Y

YEASTS

1. Introduction

Yeasts are probably the oldest deliberately cultivated microorganisms: first described before 3000 B.C. (recorded history began with the Pharoah Menes ~5000 B.C.). There are currently >100 genera comprising >800 separate species assigned to the family of yeasts (according to the Code 1994 of the ICBN). Yeasts are eukaryotic organisms, having a defined nucleus surrounded by a nuclear membrane and organized cytoplasmic organelles (mitochondria, peroxisomes, vacuoles, Golgi bodies). Yeast are fungi that exist as single cells during at least some stage of their life cycle, properly described as those fungi where vegetative growth results from budding or fission and do not form sexually or within or on fruiting bodies. Yeast lack the capacity for photosynthesis as they do not have chloroplasts; neither are they motile. All the major groups of fungi, *Basidiomycetes*, *Ascomycetes*, *Phycomycetes*, and the *Fungi imperfecti* include species that have adopted a yeast-like phase. Yeasts usually reproduce vegetatively by budding or fission, but parasexual and sexual reproduction occurs in most yeasts, although the sexual phase may remain hidden.

Among the many yeasts described, the genus *Saccharomyces* is of greatest interest and of greatest practical and economic importance because of its long-term use in bread making, brewing, and wine fermentation industries, as well as for the production of biomass, where the yeast is prepared as solids and used to provide the yeast used in the baking and brewing themselves; also used in the production of yeast based foods (eg, Vegemite, a peculiarly Australian institution) and domestic uses (cooking, ginger-bread plants where yeast is used to produce ginger beer). Products extracted from yeast are used in the vitamin supplement industries as yeast are an excellent source of vitamin B and

coenzyme Q. Yeast are also used to produce bulk biochemical products such as citric acid. Yeasts other than of the genus *Saccharomyces* also participate in alcoholic fermentations as they are found naturally on vines and both fruit and leaf (*Torulopsis* and *Kluyveromyces* species are common) although the cultures may not be well characterized. Fermentation of cane sugar and production of ethanol is already in widespread use in some countries, especially Brazil, as a biofuel, a clean alternative to petroleum.

Despite the many useful benefits of yeast, some yeasts may not be beneficial at all. Apart from yeasts causing spoilage of foodstuffs, at least two yeast genera, *Candida* and *Cryptococcus* are prevalent opportunistic pathogens, particularly *Candida* and most commonly *sp. albicans*, which can cause cystitis, dermatitis, myotitis, vulvo-vaginitis as well as causing other problems. Infection probably results where the patient's immune system is compromised and with the rise of human immunodeficiency virus (HIV) infections worldwide, infection by other yeast, eg, *Candida dubliniensis*, not normally associated with infection, are occurring. The other major yeast pathogen, the yeast *Cryptococcus neoformans*, can cause nasty infections, usually in the lungs but often on limb extremities. Specific antifungal agents capable of controlling fungal infections remain problematic, despite a few products being available in the market.

Yeasts particularly *Saccharomyces cerevisiae*, but also *Schizosaccharomyces pombe* and *Kluyveromyces lactis*, have for long provided a model system for the study of eukaryotic cell biology. With the rapid advances in technology since the advent of the revolution in molecular biology in 1975 yeast has maintained its lead as the model organism for the study of eukaryotic cell biology. A yeast (*S. cerevisiae*) was the first eukaryotic organism for which the DNA sequence of its entire genome was determined. The genomes of at least six other yeast species have been completed. As a consequence yeast remains the leading eukaryotic organism pioneering the pathways of "-omics" (Genomics, Proteomics, Metabolomics, etc). Related to these applications is the industrial use of yeast as a host for production of pharmaceuticals and other valuable proteins (eg, subunit vaccines for Hepatitis and IBDV). Due to its importance in the study of basic cell biology, reviews of Saccharomyces genetics and molecular biology appear regularly; Reference 1 is the most comprehensive current text on yeast taxonomy, Ref. 2 contains the most recent review of yeast genomics and proteomics; while Refs. 3 and 4 provide minireviews of the use of yeast in fundamental and applied research. References 5–9 provide URLs for various yeast databases available on line on the internet. References 10–22 provide older texts that are still useful in many cases (eg, *Methods in Enzymology*). Various laboratory home pages can also be accessed on line. A list of the yeast referred to in this review and a summary of their attributes can be found in Table 1.

2. Morphology, Reproduction, and Life Cycle

Single yeast cell are usually spherical to ellipsoidal in shape, but may also be cylindrical, ogival, pyramidal, or apiculate. The shape often reflects the specific site of bud formation. Apiculate or lemon-shaped forms arise from bipolar budding, ie, buds form usually at one end of the long polar axis of an elliptical

Table 1. **Some of the Yeast Species Mentioned in the Text**

Ascomycetes	budding is polar adjacent to previous buds. Spores bound in asci
Candida albicans	the most common yeast species causing disease in humans
Candida dubliniensis	one of many *Candida* species capable of infection (rare) but used for production of the food sweetening agent D-arabitol
Candida lusitaniae	cellobiose fermentation to ethanol
Candida lipolytica	production of citric acid
Candida membranaefaciens	production of L-threonine
Candida pseudotropicalis	lactose and milk fermentation
Candida shehatae	D-xylitol fermentation
Candida utilus	used as fodder, grown on sulfite liquor wastes or ethanol
Hansenula anomala	production of tryptophan
Kluyveromyces fragilis	lactose and milk fermentation
Kluveromyces lactis	lactose and milk fermentation, grown on cheese, whey or whey permeate
Kluveromyces marxianus	new name *K. fragilis*
Kluyveromyces polysporus	produces eight spores
Pachysoklen tannophilus	D-xylose fermentation of ethanol
Phaffia rhodozyma	feed base for fish and poultry meal/source of astaxanthin pigment
Pichia pastori	methanol fermentation
Pichia stipitis	xylitol (sweetner) production
Pichia segobiesis	D-xylose fermentation
Saccharomyces exiguus	new name *S. cerevisiae*
Saccharomyces bayanus	wine fermentation
Saccharomyces carlsbergensis	new name *S. pastorianus*
Saccharomyces cerevisiae	fermentation of ale, lager, leavening of bread, and dough fermentation of ethanol
Saccharomyces fermentii	new name *Torulaspora delbrueckii*
Saccharomyces pastorianus	lager beer
Saccharomyces rouxii	new name *Zygosaccharomyces rouxii*
Saccharomyces rosei	new name *Torulaspora delbrueckii*
Saccharomyces uvarum	wine fermentation, possible new name *Saccharomyces bayanus*
Schizosaccharomyces pombe	fission yeast, research model for cell division, African beer yeast
Schizosaccharomyces octosporus	produces eight spores
Torulaspora delbrueckii	wine fermentation
Zygosaccharomyces rouxii	shoyu (soy) and Misu (paste)
Basidiomycetes	budding from same locus with repetitive budding producing ruffling of colony morphology, external spores
Cryptococcus neoformans	a very infectious agent, lung infections often fatal, amputation of infected limb extremities often required, in Australia found on Murray River Eucalyptus Gum Trees

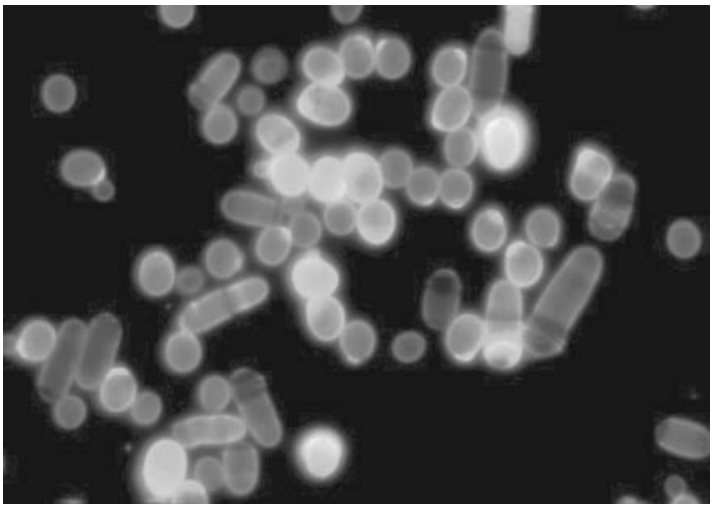

Fig. 1. A mixed culture of *S. cerevisiae* (budding yeast, observed with oval shapes) and *S. pombe* (fission yeast, observed with rod-like shapes) stained with DAPI and calcofluor. (Photo: M. Jagadish, CSIRO.)

cell, typically leaving a bud scar. In *S. cerevisiae*, the maximum number of buds a mother cell might produce appears to be ~16. Yeast cells vary in size even within the same species, with the more mature individual cells being generally larger. For ellipsoidal type cells, eg, those of *S. cerevisiae*, the length of the long axis may be 5–10 μm and of the short axis 3–5 μm (Fig. 1). The volume of such a cell may be 40 μm^3 with a mass of 10 pg and a density of 1.01–1.03 g/cm^3 (13). Some yeasts including *S. cerevisiae* may form a pseudomycelium consisting of single or branched chains of cells where the daughter cells do not separate from their mothers. Nitrogen starvation induces such a morphology in *S. cerevisiae* as does expression of some heterologous proteins. It also seems that the mother cell uses the budding cell as a recycling bin, as a repository for unfolded or misfiled proteins.

The yeast cell is surrounded by a mechanically refractory cell wall that may account for 20–30% of the cell solids (14). The cell wall surrounds the plasma membrane to produce a periplasmic space. Together they regulate the transport of chemical compounds into and out of the cell either by simple diffusion or by active transport. The active transport is mediated by proteins such as amino acid permeases, ion transporters (potassium, copper, iron, zinc, phosphate) and glucose transporters, or it may involve active uptake by clathrin coated vesicles.

The cell wall consists of alkali-soluble β-glucan, alkali-insoluble glucan, and glucan- and mannan-containing glycoproteins. Linkages within glucans are generally of the form β-(1→3) and β-(1→6). Yeast colonies are often smooth but some yeasts secrete material that form viscous colonies covered with a sticky capsular material on the outside of the cell wall. Presumably this is a protective measure to keep the colonies moist or together with the other colony forming agents. Fimbriae (small, hairlike structures) may also be present on the outer-cell wall and may play a role in flocculation. Apart from glycation yeast are

capable of many post-translational modifications of proteins, eg, glycosylation (both N- and O-linked), acetylation (of histones), prenylation, and amidation of small peptides and of course proteolysis. They are also able to secrete proteins, eg, mating factors into growth media.

The small nucleus of the yeast cell is surrounded by a membrane or tonoplast, containing many pores used for nuclear protein uptake. Several proteins such as those involved in DNA replication (DNA polymerases, primases, topoisomerases, helicases, etc), or with messenger ribonuclic acid (mRNA) transcription (polymerases), are synthesised in the cytoplasm and are transported to the nucleus via these nuclear membrane pores. The average diameter of a nucleus is $\sim$0.085 μm. In haploid cells of *S. cerevisiae*, the nucleus contains 90% of the DNA of the cell.

