Yeasts

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Abstract

Yeasts are a group of eukaryotic microfungi with a well-defined cell wall whose growth is either entirely unicellular or a combination of hyphal and unicellular reproduction. The approximately 1500 known yeast species belong to two distinct fungal phyla, the Ascomycota and the Basidiomycota. Within each these phyla, yeasts can be found in several subphyla or classes, reflecting the enormous diversity of their evolutionary origins and biochemical properties. In nature, yeasts are found mainly in association with plants or animals but are also present in soil and aquatic environments. Yeasts grow rapidly and have simple nutritional requirements, for which reason they have been used as model systems in biochemistry, genetics and molecular biology. They were the first microorganisms to be domesticated for the production of beer, bread or wine, and they continue to be used for the benefit of humanity in the production of many important health care and industrial commodities, including recombinant proteins, biopharmaceuticals, biocontrol agents and biofuels. The best-known yeast is the species *Saccharomyces cerevisiae*, which may be regarded as the world's foremost industrial microbe.

Key Concepts:

- Yeasts share in common a primarily unicellular mode of reproduction.
- Yeasts are phylogenetically diverse, being classified in several classes of two fungal phyla.
- The concept of yeast is rooted in history and does not always follow logical biological lines.
- Yeasts have a rigid cell wall primarily made up of β-(1–3)-glucan.
- Sexual reproduction in yeasts involves the formation of internally formed ascospores or externally formed basidiospores.
- Current yeast classification is based on phylogenetic relationships inferred by gene sequencing.

- Yeasts grow in nature primarily in association with the plant–insect interface or with warm-blooded animals, but also occur in soil and aquatic habitats.
- Yeasts serve as important industrial model systems in research.
- Yeasts are of great importance in biotechnology. The main products are alcohol, yeast biomass and recombinant proteins. *Saccharomyces cerevisiae* is the premier industrial microorganism.
- A few yeast species cause infections in humans and other animals, but most yeasts act in an opportunistic fashion, in individuals that are immunocompromised.

Keywords:

- reproduction;
- fungi;
- systematics;
- ecology;
- application;
- biotechnology;
- genetics;
- spores;
- budding

Introduction: Overview of Yeasts as Unicellular Fungi

Unicellular reproduction in yeasts can take place by budding, by fission or by a combination of the two processes, called bud fission. Sexually reproducing yeasts are members of two large phyla of the fungi characterised by different modes of sporulation. Ascomycetous yeasts produce variously shaped ascospores following meiosis (reduction division) of a diploid nucleus inside an ascus (a sac-like structure) that is not enclosed in a complex fruiting body or an ascocarp. Basidiomycetous yeasts exhibit a wide variety in morphology of the basidium, where meiotic spores are formed externally. Many unicellular fungi lack a sexual cycle or their sexual cycle has yet to be discovered. In heterothallic yeasts the lack of a sexual cycle may be due to absence of a compatible mating type. In this case fusion or conjugation of two haploid cells of opposite mating type (variously designated *a* and α or h^+ and h^-) must occur before meiosis and spore development can take place. Asexual, imperfect or anamorphic yeast species are usually assigned to separate genera pending the discovery

of their sexual cycle, although there is now a movement afoot to adopt a single classification for both sexual and asexual forms. The large genera *Candida* and *Cryptococcus* are important examples of taxa that contain asexual species that may have sexual counterparts in other genera. The affinity of such yeasts to the sexually reproducing or teleomorphic yeasts can be ascertained by deoxyribonucleic acid (DNA) sequence comparison. In addition, ascomycetous yeasts usually undergo holoblastic, multilateral or bipolar budding as opposed to enteroblastic budding as in many basidiomycetous yeasts. Ascomycetous and basidiomycetous yeasts often differ in the composition of their cell wall polysaccharides and in the morphology of their hyphal septa, when present. Ecologically, ascomycetous yeasts have a general tendency to be copiotrophic, adapted to submerged liquid habitats and dependent on animal vectors for dispersal. By contrast, basidiomycetous yeasts often have the ability to scavenge a broad array of nutrients present at low concentrations and exhibit surface growth adaptations and air dispersal. For example, many basidiomycetous yeasts form a mucilaginous capsule that may protect the cells from desiccation and others spread their buds by discharging them forcibly (ballistospores). See also Anamorphic Fungi, Ascomycota, Basidiomycota, Budding, Fungal Cells, Fungal Ecology, Fungal Physiology, Fungi and the History of Mycology, Meiosis, and Yeast Mating Type

The question may be asked why yeasts should be treated as a separate group of microorganisms. Historically, the term ‘yeasts’ was confined to the unicellular microbes responsible for the alcoholic fermentation of wine, beer and similar beverages as well as for the leavening of dough during bread baking. It was later realised that among food spoilage organisms many nonfermentative yeast species were present as well. Yeasts were also found to be responsible for certain pathological conditions in humans and animals. Sexually reproducing yeasts have been used in a great variety of genetic studies that gave much more rapid results than experiments in plant or animal genetics. Several yeast species have become model systems in various fields of biological research. Yeast taxonomy has evolved from an empirical approach to a sophisticated science based on molecular phylogenetics. Yearly international symposia sponsored by the International Commission on Yeasts of the International Union of Microbiological Societies take place in different countries. Some of the factors outlined here in combination with others have led to the treatment and study of yeasts as a subject separate from the filamentous fungi. It is important to distinguish between ‘yeasts’ (plural), as used here, and ‘yeast’ (singular), which often applies to the single species *Saccharomyces cerevisiae*. The latter is also referred to as ‘baker's yeast’ or ‘budding yeast’, although over a thousand species of yeasts capable of budding have been described. A large research community interested in ‘yeast’ as a model system in cell biology, genetics, development or biochemistry holds an international meeting titled ‘Yeast Genetics and Molecular Biology’. See also Fungal Fermentation: Industrial, Fungi and the History of Mycology, Budding Yeast *Saccharomyces Cerevisiae* as a Model Genetic Organism, and Yeast Cell Culture

The demarcation between yeasts and other forms of life is not always clear. For example, the current taxonomic treatise, 'The Yeasts, A Taxonomic Study, 5th edition' (Kurtzman *et al.*, 2011) includes a discussion of the genus *Prototheca*, which consists of achlorophyllous species of the green alga *Chlorella* that live like yeasts and form yeast-like colonies on agar plates. The 'black yeasts' includes a vast array of organisms that look and behave like yeasts, but are not close relatives of taxa that are normally regarded as yeasts. The genus *Moniliella*, whose exact phylogenetic position within the Basidiomycota is not clear, is included in the yeasts, but other genera such as *Aureobasidium* or *Exophiala*, which are related to the filamentous Ascomycota, are not. See also Systematics: Relevance to the Twenty-first Century

Asexual Reproduction

The most common form of cell growth is by multilateral budding, that is, the repetitive formation of daughter cells, occurring always at different locations on the surface of the mother cell. In some species the daughter cells become detached at maturity so that the population consists mainly of single cells, pairs or small clusters. In other species the daughter cells and subsequent buds remain attached to each other and form a branched cell structure. As older cells may also grow lengthwise, the structure develops into an extensive tree-like outgrowth, which is then called a pseudomycelium. The cell morphology may range from nearly spherical to elongate or cylindrical. The axial cells and their branches constitute a pseudohypha and outer cells are called blastoconidia (Figure 1). Species of *Saccharomyces* produce few or no pseudohyphae, whereas some species of *Candida* and other genera may form extensive pseudohyphal networks. Some genera of the ascomycetous yeasts have lemon-shaped or apiculate cells where budding occurs repeatedly at the tips of the cells (bipolar budding), as in the genera *Hanseniaspora* and *Saccharomycodes*. As a result of this type of budding older cells, having produced many buds, have the most pronounced apiculate shape (Figure 2). Another ascomycetous yeast genus, *Schizosaccharomyces*, lacks budding altogether and vegetative growth occurs exclusively by fission or crosswall (or septum) formation. Each cell grows by apical elongation as observed in true hyphae typical of most fungi. The resulting cells break apart and are referred to as arthroconidia (Figure 3). In other genera, such as *Saccharomycopsis*, cells may continue to grow lengthwise and become thread-like elements, or true hyphae, with crosswalls that make up the mycelial structure of such yeasts. Besides septa, such hyphae may also produce budding cells (blastoconidia) or may break apart at the septa and form arthroconidia. Septa in yeasts with a hyphal structure are not solid separations as they contain a variety of pores that allow cell-to-cell contact (van der Klei *et al.*, 2011). Budding in basidiomycetous yeasts differs from that in ascomycetous species in that repeated bud formation at the same site is the usual mode of reproduction. The cell walls of successive buds arise each time underneath the original cell wall, giving rise to concentric collars that are best observed by electron microscopy. This type of budding is called enteroblastic. Septa in

hyphal structures of basidiomycetous yeasts often differ from those in ascomycetous yeasts by having a complex pore structure (the dolipore) with differentiated membrane organelles (called parenthesomes) that delimit the pore domain on both sides of the septum. See also Budding, and Hyphae

