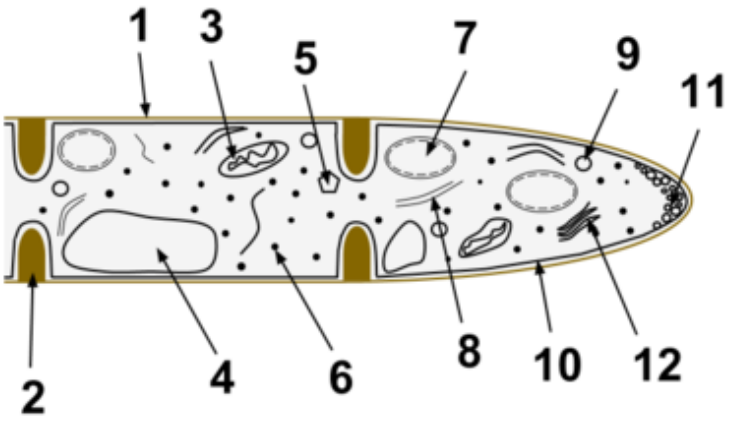
Lab-1-
Fungal physiology is the study of living fungi , their functions and activities to their environments.
The more understand their life and physiology, the better we can utilize them in agriculture , industry and medicine , and at the same time the greater will be our control over their harmful actives as pathogens of plants, animals and man , and as destroyers of timber , textiles food and feed.

Ultra structure of fungi

Most fungi grow as hyphae, which are cylindrical, threadlike

structures 2–10 μm in diameter and up to several centimeters in length.



1- Hyphal wall
2- Septum
3- Mitochondrion
4- Vacuole
5- Ergosterol crystal
6- Ribosome
7- Nucleus
8- Endoplasmic reticulum
9- Lipid body
10- Plasma membrane
11- Spitzenkorper
12- Golgi apparatus

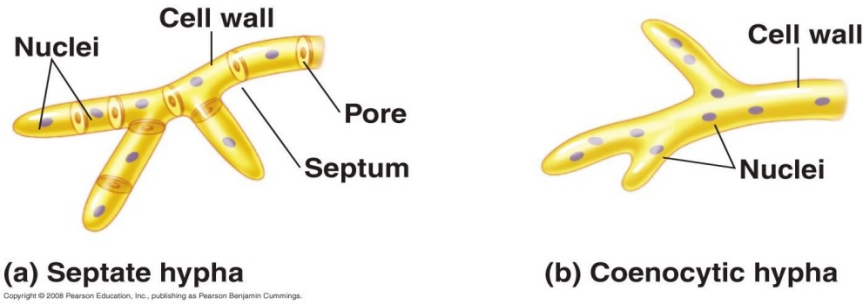
Hyphae

Hyphae grow at their tips. New hyphae are typically formed by emergence of new tips along existing hyphae by a process called branching, or occasionally growing hyphal tips bifurcate (fork) giving rise to two parallel growing hyphae.

The combination of apical growth and branching/forking leads to the development of a

mycelium, an interconnected network of hyphae.

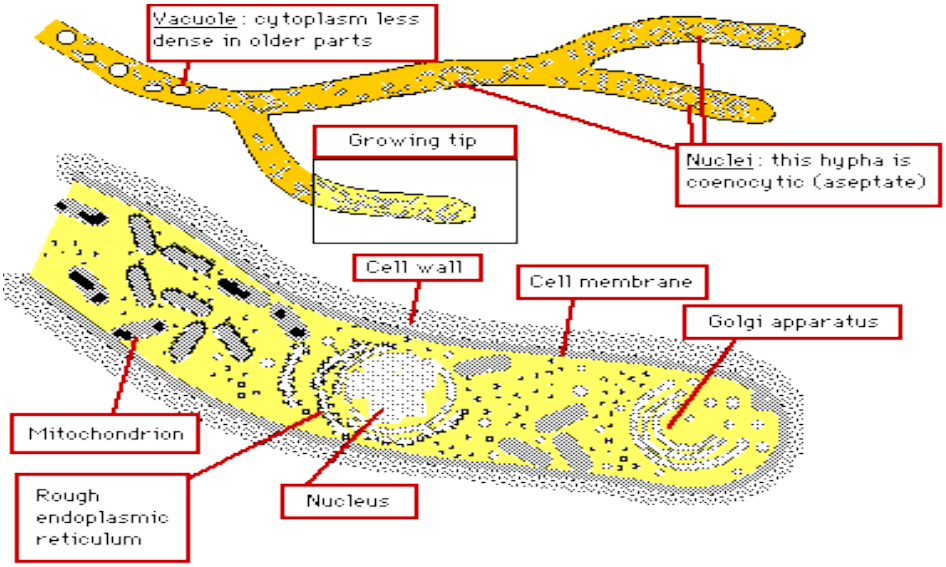
Hyphae can be either septate or coenocytic: septate hyphae are divided into compartments separated by cross walls (internal cell walls, called septa, that are formed at right angles to the cell wall giving the hypha its shape), with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized.



(a) Septate hypha (b) Coenocytic hypha

Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass through; an example is the dolipore septum in the fungi of the phylum Basidiomycota.

Coenocytic hyphae are essentially multinucleate supercells.



Cell structure	Description	Function
Cell wall	Freely permeable layer surrounding cell membrane	Supports and strengthens cell
Cell membrane	Selectively permeable double layer of lipid and protein molecules	Controls which substances can enter and leave the cell
Cytoplasm	Fluid enclosed by the cell membrane, containing organelles and ribosomes	Location of many chemical reactions
Mitochondria	Rod-shaped structures found in cytoplasm	Location where aerobic respiration occurs and most of the cell's ATP is produced
Ribosomes	Small complexes found in cytoplasm	Location where amino acids are connected together to produce proteins
Plasmids	Small circular DNA molecules	Contains genes that help cell to function, eg genes for antibiotic resistance
Nucleus	Compartment in cell where DNA is stored as chromosomes	Controls activities of cell

Fungal Cell Structure

Fungal cells are organized a little differently from animal and plant cells. Like plant cells, they have a cell wall; however, unlike plant cells, the cell wall lacks cellulose (in true fungi) and there are no chloroplasts.

The typical fungal structure is that of a colony of cells strung together in a filament called a **Hypha** (plural, hyphae). The cells in a hypha are separated by a cross-wall called a **Septum**. Hyphae tend to form a larger network of cells called a **Mycelium**. Fungal hyphae have a number of unique features:

Apical Vesicular Complex: The AVC is found at the actively growing hyphal tip (the apex) and consists of a mass of vesicles surrounding the **Spitzenkörper**, an opaque structure which can be seen with phase contrast microscopy. Further back from the centre of the AVC, several **mitochondria** are seen. The AVC can be seen in ascomycetes, basidiomycetes and oomycetes; in fact, in all the fungal species which grow as **mycelia**.

