1. Introduction

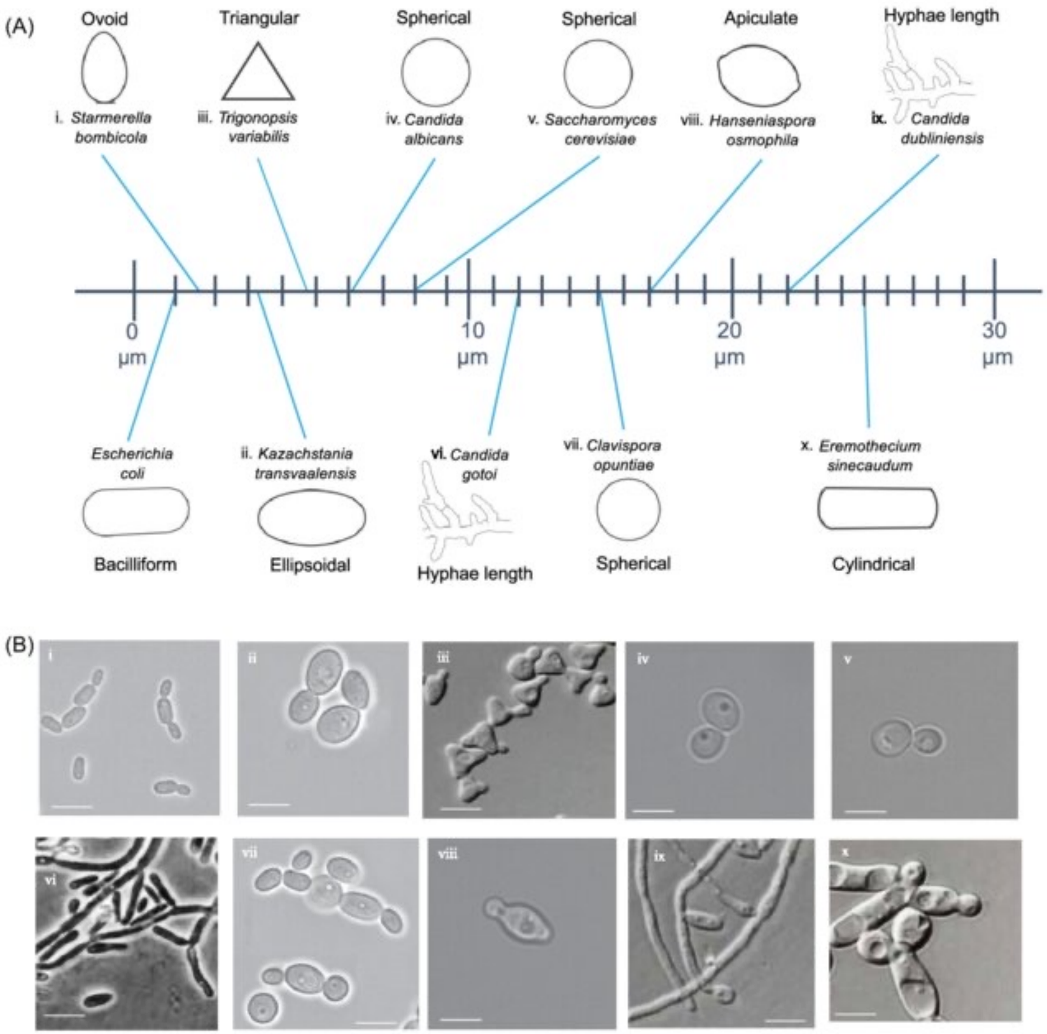
Dr. Ariyah Terasawat

1.1 Introduction

1.2 Morphology of Yeasts and Fungi

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*

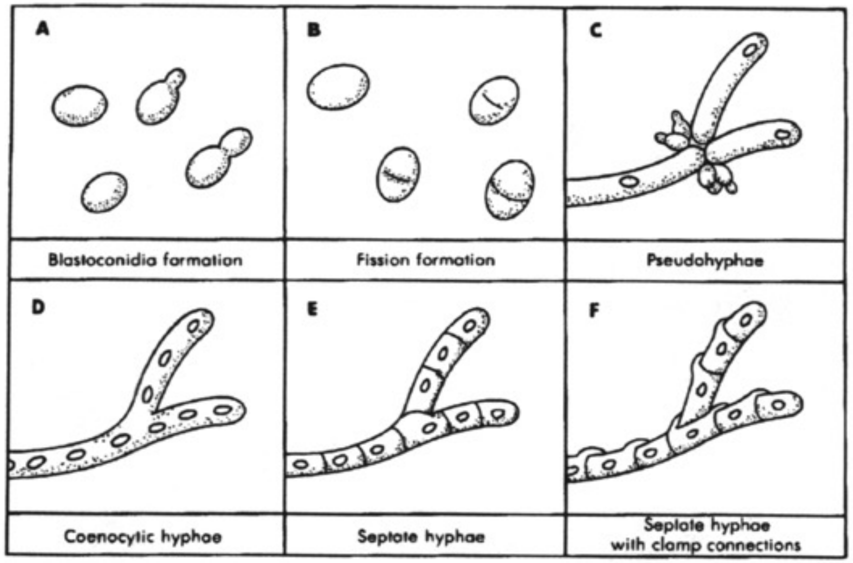


Chavez, Christina & Groenewald, Marizeth & Hulfachor, Amanda & Kpurubu, Gideon & Huerta, Rene & Hittinger, Chris & Rokas, Antonis. (2023). The cell morphological diversity of Saccharomycotina yeasts. FEMS Yeast Research. 24. 10.1093/femsyr/foad055.

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).



1.3 Ultrastructure and Function of Fungal Cells

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).

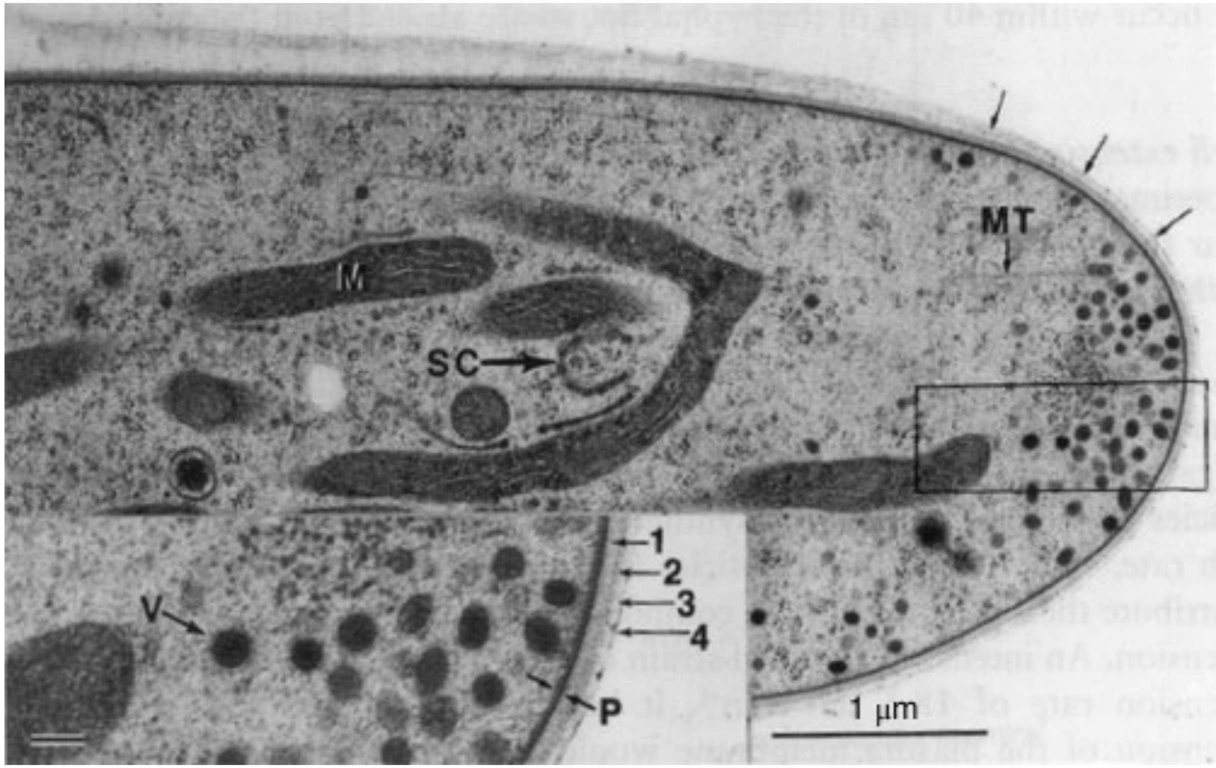


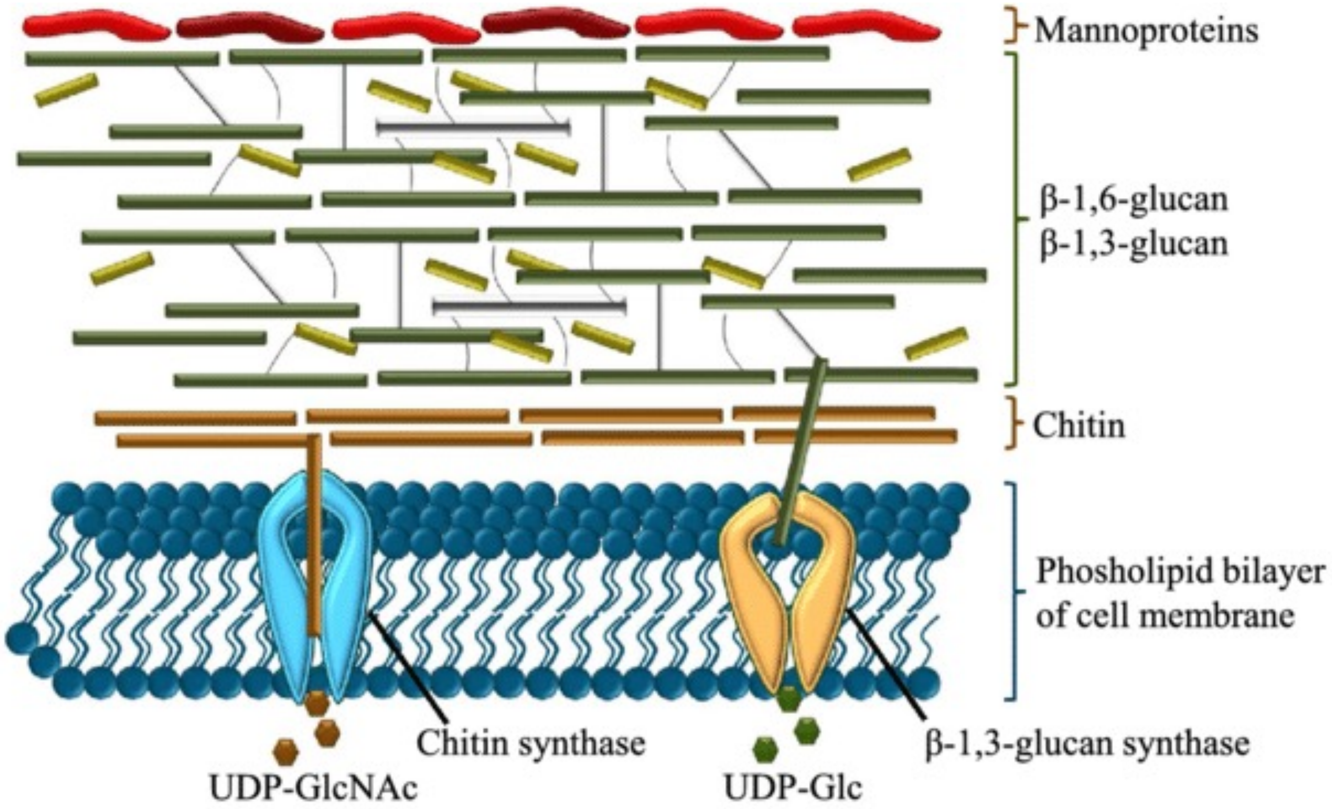
Figure 1.2 Transmission electron microscopy of ultrathin sections of a hyphal tip of *Fusarium* reveals intracellular fine structure. Layers of cell wall are shown in greater detail in lower image. M, Mitochondrion; V, vesicles; P, plasma membrane; MT, microtubules; SC, smooth Golgi cisternae; 1, 2, 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).)

1.3.1 The Fungal Cell Surface

In yeasts, the cell wall provides stability and protection to the cells and its structure comprises

1. polysaccharides (predominantly β-glucans for rigidity),
2. proteins (mainly mannoproteins on the outermost layer for determining porosity),
3. some lipid,
4. chitin (e.g. in bud scar tissue),
5. 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.



The chitin building block uridine diphosphate-N-acetylglucosamine

The glucan building block uridine diphosphate-N-glucose

Fesel, Philipp & Zuccaro, Alga. (2015). β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genetics and Biology. 90. 10.1016/j.fgb.2015.12.004.

