Physiology of fungi

For 4th year of B.Sc. students

Prepared by

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Culture media

Classification of culture media

Criteria used for classifying culture media includes their chemical composition, physical properties and their use. Every culture medium is designed for a definite use and hence its physical and chemical characteristics depend on its application and function.

I- Classification of culture media according to their use

According to their use culture media are divided into the following types:

1- **Routine laboratory media**: These media contain certain complex raw materials of plant or animal origin such as yeast extract, malt extract, peptone etc., and are employed for routine cultivation and maintenance of a wide variety of fungi.

2- Enriched media: These media are prepared by supplementing the routine laboratory media with some specific substances such as vitamins and amino acids to meet the nutritional requirements of more fastidious of fungi and are employed for their cultivation.

3- Selective media: These media facilitate the isolation of a particular group or species of microorganisms from mixed cultures. Such media contain substances which inhibit microorganisms except the desired group or species, such as mannitol salt agar and tellurite media.

4- Differential media: These media are supplemented with certain reagents or chemicals for differentiating between various kinds of microorganisms on the basis of visible differences in their growth patterns. Such type of media is used more often in bacteriological studies such as eosin methylene blue agar and deoxycholate citrate agar.

5- Assay media: These type of media is specifically employed for the assay of some metabolites such as enzymes, vitamins, amino acids, antibiotics, disinfectants etc., and are of definite composition.

6- Biochemical media: These media are generally used for the differentiation of microorganisms on the basis of their biochemical activities, and are helpful in the study of their metabolic processes.

II- Classification of culture media according to their chemical composition: According to their chemical composition media are classified into the following types:

1- Natural media: The natural medium comprises entirely complex natural products of unknown composition. The raw material of a natural medium may be of plant or animal origin, and some of the common ingredients employed for this purpose include extracts of plant and animal tissues, e.g., fruits, vegetables, egg, milk, blood, body fluids, yeast, malt and manure extracts etc. Obviously, the chemical composition and concentration of a natural medium is not well defined. On account of their complex nature, these media are able to support a variety of organisms, and hence are quite useful for routine laboratory cultures of fungi.

2- Semisynthetic media: These media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources with unknown composition. The chemical composition of a semisynthetic medium is partly known. The medium is a best serve as a routine medium and sometimes for physiological studies. Potato dextrose agar (PDA) is one of the popular media.

3- Synthetic media: These are chemically defined media of known composition and concentration. The media are exclusively composed of pure chemical substances. However, absolute purity of the ingredients is achieved, although substances of only analytical reagent quality are used for such purposes. One account of their known composition as well as being in solution, these media are quite useful for nutritional and metabolic studies of fungi. The composition of

these media may be amended as per requirement and as such they may be simple or complex in composition. A simple synthetic medium contains a single carbon and energy source, a nitrogen source, generally as ammonium salt, some sulphur and phosphorus sources and various minerals. All these ingredients are dissolved in a buffered aqueous base. However, for more fastidious organisms, a complex synthetic medium is designed by incorporating some additional factors such as certain vitamins, amino-acids, purines, pyrimidines etc., or by employing a multitude of carbon and nitrogen sources together.

C- According to their physical states: Media are classified into the following types:

1- Solid media: Media in solid state are in use since the beginning of laboratory studies of fungi. The first laboratory culture of fungi was obtained on a solid media such as fruit slices. Some common examples of such media are nutrient impregnated slices of potato, carrot, sugar-beet etc. and coagulated egg or serum. However, with the advent of agar as a solidifying agent, such media have largely been replaced by agar media. Use of fruits and vegetable slices in the cultivation of fungi is now more or less restricted to the baiting technique employed for isolation of some specific organisms.

2- Solid-reversible to liquid media: Such reversible media were first introduced by Koch (1881) who observed that addition of 2 to 5 percent of gelatin to the commonly employed media rendered them a semi-solid consistency. However, gelatin could not find a wide application on account of its low melting point (37˚C), and also because it is hydrolyzed by many proteolytic bacteria at ordinary temperature. The use of agar for solidifying culture media was also initiated the same year and in the same laboratory.

3- Semi-solid media: These are media with gelatinous consistency and are employed for specific purpose. They contain a small amount of agar or some other solidifying agent like corn meal. These media are sometimes used for the study of motile reproductive structures of fungi.

4- Liquid media: These are media without any solidifying agent, and are indispensable for most of the quantitative studies of fungi. Nutritional and metabolic studies of fungi, as well as microbiological assays are invariably carried on liquid media. Some of the advantage of liquid media is that they permit the cultures to be aerated, the mycelium to be weighed and the metabolic products to be analyzed easily. However, with respect to routine studies, liquid media have some distinct disadvantages. Growth in liquid media does not manifest the morphological characteristics of microorganisms. They are also difficult to handle without disturbing the culture. Moreover, liquid media are least helpful in the purification of microorganisms from a mixed culture. For an even distribution of nutrients and for providing uniform aeration to growing fungus, the liquid cultures are sometimes put to constant mechanical shaking.

Sterilization

Sterilization refers to the process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and spore forms etc.) from a surface, equipment, foods, medications, or biological culture media.

Sterilization can be achieved through application of heat, chemicals, irradiation and filtration.

There are three main methods for sterilization

1- Physical methods

2- Chemical methods

3- Mechanical methods

1- Physical methods

Sterilization by heat

Heat may be utilized for sterilization either in dry or moist form. However, moist heat is much more effective and requires both shorter duration and lower temperature. Sterilization by moist heat generally is complete at 121°C for 15-30 minutes of exposure. On contrast, sterilization by dry heat requires a temperature

of 160°C for 60 minutes. The two kinds of heat treatments kill the microorganisms by coagulating and denaturing their enzymes and other proteins.

Application of dry heat

a- Flaming

b- Hot-air oven

c- Radiation (Infra-red or Ultra violet)

Application of moist heat

The use of the Autoclave for sterilization

Chemical methods

Using of chemical substances as agents, like chloroform, mercuric chloride, formaldehyde and ethyl alcohol.

3- Mechanical methods

Sterilization by filtration

This technique employs special type of filters having pores so small that ordinary bacteria are arrested. This method is particularly useful for sterilizing heat sensitive materials, such as culture media containing serum, antibiotic solutions, culture filtrates etc. The most common filters are Seitz filters and Cellulose membrane filters.

Fungal cell structure

Fungi are eukaryotic organisms that include microorganisms such as yeasts, molds and mushrooms. These organisms are classified under kingdom fungi. They are classified as heterotrophs among the living organisms. They are also found in most skin infections and other fungal diseases. Fungi usually grow in places which are moist and warm enough to support them. The structure of fungi can be explained in the following points:

1. Almost all fungi have a filamentous (multicellular) structure except the yeast which are unicellular microorganisms.
2. Fungi consist of long thread-like structures known as hyphae. These hyphae together form a mesh-like structure called mycelium.
3. Fungi possess a cell wall which is made up of chitin and polysaccharides.
4. The cell wall comprises a protoplast, which is differentiated into other cell parts such as cell membrane, cytoplasm, cell organelles and nuclei.
5. The nucleus is dense, clear, with chromatin threads. The nucleus is surrounded by a nuclear membrane.
6. Fungi are eukaryotic, non-vascular, non-motile and heterotrophic organisms.
7. They reproduce by means of spores (sexual or asexual).
8. Fungi exhibit the phenomenon of alternation of generation.
9. Fungi lack chlorophyll and hence cannot perform photosynthesis.

Based on mode of nutrition, fungi can be classified into 3 groups.

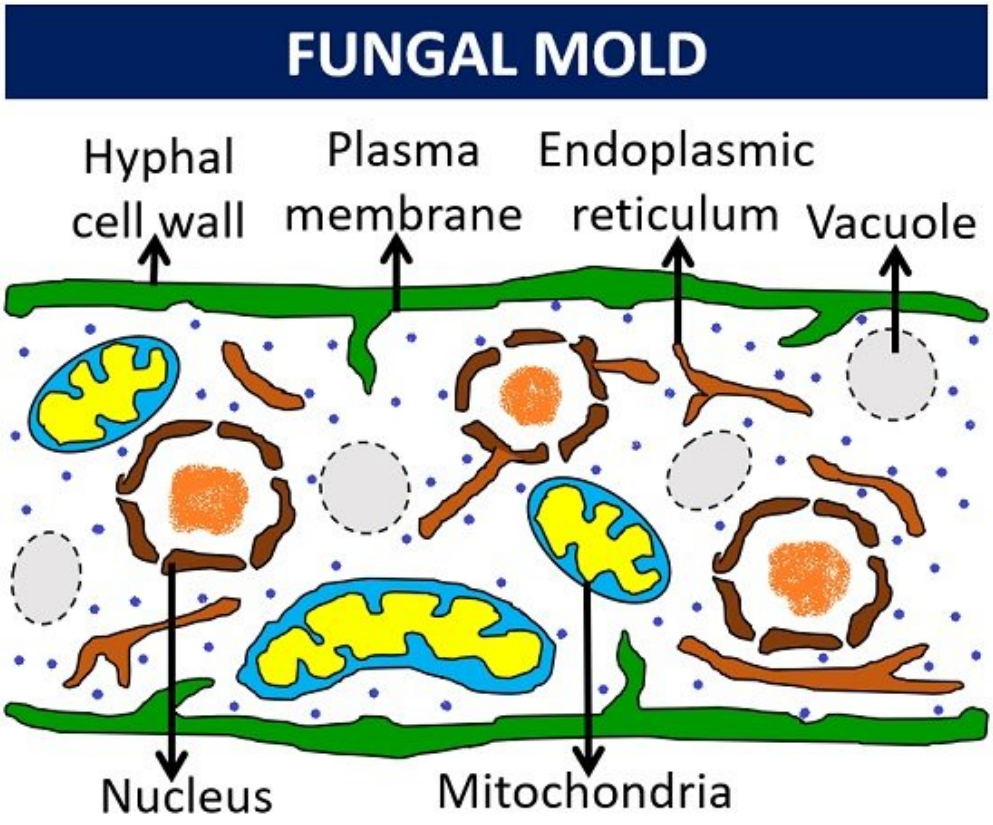
1. **Saprophytic fungi** – The fungi obtain their nutrition by feeding on dead organic substances such as *Aspergillus*, *Penicillium* and *Rhizopus*.
2. **Parasitic fungi**– The fungi obtain their nutrition by living on other living organisms (plants or animals) and absorb nutrients from their host such as *Taphrina* and *Puccinia*.
3. **Symbiotic fungi**–These fungi live with other species in which both are mutually benefited such as Lichens and mycorrhiza. Lichens are the

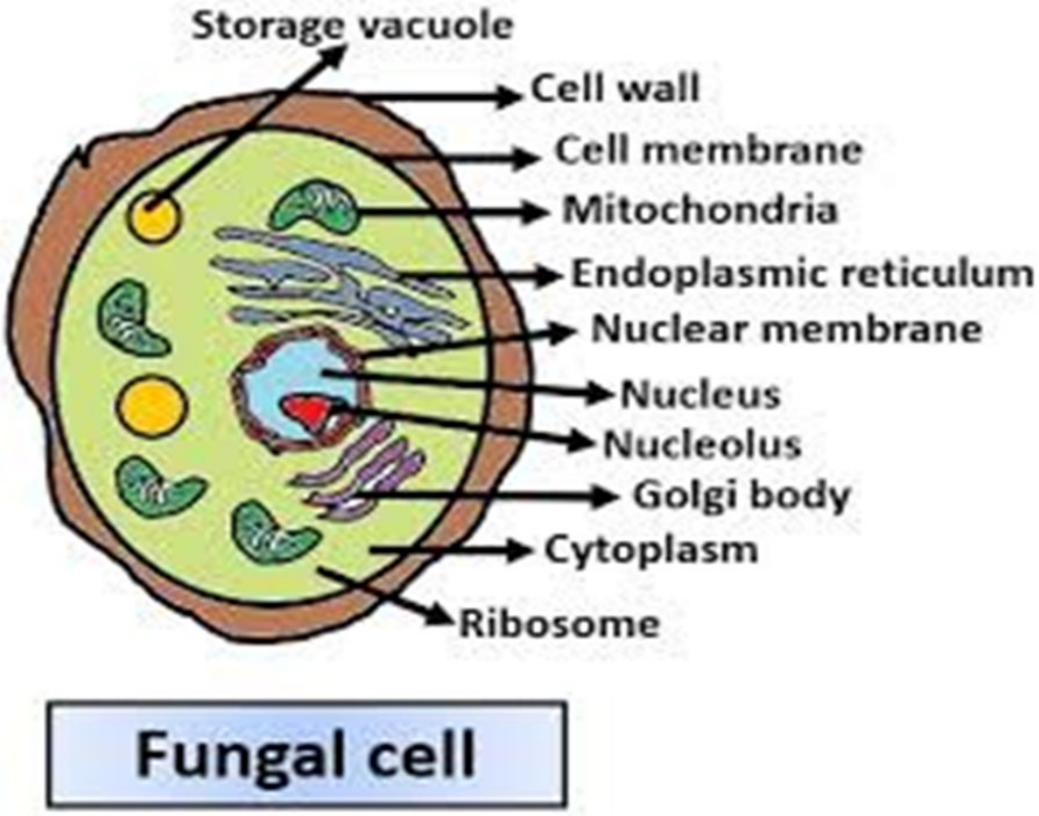
symbiotic association between algae and fungi. Here both algae and fungi are mutually benefited as fungi provide shelter for algae and in reverse algae synthesis carbohydrates for fungi. Mycorrhiza is the symbiotic association present between fungi and plants. Fungi improve nutrient uptake by plants, whereas, plants provide organic molecules like sugar to the fungus.

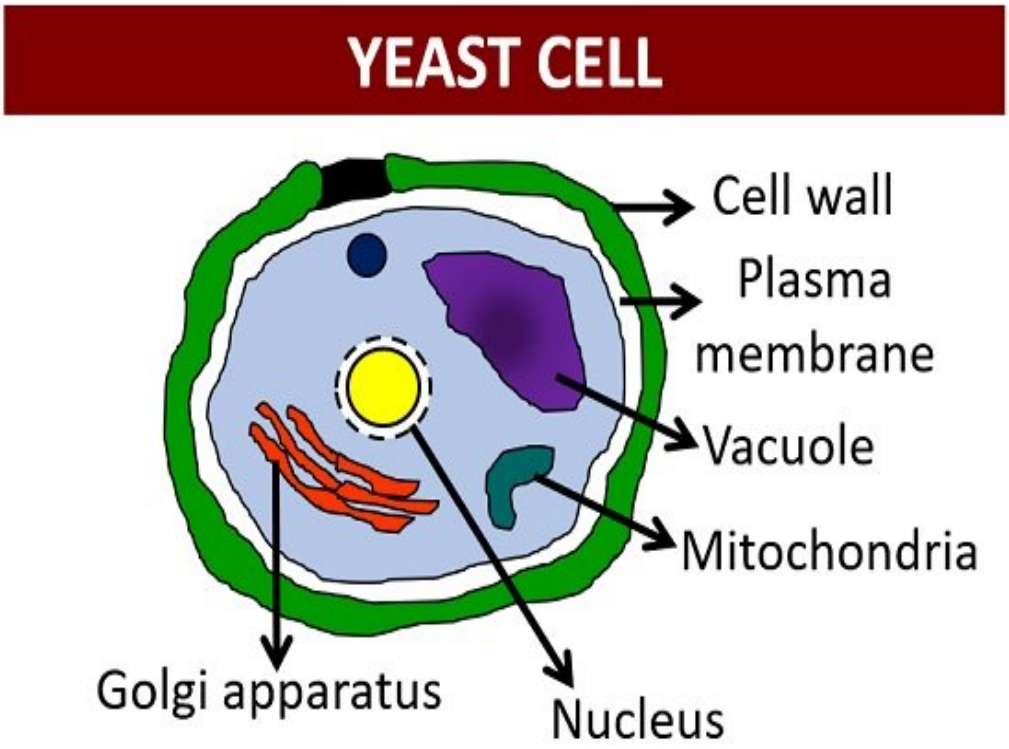
Based on spore formation, Kingdom fungi are classified into the following:

1. **Zygomycetes** – These are formed by the fusion of two different cells. The sexual spores are known as zygospores, while the asexual spores are known as sporangiospores. The hyphae are without septa. Example *Mucor* and *Rhizopus.*
2. **Ascomycetes** – They are also called sac fungi. They can be coprophilous, decomposers, parasitic or saprophytic. The sexual spores are called ascospores. Asexual reproduction occurs by conidiospores. Example – *Saccharomyces*, *Aspergillus* and *Penicillium.*
3. **Basidiomycetes** – Mushrooms are the most commonly found basidiomycetes and mostly live as parasites. Sexual reproduction occurs by basidiospores. Asexual reproduction occurs by conidia, budding or fragmentation. Example- *Agaricus.*
4. **Deuteromycetes** – They are otherwise called imperfect fungi as they do not follow the regular reproduction cycle as the other fungi. They do not reproduce sexually. Asexual reproduction occurs by conidia. Example – *Alternaria* and *Trichoderma*.

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.


FUNGAL MOLD
Hyphal cell wall
Plasma membrane
Endoplasmic reticulum
Vacuole
Nucleus
Mitochondria

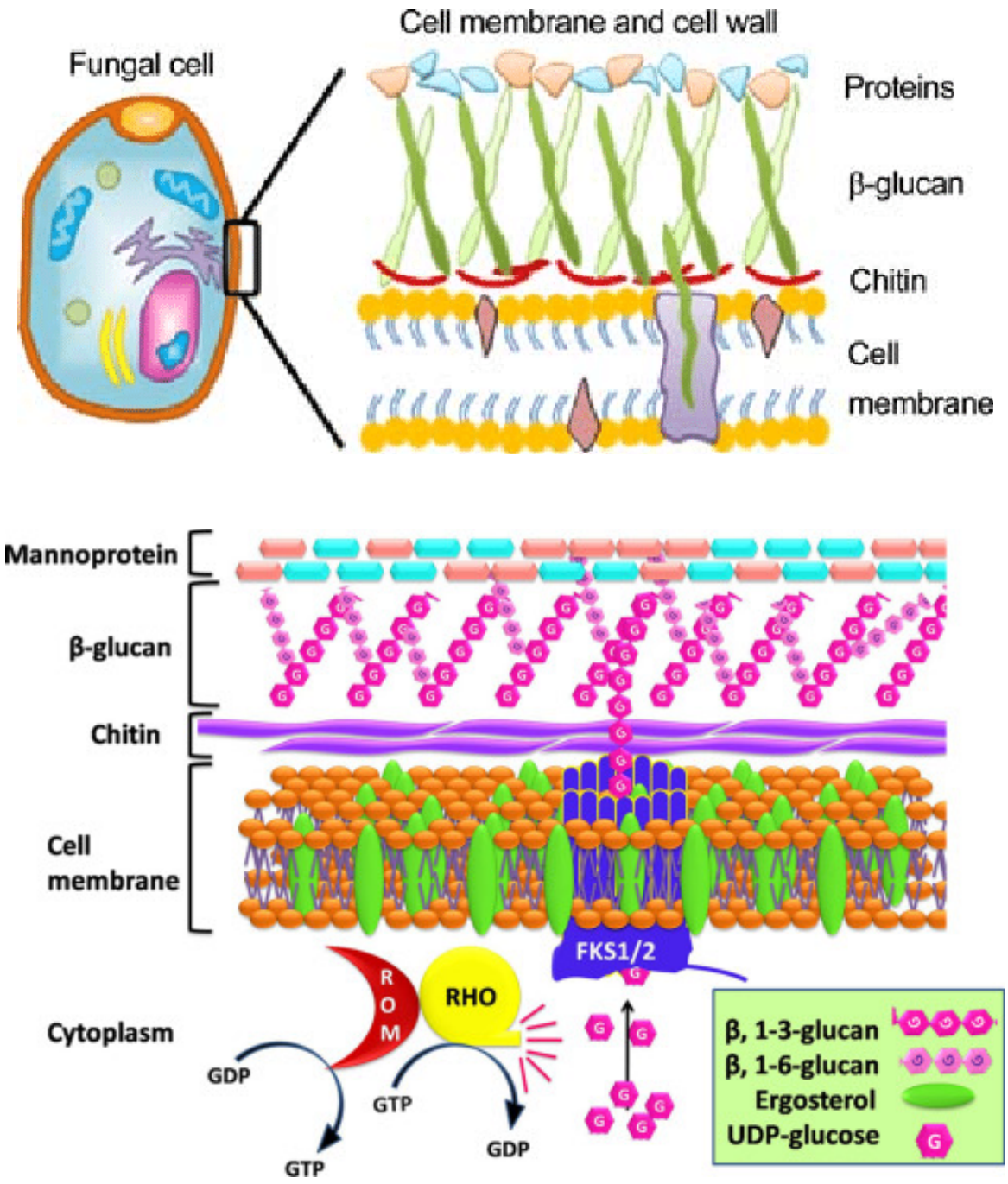

Storage vacuole
Cell wall
Cell membrane
Mitochondria
Endoplasmic reticulum
Nuclear membrane
Nucleus
Nucleolus
Golgi body
Cytoplasm
Ribosome
Fungal cell



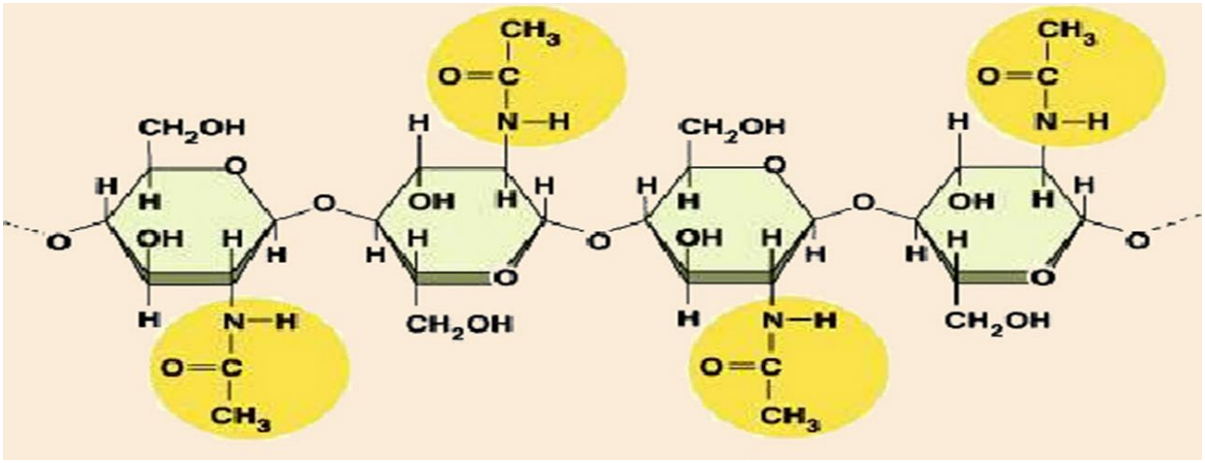
Unlike plant cells, fungal cells do not have chloroplasts or chlorophyll. Many fungi display bright colors arising from other cellular pigments, ranging from red to green to black. The poisonous *Amanita muscaria* (fly agaric) is recognizable by its bright red cap with white patches. Pigments in fungi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

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.



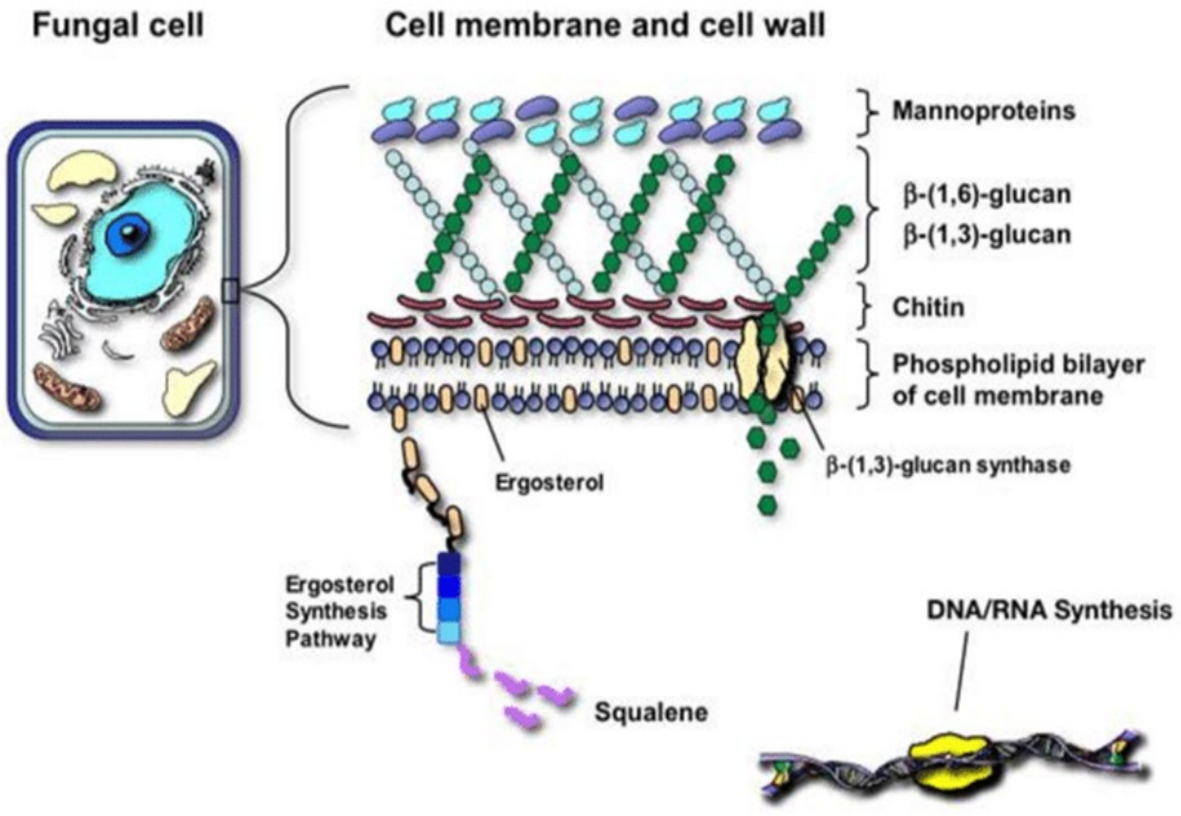
The rigid layers of fungal cell walls contain complex polysaccharides called chitin and glucans. Chitin, also found in the exoskeleton of insects, gives structural strength to the cell walls of fungi. The wall protects the cell from desiccation and predators. Fungi have plasma membranes similar to other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



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.



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. *Saccharomyces* cerevisiae, (n=18); *Trichophyton mentagophytes*, (n=4).

Fungal growth and nutrition

When a fungus is added to a suitable liquid medium and incubated at a suitable growth conditions, its growth follows a definite course. If the fungal counts are made at intervals after inoculation and plotted in relation to time, a growth curve obtained shows 4 phases:

Lag phase

Log or Exponential phase

Stationary phase

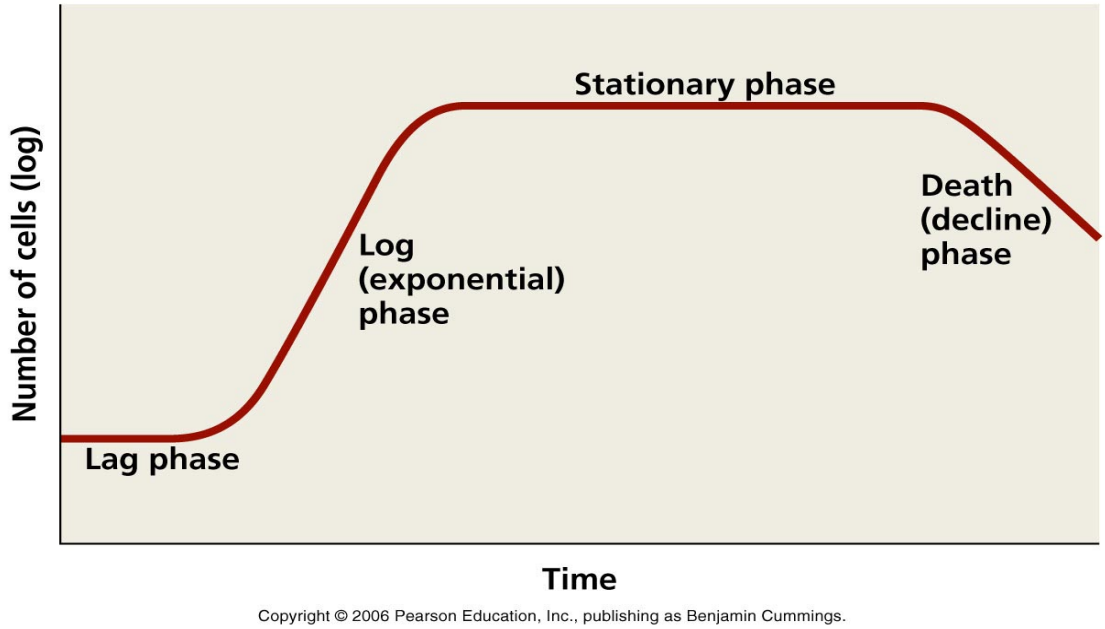
Decline phase

1. Lag phase – No increase in the cell number but there is an increase in the size of the cell. Maximum cell size towards the end of the lag phase

2. Log or exponential phase: cells start dividing and their number increases exponentially. Smaller cells, stain uniformly.

3. Stationary phase: cell division stops due to depletion of nutrients & accumulation of toxic products. Equilibrium exists between dying cells and the newly formed cells, so viable count remains stationary. Irregular staining, sporulation and production of secondary metabolites such as exotoxins & antibiotics.

4. Decline phase: population decreases due to the death of cells – autolytic enzymes. Involution forms (with ageing).



Growth curve of fungi

Factors affecting fungal growth

1- Availability of nutrients & H_2O
2- Temperature
3- Atmosphere – O_2 & CO_2
4- H-ion concentration (pH)

Functions of nutrients

Generation of energy and synthesis of cellular materials.

Essential nutrients (basic bioelements needed for fungal growth).

H_2O: universal solvent; hydrolyzing agent

Carbon: food & energy source; in the form of carbohydrates, proteins and lipids.

Nitrogen: for amino acids and protein synthesis; nucleic acids synthesis (purines & pyrimidines).

Sulfur (sulfate): Some amino acids synthesis such as cystine and methionine.

Phosphate: key component of DNA, RNA and ATP in addition to the formation of phospholipids of the cell membrane.

Minerals: associated with protein (i.e., Fe:PRO); common component of enzymes.

Macronutrients – needed in large quantities for cellular metabolism and basic cell structure such as C, N, H, P and O.

Micronutrients – needed in small quantities; more specialized for enzymes and pigments structure and function such as Fe, Cu, Mn and Zn.

Fastidious fungi: microbes that require other complex - nutrients/growth factors such as vitamins or amino acids.

Factors affecting fungal growth

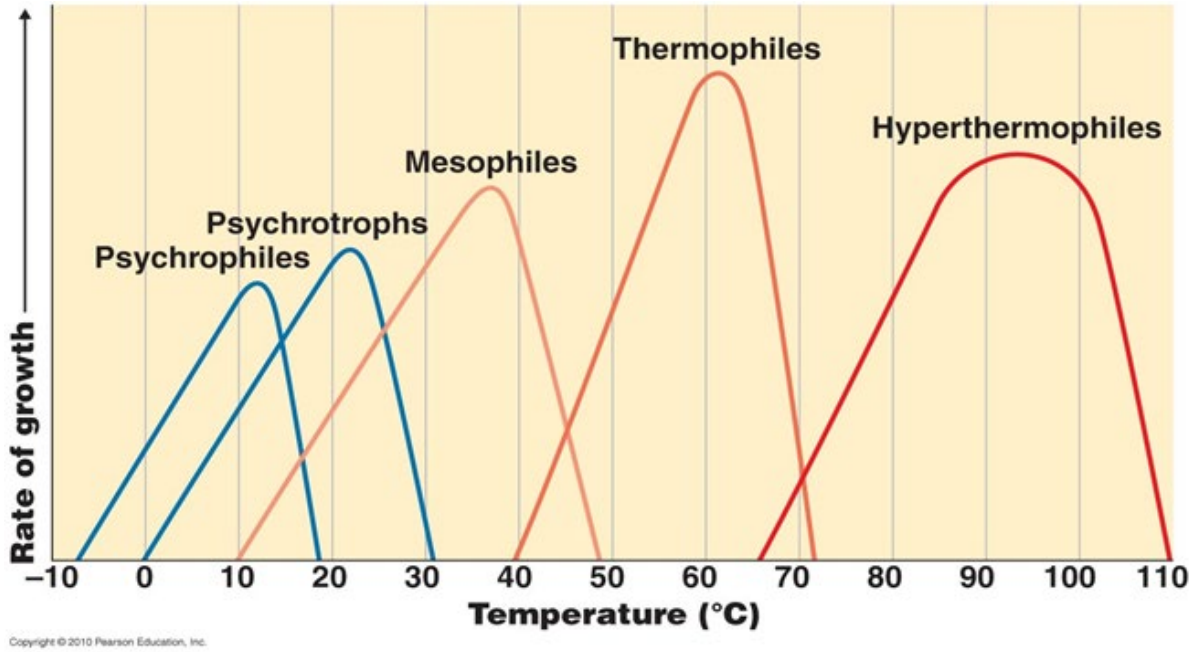
Temperature

Temperature is an important environmental factor affecting growth of molds. Fungi are capable of surviving under the full range of temperatures normally experienced in environments in which they live. The temperature ranges usually reported for fungal growth is broad (10-40°C), with a few species capable of growth below or above this range. Fungi can be divided according to their tolerance to temperature in psychrophilic, mesophilic and thermophilic fungi. Fungi are vary for their temperature requirements. Temperature range - growth does not occur above the maximum or below the minimum.

Minimum temperature – which fungi cannot grow below the minimum temperature.

Optimum temperature – which are the best for fungal growth and metabolism usually within 20-30ºC for most fungi (mesophilic fungi).

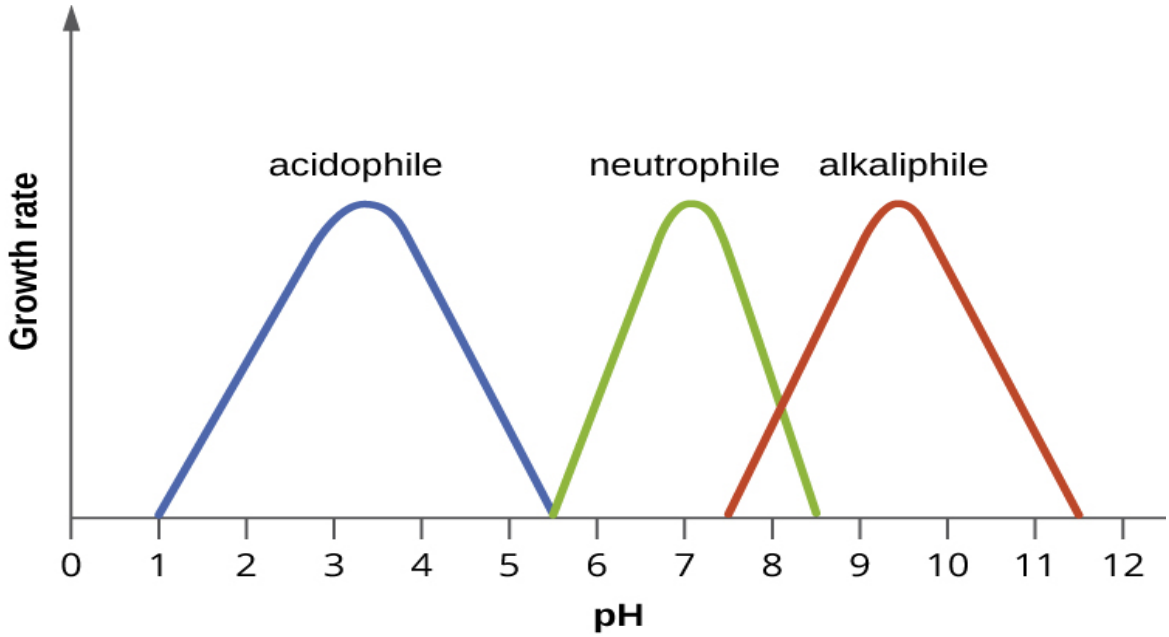
Maximum temperature - which fungi cannot grow above the maximum temperature.



Hydrogen-ion concentration (pH)

Some products such as yogurt, pickles, sauerkraut, and lime-seasoned dishes all owe their tangy taste to a high acid content. The acidity is a function of the concentration of hydrogen ions [H^+] and is measured as pH. Environments with pH values below 7.0 are acidic, with a high concentration of H^+ ions. However, those with pH values above 7.0 are considered basic. Extreme pH affects the structure of all macromolecules. The hydrogen bonds holding together strands of DNA break up at high pH values. Lipids are hydrolyzed by an extremely basic pH. The proton motive force responsible for production of ATP in cellular respiration depends on the concentration gradient of H^+ across the plasma membrane. If H^+ ions are neutralized by hydroxide ions, the concentration gradient collapses and impairs energy production. But the component most sensitive to pH in the cell is its workhorse, the protein. Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn, promotes changes in the folding of the molecule, promoting denaturation and destroying activity.

The optimum growth pH is the most favorable pH for the growth of microorganisms. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH. These values can cover a wide range, which is important for the preservation of food and to the microorganism survival in the nature.



The curves show the approximate pH ranges for the growth of the different classes of microorganisms. Each curve has an optimal pH and extreme pH values at which growth is much reduced. Most fungi are neutrophiles and grow best at near-neutral pH. Acidophiles have optimal growth at pH values near 3 and alkaliphiles have optimal growth at pH values above 9.

Neutrophiles

Most fungi are neutrophiles, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7, between 5 and 9. Also most familiar bacteria, like *Escherichia coli*, *Staphylococci*, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Acidophiles

Microorganisms that grow optimally at a pH less than 5 are called acidophiles. For example, the sulphur-oxidizing *Sulfolobus* spp. isolated from sulphur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5. Species of the archaean genus *Ferroplasma* live in acid mine drainage at pH values of 0–2.9. *Lactobacillus* bacteria, which are an important part of the normal microbiota of the vagina, can tolerate acidic environments at pH values 3.5–6.8 and also contribute to the acidity of the vagina (pH of 4, except at the onset of menstruation) through their metabolic production of lactic acid. The vagina's acidity plays an important role in inhibiting other microbes that are less tolerant of acidity. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. While the membrane is slightly leaky to protons, the cytoplasmic pH of most acidophiles is generally only slightly acidic. One of the major reasons for this is their ability to actively transport of H^+ ions out of the cell. In addition, cytoplasmic proteins have evolved to function better at a slightly acidic pH with increased negative surface charges compared to their neutrophilic homologues. The ether linkage of the archaeal membrane lipids is more acid stable than the typical ester linked phospholipids, but in addition, acidophilic archaea typically possess tetra ether membrane lipids. The resulting monolayer structure makes their membranes a much better barrier to proton leakage in extremely low pH environments. Since these organisms may also be adapted to growing at high temperatures, the membranes also maintain their semi-fluid consistency. While the cytoplasmic proteins of acidophiles have relatively normal pH optima, those that are secreted have acidic pH optima compared to their neutrophile homologues. The gene sequences for acidophilic secreted proteins have evolved to give secondary, tertiary and quaternary structures that are resistant to the protonating effects of the acidic environment. These proteins are of great interest for their possible biotechnological applications.

Alkaliphiles

Alkaliphiles microorganisms that have pH optima between 8.0 and 11. Vibrio cholerae, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0 but is inactivated by the acid of the stomach. When it comes to survival at high pH, the bright pink halophilic archaeon Natronobacterium, found in the soda lakes of the African Rift Valley, may hold the record at a pH of 10.5. Extreme alkaliphiles have adapted to their harsh environment through various evolutionary modifications. Alkaliphilic archaea have diether lipid membranes. The ether linkage is more resistant to chemical or thermal degradation compared to the ester-linked phospholipids. Given the paucity of protons in alkaline environments, maintaining a proton motive force is probably the most pressing challenge for alkaliphiles. One of the adaptations of alkaliphilic halophilic bacteria and archaea in soda lakes and other highly salty environments is the evolution of coupled transporters and flagella that exploit sodium motive force, thus conserving the PMF for oxidative and photophosphorylation by the ATP synthase. The cell surface of alkaliphiles has a high concentration of acidic (i.e. negatively charged) molecules and it has been suggested this acts as a "proton sponge", allowing a more rapid lateral diffusion of protons from the ETS, to the ATP synthase, compared to the rate of diffusion into the surrounding waters. Finally, alkaliphiles may use Na^+/H^+ antiport to create a sodium motive force. For example, the alkaliphile *Bacillus firmus* derives the energy for transport reactions and motility from SMF rather than a proton motive force. As with the acidophiles, the genes for secreted proteins of alkaliphiles have evolved to give enzymes that resist deprotonation/denaturation and chemical degradation at the high pH of their environment. These enzymes are also of interest to biotechnology companies. In fact, laundry detergents, which are alkaline in nature, contain alkaliphilic lipases and proteases to improve their stain-removing abilities.

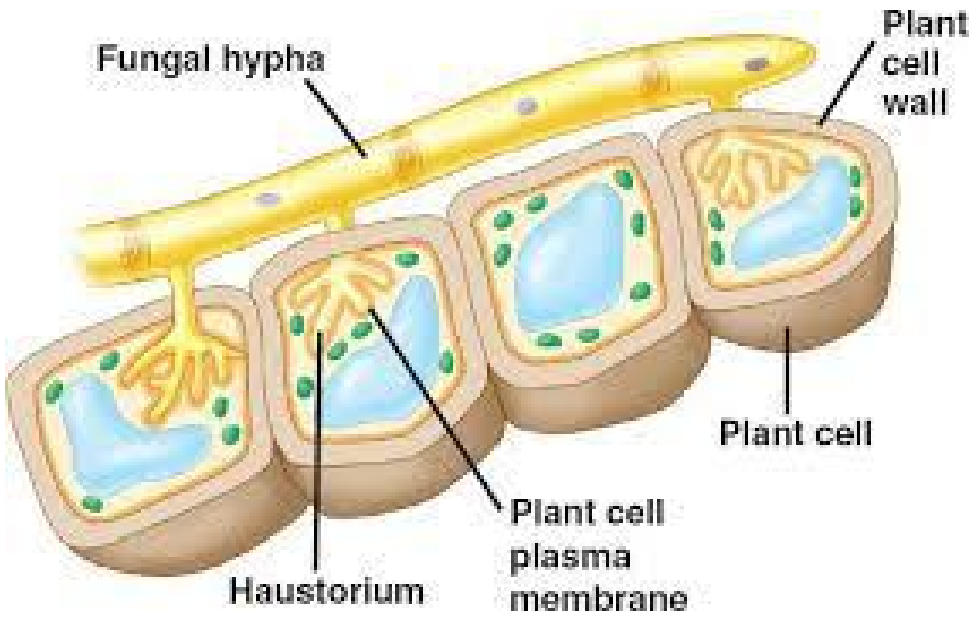
Fungal nutrition

Fungi get their nutrition by absorbing organic compounds from the environment. Fungi are heterotrophic: they rely solely on carbon obtained from other organisms for their metabolism and nutrition. Fungi have evolved in a way that allows many of them to use a large variety of organic substrates for growth, including simple compounds such as nitrate, ammonia, acetate, or ethanol. Their mode of nutrition defines the role of fungi in their environment.

Fungi obtain nutrients in three different ways:

1- They decompose dead organic matter. A saprotroph is an organism that obtains its nutrients from non-living organic matter, usually dead and decaying plant or animal matter, by absorbing soluble organic compounds. Saprotrophic fungi play very important roles as recyclers in ecosystem energy flow and biogeochemical cycles. Saprophytic fungi, such as shiitake (*Lentinula edodes*) and oyster mushrooms (*Pleurotus ostreatus*), decompose dead plant and animal tissue by releasing enzymes from hyphal tips. In this way they recycle organic materials back into the surrounding environment. Because of these abilities, fungi are the primary decomposers in forests.

2- They feed on living hosts. As parasites, fungi live in or on other organisms and get their nutrients from their host. Parasitic fungi use enzymes to break down living tissue, which may cause illness in the host. Disease-causing fungi are parasitic. Recall that parasitism is a type of symbiotic relationship between organisms of different species in which one, the parasite, benefits from a close association with the other, the host, which is harmed.



3- They live mutualistically with other organisms. Mutualistic fungi live harmless with other living organisms. The mutualism is an interaction between individuals of two different species, in which both individuals benefit.

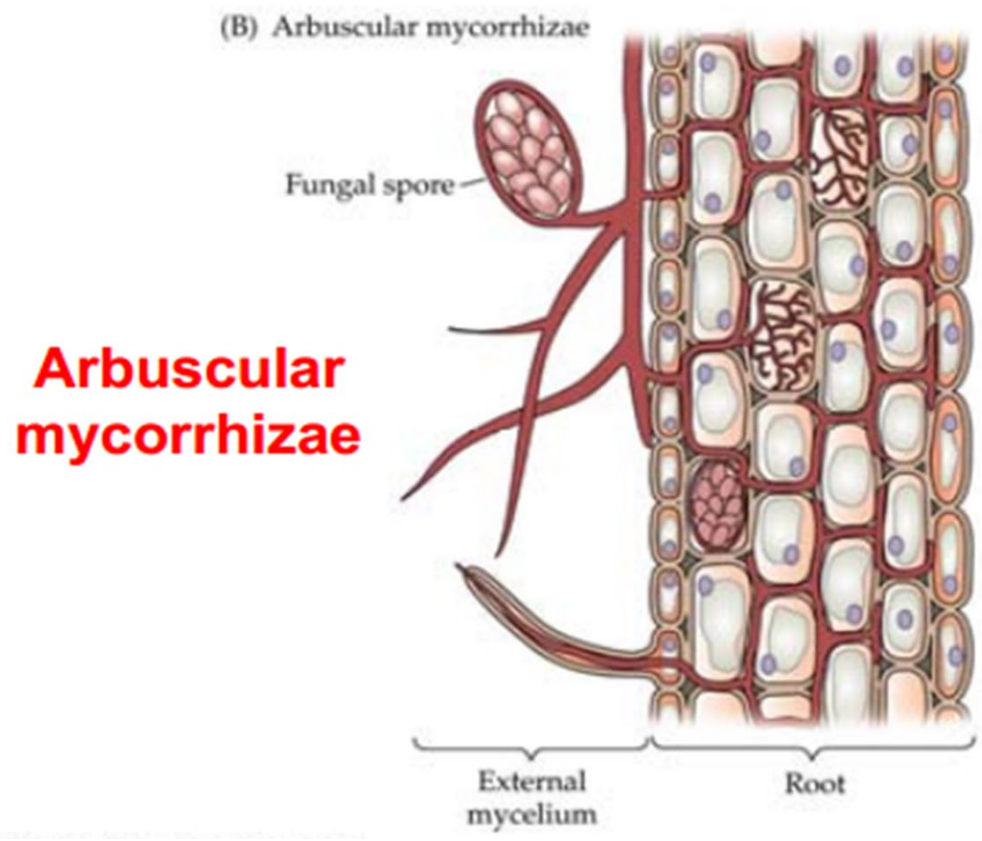
Both parasitism and mutualism are classified as symbiotic relationships, but they are discussed separately here because of the different effect on the host.

Fungal hyphae are adapted to efficient absorption of nutrients from their environments, because hyphae have high surface area-to-volume ratios. These adaptations are also complemented by the release of hydrolytic enzymes that break down large organic molecules such as polysaccharides, proteins, and lipids into smaller molecules. These molecules are then absorbed as nutrients into the fungal cells. One enzyme that is secreted by fungi is cellulase, which breaks down the polysaccharide cellulose. Cellulose is a major component of plant cell walls. In some cases, fungi have developed specialized structures for nutrient uptake from living hosts, which penetrate into the host cells for nutrient uptake by the fungus.

Fungi absorb nutrients from the environment through mycelia.

Mycorrhiza

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.

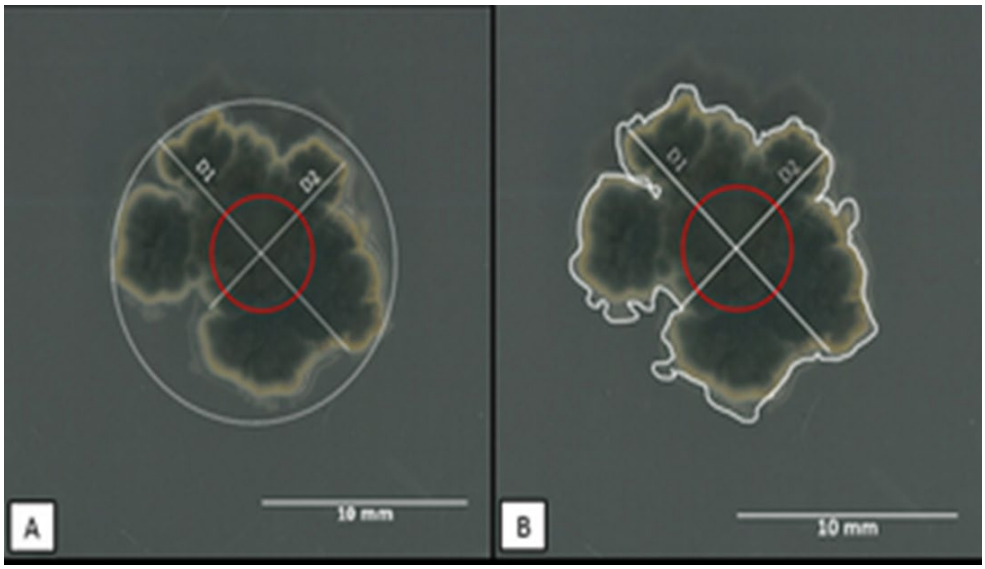


Measuring of fungal growth

The following points highlight the two methods used for measuring the growth in fungi. These methods are: 1. Linear Method (Agar Plate). 2. Mycelial Dry Weight.

1. Linear Method (Agar Plate)

After the fungal inoculum kept in the center of the agar plate, the radial growth of fungal colony can be measured and the rate of growth can be measured each 24 hours.



- Average of colony growth (diameter) = $\frac{D1+D2}{2}$

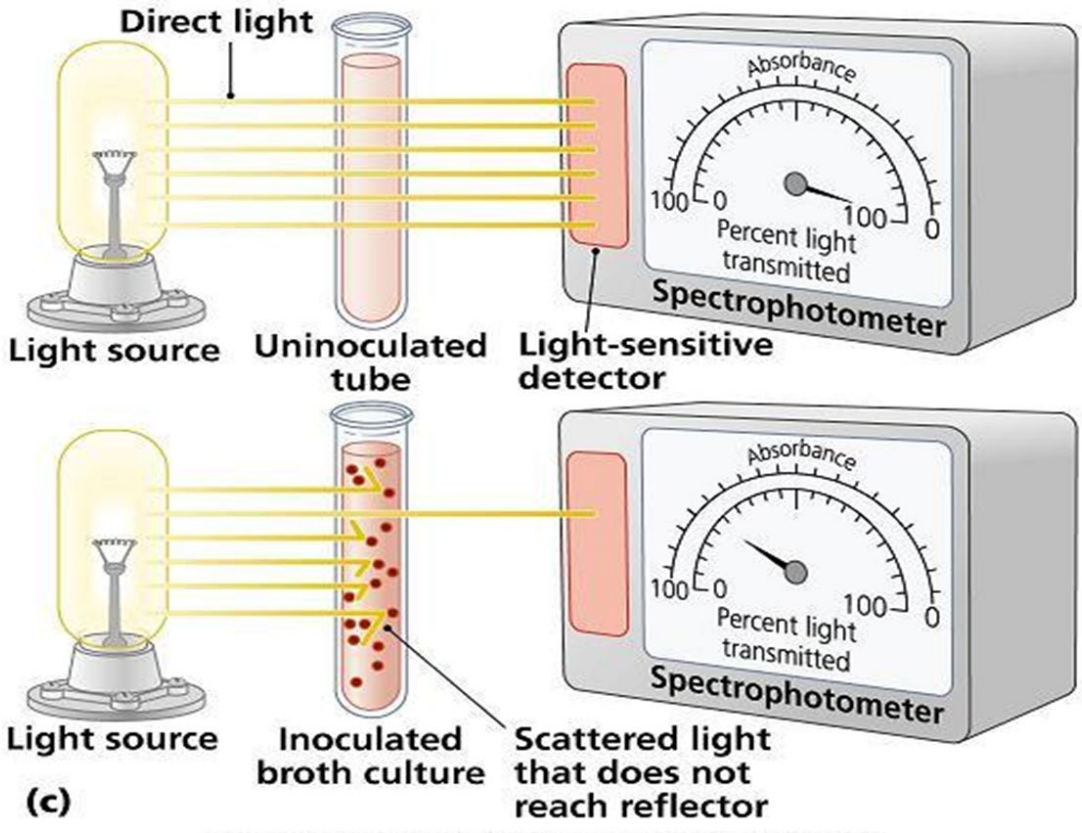
- Percentage of colony growth = $\frac{D1+D2}{2}$ X 100

2. Mycelial Dry Weight

On liquid medium (both stationary and aerated agitated cultures) the mycelial growth can be measured as dry weight. After inoculation of cultures, the cultures must be incubated at a suitable temperature and then the mycelial growth can be determined after filtration and drying of mycelia.

3- Spectrophotometric method

This method is used to measure the growth of unicellular organisms such as yeast and bacterial species using spectrophotometric analysis. Significant variations are found in the growth patterns of budding and fission of yeast. The spectrophotometer absorbance depends on the turbidity in the liquid medium due to the growth rate of yeast.



Carbon metabolism

Metabolism: The entire spectrum of living chemical reactions, occurring in living system. Metabolism is broadly classified into two:

Anabolism: biosynthetic reactions involving in the formation of complex molecules from simple precursors.

Catabolism: degradation processes concerned with the breakdown of complex molecules to simpler ones with release of energy.