The complete sequence of *S. cerevisiae* is now known. Its genome is organized into 16 linear chromosomes ranging in size from 230 to 1532 kb. The total DNA content is 12069 kb, or about three times the size of the *Escherichia coli* genome (5000 kb). The individual chromosomes can be separated electrophoretically using a variety of pulsed-field electrophoresis systems, eg, OFAGE, FIGE, CHEF, and TAFE (15,16). The genomic organization in some other yeast are shown in Table 2. Comparative genomics allows evolutionary pathways to be determined such that for instance *S. pombe*, which possesses only three chromosomes, actually has larger total size (kb) containing a total of $\sim 1.4 \times 10^7$ kb. *Saccharomyces cerevisiae* appears to contain an almost duplicate set of genes, one for growth on sugars (principally glucose), the other for growth on ethanol. This is perhaps as a result of its long use in bread and beer-making (17). Table 2 provides a comparison of the genomes from a number of yeasts for which the complete sequences have been obtained (not including *Pichia pastoris*, which is commercially available) (23–26).

S. cerevisiae cells contain one or more mitochondria carrying $\sim$5–10% of the cellular DNA, consisting of one or more copies of a single linear molecule. It is notable that for many decades it was erroneously considered to be a circular molecule! In *S. cerevisiae*, the mtDNA is 86 kb. Mitochondria provide the cell's respiratory function and contain enzymes encoded by nuclear and mitochondrial genes. The mitochondrial genome encodes a set of subunits vital for the structure and function of the mitochondrial electron transport chain (ETC) and generation of adenosine triphosphate (ATP). The mtDNA also encodes genes for mt–transfer RNAs (tRNAs) including one tRNA that recognizes and translates the codon UGA as tryptophan (in *S. cerevisiae*), rather than as a translational stop codon as it functions in the nucleus. The ribosomal RNAs (rRNAs) for the 16 and 28S subunits of the mtRNA polymerase are also encoded on the mtDNA genome. Under anaerobic conditions, the mitochondria appear to degenerate, but re-form if the cells are aerated.

Yeast carrying fully functional mitochondria are called "grandes" as they form larger colonies due to their ability to use sugars and ethanol for growth. The mitochondrial chromosome of *S. cerevisiae* is unstable as it is replicated by mtDNA polymerase that is more error prone than its nuclear counterpart. Replication of mtDNA occurs throughout the cell cycle, unlike nuclear DNA replication, and there are multiple origins of replication in yeast mtDNA, unlike mammalian mtDNA. About 1% of the cells of most laboratory strains in cultures

Table 2. **Comparison of Yeast Genomes for Species Where the Complete Genome Sequence has been Determined**[a]

	Candida glabrata	*Cryptococcus neoformans*	*Debaryomyces hansenii*	*Schizosaccharomyces pombe*	*Yarrowia lipolytica*	*Saccharomyces cerevisiae*	*Kluyveromyces lactis*
total No. bases	12,280,357	19,051,922	12,220,823	12,515,113	20,502,981	12,162,908	9,269,136
coding No. bases	8,008,142	12,354,976	9,287,388	7,252,937	9,838,860	8,827,555	2,375,388
% coding bases	65.21	64.85	76.00	57.97	47.99	72.58	60.57
G + C No. bases	4,741,203	9,247,650	4,426,787	4,511,499	10,056,963	4,639,973	3,614,962
% G + C	38.61	48.54	36.28	36.06	49.05	38.15	38.76
scaffolds	13	14	7	4	6	18	7
genes	5489	6744	7121	5275	7389	6272	5519
proteins	5272	6594	6896	5020	6666	5870	5331
% protein coding	96.05	97.78	96.85	95.71	90.22	93.59	70.69
size bp mtDNA[b]	20,063	24,874	N.D.[c]	19,430	18,431	85,779	40,281

[a]Data from Refs. 23–26.
[b]Mitochondrial DNA = mtDNA.
[c]Not determined = N.D.

contain defective mitochondrial genomes. These yeast are known as "petites" and can grow on sugars, but not on ethanol. The type of defects in petites usually range from large deletions and rearrangements (ρ^-) to complete loss of mtDNA (ρ^0). When deletions occur the remaining mtDNA sequence and its replication origins are reamplified creating chromosomes equivalent to the original size. Over many years petites were useful in mitochondrial genetics studies and early sequencing of the mitochondrial genome by the Maxam-Gilbert chemical cleavage method. Under commercial and industrial situations the phenomenon of petite formation is not considered a problem. Less frequent mitochondrial defects arise from point mutations in the mitochondrial genome (*mit*$^-$ mutants) or in the nuclear genome (*pet*$^-$ mutants). These mutants involve the loss of a single mitochondrial function, while petite mutants lose all functions encoded by the mitochondrial genome.

The largest organelle in the cell is the vacuole, which serves both hydrolytic and storage functions. Depending on culture and cell cycle conditions, one to four vacuoles may be found in a cell. Peroxisomes help maintain the oxidative state of the environment. Proteins destined for these organelles are in most cases directed to the target organelle by specific signal sequences. Thus mitochondrial proteins are targetted to dock with receptor complexes in the mitochondria by a short ampipathic leader sequence. Nuclear-located proteins are similarly taken up through the interaction of the nuclear pore receptor with a nuclear localization signal (NLS). Other proteins targetted for other membrane-bound organelles are usually processed through the secretory pathway. This consists of laminar layers of membranes through which proteins proceed and are processed, folded, and modified. The mRNA for these proteins is translated on the rough endoplasmic reticulum (RER) the membrane of which is a continuum of the nuclear envelope. Signal sequences (short, hydrophobic, and charged) direct the protein to the Golgi bodies. Passage though the various layers of membrane in the Golgi may be accompanied by glycosylation. The extent of branching during glycolysation appears to be greater in *S. cerevisiae* than in other yeast. Having entered the pathway, proteins continue until their progress is stopped by interaction of specific protein retention signals with receptor complexes that determine how far the proteins proceed. The processing of proteins may involve modifications that include proteolysis, phosphorylation, acetylation, glycosylation (N- and O-linked) prenylation, and ubiquitination.

The cytoplasm of the cell contains most of the RNA, which may account for 7–12% of total cell solids, mostly as ribosomes. Lipid globules are also found in the cytoplasm, as well as carbohydrate storage materials in the form of glycogen and trehalose, which may account for up to 23% or more of cell solids, depending on culture and metabolic conditions. The ascospores of some yeast also harbor unusual products (eg, di-tyrosine). In some species (*S. cerevisiae*, *K. lactis*, *Zygosaccharomyces rouxii*), the cytoplasm also contains plasmid DNA. These plasmids have proved to be very useful in the development of multicopy cloning systems and systems for heterologous gene expression. If a yeast species cannot be transformed by such plasmids the DNA can be introduced into chromosomal DNA directly by homologous recombination.

Some yeast, eg, *S. cerevisiae*, may also contain double-stranded RNA (dsRNA), which forms virus-like particles (VLPs) and carries genes for

killer-toxin production and self-immunity. Killer toxins are a problem in brewing as they can kill the starter cultures during production. Virus like particles are also produced by retroviral-like transposons (Ty elements), inserted at different places in nuclear DNA. Unlike retroviruses they lack coat envelope proteins and are not infectious.

Vegetative reproduction in yeast occurs mostly by budding or, in the instance of *Schizosaccharomyces* and *Endomycopsis*, by fission. The nuclear membrane remains intact during this process. During budding, the nucleus of the mother cell divides and one portion enters the newly formed bud. The bud grows until it has reached a size almost as large as the mother cell and then separates from it, forming a bud scar on the surface of the mother cell and a birth scar on the surface of the bud. The number of bud scars can be counted, and the oldest cells of a population in stationary phase typically have 12–15 bud scars, apparently the maximum number of daughter cells that can be produced by a single mother cell. The genetic control of the cell division cycle is a major topic of yeast research, but is only briefly reviewed here. However, note that scientists working with yeast model systems in this area of research were awarded the 2001 Nobel Prize in medicine. Other yeast (eg, *Schizosaccharomyces* and *Endomycopsis*) undergo cell division by fission. In this process, a septum (cross-wall) is formed across the mother cell without constriction of the cell. Division at the cross-wall or septum forms two separate cells. Mother cells that have reached their maximum number of buddings become quiescent and can apparently remain in this state for a very long time.

Yeasts of the family *Basidiomycetes* form external spores. In *Ascomycetes*, sexual reproduction occurs with the formation of spores in a cell that serves as a spore sac or ascus. Generally four, but sometimes eight or more, spores are formed in each ascus as reflected in the species name, eg, *Schizosaccharomyces octosporus*, and *Kluyveromyces polysporus*. Sporulation in *S. cerevisiae* is induced by starvation for nitrogen in the presence of a nonfermentable carbon source (eg, acetate). In principle, sexual reproduction in yeasts consists of an alternation between the haploid phase (one set of chromosomes) and the diploid phase (two sets of chromosomes). However, production strains of bakers' yeast (*S. cerevisiae*) are generally polyploid (often tetraploid, containing four sets of chromosomes and producing diploid spores). Although sporulation in laboratory strains of bakers' yeast is quite efficient, with at least 70–80% of cells forming asci and most (90% or more) spores viable, production strains sporulate with low efficiency. At most, 10% of cells form asci and spore viability is generally 1% or less. The four ascospores of *S. cerevisiae* remain together within the wall of the mother cell. After removal of the wall by enzymatic digestion, they may be separated individually by micromanipulation and grown into colonies for genetic or physiological characterization. [Commercially available digestive enzymes include Zymolyase, isolated from *Arthrobacter luteus* and Glusulase (snail gut enzyme), isolated from snail crop.] Other yeasts may exist predominantly in the haploid state, eg, *Zygosaccharomyces rouxii*; in the diploid state as for *S. cerevisiae*; or in both states, as in *Saccharomycopsis lipolytica*. An alternation between the haploid and the diploid state may also occur without production of sexual spores (parasexuality).

3. Strain Improvement and Development

A variety of transformation techniques using *E. coli*–yeast shuttle vectors and yeast selectable markers, as well as efficient yeast promoters and signal sequences, are generally available. (References 14–17 contain general reviews and descriptions of such procedures.) For industrial strains, the rarity of dominant selectable markers for transformation sometimes poses a problem. Public disinclination to purchase foods associated with genetically engineered organisms has limited the use of such strains in most of the industry. Generally, work on strain improvement is still carried out using the classical genetic approaches of hybridization and mutagenesis. However, with the complete DNA sequence of *S. cerevisiae* now available, it is possible to manipulate the genome in a more sophisticated manner. Specific mutagenic events such as might occur by insertion into, deletion from, or rearrangements of the yeast genome or by combinations of these changes, can now be very specifically defined in yeast. The results of such events can be investigated for every gene and under defined conditions of pH, temperature, growth rate, oxygen levels, and pressure or media composition. The target nucleic acids (NA) (DNA, RNA, or cDNA), or target product are prepared as arrays and screened over real time. Profiles are generated for changes (up-regulated, down-regulated, unchanged) to gene expression levels and linkage maps are developed to elucidate pathways and processes.

