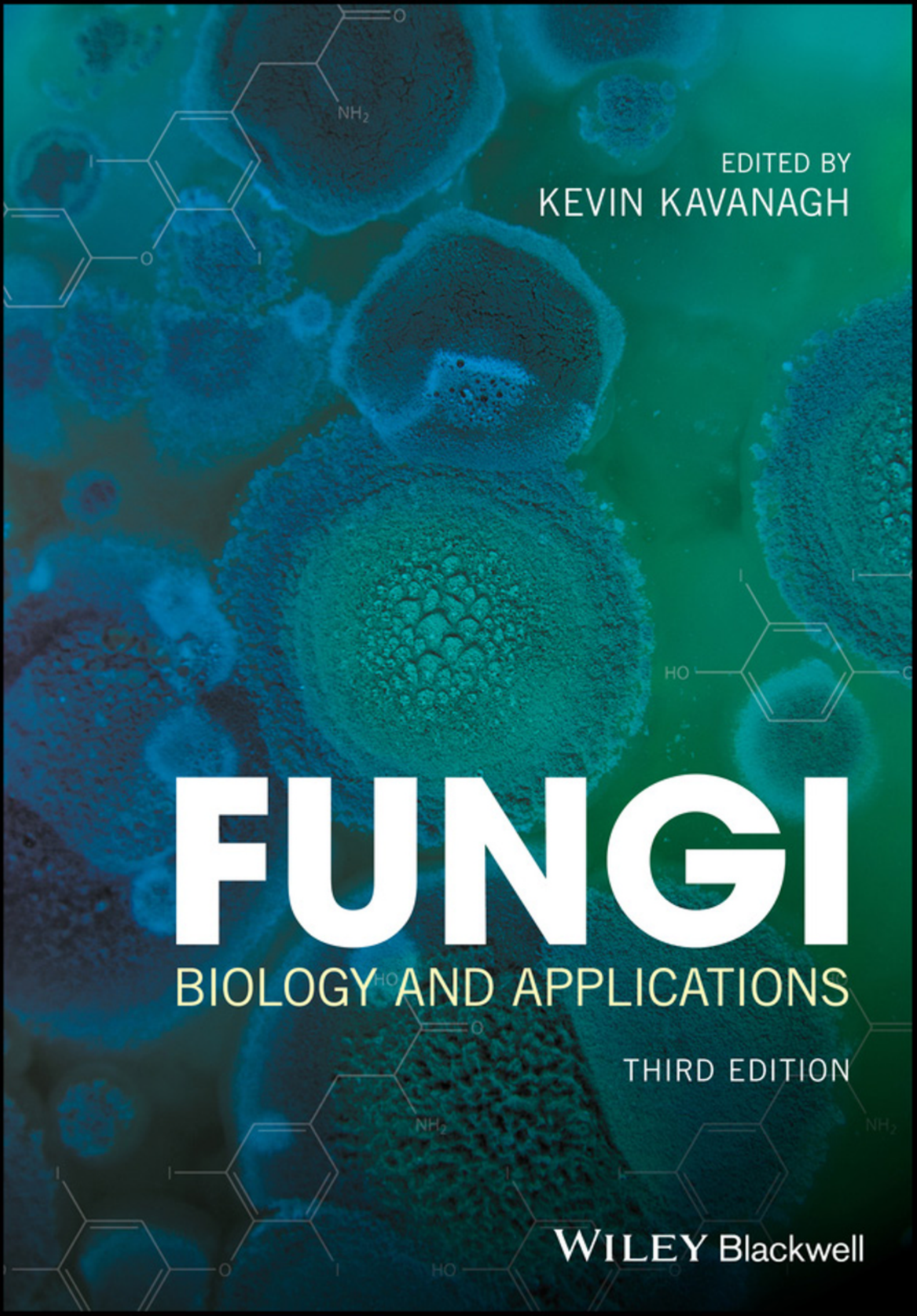
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Fungi

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Biology and Applications

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Preface

Fungi make an enormous contribution to our life. The role of yeast in the production of alcohol and bread is well characterized. We consume fungi directly in the form of edible mushrooms and in "blue cheeses" which get their characteristic flavor and aroma from the presence of fungi. Fungi are also used for the production of antibiotics, such as penicillin, and enzymes for use in the food industry. Since the 1990s, fungi have been utilized for the production of recombinant proteins, some of which have great therapeutic potential. Although infrequently recognized as important decomposers of organic detritus, fungi play a significant role in degrading biological matter, such as fallen leaves. On a more negative note, some fungi (for example members of the genus *Candida* and *Aspergillus*) are capable of causing serious life-threatening infections in immunocompromised patients, and other fungi can be serious plant pathogens.

This is the third edition of *Fungi: Biology and Applications* which was first published in 2005. Since that date there have been enormous strides in our understanding of the biology of fungi, and their contribution to our life is becoming increasingly important. The aim of the current edition is to provide a detailed description of the biology, biotechnological applications, and medical significance of fungi. The book commences with an in-depth description of the physiology of fungi in which the structure, metabolism, and growth of fungi are described. This is followed by a chapter dedicated to the genetics of fungi in which the lifecycles of a number of representative fungi are described and the use of fungi for genetic analysis is outlined. The advent of genomics and proteomics has revolutionized our study of the cell. Chapters 3, 4, and 5 describe how genomics, transcriptomics, and proteomics, respectively, have increased our knowledge of fungi and made available new opportunities for exploiting fungi for the good of humanity. Chapter 6 describes the importance of fungi as food and highlights the different techniques for the commercial production of edible fungi. Chapters 7 and 8 describe how fungi can be utilized for producing commercially important antibiotics, enzymes, and a range of chemical

products such as citric acid. Chapter 9 focuses on the exploitation of fungi for the production of heterologous proteins and illustrates how yeast has been used for the production of hepatitis B antigens. Chapter 10 describes the main fungal pathogens of humans and Chapter 11 outlines the human immune response to fungi that restricts infection. Chapter 12 describes the main classes of antifungal drugs and their modes of action. Chapter 13 outlines the role of fungi in the environment where they play a significant role in recycling nutrients. Chapter 14 describes the main fungal pathogens of plants and assesses the impact of such pathogens on the global supply of food.

This book gives a comprehensive introduction to fungi in terms of their biology, genetics, medical significance, and biotechnological potential. Each chapter is written by internationally recognized experts, so the reader is given an up-to-date and detailed account of our knowledge of the biology and various applications of fungi.

Kevin Kavanagh

1

Introduction to Fungal Physiology

Graeme M. Walker and Nia A. White

1.1 Introduction

Fungal physiology refers to the nutrition, metabolism, growth, reproduction, and death of fungal cells. It also generally relates to interaction of fungi with their biotic and abiotic surroundings, including cellular responses to environmental stress. The physiology of fungal cells impacts significantly on the environment, industrial processes, and human health. In relation to ecological aspects, the biogeochemical cycling of carbon in nature would not be possible without the participation of fungi acting as primary decomposers of organic material. Furthermore, in agricultural operations fungi play important roles as mutualistic symbionts, pathogens, and saprophytes, where they mobilize nutrients and affect the physicochemical environment, or can be exploited as agents of biocontrol or as biofertilizers. Fungal metabolism is also responsible for the detoxification of organic pollutants and for bioremediating heavy metals and other recalcitrant chemicals in the environment (including wastewaters and groundwaters). The production of many economically important industrial commodities relies on the exploitation of yeast and fungal metabolism and these include such diverse products as whole foods, food additives, fermented beverages, antibiotics, probiotics, pigments, pharmaceuticals, biofuels, enzymes, vitamins, organic and fatty acids, and sterols. More negatively, fungi can cause considerable disease, spoilage, and decay of important artefacts, commodities, and materials, buildings, and of course food supplies.

In terms of human health, some yeasts and fungi represent major opportunistic life-threatening pathogens, while others are life-savers as they provide antimicrobial and chemotherapeutic agents. In modern biotechnology, several yeast

Fungi: Biology and Applications, Third Edition. Edited by Kevin Kavanagh.

species are being exploited as hosts for the expression of human therapeutic proteins following recombinant DNA and gene editing technologies (see Chapter 9). Recently, the application of gene editing using CRISPR/Cas is leading to a revolution in fungal genetic engineering (see Chapter 2). Furthermore, an international synthetic biology research consortium, called Sc-2.0, has embarked on the construction of a completely synthetic version of *Saccharomyces cerevisiae*. This would represent the world's first fully synthetic eukaryotic genome! In addition to the direct industrial exploitation of yeasts and fungi, it is important to note that these organisms, most notably the yeast *S. cerevisiae*, play increasingly significant roles as model eukaryotic cells in furthering our fundamental knowledge of biological and biomedical science. This is especially the case now that numerous fungal genomes have been completely sequenced and the information gleaned from fungal genomics and proteomics is providing valuable insight into human genetics and heritable disorders. However, knowledge of cell physiology is essential if the functions of many of the currently unknown fungal genes, including "synthetic" ones, are to be fully elucidated.

It is apparent, therefore, that fungi are important organisms for human society, health, and well-being, and that studies of fungal physiology are very pertinent to our understanding, control, and exploitation of this group of microorganisms. This chapter describes some basic aspects of fungal cell physiology, focusing primarily on nutrition, growth, and metabolism in unicellular yeasts and filamentous fungi.

