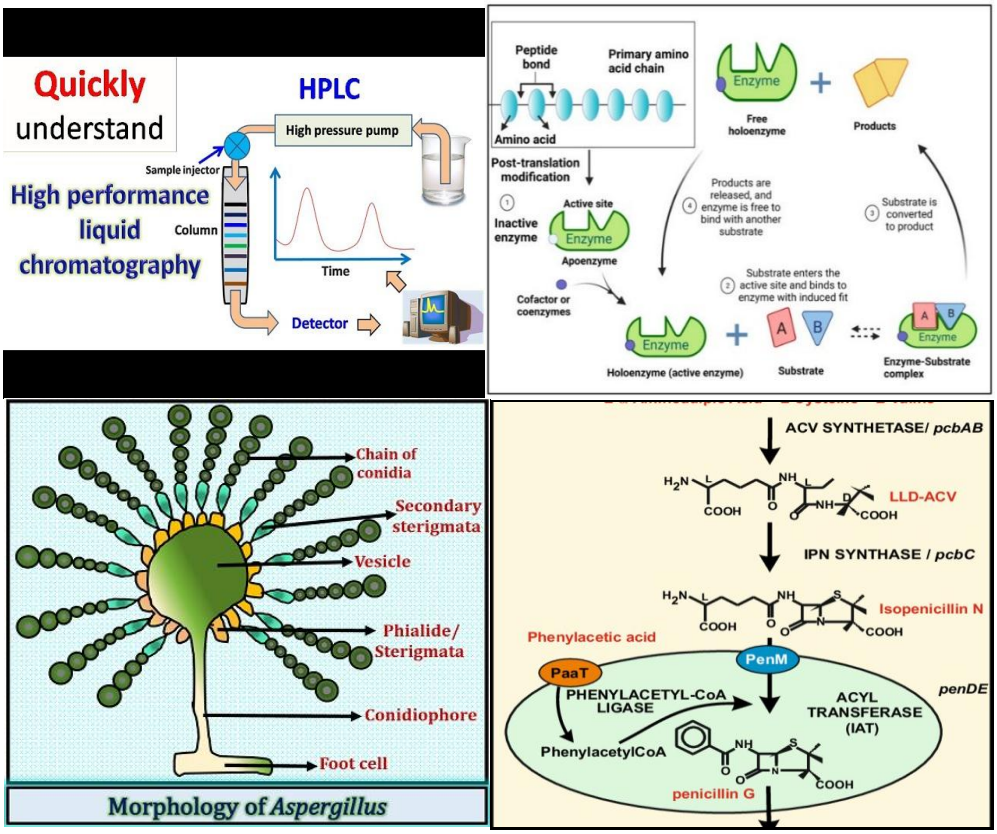
South Valley University

Faculty of Science
Department of Botany and Microbiology

Advanced Mycology



Prepared by

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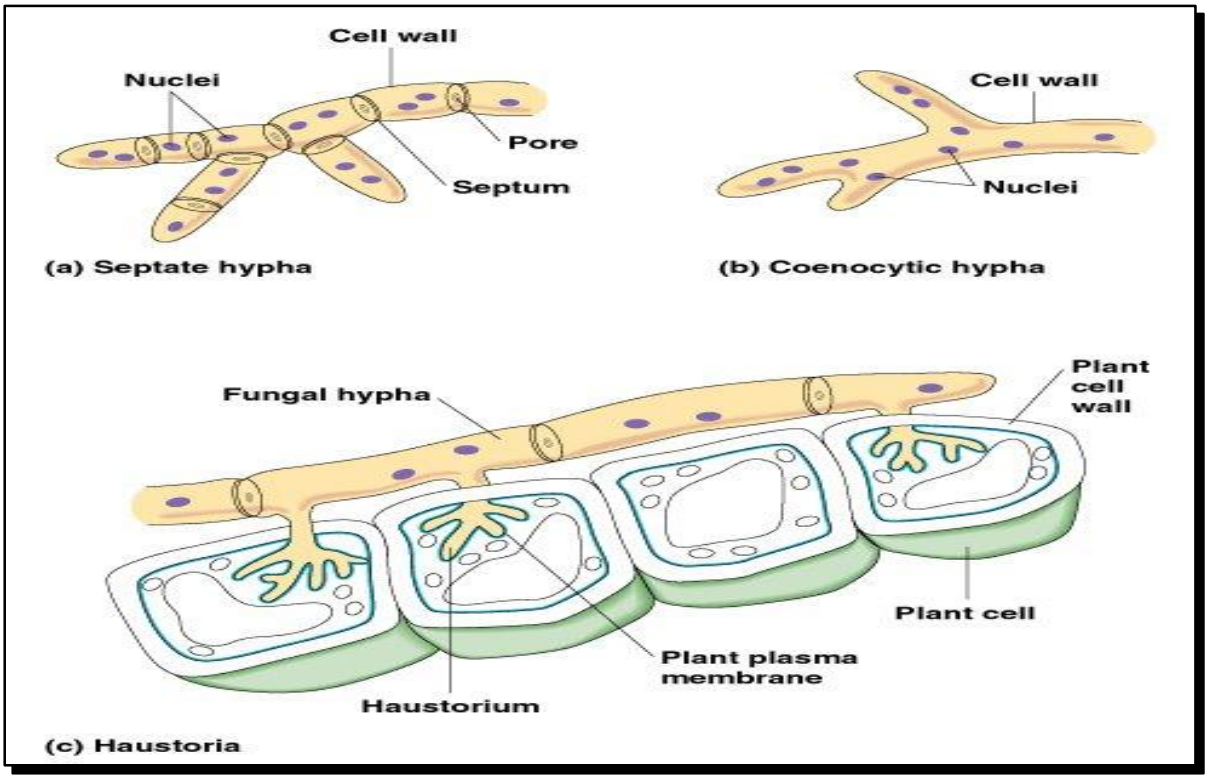
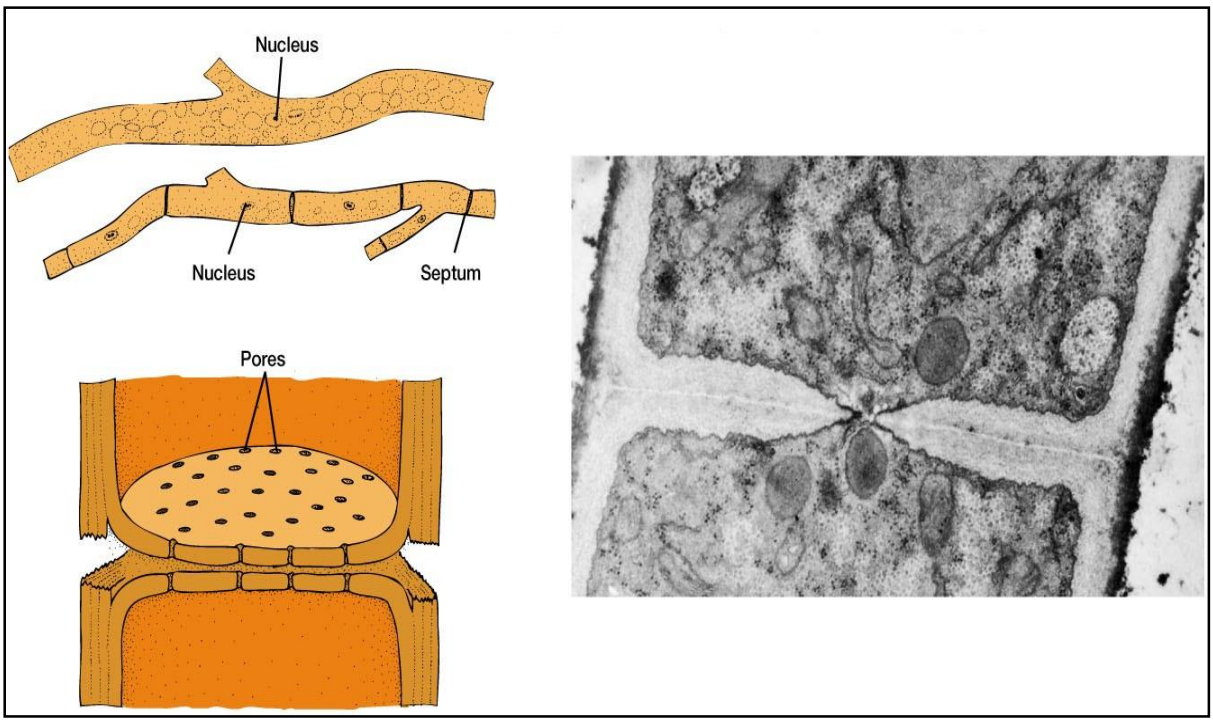
1. The vegetative structure of fungi

The vegetative body of a fungus is a unicellular or multicellular thallus. Dimorphic fungi can change from the unicellular to multicellular state depending on environmental conditions. Unicellular fungi are generally referred to as yeasts. *Saccharomyces cerevisiae* (baker's yeast) and *Candida* species (the agents of thrush, a common fungal infection) are examples of unicellular fungi.

Most fungi are multicellular organisms. They display two distinct morphological stages: the vegetative and reproductive. The vegetative stage consists of a tangle of slender thread-like structures called hyphae (singular, hypha), whereas the reproductive stage can be more conspicuous. The mass of hyphae is a mycelium. It can grow on a surface, in soil or decaying material, in a liquid, or even on living tissue. Most fungal hyphae are divided into separate cells by end walls called septa (singular, septum) (a). In most phyla of fungi, tiny holes in the septa allow for the rapid flow of nutrients and small molecules from cell to cell along the hypha. They are described as perforated septa. The hyphae in bread molds (which belong to the Phylum Zygomycota) are not separated by septa. Instead, they are formed by large cells containing many nuclei, an arrangement described as coenocytic hyphae (b). In the parasitic fungi, the hypha which penetrate the host cell is called haustorium.





Fungal hyphae structure

2. Economic Importance of Fungi

A- Negative aspects of fungi

Diseases of human

A lot of peoples around the world become infected with fungi. Many fungal infections are of skin (Dermatophytes) caused by *Trichophyton*, *Microsporum* and *Candida*, others are infected respiratory tract, lungs, bones, viscera, intestine, liver, kidney, nasal sinuses, corneal tissue of eye and urinary tract. Some species of *Rhizopus* and *Mucor* are common fungi infecting lungs and gastric tissues, whereas *Neurospora* and *Fusarium* infect corneal tissues of the eye, and Histoplasma infects spleen, liver, kidney nervous system and lymphatic system. *Aspergillus* is a common infectant of lung and nasal sinuses. Aspergillosis whose symptoms closely resemble those of tuberculosis in peoples is caused by *Aspergillus niger*, *A. flavus* and *A. terreus* whereas zygomycosis is caused by species of *Rhizopus* and *Mucor.*

Diseases of wild animals and pests

Fungal diseases are common in domestic and wild animals. Aspergillosis, candidiasis, and mycotic abortions are common in a large numbers of animals. The most prevalent fungi were the species of *Trichophyton* and *Microsporum. Microsporum canis* causes common ringworm of dog and horses and *Aspergillus fumigatus* causes bovine abortion in many animals including birds, ducks and chickens. Mycotic abortions of cattle and aspergillosis in birds are also common fungal diseases of animals.

Diseases of fishes

Many fishes,moluscs and crustaceans which are food crop of many peoples in the world are infected by fungi. Saprolegniales fungi such as *Saprolegnia* and *Achlya* are common fungal parasites of fishes.

Spoilage of food and stored grains

Food and stored grains are infected by fungi. The most common food spoilage fungi are *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus.* These fungi are able to infect several seeds and grains including wheat, maize and groundnut in storage, so the grains should therefore be dried well before storage. Some fungi also infect the food at low temperature such as *Cladosporium herbarum.* It grows on meat stored at -4°C.

Fungi and mycotoxins

Mushrooms like *Amanita phalloides*are toxic. It resulted into liver damage and kidney failure and even death. *Claviceps purpurea*, ascomycetes fungus, produce many poisonous alkaloids. *Aspergillus flavus* produce a carcinogenic toxin called aflatoxin. This toxin induces liver cancer in human and animals.

Diseases of crops

Several plant diseases are caused by pathogenic fungi. The most common diseases caused by pathogenic fungi are smuts, rusts, wilts, blights, rots and mildews.

B. Positive aspects of fungi

Fungi as research tools

Fungi are used as a basic material for the study of various fundamental biological processes. Fungi grow fast and require a short period to complete one generation, so they are good research material. *Neurospora crassa* has an ideal fungus for study of heredity and genetics. Many biochemical processes are also studied by using several fungi.

Production of antibiotics from fungi

Antibiotics are the substances produced by some living organisms which kill or inhibit other living organisms. Penicillin antibioticis produced by *Penicillium notatum* and *P. chrysogenum*. This antibiotic is used for treatment of bacterial diseases. Griseofulvin, which used against fungal diseases of skin, is extracted from *Penicillium griseofulvum*.

Preparation of organic acids from fungi

Many organic acids are produced commercially by the biochemical activities of fungi. *Aspergillus niger* is used in the production of citric acid and gluconic acid whereas *Rhizopus stolonifer* is used for the manufacture of fumaric acid, oxalic acid and lactic acid.

Fungi and alcoholic fermentation

Alcoholic fermentation takes place with fungi and is the universal basis of brewing and baking industries. These industries are based on the fermentation of sugar by yeast to produce ethyl alcohol and carbon dioxide.

Production of enzymes by fungi

Several enzymes are produced by fungi. Amylase, cellulase, invertase, lipase and protease enzymes are produced by fungi. Amylase is produced by *Aspergillus niger* and *A. oryzae* however *Saccharomyces cerevisiae* is used for production of invertase.

Production of hormones by fungi

Gibberellin, the plant hormones used to accelerate growth of crops, is produced by *Gibberella fujikuroi*. However trisporic acid sexual hormone is produced by *Mucor mucedo* and *Blakeslea trispora*.

Production of vitamins by fungi

Saccharomyces cerevisiae are good source of vitamin B-complex and riboflavin. Because of their high vitamin content and proteins, the yeasts are considered as a valuable food and good source of vitamins.

Fungi as insecticides (biological control agent)

Many fungi attack a number of insects that are harmful to the crops. Most of the insect attacking fungi belong to order Entomophthorales of class Phycomycetes or to class Deuteromycetes. Many insect pests are controlled by using fungi like *Beauveria bassiana* and *Metarhizium anisopliae*.

Fungi as food

Mushroom cultivation for food is well known these days, because of their fairly large protein content and good source of vitamins. The most common edible mushroom grown commercially is *Agaricus bisporus*. *Saccharomyces cerevisiae* is universally used for the production of yeast cake in addition to the production of yeast protein (single-cell protein). Some of other fungal-based processes are the preparation of leavened bread, cheese manufacture and clarification of fruit juices.

Mycorrhizal association

Soil borne fungi has a symbiotic relationship with the roots of plants are called mycorrhizae. There are two types of mycorrhizae known as ectomycorrizae and endomycorrhizae. The ectomycorrhizae enhance the growth of plant. Nutrients like phosphorus, nitrogen, potassium and calcium are easily absorbed by the fungal hyphae and then passed to the root of plant.

3. Fungal Cell Structure and Function

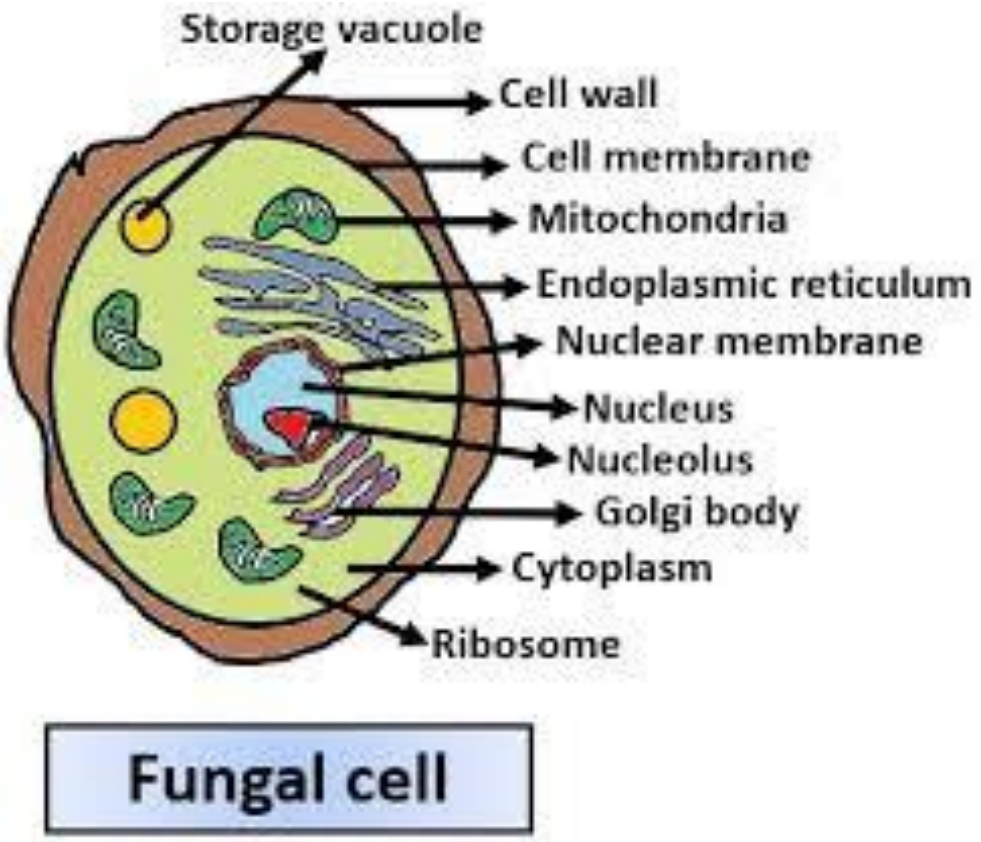
Fungi are eukaryotes and have a complex cellular organization. As eukaryotes, fungal cells contain a membrane-bound nucleus where the DNA is wrapped around histone proteins. A few types of fungi have structures comparable to bacterial plasmids (loops of DNA). Fungal cells also contain mitochondria and a complex system of internal membranes, including the endoplasmic reticulum and Golgi apparatus.

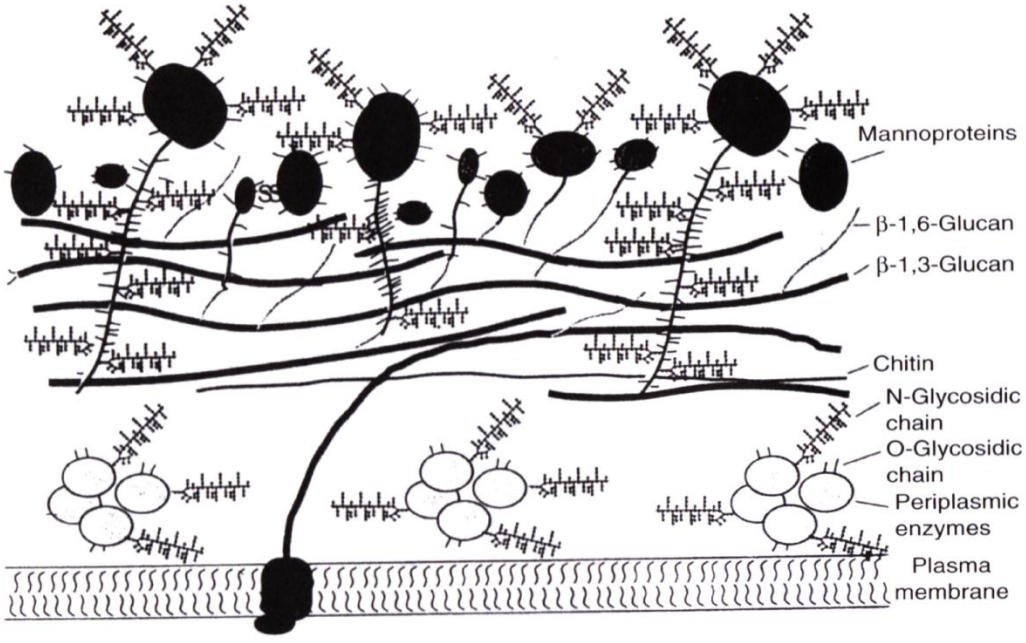
Unlike plant cells, fungal cells do not have chloroplasts or chlorophyll. Many fungi display bright colors arising from other cellular pigments, ranging from red to green to black. The poisonous *Amanita muscaria* (fly agaric) is recognizable by its bright red cap with white patches. Pigments in fungi are associated with the cell wall. They play a protective role against ultraviolet radiation and can be toxic.

Composition of fungal cell wall

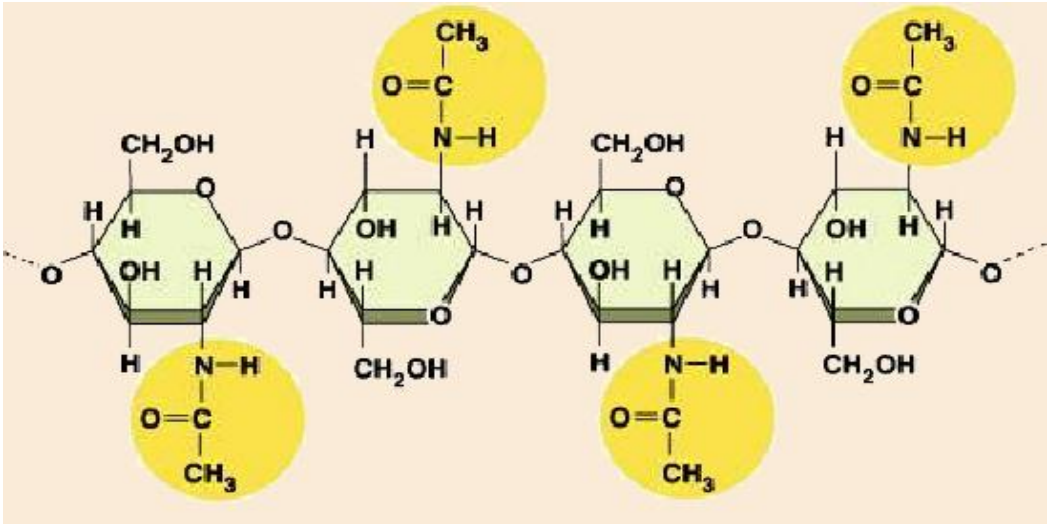
In Eumycota the hyphal cells are bounded by a cell wall. Its composition generally varies in different fungal groups. According to workers like Aronson (1965) and Bartnicki-Garcia (1970) fungal cell walls contain proteins, lipids and 80%-90% polysaccharides. Most common cell wall component is *chitin*. However, in some fungi *cellulose* or *glucans* are present. Cellulose is generally a polymer of D-glucose. According to Bartnicki-Garcia (1968) some other substances associated with the fungal cell wall in different members are cellulose-glycogen, Cellulose-glucan, cellulose-chitin, chitin-glucan, mannan-glucan, mnnan-chitin and polygalactosaminegalactan.

The rigid layers of fungal cell walls contain complex polysaccharides called chitin and glucans. Chitin, also found in the exoskeleton of insects, gives structural strength to the cell walls of fungi. The wall protects the cell from desiccation and predators. Fungi have plasma membranes similar to other eukaryotes, except that the structure is stabilized by ergosterol: a steroid molecule that replaces the cholesterol found in animal cell membranes.





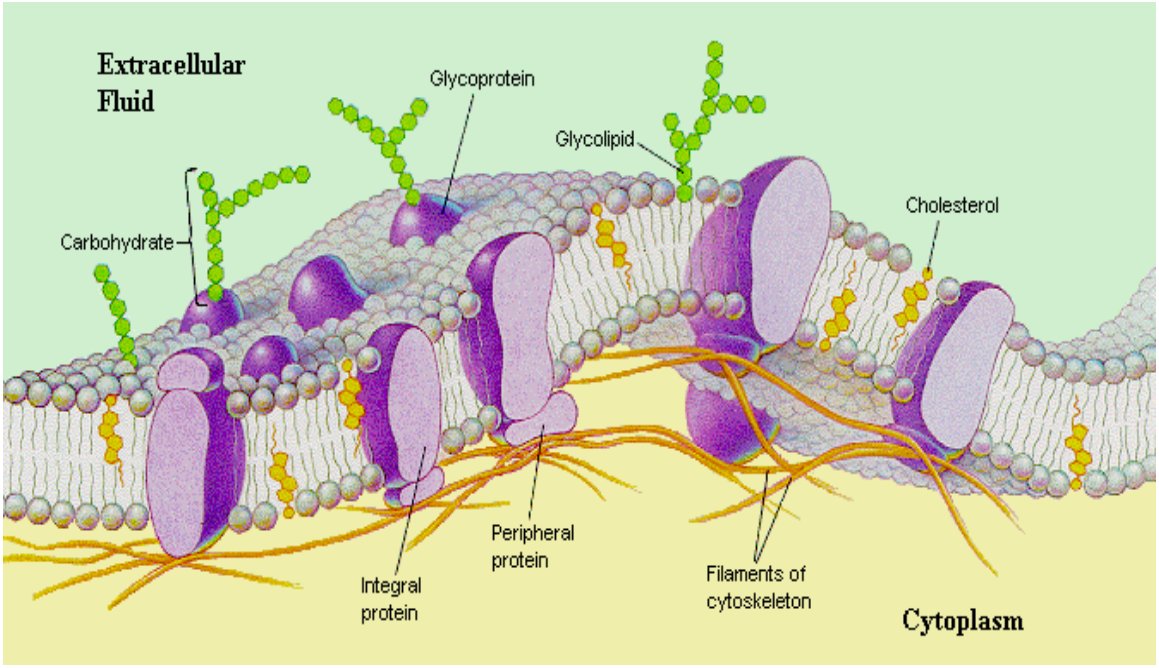
Fungal cell wall structure



Chitin

Plasma membrane

The plasma membrane, also called the cell membrane, is the membrane found in all cells that separates the interior of the cell from the outside environment. The plasma membrane consists of a bilayer of phospholipid that is semipermeable. The plasma membrane regulates the transport of materials entering and exiting the cell.



Structure of plasma membrane

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)

4. Myxomycota

Nutrition

Fungi are heterotrophs. They use complex organic compounds as a source of carbon, rather than fix carbon dioxide from the atmosphere as do some bacteria and most plants. In addition, fungi do not fix nitrogen from the atmosphere. Like animals, they must obtain it from their diet. However, unlike most animals, which ingest food and then digest it internally in specialized organs, fungi perform these steps in the reverse order: digestion precedes ingestion. First, exoenzymes are transported out of the hyphae, where they process nutrients in

the environment. Then, the smaller molecules produced by this external digestion are absorbed through the large surface area of the mycelium. As with animal cells, the polysaccharide of storage is glycogen rather than the starch found in plants.

Fungi are mostly saprobes (saprophyte): organisms that derive nutrients from decaying organic matter. They obtain their nutrients from dead or decomposing organic matter, mainly plant material. Fungal exoenzymes are able to break down insoluble polysaccharides, such as the cellulose and lignin of dead wood, into readily-absorbable glucose molecules. The carbon, nitrogen, and other elements are thus released into the environment. Because of their varied metabolic pathways, fungi fulfill an important ecological role and are being investigated as potential tools in bioremediation.

Some fungi are parasitic, infecting either plants or animals. Rust and Smut diseases affect plants, whereas athlete's foot and candidiasis (thrush) are medically important fungal infections in humans.

