### Mycology

#### For third year students, general education (Biological and Geological Sciences Division)

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### رؤية الكلية

تسعى الكلية الى مساعدة الجامعة فى تحقيق اهدافها الاسترتيجية من خلال ان تكون واحدة من الكليات المتميزة والمنافسة داخليا وخارجيا فى التعليم وخدمة المجتمع والبحث العلمى من خلال تحقيق مستوى رفيع من الاداء وتقديم خريج متميز يقابل الاحتياجات المتعددة بسوق العمل الداخلى والاقليمى والخارجى

#### رسالة الكلية

تهدف كلية التربية بالغردقة الى التميز من خلال:

- إعداد المربين والمعلمين المتخصصين والقادة إعداداً أكاديمياً ومهناً وثقافياً في مختلف التخصصات التربوية.
- تنمية القدرات المهنية والعلمية للعاملين فى ميدان التربية والتعليم بتعريفهم بالاتجاهات التربوية الحديثة.
  - إجراء البحوث والدراسات في التخصصات التربوية والمختلفة بالكلية.
  - نشر الفكر التربوى الحديث واسهاماتة لحل مشكلات البيئة والمجتمع.
- تبادل الخبرات والمعلومات مع الهيئات والمؤسسات التعليمية والثقافية المختلفة.
  - تنمية جوانب شخصية الطلاب ورعاية الموهوبين والمبعدين

### Introduction

The term "mycology" is derived from Greek word "mykes" meaning mushroom. Therefore mycology is the study of fungi.

The ability of fungi to invade plant and animal tissue was observed in early 19th century but the first documented animal infection by any fungus was made by Bassi, who in 1835 studied the muscardine disease of silkworm and proved the that the infection was caused by a fungus *Beauveria bassiana*.

In 1910 Raymond Sabouraud published his book *Les Teignes*, which was a comprehensive study of dermatophytic fungi. He is also regarded as father of medical mycology.

**Importance of fungi:** Fungi inhabit almost every niche in the environment and humans are exposed to these organisms in various fields of life.





#### Introduction

The word fungus comes from the Latin word for mushrooms. Indeed, the familiar mushroom is a reproductive structure used by many types of fungi. However, there are also many fungi species that don't produce mushrooms at all. Being eukaryotes, a typical fungal cell contains a true nucleus and many membrane-bound organelles. The kingdom Fungi includes an enormous variety of living organisms collectively referred to as Eumycota, or true Fungi.



#### **Beneficial Effects of Fungi:**

- 1. Decomposition nutrient and carbon recycling.
- 2. Biosynthetic factories. The fermentation property is used for the industrial production of alcohols, fats, citric, oxalic and gluconic acids.
- 3. Important sources of antibiotics, such as Penicillin.
- 4. Model organisms for biochemical and genetic studies. Eg: Neurospora crassa
- 5. Saccharomyces cerviciae is extensively used in recombinant DNA technology, which includes the Hepatitis B Vaccine.
- 6. Some fungi are edible (mushrooms).
- 7. Yeasts provide nutritional supplements such as vitamins and cofactors.
- 8. Penicillium is used to flavour Roquefort and Camembert cheeses.
- 9. Ergot produced by Claviceps purpurea contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
- 10. Fungi (Leptolegnia caudate and Aphanomyces laevis) are used to trap mosquito larvae in paddy fields and thus help in malaria control.



### Harmful Effects of Fungi:

1. Destruction of food, lumber, paper, and cloth.

2. Animal and human diseases, including allergies.

3. Toxins produced by poisonous mushrooms and within food (Mycetism and Mycotoxicosis).

4. Plant diseases.

5. Spoilage of agriculture produce such as vegetables and cereals in the go down.

6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues, bones and wax.



### General properties of fungi:

1. They are eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, golgi apparatus, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.

2. Have ergosterols in their membranes and possesses 80S ribosomes.

3. Have a rigid cell wall and are therefore non-motile, a feature that separates them from animals. All fungi possess cell wall made of chitin.

4. Are chemo heterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.



### General properties of fungi:

5. Fungi are osmotrophic; they obtain their nutrients by absorption.

6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).

7. All fungi require water and oxygen and there are no obligate anaerobes.

8. Typically reproduce asexually and/or sexually by producing spores.

9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.

10. Food storage is generally in the form of lipids and glycogen.



### Classification of fungi:

- Fungi were initially classified with plants and were a subject of interest for botanists; hence the influence of botany can be seen on their classification.
- In 1969 R.H Whittaker classified all living organisms into five kingdoms namely: Monera, Protista, Fungi, Plantae and Animalia.
- Traditionally the classification proceeds in this fashion: Kingdom Subkingdom Phyla/phylum Subphyla Class Order Family Genus- Species.
- This classification is too complicated to be dealt here.
- There are alternate and more practical approaches, one based on sexual reproduction and the other based on morphology of the thallus (vegetative structure).

Classification of fungi

## **Based on Sexual reproduction**

1. **Zygomycetes**: which produce through production of zygospores.

2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.

3. **Basidiomycetes**: which produce exogenous spores called basidiospores in cells called basidia.

4. **Deuteromycetes** (Fungi imperfecti): fungi that are not known to produce any sexual spores (ascospores or basidiospores). This is a heterogeneous group of fungi where no sexual reproduction has yet been demonstrated.

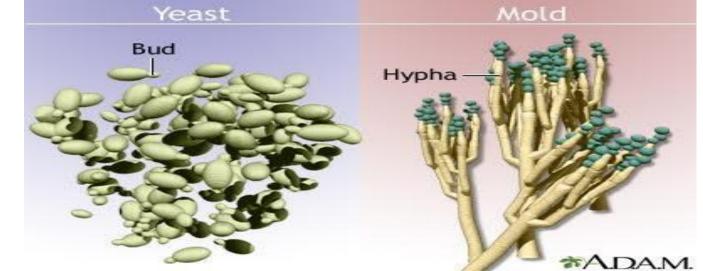
Classification of fungi

# **Based on Morphology**

- 1. Moulds (Molds): Filamentous fungi Eg: Aspergillus sps, Trichophyton rubrum
- 2. Yeasts: Single celled cells that buds Eg: *Cryptococcus neoformans*, *Saccharomyces cerviciae*
- 3. Yeast like: Similar to yeasts but produce pseudohyphae Eg: Candida albicans
- 4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions. They exist as yeasts in tissue and in vitro at 37oC and as moulds in their natural habitat and in vitro at room temperature. Eg: *Histoplasma capsulatum, Blastomyces dermatidis, Paracoccidiodes brasiliensis, Coccidioides immitis.*> Some 200 "human pathogens" have been recognized from among an estimated 1.5
  - million species of fungi.

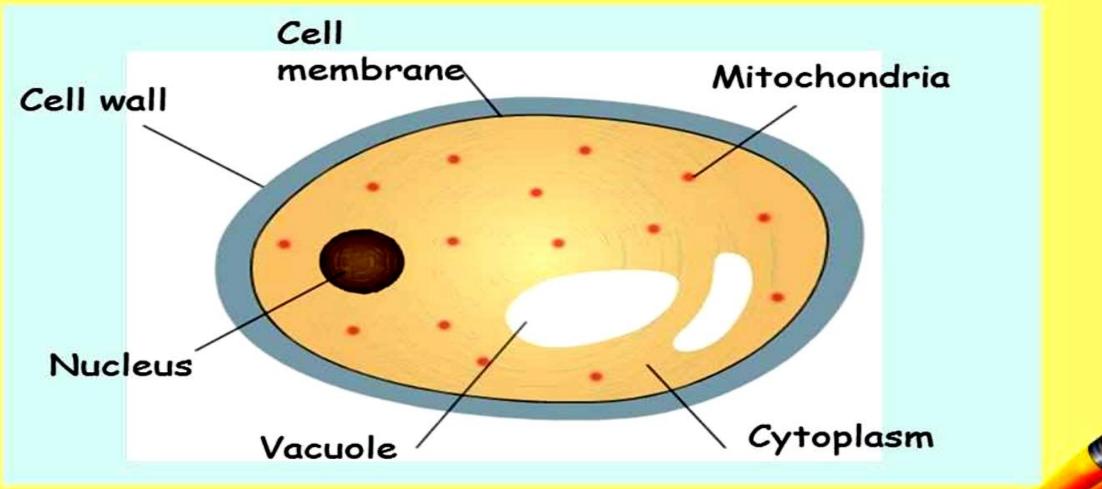
# Yeasts VS Molds





# Morphology of fungi

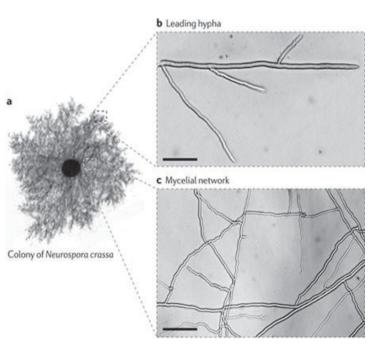
# Typical fungal cell



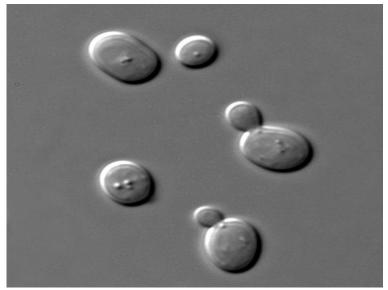


### Morphology of fungi

- Fungi exist in two fundamental forms; the filamentous (hyphal) and single celled budding forms (yeast).
- But, for the classification sake they are studied as moulds, yeasts, yeast like and dimorphic fungi. All fungi have typical eukaryotic morphology.
- They have rigid cell wall composed of chitin, which may be layered with mannans, glucans and other polysaccharides in association with polypeptides.
- Some lower fungi possess cellulose in their cell wall. Some fungi such as Cryptococcus and yeast form of Histoplasma capsulatum possess polysaccharide capsules that help them to evade phagocytosis.

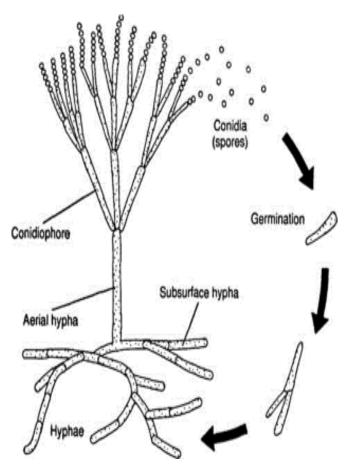






### Moulds (filamentous)

- The thallus of mould is made of hyphae, which are cylindrical tube like structures that elongates by growth at tips. A mass of hyphae is known as mycelium. It is the hypha that is responsible for the filamentous nature of mould.
- The hyphae may be branched or unbranched.
- They may be septate or aseptate.
- Hyphae usually have cross walls that divide them into numerous cells. These cross walls, called septa have small pores through which cytoplasm is continuous throughout the hyphae.
- Therefore all hyphal fungi tend to be coenocytic (multinucleate). With exception of zygomycetes (Rhizopus, Mucor), all moulds are septate.
- Non-septate hyphae are considered to be more primitive because if a hyphal strand is damaged the entire strand dies.
- When a septate hyphal strand is damaged, the pores between adjacent compartments can be plugged, thus preventing death of the whole hyphal strand.



### Mycelium are of three kinds

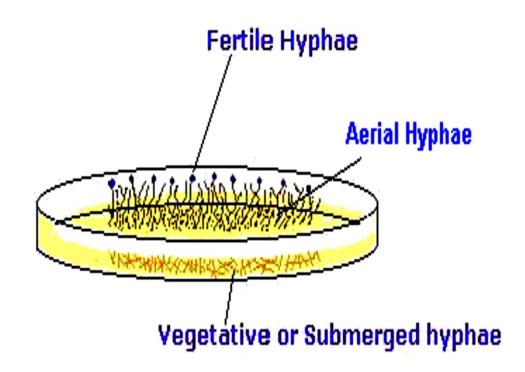
**1. Vegetative mycelium** are those that penetrates the surface of

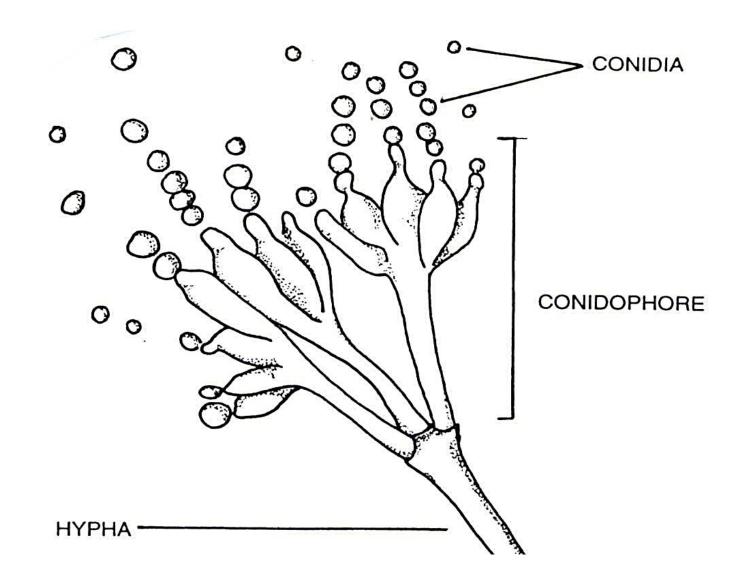
the medium and absorbs nutrients.

2. Aerial mycelium are those that grow above the agar surface

**3. Fertile mycelium** are aerial hyphae that bear reproductive

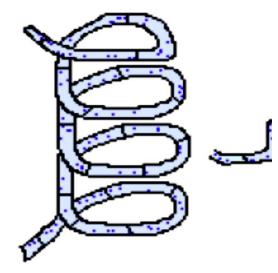
structures such as conidia or sporangia.



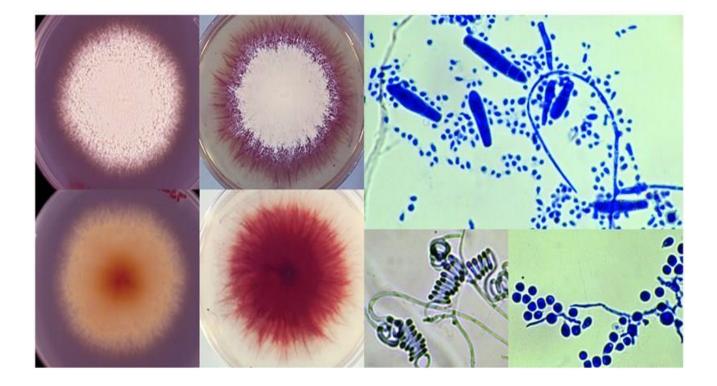


- Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony.
- Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive.
- Examples are species of *Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella* Those hyphae that don't possess any pigment in their cell wall are called hyaline.
- Hyphae may have some specialized structure or appearance that aid in identification.
- Some of these are:

a) **Spiral hyphae**: These are spirally coiled hyphae commonly seen in *Trichophyton mentagrophytes*.



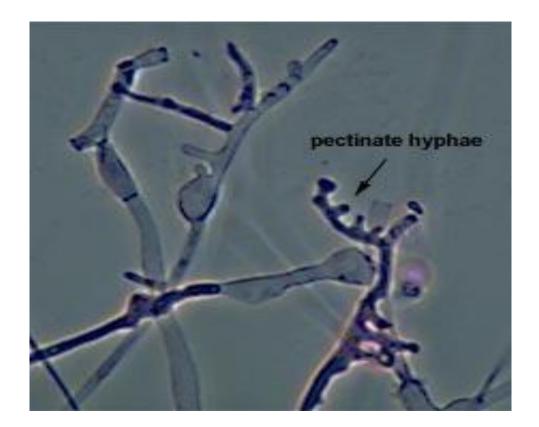
Spiral Hyphae Trichophyton mentagrophytes



b) **Pectinate body**: These are short, unilateral projections from the hyphae that resemble a broken comb. Commonly seen in *Microsporum audouinii*.

