Yeast Biotechnology: Diversity and Applications

T. Satyanarayana • Gotthard Kunze
Editors

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Brief CVs of the Editors

After completing M.Sc. and Ph.D. at the University of Saugar (India), T. Satyanarayana had post-doctoral stints at the University of Bhopal and France. In 1988, he joined the Department of Microbiology, University of Delhi South Campus as Reader and became Professor in 1998. During this period, his research efforts have been focused on understanding the diversity of yeasts and thermophilic fungi and bacteria, their enzymes and potential applications, production of ectomycorrhizal fungal inoculum and heterotrophic carbon sequestration. He has published over 140 scientific papers and reviews and edited two books. He is fellow of the Association of Microbiologists of India, Mycological Society of India and Biotech Research Society of India, and a recipient of Dr. G.B. Manjrekar award of the Association of Microbiologists of India in 2004. He is in the editorial board of Bioresource Technology and Indian Journal of Microbiology.

Gotthard Kunze studied biology at the Ernst Moritz Arndt University in Greifswald. He got a post-doctoral fellowship and a position as scientific assistant at the Department of Biology of the university. In 1986 he joined as a research associate at the Institute of Genetics and Crop Plant Research (IPK) at Gatersleben. Since 1998 he is a visiting professor at the University Greifswald and since 1998 professor at the Technical University Anhalt at Köthen. During this period, he focused his research activities on yeast genetics (construction of new yeast host vector systems, heterologous gene expression, thermo- and osmoresistance in non-conventional yeasts and microbial yeast biosensors). Prof. Gotthard Kunze is the author of about 140 publications, editor of 2 books and teaches at the universities of Greifswald and Köthen.

Foreword

The objective of the book is to give a review of knowledge on the diversity and potential applications of yeasts to the researchers in this field and to the biotechnologists. This book is a collection of articles on yeasts, which will be very useful for those who desire to have an up-to-date volume. The researchers have attempted to communicate their significant observations and ideas to the scientific community. I believe that the book will expose students to new developments in the yeast research.

Yeast communities have been found in association with plants, animals and insects. Several species of yeasts have been isolated from specialized or extreme environments. Yeasts play a vital role in food chains, carbon, nitrogen and sulphur cycles. Yeasts are now being used to express foreign genes for producing human proteins of pharmaceutical interest. The products of modern yeast biotechnologies impinge on many commercially important sectors including food, beverages, chemicals, pharmaceuticals, industrial enzymes and agriculture. The vast majority of yeasts are beneficial to human life.

This book is divided into three parts, the first part, i.e., *'Diversity and Biology'* gives information about the various yeasts species. Diversity searches in the natural environment have resulted in the description of new yeast species at a rapid pace and the field is wide open to global exploration. The second part, i.e., *'Genetic and Molecular Insights'* aims at reviewing the use of this system as an experimental tool for conducting classical genetics. Yeasts have been recognized as a very important group of microorganisms on account of their extensive use in the fermentation industry and as a basic eukaryotic model cellular system. The latest developments in genomics and micro-array technology have allowed investigations of individual gene function by site-specific deletion method. The third part, i.e., *'Biotechnology Applications'* focuses on the hydrolysis of starchy and lignocellullosic substrates to sugars and their fermentation to ethanol, yeast enzymes and their potential applications. The survey is also given here of the production, the characteristics and the potential applications of currently well studied yeast extracellular polysaccharides. It emphasizes on the biological significance and their industrial applications.

I believe that the book would provide an overview of the recent developments in the domain of yeast research with some new ideas, which could serve as an inspiration and challenge for researchers in this field.

New Delhi
Dec. 24, 2007

Prof. Asis Datta
Former Vice-chancellor, JNU
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Preface

Yeasts are eukaryotic unicellular microfungi that are widely distributed in the natural environments. Although yeasts are not as ubiquitous as bacteria in the natural environments, they have been isolated from terrestrial, aquatic and atmospheric environments. Yeast communities have been found in association with plants, animals and insects. Several species of yeasts have also been isolated from specialized or extreme environments like those with low water potential (e.g. high sugar/salt concentrations), low temperature (e.g. yeasts isolated from Antarctica), and low oxygen availability (e.g. intestinal tracts of animals). Around 1500 species of yeasts belonging to over 100 genera have been described so far. It is estimated that only 1% of the extant yeasts on earth have been described till date. Therefore, global efforts are underway to recover new yeast species from a variety of normal and extreme environments.