1.2 Morphology of Yeasts and Fungi

There are a diversity of yeast and fungal cellular morphologies. Most higher fungi are filamentous, yeasts grow as unicells, and some primitive fungi such as the Chytridomycota grow as individual rounded cells or dichotomous branched chains of cells with root-like rhizoids for attachment to a nutrient resource. Here we consider the most common growth forms, the filamentous fungi and unicellular yeasts.

1.2.1 Filamentous Fungi

The gross morphologies of macrofungi and microfungi are varied and often apparent throughout the environment (Plate 1.1). For example, we can easily recognize a variety of mushrooms and toadstools, the sexual fruiting bodies of certain macrofungi (the higher fungi Ascomycota and Basidiomycota and related forms), during a walk through pasture or woodland. Microfungi (the molds) are also diverse and are often observed on decaying foods and detritus, whereas many, including the colored rusts, smuts, and mildews, are common plant pathogens. Closer inspection of these visible structures, however, reveals that all are

composed of aggregated long, branching threads termed hyphae (singular: hypha), organized to support spores for reproduction and dissemination. The hyphae of these aerial structures extend and branch within the supporting substratum as a network, termed a mycelium, from which the apically growing hyphae seek out, exploit, and translocate available nutrients. Apically growing hyphae usually have a relatively constant diameter ranging from 1 to 30 μm or more, depending on fungal species and growth conditions.

Filamentous fungi may be cultivated within the laboratory on a variety of different liquid or solid media. On agar, the radially expanding colonial growth form of the fungal mycelium is most evident, extending from an inoculum, on, within, and sometimes above the substrate, forming a near spherical three-dimensional (3-D) colony. This radiating, circular pattern is also visible during the growth of fairy ring fungi in grassland and as ringworm infections of the skin (Plate 1.1, parts a and b).

The hyphae of individual fungi may (theoretically) extend endlessly via apical growth, provided they are supported with appropriate nutrients and other environmental conditions. Eucarpic fungi are therefore spatially and temporally indeterminate organisms, and, unlike animal, plant, and other microbial individuals, have no predetermined maximum size or age. The mycelium is not, however, simply a homogeneously extending entity, but displays considerable developmental plasticity. Different interconnected regions of the fungal mycelium may grow, branch, anastomose (fuse), age, die, sporulate, and display varying physiological and biochemical activities at different times or even simultaneously, depending on local micro-environmental conditions. Thus, colonies growing on relatively homogeneous media may be pigmented, exhibit different morphological sectors, produce aerial structures, grow as fast-effuse or slow-dense forms, and even exhibit rhythmic growth.

As well as reproductive structures and substrate mycelium, certain higher fungi, most notably the basidiomycetes, when growing within an environment where nutrients are distributed heterogeneously, can differentiate into long string-like structures called rhizomorphs or cords. These linear organs have evolved to rapidly explore for, connect, and translocate water and nutrients between patches of resource (e.g. pieces of fallen timber on the forest floor or from tree root to tree root). Accordingly, many, particularly mature rhizomorphs, contain internal vessel hyphae which possess a wide diameter, forming a channel running along the organ. The peripheral hyphae are often closely packed and melanized for insulation (Plate 1.1, parts l and m).

Filamentous fungi and yeasts are simply different styles of fungal growth suitable for occupation of different habitats and produced by differing cell growth polarities. Many species termed dimorphic fungi can adopt either the hyphal or unicellular yeast forms according to environmental circumstances. For example, certain important human and animal pathogens exist as yeast forms mobilized in body fluids but are able to form hyphae or pseudohyphae for tissue invasion.

1.2.2 Yeasts

Yeasts are unicellular (mostly ascomycete, basidiomycete, or members of the deuteromycete group) fungi that divide asexually by budding or fission and whose individual cell size can vary widely from 2–3 μm to 20–50 μm in length and 1–10 μm in width. *Saccharomyces cerevisiae*, commonly referred to as brewer's or baker's yeast, is generally ellipsoid in shape with a large diameter of 5–10 μm and a small diameter of around 5 μm (Figure 1.1). There is great diversity in cell shapes and modes of cellular reproduction in the yeasts, as summarized in Table 1.1.

The morphology of agar-grown yeasts shows great diversity in terms of color, texture, and geometry (peripheries, contours) of giant colonies. Several yeasts are pigmented and the following colors may be visualized in surface-grown colonies: cream (e.g. *S. cerevisiae*); white (e.g. *Geotrichum candidum*); black (e.g. *Aureobasidium pullulans*); pink (e.g. *Phaffia rhodozyma*); red (e.g. *Rhodotorula rubra*); orange (e.g. *Rhodosporidium* spp.), and yellow (e.g. *Cryptococcus laurentii*). The pigments of some yeasts have biotechnological uses, including astaxanthin from *P. rhodozyma* in aquacultural feed supplements for farmed salmon (that are unable to synthesize these natural pink compounds).

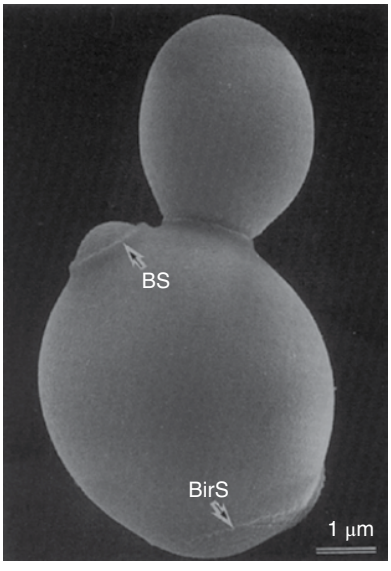

Figure 1.1 Scanning electron micrograph of a typical yeast cell (×10,000). BS, Bud scar; BirS, birth scar. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)

Table 1.1 Diversity of yeast cell shapes.

Cell shape	Description	Examples of yeast genera
Ellipsoid	Ovoid-shaped	*Saccharomyces*
Cylindrical	Elongated cells with hemispherical ends	*Schizosaccharomyces*
Apiculate	Lemon-shaped	*Hanseniaspora*, *Saccharomycodes*
Ogival	Elongated cell, rounded at one end and pointed at other	*Dekkera*, *Brettanomyces*
Flask-shaped	Cells divide by bud-fission	*Pityrosporum*
Miscellaneous shapes	Triangular	*Trigonopsis*
	Curved	*Cryptococcus* (e.g. *C. cereanus*)
	Spherical	*Debaryomyces*
	Stalked	*Sterigmatomyces*
Pseudohyphal	Chains of budding yeast cells which have elongated without detachment	*Candida* (e.g. *C. albicans*)
Hyphal	Branched or unbranched filamentous cells which form from germ tubes. Septa may be laid down by the continuously extending hyphal tip. Hyphae may give rise to blastospores	*Candida albicans*
Dimorphic	Yeasts that grow vegetatively in either yeast or filamentous (hyphal or pseudohyphal) form	*Candida albicans*, *Saccharomycopsis fibuligera*, *Kluyveromyces marxianus*, *Malassezia furfur*, *Yarrowia lipolytica*, *Histoplasma capsulatum*

1.3 Ultrastructure and Function of Fungal Cells

1.3.1 The Fungal Cell Surface

The cell envelope in yeasts and fungi is the peripheral structure that encases the cytoplasm and comprises the plasma membrane, the periplasm, the cell wall, and additional extracellular structural components (such as fimbriae and capsules). The cell wall represents a dynamically forming exoskeleton that protects the fungal protoplast from the external environment and defines directional growth, cellular strength, shape, and interactive properties (Figure 1.2).

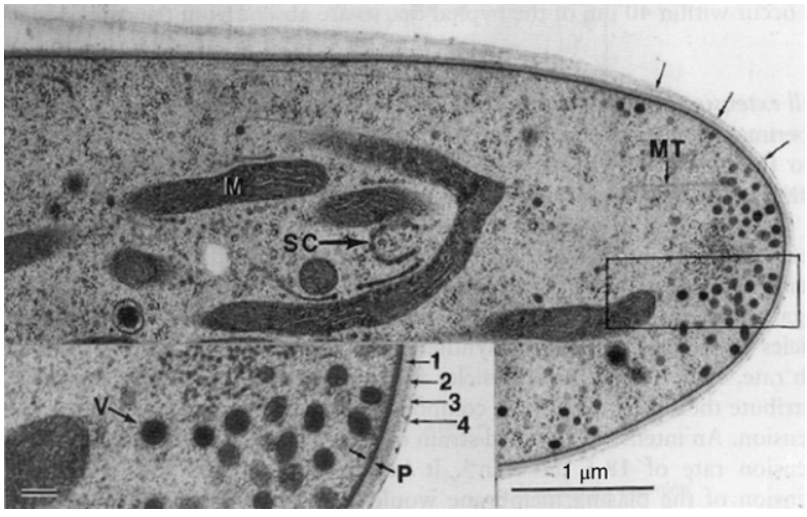

Figure 1.2 Transmission electron microscopy of ultrathin sections of a hyphal tip of *Fusarium* reveals intracellular fine structure. Layers of cell wall are shown in greater detail in lower image. M, Mitochondrion; V, vesicles; P, plasma membrane; MT, microtubules; SC, smooth Golgi cisternae; 1, 2, 3, 4, four layers of the cell wall. The Spitzenkörper appears as a region surrounded by vesicles containing many small particles (rectangle). (From Carlile *et al.* (2001).)

