Pietro Buzzini · Marc-André Lachance
Andrey Yurkov *Editors*

Yeasts in Natural Ecosystems: Ecology

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Preface

Yeasts are the smallest eukaryotic organisms. They are fungi that share the unique morphological adaptation of growing as predominantly unicellular organisms and multiplying through budding. Since their first discovery as the fermentative agent in wine and beer, yeasts have been used widely for the production of beverages, food, organic acids, enzymes, proteins, lipids, and pigments. However, unlike the domesticated yeast *Saccharomyces cerevisiae* (baker's yeast), other species do have habitats in nature. Ecology explores organisms in the context of their environment. This includes the chemical, physical, and psychological properties which together describe habitats in which an organism can live. However, successful colonization and persistence in a habitat depends on other organisms and their interactions. Each species constantly modifies the environment through its own activities that make a habitat a dynamic system. Accordingly, a yeast does not occur as a pure culture but coexists with other species in a microbial community or microbiome. Although yeasts are among the earlier colonizers of nutrient-rich substrates, their role in ecosystem processes is not limited to the consumption and transformation of simple sugars. Yeasts participate in the degradation of complex organic substances and also synthesize, accumulate, and release organic molecules into the environment. They also act as primary and secondary decomposers in ecosystems and serve as a source of nutrients for micro- and macroorganisms in the food web.

Why did we decide to assemble this book? Almost every book dealing with the biology of yeasts also introduces the reader to ecology. The large taxonomic compendium *The Yeasts: A Taxonomic Study* included in its more recent editions a chapter on yeast ecology. Several books and book series such as *The Life of Yeasts* (Phaff, Miller, Mrak), *The Yeasts* (Rose and Harrison), *Yeasts in Natural and Artificial Habitats* (Spencer and Spencer), and *Biodiversity and Ecophysiology of Yeasts* (Rosa and Peter) published in the last four decades covered major advances in the ecology of yeasts. With this book, we attempt to give an update on topics covered in previous books, introduce new subjects, and provide novel views on selected aspects of yeast ecology.

Our knowledge of yeast ecology derives from studies of yeast properties and available records of isolation sources. Research on applications of yeasts in food production and biotechnology or as model organisms in science overshadows ecological studies. As a result, the physiological properties of yeast species are better known than their habitats. Many species are documented from only a limited number of strains, and their original taxonomic descriptions do not always describe the habitat or community and biotic interactions. Yeasts constantly interact with animals, plants, and other fungi in the environment. They also engage in close relationships with other living organisms as mutualists, competitors, parasites, and pathogens. Thus, alterations of the environment may lead to rapid changes in local yeast communities—for example, a yeast species may become extinct in the absence of its host or vector. Indirect effects of climate and vegetation type on belowground yeast communities have been also demonstrated.

The book presents a comprehensive overview on different aspects of yeast ecology and constitutes the first volume of a whole monograph on *Yeasts in Natural Ecosystems*, of which the second volume (assembled by the same editors) is dedicated to yeast diversity. It shows how views on yeasts have changed with the discovery of new species and new methods to study them. All chapters review the knowledge accumulated during research carried out in the past decades. Yeast species cited in these works were often identified by different techniques and criteria that may not be as accurate as the current sequence-based approaches. Many species names cited in the early literature are not current. Accordingly, all original taxonomic designations reported in the cited references were checked and, if necessary, updated following the latest taxonomic guidelines published in Kurtzman et al. (2011), Liu et al. (2015), Wang et al. (2015a, 2015b), or more recent literature. A unified list of abbreviations was prepared to assist readers in following species names throughout the book.

The selection of topics and invitation of potential contributors were made by the three editors. Chapters were edited and managed by P. Buzzini and A. Yurkov. The editors thank all the authors for their excellent contributions. We also acknowledge researchers for granting access to public repositories of publications and sharing unpublished results.

P. Buzzini is grateful to Ann Vaughan-Martini and dedicates this book to the memory of his teacher (and friend) Alessandro Martini.

A. Yurkov is grateful to his teachers, soil microbiologists, and yeast ecologists Inna Babjeva and Ivan Chernov. A few sections of the book review their work and are dedicated to the memory of Ivan Chernov, who studied the distribution of yeasts across many terrestrial biomes.

A. Yurkov acknowledges the research network of yeast scientists promoted by the van Uden International Advanced Course on Molecular Ecology, Taxonomy and Identification of Yeasts. Many of the authors of this book were participants and later lecturers in this course in various years.

M. A. Lachance is grateful to P. Buzzini and A. Yurkov for their invitation to join the editorial team in a mostly advisory capacity.

Finally, the editors would like to thank the Springer team, especially Isabel Ullmann and Dr. Andrea Schlitzberger, for their valuable and continuous support during the preparation of this book.

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Abbreviations

The following abbreviations are used for names of microbial genera (including synonyms) cited in the text.

Acinetobacter	*Acin.*
Anthracocystis	*Anthr.*
Apiotrichum	*Ap.*
Babjeviella	*Bab.*
Candida	*C.*
Clavispora	*Cl.*
Colacogloea	*Col.*
Cryptococcus	*Cr.*
Cutaneotrichosporon	*Cut.*
Cyberlindnera	*Cyb.*
Debaryomyces	*Deb.*
Dioszegia	*Di.*
Eremothecium	*Er.*
Filobasidiella	*Fil.*
Filobasidium	*F.*
Goffeauzyma	*Goff.*
Hanseniaspora	*H'spora*
Hansenula	*H.*
Itersonilia	*It.*
Kluyveromyces	*K.*
Kodamaea	*Kod.*
Komagataella	*Kom.*
Leucosporidium	*Leuc.*
Macrorhabdus	*Mac.*
Malassezia	*Mal.*
Metschnikowia	*M.*
Meyerozyma	*Mey.*

Millerozyma	*Mill.*
Mycosarcoma	*Mycos.*
Naganishia	*Na.*
Ogataea	*O.*
Phaffia	*Ph.*
Phaffomyces	*Phaff.*
Pichia	*P.*
Pleurococcus	*Pl.*
Pseudomonas	*Pseudom.*
Rhodotorula	*Rh.*
Saccharomyces	*S.*
Saccharomycopsis	*Sacch.*
Saitozyma	*Sa.*
Solicoccozyma	*Sol.*
Sporobolomyces	*Sp.*
Starmerella	*Starmer.*
Streptococcus	*Str.*
Takashimella	*Tak.*
Taphrina	*Taph.*
Tausonia	*Ta.*
Tetragoniomyces	*Tetrag.*
Tilletiopsis	*Till.*
Trichosporon	*Tr.*
Ustilago	*U.*
Ustilentyloma	*Ust.*
Vanrija	*Va.*
Vishniacozyma	*Vishn.*
Wickerhamiella	*Wick.*
Wickerhamomyces	*W.*
Zygosaccharomyces	*Zygosacch.*

Chapter 1
Yeasts as Distinct Life Forms of Fungi

Cletus P. Kurtzman and Teun Boekhout

Abstract Detection, identification, and classification of yeasts have undergone major changes since application of gene sequence analyses and genome comparisons. Development of a database of barcodes consisting of easily determined DNA sequences from domains 1 and 2 (D1/D2) of the nuclear large subunit rRNA gene and from ITS now permits many laboratories to identify species quickly and accurately, thus replacing the laborious and often inaccurate phenotypic tests previously used. Phylogenetic analysis of gene sequences is leading to a major revision of yeast systematics that will result in redefinition of nearly all genera. This new understanding of species relationships has prompted a change of rules for naming and classifying yeasts and other fungi, and these new rules were recently implemented in the *International Code of Nomenclature for algae, fungi, and plants* (Melbourne Code). The use of molecular methods for species identification and the impact of Code changes on classification will be discussed.

Keywords Yeasts • Taxonomy • Molecular systematics • Evolution

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1.1 Introduction

The title of this chapter, "Yeasts as Distinct Life Forms of Fungi," challenges us to ask if yeasts represent a unique phylogenetic group or whether the yeast morphotype is common in many lineages of the Mycota. The most commonly known yeast, the ascomycete *Saccharomyces cerevisiae*, is widely used for production of bread, beer, wine, and many other fermentation products. The simplicity of its life cycle, asexual reproduction by budding and a sexual cycle represented by ascospore formation in a single cell ascus, suggests that it must be a primitive fungus (Guilliermond 1912), or could it be a reduced form among more mycelial taxa, as proposed by Cain (1972) and von Arx and van der Walt (1987)? Initially, it appeared that all yeasts were ascomycetes, but that belief changed with the discovery that some yeasts are basidiomycetes (Kluyver and van Niel 1924, 1927; Banno 1967; Nyland 1949).

The first indication of the phylogenetic placement of ascomycete yeasts among the fungi came from the study of Walker (1985), who proposed from analysis of 5S rRNA sequences that Ascomycota is comprised of three major groups: (1) *Schizosaccharomyces* and *Protomyces* (Taphrinomycotina), (2) budding yeasts (Saccharomycotina), and (3) the "filamentous fungi" (Pezizomycotina). Some species of the Pezizomycotina are dimorphic, but have sexual states that are formed in or on a fruiting body, which is typical of this subphylum. A multitude of additional studies, ranging from single genes to whole genomes, have shown these same basic relationships among the Ascomycota (e.g., Kurtzman and Robnett 1998, 2013; Fitzpatrick et al. 2006; James et al. 2006; Hittinger et al. 2015; Shen et al. 2016). For the Basidiomycota, single gene sequences and whole genome analyses have demonstrated placement of yeast forms in all of the major lineages. An overview of the phylogeny of the Mycota that was determined from whole genome analyses is given in Fig. 1.1.

In an effort to explain the basis for budding versus filamentous growth, Nagy et al. (2014) compared 59 genomes of filamentous, dimorphic, and yeast-forming fungi. It appears that expression of the zinc cluster transcription factors regulates which morphotype predominates. This mechanism may have arisen at the base of the *Dikarya* and shows varying expression in different lineages of the Mycota. However, this finding alone does not explain why yeasts of various lineages form sexual states not enclosed in a fruiting body, whereas dimorphic fungi in nearby

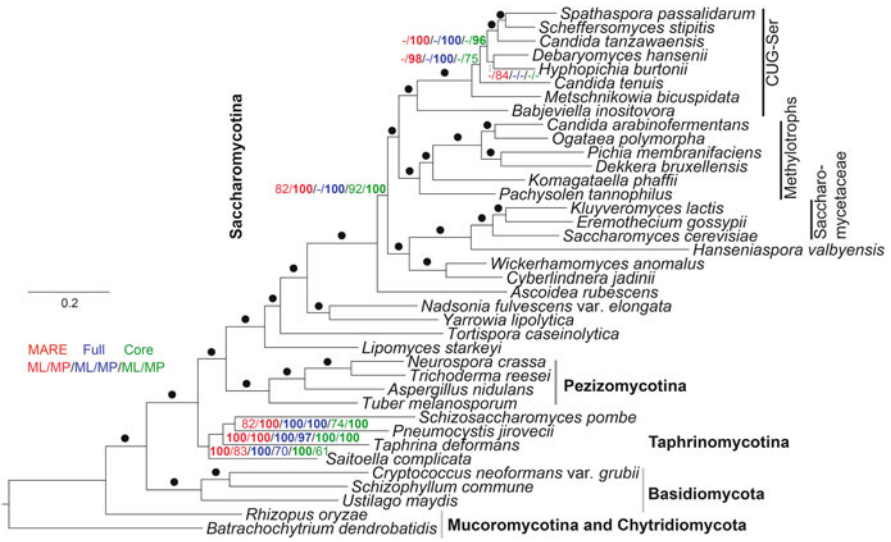

Fig. 1.1 Phylogenetic tree inferred from the MARE-filtered supermatrix (364,126 aligned amino acid residues) using maximum likelihood (ML) and rooted with *Batrachochytrium*. *Numbers* on the branches indicate ML and maximum parsimony (MP) bootstrap support values for the MARE-filtered (*red*), full (*blue*), and core genes (*green*) supermatrices. Values less than 60% are shown as *dashes; dots* indicate branches with maximum support under all settings. Yeasts in the CUG-Ser clade use an altered genetic code in which CUG codons are translated as serine rather than the canonical leucine (Santos et al. 1997, 2011). One further modification was found for *Pachysolen tannophilus*, in which CUG codes for alanine (Riley et al. 2016). (Modified from Riley et al. 2016, with permission)

lineages do form fruiting bodies. Because yeasts can occur in various lineages of the Mycota, the definition of yeasts is presently based on morphology and has become fungi, ascomycetes or basidiomycetes, that asexually reproduce by budding or fission and that have a sexual state not enclosed in a fruiting body.

In the following sections, we will discuss placement of yeasts within the taxonomic framework of the Ascomycota and Basidiomycota. Application of molecular methods for species identification has resulted in discovery of a large number of new species and genera. For comparison, the fourth edition of *The Yeasts: A Taxonomic Study* (Kurtzman and Fell 1998) listed 97 genera and 700 species, whereas the fifth edition (Kurtzman et al. 2011a) includes 150 genera and 1500 species. Since the publication of the fifth edition in 2011, many new species and genera of both ascomycetes and basidiomycetes have been described.

In addition to the substantial changes in classification brought by DNA sequence comparisons, recent changes in the rules for classification of fungi are having a major impact on naming of taxa. The classification of yeasts and other fungi previously was governed by the rules of the *International Code of Botanical Nomenclature* (e.g., McNeill et al. 2006), which based taxonomy on sexual states of fungi and required separate names for asexual "form genera." The ability to

group sexual and asexual states within a clade using DNA characters eventually led to a logical change in the rules of nomenclature, and the latest edition of the Code (*International Code of Nomenclature for algae, fungi, and plants*) (McNeill et al. 2012) ends "dual" nomenclature for fungi and requires inclusion of sexual and asexual states within monophyletic groups, which then have a common genus name.

1.2 Ascomycota

1.2.1 Saccharomycotina

For the Ascomycota, yeast species are found exclusively in the subphyla Saccharomycotina and Taphrinomycotina (Fig. 1.1), and a listing of currently accepted genera is given in Table 1.1. Phylogenetic relatedness among genera was examined by Kurtzman and Robnett (2013) from analysis of concatenated sequences from the nearly entire genes for nuclear large subunit rRNA, nuclear small subunit rRNA, translation elongation factor EF-1α, and RNA polymerase II subunit 1 and subunit 2 for type species (Fig. 1.2). Not surprisingly, the five-locus dataset shows much lower bootstrap support of basal branches than seen in whole genome analyses (e.g., Fig. 1.1), but the overall topology is similar for both trees, although many species are not represented in the whole genome tree. In both trees, *Lipomyces* is the earliest diverging genus in the Saccharomycotina, and perhaps of significance, species of *Lipomyces* are predominantly isolated from soil.

One of the most urgent problems for classification of ascomycete yeasts is the asexual genus *Candida*, which has over 400 described species (Lachance et al. 2011; Daniel et al. 2014). The genus was circumscribed on *Candida vulgaris* (= *Candida tropicalis*), although the majority of *Candida* species are not members of this clade, which also includes *Candida albicans* and *Candida dubliniensis*. Previously, many unrelated yeasts without known sexual reproduction were placed in *Candida*, often because of lack of taxonomic characters needed to group related species. Phylogenetic analyses of molecular characters led to the early recognition that species of the genus *Candida* are distributed throughout the Saccharomycotina with some species as members of ascosporic (sexual) clades, whereas others form isolated lineages that appear to correspond to independent genera without known sexual states (Kurtzman and Robnett 1998). Where sufficient DNA data are available, some divergent species already have been assigned to new genera (Table 1.1), such as *Alloascoidea*, *Danielozyma*, *Deakozyma*, *Diddensiella*, *Diutina*, *Groenewaldozyma*, *Hagleromyces*, *Hemisphaericaspora*, *Martiniozyma*, *Middelhovenomyces*, *Spencermartinsiella*, *Suhomyces*, *Teunomyces*, *Tortispora*, and *Yueomyces*, and other species await transfer as more robust datasets are developed. It is anticipated that a recircumscribed *Candida* will include the approximately 40 species that now form the *C. tropicalis* clade.

Table 1.1 Presently accepted genera of Saccharomycotina and Taphrinomycotina

Subphylum Saccharomycotina[a]	
Aciculoconidium D.S. King & S.-C. Jong (1976)	*Metschnikowia* Kamienski (1899)
Alloascoidea Kurtzman & Robnett (2013)	*Meyerozyma* Kurtzman & M. Suzuki (2010)
Ambrosiozyma van der Walt (1972)	*Middelhovenomyces* Kurtzman & Robnett (2014)
Ascoidea Brefeld & Lindau (1891)	*Millerozyma* Kurtzman & M. Suzuki (2010)
Babjeviella Kurtzman & M. Suzuki (2010)	*Myxozyma* van der Walt, Weijman & von Arx (1981)
Barnettozyma Kurtzman, Robnett & Basehoar-Powers (2008)	*Nadsonia* Sydow (1912)
Blastobotrys von Klopotek (1967)	*Nakaseomyces* Kurtzman (2003)
Botryozyma Shann & M.Th. Smith emend. Lachance & Kurtzman (2013)	*Nakazawaea* Y. Yamada, Maeda & Mikata (1994)
Brettanomyces Kufferath & van Laer (1921)	*Naumovozyma* Kurtzman (2008)
Candida Berkhout (1923)	*Ogataea* Y. Yamada, K. Maeda & Mikata (1994)
Cephaloascus Hanawa (1920)	*Pachysolen* Boidin & Adzet (1957)
Citeromyces Santa María (1957)	*Peterozyma* Kurtzman & Robnett (2010)
Clavispora Rodrigues de Miranda (1979)	*Phaffomyces* Y. Yamada (1997)
Coccidiascus Chatton emend. Lushbaugh, Rowton & McGhee (1976)	*Phialoascus* Redhead & Malloch (1977)
Cyberlindnera Minter (2009)	*Pichia* E.C. Hansen (1904)
Cyniclomyces van der Walt & D.B. Scott (1971)	*Priceomyces* Kurtzman & M. Suzuki (2010)
Danielozyma Kurtzman & Robnett (2014)	*Saccharomyces* Meyen (1870)
Deakozyma Kurtzman & Robnett (2014)	*Saccharomycodes* E.C. Hansen (1904)
Debaryomyces Lodder & Kreger-van Rij (1952)	*Saccharomycopsis* Schiönning (1903)
Diddensiella Péter, Dlauchy & Kurtzman (2012)	*Saprochaete* Coker & Shanor ex D.T.S. Wagner & Dawes (1970)
Dipodascopsis Batra & P. Millner emend. Kurtzman, Albertyn & Basehoar-Powers (2007)	*Saturnispora* Liu & Kurtzman (1991)
Dipodascus de Lagerheim (1892)	*Scheffersomyces* Kurtzman & M. Suzuki (2010)
Diutina Khunnamwong, Lertwattanasakul, Jindamorakot, Limtong & Lachance (2015)	*Schwanniomyces* Klöcker emend. M. Suzuki & Kurtzman (2010)
Endomyces Reess (1870)	*Spathaspora* N.-H. Nguyen, S.-O. Suh & M. Blackwell (2006)
Eremothecium Borzi emend. Kurtzman (1995)	*Spencermartinsiella* Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki & Kurtzman (2011)
Galactomyces Redhead & Malloch (1977)	*Sporopachydermia* Rodrigues de Miranda (1978)
Geotrichum Link:Fries (1832)	*Starmera* Y. Yamada, Higashi, Ando & Mikata (1997)
Groenewaldozyma Kurtzman (2016)	*Starmerella* Rosa & Lachance (1998)
Hagleromyces Sousa, Morais, Lachance & Rosa (2014)	*Sugiyamaella* Kurtzman & Robnett (2007)

(continued)

Table 1.1 (continued)

Hanseniaspora Zikes (1912)	*Suhomyces* M. Blackwell & Kurtzman (2016)
Helicogonium W.L. White (1942)	*Tetrapisispora* Ueda-Nishimura & Mikata emend. Kurtzman (2003)
Hemisphaericaspora Hui, Ren, Chen, Li, Zhang & Niu (2014)	*Teunomyces* Kurtzman & M. Blackwell (2016)
Hyphopichia von Arx & van der Walt (1976)	*Tortispora* Lachance & Kurtzman (2013)
Kazachstania Zubkova (1971)	*Torulaspora* Lindner (1904)
Kluyveromyces van der Walt (1971)	*Trichomonascus* H.S. Jackson emend Kurtzman & Robnett (2007)
Kodamaea Y. Yamada, T. Suzuki, Matsuda & Mikata emend. Rosa, Lachance, Starmer, Barker, Bowles & Schlag-Edler (1999)	*Trigonopsis* Schachner emend. Kurtzman & Robnett (2007)
Komagataella Y. Yamada, Matsuda, Maeda & Mikata (1995)	*Vanderwaltozyma* Kurtzman (2003)
Kregervanrija Kurtzman (2006)	*Wickerhamia* Soneda (1960)
Kuraishia Y. Yamada, Maeda & Mikata (1994)	*Wickerhamiella* van der Walt (1973)
Kurtzmaniella Lachance & Starmer (2008)	*Wickerhamomyces* Kurtzman, Robnett & Basehoar-Powers (2008)
Lachancea Kurtzman (2003)	*Yamadazyma* Billon-Grand (1989)
Lipomyces Lodder & Kreger-van Rij (1952)	*Yarrowia* van der Walt & von Arx (1980)
Lodderomyces van der Walt (1971)	*Yueomyces* Q.M. Wang, L. Wang, M. Groenewald & T. Boekhout (2015)
Macrorhabdus Tomaszewski, Logan, Snowden, Kurtzman & Phalen (2003)	*Zygoascus* M.Th. Smith (1986)
Magnusiomyces Zender (1977)	*Zygosaccharomyces* Barker (1901)
Martiniozyma Kurtzman (2015)	*Zygotorulaspora* Kurtzman (2003)
Subphylum Taphrinomycotina	
Archaeorhizomyces Rosling & T. James (2011)	*Saitoella* S. Goto, Sugiyama, Hamamoto & Komagata (1987)
Burenia M.S. Reddy & C.L. Kramer (1975)	*Schizosaccharomyces* Lindner (1893)
Neolecta Spegazzini (1881)	*Taphridium* Lagerheim & Juel ex Juel (1902)
Pneumocystis Delanoë & Delanoë (1912)	*Taphrina* Fries (1832)
Protomyces Unger (1833)	*Volkartia* Maire (1907)
Protomycopsis Magnus (1905)	

[a]Recent and expected transfer of species to comply with the Melbourne Code:
Trichomonascus species to *Blastobotrys*
Ascobotryozyma species to *Botryozyma*
Dekkera species to *Brettanomyces*
Kloeckera species to *Hanseniaspora*
Schizoblastosporion species to *Nadsonia*
Candida to be comprised of species of the *Candida tropicalis* clade
Saprochaete species to *Magnusiomyces*

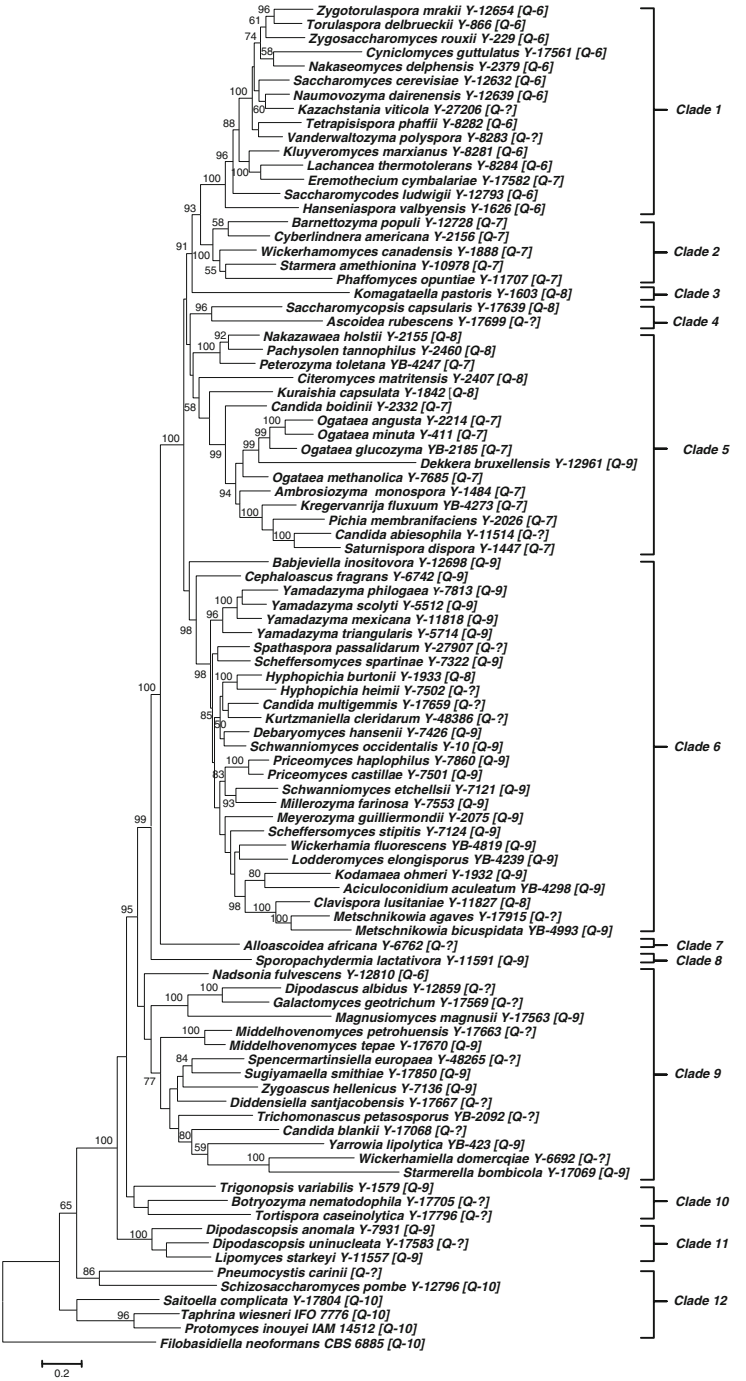

Fig. 1.2 Phylogenetic relationships among type species of ascomycete yeast genera and reference taxa determined from ML analysis using concatenated gene sequences for LSU rRNA, SSU rRNA, EF-1α, RPB1, and RPB2. *Filobasidiella neoformans* was the designated outgroup species in the

In addition to identifying clades of related species, the question arises as to which of these monophyletic lineages represent genera and families. Species in Fig. 1.3 are grouped from whole genome analysis, and this analysis presents a preview of what we might expect to see following more inclusive sequencing. For example, in this analysis Pichiaceae includes the genera *Pachysolen*, *Komagataella*, *Kuraishia*, *Ogataea*, *Brettanomyces*, and *Pichia*. The currently described Saccharomycetaceae shows a noticeable dichotomy, which may indicate that *Lachancea*, *Eremothecium*, and *Kluyveromyces* belong in a separate family sister to the Saccharomycetaceae. It is anticipated that whole genome sequencing of all known species will allow a better prediction of generic and family boundaries. Another issue is placement of newly described species in the correct genus. When these species clearly fall within the confines of a described genus by virtue of being closely related to known species, genus assignment should not be a problem. For more divergent species, the sequence from the commonly used D1/D2 domains of the nuclear large subunit rRNA gene that is used for species identification may not have enough resolution for genus assignment. Most taxonomists are unlikely to determine the genome sequence of each new species, so perhaps a selection of 5–10 genes would suffice for reliable genus placement of divergent species.

1.2.2 Taphrinomycotina

A major surprise from DNA sequence comparisons was the discovery that the genus *Schizosaccharomyces* is not in the same subphylum as *Saccharomyces cerevisiae* (e.g., Fig. 1.1). Besides *Schizosaccharomyces*, the subphylum Taphrinomycotina includes such diverse genera as *Taphrina*, *Protomyces*, *Pneumocystis*, and *Archaeorhizomyces*, the latter a slow-growing fungus associated with pine rootlets (Rosling et al. 2011) but previously detected in soil through metagenomic analyses. Many of the genera (Table 1.1, Kurtzman et al. 2011a) assigned to Taphrinomycotina are plant pathogens, but *Pneumocystis* is a common cause of pneumonia in HIV/AIDS patients. In contrast to Saccharomycotina, relatively few species of Taphrinomycotina are known, which suggests that many more species may yet be found in this earlier diverging subphylum.

Fig. 1.2 (continued) analysis. *Names in bold font* are type species of currently recognized genera, whereas names in standard font are not type species. Data for *Pneumocystis*, *Protomyces*, and *Taphrina* are from James et al. (2006). *Pneumocystis* is represented by the type species, but not the type strain. *Protomyces* and *Taphrina* are not represented by type species. Bootstrap values (1000 replicates) >50% are given at branch nodes. Strain accession numbers are NRRL unless otherwise indicated. *Designations in brackets* indicate the coenzyme Q value for each species. (from Kurtzman and Robnett 2013)

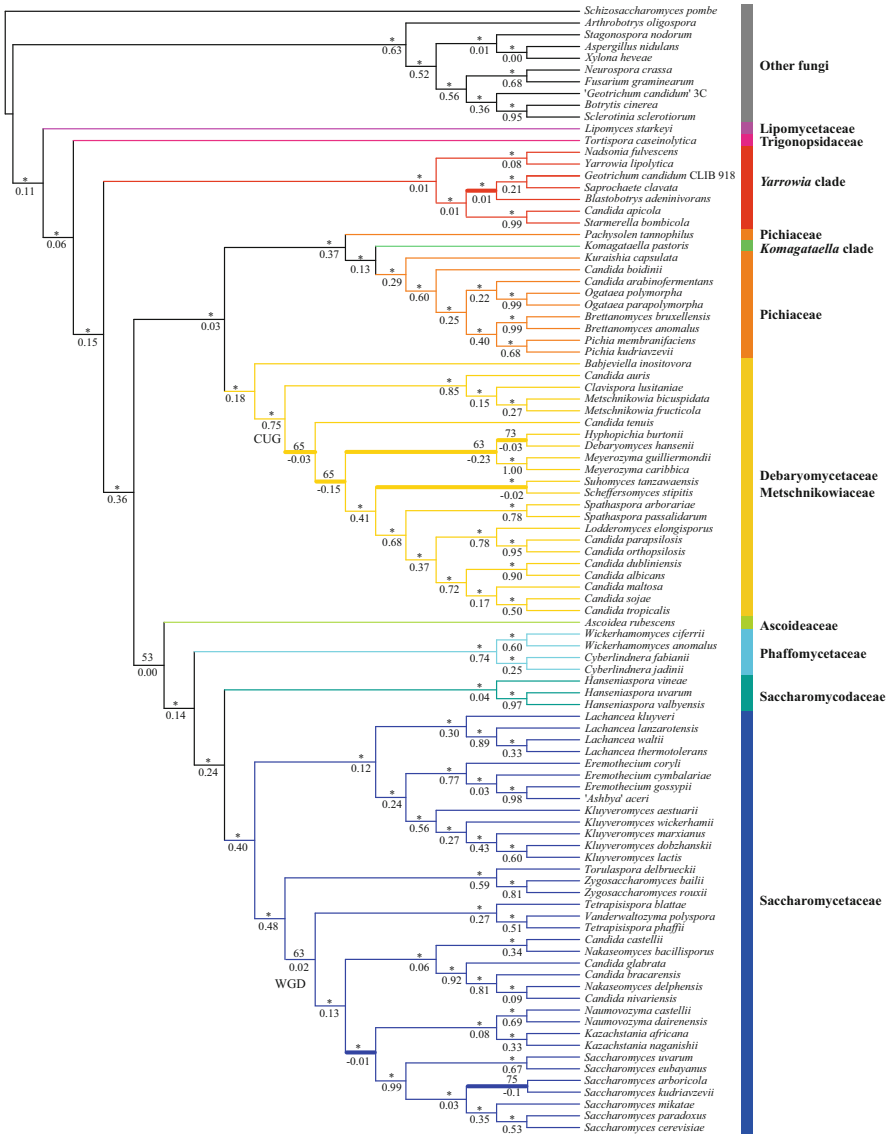

Fig. 1.3 The phylogenetic relationships of Saccharomycotina yeasts inferred from the coalescence-based analysis of a 1233 single-copy BUSCO gene amino acid (AA) data matrix. The coalescence-based phylogeny estimation was conducted using ASTRAL version 4.7.7 (Mirarab et al. 2014). Branch support values near internodes are indicated as bootstrap support value (*above*) and internode certainty (*below*), respectively. *Asterisk* indicates bootstrap support values greater than or equal to 95%. *Thicker branches* show conflicts between coalescence-based phylogeny and concatenation-based phylogeny (Shen et al. 2016, with permission)

1.3 Basidiomycota

The basidiomycetous nature of some yeasts was first suggested by the presence of ballistoconidia in the red yeast *Sporobolomyces* (Kluyver and van Niel 1924, 1927), later by the presence of budding cells, ballistoconidia, clamp connections, and smut-like teliospores in *Sporidiobolus* (Nyland 1949), and convincingly by the discovery of mating and a sexual state in *Rhodotorula glutinis* (Banno 1967). Unicellular stages, or yeasts, occur in all three lineages of phylum Basidiomycota, namely, Agaricomycotina, Pucciniomycotina, and Ustilaginomycotina (Hibbett et al. 2007; Boekhout et al. 2011). Many species are dimorphic and alternate yeast and hyphal stages throughout their life cycle (Bandoni 1995). Today the recognition of a yeast as belonging to the Basidiomycota is mainly done by the analysis of gene sequences, most notably the D1/D2 domains of the large subunit rDNA (LSU rDNA), the internal transcribed spacers 1 and 2 (ITS) of rDNA, or the small subunit (SSU) rDNA. A number of morphological, biochemical, ultrastructural, and physiological criteria indicate basidiomycetous affinity as well, such as a positive diazonium blue B reaction, urease activity, enteroblastic budding, presence of ballistoconidia and/or red carotenoid pigments, a lamellate cell wall ultrastructure, presence of a dolipore septum, the biochemical composition of the cell wall, and a high mol% G + C of genomic DNA (usually >50%) with the majority of basidiomycetous yeasts above 50% (see Boekhout et al. 2011).

Early molecular evolutionary studies of 5S rRNA indicated two phylogenetic lineages that correlated with septal ultrastructure (Walker and Doolittle 1982; Templeton 1983). This was confirmed by the observation made by Blanz and Gottschalk (1984) who distinguished a lineage that is now recognized as Pucciniomycotina and a second one now known as Agaricomycotina. Molecular phylogenetic studies using SSU rDNA revealed one more lineage (Swann and Taylor 1995; Sugiyama 1998) that is presently known as Ustilaginomycotina. Thus, these early molecular evolutionary studies revealed the presence of yeast and yeastlike fungi in all three domains of Basidiomycetes. The presence of these three subphyla in Basidiomycota and the presence of yeasts, therein, are widely accepted and supported by many molecular phylogenetic studies (Fell et al. 1995, 2000; Begerow et al. 1997; Scorzetti et al. 2002; James et al. 2006; Liu et al. 2015a, b; Wang et al. 2014, 2015a, b, c). All three subphyla are species rich and show a great amount of organismal complexity that ranges from unicellular yeasts to hyphally growing or multicellular life forms, such as mushrooms, and the plant pathogenic rusts and smuts. With respect to the taxonomy of the basidiomycetous yeasts, the most important observation resulting from these molecular studies was the lack of concordance between the previously recognized taxa, especially genera, and the molecularly defined clades. Notably, many so-called anamorphic (=asexual) genera, such as *Cryptococcus*, *Bullera*, *Sporobolomyces*, *Bensingtonia*, and *Rhodotorula* were highly polyphyletic. Therefore, a multigene-based effort was made to bring the taxonomy in line with the results of molecular phylogeny studies (Wang et al. 2014, 2015a, b, c; Liu et al. 2015a, b). Probably the most relevant

taxonomic rearrangements relate to the reclassification of species of these previously highly polyphyletic genera, such as *Rhodotorula* and *Bensingtonia*.

Pucciniomycotina seem ancestral to both the Ustilaginomycotina and Agaricomycotina (James et al. 2006). Yeast taxa form a minor part of the ca. 8000 species described (Kirk et al. 2001) with the majority (ca. 95%) known as the rusts (Pucciniales). Species in this lineage also show a great diversity of ecological interactions. As indicated, the majority of species are plant pathogens, but others are mycoparasites, insect pathogens, or saprotrophs. Until recently, eight major clades were recognized as classes (Weiss et al. 2004; Aime et al. 2006; Bauer et al. 2006; Hibbett et al. 2007), but recently a new class Spiculogloeomycetes was added (Wang et al. 2015b). Classes Agaricostilbomycetes, Spiculogloeomycetes, Cystobasidiomycetes, Microbotryomycetes, and Mixiomycetes contain yeast taxa. Mixiomycetes contain only one species, *Mixia osmundae*, that is a fern parasite that forms cream yeast colonies in culture (Nishida et al. 1995).

Spiculogloeomycetes contain three genera (Table 1.2) with yeast taxa, *Spiculogloea*, *Mycogloea* (in part), and *Phyllozyma* (Wang et al. 2015b). Agaricostilbomycetes contain ten genera with yeasts belonging to four families, Agaricostilbaceae, Chionosphaeraceae, Kondoaceae, and Ruineniaceae (Wang et al. 2015b). The phylogenetic affiliation of the genus *Jianyunia* within Agaricostilbomycetes is not yet solved (Wang et al. 2015b). Cystobasidiomycetes contain 11 genera with yeast taxa classified in seven families, Cystobasidiaceae, Erythrobasidiaceae, Naohideaceae, Symmetrosporaceae, Buckleyzymaceae, Microsporomycetaceae, and Sakaguchiaceae (Wang et al. 2015b). Several species are able to form mycosporines, which are low molecular weight water-soluble pigments that are capable of absorbing UV radiation (Libkind et al. 2005). Most pucciniomycetous yeasts belong to class Microbotryomycetes that contains 25 genera affiliated to six families, namely, Kriegeriaceae, Camptobasidiaceae, Leucosporidiaceae, Sporidiobolaceae, Colacogloeaceae, and Chrysozymaceae (Wang et al. 2015b). Yeast forms occur in the orders Kriegeriales, Leucosporidiales and Sporidiobolales (Wang et al. 2015b), whereas plant and mycoparasitism mainly belong to orders Microbotryales and Heterogastridiales, respectively.

Agaricomycotina contains five classes, viz., Agaricomycetes, Dacrymycetes, Tremellomycetes, Wallemiomycetes, and Geminibasidiomycetes (Zalar et al. 2005; Hibbett 2006; Matheny et al. 2006; Hibbett et al. 2007; Nguyen et al. 2015). In Tremellomycetes, yeasts or dimorphic taxa occur only in five orders, Cystofilobasidiales, Filobasidiales, Holtermanniales, Tremellales, and Trichosporonales (Fell et al. 2000; Sampaio 2004; Wuczkowski et al. 2011; Liu et al. 2015a, b; Table 1.3). Trichosporonales, however, is not accepted as a separate order from Tremellales by some authors (Weiss et al. 2004; Hibbett et al. 2007), but recent multigene-based phylogenies suggested otherwise (Liu et al. 2015a, b). Similar to Pucciniomycotina, the reclassifications made of highly polyphyletic genera, such as *Cryptococcus* and *Bullera*, can be seen as the most significant contribution to the restructuring of the taxonomy of yeasts in Agaricomycotina (Tables 1.3 and 1.5; Liu et al. 2015b). Within Tremellales, Cystofilobasidiales

Table 1.2 Accepted yeast genera of Pucciniomycotina

Class	Order	Family	Genus	Type species	Other (yeast) species
Agaricostilbomycetes	Agaricostilbales	Agaricostilbaceae	*Sterigmatomyces*	*Sterigmatomyces halophilus*	
			Pseudobensingtonia	*Pseudobensingtonia ingoldii*	
		Chionosphaeraceae	*Ballistosporomyces*	*Ballistosporomyces xanthus*	
			Chionosphaera	*Chionosphaera apobasidialis*	
			Cystobasidiopsis	*Cystobasidiopsis nirenbergiae*	
			Kurtzmanomyces	*Kurtzmanomyces nectairei*	
		Incertae sedis Chionospheariaceae			*Mycogloea nipponica*
		Kondoaceae	*Bensingtonia*	*Bensingtonia ciliata*	
			Kondoa	*Kondoa malvinella*	
		Ruineniaceae	*Ruinenia*	*Ruinenia rubra*	
	Incertae sedis Agaricostilbales		*Jianyunia*	*Jianyunia sakaguchii*	
Spiculogloeomycetes	Spiculogloeales	Spiculogloeaceae	*Spiculogloea*	*Spiculogloea occulta*	
			Mycogloea(pro parte)	*Mycogloea carnosa*	
			Phyllozyma	*Phyllozyma subbrunnea*	
Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae	*Cystobasidium*	*Cystobasidium fimetarium*	
			Occultifur	*Occultifur internus*	
	Erythrobasidiales	Erythrobasidiaceae	*Bannoa*	*Bannoa hahajimensis*	
			Erythrobasidium	*Erythrobasidium hasegawianum*	

	Incertae sedis Erythrobasidiales		*Cyrenella*	*Cyrenella elegans*	
			Hasegawazyma	*Hasegawazyma lactosa*	
	Naohideales	Naohideaceae	*Naohidea*	*Naohidea sebacea*	
Incertae sedis Cystobasidiomycetes		Symmetrosporaceae	*Symmetrospora*	*Symmetrospora gracilis*	
		Buckleyzymaceae	*Buckleyzyma*	*Buckleyzyma aurantiaca*	
		Microsporomycetaceae	*Microsporomyces*	*Microsporomyces magnisporus*	
		Sakaguchiaceae	*Sakaguchia*	*Sakaguchia dacryoidea*	
Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Kriegeria*	*Kriegeria eriophori*	
			Meredithblackwellia	*Meredithblackwellia eburnea*	
			Phenoliferia	*Phenoliferia psychrophenolica*	
			Yamadamyces	*Yamadamyces rosulatus*	
		Camptobasidiaceae	*Camptobasidium*	*Camptobasidium hydrophilum*	
			Glaciozyma	*Glaciozyma antarctica*	
	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*	*Leucosporidium scottii*	
	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	*Rhodotorula glutinis*	
			Rhodosporidiobolus	*Rhodosporidiobolus nylandii*	
			Sporobolomyces	*Sporobolomyces roseus*	
Incertae sedis Microbotryomycetes		Colacogloeaceae	*Colacogloea*	*Colacogloea peniophorae*	
		Chrysozymaceae	*Bannozyma*	*Bannozyma yamatoana*	
			Chrysozyma	*Chrysozyma griseoflava*	
			Fellozyma	*Fellozyma inositophila*	

(continued)

Table 1.2 (continued)

Class	Order	Family	Genus	Type species	Other (yeast) species
			Hamamotoa	*Hamamotoa singularis*	
			Pseudohyphozyma	*Pseudohyphozyma buffonii*	
			Pseudoleucosporidium	*Pseudoleucosporidium fasciculatum*	
			Oberwinklerozyma	*Oberwinklerozyma yarrowii*	
			Sampaiozyma	*Sampaiozyma ingeniosa*	
			Spencerozyma	*Spencerozyma crocea*	
			Slooffia	*Slooffia tsugae*	
			Trigonosporomyces	*Trigonosporomyces hylophilus*	
			Yunzhangia	*Yunzhangia auriculariae*	
			Vanudeniozyma	*Vanudeniozyma ferulica*	
			Vonarxula	*Vonarxula javanica*	
Mixiomycetes	Mixiales	Mixiaceae	*Mixia*	*Mixia osmundae*	

Table 1.3 Accepted yeast genera of Agaricomycotina

Class	Order	Family	Genus	Type species	Other (yeast) species
Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Itersonilia*	*Itersonilia perplexans*	
			Krasilnikovozyma	*Krasilnikovozyma huempii*	
			Mrakia	*Mrakia frigida*	
			Phaffia	*Phaffia rhodozyma*	
			Tausonia	*Tausonia pamirica*	
			Udeniomyces	*Udeniomyces pyricola*	
		Cystofilobasidiaceae	*Cystofilobasidium*	*Cystofilobasidium capitatum*	
	Filobasidiales	Filobasidiaceae	*Filobasidium*	*Filobasidium floriforme*	
			Goffeauzyma	*Goffeauzyma gastrica*	
			Heterocephalacria	*Heterocephalacria solida*	*Heterocephalacria arrabidensis*
			Naganishia	*Naganishia globosa*	
			Syzygospora	*Syzygospora alba*	
		Piskurozymaceae	*Piskurozyma*	*Piskurozyma cylindrica*	
			Solicoccozyma	*Solicoccozyma aeria*	
	Holtermanniales	Not assigned	*Holtermannia*	*Holtermannia corniformis*	
			Holtermanniella	*Holtermanniella takashimae*	
	Tremellales	Bulleribasidiaceae	*Bulleribasidium*	*Bulleribasidium oberjochense*	
			Derxomyces	*Derxomyces mrakii*	
			Dioszegia	*Dioszegia hungarica*	

(continued)

Table 1.3 (continued)

Class	Order	Family	Genus	Type species	Other (yeast) species
			Hannaella	*Hannaella sinensis*	
			Nielozyma	*Nielozyma melastomae*	
			Vishniacozyma	*Vishniacozyma carnescens*	
		Tremellaceae	*Tremella*	*Tremella mesenterica*	
		Rhynchogastremaceae	*Papiliotrema*	*Papiliotrema bandonii*	
			Rhynchogastrema	*Rhynchogastrema coronatum*	
		Bulleraceae	*Bullera*	*Bullera alba*	
			Fonsecazyma	*Fonsecazyma mujuensis*	
			Genolevuria	*Genolevuria amylolytica*	
			Pseudotremella	*Pseudotremella moriformis*	
			Tremella clade I		*Cryptococcus cuniculi* pro tem.
		Sirobasidiaceae	*Fibulobasidium*	*Fibulobasidium inconspicuum*	
		Incertae sedis Sirobasidiaceae			*Sirobasidium japonicum* pro tem.; *Sirobasidium magnum* pro tem.
		Cuniculitremaceae	*Fellomyces*	*Fellomyces polyborus*	
			Kockovaella	*Kockovaella thailandica*	
			Sterigmatosporidium	*Sterigmatosporidium polymorphum*	
		Naemateliaceae	*Naematelia*	*Naematelia encephala*	

		Carcinomycetaceae	*Carcinomyces*	*Carcinomyces effibulatus*	
		Trimorphomycaceae	*Saitozyma*	*Saitozyma flava*	
			Sugitazyma	*Sugitazyma miyagiana*	
			Trimorphomyces	*Trimorphomyces papilionaceus*	
		Cryptococcaceae	*Cryptococcus*	*Cryptococcus neoformans*	
			Kwoniella	*Kwoniella mangroviensis*	
		Phaeotremellaceae	*Gelidatrema*	*Gelidatrema spencermartinsiae*	
			Phaeotremella	*Phaeotremella pseudofoliacea*	
	Trichosporonales	Trichosporonaceae	*Apiotrichum*	*Apiotrichum porosum*	
			Cutaneotrichosporon	*Cutaneotrichosporon cutaneum*	
			Haglerozyma	*Haglerozyma haglerorum*	
			Trichosporon	*Trichosporon ovoides*	
			Vanrija	*Vanrija humicola*	
		Tetragoniomycetaceae	*Bandonia*	*Bandonia marina*	
			Cryptotrichosporon	*Cryptotrichosporon anacardii*	
			Takashimella	*Takashimella formosensis*	
			Tetragoniomyces	*Tetragoniomyces uliginosus*	

Pro tem. = pro tempore, indicating that the final taxonomic placement of the species is not yet known and, hence, it is left in the polyphyletic genus from where it should be removed when more evidence becomes available

forms the basal lineage (Liu et al. 2015a, b) with yeast taxa belonging to eight genera of two families, Mrakiaceae and Cystofilobasidiaceae (Liu et al. 2015b). Species of *Mrakia* and *Phaffia* are able to ferment, and the latter forms astaxanthin (Johnson 2003; Johnson and Echavarri-Erasun 2011) that is widely used as a colorant for salmon grown in aquaculture. The elongate basidia of *Phaffia* species are formed after cell-bud mating and produce apical basidiospores (Golubev 1995). Strains of *Phaffia* came from tree sap in temperate regions of the Northern Hemisphere and from the sugar-rich stromata of *Cyttaria* spp. that parasitize *Nothofagus* trees in the Southern Hemisphere (Libkind et al. 2007). Species of *Mrakia* may also cause spoilage of refrigerated citrus juice that may cause significant economic damage (Houbraken, unpublished data). Filobasidiales has seven yeast species containing genera that cluster into two families, Filobasidiaceae and Piskurozymaceae (Liu et al. 2015b). Holtermanniales has only two genera, namely, *Holtermannia* that forms mushroom-like gelatinous fruiting bodies formed by aggregates of erect, simple, or branched teeth of which the basidiospores germinate with yeast cells and *Holtermanniella* that contains only yeasts and one filamentous growing species.

Most yeast containing genera belong to Tremellales, namely, 28 that are classified into 11 families (Table 1.3; Liu et al. 2015b). Note that *Tremella*, a genus containing highly typical mushroom-like fruiting bodies known as jelly fungi, turned out to be polyphyletic (Chen 1998; Scorzetti et al. 2002; Liu et al. 2015a, b) with clades belonging to different families. Hence, these clades were assigned to different genera (Liu et al. 2015b), but some lineages still remain to be reclassified due to uncertainties in the phylogenetic positions of key species that may only be available as herbarium specimens. Mycoparasitism seems to occur commonly in this order based on the presence of so-called haustorial branches in many genera and species, e.g., *Papiliotrema flavescens* (cited as *Cryptococcus laurentii*, Kurtzman 1973), *Auriculibuller* (Sampaio et al. 2004), *Bulleromyces* (Boekhout et al. 1991), *Sterigmatosporidium* (cited as *Cuniculitrema*, Kirschner et al. 2001), and *Bulleribasidium* and *Papiliotrema* (Sampaio et al. 2002). Due to the recent update on its taxonomy, the previously highly polyphyletic genus *Cryptococcus* is presently confined to the human pathogens *Cryptococcus neoformans* and *Cryptococcus gattii* and related species (Hagen et al. 2015; Liu et al. 2015a, b). Next to the dimorphic yeast species, this clade also contains some filamentous growing species, previously classified in *Filobasidiella*, namely, *Cryptococcus depauperatus* and *Cryptococcus luteus* (Liu et al. 2015b). *Filobasidiella* is now considered a synonym under *Cryptococcus*. The basidia formed by *Cryptococcus sensu stricto* species are elongated holobasidia with terminal sessile basidiospores in basipetal chains and occur in both the filamentous and dimorphic representatives. Order Trichosporonales contains members of the anamorphic genus *Trichosporon* that to a large extent forms true hyphae and arthroconidia (Fell et al. 2000). Over time, several species of *Cryptococcus*, *Bullera*, and *Cryptotrichosporon* were added (Sugita et al. 2001; Takashima et al. 2001; Nakase et al. 2002; Middelhoven et al. 2003; Fungsin et al. 2006; Okoli et al. 2007) thus questioning the circumscription of genera in this order. Prillinger et al. (2007) proposed *Asterotremella* to

accommodate *Cryptococcus* species belonging to the *Humicola* clade. However, from a nomenclatural point of view, the use of the name *Vanrija* has priority over *Asterotremella* (Okoli et al. 2007; Liu et al. 2015b). A recent reclassification resulted in the recognition of nine genera that belong to the families Trichosporonaceae and Tetragoniomycetaceae (Liu et al. 2015b; Tables 1.3 and 1.5).

The subphylum Ustilaginomycotina currently comprises four classes, namely, Ustilaginomycetes and Exobasidiomycetes (Begerow et al. 2000; Hibbett et al. 2007) and the recently added Malasseziomycetes and Moniliellomycetes (Wang et al. 2014). Classes Ustilaginomycetes and Exobasidiomycetes comprise mainly plant pathogens, but asexual states of some of these may grow on agar media. Classes Malasseziomycetes and Moniliellomycetes comprise the genera *Malassezia* and *Moniliella*, respectively. A recent multigene-based phylogenetic analysis of asexual growing yeasts and yeastlike fungi from the four classes and a comparison with LSU rDNA data from sexually growing taxa, mainly plant pathogens, indicated that several asexual species belong to genera of Exobasidiomycetes and Ustilaginomycetes (Wang et al. 2015c; Tables 1.4 and 1.5). Thus, similar to the situation in Pucciniomycotina and Agaricomycotina, the polyphyletic nature of genera such as *Pseudozyma* and *Tilletiopsis* was largely reduced. Next to containing plant pathogens, some of the asexual genera previously classified in *Pseudozyma*, *Tilletiopsis*, *Meira*, and *Acaromyces ingoldii* showed biocontrol capabilities (Urquehart et al. 1994; Belangér et al. 1998; Boekhout et al. 2003). Ustilaginomycotina are also highly polyphyletic, and this prompted the reclassification of the well-known model organism *Ustilago maydis*, as *Mycosarcoma maydis* (McTaggart et al. 2016). Among Ustilaginomycotina two orders have yeasts or yeastlike species, namely, Urocystales (genus *Fereydounia*) and Ustilaginales with the genera *Farysia*, *Anthracocystis*, *Dirkmeia*, *Kalmanozyma*, *Langdonia*, *Moesziomyces*, *Ustilago*, *Mycosarcoma*, and some single species lineages (Table 1.4; Wang et al. 2015c). Note that *Fereydounia khargensis*, the first yeast found in Urocystales and originally described from soil in Iran (Nasr et al. 2014), has been found as an emerging human pathogen in Malaysia (Tap et al. 2016). Many anamorphic *Pseudozyma* species could be transferred to plant pathogenic teleomorphic genera of smuts (Tables 1.4 and 1.5), but five species could not yet be ascribed to any teleomorphic smut genus, and these were *Pseudozyma alboarmeniaca*, *Pseudozyma hubeiensis*, *Pseudozyma pruni*, *Pseudozyma thailandica*, and *Pseudozyma tsukubaensis*. These were left provisionally in the genus *Pseudozyma*, despite that the type species of the genus, *Pseudozyma prolifica*, was made a synonym of *Mycos. maydis* (Wang et al. 2015c; McTaggart et al. 2016). In the Exobasidiomycetes, yeastlike species belonged to six orders, Entylomatales, Exobasidiales, Georgefischeriales, Golubeviales, Microstromatales, and Robbauerales, and two genera, *Jaminaea* and *Sympodiomycopsis*, remained *incertae sedis* in Microstromatales (Tables 1.3 and 1.4; Wang et al. 2015c). Some anamorphic *Tilletiopsis* species could be transferred to teleomorphic genera, such as *Gjaerumia*, *Phragmotaenium*, and *Tilletiaria* (Wang et al. 2015c). Only the *Tilletiopsis washingtonensis* complex remained in

Table 1.4 Accepted genera of Ustilaginomycotina with yeast or yeastlike stages

Class	Order	Family	Genus	Type species	Other (yeast) species
Exobasidiomycetes	Entylomatales	Entylomataceae	*Tilletiopsis*	*Tilletiopsis washingtonensis*	
	Exobasidiales	Brachybasidiaceae	*Meira*	*Meira geulakonigii*	
		Cryptobasidiaceae	*Acaromyces*	*Acaromyces ingoldii*	
	Georgefischeriales	Gjaerumiaceae	*Gjaerumia*	*Gjaerumia minor*	
		Tilletiariaceae	*Phragmotaenium*		*Phragmotaenium derxii*, *Phragmotaenium fulvescens*. *Phragmotaenium flavum*, *Phragmotaenium oryzicola*
			Tilletiaria	*Tilletiaria anomala*	
	Golubeviales	Golubeviaceae	*Golubevia*	*Golubevia pallescens*	
	Microstromatales	Microstromataceae	*Microstroma*	*Microstroma album*	
	Microstromatales *incertae sedis*		*Jaminaea*	*Jaminaea angkorensis*	
			Sympodiomycopsis	*Sympodiomycopsis paphiopedili*	
	Robbauerales	Robbaueraceae	*Robbauera*	*Robbauera albescens*	
Ustilaginomycetes	Urocystales	Fereydouniaceae	*Fereydounia*	*Fereydounia khargensis*	
	Ustilaginales	Anthracoideaceae	*Farysia*	*Farysia javanica*	*Farysia acheniorum*, *Farysia chardoniana*, *Farysia itapuensis*, *Farysia setubalensis*, *Farysia taiwaniana*
		Melanotaeniaceae	*Anthracocystis*	*preceed name with 'Pseudozyma' flocculosa*	
		Ustilaginaceae	*Dirkmeia*	*Dirkmeia churashimaensis*	

			Kalmanozyma	*Kalmanozyma fusiformata*	*Kalmanozyma brasiliensis*, *Kalmanozyma vetiver*
			Langdonia	*Langdonia jejuensis*	
			Moesziomyces	*Moesziomyces bullatus*	*Moesziomyces antarcticus*, *Moesziomyces aphidis*, *Moesziomyces parantarcticus*, *Moesziomyces rugulosus*
			Ustilago	*Ustilago hordei*	*Ustilago abaconensis*, *Ustilago shanxiensis*, *Ustilago siamensis*
			Mycosarcoma	*Mycosarcoma maydis* (= *Ustilago maydis*)	
			Single species lineages		*Pseudozyma alboarmeniaca* pro tem., *Pseudozyma hubeiensis* pro tem.
	Incertae sedis Ustilaginales				*Pseudozyma pruni* pro tem.' *Pseudozyma thailandica* pro tem., *Pseudozyma tsukubaensis* pro tem.
Malasseziomycetes	Malasseziales	Malasseziaceae	*Malassezia*	*Malassezia furfur*	
Moniliellomycetes	Moniliellales	Moniliellaceae	*Moniliella*	*Moniliella acetoabutens*	

Pro tem. = *pro tempore*, indicating that the final taxonomic placement of the species is not yet known and, hence, it is left in the polyphyletic genus from where it should be removed when more evidence becomes available

Table 1.5 New versus old names of genera in Ustilaginomycotina, Pucciniomycotina, and Agaricomycotina

Subphylum	Class	Order	Earlier genus names	Current genus name
Ustilaginomycotina	Exobasidomycetes	Georgefischerales	*Tilletiopsis* p.p.	*Gjaerumia*, *Phragmotaenium*, *Tilletiaria*
		Golubeviales	*Tilletiopsis pallescens*	*Golubevia*
		Robbauerales	*Pseudozyma churashimaensis*	*Dirkmeia*
			Pseudozyma p.p.	*Kalmanozyma*
			Tilletiopsis albescens	*Robbauera*
		Microstromatales	*Rhodotorula phylloplana*	*Microstroma*
	Ustilaginomycetes	Ustilaginales	*Farysizyma*	*Farysia*
			Pseudozyma jejuensis	*Langdonia*, *Moesziomyces*, *Mycosarcoma*, *Sporisorium*, *Triodiomyces*, *Ustilago*
			Ustilago (*Ustilago maydis* clade)	*Mycosarcoma*
			Ustilago hordei clade	*Ustilago*
Agaricomycotina	Tremellomycetes	Cystofilobasidiales	*Udeniomyces pannonicus*	*Itersonilia*
			Cryptococcus p.p.	*Krasilnikovozyma*
			Mrakia curviuscula	*Krasilnikovozyma*
			Mrakiella	*Mrakia*
			Xanthophyllomyces, *Rhodomyces*	*Phaffia*
			Guehomyces pullulans	*Tausonia*
			Trichosporon pullulans	*Tausonia*

		Filobasidiales	*Cryptococcus* p.p.	*Filobasidium, Goffeauzyma, Heterocephalacria, Naganishia, Piskurozyma, Solicoccozyma, Vishniacozyma*
			Syzygospora bachmannii	*Heterocephalacria*
			Bullera taiwanensis	*Piskurozyma*
			Filobasidium capsuligenum	*Piskurozyma*
			Syzygospora sorana	*Piskurozyma*
		Holtermanniales	*Cryptococcus* p.p.	*Holtermanniella*
		Tremellales	*Bulleromyces*	*Bullera*
			Bullera p.p.	*Bulleribasidium, Carcinomyces, Carlosrosaea, Derxomyces, Dioszegia, Hannaella, Nielozyma, Papiliotrema, Saitozyma, Sugitazyma, Takashimella, Trimorphomyces, Vishniacozyma*
			Mingxiaea	*Bulleribasidium*
			Syzygospora effibulata	*Carcinomyces*
			Filobasidiella	*Cryptococcus*
			Tsuchiyaea wingfieldii	*Cryptococcus*
			Cryptococcus cistialbidi	*Dimennazyma*
			Cryptococcus p.p.	*Dioszegia, Fonsecazyma, Geladitrema, Genoluvuria, Kwoniella, Papiliotrema, Phaeotremella, Pseudotremella, Rhynchogastrema, Saitozyma, Takashimella, Tremella, Vanrija, Vishniacozyma*
			Kwoniella betulae	*Fonsecazyma*
			Fellomyces p.p.	*Kockovaella*
			Tremella p.p.	*Carcinomyces, Naematelia, Phaeotremella, Pseudotremella, Tetragoniomyces*

(continued)

Table 1.5 (continued)

Subphylum	Class	Order	Earlier genus names	Current genus name
			Auriculibuller fuscus	*Papiliotrema*
			Bandoniozyma	*Rhynchogastrema*
			Cuniculitrema	*Sterigmatosporidium*
			Trimorphomyces nebularis	*Vishniacozyma*
		Trichosporonales	*Cryptococcus* p.p.	*Bandonia, Cutaneotrichosporon, Vanrija*
			Trichosporon p.p.	*Cutaneotrichosporon, Effuseotrichosporon, Haglerozyma*
Pucciniomycotina	Agaricostilbomycetes	Agaricostilbales	*Sporobolomyces* p.p.	*Ballistosporomyces, Cystobasidiopsis, Ruinenia*
			Bensingtonia p.p.	*Pseudobensingtonia, Kondoa*
			Ballistosporomyces p.p.	*Ruinenia*
			Agaricostilbum	*Sterigmatomyces*
		Incertae sedis Agaricostilbales	*Bensingtonia sakaguchia*	*Jianyunia*
	Spiculogloeomycetes	Spiculogloeales	*Sporobolomyces* p.p.	*Phyllozyma*
	Cystobasidiomycetes	Cystobasidiales	*Rhodotorula portillonense*	*Cystobasidium*
		Erythrobasidiales	*Sporobolomyces* p.p.	*Bannoa, Erythrobasidium*
		Incertae sedis Erythrobasidiales	*Rhodotorula lactosa*	*Hasegawazyma*
	Incertae sedis Cystobasidiomycetes		*Sporobolomyces* p.p.	*Buckleyzyma, Microsporomyces*
			Rhodotorula p.p.	*Buckleyzyma, Microsporomyces, Sakaguchia*
			Sporobolomyces p.p.	*Symmetrospora*
	Microbotryomycetes	Kriegeriales	*Zymoxenogloea*	*Kriegeria*
			Rhodotorula p.p.	*Phenoliferia, Yamadamyces*

		Leucosporidiales	*Leucosporidiella*	*Leucosporidium*
			Mastigobasidium	*Leucosporidium*
		Microbotryales	*Rhodotorula hordea*	*Ustilentyloma*
		Sporidiobolales	*Rhodosporidium* p.p.	*Rhodosporidiobolus, Rhodotorula*
			Rhodotorula p.p.	*Rhodosporidiobolus*
			Sporidiobolus p.p.	*Rhodosporidiobolus, Sporobolomyces*
			Sporobolomyces p.p.	*Rhodotorula*
	Incertae sedis Microbotryomycetes		*Bensingtonia* p.p.	*Bannozyma*
			Sporobolomyces p.p.	*Chrysozyma*
			Rhodotorula p.p.	*Bannozyma, Colacogloea, Curvibasidium, Hamamotoa, Oberwinklerozyma, Pseudohyphozyma, Sampaiozyma, Slooffia, Spencerozyma, Trigonosporomyces, Udeniozyma, Vonarxula, Yungzhangia*
			Sporobolomyces p.p.	*Fellozyma, Hamamotoa, Oberwinklerozyma, Slooffia*
			Leucosporidium fasciculatrum	*Pseudoleucosporidium*

its original genus, and some species were placed in novel genera, viz., *Golubevia* and *Robbauera* (Wang et al. 2015c). Malasseziomycetes contain only one genus, *Malassezia*, that is a well-known colonizer of human and animal skin, and also implicated in several skin disorders (Gupta et al. 2004; Batra et al. 2005; Gaitanis et al. 2012; Wang et al. 2014; Wu et al. 2015). *Malassezia furfur* and *Malassezia pachydermatis* may also be implicated in sepsis, especially in neonates that receive intravenous lipid supplementation (Gaitanis et al. 2012). All species, including the so-called non-lipid-dependent *Mal. pachydermatis*, lack a fatty acid synthase gene and hence depend for their lipids on the host (Wu et al. 2015). It has been suggested that the non-lipid-dependent *Mal. pachydermatis* may survive on common media, such as Sabouraud dextrose agar (SDA), using the small amounts of lipids present in, e.g., peptone (Wu et al. 2015). Despite their lipid auxotrophy, DNA of various species has been observed in environmental samples, such as from nematodes, soil, corals, and sponges (Amend 2014). Moniellomycetes also contain only one genus, the so-called black yeast *Moniliella* (Wang et al. 2014). Until recently, the phylogenetic affiliation of this genus was unknown, except that it was recognized as a basidiomycetous yeastlike fungus (de Hoog et al. 2011). Several species produce erythritol that is used as a low-calorie sweetener (Cho et al. 1998). Moreover they can cause spoilage in food products with a low pH (Samson et al. 2000).