1.3.2 Subcellular Architecture and Organelle Function

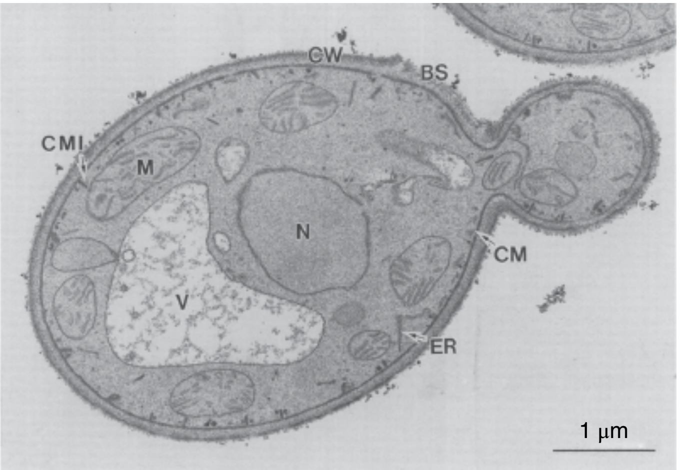


Figure 1.4 Electron micrograph of a typical yeast cell. CW, Cell wall; CM, cell membrane; CMI, cell membrane invagination; BS, bud scar; M, mitochondrion, N, nucleus; V, vacuole; ER, endoplasmic reticulum. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)

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

1.4 Fungal Nutrition and Cellular Biosyntheses

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.

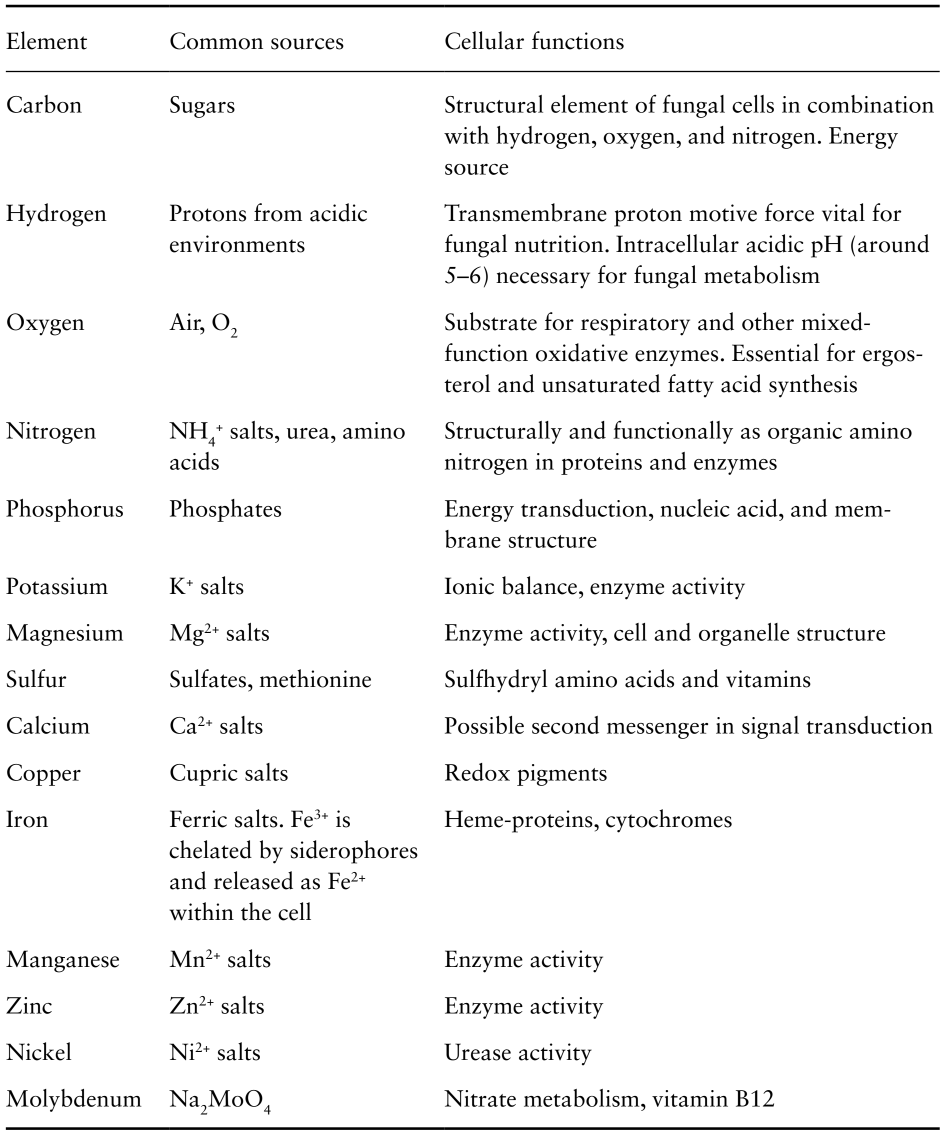
exceptions to this would include, for example, obligate symbionts such as the **vesicular-arbuscular mycorrhizal (VaM)** **(Q1)** fungi which require growth of a plant partner for cultivation.

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.

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

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
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).

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).

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).

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 ricestraw and in Hong Kong on cotton waste; and in Japan, the **shiitake mushroom** (*Lentinus edodes*) is cultivated on fresh oak logs.

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).

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

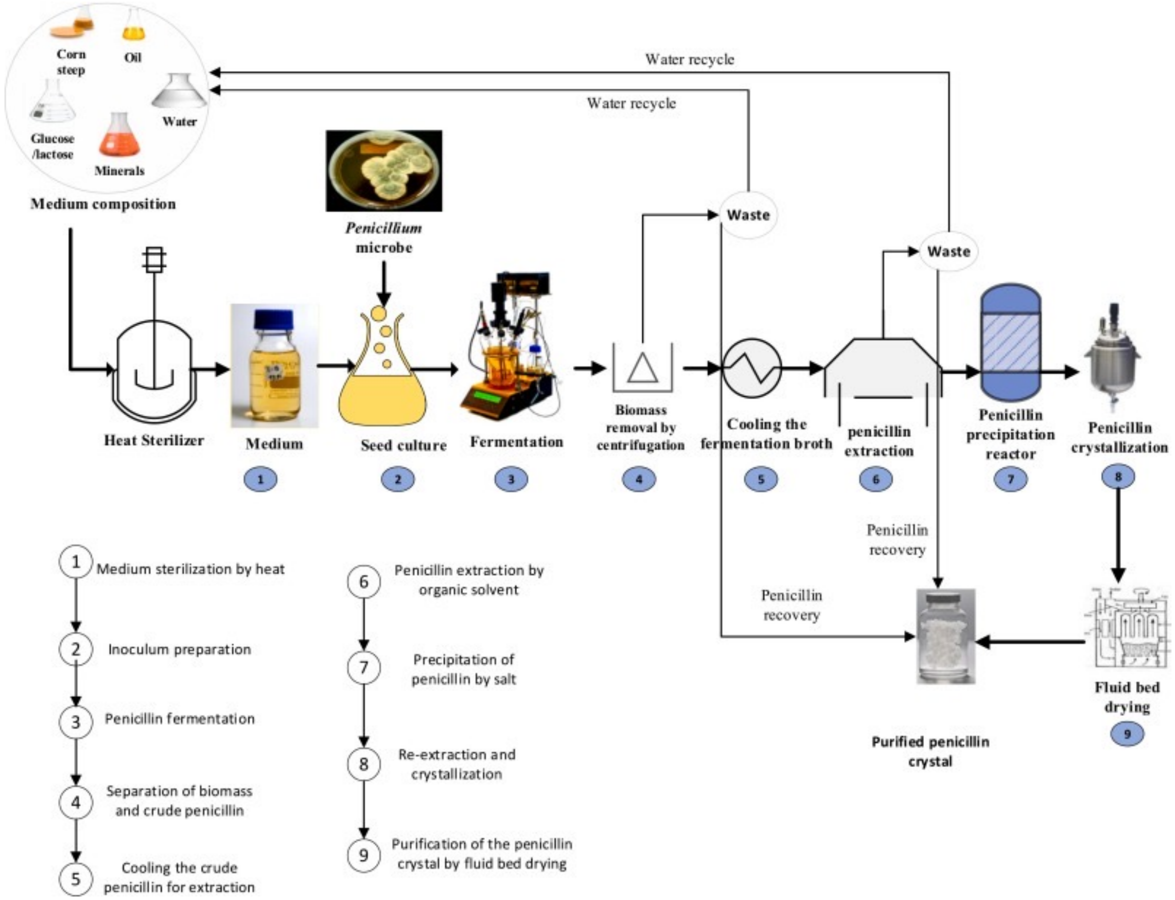
Components	Molasses	Malt wort	Wine must	Cheese whey	Corn steep liquor
Carbon sources	Sucrose Fructose Glucose Raffinose	Maltose Sucrose Fructose Glucose Maltotriose	Glucose Fructose Sucrose (trace)	Lactose	Glucose, other sugars
Nitrogen sources	Nitrogen compounds as unassimilable proteins. Nitrogen sources 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

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),
- KH2PO4 (1 g/L),
- (nh4)2so4 (12 g/L),
- CaCl2.2H2O (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**.



Md Ariful Haque, Nirmalendu Deb Nath, Tony Vaughn Johnston, Samuel Haruna, Jaehyun Ahn, Reza Ovissipour, Seockmo Ku,(2024), “Harnessing biotechnology for penicillin production: Opportunities and environmental considerations”, Science of The Total Environment, Volume 946,

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 sometime 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 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.

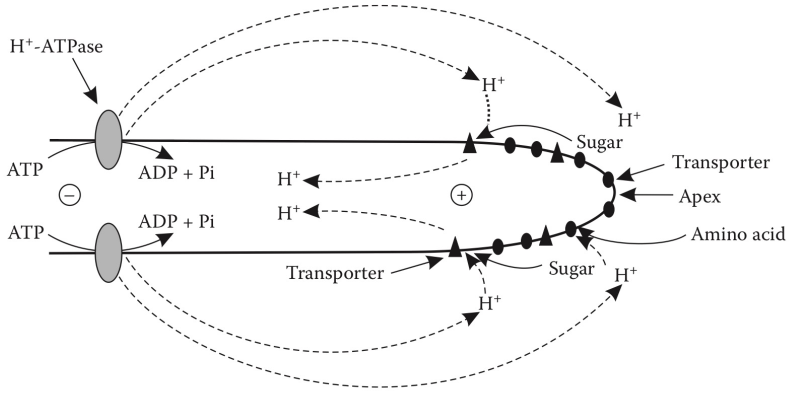
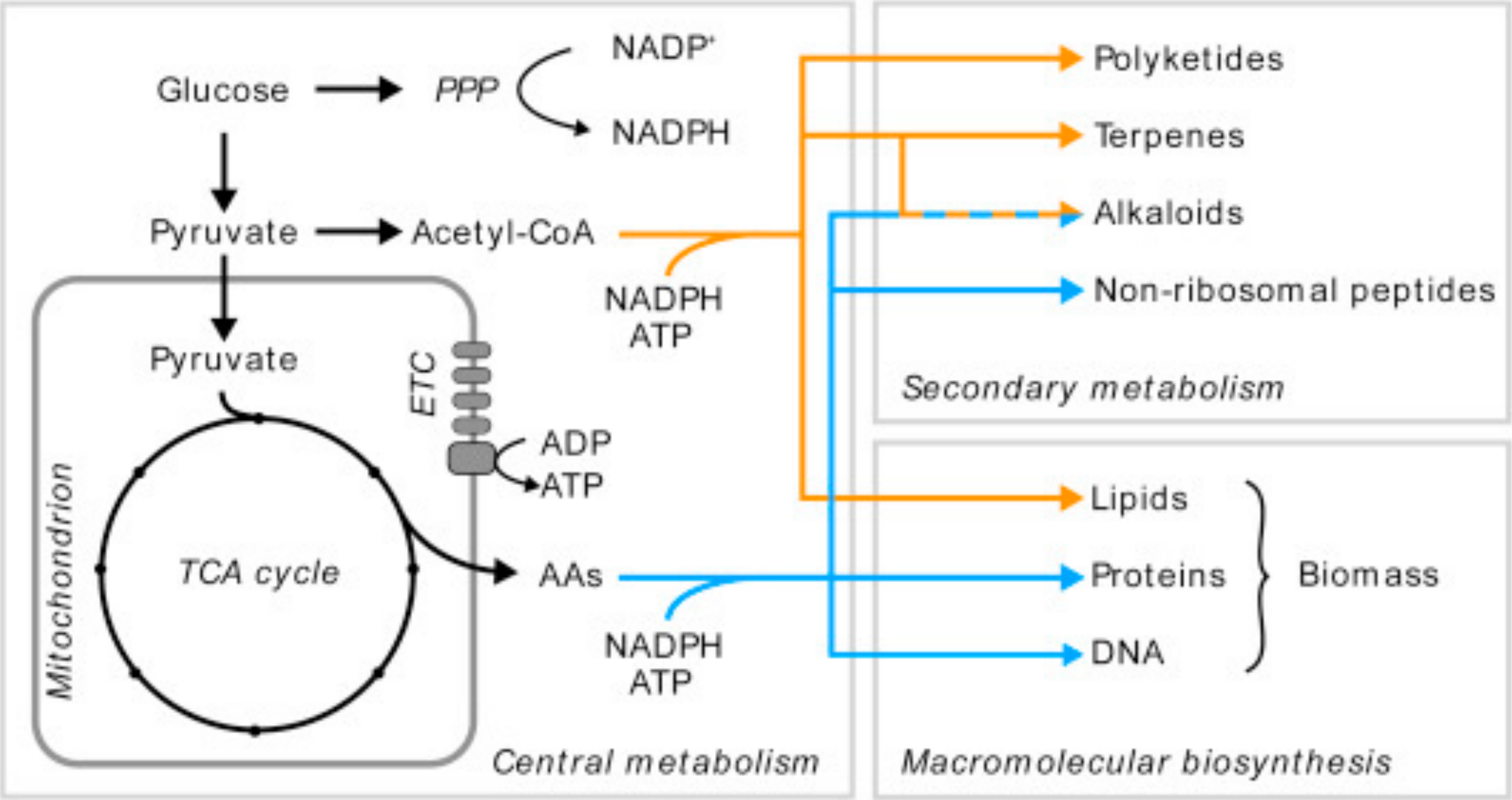


Figure 1.15 Diagram of proton pump and symport in hypha. (Based on Harold, F.M., *Fung. Genet. Biol.* 27, 128–133, 1999.)

