

UNIT:C: BACTERIA AND MOLLICUTES:

- **PART:1:** General characteristics,Morphological characters
- **PART:2:** Basic methods of classification and Reproduction

PART:1

A.Bacteria: Definition: Bacteria are microscopic unicellular,prokaryotic organisms devoid of chlorophyll,most commonly reproduce by transverse binary fission and the resulting cells are identical in size and morphology(Shape:spherical/rod,spiral or curved spherical arrangements of cell(coccus/micrococcus/diplococcus(paired),tetraacoccus(arranged in 4)).

B.General characteristics of Phytopathogenic bacteria

1. Bacteria are small: 0.6-3.5 microns in length, 0.5-1.0 microns in diameter
2. Most plant pathogenic bacteria are facultative saprophytes and can be grown artificially on nutrient media; however, fastidious vascular bacteria are difficult to grow in culture and some of them have yet to be grown in culture.
3. Bacteria may be rod shaped, spherical, spiral, or filamentous (threadlike).
4. Mostly flagellate hence motile.Some bacteria can move through liquid media by means of flagella, whereas others have no flagella and cannot move themselves.
5. Some can transform themselves into spores, and the filamentous bacteria *Streptomyces* can produce spores, called conidia, at the end of the filament. Other bacteria, however, do not produce any spores.
6. The vegetative stages of most types of bacteria reproduce by simple fission.
7. Bacteria multiply with astonishing rapidity they can produce tremendous numbers of cells in a short period of time.
8. Carbohydrate decomposition is mostly aerobic or oxidative (Except, Erwinia,which is facultative anaerobe)
9. Mostly gram-ve,rarely gram+ve(gram+ve genera:Streptomyces,Corynebacterium,Clavibacter,Curtobacterium)
10. PPB are passive invaders,i.e. enters plants through wounds or natural openings
11. Survive in/on debris and spread by means of water,rain,insects and agricultural implements.
12. Susceptible to phages,none of them cause human or human diseases.
13. Cellwall is rigid
14. Slow growth compared to other saprophytic bacteria.
15. Incubation period: 36-48hrs 25°C
16. Other distinguished features;
 - Their genetic material (DNA) is not enclosed within a membrane.
 - They lack other membrane-bounded organelles

- Their DNA is not associated with histone proteins (special chromosomal proteins found in eukaryotes).
- Their cell walls most of the time contains the complex polysaccharide peptidoglycan.
- They usually divide by binary fission. During this process, the DNA is copied and the cell splits into two cells.

Detailed description of the bacteria cell

A bacteria cell is protected by cell envelop which made-up of capsule,a cellwall and plasma membrane.In some cells slime cover instead of capsule,outer plasma membrane between capsule and cellwallA variety of structures are found in prokaryotic cells. The cells are always bounded by a chemically complex cell wall. In between the cell wall and the periplasmic space lies the plasma membrane . Internal membrane bound organelles are absent in prokaryotes and hence the internalmorphologically is very simple.The genetic material is localized ina discrete region called nucleoid and not separated from the surrounding cytoplasm by membrane. Ribosomes and inclusions are found scattered in the cytoplasmic matrix. Bacteria use flagella for locomotion, and in addition, many cells are surrounded by a capsule or slime layer external to the cell wall.

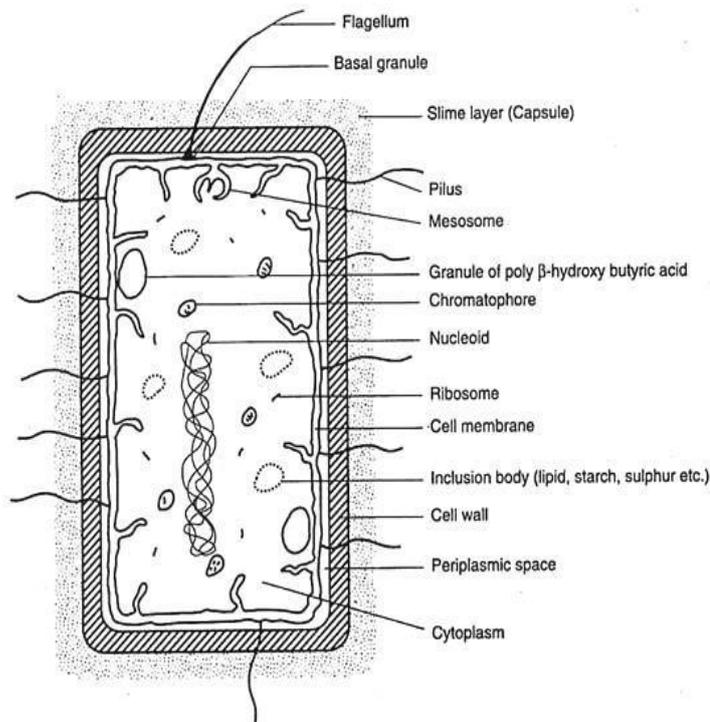


Fig: Detailed structural composition of bacterial cell

□ **The bacterial cell wall**

It's a complex, semi-rigid structure that surrounds the underlying plasma membrane and protects the cell and the internal parts from adverse surrounding environment. Except for Mycoplasmas and some Achaea most bacteria have strong walls that give them shape and protect them from osmotic lysis; wall shape and strength is primarily due to peptidoglycan. The cell walls of pathogenic bacteria have components that contribute to their pathogenicity and protect a cell from toxic substances and are the site of action of several antibiotics. Major functions – prevent cells from rupturing, maintains shape and serves as a point of anchorage for flagella. Christian Gram developed the Gram stain in 1884, and it became evident that bacteria could be divided into two major groups based on their response to staining. Gram positive bacteria stained purple and the gram negative ones colored pink or red by the technique. The gram positive cell wall consists of a single thick layer of 20 to 80 nm of peptidoglycan or murein layer lying outside the cell membrane. In gram negative cells, the peptidoglycan layer is thin of about 2 to 7 nm surrounded by a 7 to 8 nm thick outer membrane. The gram positive cells are stronger than gram negative ones because of the peptidoglycan layer.

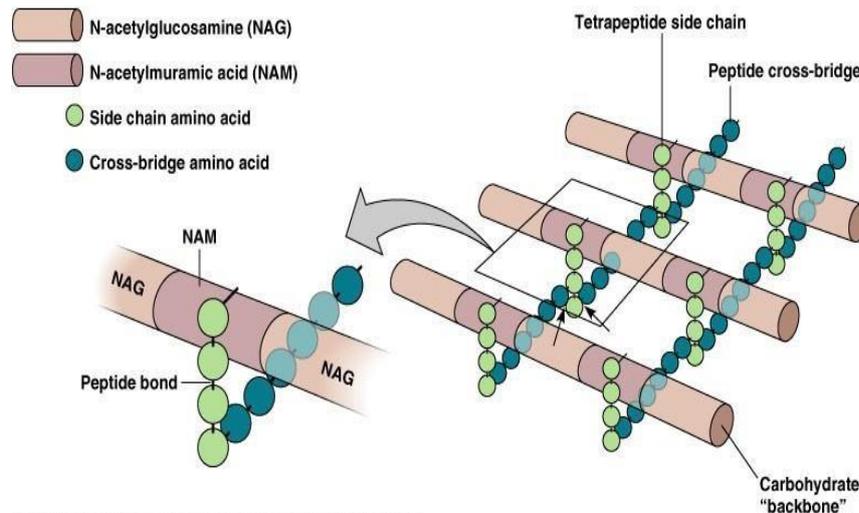
A space is seen between the plasma membrane and the outer membrane in electron micrographs of gram negative bacteria and a similar one but smaller gap may be observed between the plasma membrane and wall in gram positive bacteria. This space is called **periplasmic space**. The substance that occupies the space is the periplasm. The periplasmic space in gram negative bacteria ranges from 1nm to as great as 71 nm. It contains many proteins and participates in nutrient acquisition, hydrolytic enzymes attacking nucleic acids and phosphorylated molecules. The periplasmic space contains enzymes involved in peptidoglycan synthesis and modification of toxic compounds that could harm the cell. Gram positive bacteria may not have a visible periplasmic space but appear to have as many periplasmic proteins and several enzymes that are secreted are often called **exozymes**.

The Archaea differs from other prokaryotes. The walls lack peptidoglycan and are composed of proteins, glycoprotein or polysaccharides.

Structure of Peptidoglycan

The cell wall of bacteria is composed of a macromolecular network of polymer called **peptidoglycan (murein)**. The polymer contains two sugar derivatives, Monosaccharides- **N-acetylglucosamine (NAG)** and **N-acetylmuramicacid (NAM)**. In addition, several different amino acids, three of which – D-Glutamic acid, D-alanine, and mesodiaminopimelic acid – not found in proteins are present. The presence of D-amino acids protects against attack by most peptidases. The backbone of this polymer is composed of alternating N-acetylglucosamine and N-acetylmuramic

acid residues. A peptide chain of four alternating D- and L- amino acids is connected to the carboxyl group of N-acetylmuramic acid. Many bacteria substitute another diaminoacid, usually L-Lysine, in the third position for meso-diaminopimelic acid. Chains of linked peptidoglycan subunits are joined by cross-links between the peptides. Often the carboxyl group of the terminal D-alanine is connected directly to the amino group of diaminopimelic acid, but a peptide interbridge may be used instead . Most gram- negative cell wall peptidoglycan lacks the peptide interbridge.



(a) Structure of peptidoglycan in gram-positive bacteria

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Fig:Peptidoglycan structure in gram-positive cell

Gram positive cell walls

The cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure, which contains a peptide interbridge. Gram positive cell walls also contain **teichoic acids**, polymers of glycerol and ribitol joined by phosphate groups. To the glycerol and ribitol groups, the amino acids such as D-alanine or sugars like glucose are attached (Fig. 7). There are two classes to

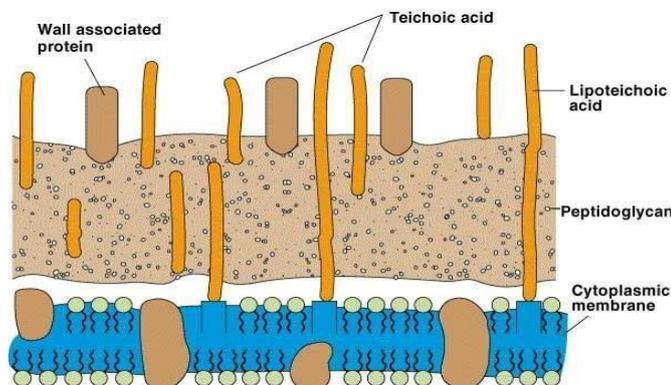


Fig: Cell wall of Gram positive bacteria

teichoic acids; lipoteichoic acid (if they are attached to the lipid of the plasma membrane) and wall teichoic acid (which extend to the surface of the peptidoglycan and are negatively charged). Teichoic acids are not found in gram-negative bacteria.

Some bacteria like *Staphylococci* and most other gram-positive bacteria have a layer of proteins on the surface of their cell wall peptidoglycan. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids and other receptors and these proteins are involved in the interactions of the cell with its environment. Ex. S-layer proteins. Other surface proteins are covalently attached to the peptidoglycan and in gram positive pathogens these have roles such as aiding in adhesion to host tissues, preventing opsonization and blocking phagocytosis.

Gram negative cell walls

The cell wall contains only a thin layer of peptidoglycan. In *E. coli*, it is about 2- 3nm thick and contains only one or two layers of sheets of peptidoglycan. The peptidoglycan is bounded to lipoproteins in the outer membrane and is embedded in a soft material, the **periplasmic gel**. Gram negative cell walls do not contain teichoic acids. They are more susceptible to mechanical breakage because of small amount of peptidoglycan.

❖ **Outer membrane** - found primarily in gram-negative cell consists of lipoproteins, liposaccharides and phospholipids and lies outside the thin peptidoglycan layer. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane and plasma membrane appear to be in direct contact at many locations in the cell wall. The adhesion sites may be regions of direct contact or possibly true membrane fusions. It is proposed that substances can move into the cell through these adhesions sites rather than travelling through the periplasm.

The most unusual constituents of the outer membrane are its lipopolysaccharides. (LPSs). These contain both lipid and carbohydrate and consist of three parts: 1. Lipid A, 2. the core polysaccharide, and 3. the O side chain. The lipid A region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. This region is buried in the outer membrane and the remaining portion projects from the surface. The second region or the core polysaccharide is joined to lipid A and the third region or the O side chain which is a polysaccharide chain extends outward from the core. The O side chain is readily recognized by host antibodies, gram negative bacteria may thwart host defenses by readily changing the nature of their O side chains to avoid detection. The core polysaccharide contains charged sugars and phosphate, LPS

contributes to the negative charge on the bacterial surface (Fig. 8). Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize the membrane structure. Lipid A is often toxic and as a result LPS can act as an endotoxin and cause some symptoms that arise in gram-negative bacterial infections. The function of the outer membrane is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. Provides a barrier to certain antibiotics (like penicillin), lysozyme detergents, heavy metals, bile salts and digestive. **Porins** are proteins that permit small molecules to pass through the outer membrane (about 800 MW). **Specific channel proteins** – allow specific substances like vitamin B12, iron, nucleotides and maltose.

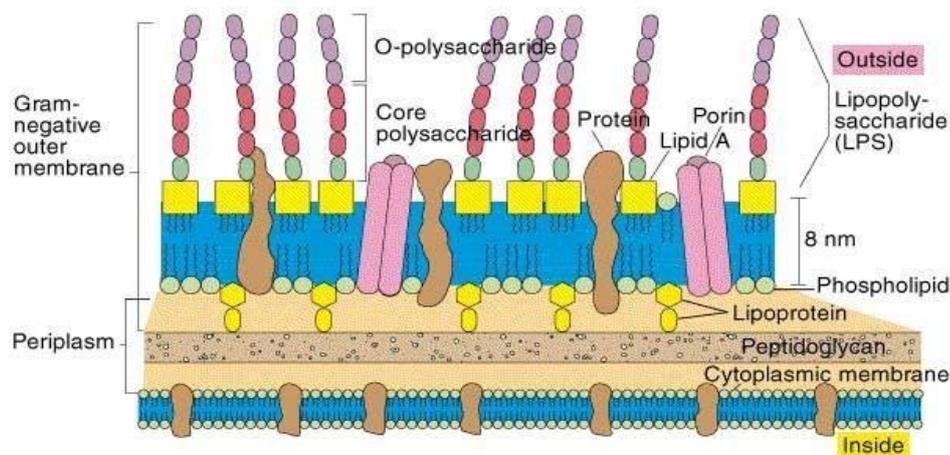


Fig: Cell wall of gram negative bacteria

Atypical cell walls:

- *Mycoplasma* – is a bacterial genus that naturally lacks cell walls. Their plasma membranes have lipids called sterols, which protect them from osmotic lysis.
- Archaeobacteria – have pseudomurein (N-acetylalosaminuronic acid) but no peptidoglycan.
- L forms - are mutant bacteria with defective cell walls.