1.4 Species Recognition

Rapid identification of yeasts species is now routinely determined from DNA sequence analyses, and some of the more widely used techniques are briefly described below. The most commonly used comparisons include nucleotide sequence divergence in domains 1 and 2 (D1/D2) of the nuclear large subunit (LSU) rRNA gene and from the internal transcribed spacer (ITS), which is located between the SSU and LSU rRNA genes (Kurtzman and Robnett 1998, 2003; Sugita et al. 1999; Fell et al. 2000; Scorzetti et al. 2002). The ITS sequence is divided into two sections (ITS1, ITS2) by the 5.8S gene, which is highly conserved and should not be included when comparing substitutions in ITS. In terms of sequencing effort, these two diagnostic gene sequences are easily obtained as a single amplicon, e.g., using primers NS-7A and either NL-4 or NL-5A (Kurtzman and Robnett 2003), and the species can then be quickly identified from a BLAST search of the sequences.

D1/D2 and ITS sequences have been quite useful for rapid identification of species, but reliance on a single gene for species identification can lead to errors because of the presence of interspecific hybrids, different substitution rates, or other genetic changes. Among these exceptions are *Saccharomyces bayanus* and *Saccharomyces pastorianus*, which share the same rRNA gene repeat (Peterson and Kurtzman 1991; Groth et al. 1999), and *Clavispora lusitaniae*, in which some strains show greater than 1% divergence in D1/D2 (Lachance et al. 2003). Rates of substitution among genes commonly used for species identification may differ considerably. The sister species *Komagataella pastoris* and *Komagataella phaffii*

are resolved by D1/D2 sequences, but just barely so, whereas the two species differ by a much greater number of substitutions when compared from the genes for translation elongation factor-1α and RNA polymerase II, subunit 1 (Kurtzman 2009). Similar results were shown in comparisons of *Lipomyces* species (Kurtzman et al. 2007). *Lipomyces mesembrius* and *Lipomyces doorenjongii* had the same D1/D2 sequences, but when compared from translation elongation factor-1α gene sequences, the two species were clearly separated. There is a perception that ITS sequences may be more resolving than those from D1/D2, but this seems to depend on the species group. Several *Bensingtonia* species were barely resolved from D1/D2, but easily separated by ITS, whereas the opposite was true for certain species of *Trichosporon* (Scorzetti et al. 2002; Kurtzman et al. 2011b). Consequently, a much better perspective is obtained if species identifications are based on multigene (multi-locus) analyses.

A number of alternatives exist to actual sequencing and can be used when sequences are available from other studies. Usually these techniques are based on a single gene and also subject to errors from the genetic changes noted above. The following is a brief description of some of the more commonly used methods.

1.4.1 Species-Specific Primers

The use of species-specific primer pairs is effective when used for PCR-based identifications involving a small number of known species or when a particular species is the subject of the search (Fell 1993; Mannarelli and Kurtzman 1998; Chapman et al. 2003; Hulin and Wheals 2014). Following the PCR reaction, the mixture is separated by gel electrophoresis to visually detect the band that identifies the target species.

1.4.2 PNA

Peptide nucleic acid (PNA) probes offer a means for detection and quantification of species in clinical samples, food products, and other substrates through fluorescence in situ hybridization (FISH). PNA probes have a peptide backbone to which nucleotides complementary to a species-specific target sequence are attached, and a fluorescent label is added for detection by fluorescence microscopy (Stender et al. 2001; Rigby et al. 2002). If probes are complementary to rRNA, the whole cell of the target species will be illuminated when visualized, which will also allow quantification by cell counts.

1.4.3 RAPD/AFLP

Microsatellite-primed RAPDs (Gadanho et al. 2003) and AFLP fingerprints (de Barros Lopes et al. 1999; Illnait-Zaragozí et al. 2012) have been effectively used for rapid preliminary identification of large numbers of isolates, and the pattern-based identification is then often followed by gene sequencing of representative strains from each group that has a unique pattern. One concern in using pattern-based identification techniques is reproducibility between laboratories, because small differences in PCR conditions may impact the species-specific patterns that serve as reference.

1.4.4 Real-Time PCR

The technique of real-time PCR has been widely studied for applications in medical mycology (Loeffler et al. 2000; Klingspor and Jalal 2006; Bergman et al. 2007; Khlif et al. 2009; Wellinghausen et al. 2009) and to detect the cause of food and beverage spoilage (Cocolin et al. 2001; Casey and Dobson 2004). Commonly used primers have been based on sequences of the rDNA repeat, such as D1/D2, ITS 1 and 2, or the SSU rRNA gene. In typical assays, 5 cfu ml^{-1} could be detected.

1.4.5 DGGE

Denaturing gradient gel electrophoresis (DGGE) is a method that has been used for species identification and quantification of yeast populations in foods and beverages. The technique is based on separation of DNA fragments that differ in nucleotide sequences (e.g., species-specific) through decreased electrophoretic mobility of partially melted double-stranded DNA amplicons in a polyacrylamide gel containing a linear gradient of DNA denaturants (i.e., a mixture of urea and formamide). A related technique is temperature gradient gel electrophoresis (TGGE), in which the gel gradient of DGGE is replaced by a temperature gradient (Muyzer and Smalla 1998). Applications of DGGE have included identification and population dynamics of yeasts in sourdough bread (Meroth et al. 2003), in coffee fermentations (Masoud et al. 2004), and on wine grapes (Prakitchaiwattana et al. 2004). Levels of detection are often around 10^3 cfu ml^{-1}, but 10^2 cfu ml^{-1} have been reported, which compares favorably with standard plate count methods (Prakitchaiwattana et al. 2004).

1.4.6 Flow Cytometry

High-throughput probe hybridization methods are available for detection of multiple species in multiple samples. One method that is effective for yeasts (Diaz and Fell 2004; Page and Kurtzman 2005) is an adaptation of the Luminex xMAP technology (Luminex Corp), which consists of a combination of 100 different sets of fluorescent beads covalently bound to species-specific DNA capture probes. Upon hybridization, the beads bearing the target amplicons are classified in a flow cytometer by their spectral addresses with a 635 nm laser. The hybridized biotinylated amplicon is quantified by fluorescent detection with a 532 nm laser. Strains that differ by one nucleotide often can be discriminated, and the assay can be performed, after amplification, in less than 50 min in a 96-well format with as many as 100 different species-specific probes per well.

1.4.7 Metagenomics

Metagenomic analyses provide a means for identifying essentially all species present in a substrate sample (Cuadros-Orellana et al. 2013; Tonge et al. 2014). Because of specific growth requirements or occurrence of some species in small numbers, culture plating methods will not detect the presence of these species in a sample when cultivation is attempted on standard media. Consequently, metagenomic methods are likely to reveal the presence of considerable unsuspected biodiversity.

1.4.8 MALDI-TOF Mass Spectrometry: An Alternative Identification Method for Yeasts

MALDI-TOF MS-based identification has revolutionized microbial identification, including yeasts, in many laboratories worldwide. In comparison with DNA-based identification methods, such as sequence analysis of the D1/D2 domains of the LSU rDNA and the ITS 1 and 2 regions of the rDNA, MALDI-TOF MS gives identifications in short turnaround times (Tan et al. 2012; Cassagne et al. 2013). MALDI-TOF MS has been successfully applied to identify isolates of many clinically relevant yeasts, e.g., *Cr. neoformans*/*Cr. gattii* species complex, *C. albicans* and non-*albicans Candida* species, and arthroconidial yeasts of *Geotrichum* and *Trichosporon* spp. and *Malassezia* spp. (Marklein et al. 2009; McTaggart et al. 2011; Cendejas-Bueno et al. 2012; Firacative et al. 2012; Kolecka et al. 2013, 2014; Hagen et al. 2015). With an increasing coverage of yeast species in the databases, the utility of the technique will further increase.

1.5 Concluding Remarks

During the past decade and a half, DNA sequence comparisons have provided an accurate means for species identification and have more than doubled the number of known yeasts. Analyses of single genes, as well as whole genomes, have brought an overall understanding to placement of yeast lineages among the Mycota. Continued whole genome sequencing will finally provide a natural system of classification for the yeasts and reveal important information on evolutionary history (Rokas 2016). Use of metagenomic analyses will help broaden our understanding of the yeasts through detection of taxa that are presently unknown.

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Chapter 2
Yeast Habitats: Different but Global

Gábor Péter, Masako Takashima, and Neža Čadež

Abstract Yeasts, a taxonomically heterogenic group of unicellular fungi, populate many different habitats on our planet. They occur in aquatic and terrestrial environments and also in the atmosphere; however, they are not evenly distributed. While some species are ubiquitous generalists occurring in wide geographic range and dwelling in different habitats, others may have more restricted distribution either geographically or by habitats. Some are known from very few isolates, and about one third of the known yeast species are represented by only one strain. In these cases their ecology remains to be elucidated. As nonmotile organisms their dispersal depends on the vectors carrying them. Insects are of outstanding importance among yeast vectors. Several exciting questions can be raised about the habitat-yeasts-vector associations. For example, which yeasts are there? Why are they only there? How did they get there? What are they doing there?

The last two decades witnessed the widespread application of DNA sequencing, providing quicker and more reliable yeast identification than earlier phenotype-based methods. Nowadays, the culture-independent methods are gaining ground in the study of biodiversity and ecology of yeasts.

In this chapter some new achievements from the field of habitat-yeasts-vector system are introduced and are embedded in a broader context.

Keywords Biodiversity hotspot • Ecological factors • Abiotic • Biotic • Extremophilic yeasts • Generalist • Specialist • Yeast dispersal forces

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Contents

2.1 Introduction

Yeasts are distributed throughout our entire planet. Their intrinsic characteristics determine their ability to populate a habitat and, thereby, describe their fundamental niche (Starmer and Lachance 2011 and the references therein). The realized niche is the "part of the fundamental niche actually occupied by a species in the presence of competitive or interactive species" (Lincoln et al. 1998). The microorganisms, including yeasts, populating a habitat first must enter the habitat. Different mechanisms have been proposed for the dissemination of microorganisms. Baas Becking's principle, which was inspired by Beijerinck, "Everything is everywhere, but the environment selects" (de Wit and Bouvier 2006) has been advocated by Fenchel and Finlay (2004). They expressed the opinion that microorganisms (i.e. small organisms less than 1 mm in length), as a consequence of their huge population sizes, tend to have cosmopolitan distribution and that the "microbial species found in a given habitat are a function only of habitat properties and not of historical factors" (evolutionary history). According to this assumption, the driving forces of the dispersal of small organisms are mainly natural phenomena such as hurricanes, global oceanic circulation, groundwater networks and damp fur and feathers (Finlay 2002). Conversely to the above notion, Ganter (2011) believes that the conception of microbial ubiquity ("Everything is everywhere, but the environment selects") is not a generally valid theory to describe distribution of microorganisms. Based on the analysis of the large amount of data accumulated for cactophilic yeasts, he argued that cactophilic yeast distributions are not global, remarkable level of endemism has been observed, and only two of the 25 cactophilic yeast species (*Pichia cactophila* and *Candida sonorensis*) are ubiquitous. One of the numerous examples of the endemism can be observed in case of the distribution of *Phaffomyces* species. Many regions have no members of this genus, although suitable host plants are present. Instead of the above-noted abiotic dispersal forces, the cactophilic yeasts are dispersed by arthropod vectors, primarily by *Drosophila* species, which are the cause of the restricted distribution patterns of cactophilic

yeasts, i.e. “Everything is not everywhere” (Ganter 2011). Besides the cactophilic community, the available data suggest that there are at least three additional habitats where dispersal of yeasts takes place primarily by animal vectors: wood, flowers and slime fluxes of trees (Ganter 2011). Other examples of yeast distribution patterns in space and time are reported in Chap. 4 of this book.

2.2 Biodiversity Hotspots

Organisms populating the Earth are not evenly distributed. Biodiversity hotspots, areas characterized by outstanding concentrations of endemic species and experiencing exceptional loss of habitat (Myers et al. 2000), are currently defined from the viewpoint of terrestrial plants. According to the definition of the webpage of Conservation International (2016), a biodiversity hotspot “must have at least 1500 vascular plants as endemics”, and “it must have 30% or less of its original natural vegetation”, i.e. it must be threatened. Thirty-five areas around the world are classified as biodiversity hotspots. They occupy merely 2.3% of Earth’s land surface, but include more than half of the world’s endemic plant species (Conservation International webpage 2016). Among them, tropical forests support approximately three-quarters of the world’s terrestrial biodiversity (Roy 2016). Although biodiversity hotspots are defined using plant inventories, they also have implications on other kingdoms of living organisms. In different habitats the diversity of animals, including arthropods, significantly increases with plant diversity (Castagneyrol and Jactel 2012). During their evolution the enormous rise in the diversity of phytophagous beetles, which account for over half of all beetle species, likely reflects the exponential rise in angiosperm diversity. The increase in beetle diversity seems to be a direct consequence of subsequent adaptive radiations onto angiosperm species (Farrell 1998). To some extent, the hotspot thesis can be extended to invertebrates as well. If we were to lose a portion of endemic plant species, we could perhaps lose a similar proportion of insect species (Myers et al. 2000) and yeasts associated with both of these habitats. Yeast–insect associations are well documented and were thoroughly reviewed by Ganter (2006) and by Blackwell (2017). The gut of beetles has been found to be a hyperdiverse source of novel yeast species (Suh et al. 2005). Among the 650 yeast strains isolated from beetles distributed in 27 families, they distinguished 290 D1/D2 sequence-based genotypes. According to their conservative estimation, 68% of the genotypes represented undescribed yeast species. Almost 200 new species were found in that single study, and the existence of an even greater number of yet unknown yeasts has been predicted (Suh et al. 2005). According to this estimation, the group of erotylid beetles alone could host at least 4500 additional undescribed yeast species. It seems that their prediction is being realized. Since their milestone publication, a large number of insect-, including beetle-associated yeast species have been described. Consequently, Boekhout (2005) characterized the gut of beetles as a yeast diversity hotspot.

One can argue about whether or not the biodiversity hotspots (according to the above cited phytocentric definition) host more yeast species than other natural habitats. The growing amount of data will provide a reliable answer, but considering the above-noted correlations between the diversity of plants, insects and the yeasts they harbour, a positive answer is foreshadowed. The Mediterranean basin has been recognized as one of the biodiversity hotspots (Roy 2016) and the only one in Europe. Yurkov et al. (2016a) published a culture-based inventory of the soil yeasts in the Mediterranean forests, woodlands and scrub biome, in Portugal. They detected different yeast community structures in the soils originating from three plots exposed to divergent levels of precipitation and, as a consequence, with different plant coverage and above ground biomass. Sequence-based identification revealed the existence of 57 yeast species, including 20 potentially novel taxa, among the isolated strains. The total number of yeast species was estimated to be 80. Either based on the number of isolated species or species richness estimations, these Mediterranean forest soils supported substantially higher yeast species richness than soils under broadleaf vegetation in Central Europe (e.g. Yurkov et al. 2012a). However, due to different sampling regimes and species recognition approaches, it is difficult to compare the species spectra obtained in different studies (Yurkov et al. 2016a). Therefore, comparisons of the available data on the soil yeasts from a few biodiversity hotspots are inconclusive. The generalization of high yeast diversity detected in the soils of the Mediterranean basin would be premature, considering that Vishniac (2006) reported the isolation of only 1–11 yeast species from tropical forest soils collected in Costa Rica, which is part of the Mesoamerica hotspot. Regarding unexplored diversity, soils remain a promising substrate for yeast isolation, and the proportion of potential new taxa has been reported to reach 30% in temperate forest soils (Yurkov et al. 2016b).

2.3 Ecological Factors Limiting the Distribution of Yeasts

Numerous intrinsic morphological, physiological and biochemical characteristics of yeasts are routinely determined and are available for the majority of the recognized species from the latest edition of *The Yeasts, a Taxonomic Study* (Kurtzman et al. 2011) and from the descriptions of newly discovered yeast species. The intrinsic characters of the autochthonous members of a habitat are expected to be in accord with the prevailing environmental conditions of that habitat. The environmental factors determining the metabolic activity, growth and survival of yeasts are both abiotic and biotic. The most important abiotic—physical and chemical—factors are temperature, light and solar radiation, pressure, the availability of nutrients and water (water activity), pH, oxygen relations and the presence or absence of antimicrobial compounds (Deák 2006). In natural ecosystems yeasts are always subjected to interactions with other organisms (biotic factors): bacteria, yeasts, moulds, plants, animals (Deák 2006), protists and viruses. These interactions can be mutual or unidirectional, neutral, synergistic or antagonistic (Lachance

and Starmer 1998). A well-known example of antagonistic interactions among yeasts is the production of killer toxins—proteinaceous compounds having fungicidal or fungistatic action, produced by yeasts which are coded by double-stranded RNA (dsRNA) viruses (Golubev 2006; see also Chap. 9 of this book). This type of killer system, which provides the genetic basis of the killer phenotype, e.g. in *Saccharomyces cerevisiae*, is an example of a mutualistic relationship between a yeast and its virus (Pieczynska et al. 2016). The outcome of species interactions also depends on the order of their arrival to a habitat, known as the priority effect. In case of six nectar yeasts, investigated pairwise in laboratory microcosms, it was found that late-arriving species experienced strong negative effects from early-arriving ones (Peay et al. 2012). The resulting community composition is constrained by the yeast species first inoculated (Peay et al. 2012, Mittelbach et al. 2016). Due to their higher ecological similarity, the competition between more closely related yeast species was more intense (Peay et al. 2012). Other examples of yeast interactions are reviewed in Chaps. 3, 6, 7 and 9 of this book. The effects of the above-listed environmental factors on yeasts can relatively easily be studied in the laboratory, especially that of the abiotic ones. In practise these environmental factors interact and may modify each other's effect on the metabolic activity, growth and survival of yeasts. For example, cryoprotective materials reduce the detrimental effect of cooling and freezing and increase the survival rate of microorganisms during freeze-thaw cycles. The geographic and climatic factors, affecting the spatial patterns and species richness of yeasts, only partly overlap with the above-noted well-defined abiotic factors. The major ecological factors include area, latitude, elevation, temperature, precipitation, relative humidity, wind and solar radiation (Lachance et al. 2001; Delort et al. 2010). Importantly, geographic factors may exert their effect not only directly on the yeasts but also through their interactions with insect vectors (Lachance et al. 2001). The importance of insects in vectoring yeasts was demonstrated by flower bagging experiments. Some buds of a morning glory (*Ipomoea acuminata*) flower were bagged to prevent their visit by insects. Unlike the unbagged ones, flowers that were covered with bags at the bud stage did not contain any yeast after opening (Lachance et al. 1989).

2.4 Extremophilic Yeasts

According to Rothschild and Mancinelli (2001) "an organism that thrives in an extreme environment is an extremophile". Extreme environmental conditions include extreme temperature, radiation, pressure, desiccation, salinity, pH, nutrient availability, oxygen species or redox potential. Although the organisms equipped with the highest thermotolerance are prokaryotes, eukaryotes are common among the psychrophiles, acidophiles, alkaliphiles, piezophiles (thriving under extremes of pressure), xerophiles and halophiles (Rothschild and Mancinelli 2001; Gadanho and Sampaio 2005). Ecological studies have shown that many yeasts species live

under various "extreme" conditions, like oligotrophic (e.g. open sea water), high pressure (deep sea), low temperature (e.g. polar and non-polar cold regions), low or high pH, low water availability and hypersaline environments. Due to their usually shorter generation time and high adaptability, bacteria easily outnumber yeasts in many natural and man-made environments. However, the tolerance of a few "extreme" environmental factors, e.g. low pH and water activity, may be stronger in some yeasts than in the majority of competing bacteria. Extremophilic yeast species and their adaptation to the environments have been broadly discussed (Raspor and Zupan 2006; Buzzini et al. 2017b; Sannino et al. 2017; Zajc et al. 2017). Therefore, in this chapter, we only briefly mention some recent developments on thermotolerant, acidotolerant, alkalitolerant and xerophilic (osmophilic) yeasts.

2.4.1 Thermotolerant Yeasts

Thermophilic yeasts are defined as those that have their lower growth limit above 20 °C with no restriction to the maximum growth temperature (Watson 1987). Another definition, adopted from Cooney and Emerson (1964), is used by Mouchacca (1997). According to that definition, the maximum growth temperature of thermophilic fungi is 50 °C or above, and the minimum is 20 °C or higher, while thermotolerant fungi have a maximum growth temperature at about 50 °C and a minimum below 20 °C. Considering any of the two above-noted definitions, those few yeast species reported to grow at 48–50 °C or even above are rather thermotolerant than thermophilic because they can grow also below 20 °C.

It was reported that *Candida thermophila*, a yeast isolated from Korean soil, can grow at 50–51 °C (Shin et al. 2001). Later, ascosporulation was observed in the type strain of this species, and an additional sporulating conspecific strain, with 49 °C as the upper limit of growth, was recovered from rotten willow wood in Europe. Therefore, the species *Ogataea thermophila* was proposed to accommodate the above-noted two strains (Péter et al. 2007). This species is now classified as *Ogataea polymorpha* based on ITS and D1/D2 LSU rRNA gene sequence analyses (Kurtzman and Robnett 2010; Suh and Zhou 2010). Using the same loci Suh and Zhou (2010) carried out a phylogenetic study of *O. polymorpha* species complex. They proposed a new combination, *Ogataea angusta*, for the type strain of *Hansenula* (*Pichia*) *angusta* and described a new species, *Candida parapolymorpha*. The latter was transferred to the genus *Ogataea* as *Ogataea parapolymorpha* because ascosporulation was observed in several strains (Kurtzman 2011). As a result, the *O. polymorpha* complex currently contains three closely related species, *O. polymorpha*, *O. parapolymorpha* and *O. angusta*, which are difficult to distinguish from each other based on phenotype. Our study (Takashima, unpublished data) indicates that growth characteristics at high temperature provide a useful tool to distinguish *O. polymorpha* from *O. angusta* and *O. parapolymorpha*. *O. polymorpha* grows well at 48 °C and in liquid culture the

absorbance at 660 nm (OD_{660}) reached about 2.5 within 48 h, whereas very weak or no growth was detected in case of *O. parapolymorpha* and *O. angusta*, respectively (Fig. 2.1). Vigorous growth within 48 h at 48 °C, therefore, seems to be a useful phenotypic character for recognizing *O. polymorpha* within this species complex.

Kluyveromyces marxianus is also a well-known thermotolerant ascomycetous yeast; several strains of this species can grow up to 45–47 °C (Deák 2006). For example, *K. marxianus* JCM 1630 grows up to 47.4 °C. Banat et al. (1992) reported the isolation of some yeast strains from an Indian distillery environment (molasses, rice husks and cane bagasse) that grew up to 52 °C and fermented up to 50 °C. Two of the five strains were identified as *K. marxianus*.

Unfortunately, in the descriptions of novel yeast species, the upper temperature limit of growth is often not determined, and this information is not available for many species even from the latest edition of *The Yeasts, a Taxonomic Study* (Kurtzman et al. 2011). According to Barnett et al. (2000) in addition to *O. polymorpha* (then listed as a synonym of *P. angusta*) and *K. marxianus*, numerous additional ascomycetous yeasts can grow at 40 °C and a few also at 45 °C, whereas the upper growth temperature limit of basidiomycetous yeasts is generally lower than that of the ascomycetous ones. Only a few basidiomycetous yeasts, including some *Trichosporon* and *Malassezia* species and *Filobasidiella* (*Cryptococcus*) *neoformans*, were listed by Barnett et al. (2000) among the species capable of growing at 40 °C, while none was reported to grow at 45 °C. Subsequent studies of yeast biodiversity revealed additional yeast species, including some basidiomycetous ones, which can grow above 40 °C. The two former *Rhodotorula* species, *Cystobasidium benthicum* (formerly *Rhodotorula benthica*) and *Cystobasidium* (*Rhodotorula*) *calyptogenae*, isolated from deep-sea sediment, were reported to grow at 41–44 °C (Nagahama et al. 2003), and *Takashimella tepidaria* (*Cryptococcus tepidarius*) grew at 47.7 °C (Takashima et al. 2009).

2.4.2 Acidotolerant and Alkalitolerant Yeasts

The general pH range for growth of yeasts is between the values of 3 and 8, with an optimum between pH 4.5–5.5. The tolerance to low pH depends on the type of the acidulant. Organic acids possess stronger inhibitory effect than inorganic acids (Deák 2008). Some yeasts, e.g. *Pichia kudriavzevii* (*Candida krusei*), *Pichia membranifaciens*, *S. cerevisiae* and *Zygosaccharomyces rouxii*, can grow at or below pH 2 and *Kazachstania exigua* down to pH 1.5 (Pitt and Hocking 2009). As pH drops below about 5, the growth of bacteria, except for lactic acid bacteria, is progressively discouraged (Pitt and Hocking 2009). Because of their tolerance to low pH values, yeasts can be cultivated under pH conditions where many other microorganisms, including the majority of bacteria, cannot grow, and this provides the theoretical basis for application of yeast isolation media with reduced pH. Figure 2.2 shows the effect of pH on growth of some yeast strains (their isolation sources are indicated in the legends). Strains isolated from human skin

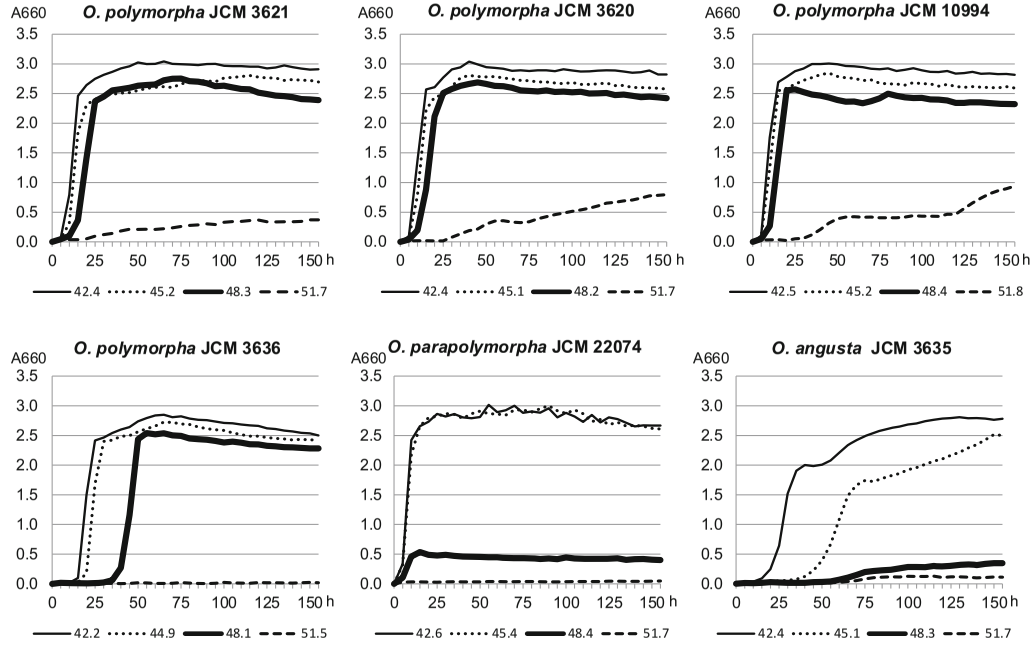

Fig. 2.1 Growth curves of yeast strains in the *Ogataea polymorpha* species complex determined at different temperatures. Cell concentrations as a function of time at different temperatures were measured using a temperature-gradient shaking incubator (TVS 126MA; Advantec Toyo). Cells were grown at 12 temperature values between 24 and 60 °C in YM broth. Optical density at 660 nm was monitored with the apparatus. Of 12 growth curves obtained per strain, those determined at higher than 40 °C, namely, 42.2–42.6 °C (*thin solid line*), 44.9–45.4 °C (*dotted line*), 48.1–48.4 °C (*thick solid line*) and 51.5–51.8 °C (*dashed line*), are shown. The following strains were investigated (the isolation sources obtained from http://jcm.brc.riken.jp/en/catalogue_e are indicated in *parenthesis*): *Ogataea polymorpha* JCM 3621 (type strain of *Hansenula polymorpha*) (soil irrigated with wastewater from distilleries); JCM 3620 (spoiled concentrated orange juice); JCM 10994 [type strain of *Ogataea* (*Candida*) *thermophila*] (soil, china clay), JCM 3636 (swine intestinal tract); *Ogataea parapolymorpha* JCM 22074 (soil); *Ogataea angusta* JCM 3635 (type strain) (fruit fly, *Drosophila pseudoobscura*)

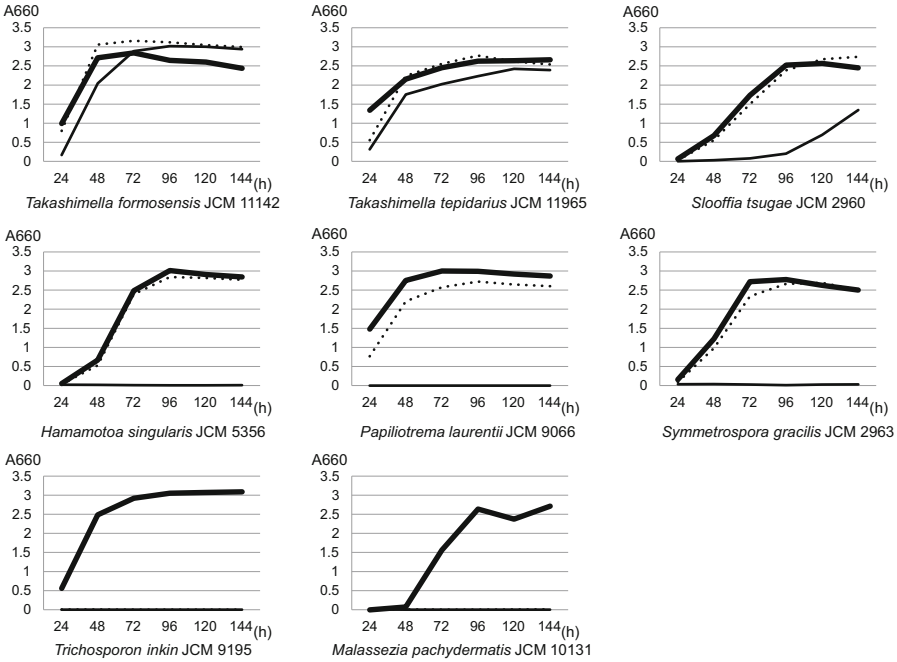

Fig. 2.2 Growth curves of some basidiomycetous yeast strains determined at different pH. Cell concentrations as a function of time at different pH values were measured in YM broth after adjusting the pH to 5.82 (*thick solid line*), 2.95 (*dotted line*) and 2.01 (*thin solid line*) using HCl. Culture tubes were incubated with agitation and the optical density at 660 nm was monitored in a temperature-gradient shaking incubator (TVS 126MA; Advantec Toyo) at a constant temperature of 25 °C. The following strains were investigated (the isolation sources obtained from http://jcm.brc.riken.jp/en/catalogue_e are indicated in *parenthesis*): *Hamamotoa singularis* JCM 5356 (frass of bark beetle, *Scolytus tsugae* in *Tsuga heterophylla*); *Malassezia pachydermatis* JCM 10131 (ear of dog with otitis externa); *Papiliotrema laurentii* JCM 9066 (palm wine); *Slooffia tsugae* JCM 2960 (frass in western hemlock *Tsuga heterophylla*); *Symmetrospora gracilis* JCM 2963 (decaying leaves); *Takashimella formosensis* JCM 11142 (leaf of *Lophaterum gracile*); *Takashimella tepidaria* JCM 11965 (water sample from a small stream at Owakudani, hot spring area); *Trichosporon inkin* JCM 9195 (human skin)

or from the ear of a dog do not grow at pH 3, whereas other environmental strains grew at pH 3 or even at pH 2. Strains capable of growth at pH 2 are *Takashimella formosensis* JCM 11142 isolated from leaf of *Lophatherum gracile*, Thailand; *Slooffia tsugae* JCM 2960 isolated from frass in western hemlock (*Tsuga heterophylla*), USA; and *Tak. tepidaria* isolated from a water stream in Owakudani, Hakone, a hot spring area in Japan, which was reported to grow at pH 1.15 (Takashima et al. 2009). The data in Fig. 2.2 indicate that some basidiomycetous yeasts might also be able to grow in acidic condition around pH 2. The yeast communities of acidic rivers and lakes were dominated by basidiomycetous species (Gadanho et al. 2006; Russo et al. 2008; Libkind et al. 2014). Gadanho et al. (2006)

reported an undescribed yeast "*Cryptococcus* sp." (related to the *Phaeotremella* species), from an acidic pond. Strains of this species not only tolerated but even required low pH for their growth, and therefore this species was considered as a putative acidophilic yeast species.

Although acidic conditions are better tolerated by yeasts than alkaline ones, numerous yeast species thrive at pH above 10 (Deák 2008). According to Pitt and Hocking (2009), the pH range for growth of *Wickerhamomyces anomalus* (*Pichia anomala*) spans from 2 to 12.4. Wide range of taxonomic distribution of alkalitolerant yeasts was reported by Aono (1990). Aono studied the alkalitolerant characteristic of species belonging to the former genus *Hansenula* and discussed the relationship between the phylogenetic position and the upper pH limit for growth. Table 2.1 contains 35 of the 37 strains of Aono (1992) with their isolation source and current classification. Strains which grew at pH higher than 10 are now classified in genera *Barnettozyma*, *Cyberlindnera* and *Wickerhamomyces*. Basidiomycetous yeasts may also tolerate pH above 10. Lisichkina et al. (2003) isolated only basidiomycetous yeasts belonging to the genera *Cryptococcus*, *Naganishia*, *Rhodotorula* and *Sporobolomyces* from soda-rich saline soils characterized by pH 10–10.5.

2.4.3 Osmophilic Yeasts

Water availability, generally expressed in physical terms, such as water activity (a_w), is an important factor affecting the growth of microorganisms in nature (Madigan et al. 2000). The usage of the terms to characterize microorganisms able to grow or even prefer to grow in reduced a_w medium is not standard. Some of the interpretations were briefly overviewed by Čadež et al. (2015) and will not be repeated here. According to Pitt and Hocking (2009), a fungus capable of growth, under at least one set of conditions, at a water activity below 0.85 is xerophile, irrespective whether it tolerates or requires reduced a_w. To the contrary, the terms osmophilic for those microorganisms which have an absolute requirement for non-ionic solutes and osmotolerant for those with no absolute requirement of non-ionic solutes were recently advocated by Dakal et al. (2014). According to early literature, the lowest a_w values of the media supporting the growth of a yeast, *Zygosaccharomyces rouxii*, are 0.62–0.65; however, more recent studies could not confirm these data (Jermini and Schmidtlorenz 1987; Deák 2008; Pitt and Hocking 2009).

Among all yeast species treated in the latest edition of *The Yeasts, a Taxonomic Study* (Kurtzman et al. 2011), only *Candida glucosophila* (Tokuoka et al. 1987) requires reduced water activity for its growth, and therefore it was the only species which was considered osmophilic. *C. glucosophila* was recovered from brown sugar. Recently, an additional osmophilic yeast species *Zygosaccharomyces favi* was described based on strains isolated from bee bread and honey (Čadež et al.

Table 2.1 Upper pH limit for growth of yeast species formerly classified in genus *Hansenula*. Data from Aono (1992), modified

Scientific name used by Aono (1992)	Strain designation	Upper pH limit	Current classification[a]	Isolation source[a]
Hansenula californica	NRRL Y-1680	10.5	*Barnettozyma californica*	Swamp soil
Hansenula dimennae	NRRL YB-3239	10.5	*Barnettozyma californica*	Soil
Hansenula dimennae	NRRL Y-5863	10.5	*Barnettozyma californica*	Soil
Hansenula petersonii	NRRL YB-2807	10.5	*Candida homilentoma* (*Hyphopichia* clade)	Frass, *Zelkova serrata*
Hansenula bimundalis	IFO 1366	8.0	*Cyberlindnera bimundalis*	Larvae, *Ergates faber*
Hansenula bimundalis	NRRL Y-5343	7.5	*Cyberlindnera bimundalis*	Insect larva of *Ergates faber*
Candida utilis	IAM 4961	10.5	*Cyberlindnera jadinii*	
Hansenula jadinii	NRRL Y-1542	10.5	*Cyberlindnera jadinii*	Pus, human abscess
Hansenula mrakii	NRRL Y-1364	10.0	*Cyberlindnera mrakii*	Soil
Hansenula petersonii	NRRL YB-3808	10.5	*Cyberlindnera petersonii*	Cadaver lung
Hansenula beijerinckii	NRRL Y-4818	10.0	*Cyberlindnera saturnus*	Elephant dung
Hansenula saturnus var. *saturnus*	NRRL Y-1304	9.0	*Cyberlindnera saturnus*	Soil
Hansenula saturnus var. *subsufficiens*	NRRL YB-1657	10.0	*Cyberlindnera subsufficiens*	Soil
Hansenula saturnus var. *subsufficiens*	NRRL YB-1718	10.0	*Cyberlindnera subsufficiens*	Soil
Hansenula capsulata	IFO 0984	7.5	*Kuraishia capsulata*	Frass on conifer
Hansenula holstii	NRRL Y-2448	8.5	*Nakazawaea holstii*	From mix of NRRL Y-2154 x NRRL Y-2155
Hansenula glucozyma	IFO 1472	7.5	*Ogataea glucozyma*	Frass of bark beetle, *Ips* sp. on *Picea engelmannii* (Engelmann spruce)
Hansenula henricii	IFO 1477	7.5	*Ogataea henricii*	Dropping of bird
Hansenula minuta	IFO 0975	7.5	*Ogataea minuta*	Fermenting fungus, *Mycena pura*
Hansenula nonfermentans	IFO 1473	7.5	*Ogataea nonfermentans*	Water

(continued)

Table 2.1 (continued)

Scientific name used by Aono (1992)	Strain designation	Upper pH limit	Current classification[a]	Isolation source[a]
Hansenula polymorpha	NRRL Y-7560	9.0	*Ogataea parapolymorpha*	Soil
Pichia pini	IFO 1342	7.5	*Ogataea pini*	Bark beetle, *Dendroctonus brevicomis*
Hansenula polymorpha	IFO 1476	8.5	*Ogataea polymorpha*	Soil
Hansenula wickerhamii	NRRL Y-4943	8.5	*Ogataea wickerhamii*	Soil in swamp with trees
Hansenula anomala var. *anomala*	NRRL Y-366	10.0	*Wickerhamomyces anomalus*	Unknown
Hansenula anomala var. *schneggii*	IFO 0806	9.0	*Wickerhamomyces anomalus*	
Hansenula beckii	NRRL Y-1482	7.5	*Wickerhamomyces bisporus*	Frass, spruce bark (*Abies* sp.)
Candida melinii	IFO 0747	8.5	*Wickerhamomyces canadensis*	Wood pulp
Hansenula canadensis	NRRL Y-1888	8.0	*Wickerhamomyces canadensis*	Frass, *Pinus resinosa* (Red Pine)
Hansenula wingei	NRRL Y-2340	9.0	*Wickerhamomyces canadensis*	Frass, *Pinus contorta*
Hansenula ciferrii	IFO 0793	10.5	*Wickerhamomyces ciferrii*	Fruit of tonka-bean
Hansenula ciferrii	IFO 0905	10.5	*Wickerhamomyces ciferrii*	Mycelial form of NRRL Y-1031
Hansenula muscicola	IFO 1383	8.0	*Wickerhamomyces silvicola*	Moss
Hansenula silvicola	NRRL Y-1678	8.5	*Wickerhamomyces silvicola*	Gum of wild black cherry trees (*Prunus serotina*)
Hansenula subpelliculosa	NRRL Y-1822	8.5	*Wickerhamomyces subpelliculosus*	Cucumber brine

[a]Data from NRRL website http://nrrl.ncaur.usda.gov/database.html and NBRC website http://www.nbrc.nite.go.jp/NBRC2/NBRCDispSearchServlet?lang=en

2015). Not surprisingly, all known strains of these two osmophilic yeast species were isolated from high sugar content substrates.

Some yeasts grow also in media with elevated salt content. Numerous ascomycetous and basidiomycetous yeast species are able to grow with 10% NaCl while many fewer with 16% (Barnett et al. 2000). Mokhtarnejad et al. (2016) recovered from Iranian hypersaline soils only basidiomycetes yeasts belonging to the following genera: *Cystobasidium*, *Holtermanniella*, *Naganishia*, *Rhodotorula*, *Saitozyma*, *Solicoccozyma*, *Tausonia*, *Vanrija* and *Vishniacozyma*.

It is supposed that growth in "extreme" environments might be the result of adaptation to the environment from which the yeasts were isolated. Detailed studies including comparative genomics will clarify the mechanisms of adaptation and provide important and helpful information on these "extreme" yeasts.

Extremophiles are organisms, which grow best under conditions that are unsuitable for most microorganisms (Madigan et al. 2000). Therefore, extremophilic (or extremotolerant) microorganisms, including yeasts, can be cultivated under conditions where other microorganisms cannot grow, a situation which can be attractive in the event of their potential industrial application (Takashima et al. 2009).

2.5 Yeast Habitats

Yeast habitats can be categorized to atmospheric, aquatic and terrestrial (Starmer and Lachance 2011). A few of these habitats and the yeasts living there will be briefly touched upon in this chapter; while some other important ones are detailed in other chapters of this book (see also Buzzini et al. 2017a).

2.5.1 The Atmosphere

While a huge amount of data has been accumulated on the occurrence and the role of yeasts in many terrestrial and aquatic habitats, fewer publications deal with yeasts in the atmosphere. Although some reports have suggested that yeasts reproduce in fog, the atmosphere is rather a reservoir than a site for growth and reproduction of yeasts. Pigmented yeasts especially have outstanding abilities to survive in the atmosphere (Starmer and Lachance 2011). Microorganisms, including yeasts, enter the air mainly from soil, vegetation or water (aerosolization) (Delort et al. 2010). In some cases actively discharged ballistoconidia produced by some basidiomycetous yeasts, often found on leaf surfaces, are dispersed via air currents (Starmer and Lachance 2011). In a recent survey 13 of the 17 cloud water samples collected at the Puy de Dôme Mountain, France, 1465 m above sea level, have yielded yeast colonies. One hundred and fifty yeast strains were isolated and most of them were successfully identified to genus level based on LSU rRNA gene sequencing. The vast majority of the isolated yeasts belonged to basidiomycetous genera. Strains of the genera *Dioszegia* and *Udeniomyces*, which contain ballistoconidium-forming species, were most frequently isolated (Vaitilingom et al. 2012). The number of yeasts depositing from the air is much smaller than that of bacteria and filamentous fungi. Culture medium containing petri dishes exposed to air in a desert are colonized by bacteria and filamentous fungi but not by yeasts (Ganter 2011). In view of the preceding information in this section, somewhat unexpectedly, following Koch sedimentation, numerous ascomycetous

yeast strains were isolated from air in Olsztyn, Poland. Based on phenotype the isolates were assigned to 12 ascomycetous species distributed among different families of Saccharomycotina, while the isolation of only one basidiomycetous yeast, *Leucosporidium scottii*, was reported (Ejdys et al. 2014).

Once in the air, microorganisms are exposed to hostile conditions, including solar radiation (especially UV), desiccation, low temperatures, oxidants, low nutrient availability, acidity and rapid variations of salinity. Microorganisms can be deposited from the atmosphere by sedimentation or with precipitation. The concentration of fungi, including yeasts (10^2–10^4 cell mL^{-1}), in the atmospheric water phase (fog and clouds) was found to be one order of magnitude lower than that of bacteria (Delort et al. 2010). It is likely that not all microorganisms entering the atmosphere can survive the rapidly changing and unfavourable conditions they are faced with in the air. During long-distance air dispersion, the atmosphere certainly selects the microorganisms capable of surviving in such an environment (Vaitilingom et al. 2012). It is generally assumed and seems to be supported by many data that pigmented microorganisms have good survival abilities in the atmosphere and in other habitats exposed to the sunlight, e.g. in the phylloplane. Based on the investigation of the effect of some simulated atmospheric stress factors on the survival of a few airborne bacterial strains and one yeast (*Dioszegia hungarica*) strain, Joly et al. (2015) came to the conclusion that probably freeze-thaw cycles and osmotic shocks constitute the most stringent selective factors on the microorganisms in the atmosphere, while the impact of solar light is limited. Klaric and Pepeljnjak (2006) carried out a year-round aeromycological study in the city of Zagreb, Croatia (two sampling sites), and in the nearby Medvednica mountain (one sampling site). Air samples were taken by an air sampler (hole-to-agar impactor). Numbers of airborne yeasts were lower than those of airborne moulds. Depending on the sampling site and the time of sampling, the number of cultivated yeasts was 0.62–46 cfu m^{-3}. The number of culturable airborne yeasts exhibited seasonal patterns, but the dynamics of the changes were different among the sampling sites. In cases, where significant differences were detected, the air in the city contained more yeast cells than the air in the mountain, which was covered with plant associations. Unfortunately, the yeast strains were not identified, but the effect of some meteorological factors on the number of airborne yeasts was investigated. The average relative humidity positively correlated with the number of airborne yeasts, while the effect of average temperature and solar radiation was inconsistent, i.e. varied with different sampling site.

2.5.2 Aquatic Habitats

Aquatic, i.e. freshwater, marine and estuary habitats have been known for a long time to harbour yeasts. The biodiversity and ecology of aquatic yeasts were recently reviewed by Nagahama (2006). Since then, novel aquatic yeast habitats have been discovered. Butinar et al. (2007) and de García et al. (2007) reported the isolation of

numerous, first of all basidiomycetous, yeasts from ice of high Arctic glaciers in Northern Europe and from glacial meltwater in South America. About 10% of the recently isolated yeasts from glacial environments belong to undescribed species (Turchetti et al. 2013). The diversity, cold adaptation strategies and biotechnological potential of yeasts from glacial habitats have been reviewed by Buzzini et al. (2012, 2017b) and by Sannino et al. (2017).

An additional new aquatic yeast habitat, water surrounding hydrothermal vents, has recently been identified by Gadanho and Sampaio (2005). Six of the seven water samples collected in the Mid-Atlantic Ridge hydrothermal fields near the Azores Archipelago yielded yeast strains. Non-pigmented yeasts were more abundant than pigmented ones and about one third of the detected yeast species represented undescribed taxa. Yeasts occurring both in "conventional" and "non-conventional" aquatic habitats have been reviewed by Hagler et al. (2017) and by Libkind et al. (2017).

2.5.3 Terrestrial Habitats

The association of terrestrial yeasts with plants and animals has been studied for a long time, while mushrooms, as a habitat for yeasts, have not attracted special attention. Plants as primary producers supply organic compounds necessary for the existence of heterotrophic organisms, like animals, yeasts and other fungi. Soil, a further habitat for terrestrial yeasts, is the "ultimate repository for organic and inorganic materials and constitutes a suitable medium for storage and even development of certain species of yeasts" (Phaff and Starmer 1987). Several important terrestrial yeast habitats have been thoroughly reviewed by Buzzini et al. (2017a); therefore, they will not be discussed here.

2.5.3.1 Flowers

Flowers have been considered an ideal yeast habitat for a long time (reviewed in Phaff and Starmer 1987). Nectars are especially suitable for yeasts because of their high sugar content and because of the frequent visits of pollinating insects transporting the yeasts between flowers of different host plants (Phaff and Starmer 1987). Flowers are usually ephemeral organs of plants providing transient habitats for yeasts; therefore, the vectors, mainly insects, introducing the yeasts to this habitat have an outstanding role in maintaining yeast communities in this environment. For a recent review of the microbial ecology of flowers, not restricted to yeasts, see Aleklett et al. (2014).

Flower yeast communities are generally dominated by ascomycetous yeast species, although recently during a 2 years study conducted on the Canary Islands, bird-pollinated flowers contained unexpectedly high numbers of basidiomycetous yeasts. In addition to *Metschnikowia gruessii*, *Vishniacozyma* (former *Cryptococcus*)

carnescens was the most widespread species in floral nectars. Less concentrated hexose-dominant nectars facilitated the colonization of flowers by basidiomycetous yeasts (Mittelbach et al. 2015). Insect-vectored yeast communities of ephemeral flowers mostly contain yeasts of the *Metschnikowia*, *Kodamaea*, *Wickerhamiella* and *Starmerella* clades. Members of the first three clades are vectored mainly by beetles and flies, while yeasts in the *Starmerella* clade are mostly carried by bees (Lachance et al. 2001). Novel yeast species and genera, e.g. *Metahyphopichia* (Sipiczki et al. 2016), are continuously being described from flowers and flower-associated insects. In addition to discovering novel taxa, several reports have been published recently on the population structure and on the function of flower-associated yeasts.

Metschnikowia reukaufii is a very frequently isolated yeast species from floral nectar (e.g. Brysch-Herzberg 2004). Pozo et al. (2012) observed that among strains isolated from nectars of *Digitalis obscura* and *Atropa baetica* as well as related habitats (bees, air, corolla and pollen), *Metschnikowia* strains did not possess outstanding resistance to plant secondary compounds and high sugar concentrations. However, they exhibited higher growth rates on culture media with elevated sugar contents at different temperatures (see also Mittelbach et al. 2015). The observed genetic diversity of *M. reukaufii* strains in Sierra de Cazorla region, southeastern Spain, was found to be host plant mediated (Herrera et al. 2014). Environmentally induced DNA methylation also contributes to the growth success of *M. reukaufii* in patchy environment where sugar composition and concentration can be extremely variable from flower to flower (Herrera et al. 2012). Herrera (2014) demonstrated that the nectars collected from eight host plants and characterized with different sugar content and composition, different amino acid content and pH had significant effect on the growth of selected *M. reukaufii* strains representing different genotypes. Furthermore, on average, the nectar of *Helleborus foetidus* best supported the growth of *M. reukaufii* strains isolated from the nectar of the same plant species. It was also observed that different yeast species, e.g. *M. reukaufii* and *M. gruessii* exerted different effects on the composition of the nectar of *Hell. foetidus* and that differential yeast occurrence across host plants may modify the interaction between the plant and its pollinators (Canto et al. 2015).

Due to the intimate association of flowering plants, nectar-dwelling yeasts and their insect vectors, it is reasonable to suppose that the organisms involved in these associations may have co-evolved. This hypothesis was tested and not supported by comparisons of divergence time estimates concerning the yeast family Metschnikowiaceae and angiosperms (Guzman et al. 2013).

2.5.3.2 Fruits

Yeast communities of fruits are dynamic in time. Unripe fruits harbour a yeast population similar to that of the leaf or stem surfaces (reviewed in Fonseca and Inácio 2006), mostly oligotrophic, non-fermentative species previously classified in polyphyletic genera *Cryptococcus* and *Sporobolomyces*, and yeast-like black yeasts

Aureobasidium (de la Torre et al. 1999; Prakitchaiwattana et al. 2004). During fruit ripening one or more pericarp layers become soft which can cause spatial leaking of sugar-rich juice as an attractive food for yeasts. With this event yeast diversity increases, and the fast-growing yeasts of genera *Hanseniaspora*, *Metschnikowia*, *Pichia* and *Starmerella* (Morais et al. 1995; Prakitchaiwattana et al. 2004; Barata et al. 2008b; Čadež et al. 2010) swiftly outnumber the earlier residents. These species are characterized by a narrow physiological profile as they assimilate only few simple sugars. With fruit deterioration, sugars and microbial metabolites become commonly available and the diversity of yeasts further increases. Beside species of the genus *Pichia* (including species previously assigned to *Issatchenkia*), *Meyerozyma*, *Saturnispora*, *Saccharomycopsis*, *Zygoascus* and *Zygosaccharomyces* become predominant (Morais et al. 1995; Nisiotou and Nychas 2007; Barata et al. 2008a). The general community structures are similar regardless of fruit type, geographical origin and microclimatic conditions (Miller and Phaff 1962; Starmer et al. 1987; Morais et al. 1995; Prada and Pagnocca 1997; for grapes reviewed by Barata et al. 2012a; Kachalkin et al. 2015). Yeasts found in tropical fruits have been reviewed by Ganter et al. (2017).

Due to their importance for wine production, grapes became a model fruit system for studying the succession of yeast communities on ripening fruits. Recent studies employing DNA deep community sequencing approaches of grape microbiota showed that the general fungal species composition associated with grapes show considerable spatial heterogeneity and might be responsible for metabolic footprint or geographical character of wines (Bokulich et al. 2014; Taylor et al. 2014; Setati et al. 2015). However, in contrast to the commonly used culture-based approaches, filamentous fungi were the predominant taxa when deep community sequencing was employed.

The evolutionary model of *S. cerevisiae* is based on an assumption that when fruits ripen, a fierce competition for the sugars starts between *S. cerevisiae* or its close relatives and other species of the community. An outcome of this battle might be the predominance of *S. cerevisiae* with its evolutionary acquired trait of "make-accumulate-consume (ethanol)" strategy (Piškur et al. 2006; Rozpedowska et al. 2011) which could be a reason for its predominance over yeast communities during traditional beverage fermentations (Heard and Fleet 1985; Romano and Marchese 1998; Morrissey et al. 2004; Duarte et al. 2010). However, as Goddard and Greig (2015) pointed out, *S. cerevisiae* is found in extremely low numbers on various fruits in different stages of their ripening (Morais et al. 1995; Mortimer and Polsinelli 1999; Barata et al. 2012b; Taylor et al. 2014); therefore, its adaptive evolution is difficult to simplify to the level of organism-substrate. Such interactions, studied on experimental populations of the deletion mutants of *S. cerevisiae* (MacLean and Gudelj 2006), showed that when resources are spatially structured in the environment, cooperation between two cocultures with different metabolic backgrounds wins. On the contrary, when resources are homogenous, the "cheater" or the culture which can switch between respiration and fermentation wins. The ecology of *S. cerevisiae* is discussed in more details in Chap. 5 of this book.

2.5.3.3 Tree Fluxes

Tree fluxes occur where the bark has been mechanically injured but their composition varies seasonally. During early spring xylem sap flows from roots towards dormant buds of deciduous trees as an aqueous solution of sugar, primarily the disaccharide sucrose as well as minerals, amino acids and hormones leak. When injured, this spring sap is nitrogen poor (Golubev et al. 1977), but the sucrose concentration may be as high as 30% by weight, giving the sap a syrupy thickness. As such it is heavily contaminated with bacteria, protozoa and yeasts, some of which can later produce exopolysaccharides forming a thick material named slime flux. The established yeast communities of slime fluxes were shown to have little seasonal variation (Phaff et al. 1964; Lachance et al. 1982; Bowles and Lachance 1983), but seem to be host specific regardless of geographic location (Lachance and Starmer 1982). As shown in Table 2.2, none of the yeast species could be regarded as specific for slime flux habitat. However, some species like *P. membranifaciens*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Komagataella pastoris*, *Kregervanrija fluxuum* and *Torulaspora delbrueckii* were found as frequently isolated yeast species (Golubev et al. 1977; Lachance et al. 1982; Ganter et al. 1986; Spencer et al. 1996). The community structure is most probably shaped by the sugar composition of the fluxes as well as by the presence of tannins, alkaloids and other nitrogenous compounds (Spencer et al. 1996). Species composition of the communities could be classified by their physiological attributes into several groups like generalist basidiomycetous species, fermenting ascomycetous yeasts *Kluyveromyes*, *Torulaspora*, *Saccharomyces* and *Lachancea* as well as methanol-assimilating yeasts of the genera *Ogataea* and *Komagataella*. The latter were present in tree fluxes of every host species except birch, in spring sap (Table 2.2). During the course of our own studies applying enrichment in methanol-containing broth, methanol-assimilating yeast species were regularly isolated from slime fluxes collected in Hungary. Seventy-five percent of the 186 samples, collected from different tree species, yielded 150 methylotrophic yeast strains. *Kom. pastoris* was far the most frequent yeast in slime fluxes (63% of all strains), followed by *Ogataea populialbae*, *Candida boidinii*, *Komagataella pseudopastoris* and a few more species in the *Ogataea* and *Kuraishia* clades. In some cases host specificity was observed. For example, while *Kom. pastoris* occurred commonly in slime fluxes of oak trees (*Quercus* spp.), *Kom. pseudopastoris* has never been isolated from oak. Oaks are known to have high tannic acid content. In agreement with the chemistry of the habitats, *Kom. pseudopastoris* proved to be less tolerant to tannic acid than *Kom. pastoris* (Dlauchy et al. 2003).

In contrast to slime fluxes, the spring sap flows are restricted to the period of bud break when xylem sap flows upward from the roots. If the trunk is injured, some tree species like birch (*Betula* spp.) produce exudates abundantly. Golubev et al. (1977) revealed that in these types of tree exudates, yeast communities are dynamic in time as they are primarily rich in several different basidiomycetous yeast species like *Bullera*, *Cystofilobasidium*, *Tausonia*, *Hannaella*, *Mrakia*, *Naganishia*,

Table 2.2 Yeast species isolated from tree exudates of different tree species

Slime flux					Spring sap
Oak (*Quercus* spp.)[a,b]	Elm (*Ulmus* spp.)[a]	Cottonwood (*Populus* spp.)[a]	Douglas fir (*Pseudotsuga*)[a]	Algarrobo (*Prosopis alba*)[b,c]	Birch (*Betula* spp.), Beech (*Fagus* spp.)[d,e]
					Aureobasidium pullulans
		Barnettozyma salicaria			
				Bulleribasidium variabile	*Bullera* sp.
Candida (*Barnettozyma*) *norvegica*	*Candida* (*Barnettozyma*) *norvegica*	*Candida* (*Barnettozyma*) *norvegica*			
Candida (*Ogataea*) *boidinii*		*Candida* (*Ogataea*) *boidinii*	*Candida* (*Ogataea*) *boidinii*		
				Candida (*Yamadazyma*) *insectorum*	
				Candida (*Starmerella*) *magnoliae*	
			Candida (*Cyberlindnera*) *maritima*		
			Candida (*Kodamaea*) *mesenterica*		*Candida* (*Kodamaea*) *mesenterica*
				Candida (*Hyphopichia*) *rhagii*	
Candida sake				*Candida sake*	*Candida sake*
	Goffeauzyma gastrica				
Papiliotrema laurentii					

(continued)

Table 2.2 (continued)

Slime flux					Spring sap
Oak (*Quercus* spp.)[a,b]	Elm (*Ulmus* spp.)[a]	Cottonwood (*Populus* spp.)[a]	Douglas fir (*Pseudotsuga*)[a]	Algarrobo (*Prosopis alba*)[b,c]	Birch (*Betula* spp.), Beech (*Fagus* spp.)[d,e]
					Cystofilobasidium capitatum
					Cystofilobasidium infirmominiatum
					Cystofilobasidium macerans
Debaryomyces hansenii				*Debaryomyces hansenii*	
Schwanniomyces polymorphus					
					Filobasidium chernovii
	Filobasidium magnum				
					Filobasidium stepposum
				Filobasidium uniguttulatum	
Geotrichum klebahnii			*Geotrichum klebahnii*		
					Goffeauzyma gastrica
					Tausonia pullulans
	Hannaella luteola				*Hannaella luteola*
	Hanseniaspora uvarum				
					Holtermanniella festucosa
Kluyveromyces lactis	*Kluyveromyces lactis*		*Kluyveromyces lactis*	*Kluyveromyces* sp.	
			Kluyveromyces wickerhamii		
Komagataella pastoris	*Komagataella pastoris*	*Komagataella pastoris*	*Komagataella pastoris*		
		Kregervanrija delftensis			

Kregervanrija fluxuum			*Kregenvanrija fluxuum*	*Kregervanrija fluxuum*	
			Lachancea kluyveri		
				Lachancea thermotolerans	
					Leucosporidium scottii
Metschnikowia pulcherrima					
				Millerozyma farinosa	
					Mrakia blollopis
					Mrakia gelida
					Mrakia robertii
					Nadsonia fulvescens
				Naganishia albida	*Naganishia albida*
		Ogataea minuta			
Ogataea pini					
				Ogataea polymorpha	
					Papiliotrema laurentii
				Phaeotremella skinneri	*Phaeotremella skinneri*
Pichia membranifaciens	*Pichia membranifaciens*		*Pichia membranifaciens*	*Pichia membranifaciens*	
					Phaffia rhodozyma
Priceomyces carsonii				*Priceomyces carsonii*	
					Rhodosporidiobolus colostri
					Rhodotorula babjevae
Rhodotorula glutinis					*Rhodotorula glutinis*
					Cystobasidium minutum
					Rhodotorula mucilaginosa

(continued)

Table 2.2 (continued)

Slime flux					Spring sap
Oak (*Quercus* spp.)[a,b]	Elm (*Ulmus* spp.)[a]	Cottonwood (*Populus* spp.)[a]	Douglas fir (*Pseudotsuga*)[a]	Algarrobo (*Prosopis alba*)[b,c]	Birch (*Betula* spp.), Beech (*Fagus* spp.)[d,e]
Saccharomyces cerevisiae			*Saccharomyces cerevisiae*		
					Schwanniomyces vanrijiae
				Solicoccozyma terreus	
					Sporidiobolus salmonicolor
					Sporidiobolus pararoseus
					Sporobolomyces roseus
Sporopachydermia quercuum					
					Sterigmatomyces sp.
Torulaspora delbrueckii	*Torulaspora delbrueckii*			*Torulaspora delbrueckii*	
Cutaneorichosporon cutaneum					
					Vishniacozyma tephrensis
				Wickerhamomyces ciferrii	
			Wickerhamomyces strasburgensis		
				Wickerhamomyces sydowiorum	

[a]Lachance et al. (1982)
[b]Ganter et al. (1986)
[c]Spencer et al. (1996)
[d]Golubev et al. (1977)
[e]Yurkov, unpublished data
Genus names in parenthesis indicate the affiliation of the *Candida* species

Papiliotrema, *Phaeotremella*, *Rhodotorula*, *Sporobolomyces* and *Sporidiobolus*, which are mostly characterized as generalists and producers of either exopolysaccharides or pigments or both. Ascomycetous yeasts that are present as early colonizers were identified as apiculate yeasts of the genus *Nadsonia*. The second phase is characterized by an elevation of air temperature and consequently by the dominance of two other yeast species, *Tausonia* (former *Guehomyces* or *Trichosporon*) *pullulans* and *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*) (Golubev et al. 1977; Weber et al. 2006). The last two species were further characterized for their role in winning the competition with early community yeast species in which their faster growth rates or the ability of *Ta. pullulans* to produce killer toxin were determined (Golubev et al. 2002). *Ph. rhodozyma* today is an important industrial microorganism since it produces astaxanthin and was mostly isolated from slime fluxes on birch and other trees. *Ph. rhodozyma* possesses unique characteristics for yeasts belonging to Basidiomycota, among which are the ability to ferment sugars, Crabtree-positive character and the induction of sexual cycle by polyols that were shown to be present in tree exudates colonized by this species (Golubev 1995; Reynders et al. 1997; David-Palma et al. 2014).

2.5.3.4 Endophytic Yeasts

The term endophytes was coined 150 years ago for organisms colonizing internal plant tissues (De Bary 1866). One of the many subsequent definitions which is commonly used (Hyde and Soytong 2008) was formulated by Petrini (1991). According to this, endophytes are "all organisms inhabiting plant organs that at some time in their life, can colonize internal plant tissues without causing apparent harm to their host." Circumstantial evidence suggests that some endophytes become primary saprobic decomposers (Hyde and Soytong 2008 and the references therein).