In filamentous fungi, cell wall formation and organization is intimately bound to the process of apical growth. Thus, for example in *Neurospora crassa*, the wall is thin (approximately 50 nm) at the apex but becomes thicker (approximately 125 nm) at 250 μm behind the tip. The plasma membrane component of the fungal cell envelope is a phospholipid bilayer interspersed with globular proteins that dictates entry of nutrients and exit of metabolites and represents a selective barrier for their translocation. Ergosterol is the major sterol found in the membranes of fungi, in contrast to the cholesterol found in the membranes of animals and phytosterols in plants. This distinction is exploited during the use of certain antifungal agents used to treat some fungal infections, and can be used as an assay tool to quantify fungal growth. The periplasm, or periplasmic space, is the region external to the plasma membrane and internal to the cell wall. In yeast cells, it comprises secreted proteins (mannoproteins) and enzymes (such as invertase and acid phosphatase) that are unable to traverse the cell wall. In filamentous fungi, the cell membrane and wall may be intimately bound as hyphae are often resistant to plasmolysis.

Fungal cell surface topological features can be visualized using scanning electron microscopy (SEM) and nanometre resolution achieved using atomic force microscopy (AFM). The latter is beneficial as it can be employed with unfixed, living cells and avoids potentially misleading artefacts that may arise when preparing cells for electron microscopy.

Table 1.2 Major polymers found in different taxonomic groups of fungi and fungus-like organisms, together with presence of perforate septa in these groups.

Taxonomic grouping	Fibrillar polymers	Matrix polymers	Perforate septa present or absent
Oomycetes (no longer considered to be true fungi)	β(1,3), β(1,6)-Glucan; cellulose	Glucan	Absent
Chytridomycetes	Chitin; glucan	Glucan	Absent
Zygomycetes	Chitin; chitosan	Polyglucuronic acid; glucuronomannoproteins	Absent
Basidiomycetes	Chitin; β(1,3)-β(1,6) glucans	α(1,3)-Glucan; xylomannoproteins	Present (mostly Dolipore)
Ascomycetes/ Deuteromycetes	Chitin; β(1,3)-β(1,6) glucans	α(1,3)-Glucan; galactomannoproteins	Present (mostly simple with large central pore)

Adapted from Deacon (2000); Carlile *et al*. (2001).

Ultrastructural analysis of fungal cell walls reveals a thick, complex fibrillar network. The cell walls of filamentous fungi are mainly composed of different polysaccharides according to taxonomic group. For example, they may contain chitin, glucans, mannoproteins, chitosan, polyglucuronic acid, or cellulose (absent from true fungi), together with smaller quantities of proteins and glycoproteins (Table 1.2). Generally, the semicrystalline microfibrillar components are organized in a network mainly in the central cell wall region and are embedded within an amorphous matrix. Bonding occurs between certain components behind the extending hyphal tip, thereby strengthening the entire wall structure. The processes of endocytosis and exocytosis occur around apical and subapical regions and serve to shape both hyphal growth and interactions with the environment (Figure 1.2). There is evidence to suggest that the cell wall is a dynamic structure where considerable quantitative and qualitative differences occur not only between different fungal species, but also between different morphological forms of the same species and even in response to environmental stress. For example, a class of hydrophobic proteins called hydrophobins are localized within the aerial growth or appressoria (terminal swellings involved in infection) of certain fungi, whereas pigmented melanins are often found within some fungal cell walls to insulate against biotic and abiotic stresses.

The hyphae of higher fungi extend via tip growth followed by cross-wall formation or septation, whereas the lower fungi remain aseptate (except when segregating spores or in damaged colony regions). Septa may offer some structural support to hyphae. Significantly, septa serve to compartmentalize hyphae

but are typically perforated, thereby permitting passage and communication of cytoplasm or even protoplasm between compartments. However, septal pores can become blocked by Woronin bodies or other materials. This aids morphological and biochemical differentiation and serves to seal-off stressed or damaged hyphae from undamaged colony regions. Again, different pore types are representative of different taxonomic groups and species (Table 1.2).

In yeasts, the cell wall provides stability and protection to the cells and its structure comprises polysaccharides (predominantly β-glucans for rigidity), proteins (mainly mannoproteins on the outermost layer for determining porosity), together with some lipid, chitin (e.g. in bud scar tissue), and inorganic phosphate material. Figure 1.3 shows the composition and structure of the *S. cerevisiae* cell wall. Hyphal cell walls generally contain fewer mannans than yeast cell forms, and such changes in composition are even observed during the transition from unicellular to mycelial growth of dimorphic fungi.

Chitin is also found in yeast cell walls and is a major constituent of bud scars (Figure 1.1). These are remnants of previous budding events found on the surface of mother cells following birth of daughter cells (buds). The chitin-rich bud scars of yeast cells can be stained with fluorescent dyes (e.g. calcoflour white) and this can provide useful information regarding cellular age, since the number of scars represents the number of completed cell division cycles. Outside the cell wall in fungi, several extramural layers may exist, including fimbriae and capsules. Fungal fimbriae are long, protein-containing protrusions appearing

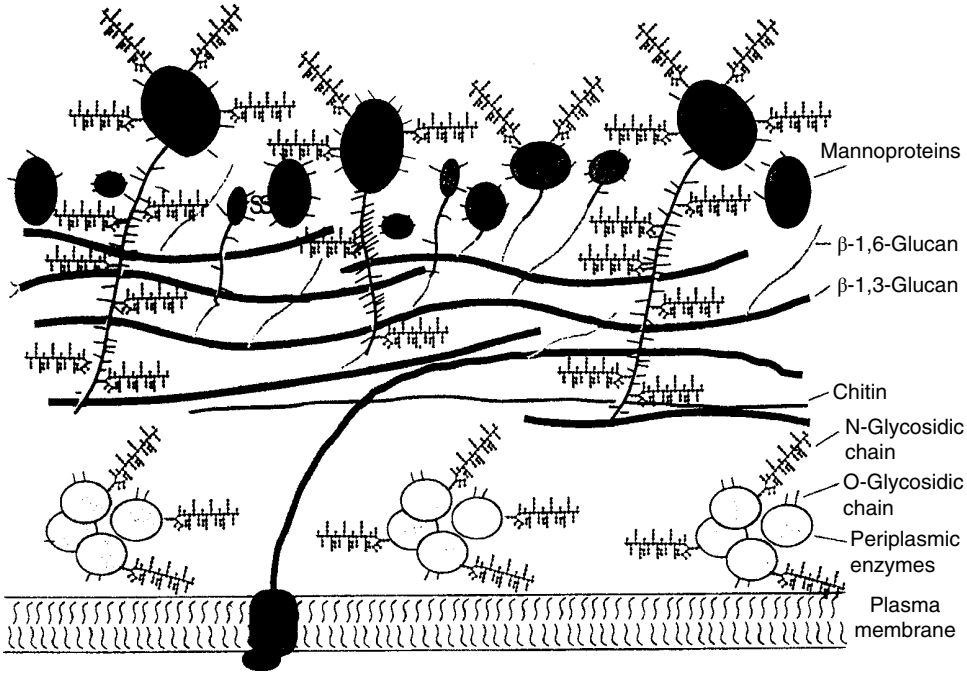

Figure 1.3 Cell envelope structure of the yeast *S. cerevisiae*. (From Walker (1998).)

from the cell wall of certain basidiomycetous and ascomycetous fungi that are involved in cell–cell conjugation. Capsules are extracellular polysaccharide-containing structures found in basidiomycetous fungi that are involved in stress protection. In *Cryptococcus neoformans* (the pathogenic yeast state of *Filobasidiella neoformans*) the capsule may determine virulence properties and evasion from macrophages. One extrahyphal substance, the polymer pullulan, is produced commercially from *Aureobasidium pullulans*, and is used in the production of oral hygiene products.

1.3.2 Subcellular Architecture and Organelle Function

Transmission electron microscopy of ultrathin sections of fungal cells reveals intracellular fine structure (Figures 1.2 and 1.4). Subcellular compartments (organelles) are bathed in an aqueous cytoplasm containing soluble proteins and other macromolecules together with low-molecular weight metabolites.