Structures internal to the cell wall

In this lecture we shall be dealing with the structures internal to the cell wall of a bacterial cell. They include plasma membrane, organelles in the cytoplasm like nuclear area, ribosomes, inclusion bodies and endospores.

Plasma (cytoplasmic) membrane

Membranes are absolute requirement of all living organisms. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. Plasma membrane – encloses the cytoplasm and consists of phospholipids and proteins (**fluid**

mosaic model). Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends. The polar ends interact with water and are hydrophilic and the nonpolar hydrophobic ends are insoluble in water. The lipid composition of bacterial membranes varies with environmental temperature in such a way that the membrane remains fluid during growth. Bacterial membranes usually differ from eukaryotic membranes in lacking sterols such as cholesterol and they contain pentacyclic sterol-like molecules called hopanoids and these are said to stabilize the bacterial membranes. Cell membranes are very thin structures about 5 to 10 nm thick and can be seen only with electron microscope. Plasma membranes have a complex internal structure; the small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer.

The most widely accepted current model for membrane structure is the fluid mosaic model of S. Jonathan Singer and Garth Nicholson. Two types of membrane proteins are seen, **Peripheral proteins** - which are loosely connected to the membrane and can be easily removed and are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These cannot be easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings. Plasma membranes serve as a selectively permeable barrier; it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The plasma membrane also is the location of a variety of crucial metabolic processes; respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation.

The bacterial plasma membrane can be destroyed by alcohols and polymyxins which cause leakage of intracellular contents and subsequent cell death of the organism.

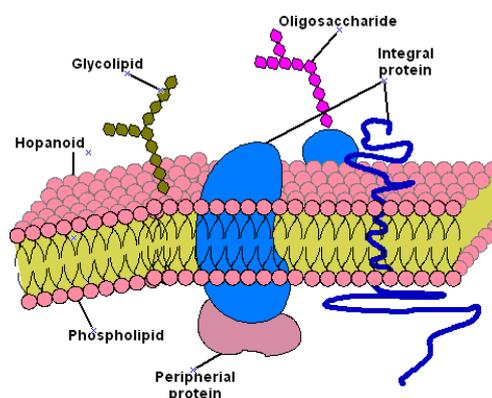


Fig: Plasma membrane structure

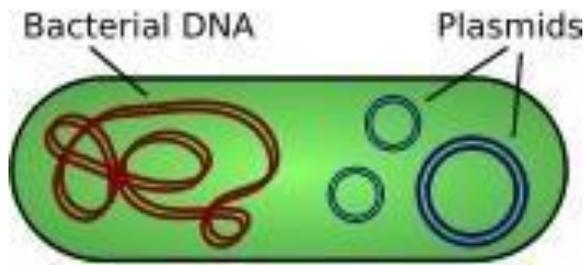


Fig: Bacterial DNA and Plasmid

❖ **Ribosomes:** The cytoplasmic matrix is also packaged with ribosomes, they also may be loosely attached to the plasma membrane. They look like small, featureless particles at low magnification electron microscope. They are made up of both protein and ribonucleic acid (RNA). Ribosome's function as sites of protein synthesis; matrix ribosomes synthesize proteins destined to remain within the cell, whereas the plasma membrane ribosomes make proteins for transport to the outside. The shape of each protein is determined by its amino acids sequence and the special proteins called molecular chaperones or chaperones aid the polypeptide in folding to its proper shape. Prokaryotic ribosomes are smaller than eukaryotic ribosomes (Fig. 11). Ribosomes are composed of two subunits, each subunit being composed of protein and a type of RNA called ribosomal RNA (rRNA). They are comm. Only 70S: 30S subunit (1 molecule of rRNA) and 50S subunit (2 molecules of rRNA) and have dimensions of about 14 to 15 nm, a molecular weight of approximately 2.7 million. The S in 70S stands for Svedberg value or sedimentation coefficient. It is the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater is its Svedberg value. The Sedimentation coefficient is a function of a particle's molecular weight, volume and shape. Several antibiotics, such as streptomycin, neomycin and tetracyclines, exert their antimicrobial effects by inhibiting protein synthesis on ribosomes.

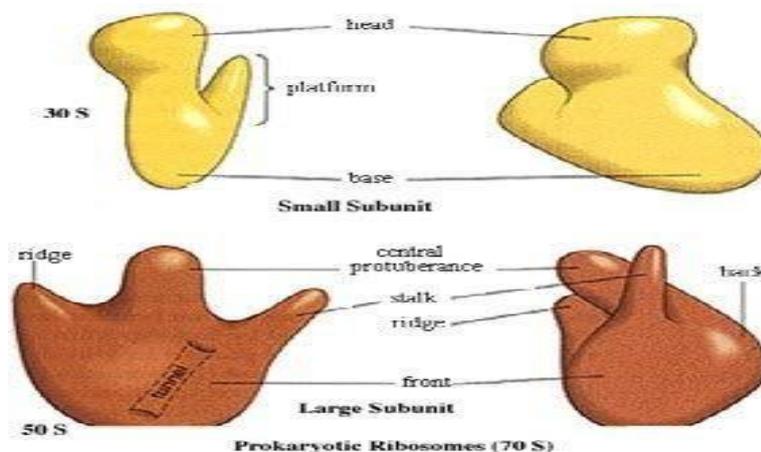


Fig: Ribosomes in bacteria

❖ **Inclusion bodies:**

Inclusion bodies can be divided into two types:

- Inclusion bodies not bounded by a membrane and lie free in the cytoplasm. Ex. Polyphosphate granules, cyanophycin granules and some glycogen granules.
- Inclusion bodies enclosed by a membrane about 2-4nm thick. Eg. Poly-β-hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes and gas vacuoles.

□ **Organic inclusion bodies: Glycogen:**

Polymer of glucose units composed of long chains formed by alpha (1-4) glycosidic bonds and branching chains connected to them by alpha (1-6) glycosidic bonds. Eg. glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells (glycogen granules appear reddish brown and starch granules appear blue).

Poly β- hydroxybutyrate: Contains beta-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Appear in various species of *Mycobacterium*, *Bacillus*, *Azotobacter*, *Spirillum* and other genera. Lipid inclusions are revealed by use of fat-soluble dyes, such as Sudan dyes.

□ **Carboxysomes:**

These are polyhedral and hexagonal inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. Bacteria that use carbon dioxide as their sole source of carbon require this enzyme for carbon dioxide fixation during photosynthesis (Ex. nitrifying bacteria, cyanobacteria, and *Thiobacilli*).

□ **Gas vacuoles:**

These are hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria. Each vacuole consists of rows of several individual gas vesicles, which are hollow cylinders covered by protein. Their function is to maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light and nutrients. They are impermeable to water and permeable to atmospheric gases.

□ **Inorganic inclusion bodies:**

Polyphosphate granules or Metachromatic granules:

Linear polymer of organo phosphates joined by ester bonds. Reservoirs for phosphate, an important component of cell nucleic acids and also energy reserves. Represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. Stain red with certain blue

dyes, such as methylene blue, and are collectively known as volutin. Found in algae, fungi and protozoans, as well as bacteria.

These granules are quite large and are characteristic of *Corynebacterium diphtheriae*, the causative agent of diphtheria, thus they have diagnostic significance.

Sulphur granules:

Sulphur bacteria, which belong to the genus *Thiobacillus*, derive energy by oxidizing sulfur and sulfur containing compounds. These bacteria may deposit sulfur granules in the cell, where they serve as an energy reserve. Purple photosynthetic bacteria use H₂S as electron donor and accumulate resulting sulfur in either the periplasmic space or in special cytoplasmic globules.

❖ Endospores “An escape pod for DNA”

Endospores are a survival mechanism: they are triggered to form during adverse environmental conditions. They are NOT reproductive structures as only one cell gives rise to one spore and endospores can be identified with special stains and differentiated from the vegetative cell (Fig. 14). Endospores are resistant to: heat: withstand boiling for over one hour; desiccation, UV radiation and chemical disinfectants. The resistance of these spores has serious consequences and some very pathogenic bacteria have the ability to produce such spores

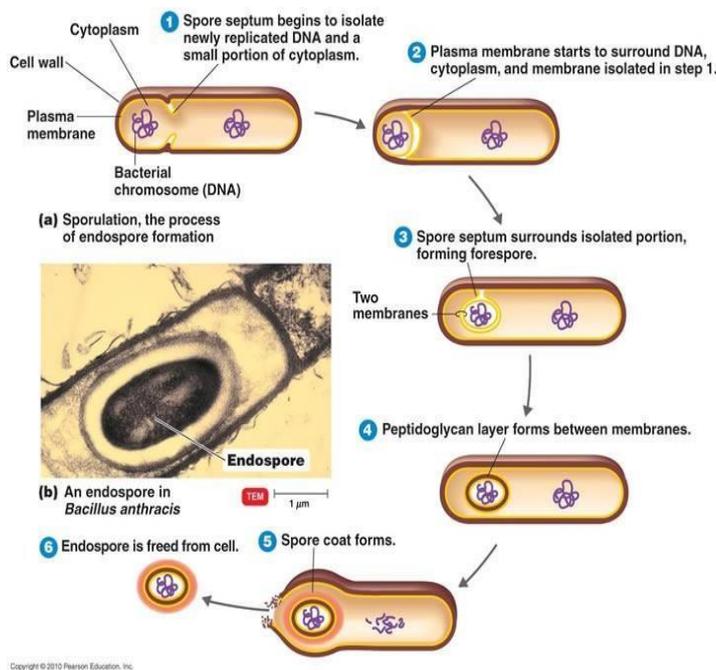


Fig: Formation of endospore

□ Structures external to the cell wall

the structures external to the cell wall of bacterial cells includes glycocalyx, fimbriae, pili, flagella, axial filaments

Glycocalyx (Capsules, Slime layers and S-layers)

It is a viscous (sticky), gelatinous polymer composed of polysaccharide, polypeptide or both. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule** (negative staining). If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer**. Capsules protect pathogenic bacteria from phagocytosis (process by which certain white blood cells engulf and destroy microbes) and contribute to virulence. Capsules are clearly visible in the light microscope by using stains or special capsule stains.

A regularly structured layer called S-layer is usually seen in many gram positive and gram negative bacteria. It consists of proteins or glycoproteins and resembles a pattern something similar to floor tiles. The S-layer adheres directly to the outer membrane in case of gram negative bacteria and with the peptidoglycan surface in gram positive bacteria. These protect the bacteria against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio*. The S layer also helps maintain the shape and envelope rigidity of at least bacterial cells and also promotes cell adhesion to surfaces. Sometimes, the layer also seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

□ **Fimbriae and Pili:**

Many gram negative bacteria have hairlike appendages that are shorter, straighter and thinner than flagella and are used for attachment rather than for motility. They are usually called fimbriae. These structures contain a protein called pilin. **Fimbriae** - occur at the poles of the bacterial cell, or they can be evenly distributed over the entire surface of the cell. Fimbriae of *Neisseria gonorrhoeae* the causative agent of gonorrhoea help the microbe to colonize mucous membranes to cause the disease. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

❖ **Pili or sex pili or pilus**- usually longer than fimbriae and number only one to ten per cell. Pili function to join bacterial cells prior to the transfer of DNA from one cell to another (sometimes called sex pili). They are genetically determined by sex factors or conjugative plasmids and are required for bacterial mating. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

□ **Flagella:**

Motile bacteria move by use of flagella, threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20 μm long. Bacterial species often differ distinctively in their patterns of flagella distribution.

Structure:

Transmission electron microscopic studies have shown that the bacterial flagellum is composed of three parts. 1) **Filament**– outermost region and contain the globular protein flagellin 2) **Hook**– the filament is attached to hook, which consists of a different protein

3) **Basal body**- which anchors the flagellum to the cell wall and plasma membrane. It consists of a small central rod inserted into it are a series of rings. The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin** (MW from 30,000 to 60,000). Some bacteria have sheaths surrounding their flagella.

The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex structure of the flagellum. In *E.coli* and Gram negative bacteria, the body has four rings connected to central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers. The inner M ring connects the plasma membrane. Gram positive have only two basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan. The synthesis of flagella is a complex process involving at least 20 to 30 genes. Flagellin subunits are transported through the filament's hollow internal core. When they reach the tip, the subunits spontaneously aggregate under the direction of a special filament cap so that the filament grows at its tip rather than at the base. Filament synthesis is an excellent example of self-assembly.

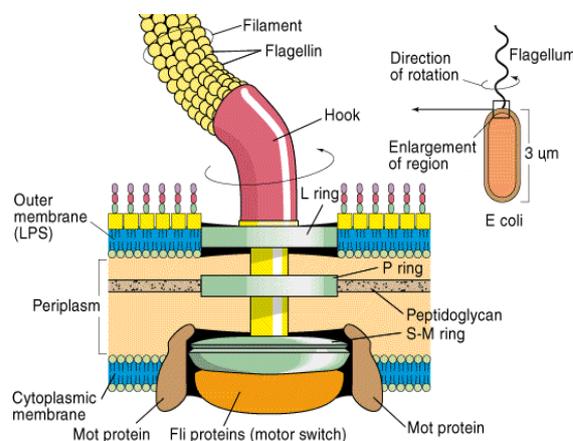


Fig:Structure of bacterial flagella (Gram negative)

Flagellar movement:

The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial movement. The movement in monotrichous bacteria stop and tumble randomly by reversing the flagellar rotation. The polar flagella, rotate counter clockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. Peritrichous bacteria also operate in a similar way. To move forward, the flagella rotate counter clockwise. As they do so, they bend at their hooks to form a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles. Motility enables the bacterium to move toward a favorable environment or away from a particular stimulus called taxis. Chemotaxis (include chemicals) and phototaxis(include light). Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as **chemotaxis**. The mechanism of chemotaxis in *E.coli* has been studied most. Forward swimming is due to counterclockwise rotation of the flagellum, whereas tumbling results from clockwise rotation. The bacteria must be able to avoid toxic substances and collect in nutrient-rich regions and at the proper oxygen levels. *E.coli* has four different chemoreceptors that recognize serine, aspartate and maltose, ribose and galactose and dipeptides respectively. These chemoreceptors often are called methyl-accepting chemotaxis proteins (MCPs)

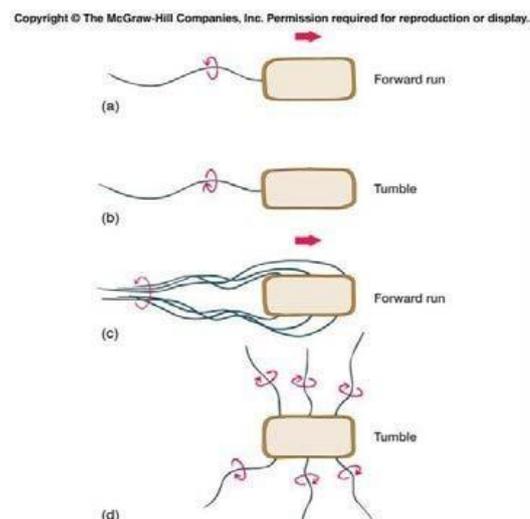


Fig: Bacterial flagellar movement

(This part for brief note purpose)

❖ Prokaryotes – Structure/Function

Prokaryotes are distinguished from eukaryotes by their smaller size (0.2-10µm), their lack of internal organelles (*e.g.*, mitochondria), the presence of a cell wall and their cell division by binary fission rather than mitosis. They lack introns, are not capable of endo/exocytosis and have single-stranded circular DNA rather than multiple discrete chromosomes. Bacteria share a number of common structures that are briefly described below.