It is possible to isolate individual ascospores or vegetative cells of yeasts, eg, *S. cerevisiae*, with a micromanipulator. If individual cells of one strain are placed next to cells of another strain, conjugation or mating may occur. The characteristic zygote formed is easily identifiable and may be isolated in turn. Such hybrids can also be produced by conjugation of spores of different species of the same genus (differences between species of *Saccharomyces* are usually minor, defined by carbon source utilization, and may not reflect large genetic differences). Ability to mate in *S. cerevisiae* is determined by a single locus called *MAT* (mating type) with two alleles. Cells expressing *MATa* are capable of mating with cells expressing *MAT*α, and vice versa, but are not capable of producing ascospores. Cells expressing both mating types (*MATa*/*MAT*α), eg, produced by mating *MATa* and *MAT*α cells, are not capable of mating, but can produce ascospores. Strains of bakers' yeast are generally heterothallic: *MATa* and *MAT*α strains display a stable mating phenotype. Many other strains, including some wine strains, are homothallic: cells of one mating type change to the other mating type after producing a bud and are capable of mating with their buds. The resulting zygote and its descendants are no longer capable of mating. Typically, only spore–spore matings are successful with homothallic strains.

Heterothallic and homothallic strains of *S. cerevisiae* differ at a single locus, *HO* (homothallism), which encodes a site-specific endonuclease. The change in mating type in homothallic strains begins when the HO endonuclease cleaves a site within the mating-type locus, located on Chromosome III. This leads to the replacement of the resident mating-type allele with a copy of the other, through a process of gene conversion. Copies of both *MATa* and *MAT*α are also

present on Chromosome III at loci known as *Hma* and *Hm*α, respectively. Only the allele present at *MAT*, however, is expressed.

Hybridization of incompatible strains is carried out by selecting rare matings or by protoplast fusions. Both procedures require the presence of selectable markers in both parent strains. Since dominant selectable markers in nuclear genes are hard to come by in industrial strains, use of mitochondrial antibiotic-resistance markers (eg, oligomycin or erythromycin resistance) is common. Rare matings are selected simply by mixing two appropriately marked parent strains, plating on nutrient agar, and then replica plating to a selective medium on which neither parent strain can grow, but rare hybrids can. Confirmation of hybrid identity by karyotyping is generally required. Cross-species mating is very common in the wine industry as growers seek to produce improved yeast that have attendant benefits of enhanced or improved production or new flavors, or higher yields in shorter times for lower costs. Such hybrids can be produced by forced mating between related wine yeasts.

Protoplast fusion is carried out by digesting the cell walls of marked strains with hydrolytic enzymes (eg, Zymolyase) and mixing the resulting protoplasts in buffer to permit membrane fusion. Following plating on selective medium in an osmotically supportive top agar and cell wall regeneration, fusions can be recovered at low efficiency. Hybridizations between species of *Saccharomyces* and *Kluyveromyces* have been achieved, as well as between *Candida* and *Pichia* species.

4. Mutation

In the past, mutagenesis was more often than not a random event. For industrial applications, mutations are induced by X-rays, ultraviolet (uv) irradiation or chemicals (nitrosoguanidine, ethyl methane sulfonate, methyl methane sulfonate, etc), but now mutants strains can be produced more quickly and exactly as selected by use of deselected recombination or by PCR *in situ* using specific oligonucleotides. Mutant selections based on amino acid or nucleotide base analogue resistance or treatment with Nystatin or 2-deoxyglucose to select auxotrophs or temperature-sensitive mutations are easily carried out.

Examples of useful mutants generated by mating are strains of: *Candida membranaefaciens*, which produce L-threonine; *Hansenula anomala*, which produces tryptophan; or strains of *Candida lipolytica*, which produce citric acid. An auxotrophic mutant of *S. cerevisiae*, which requires leucine for growth, has been produced for use in wine fermentations. This yeast produces only minimal quantities of isoamyl alcohol, a fusel oil fraction derived from leucine by the Ehrlich reaction (18,19). A mutant strain of bakers' yeast with cold-sensitive metabolism shows increased stability and has been marketed in Japan for use in doughs stored in the refrigerator (20).

5. Fermentative and Respiratory Metabolism

Although most yeast species are strict aerobes, strongly fermenting yeasts, eg, *S. cerevisiae*, grow well under anaerobic conditions, but with a lower yield. The

yield of yeast based on weight of sugar consumed is greater in the presence of oxygen, and oxygen decreases the rate of sugar consumption. Under aerobic conditions, alcohol is produced as the end product of metabolism if the sugar concentration exceeds 0.1–0.2% by weight. In the presence of higher sugar concentrations, synthesis of respiratory enzymes, eg, cytochromes, is inhibited.

The anaerobic pathways for glucose utilization by yeasts and other microbes lead to the formation of various alcohols and organic acids. Ethanol is the principal product of yeasts used in the beverage industry, formed from pyruvate via acetaldehyde by the enzyme alcohol dehydrogenase. Pyruvate itself is created in the Embden-Meyerhof-Parnass (EMP) pathway and the related hexose monophosphate (HMP) pathway. In either case, anaerobic fermentation results in 2-mol ethanol and 2-mol carbon dioxide from 1-mol glucose. This sequence also produces 2-mol ATP, supplying the energy for anaerobic growth. Common fermentable substrates are glucose, fructose, galactose, mannose, maltose, lactose, cellobiose, sucrose, and some trisaccharides.

Respiratory, or oxidative, metabolism produces more energy than fermentation. Aerobic respiration of 1 mol of glucose leads to maximum yield of ~36–38 mol of ATP. More substrates can be respired than fermented, including pentoses (eg, by *Candida* species), ethanol (eg, by *Saccharomyces*), methanol (eg, by *P. pastoris*), and alkanes (eg, by *Saccharomyces lipolytica*).

6. Composition, Nutrients, and Growth Rate of Yeast

The elemental and vitamin compositions of some representative yeasts are listed in Table 3. The principal carbon and energy sources for yeasts are carbohydrates (usually sugars), alcohols, and organic acids, as well as a few other specific hydrocarbons. Nitrogen is usually supplied in the form of ammonia, urea, amino acids, or oligopeptides. The main essential mineral elements are phosphorus (supplied as phosphoric acid or phosphates), and potassium, with smaller amounts of magnesium and trace amounts of copper, zinc, and iron. These requirements are characteristic of all yeasts. The vitamin requirements, however, differ among species. For laboratory and many industrial cultures, a commercial yeast extract contains all the required nutrients.

The specific growth constant for exponential growth, μ, is defined as $\mu \times dt = dM/M$, where M is the yeast mass and t is time. Growth is also measured by either the generation time, ie, the time required for a doubling of the yeast population, or as the hourly growth rate. The relationship of these expressions is shown in Table 4. Exponential growth ceases when one or more nutrients become limiting. For aerobic cultures, the deficiency may be in oxygen, which is difficult to supply in large amounts because of its poor solubility.

7. Yeast-Fermented Foods and Beverages

Yeasts are used for production of alcoholic beverages (beer, wine, cider, mead), baked goods, yeast biomass, and the production of fuel alcohol by fermentation. In the production of alcoholic beverages, there is a 5- to 10-fold increase in yeast

Table 3. **Composition of Yeast**[a]

Components	(wt%) C	H	N	O	Ash	(mg/kg) Fe	Ca	Mg	P	K	Na	Cu	Mn	Zn
bakers' yeast	47.0	6.0	8.5	32.1	6.0	0.003	0.060	0.13	1.0	2.0	0.03	8.0	5.9	197.0
brewers' yeast					6.4	0.010	0.130	0.23	1.4	1.7	0.07	33.0	5.7	38.7
Candida sp.	45.9	6.7	7.3	32.5	7.8	0.010	0.570	0.13	1.7	1.9	0.01	12.4	38.7	99.2

Components	(wt%) Dry matter	Crude fiber	Protein (N × 6.25)	(mg/kg) Thiamine	Riboflavin	Biotin	Pyridoxine	Panthenate	Nicotinic acid	Folic acid	Choline
baker's yeast	94.0		45.0	90.0	45.0	1.30	44.0	65.0		15	4000
brewer's yeast	93.0	3.0	44.6	91.7	35.0		43.0	110.0	448	10	3885
Candida sp.	93.0	2.0	48.3	6.2	34.0	1.10	30.0	83.0	500	23	2911

[a] References 21,22, and 27.

Table 4. **Relation of Generation Time to Hourly Growth Rate and Specific Growth Rate**

Generation time, h	Hourly growth rate, Ct/C0[a]	Specific growth rate, μ, h^{-1}
1	2.00	0.693
2	1.41	0.347
3	1.26	0.230
4	1.19	0.173
5	1.15	0.139

[a]Concentration at time t vs. Concentration at time 0.

cells during fermentation. Bakers' yeast is supplied as a thick suspension (yeast cream, ~17% solids), as a moist press cake (~30% solids), or as an active dry yeast (~93–98% solids). There is little or no growth of yeasts during dough fermentation. For *Candida utilis* (torula) biomass cells are grown on sulfite waste liquors or ethanol and for *Kluyveromyces fragilis* the growth substrate is cheese whey or whey permeate. These products are generally used as inactive, food-grade yeasts. Some bakers' yeast is also used to produce food-grade, inactive dried yeast. Yeast residue from the brewing process is also used as the basis of food extracts, eg, Vegemite (peculiar to Australia). Yeasts are also being investigated as probiotics for prevention of gastrointestinal infection, and they are given as vitamin supplements.

Yeasts grown in clear media, eg, clarified molasses or brewers' worts, can be recovered by centrifugation or filtration followed by pressing if desired. Yeasts grown in distillers' mashes or grape musts, contain insoluble particles that cannot be separated economically. All the yeast produced in wine fermentations is either discarded as waste or spread with the lees or still residues as fertilizer. Excess yeast produced during fermentation of distilled beverages and in fuel-ethanol production is recovered with the spent grains and sold as feed. Yeast produced during beer fermentations is largely combined with the spent grains and sold as feed as well; however, much is recovered separately by centrifugation and used for the production of either food-grade, inactive brewers' yeast, or yeast extracts (Vegemite).

The fermentative activity of the yeasts is almost the same in all industries. Differences in fermentation times arise mainly from differences in temperature. For the baking industry, temperature is high, therefore fermentation time is short, with little time for cell multiplication and very high cell counts at the start.

8. Bakers' Yeast Production

Bakers' yeast is grown aerobically in fed-batch fermentors under conditions of carbohydrate limitation, maximizing yield of yeast biomass and minimizing production of ethanol. Yeasts grown under these conditions have excellent dough leavening capability and perform much better in the bakery than yeast grown under anaerobic conditions.

8.1. Strains and Their Maintenance. All bakers' yeast strains are *S. cerevisiae*. They appear to descend from wine yeasts. Although many hybrid and mutant strains of bakers' yeast have been patented, few are in production.

Pure cultures of yeast strains may be maintained on agar slants with frequent transfers, as frozen liquid stocks in 50% glycerol or 7% dimethyl sulfoxide (DMSO), or in lyophilized form. Suitable strains may be obtained from any culture collection. Additionally, since yeast sold to bakers and the general public is alive, it may be isolated from commercial cream, compressed, or dry yeast products. Strains differ in their ability to ferment in doughs containing different sugar levels. Some are best in "lean" doughs (ie, with no added sugar); some perform best with sugar additions of 6–8% of flour weight ("regular" doughs) or 20% of flour weight ("sweet" doughs). Yeasts used for the production of compressed or cream yeast (fresh yeast) in the United States, or for the so-called fast compressed yeasts of Europe, are ovoid with a cell diameter of 4–6 μm. In contrast, yeasts used for the production of active or instant dry yeast are larger, ie, 5–8 μm in diameter. Although dry yeast strains generally display lower leavening power on an equivalent solids basis when used as fresh yeast, they are more tolerant of drying conditions.