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)

1.4.4 Overview of Fungal Biosynthetic Pathways



Ens Christian Nielsen, Jens Nielsen, Development of fungal cell factories for the production of secondary metabolites: Linking genomics and metabolism, Synthetic and Systems Biotechnology, Volume 2, Issue 1, 2017, Pages 5-12.

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

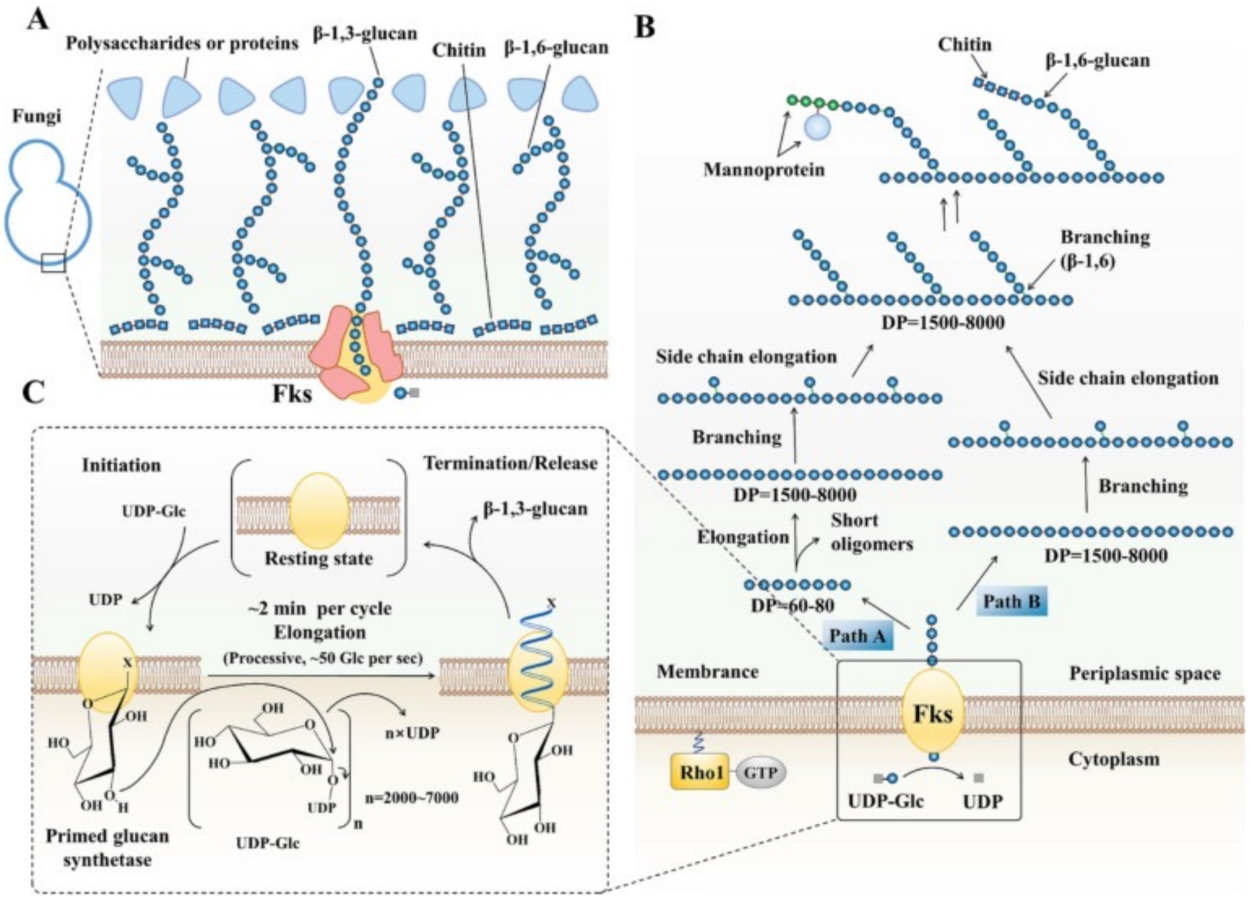
1.4.5 Fungal Cell Wall Growth

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:

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

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

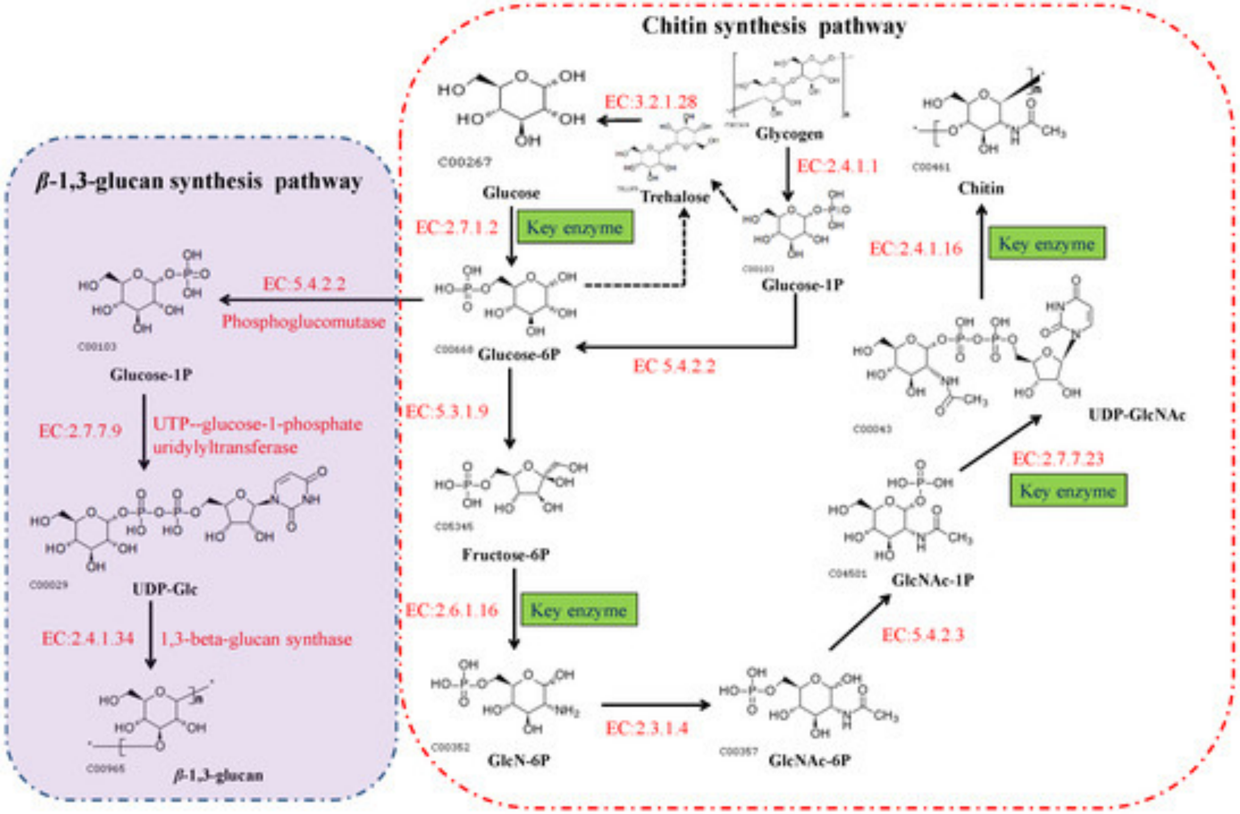
Glucan synthesis involves plasma membrane-associated glucan synthetases for assembly of β-1,3 linkages and β-1,6 branches of cell wall glucan.



Jin-Jin Liu, Yu-Ke Hou, Xin Wang, Xing-Tao Zhou, Jun-Yi Yin, Shao-Ping Nie, Recent advances in the biosynthesis of fungal glucan structural diversity, Carbohydrate Polymers, Volume 329, 2024, 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 preassembled 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.



Brauer, V. S., Pessoni, A. M., Freitas, M. S., Cavalcanti-Neto, M. P., Ries, L. N. A., & Almeida, F. (2023). Chitin Biosynthesis in *Aspergillus* Species. *Journal of Fungi*, *9*(1), 89. https://doi.org/10.3390/jof9010089

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:

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

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

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

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

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

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

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

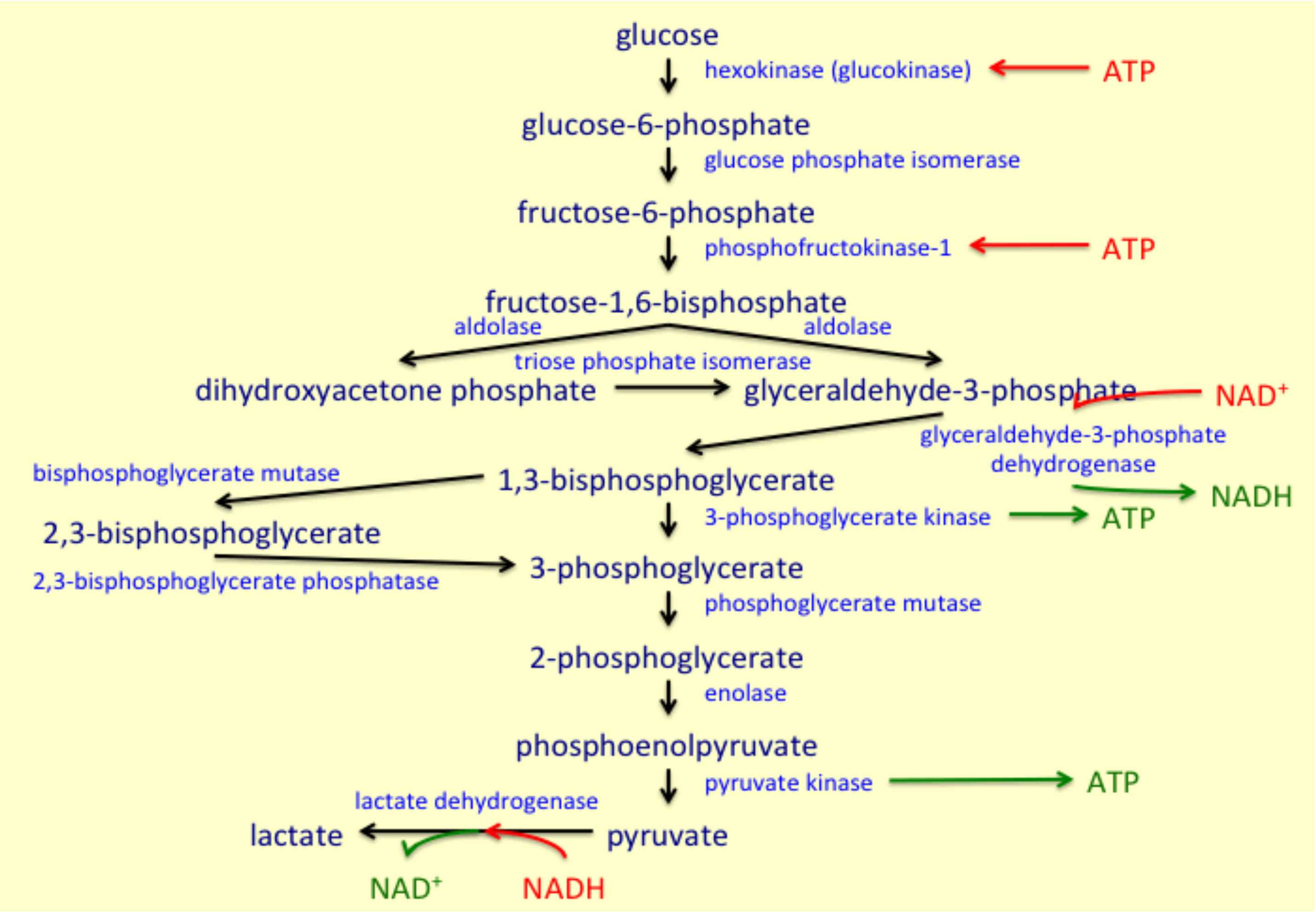
$\text{Chitin} \xrightarrow{\text{Chitinase}} N\text{-acetylglucosamine}$

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

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:



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

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 produc- tion 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*.

