4

# Yeasts Biodiversity and Its Significance: Case Studies in Natural and Human-Related Environments, *Ex Situ* Preservation, Applications and Challenges

Enrique Javier Carvajal Barriga et al.*
*Centro Neotropical para la Investigación de la Biomasa, Colección de Levaduras Quito Católica, Pontificia Universidad Católica del Ecuador, Quito Ecuador*

## 1. Introduction

Yeasts are a group of microorganisms that belongs to the Fungal Kingdom. These unicellular fungi are distributed between the Basidiomycota and Ascomycota Phyla, being a paraphyletic group. Since 1865, its study has experienced a very important advance in terms of its understanding, characterization and taxonomic accommodation. Nevertheless, it is estimated that about 99% of the potential biodiversity of this group of eukaryotic microorganisms is still unknown. That is why there is a need for increasing efforts to study yeast biodiversity, especially in mega diverse countries from the tropical regions of the planet.

To date, the majority of yeast species catalogued have been discovered in countries from the Northern hemisphere. Relatively few studies dedicated to yeast biodiversity have been done in tropical zones of the planet and in Southern hemisphere countries that embrace abundant and diverse ecosystems. A number of case studies of these approaches to yeast biodiversity are presented in this chapter, including the discovery and subsequent description of novel yeast species recently isolated in Ecuador, Brazil and Argentina. The chapter will also deal with the biodiversity of yeasts found in industry-influenced environments in Spain.

Moreover, *ex situ* preservation of yeast isolates for further characterization by physiological, morphological and molecular techniques is a fundamental issue in terms of the

* Diego Libkind[2], Ana Isabel Briones[3], Juan Úbeda Iranzo[3], Patricia Portero[1], Ian Roberts[4], Steve James[4], Paula B. Morais[5] and Carlos A. Rosa[6]
[1]*Centro Neotropical para la Investigación de la Biomasa, Colección de Levaduras Quito Católica, Pontificia Universidad Católica del Ecuador, Quito, Ecuador*
[2]*Laboratorio de Microbiología Aplicada y Biotecnología, Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), CONICET-UNComahue, Bariloche, Argentina*
[3]*Laboratorio de Biotecnología en Levaduras, Universidad de Castilla La Mancha, Ciudad Real, España*
[4]*Institute of Food Research, National Collection of Yeast Cultures, Norwich, United Kingdom*
[5]*Laboratório de Microbiologia Ambiental e Biotecnologia, Campus Universitário de Palmas, Universidade Federal do Tocantins, Palmas, TO, Brazil*
[6]*Departamento de Microbiologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*

understanding and preservation of the biodiversity. Yeast culture collections play a fundamental role not only as the repositories for invaluable yeasts strains (germplasm), but also as platforms for biotechnology exploitation. Experiences and challenges that several yeasts collections are facing will also be discussed in this chapter.

## 2. Biodiversity of Yeast, ¿What does it mean?

The term biodiversity is an abstract expression of all aspects of the variety of life (Gaston, 1996); from bio-molecules to the variety of different species populations and communities of species. Variation is the essence of biology. Thus, biodiversity is an intrinsic feature of life.
Under this focus, biodiversity loss is one of the main global concerns. This, loss can be produced by a number of different factors related to human activities and to natural events; where competition at the intra- or inter-specific levels and even at the molecular scale, reminds us the real drama of life, where Darwin's "The Origin of Species" reaches the nerve of this fundamental issue of living organisms.
Nonetheless, an undefined number of yeast species losses can be caused due to the perturbation of habitats by humankind. As Dr. Steve James from the National Collection of Yeast Cultures in the United Kingdom points out, talking about the importance of yeast biodiversity surveys: "It's a race against time. We know that massive loss of species diversity is occurring worldwide. Our efforts are thus focused on characterizing and subsequently preserving what remains."
Global-scale conversion of tropical rainforests and agricultural intensification are major causes of biodiversity loss (Chapin et al, 2000; Hoekstra et al, 2005). Extinction is the final result of a process that starts with the vigor's weakening of certain populations. The most undesirable and irreparable effect is the complete loss of all (component) populations of a single species. This effect is uniquely evident when the fragmentation and perturbation degree of natural micro- and macro-landscapes overwhelms the "decisive threshold" (Pimm and Raven, 2000).
Some microbes do seem to be restricted to very particular environments and are endangered in as much as these environments are threatened; microbes intimately associated with other organisms share (partially) the biogeographies of their hosts. As far as they are species-specific, they could potentially become extinct along with their hosts (Weinbauer and Rassoulzadegan, 2007).
We still do not have any definitive evidence of the extinction of any yeast species: it is very hard to determine that. Nonetheless, we can presume that a number of co-evolving yeasts species have probably become extinct along with their plant or animal hosts. Studies made on bumble bees demonstrate that insects play a crucial role, not only in yeast dispersion, but also acting as a type of "wet nurse" during winter, when environmental conditions are very harsh and no flowers are present in the fields (Byrsch, 2004). In this way, extinction or weakening of insects populations can ultimately lead to the extinction of certain insect-dependent yeasts species, at least locally.
As biodiversity is not only referent to the living organism by itself, but also to the diversity of its strains and varieties. Likewise, the molecular variety of yeasts is both huge and extremely dynamic. The occurrence of a great variety of yeast strains is the result of the high mutation rate that provides these microorganisms with the ability to adapt to different environments. Mutation rate is an important parameter in evolution. It dictates the speed of adaptation in populations with beneficial mutations; in the absence of such mutations it sets

the equilibrium fitness of the population (Gregory et al, 2007). The loss of varieties in yeast strains is also a concern, but, at the same time is an issue that we cannot solve and probably we don't need to try solving.

The search for yeasts species/strains with economic potential is a way to preserve those genetic varieties that are worth being kept and used in a wide range of applications. Gregory et al in 2007 found that in *S. cerevisiae* the mutation rate per gene is in the order of $10^{-10}$/base pair/generation. It is important to note that different DNA regions in living organisms have different variability. These mathematical approaches can be used to estimate the evolutionary distance in terms of time between different strains. As for *S. cerevisiae*, it has been possible to determine, based upon the nucleotide variations of several genes belonging to different strains, that this yeast species was most likely first domesticated about 11,900 years ago (Fay and Benavides, 2005). The study of ancient dormant yeast strains/species, although still in its infancy, is nevertheless a field that offers the opportunity to help better understanding of microbial biodiversity over time (Gomes *et al*., 2010).

Dormancy in yeasts and other microorganisms plays a key role to help keep a seed bank for the future (Jones and Lennon, 2009). The biodiversity of microbial communities, of which yeasts are an integral part of has important implications for the stability and functioning of managed and natural ecosystems. Dormancy is one trait that allows species to contend with temporal variability of environmental conditions. This "bet-hedging" strategy allows dormant individuals to become members of a "seed bank", which can contribute to the diversity and dynamics of communities in future generations (Turner et al. 1998; Caceres and Tessier, 2003). The recovery of dormant yeast species from archaeological pieces as well as paleontological rests provides a means of reviving species or strains that were probably destined to become extinct (Gomes *et al.*, 2009). The techniques and the approaches already done in this field will be outlined later in this chapter.

Yeasts are also adapted to dispersion and then survival. One example of this is the cross-shaped yeast *Metchnikowia gruessii* that is dispersed by bees visiting flowers during its feeding periods in the day. This cross-formed yeast species is adapted to the glossa or tongue of the bees and so use the insect as a means of dispersal from one flower to the next. As for the studies carried out by Byrsch in 2004, this species is highly successful, very common and forms predominant populations in nectar of certain central European flowers.

The best way to get into the study of yeasts in natural environment is using ecological criteria: yeasts occupy a diverse variety of micro-ecosystems and are well adapted to a wide range of weathers, altitudes, substrates and geographical locations. It is possible to find yeasts in glaciers, high salinity lakes, water, soil, air, intestines of a variety of vertebrates and invertebrates, and even in acid waters (Russo et al, 2010) and marine deep-sea environments (Nagahama Takahiko, Biodiversity and Ecophysiology of yeast). The proper way to study yeast diversity and its function in communities is by gaining an understanding of their role in communities, so we can predict the occurrence of certain species based on the features of the micro- and macro-landscapes.

Yeast species such as *S. cerevisiae* have been used by humankind throughout history for the production of fermented foods and beverages around the world. That is why yeasts are intimately linked to our day-to-day activities related with culture, economy and nutrition. Moreover, certain yeast species are linked to human diseases, while others form part of the intestine's micro-flora in both vertebrates and invertebrates.

Nevertheless, a relatively small number of yeast species are currently being used in industry, while, a large number of species collected from natural environments and human-related micro-ecosystems are still being studied and classified. Yeast taxonomy deals with the classification and accommodation of species that are being discovered in an ever increasing number year after year.

### 2.1 Yeast diversity in numbers

It is believed that only 1% of all extant yeast species is currently known. From 1820 up to 2011 the number of described yeasts has increased dramatically. By 2005, more than 2500 yeast species were published. This number of species already named includes synonyms which are being taxonomically re-accommodated. Currently there are approx. 1500 recognised yeast species, which means the expected number of yeast species on Earth would be around 150,000. Large territories of Africa, Antarctica, Asia, Australia and Latin America are mainly virgin (Hawksworth, D.L, 2004). These new and hardly explored habitats represent rich sources of fungal biodiversity still awaiting discovery. To date, relatively little work has been carried out in this field in South American countries like Argentina, Brazil and Ecuador. The yeast diversity in such countries is potentially huge. For example, over 200 new species of yeasts have been found amongst 650 isolates from the guts of beetles (Suh et al. 2004, Suh & Blackwell 2005). Coleoptera species are floricolous insects and tree flux communities whose species number about 350,000. Nevertheless, not all beetle species harbour yeasts, and so its number must to be first established to predict a possible overall estimate of yeast related with them (Lachance, 2005 Yeast biodiversity and Ecophysiology).

Molecular techniques used since 2000 have greatly boosted the number of new species identified. Molecular analyses of the variable D1/D2 regions of the 26S rDNA, 18S, 5.8S and mitochondrial small subunit rDNAs gene, as well as ITS sequencing and RFLP-ITS are very useful ways to identify yeast species and invaluable tools for phylogenetic studies (Kurtzman and Fell, 2005 biodiversity and Ecophysiology). These molecular techniques, combined with microbiological and physiological tests, are being used to characterize yeast isolates and species. Most of the analyses have used rDNA sequences, however, we now know that there are no universal criteria to distinguish between genera.

Communities of yeasts are affected by natural selection which eliminates deleterious mutations and rapid fixation of adaptive alleles, just as the environment determines whether or not a species can become established within a community (Lachance, 2006). In these terms, we can understand that events of speciation and/or extinction are occurring in yeasts around us all the time at a relatively high rate due to its remarkable rate of reproduction, mutation and adaptation to changing situations.

Diversity between strains of the same species, such as *Saccharomyces cerevisiae*, has also been studied by molecular methods. It is well known that there are variations in strains and some metabolic abilities/disabilities are not necessarily linked to the species but rather to the strains (i.e. strain variable). With few exceptions, only one strain or an individual of a particular species is sequenced while hundreds of other variants, which may be important to public health, scientific research, or commercial applications, remain un-deciphered (Winzeler et al, 2002). The use of microarrays of whole genomes divided into 25mers helps to find variations between different strains of a single species, in as much as a single base substitution in these 25mers (especially those found in the center of the sequence) disrupts

hybridization (Chee et al. 1996; Gingeras et al. 1998; Troesch et al. 1999; Lockhart and Winzeler 2000). Single Feature Polymorphism SFP assessment carried out on 14 different strains of *S. cerevisiae* yielded 11,115 variations, which demonstrates the huge genotypic variation between strains of a single species and the opportunities these variations offer for the research (Winzeler et al, 2002).

Furthermore, Single Nucleotide Polymorphism (SNP) analysis is revealing relationships within strains of a single species. Moreover, the analysis of variation in gene content, nucleotide insertions and deletions, copy numbers and transposable elements are all contributing to reveal the intricate relationships between yeast species and strains (Liti, 2009). In other words, biodiversity of yeasts at intra- and inter-specific levels is a big endeavor that is still very much in its infancy taking into account the huge diversity of yeasts.

In order to ascertain and classify the yeast biodiversity in nature in an affordable way it is necessary to investigate the multiple and varied micro-ecosystems represented by substrates that may be used as a source of nutrients by yeast as well as platforms for their dispersion. From beetle guts, to flower nectar or rotten woods, there still remains a huge field to be examined in order to identify and characterise novel and known yeast species: their distribution, ecologic relationships and the understanding of the aspects involving the yeasts natural history, and feasible uses as biotechnological work horses. South America is a region that offers great potential in terms of biodiversity (macro and micro), where yeasts are being isolated from habitats that never were sampled before. Initial results from a survey run by an international consortium from Brazil, Spain and Ecuador in the Galapagos Islands at the end of 2009 (data not published) is beginning to reveal the diversity of yeasts present in various substrates such as flowers, cacti, rotten wood, turtle's faeces, marine iguana faeces, and other substrates located in four different islands. This kind of expedition has also been done in other South American countries such as Argentina and Brazil. Several novel species have been identified in such surveys.

At this point, the identification and characterization of yeast isolates and its preservation is a task that may be accomplished by researchers. Yeast culture collections play the leading role in keeping the rich diversity of yeasts for current and future applications as well as genetic reserve. Some aspects of the biodiversity studies, yeasts preservation, novel yeast species description and its taxonomic accommodation and biotechnology applications will be developed in this chapter.

