Chapter 8
Ecology and Biodiversity of Yeasts with Potential Value in Biotechnology

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Contents

Abstract In the latest edition of the standard treatise of yeasts, in 1998, 700 species were described. Since then, the number of recognized yeast species has doubled, with a steep increase particularly in the number of the basidiomycetous yeasts. Of all these yeast species, only about a dozen is used at industrial scale, and some 70–80 species have been shown at laboratory scale to possess potential value in biotechnology; their ratio is, in the best case, 5–10%. If it is accepted, that according to a modest estimate, the known yeast species represent only 5% of the total number which may inhabit the Earth, then there is ample room to search for new species with novel potential to exploit. Where could these yeasts be discovered?

In recent years we are witnessing great progress in exploring the diverse ecological niches of yeasts, and revealing the great diversity of species living in the various habitats. Still, compared to the profusing metabolic capability of bacteria living in the soil, surprisingly less is known about the soil yeasts. Much remains to be learned on yeasts associated with insects, invertebrates and fishes in the deep ocean, inhabiting tropical forests, or striving in extreme environments. It could reasonably be expected, that among the numerous species to be discovered in specific and unusual habitats, many will be found to possess enzymes, carry out metabolic routes and show physiological properties which hold out promises to be valuable for biotechnological applications. This chapter will examine these potential values from the point of view of ecology and biodiversity of yeasts.

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8.1 Introduction

Yeasts have been used for making bread, beer and wine since ancient ages. Their role in fermentation was recognized by Pasteur, and the first pure cultures (starters) of brewer's and wine yeast were obtained by Hansen and Müller-Thurgau, respectively, at the end of the 19th century. Since then the application of yeast starters has become a standard practice in the industrial fermentation not only for food and beverages but also for a broad variety of other products made by yeasts or from yeast cells.

The traditional fermentation processes are carried out by a single species of yeasts, *Saccharomyces cerevisiae*, hence for many, its name is synonymous with yeasts, and it is thought that all yeasts are fermentative. Contrary to general belief, there have been described more than thousand species of yeasts, about half of them not being able to ferment; nevertheless many of these have gained a significant role in biotechnology.

Improvement of starter cultures relies on classical genetic techniques such as hybridization and mutagenesis followed by selection. Recently, it has become possible to tailor production strains for purpose by methods of recombinant gene technology (genetic engineering). Public concern and legal regulation may raise difficulties in commercial application of genetically modified organisms and their products. However, there is an alternative way of finding novel strains with better producing properties, and it is to search for them among the existing organisms.

In recent years we are witnessing an increasing awareness of the importance of the biodiversity in nature, and of conserving and sustainable utilizing it. Beyond plants and animals, great progress has been made in exploring the diverse ecological niches of microorganisms, among them yeasts. Much remains to be learned about the great diversity of species and the profusing metabolic capabilities of yeasts striving in extreme environments. It could be reasonably expected that among the numerous species living in specific and unusual habitats – many of them are to be discovered – several strains would be found possessing enzymes, carrying out metabolic routes and showing physiological attributes which hold out promises to be valuable for biotechnological exploitation.

This chapter will examine these potential values from the point of view of ecology and biodiversity of yeasts. Some recent overviews of the subject are Demain et al. (1998), Walker (1998), Buzzini and Vaughan-Martini (2006).

8.2 Biodiversity of Yeasts

Biodiversity of yeasts can be characterized from taxonomic and ecological point of view. Taxonomically, yeasts form an artificial group of fungi comprising mostly unicellular organisms reproducing vegetatively by budding (blastoconidia).

However, several yeasts can develop true hyphae similar to those of moulds, whereas other species may form pseudohyphae from elongated cells remaining attached together after budding. Instead of budding, some yeasts propagate by arthroconidia arising from cell division or splitting of hyphae. Sexual reproduction is known in less than half of yeast species; this may result in the generation of ascospores or basidiospores, with or without preceding conjugation. In all, yeasts represent a phylogenetically diverse group of fungi, that can be classified either to Ascomycetes (e.g. *Saccharomyces, Candida*) or Basidiomycetes (e.g. *Filobasidiella, Rhodotorula*); moreover the small genus of *Schizosaccharomyces* and few other species belong to neither, formerly was regarded a separate group called Archiascomycetes.

