Chapter 18
Biotechnologically Relevant Yeasts from Patagonian Natural Environments

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Abstract The Patagonia region constitutes a vast geographic area with multiple extreme environments having one or more of these stress factors: cold, high UV incidence, desiccation, ultra-oligotrophy, acidity, and the presence of heavy metals, among others. Yeasts that constantly live under stress conditions evolve adaptive mechanisms to minimize or resist their negative effects and thus permit survival and reproduction. These specific mechanisms are promising sources of biotechnologically relevant molecules or genes. Here we summarize numerous yeast bioprospection studies performed in the conventional and extreme environments of the Argentinean Patagonia. More than 1000 yeasts and dimorphic fungi were collected and molecularly identified; when possible, relevant secondary metabolites were screened, as well as their ability to tolerate several types of stress in laboratory conditions. Screened metabolites include carotenoid pigments, mycosporines (UV sunscreens), and cold-active enzymes. In some cases, these traits could be correlated to habitat characteristics and for those (e.g., mycosporines, carotenoid pigments, heavy metal tolerance) their potential role in the adaptive mechanisms to specific stress factors was evaluated. Genome sequencing and analyses were performed for biotechnologically relevant isolates such as *Saccharomyces eubayanus*, *Saccharomyces uvarum*, and *Phaffia rhodozyma* (synonym of *Xanthophyllomyces dendrorhous*). The biotechnological potential of selected species is addressed as specific study cases. The present work represents an overview of our findings related to biotechnologically relevant yeasts from Patagonian natural environments.

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18.1 Introduction

The Patagonian Andes possess unique physical and environmental characteristics (Villarosa et al. 2008), and its natural substrates have been poorly studied at the microbiological level. Pristine natural environments are common in Patagonia, with a higher diversity of fauna and flora toward the more humid and higher areas of Andean Patagonia. Regions under very harsh environmental conditions also exist in Patagonia and can be considered as extreme environments. These environments impose difficulties for microbial colonization and growth, selecting for microorganisms adapted to such conditions that frequently have evolved special metabolic abilities, many of which may be of interest for industrial exploitation. High ultraviolet radiation (UVR), high altitude, extreme desiccation, very cold or freezing temperatures, freezing and thawing cycles during the day, ultra-oligotrophic conditions, and volcanic activity, singly or combined, are among the stress factors that influence the microbial communities in the Patagonian natural environment, in particular Andean Patagonia. Yeasts belonging to either the Ascomycota or the Basidiomycota have proven to be able to adapt to multiple types of substrates and environments, including those regarded as extreme, and display an amazing diversity of highly plastic phenotypes. Several novel species have been obtained and formally described from those environments, and their screening for industrially relevant traits has been carried out. This chapter summarizes research carried out mainly on the biodiversity and biotechnology of Patagonian native yeasts, and how their adaptive characteristics are exploited for industrial applications.

18.1.1 Yeasts in Extreme Environments

Certain yeast species have evolved metabolic adaptations that allow them to colonize extreme natural habitats such as the deep sea (Nagahama et al. 2001; Gadanho and Sampaio 2005), glacial meltwaters (Vishniac 2005; Branda et al. 2010), hypersaline lakes (Butinar et al. 2005), ultra-oligotrophic mountain lakes exposed to increased UV radiation (Libkind et al. 2009a), and natural and anthropic hyperacidic (pH<3) aquatic environments (Gadanho et al. 2006), among others. Yeasts living in these environments are often polyextremophiles, that is, they tolerate many different extreme conditions (low pH, high temperatures, osmotic pressures, high concentration of heavy metals, chemolithotrophic microbial activity). In this section we review the research on yeasts isolated from extreme environments in Patagonia, with special emphasis in ultra-oligotrophic mountain lakes with high UVR, highly acidic waterbodies of volcanic origin, and very cold environments (glaciers and meltwater). Table 18.1 summarizes the principal yeast species recovered from Patagonian natural environments, including those considered extreme.

Table 18.1 Yeast species in Patagonian extreme environments and their biotechnological relevance

Habitat	Substrate	Predominant species	Novel yeasts described	Biotechnologically relevant species
Freshwater bodies	Lakes, lagoons, rivers	*R. mucilaginosa*[a] *Rh. babjevae* *Rh. kratochvilovae* *Sp. salmonicolor* *Sporobolomyces*[b]	*Sp. longiusculus* *S. patagonicus* *Cy. lacus-mascardii* *Cy. macerans* *R. meli*	Pigmented yeasts, sources of carotenoids, mycosporines, polyunsaturated fatty acids, and other lipids
	High-altitude lakes (>1400 m a.s.l.)	*R. mucilaginosa* *Rh. babjevae*	*H. takashimae*	
	Oligotrophic lakes	*R. mucilaginosa*[a] *Cr. victoriae*	*Dioszegia* sp. 1[c]	
	Acidic lakes and rivers	*Cr. agrionensis* *R. mucilaginosa* *Rh. toruloides*	*Cr. agrionensis*	Acid- and metal-tolerant yeasts: bioremediation of heavy metals
Glaciers	Meltwater	*Cr. spencermartinsiae* *L. fragarium*	*Cr. spencermartinsiae* *Cr. frias* *Cr. tronadorensis*	Cold-adapted yeasts: sources of cold-active enzymes, antifreeze proteins, lipids
	Ice	*D. crocea*, new genus[c] *S. ruberrimus* *D. fristingensis*		
Nothofagus forest	*Cyttaria* spp. fungal stromata, sap exudates, bark, leaves	*Saccharomyces* spp. *Hanseniospora* spp. *Pichia* spp.[d]	*W. patagonicus* *S. eubayanus* *Cys. psychroaquaticum*	Yeasts for fermentation processes (beer, wine)
	Soil	*Cr. podzolicus* (BS, R, E) *Cr. phenolicus*, *Cr. terreus*, and *T. porosum* (BS) *Cr. aerius* and *C. maritima* (R) *Cr. aerius*, *Cr. phenolicus* and *H. wattica* (E)	*La. nothofagi* [e] *Li. rizospherae*	Yeasts for enhancing plant growth and biocontrol
	Seeds, fruits	*A. pullulans* *Cr. heveanensis*		

A., Aureobasidium; C., Candida; Cr., Cryptococcus; Cy., Cystofilobasidium; Cys., Cystobasidium; D., Dioszegia; H., Holtermanniella; L., Leucosporidium; La., Lachancea; Li., Lindnera; R., Rhodotorula; Rh., Rhodosporidium; S., Sporobolomyces; Sp., Sporidiobolus; T., Trichosporon; W., Wickerhamomyces; BS, bulk soil; E, ectomycorrhizosphere; R, rhizosphere

[a]Frequently associated with anthropically influenced lakes or sites

[b]Species of this genus were predominant in lakes with low anthropic influence

[c]Formal description in progress

[d]Selective isolation of fermentative species (Ulloa et al. 2009)

[e]Strains also found in sap and bark of *Nothofagus* sp. (Mestre et al. 2010)

18.1.1.1 Ultra-oligotrophic Aquatic Environments Under High UVR

Yeasts are common inhabitants of aquatic environments, and their density and species diversity depend on water type and purity (Hagler and Mendoça-Hagler 1981). The northern part of Andean Patagonia offers a great variety of pristine, glacially formed waterbodies, covering an ultra- to mesotrophic range of small and large lakes including small high-elevation lakes or wetlands. These habitats are normally exposed to extended daylight (latitude 41–45°) and consequently increased UVR, also the result of ozone layer depletion and a clean atmosphere. Many Patagonian aquatic environments are still further affected by these environmental factors because of their transparency and ultra-oligotrophic character (Villafañe et al. 2001). Thus, yeast survival in pristine waters in Patagonia is conditioned by low temperature, ultra-oligotrophic conditions, and, more significantly, high UVR doses (Libkind et al. 2006; de Garcia et al. 2014).

