

## Review

# Cold-Adapted Yeasts from Patagonia Argentina

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## Abstract

The Patagonian Andes possess a great variety of glaciers, large temperate ice masses, and glacially formed water bodies. Most of these environments are included in protected areas characterized by low anthropogenic impact and minimal atmospheric pollution. Studies on occurrence of psychrotolerant (cold-adapted) and psychrophilic yeasts in these natural environments have allowed the characterization of native cold-adapted yeast communities, the description of six novel species, as well as description of the capability of these yeasts to produce extracellular enzymes, and other metabolites (carotenoids and mycosporines). Cold-adapted yeasts from Patagonian glaciers were able to hydrolyze natural polymers (casein, lipids, starch, pectin and carboxymethylcellulose) at low temperatures and some were able to produce photo-protective compounds. These results suggest a significant ecological role of these organisms as organic matter decomposers and nutrient cyclers, also reveal that some of these yeasts could be used for further metabolic, ecological and biotechnological studies. The objective of this review is to summarize all the findings related to cold adapted yeasts from glacial environments of the South Hemisphere (outside Antarctica), also to highlight the importance of continental ice masses from the microbiological perspective.

**Keywords:** yeasts, psychrophilic, glaciers, extracellular enzymes.

## Glaciers: general characteristics

Snow and glacial ice are interrelated ecosystems, glacier ice is formed from snow as a result of gradual compression and burial for hundreds of thousands of years (Miteva 2008).

A glacier can change its size by accumulation, which occurs by addition of snowfall, compaction and recrystallization, or by ablation, which is the loss of mass resulting from melting. Many glaciers from temperate, alpine and seasonal polar climates, store water as ice during the colder seasons and release it later in the form of melt water. Today glacial ice

is the largest reservoir of freshwater on Earth (Bahr & Radic 2012; Brown et al. 2010).

The climate in Patagonia is described as temperate or cold-temperate. Southern Patagonia has the influence increased of polar winds, so that the summer is cooler and wetter than further north and snowfall is a common phenomenon in this region (Correa 1998).

In Argentina there exists a great variety of glaciers and glacially formed water bodies (Ruiz et al. 2012). Monte Tronador is an old volcano that was degraded by intensive glacial erosion. Its main summit is 3,478 meters above sea level

and the upper most part of the covered by a thick permanent cap of ice that is the source-area for 10 glaciers. Four of these are located in Argentina (Río Manso, Castaño Overo, Alerce, Frías, in Nahuel Huapi National Park), the remaining six are in Chile (Rabassa et al. 1978; Villarosa et al. 2008). Los Glaciares National Park (Argentina) and Patagonian icefields (Hielos Patagónicos) are the largest temperate ice masses in the Southern Hemisphere and account for more than 60 % of the Southern Hemisphere's glacial area outside Antarctica. Perito Moreno glacier is located within these icefields (Skvarca et al. 2003; Stuefer et al. 2007).

Glaciers in many mountainous regions of the world have shown a generalized pattern of recession during the 20th century. The recent pattern of glacier mass losses is concurrent with a period of increasing temperatures in most regions, supporting the notion that glaciers can be used as key indicators of regional and larger-scale climatic changes (Delgado Granados et al. 2007). The glaciers of Patagonia Argentina are not an exception. Monte Tronador Patagonia Argentina glaciers are a clear example of this situation, having experienced a constant retreat for the past 100 years (Villarosa et al. 2008). Perito Moreno glacier, however, is one of the few glaciers that remains stable, i.e., it is neither advancing nor retreating (Stuefer et al. 2007).

Glacier/climate interactions in the Patagonian region are of relevance to understanding the global climate change pattern. In addition, icefields and periglacial areas hold valuable information for Quaternary paleo-environments (Stuefer et al. 2007). Lakes, rivers and peat-lands are the main landforms that create conditions for the study of past and present environmental variations in the region (Villarosa et al. 2008) and there is also dense forest-cover associated with recent glacial deposits. The relevance of studies on the effects of climatic and environmental changes on continental glaciers has been shown elsewhere, especially in the northern hemisphere (Branda et al. 2010; Villarosa et al. 2008).

### **Glaciers as microbial reservoirs**

Glacial ice is a substrate that can act as long-term, chronological repository of viable microorganisms (Butinar et al. 2007). Due to harsh conditions (high pressure, darkness, acidity and low water activity), these environments can be considered extreme habitats (Price 2007). In temperate glaciers, habitats include crevasses and water channels can act as growth substrates for microbial communities while, at the same time, convey water, nutrients, atmospheric gases, and microbiota into the glacier (Price 2007).

Psychrotolerant and psychrophilic microorganisms have been shown to inhabit different types of ice (i.e. snow, sea ice, glacial ice; Margesin et al. 2007; Turchetti et al. 2013; Zalar & Gunde-Cimerman 2013). These microorganisms can use complex biopolymers as energy sources, by synthesizing extracellular enzymes active at low temperatures (Frisvad 2007; Margesin et al. 2007).

### **Cold-adapted microorganisms**

Microorganisms inhabiting cold environments can be divided into obligate and facultative psychrophiles, and are defined by their growth temperature. Microorganisms can grow at 0 - 5 °c with an optimal growth temperature of 20–30 °c are designated facultative psychrophiles (psychrotolerant or cold-adapted). In contrast, obligate psychrophiles exhibit minimal growth temperature at 0 °c or lower, an optimal temperature ~ 15 °c and a maximum growth temperature of 20 °c (buzzini et al. 2012).