Unlike with other fungi, data published on the endophytic yeasts are rather sporadic. For a recent review see Doty (2013). Numerous studies aiming at the isolation of endophytic fungi failed to obtain any yeast cultures or the yeasts accounted only for a small minority of all isolates. In the case of culture-based approaches, the failure to detect yeasts can partly be the consequence of the applied methodology. The commonly used fragment plating method, i.e. the incubation of small pieces of surface-sterilized plant tissues on the surface of suitable agar plates, does not favour the isolation of yeasts as they do not grow out of plant tissues like filamentous fungi. By using the dilution-to-extinction protocol for foliar endophytes which includes a homogenization and a particle filtration step, Unterseher and Schnittler (2009) were able to isolate 24 endophytic yeast strains from leaves of *Fagus sylvatica*, while no yeast strains were recovered by the fragment plating method in the same study. The yeasts were not identified at species level but were designated as member of polyphyletic genera (e.g. *Rhodotorula* sp.) or as "white yeast". With the aid of the above-noted dilution-to-extinction method, Solis et al. (2015) isolated 191 endophytic yeast strains from leaves of three different *Ficus*

species collected at two locations from greenhouse-grown plants in Germany. The isolates were identified as 23 basidiomycetous yeast species assigned to the genera *Cystobasidium*, *Filobasidium*, *Naganishia* and *Rhodotorula*. No ballistoconidium-forming strains were isolated. In a study in Thailand, utilizing a culture-independent approach, 12 yeast operational taxonomic units (OTUs) were detected, in addition to 112 OTUs corresponding to filamentous fungi from rice leaves. Endophytic yeasts were detected in 19 of the 46 leaf samples. The 12 OTUs are composed of 28 different phylotypes, three-quarters of which corresponded to basidiomycetous yeasts. Sequence comparisons of the D1/D2 domains of the LSU rRNA gene indicated that several undescribed basidiomycetous yeast species were detected (Tantirungkij et al. 2015). Given the limited amount of the data on the occurrence of endophytic yeasts, no plant-specific associations have yet been revealed. Likewise, Isaeva et al. (2009) suggested that the existence of highly specialized yeast species associated with tissues of certain plants is unlikely.

Endophytic yeasts may exert plant growth-promoting effects. In vitro production of indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) by *Cyberlindnera* (*Williopsis*) *saturnus* endophytic in maize roots (Nassar et al. 2005) and IAA production by *Rhodotorula graminis* and *Rhodotorula mucilaginosa* originating from *Populus* spp. (Xin et al. 2009) was observed. L-tryptophan content of the medium was either a criterion of IAA production or enhanced its production several fold. Three rice leaf endophyte *Saitozyma flava* strains produced more than 4.8 mg IAA g^{-1} dry cell weight (Nutaratat et al. 2014). The application (to the seed) of a heavy metal-resistant, week IAA producer endophytic *Cryptococcus* (according to the DNA sequence of its ITS region probably *Filobasidium magnum*) strain promoted the survival and growth of *Brassica alboglabra* in multi-metal contaminated soil and also enhanced heavy metal extraction from the soil (Deng et al. 2012).

2.5.3.5 Mushrooms

Mushrooms are poorly sampled yeast habitats as the systematic reports correlating yeast community structure with host species, geographical origin or fruiting body maturation stage are lacking. However, based on the observations of Pimenta et al. (2009), yeast communities differ from other yeast communities associated with other substrates, like fruit, tree exudates or *Drosophila* of tropical forest. Community structure of yeasts is in the first place shaped by food source of basidiocarps which are composed of alcoholic sugar mannitol and the disaccharide trehalose and polysaccharides such as glycogen and chitin (Kalac 2013). However, complex microbial communities are hosted on fruiting bodies and interactions between them are interesting from the view of the health of fruiting bodies where yeasts can either inhibit or stimulate mycoparasitic fungus (Yurkov et al. 2012b).

In general, yeast communities associated with mushrooms as determined by Babjeva and Reshetova (1998), Middelhoven (2004), Pimenta et al. (2009) and Yurkov et al. (2012b) seem to be composed of generalist yeast species of

Aureobasidium pullulans; ascomycetous *Candida parapsilosis*, *C. krusei* (*P. kudriavzevii*) and *P. membranifaciens*; and basidiomycetous polyphyletic phenotypic genera, i.e. *Rhodotorula*, *Trichosporon* and *Cryptococcus* (and related species) associated with plants; species generally associated with soil *Apiotrichum* (*Trichosporon*) *porosum* and *Apiotrichum* (*Trichosporon*) *laibachii* and specialist species. Among the latter are three groups of phylogenetically closely related species of which many have only recently been described. The first belongs to the basidiomycetous yeast species *Vanrija humicola*, *Vanrija albida*, *Vanrija meifargana* and *Vanrija nanouana*, which were most frequently isolated from soils, basidiocarps or beetles feeding on them (Liu et al. 2011; Prillinger et al. 2007; Takashima et al. 2001). The second group of phylogenetically closely related species belong to the genus *Kodamaea* in which *s*pecies from mushrooms or basidiocarp-feeding beetles cluster separately from other species of this genus (Hsieh et al. 2010). Members of this group belong to the following species "*Endomyces scopularum*", *Kodamaea laetipori*, *Candida sagamina*, *Candida fukazawae*, *Candida fungicola* (Nakase et al. 1999; Suh et al. 2001; Suh and Blackwell 2005) and four recently described species *Candida plutei*, *Candida kaohsiungensis*, *Candida lidongshanica* and *Candida smagusa* (Hsieh et al. 2010). All the last four species can ferment trehalose which could be an indication of their substrate specificity. The third group of specialized yeast species is associated mostly with basidiocarp-feeding beetles and belongs to a large group of 16 recently described *Suhomyces* (former *Candida tanzawaensis* clade) species (Suh et al. 2004).

Recently, a fungal parasite of *Nothofagus*, an endemic forest tree of Southern hemisphere, *Cyttaria hariotii*, attracted attention as it harboured a novel psychrophilic yeast species *Saccharomyces eubayanus* (Libkind et al. 2011), *Ph. rhodozyma* (Libkind et al. 2007; David-Palma et al. 2014) and a few fermenting ascomycetous yeast species. The reason for the association of a fermentative yeast community with mature *Cytt. hariotii* is its sugar composition, which consists of up to 10.2% of simple sugars (fructose, glucose and sucrose) (Gamundí and De Lederkremer 1989) and as such resembles to the composition of grape juice.

2.6 Concluding Remarks

According to recent estimations, millions of fungi are waiting for their discovery (Blackwell 2011). Considering the ratio of currently described yeasts and the total number of known fungi, the above estimation, if correct, implies tens of thousands of novel yeast species to be discovered in the future. Current yeast species description rates support the estimations suggesting at least the above-noted magnitude of yeast species. The creation (Kurtzman and Robnett 1998; Fell et al. 2000) and continuous updating of a barcoding sequence database for yeasts has provided an excellent tool for rapid, reliable identification of yeasts enhancing the exploration of their biodiversity and understanding their ecology. Recently, the application of

DNA-based culture-independent methods has taken an increasing role in the investigation of yeast biodiversity and ecology. In addition to the continuous discovery of novel yeast species, significant effort is being made to understand their function in their natural habitats.

On the local scale, even within a very small physical space in a given ecosystem, very different habitats may co-exist, supporting dramatically different yeast communities. Different organs of a single plant and even different parts of a single organ (e.g. within a flower) may provide different microhabitats for yeasts. It is also documented that the vertical or horizontal position of a given organ may influence the yeast community it harbours. On the global scale, distantly located but similar habitats may support ubiquitous yeast species or species with similar fundamental niches. The latter sometimes prove to be cryptic. Authors describing novel yeast species are often struggling with finding phenotypic characters to distinguish the novel species from closely related taxa, successfully recognized by DNA-barcoding sequences. In their review on the phylloplane yeasts Fonseca and Inácio (2006) noted that phenotype-based yeast identification, a general practice till the late 1990s, in many cases can be considered merely tentative. Molecular phylogenetic analyses shed light on the taxonomic heterogeneity of some former ubiquitous phylloplane yeast species. Similarly, Yurkov et al. (2015) revised the species assignments of yeast strains isolated from Russian birch forests and earlier identified from phenotype. PCR fingerprinting and rDNA sequence-based identification revealed that instead of the originally supposed 9 species, the strains represent 21 yeast species, including 3 undescribed taxa. Therefore, due to the widespread application of accurate, sequence-based identification in the last two decades, it has gradually become obvious that in many habitats cryptic and phenotypically indistinguishable yeasts are replacing previously recognized ubiquitous ones.

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Chapter 3
Yeast Community Composition and Structure

Andrey Yurkov and María I. Pozo

Abstract Yeasts are globally distributed, but different species occur in different climates and environments. With a few exceptions, yeasts do not occur in their natural environments as a pure culture but co-occur with other microscopic eukaryotes and prokaryotes and comprise microbial communities. The observed yeast diversity in natural environments is a combined result of the response of each species to habitat conditions, including arrival, growth, and further dispersal, and the biotic interactions among species. In this chapter, we review some recent concepts and tools developed in community ecology and discuss how they may help understand yeast diversity in nature. We address species recognition approaches and the effects of the intraspecific variation and application of molecular operational taxonomic units on the yeast community parameters. Community ecology tools discussed in this chapter include diversity (taxonomic and functional), quantity, priority effects, species richness estimators, and species-abundance distribution. Additionally, we compare the use of community composition and community structure parameters in the literature. Concepts such as frequent (vs. rare), autochthonous (vs. transient or allochthonous) and specialist (vs. generalist) yeast species are also discussed through this chapter.

Keywords Species • Diversity • Quantity • Community parameters • Ecology tools

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3.1 Introduction

Yeasts are globally distributed, but different species occur in different climates and environments, and they also vary in the morphological (e.g. pigmentation, forcibly ejected propagules, hyphal growth, chlamydospores), physiological (e.g. fermentation, utilisation of low-weight aromatics, vitamin-free growth) and physiochemical (e.g. psychrophily, production of siderophores and acids) traits. Linking these features to the spatial distributions of species is fundamental to the understanding of yeast diversity. With a few exceptions, yeasts do not occur in their natural environments as a pure culture but co-occur with other microscopic eukaryotes and prokaryotes. Thus, yeast diversity in natural environments is a combined result of the response of each species to habitat conditions, including arrival, growth, and further dispersal, and the biotic interactions among species.

For example, flowers, rotting cacti, and tree fluxes are rich with simple sugars or alcohols and offer certain groups of yeasts a suitable habitat. These substrates are frequently visited by insects that vector yeasts, thereby, largely determining the membership of the yeast community. In the absence of vectors, these habitats might not bear any yeasts (reviewed by Starmer and Lachance 2011). To ensure transportation between mosaic habitats, yeasts steer the dispersal by targeting attraction of the vector with volatiles (e.g. Becher et al. 2012; Davis 2015; Holighaus and Rohlfs 2016) or, conversely, by masking their presence in the habitat (Mittelbach et al. 2016a). Passive propagation can also be facilitated by several morphological properties, including formation of ballistospores by phylloplane yeasts (also some mycoparasites), “aeroplane” (cross-like configuration) cells of the nectar yeast *Metschnikowia gruessii* and hydrophobic cells (rotting cacti), among them (Brysch-Herzberg 2004; Fonseca and Inácio 2006; Starmer and Lachance 2011; Pozo et al. 2012). Research on yeast co-growth in the habitat received some attention in the past, and studies report a broad range of naturally occurring interactions between species (reviewed by Starmer and Lachance 2011). Recently, environmental alteration (also called priming) by early-arriving yeasts was also

recognised as an important factor influencing flower nectar communities (see below).

In this chapter, we review some recent concepts and tools developed in community ecology and discuss how they may help understand yeast diversity in nature. Although we mainly focus on synecology (community ecology), we recommend reading book chapters summarising the knowledge on yeast autecology (species ecology) and diversity (Lachance and Starmer 1998; Lachance 2006; Starmer and Lachance 2011; Buzzini et al. 2017). Through the chapter, we shall frequently refer to the niche concept described previously by Lachance and Starmer (1998) and Starmer and Lachance (2011). The chapter is composed of two sections: we will first review the ways in which the structure (such as species composition and diversity) of communities is described and then discuss the ecological processes that are thought to determine community structure.

3.2 Community Composition and Diversity

Biodiversity has been a hot topic in community ecology for a long time, and studies addressing diversity in microbial ecosystems abound. The majority of studies listed yeast species isolated from different habitats (Fig. 3.1). As the approaches to species identification and circumscription evolved, the definition of the species as an ecological unit was changing gradually with more sophisticated techniques involved in the characterisation of yeast isolates (Barnett 2004; Lachance 2006).

3.2.1 Species Recognition

Ecological interactions among different taxa determine diversity of a community and ecosystem functioning. By "taxa", we normally refer to genus and species, and those are characterised by cell morphology (e.g. spore shape, cell division), physiology (e.g. carbon and nitrogen source assimilation tests) and phylogenetic relatedness, which is mostly estimated, in the case of yeast, by comparing sequences of the D1/D2 domains of the 26S (or LSU) rDNA gene (e.g. Kurtzman and Robnett 1998; Fell et al. 2000; Scorzetti et al. 2002). In the *Origin of the Species*, Darwin stated that "I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other, and that it does not essentially differ from the term variety, which is given to less distinct and more fluctuating forms. The term variety, again, in comparison with more individual differences, is also applied arbitrarily, and for mere convenience sake" (Darwin 1872). Like other organisms, yeast species should represent cohesive evolutionary units. Currently, there is considerable controversy on the best way of documenting the boundaries of such units (e.g. Lachance 2006, 2016; Lachance et al. 2010). Kurtzman and Robnett (1998), by conducting a large-scale study of D1/D2

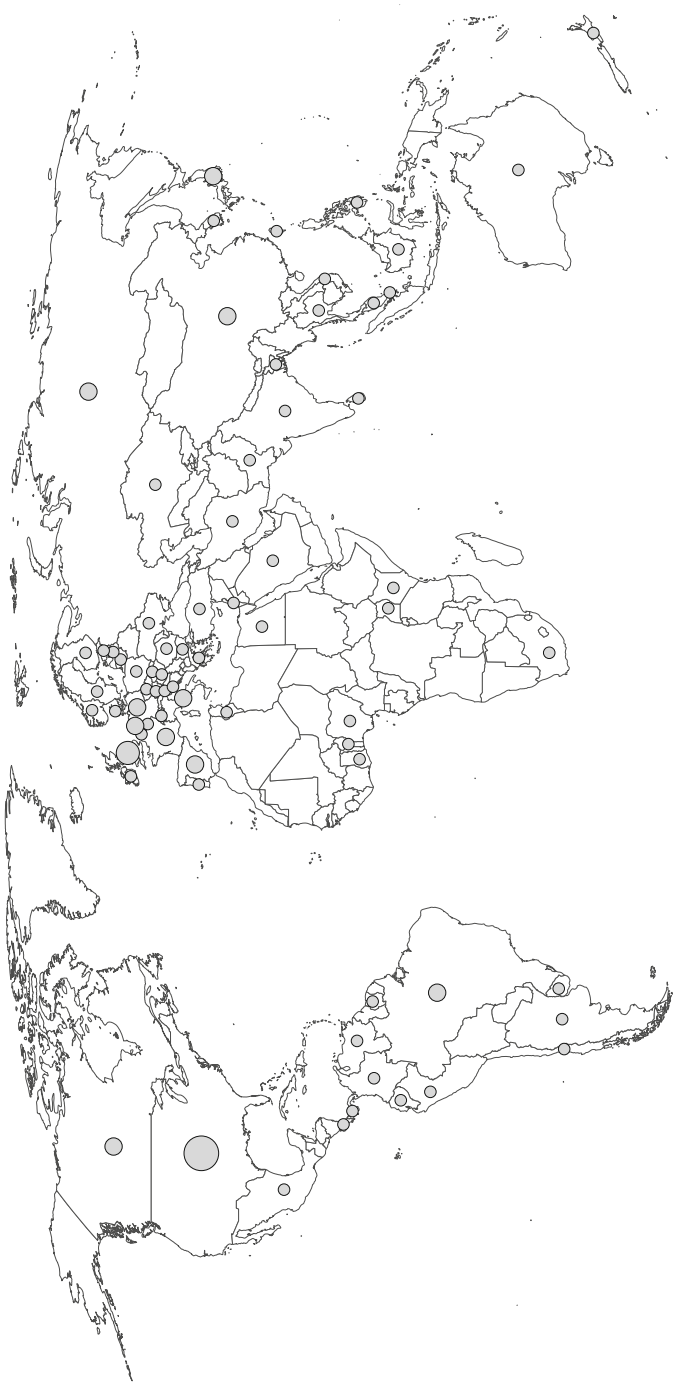

Fig. 3.1 Geographic distribution of publications in which natural yeast communities have been surveyed. A total of 1528 references have been collected using Web of Science and Google Scholar databases (citations from the English-speaking literature published from 1980 onwards have only been considered, stand December 2016). The size of *circles* is proportional to the number of publications

ribosomal LSU sequences of 500 species of ascomycetous yeasts, showed that strains that differ by less than 1% nucleotide substitutions are likely to be members of the same species. As a result, this threshold has been widely accepted as a rule to delineate species of yeast, instead of the 3% prevailing rule for many other organisms. Despite distant evolutionary relationships between ascomyceteous and basidiomycetous yeasts, the "1% rule" was used as an argument to delimit species in the latter group. However, another large-scale study of LSU sequence heterogeneity in Basidiomycete yeasts performed by Fell et al. (2000) did not show any suitable cut-off value. Similarly, the follow-up study did not reveal a common threshold, neither in LSU nor in ITS, in this group of fungi (Scorzetti et al. 2002). As new molecular identification methods develop, Lachance and Starmer (1998) foresaw that "one can easily conceive of the possibility that a primary isolation plate, a microscope slide, or even a fixed section of a yeast habitat could be treated with a mixture of DNA probes, each tagged with a different chromophore, in such a way that direct identification of individual colonies or even single cells in situ would be achievable". Although quick and affordable identifications of yeasts based on the DNA sequences have become feasible, the determination of a yeast's physiological characteristics will never cease to be of importance in understanding its ecology.

The species concept has a great influence on diversity studies because different approaches to yeast species recognition often result in different entities. A review that aimed at resolving the impact of the species concept on biodiversity studies showed remarkable differences, with surveys based on a phylogenetic species concept detecting 48% more species (300% more for fungi) and an associated decrease in population size and range (Agapow et al. 2004). The same holds true for widespread basidiomycetous yeasts identified with physiological and phylogenetic approaches (Yurkov et al. 2015a). Specifically, several phylogenetic species of the genera *Filobasidium* and *Vishniacozyma* were nested in "phenotypic" *Cryptococcus albidus* and *Cryptococcus laurentii* (Yurkov et al. 2015a). This study also showed that species recognition approach might affect our understanding of community structure and the distribution range of yeast species and require a larger sampling effort.

3.2.2 Strain Variation

Dissimilarities between isolates of the same species may represent an annoyance in practical taxonomy, is also a component of biodiversity, and communities must comprise this level of sampling. The conventional approach of taking one colony as a representative of a species (Lachance and Starmer 1998) does not capture functional diversity within species. Researchers are becoming increasingly conscious about intraspecific variance. For example, the general occurrence of within-species variation was detected by a PCR fingerprinting in sea water basidiomycete communities (Gadanho et al. 2003). Similarly, strains of the same species isolated

from forests in Germany, Portugal and Russia showed variation in ribosomal gene sequences (Yurkov et al. 2012a, 2016) and PCR fingerprints (Yurkov et al. 2015a). Yeasts have quick life cycles, and a larger number of offspring can be produced asexually instead of following a sexual cycle. The resulting yeast populations may constitute distinct biotypes subject to unusual forms of selection. In this scenario, mutation can be easily fixed as a result of diversifying selection, as shown in ascomycetous yeasts (Herrera et al. 2011). Although the sexual cycle of ascomycetous yeasts is commonly observed in the laboratory, teleomorphic stages of many basidiomycetous yeasts are known from the field studies only (e.g. Tremellomycetes, Ustilaginomycetes) and are rarely observed under laboratory conditions. Thus, in many cases, the sexual cycle remains little understood in basidiomycetes, although the sexual recombination can be revealed with a genomic approach (Coelho et al. 2011; Yurkov et al. 2015b). A diverse origin of the isolates, which also reflects different environmental constraints, typically favours high levels of intraspecific variation in wild yeast populations (Pozo et al. 2015; Yurkov et al. 2015a, b).

3.2.3 Units Other than Species and Operational Taxonomic Units (OTUs)

Although community analyses are commonly made using species, other units can be also used. Yeasts were the first group of fungi, where a DNA barcoding was adopted for species identification (Kurtzman and Robnett 1998; Fell et al. 2000; Schoch et al. 2012). Fast development of sequencing techniques and availability of sequence databases resulted in an enormous increase in the ease and speed of identification, making intense biodiversity surveys almost manageable.

Once taxa are delineated, the characterisation of yeast communities encounters additional difficulties. Biodiversity estimates are often biased on taxa that are easily cultivable in the lab (see the discussion on rare species below). Only less than 1% of the estimated microbial diversity is thought to be cultivable in laboratory conditions due to the low growth rate of many environmental microorganisms (Amann et al. 1995). To what extent this constraint can be applied to yeasts is yet unknown, although the majority of the lineages comprised by yeasts are cultivable. The description of a microbial community and the quantification of diversity associated with several habitats such as soil, phyllosphere, insect-flower system and aquatic environments have increasingly applied culture-independent methods (Steven et al. 2007; Jumpponen and Jones 2010; Redford et al. 2010; Mašínová et al. 2017). Culture-independent methods include denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (T-RFLP) and clone libraries, which are based on a DNA barcoding fragment of a conserved gene and can be used to quickly and cheaply determine the main components of fungal communities. High-throughput

sequencing with meta-barcoding of DNA has improved species detection by providing a large amount of sequence data, although all from short sequencing reads.

Computational issues nowadays compromise the efficiency of culture-independent methods and thus make it difficult to arrange large numbers of sequence reads into biologically meaningful information (e.g. classification). An automated identification of sequence data relies on a reference database, variability of the selected DNA marker and a clustering algorithm. Each of these steps has its own limitation (e.g. Amend et al. 2010) that results in an incomplete identification (assignment to the known reference sequences) of the pool of nucleotide sequences. Therefore, a pragmatic approach based on operational taxonomic units (OTUs) is commonly used. Nucleotide sequences are grouped according to their similarity (95–99% threshold) and analysed using common ecological tools implemented in various software packages. However, a reliable use of molecular OTUs as yeast species proxies is presently unlikely. Cut-off values are selected artificially and may not reflect the actual variability of the genetic marker across different phylogenetic lineages (e.g. Ascomycota and Basidiomycota). The size of the most commonly analysed DNA marker, the ribosomal ITS region, is rather stable in basidiomycetous yeasts but varies in Saccharomycetes ranging from about 300 to 1000 nucleotides. Also, the interspecific polymorphism of ribosomal gene regions (e.g. *Clavispora lusitaniae*, *Barnettozyma californica*) complicates the application of molecular OTUs. Although yeasts were detected among fungi in almost every sampled substrate, most of them were not identified to a certain species or even to the genus level. Apart from species inventories, culture-independent studies often detect thousands of units exceeding by far the number of all described fungi or yeasts (e.g. Blackwell 2011; Mašínová et al. 2017). How these molecular OTUs correspond to the species and to what extent the yeasts detected with these techniques are cultivable are a matter of debate. A recent study that analysed soil yeasts with an amplicon sequencing technique showed that several OTUs defined with a conservative 97% threshold were matched to the same yeast species (Mašínová et al. 2017). Thereby, common identification pipelines can potentially overestimate yeast diversity.

3.3 Community Composition and Diversity

By revisiting the classic literature on yeasts, we can see that communities that are studied nowadays have also been studied in the past (Figs. 3.1 and 3.2). The main criticism against past studies is that yeast isolation has not gone much beyond the mere nomenclatural description of new species, together with the assessment of species richness. Below, we summarise some of the ecological concepts that can be assessed by studying yeast communities in their natural habitats.

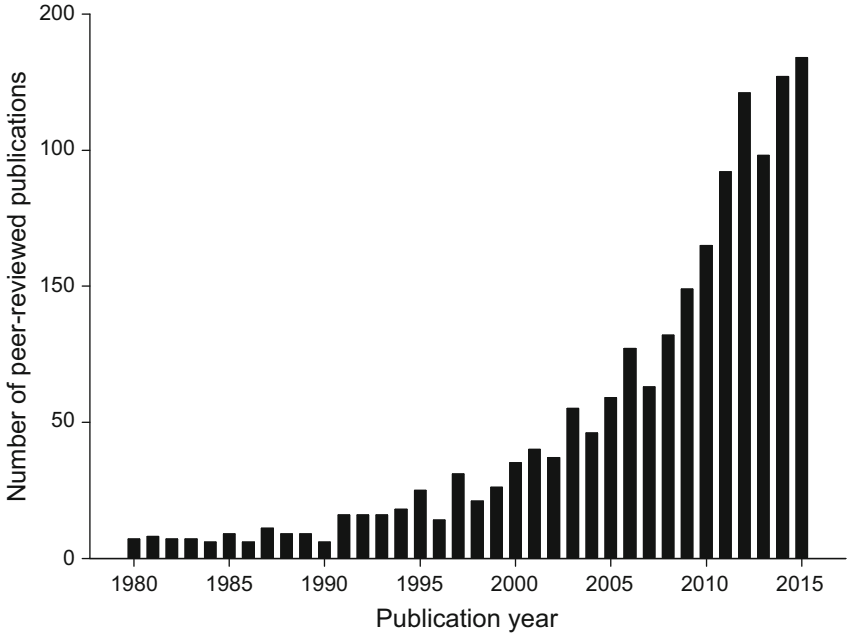

Fig. 3.2 Number of peer-reviewed studies dealing with natural yeast communities published since 1980. Other details as for Fig. 3.1

3.3.1 General Parameters

Community assembly rules aim to predict spatial species distributions and the mechanisms underlying the distribution patterns. One of the early proposed rules is that competition is responsible for determining the patterns of assemblage composition (Diamond 1975). However, their generality has been debated for over 20 years, and the debate continues today (Gotelli and McCabe 2002). Their strong spatial reference makes them highly amenable to phyllosphere studies and less amenable for aquatic environments, for instance. Below, we address a few empirical community distribution patterns in the section dedicated to the structure of yeast communities. Spatial aspects of the distribution of yeasts are reviewed in Chap. 4 of this book.

Diversity encompasses species richness (simple species count) and heterogeneity (relative abundance of each species in a community). Both types of information can be summarised in a rank abundance curve, which displays species richness and species evenness (see below). Diversity can be statistically partitioned into three hierarchical components: alpha, beta and gamma diversity (Whittaker 1960). Following the original idea, the hierarchically organised diversity partitioning is commonly used on a large spatial scale and includes local, regional and global diversity. The question is, however, whether or not the same approach can be

applied to the microbial systems, which are smaller in size than those of plants and animals. If one considers flower nectar community, which is discrete in space and is spatially limited due to the range of animal vectors, the three diversity components can be reasonably translated into the following categories: (1) nectar in a given flower (alpha diversity), (2) nectar from different plant species growing together (beta diversity) and (3) nectar of flowers pooled from different parts of an agricultural field or forest (gamma diversity). One example of this study for the given microsite could be the work by Pozo et al. (2011), who combined the study of yeast alpha and beta diversities with the assessment of their sampling effort. While the alpha diversity assessment showed that most of the individual flowers were dominated by a few *Metschnikowia* species, differences at the plant species level (beta diversity) revealed the occurrence of rare species that also suggested a need for more intense sampling at this scale.

Average number of species is a potentially useful measure to characterise species richness of a yeast community. Because the number of observed species naturally depends on the sampling intensity, species recovery depends on the community structure and the proportion or frequency of detection of each species. Therefore, Chernov (2005, 2013) introduced an index that reflects species diversity in relation to the intensity of sampling. Instead of using the total species richness values, a proportion of the observed number of species to analysed (purified) colonies or plates was calculated. Absolute and relative numbers of species differed substantially in samples with a few dozens of colonies per plate, whereas in samples with more than 100 colonies, there was no statistically significant difference detected between the estimates of these two parameters.

3.3.2 Functional Diversity

In ecology, the group of species that use the same resources in a similar manner is called a guild. Although yeasts share a common adaptation, the ability to grow in a unicellular manner, they differ in their ability to use different nutrients. The habitat has the largest effect on the composition of species, all of which should possess a pool of adaptations to colonise it. Thus, the yeast community can be viewed from the position of these capabilities or potential functions in the habitat. Species descriptions provide a set of characters, which can be used to assess the physiological profiles of the communities. A study of yeast communities associated with *Drosophila* flies across the USA showed that the habitat was the major factor influencing the physiological ability of the community (Lachance et al. 1995). Observation of species, almost indistinguishable physiologically (identical fundamental niche), in similar habitats was a driving force for understanding the ecology and distribution of yeasts (e.g. di Menna 1965; Babjeva and Chernov 1995; Starmer et al. 2003; Buzzini et al. 2012). A few approaches have been made to classify yeasts into functional groups, which would reflect the common physiological (or morphological) adaptations for the habitat or lifestyle. Babjeva, Chernov and

co-workers (Babjeva and Chernov 1995) studied distributions of yeasts across most typical biomes in the USSR and categorised them according to their phenotype and occurrence in the substrates, i.e. species assemblages (originally: complexes) in phyllosphere (e.g. *Vishniacozyma* spp. as "phenotypic *Cr. laurentii*"), litter (e.g. *Tausonia pullulans*) and soil (e.g. *Lipomyces* spp., *Solicoccozyma* spp.). This approach was based on the previous classification of the life strategies of yeasts, i.e. phytobionts (living plant material), pedobionts (soils), saprobionts (decomposing material) and humidobionts (humid sugar-rich substrates). The ability to assimilate complex substances was more pronounced in species inhabiting litter and soils. This adaptation is presumably linked to the dependency on the derivatives of plant decomposition (reviewed by Botha 2006) and was found to be common for several soil yeasts (Di Menna 1965; Sláviková and Vadkertiová 2000; Botha 2006; Mestre et al. 2011). Physiological profiles of the community can be also analysed qualitatively. Chernov (2005) studied the number of assimilated compounds (index of polytrophy) by yeast assemblages in the biogeographic context in the most common types of substrates. As a result, most polytropic communities were observed in tundra, and the number of assimilated substances decreased towards the lower latitudes. Among the surveyed substrates, availability of simple sugars strongly affected polytrophy of the communities as the number of assimilated compounds (also complex substances) was increasing in litter and soils.

Recently, a tool FUNGuild was developed to parse fungal molecular OTUs into ecologically meaningful categories such as functional guilds (Nguyen et al. 2016). The original selection of the ecological categories highlights the ecological diversity of yeasts as they can be assigned to the 7 out of 12 guilds, namely, animal pathogens, lichenicolous fungi, mycoparasites, plant pathogens, undefined root endophytes, undefined saprotrophs and wood saprotrophs. But even as saprotrophs, yeasts should not be synonymised with the saccharolytic lifestyle only. Because of their taxonomic complexity and heterogeneity, yeasts display a multitude of metabolic properties, which are routinely recorded for every described species. This makes yeasts an attractive object to study functional aspects of the community ecology.

3.3.3 Diversity and Quantity

The composition of an ecological community depends on the compatibility of species to the local environment. Moreover, their abundances in the community can be the result of complex interactions and processes (Fukami 2015). Concepts in ecology that might explain yeast coexistence and therefore the conformation of microbial communities are neutralism, commensalism, amensalism, predation and parasitism (Atlas and Bartha 1993; Starmer and Lachance 2011). Neutralism describes the occurrence of sparse-independent populations sharing the same habitat. This can be exemplified by lack of direct contact in the particular case of simple, low populated communities such as soils, in which samples with higher cell

numbers (CFU) are more species rich than those with lower cell counts (e.g. Yurkov et al. 2011; Chernov 2013). The number of isolated species positively correlated with cell density in species-poor communities (e.g. soils) following a logarithmic curve (Chernov 2005, 2013). Other yeast habitats with simple communities, such as floral nectar, are also amenable for neutralism, despite harbouring denser yeast populations (Pozo et al. 2016). Irrespective of host plant species, yeast communities of floral nectar in Europe are largely composed by two species of the genus *Metschnikowia* (Brysch-Herzberg 2004; Pozo et al. 2011). On one hand, nectar habitats are sugar rich and might be not limited with nutrients as soils. On the other hand, the co-growth of the ecologically similar species might be due to the fine niche partitioning, either in the consumption of resources or physical growth preferences. For example, physiological profiles indicated that *Metschnikowia reukaufii* and *M. gruessii* did not compete for most carbon and nitrogen sources but even support co-growth in a more restrictive culture medium (Pozo et al. 2016). Likewise, several *Saccharomyces* species co-occur on Mediterranean oaks, and their niches are strongly determined by growth temperature preferences (Sampaio and Gonçalves 2008; see also Chap. 5 of this book). The cross-feeding (syntrophy) and substrate priming further promote efficient nutrient utilisation by yeast communities (see below).

We should admit, however, that the neutral model in yeast community ecology has been met with some scepticism (Lachance 2006). As in the aforementioned examples, niche occupancy by yeasts is not random but is steered by interspecific interactions and, more importantly, by interactions with their vectors or hosts (reviewed by Starmer and Lachance 2011). Besides neutral interactions, competition, amensalism, predation, and parasitism are considered in the literature (Atlas and Bartha 1993; Starmer and Lachance 2011). The first one, competition, refers to a wide array of antagonistic interactions, including growth inhibition, contact inhibition and competition for nutrients, among others. The diversity of mechanisms used by yeasts to outcompete other species is broad and involves killer activity (killer proteins and mycocines), substrate depletion (nitrogen or vitamins), acidification, ethanol production and mineral sequestration (iron acquisition with pulcherrimin). These abilities have been reviewed previously (e.g. Golubev 2006; Starmer and Lachance 2011) and are addressed in more details in the following chapters of this book.

3.3.4 Priority Effects

The history of the substrate also plays an important role in community composition and can affect the growth of arriving yeasts. For example, the two yeasts *Hanseniaspora vineae* and *Metschnikowia pulcherrima*, which naturally coexist during wine fermentation, affected the fermentation kinetics by having a superior nutrient intake rate compared to *Saccharomyces cerevisiae* (Medina-Rland et al. 2012). The effect was even more pronounced when *S. cerevisiae* was inoculated

24 h after the initial stage of fermentation with a non-*Saccharomyces* yeast, compared to co-inoculation. This temporal succession affects the strength of interference, or facilitation, and is, therefore, named as a priority effect (Chase 2003). The aforementioned timing of the species inoculation and the subsequent modification of the media that, in turn, favours or inhibits later-arriving species change, thereby, the yeast community composition. This approach overlies the specific history of community assembly into the known rules of community ecology (Fukami 2015). The relevance of this ecological theory in the particular case of microbial ecology has been recently acknowledged, and the number of known examples is steadily growing. Specifically, nectar yeast communities have been repeatedly used as a model to test priority effects. Although secreted flower nectar does not carry yeasts, floral visitors such as bees, birds, bats or ants vector yeasts propagules to this specific habitat. Those yeasts may or may not establish and produce substantial changes (e.g. amino acid and sugar composition and concentration) in nectar as a substrate for future incoming taxa (Herrera et al. 2008; Peay et al. 2012; Vannette and Fukami 2016; Mittelbach et al. 2016b). By using experimental manipulation in microcosms, Peay et al. (2012) demonstrated the strength of priority effects for most of the yeast species found in the floral nectar of a hummingbird-pollinated shrub in California. This study concluded that late-arriving species experienced strong negative effects from early-arriving species, but also warned about the relevance of the species phylogenetic relatedness. Variation on the strength of priority effects was stronger between closer relatives. Such an outcome can be explained by changes in functional diversity. Both carbon and amino acid consumption profiles indicated that competition between closer relatives was more intense owing to higher ecological similarity (Peay et al. 2012). In another experiment that investigated the growth of nectar yeasts, *Candida rancensis* was not affected, whereas cell densities of *M. reukaufii* increased when the microcosm was inoculated with a second species (Mittelbach et al. 2016b). The basidiomycetous yeasts *Vishniacozyma victoriae* and *Itersonilia pannonica* (originally *Udeniomyces pannonicus*) showed a negative effect of a priming species. Killer yeasts are often preponderant in the very sugar rich and favourable for yeast development substrate, i.e. decaying fruits. Because the antagonistic activity of the killer toxins has a specific taxonomic range (Golubev 2006; see also Chap. 9 of this book), the first-arriving yeast would largely determine species composition in the community. This would usually result in a typical fruit yeast community, but depending on the local conditions (e.g. prevalence of another community type), the community type could change it towards allochthonous, untypical for fruits, species (Starmer, personal communication).

The cross-feeding (syntrophy) phenomenon encompasses the associations where the growth of one partner is improved or depends on the nutrients, growth factors or substrate provided by the other partner. The nutritional interdependence is known in microbiology, especially between symbiotic prokaryotes and some microbial consortia (e.g. Seth and Taga 2015). Although the topic is less studied for yeasts, cross-feeding may play an important role in the composition of yeast communities. In the aforementioned example, frequently vectored to flowers, basidiomycete yeast

It. pannonica did not grow in artificial nectar without an accompanying species (Mittelbach et al. 2016b). A similar situation may be present in the yeasts found in soapberries (*Sapindus* sp.) in Hawaii where some yeast species co-occur more often than expected, and corresponding physiological tests suggest cross-feeding relying on starch use may be involved (Starmer, personal communication). Likewise, yeasts that excrete riboflavin into the medium imply that the riboflavin may be a useful vitamin source for other organisms as well as co-occurring yeasts (Starmer, personal communication). The phenomenon of cross-feeding is probably involved in some other cases of yeast co-growth in the environment.

3.3.5 Community Stability

Community stability can be measured as resilience or resistance of a community to perturbations, either mechanic or with the introduction of an exotic species (Grimm and Wissel 1997), which does not originate from the same environment. The relationship between community stability and ecosystem functioning is a matter of lively discussions in ecology (Bezemer and van der Putten 2007).

Some of the most prominent efforts to understand diversity in higher organisms, such as mammals, are derived from the original Lotka-Volterra model (1925–1926). These assumptions converged into the concepts of "bottom-up" and "top-down" regulations of community diversity during the twentieth century. While "top-down" effects refer to the control that consumers exert on the remaining community members, "bottom-up" effects focus on how access to resources may affect community composition. Most researchers emphasised initially the association between the species richness and the availability of resources ("bottom-up" regulation); however, the recognition of the role of consumers and predators is gaining strength (reviewed by Leroux and Loreau 2015). Although the dichotomy between the two forces has motivated ecological research over the last century, microorganisms have received little attention to date (Meyer and Leveau 2012). The study of both regulatory forces in microbial communities can be seen as the global ("top-down") versus temporal ("bottom-up") resource limitation (Crowther and Grossart 2015). For example, strong nutrient limitation in soils could exemplify the "top-down" processes, whereas phylloplane communities constitute a good model to study temporal ("bottom-up") limitation.

3.3.6 Species Richness Estimators and Sampling Effort

In most of their natural habitats, yeasts hardly occur as a pure culture. The ultimate importance of sampling and isolation approaches has been highlighted repeatedly (Lachance and Starmer 1998; Boundy-Mills 2006). Cultivation techniques still provide us with the majority of data on yeast biodiversity and distribution. During

the twentieth century, these methods have been substantially improved to facilitate the discovery of yeasts (Boundy-Mills 2006). Because ecological surveys need to find an optimal sample size to reveal most of the real diversity with a bearable sampling intensity, sampling strategies have been studied for yeasts (reviewed by Boundy-Mills 2006). Sample size will depend on the richness and heterogeneity of those yeast communities. Lachance and Starmer (1998) determined empirically that a minimum of 15 independent samples is required to obtain an accurate reflection of community composition for cactus necroses, insects, tree exudates or flowers. Those are all species-poor communities, heterogeneous in terms of the abundance of single species. The number of samples also varies according to the frequency of empty samples. For example, the authors suggested the sampling size should be corrected to obtain a minimum of eight non-empty samples, when empty samples are present (Lachance and Starmer 1998). Rotting cacti, tree exudates and flowers are fairly homogeneous substrates compared with phylloplane and soils. The number of analysed samples should be further increased for heterogeneous and species-rich substrates. Insufficient sampling intensity and geographical sampling bias (Fig. 3.1) both compromise our knowledge of natural yeast communities. A few available estimations suggest that only a small fraction (approximately 5–10% depending on the habitat) of the total diversity of fungi is known (e.g. Hawksworth 2001; Blackwell 2011). The same is true for yeasts even though the number of described species has increased tenfold during the last 60 years (Lachance 2006). In our opinion, these numbers reflect the fact that the majority of the total yeast diversity is not known. This also justifies the need for further sampling in different habitats and regions.

Species richness is an intuitive measure of diversity and community composition, but despite its simplicity, this index can differ substantially from the real diversity. The problem arises when the community inventory is growing steadily with the number of samples analysed—the more samples are processed, the more species that will be found. As a result, species richness values presented in different publications (all of which used a different sampling intensity) cannot be directly compared. Another methodological problem is that both the expected species richness and the number of samples sufficiently describing the community are unknown. Thus, rarefaction curves provide a more reliable estimate of the "real" species richness, by plotting the number of species as a function of the number of analysed samples or purified (also identified) colonies. Those curves are created by randomly resampling (with replacement) the pool of N samples and plotting the average number of species (calculated after several rounds of resampling) found in each sample, i.e. in one sample, two samples, etc. (Sanders 1968). Rarefaction curves generally grow rapidly at first, as the most common species are found, but approach a plateau as only rare community members remain to be sampled (see Magurran 2004). Statistically, the use of rarefaction controls for differences in species richness values (Gotelli and Colwell 2011). For example, the analysis of rarefaction curves demonstrated that the yeast community is more species rich on

plant material than in soils irrespective of the sampling depth (Babjeva et al. 1999). Also, the use of this approach showed that yeast communities are more species rich in forest biotopes than in tundra, steppe or deserts (Maksimova and Chernov 2004). Likewise, Takashima et al. (2012) demonstrated that subtropical forests were more species rich than temperate forests above ground, whereas below ground, the opposite trend was observed.

Many attempts have been made to correct the sampling bias in species estimations. Statistical approaches to describe the species richness (or other units) have been successfully adapted to bacterial or fungal communities (Hughes et al. 2001; Bohannan and Hughes 2003; Unterseher et al. 2005, 2011; Schnittler et al. 2006). However, less than 10% from about 1500 papers (Fig. 3.2) studying yeast communities reported their sampling efficacy in soils (Yurkov et al. 2011, 2016; Orgiazzi et al. 2012; Takashima et al. 2012; Bellemain et al. 2013; Taylor et al. 2014), plant-related substrates (Pereira et al. 2002; Glushakova and Chernov 2010; Pozo et al. 2011; Takashima et al. 2012; Alvarez-Perez and Herrera 2013; Jacquemyn et al. 2013a, b; Morais et al. 2013) and insects (Ort et al. 2012; Niu et al. 2015). A few studies did not focus on a specific habitat but analysed the total yeast richness in a biotope (Maksimova and Chernov 2004; Yurkov et al. 2004) or compared species richness in different substrates (Babjeva et al. 1999). In aquatic environments, best sampling strategies still led to the assessment of just 60% of the species present (Kutty and Philip 2008; Fell 2012), whereas soils can be fairly well sampled (e.g. Babjeva et al. 1999; Yurkov et al. 2011). The utility of the rarefaction has been also shown for nectar yeast communities in Europe, which were studied with a different sampling intensity by the four aforementioned studies, whereas three of four nectar surveys observed less than 10 yeast species in a maximum of 120 samples, whereas sampling of a total of 600 flowers doubled the species richness values (Alvarez-Perez and Herrera 2013).

Next to the rarefaction, species richness estimators represent a useful tool in community ecology. The main difference between the two approaches is that estimators use the ratio of species discovery to predict the number of species by using different computational algorithms. Thus, the application of a species richness estimator can show the number of species retrieved with increased sampling effort. Alternatively, it can answer the question on whether the present sampling effort is sufficient to discover the majority of expected species in a community. In other words, it tests for undersampling. Insufficient sampling depth is an important concern in heterogeneous habitats, like soils, which require higher sampling intensities due to rare species (e.g. Yurkov et al. 2011, 2016). These infrequent yeasts may constitute the majority of the population in some environments, such as nectar (Alvarez-Perez and Herrera 2013), rotting cacti (Starmer et al. 2005) and soils (e.g. Yurkov et al. 2011, 2016). The estimators ICE and ACE improve rarefaction curves by taking into account the minimum number of samples that allow us to identify rare (in ICE) and minor (ACE) species (reviewed in Gotelli and Colwell 2011). The bootstrap estimator does not differentiate the species frequency and the first-order jackknife, and Chao 1 richness estimators additionally rely on the number of species only found once. Chao 2 estimator is distinct from the other

species richness estimators as it is an incidence-based estimator of species richness, which relies on the number of singletons and doubletons, i.e. species found in only one and two sample units.

3.4 Community Structure

The simplest description of diversity is a species inventory such as a list of yeasts isolated in a study. In community ecology, this is also called community composition. These data can be further analysed as presence or absence of a species in a sample. It does not include any quantitative information such as dominant or frequent species. Additional information about species abundances describes the structure of communities. Depending on the study design, it can include either incidence or abundance data, or both. Abundance-based community structure is usually based on colony counts, namely, number of colonies of each yeast species observed in cultivation experiments (see also Boundy-Mills 2006). Incidence-based community structure corresponds to the frequency of isolation of a species. It does not take into consideration the number of colonies but counts the detection of a yeast in the sample only. Both these approaches have their own limitations. Isolation of yeasts is usually made on solid media, where a sample is inoculated. Inoculated plates are incubated, and growing yeast colonies can be differentiated into morphological groups based on their appearance on the medium by recording, for instance, colour, shape, texture, formation of filaments and media colourisation. Representative colonies are then picked, transferred to pure culture and identified. Even though morphological characters are often not unique, their combination can often provide a suitable differentiation of yeasts in a sample. It is important to document, however, that closely related yeast species may show similar or even indistinguishable colony morphology and, thus, can be mistaken when counted on plates. Compared to counts based on colony-forming units, incidence-based community structure does not extrapolate identification results to abundance but records a species detection only. Representative cultures or even randomly purified colonies can give an impression on how frequent is the certain yeast species in a sample. This approach is likely to provide fair estimates in communities composed of species, which can be mistaken based on the morphological characters. When yeast incidence is recorded, the community structure can be expressed as frequency of occurrence. It doesn't provide information about the dominance but aims to detect most common species within sampled colonies.

When the composition of a yeast community is well characterised and rather stable and can be assessed with a reasonable sampling effort, selective isolation can be further employed to quantify morphologically indistinguishable species. Selective isolation involves any unique physiological condition (either of a yeast or habitat) and tolerance to inhibitors. This cultivation method can also reveal rare and minor species (see below). But it is important to remember that an additional replicate with a complete medium should be always used to ensure the complete

assessment of the community. This approach was successfully used to study yeast communities in rotting cactus tissues (Starmer, personal communication).

3.4.1 Diversity Indices

A quantitative measure that reflects how many different species there are in a habitat and simultaneously takes into account the proportion of each entity in the sampled community is known as a diversity index. Diversity indices, the Shannon diversity index (also known as Shannon entropy, the Shannon-Wiener or sometimes erroneously the Shannon-Weaver index) and the Simpson diversity index (also known as the inverted Simpson index) are among the most frequently used in the literature. They both take into account species richness and proportion (number of individuals or relative abundance) of species. Sometimes, values given by diversity indices are used as a synonym of alpha diversity. This is not entirely correct, as the diversity indices do not consider rarity of species in a community. Diversity values are commonly used to make comparison between habitats to reflect the degree of the diversity alteration (e.g. succession) or to provide a quantitative measure of differences in the structure of communities between samples. Importantly, the indices reflect changes in both the species richness and the evenness of the community. The latter is used to measure how close in numbers (or as a proportion) each species in a community is. Among communities with the same number of species, the one with more similar proportions of members would be characterised by a higher diversity value. Thus, the decrease or decline of the diversity can be caused by either of these parameters, i.e. community composition (species richness) and structure (evenness).

For example, diversity of yeasts in oligotrophic lakes was higher off the coast than in the coastal zone and resulted from a more even species distribution (Brandão et al. 2011). Likewise, the Shannon diversity values calculated for phyllosphere yeasts on *Sphagnum* moss were higher in swamp than in forest biotopes, although both yielded the same number of species (Kachalkin and Yurkov 2012). On the contrary, higher species richness values in the rhizosphere and bulk soil underneath *Nothofagus pumilio* resulted in a higher diversity in these soil fractions than in the ectomycorrhizosphere (Mestre et al. 2011). An interesting observation was made by Starmer and co-workers in the review of the biogeographic diversity of cactophilic yeasts (Starmer et al. 2005). Communities of yeasts in rotting cactus tissues are highly specific and composed of a small number of dominant species, all of which have shown a restricted geographic range that is determined by insect vectors and their hosts. These communities occasionally include rare non-cactus-specific yeasts, which are regionally diverse (dissimilar between locations) and are randomly transported from the surrounding environments. Because the core cactus-rot communities are very similar among locations, diversity on a regional scale is largely determined by an almost random subset of

rare non-cactus-specific yeasts. Consequently, communities in single location show a lower diversity than those on a regional scale.

Not only the number of individuals affects the observed species diversity but also the size and heterogeneity of the sample. Homogenisation of large and heterogeneous samples can potentially equalise the proportions of yeast species in cultivation experiments. This approach has been applied repeatedly for the investigation of phylloplane and soil yeasts. For example, several individual leaves pooled in a composite sample can be further subsampled (e.g. cut into pieces), washed and used to inoculate plates instead of plating leaf washings of a single leaf (e.g. Inácio et al. 2010). Likewise, soils can be homogenised (mixed) and sieved to reduce the heterogeneity and dissimilarities between subsamples and replicates (e.g. Yurkov et al. 2011).

3.4.2 Species-Abundance Distribution

Species-abundance (SAD) and rank-abundance distributions (RAD) are helpful to understand the structure of yeast communities and can be used as an additional tool in community analyses. As such, SAD and RAD are the most basic way to describe the abundance of each species in a community (reviewed by McGill et al. 2007). Abundance values, either absolute (colony counts) or relative values (proportion of each species), are arranged in descending order and plotted as a hollow-curve histogram. A visual comparison of community structure across samples implies a substantial overlap in species lists among the samples and can be done either with SAD or RAD. When a few or even no species are found in common, RAD is employed and the order or rank of a species in a community is recorded but not its identity. For example, soil yeast communities in grasslands and forests in Germany had only one species, *Apiotrichum dulcitum* (originally *Trichosporon dulcitum*), in common (Yurkov et al. 2012a). Nevertheless, the application of the RAD for the analysis showed the influence of the vegetation type on yeast communities.

Species lists and colony counts are commonly collected data during yeast surveys. At the same time, SAD is different from an abundance table as it enables easy research of community properties such as evenness and proportion of rare species. When plotted as a histogram, SAD typically produce the typical hollow curve, which is one of ecology's oldest and most universal laws (McGill et al. 2007). Several models have been proposed to describe distribution of plant and animal species in forms of SAD and RAD, including logseries, lognormal and geometric and broken stick, among others (reviewed by McGill et al. 2007). These models tried not to just describe empirical curves but explain the composition of the communities using a set of ecological tools such as species niche concepts, competition for resources, dispersal and reproductive strategy. Babjeva, Chernov and co-workers analysed yeast population on living plant material (vascular plants, mosses and lichens), senescent plants, litter and soils collected on the territory of the former USSR (reviewed by Babjeva and Chernov 1995). Later, Chernov (2005,

2013) studied the distribution of species ranks (RAD) obtained from ca. 7000 samples and employed Pearson's chi-squared tests to predict distribution models' characteristic for each substrate. Communities in decomposing plant material (forest litter, peat) were well described with the geometric distribution, whereas yeast assemblages on fresh and senescent leaves showed more flat RAD curves corresponding to the broken stick (random niche appointment) model (Chernov 2005, 2013). The authors suggested the shape of a SAD reflects the availability of resources in the community and the competition between single species.

The incline of the hollow curve reflects the evenness of the community with more even communities resulting in more gentle slopes of the SAD. McGill et al. (2007) reviewed several empirical patterns of SADs such as incline, skew and modality. In a series of experiments, more flat curves corresponded to communities with a higher productivity, for example, plant communities in late successional stages. To what extent these observations can be applied to yeast communities is yet unclear. As in the case of the observations by Chernov (2005, 2013), the steep incline of the curve could suggest a strong limitation of the resources, which is a very likely scenario for decomposed organic substrates such as litter and soils. On plant surfaces, sugars and other low-weight carbon sources are not as limited as in soils, so the phylloplane generally supported a more even yeast community, i.e. broken stick vs. a geometric model. Recent analysis of soil yeasts along a land-use gradient confirmed the previous rule regarding the shape of SADs below ground but also showed that more even communities were found under intensively managed grasslands, which were subjected to fertilisation in the past (Yurkov et al. 2012a). In another study, yeast communities underneath decomposing wood logs were more even than those sampled just 1 m apart under forest litter (Yurkov et al. 2012b). The observed difference also correlated with the higher amount of the dissolved organic carbon in soils underneath dead wood logs. Although SADs of yeast fungi in their natural habitats did not receive much attention, we believe that this tool has potential usefulness for community analyses.

3.4.3 Frequent and Rare Species

It is intuitive and understandable that some yeasts are observed in the environment more often than the others (Magurran and Henderson 2003). However, it is extremely difficult, if at all possible, to suggest any artificial cut-off values for frequent and rare species. Obviously, different ecosystems and substrates provide diverse conditions for yeast communities. Therefore, it is important to sample the habitat sufficiently to reveal the structure of the yeast community. It has been repeatedly documented that frequent (core community) and rare species also show different distribution patterns (Magurran and Henderson 2003; Unterseher et al. 2011). Although the importance of rare (or minor) species is not well understood, a few studies suggest that their diversity is important for ecosystem

functioning and stability, e.g. resistance against colonisation by exotic plants in a grassland system (Lyons and Schwartz 2001; Lyons et al. 2005).

To our knowledge, little effort has been made to distinguish ecological roles of frequent and rare species of yeasts in their habitats. While frequent species are usually well studied, rare community members, though often outnumbering the core group, are recovered as single isolates. The situation is further complicated by the extraordinary dispersal abilities of yeast cells, so rare resident species are difficult to distinguish from the transient species from outer sources (see the below discussion). A good understanding of yeast autecology could help to answer the question whether a yeast might or might not be able to live in the sampled environment. This might be, however, more difficult for yeasts that show broad chemical, physical and physiological capabilities (broad fundamental niche), which also allow them to colonise diverse habitats. Although our knowledge of distribution patterns of rare and frequent species is scarce, this community parameter can be potentially useful. For example, forest stand properties such as projective cover or a management history affect soil yeast communities (Yurkov et al. 2012a, 2016). Compared with managed areas, low-managed and near-natural forests harbour a higher number of rare yeast species, which also results in higher estimations of species richness values (Yurkov et al. 2011). Likewise, the fragmentation of the forest tree cover also increases the number of rare yeasts in soils (Yurkov et al. 2016). Whether or not the diversity and proportion of rare species show a meaningful trend in other yeast habitats should be addressed in future studies.

3.4.4 Resident (Autochthonous and Indigenous) and Transient (Allochthonous and Alien) Species

Although the terminology may differ between studies, the main intention to distinguish true inhabitants from alien or "accidentally observed" species is intuitively clear. As in the case of frequent and rare species, this question can be addressed from two different perspectives, namely, autecology and synecology (community ecology). Laboratory observations of autecological properties can help predict the range of suitable habitats for a yeast. For example, fast-growing fermenting ascomycetes are better adapted to sugary substrates, while soilborne basidiomycetes often possess the ability to assimilate low-weight aromatic compounds (Botha 2006; Starmer and Lachance 2011). Carotenoid pigmentation together with the dispersal by the forcibly ejected buds (ballistoconidia) is believed to be an adaptation to exposed environments such as plant surfaces (Fonseca and Inácio 2006; Starmer and Lachance 2011). The adaptations can be partitioned further to consider conditions of a single microhabitat or a yeast's potential interactions with vectors. In this regard, the development of more sensible isolation techniques allowed to discern tongue, crop and gut yeast communities—all of which were previously considered as an insect community (Malloch and Blackwell 1992; Hu et al. 2015).

Similarly, floricolous yeast can be separated onto nectar, corolla and anther communities (Pozo et al. 2012). A further downsizing of a yeast habitat is also feasible for nectar habitats where the oxygen gradient from bottom to top of the nectar content would allow the establishment of microaerophilic and aerobic communities, respectively (Lachance 2006).

A combination of abundance- and incidence-based approaches to describe the structure of yeast communities can be used to uncover ecological preferences of isolated yeasts. Indigenous (autochthonous) community members are species that are expected to be both abundant and frequent in a habitat (see also Lachance and Starmer 1998; Starmer and Lachance 2011). These yeasts usually comprise a core community and are easy to isolate even with a limited effort. Yeasts frequently detected in low numbers could represent minor but indigenous community members. For example, basidiomycetous yeasts were commonly considered as resident (allochthonous) in the nectar environment (e.g. Brysch-Herzberg 2004). However, the species *Cystofilobasidium alribaticum* (originally cited as *Cystofilobasidium capitatum*) and the members of the genus *Vishniacozyma* (originally cited as *Cryptococcus carnescens*, *Cryptococcus heimaeyensis* and *Cryptococcus victoriae*) were frequently observed in association with bird-visited flowers and were able to grow in artificial nectar (Mittelbach et al. 2015). Similarly, orchids' nectar contained large number of basidiomycete species (Jacquemyn et al. 2013b). In phylloplane and soils, Basidiomycetes commonly prevail over the ascomycete yeasts (e.g. Botha 2006; Fonseca and Inácio 2006; Starmer and Lachance 2011). However, infrequent ascomycetous species were successfully linked to plant surfaces (e.g. *Candida oleophila* and *M. pulcherrima*) and forest soils (*Candida vartiovaarae* and *Kazachstania piceae*) based on their occurrences in the environment (e.g. Glushakova et al. 2007; Glushakova and Chernov 2010; Yurkov et al. 2012a). In contrast to minor community members, infrequent but numerous yeasts may originate from sources other than the studied substrate (transient or allochthonous) or be restricted to a certain microhabitat in a heterogeneous environment. Isolation of rare species in low numbers is difficult to interpret due to the risk of undersampling. Often these species are minor transient community members from neighbouring substrates, and their origin can be revealed in a biotope-wide analysis including various substrates. Because the place of yeast isolation is not necessarily where they live, a careful examination of substrates that serve naturally as reservoirs (e.g. soil and water) is important to distinguish autochthonous from allochthonous species (see also Lachance and Starmer 1998; Starmer and Lachance 2011). We believe that a repeated sampling with a sufficient sampling effort can help to reveal true habitats of many species in the future.

Variation of frequency and abundance of yeasts between single samples can further complicate community analyses when occurrence and colony counts of a species differ substantially. A useful tool to account for spatial and temporal variation is the probability of dominance, which is calculated as the number of samples, where a species showed the highest abundance, relative to the total number of samples, where this species was observed (e.g. Maksimova and Chernov 2004; Glushakova and Chernov 2010; Yurkov et al. 2012a). This measure records

the cases when a yeast becomes dominant in a community, thereby accounting for heterogeneity between habitats, samples or replicates. For instance, despite little overlap between yeast communities on plant material, litter and soils, Maksimova and Chernov (2004) revealed the most typical yeasts for each type of substrate using the aforementioned parameter. When the probability of dominance is additionally considered, it is possible to distinguish rare minor community members from rare yeasts, which colonise the substrate only in a certain period of time.

3.4.5 Specialists and Generalists

A combination of physiological abilities, metabolic capacities and physical-chemical limitations determined in laboratory experiments defines a potential habitat where a yeast species might live—its fundamental niche. Narrow fundamental niches define physiological specialists. For example, *Hanseniaspora* yeasts commonly found on fruits and berries have limited physiological abilities. They ferment and respire glucose vigorously, utilise cellobiose as a source of carbon and require an external supply of vitamins (Starmer and Lachance 2011). Likewise, widespread nectar-borne yeasts *M. gruessii*, *M. reukaufii* and *C. rancensis* show a narrow spectrum of assimilated compounds. Although these species have a narrow fundamental niche, they still have a wide distribution in nature, due to the common occurrence of the corresponding habitat. Fruits and berries of different decay stages are common in nature and are regularly visited by insects that transmit yeasts between them. Even though flowers represent a short-lived and very fragmented habitat, the aforementioned nectar yeasts show no preference to a particular group of plants and are commonly found in an association with different insect vectors. The narrow fundamental niche can be further constrained by the limited distribution of its vector that would result in highly endemic yeast species (e.g. Lachance et al. 2003).

Alternatively to a narrow fundamental niche, some yeasts were found in different environments in considerable numbers and are believed to be adapted to a broader range of environmental conditions. For example, *Naganishia albida* (originally *Cr. albidus*) was isolated from soils, plants, water and cold habitats and displayed the capability to sustain cold, desiccation and oligotrophic conditions. Other examples include *Aureobasidium pullulans*, *Filobasidium magnum* (*Cryptococcus magnus*), *Vishn. victoriae* (*Cr. victoriae*), *Debaryomyces hansenii* and *Meyerozyma* (*Pichia*) *guilliermondii*, among others. These species may show opportunistic distribution, which is not restricted to a particular region or habitat. They usually possess broader physiological abilities and utilise diverse carbon sources (polytrophic). Due to the ability to live in various habitats and conditions, such yeasts are referred to in the literature as eurybionts, opportunists or generalists. They are opposed to yeasts with a narrow, geographical or fundamental niche, which are called stenobionts or specialists. To what extent the distribution of the polytrophic species is constrained by autecology and dispersal abilities is a matter of debate.

3.5 Concluding Remarks

Community ecology tools allow researchers to move from simple yeast inventory reports towards a better understanding of mechanisms structuring yeast communities. Yeast communities in their natural environments represent dynamic and open systems. Thus, it is important to distinguish typical inhabitants of the habitat from transient colonists, which may not able to grow properly in the given environment. The available knowledge suggests that properties of transient and resident community members in the habitat are different and are reflected in their distribution patterns. Application of community ecology tools also allows to study yeast observations in a larger context and compare studies across climatic and spatial transects. Because yeast habitats are often too different in respect to the number of species and their proportions in the community, it is important to ensure adequate sampling effort, which catches the majority of residing species. Physiological data traditionally collected for yeast species opens the possibility to describe additionally functional diversity of the community and analyse quantitatively relevant ecological traits and functional redundancy of the ecosystem.

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Chapter 4
Temporal and Geographic Patterns in Yeast Distribution

Andrey Yurkov

Abstract The famous hypothesis formulated by Beijerinck and Baas Becking, 'Everything is everywhere, [but] the environment selects', has dominated microbiological research and directed it towards the search of ecological factors as the main determinants of microbial community composition. The apparent lack of geographic distribution patterns in microorganisms (ubiquity) is traditionally explained by their adaptive (physiological) flexibility and ease of dispersal. Strong disproof of yeast ubiquity comes from studies on yeasts associated with beetles, drosophilids, bees, and short-lived flowers. The current knowledge suggests that geographical barriers, insect vectors, and host plants are important factors determining distribution of yeasts in their natural habitats. This chapter provides examples of the larger-scale distribution of yeasts in the environment, including endemism, latitudinal gradients, distance-decay relationships, and Holarctic and bipolar distributions. The influence of geographic factors on reproductive isolation in yeast populations is additionally addressed in this chapter. Temporal changes such as ecological successions and seasonal dynamics of yeast communities are also discussed.

Keywords Endemism • Latitudinal gradients • Distance-decay relationships • Historical factors • Succession

Contents

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4.1 Introduction

As for many other microscopic organisms, yeasts have been observed repeatedly in different natural and domestic habitats such as fermented products, soil, fruit, or air (Guilliermond 1920). The diversity of yeasts has grown rapidly as the discovery of new species has benefited from improved identification techniques. Compared to most microorganisms, yeasts are easy to culture as they often do not require special media and selective conditions of temperature, acidity, or gas phase. However, many yeast species are known from a limited number of isolates and often described based on a single strain. The repeated collection of a rare species seldom warrants a separate publication, with the result that reports on species distribution are often restricted to the original discovery. With approximately 70 taxa described in over 30 publications in 2016 (Google Scholar query, December 2016), studies reporting the isolation of new taxa outnumber broader biodiversity surveys. The latter often deal with a single habitat and sampling time and a unique set of methods, which makes it difficult to integrate studies. Because description of novel species does not necessarily involve examination of several strains of diverse origins, the ecology of newly discovered yeasts often remains unclear, and it is difficult to infer their distribution patterns from their descriptions alone.

In this chapter, I review our knowledge of the larger-scale distribution of yeasts in the environment. I aim to provide examples on how to put single observations into a larger context by using ecological tools developed for plants, animals, and, more recently, microorganisms. Ecological theory and the relevant terminology are used to show the appropriate approaches to be taken to study yeast communities on different geographical scales. Throughout this chapter, I shall frequently refer to information and ideas presented in previous reviews of yeast ecology (Phaff and Starmer 1987; Lachance and Starmer 1998; Starmer and Lachance 2011). Furthermore, a particular focus in the chapter is dedicated to the review of studies by Babjeva, Chernov, and co-authors, who, over a period of 50-some years, proposed several models and theoretical concepts in the description of yeast distributions in their natural habitats. Sadly, these studies are almost unknown outside the Russian-speaking scientific community (Babjeva and Chernov 1995; Fonseca and Inácio 2006). I also review various biogeographic concepts and approaches and illustrate them with examples from the recent literature, including several book chapters on yeasts of phylloplane (Fonseca and Inácio 2006), cactophilic yeasts (Ganter 2011), and cold-adapted yeasts (Vishniac 2006a; Buzzini and Margesin 2014).