Cell Wall: This structure prevents the hyhae from bursting due to the processes of osmosis. The cell wall in mycelial fungi varies in thickness from about 50 nm (50 billionths of a metre) in the recently synthesized areas around the apex to 250 nanometres (250 billionths of a metre). The cell wall has four main biochemical constituents:

Chemical composition of the wall:

- POLYMERIC FIBRILS
  - chitin
  - cellulose (in the Oomycota)
- AMORPHOUS MATRIX COMPONENTS
  - glucans
  - proteins
  - lipids
  - heteropolymers (mixed polymers) of mannose, galactose, fucose and xylose
- The types and amounts of these various components vary amongst different groups of fungi and may even vary during the life cycle of a single species.

Functions The Fungal Wall

- PROTECTS the underlying protoplasm;
- determines and MAINTAINS THE SHAPE of the fungal cell or hypha; if you remove the wall the resulting protoplast will always assume a spherical shape;
- acts as an INTERFACE between the fungus and its environment;
- acts as a BINDING SITE for some enzymes;
- possesses ANTIGENIC properties - which allow interactions with other organisms.

Arrangement of the wall components:

- The diagram above represents a section through the mature lateral wall of hyphae of Neurospora crassa.
- In general, the inner part of the wall consists of POLYMERIC FIBRILS embedded in an AMORPHOUS MATRIX and this is covered by further layers of matrix material.
- At the HYPHAL TIP the wall is thinner and simpler in structure, consisting of only TWO LAYERS - an inner layer of fibrils embedded in protein and outer layer of mainly protein.
- EXTRA LAYERS of wall material are deposited in the lateral walls behind the extending apex - strengthening the wall as the hypha matures.
- In the oldest parts of the hyphae (and in many fungal spores) LIPIDS and PIGMENTS may be desposited in the wall:
  - LIPIDS serve as a nutrient reserve and help prevent desiccation
  - PIGMENTS, such as MELANIN, help protect the protoplast against the damaging effects of UV radiation.

- N.B. Although represented as distinct layers in the diagram above, these four zones merge into one another.

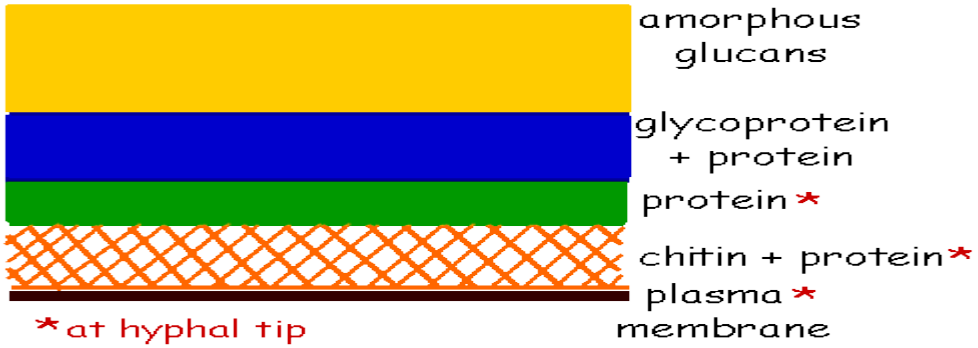


Table 1: Common wall constituents found in each division of fungi

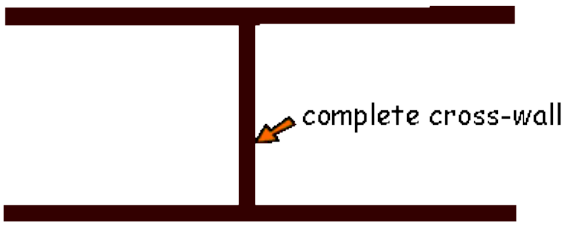
Division	Fibrous	Gel-like Polymer
Basidiomycota	Chitin β -(1-3), β-(1-6) Glucan	Xylomannoproteins α (1-3) Glucan
Ascomycota	chitin β -(1-3), β-(1-6) Glucan	Galactomannoproteins α (1-3) Glucan
Zygomycota	Chitin Chitosan	Polyglucuronic acid Glucuronomannoproteins Polyphosphate
Chytridiomycota	Chitin Glucan	Glucan

Lab-2-

Septa

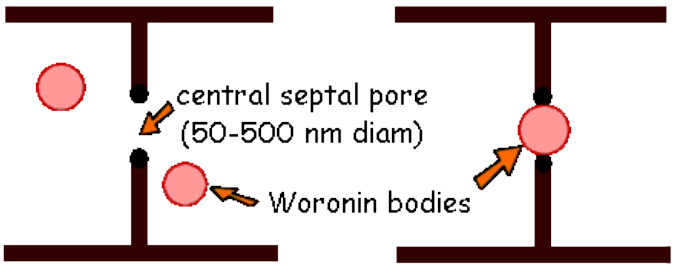
Septa (cross-walls) can be seen by light microscopy, as illustrated in this series of images and in this movie clip from the Fungal Cell Biology Group, University of Edinburgh. But electron microscopy has revealed that several different types of septa exist among the major taxonomic groups of fungi.

- Oomycota and Zygomycota:



  - In general, the hyphae of fungi belonging to these groups are not regularly septate (although there are some exceptions).
  - But septa in the form of COMPLETE CROSS-WALLS are formed to isolate old or damaged regions of the mycelium or to separate reproductive structures from somatic hyphae.

Ascomycota and some mitosporic fungi:

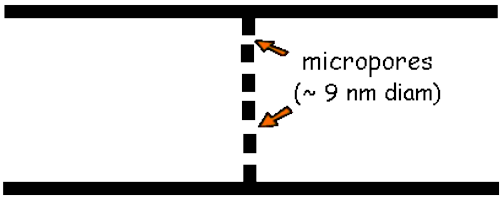


  - Hyphae of fungi belonging to these groups (and the Basidiomycota) possess perforated septa at regular intervals along their length.
  - The septum consists of a simple plate with a relatively LARGE CENTRAL PORE (50-500 nm diameter) - this allows cytoplasmic streaming (the movement of organelles, incl. nuclei) between adjacent hyphal compartments.
  - Cytoplasmic streaming enables sub-apical and intercalary (central) compartments of young hyphae to contribute towards growth of the

hyphal tip - transporting nutrients and essential enzymes to the apex - so maximizing the capacity for somatic growth.