The number of recognized yeast genera and species are increasing steadily. In a little more than 50 years, between the appearance of the 1st edition and the 5th edition of the taxonomic monograph of yeast, the number of genera increased from 26 to 133, and the number of species described from 164 to more than one thousand

Table 8.1 Yeast species of current and potential use in biotechnology

Species	Application
Candida milleri	Sourdough
C. shehatae	Bioethanol
C. sake	Biocontrol
C. oleophila	Biocontrol
C. maltosa	SCP on hydrocarbons
Debaryomyces hansenii	Cheese, sausage ripening, proteases
D. (Schwanniomyces) occidentalis	Amylase
Eremothecium ashbyi	Riboflavin
Geotrichum candidum	Cheese ripening
Hanseniaspora uvarum	Wine fermentation
Kluyveromyces marxianus	Milk fermentation, SCP from whey
K. lactis	Milk fermentation, SCP from whey
Pachysolen tannophilus	Bioethanol
Phaffia rhodozyma	Astaxanthin
Pichia angusta (Hansenula polymorpha)	Bioethanol
P. anomala	Biocontrol
P. jadinii (C. utilis)	Feedstock
P. pastoris	Heterologous proteins
P. stipitis	Bioethanol
Pseudozyma flocculosa	Biocontrol
Rhodotorula glutinis	Carotene
Schizosaccharomyces pombe	Cider fermentation
Saccharomyces cerevisiae	Brewer's, baker's, wine yeast, bioethanol, invertase, heterologous proteins
S. exiguus	Sourdough
S. boulardii (S. cerevisiae)	Probiotics
Saccharomycopsis fibuligera	Amylase
Torulaspora delbrueckii	Sourdough
Zygosaccharomyces rouxii	Soy sauce

Data from Abbas (2006); Buzzini and Vaughan-Martini (2006); Walker (1998); Demain et al. (1998)

(Lodder, 1970; Kurtzman, Fell and Boekhout, 2006), at the time of writing, the 5th edition has not appeared, and the exact figure is not known). However, the real number of existing yeast species may well exceed that of already described ones. Prudent attempts only try to estimate the existing number of fungal species as 1.5 million of which 72000 (4.8%) described (Hawksworth, 2001; Hammond, 1995). If yeasts make up about 1.0–1.5% of all known fungi, then the number of their existing species would approximately fall between 15 to 24 thousand. Of all these yeast species, only about a dozen is currently used at industrial level, and some 70 to 80 species have been tested at laboratory scale showing potential value in biotechnology application (Table 8.1). The questions arise: how much more could be exploited, and where could these yeasts be discovered? Part of the answer lies in exploring the ecology of yeasts.

8.3 Ecology of Yeasts

Yeasts live in community or biocoenosis with other organisms, which is the biotic component making up an ecosystem together with its abiotic components. The abiotic (physical and chemical) components of the ecosystem is frequently referred to as the environment. The physicochemical attributes of the environment act on the organisms as intrinsic and extrinsic ecological factors, and define the habitat in which they could exist. The properties of organisms (implicit ecological factors) as opposed to those factors provided by the environment would determine how the organisms could strive, survive or die in a given habitat (Boddy and Wimpenny, 1992). The biotic component also includes the sources and vectors contributing to the colonization of habitats, as well as the interactions between the members of the community, which are sometimes the most influencing ecological factors (e.g. synergistic or antagonistic) of an ecosystem.

Ecosystems differ in kind and size. The soil, the sea, a forest, or an animal body are natural ecosystems, an arable land, an orchard or a cow in stable are under the impact of human influence, whereas foods can be entirely artificial – nevertheless, they can be considered for ecosystems, and from microbiological point of view, they are certainly those. The extension of an ecosystem may be as large as the ocean, or as small as a leaf of a plant or a morsel of soil, but they provide equally habitats for microorganisms under their characteristic impact of ecological factors.

Ecological factors exert limits on microbial biodiversity. Microorganisms exist everywhere on Earth when physical or chemical conditions permit. One of the most surprising outcomes of the recent exploration of microbial biodiversity has been the recognition of the wide range of physiological conditions under which microbes flourish. Microorganisms have been discovered in niches not conceived to be habitable. Some thrive at temperatures close to 100°C in hot springs and at temperatures above the boiling point of water in submarine hot vents. Others are found in the ice of both Poles, some live in saturated salt brines or at pH extremes lower than 1 or

Table 8.2 Examples of extremophiles in natural habitats and foods

Environmental parameter	Microbial type	Natural habitat	Foods
Temperature			
Low < 10°C	Psychrophile	*Psychrobacter*	Some *Pseudomonas*
High > 50°C	Thermophile	*Synechococcus*	*Bacillus, Clostridium*
> 80°C	Hyperthermophile	*Pyrobolus*	None
pH			
Low ≤ 1	Acidophile	*Thiobacillus*	*Acetobacter*
High ≥ 9	Alkalophile	*Natronbacterium*	*Metschnikowia*
Low a_w < 0.85	Xerotolerant	*Penicillium*	*Debaryomyces*
< 0.65	Xerophile	*Xeromyces*	*Zygosaccharomyces*
Oxygen			
Cannot tolerate	Obligate anaerobe	*Methanococcus*	*Clostridium*
Tolerate low O_2	Microaerophile	*Spirillum*	*Campylobacter*
Neutral	Facultative	*Enterobacter*	*Saccharomyces*
Require O_2	Obligate aerobe	*Macrococcus*	*Rhodotorula*
Radioactivity	Radioduric	*Deinococcus*	None
High hydrostatic pressure	Barophilic	*Shewanella*	None