<FIGURES 1, 2 AND 3 NEAR HERE>

Another kind of asexual reproduction found in several genera of basidiomycetous yeasts is the formation of ballistospores. Such spores are borne, one at a time, on pointed stalks (sterigmata), from which they are discharged with considerable force by a peculiar droplet mechanism. A small droplet of fluid is exuded by the stalk at the base of the spore; the droplet is discharged carrying the spore with it (Figure 4). See also Fungal Spores

<FIGURE 4 NEAR HERE>

Yeast Cell Walls

Considerable qualitative as well as quantitative variations exist among different yeast species in the chemical and structural composition of the cell wall. By far the most detailed studies of the yeast wall have been done with strains of *Saccharomyces cerevisiae* and closely related species. The reader is referred to van der Klei *et al.* 2011 for a detailed treatment of this subject. These authors and others have made clear that yeast cell walls isolated from a disintegrated cell mass of compressed baker's yeast (stationary phase cells) do not have the same composition as cells going through an exponential growth phase of the cell cycle. Nevertheless, there is now a general understanding of the composition and structure of cell walls of *Sa. cerevisiae*. A brief account of these findings follows. See also Fungal Cell Walls, and Budding Yeast *Saccharomyces Cerevisiae* as a Model Genetic Organism

When isolated washed cell walls of baker's yeast are extracted with hot alkali, the insoluble residue (about 35%) retains the rigidity and shape of the yeast wall. This fraction, when extracted thoroughly with hot dilute acetic acid, yields another minor component (about 5% based on the original wall material) that is made up of a highly branched β-(1,6)-glucan with some β-(1,3)-linkages. The residue (called the acid- and alkali-insoluble glucan) still retains the shape of the original cell wall and is made up of β-(1,3)-glucan microfibrils with some β-(1,6)-linkages as branch points. The original hot alkali extract of the cell wall material has been found to contain a mixture of alkali-soluble glucan and mannan–protein complexes. The alkali-soluble glucan, which is insoluble in water, is slightly branched and largely β-(1,3)-linked except for the presence of a number of unsubstituted β-(1,6)-linked residues within the long chains of β-(1,3)-linked glucose residues. This glucan represents about 20% of the original cell wall. Some investigators have provided evidence that part of it might act as a

precursor of the alkali-insoluble glucan component. There is also evidence that the β-(1,6)-portion of the alkali-soluble glucan is linked to mannan. The manno-proteins constitute about 40% of the original cell wall and together with the alkali-soluble glucan form the outer layer of the cell wall. See also Glycoproteins, and Polysaccharides

The final component of mature cell walls is chitin, a linear polymer of *N*-acetyl-D-glucosamine linked by β-(1,4)-bonds, which occurs in concentrations varying from traces to 2%. Most of the cell wall chitin in multilaterally budding cells (e.g. baker's yeast) is found in the bud scar region of the wall, which suggests that young unbudded daughter cells lack chitin. This has proven to be only partially true since it was shown more recently that lateral walls also develop small amounts of chitin late in the cell cycle, where it appears to be linked to the alkali-insoluble wall layer. This linkage may be one of the reasons for its insolubility. See also Chitin: A Structural Biopolysaccharide with Multiple Applications

A few examples will illustrate the variability in cell wall composition among yeast species in different genera as compared with *Sa. cerevisiae*. Among the ascomycetous yeast genera, the fission yeast *Schizosaccharomyces* is unique because of the deposition of α-(1,3)-glucan or pseudonigeran in the cell wall in addition to the better known β-glucans and the virtual lack of chitin. Species of this genus also differ in mannan composition, which shows terminal D-galactose sugars in the side-chains of their mannans. Pseudonigeran is also common in species of basidiomycetous yeasts (e.g. *Cryptococcus*) and in many genera of the higher fungi. In filamentous yeasts (e.g. *Saccharomycopsis*) and the hyphal state of the pathogenic yeast *Candida albicans*, there is a large increase in chitin content of the cell wall as compared to the budding or yeast phase of that species. An overview of the variation in cell wall structure and composition across ascomycetous and basidiomycetous yeasts is given by van der Klei *et al.* 2011.

Sexual Reproduction

Many yeasts do not produce sexual spores on the common media used for vegetative growth. Over the years a large number of special media have been devised to stimulate the production of ascospores and basidiospores. Some of these are useful mainly for species of specific genera (Kurtzman *et al.*, 2011). Sporulation in diploid ascomycetous yeasts, for example *Saccharomyces* and *Saccharomycodes*, requires meiosis of the diploid nucleus, resulting in four haploid nuclei (up to eight or more by additional mitotic divisions in certain other genera) which give rise to four or more ascospores. The spore morphology may vary among the different genera. Spore shapes include spheroidal (smooth or with various ornamentations), ovoid, bean-shaped, hat-shaped, sickle-shaped, needle-shaped and elongate. In some species the asci remain intact at maturity, whereas in others the ascus wall deliquesces owing to endogenous glucanases and spores are rapidly released from the asci.

Ascosporic yeasts whose vegetative cells are primarily haploid, with the basic (1*n*) number of chromosomes, must first fuse or conjugate so that a diploid nucleus is formed before meiosis and sporulation can take place. Examples are species of the genera *Zygosaccharomyces* and *Schizosaccharomyces*. In homothallic haploid yeasts zygotes can arise in a single population and may even involve the conjugation of a mother cell with its own bud, as for example in the genera *Debaryomyces* or *Nadsonia*. In heterothallic haploid yeasts two compatible mating types are required for conjugation, usually designated as *a* and α or h^- and h^+. Heterothallism is common among species of *Clavispora*, *Kodamaea*, *Phaffomyces*, *Sugiyamaella* and *Wickerhamiella*, but generally varies among species and may even be strain dependent as in *Sa. cerevisiae*. See also Ascomycota, and Meiosis

Life cycles of basidiomycetous yeasts are more complex but have in common the production of sexual basidiospores or sporidia, which are formed externally on some form of basidium, also called a metabasidium. Such basidiospores are sessile and thus not forcibly discharged. Mating systems are occasionally multiallelic, where more than two mating types are present, or tetrapolar, meaning that mating requires compatibility at two loci. A complete coverage of all known life cycles of basidiomycetous yeasts is not possible in the available space but a few examples follow. For more information the reader is referred to Kurtzman *et al.* 2011. See also Basidiomycota

The genus *Rhodosporidium* was based on the discovery by I. Banno that some strains of *Rhodotorula glutinis* (a red, budding, carotenoid-containing yeast) are able to mate and produce a binucleate mycelium with clamp connections at the septa (Banno, 1967). Such a phenomenon was previously known only in filamentous Basidiomycetes. Ultimately some of the dikaryotic cells transform into thick-walled teliospores in which the nuclei undergo karyogamy and form a 2*n* nucleus. Under suitable environmental conditions meiosis takes place and the teliospore germinates into a metabasidium consisting of two to four cells on which lateral basidiospores are formed. The basidiospores in turn germinate by budding. A simplified life cycle is shown in Figure 5. Banno named the new species *Rhodosporidium toruloides*. Currently the genus contains several species that show some variation in details of mating type and life cycles. A genus with a similar life cycle is *Sporidiobolus*, but its species often also propagate asexually by the formation of ballistoconidia. The anamorphs of *Sporidiobolus* are species of the genus *Sporobolomyces*. The genus *Leucosporidium* and other related genera also resemble *Rhodosporidium* but the species lack carotenoid pigments and colonies are therefore pale white. Most basidiomycetous genera belong to the subphylum Pucciniomycotina. Other yeasts able to form basidia or their relatives belong to the subphyla Agaricomycotina and Ustilaginomycotina. Two teleomorphic genera in the Agaricomycotina should be mentioned. The life cycle of the genus *Filobasidiella* is characterised by conjugation of cells of opposite mating type. The zygotes produce dikaryotic hyphae with clamp connections. The septa have dolipores but lack parenthesomes. A slender holobasidium with an expanded apex arises directly from

a dikaryotic hypha. Karyogamy takes place in the metabasidium followed by meiosis in the apical area and haploid basidiospores bud basipetally from the apex of the metabasidium to form chains of basidiospores. *Filobasidiella neoformans*, *Filobasidiella bacillispora*, and their anamorphs *Cryptococcus neoformans* and *Cryptococcus gattii* are the only serious human pathogens among the heterobasidiomycetous yeasts (see section on ecology, below). The closely related, nonpathogenic genus *Filobasidium* (with four species) has a similar life cycle to *Filobasidiella* except that the basidiospores are produced singly and not in chains (Figure 6). For further details see Kurtzman *et al.* 2011. Yeasts in the subphylum Ustilaginomycotina are almost exclusively asexual. See also Fungal Genetics, and Meiosis