1) Slime (extracellular polysaccharide): This is extracellular material, loosely associated with the bacteria, that is elaborated by some bacterial species that facilitates colonization of smooth, prosthetic surfaces such as intravascular catheters.

2) Capsule: This polysaccharide outer coating of the bacterial surface often plays a role in preventing phagocytosis of bacteria.

3) Peptidoglycan (cell wall) Provides bacterial shape and rigidity. The cell wall consists of alternating units of N-acetylglucosamine and N-acetylmuramic acid. The polysaccharide chains are cross-linked by a peptide bridge. It is a primary target of antimicrobial therapy – because it is specific to prokaryotes.

❖ Assembly of the peptidoglycan:

This is a critical step for bacterial survival. The sequence of events is outlined below.

- I. Synthesis begins with formation of a water soluble, nucleotide-linked precursor (N-acetylmuramic acid - NAM) also carrying a pentapeptide in the cytoplasm.
- II. ii. The precursor is then linked to a lipid-like carrier in the cell membrane (bactoprenol) and N-acetyl glucosamine (NAG) is added to the NAM. This complex is mobilized across the cytoplasm
- III. iii. The disaccharide subunit (NAM-NAG) is then added to the end of a glycan strand.
- IV. iv. The final step is the transpeptidation reaction catalyzed by a transpeptidase enzyme (also called penicillin binding proteins) that crosslinks the growing strand with others.

4) Cytoplasmic membrane: This is a phospholipid bilayer that assumes many of the functions of eukaryotic organelles such as the biosynthetic processes.

5) Flagella: These provide bacteria with the capacity for locomotion. They vary in number and location.

6) Pili: These structures project from the cell surface enabling bacteria to adhere to host tissue surfaces. Based on their amino acid structure their affinity for particular host tissue surfaces can be remarkably specific.

6) Secreted products: There are a variety of these products including exotoxins that are proteins grouped into A-B toxins, membrane damaging toxins (*e.g.*, hemolysins) and hydrolytic enzymes capable of destroying host tissues and extracellular matrices.

C. Morphological characteristics

❖ Size and Shape of Bacteria

Prokaryotes are among the smallest of all organisms (0.5 to 2.0 μm). Because of their small size, bacteria have a large surface-to-volume ratio. The smallest member of the genus is about 0.3 μm in diameter. Even smaller cells have been reported like the nanobacteria or ultramicrobacteria appear to range from around 0.2 μm to not less than 0.05 μm . *E. coli*, a bacillus of about average size is 1.1 to μm wide by 2.0 to 6.0 μm long. Spirochaetes occasionally reach 500 μm in length and the cyanobacterium *Oscillatoria* is about 7 μm in diameter. The bacterium, *Epulosiscium fishelsoni*, can be seen with the naked eye (600 μm long by 80 μm in diameter). Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 μm in diameter).

Shape and arrangement:

Typically bacteria have three basic shapes – spherical, rod like and spiral.

Spherical bacteria:

Coccus (plural cocci) in pairs – **diplococci**; in chains – **Streptococci** (*Streptococcus*); in cube like groups of eight – **Sarcinae** (*Sarcina*); in grape like structures – **Staphylococci** (*Staphylococcus*)

Rod like bacteria:

Bacillus (plural bacilli) in pairs – **diplococci**; in chains – **streptococci**. Still others look like cocci and are called **coccobacilli**

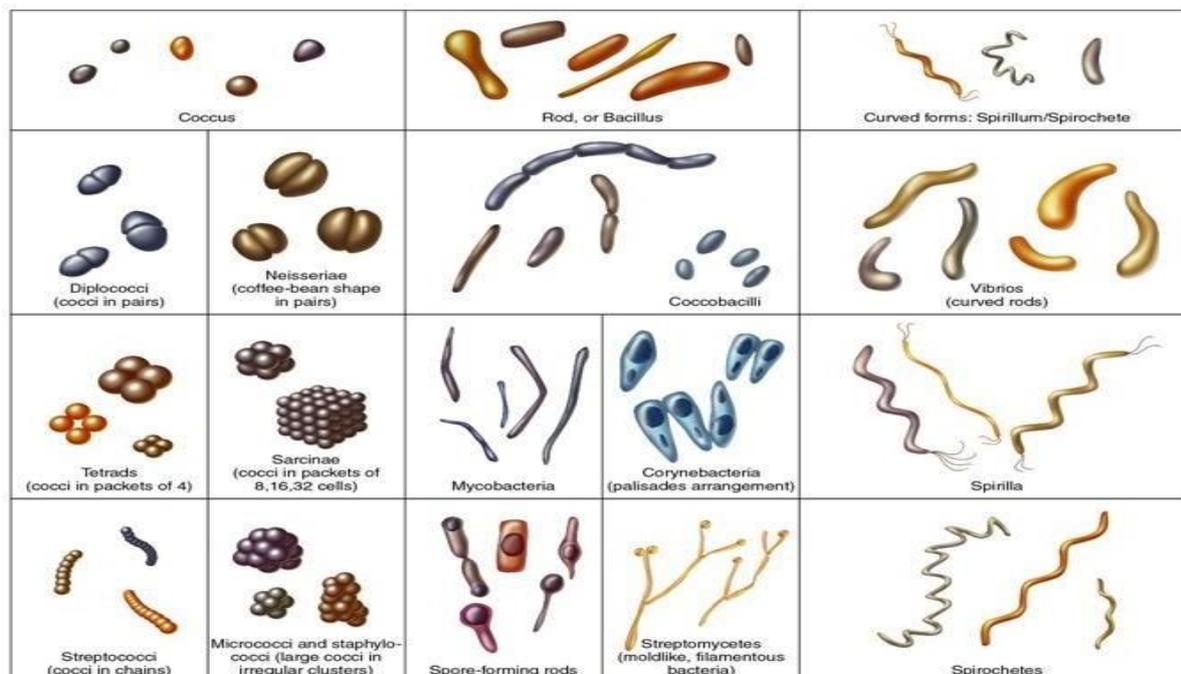


Fig: Different morphological characteristics and diversity of bacteria

Spiral bacteria

Comma shaped – *Vibrio (Vibrio)*; **Helical, long and curved** – *Spirilla (Rhodospirillum)*;

Helical and flexible – Spirochetes (*Treponema* and *Borrelia*) movement by axial filaments

In addition there are: Star shaped cells (genus *Stella*); Square flat cells (halophilic archaeobacteria); Triangular cells (*Haloarcula*); Branching mycelium – like filaments (*Streptomyces*)

□ Genetically most bacteria are monomorphic (single shape), but some have many shapes and are called pleomorphic (*Rhizobium* and *Corynebacterium*)

PART 2

Basic methods of classification

❖ Classification of phytopathogenic prokaryotes, according to Bergey's manual of Systematic Bacteriology

A. Phenotypic classification

- I. Morphological
- II. Anatomical
- III. Staining
- IV. Cultural characteristics
- V. Nutrition
- VI. Environmental factors
- VII. Biochemical reactions
- VIII. Antigenic structure

B. Genotypic classification

- I. DNA-DNA hybridization
- II. G+C content

VI. Environmental factors

-TEMP

- Psychrophiles (15-20°C)
- Mesophiles (20-40°C)
- Thermophiles (50-60°C)
- Extreme thermophiles (As high as 250°C)

-O₂ DEPENDANCE

- Aerobe
- Obligate Aerobe
- Microaerophilic
- Facultative Anaerobe
- Obligate Anaerobe
- Capnophilic
- Aerotolerant

-pH

- Acidophiles
- Alkaliphiles
- Neutrophiles

-Salt conc.

- Halophiles
- Non halophiles

-Atm. pressure

A.I. Morphological: Based on shape and size

- Rod shaped (Bacillus)
- Spherical (Coccus)
- Spiral (Spirillum)
- Curved (Vibrio)

II. Anatomical: Based on Flagellation

-FLAGELLATE

- Monotrichous
- Lophotrichous
- Amphitrichous
- Peritrichous

-AFLAGELLATE

Spore

- Spore former
- Non spore former

V. Based on nutrition:

- Autotroph
- Heterotroph

III. Staining:

- GRAM STAINING

- Gram positive cocci
- Gram negative cocci
- Gram positive rod
- Gram negative rod

- ACID FAST STAINING

- Acid fast bacilli
- Non acid fast bacilli

IV. Cultural characteristics

-EXTRA GROWTH

FACTOR REQUIREMENT

- Fastidious
- Non fastidious

-UTILIZATION OF CARBOHYDRATE

- Oxidative
- Fermentative

-GROWTH RATE

- Rapid growers
- Slow growers

-PIGMENT PRODUCTION

- Pigment producer
- Pigment non producer

❖ Genetic characteristics:

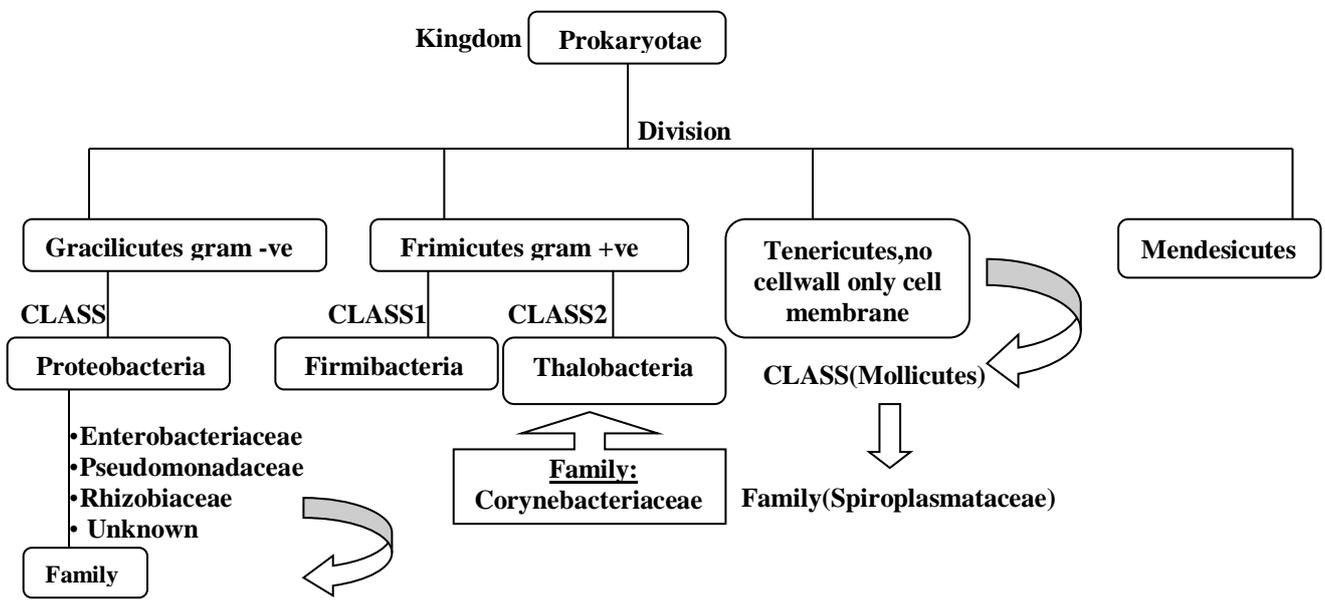
-**DNA Base composition:** DNA molecules made up of base pairs guanine-cytosine and adenine-thymine-base of classification-----

-The sequence of nucleotides base in the sequence of nucleotide is unique for each type of organism most fundamental of all

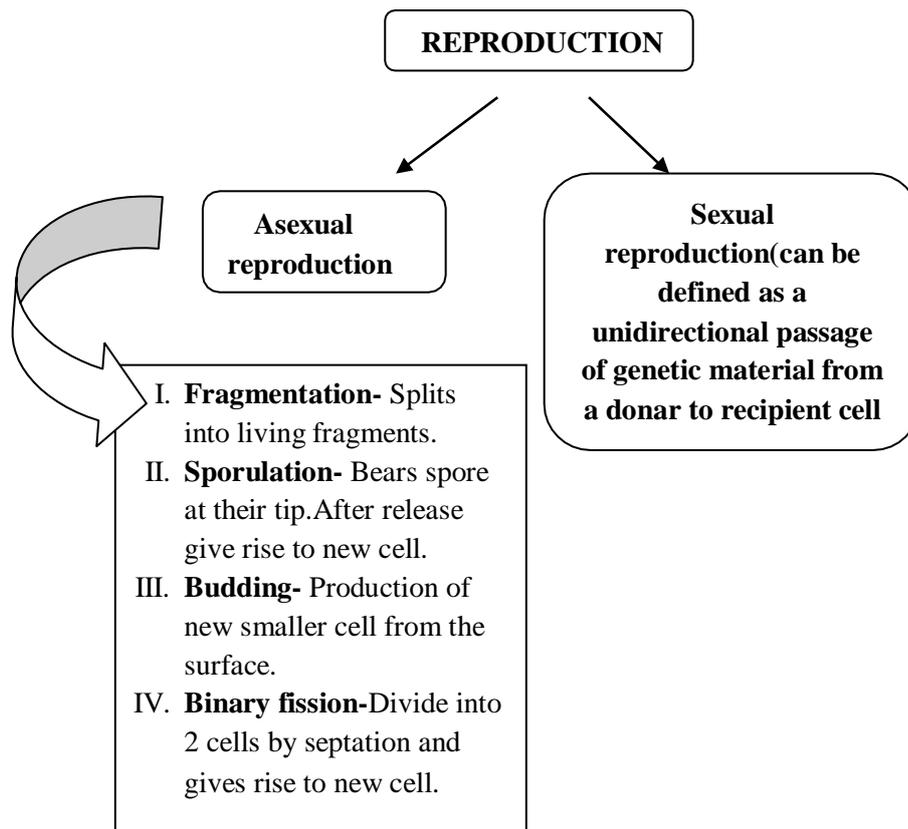
-**Pathogenicity-** Ability to cause disease-Pathogenic to plants and to microorganism.