higher than 12, still others bear high hydrostatic pressure or high radioactivity. These various forms are collectively called as extremophiles. Among them several new species have been found, moreover, new classes and phyla of bacteria have been recognized, most of them placed in the recently recognized third domain of life, the Archaea (Woese and Fox, 1977; Woese et al.,1990; Staley et al., 1997; Hugenholtz et al., 1998). Extreme physiological properties are, however, not limited to prokaryotes, the same peculiar characteristics are found among eukaryotes, as well (Roberts, 1998; Rotschild and Mancinelli, 2001; Moreira and López-Garcia, 2002). Moreover, extreme living conditions are not restricted to natural habitats, similar conditions are provided by some preserved foods, and the microorganisms striving them can be also considered extremophiles. For example, most of the major food spoilage yeasts could be termed such, they are extremely osmophilic (e.g. *Zygosaccharomyces rouxii*), halotolerant (*Candida etchellsii*), ethanol-tolerant (*Saccharomyces cerevisiae*), resistant to weak-acid preservatives (*Zygosaccharomyces bailii*) or others (Table 8.2) (Stratford, 2006; Raspor and Zupan, 2006).

8.4 Diversity of Yeast Used in Industrial Fermentation

The term ‘industrial fermentation’ is meant in the present context all kinds of processes from the traditional alcoholic fermentation of beer and wine, to the aerobic propagation of baker’s yeast, and to novel products of biotechnology made with so-called non-conventional yeasts, other than strains of *Saccharomyces*, including not only industrial, but also agricultural, environmental and medical applications and utilizations.

In this brief overview, the enormously broad and vast field of actual and potential use of yeasts cannot be covered exhaustively. Some examples will only be given to illustrate current and future trends in five major fields of exploitation: 1. Food and beverage fermentations, 2. Products of cell mass and cell constituents, 3. Bioethanol, 4. Pharmaceutical and bioactive substances, and 5. Other uses. In discussing the improvement of production strains, special attention will be paid to the potential exploitation of yeasts hidden in the biodiversity as contrasted to improvement by genetic modification.

8.4.1 Food and Beverages

The traditional use of yeasts for the production of bread, beer and wine has been comprehensively reviewed (Dequin et al., 2003; Pretorius, 2000; Bonjean and Guillaume, 2003; Dufour et al., 2003). Since the beginning of the 20th century, the application of yeast starters has become a standard practice in the industrial fermentation of these products.

In brewing, where the malt is fermented practically by the starter alone, the pivotal role of *Saccharomyces cerevisiae* as pitching yeast is unquestioned. Efforts have been directed only to improve the performance of this starter, as will be discussed below. Use of other starters at the previous stage of steeping and malting have been considered only recently. Sometimes the barley is heavily contaminated by mycotoxin producing fusaria. It has been shown that *Geotrichum candidum* starter culture can be used for the protection of barley, and its presence also increases the enzymatic potential of malt (Linko et al., 1998; Foszczynska et al., 2004).

The situation is different in the fermentation of must and in the leavening of bread, where the pure starter, when used, does not remain alone but a mixed association with other yeasts develops, often together with lactic acid bacteria and accompanied by other bacteria and molds. In enology, it has been long debated to what degree the autochthonous yeasts may contribute in the fermentation of aroma and 'bouqet' of the wine. Some even question the use of starter strain and prefer the indigenous yeasts to maintain the specific character of the 'terroir'. Experiments on lab scale and by mini-vinification have been conducted showing the potential role of yeasts other than *Saccharomyces cerevisiae* in wine making. Among these, *Hanseniaspora guilliermondii* and its anamorph *Kloeckera apiculata*, *Pichia fermentans, Candida stellata* and others have been suggested as novel adjuncts in simultaneous or sequential mixtures with *Saccharomyces cerevisiae* (Clemente-Jimenez et al., 2005; Moreira et al., 2005).

Starters are intensively used in the leavening of bread and various other baked goods. The baker's yeasts also belong to the species of *S. cerevisiae*, being special strains of it. Lactobacilli play also an important role in sourdough, and are often associated with yeasts. In addition to *Saccharomyces cerevisiae*, which can be added as baking yeasts, at least 25 different yeast species has been described from sourdough (Meroth et al., 2003), among them *Candida milleri, C. glabrata,*

C. krusei may become dominant, however, further studies are necessary to determine their importance in the fermentation and to select appropriate species for use as starter culture (Vogel, 1997). Currently, *Saccharomyces exiguus* and *Candida humilis* have been considered in commercial sourdough preparations (Hammes et al., 2005; de Wuyst and Neysens, 2005).