Cold-adapted microorganisms are generally predominant in ecosystems periodically exposed to low temperatures, whereas psychrophilic microorganisms dominate in permanent cold habitats. Both are believed to play key roles in the biodegradation of organic matter and the cycling of essential nutrients at the cryosphere level (Buzzini et al. 2012).

Studying microbial diversity in extreme environments, such as ice environments is interesting because these microorganisms exhibit metabolic adaptations to extreme

conditions, and may provide insight to potential biotechnological applications. Furthermore, psychrophilic and psychrotolerant microorganisms may be used as bio-indicators in studies monitoring global warming. In addition, changes in the microbial community may occur as these microorganisms are released into soil, rivers and oceans (Butinar et al. 2007; de Garcia et al. 2012a; Kuhn 2007).

### **Yeast from glacial meltwaters and glacial ice**

Yeasts exhibit excellent survival in water, they occur in a variety of freshwater systems, including eutrophic to ultra-oligotrophic lakes, lagoons, rivers, groundwater, glaciers and glacial melt-water (Starmer & Lachance 2011). The yeast diversity in these ecosystems is highly affected by a variety of abiotic and biotic factors, such as temperature, pressure, UV radiation (UVR), salinity, fauna, flora, soil runoff and anthropogenic effluents (Medeiros et al. 2011).

Ice has long been considered only as a refugia for microorganisms that have been randomly deposited on its surface. However, it is now known that different types of ice provide biomes that can support active microbial growth and reproduction (Gostinčar et al. 2010). Initially, the microbial presence was investigated only at the prokaryotic level. However, more recent studies have shown that fungi, primarily basidiomycetous yeasts, also represent an important part of glacial microbial communities in both polar and mountainous glacial environments worldwide (Bergauer et al. 2005; Branda et al. 2010; Buttinar et al. 2007; Connell et al. 2008; de Garcia et al. 2012a; Turchetti et al. 2008; Vaz et al. 2011).

### **Yeasts from Patagonian glaciers**

#### ***Biodiversity, ecology, and taxonomy***

Yeast studies in glacial environments of Patagonia were isolated filtering 100 and 150 mL of water or melted ice through Millipore® nitrocellulose membranes, using a sterilized Nalgene® device, membranes were then placed in general media for yeasts (MYP and YM), incubated at 5°C for up to one month (de Garcia et al. 2007). Physiological,

morphological and biochemical characterization of the isolated yeast strains was carried out according to the techniques described by Kurtzman *et al.* (2011). Molecular identification was carried out by sequencing D1/D2 domain of the LSU rRNA gene and the internal transcribed spacer region (ITS1, 5.8S and ITS2) (de Garcia et al. 2012a).

Obtained results have reported average counts from 1 to 3 x 10<sup>2</sup> colony forming units (CFU) L<sup>-1</sup> in melt-water rivers (de Garcia et al. 2007) from 1 to 5 x 10<sup>3</sup> CFU L<sup>-1</sup> for continental glacial ice (de Garcia et al. 2012a). Values obtained were similar to those from aquatic Patagonian environments (freshwater mountain lakes) (1 x 10<sup>2</sup> to 1 x 10<sup>3</sup> CFU) L<sup>-1</sup> (Brandao et al. 2011; Libkind et al. 2003; 2009), and for Italian Alps glacial environments of 1 x 10<sup>1</sup> and 4 x 10<sup>3</sup> CFU L<sup>-1</sup> (Branda et al. 2010; Buzzini et al. 2005; Turchetti et al. 2008).

When yeast diversity from Patagonian Glaciers was analyzed with the Shannon-Weaver index, diversity obtained showed higher values for ice samples from Frias glacier and meltwaters from Monte Tronador (H = 2.23 and H = 2.52, respectively), than for ice samples from Perito Moreno (H = 1.72), although there were no significant differences in Shannon-Weaver diversity indices among all compared communities (P > 0.05). Other community similarity analysis (Jaccard index) showed that yeast communities from meltwater were the most similar with communities from ice from the Frias glacier and Perito Moreno (J = 0.15 and J = 0.13, respectively), however no similarity was found when ice yeasts communities from Perito Moreno and Frias were compared (de Garcia et al. 2012a). Richness values were similar to those reported by Brandao et al. (2011) from water samples from Nahuel Huapi Lake (coast sites H= 2.2 and pelagic sites H= 2.8).

Basidiomycetous yeasts were the predominant group (90 %) in glacial environments from Patagonia (de Garcia et al. 2007; 2012a), this is in agreement with results from similar environments of the world (Antarctica, Alpine glaciers, Alaska, and Arctic;

Buzzini et al. 2012; Uetake et al. 2011; Vaz et al. 2011; Zalar & Gunde-Cimerman 2013). Also it has been suggested that the predominance of Basidiomycetous yeasts in these cold extreme environments is due to a more nutritionally versatile and a higher tolerance to extreme environmental conditions than other groups of yeasts (Frisvad 2007; Sampaio 2004; Zalar & Gunde-Cimerman 2013). In this regard, species occurring in several phylogenetic lineages of the Agaricomycotina, appear to be predominant (isolated in the highest numbers and are among the most frequently isolated species) in these environments (Connell et al. 2008; Turchetti et al. 2008; Zalar & Gunde-Cimerman 2013).

