like fungi prevailed, with CFU as high as $6x10^5$ l^{-1} (Fig. 2). There are no reports in the literature on the frequency of occurrence of fungi in sea or glacier ice, apart the infrequent sporadic isolations of individual fungal species, deposited by wind or snowfall into the ice (Abyzov, 1993; Christner et al., 2003; Ma et al., 1999a, 1999b; Price, 2000).

The frequency of occurrence of fungi in sea water and glacial ice enumerated on salt-based media is presented in Fig. 3. Isolations from sea ice are not presented, since they were performed only using 17% NaCl medium, on which primarily *Penicillium* and black yeast-like fungi were isolated with low frequencies (up to 25 CFU l^{-1}). On selective saline media the highest fungal CFU was detected on medium with 5% NaCl added. Counts occasionally increased up to 1.3×10^4 l^{-1} for glacier-ice samples. With increasing salinity, the number of fungal CFU decreased. Therefore, the upper salinity range for the detection of fungi was 24% NaCl with CFU values up to 5 CFU l^{-1} only.

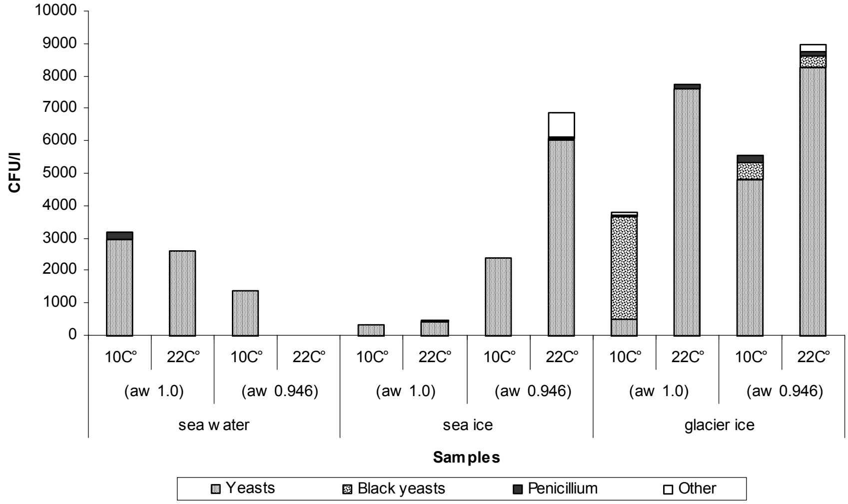

Figure 1. Colony forming units (CFU l^{-1}) of fungi on enumeration media.

Figure 2. Sample of glacier ice with gypsum inclusions.

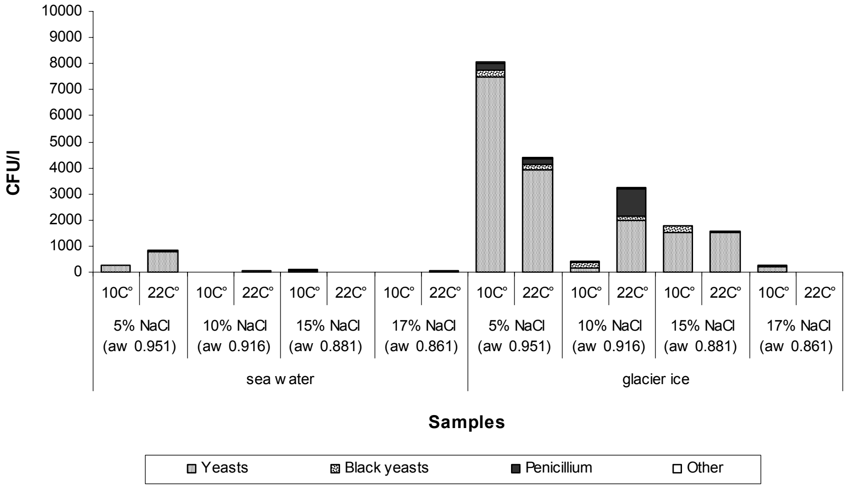

Figure 3. Colony forming units (CFU l^{-1}) of fungi on malt extract agar medium with various NaCl concentrations added (5, 10, 15, 17%).

At lower salinities non-melanized yeasts dominated, but with increasing salinity the proportions changed in favour of melanized fungi and *Penicillium* spp., with *P. crustosum* being the most frequently isolated species (Fig. 4 and 5).

Figure 4. Non-melanized yeasts on 5% NaCl medium after filtration of 0.1 ml of molten glacial water.

Figure 5. *Penicillium crustosum*, the *Penicillium* species most frequently isolated from glacial ice.

6. Diversity of Fungi

A list of fungal genera isolated in diverse ecological niches in Kongsfjorden is presented in Table 1.

The main taxa of halotolerant/halophilic fungi isolated in Kongsfjorden appear as well in hypersaline environment of solar salterns and hypersaline lakes worldwide. These isolates include melanized fungi that were mainly represented by the oligotrophic genus *Cladosporium*, taxonomically and phylogenetically closely related to the black yeast-like halotolerant genus *Aureobasidium*. Among melanized fungi the genera *Alternaria* and *Phoma* occur both in the ice and in the solar salterns, although in both cases with low frequencies. Filamentous fungi, isolated with high frequency from both environments, were represented by the cosmopolitan anamorphic genera *Aspergillus* and *Penicillium*, together with the teleomorphic form *Eurotium* (Butinar et al., 2005a, 2005b), although in the polar environment *Penicillium* prevailed over *Aspergillus* and *Eurotium*. Other filamentous genera that appeared in both habitats with low frequency were *Mucor* and *Trichoderma* (Gunde-Cimerman et al., 2001).

Non-melanized yeasts that were detected recently in hypersaline waters around the world as well as in Konggsfjorden include *Cryptococcus*, *Debaryomyces*, *Filobasidium*, *Metschnikowia*, *Pichia*, *Rhodosporidium*, *Rhodotorula* and *Trichosporon* (Butinar et al., 2005c). Genera that have been isolated only in the Arctic niches, but not in the hypersaline waters of the salterns include *Cadophora*, *Geomyces* and *Phialophora* (data not published). When strains of these genera were tested for halotolerance, they could all grow on media with at least 10% NaCl. The halophilic black yeast-like species *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum* and the halophilic genus *Wallemia*, fungi that dominate in hypersaline waters of solar salterns, were not detected in the Arctic environment (Gunde-Cimerman et al., 2000; Zalar et al., 2005).

The diversity of species was highest in sea water but decreased in sea ice, where fewer detected species were represented in higher numbers. Species diversity in glacier ice depended considerably on the occurrence of mineral inclusions. In samples with visible sediments penicillia prevailed, and their species diversity was high, while in samples of clear ice the species diversity was low, although individual species of non-melanized yeasts occurred with high frequency (Gunde-Cimerman et al., 2003).

TABLE 1. List of fungal genera isolated from diverse ecological niches in Kongsfjorden.

Species	Seawater	Sea ice	Ponds on sea ice	Snow/ice in tidal zone	Glacier ice	Glacier melt water	Antarctic ice (literature data)
Acremonium				x			x
Alternaria		x					
Ameolosidium						x	
Aspergillus		x	x		x		x
Aureobasidium	x	x	x	x	x	x	
Cadophora			x		x		x
Cladosporium	x	x	x	x	x	x	x
Cryptococcus	x	x	x		x	x	x
Debaryomyces	x				x	x	
Eurotium	x	x	x	x	x		x
Filobasidium	x	x	x		x		
Geomyces					x		x
Metschnikowia	x	x					
Mucor		x	x		x		x
Penicillium	x	x	x	x	x	x	x
Phialophora				x			x
Phoma		x	x			x	
Pichia	x				x		
Rhodosporidium					x		
Rhodotorula	x	x			x	x	x
Tricellula				x			
Trichoderma			x			x	
Trichosporon					x		

Aureobasidium

Aureobasidium is a genus of xerotolerant fungi, primarily inhabiting oligotrophic environments. The most ubiquitous species is the halotolerant *A. pullulans*, which is regularly detected in salterns, where it represents one of the core species of saltern mycobiota at lower salinities (up to 10% NaCl). The salinity range of growth for *A. pullulans*, defined *in vitro*, was from 0-18% NaCl, while it grew optimally on medium without NaCl (Butinar et al., 2005a). Besides salterns and sea water its ecological niches are damp inert surfaces such as glass, stone or the phylloplane of plants. It is also known for its ability to grow at high levels of radioactive gamma contamination, since some strains have been isolated even from the walls of the Chernobyl reactor. In polar environments *A. pullulans* has been previously isolated from continental Antarctic soil samples (Onofri et al., 2004).