Over the years, the use of yeasts for the production of food and beverages has been broadened to include dairy, meat and bakery products, spirits and alcoholic beverages other than wine (Fröhlich-Wyder, 2003; Samelis and Sofos, 2003; Hammes et al., 2005). The role of yeasts in the fermentation of some of these products has been well known for long, e.g. in kefyr and sourdough. In most cases, however, yeasts were considered in these products as spoilage organisms, or, in the best case, as innocuous, allochthonous members of the microbiota. Studies disclosing the rich biodiversity of yeasts in many of these products have also revealed, that certain yeasts may play a beneficial role in fermentation and ripening.

In the fermentation of dairy products and some kind of meat products starters are used as well. In these, the dominant microorganisms are lactic acid bacteria, however, yeasts and other microbes may join them contributing in the development of flavour and texture. In kefyr grains, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Torulaspora delbrueckii* live in strong symbiotic association with lactic acid bacteria (Narvhus and Gadaga, 2003). Although lactic acid starters are used primarily for the fermentation of cheeses, adventitious yeasts always participate in their ripening and maturation (Ferreira and Viljoen, 2003; Das et al., 2005; Leroy et al., 2006). In addition to *Saccharomyces cerevisiae*, due to their proteolytic and lipolytic activity, *Debaryomyces. hansenii* and *Yarrowia lipolytica* are regarded as good candidates for ripening agents in soft cheeses (van den Tempel and Jakobsen, 2000; Guerzoni et al., 2001; Suzzi et al., 2001; Ferreira and Viljoen, 2003), and *Geotrichum candidum* in the production of Camembert cheese (Molimard et al., 1994; Boutrou and Guéguen 2005). Of these, *Debaryomyces hansenii* has already been commercialized as potential adjunct culture (Durá et al., 2004; Flores et al., 2004). Less is known about the involvement of yeasts in the ripening of sausages. *Debaryomyces hansenii* or other lipolytic yeasts may be considered as commercial starter cultures (Olesen and Stahnke, 2000).

In the fermentation of the dairy and meat products, yeasts are only second to lactic acid bacteria, whereas in the alcoholic fermentation of various beverages other than wine, yeasts play a significant role. In many of them, e.g. cider, sake, tequila, rum and others, beyond *S. cerevisiae*, other yeasts can be dominant in developing characteristic flavor and aroma.

In recent years, the rich and varied microbiota participating in various other food and beverage fermentations has been the subject of detailed studies, and it has emerged that some species may be applied as adjuncts to improve the quality of product. Several yeast species are noted among the potent candidates. In pickled cucumbers, the mixed fermentation of *Saccharomyces rosei* (now *Torulaspora delbrueckii*) with lactic acid bacteria has been considered (Passos et al., 1997). The fermentation of coffee, cocoa, cider, olives and a number of various indigenous traditional products have recently been the subject of intensive studies, which shed

light of the complex microbial interactions and most important species (Jespersen et al., 2005). Among them there are several yeast species with the potential to be developed into a starter culture (Schwan and Wheals, 2003; Coton et al., 2006). The participation and role of yeasts of mixed fermentation, such as soy sauce, oriental products, coffee and cacao awaits further exploratory studies. Of these fermentations, with the participation of mixed microbial associations, it is perhaps the production of soy sauce the microbiology of which is best known. The process is controlled by the starters of the koji mold *Aspergillus oryzae* or *A. sojae* and the moromi yeast, *Zygosaccharomyces rouxii* (Hanya and Nakadai, 2003). The 'soy yeast', *Zygosaccharomyces rouxii*, is undoubtedly one of the main producer of aroma compounds, however, less is known about the contribution of some 20 to 25 other yeast species isolated from various stages of soy sauce production. The microbiota of indigenous (traditional, oriental) fermentations is so variable that no definite picture can be drawn on the yeasts (and other microorganisms) present in these products. Preparation of most indigenous fermented products is still a traditional art at small scale rather than a controlled process. At least 20 to 30 yeast species may participate to some degree in the development of the characteristic quality of these naturally fermented foods (Narvhus and Gadaga, 2003; Sanni and Lönner, 1993), while in the fermentation of several commodities it is the lactic acid and other bacteria and/or molds which play the determining role.