4.2 Environmental Parameters and Historical Factors

Historically, studies on the geographical distribution of microorganisms, including yeasts, have not paralleled phytogeography or zoogeography, instead attempting to identify ecological factors and properties of species that allow them to colonise a habitat (see Starmer and Lachance 2011 for discussion). The habitat is described as a pool of measurements, ignoring the processes and events that have shaped it in the past. Temperature (e.g. annual average, highest, lowest), acidity (e.g. soil pH), water availability (e.g. humidity, rainfall, osmotic conditions), and organic carbon (e.g. oligotrophy, soil organic carbon) are among most commonly used environmental parameters. The observation of a species from a habitat is taken to imply physiological adaptations that enable survival and proliferation. For example, the utilisation of low molecular weight aromatic carbon compounds and lignin degradation products appears to be adaptive for soil yeasts (e.g. Botha 2006; Mestre et al. 2011). The ability to grow rapidly under osmotic conditions and to ferment sugars is believed to help ascomycetous yeasts proliferate in sugar-rich ephemeral substrates (e.g. Herrera et al. 2010). Cold-adapted enzymes allow psychrophilic yeasts to survive under ice, in glacier sediments, and in polar soils (e.g. Vishniac 2006a). The chemical, physical, and physiological properties of a yeast species can be used to describe its potential habitat by defining its fundamental niche. Competition with other species, interactions with potential vectors, or predator-prey relationships further narrow down the effective distribution of a species, in other words its realised niche. While the fundamental niche predicts the environment in which a species might be able to live, the realised niche defines where a yeast actually lives (see Starmer and Lachance 2011).

The ultimate dependence of microbial species distributions on the environment underlies the choice of laboratory practices used to isolate and later study microorganisms. Media and growth conditions are designed to reflect the physical and chemical conditions observed in the habitat. The notion of 'environmentally determined ubiquity' (O'Malley 2008) is epitomised in the famous hypothesis formulated by Beijerinck and Baas Becking: 'Everything is everywhere, [but] the environment selects' (de Wit and Bouvier 2006). The hypothesis holds that ecological factors as the main determinants of microbial community composition. The apparent lack of geographic distribution patterns in microorganisms is traditionally explained by their adaptive (physiological) flexibility and ease of dispersal (reviewed by O'Malley 2008). However, many yeasts do not possess broad physiological capabilities that would potentially allow them to survive outside their primary habitat. For instance, ascomycetous yeasts isolated from ephemeral substrates such as rotting cactus tissues, flower nectar, and fruit that usually grow on very few sugars may be short-lived. On the contrary, many basidiomycetous yeasts have broader assimilation profiles but do not grow at elevated temperatures (e.g. Fonseca and Inácio 2006).

The local distribution of yeasts in a particular habitat can be mediated by environmental parameters in a manner such that even closely related species may

coexist in the same habitat that would have independent niches. For example, species of the genus *Saccharomyces* found in the bark of Mediterranean oaks display different growth preferences. The cold-adapted species *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* were enriched when isolation was conducted at 10 °C, whereas *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* appeared after incubation of samples at 30 °C. Different *Saccharomyces* species form sympatric populations in one locality and even the same bark sample (Sampaio and Gonçalves 2008). Similarly, variation of cultivation conditions (e.g. temperature, available sugars, enrichment) can elicit the presence, in the environment, of low numbers or dormant species, consistent with the 'rare biosphere' and 'microbial seed bank' theories (Sogin et al. 2006; Lennon and Jones 2011; Lynch and Neufeld 2015).

Yeasts isolated from extreme environments are often considered to be adapted to specific environmental conditions and, thus, expected to show a narrow distribution range. Among extreme environments, cold habitats such as ice cores, meltwaters, sediments, soils, and rocks have been studied intensively during the last decade (e.g. De García et al. 2007; Connell et al. 2008; Branda et al. 2010; Butinar et al. 2011; Selbmann et al. 2014). Other extreme environments studied for yeasts are acidic environments, hot springs, salters, alkali, and desert soils (Raspor and Zupan 2006; Mokhtarnejad et al. 2016). Species isolated from these sources are commonly tested for properties (e.g. growth rates and enzymatic capabilities) that potentially allow yeasts to survive and propagate under unfavourable conditions. As the distribution of extremophilic yeasts is restricted to localities that exhibit physically or geochemically extreme conditions, patterns of distribution of these species in space may be difficult to detect due to the fragmented nature of the habitat (Buzzini et al. 2012). Among the yeast species isolated from cold habitats, basidiomycetous yeasts of the genera *Glaciozyma*, *Leucosporidium*, *Mrakia*, *Naganishia* (e.g. former *Cryptococcus antarcticus*), *Goffeauzyma* (e.g. former *Cryptococcus gilvescens*), and *Vishniacozyma* (e.g. former *Cryptococcus victoriae*) are on average more frequent although not always unique to the cryosphere. Thus, the question of possible endemism in these yeasts remains unresolved.

Geographical barriers for the distribution of yeasts are often difficult to imagine considering their potential borderless transportation by air currents (see Starmer and Lachance 2011), organismal vectors, and their dispersal with plant material as epiphytes or endophytes (e.g. Fonseca and Inácio 2006; Zhang et al. 2010; Francesca et al. 2014). Nevertheless, distinct yeast populations do occur in different localities, habitats, and seasons (e.g. Fonseca and Inácio 2006; Starmer and Lachance 2011). Strong disproof of yeast ubiquity comes from studies on yeasts associated with beetles, drosophilids, bees, and short-lived flowers (Lachance et al. 2001; Starmer and Lachance 2011). Historical factors play important role in the dispersal of ascomycetous yeasts, which is constrained by the habits of the vector and the yeast's adaptation to being transported. When the vector reaches its distribution limit, the yeasts it carries may not be able to colonise substrates that they typically occupy. Drosophilids were extensively studied as vectors of highly specific yeast communities to the habitats where they feed, for example, tree fluxes

and rotting tissues of cacti (Starmer and Lachance 2011). When plants are moved outside of the natural distribution and the vector is lacking, these habitats are not colonised by local yeasts or contain a few widespread species only (Starmer and Lachance 2011). Unlike the yeasts of boreal and temperate forests, ascomycetous yeasts associated with bark beetles were not observed among the communities in the tundra and polar regions possibly due to the lack of target substrate (trees) and vectors (Chernov 2005). The biogeography of these specialised yeasts is highly correlated with the biography of their hosts. Among basidiomycetous yeasts, asexual (anamorphic) states of plant pathogens (members of phyla Ustilaginomycotina and Pucciniomycotina) and mycoparasites (e.g. members of classes Tremellomycetes and Cystobasidiomycetes) were previously classified in the polyphyletic genera *Cryptococcus* and *Rhodotorula* (e.g. Liu et al. 2015; Wang et al. 2015). Although the asexual morphs are found outside the hosts (e.g. Begerow et al. 2014) and display nearly cosmopolitan distributions (e.g. Fonseca and Inácio 2006; Yurkov et al. 2015a), the degree to which the host and environment influence the survival of yeast states is unknown.

The distribution of organisms in space can be either random or structured. One example of a nonrandom distribution is an aggregated distribution, which can be caused naturally by two independent factors. In the first instance, if the environment in which the yeasts are proliferating is spatially heterogeneous, the survival of incoming cells will vary in space due to differences in habitat properties. For instance, soil-like habitats are heterogeneous and contain micro-environments characterised by different, and sometimes contrasting, properties such as acidity, conductivity, density, redox potential, and availability of water and air. Major differences between micro-environments would result in different yeast communities consisting of species, each adapted to the specific properties of its microhabitat. Similarly, an aggregated distribution of microbial cells (including yeasts) has been observed on individual leaf surfaces (e.g. Fonseca and Inácio 2006; Inácio et al. 2010, and references therein). Another case factor capable of generating an aggregated distribution is variation in modes of proliferation. Yeasts are not restricted to the unicellular form but grow also as filaments or pseudo-filaments. Whereas single cells are easily transmitted with air and water currents, hyphal chains attached to a substrate have a lower probability of distribution. Notably, the ability of basidiomycetous yeasts to produce forcibly ejected spores has the potential to increase their ability to disperse. It is important to distinguish horizontal and vertical distributions of yeasts in a biotope. Available studies suggest that yeast cells can be moved passively by animals over longer distances aboveground (e.g. Belisle et al. 2014; Francesca et al. 2014; Kokurewicz et al. 2016). Similarly, cells can easily be washed down to the litter and further to the soil by the action of the rain. As a result, upper soil horizons always harbour transient species originating from leaf material (e.g. Phaff and Starmer 1987; Maksimova and Chernov 2004; Yurkov et al. 2008, 2012b). The horizontal distribution of soil yeasts is more complex. Hypothetically, filamentous species have better chances to proliferate in soils, whereas unicellular forms are more likely to be washed down into mineral horizons, where they die or are preyed upon (see also Yurkov 2017). The total number and

diversity of yeasts decrease rapidly with soil depth (e.g. Babjeva and Chernov 1995). Species composition in mineral horizons is different from that of the topsoil, featuring an abundance of typical soil ascomycetes such as *Lipomyces* spp. (Vinovarova and Babjeva 1987; Babjeva and Chernov 1995). However, the mechanisms of migration and dissipation of yeast cells in the soil profile are unknown.

4.3 Habitats and Niche Concepts

One often assumes that each yeast species has its niche, habitat, and geographic distribution. This assumption, however, does not extend to widespread species that are sometimes referred to as eurybionts, ubiquists, or cosmopolitan. Despite their broad spatial distribution, these species may still display a preference to a particular habitat with more data analysed. For example, *Cr. victoriae* (currently *Vishniacozyma victoriae*) has been originally described from soil at Victoria land in Antarctica. Later this yeast was frequently observed on plant material in different regions and is currently regarded as a phyllosphere yeast (e.g. Glushakova and Chernov 2010; Fonseca et al. 2011; Yurkov et al. 2015a). Lichens and mosses also harbour *Vishn. victoriae* and substitute vascular plants as the substrate in extremely cold climates (e.g. Santiago et al. 2015; Duarte et al. 2016; Vasileva-Tonkova et al. 2014). *Candida podzolica* (formerly *Cryptococcus podzolicus* and currently *Saitozyma podzolica*) was described from podzols and long believed to be closely associated with this type of acid soil (e.g. Babjeva and Chernov 1995; Maksimova and Chernov 2004). However, recent studies revealed an affinity of this species for moist, acid environments, including acid tropical soils (Vishniac 2006b), *Sphagnum* moss (reviewed in Yurkov et al. 2012b), and water tanks of bromeliads (Gomes et al. 2015). Although *Vishn. victoriae* and *Sa. podzolica* can be isolated from both above- and belowground sources worldwide, each of the two yeasts shows preferential affinity to a certain environment, i.e. phyllosphere and acid soils, respectively.

Traditionally, reviews of yeast diversity, including the present book, involve differentiation to type of substrates (e.g. plant surfaces, fruits, soils) or types of habitats (e.g. aquatic, terrestrial, cold) each of which consists of micro-environments with a unique combination of environmental parameters. Because neither the complexity of the habitat nor the interactions of yeasts are known, ecological studies follow a pragmatic approach where one describes a community in a particular substrate. Although this would provide a reliable overview of yeasts in a single location, the analysis of the results in a larger spatial context is complicated as habitat properties may vary between locations. A broader survey across several biotopes would benefit from an attempt to define habitats and substrates. The key assumption is that habitats with similar properties are likely to harbour similar yeast communities. Of course such an approach would result in some averaging of substrate properties and characteristics to account for the heterogeneity within and between locations. For example, yeasts on leaves are

usually studied on a few individual plants, and sometimes plants of different species grow close together in a biotope. Samples are collected and analysed to consider variability of the plant composition, leaf age, and light exposure. However, information reflecting the local heterogeneity may be lost when the sampling did not follow the same scheme, for instance, due to the lack of the same plant species. Ideally, a study should analyse distribution of yeasts in the same or very similar set of substrates as, for example, decaying tissues of cacti (e.g. Starmer et al. 2006), flowers (Lachance et al. 2003a), and fruit (Čadež et al. 2003; Maksimova et al. 2009). When it is not possible to survey exactly the same substrate in different locations, a broader definition of the substrate can be used. For example, plant surfaces in a broad sense may include different groups of plants (e.g. deciduous, coniferous, mosses), plant organs (e.g. stems, leaves), and developmental stages (e.g. buds, leaves). Other examples of broadly defined types of substrates include soils, decaying plant material, and fruits, among others.

Categorising habitats formally into type substrates can aid to distinguish better between ecological generalists and specialists (see for discussion Chap. 3 in this book) and give a hint about the association of a yeast to a particular substrate. For example, Babjeva, Chernov, and co-workers simultaneously analysed several broadly defined types of substrates to identify most typical yeast species for each of them (Babjeva and Chernov 1995; Maksimova and Chernov 2004; Chernov 2005; Yurkov et al. 2015a). The approach of the authors was strongly influenced by phytogeography, Dokuchaev's concept of natural zones and the emerging biogeography of soil microorganisms (e.g. Mishustin 1975; Aleksandrova 2012; Oldfield and Shaw 2015). The first group of substrates corresponded to a vertical stratification of each type of ecosystem studied (tundra, forest, steppe, and desert): the epiphytous complex, i.e. (1) the yeasts that occur on living, green aboveground plant parts (mainly the phylloplane); (2) the litter complex, i.e. the yeasts that are present on senescent leaves and leaf litter; and (3) the soil complex, i.e. the species inhabiting mineral soil horizons (Babjeva and Chernov 1995; Chernov 2005; Fonseca and Inácio 2006). Surfaces of mosses and lichens were sometimes regarded as a different type of substrate from those of vascular plants (e.g. Kachalkin et al. 2008). The vertical sequence of substrates was additionally thought to reflect a temporal change, or succession, of the plant material from the living plant to the decomposed organic matter. Several ephemeral sugar-rich habitats (i.e. flower nectar, fruit, and tree fluxes) and insect-related substrates (i.e. insect frass, honey, and honeydew) have been considered, although they were not present in every climatic zone. Despite being little known in the English-language literature, the authors have surveyed yeast communities in the aforementioned substrates for over 50 years. Because the techniques employed for sampling and community assessment remained uniform during that period, Babjeva and Chernov (1995) and Chernov (2005) were able to summarise the collected data and re-analyse them statistically to assess the substrate-related and latitudinal distribution of yeasts. Some of their findings are discussed below.

4.4 Examples of Distribution Patterns

4.4.1 Latitudinal Gradients

Larger organisms commonly exhibit latitudinal gradients in species diversity, with a larger biodiversity existing in the tropics and decreasing towards more temperate and polar regions. According to the hypothesis known as Rapoport's rule (Stevens 1989), organisms are expected to live within smaller geographical ranges at lower latitudes (near the equator) than at higher latitudes (near the poles). The theory explains the wide occurrence of eurybiont species with diverse ecological adaptations at high latitudes. If the same is true for yeasts, a few generalist species can potentially colonise different habitats, so high-latitude communities would contain a few dominant yeasts shared across different substrates and over a broad geographic range. On the contrary, yeast communities closer to the equator are expected to be species-rich due to higher ecosystem productivity and habitat fragmentation. In this scenario, yeast diversity is increasing because speciation favours a narrowing of the ecological niche and an increased specialisation. Successful colonisation and survival imply that yeasts must develop physiological adaptations that facilitate growth and the utilisation of nutrients, as well as increased competition through modification of the environment, also known as the priming (e.g. Kuzyakov et al. 2000; Kuzyakov 2010) and priority (e.g. Fukami 2015) effects. A close association with insect or animal vectors might additionally increase a yeast's specialisation and narrow its distribution. Taken together, yeast communities in lower latitudes would be more diverse with a higher amount of endemism. The diversity of yeasts along a latitudinal gradient has been investigated in various habitats, including ephemeral flowers (Lachance et al. 2001), soils (Babjeva and Chernov 1995; Chernov 2005; Vishniac 2006b), and plant-related substrates (Babjeva and Chernov 1995; Chernov 2005).

Lachance et al. (2001) studied yeast communities vectored by beetles, drosophilids, and bees to ephemeral flowers. Over 1000 samples were collected over a period of 13 years in the Neotropical, Nearctic, and Australian biogeographic regions. The frequency of observation of yeasts in flowers was used to assess the effects of area, latitude, and elevation on the diversity, distribution, and abundance of ascomycetous yeasts, most of which are specific to the insect-flower habitat (Lachance et al. 2001). Despite substantial differences in yeast community composition of different flowers (cactus vs. Convolvulaceae and Malvaceae flowers) and between biogeographic regions (New World vs. Australian), a few interesting north-south gradients were observed at several levels. Among species frequently found in ephemeral flowers in the New World, three species, *Candida azyma* (later found to be mostly *Candida parazyma*), *Metschnikowia* (originally *Candida*) *ipomoeae*, and *Wickerhamiella occidentalis* were not found northward of Tennessee (approx. 36°N), which can be interpreted as an example of loss of species diversity in northern latitudes. Large-spored *Metschnikowia* species in general follow such a pattern at the global level (Lachance et al. 2016a) Similarly, the

low frequency of observation of *Candida tolerans* and the absence of *Kodamaea kakaduensis* in New South Wales and Queensland in Australia could be viewed as another example supporting that trend. However, the authors suggested that geographic factors may not act directly on the yeasts themselves but rather influence the distribution of their vector insects, i.e. beetles, drosophilids, or bees (Lachance et al. 2001).

Vishniac (2006b) analysed soils taken over a period of nearly 30 years along a latitudinal gradient in western North America covering the following climatic zones: polar (tundra; Alaska), subarctic (subalpine forest; Rocky Mountains, Colorado), humid subtropical (pasture; Oklahoma), semiarid (desert; Chihuahuan Desert, Mexico), and tropical (rainforests; Costa Rica). Additionally, soils were collected in other regions with polar (Antarctica, Iceland, Chukotka Peninsula) and desert (Gobi Desert in China and Atacama Desert in Chile) climates. Ecosystems with boreal and temperate climates were not sampled in this study. Basic environmental parameters such as temperature (mean annual values), rainfall (total annual values), net primary productivity (NPP), acidity (pH), and electrical conductivity (EC) were examined as factors that may bear upon the distribution of yeasts (as frequencies) along the latitudinal gradient. A multivariate statistical analysis showed that the combination of mean annual temperature, annual rainfall, and electrical conductivity could explain up to ca. 44% of the distribution of the prominent yeast species (Vishniac 2006b). It is important to note that the frequent observation of a yeast species (incidence) was reported as dominance by the author. The latter term is normally used to designate local abundance, expressed in weight of biomass or in numbers of cells (see also Chap. 3 of this book). Vishniac (2006b) reported the relative frequency of species rather than abundance.

Despite the extraordinary size of Vishniac's sampling (2006b), only a few latitudinal trends were detected. Species of the genus *Naganishia* (*Cryptococcus* spp. in the albidus clade, Filobasidiales, *Tremellomycetes*) dominated in desert soils. Soils under better-developed vegetation favoured yeasts from the orders Filobasidiales and Tremellales, with the latter predominating in soils of low pH or higher EC. The typical soil yeast *Sa. podzolica* (formerly *Cr. podzolicus*) was more frequent in moist soils under higher-NPP vegetation in cloud and rainforests of Costa Rica. The two species *Naganishia albida* and *Filobasidium chernovii* (formerly *Cryptococcus* spp.) responded to elevated temperatures, while *Goffeauzyma gilvescens* (*Cr. gilvescens*) occurred with higher incidence in cold regions. The distribution patterns of other common species (Filobasidiales, Tremellales, Trichoporonales) were not resolved, and the quantity nor the diversity of soil yeasts was not discussed. The potential loss of species diversity in northern latitudes cannot be inferred from the data presented. An interesting observation, however, is that tundra and rainforest soils yielded a similar total number of yeast colonies.

Babjeva, Chernov, and co-workers studied the latitudinal distribution of yeasts found on selected substrates in the framework of Dokuchaev's natural zones. The authors emphasised that correct conclusions regarding the geographical distribution of yeasts can be made between samples representing the same type of habitat or substrate. They summarised observations of yeast species made starting in the

1960s and intensified in 1980–1990 on the territory of the former USSR. The concept of gradual change of yeast communities with latitude and the first descriptive summary of the observed trends were introduced by Babjeva and Chernov (1995). Later, Chernov (2005, 2013) unified data derived from a total of 114 localities and ca. 7000 samples into a similarity matrix (Sørensen-Dice coefficient) and performed a multifactorial and multidimensional analyses of geographic latitude, natural zone (i.e. polar deserts, tundra, boreal forests, temperate forests, broadleaf forests, steppe, semiarid deserts, and deserts), and type of substrate. The parameters analysed were total quantity (as CFU g^{-1}), isolation success (number of samples yielding yeasts), average number of species in the sample, diversity (as Shannon index), relative abundance of each species (also in the form of a species abundance distribution (SAD) curve), frequency of observation of each species, and physiological properties (proportion of thermophiles and psychrophiles, and number of utilised carbon sources).

Despite substantial variability among samples, yeast quantity showed a unimodal distribution. The highest values were observed in boreal and temperate climates and rapidly declined towards the north and the south. The number of observed yeast colonies (logarithmic values) in samples collected on the East European Plain grew linearly from 45°N to 65°N. Because empirical species discovery rates varied between substrates and depended on the sampling effort (see for discussion Chap. 3 of this book), Chernov (2005, 2013) suggested to replace species richness values with the 'species density', which is calculated as the number of yeast species to the number of colonies examined. This parameter showed a latitudinal trend increasing from lower to higher latitudes, although declining in the cold Arctic biotopes. Similarly, the diversity of yeast communities increased from subtropical deserts to the tundra, but most of increase was observed between forest biotopes and the tundra (Chernov 2013). This trend has been observed in all substrates. Communities in the tundra and in hot deserts were characterised by a strong dominance of a single species that constitutes up to 90% of the total abundance (Chernov 2013). Ascomycetous yeasts were extremely rare in these regions but become more prominent in forests, where they often inhabit ephemeral sugar-rich habitats. It is important to note that ascomycetous yeasts were not rare in the deserts of the Northern and Central America, where they are found in association with cacti (e.g. Starmer and Lachance 2011). Chernov (2005) used the polytrophic index as the number of carbon sources a yeast species could grow on. This idea has been used before to study yeast communities associated with trees (bark, fluxes, and rots) and *Drosophila* flies (Starmer 1981; Lachance and Starmer 1982, 1986, 1998; Morais et al. 1992). Physiological profiles of yeasts analysed quantitatively were found to be good predictors of habitat characteristics (e.g. families of trees) and reveal functional redundancy in the community (Lachance and Starmer 1998). Chernov (2005) used a simplified approach recording only the number of assimilated compounds but put it into spatial context. The ability of yeasts to colonise diverse habitats due to broader metabolic capacities was studied in relation to the geographic location of the habitat. In soils, the number of utilised sources by a local community increased

from south to north (Chernov 2005). Although not recognised by the author, this observation would fit the expectation of species with broad fundamental niches in higher latitudes (see Rapoport's rule). This observation goes in line with the previous report of yeasts with diverse physiological capabilities showing a broader distribution range across substrates visited by *Drosophila* flies (Starmer 1981). As expected from sampling along latitudinal gradients, the occurrence of psychrophilic (maximum growth temperature below 25 °C) species increased in northern latitudes, while thermophilic (maximum growth temperature above 35 °C) yeasts were more frequent in southern latitudes (Chernov 2005).

All studies of yeast ecology based on data collected over the last half century suffer from the caveat that the methods and criteria used in identifying and delineating species have changed considerably over that period. A species identified by growth characteristics may now be known by sequence-based methods to represent numerous genetic species (e.g. Barnett 2004; Kurtzman and Fell 2006). For example, the former phenotypic species *Cryptococcus albidus* and *Cryptococcus laurentii* (e.g. Babjeva and Chernov 1995; Glushakova and Chernov 2004; Maksimova and Chernov 2004), as previously circumscribed, comprise an eclectic array of species that may now be considered members of different genera. With the growing evidence for the taxonomic heterogeneity of widespread basidiomycetous yeasts (e.g. Fonseca et al. 2000; Takashima et al. 2003), Chernov's approach was repeatedly criticised as oversimplified (e.g. Vishniac 2006a; Wuczkowski and Prillinger 2004; Golubev et al. 2006). It is a matter of debate whether cryptic species always coexist in the same habitat, thereby introducing a bias to diversity estimations. A few studies where previously studied habitats were resampled and reassessed concluded that misidentification of species, as opposed to the underestimation of their diversity, is more likely to happen in phylloplane and soil substrates (Yurkov et al. 2011, 2015a; Glushakova et al. 2013).

4.4.2 Bipolar Latitude Distributions

Apart from presumably eurybiont species, a few yeasts exist in opposite hemispheres and at complementary latitudes. Such a 'bipolar' distribution often mirrors the environment, climatic conditions, or host association. For example, the astaxanthin-producing red yeast *Phaffia rhodozyma* was isolated in the temperate zone of both the Northern and Southern Hemispheres. The species was repeatedly isolated from tree fluxes in Europe, Asia, and North America, as well as from fruiting bodies of tree parasite *Cyttaria* in Patagonia. However, subsequent phylogenetic analyses showed that the species consists of genetically distinct geographical populations of *Ph. rhodozyma* distributed separately in host trees of the genera *Betula*, *Cornus*, *Fagus*, and *Nothofagus* (Libkind et al. 2007). The yeast is psychrophilic and has not been isolated in warmer climates, but this does not rule out that their distribution could be influenced by both geographic and climatic conditions. *Glaciozyma litoralis*, described (Kachalkin 2014) from macroalgae and

littoral sediments of the White Sea coast near the Arctic Ocean, was recently found to predominate also on Antarctic macroalgae (Duarte et al. 2016). Samples of ice, snow, superficial debris, and melting waters collected from Arctic (circumpolar), Alpine, and Antarctic glaciers yielded the novel psychrophilic species *Naganishia vaughanmartiniae*, formerly *Cryptococcus vaughanmartiniae* (Turchetti et al. 2015). In view of the increasing interest of ecologists in cold environments, it is likely that the number of known species inhabiting high latitudes at both poles of the Earth will be increasing in the future. The prospective studies should be directed to link potential dispersal routes to the substrates yielding yeast cells. This could answer the question whether or not the observed yeasts permanently inhabit in polar regions. To what extent the spatial separation contributes to speciation is another matter worthy of study, where yeast isolates from spatially remote regions may serve a good model.

4.4.3 Holarctic and Antarctic Distribution

A variety of animal species (e.g. brown bear, grey wolf, red fox) are distributed across the northern continents of the world, known as the Holarctic ecozone. The continuity of communities on the northern part of the Holarctic results from their shared glacial history (periods of repeated glaciations) and movement between continents allowed by the Bering land bridge. *Goff. gilvescens* (cited in the literature as *Cr. gilvescens*) is a good example of Holarctic yeast species, which was found in cold Arctic environments in Eurasia and North America (Babjeva and Chernov 1995; Polyakova et al. 2001; Vishniac 2006a, b; Buzzini et al. 2012). Although, this yeast was not observed in boreal and temperate forests south of the Arctic region, it was isolated from Alpine glaciers in high frequency (e.g. Turchetti et al. 2008; Buzzini et al. 2012). Alpine isolates of *Goff. gilvescens* displayed a remarkable ability to grow and produce enzymes at low temperatures in the laboratory, which demonstrates adaptation of the species to cold habitats. Although biodiversity surveys have repeatedly recovered yeasts from Antarctica, most are not endemic, being found in other regions and climates (e.g. Vishniac 2006a; Buzzini et al. 2012). Nevertheless, a few species are only known from Antarctica. For example, the psychrophilic yeasts *Glaciozyma antarctica*, *Metschnikowia australis*, *Naganishia antarctica*, and *Naganishia friedmannii* (cited as *Cr. antarcticus* and *Cryptococcus friedmannii*, respectively) have been repeatedly found in this ecozone and not in neighbouring ecoregions or other cold environments (Buzzini et al. 2012).

4.4.4 Distance-Decay Relationships

A decline in similarity with increasing geographic distance, known as a distance-decay relationship, has repeatedly been observed for microorganisms (reviewed by

Hanson et al. 2012). The key assumption here is that neighbouring communities are likely to have similar compositions resulting from the continuous exchange of cells. This assumption was frequently tested in biology and ecology with statistical tools such as Mantel test, which applicability has been recently criticised (e.g. Legendre and Fortin 2010; Guillot and Rousset 2013; Legendre et al. 2015). The similarity (or dissimilarity) of communities results from dispersal, colonisation, propagation, and death of yeast cells. These processes are controlled by the environment (habitat filtering ability) and interactions with other organisms (competition, predation, vectoring). Together, these events turn our understanding of the community from static into continuously changing system, where each community analysis is only a snapshot of the local community. While dynamic models explaining the richness, composition, and structure of animal and plant communities are known for decades (e.g. Rosindell et al. 2011), the persistence of yeast communities over long periods of time has not been extensively studied (Starmer and Lachance 2011). The view on yeast communities as a dynamic cycle and the relevance of species dispersal mechanisms have been discussed previously by Starmer and Lachance (2011).

Yeast populations are distributed unevenly even among adjacent samples on plant material (e.g. Inácio et al. 2010), fruits (e.g. Maksimova et al. 2009), flowers (Lachance et al. 2003a), and soils (e.g. Yurkov et al. 2011). Species richness values and colony numbers often vary substantially between samples and replicates in cultivation experiments. The uneven distribution of yeasts is to some degree due to the unique properties of each microhabitat sampled. A sample of soil is naturally heterogeneous as to its content of clay particles, sand, fine roots, as well as plant, animal, and microbial residues. A combination of these components would determine physical (water and air), chemical (salinity and acidity), and biotic (availability and quality of nutrients) properties for each of the microhabitats. Organic soil horizons, mineral horizons, the rhizosphere, and the ectomycorrhizosphere all contain different yeast species, at least to the extent of available comparisons (e.g. Botha 2006, 2011; Golubtsova et al. 2007; Mestre et al. 2011). Light exposure, temperature, photosynthesis, and sugar contents vary with both leaf position and age and are believed to influence yeast distribution in the phyllosphere (e.g. Fonseca and Inácio 2006; Nix et al. 2008; Nix-Stohr et al. 2008; Glushakova and Chernov 2010; Inácio et al. 2010). Traditional ecological niche theory assumes that each species plays a unique role, so that each is limited by a unique combination of factors. On contrary, the unified neutral theory of biodiversity and biogeography (Hubbell 2001) assumes that all individuals within the same trophic level are equivalent and have same chances of reproduction and death (Rosindell et al. 2011). When one individual from a local community dies, it is replaced by an offspring of another randomly chosen species from the local community or from an outside pool depending on the measure of dispersal limitation. The theory has been demonstrated to predict both richness and species abundance parameters. As applied to yeast communities, the abundance of a species is likely to influence the probability of dispersal, and functional redundancy may explain the probability of colonisation. Natural heterogeneity of the environment positively contributes to the variability of yeast communities, although the above factors should be kept in mind.

Above- and belowground yeast communities respond to microclimatic changes even on a small scale (Inácio et al. 2002; França et al. 2016; Yurkov et al. 2016), although metrics such as total abundance, species richness, and species composition are probably not sensitive enough to reflect those changes. Local community structure changes with aridity, temperature, sun exposure, and availability of nutrients. The determination of diversity indices may provide insights on spatial variation. Commonly used in ecology are Shannon's index and Simpson's index, which summarise species proportions in a community and thus reflect both species richness and species evenness. Community evenness in yeast communities was found to change as a function of environmental variables in a swamp to forest ecocline (Kachalkin and Yurkov 2012), in the seawater column (Gadanho et al. 2003), in insect-associated substrates (Rosa et al. 1994) in forest soils subjected to different management types (Yurkov et al. 2012a), and in grapes collected from vineyards with different farming systems (Setati et al. 2012). Similarity (or dissimilarity) among local yeast communities has been used as a measure of spatial distance and temporal variation in the community. The Sørensen-Dice coefficient, Jaccard's index, Pearson's correlation coefficient, Bray-Curtis dissimilarity, and Euclidean distance are suitable metrics that are implemented in biological software packages. The results of pairwise comparisons with the Sørensen-Dice or Jaccard's indices can be visualised by multidimensional scaling (e.g. nonmetric multidimensional scaling or NMDS) in a two-dimensional plot (e.g. Yurkov et al. 2012b; Gomes et al. 2015). A similar representation of the results can be achieved with ordination techniques such as principal components analysis (PCA) and redundancy analysis (RDA), among others (e.g. Yurkov et al. 2008; Glushakova and Chernov 2010; Mestre et al. 2011; Turchetti et al. 2013; França et al. 2016). A detailed overview of the use of diversity indices is provided in Chap. 3 of this book.

Starmer et al. (2006) found that species richness in cactophilic yeast communities increases from local communities to regional and continental ones. A similar observation was reported for yeasts on berries of the mountain ash (*Sorbus aucuparia*), which were significantly more species rich at the tree or locality levels than on neighbouring berries (Maksimova et al. 2009). Rare transient species occasionally occupy sugar-rich substrates such as fruits or decaying cactus tissues. Because their transition between sites is random and strongly depends on the surrounding environments, the dissimilarity between these communities declines with distance and parallels an increase of species richness values (Starmer et al. 2006). In contrast, Shannon's diversity index values largely depend on the proportion of dominant resident species. The diversity of cactophilic yeast communities did not change with distance (Starmer et al. 2006) because the local communities, which are shaped through a long history of interactions between yeasts and their vectors, their environment (including plants), and other resident microorganisms, already feature a substantial heterogeneity. An assemblage of mountain ash berries (corymb) showed a significantly higher diversity than did single berries (Maksimova et al. 2009). However, the diversity declined at the tree level due to averaging of the community structure and increased again on the regional level, possibly in response to climatic differences (Moscow region and Moldavia). The

similarity among these communities calculated with the Sørensen-Dice coefficient showed a gradual decline with increasing distance between samples (Maksimova et al. 2009).

4.4.5 Endemism

Recently, Ganter (2011) summarised the present knowledge of endemism in yeasts in the context of the sometimes expressed view that microorganisms are generally ubiquitous (e.g. Fenchel and Finlay 2004). Yeasts on decaying cactus tissues and in ephemeral flowers are good examples of endemism. Both types of communities are specific and strongly delimited by a combination of habitat availability (plant) and dispersal by vectors. Both parameters impose a historical framework upon the distribution of yeasts. It is important to state that the cactophilic community is dominated by species found only in cactus rots but it is not exclusive of yeasts from other habitats (Ganter 2011; Starmer and Lachance 2011). Of the 25 cactophilic yeasts, only a small minority appear ubiquitous within the habitat, namely, *Pichia cactophila* and *Candida sonorensis*, which account for about 40% of all cactophilic isolates (Ganter 2011). Another widespread species, *Sporopachydermia cereana*, is in fact a complex of closely related species with restricted geographic ranges (Lachance et al. 2001). Despite their strong association to cacti, the physiological capabilities of cactophilic yeasts allow them to survive outside their primary habitat, for example, in tree fluxes (Ganter 2011; Starmer and Lachance 2011). Yeasts of the genus *Starmera* have been found in almost every region and cactus type, while the distribution of *Phaffomyces* species is narrower (Ganter 2011). All of the taxa within the genus are distributed almost without overlap, and with a few exceptions, they are limited to the regions where cacti have been introduced (Ganter 2011). Two close relatives of *P. cactophila*, *Pichia pseudocactophila* and *Pichia insulana*, are restricted to very narrow geographic limits, i.e. the northern Sonoran Desert and Curaçao, respectively. For this reason, cactophilic yeasts exemplify narrowly endemic species.

Similarly to the cactophilic habitat, the yeast communities of wood, flowers, and sap (slime) fluxes are shaped by insect vectors. Although the potential diversity of yeasts associated with wood boring beetles is estimated to be enormous (Boekhout 2005; Suh et al. 2005; Blackwell 2011). The biogeography of yeasts from this habitat has not been well investigated, and so its level of endemism is not well understood. The biogeographic patterns of the yeast communities of slime fluxes are better known. Similarly to cactus rots, they are the breeding sites for various insects, including drosophilids (Ganter 2011; Starmer and Lachance 2011). Therefore, tree fluxes were repeatedly studied as a source of novel yeast species. Unlike flowers, tree fluxes represent extremely fragmented and irregular habitat, such that examples of large-scale sampling efforts are rare. Ganter (2011) reviewed the results of two collections from both oak (*Quercus* sp.) and poplar (*Populus* sp.) trees in Arizona and the Great Lakes region. Oaks yielded 50 and 85 isolates

representing 12 and 24 species in Arizona and the Great Lakes region, respectively. The two studies had only three yeasts in common (Lachance et al. 1982, 1995). Out of 12 and 6 species (Arizona and the Great Lakes region, respectively) isolated from tree fluxes of poplar, a single yeast species was shared between the two regions.

Flowers and nectar represent a more widespread and a more complicated system than cactus rots and tree fluxes. Among yeasts commonly found in nectar, a few have been considered widespread within the habitat, i.e. *Metschnikowia reukaufii*, *Metschnikowia gruessii*, *Starmerella bombicola*, and *Candida rancensis* (Brysch-Herzberg 2004; Starmer and Lachance 2011; Pozo et al. 2011). A combination of plant and animal vectors enhance geographic endemism of flower yeasts within this system. In a study of yeasts in ephemeral flowers in the New World and the Australian biogeographic regions, members of the large-spored *Metschnikowia* clade dominated in both Neotropical and Nearctic regions, whereas *Kodamaea anthophila* was prominent in the Australian region (Lachance et al. 2001). The same biogeographic pattern was also observed at the level of the *Wickerhamiella* clade, whereby one species (*Wickerhamiella australiensis*) was exclusive to the Australian region and another (*Wick. occidentalis*) was clearly Pan-American (Lachance et al. 2001). The major division between the two biogeographic regions is largely attributed to the prevalence of two major types of Coleoptera, namely, *Conotelus* species in the New World and *Aethina* species in the Australian region. Hawaiian samples contained yeasts typical of both biogeographic regions that was consistent with the observation of both vectors in the Hawaiian fauna. Further investigation of the yeast-insect-morning glory flowers system (Lachance et al. 2003a) showed that vectoring by certain nitidulid beetles explains both the long-range and short-range dispersal of yeasts on Hawaiian islands (Lachance et al. 2003a). The yeasts *Metschnikowia lochheadii* and *M. ipomoeae* (cited as *C. ipomoeae*) are abundant and show a global distribution following that of *Conotelus mexicanus*, a nitidulid introduced to Hawaii from the American continent early in the middle of the twentieth century. In contrast, *Metschnikowia hawaiiensis* and *Metschnikowia* (formerly *Candida*) *kipukae* are endemic to the island of Hawaii and are nearly exclusive to insects or flowers found in the Kipuka Puaulu site. Furthermore, *M. hawaiiensis* is one of six related species that are endemic to specific regions within Hawaii, ostensibly due to the endemicity of their vector beetles (Lachance et al. 2005). Of these, *M. hawaiiensis* and *Metschnikowia hamakuensis* are restricted to a single (Hawaii) island, where they occupy, respectively, southern and northern beetles and flowers (Lachance et al. 2016a).

4.4.6 Reproductive Isolation and Speciation

Understanding the dispersal mode of individuals of a species is the key to understanding its distribution range, reproductive isolation, and speciation. In their influential paper, Fenchel and Finlay (2004) proclaimed that eukaryotic microorganisms have global geographic ranges that contrast with the patterns known for

macro-organisms. In response to this study, Taylor et al. (2006) demonstrated that inferred geographic range of a fungal species depends upon the method of species recognition. While some fungal species defined by morphology show global geographic ranges, when fungal species are recognised phylogenetically, they often exhibit endemism. This observation illustrates well the lack of concordance between species concepts in ecological studies that claim to follow a 'natural' (i.e. phenotypic) species concept as opposed to one based on 'genotypic' properties, as adaptation to environmental conditions is mainly the result of phenotypic adaptation (see Barnett 2004; Yurkov et al. 2015a). With the development of DNA-based identification and species delineation, researchers can elucidate stable characteristics that differentiate morphologically indistinguishable but genetically distant yeasts. As a consequence, the circumscription of yeasts in collections from different sources and locations has become narrower, as has the extent of their known realised niche and geographic distribution. The extent of divergence in ribosomal gene sequences (LSU rRNA gene and later the ITS region) has become a widespread, often stand-alone criterion for the delineation of yeast species, following the observation that these sequences are mostly invariant within species and divergent between species (e.g. Kurtzman and Robnett 1998; Fell et al. 2000; Scorzetti et al. 2002). However, the degree of sequence variability and the significance of polymorphism are difficult to estimate for many species known from a few strains only (see also Lachance et al. 2010, 2011). In some cases where sampling is adequate, sequence polymorphisms within species can be considerable (Lachance et al. 2003b, 2010, 2011).

Ascomycetous yeasts with a bipolar mating system potentially provide a good model to study allopatric speciation and approach the biological species concept in microorganisms from a biogeographic perspective. The distribution of mating types may in some cases help to understand the colonisation of new habitats, the rules of niche occupancy, or even extinction processes in yeast populations. If the two mating types occur in equal proportions near the centre of origin of a species, a bias towards a single mating type may arise in peripheral localities due to extreme founder effects. For example, *Kod. anthophila* and *Wick. australiensis* have been frequently isolated from flowers in Australia, New Caledonia, Fiji, and Rarotonga (Lachance et al. 2001). The distribution of the two mating types of *K. anthophila* was balanced in Australia, New Caledonia, and Fiji, but only one mating type was recovered in Rarotonga, the smallest of those land masses and the most distant from Australia, the presumed centre of origin. Similarly, both mating types of *Wick. australiensis* were well represented in Australia; they were imbalanced in Fiji, and only one mating type was recovered on Rarotonga. Mating success, assessed from the observation of conjugation between compatible strains, the subsequent formation of a mature ascus, and the viability of the meiotic progeny, can be used as a measure of genetic compatibility and serve as a solid basis for species delimitation according to reproductive isolation (e.g. Lachance et al. 2005, 2010, 2011, 2016b).

Because the observation of yeasts in nature is often far from random, a few studies have focused on widespread ascomycete species, either ubiquitous within the habitat or showing a globally cosmopolitan distribution. A range of molecular

and genetic tools were used to explore distribution ranges below the species level. Lachance and co-authors (2011) studied polymorphisms in ribosomal DNA sequences and mating compatibilities in two ascosporic species, *Metschnikowia agaves* and *Starmer. bombicola*, isolated from different regions. Of 36 *Starmer. bombicola* strains connected in the 95% parsimony-rule haplotype network based on ribosomal ITS and LSU (D1/D2 domains) sequences, 35 were able to mate with at least 1 compatible partner. Mature asci were observed in crosses between individuals differing by as many as five, but not six or seven substitutions in the LSU rRNA gene. In many instances, isolates obtained from the same locality represented the same ITS-LSU haplotype or differed only in a few nucleotide positions in the network. The influence of reproductive barriers on the distribution of basidiomycetous yeasts is less well understood. A more complex sexual cycle with a tetrapolar mating system (e.g. Coelho et al. 2010; Guerreiro et al. 2013; Yurkov et al. 2015b) and a presumably parasitic lifestyle (e.g. Sampaio 2004; Weiss et al. 2014; Liu et al. 2015) further complicate such studies in the Agaricomycotina and the Pucciniomycotina. For example, sexually compatible strains of *Papiliotrema* (formerly *Cryptococcus*) *flavescens* as ascertained from mating gene sequences did not produce any teleomorphic structures in crosses on artificial media (e.g. Yurkov et al. 2015b). Conversely, the observed mating reactions in *Leucosporidium scottii* did not correlate well with the phylogenetic position of the respective alleles in the mating loci (de Garcia et al. 2015).

Correct identification of species is critical to our ability to recognise spatial patterns in basidiomycetous yeasts. Yeast communities of forest biotopes extensively sampled by Babjeva, Chernov, and co-workers were uniform within a substrate and did not change with forest type (e.g. Maksimova and Chernov 2004), plant species (e.g. Glushakova and Chernov 2010), or distant regions (Yurkov et al. 2004). However, with sequence-based identification, plant-associated communities in Siberia and the Moscow region were distinct in relative proportion of *Filobasidium magnum* (formerly *Cryptococcus magnus*) and *Sporobolomyces roseus* (Yurkov et al. 2015a). A few species were isolated from one region only. The number of distinct phylogenetic species identified among the isolates of the common phylloplane-related phenotypic species *Cr. albidus*, *Cr. laurentii*, and *Rhodotorula minuta* ranged from two to seven. The vast majority of isolates were identified to a very few phylogenetic species. *Filobasidium wieringae* and *F. magnum* accounted for more than 90% of strains identified from growth tests as phenotypic *Cr. albidus*. Molecular reassessment of the strains identified about 90% of phenotypic *Cr. laurentii* isolates as *Vishn. victoriae* and *Vishniacozyma tephrensis*. All but one isolate of phenotypic *Rh. minuta* was identified as *Cystobasidium slooffiae*. Other phylogenetic species were isolated as single strains. Identification of soilborne yeasts with growth tests was consistent with rDNA sequencing results except for *Na. albida*, which had previously been misidentified as *Cryptococcus diffluens*. Similarly, resampling and sequence-based identification of the yeast community of the Taymyr tundra confirmed that *Vishn. victoriae* is the prevalent species and not the phenotypic species *Cr. laurentii* (Glushakova et al. 2013).

The population structure of yeast species in relation to isolation locality has been studied with fingerprinting techniques such as RAPD and MSP-PCR (e.g. Naumova et al. 2000; Fernández-Espinar et al. 2003; Ganter et al. 2004; Yurkov and Chernov 2005). Both techniques use primers that target sites that are not associated with specific genes. The result, usually visualised by gel electrophoresis, is a profile of several DNA fragments of different sizes and intensities that can be unique to individual strains. Electrophoretic profiles can be analysed to generate a measure of genetic similarity between closely related isolates. For example, when characterised with several primers and principal components analysis, the common cactophilic yeasts *P. cactophila* and *C. sonorensis* gave patterns where geographic location was the main predictor of RAPD variation (reviewed by Ganter 2011). The strains of *P. cactophila* were isolated from Florida, Antigua, Argentina, Australia, and the Sonoran Desert, and the distance effect was strong enough to separate strains from North and South Florida. Similarly, variation in both DNA karyotypes and RAPD profiles of *C. sonorensis* was correlated with geographical distance between *Opuntia* roots in Australia, Florida, and Texas (Ganter et al. 2004).

PCR fingerprinting profiles were correlated with geographic location in isolates from birch forests in Western Siberia and in the Moscow region (Yurkov and Chernov 2005; Yurkov et al. 2015a). The forests of Moscow region, first studied by Maksimova and Chernov (2004), and those of Novosibirsk (Western Siberia) were similar in climatic conditions (average summer and winter temperatures, rainfall), soil type, and vegetation (dominating plant species). Yeast community composition as determined from conventional keys were notably alike, even though the two sampling regions were 3500 km apart. Strains of the ascomycetous yeasts *Debaryomyces hansenii*, *Hanseniaspora guilliermondii*, and *Torulaspora delbrueckii* clustered by locality when characterised by micro- and minisatellite primer profiles (Yurkov and Chernov 2005). The effect of geographic distance on MSP-PCR pattern divergence was not as strong in basidiomycete species (Yurkov et al. 2015a). Three different types of distribution were found: (a) variability linked to geographical factors (*Curvibasidium cygneicollum*, *Sa. podzolica*, *Vishn. victoriae*), (b) identity across regions (*Solicoccozyma terricola*, *Cystobasidium pinicola*), and (c) high variability that is uncorrelated to sampling region (Filobasidiales: *Na. albida*, and *F. magnum*).

4.5 Temporal Changes: Succession

Ecological succession is the process of change in community composition and structure with time. Succession may be initiated by the formation of a new, unoccupied habitat such as a tree flux, a fruit, or a flower. Alternatively, the succession can start with a modification of the existing environment and its properties or composition, due either to intrinsic factors such as leaf ageing or to the effects of early-arriving species (e.g. priority effects by nectar yeasts) and nutrient depletion (e.g. decomposing activities of fungi and bacteria in litter). It is

important to document that the largest body of evidence concerns long-term patterns that were made on discrete observations and reported dissimilarities of yeast population made after a rather prolonged time, e.g. seasonal changes. Studies designed to monitor continuous changes in a yeast habitat are rare. Successional stages of yeast communities have been observed in slime fluxes (Phaff et al. 1964; Golubev et al. 1977), amapa fruit (Morais et al. 1995), fermentations (Morais et al. 1997; Schwan and Wheals 2004), and degrading leaf litter and wood (Gonzalez et al. 1989; Yurkov et al. 2008). Each of these successions has its own trigger and mechanisms underlying the alteration of the substrate (e.g. availability of nutrients, pH) and the community (e.g. insect vectors). The succession starts with the rapid depletion of simple sugars by fast-growing (and often fermenting) yeasts, which may not experience much competition during the initial phase. Chemical modification of the substrate and increasing cell density in the intermediate phase stimulate killer activity, protecting the community from arriving transient species (e.g. Morais et al. 1995, 1997). Widespread species with broad physiological capabilities (often basidiomycete yeasts) gain more importance in later phases when nutrients are heavily depleted.

Surveys of soil yeasts started in the Soviet Union in the late 1950s and continued in the 1960s by Babjeva and co-workers provided fairly good overview on communities of major soil types (e.g. Babjeva and Golovleva 1963). It is important to note that the evidence for soil origin of many isolated species was insufficient at that time. Also, it has been repeatedly argued that most of yeasts found in soils are not immanent but originate from aboveground sources (e.g. discussed by Danielson and Jurgensen 1973). Thus, the studies conducted in the 1970s–1980s often included the aboveground substrates such as living plants and litter to consider an outside pool of yeasts entering soils (e.g. Babjeva et al. 1973, 1976; Chernov 1985; Vinovarova and Babjeva 1987). Babjeva, Chernov, and co-workers dedicated much of their studies refining the approach, which they later called spatial succession or, closer to the original translation, spatio-successional series (for a review in English, see Babjeva and Chernov 1995). They followed yeast community composition in substrates that represent successional stages of the decomposition of plant material, i.e. green leaves, senescent leaves, fresh litter, topsoil, and mineral soil horizons. Thus, even when yeasts are surveyed at a single time, these substrates would give the impression of a series of changes in the yeast community associated with plant decomposition on a discrete timescale. The approach resembles the chronosequence, a set of experimental plots of different ages, used in forest science to study plant communities. Considering both morphology (e.g. dimorphic growth, ballistospores, chlamydospores) and the spectrum of assimilated carbon and nitrogen sources, the yeasts typically associated with each type of substrate were arranged into functional guilds such as phylloplane-related, litter-inhabiting, and soilborne species. Using the spatio-successional series approach, the composition of yeast communities typically found in tundra, forest, steppe, and desert biotopes of the territory of the former USSR was characterised (Babjeva and Chernov 1995; Chernov 2005). Yeast population sizes usually increased with plant senescence and decay; non-lignified plant parts, dry but still attached, yielded the highest yeast cell

counts. This trend became increasingly pronounced in dry climates (steppe and subtropical deserts), where the population density on dry plants was sometimes as high as that of fruits (Babjeva and Chernov 1995; Chernov 2005). The yeast communities of the phylloplane of living plants and of dry material were alike and were represented by members of the genera *Cystobasidium* (cited as *Rh. minuta*), *Dioszegia* (e.g. *Dioszegia hungarica*), *Filobasidium* (cited as *Cr. albidus*), *Leucosporidium* (e.g. *Leuc. scottii*), *Rhodotorula* (e.g. *Rhodotorula babjevae*, *Rhodotorula mucilaginosa*), *Sporobolomyces* (e.g. *Sp. roseus*), and *Vishniacozyma* (cited as *Cr. laurentii*). Yeast numbers decreased in the litter, and the community still contained a high proportion of phylloplane-related species. Other yeasts were dimorphic basidiomycetes of the genera *Apiotrichum* (e.g. *Trichosporon porosum*), *Curvibasidium* (e.g. *Rhodotorula fujisanensis*), *Cystofilobasidium* (e.g. *Cystofilobasidium capitatum*, *Cystofilobasidium macerans*), and *Tausonia* (*Trichosporon pullulans*). Yeast numbers and diversity both dropped in the deeper litter layers closer to the topsoil. The final stage in the spatio-successional series is represented by deeply decayed organic matter in topsoils and mineral soil layers. These communities are characterised by a low population density, an uneven distribution in space, and species-poor assemblages consisting largely of yeasts of the genera *Lipomyces* (e.g. *Lipomyces starkeyi*), *Nadsonia* (*Schizoblastosporion starkeyi-henricii*), *Naganishia* (e.g. *Cr. diffluens*), *Saitozyma* (e.g. *Cr. podzolicus*), and *Solicoccozyma* (e.g. *Cryptococcus aerius*, *Cryptococcus terricola*). Taking into account changes in community structure (i.e. evenness and species abundance distribution) and the increasing proportion of polytrophic species (i.e. yeasts with broad physiological capabilities), the authors concluded that nutrient depletion (especially availability of simple sugars) is the main driver of the plant-litter-soil yeast succession.

Another example of the analysis of temporal changes made using substrates that represent successional stages is the study of yeast communities associated with ephemeral flowers on Hawaii (Lachance et al. 1989). Soon after opening, the short-lived flowers are visited by a number of insects. The same authors surveyed flowers that had been tagged as buds so that their age would be known or bagged as buds to ensure that they would open without being visited by insects. Bagged flowers as well as flowers open for a certain period of time (or number of visits) have been also used in studies addressed at plant-yeast-pollinator interactions (e.g. Vannette et al. 2013; Mittelbach et al. 2016). This method is useful to detect yeast species vectored by insects.

Epiphytic microbial populations, including both bacteria and fungi, depend on many intrinsic (e.g. abundance and composition of plant exudates) and extrinsic (e.g. temperature, humidity, solar radiation) factors. These parameters undergo seasonal and ontogenetic changes, thus causing pronounced temporal shifts in the species composition of phylloplane communities (Fonseca and Inácio 2006). Studies that evaluated the dynamics of yeast populations on different plant types provided evidence for the increase of population sizes with leaf age. In particular, a continuous increase in population size from spring to autumn and winter was the general rule with a few exceptions, which are reviewed by Fonseca and Inácio

(2006). Seasonal dynamics of epiphytic yeasts on 25 plant species confirmed the trend and showed that the total yeast population increased from spring to autumn followed by a decline during the winter months (Glushakova and Chernov 2007). The increase in population size later in the growing season was attributed to plant ontogenesis (leaf senescence) rather than environmental factors, as the same pattern was also observed for ephemeral plants, e.g. *Ficaria verna* and *Impatiens noli-tangere*. A notable deviation from the rule was observed on evergreen plants (xerophilic herbs and conifers), where the yeast population remains stable through the year. In contrast to other conifers, the quantity of epiphytic yeasts on the deciduous conifer *Larix decidua* rapidly increased with leaf senescence in the fall. Despite the pronounced effect of plant ontogenesis on yeast populations, a positive impact of the more favourable environmental factors that prevail in autumn, such as higher humidity and milder temperatures, cannot not be ruled out for other ecosystems (Fonseca and Inácio 2006). Another prevalent trend in many of the studies reviewed by Fonseca and Inácio (2006) was the increase in species richness at the end of the growing season. This trend was also observed by Glushakova and Chernov (2010), based on Shannon's diversity index values. The distribution of single species varied substantially during the year, and the peak relative abundances of many yeasts did not coincide. The proportion of ascomycetous yeasts in the community increased from spring to autumn followed by a decline during the winter months. At the species level, *Candida oleophila* and *Kazachstania barnettii* were more prominent in autumn and winter, respectively (Glushakova et al. 2007; Glushakova and Chernov 2010). The abundance of basidiomycetous species did not change throughout the year. The only significant shift was the increased proportion of the 'phenotypic' *Cr. laurentii*, which was made even with *Vishn. victoriae* in that study (Glushakova and Chernov 2010). Conversely, a later investigation suggested that yeasts identified as *Cr. laurentii* were likely to comprise several species of the genus *Vishniacozyma* and not only *Vishn. victoriae* (Yurkov et al. 2015a). Therefore, it remains unclear whether or not basidiomycetous yeasts undergo temporal changes in phylloplane communities.

In soils, temporal changes have been reported repeatedly based on discrete samplings, and a continuous increase in population size from spring to autumn was the most commonly reported trend (e.g. Starkey and Henrici 1927; Jensen 1963; Slávikovᡠand Vadkertiovᡠ2000). The only study focused on short-term seasonal dynamics of yeast soil communities has been performed on the rhizosphere of *Ajuga reptans* and *Taraxacum officinale* in a birch forest on podzolic soil (Golubtsova et al. 2007). Similarly to the phylloplane study (Glushakova and Chernov 2007), identification was based on growth tests. Although dominant soilborne species such as *Sol. terricola* and *Sa. podzolica* can be identified using that approach (e.g. Yurkov et al. 2015a), other identifications in that study are questionable. Unlike plant surfaces, soils and the rhizosphere harboured a rather homogeneous yeast population, and belowground seasonal dynamics were not as pronounced as aboveground (Golubtsova et al. 2007). The authors did not confirm the influence of root exudates on yeast population densities in soils and reported a meagre increase in yeast numbers during the active growth period. Interestingly,

phylloplane-related yeasts successfully colonised root surfaces during the period of active plant growth. During the growing season, the proportion of the epiphytic species increased on root surfaces but not in soils.

4.6 Concluding Remarks

Much of this chapter was directed at showing the potential use of ecological tools aimed at putting single observations into a larger context and exploring distribution patterns of yeasts in the same manner that it has been done for larger organisms. The distribution of yeasts in natural habitats is anything but random. Some species rely on dispersal by insect vectors, and others grow under highly selective environmental conditions. Therefore, the correct interpretation of species occurrence in an environment, with or without culturing, should take into account the yeasts' physiological abilities (autoecology), sexuality, dispersal modes, and potential interactions with other organisms. Likewise, a better understanding of the relevant properties and history of a habitat is essential to define the niche of yeast species and reveal their role in the environment. By virtue of the ease with which they can be handled in the laboratory, yeasts are amenable to the application of macroecological (biogeography and landscape ecology) tools to microscopic living objects.

I have shown that the distribution patterns of basidiomycetous yeasts, although documented, are less well understood. Yeasts that are presumed to be ubiquitous often show a rather patchy distribution and complex intraspecific structure. Whether ubiquitous, globally distributed yeast species do in fact exist is unclear at best. Reports of ubiquity may be the result of inadequate identification. The distribution patterns of closely related and phenotypically indistinguishable (cryptic) yeast species in their natural habitats are worthy of further study. Last but not least, further studies of dispersal mechanisms and survival outside the primary habitat are appropriate for both ascomycetous and basidiomycetous yeasts.

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Chapter 5
Biogeography and Ecology of the Genus *Saccharomyces*

José Paulo Sampaio and Paula Gonçalves

Abstract The genus *Saccharomyces* is intimately associated with alcoholic fermentations conducted by humans. Its most emblematic species, *Saccharomyces cerevisiae*, represents not only an important case of microbe domestication, exemplified by products such as wine, beer, and bread, but also a case of complex and yet poorly understood ecological adaptations and interactions in natural systems. Moreover, the long coexistence with humans in wineries and breweries has fostered anthropocentric concepts that are difficult to eradicate. Here we critically review, using a historical perspective, recent findings on the natural ecology and biogeography of the different species that form the genus *Saccharomyces*.

Keywords *Saccharomyces* • Ecology • Biogeography • Population genomics • Microbe domestication

Contents

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5.1 Introduction

The genus *Saccharomyces* was created in the first half of the nineteenth century and has endured innumerous changes that epitomize the turbulent history of yeast taxonomy. In the 1970s, it attained its broadest circumscription, labeled *Saccharomyces sensu lato*, and included 41 species. Those species closely related to *Saccharomyces cerevisiae* constituted the so-called *Saccharomyces sensu stricto* (van der Walt 1970). In the 1980s the species of the *sensu stricto* group were merged into a single taxon, *S. cerevisiae* (Yarrow 1984). When a few years later this concept was revised, four species were recognized (*S. cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces pastorianus*, and *Saccharomyces paradoxus*) (Vaughan-Martini and Kurtzman 1985; Martini 1989). This monophyletic group was enlarged by Naumov et al. (2000) who described *Saccharomyces cariocanus* (now viewed as representing a population of *S. paradoxus*), *Saccharomyces kudriavzevii*, and *Saccharomyces mikatae*. The genus received two new additions when researchers in China isolated strains associated with oak trees that they named *Saccharomyces arboricola* (Wang and Bai 2008) and when a natural population associated with *Nothofagus* (Southern beech) was found in Patagonia, Argentina, and described as *Saccharomyces eubayanus* (Libkind et al. 2011).

In the last two decades, whole genome analyses have provided a more detailed understanding of species delimitations (e.g., Cliften et al. 2003; Kellis et al. 2003; Liti et al. 2009) and have demonstrated the hybrid nature of some of them (Dunn and Sherlock 2008; Nakao et al. 2009; Libkind et al. 2011). As summarized in Table 5.1, the genus presently includes seven natural species (*S. cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. kudriavzevii*, *S. arboricola*, *S. eubayanus*, and *Saccharomyces uvarum*), together with two artificial and hybrid species exclusively associated with human-made fermentative environments (*S. pastorianus* and *S. bayanus*).

5.2 Wild and Domesticated

The genus *Saccharomyces* is intimately associated with alcoholic fermentations conducted by humans. Not only did the first cultures originate from well-known alcoholic beverages, but also early scientific research aimed almost exclusively at understanding the roles that these yeasts played in those fermentations. Therefore, it comes as no surprise that fruits, wineries, and breweries were viewed as the natural environments of *Saccharomyces*. However, a different range of environments

Table 5.1 Presently recognized species in the genus *Saccharomyces* and key features of their ecology and biogeography

Species	Ecology	Biogeography
Saccharomyces arboricola	Associated with Fagales (*Quercus, Cyclobalanopsis, Juglans, Nothofagus*)	Asia (China, Taiwan) and Australasia (New Zealand)
Saccharomyces bayanus	Domesticated; associated with the brewing environment; no natural ecology	Most strains were isolated in Europe
Saccharomyces cerevisiae	Wild and domesticated species. Most wild strains are associated with Fagaceae (*Castanea, Castanopsis, Cyclobalanopsis, Lithocarpus, Quercus*), but other possible sources are other families of Fagales (Betulaceae and Juglandaceae) and noncultivated fruit trees	Wild strains from Asia (China, Japan, Malaysia, the Philippines), Europe, North and South America
Saccharomyces eubayanus	Associated mainly with *Nothofagus*	South and North America, Asia (China), and possibly Australasia
Saccharomyces kudriavzevii	Associated with Fagaceae, mostly *Quercus* spp.	Asia (Japan, Taiwan) and Southern Europe
Saccharomyces mikatae	Possibly associated with Fagaceae (or Fagales)	Asia (China and Japan)
Saccharomyces pastorianus	Domesticated; associated with the brewing environment; no natural ecology	Most available strains were isolated in Europe
Saccharomyces paradoxus	Associated with Fagaceae, mostly *Quercus* spp.	Eurasia and North America; possible distribution in South America needs to be confirmed; European strains were detected in New Zealand, but human-aided dissemination is likely
Saccharomyces uvarum	Mostly a wild species associated mainly with *Nothofagus*; includes domesticated wine and cider strains isolated in Europe	South and North America, Eurasia, and Australasia

gradually emerged as a *Saccharomyces* habitat—temperate forest soil, particularly near oak trees (*Quercus* spp.), and the exudates of these trees. Although the taxonomy of the *Saccharomyces* representatives from such environments was contentious until *S. paradoxus* was established as an independent species (Martini 1989), early isolations date from 1914 (Bachinskaya 1914) and were further expanded in the 1950s and 1960s through the work of Capriotti, among others, and by Naumov and collaborators in the 1980s and 1990s who isolated mostly *S. paradoxus*, but also *S. cerevisiae* and *S. uvarum* from oak exudates.

In the case of *S. cerevisiae*, its association to fermented beverages, and its scarcity in other substrates, including vineyards and healthy grapes, gave rise to the notion that this species was absent in natural environments, being a purely

domesticated organism whose habitat was the wine and the winery (Martini 1993; Vaughan-Martini and Martini 1995). Consequently, any strain found in a natural environment was viewed as a domesticated strain that escaped its original habitat (Naumov et al. 1992). *S. paradoxus*, on the contrary, exemplified a wild yeast that is never found in man-made fermentations (Vaughan-Martini and Martini 1995).