Because of their differential ability to assimilate a larger number of complex carbon sources and a better capacity to cope with harsh conditions (Sampaio 2004), basidiomycetous yeasts predominate in the ultra-oligotrophic waterbodies of Patagonia (Libkind et al. 2003; Brandão et al. 2011). The biodiversity of basidiomycetous yeasts in certain lakes in Andean Patagonia (Argentina) was investigated, focusing on species producing photoprotective compounds such as carotenoid pigments and UV sunscreens (mycosporines) (Libkind et al. 2003, 2005a, 2009a; Brandao et al. 2011), both known strategies for the minimization of UV-induced damage in organisms (Roy 2000). These secondary metabolites, which are of industrial interest for many reasons, are discussed in Sect. 2 of this chapter. These studies indicated UVR as a selective factor that favors the occurrence of more UV-resistant yeast species/strains in these kinds of lakes and thus determining their yeast community structure (Libkind et al. 2009a; Moliné et al. 2009; Moliné 2010). Also, several novel species were described, including *Rhodotorula meli*, *Sporidiobolus longiusculus*, *Sporobolomyces patagonicus*, and *Cystofilobasidium lacus-mascardii* (Libkind et al. 2005b; 2009b; 2010) (Table 18.1). These species are interesting for their ability to accumulate carotenoid pigments, and some were regarded as biotechnologically relevant (Libkind et al. 2006; Libkind and van Broock 2006; Moliné et al. 2012).

Yeast diversity and distribution, including the entire cultivable yeast community, were evaluated in the pristine water of Nahuel Huapi (NH) Lake, one of the largest lakes in Patagonia (Brandão et al. 2011). Yeast counts ranged from 22 to 141 cfu l^{-1}, typical of clean lakes (Hagler and Ahearn 1987), with the highest values corresponding to the most anthropogenically influenced sites. Isolates from NH Lake were identified as belonging to 13 genera and 34 species, with 73.8 % being basidiomycetous. *Rhodotorula mucilaginosa* and *Cryptococcus victoriae* were the most frequently found species. Some yeast species were more represented in anthropogenically influenced sites (such as *Aureobasidium pullulans* and *R. mucilaginosa*) whereas the most represented species in sites considered less affected by human activity were also components of the community of the surrounding *Nothofagus* phylloplane. The occurrence and distribution of yeasts at the studied sites showed peculiar distributional patterns that are probably influenced by inputs of allochtho-

nous organic matter from the borders of the lake and by abiotic factors such as UVR. Photoprotective compound-producing yeasts were mainly found in pelagic points of the lake, suggesting that both carotenoids and mycosporines production capacities are important for yeast survival under high-UVR conditions.

18.1.1.2 Acidic Environments

Acidic environments can harbor numerous microorganisms, including algae, bacteria, and fungi, with distinctive capacities to survive the acidic conditions and the resulting high metal concentrations. The yeast community of a natural acidic environment located in Northwestern Patagonia was analyzed (Rio Agrio and Lake Caviahue system) (Russo et al. 2008). Yeasts were isolated from water sites with different pHs, ranging from 1.6 to 6.7. The recovery of putative autochthonous yeasts was enhanced when water from the sampling site was used in the formulation of the isolation media, compared to use of conventional yeast media. In total, 25 different species were identified, with 99% of the isolates being Basidiomycetes. *Rhodotorula mucilaginosa*, *Rhodosporidium toruloides,* and two novel *Cryptococcus* species were the most adapted species. One of the novel species, named *Cryptococcus agrionensis* (Russo et al. 2010), is highly resistant to heavy metals and belongs to the acid rock drainage (ARD) ecoclade (Gadanho and Sampaio 2009; Libkind et al. 2014; Russo et al. 2016) (Table 18.1). The second *Cryptococcus* species (referred to as *Cryptococcus* sp. 2) was able to grow in a very narrow pH range (2.5–4.5), with an optimum at pH 3, and thus could be regarded as an acidophilic yeast. Both *R. mucilaginosa* and *Cr. agrionensis* showed wider pH growth ranges. Ongoing studies aim to analyze the molecular basis of such atypical phenotypes. Interestingly, the yeast community of the naturally originated Patagonian acidic environment resembled that of acidic aquatic environments resulting from anthropic activities such as the São Domingos mines in Portugal and the Rio Tinto in Spain (Gadanho et al. 2006). The current knowledge of yeast diversity and ecology in acidic aquatic environments is scarce and limited. Detailed studies on their metabolic features, including assessment of their ability to bioremediate heavy metals, will give further insights into their biotechnological potential. In a recent study some of the aforementioned yeasts were evaluated for their ability to capture heavy metals (such as Cu^{2+}, Ni^{2+}, and Zn^{2+}) in solution at low pH, with promising results (Russo et al. 2016).

18.1.1.3 Cold Environments

Andean Patagonia in Argentina offers a great variety of glaciers and glacially formed waterbodies that are still glacier fed. The latter include small and large oligotrophic to ultra-oligotrophic lakes, including small high-elevation lakes, sometimes surrounded by a dense native forest of trees of *Nothofagus* spp. and *Austrocedrus chilensis* trees (Quirós and Drago 1985; Díaz et al. 2000). In Mount Tronador alone, in Nahuel Huapi National Park, there are ten different glaciers

(Rabassa et al. 1978). Los Glaciares National Park (Argentina) and Patagonian Icefields (Hielos Patagónicos) are the largest temperate ice masses in the Southern Hemisphere, accounting for more than 60 % of the Southern Hemisphere glacial area outside Antarctica. Perito Moreno Glacier is located within these icefields (Stuefer et al. 2007).