8.2. Raw Materials for Yeast Growth. Until the 1930s, grain worts were the principal carbon and energy sources for yeast production. Since then, cane and beet molasses have been used as they are much less expensive sources of fermentable sugars. Increases in the cost of beet molasses and more efficient extraction of sugars from beet juice have caused yeast manufacturers to consider supplementing worts with additional carbohydrate sources, eg, corn syrup. Molasses contains ~50–55% fermentable sugar in the form of sucrose, glucose, and fructose. It is also a source of potassium and other minerals, and cane molasses is the principal source of biotin, a required nutrient for bakers' yeast production. The principal nitrogen source is ammonia, added as ammonium sulfate, or as liquid, or anhydrous ammonia. Urea and many of the amino acids present in molasses are also sources of assimilable nitrogen. Since bakers' yeast contains ~3% phosphorus (as P_2O_5), based on yeast solids, phosphate is added either as phosphoric acid or phosphate salts. Calcium and magnesium salts in smaller concentrations are also added. Iron, zinc, copper, manganese, and molybdenum are required as trace minerals. The vitamin requirements for biotin, inositol, and pantothenic acid are usually provided by molasses. Thiamine beyond that required for growth is usually added as it contributes to the fermentation activity of the final yeast.

8.3. Growth of Baker's Yeast. Fermentations proceed from a series of smaller scale fermentations, beginning with small flasks followed by transfer to larger and larger vessels (see Ref. 28). There may be as many as eight stages in this sequence, producing perhaps 0.8, 3.5, 25, 120 kg, 2.5, 15, and 100 tons of yeast from a single colony isolated from a pure-culture slant. The earliest stages may be carried out under conditions approaching pure-culture fermentations, ie, with sterile media, sterile air, and presterilized vessels. For large-scale fermentations, molasses at 80° Brix is diluted to ~30° Brix and adjusted to pH 5. It is clarified by centrifugation to remove insoluble solids and sterilized by brief, high temperature treatment. The other nutrients are either included in the molasses wort or added separately during the fermentation. Thus ammonia is generally added separately because it is used both as a nutrient and as a means of pH adjustment. The fermentation is carried out at 30°C and pH 4.5–6.5; higher pH levels produce faster yeast growth, but are conducive to the growth of contaminants as well.

The large-scale fermentations are carried out as fed-batch processes (formerly known as the Zulauf process). Medium is added to the seed yeast (pitch) at a slow rate to maintain sugar concentrations in the fermentor at $<0.1\%$. At higher sugar concentrations, excess alcohol forms, lowering the yeast yield and increasing the biological oxygen demand (BOD) of the effluent. Wort addition may follow a preset program (scheduled feed) or may be controlled in response to culture conditions, eg, ethanol levels (demand feed). With sufficient aeration, it is possible to greatly extend the exponential phase, but in practice this is rarely done. Production cultures are normally harvested at yeast concentrations of $\sim$17–20% (ie, 4–5% by weight yeast solids). In practice, the yield of yeast cell mass is 50% by weight of the sugar used.

Oxygen is a critical nutrient for yeast growth. One kilogram of oxygen is required for the growth of 1 kg of yeast solids. This oxygen is supplied by forcing air through a series of pipes with small holes, arranged near the bottom of the fermentor. The distribution of air and the formation of very small bubbles (to increase surface transfer area) are often aided by agitators. Oxygen is only sparingly soluble in water, and the saturation concentration at 30°C is only 7 ppm. During oxygen uptake by concentrated yeast suspensions, the oxygen level may decrease to only 5% of saturation. In large fermentors, the rate of air supply is about one volume of air per fermentor volume per minute (VVM). Alcohol is formed if the oxygen supply is limiting.

Finally, the rate of yeast growth must be limited to the specific growth rate constant of 0.23, again to limit alcohol formation. For an hourly specific growth rate of 0.23, the hourly increase in cell mass is 1.26 and the generation or doubling time is 3 h. During yeast growth, heat is liberated and must be removed from the fermentor by internal cooling tubes or by pumping the fermentor contents through external heat exchangers.

The fermentation period is usually 12–18 h. For a 15-h fermentation, the generation time may increase from 3 to 5 and finally to 7 h, allowing for roughly an eightfold increase in cell mass. Generally, aeration continues briefly after wort additions have been stopped to allow the yeast to mature. This results in a reduction in the number of budded cells as cells complete their last division cycle and increases storage stability of the final product. At the end of the growth period, the yeast solids concentration is $\sim$5%. Experimentally, the solids concentration may be increased to 10% or higher, but in commercial practice oxygen demand is limiting.

Fermentations in larger vessels and the final trade fermentation are conducted under quasisterile conditions, and yeast growth is accompanied by some growth of contaminant bacteria. These are generally lactic acid producing organisms, but are sometimes coliform bacteria; the occurrence of *Salmonella* in fermentor liquids has not been reported. Massive contamination with *Oidium lactis* or wild yeasts has been reported.

9. Preparation of Yeast in the Food Industry

9.1. Cream Yeast. At a 5% solids concentration in the fermentor, the yeast occupies $\sim$12% of the fermentor volume. It is harvested by centrifugation

in nozzle centrifuges and washed several times with water. The final centrifugate is cooled and stored in refrigerated tanks. This yeast cream (so called because of its off-white color) may be sold directly in this form, since in large baking facilities it may be piped directly to any desired location.

9.2. Compressed Yeast. A moist press cake is obtained by pressing yeast cream in plate and frame filter presses or filtration through a rotary vacuum filter. The press cake is mixed with emulsifiers (0.1–0.2 wt%) and its moisture content is adjusted to 70%. To form block yeast, the cake is then extruded through Teflon nozzles in the shape of thick strands with a rectangular cross-section and is typically cut into 0.45-kg cakes, which are wrapped in wax paper. Compressed yeast is also prepared as crumbled yeast in the form of irregularly shaped pieces packed in bulk containers. During pressing, mixing, and extruding, the temperature of the yeast cakes is 10–15°C. They must be cooled for ~2 days before shipment in refrigerated containers. Compressed yeast is a perishable commodity and must be refrigerated at all times. At a storage temperature of 5–8°C, there is a 3–5% loss of activity per week. Compressed yeast may be kept frozen for several months. However, after thawing, some browning and softening of the cake occur.

Compressed yeast is received by the baker in 0.45-kg cakes or as crumbled yeast in 22.7-kg bags and refrigerated before use. Deliveries may be on alternate days or once a week, and the stock must be rotated so that the yeast is never kept longer than 1–2 weeks in the bakery. The yeast cakes may be mixed directly into the dough by being placed directly on top of the flour in the mixer in a straight dough fermentation. More commonly, crumbled yeast (or cream yeast) is used in the preparation of preferments. The crumbled yeast is slurried with a small portion of the water and other ingredients (oxidants, enrichment tablets, and yeast nutrients) in a premix tank, allowed to ferment and then pumped into the mixer.

Compressed yeast is also sold in supermarkets in 18- and 56-g packages. Since this product contains ~10% added starch to increase its shelf life, it has a lower protein content and fermentative activity than the compressed yeast sold to bakeries.

9.3. Active Dry Yeast. The production of active dry yeast (ADY) is very similar to the production of compressed yeast. However, a different strain of yeast is used and the nitrogen content is reduced to 7% of solids compared with 8–9% for compressed yeast. The press cake made with the active dry yeast strain is extruded through a perforated plate in the form of thin strands with a diameter of 2–3 mm and a length of 3–10 mm. The strands are dried on endless belts of steel mesh in drying chambers (a continuous process) or in roto-louvre dryers (a batch process), with the temperature kept <40°C. Drying time in drying chambers is 3–4 h and in roto-louvre dryers is 6 h or more. The final moisture level attained is 7.5–8%.

Where storage stability and convenience are important considerations, ADY has largely replaced compressed yeast. The yeast is packaged in hermetically sealed aluminum foil pouches for domestic use, in 0.45–0.91-kg cans for institutional use, or in 10-kg foil pouches. Since a minimum storage life of 1 year is guaranteed, the yeast is packed in a nitrogen atmosphere or under vacuum, and the integrity of the package is at least as important as the original activity of the

yeast. For use in wholesale bakeries, active dry yeast is usually delivered in polyethylene-lined drums and is not protected by an inert atmosphere; accordingly, it should be used within 2–3 months. The yeast requires separate hydration in water, preferably at 35–40°C before addition to other dough ingredients. A period of 5–10 min is sufficient for full rehydration and optimum activity.

9.4. Instant Active Dry Yeast. Instant ADY (IADY or HADY) production is similar to ADY production, but requires a different strain of yeast. After pressing, the yeast is extruded into noodles 0.2–0.5 mm in diameter and 1–2 cm long and deposited on a metal screen or perforated plate in a fluid-bed air dryer. Drying time is shorter than with ADY, ~1–2 h in practice, with a final moisture level of 4–6%. Instant active dry yeast does not require separate rehydration. It is always packaged in a protective atmosphere or under vacuum. On an equivalent solids basis, the activity of IADY is greater than that of regular ADY, but still less than that of compressed yeast.

All active dry yeasts make doughs softer, more extensible and less elastic. This slackening is more pronounced with the regular ADY than with IADY. The effect is believed to be due to the leaching of reducing agents, especially glutathione, from the dried yeast during rehydration, before the cells have fully recovered their activity. Up to 50 ppm (yeast solids basis) of glutathione can be leached from frozen yeast (29), although this may not be enough by itself to have a substantial effect. Slackening can be counteracted with higher levels of oxidants in the dough.

9.5. Yeast in Baked Goods. Baked goods can be leavened with yeast, chemicals, the foam of egg white, or steam. There is a preference for yeast-leavened baked goods because of their desirable flavor. Therefore, only those products are chemically leavened in which yeast does not perform well, generally as a result of high osmotic pressure. The osmotic pressure of doughs depends inversely on moisture content and directly on the concentration of salt and sugars. Cookies and highly sweetened cake doughs have an osmotic pressure too high for adequate fermentation by yeasts. Although some attempts have been made to grow yeasts with exceptional tolerance to high osmotic pressure, none has been used commercially. There are some products in which either yeast or chemical leavening, or both, are used, eg, doughnuts and pizza dough.

Another requirement for proper leavening is the presence of a protein matrix sufficiently elastic to trap small carbon dioxide bubbles. Wheat gluten fulfils this requirement. Rye protein is less suitable and the proteins of other cereals, eg, rice, oats, or corn, are practically useless.

9.6. White Pan Bread. More than 50% of all the flour used in baked food production is made into bread, rolls, and buns, and white bread is the predominant dough type. The basic method of production is the sponge-and-dough method. The sponge generally contains 60–70% of the flour, all of the yeast, and yeast food. It undergoes a 3–4-h fermentation, when the sponge, the remainder of the flour, and all other ingredients (salt, sugars, fat, etc) are mixed into a dough and permitted to rest for 20–30 min. The dough is divided, rounded, molded, and panned. The pans are placed in a proofing cabinet where the final fermentation takes place before baking. Mixing of the sponge, the sponge fermentation and the mixing of the dough are batch processes. From the dough dividing step to the final baking, the process is generally continuous.