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

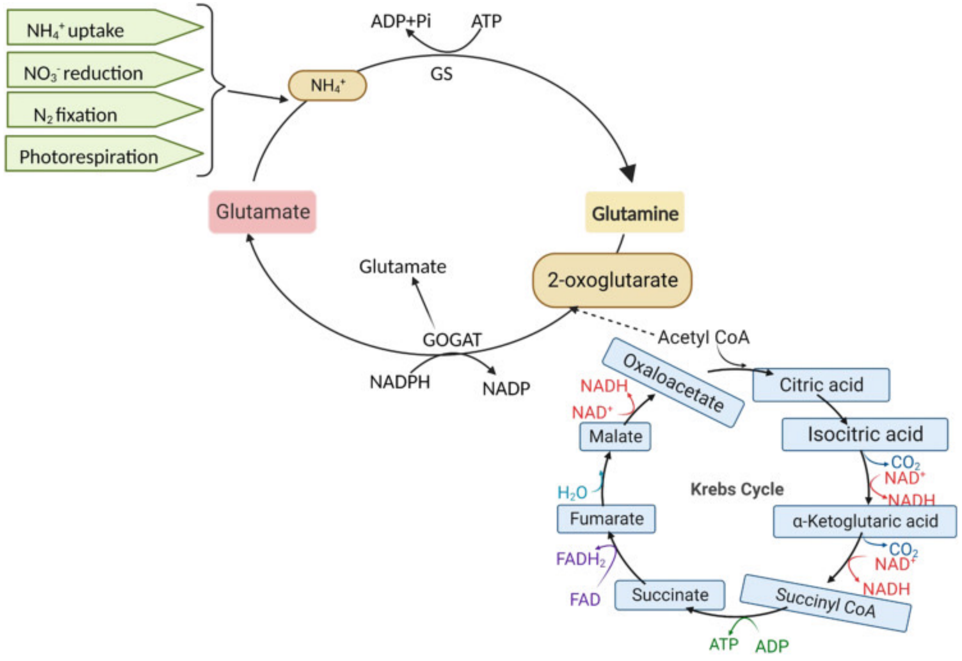
[1] The azide-sensitive pathway lacks proton transport capability and accepts electrons from NADH but not from succinate.

[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).

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 avail- able ammonium ions and the intracellular amino acid pools.



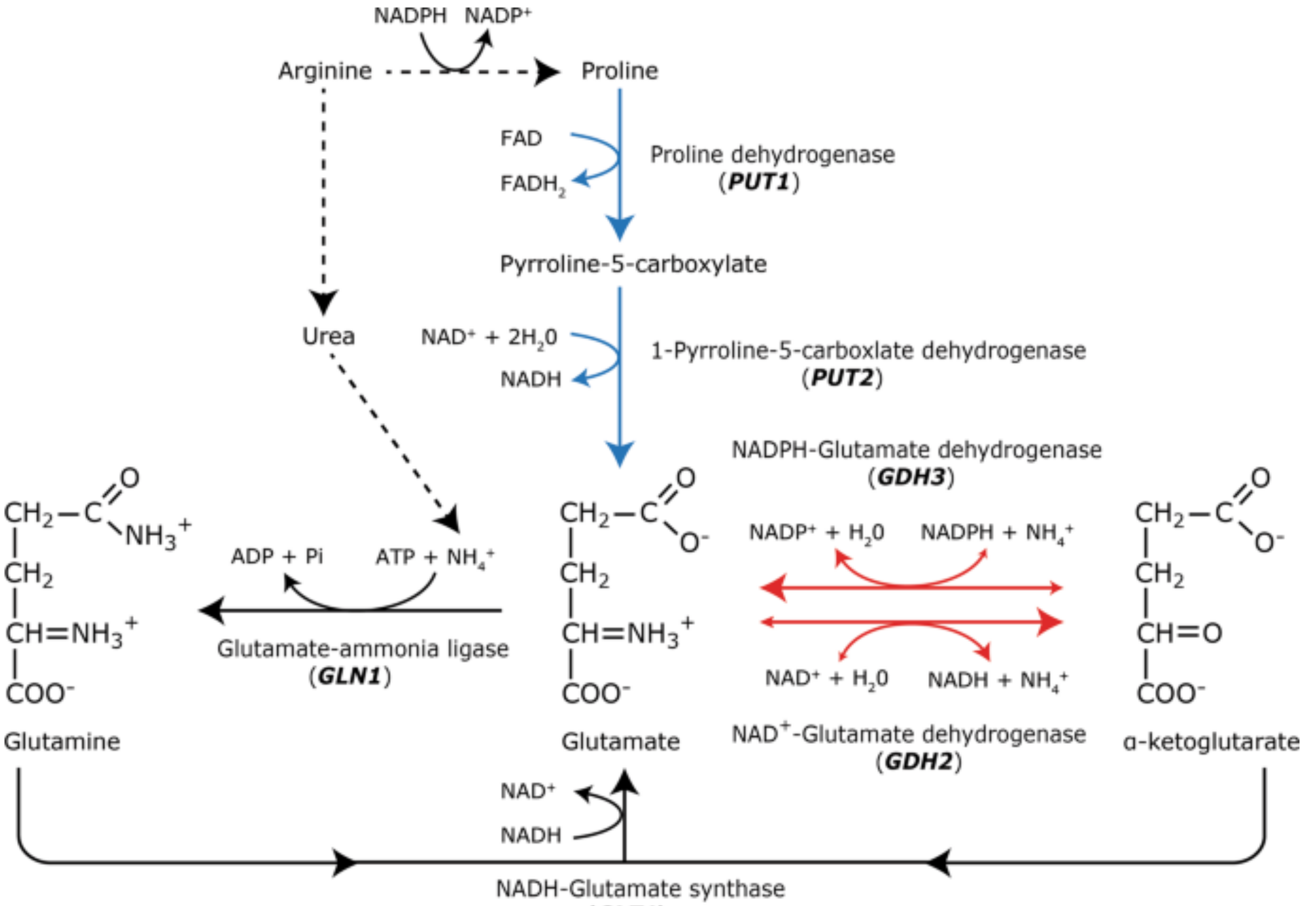
Rhydum Sharma, Richa Salwan, Vivek Sharma, Chapter 20 - Biology of nitrogen fixation in Frankia, Editor(s): Vivek Sharma, Richa Salwan, Ewa Moliszewska, David Ruano-Rosa, Małgorzata Jędryczka, The Chemical Dialogue Between Plants and Beneficial Microorganisms, Academic Press, 2023, Pages 271-281, ISBN 9780323917346, 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.



Han, TL., Cannon, R.D., Gallo, S.M. *et al.* A metabolomic study of the effect of *Candida albicans* glutamate dehydrogenase deletion on growth and morphogenesis. *npj Biofilms Microbiomes* **5**, 13 (2019). https://doi.org/10.1038/s41522-019-0086-5 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.

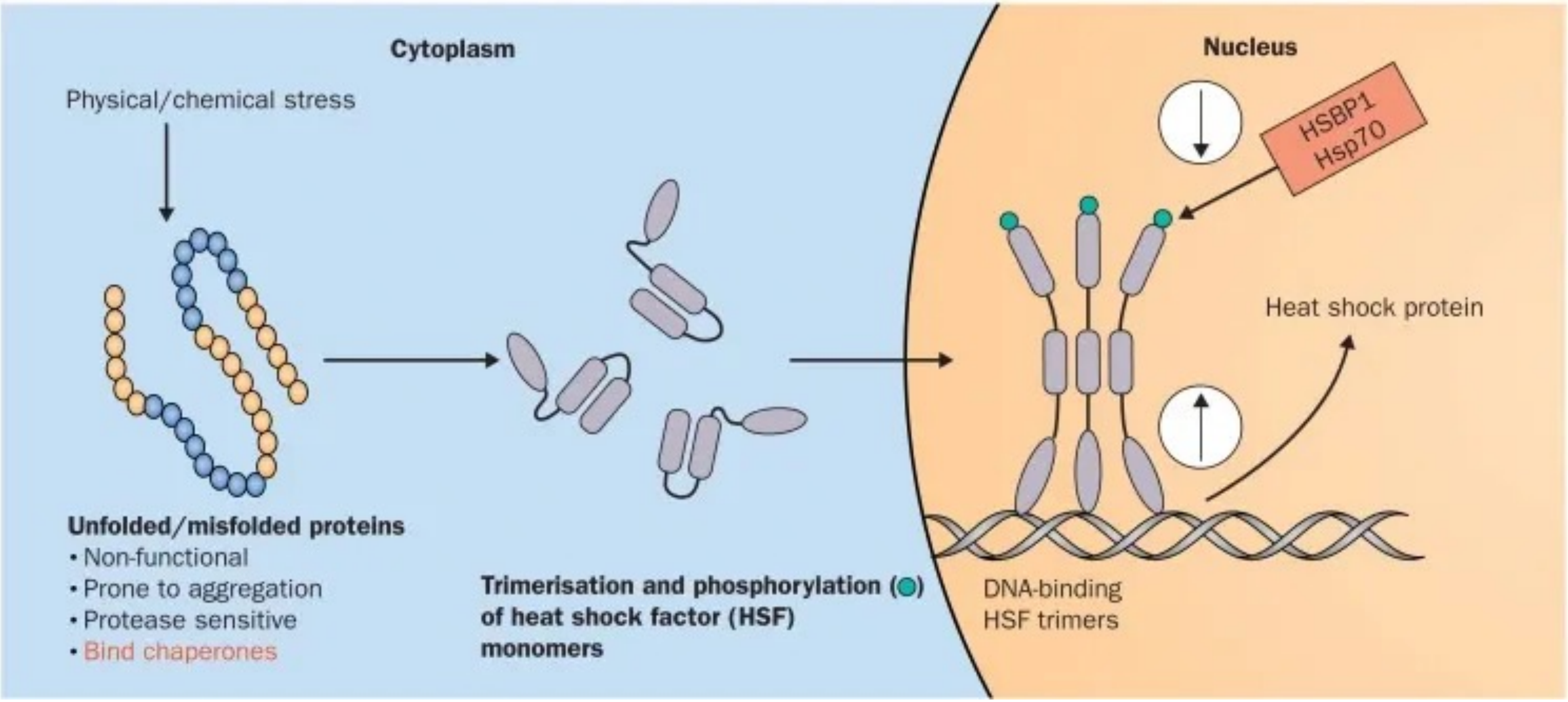
1.6 Fungal Growth and Reproduction

1.6.1 Physical Requirements for Growth

Temperature

- 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.

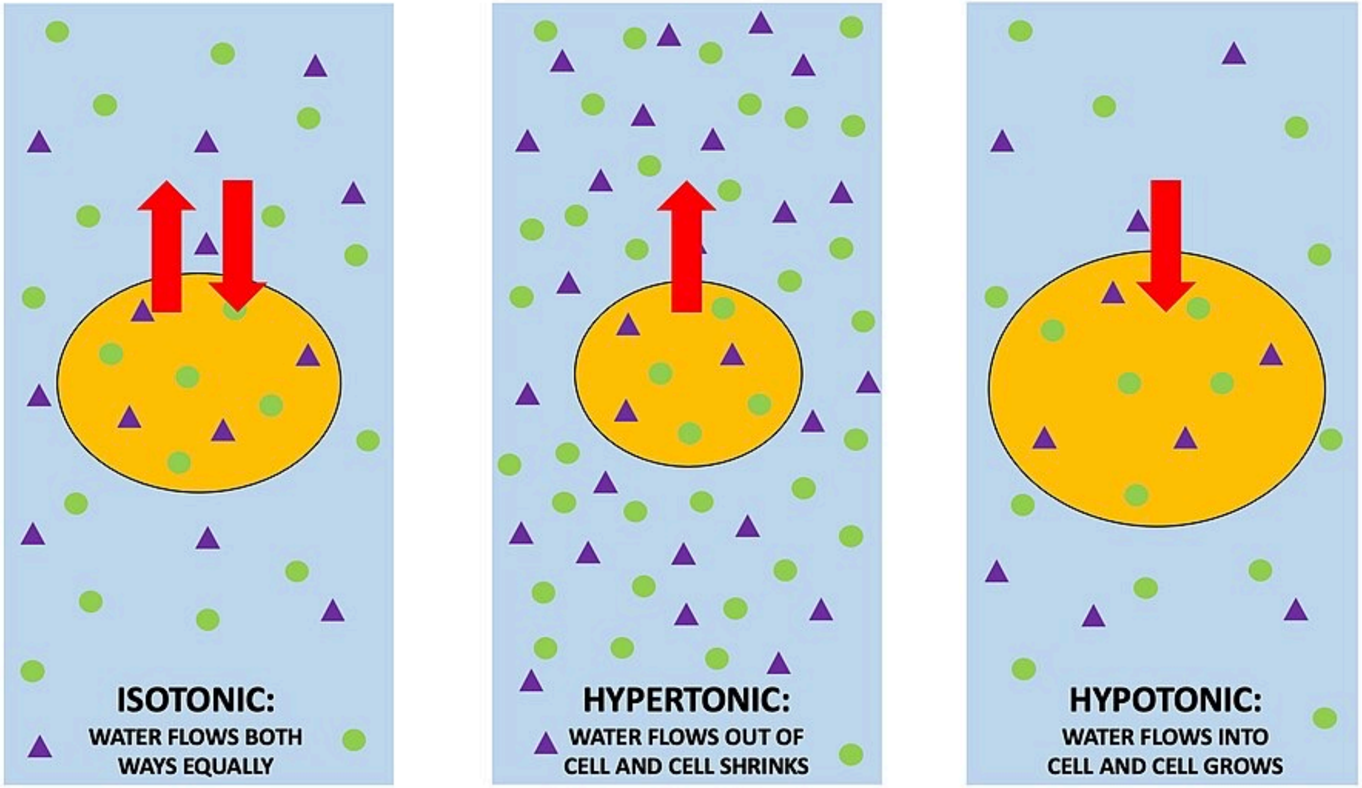
- Fungi, of course, have no means of regulating their internal temperature, and the higher the temperature, the greater the cellular damage, with cell viability declining when temperature increases beyond growth optimal levels.
- Temperature optima vary greatly in fungi, with those termed "thermotolerant" 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)** **(Q2)**– occurs. HSPs play numerous physiological roles, including thermoprotection.