<FIGURES 5 AND 6 NEAR HERE>

Systematics

The grouping of yeasts in the phyla Ascomycota and Basidiomycota has been described briefly in the first section of this article. Criteria for differentiating genera have evolved over many years with a major emphasis on spore and cell morphology and life cycles, as discussed above. The recent advent of DNA sequencing technology has changed this. Systematists generally agree that taxa of all ranks, including the rank of genus, should be monophyletic. They further trust that sequence-based phylogenies can be used to establish monophyly. Consequently, many yeast genera have been re-defined on the basis of DNA sequences. Species circumscriptions were once based on physiological or metabolic characteristics that increased from just a few sugar fermentation tests to more than 50 growth responses on different substrates and under various environmental conditions. The greatly expanded spectrum of physiological tests improved differentiation between species and provided clues that some previously accepted species represented more than a single taxon. The introduction of nuclear DNA base composition determination or G+C content in the 1960s also contributed significantly to species differentiation, although its use is mainly exclusionary and the approach has been supplanted by sequence-based methodologies. DNA reassociation methods also contributed greatly to the interpretation of relatedness among species of yeast. Members of a reproductive community, termed biological species, usually show DNA relatedness values of 70–100%. Because DNA reassociation experiments do not provide useful information on phylogeny and thus do not answer the question of how more highly divergent species are related, investigators have turned to DNA sequence comparisons to assess both phylogenetic relationships between yeasts and species boundaries. Sequences of the D1/D2 variable regions of the large subunit ribosomal ribonucleic acid (RNA) gene have been determined for all described yeast species and serve as a barcode database for identification. Kurtzman and Robnett 1998 further showed that strains of ascomycetous yeasts that are known to be members of the same species rarely differ by more than three nucleotide substitutions in that region, whereas strains of species that are reproductively isolated differ by 1% substitutions or

more (4–6 substitutions). Exceptions exist, but the criterion is widely applicable and has greatly facilitated the discovery of new yeast species. See also Cladistics, DNA Sequence Analysis, Molecular Phylogeny Reconstruction, Species Concepts, and Systematics: Relevance to the Twenty-first Century

As a result of phylogenetic sequence analyses, we now know that ascomycetous yeasts are not reduced forms derived from the more complex filamentous Ascomycetes (Pezizomycotina). Instead the budding yeasts of the subphylum Saccharomycotina form a monophyletic group, or clade, that is distinct from other Ascomycota. The fission yeasts of the genus *Schizosaccharomyces* have been assigned to the subphylum Taphrinomycotina along with a number of parasitic fungi that are not always treated as yeasts. By contrast, basidiomycetous yeasts are distributed among three major subphyla. Many changes in the taxonomy of yeasts have occurred recently and are likely to continue in the coming years. The reader is referred to Kurtzman *et al.* 2011 for a current treatment.

Yeasts as Genetic Models

Because yeasts lend themselves well to both classical and molecular genetics and have the potential for rapid alternation between diplo- and haplophase, these unicellular fungi have become ideal models for genetic research. Work in this area started in the late 1930s, when Winge in Denmark and the Lindegrens in the USA initiated studies on yeast genetics (Barnett, 2007). Since then this field has advanced rapidly into highly specialised areas of genetic research, the results of which are regularly reported at national and international meetings. Mendelian genetics that started with strains of *Sa. cerevisiae* later expanded to include species of *Schizosaccharomyces*, *Kluyveromyces*, *Wickerhamomyces* (previously known as *Hansenula* or *Pichia*), the species *Yarrowia lipolytica*, *Pachysolen tannophilus* and others. *Kluyveromyces lactis* is a serious competitor to *Sa. cerevisiae* and *Schizosaccharomyces pombe* as a model organism. *Sa. cerevisiae* was the first eukaryote to have its entire genome sequenced. Yeasts remain at the forefront of research in genetics and cell biology. Areas of rapid development include the biology of DNA replication and repair, genome structure, molecular signalling, mitotic recombination, stress responses, control of gene expression or the molecular basis of aging. Leland Hartwell, Paul Nurse and Timothy Hunt were awarded the 2001 Nobel Prize in Physiology or Medicine for their research on the yeast cell cycle. Subsequent Nobel laureates who worked with yeast include Randy Schekman (2013) for his research in protein trafficking and Yoshinori Ohsumi (2016) for his discoveries of the mechanisms of autophagy. See also Gene Expression in Yeast, Microorganisms: Applications in Molecular Biology, *Saccharomyces cerevisiae*: Applications, Yeast as a Model for Human Diseases, Budding Yeast *Saccharomyces Cerevisiae* as a Model Genetic Organism, and Two-Hybrid Systems to Measure Protein–Protein Interactions

Noteworthy developments have taken place in non-Mendelian genetic elements. These include the double-stranded RNA genomes responsible for the killer phenotype, discovered originally in certain strains of *Sa. cerevisiae*. These elements, also referred to as RNA viruses or mycocins, code for the synthesis and excretion of peptides that are lethal to other strains or species of yeast. Strains of *Kl. lactis* contain linear DNA killer plasmids that code for toxic peptides. The killer phenotype is now known to occur in many other species of yeasts, including basidiomycetous species (Golubev, 1998; Muccilli and Restuccia, 2015). Other non-Mendelian genetic elements are represented by mitochondrial DNA (mtDNA), which may constitute 5–20% (and occasionally more) of the total DNA of yeasts. mtDNA is present in a variable number of copies of closed circles of somewhat various contour lengths. Their G+C contents always appear to be lower than that of the nuclear DNA of the same yeast. It is now known that mitochondrial genomes of most living species are AT-rich (Knight *et al.*, 2001), but for yeasts, this phenomenon is remarkable given that the nuclear base content varies over a broad range from 27 to 70 mol%. Mutations in the mtDNA affect the synthesis of certain respiratory enzymes and the ability of such mutants to grow on nonfermentable substrates such as glycerol or organic acids. Yet another non-Mendelian element is the native 2 μm DNA, made up of double-stranded circular DNA molecules, 50–100 copies per cell that replicate stably during the cell cycle. Two micrometer DNA is used in constructing cloning vectors in recombinant DNA technology. See also Mitochondrial Non-Mendelian Inheritance: Evolutionary Origin and Consequences, Mitochondrial Proteome: Origin, and Plasmids

Yeast Ecology

The field of yeast ecology deals with the distribution of yeasts in nature, the specificity of their habitats, and the reasons for the highly specific habitats of many species. It also deals with the interaction between yeasts and their plant and animal hosts as well as the microbial communities that are part of their host tissues, such as filamentous fungi, bacteria, algae and even insects that often share certain niches with yeasts. Frequently, one group of microorganisms may supply nutrients to another group by enzymatic breakdown of host tissue, for example, cellulose that cannot be utilised by yeasts. A similar example, from the production of Japanese rice beer (sake), is the hydrolysis of rice starch to simple sugars by the fungus *Aspergillus oryzae*, after which the sake yeasts can ferment the sugars. Yeasts may also exhibit antagonism to other yeasts, either by the secretion of specific killer toxins (mycocins) that disrupt the ion permeability of the cytoplasmic membrane, or by the erection of infection pegs that penetrate indiscriminately the walls of neighbouring yeast cells. The latter activity is characteristic of the ascomycetous genus *Saccharomycopsis* (Lachance *et al.*, 2000). Similar structures, termed haustorial branches, have been observed in a number of basidiomycetous species of the class Tremellomycetes, suggesting that they too are capable of mycoparasitism. Penetration of hyphae of a *Verticillium* species by *Filobasidiella depauperata* has been observed