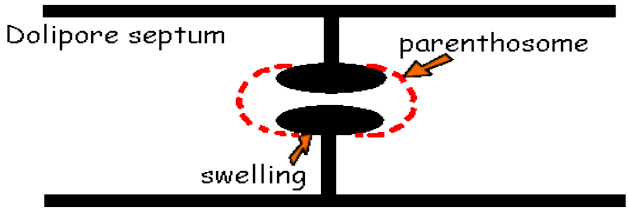
- Associated with each septum are spherical, membrane-bound organelles called WORONIN BODIES that .....
  - are composed of protein;
  - remain close to the septal pore and tend not to be disturbed by the cytoplasmic streaming taking place;
  - tend to be of the same or larger diameter than the septal pore and are, therefore, capable of blocking the pore;
  - will block the septal pore if the adjacent hyphal compartment is damaged or ageing and becoming highly vacuolated.
- Not all fungi belonging to the Acomycota possess Woronin bodies - those that don't often possess LARGE HEXAGONAL CRYSTALS OF PROTEIN in the cytoplasm that are capable of serving the same function, i.e. they can seal the septal pores of damaged or ageing hyphae.

Some other mitosporic fungi:



- A number of mitosporic fungi possess septa with a single central pore, similar to that observed in the Ascomycota.
- But other mitosporic fungi may possess MULTIPERFORATE SEPTA.
- E.g. the septa of Geotrichum candidum (illustrated above) possess characteristic MICROPORES (approx. 9 nm diameter).
- The number of pores in each septum can vary up to a maximum of approx. 50.
- These micropores allow cytoplasmic continuity between adjacent hyphal compartments, but are too small to allow cytoplasmic streaming to occur to the extent observed in fungi possessing larger septal pores.

Basidiomycota:



- The most complex type of septum is found in fungi belonging to the Basidiomycota.

- Each septum is characterized by a swelling around the central pore (DOLIPORE) and a hemispherical perforated cap (PARENTHOSOME) on either side of the pore - illustrated above.
- The perforated parenthosome allows cytoplasmic continuity but prevents the movement of major organelles.
- The plasma membrane lines both sides of the septum and the dolipore swelling, but the membrane of the parenthosome is derived from endoplasmic reticulum.

Functions of septa:

- Act as STRUCTURAL SUPPORTS
    - The addition of plate-like cross-walls to what is essentially a long tube-like structure (hypha) will help stabilize it.
- Act as the FIRST LINE OF DEFENCE when part of a hypha is damaged
    - Large-pored septa that have Woronin bodies or large proteinaceous crystals associated with them have the advantage that cytoplasmic streaming can occur between adjacent compartments.
    - But at the same time a mechanism exists for rapidly sealing the septal pore under conditions of stress (e.g. if the hypha is damaged) thereby helping protect the mycelium.
- Facilitate DIFFERENTIATION in fungi
    - Septa can isolate adjacent compartments from one another so that different biochemical and physiological processes can occur within them - these may result in differentiation of the hyphae into specialized structures, such as those associated with sporulation.
    - It's unlikely to be coincidental that the most complex and highly differentiated sporulating structures we see are those produced by fungi possessing the most complex types of septa, i.e. fungi belonging to the Basidiomycota

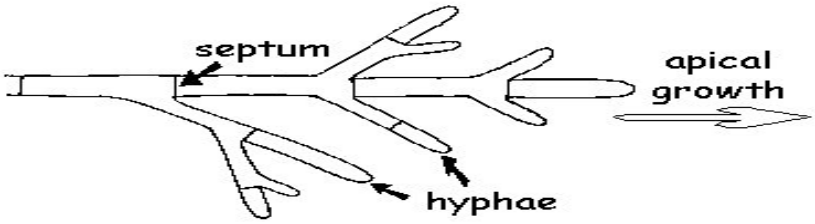
Lab-3-

How to look like fungus ?

Many of us are familiar with the appearance of mushrooms and toadstools. But these structures are simply the large, macroscopic fruiting bodies produced by some groups of fungi. The actively growing and reproductive structures of most species are microscopic, and although most fungi are mycelial (filamentous), there are some exceptions to this growth form.

1- Mycelial (filamentous)
2- Unicellular and primitively branched Chytrids
3- Yeasts (unicellular)
4- Dimorphism

1- Mycelial (filamentous):

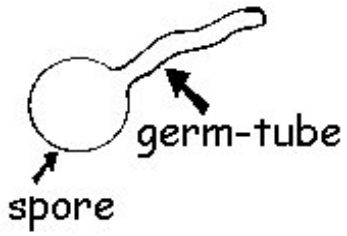


Most fungi are composed of microscopic filaments called HYPHAE, which branch to eventually form a network of hyphae, called a MYCELIUM (colony). The mycelium extends over or through whatever substrate the fungus is using as a source of food.

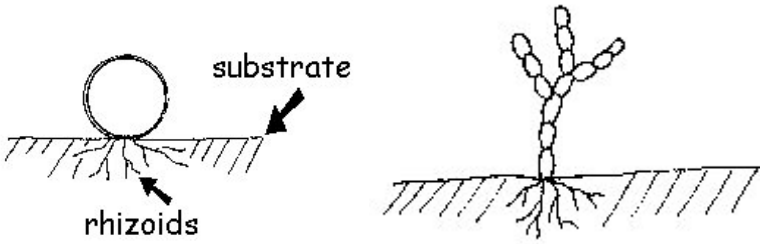
Each hypha is essentially a tube, containing PROTOPLASM surrounded by a RIGID WALL. Depending upon the species, the protoplasm may form a continuous, uninterrupted mass running the length of the branching hyphae, or the protoplasm may be interrupted at intervals by cross-walls called SEPTA. Septa divide up hyphae into individual separate cells or interconnected HYPHAL COMPARTMENTS.

Hyphae exhibit APICAL GROWTH (i.e. they elongate at their tips) and, at least in theory, are capable of growing indefinately, provided that environmental conditions remain favourable for growth. In reality, of course, their environment eventually limits or restricts their growth.

Hyphae may initially develop from a GERM-TUBE (a short, immature hypha) that emerges from a germinating spore. Spores are the microscopic dispersal or survival produced by many species of fungi.