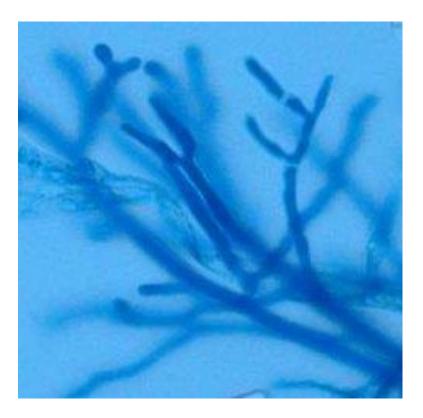


Pectinate Body Microsporum audouinii

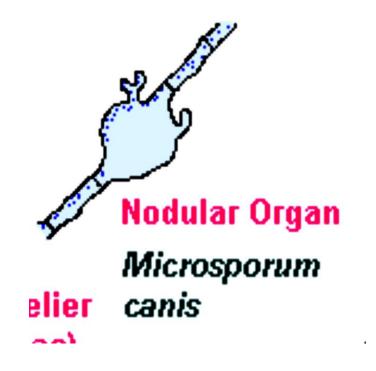


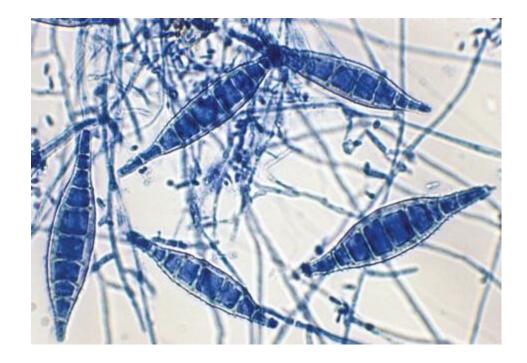
c) **Favic chandelier**: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (antler hyphae). They occur in *Trichophyton schoenleinii* and *Trichophyton violaceum*.





d) **Nodular organ**: This is an enlargement in the mycelium that consists of closely twisted hyphae. Often seen in *Trichophyton mentagrophytes* and *Microsporum canis*.



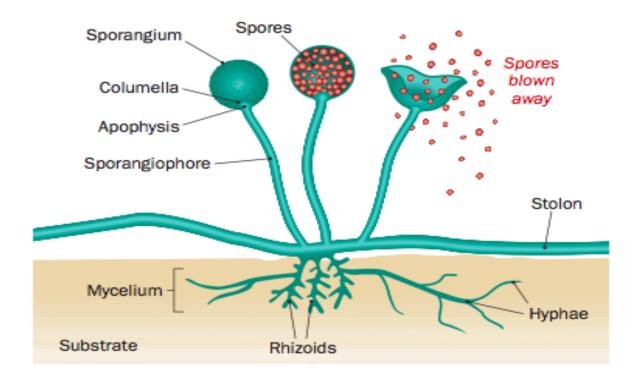


e) **Racquet hyphae**: There is regular enlargement of one end of each segment with the opposing end remaining thin. Seen in *Epidermophyton floccosum, Trichophyton mentagrophytes.* 

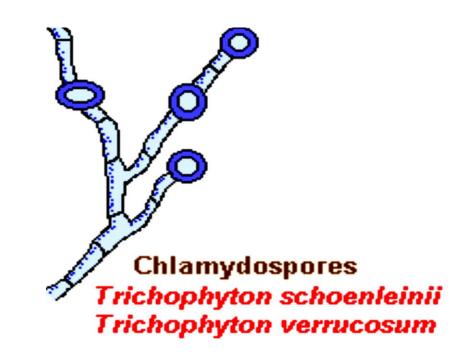
n **Racquet Hyphae** Epidermophyton floccosum



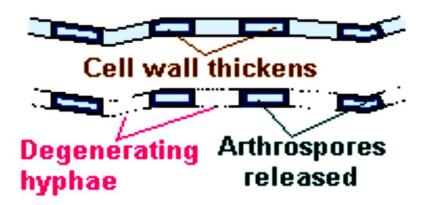
F) **Rhizoides**: These are the root like structures seen in portions of vegetative hyphae in some members of zygomycetes.



- There are structures in the hyphae, which arise out of modification of a single cell and transform into thick walled resting cells.
- Chlamydospore (or chlamydoconidia), which are produced by *Trichophyton schoenleinii* and *Trichophyton verrucosum* are thick walled cells that are larger than other cells and arranged singly or in groups.
- In some fungi such as *Trichosporon beigeilli* and *Coccidioides immitis* some alternating cells become thick walled and subsequently the intervening cells disintegrate leaving behind arthrospores (or arthroconidia).

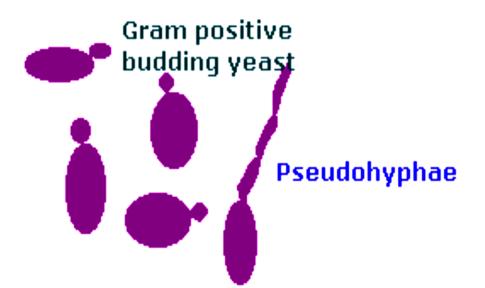


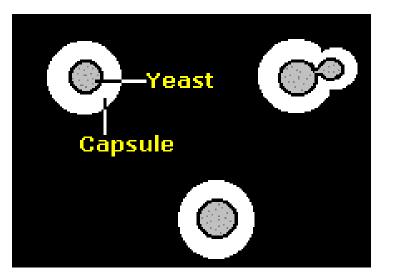




### **Yeasts**

- Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which result in blastospore (blastoconidia) formation.
- In some cases, as the cells buds the buds fail to detach and elongate thus forming a chain of elongated hyphae like filament called pseudohyphae.
- This property is seen in Candia albicans. The same species also have the ability to produce true hypha, which is seen as germ tube. The difference between the two is that there is a constriction in psueudohyphae at the point of budding, while the germ tube has no constriction.

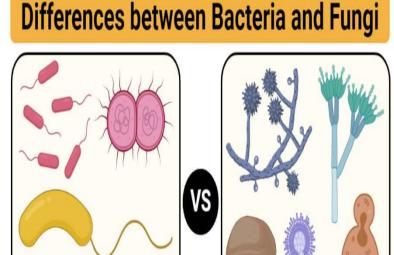


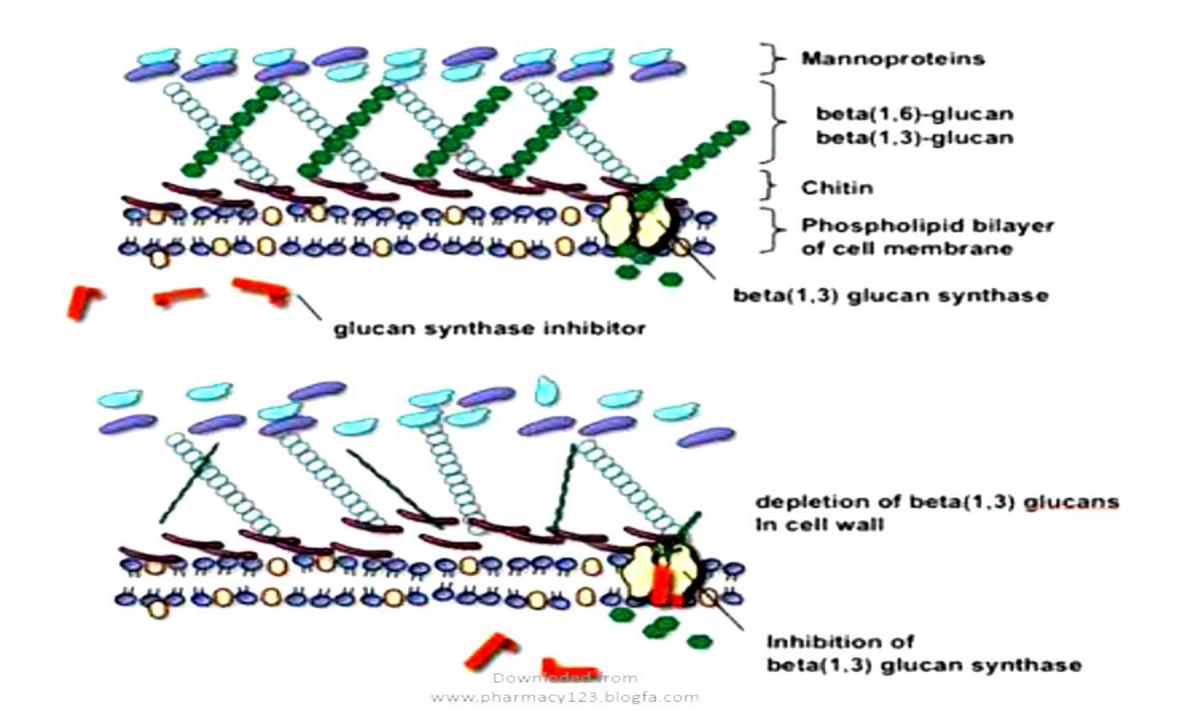


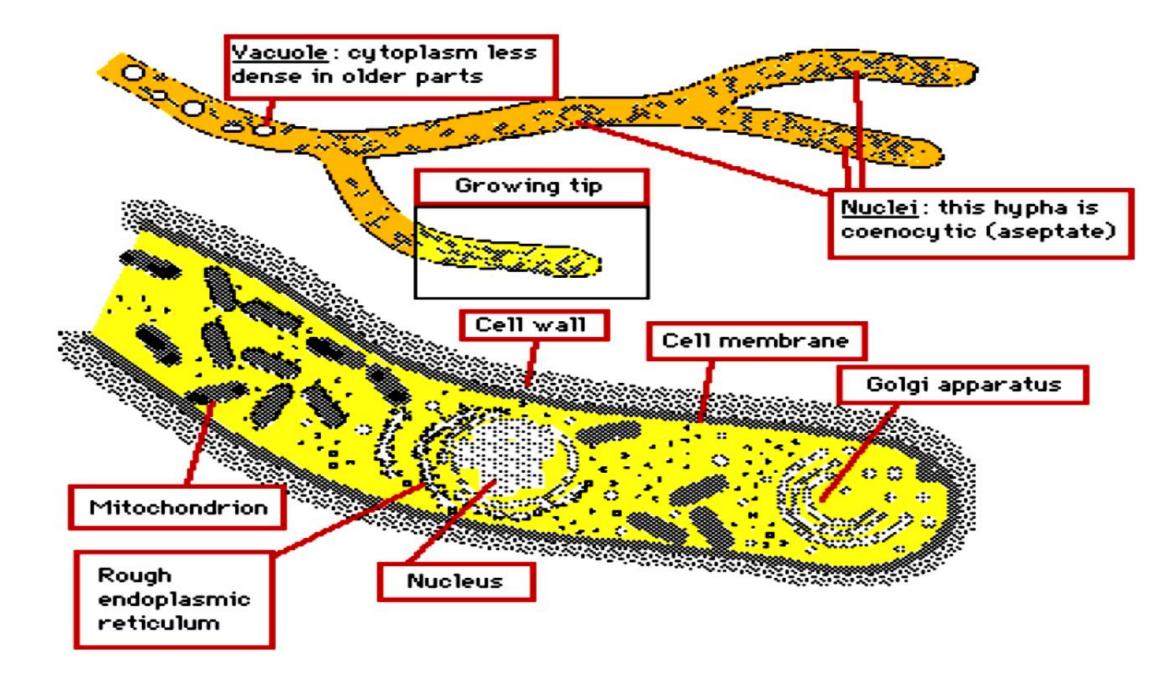
Some yeast such as Cryptococcus and the yeast form of *Blastomyces dermatatidis* produce polysaccharide capsule. Capsules can be demonstrated by negative staining methods using India ink or Nigrosin. The capsule itself can be stained by Meyer Mucicarmine stain. Some yeasts are pigmented. *Rhodotorula sps* produces pink colonies due to carotenoid pigments while some yeasts such as *Phaeoannellomyces* werneckii and Piedraia hortae are dematiaceous, producing brown to olivaceous colonies. True yeasts such as *Saccharomyces cerviciae* don't produce pseudohyphae. Yeast-like fungi may be basidiomycetes, such as *Cryptococcus neoformans* or ascomycetes such as Candida albicans.

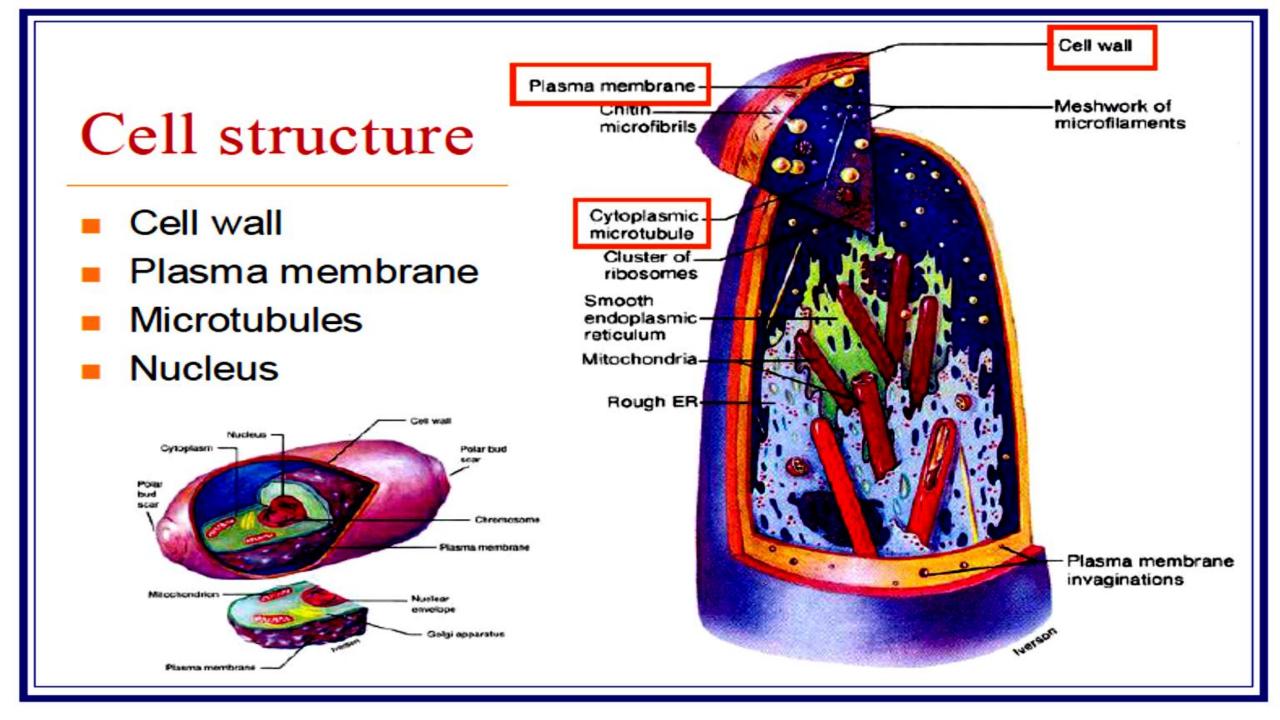
### **Difference from Bacteria**

- Cell wall consists of chitin not peptidoglycan like bacteria
- Thus fungi are resistant to antibiotics as penicillins
- Chitin is a polysaccharide composed of long chain of nacetylglucosamine.
- Also the fungal cell wall contain other polysaccharide, β-glucan, which is the site of action of some antifungal drugs.
- Cell membrane consist of ergosterol rather than cholesterol like bacterial cell membrane
- Ergosterol is the site of action of antifungal drugs, amphotericin B & azole group







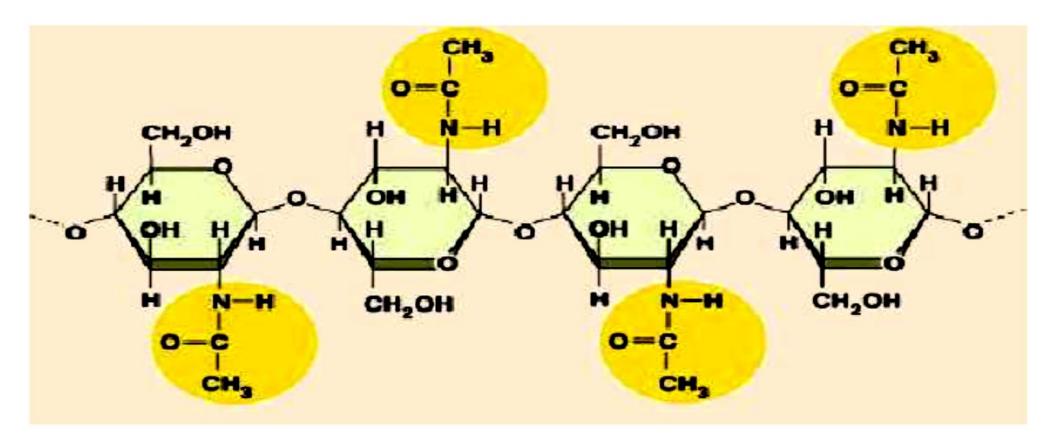


### Fungal cell wall function

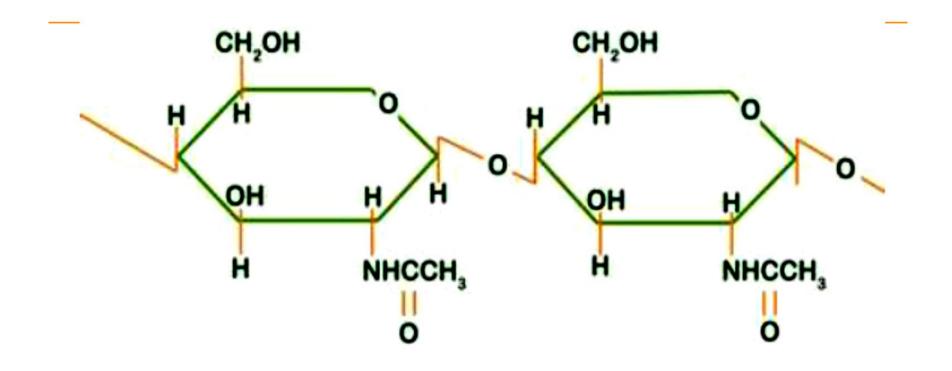
- Shape of fungi.
- Protect against osmotic lysis.
- The wall contains pigments (melanin) → protect the cell against ultraviolet radiation or the lytic enzymes of other organisms.
- It can have antigenic properties.