(Ginns and Malloch, 2003). Although this fungus is strictly hyphal, it is closely related to some yeast species of importance as human pathogens, indicating that animal pathogenecity may be linked evolutionarily to mycoparasitism. See also Fungal Ecology

Yeasts are rarely dispersed in nature by air currents as are the conidia of many filamentous fungi. Insects are by far the most important vectors for the distribution of yeasts, sometimes passively by adherence to their body parts, but more often because insects and their larval stages consume yeasts as part of their diet (fruitflies, beetles, bees, wasps, etc.). Yeasts are also distributed by water currents from surrounding soils and plant debris and in this way arrive in lakes and even coastal areas of oceans. Yeast nutrients in aquatic habitats are usually derived from plant or algal growth. The classical approach to determining the composition of a community suspected to be a yeast habitat begins with the isolation of pure cultures of representative yeasts, followed by their identification. Isolation should preferably be done by direct plating of the source material on agar media as this can give information on quantitative species distribution in the community. Enrichment of the original yeast population in a liquid medium generally favourable for yeast growth can lead to a distorted picture of the community composition because of vastly different growth rates of the original yeast biota. If bacterial growth is a problem during yeast isolation, antibiotics such as chloramphenicol can be added to media to control bacterial competitors. Adjustment of the pH to approximately 3.8 (hydrochloric acid is commonly used) can also be used to inhibit or reduce the growth rate of many competing bacteria. Controlling spreading mould growth is more difficult. Propionic acid is effective in the lower pH range, but can also affect growth of some respiratory yeast species. The fungicide dichloran and the dye Rose Bengal are also used for certain applications, but the risk of inhibiting yeasts of interest is real. When moulds are present in low numbers, it may be possible to remove them from young agar plates by excision with a spatula. When studying yeasts of soil or decaying leaf litter, samples can be incubated in shake cultures where budding yeasts can grow, but fungal conidia germinate to small mycelial balls. These can be filtered off over sterilised glass fibres, allowing yeast cells to pass in the filtrate, and can then be streaked on agar plates. The only way to correlate the identity of colonies appearing on plates with the original yeast population in the sample is to estimate the proportion of each colony type present. Many species exhibit pronounced or subtle differences in colony morphology, making it possible to pick representative types for identification tests. Experience has shown that because of habitat specificity, individual samples from a particular substrate rarely contain more than three or four different species. See also Yeast Cell Culture

Because of the greatly differing growth rates of some species, plates should be inspected daily at first, and then periodically for up to 1 or 2 weeks so that very slow growing species (e.g. species of *Brettanomyces*) are not overlooked. If it is the intention to isolate a particular species from a habitat where it is known to occur, it is sometimes possible to use selective conditions by using mineral media with a special carbon and/or nitrogen source (e.g. D-xylose plus nitrate), which the desired

isolate can utilise in contrast to most competitors. Other variables that can be used are temperature of incubation, keeping in mind that minimum and maximum temperatures for growth of various yeasts range from 0 to 47°C, and osmotic pressure of the medium (sugar or salt). Yeast communities can also be characterised without recourse to strain purification, using whole community DNA amplification and sequencing. DNA from samples is extracted, amplified by PCR and either cloned to be sequenced or subjected to denaturing gradient gel electrophoresis, which allows the visualisation of individual DNA bands that each represents a different species. This approach is particularly beneficial in studies of the progression the members of a yeast community through time. See also DNA Profiling in Ecology

Habitats of yeasts

Numerous surveys of yeast habitats by many investigators have shown that yeast species are not distributed randomly in nature but generally have highly specific habitats and are frequently associated with plant and animal hosts.

Yeasts associated with plants

Because of their photosynthetic ability and mineral uptake from the soil, plants can offer a great variety of substrates for yeast growth. Yeasts are very abundant in sap exudates or slime fluxes of certain trees. This condition is most common in spring and is usually associated with physical injury or caused by boring insects. The flowing sap becomes heavily infected with bacteria and yeasts. Many insects use such exudates for ovipositing and actual feeding. Numerous new species of yeasts were first described from samples of exudate on trees growing in a particular geographic area. Examples are *Komagataea (Pichia) pastoris* from broad-leaved trees in Europe and the western USA, *Nadsonia elongata*, *Dipodascus aggregatus* and *Phaffia rhodozyma* in eastern Europe and Japan.

An interesting and unique yeast community has been found in tanning liquors used for the tanning of animal hides. Tanning fluids are made from the bark of oaks, acacia and other trees where these yeasts originated as these species have not been found in other habitats. Examples are *Wickerhamomyces (Pichia) chambardii* and *Candida boidinii*. The natural habitat of *Sa. cerevisiae* continues to be elusive, although several researchers have successfully isolated this closely related species and from the bark of oak trees using a liquid enrichment procedure. Sampaio and Gonçalves 2008 demonstrated that the presence of several *Saccharomyces* species in this material is correlated with the sugar composition of the bark of various oak species. *Sa. eubayanus* is known to be the parental wild type of the interspeices hybrid lager beer yeast, *Sa. pastorianus*, and was first isolated from trees in Patagonia (Libkind *et al*, 2011). More recently, *S. eubayanus* has also been isolated from natural

sources in Tibet (Bing *et al*, 2014) and the USA (Peris *et al*, 2014) and shows near genetic identity to the non-*Sa. cerevisiae* DNA of the *Sa. pastorianus* genome.

Another interesting group of yeasts has been recovered from necrotic (rotting) tissue of various cacti. Cactus species, which are endemic to the New World and later introduced elsewhere, are susceptible to a rotting process of the succulent tissue caused by pectinolytic soft-rot bacteria, followed by the growth of various yeast species introduced by desert-adapted *Drosophila* species and other insects that use the macerated soft tissue for feeding and breeding purposes. The yeast biota found in such tissue has evolved following the evolution of the Cactaceae. Columnar cacti are divided into two subtribes, the Pachycereinae (which lack triterpene glycosides in their tissue) and the Stenocereinae (which contain these toxic compounds). Some yeast species (e.g. *Pichia heedii*) are sensitive to triterpene glycosides and are found only in members of the Pachycereinae such as the saguaro cactus and related species, whereas others are resistant to these compounds (e.g. *Starmera (Pichia) amethionina* var. *amethionina*) and are typically found in the Stenocereinae, such as the organ pipe cactus. The vectors of the two yeast species are different *Drosophila* species that are similarly sensitive and resistant to these toxins. *Pichia cactophila* and *Candida sonorensis* are two of the most widespread cactophilic yeasts. They are both resistant to triterpene glycosides and are common in both cactus subtribes as well as in rots of *Opuntia* species. At least 20 new cactus-specific yeast species have been described. A few species common in cactus necroses have also been isolated from other sources (soil or tree exudates), for example, *Ogataea polymorpha* (syn. *Pichia angusta* or *Hansenula polymorpha*). A most interesting dual habitat has been found for *Pichia norvegensis*, which occurs in clinical material from humans but also in *Opuntia* necroses in the Sonoran desert and on islands of the Caribbean Sea. See also Glycosides: Naturally Occurring, Plant Defences against Fungal Attack: Biochemistry, and Plant Defences against Herbivore Attack

Other rich sources of yeasts are plant leaves, flowers and fruits. The external surface of leaves, or phyllosphere, provides a habitat for species of *Cryptococcus*, *Rhodotorula* and *Sporobolomyces*. Occasionally fermentative species are found such as *Torulaspora delbrueckii*. Yeast populations usually increase during leaf senescence in late summer. Yeasts associated with flowers are particularly common in those which are pollinated by insects (bees, butterflies and others). Frequently isolated species include *Metschnikowia reukaufii* and other yeasts with needle-shaped ascospores. Decaying pome fruits and berries are a rich source of yeast species, usually introduced by scavenging beetles and wasps. Species of *Hanseniaspora*, *Pichia kluyveri*, *Pichia fermentans* and various *Candida* species are prevalent.