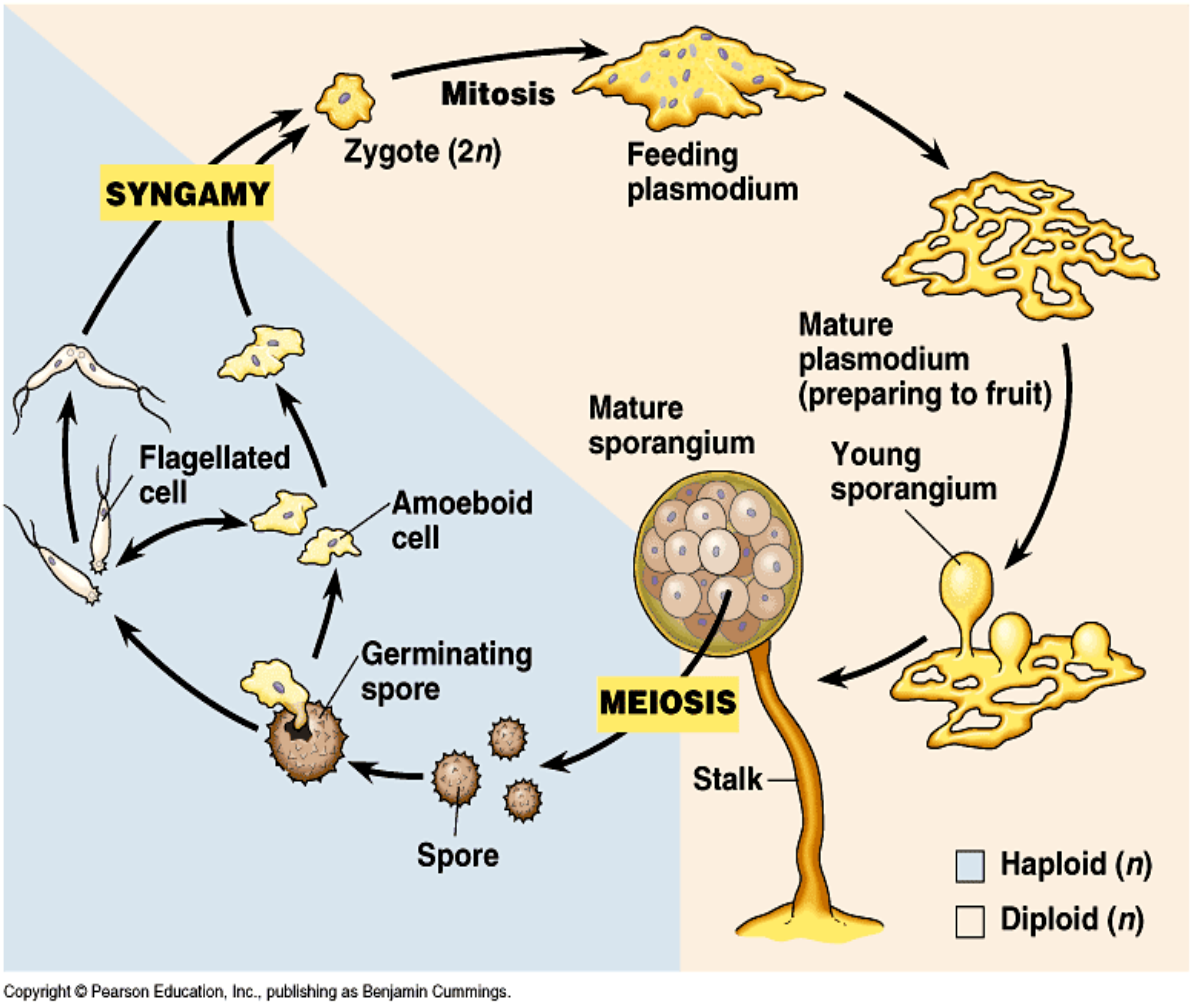
Myxomycetes

General characters

1. Myxomycetes fungi occur commonly in cool and moist shady places on decaying woods, leaves, animal dung, plant debris and other organic substrates.
2. They are represented by about 450 species, the majority of which are universally distributed.
3. The vegetative phase is in the form of a free-living plasmodium, which is simply a multinucleate, naked and a cellular mass of protoplasm.

4. The plasmodium in different members may be a very small, microscopic body called *aphanoplasmodium*, or consists of a large sheet called *phaneroplasmodium.*
5. The plasmodial protoplasm shows a rhythmic reversible streaming.
6. Under favourable conditions of moisture and temperature, the plasmodium gives rise to *sporophore* or fructifications of different shape.
7. A specialized layer (hypothallus) is deposited by the plasmodium just beneath the developing sporophores.
8. The hypothallus maybe membranous, spongy, horny or disc-like in form.
9. Wind helps in spore dispersal.
10. Spore germination results into the formation of *myxamoebae* or zoospores. Zoospores are uniflagellate or biflagellate.
11. The flagella are generally anteriorly attached and of whiplash type.
12. Asexual reproduction of plasmodium and myxamoebae may be fragmentation and fission.
13. At the time of sexual reproduction, the zoospores or myxamoebae may fuse to form zygotes.
14. The development of zygote results in the formation of plasmodia.

Slime mould plasmodium



Life cycle of myxomycetes fungi

1. Once a spore is released from the fruiting body it's dispersed, either by insects, animals, and rain or air movement. On landing on a suitable location with appropriate moisture and temperature, spores are germinated.

2. The protoplasts once released from the spores wall through a pore will be either a flagellated swarm cell if conditions are wet, or a non-flagellated myxamoebae cell in dry conditions.

3. If conditions for growth are not suitable, the cells can become microcysts to survive long periods of time.

Plasmodiophoromycetes

General characters

1. Members are obligate endoparasites, attacking many plants of economic importance like cabbage and potato. Some species also attack many aquatic pteridophytes, angiosperms, some algae (*Vaucheria*) as well as some fungi (*Saprolegnia and Pythium*).
2. The infection results into the *hypertrophy* (abnormal enlargement of host cells) and *hypertroplasia* (abnormal multiplication of host cells) in the host. Disruption in the vascular elements of host result into its general stunting.
3. A characteristic *cruciform-type* of nuclear division is found only in Plasmodiophoromycetes.
4. The life-cycle includes two distinct plasmodial phases.
5. The plasmodium is parasitic within the cells of the host plant.
6. Biflagellate zoospores contain flagella of unequal length. The flagella are anterior and of whiplash type.

Classification of *Plasmodiophora brassicae*

Division: Myxomycota

Class: Plasmodiophoromycetes

Order: Plasmodiophorales

Family: Plasmodiophoraceae

e.g. *Plasmodiophora brassicae*

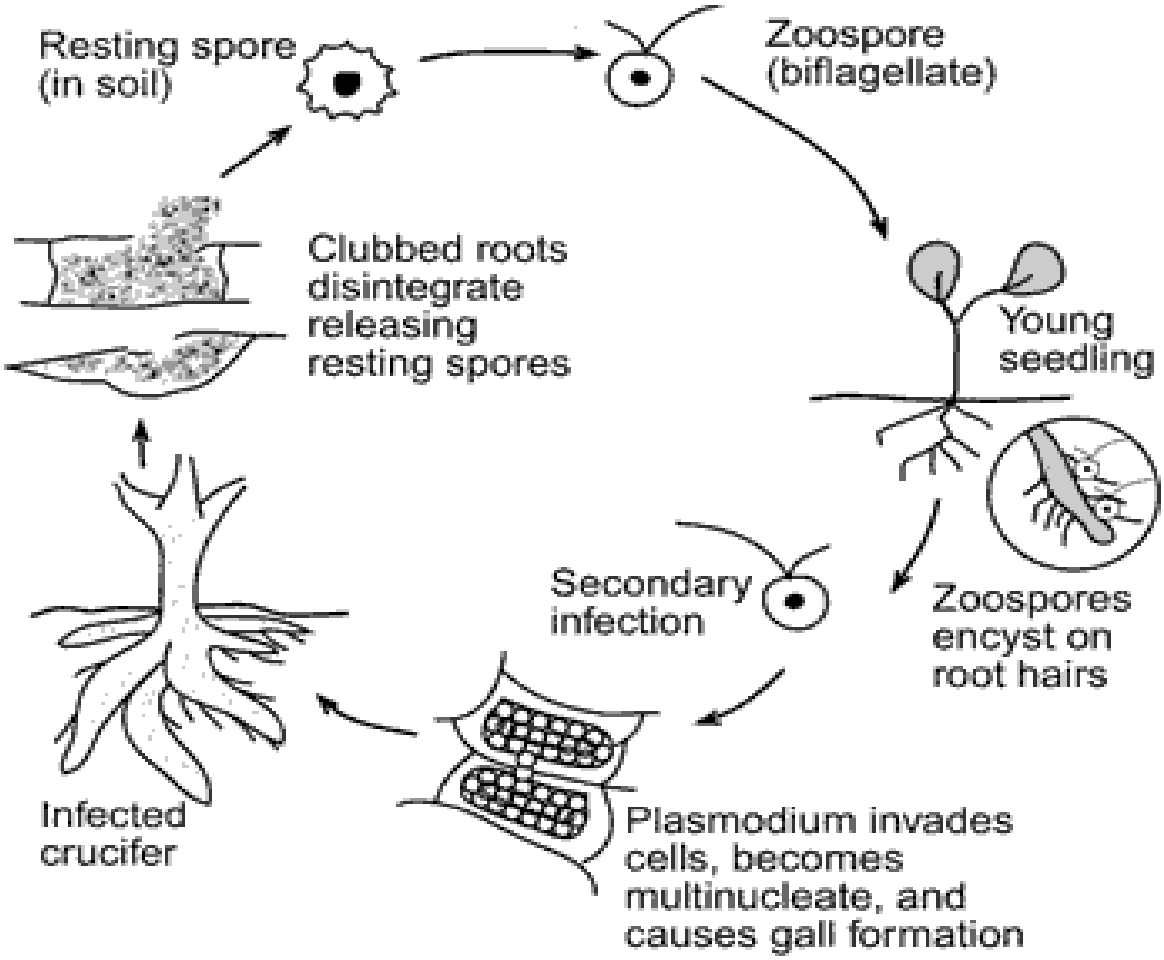
Plasmodiophora brassicae is an obligate endoparasite in the root of Crucifera plants, attacking both cultivated and wild members. It causes club-root disease of brassicas. This disease is universal and commonly attacked cabbage, rape, mustard and turnip plants.

Clup root of cabbage

- *Plasmodiophora brassicae* is an obligate parasite. It survives in the soil only as dormant cysts.
- Primary zoospores released from germinating cysts infect host root hairs by encysting on the root surface and entering through developing epidermal cells in the form of an amoeba like cell. Older roots can also be infected if wounding is present to provide an entrance to the pathogen.
- In the root hairs, amoeboid cells of the pathogen join together to form a multinucleate plasmodium. This plasmodium divides and forms multiple secondary zoospores, which are released into the soil. Secondary zoospores infect healthy parts of the initial host or infect nearby plants. These zoospores also enter through the host root hair. The infecting amoeboid cells migrate into the cortical cells of the host.
- Once in the cortex, the amoeboid pathogen infects one host cortical cell where it may multiply or join with other amoeboid cells to form a plasmodium. As the plasmodium develops, it releases plant hormones

which cause the host cells to enlarge up to 20 times of its normal size. As the plasmodium grows, it divides and infects neighboring cells causing them to enlarge. Clusters of these enlarged cells are responsible for the clubbing on the roots.

- Plasmodium in the host cells eventually undergo meiosis and develop into resting cysts. These new cysts will be released into the soil as other soil microorganisms decompose the club root.



Life cycle of *Plasmodiophora*

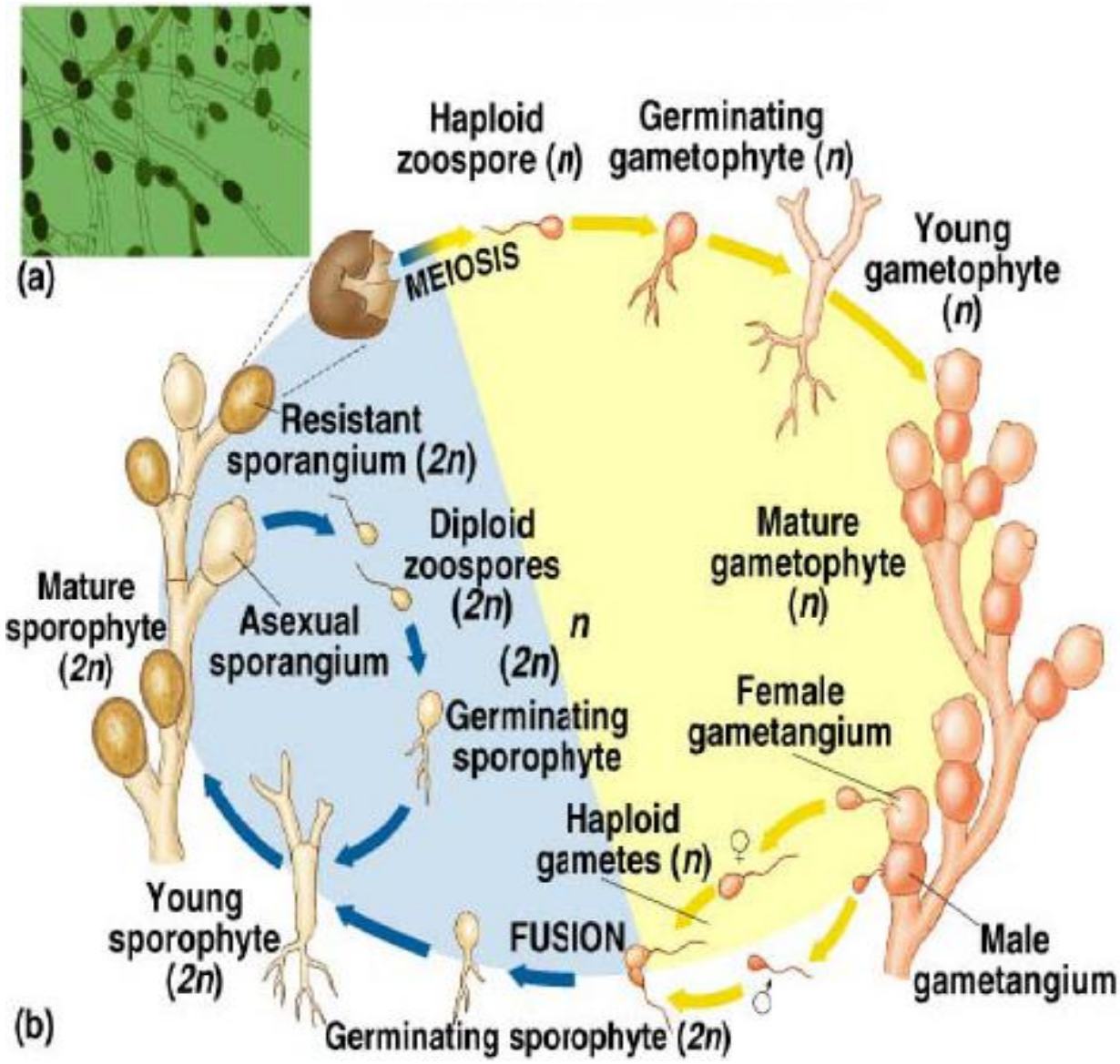
5. Chytridiomycota

- Members of the division have unicellular rudimentary to mycelial thalli.
- Their cell wall composition is mostly chitin.
- Flagellated spores and gametes are produced. Gametes and zoospores have a single, posterior whiplash flagellum.
- Sexual reproduction is variable and may be isogamous, anisogamous or oogamous.
- The ultrastructure of zoospore is a definitive characteristic of Chytridiomycota.
- The division has a single class, Chytridiomycetes, and three orders: Chytridiales, Blastocadiales, and Monoblepharidales.

Order: Chytridiales

- The most primitive members of the Chytridiomycota.
- Commonly referred to as "chytrids".
- Habitat : Fresh water.
- The thallus is commonly unicellular and may have limited hyphal growth, but is not considered to be mycelial. Hyphal cells are coenocytic except where there are reproductive structures.
- Asexual reproduction, Chytridiomycota reproduce through the use of zoospores.
- In asexual reproduction, zoospores will swim until a desirable substrate is located.
- The zoospore attaches itself, feeds off its host; the cytoplasm grows, meiotic divisions occur, and a cell wall forms around the original zoospore.

- Protoplasm increases as the cell continues to develop. Finally, cleavage of the protoplasm occurs, which produces individual zoospores that are released through a pore.
- Sexual reproduction is haploid dominant. It also depends on the isomorphic alternation of generations. The haploid thallus, called the gametothallus, produces female and male gametes.
- These occur in pairs and are terminal and subterminal. Male gametes are orange-colored, while female gametes are colorless.
- In addition, female gametes are much larger than male gametes.
- Males are attracted to females when they produce the hormone sirenin, and females are attracted to males when they produce the hormone parisin.
- The diploid thallus is called the sporothallus.
- The sporothallus produces two types of zoosporgia: zoosporangium (meitosporangium) and resistant sporangium (meiosporangium).
- Zoosporangia produce diploid zoospores, which can function as a means of asexual reproduction.
- Sexual reproduction may be isogamous, anisogamous, or oogamous. One species, *Allomyces macrogynus*, has a sporic life cycle.
- *Allomyces macrogynus* is an example of an anisogamous species.



Life cycle of *Allomyces*

6. Zygomycetes

General characters

1. The majority of the members are saprobic. Some occur on dung, showing coprophilous nature, and some members attack other fungi. A few Zygomycetes are weak parasites, attacking plants and animals.
2. Most Zygomycetes produce a well-developed and branched mycelium, consisting of course, grey or white, coenocytic hyphae. A few members, however, contain a highly reduced mycelium, having septa at definite intervals.
3. Cells contain all typical cellular organelles including mitochondria, nuclei, ribosomes, lipid granules and endoplasmic reticulum.
4. Cell wall is mainly composed of chitosan-chitin. Chitin has been reported in Mucorales and Entomophthorales but not in Zoopagales.
5. Motile cells or zoospores are absent.
6. Asexual reproduction takes place by non-motile sporangiospores, called aplanospores. They are produced in large number within the sporangia.
7. Some reproduce by chlamydospores and a few also by oidia.
8. Many Zygomycetes reproduce by modified sporangial units functioning as conidia, or by true conidia. In Entomophthorales the sporangium gets reduced and functions as a single conidium. Conidia are borne singly or in chains Zoopagales.
9. Sexual reproduction takes place by gametangial fusion. Two fusing gamete may arise from the same mycelium or from different mycelia.
10. Gametangial fusion results in the production of a thick-walled resting spore, called zygospore.
11. The zygospore remains surrounded by a very thick wall, which is highly resistant to desiccation and other unfavourable conditions.

12. At the time of germination of the zygospore a hypha emerges and bears a terminal sporangium. It is believed that meiosis occurs during germination.

13. Regarding the nutrition in zygomycetes, no vitamins or growth factors are required by primitive Moucorales. Only inorganic nitrogen with minerals and sugars are required. Higher forms like *Pilobolus* require growth factors such as ferrichrome (Coprogen). Entomophthorales require complex nutrition medium.

Classification of Zygomycetes

Heseeltine and Ellis (1973) divided Mucorales into 14 families includes Choanephoraceae, Cunninghamelaceae, Dimargaritaceae, Endogonaceae, Helico-cephalidaceae, Kickxellaceae, Mortierellaceae, Mucoraceae, Piobolaceae, Piptocephalidaceae, Radiomycetaceae, Saksenaeaceae, Syncephalastraceae and Thamnidiaceae. Only Mucoraceae is discussed in the forthcoming account.

Zygomycetes

Hesseltineand Ellis (1973) 120 species and varieties have been described. Of these *R*. *stolonifer* (syn. *R. nigricans*) is most common, and can be considered as a type species representing the genus.

Occurrence

Rhizopus stolonifer occurs very frequently on bread, and is therefore commonly called 'bread mould'. It is so frequent a contaminant of laboratory cultures of bacteria and fungi that it is considered a weed of laboratory. *Rhizopus* occurs

worldwide in soil, on decaying fruits, dung and vegetation. *R. stolonifer* also may be behaves parasitically.

***Rhizopus stolonifer* systematic position**

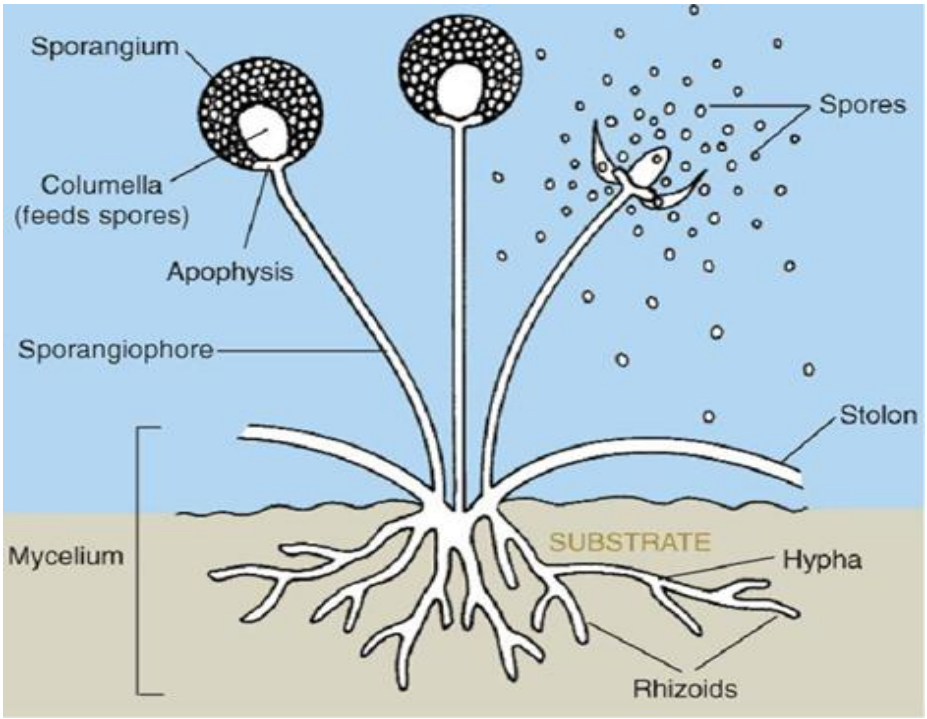
Division : Eumycota

Class : Zygomycetes

Order : Mucorales

Family : Mucoraceae

e.g. : *Rhizopus stolonifera*



Vegetative structure of *Rhizopus*

Nutrition

Certain enzymes are secreted by the rhizoidal hyphae. These enzymes convert starch into soluble carbohydrates, which are readily absorbed by the fungus. Utilization of some inorganic and organic nitrogenous compounds also enables *Rhizopus* in synthesizing the proteins.

Asexual reproduction

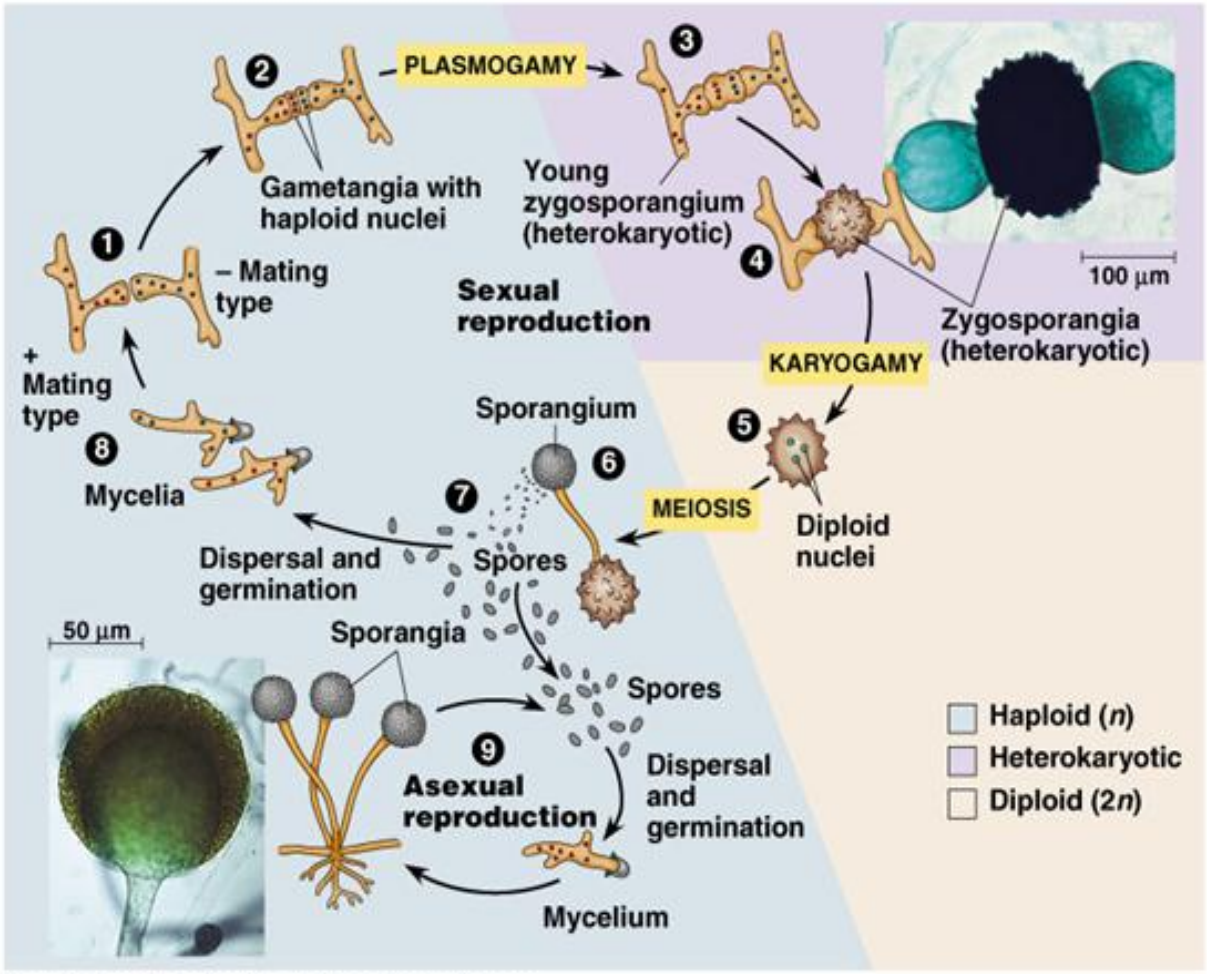
It takes place generally by the formation of sporangiospores and rarely by chlamydospores.

Sexual reproduction

Rhizopus reproduces sexually by the process of conjugation, which results in the formation of zygospores. A majority of species, including *R. stolonifer*, are heterothallic. However, species such as *R. sexualis* are homothallic. In the heterothallic species the zygospores are formed only when two compatible strains of different mating types are mated together. (Blakeslee, 1904) named these two compatible strains of Mucoraceae as + and – strains or mating types. In the homothallic species the zygospores are formed in the mycelium derived from a single sporangiospore.

In the heterothallic species the two fusing mycelia belong to two different mating types. One belong to + strain and the other is – strain. Sexual reproduction takes place only when a + strain hypha and a - strain hypha come in contact with each other.

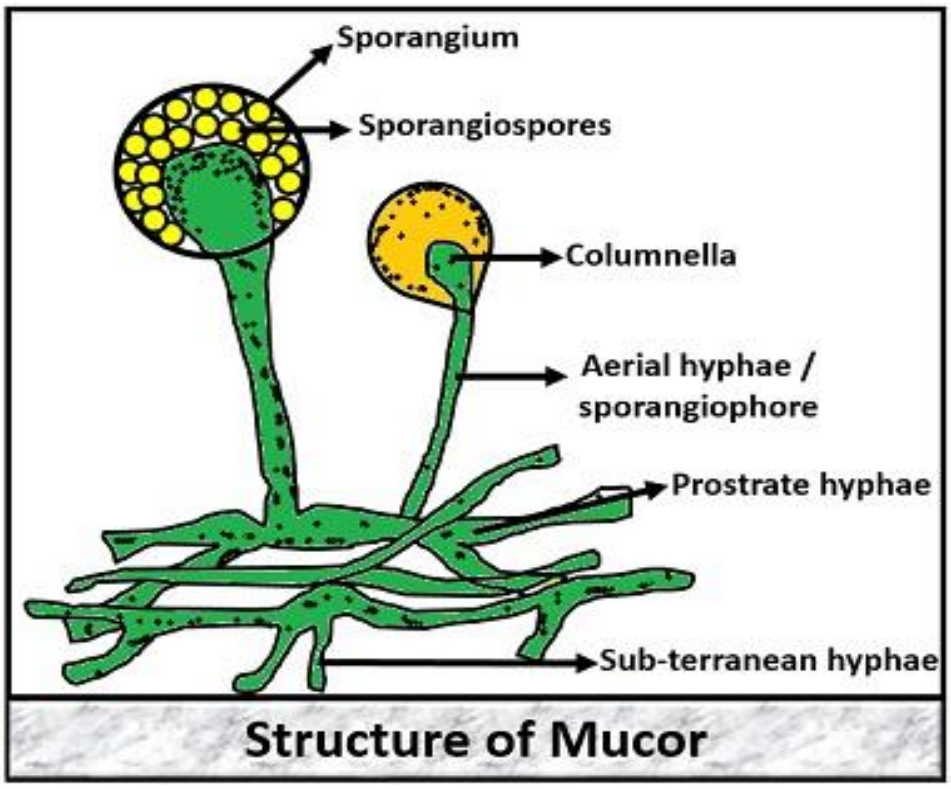
During sexual reproduction, the two compatible hyphae are attracted toward each other which are capable of the developing into progametangia and then develop to form zygospores.