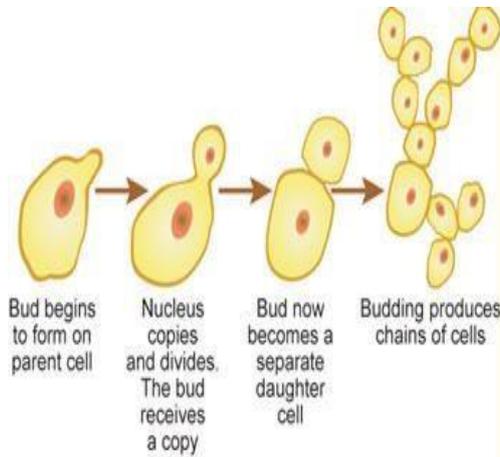
-**Ecological-** Marine microorganisms different from fresh water microorganism

Classification of phytopathogenic prokaryotes, according to Bergey's manual of systematic bacteriology, 1984

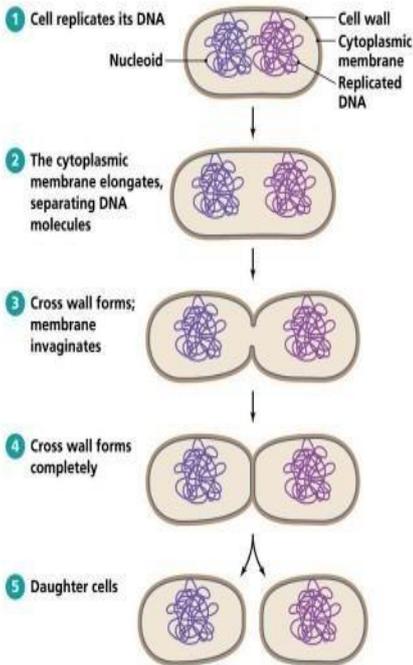


❖ Reproduction of bacteria:





BUDDING



FRAGMENTATION

ASEXUAL REPRODUCTION OF BACTERIA

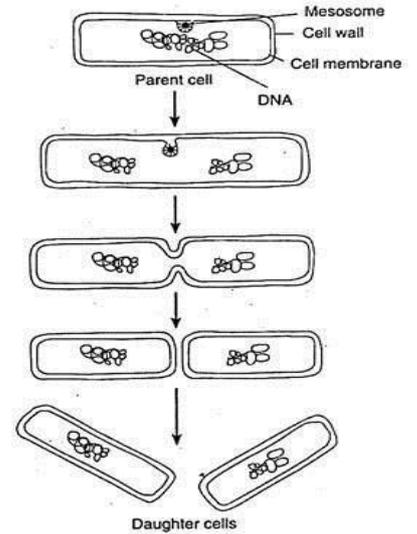
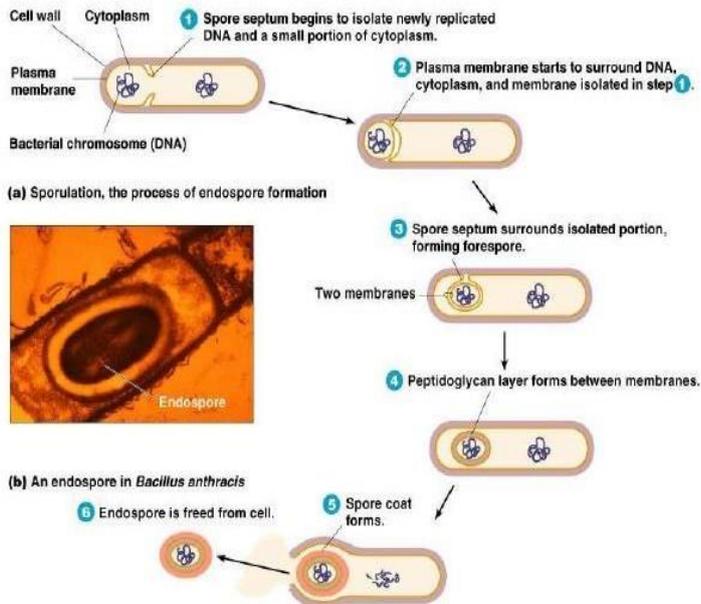


Fig. 2.20 : Binary fission of a bacterium

BINARY FISSION

Sporulation Process



Transformation

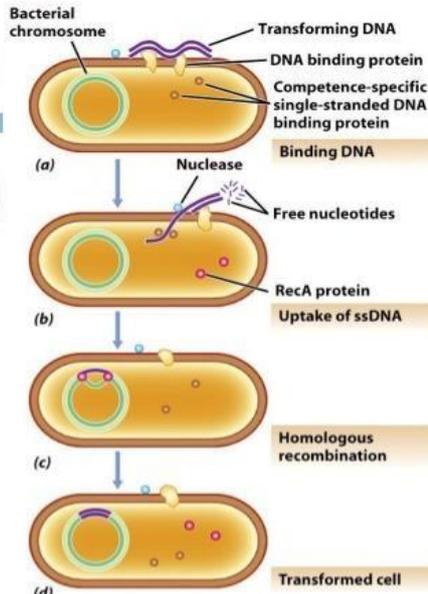
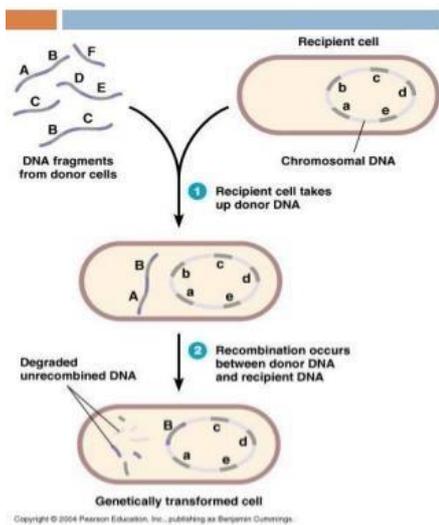
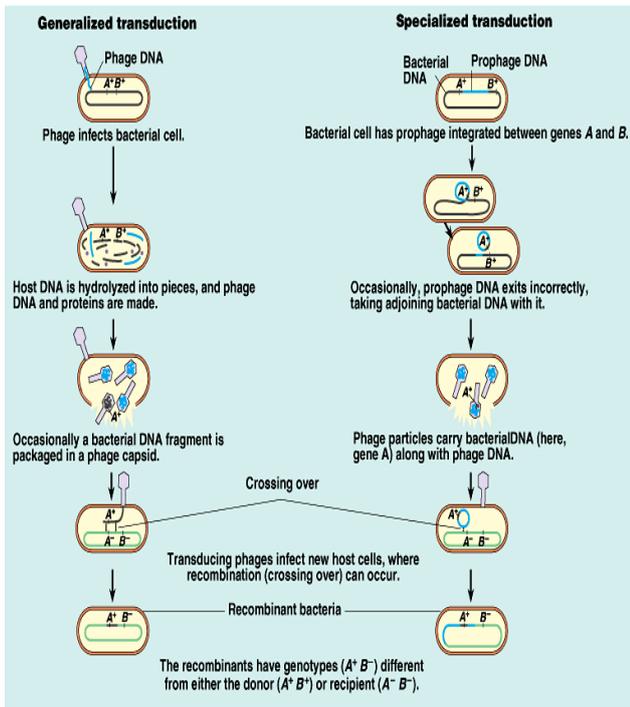
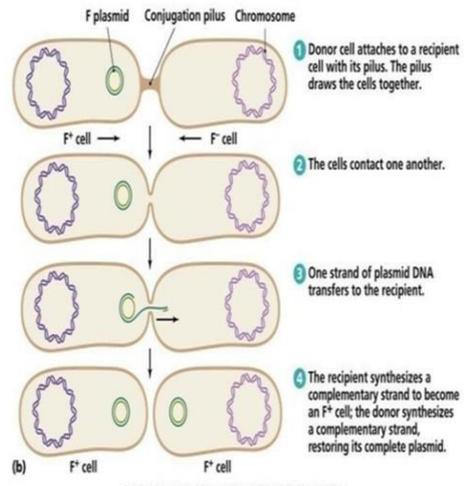


Figure 10-14 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.



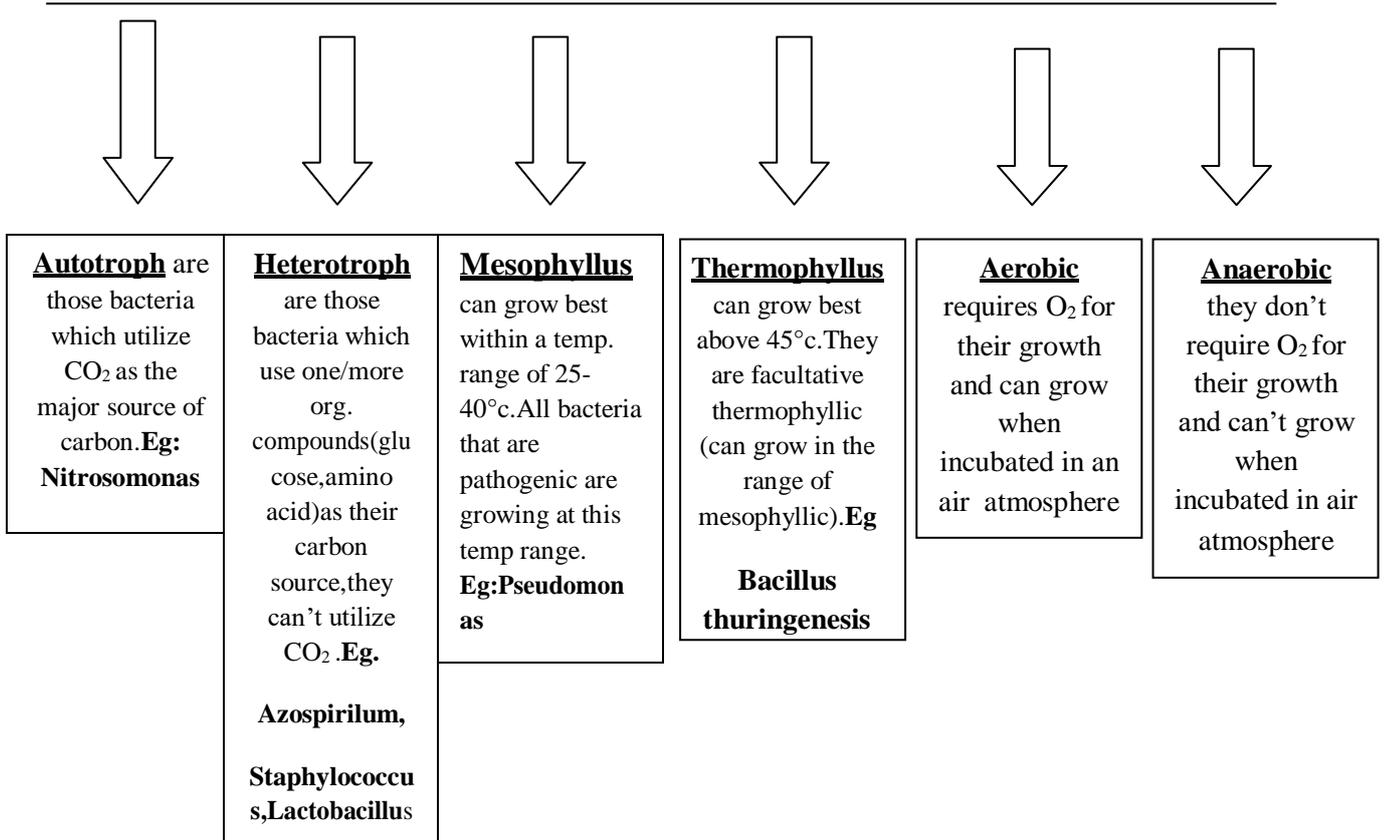
TRANSDUCTION

Bacterial Conjugation



SEXUAL REPRODUCTION IN BACTERIA

Cultivation of Bacteria

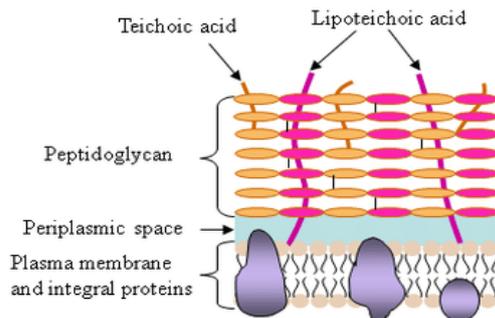


Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

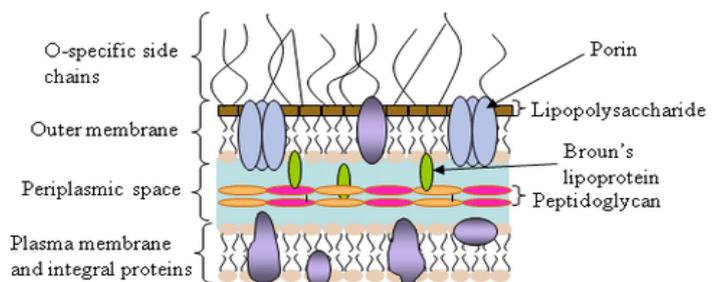
12

Characteristic	Gram-positive	Gram-negative
Gram reaction	Retain crystal violet dye and stain dark violet or purple	Can be decolorized to accept counterstain (safranin); stain red
Peptidoglycan layer	Thick (multilayered)	Thin (single-layered)
Toxins produced	Primarily exotoxins	Primarily endotoxins
Resistance to drying	High	Low
Outer membrane	Absent	Present
Lipid and lipoprotein content	Low	High
Lipopolysaccharide (LPS) content	Virtually none	High

Gram (+) cell-wall



Gram (-) cell-wall



❖ Characteristics of Bacteria Cell Structures

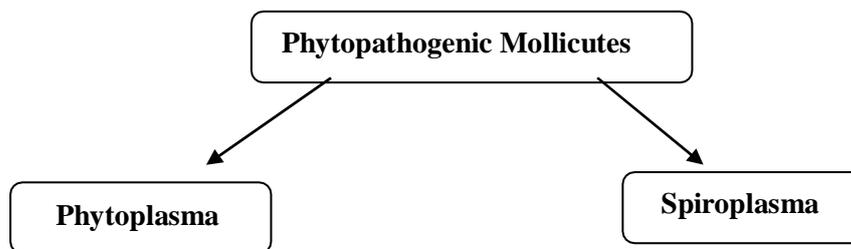
Structure	Functions(s)	Predominant chemical composition
Flagella	Swimming movement	Protein
Pili		
Sex pilus	Stabilizes mating bacteria during DNA transfer by conjugation	Protein
Common pili or fimbriae	Attachment to surfaces; protection against phagotrophic engulfment	Protein
Capsules (includes “slime layers” and glycocalyx)	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; protection against desiccation	Usually polysaccharide; occasionally polypeptide
Cell wall		
Gram-positive bacteria	confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-lipopolsaccharide “outer membrane”
Plasma membrane	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
Ribosomes	Sites of translation (protein synthesis)	RNA and protein
Inclusions	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
Chromosome	Genetic material of cell	DNA
Plasmid	Extrachromosomal genetic material	DNA

□ **MOLLICUTES:**

Mollicutes is a class of bacteria distinguished by the absence of a cell wall. The word "Mollicutes" is derived from the Latin *mollis* (meaning "soft" or "pliable"), and *cutis* (meaning "skin"). Individuals are very small, typically only 0.2–0.3 µm (200-300 nm) in size and have a very small genome size. They vary in form, although most have sterols that make the cell membrane somewhat more rigid. Many are able to move about through gliding, but members of the genus *Spiroplasma* are helical and move by twisting. The best-known genus in the Mollicutes is *Mycoplasma*.