https://vajiramandravi.com/upsc-daily-current-affairs/prelims-pointers/heat-shock-protein-70-hsp70/

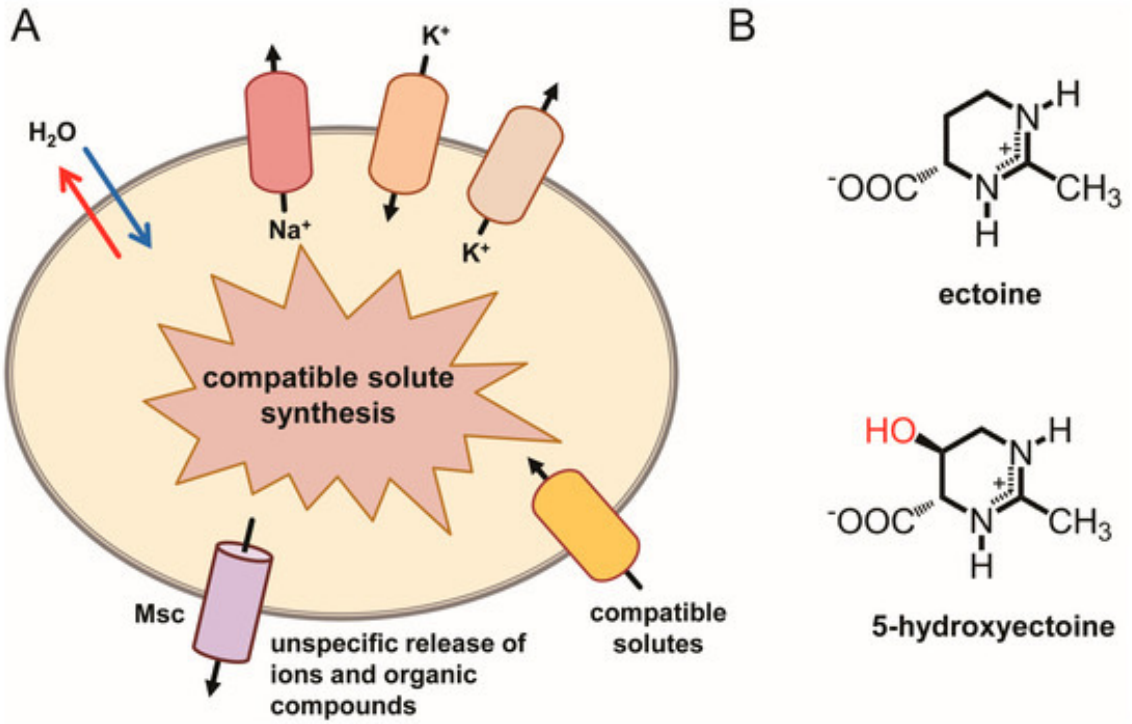
Osmotic pressure

- 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 ***hypoosmotic shock*** when introduced to a medium of higher osmotic potential (due to reducing the solute concentration).



Czech, L., Hermann, L., Stöveken, N., Richter, A. A., Höppner, A., Smits, S. H. J., Heider, J., & Bremer, E. (2018). Role of the Extremolytes Ectoine and Hydroxyectoine as Stress Protectants and Nutrients: Genetics, Phylogenomics, Biochemistry, and Structural Analysis. *Genes*, *9*(4), 177. https://doi.org/10.3390/genes9040177

- 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.

pH

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

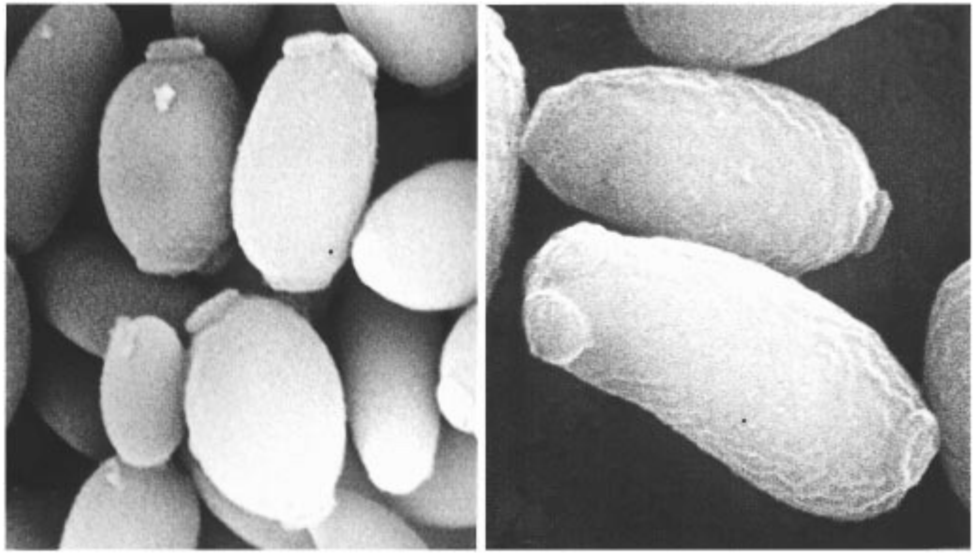
- Fungal growth involves transport and assimilation of nutrients, followed by their integration into cellular components, followed by biomass increase and eventual cell division (as in yeasts) or septation (as in higher fungi).
- The physiology of vegetative reproduction and its control in fungi has 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.

Table 1.11 Modes of vegetative reproduction in yeasts.

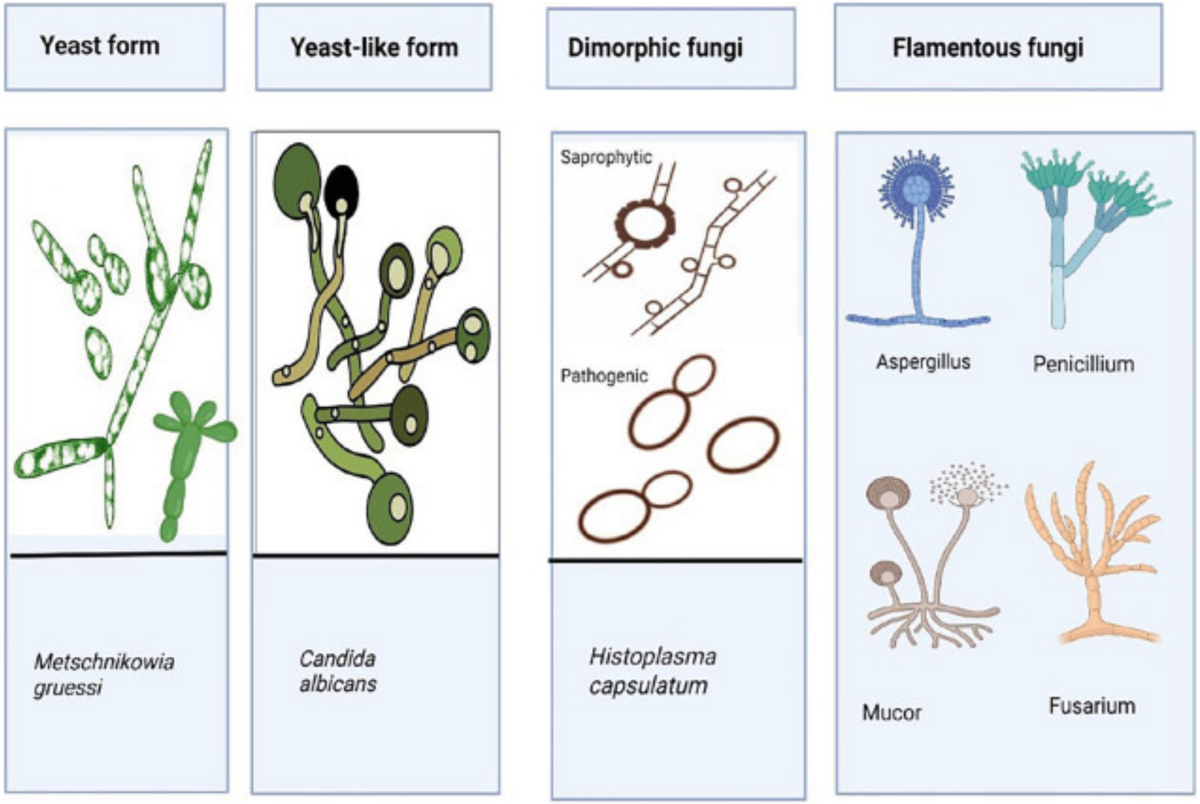
Mode	Description	Representative yeast genera
Multilateral budding	Buds may arise at any point on mother cell surface, but never again at the same site. Branched chaining may occasionally follow multilateral budding when buds fail to separate	*Saccharomyces*, *Zygosaccharomyces*, *Torulaspora*, *Pichia*, *Pachysolen*, *Kluyveromyces*, *Williopsis*, *Debaryomyces*, *Yarrowia*, *Saccharomycopsis*, *Lipomyces*
Bipolar budding	Budding restricted to poles of elongated cells (apiculate or lemon-shaped) along their longitudinal axis	*Nadsonia*, *Saccharomycodes*, *Haneniaspora*, *Wickerhamia*, *Kloeckera*
Unipolar budding	Budding repeated at same site on mother cell surface	*Pityrosporum*, *Trigonopsis*
Monopolar budding	Buds originate at only one pole of the mother cell	*Malassezia*
Binary fission	A cell septum (cell plate or cross-wall) is laid down within cells after lengthwise growth and which cleaves cells into two	*Schizosaccharomyces*
Bud fission	Broad cross-wall at base of bud forms which separates bud from mother	Occasionally found in *Saccharomycodes*, *Nadsonia*, *Pityrosporum*
Budding from stalks	Buds formed on short denticles or long stalks	*Sterigmatomyces*
Ballistoconidiogenesis	Ballistoconidia are actively discharged from tapering outgrowths on the cell	*Bullera*, *Sporobolomyces*
Pseudomycelia	Cells fail to separate after budding or fission to produce a single filament. Pseudomycelial morphology is quite diverse and extent of differentiation variable depending on yeast species and growth conditions	Several yeast species may exhibit "dimorphism," e.g. *Candida albicans*, *Saccharomycopsis figuligera*. Even *S. cerevisiae* exhibits pseudohyphal growth depending on conditions

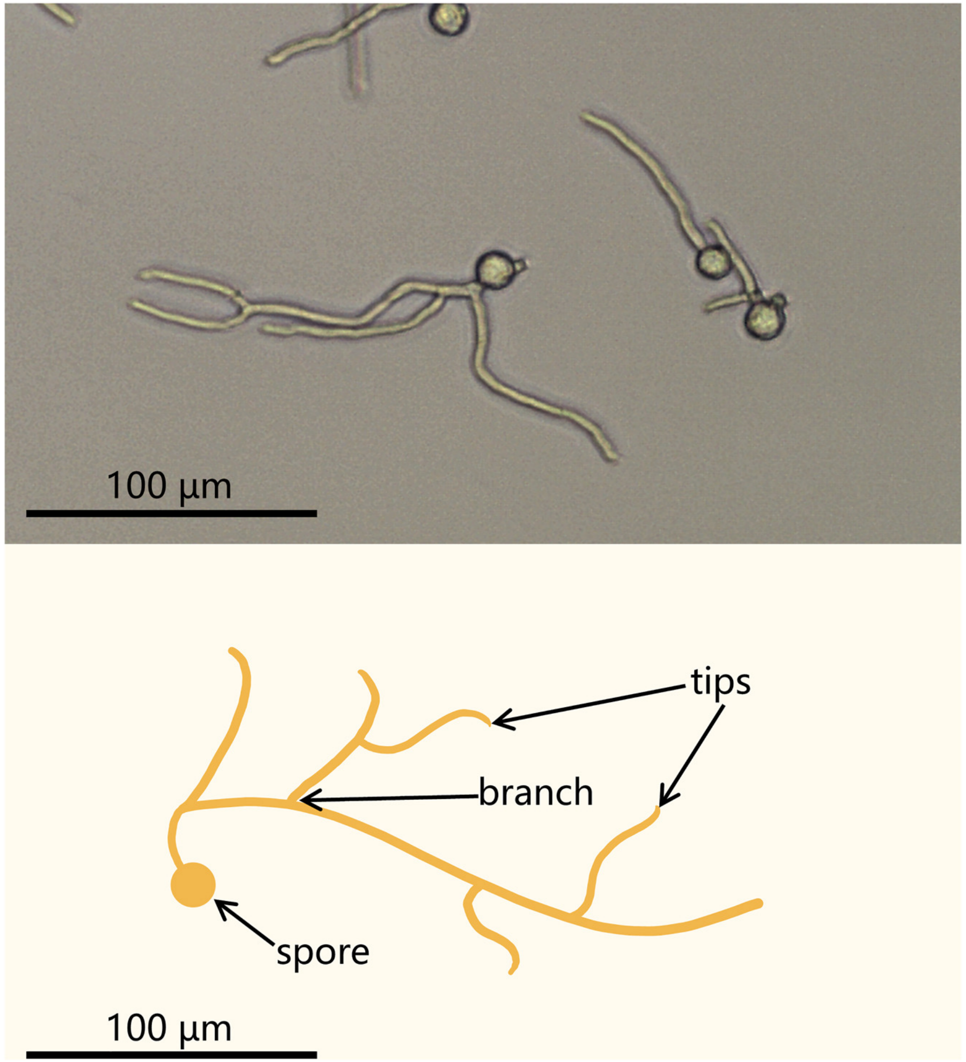
Adapted from Walker (1998).