### Major components of fungal cell walls

Division	<b>Fibrillar components</b>	Matrix components
Chytridiomycota	Chitin, glucan	Glucan
Zygomycota	Chitin, chitosan	Polyglucoronic acid,
		glucuronomannoproteins
Ascomycota	Chitin, β(1,3)-β(1,6)-	α-(1,3)-Glucan, galacto-
Deuteromycota	glucans	Mannoproteins.
Basidiomycota	Chitin, β(1,3)-β(1,6)-	
	glucans	
Oomycetes	Cellulose, β(1,3)- β(1,6)- glucans	



Chitin

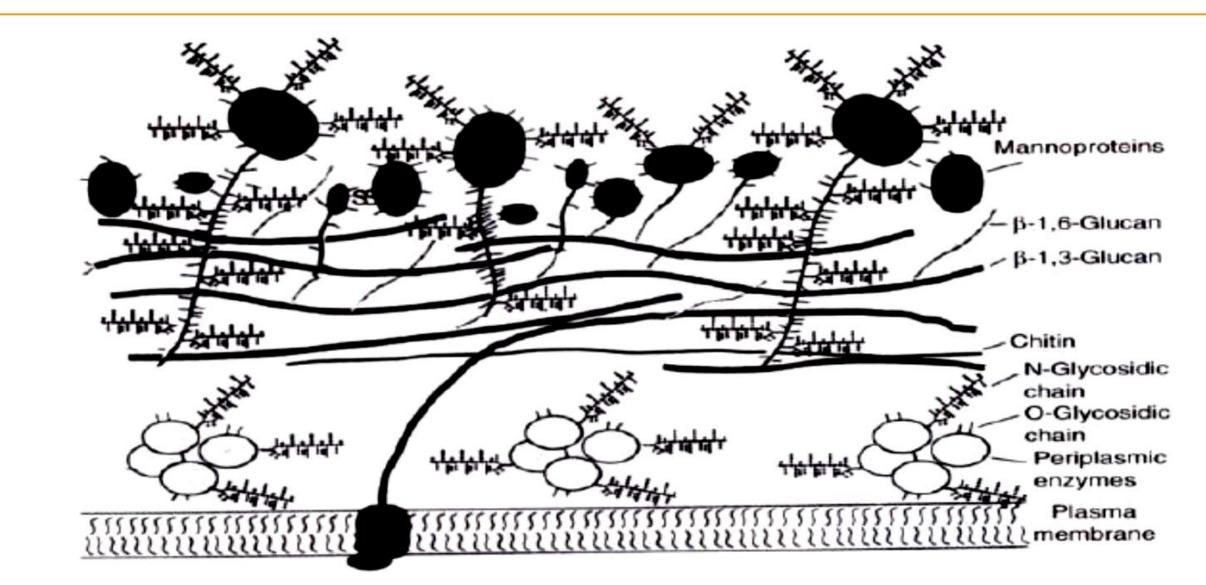


Chitin (N-Acetyl-1-4-β-D- glucosamine

### Cell wall components

- The major polysaccharides of cell wall matrix consist of glucans such as manans, chitosan, and galactans.
  - Glucan refers to a group of D-glucose polymers having glycosidic bonds.
  - Insoluble β-glucans are apparently amorphous in cell wall.
  - Mannans, galactomannans, rhamnomannans are responsible for the immunologic response to the medically important yeasts and molds.

# Structure of fungal cell wall



#### Cell wall components

- Consisting of chitinous microfibrils embedded in the matrix of small polysaccharides, proteins, lipids, inorganic salts, and pigments.
  - Chitin is a (β1-4)-linked polymer of N-acetyl-Dglucosamine (GlcNAc)
  - Produced in cytosol (from UDP GlcNAc into chains of chitin by chitin synthetase)
  - The chitin microfibrils are transported to the plasmalemma and subsequently integrated into the new cell wall.

# Cell wall components

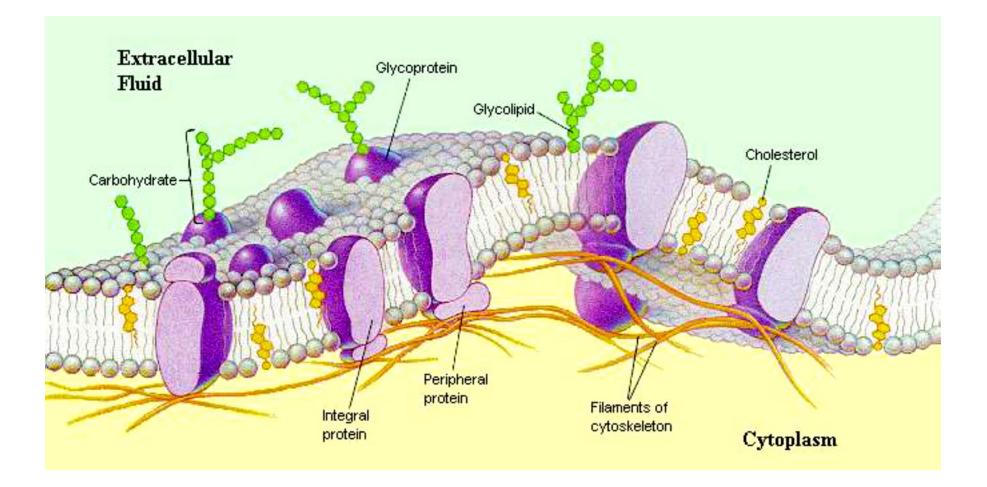
- In addition to chitin, glucan, and mannan, cell walls may contain lipid, protein, chitosan, acid phosphatase, amylase, protease, melanin, and inorganic ions (phosphorus, calcium, and magnesium).
- The outer cell wall of dermatophytes contains glycopeptides that may evoke both immediate and delayed cutaneous hypersensitivity.

# Plasma membrane

- The main role of the plasma membrane
  - To regulate the uptake and release of materials
  - Integral membrane protein (chitin syntase, glucan syntase)
  - Signal transduction.

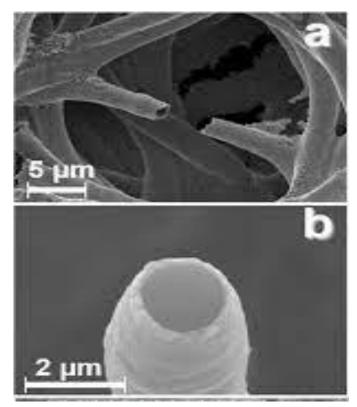
### Plasma membrane

- Similar to mammalian plasma membrane, differing in having the nonpolar sterol ergosterol, rather than cholesterol.
- regulates the passage of materials into and out of the cell by being selective permeable.
- Several antifungal agents interfere with ergosterol synthesis (i.e., amphotericin B)



# Microtubules

- Composed of the protein tubulin, which consists of a dimer composed of two protein subunits.
- Microtubules are long, hollow cylinders ~ 25 nm in diameter.
- Involved in the movement of organelles, chromosomes, nuclei, and Golgi vesicle containing cell wall precursor.

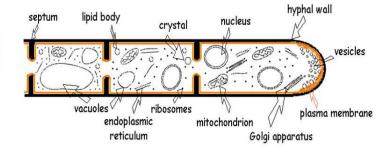


# Microtubules

- Assist in the movement of chromosomes during mitosis and meiosis.
- The destruction of cytoplasmic microtubules interferes with the transport of secretory materials to the cell periphery, which may inhibit cell wall synthesis.

# Nucleus

- The nucleus is bounded by a double nuclear envelope and contains chromatin and a nucleolus.
- Fungal nuclei are variable in size, shape, and number.
- The number of chromosomes varies with the particular fungus.
  - S. cerevisiae ; 18 (n)
  - T. mentagophytes ; 4 (n)





### **Fungal growth and nutrition**

### Fungal Growth Curve

- When a fungus is added to a suitable liquid medium and incubated, its growth follows a definite course.
- If a fungal counts are made at intervals after inoculation & plotted in relation to time, a growth curve is obtained.
- Shows 4 phases :
  - Lag phase
  - Log or Exponential phase
  - Stationary phase
  - Decline phase

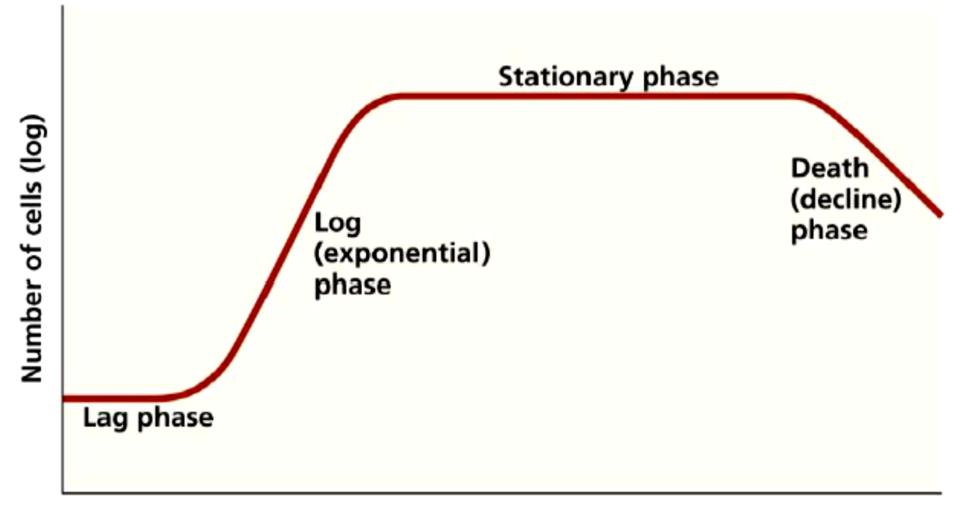
#### Phases of Growth Curve

1. Lag phase - No increase in number but there may be an increase in the size of the cell.

2. Log OR Exponential phase - cells start dividing and their number increases exponentially.

#### Phases of Growth Curve

- 3. Stationary phase cell division stops due to depletion of nutrients & accumulation of toxic products.
- equilibrium exists between dying cells and the newly formed cells, so viable count remains stationary.
- 4. Decline phase population decreases due to the death of cells - autolytic enzymes.



#### Time

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# Morphological & Physiological alterations during growth

- Lag phase maximum cell size towards the end of lag phase.
- Log phase smaller cells, stain uniformly.
- Stationary phase irregular staining, sporulation and production of exotoxins & antibiotics.
- **Decline phase** involution forms (with ageing).

# Factors affecting fungal growth

- Availability of Nutrients & H<sub>2</sub>O
- Temperature
- Atmosphere O2 & CO2
- H-ion concentration (pH)



### Nutrients

#### **Functions**

- Generation of energy
- Synthesis of cellular materials
- Essential nutrients (basic bioelements needed for fungal growth)
  - H<sub>2</sub>O: universal solvent; hydrolyzing agent
  - Carbon: food & energy source; in form of protein, sugar, lipid.
  - Nitrogen: for protein synthesis; nucleic acid synthesis (purines & pyrimidines).
  - Sulfur (sulfate): Amino acids synthesis (i.e., Cystine, methionine).
  - Phosphate: key component of DNA & RNA, ATP, and inner & outer membrane phospholipids of cell membrane.
  - Minerals: associated with protein (i.e., Fe:PRO); common component of enzymes.

#### Nutrients

#### 2 types

- Macronutrients needed in large quantities for cellular metabolism & basic cell structure
  - C, N, H, O
- Micronutrients needed in small quantities; more specialized (enzyme & pigment structure & function).
  - Fe, Cu, Mn, Zn
- Fastidious fungi: microbes that require other complex - nutrients/growth factors (i.e., Vitamins or Amino acids).

### Temperature

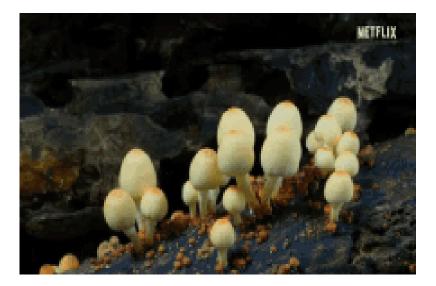
Fungi vary in the temperature requirements.

Temperature range - growth does not occur above the maximum or below the minimum.

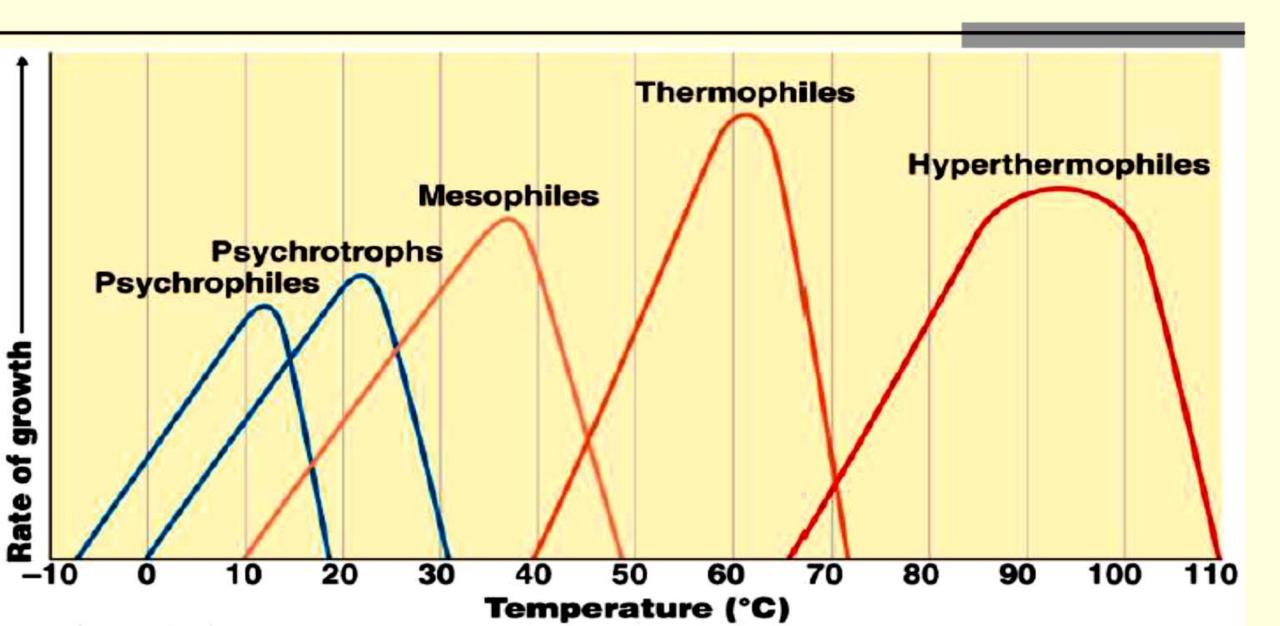
Optimum Temperature - growth occurs best, 20-30°C for most fungi.

#### Fungi differ in their temperature optimum

Psychrophiles: 1 to 20°C
Mesophiles: 20 to 40°C
Thermophiles: 40 to 70°C



#### Microbes differ in their temperature optimum



# H-ion Concentration (pH)

 Neutral or slightly acidic or alkaline pH (5-8) - majority of fungi grow well and have optimum level.

Acidic pH - little fungi grow well.

Alkaline pH - little fungi grow well.

# **Growth Factors**

- Some fungi require certain organic compounds in minute quantities Growth Factors OR Vitamins.
- It can be :
  - Essential when growth does not occur in their absence.
- Accessory when they enhance growth, without being absolutely necessary for it.