Respiration

Glycolysis (Degradation of glucose to pyruvate)

The major function of carbohydrates in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes. The carbohydrate is utilized by cells mainly as glucose. The three principal monosaccharides resulting from digestive processes are glucose, fructose, and galactose. Much of the glucose is derived from starch which accounts for over half of the fuel in the diets of most humans. Glucose is also produced from other dietary components by the liver and, to a lesser extent, by the kidneys. Fructose results in a large intake of sucrose while galactose is produced when lactose is the principal carbohydrate of the diet. Both fructose and galactose are easily converted to glucose by the liver. It is thus apparent that glucose is the major fuel of most organisms and that it can be quickly metabolized from glycogen stores when there arises a sudden need for energy. Pentose sugars such as arabinose, ribose and xylose may be present in the diet. But their fate after absorption is, however, obscure.

Glycolysis is the sequence of 10 enzyme-catalyzed reactions that convert glucose into pyruvate with the simultaneous production of ATP. The glycolytic sequence of reactions differs from one species to the other only in the mechanism of its regulation and in the subsequent metabolic fate of the pyruvate formed. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain which together harvest most of the energy contained in glucose. In fact, glycolysis is the central pathway of glucose catabolism. Glycolysis takes

place outside the mitochondria in the cytoplasm. It is frequently referred to as Embden-Meyerhof-Pathway (EMP pathway), in the honors of these pioneer workers in the field, and still represents one of the greatest achievements in the field of biochemistry. (https://www.youtube.com/watch?v=8qij1m7XUhk), (https://www.youtube.com/watch?v=UBudWWUqAmc).

Glycolysis is the metabolic process that serves as the foundation for both aerobic and anaerobic cellular respiration. In glycolysis, glucose is converted into pyruvate. Glucose is a six-membered ring molecule found in the blood and is usually a result of the breakdown of carbohydrates into sugars. It enters cells through specific transporter proteins that move it from outside the cell into the cell's cytosol. All of the glycolytic enzymes are found in the cytosol. The overall reaction of glycolysis which occurs in the cytoplasm is represented simply as:

$C_6H_{12}O_6 + 2\ NAD + 2\ ADP + 2\ P \longrightarrow 2\ Pyruvic\ acid + 2\ ATP + 2\ NADH + 2\ H^+$

Step 1

The first step in glycolysis is the conversion of D-glucose into glucose 6-phosphate. The enzyme that catalyzes this reaction is hexokinase. The glucose ring is phosphorylated. Phosphorylation is the process of adding a phosphate group to a molecule derived from ATP. As a result, at this point in glycolysis, 1 molecule of ATP has been consumed. The reaction occurs with the help of the enzyme hexokinase, an enzyme that catalyzes the phosphorylation of many six-membered glucose-like ring structures. Atomic magnesium (Mg) is also involved to help shield the negative charges from the phosphate groups on the ATP molecule. The result of this phosphorylation is a molecule called glucose 6-phosphate (G6P), because the 6′ carbon of the glucose acquires the phosphate group.

Step 2

The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase (Phosphoglucose Isomerase). The second step of glycolysis involves the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction occurs with the help of the enzyme phosphoglucose isomerase (PI). The reaction involves the rearrangement of the carbon-oxygen bond to transform the six-membered ring into a five-membered ring. To rearrangement takes place when the six-membered ring opens and then closes in such a way that the first carbon becomes now external to the ring.

Step 3

Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate. In the third step of glycolysis, fructose 6-phosphate is converted to fructose 1,6-bisphosphate (FBP). Similar to the reaction that occurs in step 1 of glycolysis, a second molecule of ATP provides the phosphate group that is added on to the F6P molecule. The enzyme that catalyzes this reaction is phosphofructokinase (PFK). As in step 1, a magnesium atom is involved to help shield negative charges.

Step 4

The enzyme Aldolase splits fructose 1,6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). This step utilizes the enzyme aldolase, which catalyzes the cleavage of FBP to yield two 3-carbon molecules. One of these molecules is called glyceraldehyde-3-phosphate (GAP) and the other is called dihydroxyacetone phosphate (DHAP).

Step 5

The enzyme triosephosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed / used in next step of Glycolysis. GAP is

the only molecule that continues in the glycolytic pathway. As a result, all of the DHAP molecules produced are further acted on by the enzyme Triosephosphate isomerase (TIM), which reorganizes the DHAP into GAP so it can continue in glycolysis. At this point in the glycolytic pathway, we have two 3-carbon molecules.

Step 6

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate. In this step, two main events take place: 1- glyceraldehyde 3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD); 2- the molecule is phosphorylated by the addition of a free phosphate group. The enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The enzyme GAPDH contains appropriate structures and holds the molecule in a conformation such that it allows the NAD molecule to pull a hydrogen off the GAP, converting the NAD to NADH. The phosphate group then attacks the GAP molecule and releases it from the enzyme to yield 1,3 biphosphoglycerate, NADH, and a hydrogen atom.

Step 7

Phosphoglycerate kinase transfers a phosphate group from 1,3-biphosphoglycerate to ADP to form ATP and 3-phosphoglycerate. In this step, 1,3 biphosphoglycerate is converted to 3-phosphoglycerate by the enzyme phosphoglycerate kinase (PGK). This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP. Since we actually have two molecules of 1,3 biphosphoglycerate (because there were two 3-carbon products from stage 1 of glycolysis), we actually synthesize two molecules of ATP at this step. With this synthesis of ATP, we have cancelled the first two molecules of ATP that we used, leaving us with a net of 0 ATP molecules up to this stage of glycolysis.

Again, we see that an atom of magnesium is involved to shield the negative charges on the phosphate groups of the ATP molecule.

Step 8

The enzyme phosphoglycero mutase relocates the P from 3-phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate. This step involves a simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate molecule, making it 2-phosphoglycerate. The molecule responsible for catalyzing this reaction is called phosphoglycerate mutase (PGM). A mutase is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another. The reaction mechanism proceeds by first adding an additional phosphate group to the 2′ position of the 3 phosphoglycerate. The enzyme then removes the phosphate from the 3′ position leaving just the 2′ phosphate, and thus yielding 2 phsophoglycerate. In this way, the enzyme is also restored to its original, phosphorylated state.

Step 9

The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This step involves the conversion of 2 phosphoglycerate to phosphoenolpyruvate (PEP). The reaction is catalyzed by the enzyme enolase. Enolase works by removing a water group, or dehydrating the 2 phosphoglycerate.

Step 10

The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10. The final step of glycolysis converts phosphoenolpyruvate into pyruvate with the help of the enzyme pyruvate kinase. As the enzyme's name suggests, this reaction involves the transfer of a phosphate group. The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP. Again, since there are two molecules of PEP, here we actually generate 2 ATP molecules.

Steps 1 and 3 = – 2 ATP, Steps 7 and 10 = + 4 ATP, Therefore Net ATP produced = 2.

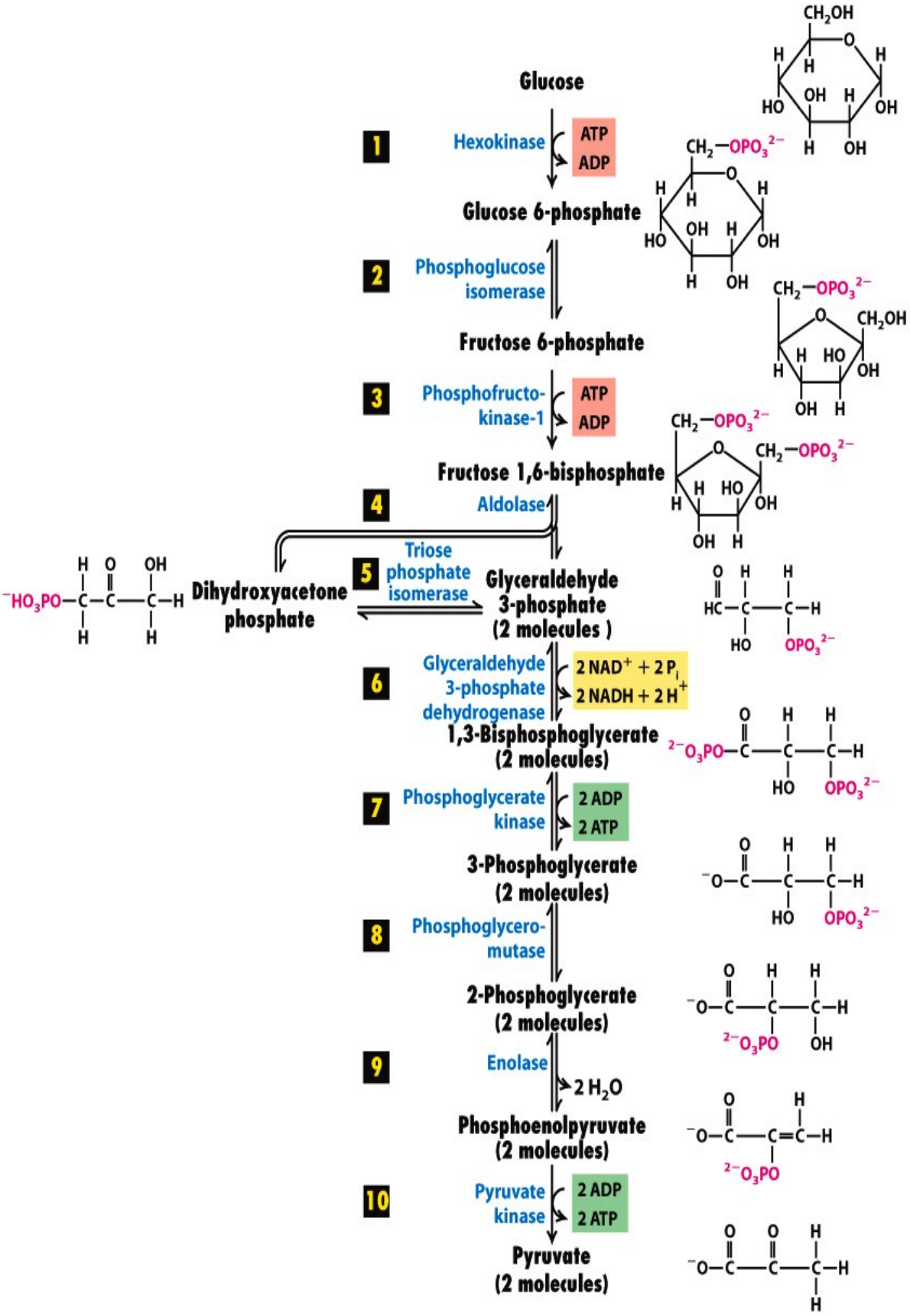


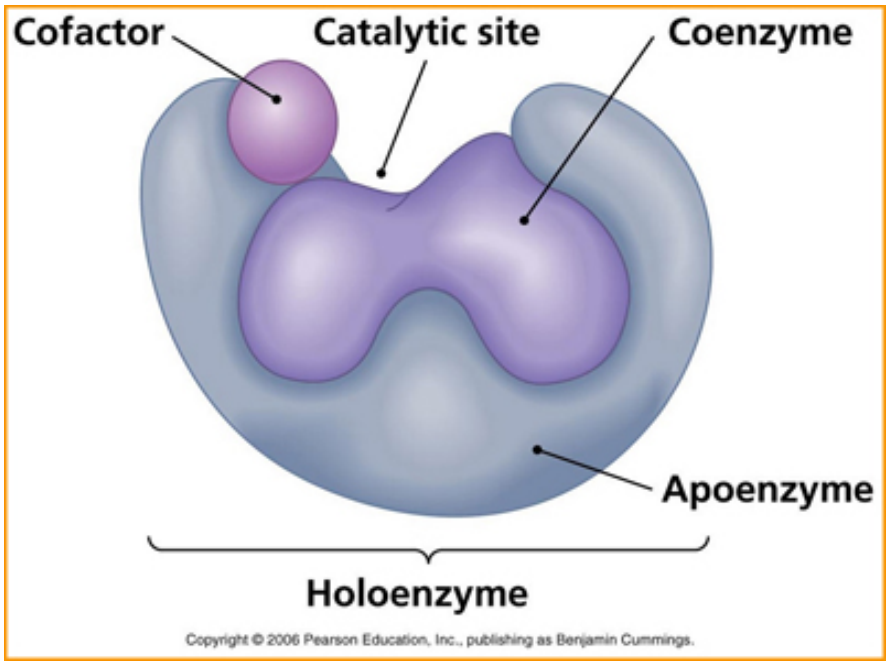
Figure 12-3
Molecular Cell Biology, Sixth Edition

Cofactors

The cofactors are non-protein compounds that assists with a biological chemical reaction. Cofactors may be metal ions, organic compounds, or other chemicals that have helpful properties not usually found in amino acids. Some cofactors can be made inside the cell, such as ATP, while others must be consumed in food. Minerals, for example, come from the environment, and cannot be made from scratch by any living cell. The organic compounds we refer to as "vitamins" are cofactors that our own bodies cannot make, so we must consume them from food in order for our cells to be able to perform essential life functions.

At the biochemical level, cofactors are important in understanding how biological reactions proceed. The presence or absence of cofactors may determine how quickly reactions proceed from their reactant to their product.

- **Cofactor**: A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- **Enzymes**: Enzymes are large biological molecules responsible for the thousands of chemical interconversions that sustain life. They are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic reactions, from the digestion of food to the synthesis of DNA.
- **Reaction**: A chemical reaction is a process that leads to the transformation of one set of chemical substances to another. Classically, chemical reactions encompass changes that strictly involve the motion of electrons in the forming and breaking of chemical bonds between atoms, and can often be described by a chemical equation.
- **Apoenzyme**: an inactive haloenzyme lacking a cofactor.


Cofactor
Catalytic site
Coenzyme
Apoenzyme
Holoenzyme
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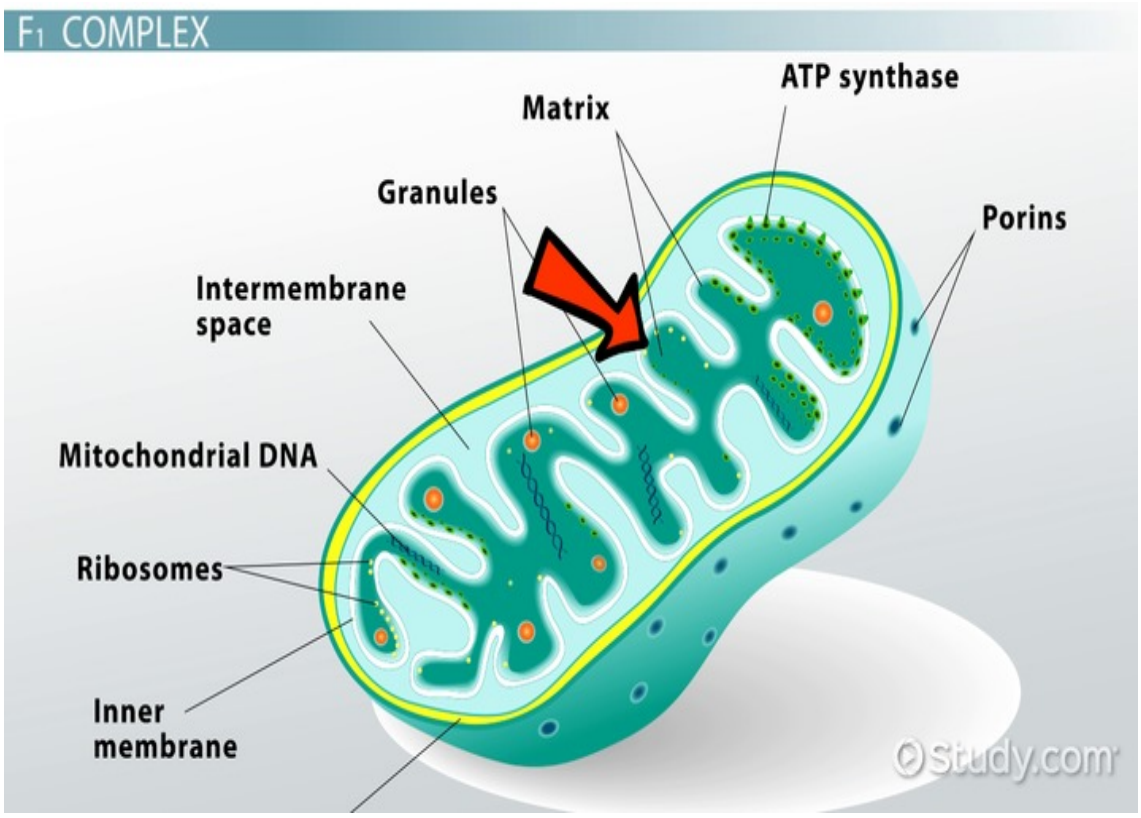
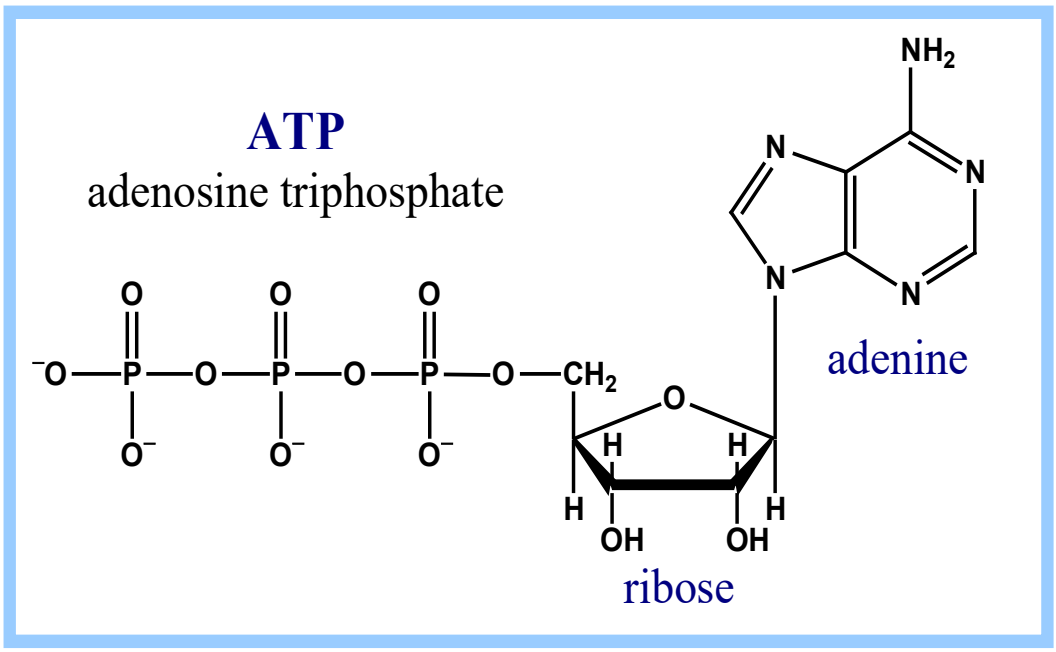
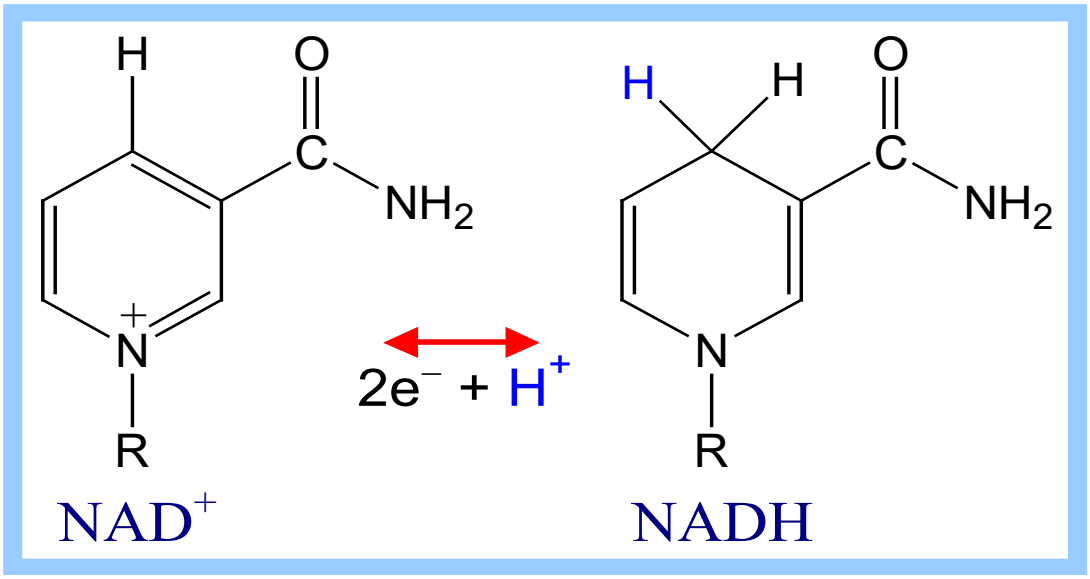

F1 COMPLEX
ATP synthase
Matrix
Granules
Porins
Intermembrane space
Mitochondrial DNA
Ribosomes
Inner membrane
Study.com



Adenosine triphosphate (ATP)



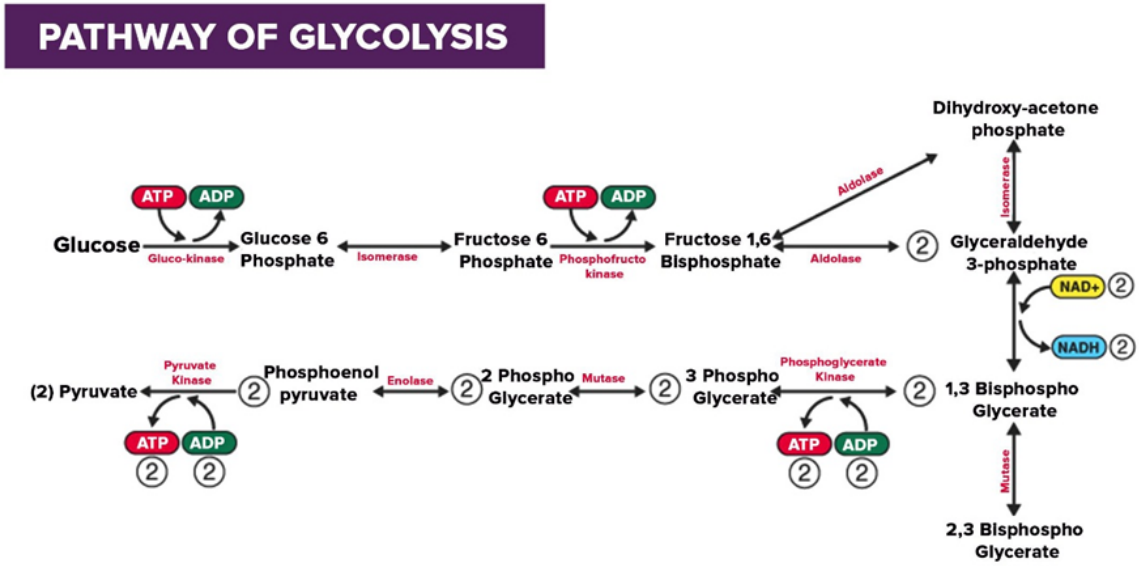
Nicotinamide adenine dinucleotide (NAD)

Nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD+) are two cofactors that are involved in cellular respiration. They are responsible for accepting "high energy" electrons and carrying them ultimately to the electron transport chain where they are used to synthesize ATP molecules. Therefore, although they themselves are not a direct source of energy, they are

used to form ATP energy molecules that are used by the cell. When these electron-carrier molecules accept the electrons, they are reduced into NADH and $FADH_2$.

ATP and NADH produced by glycolysis

It is the process of breaking down glucose to create energy. Glycolysis generates .2 ATP and 2 NADH, for a total of 8 ATP molecules.



Krebs cycle (Citric acid cycle)

The German chemist Hans Adolf Krebs discovery this cycle in 1937 marked a milestone in biochemistry. Krebs received the Nobel Prize for Physiology or Medicine in 1953 for this contribution to the study of intermediary metabolism in the oxidative breakdown of carbohydrates. Krebs and his coauthor William Arthur Johnson published their findings "The role of citric acid in intermediate metabolism in animal tissues" in Enzymologia after being rejected by Nature. That original publication was followed by many more.

Krebs or Citric acid cycle, also known as the tricarboxylic acid (TCA) cycle, is the main source of energy for cells and an important part of aerobic respiration. The cycle harnesses the available chemical energy of acetyl coenzyme A (acetyl-CoA) into the reducing power of nicotinamide adenine dinucleotide (NADH). The TCA cycle is part of the larger glucose metabolism whereby glucose is oxidized to form pyruvate, which is then oxidized and enters the TCA cycle as acetyl-CoA. Half of the intermediates on which the cycle depends are also the origin of pathways leading to important compounds such as fatty acids, amino acids, or porphyrins. If any of these intermediates are thus diverted, the integrity of the cycle is broken and the cycle no longer functions. Production of essential energy can only be resumed if the diverted intermediate or a subsequent intermediate that leads to oxaloacetate can be replenished by refilling reactions.

Krebs cycle intermediates (precursors)

These intermediates are numbered on the diagram below as Citrate, Isocitrate, Oxoglutarate, Succinyl-CoA, Succinate, Fumarate, Malate, Oxaloacetate (Oxaloacetic acid).

Krebs cycle steps

It is an eight steps process. Krebs cycle or TCA cycle takes place in the matrix of mitochondria under aerobic condition.

Step 1: The first step is the condensation of acetyl CoA with 4-carbon compound oxaloacetate to form 6C citrate, coenzyme A is released. The reaction is catalyzed by citrate synthase.

Step 2: Citrate is converted to its isomer, isocitrate. The enzyme aconitase catalyzes this reaction.

Step 3: Isocitrate undergoes dehydrogenation and decarboxylation to form 5C **α**-ketoglutarate. A molecular form of CO2 is released. Isocitrate dehydrogenase catalyzes the reaction. It is an NAD^+ dependent enzyme. NAD^+ is converted to NADH.

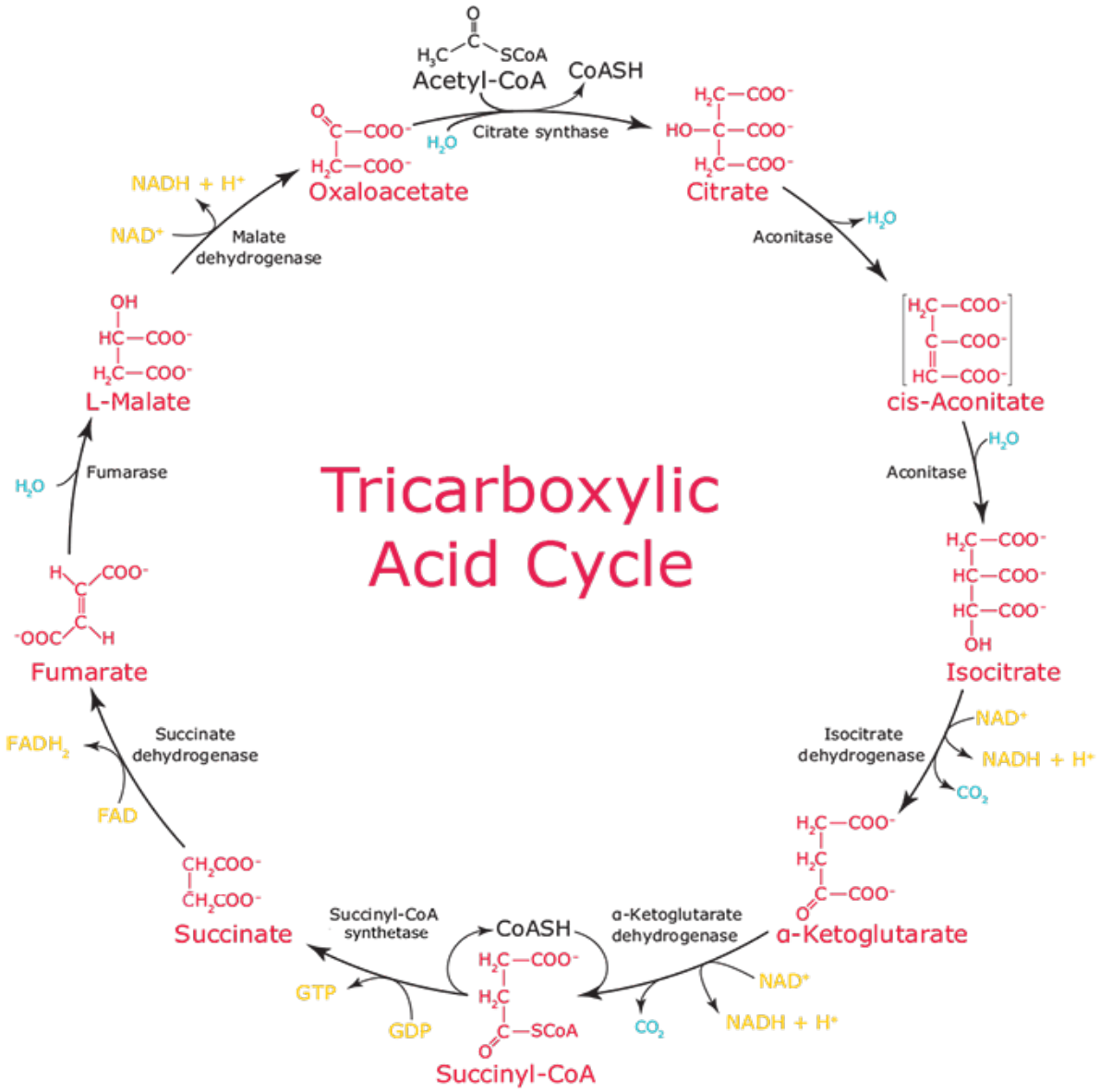
Step 4: **α**-ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by the **α**-ketoglutarate dehydrogenase enzyme complex. One molecule of CO_2 is released and NAD^+ is converted to NADH.

Step 5: Succinyl CoA forms succinate. The enzyme succinyl CoA synthetase catalyzes the reaction. This is coupled with substrate-level phosphorylation of GDP to get GTP. GTP transfers its phosphate to ADP forming ATP.

Step 6: Succinate is oxidized by the enzyme succinate dehydrogenase to fumarate. In the process, FAD is converted to $FADH_2$.

Step 7: Fumarate gets converted to malate by the addition of one H_2O. The enzyme catalyzing this reaction is fumarase.

Step 8: Malate is dehydrogenated to form oxaloacetate, which combines with another molecule of acetyl CoA and starts the new cycle. Hydrogens removed, get transferred to NAD^+ forming NADH. Malate dehydrogenase catalyzses the reaction. (https://www.youtube.com/watch?v=ubzw64PQPqM).



Summary of Krebs cycle

Location: Krebs cycle occurs in the mitochondrial matrix.

Krebs cycle reactants: Acetyl CoA, which is produced from the end product of glycolysis, i.e. pyruvate and it condenses with 4 carbon oxaloacetate, which is generated back in the Krebs cycle.

Krebs cycle products

Each citric acid cycle forms the following products:

2 molecules of CO_2 are released. Removal of CO_2 or decarboxylation of citric acid takes place at two places: In the conversion of isocitrate (6C) to **α**-ketoglutarate (5C). In the conversion of **α**-ketoglutarate (5C) to succinyl CoA (4C). 1 ATP is produced in the conversion of succinyl CoA to succinate, 3 NAD^+ are reduced to NADH and 1 FAD^+ is converted to $FADH_2$ in the following reactions:

Isocitrate to **α**-ketoglutarate → NADH

α-ketoglutarate to succinyl CoA → NADH

Succinate to fumarate → $FADH_2$

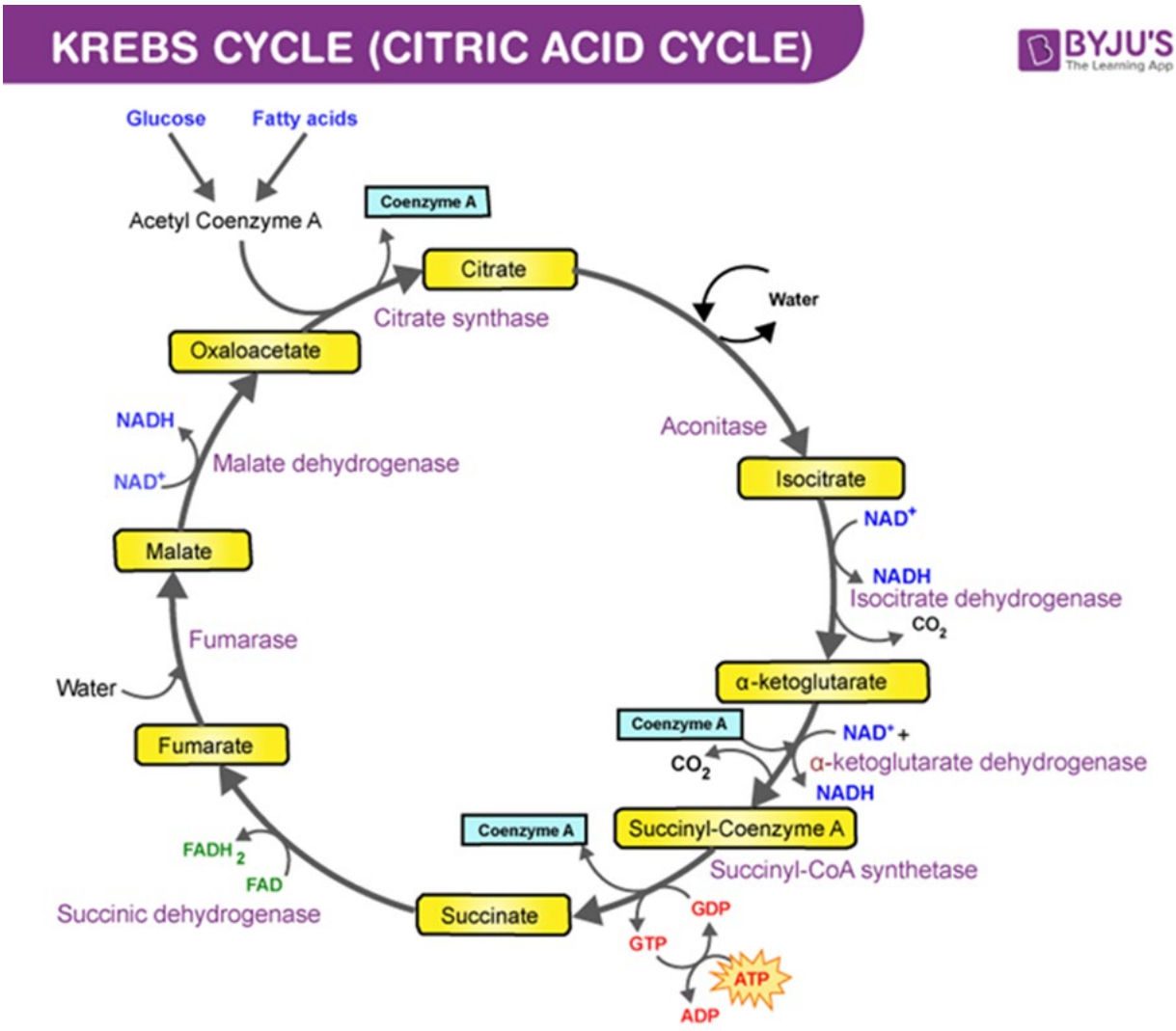
Malate to Oxaloacetate → NADH

Note that 2 molecules of Acetyl CoA are produced from oxidative decarboxylation of 2 pyruvates so two cycles are required per glucose molecule. To summarize, for complete oxidation of a glucose molecule, Krebs cycle yields 4 CO_2, 6 NADH, 2 $FADH_2$ and 2 ATPs. Each molecule of NADH can form 2-3 ATPs and each $FADH_2$ gives 2 ATPs on oxidation in the electron transport chain. It is a series of events in living organisms in which acetic acid or acetyl equivalent oxidation produces energy for storage in phosphate bonds (as in ATP).

The Krebs cycle yields three NADH molecules (two cycles) and 18 ATP molecules. In two cycles, four $FADH_2$ molecules are generated, yielding four ATP molecules. Two GTP molecules are created in two cycles, resulting in the release of two ATP molecules.

TCA cycle applications

These TCA-related metabolic applications are commonly studied using stable isotope-labeled compounds and mass spectrometry: Lipid metabolism, Amino acid metabolism, Protein metabolism (Turnover), Glucose metabolism, Energy expenditure, Metabolomics.



Significance of Krebs cycle

Krebs cycle is the final pathway of oxidation of glucose, fats and amino acids. Many organisms are dependent on nutrients other than glucose as an energy source. Amino acids (metabolic product of proteins) are deaminated and get converted to pyruvate and other intermediates of the Krebs cycle. They enter the cycle and get metabolized e.g. alanine is converted to pyruvate, glutamate to **α**-ketoglutarate, aspartate to oxaloacetate on deamination.

Fatty acids undergo ***β***-oxidation to form acetyl CoA, which enters the Krebs cycle. It is the major source of ATP production in the cells. A large amount of energy is produced after complete oxidation of nutrients. It plays an important role in gluconeogenesis and lipogenesis and interconversion of amino acids.

Many intermediate compounds are used in the synthesis of amino acids, nucleotides, cytochromes and chlorophylls, etc. Vitamins play an important role in the citric acid cycle. Riboflavin, niacin, thiamin and pantothenic acid as a part of various enzymes cofactors (FAD, NAD) and coenzyme A.

Regulation of Krebs cycle depends on the supply of NAD^+ and utilization of ATP in physical and chemical work. The genetic defects of the Krebs cycle enzymes are associated with neural damage. As most of the biological processes occur in the liver to a significant extent, damage to liver cells has a lot of repercussions.

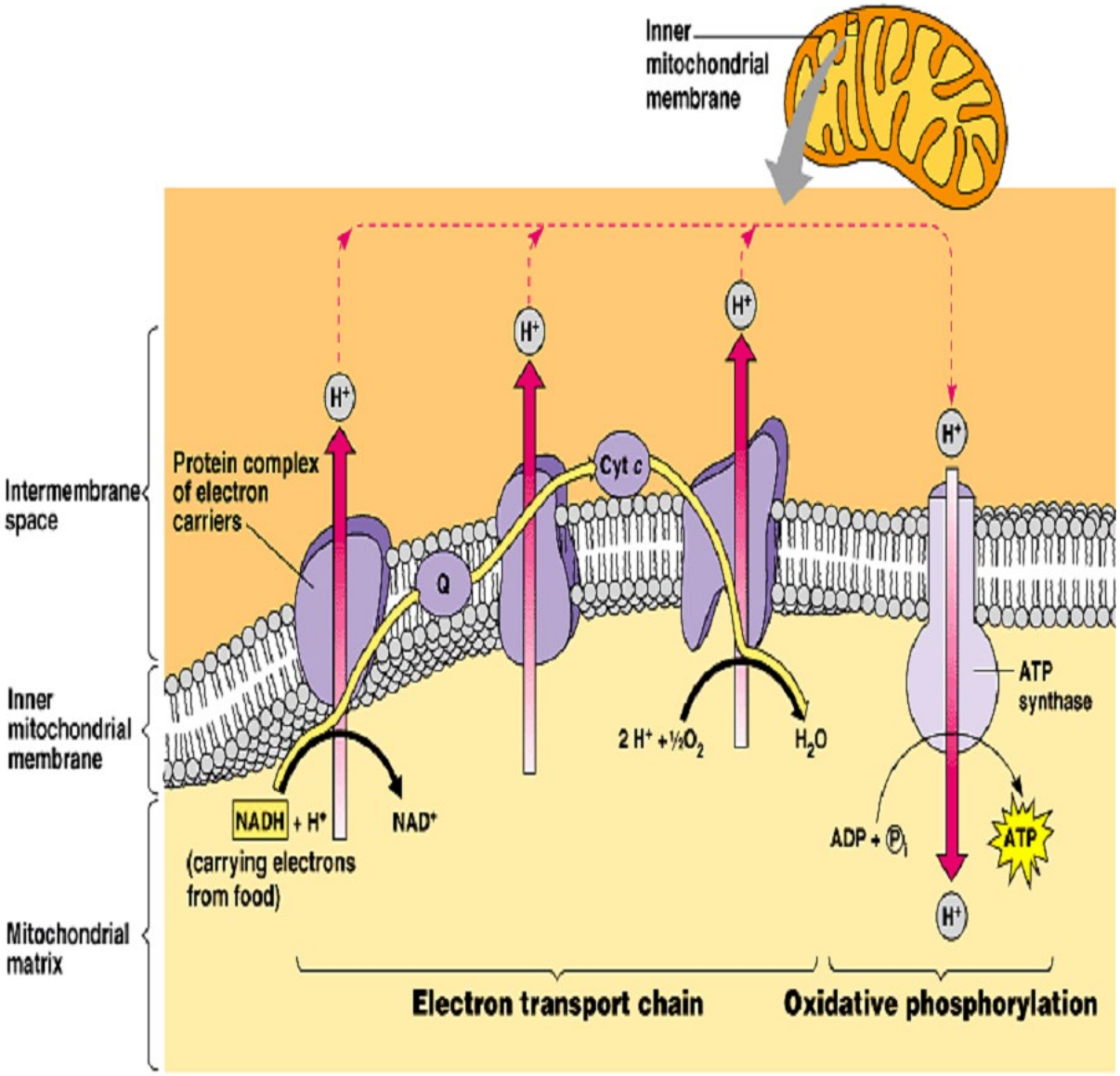
Hyperammonemia occurs in liver diseases and leads to convulsions and coma. This is due to reduced ATP generation as a result of the withdrawal of **α**-ketoglutarate and formation of glutamate, which forms glutamine.

Electron transport chain (Oxidative phosphorylation)

The electron transport chain is a series of four protein complexes that couple redox reactions, creating an electrochemical gradient that leads to the creation of ATP in a complete system named oxidative phosphorylation. It occurs in mitochondria in both cellular respiration and photosynthesis. In the former, the electrons come from breaking down organic molecules, and energy is released. In the latter, the electrons enter the chain after being excited by light, and the energy released is used to build carbohydrates.

Aerobic cellular respiration is made up of three parts: glycolysis, the citric acid (Krebs) cycle, and oxidative phosphorylation. In glycolysis, glucose metabolizes into two molecules of pyruvate, with an output of ATP and nicotinamide adenine dinucleotide (NADH). Each pyruvate oxidizes into acetyl CoA and an additional molecule of NADH and carbon dioxide (CO_2). The acetyl CoA is then used in the citric acid cycle, which is a chain of chemical reactions that produce CO_2, NADH, flavin adenine dinucleotide ($FADH_2$), and ATP. In the final step, the three NADH and one $FADH_2$ amassed from the previous steps are used in oxidative

phosphorylation, to make water and ATP. (https://www.youtube.com/watch?v=zJNx1DDqIVo).



Electron transport chain

It is the metabolic mechanism through which electrons move from one carrier to another. In two cycles, oxidative phosphorylation produces two NADH molecules while releasing six ATP molecules. All of the preceding events result in a net ATP gain of 38 molecules from a single glucose molecule.

Fermentation

Fermentation occurs in the absence of oxygen (anaerobic conditions), and in the presence of beneficial microorganisms (yeasts, molds, and bacteria) that obtain their energy through fermentation. If enough sugar is available, some yeast cells, such as *Saccharomyces cerevisiae*, prefer fermentation to aerobic respiration even when oxygen is abundant.

1. During the fermentation process, these beneficial microbes break down sugars and starches into alcohols and acids, making food more nutritious and preserving it so people can store it for longer periods of time without it spoiling.

2. Fermentation products provide enzymes necessary for digestion. This is important because humans are born with a finite number of enzymes, and they decrease with age. Fermented foods contain the enzymes required to break them down.

3. Fermentation also aids in pre-digestion. During the fermentation process, the microbes feed on sugars and starches, breaking down food before anyone's even consumed it.

Advantages of fermentation

Fermented foods are rich in probiotics, beneficial microorganisms that help maintain a healthy gut so it can extract nutrients from food.

1. Probiotics aid the immune system because the gut produces antibiotic, anti-tumor, anti-viral, and antifungal substances, and pathogens don't do well in the acidic environment fermented foods create.

2. Fermentation also helps neutralize anti-nutrients like phytic acid, which occurs in grains, nuts, seeds, and legumes and can cause mineral deficiencies. Phytates also make starches, proteins, and fats less digestible, so neutralizing them is extremely beneficial.

3. Fermentation can increase the vitamins and minerals in food and make them more available for absorption. Fermentation increases B and C vitamins and enhances folic acid, riboflavin, niacin, thiamin, and biotin. The probiotics,

enzymes, and lactic acid in fermented foods facilitate the absorption of these vitamins and minerals into the body.

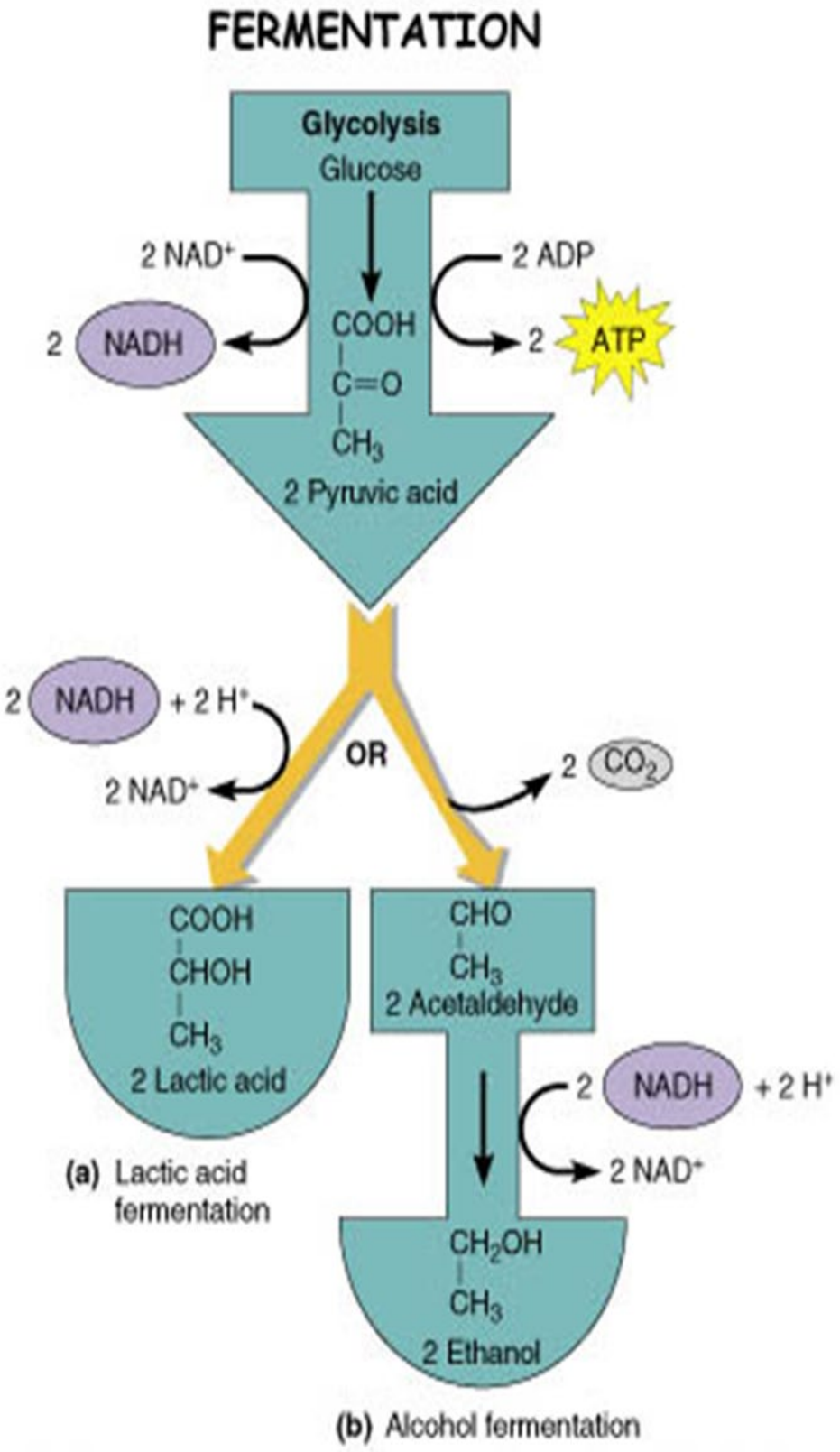
Types of fermentation

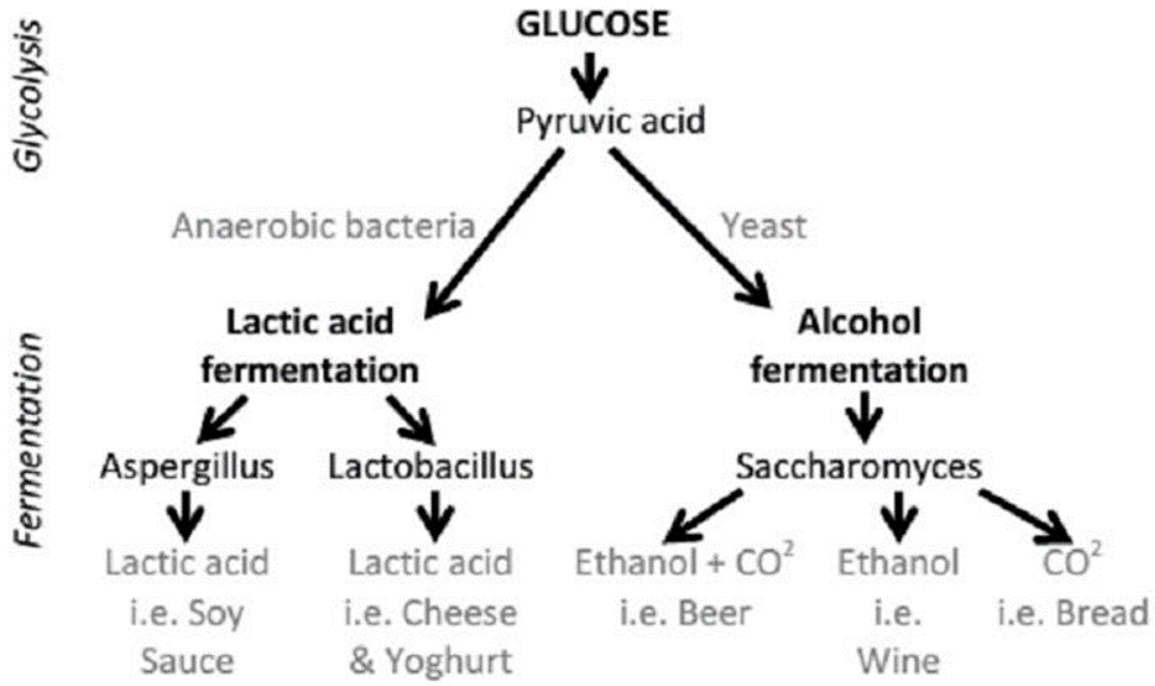
Microbes specialized at converting certain substances into others can produce a variety of foodstuffs and beverages. These are three distinct types of fermentation that people use.

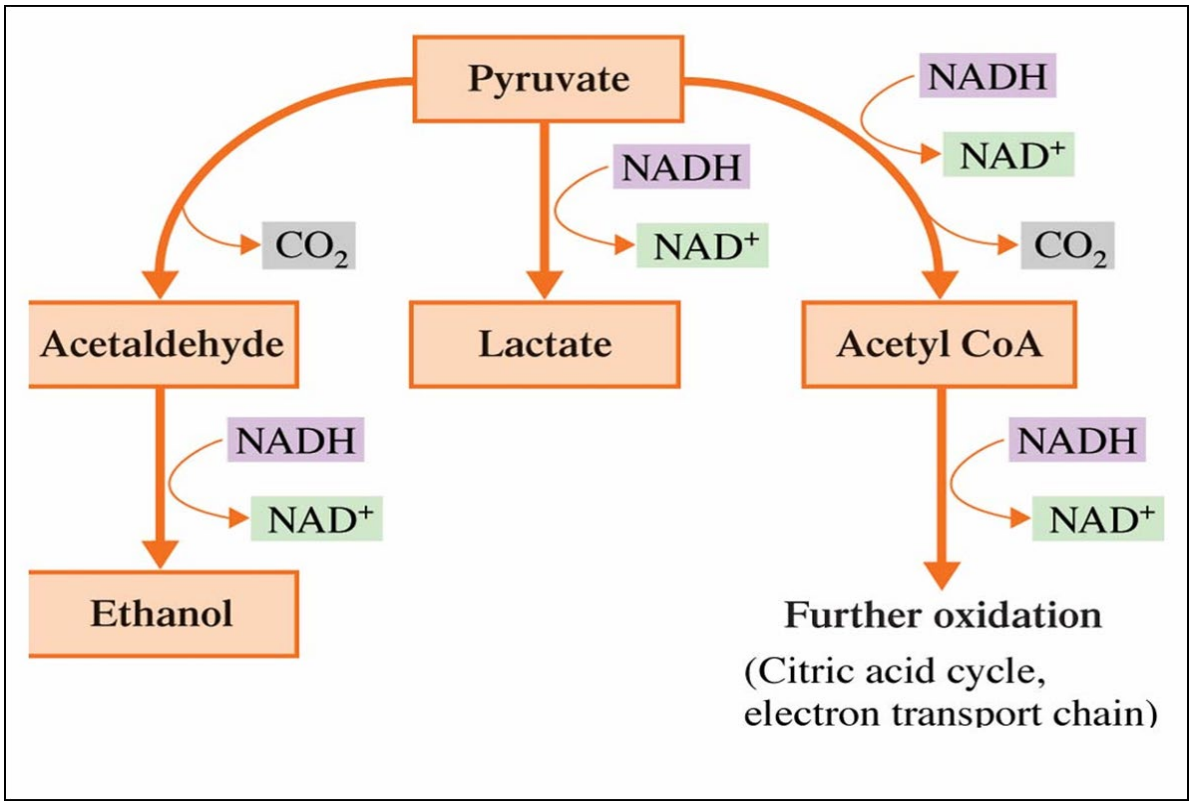
1. Lactic acid fermentation. Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation. These anaerobic chemical reactions, pyruvic acid uses nicotinamide adenine dinucleotide+hydrogen (NADH) to form lactic acid and NAD^+ (Lactic acid fermentation also occurs in human muscle cells. During strenuous activity, muscles can expend adenosine triphosphate (ATP) faster than oxygen can be supplied to muscle cells, resulting in lactic acid buildup and sore muscles. In this scenario, glycolysis, which breaks down a glucose molecule into two pyruvate molecules and doesn't use oxygen, produces ATP). Lactic acid bacteria are vital to producing and preserving inexpensive, wholesome foods, which is especially important in feeding impoverished populations. This method makes sauerkraut, pickles, kimchi, yogurt, and sourdough bread.