Extensive studies on the occurrence of psychrophilic and psychrotolerant yeasts from the cold environments of Patagonia have been carried out since the 1990s in aquatic (freshwater, meltwaters, glacial ice, seawater) and terrestrial habitats (flowers, phylloplane, sap exudates, bark, soil, rotten wood, rhizosphere, *Cyttaria* sp. stromata) (Brizzio and van Broock 1998; Libkind et al. 2003; 2004a, b; 2006; Brizzio et al. 2007; de Garcia et al. 2007; Libkind et al. 2007, 2008a, 2009a, 2011a; Mestre et al. 2011; de Garcia et al. 2012; Fernández et al. 2012). Yeasts isolated from these cold environments belong to taxa previously described as cold adapted whereas yeast species not considered as such were also present and considered transient components of the microbial community. An almost up-to-date review on cold-adapted yeasts from Patagonia was recently published (de Garcia et al. 2014). Since then a few novel investigations have been published, including the taxonomic reorganization of the psychrotolerant yeasts of the genus *Leucosporidium* (de Garcia et al. 2015). Members of this genus are particularly important as potential sources of extracellular enzymes that are active at low temperatures (cold enzymes), antifreeze proteins, and have the ability to biodegrade phenol and phenol-related compounds (Bergauer et al. 2005; Sampaio 2011a, b; de Garcia et al. 2012). In Patagonia the predominant species is *Leucosporidium creatinivorum*, a member of the *L. scotti* species complex (de Garcia et al. 2015). A considerable percentage (25–40 %) of the yeast species recovered from different cold substrates belonged to undescribed taxa. The most recently described is *Cystobasidium psychroaquaticum*, isolated in Patagonia from glacier meltwater and from the phylloplane of high-altitude trees (Yurkov et al. 2015). Including those previously mentioned, a total of 11 cold-adapted new yeast species have been formally described from Patagonian environments (Libkind et al. 2005b, 2009b; de Garcia et al. 2010a, b; Libkind et al. 2010, 2011a; de Garcia et al. 2012; Yurkov et al. 2015).

Regarding the number of cultivable yeast cells detected in aquatic cold environments of Patagonia, average yeast counts of 10^2 to 10^3 cfu l^{-1} were found in freshwater mountain lakes (Libkind et al. 2003, 2009a; Brandão et al. 2011), 1×10^2 to 3×10^2 cfu l^{-1} in meltwater rivers (de Garcia et al. 2007), and 1×10^3 to 5×10^3 cfu l^{-1} in continental glacial ice (de Garcia et al. 2012). Again, basidiomycetous yeasts are the predominant group in these environments. Similar results from different cold environments worldwide have been reported (Frisvad 2008; Buzzini et al. 2012). Notably, a relatively higher richness index of taxa among ice and meltwater samples was observed (de Garcia et al. 2012), compared to the values reported for soil samples in Patagonian forest (Mestre et al. 2011). Brandão et al. (2011) mentioned similar richness index values for water samples from Nahuel Huapi Lake (coast sites, $H = 2.2$, and pelagic sites, $H = 2.8$).

The occurrence of cold-adapted yeasts was evaluated in the dominant tree genus in Andean Patagonia, *Nothofagus* spp., in different terrestrial substrates including

leaves, seeds, bark, rotting wood, sap, soil, rhizosphere, flowers, and the stromata of *Cyttaria* spp. In contrast to the studies of oligotrophic environments, the major component of the yeast community in terrestrial substrates were ascomycetous fungi. *Aureobasidium* was the most frequently isolated genus in the phylloplane and other substrates (Muñoz et al. 2013). Other interesting yeasts of the genera *Saccharomyces* and *Phaffia* (synonym *Xanthophyllomyces*) were also detected in such forests and are reviewed in the next section.

The diversity, distribution, and physiological properties of yeasts inhabiting different substrates related to *Nothofagus* forests (seeds, bulk soil, rhizosphere, ectomycorrhizosphere) were recently published (Mestre et al. 2011; Fernández et al. 2012; Mestre et al. 2014). For example, *Cryptococcus* species such as *Cr. podzolicus*, *Cr. phenolicus*, and *Cr. aerius* were the species most frequently occurring in *N. pumilio* (Mestre et al. 2011). Recently, some of these psychrotolerant yeasts (i.e., *Aureobasidium pullulans*, *Holtermaniella takashimae*, *Candida maritima*) were shown to possess plant growth-enhancing features, such as production of auxin-like compounds and siderophores, and the ability to solubilize inorganic phosphate and to reduce the growth of common plant pathogens (Mestre et al. 2016).

18.2 Biotechnologically Relevant Traits in Patagonian Native Yeasts

18.2.1 Carotenoid Pigments: Biological Function and Biotechnological Applications

Carotenoids are yellow to red natural pigments formed by a C-40 chain, which is considered the backbone of the molecule. This chain has several conjugated double bonds (7–15) where the p electrons are highly delocalized, conferring a low-energy excited state giving their characteristic yellow to dark red color (Britton 1995). Furthermore, several modifications can occur in this basic skeleton, giving rise to more than 700 types of naturally occurring carotenes and xanthophylls (Britton 2004). Different functions have been attributed to these pigments in fungi, including protecting against reactive oxygen species (ROS) and ultraviolet radiation (UVR), being precursors of hormones (in Mucorales), being associated with membrane permeability modifications, and providing resistance to heat, radiation, and oxidation (Lampila et al. 1985; Britton 1995; Schroeder and Johnson 1995; Johnson and Schroeder 1996; Britton 2008). Regardless of their biological function, carotenoids are important for their benefits to human health, and are known to act as provitamin A (Olson 1989; Johnson and Schroeder 1996), antioxidants (Krinsky 1979; Sies and Stahl 1995), and antimutagens and anticarcinogens (Rao and Agarwal 2000; Donaldson 2004; Rao and Rao 2007). For these reasons they are of interest to the pharmaceutical, chemical, food, and feed industries (Ausich 1997; Rodríguez-Sáiz et al. 2010).

Yeast as well as other fungi can synthesize and accumulate carotenoid compounds. Most of these yeasts are called the "red yeasts," given the red to salmon-pink color of their colonies when grown in the laboratory. Despite the large number of possible pigments, red yeasts only synthesize a few carotenoid molecules, and three pigments are common for most yeast species; torulene, γ-carotene, and β-carotene. These carotenoids can be produced by species of the subphyla Agaricomycotina, Pucciniomycotina, Ustilaginomycotina, Taphrinomycotina, and Pezizomycotina (Kurtzman et al. 2011). The carotenoid pigment torularhodin (a xanthophyll product of the oxidation of torulene) is less common and is found associated with Pucciniomycotina species (Davoli et al. 2004; Buzzini et al. 2007; Sperstad et al. 2006). Torularhodin has been reported to have provitamin A activity in vitro, better antioxidant activity against singlet oxygen, and a more potent effect on the scavenging of peroxyl radicals than β-carotene (Simpson 1983; Sakaki et al. 2001; Ungureanu and Ferdes 2012). Other rare yeast carotenoids, produced by different species, are xanthophylls such as 16-hydroxytorulene, torularhodinaldehyde, plectaniaxanthin, and 2-hydroxyplectaniaxanthin, with hitherto unknown applications. Finally, the biotechnologically relevant astaxanthin can only be synthesized by one yeast species: *Phaffia rhodozyma*.