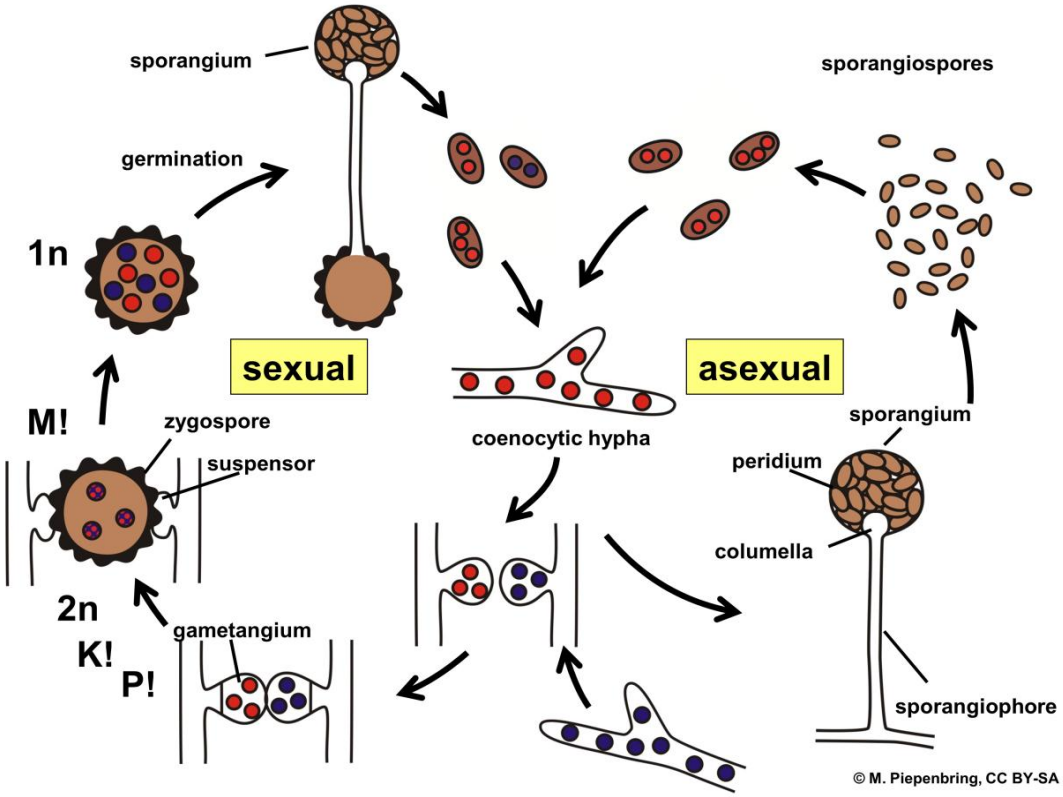


Life cycle of *Rhizopus*

Mucor systematic position

Division - Eumycota
Class -Zygomycetes
Order -Mucorales
Family -Mucoraceae
e.g - *Mucor racemosus*





Life cycle of *Mucor*

Major differences between *Rhizopus* and *Mucor*

No	***Rhizopus***	***Mucor***
1	Rhizopus (absorptive hyphae) or holdfasts are present	Rhizoids or holdfasts are generally absent or less specialized
2	stolons are present	Stolons are absent, and thus there is no differentiation of stolons and holdfast in the mycelia.
3	Food material is absorbed mainly by rhizoids	Food is mainly absorbed by the mycelia surface
4	Sporangiospores develop in well-organize groups mainly against the rhizoidal hyphae	Sporangiospore arise singly, and not in groups
5	Columella and are not easily disseminated	Spores easily blown away by wind.

7. Ascomycetes

General characters

1. Ascomycetes fungi occur in almost all climatic conditions, and in a wide variety of habitats, in soil, on dung (coprophilous), in marine as well as fresh water, as saprophytes of animal and plant remains, and also as parasites on plants as well as animals.

2. The mycelium is well-developed, profusely branched and septate. Each segment of the hypha contains several nuclei.

Types of ascocarps

1. **Cleistothecium**: Globose fruiting body with no special opening to outside as in Erysiphales, Eurotiales.
2. **Apothecium:** A saucer or cup-shaped fruiting body, as in Helotiales and Pezizales.
3. **Perithecium:** A flask-shaped fruiting body, opening with an ostiole or pore and are unitunicate, as in Pyrenomycetes (Hypocreales and Sphaeriales)
4. **Pseudothecium:** A perithecium with bitunicate as in Loculoascomycetes. Some call pseudothecium as ascostroma.

Hemiascomycetes

Saccharomyces cerevisiae systematic position

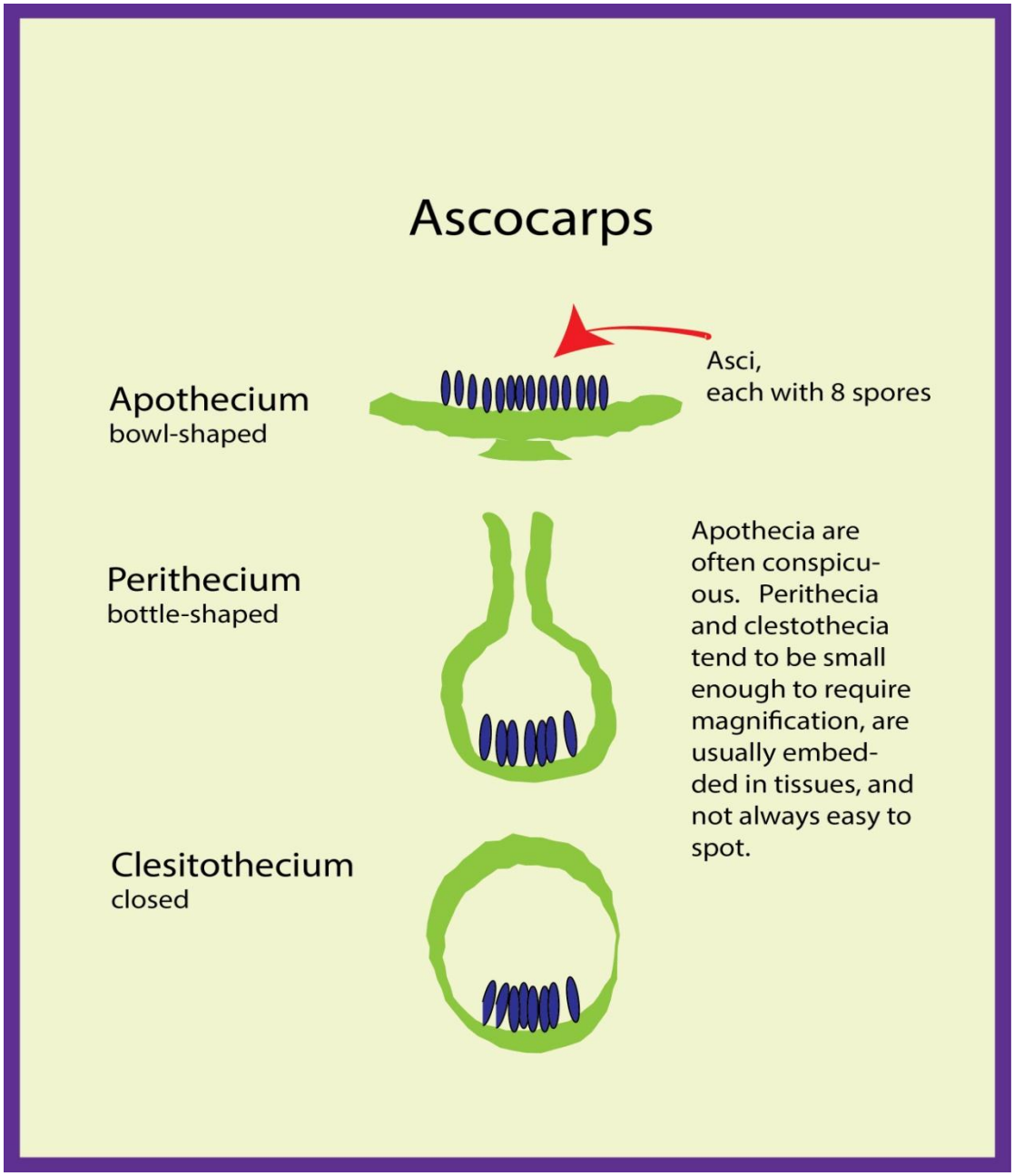
Division : Eumycota

Class : Hemiascomycetes

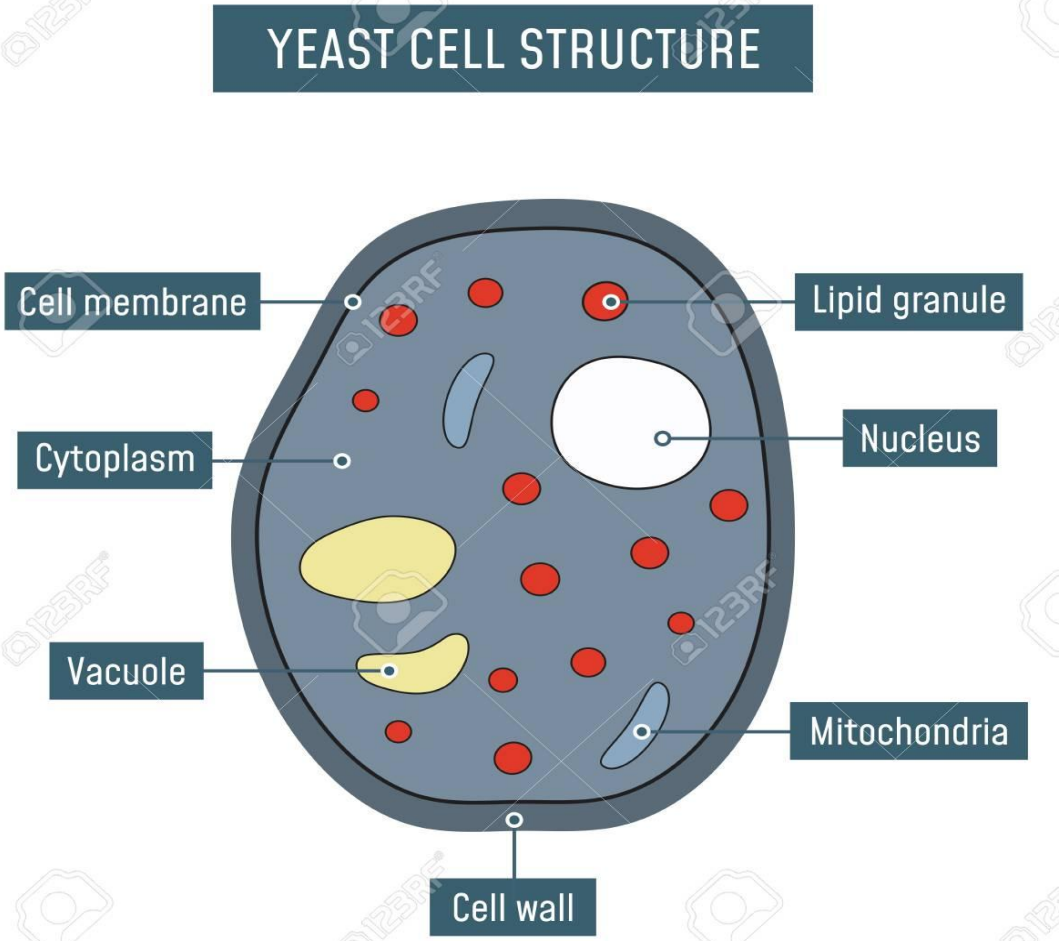
Order : Endomycetales

Family : Saccharomycetaceae

e.g. : *Saccharomyces cerevisiae*



Fruiting bodies of Ascomycetes



Nutrition

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$

However, under anaerobic conditions or when the oxygen supply is poor, yeast cells multiply very slowly. The cause is the conversion of large amount of sugar into CO_2 and ethyl alcohol.

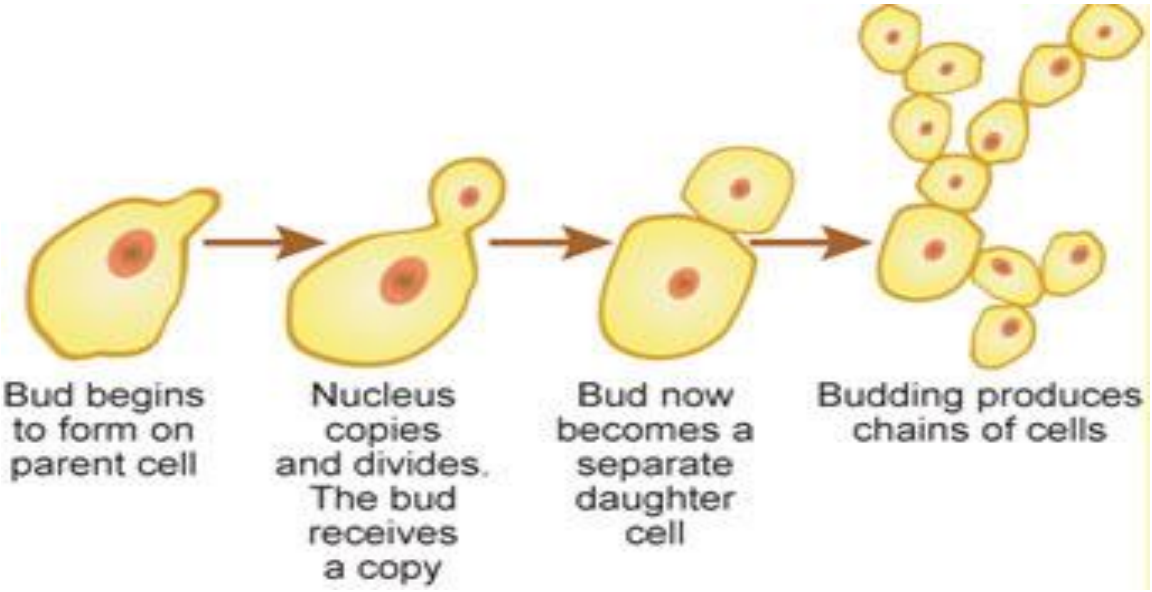
$C_6H_{12}O_6 + \text{yeast Cells} \rightarrow 2\ C_2H_5OH + CO_2 + \text{energy}$

glucose enzymes Ethyl alcohol

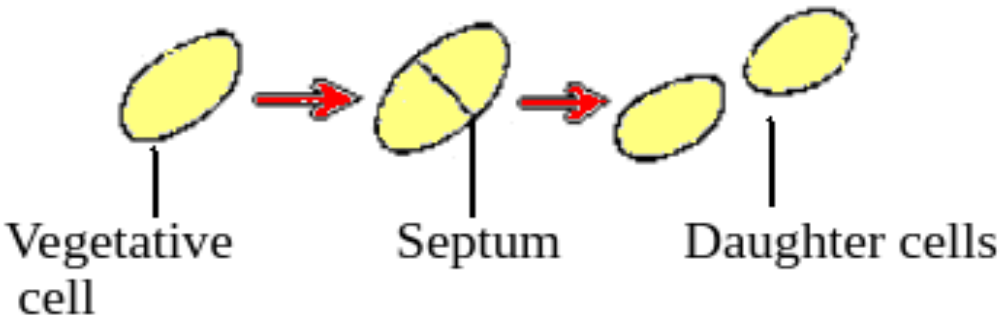
The ethyl alcohol and carbon dioxide so formed diffuse through the cell wall into the surrounding liquid. This process, in which carbohydrates (glucose) are broken by the yeast into ethyl alcohol and carbon dioxide, in the absence of oxygen, is called *alcoholic fermentation*. This liberated energy, during respiration, is used in many vital activities. The process of alcoholic fermentation by the yeasts is utilized in various industries in the preparation of bread, alcohol, wines, etc.

Budding

Budding, which is another method of asexual reproduction, occurs in most yeasts and in some filamentous fungi. In this process, a bud develops on the surface of either the yeast cell or the hypha, with the cytoplasm of the bud being continuous with that of the parent cell. The nucleus of the parent cell then divides; one of the daughter nuclei migrates into the bud, and the other remains in the parent cell. The parent cell is capable of producing many buds over its surface by continuous synthesis of cytoplasm and repeated nuclear divisions. After a bud develops to a certain point and even before it is severed from the parent cell, it is itself capable of budding by the same process. In this way, a chain of cells may be produced. Eventually, the individual buds pinch off the parent cell and become individual yeast cells. Buds that are pinched off a hypha of a filamentous fungus behave as spores; that is, they germinate, each giving rise to a structure called a germ tube, which develops into a new hypha. Although fragmentation, fission, and budding are methods of asexual reproduction in a number of fungi, the majority reproduce asexually by the formation of spores. Spores that are produced asexually are often termed mitospores, and such spores are produced in a variety of ways.



Budding in *Saccharomyces cerevisiae*



Binary Fission in Yeast

Sexual reproduction in yeasts

The yeasts do not produce definite sex organs, such as antheridia or oogonia. They reproduce sexually by the union of two somatic cells or two ascospores.

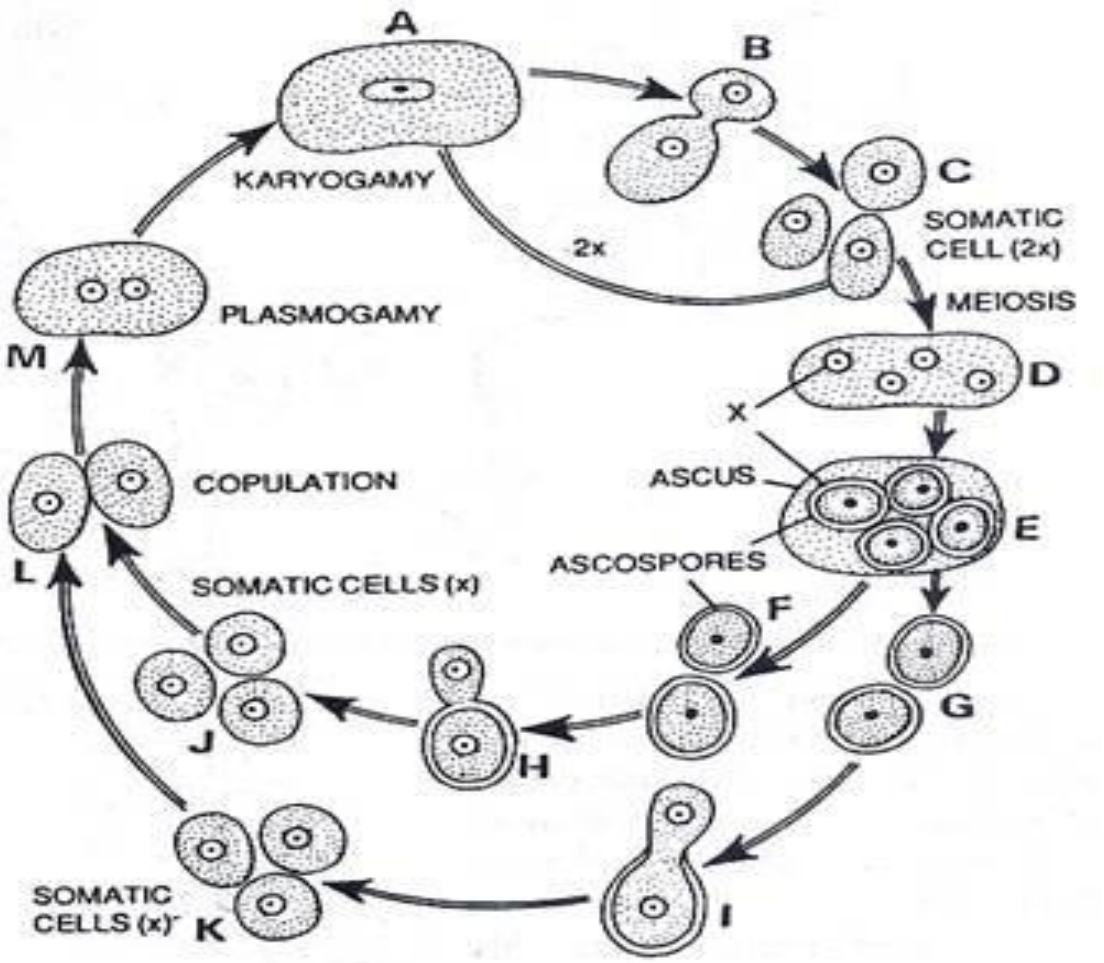
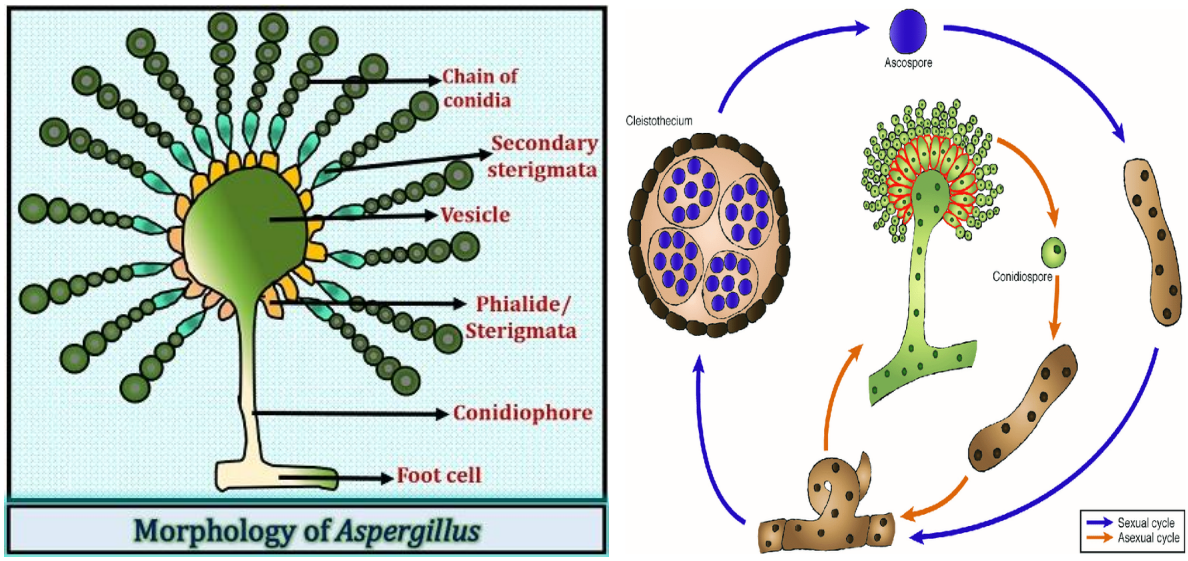


Fig. 12.23. Haplo-diplobiontic type of life-cycle in *Saccharomyces cerivisiae.*

Ascomycetes (Plectomycetes)

Aspergillus systematic position

Division : Eumycota
Class : Ascomycetes
Order : Aspergillales
Family : Aspergillaceae
Genus : *Aspergillus*



Aspergillus

Occurrence

This genus is represented by about 200 species (Raper and Fennell, 1965). It is common in the air, that if a slide or petri dish with suitable medium is exposed to the air for a few minutes, *Aspergillus* conidia appear in abundance. Many species occur also in the soil. *A. niger* is a very common contaminant of foodstuffs. Some species occur on leather and cloth fabrics. Species such as *A. flavus, A. fumigates, A. terreus* and *A. niger* occur parasitically on many animals, including man, and cause the disease *aspergillosis*.

Economic importance of *Aspergillus*

1. Food spoilage: *A. niger* and several other species cause decay of foodstuffs. It spoils nuts, bread and other foodstuffs. *A. niger* causes rot disease of fruits. They are the most frequent contaminants of food.

2. Some of *Aspergillus species* produce poisonous substance known as mycotoxins. The most important among these is aflatoxins which are carcinogenic compounds.

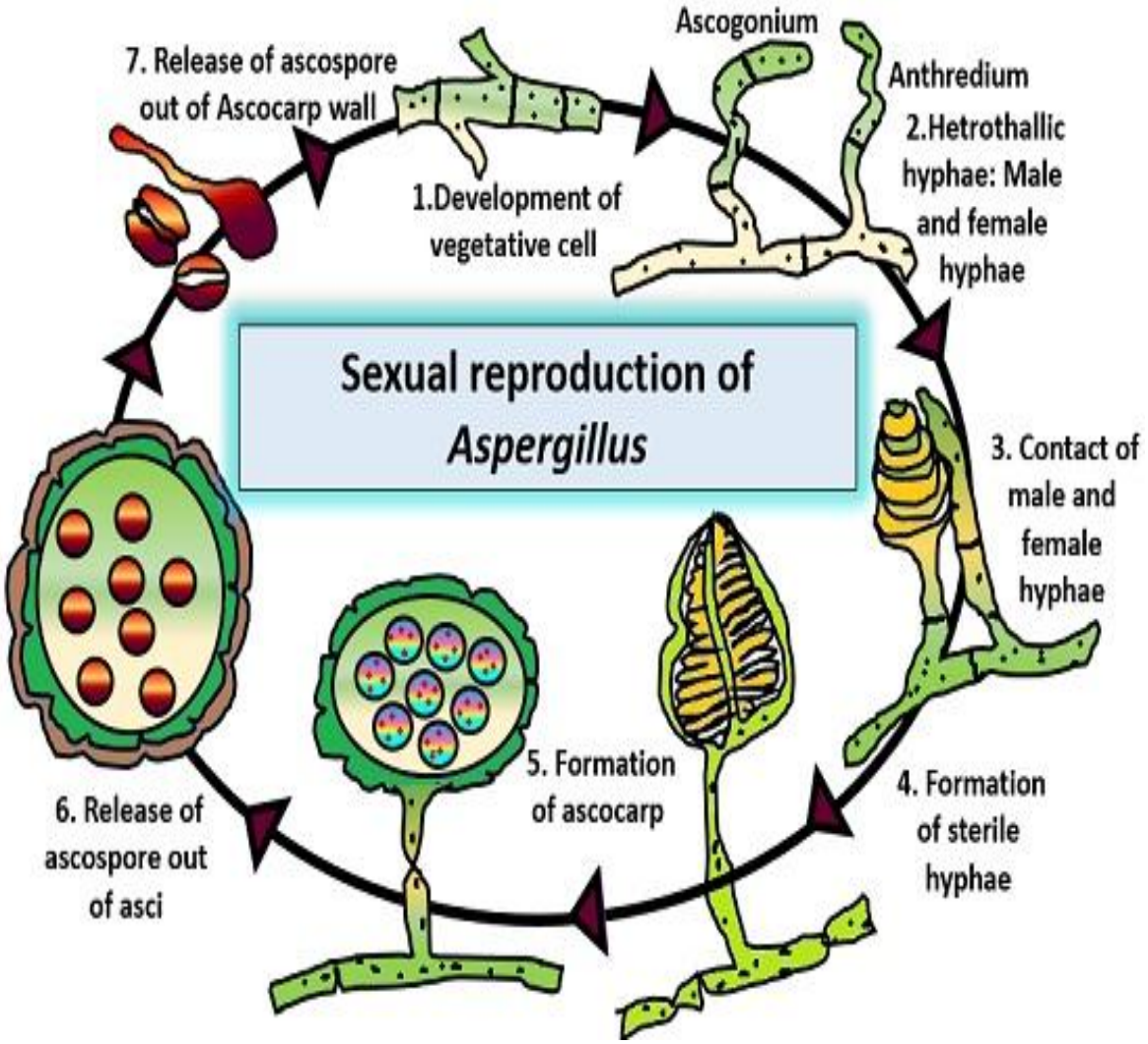
3. Some species of *Aspergillus* such as *A. fumigatus*, *A. flavus*, and *A. niger* are human pathogens. They cause disease collectively known as aspergilloses such as aspergilloses of lungs, external ear, etc.

4. Enzyme preparations: The cultures of *Aspergillus niger* and *A. oryzae* yield a wide range of enzymes used in industrial fermentation. Cultures of *A. niger* and *A. oryzae* produce a well-known amylase.

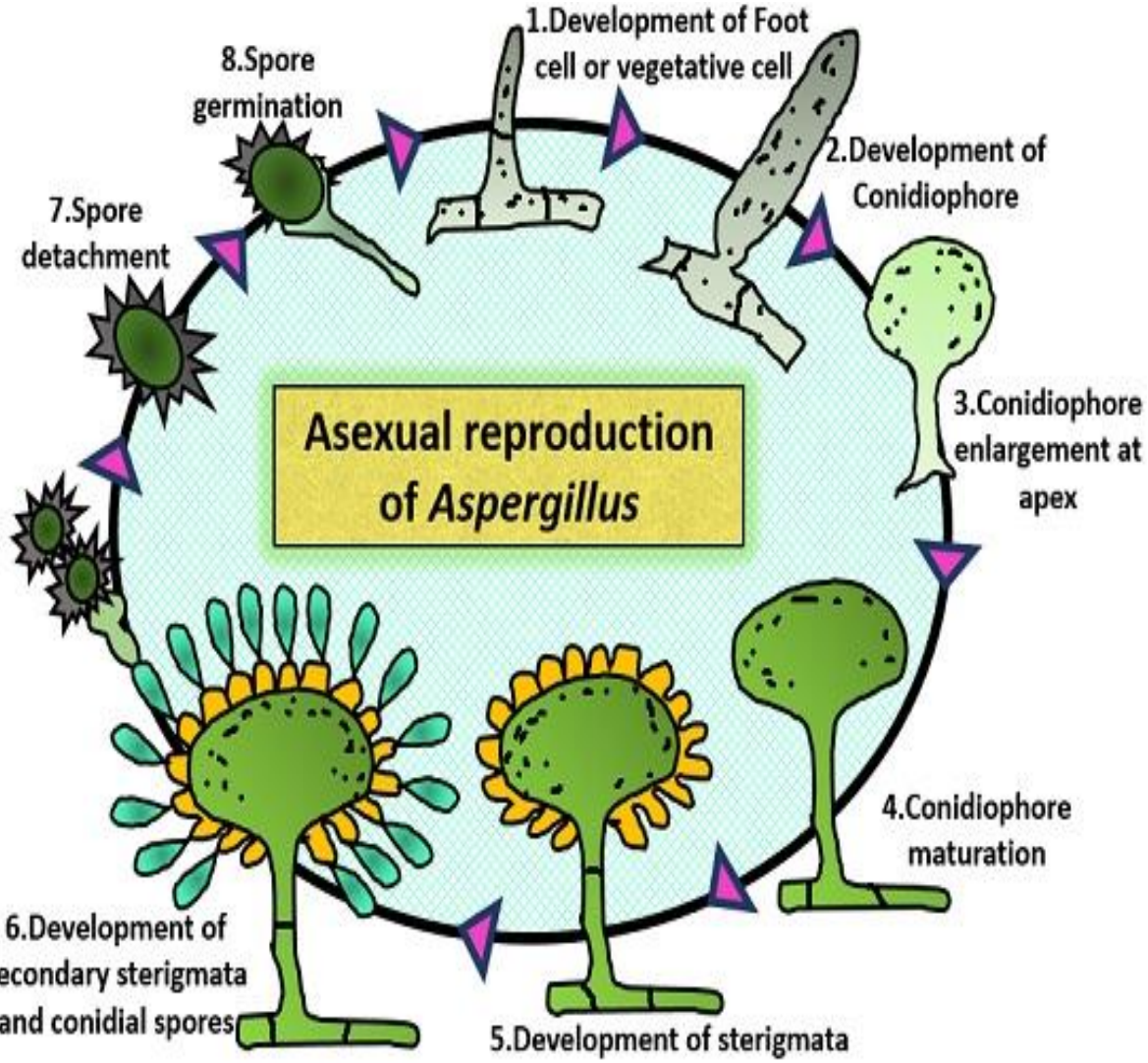
5. Preparation of organic acids: The important organic acids produced commercially as the result of the biochemical activities of molds are oxalic acid, citric acid, gluconic acid, gallic acid, fumaric acid, etc. Oxalic acid is the fermentation product of *A. niger*. Citric acid is made by mold fermentation. The acid is produced on a commercial scale and is cheaper than the acid made from the citrus fruits.