Yeasts play an important role in food chains, and carbon, nitrogen and sulphur cycles. Yeasts can be genetically manipulated by hybridization, mutation, rare mating, cytoduction, spheroplast fusion, single chromosomal transfer and transformation using recombinant technology. Yeasts (e.g. *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Pichia pastoris*) are now being used to express foreign genes for producing human proteins of pharmaceutical interest. A landmark in biotechnology was reached in 1996 with the completion of the sequencing of the entire genome of *S. cerevisiae*. The genome sequencing of three more yeasts (*Schizosaccharomyces pombe*, *Candida albicans* and *Cryptococcus neoformans*) have recently been completed. *S. cerevisiae* has now become a central player in the development of an entirely new approach to biological research – systems biology. The systems biology was made possible because the yeast genome had been sequenced, micro-array analysis of yeast mRNA had been developed, databases of protein-protein interactions were available, rapid MS analysis of protein presence and abundance was possible, and computing facilities to process all of the information were available.

The products of modern yeast biotechnologies impinge on many commercially important sectors including food, beverages, chemicals, pharmaceuticals, industrial enzymes, agriculture (e.g. *S. cerevisiae* has the potential in stimulating cereal plant defences against fungal pathogens, yeasts like *Debaryomyces hansenii* can be used in the biocontrol of fungal fruit diseases, live yeasts like *S. cerevisiae* stabilize

rumen environment, provide dicarboxylic acids to stimulate rumen bacteria) and the environment (e.g. yeasts biosorb heavy metals and detoxify chemical pollutants). Although the vast majority of yeasts are beneficial to human life, some are opportunistically pathogenic towards humans (e.g. candidiosis caused by *Candida albicans*, yeast infections in immuno-compromised individuals and AIDS patients).

The book is aimed at bringing together the scattered knowledge that has accumulated in the last few decades on aspects such as diversity of yeasts in normal and extreme environments, their ecology and adaptations, developments in their taxonomy and systematics, physiology and biochemistry, molecular aspects, and their potential biotechnological applications.

We are grateful to all the authors for readily accepting our invitation and contributing chapters for the book. We wish to thank Springer for publishing the book, and Dr. Deepak Chand Sharma, Mr. Bijender Singh and Mr. Rakesh Kumar for extending help in preparing the book.

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Part I
Diversity and Biology

Chapter 1
Antarctic Yeasts: Biodiversity and Potential Applications

S. Shivaji and G.S. Prasad

Contents

Abstract This review is an attempt in cataloguing the diversity of yeasts in Antarctica, highlight their biotechnological potential and understand the basis of adaptation to low temperature. As of now several psychrophilic and psychrotolerant yeasts from Antarctic soils and marine waters have been characterized with respect to their growth characteristics, ecological distribution and taxonomic significance. Interestingly most of these species belonged to basidiomycetous yeasts which as a group are known for their ability to circumvent and survive under stress conditions. Simultaneously their possible role as work horses in the biotechnological industry was recognized due to their ability to produce novel enzymes and biomolecules such as agents for the breakdown of xenobiotics, and novel pharmaceutical chemicals. The high activity of psychrophilic enzymes at low and moderate temperatures offers potential economic benefits. As of now lipases from *Pseudozyma antarctica* have been extensively studied to understand their unique thermal stability at 90°C and also because of its use in the pharmaceutical, agriculture, food, cosmetics and chemical industry. A few of the other enzymes which have been studied include

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extracellular alpha-amylase and glucoamylase from the yeast *Pseudozyma antarctica* (*Candida antarctica*), an extra-cellular protease from *Cryptococcus humicola*, an aspartyl proteinase from *Cryptococcus humicola,* a novel extracellular subtilase from *Leucosporidium antarcticum,* and a xylanase from *Cryptococcus adeliensis*

The ability of these yeasts to adapt to the low temperature conditions has also led to investigations directed towards characterizations of cold stress proteins and heat shock proteins so as to understand the role of these stress protein with respect to adaptation. Antarctic yeasts have also been used as model system to study the inter-relationship among free radicals, antioxidants and UV-induced cell damage.