2. Alcohol fermentation/Ethanol fermentation. Yeasts break pyruvate molecules—the output of the metabolism of glucose ($C_6H_{12}O_6$) known as glycolysis—in starches or sugars down into alcohol and carbon dioxide molecules. Alcoholic fermentation produces wine and beer.

3. Acetic acid fermentation. Starches and sugars from grains and fruit ferment into sour tasting vinegar and condiments. Examples include apple cider vinegar, wine vinegar, and kombucha.




Glycolysis
Fermentation
GLUCOSE
Pyruvic acid
Anaerobic bacteria
Yeast
Lactic acid fermentation
Alcohol fermentation
Aspergillus
Lactobacillus
Saccharomyces
Lactic acid i.e. Soy Sauce
Lactic acid i.e. Cheese & Yoghurt
Ethanol + CO2 i.e. Beer
Ethanol i.e. Wine
CO2 i.e. Bread

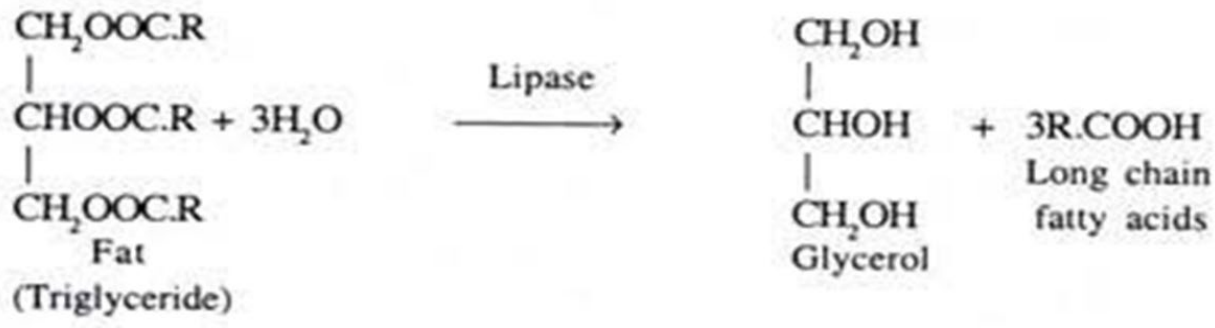

Pyruvate
NADH
NAD+
CO2
Acetaldehyde
Lactate
Acetyl CoA
Ethanol
Further oxidation
(Citric acid cycle, electron transport chain)

Lipid metabolism

Lipids are diverse group of organic compounds including fats, oils, hormones, and certain components of membranes that are grouped together because they do not interact appreciably with water. One type of lipid, the triglycerides, is sequestered as fat in adipose cells, which serve as the energy-storage depot for organisms and also provide thermal insulation. Some lipids such as steroid hormones serve as chemical messengers between cells, tissues, and organs, and others communicate signals between biochemical systems within a single cell. The membranes of cells and organelles (structures within cells) are microscopically thin structures formed from two layers of phospholipid molecules. Membranes function to separate individual cells from their environments and to compartmentalize the cell interior into structures that carry out special functions. So important is this compartmentalizing function that membranes, and the lipids that form them, must have been essential to the origin of life itself. Lipids are hydrophobic compounds. Although biological lipids are not large macromolecular polymers (e.g., proteins, nucleic acids, and polysaccharides), many are formed by the chemical linking of several small constituent molecules. Many of these molecular building blocks are similar, or homologous, in structure. The homologies allow lipids to be classified into a few major groups: fatty acids, fatty acid derivatives, cholesterol and its derivatives, and lipoproteins.

Biological fatty acids, members of the class of compounds known as carboxylic acids, are composed of a hydrocarbon chain with one terminal carboxyl group (COOH). The fragment of a carboxylic acid not including the hydroxyl (OH) group is called an acyl group. Under physiological conditions in water, this acidic group usually has lost a hydrogen ion (H^+) to form a negatively charged carboxylate group (COO^-). In addition to straight-chain hydrocarbons, fatty acids may also contain pairs of carbons linked by one or more double bonds, methyl branches, or a three-carbon cyclopropane ring near the center of the carbon chain.

Fats are degraded by lipase enzyme to produce glycerol and fatty acids according to the following equation:

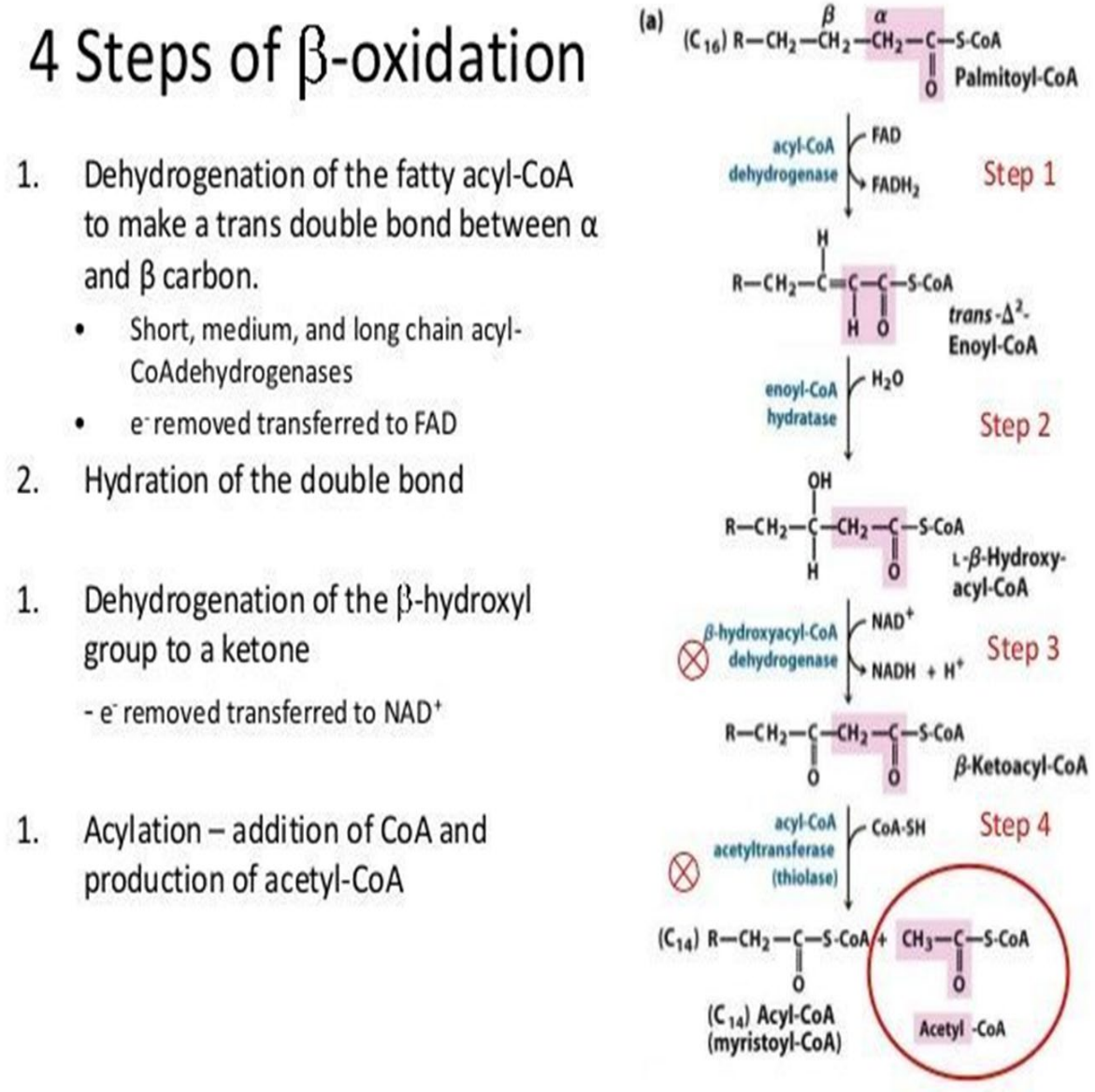


Beta oxidation

Lipids are abundant in host tissues, and fungal pathogens in the phylum basidiomycota possess both peroxisomal and mitochondrial β-oxidation pathways to utilize this potential carbon source. In addition, lipids are important signaling molecules in both fungi and mammals. They are degraded in the catabolic process called beta oxidation. During beta oxidation, the third (or beta) carbon of the saturated fatty acid chain of the fatty acyl CoA is oxidized to a ketone. β-Oxidation of fatty acids is important for the utilization of storage lipids or exogenous fatty acids to generate acetyl coenzyme A (acetyl-CoA) for central carbon metabolism. Most organisms have multiple enzymes for each of the four steps in β-oxidation to accommodate fatty acids of different chain length or saturation state. In mammals, β-oxidation occurs in both peroxisomes and mitochondria. The peroxisome is thought to be responsible for the oxidation of long-chain fatty acids, and the mitochondrion oxidizes short-chain fatty acids and also performs the final oxidation step. Fungal β-oxidation is not well characterized, and it was previously thought that fungi might have peroxisomal β-oxidation only because *Saccharomyces cerevisiae* lacks the enzymes for mitochondrial β-oxidation. However, recent in silico surveys of the pathways encoded in more than 50 fungal genomes revealed that most fungi possess both

mitochondrial and peroxisomal pathways. Mitochondrial β-oxidation has also been convincingly demonstrated in the saprophytic ascomycete *Aspergillus nidulans*.

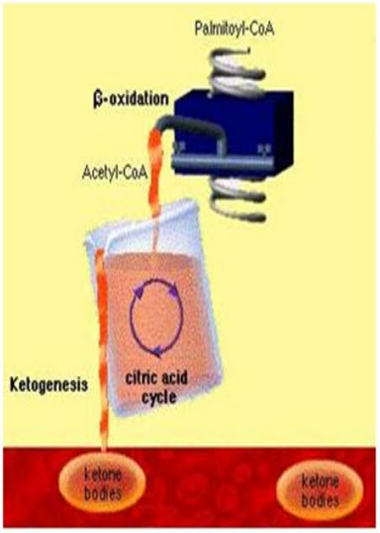
Beta oxidation is a spiral pathway. Each round consists of four enzyme-catalyzed steps that yield one molecule of acetyl CoA and an acyl CoA shortened by two carbons, which becomes the starting substrate for the next round. Seven rounds of beta oxidation degrade a C_{16} fatty acid to eight molecules of acetyl CoA. Complete oxidation of one molecule of palmitic acid to carbon dioxide and water yields 129 molecules of ATP. One round of beta oxidation yields 17 ATP.

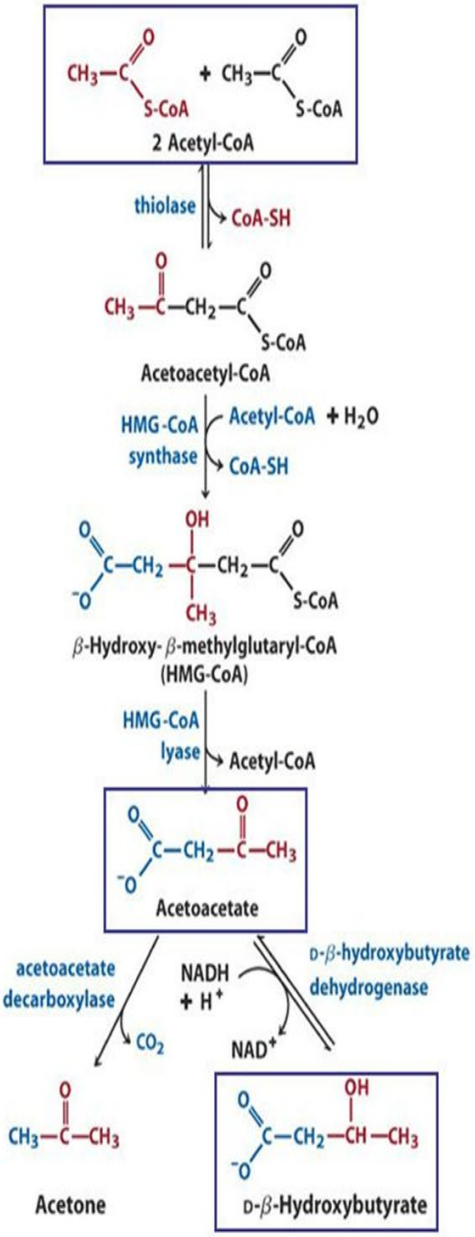


The reactions of ß-oxidation

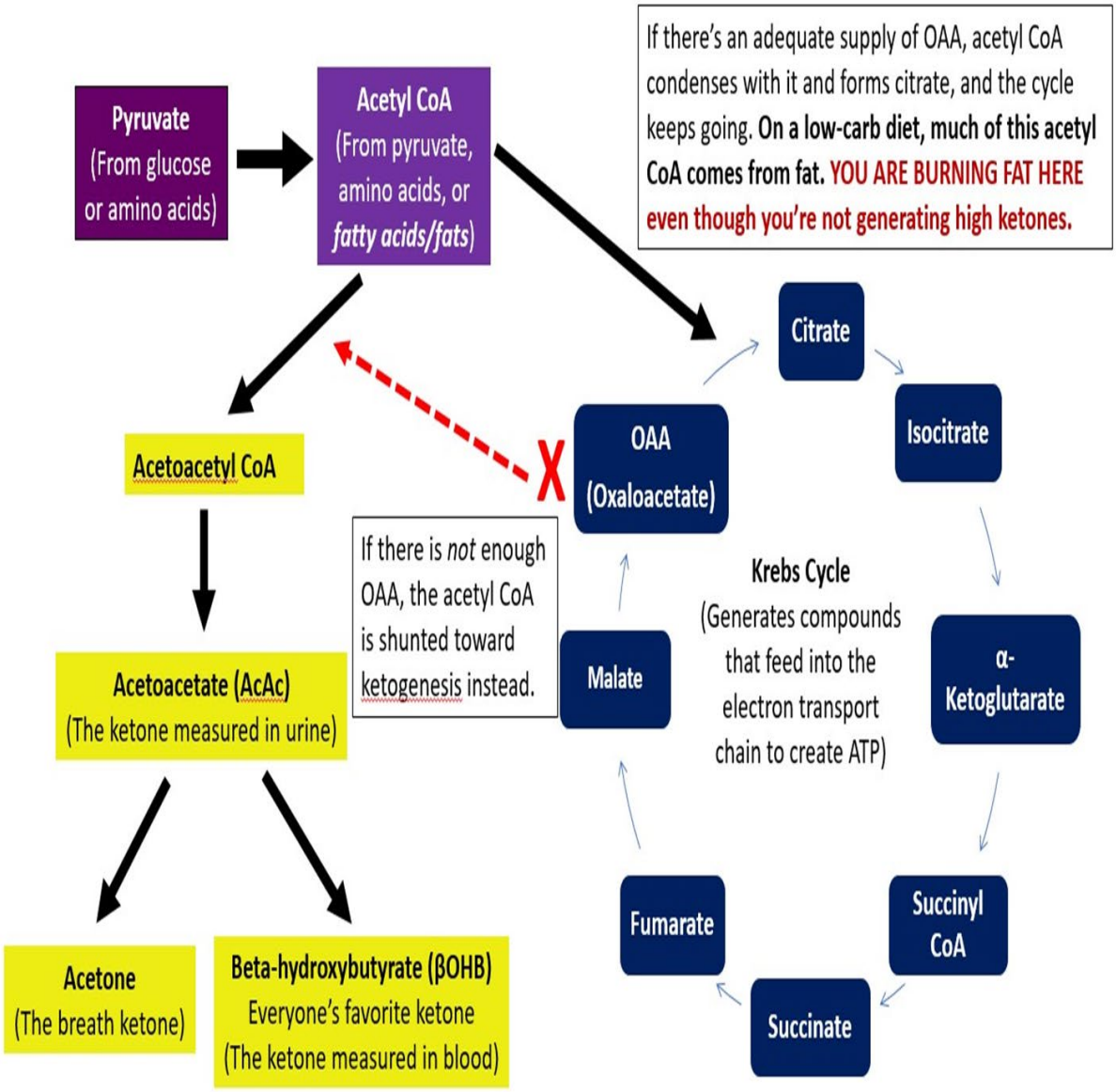
Ketogenesis

When fatty acid oxidation produces more acetyl-CoA than can be combined with OAA to form citrate, then the **"extra" acetyl-CoA** is converted to acetoacetyl-CoA and ketone bodies, including acetone. **Ketogenesis** (synthesis of ketone bodies) takes place primarily in the liver.

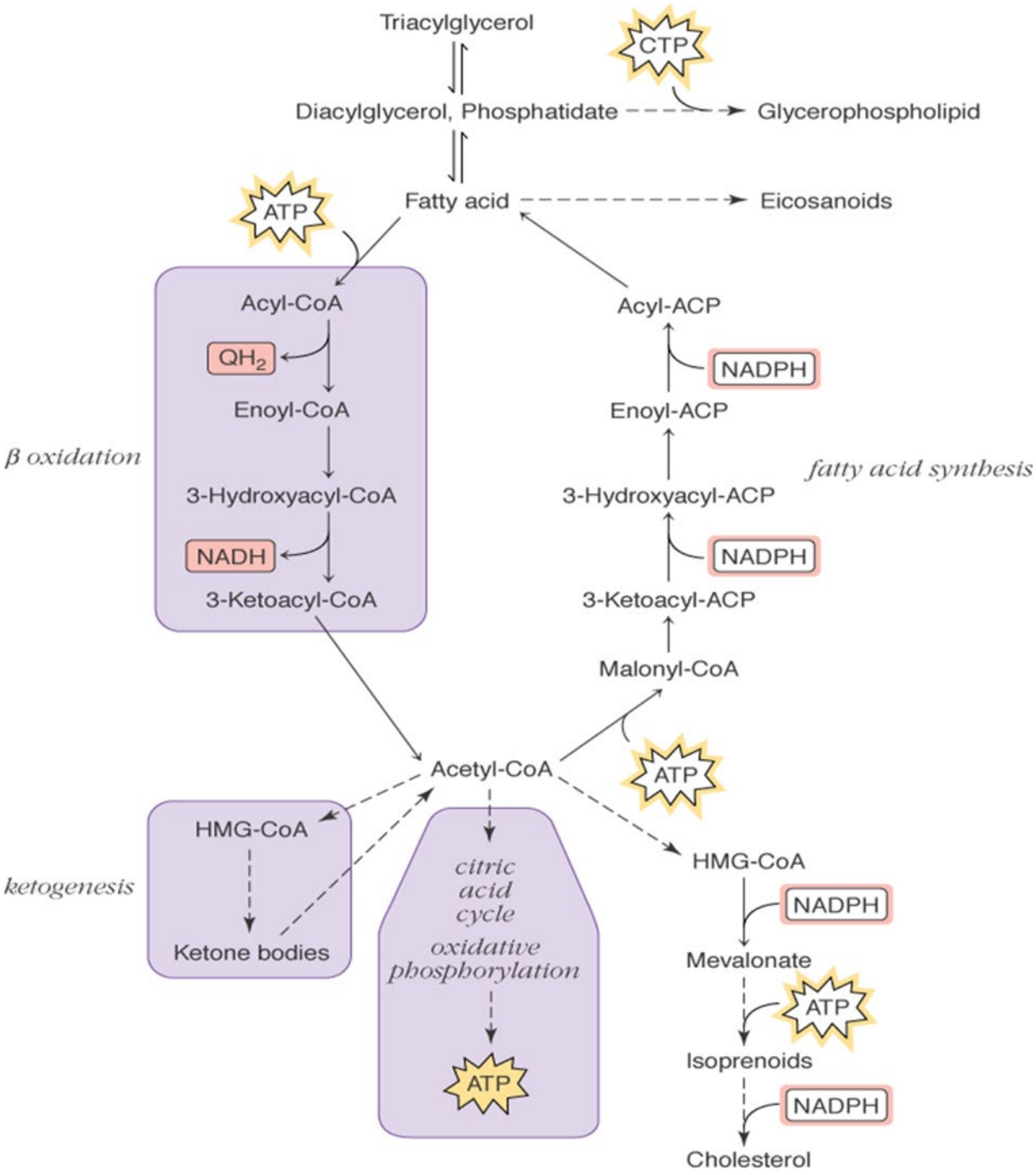




Ketogenesis and Krebs cycle



Summary of lipid metabolism



Nitrogen metabolism

Polymeric nitrogen containing compounds are proteins and nucleic acids that define the major attributes of organism such as function and structure. Operation and mechanism of metabolic pathways is provided by proteins. However genetic information is stored in nucleic acid polymers. Nitrogen is one of the most prevalent essential macro-elements which regulates fungal growth and metabolism. Anabolic processes includes: Nitrogen fixation (as in bacteria, e.g. *Rhizobium*), Amino acids synthesis, Protein synthesis. However, Catabolic processes includes: Proteolysis and amino acids destruction, Nitrification, denitrification.

Nitrification is the biological oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate.

$NO_3+O_2+2e- \rightarrow NH_2OH + H_2O$

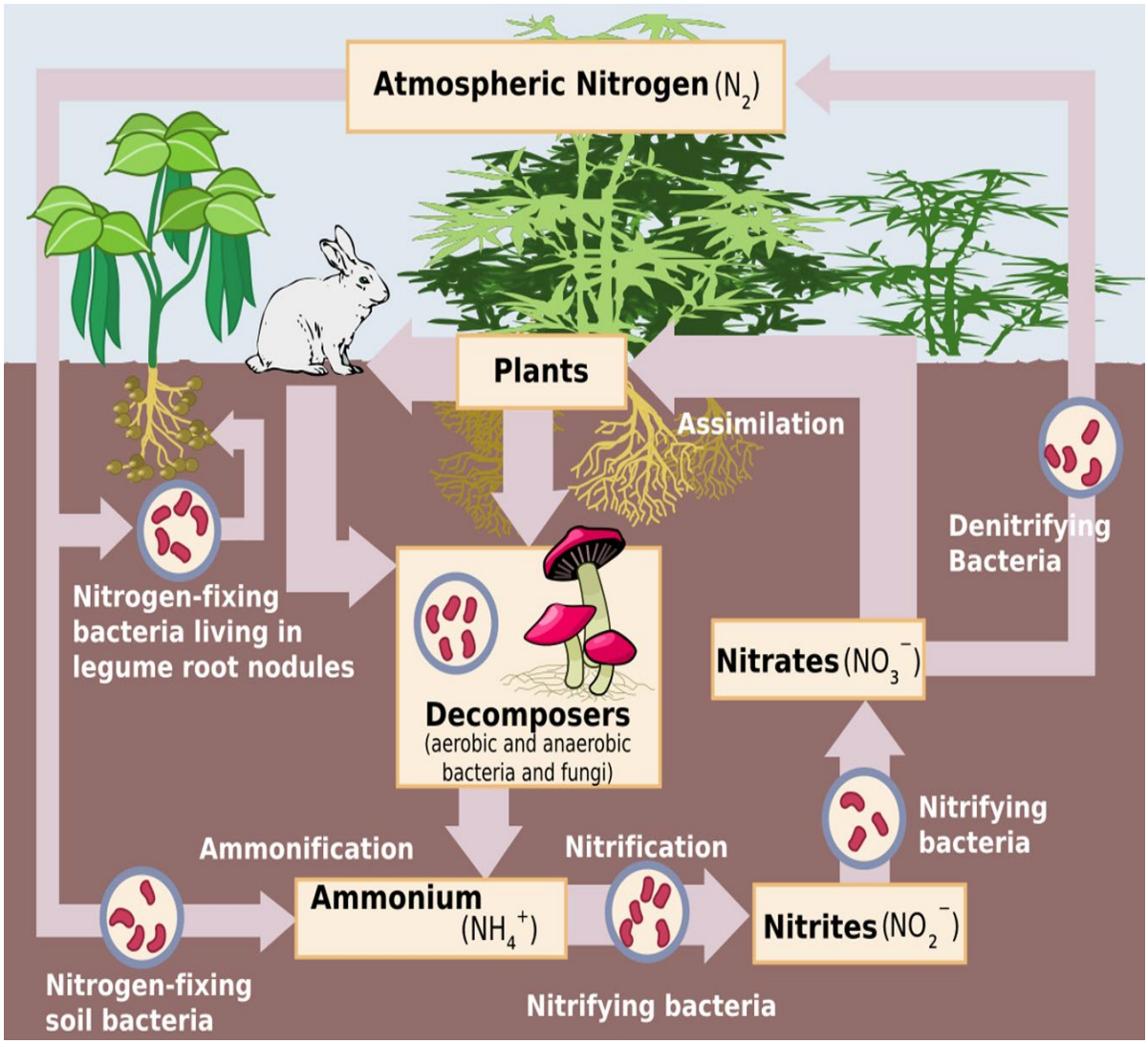
$NH_2OH +H_2O \rightarrow NO_2 +5\ H+ +4e-$

$NO_2 +O_2 \rightarrow NO_3$

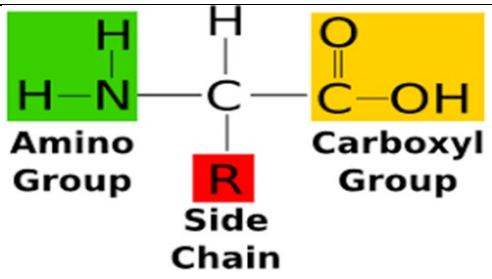
Denitrification is the process that coverts nitrate to nitrogen gas.

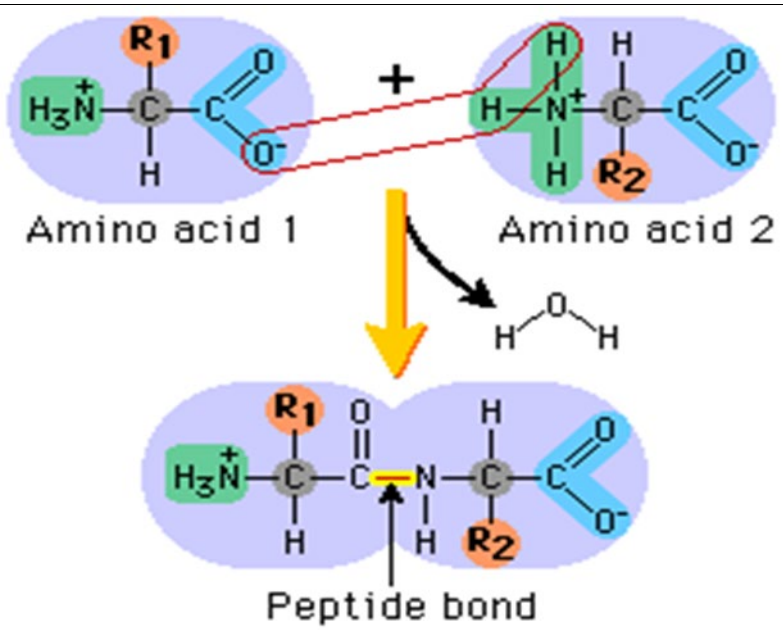
$NO_3 \rightarrow NO_2 \rightarrow NO + N_2O \rightarrow N_2$

Nitrogen cycle



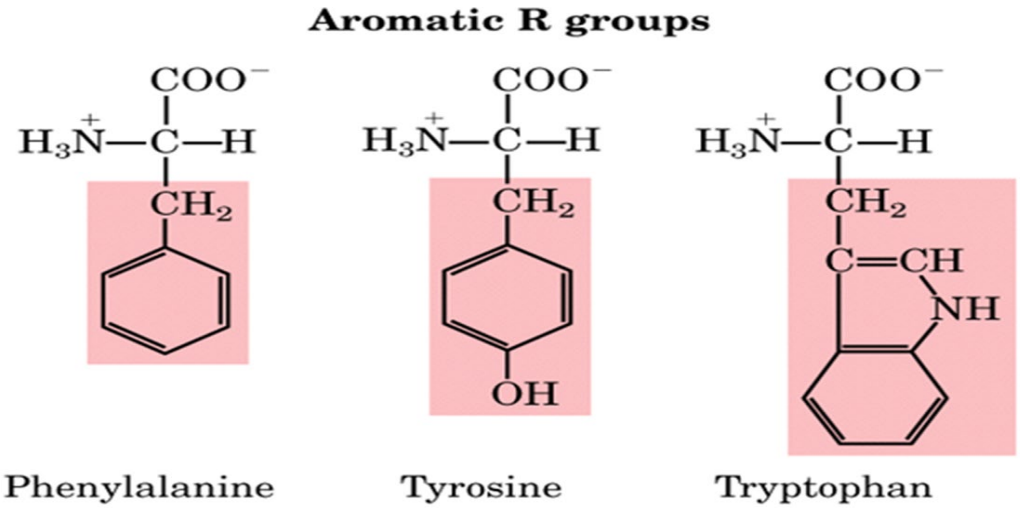
Amino acids

Amino acid structure	

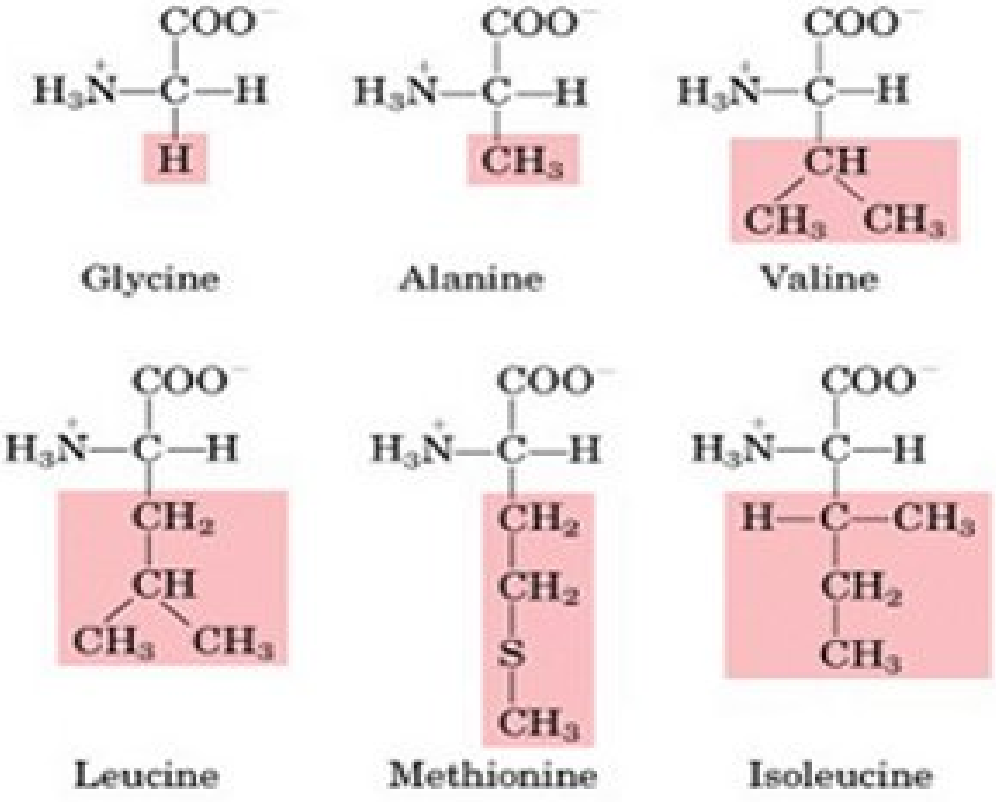
Formation of peptide bond	

Types of amino acids

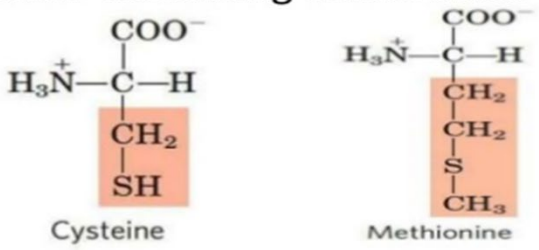
Aromatic amino acids



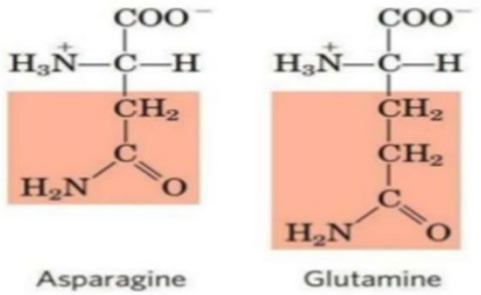
Aliphatic amino acid



❑ Sulfur-containing amino acids



❑ Amide group-containing amino acids



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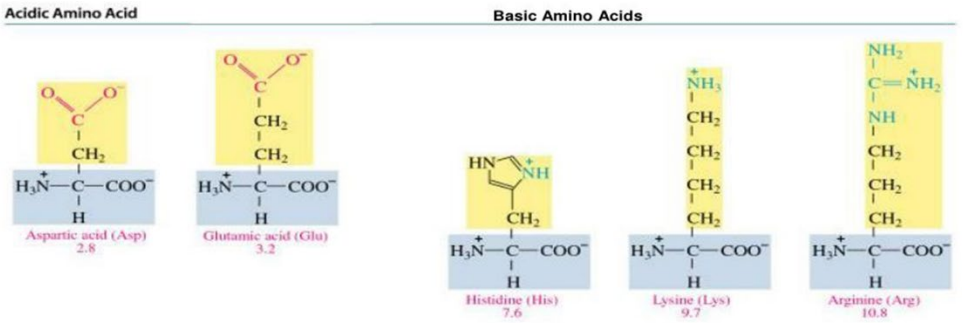
Acidic and Basic Amino Acids

An amino acid is

- Acidic with a carboxyl R group (COO^-).
- Basic with an amino R group (NH_3^+).



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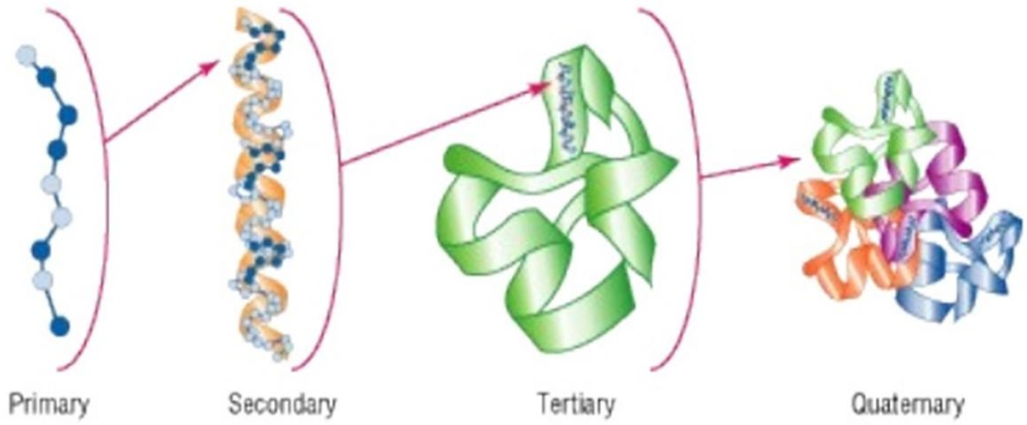
Protein structure

- Proteins, amino acid chains, can be any length and any combination.
- They have four levels of structure.



Antibiotics

Antibiotics are compounds of natural, semi-synthetic, or synthetic origin which inhibit growth of microorganisms without significant toxicity to the human or animal host. The key concept of antibiotic therapy is selectivity. The independent evolutionary history of bacterial (prokaryotic) and host (eukaryotic) cells led to a significant difference in cell organization, biochemical pathways and structures of proteins and RNA. These differences form the basis for drug selectivity.

Cell wall as antibiotic target

Most of the bacteria have a rigid cell wall which protects the cell from changes in osmotic pressure. Presence of the cell wall is critical for the survival of bacterial cell. The structure and composition of bacterial cell wall is dramatically different from the cell envelope of the eukaryotic cell. Therefore, enzymes of cell wall biosynthesis are unique to bacteria and presents an excellent target for antibiotics. According to the structure of their cell wall and staining procedure developed by Christian Gram in 1884, Bacteria are divided into Gram-positive and Gram-negative.

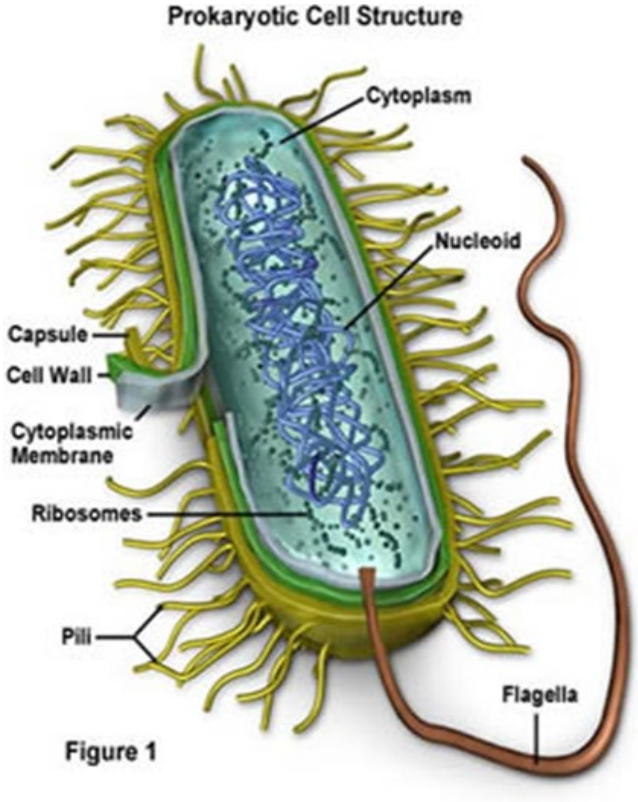
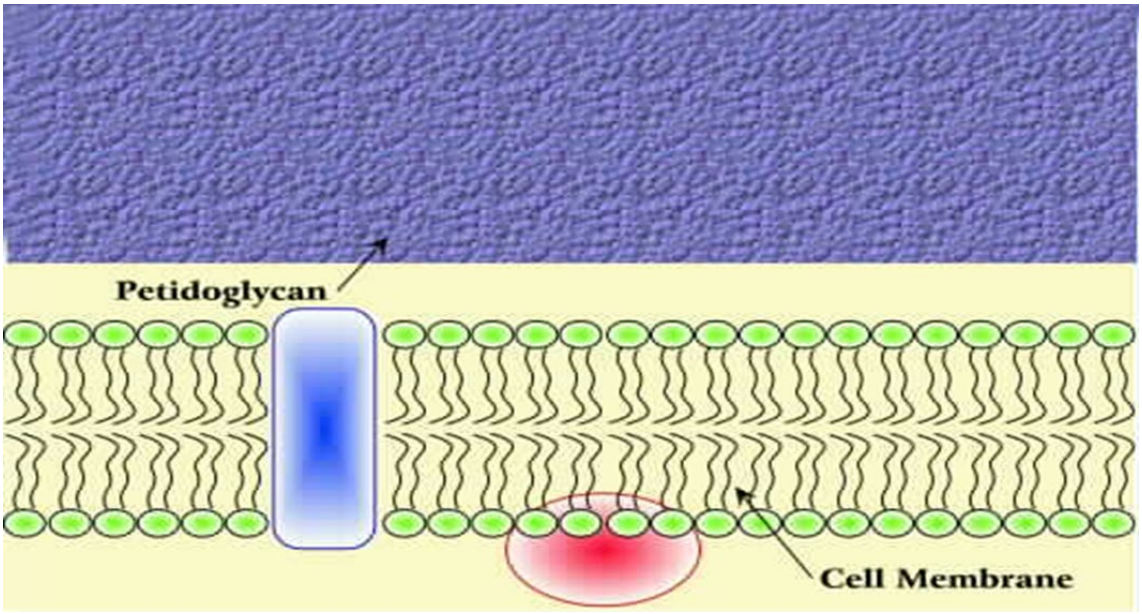


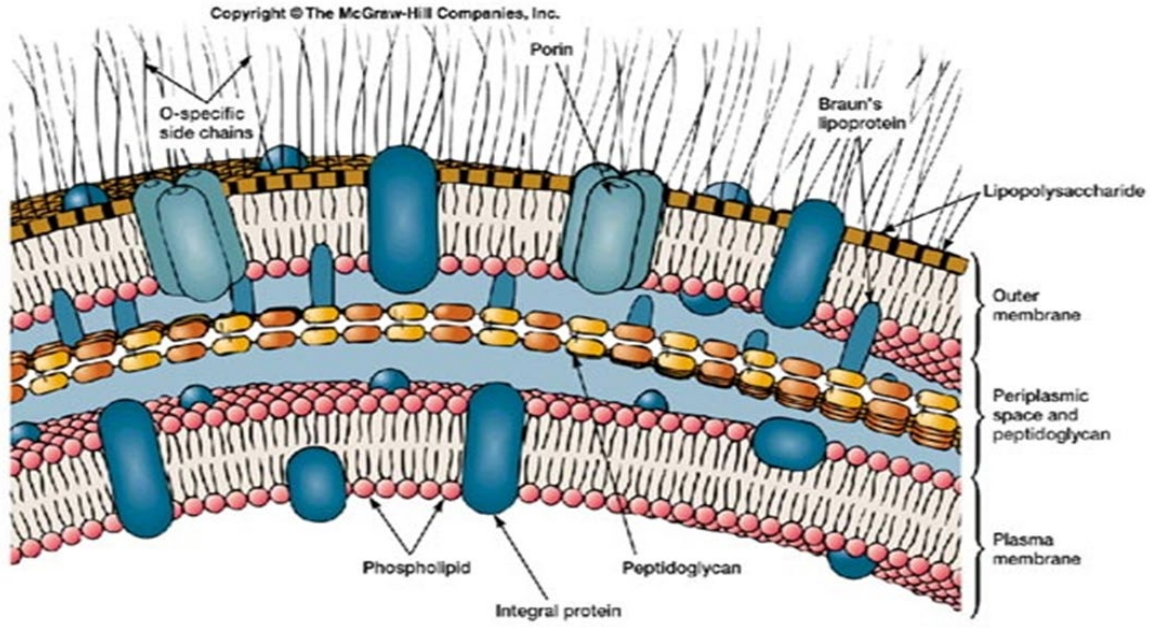
Figure 1

Cell wall of gram positive bacteria

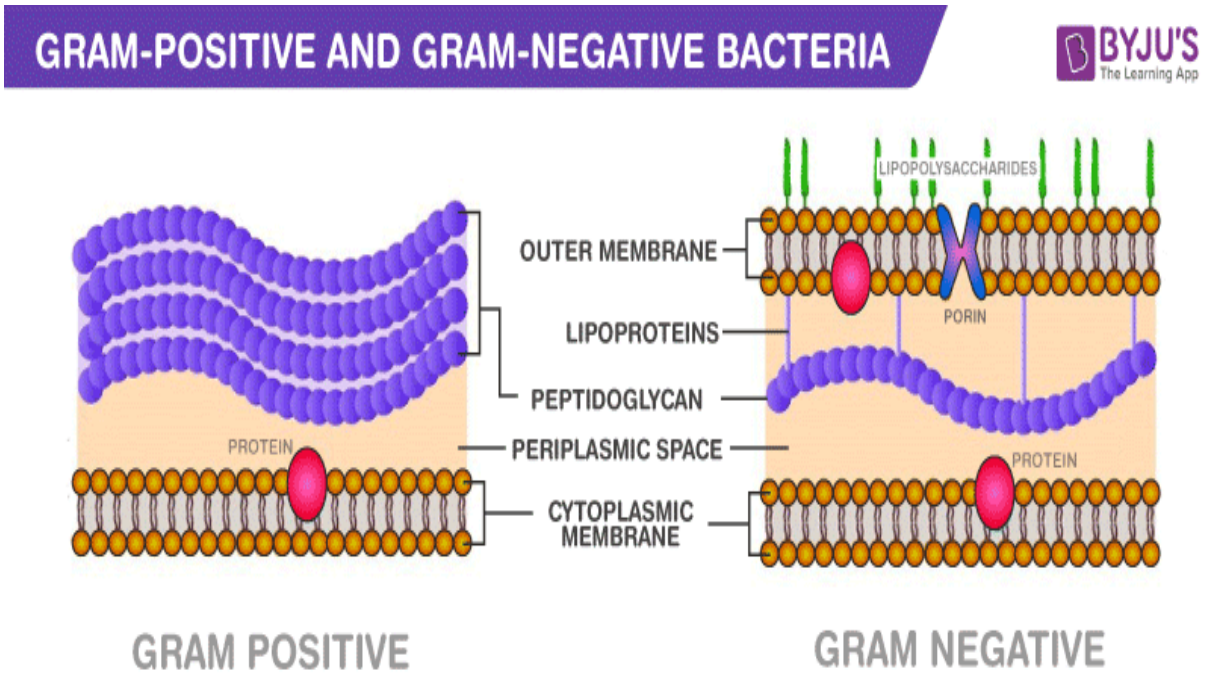


Outside of the cytoplasmic membrane of Gram-positive bacteria lies a thick layer of peptidoglycan which determines the rigidity of the cell wall. In Gram-positive bacteria, peptidoglycan accounts for 50% of the cell weight and up to 90% of the weight of the cell wall. Peptidoglycan layer is 20-80 nm thick. Peptidoglycan compose of polymer of N-acetyl glucosamine and N-acetyl muramic acid.

Cell wall of gram negative bacteria



The cell wall of Gram-negative bacteria consists of the cytoplasmic membrane, a thin layer of peptidoglycan, and an outer membrane. The area between the cytoplasmic membrane and peptidoglycan layer is called the periplasmic space.



Discovery of penicillin

The discovery of penicillin is usually attributed to Scottish Scientist Sir Alexander Fleming in 1928, though others had earlier noted the antibacterial effects of *Penicillium*. The development of penicillin for use as a medicine is attributed to the Australian Nobel Laureate Howard Walter Florey. In March 2000, doctors of the San Juan de Dios Hospital in San Jose (Costa Rica) published manuscripts belonging to the Costa Rican scientist and medical doctor Clodomiro (Clorito) Picado Twight (1887-1944). The manuscripts explained Picado's experiences between 1915 and 1927 about the inhibitory actions of the fungi of genus *Penicillium*. Apparently Clorito Picado had reported his discovery to the Paris Academy of Sciences in Paris, yet did not patent it, even though his investigation had started years before Fleming's. Fleming, at his laboratory in St. Mary's Hospital (now one of Imperial College's teaching hospitals) in London, noticed a halo of inhibition of bacterial growth around a contaminant blue-green mold *Staphylococcus* plate culture. Fleming concluded that the mold was releasing a substance that was inhibiting bacterial growth and lysing the bacteria. He grew a pure culture of the mold and discovered that it was a *Penicillium* mold, now known to be *Penicillium notatum*. Fleming coined the term "penicillin" to describe the filtrate of a broth culture of the *Penicillium* mold. Even in these early stages, penicillin was found to be most effective against Gram-positive bacteria, and ineffective against Gram-negative organisms and fungi. He expressed initial optimism that penicillin would be a useful disinfectant, being highly potent with minimal toxicity compared to antiseptics of the day, but particularly noted its laboratory value in the isolation of "*Bacillus influenzae*" (now *Haemophilus influenzae*). After further experiments, Fleming was convinced that penicillin could not last long enough in the human body to kill pathogenic bacteria and stopped studying penicillin after 1931, but restarted some clinical trials in 1934 and continued to try to get someone to purify it until 1940.

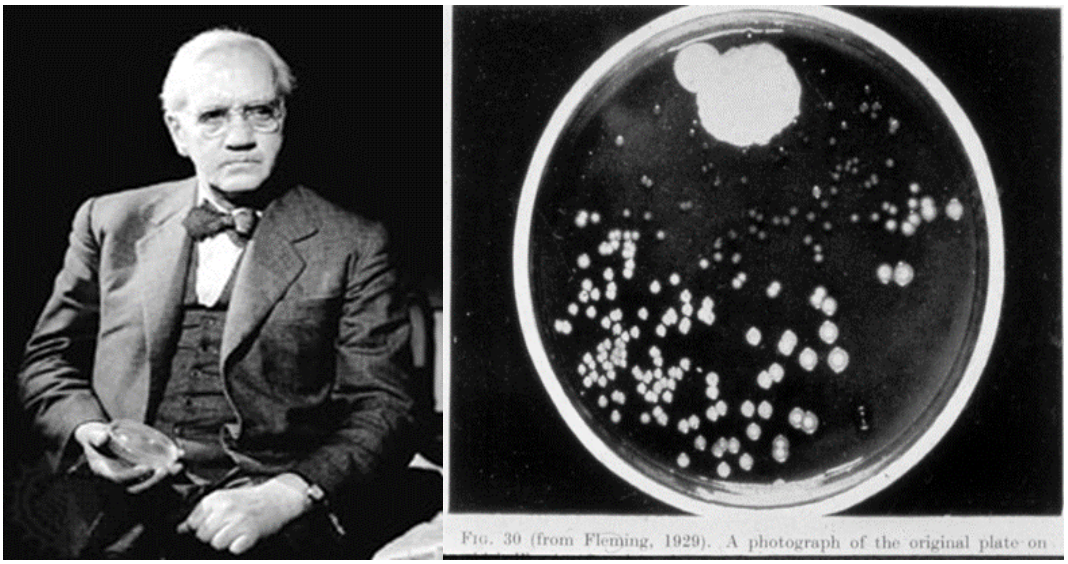


Fig. 30 (from Fleming, 1929). A photograph of the original plate on

In 1939, Australian scientist Howard Walter Florey and a team of researchers (Ernst Boris Chain, A. D. Gardner, Norman Heatley, M. Jennings, J. Orr-Ewing and G. Sanders) at the Sir William Dunn School of Pathology, University of Oxford made significant progress in showing the in vivo bactericidal action of penicillin. Their attempts to treat humans failed due to insufficient volumes of penicillin (the first patient treated was Reserve Constable Albert Alexander), but they proved its harmlessness and effect on mice.

A moldy cantaloupe in a Peoria market in 1941 was found to contain the best and highest quality penicillin after a world-wide search. Some of the pioneering trials of penicillin took place at the Radcliffe Infirmary in Oxford. On March 3, 1942 John Bumstead and Orvan Hess became the first in the world to successfully treat a patient using penicillin. Penicillin was being mass-produced in 1944.

During World War II, penicillin made a major difference in the number of deaths and amputations caused by infected wounds amongst Allied forces; saving an estimated 12-15% of lives. Availability was severely limited, however, by the difficulty of manufacturing large quantities of penicillin and by the rapid renal clearance of the drug necessitating frequent dosing. Penicillins are actively

secreted and about 80% of a penicillin dose is cleared within three to four hours of administration.

This was not a satisfactory solution, however, so researchers looked for a way to slow penicillin secretion. They hoped to find a molecule that could compete with penicillin for the organic acid transporter responsible for secretion such that the transporter would preferentially secrete the competitive inhibitor. The uricosuric agent probenecid proved to be suitable. When probenecid and penicillin are concomitantly administered, probenecid competitively inhibits the secretion of penicillin, increasing its concentration and prolonging its activity. The advent of mass-production techniques and semi-synthetic penicillins solved supply issues, and this use of probenecid declined. Probenecid is still clinically useful, however, for certain infections requiring particularly high concentrations of penicillins.

The chemical structure of penicillin was determined by Dorothy Crowfoot Hodgkin in the early 1940s. A team of Oxford research scientists led by Australian Howard Walter Florey and including Ernst Boris Chain and Norman Heatley discovered a method of mass producing the drug. Chemist John Sheehan at MIT completed the first total synthesis of penicillin and some of its analogs in the early 1950s, but his methods were not efficient for mass production. Florey and Chain shared the 1945 Nobel prize in medicine with Fleming for this work. Penicillin has since become the most widely used antibiotic to date and is still used for many Gram-positive bacterial infections.

Structure and types of Penicillin

Penicillins are a group of β-lactam antibiotics consisting of natural penicillins and semisynthetic penicillins. The basic structure of all penicillins, natural and semisynthetic, is 6-aminopenicillanic acid composed of a four membered heterocyclic β-lactam ring fused with a five membered (benzylpenicillin), penicillin V (Phenoxymethyl penicillin), thiazolidine ring.

This basic structure combines with N-acyl group which is variable and shows structural differences in different type of penicillins. The N-acyl group is the side chain attached to the amino group of 6-aminopenicillanic acid. However, there are three natural penicillins that are produced directly and can be obtained from the fermentation Iiquours of *Pencillium*.

These are penicillin G and penicillin F (phentenyl penicillin). Natural penicillins are obtained as salts of sodium (Na) or potassium (K) or procaine. The structures of natural penicillins as Na-salts.