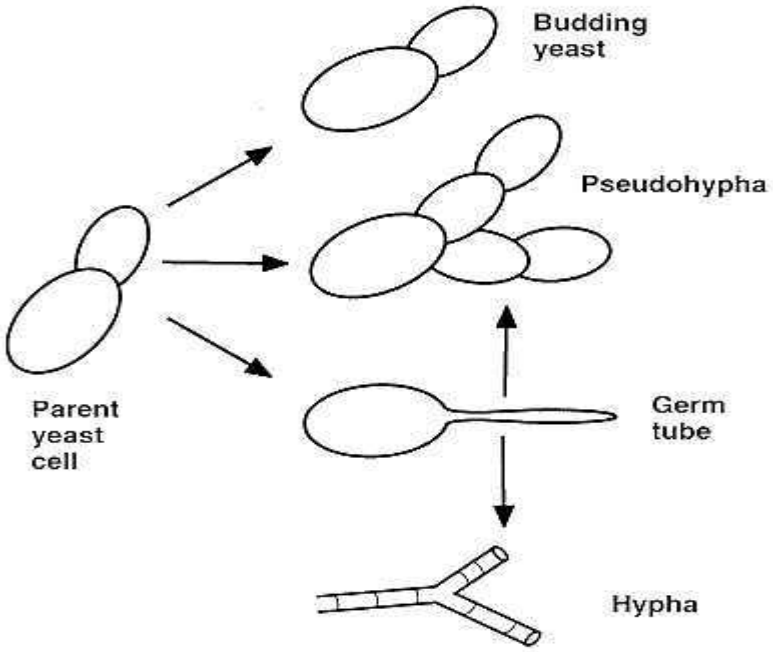
2- Unicellular and primitively branched Chytrids (Chytridiomycota):



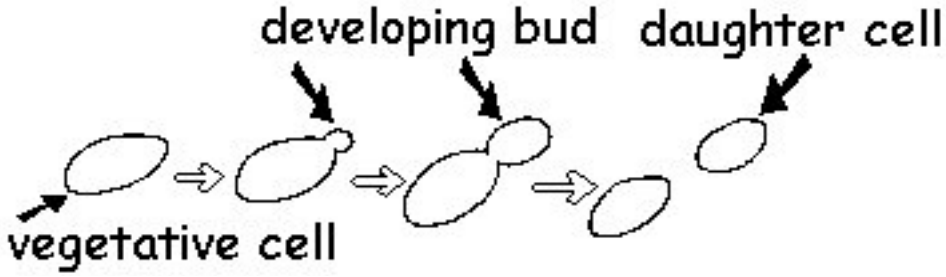
Fungi belonging to the Chytridiomycota exist as either single round cells (unicellular species) or primitively branched chains of cells. In either case, the fungus may be based to its substrate by structures called RHIZOIDS.

3- Yeasts (unicellular):

Yeasts are unicellular organisms which evolved from multicellular ancestors, with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudohyphae or false hyphae



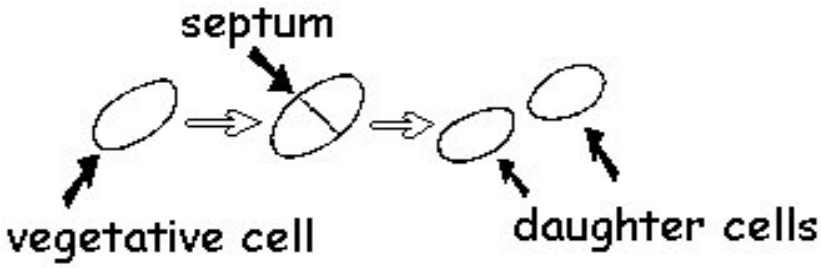
Yeasts, which are used in a variety of commercially important fermentation processes (e.g. bread-making, brewing beers and wines), are capable of reproducing asexually and sexually.



Yeasts reproduce asexually by either:

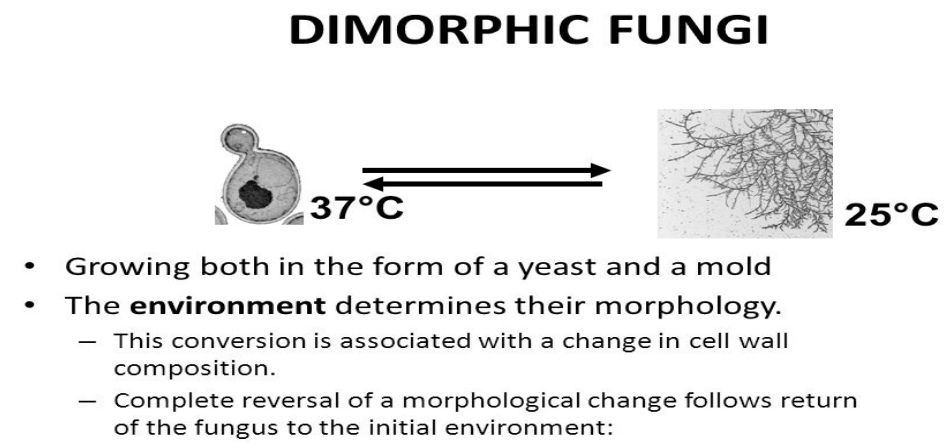
1. BUDDING (e.g. Saccharomyces cerevisiae),

2. BINARY FISSION (splitting into two equal halves; e.g. Schizosaccharomyces pombe).



4- Dimorphism (i.e. existing in two forms):

Some fungi are capable of alternating between a mycelial growth form and a unicellular yeast phase. This change in growth form is often in response to some change in environmental conditions. This phenomenon is exhibited by several species of fungi that are pathogenic in humans, e.g. Paracoccidioides brasiliensis.



Lab-4-

Introduction to Hyphal Growth

Apical Growth

Fungal hyphae extend continuously at their extreme tips, where enzymes are released into the environment and where new wall materials are synthesised. The rate of tip extension can be extremely rapid - up to 40 micrometers per minute. It is supported by the continuous movement of materials into the tip from older regions of the hyphae. So, in effect, a fungal hypha is a continuously moving mass of protoplasm in a continuously extending tube.

- You might like to begin by viewing some movies that show apical growth, hyphal branching and septum formation.
- To understand the mechanisms involved in apical growth of a hypha we need to look again at the HYPHAL TIP.
- We already know that the growing hyphal tip is structurally and functionally different from the rest of the hypha - see section on Hyphal Ultrastructure.
- BUT - the hyphal tip (like the rest of the hypha) is surrounded by a wall - although the wall may be thinner and simpler in structure than the mature lateral wall of the hypha further back - see section on the Fungal Wall.
- We also know that growth of a hypha is closely linked to the presence of vesicles which form the APICAL VESICULAR CLUSTER (AVC):
    - when a hypha stops growing, these vesicles disappear
    - when growth of the hypha resumes, the vesicles reappear.
- In addition - the position of the vesicles is linked to the direction of growth of a hypha:
    - when a hypha is growing straight ahead, the vesicles are positioned in the centre of the hyphal tip
    - movement of the vesicles to the left or right side of the hyphal tip is accompanied by a change in direction of growth of the hypha
- So it's clear that the vesicles play a key role in apical growth.

Vesicles of the AVC contain:

- wall Precursors - the sub-units or buildng blocks of the wall polymers - e.g. uridine diphosphate N-acetylglucosamine, the sub-unit of chitin

- wall Lytic Enzymes- which help breakdown and separate wall components - e.g. chitinase, glucanase
- wall Synthase Enzymes- which help assemble new wall components and so increase the size of the wall – e.g. chitin synthase, glucan synthase.