The dominant genus of black yeast-like fungi isolated from the coastal Artic environment with high abundance was *Aureobasidum*. Based on ITS sequencing, isolates were identified as diverse genotypes of *A. pullulans* (data not published). Although strains were isolated from sea ice and ponds on its surface, they prevailed in the bottom ice of polythermal glaciers containing crystal gypsum inclusions (Fig. 2). *A. pullulans* strains were primarily isolated on media with 5% NaCl after one week of incubation at 22°C, or two weeks at 10°C.

Cladosporium

The genus *Cladosporium* is taxonomically and phylogenetically closely related to black yeasts belonging to the order *Dothideales* (De Hoog et al., 1999; Sterflinger et al., 1999). It comprises about 500 plant-pathogenic and saprophytic species, many of which are among the most airborne species in the fungal kingdom.

The species *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *C. tenuissimum*, and *C. oxysporum* are often isolated from salty/sugary food (Samson et al., 2000) and other environments with low water activity such as saline (coastal) soils and salt marshes, the phylloplane of Mediterranean plants, and the rhizosphere of halophytic plants (Abdel-Hafez et al., 1978). During the study on the occurrence of fungi in hypersaline environmnts, strains from this genus were isolated from thalassohaline and athalassohaline hypersaline waters worldwide. They were in general among the most abundant and most consistently detected fungi in all natural hypersaline waters sampled, at environmental salinities between 15-25% (Butinar et al., 2005a; Gunde-Cimerman et al., 2005; Zalar et al., 2001). The majority of isolates from the salterns belong to the species *C. sphaerospermum.* Less abundant were isolates belonging to *C. cladosporoides*, *C. oxysporum* and *C. herbarum*.

The majority of cladosporia isolated from Kongsfjorden comprise the same species as most frequently isolated from Antarctic soil and hypersaline waters in the salterns: *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum* (Fig. 6), although several yet unidentified strains were isolated as well (Abyzov, 1993; Gunde-Cimerman et al., 2005; Ma et al., 1999a, 1999b), amongst them a small-spored *Cladosporium* sp. These *Cladosporium* species were primarily isolated from glacial ice samples and considerably less frequently from sea water and sea ice. The highest frequency of occurrence was obtained on media with 50% glucose added at 10°C, although all tested strains grew well on 17% NaCl media.

The genus *Cladosporium* is known for its psychrotolerance. The reported temperature range for growth of *C. cladosporoides* is from 0-32^{o}C, with slight growth possible at -3^{o}C and even at -10^{o}C. Some isolates of *C. herbarum* were shown to grow even at -6^{o}C. All three species can grow at high levels of radioactive gamma irradiation (Onofri et al., 2004).

Non-melanized yeasts

There have been only few studies on the occurrence of halotolerant/halophilic yeasts in natural environments. Most available information on the biodiversity of the few known xerotolerant yeasts is thus derived from food contaminations. Among xerotolerant food-borne yeasts ascomycetous genera prevail. They include *Candida* (*C. krusei* = *Issatchenkia orientalis* [teleomorphic state], *C. lambica*), *Hansenula* (*H. anomala*), *Debaryomyces* (*D. hansenii*), *Pichia* (*P. ohmeri*, *P. guilliermondii*) and *Zygosaccharomyces* (*Z. rouxii*, *Z. bisporus*). The only known halotolerant food-borne basidomycetous yeast genus is *Rhodotorula* (*R. glutinis*).

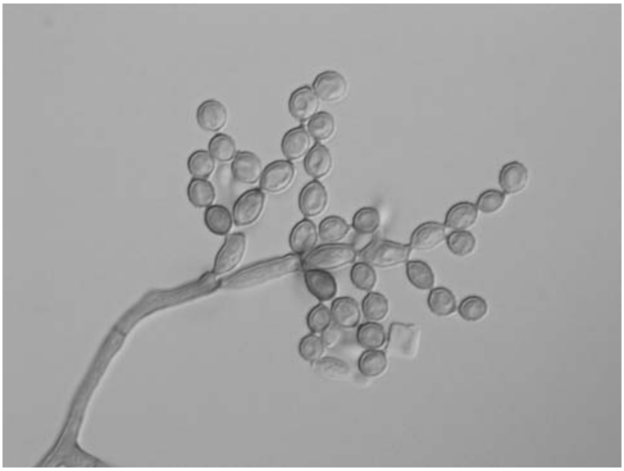

Figure 6. *Cladosporium sphaerospermum* from glacial ice.

Halotolerant yeasts in natural habitats were mainly investigated in sea water, where basidiomycetous yeasts from the genera *Rhodotorula* (*R. mucilaginosa*, *R. glutinis*), *Rhodosporidium*, *Trichosporon* and *Cryptococcus* (*Cr. laurentii*, *Cr. albidus*) prevail (Jones, 1976). Although ascomycetous yeasts are most abundant on food preserved with high concentrations of salt or sugar, they are not common in sea water. The most important ascomycetous marine genera are *Debaryomyces* represented by *D. hansenii* and *Metschnikowia*, although species of *Candida*, *Hansenula* and *Pichia* have been occasionally reported as well. Among these, *M. bicuspidata* var. *bicuspidata*, a parasite of the brine shrimp, is the only species in which a real dependency on salt has been so far discovered (Butinar et al., 2005c; Jones, 1976; Javor, 1989).

Contrary to the belief that hypersaline waters do not harbour any yeast population (Hernandez-Saavedra et al., 1995), halotolerant yeasts were recently isolated from such environments (Butinar et al., 2005c). The frequency and occurrence of halotolerant yeast species in different hypersaline environments was highly inconsistent. They were represented by diverse ascomycetous (*Pichia guilliermondii*, *Debaryomyces hansenii*, *Yarrowia lipolytica*, *Candida parapsilosis*, *C. glabrata*-like, *Metschnikowia bicuspidata*) and basidiomycetous species (*Rhodosporidium sphaerocarpum*, *R. babjevae*, *Rhodotorula laryngis*, *Trichosporon mucoides*). Notably, the species that had the highest temporal frequency of occurrence were *P. guilliermondii*, *C. parapsilosis* and *T. mucoides*. These species could be indigenous and they probably represent a stable core of fungal hypersaline communities.

The diversity of yeast in the extremely cold polar environments was before our study mainly investigated in Antarctic soil and Siberian permafrost, where basidiomycetous genera represented the dominant psychrophilic microorganisms (Abyzov, 1993; Babjeva and Reshetova, 1998; Deegenaars and Watson, 1998; Golubev, 1998; Rivkina et al., 2000; Vishniac and Onofri, 2003). The isolated species were not known or tested for their halotolerance. There were no investigations performed on the occurrence of (halotolerant/xerotolerant) yeasts in sea ice or glacial ice.

In our study, non-melanized yeasts were isolated primarily from the ice at the bottom of polythermic glaciers. Their frequency of occurrence was surprisingly high, reaching up to 3.10^6 CFU l^{-1}. Although to our knowledge ascomycetous yeasts were hardly ever isolated from the extremely cold polar regions, the majority of yeasts isolated in our study on media with low water activity were ascomycetous, while on mesophilic media basidiomycetous species prevailed.