Up to 48 different yeasts species, from these 42.5 % (17 species) represent possible new species were isolated from Patagonian meltwaters and glacial ice (Tab. 1). These results are in agreement with studies in Antarctic soils (South Victoria Land), were approximately 43% of isolates corresponded to possible previously undescribed species (Connell et al. 2008).

Fewer Ascomycetous yeast species were identified, with 12 yeasts species of total isolates, from these 7 species represent potentially previously unidentified species (Tab. 1, de Garcia et al. 2010b; 2012a).

Basidiomycetous species from Tremellales, have been recently described from cold extreme environments of Patagonia. Monte Tronador meltwaters have yielded *Cryptococcus spencermartinsiae*, *Cryptococcus tronadorensis*, and *Cr. frias* (de Garcia et al. 2010a; 2012b). *Cryptococcus fonsecae* was isolated from the Austral Sea in Patagonia Argentina, subglacial ice of Svalbard, and from geographically diverse sea-waters and *Cr. psychrotolerans* has been isolated from sea-water off of Cape Horn Meridian Argentinian Sea and from other cold marine environments (de Garcia 2012b).

Additional interesting result was the isolation and study of psychrophilic yeasts, particularly *Mrakia* and *Mrakiella* genera, and a new set of yeast identified as *Psychrophilic yeasts* sp. The latter was the most frequently recovered from Perito Moreno glacier ice (19

isolates) and was shown to be identical to isolates cultured from glacial ice in Alaska (Basidiomycota sp. GU 74, Uetake et al. 2011). It is also related to a strain isolated from Antarctica (CBS 8941), forming a new clade closely related with the *Glaciozyma* (de Garcia et al. 2012a).

Several strains of the teleomorphic genus *Mrakia* and the anamorphic related genus *Mrakiella* were isolated (de Garcia et al. 2012a). Production of mycelium and teliospores like structures was observed in *Mrakiella*. In addition in 2 strains (*Mrakiella* sp. CRUB 1272 and *Mrakiella aquatica* CRUB 1209) germination of these structures was observed. Hyphae and teliospore-like structures developed directly from a single cell, without mating, and clamp connections were not found. Germinating structures of strain CRUB 1272 produced three- to five- single celled structures, a septate structure was observed (de Garcia et al. 2012a). Results obtained from Patagonian strains of *Mrakiella* did not establish if these structures were from asexual or sexual origin. In *Mrakia* species germination of sexual teliospores is not common and in some species it has rarely been observed (*Mrakia frigida* and *M. gelida*; Fell 2011). However, teliospore production has been observed in almost all psychrophilic genera previously described (*Mrakia* and *Glaciozyma*, Thomas-Hall et al. 2010; Fell 2011; Turchetti et al. 2011). The presence of this structure could be related to enhance survival in a diverse array of harsh environmental conditions, including cold habitats (de Garcia et al. 2012a).

Most yeasts from cold (extreme) habitats have metabolic adaptations to low temperatures, these microorganisms have the ability to degrade organic macromolecules by secreting extracellular hydrolytic enzymes active to low temperatures, this suggest that these yeasts may participate in organic matter decomposition and nutrient cycling in glacial environments (de Garcia et al. 2012a; Turchetti et al. 2013). This role is also supported by the presence of organic carbon and organic and inorganic nitrogen in glacial melt-water and ice (Margesin et al. 2007).

## Cold-adapted yeasts: potential biotechnological applications

### *Cold-active enzymes*

Cold adapted yeasts possess specific adaptation mechanisms (reproduction, metabolic activities, survival and protection) that enable them to be active at low and even at subzero temperatures (Margesin & Miteva 2011). Among these adaptations are: modification in lipid membranes, extracellular polysaccharides, compatible solutes, antifreeze proteins, protection against reactive oxygen species and cold active extracellular enzymes. Enzymes are highly specific in their catalytic activity, exhibit fast reaction rates, operate under mild conditions of temperature and pH, are derived from renewable resources, and are biodegradable (Huston 2007). Cold-active enzymes can be exploited in a variety of industrial applications. For example, they can catalyze numerous reactions at low and moderate temperatures (< 40°C) more efficiently and with fewer undesired chemical reactions than those that may occur at high temperatures (Huston 2007). Thus, cold extreme environments are a potential source of new yeast taxa with desirable biological characteristics that could be exploited for biotechnology research and application (Brizzio et al. 2007; de Garcia et al. 2012a; Lee et al. 2010; Libkind et al. 2005; Margesin & Miteva 2011; Moliné et al. 2013).

Production of extracellular enzymatic activity (protease, esterase, amylase, pectinase and cellulase) at 5 and 18 °C was studied in over 200 yeast strains isolated from melt-waters and glacial ice from glaciers of Patagonia (de Garcia et al. 2007; 2012a). Results indicate a statistically significant association between basidiomycetous yeast genera and production of extracellular enzymes. This association could facilitate a directed search for genera of interest, both in culture collections and also in the environment, with the objective of finding strains with possible biotechnological applications (de Garcia et al. 2012a).