## 3. Ecology and biodiversity of yeasts

### 3.1 Yeast-insect interactions as example of biodiversity studies

In general, yeasts are suspected to engage in intimate symbiotic relationships with insects, although the nature of the interaction remains elusive in most cases (Lachance, 2006). Several examples of yeasts associated with insects have been reported in recent years (Rosa et al., 2003; Lachance et al. 2005; Starmer & Lachance, 2011). In most cases, the insects vector the yeasts and use these microorganisms as a food source. The fruit flies of the genus *Drosophila* eat yeast, digesting vegetative cells but passing spores through the gut intact and viable (Colluccio et al., 2008). Yeasts have been also described as endosymbionts in mosquito populations, lacewings, beetles and homoptera (Ganter 2006; Ricci et al. 2011). The insects rely on yeasts for various metabolic functions, including synthesis of amino acids, vitamins, lipids, sterols and pheromones, degradation of nutritional substrates, and detoxification of compounds (Suh et al. 2003; Starmer & Lachance, 2011).

Geographic gradients were identified in *Candida ipomoeae* and *Metschnikowia borealis* that are found in association with nitidulid beetles that visit short-lived flowers of morning glories and a few other plant families, indicating that historical and climatic factors play a role in shaping the populations (Lachance et al. 2001). Highly specific associations between floricolous nitidulid beetles and various yeasts, including those in the *Metschnikowia* clade, have been documented worldwide (Lachance et al. 2001b, 2005). *Metschnikowia* and related species associated with nitidulid beetles are presumed to have co-speciated with the insects (Lachance et al. 2005; Lachance, 2006). Lachance et al. (2001) suggest that *Conotelus* spp. adults feed on the nectar of ephemeral flowers and in doing so, deposit yeasts together with their fecal material in the corolla. The yeasts grow at the expense of nutrients present at the surface of the corolla. The transmission of yeasts is probably horizontal, through cross-contamination at feeding sites or possibly during copulation. One possible role of the yeasts is to assimilate low complexity carbon and nitrogen sources present in the flower and thus provide the beetle larvae with a diet that contains essential nutrients such as lipids (Nasir and Noda 2003).

More recently, a highly diverse yeast assemblage was found in the gut of various beetles families (Suh & Blackwell, 2004;) especially phytophagous Coleoptera, Homoptera, Hemiptera, Isoptera and Lepidoptera (Suh et al., 2005; Ganter, 2006; Lachance, 2006; Starmer & Lachance, 2011). In particular, it is well known that bark beetles of the weevil subfamily Scolytinae increase their host-colonizing potential by means of symbiotic relationships with fungi, which are carried within specialized structures called the mycangia, or on the body surface (Ganter, 2006). About 200 apparently undescribed species have been discovered so far from the gut of basidioma-feeding beetles, and many of those yeasts form independent clades in Saccharomycotina that have not been recognized previously (Suh et al. 2005). For example, more than 40 new beetle-associated yeast species were reported recently to form several major clades near *C. tanzawaensis*, *Meyerozyma guilliermondii*, *C. mesenterica*, and *C. membranifaciens*, and each of these clades was composed almost exclusively of insect associates (Suh & Blackwell 2004, 2005, Suh et al. 2004b, 2005).

The relationships of yeasts and insects are being discovered as studies expand: the brown planthopper (BPH), *Nilaparvata lugens*, harbors yeast-like symbiotes (YLS), especially in mycetocytes formed by fat body cells found in the abdomen. *Pichia*-like and *Cryptococcus*-like symbiotes may present a potential for biological control of this insect pest (Ganter, 2006). Also, the mutual relations of fungus-growing ants, their fungal cultivars, and antibiotic-producing bacteria suffers the interference of a black yeast counterpart that acquires nutrients from the ants' bacterial mutualist, and suppresses bacterial growth. Several yeast species were isolated from fungus garden and waste deposit of these ants, and could play an important ecological role in these substrates (Pagnocca et al. 2010).

Yeasts have also been reported associated with several species of bees, including social and solitary bees (Pimentel et al. 2005; Ganter 2006; Lachance et al. 2011). The majority of bee species, of which there are approx. 20,000 species (Michener 2000), have never been examined for the presence of yeasts (Rosa et al. 2003). The clade *Starmerella*, that includes two teleomorphic species and several asexual *Candida* species, has been isolated from honey, provisional pollen, nectar and waste deposits in hives and nests of several bee species (Rosa et al. 2003). The nature of the possible symbiosis is not known with certainty, but a role in pollen maturation is suspected (Starmer & Lachance, 2011). These yeasts were able to produce several extracellular enzymes that could metabolize the sugars and pollen stored in the nests, improving their nutritional quality (Rosa et al. 2003).

### 3.2 The yeasts in plant substrates: Leaves, flowers and fruits

All aerial plant surfaces, known as the phylloplane or phyllosphere are inhabited by diverse assemblages of microorganisms, and these have profound effects upon plant health and impact on ecosystem functions. The associations established on plant surfaces range from relatively inconsequential or transient to substantial or permanent (Fonseca & Inacio, 2006). The leaf surface characteristics may affect, both qualitatively and quantitatively, the immigration of yeasts to the phylloplane (Fonseca & Inacio, 2006). Leaf surfaces are colonized by members of several genera of saprophitic yeasts that provide a natural barrier against plant pathogens (Fokkema *et al*., 1979). Leaves are exposed to rapid fluctuations of temperature and relative humidity values, which may have an impact on the yeast population. Large fluxes of UV radiation are also one of the most prominent features of the leaf surface environment to which microorganisms have presumably had to adapt (Lindow & Brandl, 2003). Many plants contain a number of compounds whose adaptive significance may be a defense against invertebrates and microorganisms (Robinson, 1974). These compounds also act, in some cases, as selective agents which shape the yeast community composition (Starmer & Lachance, 2011). Some yeast species isolated from fruits have a potential use as antagonists and can serve as a biological control against post-harvest decay fruit diseases (Ippolito and Nigro, 2000; Seibold *et al*., 2004).

Flowers and other parts of plant species belonging to the Convolvulaceae, Bromeliaceae and Heliconiaceae families are rich sources of novel yeast species. Most of the novel yeast species isolated from these plants belong to the *Metschnikowia, Wickerhamiella* and *Starmerella* clades (Lachance et al. 2001; Ruivo et al. 2005; Rosa et al. 2007; Barbosa et al. 2011). In ephemeral flowers of the Convolvulaceae, the yeasts are transported by pollinating and non-pollinating flies, beetles and bees that deposit them in the corolla (Lachance et al. 2001). In the longer-lasting flowers of the Heliconiaceae, yeasts are probably introduced by a different and more diverse set of animal vectors and they may grow on the sugary compounds present in nectar (Barbosa et al. 2011).

Most yeast species isolated from flowers are supposedly nectar-inhabiting yeasts. Dense yeast communities often occur in the floral nectar of animal-pollinated plants, where they can behave as parasites of plant-pollinator mutualisms (Brysch-Herzberg 2004; Canto et al. 2008; Herrera et al. 2008, 2009 de Vega et al. 2009). Nectar yeasts, particularly at high densities, induce metabolic degradation of nectar, which can be detrimental to plant reproduction through reduced pollinator service (Herrera et al. 2008). This might originate selective pressures on plants to defend their nectars from exploiters through, e.g. the production of antimicrobial secondary compounds (Irwin et al. 2004). *Metschnikowia reukaufii, M. gruessii, C. bombi, K. dobzhanskii, Hanseniaspora* sp., *H. osmophila, Saccharomyces bayanus, Cryptococcus saitoi* and *Crypt. friedrichii* were the most frequent yeasts isolated from these substrates. The osmotic stress associated with the nectar high sugar concentrations is probably a limiting environmental factor together with the presence of secondary compounds (Nicolson et al. 2007; González- Teuber & Heil 2009). Since, the low species diversity prevailing in nectar yeast communities so far studied could reflect a generalized environmental filtering. Very low nitrogen content, another characteristic feature of floral nectars (Nicolson et al. 2007), may be yet another factor limiting the suitability of floral nectars as habitats for yeasts other than highly specialized nectarivores. A combination of osmotolerance, tolerance or resistance to secondary compounds and efficient nitrogen use possibly allows these specialists to exploit floral nectar.

Another aspect of the interaction of yeasts and nectar-producing plants is related to the fermentation of nectar sugars by yeasts. In cool environments floral warming can benefit both the plants (e.g. by faster growth of pollen tubes) and the pollinators (by providing a heat reward), and yeasts can become important floral warming agents for plants living in shady forest, which are unable to use direct sunshine to warm their flowers. Floral warming by yeasts and the attractiveness provides an example whereby yeasts in nectar could under some circumstances benefit plants, pollinators or both. Also, the abundant alcohol accumulating in the nectar of a tropical palm as a consequence of yeast metabolism may ultimately enhance the attractiveness of inflorescences to alcohol-seeking mammalian pollinators (Wiens et al. 2008).

Decaying fruits are an important microhabitat for several yeast species (Morais et al. 2006; Starmer & Lachance, 2011). These ephemeral substrates are among the most important sites of oviposition and sources of nutrition for larval and adult stages of insects, which vector the yeasts to new substrates (Ganter, 2006; Morais et al., 2006). Yeast communities on fruits of one development stage turn out to be more similar when they are located closer to each other. The similarity of neighboring groups of fruit and on neighboring trees depends cell migration and cross-contamination of fruits with yeast cells. So, distinctions in the yeast community structure in different geographical regions can be explained by differences in the conditions of their formation (Slávikova et al., 2009). Evidently, propagation through cell transfer should play an important role in formation of microbial groups on accessible substrata during a limited period of time, such as juicy fruits, flower nectar, animal excrement, etc. In works on yeast ecology, it was suggested that contamination plays the initial role in formation of the specific structure of yeast in such communities, in particular, directed phoretic transportation of yeast cells to invertebrates (Morais et al. 2006; Starmer & Lachance 2011).

### 3.3 Soil yeasts

Soil has been studied as a source of yeasts because of its importance in ecosystem processes (Starmer & Lachance 2011). Yeasts have been isolated from different types of soils in diverse climatatic regions (Botha 2006; Cloete et al. 2009; Vaz et al. 2011). Most studies have characterized the occurrence of yeast species, suggesting that these microorganisms are minor contributors to soil ecological processes such as carbon recycling and mineralization (Botha, 2006; Starmer & Lachance, 2011). Yeasts occur mainly in the upper surface of soil rich in organic compounds provided by the decomposition of plant materials. Typical soil yeasts include species of *Cryptococcus*, *Debaryomyces*, *Lindnera*, *Lipomyces*, *Rhodotorula* and *Schizoblastosporion* (Botha 2006, Cloete et al. 2009; Starmer & Lachance, 2011; Mestre et al. 2011, Vaz et al. 2011).

Some yeast species are associated with rhizospheric soils and can produce polyamines, such as cadaverine and spermine that could impact upon root growth (Cloete et al. 2009). The yeasts *Rhodotorula mucilaginosa, Cryptococcus laurentii* and *Saccharomyces kunashirensis* were able to produce soluble and volatile exudates that stimulated the percentage spore germination and hyphal growth of the arbuscular mycorrhizal fungus *Glomus mosseae* (Sampedro et al. 2004). Alonso et al. (2008) reported the presence of yeasts tightly associated with spores of an isolate of *G. mosseae*. These yeasts were able to solubilize low-soluble P sources (Ca and Fe phosphates) and accumulate polyphosphates. Results from inoculation experiments showed an effect of the spore-associated yeasts on the root growth of rice, suggesting potential tripartite interactions with mycorrhizal fungi and plants (Alonso et al. 2008).

Cloete et al. (2010) studied the role of rhizosphere yeasts as plant nutrient-scavenging microsymbionts in roots of a medicinal sclerophyll, *Agathosma betulina*, grown under nutrient-poor conditions, and colonized by *Cryptococcus laurentii*. The average concentrations of P, Fe and Mn were significantly higher in roots of yeast-inoculated plants, compared to control plants that received autoclaved yeast. According to the authors it was the first report describing the role of soil yeast as a plant nutrient-scavenging microsymbiont. These results suggest the potential of yeasts to improve the nutritional quality of soils for plant growth, although occurring in small numbers when compared to bacteria and filamentous fungi.

## 4. Case studies of biodiversity in natural ecosystems and human-related environments

### 4.1 Report of two novel species found in Ecuador: *Candida carvajalis* and *Saturnispora quitensis*

Ecuador is located between 1°N and 5°S on the west coast of South America. Although relatively small in size, mainland Ecuador can be subdivided nevertheless into three different and quite distinctive climatic regions: the Pacific coastal plain, the Andean highlands and the Amazon basin. In addition, Ecuador possesses a fourth region, namely the Galapagos Islands.

Climatically, the Pacific coastal plain is hot all year, with a rainy season between December and May. In the Andean highlands, the climate is markedly cooler, varying according to altitude. In contrast, the Amazon basin is hot, humid and wet all year round, while the Galapagos Islands are dry, with an annual average temperature of 25°C (77°F).

To date, very little is known about the natural yeast diversity that exists in Ecuador. In an attempt to begin addressing this scientific shortfall, and to gain a better insight into the effectsof contrasting habitats and climate variation on yeast species distribution, a survey was recently set up and initiated by the Colección de Levaduras Quito Católica (CLQCA) in Quito.

The aim of the project is to catalogue, characterise and compare the indigenous yeast species found in the different ecological habitats of the four (climatic) regions of Ecuador.

Several novel species have been found since 2006, two of them are already described. In this chapter we will be referring to these two contributions to science (James et al, 2009).