- Mollicutes are parasites of various animals and plants, living on or in the host's cells. Many *Phytoplasma* and *Spiroplasma* are plant pathogens associated with insect vectors. Whereas formerly the trivial name "mycoplasma" has commonly denoted any member of the class Mollicutes, it now refers exclusively to a member of the genus *Mycoplasma*.

- **Subcategory:** Mycoplasmataceae



A.Phytoplasma: Definition:

Prokaryotic, pleomorphic, resembles mycoplasma, wall-less, (trilamellar unit membrane) self replicating, pass through filters, sensitive to tetracyclines, transmission by leaf hoppers, found in sieve element.

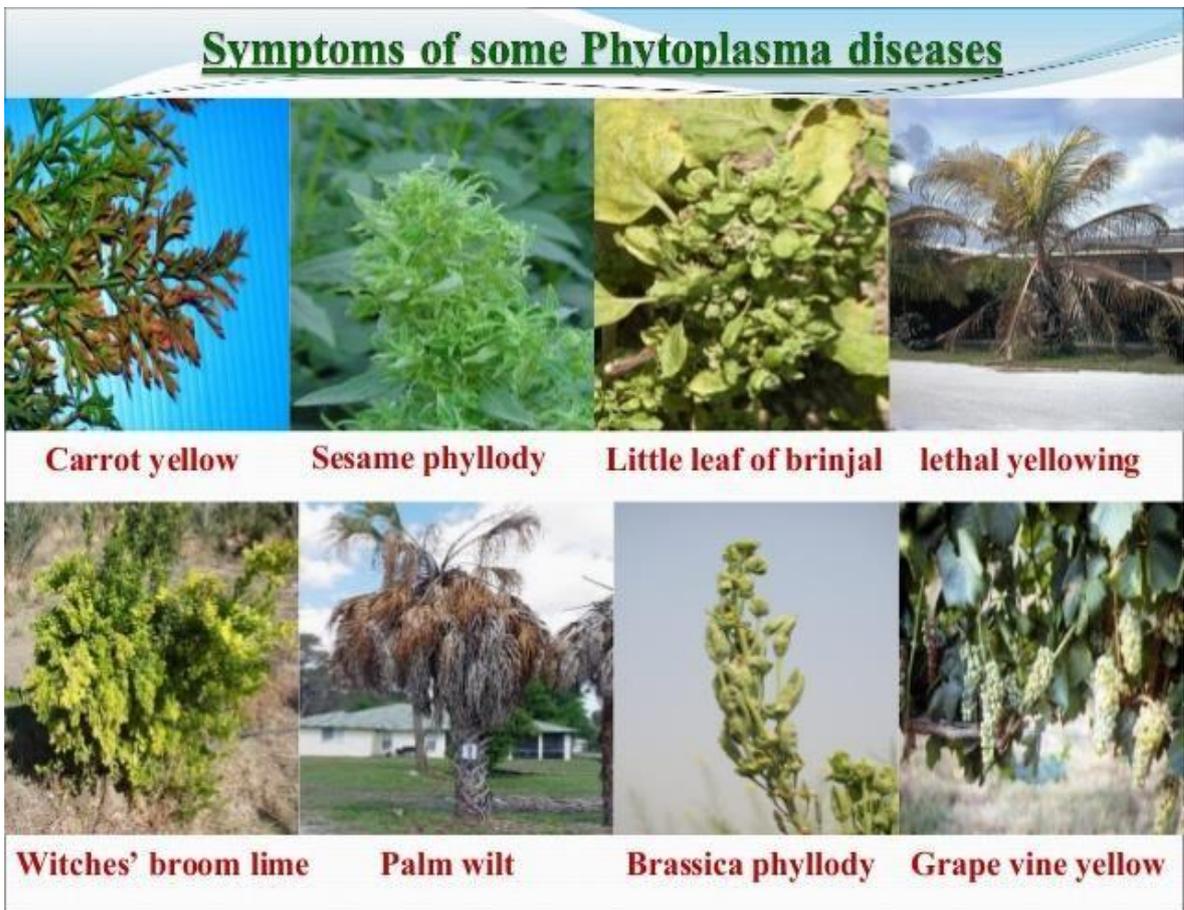
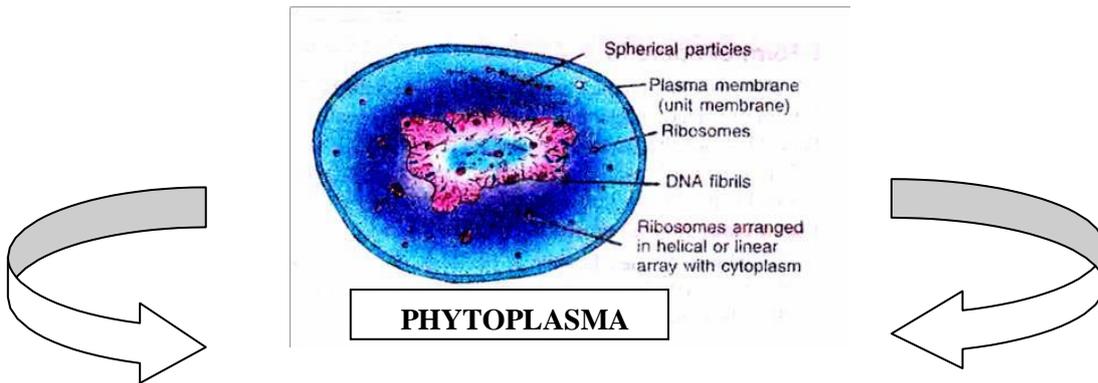
- **Characters of phytoplasma:**
 - a. Size vary from 100 nm-1µm in diameter.obligate parasite.
 - b. Wall less,covered by trilamellar nit membrane with lipoproteins.
 - c. Cells pleomorphic
 - d. Aerobic to facultative anaerobes,nonflagellate,non-spore-formers and gram -ve
 - e. Found intercellularly in phloem vessels in plant.
 - f. Ribosomes of bacteria type.
 - g. Genome is made up of double stranded DNA.
 - h. Osmotically stable.
 - i. Self-replicable by transverse binary fission.
 - j. Resistant to penicillin but sensitive to tetracycline and chloramphenicol.
 - k. Transmission by leaf hoppers and grafting.
 - l. Genetically more relates to acholeplasms than mycoplasmas.
 - m. Can be controlled by cross-protection.

- n. Requires sterol for growth.
- o. Can be controlled by thermotherapy by growing plants at 34-40°C

Eg. Mulberry dwarf

Disease caused by Phytoplasma

- I. Aster yellows (*Macrosteleles fascifrons*)(leaf hopper).
- II. Sesamum phyllody (*Orosius albicinctus, O. orientalis*)(leaf hopper)
- III. Little leaf of brinjal (*Hishimonas phycitis*)(leaf hopper)
- IV. Grassy shoot of sugarcane (*Aphis maydis*) aphid e.t.c



B. Spiroplasma:Definition:

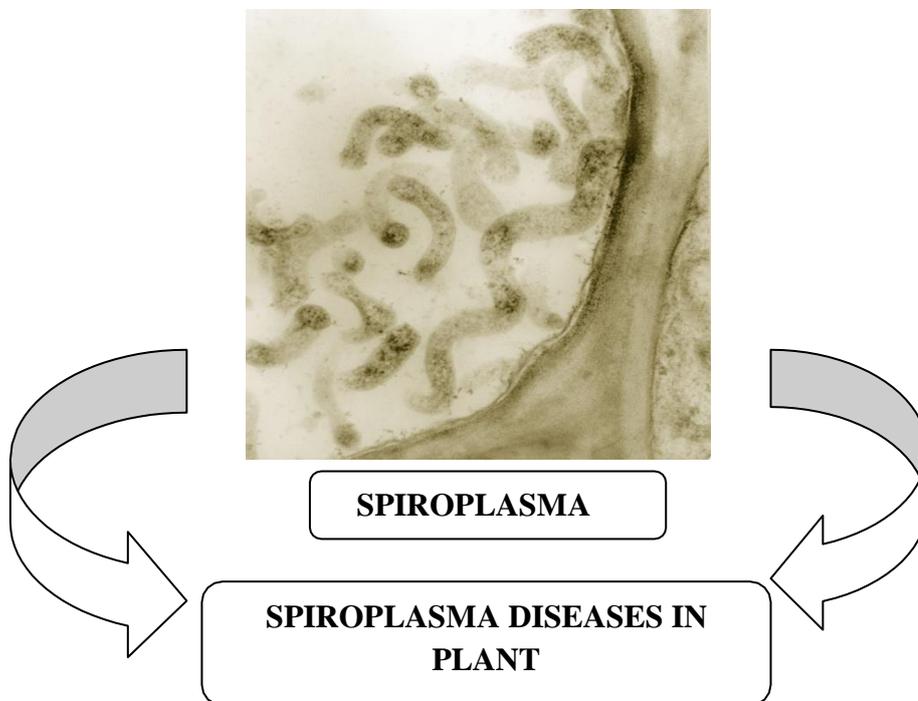
Helical, wall less prokaryotes, phloem of diseased plants, helical in culture, a kind of mycoplasma, but can't be cultured.

Important Characteristics:

- a. Helical in liquid media, gram+ve
- b. Size vary from 100-240nm in diameter, no flagella.
- c. Grown on culture medium in laboratory, colony appear like “fried egg” in culture medium.
- d. Multiplication by transverse binary fission.
- e. Resistant to penicillin but sensitive to tetracycline.

Important disease and vectors: (leaf hopper)

- I. Corn stunt (*Dalbulus elimatus*)
- II. Citrus stubborn (*Circulifer tenellus*)



Corn stunt disease



Citrus stubborn disease

References:

1. **A Text Book Of Fungi Bacteria And Viruses By H.C Dubey**
2. **Microbiology by R.P Singh**
3. **Plant Pathology by G.N Agrios(5th Edition)**
4. **Fundamentals of Plant Bacteriology by Jayashree Jayaraman and Jeevan Prakash Verma**

QUESTION BANK

A. Short Notes: (Each question carries 2marks)

1. Bacterial cellwall
2. Structure of peptidoglycan
3. Comparison between the Gram+ve and Gram –ve bacterial cellwall
4. Endospore
5. Fimbriae and pilli
6. Flagellar movement
7. Asexual reproduction of bacteria
8. Classification of phytopathogenic prokaryotes, according to Bergey's manual of systemic bacteriology, 1984(with examples)
9. Morphology based classification of bacteria with examples.
10. Difference between prokaryotes and Eukaryotes
11. Inclusion bodies
12. Slime layer

B. Broad question:(Each question carries 4marks)

1. What is bacteria? Explain general characteristics of bacteria.
2. Detailed description of Bacterial cell.along with the functions of each.
3. Distinguish between Gram positive cellwall and Gram negative cellwall with diagrammatic representation.
4. What is flagella? How does it work as the organ of locomotion incase of bacteria?
5. Describe the flagellar structure?
6. Describe the classification based on Morphology?cite examples
7. Describe the classification of bacteria on the basis of flagellation.with examples
8. What are the different modes of reproduction in bacteria?explain with diagram
9. Define mollicutes?Classification of phytopathogenic molliutes?
10. What is Phytoplasma? Briefly discuss about the general characteristics of phyoplasma.give few examples of phytoplasmal dieases in plant.
11. What is Spiroplasma? Briefly discuss about the general characteristics of Spiroplasma.give few examples of Spiroplasmal dieases in plant.

SUB- DIVISION: BASIDIOMYCOTINA

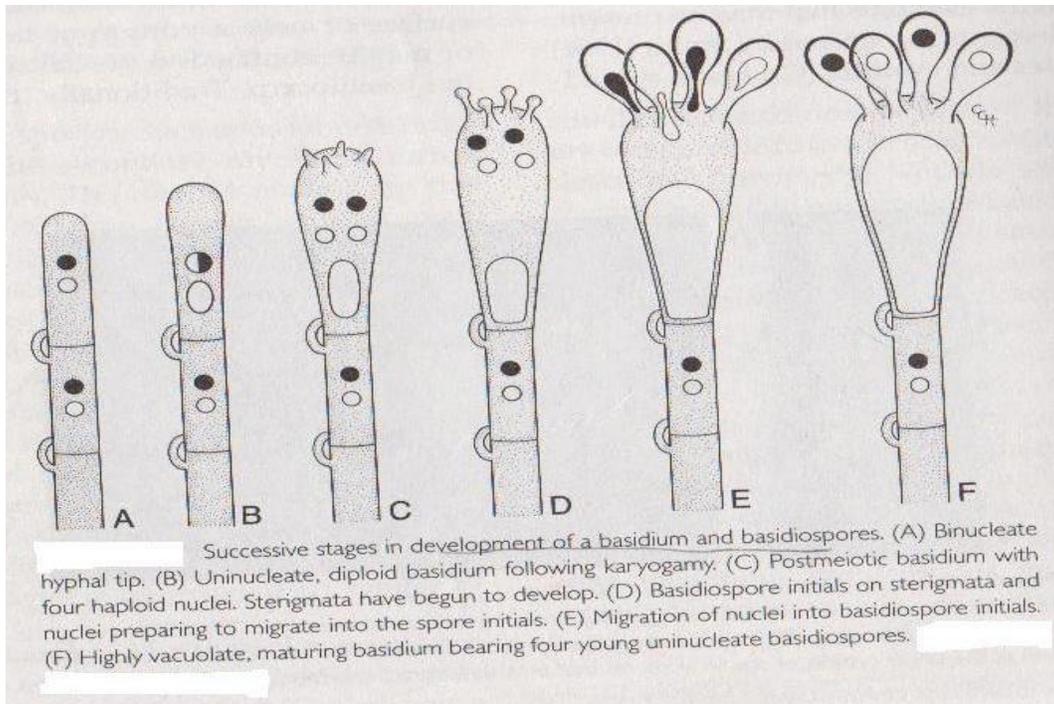
1. The name Basidiomycotina is given because the fungi produce sexual spores on a special club shaped fruiting body called **basidium**.
2. A definite number of sexual spores called basidiospores (usually **four** in number) are produced on pointed projections called sterigmata in each basidium .
3. Fungi belonging to this sub division are referred as **club fungi**.
4. The group includes mushrooms, toadstools, shelf fungi, jelly fungi, puff balls, coral fungi, bracket fungi, birds nest fungi, stink horns, rusts and smuts.