8.4.2 Yeast Cell Mass and Commodity Products

In addition to the main fermented foods and beverages, the second major group of commodities include those made from yeast cell mass or cell-derived products. Among these are pressed baker's yeast and active dried yeast, food and feed yeasts, yeasts autolysates and extracts, as well as cell components such as enzymes, vitamins, carotenoids, lipids, steroids, polysaccharides, glucans, nucleotides, flavours and many others. Several of these are important ingredients and adjuncts in the production of food and beverages, whereas others find application in chemical, pharmaceutical, cosmetic and other industries. It would be far beyond the space of this chapter to give an overview of all these, and reference is made to comprehensive reviews appeared previously and more recently (Reed and Nagodawithana, 1991; Halasz and Lasztity, 1991; Abbas, 2006).

Pichia jadinii, better known in asexual form as *Candida utilis*, is the most widely used species for the production of cell mass for animal feed. It grows abundantly on molasses, and to a certain degree also on agricultural and industrial wastes (wood hydrolysate, sulfite liquor). It has been an ongoing effort of research to find or develop a yeast species or strain being able directly utilizing lignocellulosic materials, for the bioconversion of renewable agricultural products and residues to feedstock and/or industrial fuel. Although a few yeast species has the metabolic capability to hydrolyse starch (e.g. *Debaryomyces occidentalis, Saccharomycopsis fibuligera*), and to utilize cellobiose and xylose after the partial

hydrolysis of woody materials (e.g. *Candida shehatae, Pichia stipitis*), the economically feasible solution has not yet achieved, even with genetically engineered strains (see below) (Jeffries and Kurtzman, 1994; Leathers, 2003).

A large number of yeast species has been recognized for their ability to utilize hydrocarbons as sole carbon and energy sources (e.g. *Candida maltosa, C. tropicalis, Yarrowia lipolytica* and many others; (Tanaka and Fukui, 1989; Fickers et al., 2005). In the 1970es, large industrial plants were set up to produce single cell protein on this source. After the explosion of oil prices, this technology became unprofitable and ceased. Nowadays, hydrocarbon utilizing yeasts can be used for the degradation of oil spills and remediation of the environment. A large group of yeasts, however, is capable of utilizing methanol, which could serve for an inexpensive source of producing single cell proteins from the anaerobic decomposition of agricultural wastes.

Baker's yeast is a main product as well as a source of many derived products together with spent brewer's yeast. However, beyond *Saccharomyces cerevisiae*, increasing lists of other yeast species are being exploited in producing and manufacturing these commodities. Whey is a major waste in the dairy industry, and lactose utilizing yeasts, such as *Kluyveromyces marxianus*, can be used for the production of protein-rich cell mass as well as valuable bioingredients, oligonucleotides, flavor enhancers (Belem and Lee, 1998). Further examples are: *Candida utilis* for feed, *Kluyveromyces lactis* for aromas and lactase, *Rhodotorula glutinis, Sporobolomyces pararoseus, Phaffia rhodozyma* for carotenoids and colorants, *Rhodotorula glutinis* also for lipids, *Debaryomyces (Schwanniomyces) occidentalis* for amylase, *Eremothecium ashbyi* for riboflavin, *Yarrowia lipolytica* for citric acid and lipase, *Sporidiobolus salmonicolor* for flavor compounds (Dufossé et al., 2002).

Pectinolytic enzymes are important in the food industry for improving juice extraction and clarification. Instead of addition of pectinases, *Saccharomyces cerevisiae* wine strains can be transformed to constitutively overexpress its own endopolygalacturonase gene (Fernández-González et al., 2005). Production of pectinases is not uncommon among yeasts; 7% of species belonging to six genera isolated from tropical fruits secreted pectinolytic enzymes (da Silva et al., 2005).

8.4.3 Bioethanol

Considering the exploitation of yeasts beyond the field of food and beverages, the most important biotechnological application is the production of bioethanol for gasoline additive or even substitute. In some countries, particularly Brazil, and also in the USA and Canada, considerable amount of ethanol is fermented from cane juice or other sugar-rich agricultural raw materials (Wheals et al., 1999). *Saccharomyces cerevisiae* is used for this purpose, and current interest is directed to the improvement of fermentation technology and to the utilization of cheap agro-industrial by-products or wastes. In this regard, the conversion to ethanol of

lignocellulosic hydrolysates with yeasts, such as *Pichia stipitis, Candida shehatae* or *Pachysolen tannophilus*, which can ferment cellobiose and xylose, is of primary concern. The ethanol yield is far less than in the case of *Saccharomyces cerevisiae,* and approaches have been made to the genetic transformation of *Saccharomyces cerevisiae* with genes for xylose fermentation (Kuyper et al., 2005). A multitransformant strain containing not less than four foreign genes was engineered capable of directly degrading cellulose (van Rensburg et al., 1998). Wild type of a methylotrophic yeast, *Pichia angusta* (*Hansenula polymorpha*) is able to ferment cellobiose and xylose to ethanol (Ryabova et al., 2003). *Kluyveromyces marxianus* can be used to ferment inulin and produce ethanol from many plant feedstock. Recently, a strain of *Kluyveromyces marxianus* has been used for bioethanol production also from cheese whey (Kargi and Ozmihci, 2006).