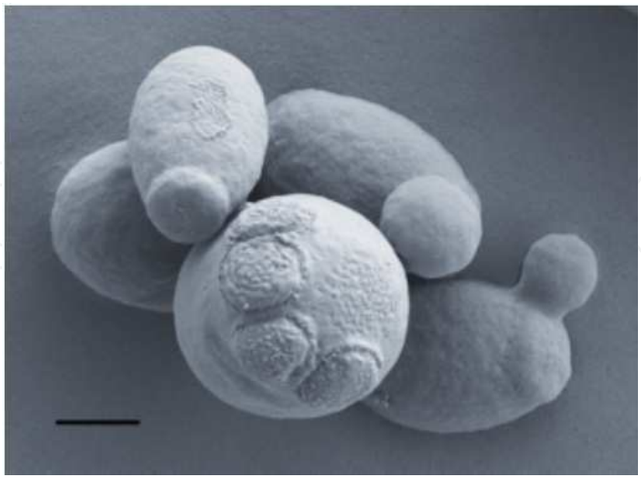
#### 4.1.1 *Candida carvajalis* sp.nov. an ascomycetous yeast species from the Ecuadorian Amazon jungle

This yeast species was isolated from rotten wood and fallen leaf debris collected at separate sites in the central Amazonian region of Ecuador. Phylogenetically, this species belongs to the *Clavispora* clade and is closely related to *Candida asparagi*, *Candida fructus*, *Candida musae* and two as yet undescribed *Candida* species, with the six taxa collectively forming a distinct species group. The phylogenetic placement of this species, coupled with the fact that it could not be induced to sporulate in pure or mixed cultures on several media, led to the conclusion that these yeast isolates belong to a novel species of *Candida* (James et al, 2009).

##### 4.1.1.1 Description of *Candida carvajalis* sp. nov.

*Candida carvajalis* – this Latin-derived epithet refers to Enrique Carvajal, father of Enrique Javier Carvajal Barriga (director at CLQCA). Although not a biologist himself, his passion for nature has nevertheless led him to become an active collaborator in the search for novel

yeast species in Ecuador. He collected these as well as other yeasts while on a number of field trips to the central Amazonian region of Ecuador (Car.va.jal.is). Figure 1.



(Image courtesy of Kathryn Cross, IFR)

Fig. 1. Scanning electron microscopic image of vegetative cells of *Candida carvajalis*strain CLQCA 20-011$^T$ grown in YM broth for 1 day at 25°Cwith agitation. Scale bar = 1 µm.

On YM agar, after 2 days at 25°C, cells are spheroidal to ovoidal (3–7 to 4–8 µm), and occur singly, in pairs or in groups. Budding is multilateral. No sexual state is observed from mixed or pure cultures plated on corn-meal agar, Gorodkowa agar, potassium acetate agar, PDA and YM agar. Pseudohyphae are formed (but only in CLQCA-20-011$^T$), but true hyphae are not formed. The type strain is CLQCA 20-011$^T$, isolated from rotten wood, collected near the town of Dayuma, in the central Amazonian region of Ecuador. Cultures of the type strain and CLQCA 20-014 have been deposited with the CLQCA, Quito, Ecuador, and the National Collection of Yeast Cultures (NCYC), Norwich, UK (CLQCA 20-011$^T$ as NCYC 3509$^T$ and CLQCA 20-014 as NCYC 3508). The type strain has also been deposited with the CBS, Utrecht, the Netherlands, as CBS 11361$^T$.

### 4.1.2 *Saturnispora quitensis* sp. nov., a yeast species isolated from the Maquipucuna cloud forest reserve in Ecuador

During a pilot study to survey the yeast diversity found in the Maquipucuna cloud forest nature reserve, located 50 miles northwest of Quito, in Ecuador, CLQCA-10-042$^T$ was isolated together with more than 70 other yeast strains. Sequence analysis of the D1/D2 domain of the LSU rRNA gene identified the isolates as belonging to 26 different species of the genera *Barnettozyma* (1), *Candida* (6), *Hanseniapora* (2), *Lachancea* (1), *Lodderomyces* (1), *Metschnikowia* (2), *Pichia* (3), *Rhodotorula* (1), *Saccharomyces* (1), *Saturnispora* (1), *Trichosporon* (2), *Wickerhamomyces* (3) and *Yarrowia* (1). Strain CLQCA-10-042$^T$ was isolated from the fruit of an unidentified species of bramble (*Rubus* sp.), and based on its physiology and ability to produce saturn-shaped ascospores was identified as representing a *Saturnispora* species (Kurtzman, 1998). Subsequent sequence analyses of the LSU D1/D2 domain and ribosomal ITS region established that this strain belongs to a genetically distinct and hitherto un-described species closely related to *S. hagleri*. The novel species is named as *Saturnispora quitensis* sp. nov., in recognition of the location in Ecuador from where it was first found.

The yeast genus *Saturnispora* is characterised by teleomorphic species that typically produce one to four spheroidal ascospores ornamented with an equatorial ledge (i.e. saturn-shaped) and have a fairly restricted physiological profile (Kurtzman, 1998). The genus is well-supported by phylogenetic analyses based on multigene sequence analysis of the small-subunit (SSU) and large-subunit (LSU) rRNA genes, and translation elongation factor-1α (EF-1 α) gene (Kurtzman *et al*., 2008). At present, the genus comprises of seven teleomorphic species, *Saturnispora ahearnii*, *Saturnispora besseyi*, *Saturnispora dispora*, *Saturnispora hagleri*, *Saturnispora mendoncae*, *Saturnispora saitoi*, *Saturnispora zaruensis, S. serradocipensis* and *S. gonsigensis* (Morais *et al*., 2005; Kurtzman *et al*., 2008). Six anamorphic species, *Candida diversa*, *Candida sanitii*, *Candida sekii*, *Candida siamensis*, *Candida silvae* and *Candida suwanaritii*, are also accommodated within the genus (Kurtzman *et al*., 2008; Boonmak *et al*., 2009; Limtong *et al*., 2010). Collectively, these yeasts have been isolated from a wide variety of different sources and habitats including *Drosophila* flies (*D. cardinae* and *D. fascioloides*), estuarine water from mangrove forest, flowers, forest soil, insect frass, marsh water, rhizosphere of oyster grass, sauerkraut, tree bark and tree exudate (*Quercus* spp.), and wild mushroom (*Hygrophorus* sp.) (Liu & Kurtzman, 1991; Kurtzman, 1998; Morais *et al*., 2005; Boonmak *et al*., 2009; Limtong *et al*., 2010)

From an ecological perspective, *S. quitensis* is most similar to *S. hagleri*, with both species being found in neotropical regions; *S. hagleri* isolated from two different species of *Drosophila* (*D. cardinae* and *D. fascioloides*) collected in an Atlantic rainforest site in Brazil, and *S. quitensis* from a bramble fruit collected in a cloud forest site in Ecuador (0°03′09′′ N; 78°41′06′′ W; 1668 m.a.s.l). In their species description, Morais *et al*. (2005) noted that of the six identified *S. hagleri* strains, four were recovered from the crops of *D. cardinae*. This led the authors to suggest that this yeast may colonize tropical fruits and substrates regularly visited by these flies and utilised as a food source. To date, only a single strain of *S. quitensis* has been isolated. However, it seems plausible to suppose that like *S. hagleri*, additional strains of *S. quitensis* could, in future, be isolated from *Drosophila* flies and other insects which visit and feed upon tropical fruits found in neotropical regions like Maquipucuna.

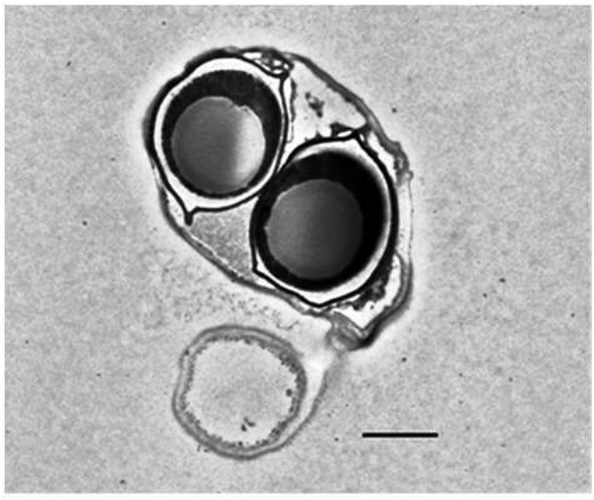
#### 4.1.2.1 Description of *Saturnispora quitensis* sp. nov.

*Saturnispora quitensis* – The specific epithet *quitensis* refers to Quito, the capital of Ecuador, near where this strain was isolated (Qui.ten.sis).

Cells are spheroidal to ovoidal (4-7 x 5-8 µm) and occur singly or in groups after growth in YM broth for 2 days at 25°C. Budding is multilateral. Sediment is formed after 1 month, but no pellicle is observed. Pseudomycelia or true mycelia are not formed. After 8 days on agar media with a low nitrogen/carbon ratio (i.e. yeast carbon base with 0.01% ammonium sulphate), conjugated cells give rise to asci containing one to two spheroidal ascospores ornamented with an equatorial ledge (i.e. saturn-shaped) Figure 2. Ascospores are not liberated. Conjugation takes place between individual cells, and more commonly between cells and their buds.

### 4.2 Yeast species described in Argentinean environments

In recent years, numerous studies have demonstrated that Patagonian natural environments harbor a broad biodiversity of yeasts with high scientific and technological value (Brizzio & van Broock, 1998; Libkind et al., 2003, 2004a, 2004b, 2006, 2007, 2008a, 2008b, 2011a, Russo et al., 2006; de García, 2007; Brizzio et al., 2007). These studies have also shown that a large proportion of the yeast species recovered belong to undescribed taxa, in general 25 to 40



(Image courtesy of Kathryn Cross, IFR)

Fig. 2. Transmission electron micrograph of a single ascus containing two ascospores, one of which is ornamented with an equatorial ledge. Scale bar=1 μm.

percent of the species obtained from a certain substrate represent novel species. This is a clear indication of the importance of conducting bioprospection studies in microbiologically unexplored habitats of Patagonia. To date, ten novel yeast species have been formally described from Patagonian natural environments and at least 20 additional undescribed taxa have been found (Libkind et al., 2005a; 2009a; 2010a; de García et al., 2010a; 2010b; Russo et al., 2010; Wuczkowski et al., 2010). Only a few salient cases are discussed here in order to show the importance of microbial surveys in unexplored habitats.

The first formal description of Patagonian autochthonous yeasts regarded two carotenoid-accumulating yeasts (also known as red yeasts) that had the ability to produce forcefully-ejected spores (ballistoconidia). These yeasts belonged to the Sporidiobolales order of the Pucciniomycotina sub-phyllum (Basidiomycota) and were described as *Sporobolomyces patagonicus* and *Sporidiobolus longiusculus* (Libkind et al., 2005a). The sexual stage (teleomorph) of *S. longiusculus* was detected and had a particular micromorphological feature: teliospore germination gave rise to an elongated basidium, which was five to six times longer (120–275um) than those of the other member species of the genus *Sporidiobolus* (Libkind et al., 2005a). Even though all known strains of both species were collected from subsurface water of Andean lakes, their suspected primary habitat is the surrounding phylloplane, probably of *Nothofagus* spp. trees.

A similar case was that of *Cystofilobasidium lacus-mascardii*, a teleomorphic species of the Cystofilobasidiales, class Agaricomycotina (Basidiomycota) of which a single isolate was first obtained from subsurface waters of the Mascardi lake (Libkind et al., 2009a). Once it was recognized as an undescribed species, attempts to obtain additional isolates using specifically designed culture media for selective isolation were performed. Thus, new isolates were found and they happened to mate with the original isolate providing the opportunity to describe its sexual stage. Again, terrestrial environments are more likely to be the habitat of this yeast species based on its low relative occurrence in freshwater and its ability to produce a wide range of extracellular enzymes (Brizzio et al., 2007).

Another interesting case is that of *Cryptococcus agrionensis*, a novel anamorphic yeast of the Filobasidiales (Agaricomycotina, Basidiomycota) associated with acidic aquatic

environments of volcanic origin in North Patagonia. Due to the high acidity, these waterbodies also contain high concentrations of toxic metals, and thus poly-extremophile microorganisms prevail. More than seventy *Crypt. agrionensis* strains were isolated, mainly from the most acidic section of the river Agrio with a pH ranging from 1.8 to 2.7 (Russo et al., 2010). More interesting was the fact that *Crypt. agrionensis* was phylogenetically related to three *Cryptococcus* species that constitute what has been described as the Acid Rock Drainage (ARD) Ecoclade (Gadanho & Sampaio, 2009). The term 'ecoclade' refers to species that are related phylogenetically and show salient physiological adaptations associated with the physicochemical conditions present in their habitats. The ARD ecoclade (including *C. agrionensis*) have a peculiar ecology and physiology: They are only known from acidic environments and are highly resistant to heavy metals such as Cd2+, Co2+, Cu2+, Li+, Ni2+ and Zn2+. The discovery of *Crypt. agrionensis* in acidic water of volcanic origin provided evidence that the ARD ecoclade was not restricted to abandoned mines of the Iberian Pyrite region (origin of the previously known species) and demonstrated that members of this ecoclade may be found in acidic environments in general, originated both naturally and anthropically.

During our yeast biodiversity survey in the Argentinean Patagonia we came upon isolates of *Phaffia rhodozyma* (sexual form, *Xanthophyllomyces dendrorhous*), a yeast that belongs to the Cystofilobasidiales order (Class Agaricomycotina, Basidiomycota). This yeast has the ability to produce astaxanthin, a carotenoid pigment with biotechnological importance because it is used in aquaculture for fish and crustacean pigmentation (Rodríguez-Sáiz et al., 2010). Known isolates of this species had been found in exudates of trees of the genera *Betula*, *Fagus* and *Cornus* in the Northern Hemisphere, mainly at high altitudes and latitudes. We isolated *P. rhodozyma*, from the Southern Hemisphere (Patagonia, Argentina), where it was associated with fruiting bodies of *Cyttaria hariotii*, an ascomycetous parasite of *Nothofagus* trees (Libkind et al., 2007). The Patagonian population besides possessing a different habitat also showed distinct genetic features based on a detailed molecular comparison with known strains from the Northern hemisphere. However, the level of genetic divergence of the Patagonian population with respect to the remaining strains was within the intraspecific level. In addition by comparing the molecular phylogenies of *P. rhodozyma* populations with that of their tree host (Betulaceae, Corneaceae, Fagaceae, and Nothofagaceae), a good concordance was found which suggested that different yeast lineages colonize different tree species (Libkind et al., 2007). Hence, we hypothesize that the association of the Patagonian *P. rhodozyma* with *Cyttaria* derives from a previous association of the yeast with *Nothofagus*. This study provided a deeper understanding of *Phaffia* biogeography, ecology, and molecular phylogeny, knowledge essential to the study of astaxanthin production within an evolutionary and ecological framework.