Brizzio et al. (2007) performed quantitative analysis of extracellular protease and lipase

activities in cell-free supernatants, of psychrotolerant yeasts isolated from aquatic cold environments of Patagonia Argentina and mesophilic yeasts from Brazilian rain forests. Protease and lipase activities from psychrophilic selected strains had significantly higher values ( $P < 0.05$ ) at 4 °C than at higher temperatures (10, 15, 20, 25 °C); while opposite results were observed for mesophilic strains where activity was affected by the drop of temperature, limited (generally) to 20 and 25 °C (Brizzio et al. 2007; de Garcia et al. 2013).

Cold-adapted enzymes are beneficial for their enhanced selectivity and high catalytic activity at low and moderate temperatures, in addition to their structural liability that can be exploited in multi-step processes requiring rapid and mild inactivation treatments (Huston 2007; Gerday 2013). Enzymatic catalysts derived from meso- and thermophilic organisms have replaced many harsh conventional processes to great economic benefit (Margesin et al. 2007). Similarly, cold-adapted enzymes could be exploited to further decrease industrial energy expenditures and costs.

### **Photo-protective metabolites**

Carotenoid pigments and mycosporines are the major groups of photo-protective compounds in cold-adapted yeasts functioning by minimize the damage caused by UVR (Moliné et al. 2009; 2013). Carotenoid pigments are potent antioxidant compounds that mainly afford photo-protection by quenching intracellular reactive oxygen species (ROS) generated by UV radiation (indirect damage), these pigments are present in a few lineages primarily of basidiomycetous yeasts of which many include cold-adapted species and genera (i.e., *Rhodotorula*, *Rhodospiridium*, *Sporobolomyces*, *Dioszegia*, *Cystofilobasidium*, *Phaffia*, etc.; Libkind et al. 2008; Moliné et al. 2010; 2011; 2013).

Mycosporines (sensu-stricto) are water-soluble molecules formed by a distinctive cyclohexenone unit and an amino acid or amino alcohol conjugated subunit. In yeast there is only one mycosporine described so far: mycosporine-glutaminol-glucoside (MGG).

Table 1: Yeasts species isolated from glaciers of Patagonia Argentina. In bold are described species and possible new ones found in this environment. CRUB, Regional University Center of Bariloche (Centro Regional Universitario Bariloche), Argentina.

Basidiomycetous	Total N° of isolates	Origin of the species	References
<i>Bensingtonia yamatoana</i>	1	Ice from Frias Glacier of Mount Tronador	1
<i>Cryptococcus adeliensis</i>	1	Melt waters of Rio Manso Glacier of Mount Tronador	2
<i>Cryptococcus cylindricus</i>	1	Melt water river from Frias Glacier of Mount Tronador	1
<b><i>Cryptococcus fonsecae</i></b>	18	Sea water, Cape Horn Meridian Argentinian Sea	3
<b><i>Cryptococcus frias</i></b>	2	Melt water river from Frias Glacier of Mount Tronador	3
<b><i>Cryptococcus psychrotolerans</i></b>	2	Sea water, Cape Horn Meridian Argentinian Sea	3
<b><i>Cryptococcus</i> sp. CRUB 1267</b>	1	Melt water river from Frias Glacier of Mount Tronador	2
<b><i>Cryptococcus spencermartinsiae</i></b>	3	Melt water river from Frias Glacier of Mount Tronador	4
<i>Cryptococcus steposus</i>	24	Melt water river from Frias Glacier of Mount Tronador	2
<b><i>Cryptococcus tronadorensis</i></b>	2	Melt water from Garganta del Diablo waterfall (Río Manso Glacier) of Mount Tronador	3
<i>Cryptococcus victoriae</i>	9	Ice and melt waters from glaciers of Patagonia Argentina	1
<i>Cryptococcus wieringae</i>	1	Melt waters of Castaño Overo glacier of Mount Tronador	2
<i>Dioszegia butyracea</i>	3	Ice from Frias Glacier of Mount Tronador	1
<i>Dioszegia crocea</i>	25	Ice and melt waters from glaciers of Patagonia Argentina	1; 2
<i>Dioszegia fristingensis</i>	13	Ice and melt waters from glaciers of Patagonia Argentina	2
<i>Guehomyces pullulans</i>	1	Ice from Perito Moreno glacier	1
<i>Holtermanniella festucosa</i>	5	Ice and melt waters from glaciers of Patagonia Argentina	1; 2
<b><i>Holtermanniella</i> sp. CRUB 1256</b>	1	Melt water river from Frias Glacier of Mount Tronador	1
<b><i>Leucosporidium creatinivorum</i>*</b>	2	Melt water river from Frias Glacier of Mount Tronador	2; 5
<b><i>Leucosporidium fragarium</i>*</b>	16	Melt water river from Frias Glacier of Mount Tronador	2; 5
<b><i>Leucosporidium intermedium</i></b>	1	Ice from Frias Glacier of Mount Tronador	1; 5
<i>Mrakia frigida</i>	3	Melt water from Garganta del Diablo waterfall (Río Manso Glacier) of Mount Tronador	2
<i>Mrakia robertii</i>	4	Ice from Frias Glacier of Mount Tronador	1
<b><i>Mrakia</i> sp. 1 CRUB 1707</b>	1	Ice from Frias Glacier of Mount Tronador	1
<b><i>Mrakia</i> sp. 2 CRUB 1706</b>	1	Melt water from Frias glacier, Mount Tronador	1
<i>Mrakiella aquatica</i>	4	Ice and melt waters from Frias glacier of Mount Tronador	1
<b><i>Mrakiella</i> sp. CRUB 1272</b>	1	Melt water from Garganta del Diablo waterfall (Río Manso Glacier) of Mount Tronador	2
<b>Psychrophilic strain sp. 1</b>	16	Ice from Perito Moreno Glacier	1
<i>Rhodotorula mucilaginosa</i>	6	Ice and melt waters from glaciers of Patagonia Argentina	1; 2
<i>Sporobolomyces ruberrimus</i>	18	Ice and melt waters from Frias glacier of Mount Tronador	1; 2
<i>Udeniomyces megalosporus</i>	3	Ice from Perito Moreno Glacier	1
<i>Udeniomyces pannonicus</i>	11	Ice and melt waters from Frias glacier of Mount Tronador	1; 2
<i>Udeniomyces pyricola</i>	5	Ice and melt waters from Frias glacier of Mount Tronador	1
<b><i>Udeniomyces</i> sp. CRUB 1695</b>	1	Melt water from Frias glacier, Mount Tronador	1
<b>Ascomycetous</b>			
<i>Candida marítima</i>	1	Melt water of Castaño Overo glacier of Mount Tronador	1
<i>Candida mesenterica</i>	4	Ice from Perito Moreno Glacier	1
<b><i>Candida</i> sp. 1</b>	3	Melt water of Castaño Overo glacier of Mount Tronador	1
<b><i>Candida</i> sp. 2 CRUB 1719</b>	1	Melt water of Castaño Overo glacier of Mount Tronador	1
<b><i>Candida</i> sp. 3 CRUB 1220</b>	1	Melt water of Castaño Overo glacier of Mount Tronador	1
<b><i>Candida</i> sp. 4 CRUB 1295</b>	1	Melt water of Castaño Overo glacier of Mount Tronador	1
<i>Debaryomyces hansenii</i>	2	Ice from Frias Glacier of Mount Tronador	1
<i>Wickerhamomyces patagonicus</i>	1	Melt water of Castaño Overo glacier of Mount Tronador	4
<b>Dimorphic fungi</b>			
<i>Aureobasidium pullulans</i>	3	Ice and melt waters from Frias and Río Manso glacier of Mount Tronador	1; 2
<b><i>Hyalodendriella</i> sp. CRUB 1755</b>	1	Ice from Perito Moreno Glacier	1
<b><i>Phaeococcomyces</i> sp. CRUB 1760</b>	1	Ice from Perito Moreno Glacier	1