Among Patagonian yeast mycobiota, species producing carotenoids were found in all the environments tested so far (water, soil, phylloplane, glaciers), and *Rhodotorula mucilaginosa* was the most common species. In our studies, the freshwater environment had the higher proportion (>50 %) of red yeasts. In a survey of five high-altitude water bodies located in the Nahuel Huapi National Park, Libkind et al. (2009b) revealed that carotenogenic yeasts prevail in lakes with higher transparency. More than 24 yeast species were recovered in this study, and 12 corresponded to red yeasts (classified into seven genera). *Rhodotorula mucilaginosa* was the most frequently isolated species (representing more than 50 % of the total isolates), followed by *Rhodosporidium babjevae*. Other less frequent pigmented species such as *Sporobolomyces ruberrimus*, *Cystobasidium laryngis*, *Cystibasidium minutum* (ex *Rhodotorula minuta*), *Sporobolomyces marcillae*, *Rhodosporidium diobovatum*, and three *Dioszegia* species have also been isolated from Patagonian lakes (Libkind, et al. 2003, 2009a). In another study Brandão et al. (2011) revealed the occurrence of 47–74 % of pigmented yeasts in pelagic sites (more transparent) of the Nahuel Huapi Lake. In Patagonian glacier meltwater and ice, the number of species bearing carotenoids was less representative; however, the isolation of species such as *Dioszegia crocea*, *Dioszegia fristingensis*, and *Sporobolomyces ruberrimus* was common, reaching more than 30 % of the isolates (de Garcia et al. 2012). In contrast, pigmented strains are unusual in soil, and only three species, namely, *Cystofilobasidium infirmominiatum*, *Cystofilobasidium capitatum*, and *Rhodotorula colostri*, were reported from this environment (Mestre et al. 2014). Given the photoprotective function of carotenoids, our results suggest that the higher abundance of carotenogenic yeasts in substrates with a higher exposition to UVR is the result of their higher tolerance to this damaging stress factor (Libkind et al. 2006, 2009a; Moliné et al. 2011a).

The proportion and type of carotenoids produced by Patagonian yeasts was highly variable and depended on the phylogenetic group to which they belonged. For example, in *Rhodotorula mucilaginosa* strains, carotenoid content ranged from 60 to 301 mg g^{-1} of dry biomass (Libkind and van Broock 2006), and torularhodin was the most important carotenoid, representing 61–98 % of the total carotenoid content (Moliné et al. 2011a, 2012). *Phaffia rhodozyma* carotenoids ranged from 98 to 415 mg g^{-1} of dry biomass, and astaxanthin was the principal carotenoid (Libkind et al. 2008b), indicating that quantitative pigment production is a strain-related feature. Moreover, the origin of the strains seemed to be a relevant aspect when considering carotenoid accumulation, because the total carotenoid content of Patagonian strains (mainly isolated from extreme environments) was in almost all cases higher than values observed for type strains of the same or related species (Libkind et al. 2004b; 2009b; Libkind and van Broock 2006; Moliné 2010; Moliné et al. 2011a).

Pigment analysis of the Patagonian yeasts revealed that torulene, torularhodin, and β-carotene were the most important carotenoids present in the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and *Sporidiobolus* (Libkind and van Broock 2006; Buzzini et al. 2007; Moliné et al. 2011a) (Fig. 18.1). Another carotenoid observed only in a few isolates was γ-carotene. Other yeast genera such as *Cystofilobasidium* and *Dioszegia* were also found to synthesize the pigments torulene and β-carotene; however, in *Cystofilobasidium* there are other unknown carotenoids representing the major compounds (probably 16′-hydroxytorulene, torularhodinaldehyde, and β-apo-2′-carotenal, based on Herz et al. (2007), and in *Dioszegia* the principal carotenoid identified was plectaniaxanthin (Moliné 2010). Finally, the most

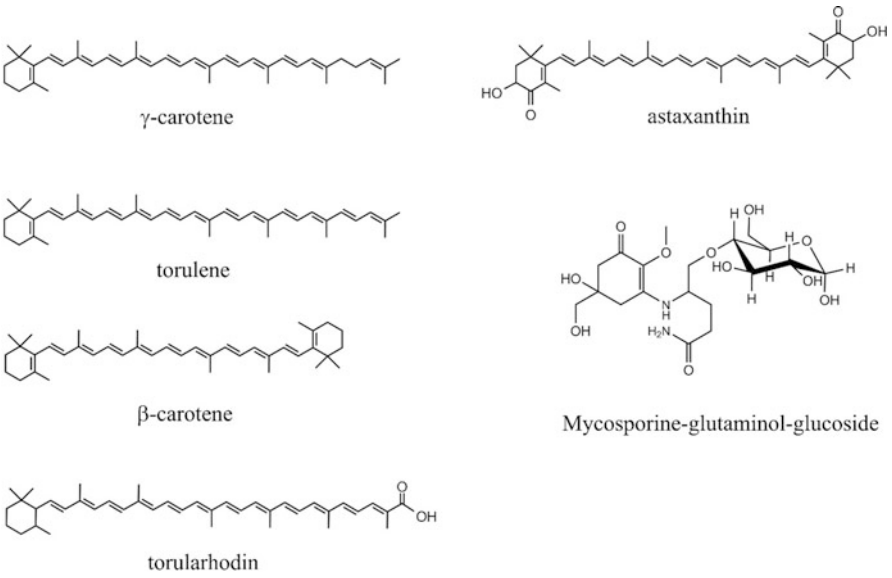

Fig. 18.1 Chemical structure of the principal carotenoids and mycosporines found in yeasts

relevant pigment identified in Patagonian yeasts was the xhantophyll astaxanthin produced by *Phaffia rhodozyma* (Fig. 18.1). Evidence has been gathered that demonstrates astaxanthin protects cells from oxidative stress including that caused by photogenerated reactive oxygen species (Schroeder and Johnson 1995). Astaxanthin is probably the most important yeast carotenoid for its high commercial value in pharmaceutics, nutraceutics, and cosmetics (Bhosale and Bernstein 2005). In addition to its antioxidant properties, astaxanthin has antiinflammatory and neuroprotective properties (Naguib 2000; Lee et al. 2011). Furthermore, astaxanthin is also used in aquaculture for pigmentation of fish and crustaceans, being the most expensive feed ingredient (Johnson and Schroeder 1995). Therefore, we address further *Phaffia rhodozyma* and its importance in aquaculture in Sect. 3.3 of this chapter and in Chapter 13, Microorganisms from Patagonian Aquatic Environments for Use in Aquaculture.

Carotenoid accumulation, the concentration and relative proportion of each pigment, is also affected by the culture media and by physical and chemical factors. For example, in *Phaffia rhodozyma*, a high carbon:nitrogen (C/N) ratio favors carotenoid production (Yamane et al. 1997; Flores-Cotera et al. 2001), and the same occurs for strains isolated from Patagonia (Moliné 2010), whereas in *Rhodotorula mucilaginosa* strains, the specific carotenoid production is not affected by the C/N ratio (Libkind et al. 2004a; Libkind and van Broock 2006). Light is one of the most important environmental forces triggering the synthesis of carotenoids in yeasts (Tada and Shiroishi 1982; An and Johnson 1990; Sakaki et al. 2001; Bhosale 2004). Exposure to photosynthetically active radiation (PAR) and UV-A was found to produce different responses in the synthesis of carotenoid pigments in yeast, with increases from 6 % up to 800 % depending on the species. The increasing effect of photostimulation in carotenoid synthesis after exposure to PAR + UV-A was negatively correlated to the basal concentration of carotenoids, suggesting that yeasts with high constitutive levels of intracellular carotenoids were less responsive (Libkind et al. 2004b). The reason is probably that they already possess sufficient carotenoids functioning as photoprotective agents to cope with the UVR-damaging effects imposed in our experiments. Evidence that one of the roles of carotenoid pigments in yeast cells could be photoprotection has been accumulated for decades (Maxwell et al. 1966; Tsimako et al. 2002). Using Patagonian carotenogenic isolates and naturally occurring albino strains, we experimentally compared pigmented and albino strains of *Cystofilobasidium capitatum* and *Sporobolomyces ruberrimus*. Albino strains invariably were less tolerant to UV-B exposure than pigmented strains, and a direct relationship between carotenoid content and survivorship in *Cy. capitatum* was observed (Libkind et al. 2006; Moliné et al. 2009). Afterward, using a set of *R. mucilaginosa* and *P. rhodozyma* strains, we established a significant positive relationship between intracellular carotenoid concentration and UV-B survival (Moliné 2010; Moliné et al. 2011a). Analysis of carotenoid content pointed out that torularhodin in *R. mucilaginosa* and astaxanthin in *P. rhodozyma* provided effective photoprotection, whereas other carotenoids such as β-carotene showed a lack of correlation with survival to UV-B.