General characteristics:

- Basidiospores are haploid, uninucleate and are the result of plasmogamy, karyogamy and meiosis.
- Presence of clamp connections on the mycelium .
- Presence of dolipore septum, except in rusts and smuts.
- Absence of motile spores.

Clamp connections: It is a hook like structure formed laterally in between the dividing nuclei in a dikaryotic hypha. It acts as a by-pass for the nuclei, as they can not pass through septal pore ie., dolipore septum. It is meant for multiplication of dikaryotic cells.

Dolipore septum: Both primary and secondary mycelium consists of dolipore septum. The septum around the central pore swells at the center forming a barrel shaped structure with open ends, thus forming a septal pore. The septal pore is surrounded by a cup like or dome shaped membrane called parenthosome or septal pore cap or nuclear pore cap. It is made up of a double membrane and its function is to shut the pore. The dolipore septum will not allow the movement of nuclei in hyphae but maintains continuity of cytoplasm.



CLASSIFICATION OF BASIDIOMYCOTINA

CLASSES

1. TELIOMYCETES

ORDER: UREDINALES

FAMILY: PUCCINIACEAE

Eg. *Puccinia*, *Uromyces*, *Hemileia*

FAMILY: MELAMPSORACEAE

Eg. *Melampsora*

ORDER: USTILAGINALES

FAMILY: USTILAGINACEAE

Eg. *Ustilago*, *Sphaecelotheca*, *Tolyposporium*

FAMILY: TILLETIACEAE

Eg. *Tilletia*, *Neovossia*, *Urocystis*

FAMILY: GRAPHIOLACEAE

Eg. *Graphiola*

2. HYMENOMYCETES

SUB CLASS: HOLOBASIDIOMYCETIDAE

ORDER: AGARICALES

FAMILY: AGARICACEAE

Eg. *Agaricus*, *Volvariella*, *Pleurotus*

ORDER: APHYLLOPHORALES

FAMILY: POLYPORACEAE

Eg. *Polyporus*, *Fomes*, *Peria*

FAMILY: GANODERMACEAE

Eg. *Ganoderma*

CLASS

1.TELIOMYCETES

In class Teliomycetes there are two orders.1.Uredinales 2.Ustilaginales

IMPORTANT CHARACTERISTICS OF ORDER UREDINALES:

The popular name for the Uredinales is the rust fungi, spores are reddish brown in colour.

Clamp connections are rare or absent.

The rusts have **polymorphic life cycle**. Production of many spore forms in the life cycle is called polymorphism. Generally 5 types of spores are seen during the life cycle viz., **spermatia** (uninucleate) in spermogonium, **aeciospores** (binucleate) in aecium, **uredospores** (binucleate) in uredium, **teleospores** (binucleate) in telium and **basidiospores** (uninucleate) on promycelium or metabasidium.

The spermogonium represents gametic stage (male gamete- spermatium, female sex organ- receptive hypha)

Autoecious rust: If all the spore stages are produced on the same host then the fungus is called autoecious and the phenomenon is called **autoecism**.

Eg. *Melampsora lini*- linseed rust, *Uromyces appendiculatus* - bean rust.

Heteroecious rust: If spore stages are formed on two unrelated hosts ie., pycnia and aecia on one host and the uredia and telia on the other host, such rusts are called heteroecious rusts and phenomenon is called **heteroecism**.

Eg. *Puccinia graminis f. sp. tritici*- black stem rust of wheat. **Primary host:** The host in which heteroecious rust produce the telial stage is called primary host (Eg. wheat).

Based on life cycle pattern, rusts are divided into macrocyclic, demicyclic and microcyclic rusts.

Macrocyclic rust: (long cycled rust): Rusts in which all 5 spore forms are produced or produce at least one type of binucleate spore in addition to teleospores are called macrocyclic rusts.

(Eg. *Puccinia helianthi*- sun flower rust) or heteroecious , macrocyclic rust
(Eg. *Puccinia graminis f.sp. tritici* - black stem rust of wheat).

Demicyclic rust: The rust in which uredial stage is absent. eg. *Gymnosporangium juniperi – virginianae*- cedar apple rust.

Microcyclic rust (short cycled rust): Rusts which produce no binucleate spore other than teleospore. i.e., teleospore is the binucleate spore produced and both aecia and uredia are lacking. Eg. *Puccinia malvacearum*- hollyhock rust.

FAMILY: PUCCINIACEAE:

Genus Puccinia:

Obligate parasites. Teleutospores are two celled and stalked.

Disease: *Puccinia graminis f.sp. tritici*- black stem rust of wheat



Genus Uromyces:

Teleutospores are single celled with a thick apex (papillum) and stalked. The stalks are fragile and short.



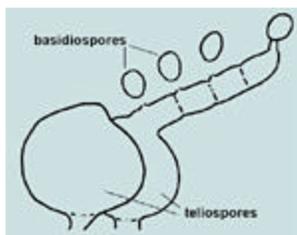
Eg. *U. appendiculatus* - bean rust

Genus Hemileia:

Uredospores are reniform, bifacially ovate resembling orange segments, concave side smooth, convex side echinulate.

Teleutospores are turnip shaped. 1 celled, stalked, smooth walled and produced on erumpent, club shaped stalks which arise through stomata.

Eg. *H. vastatrix* - coffee rust



LIFE CYCLE OF PUCCINIA GRAMINIS F. SP. TRITICI

The pathogen is an obligate parasite and causes black stem rust of wheat. It is a heterococious rust that requires 2 hosts for completion of its life cycle. The primary host

(wheat) and the secondary host (barberry). On barberry it produces pycnia and aecia while uredial telial stages are produced on wheat.

Stage	Spore	Nucleus status
O	spermagonia with spermatia (pycnia with pycniospores)	uninucleate
I	aecia with aeciospores	binucleate
II	uredia with uredospores	binucleate
III	telia with teleospores	binucleate
IV	basidia with basidiospores	uninucleate

It produces the first 2 stages in barberry and other 3 stages on wheat or other graminaceous hosts.

Significance of each stage :

Stage “ O “: The spermatia produced in spermagonia were thought to be functionless asexual spores. In 1927, Cragie found that spermatia are male gametes and are essential for spermatization of receptive hyphae (female organ) and consequent formation of aeciospores. This stage “ O” represents the sexual stage of rust fungi but the nomenclature stage “ O “ retained even today to avoid confusion.

Stage I: Aeciospores are the first binucleate spores formed in the life cycle.

Stage II: Uredospores are also called as repeating asexual spores as they function as conidia for the propagation of the rust fungus.

Stage III: Teleutospores represents the perfect stage because karyogamy and meiosis occur in them.

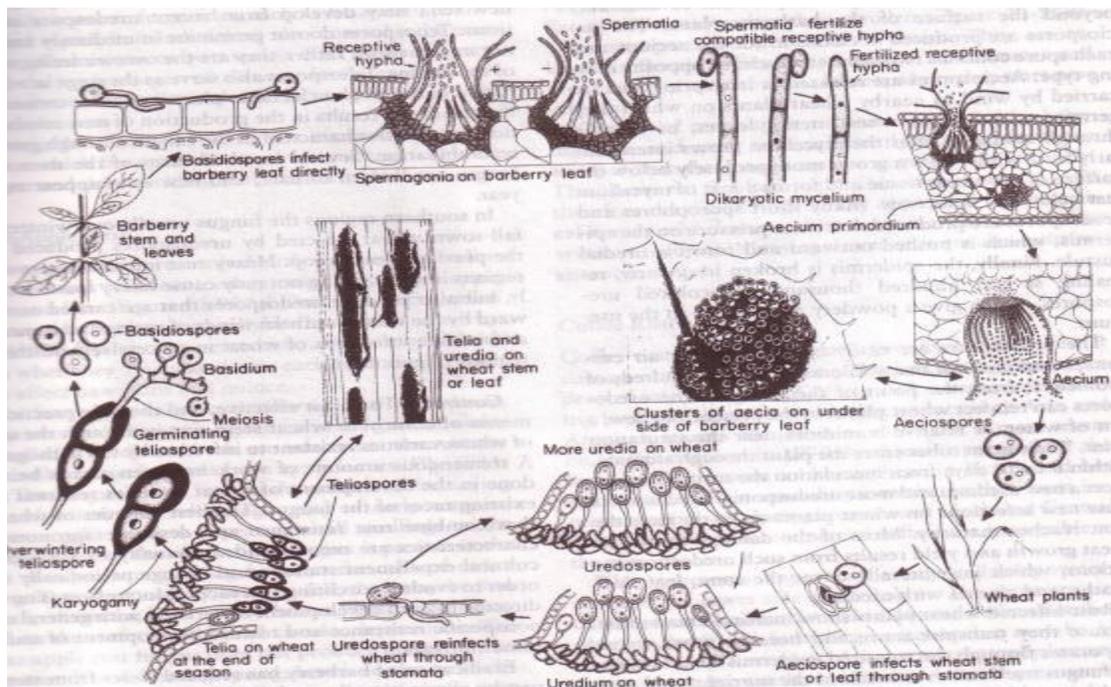
Stage IV: Basidiospores represent the sexual spores.

Stages “ O” and “ I” occur on barberry while stages “ II” and “ III “ occur on wheat.

Stage “ O”: Spermagonia with spermatia:

The spermagonia or pycnia are the structures which bear the sex organs of the pathogen. It contains spermatia which are the male sex organs and the receptive hyphae which are the female sex organs. In nature it generally happens that several basidiospores at random will reach and infect the same barberry leaf so that both + and – mycelium develop side by side and intermingle in the barberry tissue. Each spermagonium contains numerous spermatia. These are exuded (ooze out) in small droplets of nectar present in the spermagonium. Each spermatium carries + or – nucleus depending on the strain of mycelium which produced the

spermogonium. Spermatization i.e., fusion between receptive hyphae and spermatia of opposite sex takes place through agency of insects which are attracted by the honey fluid. The spermatial contents pass into the receptive hyphae. Meanwhile the mycelium penetrates the entire leaf and the hyphae near the lower epidermis develop number of aecial primordia. It is presumed that spermatial nuclei which pass from the spermatia into the receptive hyphae reach the cells of the aecial primordia rendering them binucleate. It has been demonstrated that aecial primordia fail to develop into aecia until and unless spermatization takes place.



Life cycle of *Puccinia graminis f. sp. tritici*

Stage "I" : Aecia and aeciospores are formed in the lower epidermis soon after dikaryotisation. These are the first binucleate spores produced in the life history of the fungus. An aecium is a group of binucleate hyphal cells (aeciospore mother cell) which give rise to aeciospores in chains. The aeciospores finally disseminated by the wind and under favourable conditions germinate on graminaceous host . These can not infect barberry.

Stage " II": Soon after infection by aeciospores on graminaceous host , binucleate mycelium begins to form masses of cells. These are called uredia from which binucleate

uredospores are borne on long stalks. The uredospores are one celled, oval, yellowish and spiny. They germinate readily in water and produce one or more germ tubes. The uredospores are those spores which perpetuate the fungus throughout the growing season and they are capable of reinfecting the graminaceous host on which they produce. Hence, they are also known as repeating asexual spores. They spread from plant to plant and from field to field and the disease soon becomes an epiphytotic. The uredospores upon germination produce binucleate mycelium which grows between the cells of the host and in a few days produce new uredia and uredospores.

Stage “ III” : Late in summer, at the time of ripening of grain another kind of spores known as teleospores or teleutospores are developed in the same mycelium when the uredia begin to cease. The pustules which produce teleospores are known as telia and constitute the black stage of rust. The teleospores are ellipsoidal, oblong or obclavate, typically two celled, and thick walled with slight constriction at the septum. The young teleospore is binucleate . Karyogamy eventually takes place and render the teleospores diploid and uninucleate . The teleospores are not capable of germinating immediately and should have resting period of several months and thus remain dormant until the following spring.

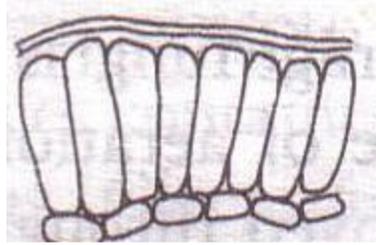
Stage “ IV” : Early in the spring each cell of teleospore germinate and produce a basidium (promycelium). The diploid nucleus in teleospore migrates in to the promycelium and undergoes meiosis and four haploid nuclei are formed. Then septa are formed, separating the nuclei from one another into four cells. Each cell of promycelium produces a sterigmata on which basidiospore is formed. The nuclei now migrate into the basidiospore. Two of the basidiospores are of one strain (+) and two are of other strain (--) . Soon after their formation ,the basidiospores are ejected and are carried away by wind. They can not infect graminaceous host, but can infect barbery and produce a well developed monokaryotic mycelium. Thus, the life cycle gets repeated on these two hosts viz., wheat and barbery.

IMPORTANT CHARACTERISTICS OF MELAMPSORACEAE :

1. Teleutospores are single celled and sessile (no stalk).

Genus Melampsora:

Teleutospores are single celled, sessile and laterally united to form layers and sub- epidermal.



Eg. *Melampsora ricini*- castor rust.

IMPORTANT CHARACTERISTICS OF ORDER USTILAGINALES:

The fungi included in this order are popularly called as smut fungi and the diseases they cause are called smut diseases.

No sex organs are produced . Plasmogamy takes place by fusion of any two basidiospores or two conidia. Somatic hyphae may also fuse resulting in dikaryotic condition.