Most of the isolated yeast genera were the same as described from temperate hypersaline environments: *Debaryomyces*, *Metschnikowia*, *Pichia*, while the prevailing basidiomycetous genera were *Cryptococcus*, *Filobasidium*, *Rhodotorula*, *Rhodosporidium* and *Trichosporon*. After identification to the species level (Yarrow, 1998), representative strains of the isolated species were tested for their halotolerance by inoculating on media with 10% and 17% NaCl. The basidiomycetous species *Cryptococcus albidus*, *Cr. liquefaciens*, *Cr. victoriae*, *Rhodosporidium diobovatum*, *Rhodotorula mucilaginosa*, *Rh. laryngis*, and *Trichosporon mucoides* and the ascomycetous yeasts *Debaryomyces hansenii*, *Pichia guilliermondii* and *Metschnikowia zobellii* were all able to grow on media with 10% NaCl added, while only *Rh. mucilaginosa*, *D. hansenii* and *P. guilliermondii* could grow at 17% NaCl (Table 2).

Cryptococcus albidus was the dominant species isolated from glacier ice, followed by *Cr. liquefaciens*. Strains in the Albidus clade are widely distributed in nature, both in natural and man-made environments (Chand-Goyal and Spotts, 1996; Middelhoven, 1997; Roostita and Fleet, 1996; Slavikova and Vadkertiova, 1997), and *Cr. albidus* is also reported as an infectious agent in humans (Horowitz et al., 1993; Loison et al., 1996). Many species have been collected from soil and plants in the polar regions (Onofri et al., 2004; Vishniac and Onofri, 2003).

The *Sporidiobolus* lineage includes species of the teliosporic genera *Rhodosporidium* and *Sporidiobolus* and their anamorphic counterparts in the genera *Rhodotorula* and *Sporobolomyces* (Fonseca et al., 2000). From clade Glutinis the halotolerant species include *Rhodosporidium diobovatum* and from clade Sphaerocarpum *Rhodotorula mucilaginosa.* The latter was obtained from sea water, sea and glacier ice. In hypersaline waste water ponds in Israel it represeted one of the two dominant microorganisms (Lahav et al., 2002). Otherwise it is rather common in diverse aquatic environments. The species *Rhodotorula laryngis* from clade Occultifur of the *Erythrobasidium* lineage was recovered in this study from glacial ice, but it was otherwise found in aquatic environments of glacial origin in Patagonia (Libkind et al., 2004), as well as in the hypersaline waters of the Dead Sea (Butinar et al., 2005c).

From the genus *Trichosporon*, the major taxon of the Trichosporonales, *Trichosporon mucoides* was the isolated halotolerant species from the clade Cutaneum. The isolates of *T. mucoides* are otherwise mostly of human origin (Kurtzman and Fell, 1998).

D. hanseni, a known halotolerant food-borne yeast has been found together with its imperfect form *Torulopsis famata* as in this study mainly in cold sea water (Norkrans, 1966). It has been isolated also from natural hypersaline habitats that are seasonally exposed to low temperatures (Butinar et al., 2005c).

TABLE 2. Occurence of non-melanized yeast in diverse ecological niches in Kongsfjorden.

Halotolerant yeast species	*Sea water*	*Sea ice*	*Ponds on sea ice*	*Snow/ ice in tidal zone*	*Glacier ice*	*Glacier ice melt-water*	*Isolation on media with low a_w*	*Growth on 10% NaCl*	*Growth on 17% NaCl*	*Growth at 10°C*	*Temperate hypersaline environments*
Cryptococcus albidus			+		+	+	+	+	-	+	-
Cr. liquefaciens					+		+	+	-	+	-
Cr. victoriae	+				+		+	+	-	+	-
Debaryomyces hansenii	+				+	+	+	+	+	+	+
Metschnikowia zobellii	+	+			+		+	+	-	+	-
Pichia guilliermondii	+				+		+	+	+	+	+
Rhodotorula laryngis					+		-	w	-	+	+
Rh. mucilaginosa	+	+			+	+	+	+	+ (25°C)	+	+
Rhodosporidium diobovatum					+		+	+	-	+	-
Trichosporon mucoides					+		+	+	-	+	+

Different species of the genus *Pichia* are frequently isolated from sea water, with *P. membranefaciens* being the most common (Soares et al., 1997). *P. guilliermondii*, identified as one of the two dominant microorganisms in hypersaline waste water in Israel (Lahav et al., 2002), was isolated from sea water and glacier ice of Kongsfjorden, although it has not been so far reported from polar regions.

The genera *Aspergillus* and *Penicillium* and their teleomorphs

Tolerance to high salt concentrations is a property found in many species of *Aspergillus* and *Penicillium.* In a survey of the NaCl tolerance encompassing 975 species of terrestrial fungi selected from the major taxonomic classes, the penicillia and aspergilli were notably the most resistant, with the majority of species able to grow in the presence of 20% NaCl or more (Tresner and Hayes, 1971). In nature their occurrence was primarily investigated in saline soil. Species belonging to the genera *Aspergillus* and *Penicillium* isolated from the natural hypersaline environments have mainly been known as contaminants of low water activity food and similar substrata (Filtenborg et al., 2000). When they were first isolated from the salterns they were considered as common airborne occurrences, able to survive prolonged exposure in hypersaline water in a dormant state. However recent results indicate that spores and mycelium of some species are able to survive prolonged suspension in brines (Butinar et al., 2005b; Kis-Papo et al., 2003). These species probably represent part of the core of indigenous hypersaline mycobiota. It seems that in saline environments with higher environmental temperatures *Aspergillus* isolates prevail, while in colder environments *Penicillium* isolates are more abundant (Samson et al., 2000).

Penicillium

The genus *Penicillium* is only associated to two teleomorphic states *Eupenicillum* and *Talaromyces*. *Eupenicillium* and its associated anamorphs *Penicillium* subgenus *Aspergilloides*, *Furcatum* and *Penicillium* are rather closely related to *Aspergillus* in a phylogenetic sense, while *Talaromyces* with its associated anamorphs in the *Penicillium* subgenus *Biverticillium* is a distinct genus closer to *Byssochlamys* and *Thermoascus* (Peterson, 2000; Pitt, 1979; Stolk and Samson, 1972). According to Pitt et al. (2000) there are 166 species in *Eupenicillium* and its related anamorphs, while 59 species are accepted in *Talaromyces* and its *Biverticillium* anamorphs. The genus *Eupenicillium* has not been found very often in saline environments, but its *Penicillium* anamorphs are common, both the food-borne forms (Frisvad et al., 2000) and the soil-borne forms (Christensen et al., 2000). Species in the genus *Talaromyces* in general thrive at higher water activities than *Eupenicillium* (Pitt and Hocking, 1997).

The *Penicillium* species isolated most frequently from salterns were *P. antarcticum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. cyclopium*, *P. glabrum*, *P. solitum*, *P. manginii*, *P. miczynskii*, *P. neodoratum* and *P. westlingii* (data not published).

All isolated halotolerant penicillia are also psychrotolerant, and they are very efficient producers of a large number of families of extrolites (Frisvad, 2004; Frisvad et al., 2000; Sonjak et al., 2005). The ubiquitous halotolerant species *P. chrysogenum*, *P. crustosum* and *P. brevicompactum* isolated during this study as well as from the Antarctic (mainly from soil) can even grow at -2°C.

The dominant *Penicillium* species in glacier ice was the halotolerant *P. crustosum* (Sonjak et al., 2005), not reported from Antarctic ice (Table 3; Fig. 5). *P. chrysogenum*, known to be xerophilic and resistant to radioactivity, survived in Antarctic ice approximately 8000 years, and was also amongst the species most frequently isolated from Kongsfjorden.

TABLE 3. List of halotolerant species of *Penicillium* isolated from diverse polar ecological niches, their occurrence in hypersaline water of the salterns and *in vitro* growth on media with 10 or 17% NaCl added.