6. Antibiotics: Some specises of *Aspergillus* are the source of certain antibiotics like Flavicein, Aspergillin, Geodin, Funagalin, Patulin, Ustin etc.

7. *A. niger* is one of the most important microorganisms used in biotechnology. *A. niger* is used in bio-assay of metals as it can detect copper even in traces. *A. niger* is used for biotransformations and waste treatment.



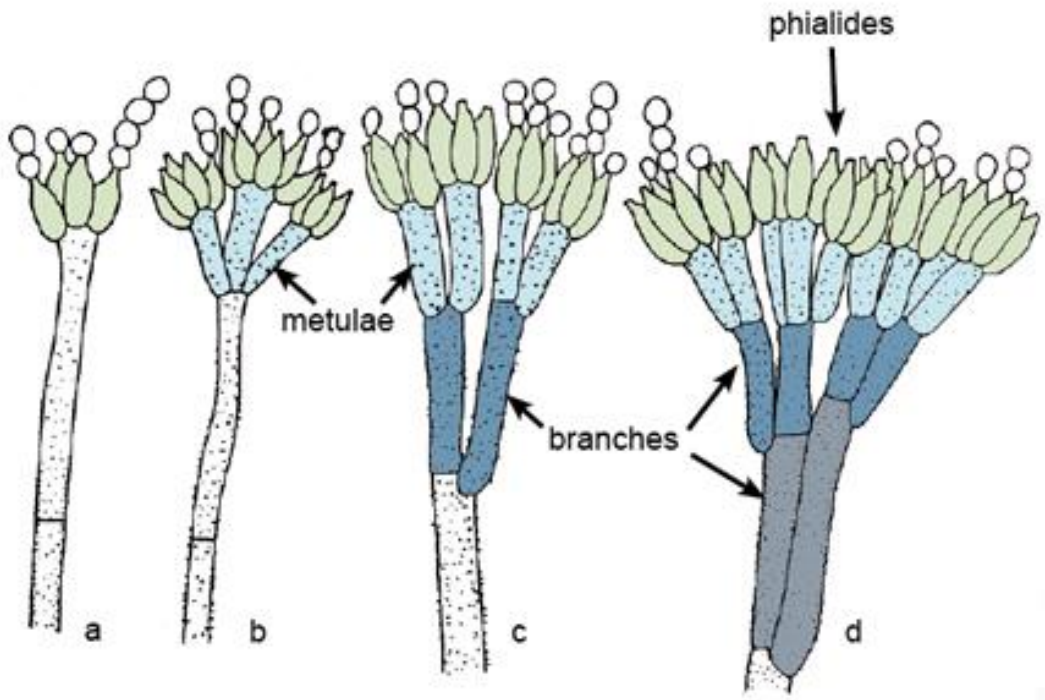
Sexual cycle of *Aspergillus*



Asexual cycle of *Aspergillus*

Penicillium **systematic position**

Division -Eumycota
Class -Ascomycetes
Order -Aspergillales
Family -Aspergillaceae
Genus -*Penicillium*



***Penicillium* structure**

Economic importance of *Penicillium*

1. *Penicillium notatum* and P. *chrysogenum* are used for the production of penicillin throughout the world.
2. *P. griseofulvum* produce the antibiotic griseofulvin which used in skin and nail infection.
3. *P. camemberti* and *P. roqueforti* are used in cheese production worldwide.
4. Decaying and rotting of fruits are caused by *Penicillium* species such as Citrus fruits rotting by *P. digitatum* and *P. italicum*, whereas apples are decayed by *P. expansum*.
5. Some species destroy the leather and fabrics, whereas other are associated with some human and animal diseases.

8. Basidiomycetes

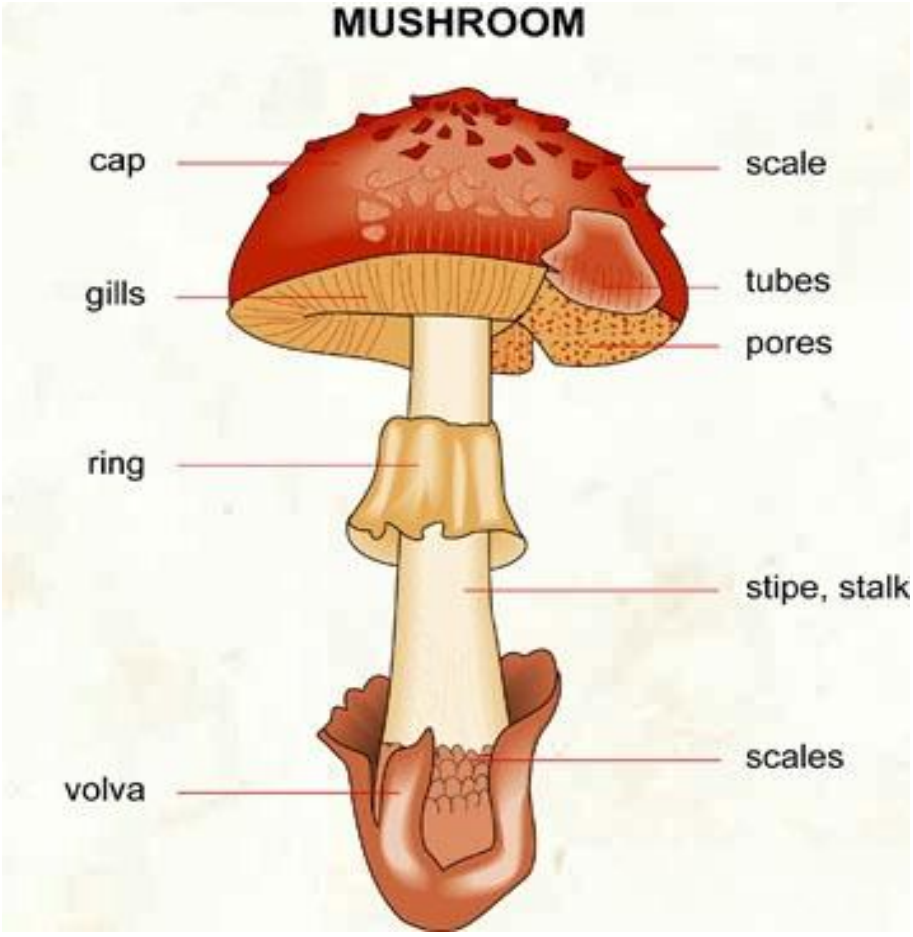
Basidiomycetes include fungi in which the perfect-state spores are basidiospores. They produce on a specialized structure called basidum.

General characters

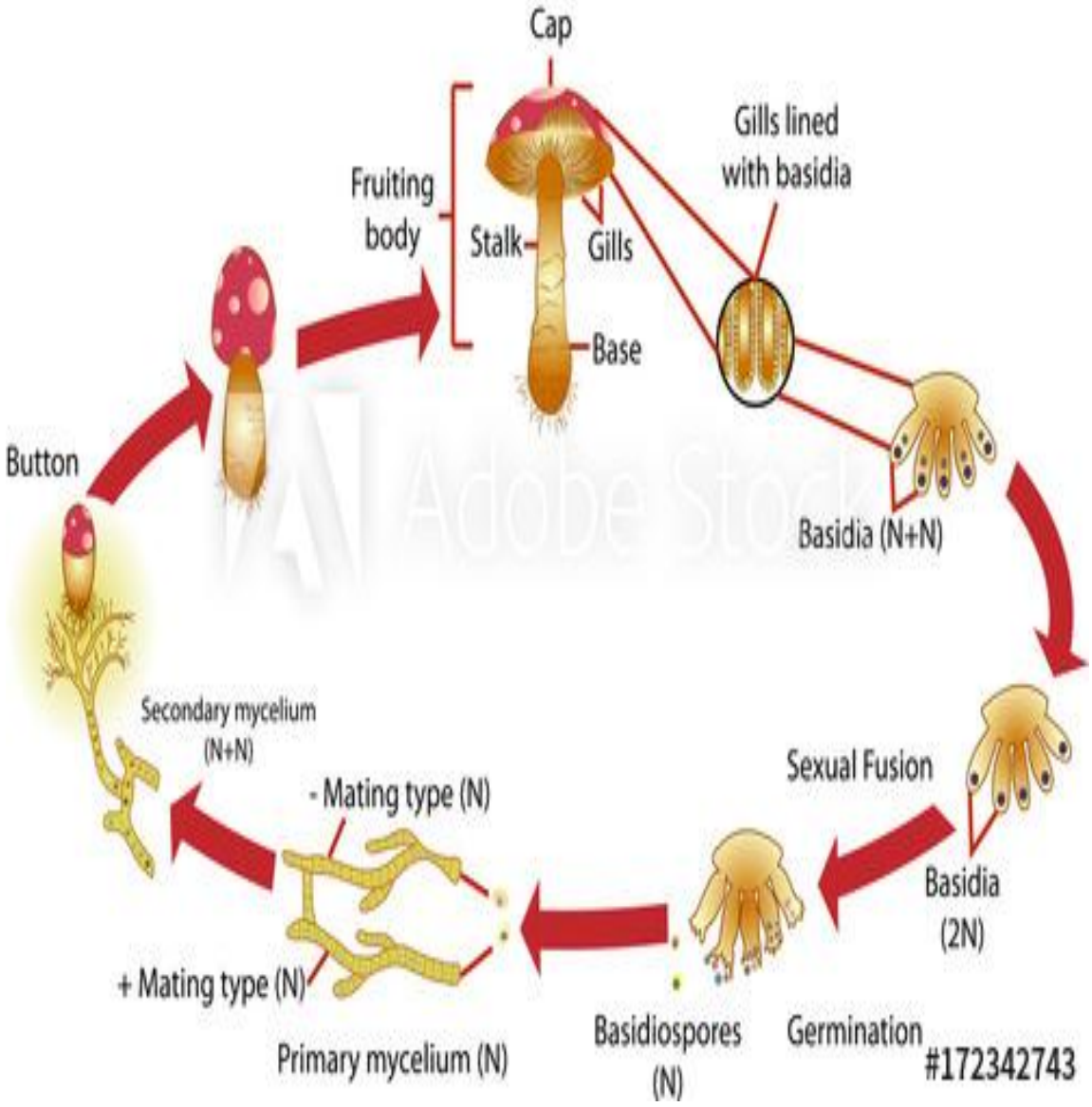
1. The members are saprophytic or parasitic (rust and smut fungi) cause serious diseases of cereal crops.
2. The mycelium is well-developed, branched and septate.
3. Cell wall consists of microfibrils of chitin.
4. Basidiomycetes reproduce asexually by conidia, arthrospores, fragmentation or budding.
5. No specialized sex organs develop in basidiomycetes. Plasmogamy takes place by somatogamy.
6. The characteristic spores are the basidiospores. They develop on a basidium.
7. Usually four basidiospores are developed on a basidium.

Mushrooms

Mushrooms are the fruiting bodies of a fungus. A mushroom is a kind of fungus with the Latin name of *Agaricus bisporus*. Other cultivated mushrooms in the Netherlands are the oyster mushroom (*Pleurotus ostreatus*) and the shiitake (Japanese mushroom) (*Lentinula edodes*).



In the vegetable kingdom the mushroom is ranked with the heterotrophic organisms (lower plants). In contrast to the higher, green plants, these heterotrophs are not capable of photosynthesis. Fungi are the scavengers of nature. In mushroom cultivation too, waste products such as chicken manure, horse manure, straw, gypsum and waste water (from their own composting) are used to produce a high-quality substrate from which the mushrooms will grow. Ammonia is removed by means of an ammonia washer from the process air before it is returned to nature. Even the ammonia from the air is used as a source of nitrogen in composting. The fungus, also called mycelium, uses the compost as a source of energy for its combustion, in which energy is released that is used for growth.


The Life Cycle of a Mushroom
Cap
Fruiting body
Stalk
Gills
Base
Gills lined with basidia
Basidia (N+N)
Button
Secondary mycelium (N+N)
- Mating type (N)
+ Mating type (N)
Primary mycelium (N)
Basidiospores (N)
Germination
Sexual Fusion
Basidia (2N)
#172342743

9. Deuteromycotina

Subdivision Deutromycotina includes the fungi in which the *'perfect stage'* is either lacking or has not been discovered. These fungi are commonly called *'imperfect fungi'*. Therefore the characteristic feature of Deutromycotina is the absence of sexual reproduction. The members reproduce only by asexual methods, and that also chiefly by conidia, which develop on conidiophores.

General characters

1- Deuteromycotina is represented by over 15,000 species, majority of which are terrestrial. Saprobes or weak parasites, causing a number of diseases of plants as well as animals.
2- Deuteromycotina have a true *mycelium*, consisting of well-developed, well-branched, septate hyphae.
3- Sexual reproduction is completely absent.
4- Reproduction takes place chiefly by the formation of special spores, called *conidia*. The *conidia* are non-motile structures which develop exogenously on the conidiophores.
5- The conidia are highly variable in shape, size, color …etc. unicellular or multicellular, and transversely septate or contain both transverse as well as longitudinal septa. They may be oval, elongated, spherical, star-shaped, curved, thread-like, coiled, and of other shape.

Classification

Criteria for classification

The shape, size, septation, color and ornamentation of conidia have been the major source for the classification of Deuteromycotina.

- **Class : Deuteromycetes (Hyphomycetes)**
- **Order : Moniliales**

This order generally divided into the following four families:

1- *Moniliaceae*: Conidiophores separate from one another or absent.
2- *Dematiaceae*: Spores and mycelium dark-colored; e.g. *Alternaria*.
3- *Tuberculariaceae*: Conidia and conidiophores are produced in sporodochium; e.g. *Fusarium*.
4- *Stilbellaceae*: Conidia and conidiophres develop in synnemata.

Alternaria

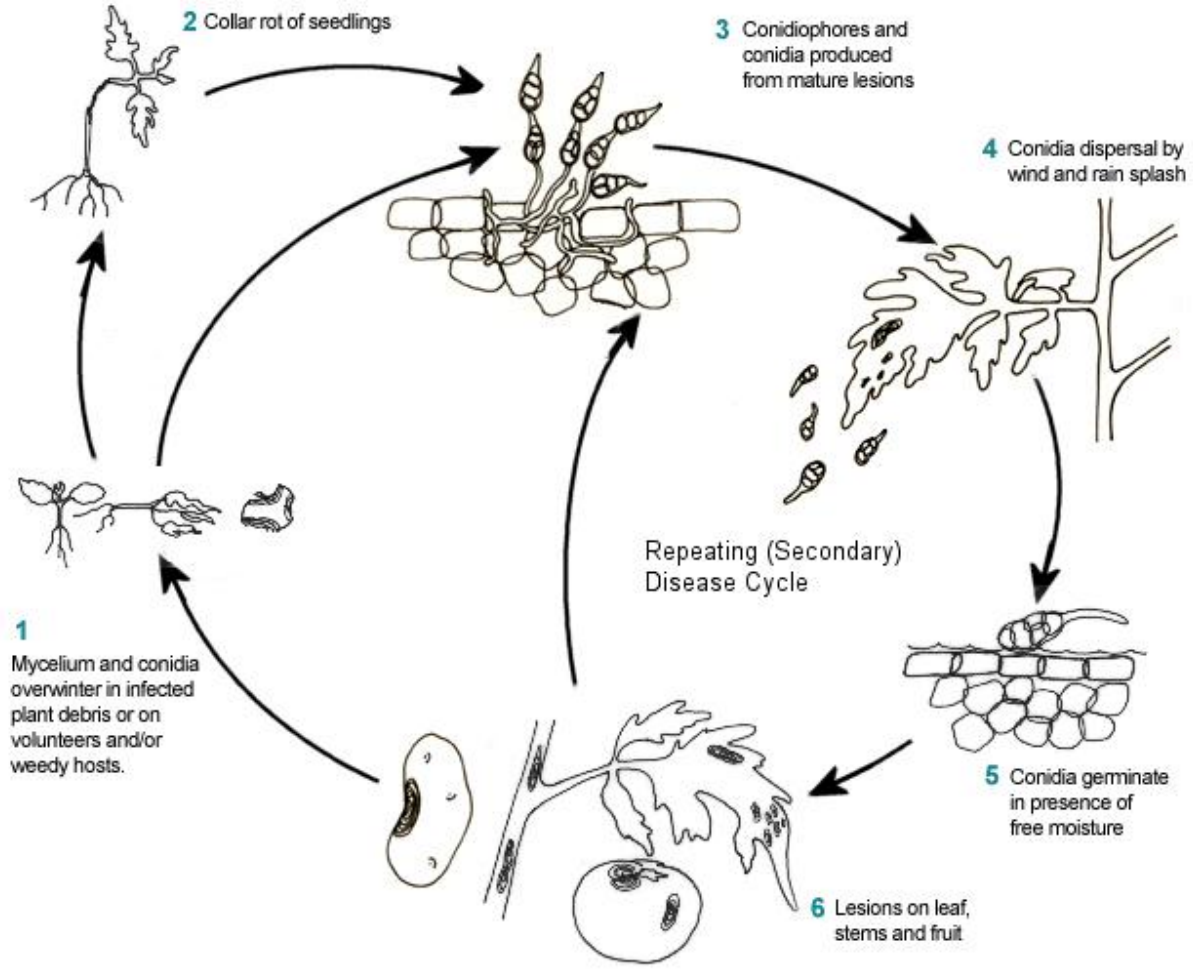
Systematic position

Division : Eumycota
Subdivision : Deuteromycotina
Class : Deuteromycetes
Order : Moniliales
Family : Dematiaceae
Genus : *Alternaria*

Occurrence

Alternaria is a very large, universally occurring fungus, as saprobes on dead parts of plants in the soil. Its conidia are very common in house dust as well as in the atmosphere, and are responsible for allergies, skin diseases. Many *Alternaria* species are parasitic on plants including members of Solanaceae (potato and tomato). *Alternaria* shows symptoms of early blight and occurs earlier than *Phytophthora*, which is the causal organism of late blight of potato. Early blight symptoms are in the form of small, yellowish brown spots on the leaves, which enlarge in size and become round to form concentric rings. Stem and even tubers or fruits are badly damaged after infection. Edible parts of tubers or fruits turn brown or black.

Alternaria early blight



Early blight of potato and tomato

Conidia of *Alternaria* develop at the tips of the conidiophores, which are short, dark colored. The young conidium first divides by transverse septa. Later on some of the cells divide by longitudinal septa. Usually the tip cell of a conidium

also shows budding, and the ultimate result is the formation of chain of conidia. A mature conidium is a multicellular body, having transverse as well as longitudinal septa. The conidia are disseminated readily by wind.

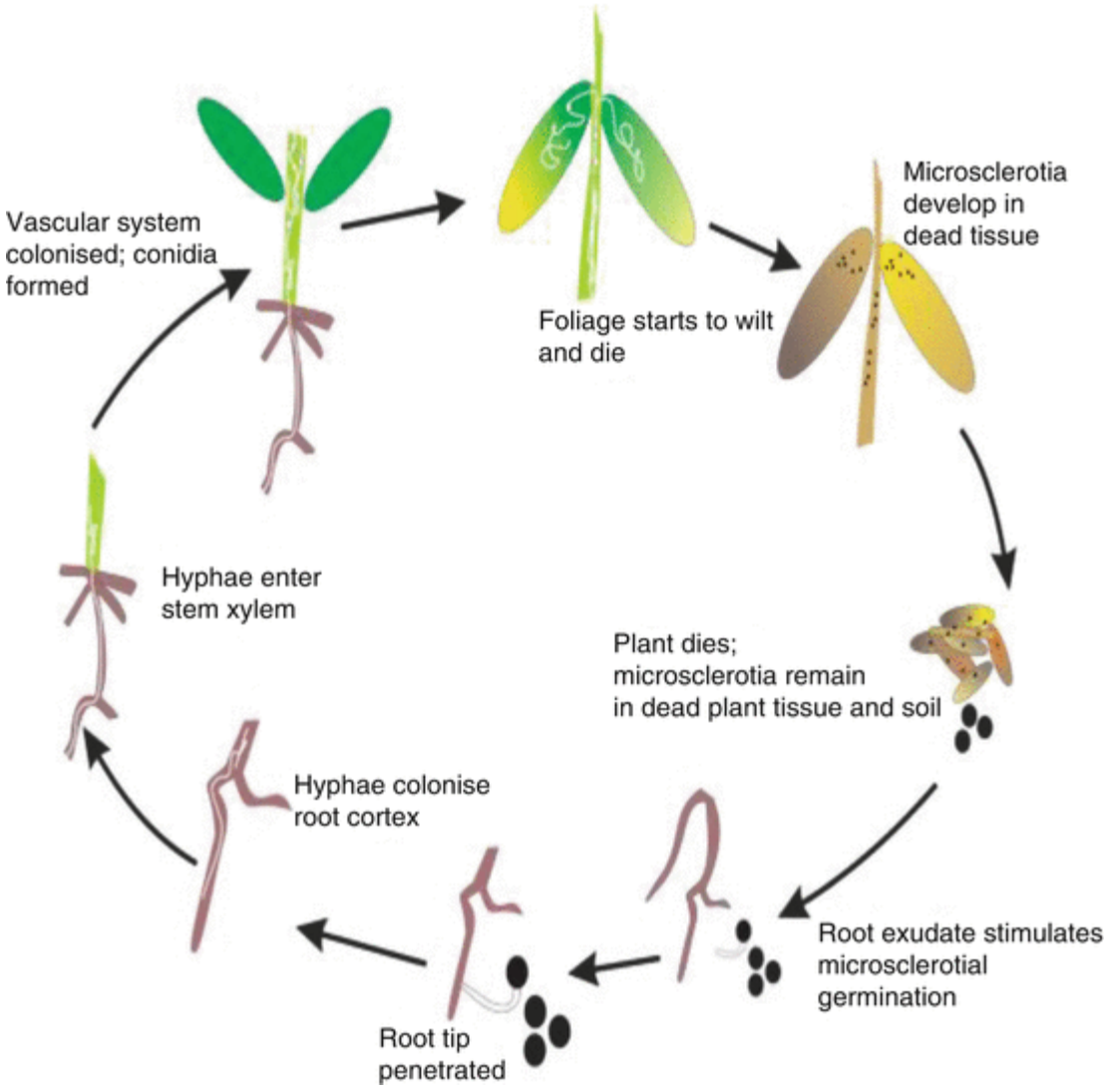
Fusarium

Systematic position

Division : Eumycota
Subdivision : Deuteromycotina
Class : Deuteromycetes
Order : Moniliales
Family : Tuberculariaceae
Genus : *Fusarium*

Occurrence

Fusarium is the largest genus of Tuberculariaceae, occurs saprophytically or parasitically on many plant crops, fruits and vegetables. It causes serious wilting of the host plants. The mycelium invades the vascular tissue and finally blocks the xylem vessels. It affects the translocation of water, leading to wilting of plants. *Fusarium* also produces some toxic compounds that secreted in the vessels of the host, which might also be the cause of wilting.



Fusarium wilt disease

Mycelium

The mycelium consists of branched, septate, often colorless hyphae, which turn brown at maturity.

Reproduction

Fusarium reproduces asexually by means of three kinds of asexual spores: macroconidia, microconidia and chlamydospores. Macroconidia are long,

multicellular, cresent-shaped. The cells on which the macroconidia develop are called phialides.

Microconidia are small, usually unicellular, spherical or oval bodies produced from simple phialides or from branched or unbranched conidiophores. Chlamydospores are round or oval, thick-walled, terminal or intercalary cells of the old hyphae. Either develops singly or in chains. Germinate by means of germ tubes, if the conditions are favorable.

10. Mycorrhizae

A mycorrhiza (Greek for "fungus roots") is a symbiotic association between a fungus and the roots of a plant. In a mycorrhizal association, the fungus may colonize the roots of a host plant by either growing directly into the root cells, or by growing around the root cells. This association provides the fungus with relatively constant and direct access to glucose, which the plant produces by photosynthesis. The mycelia of the fungi increase the surface area of the plant's root system. The larger surface area improves water and mineral nutrient absorption from the soil.

Among symbiotic fungi, those that enter into mycorrhizal relationships and those that enter into relationships with algae to form lichens are probably the best-known. A large number of fungi infect the roots of plants by forming an association with plants called mycorrhiza (plural: mycorrhizae). This association differs markedly from ordinary root infection, which is responsible for root rot diseases. Mycorrhiza is a non-disease-producing association in which the fungus invades the root to absorb nutrients. Mycorrhizal fungi establish a mild form of parasitism that is mutualistic, meaning both the plant and the fungus benefit from the association. About 90 percent of land plants rely on mycorrhizal fungi, especially for mineral nutrients (i.e., phosphorus), and in

return the fungus receives nutrients formed by the plant. During winter, when day length is shortened and exposure to sunlight is reduced, some plants produce few or no nutrients and thus depend on fungi for sugars, nitrogenous compounds, and other nutrients that the fungi are able to absorb from waste materials in the soil. By sharing the products, it absorbs from the soil with its plant host, a fungus can keep its host alive. In some lowland forests, the soil contains an abundance of mycorrhizal fungi, resulting in mycelial networks that connect the trees together. The trees and their seedlings can use the fungal mycelium to exchange nutrients and chemical messages.

There are two main types of mycorrhiza : ectomycorrhizae and endomycorrhizae. Ectomycorrhizae are fungi that are only externally associated with the plant root, whereas endomycorrhizae form their associations within the cells of the host.

Among the mycorrhizal fungi are boletes, whose mycorrhizal relationships with larch trees (*Larix*) and other conifers have long been known. Other examples include truffles, some of which are believed to form mycorrhizae with oak (*Quercus*) or beech (*Fagus*) trees. Many orchids form mycorrhizae with species of *Rhizoctonia* that provide seedlings of the orchid host with carbohydrate obtained by degradation of organic matter in the soil.

Mycorrhizae

- It literally means fungus-root
- Association between a fungus and the roots of a vascular plant
- Mutualism between:

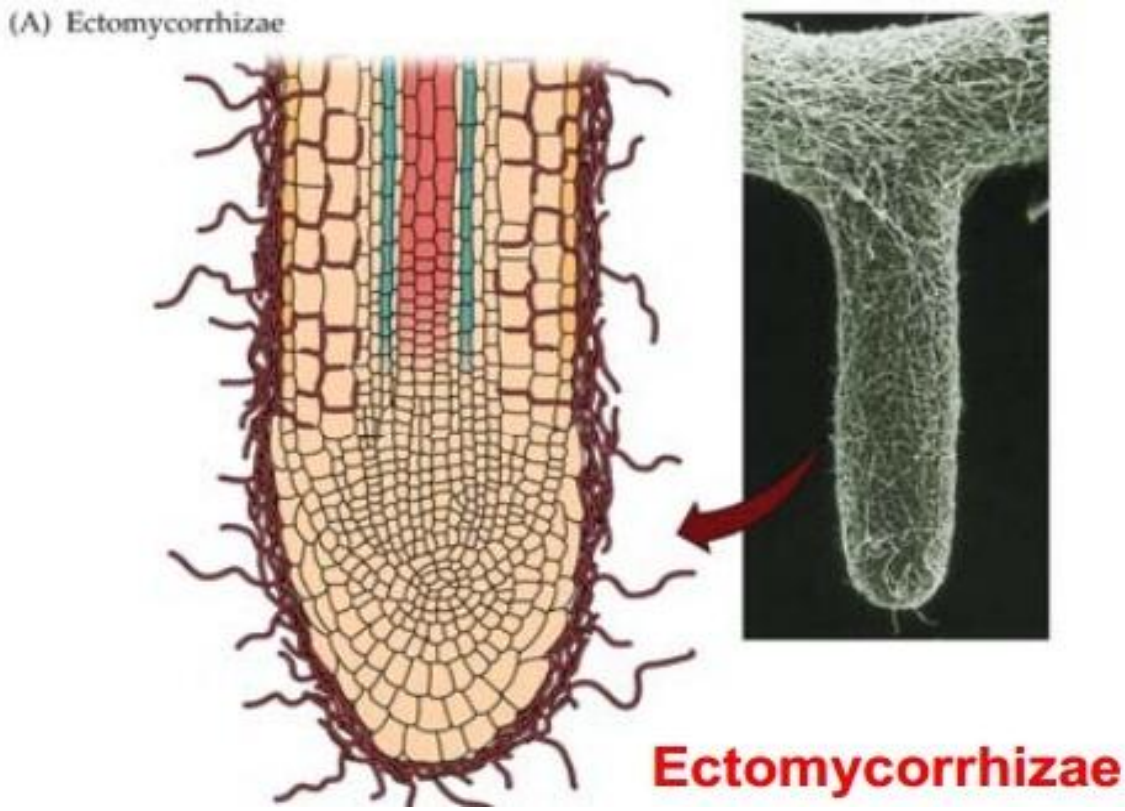
Fungus (minerals & water uptake for plant) by extending the reach of their root system.

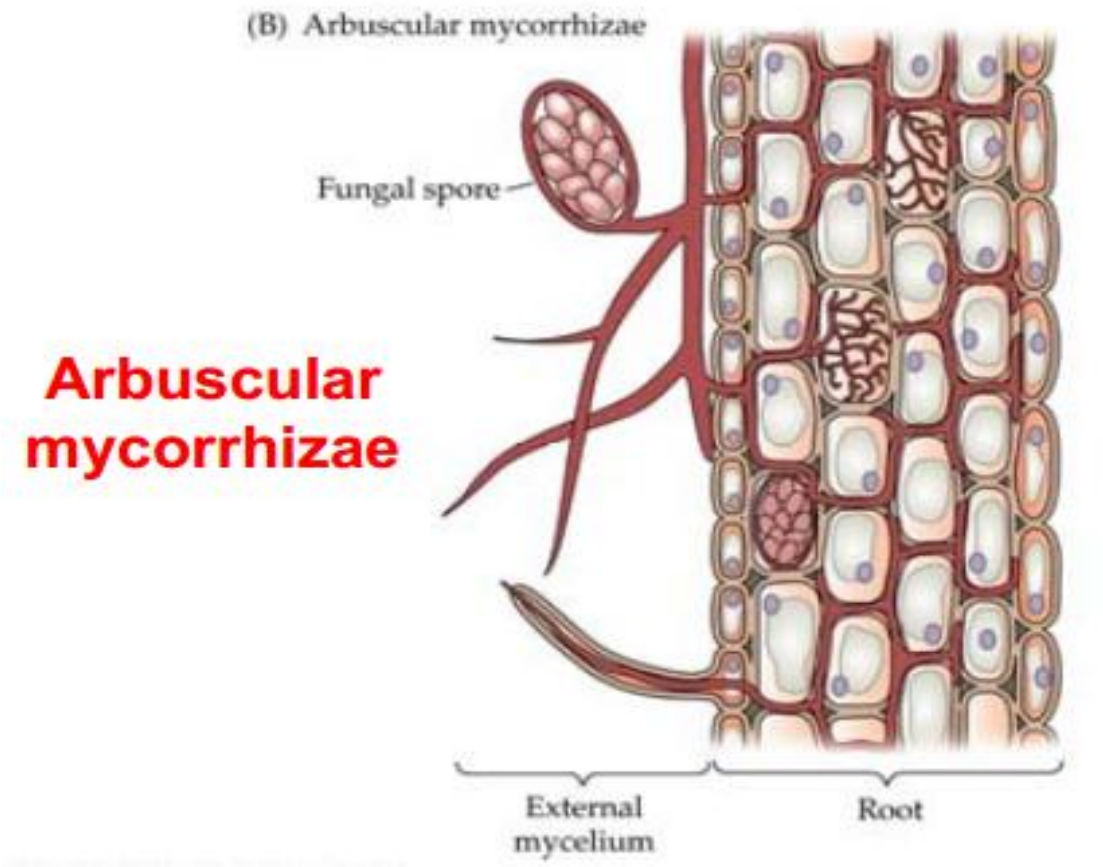
Plant (carbohydrate for fungus)

There are two main types of mycorrhizae:

Ectomycorrhizae : The partner fungi was (Ascomycota & Basidiomycota), hyphae invade root but don't penetrate cell.

Endomycorrhizae : The partner fungi was (zygomycetes), hyphae invade root cell.





Endomycorrhizae

11. Biological control of fungi

Biological control or biocontrol is a method of controlling pests such as insects, mites, weeds and plant diseases using other organisms. It relies on predation, parasitism, herbivory, or other natural mechanisms, but typically also involves an active human management role. It can be an important component of integrated pest management (IPM) programs.

There are three basic strategies for biological pest control: classical (importation), where a natural enemy of a pest is introduced in the hope of achieving control; inductive (augmentation), in which a large population of natural enemies are administered for quick pest control; and inoculative

(conservation), in which measures are taken to maintain natural enemies through regular reestablishment.

Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, pathogens, and competitors. Biological control agents of plant diseases are most often referred to as antagonists. Biological control agents of weeds include seed predators, herbivores and plant pathogens.

Biological control can have side-effects on biodiversity through attacks on non-target species by any of the same mechanisms, especially when a species is introduced without thorough understanding of the possible consequences.

Entomopathogenic fungi, which cause disease in insects, include at least 14 species that attack aphids. *Beauveria bassiana* is mass-produced and used to manage a wide variety of insect pests including whiteflies, thrips, aphids and weevils. *Lecanicillium* spp. are deployed against white flies, thrips and aphids. *Metarhizium* spp. are used against pests including beetles, locusts and other grasshoppers, Hemiptera, and spider mites. *Paecilomyces fumosoroseus* is effective against white flies, thrips and aphids; *Purpureocillium lilacinus* is used against root-knot nematodes, and *Trichoderma* species against certain plant pathogens. *Trichoderma viride* has been used against Dutch elm disease, and has shown some effect in suppressing silver leaf, a disease of stone fruits caused by the pathogenic fungus *Chondrostereum purpureum*.

The fungi *Cordyceps* and *Metacordyceps* are deployed against a wide spectrum of arthropods. *Entomophaga* is effective against pests such as the green peach aphid. Several members of Chytridiomycota and Blastocladiomycota have been explored as agents of biological control. From Chytridiomycota, *Synchytrium solstitiale* is being considered as a control agent of the yellow star thistle (*Centaurea solstitialis*) in the United States.

Fungi for the biological control of insect pests

Fungi are a diverse group of organisms with close ties to agriculture. Some fungi create devastating diseases in crops, while others are crops themselves (mushrooms). Other fungi are used successfully to protect crops from a variety of pests. Among the most prominent of these are the fungi that are used against insects and other related pests.

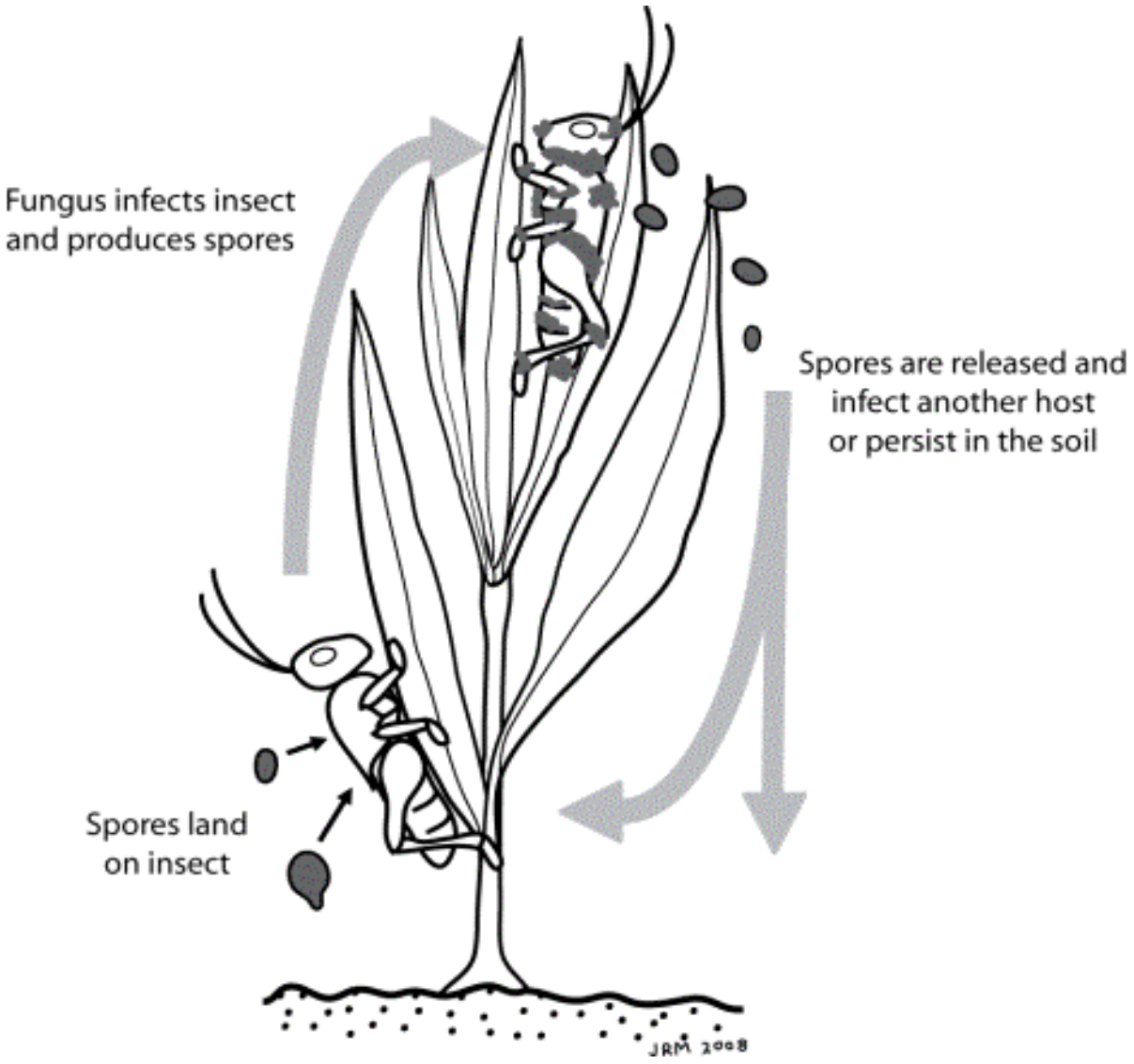
Most fungi used for the control of insect pests belong to the group hyphomycetes. Some species have been developed as commercial products because of their ability to be mass produced. Most fungi in this group are usually found in the soil and can cause natural outbreaks on their own when environmental conditions are favorable. They can infect a wide range of insect hosts. Specific fungal strains in commercial products target thrips, whiteflies, aphids, caterpillars, weevils, grasshoppers, ants, Colorado potato beetle, and mealybugs. Currently allowable products containing the hyphomycete fungus, *Beauveria bassiana*, that are commercially available include Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G) and Naturalis L (Troy BioSciences, Inc.).

There is another commonly encountered group of fungi called the entomophthorales. Fungi in this group can cause natural outbreaks in the populations of their insect hosts, but they are difficult to mass produce and as yet are not in commercial production. They tend to be much more host specific; one well known species only infects aphids. Despite the difficulties in producing them commercially, they can still have a large impact on the pest populations they infect.

Fungi that infect insects are found in the environment as spores. Insects can become infected when they come into contact with spores on the surface of plants, in the soil, in the air as windborne particles, or on the bodies of already

dead insects. Spores attach to the surface of the insect and infect by penetrating through the insect cuticle, often at joints or creases where the insect's protective covering is thinner. Once inside, the fungus grows throughout the body of the insect. Many fungi also produce toxins in the host that increase the speed of kill or prevent competition from other microbes.

Usually after the insect has died, the fungus grows out through the outer covering (exoskeleton) of the insect, usually at thinner areas like joints or creases, and begins to produce spores. The spores of commercially developed fungi in the group hyphomycetes are spread passively by the action of the wind, rain, or contact with other hosts or animals in the environment. The spores of fungi that create natural outbreaks, in the group entomophthorales, are often actively ejected from the dead insect. Since many species in this group of fungi infect insects which cluster together, like aphids, this tactic can drastically increase the spread of the infection. Insects killed by fungi often have a "fuzzy" appearance, caused by the growth of the fungus out of the exoskeleton to produce spores. Most commercial strains of fungi produce spores that are either white or green in color, although the color of the fungi can change over time as the fungus grows and ages. Spores that do not encounter a host either die or persist in sheltered areas of crop plants or in the soil. Fungi can produce spores which can persist for years in the soil.



Generalized life cycle of fungi infecting insects

Advantages and disadvantages of fungi for controlling insect pests

Advantages

Fungi make good biological control agents for a variety of reasons. They generally do not affect people or other mammals, making them extremely safe to use. It is relatively easy to mass produce spores of insect-parasitic fungi in the hyphomycete group, so they are comparably priced with other biological control agents, such as bacteria. Most commercial fungal products are

formulated as spores, which are easily adapted to existing application technology, such as spray rigs. The relatively broad host range of many fungi means one can often achieve control of multiple pests with the same product. Finally, successful infections can spread to other hosts and lead to high rates of persistence within a growing season, even if between season persistence tends to be low for most types of fungi.

Disadvantages

High concentrations of spores are often needed to get adequate control of pests in a crop, which can cut down on the cost effectiveness of fungal products. The kill time is relatively long (~1 week for most fungi), although strains used for commercial products are chosen to kill as fast as possible. Their broad host range can sometimes be a problem, especially if beneficial insects (i.e. predators, parasitoids, and pollinators) are present in a crop; non-target mortality in these populations of beneficial insects can negatively impact the success of the overall biological control program. Environmental factors can also play an important role in the success of fungi. Moist conditions or high relative humidity in the canopy of the crop are often necessary for control to be effective. Prolonged exposure to sunlight can also inactivate spores, reducing persistence in the crop. Owing to these environmental limitations, natural outbreaks of fungi tend to be sporadic and very patchy in the environment, which can limit their effectiveness in controlling pests.

Application

As with all biological control agents, fungi work best as one component of a comprehensive integrated pest management program. There are several tactics growers can us to increase their effectiveness, though, especially for applied commercial products:

- Scout consistently and often. Apply only when the target pest is seen, not as a preventive application as residues are not long-lasting. The best time to apply fungi is before pest populations reach their peak, so early application can increase their effectiveness. Also, scouting can help determine the population levels of beneficial insects and pollinators so the timing of fungal applications will not impact them as strongly. Finally, scouting can help discover natural outbreaks of fungi (e.g. aphid fungi) in time to influence control decisions.
- Time applications of fungi to coincide with host life stages that are more likely to come in contact with the spores. In general, *B. bassiana* products are more effective against earlier compared with later stages of insects. For example, applying a fungal product for grasshoppers will be most effective when there are active nymphs present that have not grown into winged adults.
- Do not apply fungal products during droughts or dry spells since the environmental conditions will decrease their effectiveness.
- Be aware of fungicide applications in the area. Even if fungicides are not directly applied to the crop, drift from nearby fields could impact the success of a fungal biological control agent.
- Apply fungal inoculum carefully to get effective coverage. Cover all plants thoroughly. Also try to reduce of spill-over into refuge areas where natural enemies may be present.
- Do not apply fungal products during the heat of the day since this will diminish the potency of the spores. There have been some reports of phytotoxicity to young vegetable transplants with products formulated as an emulsifiable suspension. Also, do not apply on rainy days, when spores

will be washed off of plant surfaces and may not come into contact with the target pest.

Use cropping practices which encourage a diverse understory and soil surface, such as cover cropping or conservation tillage. These practices will help maintain fungi in the field and could increase persistence within and between seasons.

12. Fungal Metabolism

Fungi can be found in all types of habitats. Several thousand fungal species are very diverse in morphological and physiological characters. 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 can be conveniently divided into two categories:

Anabolism - the biosynthesis of all compounds needed by the cells.

Catabolism - the breakdown of molecules to obtain energy.

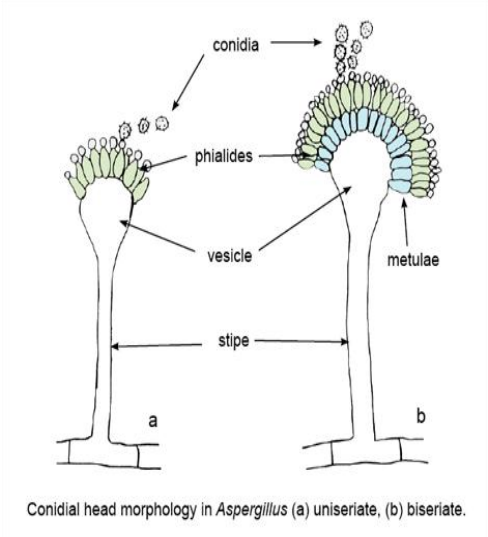
Mycotoxins

The worldwide contamination of foods and feeds with mycotoxins is a significant problem. It was estimated that 25% of the world's crops may be contaminated with these metabolites. Mycotoxigenic fungi involved with the human food chain belong mainly to three genera *Aspergillus*, *Fusarium* and *Penicillium*. The toxins produced by *Alternaria* have recently been of particular interest. The biochemistry, physiology and genetics of mycotoxigenic fungi have been discussed in several review articles. Mycotoxins diffuse into grain and can be found in all grind fractions and, due to their thermo-resistant properties, also in products subjected to thermal processing.

Aflatoxins

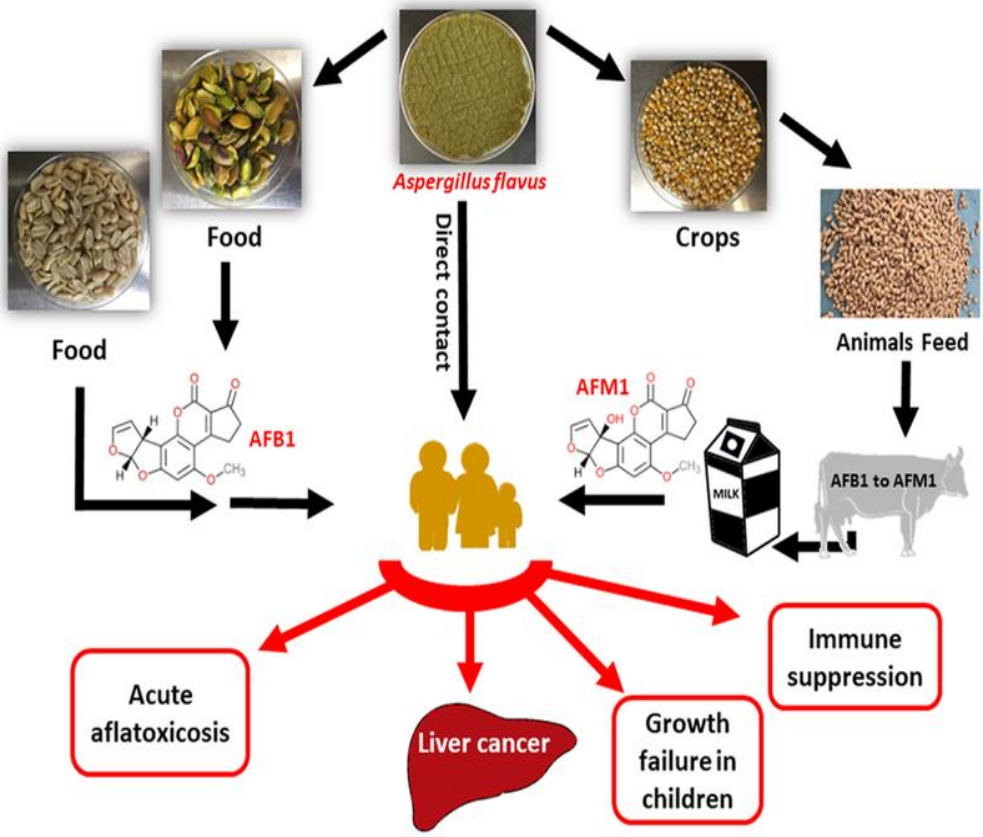
Aflatoxins are difuranocumarin derivatives. The main naturally produced aflatoxins based on their natural fluorescence (blue or green) are called B1, B2, G1, and G2. Aflatoxin M1 is a monohydroxylated derivative of AFB1 which is formed and excreted in the milk of animals. AFs are very slightly soluble in water (10–30 μg/mL); insoluble in non-polar solvents; freely soluble in moderately polar organic solvents (e.g. chloroform and methanol) and extremely soluble in dimethyl sulfoxide. They are unstable under the influence of ultraviolet light in the presence of oxygen, to extremes of pH (< 3, > 10) and to oxidizing agents.

Aflatoxins are produced maily by a closely related group of aspergilli: *Aspergillus flavus*, *A. parasiticus,* and *A. nomius* strains. These species are very widespread in the tropical and subtropical regions of the world. Aflatoxins constitute a problem concerning many commodities (nuts, spices), however, in terms of grain they are primarily problematic in case of maize because maize can be colonised by *A. flavus* and related species in the field. Out of the other grains, rice is an important dietary source of aflatoxins in tropical and subtropical regions. In regions with moderate climate, the problem is connected with imported commodities or the local crops that are wet or stored in improper conditions. The carcinogenicity, mutagenicity and acute toxicology of AFB1 have been well documented. The IARC determined it to be a human carcinogen.

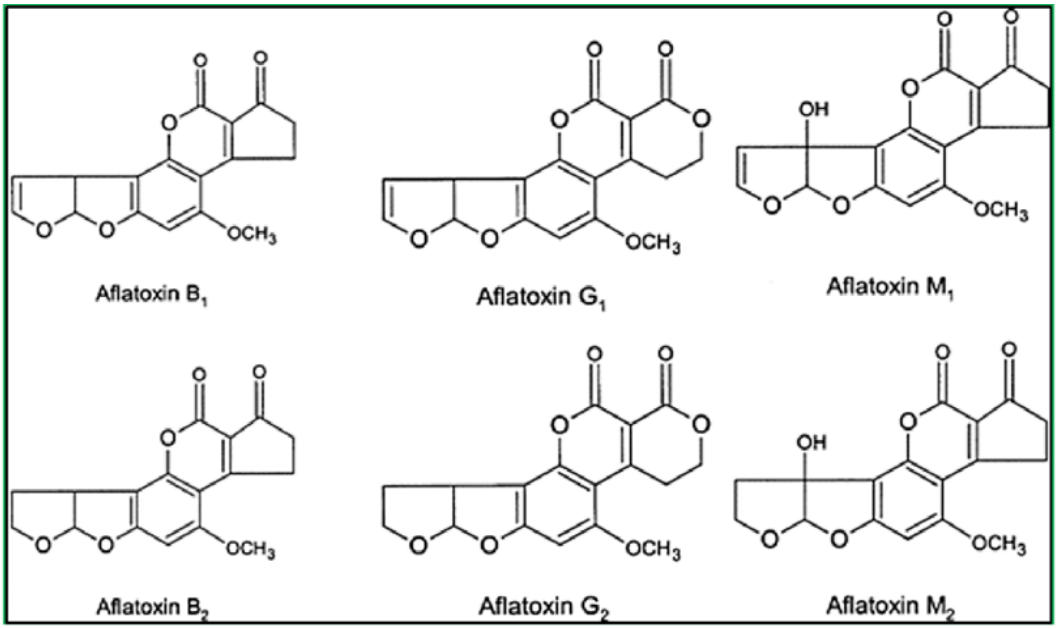


Conidial head morphology in *Aspergillus* (a) uniseriate, (b) biseriate.

Aflatoxin producing fungi



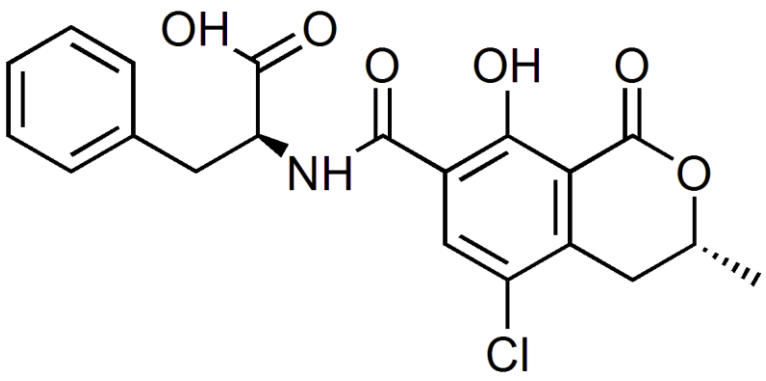
Aflatoxin contamination and their hazard effect



Chemical structure of aflatoxins

Ochratoxin A

Ochratoxin A is a chlorinated isocumarin derivative, which contains a chlorinated isocoumarin moiety linked through a carboxyl group to L-phenylalanine via an amide bond. It is colourless, crystalline, and soluble in polar organic solvents compounds. This toxin is more stable in the environment than aflatoxins. The studies reported that thermal destruction of Ochratoxin A occurs after exceeding 250°C. It is produced mainly by *Aspergillus ochraceus*, *A. melleus, A. ostanius* and *Penicillium verrucosum*, *P. auriantiogriseum*, *P. nordicum*. In moderate climates, the main producers of Ochratoxin A are *Penicillium* species, while *Aspergillus* species dominate in tropical and subtropical climates. Ochratoxin A is often found with citrinin produced by *Penicillium aurantiogriseum*, *P. citrinum*, and *P. expansum*. Significant human exposure comes from the consumption of grape juice, wine, coffee, spices, dried fruits and cereal-based products, e.g. whole-grain breads, in addition to animal origin products. The IARC determined a possible human carcinogen. Ochratoxins are the cause of urinary tract cancers and kidney damage.

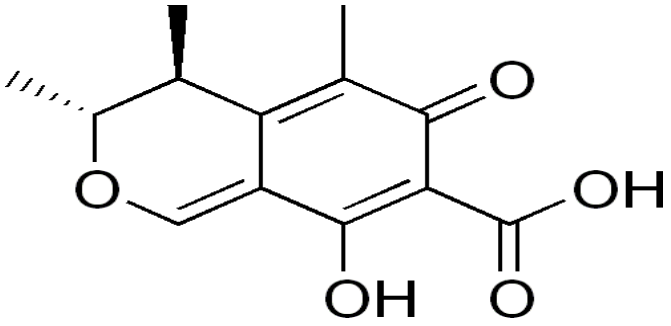


Ochratoxin A

Citrinin

Citrinin is a polyketide nephrotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. Some of the citrinin-producing fungi are

also able to produce ochratoxin A or patulin. Citrinin is insoluble in cold water, but soluble in aqueous sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most other polar organic solvents. Thermal decomposition of citrinin occurs at >175°C under dry conditions, and at > 100°C in the presence of water. The known decomposition products include citrinin H2 which did not show significant cytotoxicity, whereas the decomposition product citrinin H1 showed an increase in cytotoxicity as compared to the parent compound. The most commonly contaminated commodities are barley, oats, and corn, but contamination can also occur in case of other products of plant origin e.g. beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products.

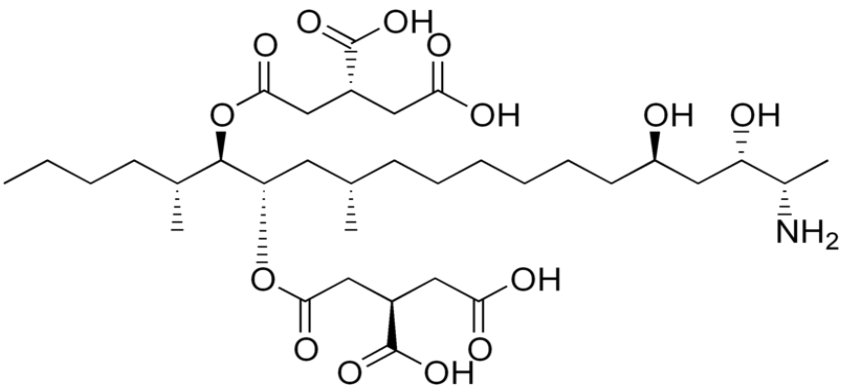


Citrinin

Fumonisins

Fumonisins are a group of diester compounds with different tricarboxylic acids and polyhydric alcohols and primary amine moiety. There are several fumonisins; such as fumonisin B1 and B2 which have been found in significant amounts. Some technological processes hydrolyze the tricarboxylic acid chain in fumonisin B1. The product of this reaction is more toxic than fumonisin. Fumonisin B1 is produced by *Fusarium*, especially by *F. moniliforme* and *F. proliferatum.* The studies suggested that the risk of contamination with *Fusarium*

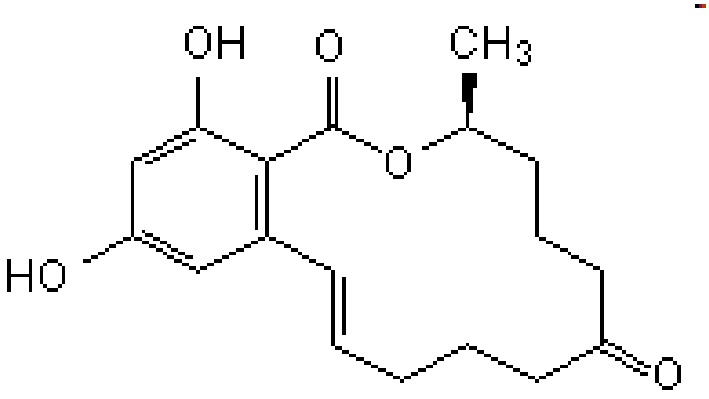
toxins is higher for maize and wheat than for soybean and pea. High concentrations of fumonisins are associated with hot and dry weather, followed by the periods of high humidity. The IARC classified *Fusarium monilliforme* toxins, including fumonisins, as potential carcinogens to humans.



Fumonisin

Zearalenone

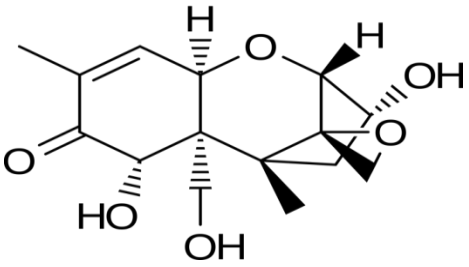
Zearalenone is a macrocyclic lactone with high binding affinity to oestrogen receptors. Zearalenone is produced mainly by *Fusarium graminearum* and *F. sporotrichoides* in the field and during storage of commodities such as maize, barley, sorghum, and soybean. The IARC has evaluated the carcinogenicity of zearalenone and found it to be a possible human carcinogen.



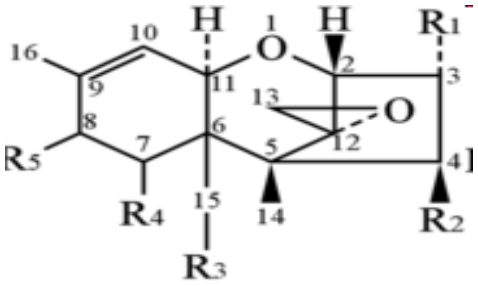
Zearalenone

Trichothecenes

Trichothecenes constitute a group of 50 mycotoxins produced by *Fusarium*, *Cephalosporium* and *Stachybotrys* genera in different commodities. There are including T-2 toxins, deoxynivalenol, nivalenol, and diacetoxyscirpenol. Beside trichothecenes, deoxynivalenol (DON, womitoxin) is probably the most widely distributed in cereal and soybean foods and feeds. In contaminated cereals, DON derivatives such as 3-acetyl DON and 15-acetyl DON can occur in significant amounts (10-20%) with DON. DON is produced by closely related *Fusarium graminearum, F. culmorum* and *F. crokwellense* species. T-2 toxin produced mainly by *F. sporotrichoides* and *F. poae* is primarily associated with mould millet, wheat, rye, oats, and buckwheat. This toxin can be transmitted from dairy cattle feed to milk.



Deoxynivalenol

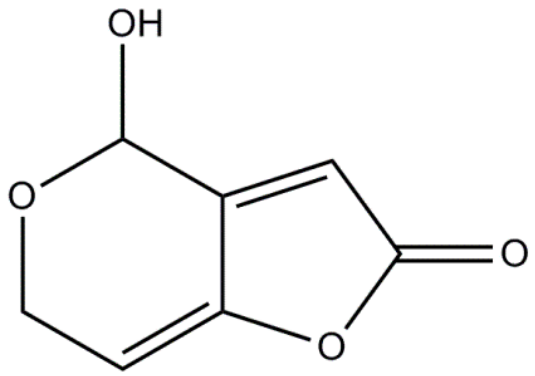


Trichothecenes

Patulin

Patulin is a mycotoxin produced by molds *Penicillium patulum, Penicillium expansum, Penicillium urticae, Penicillium claviforme, Aspergillus clavatus* and *Byssochlamys* spp., with toxically effects on human and animal body. Patulin was identified, especially, in spontaneous damaged fruits and vegetables including apples, apricots, bananas, pineapples, grapes, black currants, raspberry, strawberries, cucumbers, tomatoes, green peppers and carrots. Patulin is stable, especially in processed products from apples: apples juice, apples piuree, apples in syrup, etc. From chemical point of view, patulin is furo-piranone, with molecular mass 154, stable in acid medium, but unstable in

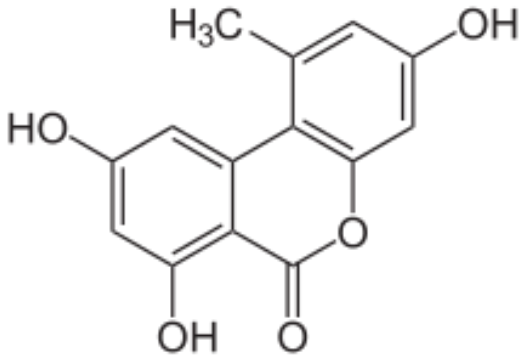
alkaline medium. It is presented as uncolored crystals form, with melting point 110.5°C, with absorption maximum at 276 nm, in alcoholic solution. In the Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.



patulin

Alternaria toxins

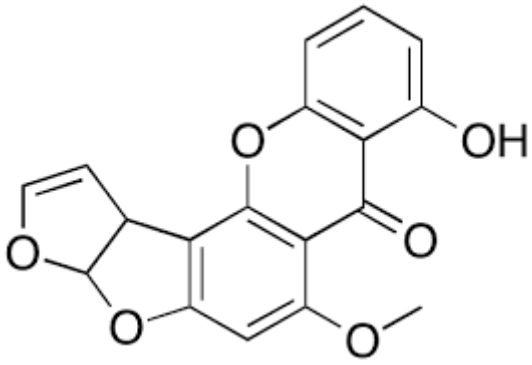
Alternaria species, besides *Fusarium,* is the most isolated fungi from soybean and cereals. Several species are known producers of toxic metabolites called *Alternaria* mycotoxins. The most important *Alternaria* toxins include alternariol (AOH), alternariol monomethyl ether (AME), altertoxins I, II, and III, tenuazonic acid (TeA). They belong to three structural classes: dibenzopyrone derivatives, perylene derivatives, and tetramic acid derivatives. Alternariol and related metabolites (AME and ALT) are produced by *Alternaria alternata, A. brassicae, A. citri, A. cucumerina, A. dauci, A. kikuchiana, A. solani,* and *A. tenuissima*. These strains are known as plant pathogens, especially fruit and vegetables. In cereals, soybean and oilseeds, AOH, AME and ALT are produced mainly by *Alternaria alternata, A. tennuisima,* and *A. infectoria.* AOH has been reported to possess cytotoxic, genotoxic, mutagenic, carcinogenic, and oestrogenic properties. Tenuazonic acid (TeA) is a mycotoxin and phytotoxin produced primarily by *Alternaria alternata* and other phytopatogenic *Alternaria* species.



Alternariol toxin

Sterigmatocystin

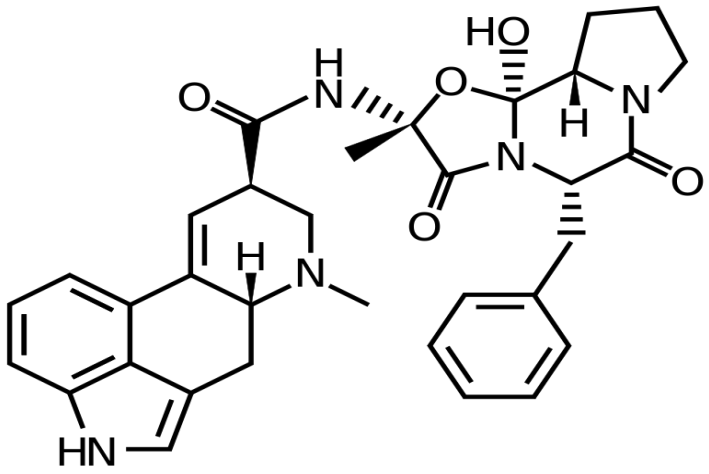
Sterigmatocystin (STC) is a precursor of the aflatoxins produced mainly by many *Aspergillus* species such as *A. versicolor, A. chevalieri, A. ruber, A. aureolatus, A. quadrilineatus, A. sydowi*, *Eurotium amstelodami,* and less often by *Penicillium*, *Bipolaris*, *Chaetomium*, and *Emericella* genera. Sterigmatocystin was reported as a fungal metabolite in moldy wheat, rice, barley, rapeseed, peanut, corn, and cheeses or salami. Sterigmatocystin is carcinogenic in mice (pulmonary adenocarcinomas) and rats (hepatocellular carcinomas at milligram doses of sterigmatocystin) following oral administration and is classified as an International Agency for Research on Cancer class 2B carcinogen (i.e., as possibly carcinogenic to humans). The toxicity of sterigmatocystin refers primarily to liver and kidney. Oral dosing of monkeys with 20 mg/kg of body weight once each fortnight for 4 to 6 months resulted in chronic hepatitis; after a 12-month exposure, hyperplastic liver nodules were observed.



Sterigmatocystin

Ergot

Ergot disease is caused by a fungus *Claviceps purpurea* that contaminates cereal crops such as rye and wheat and that produces substances (alkaloids) called ergotamines. Ergotamines constrict blood vessels and cause the muscle of the uterus to contract. They have been much used for the treatment of migraines. They have also been used and misused to induce abortion. Ergotamines can also cause symptoms such as hallucinations, severe gastrointestinal upset, a type of dry gangrene, and a painful burning sensation in the limbs and extremities. Chronic ergot poisoning (ergotism) was rife during the middle ages due to the consumption of contaminated rye. Because of the burning pain, it was known as ignis sacer (holy fire) and ignis infernalis (hell's fire), and was one of the causes of St. Anthony's fire. A form of ergot was also the original basis for the illicit drug lysergic acid diethylamide.



Ergotamine toxin

Influence of mycotoxins on human and animals

Mycotoxins enter human and animal dietary systems mainly through ingestion, but increasing evidence also points to inhalation as another entry route. Mycotoxins exhibit a wide array of biological effects including:

• Carcinogenic compounds such as aflatoxins, ochratoxins, fumonisins and patulin

• Mutagenic compounds such as aflatoxins and sterigmatocystin.

• Hematopoietic compounds such as aflatoxins and trichothecenes. Hemotopoiesis refers to the production of all types of blood cells from the primitive cells stem cells in the bone marrow. The toxic effect in cereals function of hematopoiesis leads firstly to the decrease in the number of neutrophils, thus perturbing the animal's immune system and subsequently to the decrease in red blood cells, which leads to anemia.

• Hepatotoxic compounds such as aflatoxins, ochratoxins and fumonisins. All of them induce significant liver damage in animals.

• Nephrotoxigenic compounds such as ochratoxins, citrinin, trichothecenes, and fumonisins. All of them induce significant kidney damage in animals.

• Teratogenic compounds such as aflatoxin B1, ochratoxin A, T-2 toxin, sterigmatocystin and zearalenone. These toxins affect on developing cells and tissues to initiate abnormal embryogenesis in animals.

• Oestrogenic compounds such as zearalenone. This *Fusarium* toxin affects the reproductive capacity of animals due to its negative effect on estrogens hormones in female animals.

• Neurotoxic compounds such as ergot alkaloids, fumonisins, deoxynivalenol. The effects of these mycotoxins are best evidenced by vomiting and taste aversion produced by DON, seizures, focal malata and liquefaction of the brain tissue, possibly mediated by sphingolipid synthesis under the influence of fumonisins, and other neural effects of ergot alkaloids activity resulting from the effects of the metabolite slaframine for selected receptors in the nervous system.

• Immunosupresive compounds: The predominant mycotoxins in this regard are aflatoxins, trichothecenes, and ochratoxin A. However, several other mycotoxins such as fumonisins, zearalenone, patulin, citrinin and ergot alkaloids have been shown to have some effects on the immune system.

Current EU regulations concerning mycotoxins

Since the discovery of aflatoxins in the 1960s, regulations have been established in many countries to protect consumers from harmful mycotoxins that can contaminate foods. Maximum levels of mycotoxins have been established by the European Commission after consultations with the Scientific Committee for Food, based on the analysis of scientific data collected by EFSA and the Codex Alimentarius. These data include:

• Toxicological properties of mycotoxins.

• Mycotoxin dietary exposure.

• Distribution of concentrations of mycotoxins in raw materials or a product batch.

• Availability of analytical methods.

• Regulations in other countries with which trade contacts exist.

The first two factors provide the information necessary for risk assessment and exposure assessment, respectively.

The third and fourth factors are important factors in enabling the practical enforcement of mycotoxins, through appropriate procedures as regards sampling and analysis. The last factor is the only one economic in nature, but it is equally important in decision-making to establish reasonable rules and restrictions for mycotoxins in foods and feeds.

According to the Commission Regulations, the maximum levels should be set at a strict level, which is reasonably achievable by following good agricultural and manufacturing practices and taking into account the risk related to the consumption of food. Health protection of infants and young children requires establishing the lowest maximum levels, which is achievable through the selection of raw materials used for the manufacturing of foods for this vulnerable group of consumers. Development of international trade, progress in research focused on mycotoxin food contamination and their toxicological properties cause changes in the mycotoxin-related legislation cross the European Union. There have also been established maximum levels for aflatoxins, ochratoxin A, patulin, and *Fusarium* toxin (fumonisin, deoxynivalenol, zearalenone) in different products: nuts, cereals, dried fruit, unprocessed cereals, processed cereal-based food, coffee, wine, spices, and liquorices. The number of countries that have regulations concerning mycotoxins is continuously increasing, and at least 100 countries are known to have founded specific limits for different combinations of mycotoxins and commodities, often accompanied by the prescribed or recommended procedures for sampling and analysis.

Prevention strategies of exposure to mycotoxins

Good Manufacturing Practice (GMP); a complementary management system to consider in the future is the use of Hazard Analysis Critical Control Point (HACCP). Recommendations to be taken into account before the harvest in order to reduce the risk of mould contamination and mycotoxin production include:

- Use certified seed or ensure it is free from fungal infections.
- Avoid drought stress – irrigate if possible.
- Sow the seed as early as possible, so that crop matures early.
- When practicing minimum or zero tillage, remove crop residues.
- Weed regularly.
- Control insect and bird pests.
- Rotate crops.
- Avoid nutrient stress – apply the appropriate amount of organic or inorganic fertilizer.
- Plant resistant varieties where these are available.

The main mycotoxin hazards associated with pre-harvest in Europe are the toxins that are produced by fungi belonging to the genus *Fusarium* in the growing crops. It is important to note that although *Fusarium* infection is generally considered to be a pre-harvest problem, it is certainly possible for poor drying practices to lead to crops' susceptibility in storage and mycotoxin contamination. Appropriate to reduce the prevalence of fungi belonging to the genus *Fusarium* and their mycotoxins.

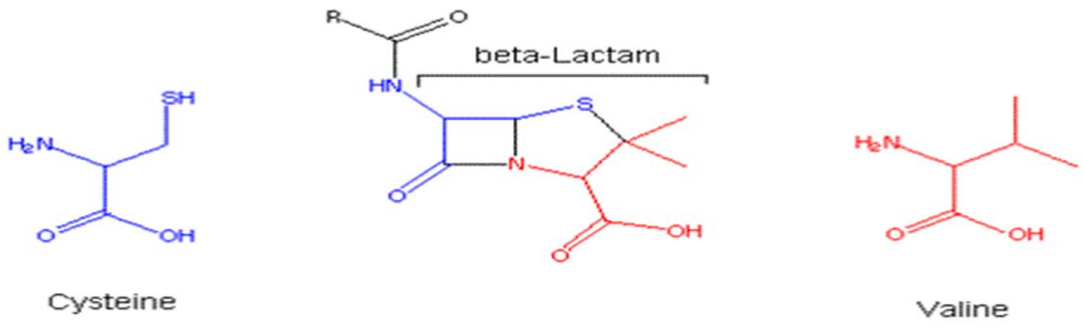
Antibiotics

Antibiotics are produced by fermentation. The process may take a few days to obtain an extractable amount of product. Antibiotic production is done by the

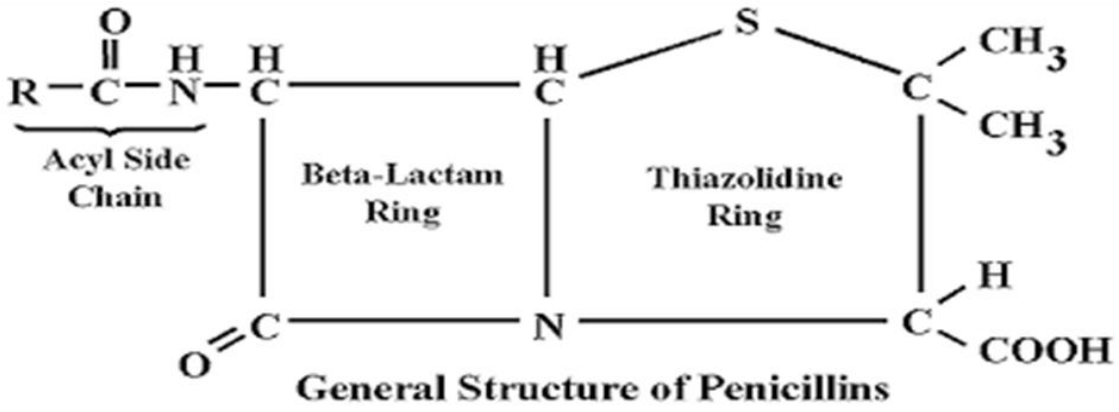
batch process. Oxygen transport is the major concern; therefor sufficient polymeric sugar and protein with a trace amount of elemental growth factors are used to enhance production. An anti-biogram test is used to observe the amount of antimicrobial agent in the fermentation broth. A bioassay determines the activity unit of the bactericides.

Biosynthesis of b-lactam antibiotics

β-lactam antibiotics are produced by fungi, some ascomycetes, and several actinomycete bacteria. Pencillins are synthesized from two amino acids (valine and cysteine).



Biosynthesis of β-lactam antibiotics



Penicillin

Penicillium notatum, was isolated by Alexander Fleming in 1928 as a contaminant of other organism culture. Then, *P. chrysogenum* are used as penicillin-producing strains. The chemotherapeutic agent obtained from secondary metabolites of living cells is known as ‘antibiotic’. The terms ‘antibiotic’ and ‘antibiosis’ were used in the early nineteenth century when Alexander Fleming in 1930s accidentally found a mold as a contaminated culture on Petri dish agar while he was cultivating microorganisms. Fleming was culturing *Staphylococcus aureus* on Petri dish with a thin agar layer. His cultured plate was contaminated with a mold. The amazing part of his work was that he found no microbial growth within a radius of 3–5 cm of the mold. Normally, any contaminated cultures are taken out of the investigation cycle, but Fleming was curious and decided to follow up his investigations. He wanted to know why there was no growth in presence of the mold. He needed to know what the toxic metabolite was that killed the organisms in the neighborhood of the mold. He found that the cell metabolites were able to lyses and dissolve the cell wall of the microorganisms. He identified the contaminants as *Penicillium* sp. Fleming named the drug penicillin, which was isolated from *Penicillium notatum*. Fleming had discovered a new antibiotic, and for his great contribution he was awarded the Nobel Prize, in the field of medicine and physiology in 1945. His discovery was the answer to the question generated in his mind, which was why the colony of *Staphylococcus* did not grow in presence of *Penicillium notatum*. The commercial production of penicillin and other antibiotics is the most dramatic example of industrial microbiology. The mould isolated by Fleming was *Penicillium notatum*. He noted that it killed his culture of *Staphylococcus aureus*. Production of penicillin has been improved by a better antibiotic-producing mold species, *Penicillium chrysogenum*. Development of submerged culture

techniques has enhanced the cultivation of the mold in large-scale operations using sterile air supply.

• Streptomycin is produced by *Actinomycetes.*

• Molasses, corn steep liquor, waste product from the sugar industry and wet milling corn are used for the production of penicillin.

• *Penicillium chrysogenum* can produce 1000 times more penicillin than Fleming's original culture. The major steps in the commercial production of penicillin are as follows.

(1) Preparation of inoculum.

(2) Preparation and sterilization of medium.

(3) Inoculation of the medium in the fermenter.

(4) Forced aeration with sterile air during incubation.

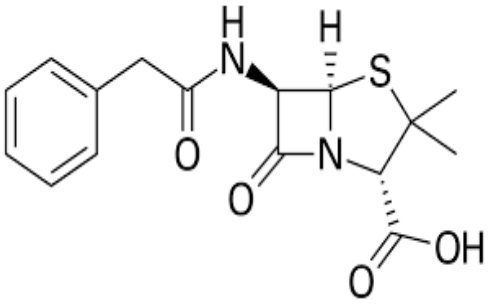
(5) Removal of mould mycelium after fermentation.

(6) Extraction and purification of the penicillin.

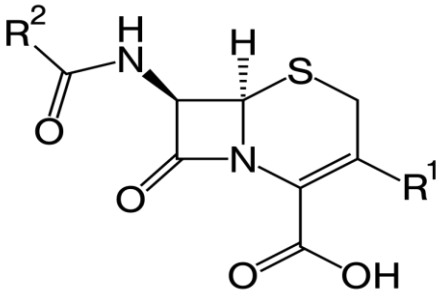
Production of Penicillin

There is only one choice for the antibiotic production process: the synthesis of benzylpenicillin (penicillin G, originally known as 'penicillin'). This, the most renowned antibiotic and the first one have been manufactured in bulk, is still universally prescribed. Although originally made by surface liquid culture, penicillin G is now produced by air-lift fermentation under aerated conditions. Penicillin G is not a typical fermentative antibiotic. It is made by a fungus, *Penicillium chrysogenum.* The antibiotics from fungal sources include penicillin G and V and cephalosporin C. These three antibiotics are the major starting materials for the semi-synthetic β-lactam antibiotics. The systemic antifungal antibiotic griseofulvine is also of fungal origin. Most antibiotics are produced by fermentation using bacteria, including streptomycin and the tetracycline family among numerous others. Streptomycin is the next important antibiotic after

penicillin G to be made available to the clinician. It has played an important role in fighting tuberculosis.



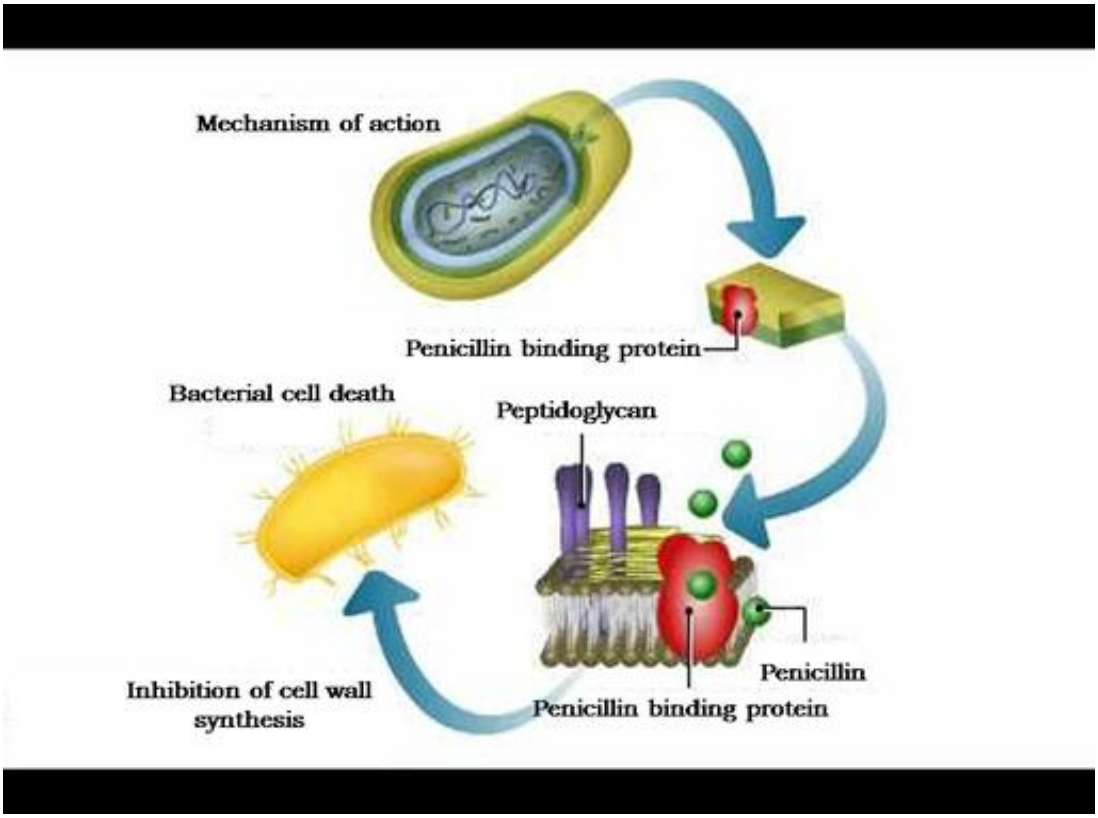
Penicillin G



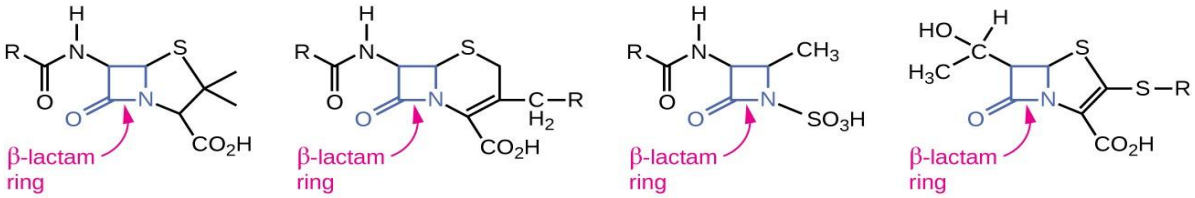
Cephalosporin C

Mode of action of penicillin

Penicillin has an interesting mode of action: it prevents the cross-linking of small peptide chains in peptidoglycan, the main cell wall polymer of bacteria. Pre-existing cells are unaffected, but all newly produced cells are abnormally grown. The newborn cells are unable to maintain their wall rigidity, and they are susceptible to osmotic lysis.



Mode of action of penicillin on bacterial cell wall



penicillin | cephalosporin | monobactam | carbapenem

R group	—CH_2—	CH_2—O—	—CH(—NH_2)—	—CH(—NH_2)—OH	CH_3O, CH_3O
Drug name	penicillin G	penicillin V	ampicillin	amoxicillin	methicillin
Spectrum of activity	G+ and a few G−	similar to penicillin G	G+ and more G− than penicillin	similar to ampicillin	G+ only, including β-lactamase producers
Route of administration	parenteral	oral	parenteral and oral	oral (better than ampicillin)	parenteral

Practical part

Experiment 1

Isolation of fungi from different sources

1- Preparation and sterilization of culture media

2- Isolation of fungi

3- Identification of fungi

4- Preservation of fungi

Table 1. Counts of fungi isolated from different sources

Sources				
Fungi				

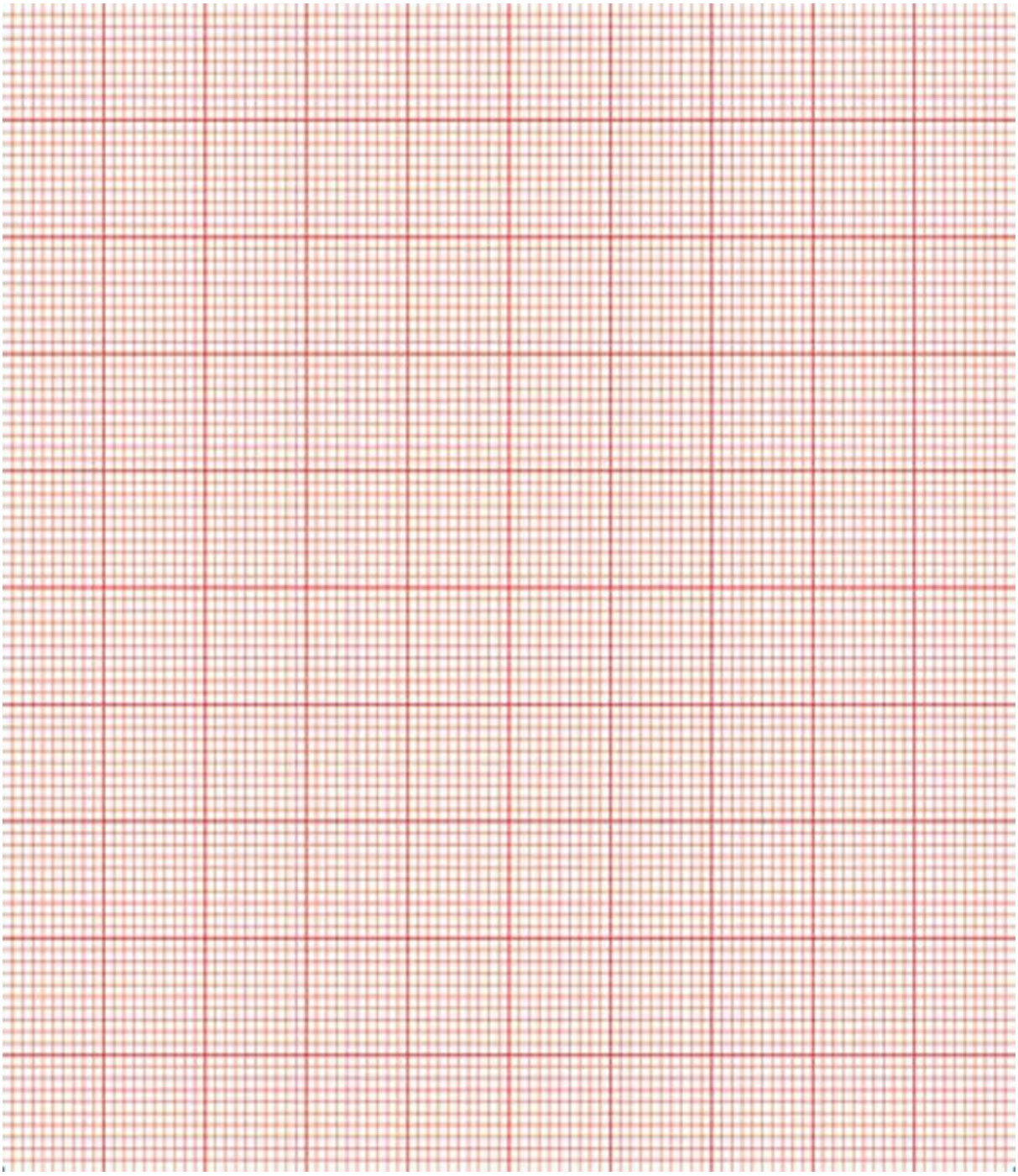
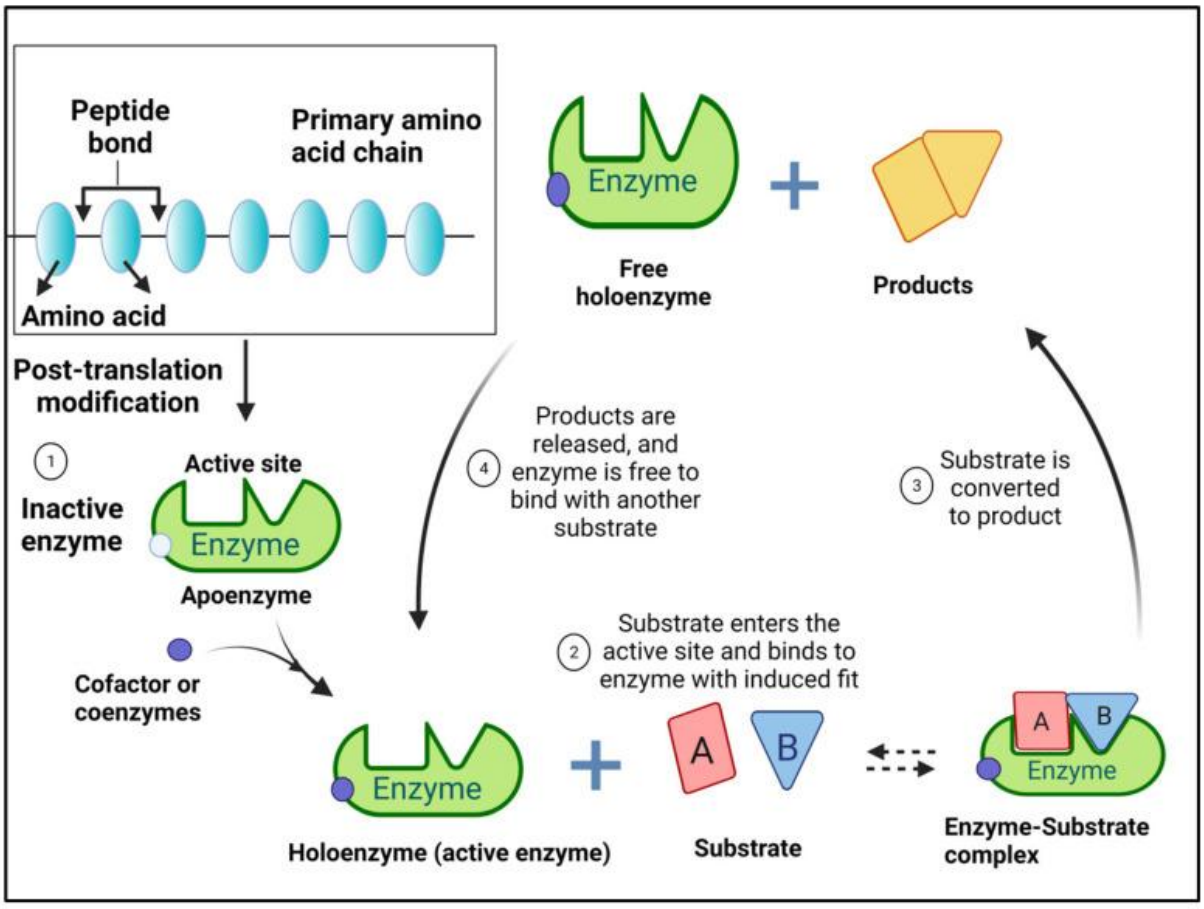


Figure 1. Fungi produced from different natural sources

Experiment 2

Fungal enzymes

Enzymes have played an important role in different types of biological systems for various applications. They are proteins that break down and convert complicated compounds to simple products. Fungal enzymes are compatible, efficient, and proper products for many purposes such as medicinal uses, industrial processing, bioremediation process, and agricultural applications. Fungal enzymes have been used in many industries, including baking, brewing, cheese making, antibiotics production, and commodities manufacturing, such as linen and leather. Furthermore, they also are used in other fields such as paper production, detergent, the textile industry, and in drinks and food technology in products manufacturing ranging from tea and coffee to fruit juice and wine. Recently, fungi have been used for the production of more than 50% of the needed enzymes. Fungi can produce different types of enzymes extracellularly, which gives a great chance for producing in large amounts with low cost and easy viability in purified forms using simple purification methods. Hydrolases are the most extensively studied groups of enzymes; they catalyze the hydrolysis of their substrate through the addition of water. Hydrolases represent the most commercially marketed enzymes due to their wide application in different industrial sectors. Fungal amylases, proteases, lipases, and cellulases represent the most commercially demanded enzymes.



Schematic illustration for enzyme structure, activation, and steps of enzyme and substrate interaction.

Amylases

Amylase enzymes are used for commercial application and was firstly applied medicinally in treating digestive disorders. Amylases could be classified into α, β, and γ-Amylases depending on the attaching site in the starch molecules and the nature of the resulting products. α-Amylases are calcium-dependent metalloenzymes that act randomly on the starchy substrates yielding maltose and maltotriose from amylose or glucose and dextrin from amylopectin. β-Amylases hydrolyze 1,4-glycosidic bonds in the carbohydrate chain, yielding one maltose unit. They are extensively important in plants, especially in the seed ripping process, but they are also reported from the microbial origin. γ-Amylases resemble the other two types of amylases in hydrolysis activity toward 1,4-glycosidic linkages, unlike the two forms characterized with 1,6-

glycosidic linkages hydrolysis activity and preferring acidic environment pH 3. *Aspergillus niger* is considered the potent commercial α-Amylase producer among all filamentous fungi. Many other fungi were reported for their capacity to produce different types of amylases, including *Aspergillus oryzae, A. terreus*, *Fusarium solani*, and *Penicillium citrinum*.

Lipases

Lipases are a group of hydrolytic enzymes that act by hydrolysis of triacylglycerol yielding fatty acid and glycerol. Lipases also catalyze the reverse reaction by esterification of glycerol and fatty acid. Fungal lipases are produced by several fungi including *Aspergillus niger*, *Penicillium verrucosum*, *Fusarium solani*, *Arthrographis curvata*, and *Rhodosporidium babjevae*. Lipases are implemented in vast commercial applications, including detergents and cosmetics additives, fine chemical production, medical application, paper pitching, leather de-fating, wastewater treatment, and biodiesel production. The application of lipase in biodiesel production, as an ecofriendly alternative for traditional fuel, intensifies the research in diminishing the production cost and enhancing the enzyme efficiency.

Proteases

Proteases play an important role in fungal physiology to digest extracellular large peptides and also in defense mechanisms against attaching pathogens. Based upon the amino acid in the enzyme active site, proteases could be categorized into different types, including serine, asparagine, cysteine, aspartic, and metalloproteases. Serine and metalloprotease are the most studied types among all proteases and are usually produced from microbial origins. Filamentous fungi, especially that of *Aspergillus* sp. are characterized by their high capacity for protease production. Other fungal genera also reported for their potency regarding proteases production, including *Penicillium* sp., *Fusarium* sp., and *Pichia farinosa*.

Cellulases

Cellulose, hemicellulose, and lignin are the main components of most agricultural wastes. Most fungi have the complete enzymatic system (Endoglucanases, Cellobiohydrolases, β-glucosidases, and Xylanases) to degrade this complex cellulosic material for nutrition. *Trichoderma reesei* is widely applied for the commercial production of cellulases, other fungi also represent potent cellulase producers, including *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis*. Xylan, a complex polysaccharide, is also a major component of hemicellulose; hence, xylanases play an important role in the efficient hydrolysis of plant cellulolytic material. Regarding the diverse and complex structure of Xylan, its hydrolysis required a group of synergistically working enzymes (xylanolytic system) for complete degradation. Filamentous fungi are characterized by the required xylanolytic system for complete xylan degradation, especially that of *Trichoderma reesei*. *Aspergillus oryzae*, and *Aspergillus flavus*.

Detection of amylase produced by fungi

Procedures

1- Preparation of culture media for amylase production and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature and pH

3- At the end of the incubation period, filtration of cultures and assay for the enzyme activity.

4- Illustrate the data and write a comment.

Table 2. Amylase activity produced by fungi

Fungi	Amylase activity

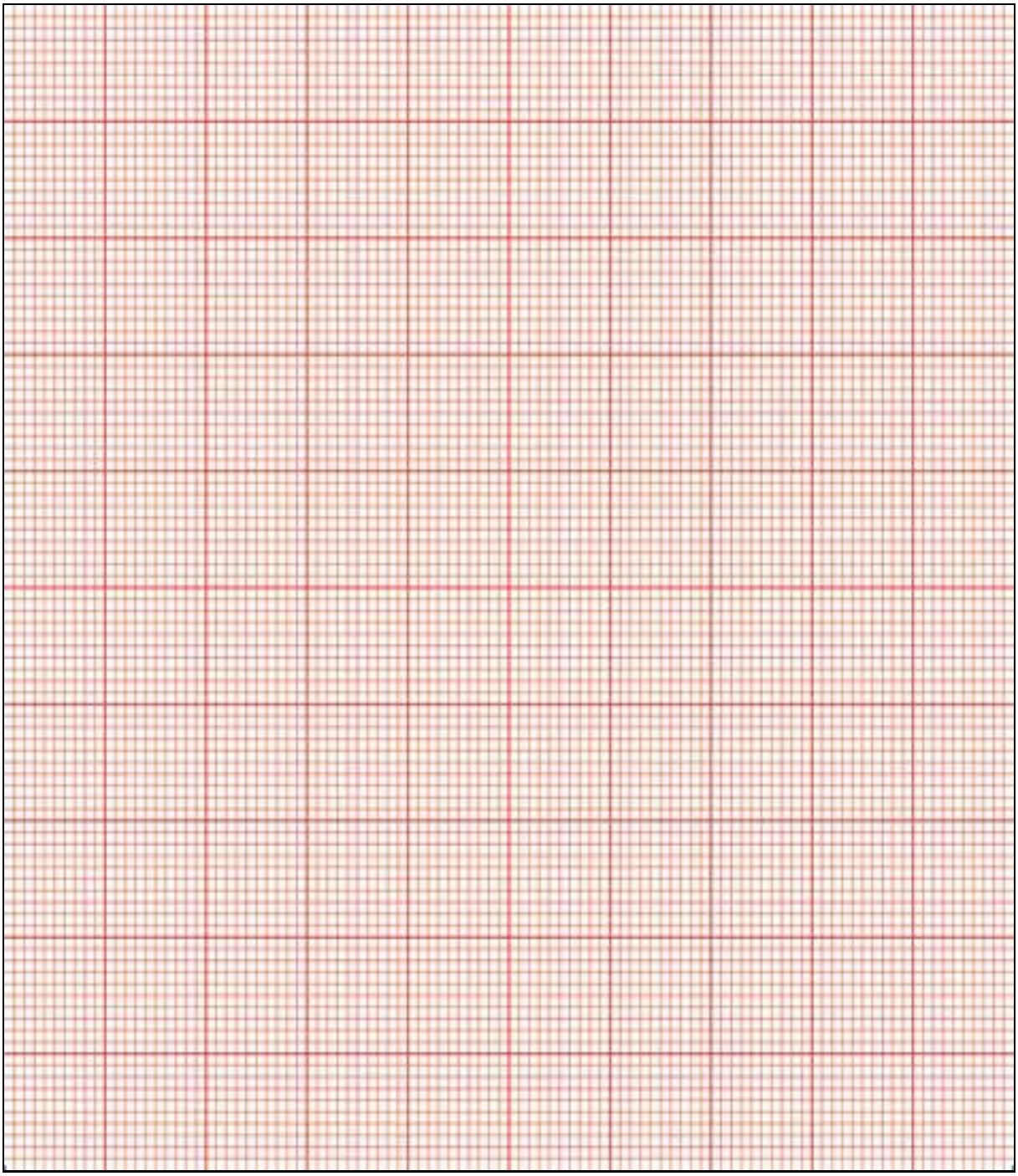


Figure 2: Amylase activity of fungi

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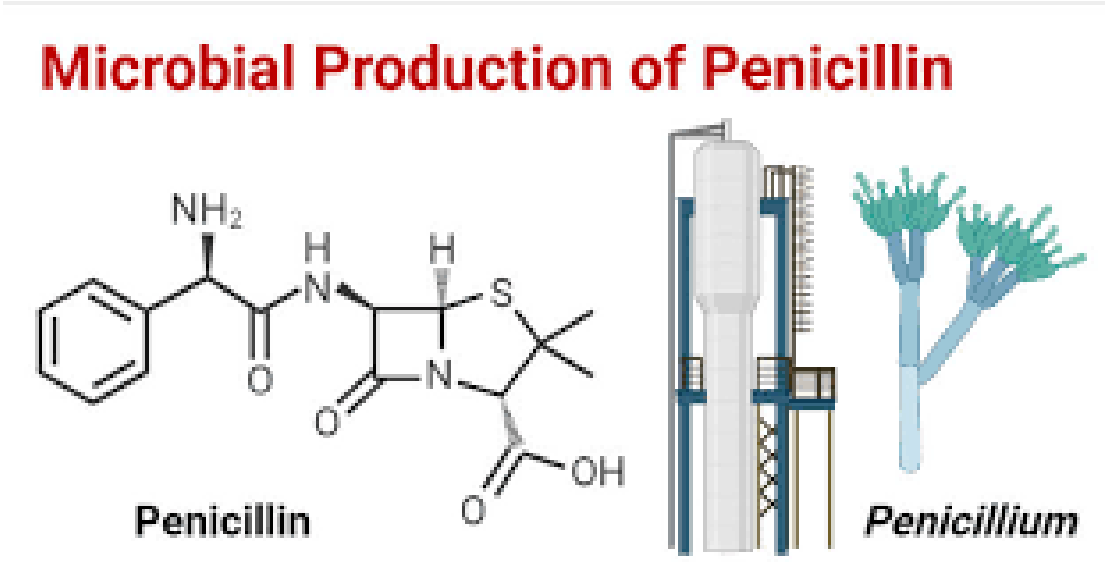
Experiment 3

Antifungal agents

A wide range of antifungal agents are used in combating biodeterioration and in preventing or treating fungal diseases of plants. In these contexts, they are commonly referred to as **fungicides**. Others are used for treating disease in animals and man, and are simply referred to as **antifungal agents.** Antimicrobial agents produced by means of a microbial fermentation, called **antibiotics**, by the plant on which the mold is growing, or added as biocides during crop management, are other factors interacting with the growth and metabolism of a mold. Antifungal agents differ widely in their chemical nature and in their properties and mode of action (Carlile and Watkinson, 1996). The effect of pesticides is interesting as they are largely used to control several diseases in plants. The correct use of fungicides to diminish fungal mycoflora could lead to a diminution in the amount of mycotoxins produced. But certain number of studies showed that the use of sub-lethal concentration could favour the production of the toxins (Moss and Frank, 1987). It is also possible that the pesticide decreases the synthesis of the mycotoxins without affecting the fungal growth (Draughton and Ayres, 1978, 1982).



Production of Penicillin by *Penicillium chrysogenum*

Procedures

1- Cultivation of fungi on a suitable media for penicillin production.

2- Incubation of cultures at suitable conditions (Temperature, pH, etc.).

3- At the end of the incubation period, filtration of cultures.

4- Extraction of penicillin using a suitable solvent.

5- Collection of solvent with the antibiotic.

6- Concentration of solvent by rotary evaporation.

7- Collection of solvent and dissolve of penicillin in methanol.

8- Analysis of penicillin for detection and concentration by TLC, HPLC, etc.

9- Illustrate the data and write a comment.

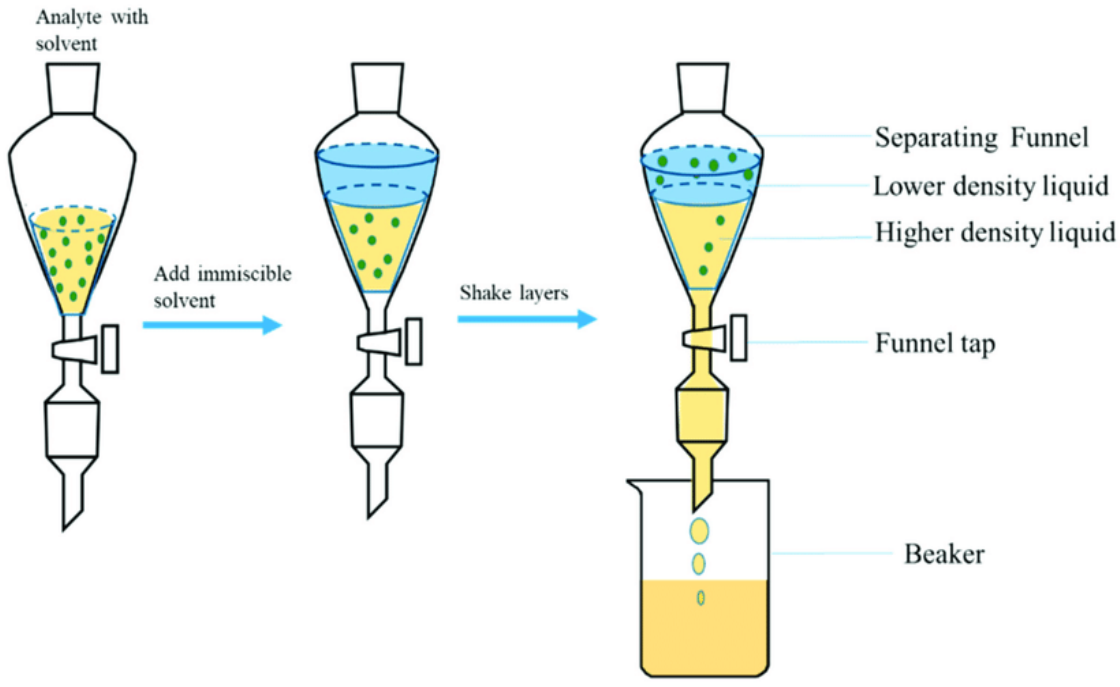


Table 3:……..…

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Penicillium strains	Penicillin activity
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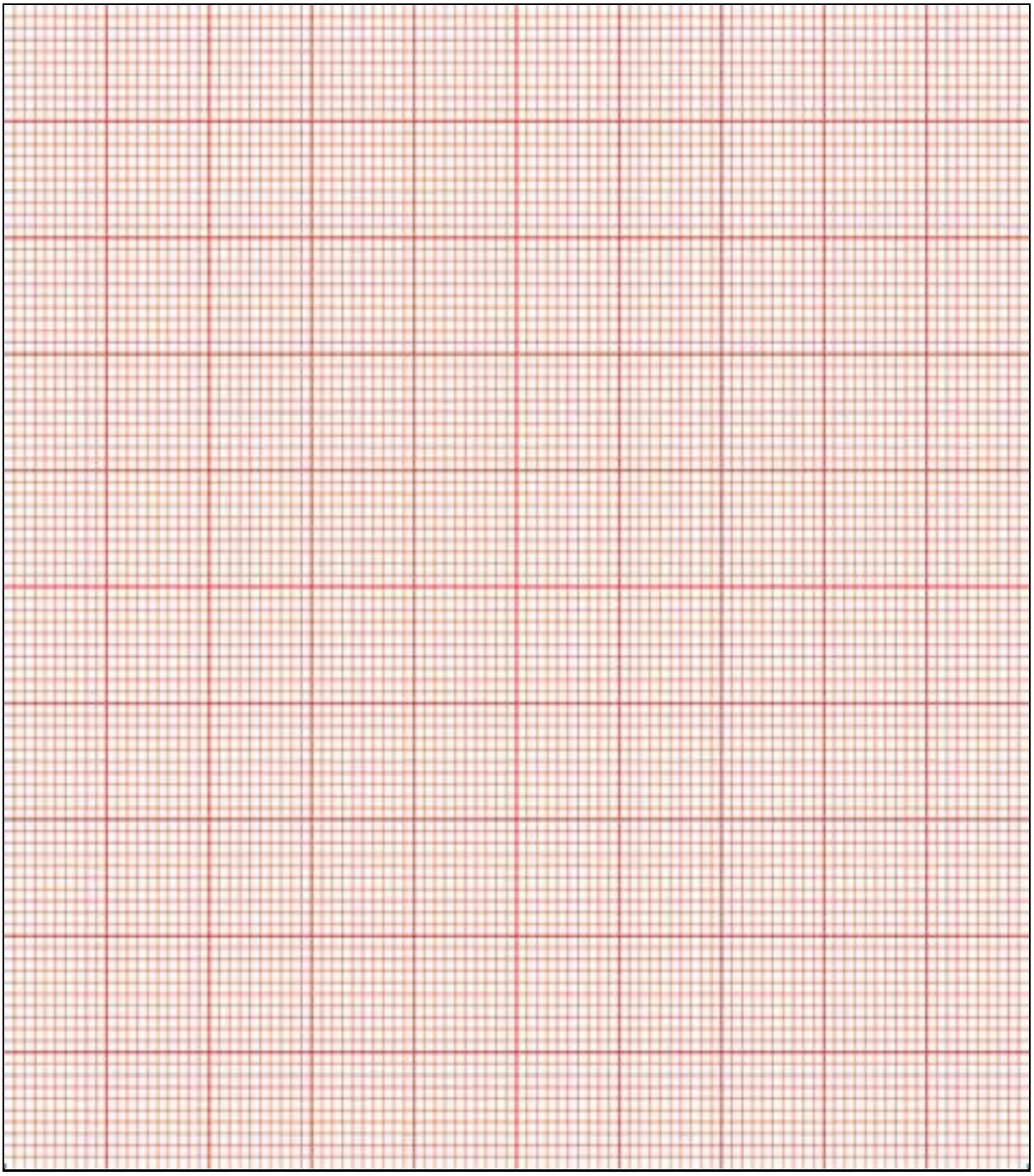

Figure 3: ……..

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Experiment 4

Methods for the analysis of antibiotics

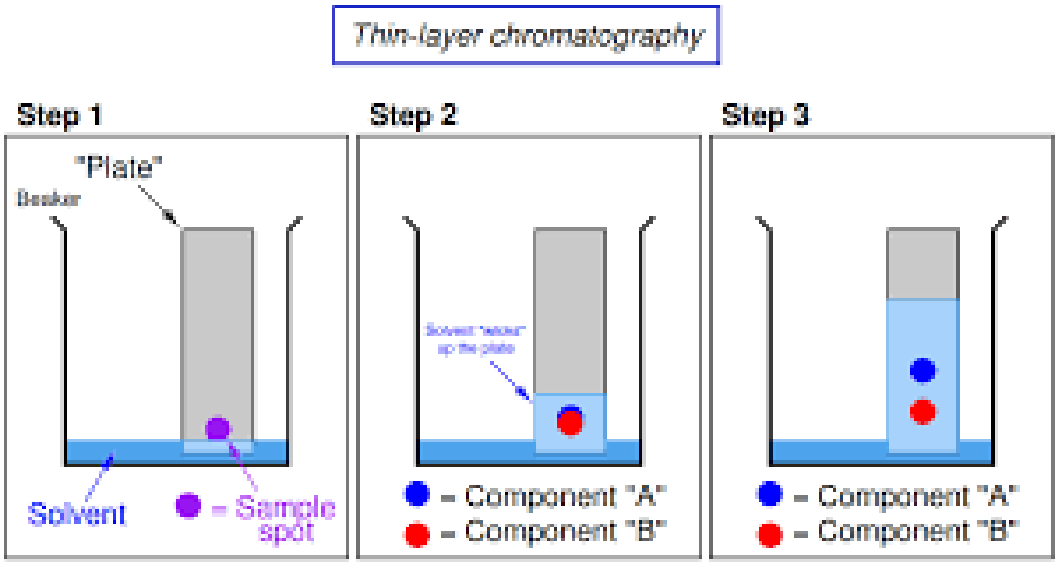
The different stages involved in the analytical process of antibiotics detection are sampling, extraction, clean-up, separation, detection and confirmation. Although many interfering compounds may be partially removed during the extraction sequence, further clean-up of the extract is normally necessary. The traditional clean-up systems generally involved either solvent portioning and/or open column chromatography on silica adsorbent. The development of solid phase extraction (SPE) cartridges containing packing with various surface chemistries allowed more rapid and efficient clean-up process. However, the introduction of the immunoaffinity columns (IAC) in which specific antibodies are bound to a solid matrix, has allowed an even more specific clean-up process. Classical analytical separation methods for antibiotics include TLC, HPLC, gas chromatography (GC) and MS. Mass spectrometry offers the ideal confirmatory technique via the detection of molecular ions at specific chromatographic retention times and via the generation of a compound specific fragmentation pattern.

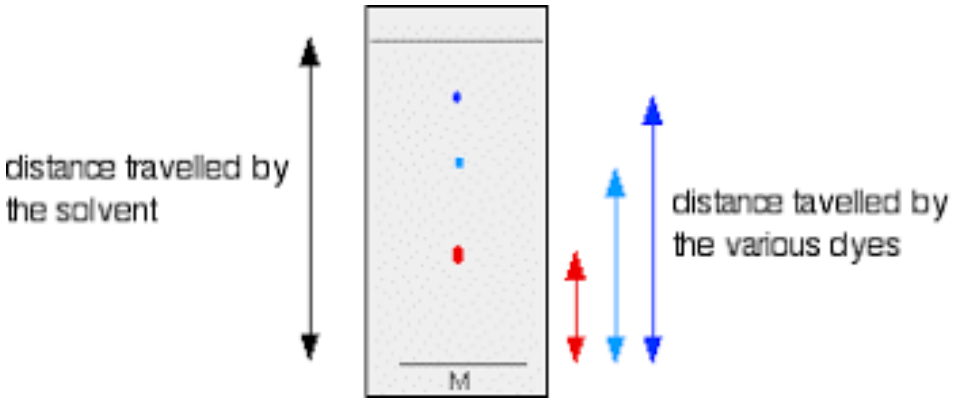
Experiment 4

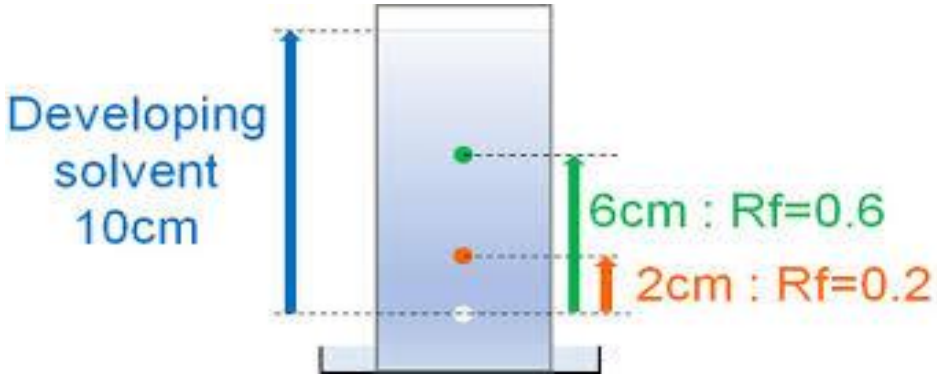
Thin Layer Chromatography (TLC) analysis

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analyzed by TLC. TLC consists of three steps: spotting, development, and visualization. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipet to transfer a small amount of the dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic or glass sheet. The spotting solvent quickly evaporates and leaves behind a small spot of

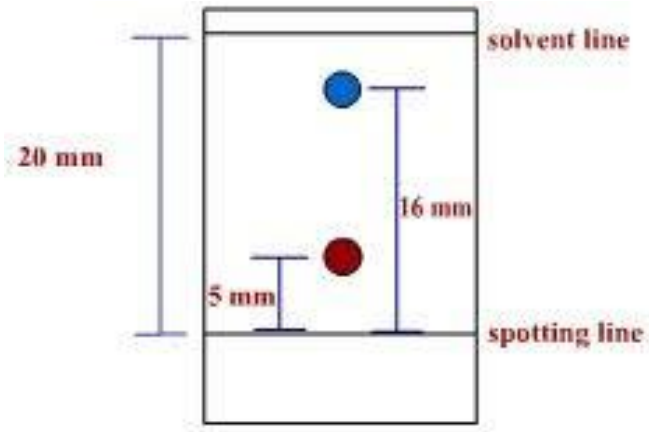
the material. Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate. Visualization of colored compounds is simple–the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. It is good practice to use at least two visualization techniques in case a compound does not show up with one particular method. The Rf value is used to quantify the movement of the materials along the plate. Rf is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always between zero and one.

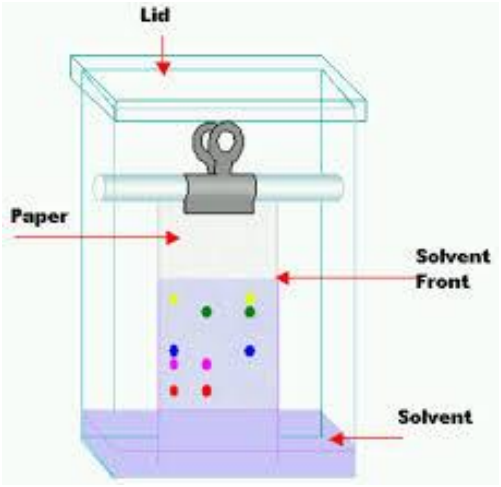






$$Rf = \frac{\text{distance moved by the compound}}{\text{distance moved by the solvent}}$$





Thin Layer Chromatography (TLC) is a technique used to separate mixtures of compounds. This technique can be performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different compounds ascend the TLC plate at different rates, separation is achieved. The ratio of the distance the compound travels to the distance the solvent travels is called the Rf value. The symbol Rf stands for "retardation factor" or "ratio-to-front". It is expressed as a decimal fraction. When the conditions are duplicated, the same average relative positions will turn up for the solvent and solute; thus the Rf value is a constant for a given compound. The Rf value is a physical property for that compound. The Rf value is useful in identifying compounds, but other properties should be used in combination with the Rf value to confirm compound identification.

Procedure for TLC analysis

1. Obtain an 18.5 x 8 cm square of TLC paper. Using a pencil, draw a line ~1 cm from the edge of bottom side of the plate.

2. Make pencil dots along this line. Underneath each dot, label the color of the sample you will test on that spot.

3. Place a few drops of each color on a spot plate. Dip a toothpick into a color and dab the color onto the pencil dot for that color. Use a clean toothpick for each color. Try to keep each dot as small as possible.

4. Allow the plate to dry, then go back and add more color to each dot, a total of three times, so you have lots of pigment in each sample.

5. When the plate is dry, insert it into a cylinder containing solvent.

6. The compounds will separate.

7. Using a pencil, mark the height in which the solvent travelled on the plate.

8. When the plate is dry measure the height of the solvent traveled as well as the height of compounds that traveled.

9. Determine which colors were present in each mixture. Calculate the Rf factor for each colored compounds.

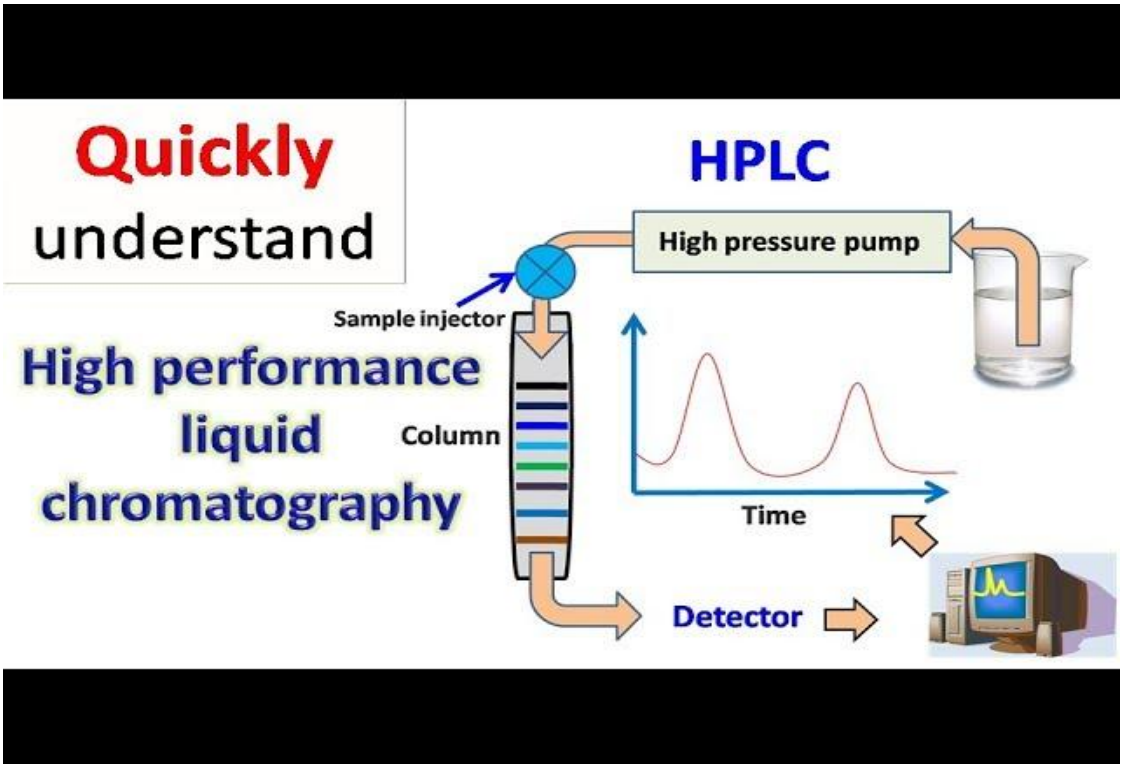
Experiment 5

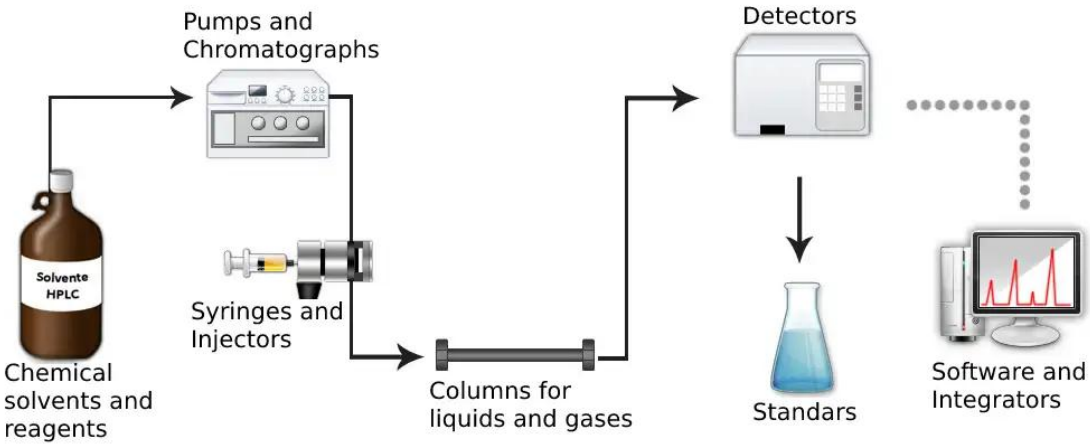
High-performance liquid chromatography (HPLC) analysis

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid

particles (*e.g.*, silica, polymers, etc.), 2–50 μm in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents (*e.g.*, water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

The schematic of an HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in an HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/V is, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature at which the separation is performed.





High-performance liquid chromatography (HPLC)

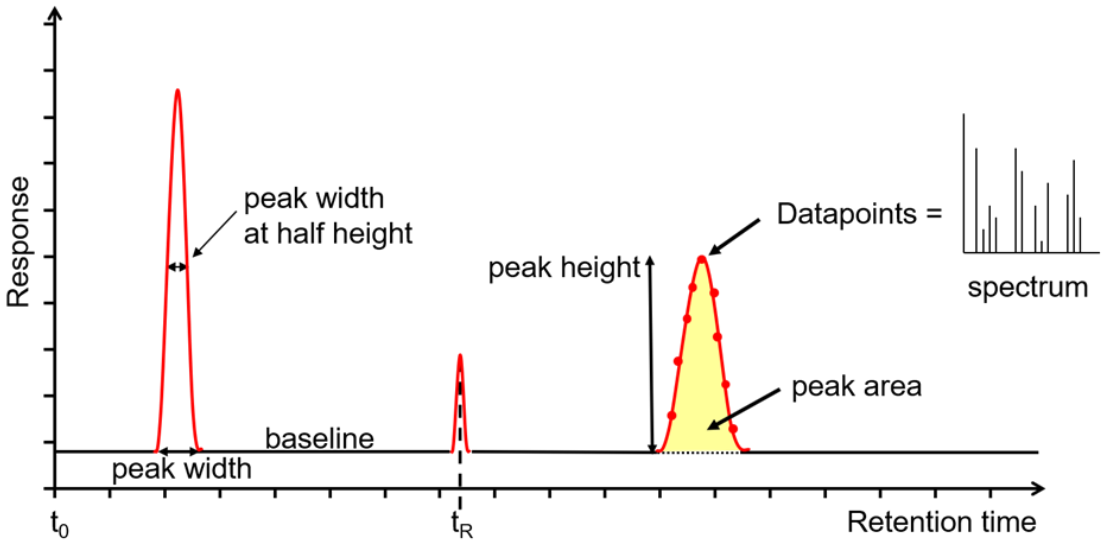
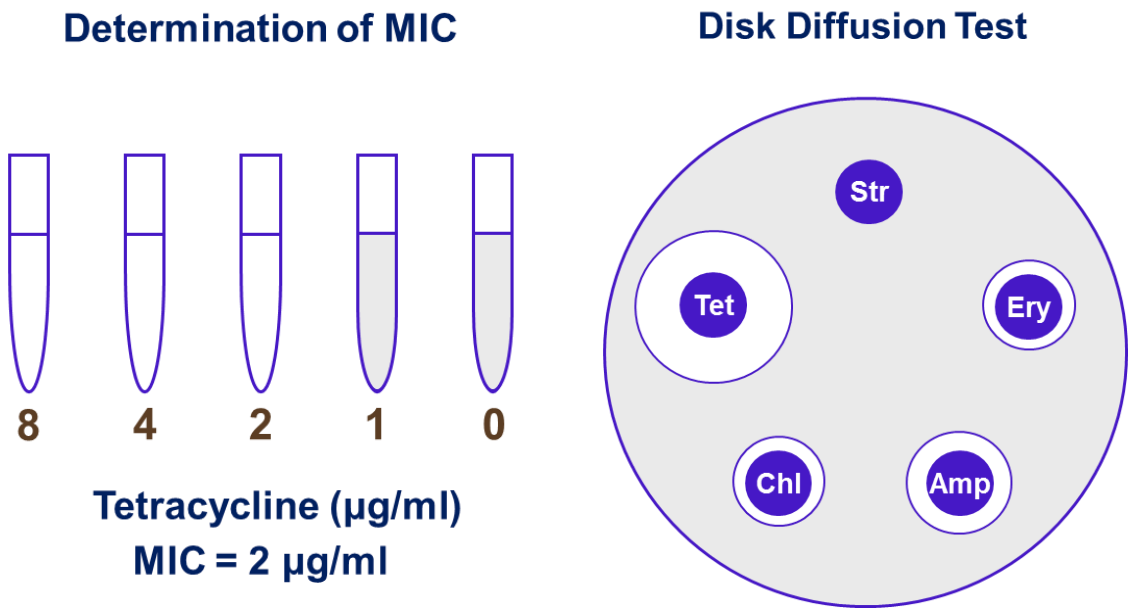
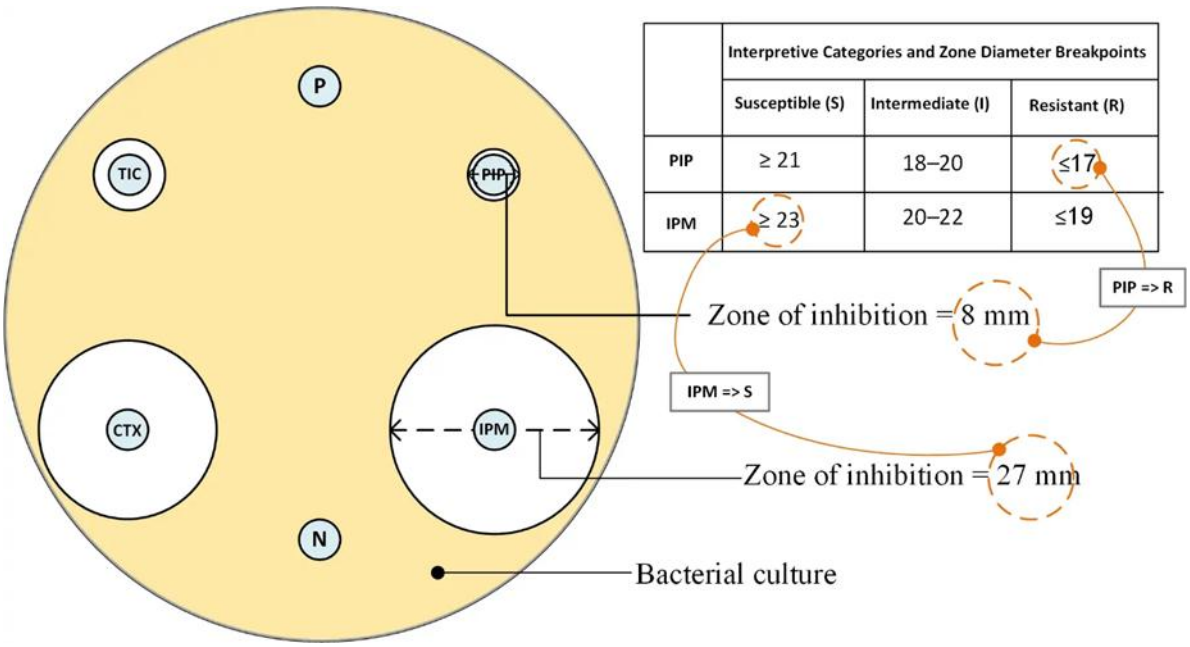


Chart of HPLC analysis

Experiment 6

Susceptibility testing of antibiotics (Minimum inhibitory concentration, MIC).





	Interpretive Categories and Zone Diameter Breakpoints		
	Susceptible (S)	Intermediate (I)	Resistant (R)
PIP	≥ 21	18–20	≤17
IPM	≥ 23	20–22	≤19

PIP => R

Procedures

1- Preparation of suitable medium for antibiotic testing.

2- Cultivation of bacteria.

3- Placed of discs on the Petri-dish and added different antibiotics or different antibiotic concentrations.

4- Incubation of dishes for 24 or 48 hours.

5- Measure the inhibition zones around the discs and calculate the averages.

6- Determine the activity and MIC of the antibiotics.

7- Illustrate the data and write a comment.

Table 4:……..……..…

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Antibiotics	Inhibition zone (mm)	MIC

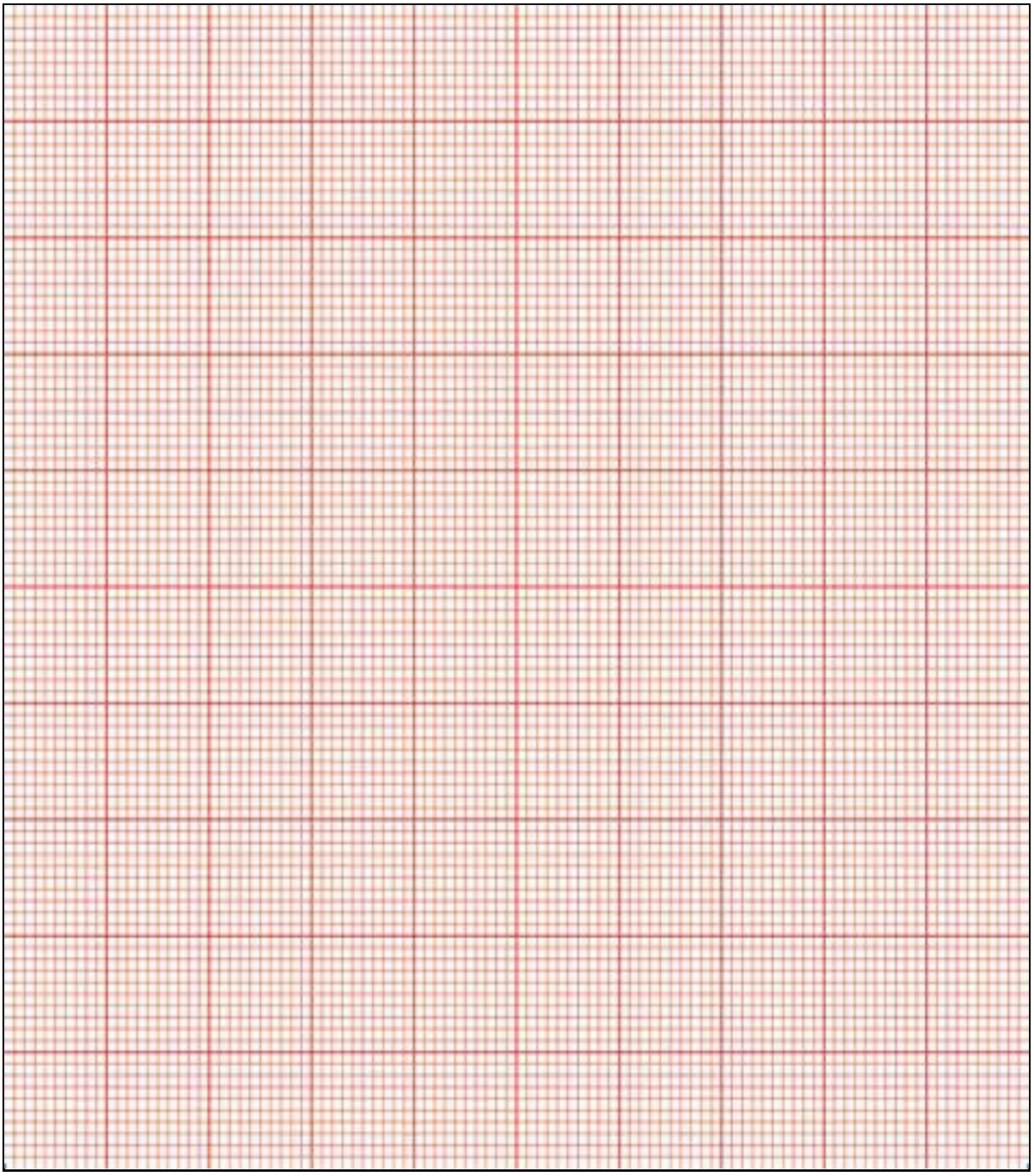

Figure 4: ……..

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Production of antibiotics by fungi

All of the above processes are operated as batch fermentations, in which a volume of sterile medium in a vessel is inoculated. The broth is fermented for a defined period. The tank is then emptied and the products are separated to obtain the antibiotic. The vessel is then recharged for batch operation with medium and the sequence repeated, as often as required. Continuous fermentation is not common practice in the antibiotics industry.

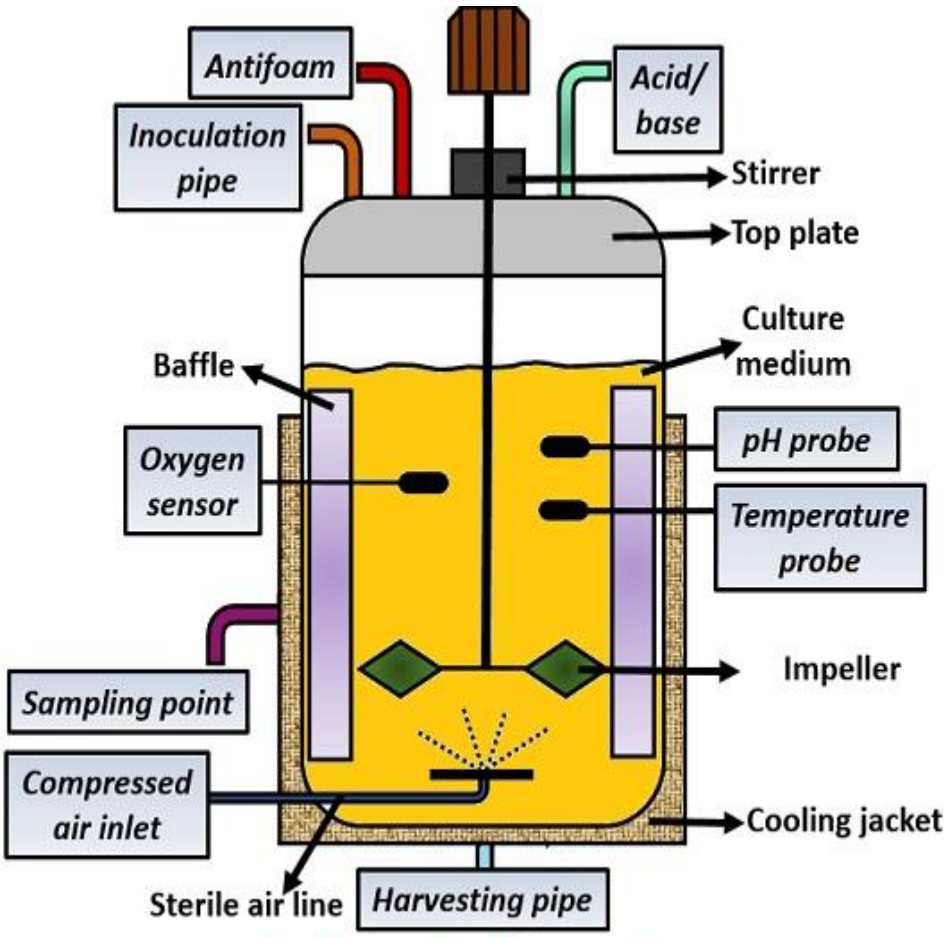
Microorganisms and media

Penicillium chrysogenum is used for these experimental studies. A complex growth medium for *P. chrysogenum* was prepared. The media contained: 20 g sucrose, 10 g lactose, 5 g peptone, 13 g $(NH4)_2SO_4$, 3 g KH_2PO_4, 0.5 g Na_2SO_4, 0.55 g EDTA, 0.25 g $MgSO_4$, 0.05 g $CaCl_2$, 0.25 g Fe_2SO_4, 0.02 g $MnSO_4$, 0.02 g $ZnSO_4$, 0.01 g Na_2MoO_4 and 0.005 g $CuSO_4$ in 1000 ml of distilled water. The media was sterilized in an autoclave at 121°C for 30 min.

Inoculum preparation

Most fungi sporulate on suitable agar media but a large surface area is required to produce sufficient spores. A roll-bottle technique was used to produce spores of *Penicillium chrysogenum*, with 300 ml of media containing 3% agar sterilized in a 1 L cylindrical bottle. After autoclave, the media was cooled at 45°C and rotated on a roller mill so that a layer of agar formed on the cylinder wall. The inoculum was of spore suspension incubated at 24°C for 6–7 days. Submerged culture is common for sporulation of fungi such as *P. chrysogenum*. The sporulation is induced by inoculating 300 ml in a 1 L shaking flask with spores from a well-sporulated agar culture and incubated for sufficient time. At this stage, a 2 L fermenter was inoculated with a pure incoluum (300 ml) and harvested in the fast-growing (logarithmic) phase, so that in the culture media a

high cell density could be obtained. The organism, *P. chrysogenum*, grows in a filamentous (hyphal) form, with branching occurring to a greater or lesser extent. The B. Braun airlift fermenter was used. Pressurized filter air was used to circulate the mycelia in the internal loop pattern. Air was continuously supplied. The bubbles lose oxygen as they rise up the column. At the same time, carbon dioxide and other gaseous metabolites diffuse into the media and are released in the overhead gas compartment. The production of penicillin G is very sensitive to temperature. Heat is generated by the metabolism of nutrients is removed by a well-controlled cooling system. Cooling coils are used for isothermal operation. The fermentation vessel is fitted with several probes to detect foaming, to monitor temperature, to control media level and to record parameters such as pH. The rate of air flow through the fermenter is measured, and the exhaust gases that emerge from the top of the vessel may also be analyzed. Originally all penicillin G was manufactured using lactose in this way, and some manufacturers still prefer this technique.



Design of a fermentor

Calcium, magnesium, phosphates and trace metals added initially are usually sufficient to last throughout the fermentation but the microorganism needs further supplies of nitrogen and sulphur to balance the carbon feed. Nitrogen is often supplied as ammonia gas. Ammonium ions can contribute to pH control, the carbon metabolism being acidogenic and balanced by the alkalinity of the ammonia. Sulphate is usually supplied in common with the sugar feed and the flow adjusted with suitable feed stream ratios. All feed streams are sterilized before being metered into the fermentation vessel. Contaminants resistant to the antibiotic rarely find their way into the fermenter. When they find a way to contaminate media, their effects are so catastrophic that prevention is of paramount importance. A resistant, β-lactamase producing, fast-growing

bacterial contaminant can destroy the penicillin. The contaminants not only consume nutrients intended for the fungus, but also cause loss of pH control and interference with the subsequent extraction process.

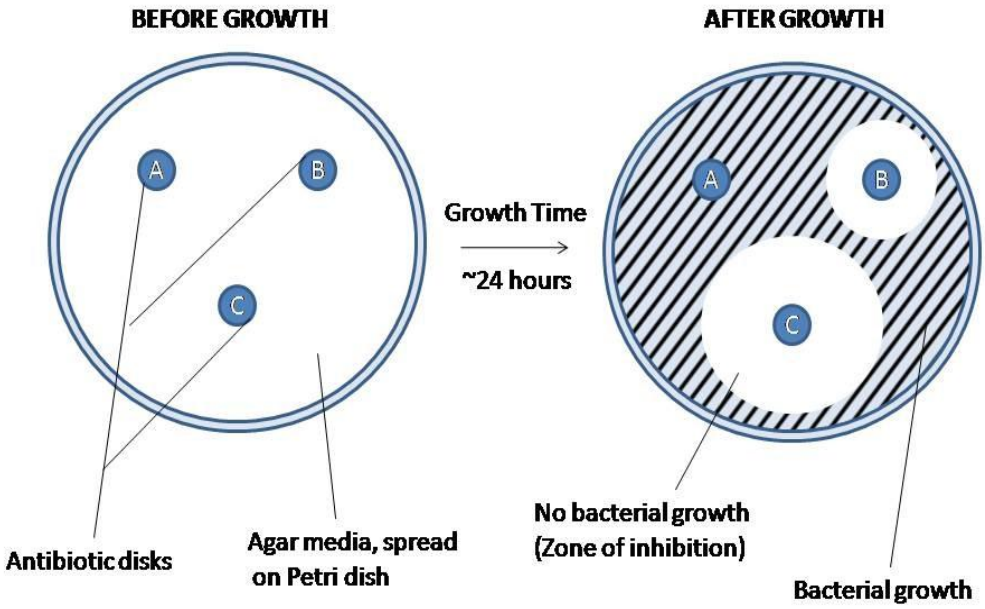
Filtration and extraction of penicillin

At harvesting time, the cells are removed. The penicillin G as the cell product is in the solution that is extracellular with a range of other metabolites and medium constituents. The first step is to remove the cells by filtration. This stage is done under conditions that avoid contamination of filtrate with enzyme, which may destroy antibiotic. The β-lactamase-producing microorganisms could react with the antibiotics, which would cause serious or total loss of product. The next stage is to isolate the penicillin G. Solvent extraction is the generally accepted process. In aqueous solution at pH 2–2.5, there is a high partition coefficient in favour of certain organic solvents such as amyl acetate, butyl acetate and methyl iso-butyl ketone. The extraction has to be done quickly since penicillin G is very unstable at those low pH values. The penicillin is then extracted back into an aqueous buffer at pH 7.5. The partition coefficient now strongly favours the aqueous phase. The solvent is recovered by distillation for re-use.

Analytical method for bioassay and detecting antibiotic

The extracted antibiotic was used in an antibiogram test. Petri dishes of *Bacillus subtilis* ATCC 6633 was cultured for bioassay of penicillin. Small circular paper filters (3–5mm) are placed at different positions on the surface of the agar. The small circular filters were sterilized earlier. A few drops of concentrated and extracted antibiotic were poured on the filter. The small circular filter will hold antibiotic after incubation for 24 hours. There will be a clear circle around the paper filter without any microbial growth. This bioassay is called antibiogram. Normally, an antibiogram is used for clinical purposes to identify a suitable

antibiotic for infected patients. High-performance liquid chromatography (HPLC) is commonly used for quantitative analysis. The carbohydrate concentration is determined by the dinitrosalicylic acid method. Biomass was evaluated by measuring the total solid concentration. Cell dry weight may represent the growth curve in the fermentation broth. The samples were centrifuged at 4500 rpm for 20 min and the sediment washed with distilled water and dried in an oven at 105°C to determine cell dry weight.



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