IMPORTANT CHARACTERISTICS OF USTILAGINACEAE:

- 1.Known as smut fungi
- 2.Promycelium is septate
- 3.Basidiospores are produced laterally from each cell of the promycelium

Character	Rusts	smuts
Systematic position Order:	Uredinales	Ustilaginales
Plant parts affected	foliar parts (leaves, stem, petiole)	floral parts(flowers)
Symptoms	reddish brown coloured pustules	ovaries turn into black sooty masses
Parasitism	obligate parasites	facultative saprophytes
Polymorphism	polymorphic	not polymorphic
Teleutospores	terminal , 1 or 2 celled, stalked or sessile	intercalary, 1 celled, sessile
Basidiospores	produced on sterigmata, discharged, 4 in number	violently sessile,not violent 4 to many(indefinite in number
Sex organs	spermatia (male) receptive hyphae(female)	no sex organs
Dikaryotisation	spermatiation	fusion of 2 basidia or Basidiospores or conidia or promycelium

IMPORTANT CHARACTERISTICS OF FAMILY TILLETIACEAE:

1. Known as bunts
2. Promycelium is non-septate



3. Basidiospores arise terminally

DISTINGUISHED CHARACTERISTICS OF GENERA:

Tilletia:

1. Teleospores singles
2. Spores dusty and escaping at maturity
3. Sporidia are fused to form H shaped structures

Neovossia:

1. Teleospores singles
2. Spores dusty and escaping at maturity
3. Sporidia do not fuse, no H shaped structures

Urocystis :

1. Teleospores in balls
2. Sori dusty, spore balls surrounded by an adhering layer of hyaline sterile cells.
3. spore balls escape from sorus

DISEASES CAUSED BY THE GENERA:

Tilletia caries and *T. foetida*- bunt of wheat

Neovossia horrida- bunt of paddy

N. indica- Karnal bunt of wheat

Urocystis cepulae- onion smut

U. tritici- flag smut of wheat

DIFFERENCES BETWEEN SMUTS AND BUNTS :

S.No.	Smuts	Bunts
1	Belongs to family Ustilaginaceae	Belongs to family Tilliteaceae
2	Promycelium is Septate	Promycelium is non-Septate and hollow tube like
3	Basidiospores are formed laterally from each cell of the promycelium	Basidiospores are formed at the tip of the promycelium
4	Basidiospores are usually four	Basidiospores are more than four usually eight
5	H shaped structures are not formed	H shaped structures are present in which plasmogamy occurs.
6	Meiosis occurs in promycelium or teliospores	Meiosis always occurs in teliospores before germination
7	No fishy odour is observed	Characteristic stinking fishy odour is observed
8	Genera included are <i>Ustilago</i> , <i>Sphaecelotheca</i> , <i>Tolyposporium</i>	Genera included are <i>Tilletia</i> , <i>Neovossia</i> , <i>Urocystis</i>

IMPORTANT CHARACTERISTICS OF DIVISIONS AND SUB-DIVISIONS

DIVISIONS :

1. **MYXOMYCOTA:** Plasmodial forms with out cell wall. Plasmodium is a naked multinucleate mass of protoplasm which moves and feeds in an amoeboid direction. Also called as slime molds.
2. **EUMYCOTA:** True fungi. Thallus is typically filamentous with cell wall. Plasmodium absent

SUB DIVISIONS OF EUMYCOTA:

1. MASTIGOMYCOTINA :

Thallus is unicellular or aseptate mycelium. Asexual spores are zoospores (motile spores). Sexual spores are oospores. Sexual reproduction by gametangial contact.

2. ZYGOMYCOTINA :

Thallus is aseptate mycelium. Motile spores are absent. Asexual spores are sporangiospores (aplanospores). Sexual spores are zygospores. Sexual reproduction through gametangial copulation.

3. ASCOMYCOTINA :

Thallus is septate mycelium. Rarely unicellular. Motile spores are absent. Asexual spores are conidia. Sexual spores are ascospores produced endogenously in an ascus. Sexual reproduction mainly by gametangial contact .

4. BASIDIOMYCOTINA:

Thallus is septate mycelium. Motile spores are absent. Clamp connections and dolipore septum are present. Sexual spores are basidiospores produced exogenously on basidium. Sexual reproduction is by spermatization and somatogamy.

5. DEUTEROMYCOTINA:

Thallus: septate mycelium . Motile spores are absent. Sexual spores are absent. Asexual spores called conidia are present.

DIVISION – MYXOMYCOTA

IMPORTANT CHARACTERISTICS OF

CLASS: PLASMIDIOPHOROMYCETES, ORDER: PLASMIDIOPHORALES

FAMILY: PLASMIDIOPHORACEAE

1. These are obligate endoparasites. Commonly called as endoparasitic slime molds. Thallus is a plasmodium
2. Zoospores are anteriorly biflagellate, whiplash type, unequal in size which are called as Heterokont zoospores.
3. After swimming for some time, the zoospore encysts on the root hair of the host. A cylindrical sharp pointed body, called **satchel**, is formed in a specialized pouch or sheath called **Rohr**.
4. Nuclear division is by cruciform division.
5. Sexual reproduction is by isogamous planogametic copulation.
6. Members cause abnormal enlargement and multiplication of host cells i.e., hypertrophy and hyperplasia.

Eg: *Plasmodiophora*, *Spongospora*

Diseases : *Plasmodiophora brassicae* : club root / finger & toe disease of cabbage / crucifers.

Spongospora subterranea : powdery scab of potato

DIVISION : EUMYCOTA

... **SUB - DIVISION: MASTIGOMYCOTINA**

IMPORTANT CHARACTERISTICS OF CLASS CHYTRIDIOMYCETES:

1. Thallus (a) primitive members - unicellular, advanced members with coenocytic mycelium.
2. Asexual reproduction is by zoospores produced in zoosporangia.
3. Sexual reproduction is by (a) planogametic copulation (isogamy, anisogamy, heterogamy) . (b) gametangial copulation.
4. Zygote is converted into resting sporangium / resting spore.
5. Zoospores produced from this resting spore infect host cell and produces pro sorous.

Eg. *Synchytrium*

Disease: Causes black wart of potato. It is prominent in hilly regions like Darjeeling.

Epidermal cells of tubers are infected by the fungus. Hypertrophy and hyperplasia takes place, as a result, out growths appear on tubers.

Diseases transmitted : *Synchytrium endobioticum* transmits potato virus - x .

IMPORTANT CHARACTERISTICS OF CLASS OOMYCETES

1. Members may be aquatic or terrestrial ,saprophytes or obligate parasites.
2. Thallus - mostly eucarpic ,coenocytic
3. Cell wall consists of cellulose. Chitin is absent.
4. Asexual reproduction is by zoospores produced in zoosporangia. Zoospores are biflagellate (whiplash and tinsel), anteriorly or laterally positioned, equal in size.
5. Sexual reproduction is oogamous type ie.,gametangial contact/ gametangy.. Heterogametangia come in contact, contents of antheridium (male gametangium) passes into oogonium(female gametangium) containing oosphere (egg) through fertilization tube.
6. Zygote resulting from sexual reproduction is called oospore.
7. Oospore is the sexual resting spore which is the characteristic of oomycetes.
8. This zygote/ oospore is diploid.Oospore which gives rise to mycelium/ gametangia is also diploid.
9. Meiosis occurs in gametangia instead of zygote.
- 10.

ORDER PERONOSPORALES :

Families: 1.Pythiaceae 2.Albuginaceae 3.Peronosporaceae

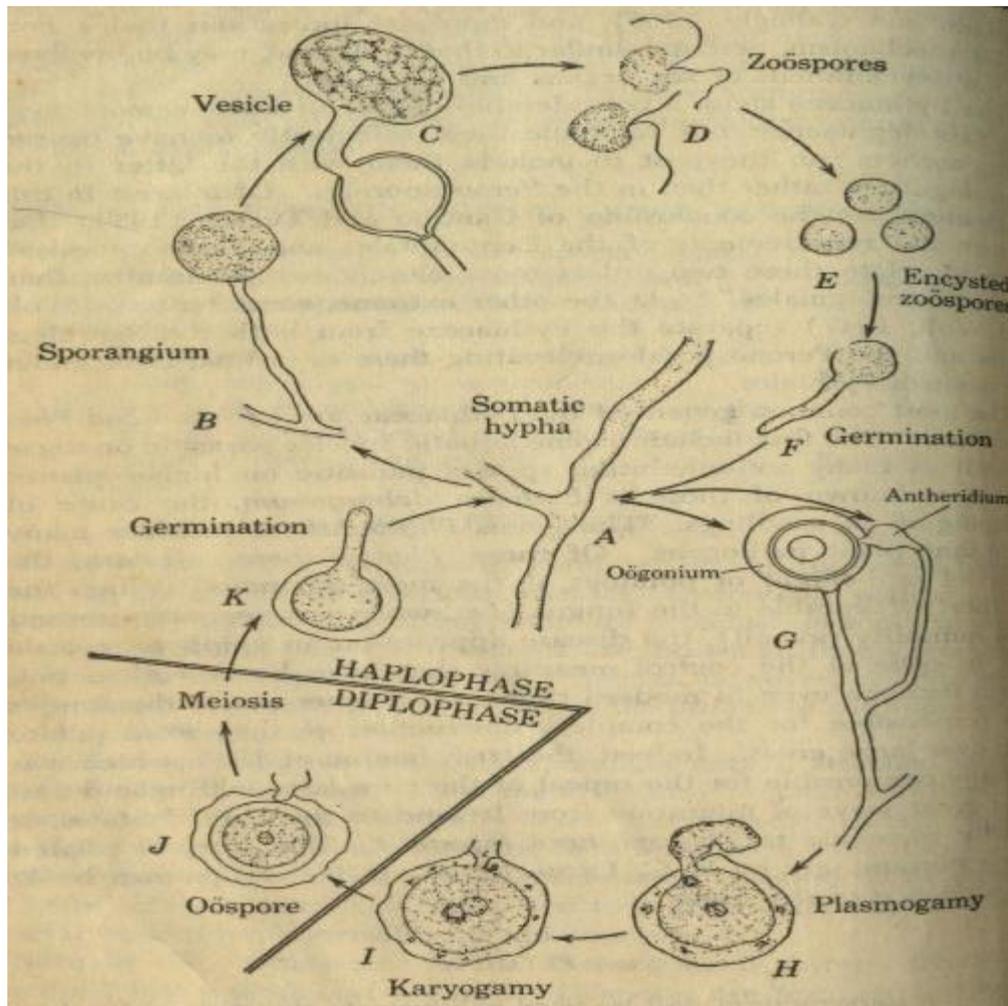
These three families are distinguished based on characteristics of sporangiophores and sporangia.

FAMILY 1. PYTHIACEAE :

Genus **PYTHIUM** :Fungus is facultative parasite and lives in soil on dead organic matter or parasitically on young seedlings of crop plants .Mycelium well developed,branched,coenocytic,hyaline,intracellular myceliumwithout haustoria. Thallus is homothallic.Asexual reproduction is through zoospores produced in zoosporangia. . Sporangia are large globose(*P. debaryanum*) or irregularly lobed (*P. aphanidermatum*) produced terminally or intercalary on somatic hyphae. Zoospores are produced in a vesicle which emerge out of sporangium.ie.,zoospore differentiation takes place in vesicle.Sexual reproduction is by gametangial contact.

Paragynous antheridium(Antheridium is by the side of oogonium).Oospores are smooth,thick walled,round,light brown.

Diseases: Damping off of vegetable seedlings of solanaceous crops caused by *Pythium debaryanum* or *P. aphanidermatum*



FAMILY : ALBUGINACEAE

GENUS: Albugo

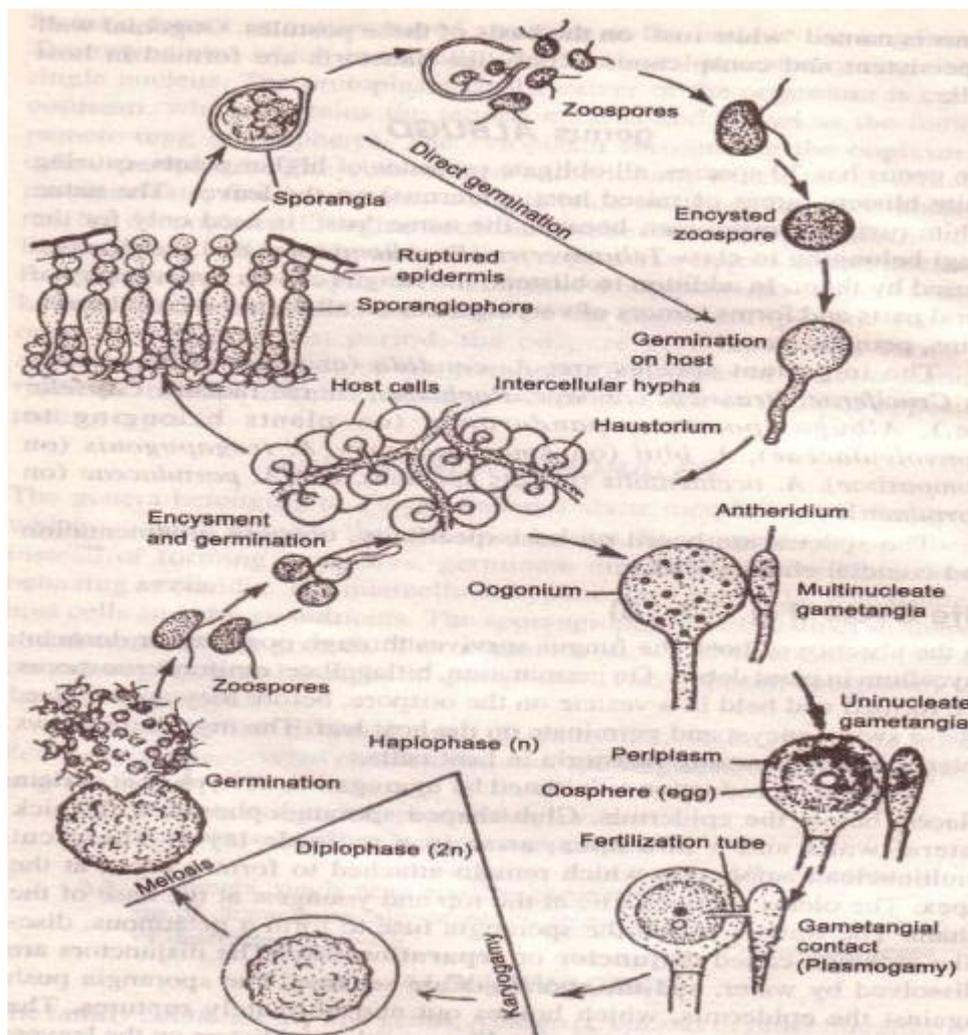
Fungus is an obligate parasite on crucifers and lives in soil in the form of oospores or parasitically on plants.

Mycelium is coenocytic, hyaline, endophytic, intercellular with knob shaped haustoria.

Asexual reproduction is by zoospores produced in sporangia. Sporangioophores are unbranched, hyaline clavate, bears sporangia in chains in basipetal succession. Sporangia are spherical, thin walled, sessile, hyaline and germinate by zoospores.

Sexual reproduction is by gametangial contact. Oospores are round, thick walled, dark brown and outer wall warty.

Diseases: White rust on mustard caused by *Albugo candida*



FAMILY:3.PERONOSPORACEAE

foliage consisting of sporangiophores of these fungi.

The members are further divided into different genera and distinguished based on two characteristics viz., 1. morphology of sporangiophore (branching pattern) 2. method of germination of sporangia.

Eg. *Peronospora*, *Pseudoperonospora*, *Peronosclerospora*, *Sclerospora*, *Plasmopara*, *Bremia*, *Sclerophthora*.

DISTINGUISHING CHARACTERISTICS OF DOWNY MILDEW GENERA:

1. **SCLESPORA**: Sporangiophores are stout, having upright branches, bearing sporangia on sterigmata. Sporangia are hyaline, ovoid, smooth walled, papillate and germinate by zoospores. Oospore is plerotic.