However, the hyphae of central (and therefore older) colony regions of filamentous fungi may become devoid of protoplasm and organelles, as protoplasmic components are driven forward or are recycled, to support the growth of actively growing hyphal tips. Cytoplasmic components additionally comprise microbodies, ribosomes, proteasomes, lipid particles, and a cytoskeletal network. The latter confers structural stability to the fungal cytoplasm and consists of microtubules and microfilaments. The following membrane-bound organelles may be found in a typical fungal cell: nucleus, endoplasmic reticulum (ER), mitochondria, Golgi apparatus, secretory vesicles, and vacuoles. Several of these

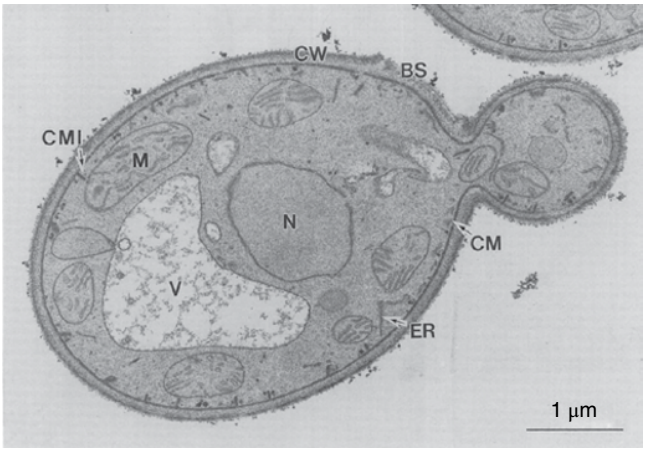

Figure 1.4 Electron micrograph of a typical yeast cell. CW, Cell wall; CM, cell membrane; CMI, cell membrane invagination; BS, bud scar; M, mitochondrion, N, nucleus; V, vacuole; ER, endoplasmic reticulum. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)

organelles form extended membranous systems. For example, the ER is contiguous with the nuclear membrane and secretion of fungal proteins involves intermembrane trafficking in which the ER, Golgi apparatus, plasma membrane, and vesicles all participate. The physiological function of the various fungal cell organelles is summarized in Table 1.3.

The nucleus is the structure that defines the eukaryotic nature of fungal cells. It is bound by a double membrane and encases the chromosomes in a nucleoplasm. Most yeasts and fungi are haploid (singular copies of each chromosome), although some (e.g. *S. cerevisiae*) may alternate between haploidy and diploidy.

Table 1.3 Functional components of an idealized fungal cell.

Organelle or cellular structure	Function
Cell envelope	Comprising: the plasma membrane which acts as a selectively permeable barrier for transport of hydrophilic molecules in and out of fungal cells; the periplasm containing proteins and enzymes unable to permeate the cell wall; the cell wall which provides protection and shape, and is involved in cell–cell interactions, signal reception, and specialized enzyme activities; fimbriae involved in sexual conjugation; capsules to protect cells from dehydration and immune cell attack
Nucleus	Relatively small. Containing chromosomes (DNA–protein complexes) that pass genetic information to daughter cells at cell division and the nucleolus which is the site of ribosomal RNA transcription and processing
Mitochondria	Site of respiratory metabolism under aerobic conditions, and, under anaerobic conditions, for fatty acid, sterol, and amino acid metabolism
Endoplasmic reticulum	Ribosomes on the rough ER are the sites of protein biosynthesis
Proteasome	Multi-subunit protease complexes involved in regulating protein turnover
Golgi apparatus and vesicles	Secretory system for import (endocytosis) and export (exocytosis) of proteins
Vacuole	Intracellular reservoir (amino acids, polyphosphate, metal ions); proteolysis; protein trafficking; control of cellular pH. In filamentous fungi, tubular vacuoles transport materials bidirectionally along hyphae.
Peroxisome	Oxidative utilization of specific carbon and nitrogen sources (contain catalase, oxidases). Glyoxysomes contain enzymes of the glyoxylate cycle

Many industrial strains of *S. cerevisiae* exhibit aneuploidy (odd numbers of chromosomes) or are polyploid (multiple chromosome copies). Chromosomes comprise DNA–protein structures that replicate and segregate to newly divided cells or hyphal compartments at mitosis. This, of course, ensures that genetic material is passed onto daughter cells or septated compartments at cell division. Yeasts usually contain a single nucleus per cell. However, the hyphal compartments of filamentous fungi may contain one or more nuclei. Monokaryotic basidiomycetes possess one nucleus per compartment, whereas dikaryons and heterokaryons possess two or more genetically distinct haploid nuclei. The maintenance of multiple nuclei within individual hyphal compartments allows fungi to take advantage of both haploid and diploid lifestyles. This is discussed further in Chapter 2.

In filamentous fungi, a phase-dark near-spherical region, which also stains with iron hemotoxylin, is evident by light microscopy at the apex during hyphal tip growth. The region is termed the Spitzenkörper, the apical vesicle cluster or centre, or apical body, and it consists of masses of small membrane-bound vesicles around a vesicle-free core with emergent microfilaments and microtubules (Figure 1.2). The Spitzenkörper contains differently sized vesicles derived from Golgi bodies, either large vesicles or microvesicles (chitosomes), with varying composition. It orientates to the side as the direction of tip growth changes, and disappears when growth ceases. This vesicle supply centre is involved in wall extension and hence tip growth, branching, clamp connection formation (in basidiomycetes), and germ tube formation.

1.4 Fungal Nutrition and Cellular Biosyntheses

1.4.1 Chemical Requirements for Growth

Yeasts and fungi have relatively simple nutritional needs and most species would be able to survive quite well in aerobic conditions if supplied with glucose, ammonium salts, inorganic ions, and a few growth factors. Exceptions to this would include, for example, obligate symbionts such as the vesicular-arbuscular mycorrhizal (VAM) fungi which require growth of a plant partner for cultivation. Macronutrients, supplied at millimolar concentrations, comprise sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, and magnesium; and micronutrients, supplied at micromolar concentrations, comprise trace elements like calcium, copper, iron, manganese, and zinc and would be required for fungal cell growth (Table 1.4). Some fungi are oligotrophic, apparently growing with very limited nutrient supply, surviving by scavenging minute quantities of volatile organic compounds from the atmosphere.

Being chemo-organotrophs, fungi need fixed forms of organic compounds for their carbon and energy supply. Sugars are widely utilized for fungal growth, and can range from simple hexoses like glucose to polysaccharides like starch and cellulose.

Table 1.4 Elemental requirements of fungal cells.

Element	Common sources	Cellular functions
Carbon	Sugars	Structural element of fungal cells in combination with hydrogen, oxygen, and nitrogen. Energy source
Hydrogen	Protons from acidic environments	Transmembrane proton motive force vital for fungal nutrition. Intracellular acidic pH (around 5–6) necessary for fungal metabolism
Oxygen	Air, O_2	Substrate for respiratory and other mixed-function oxidative enzymes. Essential for ergosterol and unsaturated fatty acid synthesis
Nitrogen	NH_4^+ salts, urea, amino acids	Structurally and functionally as organic amino nitrogen in proteins and enzymes
Phosphorus	Phosphates	Energy transduction, nucleic acid, and membrane structure
Potassium	K^+ salts	Ionic balance, enzyme activity
Magnesium	Mg^{2+} salts	Enzyme activity, cell and organelle structure
Sulfur	Sulfates, methionine	Sulfhydryl amino acids and vitamins
Calcium	Ca^{2+} salts	Possible second messenger in signal transduction
Copper	Cupric salts	Redox pigments
Iron	Ferric salts. Fe^{3+} is chelated by siderophores and released as Fe^{2+} within the cell	Heme-proteins, cytochromes
Manganese	Mn^{2+} salts	Enzyme activity
Zinc	Zn^{2+} salts	Enzyme activity
Nickel	Ni^{2+} salts	Urease activity
Molybdenum	Na_2MoO_4	Nitrate metabolism, vitamin B12

Some fungi can occasionally utilize aromatic hydrocarbons (e.g. lignin by the white-rot fungi). Table 1.5 outlines the variety of carbon sources that can be utilized by yeasts and filamentous fungi for growth.

Fungi are nondiazotrophic (cannot fix nitrogen) and need to be supplied with nitrogenous compounds, either in inorganic form such as ammonium salts, or in organic form such as amino acids. Ammonium sulfate is a commonly used nitrogen source in fungal growth media since it also provides a source of utilizable sulfur. Many fungi (but not the yeast *S. cerevisiae*) can also grow on nitrate,

Table 1.5 Diversity of carbon sources for yeast and filamentous fungal growth.

Carbon source	Typical examples	Comments
Hexose sugars	D-glucose, D-galactose,	Glucose metabolized by majority of yeasts and filamentous fungi
	D-fructose, D-mannose	If a yeast does not ferment glucose, it will not ferment other sugars. If a yeast ferments glucose, it will also ferment fructose and mannose, but not necessarily galactose
Pentose sugars	L-arabinose, D-xylose, D-xylulose, L-rhamnose	Some fungi respire pentoses better than glucose. *S. cerevisiae* can utilize xylulose but not xylose
Disaccharides	Maltose, sucrose, lactose, trehalose, melibiose, cellobiose, melezitose	If a yeast ferments maltose, it does not generally ferment lactose and vice versa. Melibiose utilization is used to distinguish ale and lager brewing yeasts. A large number of yeasts utilize disaccharides. Few filamentous fungi (e.g. *Rhizopus nigricans*) cannot utilize sucrose
Trisaccharides	Raffinose, maltotriose	Raffinose only partially used by *S. cerevisiae*, but completely used by other *Saccharomyces* spp. (*S. carlsbergensis*, *S. kluyveri*)
Oligosaccharides	Maltotetraose, maltodextrins	Metabolized by amylolytic yeasts, not by brewing strains
Polysaccharides	Starch, inulin, cellulose, hemicellulose, chitin, pectic substances	Polysaccharide-fermenting yeasts are rare. *Saccharomycopsis* spp. and *S. diastaticus* can utilize soluble starch; *Kluyveromyces* spp. possess inulinase. Many filamentous fungi can utilize these, depending on extracellular enzyme activity
Lower aliphatic alcohols	Methanol, ethanol	Respiratory substrates for many fungi. Several methylotrophic yeasts (e.g. *Pichia pastoris*, *Hansenula polymorpha*) have industrial potential
Sugar alcohols	Glycerol, glucitol	Can be respired by yeasts and a few fungi.
Organic acids	Acetate, citrate, lactate, malate, pyruvate, succinate	Many yeasts can respire organic acids, but few can ferment them
Fatty acids	Oleate, palmitate	Several species of oleaginous yeasts can assimilate fatty acids as carbon and energy sources