The cases above, describing novel yeast species/populations, clearly illustrate the need to increase the efforts to further survey the micobiota of relatively unexplored habitats such as the emblematic Patagonia.

### 4.3 Yeast biodiversity in wineries, Distillerie plants and olive oil mills in La Mancha region (Spain)

Yeast ecosystems are used as raw materials in the food industry as well as in processing: the yeasts in grapes, musts and fermented musts in wineries, and in the piquettes, bagasse, grape-skins and lees used as feedstocks in the ethanol industry provide an inexhaustible supply of microorganisms.

La Mancha is the world's largest vine-growing region, with a surface area of around 600,000 hectares, i.e. roughly 50% of the country's table wine. Annual grape production, comprising entirely winemaking varieties, is around 3.6 million tonnes. This output generates roughly 600,000 tonnes of pomace or marc, produced by pressing the fermented red or white grapes which thus contain a certain amount of sugar. Pomace generally contains plant tissue residue: skin and pips from the pressing of red grapes, as well as stalks from pressed white grapes. The ratio of pomace output to grape production varies considerably, depending on the grape variety and on growing conditions. However, pomace is estimated to account for 17% of overall grape weight; within that figure, skin accounts for 8%, pips for 5% and stalks for the remaining 4%. Pomace and/or residual sugars are used as a feedstock for ethanol production.

### 4.3.1 Wineries

Traditional wine fermentation is a complex, heterogeneous microbiological process involving the sequential development of various yeasts and other microorganisms present in musts, such as moulds as well as lactic and acetic acid bacteria. However, it is accepted that certain strains of *Saccharomyces cerevisiae*, known as "wine yeasts", are especially well adapted to this process, and play a major role in the fermentation of grape musts; for that widely studied. Nonetheless, it is important to remember that upto 15 different genera of non-*Saccharomyces* yeasts may also be present at the start of the wine-making process, and these may contribute to the special characteristics of individual types of wine (Pretorious, 2000).

Although most wineries now use commercial starter cultures, it is usually to spend over 60 million tonnes of active dry yeasts (ADYs); nevertheless, spontaneous alcoholic fermentation, that is, fermentation carried out without the addition of commercial dry yeasts, is still typical for certain wine cellars in this wine-making area. This type of fermentation is of particular interest with a view to ascertaining the ecology of fermentation processes in respect to *Saccharomyces* and non-*Saccharomyces* yeast strains. In the case of the former yeasts, it is necessary to establish whether fermentation is carried out by one or several predominant strains or whether, in contrast, there is a succession of different strains over the course of wine-making.

Extensive ecological surveys using molecular methods of identification have been carried out with the aim of studying winery biodiversity and then selecting new yeasts better adapted to local fermentation conditions (Briones et al, 1996; Fernández-Gonzalez et al, 2001; Izquierdo et. al, 1997; Querol et. al, 1992), thus allowing the behavior of the various strains to be charted throughout fermentation.

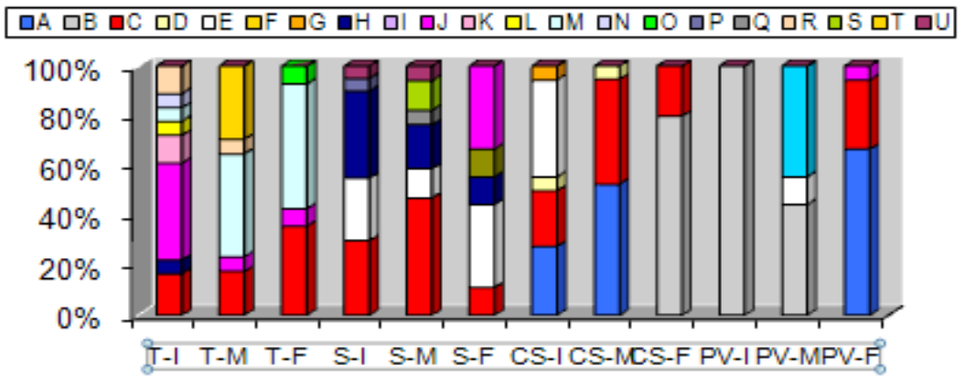
Non-*Saccharomyces* yeasts, which display low fermentative capacity and low ethanol tolerance could impart specific characteristics to the wines, and to enhance wine flavour by increasing concentrations of the volatile compounds responsible for the fruity aroma, through hydrolysis of aromatic precursors prompted by β-glucosidase enzyme activity (Arevalo-Villena et al, 2006;) or even for the production of volatile esters.

In the cellars of this area, during the early stages of wine-making there is substantial growth of non-*Saccharomyces* species. The main species found in different grapes varieties at the beginning of the process are *Candida stellata*, *Pichia membranaefaciens, Metschnikowia pulcherrima Hanseniaspora uvarum/guillermondii/osmophila, Kluyveromyces thermotolerans. Torulaspora delbrueckii* and *Debaryomyces hansenii* were isolated at the middle of the process, and *Lachancea fermentati (formerly Zygosaccharomyces fermentati)* were able to survive at the middle, or even until the end of fermentation.

The study of the enzymatic activities of non-*Saccharomyces* wine yeasts revealed that nearly 80% of the yeasts presented at least one enzyme of biotechnological interest. Polygalacturonase was the enzyme most commonly found and was secreted by 45% of the yeasts, whereas β-glucosidase was only observed in 14% of the yeasts. Proteolytic activity was also found in some species (Fernández-Gonzalez et al, 2000).

The analysis of restriction mitochondrial DNA is a suitable technique to study the biodiversity of *Saccharomyces* wine strains. With regard to the population dynamics of *Saccharomyces* strains, there was a greater variability of them at the start of fermentation; as fermentation progressed some initial strains were succeeded and displaced by others better adapted to environmental conditions; this succession of strains is common in spontaneous fermentation.

*Saccharomyces* biodiversity during vinification of the different red grape varieties is shown in Figure 3. Of the 21 genetic profiles identified, 10 contained more than four isolates. In some cases, as can be observed in the figure, the isolates are grouped in a dominant profile, i.e. 26% of isolates displayed the same profile (C), suggesting that this was a common genetic pattern in the winery; other patterns included 16% (A) or around 10% (E) of isolates, others 8% - 7%, and some of them accounted for around 2% or 3%, while the remainder contained only one or two isolates.




T: Tempranillo; S: Syrah; CS: Cabernet Sauvignon; PV: Petit Verdot. I: Beginning; M: Middle; F: End

Fig. 3. Genetic profiles of *Saccharomyces* wild strains in red grape varieties.

Some profiles were exclusive to certain varieties: profile C was isolated in abundance from all varieties; profile J was also present, though to a lesser extent, in almost all tanks; profiles A and B were characteristic of *Cabernet* and *Petit Verdot*, whilst M and T were typical of *Tempranillo,* and E and H of *Syrah*.

Other cellars showed a 65% of variability respect to a genetic patterns, found some of them repeated in some of the fermentation stages sampled. Substantial genetic differences were recorded, a customary finding for spontaneous fermentations representative for the studied region (Briones et al. 1996; Izquierdo et al. 1997).

In a study carried out in a single winery, situated around 1000 m.a.s.l. and whose wines have high quality, the sampling was done in 11 both white and red fermentations of different grape varieties (Chardonnay, Sauvignon Blanc, Cabernet sauvignon, Tempranillo, Merlot, Syrah) collecting a total of 28 samples at different stages of fermentations. The molecular analysis methods led to the determination of 23 different *Saccharomyces* mtDNA restriction patterns from 358 isolates. The degree of variability was a good parameter with

which to evaluate the number of strains actively involved in fermentation. The variability average found in this study (6.4%) was similar to those from previous studies: 8.6% (Querol *et al*., 1994); 2.2 to 4.2% (Schütz and Gafner, 1994) and clearly lower than corresponding results from 32, 42, 38% and 23, 23, 22% in three different cellars and two consecutive years (Izquierdo et al. 1997), 22% (Torija et al. 2001) and 20.7% (Nadal et al. 1996).
The majority pattern found cluster the 56% of the isolated ones, followed of others one with a 15% and a 9%. Four restriction patterns were about 3% and the rest of the patterns whose presence was limited to one or two isolates. The main pattern was isolated in all sampled vats and in all grape varieties, both white and red; that situation is not frequently in this viticulture area, where the *Saccharomyces* biodiversity is high as recorded previously by Briones et al. (1996). In three different cellars, selected at random a large number of *S. cerevisiae* strains appeared with either the same, or a very similar, karyotype, indicating that they are strains highly characteristic of these wineries.

### 4.3.2 Distillery plants

Thirty-three distilleries in Spain are licensed to produce ethanol from winemaking by-products. Thirteen of these are located in the region of La Mancha.
During harvest, fermented and "fresh" pomace (from white-wine vinification) is transported to the ethanol plant, where it is mixed and stored (generally outdoors) for between 10 and 15 days; during this period, "fresh" pomaces start to ferment. After, pomaces are washed to extract ethanol. The liquid produced by this process is known as "piquette", a mixture of alcohol (3º-4º), water and sugar; the piquette is mixed with the liquid drained off during outdoor storage-fermentation.
The piquette is fermented in stainless-steel or iron tanks for two or three days, attaining an alcohol content of between 4º and 5º (V/V). Although a few ethanol plants use active dry yeasts, fermentation is mostly spontaneous; this gives rise to a highly- varied *Saccharomyces* and non- *Saccharomyces* biota, as discussed below.
To date, little research has been carried out on the yeast biodiversity found in Spanish grape-based ethanol plants. A study of yeast populations in ethanol plants and distilleries in La Mancha sought to determine yeast biodiversity at various sites, with six different ethanol plants studied (subsequently referred to as plants A-F). *Saccharomyces* strains predominated in all ethanol plants studied; the proportion of non-*Saccharomyces* strains ranged from 14% to almost 47%. The 144 *Saccharomyces sp.* isolates matched 105 different genetic profiles; 46 profiles were from fresh piquettes, 43 from fermented piquettes and 16 from lees. In all samples and all plants, variability exceeded 50%; in five cases, variability was higher than 80%.
Fresh piquettes displayed considerable strain diversity; variability was almost 90% at plant B, and 81% at plant C. A total of 46 genetic profiles were found, 45 of which were different, while one – although infrequent (4%) – was isolated at plants A and C. Only one majority profile accounted for 22% of the yeasts isolated at plant C. Most strains were *Saccharomyces cerevisiae,* with only a small number of *S. paradoxus* and *S. bayanus* strains.
In fermented piquettes, biodiversity was greater than in fresh piquettes, and at three plants (B, D and E) different strains accounted for over 85% of the total. Plant A displayed the least genetic diversity. Piquettes at plants B and C contained negligible amounts of *S. paradoxus* and *S. bayanus,* respectively.
Lees obtained from piquette fermentation displayed less *Saccharomyces* strain diversity than either fresh or fermented piquettes. Isolates fitted 15 different genetic patterns. While a 78% strain variability was observed at plant C, lees sampled at plants A and B displayed only

50% and 57% diversity, respectively. Although patterns tended to be typical of each plant, majority profiles accounted for 57% of isolates at plant B, 33% at plant C and 30% at plant A. The greatest degree of *Saccharomyces* variability was found for fermented piquettes, although several strains co-existed in both lees and fresh piquettes. These results confirm that, whilst genetic diversity in wineries has declined considerably due to the increasingly widespread use of commercial starter cultures, *Saccharomyces* variability in ethanol plants remains considerable.

With respect to non-*Saccharomyces* yeasts, the largest percentage (49%) was found in fermented piquettes, even though the ethanol concentration varied between 4º - 5º (V/V). A total of 41% of non-*Saccharomyces* strains were isolated in fresh piquettes, and only 10% in lees. The greatest species diversity was observed in fresh and fermented piquettes, the most frequently- isolated species being *T. delbrueckii* and *C. silvae,* respectively.

Only *Pichia kudriavzevii* (formerly *Issatchenkia orientalis*) was isolated in all three types of source. *Kluyveromyces thermotolerans* and *Wickerhamomyces anomalus* (formerly *Pichia anomala*) were isolated in both fresh and fermented piquettes, while *Candida ethanolica* was isolated in fresh piquettes and lees. The remaining species were isolated in only one type of source at the various ethanol plants.

A number of species, including *Hanseniaspora. vineae, P ichia kudriavzevii* and *Torulaspora delbrueckii,* have been reported in white-wine pomace or marc used for the production of grappa (Bovo et al., 2009) while *Zygosaccharomyces bailii* and *Saccharomycodes ludgiwii* have been identified, in smaller numbers, in agave fermentation for tequila production (Lachance, 1995).

### 4.3.3 Olive oil mills

Olive-fruit spontaneous microbiota comprises non-*Saccharomyces* yeasts, lactic acid bacteria (LAB) and filamentous fungi. From other research it is known that during the olives fermentation the presence of yeasts may produce compounds with suitable organoleptic attributes determining the quality and flavour of the final product (Arroyo-López et al., 2008). However the olive oil production is also important and there are few references in the literature about yeast biodiversity present in both fresh olives intended for oil production and their sub-products. Giannoutsou et al. (2004) suggested that "alpeorujo" is a good substrate for yeast growth which could be used as a feed additive, as a fertilizer in crops or as a substrate for the growth of edible mushrooms.