Keywords Biodiversity, yeast, Antarctica, enzymes, lipase, psychrophilic

1.1 Introduction

The continent of Antarctica which occupies an area of 14 million square kilometers, is a major cold habitat, of which about 99% is covered by ice and snow (Holdgate, 1977). Apart from being very cold, this continent is considered to be a very extreme habitat due to the fact that it is also the driest (Vincent, 1988; Claridge and Campbell, 1977; Campbell and Claridge, 2000), windiest and iciest of all known habitats of the world with high solar radiation at least during the summer season (Smith et al., 1992). Despite these extreme conditions, Antarctica is host to a number of life forms demonstrated by the presence of bacteria, yeasts, fungi, lichens, small invertebrates, many species of birds and mammals (Cameron et al., 1970; Vishniac and Mainzer, 1972; Vincent, 1988; Wynn-Williams, 1990). All these life forms have evolved special mechanisms to overcome the influence of low temperature, high salinity and high radiation.

The microorganisms that thrive in the extreme environment of Antarctica are cold loving and are referred to as psychrophiles. Psychrophilic (cold-loving) organisms differ from the psychrotolerant (cold-tolerant) organisms, on the basis of their cardinal temperatures. Psychrophilic yeasts have an optimum temperature for growth at about 15°C or lower, a maximum up to 25°C but are still capable of growing at 0°C or below (Morita, 1975; Arthur and Watson, 1976); in contrast the psychrotolerant microorganisms are those that are capable of growing at 5°C and below, regardless of whether the optimum temperature was about 15°C or more (van Uden, 1984; Vishniac, 1987). Psychrophiles are unable to grow above 20°C and are widely prevalent in permanently cold habitats, such as in polar regions, at high altitudes or in the deep sea. In contrast, the psychrotolerant which grow over a wider range of temperature and show better growth rates above 20°C are predominant in environments with periodic low temperatures. In Antarctica, psychrophilic and psychrotolerant microorganisms are believed to play key roles in the biodegradation of organic matter and the cycling of essential nutrients (Russell, 1990).

Psychrophilic bacteria, yeasts and other microorganisms define the lower limits of temperature for the survival of life forms. In this context the psychrophilic bacteria and yeasts of Antarctica could serve as excellent model systems to understand the molecular basis of survival at low temperatures. As yet, biological studies in Antarctica have mostly focused on the diversity of bacteria (Shivaji, 2005; Shivaji et al., 2005a; Prabagaran et al., 2006), their taxonomic position (Shivaji et al., 2004; Shivaji et al., 2005b, 2005c), their biotechnological potential (Cavicchioli et al., 2002) and as model systems to understand adaptation of microorganisms to the low temperature (Shivaji et al., 2007; Chintalapati et al., 2006, 2007; Kiran et al., 2004, 2005; Jagannadham et al., 1991, 2000; Chattopadhyay et al., 1997; Ray et al., 1994a, b, c). However, similar studies on yeasts are very limited. This review focuses primarily on the diversity and cataloging of yeasts from Antarctica and their biotechnological potential.

1.2 Diversity of Yeasts in Antarctica

Yeasts are a versatile group of eukaryotic microorganisms which are heterogeneous in their nutritional abilities and are capable of surviving in a range of habitats (Lachance and Starmer, 1988) such as in deep sea (Seiburth, 1979; Fell, 1976), moist and uneven surfaces including polluted waters (Hagler and Ahearn, 1987), on dry substrates and in the presence of high concentrations of salt and sugar (Ingram, 1958). Turkiewicz et al. (2003) suggested that yeasts may be better adapted to low temperatures than bacteria. Therefore, it is not surprising that yeasts belonging to genera such as *Bullera, Candida, Cryptococcus*, *Cystofilobasidium, Debaryomyces, Kondoa, Leucosporidium, Metschnikowia, Mrakia, Pseudozyma*, *Rhodotorula, Sakaguchia, Sporopachydermia, Sympodiomyces* and *Trichosporon* have been identified in various habitats of Antarctica.