1- de Garcia et al. (2012a) ; 2- de Garcia et al. (2007); 3- de Garcia et al. (2012b); 4- de Garcia et al. (2010a); 5- de Garcia et al. (2015). \* *Leucosporidium* species complex

The production of MGG was studied in different *Cryptococcus stepposus* strains (isolated from melt-waters from Frias glacier, Monte Tronador) under light and dark conditions and the relationship between concentration of MGG, UVB-radiation survival, and DNA damage was measured (Moliné et al. 2011). When strains were cultured under photosynthetically active irradiation a larger accumulation of MGG occurs, and a positive relation was observed for MGG production and UVB-radiation survival. This positive MGG accumulation under light supports the idea of a photoprotective function of this molecule. Additionally, small changes in MGG accumulation clearly affected the survival of *Cr. stepposus* isolates under these conditions. Moliné et al., (2011) have stated that MGG efficiently absorbs UVB radiation, is highly stable, and could act as a natural sunscreen in these microorganisms.

The phylogenetic distribution of MGG is more widespread compared to carotenoids, although less chemically complex since only one microsporine molecule has so far been detected for yeasts. Production of MGG in yeasts is favored under high UV irradiances as denoted by the large numbers of cold-adapted species that are able to synthesis such metabolite. Given that UVR is an important force in structuring yeast community composition, the ability of yeasts to synthesize carotenoids and mycosporines becomes at least part of the adaptive suite of mechanisms needed for exploiting highly UV-exposed cold habitats (Moliné et al. 2013).

### **Final Remarks**

South America has a great potential in terms of macro- and micro-biodiversity (Caravajal et al. 2011) much of which may still be undiscovered. In this sense the Patagonian Andes possess glaciers with great potential for glaciological and paleoclimatic studies, and also for microbiological surveys (de Garcia et al. 2012a). Biodiversity studies of cold-adapted yeasts from glacial environments of Patagonia have contributed not only to the conservation of genetic resources in these extreme environments in Patagonia, but also to fungal

biodiversity, to general yeasts taxonomy and also to biogeography analysis of a few biotechnologically important species. Studies of cold-adapted yeasts from glacial melt-water and ice mentioned here were the first surveys from continental glaciers in the Southern Hemisphere (outside Antarctica). It has been clearly established that Patagonian glaciers are cold-adapted yeast reservoirs with potential biotechnological applications.