Two Models have been proposed to explain the mechanisms of apical growth - they differ in whether or not wall lytic enzymes are necessary.

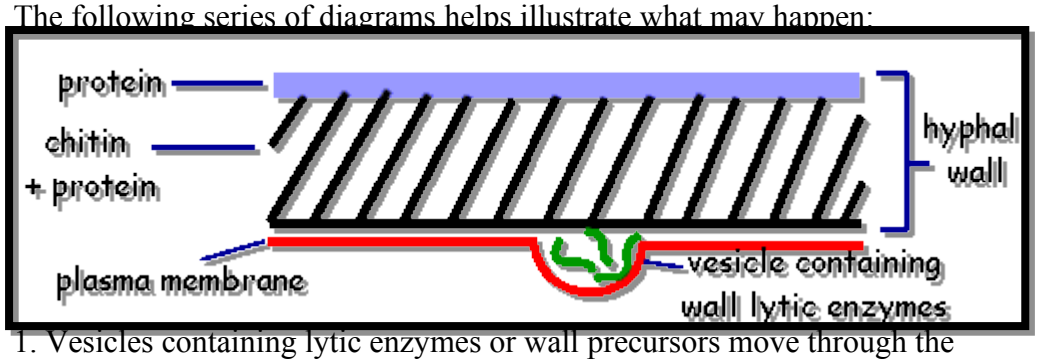
Model 1 - involvement of wall lytic enzymes:

According to this model, if the hypha is going to be able to extend at its tip, there will have to be:

- some softening (lysis) of the existing wall, and
- the synthesis and incorporation of new wall material.

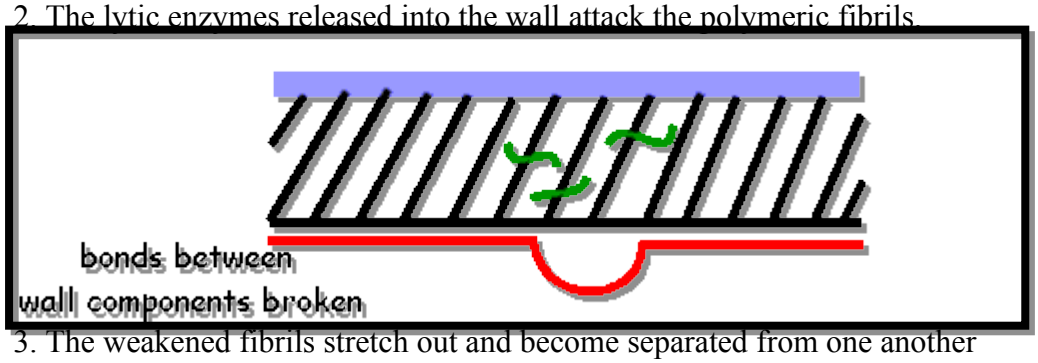
But these processes will have to be finely balanced - otherwise, the wall may become too weak or too rigid for further growth

The following series of diagrams helps illustrate what may happen:

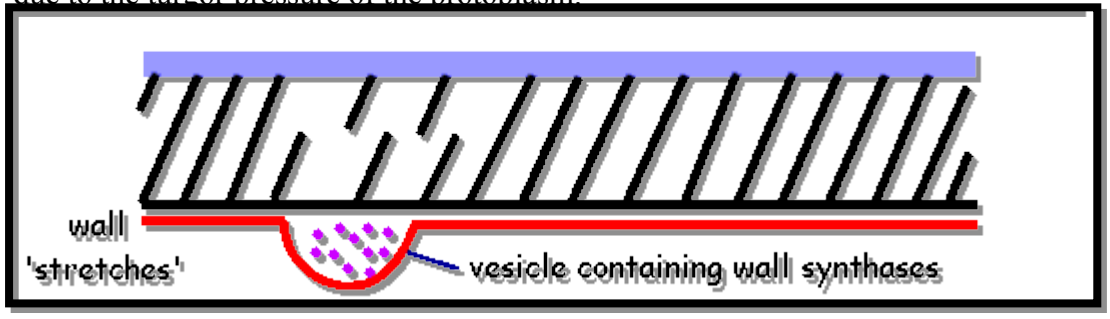


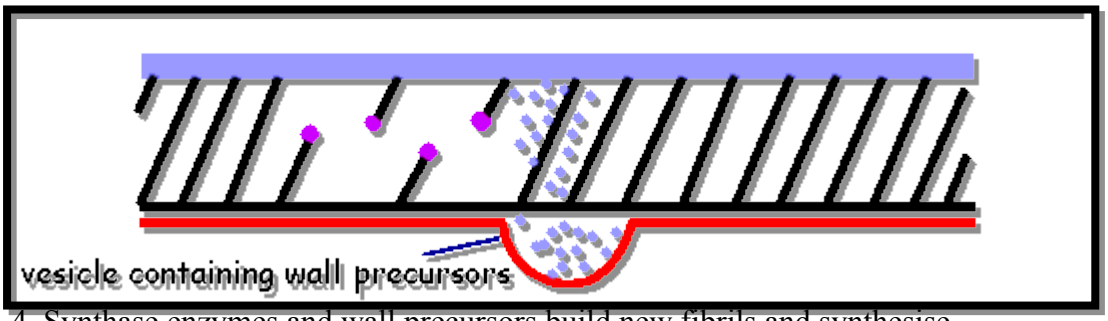
1. Vesicles containing lytic enzymes or wall precursors move through the cytoplasm towards the hyphal tip, where they fuse with the plasma membrane, releasing their contents into the wall.