A different perspective was proposed by Fay and Benavides (2005), who analyzed a diverse group of *S. cerevisiae* strains and showed that wine strains could be separated from sake strains, an indication of two independent domestication events. Moreover, wild strains isolated from oaks in North America were placed at the base of the phylogenetic tree of *S. cerevisiae*, suggesting that the domesticated groups, which had also lower genetic diversity, derived from their wild relatives. The model that emerged accommodated wild populations occupying niches that were sufficiently ancient to be compatible with the estimated age of *S. cerevisiae*, 5–10 (Kellis et al. 2003) or 0.4–3.4 million years (Liti et al. 2006). Hence, there is a dramatic difference between the age of domesticated lineages of *S. cerevisiae*, which are probably not older than 10,000 years (a rough estimate of the first human-driven fermentations), and the age of the species itself.

5.3 Generated from Domestication

As shown in Table 5.1, two species, *S. pastorianus* and *S. bayanus*, are entirely artificial. They have not been found in natural environments, i.e., in substrates with null or restricted human influence. Both species are intimately associated with beer and with the brewing environment, and it is very likely that they were formed as a consequence of the peculiar selection regimes associated with brewing. *S. pastorianus*, an allopolyploid hybrid of *S. cerevisiae* and *S. eubayanus* (Dunn and Sherlock 2008; Nakao et al. 2009; Libkind et al. 2011), ferments lager beer. *S. bayanus*, once thought to be a natural species and the non-cerevisiae progenitor of *S. pastorianus* (Vaughan-Martini and Kurtzman 1985; Turakainen et al. 1994; Montrocher et al. 1998; Nakao et al. 2009; Bond 2009), is in fact a complex hybrid with contributions from *S. cerevisiae*, *S. eubayanus*, and *S. uvarum* (Libkind et al. 2011). The absence of these two species in natural environments and their hybrid genomes are eloquent manifestations of selection in an artificially created environment. Hybrid genomes associated with *Saccharomyces* domestication have also been revealed for strains that ferment wine at low temperatures (González et al. 2006) and for some Belgian beer strains (González et al. 2008). Such hybrids include contributions from two cryotolerant species, *S. kudriavzevii* and *S. uvarum*. In one case, the *S. cerevisiae* x *S. kudriavzevii* wine hybrid produces larger amounts of a fruity thiol than non-hybrid commercial strains (Borneman et al. 2012). Another case of interspecies hybridization fostered by fermentations conducted by humans was observed for European *S. uvarum* wine and cider strains. Introgressions from *S. eubayanus* were found to be frequent in *S. uvarum* strains

obtained from those fermentations, whereas they were rare in strains isolated from natural environments (Almeida et al. 2014).

5.4 Wild and Never (or Little) Domesticated

Most members of the genus *Saccharomyces* can be viewed as purely wild species. This group includes five species, *S. arboricola*, *S. eubayanus*, *S. kudriavzevii*, *S. mikatae*, and *S. paradoxus*. Excluded from this group are the two artificial species mentioned in the previous section and *S. cerevisiae* that includes wild and a significant fraction of extensively domesticated strains. A borderline species is *S. uvarum*, for which a peculiar case of domestication of wine and cider strains has been documented (Almeida et al. 2014). For the sake of simplicity, *S. uvarum* is also treated in this section, but here we consider only strains from natural environments.

The samples from which the majority of strains of each of the species in this group have been isolated can be related to *Quercus* spp. in all cases except for *S. eubayanus* and *S. uvarum*, for which most strains are linked to *Nothofagus* spp. (Table 5.2). Throughout this chapter we use the designation "association with *Quercus*" as one of convenience. In fact, this association appears to be both broader, encompassing the family Fagaceae that besides *Quercus* includes also *Castanea* (chestnut) and *Fagus* (beech), among seven other genera, and narrower since not all *Quercus* are a preferred *Saccharomyces* habitat (Sampaio and Gonçalves 2008; Sampaio, unpublished data). Since the number of field studies is limited, the understanding of the ecology and biogeography of *Saccharomyces* in the wild progresses slowly, but a preliminary picture is emerging. The literature records on the occurrence of *Saccharomyces* in strict natural environments, i.e., in environments that have not been created by humans and that are likely to have been slightly affected by human activities (thus excluding, e.g., cultivated vineyards), show two patterns of distribution of *Saccharomyces* species. The first group includes the most basal species, *S. eubayanus* and *S. uvarum*, which have a primary distribution in the Southern Hemisphere characterized by a relatively high frequency of isolation, considerable genetic diversity, and an association with *Nothofagus*. The association with *Nothofagus* is interesting because this genus shares several features with *Quercus*. Both genera belong to the order Fagales, *Nothofagus* occupying the most basal position in the phylogenetic tree of the Fagales (Li et al. 2004), and both are typical of temperate zones, *Quercus* in the Northern Hemisphere and *Nothofagus* in the Southern Hemisphere. Large numbers of isolates, suggesting a relatively high frequency in the analyzed samples, were obtained in Patagonia for *S. eubayanus* and *S. uvarum* (Table 5.2). Although both species have been detected also in other regions, they were recovered in much lower numbers, which suggests that *S. eubayanus* and *S. uvarum* are less frequent outside South America.

Table 5.2 Relevant reports on the isolation of *Saccharomyces arboricola*, *Saccharomyces eubayanus*, *Saccharomyces kudriavzevii*, *Saccharomyces mikatae*, and *Saccharomyces uvarum* from natural environments

Species	Reference	Details on the substrate of isolation	Geographic distribution
Saccharomyces arboricola	Wang and Bai (2008)	3 strains from bark of *Quercus fabri* (1) and *Castanopsis orthacantha* (2)	Qiling Mountains, Shaanxi Province, West China, 2006
	Wang and Bai, unpublished data	**47 strains isolated from bark of *Carpinus turczaninowii* (2), bark of *Castanea henryi* (1), bark of *Corylus mandshurica* (1), bark of *Cyclobalanopsis jenseniana* (8), bark of *Cyclobalanopsis myrsinifolia* (7), bark of *Juglans cathayensis* (5), bark of *Quercus cocciferoides* (5), bark of *Quercus* sp. (1), bark of unidentified tree (9), fruit (1), rotten wood (7)**	**Liupanshan Mountain, Ningxia Province, West China, 2011 (6 strains); Qiling Mountains, Shaanxi Province, West China, 2012 (36 strains); Hubei Province, Middle-China, 2012 (5 strains)**
	Naumov et al. (2013)	1 strain isolated from the fruiting body of a basidiomycete (*Auricularia polytricha*)	Sioulin, Hualein, Taiwan, 2010
	Gayevskiy and Goddard (2016)	10 out of 731 isolates of yeasts and bacteria obtained from 442 fruit, soil, and bark samples	North Island, New Zealand, 2012
	Sampaio, unpublished data	1 strain from *Cyttaria nigra* on *Nothofagus menziesii*; total of 113 samples of *Nothofagus* spp. (bark, soil, stromata of *Cyttaria* spp.)	New Zealand
Saccharomyces eubayanus	Libkind et al. (2011)	**62 strains from bark of *Nothofagus* spp. (29), from soil underneath *Nothofagus* spp. (20), and from the stromata of its biotrophic fungal parasite *Cyttaria hariotii* (13); total of 133 samples**	**South America, Patagonia, Argentina**
	Bing et al. (2014)	**27 strains from approx. 1400 samples—bark of *Quercus* spp. (11), rotten wood (11), bark of *Rosa* sp. (2), bark of *Juglans cathayensis* (1), bark of *Salix hypoleuca* (1), unknown (1)**	**Tibet, Qinghai, Shaanxi and Sichuan provinces, China**

(continued)

Table 5.2 (continued)

Species	Reference	Details on the substrate of isolation	Geographic distribution
	Peris et al. (2014)	**18 strains from bark of *Nothofagus* spp. (9), from soil underneath *Nothofagus* spp. (4), and from the stromata of its biotrophic fungal parasite *Cyttaria hariotii* (5)**	**South America, Patagonia, Argentina**
	Peris et al. (2014)	3 strains from bark of *Fagus grandifolia* (2) and *Acer saccharum* (1)	North America, Wisconsin State, USA
	Rodríguez et al. (2014)	9/8 strains from *Araucaria araucana* (seeds and bark) from isolations carried out at 10 °C/30 °C, respectively; total of 120 samples	South America, Patagonia, Argentina
	Peris et al. (2016)	7 strains from bark of *Cedrus* sp., bark and soil of *Pinus taeda*, and bark of *Quercus rubra*	North America—Washington State, USA (1), North Carolina, USA (2), New Brunswick, Canada (4)
	Gayevskiy and Goddard (2016)	1 out of 731 isolates of yeast and bacteria obtained from 442 fruit, soil, and bark samples	North Island, New Zealand, 2012
Saccharomyces kudriavzevii	Sampaio and Gonçalves, (2008)	**15 strains from bark of *Castanea sativa* (2) and *Quercus* spp. (13); total of 61 samples that included also *Fagus sylvatica***	**Portugal, 2005–2006**
	Lopes et al. (2010)	5 strains from bark of *Quercus* spp.; total of 56 samples	Spain, 2008
	Polona Zalar, unpublished data	10 strains from oak bark	Slovenia, 2010–2011
	Sampaio, unpublished data	**65 strains from bark of *Quercus* spp.**	**Portugal, Spain, Greece and Japan, 2007–2012**
Saccharomyces mikatae	Wang and Bai, unpublished data	**31 strains isolated from bark of *Castanea mollissima* (2), bark of *Ulmus pumila* (2), bark of *Diospyros kaki* (2), bark of *Quercus wutaishanica* (1), bark of unidentified trees (13), soil under *Juglans cathayensis***	**Beijing, North China, 2008 and 2011 (22 strains); Taishan Mountains, Shandong province, North China, 2008 (1 strain); Wuyi Mountains, Fujian province, 2009 (1 strain); Fanjingshan, Guizhou**

(continued)

Table 5.2 (continued)

Species	Reference	Details on the substrate of isolation	Geographic distribution
		(3), soil under *Betula platyphylla* (2), soil (6)	**Province, Southwest China, 2011 (3 strains); Qiling Mountains, Shaanxi Province, West China, 2012 (2 strains); Hubei Province, Middle China, 2012 (2 strains)**
Saccharomyces uvarum	Libkind et al. (2011)	**47 strains from bark of *Nothofagus* spp. (26), from soil underneath *Nothofagus* spp. (10), and from the stromata of its biotrophic fungal parasite *Cyttaria hariotii* (11); total of 133 samples**	**South America, Patagonia, Argentina**
	Almeida et al. (2014)	**59 strains from *Nothofagus* spp. (bark, soil, stromata of *Cyttaria hariotii*); total of 218 samples**	**South America, Patagonia, Argentina**
	Almeida et al. (2014)	8 strains from *Nothofagus* spp. (bark, soil, stromata of *Cyttaria* spp.); total of 113 samples	Australasia, New Zealand, and Tasmania

The most expressive results are given in boldface

For *S. uvarum*, a comprehensive phylogeographic survey that analyzed whole-genome data of strains from North and South America, Eurasia, and Australasia detected a much higher genetic diversity in South America (Almeida et al. 2014). Moreover, whereas in South America two 1% divergent populations were found, all Holarctic strains could be assigned to one of these populations. Therefore, it appears that migrants from South America dispersed to North America and Eurasia and that the low genetic diversity of Holarctic *S. uvarum* is compatible with a founder effect based on a small subpopulation (Almeida et al. 2014). Interestingly, *S. uvarum* was also detected in Australasian *Nothofagus* surveyed in New Zealand and Tasmania. However the Australasian isolates were 4.4% divergent from their South American relatives and showed partial reproductive isolation, thus suggesting limited gene flow and incipient speciation between the Australasian and South American populations (Almeida et al. 2014).

A remarkably similar scenario has been recently proposed for *S. eubayanus*. In Patagonia, two 1% divergent populations were detected (Patagonia A and Patagonia B) (Peris et al. 2014). Infrequent isolations in North America correspond to

members of Patagonia B population, to admixed genotypes of the two Patagonian populations, and to a member of the Tibet lager population (Peris et al. 2016). It appears that the Patagonian B population contributed to the expansion of *S. eubayanus* to other regions since genetically close representatives have been found in New Zealand and in Tibet. In Asia, another two more divergent populations (Sichuan and West China populations) were detected (Bing et al. 2014). Like the Australasian populations of *S. uvarum*, they might represent emerging species in the *S. eubayanus* complex (Peris et al. 2016).

S. eubayanus and *S. uvarum* have their diversity hotspots in the Southern Hemisphere and in association with *Nothofagus*, *S. arboricola*, *S. kudriavzevii*, *S. mikatae*, and *S. paradoxus* appear to have their radiation centers in the Northern Hemisphere and so far have been isolated mostly or entirely from this part of the world. *S. arboricola* was originally found in China, and most strains known come from this region, but this species has also been isolated in New Zealand (Table 5.2) (Gayevskiy and Goddard 2016). Analyses of genetic diversity and population structure have not been performed yet. Most Chinese strains appear to be associated with *Quercus*, whereas an association with *Nothofagus* might occur in New Zealand. Another species that appears to be native to Asia is *S. mikatae* as all strains known to date come from China and Japan and, again, an association with *Quercus* seems plausible (Table 5.2). *S. kudriavzevii* was originally described based on two collection strains isolated in the 1970s (Naumov et al. 2000). The study of two additional strains from the same collection suggested the existence of two Japanese populations exhibiting considerable genetic divergence (~4%), both of which lost their GAL utilization pathway (Hittinger et al. 2010). A third population was later uncovered in Europe (Sampaio and Gonçalves 2008). Interestingly, although *S. kudriavzevii* is among the *Saccharomyces* species exhibiting the lowest maximum growth temperature (Gonçalves et al. 2011; Salvadó et al. 2011), it occurs in Southern Europe often in sympatry with *S. cerevisiae*, and it is likewise not found in Northern Europe. Surprisingly, the European population of *S. kudriavzevii* was found to have a functional GAL pathway. Hence the GAL pathway status seems to be a balanced polymorphism in *S. kudriavzevii*, likely to result from divergent local adaptation in Asia and in Europe. An association with oak trees is also plausible for this species, which has not been found so far in the Southern Hemisphere and in North America.

Among the *Saccharomyces* species known solely (or mostly) from wild populations, *S. paradoxus* is the one for which most information on biogeography and population structure has been obtained. It appears that low frequency of dispersion, isolation by distance, and ecological specialization have contributed to fragment the species into five populations that tend to be genetically isolated from each other and may be at an early stage of speciation. First, three allopatric populations were recognized in Far East, Europe, and America, with a candidate fourth population represented by a single strain from Hawaii (Liti et al. 2006, 2009). Subsequent studies revealed that several populations coexisted in North America. They were designated as North American populations A, B, and C, the representatives of population A being identical to European strains and viewed as recent

migrants to North America (Kuehne et al. 2007). More recent studies have confirmed the existence of multiple populations on North America, with groups B and C displaying a north–south gradient, the latter being mainly found in colder regions (Leducq et al. 2014). Whereas population B exhibits the broadest distribution in North America, having even been directly (Naumov et al. 2000) or indirectly (Barbosa et al. 2016) detected in South America (Brazil), an additional lineage, sister to population C, showed little genetic admixture with other populations and a narrow geographic distribution (Leducq et al. 2016). Robinson et al. (2016) compared the isolation frequency of *S. paradoxus* in Europe and observed that regions from southern Europe located in Portugal, Spain, France, and Greece yielded more *S. paradoxus* than regions in Northern Europe. They also observed that the optimum summer temperature for this species appears to be between 22 and 28 °C. In the same study, a worldwide literature survey for *S. cerevisiae* exposed the likely basis for partial sympatry with *S. paradoxus* since the majority of *S. cerevisiae* isolates are mapped to locations with summer temperatures of 25–38 °C. Therefore, in Europe, Asia (China), and North America while both species are present in intermediate temperature zones, *S. paradoxus* is the sole colonizer of cooler zones, while *S. cerevisiae* occurs preferably in warmer regions within the approximate range of summer temperatures mentioned above.

5.5 Niche or No Niche and the Uniqueness of *S. cerevisiae*

As discussed above, it seems reasonable to conclude that for most *Saccharomyces* species, their natural ecology revolves around the oak/Fagaceae (or *Nothofagus*) system. Although this model is widely accepted for non-*S. cerevisiae* species (Boynton and Greig 2014), a contrasting view has been proposed for this latter species. It has been suggested that *S. cerevisiae* is a nomad microbe with no niche (Goddard and Greig 2015), a proposal that is reminiscent of the model of the purely domesticated microbe with no natural ecology (Martini 1993; Vaughan-Martini and Martini 1995). The proponents of the nomad microbe model argued that in its truly natural niche *S. cerevisiae* should be abundant and therefore easily isolated without the need for enrichment culture (Goddard and Greig 2015). In fact, except for spontaneous wine fermentations and related artificial substrates, it appears that *S. cerevisiae* does not occur in cell densities that would make its direct isolation straightforward. For example, in vineyards, intuitively considered by many to be the natural *S. cerevisiae* habitat (e.g., Török et al. 1996; Pretorius 2000; Stefanini et al. 2012), *S. cerevisiae* is far from abundant. In undamaged grape berries, the frequency of *S. cerevisiae* is less than 0.1%, although in berries damaged by birds or insects, the frequency increases considerably to ~24% (Landry et al. 2006). The difficulty in finding *S. cerevisiae* outside wine fermentations led to the use of selective enrichment media for its isolation. These isolation procedures include the use of ethanol as a selective agent and anaerobic conditions. However, these procedures do not differ from those adopted for the isolation of the other

Saccharomyces species from oaks, namely, the most paradigmatic wild species, *S. paradoxus*, whose association with the oak niche is not questioned because of that (e.g., Kowallik and Greig 2016; Boynton et al. 2017). In fact, although some situations are consistent with that model, modern microbiology is probably richer in instances that dismiss it. For example, yeast communities (see Chap. 3 of this book) or, more broadly, soil or marine microbes form communities that normally include a few abundant and many rare species. Moreover, considering the approximately 2000 known yeast species, only a minority fulfill the prerequisites of the so-called adaptation model that postulates that organisms adapted to a niche should be abundant in that niche (Goddard and Greig 2015). Therefore, according to these criteria, most yeast species should be considered nomad species.

In addition, it is worth noting that like all other *Saccharomyces* species and indeed like many other yeast species (e.g., Hanson et al. 2014), *S. cerevisiae* is capable of mating-type switching which is thought to constitute a means to facilitate sexual reproduction to individuals in low-density populations. If this biological imprint of adaptation to low cell density is taken into account, it seems reasonable to assume that *S. cerevisiae* and other *Saccharomyces* species are adapted to live in environments where cell densities are low, compatible with low levels of nutrients.

In some oak environments, *S. cerevisiae* is as frequently isolated as *S. paradoxus*, as what happens in Southern Europe, or even at higher frequencies, as we observed in Japan (Sampaio, unpublished data). It was also suggested that the oak association proposed for *S. cerevisiae* might be just a consequence of oaks, not other trees, having been sampled (Goddard and Greig 2015). However, not only have other trees indeed been sampled and the results supported the oak association model (Kowallik and Greig 2016, Sampaio unpublished data), but an unparalleled genetic diversity has been revealed when the oak association was explored (Wang et al. 2012; Almeida et al. 2015), thus indicating the relevance of such association for the life history of *S. cerevisiae*.

Undeniably, the strong evidence that the oak niche is a natural habitat of *S. cerevisiae* is difficult to rime with the manifest absence of high sugar concentrations in that environment. Given its aptitude to utilize simple sugars which is no different from other *Saccharomyces* species, and the Crabtree effect, i.e., the preferential fermentation of sugars to ethanol even if oxygen is available for aerobic respiration, one would expect the natural niches of the species of the genus *Saccharomyces* to be rich in sugars and prone to favor alcoholic fermentation. Because such sugar-rich substrates, like wild fruits, are likely to be ephemeral and seasonal, it has been hypothesized that a species like *S. cerevisiae* could use oaks as a refuge from which sugar-rich environments could be colonized. This model is appealing because it not only reconciles the oak niche with the Crabtree effect but also because insects, birds, or other animals could act as vectors to and from these two radically distinct environments. Unfortunately, several lines of evidence fall outside this scenario, thus suggesting that reality might be even more complex. Before analyzing this model in more detail, we consider first in the next section what we already know concerning the wild and domesticated populations of *S. cerevisiae*.

5.6 Populations of *S. cerevisiae*

Genetic analyses, especially those relying on complete genome data, have revealed distinct populations of *S. cerevisiae* (Liti et al. 2009; Cromie et al. 2013; Almeida et al. 2015; Strope et al. 2015; Ludlow et al. 2016). These populations appear to be organized firstly according to their ecology and secondly according to their geography. However, the general perspective is that global population structure of *S. cerevisiae* is far from being understood due to a combination of factors such as incomplete sampling of extant lineages, coexistence of wild and domesticated populations, genetic admixture between populations giving rise to mosaic genotypes, and migration over long distances promoting secondary contact between different genotypes. However, a combination of phylogenetic analysis and population studies yielded valuable information concerning populations, their distribution, and their interrelatedness, which is briefly presented below.

5.6.1 Mediterranean Oaks

A wild population found in association with Mediterranean oaks (MO population) was recently revealed and so far has been only found in southern Europe (Almeida et al. 2015). It is the closest relative of the best known population of *S. cerevisiae*, that of domesticated wine yeasts, and a likely candidate to represent the extant representatives of the wild ancestors of oenological strains. Although theory predicts that the wild relatives of a given domesticate are genetically more diverse than the domesticated subpopulation, the Wine-MO system appears to deviate from the norm. In fact, whereas the nucleotide diversity based on pairwise differences (π*100) measured in the MO group is 0.00099, that of the wine group is 0.00112 (Almeida et al. 2015). Because the diversity of the Japanese population, another oak-associated wild lineage, was twice as high (0.00235), it was hypothesized that the MO population was itself the outcome of a population bottleneck and proposed to be a relatively recent arrival to the Mediterranean region (Almeida et al. 2015). Contrary to the wine yeasts that show several signatures of domestication (see Sect. 5.6.2), the MO population appears to maintain the ancestral state of these attributes. Wine-MO admixed strains were reported by Almeida et al. (2015).

5.6.2 Wine

A multitude of methodologies revealed the most emblematic domesticated breed, the wine yeasts. The monophyletic assemblage of strains encompassing wine yeasts was dubbed the “wine-European” group, a designation that unfortunately matches very poorly the most recent findings. First, as recognized from the beginning, the

wine clade gathers virtually all wine strains worldwide, from Europe but also from North and South America, South Africa, and Oceania, probably dispersed with the tools used in viticulture and winemaking and/or with the vines (Legras et al. 2007; Liti et al. 2009; Schacherer et al. 2009; Almeida et al. 2015; Borneman et al. 2016). Secondly, as discussed in the previous section, a wild South European population was uncovered in association to oaks in the Mediterranean region. This is so far the only population to which the epithet "European" can be appropriately applied, since to date all members of this population were found only in Southern Europe.

Wine yeasts exhibit several genetic changes associated with adaptation to the particular conditions of vineyards and wine must, viz., high copper and sulfite levels, high osmolarity, depletion of nitrogen compounds, and ethanol toxicity (Borneman et al. 2013; Marsit and Dequin 2015). These yeasts show also characteristics likely to have been selected during domestication because they contribute to wine quality, such as enhanced fermentative capacity under oenological conditions and production of fruity aromas (Marsit and Dequin 2015). Several signatures of domestication have been documented, such as the presence of three horizontally transferred regions from non-*Saccharomyces* yeasts (Novo et al. 2009), a chromosomal rearrangement associated to resistance to sulfite (Pérez-Ortín et al. 2002); the expansion of *CUP1*, a gene involved in copper resistance (Warringer et al. 2011); and the inactivation of the water channels, aquaporins, a condition selected for in the high osmolarity environment of wine must (Will et al. 2010).

Although it is possible to distinguish the wine yeasts as a genetically separate and homogeneous group that forms a recognizable population when compared with the other main lineages (Almeida et al. 2015), this group tends to harbor a substantial fraction of mosaic strains as shown in various studies (Liti et al. 2009; Cromie et al. 2013; Strope et al. 2015). Among wine yeasts, more recent studies have recognized some phylogenetic partitioning, and subclades typified by sherry wine strains (flor yeasts) and by champagne strain EC1118 (*prise de mousse* group) have been proposed (Legras et al. 2014; Borneman et al. 2016). Therefore it appears that specific variants of wine yeasts with particular oenological properties will be recognized in the near future.

5.6.3 North America and Japan

A wide diversity of *S. cerevisiae* wild lineages from China was revealed by multilocus sequencing (Wang et al. 2012), but has not yet been investigated by population genomics. Four of the five most divergent lineages were found on the bark of Fagaceae or on rotten wood. Other isolates from Fagaceae in Japan, possibly related to the Chinease lineages and for which complete genome data is available, were found to form multiple but closely related lineages, a group that also included the oak-associated wild lineages from North America (Almeida et al. 2015). North American and Japanese strains do not segregate according to geography. On the contrary, members of the same subpopulation were found in North America and

Japan, suggesting gene flow over long distances. Therefore, these multiple lineages are jointly designated as the North America–Japan population.

5.6.4 Sake

The so-called sake yeasts correspond to a group of strains associated with the fermentation of rice-based products such as sake, a Japanese rice wine; sochu, a distilled beverage whose fermentation is similar to that of sake; and ragi and bubod, fermented rice products used as starters in Indonesia and the Philippines, respectively (Fay and Benavides 2005). More recently, this group was enlarged with beer strains, especially wheat beer strains (Gonçalves et al. 2016). A domestication signature for this clade is the presence of *BIO1* and *BIO6*, two genes that code for enzymes involved in biotin synthesis (Borneman and Pretorius 2015). Due to the very low biotin content of sake mash, sake strains are able to synthesize biotin de novo, whereas strains in other clades are auxotrophic for biotin.

5.6.5 Beer

It was recently revealed that German, British, and American ale-type beers are fermented by a domesticated breed that is different from the wine and sake lineages (Gallone et al. 2016; Gonçalves et al. 2016). Contrary to those domesticated groups, beer yeasts have a ploidy higher than 2n and domestication signatures associated with beer brewing, for example, the inactivation of *PAD1* and *FDC1*, two genes that code for enzymes responsible for phenolic off flavors, a detrimental attribute of most beers (Gallone et al. 2016; Gonçalves et al. 2016). As in the wine group, beer strains harbored nonfunctional alleles of the two paralogous water transporters (aquaporins) *AQY1* and *AQY2*, although the inactivating mutations tended to be distinct in beer and wine yeasts, suggesting different origins of these two groups (Gonçalves et al. 2016). Gallone et al. (2016) designated this clade as beer 1 because they detected a second group of domesticated beer strains (beer 2) that included additional ale strains. This second clade is more closely related to the wine clade than to beer 1 and appears to represent a minor fraction of ale beer yeasts. Besides these two beer groups, beer strains were also found in the wine, sake, and bread groups, being therefore much more genetically diverse than wine strains (Gallone et al. 2016; Gonçalves et al. 2016). Mosaicism was also detected in beer strains, especially in strains of the beer 1 clade and in beer strains clustering in the bread clade. Nevertheless, population analyses indicated that beer yeasts represent new populations, thus adding to the complexity of *S. cerevisiae* domestication and suggesting multiple origins for this particular class of fermented beverages.

5.6.6 West Africa

A distinct lineage of yeasts responsible for the fermentation of artisanal beverages like palm wine, ginger beer, and bili-bili in Ghana, Nigeria, and Cameroon (West Africa) was originally detected by Liti et al. (2009) based on two strains. This population was expanded to eight strains (Sampaio, unpublished data), although two of them, SK1 and Y55, are laboratory strains. SK1 is a North American strain that combines African sake and wine ancestries, whereas Y55 is a French strain with African and wine ancestry. The remaining native African strains appear to be clean, or mostly clean, members of a West African population. Since all African strains originate from man-driven fermentations, it is plausible that this population results from domestication. However, the limited sampling in Africa precludes a more definitive assessment.

5.6.7 Brazil

Barbosa et al. (2016) analyzed wild Brazilian isolates and found a new population, the Brazil I population. Most strains from this population were found in association with a local tree, *Tapirira guianensis* (Anacardiaceae), a proposed tropical natural habitat for *S. cerevisiae*. Interestingly, half of the strains were mosaics, exhibiting variable but minor contributions of the wine population. A second local cluster, Brazil II, was rarely found. Overall, 54% of the wild Brazilian isolates had some proportion of ancestry in the wine group. This widespread dissemination of the wine genotype in the Brazilian wild populations questions their true natural nature as this is a probable consequence of genetic contact with domesticated strains. It was hypothesized that the South American populations derive those from North America (Barbosa et al. 2016).

5.6.8 Bread

Although bread yeasts have not been thoroughly analyzed at a population genomics level, a comprehensive survey using microsatellite data suggested that they do not form a single group (Legras et al. 2007). In that study, the main group of bread strains contained mostly tetraploid strains. In agreement to this, Gonçalves et al. (2016) observed that the genomes of bread strains had a ploidy higher than 2n. Moreover, bread strains had admixed genome compositions, whose ancestries could be traced to the beer 1, wine, and sake populations. Therefore, the distinctive characteristic of bread strains is hybridization involving wine, beer, and sake yeasts.

5.6.9 *Malaysia*

Like the West African population, the Malaysian population is based on a limited number of strains, all obtained by Lachance and isolated from the fermented floral nectar of *Eugeissona tristis*, a West Malaysian rainforest palm (Wiens et al. 2008). The Malaysian strains harbor very little genetic diversity and were found to be reproductively isolated as evidenced by low viability of meiotic offspring in crosses with other isolates (Cubillos et al. 2011). Although the first analyses suggested that the Malaysian population represented a clean lineage (Liti et al. 2009), more recent studies indicated that these strains might be admixed, combining multiple origins that include contributions from North American and Japanese oak-associated lineages and wine and sake ancestries (Almeida et al. 2015; Gonçalves et al. 2016).

5.6.10 *Philippines*

This population was discovered more recently and is currently based on ten strains obtained from fruits, sap of palm trees, and palm wine, mostly in the Philippines but also in Indonesia and Pakistan (Almeida et al. 2015; Sampaio, unpublished data). Similarly with the Malaysian population, the Philippine population reveals signs of admixture (Gonçalves et al. 2016). Besides what appears to be the contribution of a local ancestry, this population has also contributions from the sake and North America–Japan populations. Therefore, taking into account the ecological association with sugary substrates, their geographic distribution in Southeast Asia and their genetic ancestries, it appears that the Malaysian and Philippine populations share some resemblance. The wild or domesticated nature of the Philippine population is difficult to ascertain at this stage.

5.7 Migration, Mosaicism, and Ecological Consequences for *S. cerevisiae*

A hallmark of *S. cerevisiae* is its propensity for inter-lineage recombination (Liti et al. 2009). Because *S. paradoxus*, its closest relative, does not exhibit equivalent signs of recombination (Liti et al. 2009), this property appears even more striking and emerges as a distinctive apomorphy of *S. cerevisiae*. Another unique characteristic of this species is the range of natural substrates that are colonized. Although with respect to their oak niche in North America, Europe, and China, *S. cerevisiae* and *S. paradoxus* are mostly sympatric, even considering the less extreme northern boundaries of *S. cerevisiae* distribution (Charron et al. 2014; Robinson et al. 2016), *S. cerevisiae*, but not *S. paradoxus*, appears to be able to colonize other niches frequently, as, for example, fruits (Wang et al. 2012) or the wasp gut (Stefanini et al.

2012). Unexpectedly, at least in the Mediterranean region, most of the strains obtained from natural substrates other than oaks are genetically distinct from the MO population, thus contradicting the hypothesis that oaks are a refuge from which sugar-rich substrates are colonized (Boynton and Greig 2014). Almeida et al. (2015) isolated ten strains from nutrient-rich natural substrates, like wild figs and apples, in the Mediterranean region. Interestingly, only two of these strains were assigned to the MO population. The remaining strains were assigned in equal numbers to the wine population and to mosaics that combined ancestries in the wine, Mediterranean oak, sake, and North America–Japan populations. Therefore, a genetically distinct "fruit" population was neither detected in the Mediterranean region nor in the other regions where oak-associated populations have been found (Robinson et al. 2016). Moreover, in the group of Mediterranean oak isolates, the frequency of mosaic strains was 7.7%, whereas for the group of wild fruit isolates in the Mediterranean region, the frequency of mosaics was 40%. Thus, we note that although in the Mediterranean region a distinct population could be assigned to the oak niche, the same could not be done for strains isolated from fruits of noncultivated plants, a supposedly more obvious *S. cerevisiae* natural environment.

In the study of Almeida et al. (2015), amid the strains isolated from Mediterranean oaks, 71.8% belonged to the MO population. Of the remaining strains, 12.8% were assigned to the wine population and could therefore represent feral strains, i.e., strains that escaped the vineyard/winery environment. Interestingly, 15.4% of the strains isolated from Mediterranean oaks were affiliated to the North American oak population. These strains appear therefore to be migrants. The impact of migration on the population structure of wild populations of *S. cerevisiae* has not been studied yet, but this example and other instances, like migration between North and South America (Barbosa et al. 2016) and between North America and Japan (Almeida et al. 2015), suggest that this is a relatively frequent and natural phenomenon, possibly not only a consequence of human dispersion of domesticated strains, as previously thought (Liti et al. 2009).

Migration appears therefore to contribute to the global genetic cohesion of the species. In addition, migration combined with interpopulation recombination seems to be relatively common, giving rise to mosaics. These mosaics are rarely found in the oak niche and therefore do not seem to thrive there. However, certain mosaic genotypes could be particularly well adapted to nutrient-rich environments, given their relatively higher frequency of isolation from such environments. It is tempting to speculate that for *S. cerevisiae* a unique ecological strategy based on recombination between existing genotypes has emerged. This process yields transient genotypes that in some cases seem to be better adapted for the exploitation of sugar-rich substrates. Although temporally more ephemeral, the presumably significant higher number of generations and increased cell densities supported by nutrient-rich environments may provide sufficient opportunity for selection of "fruit-adapted" alleles or allele combinations. In spite of the apparent transient nature of mosaics, the propensity for inter-lineage recombination in *S. cerevisiae* may provide a means for the species to preserve these alleles or allele combinations in the context of its pan-genome. In addition, the global spread of human-driven

fermentations and the appearance of domesticated lineages only enhanced this pattern of widespread mosaicism now involving also alleles and allele combinations that result from domestication. These observations are also in line with the high degree of phenotypic variation observed in *S. cerevisiae* when compared with *S. paradoxus*, in spite of its lower genetic diversity (Warringer et al. 2011). A greater tendency for migration and interpopulation recombination in *S. cerevisiae* when compared to *S. paradoxus* might also explain the quasi-species status of most populations in *S. paradoxus*, not observed in *S. cerevisiae*.

5.8 Concluding Remarks

We have seen how the understanding of *Saccharomyces* ecology has progressed slowly and amid controversy. Besides the difficulties associated with the cryptic nature of yeasts, we suggest that our proximity to *S. cerevisiae* through the foodstuffs and beverages it ferments imparts to our view of this species, but not to its siblings, a biased perspective. The proposed *Drosophila* (fruit fly)–*S. cerevisiae* association is a good example of an assumption that is proving difficult to reconcile with experimental evidence. Viewed as a biologically relevant characteristic of *S. cerevisiae*, this supposedly mutualistic interaction involves mechanisms of molecular attraction of *Drosophila melanogaster* by *S. cerevisiae*, the fly subsequently promoting yeast dispersal (Christiaens et al. 2014; Buser et al. 2014). Yet, although several field experiments have been reported in the literature, none supports a relevant association between *Drosophila* and *S. cerevisiae*, other yeasts being in fact more frequently associated with these insects (Phaff et al. 1956; da Cunha et al. 1957; Blackwell 2017). Recently, a number of concerns have been raised regarding this fly–yeast model (Hoang et al. 2015). Other studies suggested associations of *S. cerevisiae* with other insects such as bees and wasps. In the case of wasps, it was proposed that they are a key environmental niche for *S. cerevisiae*, contributing to overwintering (when vine plants are in dormancy), dispersion, and maintenance of diversity (Stefanini et al. 2012). In the referred study, *S. cerevisiae* were found to constitute 4% of the yeast community in the wasp gut, and the wasp-associated *S. cerevisiae* strains were mainly mosaics with a minority being assigned to the wine population. A follow-up study demonstrated that *S. cerevisiae* outbreeding is promoted in the wasp gut (Stefanini et al. 2016), a finding that may be more relevant to explain the origin of mosaic genomes than to account for intra-population recombination. Indeed, since the wasp gut environment promotes interpopulation mating, thereby disrupting locally adapted genotypes, the relevance of this environment for the natural biology of *S. cerevisiae* should be analyzed under this perspective.

It is clear that the field of *Saccharomyces* ecology is lagging behind among the diversity of disciplines that generate knowledge on this model organism. One of the most important outstanding tasks consists in linking the make–accumulate–consume (ethanol) strategy (Piškur et al. 2006) with oak and other nutrient-poor

arboreal niches and with the perceived population dynamics of the species. An example of poorly explored avenues for future research relates to the Crabtree effect. This trait is invariably found in any *S. cerevisiae* strain and is considered therefore a fixed character of the species. However, when looking beyond this genetically inherited trait, Jarosz et al. (2014a) found a prion that was capable of "reversing" it. Interestingly, the frequency of appearance of prion-harboring variants insensitive to glucose repression varied depending on the ecological history of the strain. Moreover, prion appearance was triggered by several bacteria (Jarosz et al. 2014b), an effect later shown to be mediated by the production of lactic acid (Garcia et al. 2016), indicating that it is responsive to the environment. Both characteristics point to an ecologically relevant, adaptive role for this epigenetic trait. They also suggest that epigenetic traits may confer additional plasticity to the species in a manner that has been so far largely overlooked when examining apparent contradictions between the physiology of *S. cerevisiae* and its occurrence on natural substrates. Rather than being devoid of an ecology, *S. cerevisiae* and its siblings appear to have evolved sophisticated ecological strategies that still await for ecologically sensible investigations to be revealed.

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Chapter 6
Mutualism in Yeasts

Moritz Mittelbach and Rachel L. Vannette

Abstract Yeasts are often associated with macro- and microorganisms, but these interactions can vary from mutually beneficial to antagonistic. In this chapter, we review mutually beneficial interactions involving yeasts. First, we describe some ways in which yeasts may benefit from the metabolism or actions of other species. Next, we describe the characteristics of yeasts that could benefit other organisms, including rapid growth, high nutrient content, detoxification, and the production of metabolic by-products. We highlight in detail a few of the types of interactions that most resemble mutualisms between yeasts and other organisms for: (1) yeast interactions with animals (vertebrate and invertebrate), (2) yeast interactions with plants, (3) yeast interactions with other microorganisms, and (4) multispecies interactions, including pollination. We necessarily focus on recently published work. We indicate where good evidence exists for mutualism and where more results will be required to demonstrate mutual benefit. Finally, we conclude the chapter with directions for future work, including how current technological approaches may be combined with manipulative experiments to allow rigorous tests of the mutualistic nature of yeast associations.

Keywords Community ecology • Dispersal • Indirect mutualism • Syntrophy • Yeast-insect interactions

Contents

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6.1 Introduction

Yeasts are often found in close association with other organisms, including invertebrates (Do Carmo-Sousa 1969; Phaff et al. 1978; Phaff and Starmer 1987), plants (e.g., Lund 1954; Last and Price 1969; Phaff and Starmer 1987), and within complex microbial communities (e.g., Frey-Klett and Burlinson 2011), as has been documented extensively and reviewed previously (e.g., Ganter 2006; Fonseca and Inácio 2006). The effects of yeasts on their associates can be mutualistic, commensal, antagonistic, or pathogenic. Here, we are tasked with examining the mutualistic interactions that involve yeast, where both yeast and the associated species benefit from the interaction. Although yeasts are often implicated in mutualistic interactions, in many cases, more work is required to demonstrate mutualism *sensu stricto*. In this chapter, we highlight the cases with good evidence for mutualism, and also those where available evidence suggests mutualism, but more work is necessary to demonstrate mutual benefit.

6.1.1 Mutualism: Definitions and Classification

Mutualism, broadly defined, is the association between organisms of different species, where both species benefit from being involved in the interaction (Boucher et al. 1982; Bronstein 1994). Here, we distinguish the term mutualism from the related term "symbiosis" which describes species living in close proximity, although not necessarily benefiting the other. Mutualism may be defined as an increase in individual-level fitness or an increase of the fitness of a population (Boucher et al. 1982). In the case of yeasts, population-level analyses seem most relevant and are the ones typically used to describe the outcome of species interactions with yeasts. In contrast, the growth or reproductive output of a single yeast cell is rarely examined. Rather, changes in yeast occurrence or cell density compared to an initial population of cells are the parameters most commonly reported. On the other hand, effects of yeasts on other organisms are typically examined at either the level of the individual (e.g., biomass accumulation, growth, or reproductive output) or, less commonly, using population-level measurements.

Additional terms can be used to indicate the degree of reliance of one species on another. For example, facultative mutualists benefit from the presence of their partner and may experience an increase in growth or fitness but do not require this partner for growth or reproduction. In contrast, obligate mutualists require the

interaction for growth, reproduction, or other functions necessary to complete the organism's life cycle. Further, mutualisms can vary in specificity: in pairwise or species-specific mutualisms, a specific partner species is necessary to fulfill activities or actions that mediate the mutualistic exchange. By contrast, in more generalized, diffuse, or guild-specific mutualistic interactions, any organism within a guild of functionally similar species may serve as a mutualist (Stanton 2003; Crowley and Cox 2011). In this case, the species involved in a given mutualism may vary among geographic locations or, temporally, depending on the day, season, or year. Finally, interactions need not be symmetric: interactions may be obligate for one species and facultative for the other, species-specific for one and more generalized for the other.

Despite the clear definitions above, most mutualisms in nature are difficult to neatly categorize. Many interactions defined as mutualism vary in strength, specificity, and symmetry, depending on genetic background of the partner species or with environmental conditions. This variability generates a range of outcomes that may extend from mutualism to parasitism (e.g., Johnson et al. 1997). For example, in an experimentally evolved mutualism between algae and S*accharomyces cerevisiae*, the degree of reliance of one species on the other depended strongly on the environmental conditions (Hom and Murray 2014), becoming obligate when environmental conditions favored the mutualism. In another example, variation in nutrient availability shifted interactions between cross-feeding *S. cerevisiae* strains from obligate mutualism into exclusive competition (Hoek et al. 2016). Yeasts are typically thought to be involved in diffuse or guild mutualistic interactions (e.g., Ganter 2006; Starmer and Lachance 2011; Buser et al. 2014). That is, yeasts and other partner species may be ecologically or biologically dependent on a partner organism for growth or reproductive success, but any number of ecologically similar species may fill this role.

6.2 Biology of Yeasts, as Related to Mutualism

Yeasts are defined as eukaryotic single-celled fungi whose "asexual growth predominantly results from budding or fission, and which do not form their sexual states within or upon a fruiting body" (Kurtzman et al. 2011). Typically aerobic organisms, ascomycete yeasts are often capable of rapid fermentation, with the production of characteristic fermentation products including ethanol, carbon dioxide, and other fermentation volatiles. Yeasts often inhabit environments as part of complex microbial communities and exhibit adaptations for competition (e.g., killer toxins or predation) or interactions with other members of microbial communities, such as contributing to biofilm formations or cross-feeding (Golubev 2006; Starmer and Lachance 2011). For additional information on the antagonistic interactions among yeasts and on the biology and ecology of killer yeasts, see Chap. 9 of this book. Due to these biological characteristics, yeasts may benefit

from activities of other organisms and contribute to the fitness of other organisms in certain ways, as we describe below.

6.2.1 What Do Yeast Need? Biological and Ecological Considerations

Yeasts can inhabit diverse environments and utilize a range of substrates as carbon and nutrient sources (Deák 2006). However, as small single-celled organisms with no fruiting body (and therefore limited dispersal ability in an active form), yeasts often deplete nutrients rapidly in local habitats and, as a result, benefit from being vectored into new habitats suited for their particular biology. We outline requirements for yeast success below, including (1) dispersal to suitable habitats, (2) environmental conditions amenable for growth, and (3) environments that promote sexual reproduction and outcrossing.

6.2.1.1 Dispersal to Suitable Habitats

For some yeasts, ideal habitat is ephemeral and includes plant exudates, floral nectar, rotting fruit, and short-lived insects. Moreover, yeast metabolism can alter habitat characteristics by rapidly depleting nutrients or oxygen, or generating toxic by-products. Frequent dispersal to suitable habitats is likely to be important for colonization and competition of yeasts with a rapid growth strategy. In addition, evidence to date supports the hypothesis that yeast habitat specificity may be linked to its particular dispersal strategy. For example, yeast species capable of colonizing a broad variety of different substrates may disperse via various mechanisms including wind dispersal of spores or phoretic transport by a variety of organisms, whose different habits and strategies could mean an uncertain route and destination. Indeed, endophytic yeasts that can colonize a diversity of plant species (Glushakova and Chernov 2010) are thought to be dispersed in an undirected manner through nonspecialized insects or wind (Fonseca and Inácio 2006), in some cases through the formation of ballistoconidia or ballistospores (Last and Price 1969). In contrast, specialized yeasts that require certain environmental conditions might be less flexible in dispersal strategy and rely on a subset of vectors with direct routes and predictable destinations. The transportation of yeast species that associate with *Drosophila* may be highly predictable and strain-specific (Gilbert 1980). Similarly, yeasts that associate with flower-inhabiting beetles or bees are largely restricted to the floral niche (Lachance et al. 2003; Brysch-Herzberg 2004), which may enhance the probability of dispersal to suitable habitats. For yeasts characterized by slow growth rates, population densities may be low and frequent dispersal among habitats may not be as important.

6.2.1.2 Conditions for Growth

As mentioned above, suitable conditions for yeast proliferation and survival vary dramatically among species (e.g., Deák 2006). Yeasts can benefit from partners that create or locate habitats that fulfill particular habitat requirements, or that suppress competitor organisms. In some cases, ecosystem "engineers" (organisms which modify the physical characteristics of the environment) may enable or facilitate yeast growth. For example, *Drosophila* larvae moderate yeast densities through changes to fruit structure (Stamps et al. 2012), and bark beetles provide specialized spatial structures (Hofstetter et al. 2006). In other cases, organisms may directly provide specific metabolites or easily utilized substrates to yeasts (e.g., Hom and Murray 2014). Organisms that help yeasts avoid or preempt competition may also benefit yeast growth. For example, *Drosophila* avoid oviposition and yeast dispersal to habitats that emit the volatile compound geosmin, which is produced by bacteria and indicates habitats where yeasts and larvae are unlikely to successfully establish (Stensmyr et al. 2012). Finally, organisms that directly provide an environment for yeasts, including an internal or external habitat (e.g., gut or organism surface) may directly enhance yeast growth.

6.2.1.3 Conditions for Sexual Reproduction and Outcrossing

Yeasts often require specific environmental conditions for sexual reproduction and the opportunity to mate with cells from different asci (termed outbreeding or outcrossing) (e.g., Knop 2006). Species that provide opportunities for sexual reproduction or outcrossing by yeasts may act as mutualists if recombination and genetic variation are beneficial to yeasts. Sexual reproduction of yeasts has seldom been incorporated in ecological experiments, and its impact on ecological strategies and environmental needs of yeasts remains poorly understood. Asexual reproduction often seems to be the prevailing reproduction mode and might play an important role in the exploration of new habitats (Phaff et al. 1986; Herrera et al. 2011). On the other hand, sexual reproduction can purge deleterious mutations, allow for more efficient selection, and increase yeast fitness in some environments (e.g., Zeyl and Bell 1997; Goddard et al. 2005). However, mating types of a single yeast species can be distributed unequally in local populations (while the global distribution seems balanced) (Phaff et al. 1986; Lachance et al. 1994), and the frequency of compatible mating types varies among environments (Botes et al. 2009). Yeast vectors could allow for sexual reproduction among otherwise allopatric populations, or in some cases, the vectors themselves could also serve as suitable mating habitats. For example, mating, recombination, and hybridization within and between *S. cerevisiae* and *Saccharomyces paradoxus* are promoted in the intestines of *Polistes* wasps (Stefanini et al. 2012; Stefanini et al. 2016). In addition to environmental conditions amenable to sexual reproduction, vectors may also offer protection from external environmental hazards or predators (Vega and

Dowd 2004 and references therein). A prerequisite for this strategy would be the ability to survive the gut passages within insects, which in turn could directly be employed to promote outbreeding through the dissolution of spore tetrads (Reuter et al. 2007). Due to spatial distribution of mating types or environmental conditions required for mating, species that mediate sexual reproduction by yeasts might be considered mutualists, but more increased attention to this area is needed to determine the ecological trade-offs associated with different reproductive modes.

6.2.2 Characteristics of Yeasts That May Be Involved in Mutualism

Although yeasts vary to a great degree in their life history and metabolism, there are characteristics shared by many described yeast species that could be beneficial to other organisms. We highlight examples of these traits below, although there are likely many other characteristics of lesser-studied yeasts of which we are not aware or cannot cover in detail. In addition, yeast characteristics are not neatly confined to single categories, as they are part of a life history that gives rise to multiple correlated traits. As a result, multiple traits described below may be simultaneously involved in species interactions.

6.2.2.1 Rapid Growth

Many yeast species are capable of rapid multiplication in suitable conditions (Fleet 2006), which has often been exploited by humans for food and beverage preservation. Single-celled organisms often thrive in nutrient-rich or ephemeral habitats, and this trait can increase yeast competitiveness and may serve as the basis for mutualism. For example, the fast growth of yeasts could benefit other species when yeasts suppress harmful, pathogenic, or other organisms less beneficial than yeasts. The yeast *Sporobolomyces roseus* has been found to reduce the establishment of the pathogen *Bipolaris sorokiniana* (= *Cochliobolus sativus*) on leaves with aphid honeydew (Fokkema et al. 1983) and of the European corn borer on leaves from maize (Martin et al. 1993). In another system, black yeasts (closely related to fungi in the genus *Phialophora*) can suppress bacterial pathogens (*Escovopsis*) through resource competition, which may benefit the leaf-cutting attine ants in the genus *Apterostigma* (Little and Currie 2008).

For species that benefit from the outcome of yeast competition with other microorganisms, introducing yeasts to a particular environment before other species arrive may then preclude or substantially reduce the growth of later arrivers (a phenomenon called priority effects). For example, in nectar-inhabiting yeast systems, the introduction of one yeast species may inhibit the growth of later

arriving yeasts or less beneficial bacteria through resource competition (e.g., Peay et al. 2012; Vannette and Fukami 2013; Mittelbach et al. 2016), as we detail below.

6.2.2.2 Yeast as (Nutrient-Rich) Diets

Yeast cells as a component of vertebrate and invertebrate diets have been discussed in depth previously (Lipke and Fraenkel 1956; Sang 1978; Starmer and Aberdeen 1990) and suggested as the basis for many relationships between animals and yeasts (Ganter 2006). Briefly, yeasts are rich in proteins and vitamins and can serve as a supplement or even major constituent for the diet of different organisms, domesticated and wild. For many species of flies, butterflies, and beetles, yeasts are necessary for larval development and positively influence survival, resistance to parasitoids, development time, and dry weight in *Drosophila* and *Cydia* (Rohlfs and Kürschner 2010; Anagnostou et al. 2010a, b; Witzgall et al. 2012). Nutritional relationships may be extremely taxonomically diverse: vertebrate and invertebrate organisms feed on yeasts. However, this association may be characterized as predation or commensalism because, in many cases, the effects on yeast fitness are not examined (see also Blackwell 2017).

6.2.2.3 Syntrophy

In contrast to direct consumption of yeasts, syntrophy or "cross-feeding" is defined as the consumption of the products of another species. Yeasts can consume or break down a remarkable variety of carbon and nitrogen sources and in turn synthesize a variety of different substances that are released into the surrounding environment. These substances can comprise supplementary or essential parts of diets of mutualist partners or could function as substrates for other essential processes. Syntrophy is an essential trait in some insect symbionts (Moran 2007) and within microbial communities (e.g., Ren et al. 2015), but is not limited to these niches. For example, fermenting ascomycetes release ethanol into floral nectar, which is consumed by small mammals (Wiens et al. 2008). In the absence of atmospheric CO_2, *S. cerevisiae* releases CO_2, which is then fixed by the algae *Chlamydomonas reinhardtii* (Hom and Murray 2014). Although infrequently documented in natural systems with yeasts, we suspect that syntrophic interactions are likely to be common in multispecies assemblages.

6.2.2.4 Detoxification and Metabolic Breakdown

Although yeasts are not well known for the extracellular secretion of enzymes, yeasts can detoxify or convert toxic substances to tolerable doses or edible diets (see the summary in Starmer and Lachance 2011), which could serve as a basis for mutualism. Several yeast species, including from the genera *Saccharomyces*, and

basidiomycetous yeasts previously classified in polyphyletic phenotypic genera *Cryptococcus*, *Rhodotorula*, and *Trichosporon*, including *Apiotrichum dulcitum*, *Cutaneotrichosporon mucoides*, and *Oberwinklerozyma yarrowii*, can hydrolyze fungal toxins (Molnar et al. 2004; Schatzmayr et al. 2003; McCormick 2013), but its role in mutualism has not been characterized to our knowledge. In floral nectar, the yeast *Metschnikowia reukaufii* reduced the deterrent effects of secondary metabolites, such as caffeine or nicotine, and increase pollinator foraging on nectar, although effects on metabolite concentrations were minimal (Vannette and Fukami 2016). Yeast contribution to enzymatic breakdown may also be common in some environments. For example, *Candida*, *Cystofilobasidium*, *Diutina*, *Rhodotorula*, *Saprochaete*, *Cutaneotrichosporon*, and *Magnusiomyces* have been isolated from the rumen of livestock (Clarke and Di Menna 1961; Prajapati et al. 2016) and may contribute to metabolism in the rumen. In some termite species, strains of *Candida* and *Debaryomyces* contribute to degradation of cellulose through the production of hemicellulolytic enzymes in the gut (Schäfer et al. 1996).

6.2.2.5 Scent

During proliferation and metabolism, yeasts often produce odors and volatile substances, or alter scent profiles through the utilization of volatiles as carbon sources (Vishniac et al. 1997) or changing host volatile emission (Farré-Armengol et al. 2016). In some cases, volatiles can attract partner organisms to suitable substrates, which are then used as feeding or oviposition sites for the partner. In turn, the attraction of potential vectors seems to be directly linked to the efficiency of yeast dispersal (Buser et al. 2014; Christiaens et al. 2014). For example, *Drosophila* flies are attracted to yeast cultures, with ethanol, acetic acid, acetoin, 2-phenyl ethanol, and 3-methyl-1-butanol as key compounds that indicate attraction (Becher et al. 2012). Insects may also use microbial volatiles to locate plant hosts (Beck and Vannette 2017). Codling moths use yeast volatiles (phenols and terpenoids) from *Metschnikowia pulcherrima* and *Metschnikowia andauensis* to find larval host plants (Witzgall et al. 2012). Similarly, the yeast *Kodamaea ohmeri* releases volatiles that mimic the alarm pheromones of honey bees, attracting its vector the bee parasitic small hive beetle (*Aethina tumida*) (Torto et al. 2007). Nectar-borne yeast species contribute aliphatic alcohols to floral scents (Golonka et al. 2014) and attract foraging bumblebees (potential yeast vectors) to flowers (Herrera et al. 2013; Schaeffer et al. 2016). Similarly, the yeast-like fungus *Aureobasidium pullulans* growing on apple fruits attracts eusocial wasps from the genus *Vespula* through the production of 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethyl alcohol (Davis et al. 2012). Yeasts presumably benefit from attracting pollinators or wasps by dispersal to suitable habitats. In the bark beetle microbiome, yeast-derived volatiles are involved in the production of anti-aggregation pheromones, which regulate beetle densities in their tree hosts. Specifically, the yeast species *Kuraishia capsulata* and *Ogataea pini* (formerly *Hansenula capsulata* and *Pichia pini*, respectively) are capable to convert *cis*- and *trans*-verbenol into verbenone

(Hunt and Borden 1990). Finally, yeast volatiles can alter the growth of other organisms, a trait implicated in mutualistic multispecies interactions. For example, *O. pini* emits ethanol, carbon disulfide (CS_2), and Δ-3-carene, which suppress the growth of the entomopathogen *Beauveria bassiana* and increase the growth of the fungal beetle mutualist from the genus *Entomocorticium* in subcortical galleries of bark beetles (*Dendroctonus*) (Davis et al. 2011).

6.2.2.6 Temperature

Yeast assimilation is an exothermic process and may increase the local environmental temperature. The phenomenon is well studied in industrial fermentation, but ecological consequences in natural habitats or cases where this trait might even be beneficial in interactions are scarce. Herrera and Pozo (2010) found an increase of inner floral temperature by 3–5 °C through the assimilation processes of *M. reukaufii*. This temperature shift most probably suits pollinators (Dyer et al. 2006) and may enhance pollination success of the respective flowers in cold conditions (Herrera and Medrano 2016).

6.2.2.7 Inhibition and Killer Toxins

Yeasts also release allelopathic or toxic compounds to suppress competitors (see Chap. 9 of this book). This trait is hypothesized to play a role in mutualistic interactions. Several yeast species from the microbiome of leaf-cutting ants can suppress fungi and insect pathogens through various mechanisms, promoting the health of the ant-fungi symbiosis (Rodrigues et al. 2009). Moreover, yeast species have been thought to assist with the breakdown of polysaccharides and involved in the regulation of fungal populations in the ant gardens (Arcuri et al. 2014). The yeast *M. pulcherrima* strongly inhibits the growth of molds through the emission of pulcherriminic acid (MacWilliam 1959) or the depletion of free iron in the media (Golubev 2006 and references therein; Sipiczki 2006). Affiliation with this yeast therefore could provide an advantage to the larvae of the codling moth to reduce mold contamination of apple fruits (Witzgall et al. 2012).

6.3 Examples and Case Studies

As outlined above, yeasts can benefit from the activities of other organisms and in turn facilitate the growth or performance of other species in a number of ways. Below, we overview documented or likely examples of yeast mutualism. We focus on well-studied examples of mutualism like yeast-Drosophilid interactions, but also highlight other putative mutualisms and suggest where more research could move forward this field.

6.3.1 Mutualisms Between Yeasts and Animals

6.3.1.1 Invertebrate-Yeast Mutualisms

Yeast associations with invertebrates have been extensively cataloged (Vega and Dowd 2004; Ganter 2006; Starmer and Lachance 2011), and evidence to date indicates that yeast associations are often beneficial, if not essential for the nutrition and development of many insect species. Beetle associations with yeasts and fungi have been well studied. In particular, bark beetles rely on yeasts and other fungi for growth, metabolism of substrate, and detoxification of host metabolites, while fungi are dispersed in specialized mycangia. As this mutualism has been reviewed recently (Davis 2014; Douglas 2016; Moller et al. 2016), we do not cover this topic here. Instead, we highlight a few studies from selected interactions that provide ecological or evolutionary evidence for mutualism. Yeasts associated with insects and invertebrates have been exhaustively reviewed by Blackwell (2017).

6.3.1.2 Ants

Although many neotropical ants cultivate fungi (Weber 1972; Mueller et al. 2005), one rather unusual association involves ant-yeast interactions. *Cyphomyrmex rimosus* ants cultivate basidiomycetous fungi of the genera *Leucoagaricus* and *Leucocoprinus* in a yeast-like form. Ants rely on yeast gardens as a food source for larvae and adults (Mueller et al. 1998; Lange and Grell 2014). Molecular evidence indicates the leucocoprineous yeast-like fungi are largely confined to their cultivated habitats, but close relatives can be found in a nonsymbiotic form (Vo et al. 2009). However, the fitness consequences of the two lifestyles remain unknown. We speculate that the yeast likely benefits from growth conditions maintained by attine ants; in addition, *Cyphomyrmex* ants are known to associate with the antibiotic-producing bacteria of the genera *Pseudonocardia* (Currie et al. 2006). If leucocoprineous yeasts exhibit resistance to these compounds, they may benefit from suppression of competitors by *Pseudonocardia*, but this complex relationship remains to be examined from the yeast perspective.

Despite years of documenting patterns of yeast associations with insects, clear evidence of mutualism in insects-yeast interactions has been documented in only a minority of cases. We call for increased ecological work to examine the nature of insect-yeast interactions, to focus on their specificity and degree of reliance on partners. This may lead to enhanced control of pests in managed systems (e.g., Beck and Vannette 2017) and improved conservation efforts in natural systems.

6.3.1.3 Drosophila-Yeast Mutualism: What's New?

Probably the best-studied system of yeast-invertebrate interactions is between yeast and invertebrate fruit flies (Drosophilids), and these interactions have been reviewed extensively in the past (Begon 1982; Phaff and Starmer 1987; Ganter 2006; Kurtzman et al. 2011). Although many aspects of this system would still benefit from additional work, a preponderance of evidence indicates that interactions between yeast and Drosophilids are mutualistic. In general, Drosophilids vector yeasts to suitable habitats, deposit yeasts with eggs, and larvae benefit from nutrition or detoxification that the yeast provide; in many cases, the flies cannot complete development without yeasts (Kircher 1982; Starmer and Fogleman 1986; Starmer et al. 1988; Starmer and Aberdeen 1990). Previous work has produced clear evidence of larval benefit from yeasts. Recent work found that insects benefit from more diverse yeast communities, as more speciose yeast communities better suppressed the growth of the mold *Aspergillus nidulans*, increasing the survival and decreasing development time of *Drosophila melanogaster* larvae (Rohlfs and Kürschner 2010). Indeed, drosophilids are so reliant on yeasts that fermentation odors have evolved in deceptive pollination systems, including in the Solomon's lily (*Arum palaestinum*), which is pollinated by drosophilids (Stökl et al. 2010). On the other hand, there is mounting evidence that yeasts also benefit. As discussed previously, fly vectors can avoid habitats colonized by other microorganisms based on microbial volatiles like geosmin (Stensmyr et al. 2012) and alter the environment to favor yeast growth (e.g., Stamps et al. 2012), giving yeasts a competitive advantage. In addition, fly-associated yeasts appear adapted to being consumed by flies. For example, many ascomycetes are capable to survive the gut passage in flies by forming spores (Coluccio et al. 2008) and seem to require these adverse environmental conditions to enhance outbreeding rates (Reuter et al. 2007).

Yeast-drosophilid interactions are thought to be guild mutualisms with limited specificity. Recent work by Buser et al. (2014) found that *S. cerevisiae* strains, which were more attractive to the fly *Drosophila simulans*, were also dispersed to a greater extent and promoted higher egg deposition by flies, suggesting the possibility for specificity to arise. However, intensive sampling of orchards reveals that many different species of yeasts are found to associate with drosophilids, and even putative specialist yeasts like *Hanseniaspora uvarum* are not consistently isolated (e.g., Lam and Howell 2015). It is likely that the specificity of the interaction may vary depending on the species considered: cactophilic yeasts show some specificity to fly vectors, while yeast strains dispersed by vinegar flies show relatively little specificity and poor correspondence between fly preference and performance among yeast strains (Ganter 2006; Hoang et al. 2015). Nevertheless, increased work on non-model systems or more realistic species combinations in Drosophilids and other invertebrates is likely to improve our understanding of the specificity and degree of reliance of each partner species.

6.3.1.4 Marine Invertebrates

In marine environments, red yeasts from the order Sporidiobolales and *Erythrobasidium* yeasts (among others) are frequently isolated from invertebrates, including clams, sponges, and other invertebrates (Nagahama 2006). Together with yeast traits and putative function in these environments, frequent isolation with particular hosts suggests that some interactions may be mutualistic (Galkiewicz et al. 2012; Webster and Taylor 2012; Vaca et al. 2013; Flórez et al. 2015). A strain of *Filobasidium* isolated from the scleractinian coral *Pocillopora damicornis* was found to extend the longevity of coral cells following damage (Domart-Coulon et al. 2004). Due to the difficulty of manipulative experiments in realistic marine conditions, ecological studies of these interactions remain rare, but marine systems may be a fruitful avenue of future study of yeast-invertebrate mutualisms.

6.3.1.5 Vertebrate-Yeast Mutualisms

Interactions between yeasts and vertebrates have been characterized as largely parasitic and outside human domestication of yeast; evidence for mutualistic interactions is generally lacking. Studies surveying the fungal diversity of different groups of vertebrates regularly report abundant yeast populations from mammals (Lund 1974), fish and other aquatic animals (Hagler and Ahearn 1987), amphibians (Poonlaphdecha and Ribas 2016), and birds (Cafarchia et al. 2006; Francesca et al. 2014), suggesting that yeasts may be associated with many groups of vertebrates. Notably, yeasts have been proposed to play a role in fish health and metabolism (Gatesoupe 2007). For example, *Debaryomyces hansenii* and *Rhodotorula mucilaginosa* are frequently isolated from the gut of carnivorous salmonid fishes; and many of these yeast strains produce aminopeptidases and lipases that may be involved in metabolism (Raggi et al. 2014). Building evidence suggests that fish likely benefit from the interaction, but the ecological or fitness consequences of this interaction for yeasts have not been explored. Other examples from aquatic and terrestrial systems are likely to emerge as the microbiome composition of more vertebrate species is characterized.