La Mancha is the second largest olive growing region in Spain (350,000 ha) and a major olive oil producer. No previous studies have dealt with yeast populations in local olives, nor in the by-products of olive processing, i.e. paste and pomace. Olive fruits from two varieties of *Olea europaea L.* (Arbequina and Cornicabra) were randomly picked at various olive groves; likewise, olive paste and olive pomace were also collected from different oil mills.

Fourteen different species of yeasts were identified, belonging to eight different genera (*Zygotorulaspora, Nakazawaea*, *Pichia, Lachancea, Kluyveromyces, Saccharomyces, Candida* and *Torulaspora*), thus demonstrating considerable species diversity. In fresh olive fruits, yeasts were largely outnumbered by moulds and bacteria probably due to the fact that they had not been processed (i.e. were collected straight off the tree), so the only contribution was the environmental biota. Although a similar number of isolates were obtained from paste and pomace, the latter displayed greater species diversity, with 11 different species identified. Some species were typically found in olive paste (*Nakazawaea holstii, Pichia. mississippiensis* and *Lachancea sp*.), whilst *S. cerevisiae*, *Kazachstania rosinii* (formerly *Saccharomyces rosinii*),

*Candida sp.* and *C. diddensiae, Zygotorulaspora florentinus* (formerly *Zygosaccharomyces florentinus*) and *Torulaspora delbrueckii* were found only in pomace.

The species most commonly isolated in the Cornicabra variety was *Pichia holstii* (39%), followed by *Lachancea fermentati* (25%), whilst the predominant species in Arbequina was *Pichia caribica* (59%) followed by *Lachancea fermentati* (23%). The remaining 11 species did not exceed 8% in either variety. *Candida diddensiae* was found in Arbequina olive variety, and similar results were obtained by Hurtado et al. (2008) who also isolated this species in Arbequina fruits. *Nakazawaea holstii* (formerly *Pichia holstii*) and *Lachancea fermentati* are yeasts associated with wastewater from continuous olive mills in Southern Italy and Spain (Barnett et al., 2000), while *Meyerozyma caribbica* (formely *Pichia caribbica*; anamorph: *Candida fermentati*) is involved in artisanal cachaca fermentation in Brazil and is found in soils in China (Barnett et al., 2000). The other species isolated are found chiefly in soils, although *C. diddensiae* has also been reported in olives in Italy.

All species isolated were fermentative to a varying degree. *S. cerevisiae* was a striking finding in this respect, and might represent a potential spoilage organism during olive oil storage. However, this should not be a problem since none of these yeasts have lipolytic activity.

*Lachancea* genus was isolated from olive paste of both varieties. This new genus was formed on the basis of five species; *L. thermotolerans* was chosen as type species and has been isolated from mushrooms, flowers, leaves and oil wastewaters (Naumova et al., 2007).

Biodiversity was greater in olive by-products than olive fruits, and greater in Cornicabra than in Arbequina (11 species vs. 6). Three species were common to all olive fruits and both by-products (*Lachancea fermentati, M. caribbica, Lachancea* sp.).

*Candida spp*. were isolated from olive paste (Torres-Vila et al., 2003), other authors have isolated yeasts in olive fruits and brines during fermentation process, including *T. delbrueckii*, *Candida boidinii*, *Cryptococcus spp*., *Wickerhamomyces anomalus*, *Kluyveromyces marxianus* (Marquina et al., 1992; Coton et al., 2006; Hernández et al., 2007).

With regard to yeast biodiversity in oil mills, species distribution was very much dependent upon the oil mill plant. In some mills, 4 to 5 different species were identified, whereas in others (4 mills) only 2 species were isolated. *N. holstii* was isolated in all samples from Cornicabra except in one, and was not detected in the oil mills from Arbequina. Nevertheless *Lachancea fermentati* was present in the majority of mills.

Our work also shows the potential of these strains isolated from olive by-products, i.e. olive paste and pomace, suggesting that these olive wastes can also be used for industrial biotechnological purposes, for the production of enzymes, commercial preparations or fermentative processes in different industry sectors.

Characterization of these resources can also contribute to the development of a microbial bank, providing data on technological properties and enzyme characteristics for potential industrial applications. On the other hand, the quality and yield in olive oil extraction may be influenced by the presence of some yeasts with high or moderate enzymatic activities such as lipases, glucanases, cellulases, glucosidases or polygalacturonases.

## 5. The role of yeast culture collections: Preservation, applications and challenges

### 5.1 The Catholic University yeast Collection (CLQCA)

The CLQCA, or Catholic University Yeast Collection, was originally set up to carry out a survey of environmental biodiversity of yeasts in Ecuador in 2006, as a pioneer bioprospecting study. Over the last five years this collection has developed a diverse range

of techniques in order to identify and characterize yeast biodiversity. This collection has had agreements and tight collaboration with other collections such as the National Collection of Yeast Cultures (NCYC) in the United Kingdom, and the UFMG yeast collection in Brazil.
Currently, this yeast collection is the only one of its kind in Ecuador, and one of the few in South America. To date, more than 15 novel yeast species have been isolated and three of them have already been formally described and published (*Candida carvajalis*, *Saturnispora quitensis*) in collaboration with the NCYC (UK). More than 2000 yeast isolates from the 24 provinces of Ecuador have been preserved at the CLQCA. One of the most important surveys related to biodiversity was carried out in 2008 in the Galápagos Islands, where more than 800 isolates were collected from four different islands. Other Ecuadorian environments such as the high Andes, the Amazonia, and the Pacific Coast have also been sampled.
Approximately 1/3 of the isolates are already identified by ribosomal DNA (rDNA) sequencing and/or RFLP-ITS method. So far, the predominant species registered are *Candida tropicalis* (142 strains) and *Saccharomyces cerevisiae* (100 strains). However, yeasts from *Hanseniaspora*, *Pichia*, *Rhodotorula* and other genera are also well represented, as shown in Figure 4.
The CLQCA is not only a bank for the yeast biodiversity, but a biotechnology exploitation platform, where several projects are being carried out. Some of the most important ones are focused on second generation bioethanol production, biocontrol of molds, microbial archaeology and beer production.

### 5.2 Yeast culture collection in Patagonia

The CRUB (Centro Regional Universitario Bariloche) yeast collection is a research culture collection kept at the Applied Microbiology and Biotechnology Lab. which is held at the Biodiversity and Environmental Research Institute (INIBIOMA, CONICET-UNComahue) in Bariloche, Argentina (Northwestern Patagonia). Certainly is the most southern culture collection devoted to the preservation of native yeasts. Its collection derive from studies of yeast diversity in Patagonian natural substrates that have been mainly focused on environments with extreme conditions which impose a selective pressure towards the prevalence of adapted microorganisms with innovative physiological characteristics that can be biotechnologically relevant. Extreme environments such as glacial ice and meltwater (de Virginia et al., 2007), and acidic waterbodies of volcanic origin that have high concentrations of toxic metals (Russo et al., 2008) are being studied. Many strains have been proved to be interesting as producers of psycro-enzymes (de Virginia et al., 2007; Brizzio et al., 2007; Brandao et al., 2011), poly-unsaturated fatty acids (Libkind et al., 2008b) or because of their tolerance to heavy metals (Russo et al., 2010). However, the studies in Patagonia have been mostly concentrated in environments exposed to increased UV radiation (UVR) such as transparent mountain lakes or the phylloplane of high altitude forests. Yeasts adapted to high UVR exposure have shown to produce large quantities of photoprotective compounds (PPC) which are necessary to reduce the detrimental effect of the damaging wavelengths of UVR (Libkind et al., 2006). The synthesis of metabolites that have antioxidant and/or UV screening activities are among the strategies commonly seen in yeasts for photoprotection.

## 6. Biodiversity and biotechnology

Yeasts biotechnology is a growing field where novel species and its physiological abilities are potentially useful in the search of new products by means of the metabolic engineering

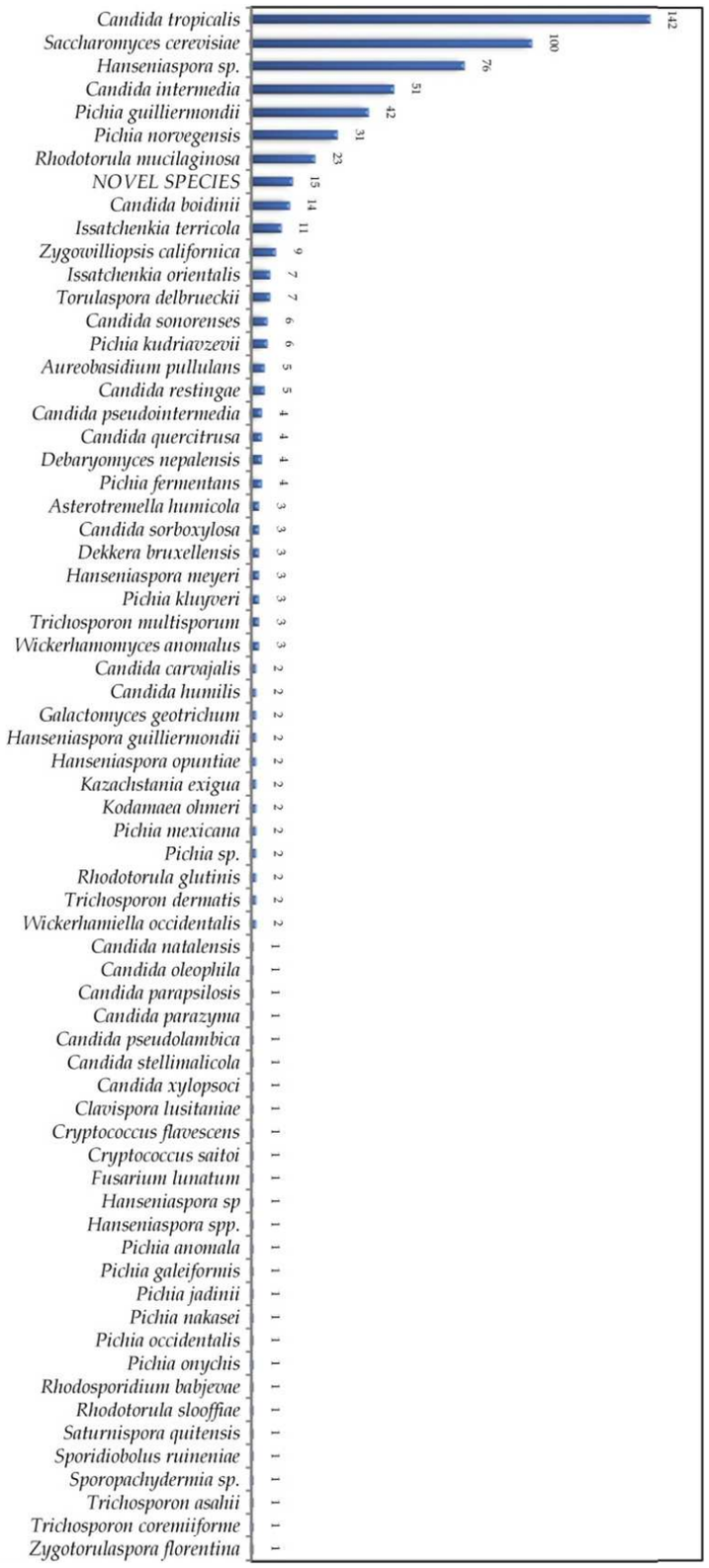



Fig. 4. Number of identified yeast isolates preserved at CLQCA up to 2011.

approach and the application of novel species for industrial production, not only as fermenting organisms or molecules producers, but as sources of molecules that can be purified from the structures of the yeast cell. As an example we can talk about β-glucans and mannans from cell walls that are being used as food additives for animal feed. Other examples are the partially hydrolysate yeast cells used in animal feed as well with desirable results in terms of weight and health improvements.

Biodiversity of yeasts is being studied not only to catalogue life on Earth; one of the most promising fields related to the characterization and identification of yeast diversity is related to the potential use of them in producing novel enzymes and chemicals. Psycrophilic yeasts from Antarctic substrates as well as those from high altitudes or glaciers are potential work horses in biotechnology industry to produce the breakdown of xenobiotics and pharmaceutical novel variations of molecules. Lipases from *Pseudozyma antarctica* have been extensively studied to understand their unique thermal stability at 90°C and also because of its use in the pharmaceutical, agriculture, food, cosmetics and chemical industry. Other enzymes which have been studied include extracellular alpha-amylase and glucoamylase from the yeast *Pseudozyma antarctica* (*Candida antarctica*), an extra-cellular protease from *Cryptococcus humicola*, an aspartyl proteinase from *Cryptococcus humicola*, a novel extracellular subtilase from *Leucosporidium antarcticum*, and a xylanase from *Cryptococcus adeliensis* (Shivaji and Prasad, 2009).

Other common use of the yeast biodiversity—as part of microbial communities—is in the bioremediation of oil spills. Yeasts are able to use various petroleum components as sole carbon source, showed that their biodegradability decreases from n-alkanes to high molecular weight aromatic and polar compounds. The alkanes are mainly degraded using the monoterminal oxidation pathway through cytochrome P450 system, and transformed into fatty acids with the same length of the carbon chain. Extensive studies showed that there are more than 80 genes involved in obtaining the alkane specific phenotype. Up to date, about 14 different genera of yeasts have been reported to consume hydrocarbons, exhibiting bioremediation potential uses. The abovementioned genera are: *Candida,Clavispora, Debaryomyces, Leucosporidium, Lodderomyces, Metschnikowia, Pichia, Rhodosporidium, Rhodotorula, Sporidiobolus, Sporobolomyces, Stephanoascus,Trichosporon* and *Yarrowia* (Mauersberger, 1996; Scheller, 1998).