(*continued*)

Table 1.5 (*Continued*)

Carbon source	Typical examples	Comments
Hydrocarbons	n-Alkanes	Many yeast and a few filamentous species grow well on C_{12}-C_{18} n-alkanes
Aromatics	Phenol, cresol, quinol, resourcinol, catechol, benzoate	Few yeasts can utilize these compounds. Several n-alkane-utilizing yeasts use phenol as carbon source via the β-ketoadipate pathway
Miscellaneous	Adenine, uric acid, butylamine, pentylamine, putrescine	Some mycelial fungi and yeasts, e.g. *Arxula adeninivorans* and *A. terestre*, can utilize such compounds as sole source of carbon and nitrogen
	Lignin	Can be decayed only by white-rot fungi (basidiomycotina). Little net energy gained directly, but makes available other polysaccharides such as cellulose and hemicellulose
	"Hard" keratin	Keratinophilic fungi

Adapted from Walker (1998).

and if able to do so may also utilize nitrite. Nitrate reductase, followed by nitrite reductase, are the enzymes responsible for converting nitrate to ammonia. Most fungi can assimilate amino acids, amines, and amides as nitrogen sources. Most fungi (but not many yeasts) are also proteolytic and can hydrolyze proteins (via extracellularly secreted proteases) to liberate utilizable amino acids for growth. Urea utilization is common in fungi, and some basidiomycotenous yeasts are classed as urease-positive (able to utilize urea), while several ascomycotenous yeasts are urease-negative.

In terms of oxygen requirements, most fungi are aerobes and are often described as being microaerophilic (preferring an oxygen tension below that of normal atmospheric). Although yeasts like *S. cerevisiae* are sometimes referred to as facultative anerobes, they cannot actually grow in strictly anaerobic conditions unless supplied with certain fatty acids and sterols (which they cannot synthesize without molecular oxygen). In fact, there are thought to be very few yeast species that are obligately anaerobic. Unsaturated fatty acids (e.g. oleic acid) and sterols (e.g. ergosterol) are important constituents of the yeast cell membrane, and oxygen is required for their synthesis and to maintain membrane functional integrity and stress resistance. For aerobically respiring yeasts and fungi, oxygen is required as the terminal electron acceptor, where it is finally reduced to water in the electron transport chain. Different fungal species respond to oxygen availability in diverse ways and Table 1.6 categorizes fungi into different groups on this basis.

Table 1.6 Yeast and fungal metabolism based on responses to oxygen availability.

Mode of energy metabolism	Examples	Comments
Obligate fermentative	Yeasts: *Candida pintolopesii* (*Saccharomyces telluris*)	Naturally occurring respiratory-deficient yeasts. Only ferment, even in presence of oxygen
	Fungi: facultative and obligate anerobes	No oxygen requirement for these fungi. Two categories exist with respect to the effects of air: facultative anerobes (e.g. *Aqualinderella* and *Blastocladia*) and obligate anerobes (e.g. *Neocallimastix*)
Facultatively fermentative		
Crabtree-positive	*Saccharomyces cerevisiae*	Such yeasts predominantly ferment high sugar-containing media in the presence of oxygen
Crabtree-negative	*Candida utilis*	Such yeasts do not form ethanol under aerobic conditions and cannot grow anaerobically
Nonfermentative	Yeasts: *Rhodotorula rubra*	Such yeasts do not produce ethanol, in either the presence or absence of oxygen
	Fungi: *Phycomyces*	Oxygen is essential for such (obligately oxidative) fungi
Obligate aerobes	*Gaemannomyces graminis* (the take-all fungus)	Growth of these is markedly reduced if oxygen partial pressure falls below normal atmospheric

Adapted from Walker (1998), Deacon (2000), and Carlile *et al*. (2001).

Sulfur sources for fungal growth include sulfate, sulfite, thiosulfate, methionine and glutathione, with inorganic sulfate and the sulfur amino acid methionine being effectively utilized. Virtually all yeasts can synthesize sulfur amino acids from sulfate, the most oxidized form of inorganic sulfur.

Phosphorus is essential for biosynthesis of fungal nucleic acids, phospholipids, adenosine triphosphate (ATP), glycophosphates, and polyphosphates. Hence, the phosphate content of fungi is considerable (e.g. in yeast cells, this accounts for around 3–5% of dry weight; the major part of this is in the form of orthophosphate (H_2PO_4–) which acts as a substrate and enzyme effector). The fungal

Table 1.7 Metals required for fungal growth and metabolic functions.

Metal ion	Concentration[1]	Main cellular functions supplied in growth medium
Macroelements		
K	2–4 mM	Osmoregulation, enzyme activity
Mg	2–4 mM	Enzyme activity, cell division
Microelements		
Mn	2–4 μM	Enzyme cofactor
Ca	<1 μM	Second messenger, yeast flocculation
Cu	1.5 μM	Redox pigments
Fe	1–3 μM	Heme-proteins, cytochromes
Zn	4–8 μM	Enzyme activity, protein structure
Ni	~10 μM	Urease activity
Mo	1.5 μM	Nitrate metabolism, vitamin B12
Co	0.1 μM	Cobalamin, coenzymes

[1] Concentration figures relate to yeast (*S. cerevisiae*) growth stimulation, and are dependent on the species/strain and conditions of growth, but they would be generally applicable for fungal growth. Adapted from Walker (2004).

vacuole can serve as a storage site for phosphate in the form of complexed inorganic polyphosphates (also referred to as volutin granules). Both nitrogen and phosphorus availability may be growth limiting in nature. Filamentous fungi have evolved a number of biochemical and morphological strategies allowing capture of often poorly available phosphorus within the natural environment. Plants exploit such efficiency during symbioses between their roots and certain mycorrhizal fungi. The major storage form of phosphorus in plants is phytic acid (myo-inositol hexa-dihydrogenphosphate) which is poorly utilized by monogastrics (e.g. humans, pigs, poultry), and fungal (and yeast) phytases have applications in reducing phytate content of foods and feeds (see Chapter 8).

Concerning requirements for minerals, potassium, magnesium, and several trace elements are necessary for fungal growth. K and Mg are macroelements required in millimolar concentrations primarily as enzyme cofactors, whereas other microelements (trace elements) are generally required in the micromolar range. These include Mn, Ca, Fe, Zn, Cu, Ni, Co, and Mo. Table 1.7 summarizes the main metals required for fungal growth. Toxic minerals (e.g. Ag, As, Ba, Cs, Cd, Hg, Li, Pb) adversely affect fungal growth generally at concentrations greater than 100 μM.

Fungal growth factors are organic compounds occasionally needed in very low concentrations for specific enzymatic or structural roles, but not as energy

sources. These include vitamins (e.g. thiamine, biotin), purines, pyrimidines, nucleosides, nucleotides, amino acids, fatty acids, and sterols. For fungi to have a growth factor requirement, this indicates that cells cannot synthesize the particular factor, resulting in the curtailment of growth without its provision in culture media. Some fungi (e.g. *Aspergillus niger*, *Penicillium chrysogenum*) have very simple nutritional needs and are able to synthesize their own growth factors from glucose.

1.4.2 Fungal Cultivation Media

Fungal nutritional requirements are important not only for successful cultivation in the laboratory but also for the optimization of industrial fermentation processes. In the laboratory, it is relatively easy to grow yeasts and fungi on complex culture media such as malt extract or potato-dextrose agar or broth, which are both carbon rich and in the acidic pH range. Mushrooms are cultivated on various solid-substrates depending on provincial availability. Therefore, *Agaricus bisporus* (common button mushroom) is grown in the United Kingdom, United States, and France on wheat-straw; the padi-straw mushroom (*Volvariella volvacea*) is grown in South-east Asia on damp rice-straw and in Hong Kong on cotton waste; and in Japan, the shiitake mushroom (*Lentinus edodes*) is cultivated on fresh oak logs (see Chapter 6). In industry, media for fungal fermentation purposes need to be optimized with regard to the specific application and production process. For some industrial processes, growth media may already be relatively complete in a nutritional sense, such as malt wort or molasses for brewing or baker's yeast production, respectively (Table 1.8). However, for other processes, supplementation of agriculturally derived substrates like corn steep liquor, molasses or malt broth with additional nutrients and growth factors may be necessary. For example, for penicillin production by *Penicillium* spp. the following may constitute a suitable fermentation medium – sucrose (3 g/L), corn steep liquor (100 g/L), KH_2PO_4 (1 g/L), $(NH_4)_2SO_4$ (12 g/L), $CaCl_2.2H_2O$ (0.06 g/L), phenoxyacetic acid (5.7 g/L) – whereas other industrial processes such as the growth of *Fusarium graminarium* for the production of Quorn™ mycoprotein require culture on a completely defined medium.