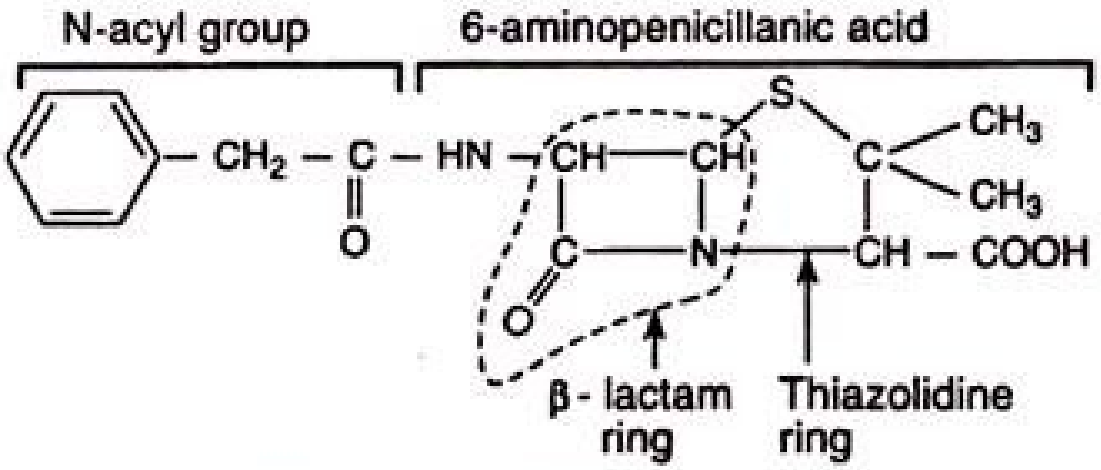


FIG. 45.6. Basic structure of penicillins.

Developments from penicillin

The narrow spectrum of activity of the penicillins, along with the poor activity of the orally-active phenoxymethylpenicillin, led to the search for derivatives of penicillin which could treat a wider range of infections.

The first major development was ampicillin, which offered a broader spectrum of activity than either of the original penicillins. Further development yielded beta-lactamase-resistant penicillins including flucloxacillin, dicloxacillin and methicillin. These were significant for their activity against beta-lactamase-

producing bacteria species, but are ineffective against the methicillin-resistant *Staphylococcus aureus* strains that subsequently emerged.

The line of true penicillins were the antipseudomonal penicillins, such as ticarcillin and piperacillin, useful for their activity against Gram-negative bacteria. However, the usefulness of the beta-lactam ring was such that related antibiotics, including the mecillinams, the carbapenems and, most importantly, the cephalosporins, have this at the center of their structures.

Mechanism of action of beta-lactam antibiotic

β-lactam antibiotics work by inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall. The β-lactam moiety (functional group) of penicillin binds to the enzyme (DD-transpeptidase) that links the peptidoglycan molecules in bacteria, and this weakens the cell wall of the bacterium (in other words, the antibiotic causes cytolysis or death). In addition, the build-up of peptidoglycan precursors triggers the activation of bacterial cell wall hydrolases and auto lysins which further digest the bacteria's existing peptidoglycan.

When the bacteria lose their cell walls they are then called spheroplasts. Penicillin shows a synergistic effect with aminoglycosides since the inhibition of peptidoglycan synthesis allows aminoglycosides to penetrate the bacterial cell wall more easily, allowing its disruption of bacterial protein synthesis within the cell. This results in a lowered MBC for susceptible organisms.

Benzylpenicillin, commonly known as penicillin G, is the gold standard penicillin. Penicillin G is typically given by a parenteral route of administration (not orally) because it is unstable in the hydrochloric acid of the stomach. Because the drug is given parenterally, higher tissue concentrations of penicillin G can be achieved than is possible with phenoxymethylpenicillin. These higher concentrations translate to increased antibacterial activity.

Phenoxymethylpenicillin, commonly known as penicillin V, is the orally-active form of penicillin. It is less active than benzylpenicillin, however, and is only appropriate in conditions where high tissue concentrations are not required.

Semi-synthetic penicillins

Structural modifications were made to the side chain of the penicillin nucleus in an effort to improve oral bioavailability, improve stability to beta-lactamase activity, and increase the spectrum of action.

Narrow spectrum penicillinase-resistant penicillins

This group was developed to be effective against beta-lactamases produced by *Staphylococcus aureus*, and are occasionally known as anti-staphylococcal penicillin. Penicillin is rampantly used for curing infections and to prevent growth of harmful mold.

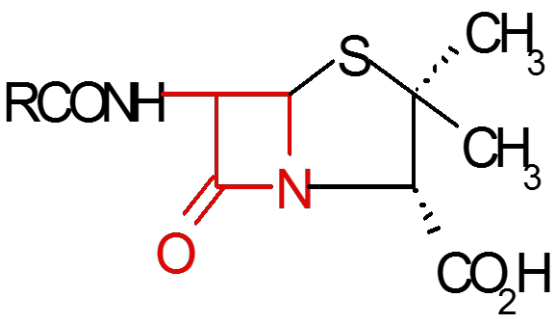
Narrow spectrum β-lactamase-resistant penicillins

This molecule has a spectrum directed towards Gram negative bacteria without activity on *Pseudomonas aeruginosa* or *Acinetobacter* spp. with remarkable resistance to any type of β-lactamase.

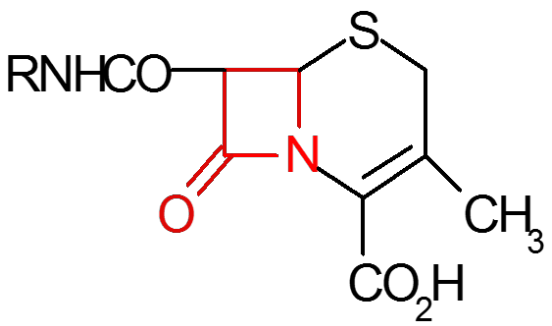
Commercial production of penicillin

Development of methods for growing *Penicillium notatum* and purifying Penicillin and chain made it into a drug. The deep fermentation method, the use of corn steep liquor and the discovery of *P. chrysogenum* made the commercial production of penicillin possible.

β-lactam antibiotics



Penicillins

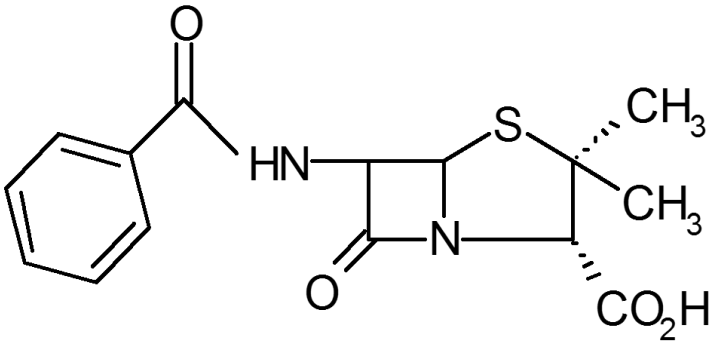


Cephalosporins

The most important class of antibiotics affecting cell wall biosynthesis are β-lactams. β-lactam group (a four-atom cyclic amide) is the pharmacophore of all β-lactam antibiotics. β-lactam rings were unknown before the discovery of penicillin and it took big effort to determine the structure of the drug. The most important classes of β-lactam antibiotics are penicillins and cephalosporins.

Penicillin G

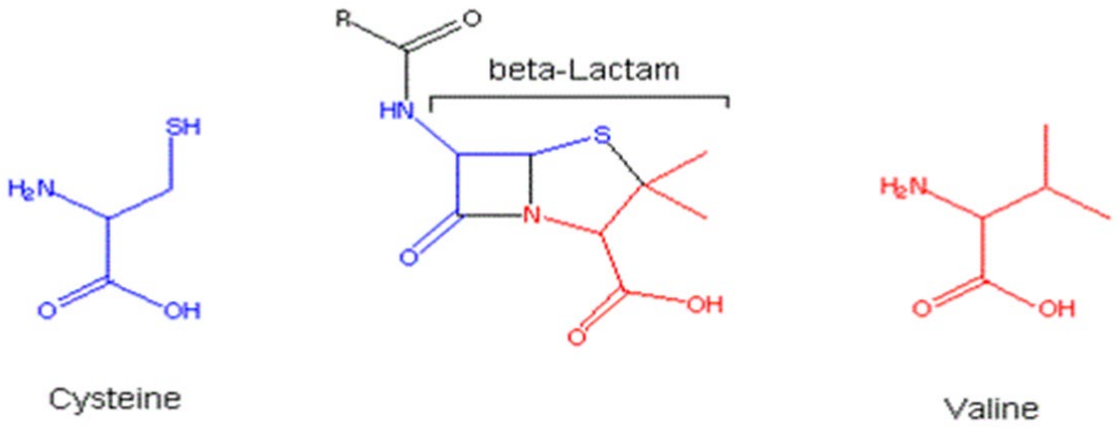
In penicillins, the β-lactam ring is fused to thiazolidine ring. Originally, penicillin was produced in the form of Penicillin G (benzylpenicillin) by fermenting *Penicillium* mold in the presence of phenyl acetic acid as a precursor. It has good activity against Gram-positive bacteria.

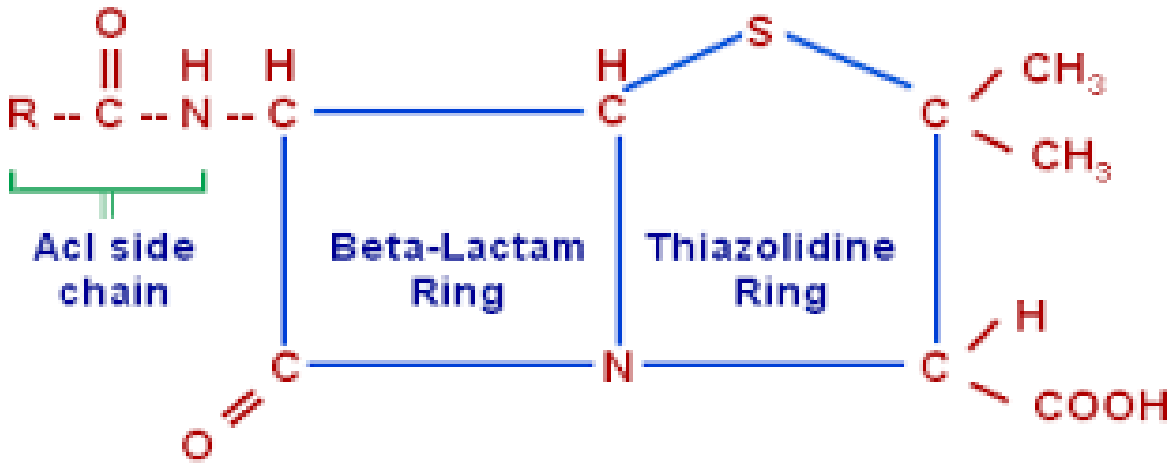


Benzylpenicillin (penicillin G)

Biosynthesis of Penicillins

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





General Structure of Penicillins

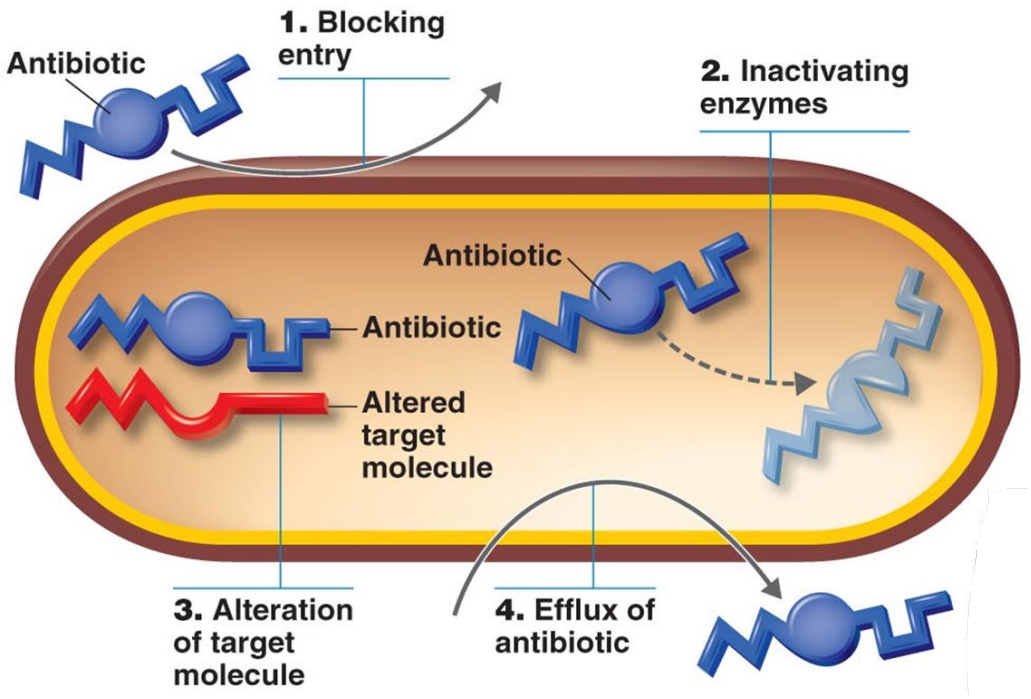
6-Aminopenicillanic acid (6-APA)

Presently, many penicillins are produced semisynthetically starting from 6-aminopenicillanic acid (6-APA) as a precursor. 6-APA can be generated from penicillin G by cleaving off the benzyl moiety of penicillin G. Various new side chains can be then attached to the penicillin molecule through the amino group of 6-APA.

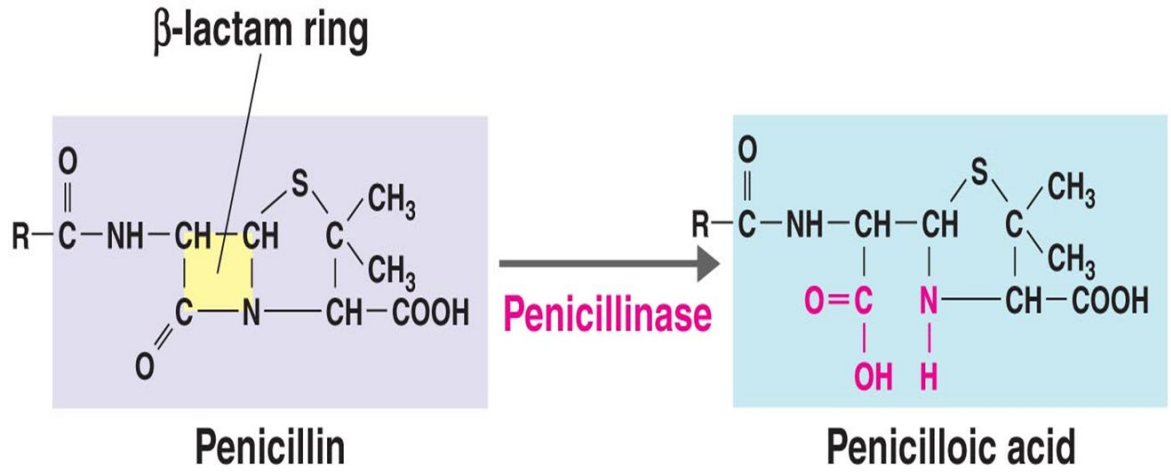
Mechanisms of antibiotic resistance

1- Enzymatic destruction of drug
2- Prevention of penetration of drug
3- Alteration of drug's target site
4- Rapid ejection of the drug

Resistance genes are often on plasmids or transposons that can be transferred between bacteria.

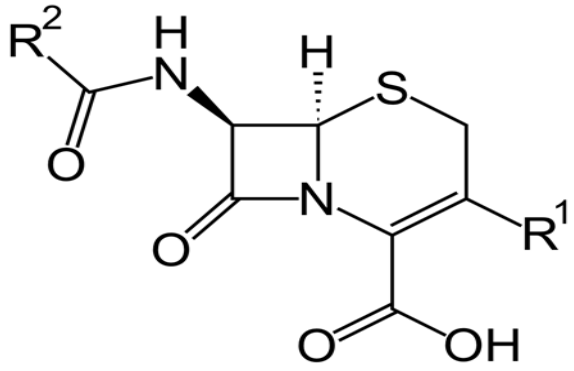


Penicillinase (β-lactamase): bacterial enzyme that destroys natural penicillins



Cephalosporins

Cephalosporins have been first obtained from a fungus *Cephalosporium acremonium*. Similar to penicillins, many cephalosporins are produced semi-synthetically either starting from 7-aminocephalosporanic acid (7-ACA) or by converting relevant penicillins into cephalosporins.



Antibiotic Susceptibility Testing (Minimum inhibitory concentration, MIC)

Susceptibility is a term used when microbe such as bacteria and fungi are unable to grow in the presence of one or more antimicrobial drugs. Susceptibility testing

is performed on bacteria or fungi causing an individual's infection after they have been recovered in a culture of the specimen. Testing is used to determine the potential effectiveness of specific antibiotics on the bacteria and/or to determine if the bacteria have developed resistance to certain antibiotics. The results of this test can be used to help select the drug(s) that will likely be most effective in treating an infection.

Bacteria and fungi have the potential to develop resistance to antibiotics and antifungal drugs at any time. This means that antibiotics once used to kill or inhibit their growth may no longer be effective.

During the culture process, pathogens are isolated (separated out from any other microbes present). Each pathogen, if present, is identified using biochemical, enzymatic, or molecular tests. Once the pathogens have been identified, it is possible to determine whether susceptibility testing is required. Susceptibility testing is not performed on every pathogen; there are some that respond to established standard treatments. For example, strep throat, an infection caused by *Streptococcus pyogenes* (also known as group A streptococcus), can be treated with ampicillin and does not require a test to predict susceptibility to this class of antibiotics.

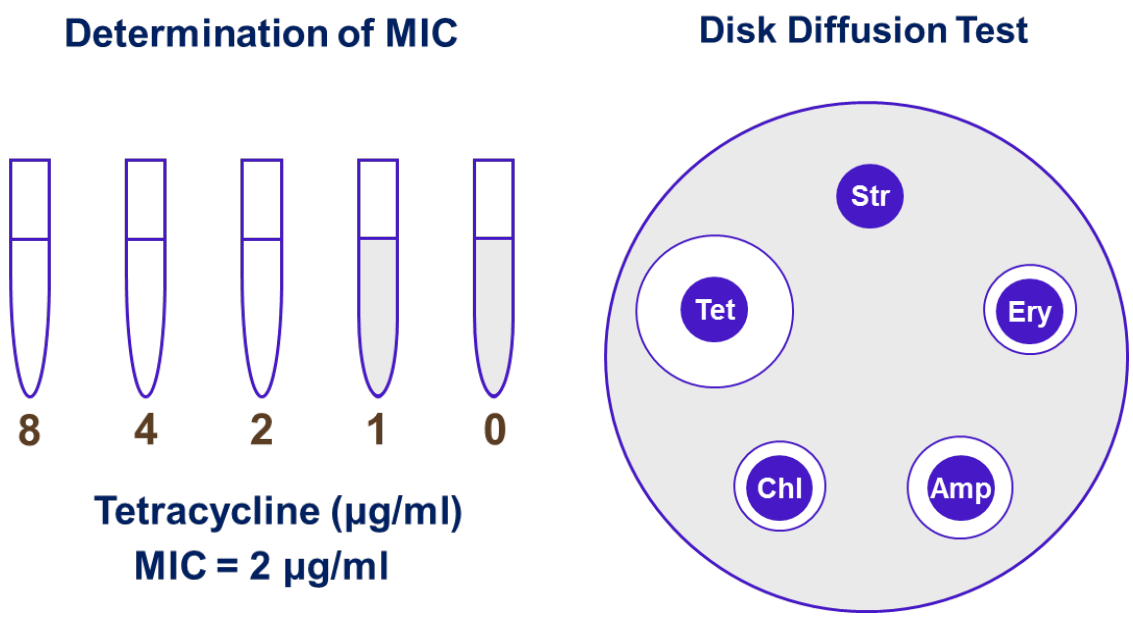
Susceptibility testing is performed on each type of bacteria or fungi that may be relevant to the individual's treatment and whose susceptibility to treatment may not be known. Each pathogen is tested individually to determine the ability of antimicrobials to inhibit its growth. This is can be measured directly by bringing the pathogen and the antibiotic together in a growing environment, such as nutrient media in a test tube or agar plate, to observe the effect of the antibiotic on the growth of the bacteria. Resistance can also be determined by detection of a gene that is known to cause resistance to specific antibiotics.

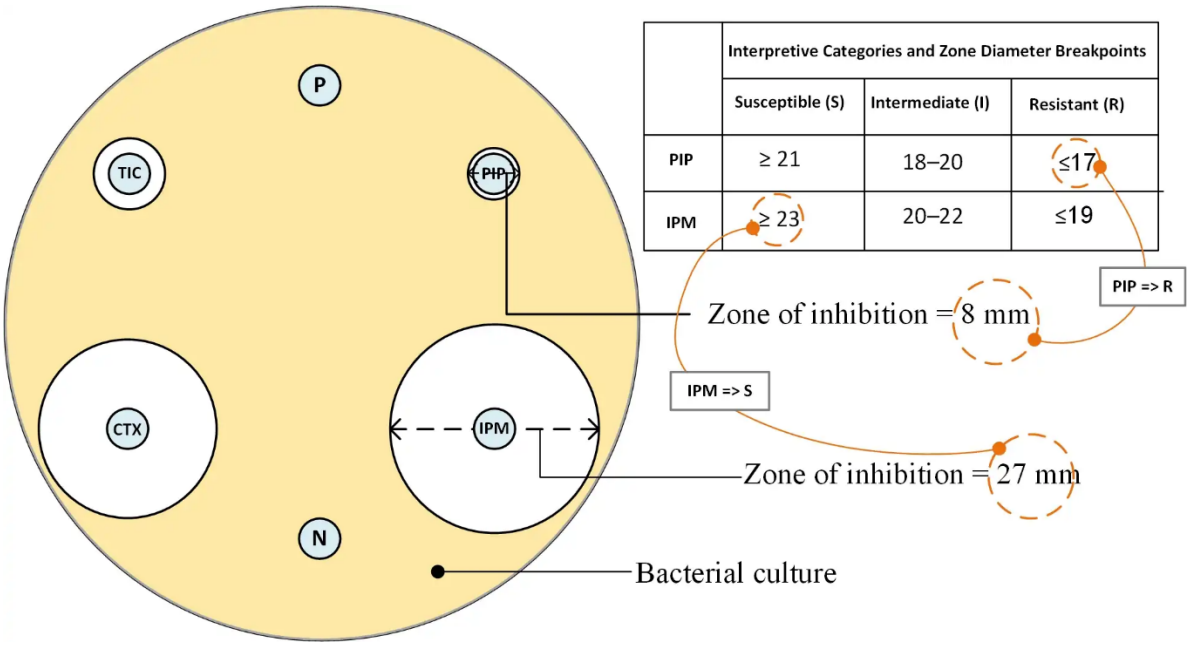
Susceptibility testing is used to determine which antimicrobials will inhibit the growth of the bacteria or fungi causing a specific infection. The results from this

test will help a health care practitioner determine which drugs are likely to be most effective in treating a person's infection.

Some types of infections may require testing because the bacteria or fungi isolated from an infection site are known to have unpredictable susceptibility to the drugs usually used to treat them. Some examples include staphylococci ("staph") and *Pseudomonas aeruginosa*.

Sometimes there may be more than one type of pathogen isolated from an infected site, such as a wound infection. Susceptibility testing may be used to determine which antibiotic or antibiotic combinations will be most effective in treating all the different types of bacteria causing the infection.

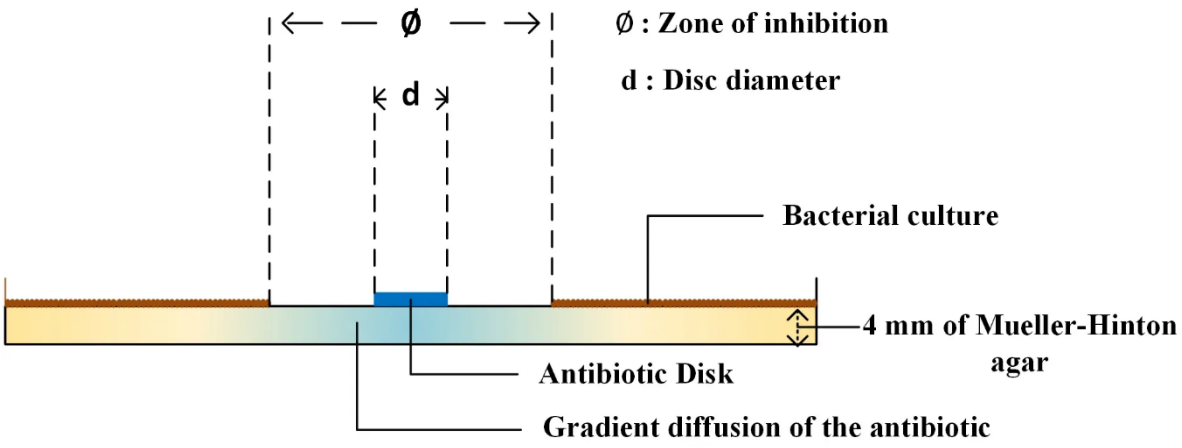




	Interpretive Categories and Zone Diameter Breakpoints		
	Susceptible (S)	Intermediate (I)	Resistant (R)
PIP	≥ 21	18–20	≤17
IPM	≥ 23	20–22	≤19

The medium used for the majority of bacterial species is Mueller-Hinton agar (plus 5% blood for fastidious germs):

- It shows acceptable lot-to-lot reproducibility for susceptibility testing.
- It is low in inhibitors which affect sulfonamide, trimethoprim and tetracycline susceptibility test results.
- It supports satisfactory growth of most pathogens.
- A large amount of data and experience has been collected on sensitivity tests carried out with this medium.



South Valley University

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MYCOLOGY

(Plant Pathology)

4th year

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Contents

ACKNOWLEDGEMENT

To my parents

1 What is disease?

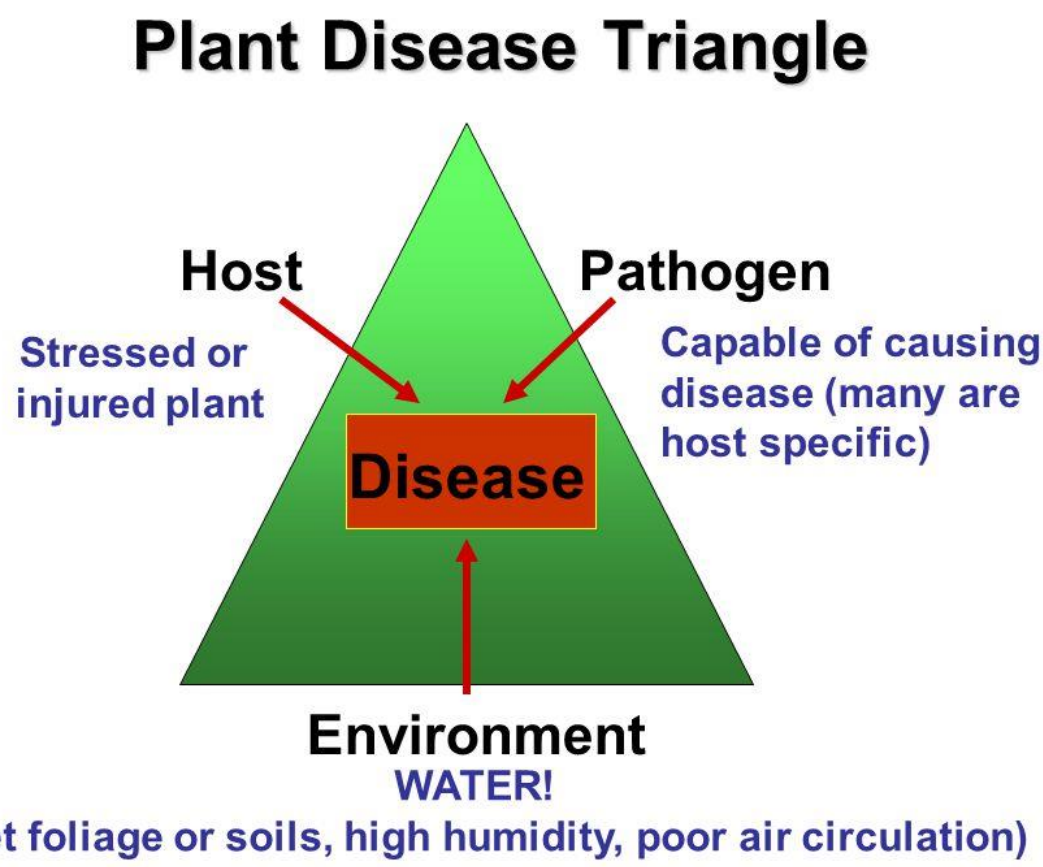
Disease can be defined as "any disturbance brought about by a pathogen (organism that causes disease) or an environmental factor (water, nutrients etc…) yields less than a normal, healthy plant of the same variety.

In order to understand how infection by microorganisms can cause disease, one must first understand a little about their modes of nutrition. Most microorganisms are beneficial to man in that they break down dead and decaying organic material. Such substances utilized by microorganisms for growth are called substrate. Organisms feed in this way are saprophytes. However, there are other microorganisms which live on other living organisms, their hosts. They take nutrients from their hosts in a way that does not benefit the host at all. Such organisms are parasites. The term parasite is often used interchangeably with pathogen. If there is mutual benefit in the relationship between microorganism and host the relationship is symbiotic.

Parasites have been divided into obligate and facultative parasites. An obligate parasite can live and reproduce only by feeding on living plant materials. Obligate parasites are often referred to as biotrophs. In practice it is difficult or impossible to grow obligate parasites such as *Puccinia striiformis* (causing yellow rust of wheat) in the laboratory on agar or other media. In contrast facultative parasites can feed equally well on dead decaying materials or living plants. Facultative parasites may be necrotrophs, i.e organisms which kill host tissues then utilize the dead host tissues as substrate.

Factors affecting disease development

Disease is a complex interaction of factors associated with the pathogen, host and environment. Indeed the disease will not build up unless there is an active pathogen, a susceptible host and suitable environmental conditions for infection, colonization and reproduction of the pathogen.



Symptoms of plant disease

The different types of external and internal symptoms have been grouped into three categories, which are:

1- **Hyperplasia**: over development of tissues due to stimulation of cells.
2- **Necrosis**: decay or rotting of tissues due to derangement of cells. The various necrotic symptoms are as follows:
   - **Rot**: here the necrosis is not localized and parenchyma, collenchymas and pith tissues all are infected.
   - **Canker**: this type of corky necrosis usually results in the destruction of woody tissues. Cankerous growth occurs due to the hypertrophy of cambium tissues.
   - **Blights**: these are characterized by very rapid and extensive necrosis of the whole plant or plant parts. On the basis of infected plant parts names like leaf blight, shoot blight.
   - **Spots**: the necrosis may be local. Spots develop on leaf, stem and fruit. The killed tissues get colored.

- **Damping off**: tissues in the basal portion of the stem or crown region are attacked. Infected tissues become too weak to support the stand to the plant which topples down.
- **Wilts**: in wilts vascular tissues may be affected. In cabbage yellows the vascular tissues become from yellow to dark brown.
- **Scab**: necrosis is usually superficial and restricted mainly to the epidermal region.

3- **Hypoplasia**: in hypoplastic diseases reduction in size of the whole plant or plant parts or reduction in chlorophyll may occur. Hypoplasia can be expressed in the forms of stunting, small leaves or colour changes in leaves.

Classification of plant diseases:

Based on plant part affected

- **Localized**, if they affect only specific organs or parts of the plants.
- **Systemic**, if entire plant is affected. or

They can be classified as root diseases, stem diseases, foliage/foliar diseases, etc.

Based on perpetuation and spread

- **Soil borne** -when the pathogen perpetuates through the agency of soil.
- **Seed borne** -when the pathogen perpetuates through seed (or any propagation material).
- **Air borne** -when they are disseminated by wind e.g. rusts and powdery mildews.

Based on the signs and symptoms produced by the pathogens

Diseases are classified as rusts, smuts, powdery mildews, downy mildews, root rots, wilts, blights, cankers, fruit rots, leaf spots, etc. In all these examples, the diseases are named after the most conspicuous symptom of the disease appearing on the host surface.

Based on the host plants affected

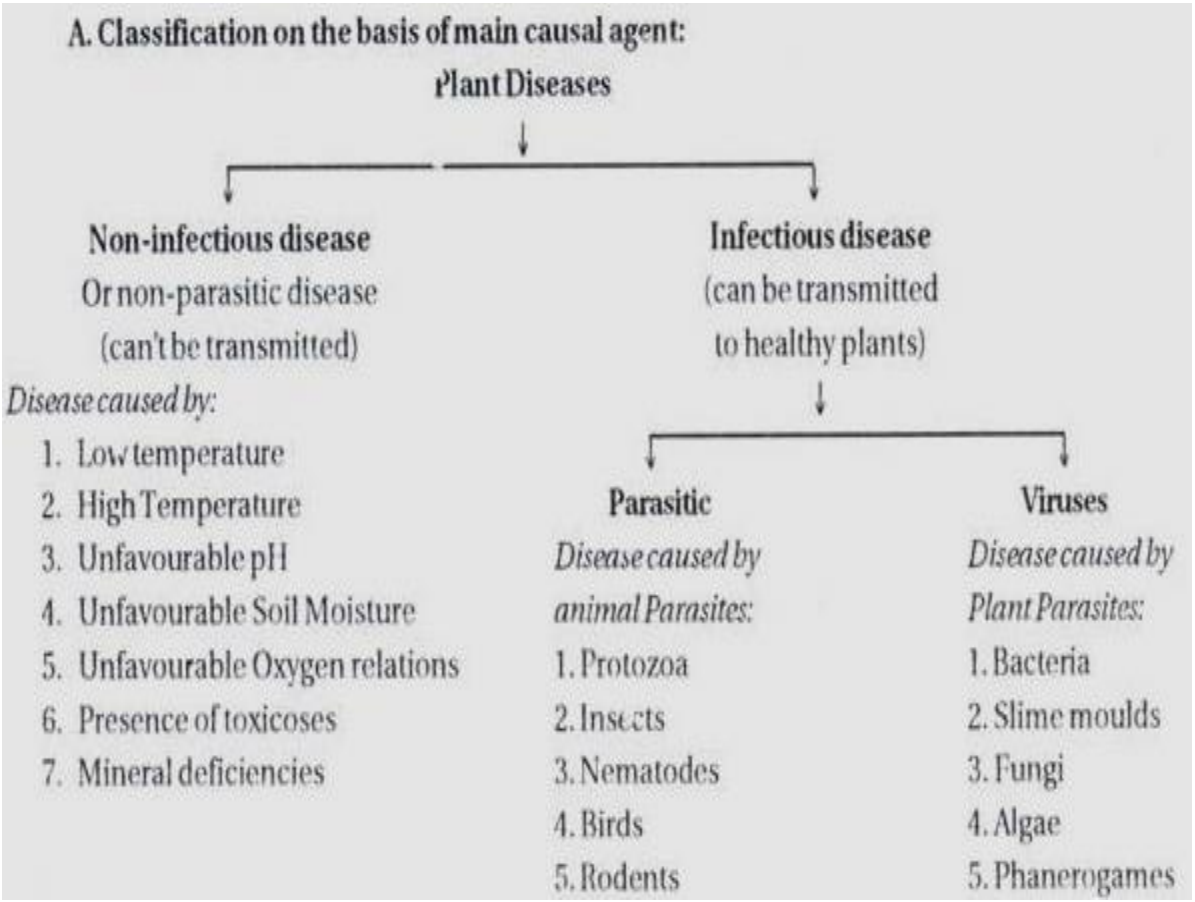
They can be classified as cereal crop diseases, forage crop diseases, flax diseases, millet diseases, plantation crop diseases, fruit crop diseases, vegetable crop diseases, flowering plant diseases, etc.

Based on major Causes

They can be classified as fungal diseases, bacterial diseases, viral diseases, mycoplasmal diseases, etc.

Based on Infection Process

- **Infectious** -All the diseases caused by animate causes, fungi, viruses and viroids can be transmitted from infected host plants to the healthy plants and are called infectious.
- **Non-infectious**- Non-infectious diseases can not be transmitted to a healthy plant. Also referred as non-parasitic disorders or simply physiological disorders, and are incited by abiotic or inanimate causes like nutrient deficiency or excess or unfavorable weather conditions of soil and air or injurious mechanical influences.



Classification of Animate Diseases in Relation to Their Occurrence

- **Endemic diseases** -which are more or less constantly present from year to year in a moderate to severe form in a particular geographical region, i.e. country, district or location.
- **Epidemic or epiphytotic diseases** - which occur widely but periodically particularly in a severe form. They might be occurring in the locality every year but assume severe form only on occasions due to the favourable environmental conditions occurring in some years.
- **Sporadic diseases** occur at irregular intervals and locations and in relatively few instances.
- **Pandemic diseases:** A disease may be endemic in one region and epidemic in another. When epiphytotics become prevalent through out a country, continent or the world, the disease may be termed as pandemic.

2 Pathogenicity, Infection and Disease Development

Pathogenicity: disease is a complex of interactions of host, pathogen and environment. Usually this term is used for living entities that incite disease. The property of a pathogen to incite disease is known as pathogenicity. The parasite is different from the pathogen, the former lives and obtains food from other organism without inciting disease.

Infection: infection means the establishment of pathogen within the host. To establish within the plant the pathogen must enter the plant. Its entry into the plant or penetration constitutes the initial step of infection. The transference of pathogen from the source of inoculums to the invasion court constitutes inoculation. The inoculums of fungi may be spores, mycelium, sclerotia etc.

The phenomenon of infection is influenced by several factors. Important environmental factors which govern infection are moisture, temperature, light and pH. Water congestion may increase number of infections by fungi.

Infection process: the development of pathogen on the surface of the host after contact, penetration into the host and very early stages of establishment within the host tissues constitute the process of infection.

1- The prepenetration phase: the stage at which the pathogen is present on the host surface but has not entered the plant.
2- Penetration phase: the pathogen penetrates the plants mainly through uninjured plant surface, pre-existing gaps provided by stomata, hydathodes and lenticels and wounds.

Disease development: the chain of events occurring between the time of infection and full expression of disease constitute the disease development. The disease cycle involves the following events

1. Inoculation. Spore, mycelium, sclerotia etc.
2. Penetration. Wound, stomata etc.
3. Infection.
4. Incubation.
5. Invasion. The pathogen spreads after the establishments of infection. Fungal invasions could be inter or intracellular
6. Reproduction of incitant. Mycelium produce spores.

7. Dissemination of incitant. Wind, water, insect, animal and men.
8. Overwintering or oversummering of inctant. Resting bodies, seeds or eggs.

Attachment, Penetration, Infection

- Fungal inoculum lands on plant surface, and adheres either by present moisture or excretion of enzymes and mucilaginous substances
- Bacterial and viral cells can be washed in by water droplets or insects

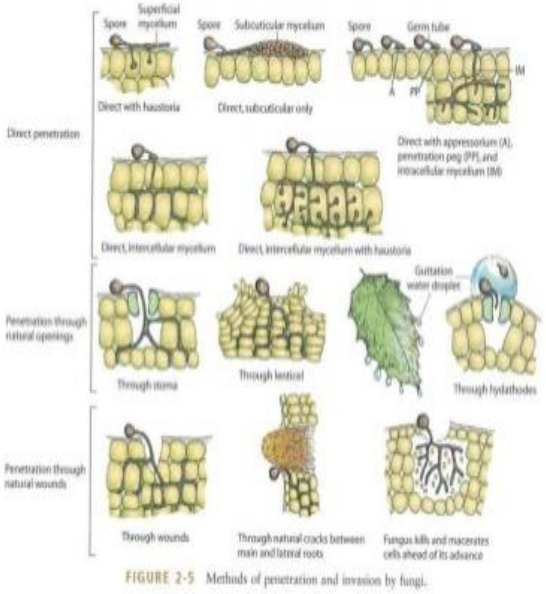
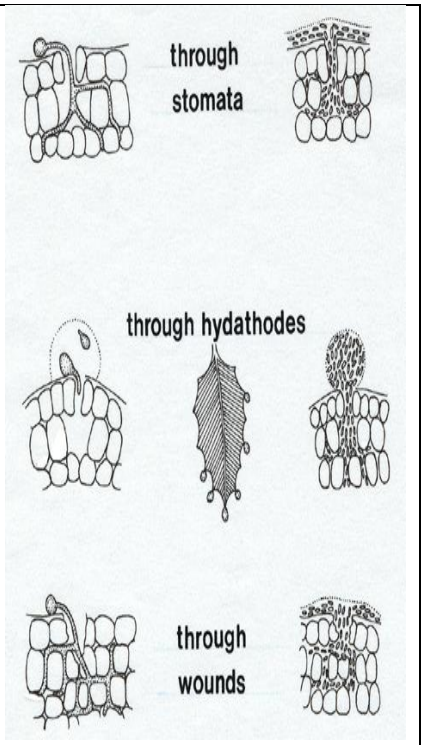


FIGURE 2-5 Methods of penetration and invasion by fungi.



3Host Plant in Defence

The ability of plants to overcome wholly or in part the effect of the pathogen or other harmful factors is called resistance. Resistance can be functional chemical or mechanical. We shall discuss resistance in two phases resistance before penetration and resistance after penetration.

A- Resistance before penetration: resistance to penetration could be structural functional and biochemical. Thickness of the cuticle and presence of the wax layer may protect plants from penetration. Wax-layer and hairs make adherence very difficult. Cuticle and tough outer wall serve as barriers to penetration in certain cases. Certain plant releases some chemicals which interfere with the germination of spores.

B- Resistance after penetration: **cork** layers and **abscission** layers are formed is deposited by certain plants to resist farther spread of the pathogen. Cork layers formed around the point of infection check further invasion. These layers do not allow the pathogen to penetrate further, block toxic substances of the pathogen and check the supply of nutrients and water from the healthy to the infected cells.

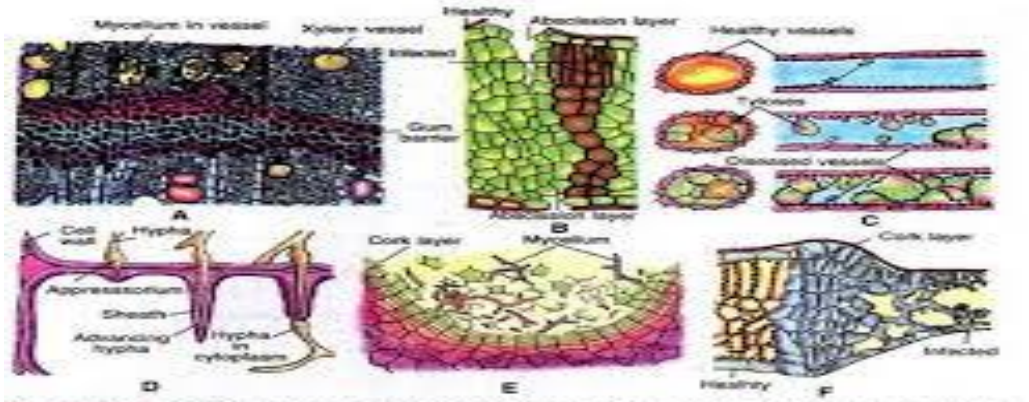


Fig. 21.1. (A-F). Different defence structures developed after infection : (A) Gum deposition in cells ; (B) Abscission layer ; (C) Tyloses ; (D) Formation of sheath around developing hyphae (E, F) Development of cork layer in tuber (D) and leaf (F).

In forming abscission layers the plant discards the infected area which may be sloughed off and the rest of the leaf area is then protected from the infection or from toxic substances released by the pathogen.

In some plant diseases the outer walls of epidermal or subepidermal cells swell and limit spread of the pathogen. Swelling of the cell wall is known in cucumber varieties resistant to *Cladosporium cucumerinum (*scab disease). The hyphae

penetrating the cell wall are covered by a sheath formed by the host; this may slow down the progress of pathogen. The peg like proliferation of the host cell wall called lignituber. It is composed of lignin.

C- Biochemical resistance in plants

Various known biochemical mechanisms in the host induced in the pathogen can be classified into six categories: production of inhibitors, detoxification of toxins of the pathogen, alteration in respiration, alteration in biosynthetic pathways, hypersensitive reaction and inactivation of the pathogen enzymes.

Phenolic compounds are important inhibitors. The fungitoxic compounds produced in plant as a result of interaction of two metabolic systems, the host and the parasite or produced due to the stimulation by mechanical and chemical injuries are called phytolexins.

D- Hypersensitivity

The term hypersensitivity means increases sensitivity as in the rapid death of a host cell in the immediate vicinity of the site of infection. Hypersensitivity has been studied chiefly in rusts, powdery mildew and potato blight. Hypersensitive reaction takes place through quick necrosis of the infected cells.

4Physiological Cellular and Molecular Changes in Diseased Plant

The interface

The site of contact between a pathogen and the host cell or protoplasm is called the host pathogen interface. At this site the materials are taken up or exchanged. The types of host pathogen interface are tabulated below.

Character	Types of host pathogen interface
Pathogen is intercellular	Necrotrophic fungi
Pathogen is partially intracellular	Biotrophic fungi
Pathogen is entirely intracellular	Plasmodial fungi

- **Biotrophs** derive energy from living cells, they are found on or in living plants, can have very complex nutrient requirements and do not kill host plants rapidly.
- **Necrotrophs** derive energy from killed cells; they invade and kill plant tissue rapidly and then live saprotrophically on the dead remains.
- **Hemibiotrophs** have an initial period of biotrophy followed by necrotrophy.

Haustoria

The haustoria penetrate host cells and enter the epidermis. The host cell wall is breached by a narrow hypha which subsequently inflates within the cell lumen.

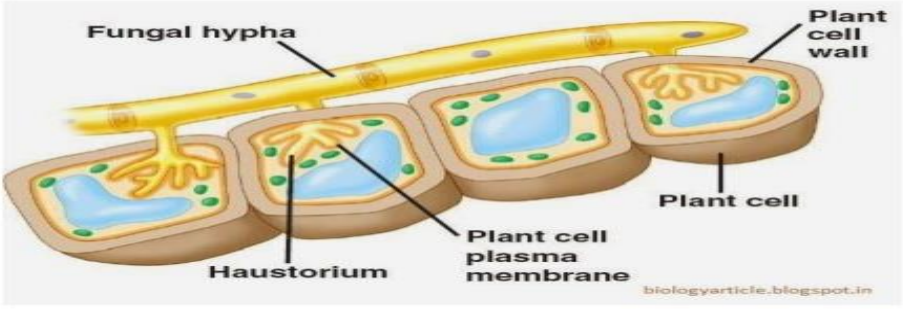


Fig : Showing typical fungal haustorium

Change in host cell walls

The first response of a plant cell to an invading microorganisms is an alteration in the appearance or properties of the cell wall. In cereal leaves penetrated by virulent fungi there is a deposition of plug of material known as a papilla directly beneath the penetration site. Similar thickening and modification of host cell walls occur in other plant fungus interaction.

The possible functions of the cell wall changes in host resistance could be as follow:

1- Mechanical barrier
2- Increased resistance to cell wall degrading enzymes.
3- Reduced diffusion of compounds from host to pathogen or vice versa.
4- Direct toxicity of wall precursors e.g. phenols to pathogen.

Photosynthesis in diseased plants

The capture of solar energy by chlorophyll and its subsequent utilization to fix carbon dioxide into organic compounds is the basis of the life on this planet. The overall chlorophyll content of diseased leaves is known to be reduced. Chlorosis is one of the most common symptoms of plant disease. It is indicative of a reduction in chlorophyll content of green tissue. Reduced chlorophyll content could be due either to the breakdown of chlorophyll or to the inhibition of chlorophyll synthesis.

Respiration in Diseased Plant

In infected plants the rate of respiration generally increases. The increased rate of respiration appears shortly after infection, continues to rise during the multiplication and sporulation. After that respiration declines to normal levels or to levels lower than those of healthy plants.

Translocation in Diseased plants

Destruction of roots, inhibition of root hair and alteration of permeability of root cells caused by pathogen like root rotting fungi and damping off fungi, ultimately interfere with the absorption of water by roots of the infected plant.

Translocation through xylem may be influenced in various ways. Pathogens like *Fusarium* and *Verticillium* attack xylem tissues. In fungal wilts mass of mycelium and spores reduce the flow of liquid in the vessels to some extent.

Translocation of nutrients through phloem maybe affected by the plant pathogens. Accumulation of nutrients and products of photosynthesis in regions invaded by mildew and rust fungi.

Nitrogen metabolism in diseased plant

The genetic information contained in nucleic acids is expressed in the cell via the synthesis of protein. In many fungal infections protein synthesis is greatly stimulated. Infection by certain fungi can also result in destruction of the host protein. Proteins metabolism seems to be involved in resistance or susceptibility of the host to fungal infection.

Changes at the molecular level

Genetic information contained in the DNA genome is expressed via RNA and protein synthesis. Alteration of genetic activity could occur either in the regulation of transcription itself, i.e. at the level of DNA or in the translation of genetic messengers on the ribosome.

The acceleration of metabolism in diseased tissues is usually accompanied by an increase in synthesis of RNA, and consequently of protein. Thus cells penetrated by fungal haustoria often contain more polyribosomes than healthy cells suggesting that protein synthesis is enhanced. Further, nucleoli of infected cells are swollen indicating an accumulation of RNA.

5Plant disease control

One early proposal by H. H. Whetzel included four general disease control principles, **exclusion**, **eradication**, **protection** and **immunization** (the latter principle is more appropriately called **resistance** since plants do not have an immune system in the same sense as animals). These principles have been expanded or altered to some extent by others. They are still valid and are detailed here but students should investigate other systems such as those proposed by Gäumann, Sharvelle, or the National Academy of Science and use the one(s) that they believe are applicable. These and other disease control principles are discussed in Maloy, Plant Disease Control (1993) cited in the general references of this lesson.

EXCLUSION

This principle is defined as any measure that prevents the introduction of a disease-causing agent (pathogen) into a region, farm, or planting area. The basic strategy assumes that most pathogens can travel only short distances without the aid of some other agent such as humans or other vector, and that natural barriers like oceans, deserts, and mountains create obstacles to their natural spread. In many cases pathogens are moved with their host plants or even on non host material such as soil, packing material or shipping containers. Unfortunately, exclusion measures usually only delay the entry of a pathogen, although exclusion may provide time to plan how to manage the pathogen when it ultimately arrives.

ERADICATION

This principle aims at eliminating a pathogen after it is introduced into an area but before it has become well established or widely spread. It can be applied to individual plants, seed lots, fields or regions but generally is not effective over large geographic areas.

Two programs that are actually forms of protection and not pathogen eradication are barberry eradication for reducing stem rust (caused by *Puccinia graminis*) of wheat and *Ribes* eradication for preventing white pine blister rust. The strategy is that removing these alternate hosts breaks the disease cycles and prevents infection of the economically more valuable host. These two examples are mentioned here because they are frequently cited as eradication measures.

Eradication may also be accomplished by destroying weeds that are reservoirs of various pathogens or their insect vectors.

Soil fumigation has been a widely used eradication strategy. This technology involves introducing gas-forming chemicals such as carbon disulfide, methyl bromide, or chloropicrin into soil to kill target pathogens. However, undesirable side effects such as killing beneficial organisms, contamination of groundwater, and toxicity of these chemicals have resulted in less reliance on this approach for disease management. Volatile fumigants like methyl bromide are injected into soil and sealed with a plastic film (Figure 6). Some water-soluble fumigants like metam-sodium can be injected into the soil and the soil simply compacted to form a seal (Figure 7).

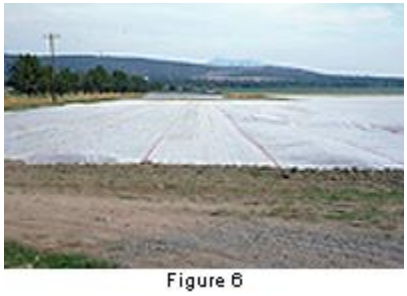


Figure 6


Figure 7

Crop rotation is a frequently used strategy to reduce the quantity of a pathogen, usually soil-borne organisms, in a cropping area. Take-all of wheat (caused by *Gaeumannomyces graminis*) and soybean cyst nematode (*Heterodera glycines*) (Figure 8) are two examples of soilborne diseases that are easily managed by short rotations of 1 and 2 years, respectively, out of susceptible crops, which may include susceptible weed hosts such as grasses in the case of take-all.


Figure 8

Burning is an effective means of eradicating pathogens and is often required by law to dispose of diseased elm trees affected by Dutch elm disease (DED) (Figure 9) However, burning agricultural fields is controversial because the smoke creates human health and safety and environmental concerns.


Figure 9


Figure 10


Figure 11

PROTECTION

This principle depends on establishing a barrier between the pathogen and the host plant or the susceptible part of the host plant. It is usually thought of as a chemical barrier, e.g., a fungicide, bactericide or nematicide, but it can also be a physical, spatial, or temporal barrier. The specific strategies employed assume that pathogens are present and that infection will occur without the intervention of protective measures. For example, bananas are covered with plastic sleeves as soon as the fruit are set (Figure 12) to protect the fruit from various pests including fruit decay fungi.

Figure 12

Protection often involves some cultural practice that modifies the environment, such as tillage, drainage, irrigation, or altering soil pH. It may also involve changing date or depth of seeding, plant spacing, pruning and thinning, or other practices that allow plants to escape infection or reduce severity of disease. Raising planting beds (Figure 13) to assure good soil water drainage is an example of cultural management of plant diseases such as root and stem rots.

Figure 13

Fungicides have been used for more than a hundred years and new fungicides continue to be developed. In the early to mid-1900s organic fungicides such as thiram, captan, and the bisdithiocarbamates were developed. These are broad-spectrum, contact or protectant fungicides that control a wide range of fungal diseases. A recent group of systemic fungicides are the strobilurins. Some fungicides have narrow ranges of activity and are used primarily for control of specific groups of diseases such as downy mildews, rusts, smuts or powdery mildews while others are active against a wider range of diseases.

Fungicides can be applied by any of several methods: ground sprayers (Figure 14), airplanes (Figure 15) or through irrigation systems, but to be effective applications must be done properly. First, the fungicide must be legally registered for use on the plant involved and against the target disease. Several different chemicals may be registered for the same crop or disease. If the different fungicides are similar in effectiveness, cost, ease of application, and safety, then timing of application becomes the most critical factor. If applied too early much of the chemical will be wasted before it can be effective; if applied too late, it will be largely ineffective. The benefits of properly applied fungicides can often be striking (Figure 16). Distribution of the spray droplets is important; the finer the spray the more

complete the coverage on the plant surface (Figure 17). However, very small droplets form a mist that is easily displaced by wind.