Since the 1950s and 1960s other production methods have been used. For example, the sponge can be replaced by a liquid sponge or preferment, which contains little or no flour, but most or all of the water, yeast, yeast nutrients, and a suitable buffer. Addition of the buffer prevents the pH from dropping <4.5. The preferment must remain sufficiently fluid that it can be pumped. The temperature is generally set at 25–30°C and fermentation time is 2–4 h. Another method is the use of continuous dough mixing, which must be combined with the use of liquid preferments. Continuous high speed mixing produces a very extensible, slack dough with a uniform distribution of fine bubbles of carbon dioxide. The resulting bread has a very fine grain and is very soft.

All the preceding methods have the advantage of flexibility. Depending on the course of the sponge fermentation, alterations can be made in dough mixing time or in the addition of other ingredients, eg, oxidants, to the dough. In case of equipment failure, sponges can be held for several hours without spoiling, and preferments can be cooled and stored overnight. Wholesale bakers also use straight dough methods, in which all of the ingredients of a bread formula and the water are mixed in a single operation. The dough is then fermented for 2–4 h. The operations following fermentation are then the same as those for sponge and dough methods. More detailed descriptions of actual bakery methods are found in Ref. 30.

The function of yeast in bread making is threefold: it serves as a leavening agent, it contributes to flavor, and it matures the dough. In contrast to chemical leavening, which is a rapid reaction, yeast leavening is slow and proceeds as long as fermentable sugar is present. Flour contains ~1% fermentable sugar, mostly glucose and maltose, which is generally fermented within the first 1–1.5 h. However, flour also contains damaged flour granules (6–12% by weight), which are hydrolyzed by amylases to maltose. A β-amylase is present in the flour, and α-amylase derived from barley malt or fungi may be added as well. In doughs without added sugar, glucose is fermented before fructose; maltose fermentation is initiated only after glucose is essentially exhausted, and continues as long as maltose is produced from starch.

In practice, most bread doughs contain 4–8% (based on flour weight) added sugar, usually sucrose, glucose, corn syrup, or high fructose corn syrup. Most of these sugars are consumed during fermentation, but some residual sugar appears in the bread. With the addition of 6.7% sucrose, eg, the residual sugars in the baked bread are fructose (2.6%), glucose (1.7%), and maltose (0.7%). Lactose (milk sugar) is not fermented by bakers' yeast, and addition of milk solids or whey results produces residual lactose in the bread.

The pH of doughs or preferments has little effect on yeast fermentation rates unless the pH drops <4.5. Prior to fermentation, the pH of a dough is 5–6. During fermentation it drops to ~4.8 due to production of carbonate (from dissolved carbon dioxide) and organic acids. During baking there is a rise in pH as carbon dioxide is driven off. Temperature has a considerable effect on yeast activity: up to 38°C, activity increases, but diminishes at higher temperatures. At 52°C, bakers' yeast cells die within 10 min. Bakery sponges, straight doughs, and preferments are generally set at 24–26°C. During fermentation there is a rise of 2–5°C resulting from the metabolic activity of the yeast. In the case of continuously mixed doughs, the temperature may reach 35°C

outside the mixer. Proof box temperatures are generally 30–40°C. During baking, the dough temperature rises rapidly and yeast activity ceases after ~10 min. During this time, expansion of the dough in the pan (the so-called oven spring) occurs, resulting from continued fermentation, reduced solubility of carbon dioxide in the dough water, thermal expansion of carbon dioxide bubbles in the dough water, and evaporation of ethanol and volatile organic acids.

The effect of osmotic pressure on yeast activity is of great importance, and is often overlooked. At salt concentrations up to 1.5%, the effect is slight; salt concentrations of 2–2.5%, which are common in bread doughs, inhibit yeast activity considerably. Likewise, sugar concentrations ~4% produce apparent inhibition. Consequently, yeast-raised sweet doughs (15–20% sugar), contain very high yeast concentrations.

Ethanol inhibits yeast fermentation. Mould inhibitors such as calcium propionate (added at 0.25–0.50% of the flour) or small amounts of vinegar are commonly added to bread doughs. However, mould inhibitors may also inhibit fermentation to a slight degree unless the yeast is specially treated by the manufacturer during growth. Although dough alcohol concentrations rarely exceed 3% and are not inhibitory, increasing the alcohol concentrations to higher levels equivalent to concentrations achieved during wine fermentation (11–15%), may be important in inhibiting yeast fermentation in dough production.

There is little or no growth of yeast during dough fermentation. However, budding can be observed during sponge fermentation or during the proof stage of a straight dough fermentation. To boost the yeast the addition of yeast nitrogen nutrients (~0.05% ammonium sulfate or ammonium chloride) is commonly practiced.

The effectiveness of yeast leavening depends on the development of a protein matrix to retain carbon dioxide. The matrix can be obtained with wheat flours, which contain 10–14% protein. A portion of the carbon dioxide produced during the fermentation escapes from the dough and is not available for leavening. This is important during proofing when the volume of the bread is determined. The amount of high speed mixing is critical to development of the elastic protein matrix. Undermixed or overmixed doughs do not retain gas well. The protein matrix does not develop with corn flour and consequently cornbreads have a coarse, grainy crumb.

Yeast fermentation is also central to flavor development. Flavors arise from the by-products of fermentation such as, higher alcohols, esters, aldehydes, and other carbonyl compounds. However, in contrast to alcoholic beverages, the flavor of baked bread is produced by the interaction of these fermentation by-products with other dough constituents, particularly during baking with considerable portion of the bread flavor being formed in the crust during browning. After baking and cooling, these flavors diffuse into the bread crumb. Despite the identification of >100 flavor compounds, very little is known about these reactions.

The role of yeast in fermenting dough maturation is even less clear. The alcohol and carbon dioxide developed during fermentation must influence the elastic properties of the protein matrix. However, experimental procedures that would permit this to be checked in the absence of yeast have not been developed.

9.7. Sourdoughs. The microflora of flour includes large numbers of lactic acid producing bacteria. In the past, bread dough fermentations were mixed yeast and bacterial fermentations. This is still true of breads made exclusively from rye dough (in the United States, rye bread generally contains only small amounts of rye flour mixed with large amounts of wheat flour). Sour rye doughs and wheat doughs are inoculated with bacterial starter cultures as well as yeast. The lactic acid bacteria normally used in sourdough fermentations are *Lactobacillus plantarum*, *L. brevis*, or *L. fermenti*. The production of organic acids by these bacteria causes a much larger drop in the pH of the dough than occurs in a fermentation using yeast only.

The production of San Francisco sourdough bread is a typical sponge-and-dough fermentation, and a portion of each sponge is retained for inoculation of the succeeding sponge. The yeast used in this fermentation is *Saccharomyces exiguus* (31), the bacterium is *Lactobacillus sanfrancisco* (32). The same yeast is also responsible for the fermentation of the Italian pannetone, a traditional Christmas fruitcake.

The production of soda crackers is also based on a mixed fermentation. Doughs for cracker production are inoculated with very small amounts of bakers' yeast. During the first 3–5 h of the 18-h fermentation, yeast activity predominates; thereafter bacterial fermentation causes a rapid decrease in pH through formation of lactic acid.

10. Brewing

Beer brewing has been traced back to ancient Egypt (at least to ~1000 B.C.), in a form related to modern practice. Recent findings suggest that sprouted barley, dried, cooked, and ground, along with crushed uncooked wheat or malt was treated with hot water (33). The filtered extract was then inoculated with yeast. Because the extraction was inefficient, the same mixture of grains may have been extracted repeatedly. It is not clear whether the beer was flavored, as modern beers are by the addition of hops.

The basic raw materials for the production of beer are sweet worts formed by enzymatic hydrolysis of cereal starches. The principal cereal is barley which, after malting, is also the source of enzymes that hydrolyze starches, glucans, and proteins. In some countries, eg, Germany, the mash bill consists entirely of malted barley. In other countries, adjuncts, eg, corn, rice, corn syrup, or glucose, are common. In the United States, the mash bill typically contains 60 wt% barley malt and 40 wt% corn or rice. In the United Kingdom, sugar syrups are often used.

Malt is produced by steeping barley in water for several days. This initiates germination, which activates several enzymes important in the digestive process that occurs in malt. The germinated barley is then dried and kilned at ~80°C, or higher for darker malts.

Brewers' wort is made by mashing the barley malt, or malt plus adjuncts, with water. In the infusion method, the temperature of the mash is gradually raised to 60–65°C through a number of steps. Heating is gradual, and the mash is maintained for some time at ~52°C to permit the proteolytic enzymes

to act. At the final temperature, amylases catalyze the hydrolysis of starches to maltose, oligosaccharides, and dextrins. Adjuncts are generally heated to boiling to gelatinize their starches. The boiled adjuncts are added slowly to the malt mash to raise its temperature in the desired sequence. Thus, they contain no essential enzymes that must be preserved for mashing; their starch is readily hydrolyzed by the amylases of the malt mash. Finally, the mash is filtered to separate the insoluble constituents such as barley hulls from the soluble constituents. The filtrate or sweet wort is then pumped into the brew kettles where it is boiled with hops. This inactivates the enzymes, extracts the bitter hops flavor, and sterilizes the wort.

Brewery fermentations are carried out with pure-culture yeasts. Stock cultures of brewers' yeasts may be maintained on agar-containing solid media containing malt extract, glucose and peptone, or in a lyophilized form. During the aseptic propagation of the seed yeast, there is a 10-fold increase in cells from one step to the next. Often, yeast from a preceding commercial beer fermentation is inoculated into one or several (up to 10) succeeding fermentations. The fermentations are not carried out under strictly aerobic conditions. Although some sterile air is forced through the wort, the yeasts' metabolism is not respiratory because of the high concentration of sugar.

Two pure-culture yeasts, top and bottom fermenting, are used in brewing. Bottom-fermenting yeasts, *S. pastorianus* (formerly *S. carlsbergensis*) and *S. uvarum*, are used for the production of lagers while the top-fermenting yeast, *S. cerevisiae*, is used for the production of ale. The yeasts are indistinguishable microscopically. The presence of melibiase (α-galactosidase), which permits complete fermentation of the trisaccharide raffinose, characterizes *S. uvarum* with *S. cerevisiae* only able to ferment the fructose portion. This diagnostic feature is of no practical importance for brewing, since worts contain little or no raffinose. In general, top-fermenting yeasts have stronger respiratory capacity than bottom-fermenting yeasts and are generally less flocculent.