### **References**

- Bahr, D.B. & Radic, V. 2012. Significant total mass contained in small glaciers. *The Cryosphere Discussions*, 6: 737-758.
- Bergauer, P.; Fonteyne, P.A.; Nolard, N.; Schinner, F. & Margesin, R. 2005. Biodegradation of phenol and phenol-related compounds by psychrophilic and cold-tolerant alpine yeasts. *Chemosphere*, 59: 909-918.
- Brown, M.E.; Ouyang, H.; Habib, S.; Shrestha, B.; Shrestha, M.; Panday, P.; Tszortziou, M.; Policelli, F.; Artan, G.; Giriraj, A.; Bajracharya, S.R. & Racoviteanu, A. 2010. HIMALA: Climate Impacts on Glaciers, Snow, and Hydrology in the Himalayan Region. *Mountain Research and Development*, 30(4): 401-404.
- Branda, E.; Turchetti, B.; Diolaiuti, G.; Pecci, M.; Smiraglia, C. & Buzzini, P. 2010. Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). *FEMS Microbiology Ecology*, 72: 354-369.
- Brandao, R.L.; Libkind, D.; Vaz, B.M.A.; Espirito Santo, L.C.; Moliné, M.; de Garcia, V.; van Broock, M. & Rosa, C.A. 2011. Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes. *FEMS Microbiology Ecology*, 76: 1-13.
- Brizzio, S.; Turchetti, B.; de Garcia, V.; Libkind, D.; Buzzini, P.; Gasparetti, C. & van Broock, M. 2007. Extracellular enzymatic activities (EEA) in basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). *Canadian Journal of Microbiology*, 53: 519-525.

- Butinar, L.; Spencer-Martins, I. & Gunde-Cimerman, N. 2007. Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. *Antonie van Leeuwenhoek*, 91(3):277-289.
- Buzzini, P.; Turchetti, B.; Diolaiuti, G.; D'Agata, C.; Martini, A. & Smiraglia, C. 2005. Cultivable yeasts in melt waters draining from two glaciers in the Italian Alps. *Annals of Glaciology*, 40(1): 119-122.
- Buzzini, P.; Branda, E.; Goretti, M. & Turchetti, B. 2012. Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. *FEMS Microbiology Ecology*, 82: 217-241.
- Carvajal, J.; Libkind, D.; Briones, A.; Úbeda, J.; Roberts, I.; James, S.; Portero, P.; Alejo, J. & Rosa, C. 2011. Yeasts biodiversity significance: case studies in natural and human-related environments, ex situ preservation, applications and challenges. In: Grillo, O. & Venora, G. (Eds.) *Changing Diversity in Changing Environment*. InTech, Croatia, pp. 55-86.
- Connell, L.B.; Redman, R.; Craig, S.; Scorzetti, G.; Iszaard, M. & Rodriguez, R. 2008. Diversity of soil yeasts isolated from South Victoria Land, Antarctica. *Microbial Ecology*, 56: 448-459.
- Correa, M.N. 1998. Flora Patagónica (República Argentina) Tomo VIII. In: Correa, M. N. (Ed.) *Flora Patagónica (República Argentina)*, Colección científica del INTA, Buenos Aires, pp 31-38.
- Delgado Granados, H.; Miranda, P. J.; Huggel, C.; Ortega del Valle, S. & Alatorre Ibarquengoitia, M.A. 2007. Chronicle of a death foretold: Extinction of the small-size tropical glaciers of Popocatepetl volcano (Mexico). *Global Planet Change*, 56:13-22.
- de Garcia, V.; Brizzio, S.; Libkind, D.; Buzzini, P. & van Broock, M. 2007. Biodiversity of cold-adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. *FEMS Microbiology Ecology*, 59(2): 331-341.
- de Garcia, V.; Brizzio, S.; Russo, G.; Rosa, C.A.; Boekhout, T.; Theelen, B.; Libkind, D. & van Broock, M. 2010a. *Cryptococcus spencermartinsiae* sp. nov., a basidiomycetous yeast isolated from glacial waters and apple fruits. *Journal of Systematic and Evolutionary Microbiology*, 60(3): 707-711.
- de Garcia, V.; Brizzio, S.; Libkind, D.; Rosa, C.A. & van Broock, M. 2010b. *Wickerhamomyces patagonicus*, a novel yeasts isolated from Patagonia, Argentina. *Journal of Systematic and Evolutionary Microbiology*, 60(7): 1693-1696.
- de Garcia, V.; Brizzio, S. & van Broock, M. 2012a. Yeasts from Glacial Ice of Patagonian Andes, Argentina. *FEMS Microbiology Ecology*, 82(2): 540-50.
- de Garcia, V.; Zalar, P.; Brizzio, S.; Gunde-Cimerman, N. & van Broock, M. 2012b. *Cryptococcus* species (Tremellales) from glacial biomes in the southern (Patagonia) and northern (Svalbard) hemispheres. *FEMS Microbiology Ecology*, 82(2): 523-39.
- de Garcia, V.; Libkind, D.; Moliné, M.; Rosa, C.A. & van Broock, M. 2013. Cold-Adapted Yeasts in Patagonian Habitats. In: Buzzini, P. & Margesin, R (Eds.) *Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance*. Springer-Verlag, Berlin, pp. 123-148.
- de Garcia, V.; Coelho M.; Maia T.; Rosa L.; Martins Vaz A.; Rosa C.A.; Sampaio J.; Goncalves P.; van Broock M.; Libkind D. 2015. Sex in the cold: taxonomic reorganization of psychrotolerant yeasts in the order Leucosporidiales. *FEMS Yeasts Research*, 15(4): pii: fov019. doi: 10.1093/femsyr/fov019.
- Fell, J.W. 2011. *Mrakia* Y. Yamada & Komagata (1987). In: Kurtzman, C.P.; Fell, J.W. & Boekhout, T. (Eds.) *The Yeasts: a Taxonomic Study*, 5th edn, Elsevier, Amsterdam, pp. 1503-1510.
- Frisvad, J.C. 2007. Fungi in cold ecosystems. In: Margesin, R.; Schinner, F.; Marx, J.C. & Gerday, C. (Eds.) *Psychrophiles: From Biodiversity to Biotechnology*. Springer-Verlag, Berlin, pp. 137-156.
- Gerday, C. 2013. Fundamentals of Cold-Active Enzymes. In: Buzzini, P. & Margesin, R. (Eds.). *Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance*, Springer-Verlag, Berlin, pp. 325-352.