Herrero, Ana & López, M. & Fernández-Lago, Luis & Domínguez, A. (1999). Candida albicans and Yarrowia lipolytica as alternative models for analysing budding patterns and germ tube formation in dimorphic fungi. Microbiology (Reading, England). 145 (Pt 10). 2727-37. 10.1099/00221287-145-10-2727.

- In certain yeast species, the presence or absence of pseudohyphae and true hyphae can be used as taxonomic criteria (e.g. the ultrastructure of hyphal septa may discriminate between certain ascomycetous yeasts). some yeasts grow with true hyphae initiated from *germ tubes* (e.g. *Candida albicans*), but others (including *S. cerevisiae*) may grow in a **pseudohyphal** fashion when starved of nutrients or when subjected to environmental stress.
- In other words, cells can revert to yeast unicellular growth in more conducive growth conditions, indicating that a filamentous mode of growth represents an adaptation by yeast to foraging when nutrients are scarce.



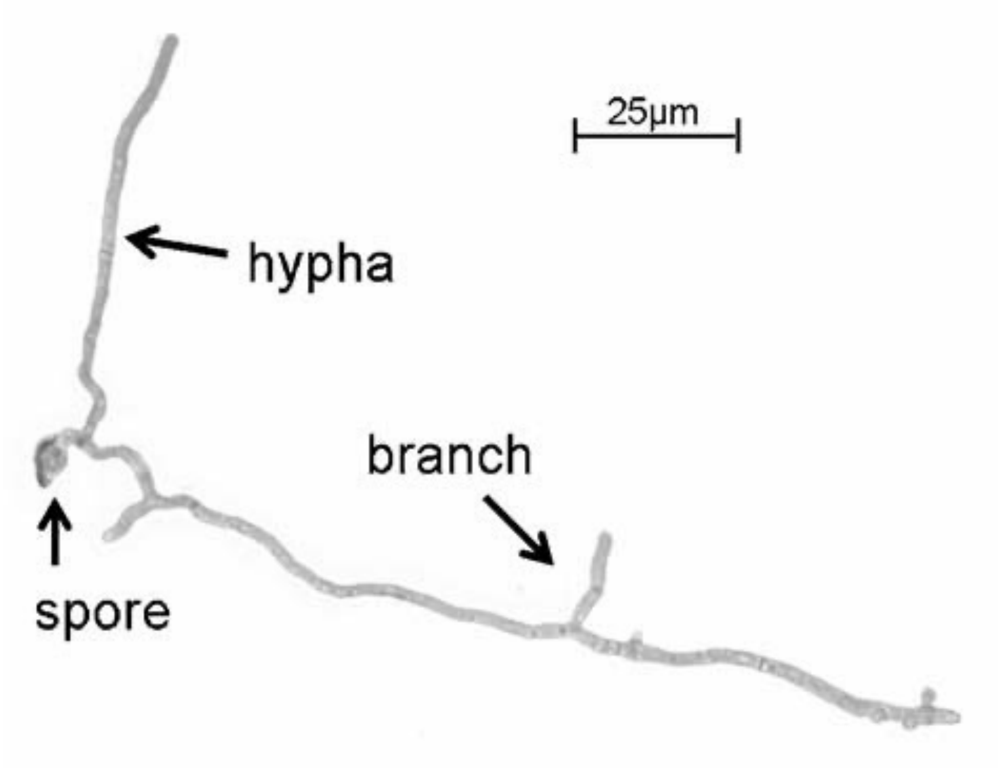


Dinius, A., Kozanecka, Z., Hoffmann, K. & Krull, R. (2024). Intensification of bioprocesses with filamentous microorganisms. *Physical Sciences Reviews*, *9*(2), 777-823. https://doi.org/10.1515/psr-2022-0112

- What constitutes a cell in filamentous fungi is ambiguous. The apical compartments of higher filamentous fungi are often multinucleate, and so the process of nuclear replication and segregation into a newly extended **septated hyphal** compartment is known as the duplication cycle.
- Thus, *Aspergillus nidulans* apical compartments contain approximately 50 nuclei per compartment produced during a 2-hour duplication cycle period. continued septation results in the formation of subapical compartments containing fewer nuclei.
- Hyphae also commonly branch, usually at some distance behind the leading growing hyphal tip and often just behind a septum in higher fungi.

Rates of branching and tip growth are related to the cytoplasmic volume. Thus, the hyphal growth unit is a measure of the average length of hypha required to support hyphal tip growth.

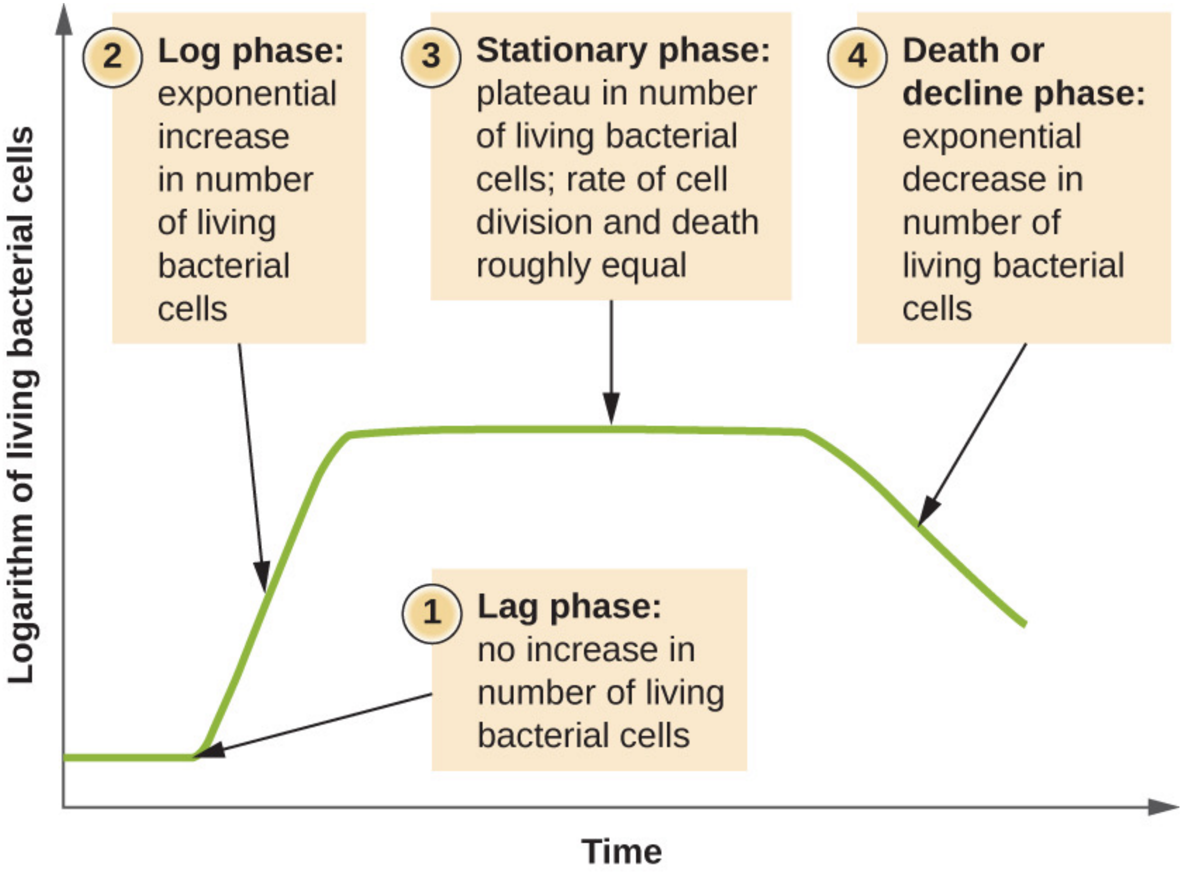
It can be calculated from microscopic preparations growing on agar media as the ratio between the total length of mycelium and the total number of tips. The ratio becomes constant after the initial stages of growth, and is characteristic of each fungal species or strain.



Grimm, L & Kelly, Sven & Krull, Rainer & Hempel, D. (2006). Morphology and productivity of filamentous fungi. Applied microbiology and biotechnology. 69. 375-84. 10.1007/s00253-005-0213-5.

1.6.3 Population Growth

When yeast or fungal cells are inoculated into a nutrient medium and incu- bated under optimal physical growth conditions, a typical batch growth curve will result comprising lag, exponential, and stationary phases.



The *lag phase* represents a period of zero population growth and reflects the time required for inoculated cells to adapt to their new physical and chemical growth environment (by synthesizing ribosomes and enzymes).

The *exponential phase* is a period of logarithmic cell (or mycelial biomass in the case of filamentous growth) doublings and constant, maximum specific growth rate (μmax, in dimensions of reciprocal time, per hour), the precise value of which depends on the prevailing growth conditions. If growth is optimal and cells double logarithmically, then