#### **Growth Factors**

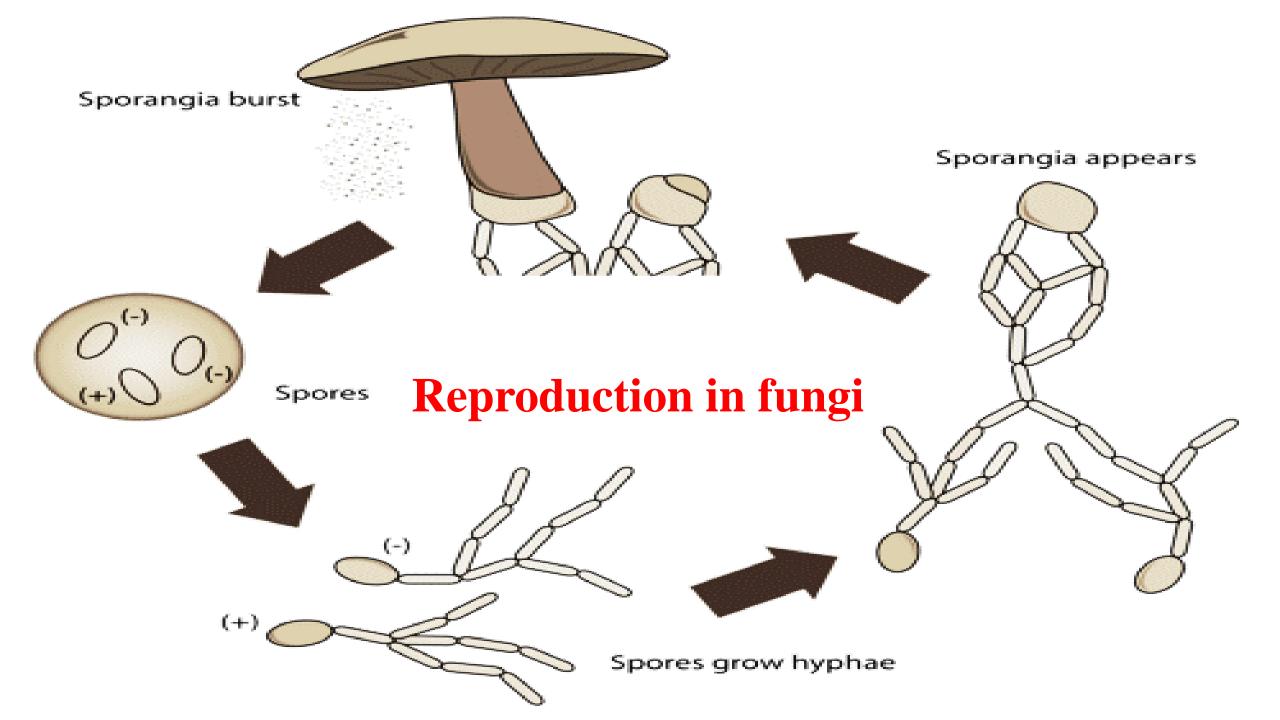
Identical with eukaryotic nutrition

- Vitamin B complex -
- thiamine
- riboflavine
- nicotinic acid
- pyridoxine
- folic acid
- Vitamin B 12

#### Presence or Absence of Gases

- Primary gases =  $O_2$ ,  $N_2$ , &  $CO_2$ 
  - > O<sub>2</sub> greatest impact on microbial growth (even if the microorganism does not require it)
- Aerobic respiration terminal electron acceptor is oxygen.
- Anaerobic respiration terminal electron acceptor is an inorganic molecule other than oxygen (e.g. nitrogen).



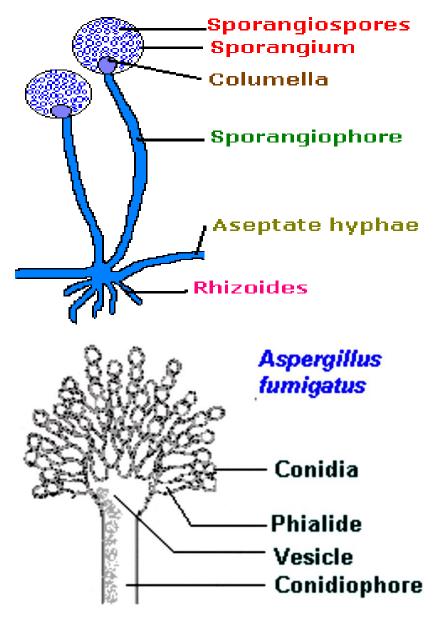


# **Reproduction in fungi**

- Fungi reproduce by asexual, sexual and parasexual means.
- Asexual reproduction is the commonest mode in most fungi with fungi participating in sexual mode only under certain circumstances.
- The form of fungus undergoing asexual reproduction is known as anamorph (or imperfect stage) and when the same fungus is undergoing sexual reproduction, the form is said to be teleomorph (or perfect stage).

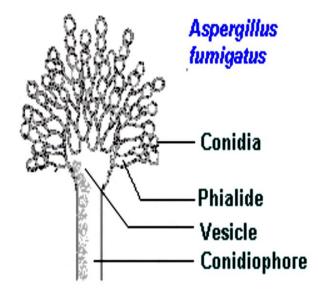
# **Asexual reproduction**

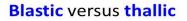
- Asexual propagules are termed either spores or conidia depending on their mode of production.
- Asexual spores are produced following mitosis where as sexual spores are produced following meiosis.
- The asexual spores of zygomycetes, which are known as sporangiospores form within sac like structure known as sporangia.
- The sporangiospores result from the mitotic cleavage of cytoplasm in the sporangium.
- The sporangia are borne on special hyphae called sporangiophore. This endogenous process of spore formation within a sac is known as sporogenesis.

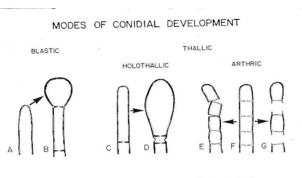


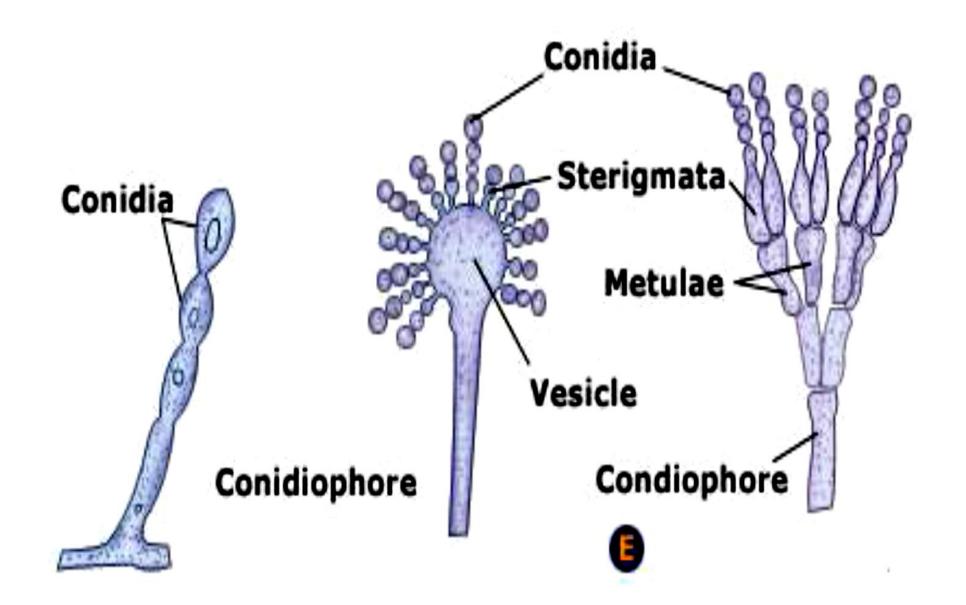
#### **Asexual reproduction**

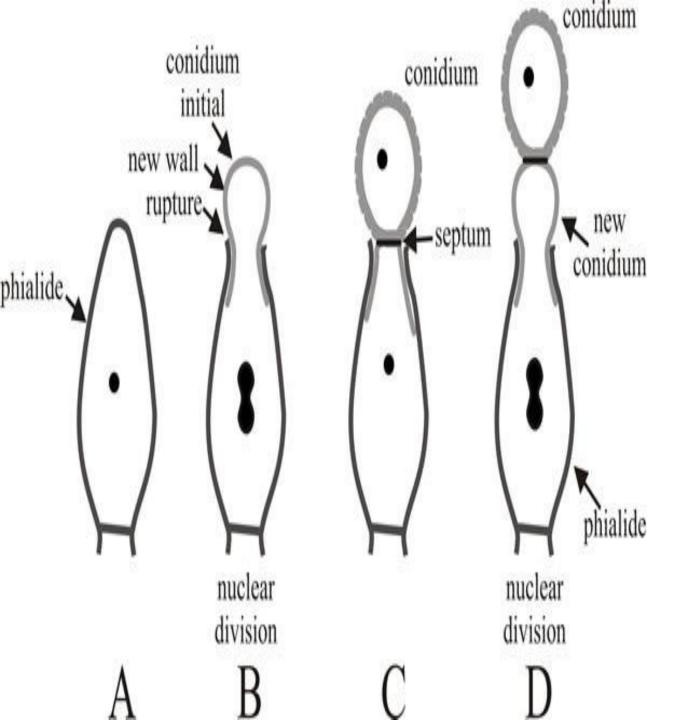
- Conidia arise either by budding off conidiogenous hyphae or by differentiation of preformed hyphae.
- These develop following mitosis of a parent nucleus and are formed in any manner except involving cytoplasmic cleavage. This exogenous process is known as conidiogenesis, a process that occurs both in yeasts and moulds. Conidia are borne on specialised structures called conidiophore. Conidia production may be blastic or thallic. In blastic development the conidium begins to enlarge and a septum is formed. Here the conidium originates from part of parent. In thallic mode of development the conidium is differentiated by <sup>@</sup> septum before its differentiation. Thus the conidium results from the conversi of entire parent cell into the conidium.
- The cell that gives rise to a conidium is called a conidiogenous ce Conidiophores are specialised hyphae that bear conidia or conidiogenous cells. many cases conidiogenous cells are referred as phialides.

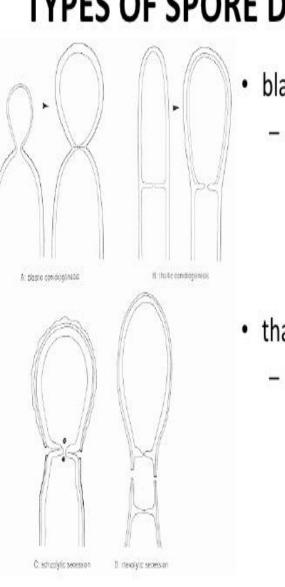












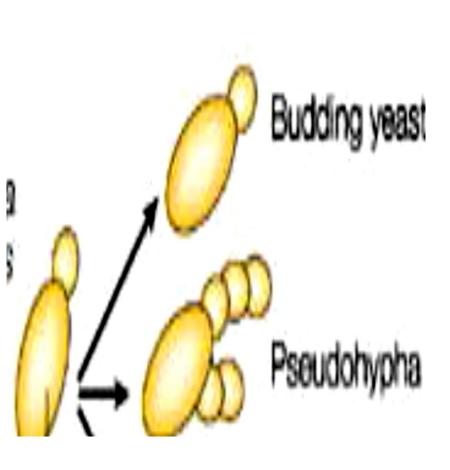
#### **TYPES OF SPORE DEVELOPMENT**

- blastic conidiogenesis
  the young conidium is
  - recognizable before it is cut off by a cross-wall (BUDDING)
- thallic conidiogenesis
  - cross-wall is laid down before differentiation of the conidium begins

# Asexual reproduction

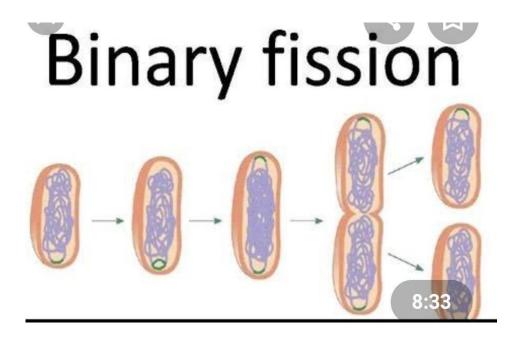
# 1. Budding

• The initial events of budding can be seen as the development of a ring of chitin around the point where the bud is about to appear. This reinforces and stabilizes the cell wall. Enzymatic activity and turgor pressure the act to weaken and extrude the cell wall. New cell wall material incorporates during this phase. Cell contents forces into the progeny cell, and as the final phase of mitosis ends a cell plate, the point at which a new cell wall will grow inwards from, forms. Separation of the bud from the parent leaves a scar. When chains of yeast cells do not fully separated this can create a pseudo mycelium



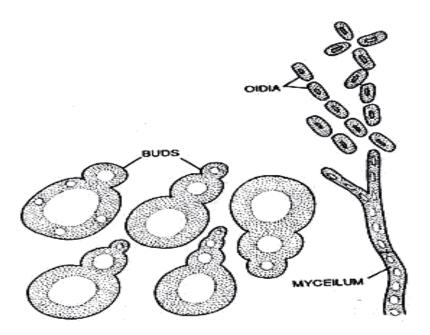
# 2. Binary fission

• Binary fission is the form of asexual reproduction most prokaryotes and protists used by to reproduce. This process results in the reproduction of a living cell by division into two equal or nearequal parts. Binary fission begins when the DNA replication occurs. Each circular DNA strand then attaches to the cell membrane. The cell elongates, causing the two chromosomes to separate. The cell membrane then invaginates (grows inwards) and splits the cell into two daughter cells through a process called cytokinesis



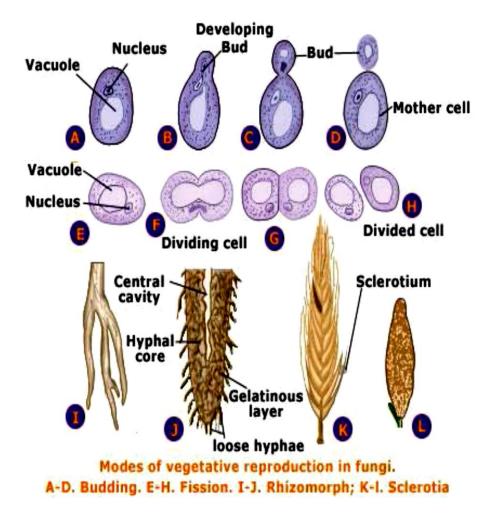
### 3. Fragmentation

• Many fungi can reproduce by fragmentation. Any mycelium that is fragmented or disrupted, if the fragment contains the equivalent of the peripheral growth zone, can grow into a new colony. Many fungi sub-cultured using this hyphal fragment technique. Cut mycelial tips do not regenerate, but branches can form some distance from the damage point.



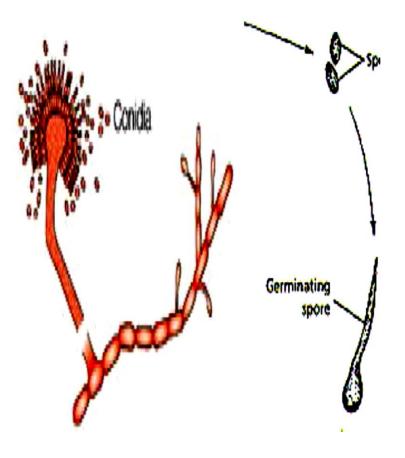
### 4. Sclerotia

• In some cases, as in *Claviceps*, the hypha becomes intervoven to form a compact mass and surrounded by a hard covering or rind. Such structure called sclerotia. They remain dormant under unfavorable conditions and germinate into new mycelia on the return of favorable conditions



## **5. Sporulation**

• By far the most important type of asexual reproduction is that of spore formation. Asexual reproduction is extremely important to fungi. It is responsible for the production of large numbers of spores throughout the year. These asexual spores formed on a phase of the fungal life cycle termed in some texts as the mitosporic, or anamorphic phase. There can be more than one mitosporic state for each species of fungus, and in some cases the mitosporic state of very different species can look very similar, any spore germinate to giving germ tube, the elongate to giving new mycelium



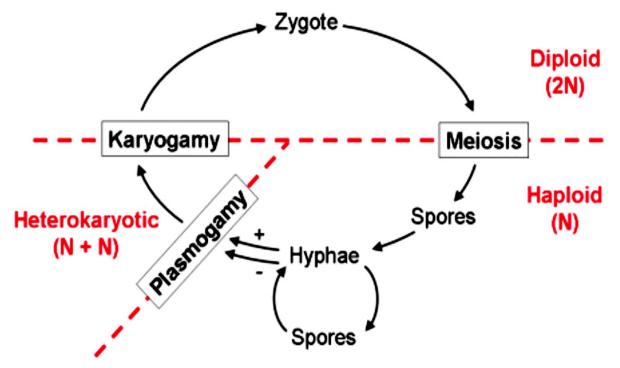
# Sexual reproduction

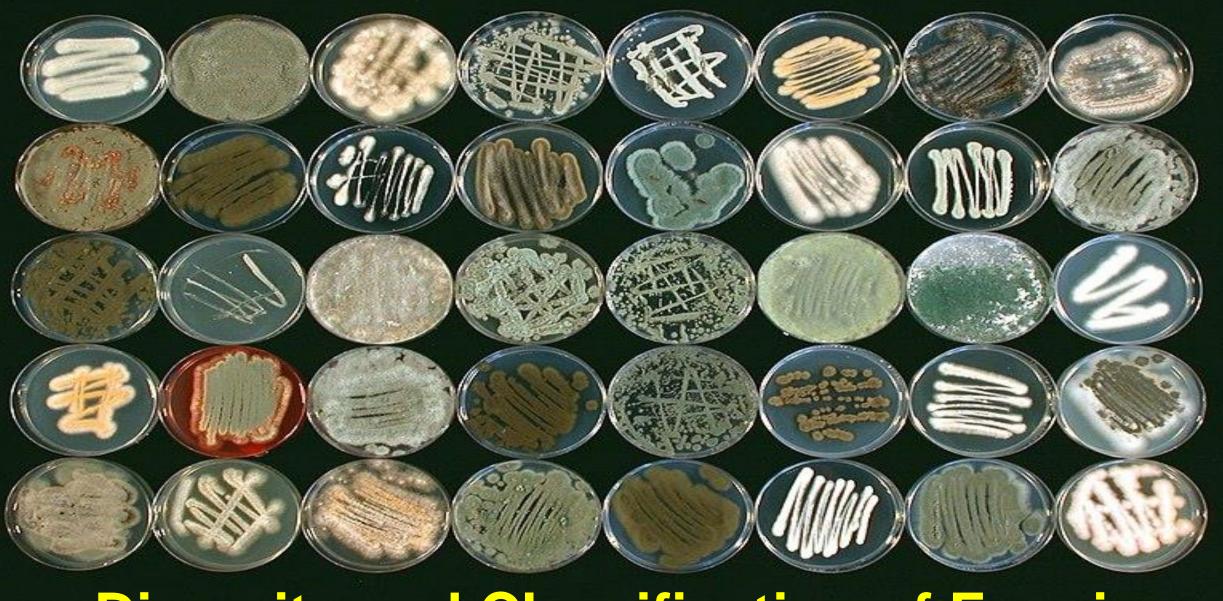
### **Sexual reproduction**

• In general, the sexual reproduction life cycle, involves the fusion of hyphae from two individuals, forming a mycelium that contains haploid nuclei of both individuals. There are typically three phases in the sexual reproduction of fungi: plasmogamy, karyogamy and meiosis. 1- **Plasmogamy** is a stage in the sexual reproduction of fungi. In this stage, the cytoplasm of two parent mycelia fuse together without the fusion of nuclei, as occurs in higher terrestrial fungi. After plasmogamy occurs, the secondary mycelium forms. The secondary mycelium consists of dikaryotic cells, one nucleus from each of the parent mycelia.