2. The lytic enzymes released into the wall attack the polymeric fibrils

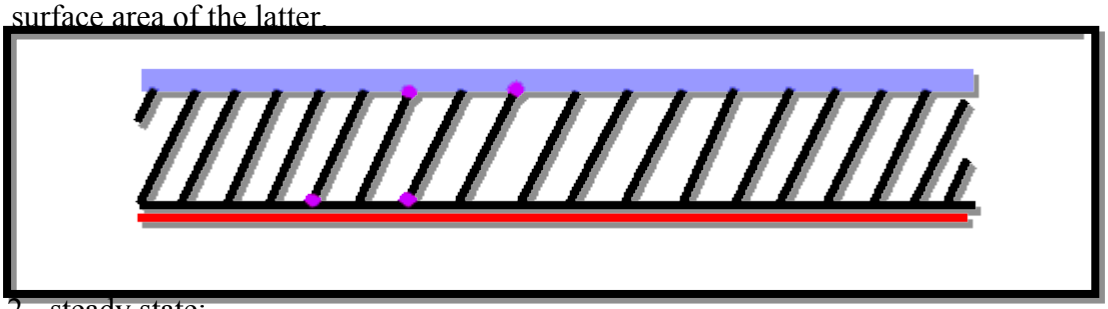


3. The weakened fibrils stretch out and become separated from one another due to the turgor pressure of the protoplasm.

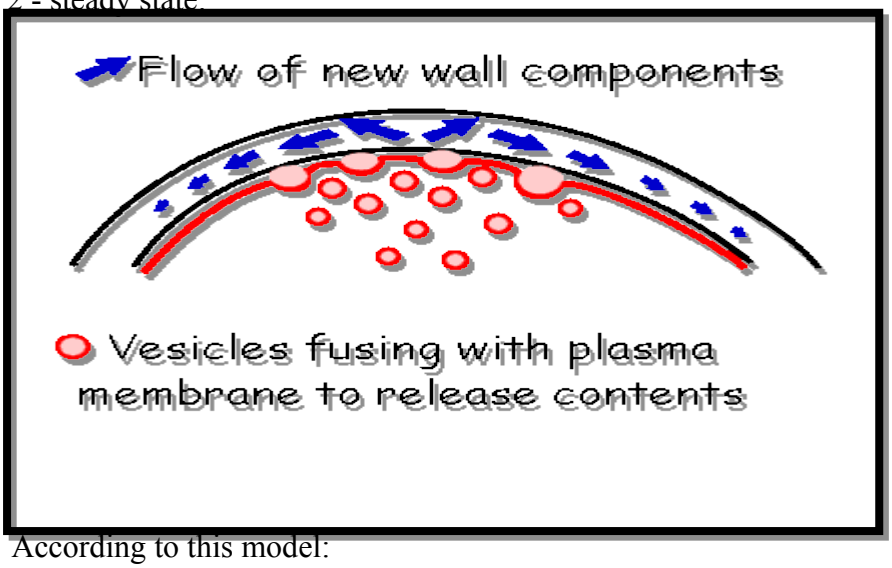




4. Synthase enzymes and wall precursors build new fibrils and synthesise additional amorphous components of the wall.
5. The surface area of the hyphal wall increases. Fusion of the vesicles with the plasma membrane ensures that the fomer contribute to the increase in surface area of the latter.



Model 2 - steady state:



According to this model:

- lytic enzymes are NOT involved in apical growth
- because the newly formed wall at the extreme tip of the hypha is VISCOELASTIC (essentially fluid)
- so that as new wall components are added at the tip, the wall flows outwards and backwards (see adjacent diagram)
- and the wall then rigidities progressively behind the tip by the formation of extra chemical bonds.

Hyphal Branching

Although each hypha exhibits apical growth (i.e. extends at its tip), it doesn't continue growing as just a single filament - it will eventually BRANCH and as the branches become progressively longer they too will branch,

Features:

- ❖ Hyphal branching is necessary for efficient colonization and utilization of the substrate upon which the fungus is growing.
- ❖ A branch arises when a NEW GROWTH POINT is initiated in the existing lateral wall of the hypha - this is accompanied by the ACCUMULATION OF VESICLES.
- ❖ Branch formation almost certainly involves wall lytic enzymes (model 1), since the branch will emerge through a mature, rigid area of the hypha's lateral wall.
- ❖ Branches normally EXTEND AWAY FROM ONE ANOTHER, filling the gaps between existing hyphae, because they're:

- responding to nutrient gradients - growing out of areas where nutrients have become limited around existing hyphae, into areas where nutrients are more plentiful
- growing away from areas which have become staled by the metabolic by-products of existing hyphae.

- ❖ The extent of hyphal branching, i.e. the density of a fungal colony (number of hyphal branches formed per unit area), is directly related to the concentration of nutrients in the substrate or growth medium:

- a sparsely branched colony (low hyphal density) will develop on a nutritionally weak substrate or growth medium
- a densely branched colony will develop on a nutritionally rich substrate or growth medium.

Lab-5-

Dimorphic fungi : fungi that can reproduce as either a mycelial or a yeast-like state. Generally the mycelial form grows at 25° C, and the yeast-like pathogenic form at 37° C. This dimorphism is important in the identification of mycoses, as it makes rapid identification of many pathogenic organisms possible.

Environmental factors control the dimorphism

1- Temperature –dependence dimorphism :

Examples :

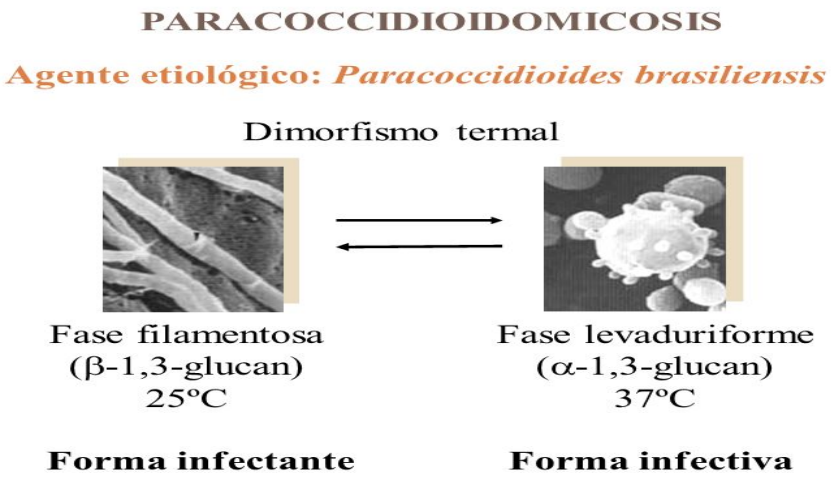
a- ***Blastomyces dermatitidis*** **35 - 37c°**

b- ***Paracoccidioides brasiliensis*** **37c°**

The mycelial form of *P. brasiliensis* can be converted to the yeast form in vitro by growth on brain heart infusion agar or blood-glucose-cysteine agar when incubated for 10–20 days at 37 °C, Under these conditions, hyphal cells either die or convert to transitional forms measuring 6–30 µm in diameter, which ultimately detach or remain on the hyphal cells, yielding buds . New buds develop mesosomes and become multinucleated. In contrast, yeast-like cultures can be converted to the mycelial form by reducing the incubation temperature from 37 to 25 °C. Initially it, nutritional requirements of both the yeast and mycelial phases of *P. brasiliensis* were thought to be identical; however, later studies demonstrated the yeast form to be auxotrophic, requiring exogenous sulfur-containing amino acids including cysteine and methionine for growth

Note

The main component of the cell wall in yeast cells is a-*1,3-glucan* while the mycelium is β-*Glucan* as well as the presence of other components such as chitin .