Halotolerant *Penicillium* spp.	Sea water	Sea ice	Ponds on sea ice	Coastal snow	Glacier ice	Glacier Melt water	Isolation on media with NaCl	10% NaCl	17% NaCl	Salterns	10°C
"P. arcticum"	x	x			x	x	x	x	x		x
P. bialowiezense	x							x	x		
P. brevicompactum					x			x	x	x	
P. chrysogenum	x	x	x	x	x		x	x	x	x	x
P. corylophilum					x			x	x	x	x
P. crustosum	x	x	x	x	x	x	x	x	x	x	x
P. decumbens	x		x				x	x	x		
P. echinulatum			x		x		x	x	x		x
P. olsonii					x			x	x		x
P. polonicum					x		x	x	x		x
P. resedanum			x				x	x	x		
P. solitum		x		x	x		x	x	x	x	x
"P. svalbardense"					x		x	x	x		x

"P. arcticum" and "P. svalbardense" are new species, so far undescribed.

Halotolerant *P. antarcticum* and P. *aurantiogriseum* that survived in the central Antarctic sheet for 10,000 years (Onofri et al., 2004) were not isolated during our study.

Compared to the fungi found in glacier ice, melted ice and sea water from Svalbard, the mycobiota found in Antarctica are not very different. McRae (1999) found in Antarctica the following halotolerant penicillia: *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. echinulatum*, and *P. solitum*, in addition to the Aspergilloides and Furcatum species *P. corylophilum* and *P. waksmanii*. Most seem to be panglobal except for *P. svalbardense* and *P. bialowiezense* (Frisvad, 2004).

Aspergillus

The genus *Aspergillus* and its teleomorphic states contains 254 broadly accepted species (Pitt et al., 2000). Many of these are known for their xerotolerance and frequent occurrence as contaminants of food preserved with high concentrations of salt or sugar, primarily in warmer climates. Amongst the species of the genus *Aspergillus* most frequently isolated from the salterns are *A. candidus*, *A. fumigatus*, *A. melleus*, *A. niger*, *A. ochraceus*, *A. sydowii*, *A. terreus*, *A. sclerotiorum*, *A. versicolor* and *A. wentii* (data not published). Amongst these *A. sydowii*, *A. terreus* and *A. versicolor* were frequently detected in the Dead Sea as well (Wasser et al., 2003).

In spite of their ubiquitious nature and preference for low water activity, only few halotolerant *Aspergillus* species were isolated from Kongsfjorden: *A. niger*, *A. tubigensis*, *A. sydowii* and *A. versicolor*. *A. niger* and *A. sydowii* have been repored world-wide from sand dunes, salt marshes, estuaries, mangrove mud and other marine environments

(Gunde-Cimerman et al., 2005). *A. sydowii* spores survived prolonged suspension in Dead Sea water (Kis-Papo et al., 2003). This species is also one of the few filamentous fungi reported to have survived in the Antarctic ice sheet for 10,000 years or more. *A. versicolor*, isolated most frequently from diverse ice samples in Kongsfjorden, was repeatedly isolated as well from Antarctic soils, and is also common in diverse low a_w environments in temperate zones. It belongs to the most xerophilic Antarctic species, able also to grow at high levels of radioactive contamination (Onofri et al., 2004).

Eurotium

The genus *Eurotium* is the holomorphic ascomycete genus for *Aspergillus* sections *Aspergillus* and *Restricti*. Species in this genus are particularly xero- and halophilic (Pitt and Hocking, 1985a, 1985b). Its representatives have been reported to live in concentrated salt or sugar solutions at a_w as low as 0.7 (Martín et al., 1998; Wheeler and Hocking, 1993). Different species of the teleomorphic genera *Eurotium* were often isolated from natural hypersaline water environments such as Dead Sea water and hypersaline waters of salterns around the globe. *Eurotium amstelodami* was isolated most frequently, followed by *E. herbariorum* and *E. repens*, *E. rubrum* and *E. chevalieri* were isolated with lower frequency (Butinar et al., 2005b).

In Kongsfjorden *Eurotium* species were rarely isolated, although more frequently than *Aspergillus* spp. They were primarily derived from glacial and sea ice. The most frequent isolate was *E. repens*, followed by *E. amstelodami* and *E. rubrum*. *E. repens* and *E. rubrum* were previously isolated from 8,150 years old ice and Antarctic soil (Onofri et al., 2004).

Alternaria

The most frequently isolated species from hypersaline environments is *Alternaria alternata.* It was repeatedly isolated from water in salterns on three different continents, as well as from Dead Sea water, salt marshes, desert soil and low a_w food (Gunde-Cimerman et al., 2005).

Besides our isolations from sea ice, sea water and glacial ice, *A. alternata* was reported from maritime Antarctica, Alaska and Spitsbergen. It can grow at least at 0^oC, at high levels of radioactive contamination, and is UV resistant (Onofri et al., 2004).

Phoma

Phoma species are common coelomycetous soil fungi, which are relatively often obtained from deserts. *Phoma herbarum*, reported from maritime Antarctica, Alaska and Spitsbergen, was isolated from sea ice and ponds on its surface. It has been reported as one of the xerotolerant Antarctic species, and is able to grow at 0^oC (Onofri et al., 2004).

7. Cellular Responses to High Salinity and Low Temperatures

Studies performed thus far on the presence of fungi in extreme environments have shown that several species are well adapted to stressful conditions (Onofri et al., 2004). Fungi can typically grow at much lower minimal a_w than prokaryotes, and they also appear to be able to survive strong fluctuations in osmotic and thermal conditions, low environmental temperatures, high UV irradiance and low nutrient availability.

Black multi-cellular thick-walled spores and meristematic endoconidia are probably the most stress-tolerant fungal structures known, but even the hyphae of dematiceous fungi show considerable resistance. In polar regions, thick cell walls and surrounding polysaccharides help overcome the stress associated with low temperatures and consequential water loss, causing increased intracellular solute concentrations, decreased cell size, weakening of the cell membrane, and physical cell rupture which can be caused by freezing and thawing (Christner et al., 2000; Krembs et al., 2002). Adaptations experienced at low temperatures can be related to other environmental challenges such as salt stress, and may therefore reveal new links between psychrophily and halophily (Deming, 2002).

Extracellular freezing leads to cellular dehydration, and chilling temperatures reduce water absorption (Zhu, 2001). Dehydration caused by high salinities and low a_w, is therefore a major stress for ice-trapped organisms. Conversely, when the ice melts, the released organisms are suddenly exposed to hyposaline conditions close to freshwater values (Thomas and Dieckmann, 2002), giving advantage to halotolerant organisms with a wide range of amplitude. Although salt, water and cold stresses are clearly different from each other in their physical nature, each eliciting specific responses, they also activate some common reactions. Salinity creates both ionic and osmotic stress, and drought and cold cause osmotic and oxidative stress. Accordingly, they can induce some common responses. At very low temperatures one of the threats represents ice formation within the cell, which might lead to cell lysis because of the volume increase on expansion of water as ice is formed. The second is increased salinity outside the cell, as ice formation leads to the separation of salt out of pure water (as ice) and a corresponding increase in salt concentration, leading to an osmotic gradient across the cell membrane. It has been shown that mechanisms of freezing and dehydration tolerance in plants and arthropods may involve common gene products and cell signalling and regulatory pathways, and it is likely that these occur in other organisms too. These diverse stresses often activate the production of stress proteins (Cheng, 1998), up-regulation of anti-oxidants and accumulation of compatible solutes, which are at the same time osmolytes and cryoprotectants (Wang et al., 2003). Since protection against dehydration damage is correlated with intracellular accumulation of compatible solutes, an increase in the amount of unfreezable water may be achieved by their production (British Antarctic Survey, 1995; Mindock et al., 2001). Glycerol acts as the primary compatible solute in response to high osmolarity and low temperature in fungi (Gunde-Cimerman et al., 2005), while glycine betaine is synthesized by diverse organisms, including several ascomycetous and basidiomycetous fungi, under conditions of environmental stress such as drought, low temperatures and high salinity (Blackwell et al., 2001).

Water transport through the plasma membrane is important in determining freeze tolerance and halotolerance. Aquaporins, channel membrane proteins with transmembrane domains, are involved in the transport of water and/or small neutral solutes such as glycerol. The precise physiological functions of the fungal aquaporins (and others) have remained unknown so far, although in *S. cerevisiae* a correlation was found between resistance to freezing and the presence of aquaporin genes. Overexpression improved freeze tolerance, while deletion had the opposite effect. A rapid osmotically driven efflux of water during the freezing process reduced intracellular ice crystal formation and the resulting cell damage (Rodriguez-Vargas et al., 2002; Tanghe et al., 2002). On the other hand, osmotic stress induced a decrease of water in the cytoplasm by efflux of water through aquaporins, with a resultant increase in intracellular ion concentrations (Allakhverdiev et al., 2000).