The studies described here show that the environmental niches for the isolation of red yeasts were identified, as well as the carotenoid pigments synthesized, and

the biological function of such pigments was experimentally confirmed. In summary, red yeasts from Patagonia represent an interesting source of carotenoid pigments with biotechnological value, and further studies are needed to determine their potential for industrial applications.

18.2.2 Natural Sunscreens: Mycosporines

Mycosporines are water-soluble compounds composed by a cyclohexenone attached to an amino acid (or amino alcohol). There are more than a dozen different mycosporines, but in fungi only mycosporine serine, alanine, α-amino alcohol serinol, pyroglutamic acid, and the related pairs glutamine–glutaminol and glutamic acid–glutamicol were described (Young and Patterson 1982; Bernillon et al. 1984; Leite and Nicholson 1992; Volkmann et al. 2003; Sommaruga et al. 2004). Because these compounds absorb light in the UV spectrum with a maximum at 310 nm, the primary function of mycosporines was to act as photoprotective UV filters (Shick and Dunlap 2002; Torres et al. 2004). However, other functions were also attributed, including antioxidant activity, osmoregulation, resistance to thermal stress, and serving as intracellular nitrogen storage (Oren and Gunde-Cimerman 2007). Only recently was mycosporine synthesis reported for yeasts by our laboratory. Different basidiomycetous yeasts, most isolated from Patagonia lakes, were found to synthesize a UV-absorbing compound when grown under photosynthetically active radiation (PAR) (Libkind et al. 2004b). In basidiomycetous yeasts, mycosporines were reported in different species of the subphyla Pucciniomycotina (Libkind et al. 2011b) and Agaricomycotina (Libkind et al. 2005a, 2011c). In both groups there are taxa with and without the ability to produce mycosporines, suggesting that this trait might be plesiomorphic. Yeasts and dimorphic fungi of the Ascomycota able to produce mycosporines are classified in the orders Dothideales, Capnodiales, and Taphrinales (Gunde-Cimerman and Plemenitaš 2006; Kogej et al. 2006). In most yeasts so far tested a main mycosporine was detected, mycosporine-glutaminol-glucoside (MGG) (Sommaruga et al. 2004), which consists of a cyclohexanone attached to a glutaminol and glucose molecule, with a molecular weight of 464.5 g mol^{-1} and a characteristic absorbance at 310 nm (Fig. 18.1).

The biodiversity of basidiomycetous MGG-producing yeasts was investigated by Libkind et al. (2003, 2005a, 2009a) in certain lakes in Andean Patagonia (Argentina). The occurrence of MGG-positive yeast in waterbodies goes through a wide range, from 14 % to near 90 % of total cultivable yeast community. MGG synthesis was more frequent in yeasts that were not able to accumulate carotenoid pigments, and only red yeasts such as *Rhodotorula minuta*, *R. laryngis*, and *Dioszegia* spp. were positive for MGG. As for red yeasts, the abundance of mycosporine-positive species was higher in highly transparent lakes or in pelagic zones (Brandão et al. 2011). In glacier meltwater and ice, yeasts able to synthesize MGG are less frequent, including such species as *Dioszegia crocea* and *D. fristingensis* as the most important ones (de Garcia et al. 2012).

As for carotenoids, the biological role of mycosporines in yeasts was evaluated. Libkind et al. (2004b) suggested a photo-protective role, based in the strong response of mycosporine production to radiation. Using a set of *Cryptococcus stepposus* and *Phaffia rhodozyma* strains isolated from different Patagonian environments, we found a high positive correlation between survival to UV-B and MGG concentration. The fact that MGG accumulation protects yeasts against the effects of UVR avoiding the direct damage of DNA was also experimentally demonstrated (Moliné 2010; Moliné et al. 2011b). In addition, biochemical characterization of MGG from yeast revealed that it possesses high photostability and antioxidant properties (Moliné et al. 2011b). Thus, MGG appears as an interesting compound for multipurpose UV sunscreens, and yeasts become a valuable biotechnological source of these natural UV protectants. Several patents related to the production and usage of mycosporines sensu lato from different types of microorganisms have been published (for review, see Colabella et al. 2014) among which so far only one covers the use of yeasts (van Broock et al. 2009). In this patent, MGG from yeasts was purified and incorporated into base creams that were tested for UVR sunscreen. The product, with MGG concentration between 0.1 % and 5 %, showed a reduction in the UV-B flux (315 nm) for all the concentrations tested. UVB reduction values for 5 % were comparable with those obtained for commercial sunscreens with SPF 15 and 30.

These results are promising and lead to the investigation of the ability of different yeast species and strains from Patagonia to produce MGG in laboratory conditions. The concentration of MGG produced by Patagonian yeasts was highly variable; quantitative studies on MGG accumulation showed that differences in the production of this compound occur between different yeast species but also between strains. MGG accumulation varied among species, ranging from 2.5 to more than 50 mg g^{-1} dry weight. For example, different strains of *Cryptococcus stepposus* produce from 2.5 to a maximum of 5 mg g^{-1} whereas other species of the genera *Dioszegia* and *Aureobasidium* produce between 35 and more than 50 mg g^{-1} (Libkind et al. 2005a; Moliné 2010; Moliné et al. 2011b; Muñoz et al. 2013). Such production yields are larger than those observed for similar compounds (other mycosporines) in Cyanobacteria (2–9 mg g^{-1}) (Scherer et al. 1988; Portwich and Garcia-Pichel 1999). Using a set of 20 strains of *Phaffia rhodozyma* obtained either from culture collections or from Patagonian natural environments, we observed that the MGG production from the latter source ranged from 16 to 39 mg g^{-1} (average, 25 mg g^{-1}), whereas in the former MGG production ranged from 8 to 26 mg g^{-1} (average, 18 mg g^{-1}) (Moliné 2010). Thus, certain Patagonian yeasts are interesting sources of natural sunscreens for use in human photoprotection products.