6.3.2 Plant-Yeast Mutualisms

Yeasts are commonly isolated from plant roots, leaves, flowers, and fruits, but documented mutualisms between plants and yeasts remain uncommon. Epiphytic yeasts (those occurring on plant surfaces) have been historically viewed as commensal, where yeasts benefit from associating with plants, but have no effect on plant fitness. However, evidence is accumulating that in some cases, yeasts may also benefit the plant. In a survey of 114 yeast isolates from plant leaves in

Thailand, approximately 35% of phylloplane yeast species tested produced the plant hormone indole acetic acid (IAA), with some strains producing high levels of the hormone (Limtong and Koowadjanakul 2012). Streletskii et al. (2016) even found 92% of 124 yeast stains isolated from different plant substrates capable to synthesize IAA. Production of IAA has been suggested to promote plant growth but is involved in interactions that range from mutualistic to pathogenic (Spaepen and Vanderleyden 2011), so we caution that ecological studies are required to assess fitness benefits of IAA production. Regardless of the mechanism, a few studies document yeast enhancement of plant growth. In maize, the root endophytic yeast *Cyberlindnera saturnus* (formerly *Williopsis saturnus*) produces IAA and increased plant growth under some environmental conditions (Nassar et al. 2005). In the shrub *Agathosma betulina*, inoculation with the yeast *Papiliotrema laurentii* (*Cryptococcus laurentii*) on roots also increased root biomass (Cloete et al. 2009). When associated with sugar beet roots, *Pichia membranifaciens* (*Candida valida*), *Rhodotorula glutinis*, and *Trichosporon asahii* improved plant growth through reduced postemergence damping off disease (*Rhizoctonia solani*) in seedlings, crown, and root rots of mature plants (El-Tarabily 2004), suggesting that competition in the rhizosphere may provide protection against root pathogens. However, few other examples of yeast epiphytes on plant growth or performance have been documented, and documenting benefit for both plants and yeasts simultaneously remains elusive.

Yeasts and plants may benefit each other through more complex interactions, which could result in indirect mutualism. For example, nectar-inhabiting yeasts can increase foraging by bees (Herrera et al. 2013; Schaeffer and Irwin 2014; Schaeffer et al. 2016; Vannette and Fukami 2016), with possible fitness effects on plant hosts. More complex interactions are also possible. For example, bacteria also frequently colonize floral nectar and reduce pollinator foraging (Vannette et al. 2013; Good et al. 2014). Yeasts have been shown to suppress the growth of deterrent bacteria in floral nectar and may thereby positively influence pollination. Laboratory experiments indicate that nectar-inhabiting yeasts *M. reukaufii* or *Candida rancensis*, if introduced to nectar first, could inhibit the growth of bacteria in the genera *Gluconobacter* and *Asaia* (Tucker and Fukami 2014). By reducing the growth of bacteria, which are distasteful to pollinators (Vannette et al. 2013; Good et al. 2014), yeasts may indirectly improve pollination within individual flowers, or as modeling results suggest, over the duration of the flowering season (Song, unpublished data). Building evidence suggests that nectar specialist species (e.g., *M. reukaufii*, *Starmerella bombicola*, *Metschnikowia koreensis*, and others) are reliant on pollinator vectors to access flowers (Mittelbach et al. 2016; Vannette and Fukami, unpublished data). Taken together, strong reliance on pollinators and the suppression of microbes that are not beneficial to pollinators suggest a facultative mutualism between plants, pollinators, and nectar-borne ascomycetous yeasts. However, nectar yeasts sometimes decrease pollen germination (Eisikowitch et al. 1990) or plant reproduction in some plant species (Herrera et al. 2013). Further work to examine the range and frequency of outcomes in this system will be required.

Most work on yeast-plant interactions suggest facultative and guild interactions rather than specialized or obligate mutualism. This may be due to the nature of plant acquisition of microbial partners in the environment, limited study, or that yeasts and plants, as largely immobile producers, do not offer complementary traits. However, the evolved mutualism between syntrophic algae and yeasts (Hom and Murray 2014), or co-option of yeast scent by deceptive flowers (Stökl et al. 2010) suggest yeast traits may in some cases complement those of plants. Further work will elucidate the range, specificity and frequency of these interactions.

6.3.3 Microbe-Yeast Mutualism

Yeasts typically inhabit environments with complex communities of microorganisms, often as part of the microbiome of macroorganisms. Mutualisms between yeasts and other species of fungi, bacteria, or even viruses are common and have been best studied in clinical and food production environments. We highlight a range of interactions, where partners benefit from rapid yeast growth, metabolism, biofilm formation, or syntrophy.

6.3.3.1 Yeast-Virus Mutualism

Mutualism between yeasts and viruses is well documented in the "killer yeast" phenomenon (see also Chap. 9 of this book). Although toxins are sometimes encoded on plasmids or chromosomal DNA, toxin-encoding genes can be carried by double-stranded RNA viruses in the family Totiviridae. When infected by the dual viruses, yeast species including *S. cerevisiae* produce a killer toxin and are conferred self-protection against the toxin through immunity. Toxin secretion often leads to rapid competitive dominance by the infected yeast strain. In turn, the virus receives a host and opportunities to spread through cell-cell mating or heterokaryon formation, as reviewed by Schmitt and Breinig (2006). Yeasts that carry the virus include *S. paradoxus*, *Saccharomyces mikatae*, *Saccharomyces bayanus*, *Zygosaccharomyces bailii*, and *H'spora uvarum* (Drinnenberg et al. 2011) and have been described in environments where competition among strains is strong. In this case, the association is obligate for the virus but facultative for the yeast.

6.3.3.2 Biofilm Formation

Close physical association between yeasts and other microorganisms could enhance the success of both species. Many microorganisms are aggregated into biofilms, where cells stick together and/or to a surface. Many yeast species can initiate biofilms, although they have been best studied in *Candida albicans* in the clinical setting, *S. cerevisiae* in fermentation, and diverse *Candida* species used in biofuel

production (Blankenship and Mitchell 2006). In the clinical environment, *S. cerevisiae* can initiate (multispecies) biofilms through the production of a cell surface glycoprotein, even when bacterial adhesins are not effective (Reynolds and Fink 2001). In medical infections in humans, multispecies biofilms are often more resistant than are planktonic cells to antimicrobial drugs, including antifungals. Resistance has been linked to structural complexity, the presence of an extracellular matrix, and upregulation of efflux pump genes in biofilms (e.g., Elias and Banin 2012; Fanning and Mitchell 2012). Antimicrobial resistance has consequences for yeast persistence in a clinical setting: in vivo wound healing was delayed even with antimicrobial treatment when subjects were coinfected with *Staphylococcus aureus* and *C. albicans* (Peters et al. 2010). In this example, in vitro experiments show close physical association between fungal and bacterial cells, which was associated with changes in gene expression that promoted virulence and is thought to promote invisibility of these dual-species biofilms (Peters et al. 2010; Burmølle et al. 2014). In this way, yeast interactions with other microorganisms may benefit both microbial species involved, although certainly not the host. Biofilm-based mutualism involving yeasts is perhaps best described in the clinical context, but similar interactions are likely to occur in many additional environments and will require further study through sequencing, co-culture, and in situ examination of microbial consortia.

6.3.4 Multispecies Microbial Interactions

In some cases, yeasts have been found to benefit either other microbial community members or indirectly benefit their host. Most of these associations do not represent mutualism as strictly defined, but may convey indirect benefits. For example, yeasts can improve each other's growth in rotting cactus tissues (Starmer and Fogleman 1986). Yeasts are involved in multispecies interactions in the rhizosphere, where they release vitamin B_{12}, promoting the growth of plant-associated arbuscular mycorrhizal fungi (Fracchia et al. 2003), which may indirectly improve plant growth (Singh et al. 1991; Boby et al. 2008). In bark beetle galleries in tree phloem, the yeasts *Yamadazyma scolyti* and two unidentified species (one basidiomycete and *Candida* sp.) imposed divergent effects on beetle-associated fungi: while *Ophiostoma montium* benefits from yeast growth through unknown mechanisms, *Grosmannia clavigera* is most probably outcompeted by yeast growth (Adams et al. 2008). Besides the diverse interactions of yeasts to the beetles itself, the yeast *O. pini* enhances the growth of some mycangial fungi by metabolizing carbohydrates or terpenoids (Davis et al. 2011). Yeasts comprise part of the microbiome of *Drosophila* and many other organisms (Hoffmann et al. 2013), which are thought to benefit the host while also providing a habitat for microbes. Interactions among microorganisms in a multispecies context have not been addressed intensively or rigorously with respect to mutualism, but with enhanced focus on the microbiome, understanding of yeast interactions with hosts and other microorganisms within the

microbiome is likely to improve. As an increasing number of surveys include bacteria, fungi, yeasts, and viruses as part of complex microbial communities, co-occurrence patterns may suggest strains for further experimental analysis and may reveal other microbial interactions and suggest where they may be important.

6.4 Concluding Remarks

As we have described, yeast interactions often resemble mutualism, but the nature of these relationships can be quite variable. We note the prevalence of a few different strategies documented so far and common themes among them.

In one presumably common type of interaction, yeasts are found in association with mobile organisms—primarily insects—with specific habitat preferences and keen olfaction. Here, rapidly growing yeasts often provide nutrition, detoxification, and competitive or inhibitory activity that suppresses the growth of other non-preferred microorganisms. Yeast volatiles serve frequently as signaling chemicals that indicate favorable habitat or aggregation locations for insects, or to attract vectors for yeast transportation. Yeasts often benefit from transportation to and early arrival at suitable environments and, in some cases, environmental modification by their vector. In many cases, yeasts may also benefit from outbreeding, preparation for dormant phases, or ability to adapt to changes in habitat, but these are only infrequently quantified. To date, such relationships seem facultative or generalized, but including other benefits in ecologically realistic experiments may indicate greater degrees of specialization and mutual dependence.

In contrast, association between yeasts and largely sessile organisms like plants or microorganisms is characterized by increased importance of syntrophy, the production of specific metabolites, physical associations like biofilms, or, in some cases, strong effects of yeast reproduction and competitive suppression. Yeasts can benefit from partners that provide chemical substrates, enhance their own competitive ability, or improve local environmental conditions. Similar traits may dominate interactions within the microbiome, but yeast contribution to and benefit from them microbiome remains to be demonstrated empirically. The broad characterization above neglects that nuance of many unique interactions characterized to date, but generates hypotheses for the types of interactions we expect, given species traits. Overall, few cases of obligate mutualism have been documented (although many have been postulated, see Ganter (2006) and references therein). These broad patterns should be tested more rigorously.

Many mutualistic species and interactions remain to be uncovered. In many cases, associations are documented but experiments demonstrating mutualism have not yet been performed. More basic research, including traditional experiments, is sorely needed on a variety yeast interactions with invertebrates from sponges to lacewings, which may lead to improved conservation efforts or management of insect populations. Although effects on yeast fitness and biology are typically overlooked in these studies, including them will not only enhance our

understanding of mutualism, but may be essential in understanding the feasibility of any strategies developed. Moreover, dissecting the nature of interactions within complex microbial communities has been challenging, but new techniques are likely to invigorate this field. Increased research on the role of yeasts in the microbiome and in other complex communities is likely underway. In this area, transcriptomic analyses will be useful for hypothesis generation, while improved methods for selective removal of microbiome members can be used to assess function in complex communities.

Finally, given that perhaps 90% of yeast diversity has not been described (Kurtzman and Fell 2006), efforts to describe and assess the taxonomy of yeasts are sorely needed. For species involved in mutualism, methodology may have particularly constrained species detection and description. Yeast species or strains that require specific environmental conditions or a partner organism for growth are less likely to be amenable to traditional culture methods, as has been suggested for some pathogenic species of *Metschnikowia* (e.g., Weiser et al. 2003; Kurtzman et al. 2011; Lachance 2016). Yeast inhabitants of intestines or other specific environments still evade cultivation (Ebbert et al. 2003). Identifying the specific growth conditions for particular species may require in-depth knowledge about their habitat requirements (Boundy-Mills 2006), novel culturing methods recently employed for bacteria (e.g., Ling et al. 2015), or culture-independent approaches targeting function-specific genes (Prajapati et al. 2016). Culture-independent approaches have suggested key roles of yeasts in diverse environments, including that of the tremellomycetous yeast *Dioszegia* in community assembly of the plant microbiome (Agler et al. 2016), in sediments of estuarine systems (Burgaud et al. 2013), and in the human microbiome (Hoffmann et al. 2013). Culture-independent approaches are beginning to recognize the contribution of fungi to microbiome structure and function (Huffnagle and Noverr 2013). However, far fewer studies characterize fungal communities, so the understanding of fungal and yeast contribution lag behind bacterial characterization. Moreover, the most frequently used primer sets for environmental sequencing of fungi (ITS1f-ITS2) discriminate against many groups of yeasts, including those in the Saccharomycetales and Tremellales (Tedersoo and Lindahl 2016). Shotgun (primer-free) metagenomic characterization also fails to adequately fungal diversity and function (Tedersoo et al. 2015). Although primer sets exist that better capture focal yeast clades, they are in use by a smaller community of researchers. Improved methods, including primer sets, primer-free sequencing techniques, and reference databases are therefore a high priority for discovery and hypothesis generation in the study of yeast mutualisms.

We hope that improved methods for detection and current interest in the microbiome will spur new research on this topic. We emphasize that measuring yeast fitness and traits within this context, including traditional culture-based methods and ecologically relevant experiments, will greatly enhance our understanding of the range and frequency of yeast mutualisms.

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Chapter 7
Parasitism in Yeasts

Dominik Begerow, Martin Kemler, Anja Feige, and Andrey Yurkov

Abstract Yeasts are common in all habitats and interact with dead and living substrates such as plants, animals, and fungi. Besides their saprobic capabilities, parasitic interactions of yeasts and yeast-like organisms were brought into focus through enhanced/new species discovery that expanded our knowledge about phylogenetic relationships of yeasts and parasitic fungal lineages. Especially common dimorphism of many Basidiomycota goes along with an alternating saprobic yeast stage and parasitic filamentous stage. Interestingly, this seems to be a common feature not only for plant parasites but also for animal and fungal parasites. Even some Ascomycota share this character.

The chapter aims to provide an overview of the most relevant parasites among yeast species and lineages. For this we summarize the most recent literature to initiate further studies and to provide ideas for common patterns and strategies. As can be seen in this chapter, the knowledge differs between animal parasites, plant parasites, and mycoparasites leaving space for new research and hypotheses. However, it is apparent that the comparison of the three different host groups provides interesting insights of common features and concepts.

Keywords *Candida* • *Cryptococcus* • Smuts • *Ustilago* • *Microbotryum* • Mycoparasites • Lichenicolous fungi

Contents

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7.1 Introduction

Yeasts inhabit many ecological niches and, like filamentous fungi, can be isolated from almost every habitat worldwide. As heterotrophs, they rely on different biologically accessible nutrients but are mainly limited in their growth by carbohydrate and nitrogen resources, whereas most other elements are not limiting factors in most habitats. Many fungi have solved the problem of nutrient availability by interacting with other organisms. This has been implicated as one of the reasons for high species diversity in fungi, and especially plant-fungus interaction might have played a key role in the early radiation of fungi (Redecker et al. 2000; Begerow et al. 2004). But fungal interactions are not restricted to plants, and fungi interact with many eukaryotic lineages, including prokaryotes, several protist lineages, plants, and animals. The spectrum of interactions range from neutralism, commensalism, and mutualism to parasitism (e.g., Starmer and Lachance 2011) and are reviewed in other chapters of this book. Here, we will focus on parasitism of yeasts and yeast-like organisms (Fig. 7.1). Reviewing the broad literature, we have to acknowledge that the terms "parasite" and "pathogen" are not clearly separated in the various fields of research. While "pathogen" is broadly used in plant-centered publications, animal literature seem to have a preference for "parasite." In general, the term "pathogen" is emphasizing the causal agent of a disease, and the term "parasite" is highlighting the form of interaction. Without neglecting the difference between the two terms, we decided to use "parasite" throughout the text for simplicity.

A major character of yeasts, which has been always stressed in research, is their ability to grow on artificial media. Therefore, their saprobic capabilities (e.g., spectrum of utilized compounds) have been in focus for a long time (reviewed in Barnett 2004). In the middle of the nineteenth century, yeasts were recognized as living organisms and assigned to fungi (Barnett 1998). The first described yeast species originated from fermenting products, a substrate where they often occur in a single-celled form. It is however well accepted nowadays that "yeast" represents a live stage or live form (characterized by singe cells proliferating by budding or fission) that has evolved early in fungal evolution and has been retained in several

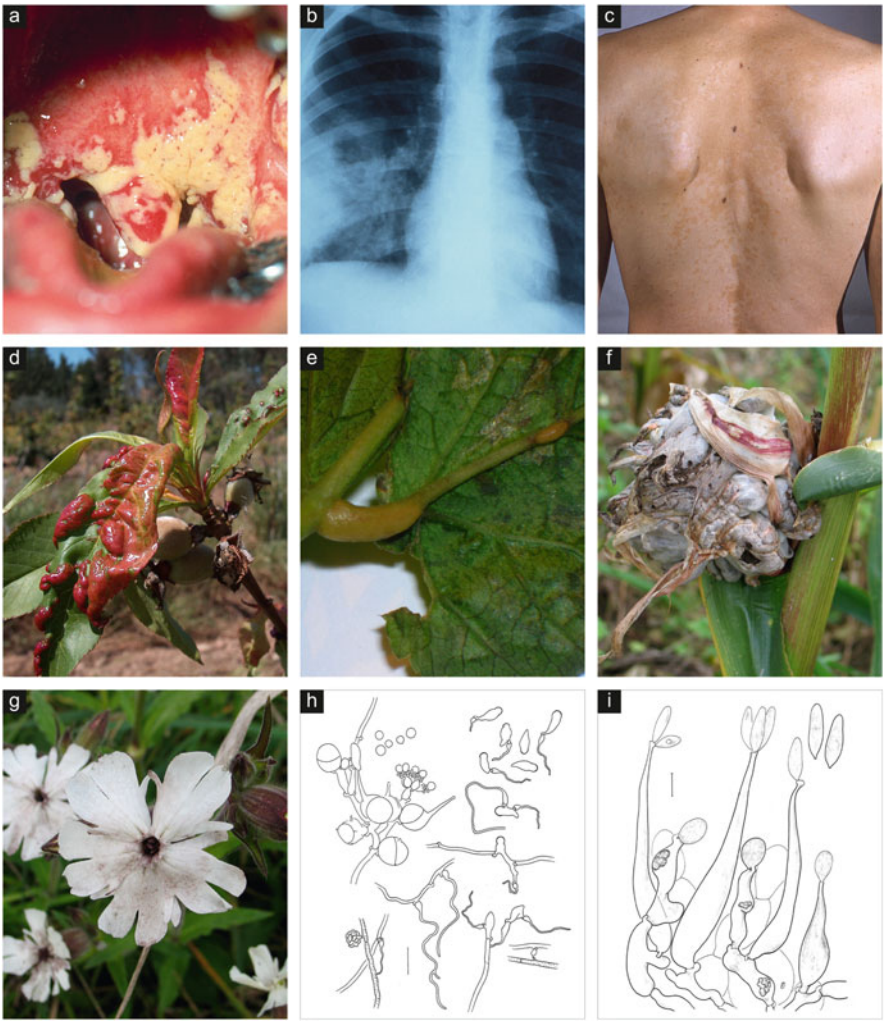

Fig. 7.1 Pathogenic stages of yeasts. (**a**) Oral thrush caused by *Candida albicans* (Photo: CDC and PHIL © CC-BY-SA 2.0). (**b**) X-ray of the thorax reveals the telltale signs of non-encapsulated pulmonary cryptococcosis in a patient infected with *Cryptococcus* sp. (Photo: Centers for Disease Control (CDC) and Prevention's Public Health Image Library (PHIL) © CC-BY-SA 2.0). (**c**) Posterior view of a man's torso reveals the presence of a patchy, erythematous rash caused by *Malassezia furfur* (Photo: CDC/Dr. Lucille K. Georg © CC-BY-SA 2.0). (**d**) Peach leaf curl caused by *Taphrina deformans* (Photo: Giancarlo Dessì © CC-BY-SA 2.0). (**e**) *Protomyces macrosporus* gall as seen on the lower surface of *Aegopodium podagraria* (Photo: Roger Griffith © public domain). (**f**) *Mycosarcoma* (*Ustilago*) *maydis* sporulating in tumors substituting fruits of *Zea mays* (Photo: Domink Begerow). (**g**) *Microbotryum lychnidis-dioicae* sporulating on the anthers of *Silene latifolia* (Photo: Angela Schäfer). (**h**) Various stages of *Sterigmatosporidium polymorphum* including conidiophores, basidia, hyphae with clamp connections, and tremelloid haustoria from yeasts cells and hyphae (Modified from Kirschner et al. 2001). (**i**) Sectional view of *Cryptomycocolax abnorme* including basidia, parasitic hyphae with clamp connections, host cells, and botryose structures of interaction (F. Oberwinkler as published in Oberwinkler and Bauer 1990)

phylogenetic lineages (Nagy et al. 2014). In many lineages, it represents the only growth form the fungus can exhibit, but researchers have observed filamentous fungi or their spores giving rise to cultures proliferating as yeasts by cell budding. Under certain conditions, yeast-like states could be observed in molds (e.g., *Mucor* and *Penicillium*), parasitic ascomycetes (e.g., *Taphrina* and *Kabatiella*), jelly fungi (e.g., Auriculariales, Dacrymycetales, Tremellales), and smuts (e.g., *Ustilago*). Yeast stages of the intensively studied smut fungi were regularly observed and used in experiments, even though budding yeast state was not characterized or explicitly mentioned in the descriptions.

The term "dimorphic", a term introduced by Brefeld in the 1880s (reviewed in Bandoni 1995), was used to contrast the yeast stage of basidiomycetous fungi having also a dikaryotic hyphal phase from the typical unicellular morphology of ascomycetous yeasts. The life histories of most dimorphic taxa consist of mating of compatible (heterothallic) yeast states to produce dikaryotic mycelia. Haploid conidial development can again result in a yeast state, as it may direct budding of basidia in some taxa. The presence or absence of haploid and/or diploid conidia is a common variation in the life histories (Bandoni 1995). Variations in mating systems are also common, and homothallism is present in some species (e.g., Lin and Heitman 2007; David-Palma et al. 2016).

Fungal parasites of plants were among the first organisms where understanding the role of resting structures and saprobic asexual states in survival and dispersal was achieved. Even though the ability to produce a yeast stage was often viewed from the perspective of systematics rather than from its functional side, mycologists accumulated substantial numbers of examples of parasitic fungi with predominantly unicellular (or yeast-like) asexual states. Most of our present knowledge about so-called heterobasidiomycetes and dimorphic fungi is coming from the research made by Robert J. Bandoni (University of British Columbia, Canada) and Franz Oberwinkler (University of Tübingen, Germany) with collaborators. More recent studies on yeasts in their natural habitats showed complex interactions with the environment, their vectors, and hosts (reviewed by Starmer and Lachance 2011).

Dimorphism is often associated with a change in nutrient acquisition, whereby the yeast stage grows saprobically and the hyphal phase is parasitic. As detailed in this chapter, this behavior has evolved in various lineages and is independent of the host's phylogenetic position. Especially sequencing technologies and molecular identification (e.g., Begerow et al. 2010; Schoch et al. 2012) have made it possible to recognize the link between yeast and parasitic stages in several lineages, thereby changing our understanding of yeast biology in many ways (Begerow et al. 2014; Begerow and Kemler 2017). Subsequent studies showed that many known basidiomycete yeasts are phylogenetically related to parasitic fungi (Fell et al. 2000; Scorzetti et al. 2002; Liu et al. 2015; Wang et al. 2015a, b).

Recent advances in high-throughput DNA sequencing of fungi showed the potential to develop yeast type of proliferation being widely distributed across Fungi (Nagy et al. 2014). This ability arose early in fungal evolution and became dominant independently in different phylogenetic groups being, possibly, results

from the diversification of the gene family (Zn-cluster transcription factors) that are responsible for yeast-filamentous switches (Nagy et al. 2014). Although the transition between filamentous and yeast growth forms (morphs) has been known for more than a century, the importance and role of this adaptation in *Fungi* are still a matter of scientific debates.

Below we review the knowledge about yeasts as parasites; focus on animal, plants, and fungi as hosts; and follow a phylogenetic approach within each of three ecological groups (Fig. 7.2). However, yeasts associated with humans and invertebrates are discussed in more detail elsewhere (see Chap. 8 of this book and Blackwell 2017, respectively) and therefore are mentioned only briefly. As the majority of our knowledge on parasitism of yeasts is probably based on studies of dimorphic Basidiomycota in the Ustilaginomycotina and some lineages of Pucciniomycotina, these will be reviewed extensively. Where possible, we additionally provide an overview on parasitic stages of fungi in their yeast stage.

7.2 Animal and Human Hosts

With respect to ecology and growth optima, many yeast species are saprobic and mesophilic (Lachance and Starmer 1998). Although yeasts grow as soon as carbohydrates and nitrogen are available, they do not cope well with elevated temperatures. Therefore, growth at the body temperature of warm-blooded animals is unusual for these fungi. However, some yeasts have adapted to grow at these temperatures and contain some of the most serious fungal pathogens of humans and animals. Their phylogenetic placement in the *Dikarya* is shown in Fig. 7.2.

7.2.1 Candida *and Former* Candida *Species*

The polyphyletic genus *Candida* comprises some of the most widely studied yeasts with relevance to human health (Daniel et al. 2014). Several species of the genus are part of the mycobiome associated with healthy individuals, and at least *Candida albicans* is ubiquitous in the human gut. However, people with compromised immune system might suffer from serious health issues (e.g., Fig. 7.1a; Cui et al. 2013; Huffnagle and Noverr 2013; Smeekens et al. 2016). Most indications are associated with dysfunctions of the intestinal tract. As saprotrophic ascomycetous yeasts, *Candida* spp. grow well in high sugar concentrations, and dietary shifts are the primary advice in such cases. However, severe infections are also reported for almost all organs, including skin, vagina, lung, heart, and brain. Especially, infection via the bloodstream causes severe secondary invasive infections. These represent some of the most serious complications during organ transplantations or HIV infections and are still difficult to treat medically (e.g., Miceli et al. 2011; Clancy and Nguyen 2013; Silveira and Kusne 2013).

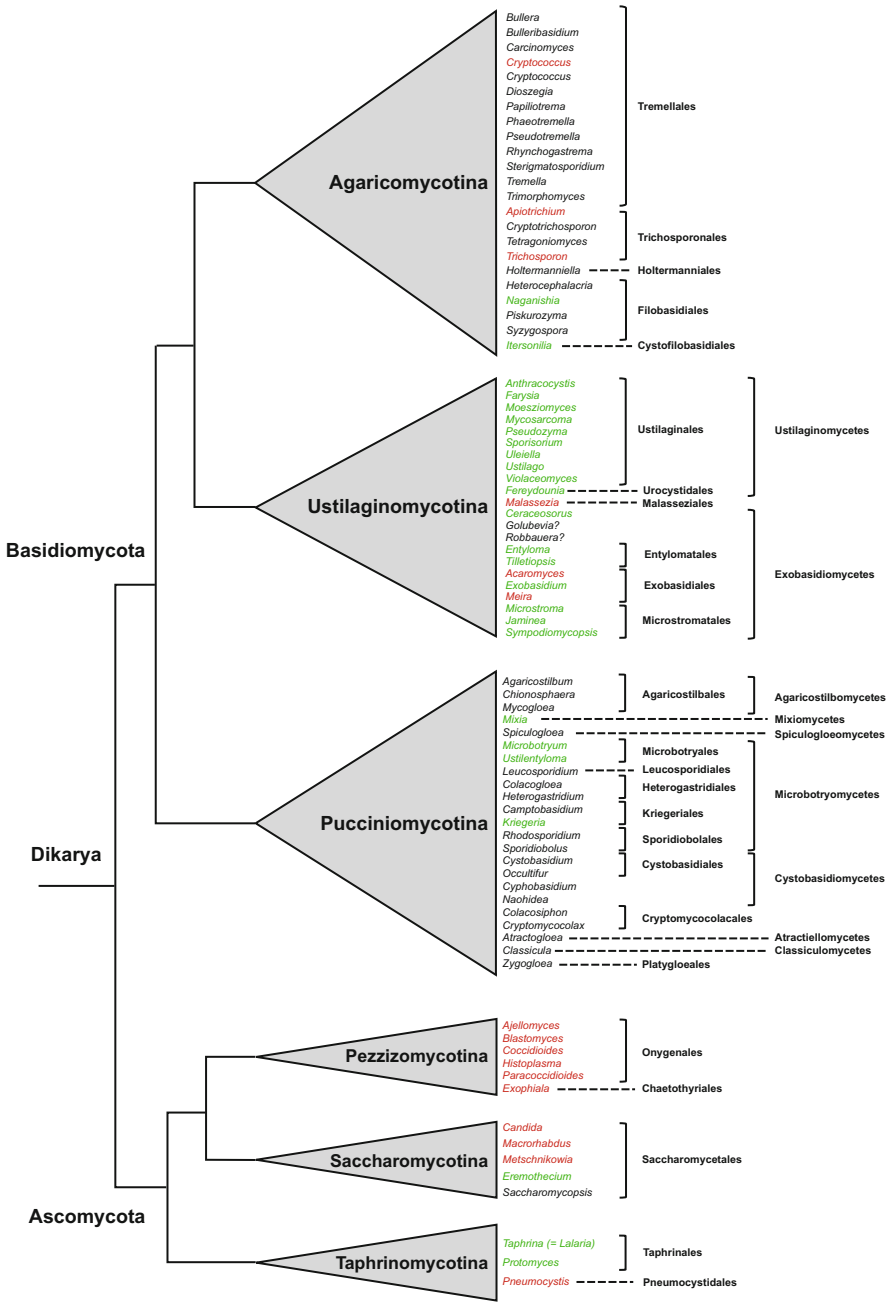

Fig. 7.2 Overview of the phylogeny of *Dikarya* including yeast lineages with known parasitic interactions. Fungal genera with yeast states reported as parasites of animals (red, see Sect. 7.2), plants (green, see Sect. 7.3), and other fungi, including lichens (black, see Sect. 7.4)

Taxonomically, *Candida* has been a catchall genus for white ascomycetous yeasts that were distinct from *Saccharomyces*. Although major efforts have been made to resolve the phylogeny and taxonomy in the last decade, it is still a large genus containing several polyphyletic lineages (Daniel et al. 2014). Besides *C. albicans*, four species (*Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*, the last currently *Pichia kudriavzevii*) account for 90% of diagnosed cases of invasive candidiasis (but see Clancy and Nguyen 2013). Because proper species identification of the pathogen is still rare, most treatment advices are based rather on the clinical syndrome than on the causative strain or species (Antinori et al. 2016). However, among the possible threats of such symptomatic treatments is missing out on potential multi-resistant species such as *Candida auris* (e.g., Lockhart et al. 2017).

The clade including *C. albicans* is currently well circumscribed, but others are still in need of thorough analysis. Multigene phylogenies are needed to resolve the various clades, and often new genera need to be established to provide a taxonomy based on monophyletic lineages (e.g., Kurtzman and Robnett 2014; Shen et al. 2016). However, so far it seems that pathogenicity is restricted to the *C. albicans* clade and other lineages are not hazardous to humans. Yeasts found in the association with candidiasis in humans are discussed in detail in Chap. 8 of this book.

7.2.2 Cryptococcus

Serious human infections can also be caused by *Cryptococcus* spp. (Fig. 7.1b), which occur in other mammals, besides humans as well. Although most relevant in the tropics and the Southern Hemisphere, cryptococcosis received only broader attention after an outbreak on Vancouver Island in 1999 (Galanis and MacDougall 2010). The causative agent was identified as a single species, *Cryptococcus gattii* (currently *Cryptococcus bacillisporus*). Detailed studies in the last decades however revealed a complex of several species and hybrids to be involved in cryptococcosis in general. In the meanwhile, *Cryptococcus neoformans* (formerly *Filobasidiella neoformans*) s.l. is probably the best-studied yeast in terms of its mating system and variation of its life cycle (e.g., Hull and Heitman 2002; Heitman et al. 2011). Due to its importance as human pathogen and model organism, a separate chapter addresses the most recent advances. We therefore refer the reader to Chap. 8 of this book and earlier reviews on the topic (e.g., Idnurm et al. 2005; Lin and Heitman 2006; Heitman et al. 2011). However, it is important to document that species of the *Cr. neoformans* species complex are not obligate pathogens and human-to-human transmission of the infection is either absent or rare (e.g., Gerstein and Nielsen 2017). Since these yeasts are present in the environment, biotic and abiotic factors are responsible for strain diversity of *Cr. neoformans* s.l. in terms of physiological properties and virulence traits (e.g., Gerstein and Nielsen 2017).

For a long time, the genus *Cryptococcus* has been used to accommodate diverse and often distantly related species of Tremellomycetes, which often resulted in

confusion between pathogenic and nonpathogenic species. Recent changes in the taxonomy of basidiomycetous yeasts have restricted the genus *Cryptococcus* to the *Filobasidiella* clade (see Fonseca et al. 2011). This clade currently comprises seven clinically relevant (Hagen et al. 2015) and two presumably saprobic species, *Cryptococcus amylolentus* and *Cryptococcus depauperatus* (Liu et al. 2015). Another species, *Cryptococcus luteus* (originally *Filobasidiella lutea*), has been placed in the genus based on the morphological similarity of the sexual stage, but cultures and sequence data for this species are currently missing (see Liu et al. 2015). The two species *Cr. depauperatus* and *Cr. luteus* were found as parasites of other fungi, i.e., *Lecanicillium lecanii* (now *Cordyceps confragosa*) and *Granulobasidium vellereum*, respectively. Examples of mycoparasites of this lineage are discussed below in this chapter.

7.2.3 Cutaneous Yeasts

7.2.3.1 *Malassezia*

The human skin is the most relevant organ besides the intestinal tract in terms of microbes including fungal diversity. Millions of microbial cells inhabit the skin, and most form part of the healthy skin microflora (see Chap. 8 of this book for more details). Although the research on the diversity and function of this microbiome is still in its infancy, several studies highlight the relevance of certain fungal taxa. *Malassezia* (Malasseziomycetes, Ustilaginomycotina) is a common genus found in many skin microbiome studies (e.g., Findley et al. 2013; Cabañes 2014). Skin colonization by *Malassezia* species of healthy humans starts at birth, and abundance increases in the first weeks of life. As with other described yeast taxa, only few healthy people suffer from infections caused by *Malassezia* (see Cabañes 2014). The clinical syndromes are skin discolorations (Fig. 7.1c) and sometimes itchiness, and some species have been related to dandruff. Due to the relative harmlessness, *Malassezia* has received less attention than to other human pathogens. However, two species, *Malassezia furfur* and *Malassezia pachydermatis*, have been reported to cause yeast systemic infections at a low percentage. However, frequency of these infections could be underestimated as not all commonly used culture media contain lipids, which are essential for isolation and detection of these fungi (see below). *Mal. pachydermatis* is considered to be zoophilic and is frequently found on wild and domestic carnivores. This species is usually associated with *otitis externa* and different kinds of dermatitis in domestic animals, especially in dogs (see Cabañes 2014). This yeast is occasionally found on the human skin, and its zoonotic transfer from dogs has been documented (see Cabañes 2014).

Biology and evolutionary relationships of these yeasts are highly interesting. It is known as anamorphic yeast only and forms a monophyletic lineage in the Ustilaginomycotina (Begerow et al. 2000; Wang et al. 2015a). No sexual structures could be identified so far, but genome sequencing of this lineage suggests that a

sexual state might exist (reviewed in Begerow et al. 2014). All of the known species are bound to warm-blooded animals and especially to respiratory glands. They are lipophilic and require special media for growth. All but one species (*Mal. pachydermatis*) do not grow in pure culture without external oils added into the medium and are therefore often not detected in culture-dependent surveys. They are, however, detected by analysis of DNA amplicon libraries (e.g., Cabañes 2014; Findley and Grice 2014). Not only isolation but also proper preservation and identification of *Malassezia* isolates can pose some difficulties.

Malassezia species have been isolated from almost all domestic animals, from different wild animals held in captivity, and also from wildlife (see Cabañes 2014). However, these reports are often fragmentary to provide a good overview on the occurrence of these yeasts on the skin of different animals. In spite of the limited physiological abilities of these yeasts, the detection of *Malassezia* in environmental samples is of special interest. In a few cases, like in the sequence libraries prepared from deep-seawater samples, *Malassezia*-related sequences were surprisingly prominent (Bass et al. 2007). Likewise, these yeasts were detected among marine fungal communities associated with corals and sponges (Gao et al. 2008; Amend et al. 2012). Amplified fungal ITS1 fragments subjected to RFLP analysis indicate the presence of *Malassezia restricta* and *Malassezia globosa* in soil nematodes (Renker et al. 2003).

7.2.3.2 *Trichosporon* and Former *Trichosporon* Species

Dimorphic yeasts producing hyphae breaking into segments (arthroconidia) and lacking sexual stages formerly classified in the phenotypic genus *Trichosporon* (Trichosporonales, Tremellomycetes, Agaricomycotina) are another group of yeasts commonly reported as a part of the skin mycobiome (e.g., Guého et al. 1994; Mariné et al. 2015). About one third of known former *Trichosporon* species are correlated with human infections or allergies (Weiss et al. 2014). *Trichosporon cutaneum* (currently *Cutaneotrichosporon cutaneum*), *Trichosporon inkin*, *Trichosporon loubieri* (*Apiotrichum loubieri*), and *Trichosporon ovoides* are the most prominent species involved in superficial trichosporonosis (e.g., white piedra), while *Trichosporon asahii*, *Trichosporon asteroides*, and *Trichosporon mucoides* (*Cutaneotrichosporon mucoides*) are associated with invasive infections in immunocompromised patients (e.g., Guého et al. 1994; Miceli et al. 2011; Mariné et al. 2015). These yeasts are also frequently mentioned among non-*Candida* and non-*Cryptococcus* yeasts in the clinical practice (e.g., Miceli et al. 2011; Chitasombat et al. 2012). For instance, *Trichosporon dermatis* (*Cutaneotrichosporon dermatis*) has been shown to be involved in summer-type hypersensitivity pneumonitis (SHP), an allergic disease occurring in hot and humid seasons in Asia that is caused by inhalation of arthroconidia (Sugita 2011). Another species, *Tr. asahii*, colonizes the gastrointestinal tract of healthy subjects (Cho et al. 2015). The detected genotypes were almost identical to those of reported clinical isolates suggesting that the development of trichosporonosis is probably caused by the normal fungal gut

microbiota together with additional unknown factors. However, pathogenicity cannot be unequivocally demonstrated for all former *Trichosporon* species, as many of them do not grow at 37 °C.

Trichosporon yeasts have been reported from different habitats (e.g., plant material, soils, insects) and geographical regions. The taxonomic heterogeneity of arthroconidia-forming yeasts in the genus *Trichosporon* has been demonstrated with phylogenetic analyses based on the ribosomal LSU and ITS sequences (Fell et al. 2000; Scorzetti et al. 2002; Middelhoven et al. 2004). As a result, *Trichosporon pullulans* (currently *Tausonia pullulans*, Mrakiaceae, Cystofilobasidiales, Tremellomycetes) has been removed from the genus to restrict it to the members of the Trichosporonales (Fell and Scorzetti 2004). As for 2014, the order Trichosporonales comprised several clades (Fell and Scorzetti 2004; Middelhoven et al. 2004; Sugita 2011; Weiss et al. 2014) with dimorphic *Trichosporon* yeasts and predominantly unicellular *Asterotremella*, *Bullera*, and *Cryptococcus* species (Liu et al. 2015). In spite of the taxonomic complexity, the genus *Trichosporon* was recently reclassified into six monophyletic lineages (Liu et al. 2015). Clinically relevant species are currently accommodated in the genera *Cutaneotrichosporon* (*Cut. cutaneum*, *Cut. dermatis*, and *Cut. mucoides*) and *Trichosporon* (*Tr. asahii*, *Tr. asteroides*, *Tr. inkin*, and *Tr. ovoides*), whereas most of the saprobic species have been transferred in the reinstated genus *Apiotrichum* (Liu et al. 2015).

Until the end of the twentieth century, a wide range of species was included under the name of *Trichosporon* (*Pleurococcus*) *beigelii* or synonyms, which were later shown to be phylogenetically distinct (Mariné et al. 2015). The literature lists *Cut. cutaneum*, *Cutaneotrichosporon moniliiforme*, and *Tr. ovoides* as synonyms of *Tr. beigelii*. It is important to notice that both the name *Pl. beigelii* and neotypification of *Tr. beigelii* have been rejected by Guého and colleagues in the beginning of the 1990s (reviewed in Liu et al. 2015). Nevertheless, the name *Tr. beigelii* is still being reported from clinical samples without a possibility to attribute its detection to any of the recognized yeast species. Both *Tr. beigelii* and *Cut. cutaneum* have been reported from environmental samples such as soil, litter, and invertebrates in older studies, which used a limited set of physiological tests for species identification (e.g., Di Menna 1965; Byzov et al. 1993; Carreiro et al. 1997; Sláviková and Vadkertiová 2000). Application of nucleotide sequencing of the ribosomal gene regions identified *Apiotrichum porosum* and *Apiotrichum dulcitum* as possible species behind the phenotypic *Tr. cutaneum* (see for discussion Yurkov et al. 2012; Yurkov 2017). Recently, *Trichosporon lactis* has been reported colonizing exoskeletons of various dung beetle species of the genus *Onthophagus* (Górz and Boroń 2016). The yeast grew as unusual epizoic excrescences on the elytra, prothorax, and head of the studied beetles. A few species previously classified in the genus *Trichosporon* have been isolated from insects, including Scarabaeoidea and identified as *Apiotrichum scarabaeorum* (Middelhoven et al. 2004). However, yeast proliferation on insect bodies has not been reported before. Whether or not

Trichosporon and its relatives are insect pathogens requires additional studies in the future.

7.2.4 Other Important Pathogenic Yeasts on Humans and Animals

The abovementioned species and genera contain probably more than 90% of the described human pathogenic yeasts, but there are many more yeast species known to result in serious infections. Most of them are found frequently in environmental samples and, like many filamentous fungi, seem to be opportunistic pathogens. Disease-causing dimorphic ascomycetes such as *Ajellomyces*, *Histoplasma*, *Coccidioides*, *Paracoccidioides*, and *Blastomyces* (Ajellomycetaceae, Onygenales, Pezizomycotina) are known from warm, moist climates and can be found in soils, decaying wood, and bird droppings. The switch from filamentous to yeast growth plays an important role in the infection process, and asexual spores are supposed to be the primary agent. Inability to grow on artificial media hinders our understanding of the infection routes and additionally complicates estimation of the biodiversity of these fungi.

Pneumonia caused by *Pneumocystis* (Pneumocystidomycetes, Taphrinomycotina) is an important disease of immunocompromised patients. Like the abovementioned ascomycetes, *Pneumocystis* species cannot be grown in culture, and all stages of the life cycle have to be studied directly in lung tissues. Despite clinical relevance of this dimorphic taxon, profound basic research is lacking. Even diagnostics of many potentially deadly yeast infections are insufficient, and it is currently unknown how many of deathly pneumonias are caused by *Pneumocystis*, other yeasts, or bacteria as most of them occur in regions with insufficient health care, especially regarding HIV-infections (Thomas and Limper 2007; Skalski et al. 2015).

The asexual ascomycete *Macrorhabdus ornithogaster* has been isolated from a number of birds, where it infects the stomach. Although this organism was first reported as a yeast in 1980, subsequent studies wrongly named it a "megabacterium" until the identification with DNA sequencing placed this yeast as a member of Saccharomycetales (Tomaszewski et al. 2003). Because cultivation of *Mac. ornithogaster* was not successful, little is known about its physiological properties and requirements. This yeast however causes widespread infections and occasionally results in devastating disease outbreaks in some bird species (Phalen et al. 2011).

So-called black yeasts represent a heterogenic group in terms of both taxonomy and ecology. Many of these species show pronounced dimorphic growth and are capable of sustaining unfavorable environmental conditions such as desiccation and salinity (e.g., Gostinčar et al. 2010). Members of the genus *Exophiala* display

pathogenicity toward humans and animals (including cold-blooded animals) (e.g., de Hoog et al. 2011; Chowdhary et al. 2014). Clinically relevant species include *Exophiala dermatitidis*, *Exophiala xenobiotica*, *Exophiala spinifera*, and *Exophiala oligosperma* (Chowdhary et al. 2014). Less common are *Exophiala lecanii-corni*, *Exophiala asiatica*, *Exophiala phaeomuriformis*, *Exophiala jeanselmei*, *Exophiala bergeri*, and *Exophiala mesophila* (Chowdhary et al. 2014). It is important to document that black yeasts can be also isolated from environmental samples (Buzzini et al. 2017; Sannino et al. 2017), as well as in polluted and anthropogenic environments (Novak Babič et al. 2017).

Yeasts have been isolated from various aquatic animals, including clams, mussels, shrimps, isopods, amphipods, crabs, sponges, sea urchins, polychaete worms, fish, and marine mammals (e.g., Hagler and Ahearn 1987; Starmer and Lachance 2011). At least one of them, *Metschnikowia bicuspidata*, is pathogenic to crustaceans and fish (Lachance 2016). By using its needle-shaped ascospores, the yeast is believed to invade host bodies (reviewed by Lachance 2016). Although some other *Metschnikowia* species also produce long needle-shaped spores, there is little evidence that species outside the *M. bicuspidata* clade display parasitic relationships. Lachance (2016) reported the observation of a spore of *Metschnikowia hawaiiensis*, which is associated with the nitidulid beetle *Conotelus obscurus*, inside a nematode. The possibility that the nitidulid beetle benefits from the presence of *M. hawaiiensis* ascospores because the latter might curb infection by parasitic aphelenchoid nematodes was investigated, but could not be confirmed.

Finally, two genera of Ustilaginomycotina, *Acaromyces* and *Meira*, have been studied as potential biocontrol agents against citrus mites (Boekhout et al. 2003). Yeasts were found growing on dead insect larvae and endophytically in plant tissues. Their potential parasitism has been hypothesized at the beginning, but further studies showed these yeasts producing glycolipid compounds with a broad inhibition range, which included several fungi pathogenic to plants. The compound has been also shown to have a toxic effect on insect larvae (reviewed in Begerow et al. 2014).

7.3 Plant Parasites

In contrast to animal hosts, plant hosts are much more common among fungal and yeast parasites. Phylogenetic analyses based on genome scale data (Spatafora et al. 2016) support the close interaction between the radiation of fungi and land plants. Although early fungi might have been mutualists of fungi (Redecker et al. 2000), the transition to a parasitic lifestyle is very common and is realized in several lineages. Here we focus on the most relevant yeast lineages in terms of parasitism instead of following a detailed phylogenetic approach analyzing all lineages of Ascomycota and Basidiomycota.

7.3.1 *Ascomycota:* Taphrina, Protomyces, *and* Eremothecium

7.3.1.1 *Taphrina*

The plant pathogenic genus *Taphrina* is part of the early diverging lineage Taphrinomycotina of Ascomycota (Fig. 7.2), which also includes the fission yeast *Schizosaccharomyces pombe*, the saprobic yeast *Saitoella*, the tree-associated *Neolecta*, the human pathogen *Pneumocystis*, and the second plant pathogenic genus *Protomyces*. Most of our present knowledge of *Taphrina* is derived from the works of Arthur J. Mix, who collected and reviewed older works and provided the first comprehensive list of these fungi (Mix 1949). Verona and Rambelli were the first authors who found the need to provide a formal name (*Saprotaphrina*) for the asexual states of *Taphrina*, which they have isolated from flowers and leaf litter (reviewed by Fonseca and Inácio 2011). Because Verona and Rambelli did not provide either a Latin diagnosis of the genus or designated the type species, the genus *Saprotaphrina* remained invalid, and Moore (1990) proposed the genus *Lalaria* to accommodate the yeast states of Taphrinales, introducing 23 new species all representing anamorphic forms of previously known *Taphrina* spp. (Fonseca and Rodrigues 2011). Several *Lalaria* species have been isolated from the environment, either from healthy plants (see also Kemler et al. 2017) not known as *Taphrina* hosts or other substrates such as litter and soil (Rodrigues and Fonseca 2003; Fonseca and Inácio 2011). Phylogenetic relationships among the extant authentic *Taphrina* cultures have been studied using ribosomal gene sequences: the SSU rRNA gene (Sjamsuridzal et al. 1997) and the D1/D2 domains of the LSU rRNA gene and the internal transcribed spacer region (Rodrigues and Fonseca 2003). The existing *Taphrina* cultures correspond to yeast states that were, in most cases, isolated from infected plant material using the spore-fall method (e.g., Mix 1953). Inácio et al. (2004) discussed the redundancy of the genus *Lalaria* for the original 23 species proposing to legitimize the use of this genus name only for species isolated in the yeast state where an unequivocal connection to a *Taphrina* teleomorph could not be established. The number of species accommodated in *Lalaria* was reduced from 23 to 5 (Fonseca and Inácio 2011), and the subsequent recent changes in the taxonomy of fungi discontinued the practice of using dual names for sexual and asexual morphs, so a few *Lalaria* species listed by Fonseca and Inácio (2011) have been transferred into the genus *Taphrina* (Selbmann et al. 2014b). Most *Taphrina* species and their asexual counterparts display a narrow spectrum of utilized carbon sources, thereby supporting the parasitic lifestyle (Inácio et al. 2004). The species *Taphrina inositophila* and *Taphrina veronaerambellii* were frequently isolated from the surfaces of Mediterranean plants not known as *Taphrina* hosts. They were considered to be true phylloplane yeasts. Interestingly, the two species utilized diverse carbon sources like other phylloplane yeasts. It has been suggested that these two yeasts may have a predominantly saprobic lifestyle unlike other *Taphrina* species, which are

commonly assumed to have a parasitic stage, even if the host and symptoms are not known so far (Moore 1990; Inácio et al. 2004). The genus seems to be distributed worldwide, and even Antarctica harbors at least one species (Selbmann et al. 2014a, b).

The plant pathogenic species in *Taphrina* cause various symptoms (Fig. 7.1d) on several quite unrelated plant families. While it is best known for the symptoms on Rosaceae, where it causes curly leaves or deformed and aborted fruits like *Taphrina deformans* on *Prunus persica* or *Prunus dulcis* (Cissé et al. 2013), infections of members of other host families result in different symptoms like the witches' brooms in Fagaceae (mainly *Alnus*, *Populus*, and *Betula*) or just withered leaves in some ferns and/or herbaceous Rosaceae. Infections caused by *Taph. deformans* and *Taphrina betulina* are of economic relevance. Most of the studies focused on diseases of economically important fruit trees such as peach, plum, and cherry (*Prunus* spp.) infected by *Taph. deformans*. It has been shown that *Taph. betulina* infection reduces the mean height and diameter of infected *Betula pubescens* substantially (Spanos and Woodward 1994) and thereby may cause potential losses in timber production within short rotation forestry (McKay 2011).

The unusual ascus formation in and on the plant epidermis is directly followed by a haploid yeast stage, which can reinfect the plant probably after mating. The plant-parasitic hyphal stage of *Taphrina* is dikaryotic like in members of Basidiomycota, which is not known from other Ascomycota (Kramer 1960).

The genomes of various *Taphrina* species have been sequenced recently. It encodes a full repertoire of carbohydrate-active enzymes, including those required for degrading plant cell wall components such as cellulose, hemicellulose, and pectin. However, genes for lignin-degrading enzymes are lacking (Cissé et al. 2013; Tsai et al. 2014). Interestingly, the genome encodes targets for known antifungal drugs like azoles, although they are known to be resistant. Thus, additional resistance mechanisms might play a role during the epiphytic stage on leaves (Cissé et al. 2013). In addition, the genome harbors genes putatively involved in the biosynthesis of plant hormones like indole-3-acetic acid (IAA) and cytokinin that might be responsible for the formation of characteristic leaf deformation symptoms (Cissé et al. 2013; Tsai et al. 2014). Another peculiarity of the *Taphrina* genome is the occurrence of a single copy of the rRNA cistron, which is usually present in several copies in other fungi (Cissé et al. 2013). Environmental sequencing using high-throughput methods revealed members of the *Taphrina* in analyzed sequence libraries as being among the most frequently observed fungi in some tree leaves (see also Kemler et al. 2017).

7.3.1.2 *Protomyces*

Reddy and Kramer (1975) reviewed earlier ideas on phylogenetic relationships noting that many other mycologists thought that *Protomyces* was a "primitive" ascomycete. They also revised morphologically similar fungi (i.e., *Burenia*, *Protomycopsis*, *Taphrinidium*, and *Volkartia*) and placed them in Protomyceteacea.

It is important to document that nucleotide sequences or cultures of other members of Protomyceteacea are lacking and their relationships with the genus *Protomyces* are thus putative (Kurtzman 2011). Species of the genus *Protomyces* cause symptoms similar to those caused by *Taphrina* (Fig. 7.1e). All known *Protomyces* species are plant parasites causing galls on stems, leaves, and fruits, mainly of Apiaceae and Asteraceae. Similar to *Taphrina*, members of *Protomyces* produce a budding yeast-like culture on laboratory media but do not develop hyphae or sexual states unless infecting their host plants (Kurtzman 2011). The yeast colonies are colored in reddish or salmon-like due to the synthesis of carotenoid pigments. Unlike *Taphrina*, saprobic states of *Protomyces* are rarely reported from studies, which employed either cultivation or culture-independent approaches. Therefore, our knowledge about asexual yeast states of these plant pathogens is scarce. Substantial economic losses caused by these fungi are rare.

7.3.1.3 *Eremothecium*

A filamentous member of Saccharomycetaceae, *Eremothecium gossypii* (syn. *Ashbya gossypii*), was originally isolated from cotton plant as a pathogen causing stigmatomycosis (Kurtzman and de Hoog 2011). This fungal disease, caused mainly by the two species *Er. gossypii* and *Eremothecium coryli* (syn. *Nematospora coryli*), results in severe economic losses of cotton, coffee, pistachio, and citrus plants in tropical and subtropical areas of the world (Kurtzman and de Hoog 2011). The genus is now comprised by five species, all of which are recognized parasites of plants. Elongated needle-shaped spores of *Eremothecium* are transmitted to plants by hemipteran insect pests (Kurtzman and de Hoog 2011). Control of the transmitting insects is used to protect crops from stigmatomycosis. *Er. gossypii* is currently used for industrial production of riboflavin (vitamin B2), which is naturally accumulated by the fungus to protect its spores against ultraviolet light. As the genome has been sequenced, *Er. gossypii* became also a model to study filamentous growth using the knowledge derived from the first model fungus, *Saccharomyces cerevisiae* (Wendland and Walther 2005; Perez-Nadales et al. 2014).

7.3.2 *Smuts:* Ustilago *and Other Members of Ustilaginomycotina*

Probably the best-studied plant parasites among yeasts are the smut fungi (Fig. 7.1f). The capacity to cultivate them on artificial media is known for more than 100 years and has made them a model system in plant pathological research since then. However, major results and knowledge have often not been incorporated into yeast literature because of unknown links between traditional yeast species and smut fungi and the resulting dual nomenclature of asexual and sexual morphs

(Begerow and Kemler 2017). Here, we describe shortly most recent results and the challenges for upcoming yeast research.

Traditionally, only few yeast genera and species have been recognized within the Ustilaginomycotina. Cell wall sugar composition has been used in yeast systematics to characterize yeasts of this subphylum of Basidiomycota. The two major genera *Pseudozyma* and *Tilletiopsis* are very vague in their morphological and physiological properties, and a detailed systematic and taxonomic treatment of these yeast taxa was only possible using molecular phylogenetics (Boekhout et al. 1995; Begerow et al. 2000). Since *Pseudozyma* and *Tilletiopsis* could not be linked to the genera of their probable teleomorphs, they became catchall genera for yeast strains belonging to Ustilaginomycotina. This resulted in highly polyphyletic assemblages (reviewed in Begerow et al. 2014; Wang et al. 2015a). Although *Pseudozyma* and *Tilletiopsis* fulfilled the need formally to distinguish yeast states related to Ustilaginomycetes and Exobasidiomycetes, respectively, these large asexual genera could not accommodate all yeasts in Ustilaginomycotina. As a result, new asexual genera (*Farysizyma*, *Jaminaea*, and *Sympodiomycopsis*) have been described (Sugiyama et al. 1991; Inácio et al. 2008; Sipiczki and Kajdacsi 2009). In the case of *Farysizyma*, the new genus additionally reduced polyphyly in the yeast genus *Rhodotorula* (Inácio et al. 2008). As phylogenetic data accumulated, Wang et al. (2015a) consequently proposed to separate monophyletic lineages and either incorporate them directly into teleomorphic genera (or species) or propose new genera for taxa when a direct link to a teleomorph was not known. The teleomorphic genera *Sporisorium* and *Ustilago* have been also reclassified and are now restricted to clades with the respective type species (McTaggart et al. 2012, 2016). Out of 20 described *Pseudozyma* species, 5 could not be assigned unambiguously to any genus and were temporarily (*pro temp*.) retained as *Pseudozyma* (Wang et al. 2015a). The biocontrol yeast *Pseudozyma flocculosa* has been transferred to the genus *Anthracocystis* (*Anthracocystis flocculosa*, parasites on Cyperaceae), and the biotechnologically relevant *Pseudozyma antarctica* has been accommodated in the genus *Moesziomyces* (*Moesziomyces antarcticus*, parasites on Poaceae).

Unlike Agaricomycotina, members of the Ustilaginomycotina differ not only in their basidia, fruiting body morphology, and color, but in their ultrastructure as well. The latter resembles the situation within Pucciniomycotina, where several types of septal pores have been established, although the functional relevance of this diversity is unknown (Bauer et al. 1997). Most striking are the differences of cellular interactions with their plant hosts, which are interpreted as driving forces for the adaptive radiation of the whole subphylum Ustilaginomycotina (Bauer et al. 1997; Begerow et al. 2014).

Several studies based on massive isolation of yeasts from the environment have revealed a species diversity, which seems even larger as the current amount of species of smut fungi (e.g., Boekhout et al. 2006). Especially, lineages within inconspicuous plant pathogens like *Microstroma* or *Entyloma* could harbor an unknown species diversity. For instance, the recently described species of the genera *Jamineae* and *Sympodiomycopsis* in Microstromatales might be just the

tip of the iceberg (Wang et al. 2015a; Francesca et al. 2016; Kijpornyongpan and Aime 2017). These yeasts originate from tropical and subtropical regions of the worlds. Their primary habitat is still unknown; however, plant association is likely, as the isolation sources were flowers, wood material, and herbivore insect frass. Boekhout et al. (2006) reported surprisingly high diversity of previously unknown *Tilletiopsis* species isolated from apple surfaces. Some of these yeasts were phylogenetically placed in the genus *Entyloma*, but anamorph-teleomorph relationships could not be resolved unequivocally so far. Asexual states of species of *Exobasidium* (parasitic fungi of Ericaceae) can be obtained in culture by the spore-fall method (Boekhout 1991). Like other anamorphic Ustilaginomycotina, the yeasts belonging to the genus *Exobasidium* are reported from healthy plants, which are not known as hosts of the parasitic stages (e.g., Inácio 2003). Similarly, yeast states of the grass parasites *Farysia* were isolated from nonhost plants, flowers, and even fruits (Inácio et al. 2008; Begerow et al. 2014). Recent studies suggest large number of Ustilaginomycotina with saprobic asexual states to be discovered, as exemplified with new genera: *Ceraceosorus*, *Fereydounia*, *Uleiella*, and *Violaceomyces* (Nasr et al. 2014; Albu et al. 2015; Kijpornyongpan and Aime 2016; Riess et al. 2016). These new fungal lineages were discovered in highly diverse ecosystems, most of which have yet not been intensively explored for their yeast flora.

7.3.3 *Anther Smuts:* Microbotryum *and Allied Fungi*

Yeasts in the plant pathogenic lineage Microbotryales (Microbotryomycetes, Pucciniomycotina) have only recently became more prominent in the yeast literature. Only a single species, *Rhodotorula hordea* (currently *Ustilentyloma graminis*), was placed in this order. Another yeast from the intestine of a plant bug, *Collaria oleosa* (Heteroptera, Miridae), collected in Costa Rica was described in the new genus *Microbotryozyma* (Suh et al. 2012) within the Microbotryales. Unlike other plant parasites, members of Microbotryales are not commonly isolated as epiphytes from plant surfaces (but see Wang et al. 2016). However, they have been isolated from nectar (e.g., Golonka and Vilgalys 2013), and it has been shown that yeasts can proliferate within flowers before infecting (Schäfer et al. 2010). The Microbotryales comprise two families, the Ustilentylomataceae mainly infecting Poaceae and Cyperaceae and Microbotryaceae on dicots including Polygonaceae and Caryophyllaceae (Bauer et al. 2006). So far, it is not clear if all species can be cultivated and include a yeast stage, but at least some members of the genus *Microbotryum* sporulating in the anthers of Caryophyllaceae (Fig. 7.1g) are easy to cultivate. This made it possible to establish them as a model system for the evolution of host specificity and sexually transmitted disease, as their infection results in male sterile plants (Schäfer et al. 2010). Cultivation success of *Microbotryum* parasites on Polygonaceae is much lower, and only a few cultures of these species have been reported (e.g., Wang et al. 2015b). Besides the already

described yeast *Ust. graminis*, a potential new *Ustilentyloma* species has been recently isolated from bean phylloplane (Prior et al. 2017).

While the genus *Microbotryum* comprises approx. 90 species, the order itself might only include approx. 115 known species in total (Vánky 2012). However, some of the grass-infecting species are very inconspicuous, and there might be a so far overlooked diversity. The phylogeny of Microbotryales is well studied. Dichotomy between monocot- and dicot-infecting lineages has been discussed (Bauer et al. 2006). Within dicot-infecting lineages, radiation on major plant lineages has occurred with an additional specialization to various host tissues (Lutz et al. 2005, 2008; Kemler et al. 2006, 2013; Le Gac et al. 2007; Refregier et al. 2008; Piątek et al. 2011, 2013). Besides being a model system in population genetics, phylogenetics, and infection biology (Schäfer et al. 2010), *Microbotryum* became also relevant for genomics of plant pathogens. The genome has similar properties as genomes of members in the Ustilaginomycotina, such as amount of genes, paucity of cell wall-degrading enzymes, and a large amount of genes coding for secreted effectors (Kämper et al. 2006; Perlin et al. 2015; Toh et al. 2016), but it also shows some peculiarities (Perlin et al. 2015) which could be related to its different mode of infection (Bauer et al. 1997). The genome comprises, for instance, a large number of genes coding lipases and additionally a repertoire of enzymes that could infer with organ development of the host (Perlin et al. 2015).

7.3.4 Other Basidiomycetes

The dimorphic fungus *Itersonilia perplexans* (Cystofilobasidiales, Tremellomycetes) causes flower blight in anemone, dahlia, chrysanthemum, and globe artichoke (McGovern et al. 2006). Other symptoms include seedling blight, leaf spots, necrosis, and root cankers in dill, edible burdock, parsnip, and sunflower. Infections caused by *It. perplexans* resulted in extensive post-harvest losses in cut flower production of China aster and sunflower (reviewed in McGovern et al. 2006). Serious infections by the pathogen were also observed on different herbs including carrot, dill, coriander, parsley, and parsnip in European countries. Growth, sporulation, and infection of *It. perplexans* are favored by high humidity and cool temperatures. Therefore, post-harvest damage from this fungus may occur in cut flowers held under refrigeration (reviewed in McGovern et al. 2006).

Recently, the yeasts *Naganishia adeliensis* and *Naganishia uzbekistanensis* (Filobasidiales, Tremellomycetes, Agaricomycotina) were reported to cause stem and branch canker on stone fruit trees in Iran (Dehghan-Niri et al. 2015; Borhani and Rahimian 2016). These species are known from live and senescent plant material and soils (e.g., Pozo et al. 2011; Yurkov et al. 2015; Mokhtarnejad et al. 2016) and were not previously associated with any disorder of plants.

Kriegeria eriophori (Kriegeriales, Microbotryomycetes, Pucciniomycotina) is phytoparasitic, and the sexual stage develops only on the host plant (Cyperaceae). A few yeasts in genera *Meredithblackwellia*, *Phenoliferia*, and *Yamadazyma*

phylogenetically related to *Kriegeria* were described from plants, soil, and glaciers (e.g., Branda et al. 2010; Wang et al. 2015b). The family Kriegeriaceae is a sister to Camptobasidiaceae, which is comprised by the predominantly psychrophilic yeasts *Glaciozyma* and putative aquatic mycoparasite *Camptobasidium hydrophilum* (Toome et al. 2013). *Mixia osmundae* (Mixiomycetes, Pucciniomycotina) is a rare plant parasite, which is found only on ferns in the genus *Osmunda* (Nishida et al. 2011). It is currently known from Japan and Taiwan only.