The plasma membrane is the primary structure through which the cell maintains contact with its environment. Changes in its composition therefore affect its integrity and stability. Membrane characteristics most important for cellular functions are passive and active permeability, nutrient uptake, electron transport, environmental sensing, and recognition (Georlette et al., 2004). Since extreme environmental conditions such as high salinity and low temperatures influence membrane properties, several studies on their adaptations have been performed.

So far the response of the membrane lipid composition of psycrophilic bacteria to changes in temperature has been well documented. In studies of the properties of bacterial membranes at low temperatures, especially alterations in fatty acid composition have been observed. A decrease in temperature led to one or more changes, including a decrease in average fatty acid chain length, an increase in fatty acid unsaturation, an increase in methyl branching, and an increase in the ratio of *anteiso*-branching relative to *iso*-branching. This termal effect was mediated via temperature-dependent changes in the activity of the enzymes involved in fatty acid and lipid biosynthesis (Chattopadhyay and Jagannadham, 2001; Russell, 1997). The fatty acid composition of membrane phospholipids regulates the membrane fluidity; hence, shorter and more unsaturated fatty acids including polyunsaturated fatty acids (PUFAs) are central in retaining membrane fluidity at low temperature. A novel enzyme family, the polyketide synthases, which are active at low temperatures and are required for the biosynthesis of PUFAs, have recently been found in several psychrophilic bacteria isolated from sea ice (Thomas and Dieckmann, 2002).

Only few such studies have yet been performed with fungal species. In the few studies dealing with the membrane adaptation of psychrophilic/psychrotolerant fungi, such as *Mortierella antarctica* and *Cadophora fastigiata* isolated in the Antarctic, it was observed that at low temperatures the amounts of linoleic and arachidonic acid increased respectively. In *Cryptococcus albidus*, *Cr. laurentii*, and *Rhodotorula mucilaginosa*, so far only known for their psychrotolerance, at lower temperatures unsaturated fatty acids predominated in the membranes (Zlatanov et al., 2001).

Studies on the adaptation of membranes of halophilic black yeast-like fungi at high salinity have only recently been performed. Before that the effect of salt stress on lipid composition and membrane fluidity had been investigated only in a restricted group of salt-sensitive fungi. The study on the influence of increased NaCl concentration on membrane lipid composition and fluidity in the halophilic *H. werneckii* and *P. triangularis* and the halotolerant *A. pullulans* showed an increase in the phospholipid-esterified fatty acid unsaturation and the maintenance of a low sterol-to-phospholipid ratio compared to the salt-sensitive *S. cerevisiae.* The total sterol content, including ergosterol as the major sterol, did not change significantly in response to raised NaCl concentrations in the halophilic/halotolerant melanized fungi studied. The major classes of phospholipids were phosphatidylcholine and phosphatidylethanolamine, followed by anionic phospholipids. The most abundant fatty acids in the phospholipids were 16- and 18-carbon fatty acids with a high percentage of $C18{:}2^{\Delta 9,12}$. These data agreed with electron paramagnetic resonance spectroscopy measurements, which showed that the membranes of the halophilic/halotolerant fungi remained fluid over a much broader range of salinities than membranes of salt sensitive fungi (Turk et al., 2004).

When adaptations to low temperature and high salinity were compared, similar mechanisms of membrane fluidity regulation could be observed. A reduction in temperature slowed down most physiological processes, reduced membrane fluidity, and caused an increased viscosity of water. Thus, tight regulation of membrane fluidity and ion channels permeability are needed, as shown earlier in halophilic organisms (Georlette et al., 2004; Turk et al., 2004). At low temperatures and high salinities, the microbe's ability to modulate the fluidity of its membranes is crucial for its survival. The interrelationship of low temperature and salt stress has been already shown in cyanobacterial cells, in which unsaturated fatty acids in membrane lipids play important roles in the tolerance to salt stress as well as to cold stress. In response to a decrease in ambient temperature or to an increase in salt concentration, psychrophiles or halophiles increase the degree of unsaturation of their fatty acids, thus maintaining the appropriate fluidity of their membrane lipids.

Further investigations are needed to unravel and compare the mechanisms which enable life in some of the most extreme environments on our planet, perhaps on others as well: the hypersaline and extremely cold areas.

8. Conclusions

Fungi, one of the ecologically most successful eukaryotic lineages, have been discovered in diverse extreme environments on Earth. Halotolerant and halophilic fungi represent an integral part of the microbial communities inhabiting hypersaline waters of salterns and hypersaline lakes worldwide, while psychrotolerant and psychrophilic fungi in extremely cold polar areas play important roles in geomicrobiological processes.

Ice, whether in the form of tundra, glacier, snow, lake- or sea ice, presents a special environment for microbial life. Ice combines several stressful factors such as freezing, desiccation, occasional increased salinities, low nutrient availability, thawing and solar irradiation. The occurrence of microorganisms in diverse types of ice has not much been investigated. Occasional fungal isolates from ice were interpreted as random deposits that in some cases survived prolonged periods in the frozen state. However, recent studies have revealed the existence of microbial communities able to retain their viability through dynamic processes of ice melting, followed by micobial enrichments and refreezing. Such studies have so far unraveled only the presence of Bacteria and Archaea, but the presence and role of fungi and other eukaryotic microorganisms have not been investigated.

Certain species of fungi display a general xerophylic phenotype, determined primarily by the low water activity (a_w) potential, which could represent an advantage in ice polar environments. Particularly for halotolerant/xerotolerant and halophilic/xerophilic fungi, adapted to ionic, osmotic or matric water stress, ice could therefore represent a potential habitat. Although halophilic fungi dominant in hypersaline environments (black yeast-like species and *Wallemia*) were not detected in ice, the main taxa of halotolerant fungi isolated from hypersaline environments were isolated either from diverse types of ice or its meltwater. These fungi include the non-melanized basidiomycetous yeast genera *Cryptococcus*, *Rhodosporidium*, *Rhodotorula* and *Trichosporon*, as well as the ascomycetous yeast genera *Debaryomyces*, *Filobasidium*, *Metschnikowia* and *Pichia.* Filamentous fungi prevailing in both habitats belong to the cosmopolitan anamorphic *Penicillium*, *Cladosporium* and the phylogenetically related *Aureobasidium pullulans*. The genera *Alternaria*, *Phoma* and *Aspergillus* were present to a lesser extent, together with *Eurotium* – the teleomorphic form of *Aspergillus*, as well as *Mucor* and *Trichoderma*.

It seems that fungal adaptations to low temperatures can be related to salt stress, revealing new links between psychrophily and halophily. Both extracellular freezing and hypersaline stress lead to cellular dehydration, and both can therefore activate some common responses. These include among others the level of compatible solutes, ion transport across membranes, regulation of water efflux, the composition and fluidity of cell membranes responsible for cellular functions such as passive and active permeability, nutrient uptake, electron transport, environmental sensing, and recognition.

These salt- and cold-adapted fungi represent a new world of eukaryotic extremophiles, a world of organisms that have developed strategies different from those previously investigated. The ability of fungi to adapt to changing water activity conditions, whether due to low temperatures or high salinities, is crucial for their successful survival in some of the harshest extreme environments on our planet.

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References

Abdel-Hafez, S.I.I., Moubasher, A.H. and Abdel-Fattah, H.M. (1978) Cellulose-decomposing fungi of salt marshes in Egypt. Folia Microbiol. **23,** 37-44.

Abyzov, S.S. (1993) Microorganisms in the Antarctic Ice, In: E.I. Friedmann (ed.), *Antarctic Microbiology*. Wiley-Liss, New York, pp. 265-285.

Aguilar, A. (1996) Extremophile research in the European Union: from fundamental aspects to industrial expectations. FEMS Microbiol. Rev. **18**, 89-92.