Yeast can be used in foods and chemical production as they were probably one of the first organisms domesticated by humankind. Production of wine, beer and bread are three examples of the importance of yeasts in human nutrition and culture. More and more applications for yeast will arise in the next future. The diversity of yeast had been the answer to fulfill human necessities in the early times of civilization, and, undoubtedly, it is going to continue being a source of new solutions in the future.

### 6.1 From biodiversity to biotechnology: the case study of photoprotective compounds

Carotenoids are a group of valuable molecules for the pharmaceutical, chemical, food and feedindustries, not only because they can act as vitamin A precursors, but also for their antioxidant and possible tumour-inhibiting activity (Johnson & Schroeder, 1995). Many yeasts accumulate a variety of carotenoid pigments intracellularly are commonly known as carotenogenic or red yeast. Red yeasts were found to occur widely in aquatic environments in Patagonia, and many pigmented strains of the carotenogenic genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, *Sporidiobolus*, *Dioszegia*, *Cystofilobasidium* and *Xanthophyllomyces*

were isolated and are kept at the CRUB collection. These new yeast isolates from Patagonian habitats were studied for the production of biomass and carotenoids as the first step towards the selection of hyper-producing strains and the design of a process optimization approach. Patagonian yeast isolates considered as potential biomass and carotenoid sources were studied using conventional media or semi-synthetic medium employing agro-industrial byproducts (cane molasses, corn syrup, raw malt extract) as carbon sources (Libkind et al., 2004a; Libkind & van Broock, 2006). Maximum pigment production (400 ug $g^{-1}$ cell dry weight) was achieved after optimization through a factorial design with the yeast *Cystofilobasidium lacus-mascardii*. β-carotene, torulene and torularhodin were the major carotenoids found in most yeasts (Buzzini et al., 2006; Libkind & van Broock, 2006). The exceptions were *Phaffia rhodozyma* strains which produced the biotechnologically relevant pigment astaxanthin (Libkind et al., 2008a). Moreover, photobiological studies were performed that demonstrated the photoprotective role of these carotenoid pigments in yeasts (Moliné et al., 2009), in particular torularhodin in the ubiquitous yeast *Rhodotorula mucilaginosa* (Moliné et al., 2010). Thus, the CRUB collection represents an interesting source of carotenogenic yeast strains already characterized regarding their biomass and carotenoid production performance at Lab scale and providing a variety of pigments for diverse applications.

In contrast to carotenoid pigments, Mycosporines, are water soluble UV-absorbing (310–320 nm) and very less known. They are compounds containing an aminocyclohexenone unit bound to an amino acid or amino alcohol group (Bandaranayake, 1998). Although mycosporines were initially discovered in fungal sporulating mycelia (Leach et al., 1965), it was not until recently that their synthesis was reported in yeasts by us (Libkind et al., 2004b). A number of basidiomycetous carotenogenic yeasts were found to synthesize a UV-absorbing compound (peak absorption at 309–310 nm) when grown under photosynthetically active radiation (PAR, 400–750 nm). The compound was afterwards identified as mycosporine-glutaminol-glucoside (MGG) (Sommaruga et al., 2004). More recently the MGG was confirmed as a photoprotective agent in yeasts (Moline et al., 2011) and its possible use in human sunscreens has been tested (Libkind et al., 2009c). To date, many yeast species have been detected as MGG producers (Libkind et al., 2005b; 2011b) and the level of synthesis appear to be related to the solar exposition history in the habitat of origin (Libkind et al., 2006). Thus, the diversity surveys in highly UV exposed habitats have rendered valuable isolates able to accumulate large quantities of MGG with concentrations above 5% of the dry weight (Libkind et al., 2005b, 2011a; Brandao et al., 2011). These MGG producing strains are conserved in the CRUB collection and are used in studies related to the elucidation of the genetic bases of MGG synthesis in yeasts and fungi in general.

### 6.2 Innovative biotechnological method to resuscitate ancient yeasts from fermenters useful in microbial archaeology

Fermenters from ancient cultures are suitable substrates to keep dormant yeasts within its pores. Both in Ecuador and Spain, yeasts isolates were recovered from archaeological pieces belonging to fermenters used by ancient cultures. The most remarkable ones were vessels from about 2500 b.C. belonging to the Iberos culture (Spain), and Sierra Norte (Ecuador) from about 200 a.C. Other remarkable yeast recovered from ancient fermenters belonged to the first brewery founded in America in 1566 (Ecuador), where wooden vessels were sampled as well.

The method developed to recover these yeast strains is based upon the so-called "resuscitation triangle", where cell walls restoring and membranes fluidization is carried out firstable; after that, a hydration step is performed; and, finally, a metabolic activation step is accomplished to resuscitate ancient and valuable yeast strains. Underlying these techniques is the nascent Microbial Archaeology that pursues an understanding of the ancient microflora and its implications for human beings by using archaeological rests as sources of ancient microorganisms. This method, allowed retrieving and isolating dozens of different yeasts, most of them belonging to species such as *Saccharomyces cerevisiae, Clavispora luisitaniae, Cryptococcus saitoi, Rhodotorula mucilaginosa*, *Meyerozyma guillermondi, Cr. diffluens, Candida parapsilosis,* and *C. tropicalis* and other undescribed species.
The method used to recover these ancient yeasts is a trade secret belonging to the Pontificia Universidad Católica del Ecuador, where the CLQCA is placed.

## 7. Conclusion

The yeast biodiversity study is currently being boosted by more and more groups that show interest in discover novel yeasts species and understand the ecology, physiology, and evolutionary aspects of yeasts.
As pointed out previously, there is still much to be knonwn about yeast biodiversity in vast zones of the planet. In our understanding, this gap will take a long time to be closed taking into account the still unexplored habitats occupied by these organism.
Moreover, taxonomy of yeasts faces challenges in terms of the accomodation of species inasmuch as frequently yeasts are being re-clasified. The genus *Candida* is the more extended in yeasts, nevertheless, this genus is only created to accommodate those yeasts that haven't shown teleomorphic (sexual) phase. But this characteristic can be reverted if an appropriate medium allows the yeast sporulation.
On the other hand, molecular techniques developed during the last 30 years are very valuable tools for research. Molecular approaches are fundamental in current studies of yeast for its identification and classification. Concomitantly, chemotaxonomic methods are complementary to characterize yeast strains. These methods allow the researchers find out the metabolic abilities of yeast strains whose understanding is the first step to potential use of in biotechnology and industry.
Yeast collections play a fundamental role in preservation, identification and characterization of these microorganisms; represent safe repositories where biodiversity is preserved for the future. The *ex situ* preservation of yeasts is a big effort not only in economical, but also in technical terms. Qualified personnel are needed as well as economic sources to carry out the Contamination and loss of viability are two main concerns of curators in yeast collections, that is why the ex situ preservation of yeasts is a big endeavour, even though yeasts themselves are quite small.

## 8. Acknowledgments

The authors would like to acknowledge the Pontificia Universidad Católica del Ecuador as 38 well as the SENESCYT and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico 39 (CNPq) for founding the research of yeast in Ecuador and Brazil, respectively. INR and SAJ are supported by the BBSRC in the UK.

## 9. References


Adler, L.S., (2000). *The ecological significance of toxic nectar*. Oikos 91, 409–420.

Alonso, L. M., Kleiner, D., Ortega, E. (2008). *Spores of the mycorrhizal fungus Glomus mosseae host yeasts that solubilize phosphate and accumulate polyphosphates*. Mycorrhiza, 18:197-204.

Arroyo-López, F.N., Querol, A., Bautista-Gallego, J., Garrido-Fernández, A. (2008). Role of yeasts in table olive production. *International Journal of Food Microbiology*, 128, 189-196.

Bandaranayake, W. (1998) Mycosporines: are they nature's sunscreens?, Nat. Prod. Rep. 15, 159–172.

Barbosa, A., Morais, C, Morais, P., Rosa, L., Pimenta, R., Lachance, M-A., Rosa, C.A. (2011). *Wickerhamiella pagnoccae* sp. nov. and *Candida tocantinsensis* sp nov., two ascomycetous yeasts from flower bracts of *Heliconia psittacorum* (Heliconiaceae) *Int J Syst Evol Microbiol*., in press. DOI 10.1099/ijs.0.032466-0

Barnett, J.A., Payne, R.W., Yarrow, D. (2000). *Yeasts: Characteristics and Identification*, (third ed.) Cambridge University Press, pp. 163, 172, 530, 792. 0 521 57396 3

Barth G., Gaillardin C. (1996). *Nonconventional yeasts in biotechnology*. A handbook, K. WOLF, ed., Springer, pp.313 - 383.

Botha, A. (2006). Yeasts in Soil. In: Rosa, C. A. & Peter, G. (Ed.) *Biodiversity and Ecophysiology of Yeasts*, The Yeast Handbook. Heidelberg, Springer, pp. 221-240.

Bovo B, Andrighetto C, Carlot M, Corich V, Lonbardi A, Giacomini A (2009) Yeast population dynamics during pilot-scale storage of grape marcs for the production of Grappa, a traditional Italian alcoholic beverage. *International Journal of Food Microbiology*, 129, 221–228.

Brandão, L.R.; Libkind, D.; Vaz, A.B.M.; Espírito Santo, L.; Moliné, M.; de García, V.; van Broock, M.; Rosa, C.A. 2011. Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photo-protective compounds and extracellular enzymes. *FEMS Microbiology Ecology*. 76:1-13.

Briones, A., Úbeda, J. , Grando, S. (1996). Differentiation of Saccharomyces cerevisiae strains isolated from fermenting must according to their karyotype patterns. *International Journal of Food Microbiology*,. 28, 369-377

Brizzio, S.; Turchetti, B.; de García, 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.

Brysch-Herzberg M, Lachance MA (2004). *Candida bombiphila* sp. nov., a new asexual yeast species in the Wickerhamiella clade. *Int J Syst Evol Microbiol* 54: 1857–1859

Brysch-Herzberg, M. (2004). *Ecology and Taxonomy of yeast associated with the plant-bumblebee mutualism in Central Europe*. Doctorate Thesis, University of Marburg, Marburg, Germany.

Buzzini, P., Innocenti, M.; Turchetti, T.; Libkind, D.; van Broock, M.; Mulinacci, N. 2007. Carotenoid profiles of yeasts belonging to the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces* and *Sporidiobolus. Canadian Journal of Microbiology,* 53: 1024-1031.

Caceres CE, Tessier AJ. 2003. *How long to rest: The ecology of optimal dormancy andenvironmental constraint. Ecology* 84:1189–1198.

Canto, A., Herrera, C.M., Medrano, M., Pérez, R., García, I.M., (2008). Pollinator foraging modifies nectar sugar composition in *Helleborus foetidus* L. (*Ranunculaceae*): an experimental test. *American Journal of Botany* 95, 315–320.

Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.V. y Díaz, S. (2000). *Consequences of changing biodiversity*. Nature 405: 234-242.

Charoenchai, C., Fleet, G.H., Henschke, P.A. and Todd, B.E.N. (1997) Screening of *non-Saccharomyces* wine yeasts for the presence of extracelullar hydrolytic enzymes. *Australian Journal Grape and Wine Research*, 3, 2–8.

Chee M., Yang R., Hubbell E., Berno A., Huang X. C. et al. (1996*). Accessing genetic information with high-density DNA arrays.* Science 274: 610–614.

Ciani, M.; Picciotti, G. (1995) The growth kinetics and fermentation behaviour of some *non-Saccharomyces* yeasts associated with wine-making. *Biotechnology Letters*, 17, 11, 1247-1250

Cloete KJ, Przybylowicz WJ, Mesjasz-Przybylowicz J, Barnabas AD, Valentine AJ, Botha A. (2010). Micro-particle-induced X-ray emission mapping of elemental distribution in roots of a Mediterranean-type *sclerophyll, Agathosma betulina* (Berg.) Pillans, colonized by *Cryptococcus laurentii*. *Plant Cell Environ*. 33:1005-1015.

Cloete, K. J., Valentine, A. J., Stander, M. A., Blomerus, L. M. & Botha, A.(2009) Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a *sclerophyllous medicinal shrub*, *Agathosma betulina* (Berg.) *Pillans. Microb. Ecol*. 57, 624–632.

Coluccio, A.E., Rodriguez R.K., Kernan M.J., Neiman A.M. (2008). The yeast spore wall enables pores to survive passage through the digestive tract of *Drosophila*. *PLoS ONE* 3(8): e2873.

Cordero Otero, R., Úbeda- Iranzo, J., Briones-Perez, A., Potgieter, N., Arévalo-Villena, M., Pretorius, S. Van Rensburg, P. (2003) .Characterization of the b-glucosidase activity produced by enological strains of *non- Saccharomyces* yeasts. *Journal of Food Science*, 68, 2564- 2569

Coton, E., Coton, M., Levert, D., Casaregola, S., Sohier, D. (2006). Yeast ecology in French cider and black olive natural fermentations. *International Journal of Food Microbiology*, 108, 130- 135.

de García, V.; Brizzio, S.; Libkind, D.; Buzzini, P.; van Broock, M. 2007. Biodiversity of cold-adapted yeasts from runoff glacial rivers in Patagonia, Argentina.*FEMS Microbiology Ecology*,59(2):331-341.

de Garcia, V.; Brizzio, S.; Libkind, D.; Rosa, C. & van Broock, M. 2010. *Wickerhamomyces patagonicus* sp. nov., a novel teleomorphic ascomycetous yeast from Patagonia, Argentina. *International Journal of Systematic and Evolutionary Microbiology*. 60: 1693-1696.

de Garcia, V.; Brizzio, S.; Russo, G.; Rosa, C.A.; Boekhout, T.; Theelen, B.; Libkind, D. & M. van Broock. 2010. *Cryptococcus spencermartinsiae* sp. nov., a basidiomycetous yeast isolated from glacial waters and apple fruits. *International Journal of Systematic and Evolutionary Microbiology*. 60: 707-711

de Vega, C., Herrera, C. M., Johnson, S.D. (2009) Yeasts in floral nectar of some South African plants: Quantification and associations with pollinator type and sugar concentration *South African Journal of Botany* 75 (2009) 798–806

Fay, J. y Benavides, J. (2005). Evidence for Domesticated and Wild Populations of Saccharomyces cerevisiae. *PLoS Genet*, 1, 66-71.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Fernández, M., Úbeda Iranzo, J.F., Briones Pérez, A.I., (2000). Typing of *non-Saccharomyces* yeasts with enzymatic activities of interest in winemaking. *International Journal of Food Microbiology*, 59, 29-36.