7.4 Mycoparasites

The importance of fungi as parasites of plants and animals is well established. But fungal species in general co-occur together with other fungal species in the same community. It therefore should not come as a surprise that some fungal species have evolved the ability to gain their nutrients completely or in parts from other fungi. Considering its potential ecological and evolutionary importance (e.g., Howe and Suberkropp 1993), the knowledge about fungal-fungal interaction is sparse and mostly limited to potential biocontrol fungi, such as species in the genus *Trichoderma* (e.g., Harman 2006). By involving direct cell contact, mycoparasitism is going beyond the fairly well-reviewed antagonistic activity of yeasts, which involves killer proteins, glycolipids, and other agents (e.g., pulcherrimine) (Vustin et al. 1990; Golubev 2006; Sipiczki 2006, see also Chap. 9 of this book). Below, we summarize the current knowledge about mycoparasitic interactions involving yeasts and yeast-like fungi.

7.4.1 Diversity and Interactions

Yeasts can be parasites or predators on other fungi (Lachance and Pang 1997; Lachance et al. 2000). Ascomycetous yeasts in the genus *Saccharomycopsis* and related *Candida* species have been found to share the ability to form infection pegs that penetrate the wall of various other yeast species as well as some molds (Starmer and Lachance 2011). However, comparison of the response of various predacious species to different nutrient regimes disputed this idea (Lachance et al. 2000). These studies instead indicate that the necrotrophic destruction of prey could be a form of competitive exclusion and not the result of a physiological deficiency in essential nutrients like sulfur, as has been hypothesized earlier (Lachance and Pang 1997).

The number of known and potential mycoparasitic yeasts is larger in Basidiomycota than in Ascomycota (comp. Fig. 7.2). Hosts of the basidiomycetous mycoparasites are either Ascomycota or Basidiomycota, and mycoparasitism on Chytridiomycota or Zygomycetes is unknown (Bauer and Oberwinkler 2008). Phylogenetic correlation between the basidiomycetous mycoparasites and their

respective host fungi has not been reported (Bauer and Oberwinkler 2008). Parasitic interactions are associated with the sexual (teleomorphic) stage of the life cycle in the Tremellomycetes (Agaricomycotina), Agaricostilbomycetes (Pucciniomycotina), Microbotryomycetes (Pucciniomycotina), Spiculogloeomycetes (Pucciniomycotina), and Cystobasidiomycetes (Pucciniomycotina), which have been studied as dimorphic heterobasidiomycetes (e.g., Bandoni 1995).

Studies of dimorphic heterobasidiomycetes followed two different directions. The first way, taken by traditional mycologists, relied on mycoparasites sampled in the field, which were investigated in the laboratory, including cultivation (or germination) experiments and research of the interaction modes between the parasite and host. As a result, we know that sexual structures of some mycoparasites (e.g., *Tremella*, *Rhynchogastrema*, *Trimorphomyces*) germinate with yeast states. The second way undertaken by yeast researchers included mating experiments to obtain teleomorphic state on the laboratory media (e.g., *Bulleromyces*, *Curvibasidium*, *Leucosporidium*, and *Papiliotrema*) and the subsequent description of the relevant morphological characters, such as basidial and hyphal morphology. It turned out that species commonly considered as yeasts form a sexual cycle ex situ and display features previously described as an adaptation to mycoparasitism. We also suggest readers to consider earlier reviews on heterobasidiomycetes and mycoparasites by Bandoni (1987, 1995), Bauer and Oberwinkler (2008), and Weiss et al. (2014).

Basidiomycota exhibit two different types of cellular interaction between parasite and host, namely, parasitism with tremelloid haustoria (or fusion interaction) and parasitism involving colacosomes (Bandoni 1995; Bauer and Oberwinkler 2008). Haustoria, thin hyphae growing in close contact with the host hyphae to draw the nutrients (Fig. 7.1h), have been observed in many Tremellomycetes (Agaricomycotina), including the species *Bullera alba* (originally *Bulleromyces albus*), *Bulleribasidium oberjochense*, *Dioszegia antarctica*, *Cr. neoformans*, *Holtermanniella mycelialis*, *Rhynchogastrema coronatum*, *Sterigmatosporidium polymorphum* (*Cuniculitrema polymorpha*), *Syzygospora alba*, *Syzygospora pallida* (*Christiansenia pallida*), *Tetragoniomyces uliginosus*, and *Tremella* sp. (e.g., Metzler et al. 1989; Kirschner et al. 2001; Golubev and Golubev 2003). Haustoria-like hyphae have been also reported for Pucciniomycotina, e.g., *Classicula*, *Cyphobasidium*, *Cystobasidium*, *Mycogloea*, *Naohidea*, *Occultifur*, *Spiculogloea*, and *Zygogloea* (Bauer and Oberwinkler 2008). The interaction with tremelloid haustoria involves the fusion of parasite and host cell membranes, inducing direct contact of both partners' cytoplasm (Bauer and Oberwinkler 2008).

Another type of structures responsible for host-parasite interaction observed in culture are colacosomes (Bauer and Oberwinkler 2008). This organelle is formed at the interface between the parasite and its fungal host (Fig. 7.1i). This mycoparasitic organelle was first described in detail from the interaction of the parasite *Colacogloea peniophorae* (Microbotryomycetes, Pucciniomycotina) and its host *Hyphoderma praetermissum* (Bauer and Oberwinkler 1991). Colacosomes develop in the contact area between the parasite and its host and are positioned at the inner

surface of the parasite cell outside the cytoplasm but inside the cell wall (Bauer and Oberwinkler 2008). These organelles have so far been only found within the Microbotryomycetes and seem to occur in several, distantly related families within this class, thereby indicating a potential early origin or convergent evolution of this trait within Microbotryomycetes. Yeasts commonly thought to be pure saprobes, i.e., in *Leucosporidium*, *Rhodosporidium* (currently *Rhodotorula*), and *Sporidiobolus* (currently *Sporobolomyces*), are also known to contain colacosomes, as well as former *Rhodotorula* yeasts, which are phylogenetically related to the parasite *Col. peniophorae* (Sampaio et al. 2003, Bauer and Oberwinkler 2008; Wang et al. 2015b).

7.4.2 *Pucciniomycotina*

Mycoparasites in this lineage include the genera *Agaricostilbum*, *Atractogloea*, *Camptobasidium*, *Chionosphaera*, *Classicula*, *Colacogloea*, *Colacosiphon*, *Cryptomycocolax*, *Cyphobasidium*, *Cystobasidium*, *Heterogastridium*, *Mycogloea*, *Naohidea*, *Occultifur*, *Rhodotorula*, *Spiculogloea*, *Sporobolomyces*, *Zygogloea*, and some species of *Platygloea* (reviewed in Bauer and Oberwinkler 2008). Some of these fungi (genera *Cyphobasidium* and *Cystobasidium* in Cystobasidiomycetes) are lichenicolous, but the spectrum of lichen-associated taxa is most likely much larger as suggested by recent observations of yet undescribed yeasts in Cystobasidiomycetes in the cortex of lichens collected from different regions of the world (Spribille et al. 2016). Unfortunately, the performed phylogenetic analyses do not allow a solid interpretation of the taxonomic position of these yeasts. The authors indicated lichenicolous parasites of the species *Cyphobasidium usneicola* and *Cyphobasidium hypogymniicola* (both formerly classified as belonging to the genus *Cystobasidium*) as the closest match. However, the genus *Cyphobasidium* is not monophyletic as demonstrated by Millanes et al. (2016), and its position within Cystobasidiomycetes has not been resolved. The observation of the Cystobasidiomycetes yeasts with lichens showing no parasitic interactions goes in parallel with the detection of *Fellomyces*-related sequences (originally Tremellales sp. A/B) from necrotic parts of the lichen thalli, without any basidiomata or basidiomyceteous hyphae visible (Lindgren et al. 2015). This data indicates that lichens can be a promising source of a yet unknown basidiomycetous, mycoparasitic, yeast diversity.

7.4.3 *Ustilaginomycotina*

Several ecological adaptations facilitate the ability of asexual Ustilaginomycotina saprobic stages to grow and survive in their natural habitats. Among them is the ability to sustain low temperature, microaerophilic conditions, and antagonistic

activity directed toward bacteria and other fungi. Such a behavior is reported for yeasts previously classified in the genera *Pseudozyma* and *Tilletiopsis* (reviewed in Begerow et al. 2014). However, it must be emphasized that most of this behavior does not classify as mycoparasitism in the strict sense and is only included for the sake of completeness. Antagonistic reactions toward other fungi were reported for *Anthr. flocculosa*, *Kalmanozyma fusiformata*, *Sporisorium graminicola*, *Golubevia pallescens*, *Phragmotaenium flavum*, and *Robbauera albescens* (formerly *Pseudozyma fusiformata*, *Pseudozyma graminicola*, *Tilletiopsis pallescens*, *Tilletiopsis flava*, and *Tilletiopsis albescens*, respectively) which were reported to secrete glycolipids and modified long-chain fatty acids (reviewed in Begerow et al. 2014). Consequently, some Ustilaginomycotina yeast species might even have evolved a mycoparasitic lifestyle, as has been suggested for *Till. pallescens*, which was repeatedly isolated from basidiocarps of other fungi (Boekhout 2011). Also, GFP-labeled *Anthr. flocculosa* observed on plants infected with mildew showed strong association with mildew colonies in laboratory experiments (Neveu et al. 2007). It is however unclear whether *Anthracocystis* (teleomorph is a grass pathogen) and *Golubevia* species compete for the resources on infected plants or act as hyperparasites.

7.4.4 Agaricomycotina

In the circumscription of the order Tremellales, Bandoni (1984, 1987) indicated mycoparasitism among other characters common for this group of fungi. This view received additional support with time. As reviewed by Weiss et al. (2014), the mycoparasitic lifestyle is a distinctive feature of the teleomorphic stages for many, if not all, members of the Tremellomycetes. Obvious host specificity and morphological evidence, such as the presence of host hyphae inside fruiting bodies of Tremellomycetes and the presence of tremelloid haustoria, indicate such a lifestyle as ancestral in this lineage. Additional evidence of the presumably parasitic lifestyle of many tremellaceous yeasts comes from phylogenetic studies, which showed yeasts previously classified in the genera *Bullera* and *Cryptococcus* intermixed with *Tremella* species (e.g., Fell et al. 2000; Scorzetti et al. 2002; Millanes et al. 2011; Weiss et al. 2014). The recent analysis of 286 type strains of yeast species and 47 basidiocarp-forming Tremellomycetes supported the previous observation regarding close relationships between yeasts and mycoparasites in Filobasidiales, Holtermannialles, Tremellales, and Trichosporonales (Liu et al. 2015).

In Filobasidiales, yeasts related to *Cryptococcus arrabidensis* are clustered with the lichenicolous genus *Heterocephalacria* (former *Syzygospora*) (Liu et al. 2015). The fungicolous species *Syzygospora sorana* was transferred into the genus *Piskurozyma* together with *Filobasidium capsuligenum* (*Piskurozyma sorana* and *Piskurozyma capsuligena*, respectively) and several *Cryptococcus* species (Liu et al. 2015). In the Tremellales, yeasts were observed in almost every clade containing known and putative mycoparasites, i.e., *Bulleribasidium* (former

Mingxiaea), *Dioszegia* (*Di. antarctica*), *Carcinomyces* (former *Bullera*), *Cryptococcus* (*Fil. lutea* and *Filobasidiella depauperata*), *Papiliotrema* (former *Auricullibuller*, *Bullera*, and *Cryptococcus*), *Phaeotremella* (former *Cryptococcus*), *Pseudotremella* (former *Cryptococcus*), *Rhynchogastrema* (*Bandoniozyma* yeasts), *Tremella sensu stricto* (former *Cryptococcus*), and *Tremella* clades I and II (*sensu* Millanes et al. 2011). Other mycoparasitic genera in the Tremellomycetes [i.e., *Fibulobasidium*, *Sirobasidium*, *Sterigmatosporidium* (former *Cuniculitrema*), and *Trimorphomyces*] were already known to produce yeast stages in culture. Although members of the *Tremella encephala* clade (currently *Naematelia encephala*) are cultivable, no previously described yeast species have been found in this clade (Liu et al. 2015). Yeast stages are also not yet known for the lichenicolous *Tremella* clade III (*sensu* Millanes et al. 2011), which also contains the genus *Biatoropsis* (Liu et al. 2015; Millanes et al. 2016). Both *Holtermannia* and its putative asexual counterpart *Holtermanniella* are known to form tremelloid haustoria. The only known sexual species in Trichosporonales, the fungicolous parasite *Tetrag. uliginosus*, showed weak relationships to the yeast genus *Cryptotrichosporon* (Liu et al. 2015).

The study performed by Liu et al. (2015) provides a good overview on the phylogeny of Tremellomycetes. As the study reduced polyphyly in this class and attempted to unify taxonomy of sexual and asexual fungi, it provides an excellent starting point to study the evolution of mycoparasitism in this group of Basidiomycota.

7.5 Concluding Remarks

In the eyes of non-specialists, yeasts are often synonymized with the fermenting ascomycete *Saccharomyces*. However, a unicellular yeast stage is realized in many fungal lineages, and most of these yeast species do not share the typical saccharolytic (or sometimes copiotrophic, i.e., the preference of nutrient-rich environments) lifestyle. Among the growing number of yeast species studied, we observe an increasing percentage of parasitic species—especially dimorphic species tend to be pathogenic at least in parts of their life cycle.

While some species like *Candida* or *Exophiala* are prominent opportunistic (not obligatory) parasites in immunocompromised patients, other yeasts have evolved a parasitic stage and cannot complete their life cycle without it. Thus, it becomes increasingly important and more relevant to understand yeasts as a pluripotent unicellular stage of fungi being highly diverse in terms of nutrition mode and ecology.

While human parasites are well recognized in yeast research, yeasts infecting plants and especially fungi are rarely studied, and our knowledge is very scarce. Herewith, we aimed to summarize some of the current knowledge in this field and to point toward further research directions. We would like also to motivate others to study yeasts with respect to their pathogenic capabilities.

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Chapter 8
Commensalism: The Case of the Human Zymobiome

João Inácio and Heide-Marie Daniel

Abstract The mycological community of humans is subject to numerous interactions, for example, among cohabitating fungi, other microbes, their hosts, as well as biotic and abiotic factors the host is exposed to. Yeasts form an important part of this community. While human-colonising yeasts receive high attention as opportunistic pathogens, they are less recognised as commensals. The ecology of the yeast-human relationship bears many open questions. This includes the potential effects of colonising yeasts on humans. Negative effects may be linked to an imbalance of total microbiota, and literature often associated the state of health with high mycological diversity. The mycological communities are less well studied compared to the bacterial components, and a systematic evaluation of the fungal diversity that colonises humans is still difficult. Literature suggests that the same yeast species that are known as frequent opportunists (e.g. *Candida albicans*) may also play beneficial roles, while dominantly as beneficial recognised yeasts (e.g. *Saccharomyces cerevisiae*) may turn infective in states of immune impairment. The yet incomplete list of factors that influence yeast diversity in humans includes age, diet, body site, medical treatments, bacterial community composition, and immune status. Further studies of this area are hoped to extend the knowledge on healthy yeast diversity and the interactions in which yeasts participate.

Keywords Yeast • Zymobiome • Fungi • Mycobiome

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Contents

8.1 Introduction

Humans, as other animals, are host to various microbial communities (microbiomes) that interact with each other and the host. Microbes cause in most cases no harm to their host while benefiting from the sheltered ecological niche, a relation that may be called commensalism. While some microbes undoubtedly are able to cause disease and are considered parasites (or pathogens), emerging evidence indicates that some so-called microbial commensals may be beneficial to the host and may represent mutualists. However, microbial relations may take different significance for the host under different conditions, and the strict separation of commensal, mutualistic, or parasitic relations may be difficult. Microbial communities influence processes ranging from digestion to behaviour, and at least some are thought to be fundamental components for homeostasis (Clemente et al. 2012; Tremaroli and Bäckhed 2012; Erturk-Hasdemir and Kasper 2013). It is established that the bacterial microbiome has a strong influence on health and disease, with many bacteria capable to influence fungal growth (Kerr 1999; Hogan et al. 2004; Cogen et al. 2008). The study of the fungal component of the microbiome (mycobiome), including yeasts (which we designate here as zymobiome), lags behind the study of the bacterial microbiome (Huffnagle and Noverr 2013; Underhill and Iliev 2014).

The human zymobiome is frequently understood as a bloom of opportunistic pathogens such as some species of the genera *Candida* (notably *Candida albicans*), *Malassezia*, and the species complex represented by *Cryptococcus neoformans*. The yeasts detected in a clinical, hence disease-linked context have received high interest. Pfaller et al. (2012) reviewed candidemia surveys in the United States and listed the most frequently detected species as *C. albicans*, *Candida glabrata* (*iter. nom.*[1] *Nakaseomyces*), *Candida parapsilosis*, *Candida tropicalis*, and *Pichia kudriavzevii* (= *Candida krusei* = *Issatchenkia orientalis*). Data on the yeasts harboured by healthy humans are restricted by far lower study and sample numbers, but the range of yeasts frequently, although not systematically, overlap with those detected in clinical contexts (Cui et al. 2013). While yeast blooms may cause a range of conditions from skin affectations to serious disease, the involved yeast

[1] *Iterum nominanda* (to be renamed).

species may have been present as commensals in or on the body over a long time without compromising the host. The ability of certain yeasts to invade the host in conditions such as imbalanced immunity, altered microbiota or exposure to unusual inoculants (high loads, indwelling catheters) qualifies them as opportunistic pathogens that are able to use a change in the host's system to become pathogen. Virulence factors that enable yeasts to become invasive in warm-blooded hosts are intensely studied and include temperature relation, dimorphism, biofilm formation as well as biochemical properties such as polysaccharide capsule, melanin and phospholipase in *Cr. neoformans* (Casadevall 2007). It is recognised that a yeast's ability to become a pathogen is due to multiple functions and regulations that need further study (Roetzer et al. 2011; Neville et al. 2015) and that differ between species. Due to clinical importance, for example, on indwelling catheters, prostheses and dental surfaces, microbial biofilms have attracted high interest, and many of them involve yeasts and bacteria. While diverse biofilm forming bacteria have been studied, mixed biofilm models mostly use *C. albicans* as yeast component. Studies have pointed towards synergistic and antagonistic interactions that influence adhesion, may be based on metabolism and on quorum sensing and may contribute to the conversion of the yeast from commensal to pathogen (d'Enfert 2009; Shirtliff et al. 2009). A particular case is the bidirectional interaction between *Acinetobacter baumannii*, an emerging opportunistic pathogen, and *C. albicans*. The bacilli may inhibit filamentous yeast growth and kill the yeast, while *C. albicans* in a sufficient population density as encountered in biofilms may inhibit *Acin. baumannii* (Peleg et al. 2008; Kostoulias et al. 2016). The potential impact of *Acin. baumannii* on human health was recognised by its listing among the highest priority target bacteria to guide antibiotic development (World Health Organization 2017). The reproducible isolation of *C. albicans* from oxygen-limited zones of a sewage-polluted river inspired research that showed growth inhibition of the yeast by co-isolated Enterobacteriaceae in aerobe, but not anaerobe conditions (Benadé et al. 2016). The authors linked the observation to extracellular yeast cell wall proteolytic bacterial enzymes that were produced under aerobe conditions. An example for yeast-host interaction is the response of *C. albicans* to the immune status of the host through the transcription factor Efg1p (Tyc et al. 2016).

Research into the species diversity, interspecies relationships and interactions of the mycobiome with their host is still relatively scarce. Available data on the mycobiome (and zymobiome) diversity is likely to be incomplete because of the reliance of most studies on culture-based methods. Advances in next-generation sequencing (NGS) approaches and metagenomics have highlighted a previously unrecognised diversity of fungi as part of the human microbiome (Ghannoum et al. 2010; Delhaes et al. 2012; Park et al. 2012; Findley et al. 2013; Hoffmann et al. 2013; Dupuy et al. 2014), although its role in host metabolism and immunity remains largely unknown. Unknown or uncultivable fungi formed large fractions of the recovered DNA (Ghannoum et al. 2010; Chen et al. 2011; Delhaes et al. 2012; Hoffmann et al. 2013), and although some of them might be resolved as well-known taxa by improved database annotations, others may not, and they deserve further investigation of potential impact on health and disease.

There are some limitations when analysing NGS-derived fungal sequence data (Nilsson et al. 2006; Dupuy et al. 2014; Tang et al. 2015). Although species coverage in NCBI GenBank databases should be better for yeasts than they are for filamentous fungi, sequence annotations are still problematic and should be judged carefully, ideally in reliance on type strain sequences. One important issue is the dual-name anamorph/teleomorph nomenclature in fungi. In following the 'one fungus, one name' principle, members of the commonly reported artificial genera *Candida* and *Cryptococcus* have been or will be assigned to their phylogenetically most closely related or to new genera (Daniel et al. 2014; Liu et al. 2016). An unawareness of nomenclatural issues and a failure to appropriately collapse synonymous taxa may result in a misleading view of the species richness and relative abundance of fungal communities.

We raise some considerations about the zymobiome of distinct anatomical environments of humans for which the respective inhabiting fungal communities have been studied recently using molecular-based, NGS and/or metagenomics tools: the gastrointestinal tract (mainly comprising the gut), the oral cavity (mouth), the genitourinary tract, the respiratory tract (lungs) and the skin/scalp.

8.2 The Gastrointestinal Tract

Most studies on the human microbiome have focused on gut bacteria, particularly inhabiting the colon and recovered from faeces (Gill et al. 2006; Arumugam et al. 2011). Fungi are also found in the intestinal tract, and yeasts have been detected in human stool since the beginning of the last century (Ashford 1915; Anderson 1917). However, while the diversity and function of gut bacteria have been broadly characterised, little is known about the composition and dynamics of commensal mycobiota in this environment and its role in health and disease. The human gut harbours hundreds of fungal taxa, with their majority reported in single or a low number of studies (Suhr and Hallen-Adams 2015). Recent reports using NGS approaches showed, for example, 66 genera detected by Hoffmann et al. (2013). While yeasts represent approximately 40% of the fungal gut taxa, with *C. tropicalis* and *C. albicans* as the most commonly reported commensal fungi from the intestine (Hallen-Adams and Suhr 2017), yeasts appear as more abundant than filamentous fungi by NGS (Sokol et al. 2017). Other yeasts from the gut belong to some genera and species such as *Nakaseomyces* (*C. glabrata*), *Clavispora lusitaniae* (= *Candida lusitaniae*), *Naganishia globosa* (= *Cryptococcus saitoi*), *Cystofilobasidium capitatum*, *Debaryomyces hansenii*, *Galactomyces geotrichum*, *Galactomyces candidus* (= *Geotrichum candidum*), *Malassezia restricta*, *Malassezia globosa*, *Meyerozyma guilliermondii* (= *Pichia guilliermondii*), *P. kudriavzevii*, *Pichia fermentans*, *Pichia manshurica*, *Pichia kluyveri*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*, *Trichosporon asahii* and others (Angebault et al. 2013; Hoffmann et al. 2013; Gouba et al. 2014; Hallen-Adams et al. 2015; Suhr and Hallen-Adams 2015; Hallen-Adams and Suhr 2017; Strati et al. 2016; Suhr et al.

2016). Which of these taxa are true residents and which are transients originating from food, probiotic food complements or the environment is unknown. A majority of transient fungi in the gut may also explain the fact that the composition of the gut mycobiome does not seem to be stable over time in the same individual (Hallen-Adams et al. 2015; Hallen-Adams and Suhr 2017). *C. albicans* is regarded as the most prevalent yeast species in humans, but recent studies highlighted that different yeast colonisation patterns may exist. *P. kudriavzevii*, *S. cerevisiae* and *C. tropicalis*, possibly derived from food or environment, were predominant among a remote community of Amerindians living in French Guiana (Angebault et al. 2013). A high incidence of *S. cerevisiae* was tentatively accounted to the ubiquity of this species DNA in food (Hoffmann et al. 2013; Suhr and Hallen-Adams 2015; Sokol et al. 2017). Alterations in diet have been shown to affect the composition of the gut mycobiota, and a carbohydrate and plant-based diet has been linked to an increase in *Candida* prevalence (David et al. 2013; Hoffmann et al. 2013). Unfortunately, without identification of the *Candida* members to the species level, this result does not allow hypotheses on the origin or role of these yeasts in view of the large phylogenetic and hence ecologic divergence covered by the genus (Daniel et al. 2014).

The study of the relationships between gut yeasts and the human host is in its early stages, sample numbers are restricted, mechanistic studies are based on animal models, and therefore interpretations require great care. Anti-*Saccharomyces* antibodies against yeast mannan, which have been shown to be also reactive to *Candida* species (Standaert-Vitse et al. 2006), have been detected in inflammatory bowel disease patients and may be useful to judge disease progression (Seibold et al. 2001; Murdoch et al. 2012). Fungal and bacterial microbiota in humans afflicted by inflammatory bowel disease compared to healthy subjects showed interdependent modifications and suggest a role of gut fungi in gut health (Moyes and Naglik 2012; Mukherjee et al. 2015; Richard et al. 2015; Sokol et al. 2017; Liguori et al. 2016). Basidiomycota abundance (e.g. Malasseziales, Filobasidiaceae) was increased at the cost of Ascomycota (dominated by *Saccharomyces* and *Debaryomyces*) during active disease phases. In addition, *S. cerevisiae* was decreased, and *C. albicans*, although an overall minor component, increased. Crohn's disease was characterised by an overrepresentation of Cystofilobasidiaceae and *C. glabrata* (Liguori et al. 2016).

Gut mycobiota may be a source of fungaemia (e.g. by *C. albicans*) when systemic or local mucosal immune functions are disturbed by extrinsic factors (Nucci and Anaissie 2001; Miranda et al. 2009; Gouba and Drancourt 2015). Disruption of the indigenous microflora in mice by antibiotics allowed *C. albicans* to proliferate in the gut and to spread to other organs (Kennedy and Volz 1985). On the other hand, also intrinsic host or fungal features such as polymorphisms in receptor or signalling pathways or strain-specific cell surface differences may contribute to susceptibility differences to fungal infection (Underhill and Pearlman 2015). Dectin-1, a glucan-recognising immune receptor, deficiency increased severity of chemically induced intestinal inflammation in mice by *C. tropicalis*, but not by *Saccharomycopsis fibuligera* (Iliev et al. 2012).

Antifungal treatment in dectin-1 competent mice was effective to decrease the load of *Penicillium brevicompactum* and *C. tropicalis* in the gut, but *Aspergillus amstelodami*, *Epicoccum nigrum* and *Wallemia sebi* expanded upon this treatment, and bacterial communities also changed. The antifungal treatment and also oral supplementation of mice with the latter three fungi exacerbated house dust mite-mediated allergic airway disease without the presence of these fungi in the lung (Wheeler et al. 2016).

Attention needs to be extended from fungal-host to bacterial-fungal interactions that may occur in the host regulated by quorum sensing, involving biofilm formation and other mechanisms (Klotz et al. 2007; De Sordi and Mühlschlegel 2009; Peleg et al. 2010; Wang et al. 2014). Fungi, in particular yeasts, showed a potential to exert marked effects on the bacterial community reassembly after antibiotic (antibacterial) treatment (Mason et al. 2012; Erb-Downward et al. 2013). In eradiating gut bacteria, such treatment elevated fungal colonisation and promoted lung inflammation in mice (Noverr et al. 2004, 2005). This effect has been linked to the fungal-driven conversion of host arachidonic acid into prostaglandin a potent immunomodulatory (Noverr et al. 2001; Kim et al. 2014). All of the above suggest that normal fungal, yeast and bacterial populations of the gut help to maintain a healthy intestinal homeostasis including the regulation of immune response.

The knowledge on the beneficial effects of living yeast cells or extracted compounds has led to the development of commercialised preparations. Yeast-derived cell wall glucans as immune modulatory have received intense interest (Samuelsen et al. 2014; Stier et al. 2014). A subpopulation of *S. cerevisiae*, known as *Saccharomyces boulardii* (*nom. nud.*) or *S. cerevisiae* subsp. *boulardii*, was found to have positive influence on the recovery from gastroenteritis and is now well studied for its probiotic aspects (McFarland 2010). Other yeast species have been explored for similar properties (e.g. *Deb. hansenii*, *Kazachstania lodderae*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Yarrowia lipolytica*) (Hatoum et al. 2012). A recent patent application secured the use of another commensal gut yeast, *Sacch. fibuligera*, for prophylaxis and treatment of intestinal disorders, obesity and colon cancer (Underhill and Iliev 2013). The application of especially living cell preparations is an intervention towards the existing, (im)balanced microbiota that requires the consideration of possible side effects, including rare invasive yeast infection (Enache-Angoulvant and Hennequin 2005; Martin et al. 2017).

8.3 The Oral Cavity

The human mouth is colonised by a variety of microorganisms, including bacteria and fungi. Yeasts were established as normal inhabitants of the mouth in about 50% of healthy college students with a higher incidence in acidic conditions and a prevalence of *C. albicans* higher than 90% (Young et al. 1951). Yeasts are carried by most individuals asymptomatically, with the prevalence increasing with age

(Wade 2013). NGS and metagenomic approaches considerably extended the list of fungi detected in the human mouth mycobiome (and zymobiome), while the findings of culture-based studies principally confirmed the presence of *Candida*, with emphasis for *C. albicans*. Other yeasts, which are often recovered from the oral cavity, particularly in immunosuppressed individuals, are *C. glabrata*, *Candida dubliniensis*, *C. parapsilosis*, *C. tropicalis*, *Diutina rugosa* (= *Candida rugosa*), *P. kudriazevii*, *Mey. guilliermondii*, and *Cl. lusitaniae* (Diaz et al. 2017). Additional potential members of the mouth zymobiome disclosed by NGS-based studies are *Cryptococcus*, *Cyberlindnera* (e.g. *Cyberlindnera jadinii*), *Hyphopichia* (including the phylogenetically related *Candida khmerensis*), *Malassezia*, *Saccharomyces*, *Schizosaccharomyces*, *Trichosporon* and *Zygosaccharomyces* (Ghannoum et al. 2010; Dupuy et al. 2014; Mukherjee et al. 2014; Diaz et al. 2017; Imabayashi et al. 2016). Such studies are based on low sample numbers, and large differences between samples were noted. The high prevalence and abundance of *Malassezia*, common skin commensals and pathogens, found in some studies suggest the potential underestimation of this genus in oral samples due to nutritional requirements and ineffective DNA extraction protocols (Dupuy et al. 2014; Diaz et al. 2017). Not all detected fungi are true colonisers, and many represent transient ubiquitous organisms acquired from food or from the environment (Ghannoum et al. 2010; Diaz et al. 2017). Although the presence of fungi with invasive potential in the mouth was suggested as a predisposing factor, their high frequency in healthy individuals suggests that additional factors are necessary before fungal invasion may take place. A comparison of the oral mycobiome of healthy and oral candidiasis-afflicted persons showed a prevalence of *C. albicans* in both groups (Imabayashi et al. 2016). The study suggests that an increase in fungal load and a shift in species composition may be linked to the appearance of clinical symptoms. A possible link of low *Candida* colonisation with the presence of *Mey. guilliermondii*, *Hyphopichia* and *Cyb. jadinii* was suggested by Mukherjee et al. (2014). Their elucidation of the potential inhibition mechanisms showed that *C. albicans* growth, biofilm formation, germ tube formation and catheter adherence were all reduced in co-culture with *Millerozyma farinosa* (= *Pichia farinosa*) or if exposed to spent *Mill. farinosa* growth medium. Mukherjee et al. (2014) also found a higher fungal diversity in healthy individuals when compared to HIV-infected individuals. Nevertheless, it was also demonstrated that different members of the oral mycobiota may interact to establish infectious processes. A recent study showed that *C. glabrata* is able to cause oral thrush after binding to the hyphae of *C. albicans* (Tati et al. 2016). In the field of bacterial-fungal interactions, the presence of *C. albicans* in biofilms with *Streptococcus mutans* has been shown to amplify dental caries (Falsetta et al. 2014). The effect was observed under the conditions of sucrose presence and restricted saliva access that lead to a strong production of exopolysaccharides by bacterial excreted enzymes on the surface of the yeast cells and hyphae. It is conceivable that the co-occurrence of *C. albicans* and *Str. mutans* is a precursor for severe dental caries provoked by microbial metabolic responses to their environment. In another remarkable example, the presence of oral commensal bacteria such as *Streptococcus oralis* seems to enhance

the severity of oropharyngeal candidiasis in murine models, due to the intensification of local pro-inflammatory responses during co-infection (Xu et al. 2013; Diaz et al. 2014).

8.4 The Genitourinary Tract

Similarly to other mucosal anatomical locations of the human body, the vagina is colonised by a beneficial microbiome, notably by lactic acid bacteria (LAB) that maintain a low pH and prevent the development of pathogens in this environment (White et al. 2011). Yeasts, particularly *C. albicans*, colonise about one fifth of women asymptomatically, although yeasts are also a leading cause of infectious vaginitis (Sobel 2007). A cultivation-independent 18S rRNA gene clone library, confirmed by cultures, showed *C. albicans*, *S. cerevisiae* and *C. tropicalis* in similar abundance in healthy women (Guo et al. 2012). Other yeasts were found less frequently in healthy women, such as *Candida sake* and unidentified basidiomycetous yeasts (Guo et al. 2012). The study compared the healthy state to vaginal disease and allergic rhinitis and detected species shifts, for example, increased *C. albicans* and decreased *S. cerevisiae* loads in the diseased state (Guo et al. 2012). The authors concluded that fungal dysbiosis is linked with disease states. An NGS-based study of healthy women reported 161 unique species matches in the UNITE database with the most abundant taxa: *C. albicans*, *Cladosporium* (including those reported as Davidiellaceae sp. PIMO_97) and *Exophiala* (reported as uncultured fungus_1) (Drell et al. 2013). Other reported yeasts included *Candida alimentaria* (*iter. nom. Yarrowia*, reported as *Candida* sp. VI04616), *C. dubliniensis*, *C. parapsilosis*, *P. kudriavzevii* and *Rhodotorula*. Despite the far larger diversity detected by NGS, the dominance of yeasts, particularly of *C. albicans*, was confirmed in 65% of the women, more than previously estimated. The conditions in the human vagina are unique compared to other body sites. Apart from commonly encountered low pH and presence of LAB, particular conditions include the production of glycogen and its breakdown by alpha amylases, epithelium shedding in response to the cycles of reproductive hormones and primarily innate and not adaptive immunity (Bradford and Ravel 2017). Knowledge on the *C. albicans* colonisation of the vagina in healthy conditions includes increase during pregnancy, estrogenic influences of yeast adherence to epithelial cells and lowered yeast uptake by immune cells with lactic acid as sole carbon source and points towards a high adaption of the yeast to this niche in interaction with the human host and other commensals such as lactic acid bacteria (Bradford and Ravel 2017). The transition from yeast to hyphal growth was linked to inflammation (Peters et al. 2014). Interestingly, the yeast-hyphae switch was inhibited in *C. albicans* by metabolic products of LAB such as short chain fatty acids and lactic acid as well as by LAB culture supernatants and live LAB (Noverr and Huffnagle 2004). Findings of low LAB numbers during yeast vaginitis seem to substantiate the protective power of a balanced micro- and mycobiome (Bradford and Ravel 2017).

8.5 The Respiratory Tract and the Lungs

The lungs of healthy individuals were believed to be sterile until a few years ago, but several more recent studies have evidenced that this environment may also contain microbial inhabitants (Nguyen et al. 2015; Dickson et al. 2016; Tipton et al. 2017; Krause et al. 2017). Bacteria seem to predominate at this anatomical site, but viruses, fungi and other eukaryotes may be also present (Charlson et al. 2012; Delhaes et al. 2012; Bittinger et al. 2014; Nguyen et al. 2015; Tipton et al. 2017). Whether these organisms are true commensals or just transients acquired from the environment is mostly unknown. Fungi and their spores are ubiquitous in the environment, and thus, it is no surprise that most fungal species detected in the lungs are widespread environmental moulds such as *Cladosporium*, *Penicillium*, *Aspergillus* and *Eremothecium* (Charlson et al. 2012; van Woerden et al. 2013; Bittinger et al. 2014; Kramer et al. 2015; Tipton et al. 2017). Yeasts and yeast-like fungi are not (Bittinger et al. 2014; Krause et al. 2016) or less commonly found in the healthy lungs and include *Candida*, *Malassezia*, *Kluyveromyces*/*Vanderwaltozyma* and *Pneumocystis* (Delhaes et al. 2012; van Woerden et al. 2013; Tipton et al. 2017). The lung mycobiome seems highly variable among different individuals, further supporting its transient nature and environmental origin (Tipton et al. 2017). The lung microbiome, including its fungal component, is mostly assessed by DNA-based approaches since the culture from samples of healthy lungs, such as bronchoalveolar lavage (BAL) fluids or induced sputum, is often unsuccessful. Additional methodological difficulties for studying the lung mycobiome are the need for invasive procedures to collect the samples, the likelihood of these samples being contaminated by the oral mycobiome during the collection and the low load of fungal DNA in these samples, which are dominated by the presence of human DNA (Tipton et al. 2017; Krause et al. 2017). Despite the apparent low fungal load in healthy lungs, their populations are affected by different respiratory disorders including cystic fibrosis, asthma, lung transplant and chronic obstructive pulmonary disease (COPD) (Charlson et al. 2012; Delhaes et al. 2012; Cui et al. 2015). As a general trend, these diseases are often accompanied by an increase of the fungal load but also by a reduced fungal diversity in the lungs, when compared with healthy individuals, with a prominence of *Candida*, particularly *C. albicans* (Charlson et al. 2012; Delhaes et al. 2012; Bittinger et al. 2014; Cui et al. 2015; Kim et al. 2015; Nguyen et al. 2015; Tipton et al. 2017; Krause et al. 2017). *Candida* dominance in the lungs was linked to intensive care unit treatment including the intubation and mechanical ventilation, but not to antimicrobial therapy or pneumonia (Krause et al. 2016). Other environmental widespread filamentous fungi such as *Aspergillus*, *Penicillium* and *Scedosporium* and the yeasts *Cryptococcus*, *Saccharomyces* and *Malassezia* are also commonly found in compromised lungs (Delhaes et al. 2012; van Woerden et al. 2013; Bittinger et al. 2014; Kim et al. 2015; Kramer et al. 2015; Nguyen et al. 2015). Some of these, particularly *Mal. restricta* and *Mal. globosa*, *Aspergillus* and *Cryptococcus*, seem also frequent in the nasal vestibule mycobiome (Jung et al.

2015). The oropharynx was well distinguished in terms of a high *Candida* abundance also in healthy subjects, with other yeasts such as *Debaryomyces*, *Malassezia*, *Pichia*, *Rhodotorula* and *Saccharomyces* also detected (Bittinger et al. 2014).

It remains to be further evaluated how cross-kingdom microbial interactions may influence the composition and stability of the lung microbiota. Antimicrobial therapy did not lead to a loss of fungal diversity and influence *Candida* colonisation, and no bacterial communities were linked with *Candida* in the lung (Krause et al. 2016). Nevertheless, *Candida* abundance in the oropharynx was correlated with its bacterial community structure, in particular with streptococci, *Rothia* and *Veillonella* (Bittinger et al. 2014). *Pseudomonas aeruginosa* and *C. albicans* were often co-isolated from the same cystic fibrosis-suffering patients (Lindsay and Hogan 2014). *Pseudom. aeruginosa* affects the transition to the hyphal morphology in *C. albicans* (Hogan et al. 2004; Morales et al. 2013), thereby potentially preventing the overgrowth and invasiveness of this opportunistic fungal pathogen in the lungs. Remarkable strains of *C. albicans* resistant against the filamentation-inhibitory effects of *Pseudom. aeruginosa* were also recovered from the lungs of cystic fibrosis patients (Kim et al. 2015).

8.6 The Skin and the Scalp

The human skin is the first line of defence against invasion by harmful microorganisms but is also the home for a complex community of resident bacteria, viruses and eukaryotes, including fungi. The skin microbiome varies between different anatomical locations, in function of the different microhabitats providing a range of water availability, temperature, pH, presence of antimicrobial compounds and structures such as glands and hair follicles (Costello et al. 2009; Schommer and Gallo 2013). It also varies between individuals but remains relatively stable over long periods of time (Costello et al. 2009). Former culture-based studies have reported *Malassezia*, *Rhodotorula*, *Cryptococcus*, *Debaryomyces* and *Candida* as members of the skin fungal community (Roth and James 1988). Combined amplicon sequencing and culture-based studies showed the predominance of *Malassezia* species on body skin (Findley et al. 2013; Leung et al. 2016; Sugita et al. 2016). *Mal. globosa*, *Mal. restricta* and *Malassezia sympodialis* seem to dominate the skin zymobiome, with more than ten *Malassezia* species detected in total (Findley et al. 2013; Sugita et al. 2016). Other yeasts found were *C. albicans*, *C. tropicalis*, *Naganishia albida* (= *Cryptococcus albidus*), *Papiliotrema laurentii* (= *Cryptococcus laurentii*), *Cyb. jadinii*, *P. kudriavzevii* and *Tr. asahii*. Distinct *Malassezia* species seem to show some tropism for different areas of the skin, for example, with *Mal. globosa* more associated to the back and occiput and *Mal. restricta* mostly found on the scalp and in the external auditory canal (Findley et al. 2013; Leung et al. 2016). Such tropism may reflect species-specific lipid requirements that may have masked some species in culture-based studies. Age-related

differences of lipid profiles may explain the differences found among the skin mycobiota of children and adults, with children usually colonised by more diverse fungal communities and *Malassezia* predominance on adults (Jo et al. 2016, 2017).

The foot was found to be colonised by a tremendously diverse and temporally rather instable community dominated either by *Malassezia* or filamentous fungi (notably *Aspergillus, Epicoccum*), including yeasts such as *C. tropicalis*, *C. parapsilosis*, *Candida orthopsilosis*, *Naganishia diffluens* (= *Cryptococcus diffluens*), *Rhodotorula*, *Saitozyma flava* (= *Cryptococcus flavus*) and *Vishniacozyma dimennae* (= *Cryptococcus dimennae*) (Findley et al. 2013). Black yeast-like fungi such as *Knufia epidermidis* (= *Coniosporium epidermidis*), *Exophiala lecanii-corni*, *Cyphellophora europaea* (= *Phialophora europaea*) and *Cladophialophora boppii* may be also found in the skin environment as potential colonisers (Saunte et al. 2012).

The scalp is colonised by a diverse fungal community, including yeasts such as *Malassezia*, *Naganishia albidosimilis* (= *Cryptococcus albidosimilis*), *Na. albida*, *Na. diffluens, Naganishia liquefaciens* (= *Cryptococcus liquefaciens*), *Papiliotrema flavescens* (= *Cryptococcus flavescens*), *Filobasidium floriforme*, *Filobasidium magnum* (= *Cryptococcus magnus*), *Filobasidium oeirense* (= *Cryptococcus oeirensis*) and *Rh. mucilaginosa* (Park et al. 2012).

Malassezia members are lipophilic fungi that are frequently associated with sebum-rich areas of the skin. They are postulated to be involved in conditions and skin diseases such as pityriasis versicolor, seborrheic dermatitis, psoriasis and dandruff as the various conditions can be improved by antifungal, but not by antibacterial treatment (Gaitanis et al. 2012; Clavaud et al. 2013; Jo et al. 2017). Impaired skin barrier function facilitates the disease (Harding et al. 2002). Although mechanisms are not yet understood, it was speculated that the fungus changes role from commensal to pathogen via a lack of growth control by yet unknown factors (Schommer and Gallo 2013). These factors may eventually promote the development of more virulent mycelial forms of *Malassezia* on skin lesions, as opposed to the yeast forms which seem to be more commonly found on healthy skin (Prohic and Ozegovic 2007). Other members of the skin mycobiome may be associated with poor clinical outcomes in the healing process of chronic wounds (Kalan et al. 2016). These authors used NGS-based approaches to detect a diverse fungal community thriving on diabetic foot ulcers, including yeasts such as *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *Rhodosporidium* spp. and *Trichosporon* spp., among other species. *Malassezia* was found less frequently on these lesions, which may be also correlated to the lower abundance of these yeasts on the foot skin (Findley et al. 2013; Kalan et al. 2016).

8.7 Concluding Remarks

Humans are constantly exposed to microorganisms and have evolved to live a most often healthy relationship with them. However, commensals may become pathogens by host impairment that permits invasion. The bacterial microbiome and the

mycobiome in different body sites form an interconnected landscape that provides each other with a pool of available colonists. Understanding the interactions within and between niches could help to prevent disease and, where necessary, design treatment dedicated to minimise collateral damage (Costello et al. 2009). Evidence is beginning to accumulate that a diverse mycobiome, also comprising the zymobiome, may play a greater role than previously attributed in human hosts, particularly in the gut environment, in host immune regulation, chronic inflammatory diseases and metabolic disorders, as well as in other physiological processes, including recovery from antibiotics, although the mechanisms remain unclear. The reasons for the frequent association of *C. albicans* with humans as commensal are worth exploring. Although the human mycobiome is more diverse than previously thought, high-throughput approaches mostly confirm the core mycobiota known already from culture-based studies. More work is required to catalogue this mycobiome and to analyse these communities from the perspective of microbial ecology to elucidate the function and relationships with their microbial neighbours and the hosts, in states of health and disease.

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Chapter 9
Antagonistic Interactions and Killer Yeasts

Roland Klassen, Raffael Schaffrath, Pietro Buzzini, and Philip F. Ganter

Abstract Antagonistic interactions occur between yeasts and other competing microorganisms. These interactions may rely on non-proteinaceous compounds or proteins called killer toxins. A large variety of structurally and functionally diverse toxins released from killer yeasts are known. In addition to chromosomally encoded toxins, several well-characterized toxins are encoded by selfish extrachromosomal DNA or RNA molecules of viral origin. Despite their structural diversity, only a handful of toxic strategies are utilized by structurally distinct killer toxins, and multistep modes of cell killing involve common steps, such as the binding of different cell wall receptors. In addition, distinct toxin types are known to rely on common mechanisms for maturation, structural stabilization, and release from producer cells. In case of the extrachromosomally encoded toxins, specific immunity mechanisms are linked to toxin production. In these cases, toxins are assumed to provide a positive selection mechanism for the genetic system encoding both toxin and immunity. Hence, release of killer toxins might benefit both the toxin producer and the selfish genetic element in the producer cell.

Killer yeasts display broad taxonomic diversity, including basidiomycetes and ascomycetes. Target species may not only include yeasts of both fungal phyla but also other microorganisms such as bacteria or protozoa that may compete in certain natural habitats with the killer yeast. Although killer systems are assumed to be competitive mechanisms, their role in natural yeast communities is not yet well understood. Theoretical approaches have, in general, failed to predict the coexistence of killer, non-killer, and target strains that occurs with regularity in nature. The few empirical studies of natural killer systems have confirmed the ecological

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importance of killer toxins but have uncovered differences in the exact role the toxins play in yeast ecology.

Keywords Killer yeast diversity • Ecology • Toxin mode of action • Toxin immunity

Contents

9.1 Introduction

Antagonistic interactions between strains of *Saccharomyces cerevisiae* and potential competitors based on secreted toxins were first described at a conference in the Netherlands in 1963 (Bevan and Makower 1963; Makower and Bevan 1963). The discoverers dubbed the phenomenon the killer character and suggested that, in addition to nuclear genes, a cytoplasmic element was required. In 1968, the soluble toxin was found to be a protein (Woods and Bevan 1968). From the start, the killer character was assumed to be a mechanism of interference competition that allowed strains with the ability to gain access to resources by killing rivals sensitive to their toxin. Since that time, our knowledge of the number of killer species, types of killer toxin, and modes of killing has expanded greatly. Here we review the diversity of killer types, species, and modes of action and discuss the ecological implications of the killer phenomenon.

For this chapter, we will primarily restrict the use of the term "killer factor" to proteins, but there are soluble antimicrobial secretions produced by yeast that are not proteinaceous in nature. *Ogataea pini*, a yeast commonly found in mycangia of *Dendroctonus* bark beetles in North America, secretes organic volatiles that suppress the growth of fungal pathogens of the beetle (Davis et al. 2011). The mechanism of suppression is not known, and the same volatiles promote the growth of the beetle's fungal mutualist. *Metschnikowia pulcherrima* has long been known to inhibit the growth of many bacteria and yeasts (Viljoen 2006). The yeast produces a reddish pigment, pulcherrimin, a compound that chelates two iron ions. The pigment's unchelated state, pulcherrimic acid, is the active agent (Kántor et al. 2016).

Both ascomycetous and basidiomycetous yeasts secrete biosurfactant glycolipids, presumably to free hydrophobic compounds bound to substrates for subsequent consumption by the yeast (Sáenz-Marta et al. 2015). Some basidiomycetous yeasts secrete fungicidal glycolipids consisting of at least a cellobiose and a fatty acid moiety, both of which are necessary for fungicidal activity (Kulakovskaya et al. 2010). The glycolipid has detergent-like properties (Puchkov et al. 2001, 2002) and disrupts fungal cell membranes in a wide spectrum of susceptible species (Golubev et al. 2001, 2004, 2006). Greater activity has been reported against basidiomycetes, including yeast, yeastlike, and mycelial fungal species, than against ascomycetous yeasts. Little is known about the mechanisms of immunity to the effect of the fungicidal glycolipids.

Pseudozyma fusiformata (currently *Kalmanozyma fusiformata*), *Pseudozyma tsukubaensis*, *Pseudozyma graminicola* (*Sporisorium graminicola*), *Sympodiomycopsis paphiopedili*, *Kwoniella pini* (putatively a glycolipid), *Cryptococcus humicola*, and *Trichosporon porosum* (*Vanrija humicola* and *Apiotrichum porosum*, respectively) all secrete glycolipid versions (Golubev et al. 2001, 2004, 2006; Kulakovskaya et al. 2003; Golubev 2009; Morita et al. 2011). Ustilagic acid, a biosurfactant secreted by *Ustilago maydis* (*Mycosarcoma maydis*), is similar to the fungicidal glycolipids but has not been associated with any fungicidal activity. The lack of activity may be due to the test conditions. The fungicidal glycolipids are active only under fairly acidic conditions (circa pH 4.5), but ustilagic acid has not been tested for activity under these conditions (Kulakovskaya et al. 2005). There seems no single phylogenetic or ecological connection between the fungicidal glycolipid-secreting species other than all are basidiomycetes. Three of the five species listed above belong to the Ustilaginomycotina (along with *U. maydis*), while *Ap. porosum* and *Va. humicola* are both members of the Agaricomycotina (Ebersberger et al. 2009). All are associated with plants or soils, but no more specific habitat description would apply to all of them.

Most work on the killer factor investigates the toxin's ability to kill other yeast. Not all antagonistic toxins target yeast. Observations of the ability of yeast to inhibit bacterial growth have a long history (Viljoen 2006), but isolation of the active agent is more recent. Interest in the potential of yeasts as a source of antibiotics leads to isolation of proteins secreted by *S. cerevisiae* in 1962 (Robinson et al. 1962). In 1974, it was demonstrated that a soluble secretion from a *Candida albicans* strain inhibited the growth of some bacterial strains of *Neisseria gonorrhoeae* (Hipp et al. 1974). Polonelli and his coworkers subsequently reported that killer strains from a number of yeast species could also kill bacteria, mycelial fungi, and yeastlike achloric saprophytic algae of the genus *Prototheca* (Polonelli and Morace 1986). The inhibitory molecule was not isolated, but the specificity of the inhibition (often only some strains of a yeast were killers, and only some strains of many bacterial species were susceptible) argued for something other than a generic toxic yeast waste product as the active agent. Dick et al. (1992) demonstrated that *S. cerevisiae* produced a protein that was responsible for inhibition of strains from several genera of bacteria, some responsible for the malolactic fermentation important in wine production. The protein was active over a wide range

of temperatures but only under acidic conditions (although a second peak of activity at high pH was suggested).

9.2 General Aspects in the Biology, Genetics, and Biochemistry of Killer Yeasts

In the following, we will focus on proteinaceous killer toxins from yeast that are active mainly against other yeast species and describe current knowledge of their genetic basis, biogenesis, and molecular mechanisms of target cell killing.

9.2.1 Yeast Killer Toxin Diversity

Yeast killer toxins represent a quite heterogeneous group of proteins. While all killer toxins kill nonself yeast species or variants in close proximity (see Sect. 9.4), the variety of structures and modes of killing suggest they were repeatedly and independently invented during yeast evolution. From a structural point of view, killer toxins can be grouped into three distinct classes: small single-subunit proteins, (hetero)dimeric proteins, and multimeric protein complexes. For some killer toxins, three-dimensional structures have been determined and diverse folding patterns observed. For example, *Cyberlindnera mrakii* (formerly known as *Hansenula mrakii*) HM-1 toxin and *Millerozyma farinosa* (formerly known as *Pichia farinosa*) SMKT are both small basic proteins (<20 kDa), but, structurally, they turned out to adopt completely different folding patterns. HM-1 displays an all-β-fold similar to eye lens crystallins (Antuch et al. 1996), whereas SMKT is a two-subunit protein with an α-β-sandwich fold (Fig. 9.1). A common feature, however, is the presence of an extensive number of intramolecular disulfide bonds (Fig. 9.1) which likely contribute to the extreme thermo- and pH stability characteristic of some of the toxins, in particular HM-1 (Yamamoto et al. 1986a, b).

9.2.2 Classification of Killer Systems Based on Gene Localization

An alternative criterion for classification of the diverse killer toxins is the genetic basis of toxin production. While a number of well-characterized toxins in different yeast species appear to be encoded by the nuclear genome (Table 9.1), extrachromosomal localization of toxin genes is also common (Table 9.2). In the latter case, toxin genes may be localized on cytoplasmic RNA viruses or cytoplasmic dsDNA molecules that display a linear configuration. The toxin-encoding RNA viruses are

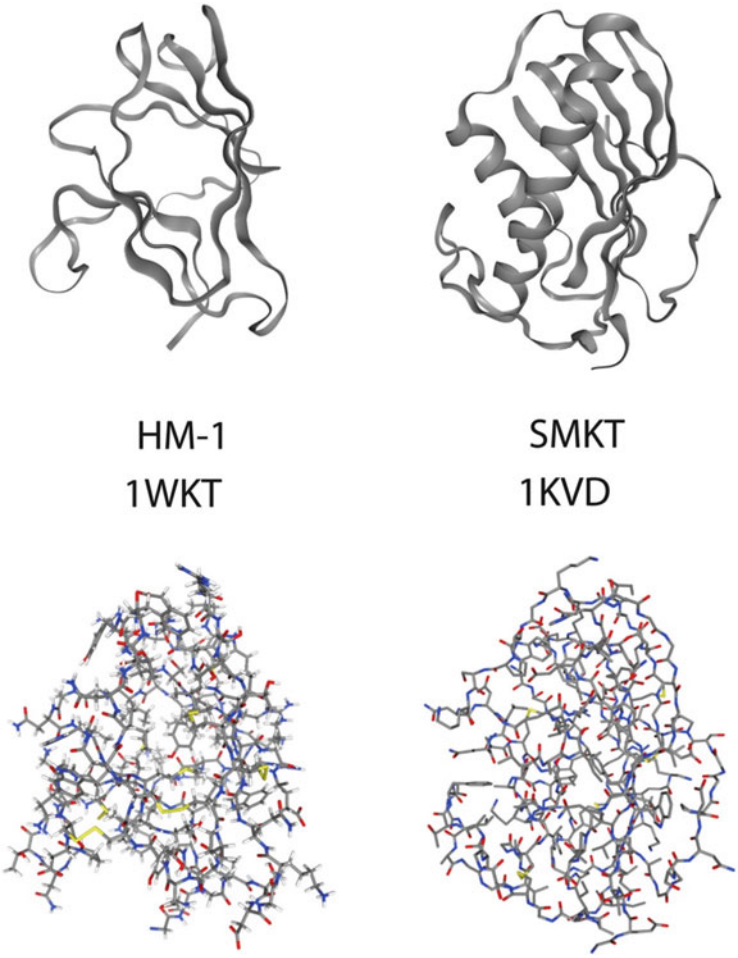

Fig. 9.1 Three-dimensional structures of small basic killer toxins HM-1 and SMKT. Top: Cartoon style representation of protein data bank (pdb) structures 1WKT and 1KVD. Bottom: Licorice style representation of the same structures. Images were generated using the NGL (WebGL) viewer (Rose and Hildebrand 2015)

common among *S. cerevisiae* killer strains (reviewed in Schmitt and Breinig 2002), even though two chromosomally encoded toxins (KHR and KHS) are known as well (Goto et al. 1990, 1991).

9.2.2.1 dsRNA Viruses Encoding Killer Toxins

The extrachromosomal elements encoding killer toxin genes are routinely nonautonomous dsRNA or dsDNA molecules that require the presence of a helper

Table 9.1 Selected chromosomally encoded toxins with known subunit structure and mode of action

Species/mode of action	Original taxonomic designation cited in the literature	Toxin	Size (kDa)	Receptor	References
Cell wall synthesis inhibitor					
Cyberlindnera mrakii	*Williopsis saturnus* var. *mrakii*, *Hansenula mrakii*	HM-1	10.7	β-glucan	Ashida et al. (1983), Kasahara et al. (1994)
		HYI	9.5		Komiyama et al. (1995, 1998)
Ionophores					
Millerozyma farinosa	*Pichia farinosa*	SMKT	α (6.6) β (7.9)		Suzuki and Nikkuni (1994)
Pichia kluyveri			11		Kagan (1983)
Pichia membranifaciens		PMKT	18	β-1,6-glucan	Santos et al. (2000)
Schwanniomyces occidentalis		–	α (7.4) β (4.9)	Mannan	Chen et al. (2000)
Glucanases					
Wickerhamomyces anomalus	*Pichia anomala*, *Hansenula anomala*	K5/ panomycocin	49	β-1,3-glucan β-1,6-glucan[a]	Izgü and Altinbay (2004), Muccilli et al. (2013), Wang et al. (2007a,b)
Tetrapisispora phaffii	*Kluyveromyces phaffii*	KpKt	33	β-1,6−/β-1,3-glucan	Comitini et al. (2004b)
Cell cycle inhibitor					
Pichia membranifaciens		PMKT2	30	Cell wall mannoprotein	Santos et al. (2009, 2013)

[a]Results obtained with BCA15, BCU24, and BS91 strains (Mucilli et al. 2013)

Table 9.2 Selected extrachromosomally encoded toxins

Species	Original taxonomic designation cited in the literature	Toxin	Size	Receptor	Toxic activity	References
Cytoplasmic RNA encoded toxins						
Saccharomyces cerevisiae		K1	α (9.5) β (9)	β–(1–6)-glucan	Ionophore	Young and Yagiu (1978)
		K28	α (10) β (11)	Mannoproteins	Inhibition of DNA synthesis	Schmitt and Tipper (1990)
Mycosarcoma maydis	*Ustilago maydis*	KP4	13.6		Inhibition of calcium uptake	Park et al. (1994)
		KP6	α (8.6) β (9.1)		K^+ depletion	Tao et al. (1990)
Hanseniaspora uvarum		–	18	β–(1–6)-glucan		Schmitt and Neuhausen (1994)
Zygosaccharomyces bailii		Zygocin	10	Mannoprotein	Ionophore	Schmitt and Neuhausen (1994)
Cytoplasmic DNA encoded						
Kluyveromyces lactis		Zymocin	α (99) β (30) γ (28)	Chitin	$tRNA^{Glu}$ cleavage	Gunge et al. (1981)
Millerozyma acaciae	*Pichia acaciae*	PaT	α (110) β (39) γ (38)	Chitin	$tRNA^{Gln}$ cleavage	Worsham and Bolen (1990)
Debaryomyces robertsiae		DrT	>100	Chitin	$tRNA^{Gln}$ cleavage	Klassen and Meinhardt (2002)
Babjeviella inositovora	*Pichia inositovora*	PiT	>100	Chitin	rRNA cleavage	Hayman and Bolen (1991)

element for stable propagation (reviewed in Wickner 1992; Schmitt and Breinig 2002, 2006). For the *S. cerevisiae* toxin-encoding dsRNA viruses, the killer toxin-encoding satellite viruses M1, M2, and M28 need to be accompanied by an L-A helper virus (Bostian et al. 1980). The L-A and M viruses are separately encapsidated into icosahedral capsids (Bostian et al. 1980; Esteban and Wickner 1986; Cheng et al. 1994; Castón et al. 1997). L-A encodes the major capsid protein Gag that is required for encapsidation of both L-A and M viruses (Fujimura and Wickner 1988; Icho and Wickner 1989). Both cytoplasmic gene expression and the dsRNA replication cycle are facilitated by an RNA-dependent RNA polymerase (Pol), which is also encoded by the L-A element and expressed as a Gag-Pol fusion protein (Fujimura and Wickner 1988; Dinman et al. 1991). The Pol-encoding ORF overlaps the Gag-encoding ORF on the L-A virus and requires −1 ribosomal frameshifting for translation (Dinman et al. 1991). During the replication cycle L-A- and M-derived ssRNA transcripts are encapsidated with at least one copy of the Gag-Pol fusion protein that converts the ssRNA transcripts into dsRNA and subsequently generates multiple ssRNA copies for translation of viral gene transcripts and the following replicative cycles (Fujimura et al. 1992). Hence, all toxin-encoding M viruses critically depend on the presence of an L-A virus, while the latter can propagate in the absence of M viruses. Related dsRNA viruses encoding killer toxins were described in other species such as *U. maydis* and *Zygosaccharomyces bailii* (Koltin and Day 1976; Tipper and Bostian 1984; Schmitt and Neuhausen 1994). As for the *S. cerevisiae* viruses, these toxins are encoded by satellite viruses that depend on the presence of a helper virus. It is assumed that dsRNA killer viruses lack an infectious cycle and are transmitted via mating. This may also involve hyphal/cell fusions between distinct species, possibly explaining the presence of related dsRNA killer viruses in yeast taxa as diverse as *Saccharomyces* (Ascomycota) and *Ustilago* (a basidiomycetous yeastlike dimorphic fungus).

9.2.2.2 dsDNA Viruslike Elements Encoding Killer Toxin Genes

A situation reminiscent of the L-A dependence of M viruses is encountered in case of the toxins encoded by yeast linear DNA plasmids, which are also known as viruslike elements (reviewed in Meinhardt and Schaffrath 2001; Schaffrath and Meinhardt 2005; Meinhardt and Klassen 2009; Satwika et al. 2012). These cytoplasmic dsDNAs typically occur in pairs or triplets which can be classified into autonomous and nonautonomous elements as well. As for the L-A virus in dsRNA killer systems, the autonomous element in dsDNA killer systems contributes essential factors to ensure replication and gene expression (transcription) in the cytoplasm (Schaffrath and Meacock 1995, 1996; Schaffrath et al. 1995a, b, 1999). However, compared to L-A, where a single Gag-Pol fusion protein is sufficient for both mRNA generation and conservative replication, a number of different proteins are required for gene expression and replication in case of dsDNA plasmids (Schaffrath et al. 1996, 1999, 2000; Schaffrath and Meacock 2001). These include a plasmid-encoded DNA polymerase, which has an N-terminal domain that remains

covalently attached to the 5′ ends of the dsDNAs after replication, a single-stranded DNA-binding protein, a putative RNA polymerase, an mRNA capping enzyme, and additional proteins of unknown function that are essential for maintenance. For a detailed description of these functions, we refer to recent reviews (Klassen and Meinhardt 2007; Meinhardt and Klassen 2009; Satwika et al. 2012). In essence, the maintenance functions provided by autonomous linear plasmids can be divided into those related to the replication of the entire set of dsDNAs (autonomous and nonautonomous elements) and those related to the generation or modification (capping) of mRNAs transcribed from cytoplasmic genes (Klassen and Meinhardt 2007; Satwika et al. 2012). Killer plasmid systems of such type have been described in strains of *Kluyveromyces lactis*, *Pichia acaciae*, *Pichia inositovora* (now *Millerozyma acaciae* and *Babjeviella inositovora*, respectively), and *Debaryomyces robertsiae* (Gunge et al. 1981; Worsham and Bolen 1990; Hayman and Bolen 1991; Klassen and Meinhardt 2002). In all cases, toxin-encoding genes are located on nonautonomous elements that do not provide a maintenance function, and autonomous elements must accompany such killer plasmids (Stark et al. 1984, 1990; Sor and Fukuhara 1985; Klassen and Meinhardt 2003; Klassen et al. 2004). As for the L-A virus, autonomous dsDNA plasmids may occur without a killer plasmid, and the genetic organization and deduced proteins from autonomous elements of different species have been found to be highly similar (Klassen et al. 2001; Jeske and Meinhardt 2006). Interestingly, several of the maintenance-related proteins encoded by the autonomous elements are related to similar proteins of viral origin, and a direct evolutionary link between cytoplasmic dsDNA viruses and dsDNA killer plasmids has been suggested (Jeske et al. 2006; Klassen and Meinhardt 2007; Satwika et al. 2012). Since the linear cytoplasmic killer plasmids are in numerous ways more similar to viruses than to plasmids (which are usually circular and replicate in the nucleus), the term viruslike elements was introduced (Jeske et al. 2006; Klassen and Meinhardt 2007; Satwika et al. 2012).