Allakherdiev, S.I., Sakamoto, A., Nishiyama, Y. and Murata, N. (2000) Inactivation of photosystems I and II in response to osmotic stress in *Synechococcus*. Contribution of water channels. Plant Physiol. **122,** 1201-1208.

Babjeva, I. and Reshetova, I.. (1998) Yeast resources in natural habitats at polar circle latitude. Food Technol. Biotechnol. **36,** 1-5.

Baublis, J.A., Wharton, R.A. and Volz, P.A. (1991) Diversity of micro-fungi in an Antarctic dry valley. J. Basic Microbiol. **31,** 3-12.

Blackwell, M., Longstretch, D.J., David, C. and Barker, S.A. (2001) Glycine betaine in fungi. Inoculum **52,** 22.

British Antarctic Survey (1995) *Dynamics of Antarctic terrestrial and Freshwater Ecosystems – Survival Strategies. Report of Activities* http://www.anta,canterbury.ac.nz/Subfolder/science/biotas/newsletter/number_9/bas.html

Brown, M.V. and Bowman, J.P. (2001) A molecular phylogenetic survey of sea - ice microbial communities (SIMCO). FEMS Microbiol. Ecol. **35,** 267-275.

Buchalo, A.S., Nevo, E., Wasser, S.P., Oren, A. and Molitoris, H.P. (1998) Fungal life in the extremely hypersaline water of the Dead Sea: first records. Proc. R Soc. London B **265,** 1461-1465.

Butinar, L., Sonjak, S., Zalar, P., Plemenitaš, A. and Gunde-Cimerman, N. (2005a) Population dynamics of melanized halophilic fungi in solar salterns. Bot. Mar. (in press).

Butinar, L., Zalar, P., Frisvad, J.C. and Gunde-Cimerman, N. (2005b) The genus *Eurotium* – members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol. Ecol. **51**, 155-166.

Butinar, L., Santos, S., Spencer-Martins, I., Oren, A. and Gunde-Cimerman, N. (2005c) Yeast diversity in hypersaline habitats. FEMS Microbiol. Lett. (in press).

Casamayor, E.O., Massana, R., Benlloch, S., Øvreås, L., Diez, B., Goddarad, V.J., Gasol, J.M., Joint, I., Rodríguez-Valera, F. and Pedrós-Alió, C. (2002) Changes in archeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. Environ. Microbiol. **4**, 338-348.

Chand-Goyal, T. and Spotts, R.A. (1996) Enumeration of bacterial and yeast colonists of apple fruits and identification of epiphytic yeasts on pear fruits in the Pacific Northwest United States. Microbiol. Res. **151,** 427-432.

Chattopadhyay, M.K. and Jagannadham, M.V. (2001) Maintenance of membrane fluidity in Antarctic bacteria. Polar Biol. **24,** 386-388.

Cheng, C.C. (1998) Evolution of diverse antifreeze proteins. Curr. Opin. Genet. Dev. **8,** 715-720.

Christensen, M., Frisvad, J.C. and Tuthill, D.I. (2000) *Penicillium* species diversity in soil and some taxonomic and ecological notes, In: Samson, R.A. and Pitt, J.I. (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 309-320.

Christner, B.C. (2002) Incorporation of DNA and protein precursors into macromolecules by bacteria at -15°C. Appl. Environ. Microbiol. **68,** 6435-6438.

Christner, B.C., Mosley-Thompson, E., Thompson, L.G. and Reeve, J. (2003) Bacterial recovery from ancient glacial ice. Environ. Microbiol. **5,** 433-436.

Christner, B.C., Mosley-Thompson, E., Thompson, L.G., Zagorodnov, V., Sandman, K. and Reeve, J.N. (2000) Recovery and identification of viable bacteria immured in glacial ice. Icarus **144,** 479-485.

De Hoog, G.S., Zalar, P., Urzi, C., de Leo, F., Yurlova, N.A. and Sterflinger, K. (1999) Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 rDNA sequence comparison. Stud. Mycol. **43,** 31-37.

De Wit, R., Dyer, P., Genilloud, O., Goetlich, E., Hodgson, D., de Hoog, S., Jones, B., Laybourn-Parry, J., Marinelli, F., Stackebrandt, E., Swings, J., Tindall, B.J., Vyverman, W. and Wilmotte, A. (2003) Antarctic lakes – 'hot spots' for microbial diversity and biotecnological screening, In: *1st FEMS Congress, June 29-July 3, Abstract book*. Ljubljana, Slovenia, p. 228.

Deegenaars, M.L. and Watson, K. (1998) Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. Extremophiles **2,** 41-49.

Deming, J.W. (2002) Psychrophiles and polar regions. Curr. Opin. Microbiol. **5,** 301-309.

Dmitriev, V.V., Gilichinsky, D.A., Faizutdinova, R.N., Shershunov, I.N., Golubev, W.I. and Duda, V.I. (1997a) Occurrence of viable yeasts in 3-million-year-old permafrost in Siberia. Mikrobiologiya **66,** 655-660.

Dmitriev, V.V., Gilichinsky, D.A., Faizutdinova, R.N., Ostroumova, N.V., Golubev, W.I. and Duda, V.I. (1997b) Yeasts in late Pleistocene-early Pleistocene Siberian permafrost. Cryosphere of the Earth **1,** 67-70.

Domsch, K.H., Gams, W. and Anderson, T.H. (eds.) (1980) *Compendium of Soil Fungi*. Academic Press, London.

Ekström, G., Nettles, M. and Abers, G.A. (2003) Glacial earthquakes. Science **302,** 622-624.

Fahnestock, M. (2003) Geophysics: glacial flow goes seismic. Science **302,** 578-579.

Filtenborg, O., Frisvad, J.C. and Samson, R.A. (2000) Specific association of fungi to foods and influence of physical environmental factors, In: R.A. Samson, E.S. Hoekstra, J.C. Frisvad and O. Filtenborg (eds.), *Introduction to Food- and Airborne Fungi*. Centraalbureau voor Schimmelcultures, Utrecht, pp. 306-320.

Foght, J., Aislabie, J., Turner, S., Brown, C.E., Ryburn, J., Saul, D.J. and Lawson, W. (2004) Culturable bacteria in subglacial sediments and ice from two southern hemisphere glaciers. Microb. Ecol. **47,** 329-340.

Fonseca, A., Scorzetti, G. and Fell, J.W. (2000) Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. Can. J. Microbiol. **46,** 7-27.

Frisvad, J.C., Filtenborg, O., Lund, F. and Samson, R.A. (2000) The homogeneous species and series in subgenus *Penicillium* are related to mammal nutrition and excretion, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 265-283.

Frisvad, J.C. (2004) Chemical diversity of psychrotolerant fungi, In: M.M. Watanabe, K. Suzuki and T. Seki (eds.), *Innovative Roles of Biological Resource Centers*. Tsukuba, Japan, pp. 165-167.

Gaidos, E., Lanoil, B., Thorsteinsson, T., Graham, A., Skidmore, M., Han, S.-K., Rust, T. and Popp, B. (2004) A viable microbial community in a subglacial volcanic crater lake, Iceland. Astrobiology **4,** 327-344.

Georlette, D., Blaise, V., Collins, T., D'Amico, S., Gratia, E., Hoyoux, A., Marx, J.C., Sonan, G., Feller, G. and Gerday, C. (2004) Some like it cold: biocatalysis at low temperatures. FEMS Microbiol. Rev. **28,** 25-42.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J.P., Claverie, P., Collins, T., D`Amico, S., Dumont, J., Garsoux, G., Georlette, D., Hoyoux, A., Lonthienne, T., Meeuwis, M.A. and Feller, G. (2000) Cold adapted enzymes: from fundamentals to biotechnology. Tibtech **18,** 103-107.

Golubev, W.I. (1998) New species of basidiomycetous yeasts, *Rhodotorula creatinovora* and *R. yakutica*, isolated from permafrost soils of Eastern-Siberian Arctic. Mykologiya Phytopathologiya **32,** 8-13.