MGG production in yeasts depends on illumination conditions and culture media composition. Yeast mycosporinogenesis is a phenomenon triggered by photostimulation (Libkind et al. 2004b; 2011c; Moliné 2010); however, significant constitutive synthesis has been found for certain yeast species. In *Cryptococcus stepposus*, increase in MGG accumulation after photostimulation (PAR) is 2- to 3 fold (Moliné et al. 2011b), whereas in *P. rhodozyma* increases are 10 fold and in *Cystobasidium minutum* (ex *Rhodotorula minuta*) the increases are 20 fold higher. When UVR rather than PAR is used, the latter increase goes up to 34 fold, indicating that the type and intensity of light modulates the response in MGG production in yeasts (Libkind et al. 2006).

Active compounds used in commercial sunscreens are, in general, lipophilic organic molecules produced by chemistry synthesis. These compounds absorb light in the UV-A to UV-C spectrum and are commonly added in concentrations up to 15 % to sunscreen products for skin protection (Wang et al. 2010; Loden et al. 2011). Because of growing public concern about skin damage by UV light, the demand for UV screens is increasing; however, several studies have brought to light the negative effects of these compounds to the environment and human health. Chemically synthesized UV screens are relevant environmental contaminants (Balmer et al. 2005), and for some active compounds androgenic and estrogenic effects have been detected (Krause et al. 2012). Further, common active ingredients such as 3-benzophenone of zinc oxide have been shown to produce reactive oxygen species and a photoallergenic effect in humans (Brezová et al. 2005; Hanson et al. 2006; Scheuer and Warshaw 2006). Consequently, the industry is seeking for more innocuous and natural compounds to replace current chemically synthesized and controversial ingredients. Today, a few examples of sunscreen products containing mycosporine-like amino acids are known, such as 'Helioguard' and 'Helionori' (Colabella et al. 2014). Although none of these contains MGG or any yeast-derived compound, we anticipate that natural sunscreen compounds such as yeast MGG represent a new and natural alternative to be used in commercial photoprotection products.

18.2.3 Cool Applications from Cold-Adapted Yeasts: Extracellular Enzymes

Cold-adapted microorganisms, including bacteria, archaea, filamentous fungi, algae, and yeasts, are being studied as sources for cold-active enzymes. The biotechnological value of cold-adapted enzymes stems from their high catalytic activity at low to moderate temperatures, their high thermolability at elevated temperatures, and their ability to function in organic solvents (Gerday et al. 1997). Applications include their use in cheese, wine, and juice production (pectinases), dough fermentation (xylanases), animal feed (cellulases), pulp bleaching (alpha amylases), detergents (lipases, peptidases), molecular biology (DNA, RNA polymerases), and nutrition (phytases) (Cavicchioli et al. 2011). Yeasts are heterotrophic organisms with the ability to degrade organic macromolecules by means of extracellular hydrolytic enzymes. Low molecular weight compounds are subsequently transported to serve in both catabolic and anabolic reactions. Cold-adapted yeasts secrete extracellular enzymes that can catalyze these reactions at low temperatures, mainly because of their highly flexible structures (Gerday et al. 1997). Other extracellular proteins function in cell-wall remodeling and anti-freezing, among other reactions (Crevel et al. 2002). The presence of some of these proteins was evaluated in a number of surveys with yeasts isolated from cold environments in Patagonia. In a first survey, 78 yeast strains were analyzed for extracellular enzymatic activities (EEA) (de Garcia et al. 2007). The ability of the strains to degrade starch, proteins, lipids, pectin, cellulose, and chitin was evaluated. Ninety-five percent of the tested strains showed at least one extracellular enzyme activity at either 4 °C or 20 °C. Lipolysis was the most frequent extracellular enzyme activity whereas none

of the strains showed the ability to hydrolyze chitin or cellulose. In a subsequent screening, yeasts from glacial and subglacial water (belonging to the genera *Cryptococcus*, *Leucosporidiella*, *Dioszegia*, *Mrakia*, *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, *Sporidiobolus*, *Cystofilobasidium*, and *Udeniomyces*) were studied (Brizzio et al. 2007). Most of the 91 studied isolates exhibited amilolytic, protease, and lipase activities that were higher at 4 °C than at 20 °C. In a more recent survey, five enzymatic activities were analyzed in 212 yeast strains isolated from ice and meltwater (de Garcia et al. 2012). At least one enzymatic activity was present in 85 % of the strains, whereas 18 % showed positive tests for all the five activities (degradation of starch, caseine, pectin, carboxymethyl cellulose, and Tween-80). As a consequence of these studies, a number of biotechnologically relevant strains have emerged. Ongoing studies, including genomic and proteomic analyses of the extracellular proteins, will allow finding interesting new cold enzymes. Detailed studies of their enzymatic capabilities are also necessary to reveal possible applications.

18.3 Biotechnologically Relevant Yeasts from Patagonia: Three Hot Cases from the Cold

In this section we review the three most interesting cases of yeasts isolated from Patagonian natural environments that clearly have potential for biotechnological exploitation: these include the recently described new species of *Saccharomyces*, *S. eubayanus*, the ancestor of the lager brewing hybrid yeast. Also, the discovery of novel populations of *S. uvarum* in Patagonia is addressed together with their contribution to the origin of domesticated lineages used for wine and cider production. Finally, the finding and characterization of the astaxanthinogenic and mycosporinogenic yeast *P. rhodozyma* from Patagonia are reviewed.

18.3.1 The Discovery of S. eubayanus*, the Ancestor of the Lager Brewing Yeast*

Beer is the most common fermented beverage in the world and can be classified as ale or lager, depending on the fermentation conditions and yeasts used. Lager beer is the most common commercially produced beer worldwide (94 % of total beer market) and yet, the genetic origin of the yeast strains that brew them has been full of mystery and controversy. Compared with conventional ale-style beers, which are generally brewed with *Saccharomyces cerevisiae* (Hornsey 2003), lagers are brewed at colder temperatures with allopolyploid hybrid yeasts of *Saccharomyces cerevisiae* × *S. eubayanus* (known as *S. pastorianus*). *S. eubayanus* was only recently discovered and formally described as a result of yeast biodiversity surveys in Patagonian *Nothofagus* forests, from which two cold-adapted *Saccharomyces*

species were recovered mainly from bark and soil samples but particularly from the stromata of *Cyttaria* fungus (Libkind et al. 2011a). *S. eubayanus* was the first yeast species to be formally described based on a complete genome sequence (Libkind et al. 2011a). The first draft genome of the type strain (PYCC 6148) was obtained for comparison with the non-*S. cerevisiae* subgenome of the lager yeast (see Sect. 4.1), revealing the 99.5 % similarity that lead to the conclusion that *S. eubayanus* was the ancestor species. Today, additional isolates have been obtained from other parts of the world, generating discussion about the actual origin of the population that gave rise to the hybdrid. However, so far none of the new sources of *S. eubayanus* showed the high frequency of recovery and the large genetic diversity found in Patagonia (addressed in Sect. 4.1). The second species of *Saccharomyces* found in Andean Patagonia *Nothofagus* forests was *S. uvarum*, the sister species of *S. eubayanus*, which is discussed next.