Figure 14


Figure 15


Figure 16


Figure 17

Many cultural practices can be modified to manage the occurrence, intensity or severity of plant diseases. These include selection of suitable growing sites for the crop, adequate tillage to bury pathogen-infested plant residues, rotation to nonsusceptible crops, selecting pathogen-free planting stocks, orientation of plantings to improve exposure to sun and air currents, pruning and thinning to eliminate sources of infection and improve aeration in and around susceptible plants, water management on both plants and in soil, adequate nutrition, proper cultivation to improve root growth and avoid plant injury, and sanitation procedures to eliminate sources of inoculum.

RESISTANCE

Use of disease-resistant plants is the ideal method to manage plant diseases, if plants of satisfactory quality and adapted to the growing region with adequate levels of durable resistance are available. The use of disease-resistant plants eliminates the need for additional efforts to reduce disease losses unless other diseases are additionally present. Resistant plants are usually derived by standard breeding procedures of selection and/or hybridization. A few disease-resistant lines have been obtained by inducing mutations with x-rays or chemicals. There is also interest in chemicals called "plant activators" that induce plant defense responses called systemic acquired resistance (SAR) and induced resistance. Recently, resistant plants have been developed through the use of genetic engineering (e.g., resistance to the *Papaya ringspot virus*).

Selection of resistant plants involves subjecting plants to high levels of disease pressure (Figure 18) and using the surviving plants as sources of disease resistance. Plants that survive this pressure often have genetic resistance that can be utilized directly by propagation or as sources of resistance to develop resistant plants that also have the requisite qualities for that crop

Figure 18

6 Diseases of Small Grains Cereals

(1) Powdery mildew

(i) Causal fungus *Erysiphe graminis*

(ii) Host range

E. graminis f.sp. *tritici* affects wheat
E. graminis f.sp. *hordei* affects barley
E. graminis f.sp. *avenae* affects oats
E. graminis f.sp. *secalis* affects rye

(iii) Symptoms

Symptoms of powdery mildew can be found on all aerial parts of cereals leaves and stems but leaves are most commonly infected the first symptoms of infection, which can easily be detected are **chlorotic flecks** on plant tissue. A white **mildew pustules** soon develops which quickly produces masses of spores and assumes a powdery appearance. As the mildew pustules become older they often change colour slightly and acquire grey or brown tings. Towards the end of the season dark coloured sexually produced spore cases (cliestothecia) may be found embedded in the mildew pustules.

(iv) Life cycle

Knowledge of the life cycle of a powdery mildew fungus can provide important clues to epidemiology and disease control. A complete life cycle includes both asexual and sexual reproduction.

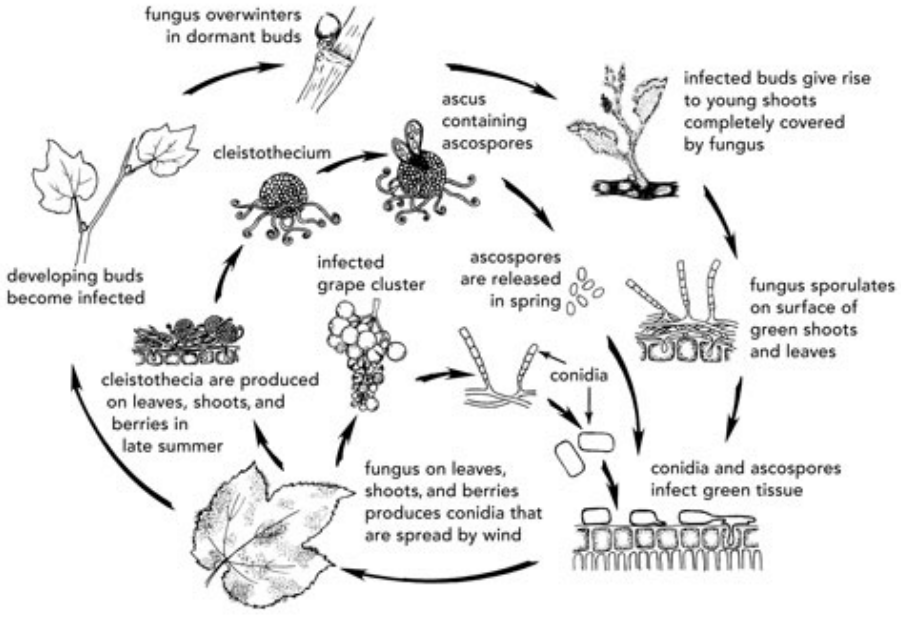
The asexual state produces conidia (asexual spores).

The sexual state includes the ascocarps (cleistothecia,) that contain asci with ascospores. When both states occur, ascocarps usually are important in perennation (survival in the absence of a live host) and disease establishment, whereas the conidial state serves to spread and intensify the disease.

Powdery mildews produce conidia in enormous numbers during the growing season, typically within 3–7 days after infecting the host under disease-conducive conditions. The rapid rate of asexual reproduction can lead to exponential growth of powdery mildew populations resulting in **epidemics**.

Sexual reproduction of powdery mildew often is initiated in many plant species after flowering or late in the growing season. Ascocarps are resistant to low temperatures and drought, which enables the ascospores they contain to survive harsh conditions. Genetic recombination resulting from sexual reproduction can produce new genotypes resistant to fungicides or that display greater virulence than parental genotypes. Ascocarps also can initiate new epidemics (typically in the spring following the growing season during which they were formed) when, following a rainstorm or irrigation event, they discharge ascospores. Ascospores that land on susceptible and unprotected host tissue can establish primary infections to initiate the epidemic.

Powdery mildews also can survive winter in the form of dormant mycelium within buds of infected plants.



Control

This handbook includes recommendations for control of specific powdery mildew diseases. It is difficult to make general recommendations because of the large numbers of growing or cropping systems that are involved, and the diversity of hosts and powdery mildew species that occur in the Pacific Northwest. Effective disease control will depend on using appropriate strategies for a given powdery mildew fungus and host combination in a particular growing situation. In general, the following approaches, frequently used in combination, can be effective in controlling powdery mildew diseases:

Exclusion

The use of healthy planting stock can delay or prevent development of damaging epidemics.

Eradication

In some situations (such as encountered by homeowners growing susceptible cultivars of roses or other species) reasonably effective control can be attained by removing and burying or otherwise destroying infected plant tissue. This approach demands constant, vigilant attention and aggressive action and generally will not be economically feasible in commercial production systems.

Cultural Practices

In landscapes, experience generally will enable a grower to learn whether a particular area in a yard or garden is conducive to powdery mildew diseases. For example, the higher relative humidity of shady areas often favors powdery mildew infections. In such situations an effective control strategy could be to plant only resistant species or cultivars. In some commercially-grown crops, such as grapes and cherries, pruning, plant spacing, and managing irrigation in order to avoid

dense canopies can be effective in reducing humidity to prevent infection. Pruning for enhanced light penetration is also utilized in perennial crops.

Resistance

In areas conducive to epidemic development, growing resistant plant species or cultivars may be an effective strategy for controlling the disease. Resistant cultivars might not be an option for all crops, for example when a grower specializes in a particular apple or grape cultivar.

Biological Control

In recent years several commercially available preparations of mycoparasitic fungi (fungi that parasitize fungi) and culture filtrates, derived from bacteria, have been labeled for use in controlling powdery mildews. As is the case for chemical control agents, users of biological control agents should carefully follow all label instructions.

Chemical Control

A variety of effective chemical controls are available for managing powdery mildew diseases. Users should be careful to follow label recommendations for the chemicals listed in this manual. Recommendations for fungicide use on some crops include information on disease monitoring systems and predictive models that can reduce fungicide use by ensuring that applications are made when they will be most effective. In general, the sulfur, petroleum-derived spray oils, sterol-biosynthesis inhibitors (DMI), quinone outside inhibitors (QoI), and quinoline fungicides are effective in powdery mildew management programs. Users should pay strict attention to resistance management guidelines.

(2) Ergot

(i) Causal fungus; *Clavicepes purpurea*

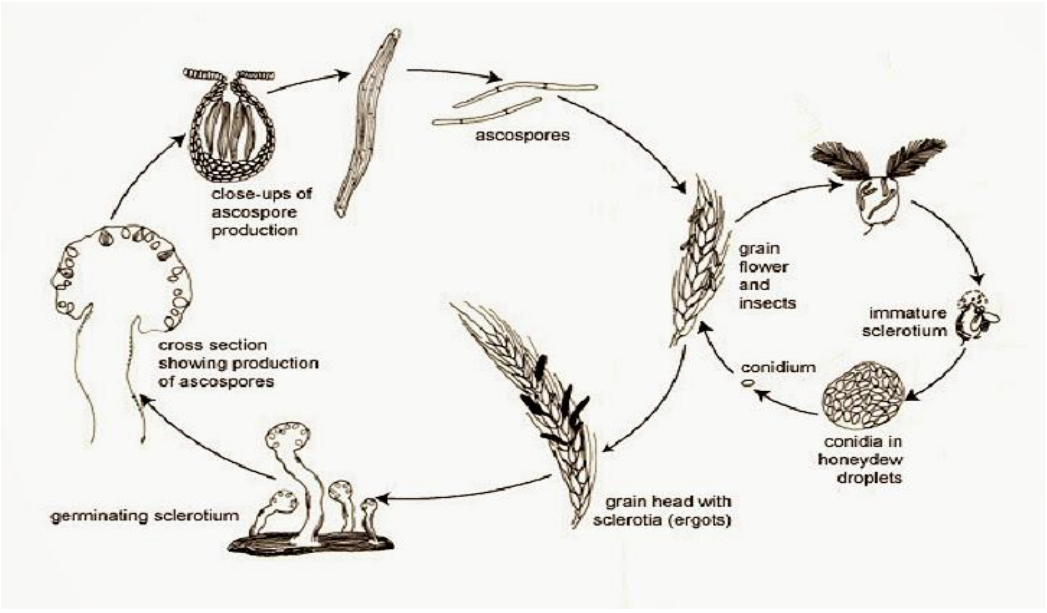
(ii) Host range: wheat, barley, rye and many grass species.

(iii) Symptoms:

The first symptoms of ergot are easily overlooked. Creamy to golden colourd droplets of honeydew exude from young florets in affected cereal ears. The more obvious symptoms of ergot are the horn shaped, purplish black ergots or sclerotia which appear as individual replacements to grains.

(iv) Disease cycle:

Ergots or sclerotia are hard, compact masses of fungal tissue which are the overwintering bodies of the fungus. Ergots overwinter either in the soil or as contaminants of grains. The ergot requires a dormant period at low temperatures before it germinates in the spring. The resulting perithecia release windblown ascospres. If ascospres alight on a susceptible floret, they quickly germinate and penetrate the ovary. Within a few days of infection, conidia are formed in honeydew which serves as secondary inoculums. Conidia are transferred to healthy florets by wind, splashing rain and insects. Honeydew formation and build-up of disease in the crop is favored by cool wet weather around flowering time. As the crop matures, infected ovaries enlarge and are converted into sclerotia.



(v) Control :

1- Deep ploughing of land in order to bury ergots to a depth in excess of 70 mm. this stop perithecial development.
2- Rotations may also help to reduce the number of viable ergots in the soil.
3- Grass weeds should be eliminated to reduce the possibility of ergot spread from the grass within the crop.
4- Remove ergot from grains by sieving or floating.
5- Disease resistance and chemical control methods are not widely practiced.

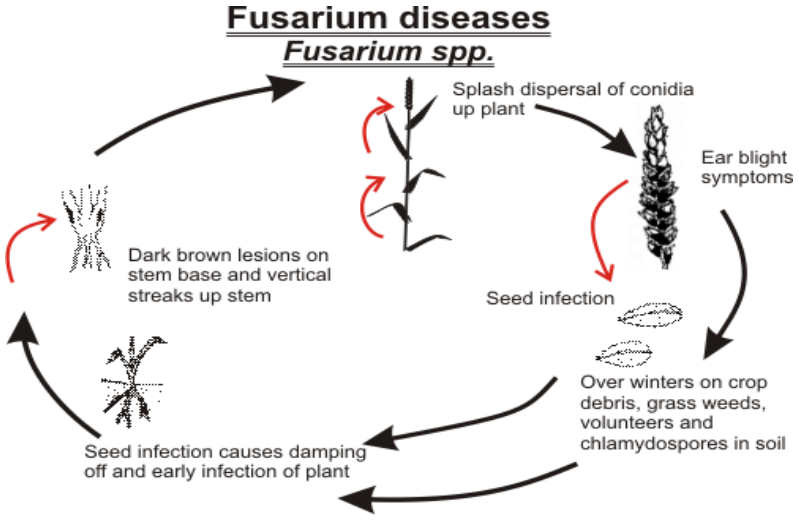
(3) *Fusarium* ear blight (scab)

(i) Causal fungus: *F. culmorum*, *F. avenaceum*, *F. graminearum* and *F. poae*.

(ii) Host range: wheat and barley.

(iii) Symptoms: individual spikelets become bleached as a result of infection. White mycelium, together with pink orange spore masses. *F. poae* produces different symptoms pale lesions on glumes with a dark border.

(iv) Disease cycle: the disease cycle of *Fusarium* ear blight is not well documented. It is generally assumed that ear infections arise primarily from the spores splashed from infections at the stem base. There is a little evidence to suggest systemic growth of fungus in the plant. The warm humid weather at the flowering is necessary for disease development.



(v) Control

1- Disease resistance is available in winter wheat varieties (NIAB Farmers Leaflet No. 8).

2- Fungicides should be applied before the disease is seen (if more than 10% of the area of the ear is affected, control may be ineffective {e.g. Vassgro Manex (maneb+zinc)}.

7 Diseases of field peas and beans

(1) Foot and root rot

(i) Causal fungus: *Fusarium solani* f.sp. *pisi* (peas) and *F. solani* f.sp.*phaseoli* (beans).

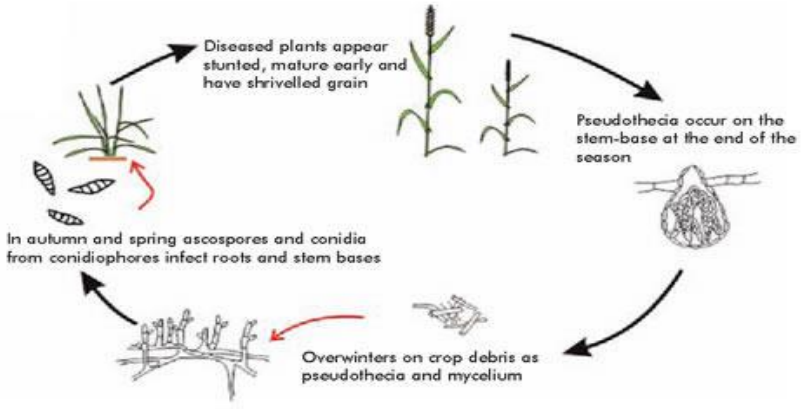
(ii) Host range: the formae specialis pathogens listed above are specific to peas and beans.

(iii) Symptoms: most of the above pathogens can cause damping-off in peas and beans, especially if the weather is cold and wet and germination is slow. Wilting may occur after warm periods and patches of stunted plants can be seen in the crop. Infections can lead to pink orange spore masses developing in the surface of the stem base.

(iv) Disease cycle: most of the pathogens are soil borne and can form specialized resting structures (chlamydospres). Sporulation of fungi can lead to spread of the disease in the crop. Generally warm wet weather favours diseases development.

(v) Control: cultural control involving good crop rotations helps to reduce disease.

Chemical control is aimed at eliminating the problem on the seed and allowing the plant to establish itself. Fungicides seed treatments approved for the control of damping off in peas and beans is Aliette Extra (active ingredients are captan + fosetyl aluminium + thiabendazole).



(2) Wilt

(i) Causal fungus: *Fusarium oxysporum* f.sp. *pisi* (peas), *F. oxysporum* f.sp. *fabae* (beans).

(ii) Host range: generally fungi are specific to their forma specialis host.

(iii) Symptoms: first symptoms of *Fusarium* wilt usually become apparent during early summer. Individual plants wilt, especially during hot dry weather, and recover during the night. Later lower leaves of the plant become chlorotic and eventually die and a permanent wilt occurs from the base of the plant upwards. The death can occur quite quickly during dry periods around the flowering time. The xylem of the infected plants develops a brown red coloration.

(iv) Disease cycle; the fungi exist as chlamydospores in the soil and on plant debris. These are stimulated to germinate by the presence of rootlets and hyphae penetrate the rootlets. The fungus then rapidly grows towards the xylem. In the xylem, both mycelium and spores are produced. Rapid colonization of considerable lengths of xylem may be effected by the production of microconidia which are transported in the transpiration stream. Wilting is caused both by physical blockage of xylem by the fungus and probably the production of toxins. The fungus remains confined to xylem vessels until the plant dies, when it grows out of the dead tissue and produces more chlamydospores, which are released into the soil.

(v) Control: disease resistance is the main and most effective control method.

(3) Downy mildew:

(i) Causal fungus: *Peronospora viciae*.

(ii) Host range: peas and beans.

(iii) Symptoms: young plants may be systemically colonized by *P. viciae*. Plants are stunted and chlorotic and the undersides of the leaflets become covered with dense grey-white fungal hyphae. Systemically colonized plants usually die. Disease in mature plants results in chlorotic patches on upper leaf surfaces with corresponding grey white fungal growth on the underside of the leaf. Pods and seeds can also become contaminated with downy mildew. Pods develop a yellow blotchiness externally, leading to white fungal growth inside the pod and discoloration of seeds.

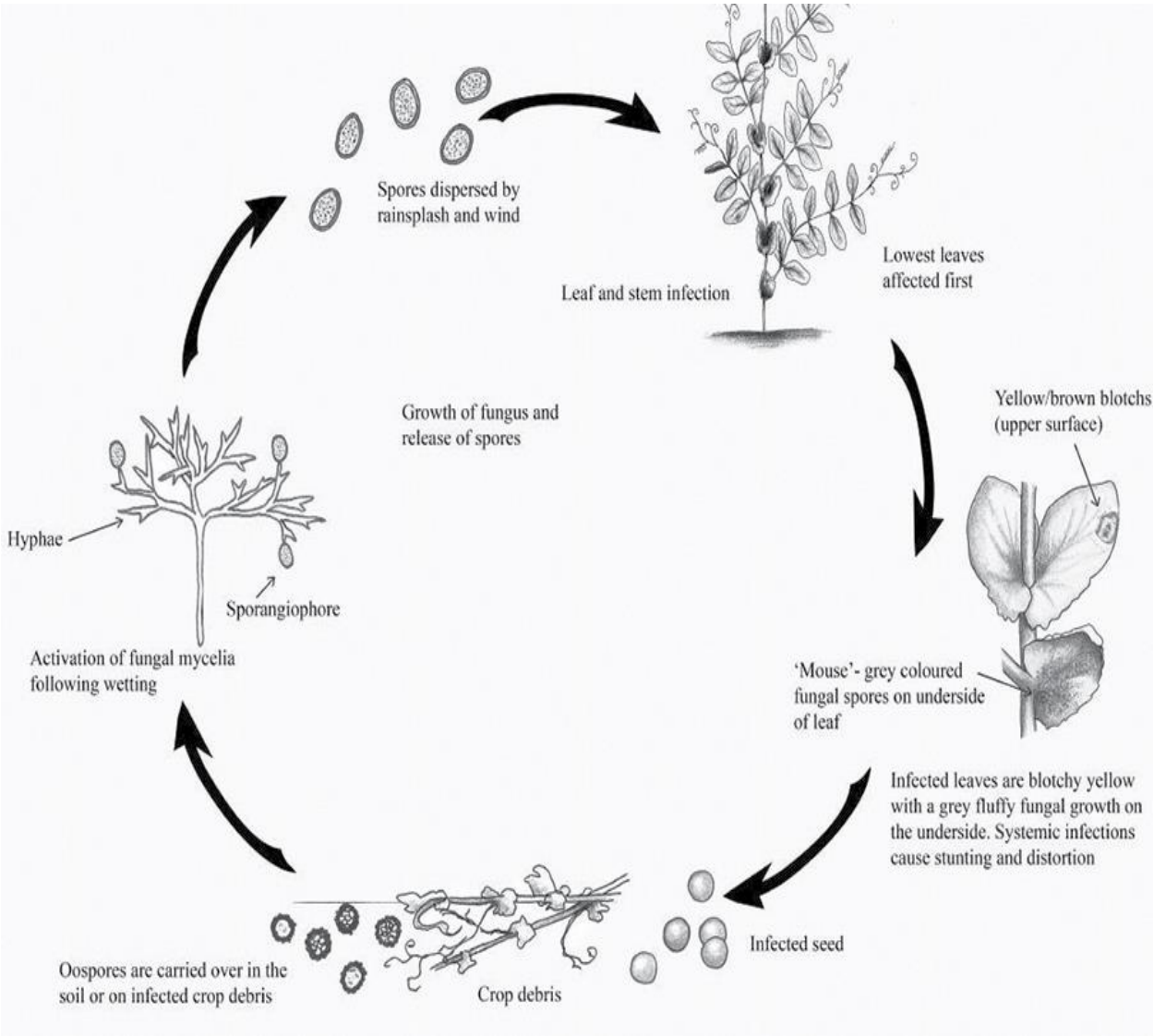
(iv) Disease cycle: the main source of overwintering inoculums is oospores in contaminated debris (soil and seeds). Oospores in the surface layers of soil or debris germinate and infect plants, resulting in systemic colonization, or produce sporangia which initiate infections on aerial parts of the plant. Further sporangia are produced on the undersides of the leaves and are dispersed by rain and wind. On susceptible host tissue sporangia germinate directly leading to penetration of the leaf surface. Spore germination is optimal at temperature between 4 and 8^{o} C and long periods of high humidity are also required.

(v) Control:

Cultural control includes good rotations, allowing at least four years between pea crops in badly infested land. Debris should be disposed by burning.

Disease resistance, Recommended Varietires of Field Peas and Field Beans.

Chemical control methods are available (Aliette Extra).



life cycle of *Peronospora viciae*

8 Disease of potatoes

(1) Potato blight (late blight)

(i) Causal fungus: *Phytophthora infestans*

(ii) Host range: members of *Solanaceae*, especially potato and tomato.

(iii) Symptoms:

a- On haulm: the first signs of potato blight are circular or irregular water-soaked patches, often at the tips or edges of lower leaves. Large brown dead areas of leaves may quickly develop and a zone of white downy fungal growth may occur at the edge of the lesion on the underside of the leaf.

b- On tuber: the first signs of blight in potato tubers are unobstructive brown or black irregular blotches. Tubers may also appear "marbled" in patches where internal streaks tissue can be seen through the skin. Blight symptoms are best seen when tubers open. The first symptom is a brown discoloration of the outside layers of tuber tissue. This can be quickly progress inwards resulting in large areas of firm but brown rotten tissue. Blight can progress in infected tubers in potato stores but it does not usually spread to healthy tubers. Blighted tubers are frequently colonized by secondary bacterial pathogens in poorly ventilated warm humid potato stores.

(iv) Disease cycle: Late blight has two ways of reproducing; (i) spores called "sporangia" are produced without going through a sexual process or (ii) spores called "oospores" are produced when two strains (A1 and A2) come together and mate.

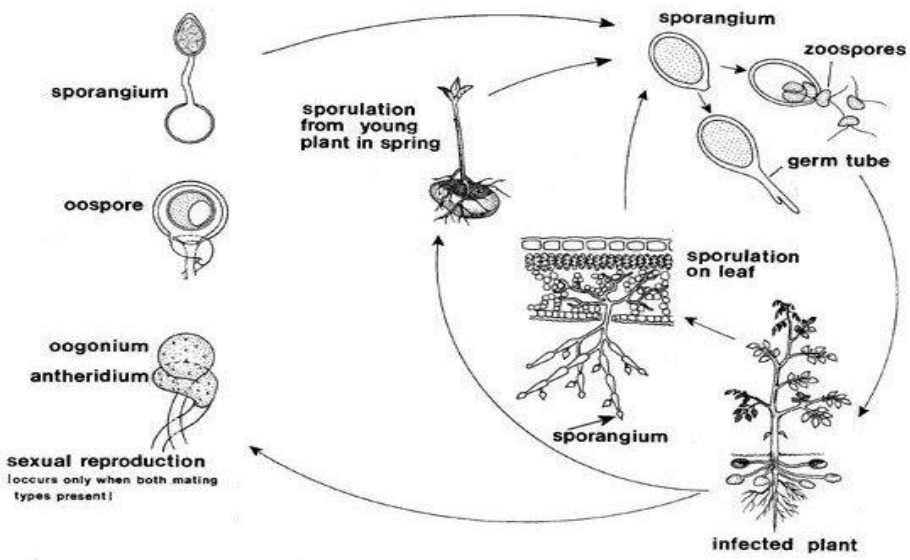
The **sporangia** on the leaves, stems and tubers within the cottony growth are spread by wind, rain and water splash. If the sporangia land on a potato plant, and the surface is wet, they germinate. There are two ways of doing this depending on temperature: (i) in warmer conditions (21-26^{o}C), the spores germinate like seeds and infect the plant directly, or (ii) in cooler conditions (18-22^{o}C) each spore produces 6-8 smaller spores (called "zoospores") which burst out of the larger spore and swim short distances over the plant surface before germinating and infecting. Sporangia and zoospores survive only for a few days in plant remains or in the soil.

By contrast, **oospores** are resting spores; they have thick walls for survival either in plant remains, in the soil or in the tubers. Oospores germinate and produce sporangia.

Epidemics of late blight occur when night temperatures are cool, followed by warm days with mists and rains. Under those conditions, sporangia and zoospores spread the disease rapidly and fields of potato are destroyed in a less than 2 weeks.

Spread of late blight over short to moderate distances between plants and fields occurs as sporangia and zoospores in wind or wind-driven rain. Sporangia can travel as far as 15-20 km. Spread over longer distances, across countries and continents, occurs in tubers used as "seed" for planting.

Survival of late blight between crops occurs as oospores, where A1 and A2 strains occur together, but this is not as important as survival in tubers. Spores are produced on the surface of tubers in the cull piles (reject tubers), and on volunteer plants from tubers left in the ground. See life cycle (Diagram).



Life cycle of *Phytophthora infestans*

(v) Detection & Inspection

Look for spots and patches on the leaves which grow rapidly and produce a furry white growth on the underside. Look for the white growth after putting the leaves in a plastic bag overnight. Check the plants regularly for infections, especially when the days are cool and wet, or overcast with heavy dews, fogs or mists.

(vi) Control

QUARANTINE

Until recently the movement of tubers from countries where the A2 strain existed was regulated, but with the wide distribution of that strain in recent year, the policy has been reassessed.

CULTURALCONTROL

Note it is important for all growers in a region to cooperate in getting rid of the sources of the disease. This means getting rid of the cull piles, and also any other unharvested tubers left in the fields.

Before planting:

- It is important to use certified seed, i.e., that which is free from late blight infection, and from viruses. If unsure about freedom from late blight, test a few seed tubers by lettings them sprout at 15-20^{o}C for 10-15 days. If infected by late blight they will rot.
- Check for volunteer plants from the previous crop and, if found, remove and burn them.
- Select sites where there is good drainage, and where there is good air movement, so that leaves dry quickly after rain and dews. Avoid fields surrounded by trees.
- Practice crop rotation. Do not plant crops where they were grown in the previous season, or year. Use a 2-3-year crop rotation.
- Choose a short duration, "early" variety that set fruits and matures quickly to produce a crop in the shortest time possible, and potentially avoid serious build up of disease.

During growth:

- Plant the seed potatoes on ridges so that the spores have further to travel to reach the tubers.
- Avoid overhead irrigation; otherwise, conditions will be created for the production of spores and their infection of both leaves and tubers.
- Remove self-grown potatoes and *Solanum* weeds (i.e., volunteer plants) as they may have late blight infections.
- Do not apply too much nitrogen fertilizer as this will increase the growth of leaves, and also delay the time to crop maturity.
- Frequently, inspect the crop for spots on the leaves, especially if fungicides are not being used routinely to prevent infection (see under Chemical Control).
- Destroy the leaves before harvest if late blight is present to avoid the infection of tubers when they are lifted. Use a herbicide to kill the leaves.

After harvest:

- Do not leave rejected tubers in cull piles in the field, otherwise they will provide a source of spores for the next crop

RESISTANT VARIETIES

Late blight resistant varieties are available. They are being bred continually by CIP, so check if they are available in your country.

CHEMICAL CONTROL

The following points are important:

- Use a preventative spray of a contact fungicide before symptoms are seen, e.g, a copper product, chlorothalonil, mancozeb or polyram.
- If using a systemic product that may control the disease after infection, e.g., metalaxyl, cymoxanil, dimethomorth, or a strobilurin, alternate single sprays with two sprays of a preventative product to avoid the development of resistant strains of late blight.
- Manufacturers of cymoxanil and dimethomorph recommend the addition of either mancozeb or polyram to these products.
- It is very important to follow manufacturers recommendations if using systemic products.
- Copper fungicides - copper hydroxide and copper sulfate - can be used on organic crops, although because of copper toxicity, alternatives, such as phosphorous acid and horticultural oils may be necessary.
- The frequency of spray application will depend on the susceptibility of the potato variety and whether environmental conditions favour late blight disease.

(2) Early blight (target spot):

(i) Causal fungus: *Alternaria solani*

(ii) Host range: Solanaceae including potato an tomato.

(iii) Symptoms:

Symptoms of early blight occur on fruit, stem and foliage of tomatoes and stem, foliage and tubers of potatoes. Initial symptoms on leaves appear as small 1-2 mm black or brown lesions and under conducive environmental conditions the lesions will enlarge and are often surrounded by a yellow halo (Figures 2 and 3). Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. This so-called "bulls eye" type lesion is highly characteristic of early blight (Figure 4). As lesions expand and new lesions develop entire leaves may turn chlorotic and dehisce, leading to significant defoliation. Lesions occurring on stems are often sunken and lens-shaped with a light center, and have the typical concentric rings (Figure 5). On young tomato seedlings lesions may completely girdle the stem, a phase of the disease known as "collar rot," which may lead to reduced plant vigor or death.

Figure 2

Figure 3

Figure 4

Figure 5

Infection of both green and ripe tomato fruit normally occurs through the calyx with lesions sometimes reaching a considerable size (Figure 6). The lesions appear leathery and may have the characteristic concentric rings. Infected fruit will frequently drop prematurely. Symptoms on potato tubers are characterized by sunken, irregular lesions (Figure 7), which are often surrounded by a raised purple border. Beneath the surface of the lesion the tuber tissue is leathery or corky with a brown discoloration. Early blight lesions on tubers tend to be dry and are less prone to invasion by secondary organisms than lesions of other tuber rots. After prolonged storage severely diseased tubers may become shriveled.

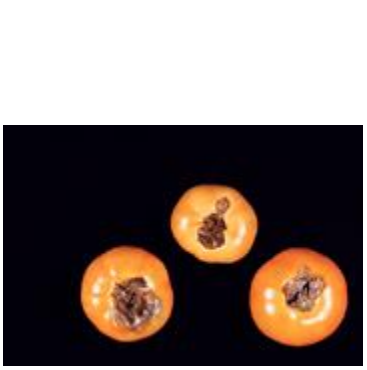
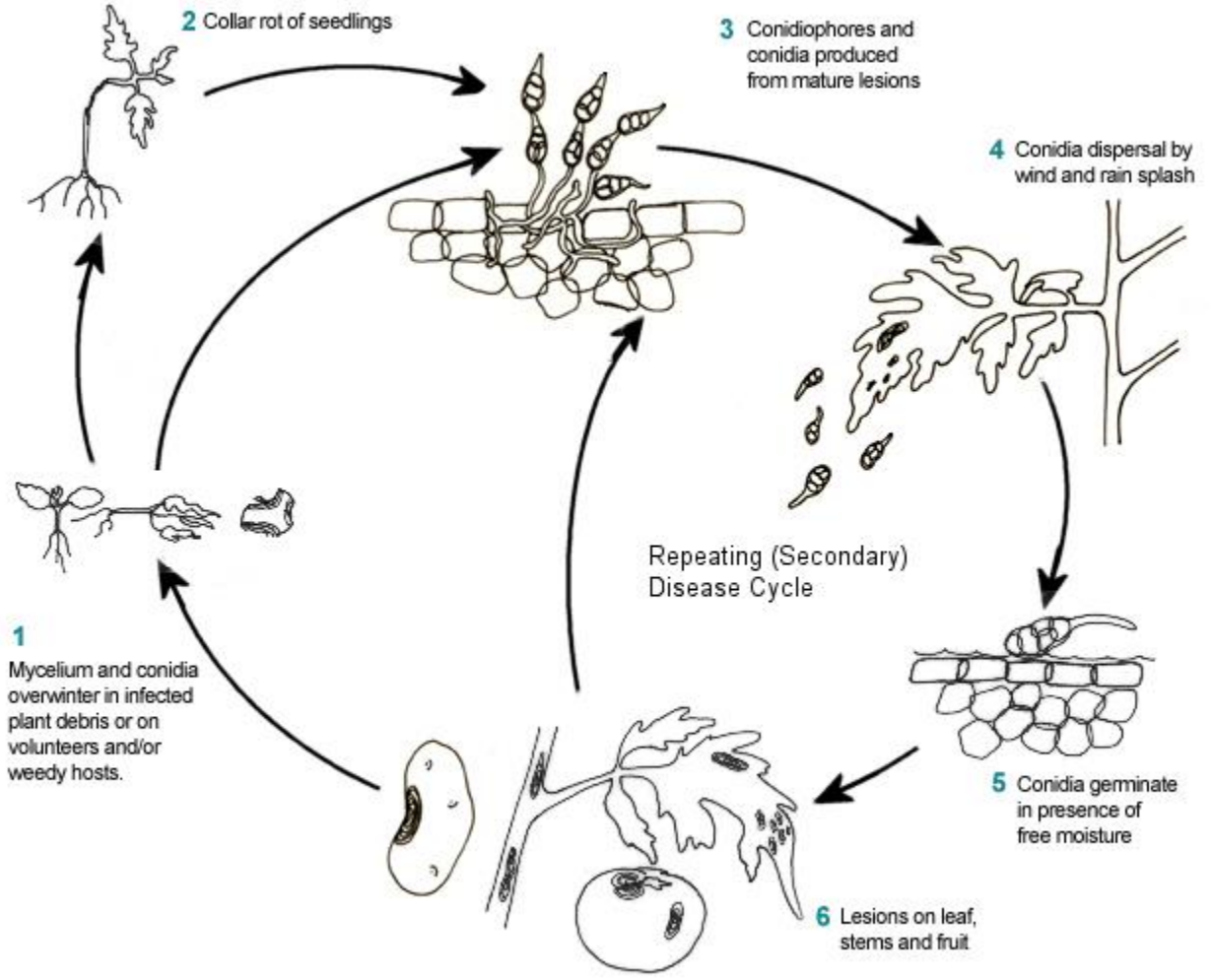


Figure 6

Figure 7

(iv) Disease Cycle and Epidemiology



Alternaria solani overwinters primarily on infected crop debris. The dark pigmentation of the mycelium increases resistance to lysis which extends the survival time in the soil to several years. Thick-walled chlamydospores have been reported, but they are found infrequently. In mild climates the pathogen can survive from season to season on volunteer tomato and potato plants as well as other weedy Solanaceous hosts such as horsenettle and nightshade.

Warm, humid (24-29°C/ 75-84°F) environmental conditions are conducive to infection. In the presence of free moisture and at an optimum of 28-30°C (82-86°F), conidia will germinate in approximately 40 min. Desiccated germ tubes are able to renew growth when re-wetted, and, hence, infection can occur under conditions of alternating wet and dry periods. Germ tubes penetrate the leaf

epidermis directly or enter through stomata. Infection of potato tubers usually occurs through wounds in the tuber skin inflicted during harvest. Wet conditions at harvest provide a favorable environment for spore germination as well as causing swollen lenticels on the tubers which are easily invaded.

Time from initial infection to appearance of foliar symptoms is dependent on environmental conditions, leaf age, and cultivar susceptibility. Early blight is principally a disease of aging plant tissue. Lesions generally appear quickly under warm, moist conditions on older foliage and are usually visible within 5-7 days after infection.

A long wet period is required for sporulation but it can also occur under conditions of alternating wet and dry periods. Conidiophores are produced during wet nights and the following day light and dryness induce them to produce spores, which emerge on the second wet night.

Secondary spread of the disease results from conidia being dispersed mainly be wind and occasionally by splashing rain or overhead irrigation. Early blight is considered polycyclic with repeating cycles of new infection. This is the period when the disease has the potential to spread rapidly and build up to damaging levels in the crop.

(v) Disease Management

Cultural practices

In many cases employing sound cultural practices that maintain potato and tomato plants in good health will keep early blight losses below economic levels. Because the pathogen over winters on infected crop debris, in-field sanitation procedures that reduce initial inoculum in subsequent crops are beneficial. Consideration should be given to removing potentially infected material such as decaying vines and fruits from the vicinity of production fields. Controlling volunteers and weeds, such as nightshade and horsenettle which serve as alternative hosts for the disease, prior to planting the new crop will help to reduce the risk of transmission of disease. Ensuring seed or transplants are pathogen free before placing out in the field and rotating fields to a non susceptible host crop will also help to reduce buildup of inoculum in the soil. Optimal tuber maturity is the most important factor for control of tuber infection. Tubers

harvested before maturing are susceptible to wounding and infection. Tuber infection can be reduced by careful handling during harvest to minimize wounding as well as avoiding harvesting during wet conditions if possible. Tubers should be stored at 50 to 55 F, at high relative humidity and with plenty of aeration to promote wound healing which will reduce the amount and severity of tuber infections that develop in storage.

Figure 9

During the season, overhead irrigation schedules should minimize the duration of leaf wetness in the crop (Figure 9). Avoid irrigation in cool, cloudy periods or late in evening when foliage may stay wet for extended periods. Selecting fields with good drainage and an absence of natural impediments to air flow over the crop, e.g. rows of trees, will reduce periods of leaf wetness. Maintenance of adequate soil fertility levels is also critical for managing early blight. The disease is often associated with crops suffering from a lack of nitrogen, particularly towards the end of the growing season on older senescing foliage. Management of other diseases such as Verticillium wilt will reduce plant stress and hence, early blight severity.

Resistant cultivars

Complete resistance to early blight does not exist in commercial potato or tomato cultivars. Using wild *Lycopersicon* species which show a high degree of resistance in breeding programs has led to the release of a number of cultivars of potato and tomato with a degree of resistance to early blight. Apparent levels of resistance are often correlated with plant age. Immature potato and tomato plants are relatively resistant to early blight but, after tuber and fruit initiation, susceptibility increases gradually, and mature plants are very susceptible to the pathogen.

Figure 10

Chemical control

Fungicides with protectant and curative properties are registered for use against early blight on tomato and potato (Figure 10). The cheaper protectant fungicides such as mancozeb and chlorothalonil are the foundation of most early blight management programs. These fungicides must be reapplied every 7-10 days to provide protection of new growth as well as to counter the effects of weathering which progressively removes the chemical from the leaf surface. Advantages of these types of products include their reliable efficacy and multi-site mode of action, which reduces the risk of resistant isolates developing in the pathogen population; therefore, they are useful as tank mix partners or used in rotation with other fungicides. Disadvantages include the need to apply regularly and their relatively high use rates.

The so-called Quinone Outside Inhibitors (QoI) class of fungicides (FRAC code #11) which inhibit fungal respiration are highly active against *Alternaria* species. Molecules from this very important class of fungicides which are registered for *Alternaria* control in potato and or tomato include azoxystrobin, pyraclostrobin, trifloxystrobin, fenamidone and famoxidone. In general QoIs are readily taken up into the plant tissue and work preventively to stop infection by inhibiting spore germination. They are weakly curative and use rates are considerably lower than the traditional protectant products, although cost per acre is typically higher. QoIs are high-risk fungicides with respect to resistance development, and isolates of *A. solani* which possess the F129L mutation have been isolated from the field. These isolates show significantly reduced levels of sensitivity to the QoI fungicides (Pasche and Gudmestad, 2008). Isolates of *A. solani*bearing the G143A mutation which confers high levels of resistance to QoIs have also been detected in routine monitoring in Europe. The Fungicide Resistance Action Committee (FRAC) discourages the use of QoIs in a

curative manner and recommends that this fungicide class should make up no more than six applications or 50% of the total spray program in a single season providing the material is applied with another fungicide having an alternative mode of action, either as a tank mixture or in a co-formulated product. If the QoI is applied solo to the crop then the grower should not exceed 4 sprays or 33% of the total spray program.

Growers also have new weapons in their chemical arsenal for control of early blight in the shape of fluxapyroxad, fluopyram and penthiopyrad which are all succinate dehydrogenase inhibitors (SDHIs) -FRAC code #7. SDHIs are also inhibitors of fungal respiration although they bind to a different target site than the QoIs and hence are not cross resistant to the latter. These materials are new in the potato and tomato market in 2012 and will give growers a welcome additional mode of action for managment of early blight. As single-site inhibitors they are consider by FRAC as at medium to high risk of resistance development and will require careful management of the resistance risk via appropriate tank mixing and alternation with other effective modes of action.

Timing of fungicide sprays relative to environmental conditions and subsequent potential for disease development is critical if good control is to be attained. Use of disease forecasting programs such as FAST in tomatoes and P-DAY in potatoes to correctly time application of sprays as well as thorough scouting of fields increases efficacy of the fungicide products as well as helping to cut down on unnecessary applications and costs.

(3) Stem canker and black scurf:

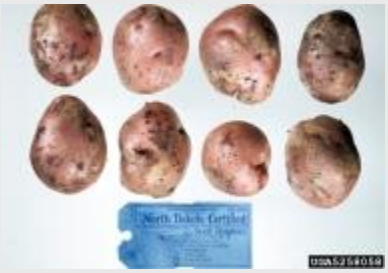
(i) Causal fungus: *Rhizocotina solani*

(ii) Host range: *Brassica* species beans sugar beet, tomatoes and potatoes.

(iii) Symptoms:

Symptoms can be observed on above and below ground plant parts. Symptoms observed above ground early in the season include necrosis at the tips of the sprouts (which may eventually cause the emerging plant to die) and sunken lesions on stolons, roots, and stems. Later in the season, sclerotia are produced in the

tubers creating a sign called black scurf which is simply, sclerotized mycelium (5, 9). Stems with cankers can become girdled, resulting in stunted plants. Leaves of infected plants develop a purplish and chlorotic coloration. In severe infections, green tubers develop above the ground. Affected tubers are deformed and can produce sclerotia on the surface (9).



(iv) Ecology and Spread

Rhizoctonia solani is a soil pathogen and thus is affected by soil conditions. Black scurf and stem canker are more severe in soils that are cool and moist. Development of these diseases is favored by soil temperatures between 16 to 23 °C, while soil temperatures above 25°C reduce the severity of canker (1). The disease also tends to be more severe on dry light soils. The sexual stage of this pathogen can be found in infected potato plants but is not clear the role of basidiospores in the epidemiology of stem canker and black scurf in potato (7). It is believed that *R. solani* sclerotia (i.e., resistant structures) are the structures responsible for long distance dispersal (2).

Life cycle of *R. solani* on potato: Production of asexual spores is not observed in *R. solani* (3). Sclerotia formed at the end of the cropping season, allow the fungus to overwinter. The fungus can also survive on infested tubers left on the ground, soil, and debris. During the spring, potato plants from any stage can become infected by mycelia of the pathogen that spread underground from plant to plant.

Geographic Distribution

Rhizoctonia solani is a cosmopolitan fungus and infects a wide variety of plant families (4). Most of the isolates responsible for potato stem canker and black scurf are found ubiquitously and within the AG-3 (9).

(v) Control

Management of the disease should not rely solely on chemical control. It must be achieved through the combination of multiple management strategies:

- Conduct a soil test prior to establishment of the crop to know nutrient content and soil pH. Low pH is not conducive for disease development.
- Use disease-free soil. Avoid areas with history of potato production or history of potato scurf and stem canker.
- Use certified potato seed and assure disease-free propagation material. If seed is not certified, it should be treated with antagonists or fungicides before planting.
- Perform long rotations (i.e., 3 or more years) to produce a significant reduction on the inoculum of *R. solani*. Rotations with canola, barley, or sweet corn have been recommended in the literature for reduction of *Rhizoctonia* inoculum and enhancement in potato quality.
- Separate tubers from crop residues after destruction of the stem to prevent an increase in black scurf.
- Plant less susceptible cultivars. There are differences in susceptibilities among potato cultivars, however resistant cultivars have not been developed yet.
- Consult your local extension specialist for legal and efficacious fungicide products available in your state. Remember, the label is the law and the product applicator is responsible for reading and following all chemical labeling.

9 Diseases of sugar beet

(1) *Phoma* disease

(i) Causal fungus: *Phoma betae*

(ii) Host range: beet

(iii) Symptoms: the pathogen can attack beet at all stages of its development. Initially P. betae causes blackleg (pre-emergence damping-off). Mature leaves of beet can also be infected by *Phoma*, causing leaf spot. The resulting light brown circular lesions contain black pycnidia arranged in concentric rings near the lesions edge. *Phoma betae* is also one of the major causes of storage rots of beet in USA, after storage for about 80 days or more the rot usually begins in the centre of the crown and spreads downwards into the beet, resulting in a cone- shaped area of black rotten tissue. Pockets lined with white mycelium may develop and black pycnidia are also formed.

(iv) Disease cycle: the fungus overwinters on seed debris. The pycnidia formed release spores during wet weather, which are spread mainly by rain-splash and insects. Spread of the pathogen in seed crops may also be systemic.

(v) Control: crop rotation. Storage rots are reduced by application of thiabenzol, avoiding wounds at harvesting and allowing beet to heal before being put into piles.

(2) *Alternaria* leaf spot

(i) Causal fungus: *Alternaria alternate*

(ii) Host rang: numerous

(iii) Symptoms: symptoms appear late in the growing season. Leaves turn brown from the edge inwards. Severe attack can kill leaves. Characteristics *Alternaria* target spots may be seen. *Alternaria* may rot beet roots stored in clamps.

(iv) Disease cycle: the fungus is weakly pathogenic and can attack only senescing tissue. Its large club-shaped spores are windblown onto beet from any contaminated decaying plant material.

(v) Control: control of the disease is currently not considered worthwhile.

(3) Rust :

(i) Causal fungus : *Uromyces betae*

(ii) Host range: beet species

(iii) Symptoms: the disease is perhaps most obvious during late summer on sugar beet leaves, although early summer infections may also occur. Primary symptoms consist of small red-orange pustules surrounded by chlorotic haloes scattered over both leaf surfaces, sometimes occurring in clusters. Late summer symptoms consist of darker brown, more evenly spread pustules. Severe attacks can give leaves an overall brown appearance and may result in death.

(iv) Disease cycle:

The fungus overwinters primarily on seed crops or groundkeepers as the teliospore stage (end of season symptoms). Teliospores may also contaminate seeds. The optimum temperature for rust development occurs between 15 and 22^{o} C, and temperatures above 22^{o} C inhibit rust development.

(v) Control: elimination of groundkeepers, will help to reduce the disease. Triadimenol (Spinnaker) is an approved fungicide for control of the disease.

10 Diseases of soybeans

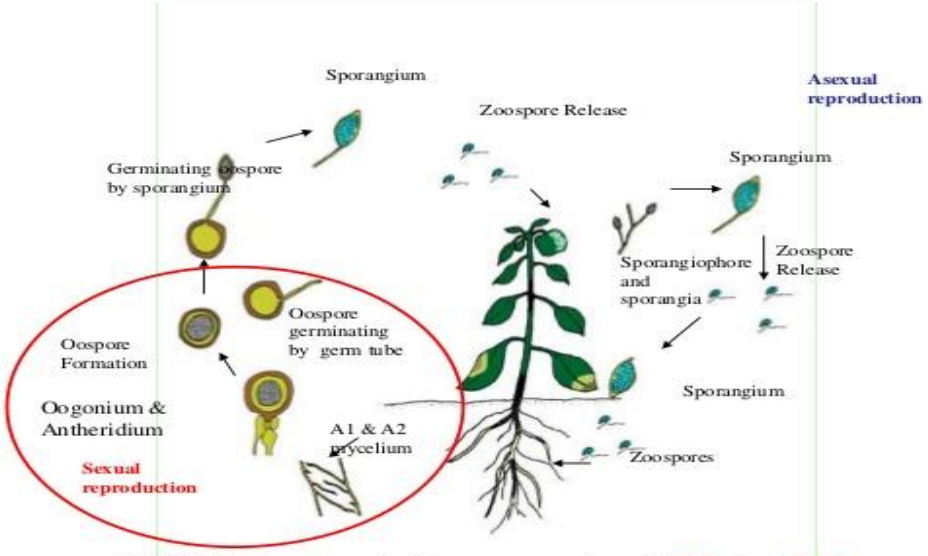
(1) *Phytophthora* root and stem rot

(i) Causal organism: *Phytophthora megaserma* f.sp. *glycinea*.

(ii) Host range: soybean, tomato, alfalfa, clover and garden pea.

(iii) Symbtoms: the fungus can cause pre- and post-emergence damping-off in seedlings. In mature susceptible plants, a brown girdling rot develops, followed by wilting and death. More resistant plants may survive an attack but brown sunken lesions may develop on stems. Diseased tap roots then become covered with orange masses of spores of a secondary pathogen, *Fusarium* spp.

(iv) Disease cycle: the pathogen overwinters primarily as oospores in debris and soil. When temperatures rise and abundant moisture becomes available, oospores germinate to form sporangia which then release swimming zoospores. Zoospores are attracted to germinating seeds or rootlets where they encyste, germinate and penetrate plant tissues. Secondary infections occur when sporangia produced on rotting rootlets, release zoospores into flooded soils. Leaf and stem infection may also occur if contaminated soil is blown or splashed onto aerial plant parts and humid damp conditions prevail. The disease is most common in a heavy, wet clay soils and on farms where minimum cultivation is practiced. A temperature range between 25 and 30^{o} C favors disease development.



Life history and disease cycle of *Phytophthora*

(v) Control:

- Cultural control. Direct improvement of drainage will reduce the disease. Ploughing soil also may indirectly improve drainage. Crop rotation is also advisable.
- Disease resistance. Breeding for resistance to P. *megasperma* f.sp. *glycinea* was initiated in themed-1950s and is race specific.
- Chemical control: seed treatments are available for the reduction of early attacks of *P. megasperma* f.sp. *glycinea*. Metalaxyl (Apron) and pyroxyfur (Grandstand) are systemic seed treatments with efficacy against the pathogen. Metalaxyl may beincorporated into soil or used as foliar spray.
- Biological control: has been investigated and may prove feasible in the future. Hyperparasitic fungi on seed coats have been found to reduce oospore populations in soil (Filonow &Lockwood,1985).

(2) Pod and stem rot:

(i) Causal fungus: *Diaporthe phaseolorum* var. *sojae*.

(ii) Host range: mainly soybeans, but other crops and weeds may also be infected.

(iii) Symptoms: all aerial parts of the soybean plant may be attacked, resulting in stunting and the production of numerous black pycnidia in bleached dead patches of leaf, stem and pod tissues. The disease on leaves usually starts at the margin and quickly progresses until the leaf is killed, rather than resulting in discrete lesions. Pycnidia develop in a linear fashion on stems and pods.

(iv) Disease cycle: the fungus overwinters as dormant mycelium in debris and seed. Primary infections are initiated by either asexual spores produced in pycnidia or sexual spores from perithecia. Free water is essential for spore germination. It is now known that the fungus infects at any time during the growing season and remains latent or symptom less until plants begin to senesce. It is at this stage that symptoms develop and fruiting structures are formed. However if plants are stressed earlier in the season to cause early senescence, symptoms and fruiting structures will develop earlier. Temperature above 20^{o} C together with prolonged wet periods tends to favour disease development. Secondary infections occur as a result of splash dispersed asexual conidia. Colonization of pods frequently leads to seed contamination particularly if harvest is delayed as a result of wet weather. Contaminated seed can result in the introduction of the disease into new planting areas.

(v) Control:

Cultural control. Soybeans should be harvested promptly at maturity. Crop rotation and ploughing will also help to reduce the disease.

Disease resistance is available in some soybean varieties.

Chemical control using both seed treatments and foliar sprays can also be cost-effective on farms with a history of the problem. Products approved for the control of pod and stem rot include Benlate (active ingredient benomyl) and Bravo 500 (chlorothalonil).

Biological control using bacterial seed treatments.

(3) Anthracnose

(i) Causal fungus: *Colletotrichum truncatum* and *Glomerella glycines*.

(ii) Host range: neither pathogen is specific to soybean and some weeds may be infected.

(iii) Symptoms: the first symptom to be seen if contaminated seed is planted is damping-off. Dark sunken lesions, which develop on cotyledons of emerging seedlings, may cause defoliation and death. Symptoms of disease may or may not be seen on mature plant stems, pods, leaves and petioles. Irregular brown lesions develop and particularly towards the end of the season, small black acervuli or prethecia develop in a random fashion on affected tissues.

Symptoms of Anthracnose on soybean

(iv) ى

(v) Control: cultural control methods include crop rotation, correct disposal of debris by ploughing and use of high quality pathogen free seeds.

Chemical control: both fungicide seed treatments and foliar sprays are available. Benomyl has proved effective if applied between flowering and pod-fill. Products approved for the control of anthracnose are the same as those approved for the control of pod and stem rot.