1.2.1 Distribution of Yeasts in Antarctica

The entire Antarctic region is cold and therefore the distribution of yeasts in Antarctica if dependent only on temperature one should be able to see yeasts uniformly distributed. But this is not so. The most northern and southern sampling sites in Antarctica are separated by 10° of latitude. Despite this separation, psychrophiles appeared to be random in their distribution and did not increase with latitude (di Menna, 1960, 1966a). The possible reasons for not obtaining yeasts from some Antarctic samples could be due to the fact that the isolation methods were unsuitable, the incubation temperatures being too high or too low, the incubation time too short, or the medium is too acidic or because of too low osmotic pressure (di Menna, 1966a). Yeasts were usually found in substrates which are acidic rather than alkaline, but inspection of the results showed that high pH values were

not in themselves inhibitory. It was also observed that the yeasts found in Antarctic soils appeared to be dependent on plants. Babyeva and Golubev (1969) isolated more yeasts at 5°C than at higher temperature and showed that forty percent of their 63 isolates were "obligate psychrophiles", failing to grow above 20°C. Vishniac (1996) concluded that the biodiversity of yeasts and filamentous fungi in terrestrial Antarctic ecosystems increases with the availability of water and energy. Further it was also suggested that yeasts predominate in continental Antarctica compared to maritime and sub-Antarctic habitats (Vishniac, 1996).

1.2.2 Survival of Yeasts in Antarctica

Over the years attempts have been made to understand as to how psychrophilic yeasts survive at low temperatures (< 20°C) (Inniss, 1975; Larkin and Stokes, 1968).On the basis of melting points of major fatty acids present in yeasts, it was proposed that the psychrophilic yeasts would be able to grow at temperatures as low as −10°C. Further thermotolerance to temperatures > 20°C may be attributed to the capacity of these yeasts to synthesize heat shock proteins (hsp) and (or) trehalose accumulation as in *Mrakia frigida, Leucosporidium fellii* and *L. scottii* but not in *L. antarcticum* (Deegenaars and Watson, 1997, 1998). In fact based on these studies it was speculated that hsp 110 may play a role in stress tolerance in psychrophilic yeasts, similar to that of hsp 104 in mesophilic species.

1.2.3 Lipid Composition of the Membranes and Psychrophily

Several studies have clearly indicated that the ability to modulate membrane fluidity by regulating the synthesis of fatty acids is very crucial for low temperature adaptation (Shivaji et al., 2007; Chintalapati et al., 2005). As a thumb rule, low growth temperature increases the proportion of unsaturated fatty acids compared to the saturated fatty acids. This phenomenon applies to bacteria or yeasts (Shivaji et al., 2007; Chintalapati et al., 2005; Sato and Murata, 1980; Sato et al., 1979; Murata et al., 1992; Wada and Murata, 1990; Arthur and Watson, 1976) and in several species of psychrophilic yeasts the unsaturated fatty acids, constituted 50–90% of the total fatty acid composition as in species of *Mrakia, Candida, Torulopsi, Leucosporidium*, and *Cryptococcus* (Watson, 1987; Thomas-Hall and Watson, 2002). Sabri et al. (2001) showed that the inability of *Rhodotorula aurantiaca* to grow at temperatures close to 20°C was due to high accumulation of myristoyl-CoA (C_{14}-CoA), (28-fold higher than in cells cultivated at 0°C temperature). Silver et al. (1977), observed that the cessation of growth at temperatures above 20°C in the psychrophilic yeast *Leucosporidium stokesii* is due to the inability of the yeast to complete an event(s) associated with nuclear division

such as DNA synthesis and normal cell division cycle (Silver and Sinclair, 1979). Meyer et al. (1975) observed that the psychrophilic yeasts are more sensitive to freeze-thaw cycles compared to mesophilic yeasts.