The suitability of brewers' yeast strains depends largely on the rate and extent of growth, rate, and extent of fermentation, degree of flocculence, and influence of strains on the flavor and aroma of the beer. Fermentation rates of brewers' yeasts are of the same order of magnitude as wine, bakers', distillers' or sake yeasts. Typical activities are 600 and 700 mL of CO_2/h/g-yeast solids in glucose and maltose medium at 25°C (34), and 265 mL/h/g at 25°C in wort (35). The concentration of fermentable sugars in worts varies with the concentration of the wort and with the composition of the mash. About 90–92% of the wort solids are carbohydrates, of which 70–80% are fermentable. As a rule, a barley wort contains 0.5–1.0% glucose, 3.5–6% maltose, 1.1–1.8% maltotriose, and 0.1–0.5% sucrose and fructose (36). The transport of glucose, sucrose, and fructose into the yeast and their rate of fermentation are similar. However, the fermentation of maltose undergoes adaptation and deadaptation (although some strains are constitutive for maltose fermentation). The rate-limiting step appears to be transport of maltose across the membrane. The synthesis of the maltose transport system is repressed by glucose in the medium. Adaptation begins when the concentration of glucose in the wort decreases below 0.6%. Maltotriose fermentation takes place when the concentration of maltose falls to a low level; some strains do not ferment maltotriose and consequently produce

beers with a high concentration of the trisaccharide. Maltotetraose, isomaltose, and higher glucose polymers are not fermented.

10.1. Fermentation. Lager beer fermentations with the bottom-fermenting *S. uvarum* are carried out at 10–16°C for 6–10 days. Fermentation is followed by a lager period of up to several months at 2–5°C. Ale fermentations with the top-fermenting *S. cerevisiae* are carried out at 15–22°C for 3–5 days. The rate of fermentation is highly dependent on temperature. For example, at 25°C, the rate of fermentation is about three to four times faster than at 10°C. The pH drops from ~5.5 to ~4.3–4.5 during the course of fermentation. The rate of maltose fermentation is almost constant at pH 3.5–5.0 (although it drops sharply outside this range) and the optimum pH for maltotriose fermentation is 4.3–5.4. Thus, the rate of wort fermentation is not greatly affected by this pH drop. Fermentation and yeast growth are inhibited by high concentrations of ethanol and dissolved carbon dioxide. At ethanol concentrations of 5–6% and carbon dioxide levels of 0.4–0.5%, the inhibition is minimal. Since the alcohol content of U.S. lager beers ranges from 3.4–3.8%, and only reaches ~8.7% in British stout, any inhibition is slight. Initially, brewers' fermentations contain $5–10 \times 10^6$ yeast cells/mL, corresponding to ~0.75-g yeast solids/L. Fermentation results in a four- to eightfold increase in cell mass. The basic carbon source for this growth is carbohydrate. Some oxygen is available at the start of the fermentation, by aeration of the wort. Very little oxygen, however, is required for fermentative yeast growth. Nitrogen is supplied by the amino acids in the wort. Some amino acids, particularly asparagine, serine, threonine and lysine, are assimilated rapidly, while others are absorbed more slowly. Proline is not absorbed, and the resulting beer contains a relatively high concentration of this amino acid. Brewers' worts usually contain sufficient minerals and vitamins to support yeast growth and fermentation.

10.2. Flocculation. The exact degree of flocculence is very important for both top- and bottom-fermenting yeasts. If the yeast is too flocculent, a large portion rapidly settles out, delaying fermentation. If the yeast is too powdery, the fermentation is rapid, but the yeast does not settle out at the end of fermentation. Genes involved in flocculation have been identified; physical and chemical factors, eg, divalent cation (Ca and Mg) concentration, pH, temperature, and net cell surface charge also play a role. In some strains, flocculation is apparently affected by hair like appendages, fimbriae, on the cell wall.

10.3. Flavor and Aroma. The flavor compounds, fermentation by-products, are higher alcohols (known as fusel oils), esters, diketones, aldehydes, organic acids, and sulfur compounds. The particular yeast strain used affects the formation of higher alcohols and esters. Although the flavor compounds can be identified analytically, it is not always possible to specify their effect on flavor. Thus, the higher alcohols are individually present in concentrations below their threshold of perception, but the combination affects flavor. Their total concentration may be 50–100 ppm; ~50–66% of this consists of isoamyl alcohol, isobutanol, and propanol. Amyl and butyl alcohol are negligible. The concentration of esters in U.S. lagers is 25–50 ppm. Ethyl, isoamyl, and phenethyl acetates contribute a fruity flavor. Ethyl acetate forms from the reaction of coenzyme A with ethanol at a rate greatly dependent on temperature. Maximum formation occurs at 20–25°C, and top-fermented ales contain large amounts of this ester.

Diacetyl, acetoin, and diketones form during fermentation. Diacetyl has a pronounced effect on flavor, with a threshold of perception of 0.1–0.2 ppm; at 0.45 ppm it produces a cheesy flavor. U.S. lager beer has a very mild flavor and generally has lower concentrations of diacetyl than ale. Diacetyl probably forms from the decarboxylation of α-ethyl acetolactate to acetoin and consequent oxidation of acetoin to diacetyl. The yeast enzyme diacetyl reductase can irreversibly reduce diacetyl to acetoin. Aldehyde concentrations are usually 10–20 ppm. Their effects on flavor must be minor, since the perception threshold is ~25 ppm.

Organic acids, including carbon dioxide, lower the wort pH during fermentation. The principal acids formed are lactic, pyruvic citric, malic, and acetic acids, at concentrations ranging from 100 to 200 ppm. The main sulfur compounds formed during fermentation and their perception thresholds are as follows: H_2S (5–10 ppb); ethanethiol (5–10 ppb); DMSO (35–60 ppb); and diethyl sulfide (3–30 ppb). At low levels, these may have a desirable flavor effect; at higher levels they are extremely undesirable. Sulfur dioxide also forms during fermentation, at concentrations of 5–50 ppm; its presence can be tasted at levels >50 ppm.

Beer taste can be spoiled by contaminating bacteria or yeasts. The most common bacteria are lactic and acetic acid producers and *Zymomonas*. Wild yeasts can be anything other than the intended strain: *S. uvarum* is considered a contaminant of ale fermentations and *S. cerevisiae* a contaminant of lager fermentations. The common wild yeast contaminants are *S. diastaticus* and species of *Pichia*, *Candida*, and *Brettanomyces*. Note that the flavor of beer may be improved by the ability of yeast to adsorb bitter substances extracted from hops, eg, humulones and isohumulones.

11. Wine

The earliest known wines were made in Iran ~5400–5000 B.C. (37). The species of grape used is unknown and may have been either the wild grape *Vitis viniferus sylvestris* or a cultivated precursor of the modern wine grape *V. viniferus viniferus*. The source of the yeast used, and the procedures used are completely unknown. In modern times, grapes (~21–23% sugar) are pressed; the liquid must is either separated and allowed to settle for 1–2 days (for white wines) before inoculation with yeast, or the whole mass is directly inoculated with yeast (for red wines). In either case, while the initial fermentation takes place, the carbon dioxide formed by fermentation excludes air and prevents oxidation. White wines are transferred to a second fermentor (racked) near the end of fermentation and kept isolated from the air while solids, including yeast, settle out, a process that requires about six weeks. Additional rackings, filtration or centrifugation may be required to clarify the wine and remove tartrates prior to bottling.

Red wines are fermented in the presence of the grape skins to extract pigments and tannins, until 70–90% of the sugar has been fermented, and then pressed. The juice undergoes a residual fermentation to consume the remaining sugar. At this point the wine is racked to separate the yeast; a second, bacterial malolactic fermentation, which transforms malic acid to lactic acid,

occurs at this stage and reduces the acidity of the wine. Red wines may be aged in wooden casks to improve flavor and precipitate tannins and tartrates. Additional filtrations or centrifugations may be employed before bottling to clarify the wine.

Addition of up to 200 ppm sulfur dioxide to grape musts is customary. Strains of *S. cerevisiae* and *S. bayanus*, grown in the presence of sulfite, become tolerant of fairly high concentrations of SO_2. Cultures propagated in the winery are added in liquid suspension, usually at 1–2% of the must volume. Many strains are available in pure culture. Factors, eg, flocculence, lack of foaming, fast fermentation, lack of H_2S and SO_2 formation, resistance to sulfur dioxide and other inhibitors, and flavor production, will affect strain choice. No strain possesses all the desired properties.

Pure culture yeasts are available on slants or in lyophilized form. They may be propagated in wineries without complete asepsis (38,39), since the low pH of grape must inhibits many bacteria; wine fermentations are mixed cultures naturally. Production of active dry wine yeasts (WADY) began in the 1960s. These are produced by bakers' yeast companies and are grown by methods resembling those used for bakers' yeast production, described earlier. The dry yeasts have excellent storage stability, up to a year or more if packaged under an inert atmosphere (N_2, CO_2, or vacuum). First introduced into the United States and then Australia, they are now being introduced into European winemaking as well. A number of strains of *S. cerevisiae*, *S. bayanus*, and *Torulaspora delbrueckii* (formerly *S. fermentati*) are available.

In addition to strains of *S. cerevisiae*, the term wine yeast refers to genera that occur naturally in grape musts and participate in spontaneous fermentation in wine. The number of yeast cells on intact young grape berries is not large. However, it increases as the grapes mature and there are many on injured grapes leaking juice. Essentially none of these is *S. cerevisiae*, and most yeasts that take part in spontaneous wine fermentations derive from cells adhering to wine-making equipment, eg, crushers and presses. Generally, the early fermentation is dominated by *Kloeckera apiculata*, *Metschnikova pulcherrima*, and *Torulopsis stellata*. *Torulaspora delbruekii* (formerly *S. rosei*) appears between the start and the main fermentation, which is dominated by *S. cerevisiae* and *S. uvarum*. *Saccharomyces cerevisiae* and *S. bayanus* also dominate the final stages. The succession of species described here depends largely on their alcohol tolerance; less tolerant species disappear as the alcohol content increases.

Spontaneous fermentations are used for wine production in France, some other European countries, South America and in some wineries in Australia. In recent years, smaller California wineries have begun experimentation with spontaneous fermentations as well. They generally start more slowly than fermentations inoculated with commercial dried yeast, are more difficult to control, and may suffer from growth of undesirable contaminants. However, it is claimed that the resulting wines possess better organoleptic properties, particularly more complex flavor and aroma.

A number of different species dominate the fermentation at different times as listed below. *Saccharomyces* is dominant only toward the end. *Kloeckera apiculata*, with cell dimensions of $(2–4.5) \times (5–8)$ μm, usually dominates the early stages, along with *Metschnikova pulcherrima* and *Torulopsis stellata*. It stops fermenting and growing at ethanol concentrations of 4–5%. Cells of

K. corticus and *K. africana* may also be observed. *Kloeckera* is the imperfect form of *Hanseniaspora* and does not form ascospores. The small cells are pointed or lemon-shaped and show bilateral budding. *M. pulcherrima* (*Candida pulcherrima*) propagates by multilateral budding and forms large asci with needle-shaped ascospores. Growth is inhibited at 4% alcohol as well. *Torulopsis stellata* (*T. bacillaris*) is an imperfect fungus whose cells are ellipsoidal. It propagates by multilateral budding and its alcohol-producing ability varies. Of the *Pichia* yeasts, the predominant is *P. membranaefaciens*, a film-forming yeast. It forms a continuous film on the surface of young wines. Strictly aerobic, it does not produce ethanol, but tolerates high concentrations. The cells are ellipsoid to cylindrical (2.5–4.5) μm × (5–14) μm. Vegetative reproduction is by multilateral budding; 2–4 sexual spores are formed. *Hansenula* yeasts propagate vegetatively by multilateral budding and form 2–4 hat- or saturn-shaped ascospores. Cells are (2.5–5) μm × (5.5–20) μm. Some species may produce up to 10% ethanol as well as ethyl acetate and other esters. *Candida krusei*, *C. vini*, and *C. valida* are film-forming wine spoilage yeasts. *Saccharomycodes ludwigii*, the only species in its genus, is another wine spoilage yeast and is highly resistant to sulfur dioxide. The cells are lemon-shaped (5–8) μm × (10–30) μm, and four spherical ascospores may be formed. *Schizosaccharomyces* species are spore-forming yeasts that divide by fission rather than budding. *Schizosaccharomyces pombe* is known for its ability to degrade malic acid to ethanol and carbon dioxide under anaerobic conditions. Although not often found in spontaneous fermentations, it has been added to fermentations as a pure-culture yeast. *Brettanomyces* cells are ovoid or pointed at one end. *B. intermedius* is a wine-spoilage yeast. During fermentation a strong characteristic odor is observed, and under aerobic conditions, large concentrations of acetic acid are produced. It can tolerate up to 12% ethanol, but is very sensitive to sulfur dioxide.