8.4.4 Pharmaceutical and Bioactive Products

As a further biotechnological extension, yeasts can be utilized for the production of compounds of pharmaceutical value. Few of these can be obtained from natural strains of *S. cerevisiae* or non-conventional yeasts, and more by genetically modified (GM) strains expressing heterologous proteins. Since the beginning of the 1980s a number of vaccines, antigens, hormones and other biotherapeutic compounds have been cloned into yeasts and expressed at laboratory scale, and some of these (e.g. insulin, interferon, hepatitis A antigen) have reached commercial production as well. GM yeasts will be discussed below in more details. In biotherapeutic respect, the potential use of yeast as probiotics should be mentioned. Compared with the widely accepted probiotic activity of lactic acid bacteria and bifidobacteria, yeasts are less recognized although some strains of *Saccharomyces cerevisiae* referred to as *'Saccharomyces boulardii'* have been used to control gastrointestinal disorders (McFarland et al., 1993). More recently, it has been shown that viable and dead cells, in particular cell wall preparates (glucomannans) can be applied to bound and remove mycotoxins from the intestine of poultries and also from juices (Bejaoui, 2004; Yiannikouris et al., 2004; Basmacioglu et al., 2005), moreover, a new yeast species, *Trichosporon mycotoxinovorans*, was described with the ability to degrade mycotoxins (Molnar et al., 2004).

8.4.5 Other Uses of Yeasts

Miscellaneous further, potential applications of yeasts relate to both foods and other biotechnological products and processes. Yeasts of certain capability of biodegradation have been considered for bioremediation and action in environmental protection. Hydrocarbon assimilating yeasts may be useful for the degradation of oil spills, yeast cells as biosorbent can be used for the removal of heavy metals and

radioactive isotopes, and stains of *Trichosporon cutaneum* and the yeast-like *Aureobasidium pullulans* able to degrade phenols and other aromatic compounds can be used for their removal from industrial effluents. Olive oil manufacture results in large quantities of black wastewaters due to phenolic compounds which could be decolorized by depolymerization of the phenolics by *Geotrichum candidum* (Ayed et al., 2005). Another case is the reduction of the pesticide, glyphosphate residues in wheat flour during proofing of yeasted dough (*Saccharomyces cerevisiae*) as demonstrated by Low et al. (2005).

An area attracting growing interest is the application of yeasts for biocontrol. Some yeast species, in particular *Pichia guilliermondii, P. anomala*, and *Debaryomyces hansenii* inhibit the growth of certain moulds attacking fruits and grains. The possible use of antagonistic yeasts to control post-harvest diseases and production of mycotoxins has been reviewed (Wisniewski and Wilson, 1992; Druvefors and Schnürer, 2005; Suzzi et al., 2005).

8.5 Improvement of Yeast Strains Used in Production

Since the creation of the first pure cultures of yeast, intensive research has been carried out leading to production of industrial strains with improved properties, made first by selection and hybridization, later by protoplast fusion and cytoduction, and from the 1980s on, by genetic engineering. These studies have been excellently reviewed by Hammond (1995); Dequin (2001); Pretorius and Westhuizen (1991); Schuller and Casal (2005).

In the field of traditional food and beverage fermentations, only a few will be mentioned of the broad purposes of improvement for technologically important properties. Among these were in brewing: carbohydrate utilization, fermentation of dextrins, flocculation and filtration, reduction of H_2S and diacetyl production, osmotolerance (high gravity wort); in baking: fast dough raising, organic acid resistance, rehydration tolerance; in wine making: ethanol tolerance, fermentation capacity, absence of off-flavours. These targets have been achieved with some success by the application of classical genetic techniques such as mutagenesis and hybridization followed by selection, and more recently protoplast fusion and cytoduction. However, a major limitation of these classical genetic techniques has been in general the difficulty of adding or removing one feature without altering gross performance. In particular, the stable genetic constitution of polyploid/aneuploid industrial strains, lack of mating type characteristics, and poor sporulation all restricted the possibilities of broad strain improvement. The potential of recombinant gene technology (genetic engineering) has provided more possibilities, and holds out much promises of specific modifications.