2- Temperature and nutrient–dependence dimorphism :

Examples :

Histoplasma capsulatum

This fungus grows mycelial form at temperature 25c°, but raise the temperature to 37c° is not enough to turn into yeast, Only if processing of culture media amino acid . (cysteine)

3- Nutrient–dependence dimorphism :

Examples :

Candida albicans

Candida tropicalis

The dimorphism here depends on the specific nutrition , such as cysteine , NH4CL ,Glucose ,*Candida albicans* grow as yeast at a temperature of 37 or 25 c° when a carbon source of glucose, but in the case to replace the carbon source starch or glycogen consists the mycelium

-Lab-6

Fungi growth : Growth may be defined as an irreversible increase in the volume of an organism, usually accompanied by an increase in .biomass

Laboratory methods for measuring the growth rate of :fungi

To measure the rate of fungal growth on solid media use measure the diameter of the colony While on liquid media we use the method of dry .weight

1- Measure the diameter of the colony:

It is one of the most common ways are made through pollination mid dishes containing Media PDA by regular discs from fungal culture modern age between the ages (7-14 days) and then incubated at

a temperature of 25 ° C or in an atmosphere lab for a week, taking into account taking measure the diameter colony at regular intervals and that the work of the two lines perpendicular to the base plate and measure both of them and take the average.

The downside of this method is that it ignores the thickness of the developing mycelium in the dish. Some fungi may not expand in growth horizontally as much as it expands vertically.

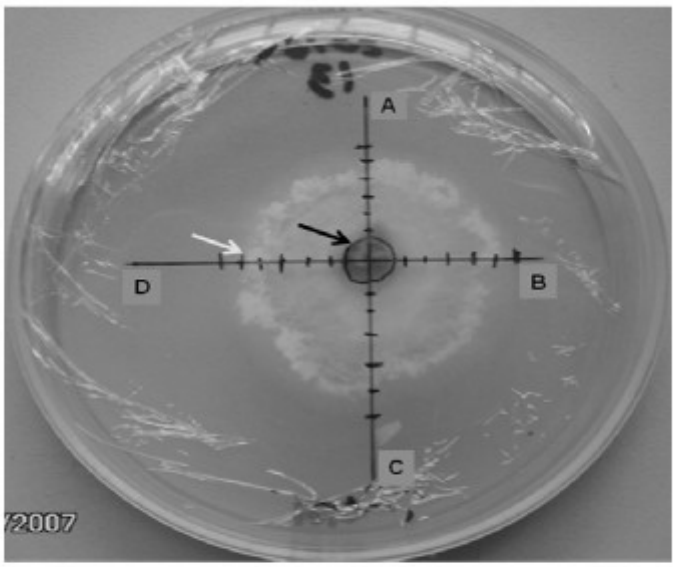


Figure 1. Petri dish used for fungus growth bioassay. Black arrow indicates the edge of initial inoculum. White arrow indicates the edge of fungi radial growth six weeks after bioassay start. Letters A, B, C, D correspond to the four segments used for growth measurements.

2- The method of measuring dry weight and wet weight:

This method is considered one of the best ways and the most accurate and which weighed mycelium and that first filtration of media components with the fungal growth and should be considered wash mycelium and to get rid of the remnants sticking with the media , weighed mat fungal wet and then dried mycelium in special oven at a temperature of 80 ° C until constancy weight and weighed mycelium after drying on a sensitive balance and sufficient weight to approximate the nearest mg. This method is used with the liquid media.

The problem of this method is that the weight may reflect the accumulation of multiple sugars or other materials .

3- Turbidity method

This method for estimating private growth unicellular fungi, such as yeast, can be made a count by using a hemacytometer slide,also spectrophotometer device used to estimate the number of cells through the turbid measure where the turbid turn into a number of cells through use of standard curve. The problem with this method .is that it is estimated the living and the dead cells together

4- Measure the concentration of the cell components

Some of the cell components such as chitin, glucosamine, .is considered as a standard for growth

It is a useful way to measure the growth of pathogenic fungi for the plant, after the hydrolysis of the infected tissue is released glucose- amin, which is separated chromatography, separated quantity commensurate with the dry weight, which can determine the severity of the infection in the plant tissue. The membrane lipid ergosterol is found almost exclusively in fungi, and is frequently used by environmental microbiologists as an indicator of living .fungal biomass

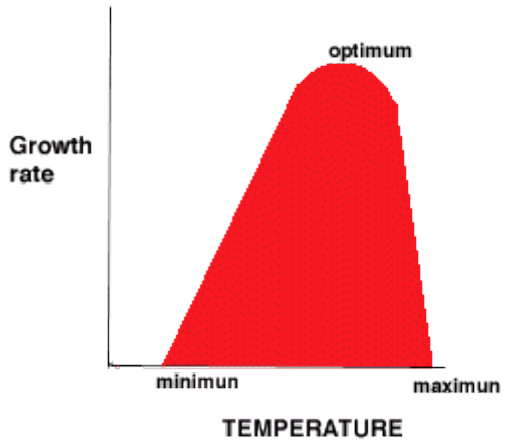
5- Metabolism measurement

Metabolic activities are used as a measure of growth, such as the production of co2, to estimate the growth of some fungi in the fermentation (Brewers)

-Lab-7

a-Temperature and growth

Temperature affects the growth, germination of spores,
. reproduction and truth in all vital activities
There is a curve represents the relationship between growth and temperature-fungal, In this curve there a linear portion and which the growth increases with increasing temperature , There are optimum range , which is either a narrow or broad, and there are descending limb (landing stage) with a high increase of
. temperature



:Examples of the temperature of some fungi

- ***Phycomyces blakesleeanus***

Faster growth at a temperature of 20-25 c°, but the highest rate of growth occurs at a temperature of 10 c° With a limited level of thiamine, and the effect of temperature is . often absent (wiped out) when the nitrogen level is limited

- ***Coprinus fimetarius***

Very weak(poorly) growth at a temperature of 44 c° due to failure of methionine biosynthesis but Provide methionine . externally back normal growth

- ***Sclerotinia fructicola***

. Optimum temperature linked with the pH of the culture

- **Temperature determining factor for the spread of some fungi in nature**
  - **Allomyces in Tropical regions**
  - ***Phymatotrichum omnivorum* in Cold regions**
  - ***Fusarium nivale and Typhula sp.* in snow molds**
  - ***Fhacidium infestans***

The optimum temperature of 15 c° has the ability to .grow at temperatures -3 c° and infects pine

  - ***Cladosporium* sp. *sporotrichum* sp.**

Causing spoilage refrigerater food , Grow at a °temperature less than zero Up to -5 c

- **Dermatophytic fungi :**

- ***Tinea Capitis***

disease that affects the hair follicles in culture
°media growth in temperature 25-35 c