Fernandez-González, M., Espinosa, J.C., Ubeda, J., Briones, A.I. (2001). Yeasts presentduring wine fermentation: comparative analysis of conventional plating and PCR- TTGE. *Systematic and Applied Microbiology* 24, 639-644.

Fokkema N.J., den Houter J.G., Kosterman Y.J.C., Nelis A.L. (1979). Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum. Trans. Br. Mycol. Soc.,* 72: 19-29.

Fonseca, A., Inacio, J. Phylloplane Yeasts. (2006) . In: Rosa, C. A. & Peter, G. (Ed.) *Biodiversity and Ecophysiology of Yeasts*, The Yeast Handbook. Heidelberg, Springer, pp. 263-302

Gadanho, M. & Sampaio, J. P. (2009). Cryptococcus ibericus sp. nov., Cryptococcus aciditolerans sp. nov. and Cryptococcus metallitolerans sp. nov., a new ecoclade of anamorphic basidiomycetous yeast species from an extreme environment associated with acid rock drainage in Sao Domingos pyrite mine, Portugal. *International Journal of Systematic and Evolutionary Microbiology* 59, 2375–2379.

Ganter, P. F. (2006). *Yeast and invertebrate associations*. In: Rosa, C. A. & Peter, G. (Ed.) *Biodiversity and Ecophysiology of Yeasts,* The Yeast Handbook. Heidelberg, Springer, pp. 303-370.

Giannoutsou, E.P., Meintanis, C., Karagouni, A.D. (2004). Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. *Bioresources and Technology*, 93, 301- 306.

Gingeras T. R., Ghandour G., Wang E., Berno A., Small P. M. et al. (1998) Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic Mycobacterium DNA arrays. *Genome Res*. 8: 435–448.

Gomes, F., Lacerda, I., Libkind, D., Lopes, C., Carvajal, J. y Rosa, C. (2009). Traditional Foods and Beverages from South America: Microbial Communities and Production Strategies. *Nova Science Publishers*, Inc. pp. 2-27

González-Teuber, M. & Heil, M. (2009) Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal*. Behav. 4.

Gregory I. Lang and Andrew W. Murray, (2007). Estimating the Per-Base-Pair Mutation Rate in the Yeast *Saccharomyces cerevisiae*. *Genetics* 178: 67–82.

Guillamón, J.M., Sabaté, J., Barrio, E., Cano, J. y Querol, A. (1998) Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Archives of . Microbiology* , 69, 387–392.

Hawksworth, D.L. (2004), Fungal diversity and its implications for genetic resource collections. *Studies in Mycology*, 50; 9-18.

Hernández, A., Martín, A., Aranda, E., Pérez-Nevado, F., Córdoba, M.G., (2007). Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. *Food Microbiology* , 24, 346-351.

Herrera, C., De Vega, C., Canto, A., Pozo, M.I., (2009). Yeasts in floral nectar: a quantitative survey. *Annals of Botany* 103, 1415–1423.

Herrera, C.M., García, I.M., Pérez, R., (2008). Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology* 89, 2369–2376.

Hoekstra, J.M., T.M. Boucher, T.H. Ricketts, and C. Roberts (2005). Confronting a biome crisis: Global disparities of habitat loss and protection. *Ecology Letters* 8:23-29.

Hurtado, A., Reguant, C., Esteve-Zarzoso, B. (2008). Microbial population dynamics during the processing of Arbequina table olives. *Food Research International*, 41, 738-744.

Ippolito A., Nigro F. (2000). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection*, 19: 715-723.

Irwin, R. E., Adler, L. S. & Brody, A. K. (2004). The dual role of floral traits: pollinator attraction and plant defense. *Ecology* 85, 1503–1511.

Izquierdo, P. M., Úbeda, J. F. and Briones, A. I. (1997). Study of the karyotype of wine yeasts isolated in the region of Valdepeñas in two consecutive vintages. *Food Microbiology*, 14, 221-225

James SA, Collins MD & Roberts IN (1996) Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera *Zygosaccharomyces* and *Torulaspora*. *Int J Syst Bacteriol* 46: 189–194.

James S., Carvajal J., Bond C., Cross K., Nunez N., Portero P., Roberts I. (2009). "*Candida carvajalis* nov.sp,an ascomycetous yeast species found in Ecuador Rain Forest". *FEMS Yeast Reasearch* 9: 784-788

Johnson, E.A.&Schroeder,W.A. (1995).Microbial carotenoids.*Advancesin Biochemical Engineering and Biotechnology*53, 121–178.

Jones S. and Lennon J. (2009). Dormancy contributes to the maintenance of microbial diversity. *www.pnas.org/cgi/doi/10.1073/pnas.0912765107.*

Kurtzman CP & Robnett CJ (1997) Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. *J Clin Microbiol* 35: 1216-1223.

Kurtzman CP & Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* 73: 331-371.

Lachance MA (1995) Yeast communities in a natural tequila fermentation. *Antonie van Leeuwenhoek*, 68,151–160.

Lachance MA, Daniel HM, Meyer W, Prasad SP & Boundy-Mills K (2003) The D1/D2 domain of the large-subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. *FEMS Yeast Res* 4: 253-258.

Lachance M-A, Ewing CP, Bowles JM, Starmer WT, (2005). *Metschnikowia hamakuensis* sp. nov., *Metschnikowia kamakouana* sp. nov. and *Metschnikowia mauinuiana* sp. nov., three endemic yeasts from Hawaiian nitidulid beetles. *Int. J. Syst. Evol. Microbiol.* 55: 1369–1377.

Lachance MA, Starmer WT, Rosa CA, Bowles JM, Barker JS, Janzen DH (2001) Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Res* 1:1–8.

Lachance, M.-A. (2006). *Yeast diversity: how many and how much? In*: Rosa, C. A. & Peter, G. (Ed.) *Biodiversity and Ecophysiology of Yeasts*, The Yeast Handbook. Heidelberg, Springer, pp. 1-10

Lachance, M-A., Wijayanayaka, T. M., Bundus, J. D., Wijayanayaka, D. N. (2011) Ribosomal DNA sequence polymorphism and the delineation of two ascosporic yeast species: *Metschnikowia agaves* and *Starmerella bombicola*. *FEMS Yeast Res.*, in press. DOI: 10.1111/j.1567-1364.(2011).00718.x

Leach, C.M. (1965)Ultraviolet-absorbing substances associated with light-induced sporulation in fungi, Can. J. Bot. 43, 185–200.

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(4): 313-322.

Libkind, D.; Brizzio, S.; van Broock, M.R. 2004a. *Rhodotorulamucilaginosa*, a carotenoid producing yeast strain from a Patagonian high altitude lake.*Folia Microbiologica* 49(1): 19-25.

Libkind, D.; Pérez, P.; Sommaruga, R.; Diéguez, MC.; Ferraro, M.; Brizzio, S.; Zagarese, H.; van Broock, MR. 2004b. Constitutive and UV-inducible synthesis of photoprotective compounds (carotenoids and mycosporines) by freshwater yeasts. *Photochemical and Photobiological Sciences* 3: 281-286.

Libkind, D.; Gadanho, M.; van Broock, M.R.; Sampaio, J.P. 2005a. *Sporidiobolus longiusculus* sp. nov. and*Sporobolomyces patagonicus* sp. nov., novel yeasts of the Sporidiobolales isolated from aquatic environments in Patagonia, Argentina. *International Journal of Systematic and Evolutionary Microbiology*. 55(1): 503-509.

Libkind, D.; Sommaruga, R.; Zagarese, H.; van Broock, MR.2005b. Mycosporines in carotenogenic yeasts.*Systematic and Applied Microbiology* 28: 749-754.

Libkind, D.; Diéguez, M.; Moliné, M.; Pérez, P.; Zagarese, H. & van Broock, M. 2006. Occurrence of photoprotective compounds in yeasts from freshwater ecosystems of northwestern Patagonia (Argentina). *Photochemistry and Photobiology,* 82: 972-980.

Libkind, D. & van Broock, M.R. 2006.Biomass and carotenoid pigments production by Patagonian native yeasts.*World Journal of Microbiology and Biotechnology,* 22(7): 687-692.

Libkind, D.; Ruffini, A.; van Broock, M.; Alves, L.; Sampaio, J.P. 2007.Biogeography, host-specificity, and molecular phylogeny of *Phaffia rhodozyma* and its sexual form, *Xanthophyllomyces dendrorhous.Applied and Environmental Microbiology*, 73 (4): 1120-1125.

Libkind, D.; Moliné, M; de García, V.; Fontenla, S.; van Broock, M. 2008a. Characterization of a novel South American population of the astaxanthin producing yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). *Journal of Industrial Microbiology and Biotechnology*. 35(3):151-158.

Libkind, D., Arts, M., van Broock, M. 2008b. Fatty acid composition of cold-adapted carotenogenic basidiomycetous yeasts.*Revista Argentina de Microbiología*. 40: 193-197.

Libkind, D.; Gadanho, M.; van Broock, M.; Sampaio, J.P. 2009a. *Cystofilobasidium lacus-mascardii* sp. nov., a new basidiomycetous yeast species isolated from aquatic environments of the Patagonian Andes and *Cystofilobasidium macerans* sp. nov., the sexual stage of *Cryptococcus macerans. International Journal of Systematic and Evolutionary Microbiology*. 59(3):622-630.

Libkind, D.; Moline, M.; Sampaio, J.; van Broock, M. 2009b. Yeasts from high altitude lakes: influence of UV radiation. *FEMS Microbiology Ecology*. 69:353–362.

Libkind, D.; Moliné, M.; van Broock, M.R. (2009c). Composiciones que absorben UVB y antioxidantes. Procedimientos y usos.Patent application AR073777A1.

Libkind, D., Sampaio, J.P., & M. van Broock. 2010. Cystobasidiomycetes yeasts from Patagonia (Argentina): description of *Rhodotorula meli* sp. nov. from glacial meltwater. *International Journal of Systematic and Evolutionary Microbiology* 60(9), 2251-2256.

Libkind, D.; Moliné, M.; van Broock, MR. 2011a. Production of the UVB absorbing compound Mycosporine-glutaminol-glucoside by *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).*FEMS Yeast Research*. 11(1), 52-59.

Libkind, D.; Moliné, M.; Sommaruga, R.; Sampaio, J.S.; van Broock, M. 2011b.Phylogenetic distribution of fungal mycosporines within Pucciniomycotina (Basidiomycota).*Yeast.In press*.

Lindow S.E., Brandl M.T. (2003). Microbiology of the phyllosphere. *Appl. Environ. Microbiol*., 69: 1875-1883.

Liti G., Carter M., Moses A., Warringer J., Parts L, James S., Davey R., Roberts I., Burt A., Koufopanou V., Tsai I., Bergman C., Bensasson D., O'Kelly M., van Oudenaarden A., Barton D., Bailes E., Nguyen Ba A., Jones M., Quail M., Goodhead I, Sims S, Smith F., Blomberg A., Durbin R., Louis E. (2009). *Nature*, Vol. 458.

Lockhart D. J., and E. A. Winzeler (2000) Genomics, gene expression and DNA arrays. *Natur*e 405: 827–836.

Lu H-Z, Jia J-H, Wang Q-M & Bai F-Y (2004) *Candida asparagi* sp. nov., *Candida diospyri* sp. nov. and *Candida qinlingensis* sp. nov., novel anamorphic, *ascomycetous* yeast species. *Int J Syst Evol Microbiol* 54: 1409-1414.

Manzanares, P., Rojas, V., Genovés S. and Vallés, S. (2000). A preliminary search for anthocyanin-b-D-glucosidase activity in *non-Saccharomyces* wine yeasts. *International Journal Food Science Technology*, 35, 95-103

Markus G. Weinbauer*, Fereidoun Rassoulzadegan, (2007). Extinction of microbes: evidence and potential consequences. *Endang Species Res* Vol. 3: 205–215.

Marquina, D., Peres, C., Caldas, F.V., Marques, J.F., Peinado, J.M., Spencer-Martins, M. (1992). Characterization of the yeast population in olive brines. *Letters in Applied Microbiology*, 14, 283-297.

Mauersberger S., Ohkuma M, Schunck W-H., Takagi M., *Nonconventional yeasts in biotechnology*. A handbook, K.Wolf, ed., Springer, (1996), pp.546 - 553.

Mestre, M. C., Rosa, C. A., Fontenla, S. B. (2011). *Lindnera rhizosphaerae* sp. nov., a yeast species isolated from rhizospheric soil. *Int J Syst Evol Microbiol* (2011) 61: 985-988.

Meyer SA, Payne RW & Yarrow D (1998) Candida Berkhout. *The Yeasts, A Taxonomic Study*, 4th edn (Kurtzman CP & Fell JW, eds), pp. 454-573. Elsevier, Amsterdam, the Netherlands.