In Patagonia, *S. eubayanus* seems to be partially restricted to certain species of *Nothofagus* such as *N. pumilio* and *N. antarctica*; however Rodríguez et al. (2014) found several isolates from a different tree in the north of Patagonia: *Araucaria araucana*. These tree species are endemic from Patagonia; thus in other parts of the world *S. eubayanus* was collected mainly from oak trees, or other types such as *Cedrus* sp., *Pinus taeda*, and *Fagus* sp. (Peris et al. 2014; 2016).

18.3.2 Saccharomyces uvarum: *Wine and Cider*

Thus, in Patagonian habitats *S. eubayanus* and *S. uvarum* (two sister species), exist in apparent sympatry in *Nothofagus* (Southern beech) forests, but are isolated genetically through intrinsic postzygotic barriers (Libkind et al. 2011a). Previously, it was shown that sympatric *Saccharomyces* species tend to have different growth temperature preferences, as is true for *S. cerevisiae* (thermotolerant) and *Saccharomyces kudriavzevii* (psychrotolerant) co-occurring in Mediterranean regions, as well as *Saccharomyces paradoxus* (thermotolerant) and *S. uvarum* (psychrotolerant) coinhabiting temperate Europe and North America (Sampaio and Gonçalves 2008). Another particular characteristic of Patagonian environments in contrast to North Hemisphere counterparts is the almost complete occupancy of the *Nothofagus* niche by psychrotolerant *Saccharomyces* species. Although less than 50 % of the isolates from bark and soil samples from the North Hemisphere belong to *Saccharomyces*, in Patagonia these values range from 64 % to 95 % for both *S. uvarum* and *S. eubayanus*. The substrate of greatest occupancy is *Cyttaria* stromata (~95 %), which is in agreement with its high content of simple sugars.

Furthermore, a putative ecological isolation through host preference was detected, given that *S. eubayanus* was found in association with *N. antarctica* and *N. pumilio*, whereas *S. uvarum* was associated with *N. dombeyi*. This finding might explain the co-existence of these two hitherto phenotypically undistinguishable species.

18.3.3 The Colorful Case: Phaffia rhodozyma

Phaffia rhodozyma (synonym of *Xanthophyllomyces dendrorhous*) represents an exceptional fungal case of the basidiomycota, given that it combines the production of orange-colored colonies with the ability to ferment simple sugars. The main carotenoid pigment synthesized by *Phaffia* is astaxanthin, another exclusive characteristic of this yeast species (Andrewes et al. 1976) and the main reason for which it is currently being exploited biotechnologically as a natural source of astaxanthin in aquaculture feed (Rodríguez-Sáiz et al. 2010). The first isolates of *P. rhodozyma* were found in association with spring sap flows of various broad-leaved trees in Japan, Alaska, and Russia (Phaff et al. 1972; Golubev et al. 1977). Later, more strains were recovered from beech trees in central Europe (Weber et al. 2006) and the United States (US) (Fell et al. 2007). The range of *P. rhodozyma* was significantly expanded when a South American population associated with *Nothofagus* trees (southern beech), particularly the stromata of its biotrophic fungal parasite *Cyttaria* spp., was discovered (Libkind et al. 2007, 2008b, 2011d). The Patagonian isolates were found to be genetically different from the Northern Hemisphere strains based on DNA–DNA reassociation experiments, micro/mini-satellite-primed (MSP)–polymerase chain reaction (PCR) fingerprinting, as well as internal transcribed spacer (ITS) and intergenic spacer (IGS) rRNA gene sequencing (Libkind et al. 2007, 2011b). Finally, thes differences were confirmed using multi-locus sequence typing (David-Palma et al. 2014) and later using complete genome analyzes (Bellora et al., in press). However, Patagonian strains appeared to be genetically uniform (minor differences were found using the L41 gene as marker) and could be included into a distinct population, supporting the hypothesis that geographic isolation and association with different host species has determined genetically different *P. rhodozyma* populations worldwide (David-Palma et al. 2014). Weber et al. (2008) described a novel isolate from Chile with marked ITS and LSU sequence differences from the other known populations. This single isolate was obtained from a leaf of the Tasmanian blue gum tree (*Eucalyptus globulus*) in the Mediterranean climate at Concepción, a tree species originally from Australasia. David-Palma et al. (2014) reported the isolation of highly divergent lineages of *P. rhodozyma* from *Nothofagus* forests in Australia and New Zealand, expanding the known geographic distribution of this yeast and its genetic diversity. Two of these lineages deserve the assignation to distinct species and will be described in the near future as novel taxa in the genus *Phaffia* (unpublished results).

It is evident that *P. rhodozyma* possesses a greater genetic variability and geographic distribution than previously thought, generating the necessity to uncover it and assess its potential for the astaxanthin and UV sunscreen industry. However, difficulties for the isolation of this yeast hinder extensive environmental surveys. Our group developed a new and innovative strategy for improving *P. rhodozyma* recovery rate and identification from environmental samples (Tognetti et al. 2013), as well as a new PCR-based method for the rapid identification of *P. rhodozyma* isolates (Colabella and Libkind 2016).

18.4 Genomic Approaches to the Study of Patagonian Yeasts

As already demonstrated, yeasts from Patagonian natural environments include biotechnologically useful species and strains relevant to the production of beer, wines, antioxidants, photoprotective and cryoprotective compounds, among others. Genome assemblies using next-generation sequencing (NGS) and comparative genomic analyses were performed for the most interesting species, namely, *Saccharomyces eubayanus* (brewing), *S. uvarum* (wine and cider), and *Phaffia rhodozyma* (astaxanthin and mycosporines). The phylogenetic analyses in combination with geographic information enlightened the relationships between strains from the Northern and Southern Hemispheres and contributed to a better understanding of the origin and complexity of domesticated genomes. On the other hand, genome mining allowed detecting and characterizing specific genes and variants implicated in pathways of processes of biotechnological relevance. A remarkable outcome of such studies was the discovery of the wild genetic stock of domesticated yeasts currently used in major fermented beverages industries such as beer, wine, and cider (Table 18.2).

18.4.1 Phylogenomic and Phylogeographic Studies of Industrially Relevant Yeasts from Patagonia

In recent years, several genomics studies on the phylogeography of biotechnological relevant yeasts from Patagonia were conducted, including the two psychrotolerant biologically recognized species of *Saccharomyces*: *S. eubayanus* and *S. uvarum*.