- Gostincar, C.; Grube, M.; de Hoog, S.; Zalar, P. & Gunde-Cimerman, N. 2011. Extremotolerance in fungi: evolution on the edge. *FEMS Microbiology Ecology*, 71: 2-11.
- Huston, A.L. 2007. Biotechnological aspects of cold-adapted enzymes. In: Margesin, R.; Schinner, F.; Marx, J.C. & Gerday, C. (Eds.) *Psychrophiles: From Biodiversity to Biotechnology*. Springer-Verlag, Berlin, pp. 347-363.
- Kuhn, M. 2007. The climate of snow and ice as boundary condition for microbial life. In: Margesin, R.; Schinner, F.; Marx, J.C. & Gerday, C. (Eds.) *Psychrophiles: From Biodiversity to Biotechnology*. Springer-Verlag, Berlin, pp. 3-16.
- Kurtzman, C.P.; Fell, J.W.; Boekhout, T. & Robert, V. 2011. Methods for the isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman, C.P.; Fell, J.W. & Boekhout, T. (Eds.), *The Yeasts, a Taxonomic Study*, 5th edn, pp. 87-110. Amsterdam, Elsevier.
- Lee, J.K.; Park, K.S.; Park, S.; Park, H.; Song, Y.H.; Kang, S.H. & Kim, H.J. 2010. An extracellular ice-binding glycoprotein from an Arctic psychrophilic yeasts. *Cryobiology*, 60: 222-228.
- Libkind, D.; Brizzio, S.; Ruffini, A.; Gadanho, M.; van Broock, M.R. & Sampaio, J.P. 2003. Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. *Antonie van Leeuwenhoek*, 84: 313-322.
- Libkind, D.; Sommaruga, R.; Zagarese, H. & van Broock, M. 2005. Mycosporines in carotenogenic yeasts. *Systematic and Applied Microbiology*, 28: 749-754.
- Libkind, D.; Gadanho, M.; van Broock, M.R. & Sampaio, J.P. 2008. Studies on the heterogeneity of the carotenogenic yeast *Rhodotorula mucilaginosa* from Patagonia, Argentina. *Journal of Basic Microbiology*, 48: 93-98.
- Libkind, D.; Moliné, M.; Sampaio, J. & van Broock, M. 2009. Yeasts from high altitude lakes: influence of UV radiation. *FEMS Microbiology Ecology*, 69: 353-362.
- Margesin, R.; Neuner, G. & Storey, K. B. 2007. Cold-loving microbes, plants, and animals-fundamental and applied aspects. *Naturwissenschaften*, 94: 77-99.
- Margesin, R. & Miteva, V. 2011. Diversity and ecology of psychrophilic microorganisms. *Research in Microbiology*, 162: 346-361.
- Medeiros, A.O.; Rosa, C.A.; Brandao, R.L.; Giani, A.; Gomes, A.N.L. & Libkind, D. 2011. Microbial Quality of Freshwater Ecosystems of South America. In: Ertuo, K. & Mirza, I. (Eds.) *Water Quality: Physical, Chemical and Biological characteristics*. Nova Science Publishers, Inc. New York, pp. 43-69.
- Mestre, M.C.; Rosa, C.; Safar, S.C.; Libkind, D. & Fontenla, S. 2011. Yeast communities associated with bulk-soil, rhizosphere and ectomycorrhizosphere of a *Nothofagus pumilio* forest in Northwestern Patagonia, Argentina. *FEMS Microbiology Ecology*, 78: 531-541.
- Miteva, V. 2008. Bacteria in snow and Glacier Ice. In: Margesin, R.; Schinner, F.; Marx, J.C. & Gerday, C. (Eds.) *Psychrophiles: From Biodiversity to Biotechnology*. Springer-Verlag, Berlin, pp. 31-50.
- Moliné, M.; Libkind, D.; del Carmen Diéguez, M. & van Broock, M. 2009. Photoprotective role of carotenoids in yeasts: response to UV-B of pigmented and naturally-occurring albino strains. *Journal of Photochemistry and Photobiology B: Biology*, 95(3): 156-161.
- Moliné, M., Flores, M.R.; Libkind, D.; del Carmen Diéguez, M.; Farías, M.E. & van Broock, M. 2010. Photoprotection by carotenoid pigments in the yeast *Rhodotorula mucilaginosa*: the role of torularhodin. *Photochemistry & Photobiological Sciences*, 9(8): 1145-1151.
- Moliné, M.; Arbeloa, E.M.; Flores, M.R.; Libkind, D.; Farías, M.E.; Bertolotti, S.G.; Churio, M.S. & van Broock, M.R. 2011. UVB photoprotective role of mycosporines in yeast: photostability and antioxidant activity of mycosporine-glutaminol-glucoside. *Radiation Research*, 175(1): 44-50.