Michener, C. D. (2000). *The Bees of the World*. – Baltimore and London (The John Hopkins University Press). – 913 S., 48 Farbfotos und zahlr. s/w Illustrationen. ISBN 0-8018-6133-0.

Moliné M, Libkind D, Diéguez MC, van Broock M. 2009. Photo-protective role of carotenoid pigments in yeasts: experimental study contrasting naturally occurring pigmented and albino strains. *Journal of Photochemistry and PhotobiologyB: Biology*. 95:156–161.

Moliné, M.; Regina Flores, M.; Libkind, D.; Diéguez, M.C.; Farías, M.E. & van Broock, M. 2010. Photoprotection by carotenoid pigments in the yeast *Rhodotorula mucilaginosa*: the role of torularhodin. *Photochemical and Photobiological Sciences*. 9, 1145–1151.

Moliné, M.; Arbeloa, E. M.; Regina Flores, M.; Libkind, D.; M.C.; Farías, M.E.; Bertolotti, S.G.; Churio, M.S. & van Broock, M. 2011. UV-B photoprotective role of mycosporines in yeasts: photostability and antioxidant activity of mycosporine-glutaminol-glucoside. *Radiation Research*. 175 (1), 44-50.

Morais, P. B.; Pagnocca, F. C. & Rosa, C. A. (2006). *Yeast communities in tropical rain forests in Brazil and other South American ecosystems*. In: Rosa, C. A. & Peter, G. (Ed.). The Yeast Handbook. Heidelberg, Springer, pp.461-484.

Nadal, D., Colomer, B. and Piña, B. (1996). Molecular polymorphism distribution in phenotypically distinct populations of wine yeast strains. *Applied. And Environmental Microbiology*, 62, 6, 1944-1950.

Nakase, T (1971) New species of yeasts found in Japan. J Gen *Appl Microbiol*: 17: 409-419.

Nasir H, Noda H. (2003). Yeast-like symbionts as a sterol source in anobiid beetles (*Coleoptera: Anoboiidae*): possible metabolic pathways from fungal sterols to 7-dihydrocholesterol. *Archives of Insect Biochemistry and Physiology* 52: 175–182.

Naumova, E.S., Serpova, E.V., Naumov, G.I. (2007). *Molecular systematics of Lachancea yeasts*. Biochemistry (Mosc) 72, 1659-1667.

Nicolson, S. W., Nepi, M. & Pacini, E. (eds) (2007). *Nectaries and nectar*. Dordrecht, The Netherlands: Springer-Verlag.

O'Donnell K (1993) *Fusarium and its near relatives. The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics* (Reynolds DR & Taylor JW, eds), pp. 225-233. CAB International, Wallingford, UK.

Pagnocca, F.C., Legaspe, M. F., Rodrigues, A., Ruivo, C.C.C., Nagamoto, N.S., Maurício Bacci, M. & Forti, L. C. (2010). Yeasts isolated from a fungus-growing ant nest, including the description of Trichosporon chiarellii sp. nov., an anamorphic basidiomycetous yeast *Int J Syst Evol Microbiol* 60: 1454-1459.

Pearson JR & Lipman DJ (1988) Improved tools for biological sequence comparison. *Proc Natl Acad Sci* USA 85: 2444-2448.

Pimentel, M. R. C., Antonini, Y., Martins, R. P., Lachance, M. A. & Rosa, C. A. (2005). *Candida riodocensis* and *Candida cellae*, two new yeast species from the *Starmerella clade* associated with solitary bees in the Atlantic rain forest of Brazil. *FEMS Yeast Res* 5, 875–879.

Pimm, S.L. y Raven, P. (2000). Extinction by numbers. *Nature* 403: 843-845.

Pretorius, I.S. (2000). *Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking*. Yeast 16, 675-729.

Querol, A., Barrio, E. y Ramón, D. (1994) Population dynamics of natural *Saccharomyces* strains during wine fermentation *International Journal of Food Microbiology*, 21, 315-323.

Querol, A., Barrio, E., Huerta. T., Ramón, D., (1992). Molecular monitoring of wine fermentations conducted by active dry yeast strains. *Applied and Environmental Microbiology* 58 ,9, 2948-2953.

Ricci, I., Mosca, M., Valzano, M. Damián, C., Scuppa, P., Rossi, P., Crotti, E. Cappelli, A., Ulissi, U., Capone, A., Esposito, F., Alma, A., Mandrioli, M., Sacchi, L., Bandi, C., Daffonchio, D., Favia, G. (2011) Different mosquito species host Wickerhamomyces anomalus (*Pichia anomala*): perspectives on vector-borne diseases symbiotic control. *Antonie van Leeuwenhoek*, 99:45-50.

Rodríguez-Sáiz, M., de la Fuente, J.L., and Barredo J.L. (2010) *Xanthophyllomyces dendrorhous* for the industrial production of astaxanthin.*Appl Microbiol Biotechnol* 88, 645-658.

Rosa, C. A., Lachance, M. A., Silva, J. O. C., Teixeira, A. C. P., Marini, M. M., Antonini, Y. & Martins, R. P. (2003). Yeast communities associated with stingless bees. *FEMS Yeast Res* 4, 271–275.

Rosa, C. A., Lachance, M. A., Teixeira, L. C. R. S., Pimenta, R. P. & Morais, P. B. (2007). *Metschnikowia cerradonensis* sp. nov., a yeast species isolated from ephemeral flowers and their nitidulid beetles in Brazil. *Int J Syst Evol Microbiol* 57, 161–165.

Ruivo, C. C. C., Lachance, M. A., Rosa, C. A., Bacci, M. & Pagnocca, F. C. (2005). *Candida bromeliacearum* sp. nov. and *Candida ubatubensis* sp. nov., two yeast species isolated from the water tanks of *Canistropsis seidelii (Bromeliaceae*). *Int. J. Syst. Evol. Microbiol*. 55: 2213-2217.

Russo, G.; Libkind, D.; Sampaio, J.P.; van Broock. 2008. Yeast diversity at the Volcanic acidic environment of the Lake Caviahue and Rio Agrio (Patagonia, Argentina). *FEMSMicrobiology Ecology*. 65(3), 415-424.

Russo, G.; Libkind, D.; Ulloa, RJ, de García, V. Sampaio, JP, van Broock, MR. 2010. *Cryptococcus agrioensis* sp. nov., a basidiomycetous yeast of the acidic rock drainage ecoclade, isolated from acidic aquatic environments of volcanic origin (River Agrio, Argentina). *International Journal of Systematic and Evolutionary Microbiology.* 60: 996-1000.

Saitou N & Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406-425.

Sampedro, I., Aranda, E., Scervino, J.M., Fracchia S, García-Romera I, Ocampo JA, Godeas A. (2004*) Improvement by soil yeasts of arbuscular mycorrhizal symbiosis of soybean (Glycine max) colonized by Glomus mosseae. Mycorrhiza.* (2004) Aug;14(4):229-34. Epub 2003 Dec 18.

Scheller U., Zimmer T., Becher D., Schauer, Schunck W-H. (1998). Oxygenation cascade in conversion of n-alkanes to α,ω - dioic acids catalyzed by cytochrome P450 52A3, *J. of Biol. Chem*., 273 (4), p.32528-32534.

Schütz, M. and Gafner, J. (1994), Dynamics of the yeast strain population during spontaneous alcoholic fermentation determined by CHEF gel electrophoresis. *Letters in Applied Microbiology*, 19, 253–257

Seibold A., Fried A., Kunz S., Moltmann E., Lange E., Jelkmann W. (2004). Yeasts as antagonists against fireblight. *Bulletin OEPP/EPPO Bulletin*, 34: 389-390.

Shivaji, S., and Prasad, G.S. (2009), *Antartic Yeasts: Biodiversity and Potential Applications*. Yeast Biotechnology: Diversity and Applications. I, 3-18.

Slavikova, E., Vadkertiova, S., Vranova, D. (2009). *Yeasts colonizing the leaves of fruit trees.* Annals of Microbiology, 59: 419-424

Sommaruga, R.; Libkind, D.; van Broock, M.; Whitehead, K. 2004.Mycosporine-glutaminol-glucoside, a UV-absorbing compound of two *Rhodotorula* yeast species.*Yeast* 12(13): 1077-1081.

Starmer, W. T. & Lachance, M.-A. (2011). *Yeast Ecology. In: The Yeasts A Taxonomic Study. 5th Ed*. Kurtzman, C.P., Fell, J. W., Boekhout, T. Elsevier: Amsterdam. Pp. 65-86.

Starmer, W. T., Scmedicke, R. A., Lachance, M.-A. (1988). *The origin of the cactus-yeast community*. MS Yeast Research, 3: 441-448.

Suh S, McHugh J., Pollock D., and Blackwell M., (2004). The beetle gut: a hyperdiverse source of novel yeasts, *Mycol. Res.* 109 (3): 261–265.

Suh S-O, Blackwell M, (2004). Three new beetle-associated yeasts in the Pichia guilliermondii clade. *FEMS Yeast Research* 5: 87–95.

Suh S-O, Blackwell M. (2005). *Beetles as hosts for undescribed yeasts. In: Insect-fungal associations: ecology and evolution* (FE Vega, M Blackwell, eds). Oxford University Press, Oxford: 244–256.

Suh S-O, Marshall CJ, McHugh JV, Blackwell M, (2003). Wood ingestion by passalid beetles in the presence of xylose fermenting gut yeasts. *Molecular Ecology* 12: 3137–3145.

Suh S-O, McHugh JV, Blackwell M, (2004). Expansion of the Candida tanzawaensis yeast clade: 16 new Candida species from basidiocarp-feeding beetles. *Int J Syst Evol Microbiol* 54: 2409–2429.

Suh S-O, McHugh JV, Pollock DD, Blackwell M, (2005). The beetle gut: a hyperdiverse source of novel yeasts. *Mycol Research* 109: 261–265.

Thompson JD, Higgins DG & Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680.

Torija, M.J., Rozès, N., Poblet, M., Guillamón, J.M. y Mas, A. (2001) Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years. *Antonie van Leeuwenhoek* 79, 345-352.

Torres-Vila, L.M., Rodriguez-Molina, M.C., Martínez, J.A. (2003). Efectos del daño de la mosca del olivo y del atroje sobre la microflora en pasta y la acidez del aceite virgen de oliva. *Grasas Aceites* 3, 285- 294.

Troesch A., Nguyen H., Miyada C. G., Desvarenne S., Gingeras T. R. et al. (1999) Mycobacterium species identification and rifampin resistance testing with high-density DNA probe arrays. *J. Clin. Microbiol*. 37: 49–55.

Turner MG, Baker WL, Peterson CJ, Peet RK. (1998). *Factors influencing succession: Lessons from large, infrequent natural disturbances*. Ecosystems (N Y, Print) 1:511–523.

Vaz, A. B. M., Rosa, L. H., Vieira, M. L. A., Garcia, V., Brandão, L. R., Teixeira, L. C. R. S., Moliné. M., Libkind, D., van Broock, M., Rosa, C. A. (2011). The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. *Braz. J. Microbiol*., In Press.

Wiens, F., Zitzmann, A., Lachance, M.A., Yegles, M., Pragst, F., Wurst, F.M., Von Holst, D., Guan, S.L., Spanagel, R., (2008). Chronic intake of fermented floral nectar by wild treeshrews. *Proceedings of the National Academic of Sciences* 105, 10426–10431.

Winzeler E., Castillo-Davis C., Oshiro G., Liang D., Richards D., Zhou Y., and Hartl D L., (2002). Genetic Diversity in Yeast Assessed With Whole-Genome Oligonucleotide Arrays. *Genetics* 163: 79–89.

Wuczkowski, M., Passoth, V., Turchetti, B., Andersson, A-C., Olstorpe, M., Laitila, A., Theelen, B., van Broock, M., Buzzini, P., Prillinger, H., Sterflinger, K., Schnürer, J., Boekhout, T., Libkind, D.2011. Proposal of *Holtermanniella takashimae* sp. nov., *Holtermanniella* gen. nov. and the new order Holtermanniales to accommodate Tremellomycetous yeasts of the *Holtermannia* clade.*International Journal of Systematic and Evolutionary Microbiology*. 61: 680 - 689.

Yarrow D (1998) *Methods for the isolation, maintenance and identification of yeasts. The Yeasts, A Taxonomic Study,* 4th edn (Kurtzman CP & Fell JW, eds), pp. 77-100. Elsevier, Amsterdam, the Netherlands.


**Changing Diversity in Changing Environment**
Edited by PhD. Oscar Grillo

ISBN 978-953-307-796-3
Hard cover, 392 pages
**Publisher** InTech
**Published online** 14, November, 2011
**Published in print edition** November, 2011

As everybody knows, the dynamic interactions between biotic and abiotic factors, as well as the anthropic ones, considerably affect global climate changes and consequently biology, ecology and distribution of life forms of our planet. These important natural events affect all ecosystems, causing important changes on biodiversity. Systematic and phylogenetic studies, biogeographic distribution analysis and evaluations of diversity richness are focal topics of this book written by international experts, some even considering economical effects and future perspectives on the managing and conservation plans.

**How to reference**
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Enrique Javier Carvajal Barriga, Diego Libkind, Ana Isabel Briones, Juan Úbeda Iranzo, Patricia Portero, Ian Roberts, Steve James, Paula B. Morais and Carlos A. Rosa (2011). Yeasts Biodiversity and Its Significance: Case Studies in Natural and Human-Related Environments, Ex Situ Preservation, Applications and Challenges, Changing Diversity in Changing Environment, PhD. Oscar Grillo (Ed.), ISBN: 978-953-307-796-3, InTech, Available from: http://www.intechopen.com/books/changing-diversity-in-changing-environment/yeasts-biodiversity-and-its-significance-case-studies-in-natural-and-human-related-environments-ex-s

INTECH
open science | open minds

**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen