FUNGI

For 4th year of B.Sc. students

Prepared by

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INTRODUCTION

Plant pathology has four objectives:

1. To study the structure and life cycle of the etiologic organism.
2. To study the pathogenesis and the disease symptoms.
3. To study the epidemiology and the conditions that favor spread of the pathogen.
4. To know the methods of disease control or management to reduce losses in crop yield.

Terminology of Plant Pathology

1-Disease: pathological process involving harmful physiological changes in the living plant after infection by a living organism.

2-Disorders: Physiological changes due to non parasitic agents.

3-Pathogens: living organisms (fungi, bacteria, etc.) which cause damage to the host plants

4- Parasites: pathogens deriving nutrients for growth from a living plant. They are:

* Obligate (biotrophs), restricted to living tissues.
* Facultative colonize living or dead tissues.
* Necrotrophs: grow on dead tissue; they kill in advance, thus more dangerous than biotrophs.

5- Pathogenicity: the ability to cause disease.

6- Virulence: degree of pathogenicity in a qualitative sense. Some strains of a pathogen may be avirulent

7- Aggressiveness: capacity of a parasite to invade and grow in its host plant and to reproduce on or in it.

8- Inoculum: portion of a pathogen capable of infecting a host.

9- Inoculum potential: a measure of the biological energy available for the colonization of a host. It is a function of: i - inoculum density,
ii - nutrients available to the infectious units for germination or growth,
iii - virulence of the pathogen, iv - susceptibility of the host.

10 - Immune: exempt from infection.

11 – Resistance& susceptibility: the extent to which the plant is able to prevent the entry or subsequent growth of the pathogen within it. High resistance means low susceptibility that approaches immunity. Low resistance means high susceptibility.

12 – Hypersensitivity: development of necrotic spots resulting from rapid death of cells in the vicinity of invading pathogen (confers high resistance to host plant).

13 – Entry (Penetration): direct or indirect.

14 – Infection: Establishment of nutritional relationship between the pathogen and the host.

15– Colonization: the pathogen advances through the tissues of host to varying extent.

16 – Symptoms: visible external alterations on the host by which a disease can be recognized.

General categories of symptoms are:

a- Necrosis: (death of infected tissue).

b- Hyperplasia: (increased cell division) and / or hypertrophy (increase in cell size) leading to galls, tumors, and witches ' brooms

c- Hypoplasia (reduced growth or stunting of infected plant).

Significance of Plant Diseases

1. Reduction of quality and quantity of plant products (flowers, fruits, fibers, wood, latex, etc.).
2. Limitation the kinds of plants and industries in an area.
3. Contamination of plant products with poisonous substances.
4. Responsible for direct/indirect financial losses (costs of control).

Stages in the Development of a Disease (Disease Cycle)

1- Inoculation: pathogen in contact with plant.

2- Prepenetration: germination of spores, attachment to host and recognition // host &pathogen

3- Penetration: a- Direct through cuticle.
b- Indirect through natural openings (stomata, hydathodes)
c- Indirect through wounds caused by nematodes or farming tools.

4- Infection (includes invasion): pathogen establishes contact with host cells & tissues, absorbs nutrients.

5- Colonization: growth and reproduction of the pathogen on host surface, within the plant or its vascular elements.

6- Dissemination of the pathogen: transfer of inoculum from the site of its production to the susceptible host surface either actively or passively by air, water, human, animal, insects, agricultural practice, seeds transplants etc.

7- Seasonal carryover (overwintering or over summering): survival of the pathogen in the form of hyphae, resting spores, sclerotia, chlamydospores, etc.

Classification of Plant Diseases

A- According to mode of primary infection:

1- Soil-borne diseases: due to soil-borne pathogens e.g. damping-off of seedlings, vascular wilt, root rots etc.

2- Air-borne disease: fungal spores are disseminated by wind and infect the shoot of plant e.g. rusts, downy mildews, powdery mildews, etc.

3- Seed-borne diseases: some pathogens survive as dormant mycelium in the seeds or other propagative structures of host plants e.g. many smuts.

B- <u>According to extent of occurrence and geographic distribution:</u>

1- Endemic diseases: constantly present in a particular country or part of the earth.

2- Epidemic (epiphytotic) diseases: occur periodically but in a severe form under favorable environmental conditions

3- Sporadic: occur at very irregular intervals and locations in few instances A disease may be endemic in one region and epidemic in another.

C- <u>According to disease symptoms:</u>

I- Necrosis (death of cells & tissues)

1- Rusts 2- Smuts 3- Mildews 4- Root-rots 5- Blights

6- Leaf spots 7- Wilts 8- Cankers 9- Fruit rots 10- Dieback

11- Chlorosis 12- Bloch 13- Damping-off 14 - Scab

15- Streaks or stripes 16- Burn, scald or scorch 17- White blisters or pustules

II- Hypertrophy and hyperplasia

1- Elongated internodes: rice infected with *Gibberella fujikuroi*; *Euphorbia* with *Uromyces pisi*; sugarcane with *Sclerospora sacchari.*

2- Galls and tumors: globose, elongated or irregular large sized outgrowths formed on attacked part e.g. Club root of Crucifers;

3- Witche's broom: upright cluster of small shoots contrasting with horizontal growth habit of normal shoot.

4- Curls: leaves are arched, twisted and distorted eg. peach leaf curl,

5- Floral abnormalities: enlargement of infected inflorescence which become green and fleshy with stamens converted into leafy structures.

III- Hypoplasia

1- Chlorosis: reduced development of chlorophyll (mosaic, vein clearing yellowing).

2- Reduction of individual organ: e.g. leaves, flowers, internodes as in dwarf bunt of wheat by *Tilletia contraversa.*

3-Floral abnormalities: in anther smut of Caryophyllaceae caused by *Ustilago violacea,* stamens become sterile.

D- <u>According to major Phyla of fungi:</u>

1- Diseases caused by Myxomycota
2- Diseases caused by Oomycota
3- Diseases caused by Chytridiomycota
4- Diseases caused by Ascomycota
5- Diseases caused by Basidiomycota
6- Diseases caused by Deuteromycota

I- Diseases caused by Myxomycota

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Plasmodiophora brassicae

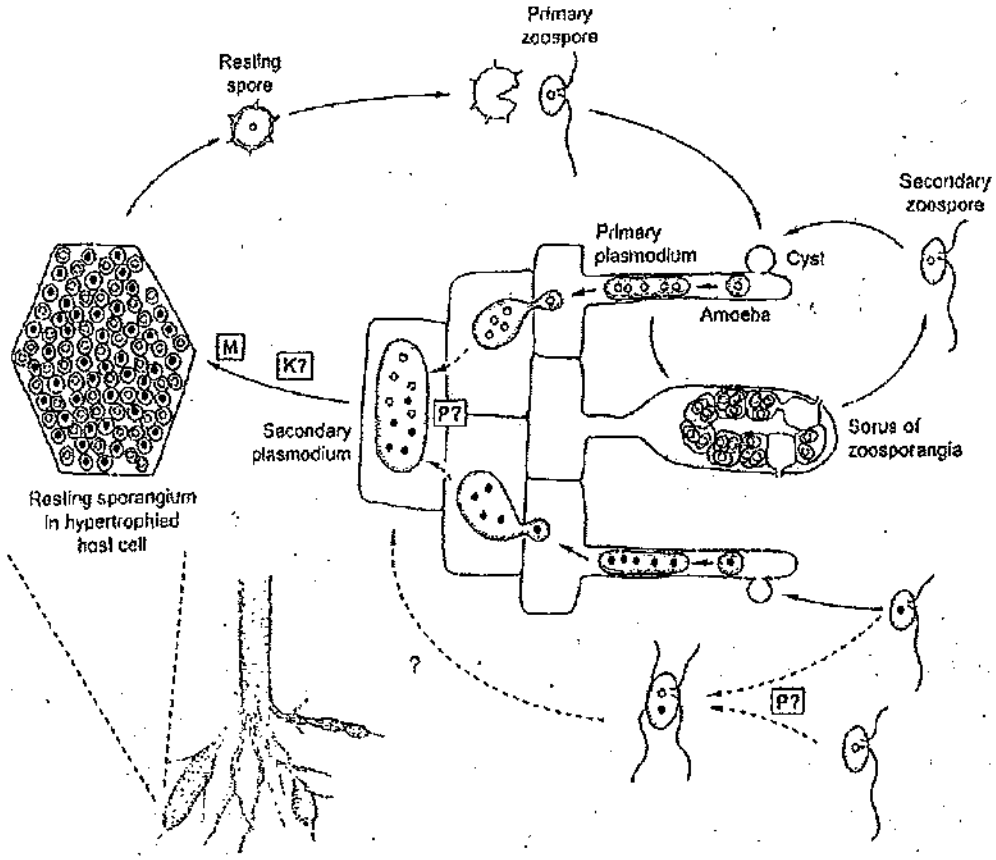
A- Club Root of Crucifers (finger and toe disease)

- Causal agent: *Plasmodiophora brassicae*
- Host plants: Cruciferous vegetables such as cabbage, cauliflower, radishes, and turnips; and field crops such as mustard.
- Symptoms:

Roots show malformation and enlargement due to spindle or club shaped swellings resulting from hypertrophy and hyperplasia of infected cells. Inside root cells, plasmodia followed by resting spores are formed. Leaves show yellowing and wilting.

- Disease cycle:

Plasmodiophora brassicae infects susceptible host plants through root hairs. It stimulates abnormal growth of affected parts, resulting in a swollen clubs. Infection is favored by excess soil moisture and low pH. Numerous resistant spores of the fungus are produced in the "clubbed" tissues. As tissues decay, spores are released into the soil where they can remain infectious for at least 10 years.



- Disease management:
  1. Eradication of cruciferous weeds.
  2. Use of well drained, pathogen free pots.
  3. Use of seedlings raised in pathogen free soil.
  4. Very long crop rotation with non cruciferous crops.
  5. Soil fumigation with volatile chemicals such as vapam, methyl dibromide etc.
  6. Alteration of soil pH to 7 or above by adding lime.
  7- Soil treatment with fungicides (e.g. PCNB)

B- Powdery scab of potatoes

- Causal agent: *Spongospora subterranea*

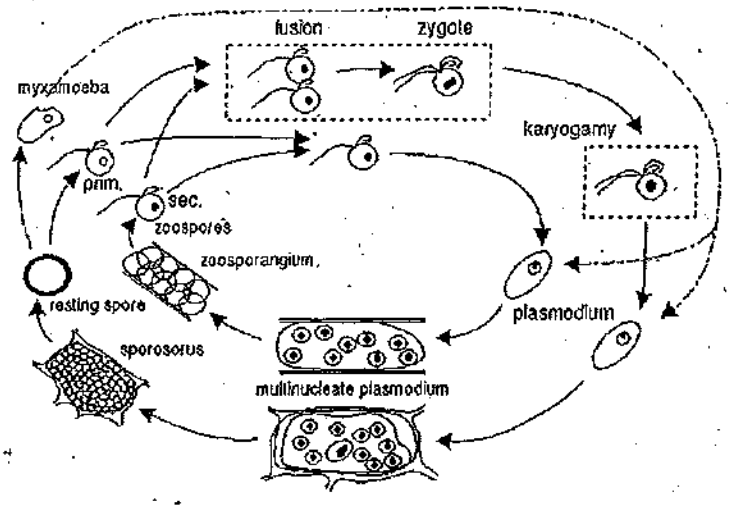
  It is generally found in wet, badly drained soils. The spores remain in the soil for several years.

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Spongospora subterranea

- Symptoms:

  On tubers, irregular brown depressions with raised papery margins (scabs) are formed. These scabs are filled with dusty brown spongy masses of spore balls. Infected young tubers show distortion and swollen outgrowths.

- Control:
  1. Healthy, powdery scab-free seeds are only planted.
  2. Infected tubers should be disposed correctly not composted.
  3. Crop rotation is useful where replanting potatoes in the same position is avoided for three years.
  4. Improved soil aeration.
  5. There are no fungicides that can be used.



II- Diseases caused by Chytridiomycota

Class: Chytridiomycetes
Order: Chytridiales
Family: *Synchytriaceae*
Synchytrium endobioticum

1- Black Wart of Potatoes

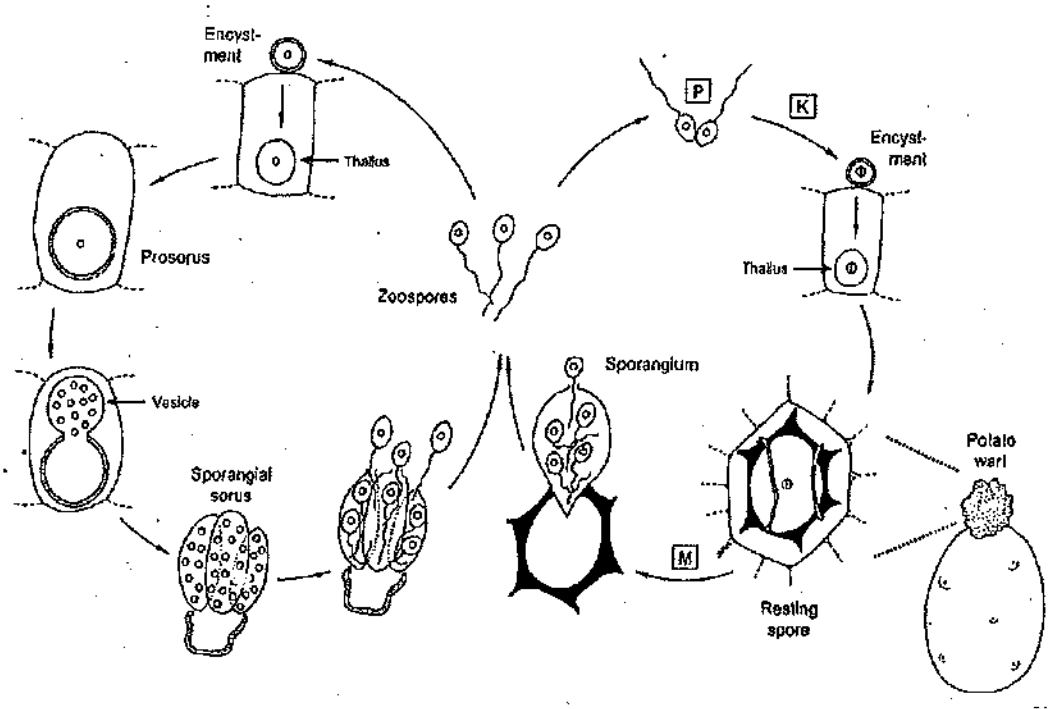
Caused by: *Synchytrium endobioticum*. It is a non mycelial, unicellular, holocarpic biotrophic chytrid fungus.

Disease Cycle:

- Infected host cells contain spherical (2n) resting sporangia (RS) of dark brown walls
- RS are released by the decay of warts and they may remain viable in soil for up to 40 years. They germinate producing prosporangia (vesicles) in which uniflagellate zoospores (n) are produced.
- Meiosis occurs during germination.
- Zoospores encyst on host epidermis before infection
- Inside the host cell the small fungal cell enlarges and the host is stimulated to enlarge.
- Zoosporangia (n) are formed producing up to 600 zoospores per spoangium.
- At later stages zoospores behave as gametes to give resting sporangia

Symptoms:

- Large irregular cauliflower-like warts or galls develop on all underground parts except roots.
- Warts at first greenish-white, becoming dark or black
- Warts develop due to hyperplasia and hypertrophy.
- The disease causes losses by reducing the quantity and quality of tubers.



III- Diseases caused by Oomycota

Class: Oomycetes
Order: Saprolegniales
Family: *Saprolegniaceae*
Aphanomyces euteiches

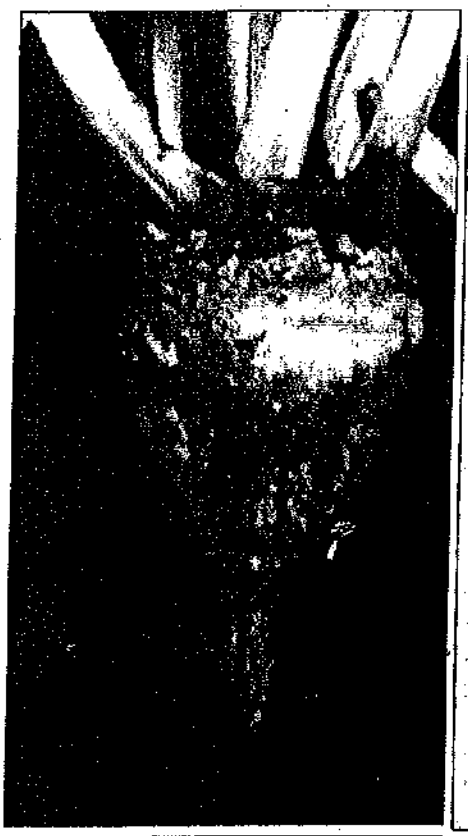
1- Aphanomyces root rot

Caused by *Aphanomyces* one of the zoosporic fungi belonging to the Family Saprolegniaceae

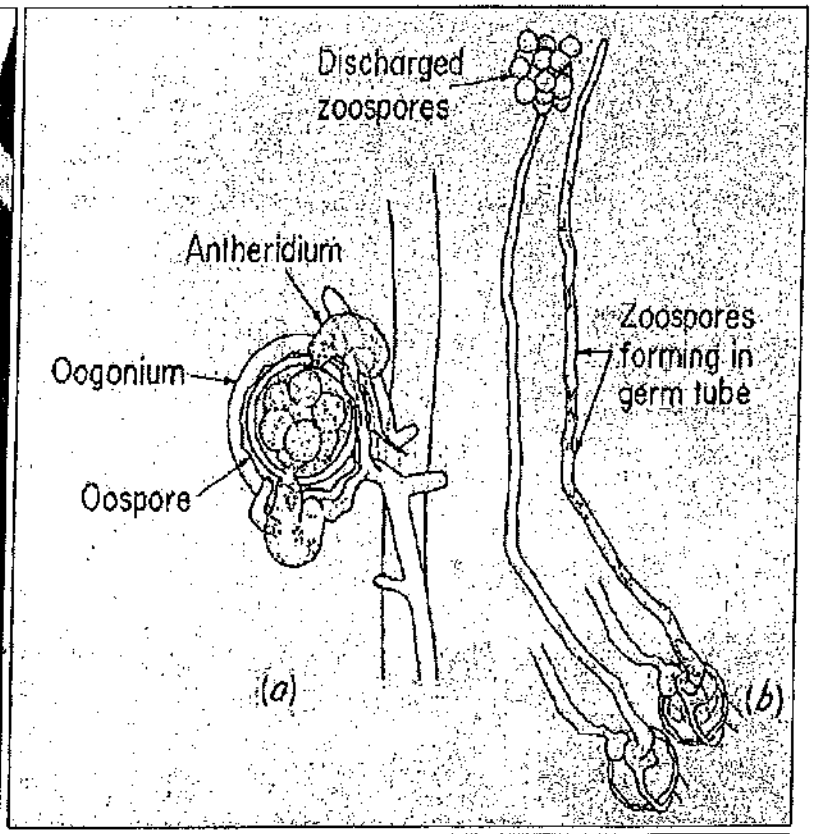
Hosts: Sugar beet, wheat, pea, etc

Symptoms:

- Soft decay of the root cortex.
- Vascular core of root tends to come out when roots are pulled up.
- Progressive death of the leaves from the base of the stem.
- General check in growth.
- Infected plants may survive but produce poorly filled pods.
- Oospores of the fungus are present in decaying tissues.
- Disease is most serious in soils with high moisture content and at 15° – 30° c.



Aphanomyces **root rot of Sugar beet**



Aphanomyces: **asexual and sexual reproductive units**

2- Damping off and seedling blight

Caused mainly by *Pythium* species, Family Pythiaceae

- Other fungal species belonging to *Phytophthora, Rhizoctonia, Fusarium, Helminthosporium and Botrytis* could be associated with damping off and seedling blight

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Pythium aphanidermatum
Pythium oligandrum

Symptoms:

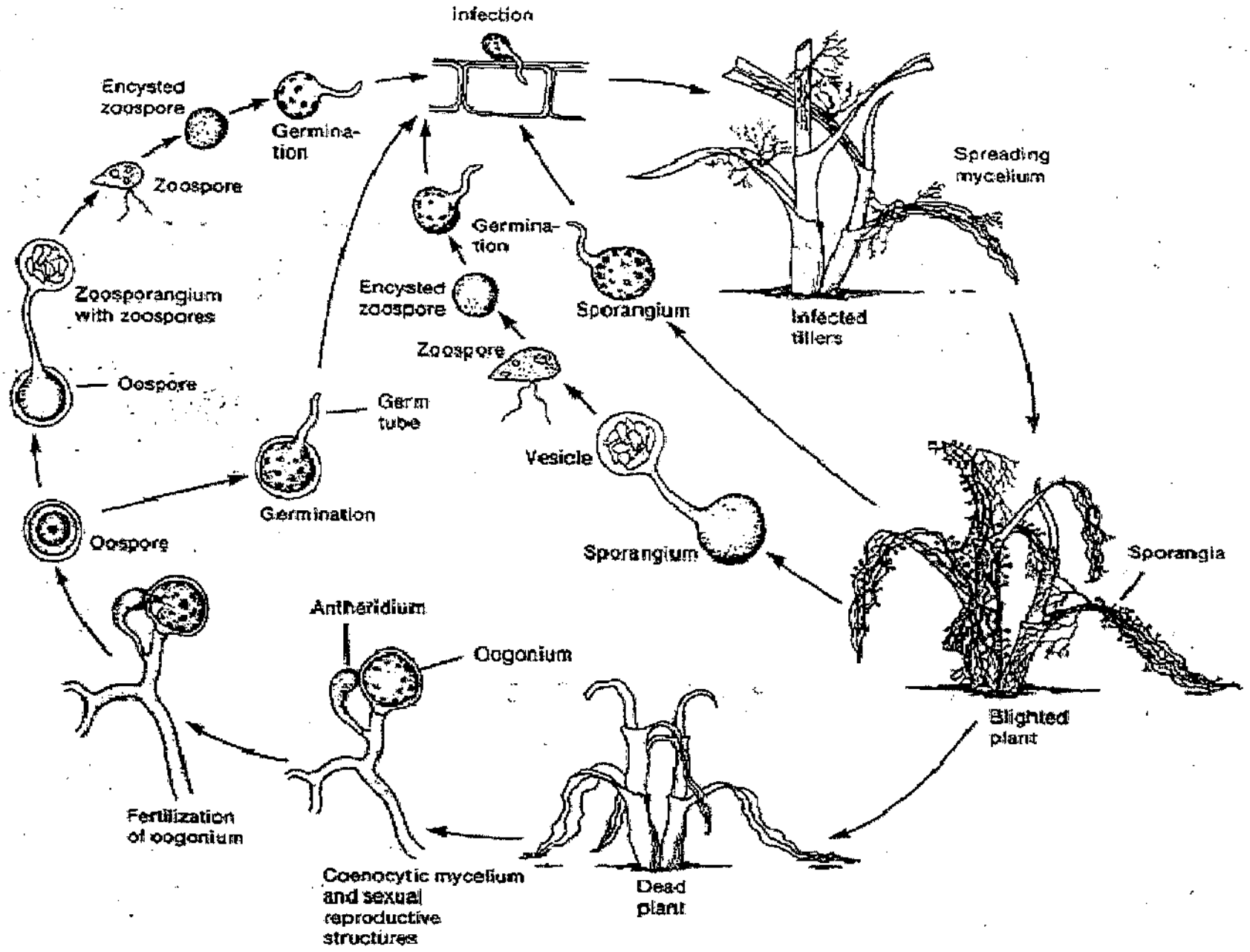
- Pre-emergence: Emergence of seedlings is poor even with seeds of high germinative capacity
- There are patches with no seedlings at all.
- Post- emergence: Seedlings that have emerged often show water soaking, browning or shriveling of the stem tissues at soil level and die.
- When plants are pulled up they show browning and rotting of the smaller roots or stem, and stem lesions at soil level.
- Plants are stunted.
- Plants wilt at midday and may recover at night.
- Plants show yellowing and die.
- Brown tissue on the outer portion of the root easily pulls off leaving a bare strand of vascular tissue exposed.
- Root tips are brown and dead
- The cells of roots contain round, microscopic, thick-walled oospores of *Pythium*

Control:

The most favorable environments for *Pythium* disease are soil treated with high-nitrogen fertilizers, alkaline soil and soil with low calcium levels.

To prevent or minimize contamination it is recommended to:

a- Utilize balanced fertilizers and keep soil pH neutral or slightly acid.
b- Prune trees and shrubs to improve air circulation.
c- Correct drainage problems, avoid over-watering,
d- Preventive fungicide treatment programs using metalaxyl, terrazole, coban, etc


Infection
Encysted zoospore
Germination
Zoospore
Spreading mycelium
Germination
Zoosporangium with zoospores
Encysted zoospore
Sporangium
Infected tillers
Oospore
Zoospore
Germ tube
Vesicle
Germination
Oospore
Sporangium
Sporangia
Antheridium
Oogonium
Blighted plant
Fertilization of oogonium
Coenocytic mycelium and sexual reproductive structures
Dead plant

3- Late blight of potato and tomato

Caused by: *Phytophthora Infestans*

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Phytophthora infestans

- Late blight epidemics in the 1840s led to the Irish Potato Famine, in which over a million people died and a million emigrated to other countries.
- Even today, *Phytophthora Infestans* poses a major threat to potato agriculture.

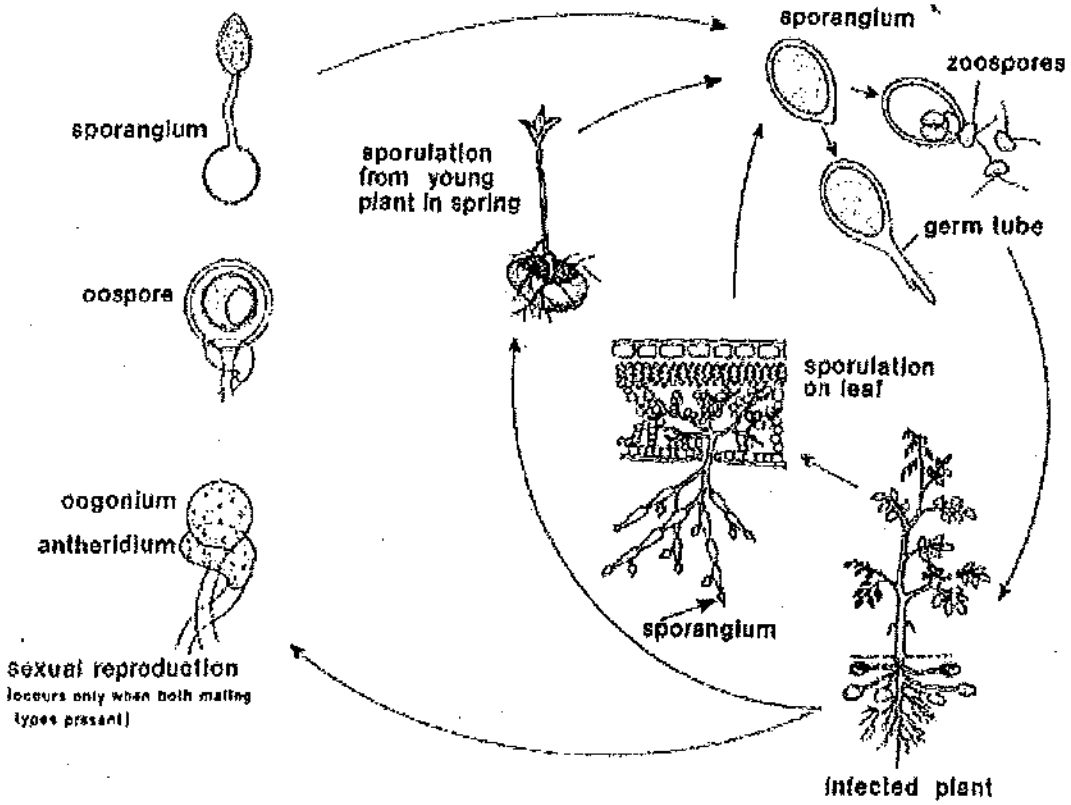
Symptoms:

On leaves and stems:

- Dark brown lesions of varying sizes and shapes.
- Under moist conditions a mass of sporangiophores
- (White bloom) develop on the lower leaf surfaces.
- Lesions increase rapidly and coalesce.
- Potato shoots are killed within 3-4 weeks.

On tubers:

- Tubers show irregular dark and sunken areas associated with brownish rot.
- Rotting often increase during storage due to further invasion by bacteria.



This is a simplified disease cycle for late blight of potato.

4- White rust Diseases

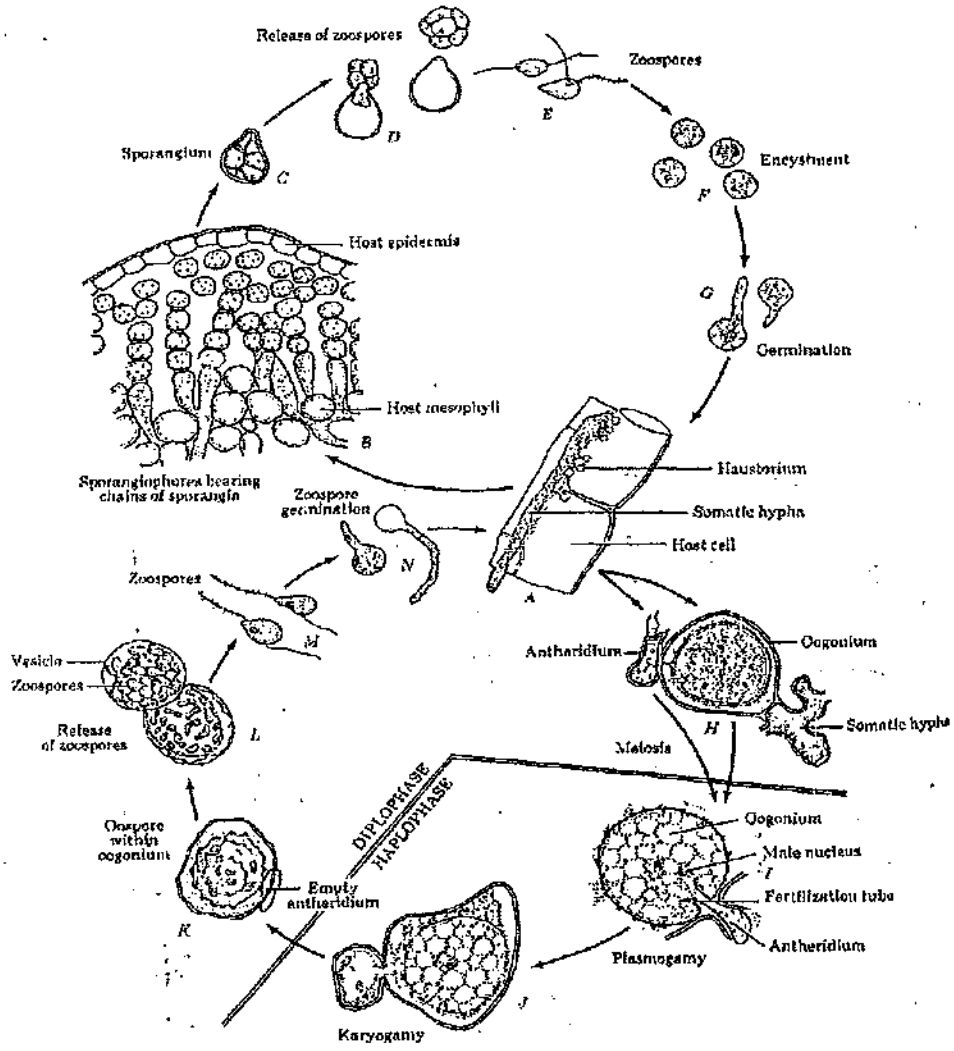
A- White rust of Crucifers by *Albugo candida* of the Hosts include radish, turnip, cabbage, cauliflower, mustard,

B- White rust of *Portulaca* by *Albugo portulacae*

Class: Oomycetes
Order: Peronosporales
Family: Albuginaceae
Albugo candida
Albugo portulacae

Symptoms:

- White pustules or sori develop on leaves and stems.
- Host epidermis ruptures exposing a white powdery mass of spores (chains of sporangia on clavate sporangiophores).
- The fungus grows inside the whole plant tissue stimulating various types of deformities.
- Inflorescences and flowers become thickened due to hypertophy and hyperplasia of affected cells.
- The swollen parts are full of oospores



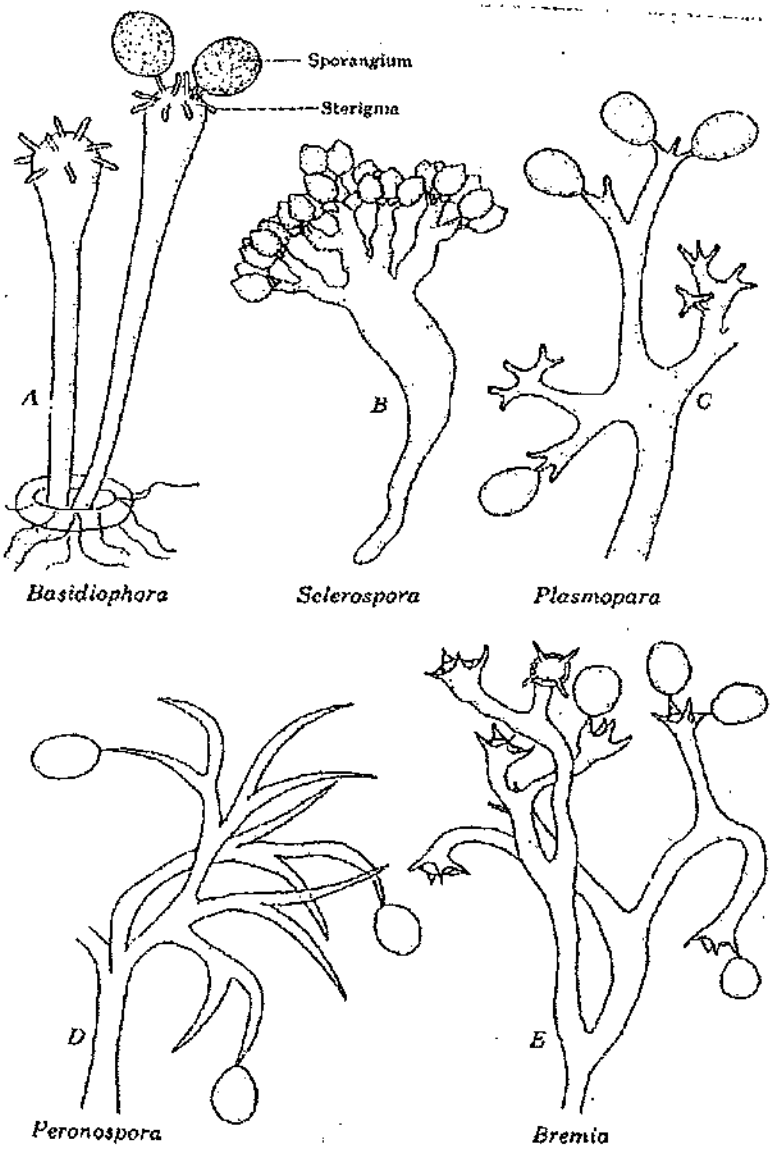
Life cycle of *Albugo candida*

5- Downy mildew diseases

Caused by members of the Family Peronosporaceae, Order Peronosporales, Class Oomycetes. Fungal species, their hosts are shown in the following table.

Pathogens	Hosts
Plasmopara viticola	grapevine
Bremia lactucae	lettuce
Peronospora destructor	onion
Peronospora pisi	peas
Peronospora parasitica	Cauliflower, Cabbage
Pseudoperonospora cubensis	Cucurbits
Sclerospora graminicola	cereals (wheat, sorghum, corn)
Basidiophora sp	Compositae (*Sonchus, Helianthus*)

The sporangiophores of downy mildew fungi are illustrated in the following figure



A- Downy Mildew of Grapevine by *Plasmopara viticola*

Class: Oomycetes
Order: Peronosporales
Family: *Peronosporaceae*
Plasmopara viticola

Signifcance of disease:

- The fungus causes direct yield losses by rotting inflorescences, clusters and shoots.
- Indirect losses can result from premature defoliation of infected vines.
- Premature defoliation predisposes the vine to winter injury.

Symptoms:

a- On leaves:

- Aappearance on the upper leaf surface of irregular pale-yellow to greenish-yellow spots up to 1/4 inch or more in diameter.
- On the underside of the leaf, the fungus mycelium (the "downy mildew") can be seen within the border of the lesion as a delicate, dense, and white to grayish, cotton-like growth.
- Infected tissue gradually becomes dark brown, irregular, and brittle.
- Severely infected leaves eventually turn brown, wither, curl, and drop.

b- On fruits:

- young berries turn light brown and soft, and under humid conditions are often covered with the downy-like growth of the fungus .
- Berries infected at late summer do not turn soft or become covered with the downy growth. Instead, they turn dull green, then dark brown to brownish-purple.
- They may wrinkle and will never mature normally

c- On shoots and tendrils:

- Early symptoms appear as water-soaked, shiny depressions on which the dense downy mildew growth appears.
- Young shoots usually are stunted and become thickened and distorted.
- Severely infected shoots and tendrils usually die.

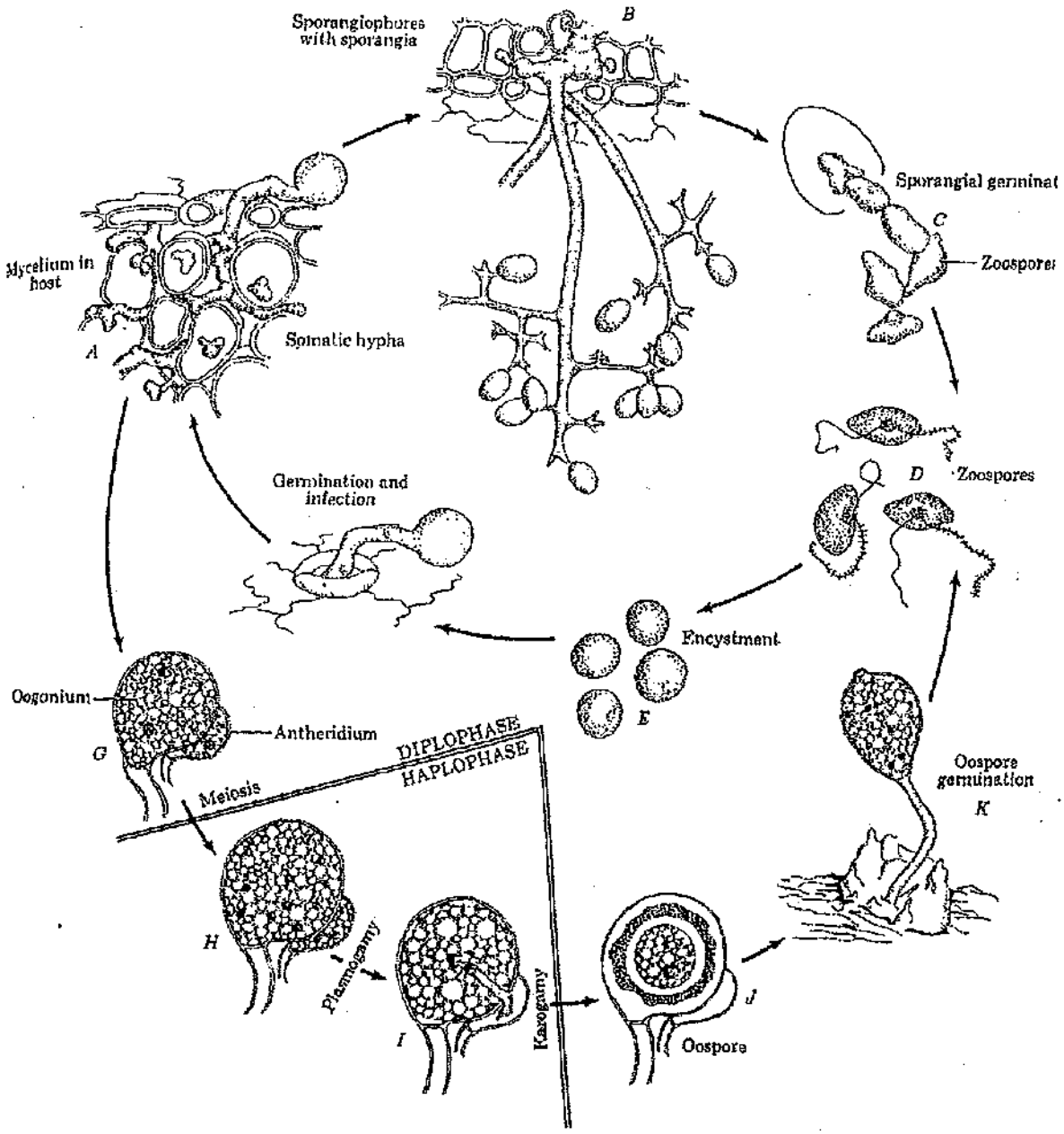
Disease cycle:

- The overwintering oospore germinates in the spring and produces a sporangium. Sporangia are spread by wind and splashing rain.
- When plant parts are covered with a film of moisture, the sporangia release small swimming spores, called zoospores.
- Zoospores, germinate by producing a germ tube that enters the leaf through stomata on the lower leaf surface.
- The optimum temperature for disease development is 18 to 25 C.
- Once inside the plant, the fungus grows and spreads through tissues.
- Infections are usually visible as lesions in about 7-12 days.
- At night during periods of high humidity and temperatures above 13 degrees C, the fungus grows out through the stomata of infected tissue and produces microscopic, branched, tree-like sporangiophores on the lower leaf surface.

- The small sporangiophores and sporangia make up the downy mildew growth.
- Sporangia cause secondary infections and are spread by rain

Control:

- Select a planting site where vines are exposed to all-day sun, with good air circulation and soil drainage.
- Proper spacing and orientation of vines in the rows to maximize air movement
- Removal of dead leaves and berries from vines and the ground after leaf drop.
- Downy mildew can be effectively controlled by properly timed and effective fungicides.



Life cycle of *Plasmopara viticola*

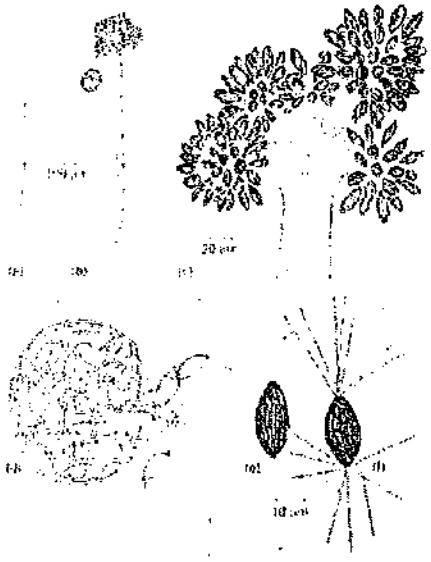
IV- Diseases caused by Zygomycota

Class: Zygomycetes
Order: Mucorales
Family: *Choanephoraceae*
Choanephora cucurbitarum

A- Choanephora rot

Symptoms: Fruits rot rapidly and white fungal mold appears on the infected area. With time, fruit look like a pin cushion with numerous small, black-headed pins stuck in it. Initially, the heads are white to brown but turn purplish-black within a few days. Affected flowers, pedicels, and immature fruit become water-soaked, and a soft, wet-rot develops. An entire fruit can rot in a 24 to 48 hour period. Symptoms usually begin on the blossom end of the fruit.

PERSISTENCE AND TRANSMISSION. The fungus overwinters as a saprophyte (living on dead plant tissue) and/or in a dormant spore form (such as a chlamydospore or zygospore). In spring, fungal spores are spread to squash flowers by wind and by insects such as bees and cucumber beetles. Infection occurs through the blossom, into the fruit and stem. Development of wet rot is favored by high relative humidity and excessive rainfall.



Choanephora cucurbitarum

Choanephora rot of squash by *Choanephora cucurbitarum*.

Other diseases by Zygomycetes:

a- Mucor rot of vegetables and fruits e.g. Guava, cucumber, grape, etc.
b- Rhizopus rot of vegetables and fruits e.g. Tomatoes, cantaloupe, mandarine, sweet potato, strawberry, etc.

V- Diseases caused by Ascomycota

Disease	Causal agent
Peach leaf curl	*Taphrina deformans*
Powdery mildews	Members of the Family Erysiphaceae
Apple scab	*Venturia Inaequalis*
Duch elm disease	*Ophiostoma ulmi*
Ergot disease	*Claviceps purpurea*
Canker of trees	*Nectria galligena*
Sclerotinia soft rot of vegetables	*Sclerotinia sclrotiorum*

1- Peach Leaf Curl

Caused by *Taphrina (Exoascus) deformans*

Class: Ascomycetes
Order: Exoascales
Family: Exoascaceae
Taphrina deformans

Taphrina has four unique features:

(A) The assimilative mycelium is dikaryotic.

(B) It produces an exposed layer of asci on the surface of the host leaf .

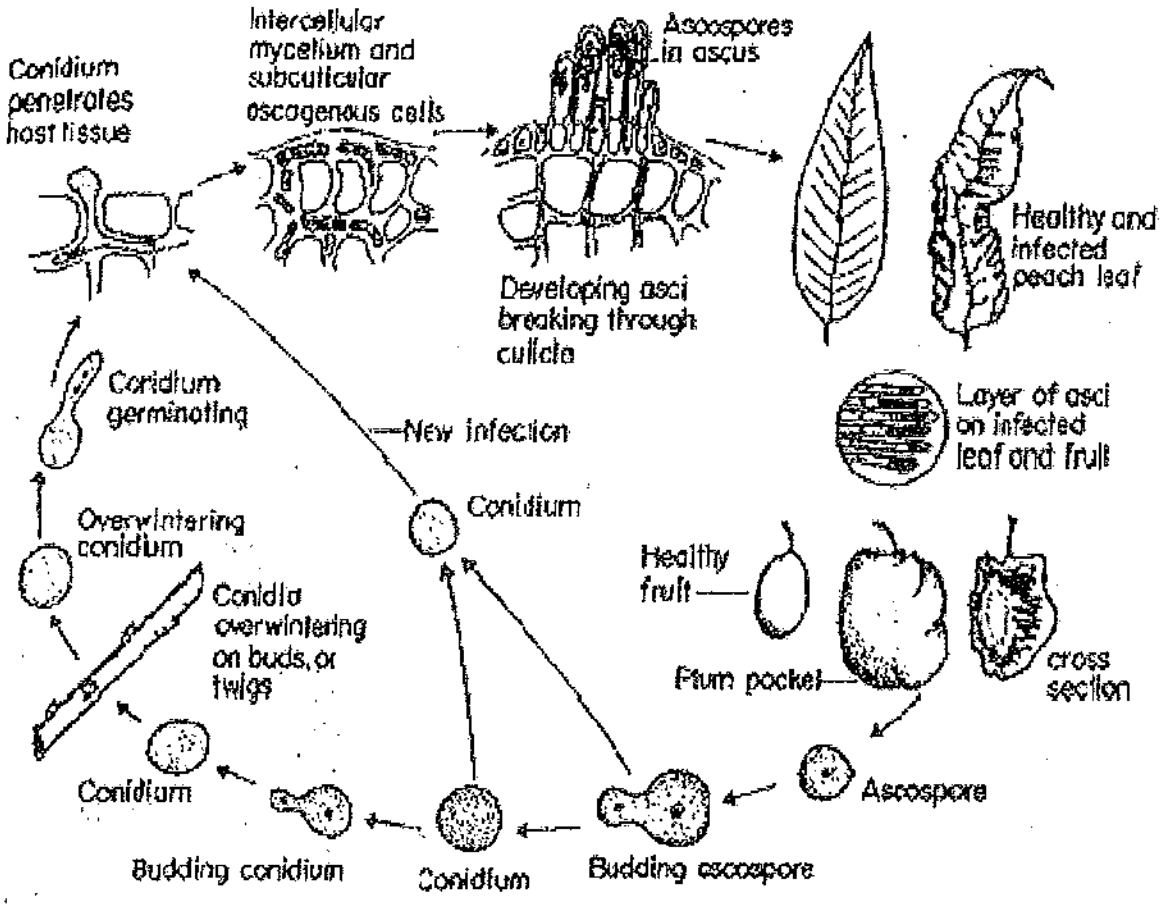
(C) Ascospores often bud in a yeast-like manner, even while still inside asci.

(D) When the asci open to release their spores, they tend to split across the tip.

(E) The anamorph of *Taphrina*, the phase in which it grows in culture, is single- celled budding yeast named *Lalaria*.

Symptoms:.

- Infected leaves are severely deformed and often display a variety of colors ranging from light green and yellow to shades of red and purple.
- The fungus causes the meristematic cells at leaf margins to proliferate quickly and randomly, which results in the leaves becoming variously wrinkled, puckered, and curled.
- As infected leaves mature, naked asci containing ascospores of the pathogen are produced on the surface giving them a dusty appearance, after which the leaves turn brown, shrivel, and drop from the tree.
- Many infected fruits drop early while others develop reddish to purple, wart-like deformities on the fruit surface.
- Infections on young peach leave occur at temperatures of 10-21 C.



Disease cycle of Peach leaf curl caused by *Taphrina deformans*

Control of peach leaf curl

1- Treat trees with a fungicide in late fall using:
   a- Copper ammonium complex
   b- 90 % tribasic copper sulfate
   c- Potassium resinate and potassium oleate
   d- Bordeaux Mixture
   e- Lime Sulfur
2- Select resistant Varieties.

2- Powdery mildews

- Caused by members of the Family Erysiphaceae of the Order Erysiphales, Class Pyrenomycetes, Phylum Ascomycota
- General characteristics of Powdery mildew fungi:

1- They are obligate, biotrophic parasites

2- They tend to grow superficially, or epiphytically, on plant surfaces producing whitish, powdery asexual structures (hyphae and conidia) on upper and lower leaf surfaces.

3- Few genera produce endophytic hyphae.

4- Infections can also occur on stems, flowers, or fruit.

5- Specialized absorption cells (haustoria) extend into the plant epidermal cells to obtain nutrients.

6- Conidia develop either singly or in chains on specialized conidiophores

7- Conidiophores arise from the epiphytic hyphae, or in the case of endophytic hyphae, the conidiophores emerge through stomata.

8- Tiny, dark sexual structures (ascomata) are produced later on infected shoots

9- Infection by these fungus is favored by high humidity but not by free water.

10- Individual species typically have a very narrow host range.

Powdery mildew fungi and their hosts

Fungal species	Hosts
Erysiphe graminis	Wheat, barley
E. cichoracearum	Cucurbits, *Sonchus,*
E. polygoni	Beans, peas
Leveillula taurica	artichoke
Sphaerotheca macularis	strawberry
Sphaerotheca pannosa	Rose, peach
Uncinula necator	Grape vine
Microsphaera alphitoides	Oak, lilac
Podsphaera leucotricha	apple
Phyllactinea corylea	Oak, elm, tulip,
Oidium mangiferae	mango

Types of conidiophores of Powdery mildew fungi

1- The oidium type: short stipe of one or more cells, conidiogenous cell and a chain of maturing conidia, e.g. ***Erysiphe, Uncinula, Microsphaera, Podosphaera and Sphaerotheca***

2- The ovulariopsis type: with clavate conidia e.g. ***Phyllactinia.***

3- The oidiopsis type: conidiophores branched arising from stomata. e.g. ***Leveillula***

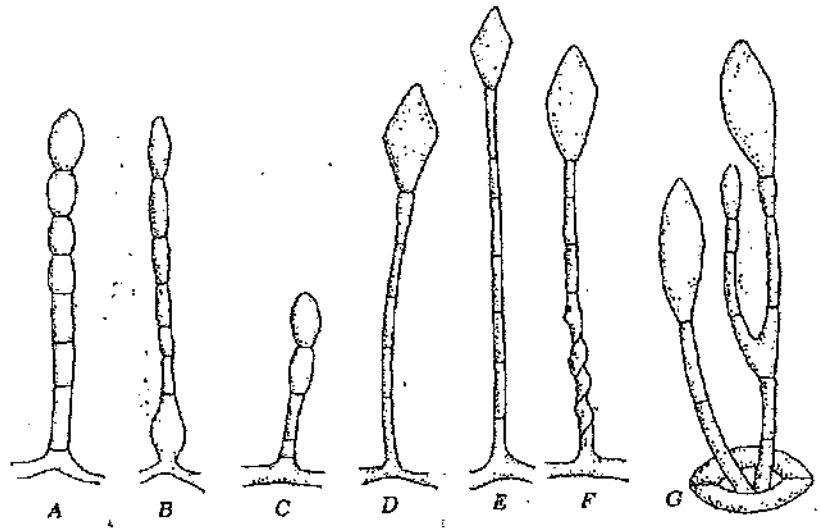


Figure 15-6 Conidiophore types. (A) *Erysiphe cichoracearum*. (B) *Erysiphe graminis*. (C) *Erysiphe polygoni*. (D) *Phyllactinia suffulta*. (E) *Phyllactinia rigida*. (F) *Phyllactinia subspiralis*. (G) *Leveillula taurica*. (Redrawn from Blumer. 1933. By R. W. Scheetz.)

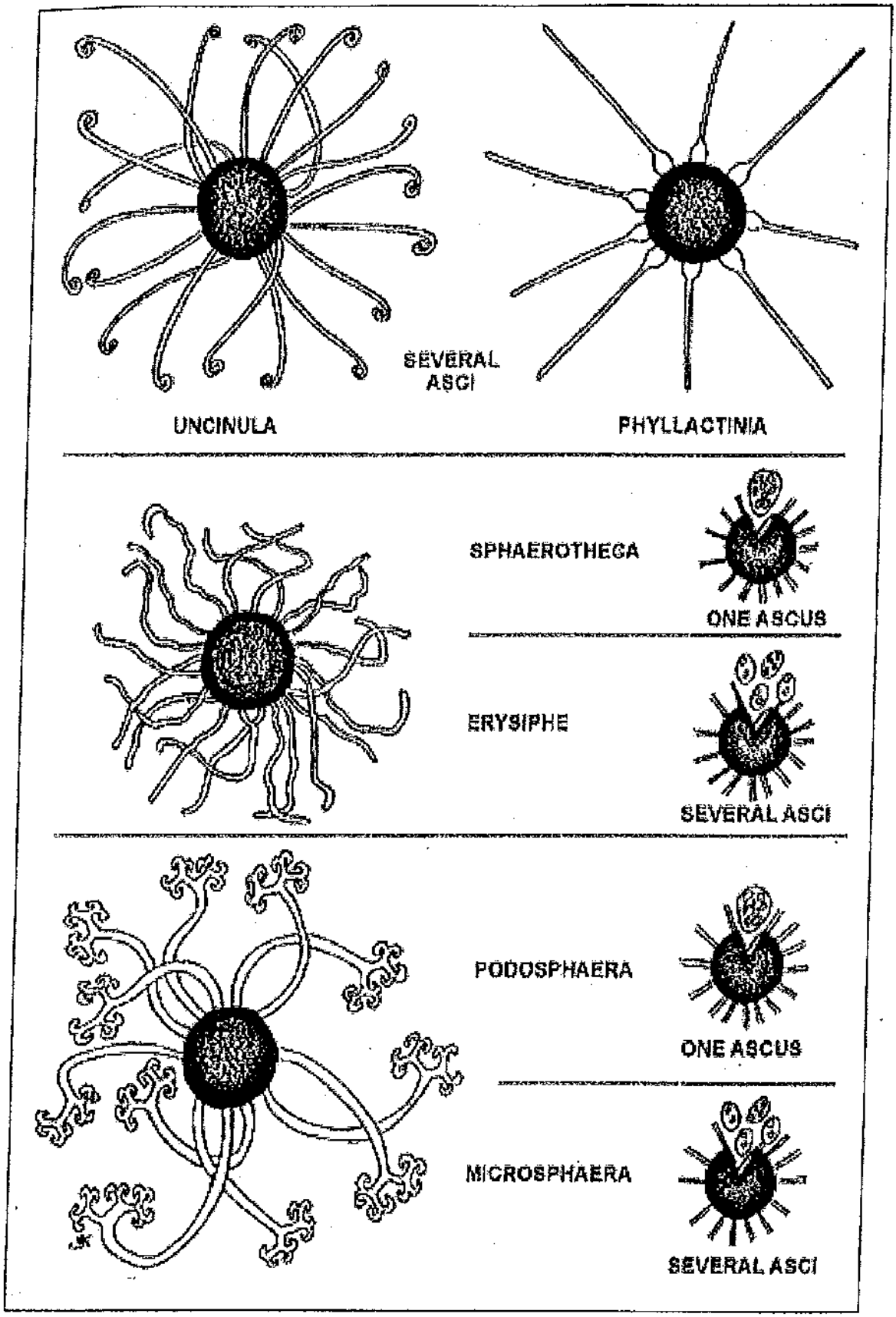
Key to Genera of Powdery Mildew Fungi (based on ascomatal appendages and number of asci)

- Appendages coiled or hooked at tip ----------*Uncinula*
- Appendages simple and straight with bulb-like base----*Phyllactinia*

- Appendages simple or irregularly branched, often enterwined
  - # Cleistothecium contains a single ascus ----------*Sphaerotheca*
  - # Cleistothecium contains several asci ----------*Erysiphe*

  (endophytic mycellum-----*Leveillula*)

- Appendages branching dichotomously at tip, Cleistothecium contains a single ascus ----------*Podosphaera*
  - # Cleistothecium contains several asci ----------*Microsphaera*



Types of Ascomata of powdery mildew fungi

Disease cycle of powdery mildew fungi

1- The fungus can overwinter as dormant mycelium or resting ascospores (in dark cleistothecia) on infected stems or leaves.

2- In spring, dormant mycelia becomes active producing asexual conidia while the cleistothecia produce ascospores.

3- Conidia and ascospores are then carried by wind to susceptible young plant parts.

4- when a conidium germinates on host leaf surface it produces a germ tube which gives an appressorium.

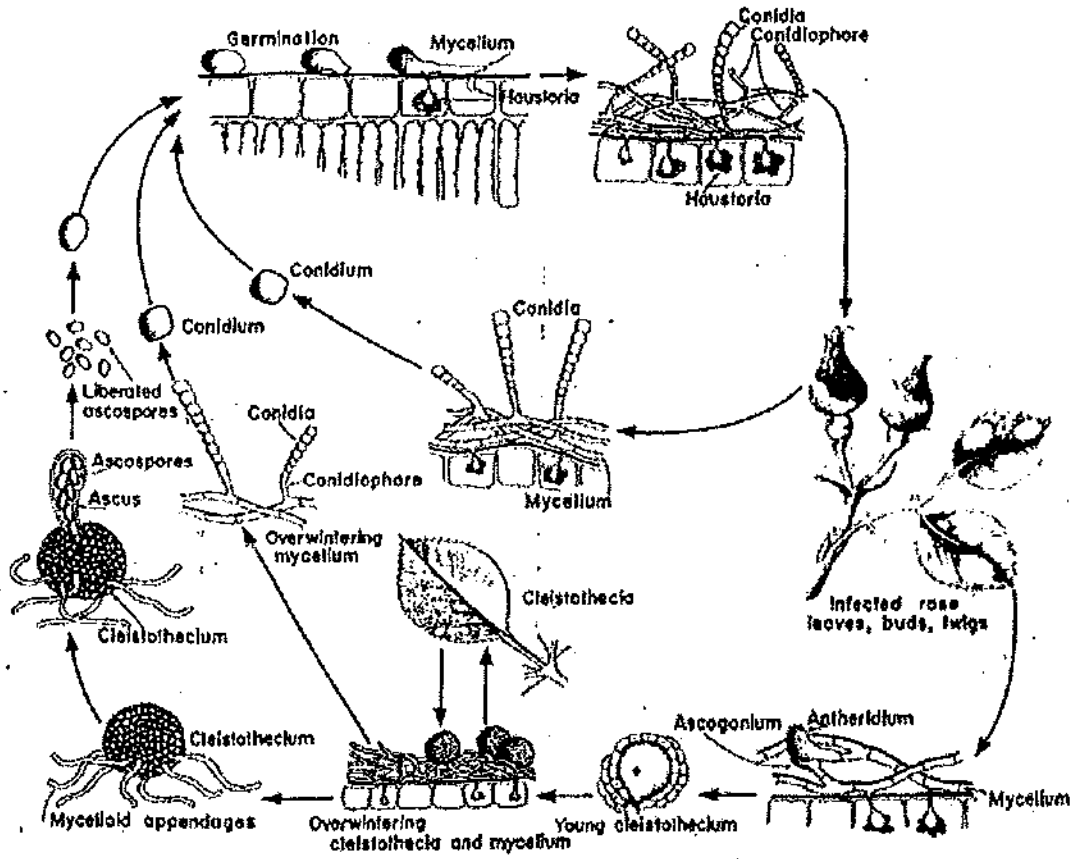
5- From the appressorium a penetration hypha grows through the cuticle and cell wall then swells out in the epidermal cell to form a haustorium (globular or finger-like in shape). The haustorium is a fungus structure that takes the nutrients from the plant

6-Further germ tubes, appressoria and haustoria are produced and the fungus grows out radially from the point of inoculums.

7- About 4 days after inoculation, sporulation starts extending outwards giving conidial chains

8- Dark pin point cleistothecia develop superficially in the same mycelial felt.

9- Cleistothecia overwinter and provide inoculums for infection of next season's crop.



Disease cycle of powdery mildew of rose by *Sphaerotheca pannosa*

Factors influencing disease development

1- Moisture: Powdery mildews are most severe in dry weather. germination of conidia is poor in free water. Spore maturation and release usually occurs during the day when relative humidity is low, at night an increase in relative humidity favors spore germination and penetration of the fungus.

2- Temperature: 11-28 C is favorable for infection. Cool damp nights and warm sunny days favor the development of Powdery Mildew.

3- Light: Higher incidence of powdery mildew on shaded than on exposed leaves. Effects of light include increased conidial germination, negative phototro-pism of germ tubes to white light (+ve to green).

4- Soil fertility: mineral nutrition (K, N, P) affects susceptibility. K- deficiency increases susceptibility.

5- Others: Closely planted gardens with some air movement are ideal conditions for spread of this disease.

Symptoms of powdery mildews:

- Slightly raised blister like areas on the upper leaf surfaces.
- Later, the young expanding leaves become twisted, distorted and covered with a white powdery mass of mycelium and spores.
- Young peduncles, sepals, petals and stems may also show distortion while growing tips and buds may be killed.
- Infected older leaves and stems may remain symptomless

Control of powdery mildew

1- Separation of new plantings from old ones.

2- Application of crop rotation of at least 1 year.

3- Control of weeds especially those related to host plants.

4- Fungicide sprays; e.g. with sulfur, karathane (0.1%), benlate (0.1%), calixin. Seed treatment with bayleton (0.1- 0.2%) or its spray on leaves (200- 500 ug /ml)

5- Breeding of resistant varieties:

3- Apple scab by *Venturia inaequalis*

Ascomycota
Dothideomycetes
Pleosporales
Venturiaceae
Venturia inaequalis

Symptoms:

Dull black or grey-brown lesions on the surface of tree leaves, buds or fruits.
Lesions may also appear less frequently on the woody tissues of the tree.
The disease rarely kills its host, but can significantly reduce fruit yields and fruit quality.
Affected fruits are less marketable due to the presence of the black fungal lesions.

Life cycle

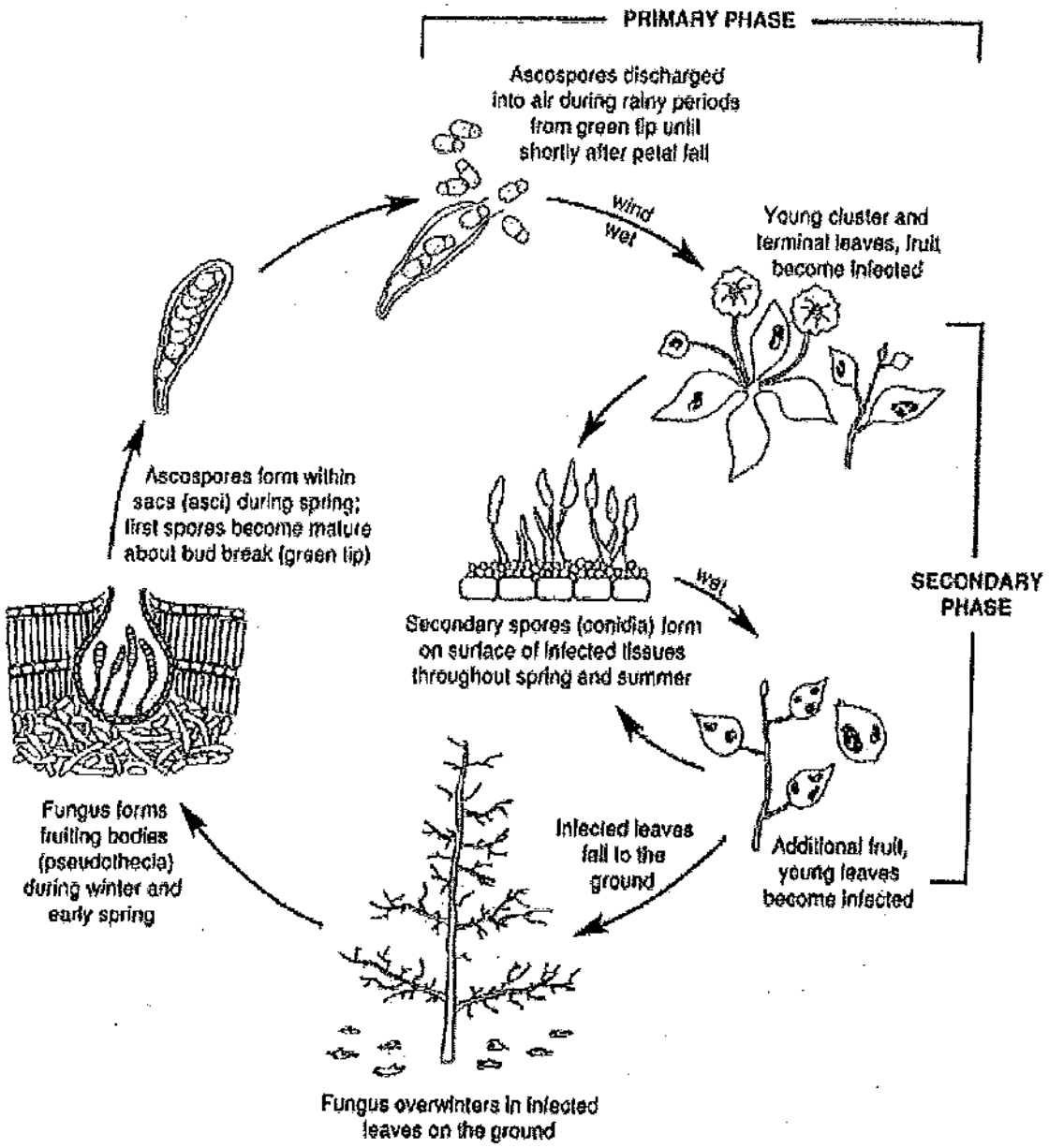
1. The infection cycle begins in the springtime, when suitable temperatures and moisture promote the release of *V. inaequalis* ascospores from leaf litter around the base of previously infected trees.
2. These spores rise into the air and land on the surface of a susceptible tree, where they germinate and form a germ tube that can directly penetrate the plant's waxy cuticle.
3. A fungal mycelium forms between the cuticle and underlying epidermal tissue, starting as a yellow spot that grows and ruptures to reveal a black lesion bearing the asexually as the conidia are released and germinate on fresh areas of the host tree, which in turn produce another generation of conidial spores.
4. This cycle of secondary infections continues throughout the summer, until the leaves and fruit fall from the tree at the onset of winter.
5. Over the winter, *V. inaequalis* undergoes sexual reproduction in the leaf litter around the base of the tree, producing a new generation of ascospores that are released the following spring.
6. Scab lesions located on the woody tissues may also overwinter in place, but will not undergo a sexual reproduction cycle; these lesions can still produce infective conidial spores in the spring.

Control:

a- Resistant cultivars: Breeding programs to develop high quality disease-resistant apple cultivars
b- Sanitation: Prevention of pseudothcial formation in overwintering apple leaves would probably eliminate scab as a serious threat to apple production. Leaf pickup and destruction in late autumn can be employed. Applications of 5% urea to foliage in autumn can hasten leaf decomposition, thus reducing formation of pseudothecia.

c- Chemical treatment: Protectant fungicides prevent the spores from germinating or penetrating leaf tissue. Postinfection fungicides control the scab fungus inside leaves and fruit. These chemicals can penetrate plant tissues to eliminate or inhibit lesion development. Several fungicides are available for controlling apple and pear scab. These include fixed copper, Bordeaux mixtures, copper soaps (copper octanoate), sulfur, mineral or neem oils, and myclobutanil. All these products except myclobutanil are considered organically acceptable

APPLE SCAB DISEASE CYCLE



4- Dutch elm disease (DED) by *Ophiostoma ulmi*

Fungi
Pezizomycotina
Sordariomycetes
Ophiostomatales
Ophiostomataceae
Ophiostoma ulmi

Symptoms:

Dutch elm disease results in the blockage of the water-conducting tissue within the tree.

Initial symptoms include discoloration and wilting of foliage.

This insect (*Scolytus scolytus)* feeds primarily on small branches high in the tree crown.

Foliage on diseased branches turns yellow. .

Wilt symptoms continue to progress on other branches in the tree crown over successive weeks or months.

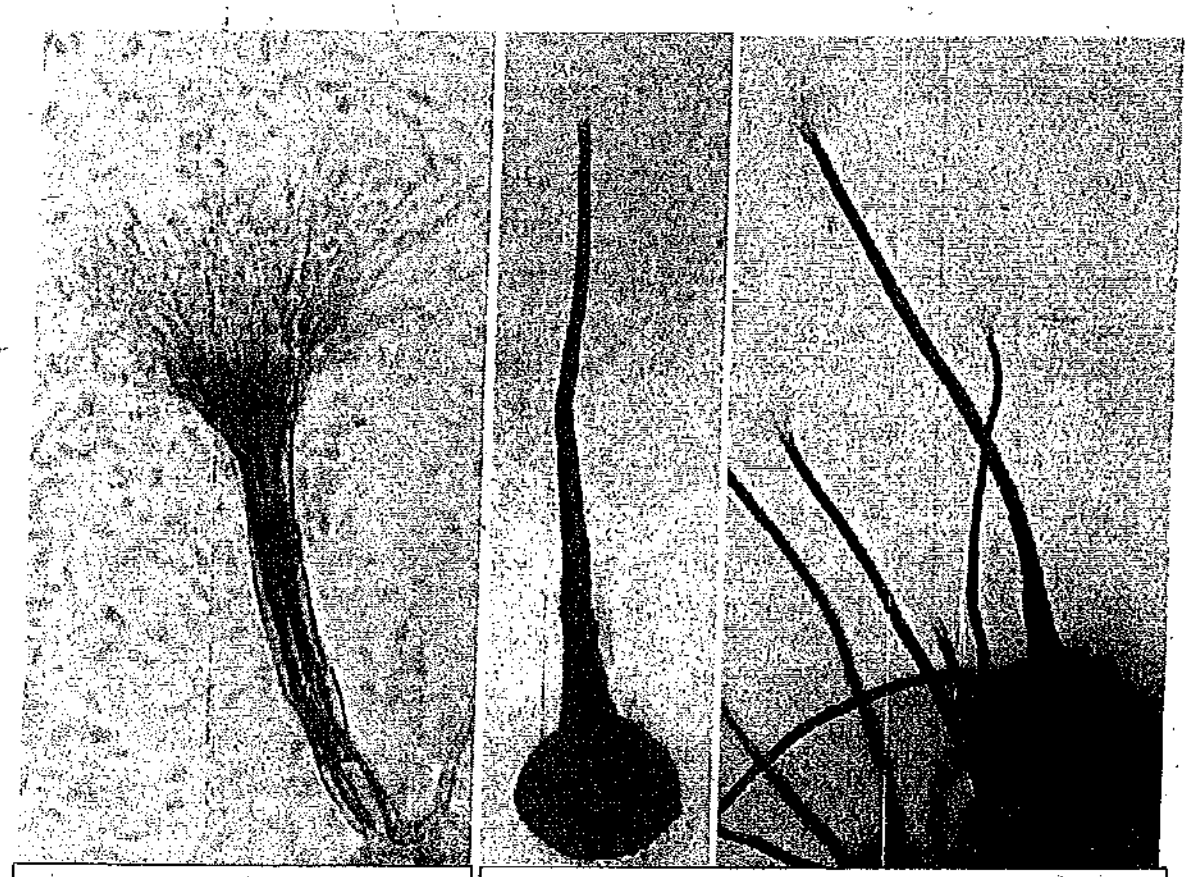
Foliage throughout the crown wilts and the tree dies.

Another diagnostic feature is the formation of brown streaks in infected sapwood.

This is common in trees where infections started by beetle transmission.

Discoloration may occur in the main trunk on trees rapidly killed by root graft infection.

Branches infected with the fungus typically have long brownish red streaks running the length of a branch section.



Graphium state of *O. ulmi*

Ascoma of Ophiostoma ulmi

Control:

Dutch elm disease control involves two different but related programs: (1) community-wide sanitation programs designed to reduce the level of elm bark beetles (principal carriers of the Dutch elm disease fungus); and (2) prevention of the spread of the disease through natural root grafts from infected trees to adjacent healthy trees.

Insecticides: Dursban insecticide spray of tree bases as part of their regular DED control program

Sanitation: destruction of all dead or dying elm wood present in the community.

The only way to prevent transmission through the roots is to create a barrier between diseased and healthy trees by severing or killing those roots between the trees

Chemical Treatment: Systemic fungicides (Arbotect) can be injected into the trunk or root-collar of the affected tree

Therapeutic tree injection is generally only effective where less than 5 percent of the crown of the tree shows symptoms.

Protective Treatment of Healthy Elms: The most effective chemical currently available is Arbotect.

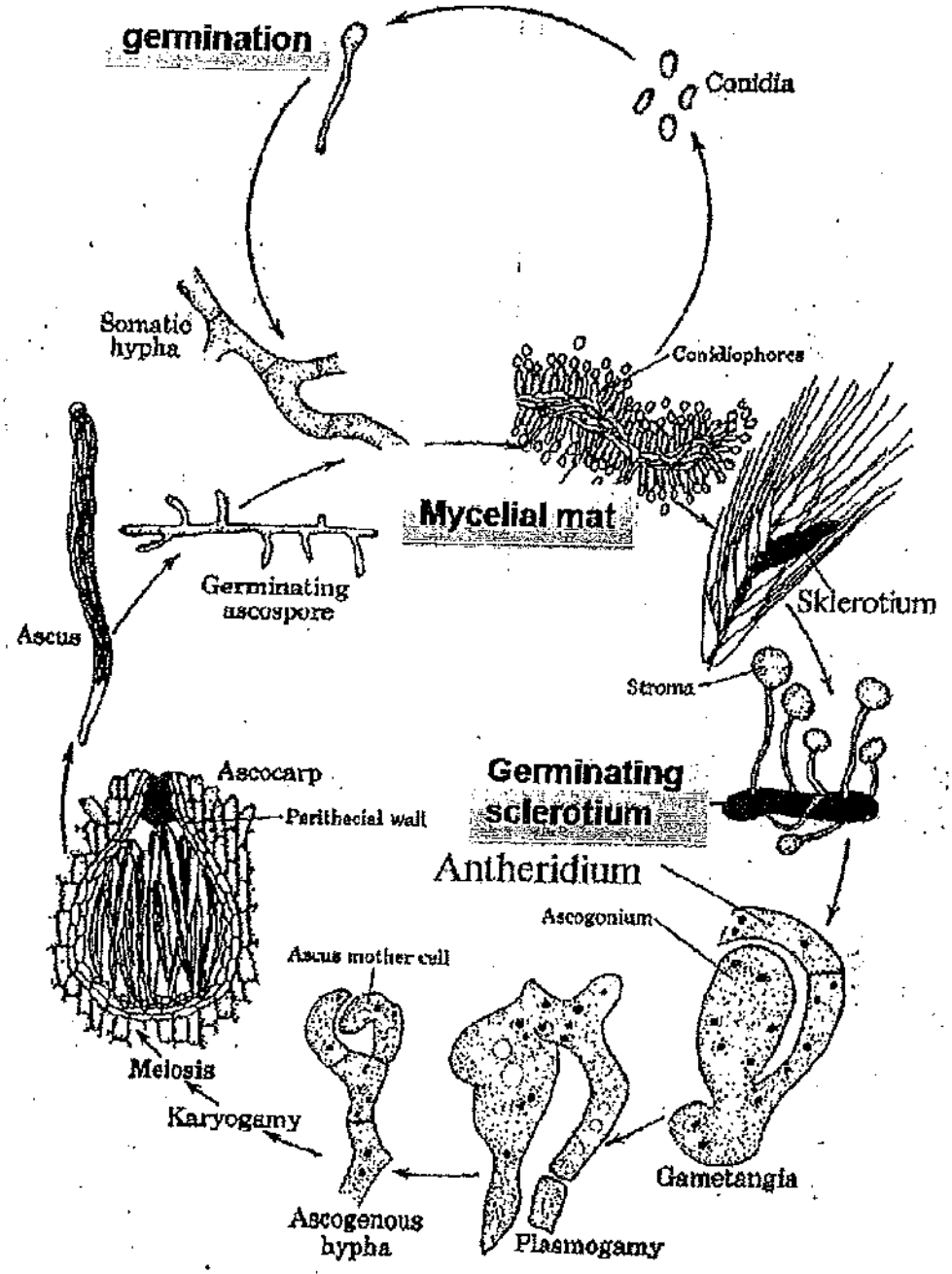
5- Ergot Disease of cereals by *Claviceps purpurea*

Symptoms and Signs

- Dark purple to black sclerotia (ergot bodies) found replacing the grain in the heads of cereals and grasses just prior to harvest.
- The ergot bodies consist of a mass of vegetative strands of the fungus. The interior of the sclerotia is white or tannish-white.
- In some grains, ergot bodies are larger than the normal grain kernels, while in other grains, such as wheat, grain kernels and the ergot bodies may be similar in size .
- Prior to development of the sclerotia bodies, the fungus develops a honey dew stage in the open floret.
- The "honey dew" consists of sticky, yellowish, sugary excretions of the fungus.

- Disease Cycle of *Claviceps purpurea*
- Sclerotia produced in small grain fields or grassy areas fall to the ground and survive on the surface of the soil.
- In the spring and early summer, the sclerotia germinate to produce tiny mushroom-like bodies (stroma) approximately the size of a pin .
- Spores (ascospores) formed by a sexual process in these bodies are shot into the air, and wind currents may carry them to grain heads.
- The first infections are from these wind-borne ascospores which invade the embryo of the developing kernel
- Soon a yellow-white, sweet, sticky fluid ("honey-dew") exudes from the infected flowers. The fluid contains a large number of asexually produced fungus conidia.

- Many species of insects visit the "honey-dew" and become contaminated with the fungus spores.
- These insects visit other grass flowers and spread the fungus.
- Spores may be transferred to other grain heads by rain-splash and direct contact, as well.
- Once the fungus becomes established in the florets, it grows throughout the embryos and replaces them, later producing the dark sclerotia.
- Many sclerotia fall to the ground before harvest and overwinter on the soil surface, serving as potential sources of spores the following year.



Life cycle of *Claviceps purpurea*

6- Nectria canker of hardwoods

Causal agent: *Nectria galligena* Bres.

Host: beech, white and yellow birch, red and sugar maple, poplar, and willow.

Symptoms:

A depressed or flattened area of bark near small wounds or at the base of dead twigs or branches is the first indication of the disease.

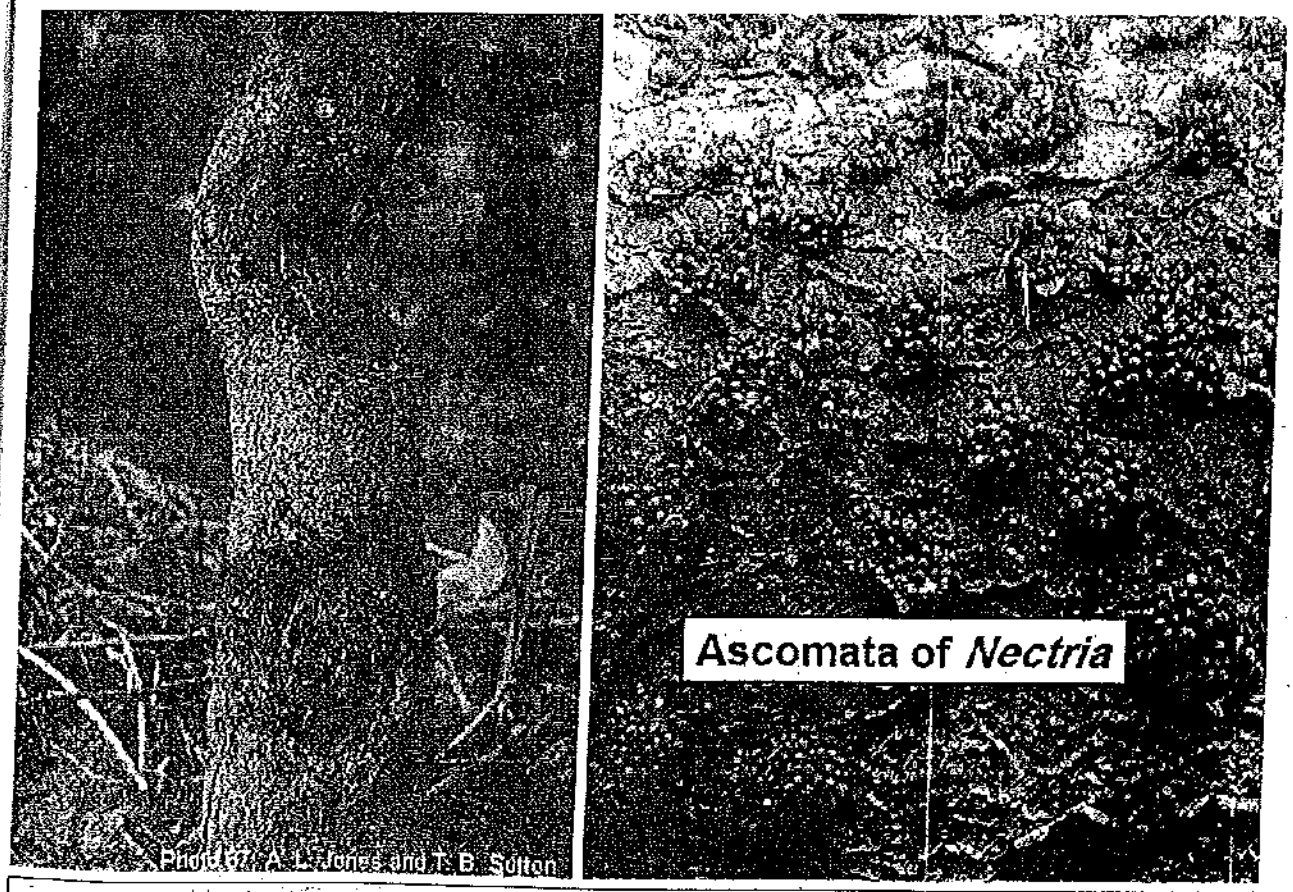
These areas may have a darker color and a water-soaked appearance.

The older and larger cankers may be concentric or target-shaped with callous ridges evident and the bark completely sloughed off or irregular in shape and lacking evidence of callous tissue.

The tiny, red, balloon-like fruiting bodies may be evident on the canker margin.

Cankered area is partially or completely covered by a roll of callus, (the tree is overcoming the infection.

The resulting deformation reduces the value of the tree



Nectria canker on apple tree caused by *Nectria galligena*

7- Soft rot Diseases by *Sclerotinia sclerotiorum*

Hosts: Cabbage, bean, citrus, celery, coriander, melon, squash, soybean, tomato, lettuce, carrots,, onions, peas, pumpkins and cucumber.

Symtoms:

Water-soaked spots on fruits, stems, leaves, or petioles which usually have an irregular shape.
These spots enlarge and a cottony mycelium covers the affected area.
The fungus spreads and the plant becomes a soft, slimy, water-soaked mass.
The cottony mycelium usually produces numerous sclerotia, black seed-like reproductive structures, a reliable diagnostic sign of *Sclerotinia* (these usually do not form until after host death).
In contrast to the water-soaked symptoms, the host may exhibit "dry" lesions on the stalk, stems, or branches, with an obvious definition between healthy and diseased tissues.
The lesions enlarge and girdle the plant part.

Distal portions of the plant become yellow, then brown, then die.

The girdled portion is often the base of the plant which causes the plant to die.

Sclerotia form within the stem pith cavities, fruit cavities, or between tissues (i.e., bark and xylem).

Apothecia of *Sclertiorum*

VI- Diseases caused by Basidiomycota

- These are the most structurally complex fungi, and include what we commonly call mushrooms, toadstools and bracket fungi. Rust and smut fungi are plant parasitic basidiomycetes.
- Basidiomycetes are characterized by a septate mycelium.
- The septa are highly complex and are pierced by a particular kind of pore termed a dolipore.
- The dolipore does not allow nuclei to pass through the septum.
- Consequently, hooked outgrowths called clamp connections are formed to ensure the proper distribution of nuclei as the hyphae grow.

The Basidiomycota have three classes:

a) Hymenomycetes

- Mushrooms and toadstools, composed of highly complex fruiting bodies (basidioma) and networks of dikaryotic mycelia.
- Basidioma have pores or gills, which are lined with basidia.
- Mushrooms as *Armillaria mellea* attack roots and trunks of many trees.
- Bracket fungi as *Ganoderma* grow on solid substrates such as tree trunks.

b) Uredinomycetes

- These are highly specialized plant pathogens which can only grow and reproduce on their host species or closely related species.
- Over 6000 members of the Uredinomycetes (commonly known as rusts) are important members of these sub-phyla.
- Wheat and bean rusts are economically important diseases.

Rust Diseases caused by Order: Uredinales

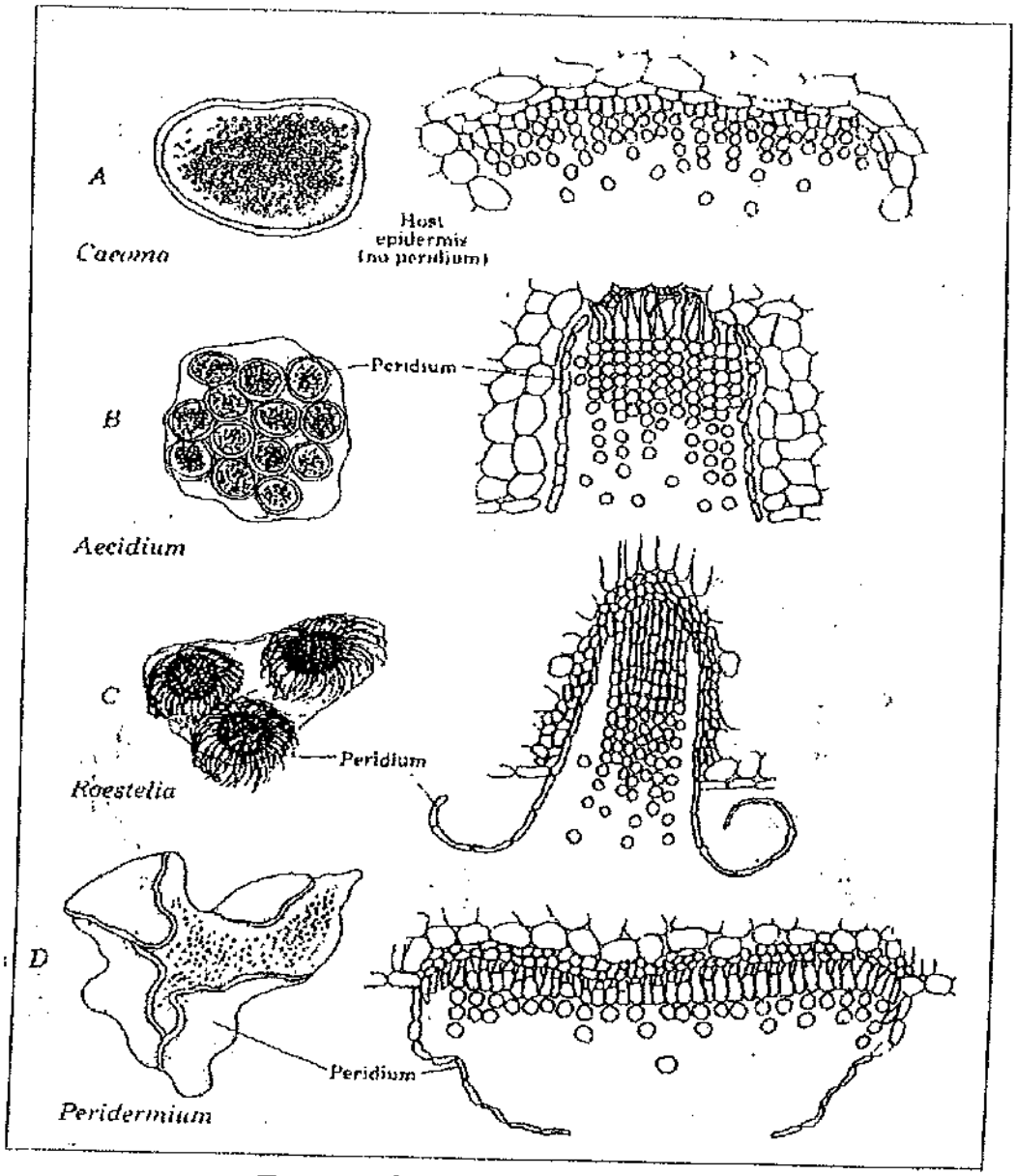
1- *Family Pucciniaceae*: (teliospores stalked)
   a. *Uromyces fabae, U. appendiculatus*
   b. *Puccinia graminis tritici*
   c. *Hemilea vastatrix*
   d. *Gymnosporangium junperi- virginianae*
   e. *Phragmidium mucronatum*

2- *Family Melampsoraceae*: (teliospores sessile)
   a. *Melampsora lini*
   b. *Cronartium ribicola*

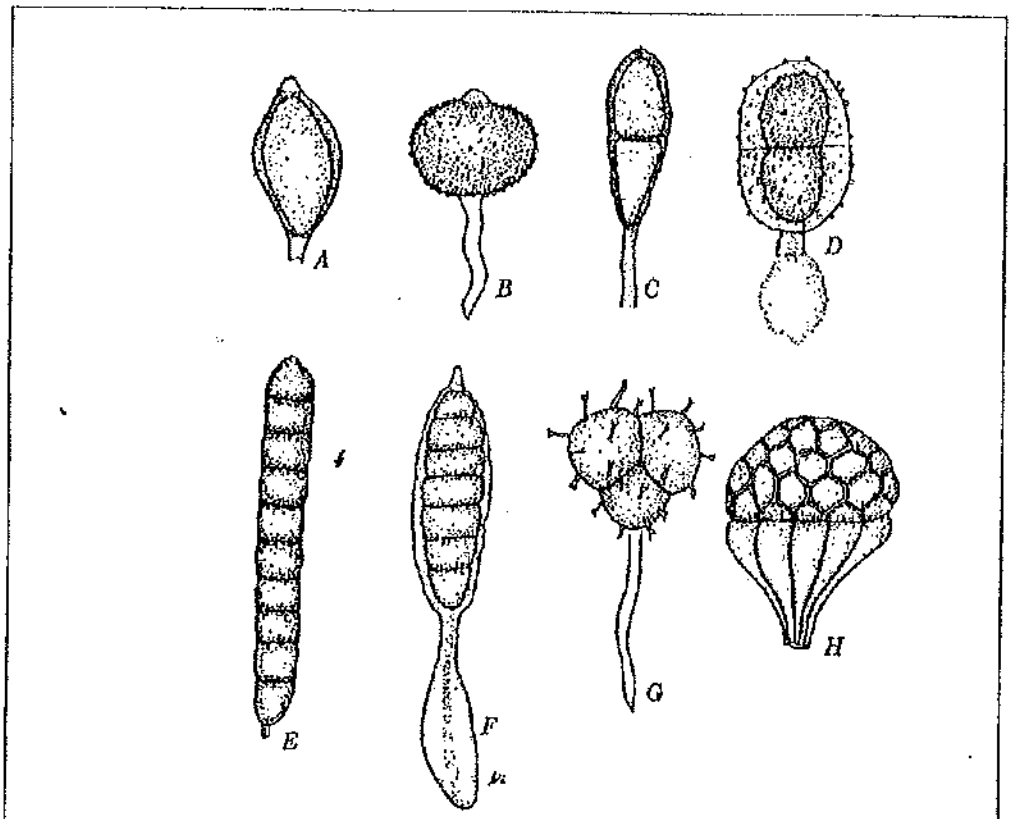
The life cycle of a typical rust species is among the most complex found anywhere in nature, consisting of five different spore stages (macrocyclic) on two plant hosts which are taxonomically entirely unrelated to each other.

<u>The macrocyclic life cycle is consisting of:</u>

A- Spermogonium or pycnial stage
B- Aecial stage
C- Uredial stage
D- Telial stage
E- Basidial stage (in soil)

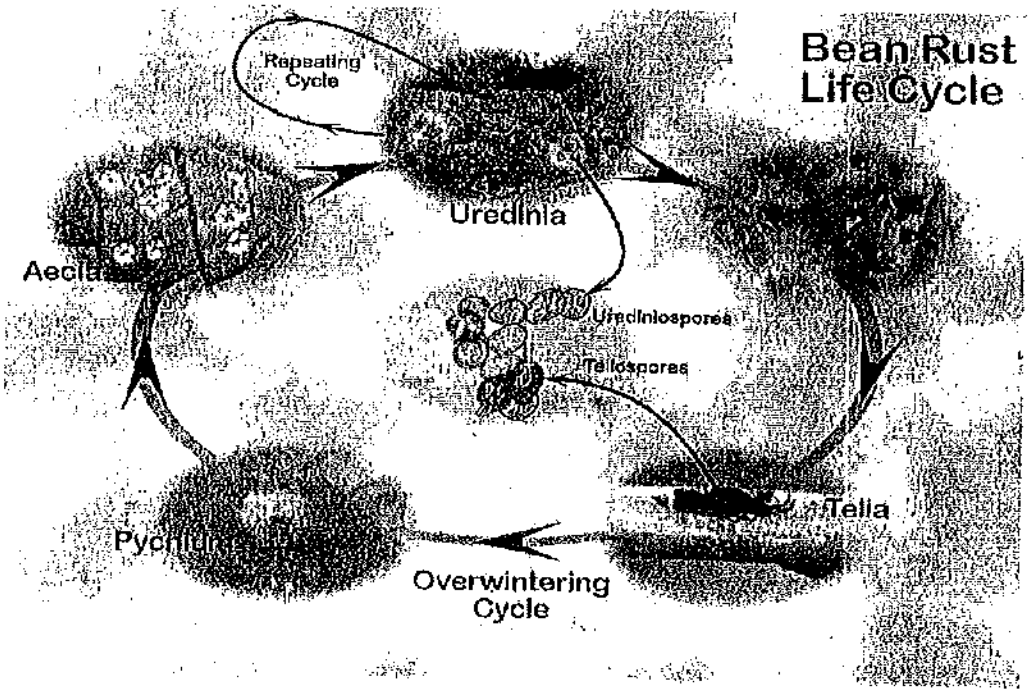


Types of aecia of rust fungi



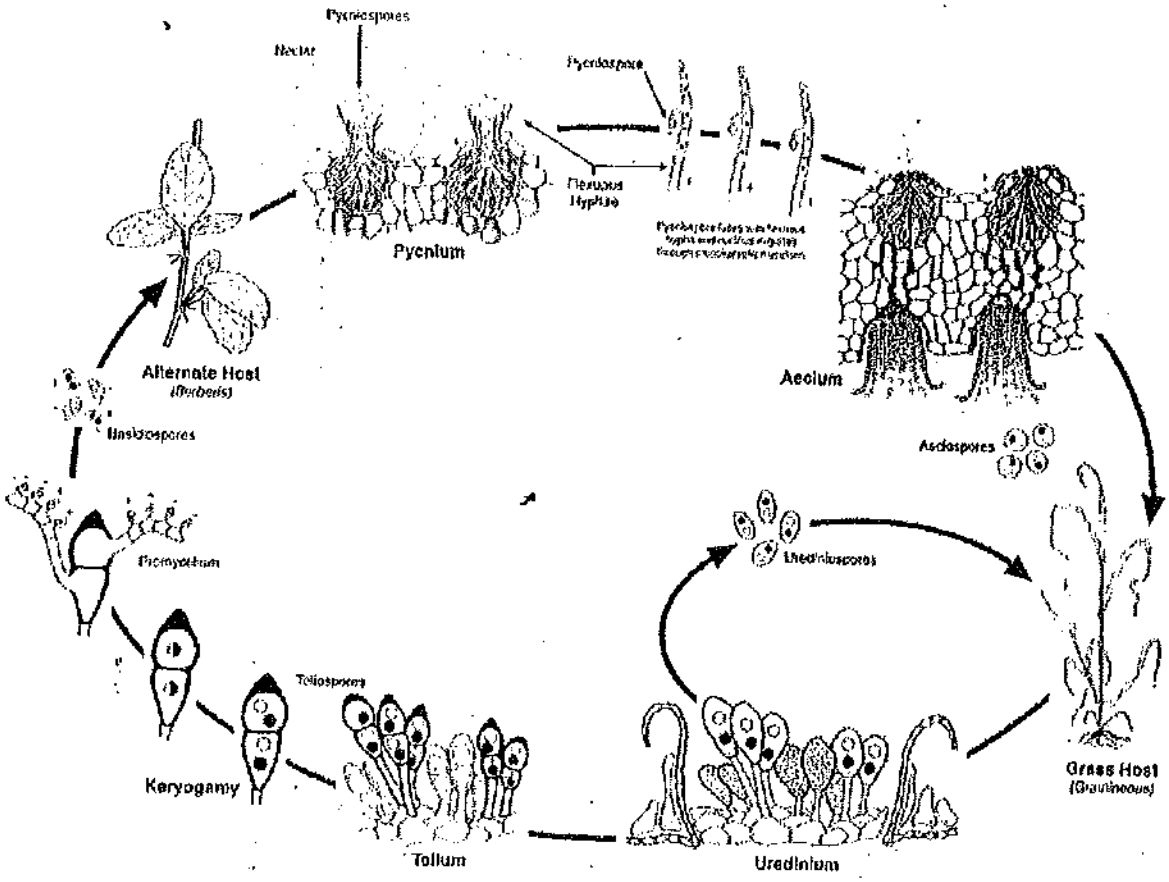
Various types of teliospores representing eight genera of rusts: (A) *Uromyces*, (B) *Pileolaria*, (C) *Puccinia*, (D) *Uropyxis*, (E) *Xenodochus*, (F) *Phragmidium*, (G) *Nyssospora*, (H) *Ravenelia*. [(B, D, E, H) Redrawn from Cummins by permission from *Manual of the Rusts of the United States and Canada*, by J. C. Arthur (1934). Purdue Research Foundation, Lafayette, IN.]

A- Rust of broad bean by *Uromyces fabae*



B- Black stem rust of wheat caused by *Puccinia graminis*

- Life cycle: (macrocyclic, 5 stages)
  - A- Spermogonium or pycnial stage: on upper srface of *Berberis vulgaris* leaves
  - B- Aecial stage :(on lower surface of *Berberis* leaves)
  - C- Uredial stage (early on wheat stem)
  - D- Telial stage (late on wheat stem)
  - E- Basidial stage (In soil)



Disease cycle of black stem rust of wheat caused by *Puccinia graminis tritici*

C- Yellow (stripe) rust of wheat by *Puccinia striiformis*
D- Orange rust on wheat leaves by *Puccinia recondite*
E- Rust of garlic by *Puccinia allii*
F- Peanut rust by *Puccinia arachidis* on the underside of leaves
G- Aple rust by *Gymnosporangium clavariaeforme*
H- Rust of rose by *Phragmidium mucronatum*
I- White pine rust by *Cronartium ribicola*
J- Rust of flax by *Melampsora lini*

c) Ustilagomycetes

They are commonly known as smuts, and over 1000 members of this class live in a similar manner to the rusts, as obligate biotrophic fungi – they can only grow on living plants.

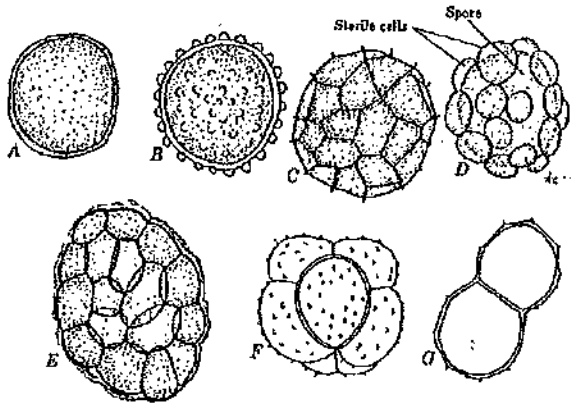
Maize Smut, caused by *Ustilago maydis* is an economically important disease

Smut Diseases caused by Order: Ustilaginales

1- *Family Ustilaginaceae*
Ustilago maydis, Ustilago tritici,
Ustilago hordei, Ustilago avenae,
Sphacelotheca sorghi, S. reliana
Tolyposporium ehrenbergii

2- *Family tilletiaceae*
Tilletia caris
Urocystis cepulae

3- *Family: Graphiolaceae*
Graphiola phoenicis



Modes of infection by smut fungi

1- Embryo infection:
- Loose smut of wheat by *U. nuda*

2- Seedling infection:
- Loose smut of oats by *U. avenae*
- Covered smut of barley by *U.hordei*
- Stripe smut of grasses by *U. striiformis*
- Dwarf bunt of wheat by *Tilletia contraversa*
- Onion smut by *Urocystis cepulae*

3- Shoot or local infection:
- Smut of anthers of *Melandrium album* by *U. violaceae.*
- Sugarcane smut by *U. scitaminae*
- Long smut of sorghum by *Tolyposporium ehrenbergii*
- Common smut of corn by *U. maydis*
- Rice bunt by *Tilletia barclayana*

Examples of smut diseases

1- Loose smut of wheat and barley by *U. nuda*

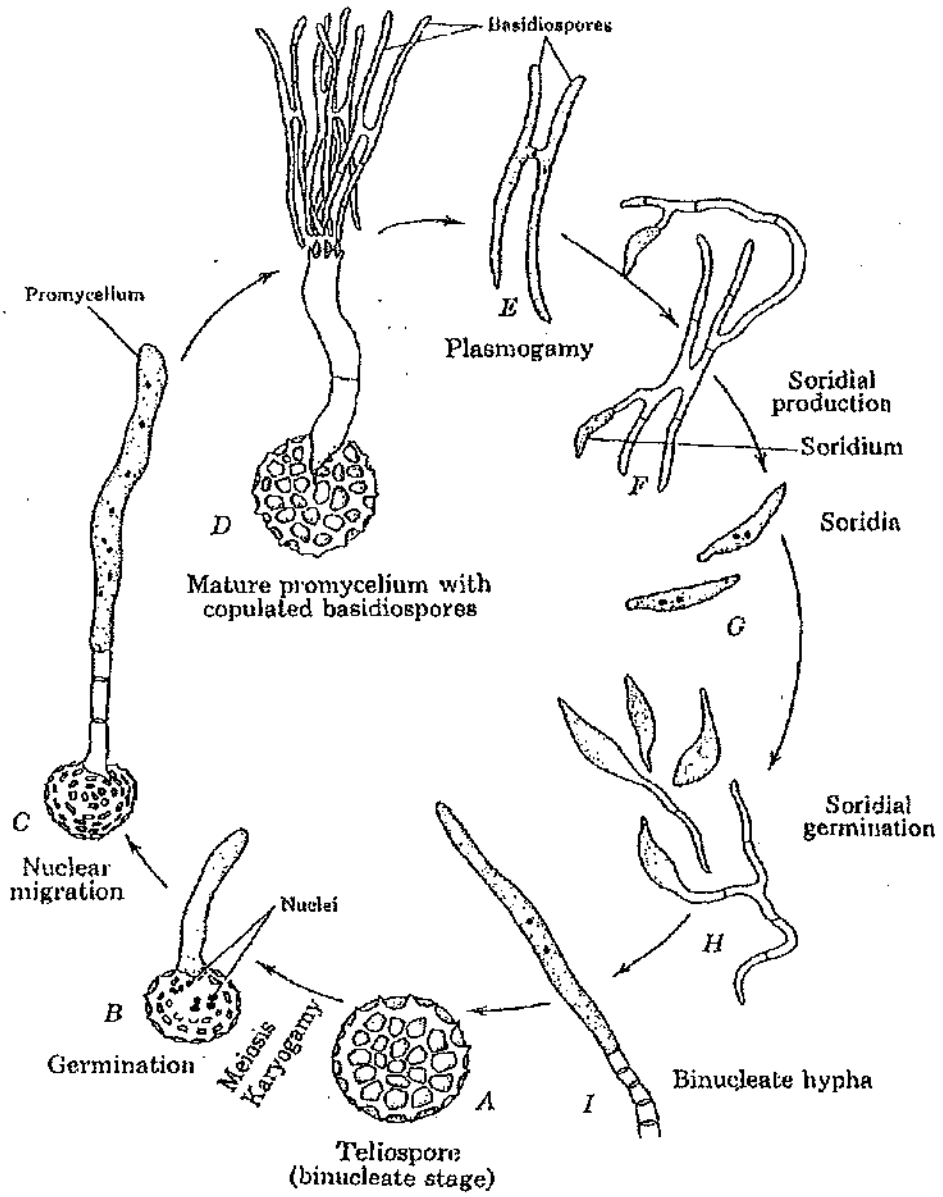
- Embryonic infection.
- It is not possible to determine whether a plant is diseased or not until the ears emerge when in infected plants the inflorescence is replaced by a mass of black, smut spores.
- Flag leaf may be infected in highly susceptible plants.
- Only glumes are affected in resistant plants
- Once spores are exposed they are blown by wind to flowers of healthy plants.
- Infection by *U. nuda* occurs through ovary walls, hyphae cross pericarp, enter the testa (intracellular), move towards the embryo (intercellular).
- Infected grains appear as healthy.
- When grains germinate, fungal mycelium becomes active, passes into the crown node of the seedling and is carried up during growth to the inflorescence primordea.
- Spore formation begins some weeks before the ears emerge and is complete at emergence.

2- Loose smut of oat:

- caused by *Ustilago avenae*
- Seedling infection.
- Spores are dispersed at flowering.
- On germination, the mycelium becomes established in glumes and pericarp.
- Embryo not invaded.
- When seeds are planted the dormant mycelium becomes active and invades the young seedlings.
- Subsequent development of the fungus is similar to that of *U. nuda*.

3- Bunt of wheat (stinking smut)

- caused by *Tilletia caries*. Seedling infection.
- Spores have an odor of bad fish (trimethylamine).
- All parts of the grain except the coat are replaced by smut spores.
- Infected grains (Bunt balls) are shorter and plumper than healthy.
- Broken bunt balls release millions of spores which contaminate healthy grains
- When contaminated grains are sown, spores on the grain coat germinate and the binucleate hyphae formed by fusion of sporedial cells infect the young coleoptile.
- Subsequent events are similar to those in U. nuda

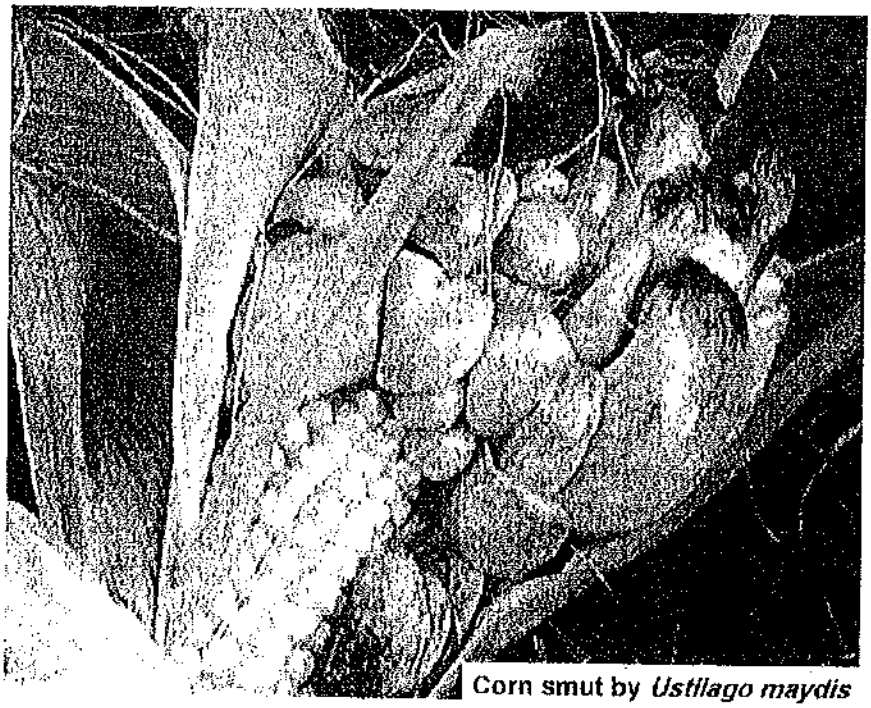


Life cycle of *Tilletia carie* cause of bunt of wheat

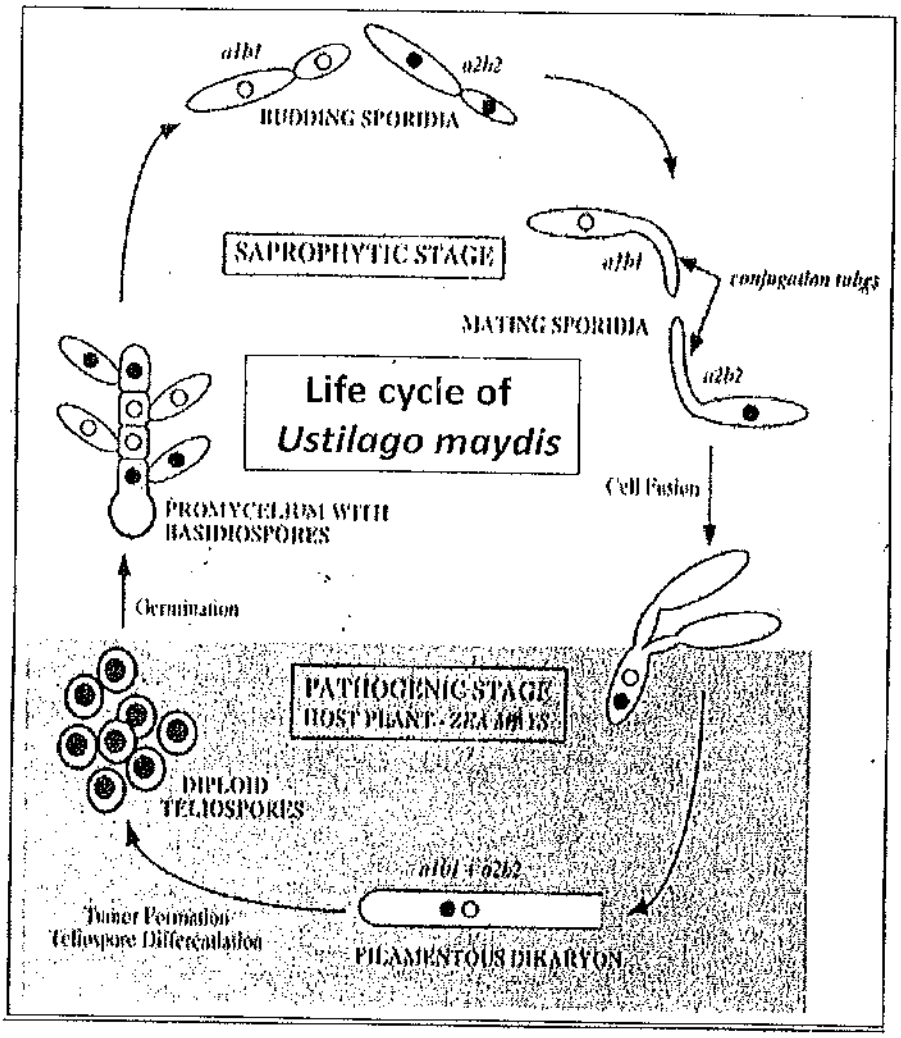
4- Sugarcane smut: Black, whiplike sorus arising from the terminal meristem of a stalk infected by *Ustilago scitaminea.*

5- - Onion smut by *Urocystis cepulae*

6- Common smut of corn by *Ustilago maydis*



Corn smut by *Ustilago maydis*



VII- Diseases caused by Deuteromycetes

1- Fusarium and Verticillium wilts

Fungus	Host
F. oxysporum f.sp. lycopersici	Tomato, potato, eggplant
F. oxysporum f.sp. vasinfectum	Cotton
F. oxysporum f.sp. conglutinans	Cabbage
F. oxysporum f.sp. Cubense	banana
F. oxysporum f.sp. betae	sugarbeet
Verticillium alboatrum (dark resting mycelium)	Tomato, alfalfa
Verticillium dahliae (microsclerotia)	*Potato, tomato,*
Verticillium nigrescens (chlamydospores)	Tomato

Mode of infection and survival:

- Wilt inducing forms of *Fusarium* and *Verticillium* enter their hosts through uninjured young roots or injured old roots.
- Plants infested by nematodes are severely attacked by wilt fungi.
- Fungi invade root cortex but do not damage it to a great extent. They become established in xylem vessels.
- Fungal enzymes disintegrate walls of xylem vessels.
- Fungal toxins are considered as a cause of wilt.
- Survival: by resting mycelium, chlamydospores or microsclerotia.

Disease symptoms:

- Lower leaf-petioles bend downwards (epinasty)
- Slight vein clearing and yellowing of the lower leaves.
- Chlorosis and death of leaves.
- Similar symptoms develop on younger leaves.
- During hot days, leaves wilt ,then recover at night.
- Wilt becomes permanent and plants die.
- Browning of vascular system.
- Water supply to leaves is plugged with fungal mycelium, conidia, tyloses and gums
- Some collapse of the vessels and disintegration of adjoining parenchyma.

4- Bean Anthracnose by *Colletotrichum lindemuthianum*

- Symptoms and Signs
- Seedlings grown from infected seeds often have dark brown to black sunken lesions on the cotyledons and stems.
- Severely infected cotyledons senesce prematurely, and growth of the plants is stunted. Diseased areas may girdle the stem and kill the seedling.
- Under moist conditions, small, pink masses of spores are produced in the lesions. Spores produced on cotyledon and stem lesions may spread to the leaves.
- Symptoms generally occur on the underside of the leaves as linear, dark brick-red to black lesions on the leaf veins. As the disease progresses, the discoloration appears on the upper leaf surface.
- On pods. Small, reddish brown to black lesions.
- Mature lesions are surrounded by a circular, reddish brown to black border.
- During moist periods, the interior of the lesion may exude pink masses of spores.
- Severely infected pods may shrivel, and the seeds they carry are usually infected.
- Infected seeds have brown to black sunken lesions.

5- Post harvest diseases

Disease	Causal agent
Spoilage of corn grains	*Aspergillus flavus,*
Rot on peanut kernels	*Aspergillus flavus*
Blue rot of apple fruits	*Penicillium expansum*
Blue rot of Citrus fruits	*Penicillium italicum*
Green rot of Citrus fruits	*Penicillium digitatum*
Cladosporium rot of corn	*Cladosporium cladosporioides*
Penicillium rot of corn	*Penicillium oxalicum*
Fusarium rot of corn	*Fusarium graminearum*

Physiology of fungi

For 4th year of B.Sc. students

Prepared by

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Physiology of fungi

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.

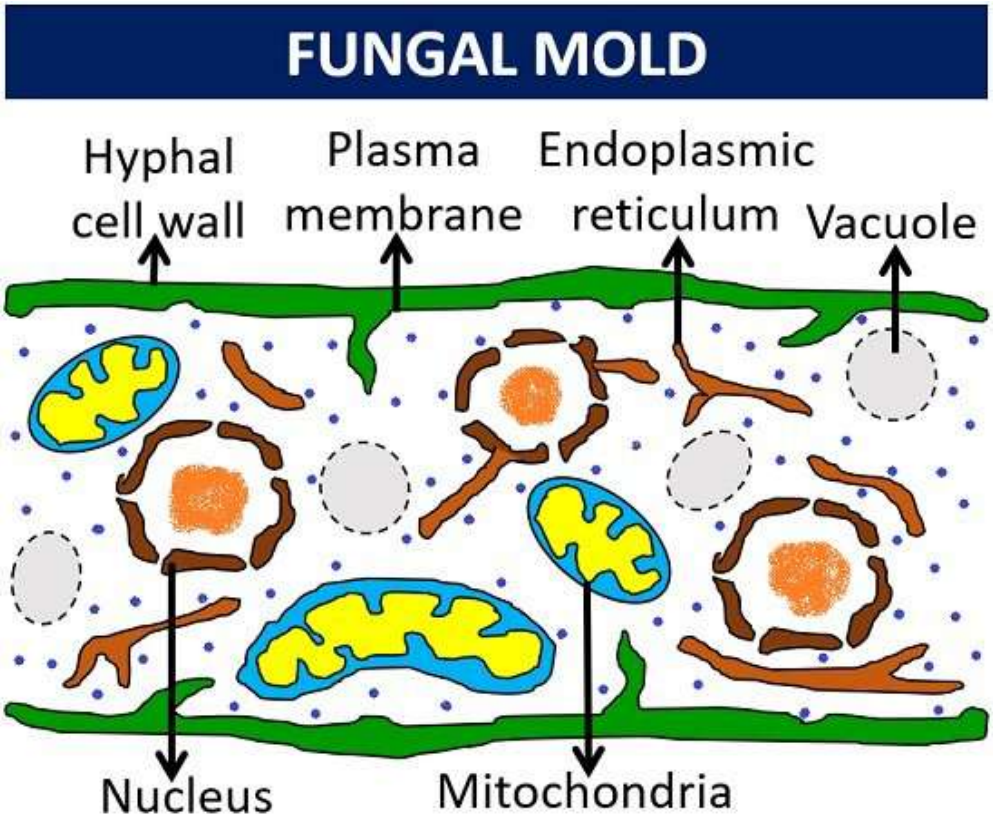
1. **Saprophytic fungi** – The fungi obtain their nutrition by feeding on dead organic substances such as *Aspergillus*, *Penicillium* and *Rhizopus*.
2. **Parasitic fungi**– The fungi obtain their nutrition by living on other living organisms (plants or animals) and absorb nutrients from their host 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

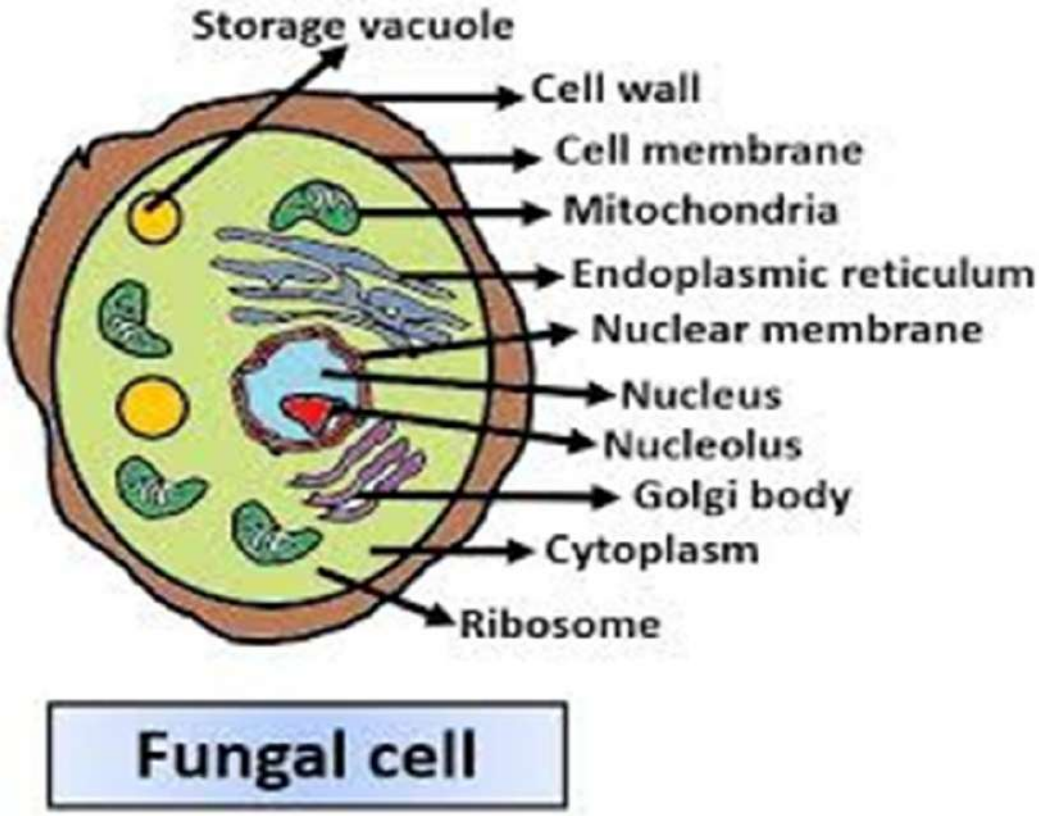
symbiotic association between algae and fungi. Here both algae and fungi are mutually benefited as fungi provide shelter for algae and in reverse algae synthesis carbohydrates for fungi. Mycorrhiza is the symbiotic association present between fungi and plants. Fungi improve nutrient uptake by plants,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

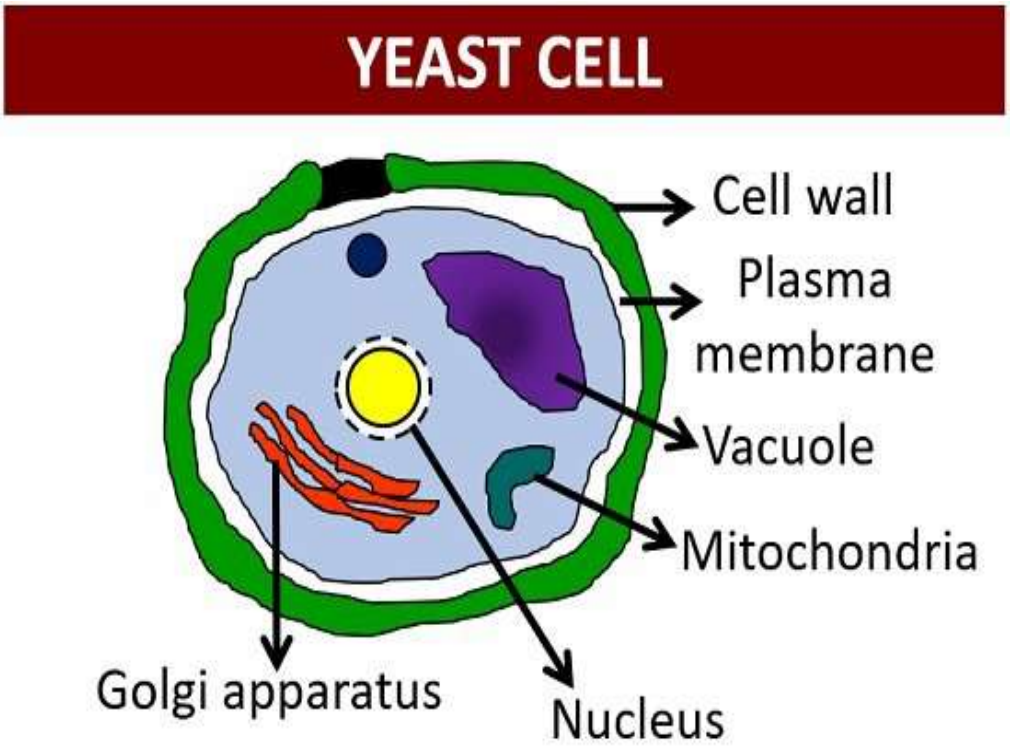
Based on spore formation, Kingdom fungi are classified into the following:

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

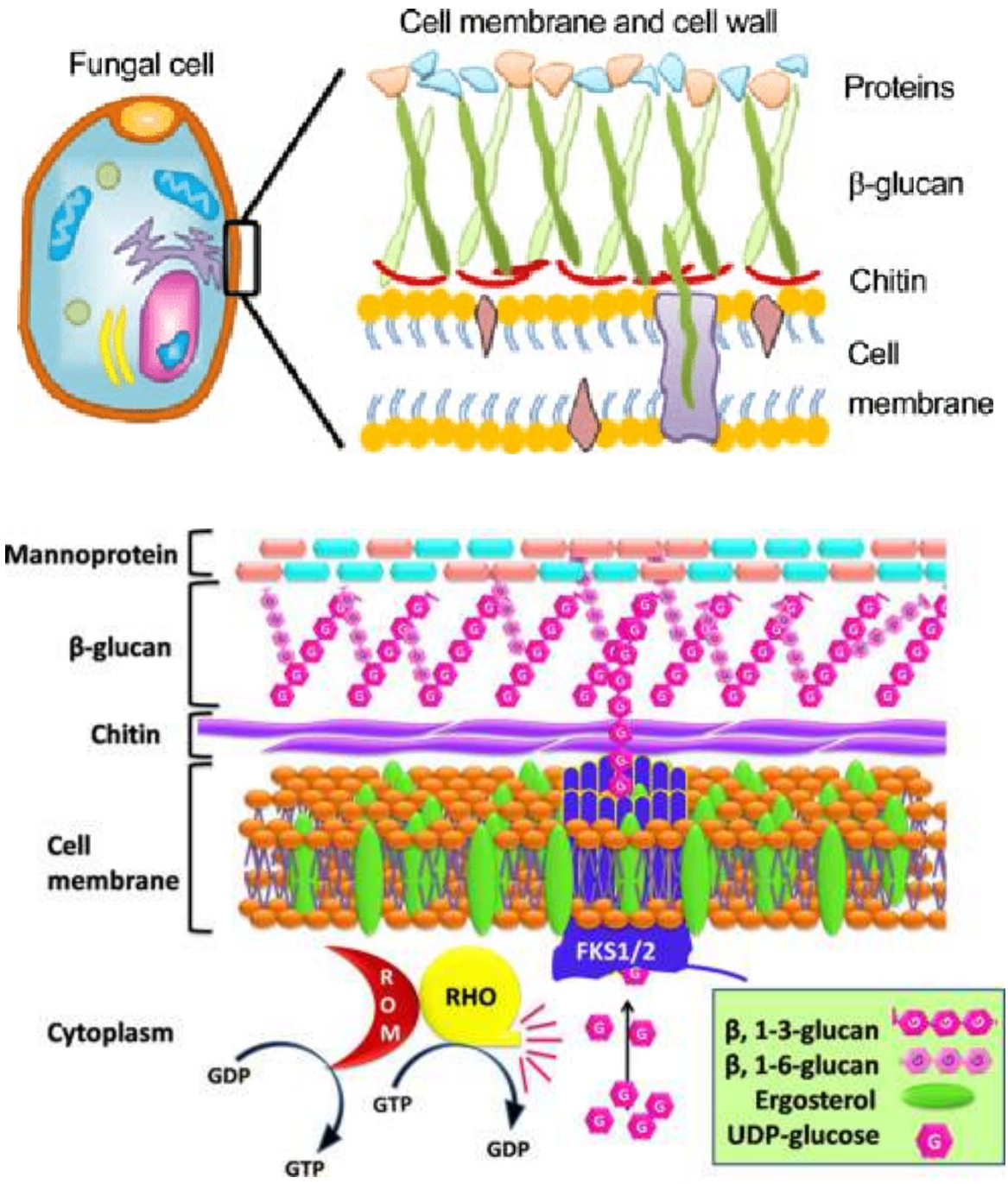

Storage vacuole
Cell wall
Cell membrane
Mitochondria
Endoplasmic reticulum
Nuclear membrane
Nucleus
Nucleolus
Golgi body
Cytoplasm
Ribosome
Fungal cell



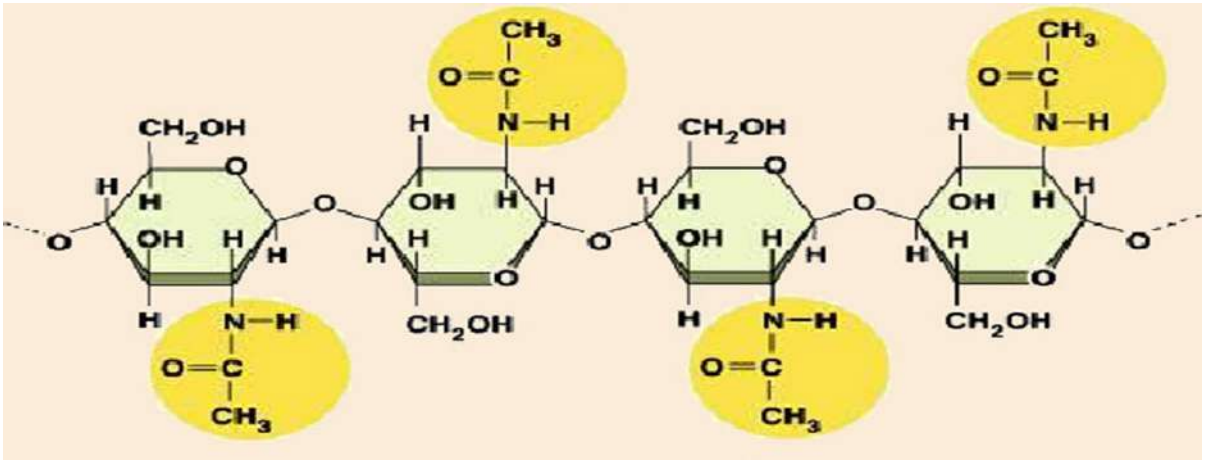
Unlike plant cells, fungal cells do not have chloroplasts or chlorophyll. Many fungi display bright colors arising from other cellular pigments, ranging from red to green to black. The poisonous *Amanita muscaria* (fly agaric) is recognizable by its bright red cap with white patches. Pigments in fungi 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.



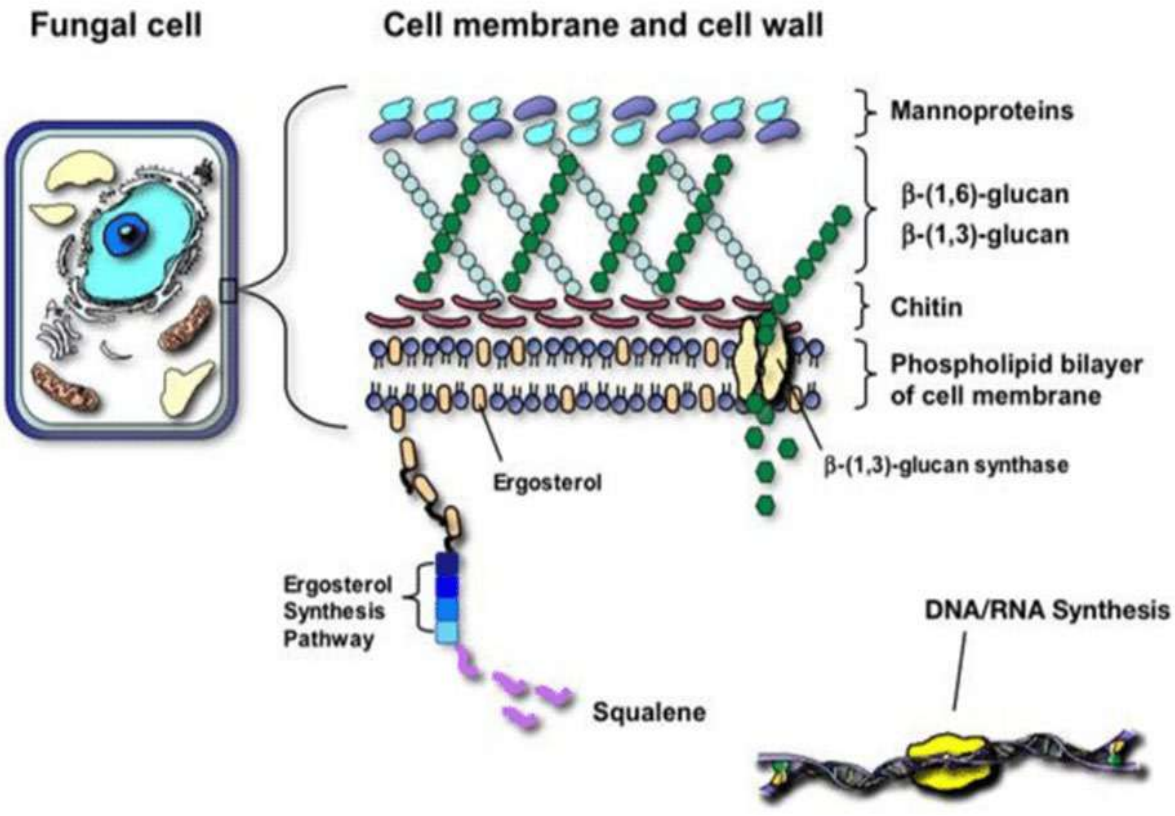
The rigid layers of fungal cell walls contain complex polysaccharides called chitin and glucans. Chitin, also found in the exoskeleton of insects, gives structural strength to the cell walls of fungi. The wall protects the cell from desiccation and predators. Fungi have plasma membranes similar to other 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

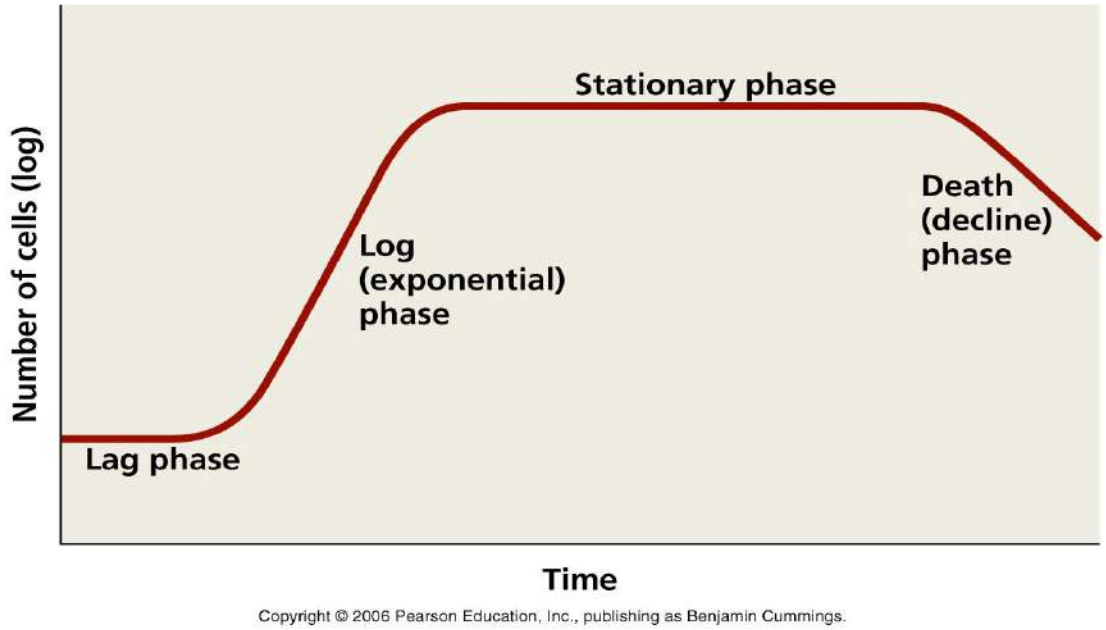
Decline phase

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

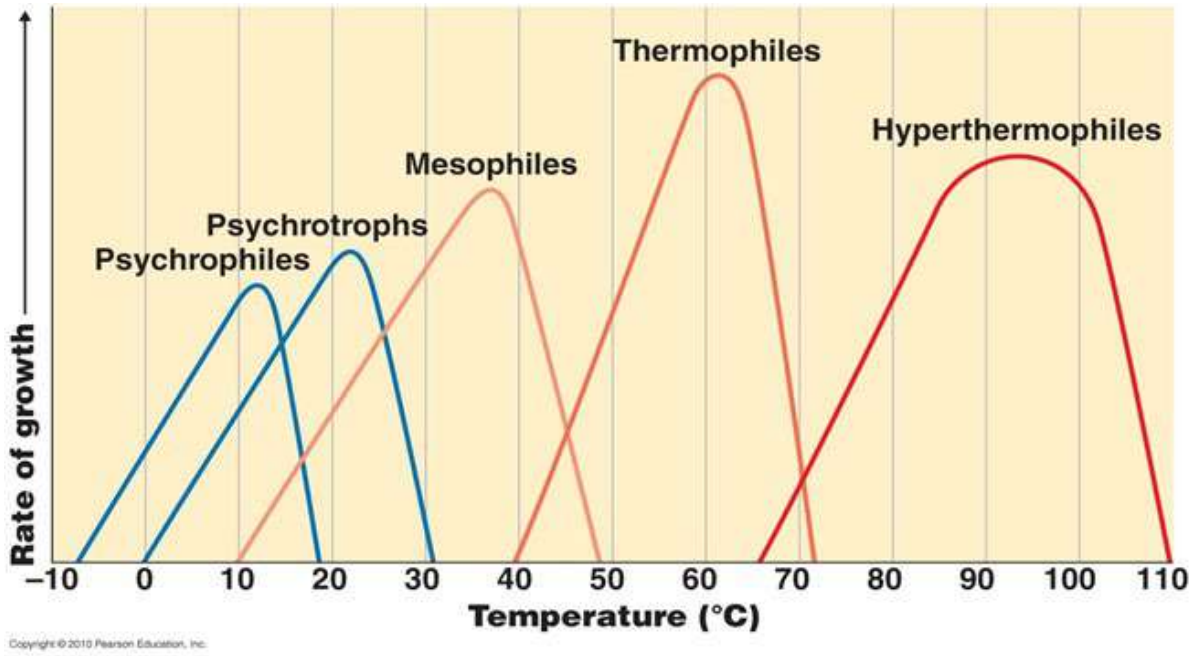
Temperature

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).

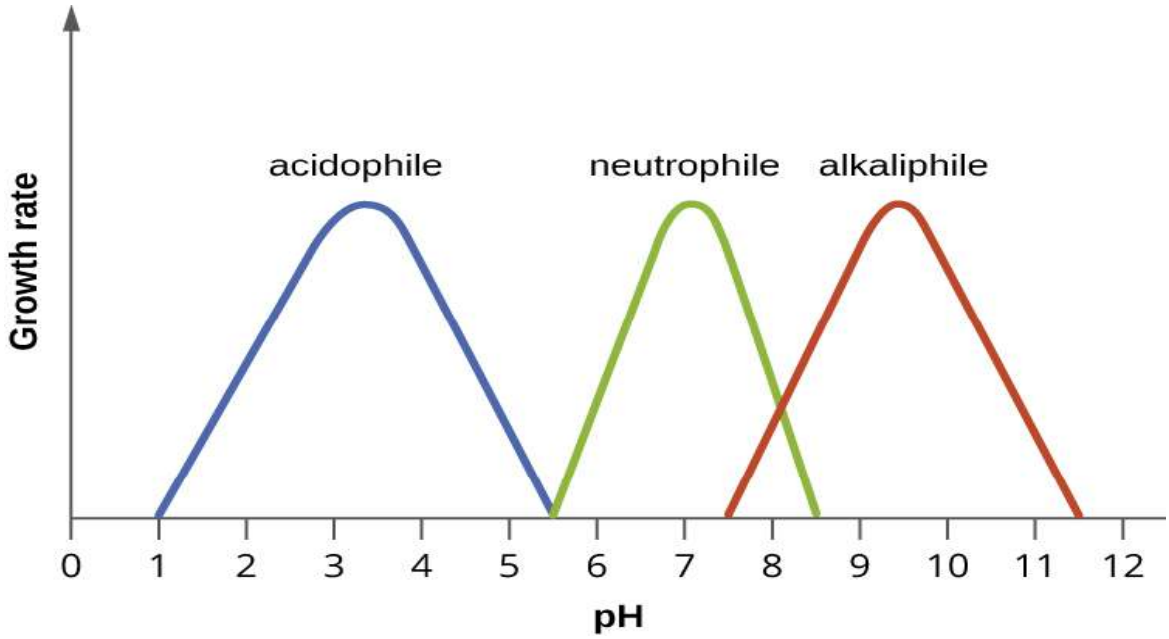
Maximum temperature - which fungi cannot grow above the maximum temperature.



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.

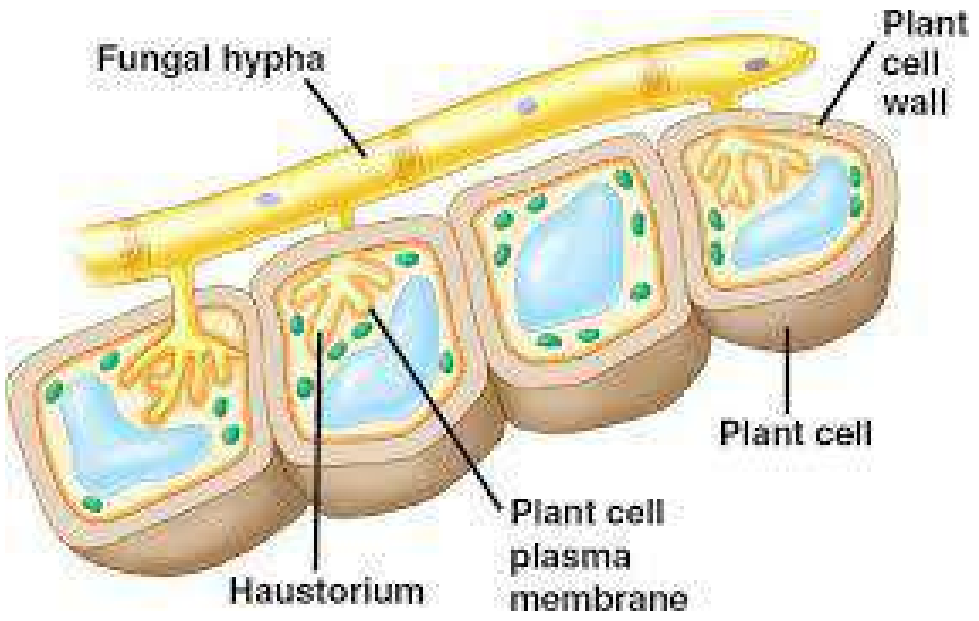
Fungal nutrition

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.

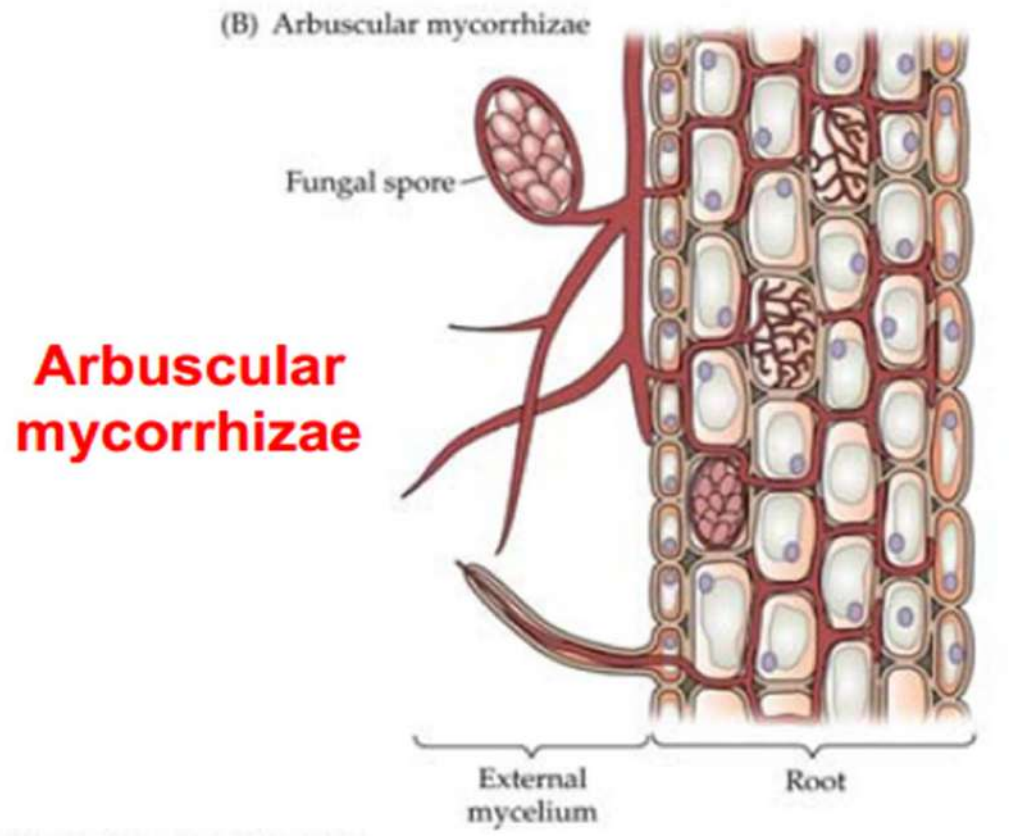
Both parasitism and mutualism are classified as symbiotic relationships, but they are discussed separately here because of the different effect on the host.

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.

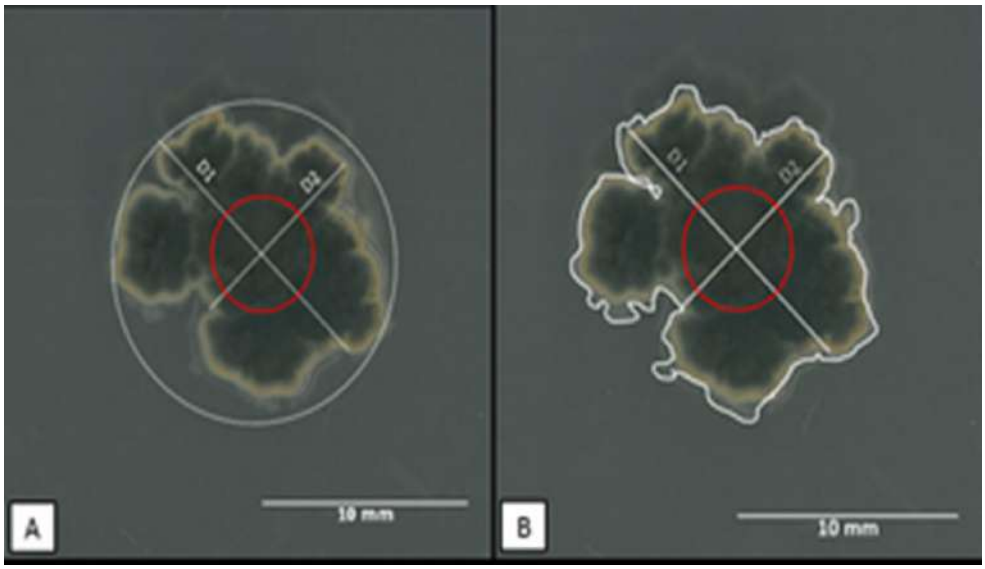


Measuring of fungal growth

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) = $\frac{D1+D2}{2}$

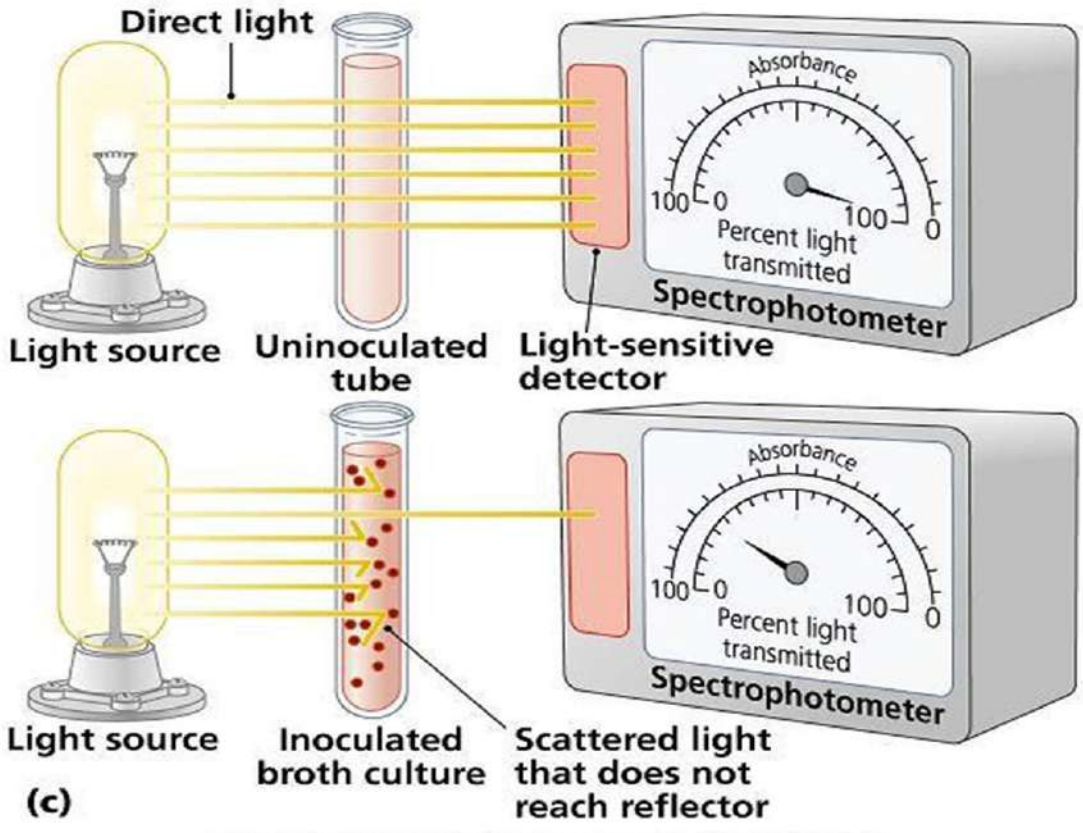
- Percentage of colony growth = $\frac{D1+D2}{2} \times 100$

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.

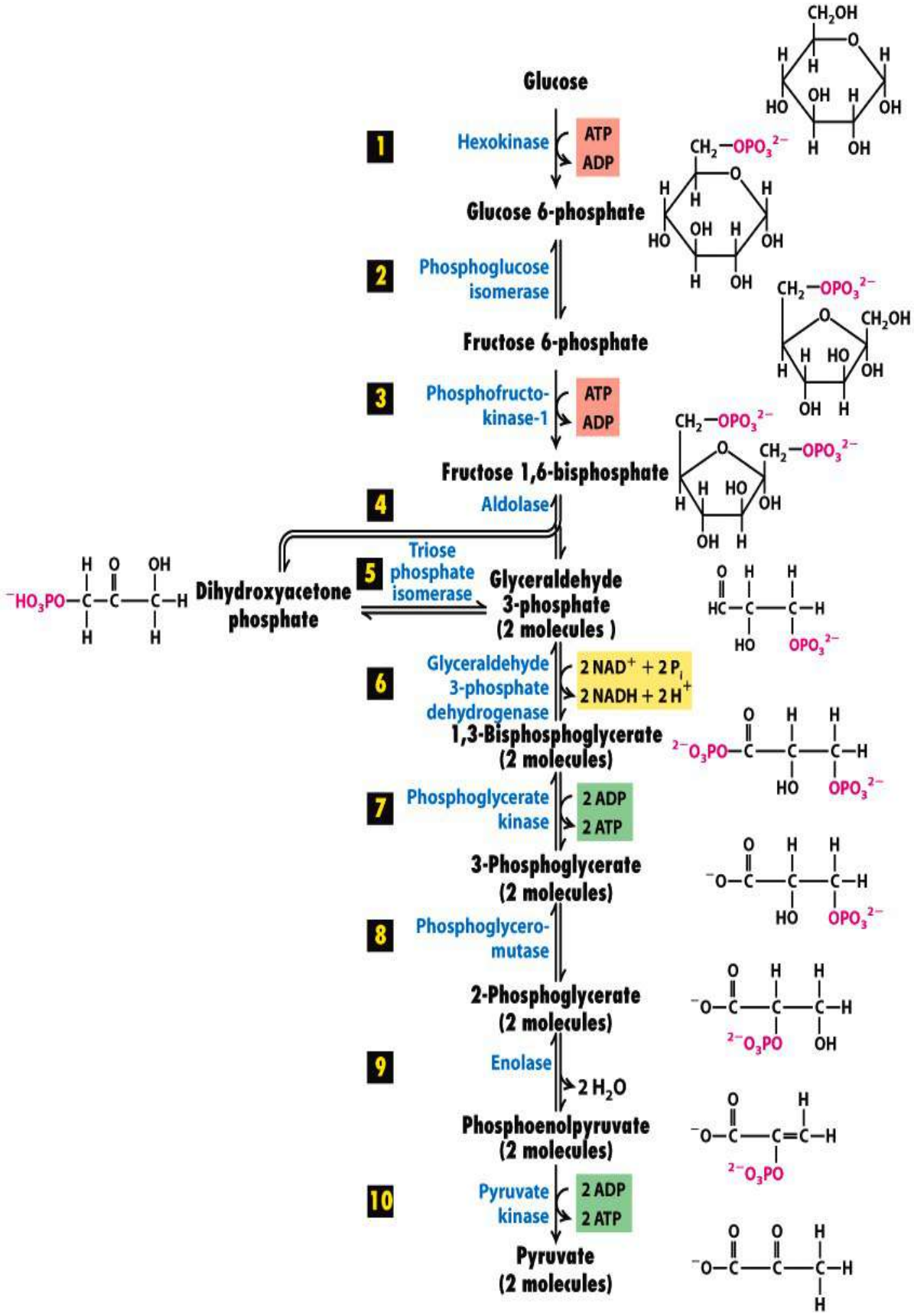


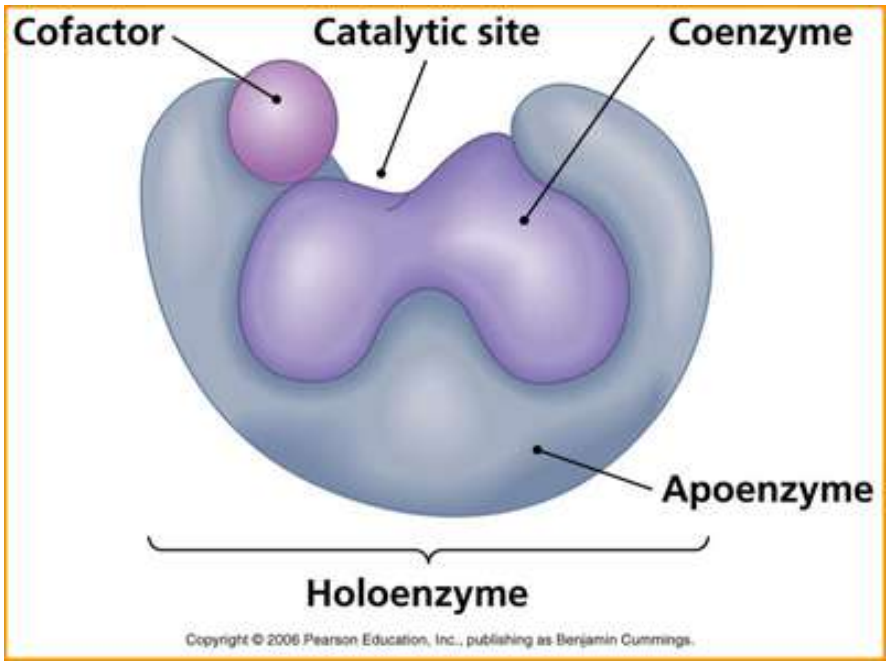
Figure 12-3
Molecular Cell Biology, Sixth Edition

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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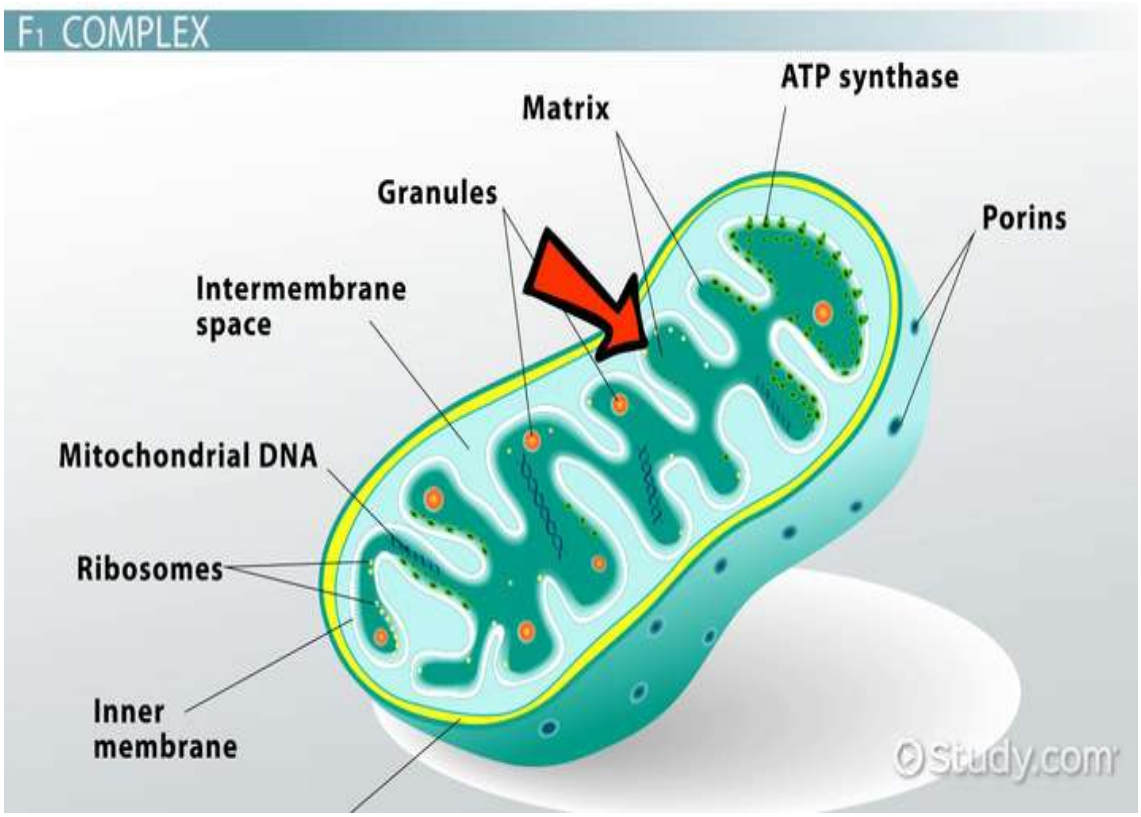
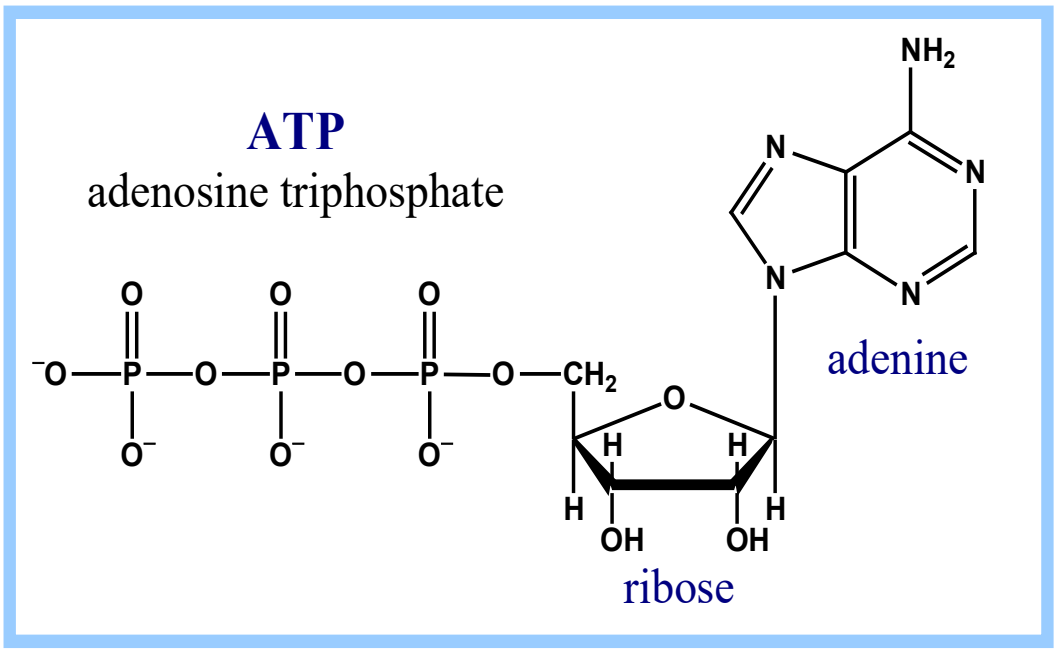
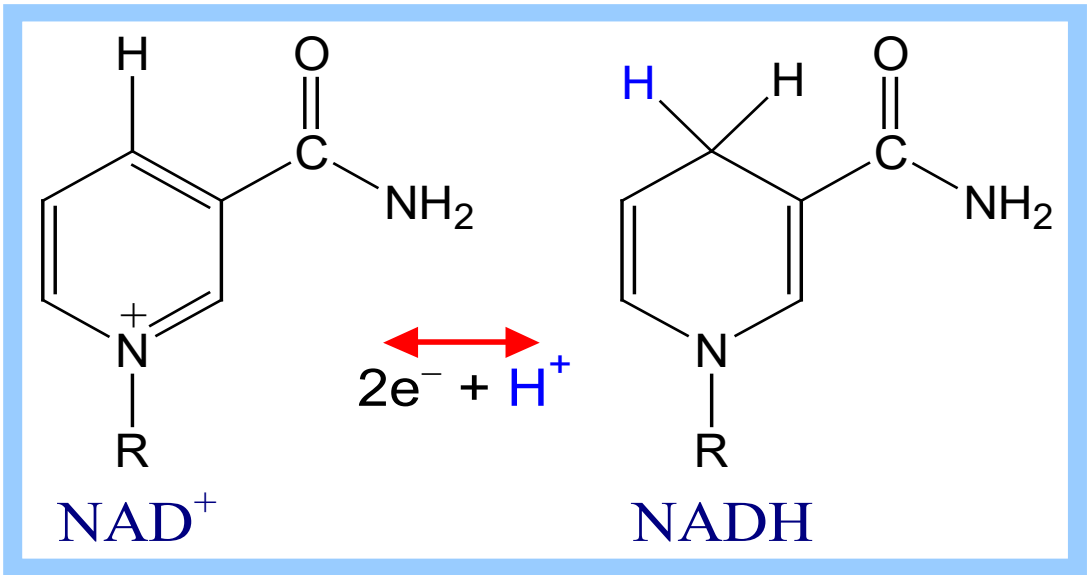

F1 COMPLEX
ATP synthase
Matrix
Granules
Porins
Intermembrane space
Mitochondrial DNA
Ribosomes
Inner membrane
Study.com



Adenosine triphosphate (ATP)



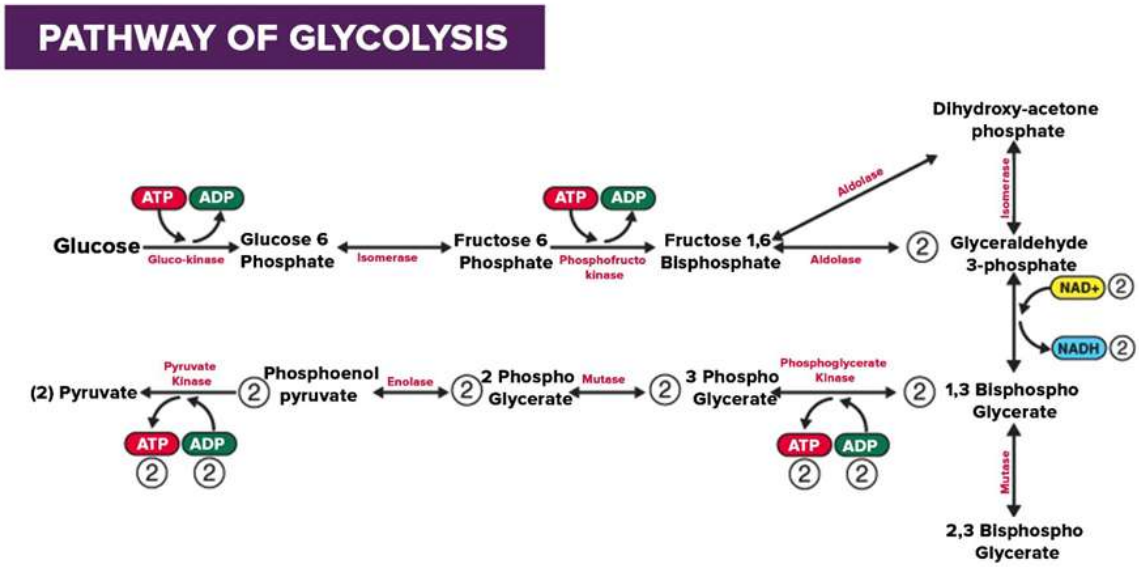
Nicotinamide adenine dinucleotide (NAD)

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.

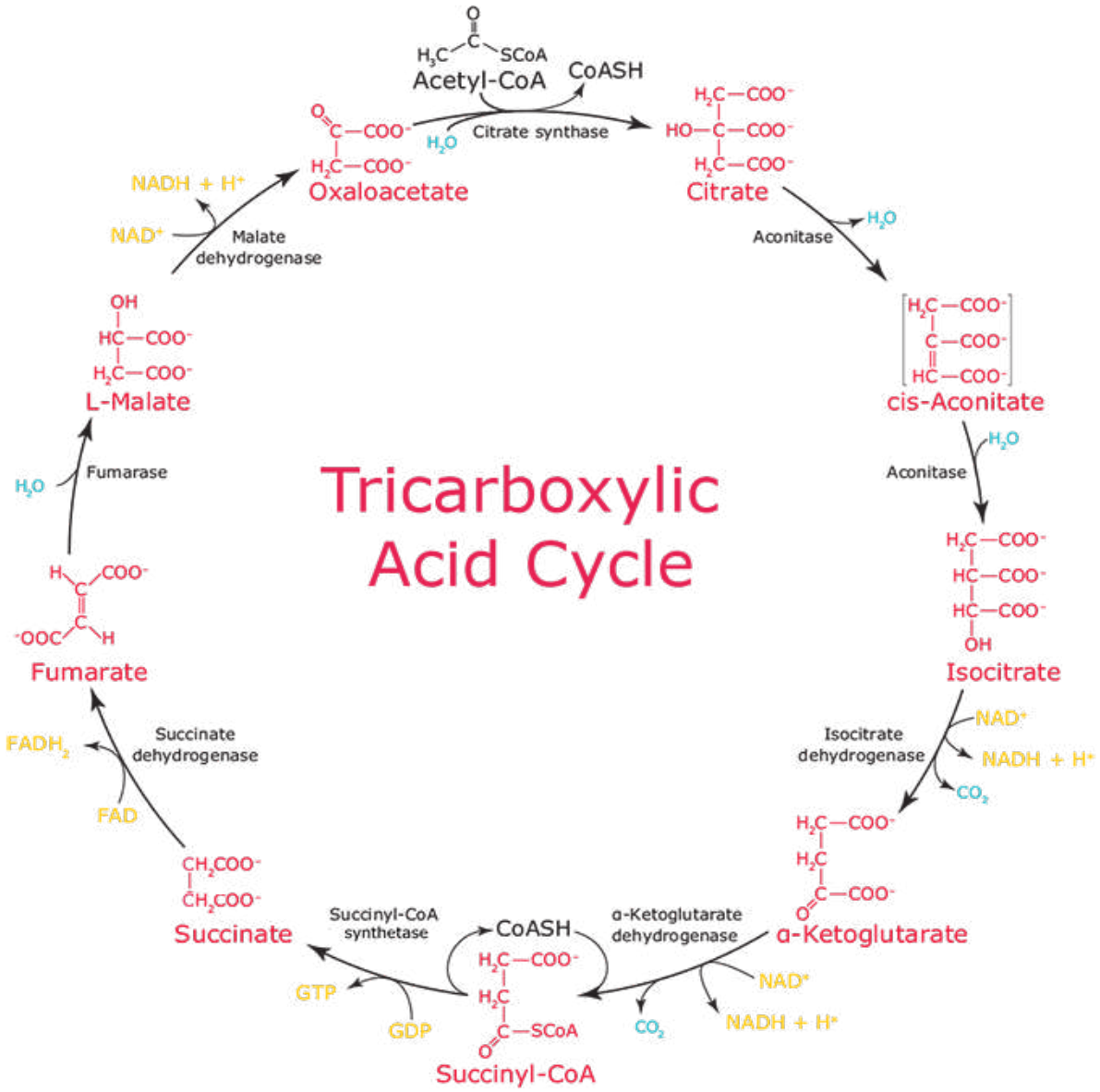
Step 4: **α**-ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by the **α**-ketoglutarate dehydrogenase enzyme complex. One molecule of CO_2 is released and NAD^+ is converted to NADH.

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 → $FADH_2$

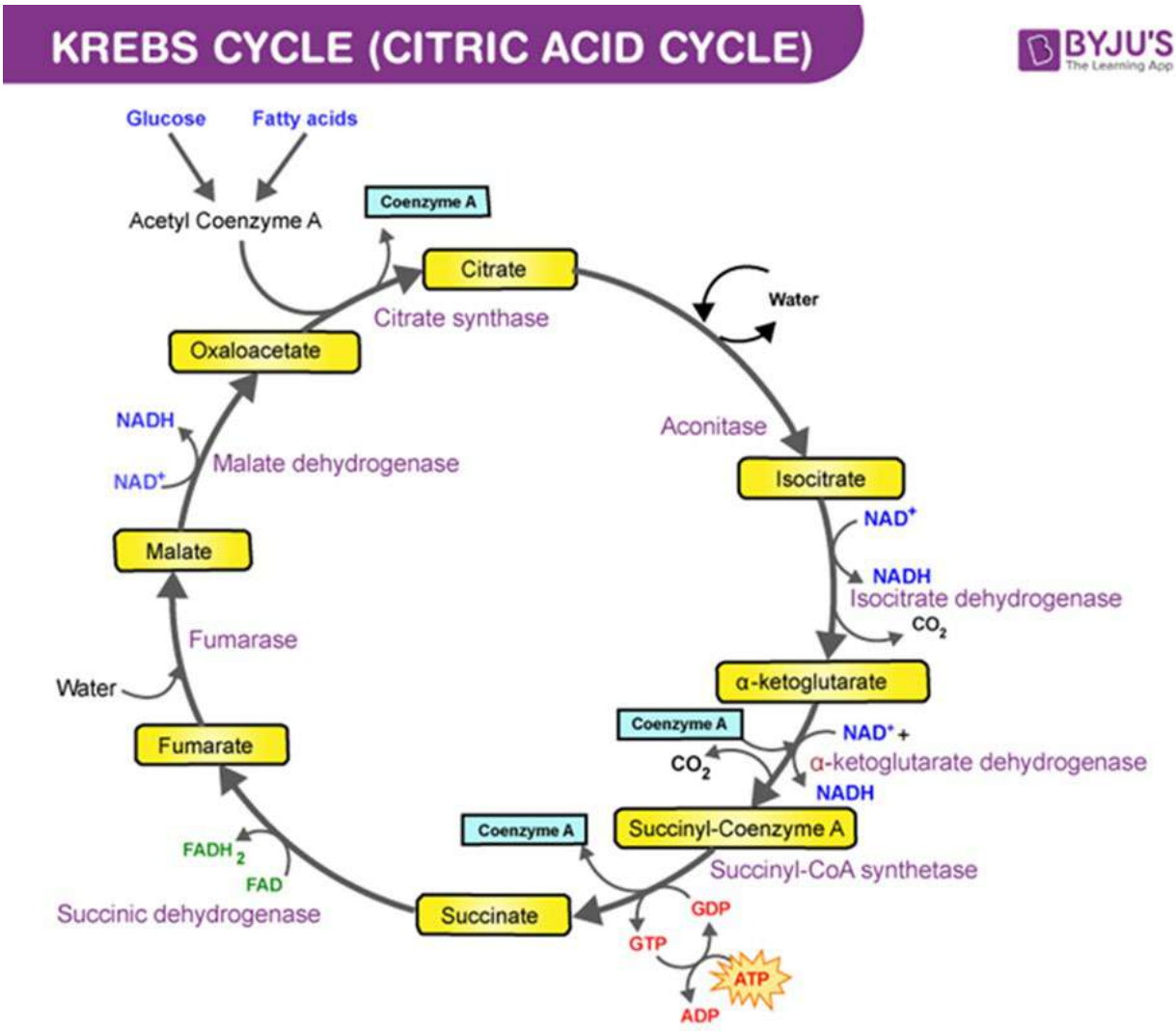
Malate to Oxaloacetate → NADH

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.

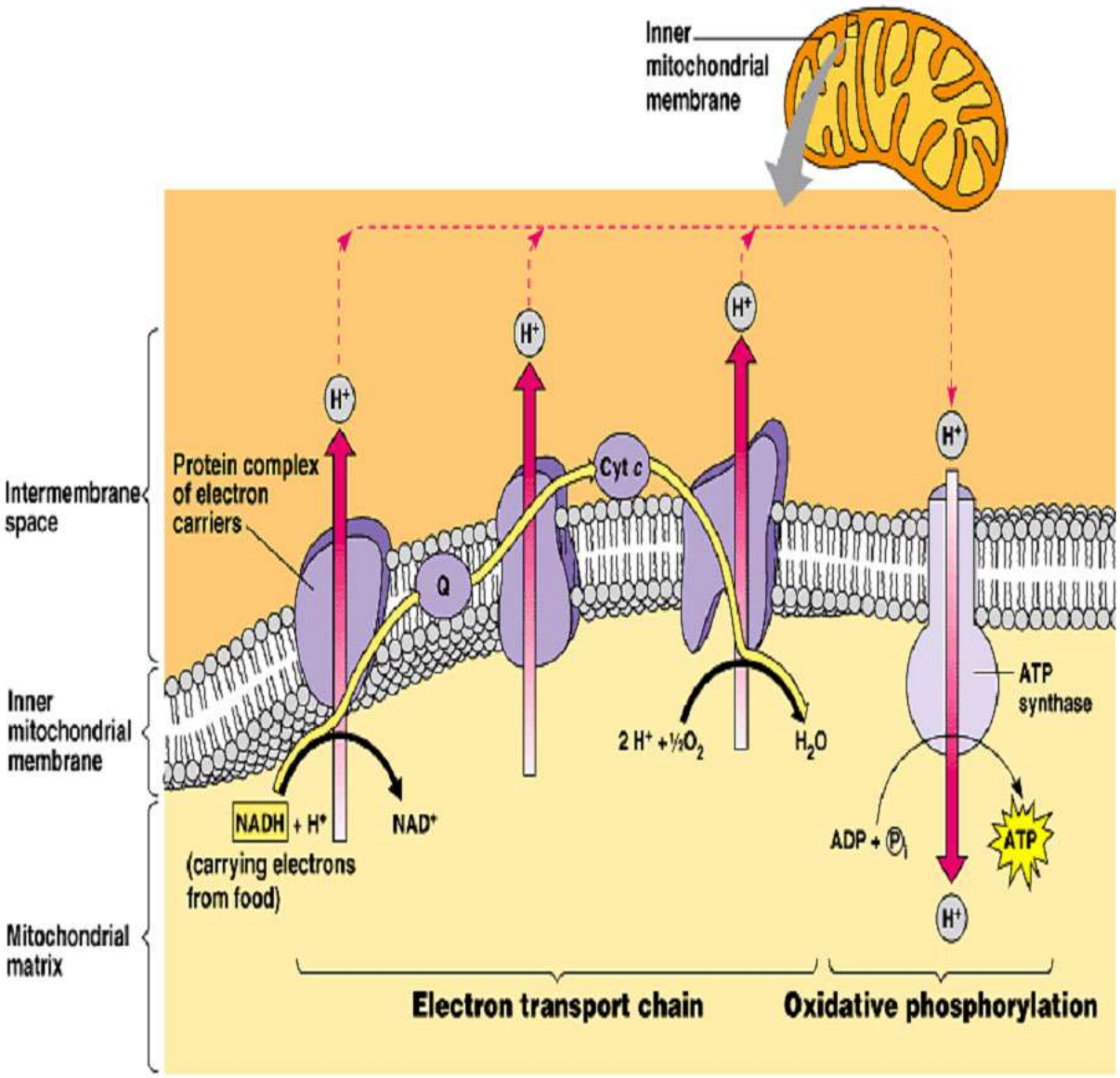
Hyperammonemia occurs in liver diseases and leads to convulsions and coma. This is due to reduced ATP generation as a result of the withdrawal of **α**-ketoglutarate and formation of glutamate, which forms glutamine.

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.

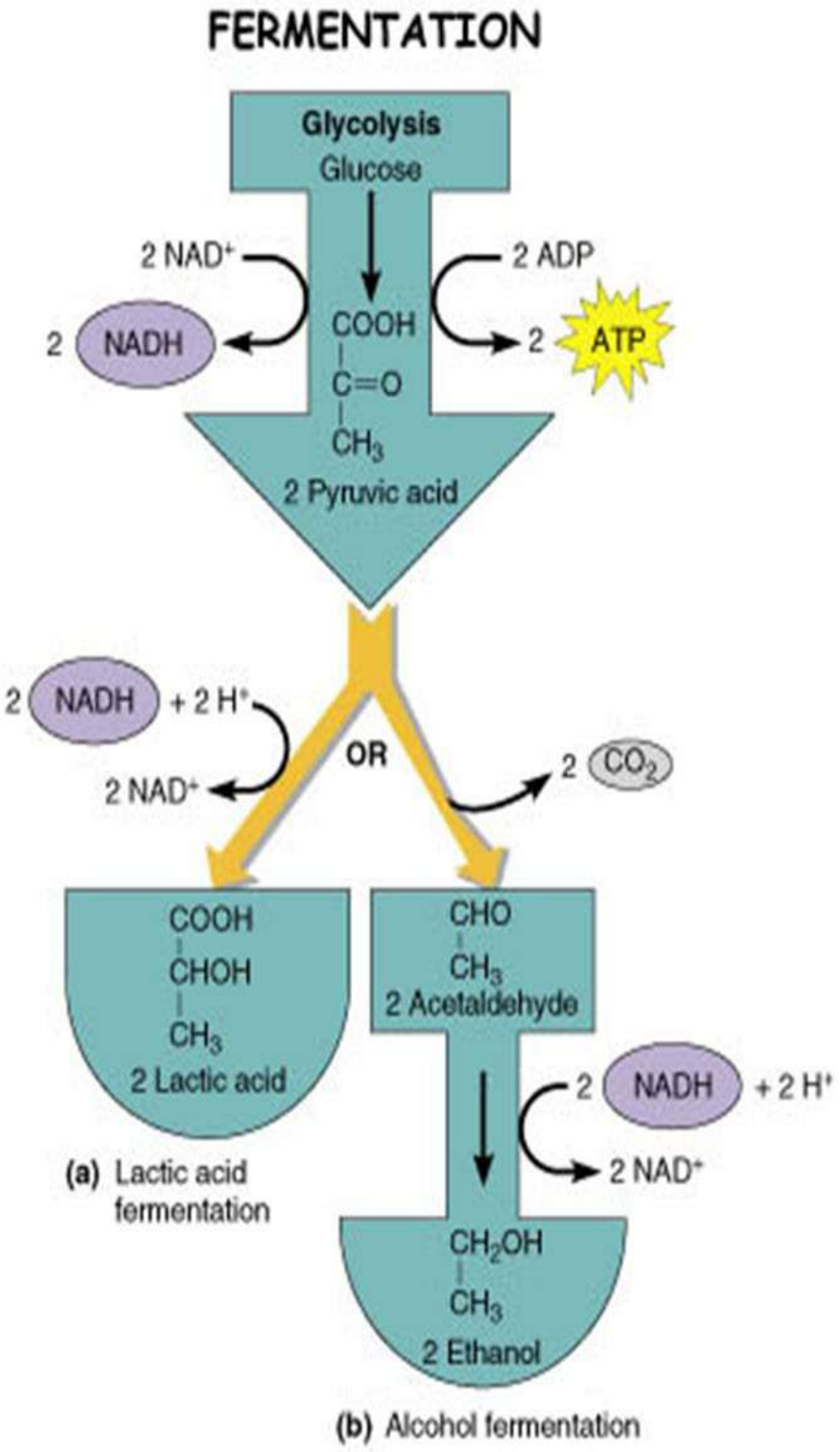
Types of fermentation

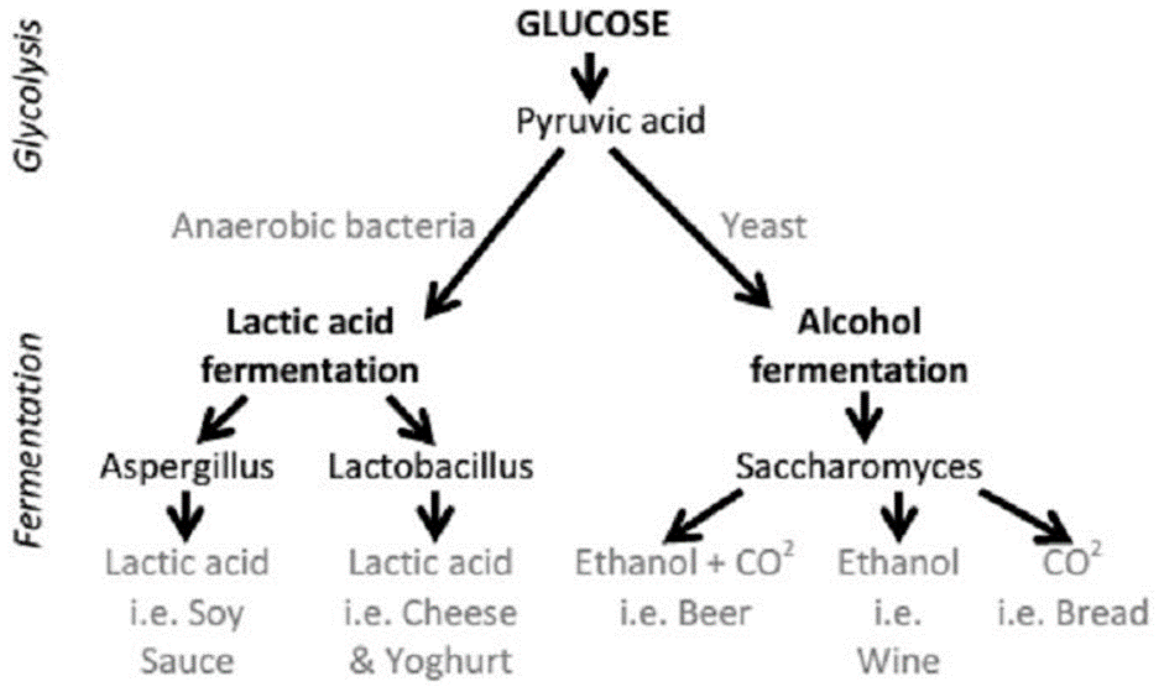
Microbes specialized at converting certain substances into others can produce a variety of foodstuffs and beverages. These are three distinct types of fermentation that people use.

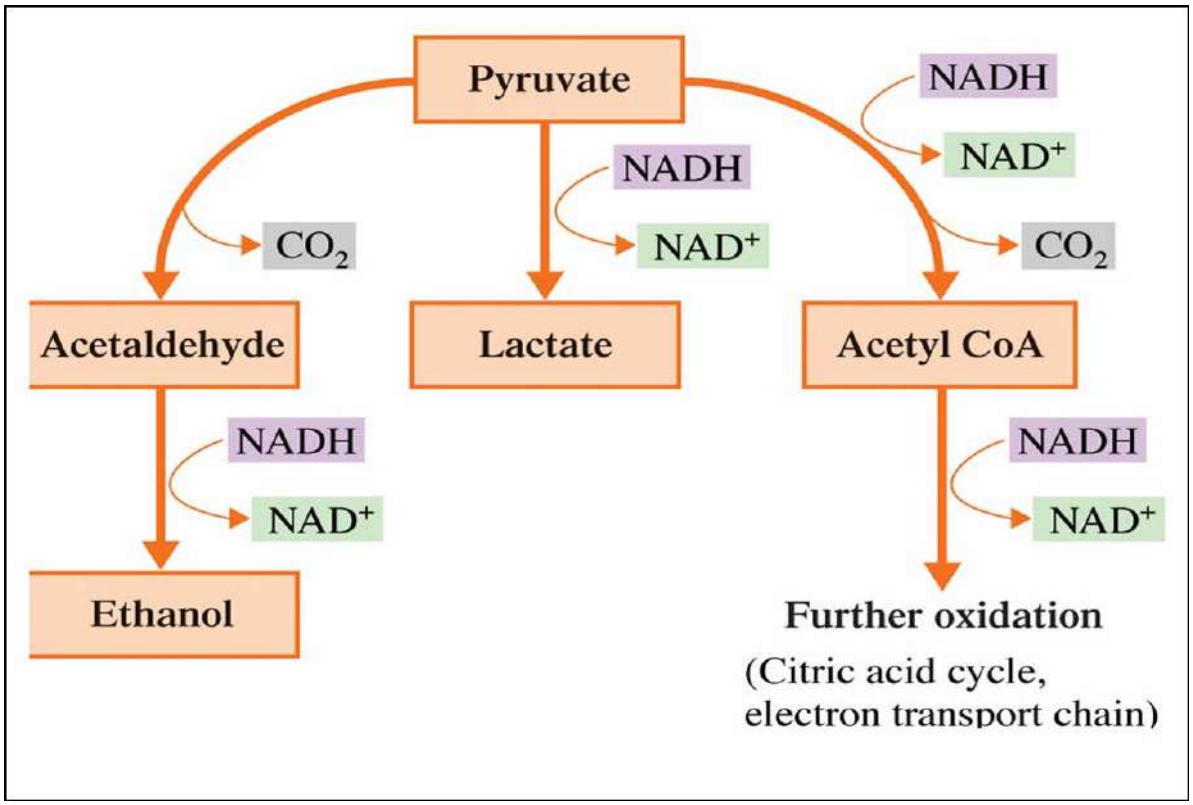
1. Lactic acid fermentation. Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation. These anaerobic chemical reactions, pyruvic acid uses nicotinamide adenine dinucleotide+hydrogen (NADH) to form lactic acid and NAD^+ (Lactic acid fermentation also occurs 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

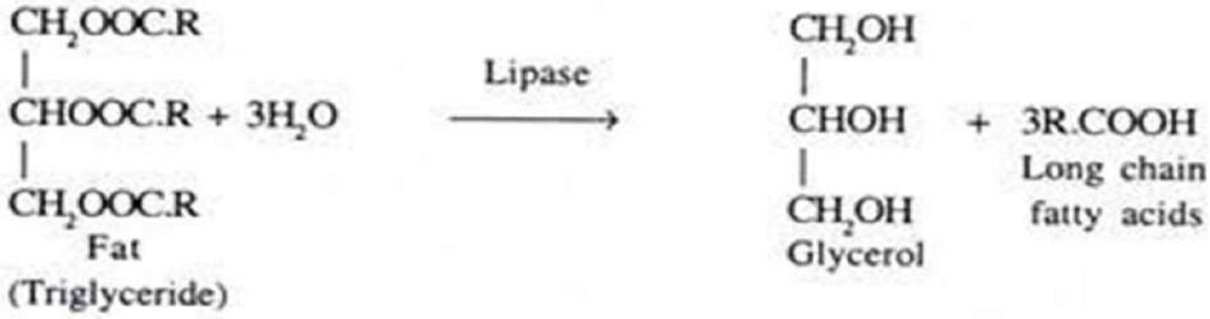

Pyruvate
NADH
NAD+
CO2
Acetaldehyde
Lactate
Acetyl CoA
Ethanol
Further oxidation
(Citric acid cycle, electron transport chain)

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:

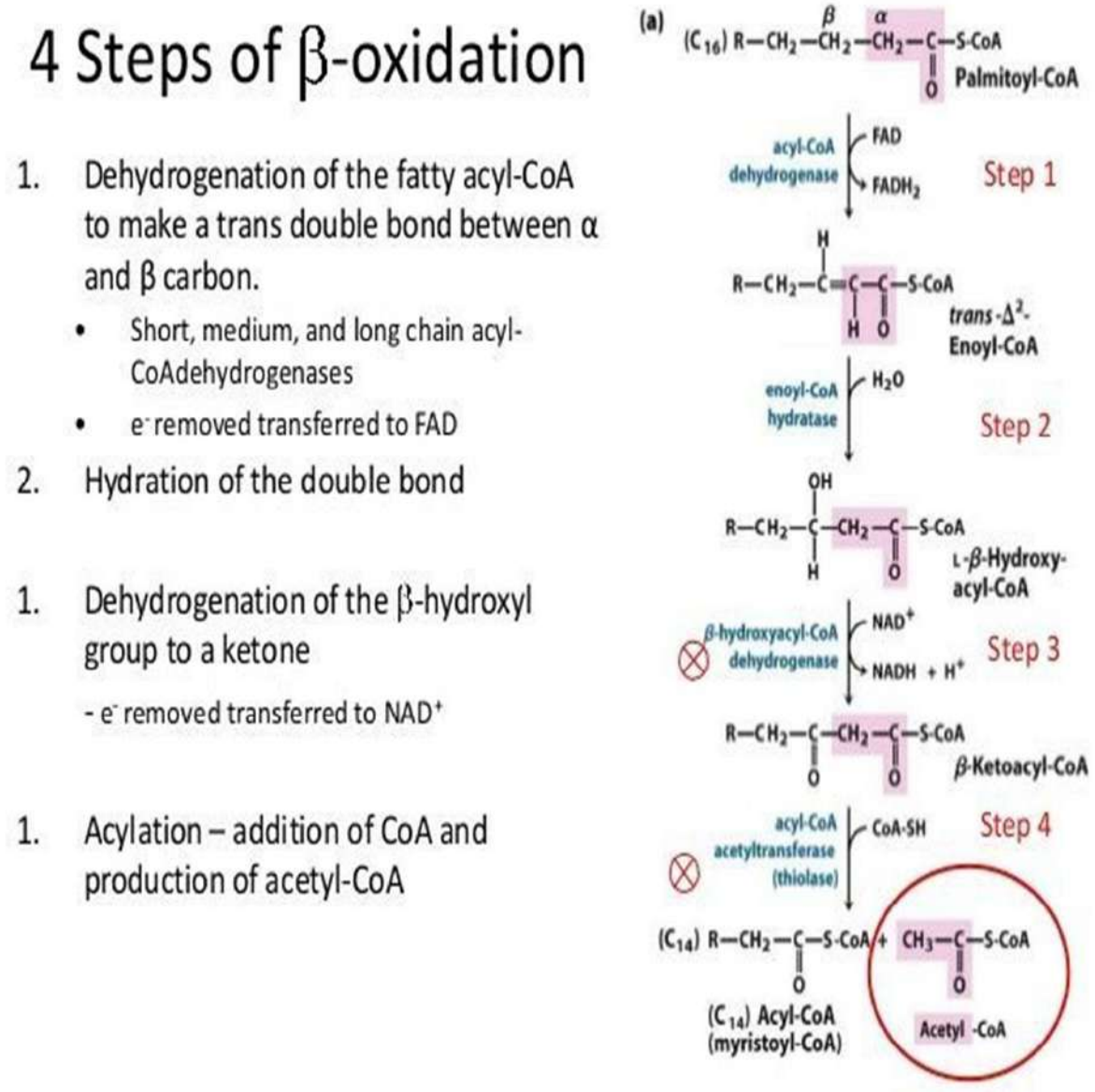


Beta oxidation

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*.

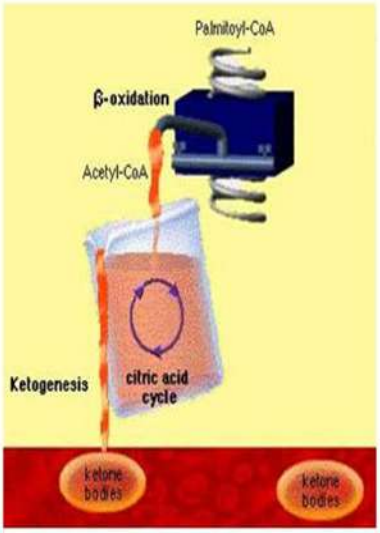
Beta oxidation is a spiral pathway. Each round consists of four enzyme-catalyzed steps that yield one molecule of acetyl CoA and an acyl CoA shortened by two carbons, which becomes the starting substrate for the next round. Seven rounds of beta oxidation degrade a C_{16} fatty acid to eight molecules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

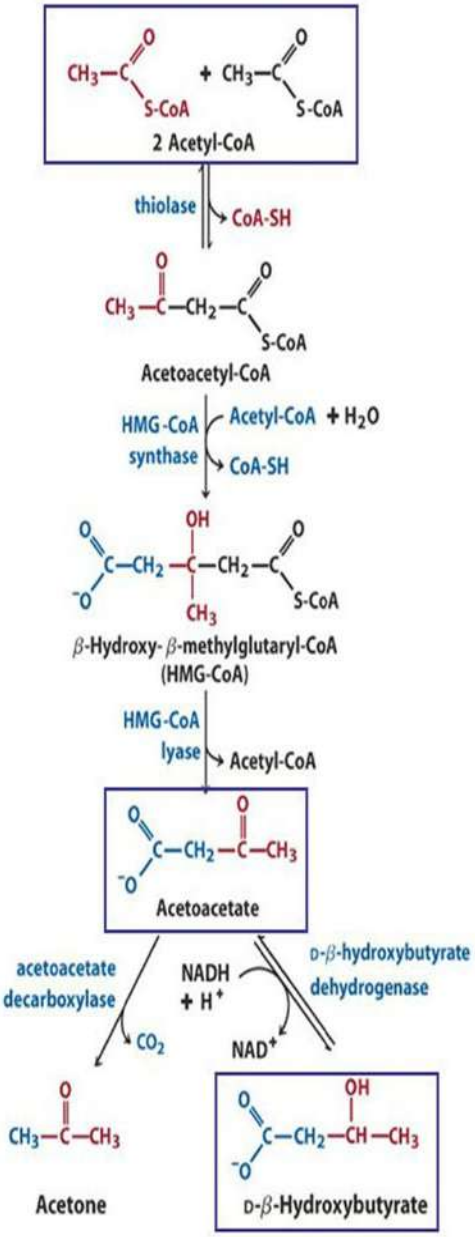


The reactions of ß-oxidation

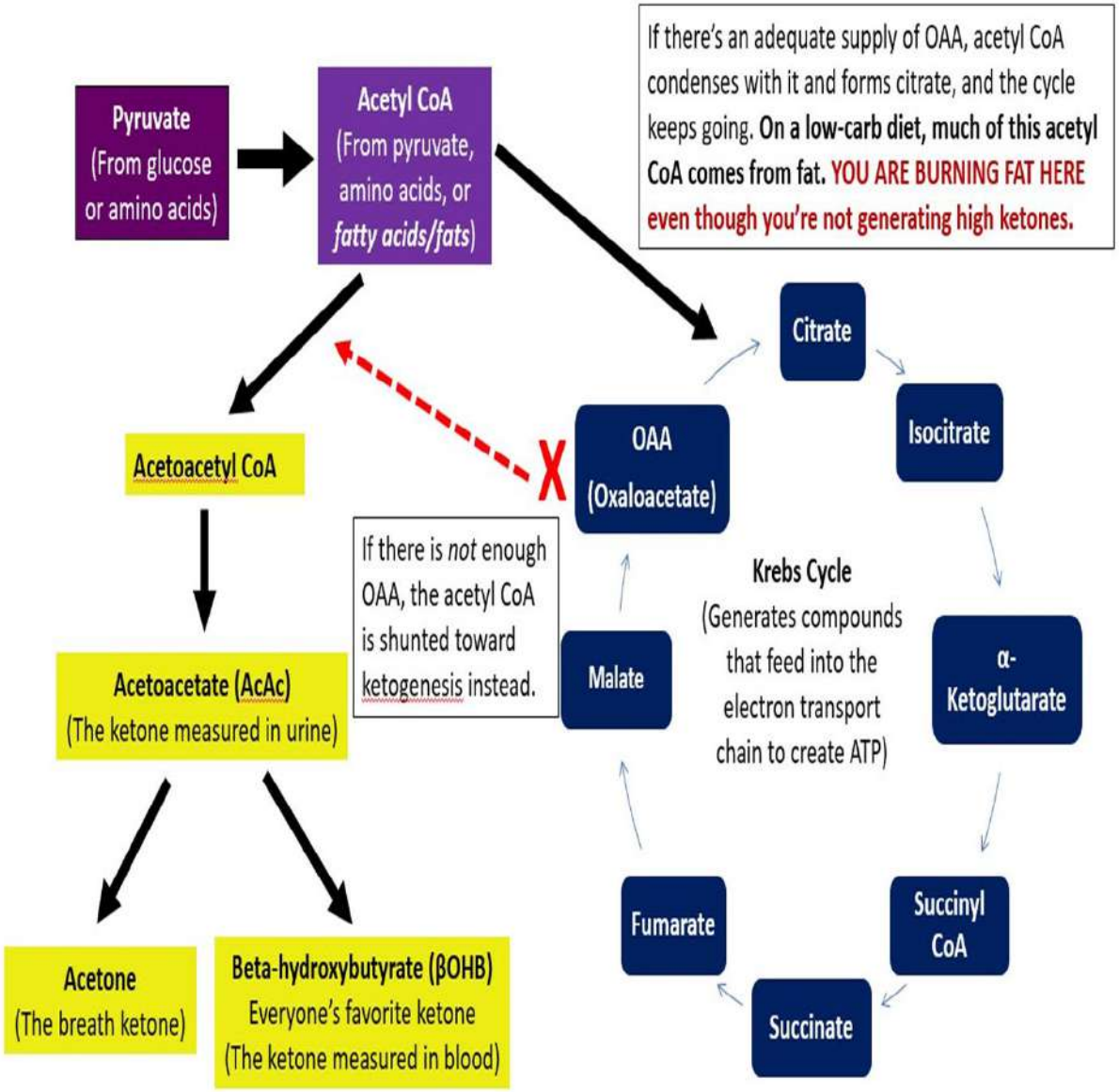
Ketogenesis

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.

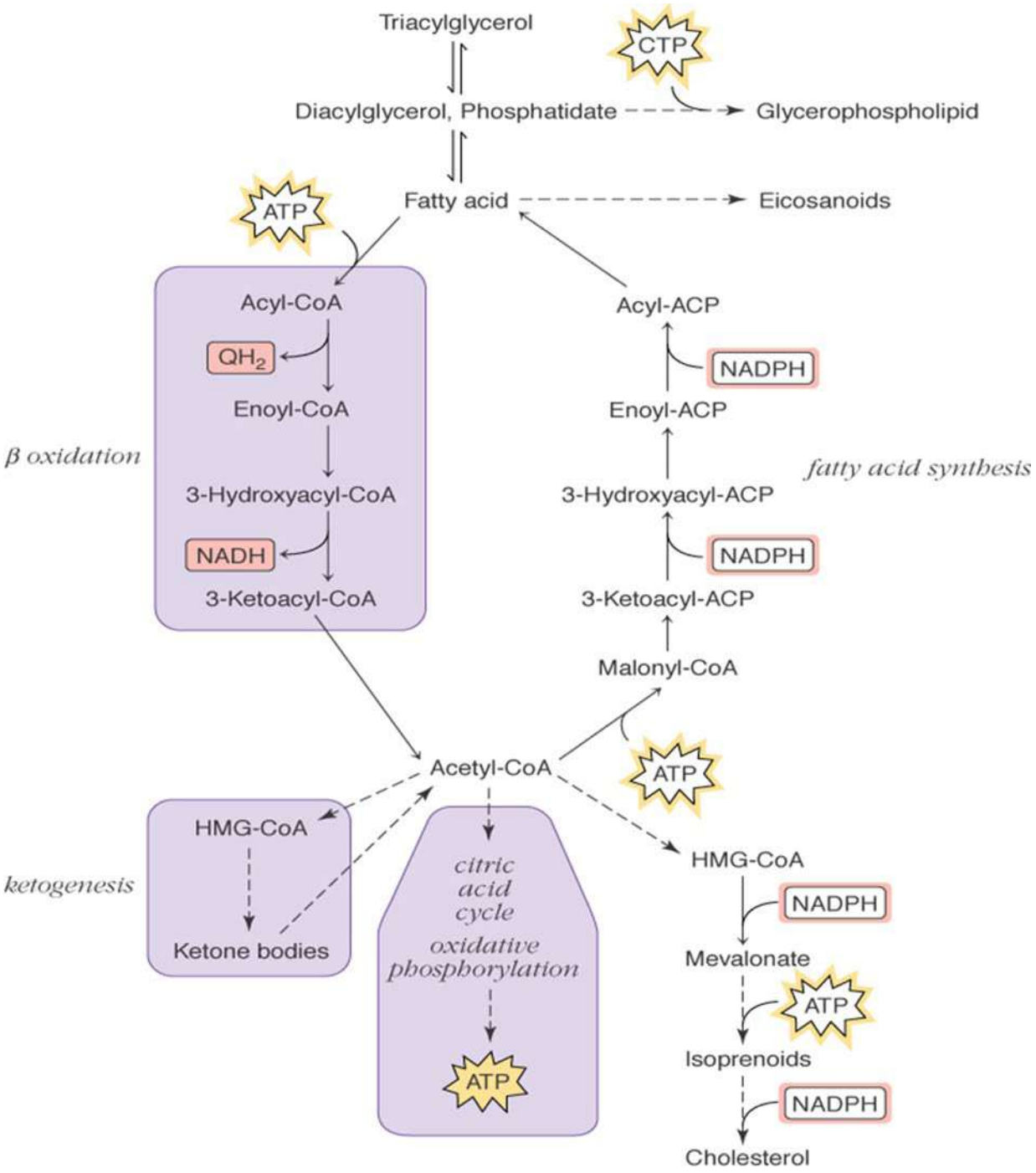




Ketogenesis and Krebs cycle



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.

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

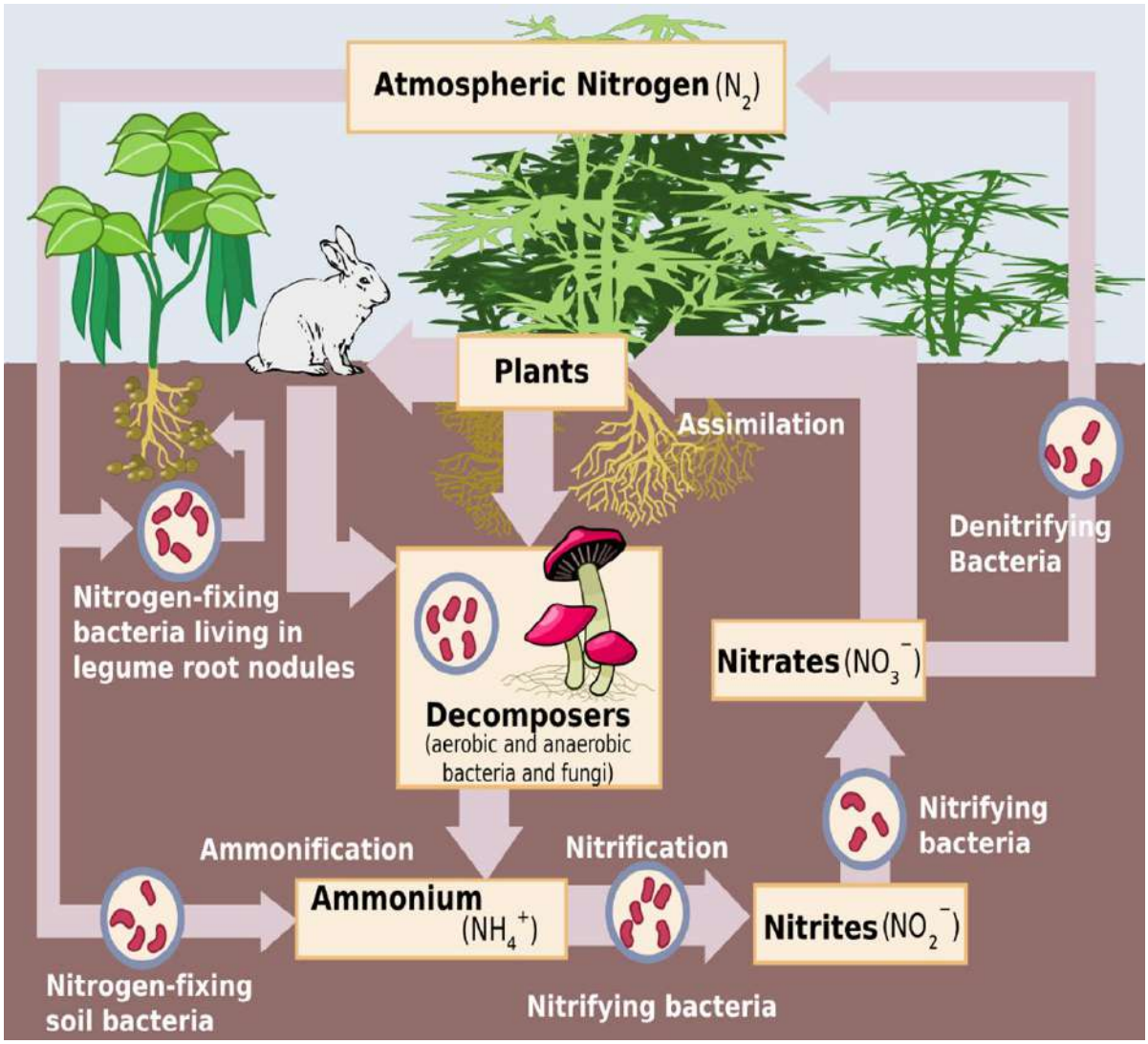
$NH_2OH +H_2O \rightarrow NO_2 +5\ H+ +4e-$

$NO_2 +O_2 \rightarrow NO_3$

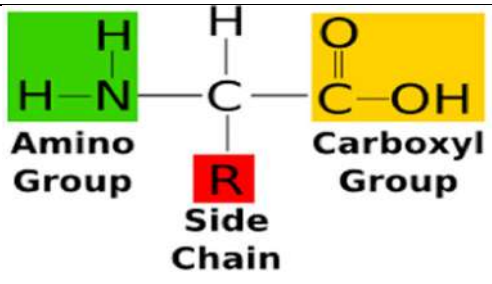
Denitrification is the process that coverts nitrate to nitrogen gas.

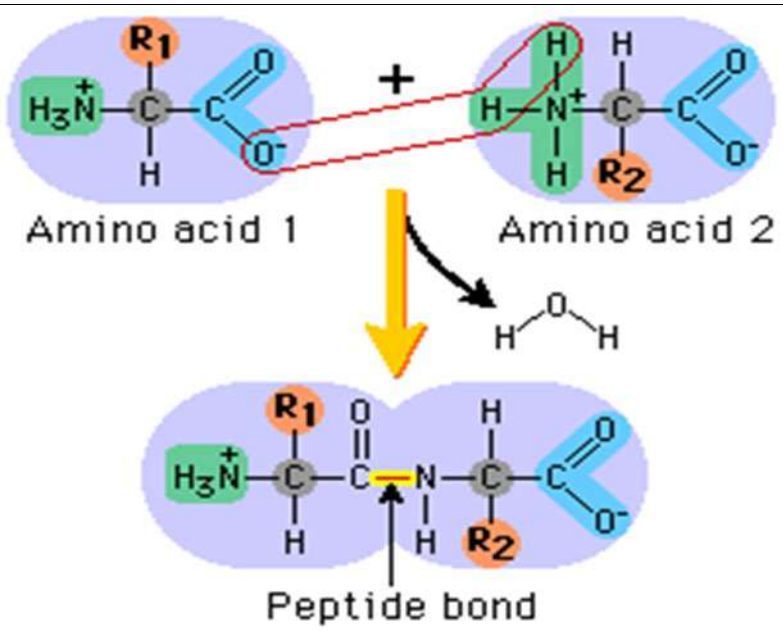
$NO_3 \rightarrow NO_2 \rightarrow NO + N_2O \rightarrow N_2$

Nitrogen cycle



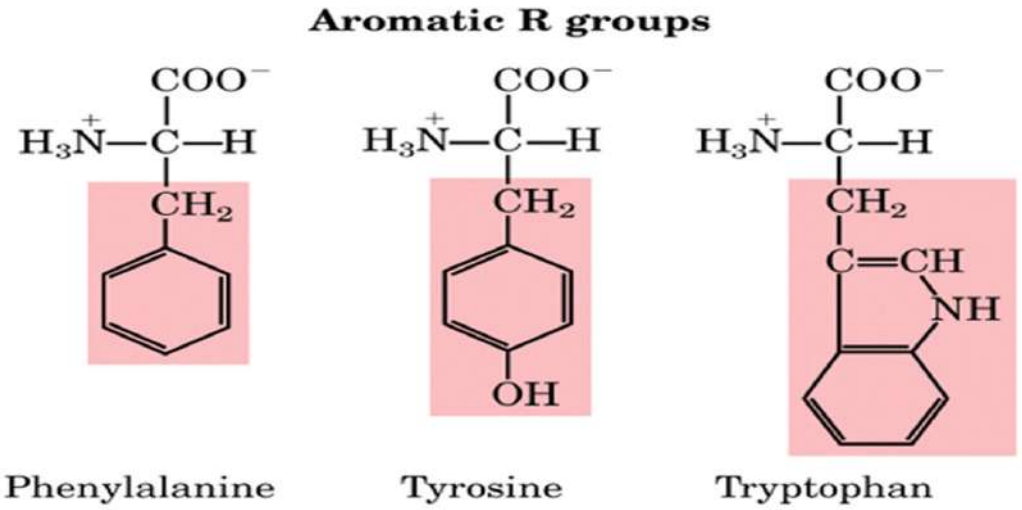
Amino acids

Amino acid structure	

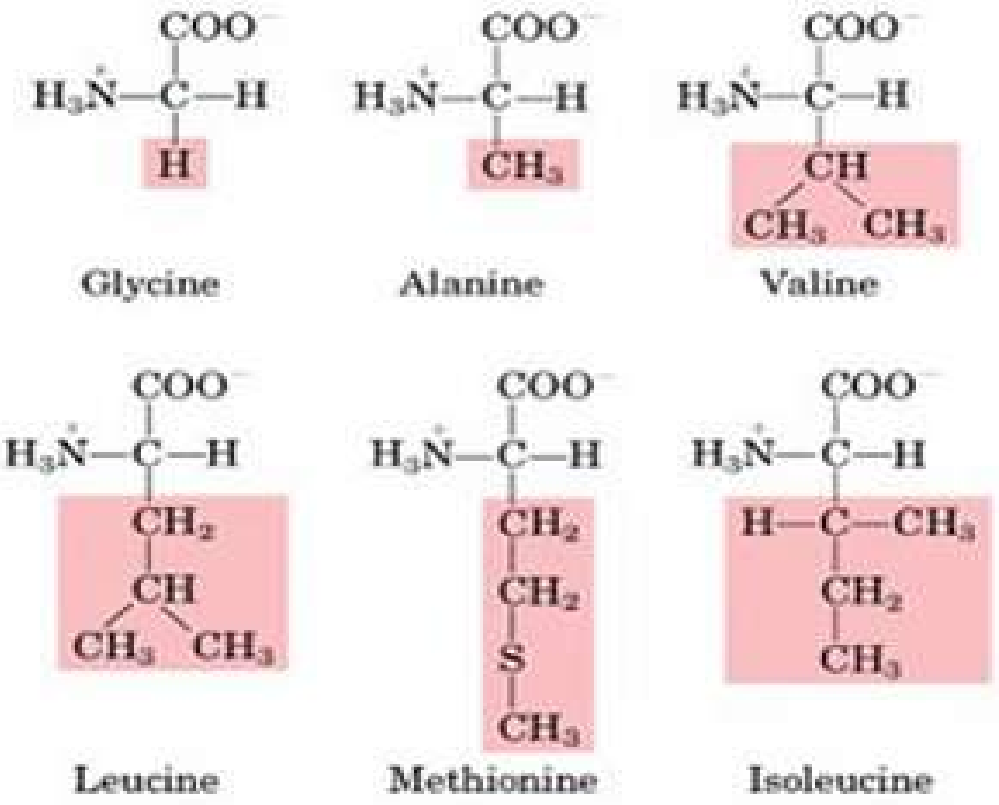
Formation of peptide bond	

Types of amino acids

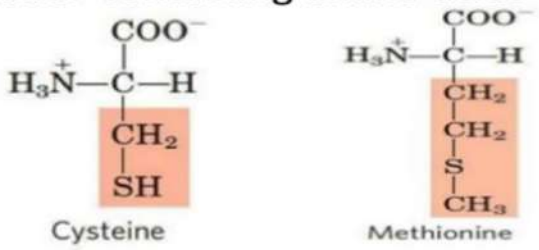
Aromatic amino acids



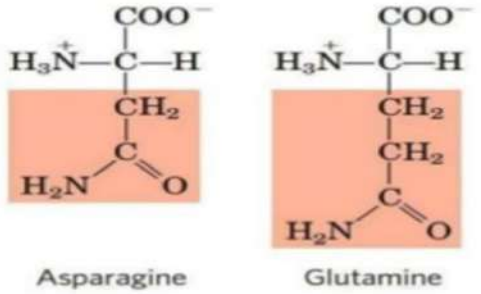
Aliphatic amino acid



❑ Sulfur-containing amino acids



❑ Amide group-containing amino acids



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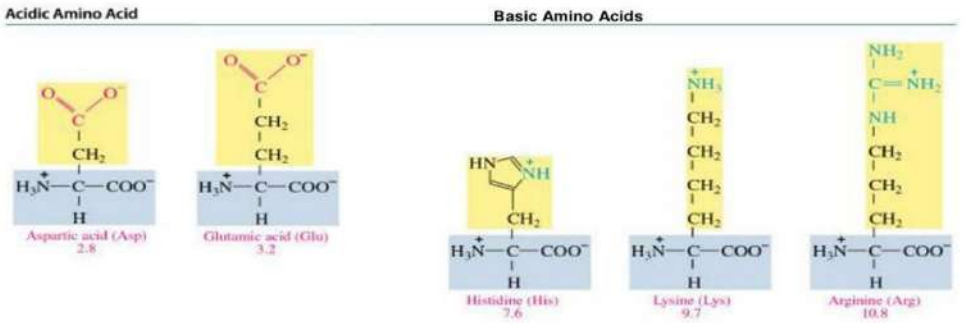
Acidic and Basic Amino Acids

An amino acid is

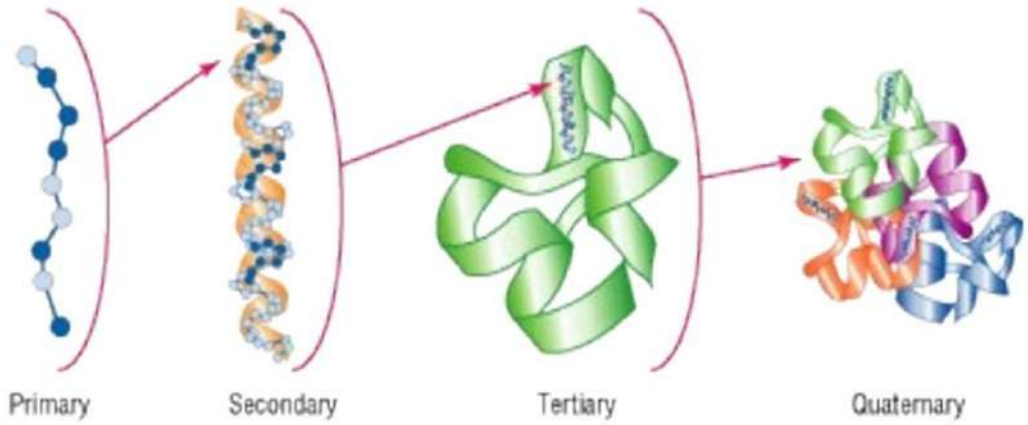
- Acidic with a carboxyl R group (COO^-).
- Basic with an amino R group (NH_3^+).



8

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.

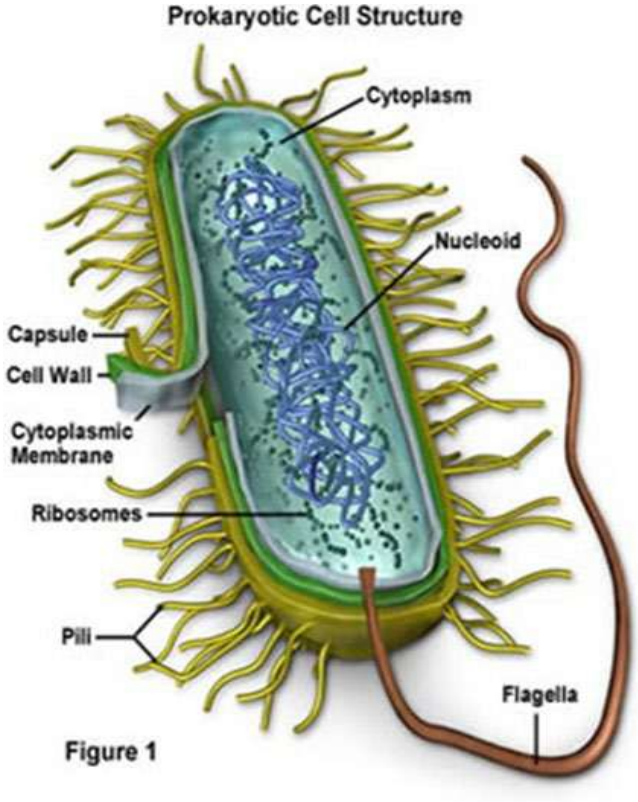
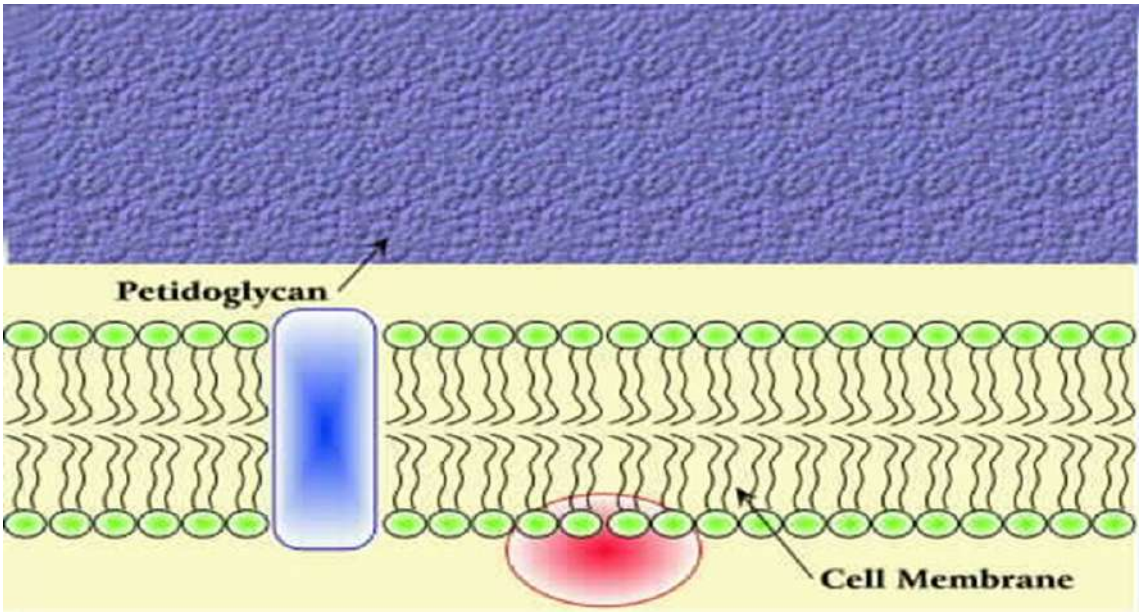


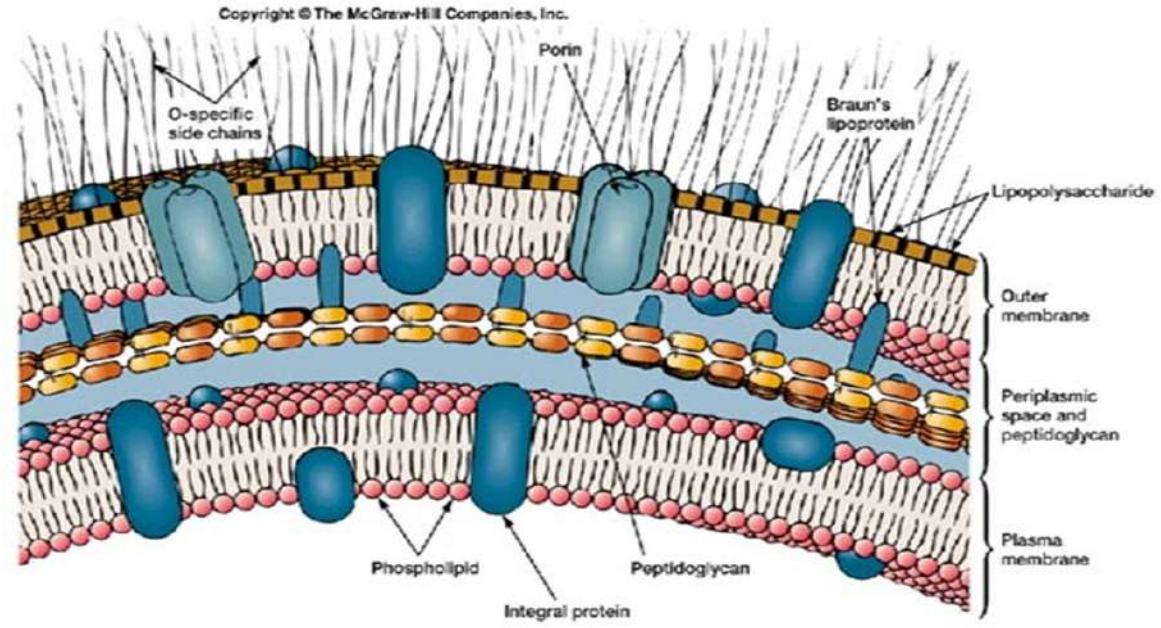
Figure 1

Cell wall of gram positive bacteria

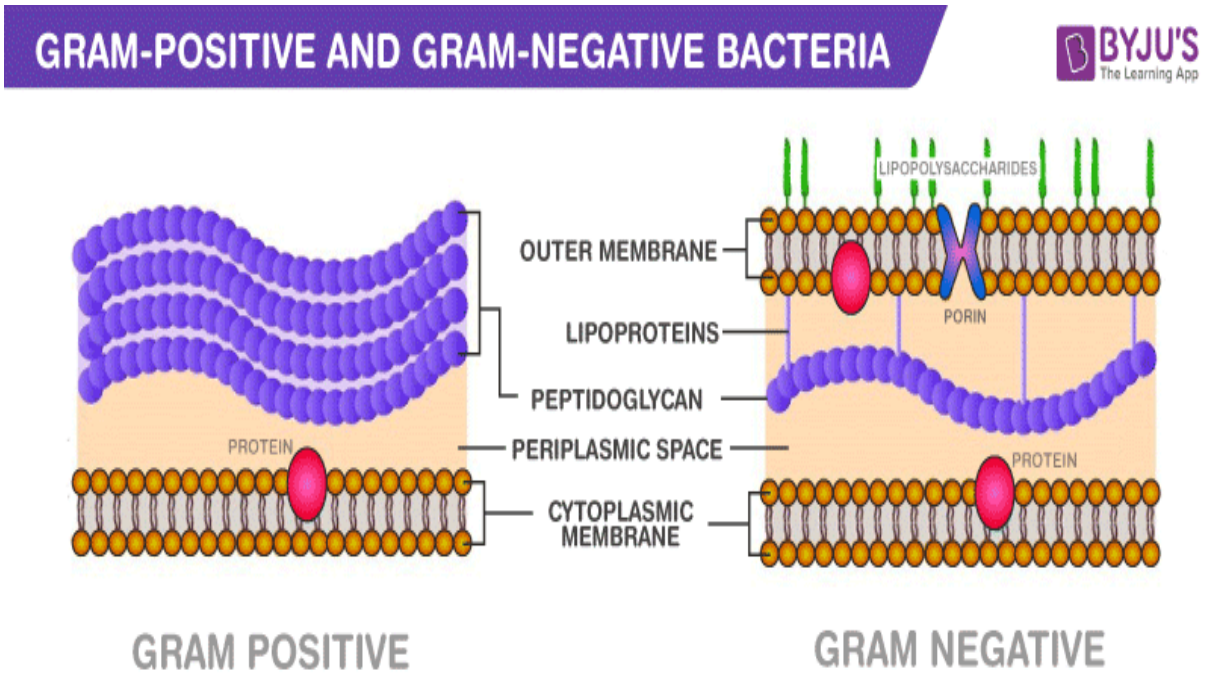


Outside of the cytoplasmic membrane of Gram-positive bacteria lies a thick layer of peptidoglycan which determines the rigidity of the cell wall. In Gram-positive bacteria, peptidoglycan accounts for 50% of the cell weight and up to 90% of the weight of the cell wall. Peptidoglycan layer is 20-80 nm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

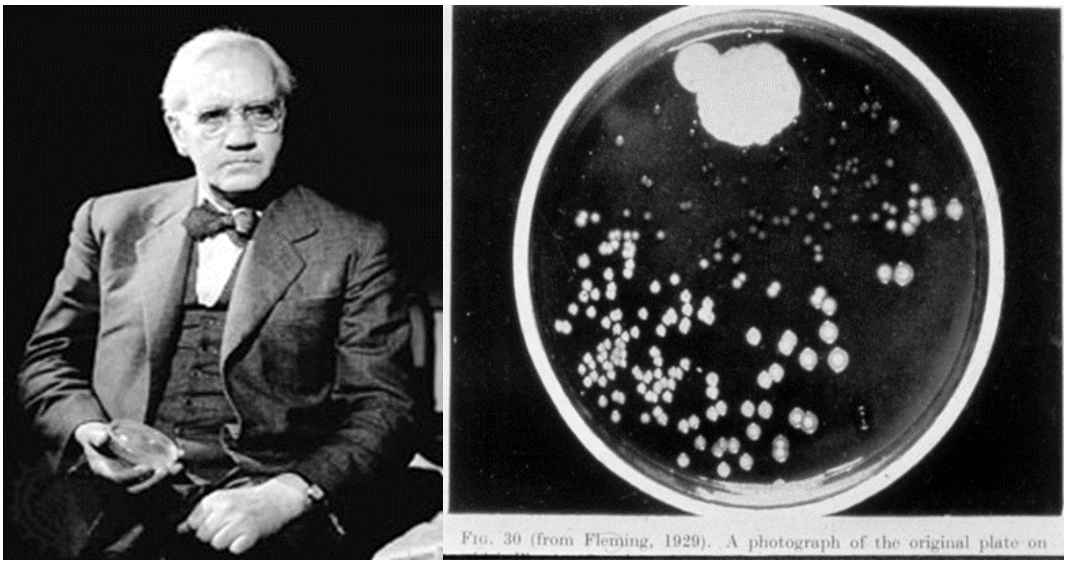


Fig. 30 (from Fleming, 1929). A photograph of the original plate on

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.

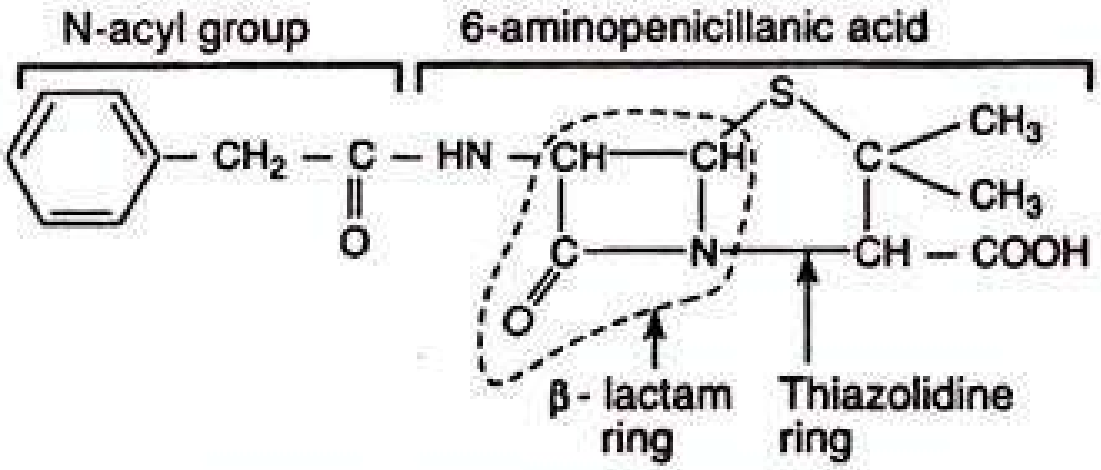


FIG. 45.6. Basic structure of penicillins.

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.

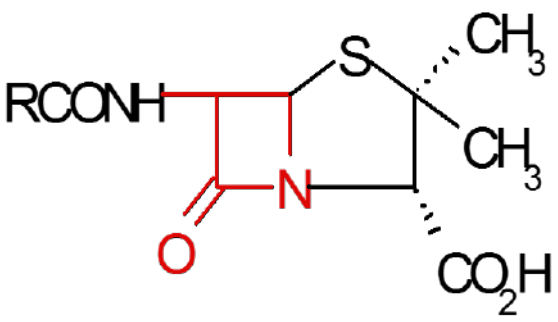
Narrow spectrum β-lactamase-resistant penicillins

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.

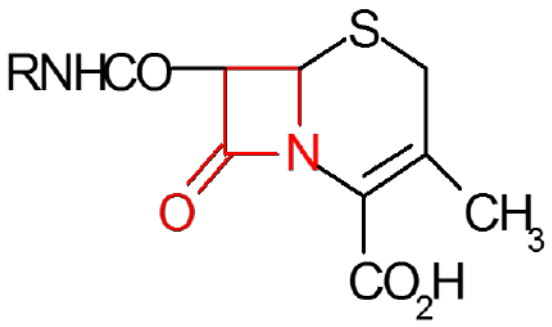
Commercial production of penicillin

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

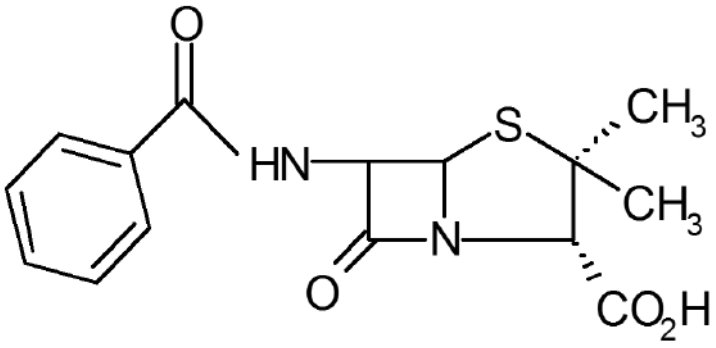


Cephalosporins

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

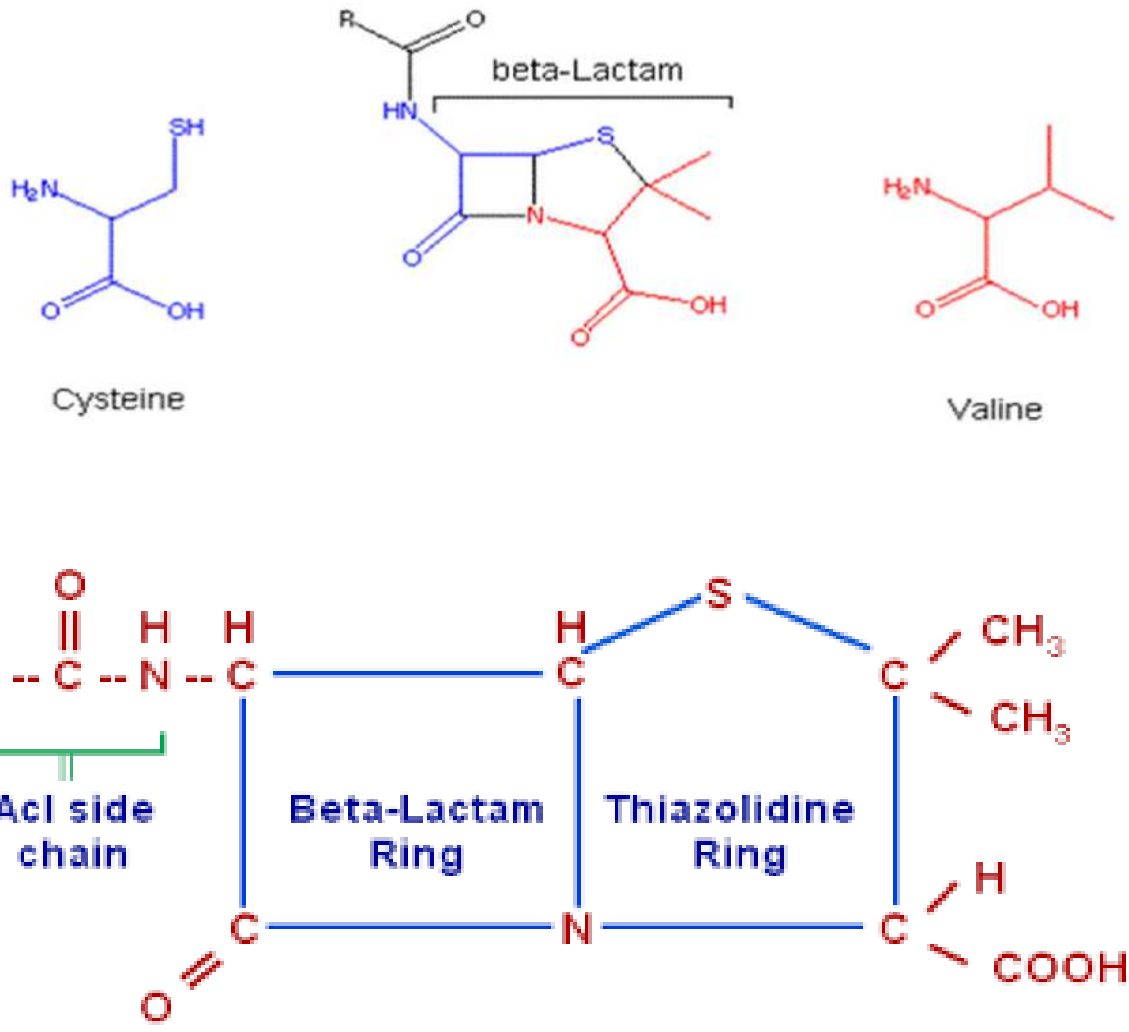
In penicillins, the β-lactam ring is fused to thiazolidine ring. Originally, penicillin was produced in the form of Penicillin G (benzylpenicillin) by fermenting *Penicillium* mold in the presence of phenyl acetic acid as a precursor. It has good activity against Gram-positive bacteria.



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).



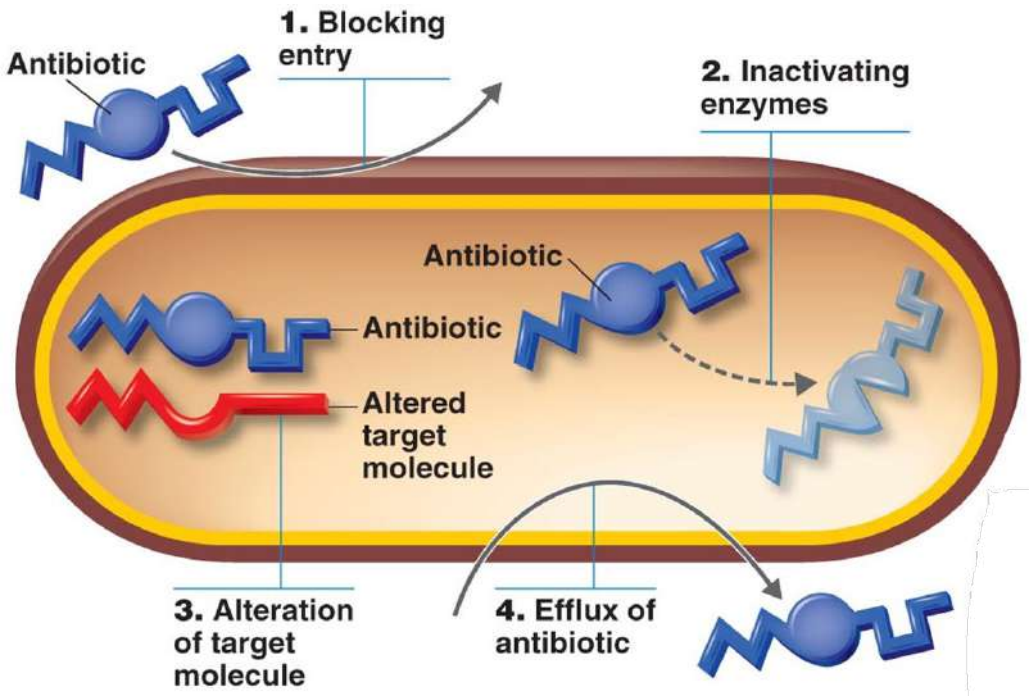
6-Aminopenicillanic acid (6-APA)

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.

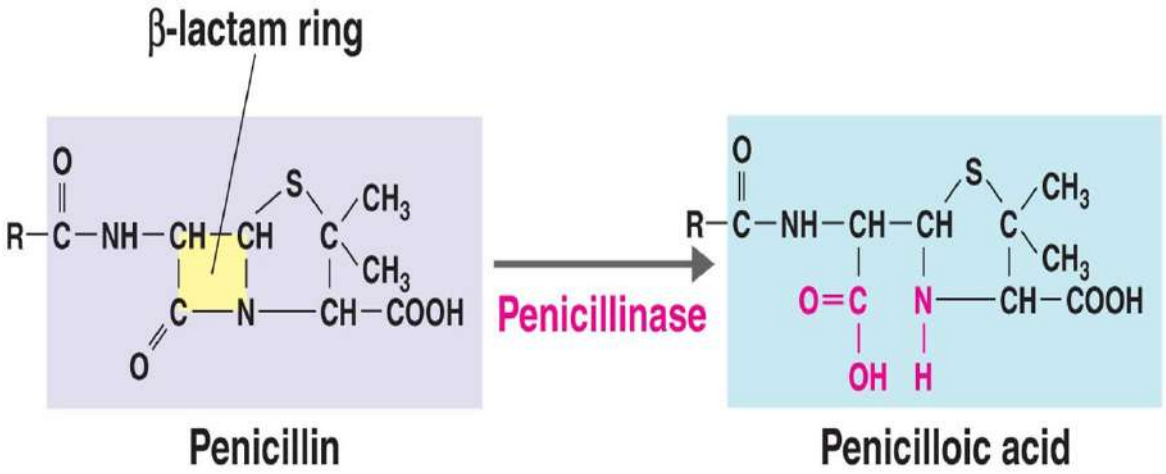
Mechanisms of antibiotic resistance

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.

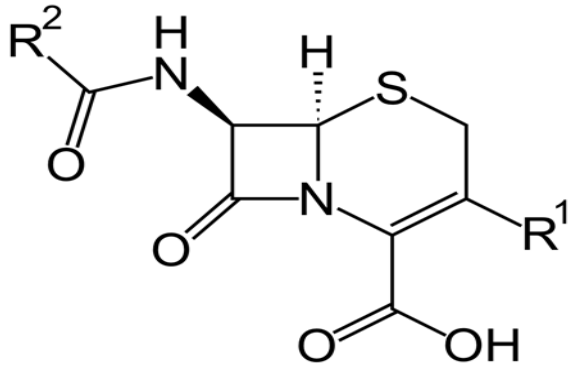


Penicillinase (β-lactamase): bacterial enzyme that destroys natural penicillins



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.

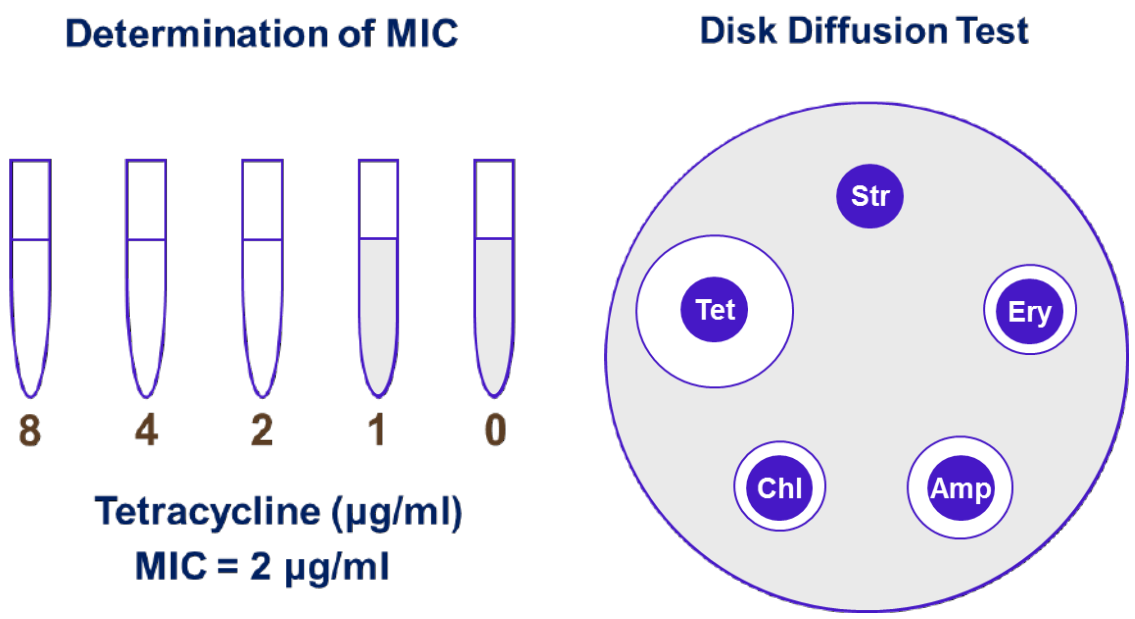
Susceptibility testing is performed on each type of bacteria or fungi that may be relevant to the individual's treatment and whose susceptibility to treatment may not be known. Each pathogen is tested individually to determine the ability of antimicrobials to inhibit its growth. This is can be measured 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.

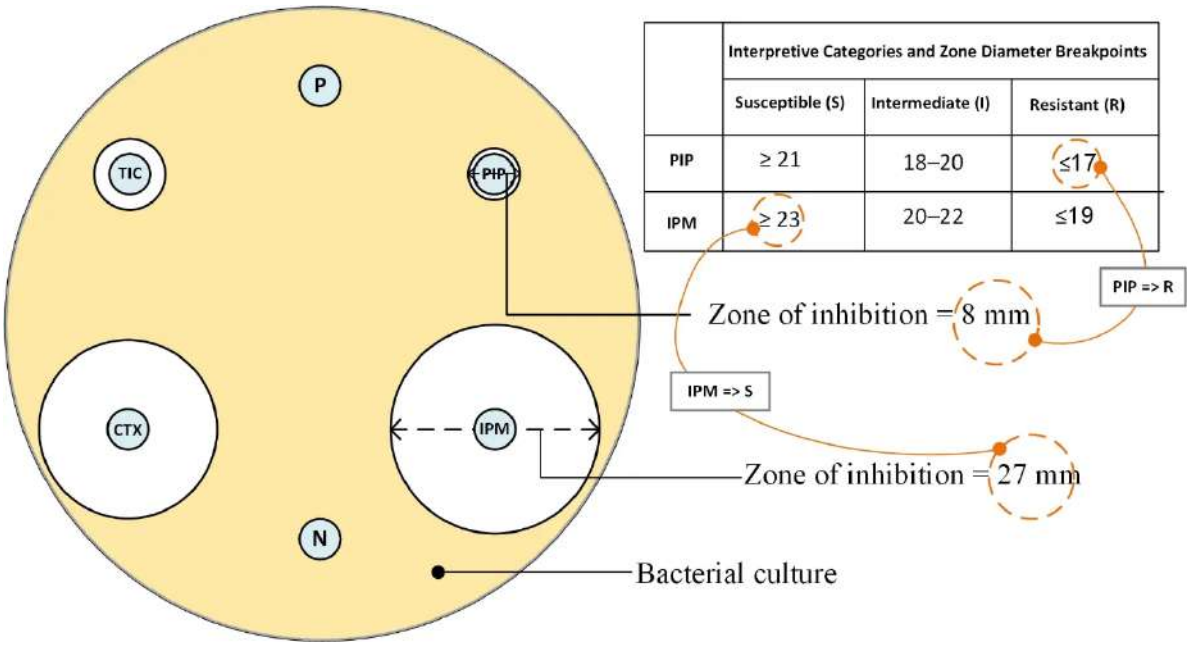
Susceptibility testing is used to determine which antimicrobials will inhibit the growth of the bacteria or fungi causing a specific infection. The results from this

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.

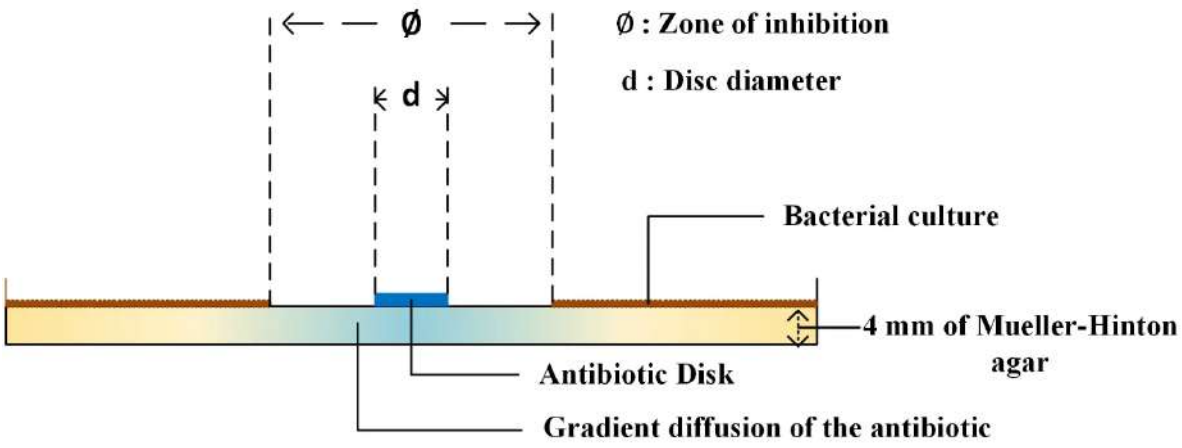




	Interpretive Categories and Zone Diameter Breakpoints		
	Susceptible (S)	Intermediate (I)	Resistant (R)
PIP	≥ 21	18–20	≤17
IPM	≥ 23	20–22	≤19

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.



جامعة جنوب الوادى
كلية العلوم بقنا
قسم علم النبات

KEY

For Identification OF Aspergillus& Penicillium And Other Genera

BY
Mycological Laboratory
Qena Univ. Faculty

2023/2024

Identification

K E Y T O GROUPS
Based primarily on morphology

I. sterigmata strictly uniseriate

A - C onidial heads clavate with spore masses splitting at maturity, in blue-green shades; vesicles strongly clavate ..... A- clavatus group

B. Conidial heads radiate to columnar, variable in color; vesicles variable, from globose or nearly so to subclavate or turbinate

1. Conidial heads radiate, variable in size, in bluish green or olive green shades (brown in one species); osmophilic bright yellow cleistothecia abundant in most species .. .. A. glaucus group

2. C onidial heads radiate to very loosely columnar, comparatively large, in grayish or yellowish green to olive-br shades; white to purplish or olive cleistothecia produced in three species ..... A. ornatus group

3. C onidial heads radiate (short columnar in one species), small, in pinkish fawn shades; cleistothecia lacking ..... A. cervinus group

4. Conidial heads loosely to definitely columnar, often long, thin and twisted, in green shades; conidia cylindrical when young; osmophilic; cleistothecia lacking ..... A. restrictus group

5. conidial heads compactly columnar, in pale gray-green to dark blue-green shades; conidial not cylindrical when young; not osmophilic ..... A. fumigatus group

A. Cleistothecia lacking ..... A fumigatus series

B. Cleistothecia present, white to yellowish.. fisheri series

II. Sterigmata biseriate, or uniseriate(the former predominat) or with both conditions in the same head

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A. Cleistothecia lacking ..... A fumigatus series

B. Cleistothecia present, white to yellowish.... fisheri series

II. Sterigmata biseriate, or uniseriate(the former predominat) or with both conditions in the same head

A. conidial heads usually globose when young, radiate or splitting in age, rarely losely columnar; vesicles globose to subglobose ..... or somewhat elongate; conidio-phores not constricted below the vesicle; sclerottia produced in many species

I. Conidial heads globose when young, sometimes remaining so but usually splitting into more or less well-defined columns at maturity

a. Conidial heads in yellow, buff, or ochraceous shades; conidiophores commonly roughened and often pigmented; cleistothecia in one species .....A. ochraceus group

b. Conidial heads in shades of black; conidiophores usually smooth and colorless or becoming pigmented below the vesicle ..... A. niger group

C. Conidial heads white or cream colored; conidiophores smooth and colorless .....A. candidus group

2- Conidial heads typically radiate with spore chains usually separate, sometimes forming poorly fefined columns

A. Conidial heads in yellow-green to deep olive-brown shades; conidiophores usually roughened, colorless A. flavas group

B. Conidial heads in yellow-brown to dull buff shades; Condidiophores smooth or delicately

A. wentii Group

roughened, coloyless or lightly pigmented ..A. wentii group

B. conidial heads large, radiate; vesicles strictly globose; conidiophores definitely constricted below the vesicles; sclerotia lacking

I. Conidial heads of one type, buff-brown, pale yellow-green or blue-green; conidiophores usually colorless smooth; o-smophilic; cleistothecia produced in two species

A. cremeus group

2- conidial sttructures of two types; large heads light gray, green , or olive-buff with conidiophores usually in brown shades and encrusted; fragmentary structures borne near or beneath the agar surgace .....A. sparsus group

III . Sterigmata strictly biseriate

A. Conidial heads typically in definite green shades; hulle cells usually globose but sometimes irregularly ovate to pyriform

I. Conidial heads typically radiate; becoming loosely columnar in some species: conidiophores colorless or light brown. commonly exceeding 300 u in length; vesicles variable elongate, subglobse, hemispherical, or only slightly expanded hulle cells sometimes abundant, more often limited or lacking .....A. versicolor group

a. Conidial heads uniformly pigmented, small or fragmentary structures sometimes present; hyphal masses or sclerotia occasionally produced .....A. versicolor series

b. Conidial heads not uniformly pigmented, both white and green heads present (at least on some substrates) .....A. janus series

2. Conidial heads typically columnar, usually dark yellow-green but occasionally gray blue-green or brownish; conidiophores brown walled, commonly less than 300 u long; vesicles supglobose, hemispherical, or terminally fattened; hulle cells typically, produced, usually abundant, clustered, forming crusts, or enveloping ascocarbs; cleistothecia common, purplish at maturity; ascospores in orange-red to blue-violet shades ....A. nidulans group

B. Conidial heads in shades other than true green, hulle cells when present, elongate to strongly curved and twisted

I. Conidial heads radiate to broadly columnar, in drab, olive, or dull brown shades; conidiophores typically brown-walled; vesicles variable from globose to elongate or hemispherical; hulle cells elongate, often strongly curved or twisted .....A. ustus group

2. Conidial heads broadly to irregularly columnar white to avellaneous or vinaceous; conidiophores with walls brown or uncolored; vesicles subglucose to elongate; elongate hulle cells or heavy-walled hyphal elements prelent .A. flavipes group

3. Conidial heads compactly columnar, typically in cinnamon to orange - brown or pale buff shades ; conidiophores colorless; vesicles hemispherical ....A. terreus group

KEY TO GROUPS

Based primarily on color

A. Conidial heads showing some shade of green during development .....B.

AA. Conidial heads in some other color .....L.

B. Vesicles clavate or subclavate; sterigmata uniseriate ..C.

BB. Vesicles not clavate; sterigmata uniseriate or biseriate.D

C. Vesicles strongly clavate; conidial heads blue-green, becoming gray in age .....A. clavatus group

CC. Vesicles subclavate; sterigmata uniseriate; conidial heads yellow-green gray-green, or blue-green when youngdarkening in most species .....A. ornatus group

D. Conidial heads bright yellow-green when young, sometimes becoming brown in age, loosely radiate; sterigmata biseriate in most species. .....A. fiavus group

DD. Conidial heads in other green shades; sterigmata uniseriate or biseriate .....E.

E.

E. Colonies mostly showing naked yellow cleistothecia and yellow or red encrusted hyphae .....A. glaucus group

EE. Colonies lacking naked yellow cleistothecia and yellow and red encrusted hyphae .....F.

F. Conidial heads definitely columnar.....G.

FF. Conidial heads globose, radiate, or loosely columnar.I.

G. Sterigmata uniseriate .....H.

GG. Sterigmata biseriate; globose to subglobose hulle cells common; cleistothecia in some species; ascospores orange red to violet.....A. nidulans group

H. Conidial heads columnar, long, narrow(often twisted) to irregular; conidia usually formed as cylindrical segments from the sterigmata; cleistothecia lacking; typically osmophilic.....A. restrictus group

HH. Conidial heads columnar, compact and typically uniform in diameter throughout; conidia not formed ar cylindrical segments; cleistothecia in some species; not typically osmophilic.....A. fumigatus group

I. Vesicles small, variable in shape.....J.

II. Vesicles large, strictly globose; conidiophores constricted below the vesicle.....K.

J. conidial heads blue-green, dull yellow-green, or gray blue-green, radiate to loosely columnar; hulle cells globose to subglobose .....A.versicolor group

JJ. Conidial heads olive, olive-gray, dark to light brown; radiate to broadly columnar; hulle cells elongate to twisted.....A.ustus group

K. Conidial heads graying in age from blue-green or olive-buff shades .....A. sparsus group

KK. Conidial heads pale yellow-green, blue-green, or buff-brown.....A. cremeus group see also A.wentii group)

L. Growth very sparse and sporulation poor on Czapek's agar .....

LL. growth and sporulation usually abundant on CZ pek. ager

ager .....M.

M. Heads loosely to compactly columnar.....N.

MM. Heads globose to radiate.....O.

N. Heads loosely columnar, white, flesh colored, or cream-buff.....A. flavipes group

NN. Heads compactly columnar, avellaneous to cinnamon.....

A. terreus group

O. Heads persistently white; larger heads definitely globose or radiate.....A. candidus group

OO. Heads not white.....p

P. Heads in yellow, ochraceous or light brownish shades .Q.

PP. Heads in black or dark brown shades ..A.niger group

Q. Heads in sulphur yellow to ochraceous shades A. ochraceus group

QQ. Heads in yellow-brown to dull buff shades .....

.....A.wentii group (alsoA.cremeus group in part)

Aspergillus clavatus Group

clavatus

group key

C onidial structures often I to 5 or more cm.in length....

.....A. giganteus Wehmer

Conidial structures not exceeding 4.0mm. in length .....

...A. clavatus Desm.

Conidial structures less than I.O MM In length .....

.....A. clavato-nanica Batista, Maia,& Alecrim

Aspergillus glaucus Group

Group Key

A. Asc ospores lenticular 6u or less in long axis, including ridges or crestg

(I) Ascospores with convex surfaces smooth or nearly so

a. Equatorial ridges lacking, furrow absent or showing, only as a trace

(I) Conldial heads large, radiate to loosely columnar, borne above the surface layer of cleistothecia and enveloping hyphae ... A. repens DeBary. *

(2) Conidial heads small, enmeshed with the cleistothecia in a felt of sterile hyphae .. A. peseudoglaucus Blochwitz

b. Equatorial ridges lacking but with furiow definite, appearing asa a narrow shallow depression; colonies yellow, not developing red pigmentation .. A. tonophilus ohtsuki

c. Equatorial ridges low and roundded, furrow broad and shallow colonies developing a strong red or orangered pigmentation .. A. rub er (Konig, spieckeimann Bremer) thom church .

d. Equetorial ridges thin and flexuous, crestlike (ascospore resemblingapulley) .. A. chevalieri (Mangin Thom& Church

(2) Ascospores with convex surfaces rough

a. Conidia smali, smooth walled; ascospoies with definite crests .. A. chevalieri Ver. intermedius Thom & Raper

b. Conidia small, echinulate; ascospores without crests but with prominent V-shaped furrow flanked by irregularr ridges.

(I) Colonies predominantly cleistothecial .. A. amstelodomi (hangin Thomy chueck

(2) coloniespredominankly conieial .. A. monteyidensis Talice machkinnon

(I) Conidia small, not exceeding 5.5 u in diameter .....
.....A. cristatus n. sp.

(2) Conidia large, exceeding 5.5 u in diameter.

(a) Ascospores 6.5 to 7.5 u in long axis. A. mangini Thom & Kaper

(b) Ascospores 7.5 to 8.5 u in long axis .....
.A. umbrosus Bainier & Sartory

(c) Ascospores 9.0 to I0.0 u in long axis.....
.....A. echinulatus (Delacr.) Thom & Church

B. Asci ripening more slowly, colonies favored by media containing 40 per cent sugar or more.

(I) Ascospores roughened in equatorial area with ridges and furrow.

(a) Ascospores 8.8 to 9.6 u by 6.0 to 6.8 u with ridges ~~relatively~~ thin and irregular; colonies developing brick red pigmentation on M 40y agar .A. medius to 7. 5u Meissner

(b) Ascspores5.5 u by 4.0 to 5.0 u (up to 9.0 by 7.0 u), with ridges low; colonies maize to buff yellow on Czapek agar with ca. 70 per cent sucrose.....
...A. halophilicus chistensen, papavizas,& Benjamin

(2) Ascospores usually without equatorial ridges and furrow...A. carnoyi (Biourge) Thom & Kaper

[illegible] DESCRIPTIONS

2. Conidial heads white.....A. niveo-glaucus Thom & Kaper

II. Cleistothecia absent, but coiled ascogonia abundant

A. Ascogonia producing naked clustered asci; conidial heads light brown .....A. athecius n. sp.

B. Ascogonia never producing asci; conidial heads green pro

ASPERGILLUS ORNATUS GROUP

GROUP KEY

I. Conidial heads yellow-green to brownish green. radiate; conidia citriform to elliptical; cleistothecia tyically produced.

A. Cleistothecia at first white and parenchymatous throughout, becoming purplish at maturity; produced in dark incubated cultures.

1. Ascospores with multiple thin flexuous crests.....
.....A. ornatus Raper, Fennell & Tresner

2. Ascospores with a single equatorial ridge(two adherent crests?) ....A. citrisporus (Von Hohnel) Raper, Fennell Tresner

B. Cleistothecia white to olive, lacking a definite wall, surrounded by loosely interwoven fine hyphae; ascospores large, spiny, without equatorial furrow or ridges .....
.....A. spinulosus Warcup n. sp.

II. Conidial heads light grayish blue-green, loosely columnar or radiate; conidia elliptical; sclerotia or compact sclerotium-like masses of hulle cells typically present and produced more abundantly in dark-incubated cultures.

A. Sclerotia present; heads columnar; conidiophores and conidia delicately roughened..A. paradorus Fennell & Raper

B. Hulle cell masses present; heads usually radiate; conidiophores and conidia smooth ..A. raperi Stolk

III. Conidial heads at first dark blue-green then brownish black, radiate; conidia globose, strongly spinulose to irregularly warty.....A. brunneo-uniseriatus Singh & Bakshi

Aspergillus ceruinus Group

GROUP KEY

I. Heads radiate

A. Conidiophores exceeding IOO, erect to terminally recurved

I. Conidiophores usually IOO to 300 ,in length, walls very thick, I.5 to 2.0 ,; heads erect.....
....A. cervinus (Massee) emend. Neill

2. Conidiophores extremely variable, IOO to 800 u, in length; large heads usually erect, smaller heads often nodding ...A. kanagawaensis Nehira

B. Conidiophores not exceeding IOO in length; vesicles upright or borne at an angle ..A. parvulus Smith

II. Heads columnar

A. Conidiophores and vesicles strongly pigmented, vesicles borne at acute angles.....
.....A. nutans Mclennan & Ducker

Aspergillus restrictus Group

GROUP KEY

I. Heads columnar; vesicles small, flask shaped. domelike or only gradual enlargements of the conidiophore apices fertile on the upper surface only

A. Conlonies 4 to 5 cm. in diameter in 3 weeks at 26 C on standard Czapek'S agar ..A. caesiellus saito

KEYS AND SPECIES DESCRIPTIONS

B. Colonies less than I.5 cm. at 3 weeks on Czapek's agar

I Rapidly growing on M 40 y agar, dark olive green; columns long, often twisted, adherent in fluid mounts .....
...A. restrictus smith

2.less rapidly growingon M40y agar, light gray green,columns more delicate

a. conidia elliptical when first formed, often remaining so,mostly 4,5uby3,0to 3.5uchains adherent in fluid mounts.....A. conicus Blochwitz

b. conidia subglobose to pyriform, mostly 3.0 to 3.5u in diameter, chains not adherent in fluid mounts.....
... .A. gracilis Bainier

II. Heads radiate when young, tardily becoming loosely or irregularly columnar; vesicles subglobose to pear-shaped, fertile over the upper half to two-thirds.....
.....A. penicilloides spegazzini

Aspergillus Fumigatus Group

GROUP KEY

Cleistothecia absant .....A. fumigatus series

A. Conidial heads erect, compart, and strongly columnar; vesicles commonly 20to 30u in diameter, upright on the conidiophore

I. conidiophores 0.5 mm.or less; conidial heads dark green; conidia globose echinulate .....A. fumigatus Fresenius

2. Conidiophores exceeding 0.5 mm.; heads light yellow-green; conidia elliptical, smooth or nearly so.....
.. ....A. fumigatus varellipticus, n.var.

B. Conidial heads often presenting a nodding appearance. smaller than the preceding and not consistently columnar; vesicles less than 20u in diameter

I. Vesicles upright but with sterigmata often borne in lateral or basal clusters to give a pseudonodding appearance..... ....A. unilateralis Thrower

2. Vesicles often borne at an angle to the conidiophore

a. Conidiophores thin walled, sinuous; vesicles uncolored and often strongly nodded, conidia in pale blue green shades.....A. viridi-nutans Ducker& Thrower

b. Conidiophores heavy walled; vesicles and sterigmata colored ; conidia in dark blue- green shades

(I) Conidia conspicuously echinulate; colony reverse uncolored or nearly so ....A. duricaulis, n.sp.

(2) Conidia finely spinulose; colony reverse in reddish brown to deep rose shades ...A. brevipes Smith

II. Cleistothecia present .....A. fischeri series

A. Cleistothecia and enveloping hybhae white to cream in color

I. Ascospores showing two distinct equatorial crests

a. Convex surfaces bearing anastomosing ridges.....

.....A. ficheri wehmer

b. Convex surfaces smooth (ornearly so) .....

..... A. ficheri var.glaber Fennell & raper

C. Convex surfaces spinulose or echinulate .....

....A. Ficheri var. spinosus, n. var.

2. Ascospores showing more than two equatorial crests

a. ascospores showing 4equatorial crests.....

.....A. Quadricinctus Guill

B. cleistothecia and enveloping hyphae in yellow, golden or orange shades

I. Colonies loose textured, growing rapidly on all media, cleistothecia large

a. Ascospores with prominent equatorial crests and

Cconvex surfaces conspicuously echinulate .....
.....A. aureolus Fennell & raper

2. Ccolonies close textured , growing very restrictedly on czapek's agar, cleistothecia small

a. ascospores with prominent equatorial crests and convex surfaces delicately echinulate.....
..... A. stramenius Novak & kaper, n. sp.

BB. Ascospores with low equatorial crests and convex surfaces finely reticulate or spinulose..A. auratus Warcup. n. sp.

Aspergillus Ochraceus Group

ochraceus group

GROUP KEY

I. CConidial heads in pale pure yellow shades

A. Sclerotia cream to pale yellow, produced in a dense layer, 300 to 450 u in diameter; conidial heads loosely radiate, spore chains adherent into narrow divergent columns.....A. sulphureus(Fres.) Thom & Church

B. Sclerotia white to cream to pale pink, produced singly, I.0 to I.5 mm. in diameter; conidial heads hemispherical to loosely columnar, or split into two or more compact .....
.. columns..A. sclerotiorum Huber

II. CConidial heads in bright golden yellow shades

A. Sclerotia black at maturity, vertically elongate,I to 3mm. high (at several months containing multiple cleistothecia); conidia smooth, oval to subglobose,2.5 to 4.0 bu 2.0 to 3.5u; c... ...h, conedial heads changing. to cinnamon buff in age...A. alliaceus Thom & C hurch

B. Sclerotia orange to rufous, globose to subglobose, 500 to 700 u in diameter, conidia heavy walled, smooth , elliptical or ovate, 3.3 to 4.4 u by 2.5 to 3.0u;

Conidial heads remaining bright in age ...A. auricomus (Guéguen) saito

III. Conidial heads in dull yellowish cream, buff, or ochraceous shades

A. Sclerotia produced in most strains

I. Sclerotia abundant, small, commonly 400 to 500u

a. Sclerotia pure yellow then brown; conidia globose, subglobose or elliptical, 2.75 to 3.5 u or 3.0 to 3.3 u by 2.5 to 2.8 u .....A. melleus Yukawa

2. Sclerotia scattered, developing late, large, commonly 500 to I000 u

a. Sclerotia pink to vinaceous purple when mature, globose, ovate to cylindrical; conidia globose to subglobose, mostly 2.5 to 3.0 u ...A. ochraceus Wilhelm

b. Sclerotia cream to buff or clay colored, globose to ovate; conidia elliptical to pyriform 4.0 to 5.0 u by 3.0 to 3.5 u .....A. ostianus Wehmer

c. Scmerptoa wjote tp cream, ovate to discoid; conidia ovate to elliptical, mostly 3.2 to 4.0 u by 2.8 to 3.2 u.....A. elegans Gasperini

B. Sclerotia unknown

I. Colonies [illegible] close textured, sporulating slowly conidial heads pinkish buff; conidia subglobose ovate or elliptical, mostly 3.0 to 4.0 u by 2.5 to 3.0 u..... .....A. petrakii Voros

Aspergillus niger Group

GROUP KEY

I. Sterigmata in two series

A. Colonies (conidial heads) on Czapek's agar appearing carbon black to the naked eye

I. Conidia 6 to IO u in diameter at maturity ..A. carbonarius (Bainier) thom

2. Conidia 5.0 u or less in diameter at maturity

a. Conidiophores not exceeding 4.0 mm. in length

(I). Colonies spreading rapidly on Czapek's agar.....A. ficuum (Reich.) Hennings

(2). Colonies growing mores slowly on Czapek's agar

(a). Conidia at maturity horizontally flattened, mostly 3.0 to 3.5 u in diameter, with longitudinal color bars or striations ....A. phoenicis (Cda.) Thom

(b). Conidia at maturity globose, mostly 4.0 to 5.0 u irregularly roughened with conspicuous ridges and echinulations not arranged as longitudinal striations.....A. niger V. Tiegh .

b. Conidiophores commonly exceeding 5 mm. and reaching I cm. but also commonly with shorter stalk bearing diminutive heads .....A. pulverulentus (Mcalp.) Thom

B. Colonies (Conidial heads) grayish olive brown or deep olive brown when young; usually becoming reddish brown to brownish black, but with olive or grayish colors often persistent.

(I). Heads quickly dark black-brown or reddish brown a.

a. Conidia under 5.0 u in diameter, horizontally flattened, and appearing striate at maturity

(I) Heads quickly dark black- brown; colony reverse uncolored; conidiophores mostly 2 to 3 mm. but up to 5.0 mm. long; conidia mostly 3.0 to 3.5 u in diameter.....A. tubingensis(Schober) Moss.

(2) Heads quickly reddish brown; colony reverse in similar shades; conidiophores mostly I.0 to 1.5 mm. long; conidia mostly 4.0 to 4.5 u in diameter..... .....A. awamori Nakazawa

b. Conidia 6.0 to 8.0 u in diameter, globose to subglobose, coarsely tuberculate ....A. flavo-furcatis Batista and Maia (see A. flavus group)

2. Heads persistently dark grayish brown or olive-brown

a. Conidia at maturity elliptical, conspicuously echinulate, 5.0 to 5.5 u by 3.3 to 3.8 u.....A. ellipticus, sp . nov.

b. Conidia at maturity globose or nearly so, sometimes elliptical when young

(I) Conidia at maturity conspicuously spinulose..... .....A. heteromorphus Batista and Maia

(2) Conidia at maturity irregularly and finely roughened

(a) Conidial heads generally small, in age on malt agar splitting into fairly numerous compact divergent columns....A. foetidus (Naka.) Thom and Raper

(b) Conidial heads large, columns few and poorly defined on malt agar

(I) Basal mycelium on malt agar uncolored or only faintly yellow..A. foetidus(Naka.) T. & R. var. N ., S.,&W.

(2) basal mycelium on malt agar bright golden yellow .....A. foetidus (Naka.) T.& R.var.acidus .N S.,& W.

II. Sterigmata uniseriate

A. Conidia globose to subglobose, conspicuously echinulate; vesicles commonly 20 to 35 u but ranging from I5 to 45 u .....A. japonicus saito

B. Conidia subglobose to definitely elliptical, conspicuously echinulate; vesicles commonly 60 to 80 u ranging from 35 to 100 u ...A. A. aculeatus Iizuka

Aspergillus flavus Group

GROUP KEY

I. Conidial heads in pale to intense yellow or yellow-green shades when young

A. Colonies not shifting to brown on Czapek's agar; conidia definitely echinulate

1. Sterigmata either single or double with the latter predominant; heads radiate or very loosely columnar ..... A. flavus Link

2. Sterigmata typically in a single series

a. Heads colunar; sterigmata usually uniseriate..... A. flavus var. columnaris, N. var.

b. Heads radiate; sterigmata uniseriate..... A. parasiticus Speare

B. Colonies shifting to light brownish green in age on Czapek's agar; conidia irregularly roughened or smooth

1. Conidia large, mostly 4.5 to 7.0 u but up to 8.0 u or 10.0 elliptical at first, then globose to subglobose, smooth to irregularly roughened

a. Conidiophores borne primarily from the substrate ..... A. oryzae(Ahib.) Cohn

b. Conidiophores borne primarily as short branches from aerial hyphae....A. oryzae(Ahlb.) Cohn Var. effusus (Tiraboschi) Ohara

2. Conidia small, oval to elliptical, mostly 3.0 to 3.5u by 2.4 to 3.0 u smooth or nearly so

a. Growth negligible on Czapek's agar; conidial structures abundant, zonately arranged on ~~mall agar coni~~ diophores smooth or nearly so ...A. zonatus Kwon & Fennell, n. sp.

b. Growth spreading on both Czapek's and malt agars; conidial structures often forming coremiform clusters; conidiophores conspicuously roughened.....A. clavato-
.... flavus, N. sp

II. Conidial heads in deep yellow-green to olive-brown shades when young; conidia conspicuously verruculose

A. Conidial heads at first deep yellow-green, shifting to brownish green or brown on czapek's agar .....
.....A. tamarii Kita

B. Conidial heads quickly olive'brown then dark brown..
....A. furcatis Batista& Maia

III. Conidial heads in pale yellowish olive or grayish olive shades; conidia smooth or nearly so

A. Conidiophores conspicuously échinulate...A. subolivaceus,n.sp.

B. Conidiophores smooth or nearly so..A.avenaceus smith

Aspergillus wentii Group

GROUP KEY

I. Conidiophores smooth or granulose

A. Conidial heads large, up to 500 u or more in diameter, on smooth or slightly granular stalks which may reach several millimeters in length...A. wentii Wehmer

B. Conidial heads smaller and borne on shorter stalks

I. Sterigmata mostly double, few single; both single and double sterigmata often observed in the same head

a. Conidia mostly 5.5 to 6.5 u coarsely echinulate...
.....A. terricola Marchal

b. Conidia mostly 4.5 to 5.5 by 3.8 to 5.0 u, rugulose.
.....A. terricola var. americana Marchal

2. Sterigmata almost entirely single, rarely double, and then at the base of the vesicle in otherwise uniseriate heads ....A. terricola var. indicus(Mehrotra&Agnihotri) n. comb .

II. Conidiophores conspicuously echinulate.....
.....A. thomii Smith

Aspergillus cremeus Group

GROUP KEY

I. Conidial heads in light green shades
A. Sterigmata biseriate; cleistothecia present .....
....A. cremeus Kwon & Fennell,n. sp.
B. Sterigmata initially uniseriate. becoming biseriate progressively from the base of the vesicle upward; dark stroma'like hyphal masses present..... ...
.....A. stromatoides, n. sp.
C. Sterigmata uniseriate; dark hyphal masses and cleistothecia lacking...A. itaconicus Kinoshita

II. Conidial heads in light brown shades
A. Sterigmata biseriate; cleistothecia present .....
.....A. chrysellus Kwon & Fennell, n. sp .
B. Sterigmata mostly uniseriate, occasionally biseriate; cleistothecia absent...A.flaschentraegeri Stolk

Aspergillus sparsus Group

GROUP KEY

I. Sterigmata in two series
A. Conidia usually of one type
1. Conidia in large heads light yellow'green to olive-buff, subglobose to elliptical, delicately roughened.....A. sparsus Raper & Thom

2. Conidia in large heads darker green, globose, conspicuously echinulate; similar conidia produced from fragmentary structures at agar surface ..A.biplanus, spnov .

B. Conidia of two types

I. Conidia in large heads gray , slightly elliptical, smooth or nearly so; conidia from low level fragmentary structures, green,globose, conspicuously roughened .....A. diversus, sp. nov.

II. Sterigmata in a single series

A. Conidia in heads borne on long stalks at first elliptical and smooth, those subsequently formed subglobose to globose, brown and coarsely roughened as are the conidia of short stalked and fragmentary heads ....A. funiculosus Smith

Aspergillus versicolor Group

GROUP KEY

I. conidial heads of one color....A.versicolor series

A. Vesicles globose to somewhat elongate, fertile over most of the vesicular surface; globose to subglobose hullecells often present; compact hyphal masses and sclerotia lacking

I. Mature conidia not exceeding 4.0 u consistently globose to subglobose

a. Conidiophores uncolored to faintly yellowish

(1) Conidial heads variable in color, light yellow-green, buff to orange'yellow, or occasionally flesh colored..... A. versicolor(Vuill. tiraboschi

(2) Cnidial heads always blue'green when young.....
....A. sydowi(Bain.&Sart.) Thom and Church

b. Conidiophores definitely brown

(I) Conidial heads radiate, very dark yellow-green; conidiophore walls smooth; Hulle cell masses conspicuous.....A. silvaticus Fennell & Raper

(2) Conidial heads radiate; very dark blue-green; conidiophore walls definitely rough; hulle cells lacking..... .A. pulvinus Kwon & Fennell. n. sp.

(3) Conidial heads variable in shape, often loosely columnar; conidiophore walls smooth but with knobby encrustments on malt agar...A. speluneus. n. sp.

2. Mature conidia usually exceeding 4.0 u globose, subglobose, or elliptical

a. Conidiophores colored in brown shades

(I) Conidia strictly globose, echinulate

(a) Conidial heads pale gray-green, globose to somewhat elongate Hulle cells in small colorless clusters .: .....A. granulosus Raper & Thom

(b) Conidial heads dark yellow-green globose to elliptical hulle cells in conspicuous hyaline to purplish masses.....A. caespitosus Raper& Thom

(2) Conidia of two types; elliptical and smooth; globose and rough .....A. asperescens Stolk

b. Conidiophores uncolored in wet mounts; conidia 4.0 to 5.0 u in long axis ...A. varians Wehmer

B. Vesicles turbinate, spathulate, or merely slight expansions of the conidiophore apices, fertile on the apex only; hulle cells lacking, or if present, pyriform to elongate; compact hyphal masses or sclerotia present

I. Soft masses of white to cream-colored, compacted, thin-walled or heavy-walled cells present, sometimes in lim-

a. Stalks long, 1 to several cm.; smooth; conidia mostly oval to elliptical, smooth or very slightly roughened; abundant sclerotium-like masses composed of globose, elongate, or pear-shaped elements resembling hulle cells ....A . malodoratus Kwon & Fennell. n. sp.

b. Stalks less than 400 u delicately roughened; conidia globose, 4 to 7 u in diameter with long echines; sclerotium-like masses sparsely produced, composed of thin-walled cells and surrounded by short sterile hyphae..... .....A. crystallinus Kwon & Fennell. n. sp.

2. True sclerotia present, cream to buff; vesicles turbinate, often borne at a slight angle to the conidiophore

a. Conidial heads dark yellow-green; conidiophores up to 600 u long; conidia globose, minutely asperulate, mostly 2.5 to 3.0 u in diameter ...A. peyronelii sappa

b. Conidial heads gray-blue-green, conidiophores up to 350u long; conidia globose to subglobose, smooth or nearly so, mostly 2.2 to 2.8 u in diameter ...A. arenarius,n.sp.

II. conidial heads of two colors; green or white ....A. JANUS series

A. Vesicles of green and white heads dissimilar

1. Vesicles of white heads conspicuously clavate, 45 to 60u by 15 to 18 u borne on conidiophores usually exceeding 2 mm. in length ....A. janus Raper& Thom

2. Vesicles of white heads not conspicuously clavate,20 to 25 u by 14 to 18 u borne on conidiophores less than 2.0 mm. in length.....A. janus var. brevis Raper& Thom

B. Vesicles of green and white heads essentially similar

I. Conidia from both white and green heads smooth walled and of similar dimensions ....A. allahabadii Mehrotra & Agnihotri

2. Conidia from green heads rugulose and larger than the smooth conidia of white heads

a. Colonies on malt agar producing cushion-like overgrowths of thick-walled hyphal elements .....
.....A. ambiguus Sappa

b. Colonies on malt agar notproducing overgrowths .....
.....A. microcysticus Sappa

Aspergillus nidulans Group

GROUP KEY

I. Ascospores present

A. Ascospores orange-red in color

I. Equatorial crests two in number, rarely lacking. not exceeding 2.0 u in width

a. Convex walls smooth

(I) Equatorial crests lacking ....A. nidulans var. acristatus Fennell& Raper
.....

(2) Equatorial crests present

(a) Conidial stage dark yellow-green, promiment on malt agar, generally arising from submerged mycelium.

(1.) Coarse, encrusted, spicular hyphae absent

(1.) Crests entire, 0.5 to I.3 u wide ...A. nidulans (Eidam) Wint

(2.) Crests entire, I.5 to 2.0 u wide

(i') Crests entire, I.5 to 2.0 u wide

(i") Cleistothecial envelope in dull shades consisting of hulle cells only .....A. nidulans var, latus Thom & Raper

(2.) Cleistothecial envelope consisting of hulle cells associated with abundant mycelium in bright yellow to

red- orange shades.....A. heterothallicus Kwon, Fennell,& Kaper, n. sp .

(c) Crest dentate.....A. nidulans var. dentatus Sandhu& Sandhu

(2) Coarse, encrusted, spicular hyphae present; cleistothecia rare .....A. unguis(Emile-weil& Gaudin) Thom & Kaper

(b) Conidial stage green , inconspicuous on malt agar, genrally arising from aerial mycelium

(1) Cleistothecia borne in mycelial tufts with few accompanying hulle cells

(a) Cleistothecia 50 to 100 u associated mycelium heavily encrusted, silvery in apperance .... A. fruticulosus n. sp.

(b) Cleistothecia less than 50u associated mycelium mostly unbranched, not encrusted ....A. parvathecius, n.sp.

(2) Cleistothecia obscured by a nearly continuous layer of hulle cells ...A. aurantiobrunneus (A., H., & K.)n. comb.

b. Convex walls not smooth

(1) walls echinulate .... A. nidulans var. echinulatus Fennell & Kaper

(2) Walls coarsely rugulose .....A. rugulous Thom & Kaper

2. Crests two in number, 3.0 u or more in width.

a. Crests dissected, stellate.....A. variecolor(B&B.) Thom & Kaper

b. Crests entire .....A. variecolor var. astellatus Fennell & Kaper

3. Crests four or more in number,

a. Crests four in number, narrow .....A. quadrilineatus Thom & Kaper

b. Crests multiple, sometimes irregularly arranged and

A. Stellatus

giving the impression of striations .....A. striatus Kai. Tewari & Mukerji

B. Ascospores blue-violet in color, with convex surfaces echinulate .....A. violaceus Fennell& Raper

II. Ascospores not present

A. Conspicuous coarse encrusted spicular hyphae present; hulle cells lacking except in one unique strain which has been known to produce occasional cleistothecia .... .A. unguis (E.W.&.G.) Thom & Raper

B. Conspicuous spicular hyphae absent

I. Hulle cells absent or limited in number

a. Hulle cells absent, yellow mycelium prominent

(i) Conidiophores short, straight; conidial heads loosely columnar to somewhat divergent; arising from a yellow submerged mycelium .....A. aureolatus Hunt. Cvet.& Bata

(2) Conidiophores short, curved or coiled;conidial heads columnar, enmeshed in a prominent yellow aerial mycelium ....A. recurvatus, n. sp.

b. Hulle cells very limited in number, scattered, seldom exceeding IO to I2 u in diameter ...A. speluneus, n. sp. (see A. versicolor group

2. Hulle cells aggregated in scattered and irregular masses suggestive of cleistothecia; not produced on malt agar ..... A. caespitosus Raper & Thom (see A. versicolor group)

3. Hulle cells a bundant, scattered throughout the mycelial felt; conidiophores very short ...A. subsessilis, n . sp.

4. Hulle cells abundant, massed to form continuous crusts on malt agar

a. Hulle cell crust blue-gray, colonies restrictedly growing ....A. crustosus,n. sp.

b. Hulle cell crust reddish purple, colonies rapidly spreading .....A. multicolor sappa

c. Hulle cell crust bronze .....A aeneus sappa

d. Hulle cell crust golden yellow ..A. silvaticus Fennell & Raper (see A. versicolor group)

e. Hulle cell crust white to creamy white, conidial heads few in number and developing tardily ....A. eburneo-cremeus sappa

Aspergillus ustus Group

GROUP KEY

======

I. Vesicles upright on the conidiophores

A. Conidial heads in olive-gray to drab or red-brown shades

1. Conidial heads variable, radiate when young to loosely or broadly columnar at maturity

a. Hulle cells typically present, scattered throughout the colonuY or forming irregular masses not associated with pigmented mycelium ...A. ustus(bain.) Thom & church

b. Hulle cells abundantly produced, forming conspicuous masses associated with brightly pigmented yellow mycelium .....A. puniceus Kwon & Fennell. n. sp.

2. Conidial heads persistently radiate

a Conidial heads reddish brown (near wood brown); hulle cells elongate, twisted, in tufts of red mycelium ....A. panamensis Raper & Thom

b. Conidial heads buffy olive; hulle cells elongate, seldom bent, associated with yellow mycelium ....A. conjunctus Kwon & Fennell, n . sp .

II. Vesicles borne at a sharp angle to the vertical axis of the conidiophore .....A. deflectus Fennell& Raper

Aspergillus flavipes Group

GROUP KEY

==

I. Cnidiophores definitely pigmented in yellow to light brown shades

A. Conidial heads usually white to very pale buff, in occasional strains darker near avellaneous .....
.....A. flavipes (Bain.& sart.) Thom& church)

II. Conidiophores unpigmented or very faintly yellowed

A. Conidial heads persistently white ... A. niveus Blochwitz

B. Conidial heads at first white, becoming vinaceous fawn ..;.....A. carneus(V. Tiegh.) Blochwitz

Aspergillus terreus Group

GROUP KEY

=========

I. Colonies velvety; conidial heads long, compactly columnar, in cinnamon to orange-brown or brown shades; borne on short conidiophores

A. Sclerotium-like masses of swollen, relatively heavy-walled cells lacking on malt and agar....A. terreus Thom

B. Sclerotium-like masses present on malt agar .....
.....A. africanus Fennell & Raper

II. Colonies floccose, aerial mycelium conspicuously golden yellow, conidial heads small, compactly columnar, cream to buff; borne on conidiophores to 500 or more long..... A .....A. terreus var. aureus Thom & Raper

Practical physiology of fungi

For 4th year of B.Sc. students

Prepared by

Prof. Dr. Abdelrahman Saleem

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.

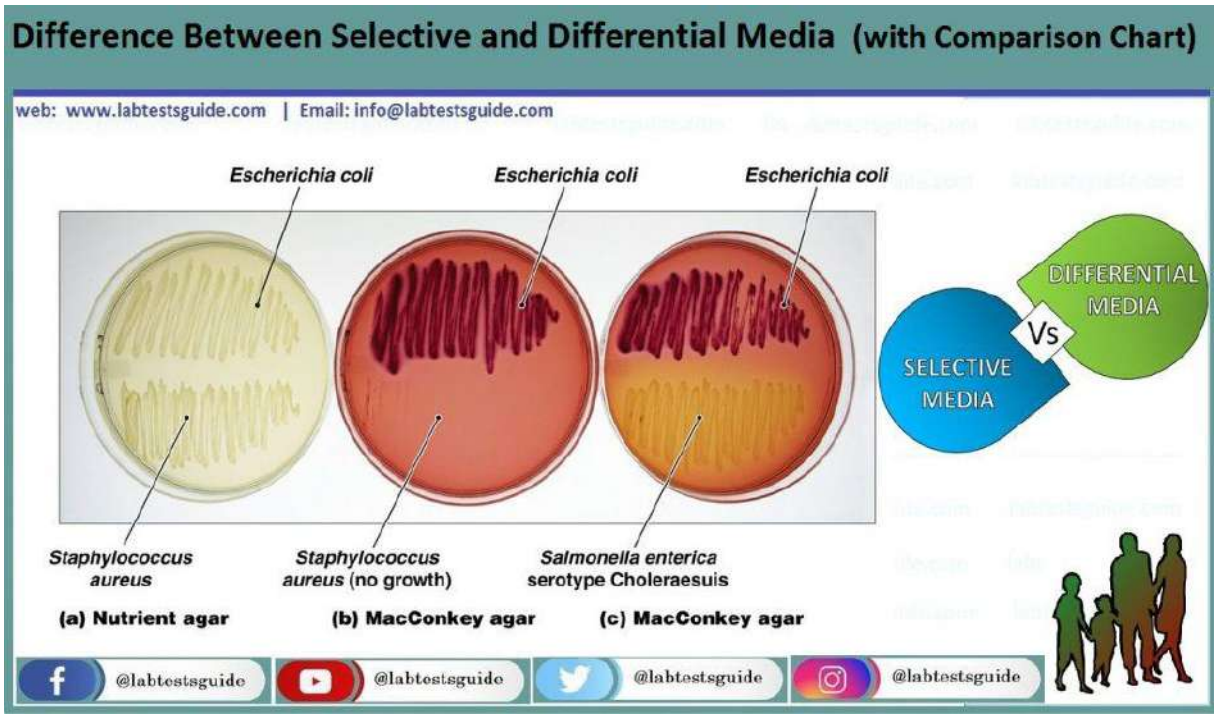
I- Classification of culture media according to their use

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

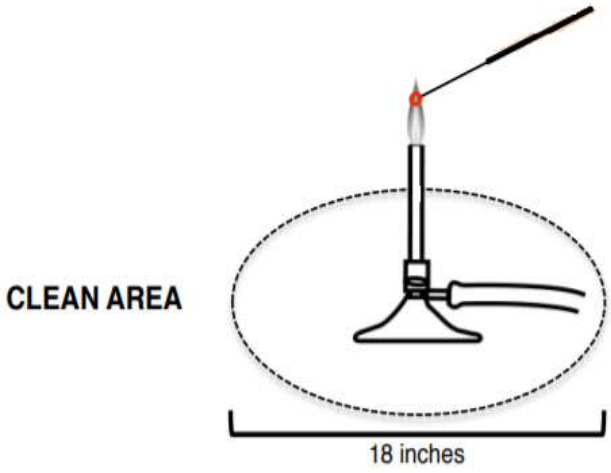
1- Physical methods

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.

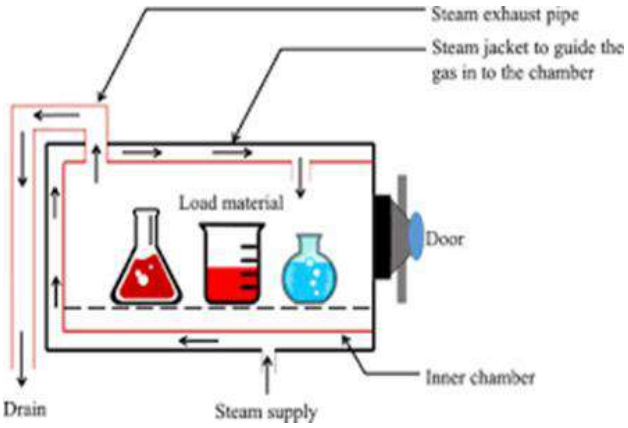
Application of dry heat

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

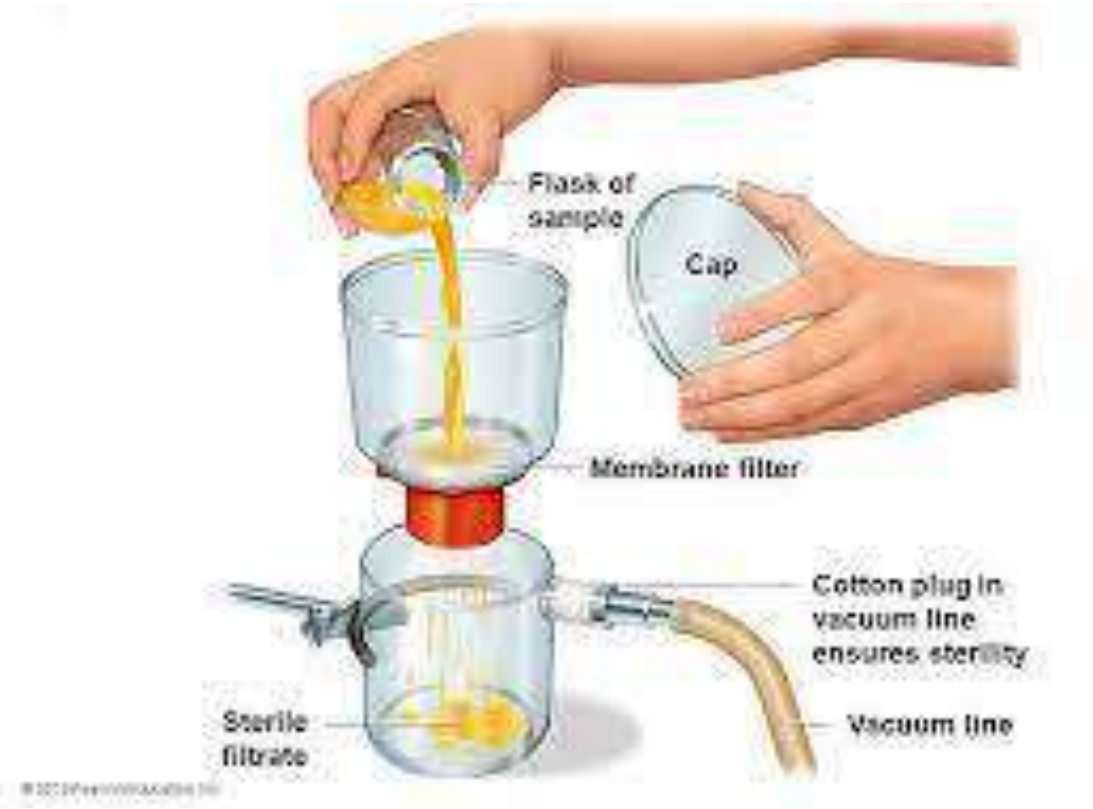
2- Chemical methods

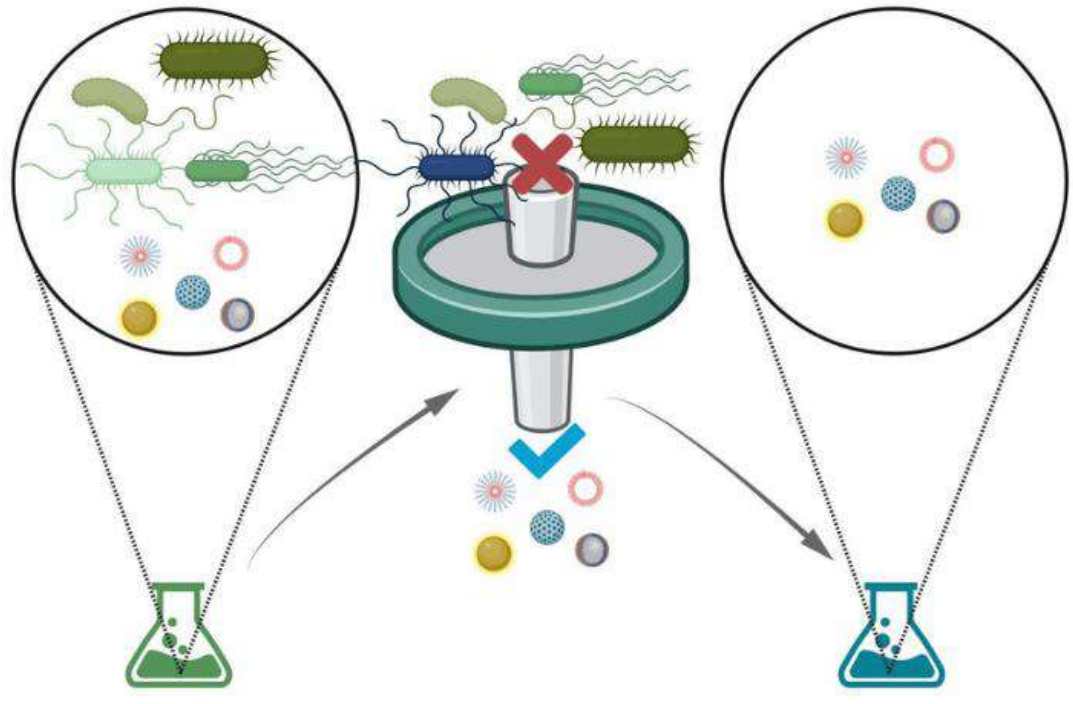
Using of chemical substances as agents, like chloroform, mercuric chloride, formaldehyde and ethyl alcohol.

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: …….

……..…….

Fungi	Counts	%	OR

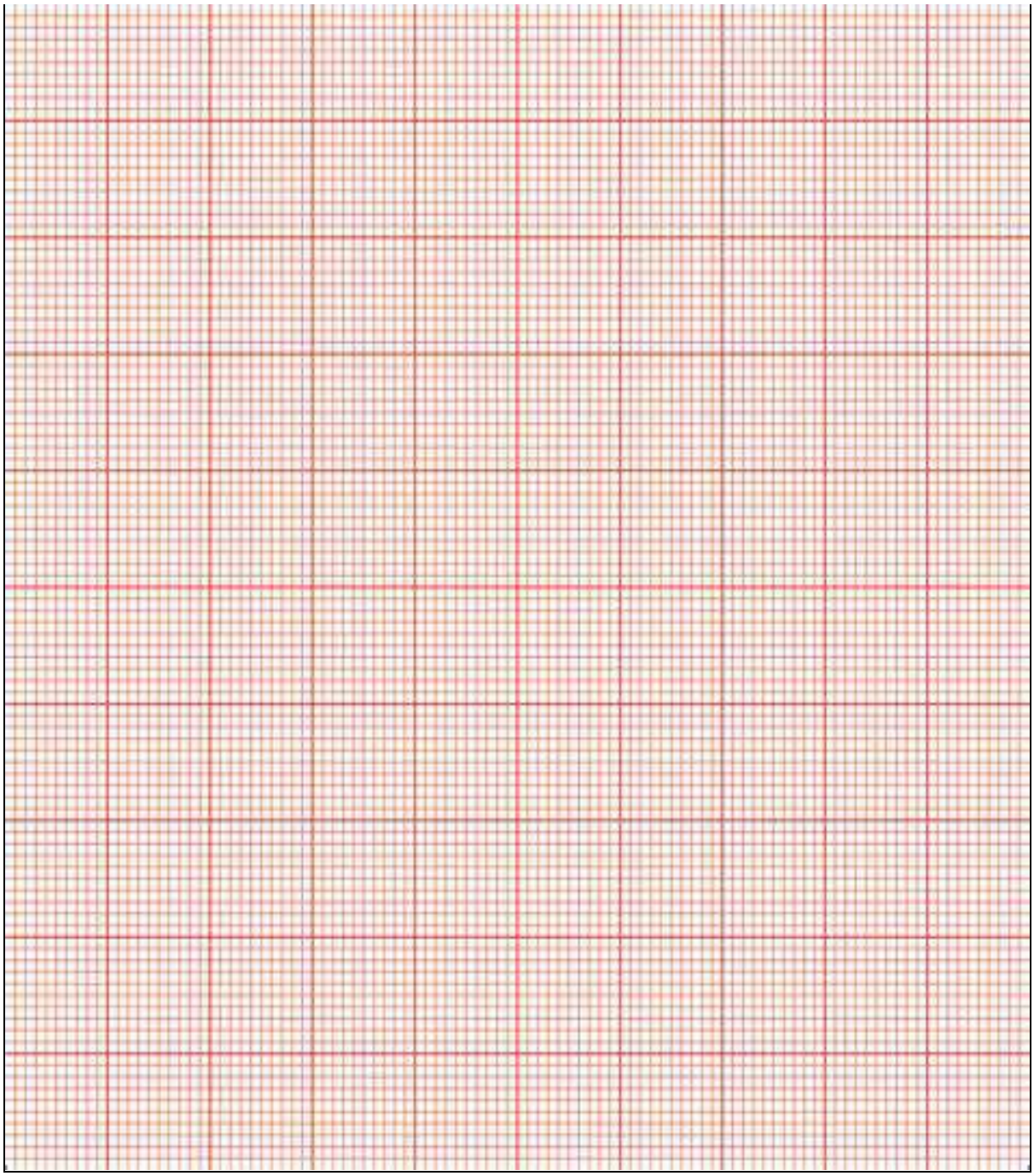


Figure 1: ……..

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Comment

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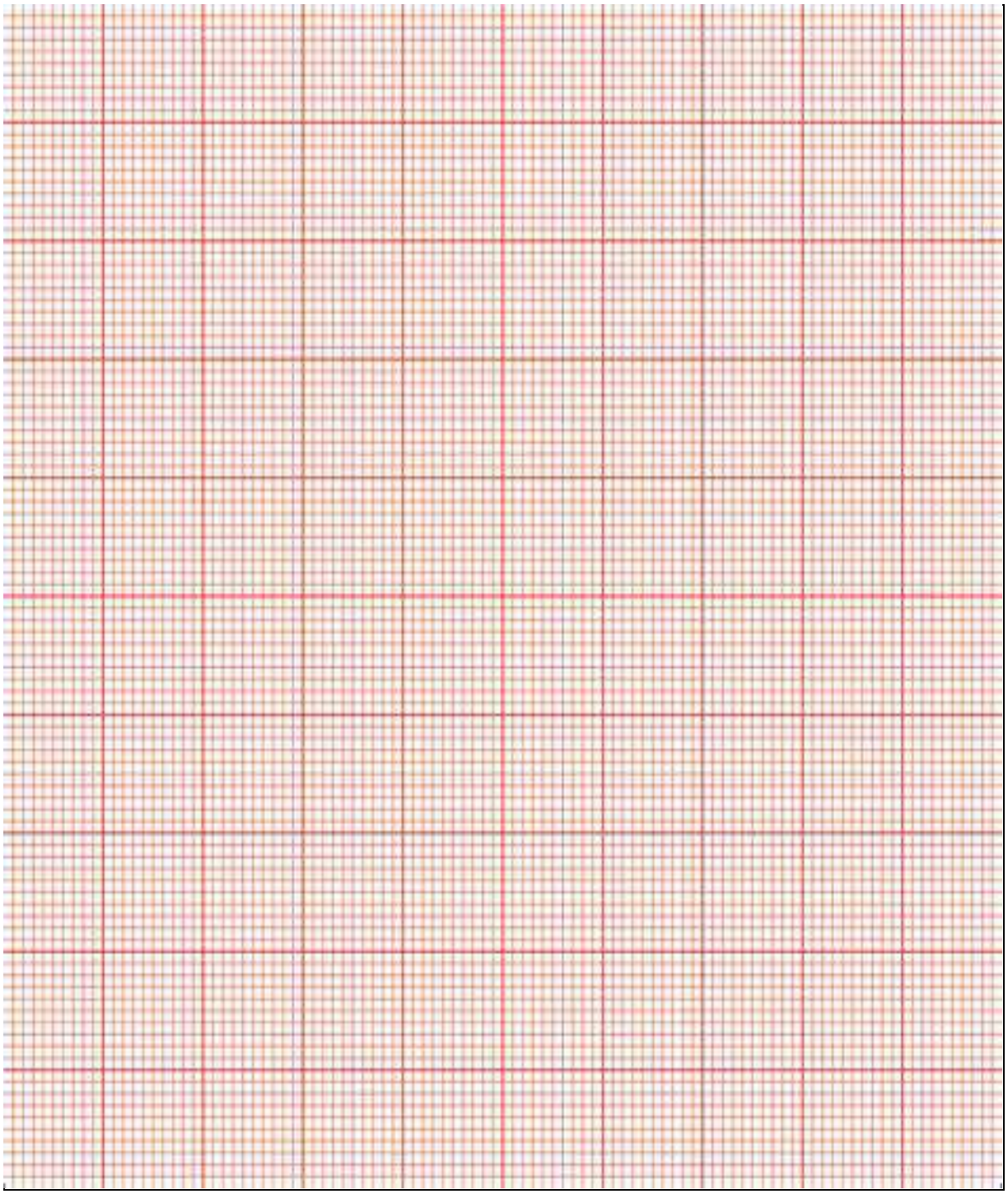
Temperature	*Aspergillus*	*Fusarium*	*Rhizopus*
15			
20			
25			
30			
35			
40			



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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*
2			
3			
4			
5			
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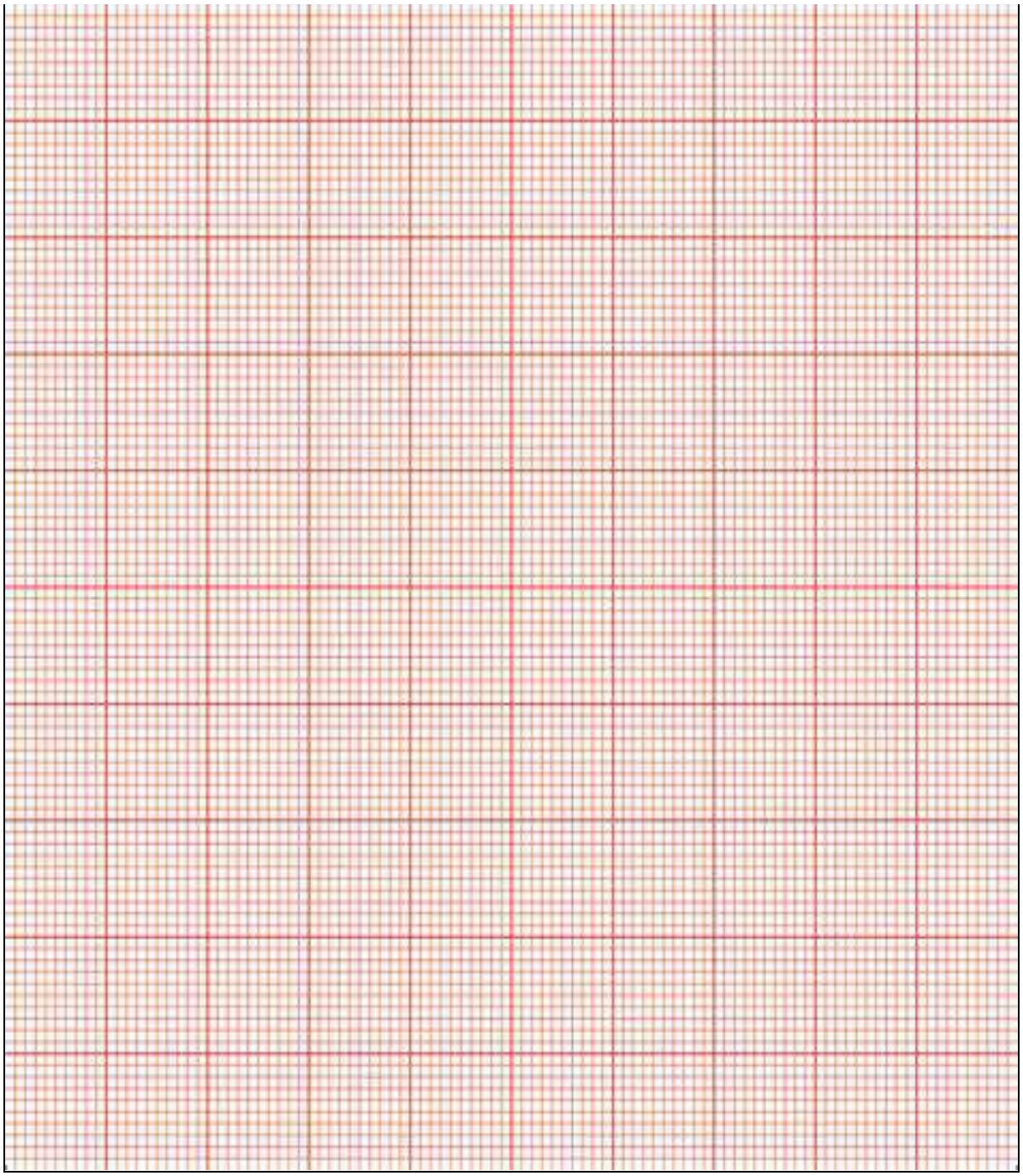

Figure 3: ……..

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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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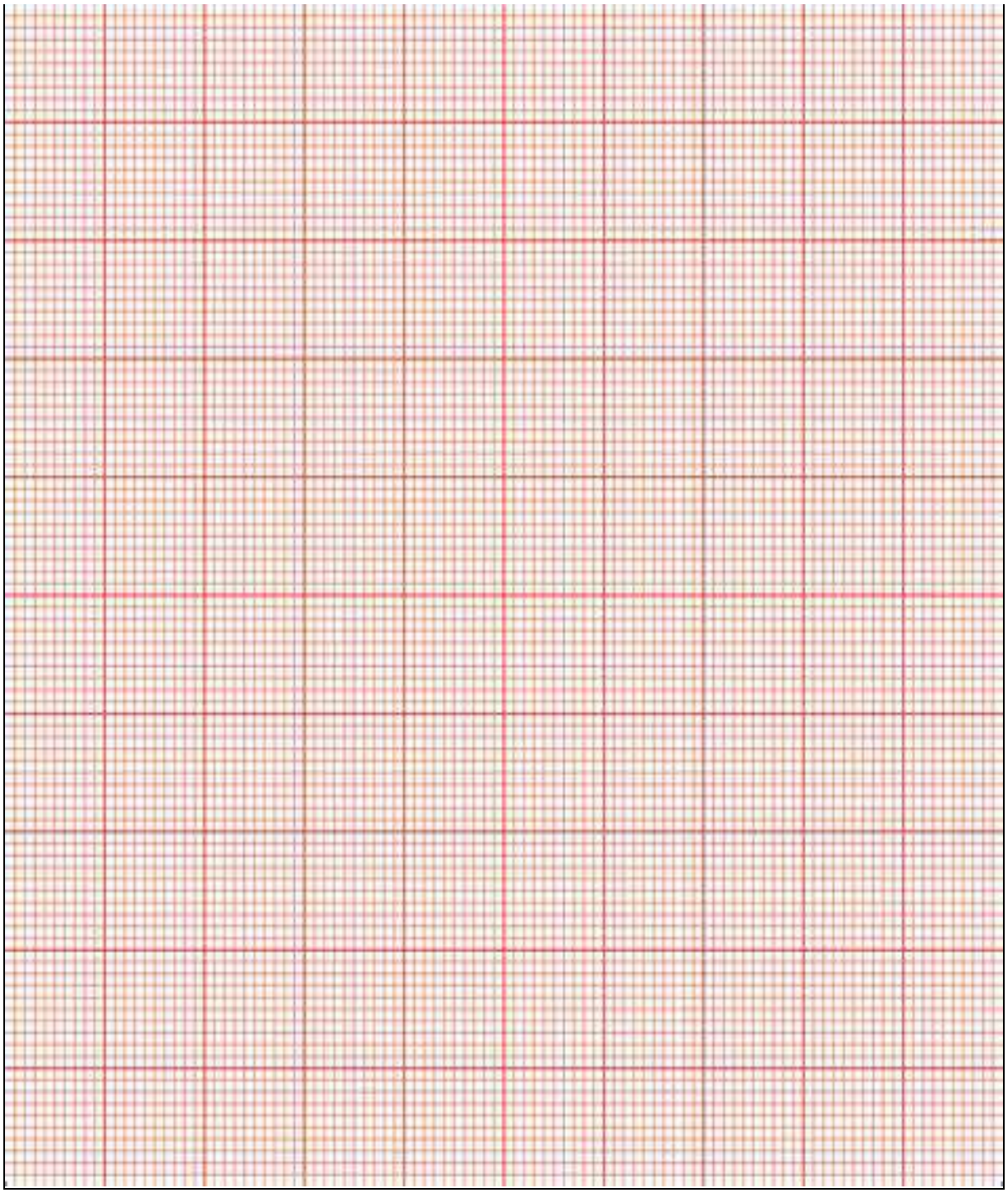
Carbon sources	*Aspergillus*	*Fusarium*	*Rhizopus*



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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*

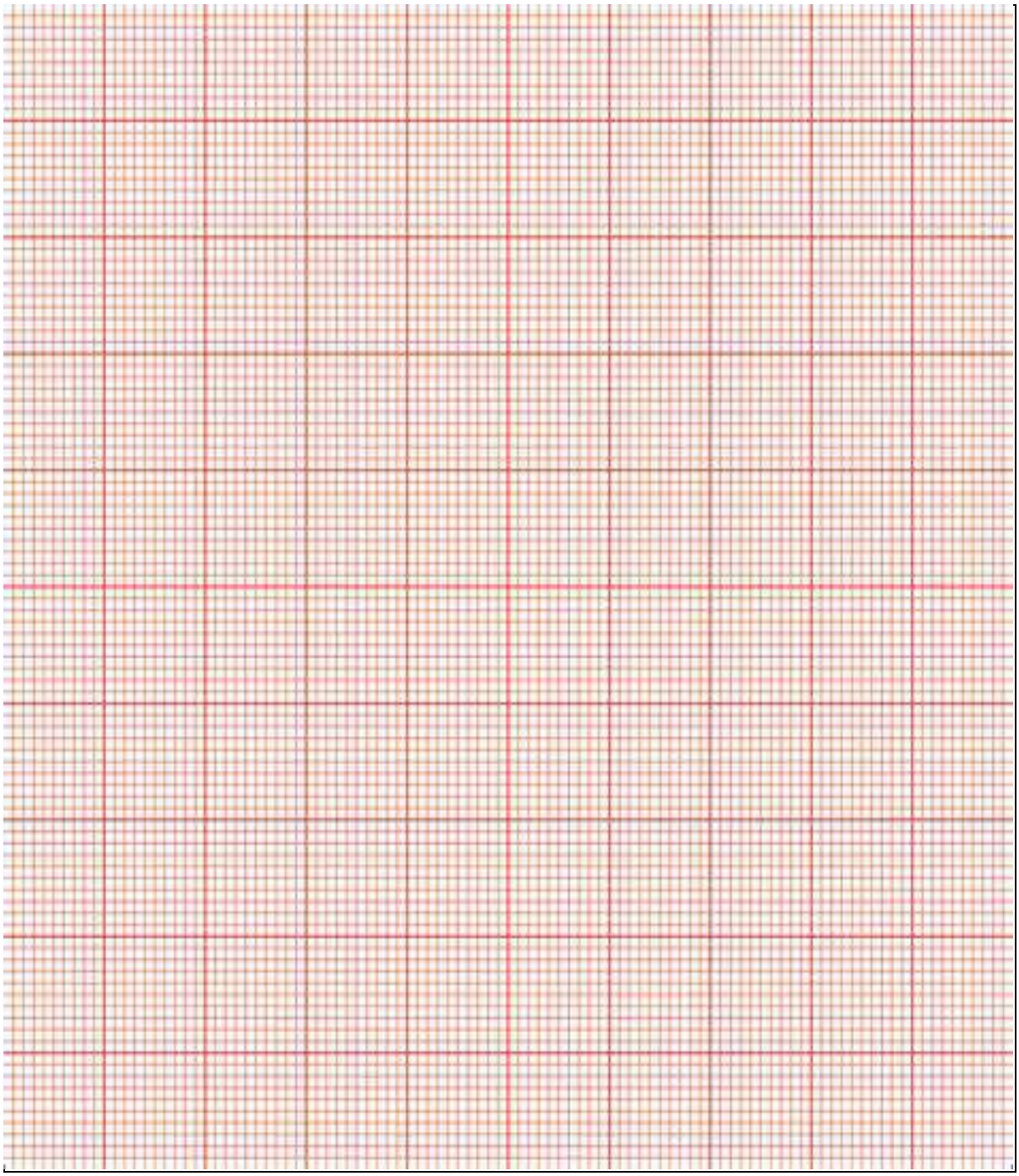

Figure 5: ……..

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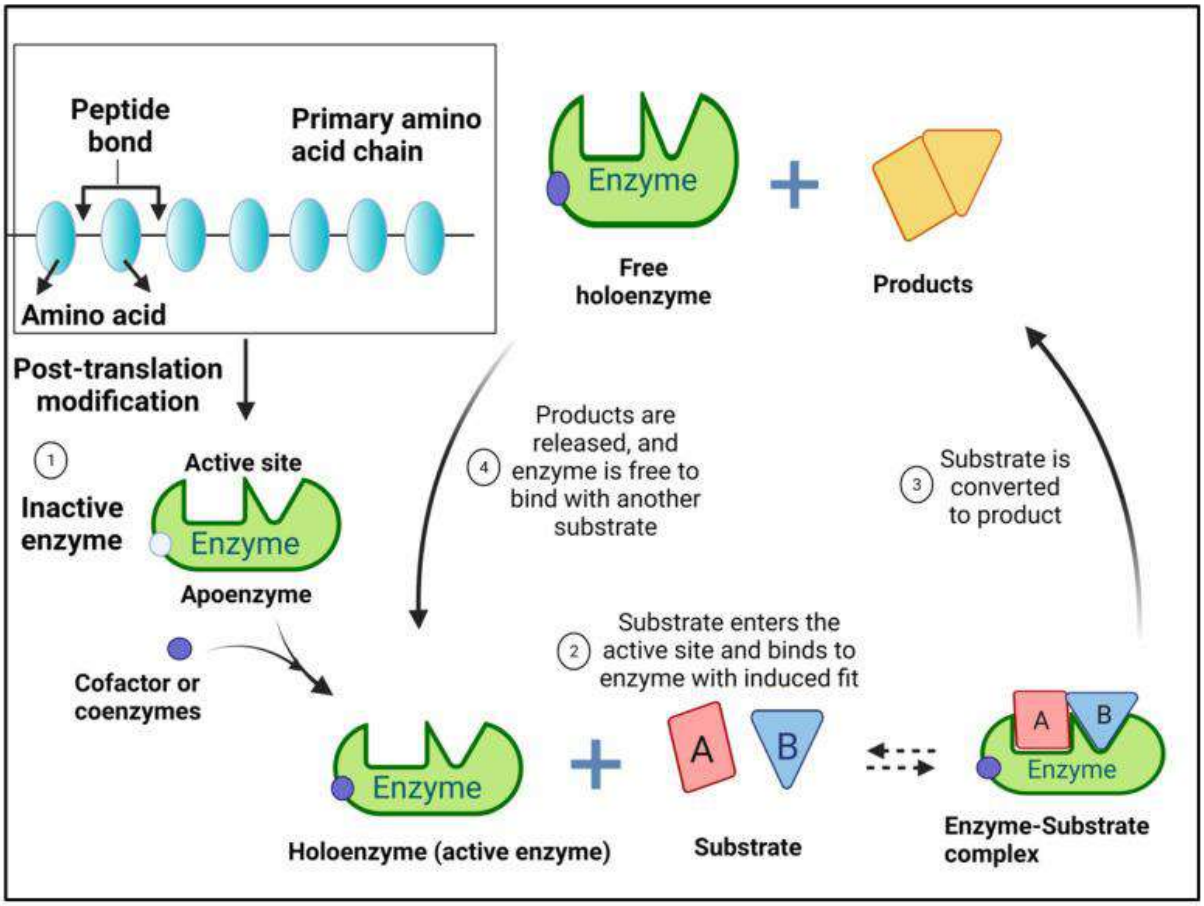
Experiment 6

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

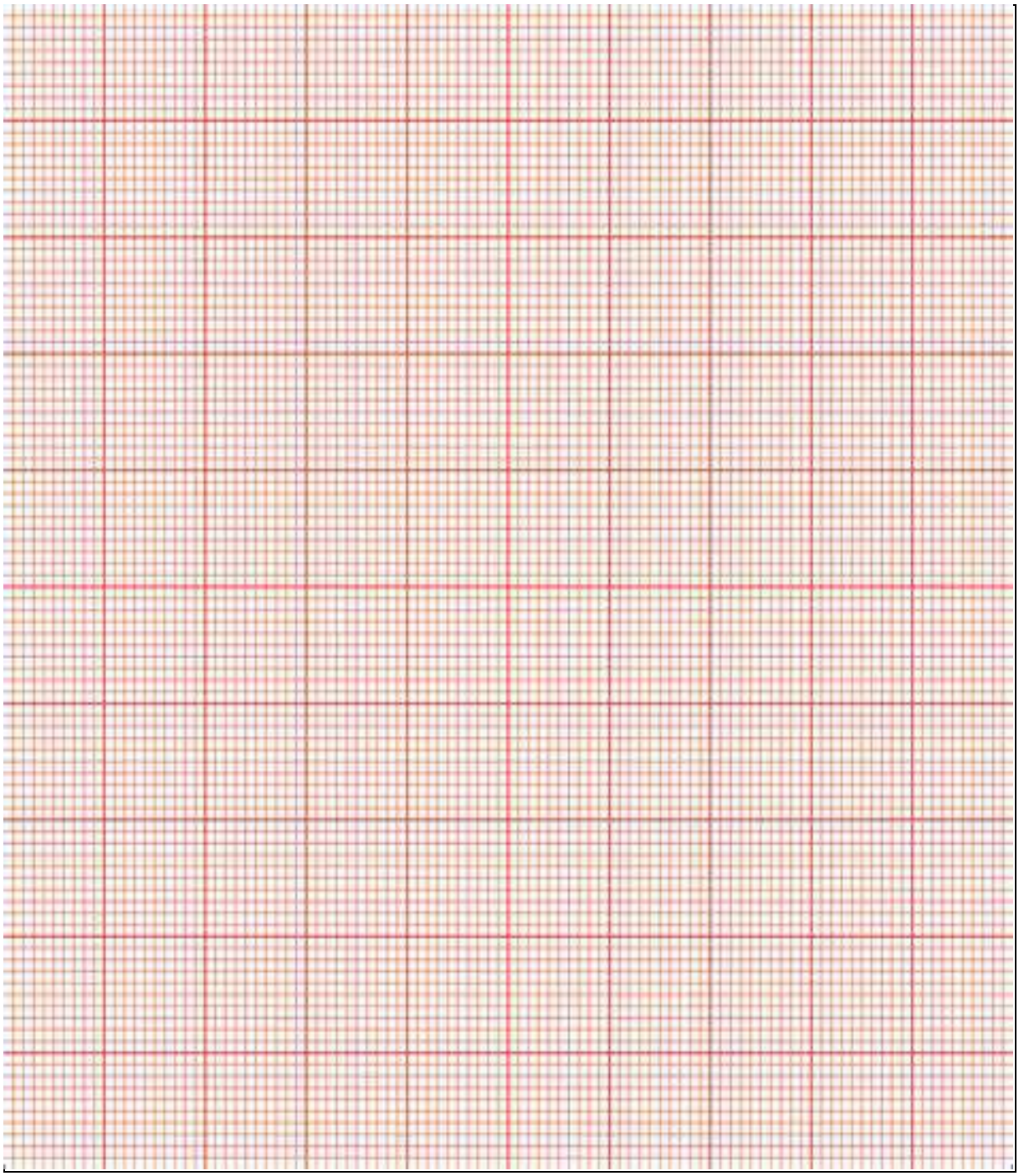

Figure 6: ……..

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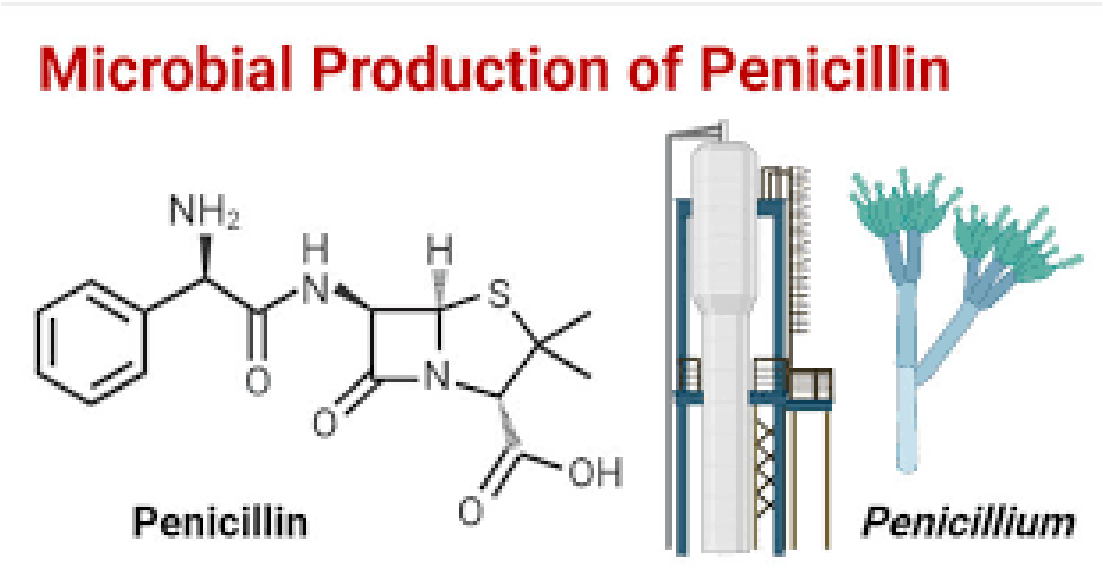
Experiment 7

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.

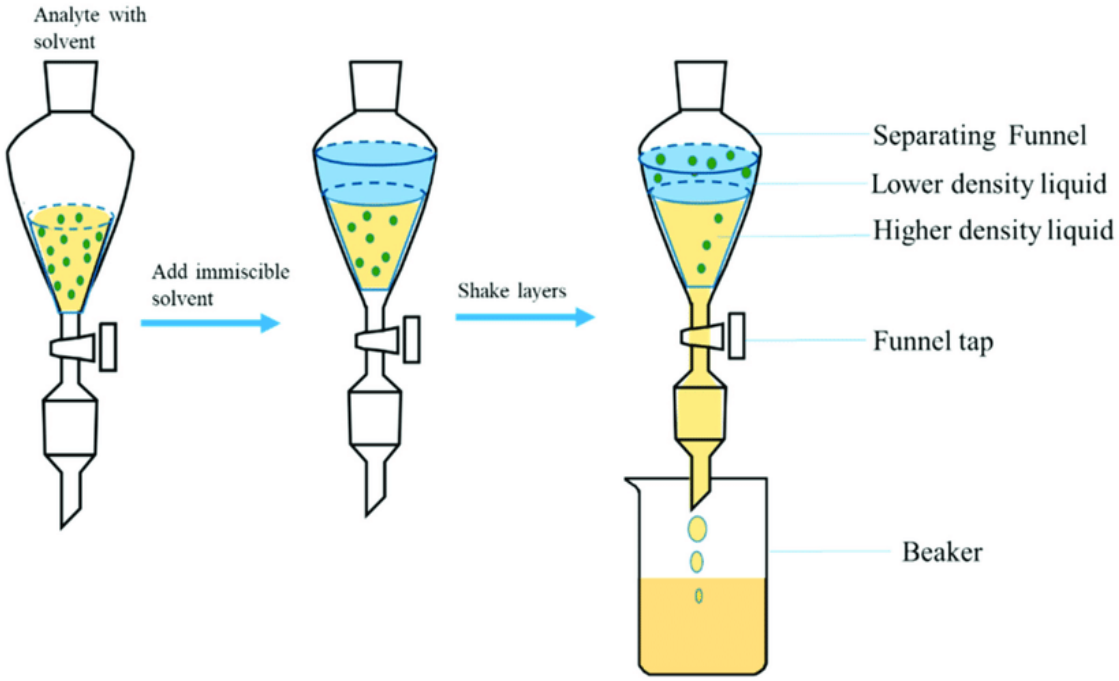


Table 7:……..……..…

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Penicillium strains	Penicillin activity
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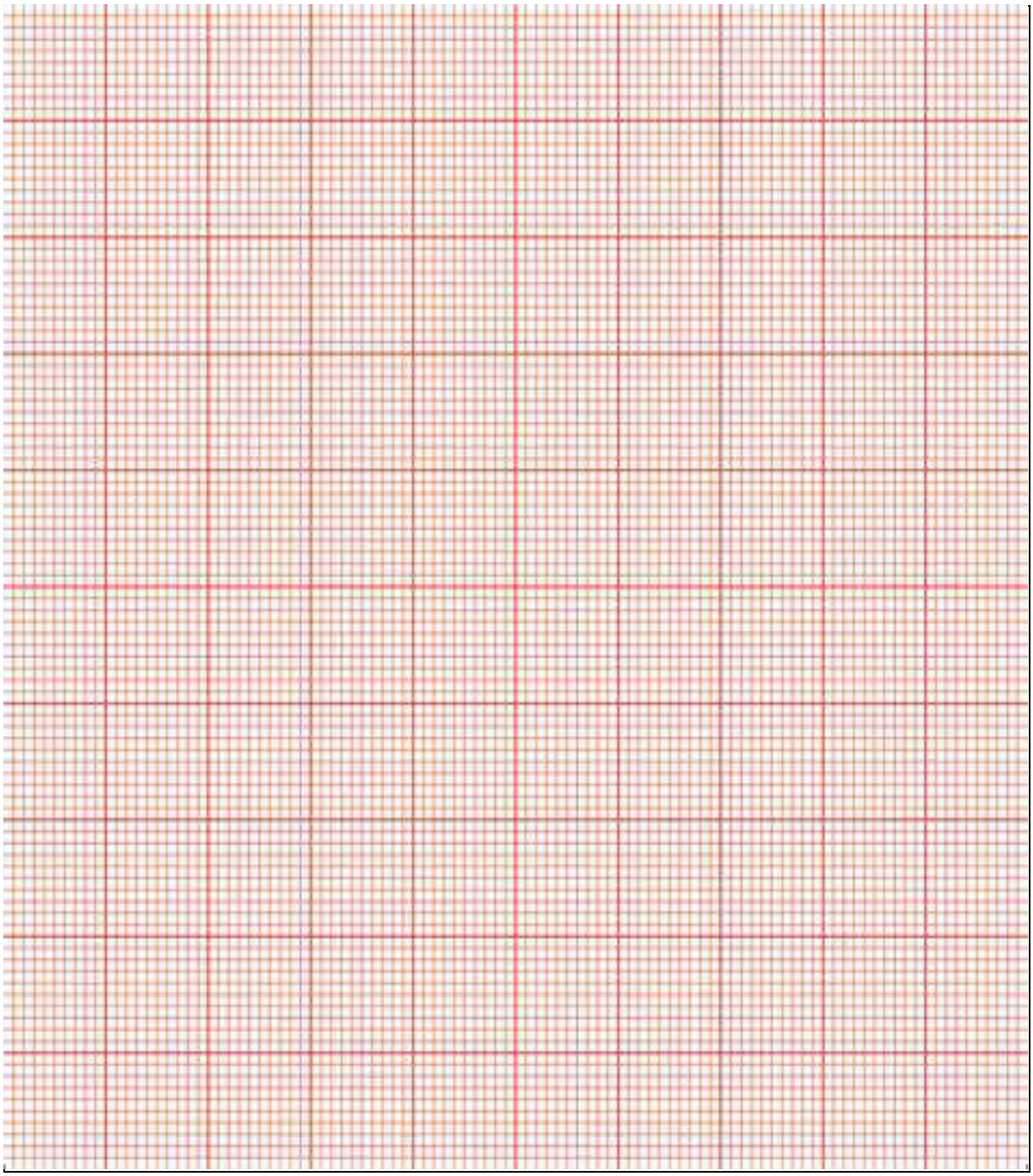

Figure 7: ……..

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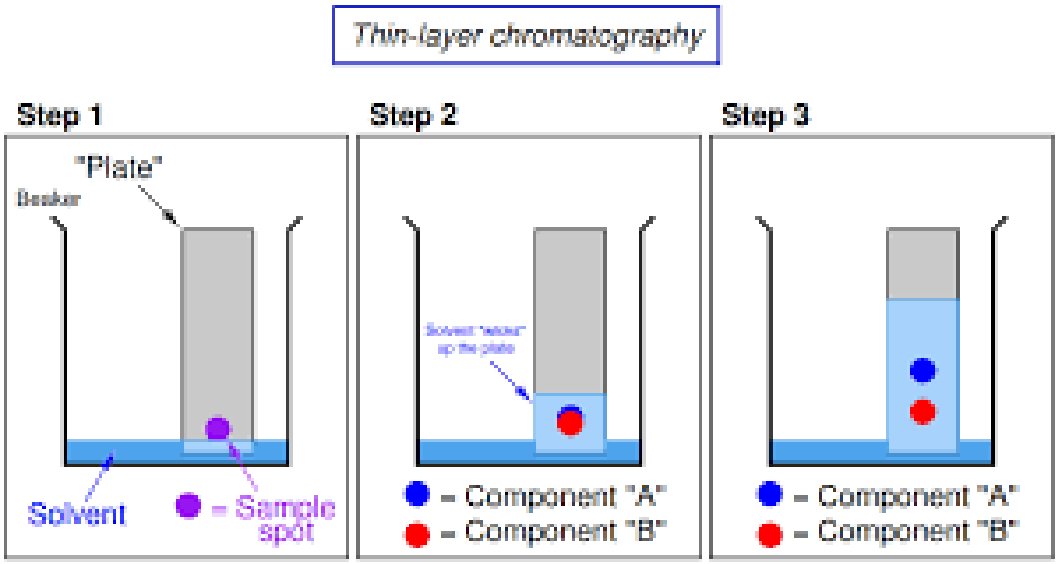
Methods for the analysis of antibiotics

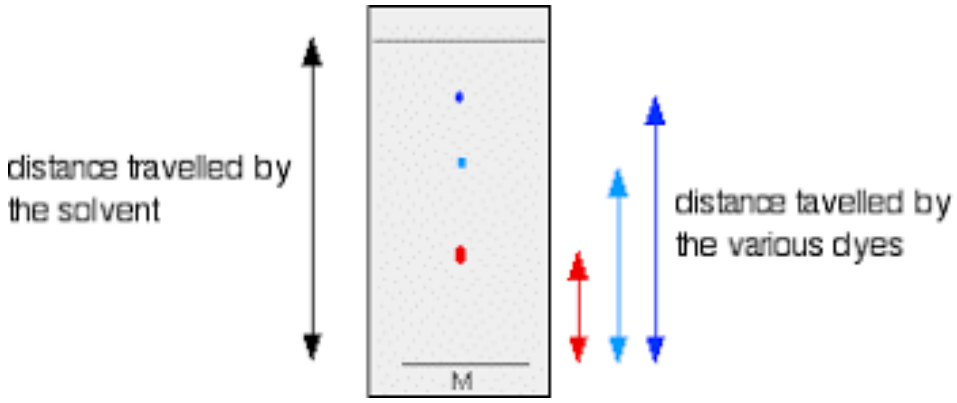
The different stages involved in the analytical process of antibiotics detection are sampling, extraction, clean-up, separation, detection and confirmation. Although many interfering compounds may be partially removed during the extraction sequence, further clean-up of the extract is normally necessary.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

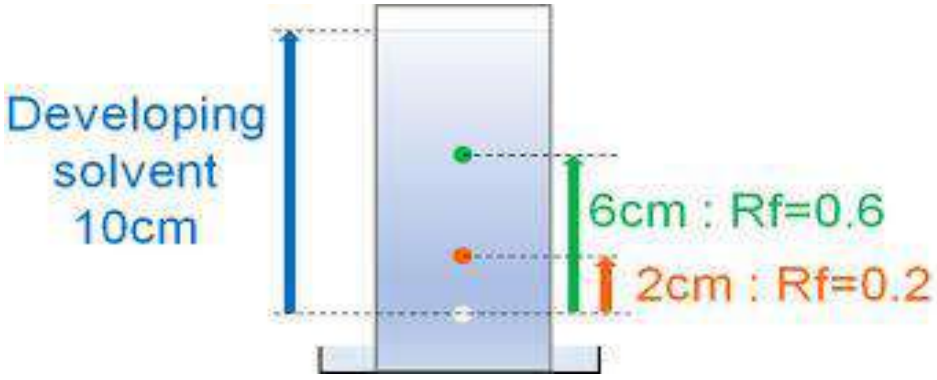
Thin Layer Chromatography (TLC) analysis

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

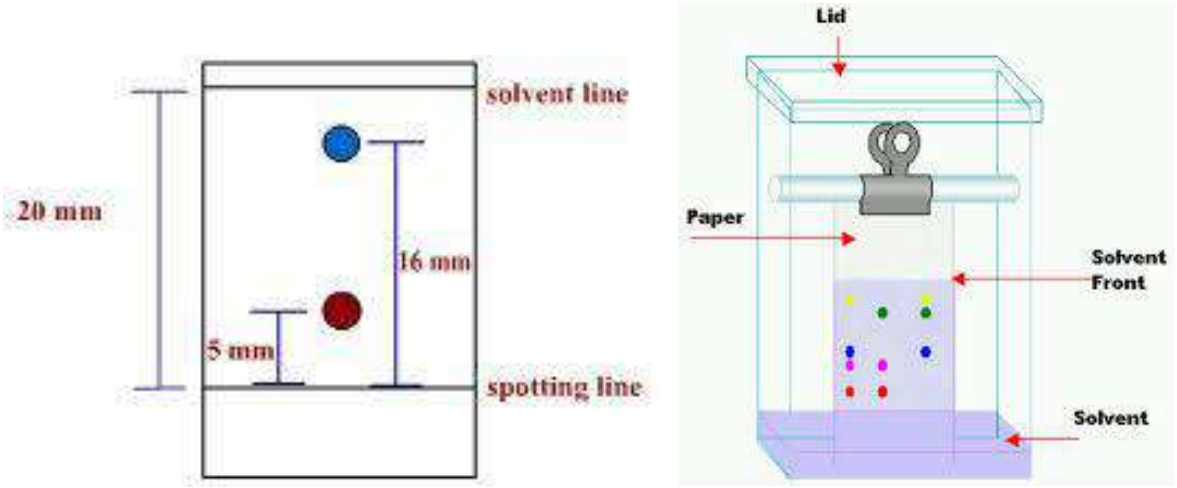
the material. Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the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







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

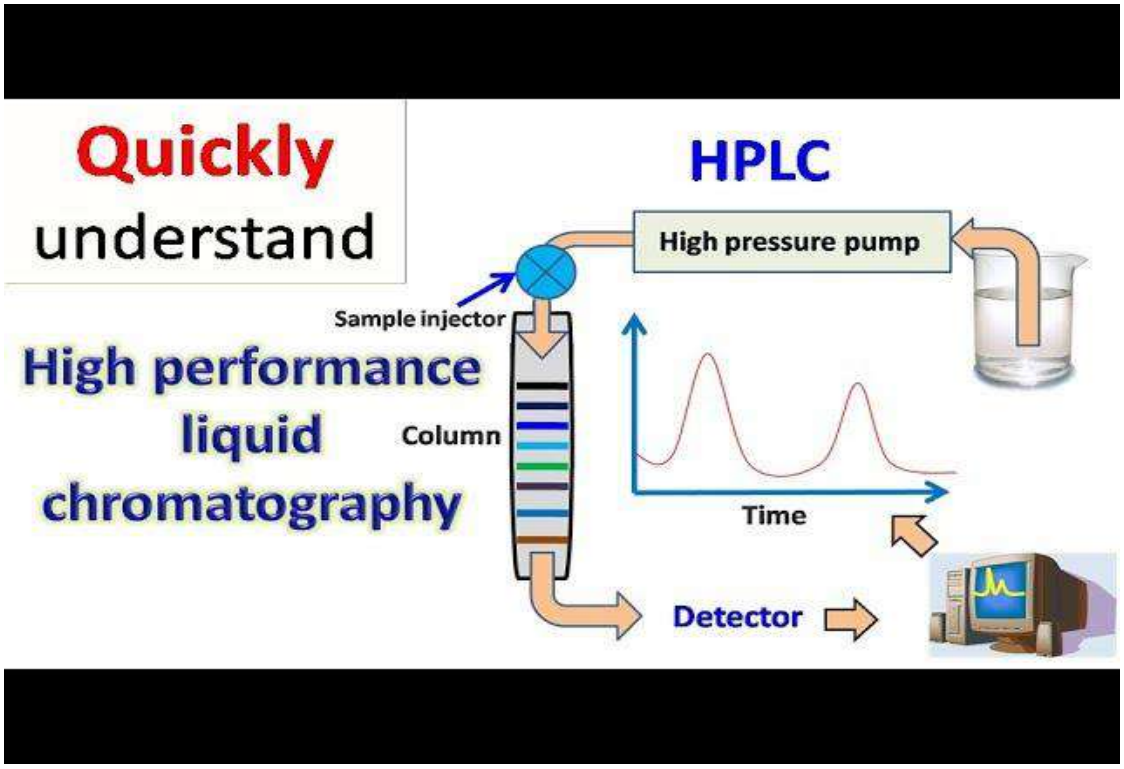


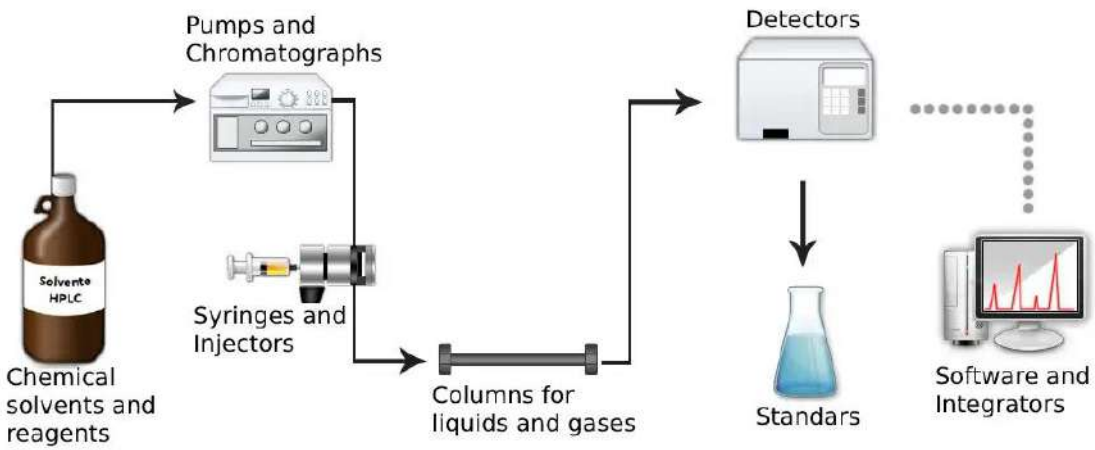
High-performance liquid chromatography (HPLC) analysis

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)

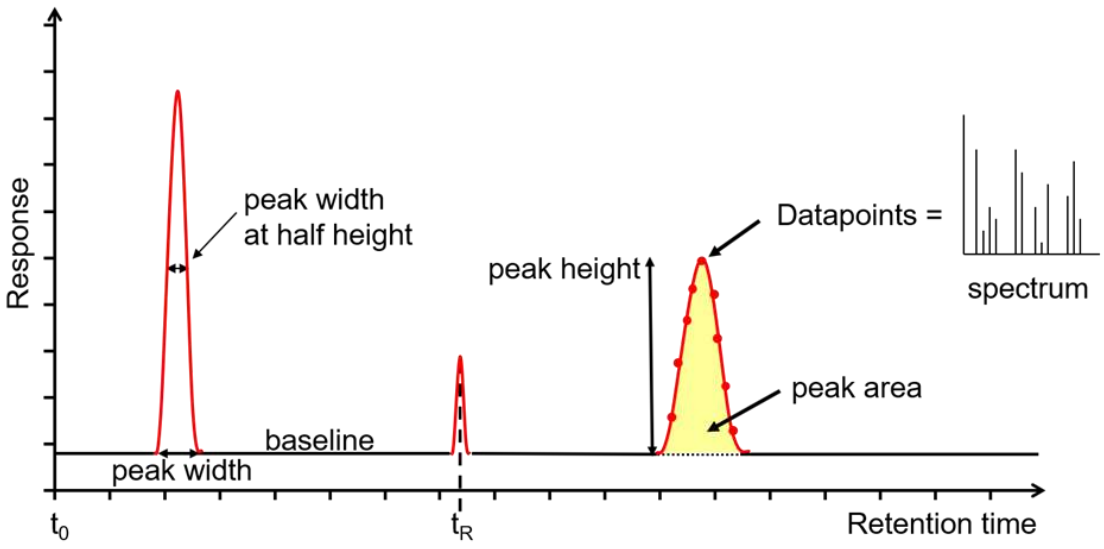
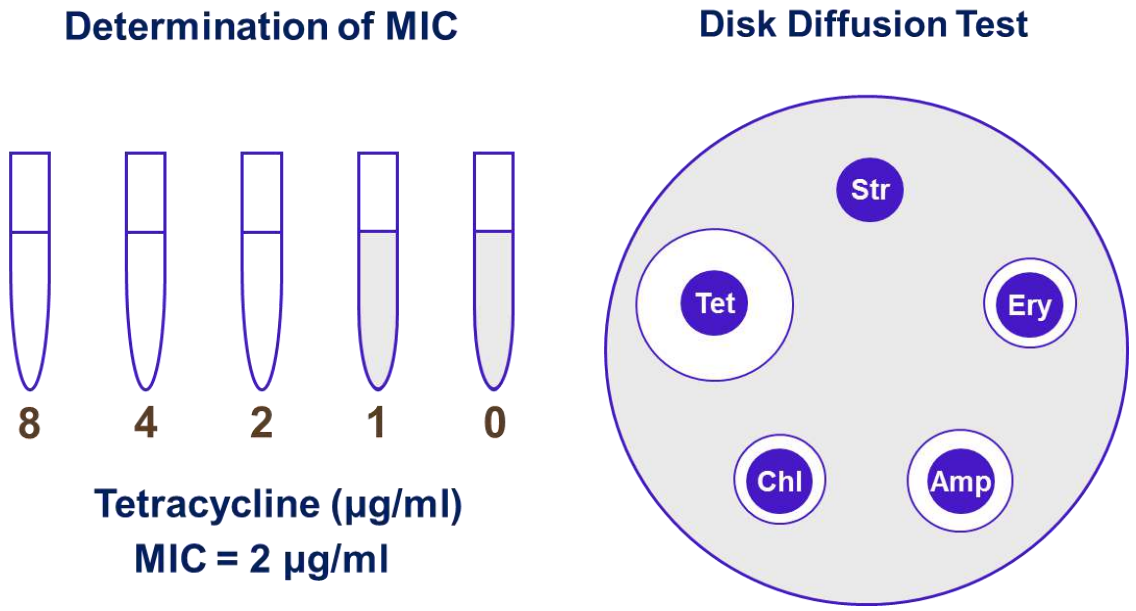
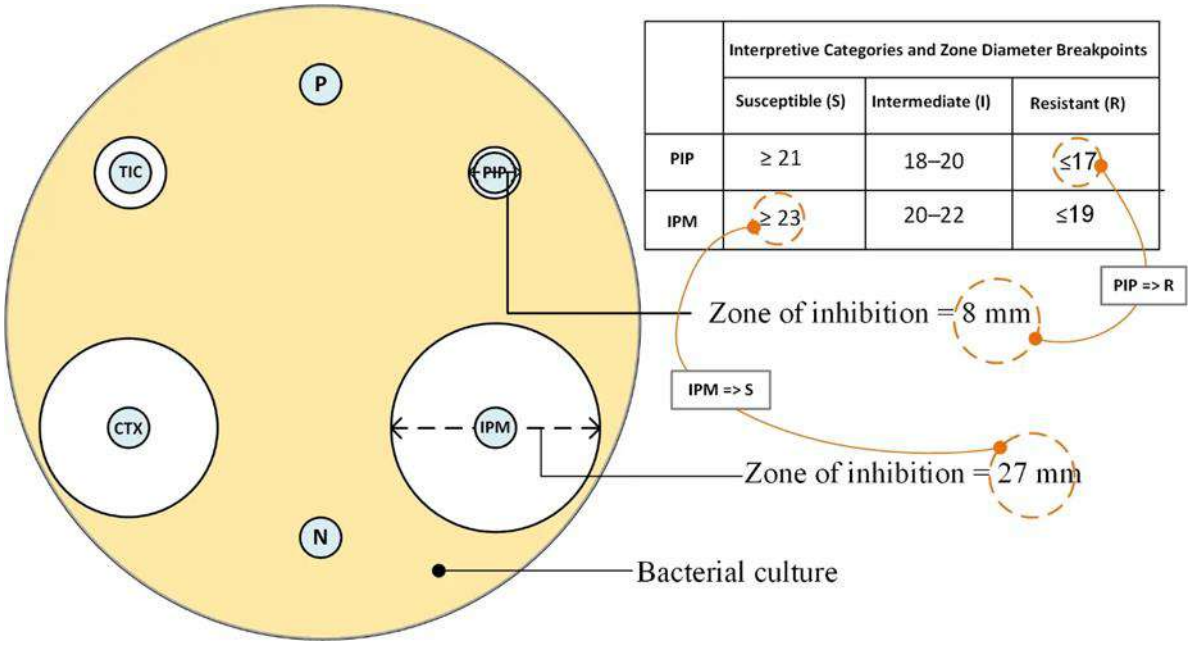


Chart of HPLC analysis

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

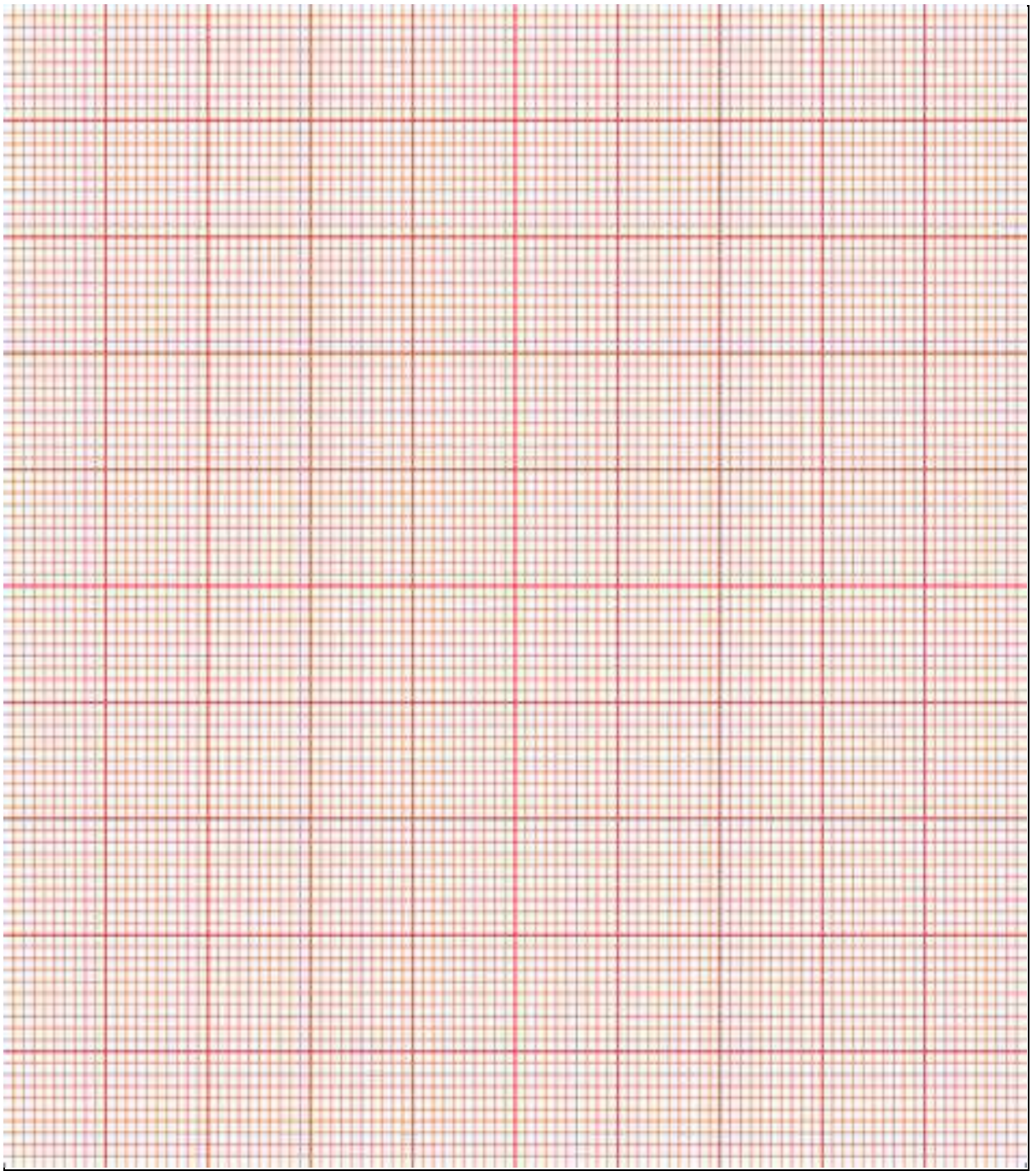

Figure 8: ……..

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Comment

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