1.4.3 Nutrient Uptake and Assimilation

Fungal cells utilize a diverse range of nutrients and employ equally diverse nutrient acquisition strategies. Fungi are nonmotile, saprophytic (and sometimes parasitic), chemo-organotrophic organisms. They exhibit dynamic interactions with their nutritional environment that may be exemplified by certain morphological changes, depending on nutrient availability. For example, the filamentous mode of growth observed at the periphery of certain yeast colonies

Table 1.8 Principal ingredients of selected industrial media for yeasts and fungi.

Components	Molasses	Malt wort	Wine must	Cheese whey	Corn steep liquor
Carbon sources	Sucrose Fructose Glucose Raffinose	Maltose Sucrose Fructose Glucose Maltotriose	Glucose Fructose Sucrose (trace)	Lactose	Glucose, other sugars
Nitrogen sources	Nitrogen compounds as unassimilable proteins. Nitrogen sources need to be supplemented	Low molecular α-amino nitrogen compounds, ammonium ions, and a range of amino acids	Variable levels of ammonia nitrogen, which may be limiting. Range of amino acids	Unassimilable globulin and albumin proteins. Low levels of ammonium and urea nitrogen	Amino acids, protein
Minerals	Supply of P, K, and S available. High K^+ levels may be inhibitory	Supply of P, K, Mg, and S available	Supply of P, K, Mg, and S available. High levels of sulfite often present	Supply of P, K, Mg, and S	Supply of P, K, Mg, and S
Vitamins	Small, but generally adequate supplies. Biotin is deficient in beet molasses	Supply of vitamins is usually adequate. High adjunct sugar wort may be deficient in biotin	Vitamin supply generally sufficient	Wide range of vitamins present	Biotin, pyridoxine, thiamin
Trace elements	Range of trace metals present, although Mn^{2+} may be limiting	All supplied, although Zn^{2+} may be limiting	Sufficient quantities available	Fe, Zn, Mn, Ca, and Cu present	Range of trace elements present
Other components	Unfermentable sugars (2–4%), organic acids, waxes, pigments, silica, pesticide residues, caramelized compounds, betaine	Unfermentable maltodextrins, pyrazines, hop compounds	Unfermentable pentoses. Tartaric and malic acids. Decanoic and octanoic acids may be inhibitory. May be deficient in sterols and unsaturated fatty acids	Lipids, NaCl. Lactic and citric acids	High levels of lactic acid present. Fat and fibre also present

growing in agar is akin to a foraging for nutrients as observed in certain eucarpic fungi. Metabolic dynamism is also evident in yeasts which, although not avid secretors of hydrolytic enzymes like higher fungi, are nevertheless able to secrete some enzymes to degrade polymers such as starch (as in amylolytic yeasts like *Schwanniomyces* spp.).

Several cellular envelope barriers to nutrient uptake by fungal cells exist, namely the capsule, the cell wall, the periplasm and the cell membrane. Although not considered as freely porous structures, fungal cell walls are relatively porous to molecules up to an average molecular mass of around 300 Da, and will generally retain molecules greater than around 700 Da. Typically, fungi absorb only small soluble nutrients such as monosaccharides and amino acids.

The plasma membrane is the major selectively permeable barrier which dictates nutrient entry and metabolite exit from the fungal cell. Membrane transport mechanisms are important in fungal physiology since they govern the rates at which cells metabolize, grow, and divide. Fungi possess different modes of passive and active uptake at the plasma membrane: free diffusion, facilitated diffusion, diffusion channels, and active transport (Table 1.9). Active transport of nutrients such as sugars, amino acids, nitrate, ammonium, sulfate, and phosphate in filamentous fungi involves spatial separation of the ion pumps mostly behind the apex, whereas the symport proteins are active close to the tip. Thus, nutrient uptake occurs at the hyphal tip as it continuously drives into fresh resource, and the mitochondria localized behind the apex supply ATP to support the ion pump and generate proton motive force.

1.4.4 Overview of Fungal Biosynthetic Pathways

Anabolic pathways are energy-consuming, reductive processes which lead to the biosynthesis of new cellular material and are mediated by dehydrogenase enzymes which predominantly use reduced $NADP^+$ as the redox cofactor. NADPH is generated by the hexose monophosphate pathway (or Warburg–Dickens pathway) which accompanies glycolysis (see Section 1.5.1). In *S. cerevisiae*, up to 20% of total glucose may be degraded via the hexose monophosphate pathway. This pathway generates cytosolic NADPH (following the dehydrogenation of glucose 6-phosphate using glucose 6-phosphate dehydrogenase and $NADP^+$ as hydrogen acceptor) for biosynthetic reactions, leading to the production of fatty acids, amino acids, sugar alcohols, structural and storage polysaccharides, and secondary metabolites. Besides generating NADPH, the hexose monophosphate pathway also produces ribose sugars for the synthesis of nucleic acids, RNA, and DNA and for nucleotide coenzymes, NAD, NADP, FAD, and FMN. This is summarized as follows:

$$\text{Glucose 6-phosphate} + 2\text{NADP}^+ \rightarrow \text{Ribulose 5-phosphate} + \text{CO}_2 + \text{NADPH} + 2\text{H}^+$$

Table 1.9 Modes of nutrient transport in fungi.

Mode of nutrient transport	Description	Examples of nutrients transported
Free diffusion	Passive penetration of lipid-soluble solutes through plasma membrane following the law of mass action from a high extracellular concentration to a lower intracellular concentration	Organic acids, short-chain alkanes, and long-chain fatty acids by fungi and export of lipophilic metabolites (e.g. ethanol) and gaseous compounds)
Facilitated diffusion	Translocates solutes down a transmembrane concentration gradient in an enzyme (permease) mediated manner. As with passive diffusion, nutrient translocation continues until intracellular concentration equals that of the extracellular medium	In the yeast *S. cerevisiae*, glucose is transported in this manner
Diffusion channels	These operate as voltage-dependent "gates" to transiently move certain nutrient ions down concentration gradients. They are normally closed at the negative membrane potential of resting yeast cells but open when the membrane potential becomes positive	Ions such as potassium may be transported in this fashion
Active transport	The driving force is the membrane potential and transmembrane electrochemical proton gradient generated by plasma membrane H^+-ATPase. The latter extrudes protons using the free energy of ATP hydrolysis that enables nutrients to enter either with influxed protons, as in "symport" mechanisms, or against effluxed protons, as in "antiport" mechanisms	Many nutrients (sugars, amino acids, ions)

and complete oxidation of glucose 6-phosphate would result in:

$$\text{Glucose 6-phosphate} + 12\text{NADP}^+ \rightarrow 6\text{CO}_2 + 12\text{NADPH} + 12\text{H}^+ + \text{Pi}$$

Fungal growth on noncarbohydrate substrates as sole carbon sources (e.g. ethanol, glycerol, succinate, and acetate) may lead to gluconeogenesis (conversion of pyruvate to glucose) and polysaccharide biosynthesis. Gluconeogenesis may be regarded as a reversal of glycolysis and requires ATP as energy and NADH as reducing power.

Concerning fungal amino acid biosynthesis, simple nitrogenous compounds such as ammonium may be assimilated into amino acid *families*, the carbon skeletons of which originate from common precursors of intermediary carbon metabolism.

The two main fungal storage carbohydrates are glycogen and trehalose. Glycogen is similar to starch with $\alpha(1 \rightarrow 4)$ glucan linear components and $\alpha(1 \rightarrow 6)$ branches. Trehalose (also known as mycose) is a disaccharide of glucose comprising an $\alpha,\alpha(1 \rightarrow 1)$ glucoside bond between two α-glucose units. Both trehalose and glycogen are synthesized following the formation of UDP-glucose, catalyzed by UDP-glucose pyrophosphorylase:

$$\text{UTP} + \text{Glucose 1-phosphate} \rightarrow \text{UDP-glucose} + \text{Pyrophosphate}$$

Glycogen is synthesized by glycogen synthase. Glycogen may be metabolized by glycogen phosphorylase when nutrients become limited under starvation conditions and this contributes to the maintenance metabolism of cells by furnishing energy in the form of ATP. In yeast cells, glycogen breakdown is accompanied by membrane sterol biosynthesis (in the presence of some oxygen) and this is important for brewing yeast vitality and successful beer fermentations. The other major storage carbohydrate, trehalose, is synthesized from glucose 6-phosphate and UDP-glucose by trehalose 6-phosphate synthase and converted to trehalose by a phosphatase.

In addition to a storage role, trehalose is an important translocation material in filamentous forms and is also involved in stress protection in yeasts and fungi, accumulating when cells are subject to environmental insults such as heat shock or osmotic stress, or during plant host–fungal parasite interactions. Trehalose acts by protecting cell membranes against desiccation or thermal damage. Polyols, such as mannitol derived from fructose phosphate and glycerol from the glycolytic intermediate dihydroxyacetone phosphate, are also translocated by fungi. In particular, glycerol is produced as a "compatible solute" in response to osmotic stress to counteract the loss of intracellular water (see Section 1.6.1). Glycerol is also a yeast fermentation by-product and contributes to the viscosity or mouthfeel of alcoholic beverages such as beer and wine.

1.4.5 Fungal Cell Wall Growth

The structural polysaccharides in fungal cell walls include mannans, glucans, and chitin and are synthesized from sugar nucleotide substrates formed by pyrophosphorylase enzymes. For example:

$$\text{Glucose 1-phosphate} + \text{UTP} \rightarrow \text{UDP-glucose} + \text{PPi}$$

$$\text{Mannose 1-phosphate} + \text{GTP} \rightarrow \text{GDP-mannose} + \text{PPi}$$

Glucan synthesis involves plasma membrane-associated glucan synthetases for assembly of β-1,3 linkages and β-1,6 branches of cell wall glucan. Chitin (a polymer

of N-acetylglucosamine) is an important fungal cell wall structural component and is involved in the yeast budding process and in dimorphic transitions from yeast to filamentous forms. Chitin synthetases catalyze the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to a growing chitin polymer within the fungal cell wall. The mannoproteins predominantly of unicellular forms are pre-assembled within the Golgi and are delivered to the cell wall via vesicles from the vesicle supply centre. Various vesicles containing cell wall-synthetic enzymes, wall-lytic enzymes, enzyme activators, and certain pre-formed wall components, are transported to the tip where they fuse with the plasma membrane and release their contents, which, together with substrates delivered from the cytosol, facilitate synthesis of the growing cell wall.

1.5 Fungal Metabolism

1.5.1 Carbon Catabolism

Being chemo-organotrophs, fungi derive their energy from the breakdown of organic compounds. Generally speaking, fungi, but few yeast species, extracellularly break down polymeric compounds by secreted enzymes prior to utilization of monomers as carbon and energy sources. Due to their relatively large size (20–60 KDa), enzymes assembled by the Golgi are transported in vesicles to be secreted from sites of cell growth, essentially from extending hyphal tips. Enzymes may either become linked to the cell wall as wall-bound enzymes, or may diffuse externally to decay substrates within the local environment.

Some examples follow of hydrolytic, oxidative, peroxidative, and free radical generating enzyme systems produced by fungi for the degradation of polymeric compounds:

$$\text{Pectin} \xrightarrow{\text{Pectin lyase, polygalactorunase}} \text{Galacturonic acid}$$

$$\text{Starch} \xrightarrow{\text{Amylases, glucoamylase}} \text{Glucose}$$

$$\text{Inulin} \xrightarrow{\text{Inulinase}} \text{Fructose}$$

$$\text{Cellulose} \xrightarrow{\text{Cellulases}} \text{Glucose}$$

$$\text{Hemicellulose} \xrightarrow{\text{Hemicellulases, xylanase}} \text{Xylose, Glucose}$$

$$\text{Lipids} \xrightarrow{\text{Lipases}} \text{Fatty acids}$$

$$\text{Proteins} \xrightarrow{\text{Proteinases}} \text{Amino acids}$$

$$\text{Chitin} \xrightarrow{\text{Chitinase}} \textit{N}\text{-acetylglucosamine}$$

$$\text{Lignin} \xrightarrow{\text{Ligninase; manganese peroxidase; laccase; glucose oxidase}} \text{Variety of largely phenolic products}$$

Several lipolytic yeasts are known (e.g. *Candida rugosa*, *Yarrowia lipolytica*) which secrete lipases to degrade triacylgycerol substrates to fatty acids and glycerol.

In wood, the cellulose and hemicellulose components are embedded within a heteropolymeric 3-D lignin matrix, thus forming a complex lignocellulose material. Only certain filamentous basidiomycete or ascomycete fungi are able to degrade the recalcitrant lignin component, making available the cellulose or hemicellulose components. These are known as white-rot fungi due to resultant coloration of the delignified wood. Such fungi employ a cocktail of oxidative (including laccases) and peroxidative enzymes, together with hydrogen peroxide generating enzyme systems, to attack at least 15 different inter-unit bond types extant within the lignin polymer. The manganese and lignin peroxidase enzyme systems operate by releasing highly reactive but transient oxygen free radicals, which bombard and react with parts of the lignin molecule, generating a chain of chemical oxidations and producing a range of mainly phenolic end products. White-rot fungi have applications in, for example, upgrading lignocellulose waste for animal feed, paper production and bleaching, the bioremediation of contaminated land and water, and (potentially) for biofuel production (e.g. pre-treatment of lignocellulosic biomass for second-generation bioethanol). Brown-rot and soft-rot (in wet wood) fungi are only able to degrade the cellulose and hemicellulose components of wood. Cellulose decomposition involves the synergistic activity of endoglucanases (that hydrolyze the internal bonds of cellulose), exoglucanases (that cleave cellobiose units from the end of the cellulose chain), and glucosidases (that hydrolyze cellobiose to glucose). Initial attack of cellulose microfibrills within the cell wall may involve the generation of hydrogen peroxide. Commercially available cellulolytic enzymes are produced from filamentous fungal cultures, notably *Trichoderma reesei*.

Catabolic pathways are oxidative processes which remove electrons from intermediate carbon compounds and use these to generate energy in the form of ATP. The catabolic sequence of enzyme-catalyzed reactions that convert glucose to pyruvic acid is known as glycolysis, and this pathway provides fungal cells with energy, together with precursor molecules and reducing power (in the form of NADH) for biosynthetic pathways. Therefore, in serving both catabolic and anabolic functions, glycolysis is sometimes referred to as an amphibolic pathway. Glycolysis may be summarized as follows:

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD}^+ \rightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH}^+ + 2\text{H}^+$$

During glycolysis, glucose is phosphorylated using ATP to produce fructose 1,6-biphosphate, which is then split by aldolase to form two triose phosphate compounds. Further phosphorylation occurs, forming two triose diphosphates from which four H atoms are accepted by two molecules of NAD^+. In the latter stages of glycolysis, four molecules of ATP are formed (by transfer of phosphate from the triose diphosphates to ADP) and this results in the formation of two molecules of pyruvic acid. ATP production (two molecules net) during glycolysis is referred to as substrate-level phosphorylation.

In yeast cells undergoing alcoholic fermentation of sugars under anaerobic conditions, NAD^+ is regenerated in terminal step reactions from pyruvate. In the first of these, pyruvate is decarboxylated (by pyruvate decarboxylase) before a final reduction, catalyzed by alcohol dehydrogenase (ADH) to ethanol. Such regeneration of NAD^+ prevents glycolysis from stalling and maintains the cell's oxidation–reduction balance and ATP production. Additional minor fermentation metabolites are produced by fermenting yeast cells, including glycerol, fusel alcohols (e.g. isoamyl alcohol), esters, (e.g. ethyl acetate), organic acids (e.g. citrate, succinate, acetate), and aldehydes (e.g. acetaldehyde). Such compounds are important in flavor development in alcoholic beverages such as beer, wine, and whisky.

Aerobic dissimilation of glucose by fungi leads to respiration, which is the major energy-yielding metabolic route and involves glycolysis, the citric acid cycle, the electron transport chain, and oxidative phosphorylation. Yeasts, in particular *S. cerevisiae*, are unique microorganisms in that they can switch from respiration to fermentation, and vice versa, depending on the prevailing growth conditions. In addition to glucose, many carbon substrates can be respired by fungi including: pentose sugars (e.g. xylose), sugar alcohols (e.g. glycerol), organic acids (e.g. acetic acid), aliphatic alcohols (e.g. methanol, ethanol), hydrocarbons (e.g. *n*-alkanes), and aromatic compounds (e.g. phenol). Fatty acids are made available for fungal catabolism following extracellular lipolysis of fats and are metabolized by β-oxidation in mitochondria.

During glucose respiration under aerobic conditions, pyruvate enters the mitochondria where it is oxidatively decarboxylated to acetyl CoA by pyruvate dehydrogenase, which acts as the link between glycolysis and the cyclic series of enzyme-catalyzed reactions known as the citric acid cycle (or Krebs cycle). This cycle represents the common pathway for the oxidation of sugars and other carbon sources in yeasts and filamentous fungi and results in the complete oxidation of one pyruvate molecule to: $2CO_2$, 3NADH, $1FADH_2$, $4H^+$, and 1GTP. Like glycolysis, the citric acid cycle is amphibolic since it performs both catabolic and anabolic functions, the latter providing intermediate precursors (e.g. oxaloacetate and α–ketoglutarate) for the biosynthesis of amino acids and nucleotides. The removal of intermediates necessitates their replenishment to ensure continued operation of the citric acid cycle. The glyoxylate cycle is an example of such an *anaplerotic* reaction and involves the actions of the enzymes pyruvate carboxylase:

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

and phosophoenolpyruvate carboxykinase:

$$\text{Phosphoenolpyruvate} + CO_2 + H_2O \rightarrow \text{Oxaloacetate} + H_3PO_4$$

During the citric acid cycle, dehydrogenase enzymes transfer hydrogen atoms to the redox carriers NAD^+ and FAD, which become reduced. On the inner membrane of mitochondria, these reduced coenzymes are then re-oxidized, and

Table 1.10 Respiratory chain characteristics of yeasts and fungi.