Gorbushina, A.A., Panina, L.K., Vlaslov, D.Y. and Krumbein, W.E. (1996) Fungi deteriorating marble in Chersonessus. Mikol. Fitopatol. **30,** 23-27.

Gunde-Cimerman, N., Zalar, P., de Hoog, G.S. and Plemenitaš, A. (2000) Hypersaline water in salterns – natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol. **32**, 235-240.

Gunde-Cimerman, N., Cerovac, S. and Zalar, P. (2001) Biodiversity of filamentous fungi in the saltpans Sečovlje. Acta Biol. Slov. **44**, 25-30.

Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J.C., Diderichsen, B. and Plemenitaš, A. (2003) Extremophilic fungi in Arctic ice: a relationship between adaptation to low temperature and water activity. Phys. Chem. Earth **28,** 1273-1278.

Gunde-Cimerman, N., Zalar, P., Petrovič, U., Turk, M., Kogej, T., de Hoog, S. and Plemenitaš, A. (2004) Fungi in the salterns, In: A. Ventosa (ed.), *Halophilic Microorganisms*. Springer-Verlag, Berlin, pp. 103-111.

Gunde-Cimerman, N., Frisvad, J.C., Zalar, P. and Plemenitaš, A. (2005) Halotolerant and halophilic fungi, In: S.K. Deshmukh and M.K. Rai (eds.), *The Biodiversity of Fungi: Their Role in Human Life*. Oxford & IBH Publishing Cp. Pvt. Ltd., New Delhi, pp. 69-127 (in press).

Hernandez-Saavedra, N.Y., Ochoa, J.L. and Vazquez-Dulhalt, R. (1995) Osmotic adjusment in marine yeast. J. Plankton Res. **17,** 59-69.

Hocking, A.D. (1993) Responses of xerophilic fungi to changes in water activity, In: Jennings, D.H. (ed.), *Stress Tolerance of Fungi*. Marcel Dekker Inc., New York, pp. 233-243.

Hodkinson, D., Webb, N.R., Bale, J.S. and Block, W. (1999) Hydrology, water availability and tundra in a changing climate: the need for a closer integration of ideas. Glob. Chang. Biol. **5,** 359-369.

Hölker, U., Bend, J., Pracht, R., Müller, T., Tetsch, L. and de Hoog, G.S. (2004) *Hortaea acidophila*, a new acidophilic black yeast from lignite. Antonie van Leeuwenhoek (in press).

Horowitz, I.D., Blumberg, E.A. and Krevolin, L. (1993) *Cryptococcus albidus* and mucormycosis empyema in a patient receiving hemodialysis. South. Med. J. **86,** 1070-1072.

Ito, H. and Koduh, S. (1997) Characteristics of water in Kongsfjorden, Svalbard. Proc. NIPR Symp. Polar Meteorol. Glaciol. **11,** 221-232.

Javor, B. (1989) *Hypersaline Environments. Microbiology and Biogeochemistry*. Springer-Verlag, Berlin.

Jones, G.E.B. (ed.) (1976) *Recent Advances in Aquatic Mycology*. The Gresham Press, Old Woking, Surrey.

Junge, K., Imhoff, F., Staley, T. and Deming, J.W. (2002) Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. Microb. Ecol. **43,** 315-328.

Kis-Papo, T., Oren, A., Wasser, S.P. and Nevo, E. (2003) Survival of filamentous fungi in hypersaline Dead Sea water. Microb. Ecol. **45,** 183-190.

Krembs, C., Eicken, H., Junge, K. and Deming, J.W. (2002) High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryopeotection of diatoms. Deep-Sea Res. I **49,** 2163-2181.

Kriss, A.E., Mitskevich, I.N., Rozanova, E.P. and Osnitskaia, L.K. (1976) Microbiological studies of the Wanda Lake (Antarctica). Mikrobiologiya **45,** 1075-1081.

Kurtzman, C.P. and Fell, J.W. (eds.) (1998) *The Yeasts, A Taxonomic Study*. Elsevier, Amsterdam.

Lahav, R., Fareleira, P., Nejidat, A. and Abeliovich, A. (2002) The identification and characterisation of osmotolerant yeast isolates from chemical wastewater evaporation ponds. Microb. Ecol. **43,** 338-396.

Lanoil, B. (2004) Microbiology of subglacial environments, In: *Extremophiles 2004*, *5th International Conference on Extremophiles, September 19-23, Abstract Book*. Cambridge, Maryland, p. 19.

Libkind, D., Brizzio, S. and van Broock, M. (2004) *Rhodotorula mucilaginosa*, a carotenoid producing yeast strain from a Patagonian high-altitude lake. Folia Microbiol. **49,** 19-25.

Loison, J., Bouchara, J.P., Guého, E., de Gentile, L., Cimon, B., Chennebault, J.M. and Chabasse, D. (1996) First report of *Cryptococcus albidus* septicaemia in a HIV pacient. J. Infect. **33,** 139-140.

López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C. and Moreira, D. (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature **409,** 603-607.

Ma, L.J., Rogers, S.O., Catranis, C.M. and Starmer, W.T. (1999a) Detection and characterization of ancient fungi entrapped in glacial ice. Mycologia **92,** 286-295.

Ma, L.J., Catranis, C.M., Starmer, W.T. and Rogers, S.O. (1999b) Revival and characterization of fungi from ancient polar ice. Mycologist **13,** 70-73.

Margesin, R., Zacke, G. and Schinner, F. (2002) Characterization of heterotrophic microorganisms in Alpine glacier cryoconite. Arctic, Antarctic and Alpine Res. **34,** 88-93.

Martin, S., Companys, E., Sanchis, V. and Ramos, A.J. (1998) Effect of water activity and temperature on competing abilities of common maize fungi. Mycol. Res. **120,** 959-964.

McRae, C.F., Hocking, A.D. and Seppelt, R.D. (1999) *Penicillium* species from terrestrial habitats in the Windmill Islands, East Antarctica, including a new species, *Penicillium antarcticum*. Polar Biol. **21,** 97-111.

Middelhoven, W.J. (1997) Identity and biodegradative abilities of yeasts isolated from plants growing in arid climate. Antonie van Leeuwenhoek **72,** 81-89.

Mindock, C.A., Petrova, M.A. and Hollingsworth, R.I. (2001) Re-evaluation of osmotic effects as a general adaptive strategy for bacteria in sub-freezing conditions. Biophys. Chem. **89,** 13-24.

Nagahama, T., Hamamoto, M., Nakase, T., Takami, H. and Horikoshi, K. (2001) Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific ocean. Antonie van Leewenhoek **80,** 101-110.

Norkrans, B. (1966) Studies on marine occuring yeasts: growth related to pH, NaCl concentration and temperature. Arch. f. Mikrobiol. **54,** 374-392.

Northolt, M.D., Frisvad, J.C. and Samson, R.A. (1995) Occurrence of food-borne fungi and factors for growth, In: R.A. Samson, E.S. Hoekstra, J.C. Frisvad and O. Filtenborg (eds.), *Introduction to Food-borne Fungi.* CBS, Delft, pp. 243-250.

Onofri, S., Selbmann, L., Zucconi, L. and Pagano, S. (2004) Antarctic microfungi as models for exobiology. Planet. Space Sci. **52,** 229-237.

Pennisi, E. (2003) Microbiology. Neither cold nor snow stops tundra fungi. Science **301,** 1307.

Peterson, S.W. (2000) Phylogenetic analysis of *Penicillium* based on ITS and lsu-rDNA nucleotide sequences, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*. Harwood Academic Publishers, Amsterdam, pp. 163-178.

Petrovič, U., Gunde-Cimerman, N. and Plemenitaš, A. (2002) Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*. Mol. Microbiol. **45,** 665-672.

Pitt, J.I. (ed.) (1979) *The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces*. Academic Press, London.

Pitt, J.I. and Hocking, A.D. (1985a) The ecology of fungal food spoilage, In: J.I. Pitt and A.D. Hocking (eds.), *Fungi and Food Spoilage*. Academic Press, Sydney, pp. 5-18.