Yeasts are generally not regarded as important plant pathogens, but members of the genus *Eremothecium* (syn. *Ashbya*, *Holleya* and *Nematospora*) are exceptions. These yeasts produce needle-shaped ascospores and their host range includes cotton plants, citrus fruit, tomatoes, lima beans,

mustard seeds and coffee berries. The symptoms usually take the form of surface lesions on fruits or a discoloration of cotton fibres in cotton bolls. Infection is caused by hemipterous insects (bugs of the genus *Dysdercus*) that act as vectors of the disease during feeding. Plant pathogens are also found among the basidiomycetous fungi that have a yeast state in their dimorphic development. Of great economic importance are the smut fungi, members of the order Ustilaginales, with more than a thousand species that are pathogenic on a large number of predominantly monocotyledenous hosts, such as corn. See also Fungal Pathogens of Plants

Endophytic yeasts

The yeast-like fungus *Aureobasidium pullulans*, a distant relative of ascomycetous yeasts, is almost omnipresent on leaf surfaces and has long been thought to grow endophytically in fruit trees and other cultivated plants. Several reports have suggested also that some yeasts live inside plant tissue and may even benefit the plants by excreting growth hormones such as indole-acetic acid. Suspected endophytic yeasts include widespread basidiomycetous species such as *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, *Sporobolomyces* and *Sporodiobolus* species, as well as ascomycetous yeasts identified as *Debaryomyces hansenii*, *Meyerozyma* (syn. *Pichia*) *guilliermondii*, *Lindnera* (syn. *Pichia*) *saturnus* and *Candida ipomoeae*. Endophytes appear to be commonplace in a wide variety of plants including fruit trees, maize and cotton. See also Mutualistic Symbioses

Yeasts associated with insects

Insects are the most important vectors in the distribution of yeasts in nature, and many insect species require yeasts in their diet for their normal life cycle from larvae to the adult stage. Yeasts serve mainly as a source of sterols, nucleic acids and vitamins. For example, most of the more than 1400 described species of *Drosophila* seek substrates in nature that contain yeasts and that can also support larval development such as fermenting fruit or tree exudates. The yeast community present in the crops of *Drosophila* has been studied extensively. The yeasts found in the domestic ‘garbage’ species *Drosophila melanogaster* are similar to those described above in decaying fruits and berries, but wild species such as *Drosophila pseudoobscura* have been shown to contain a very different yeast community that includes *Lachancea* (syn. *Kluyveromyces*) *thermotolerans*, *Kl. lactis* var. *drosophilarum* or *Pi. cactophila* in desert areas. There is no clear evidence pointing to the source of some yeast species isolated from wild *Drosophila* species collected in wilderness areas, but these flies constitute a valuable probe of what is there. It is likely that the *Saccharomces* species found in the bark of oaks are deposited there by *Drosophila* species. Another association with insects is between bark beetles that attack coniferous trees and a group of highly specific yeasts. The larval galleries of these insects are made in the phloem of the tree, where the yeasts, introduced by adults through the bark, actively multiply in the presence of oleoterpenes (pitch) from the tree. The yeasts that occur in

the larval galleries are unique species formerly assigned to the genus *Pichia*, such as *Kuraishia capsulata*, *Wickerhamomyces canadensis* and *Nakazawaea holstii*, associated with *Ips* and *Dendroctonus* beetles in pines, and *Yamadazyma scolyti* in *Scolytus* beetles, found mainly in fir trees. Another group of yeast species is associated with ambrosia beetles and other wood-boring insects belonging to the Coleoptera. These beetles, in contrast with the bark beetles, bore tunnels directly into the sapwood or hardwood of weakened coniferous or broad-leaved trees and they carry the symbiotic ambrosia fungi and yeasts that grow on the walls of the tunnels at the expense of the tree sap. Several new species of yeast have been isolated and described from this symbiotic association between higher fungi and yeasts, many of them from South Africa. A few examples of ambrosia yeasts are *Ambrosiozyma platypodis* and *Ambrosiozyma cicatricosa*. Nitidulid (sap) beetles that visit ephemeral flowers world-wide contain various species belonging to four clades with affinities to the genera *Metschnikowia, Kodamaea, Kurtzmaniella* and *Wickerhamiella*, respectively. The distribution of the *Metschnikowia* species is of special interest as it mirrors the geography of the beetle species. Wild bees also carry a specific yeast community that may consist of *Me. reukaufii* and related species as well as a diverse array of species related to the genus *Starmerella*. These include the cosmopolitan species *Starmerella bombicola* and *Candida apicola* as well as the Neotropical species *Starmerella meliponinorum*. The presence of *Me. reukaufii* in nectar of the stinking hellebore has been shown to cause a rise of as much as 6°C in nectar compared to ambient temperature (Herrera and Pozo, 2010), favouring visitation of the plant by bumblebees. See also Ecology of Invertebrate Nutrition

A final example of symbiosis is the unique association between yeast and a group of lacewing insects belonging to the Chrysopidae. *Chrysoperla carnea* adults (green lacewings) feed primarily on honeydews produced by aphids, but its larval stage is predaceous and feeds primarily on aphids. The predaceous larvae do not contain symbiotic yeasts, but adults lack a balanced diet in their honeydew food. Adult lacewings correct for this deficiency by carrying intracellular yeast symbionts, residing in the crop or tracheal trunks that provides the nutrients lacking in honeydew. The yeasts, which are close relatives of *Metschnikowia pulcherrima*, are propagated in adult insects by trophallaxis, that is, the sharing of a drop of regurgitated liquid before mating.

Intracellular symbionts

Many investigators have observed and isolated budding yeast cells inside specialised cells of insects that are called mycetocytes or mycetomes (evaginations of the midgut). Only a few species have been isolated and described in adequate detail. An example is *Nakazawaea holstii* (syn. *Candida ernobii*) from larvae and imagos of Anobiidae beetles. Such symbionts appear to supply their hosts with nutrients that are deficient in their normal diet. Another example is the observation of a budding, ascomycetous yeast in intestinal epithelial cells of *Drosophila funebris* by several investigators. This

yeast, named *Coccidiascus legeri*, has not been isolated in culture as yet. See also Mutualistic Symbioses

Yeasts occurring in soils

Soils are a rich source of yeasts that presumably live at the expense of organic matter derived from plant or animal residues. Studies of soil yeasts have shown that most isolates are specifically associated with various soils and have no direct connection with plants that grow in such soils. Exceptions to this are soils in fruit orchards or vineyards which often contain yeasts that also occur on fruits and grape berries, such as species of *Hanseniaspora* and *Metschnikowia*, or certain growth-enhancing yeast species found in association with plant roots. Species of *Schwanniomyces* have been isolated only from soils in a wide range of geographic locations. The same is true for species of *Lipomyces* and the monotypic genus *Schizoblastosporion*. Many individual species of other genera are also soil specific. A number of species from Antarctic soils are psychrophilic and belong to the genus *Leucosporidium*. For further details see Botha 2011 and Phaff and Starmer 1987. See also Soils and Decomposition

Yeasts from aquatic sources

Sampling equipment is available to collect water samples aseptically at various depths. As the yeast concentration is often less than 100 cells per litre, isolation is usually done by filtering different quantities of water through sterile membrane filters which are then placed on a nutrient agar medium. It is recommended that samples from marine sources be plated on media to which 3% sodium chloride is added to improve growth of some species of *Metschnikowia*. Strains of *Metschnikowia bicuspidata* are common in coastal waters of the Pacific Ocean and can be pathogenic to certain crustaceans, such as brine shrimp (*Artemia salina*) that are grown commercially in salt ponds or lakes. Yeasts also enter lakes, streams and coastal oceans through terrestrial run-off. Some cosmopolitan yeasts from aquatic sources include species of *Rhodotorula* and *Cryptococcus*, *Debaryomyces hansenii* and *Candida parapsilosis*. For further details see Hagler and Ahearn 1987. Of special interest is a report on yeasts collected near the sea floor along thermal vents of the Mid-Atlantic Ridge (Gadanho and Sampaio, 2005), which yielded a yeast community made up of cosmopolitan species that included *Rh. mucilaginosa* and *Ca. parapsilosis*, among others.