❖ **Systemic mycoses fungi :**

- ***Aspergillus fumigates***

Cause tuberculosis disease in humans growth in
°temperature 37 c

❖ **Wood-destroying fungi**

- ***Serpula lacrymans***

.has a preference for temperatures of 21 to 22 °C

❖ **True thermophiles :**

- ***Thermomyces lanuginous***
- ***Penicillium duponti***
- ***Thermoascus aurantiacus***
- ***Chaetomium thermophile***

.growth in the temperature range of 30 to 58 °C

❖ **Fungi that infect stored crops**

prefer a plant that will be the same temperature for
. mycelium
wilt tomato disease often have the same pattern
optimum temperature of the Fungal pathogen
.***Fusarium* sp**

-Lab-8

b - PH and growth

Under standard conditions the fungus can grow to the maximum extent within a certain range close for early acidity for culture media , fungi fails to grow upper and lower limits for acidity . fungi growth in the acidic culture or close to neutral

Lows levels of pH affect the activity of enzymes While High levels of pH . affect the systemic or soluble metal elements

Any environmental factor can changes the form of fungal growth curve . with the concentration of pH

: PH affect

- Enter the essential vitamins
- External metabolism
- Entry and movement of organic acids
- Mineral uptake

: Effect the pH of the culture media in growth of fungus

❖ **Readiness of nutrients**

Metals like Zn , Ca , Fe , Mg be ready for soluble by the fungus at . low PH but become insoluble at high PH

❖ **Affect the permeability of the cell membrane**

Effect in the mycelium and thus effect the enzymatic activity

❖ **Effect on activity of enzymes**

Enzymes have a different PH optimum for activity , Favorite range . for the activity between (4-8)

Double PH-optimum : Phenomenon registered in the number of . fungus presence of two levels of pH optimum for growth

: Oxygen and growth

Fungus aerobic organisms , There is a quantitative relationship between growth and oxygen differ between the various fungi , important note that . the number of fungi less in the depths of the soil due to lack of oxygen

Blastocladia sp. Failed to grow in containers pull them oxygen by chemical absorption pyrogallol meaning that the fungus needs oxygen to grow, even if a few

: CO_2 and growth

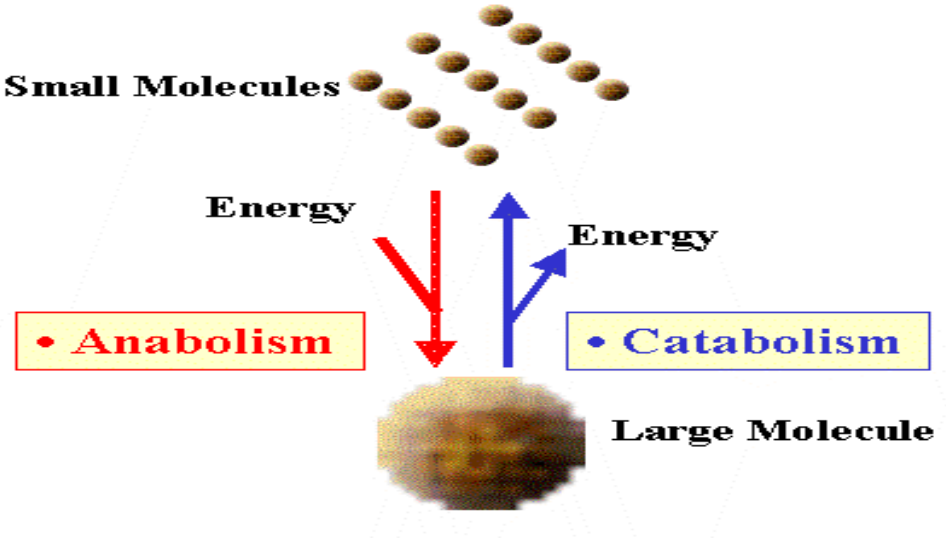
High concentrations of **CO_2** inhibit the growth but the level at which . inhibits it differs from a fungus to another

For example *Alternaria solani* , inhibits in concentrations **CO_2** 38Mm, while other fungi are not affected so much until in concentrations **CO_2** . 150Mm

Lab-9-
Metabolism :

is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism.

Metabolism is usually divided into two categories: catabolism, the *breaking down* of organic matter, for example, by cellular respiration, and anabolism, the *building up* of components of cells such as proteins and nucleic acids. Usually, breaking down releases energy and building up consumes energy.



Primary metabolites: are essential compounds for growth to occur and include proteins, carbohydrates, nucleic acids and lipids. these primary products must be synthesized if they cannot be obtained from the growth medium. These primary metabolites have essential and obvious roles to play in the growth of the fungus. Typically, primary metabolites are associated with the rapid initial growth phase of the organism and maximal production occurs near the end of this phase. Once the fungus enters the stationary phase of growth, however, primary metabolites may be further metabolized. Examples of primary metabolites produced in abundance: enzymes, fats, alcohol and organic acids as well as, low molecular weight compounds.

Primary metabolism is used for:

1 Growth and development of hyphal structure
2 Energy metabolism
3 Regulation of metabolism
4 Intermediate in biosynthesis of compound.

Secondary metabolites; Organic compounds , with low molecular weight ,which are not essential for fungal growth but their natural production have certain significances. Furthermore, secondary metabolites are derived from a few common biosynthetic pathways which branch off the primary metabolic pathways and are often produced as families of related compounds, often specific for a group of organisms.

Fungi are a rich source of secondary metabolites and have been of interest for humans for thousands of years.

Secondary metabolism is used for:

1 competition

2 antagonism

3 self-defense mechanisms against other living organisms to allow the fungus to occupy the niche and utilize the food.

Types of Fungal secondary metabolites

1. Strobilurin (antifungal)

Strobilurins are a group of chemical compounds used in agriculture as fungicides.

2. Gibberellins (growth Hormons)

Gibberellins : are plant hormones from fungal strain (*Gibberella fujikuroi*) that regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction, and leaf and fruit senescence.

3. Mycotoxins (poisneous)

Mycotoxins : is a toxic secondary metabolite produced by organisms of the fungus kingdom. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin.

Examples of common Mycotoxins

- Aflatoxins :are a type of mycotoxin produced by *Aspergillus* species of fungi.
- Ergot **Alkaloids** :are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps purpura* .

- Patulin is a toxin produced by the *P.expansum*, *Aspergillus*, and *Paecilomyces* fungal species

4. Herbicides (control weeds)

The fungi species most commonly used as herbicides in North America are *Phytophthora palmivoraa*

5. Insecticides (control insects)

The fungi species most commonly used to kill insects *are Beauveria bassiana*

6. Antibiotics (drugs)

the fungus *Penicillium chrysogenum* was first used successfully to treat an infection caused by a bacterium