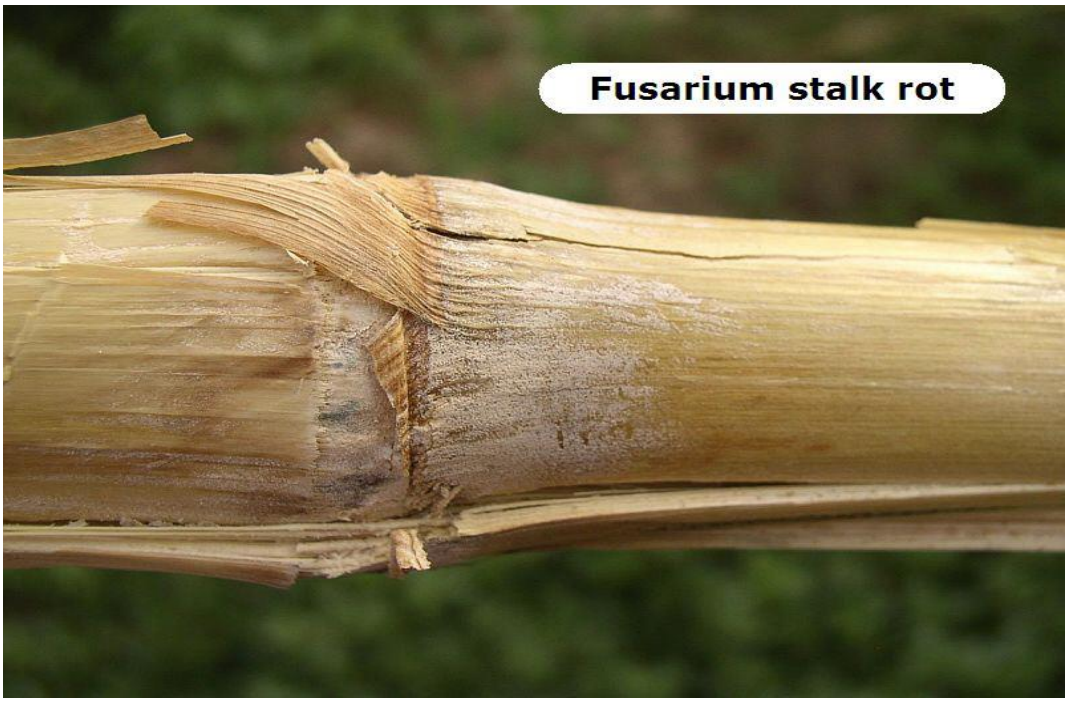
11 Diseases of maize

(1) Stalk rot

(i) Causal fungus: *Diplodia maydis*, *Fuasrium culmorum*, *F. moniliforme* and *F. graminearum*. *Pythium aphanidermatum* and *Colletotrichum graminicola*, the cause of anthracnose, may also cause stalk rot.

(ii) Host range: the main stalk rot pathogens, the *Fusarium* spp. are most important on maize and other cereals.

(iii) Symptoms: symptoms of *Diplodia* and *Fusarium* stalk rot are similar. Affected stalk tissue becomes spongy and bleached. Vascular bundles remain intact but pith tissue becomes discolored. Dark sub-epidermal *pycnidia* of *Diplodia* can often be found near nodes, compared with superficial perithecia formed in stalks affected by *Fusarium graminearum*. In addition, *Fusarium*-rotted stalks often show a pinkish-red or orange discoloration, whereas a white fungal growth sometimes occurs in *Diplodia* stalk rot.



(iv) Disease cycle: both *D. maydis* and *Fusarium* species can overwinter on seed and crop depris. Seed contamination can result in damping-off. Generally stalk rot tends to be more severe if conditions are dry during the vegetative period of growth and wet during the reproductive period. Primary infections are initiated by wind-blown ascospores in *Fusarium graminearum*, but subsequent infections by *Fusarium* spp. and infection by *D. maydis* arise mainly from rain-splashed conidia. Plants damage by pest attack or adverse weather tends to be more susceptible to stalk rot.

(v) Control; cultural control. This involves the use of balanced fertilizer programme. Excess nitrogen should not be used and any deficiencies in potassium should be corrected. Optimum seed rates should be used and overcrowded humid crops avoided.

Disease resistance varieties and hyprids is widely available.

Chemical control. Thiram and captan seed treatments will reduce seed contamination and damping-off, but do not protect plants from subsequent attacks.

(2) Storage moulds:

(i) Causal fungus: *Aspergillus*, *Penicillium* and *Fusarium*.

(ii) Host range: numerous.

(iii) Symptoms: grey, green, blue, brown, black or pink mould development leading to heating and caking of seed.

(iv) Disease cycle: affected seed may enter stores with or without disease symptoms as a result of ear rots in the field. Inoculums may also be present on debris in grain bins. Problems occur if seed has above 15% moisture content and if temperatures are allowed to exceed 21^{o} C.

(v) Control:

1. Grain for storage should be dried to a minimum of 15% moisture (13% for long term storage) as soon as possible after harvest.
2. Grain should then be cooled to 2-5^{o} C and stored below 10^{o}Cif possible.
3. Grain bins should be thoroughly cleaned before use, ventilated throughout the storage period and checked

regularly for evidence of heating, mustiness and mould development.

12 Diseases of field vegetables

(1) Club root

(i) Causal fungus: *Plasmodiophora brassicae*

(ii) Host Range and Distribution**:** *Plasmodiophora brassicae* can be found worldwide in all temperate zones. It infects over 300 species in 64 genera of crucifers and can be found in both cultivated and wild crucifers. Economically important hosts include cabbage, collards, kale, mustard, brussel sprouts, radish, turnip, rutabaga, cauliflower, broccoli, rape, and kohlrabi.

(iii) Symptoms: Symptoms vary slightly from host to host. The first observable above ground symptom is day wilting. Otherwise healthy looking plants wilt on hot dry days, recovering once the sun sets or temperatures cool. As the disease progresses, leaves yellow and die. Diseased plants are obviously stunted compared to uninfected plants and will often be localized in low, wet areas of the field. When dug up, roots exhibit a variety of symptoms. New infections cause small knot like galls on roots, where as more developed infections display long spindle shaped clubs on primary and lateral roots. Some hosts, such as turnips and radishes, do not form clubs when infected. These hosts have black sunken lesions along the root surface.

(iv) Life Cycle**:** *Plasmodiophora brassicae* is an obligate parasite. It survives in the soil only as dormant cysts. Cysts can survive for up to 6-8 years without the presence of a host, and will germinate in response to the presence of crucifer root exudates. Primary zoospores released from germinating cysts infect host root hairs by encyst 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.

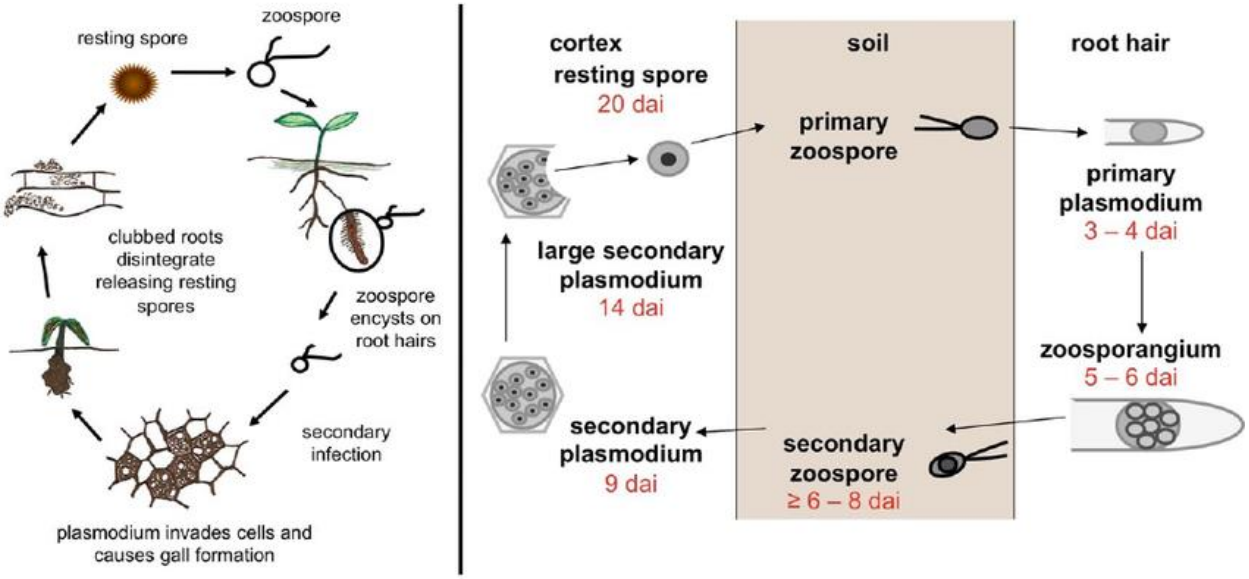
In the root hairs, amoeboid cells of the pathogen join together to form a multinucleate plasmodium. This plasmodium divides and forms multiple secondary zoospores, which are released into the soil. Secondary zoospores infect healthy

parts of the initial host or infect nearby plants. These zoospores also enter through the host root hairs, but 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 (IAA) 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 and are referred to as 'Kankheitsherd'. These Kankheitsherd are diagnostic of *P. brassicae* and can be observed in cross sections of infected roots.

Not all amoeboid cells infect cortical cells. Some move into the vascular tissue and infect the cambial cells of the host. The soft undeveloped cell walls of the cambial cells allows *P. brassicae* to easily travel up and down the root, infecting cortical cells, vascular ray cells, and cambial cells as it goes. The infection and resultant swelling of the vascular ray cells is responsible for the characteristic wilt symptoms associated with *P. brassicae*. As the ray cells swell to abnormal sizes, sections of xylem are pushed aside, and the continuity of the water column is broken.

Plasmodium in all 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.


resting spore
zoospore
clubbed roots disintegrate releasing resting spores
zoospore encysts on root hairs
secondary infection
plasmodium invades cells and causes gall formation
cortex
resting spore
20 dai
soil
root hair
primary zoospore
primary plasmodium
3 – 4 dai
large secondary plasmodium
14 dai
zoosporangium
5 – 6 dai
secondary plasmodium
9 dai
secondary zoospore
≥ 6 – 8 dai

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Practical physiology of fungi

For 4th year of B.Sc. students

Prepared by

Prof. Dr. Abdelrahman Saleem

2023/2024

Culture media

Classification of culture media

Criteria used for classifying culture media includes their chemical composition, physical properties and their use. Every culture medium is designed for a definite use and hence its physical and chemical characteristics depend on its application and function.

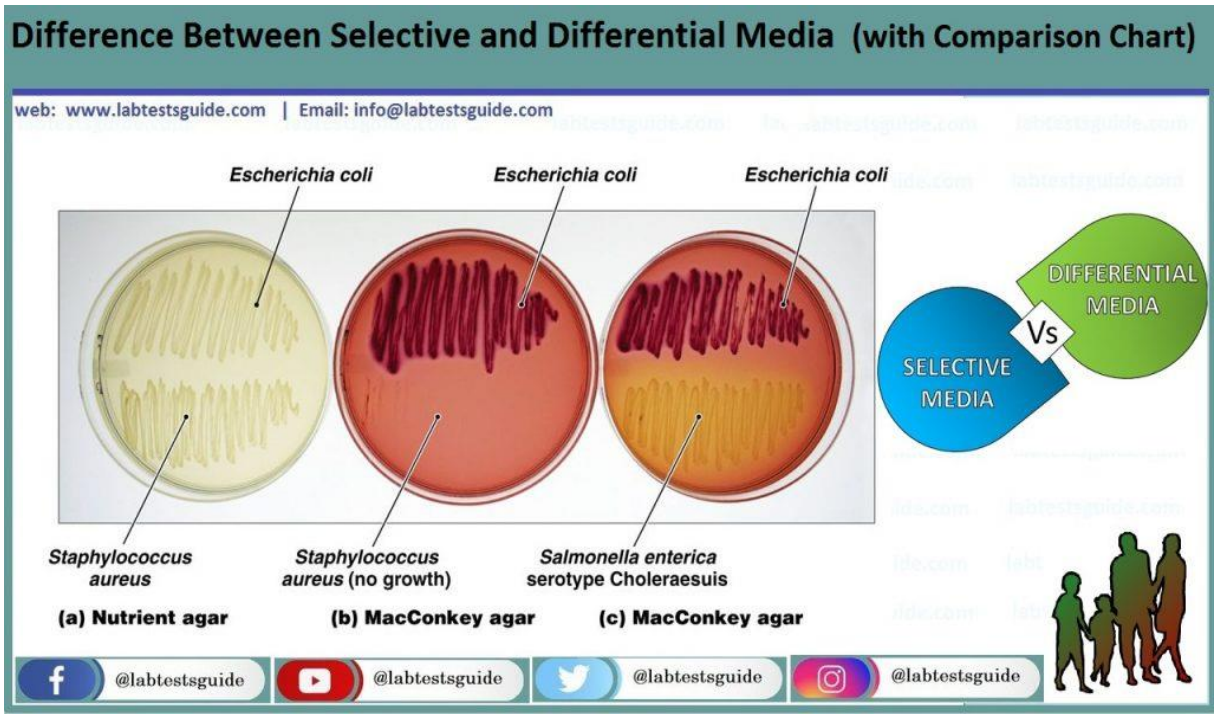
I- Classification of culture media according to their use

According to their use culture media are divided into the following types:

1- **Routine laboratory media**: These media contain certain complex raw materials of plant or animal origin such as yeast extract, malt extract, peptone etc., and are employed for routine cultivation and maintenance of a wide variety of fungi.

2- Enriched media: These media are prepared by supplementing the routine laboratory media with some specific substances such as vitamins and amino acids to meet the nutritional requirements of more fastidious of fungi and are employed for their cultivation.

3- Selective media: These media facilitate the isolation of a particular group or species of microorganisms from mixed cultures. Such media contain substances which inhibit microorganisms except the desired group or species.



4- Differential media: These media are supplemented with certain reagents or chemicals for differentiating between various kinds of microorganisms on the basis of visible differences in their growth patterns. Such type of media is used more often in bacteriological studies.

5- Assay media: These type of media is specifically employed for the assay of some metabolites such as enzymes, vitamins, amino acids, antibiotics, disinfectants etc., and are of definite composition.

6- Biochemical media: These media are generally used for the differentiation of microorganisms on the basis of their biochemical activities, and are helpful in the study of their metabolic processes.

II- Classification of culture media according to their chemical composition: According to their chemical composition media are classified into the following types:

1- Natural media: The natural medium comprises entirely complex natural products of unknown composition. The raw material of a natural medium may be

of plant or animal origin, and some of the common ingredients employed for this purpose include extracts of plant and animal tissues, e.g., fruits, vegetables, egg, milk, blood, body fluids, yeast, malt and manure extracts etc. Obviously, the chemical composition and concentration of a natural medium is not well defined. On account of their complex nature, these media are able to support a variety of organisms, and hence are quite useful for routine laboratory cultures of fungi.

2- Semisynthetic media: These media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources with unknown composition. The chemical composition of a semisynthetic medium is partly known. The medium is a best serve as a routine medium and sometimes for physiological studies. Potato dextrose agar (PDA) is one of the popular media.

3- Synthetic media: These are chemically defined media of known composition and concentration. The media are exclusively composed of pure chemical substances. However, absolute purity of the ingredients is achieved, although substances of only analytical reagent quality are used for such purposes. One account of their known composition as well as being in solution, these media are quite useful for nutritional and metabolic studies of fungi. The composition of these media may be amended as per requirement and as such they may be simple or complex in composition. A simple synthetic medium contains a single carbon and energy source, a nitrogen source, generally as ammonium salt, some Sulphur and phosphorus sources and various minerals. All these ingredients are dissolved in a buffered aqueous base. However, for more fastidious organisms, a complex synthetic medium is designed by incorporating some additional factors such as certain vitamins, amino-acids, purines, pyrimidines etc., or by employing a multitude of carbon and nitrogen sources together.

C- According to their physical states: Media are classified into the following types:

1- Solid media: Media in solid state are in use since the beginning of laboratory studies of fungi. The first laboratory culture of fungi was obtained on a solid media such as fruit slices. Some common examples of such media are nutrient impregnated slices of potato, carrot, sugar-beet etc. and coagulated egg or serum. However, with the advent of agar as a solidifying agent, such media have largely been replaced by agar media. Use of fruits and vegetable slices in the cultivation of fungi is now more or less restricted to the baiting technique employed for isolation of some specific organisms.

2- Solid-reversible to liquid media: Such reversible media were first introduced by Koch (1881) who observed that addition of 2 to 5 percent of gelatin to the commonly employed media rendered them a semi-solid consistency. However, gelatin could not find a wide application on account of its low melting point (37˚C), and also because it is hydrolyzed by many proteolytic bacteria at ordinary temperature. The use of agar for solidifying culture media was also initiated the same year and in the same laboratory.

3- Semi-solid media: These are media with gelatinous consistency and are employed for specific purpose. They contain a small amount of agar or some other solidifying agent like corn meal. These media are sometimes used for the study of motile reproductive structures of fungi.

4- Liquid media: These are media without any solidifying agent, and are indispensable for most of the quantitative studies of fungi. Nutritional and metabolic studies of fungi, as well as microbiological assays are invariably carried on liquid media. Some of the advantage of liquid media is that they permit the cultures to be aerated, the mycelium to be weighed and the metabolic products to be analyzed easily. However, with respect to routine studies, liquid media have some distinct disadvantages. Growth in liquid media does not manifest the morphological characteristics of microorganisms. They are also difficult to handle

without disturbing the culture. Moreover, liquid media are least helpful in the purification of microorganisms from a mixed culture. For an even distribution of nutrients and for providing uniform aeration to growing fungus, the liquid cultures are sometimes put to constant mechanical shaking.

Sterilization

Sterilization refers to the process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and spore forms etc.) from a surface, equipment, foods, medications, or biological culture media.

Sterilization can be achieved through application of heat, chemicals, irradiation and filtration.

There are three main methods for sterilization:

1- Physical methods

2- Chemical methods

3- Mechanical methods

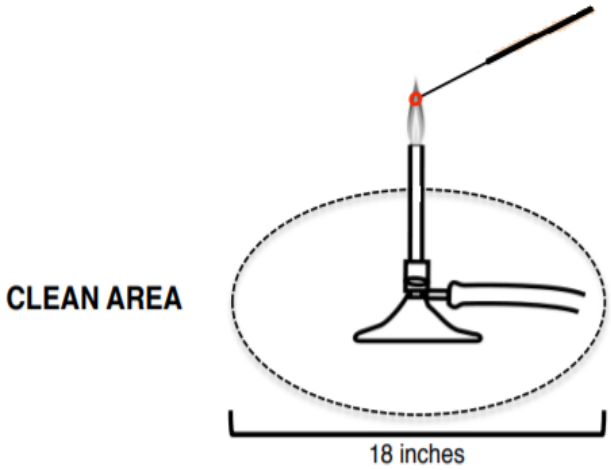
1- Physical methods

Sterilization by heat

Heat may be utilized for sterilization either in dry or moist form. However, moist heat is much more effective and requires both shorter duration and lower temperature. Sterilization by moist heat generally is complete at 121°C for 15-30 minutes of exposure. On contrast, sterilization by dry heat requires a temperature of 160°C for 60 minutes. The two kinds of heat treatments kill the microorganisms by coagulating and denaturing their enzymes and other proteins.

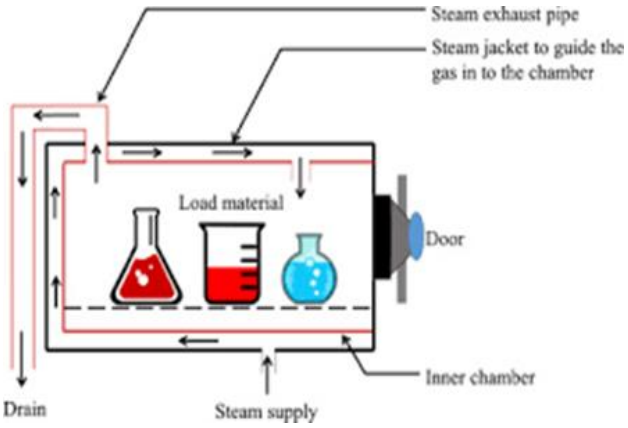
Application of dry heat

a- Flaming



b- Hot-air oven

Oven



radiation

c- Radiation (Infra-red or Ultra violet)

Application of moist heat

The use of the Autoclave for sterilization

2- Chemical methods

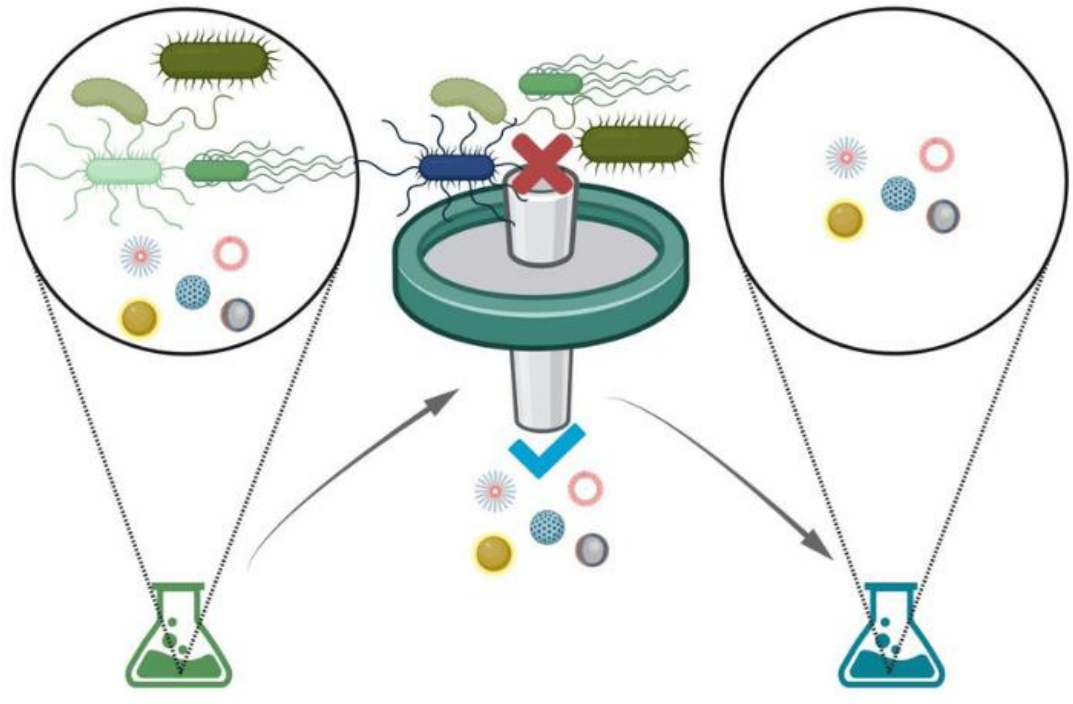
Using of chemical substances as agents, like chloroform, mercuric chloride, formaldehyde and ethyl alcohol.

3- Mechanical methods

Sterilization by filtration

This technique employs special type of filters having pores so small that ordinary bacteria are arrested. This method is particularly useful for sterilizing heat sensitive materials, such as culture media containing serum, antibiotic solutions, culture filtrates etc. The most common filters are Seitz filters and Cellulose membrane filters.


Flask of sample
Cap
Membrane filter
Cotton plug in vacuum line ensures sterility
Sterile filtrate
Vacuum line



Methods for measuring fungal growth

Growth may be defined as the orderly increase in cell components leading to an increase in biomass (Prosser, 1995). The growth form of filamentous fungi is complex; extension of individual hyphae is localized at the tip, whereas biomass synthesis supporting that growth may take place throughout the mycelium. The growth of a fungus can be measured in various ways such as increase in colony diameter, increase in dry weight, rate of production of different type of metabolites, etc. The mechanisms involved in the control and regulation of mycelial growth are better studied on solid medium than in submerged cultures, as fungi are adapted to growth on solid substrates.

Direct methods

Some of the direct methods to determine fungal growth are stated afterwards:

Growth of hyphal extension

Measure of hyphal extension rate is measured microscopically on solid medium as an increase in length, e.g. measure of the increase in radii of circular colonies, daily or every other fixed period of time, after inoculating the mold in an agar plate and incubate it at the appropriate conditions. This is probably the most common technique for estimation of growth of filamentous fungi on solid media. A growth rate function can be derived by plotting colony diameter against time and measuring the slope of the straight part of the line. Analysis is now greatly facilitated by the increased availability of image analysis systems which enable automated measurement of hyphal lengths and subsequent kinetic analysis of data (Wiebe and Trinci, 1990; Gray and Morris, 1992).

Measure of fungal biomass

Molds are usually grown on the surface of a cellophane membrane, overlaying the agar, from which the biomass can be washer or otherwise removed for the determination of the dry weight. This measure is also possible in liquid cultures. In both solid and liquid media, separation of biomass from the growth medium is slow, tedious and requires relatively large amounts of biomass for accuracy.

Turbidimetric techniques are less reliable due to the heterogeneous nature of liquid cultures of filamentous fungi.

Indirect methods

Ergosterol

Fungal plasma membranes are similar to mammalian plasma membranes, differing in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. The plasma membrane regulates the passage of materials into and out of the cell by being selectively permeable. Membrane sterols provide structure, modulation of membrane fluidity, and possibly control of some physiologic events. Fungal growth and biomass could therefore be estimated by measuring this specific component of fungi. Quantifying ergosterol production in foods has proved more difficult. Since now, ergosterol content has been mainly assayed in cereal samples. The determination of ergosterol is also valuable in correlating metabolites such as aflatoxins and OTA (Gourama and Bullerman, 1995; Saxena et al. 2001).

Impedimetry and conductimetry

Metabolites produced by growth of microorganisms in liquid media alter the medium's impedance and conductance. The use of changes in these properties has been used to estimate fungal growth. A major problem of these techniques involves the selection of suitable media, but when the method is set up, this method results rapid and effective.

Adenosine triphosphate (ATP)

Another measure of microbial biomass is the measure of the bioluminescence emitted by the molecules of fungal ATP. However, living plant cells contain also high levels of ATP and fungi are often very difficult to separate from food materials.

Pectinesterase

The fundament of this technique is that gas liquid chromatography is used to determine the amount of methanol released from pectin by the fungal enzyme

pectinesterase. This is considered a rapid method for detecting viable spores of spoilage fungi, but it needs some improvements before practical application.

Fungal volatiles

It consists in measuring the effects of fungi on foods. Fungi produce chemical volatiles during growth and particular chemicals can be detected and therefore measure fungal growth in an indirect way. Several commercial gas sensor array instruments are now available on the market covering a variety of chemical sensor principles, system design and data analysis techniques. A series of different detection principles can be used in chemical gas sensors: heat generation, conductivity, electrical polarization, electrochemical activity, optical properties, dielectric properties and magnetic properties. In principle, the results obtained from a gas-sensor array represent qualitative and quantitative information of the composition of the headspace gas mixture of a sample. The technique should therefore have a great potential in a number of applications related to food. Numerous **electronic nose** studies related to food already have been published, but the electronic nose technology applied on food must be regarded as being in its early stage. A goal of this technology is to explore the use of an electronic nose for rapid detection of food spoilers and pathogens via development of a standard curve of some potential volatile compounds that can be used to develop some specific aroma-labeled substrates.

Immunological techniques

Fungal cell wall proteins produce antigens, which can be detected by immunological methods. Some antigens are derived from components common to a wide range of fungi, and hence are indicative of general fungal growth, while others are genus or even species specific.

Molecular methods

They are based on nucleic acid sequences that are specific to the target fungi. The most known method is called **nucleic acid hybridization** and it involves the selection, cloning and chemical labelling of sequences specific to the target

organism. These are then used as probes to detect RNA or DNA of the pathogen in extracts of the substrate. DNA may be specific at almost any taxonomic level. In some instances the detection and identification of the causal agent(s) may be secondary to other consideration. For example, it may be more important to quantify the amount of pathogen present rather than just determine its identity. Several approaches have been taken to develop diagnostic assays, and are divided into immunological and DNA-based systems, this last generally being **polymerase chain reaction** (PCR). In contrast to hybridization, PCR-based assays for detecting mycotoxins in fungi have been widespread in the last years. PCR is an extremely sensitive technique and involves the enzymatic amplification of a target DNA sequence by a thermostable DNA polymerase.

Experiment 1

1- Isolation of fungi from natural sources

- Preparation of culture media and sterilization.
- Isolation of fungi on the suitable media for fungal growth.
- Identification and preservation of fungi

Table 1: …….

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Fungi	Counts	%	OR

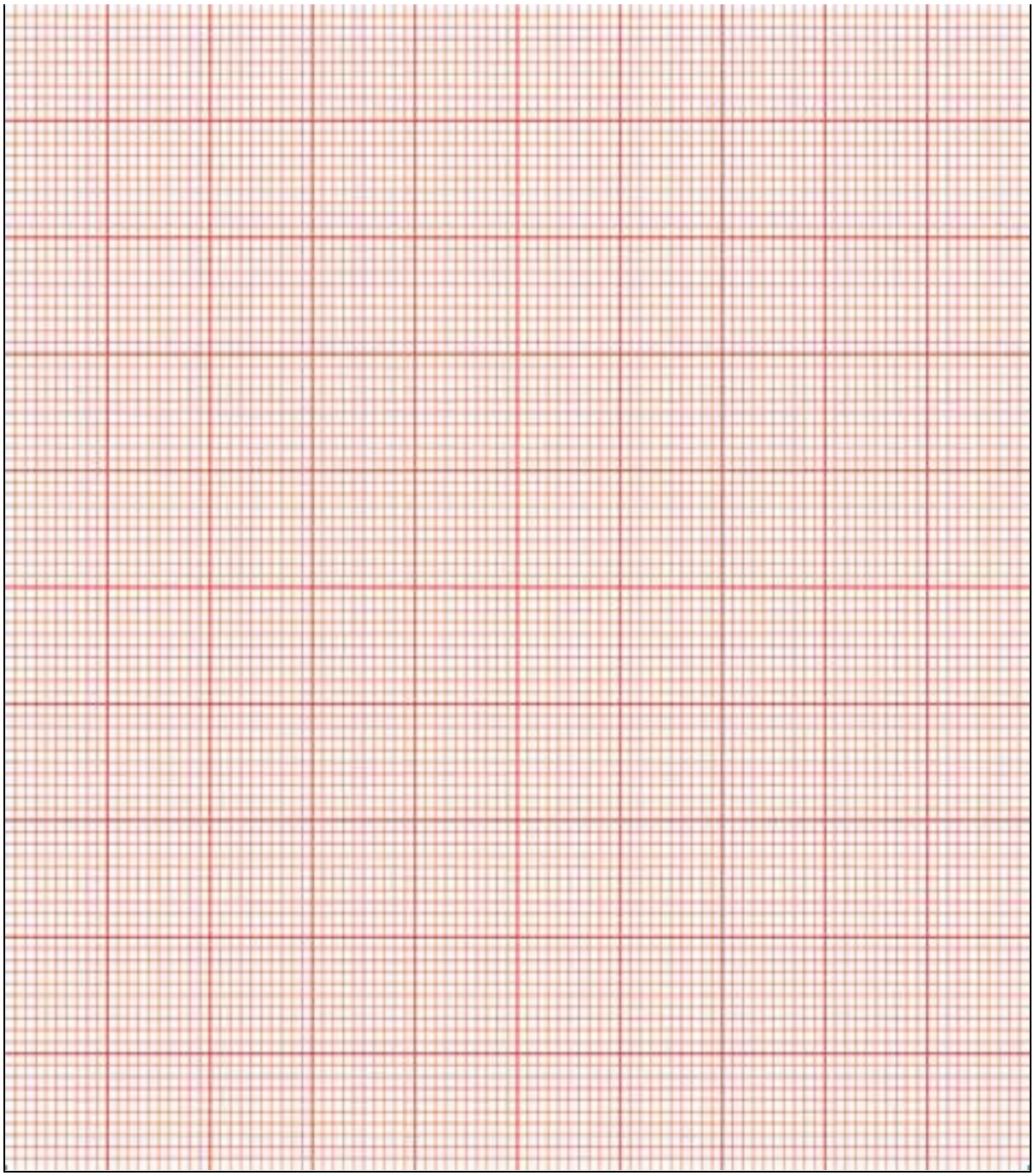

Figure 1: ……..

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2- Factors affecting fungal growth and metabolism

Fungi have in the course of evolution diversified to exploit a wide variety of habitats. Different species hence require different conditions for optimal growth. Microbial metabolism is significantly influenced by the physical and chemical environment. Thus, toxin-producing fungi may invade food at pre-harvesting period, harvest-time, during post-harvest handling and in storage. According to the site where fungi infest food, toxinogenic fungi can be divided into three groups (Suttajit, 1989).

- Field fungi, includes species of plant pathogenic fungi, usually with high requirements of water, such as *Alternaria*, *Cladosporium* and *Fusarium*.
- Storage fungi, with lower requirements of humidity, are principally the genus *Aspergillus* and *Penicillium*.
- Advanced deterioration fungi, normally do not infest intact food, but easily attack damaged one and require high moisture content. Some examples are some other aspergilli species, *Chaetomium*, *Scopulariopsis*, *Rhizopus*, *Mucor* and *Absidia*.

The main factors that influence growth of fungi include temperature, pH and moisture. But apart from environmental factors, chemical and biological factors clearly play a role (Figure 49). Under some circumstances these effects are additive. Under others, the implication is that synergistic interactions lead to a combined effect of greater magnitude than the sum of constraints applied individually. This has been described by Leistner and Rödel (1976) as the 'hurdle concept.' Moreover, hurdles are frequently combined to minimize the impact of processing on the quality and to improve the safety of ready-to-eat foods.

Environmental factors

The large and diverse group of microscopic foodborne yeasts and molds includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile, environmental requirements. There

are several major parameters governing fungal invasion, growth and production of mycotoxins:

Experiment 2

Temperature

Temperature is an important environmental factor affecting growth and mycotoxin production by molds. Fungi are capable of surviving under the full range of temperatures normally experienced in environments in which they live. The temperature range usually reported for fungal growth is broad (10-35°C), with a few species capable of growth below or above this range. Fungi can be divided according to their tolerance to temperature in psychrophilic, mesophilic, and thermophilic fungi.

Procedures

1- Preparation of culture media and sterilization

2- Cultivation of fungi

3- Incubation of cultures under various temperatures

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under various temperatures.

4- Illustrate the data and write a comment.

Table 2:……..……..…

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Temperature	*Aspergillus*	*Fusarium*	*Rhizopus*
15			
20			
25			
30			
35			
40			

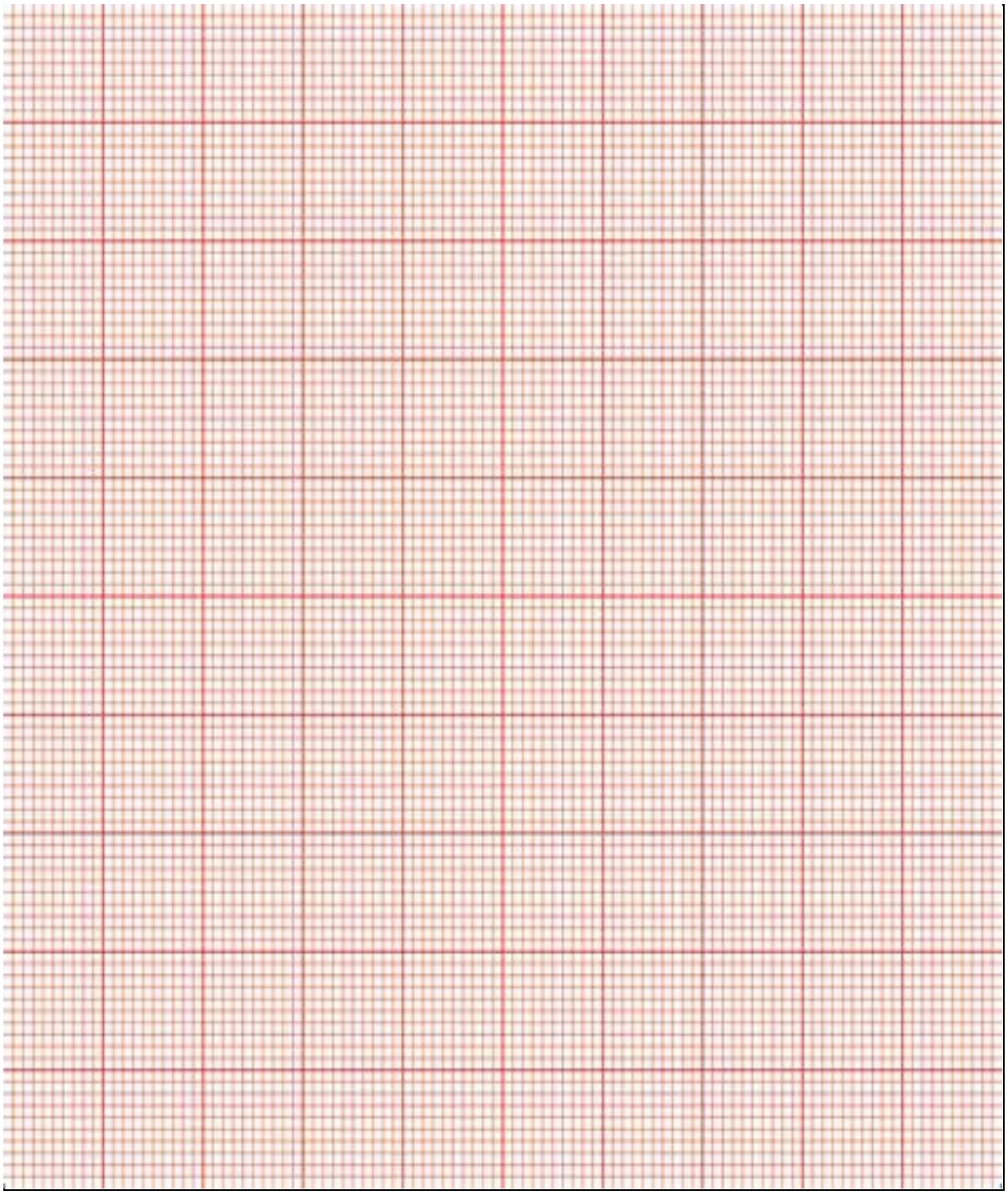

Figure 2: ……..

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Experiment 3

Hydrogen ion concentration (pH)

In general, there is a lack of information on the effect of pH on fungal growth parameters, in spite of a considerable literature on growth in relation to the initial pH of media. This data is of limited value, since fungal metabolism alters pH during their evolution. Hydrogen ion concentration in a medium could affect growth either indirectly by its effect on the availability of nutrients or directly by action on the cell surfaces. The acid/alkaline requirement for growth of all yeasts and molds is quite broad, ranging from pH 3 to above pH 8, with optimum around pH 5, if nutrient requirements are satisfied. In general, Aspergillus species are more tolerant to alkaline pH while *Penicillium* species appear to be more tolerant to acidic pH (Wheeler et al., 1991). It is seen that in situations near neutral pH, fungi must compete with bacteria for niches, and at higher aw values most fungi are not competitive in mixed culture. However, where a w is below 0.90, fungi become dominant irrespective of pH. In specialized niches where bacteria do not appear to have a role as pathogens, specific Fusarium and *Penicillium* species are dominant even at neutral pH and high a_w (Wheeler et al., 1991).

Procedures

1- Preparation of culture media with variable pH values and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under various pH values.

4- Illustrate the data and write a comment.

Table 3:……..……..…

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pH values	*Aspergillus*	*Fusarium*	*Rhizopus*
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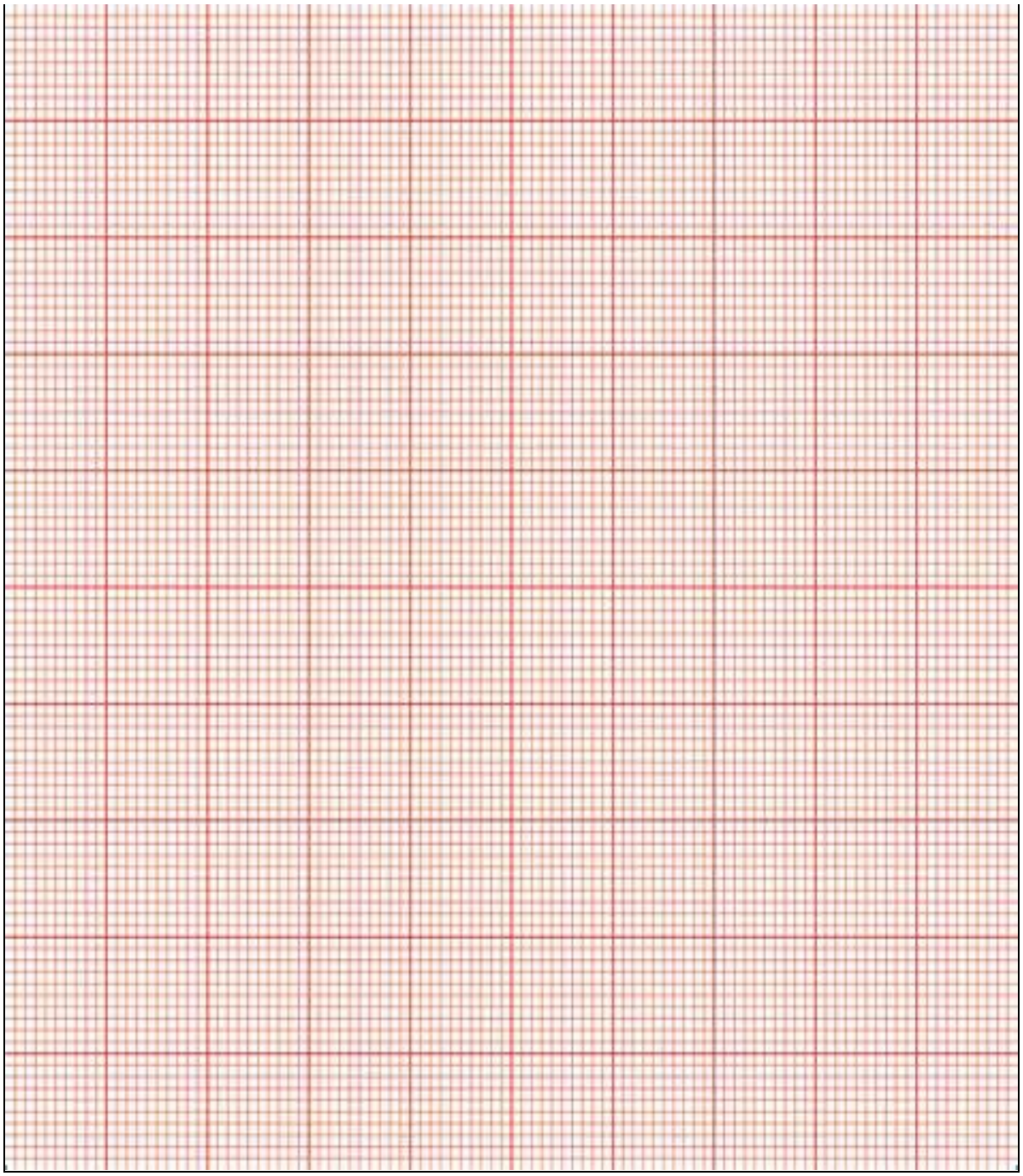

Figure 3: ……..

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Availability of water

Living organisms consist largely of water. Hence if an organism is to grow has to take up water from the environment. Whether water enters or leaves a cell depends on the difference between the water potential of the cell and that of the surrounding medium, water moving from a region of high to one of lower water potential (Carlile and Watkinson, 1996). In microbiology, three measures of the water availability have been used: water potential (ψ), water activity (a_w), and relative humidity (R.H.).

Water potential is the sum of numerous components, of which the most important are osmotic, matric and turgor potential, and is measured in units of pressure.

Water activity is a fundamental property of aqueous solutions, and by definition is the ratio of the vapour pressure of the water in the substrate (P) to that of pure water at the same temperature (P0):

$$a_w = P/P0$$

p0 p aw = Water activity ranges from zero (water absent) to 1.0 (pure water). For an ideal solution aw is independent of temperature, and in actual practice, the aw of a given solution varies only slightly with temperature within the range of temperature permitting microbial growth. The relationship between water potential and water activity is given by the next equation, where the value of k depends on temperature and is, for example, 1.37 at 25ºC and 1.35 at 20ºC.

$$\psi \text{ (Mpa)} = k \ln a_w$$

Not only is the availability of water in the surrounding liquid phase of importance to fungi, but the water content of the adjacent gas phase. The water content of the atmosphere is expressed in terms of **relative humidity**, the ratio of the water vapour pressure of the gas phase being considered, to that of a saturated atmosphere at the same temperature. It is hence the same ratio as water activity but expressed as a percentage. In most of the studies presented in this thesis, aw was used to describe the status of the water in solution or substrate in preference

to R.H., which applies more strictly to the surrounding atmosphere. Under equilibrium conditions the two terms are interchangeable. A required aw in the environment of a fungus may be obtained either by fixing the water content or the solute concentration in the culture substrate or by keeping the substrate in equilibrium with an atmosphere of controlled R.H. Moisture requirements of foodborne molds are relatively low; most species grow at a 0.85 a w or less, although yeasts generally require a higher water activity. 0.60 aw is considered the limit for cell growth, but spores of Aspergillus and *Penicillium* for example, are able to survive at lower aw for several years (Carlile and Watkinson, 1996). Moisture control is the best and most economical means to control the environment to prevent mold growth and mycotoxin production.

Light

There are some reports that illumination will increase or more commonly reduce the rate at which fungi spread across an agar surface. Such effects are sometimes due to the photochemical destruction of components of the medium but in other instances a direct effect on metabolism seems likely. The biosynthesis of pigments, mainly carotenoids, as consequence of light action has been demonstrated.

Availability of oxygen

Organisms can obtain energy by oxidative (respiratory) metabolism or by fermentation. The implications for oxygen requirements of the occurrence of respiration, fermentation or both in a fungus divided them in obligate aerobes, facultative anaerobes and obligate anaerobes (Carlile and Watkinson, 1996): Food spoilage molds, like almost all other filamentous fungi and yeasts, have an absolute requirement for oxygen. However, many species appear to be efficient oxygen scavengers, so that the total amount of oxygen available, rather than the oxygen tension, determines growth. The concentration of oxygen dissolved in the

substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Pitt and Hockings, 1997). The most oxygen demanding molds will colonize the surface of the food, while the less exigent could be found inside the food. Although probably not economically feasible, one sure way to prevent mycotoxin contamination of cereals and other food, is to store them under anaerobic conditions, e.g. CO_2 or nitrogen. For instance, this could be done in large airtight silos. The molds would not grow, but this type of environment control is sometimes understandably unrealistic.

Chemical factors (**Nutritional factors**)

Experiment 4

Effect of different Carbon sources on fungal growth

All forms of life, including molds, require exogenous materials to build into biomass. As heterotrophs, the molds require organic compounds for both the synthesis of biomass (anabolic metabolism) and to produce the energy to drive these reactions (catabolic metabolism). These aspects of metabolism are frequently referred to as primary metabolism (Smith and Moss, 1985). Fungi can use a number of different **carbon sources** to fill their carbon needs for the synthesis of carbohydrates, lipids, nucleic acids and proteins. Oxidation of sugars, alcohols, proteins, lipids, and polysaccharides provides them with a source of energy. Differences in their ability to utilize different carbon sources, such as simple sugars, sugar acids, and sugar alcohols, are used, along with morphology, to differentiate the various yeasts.

Procedures

1- Preparation of culture media with different carbon sources 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 determination of mycelial growth that grown under different carbon sources.

4- Illustrate the data and write a comment.

Table 4:……..……..…

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Carbon sources	*Aspergillus*	*Fusarium*	*Rhizopus*

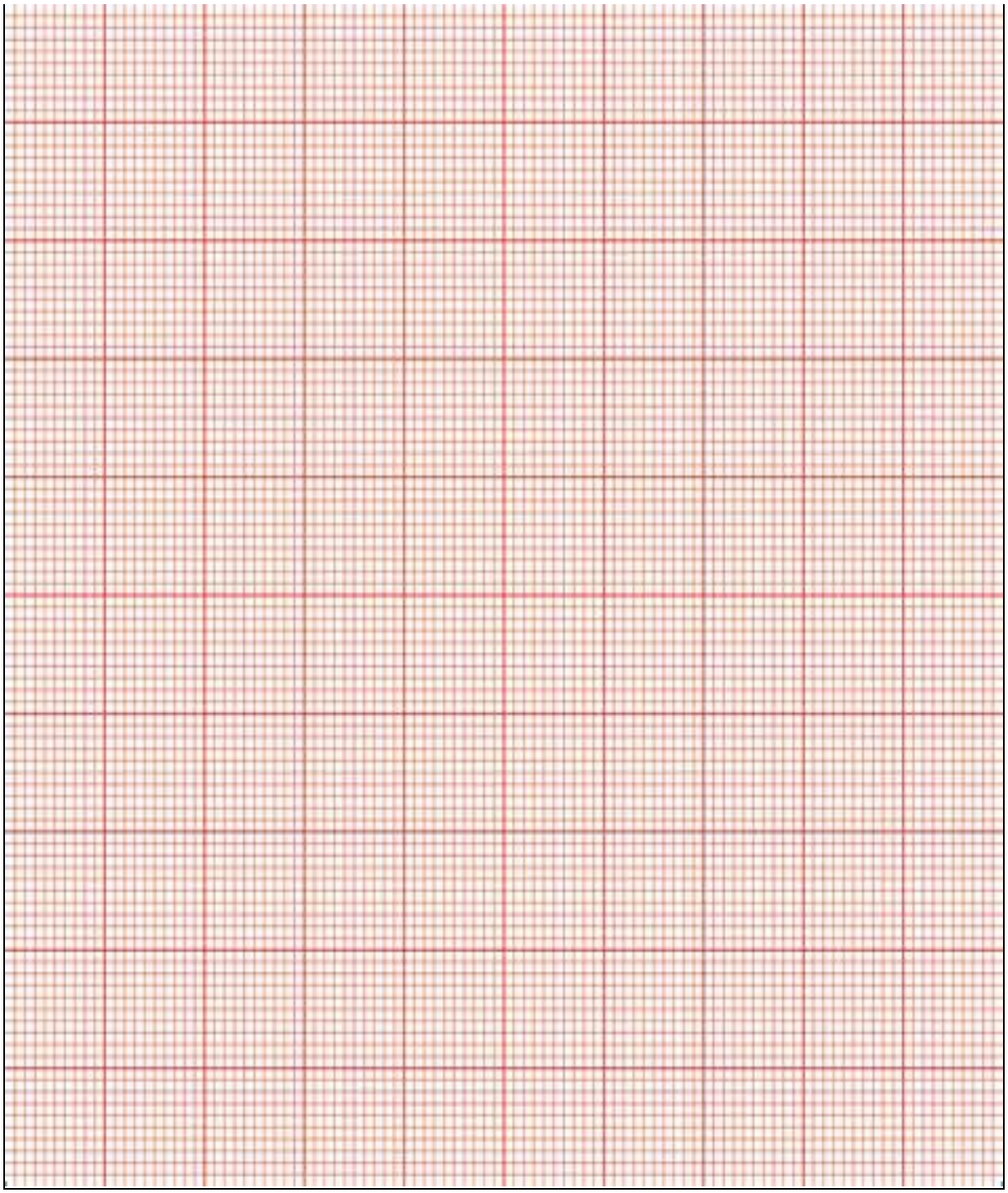

Figure 4: ……..

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Experiment 5

Effect of different Nitrogen sources on fungal growth

Fungi require a source of **nitrogen** for synthesis of amino acids for proteins, purines and pyrimidines for nucleic acids, glucosamine for chitin, and various vitamins. Depending on the fungus, nitrogen may be obtained in the form of nitrate, nitrite, ammonium or organic nitrogen as no fungus can fix nitrogen. Most fungi use nitrate, which is reduced first to nitrite and then to ammonia. Therefore, availability and type of nutritional factors such as carbon source and nitrogen source can also affect both mycotoxin production and morphological differentiation. Other major nutrients for fungi are sulphur, phosphorus, magnesium and potassium, which can be supplied to most fungi as salts. Trace elements like iron, copper, manganese, zinc and molybdenum are required by nearly all fungi as cofactors for enzymes. But in high amounts, some trace elements can become toxic for some fungi. For example, OTA production by *A. ochraceus* strains varied with the different concentrations of yeast extract (0-4%) and sucrose (0-4%) in a laboratory medium (Atalla and El-Din, 1993).

Procedures

1- Preparation of culture media with different nitrogen sources 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 determination of mycelial growth that grown under different nitrogen sources.

4- Illustrate the data and write a comment.