Yeasts associated with warm-blooded animals

This group constitutes a limited number of species with ascomycetous as well as basidiomycetous affinity that have in common the ability to grow at 37°C or above. Some species of this group form part of the normal intestinal biota, but others may be responsible for certain pathological conditions. Examples of the former category are *Kazachstania* (syn. *Arxiozyma*) *telluris* and relatives that occur

in the intestinal tract of swine, horses, cattle and small rodents, and *Cyniclomyces guttulatus*, which occurs in rabbits. All have unusually stringent growth requirements and are not normally found in the environment. Other nonpathogenic yeasts capable of growth above 37°C may be associated at times with warm-blooded hosts but these are also common in the environment. Although normally such yeasts are not pathogens in humans or animals they may become so for immunocompromised patients or debilitated individuals. Examples are *Kluyveromyces marxianus*, *Ca. parapsilosis*, *Trichosporon cutaneum* and related species. Among the yeasts known to be true pathogens some have affinity to the ascosporic genus *Lodderomyces*, the best known of which is *Ca. albicans*. These yeasts are usually considered opportunistic as they may particularly cause infection in susceptible individuals, diabetics or those treated with broad-spectrum bacterial antibiotics. Such diseases are referred to as candidiasis or candidosis. They usually involve the skin or mucous membranes (thrush and vaginitis). *Candida glabrata* is frequently associated with urinary tract infections. *Malassezia furfur* (syn. *Pityrosporum ovale*) is a commensal of the skin and may produce characteristic lesions of the skin and hair follicles (called tinea versicolor). The most serious yeast pathogens of humans and animals are the basidiomycetous species *F. neoformans* and *F. bacillispora* (anamorphs *Cr. neoformans* and *Cr. gattii*). The disease cryptococcosis occurs relatively rarely, but cases are often dramatic and fatal. When infection becomes systemic, the central nervous system becomes involved causing a chronic meningitis. *Cr. neoformans* is apparently spread by pigeons, in whose droppings the yeast has been repeatedly demonstrated. See also Antifungal Agents, Fungal Infections in Humans, Fungal Pathogens of Humans, Fungal Pathogens of Nonhuman Animals, Immunity to Fungi, Infections in the Secondary Immunocompromised Host, and Mycoses

Industrial and Agricultural Uses of Yeasts

Yeasts and their metabolic products have been used by humans in empirical ways since ancient times. In modern times they are used in many ways, usually in well-equipped plants and under proper scientific control.

Fermented beverages

Fermented beverages are made from substrates containing readily fermentable sugars such as fruit juices or from starch in cereal grains, which must first be converted enzymatically to sugars that can be fermented by yeasts, principally *Sa. cerevisiae* (Walker and Stewart, 2016). The first category is represented by wine fermentation where grape juice or juice from other fruits is directly fermented to ethanol and carbon dioxide by suitable strains of *Sa. cerevisiae* to produce table wines of 10–12% alcohol. For red table wines the skins of dark grape varieties are allowed to remain in the fermentation tank for several days during which the alcohol and carbon dioxide formed extract the red anthocyanin pigments from the skins. For true Spanish sherry wines, grapes with higher sugar content are

fermented to wine with about 15% alcohol and by different strains of yeast that rise to the surface of the vessel and form a thick surface film after the sugar is completely fermented. The subsequent oxidative stage may take up to several years and the yeast imparts flavour changes to the wine that includes an increase in acetaldehyde content. Pretorius (2016) has reviewed the role of yeasts in development of wine flavour and aroma.

The substrate for beer brewing is cereal starch (barley and sometimes other grains), which is enzymatically converted at 60–70°C to maltose, glucose and oligosaccharides by an amylase complex from germinated barley, called malt. The soluble extract from this process, that is, wort, is fermented by two different methods to produce lager beer and ale. Lager fermentation is carried out at 10°C by strains of *Saccharomyces pastorianus* (syn. *carlsbergensis*) which settle to the bottom of the tank when the fermentation is complete ('bottom yeasts'), whereas ale fermentation is done at 20–25°C by strains of *Sa. cerevisiae*, which tend to rise to the top of the fermenting liquid and are skimmed off the surface when fermentation subsides ('top yeasts'). Brewer's yeast from both processes is recovered, washed, stored and recycled if desired (Stewart, 2016). Surplus yeast from breweries constitutes a valuable by-product, being a source of protein in animal feed and for producing yeast autolysate for food flavouring. Yeast extract spreads are particularly popular in Great Britain, Australia and New Zealand.

Sake brewing is an ancient process of the Orient leading to the production of rice wine or sake. In this process rice starch is converted by amylolytic enzymes of *Aspergillus oryzae* to low-molecular weight sugars and fermented simultaneously by strains of *Saccharomyces sake* (now considered to be *Sa. cerevisiae*). The main fermentation, called moromi, is done at 10–15°C and leads to a beverage after filtration and pasteurisation with 18–20% alcohol.

Traditional fermentations of wines and some distilled beverages have relied on the spontaneous development of yeasts that are present in the immediate environment of the wineries or distilleries. In most cases, this led to products of unpredictable and variable quality. The modern approach, with some exceptions, is to introduce into the must a commercially grown yeast strain selected for the ability to support a rapid fermentation and give rise to a high quality product. See also Walker and Stewart (2016) *Beverages***2016**, *2*, 30; doi:10.3390/beverages2040030

Fuel alcohol

Fuel alcohol produced by yeast fermentation is also known as *bioethanol* which is produced at the billions of litres scale annually for use as a renewable transportation fuel in mixtures with gasoline. The major producers are the US and Brazil which converts corn starch and sugar cane juice or molasses into ethanol, respectively (Walker *et al*, 2017). Bioethanol production is made with special distiller's strains of *Sa. cerevisiae* that have been improved by

protoplast fusion, genetic manipulations or by gene editing. Commercially available GM strains of *Sa. cerevisiae* are now being used in fuel alcohol plants and their industrial exploitation represents the biggest deployment of any GM microorganism (Walker and Walker, 2018). The raw materials may be rich in sugar that is directly fermentable by the yeast, as for example the sucrose of sugar cane, or may require a chemical or enzymatic step to degrade polysaccharides such as corn starch into simpler sugars such as maltose and glucose. The conversion of lignocellulosic substrates such as woody biomass or energy crops into ethanol by yeast represents a more difficult technological challenge. For example, wood hydrolysates yield a mixture of cellobiose, D-xylose and other hydrolysis products from cellulose and hemicellulose that cannot be fermented by *Sa. cerevisiae*, but other yeast species have been discovered that can convert these to ethanol. *Clavispora lusitaniae* ferments cellobiose. *Pa. tannophilus* and several species of the *Scheffersomyces* (syn. *Pichia*) and *Spathaspora* clades can produce ethanol from D-xylose. Lactose-fermenting yeasts (certain strains of *Kl. lactis* or *Kl. marxianus*) can produce ethanol from milk whey, a by-product of the cheese industry, for use either in beverages or biofuels. See also Fungal Fermentation: Industrial, *Saccharomyces cerevisiae*: Applications, and Yeast Cell Culture

Yeasts grown for biomass

These include *Sa. cerevisiae* for the baking industry, which is sold as compressed yeast, active dry yeast and as liquid cream concentrate. Another major product is feed yeast for animal diets. Feed yeast is often prepared from the abundant by-products of the brewing and distilling industries, but strains of *Sa. cerevisiae* may be grown specifically for this purpose. In some cases, the yeast is enriched with trace nutrients such as selenium or chromium. Another species, *Candida utilis* (syn. *Lindnera jadinii*), can be grown on sulfite waste liquor (a byproduct of the paper industry) or on wood hydrolysate. Certain yeast species can also be grown on straight-chain hydrocarbons as substrates for single-cell protein production, particularly those with 10 to 18 carbons. Examples are *Yarrowia lipolytica*, *Candida maltosa* and *Candidatus tropicalis*. The biotechnology industry has invested much money and effort in developing a viable process for the production of single-cell protein from purified *n*-alkanes, but this industry has faltered for reasons of perceived safety and unfavourable economics. On the positive side, hydrocarbon-utilising yeasts have considerable potential in the microbial oxidation and cleanup of crude oil spills on land and sea. See also Bioremediation, Fungal Physiology, and History of Biotechnology