Saccharomyces yeasts are rapid fermentors. *Saccharomyces cerevisiae* and *S. bayanus* produce up to 18–20% ethanol (40). The cells are ovoid to spherical, elliptical, or elongated (especially under conditions of nitrogen starvation). Vegetative propagation is by multilateral budding. *Saccharomyces uvarum* and *Torulaspora delbruekii* occur earlier in the fermentation, when *Torulaspora delbruekii* may produce up to 6–8% ethanol before being overgrown by the other *Saccharomyces* yeasts.

12. Distilled Beverages

Distilled alcoholic beverages are made by fermenting sugars from grains or fruits followed by distillation. The common cereal grains used for whiskey are barley, wheat, corn, or rye. Vodka is made from potatoes, rum from molasses, and brandy from grape or other fruits. Grain or potato starch must be converted to a fermentable form before use. Except for some Scotch or Irish whiskeys, whole mashes of grains are fermented. That is, fermentation and conversion of starch to fermentable sugar proceed simultaneously. The cereal grains, except for barley malt, are cooked in water to gelatinize the starch. The water temperature, in pressurized vessels, may exceed 100°C. After cooling the mash to ~65°C, a slurry of malted barley is added to ~5–10% of the total mash. Amylases in the malt

convert the starch to fermentable sugars. (Although fungal amylase is sometimes used for this purpose, the barley malt also provides some flavor to the whiskey.) The mash is cooled to 25–25°C before inoculation with yeast.

Distillers' yeasts are specialized strains. Many distillers maintain their own proprietary yeasts or have them grown by commercial yeast producers. Commercial bakers' yeasts may be used for the production of neutral grain spirits. The yeast is propagated on cooled mash before inoculation into the final mash at 3–5 vol%. Sometimes the inoculum is a portion of an actively fermenting mash (backstocking). In the United Kingdom, residual brewers' yeast is sometimes used. Distillers' fermentations are generally carried out at pH 4.8–5.0, and at an initial temperature sufficiently low that the final temperature does not exceed 30°C. Fermentation time is 2–5 days; the latter stages are very slow because after 30 h the limiting factor is starch conversion (41). Alcohol concentrations at the end of fermentation are ∼7–9%.

Lactic acid bacteria are common contaminants of distillers' fermentations. *L. lactis* may produce excessive amounts of volatile acids. Some species convert glycerol to β-propionaldehyde, which may break down to acrolein during distillation, producing an acrid odor.

Fermentation of fruit juices other than grapes, principally apple juice, is the same as that in the production of grape wine. For the production of rum, cane molasses is diluted to a sugar concentration of 15–29%; the sucrose, glucose, and fructose is completely fermented within 36 h at 30–32°C.

12.1. Sake. Production of Japanese rice wine begins with the preparation of a culture of *Aspergillis oryzae* (42) by inoculating steamed polished rice. The mold produces extracellular amylases and proteases that act on the rice, liberating glucose and amino acids. This koji is inoculated into a mixture of water and additional steamed rice that is allowed to ferment for a period of ∼10 days (the moto). At this time, it may be inoculated with a culture of sake yeast, *Saccharomyces sake*, a specialized subspecies of *S. cerevisiae*. During this initial period, continued growth of *Aspergillis* and the action of *lactobacilli* produce a solution high in glucose (26–28%) and organic acids, which favors the growth of the sake yeast. After ∼2 weeks of yeast fermentation, there are three successive additions of koji, steamed rice, and water to the moto. A final fermentation of 3 weeks following the last addition produces fresh sake, with an alcohol content of 18–21% and a significant accumulation of succinic, lactic, and malic acids. This is filtered, held 1 month to allow settling of the remaining solids, pasteurized, and bottled. It is frequently diluted to an alcohol content of 15–16%.

Although *S. sake* is biologically a strain of *S. cerevisiae*, it differs in several important aspects from Western strains. Sake yeasts will grow at relatively high level of lactic acid (>2%), and excrete succinic acid while fermenting. They continue to ferment at alcohol levels much higher than tolerated by other yeasts and will grow, albeit very slowly, at the very high osmotic pressures found early in the moto stage; some strains will continue to grow at sugar concentrations of 45% (43).

12.2. Soy Sauce (Shoyu). Production of soy sauce is a two-step process in which a mixture of boiled soybeans and crushed roasted wheat is first inoculated with 1–2% of a culture of *Aspergillis oryzae* or *A. soyae* previously grown on polished rice (44). The mixture is allowed to ferment 2–3 days at 30°C and then

transferred to large vessels along with an approximately equal volume of 18–19% brine. The subsequent fermentation, which traditionally lasts 2–3 years, is first dominated by lactic acid-producing bacteria including species of *Pediococcus*, *Bacillus*, and *Lactobacillus*. As the pH of the fermenting mash (moroni) drops owing to acid produced by the bacteria, a yeast fermentation begins, dominated by strains of *Zygosaccharomyces rouxii* (formerly *Saccharomyces rouxii*) tolerant of high salt and osmotic pressure. Proper flavor development, however, requires the presence of species of *Torulopsis* as well.

The function of the *Aspergillis* fermentation appears to be primarily the breakdown of protein and polysaccharides by secreted proteases and amylases. Replacement of *Aspergillis* by chemical or enzymatic hydrolysis has no major impact on the organoleptic properties of the sauce. Likewise, inoculation with a pure culture of *Lactobacillus delbrueckii* to carry out the acetic acid fermentation produces a normal product. The *Z. rouxii* and *Torulopsis* yeasts, however, are specifically required for proper flavor development.

13. Microbial Biomass and Single-Cell Protein

In all fermented foods, microbes contribute as preservatives, ie, by lowering the pH and producing ethanol, or by making the food more palatable. The deliberate use of yeasts as food in themselves is less common. Small beer, the sediment from beer, has been traditionally used as a vitamin supplement for infants. Beginning in 1910, dried, spent brewers' yeast was developed as a food, and *Candida utilis* was used as a food supplement in Germany during World War II.

In the 1960s and 1970s, the world protein shortage stimulated the development of additional processes for producing microbial biomass both from traditional substrates, eg, carbohydrates, and from alternative materials, eg, *n*-paraffins, ethanol, and methanol. Generally, industrial processes for biomass production use yeast cultures, although methanol-based aerobic processes for *Pseudomonas methylotropha* (in the United Kingdom) and *Paecilomyces varioti* (in Finland) are also known. Yeasts are preferred both because of their relatively large size compared to bacteria, which permits recovery by centrifugation or filtering more easily, and because of their general acceptance of safety by the food industry. Historically the carbon source for biomass production is fermentable sugars. Because of their low cost and contribution of some nitrogen, minerals and vitamins, beet, and cane molasses are preferred feedstocks. Starch from rice, potatoes, corn, or cassava must be hydrolyzed to produce fermentable sugars. Likewise, cellulose may be used as a carbon source only after acid or enzymatic hydrolysis to glucose. Sulfite waste liquor, derived from wood pulp manufacture, lactose-containing whey permeate from cheese-making, ethanol, and methanol are also employed. In addition to these carbon sources, yeasts require large supplements of nitrogen, phosphorus, potassium, calcium, and magnesium, as well as trace minerals. Vitamin requirements of different yeasts vary. Bakers' yeast, eg, requires biotin (usually supplied by cane molasses), while *C. utilis* does not.

Like bakers' and brewers' yeasts, yeast for biomass need not be produced under conditions of complete sterility. Most fermentations are carried out at a

pH > 4.5, which limits bacterial growth. The most common contaminants are lactic- and acetic acid-producing bacteria. As in the production of bakers' yeast, processes designed for yeast growth are highly aerobic to maximize biomass production and require heat removal. The temperature is typically held at 30°C. The supply of oxygen is also costly, since oxygen transfer rates from air to liquid are low. In many fermentations, the rate of aeration is one volume of air per volume of fermentor liquid per minute. Such a high rate lowers the density of the gas-containing liquid and favors the production of foam, restricting the useful liquid volume of the fermentor.

Continuous processes may be used for the production of yeast biomass. Raw liquid feed is added continuously to the fermentor and an equal volume of fermentor liquid is removed to harvest the yeast cells. These may be a single homogeneous fermentation in a stirred fermentor or two fermentors in series. Growth rates are high: a typical dilution rate in the production of *C. utilis* on sulfite waste liquor is 0.25, ie, one-fourth of the fermentor volume is harvested hourly.

Thus, microbial biomass may be produced either as a by-product of a food fermentation (eg, brewers' yeast) or specifically as a food or feed supplement. In either case, its main importance is in its contribution as a protein supplement. Yeast solids contain ~7.5–9% nitrogen. Protein is crudely calculated as $N \times 6.25$ or ~45–60%, but this includes 6–12% nucleic acids, primarily RNA. A less important contribution consists of the vitamin and mineral content. Also present are 5–9% ash and 2–6% lipids (45). The essential amino acid contents of dry yeasts are quite similar regardless of the species. Lysine content is high, and sulfur-containing amino acids, eg, methionine, are low. The protein efficiency ratio (PER: the ratio of weight gain to weight fed for young rats) of bakers' yeast is 2.02; supplementation with 0.16% D,L-methionine increases this to 2.27, and with 0.5% D,L-methionine to 2.77. Yeast protein purified from most nucleic acids has a PER of 2.2. By comparison, the PER for casein is 2.5.

Brewers' and bakers' dried yeasts are used as dietary supplements. They contribute some protein and trace minerals, and some B vitamins, but no vitamin C, vitamin B12, or fat-soluble vitamins. They also provide the health food industry with glucose tolerance factor (GTF) which is claimed to potentiate the effect of insulin and be important for older people who cannot synthesize GTF from inorganic dietary chromium.

The most widely available yeast biomass is a by-product of the brewing industry, where the multiplication of yeast during brewing results in a surplus of cells. For every barrel (117 L) of beer brewed, 0.2–0.3 kg of yeast solids may be recovered. In the United States, a substantial fraction is recovered and made available: $\sim 40 \times 10^6$ kg of brewers' yeast annually. The yeast is recovered from beer by centrifuging and dried on roller drums or spray dryers and sold as animal feed or a pet food supplement. It can be debittered by alkaline extraction to remove the bitter hop residues, and is then sold mainly by the health food industry. It is available as tablets, powder, or flakes and is often fortified with additional vitamins. Distillers' yeast cannot be readily separated from the fermented mash and the mixture is sold as an animal feed supplement.