1.2.4 Yeasts of the Genus Cryptococcus

The abundance of yeast in Antarctica varies depending on the habitat. In fact even in the same habitat, such as soil, the number varied from total absence to as many as 100,000 yeasts per gram of soil (di Menna, 1966a). *Cryptococcus* is the most predominant group of yeasts in the Antarctic. In this genus C. *laurentii* and *C. albidus* are more predominant compared to *C. luteolus* and *C. diffluens* (di Menna, 1966a). Several new species of *Cryptococcus* have been reported from various habitats in Antarctic such as *Cryptococcus friedmannii* from an Antarctic cryptoendolithic community (Vishniac, 1985a); *Cryptococcus vishniacii* (Vishniac and Hempfling, 1979a, b; Vishniac and Baharaeen, 1982), *Cryptococcus antarcticus* (Vishniac and Kurtzman, 1992; Vishniac and Onofri, 2003), *Cryptococcus albidosimilis* (Vishniac and Kurtzman, 1992), *Cryptococcus socialis* (Vishniac, 1985b), and *Cryptococcus consortionis* (Vishniac, 1985b) from Arctic soils; *Cryptococcus victoriae* (Montes et al., 1999), *Cryptococcus adeliensis, Cryptococcus albidus, C. laurentii* and *Candida oleophila* (Scorzetti et al., 2000; Pavlova et al., 2001) from mosses and lichens; *Cryptococcus nyarrowii* and *Cryptococcus statzelliae* from soil and snow samples (Thomas-Hall et al., 2002). Some strains of yeasts belonging to the same species appeared to be very different morphologically. Interestingly *Cryptococcus nyarrowii* was represented by two different coloured strains CBS 8804^T (pink colonies) and CBS 8805 (yellow colonies). Other yeast strains (CBS 8908, CBS 8915 and CBS 8920) such as *Cryptococcus victoriae*, *Cryptococcus watticus* sp. nov. (CBS 9496^T) were also isolated from samples collected from the Vestvold Hills, Davis Base (Guffogg et al., 2004).

Cryptococcus laurentii and *C. albidus* are considered as ubiquitous, and are reported by almost all investigators from Antarctica. This could be due to incorrect delineation of these species, as several tests used for identifying them are variable (Fell and Statzell-Tallman, 1998; Barnett et al., 2000; Takashima et al., 2003; Fonseca et al., 2000; Sugita et al., 2000). Sequence analysis of D1/D2 domain of the large subunit rRNA gene and the ITS region has resulted in description of several new species of *Cryptococcus* which were earlier thought to be either *C. albidus* or *C. laurentii,* based on phenotypic methods (Takashima et al., 2003; Middelhoven, 2005). It is also difficult to discriminate *Cryptococcus laurentii* from *C. cellulolyticus, C. flavus, C. humicola and C. hungaricus* based on physiological characters (Barnett et al., 2000). Sugita et al. (2000) reported genetic diversity in the ITS and D1/D2 regions among the clinical isolates of *C. laurentii* and 10 isolates examined in that study were found to belong to seven different species. Similarly, Fonseca et al. (2000) examined several strains of "*Cryptococcus albidus*", using sequence

analysis of the D1/D2 domain of large subunit rRNA gene and established eight new species.

According to Vincent (1988), the *Cryptococcus* yeasts recovered in Antarctic lakes were clearly the result of wash-in from adjacent soils. Moreover, polar soil yeasts, which occur in significant numbers, were found mostly in soil samples that also contain moss, lichen or microalgal material. Vishniac (1995) demonstrated that *Cryptococcus albidus,* a dominant soil organism, was capable of rapid growth when introduced into autoclaved soil, following which viability was retained for 2 months. It was suggested that sterilization altered the nutritional value of the soil in a manner similar to natural weathering factors. Consistent with this, the growth of indigenous soil yeasts would be a function of the frequency and intensity of disturbances of the soil.