A number of specialty products are being made commercially from certain yeast species, which are briefly described below. Although riboflavin (vitamin B_2) is not required extraneously for the growth of any known species of yeast, a few species are overproducers of this vitamin, for example, *Eremothecium (Ashbya) gossypii*, *Eremothecium ashbyi* and *Candida famata*. Strain modification by mutation and protoplast fusion has led to strains of *Ca. famata* that produce at least 20 g of riboflavin

per litre of culture liquid. The principal use of riboflavin is in animal feed formulations. Baker's yeast (*Sa. cerevisiae*) can also be enriched with vitamin D following treatment with UV light. Citric acid can be produced in very high yields by the yeast *Yarrowia lipolytica*, but originates mostly from the mould *Aspergillus niger*. See also Fungal Fermentation: Industrial

Although many species of the genera *Rhodotorula* and *Cryptococcus* produce a variety of carotenoid pigments, the carotenoids of *Ph. rhodozyma* (syn. *Xanthophyllomyces dendrorhous*) consist mainly of astaxanthin, a pigment responsible for the orange-coloured flesh of salmon and trout raised in their natural environment. Most commercially raised salmon is presently grown in pens with ocean water. This type of aquaculture would yield fish with white muscle unless their diet is supplemented with a source of synthetic or microbial astaxanthin. *Ph. rhodozyma* is one of several commercial sources of the pigment, which include chemical synthesis as well as biological production by the microalga *Haematococcus pluvialis*.

Agricultural uses of yeasts

An interesting use of yeasts is in the biological control of crop plant diseases. Chemical treatments for these purposes have gradually fallen out of favour for environmental reasons. An alternative strategy to prevent fungal attack or decay relies on surface treatment of crops with yeasts. Examples include the control of powdery mildew by *Pseudozyma flocculosa* and of fruit grey mould by various species of both ascomycetous and basidiomycetous yeasts. Several yeasts can reduce post-harvest spoilage of fruit and cereal crops when applied in the field. A commercial preparation consisting of dry cells of *Candida oleophila* is used to reduce rotting of citrus fruit by moulds. The protective effect has been ascribed to nutrient competition between the yeast (a nonpathogen) and the moulds.

For information on the role of yeasts in food spoilage, the reader is directed to Deâk (2008) and Querol and Fleet (2006).

Recombinant DNA

261. Yeasts strains for industrial exploitation can be genetically enhanced in a number of ways. The methods involve a variety of classical genetics (eg. hybridisation, mutagenesis), adaptive evolution (also known as evolutionary engineering), recombinant DNA technology (genetic engineering) and gene editing (e.g. CRISPR/Cas9). Regarding recombinant DNA, there are two main methods: cisgenic and transgenic. Cisgenic methods are also known as *self-cloning* and involve yeast species-species gene transfer whereas transgenic methods involve

introduction of foreign DNA into yeast. The latter methods have many important applications in industrial and biomedical areas of biotechnology. For example, for the production of human therapeutic proteins such as insulin, hepatitis vaccine and serum albumin has been possible using transgeneic yeasts.

An important use of yeasts is to serve as hosts for expression of recombinant DNA (Romanos *et al.*, 1992; Feldmann, 2012). The introduction and expression of foreign or heterologous genes in nonpathogenic yeasts such as *Sa. cerevisiae* is preferred over the use of bacteria for that purpose. Further advantages are the high stability of its cells and ability to grow in essentially protein-free media, facilitating recovery of the foreign gene product. Yeasts can also glycosylate proteins in contrast to most bacteria. The procedure is to insert a heterologous gene in a yeast plasmid capable of replication and provided with a 5′-flanking DNA promoter sequence to increase transcription (e.g. the yeast mating factor Mfα1) and with a transcription terminator in the 3′-flanking fragment. Ideally, the gene products should be secreted from the cells, as is the case with yeast invertase and acid phosphatase. Yeast enzymes involved with the processing steps and the translocation of proteins across the cytoplasmic membrane also work with foreign gene products. Examples of foreign genes cloned in *Sa. cerevisiae* and whose products are secreted by the recombinant yeast are wheat α-amylase, human epidermal growth factor, human serum albumin and insulin, often as a significant percentage of total cell protein. *Kl. lactis* has been used to produce bovine chymosin, the rennet enzyme used as a milk coagulant in cheesemaking.

The methylotrophic yeasts *Komagataea* (syn. *Pichia*) *pastoris* and *Ogataea polymorpha* (syn. *Pichia angusta* and *Hansenula polymorpha*) have some advantages over *Sa. cerevisiae* as hosts for heterologous genes because they can be grown to extremely high densities in protein-free media, they are less inclined to overglycosylation of the recombinant protein, and foreign genes are incorporated in multiple copies into their chromosomes. The *Ko. pastoris* system uses a methanol-regulated alcohol oxidase gene promoter and the expression cassette is stably integrated into the yeast genome at specific locations. The haploid nature of *Ko. pastoris* vegetative cells makes it amenable to traditional mutagenesis with or without protoplast fusion. See also Gene Expression in Yeast, and Protein Production for Biotechnology

Yeast gene editing and synthetic biology

Modern gene editing techniques in yeast represent an alternative to recombinant DNA technology. The introduction of methods based on CRISPR (Clustered Regularly Interspaced Palindromic Repeats) and CRISPR-associated protein-9 nuclease (Cas9) have proved

beneficial in modifying gene targets irrespective of the genetic background of the yeast strain (Mans *et al*, 2015). For example, using CRISPR/Cas9 it has been shown possible to bioengineering yeasts with better stress resistance and fermentation performance (Stovicek, *et al*, 2017; Deparis *et al* (2017).

Although *S. cerevisiae* was the first eukaryote to have its genome sequenced (in 1996) and this provided abilities to *read* its genome, *synthetic biology* now provides scientists an ability to *write* its entire genetic code. An international *Saccharomyces cerevisiae* synthetic biology project, known as Sc2.0, is aiming to completely synthesise and construct this yeast's 16 chromosomes. Currently, *Sa. cerevisiae* chromosomes II, III, V, VI, X and XII have already been synthesised with the remaining ones nearing completion which would represent a landmark in biology. A defining feature of the Sc2.0 project involves introducing novel changes to the yeast genome, and at a practical level, yeast synthetic biology has great potential for development of new yeast strains with valuable properties. The technology involves designing and synthesizing DNA and applies engineering principles to biology (Cameron *et al.*, 2014). Synthetic biology can transform cellular behaviour by constructing genes, metabolic pathways and entire genomes. CRISPR/Cas9 gene editing techniques are also employed by yeast synthetic biologists to perform precisely targeted DNA modifications to improve industrially-relevant traits. This is particularly beneficial in the biofuels sector and can lead to complete bioconversion of lignocellulosic substrates (woody biomass, straw, corn stover, energy crops etc.) into fermentable substrates to produce fuel ethanol by engineered strains of *S. cerevisiae* (Liu *et al*., 2016; Walker and Walker, 2018). Other areas of synthetic yeast biology and biotechnology await further exploitation.

Glossary

Anamorph
An asexual yeast culture, usually a haploid of a single mating type.

Apiculate

A lemon-shaped yeast cell.

Arthroconidium

Also called arthrospore, a fragment formed during the breaking up of a septate mycelium.

Ascocarp

A complex fruiting body containing asci produced by ascomycetous fungi.

Ascomycetes

Fungi that reproduce sexually by formation of internal meiotic spores and assigned to the subphylum Ascomycota.

Ballistoconidium

Also called ballistospore, any spore that is forcibly discharged.

Basidiomycetes

Fungi that reproduce sexually by formation of external meiotic spores and assigned to the subphylum Basidiomycota.

Basidium

A structure bearing on its surface a definite number of basidiospores following meiosis.

Blastoconidium

Also called blastospore, a budding cell borne on a pseudomycelium.

Dikaryotic

A cell with two nuclei, usually of opposite mating types, commonly found in basidiomycetous yeasts.

Diploid

Containing the double or 2*n* number of chromosomes.

Dolipore

A septal pore where the pore wall is swollen into a barrel-like morphology.

Enteroblastic

A type of repeated budding at the same location of the cell surface in basidiomycetous yeasts.

Haploid

Containing the reduced or 1*n* number of chromosomes.

Heterothallic

Sexual reproduction requiring fusion or conjugation of compatible mating types before karyogamy.

Holobasidium

A single-celled basidium, often club shaped.