- Moliné, M.; Libkind, D.; de García, V. & van Broock, M. 2013. Production of Pigments and Photo-Protective Compounds (Carotenoids and Mycosporines). In: Buzzini, P. & Margesin, R. (Eds.) *Cold-Adapted Yeasts: Biodiversity, Adaptation Strategies and Biotechnological Significance*, Springer-Verlag, Berlin, pp. 193-224.
- Price, P.B. 2007. Microbial life in glacial ice and implications for a cold origin of life. *FEMS Microbiology Ecology*, 59(2): 217-231.
- Rabassa, J.; Rubulis, S. & Suárez, J. 1978. Los Glaciares del Monte Tronador Parque Nacional Nahuel Huapi (Río Negro, Argentina). *Anales Parques Nacionales*, 14:259–318.
- Ruiz, L.; Masiokas, M.H. & Villalba, R. 2012. Fluctuations of Glaciar Esperanza Norte in the north Patagonian Andes of Argentina during the past 400 yr. *Climate of the Past*, 8: 1079-1090.
- Sampaio, J.P. 2004. Diversity, phylogeny and classification of basidiomycetous yeasts. In: Agerer, R.; Blanz, P. & Piepenbring, M. (Eds.) *Frontiers in Basidiomycete Mycology*. IHWVerlag, Eching, pp. 49–80.
- Skvarca, L.; Raup, B. & De Angelis, H. 2003. Recent behaviour of Glaciar Upsala, a fast-flowing calving glacier in Lago Argentino, Southern Patagonia. *Annals of Glaciology*, 36:184-188.
- Starmer, W.T. & Lachance, M. 2011. Yeast Ecology. In: Kurtzman, C.P.; Fell, J.W. & Boekhout, T. (Eds.) *The yeasts, A Taxonomy study*. Elsevier, London, pp. 65-83.
- Stuefer, M.; Rott, H. & Skvarca, P. 2007. Glaciar Perito Moreno, Patagonia: climate sensitivities and characteristics preceding 2003/04 and 2005/06 damming events. *Journal of Glaciology*, 53(180):3-16
- Thomas-Hall, S.; Turchetti, B.; Buzzini, P.; Branda, E.; Boekhout, T.; Theelen, B. & Watson, K. 2010. Cold adapted yeasts from Antarctica and the Italian Alps. Description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. *Extremophiles*, 14: 47-59.
- Turchetti, B.; Buzzini, P.; Goretti, M.; Branda, E.; Diolaiuti, G.; D'Agata, C.; Smiraglia, C. & Vaughan-Martini, A. 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microbiology Ecology*, 63(1):73-83.
- Turchetti, B.; Thomas-Hall, S.R.; Connell, L.B.; Branda, E.; Buzzini, P.; Theelen, B.; Müller, W.H. & Boekhout, T. 2011. Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. *Extremophiles*, 15:573–586.
- Turchetti, B.; Goretti, M.; Buzzini, P. & Margesin, R. 2013. Cold-Adapted Yeasts in Alpine and Apennine Glaciers. In: Buzzini, P. & Margesin, R. (Eds.) *Cold-Adapted Yeasts. Biodiversity, Adaptations Strategies and Biotechnological Significance*. Springer, Berlin, pp. 99-122.
- Uetake, J.; Kohshima, S.; Takeuchi, N.; Fujita, K.; Miyake, T.; Narita, T.; Aizen, V. & Nakawo, M. 2011. Evidence for propagation of cold-adapted yeasts in an ice core from a siberian Altai glacier. *Journal of Geophysical Research*, 116: G01019.
- Vaz, A.B. M.; Rosa, L.H.; Vieira, M.; de García, V.; Brandão, R.L.; Teixeira, L.; Moliné, M.; Libkind, D.; van Broock, M. & Rosa, C.A. 2011. The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. *Brazilian Journal of Microbiology*, 42: 937-947.
- Villarosa, G.; Outes, V.; Masiokas, M.; Villalba, R. & Rivas, S. 2008. El Monte Tronador: Historias de hielo y fuego. In: Rastelli, D. (Ed.) *Sitios de Interés Geológico de la República Argentina*. SEGEMAR (Servicio de Geología y Minería de la República Argentina), Buenos Aires, pp. 627-641.
- Zalar, P. & Gunde-Cimerman, N. 2013. Cold-Adapted Yeasts in Arctic Habitats. In: Buzzini, P. & Margesin, R. (Eds.) *Cold-Adapted Yeasts. Biodiversity, Adaptations Strategies and Biotechnological Significance*. Springer, Berlin, pp. 49-74.