1.2.5 Yeasts of Other Genera

Yeasts belonging to the genus *Candida* appear to be quite common in Antarctica but not as predominant as the *Cryptococcus* yeasts. Several strains of *Candida* spp. such as *Candida nivalis, Candida gelida* and *Candida frigida*, presently known as *Mrakia frigida* (di Menna, 1966b), *Candida humicola, Cadida famata, Candida ingeniosa* and *Candida auricullariae* (Ray et al., 1989) and *Candida oleophila.* (Pavlova et al., 2001) have been isolated from soil and moss. *Candida* (*Torulopsis*) *austromarina* (Fell and Hunter, 1974) has been reclassified as *Candida sake* on the basis of identity of the D1/D2 regions of rDNA. (Kurtzman and Robnett, 1998). All these yeasts were found to be psychrophilic. *Candida* isolates were also identified in various other habitats of Antarctica such as in water, associated with algae, penguin dung etc. (Goto et al., 1969). Other yeasts isolated from Antarctica include *Leucosporidium* (Fell et al., 1969), *Debaryomyces hansenii* (Biswas et al., unpublished results), *Rhodotorula rubra*, (Ray et al., 1989), *Rhodotorula minuta* (Pavlova et al., 2001), *Rhodotorula mucilaginosa* (Pavlova et al., 2001), *Bullera alba* (Ray et al., 1989), *Mrakia frigida* (Biswas et al., unpublished results) and *Mrakia psychrophila* closely related to *Mrakia frigida* (Xin and Zhou, 2007).

1.3 Antarctic Yeasts in Culture Collections

It is interesting to note that about 90% of the yeasts isolated from Antarctica are of basidiomycetous origin (Table 1.1).

The Centraalbureau voor Schimmecultures (CBS), Utrecht, Netherlands has 125 Antarctic yeast strains and the American Type Culture Collection (ATCC), USA has 18 Antarctic yeast cultures, including type strains of nine species of *Cryptococcus* (Table 1.1). Based on the sequence analysis of D1/D2 domain of 26S rRNA gene (Fell et al., 2000) and ITS regions (Scorzetti et al., 2002) these nine type strains

Table 1.1 Antarctic yeast strains available at The Centraalbureau voor Schimmecultures, Utrecht, the Netherlands

Accepted scientific name	CBS accession number	Habitat	Site of collection
Candida davisiana Guffogg et al.	CBS 9495	Soil	Antarctica, Davis base, Vestvold Hills, Moss Cirque
Candida parapsilosis group II	CBS 8548	-	Antarctica
Candida psychrophila (S. Goto et al.) S.A. Meyer & Yarrow	CBS 5956	Dung of penguin	Antarctica, Ross Island, Cape Royds
Candida sake (Saito & Oda) van Uden & H.R. Buckley	CBS 5957	Stream water	Antarctica, Lake Bonney
Cryptococcus adeliensis Scorzetti et al.	CBS 8351	Decayed algae	Antarctica, Dumont d'Urville base
Cryptococcus albidosimilis Vishniac & Kurtzman	CBS 7711	Soil	Antarctica, South Victoria Land, Wright Valley, Linnaeus Terrace
Cryptococcus albidus (Saito) C.E. Skinner et al. var. *albidus*	CBS 9809	Soil	Antarctica, Victoria Land, Edmonson Point
Cryptococcus antarcticus Vishniac & Kurtzman var. *antarcticus* Vishniac & Kurtzman	CBS 7687	Soil	Antarctica, University Valley
Cryptococcus antarcticus Vishniac & Kurtzman var *circumpolaris* Vishniac & Onofri	CBS 7689	Soil	Antarctica, University Valley
Cryptococcus consortionis Vishniac	CBS 7159	Soil	Antarctica, South Victoria Land, Linnaeus Terrace
Cryptococcus friedmannii Vishniac	CBS 7160	Soil	Antarctica, Ross Desert
Cryptococcus humicola (Daszewska) Golubev	CBS 5958	Water	Antarctica, Lake Vanda
Cryptococcus mycelialis Golubev, V.I & Golubev, N.V	CBS 7712	Soil	Antarctica, East Falkland Island
Cryptococcus nyarrowii Thomas-Hall & Watson	CBS 8805	Soil and lichen	Antarctica, Lichen Valley, Vestfold Hills, Davis base
Cryptococcus nyarrowii Thomas-Hall & Watson	CBS 8804	Bird	Antarctic, Lichen Valley, Vestfold Hills, Davis base
Cryptococcus socialis Vishniac	CBS 7158	Soil	Antarctica, South Victoria Land, Linnaeus Terrace
Cryptococcus victoriae Montes et al.	CBS 8685	Soil	Antarctica, Victoria Land
Cryptococcus vishniacii Vishniac & Hempfling var. *vishniacii*	CBS 6808	Soil	Antarctica, Mount Baldr