Holoblastic

A type of budding involving softening of the cell wall followed by intercalary synthesis of new wall material as in ascomycetous yeasts.

Homothallic

Not sexually differentiated, thus self-compatible mating.

Karyogamy

Nuclear fusion.

Meiosis

Reduction division during which the chromosome number is reduced by one half.

Metabasidium

Also called promycelium, an outgrowth of a teliospore following meiosis and bearing basidiospores.

Monophyletic

A clade, that is, a taxon that comprises all descendents of a single common ancestor. Paraphyletic taxa are incomplete clades, whereas polyphyletic taxa are groupings of selected taxa that belong to different clades.

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Figure 1. Blastoconidia. A well-developed pseudomycelium; all cells are formed by budding, remaining attached to each other and forming a tree-like structure; the lateral and terminal cells are called blastoconidia or blastospores.

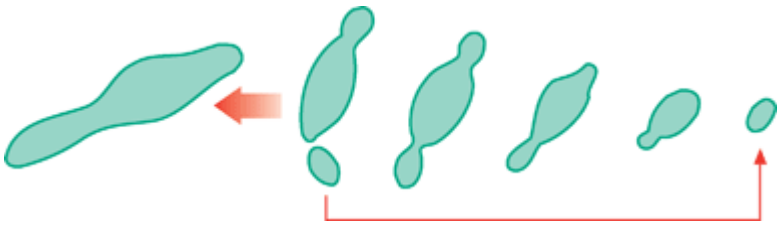


Figure 2. Apiculate cells. Ontogeny or development of characteristic cell shapes in an apiculate yeast (e.g. *Hanseniaspora*) from oval to apiculate during repeated bipolar budding.

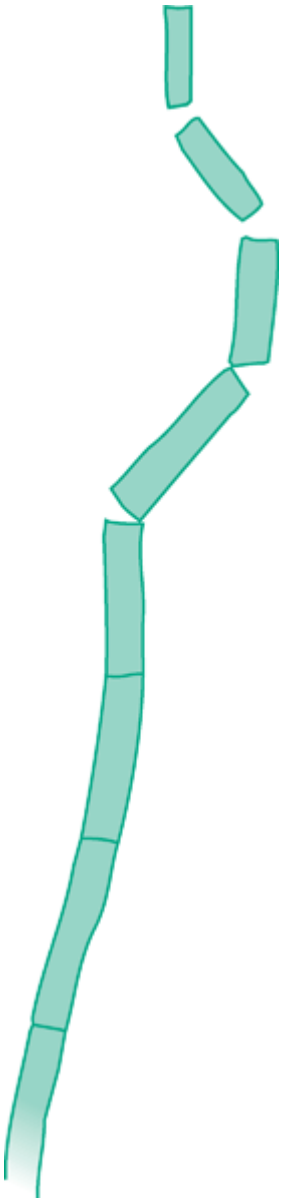


Figure 3. Arthroconidia. A hyphal (filamentous) cell undergoing fission or cross wall (septum) formation and breaking up into individual cells called arthroconidia or arthrospores (e.g. *Trichosporon*).

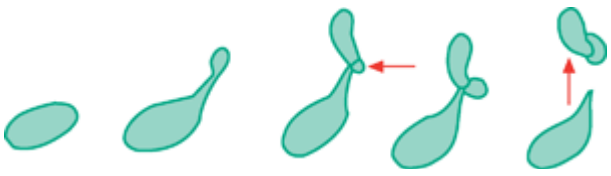


Figure 4. Ballistospores. Formation of an asymmetric balistoconidium or ballistospore on a pointed stalk or sterigma on a mother cell of a *Sporobolomyces* species. The asexual ballistospore is discharged forcefully by means of a droplet mechanism (arrows).

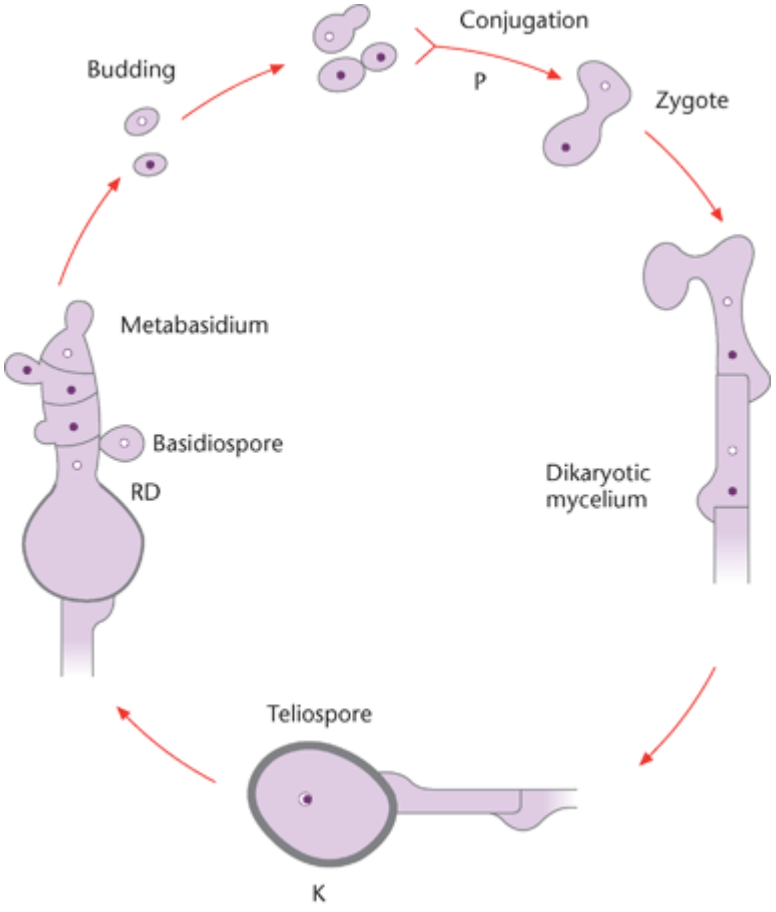
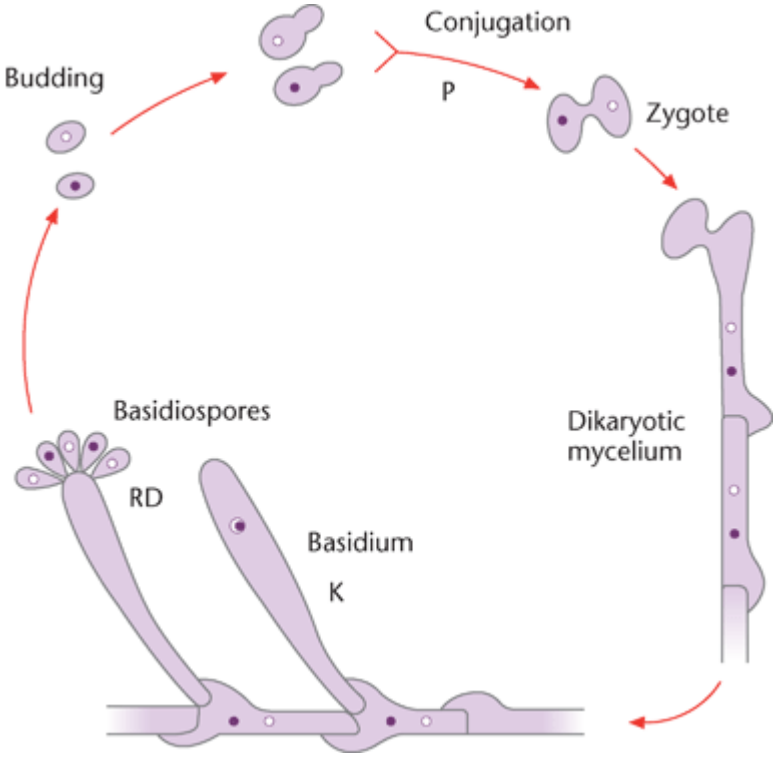


Figure 5. Life cycle of *Rhodosporidium*. Simplified life cycle of a heterothallic species of *Rhodosporidium*: K, karyogamy; P, plasmogamy; RD, reduction division (meiosis).



1 Figure 6. Life cycle of *Filobasidium*. Schematic life cycle of a heterothallic species of
2 *Filobasidium*: K, karyogamy; P, plasmogamy; RD, reduction division (meiosis).

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