Table 5:……..……..…

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Nitrogen sources	*Aspergillus*	*Fusarium*	*Rhizopus*

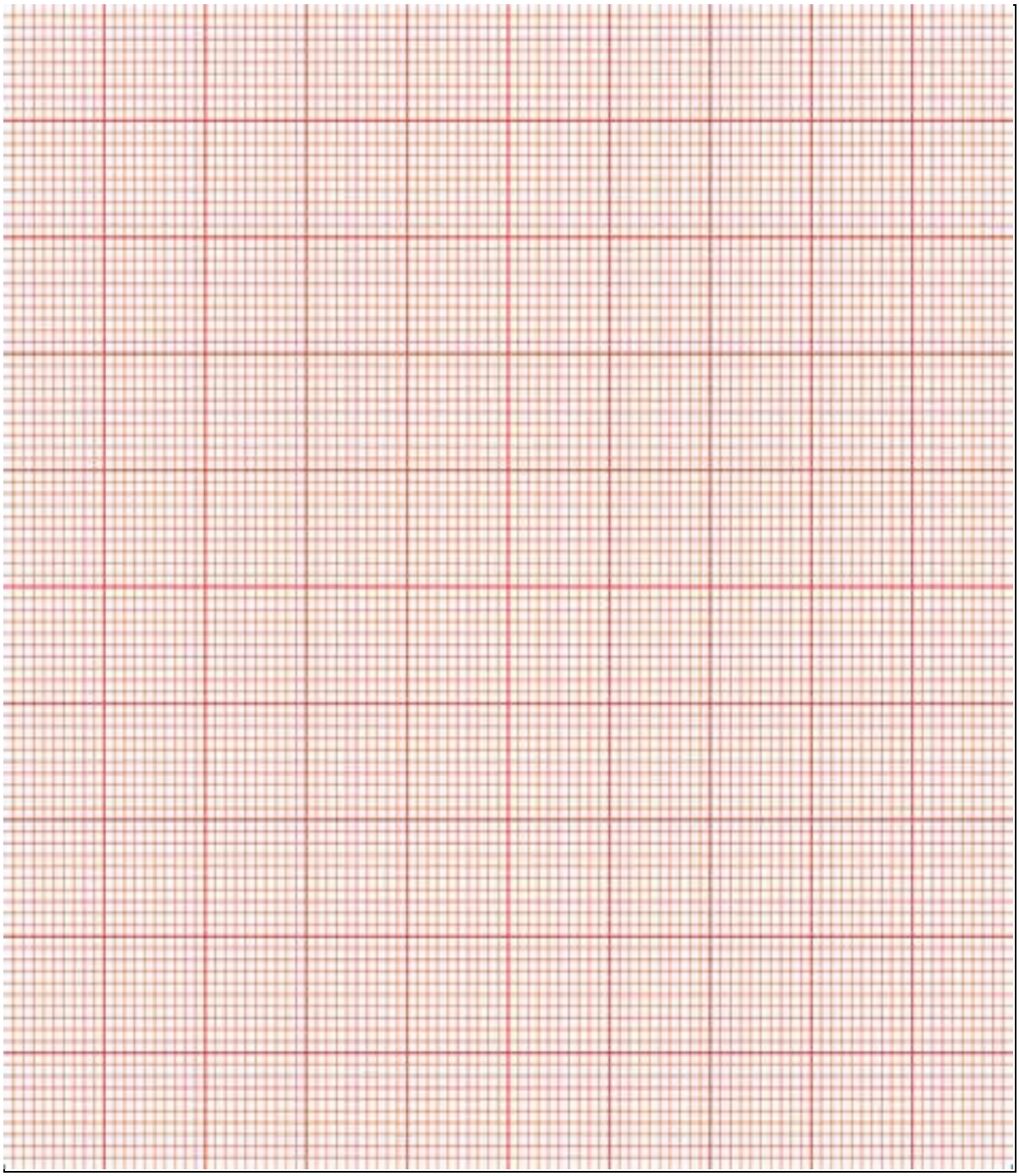

Figure 5: ……..

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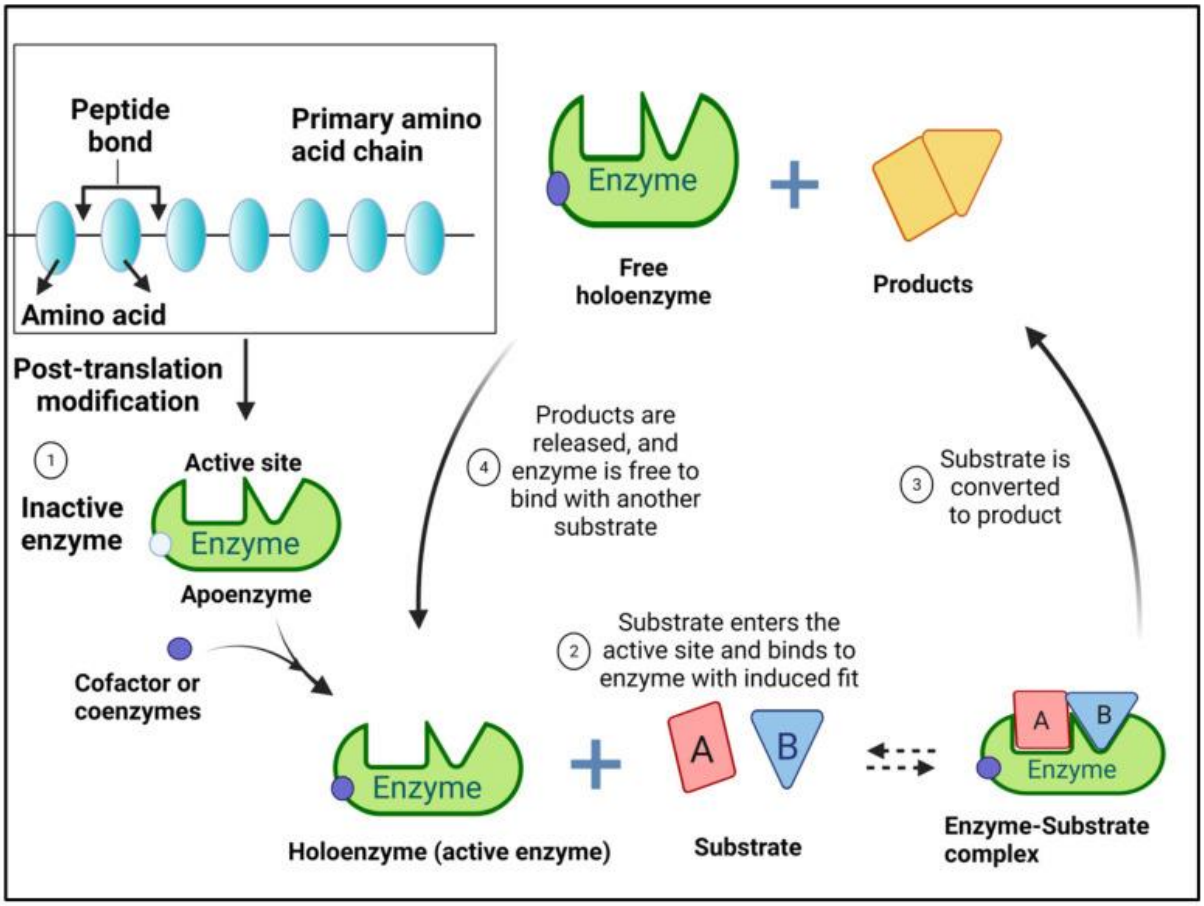
Experiment 6

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 6:……..……..…

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Fungi	Amylase activity

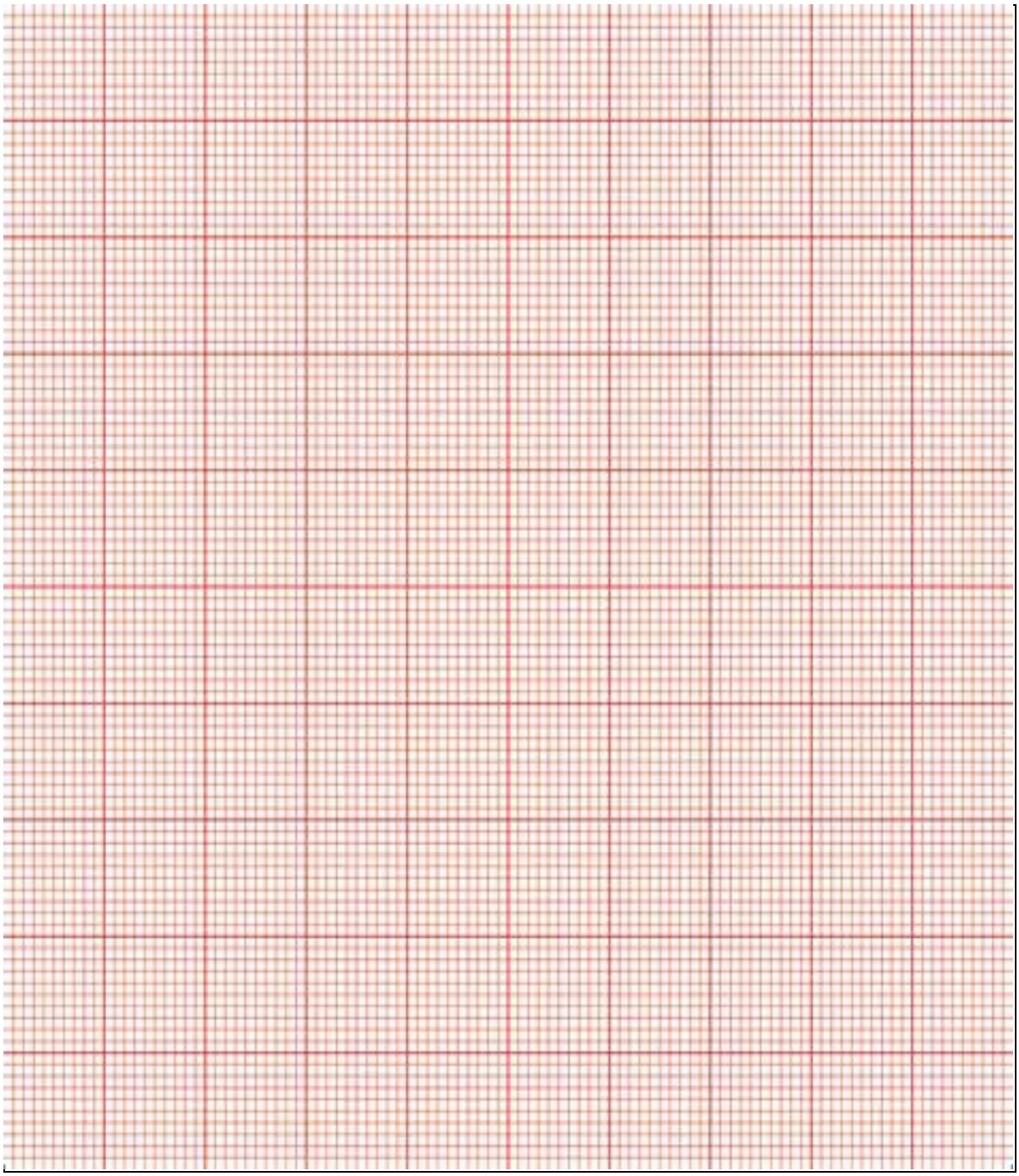

Figure 6: ……..

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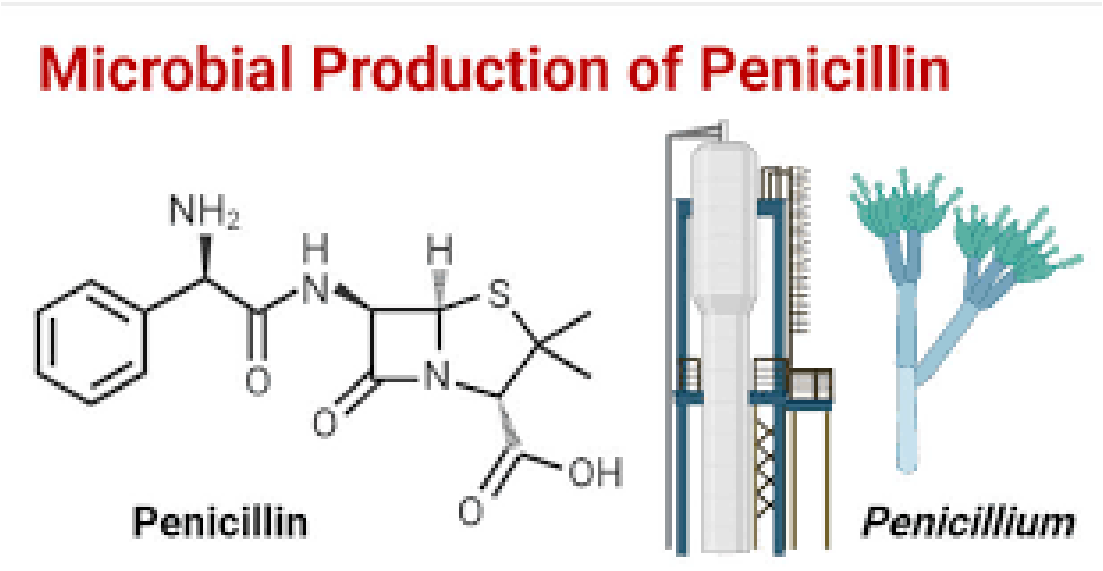
Experiment 7

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.

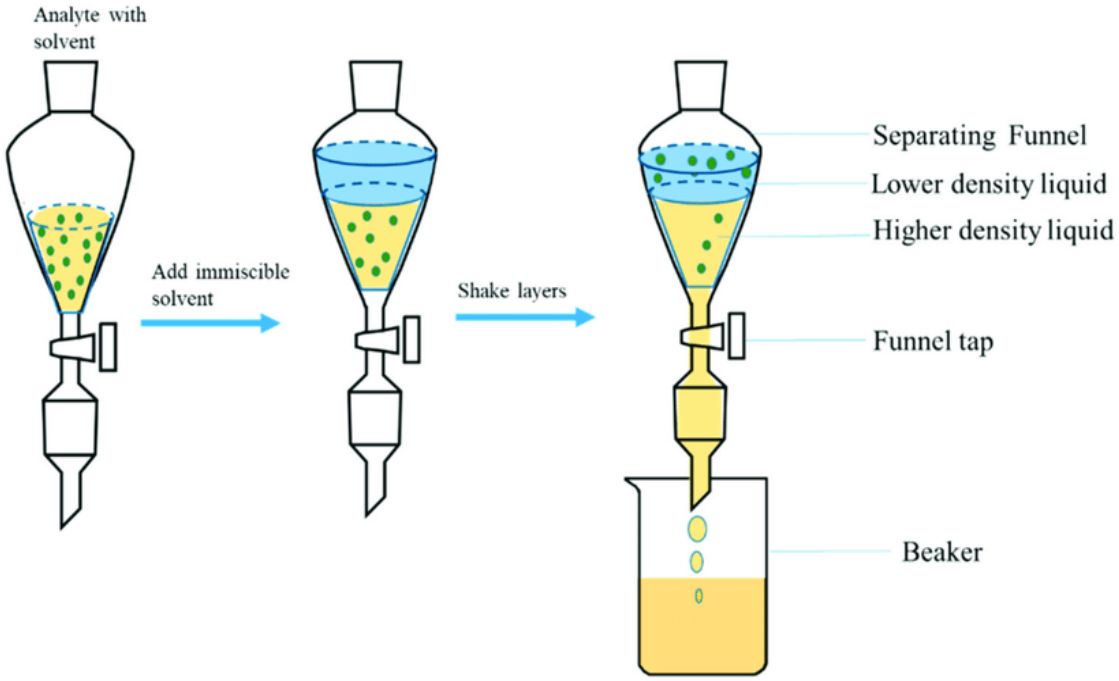


Table 7:……..……..…

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Penicillium strains	Penicillin activity
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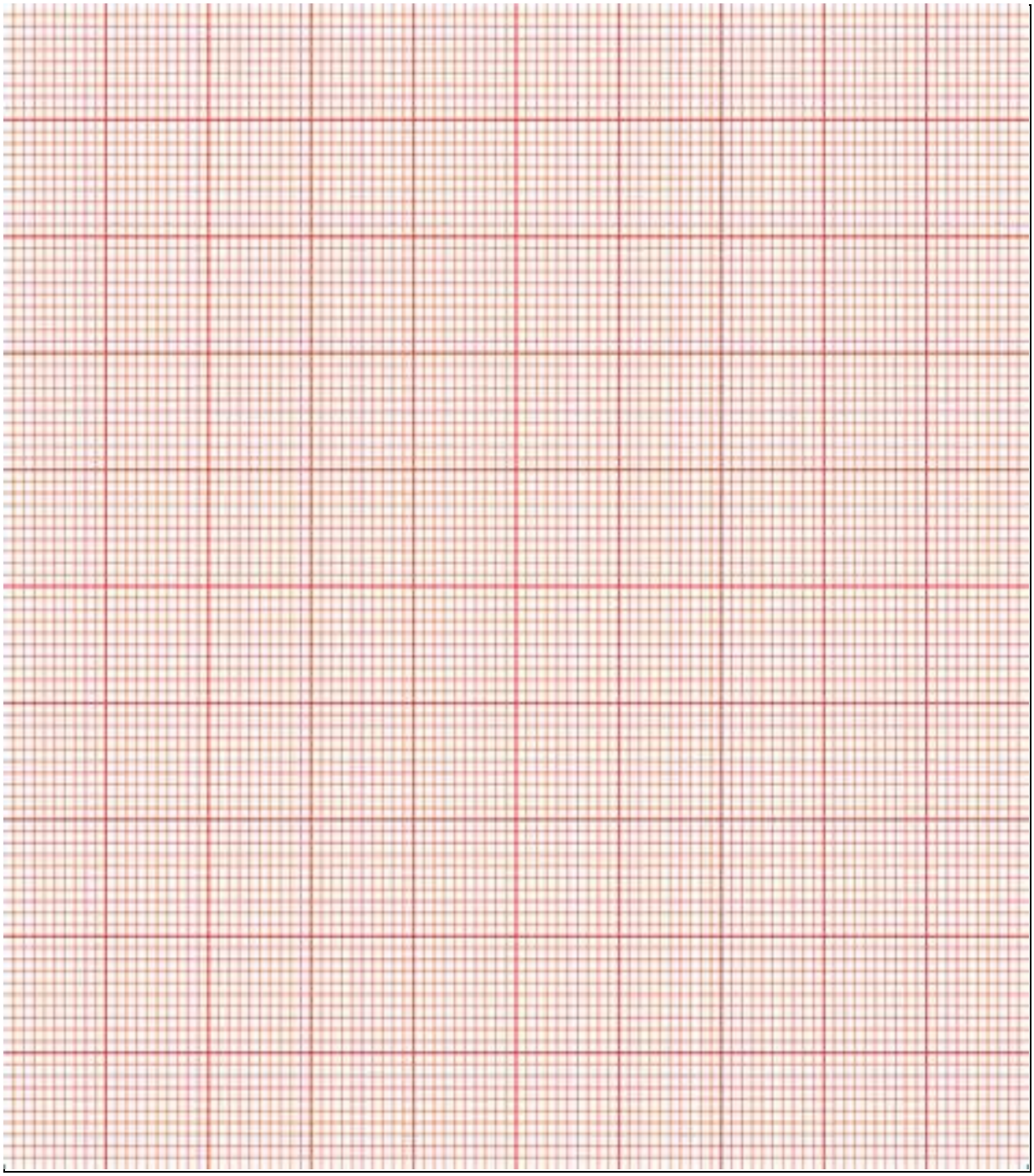

Figure 7: ……..

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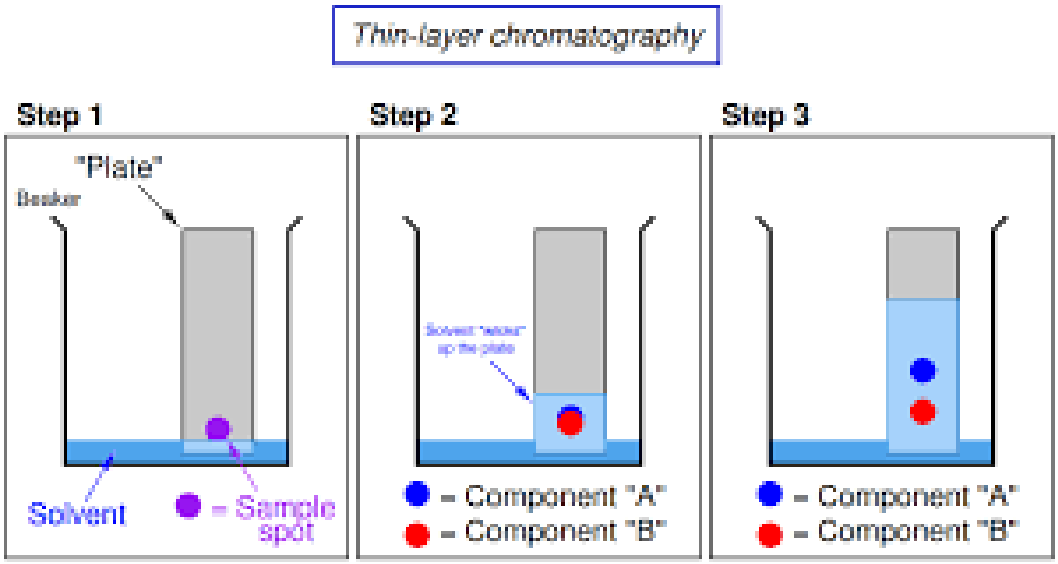
Methods for the analysis of antibiotics

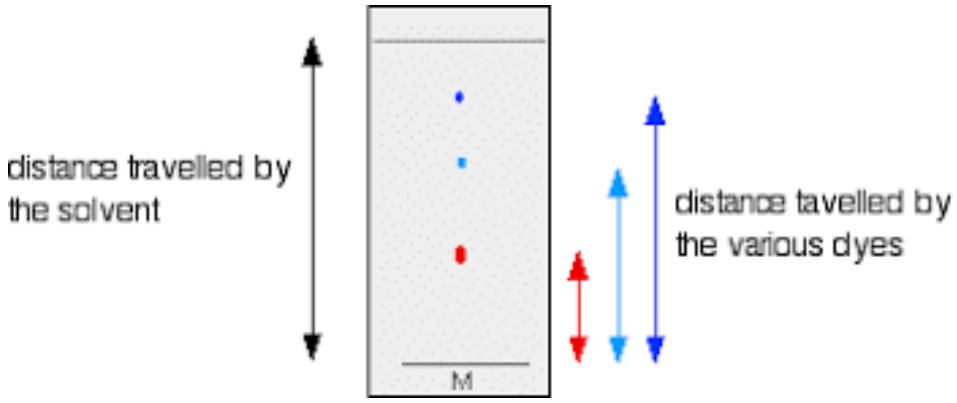
The different stages involved in the analytical process of antibiotics detection are sampling, extraction, clean-up, separation, detection and confirmation. Although many interfering compounds may be partially removed during the extraction sequence, further clean-up of the extract is normally necessary. 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.

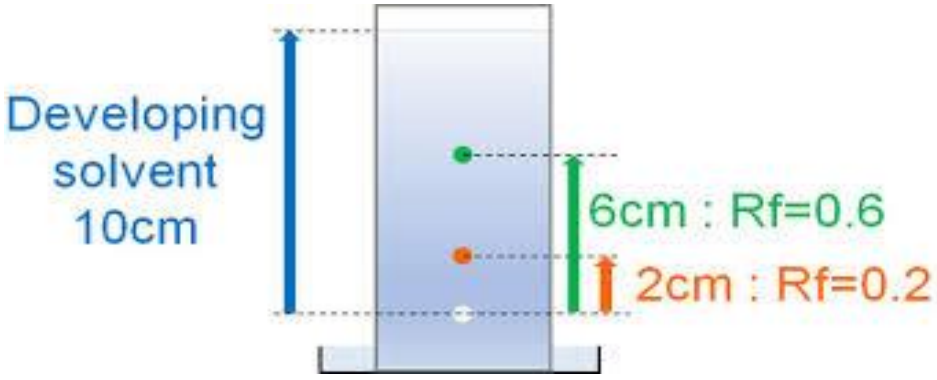
Thin Layer Chromatography (TLC) analysis

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

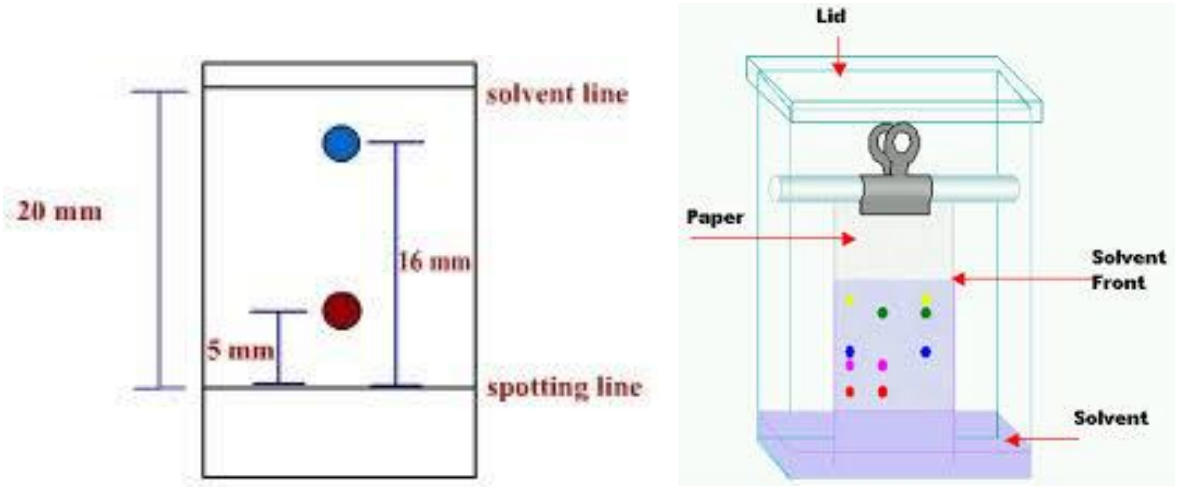
the material. Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the 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.







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

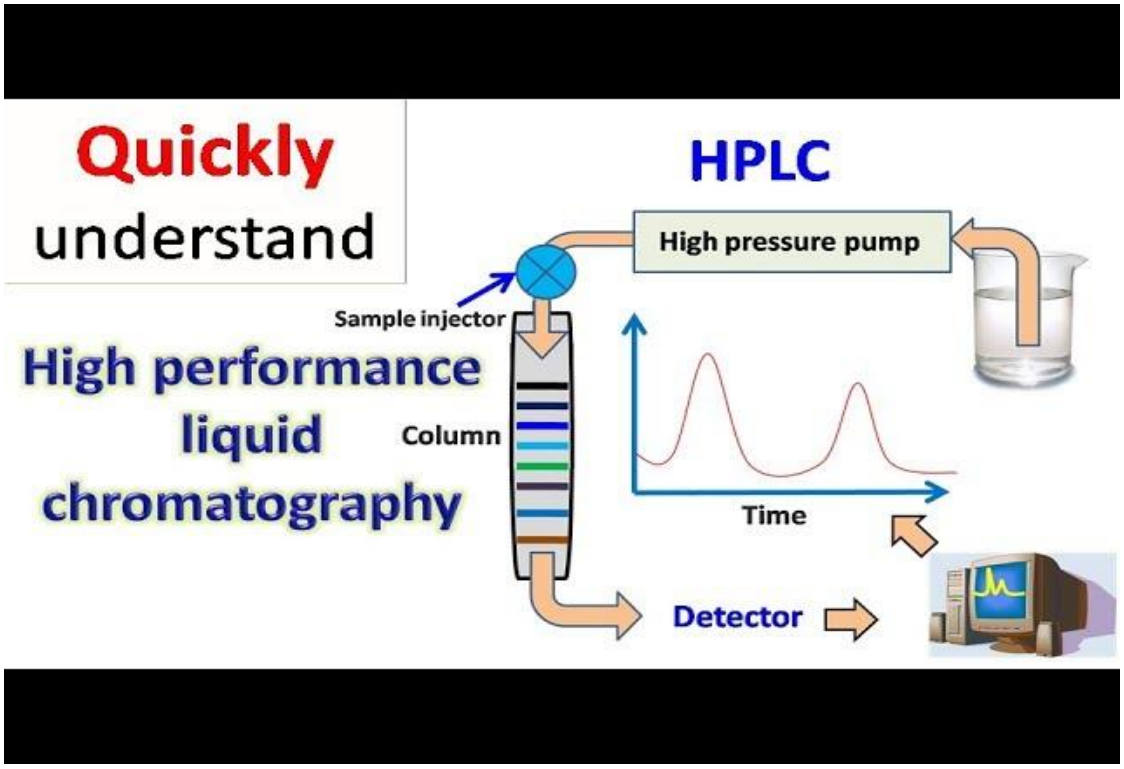


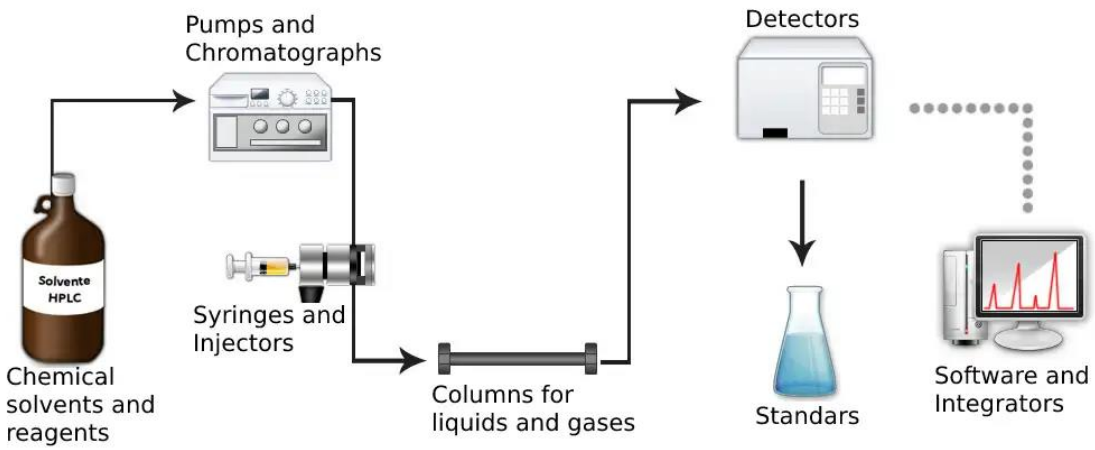
High-performance liquid chromatography (HPLC) analysis

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)

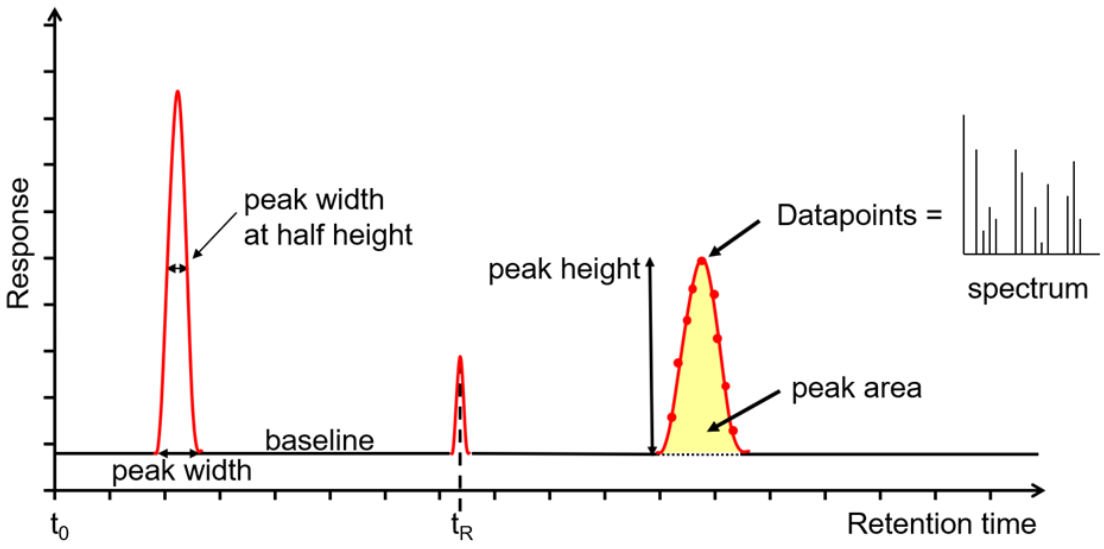
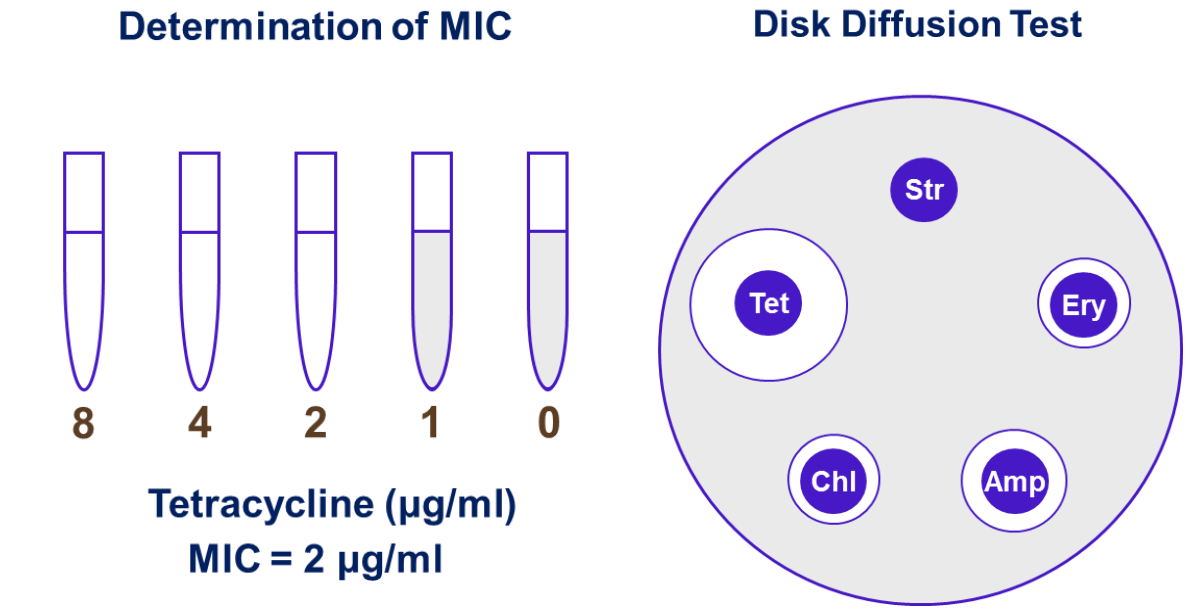
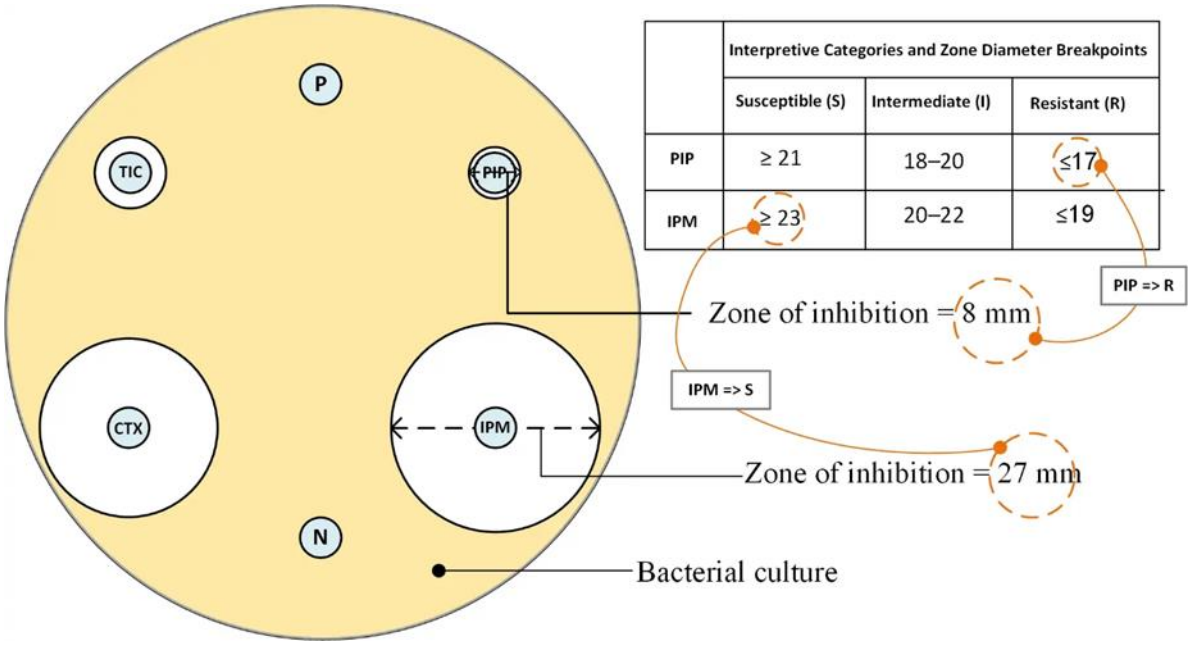


Chart of HPLC analysis

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

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 8:……..……..…

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Antibiotics	Inhibition zone (mm)	MIC

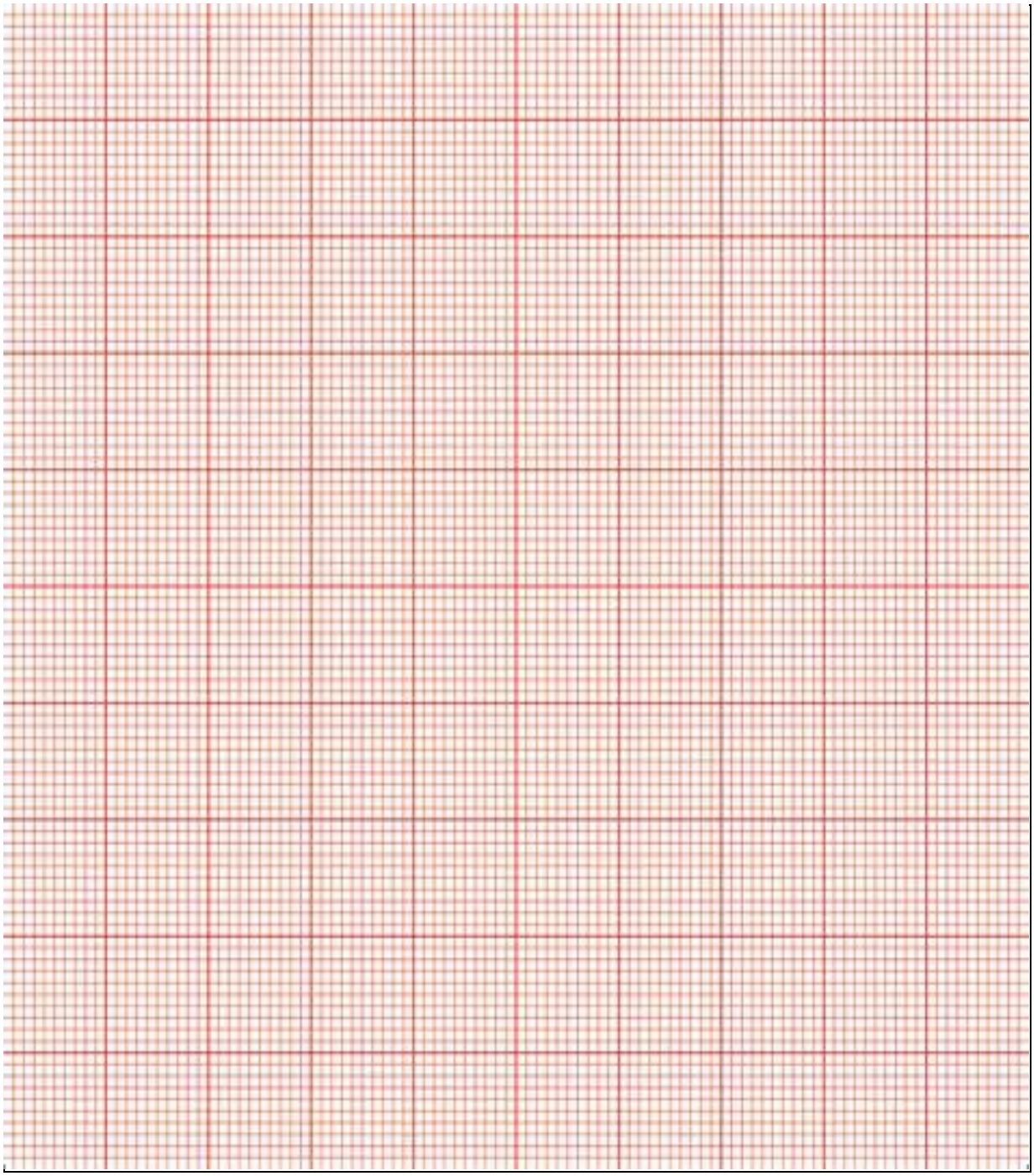

Figure 8: ……..

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Comment

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Practical part

Fungi

4th year Chemistry &Botany

Dr. Amany Atta El-Shahir

Safety Procedures for the Microbiology Laboratory

General Laboratory Safety Practices and Procedures

1. If you are **taking immune-suppressants, are pregnant,** or have a known medical condition that would prevent full participation in the laboratory, please contact the course instructor before the first day of lab.
2. Read and understand each laboratory exercise **before** you come to class.
3. Do not eat, drink, smoke, or chew pens in the laboratory.
4. You must wear close-toed shoes while in the laboratory and long pants.
5. No hats of any kind will be allowed in lab, unless allowed by University policy and cleared with the instructor.
6. Long hair should be pulled back to keep it away from bacterial cultures, bacticinerator or open flames.
7. Follow precautionary statements given in each exercise.
8. Personal electronic devices will be turned off and stored while in this laboratory. *The unauthorized use of any electronic device (phone, tablet, computer) in lab will result in a loss of course points.
9. Know where specific safety equipment is located in the laboratory, such as the fire extinguisher, safety shower, and the eyewash station.
10. Recognize the international symbol for biohazards, and know where and how to dispose of all waste materials, particularly biohazard waste. Note that all biohazard waste must be sterilized by autoclave before it can be included in the waste stream.

Figure 1: Biohazard Symbol

11. Keep everything other than the cultures and tools you need OFF the lab bench. Only necessary work material should be at or on the laboratory bench. Coats, backpacks, and other personal belongings will not be allowed on the laboratory bench top. Store them in a place designated by your instructor. This is to prevent cluttering of the workspace and to avoid exposing them to permanent stains, caustic chemicals, and microorganisms used in the exercises.
12. Leave all laboratory facilities and equipment in good order at the end of each class. Before leaving the laboratory, check to make sure the bacticinerator heat sterilizer is turned off.
13. Never, under any circumstances, remove equipment, media, or microbial cultures from the laboratory.
14. No pets are allowed in the laboratory.

Microbiology Specific Laboratory Safety Practices

During the course of the semester in the laboratory you will be taught the methods used in the proper handling of microorganisms. Although you will not be working with any that are human pathogens, exercise caution in handling all material coming in contact with live microbial cultures. All cultures should be handled with respect and proper aseptic technique *as if they were potential pathogens.* This is called **"universal precaution"**. Specific instructions that should be followed:

1. Remember that all bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. If you as a student have a compromised immune system or a recent extended illness, you should share those personal circumstances with your lab instructor.
2. Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage. Lab coats, safety glasses or goggles are also required. These will be stored in the laboratory each week in a ziplock bag.
3. Disinfect your work area both BEFORE and AFTER working with bacterial cultures.
4. Cultures of live microorganisms and any material coming in contact with live cultures must be properly sterilized after use in the laboratory. Your instructor will inform you of specific procedures. Follow the general rules outlined below.

   a. Glassware such as test tubes, bottles, and flasks may be reused and washed after sterilization. These are normally placed on a cart at the front of the laboratory after you have finished an experiment or exercise. BE SURE TO REMOVE LABELS before placing any glassware on the cart. Your instructor will sterilize and then wash these items.

   b. Some materials, such as plastic petri dishes, plastic pipettes, microscope slides, and swabs, are considered disposable. These are used once and if they become contaminated by contact with live microorganisms are sterilized and discarded. All of these disposable contaminated materials should be placed in the designated waste container containing a BIOHAZARD autoclave bag.

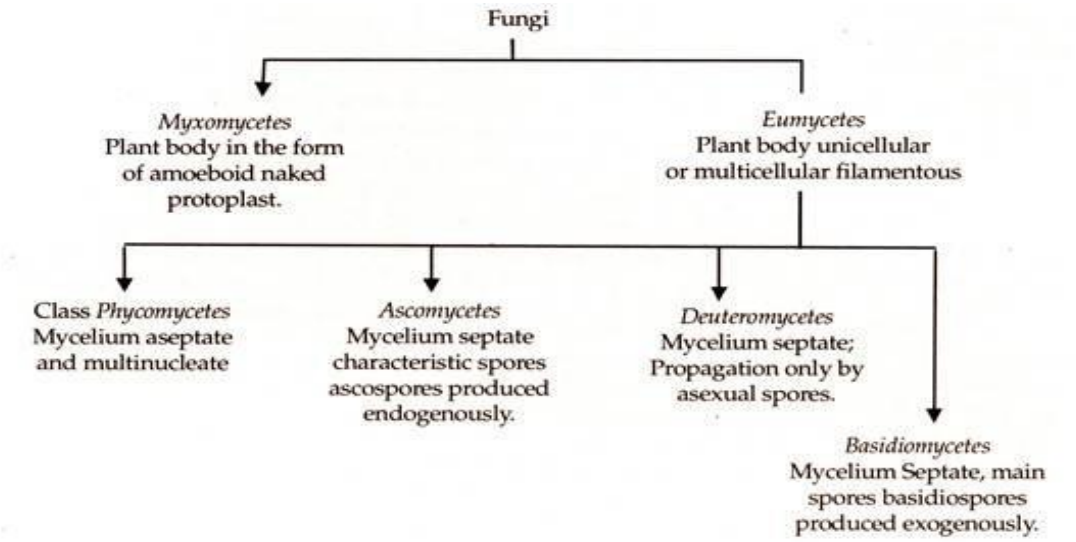
5. Never place contaminated pipette tips (or pipettes), inoculating loop, or any other contaminated material on the bench top. Sterilize loops before and after each use. Place contaminated pipette tips in the orange biohazard buckets on your bench. Place all other contaminated materials in their designated waste containers. Do not place or put anything containing live microorganisms in the sink.
6. Aerosols should be avoided by the use of proper technique for sterilizing the inoculating loops and by performing any mixing of cultures and reagents in such a way as to avoid splashing.
7. Cultures or reagents should always be transferred with an automatic pipettor that will be provided. In no case should one employ mouth pipetting.

8. Always keep cultures capped and in proper storage racks when not being used during an exercise.
9. In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant. Notify your instructor about the spill. If the chemical is marked "danger" or "caustic" you should notify the instructor who will handle this type of spill.
10. Immediately report all accidents such as spills, cuts, burns, or other injuries to the instructor
11. Make sure that lab benches are completely cleared (everything either thrown away or returned to storage area) before you leave the lab.
12. Clothing worn in the microbiology laboratory should be washed before being subsequently worn in a facility such as a hospital, clinic or nursing home, or in an area of public food preparation.
13. In the event of a fire alarm, follow the directions of your instructor, and meet at the place designated by your instructor.

Classifications

Alexopoulos (1956) places all fungi in the division Mycota. The division Mycota is divided into two subdivisions (1) Myxomycotina (2) Eumycotina (true fungi). Myxomycotina has only one class – Myxomycetes.

Eumycotina has the four classes as shown in the figure.



K. Mycophyta

D. Myxomycophyta

C. Myxomycetes

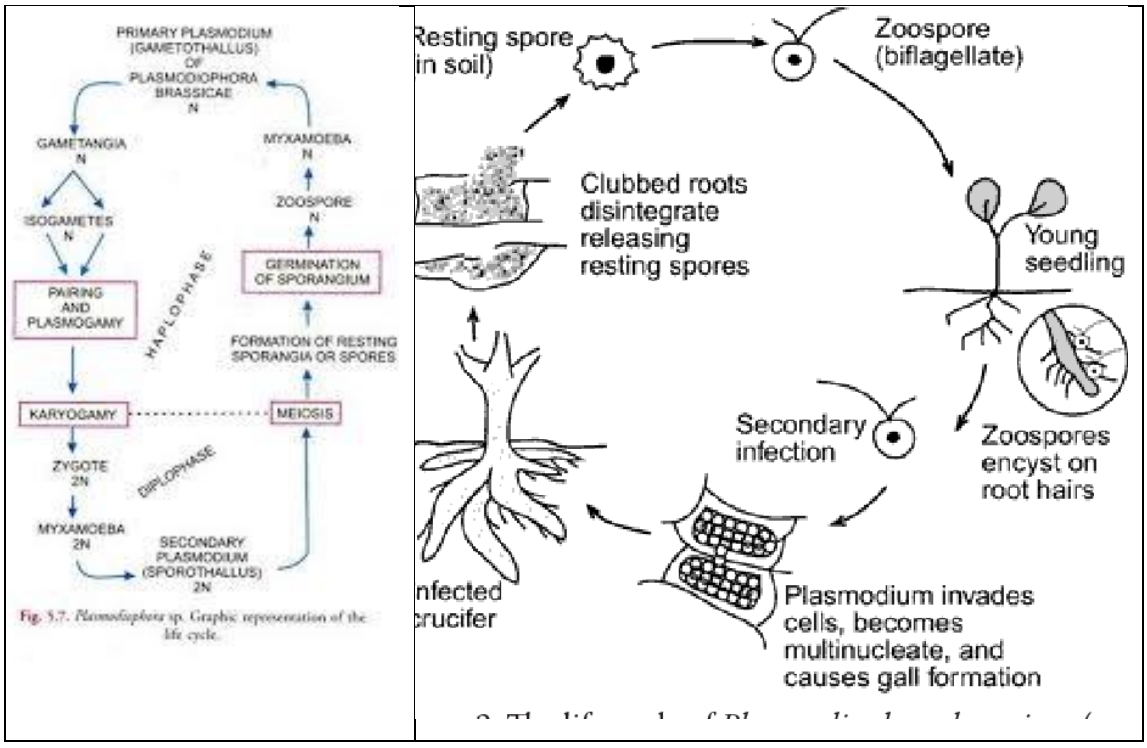
O: *Plasmodiophorales*

F: *Plasmodiophoraceae*

Ex. *Plasmodiophora brassicae*

Please check images in the following link

https://www.shutterstock.com/search/plasmodiophora-brassicae

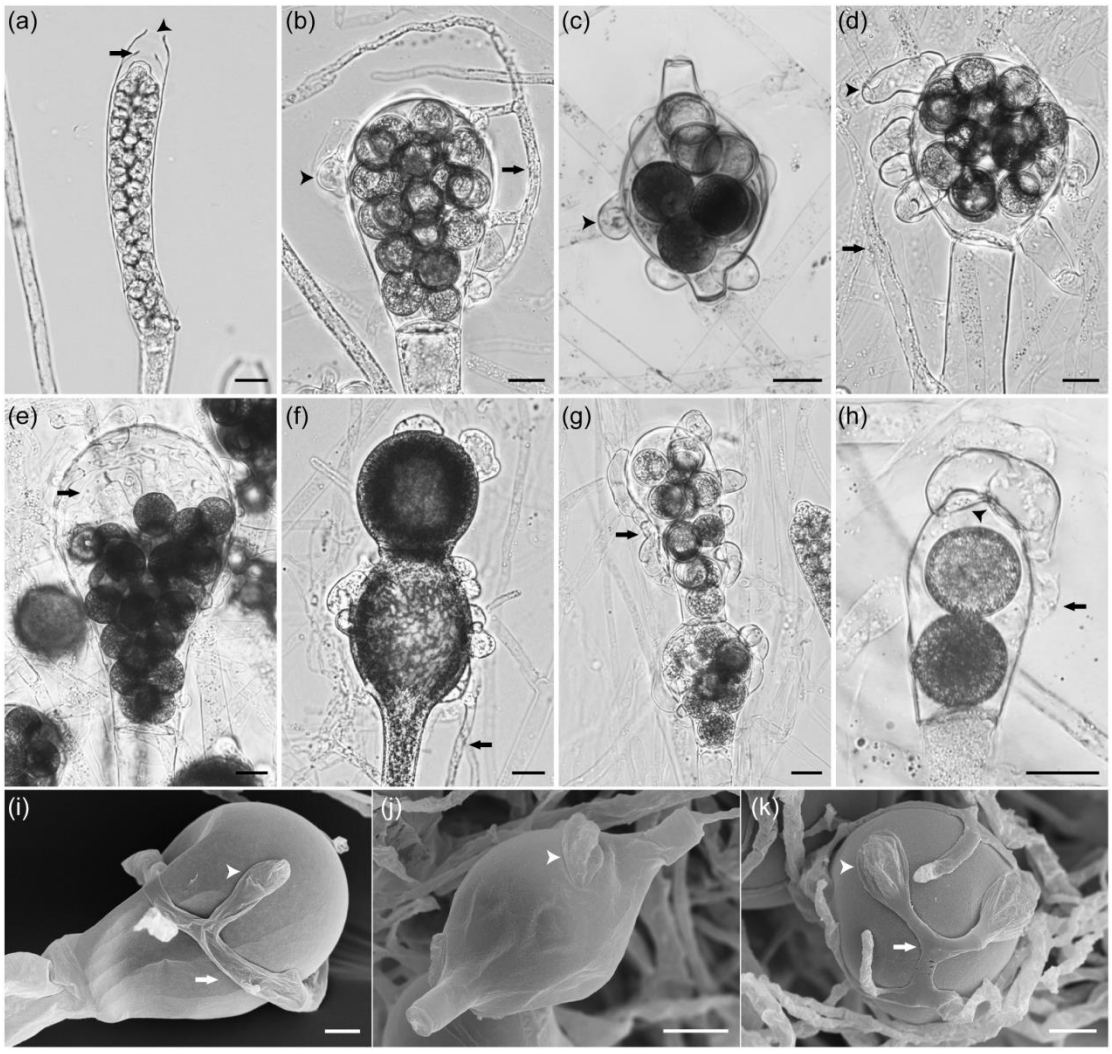


C: Phycomycetes

O: Saprolegniales

F: Saprolegniaceae

Ex.: *Saprolegnia* sp.