Bakers' inactive dry yeast is also widely used in the food industry. This yeast may be grown specifically as a food supplement and consequently there is a choice to engineer its composition by varying growth conditions and growth

substrates. It can possibly produce high levels of nicotinic acid and thiamine, the crude protein content can be raised to 50–55% and it can be used as a vehicle for the incorporation of micronutrients such as selenium or chromium into the diet.

Candida utilis is grown on sulfite waste liquor in western Europe and North America, on sugar cane molasses in Cuba and Taiwan and on cellulose acid hydrolysates in eastern Europe and the former Soviet Union. *Candida utilis* utilizes hexoses, pentoses, and many organic acids. Sulfite liquor from hardwoods contains 2–3% fermentable sugars of which 20% are hexoses and 80% pentoses; in softwood liquors the proportions are reversed. The SO_2 must be stripped out to allow yeast growth, which is carried out in large, highly-aerated fermentors. For continuous fermentations, carried out at pH 4 and 30°C, the dilution rate is 0.27–0.30 (46).

Cheese whey solids contain 70–75% lactose, which can serve as the carbon source for lactose fermenting yeasts, eg, *Kluyveromyces fragilis*. The total volume produced commercially is considerably smaller than for the other yeasts described.

Both *n*-alkanes and gas oil can be used as carbon and energy sources. Commercially, *Candida tropicalis* and *C. lipolytica* have been used (47,48). The fermentation contains two immiscible liquid phases (the alkane and the water); the semisolid yeast; and the gaseous air phase. In contrast to yeasts grown on carbohydrates, where maximum yields are 50%, yeasts grown on alkanes generally give yields of 95–105% based on the weight of the alkane. Cells can be recovered readily by centrifugation but if grown on gas oil must be extracted to remove the residual hydrocarbon. Although some plants operated during the 1970s using alkanes as the substrate, the steep rise in oil prices and regulatory problems have largely stopped this production.

The genera *Hansenula*, *Candida*, *Pichia*, *Torulopsis*, and *Trichoderma* contain facultative methylotrophs. Such yeasts will grow on methanol, carbohydrates, or organic acids. Ethanol may be used for biomass production of some species of the genera *Candida*, *Debaromyces*, *Endomycopsis*, *Hansenula*, *Mycoderma*, *Pichia*, *Rhodotroula*, and *Saccharomyces*. In the United States, *C. utilis* is grown on ethanol, and in Finland, bakers' yeast is grown on a combination of ethanol and molasses.

One of the most promising substrates for future production of microbial biomass is the cellulose contained in agricultural residues, eg, wood pulp, sawdust, feed-lot waste, corn stover, rice hulls, nut shells, and bagasse, all of which contain cellulose as the principal carbon source. Cellulose contents range from 90% in cotton to 15–20% in dicotyledon leaves. Wood residues and grasses contain mixtures of cellulose, hemicellulose, and lignin. The major impediment preventing commercial use is the difficulty in converting cellulose to glucose by enzymatic or acid treatment. As practiced in eastern Europe, acid hydrolysis is costly because the acid must be neutralized and the resulting salts disposed of. Enzymatic hydrolysis with cellulase and cellobiase is under intensive research. The most commonly used enzymes are derived from *Trichoderma reesei*. In many instances, 50% of the cellulose can be converted, and higher conversion rates can be achieved by pretreatment of the cellulose with alkali, amines, or ammonia, or by mechanical comminution. Newsprint is an attractive raw material for the production of glucose syrup, ethanol, or biomass from the glucose present, but

although progress in this area is continuing, these materials are not economical compared with the traditional substrates.

Recovery of biomass from the fermentor requires centrifugation or filtering, washing, concentration, and drying. Yeast grown on sulfite waste liquor contains lignosulfonic acids if not washed. Although suitable for agricultural feed as is, extensive washing is required to produce yeast suitable for food use.

Extraction of protein requires breaking the cell wall to release the cytoplasmic contents. This can be achieved by high speed ball or colloid mills or by high pressure (50–60 MPa) extrusion. Protein is extracted by alkaline treatment followed by precipitation after enzymatic hydrolysis of nucleic acids. Although the protein can be spun into fibers or texturized, such products are more expensive than those derived from soybean and there is no market for them.

The presence of nucleic acids in yeast is one of the main problems with their use in human foods. Other animals metabolize uric acid to allantoin, which is excreted in the urine. Purines ingested by humans and some other primates are metabolized to uric acid, which may precipitate out in tissue to cause gout (49). The daily human diet should contain no more than ~2 g of nucleic acid, which limits yeast intake to a maximum of 20 g. Thus, the use of higher concentrations of yeast protein in human food requires removal of the nucleic acids. Unfortunately, yields of protein from extracts treated as described are low, and the cost of the protein may more than double.

Other chemicals of possible concern for health and safety found in yeast proteins include tyramine (0–2.25 mg/g) and histamine (0.2–2.8 mg/g), formed by decarboxylation of the corresponding amino acids (50). These compounds are also found in other fermented (including pickled) foods. Their presence in yeast extracts used as condiments contributes very little to human intake. Likewise, the nephrotoxic compound lysinoalanine has been identified in alkali-treated yeast extracts, at a level of 0.12 mg/g. However, the chemical occurs at similar low concentrations in almost all heat- and alkali-treated foods.

13.1. Other Food Uses. Inactive dried yeasts are used as ingredients in many formulated foods: baby foods, soups, gravies, and meat extenders; as carriers of spice and smoke flavors; and in baked goods. Yeasts used in the health food industry are generally fortified with minerals and contain higher concentrations of the B vitamins, especially thiamine, riboflavin, and niacin.

Dried yeasts are used extensively in animal feeds. Most distillers' and brewers' yeasts are incorporated by codrying with the spent grains. Approximately 15,000 t of brewers' yeast is dried for this purpose. The main use is in rations for monogastric animals. Active (ie, live) dry yeast is incorporated into feed for ruminants and is believed to aid digestion. Diets supplemented with these yeasts are also claimed to lead to increases in weight gain and productivity. Appreciable amounts of brewers' yeast and torula (*C. utilis*) are also used in pet foods, principally for dogs and cats, but also in feeds for birds, fish, mink, and bees.

14. Yeast-Derived Products

14.1. Enzymes. Invertase (β-fructofuranosidase) is commercially produced from *S. cerevisiae* or *S. uvarum*. The enzyme, a glycoprotein, is not

excreted, but transported to the cell wall. It is, therefore, isolated by subjecting the cells to autolysis followed by filtration and precipitation with either ethanol or isopropal alcohol. The commercial product is available dry or in the form of a solution containing 50% glycerol as a stabilizer. The main uses are in sucrose hydrolysis in high test molasses and in the production of cream-centered candies.

Lactase (β-galactosidase) is produced commercially from the lactose fermenting *Kluyveromyces fragilis*. The enzyme has a pH optimum of 6–7 and is used in the hydrolysis of lactose in milk or skim milk.

14.2. Yeast Extracts. Autolysis in yeast cells is induced by raising the temperature of a cell suspension to 44–55°C; at which temperature, the yeast cells die, but their hydrolytic enzymes remain active. During this process, proteins, carbohydrates, and nucleic acids are hydrolyzed and solubilized. Commercial yeast autolyses generally last >10 h. At the end of autolysis, the insoluble cell wall materials can be separated from the solubilized products by centrifugation. The resulting extract is evaporated to a paste of ~75% solids or spray dried to a powder. The powder contains 50–80% oligopeptides and amino acids; amino nitrogen may account for 25–45% of the total nitrogen, with the rest consisting mainly of nucleic acids.

Commercially available yeast extracts are made from brewers' yeast, from bakers' yeast, from alcohol-grown yeast (*C. utilis*) and from whey grown yeast (*K. fragilis*). Extracts are used in fermentation media for production of antibiotics, in cheese starter cultures, and in the production of vinegar. They are also extensively used in the food industry as condiments to provide savory flavors for soups, gravies and bouillon cubes, and as flavor intensifiers in cheese products.

The nucleotides inosine-5′-monophosphate and guanosine-5′-monophosphate, produced from yeast RNA are potent flavor potentiators for meat products. They act synergistically with monosodium glutamate and are usually used in conjunction with this amino acid.

Food Preservation and Food Spoilage. Many foods, eg, alcoholic beverages, pickles, cheese, and fish sauce, are preserved by fermentation. Spontaneous fermentations by mixed populations of yeasts and bacteria are normally involved. Preservation results from a lowering of pH or the formation of ethanol. Yeasts do not produce antibiotics, although isolates of a number of species produce a toxin (killer factor) lethal to other yeasts.

Yeasts also act as spoilage organisms. Jams, jellies, and honey can be fermented by osmophilic yeasts such as *S. mellis* or *Z. rouxii*. Wild yeasts may also spoil wines or beers. Film-forming yeasts, eg, *P. membranaefaciens*, may grow on the surface of sauerkraut, pickles, or other fermented vegetables. *Kluyveromyces fragilis* or other lactose-fermenting yeasts occur in milk products. *C. lipolytica* may occasionally spoil butter or margarine.

Pathogenic Yeasts. Few yeasts are pathogenic to healthy individuals, but immunocompromised people can suffer from infections from a number of normally innocuous yeasts. *Candida albicans* is the best known of the pathogenic species. It may cause infections of the skin or of the mucous membranes, eg, in the oral cavity (thrush), intestinal tract, and vagina. Occasionally, skin infections may be caused by other species of *Candida*, eg, *C. tropicalis*. *Cryptococcus neoformans* may cause serious infections in a number of organs, particularly the

meninges, with sometimes fatal results. Some fungi grow as yeasts at body temperature in laboratory cultures, but grow as mycelia at lower temperatures. Of these, *Histoplasma capsulatum* infects the lungs and may spread to other parts of the body: this systemic disease, histoplasmosis, can be fatal.

15. Outlook

Back when the early pioneers of biochemistry, biology, microbiology, and genetics were laying the foundations of these disciplines they did not know what they would find or where the many paths might lead; they were simply curious. However, even then yeast had become an equivalent to the model prokaryote, *E. coli*. This was partly because it was like *E. coli* in being a small, easily manipulated, simple unicellular organism, and also because containing internal membrane bound organelles (nucleus, mitochondria, etc) defining it as a eukaryotic organism. Additionally, what made yeast attractive apart from its many products, were the processes required to produce these products from yeast and that yeast had already been "domesticated," "industrialized," and "commercialized" for the greater part of >6 millenia.

Then as now yeast leads the way among the eukaryotes and it was the first eukaryote to have its genome completely sequenced. Although we still do not know where and what the outcomes will be, the outlook for yeast remains bright. The development of various omics (the new ologies) and the ability to look at more than the actions of a single gene or gene product at a single point in real time for a single gene at one time, or the outcomes of a single mutation in that gene has now been improved to the point where the interplay of every single gene under any circumstances has made it possible that a single experiment may produce enough data for a lifetime's study.

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