Type	Typical species	Sensitive to	Insensitive to
Normal respiration	All aerobic fungi	Cyanide and low azide[1]	SHAM[2]
Classic alternative	*Yarrowia lipolytica* (and in stationary phase cultures of several yeast species)	SHAM	Cyanide, high azide
New alternative	*Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Williopsis saturnus*	High azide	Cyanide, low azide, SHAM

[1]The azide-sensitive pathway lacks proton transport capability and accepts electrons from NADH but not from succinate.
[2]The SHAM (salycil hydroxamate)-sensitive pathway transports electrons to oxygen also without proton transport, and therefore does not phosphorylate ADP.
Adapted from Walker (1998).

oxygen is reduced to water via the electron transport chain. Energy released by electron transfer is used to synthesize ATP by a process called oxidative phosphorylation. The chemiosmotic theory describes proton pumping across the inner mitochondrial membrane to create a transmembrane proton gradient (ΔpH) and a membrane potential difference. Together, these comprise the proton motive force that is the driving force for ATP synthesis. Each pair of electrons in NADH yields about 2.5 ATP, while residual energy is largely dissipated as metabolic heat. Since mitochondria are impermeable to NADH, this reduced coenzyme generated in the cytoplasm during glycolysis is "shuttled" across the mitochondrial membrane using either the *glycerophosphate shuttle* (that uses NADH to reduce dihydroxyacetone phosphate to glycerol 3-phosphate) or the *malate shuttle* (that uses NADH to reduce oxaloacetate to malate). These processes enable molecules to be oxidized within mitochondria to yield reduced cofactors, which in turn are oxidized by the electron transport chain.

Fungi use molecular oxygen as a terminal electron acceptor in aerobic respiration in different ways (Table 1.10). Some yeasts, including *S. cerevisiae*, exhibit *alternative respiration* characterized by insensitivity to cyanide but sensitivity to azide.

1.5.2 Nitrogen Metabolism

Fungi assimilate simple nitrogenous sources for the biosynthesis of amino acids and proteins. For example, ammonium ions are readily utilized and can be directly assimilated into the amino acids glutamate and glutamine that serve as precursors for the biosynthesis of other amino acids. Proteins can also be utilized

following release of extracellular protease enzymes. Glutamate is a key compound in both nitrogen and carbon metabolism, and glutamine synthetase is important as it catalyzes the first step in pathways leading to the synthesis of many important cellular macromolecules. Other important enzymes of fungal nitrogen metabolism include glutamate dehydrogenase and glutamate synthase (glutamine amide: 2-oxoglutarate-aminotransferase, or GOGAT), the latter requiring ATP. When glutamine synthetase is coupled with glutamate synthase this represents a highly efficient "nitrogen-scavenging" process for fungi to assimilate ammonia into amino acids and citric acid cycle intermediates. The particular route(s) of ammonium assimilation adopted by fungi depend on the concentration of available ammonium ions and the intracellular amino acid pools.

Some yeasts (but not *S. cerevisiae*) and fungi can use *nitrate* as a sole source of nitrogen through the activities of nitrate reductase:

$$NO_3^- \rightarrow NO_2^-$$

and nitrite reductase:

$$NO_2^- \rightarrow NH_4^+$$

The resulting ammonium ions can then be assimilated into glutamate and glutamine that represent end products of nitrate assimilation by yeasts.

Urea can also be utilized following its conversion to ammonium by urea aminohydrolase (urea carboxylase plus allophanate hydrolase):

$$NH_2CONH_2 + ATP + HCO_3^- \rightarrow NH_2CONHCOO^- \rightarrow 2NH_4^+ + 2HCO_3^-$$

Amino acids can either be assimilated into proteins or dissimilated by decarboxylation, deamination, transamination, and fermentation. Amino acid degradation by yeasts and fungi yields both ammonium and glutamate. During fermentation, yeasts may produce higher alcohols or *fusel oils* such as isobutanol and isopentanol following amino acid deamination and decarboxylation. These represent important yeast-derived flavor constituents in fermented beverages.

1.6 Fungal Growth and Reproduction

1.6.1 Physical Requirements for Growth

Most yeast and fungal species thrive in warm, sugary, acidic, and aerobic conditions. The temperature range for fungal growth is quite wide, but generally speaking most species grow very well around 25 °C. Low-temperature psychrophilic fungi and high-temperature thermophilic fungi do, however, exist in nature. Fungal growth at various temperatures depends not only on the genetic background of the species but also on other prevailing physical growth parameters and nutrient

availability. With regard to high temperature stress (or heat shock) on fungal cells, thermal damage can disrupt hydrogen bonding and hydrophobic interactions, leading to general denaturation of proteins and nucleic acids.

Fungi, of course, have no means of regulating their internal temperature, and the higher the temperature, the greater the cellular damage, with cell viability declining when temperature increases beyond growth optimal levels. Temperature optima vary greatly in fungi, with those termed "thermotolerant" growing well above 40 °C. Thermotolerance relates to the transient ability of cells subjected to high temperatures to survive subsequent lethal exposures to elevated temperatures, such that *intrinsic* thermotolerance is observed following a sudden heat shock (e.g. to 50 °C), whereas *induced* thermotolerance occurs when cells are pre-conditioned by exposure to a mild heat shock (e.g. 30 minutes at 37 °C) prior to a more severe heat shock. Heat-shock responses in fungi occur when cells are rapidly shifted to elevated temperatures, and if this is sublethal, induced synthesis of a specific set of proteins – the highly conserved "heat-shock proteins" (HSPs) – occurs. HSPs play numerous physiological roles, including thermoprotection.

High water activity, a_w, is required for growth of most fungi, with a minimum a_w of around 0.65. Water is absolutely essential for fungal metabolism, and any external conditions that result in reduced water availability to cells (i.e. "osmostress") will adversely affect cell physiology.

The term water potential refers to the potential energy of water and closely relates to the osmotic pressure of fungal growth media. Certain fungal species, for example the yeast *Zygosaccharomyces rouxii* and some *Aspergillus* species, are able to grow in low water potential conditions (i.e. high sugar or salt concentrations) and are referred to as osmotolerant or zerotolerant. By comparison, *S. cerevisiae* is generally regarded as a nonosmotolerant yeast. Mild water stress, or *hyperosmotic shock*, occurs in fungi when cells are placed in a medium with low water potential brought about by increasing the solute (e.g. salt, sugar) concentration. Conversely, cells experience a *hypo-osmotic shock* when introduced to a medium of higher osmotic potential (due to reducing the solute concentration).

Fungi are generally able to survive such short-term shocks by altering their internal osmotic potential (e.g. by reducing intracellular levels of K+ or glycerol). Glycerol is an example of a *compatible solute* that is synthesized in order to maintain low cytosolic water activity when the external solute concentration is high. Glycerol can effectively replace cellular water, restore cell volume, and enable fungal metabolism to continue. Trehalose, arabitol, and mannitol can similarly protect against osmotic stress. Evidence suggests that the accumulation of compatible solutes is attributed not only to their synthesis but also to control of membrane fluidity, thus preventing their leakage to the external environment.

As for pH, most fungi are acidophilic and grow well between pH 4 and 6, but many species are able to grow, albeit to a lesser extent, in more acidic or alkaline conditions (around pH 3 or 8, respectively). Fungal cultivation media acidified

with organic acids (e.g. acetic, lactic acids) are more inhibitory to growth compared with those acidified with mineral acids (e.g. hydrochloric, phosphoric acids) because organic acids can lower intracellular pH (following their translocation across fungal plasma membranes). Exposure to organic acids leads to cells exhausting their energy (ATP) when endeavouring to maintain pH homeostasis through the activities of proton-pumping ATPase in the plasma membrane. This forms the basis of action of weak acid preservatives in inhibiting the growth of food spoilage fungi. Many filamentous fungi can alter their local external pH by selective uptake and exchange of ions (NO_3^- or NH_4^+/H^+), or by excretion of organic acids such as oxalic acid.

Other physical parameters influencing fungal physiology include radiation (light or UV may elicit mycelial differentiation and sporulation in some fungi that produce airborne spores), aeration, pressure, centrifugal force, and mechanical shear stress.

1.6.2 Cellular Reproduction

Fungal growth involves transport and assimilation of nutrients, followed by their integration into cellular components, followed by biomass increase and eventual cell division (as in yeasts) or septation (as in higher fungi). The physiology of vegetative reproduction and its control in fungi has been most widely studied in two model eukaryotes, the budding yeast, *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*.

Budding is the most common mode of vegetative reproduction in yeasts and multilateral budding is typical in ascomycetous yeasts (Table 1.11). In *S. cerevisiae*, buds are initiated when mother cells attain a critical cell size and this coincides with the onset of DNA synthesis. The budding processes result from localized weakening of the cell wall and this, together with tension exerted by turgor pressure, allows extrusion of cytoplasm in an area bounded by a new cell wall. Cell wall polysaccharides are mainly synthesized by glucan and chitin synthetases. Chitin is a polymer of N-acetylglucosamine and this material forms a ring between the mother cell and the bud that will eventually form the characteristic *bud scar* after cell division. Under optimized growth conditions, budding yeasts, typified by *S. cerevisiae*, can complete their budding cell division cycle in around 2 hours.

Fission yeasts, typified by *Schizosaccharomyces* spp., divide exclusively by forming a cell septum, which constricts the cell into two equal-sized daughters. In *Schiz. pombe*, newly divided daughter cells grow in length until mitosis is initiated when cells reach a constant cell length (about 14 μm). The cell septum in *Schiz. pombe* forms by lateral growth of the inner cell wall (the primary septum) and proceeds inwardly, followed by deposition of secondary septa. Cellular fission, or transverse cleavage, is completed in a manner resembling the closure of an iris diaphragm.