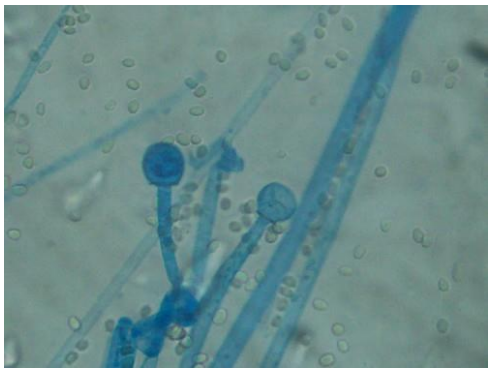


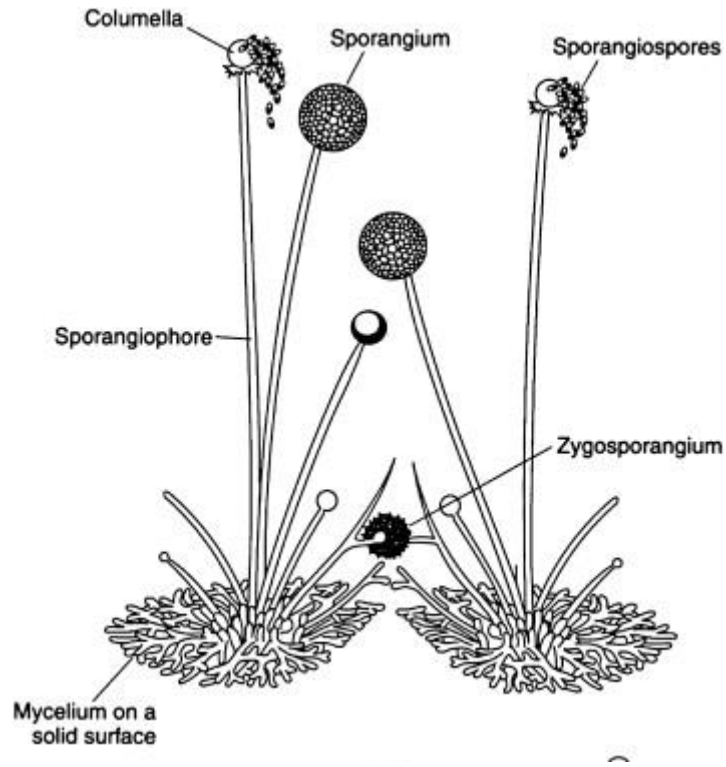
C: *Phycomycetes*

O: *Mucorales*

F: *Mucoraceae*

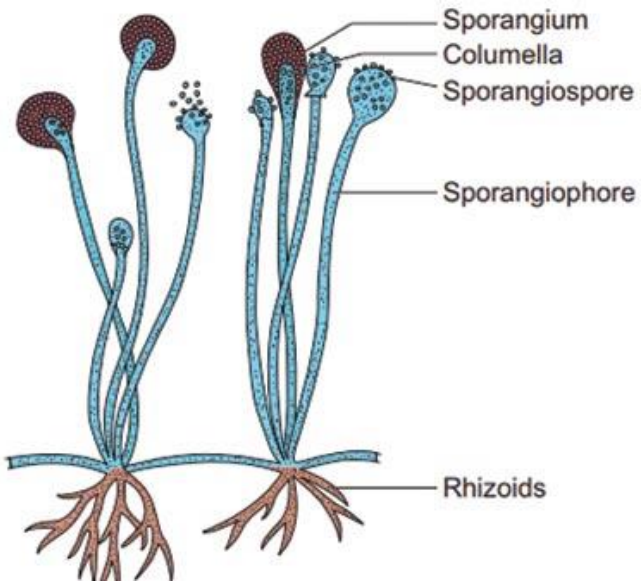
Ex.1. *Mucor racemosus*



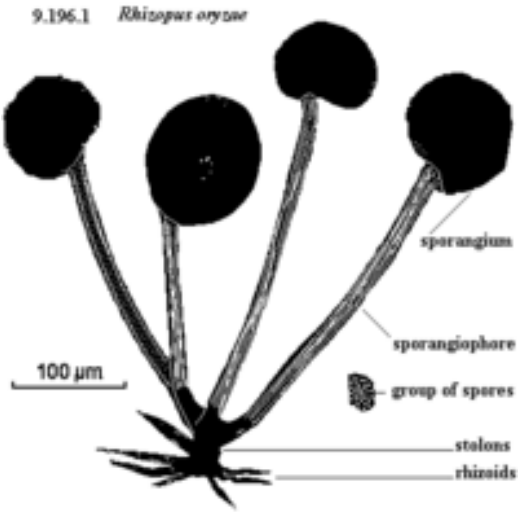


Ex. 2: *Rhizopus stolonifer*

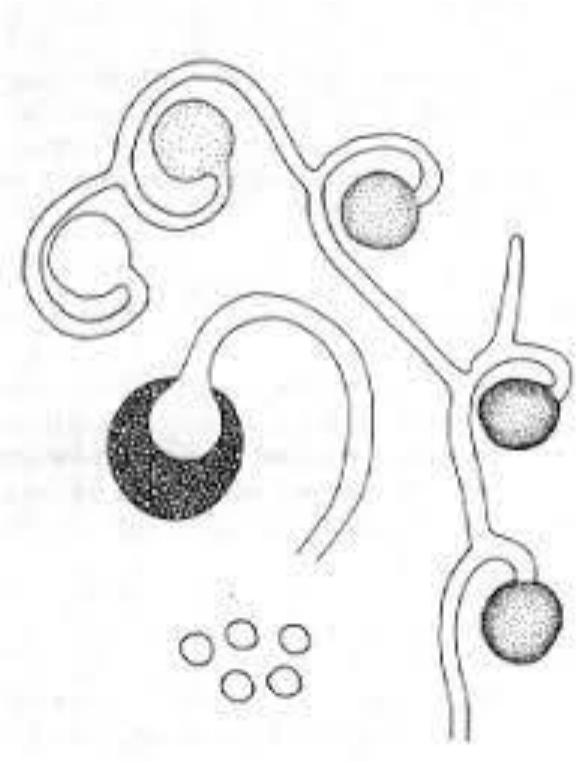
Rhizopus is **a genus of saprophytic and parasitic fungi**. They are found in moist or damp places. They are found on organic substances like vegetables, fruits, bread, jellies, etc. The vegetative structure is made up of coenocytic (multinucleated) and branched hyphae.

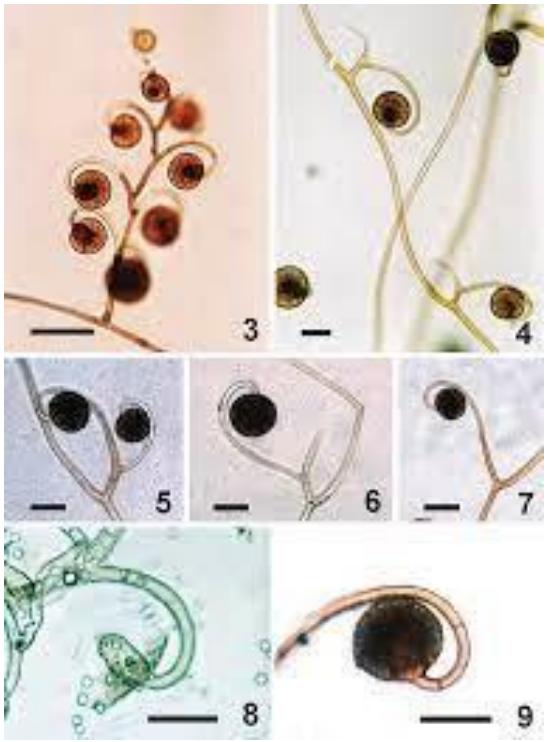


R. oryzae



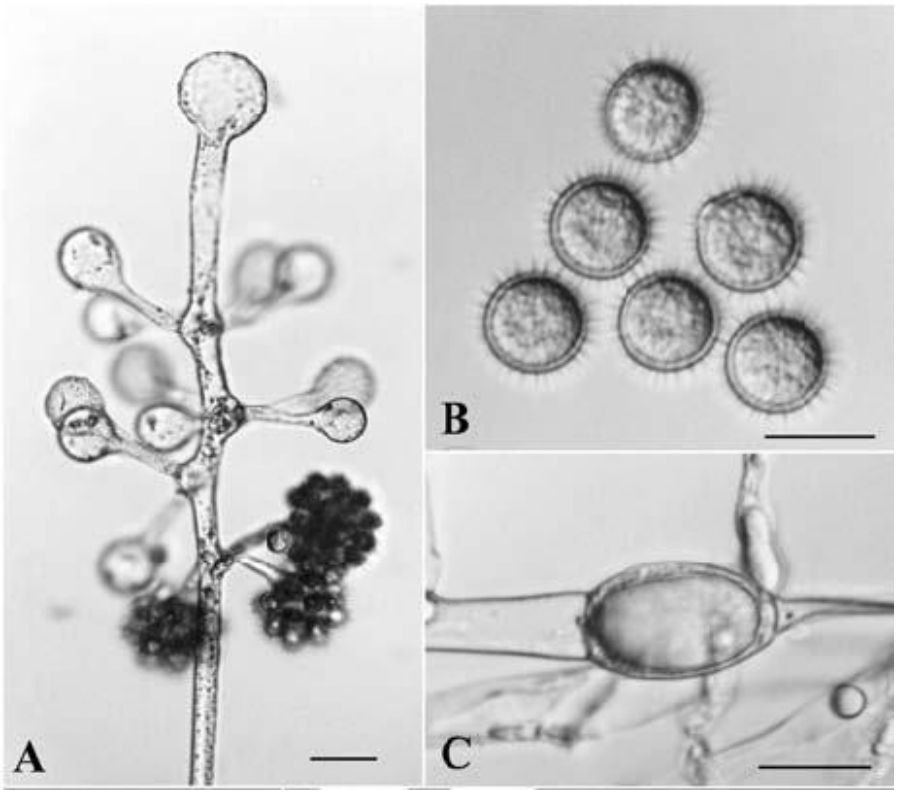
Ex. 3 *Circinella* sp.





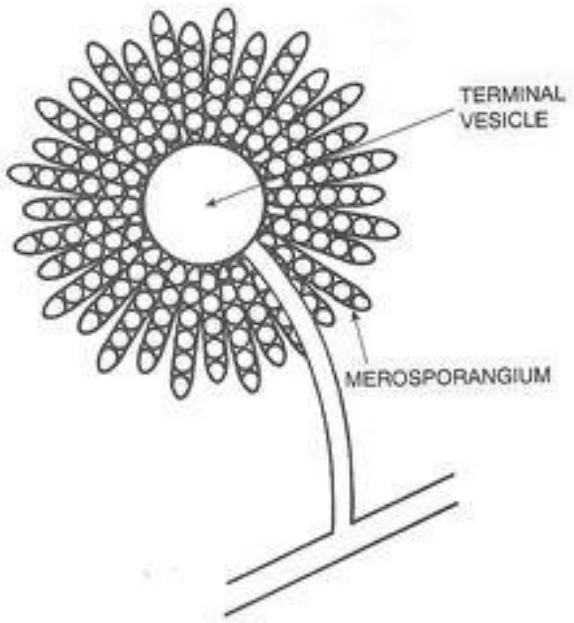
Family 2: *Choanephoraceae*

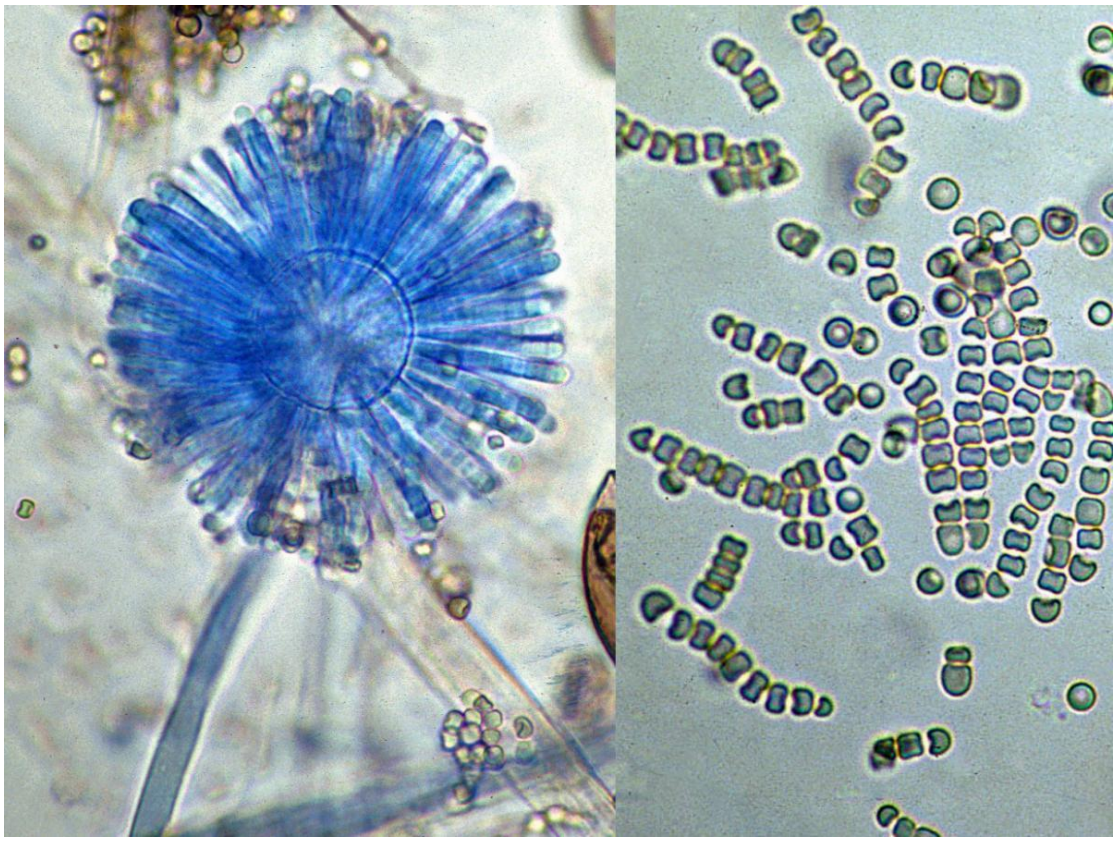
Ex. *Cunninghamella echinulata*



Family 3 : *Cephalidaceae*

Ex. *Syncephalastrum* sp.





Class 2: *Ascomtcetes*

Subclass: *Euascomycetes*

Series 1: *Plectomycetes*

Order 1: *Aspergillalaes*

Family : *Aspergillaceae*

Ex.1 *Aspergillus*

Different *Aspergillus* sp.

1	*Aspergillus clavatus*
2	*Aspergillus chevalieri*
3	*Aspergillus fumigatus*
4	*Aspergillus candidus*
5	*Aspergillus flavus*
6	*Aspergillus ochraceus*
7	*Aspergillus niger*
8	*Aspergillus versicolor*
9	*Aspergillus nidulans*
10	*Aspergillus ustus*
11	*Aspergillus flavipes*
12	*Aspergillus terreus*

General characteristics

1- Colony colour
2- Colony reverse
3- Sterigmata: Biserriate – uniserriate
4- Conidia: globose – subglobose –elliptical – ovate – rough – smooth – hyaline – pigment.
5- Vesicle : globose – subglobose – clavate.
6- Conidial head: radiate – columnar – clavate.
7- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
8- Ascospre
9- Hull cell
10- Sclerotia

Ex. 2: Penicillium

General characteristics

1- Colony colour
2- Colony reverse
3- Metulae: Present – Absent
4- Penicillin: Monoverticillata – biveticillata – symmetrica – asymmetrica – divaricate – nondivaricata (velutina – lanata – fasiculata).
5- Conidia: globose – subglobose –elliptical – ovate – rough – smooth – hyaline – pigment.
6- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
7- Ascospre
8- Hull cell
9- Sclerotia

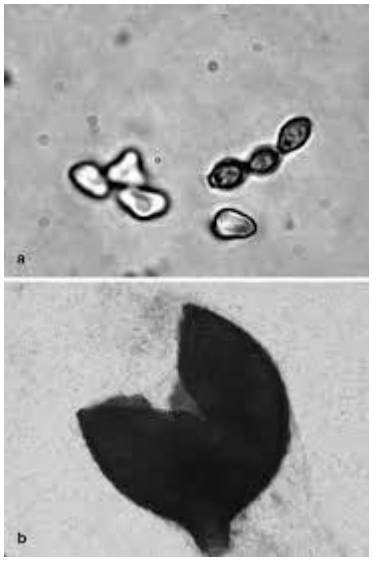
Different *Penicillium* sp.

1	*Penicillium corylophilum*
2	*Penicillium duclauxi*
3	*Penicillium funiculosum*
4	*Penicillium chrysogenum*
5	*Penicillium steckii*
6	*Penicillium waksmani*
7	*Penicillium purpurogenum*
8	*Penicillium corylophilum*
9	*Penicillium duclauxi*
10	*Penicillium funiculosum*
11	*Penicillium chrysogenum*
12	*Penicillium steckii*
13	*Penicillium waksmani*
14	*Penicillium purpurogenum*

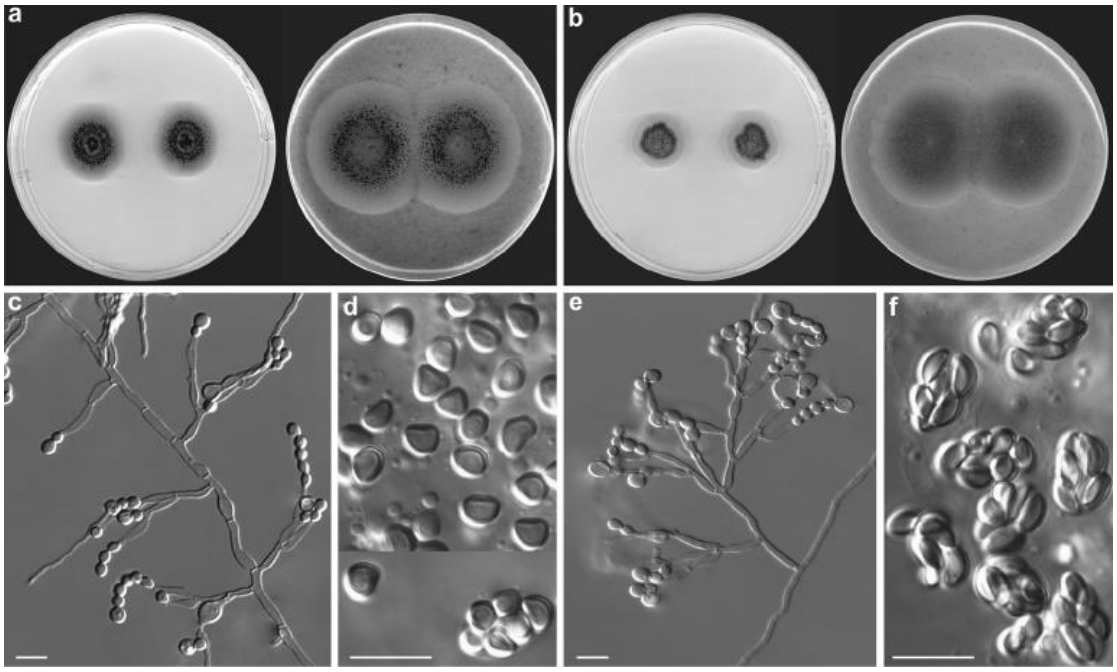
Order 2: Microascales

Family: Microascaceae

Ex. *Microascus trigonosporus* **(الجراثيم الزقية مثلثة الشكل)**



M. cinereus **(الجراثيم الزقية كلوية الشكل)**

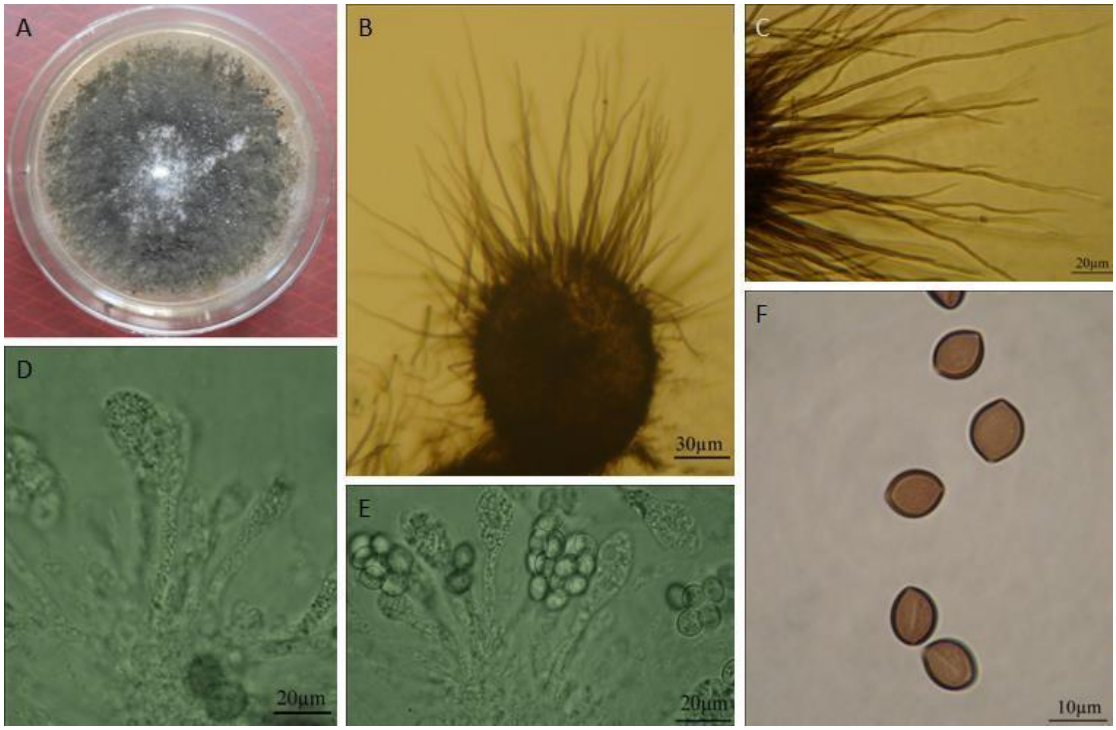


Series 2: *Pyrenomycetes*

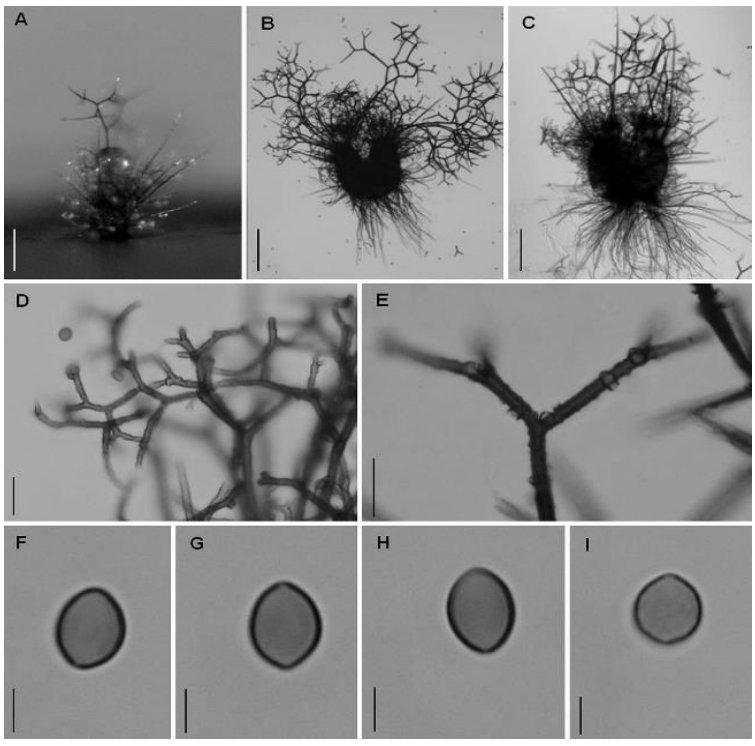
Order: *Sphaeriales*

Family: *Chaetomiaceae*

Ex.: Cheatomoium globosum



C. funicola



C. dreyfussi

C. uniporum

C. atrobrunneum

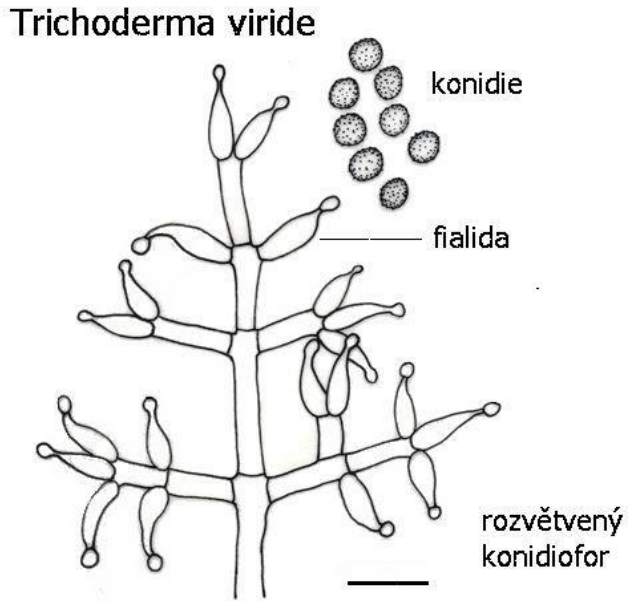
Class 3 : *Deuteromycetes*

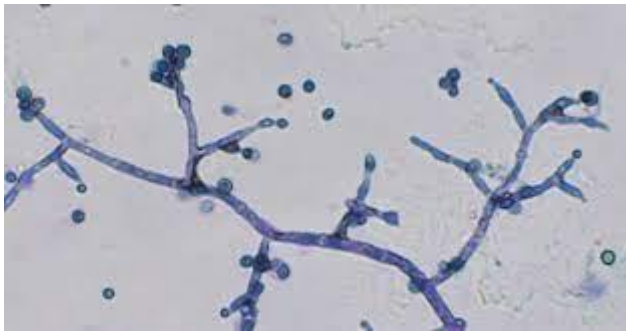
Order 1: *Moniliales*

Family: *Moniliaceae*

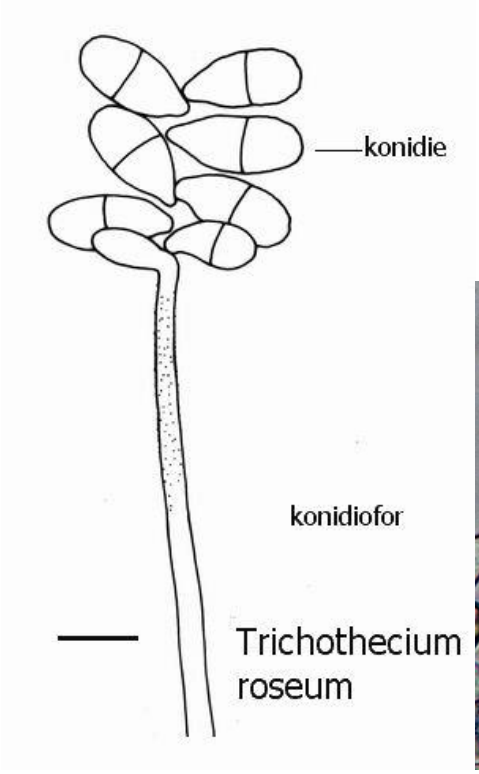
Ex. 1: *Trichoderma viride*

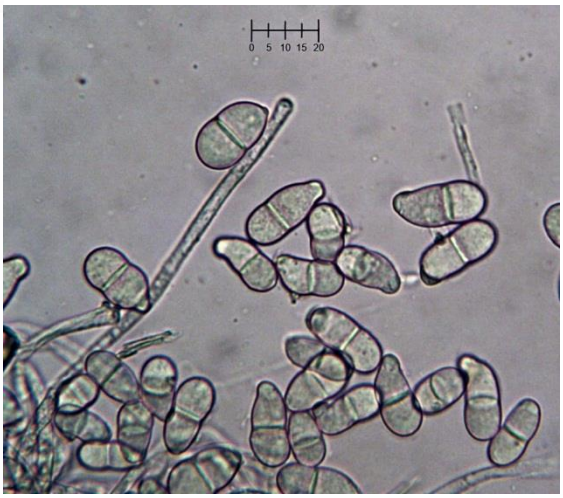
Trichoderma hamatum



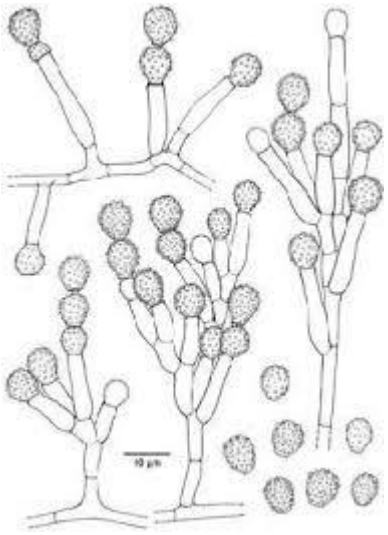


Ex. 2: *Trichothecium roseum*

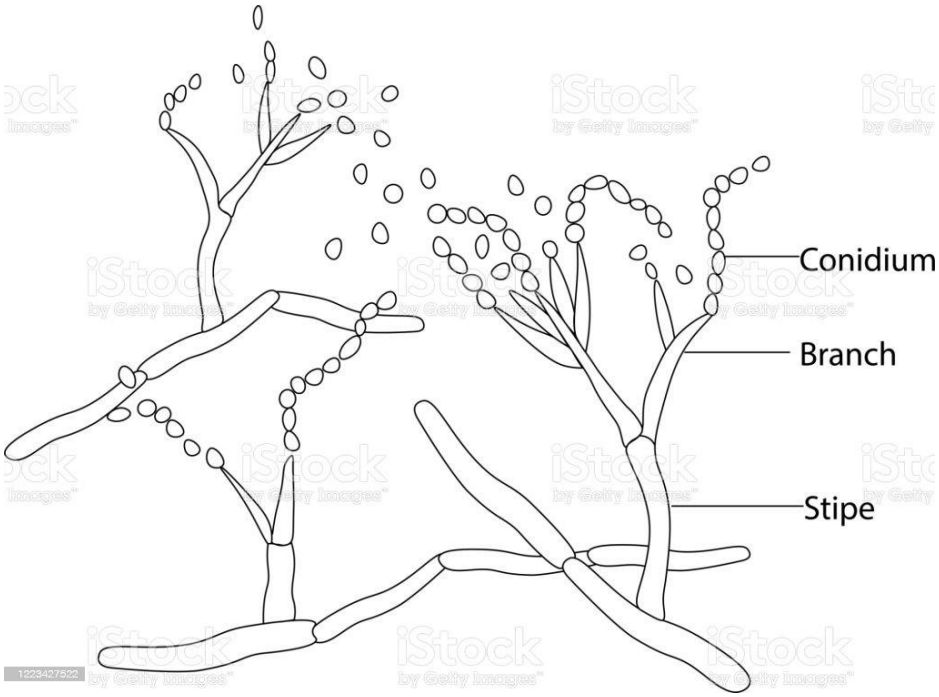




Ex. 3: *Scopulariopsis brevicaulis*

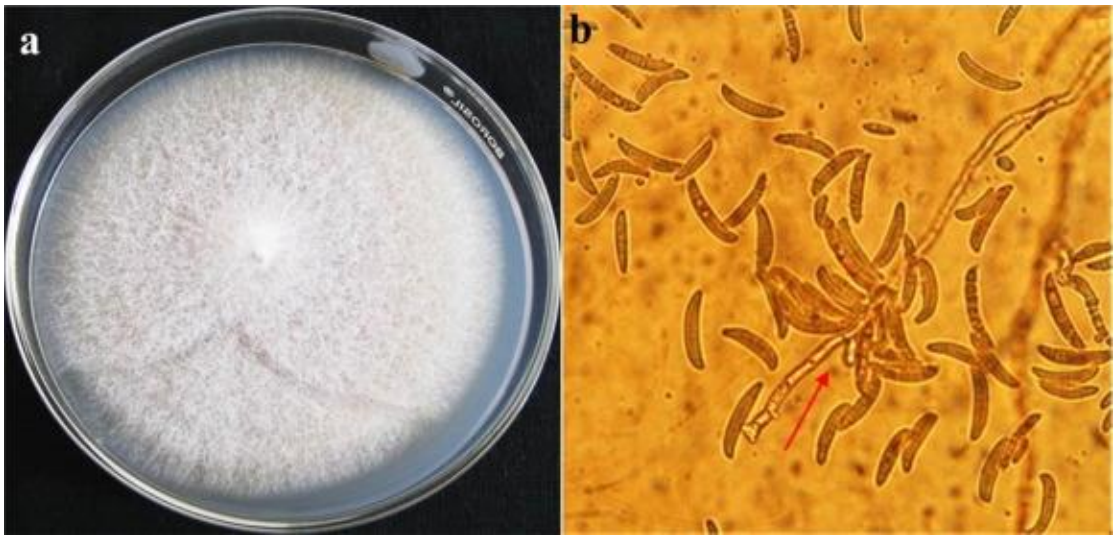


Ex. 4: *Pacielomyces variotii*

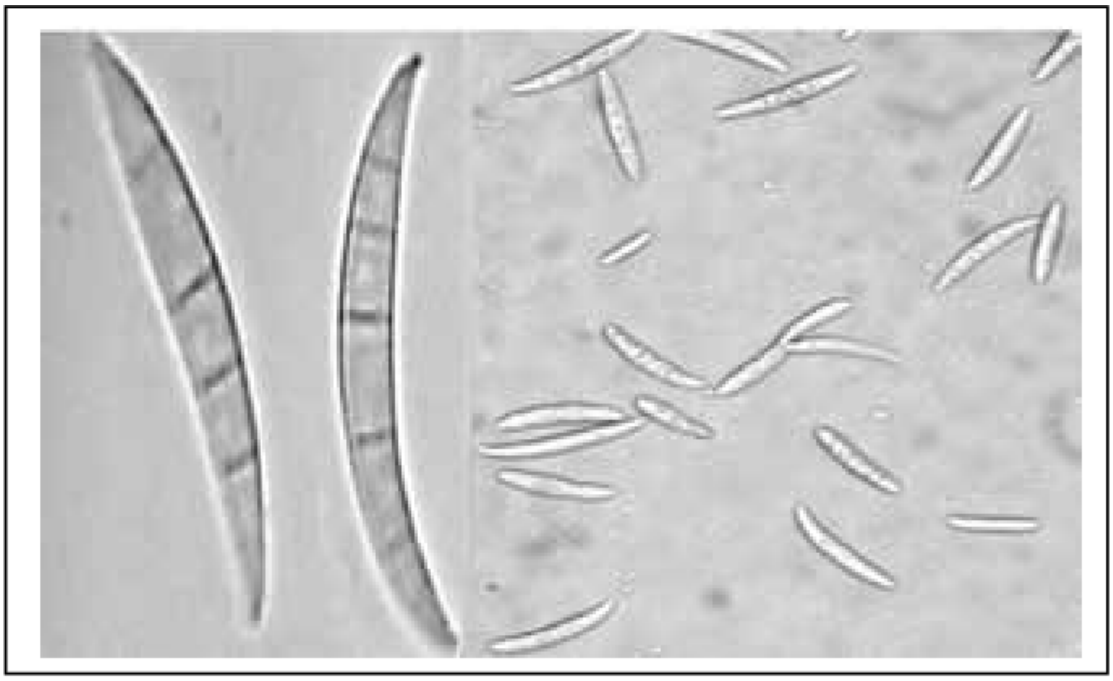


Family 2: *Tuberculariaceae*

Ex. 1: *Fusarium oxysporum*



F. solani

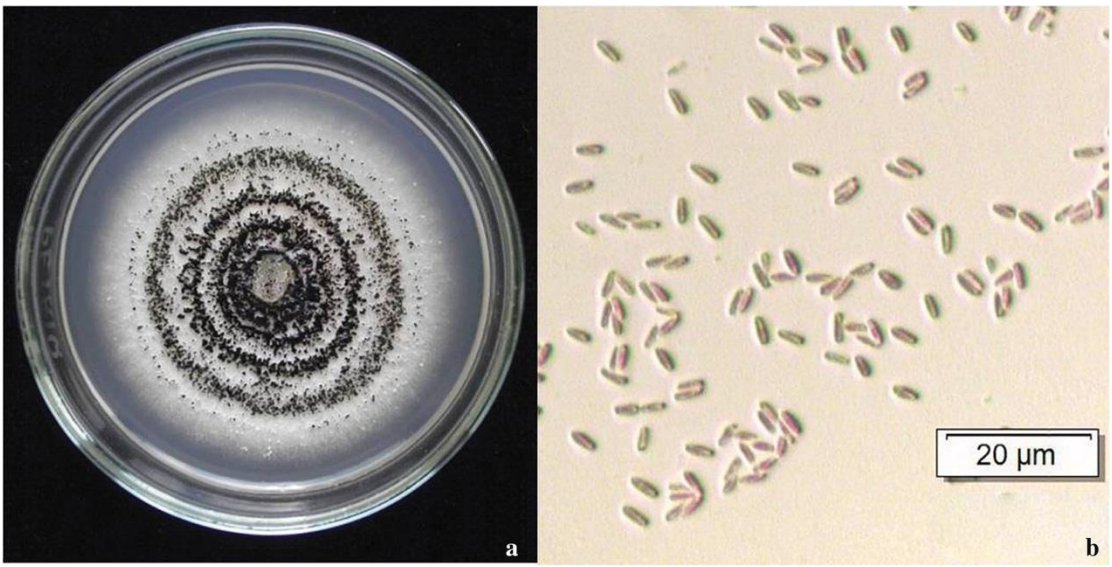


F. moniliform

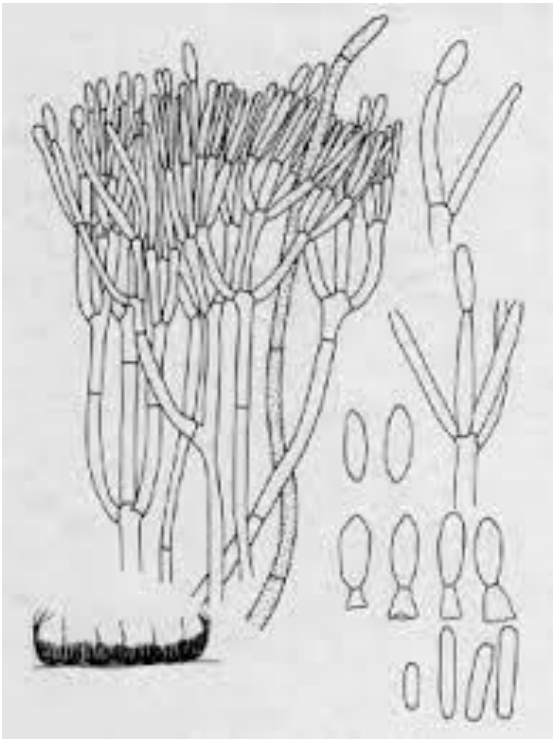
F. merismioides

F. tricinctum

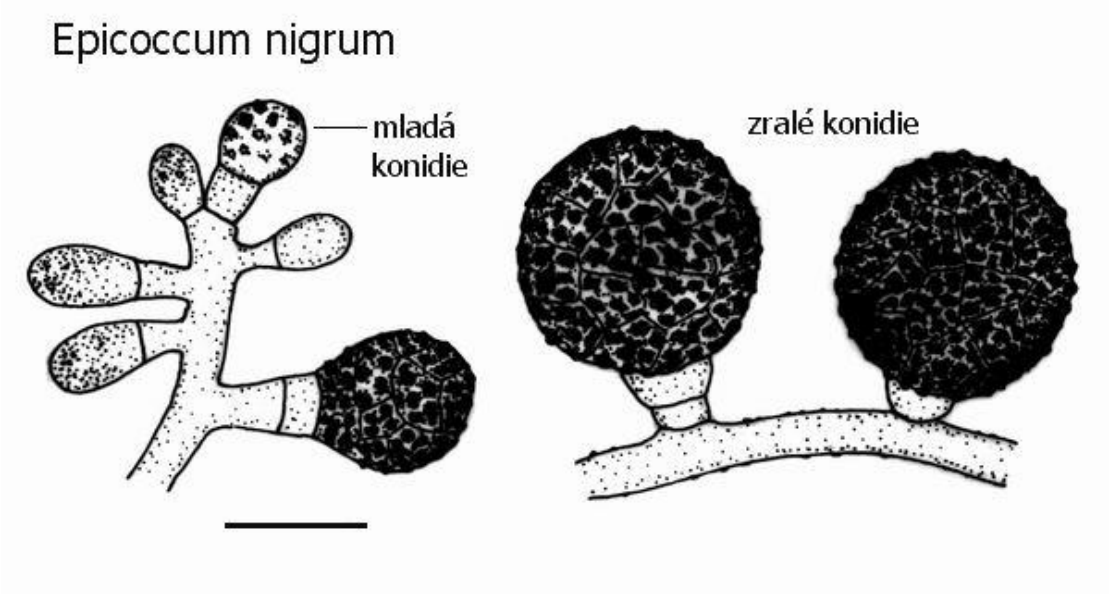
Ex. 2: *Myrothecium roridum*



M. verrucaria

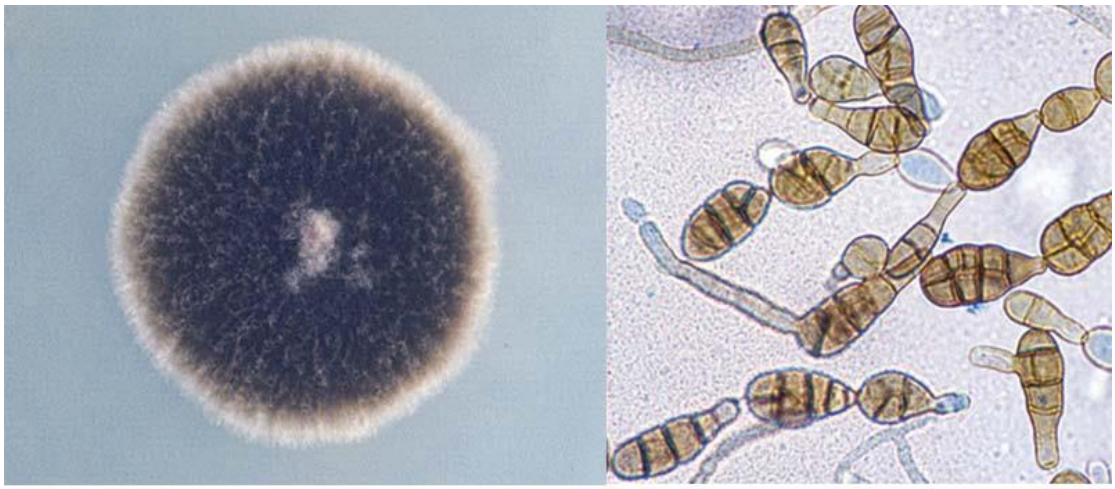


Ex. 3: *Epicoccum nigrum*



Family 3: *Dematiaceae*

Ex. 1: *Alternaria alternate*



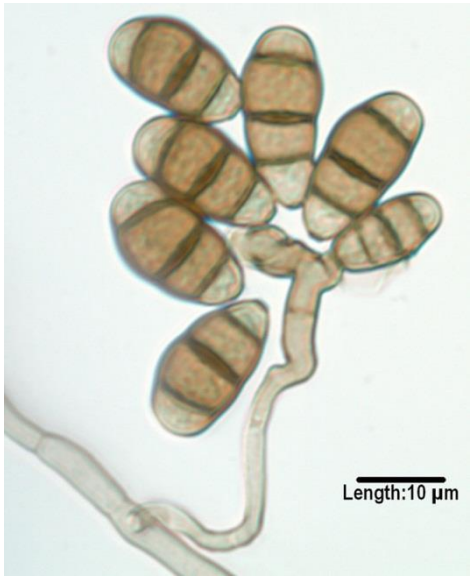
Alternaria alternata **colonies are black to olivaceous-black or greyish, and are suede-like to floccose.**

Alternaria alternata **showing branched acropetal chains and multicelled, obclavate to obpyriform conidia with short conical beaks.**

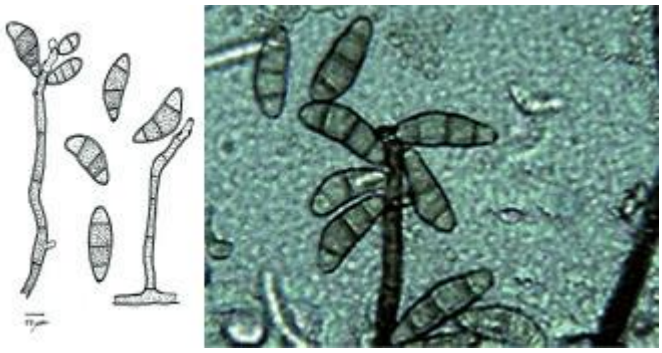
- *A. chlamydospora*
- *A. citri*

Ex. 2: *Ulocladium chartarum*

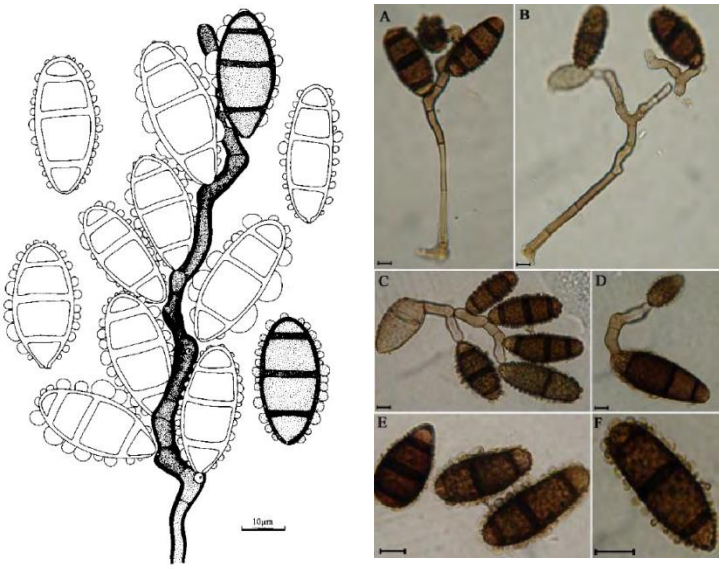
Ex. 3: *Curvularia lunata*



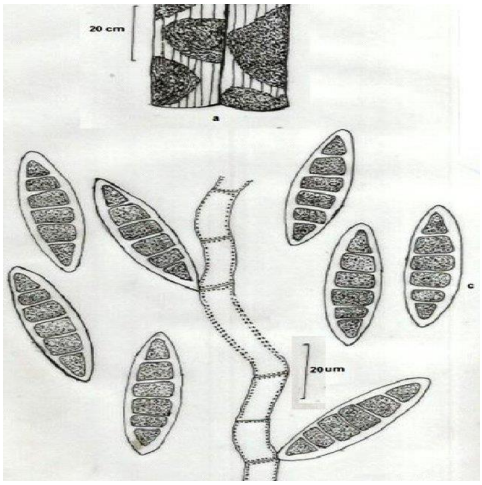
C. pallescens



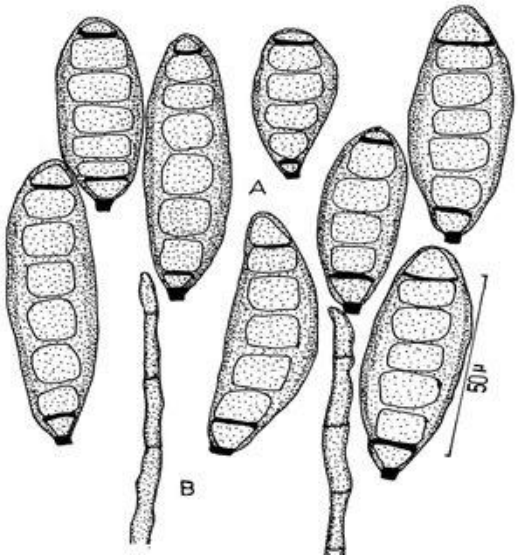
C. tuberculata



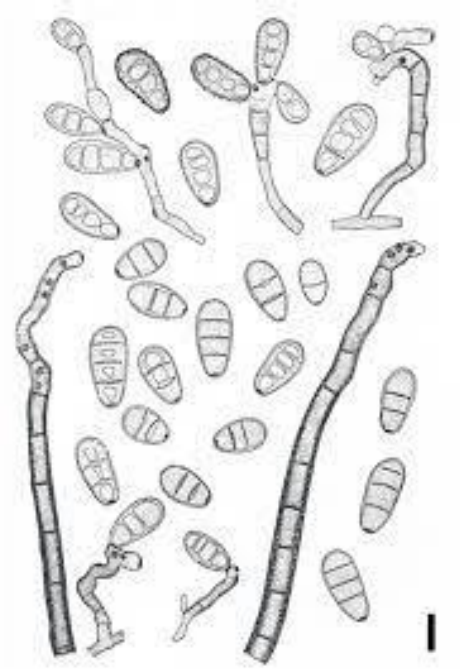
Ex. 4: *Drechslera indica*



D. rostrata



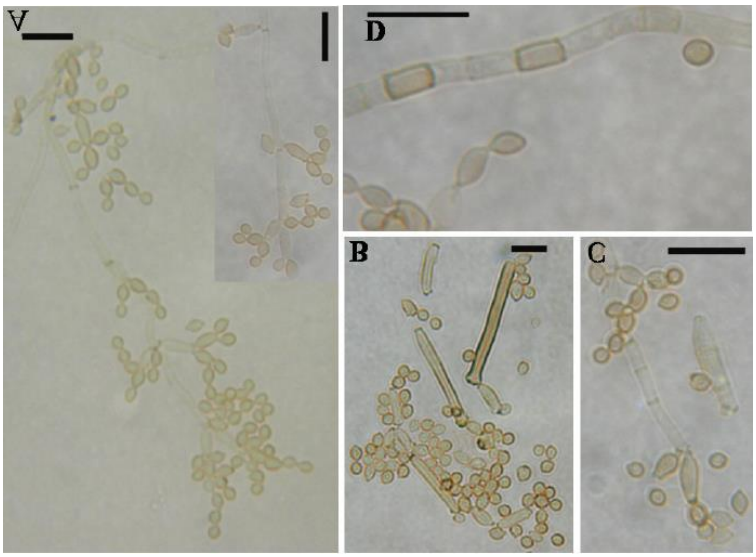
D. biseptata



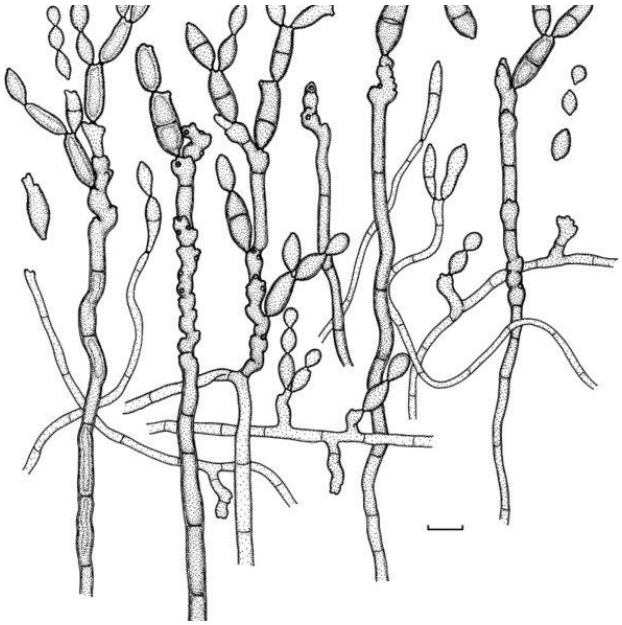
Ex. 5: *Stachybotrys chartarum*

Ex. 6: *Cladosporium cladosporioides*

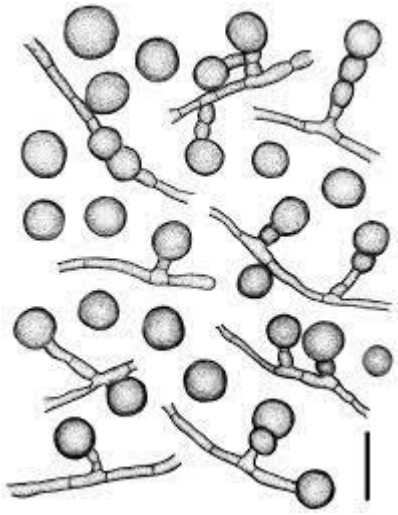
C.sphaerospermum



C.herbarum



Ex. 7*: Humicola grisea*



H. stellate الكونيده نجمية الشكل (

Family 3: *Stilbaceae*

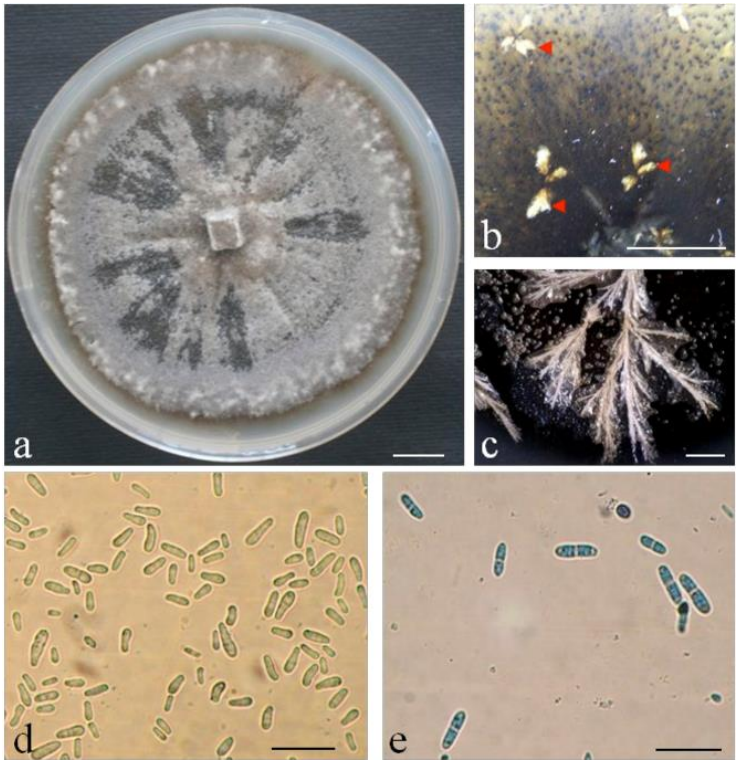
Ex. *Trichorus spirales*

Order 2: Sphaeropsidales

Family: Sohaeropsidaceae

Ex. : *Phoma eupyrena*

P. medicaginis



P. exigua

P. cylindrospora