Pitt, J.I. and Hocking, A.D. (1985b) Xerophiles, In: J.I. Pitt and A.D. Hocking (eds.), *Fungi and Food Spoilage*. Academic Press, Sydney, pp. 313-333.

Pitt, J.I. and Hocking, A.D. (eds.) (1997) *Fungi and Food Spoilage*, Blackie Academic and Professional, London.

Pitt, J.I., Samson, R.A. and Frisvad, J.C. (2000) List of accepted species and their synonyms in the family Trichocomaceae, In: R.A. Samson and J.I. Pitt (eds.), *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification.* Harwood Academic Publishers, Amsterdam, pp. 9-47.

Price, P.B. (2000) A habitat for psychrophiles in deep Antarctic ice. Proc. Natl. Acad. Sci. USA **97,** 1247-1251.

Priscu, J.C., Fritsen, C.H., Adams, E.E., Giovannoni, S.J., Paerl, H.W., McKay, C.P., Doran, P.T., Gordon, D.A., Lanoil, B.D. and Pinckney, J.L. (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science **280**, 2095-2098.

Reeve, J.N., Christner, B.C., Kvitko, B.H., Mosley-Thompson, E. and Thompson, L.G. (2002) Life in glacial ice, In: *Extremophiles 2002*, *4th International Congress on Extremophiles, September 22-26, Abstract Book.* Naples, Italy, p. 27.

Rivkina, E.N., Friedmann, E.I., McKay, C.P. and Gilichinsky, D.A. (2000) Metabolic activity of permafrost bacteria below the freezing point. Appl. Environ. Microbiol. **66,** 3230-3233.

Rodriguez-Vargas, S., Estruch, F. and Randez-Gil, F. (2002) Gene expression analysis of cold and freeze stress in baker's yeast. Appl. Environ. Microbiol. **68,** 3024-3030.

Roostita, R. and Fleet, G.H. (1996) The occurrence and growth of yeasts in Camembert and blue-veined cheeses. Int. J. Food Microbiol. **28,** 393-404.

Russell, N.J. (1997) Psychrophilic bacteria-molecular adaptations of membrane lipids. Comp. Biochem. Physiol., Part A, Physiol. **118,** 489-493.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C. and Filtenborg, O. (eds.) (2000) *Introduction to Food-and Airborne Fungi*. Centraalbureau voor Schimmelcultures, Utrecht.

Schadt, C.W., Martin, A.P., Lipson, D.A. and Schmidt, S.K. (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science **301,** 1359-1361.

Siegert, M.J., Ellis-Evans, J.C., Tranter, M., Mayer, C., Petit, J.R., Salamatin, A. and Priscu, J.C. (2001) Physical, chemical and biological processes in Lake Vostok and other Antarctic subglacial lakes. Nature **414,** 603-609.

Skidmore, M.L., Foght, J.M. and Sharp, M.J. (2000) Microbial life beneath a high Arctic glacier. Appl. Environ. Microbiol. **66,** 3214-3220.

Slavikova, E. and Vadkertiova, R. (1997) Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. Antonie van Leeuwenhoek **72,** 77-80.

Smedsgaard, J. (1997) Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. J. Chromatogr. **760,** 264-270.

Soares, C.A.G., Maury, M., Pagnocca, F.C., Araujo, F.V. and Mendoca-Hagler, L.C. (1997) Ascomycetous yeast from tropical intertidal dark mud of southeast Brazillian estuaries. J. Gen. Appl. Microbiol. **43,** 265-272.

Sonjak, S., Frisvad, J.C. and Gunde-Cimerman, N. (2005) Comparison of secondary metabolite production by *Penicillium crustosum* strains, isolated from Arctic and other various ecological niches. FEMS Microbiol. Ecol. (in press).

Sterflinger, K. and Krumbein, W.E. (1997) Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones. Geomicrobiol. J. **14,** 219-230.

Sterflinger, K., de Hoog, G.S. and Haase, G. (1999) Phylogeny and ecology of meristematic ascomycetes. Stud. Mycol. **43,** 5-22.

Stolk, A.C. and Samson, R.A. (1972) The genus *Talaromyces*. Studies on *Talaromyces* and related genera II. Stud. Mycol. **2,** 1-67.

Takano, Y., Kobayashi, K., Marumo, K. and Ishikawa, Y. (2004) Biochemical indicators and enzymatic activity below permafrost environment, In: *Extremophiles 2004, 5th International Conference on Extremophiles, September 19-23*, *Abstract Book*. Cambridge, Maryland, p. 84.

Tanghe, A., Van Dijck, P.V., Dumortier, F., Teunissen, A., Hohmann, S. and Thevelein, J.M. (2002) Aquaporin expresion correlates with freez tolerance in baker's yeast, and overexpression improves freeze tolerance in industrial strains. Appl. Environ. Microbiol. **68,** 5981-5989.

Thomas, D.N. and Dieckmann, G.S. (2002) Antarctic sea ice-a habitat for extremophiles. Science **295,** 641-644.

Tosil, S., Casado, B., Gerdol, R. and Caretta, G. (2002) Fungi isolated from Antarctic mosses. Polar Biol. **25,** 262-268.

Tresner, H.D. and Hayes, J.A. (1971) Sodium chloride tolerance of terrestrial fungi. Appl. Microbiol. **22,** 210-213.

Turk, M., Méjanelle, L., Šentjurc, M., Grimalt, J.O., Gunde-Cimerman, N. and Plemenitaš, A. (2004) Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. Extremophiles **8,** 53-61.

Vishniac, H.S. and Klinger, J. (1986). Extreme environments. Yeasts in the Antarctic deserts, In: F. Megušar and M. Gantar (eds.), *Perspectives in Microbial Ecology.* Slovene Society for Microbiology, Ljubljana, pp. 46-51.

Vishniac, H.S. and Onofri, S. (2003) *Cryptococcus antarcticus* var. *circumpolaris* var. nov., a basidiomycetous yeast from Antarctica. Antonie van Leeuwenhoek **83,** 231-233.

Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta **218,** 1-14.

Wasser, S.P., Grishkan, I., Kis-Papo, T., Buchalo, A.S., Volz, P.A., Gunde-Cimerman, N., Zalar, P. and Nevo, E. (2003) Species diversity of the Dead Sea fungi, In: E. Nevo, A. Oren and S.P. Wasser (eds.), *Fungal Life in the Dead Sea.* A.R.G. Gantner Verlag, Ruggell, pp. 203-270.

Wheeler, K.A. and Hocking, A.D. (1993) Ineteractions among xerophilic fungi associated with dried salted fish. J. Appl. Bacteriol. **74,** 164-169.

Yarrow, D. (1998) Methods for the isolation, maintenance and identification of yeasts, In: C.P. Kurtzman and J.W. Fell (eds.), *The Yeasts, A Taxonomic Study*. Elsevier, Amsterdam, pp. 77-100.

Zalar, P., de Hoog, G.S. and Gunde-Cimerman, N. (1999) Ecology of halotolerant dothideaceous black yeasts. Stud. Mycol. **43**, 38-48.

Zalar, P., de Hoog, G.S and Gunde-Cimerman, N. (2001) Fungi from the salterns: halophilic versus eurytolerant species in the genus *Cladosporium*, In: A. Ventosa and D.R. Arahal (eds.), *Abstracts of the International Conference on Halophilic Organisms, September 23-27.* Sevilla, Spain, p. 17.

Zalar, P., de Hoog, G.S., Schroers, H.-J., Frank, J.M. and Gunde-Cimerman, N. (2005) Taxonomy and phylogeny of the osmophilic genus *Wallemia* (*Wallemiomycetes* and *Wallemiales*, cl. et ord. nov.). Antonie van Leeuwenhoek (in press).

Zhu, J.-K. (2001) Cell signaling under salt, water and cold stresses. Curr. Opin. Plant Biol. **4,** 401-406.

Zlatanov, M., Pavlova, K. and Grigorova, D. (2001) Lipid composition of some yeast strains from Livingston Island, Antarctica. Folia Microbiol. **46,** 402-406.