The principal aims of genetic modification is the transformation of host cell by introduction of foreign genes. It is beyond the scope of this chapter to go into details of the techniques for transformation and cloning (only few of the extensive list of reference manuals: Broach et al. (1991); Evans (1996); Jones et al. (1992);

Pringle et al. (1997). Briefly, the major steps are: 1. identifying the target gene and obtaining the DNA fragment from a genomic cDNA library or by PCR amplification; 2. creating a suitable plasmid vector; 3. joining the DNA fragment to the vector DNA generating a recombinant DNA molecule; 4. inserting the recombinant into host cell; 5. screening transformed cells and selecting the target gene using appropriate marker system.

Yeasts are excellent hosts for the production of recombinant proteins, offering ease of genetic manipulation, and cultivation to high cell density with a fast growth rate. Moreover, yeasts are able to perform complex eucaryotic-type posttranslational modification and produce proteins similar to mammalian origin. *S. cerevisiae*, the genetically best characterized organisms, is the host used most frequently for transformation. However, the *S. cerevisiae* transformation system has some limitations in that the proteins are often overglycosylated and may contain a terminal group suspected to be allergenic; the yield of recombinant proteins is relatively low, and the narrow substrate specificity of the species limits fermentation design. Some of the non-conventional yeasts, such as *Pichia pastoris, Pichia angusta (Hansenula polymorpha*) and others, may be more advantageous host, although the number of cloned genes, the availability of molecular genetic tools, and the understanding of metabolic regulation are limited compared with *Saccharomyces cerevisiae* (Cereghino and Cregg, 2000).

The primary approaches have been directed to the genetic improvement of the production characteristics of *Saccharomyces cerevisiae* starter strains used in brewing, wine making and baking. Table 8.3 gives some examples of these. Note, that in several cases the genetic modification is achieved by self-cloning, i.e. the GM strain does not contain foreign gene from organisms other than *Saccharomyces cerevisiae*.

Developing of transgenic strains has been extended to the broader field of biotechnology, in particular for the production of bioethanol and pharmaceuticals. Sequential introduction of multiple genetic alterations into a single host genome is now not exceptional. Examples are the total biosynthesis of the steroid hydrocortisone involving as many as 13-engineered genes (Szczebara et al., 2003), and a celluluse fermenting yeast containing genes from four different organisms (van Rensburg et al., 1998).

In this regard it is worth mentioning, that screening among yeast isolates from natural sources revealed rich sources of cellulose decomposing strains, several of which turned out to be novel species (Buzzini and Martini, 2002; Nakase et al., 1994; Carreiro et al., 2004). A potential producing strains may well be found among these isolates.

Genetic modification of microorganisms and, in particular, crop plants, have been the subject of big controversy and being debated heavily both in scientific circles and by the general public. In these days, the great developments and achievements already made in the field should be taken seriously, and the issues arising from technological, environmental, economic, social, ethical and political point of views should be discussed critically and rationally (Pretorius, 2000; Schuller and Casal, 2005; Verstrepen et al., 2006). Concerns about GMOs and GM

Table 8.3 Genetically modified *Saccharomyces cerevisiae* starters for brewing, baking and wine making

Improvement	Proteins, genes	Sources
Wine yeast		
Clarification, no haze	Pectate lyase *pelA*	*Erwinia chrysanthemi*
Endopolygalacturonase	*PGU1*	*S. cerevisiae*
Flocculation	Flocculin *FLO1*	*S. cerevisiae*
Flor formation	Adhesin *FLO11*	*S. cerevisiae*
Stress tolerance	Trehalose *TPS1,2*	*S. cerevisiae*
Ethanol tolerance	Sterols *SUT1*	*S. cerevisiae*
Glycerol overproduction	Glycerol-P-dehydr. *GPD1*	*S. cerevisiae*
Resveratrol production	β-Glucosidase *bglN*	*C. molischiana*
Malolactic fermentation	Permease, *mae1* malic enzyme *mleS*	*Schizo. pombe* *Lactococcus lactis*
Brewer's yeast		
Dextrin fermentation	Glucoamylase *STA2* Amyloglucosidase *AMG*	*S. cerev.* var. *diastaticus* *Aspergillus awamori*
Flocculation	Glucanase EG1	*Trichoderma reesii*
Diacetyl elimination	Acetoacetate decarboxylase *ALDC*	*Enterobacter aerogenes*
Reduced H_2S production	Sulfuhydrase *MET25*	*S. cerevisiae*
Acetate esters production	Acetyltransferase *ATF1*	*S. cerevisiae*
Antibacterial property	Pediocin *pedA* Leucocin *lcaB*	*Pediococcus cerevisiae* *Leuconostoc carnosum*
Baker's yeast		
Melibiose utilization	α-Galactosidase *MEL1*	*S. bayanus*
Maltose utilization	Stronger promoter *ADH*	*S. cerevisiae*
Cryoresistance	Aquaporin AQY1	*Sch. pombe*
Osmotolerance	Glycerol synthesis GPD1	*S. cerevisiae*

Data from: Randez-Gil et al. (1999); Dequin (2001); Schuller and Casal (2005); Hammond (1995); Panadero et al. (2005); Pretorius (2000); Pretorius et al. (2003); Verstrepen et al. (2006); Gonzalez-Candelas et al. (1995).

products are beyond the scope of this review. Regarding microorganisms only, it should be realized, however, that vaccines, drugs, enzymes produced by genetically engineered strains have been on the market for years, and are not just beneficial but also indispensible. Several of them are produced by GM yeasts, such as interferons, somatostatin, insulin, chymosin and others.