(continued)

Table 1.1 (continued)

Accepted scientific name	CBS accession number	Habitat	Site of collection
Cryptococcus watticus Guffogg et al.	CBS 9496	Soil	Antarctic, Davis base, Vestfold Hills, Watts Lake
Cystofilobasidium bisporidii (Fell et al.) Oberwinkler & Bandoni	CBS 6346	Sea water	Antarctic Ocean
Cystofilobasidium capitatum (Fell et al.) Oberwinkler & Bandoni	CBS 6358	Zooplankton	Antarctic Ocean
Cystofilobasidium infirmominiatum (Fell et al.) Hamamoto et al	CBS 6350	Zooplankton	Antarctic Ocean
Kondoa malvinella (Fell & Hunter) Y. Yamada et al.	CBS 6082	Sea water	Antarctica
Leucosporidium antarcticum Fell et al.	CBS 5942	Sea water	Antarctica, Weddell Sea off Joinville Island
Leucosporidium scottii Fell et al.	CBS 5930	Sea water	Antarctica
Metschnikowia australis (Fell & Hunter) Mendonça-Hagler et al.	CBS 5847	Sea water	Antarctic Ocean
Metschnikowia koreensis Hong et al.	CBS 9068	-	Antarctica
Mrakia frigida (Fell et al.) Y. Yamada & Komagata	CBS 5266	Soil	Antarctica, Scott Base
Rhodosporidium sphaerocarpum S.Y. Newell & Fell	CBS 5939	Sea water	Antarctica, Marguerite Bay
Rhodotorula minuta (Saito) F.C. Harrison var. minuta	CBS 9810	Soil	Antarctica, Victoria Land, Edmonson Point
Rhodotorula sp. F.C. Harrison	CBS 8940	Water	Antarctica, Chelnok lake
Sakaguchia dacryoidea (Fell et al.) Y. Yamada et al.	CBS 6353	Sea water	Antarctic Ocean
Sporopachydermia lactativora Rodrigues de Miranda	CBS 5771	Sea water	Antarctic Ocean
Sympodiomyces parvus Fell and Statzell-Tallman	CBS 6147	Sea water	Antarctic Ocean
Trichosporon pullulans (Lindner) Diddens & Lodder	CBS 5108	Soil	Antarctica

were identified as being synonyms to *Cryptococcus vishniacii* var. *vishniacii.* The Microbial Type Culture Collection and Gene Bank (MTCC) in India has 25 Antarctic yeasts in its collection, isolated from the Schirmacher Oasis region of Antarctica (Ray et al., 1989).