9.2.3 Killer Toxin Biogenesis: Common Steps for Distinct Types

While the modes of inheritance and gene expression of the two types of cytoplasmic extrachromosomal genes are fundamentally different from each other and even more so from the chromosomally encoded toxins, the encoded killer proteins are all similar in their requirement to enter the secretory pathway in order to become externalized and interact with target cells (Fig. 9.2).

Secretion and maturation of killer toxins often requires the processing of preprotoxins during the secretory pathway. HM-1, for example, is encoded by the *HMK* gene of *Cyb. mrakii* (Kimura et al. 1993) as a precursor protein with an N-terminal signal peptide and a propeptide region that is cleaved in the Golgi apparatus by the KEX protease. Such processing may also give rise to distinct

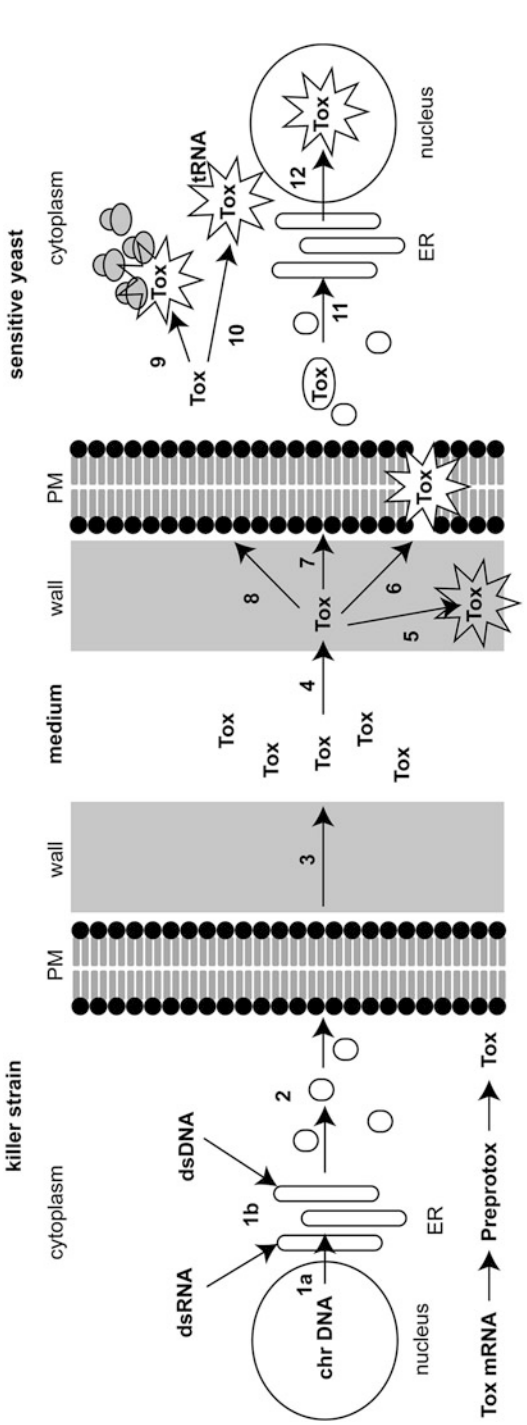

Fig. 9.2 Overview of killer toxin biogenesis and modes of action. (*1*) Transcription of toxin genes and translation of toxin-encoding mRNA (Tox mRNA), generating unprocessed toxin precursor (preprotox). (*1a*) nuclear genes, (*1b*) cytoplasmic genes on either dsRNA virus or linear dsDNA. (*2*) Processing to mature toxin (Tox) and passage of secretory pathway. (*3*) Secretion and release from killer strain. (*4*) Binding of mature toxin to primary cell wall receptor. (*5*) Induction of cell wall damage (glucanase; inhibition of glucan synthase may occur after interaction with secondary membrane receptor). (*6*) Integration into plasma membrane (PM) and formation of ion channel. (*7*) Binding to secondary membrane receptor and uptake via endocytosis. (*8*) Binding to PM and translocation into cytoplasm of target cell. (*9*) Cleavage of ribosomal RNA. (*10*) Cleavage of specific tRNAs. (*12*) Exit from ER after retrograde passage of secretory pathway, followed by entry into nucleus and inhibition of DNA synthesis. See text for further detail and references

toxin subunits that are encoded by a single gene, such as in *Mill. farinosa* SMKT encoded by the *SMK1* gene (Suzuki and Nikkuni 1994). In this case, the precursor protein is cleaved by signal peptidase and twice by the KEX peptidase, liberating the γ-peptide between the regions containing the α- and β-subunit polypeptides (Suzuki and Nikkuni 1994). This is also true in the cases of K1 and K28, where two toxin subunits (α and β) are formed via KEX protease processing of a preprotoxin within the secretory pathway, and, again, an intervening γ-peptide is released (Bostian et al. 1984; Schmitt and Tipper 1990, 1995; Zhu et al. 1992; Riffer et al. 2002). In contrast to SMKT, however, the α- and β-subunits are covalently linked to each other via disulfide bonds (Bostian et al. 1984; Riffer et al. 2002). In case of the dsDNA-encoded zymocin (Stark et al. 1990; Schaffrath et al. 1999; Schaffrath and Breunig 2000), three toxin subunits are generated from two structural genes. Here, α- and β-subunits are the product of a single gene (pGKL1 ORF2), and the subunit (γ) is encoded by a separate ORF possessing its own functional signal peptide. During secretion, Orf2 is processed by signal peptidase and KEX protease to generate α- and β-subunits. The γ-subunit is separately encoded on pGKL1 and, in the mature zymocin, covalently linked to the β-subunit via a disulfide bond (Hishinuma et al. 1984; Stark et al. 1984; Sor and Fukuhara 1985; Stark and Boyd 1986; Tokunaga et al. 1987). Mutagenesis studies revealed that this disulfide linkage is essential for the ability to assemble functional zymocin and that αβ secretion requires the cosecretion of the γ-subunit (Wemhoff et al. 2014). Overall, there are several examples of secreted heterodimeric or multimeric killer toxin complexes that are distinct in sequence and mechanism (see below) but utilize similar strategies for generation of individual subunits while passaging the secretory pathway.

9.2.4 Killer Toxins' Mode of Action

Consistent with the structural diversity, a number of different strategies to kill or inhibit the growth of competitors have been discovered (Tables 9.1 and 9.2; Fig. 9.2) and will be discussed along with some well-studied examples. After toxins are released from the producer cell, interaction with the target cell wall is generally a required step preceding the actual cell killing (Fig. 9.2). Typically, cell wall components such as glucan, chitin, or mannoproteins represent such initial binding sites. In several cases in which the toxin has a final destination other than the cell wall itself, secondary receptor proteins have been identified that may guide the toxin from the cell surface to its ultimate cellular target.

9.2.4.1 Cell Wall Synthesis Inhibitors

A well-studied toxin inhibiting cell wall synthesis is the aforementioned HM-1 toxin from *Cyb. mrakii* (*H. mrakii*). HM-1 is a small basic protein of outstanding

thermo- and pH stability, which is thought to be the result of extensive intramolecular disulfide bonds (Fig. 9.1; Yamamoto et al. 1986a, b; Ashida et al. 1983; Lowes et al. 2000). This toxin was shown to bind to and inhibit β-1,3-glucan synthase, an enzyme localized in the plasma membrane (Yamamoto et al. 1986a, b; Takasuka et al. 1995; Komiyama et al. 1996). Such inhibition results in a deficiency of cell wall resynthesis in budding regions and eventually causes pore formation and cell lysis (Takasuka et al. 1995; Komiyama et al. 1996). It was further observed that mutants defective in Alg3, an α-1,3-mannosyltransferase involved in protein glycosylation, display strongly enhanced HM-1 resistance, and several other mutants with defects in protein glycosylation also increase resistance (Kimura et al. 1999; Miyamoto et al. 2011). Since it was also shown that HM-1 binds to an unidentified protein in the membrane fraction of yeast cells (Miyamoto et al. 2006) and binding efficiency is strongly reduced in the *alg3* mutant, it is assumed that HM-1 initially binds to the glycosylated receptor protein and subsequently inhibits beta-glucan synthase (Miyamoto et al. 2011). However, screening of the genome-wide deletion library of *S. cerevisiae* also identified strong resistance in yeast cells lacking the porin channel protein Fps1, which is involved in turgor regulation. Cells defective in the high-osmolarity glycerol (HOG) and cell wall integrity (CWI) pathways are hypersensitive to the toxin (Miyamoto et al. 2011, 2012). Since *fps1* mutant cells are both more sensitive to chemical agents inhibiting β-1,3-glucan synthase and display enhanced HM-1 resistance, it may be that the main toxic effect of HM-1 is not inhibition of the synthase (Miyamoto et al. 2011, 2012). As HM-1 was shown to activate both the HOG and CWI pathway signaling, cell wall stress induced by binding of the toxin to the still unidentified mannoprotein might represent the key step of cell killing (Miyamoto et al. 2012). Toxins related to HM-1 were identified in several other *Cyb. mrakii* strains (reviewed in Meinhardt and Klassen 2009) but not in other species so far, suggesting that this type of killer toxin is restricted to this species.

9.2.4.2 Ionophoric Toxins

Several toxins encoded by either chromosomal genes or dsRNA viruses in different genera are thought to act by interaction with the target cell membrane and subsequent formation of ion channels, such as the toxin produced by *Pichia kluyveri* (Kagan 1983). A well-studied example of this toxin type is the K1 toxin encoded by a M-type dsRNA virus in *S. cerevisiae* (de la Peña et al. 1981; Bussey 1991; Breinig et al. 2002, 2004). The secreted mature toxin consists of two subunits that are generated from a single preprotoxin via KEX processing. Initially, K1 interacts with β-1,6-glucan in the cell wall (Hutchins and Bussey 1983) and subsequently interacts with the GPI-anchored Kre1 protein, which represents the secondary cell membrane receptor (Breinig et al. 2002, 2004). While both α- and β-subunits are required for cell wall binding, the hydrophobic α-subunit alone is thought to form a cation-specific membrane channel for ultimate cell killing (Bussey 1991; Zhu and Bussey 1991). The chromosomally encoded SMKT and PMKT are thought to

exhibit similar cell killing strategies, eventually resulting in permeabilization of the cell membrane (Suzuki et al. 2001; Santos et al. 2007), even though toxin sequences and structures differ from K1. In analogy to K1, PMKT was shown to work after binding to β-1,6-glucan and then a secondary GPI-anchored membrane receptor protein, which, however, is distinct from that bound by K1 (Santos et al. 2007). Thus, several structurally distinct toxins are known which have adopted related mechanisms to access and disrupt the target cell's membrane integrity and induce lethal loss of ions and other cellular molecules.

9.2.4.3 Glucanase Killer Toxin

A variety of yeast species secrete killer toxins that act as glucanases (reviewed in Meinhardt and Klassen 2009). Well-studied examples are KpKt from *Tetrapisispora phaffii* (*Kluyveromyces phaffii*) and WmKT from *Cyb. mrakii* (*Williopsis saturnus* var. *mrakii*) (Guyard et al. 2002a; Comitini et al. 2004a). A very common source for glucanase toxins is the species *Wickerhamomyces anomalus* (formerly *Pichia anomala*), since a quite large variety of glucanase killer toxins have been described in strains of this species isolated from various sources, including different agricultural or food samples (Comitini et al. 2004b; Izgü and Altinbay 2004; Wang et al. 2007a; Muccilli et al. 2013). Due to a broad antimicrobial activity, which also includes pathogenic bacteria or mycelial fungi and even protozoans, there is considerable interest in applications of these toxin types for biocontrol purposes (Sawant et al. 1989; Walker et al. 1995; Jijakli and Lepoivre 1998; Izgü et al. 2007a, b; Wang et al. 2007a; Muccilli et al. 2013; Valzano et al. 2016). The toxic principle against yeast cells usually includes the induction of cell wall damage due to hydrolysis of cell wall glucan and subsequent induction of cell death by cell lysis (Guyard et al. 2002b). Recently, *W. anomalus* glucanase killer toxin-producing strains were also isolated from malaria mosquitoes and novel strategies to utilize such strains, and the anti-plasmodial effects of encoded toxins to control the spread of plasmodium infection in malaria mosquitoes have been investigated (Valzano et al. 2016).

9.2.4.4 A/B Toxin-Type Killer Toxin: DNA Synthesis and Cell Cycle Inhibitor

The heterodimeric K28 toxin encoded by an M-type dsRNA virus in *S. cerevisiae* shares striking mechanistic similarities with other bacterial toxins of the A/B type, such as cholera toxin, diphtheria toxin or *Pseudomonas* exotoxin A (Schmitt and Tipper 1990; Eisfeld et al. 2000; Schmitt and Breinig 2006; Uthman et al. 2013; Schaffrath et al. 2014). In such toxins, the β-subunits routinely mediate cell binding, followed by endocytotic uptake and retrograde passage through the secretory pathway after which translocation to the cytoplasm occurs and lethal effects are induced by the α-subunit. As a typical A/B-type killer toxin, K28 initially binds to

mannoproteins in the cell wall and is thought to subsequently interact with Erd2, the secondary receptor at the membrane level recognizing the C-terminal HDEL motif in the β-subunit (Becker et al. 2016). Consistently, cell wall mutants in which oligosaccharide structures of mannoproteins become altered exhibit K28 resistance that can be overcome by excess of toxin or by converting cells to spheroplasts (Schmitt and Radler 1988; Breinig et al. 2002). These initial events are followed by uptake of the heterodimer toxin via endocytosis and retrograde passage through the ER, and, finally, the complex is released to the cytosol, where it dissociates and allows the α-subunit to enter the nucleus and inhibit DNA replication, causing cells to arrest in the G1/S phase of the cell cycle (Schmitt et al. 1996; Eisfeld et al. 2000; Heiligenstein et al. 2006). A distinct chromosomally encoded killer toxin (PMKT2) from *Pichia membranifaciens* shows some similarities to K28's mode of action. Similar to K28, it utilizes mannoproteins as the cell wall receptor and was shown to induce a cell cycle arrest in G1/S (Santos et al. 2013).

9.2.4.5 tRNA Endonucleases

A group of comparatively large toxins (~100 kDa and larger, Table 9.2) encoded by linear dsDNA plasmids in the cytoplasm have been shown to target different RNA species in the cytoplasm. The best-studied example is the zymocin from *K. lactis*, which is a heterotrimer with subunit sizes of 99 kDa (α), 30 kDa (β), and 28 kDa (γ) encoded by the cytoplasmic pGKL1 plasmid that requires a helper plasmid (pGKL2) for maintenance and gene expression in the cytoplasm (Stark and Boyd 1986). During zymocin maturation, the γ-subunit is attached to the β-subunit via a disulfide bond (Stark and Boyd 1986; Stark et al. 1990). In the initial steps of target cell interaction, zymocin is thought to bind to the target's cell wall chitin. This step is mediated by the α-subunit, which is a chitin-binding protein that can also hydrolyze chitin (Stark et al. 1990; Butler et al. 1991a; Schaffrath and Breunig 2000; Jablonowski et al. 2001; Jablonowski and Schaffrath 2007). Subsequently, the smallest subunit gets imported into the target cell in a poorly understood process that likely requires the aid of the hydrophobic β-subunit and is dependent on the presence of a particular membrane sphingolipid ($M(IP)_2C$) and a proton gradient generated by Pma1, a plasma membrane ATPase (Mehlgarten and Schaffrath 2004; Zink et al. 2005). The chitinase activity of the α-subunit and the disulfide bond between β and γ are essential for the killing activity of the zymocin (Butler et al. 1991a; Wemhoff et al. 2014). However, full toxicity can be recapitulated in cells conditionally expressing a version of the γ-subunit that accumulates intracellularly due to the absence of a signal peptide, indicating the key toxic activity resides in this subunit and suggests that the other two subunits are mainly needed to deliver γ-toxin into target cells (Tokunaga et al. 1989; Stark et al. 1990; Butler et al. 1991b). Once delivered to the target cell, γ specifically cleaves $tRNA^{Glu}_{UUC}$ by hydrolyzing the phosphodiester bond between the wobble nucleoside (U34) and the 3′ nucleoside (U35) (Lu et al. 2005; Jablonowski et al. 2006). This cleavage event requires the presence of a posttranscriptional modification

(5-methoxy-carbonyl-methy-2-thiouridine, mcm^5s^2U) at the wobble position of the target tRNA (Butler et al. 1994; Frohloff et al. 2001; Huang et al. 2005; Lu et al. 2005). Mutations preventing this modification abolish toxicity of zymocin, and isolated tRNA from such mutants is highly resistant to in vitro cleavage by purified γ-toxin (Lu et al. 2005; Jablonowski et al. 2006; Schaffrath and Leidel 2017). Despite the fact that three distinct tRNAs are present in yeast cells that carry the mcm^5s^2U modification and an unmodified U in position 35, γ-toxin exhibits a strong preference of cleaving $tRNA^{Glu}_{UUC}$, indicating that mcm^5s^2U and U35 are essential for cleavage but not sufficient for recognition of the target tRNA (Huang et al. 2005; Lu et al. 2005; Jablonowski and Schaffrath 2007).

Toxins related to zymocin and encoded by linear cytoplasmic dsDNAs were also identified in *Mill. acaciae* and *Deb. robertsiae* (formerly *Wingea robertsiae*) (Worsham and Bolen 1990; Meinhardt and Schaffrath 2001; Klassen and Meinhardt 2002). In both species, nonautonomous elements were found carrying genes with predicted proteins similar to the zymocin αβ precursor protein, while a γ-subunit homologue was lacking (Klassen et al. 2004). As for zymocin, these toxins (PaT, *Mill. acaciae* toxin, and DrT, *Deb. robertsiae* toxin) act by first binding to cell wall chitin and subsequently import a toxin subunit that is distinct in sequence from zymocin γ but related in both DrT and PaT (Klassen et al. 2004). In both cases, intracellular active toxin subunits target a distinct tRNA species ($tRNA^{Gln}_{UUG}$) for cleavage compared to zymocin (Klassen et al. 2008, 2014). A more detailed study of PaT's mode of action revealed that the heterologously expressed active toxin subunit (PaOrf2) cleaves $tRNA^{Gln}_{UUG}$ at position 34 as does zymocin but, unlike the latter, does not require the presence of mcm^5s^2U at this position (Klassen et al. 2008). Strikingly, however, a mutant that blocks the last step of mcm^5s^2U formation and accumulating a chemically distinct wobble base modification (5-carbamoyl-methyl-2-thiouridine, ncm^5s^2U) exhibits strong PaT resistance, and tRNA from such mutant resists cleavage by heterologously expressed PaT tRNAse subunit (Kalhor and Clarke 2003; Klassen et al. 2004, 2008; Chen et al. 2011). Thus, even though mcm^5s^2U is not a strong positive factor for cleavage, the presence of a chemically distinct modification at the cleavage site may act as a strong inhibitor. Additional evidence suggests that an isoacceptor $tRNA^{Gln}$ with a C34 is also cleaved and that $tRNA^{Gln}_{UUG}$ may be cleaved at a position upstream of U34 as well (Klassen et al. 2008). A structural study of this endonuclease revealed a folding pattern that is distinct from all known ribonucleases (Chakravarty et al. 2014).

9.2.4.6 rRNA Targeting Toxin

PiT, a third toxin of the zymocin family, is known to be encoded by a linear plasmid in *Bab. inositovora* (formerly *P. inositovora* and *Yamadazyma inositovora*) (Hayman and Bolen 1991; Klassen and Meinhardt 2003; Kast et al. 2014). As for zymocin, an αβ-like precursor protein is encoded by a nonautonomous plasmid, and a separate gene encodes a subunit that is thought to be imported into target cells and

carries the toxic activity (Klassen and Meinhardt 2003; Kast et al. 2014). Conditional expression of a modified gene encoding a signal peptide-less version of the toxic subunit induces full growth arrest. In contrast to zymocin or PaT, however, the toxic subunit of PiT was shown to induce fragmentation of both the 18S and 25S rRNAs (Kast et al. 2014). Approximate mapping of cleavage sites revealed several positions that become fragmented after induction of the toxic subunit. One cleavage site in 18S rRNA was mapped at the nucleotide level. It turned out that this cleavage occurs in a small loop of the 18S rRNA which shows some similarity to the anticodon loop of $tRNA^{Glu}_{UUC}$ that is cleaved by γ-toxin (Kast et al. 2014). Since there is also a modest sequence similarity between zymocin γ and the PiT toxin subunit, both might represent distantly related ribonucleases that developed distinct RNA substrate specificities.

9.2.4.7 Yeast Apoptosis Induced by Diverse Killer Toxins

Despite its unicellular lifestyle, *S. cerevisiae* was shown to undergo a programmed cell death (PCD) under diverse conditions, including exposure to exogenous and endogenous stresses (reviewed in Carmona-Gutierrez et al. 2010). It is assumed that yeast PCD represents an altruistic response of less-fit cells, which activate a suicide program in favor of promoting survival of fitter cells in the population under adverse environmental conditions (Carmona-Gutierrez et al. 2010). Strikingly, a number of yeast killer toxins were also found to induce PCD in sensitive *S. cerevisiae* strains (Klassen and Meinhardt 2005; Reiter et al. 2005; Santos and Marquina 2011; Santos et al. 2013). PCD induced by killer toxins was shown to exhibit the typical cytological markers of apoptosis, including mitochondria-dependent formation of reactive oxygen species (ROS), DNA fragmentation, and externalization of phosphatidylserine (Klassen and Meinhardt 2005; Reiter et al. 2005; Santos and Marquina 2011; Santos et al. 2013). Interestingly, apoptosis induction by yeast killer toxins appears to be independent of the primary killing mechanism, since it is induced by toxins exhibiting completely distinct killing strategies: K1, zygocin, and PMKT (ion channel formers), K28 (A/B toxin inhibiting cell cycle), PaT (tRNA endonuclease), and PMKT2 (monomeric toxin inhibiting cell cycle). In several cases, apoptotic cell death was specifically induced by low doses of the toxins, whereas higher doses were found to induce a rapid necrotic cell death via the different toxic strategies, such as lethal membrane pore formation (Reiter et al. 2005; Santos et al. 2013). Since low toxin doses are assumed to more realistically reflect conditions encountered in natural yeast habitats, the induction of apoptotic cell death could well be relevant for the antagonistic interaction between killer and non-killer strains (see also Sect. 9.4).

9.2.5 Toxin Immunity

Even though some killer toxins (e.g., *Wickerhamomyces* glucanases) exhibit very broad target spectra, most killer toxins are active against other yeasts, which may even include distinct variants of the same species. Therefore, a system-inherent problem is the mandatory avoidance of self-killing. One obvious strategy is the production of toxins recognizing primary or secondary receptors that are not present in the producer strain. However, such strategy generally would limit the target spectrum and cannot be applied to dsRNA virus or dsDNA encoded toxins, which are routinely active against other yeasts of the same species not carrying the extrachromosomal elements. In these cases, the killer toxin gene-encoding elements commonly encode immunity mechanisms that prevent toxin-induced suicide. While chromosomally encoded killer toxins are usually regarded as factors enhancing the ability of the producer cell to compete against other yeasts in an environmental setting with limited resources, an autoselective purpose might explain the presence of dsRNA- and dsDNA-encoded killer toxins as well. As outlined in Sect. 2.2, these extrachromosomally encoded toxins are associated with genetic elements that can be lost from the cell. Hence, the presence of a toxin effective against those cells that have lost the toxin (and immunity)-encoding element would create a strong selective pressure to maintain this nonautonomous element (autoselection).

The molecular details about the mechanism of killer toxin immunity are available in a few cases. For the virally (M28) encoded K28, which enters the cell via endocytosis, it was demonstrated that killer cells also internalize their own toxin (Breinig et al. 2006; Schmitt and Breinig 2006). Once the mature toxin arrives in the producer cells' cytoplasm, a complex between newly synthesized preprotoxin and the reinternalized mature toxin is formed after which ubiquitination and proteasomal degradation of the active mature toxin occurs (Breinig et al. 2006). Hence, immunity is tightly linked to the active production of the killer toxin in the M28 host cell. In case the nonautonomous element is eliminated from some of the host cells surrounded by cells still retaining M28, preprotoxin production is stopped, and susceptibility to the toxin present in the environment is the immediate consequence. Such a mechanism creates an elegant addiction system that positively selects for the presence of the toxin-encoding element.

Similarly, immunity genes were identified in nonautonomous dsDNA plasmids associated with production of the killer proteins zymocin, PaT and DrT (Tokunaga et al. 1987; Paluszynski et al. 2007; Kast et al. 2015). Consistent with the view that PaT and DrT represent functionally related tRNA-cleaving toxins, immunity genes of both systems show detectable similarity, and it was demonstrated that the PaT immunity gene is able to mediate full protection against PaT and reduced protection against the related DrT (Klassen et al. 2014). No similarity was observed between the PaT/DrT and the zymocin immunity factor, and, consistently, cross protection was not observed (Kast et al. 2015). Thus, immunity factors seem to recognize and prevent toxic action of the cognate toxin. Since all three immunity factors not only prevent toxic action of the exotoxin but also mediate full resistance against

intracellularly expressed tRNAse toxin subunits, a direct recognition and inhibition of the matching tRNAse subunit by the immunity factor is assumed to constitute the molecular mechanism of toxin immunity. In support of this, it was demonstrated in vivo for DrT and in vitro for PaT that tRNA cleavage is indeed prevented by the immunity factor (Chakravarty et al. 2014; Klassen et al. 2014).

9.3 Diversity of Killer Yeasts

Current literature reports discrepancies on the frequency of killer strains in surveys of killer diversity. This may be due to the differences in the origin of putative killer yeast cultures, differences in the methodology employed, or differences in the panel of yeasts used as potential susceptible strains. A few large-scale screening surveys (Vadkertiová and Sláviková 1995, 2007; Buzzini and Martini 2000) reported that the frequency of strains exhibiting killing activity in yeasts isolated from natural habitats was higher than that found in strains conserved for a long time in public or private repositories. The same studies found the occurrence of killing properties in strains belonging to a number of species never regarded as killers, thus suggesting that the killer phenomenon is much more common in natural ecosystems than previously postulated. The use of a more or less large panel of sensitive strains may be another critical bottleneck that could probably affect the frequency of killer strains found among yeasts sharing a given habitat. In fact, a wider selection of sensitive strains, and the choice of using more strains within the same species, should be considered critical in the evaluation of the occurrence of the killer phenomenon within a large number of yeasts isolates (Vadkertiová and Slavikova 1995, 2007; Buzzini and Martini 2000). As reported by Golubev (1998, 2006), most literature concerning large screening surveys of killing ability was characterized by the absence of a rational selection of sensitive strains. Accordingly, the low frequency of killer strains sometimes found in previous studies may possibly be ascribed to the use of a few (or even only one) sensitive cultures (frequently strains of *S. cerevisiae* and *Candida glabrata*). Interpreting results on the frequency of the killer phenomenon versus yeast diversity based only on one or two sensitive strains would considerably underestimate the presence of killing ability (Buzzini and Martini 2000; Vadkertiová and Sláviková 2007).

The list of yeast species reported so far as producers of killer proteins (updated from Golubev 2006) is reported in Table 9.3. On the basis of current literature, the ability to secrete killer proteins seems to be quite widespread among yeasts. Among Ascomycota, the most studied species were *C. glabrata*, *Candida maltosa*, *Cyb. mrakii*, *Cyberlindnera saturnus* (former *Williopsis saturnus*), *Cyberlindnera subsufficiens*, *Hanseniaspora uvarum* (and its anamorph *Kloeckera apiculata*), *Kluyveromyces marxianus*, *K. lactis*, *P. kluyveri*, *Pichia kudriavzevii* (*Issatchenkia orientalis*), *P. membranifaciens*, *S. cerevisiae*, and *W. anomalus*. Among Basidiomycota, the most studied species were *Papiliotrema laurentii* (former *Cryptococcus laurentii*), *Rhodotorula glutinis*, *Rhodotorula graminis*, and

Table 9.3 List of yeast species reported as producer of killer proteins (updated from Golubev 2006)

Killer species	Original taxonomic designation cited in the literature	References
Ascomycota		
Babjeviella inositovora	*Pichia inositovora*	Klassen and Meinhardt (2003)
Barnettozyma californica	*Hansenula californica*, *Williopsis californica*, *Zygowilliopsis californica*	Nomoto et al. (1984), Starmer et al. (1987), Vustin et al. (1988), Theisen et al. (2000), Buzzini et al. (2003)
Barnettozyma pratensis	*Williopsis pratensis*	Vustin et al. (1991)
Candida sp.		Yokomori et al. (1988), De Souza Cabral et al. (2009)
Candida albicans		Rogers and Bevan (1978)
Candida apis		Abranches et al. (1997)
Candida azyma		Ferraz et al. (2016)
Candida berthetii		Buzzini et al. (2003)
Candida diversa		Abranches et al. (2000)
Candida etchellsii	*Candida nodaensis*	Aguiar and Lucas (2000), Da Silva et al. (2008)
Candida freyschussii		Buzzini et al. (2003)
Candida fructus		Abranches et al. (1997)
Candida glabrata	*Torulopsis glabrata*	Sriprakash and Batum (1984), Arroyo-Helguera et al. (2012), Robledo-Leal et al. (2012)
Candida homilentoma		Carreiro et al. (2002)
Candida maltosa		Polonelli et al. (1987), Vadkertiová and Sláviková (2007)
Candida metapsilosis		Robledo-Leal et al. (2014)
Candida naeodendra		Suzuki et al. (1989)
Candida oleophila		Buzzini and Martini (2000)
Candida orba	*Pichia opuntiae* var. “hem”	Starmer et al. (1987), Ganter and Starmer (1992)
Candida parapsilosis		Zekhnov et al. (1989)
Candida pyralidae		Mehlomakulu et al. (2014)
Candida silvae		Buzzini and Martini (2000)
Candida sonorensis		Starmer et al. (1987)
Candida stellata		Abranches et al. (2000)
Candida succiphila		Mushtaq et al. (2015)
Candida versatilis		Vaughan-Martini et al. (1988)
Cyberlindnera americana	*Pichia americana*	Buzzini and Martini (2000)
Cyberlindnera bimundalis	*Pichia bimundalis*	Polonelli et al. (1987)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Cyberlindnera fabianii	*Pichia fabianii*	Polonelli et al. (1987)
Cyberlindnera jadinii	*Candida utilis* (anamorph), *Pichia jadinii*	Vaughan-Martini et al. (1988), Robledo-Leal et al. (2012)
Cyberlindnera mrakii	*Hansenula mrakii*, *Williopsis mrakii*, *Williopsis saturnus* var. *mrakii*	Ashida et al. (1983), Kimura et al. (1993, 1999), Kasahara et al. (1994), Hodgson et al. (1995), Walker et al. (1995), Lowes et al. (2000), Marquina et al. (2002)
Cyberlindnera petersonii	*Hansenula petersonii*	Nomoto et al. (1984)
Cyberlindnera saturnus	*Hansenula saturnus*, *Hansenula beijerinckii*, *Williopsis beijerinckii*, *Williopsis saturnus*	Nomoto et al. (1984), Shemyakina et al. (1991), Kimura et al. (1993, 1995), Komiyama et al. (1995), Salgado Vital et al. (2002), Marquina et al. (2002), Buzzini et al. (2003, 2004), Goretti et al. (2009), Wang et al. (2012)
Cyberlindnera subsufficiens	*Hansenula saturnus* var. *subsufficiens*, *Williopsis subsufficiens*	Nomoto et al. (1984), Shemyakina et al. (1991), Salgado Vital et al. (2002)
Debaryomyces hansenii	*Candida famata*	Suzuki et al. (1989), Llorente et al. (1997), Aguiar and Lucas (2000), Santos et al. (2002), Buzzini et al. (2003), Mushtaq et al. (2015)
Debaryomyces robertsiae	*Wingea robertsiae*	Klassen et al. (2004)
Geotrichum klebahnii		Buzzini and Martini (2000)
Hanseniaspora guilliermondii	*Kloeckera apis* (anamorph)	Abranches et al. (1997)
Hanseniaspora uvarum	*Kloeckera apiculata* (anamorph)	Rosini and Cantini (1987), Zorg et al. (1988), Schmitt and Neuhausen (1994), Abranches et al. (1997), Schmitt and Schernikau (1997), Vadkertiová and Sláviková (2007)
Hanseniaspora valbyensis	*Kloeckera japonica*	Starmer et al. (1987)
Hanseniaspora vineae	*Kloeckera africana*	Abranches et al. (2000)
Hyphopichia burtonii	*Pichia burtonii*	Buzzini and Martini (2000)
Kazachstania exigua	*Candida holmii*, *Saccharomyces exiguus*	Nagornaya et al. (1989), Salgado Vital et al. (2002)
Kazachstania lodderae	*Kluyveromyces loddereae*	Vaughan-Martini and Rosini (1989)
Kazachstania unispora	*Saccharomyces unisporus*	Nagornaya et al. (1989)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Kloeckera lindneri		Abranches et al. (2000)
Kluyveromyces aestuarii		Vaughan-Martini and Rosini (1989)
Kluyveromyces dobzhanskii		Vaughan-Martini and Rosini (1989)
Kluyveromyces lactis	*Candida sphaerica*	Panchal et al. (1985), Vaughan-Martini et al. (1988), Wilson and Whittaker (1989), Butler et al. (1991a, b, c), Stark et al. (1990), Kitamoto et al. (1999)
Kluyveromyces marxianus	*Candida pseudotropicalis*, *Kluyveromyces fragilis*, *Kluyveromyces wikenii*	Lehmann et al. (1987b), Polonelli et al. (1987), Rosini and Cantini (1987), Abranches et al. (1997), Marquina et al. (2002)
Kluyveromyces siamensis		Buzdar et al. (2011)
Kluyveromyces wickerhamii		Vaughan-Martini and Rosini (1989)
Kodamaea ohmeri	*Pichia ohmeri*	Zekhnov et al. (1989)
Komagataella pastoris	*Pichia pastoris*	Starmer et al. (1987)
Lachancea fermentati	*Zygosaccharomyces fermentati*	Buzzini and Martini (2000)
Lachancea thermotolerans	*Candida dattila*, *Kluyveromyces thermotolerans*	Choi et al. (1990)
Lachancea waltii	*Kluyveromyces waltii*	Kono and Himeno (1997)
Magnusiomyces capitatus	*Dipodascus capitatus*, *Trichosporon capitatum*	Morace et al. (1983/1984), De Souza Cabral et al. (2009)
Metschnikowia pulcherrima		Farris et al. (1991), Nguyen and Panon (1998), Vadkertiová and Sláviková (2007)
Meyerozyma guilliermondii	*Candida guilliermondii*, *Pichia guilliermondii*	Zekhnov et al. (1989)
Millerozyma acaciae	*Pichia acaciae*	Bolen et al. (1994), McCracken et al. (1994), Klassen et al. (2004)
Millerozyma farinosa	*Candida cacaoi*, *Pichia farinosa*	Suzuki and Nikkuni (1994), Price et al. (1999), Aguiar and Lucas (2000), Marquina et al. (2002)
Nakazawaea holstii	*Pichia holstii*	Polonelli et al. (1987)
Ogataea minuta	*Pichia minuta*	Polonelli et al. (1987), Buzzini et al. (2003)
Ogataea pini	*Pichia pini*	Zekhnov et al. (1989)
Phaffomyces antillensis	*Pichia antillensis*	Starmer et al. (1987), Ganter and Starmer (1992)
Phaffomyces opuntiae	*Pichia opuntiae*	Starmer et al. (1987), Ganter and Starmer (1992)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Phaffomyces thermotolerans	*Pichia thermotolerans*	Starmer et al. (1987), Ganter and Starmer (1992)
Pichia cactophila		Starmer et al. (1987)
Pichia eremophila	*P. kluyveri* var. *eremophila*	Starmer et al. (1987), Ganter and Starmer (1992)
Pichia fermentans		Marquina et al. (2002)
Pichia kluyveri		Middelbeek et al. (1980b), Zorg et al. (1988), Starmer et al. (1992), Abranches et al. (1997), Pintar and Starmer (2003), Labbani et al. (2015)
Pichia kudriavzevii	*Candida krusei*, *Issatchenkia orientalis*	Lehmann et al. (1987a), Abranches et al. (1997), Bajaj et al. (2013)
Pichia manshurica	*Pichia punctispora*	Golubev and Blagodatskaya (1994)
Pichia membranifaciens	*Candida valida*	Golubev and Blagodatskaya (1993), Abranches et al. (1997, 1998), Llorente et al. (1997), Santos et al. (2000, 2009), Santos and Marquina (2004), Alonso et al. (2015)
Pichia occidentalis	*Candida sorbosa* (anamorph), *Issatchenkia occidentalis*	Abranches et al. (1997)
Pichia scutulata	*Issatchenkia scutulata*	Buzzini and Martini (2000)
Pichia terricola	*Issatchenkia terricola*	Abranches et al. (2000)
Priceomyces carsonii	*Debaryomyces carsonii*	Polonelli et al. (1987)
Priceomyces haplophilus	*Pichia halophila*	Aguiar and Lucas (2000)
Saccharomyces sp.		Maqueda et al. (2012)
Saccharomyces cerevisiae		Schmitt and Radler (1988), Zhu and Bussey (1989, 1991), Goto et al. (1990), Walker et al. (1995), Wickner (1996), Schmitt and Schernikau (1997), Marquina et al. (2002), Bajaj et al. (2003), Satora and Tuszynski (2005), Rodríguez-Cousino et al. (2011), Maturano et al. (2012), Chang et al. (2015), Lukša et al. (2015), Orentaite et al. (2016)
Saccharomyces eubayanus		Chang et al. (2015)
Saccharomyces paradoxus		Naumov (1985), Chang et al. (2015)
Scheffersomyces spartinae	*Pichia spartinae*	Polonelli et al. (1987)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Scheffersomyces stipitis	*Pichia stipitis*	Laplace et al. (1992)
Schizosaccharomyces pombe		Bonilla-Salinas et al. (1995), Heintel et al. (2001)
Schwanniomyces capriottii	*Debaryomyces castellii*	Vustin et al. (1993), Mushtaq et al. (2015)
Schwanniomyces etchellsii	*Debaryomyces etchelsii*	Buzzini and Martini (2000)
Schwanniomyces occidentalis	*Debaryomyces occidentalis*	Vaughan-Martini et al. (1988), Chen et al. (2000)
Schwanniomyces polymorphus	*Debaryomyces polymorphus*	Vustin et al. (1993)
Schwanniomyces vanrijiae	*Debaryomyces vanrijiae*	Aguiar and Lucas (2000)
Starmera amethionina	*Pichia amethionina* var. *amethionina*	Starmer et al. (1987)
Starmera quercuum	*Pichia quercuum*	Zekhnov et al. (1989)
Starmerella bombicola	*Candida bombicola* (anamorph)	Abranches et al. (1997)
Tetrapisispora phaffii	*Kluyveromyces phaffii*	Ciani and Fatichenti (2001), Buzzini et al. (2003), Comitini et al. (2004a, b, 2009), Oro et al. (2014)
Torulaspora delbrueckii		Bonilla-Salinas et al. (1995), Villalba et al. (2016)
Torulaspora microellipsoides	*Zygosaccharomyces microellipsoides*	Buzzini and Martini (2000)
Vanderwaltozyma polyspora	*Kluyveromyces polysporus*	Kono and Himeno (1997)
Wickerhamomyces anomalus	*Hansenula anomala*, *Pichia anomala*	Nomoto et al. (1984), Kagiyama et al. (1988), Sawant et al. (1989), Walker et al. (1995), Llorente et al. (1997), Abranches et al. (1998), Buzzini et al. (2003), İzgü and Altinbay (2004), İzgü et al. (2005), Vadkertiová and Sláviková (2007), Wang et al. (2007a, b), De Ingeniis et al. (2009), Mushtaq et al. (2010), Satora et al. (2014)
Wickerhamomyces bovis		Golubev (2016)
Wickerhamomyces canadensis	*Pichia canadensis*	Lehmann et al. (1987a)
Wickerhamomyces ciferrii	*Hansenula ciferrii*	Nomoto et al. (1984)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Wickerhamomyces lynferdii	*Pichia lynferdii*	Mushtaq et al. (2015)
Wickerhamomyces subpelliculosus	*Pichia subpelliculosa*	Young and Yagiu (1978)
Yamadazyma mexicana	*Pichia mexicana*	Starmer et al. (1987)
Zygosaccharomyces bailii		Radler et al. (1993), Schmitt and Neuhausen (1994), Marquina et al. (2002), Weiler and Schmitt (2003)
Zygotorulaspora florentina	*Zygosaccharomyces florentinus*	Aguiar and Lucas (2000)
Basidiomycota		
Bullera alba		Golubev et al. (1997)
Bullera hannae		Golubev et al. (1996)
Bullera pseudoalba		Mushtaq et al. (2010)
Bullera unica		Golubev and Nakase (1998)
Curvibasidium cygneicollum	*Rhodotorula fujisanensis* (anamorph)	Golubev (1992)
Cystobasidium minutum	*Rhodotorula minuta*	Golubev (1991b)
Cystofilobasidium bisporidii		Karamysheva et al. (1991), Kulakovskaya et al. (1996)
Cystofilobasidium capitatum		Vadkertiová and Sláviková (2007)
Cystofilobasidium infirmominiatum		Golubev et al. (2003b)
Dioszegia hungarica	*Cryptococcus hungaricus*	Gacser et al. (2001)
Hamamotoa lignophila	*Rhodotorula lignophila*	Buzzini and Martini (2000)
Hannaella luteola	*Cryptococcus luteolus*	Buzzini and Martini (2000)
Hannaella sinensis	*Bullera sinensis*	Golubev and Nakase (1997)
Hasegawazyma lactosa	*Rhodotorula lactosa*	Buzzini and Martini (2000)
Kwoniella pini	*Cryptococcus pinus*	Golubev (2009)
Mrakia aquatica	*Cryptococcus aquaticus*, *Mrakiella aquatica*	Pfeiffer et al. (2004)
Mrakia frigida		Hua et al. (2010), Liu et al. (2012)
Naganishia albida	*Cryptococcus albidus*	Starmer et al. (1987), Vadkertiová and Sláviková (2007), Mushtaq et al. (2015)
Papiliotrema laurentii	*Cryptococcus laurentii*	Middelbeek et al. (1980a), Golubev and Kuznetsova (1989), Vadkertiová and Sláviková (2007)
Papiliotrema nemorosus	*Cryptococcus nemorosus*	Golubev et al. (2003a)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Papiliotrema perniciosus	*Cryptococcus perniciosus*	Golubev et al. (2003a)
Piskurozyma capsuligena	*Filobasidium capsuligenum*	Rosini and Cantini (1987), Golubev and Kuznetsova (1991)
Pseudozyma antarctica		Buzzini and Martini (2000)
Pseudozyma aphidis		Farris et al. (1991), Vadkertiová and Sláviková (2007)
Pseudozyma flocculosa		Farris et al. (1991), Vadkertiová and Sláviková (2007)
Pseudozyma prolifica		Golubev (2007)
Pseudozyma tsukubaensis		Golubev et al. (2006)
Rhodosporidiobolus colostri	*Rhodotorula colostri*	Golubev and Tomashevskaya (2009)
Rhodosporidiobolus ruineniae	*Sporidiobolus ruineniae*	Mushtaq et al. (2010)
Rhodotorula glutinis		Golubev (1989), Aguiar and Lucas (2000), Vadkertiová and Sláviková (2007)
Rhodotorula graminis		Buzzini and Martini (2000), Vadkertiová and Sláviková (2007)
Rhodotorula mucilaginosa		Golubev and Churkina (1990), Vadkertiová and Sláviková (2007)
Saitozyma flava	*Cryptococcus flavus*	Buzzini and Martini (2000)
Saitozyma podzolica	*Cryptococcus podzolicus*	Golubev (1991a)
Solicoccozyma aeria	*Cryptococcus aerius*	Carreiro et al. (2002)
Sporidiobolus pararoseus		Janderova et al. (1995)
Sporisorium graminicola	*Pseudozyma graminicola*	Golubev et al. (2008)
Sporobolomyces roseus		Abranches et al. (1997), Vadkertiová and Sláviková (2007)
Sporobolomyces salmonicolor	*Sporidiobolus salmonicolor*	Golubev and Tsiomenko (1985), Vadkertiová and Sláviková (2007)
Tausonia pullulans	*Guehomyces pullulans*, *Trichosporon pullulans*	Golubev et al. (2002)
Tilletiopsis albescens		Golubev (1998)
Tilletiopsis flava		Golubev and Churkina (2001)
Trichosporon asteroides		Buzzini and Martini (2000)
Trichosporon asteroides		Buzzini and Martini (2000)
Trichosporon insectorum		Fuentefria et al. (2008)

(continued)

Table 9.3 (continued)

Killer species	Original taxonomic designation cited in the literature	References
Trichosporon jirovecii		Carreiro et al. (2002)
Udeniomyces pyricola	*Bullera pyricola*	Mushtaq et al. (2015)
Vanrija humicola	*Cryptococcus humicola*	Golubev and Shabalin (1994)
Dimorphic fungi		
Aureobasidium pullulans		Vadkertiová and Sláviková (1995), Buzzini and Martini (2000)
Mycosarcoma maydis	*Ustilago maydis*	Gage et al. (2001)

Rhodotorula mucilaginosa. The occurrence of killer activity in dimorphic yeastlike fungi of the species *Aureobasidium pullulans* and *U. maydis* was also found (Table 9.3).

A few authors (Golubev and Boekhout 1995; Golubev 2006) suggested that the distribution of a killer protein's activity against yeast whole cells may be restricted to taxonomically related organisms, so that sensitivity patterns could be considered of taxonomic importance. This was hypothesized on the evidence that yeast cell wall variation is taxonomically constrained and the importance of cell wall binding in toxin activity (Weijman and Golubev 1987; Fleet 1991). Golubev (2006) also postulated that the diversity of cell wall receptors involved in binding killer proteins could have taxonomic significance if a taxon had a receptor both unique to it and common for all members of the taxon. This may provide the basis of differences in the killer activity spectra, but he also warned that the possible use of a given killer protein as a taxonomic tool should be preceded by a careful study of its killing pattern.

However, a number of recent studies reported that the spectrum of activity of killer proteins appears to be much broader than previously believed, with no evident distinction between the killer activity exhibited by yeasts belonging to Ascomycota and Basidiomycota; indeed, some basidiomycetous yeasts exhibited an interphylum killing activity against ascomycetous strains and vice versa. Some intraphylum interactions (i.e., Ascomycota vs. Ascomycota, Basidiomycota vs. Basidiomycota) were also found, both at intergeneric and intrageneric level (Buzzini and Martini 2000; Marquina et al. 2002; Buzzini et al. 2003, 2004; Vadkertiová and Sláviková 2007; Goretti et al. 2009; Labbani et al. 2015). These data are in agreement with the hypothesis postulated by Golubev (2006) that there are no definite taxonomic criteria for the action of killer proteins and, although they could also be active against organisms phylogenetically and taxonomically related to the killer strains, the degree of relatedness may vary from strains of the same species to species of related genera or even higher taxa. This is also true for other antagonistic interactions involving yeasts vs. bacteria and/or filamentous fungi and for the production of non-proteinaceous antimicrobial agents by yeasts.

9.4 Ecology of Killer Yeasts

In addition to its role in autoselection of toxin-encoding dsDNAs or dsRNAs (see Sect. 9.2.5), the killer phenomenon is a potential mechanism of interference competition. Its role in yeast ecology has received theoretical, observational, and experimental study. We will first look at predictions based on theoretical analysis and then empirical studies of killer ecology.

Much of the analysis of microbial antagonism has focused on bacteriocins. Chao and Levin (1981) proposed that bacteriocins would only be advantageous for cells that incur the cost of toxin production if the benefits of that toxin accrued to the producer cell and not to other nonproducing cells. They concluded that "structured environments" were most likely to promote this sort of antagonism because, in a structured environment, it is possible to restrict the toxin to the neighborhood of the cell that produced it so that the cells killed were those in direct competition with the toxin-producing cell. They equated structure with the viscosity of the medium. Liquid media are unstructured habitats; agar plates or soft-agar pour plates are structured habitats. Liquid media mix all cells together, while plates force spatial structuring by immobilizing cells so that each cell interacts most intensely with its neighboring cells.

Cell-scale spatial structure is not the only scale of structure potentially important to killer yeast ecology. Often, yeast habitat is broken into larger patches, such as individual fruit necroses or slime fluxes. These larger patches impose a second layer of spatial structure that can affect the global success of killer yeasts. With respect to this larger scale of structure, Chao and Levin (1981) established conditions under which toxin production is favorable within patches. Levin et al. (1988) further refined the model of within-patch dynamics by considering the effect of frequency on the ability of toxin-producing bacteria to invade or maintain themselves in chemostat cultures. In their analysis, toxin production was a successful strategy only if toxin producers were at high frequency. The final outcome for mixtures of killers and susceptible strains was either all toxin producers or all susceptible strains but which would prevail depending on initial conditions.

It is not clear what effects large-scale patchiness might have on killer yeast ecology. Frank (1994) constructed a more elaborate model of microbial antagonism that included resource competition and variation in environmental quality. Larger-scale spatial dynamics were the outcome of cell population diffusion across one-dimensional habitats that varied in quality (imposed by removing all individuals from portions of the space, akin to the "holes" produced by ecological disturbances). The inclusion of this variable habitat quality allowed for stable coexistence of toxin producers and susceptible strains. Other studies concluded that patchy systems are unstable and that initial conditions would determine whether killer or susceptible morphs would prevail. Iwasa et al. (1998) concluded that patchiness would lead once again to the elimination of either killers or susceptible strains except for a small section of parameter space that predicted coexistence. Durrett and Levin (1994) found that only one strategy, killer or

susceptible strains, would persist in spatially structured populations but that which strategy would prevail depended on the relative cost and benefits of being a killer, not on initial conditions. Coexistence was only predicted when a third type of cell was introduced, resistant strains that did not expend much on toxin production. Coexistence was the outcome of intransitive relationships between the three strategies (a kind of rock-scissors-stone game) (Durrett and Levin 1994; Czaran et al. 2002).

The conclusion that either killer or sensitive cells would predominate even in spatially structured systems is inconsistent with the high rate of polymorphism in natural populations (see above section or below in the discussion of empirical studies). Other approaches to modeling killer systems have predicted stable polymorphisms between killers and susceptible strains. A metapopulation model with a low cost of toxin production and local patch dynamics that allowed the killer strain to eliminate the sensitive strain whenever both occupied a patch produced coexistence of killers and susceptible strains in most situations without the presence of resistant strains (Czaran and Hoekstra 2003). Sinclair (2014) approached the question of persistence from the opposite perspective of most who have modeled microbial antagonism: how do susceptible strains persist in the presence of killers? Using an analytical approach, he found coexistence for susceptible strains even when their advantage in growth over killer strains was very small.

Frank (1994) distinguished between the indirect benefit from toxin production of suppressing the growth of competitors, which makes more of the habitat's resources available to the killer strain, and the direct benefit provided by the nutrients that are freed from sensitive cells after they are killed. Frank (1994) found that susceptible strains were favored in poor-nutrient conditions and killers in habitats with readily available nutrients. The higher growth rates of susceptible strains allowed them to gather the lion's share of limited resources, which lowered the indirect benefit of toxin production. Leisner and Haaber (2012) came to somewhat different conclusions regarding the effect of nutrient availability. At low nutrient levels, they argued that the direct benefit of killer toxin production would increase versus the fixed cost of toxin production, making killer toxin production most valuable under conditions of nutrient stress. Bucci et al. (2011) came to the conclusion that killer toxins were favored under low-nutrient conditions in biofilms and that toxin production was important to the high microbial diversity found in biofilms. When nutrients availability increased, their model predicted that sensitive strains would prevail and biodiversity would decrease.

Empirical studies of the role of killer toxins played in natural and man-made habitats can be separated into two general types: studies of toxin ecology and studies of toxin diversity. While there is ample evidence of the diversity of killer toxins, empirical studies of killer yeast ecology are neither plentiful nor comprehensive at this time. But that is true for the ecology of antagonism among microbes in general. Tremendous diversity in antibiotics and bacteriocins is known. Williams and Vickers (1986) proposed several explanations for why so little was known about the role of antibiotics in natural communities and their assessment has been echoed in several more recent publications for both antibiotics (Fierer et al. 2012;

Kumbhar and Watve 2013; Kinkel et al. 2014; Schlatter and Kinkel 2014) and bacteriocins (Riley and Wertz 2002; Ghoul and Mitri 2016; Widder et al. 2016). What follows is a summary of what is known of killer yeast ecology.

The ability of killer yeast to exclude susceptible strains from a habitat was first demonstrated in industrial fermentations using S. *cerevisiae* (Moriya et al. 1987; Vondrejs 1987). Vadasz et al. (2003) demonstrated that frequency is important under these conditions and that, at low frequency under nutrient-poor conditions, killers went extinct but coexistence resulted at intermediate frequency, as theory predicted (Levin et al. 1988; Frank 1994). The importance of the interaction of frequency and nutrient abundance was also demonstrated by Wloch-Salamon et al. (2008) under a different set of growth conditions. Once again, invasion by killer *S. cerevisiae* strains was only possible at a high frequency of killer cells and under high-nutrient conditions. Greig and Travisano (2008), working with *S. cerevisiae* grown in liquid and on solid media, confirmed the importance of frequency in liquid media and found that the killer was not able to invade when at low frequency even on solid (= structured) media, contrary to Chao and Levin's prediction (Chao and Levin 1981). In addition, Greig and Travisano (2008) found that the ability to invade was density dependent as well as frequency dependent on both liquid and solid media. The density dependence effect was felt early in population growth, when there were only few toxin-producing cells and few sensitive cells were near enough to killer cells to receive a lethal dose of toxin. Early on, toxin producers incurred the costs of toxin production in full but did not receive full benefits.

Starmer et al. (1987) reported the first large-scale survey of killer factor occurrence in three habitats associated with *Drosophila* in Arizona, USA: cactus stem necroses, cactus fruit necroses, and tree sap fluxes. The habitats surveyed were geographically interspersed, so it was not trivial to test for inter-habitat killer-sensitive relationships. Their methodology, which they termed "cross-killing," was to test each yeast strain collected for its ability to kill all other strains collected at the same time. Each of these habitats harbors a different yeast community, and they found at least one killer species among the common species in each community. Killer strains were more likely to kill strains from other communities than from their own community.

Ganter and Starmer (1992) followed up with a more detailed analysis of killer factor's role in the cactus-yeast-*Drosophila* system. An analysis of the pattern of killer toxin production and sensitivity was linked to lab experiments on the growth rates of killer strains, non-killer strains, and susceptible strains. Two different sets of cactophilic killer species were investigated. One set consisted of the varieties of *P. kluyveri* (Phaff et al. 1987), with each variety associated with a different cactus host and/or different geographic region and each variety with both killer and non-killer strains. Kurtzman et al. (2008) have since elevated the varieties to species status based on rDNA sequence divergence. However, due to their known hybrid interfertility and the high potential for gene flow resulting from habitat overlap among the strains studied by Ganter and Starmer, we will be consistent with earlier studies and refer to the types as varieties. The other set was four related species from *Phaffomyces*, each associated with different hosts and/or regions and

each with interspecific variation in killer phenotype. Analysis of a large cross-kill matrix demonstrated that the killer toxin from each strain within a variety or species tended to kill the same set of sensitives but, when comparing among varieties (for *P. kluyveri*) and species (for *Phaffomyces*), ecology was a better predictor of which strains were sensitive to the killer toxin than was phylogeny.

Killer factor's impact on *P. kluyveri*'s growth was assessed under simulated field conditions (Ganter and Starmer 1992). All experiments were conducted on the natural substrate (*Opuntia phaeacantha*) from which the yeast was collected supplemented with axenic *Drosophila arizonae* larvae, a species of *Drosophila* that breeds in the same *Opuntia* species. When grown with a sensitive strain from one of two different common cactophilic species as potential competitors, a killer strain of *P. kluyveri* grew faster and reached a greater density than did a non-killer *P. kluyveri* strain. Reciprocally, sensitive strains reached greater densities when no *P. kluyveri* at all was present but the killer strain of *P. kluyveri* reduced the sensitive's density more than the non-killer strain did (there was no effect on the sensitive's growth rate). Pintar and Starmer (2003) also looked for trade-offs in growth between killer and non-killer *P. kluyveri*. Their killer and non-killer strains were haploid clones grown from spore dissection of a single diploid strain, so genetic differences other than killing ability were minimized. They grew the strains on YM broth and, under those conditions, found that killers had a reduced growth rate compared to non-killers but no difference in maximal cell density.

Pintar and Starmer (2003) assessed the ecological impact of *P. kluyveri* killer factor in cage experiments in which a killer or a non-killer strain of *P. kluyveri* competed against a strain from a sensitive or a nonsensitive species. The substrate was grape berries, and axenic *Drosophila melanogaster* were included in the cages. The flies consumed the yeast as both larvae and adults vectored yeast to new grapes, where the adults would inoculate newly added berries and lay eggs (generation time for the flies was about 2 weeks, sampling began at 4 weeks, and samples were taken from each cage four times). When the competitor was sensitive to *P. kluyveri* killer toxin, the killer strain was more successful than the non-killer strain. When the competitor was not sensitive to the killer toxin, the non-killer strain that was consistently found as a higher proportion of the yeast present than was the killer strain.

Although the above provides evidence of the effectiveness of killer toxins in seminatural situations, analysis of the local distribution of killer, non-killer, and sensitive strains suggested that the killer factor's role in the ecology of killer yeast was not always the same (Ganter and Starmer 1992). In pairwise comparisons of the species found in individual cactus necroses (patches to a theorist), pairs of necroses were more similar to each other if both contained a killer strain or if neither did than if one contained a killer and the other did not. This difference was significant for both *P. kluyveri* varieties and *Phaffomyces* species. But a closer look at the pattern of sensitivity found that the *P. kluyveri* and *Phaffomyces thermotolerans* patterns differed. Both species were collected from the same region, the Sonoran Desert, but *P. kluyveri* is found in a wide variety of hosts, while *Phaff. thermotolerans* is restricted to cacti from the subtribe Pachycereinae (which includes the giant

saguaro). Killer strains of the generalist *P. kluyveri* occurred with strains of competitors that were less sensitive to its toxin than potential competitor strains from necroses with either non-killer *P. kluyveri* strains or from necroses on other hosts, indicating that killer *P. kluyveri* had eliminated some of the most sensitive competitors from rots it occupied. Strains of the specialist, *Phaff. thermotolerans*, were found with potential competitor strains that were more sensitive to their toxin than strains found in necroses without *Phaff. thermotolerans* or from other hosts, indicating that *Phaff. thermotolerans* had arrived more recently than its competitors. Ganter and Starmer (1992) speculated that the difference might arise from the toxin's role as either a means of fending off newly arrived competitors for an established yeast or as a means of securing entry to a necrosis already colonized by competitors. Brown et al. (2006) also considered that toxin production might be useful for both invading occupied habitats and resisting invasion by newcomers. Often killer toxin is assumed to be a method of invasion, but Frank (1994) and Levin et al. (1988) stressed its usefulness in resisting invasion.

P. kluyveri is an unusual cactophilic yeast in that it occurs regularly outside of the cactophilic system. Starmer et al. (1987) concluded that *P. kluyveri* killer factor was probably encoded at a chromosomal locus. Starmer et al. (1992) examined the killer phenotype of *P. kluyveri* in greater detail. Killer strains from non-cactophilic *P. kluyveri* were collected from across the continental United States and tested for their ability to kill 71 strains of yeast from tree sap fluxes and cactus necroses collected in the southwestern United States. Of the 167 *P. kluyveri* strains collected, 67% were able to kill at least one of the potential susceptible strains, and the probability that a *P. kluyveri* strain would kill a sensitive strain was weakly inversely related to the geographic distance between where the *P. kluyveri* strain was collected and the southwestern United States, the source of the susceptible strains. Starmer et al. (1992) identified four distinct killer phenotypes among the 167 *P. kluyveri* strains with all locations variable for killer phenotype. At least three epistatic loci with major effects were identified by analysis of matings among haploid clones derived from spore dissections of asci from ten of the collected strains.

It is difficult to know if the prevalence and diversity of killer strains are unusually high in *P. kluyveri*. Maqueda et al. (2012) found that 40% of 126 *S. cerevisiae* strains isolated from spontaneous wine fermentations had one of four killer phenotypes. Of 136 *S. cerevisiae* and *Saccharomyces paradoxus* strains tested by Pieczynska et al. (2013), only 6 of 100 *S. cerevisiae* and 4 of 36 *S. paradoxus* strains were killers. Wojcik and Kordowska-Wiater (2015) surveyed (but did not identify) 102 strains of yeast isolated from leaves, flowers, forest litter, wheat ears, and fruit for the ability to kill a panel of 12 potential sensitive strains. They found 24 strong and 10 weak killer strains, but it is not clear how many species this represents. Differences in methodology make it hard to compare the results of surveys. Starmer et al. (1992) used a panel of 71 potential susceptible strains, while Wojcik and Kordowska-Wiater (2015) used only 12. It is not surprising that the former study found a higher incidence of killing ability.

Other possible venues in which killer toxins may play a role in yeast ecology have not yet been explored, either theoretically or empirically. Rodrigues et al. (2009) found that the benefit from toxin production may be complicated by mutualistic relationships. They found that yeast in leafcutter ant (*Atta* sp.) gardens killed invading fungi harmful to the ant rather than other yeast competing for a place in the garden. Rivero et al. (2015) found that a sensitive strain of *S. cerevisiae* was harmed when in the presence of a live strain of killer *S. cerevisiae* and that it benefitted from the presence of dead cells of the same killer strain. This benefit was tied to the release of a heat shock protein by the dead cells. Another example involves the cactus-yeast-*Drosophila* system. Animal vectors are responsible for dispersing yeast among cactus necroses (Ganter et al. 1986; Fogleman and Foster 1989; Ganter 2011). Most necroses contain more than a single species of yeast (Starmer 1982; Starmer and Phaff 1983; Fogleman and Starmer 1985). Recently, it has been observed that yeasts are affected by passage through the insect gut (Reuter et al. 2007; Greig and Leu 2009). Flies pack their crops with yeast prior to leaving a patch of food. In the crop, yeasts are in close proximity, and a killer that managed to secrete toxin there might benefit disproportionately by increasing its representation in the yeast deposited by the fly in the new necrosis. Gurevitch (1984) demonstrated that, in pairwise competition experiments, final population size is positively correlated with relative inoculum size. So, the insect crop might be a place where the killer toxin can be most effective in two ways. The confined space increases the killer toxin effectiveness, and the advantage gained there becomes a key to greater population size in the new patch, thereby increasing the chance of dispersal for the killer yeast.

9.5 Concluding Remarks

In the 54 years since killer yeasts were discovered, we have learned much about killer toxin biodiversity. They are encoded on both extrachromosomal and chromosomal genes, they kill by a variety of mechanisms, and some toxins have little or no sequence similarity to others. Indeed, some killer molecules are not even proteins. Their only commonality is that they target and kill other yeast. Targeting often involves recognition of the susceptible yeast's cell wall, but secondary targets are common. Susceptible strains may be conspecifics, species from the killer's habitat, or from completely different habitats.

Killer systems have arisen multiple times within the ascomycocetous and basidiomycetous yeast lineages. There is evidence that such systems are costly in terms of fitness and the diversity in killer systems strongly implies that they often supply sufficient advantages to offset those costs. Evidence at this time suggests that the benefits accrue because toxins confer competitive advantage to producers but the general lack of understanding of yeast ecology limits our understanding of killer toxin's ecological roles. Of course, toxin production may be beneficial for reasons not related to competition (such as those provided by yeast in leaf-cutting ants'

gardens), and these benefits are not mutually exclusive of those from competition. In addition, killer toxin production may also benefit the toxin-encoding extrachromosomal genetic element by killing those strains that do not maintain it.

It is clear that there is much more to learn about killer systems and about antagonism between yeasts in general. Too little is known about how killer cells remain immune to their own toxins. There is also much to learn about resistance to killer toxins. Until our knowledge of resistance is as detailed as our knowledge of the toxins, we will not have a clear picture of their coevolutionary history nor of the role of killer systems in yeast ecology. The search for killer yeast has not been extensive, and new killer systems will be discovered if surveys are done with larger, carefully selected panels of potentially susceptible strains. Such surveys should vary the physical conditions under which the survey is conducted. The temperature, pH, salt concentration, and even the nutrient concentration of the media may affect the outcome.

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