**2-Karogamy** is the two nuclei that originated from different individuals fuse to form a diploid zygote.

**3-Meiosis** The diploid nucleus will undergo meiosis as a preliminary to the formation either four haploid nuclei or four haploid cells (ascospores or basidiospores).

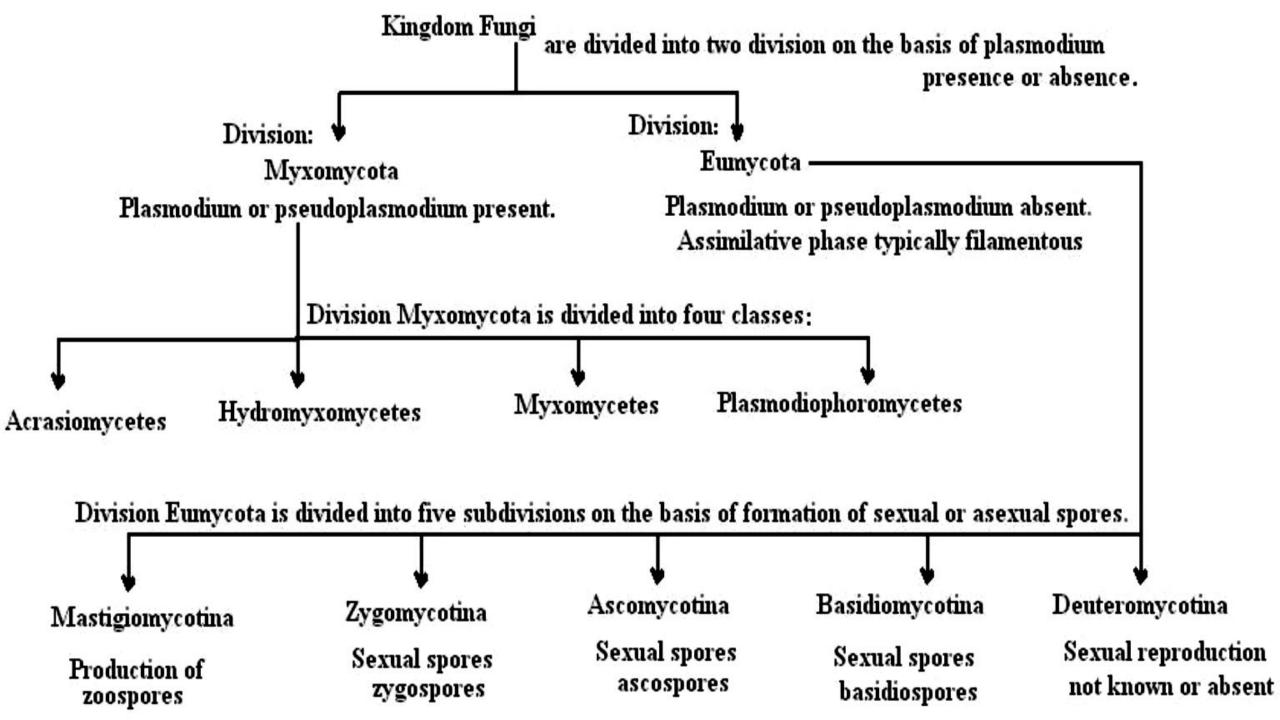


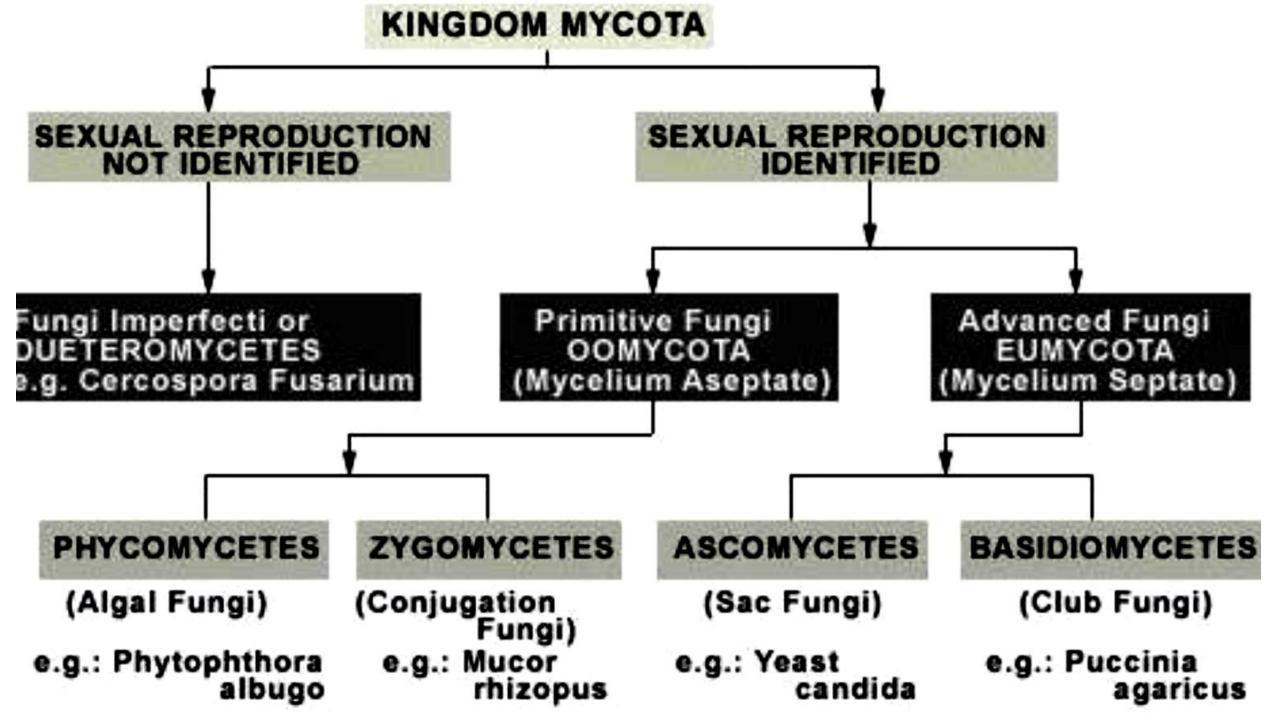


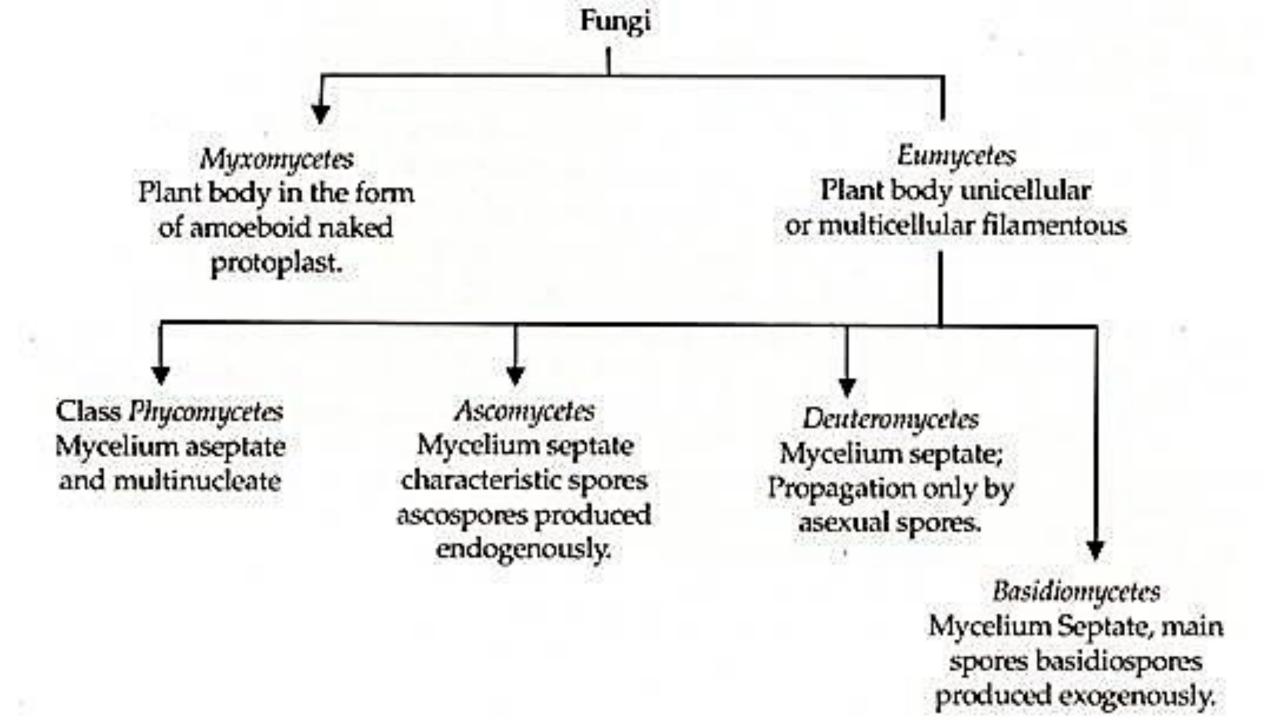
• Fungi grow in a wide range of habitats, such as extreme environments including deserts or areas with high high osmotic conditions of sugar and salt concentrations, ionizing radiation, as well as in deep-sea sediments. Some of fungi can survive the intense UV and cosmic radiation encountered during space travel. Most grow in terrestrial habitats, though many species grow partly or solely in aquatic habitats, such as the chytrid fungus Batrachochytrium dendrobatidis, a parasite that has been responsible for a worldwide decline in amphibian populations. This fungus spends part of its life cycle as a motile zoospore, enabling it to propel itself through aquatic habitats and enter its amphibian host. Other examples of aquatic fungi include those living in hydrothermal areas of the ocean. Therefore, fungi have a worldwide distribution

• Fungal species are historically been distinguished by many methods and concepts. Classification based on morphological characteristics, such as the shape and size of spores. or fruiting structures, has traditionally dominated fungal classification. Species may also distinguished by their biochemical and physiological characteristics, such as their ability to metabolize certain biochemical materials, or their reaction to chemical tests. Recently, the application of molecular tools, including DNA sequencing and phylogenetic analysis, to study diversity has greatly improved the resolution and added robustness to estimates of genetic diversity within various taxonomic groups. In addition, fungal taxonomy depends on macroscopically and microscopically observable characteristics. The phenotypic approach such as:

- a) Color of the growth
- b) Rate of growth
- c) Method of spore production
- d) Hyphal septation all aid in assigning a fungus a name
- More technologies that are newer have aided in both identifying new organisms and recognizing the various forms of the same organism.
- a) Electron microscopy allows recognition of structures not visible by light microscopy.
- b) Physiological and biochemical techniques are being applied as well as identification of secondary metabolites.
- c) Polymerase Chain Reaction (PCR) allows comparison of the DNA or RNA structure of organisms, including fungi.







# **Nyxomycetes**

# Myxomycetes

- Subdivision (1) Myxomycotina
- Class Myxomycetes
- Occurrence: Myxomycetes are found in cool places, decaying wood and humus rich soil.
- Structure: The vegetative stage in Myxomycetes has no cell wall, naked and irregular mass of protoplasm called plasmodium. The plasmodium is amoeboid in shape and multinucleate and moves with the help of pseudopodia.
- **Classification:** the class Myxomycetes divided into three orders, namely Plasmodiophorales, Stemonitales and Acrasiales



### **Order:** *Plasmodiophorales*

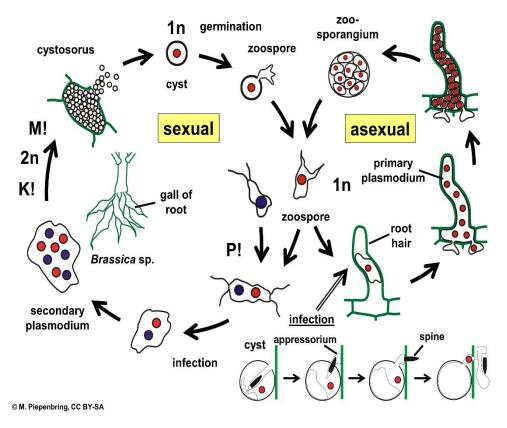
- F: Plasmodiophoraceae
- Ex. Plasmodiophora brassicae
- **Distribution**: the fungus causes disease to cruciferous plants mainly to cabbage ( club root disease).
- Occurrence: the fungus is an obligate parasite.
- **Disease symptoms**: irregular growth or hypertrophy in the root.
- **Structure of the pathogen**: naked mass of protoplasm called plasmodium.





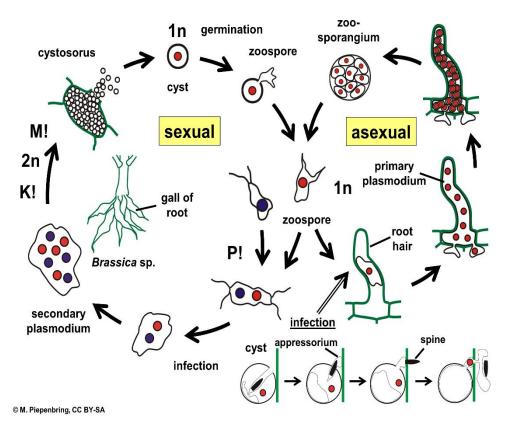
### **Asexual reproduction**

- The nuclei in the plasmodium are diploid. They undergo reduction division when the plasmodium are in the cells of root of host plant.
- Spherical non motile spores have a chitinous wall are formed. The death and decay of the root cells of the host sets the spore free.
- The spores metamorphose themselves into biflagellate zoospores infect the healthy plants forming myxmoeba, which repeatedly divided forming haploid plasmodium.



### **Sexual reproduction**

- Each nucleus of the haploid plasmodium gets isolated surround itself by a little cytoplasm and form gametangium.
- The nucleus of each divides mitotically to form 8-10 biflagellate isogametes.
- Two isogametes fuse and form a diploid zygote
- The diploid zygote divides mitotically and develops into diploid pladmodium.



# Eumycetes

## **Class 1: Phycomycetes**

- Occurrence: they are very common in occurrence. The bread mold (*Mucor*), the water mold (*Saprolegnia*), the white rust of mustard all are phycomycetes.
- Structure: they are coenocytes, aseptate much branched mycelium (septa appearing to the dead portions or at the time of formation of reproductive structures. Many primitive phycomycetes are aquatic in higher forms their distribution even (except zygomycetes) show dependence on moisture. They may be parasite or saprophyte.





# Reproduction

**Reproduction**:

#### Asexual reproduction is brought about by

1- Fragmentation 2- Spore formation

Phycomycetes produce both zoospores and conidia. *Saprolegnia*, *Phytophthora*, *Pythium* and others produce zoospores.

Albugo produces conidia.

In Mucorales, asexual reproduction is by aplanospores.

Chlamydospores also found in some members.





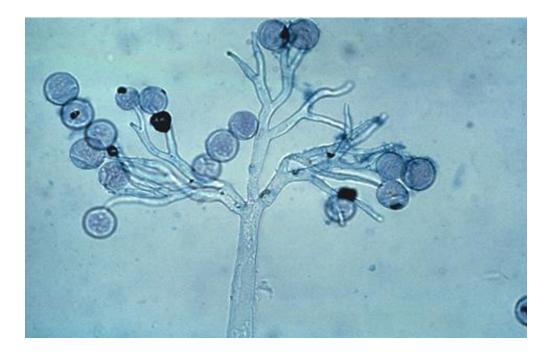
# Reproduction

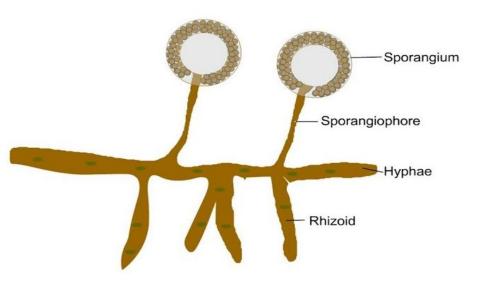
#### **Sexual reproduction**

- 1-Planogametic copulation.
- 2- Gametangial copulation.
- 3- Gametangial contact.
- Stages in sexual reproduction
- a- Plasmogamy
- b- Karyogamy
- c- Meiosis

### Classification

- The filamentous phycomycetes are divided according their mode of reproduction into 2 orders namely
- (1) **Oomycetes**: reproduction is oogamous.
- (2) **Zygomycetes**: reproduction is isogamous.
- The nonfilamentous phycomycetes having rounded lobed mycelia thallus are placed in (3) **Archymycetes**.
- The following examples are discussed here; Oreders:
- Saprolegniales ex. Saprolegina.
- Mucorales ex. Mucor.





### **Order:** *Mucorales*

C: *Phycomycetes* 

O: Mucorales

F: *Mucoraceae* 

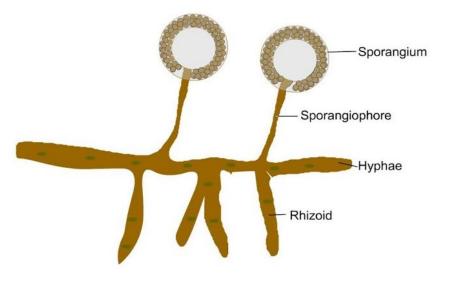
Ex. Mucor Ex: Rhizopus

Occurrence and habitat: *Mucor* lives in a habitat like

organic soil, a dead decaying matter of fruits, vegetables

and plants, it is essentially saprophyte.





### Morphological features

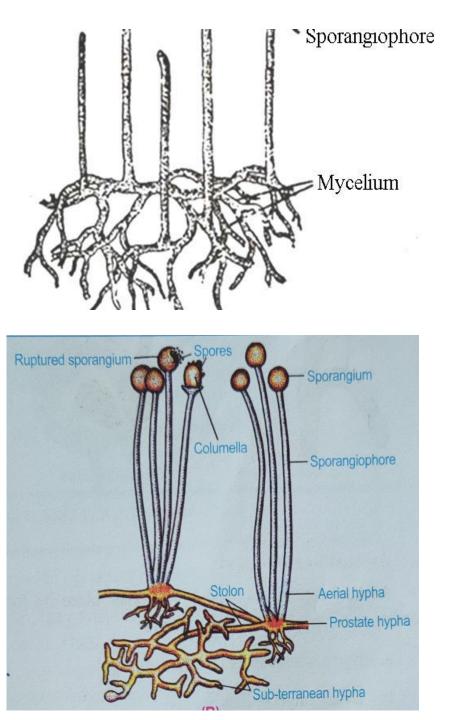
#### **D**Mycelium

The mycelium of *Mucor* is highly branched forms a fine network of hyphae. A mycelium is simply a cluster of hyphae.

#### 

These are the thread like and very fine structures that form a "Mycelial network". Hyphae of Mucor is filamentous, aseptate or coenocytic. In *Mucor*, the hyphae categorize into three types:

- 1. Sub-terranean hyphae
- 2. Prostrate hyphae
- 3. Aerial hyphae



- **Sub-terranean hyphae:** are the type which is highly branched, more penetrating and is present horizontally to the substratum.
- **Prostrate hyphae** are the type which is also present horizontally between or under the substratum. These two hyphae i.e. sub-terranean and prostrate hyphae help in absorption of water and nutrition.
- Aerial hyphae are the type, which originates vertically out from the prostrate hyphae.

## Morphological features

• Sporangiophore

It is elongated, slightly narrow in shape.

• Columella

Sporangiophore swells up to form a dome-like structure called "Columella" which can vary in both shape and size.

#### • Sporangium

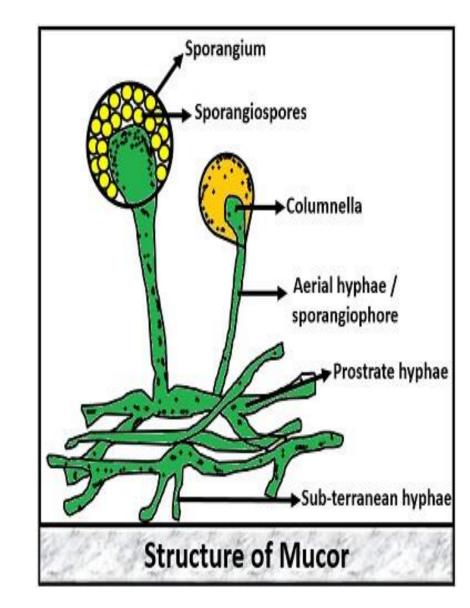
It is the round and thick outer covering which carries numerous spores inside it. It can be globose to spherical.

#### • Spores

These are the reproductive structures forms within the sporangium which are simple, flattened and variable in shape and size.

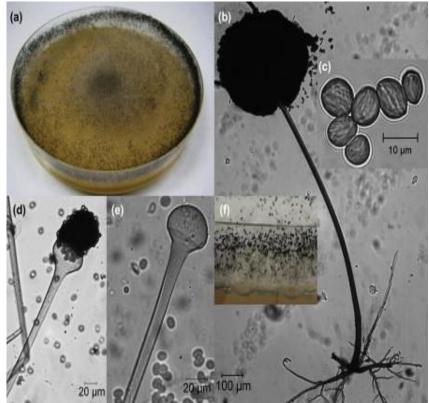
#### • Nucleus

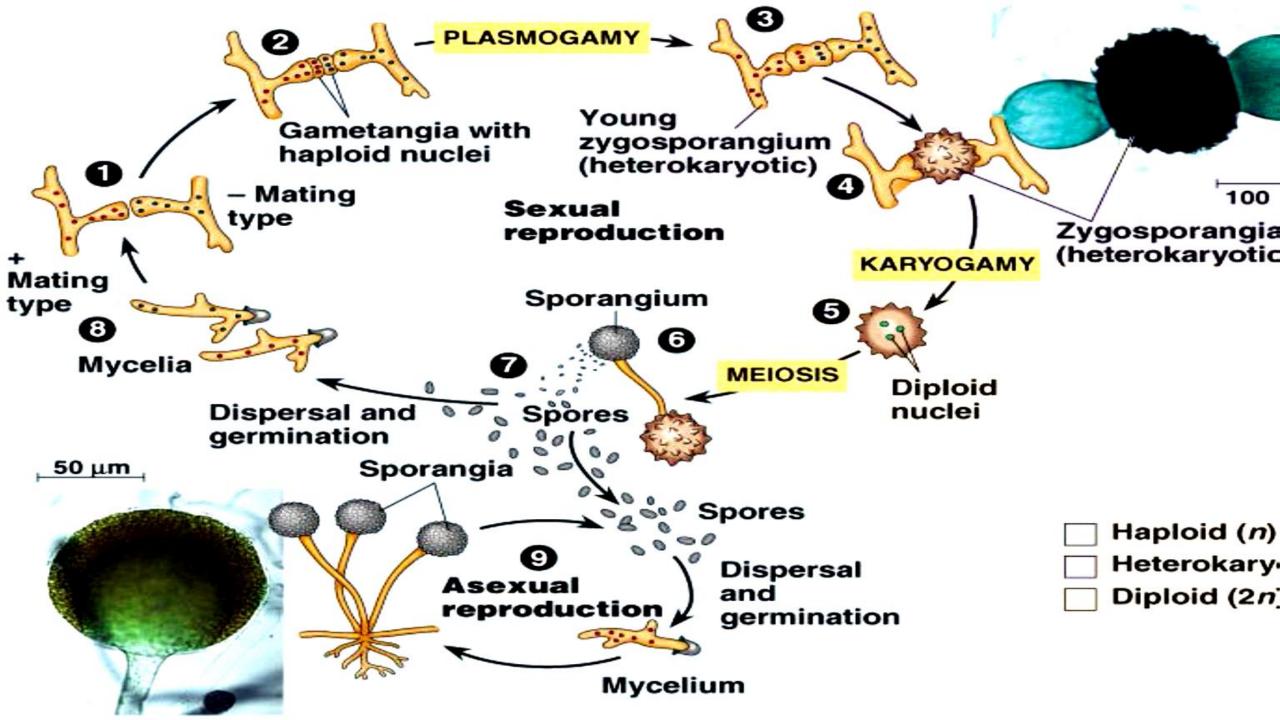
Multinucleate nuclei present in Mucor.



## Macroscopic features

- The colony of *Mucor* shows rapid growth.
- The colour of the colony is usually white to grey and turns to brown when the culture becomes old
- Hypha: Coenocytic and branched
- Spores: Generally black in colour but can vary with different species. The spores can be motile or non-motile and can exist in variable shapes.

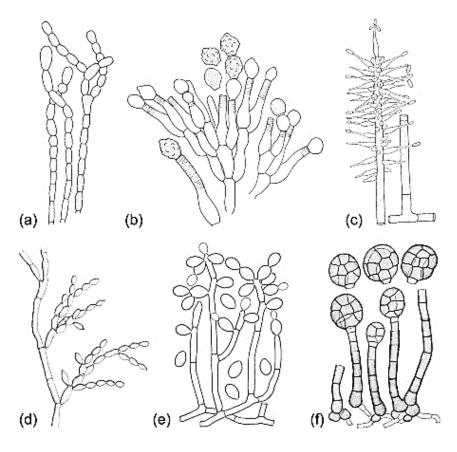




# **Division:** Ascomycota

### **Division:** Ascomycota

- Subdivision: Ascomycotina
- Class: Ascomycetes
- The subdivision Ascomycotina is equivalent to class Ascomycetes of the older classifications. Ascomycotina includes only such fungi in which the zygospores are absent and the perfect-state spores are the ascosporic.
- The Ascomycetes and the Basidiomycetes commonly called "higher fungi".
- Ascomycetes is the largest class of fungi, including more...than 15,000 species. Some of the commonly-known Ascomycetes are yeasts, black molds, green molds, powdery-mildews and morels- The characteristic ascosporic are present in a sac-like body, called accuse and therefore these fungi are called "sac fungi" (Gr. ciskos = sac).



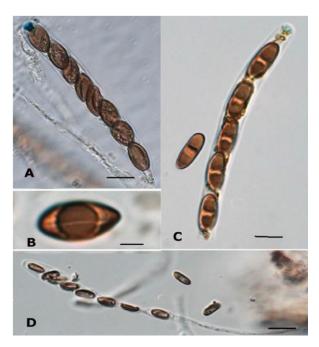
### **General characteristics**

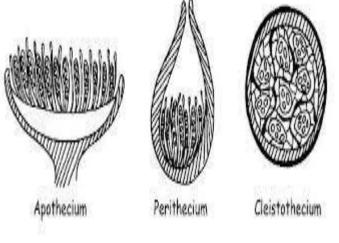
- Ascomycetes occur in almost all climatic conditions, and in a wide variety of habitats, i.e. in soil, on dung (coprophilous), in marine, as well as fresh water, as saprophytes of animal and plant remains, and as parasites on plants as well as animals. Micah of most parasitic species grows within the host tissue, but powdery mildews grow superficially upon the host showing ectoparasitic nature. Few Ascomycetes are entirely hypogeal, i.e. grow and develop only underground.
- 2. The mycelium is well developed, profusely branched and septate. Each segment the hyphen contains several nuclei. However, yeasts are single-celled organisms.
- 3. In each septum or cross wall of the mycelium there is present a simple central pore.
- The chief distinguishing character of all Ascomycetes is the presence of a sac-like body, called ascus (pi. asci). It contains sexually produced spores, called ascospores.

### **General characteristics**

5. The ascosporic are formed after karyogamy and meiosis. In an ascus, the number of ascospores is usually eight. However, in soroe species their number may vary from one to over 1000 in an ascus.

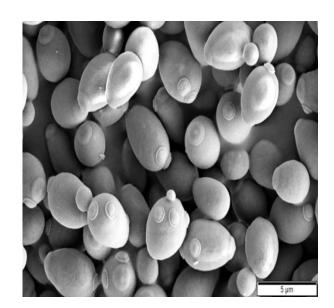
- 6. The ascospores are always endogenous in origin, and called perfect-state spores.
- 7. The asci usually grouped to form a definite type of multicellular fruiting body called ascocarp. The ascocarps remain enveloped in a sheath of sterile hyphae.
- 8. The ascocarps are either cup or saucer-shaped (apothecium, e.g. Discomycetes), flask-shaped (perithecium, e.g. Pyrenomycetes), or ovoid, spherical and indehiscent cleistothecium, e.g. many Plectomycetes).
- 9. Any type of flagellate cells is completely absent in the life cycle of all Ascomycetes.

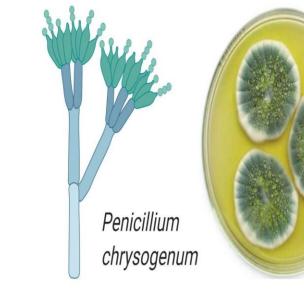




### Classification

- Many modern mycologists classify Ascomycetes as follow:
- Class: Ascomycetes
- Sub-class 1. Hemiascomycetes (no ascocarp)
- Sub-class 2. Euascomycetes (ascocarp present)
- Based on the nature of ascocarp the sub class Euascomycetes is divided into three series namely
- Plectomycetes (cliestithecium)
- Pyrenomycetes (perithecium)
- Discomycetes (apothecium)
- The following examples will discussed here:
- Hemiascomycetes: Saccharomyces (yeast)
- Plectomycetes : *Penicillium*



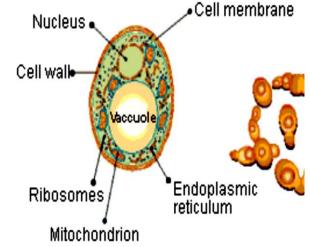


### Classification

- C: Ascomycetes
- S.C.: Hemiascomycetes
- **O:** Endomycetales
- F: Saccharomycetaceae
- Ex. Saccharomyces
- 1. Have a predominantly unicellular thallus that may produce pseudomycelium,
- 2. Reproduce asexually primarily by multilateral budding.
- 3. Produce ascospores in a free ascus that originates from a zygote or parthogenetically from a single somatic cell.

The best-known member of the family is *Saccharomyces cerevisiae*.

• *Saccharomyces cerevisiae* commonly employed in bread making and beer brewing. Therefore, it known as called the brewer's or baker's yeast.



#### • Cell structure:

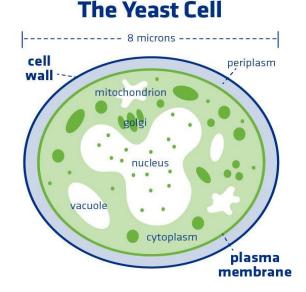
Antony Von Leeuwenhoek (1680) was the first to describe the yeast cells. Its unicellular and non-mycelial.

• The Cell Wall

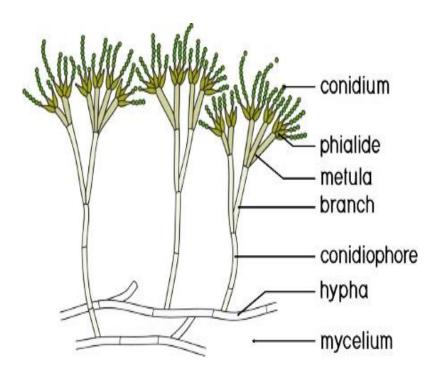
The cell wall is double layered, thin, delicate and flexible. It is composed of two complex polysaccharides, mannan (30%) and glucan (30-40%) with smaller quantities of protein (6-8%), lipid (8.5 - 10.5%) and chitin (2%). Cellulose is absent.

• The Protoplast

Inner to cell wall is a cytoplasmic membrane or plasma membrane. It surrounds the cytoplasm and a nucleus.



- C: Ascomycetes
- S.C.: Euascomycetes
- S: Plectomycetes
- **O:**Aspergillales
- F: Aspergillalaceae
- Ex. Penicillium



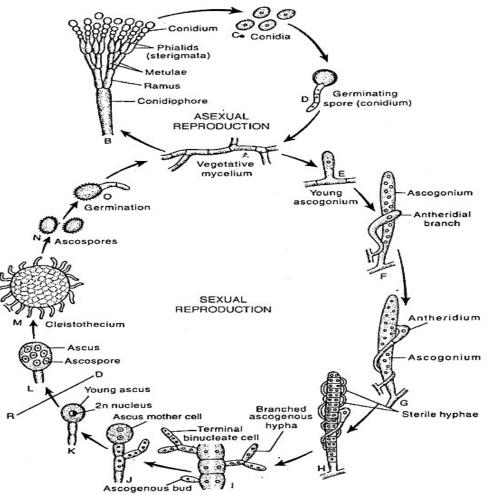
• *Penicillium* species occur on Citrus and other fruits, cheese and other foods. The mycelium is well branched, and consists of many septate hyphae, conidiophores are develops from the hyphae, the conidia developing on the erect conidiophores. The conidiophores develop from any hyphal cell but not from specialized foot cell as in *Aspergillus* sp. The conidiophores are branched but unbranched in few species, *Penicillium* characterized by the presence of primary and secondary strigmata.

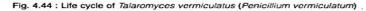
## **Asexual reproduction:**

The conidia dispersed by wind. On getting the suitable conditions of moisture and temperature, each conidium swells and germinates by producing a germ tube. The latter becomes septate and develops into the mycelium.

### **Sexual reproduction:**

The antheridial nucleus migrates into the ascogonium, the ascogonium septeted into binucleate cells function as ascogenous hypha, which contains two nuclei (one from ascogonium and the other from antheridium). The two nuclei fused to form the diploid nucleus in the young ascus mother cell, and the zygotic nucleus might have passed through meiosis to form eight ascospores in each ascus. Many sterile hyphae develop and enclose the young asci from all sides and the fruiting body is formed





## **Economic importance of** *Penicillium*

1. Several species of Penicillium play a central role in the production of cheese and of various meat products.

2. Penicillium roqueforti are the molds on Camembert, Brie, Roquefort and many other cheeses.

3. Penicillium nalgiovense used to improve the taste of sausages and hams and to prevent colonization by other moulds and bacteria.

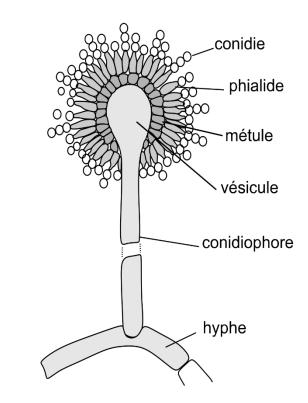
4. Species of Penicillium serve in the production of a number of biotechnologally produced enzymes and other macromolecules, such as gluconic, citric and tartaric acids, as well as several pectinases, lipase, amylases, cellulases and proteases.

5. Most importantly, they are the source of major antibiotics, particularly penicillin and griseofulvin.

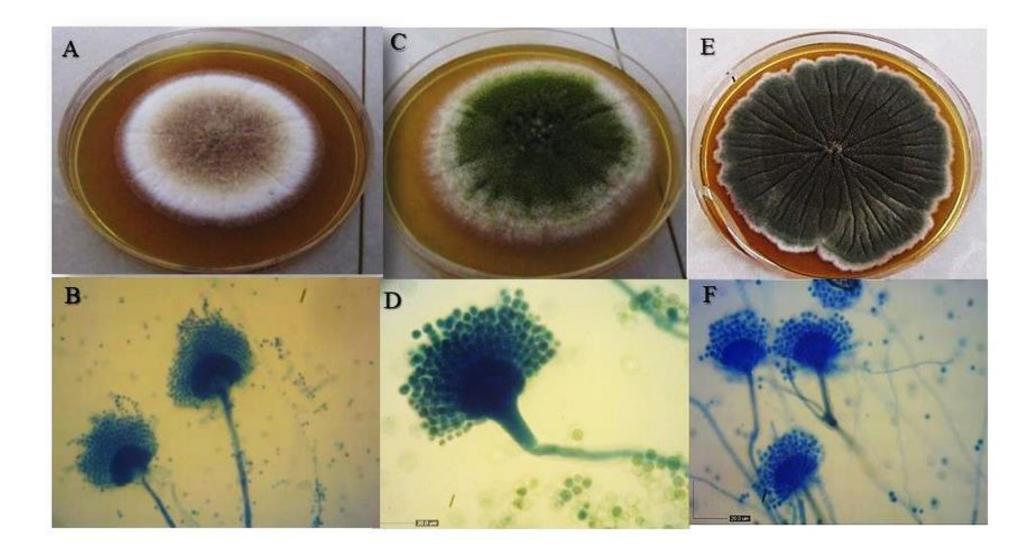
6. Some species causes animal and human diseases.



- Genus Aspergillus
- Division: Amastigmycota
- Subdivision: Ascomycotina
- Class: Ascomycetes
- Subclass: Plectomycetidae
- Order: Eurotiales (Aspergillales)
- Family: Eurotiaceae (Aspergillaceae)
- Genus: Aspergillus



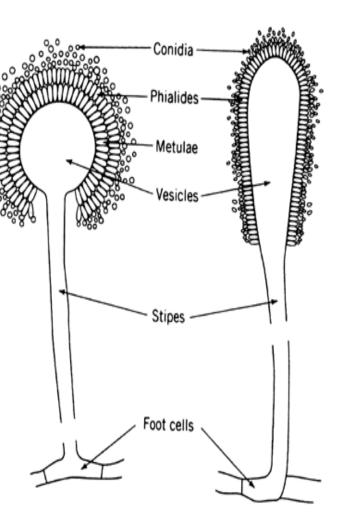
- □ The genus is widely distributed fungus from the very cold region of arctic to the tropics.
- □ The name is derived from **aspergillum**, which means a special bruch resemblance to the conidial apparatus of *Aspergillus*.
- □ *Aspergillus* colonies. (it is colour)
- □ They mostly consist of erect conidiophores.



- ✓ Aspergillus produce antibiotics and mycotoxins and cause a lot of food spoilage.
- ✓ Spores of *Aspergillus* are carried by air, everywhere, and that is why of spoilage of organic materials.
- ✓ The successful colonization of numerous types of media (active enzyme system).

## **Asexual reproduction**

- It takes place through the production of conidia. Conidiophore arises as aerial unbranched hypha. The cell from which the conidiophores emerge is known as foot cell.
- Conidiophores are produced singly and are non-septate, un-branched and each end with a head known as vesicle.
- Vesicle covered with either a single phialides (strigmata) or a layer of metulae which bear small phialides (uniserriate or biserriate). The vesicle, phialides, metulae (if present) and conidia form conidial head.
- Conidia born from phialides, are one-celled, smooth, or rough-walled, hyaline or pigmented and are basocatenate, forming long chains may be divergent (radiate), or aggregated in compact columns (columnar).
- Condia split from phialides and spread away, when find suitable substrate, it germinates forming new fungus

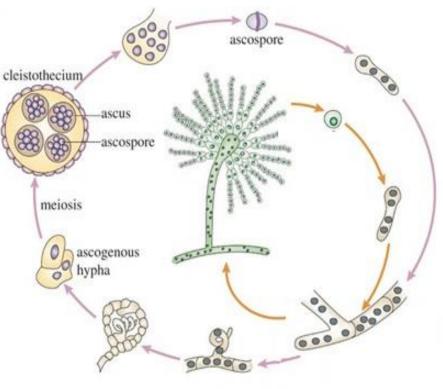


## **Sexual reproduction**

- The sexual reproduction is rare.
- *Aspergillus* is **homothallic**, a male (Antheridium) and female (Ascogonium) sex organs are developed close together on the same hypha or on separate nearby hyphae of the **same mycelium**. Both are elongate, multinucleate and generally coil around each other.
- Ascogonium: A small, loosely coiled septate hyphal branch arise from vegetative hypha. It is differentiated into three parts.
- 1) The terminal segment is generally the longest and single celled it contains up to 20 nuclei called **trichogyne**. It is functions as the **receptive part of the female organ**.
- 2) The segment below trichogyne function as the female gametangium called ascogonium.
- 3) Below ascogonium is the stalk consisting of few cells.

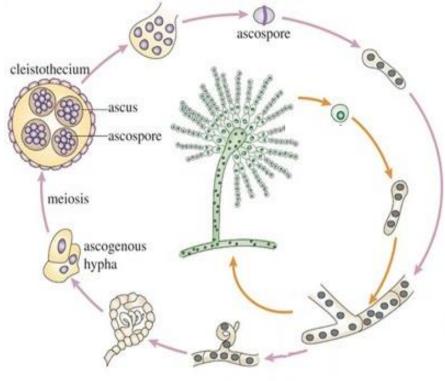
## **Sexual reproduction**

- Fusion take place between the antheridium and trichogyne. The tip of the **antheridium arches over the apex** of the trichogyne and fuse with it. The contents of antheridium then pass through the opening into the trichogyne.
- The haploid male and female nuclei in the ascogonium come to lie in pairs (dikaryons).
- After pairing of the nuclei, ascogenous hyphae arise which differentiated to form ascus mother cell which produce ascus contain 8 ascospores.
- Ascospores after liberation germinate at suitable condition producing new fungus.
- The ascocarp in *Aspergillus* is a small, rounded, yellow with smooth walls. Even at maturity it remain closed called **cleistothecium** or cleistocarp.



## **Economic importance**

- Aspergillus causes rot of dates, decay of tobacco and cigars.
- It spoils nuts, bread and other food-stuffs.
- Some of Aspergillus produce mycotoxins such aflatoxin and ochratoxin.
- They cause a number of disease grouped under the name Aspergilloses also cause ear disease called Otomycosis.
- Aspergillus used for antibiotic production such as Flavicin, Aspergillin, Geodin, Patulin and Ustin.
- Aspergillus gossypii used for production of certain vitamins such as B 12.
- Certain species used for production of many enzymes, lipids and several organic acids.



## **Deuteromycotina (Fungi imperfecti)**

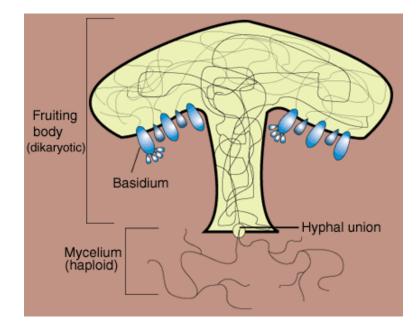
# **Deuteromycotina (Fungi imperfecti)**

- This group of fungi is reproducing by asexual means usually by conidia, but lack perfect sexual stage.
- 2) Deuteromyctes economically highly important, their number goes up to thousands.
- 3) Many of them live as saprophytes and many more as parasites.
- 4) The latter are the causative agents of diseases in plant and animals including man.
- 5) Some cause spoilage of stored products.



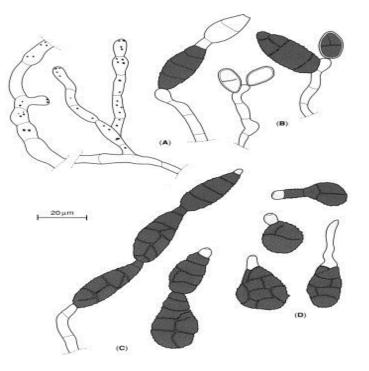
### The somatic phase

- The majority of these fungi consists only of the **haploid** mycelium. It is septate and profusely branched.
- Reproduction takes place chiefly by formation of conidia.
- Formation of oidia and chlamydospores has been also reported.



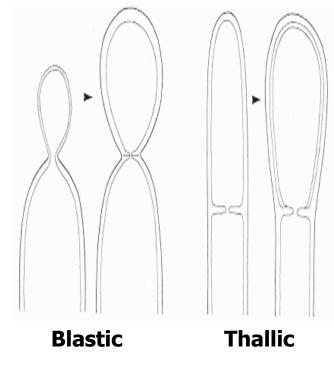
### **Conidia and conidial development**

- The conidia are exogenously produced spores. They are nonmotile and are borne externally from growing hyphae called conidiophores.
- The apical region of conidiophore may produce a single conidium or a chain of conidia usually in basipetal succession.



## **Conidia and conidial development**

- Ellis (1971) has recognized two types of conidial developments
  - **Blastic:** in this type of conidial development marked enlargement of conidial initial take place before it is delimited by a septum. It may be of two types:-
- **1. Holoblastic**: both the inner and outer wall of conidiogenous cells takes part in the formation of conidia.
- **2.** Enteroblastic: only the inner wall of conidiogenous cells takes part in the formation of conidia
  - Thallic: in this type of conidial development there is no enlargen of conidial initial. It take place after the initial has been delimited by septum.



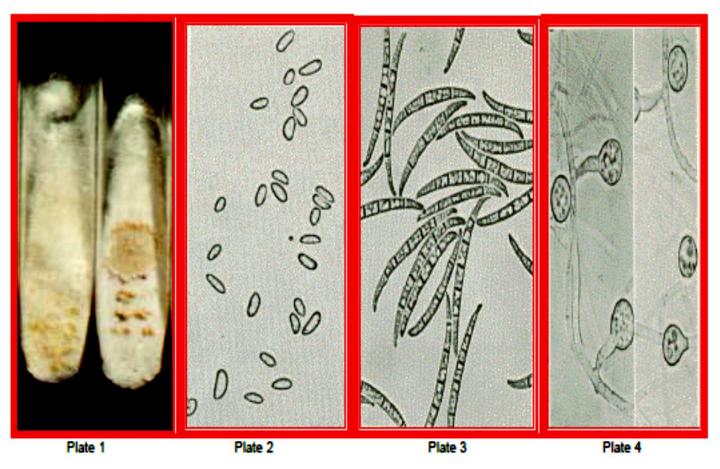
#### Genus: Fusarium

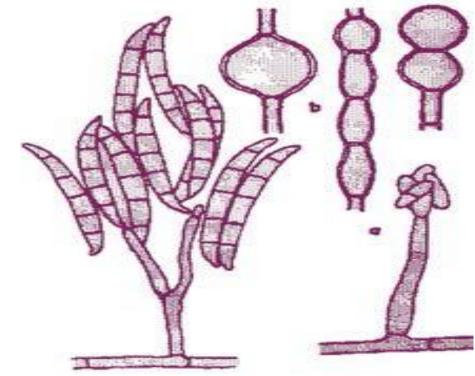
- This genus includes a large number of species and many forms within species.
- Many of these are **saprophytic**, some are **facultative parasites** and others are **parasitic**.
- **Mycelium:** it is extensive. The hyphae are septate and branched. When young they may be colourless or with a tinge of pink, purple or yellow and become dark coloured at maturity.



- **Microconidia:** Macroconidia (asexual spores) derived from conidium-producing cells called phialides. The phialides clustered together in cushion-shaped masses known as sporodochia. The macroconidia are hyaline, canoe-shaped spores usually with five or more septa.
- **Macroconidia:** Microconidia are small, usually unicellular but sometimes bicelled, spherical or oval bdies produced from simple phialides or from branched or unbranched conidiophores.
- **Chlamydospores:** Chlamydospores are round or oval, thick- walled, terminal or intercalary cells of old hyphae. They develop either singly or in chains. They get detached and germinate by means of germ tubes if the condition is favourable. The chlamydospores are very durable and remain viable for along time.

#### Sclerotia





Basidiomycetes

### **Basidiomycetes**

Basidiomycota is a major division (or phyla) of the kingdom Fungi, About 30,000 described species are placed within Basidiomycota, or about 37 percent of all described species of fungi. Basidiomycetes compose a class of fungi containing many important plant pathogens and important litter decomposing fungi. This group also contains many ectomycorrhizal fungi of trees as well as delectable mushrooms for the cook. The mycorrhizal relationship is a symbiosis that benefits both trees and fungi.



#### **General characteristics**

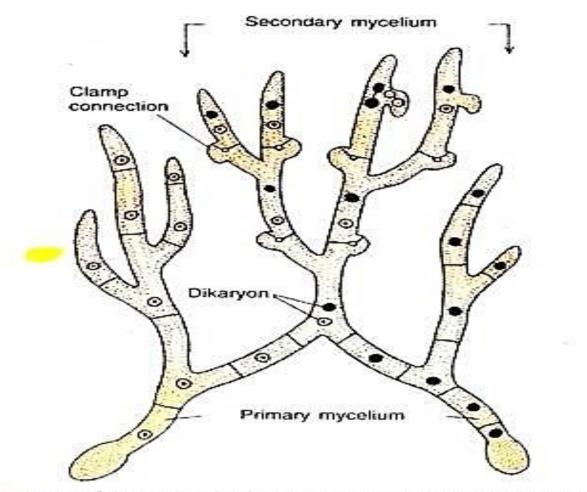
1. Facultative parasites (e.g. can live some of their life as saprophytes) to obligate parasites (rusts and smuts).

- 2. Basidiomycetes confined to only living host plants in nature.
- 3. The mycelium is well developed, branched and septate, and differentiated into primary, secondary or tertiary.
- 4. Most Basidiomycetes formed clamp connection.
- 5. Dolipore septa are present in most genera.
- 6. Cell wall in majority of basidiomycetes consists of chitin and glucans with 1,3 linked and 1,6 linked B- D glucosyl units.
- 7. No specialized sex organs formed in basidiomycetes, plasmogamy takes place by somatogamy or spermatization.

8. Basidiomycetes reproduce sexually by basidiospores borne on basidia. Some basidiomycetes reproduce asexually by spores that sometimes called conidia. Both spores can infect plants. Some wood rotting basidiomycetes also can produce rootlike strands of hyphae called rhizomorphs that can infect roots. The basidium is septated or non-septated.

9. Usually four basidiospores develop on basidium.

10. Basidiomycetes are harmful as well as useful. Their attack foods and ornamental plants, cause many different diseases including seedling diseases, wood rots, root and stem rots, seed diseases (smuts) and rusts on the other hand it used as humans foods.



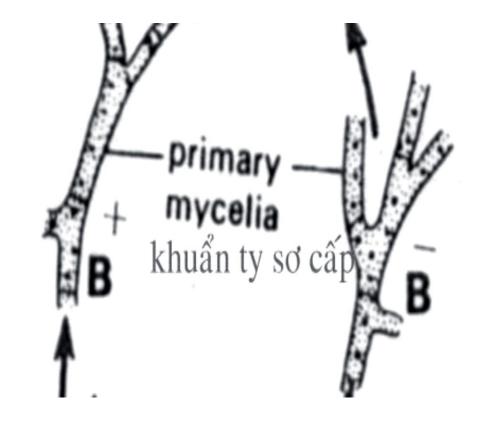
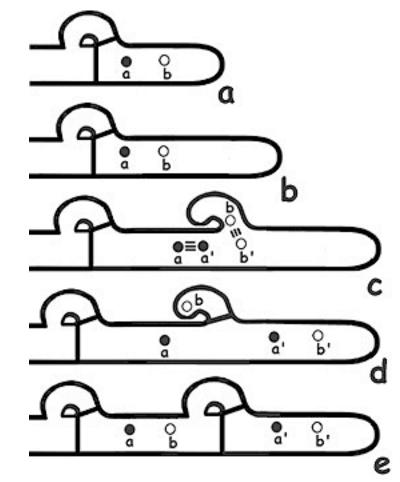


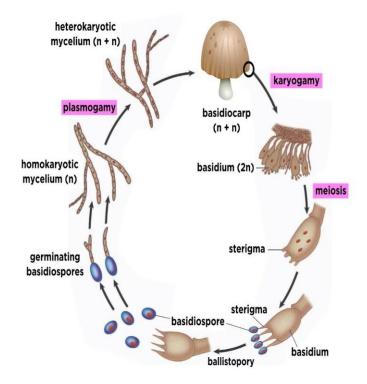
Fig. 13.2. Basidiomycetes. Sketch showing the formation of a secondary mycelium from a dikaryotised cell produced by somatogamous copulation between two uninucleate cells of primary mycelia of opposite strains.

### The clamp connection

• Thus simply functions as a bypass. It ensures that the sister nuclei formed by the conjugate division of the dikaryon separate into two newly formed daughter cells. The clamp connections are usually formed on the terminal cells of the hyphae of the secondary mycelium.



- Asexual reproduction: takes place by avariety of methods such as fragmentation, budding, conidia and arthrospores
- Sexual reproduction: there are four stages
- (1) Plasmogamy (2) dikaryotization
- (3)karyogamy (4) reduction division





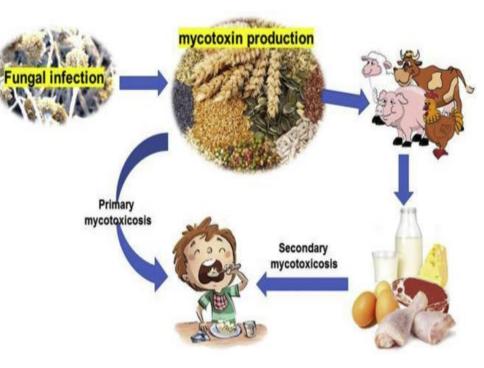
### Mycotoxin

A mycotoxin is a toxic secondary metabolite produced by organism of the fungus kingdom and is capable of causing disease and death in both humans and other animals . The term 'mycotoxins' is usually reserved for the toxic chemical products prodused by fungi that readily colonize crops .



### **General characters**

- Definition : mycotoxin combines the greek word for fungi "mykes" and the latin word " toxicum" meaning poison . there are numerous varieties of fungi ranging from those growing in woods to microscopic species .
- Mycotoxins are known to cause serious health problems in animals i. Reduced weight gain, capillary fragility, reduced fertility, suppressed disease resistance, and even death have been attributed to mycotoxins.



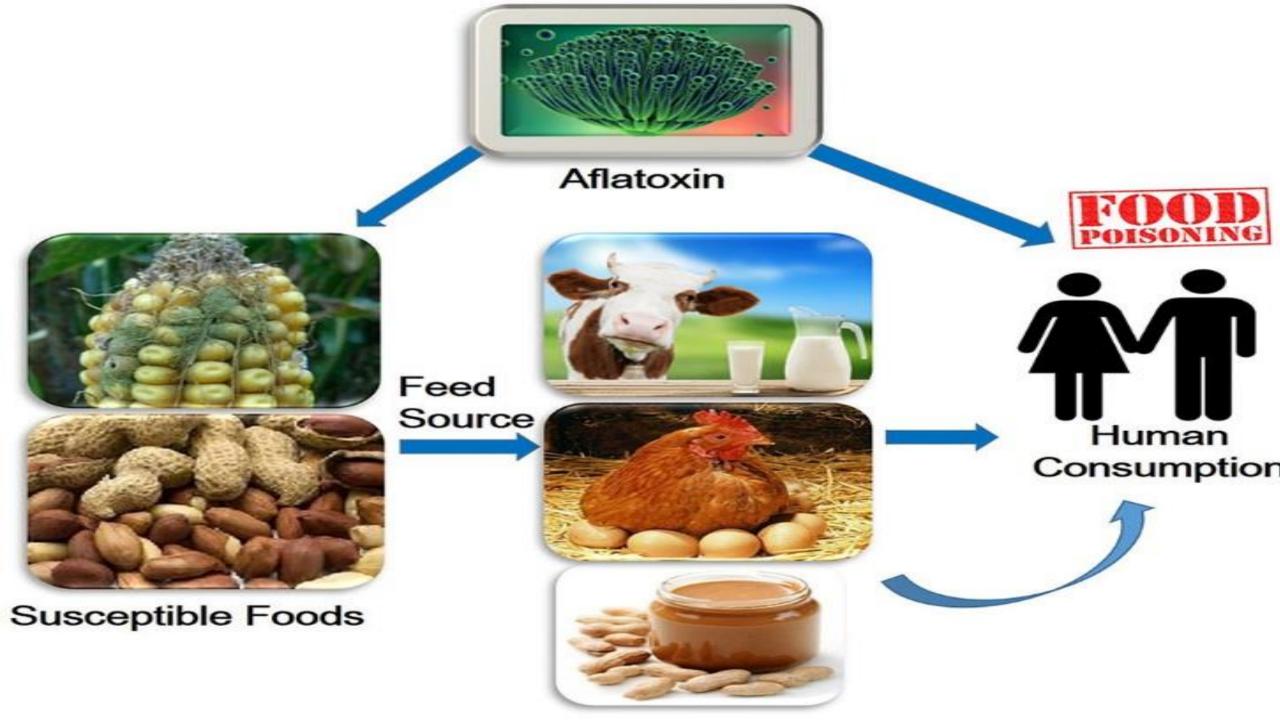
### **Examples of mycotoxins**

Examples of mycotoxins that cause disease to humans and animals include (aflatoxins, citrinin, fumonisins, ochratoxin A ,patulin, trichothecenes ,zearalenone and ergot alkaloids such as ergotamine ).

One mold species may produce many different mycotoxins, and several species may produce the

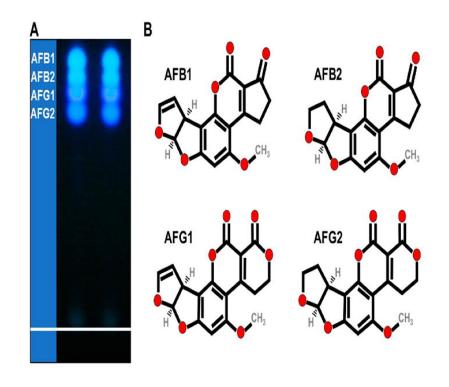
same mycotoxin.





## **Description for aflatoxin**

- Toxin is crystals solid with colorless to pale yellow
- Gel plates- have flouroscence under ultra violet on running upon silica
- AFG1 and AFG2 have fluorescence green ,, AFB1,AFB2 have fluorescence blue
- AFM1 and AFM2: fluorescence blue -violet



#### **Chemical factors destroyed**

- The Alfatoxin characterized by have lactone ring structure which open under alkaline condition and the aflatoxin destroyed .
- The reaction is reversible under acidic condition .
- Ammoniom salt results the opening of the lactone ring at high temperature
- This reaction is irreversible .

#### **Characters for Aflatoxin**

- Aflatoxins are bis-furanocoumarin metabolites produced primarily by some strains of Aspergillus flovus group .
- There are four main aflatoxins (B1,B2,G1,G2) plus two additional that are of significance and these are M1 and M2.
- Aflatoxins are potent liver toxins and also potent carcinogens.
- Alfatoxin B1 is the most toxic of the group .
- Alfatoxins are international mycotoxins intensively investigated in different parts of the world especially in warm

#### **Characters for Aflatoxin**

• There are several factors affecting aflatoxin production on foods, these are moisture content of the foods, temperature, incubation period, darkness, PH value, trace elements, and sodium chloride Nacl.

#### **Aflatoxin Derivatives**

- The aflatoxins are agroup of bis-furonocoumarin
- Four derivatives are mostly common with *A. flovus & A.parositicus*, and these are B1, B2, G1, G2
- aflatoxin B1 is the most toxic
- There are metabolites of aflatoxin described as M1 & M2, were first discovered in cow's milk ; these metabolites are also found in urine of animals including humans .

#### **Categories of grain-mycotoxin**

- A field fungi: such *as Alternaria*, *fusarium*, *cladosporium and pulluoria* which invade grains during development of plants in the field or after they have been maturated, but before they are harvested, these fungi require a moisture content in equilibrium with a relative humidity of more than 90% in starchy grains cereals this is equivalent to 24-25% moisture content on wet-weight basis.
- B storage fungi : comprise mainly several species of Asperigllus which is particularly, A. glaucus group, in addition to A. candidus, A. ochroceus & A. flavus.

#### AFLATOXIN

• Aflatoxins are poisons carcinogens and mutagens that are produced by certain molds (Aspergillus flavus and Aspergillus parasiticus ) which grow in soil ,decaying vegetation ,hay and grains . They are regularly found in improperly stored staple commodities such as cassava ,chili pepper, cotton seeds, peanut, rice, wheat, sunflower seeds and variety of spices . When contaminated food is processed, aflatoxins enter the general food supply where they have been found in both pet and human foods, as well as in feedstock for agricultural animals.





#### **Stability of the aflatoxin**

- Aflatoxins are soluble in moderately polar solvents such as chloroform , methanol and dimethylsulfoxide and dissolved in water for 10-20 mg/Litre, they fluoresce under UV radation
- The crystalle are extremely stable in the absence of light even at temperature in excess of 100 dgree
- -Unstable in Uv light in the presence of oxygen
- -Unstable in alkaline medium in extreme Ph >10, are reversible in acidi medium and irrversible in high temperature





#### **Physical and Chemical Proparites**

#### **Physicochemical Properties**

1-The <u>aflatoxins</u> are white crystalline solids that are optically active and have a strong absorbance at about 365 nm with a fluorescence emission of 415–450 nm,

2-depending on the solvent or physical status (. Fluorescence is strong especially when the aflatoxins are absorbed on <u>silica gel</u>, making this a sensitive means of detection on thin-layer <u>chromatograms</u>.

3-The aflatoxins are soluble in <u>organic solvents</u> such as <u>chloroform</u>, ethanol, methanol, and <u>acetone</u>, and insoluble in lipophylic solvents such as <u>hexane</u>, <u>petroleum ether</u>, and <u>diethyl ether</u>.



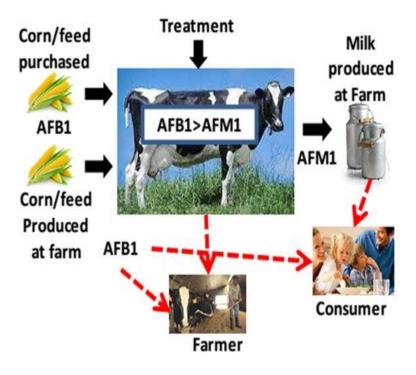
#### **Pathology**

High level aflatoxin exposure produces an acute hepatic necrosis (acute aflatoxicosis), resulting later in carcinoma of the liver . Acute liver failure is made manifest by bleeding , edema , alteration in digestion , changes to the absorption and metabolism of nutrients, and mental changes or coma. Chronic exposure increase the risk of developing liver and gallbladder cancer,



#### **Biological Activity**

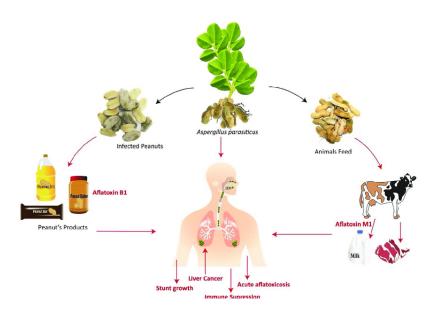
Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1, secreted in milk of mammals receiving aflatoxin B1. It is potentially hepatocarcinogenic. The LD50 value for aflatoxin M1 in day old ducklings was 16.6µg/duckling compared to 12µg/duckling for aflatoxin B1 determined simultaneously. Pathology and clinical signs similar to aflatoxin B1. Ducklings dosed with a flatoxin M1 showed liver lesions, M1 also induced a renal tubular necrosis not seen with B1.



#### **Occurrence in Food**

Aflatoxins are found in various cereals, oilseeds, spices, and

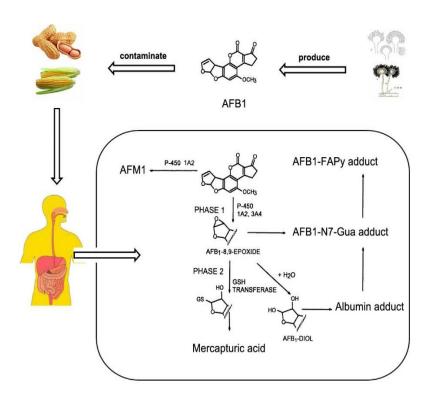
- nuts). These Aspergillus colonize and produce aflatoxins,
- which contaminate grains and cereals at various steps during
- harvesting or storage. Fungal contamination can occur in the
- field, or during harvest, transport and storage). In milk, aflatoxins is generally at 1-6% of the total content in the
- feedstuff).



#### **Mechanism of Toxicity and Health Effects by Aflatoxin**

-Aflatoxin are specifically target the liver organ. Early symptoms of hepatotoxicity of liver caused by aflatoxins comprise fever, malaise and anorexia followed with abdominal pain, vomiting, and hepatitis;

- Chronic toxicity by aflatoxins comprises immunosuppressive and carcinogenic effects.. AFT-B1 reduced anti-inflammatory cytokine IL-4 expression, but increased the pro-inflammatory cytokine IFN- $\gamma$  and TNF- $\alpha$  expression by NK cells. These findings indicate that frequent AFT-B1 exposure accelerates inflammatory responses via regulation of cytokine gene expression.



-Aflatoxins cause reduced efficiency of immunization in children that lead to enhanced risk of infections).

-The hepatocarcinogenic of aflatoxins is mainly due to the lipid peroxidation and oxidative damage to DNA).

-AFTs- $B_1$  in the liver is activated by cytochrome -enzymes, which are converted to AFTs-B1-epoxide responsible for carcinogenic effects in the kidney).

Among all major mycotoxins, aflatoxins create a high risk in dairy because of the presence of their derivative, AFTs- $M_1$ ,

- in milk, have potential health hazard for human consumption AFTs- $B_1$  is rapidly absorbed in the digestive tract and metabolized by the liver,

The other effects of AFTs- $M_1$  include liver damage, decreased milk production, immunity suppression and reduced oxygen supply to tissues due to anemia, which reduces appetite and growth in dairy cattle.

-The expression of aflatoxin toxicity is regulated by factors such as age, sex, species, and status of nutrition of infected animals). The symptoms of acute aflatoxicosis include oedema, haemorrhagic necrosis of the liver and profound lethargy, while the chronic effects are immune suppression, growth retardation, and cancer .

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