1.4 Are Antarctic Yeasts Endemic?

The larger question in microbial ecology is whether microbes are endemic? The continent of Antarctica due to its remoteness and isolation from the remaining landmass of the earth for millions of years should be amongst the first places to look for endemic organisms and also to examine the evolutionary processes that can give rise to microbial speciation. *Cryptococcus antarcticus* and *C. vishniacii* occur in Antarctica and as of now are unknown outside Antarctica (Vishniac, 1999). But this may not be sufficient evidence in support of endemism since many other yeasts are widely distributed. *Candida antarctica* (reclassified as *Pseudozyma antarctica*), was first isolated from Antarctica (Goto et al., 1969); but later it was identified from Japanese natural samples and from flowers in India (Saluja and Prasad, unpublished observations). Similarly, *Cryptococcus victoriae*, first reported from Antarctica (Montes et al., 1999) is also found in flower and soil samples in India (Saluja and Prasad, unpublished observations). The yeast genus *Leucosporidium* originally isolated from Antarctica was later isolated from temperate climates (Summerbell, 1983). However, the species of the genus *Mrakia* seems to be confined to cold habitats. Besides, Antarctica it has been reported from other cold habitats such as European Alps (Margesin et al., 2005), Hokkaido, Japan (Nakagawa et al., 2004), glacial and subglacial waters of northwest Patagonia, Argentina (Brizzio et al., 2007), Western Siberia (Poliakova et al., 2001) and Tinto river in southwestern Spain (Lopez-Archilla et al., 2004). A new species *Mrakia curviuscula* was isolated from forest substrates collected in the central part of European Russia (Bab'eva et al., 2002). It appears that organisms are extremely versatile in their adaptive capabilities and therefore would break the shackles of endemism and attain an ubiquitous distribution.

1.5 Biotechnological Potential of Antarctic Yeasts

1.5.1 Enzymes from Antarctic Yeasts

Bioprospecting for biomolecules such as enzymes, pigments, polyunsaturated fatty acids etc. from psychrophilic yeasts has gained momentum with the realization that these yeasts due to their unique ability to survive and grow at low temperatures

Table 1.2 Enzymes produced by Antarctic yeasts

Enzyme	Yeast	Reference
Proteinase	*Cryptococcus friedmannii*	Vishniac, 1985
Serine proteinase	*Leucosporidium antarctium*	Turkiewicz et al., 2003
Aspartyl proteinase	*Candida humicola*	Ray et al., 1992
Xylanase	*Cryptococcus adeliensis*	Gomes et al., 2000; Petrescu et al., 2000
Xylanase	*Cryptococcus albidosimilis* (*Cryptococcus albidus* TAE85)	Amoresano et al., 2000
Lipase	*Leucosporidium antarcticum*	Turkiewicz et al., 2003
Lipases A and B	*Pseudozyma antarctica* (*Candida antarctica*)	
α-Glucosidase	*Leucosporidium antarcticum*	Turkiewicz et al., 2003
α-Amylase	*Candida antarctica*	De Mot and Verachtert, 1987
Glucosamylase	*Candida antarctica*	De Mot and Verachtert, 1987
Acid phosphatase	*Leucosporidium antarcticum*	Turkiewicz et al., 2003
Alkaline phosphatase	*Leucosporidium antarcticum*	Turkiewicz et al., 2003
Beta-fructofuranosidase	*Leucosporidium antarcticum*	Turkiewicz et al., 2003

would be producing enzymes which are cold active and also other biomolecules so as to facilitate their survival at low temperatures. Cold active enzymes may provide interesting clues that would add to our understanding of the relationship between structure, stability and activity of enzymes at low temperatures (Gerday et al., 1997). Most biological systems show 2–3 times reduced reaction rate when the temperature is decreased by 10°C. Enzymes from psychrophilic microorganisms are thought to have evolved a more flexible structure when compared to their mesophilic and thermophilic counterparts. This character probably originates from weakening of intramolecular interactions and is supposed to be responsible for the increased catalytic efficiency and the low thermal stability of psychrophilic enzymes in general (Feller and Gerday, 1997). Several different types of enzymes have been characterized from psychrophilic yeasts (Table 1.2).

1.5.2 Lipases

Two lipases from *Pseudozyma antarctica* (*Candida antarctica*) namely CAL-A and CAL-B have been patented and used for various processes such as preparation of optically active amines, acid ethyl esters, triglycerides, alkyl ester derivatives of restaurant grease (Hsu et al., 2003), hydrolysis of fats, hydrolysis of water insoluble esters of fats, hydrolysis of a mixture of (chloromethyldimethylsilyl)-2-propenyl acetate (Rubio et al., 2001), synthesis of polyesters etc. which are useful to the detergent, food, pharmaceutical and other industries (UNEP report on Antarctic bioprospecting, 2004). Thus both these lipases have extensive applications (de Maria et al., 2005) and CAL-A is considered as the most thermostable lipase known, being able to work efficiently even at above 90°C