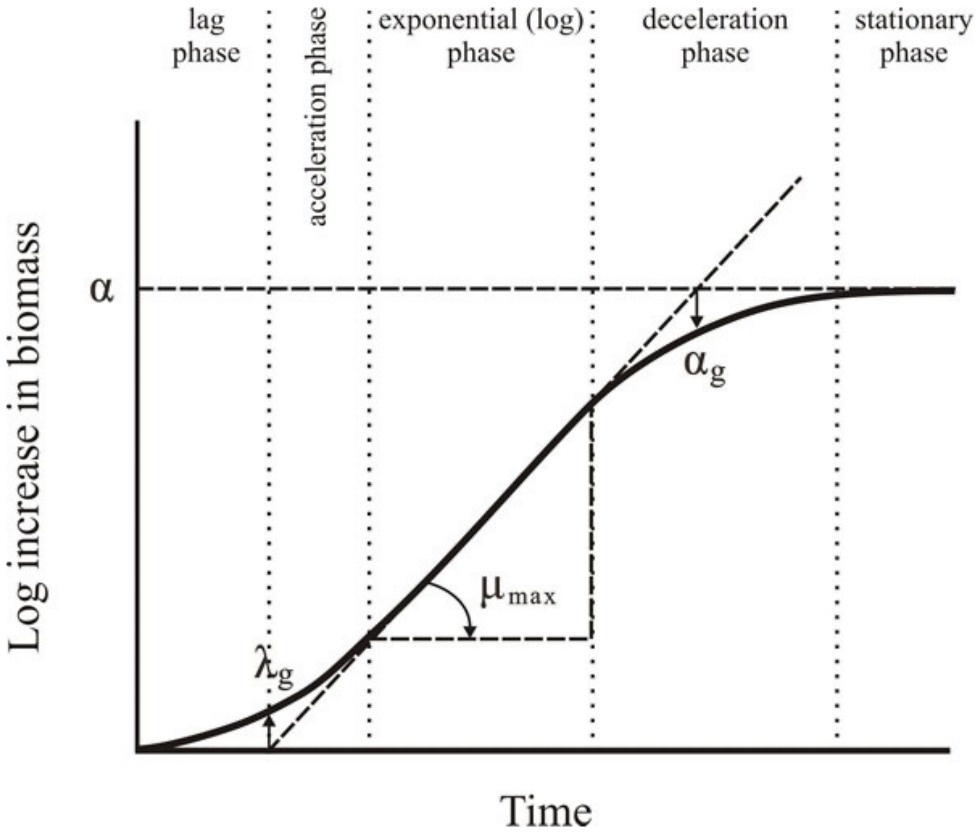
$$\frac{dx}{dt} = \mu x$$

and when integrated this yields

$$lnx - lnx_0 = \mu t$$

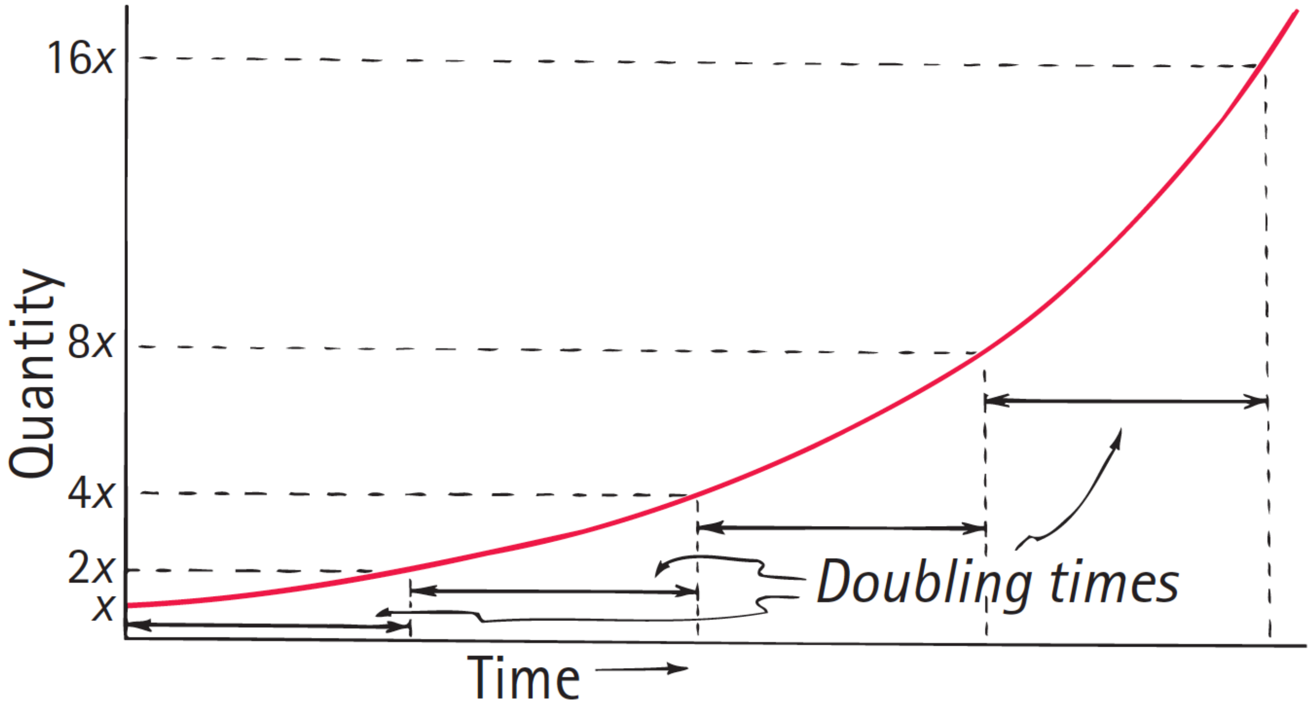
or

$$x = x_0 e^{\mu t}$$



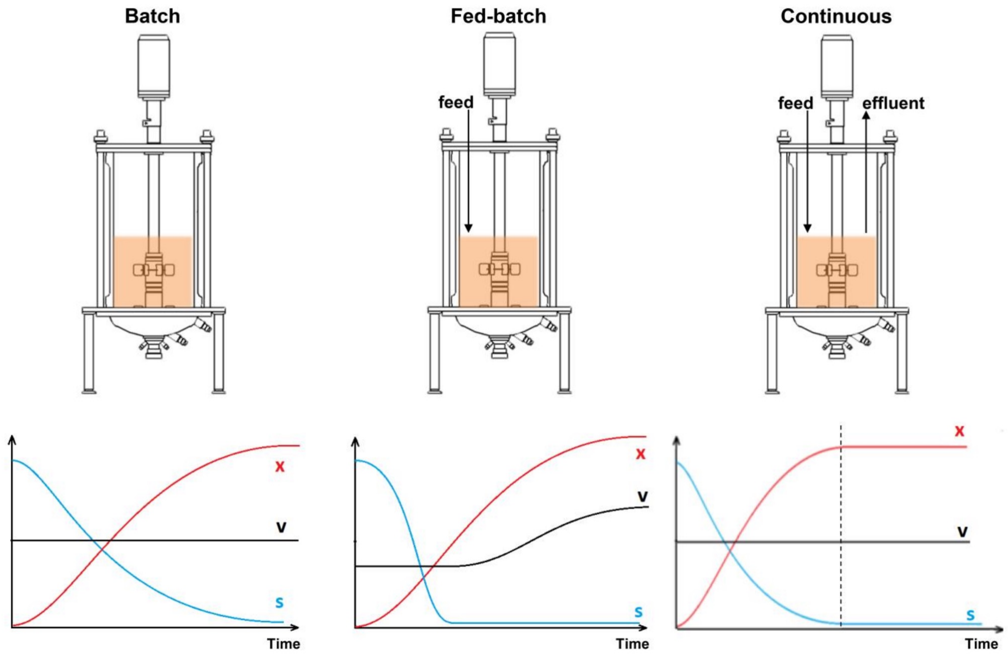
The hypothetical growth curve (‘classic growth curve’) for a cell population grown in submerged liquid culture. parameters μ_{max} (maximum specific growth rate), **λ_g (lag phase)**, α_g (end of exponential phase), and α (asymptote; which is the straight line that is the limiting value of the curve); λ_g is determined as the time where the tangent crosses the starting level, α_g is determined as the time where the tangent crosses the asymptote.

which is the fundamental equation for exponential batch growth. according to these kinetic expressions, a plot of ln *x* versus time is linear, with the slope being μ_{max}. calculation of the doubling time (t_d) of a yeast or fungal culture can be acheved from knowledge of μ_{max} as follows:

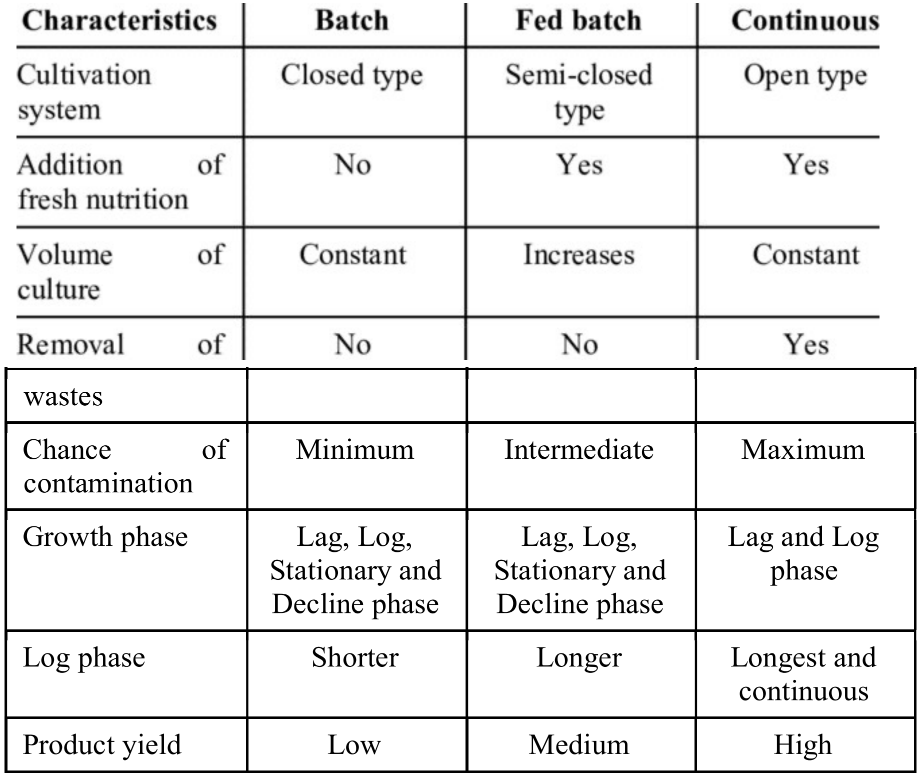


during the exponential phase of balanced growth, cells are undergoing primary metabolism, explicitly those metabolic pathways that are essential for growth of the cell.

Industrial fermentations requiring maximum cell biomass production or the extraction of primary metabolites or their products therefore aim to extend this phase of growth, often via **fed-batch** culture (incremental nutrient feeding) or **continuous culture** techniques (continuous nutrient input with concomitant withdrawal of the biomass suspension).



Bolmanis, E., Dubencovs, K., Suleiko, A., & Vanags, J. (2023). Model Predictive Control—A Stand Out among Competitors for Fed-Batch Fermentation Improvement. *Fermentation*, *9*(3), 206. https://doi.org/10.3390/fermentation9030206



Characteristics	Batch	Fed batch	Continuous
Cultivation system	Closed type	Semi-closed type	Open type
Addition of fresh nutrition	No	Yes	Yes
Volume of culture	Constant	Increases	Constant
Removal of wastes	No	No	Yes
Chance of contamination	Minimum	Intermediate	Maximum
Growth phase	Lag, Log, Stationary and Decline phase	Lag, Log, Stationary and Decline phase	Lag and Log phase
Log phase	Shorter	Longer	Longest and continuous
Product yield	Low	Medium	High

Gouthami, Y. & Gidagiri, Praveen & Bhuvaneshwari, G. & lekshmi, Geetha & Shameena, Ms. (2024). BIOETHANOL PRODUCTION FROM AGRICULTURAL WASTE. 10.58532/V3BCAG15P4CH8.

Following the exponential phase, cells enter a period of zero population growth rate, the stationary phase, in which the accumulated fungal or yeast biomass remains relatively constant and the specific growth rate returns to zero. after prolonged periods in stationary phase, individual cells may die and autolyze (see below).

The stationary phase may be defined as cellular survival for prolonged periods (i.e. months) without added nutrients.

In addition to nutrient deprivation, other physiological causes may promote entry of fungal cells into stationary phase, including

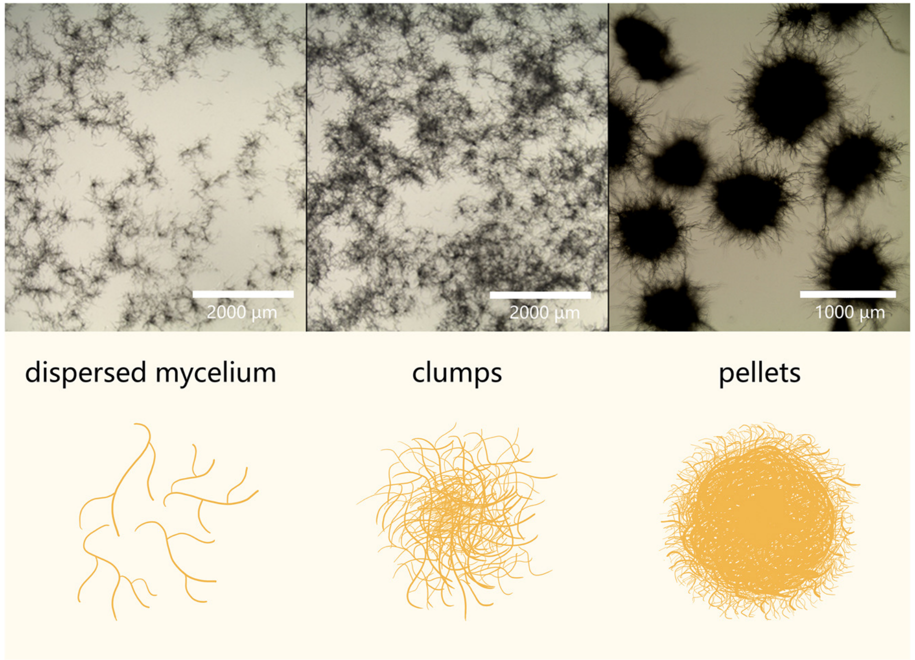
toxic metabolites (e.g. ethanol in the case of yeasts), low pH, high CO_2, variable O_2, and high temperature.

during the stationary phase of unbalanced growth, fungi may undergo secondary metabolism, specifically initiating metabolic pathways that are not essential for growth of cells but are involved in the survival of the organism.

The industrial production of **fungal secondary metabolic compounds** such as penicillin and the ergot alkaloids therefore involves the controlled maintenance of cell populations within a stationary phase of growth.

recently, *S. cerevisiae* has been grown at near-zero growth rates in specialized cultivation systems called ***retentostats***, in which cells can retain high metabolic capacities and stress resistance. retaining yeast cells under such maintenance-energy metabolic conditions may have relevance for industrial bioprocesses.

Filamentous fungi tend to grow as floating surface pellicles when cultivated in static liquid culture. In agitated liquid culture, fungi grow either as dispersed filamentous forms, or as pellets of aggregated mycelia, subject to species, inoculum size, agitation rate, and nutrient availability.