A different issue is, however, when not the purified product but the organisms itself containing foreign genes is included in the consumables or foods. Baker's yeast with high maltase activity, brewer's yeast with glucoamylase for dextrin hydrolysis, and a sake yeast with enhanced ethyl caproate flavor (Akada, 2002) have got approval by respective authorities, however, have not been commercialized because the lack of public acceptance refrained industry from putting to use (Moseley, 1999). Recently, the appearance on the US market of recombinant wine yeast capable of malolactic fermentation may sign a breakthrough in this respect (Cummins, 2005).

Examples for the expression of heterologous genes in *S. cerevisiae* and other yeast hosts are listed in Tables 8.4 and 8.5.

Table 8.4 Genetic modification of *Saccharomyces cerevisiae* expressing foreign genes

Foreign gene	Donor species	Result
β-Galactosidase	*Kluyveromyces lactis*	Lactose utilization
L-Galactose dehydrogenase	*Arabidopsis thailana*	Ascorbic acid (vitamin C)
α-Amylase^{+}	*Lipomyces kononenkoae*	
Glucoamylase	*S'copsis fibuligera*	Starch fermentation
Xylose isomerase	*Piromyces* sp. fungus	Xylose fermentation
α-Glucuronidase	*Aureobasidium pullulans*	Xylan degradation
Cellobiase	*Endomyces fibuliger*	Cellulose degradation
$^{+}$Endo-β-glucanase	*Butyrivibrio fibrisolvens*	
$^{+}$Cellobiohydrolase	*Phaanerochaete chrysosporium*	
$^{+}$Cellodextrinase	*Ruminococcus flavefaciens*	
Pectate lyase	*Fusarium solani*	Pectin hydrolysis
Eight foreign genes and disruption of five host genes	Mammalian	Hydrocortison

Data from Rubio-Teixera et al. (2000); Sauer et al. (2004); Knox et al. (2004); Kuyper et al. (2005); de Wet et al. (2006); van Rensburg et al. (1998); Szczebara et al. 2003.

Table 8.5 Examples of the production of foreign proteins in non-conventional yeasts

Yeast	Protein	Year of publication
Schizosaccharomyces pombe	Invertase from *S. cerevisiae*	1985
	α-amylase from *D. occidentalis*	1989
	Glucoamylase from *S. diastaticus*	1986
Pichia pastoris	β-galactosidase	1987
	Hepatitis B antigen	1987
	Bovine lysozyme	1989
	Human epidermal growth factor	1990
Pichia angusta	β-lactamase	1988
(*Hansenula polymorpha*)	Glucoamylase	1991
	Human serum albumin	1990
Kluyveromyces lactis	Prochymosin	1990
	Human serum albumin	1991
	α-Amylase from *D. occidentalis*	1989
Yarrowia lipolytica	Porcine α-interferon	1990
	Bovine prochymosin	1988
	Human proinsulin	1993
Zygosaccharomyces bailii	Lactate dehydrogenase	2004

Data from Romanos et al. (1992); Madzak et al. (2004)

8.6 Conclusions

The use of selected strains of *Saccharomyces cerevisiae* has provided tremendous advantages in traditional fermentation and novel biotechnology industries. The methods of conventional breeding, hybridization and selection, though have already resulted in numerous innovations and improvement in the properties of traditional starters, are nevertheless somewhat limited in their capacity. The application of

molecular techniques and recombinant gene technologies, as further possible ways for the development of novel starters will have to receive serious consideration in the future. Introduction of foreign genes into baking, brewing and wine yeasts, and to a number of non-conventional yeast species, has resulted in many improved strains genetically modified at laboratory scale. Only few of them have got legal approval but the lack of public acceptance refrained industry from commercial application. Hence, the exploration of the rich and yet only partially known biodiversity of natural ecosystems, among them indigenous fermentations, is a promising and challenging way for the quest of novel potential starters and adjuncts in the production not only of food and beverages, but also across various biotechnology sectors from bioenergy and pharmaceuticals to bioremediation and environmental protection. Yeasts have been and will continue being important contributors to benefit our life.

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