The draft genome sequence of *S. eubayanus* was reported in 2011 by Libkind and colleagues (Libkind et al. 2011a) from wild Patagonian isolates that showed some genetic resemblance to the currently controversial species *S. bayanus*. Surprisingly, the authors found a 99.5 % of identity of the wild *S. eubayanus* to the non-*S. cerevisiae* subgenome of the allopolyploid lager brewing yeast: *S. pastorianus.* No evidence of introgression, hybridization, or horizontal gene transfer indicated that *S. eubayanus* represented a pure lineage. On the other hand, *S. pastorianus* as well

Table 18.2 Available genomes of Patagonian strains that belong to biotechnologically relevant yeast species

Species	Genome size (Mb)	Genomes available for Patagonian strains (other strains)	Biotechnology application	Remarks
S. eubayanus	11.6	18 (5)	Brewing	The largest diversity is in Patagonia
S. uvarum	11.5	16 (38)	Wine, cider	The largest diversity is in Patagonia
P. rhodozyma	18.9	1 (2)	Asthaxantin, mycosporines	Unique population in Patagonia

S., Saccharomyces; P., Phaffia

as *S. bayanus* are not biologically recognized "species" but hybrids, products of the artificial brewing environment with no occurrence in nature (Libkind et al. 2011a; Baker et al. 2015). The genomic complexity of *S. bayanus*, a hybrid yeast frequently associated with contaminated beer, could be explained by the contribution of mixtures of regions from *S. uvarum*, *S. eubayanus*, and *S. cerevisiae. S. eubayanus* and *S. uvarum* are sister species and form a basal clade distant to *S. cerevisiae* within the genus. Their genome-wide divergence is ~7 % and is the lowest between *Saccharomyces* species to result in genetic isolation (Libkind et al. 2011a). The two species coexist in Patagonian *Nothofagus* forests. Later, a higher-quality genome of *S. eubayanus* was released together with the first mitochondrial complete sequence (Baker et al. 2015). In this study, *S. eubayanus* subgenomes of lager brewing yeasts were shown to have experienced increased rates of evolution since hybridization, and that certain genes involved in metabolism may have been particularly affected (see following section). Interestingly, it was demonstrated that the *S. eubayanus* subgenome underwent an especially strong shift in selection regimes, consistent with more extensive domestication of the *S. cerevisiae* parent before hybridization. In contrast to recent proposals that lager brewing yeasts were domesticated following a single hybridization event (Walther et al. 2014; Wendland 2014), the radically different neutral site divergences between the subgenomes of the two major lager yeast lineages strongly favor at least two independent origins for the *S. cerevisiae* × *S. eubayanus* hybrids that brew lager beers (Baker et al. 2015). The recent isolation of newly *S. eubayanus* strains and their genomic characterization fueled the controversy of which population gave rise to the lager hybrid. Peris et al. (2016), using genome sequence data, examined the relationships of a larger set of wild *S. eubayanus* strains to each other and to domesticated lager strains. Results supported the existence of a relatively low diversity lineage of *S. eubayanus* whose distribution stretches across the Holarctic region and includes wild isolates from Tibet (Bing et al. 2014), new wild isolates from North America (Peris et al. 2014; 2016), and the *S. eubayanus* parents of lager yeasts. This clade is closely related to a high-diversity population that is found primarily in South America but includes some widely distributed isolates in the US (Peris et al. 2016) and New Zealand (Gayevskiy and Goddard 2016). It was further shown that no single Holarctic isolate was the sole closest relative of lager yeasts and that the wild Holarctic population of *S. eubayanus* is responsible for genetic variation still segregating among modern lager brewing hybrids. These observations suggest that lager yeast origins were more complex than we thought and stress the need for further investigations in the Northern Hemisphere.

Almeida and colleagues studied 54 *S. uvarum* domesticated (from wine and cider) and wild strains from different geographic areas of the globe based on high-quality polymorphic sites and resolved the strains into three main groups (Almeida et al. 2014): a clade that contained all Holarctic isolates (including the domesticated ones) and a few from South America, a second with only South American isolates, and a third with a few recently found Australasian strains. Holarctic populations when compared to Patagonian ones were extremely low in genomic diversity. Then, the highest diversity of strains of *S. uvarum* was thus detected in Patagonian environments. The Australasian lineage instead diverged significantly from the other two groups (by

4.4%), forming a sister group to the two other clades and being closer to *S. uvarum* than any other *Saccharomyces* species. It yielded similar divergences to those found between North American and European populations of *S. paradoxus* (Liti et al. 2006), so it was considered conspecific, although possibly going through a process of allopatric speciation. Phylogenomic analyses support the view that a restricted subset of one of the two Patagonian *S. uvarum* populations gave rise to the Holarctic population, although vectors and mechanisms of this migration into the Northern Hemisphere remain to be elucidated (Almeida et al. 2014).

In a recent study, the genomes of the Patagonian and the type strain of the astaxanthin- and mycosporine-producing yeast *P. rhodozyma* were assembled and compared (Bellora et al. 2016). The Patagonian strain showed 4.4% of genomic divergence toward two sequenced Holarctic strains (0.073% between them) (CBS 7918 T and CBS 6938), indicating that an allopatric speciation process might be occurring and that the former deserves to be assigned at least to a distinct variety. A considerable number of exclusive genes were present in the Patagonian strain but not in the European strains. Other interesting observations include a high occurrence of introns in *P. rhodozyma* and other Cystofilobasidiales and new insights into fungal homothallism.

18.4.2 Genes Related to Biotechnologically Relevant Pathways and Genomic Footprints of Domestication

The study of the effect of domestication in the lager brewing yeast was only possible once the genome of the parental *S. eubayanus* become available. Genetic changes detected in the *S. pastorianus* genome in comparison to the parental strain that seem to have been favored by the brewing environment include extra copies of *S. cerevisiae IMA1* (isomaltase, cleavage of disaccharide isomaltose), inactivation of the *SUL1* gene (encode high-affinity transporters of sulfate, the metabolic precursor of sulfite, a known antioxidant and flavor stabilizer), favoring *SUL2* which improves sulfite production in brewing conditions, and several gene expression regulators related to genes that allow alcohol utilization (i.e., *ADR1*), glucose-repressed genes such as *MAL* genes (i.e., *REG2*), among others (Libkind et al. 2011a; Baker et al. 2015). Moreover, a similar mtDNA gene arrangement and sequence established *S. eubayanus* as the main mitochondrial donor of lager yeast of the Frohberg lineage, harboring CDSs with ~98.6% identity.

Almeida et al. (2014) detected that European *S. uvarum* domesticated strains (wine and cider) consistently contained several *S. eubayanus* introgressions. These introgressions were absent in the large majority of wild strains, and gene ontology analyses indicated that several genes included in the introgressed regions were relevant for wine fermentation. Many of the introgressions were subtelomeric and contained genes such as ASP1, a gene encoding the cytosolic L-asparaginase used to degrade asparagine to be used as nitrogen source, and FZF1, a transcription factor that regulates several genes, including Ssu1, that encode an efflux pump involved in

sulfite resistance (Almeida et al. 2014). These results represented the first clear indication of domestication in the yeast *S. uvarum*, used for wine and cider production worldwide. Genome mining of *P. rhodozyma* allowed detection and annotation of all genes of the astaxanthin synthesis as well as previously unknown putative regulatory enzymes of the metabolic pathway (Bellora et al., in press). Additionally, genes homologous to those reported to be implicated in the synthesis of mycosporines in Cyanobacteria (Balskus and Walsh 2010) were detected in a similar cluster disposition in the Patagonian strain of *P. rhodozyma*. One of the Holarctic strains (the type strain of the species) possessed the same cluster arrangement but a second European strain (Sharma et al. 2015) lacked the complete set of genes. In agreement with this finding, it was later demonstrated that the former *P. rhodozyma* strain (CBS 6938) lacked the ability to synthesize MGG (Bellora et al., in press).

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