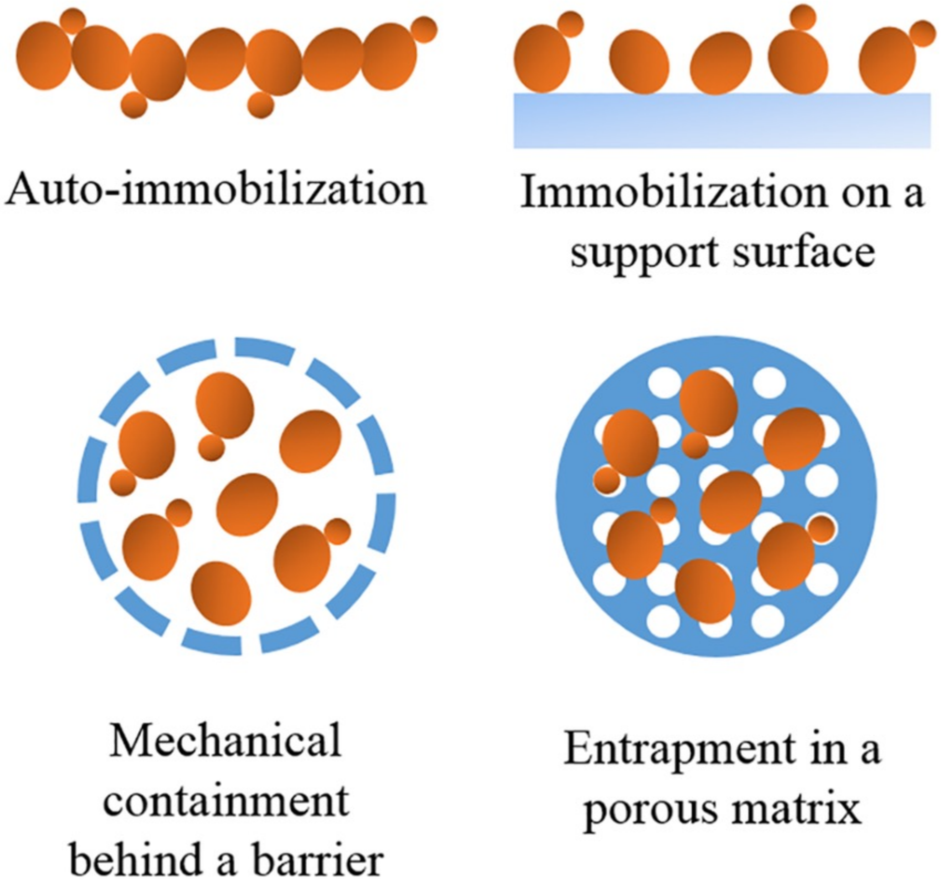


Dinius, A., Kozanecka, Z., Hoffmann, K. & Krull, R. (2024). Intensification of bioprocesses with filamentous microorganisms. *Physical Sciences Reviews*, *9*(2), 777-823. https://doi.org/10.1515/psr-2022-0112

- different growth forms will locally experience different micro-environmental conditions, which will affect fungal physiology and hence fermentation processes.
- In fungal biotechnology, cell morphology may directly influence fermentation progress.

For example, **the rheological properties** of the growth medium, **oxygen transfer**, and **nutrient uptake** may adversely affect bioproduct formation.

- In the natural environment, fungal populations interact frequently to form often complex dynamic communities, which in turn shape ecosystem functioning.
- understanding the population growth and functional (physiological) responses of fungi to their local environment is key to the development of predictive models and to our general understanding of the resilience and resistance of fungal communities to environmental perturbations such as climate change.



Moreno-García, Jaime & García-Martínez, Teresa & Mauricio, Juan & Moreno, Juan. (2018). Yeast Immobilization Systems for Alcoholic Wine Fermentations: Actual Trends and Future Perspectives. Frontiers in Microbiology. 09. 10.3389/fmicb.2018.00241.

yeast or fungal cell immobilization onto inert carriers has many advantages over free cell suspension culture in industrial processes.

cells may be successfully immobilized either by entrapment, aggregation, containment, attachment or deposition. Fungal biofilms represent a natural form of cell immobilization resulting from cellular attachment to solid support materials.

yeast biofilms have several practical applications in fermentation biotechnology and are also medically important with regard to colonization of human tissue.

regarding the former case, with dimorphic yeasts such as *Kluyveromyces marxianus*, filamentous cells with a large surface area may be better suited to immobilization compared with ellipsoidal unicellular yeast forms with a low surface area.

In this latter case of pathogenic yeast biofilms, *C. albicans* has been shown to adhere to surgical devices such as heart pacemakers and catheters, human epithelial cells, and dental acrylic.

1.6.4 Fungal Cell Death

An understanding of the death of fungal cells is important from a fundamental viewpoint because fungi, especially yeasts, represent valuable model systems for the study of cellular aging and **apoptosis** (programed cell death).

recycling and redeployment of cellular material also helps drive the apical growth of filamentous fungi and the mycelium explores and extends through the environment.

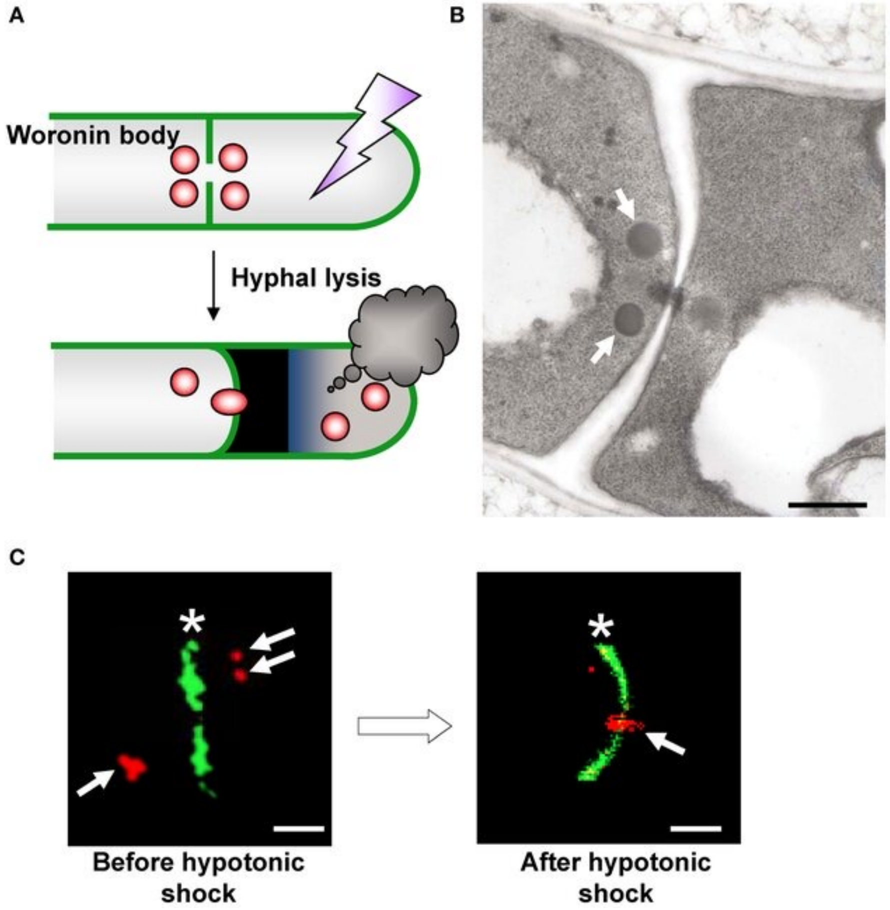
From a practical perspective, cell death in fungi is pertinent in relation to the following situations:

- industrial fermentation biotechnology (where high culture viabilities are desired),
- food preservation (regarding inhibition of spoilage fungal growth),
- food production (promotion of cellular autolysis for yeast extracts),
- and clinical mycology (where fungal death is the goal in treatment of human mycoses).

numerous physical, chemical, and biological factors influence fungal cell death, which may be defined as complete and irreversible failure of cells to reproduce.

Fungi will die if confronted with

- excessive heat,
- extreme cold,
- high-voltage electricity,
- ionizing radiation,
- high hydrostatic and osmotic pressures,
- and if exposed to chemical or biological fungicidal agents.



Maruyama, Jun-ichi & Kitamoto, Katsuhiko. (2013). Expanding functional repertoires of fungal peroxisomes: contribution to growth and survival processes. Frontiers in Physiology. 4. 177. 10.3389/fphys.2013.00177.

When the cells' physiological protection responses are insufficient to counteract the cellular damage caused by physical stress, cells will die. In industrial situations, physical treatments can be used to eradicate contaminant fungi. For example, yeasts exposed to elevated temperatures may lead to their thermal death, and this is exploited in the pasteurization of foods and beverages to kill spoilage yeasts.

There are numerous chemical factors influencing survival of fungi.

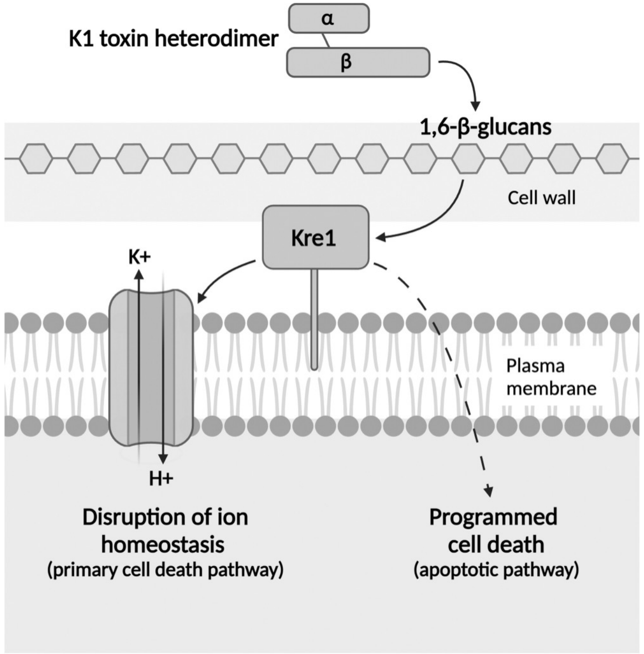
- several external chemical agents act as fungicides, including toxic organic compounds, oxygen free radicals, and heavy metals.
- chemical preservatives are commonly employed as antifungal agents in foodstuffs, including weak acids such as sorbic, benzoic, and acetic acids.
- These agents, which are generally fungistatic rather than fungicidal, act by dissipating plasma membrane proton gradients and depressing cell pH when they dissociate into ions in the yeast cytoplasm.
- similarly, sulfur dioxide which has long been used to eliminate undesirable yeasts (and bacteria) from wine, dissociates within the yeast cell to SO_2 and H_2SO_3 resulting in a decline in intracellular pH and this forms the basis of its antizymotic action. Fungicidal acids include medium-chain fatty acids (e.g. decanoic acid), which may cause rapid cell death of yeasts and fungi by disruption of cell membrane integrity.
- endogenous chemical factors such as ethanol and other toxic metabolites (e.g. acetaldehyde) produced by fermentative activity, excessive intracellular acidity or alkalinity, or inability to protect against oxidative damage or sequester toxic metals, may also prove lethal to fungi. If fungal cells are unable to detoxify or counteract detrimental effects of chemicals, they may die.

examples of lethal biotic interactions with fungi include

- direct ingestion (by insects, protozoa),
- engulfment and lysis (by mycoparasitizing fungi),
- direct predation (by haustoria-mediated processes),
- and intoxication (by killer toxin- producing yeasts).

Killer yeasts secrete proteinaceous toxins that are lethal to other yeasts but to which the killers themselves are immune. several yeast species have now been identified as possessing killer character, but the best known is the K1 system in *S. cerevisiae*.

The K1 toxin from this species acts by binding to cell wall receptors in sensitive yeast cells, followed by plasma membrane channel formation. This latter event causes disruption of membrane permeability, which leads to the death of sensitive cells.



Schematic of K1 killer toxin activity upon binding to sensitive cells, including its primary (cell wall) and secondary (plasma membrane) targets. Toxin activity proceeds either through the primary, ion homeostasis disrupting pathway resulting in H+ influx and K+ efflux, or an alternate apoptotic pathway (proceeding through a currently unknown mechanism), resulting in programmed cell death.

Chan, A., Hays, M. and Sherlock, G. (2024), The Viral K1 Killer Yeast System: Toxicity, Immunity, and Resistance. Yeast, 41: 668-680. https://doi.org/10.1002/yea.3987

With regard to endogenous biotic factors influencing fungal cell survival, several physiological, morphological, genetic, and biochemical events may take place leading to “self-inflicted” death.

For example, fungal autolysis may be described as cellular self-digestion and occurs when endogenous (vacuolar) hydrolytic enzymes, notably proteases and carbohydrases, cause dissolution of cytoplasmic proteins and cell wall polysaccharides, respectively.

autolytic enzymatic activity is encouraged during the production of yeast extracts in the food industry by using high temperatures (e.g. 45 °c), salt (to encourage plasmolysis) and solvents (to promote lipid dissolution). exogenous hydrolytic enzymes such as papain can also be used to accelerate cell wall breakdown.

Genetic factors also influence fungal cell death. For example,

- Cells may commit suicide following DNA damage, presumably to avoid the risk of producing genetically altered progeny.
- Cellular aging and apoptotic cell death has been widely studied in yeasts, especially *S. cerevisiae*, which is a valuable model organism for understanding molecular genetic basis of the aging process in eukaryotic cells.
- Beyond a certain finite limit (termed the hayflick limit) of cell division cycles (generally around 20 in *S. cerevisiae*), this yeast can generate no further progeny and cells enter a senescent physiological state leading to death.
- Aged and senescent populations of this yeast can be isolated, together with mutants displaying age-related phenotypes. In *S. cerevisiae*, ***UTH*** **(youth) genes (Q3)** have now been identified which appear to influence both stress resistance and longevity.