Eg. *Sclerospora graminicola* – downy mildew of bajra.

2. **PERONOSPORA**:Sporangiophores are dichotomously branched 2-7 times at acute angles and tips of branches are curved and pointed bearing sporangia on sterigmata.Sporangia are hyaline ,ovoid ,non-papillate and always germinate by germ tube. i.e.,sporangia behave like conidia.

Eg;*Peronospora destructor*-downy mildew of onion.

3. **PERONOSCLEROSPORA** :Fungus possess characteristics of both *Peronospora* and *Sclerospora*.Sporangiophores are erect,short,stout,widening towards upper portion,dichotomously branched 2 -5 times at apex bearing sporangia on sterigmata.Sporangia are hyaline,elliptical or ovoid,thin walled, non-papillate and germinate by germ tube like *Peronospora*. Oospore is plerotic type like *Sclerospora*.

Eg.*Peronosclerospora sorghi*-downy mildew of jowar

P.philippinensis-downy mildew of maize

4. **PSEUDOPERONOSPORA**:Sporangiophores are branched at acute angles with curved,blunt tips,bearing sporangia on sterigmata.Sterigmata are unequal (1 big and 1 small).Sporangia are greyish,ovoid,papillate and germinate by zoospores.

Eg.*Pseudoperonospora cubensis* -downy mildew of cucurbits

5. **PLASMOPARA**;Sporangiophores are branched at right angles to the main axis at regular intervals.Monopodial branching is observed.Subsequent branches are 3-6 which end in blunt sterigmata of 3 in number.Sporangia are ovoid and germinate by zoospores.

Eg.*Plasmopara viticola*-downy mildew of grapes.

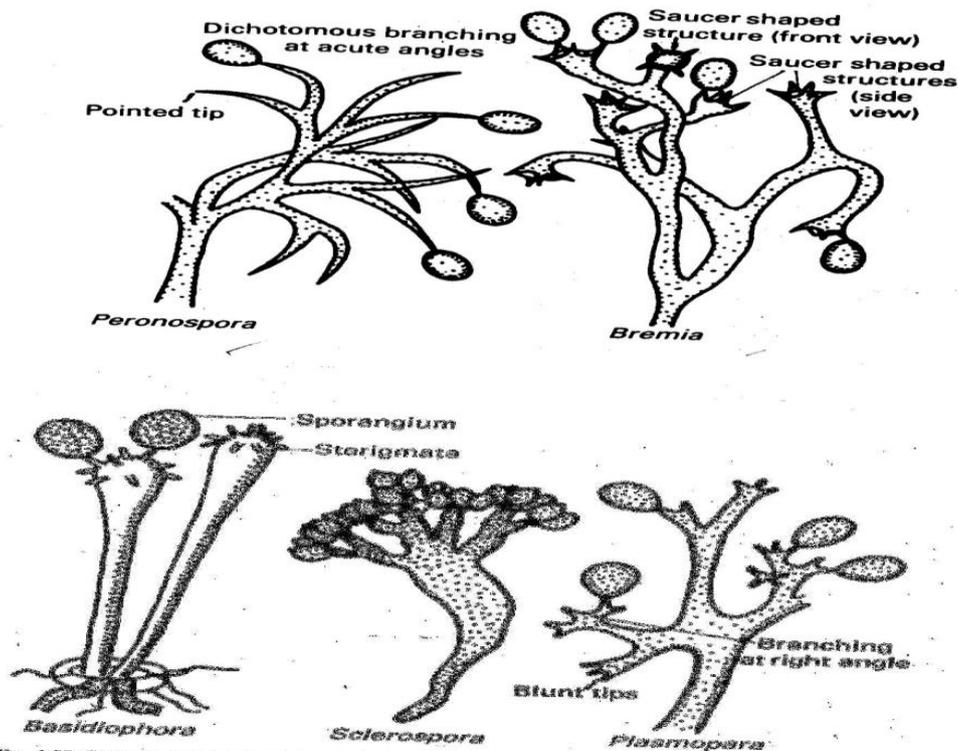


Fig. 4.59: Sporangiohores characteristic of five genera of the Peronosporaceae (Redrawn from Alexopoulos and Mims, 1978).

1. **BREMIA** :Sporangiophores are dichotomously branched ,tips of branches are expanded to cup shaped apophysis with four sterigmata bearing sporangia.Sporangia are ovoid,papillate and germinate by zoospores.

Eg:*Bremia lactucae*-downy mildew of lettuce.

SUB-DIVISION: ZYGOMYCOTINA
CLASS: ZYGOMYCETES
ORDER: MUCORALES:

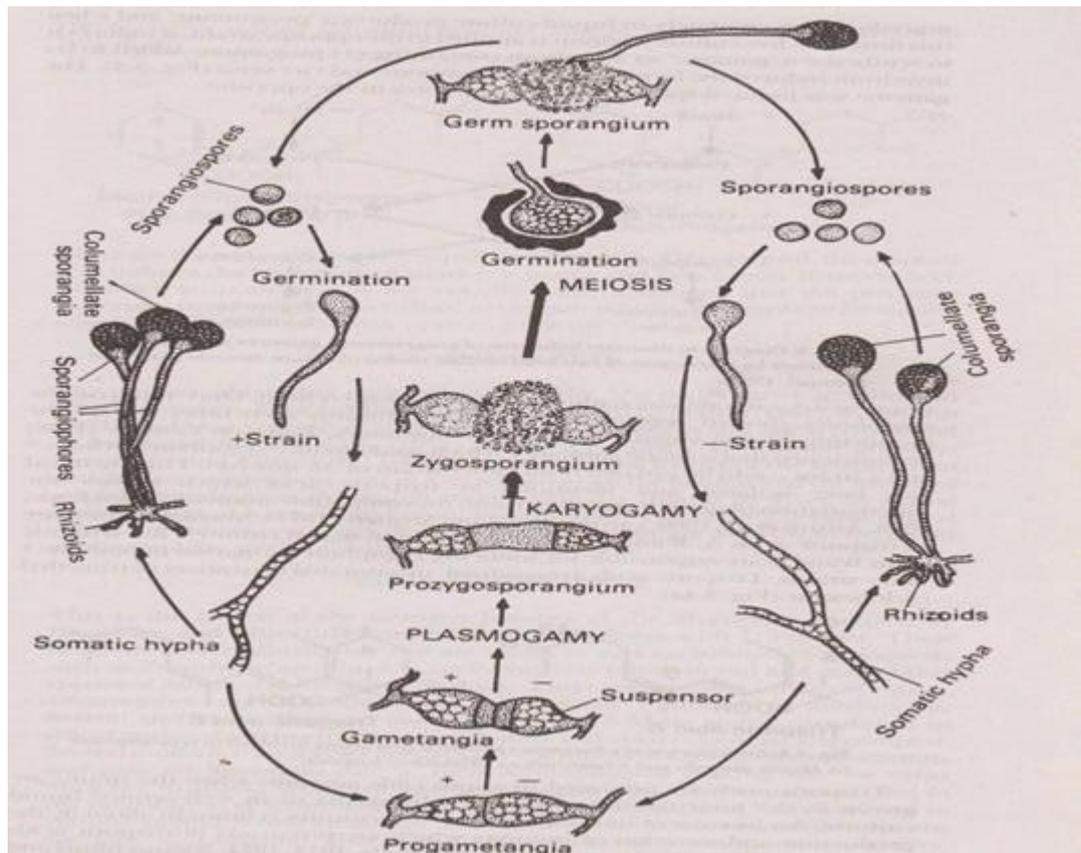
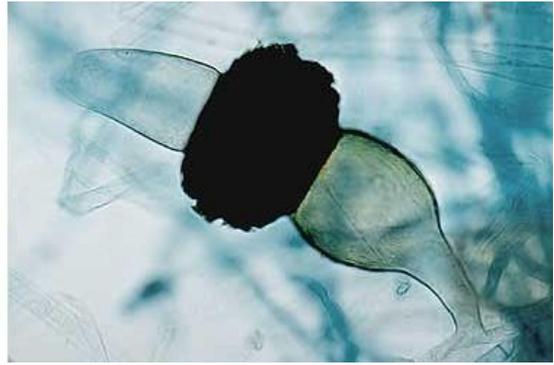
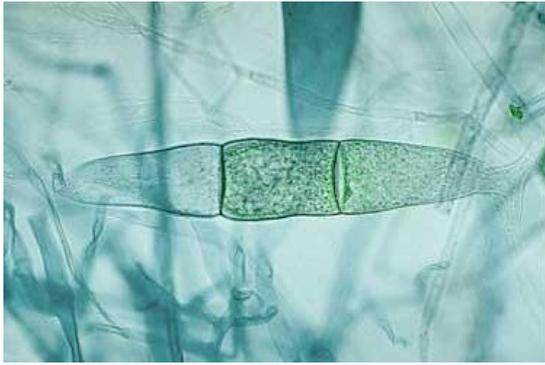
FAMILY: MUCORACEAE:

Eg. Rhizopus stolonifer

Genus: *Rhizopus*:

Rhizopus stolonifer, commonly called as bread mold is a general contaminant of several food materials. The fungus is mostly saprophytic but is a weak parasite.

1. Well developed coenocytic mycelium. Mycelium is differentiated into rhizoids, stolons and sporangiophores
2. Rhizoids / holdfast are a cluster of brown, slender, branched root like structures which arise from the lower side of stolons and penetrate into the substratum. These are useful for anchoring the thallus into the substratum and for absorption of nutrients.
3. Stolons or runners are aerial hyphae which grow on the surface of substratum horizontally and connect the two nodal points (the junction of stolon and rhizoid or the point from which rhizoids are produced is called node).
4. Sporangiophores are erect, unbranched hyphae usually produced in fascicles (groups) only from the nodes during asexual reproduction. Each sporangiophore bears a single sporangium at its tip. Sporangia are large, globose, many spored with a sterile structure called columella.
5. Asexual reproduction is by non-motile sporangiospores.
6. Fungus is heterothallic. Phenomenon of Heterothallism was discovered by in 1904 by A.F. Blakeslee.
7. Sexual reproduction is by isogametangial copulation. Zygosporangia are thick walled, dark, warty sexual resting spores which develop in a zygosporangium.
8. Diseases: Soft rot of sweet potato, fruits and vegetables.



SUB DIVISION: ASCOMYCOTINA

The members of sub division Ascomycotina are commonly called as sac fungi, because of production of sexual spores, ascospores in a sac like structure called ascus. The members of sub divisions Ascomycotina, Basidiomycotina and Deuteromycotina are also considered as higher fungi.

IMPORTANT CHARACTERISTICS OF SUB -DIVISION ASCOMYCOTINA :

1. Produce definite number (usually eight) of sexual spores (ascospores) in a sac like structure called ascus.
2. Mycelium is septate, branched and organized into tissues known as plectenchyma.
3. Production of sexual fruiting body called ascocarp in which asci are produced.
4. Absence of motile spores and presence of asexual spores called conidia.
5. Presence of a short dikaryotic phase in ascogenous hypha or ascogenous cell.

1. **Somatic characteristics:** Mycelium is septate and organized into fungal tissues. Some consists of septate hyphae except in a few cases like yeasts which are single celled. Mycelium is profusely branched (except in yeasts) and the hyphal walls containing chitin. The septum is simple, incomplete, perforated and have a central pore/ septal pore through which cytoplasm and nuclei move from one cell to another cell thus, cytoplasmic continuity is maintained.

2. **Fungal tissues:** Mycelium is mostly organized into fungal tissues known as plectenchyma. These tissues are chiefly associated with fruiting bodies called ascocarps viz., cleistothecium, perithecium, apothecium and ascostroma.

Asexual reproduction: Asexual stage is also called as anamorph or imperfect stage. Asexual reproduction occurs by fission (Yeasts), budding (blastospores) (Yeasts), fragmentation (majority of fungi) chlamydospore and conidia. Conidia are short lived, indefinite (enormous) in number, may be produced directly from somatic hyphae, or from conidiogenous cells or from specialized hyphae called conidiophores. Conidiophores may be short or long, branched or unbranched or may form complex asexual fruiting bodies viz., pycnidium, acervulus, sporodochium and synnemata and the method vary with species and environmental conditions.

3. **Sexual reproduction:** Sexual stage is also called as teleomorph or perfect stage or ascigerous stage / state. Sexual spores are called ascospores which are produced in a sac like structure called ascus. The methods of sexual reproduction

are gametangial copulation, gametangial contact, spermatization and somatogamy. The gametangia are antheridium (male sex organ) and ascogonium(female sex organ). Ascogonium is provided with a hair like structure called trichogyne

(receptive neck of ascogonium), which is often long and functions as a fertilization tube.

4. The asci may be formed by any of the following methods.

A.Direct development of zygote into ascus - eg. In yeasts, the compatible nuclei brought together during plasmogamy, fuse (karyogamy) and the cells containing single diploid nucleus (zygote) directly develops into ascus.

B.Indirect Development of asci from ascogenous hyphae- eg. sexual reproduction and ascus development as exemplified by general life cycle pattern in *Pyronema omphalodes*.

5.Short dikaryotic phase is seen.Plasmogamy and karyogamy are separated both by space and time.

DEVELOPMENT OF ASCUS INDIRECTLY FROM ASCOGENOUS HYPHAE OR LIFE CYCLE OF *PYRONEMA OMPHALODES* :

1. The male sex organ is known as antheridium and the female sex organ is known as ascogonium.

2.During sexual reproduction,the male nuclei from the antheridium pass through the trichogyne into the ascogonium and pairs up with female nuclei to form dikaryon. (they do not fuse thus delaying karyogamy) .

3. The sexual act stimulate the ascogonium to produce a number of swellings/papillae just opposite to groups of nuclei located in the periphery of the ascogonium.

4. The dikaryons in ascogonium multiply by conjugate division and as these swellings enlarge, the daughter nuclear pair from ascogonium begin to pass into swellings one by one.

5. Eventually, the swellings elongate into ascogenous hyphae. The nuclear pair or dikaryon in ascogenous hyphae undergoes conjugate divisions.Later, septa are formed in ascogenous hyphae in such a way that each cell of ascogenous hyphae is dikaryotic,except the terminal cell which is uninucleate.Thus,the dikaryotic phase in Ascomycotina is represented by ascogenous hyphae where in one

nucleus is ascogonial origin and the other antheridial.

6. The penultimate binucleate cell of ascogenous hypha elongates and bends over to form a hook like cell called as 'hook cell' or 'crozier'. The two nuclei in crozier cell undergoes conjugate division to form 4 nuclei.

7. Now septa are formed in hook cell in such a way that basal and apical cells consist of single nucleus each, and the middle cell consists of two nuclei. This binucleate middle cell is known as 'Crook cell'.

8. Crook cell enlarges and converted into 'Ascus mother cell'. Karyogamy takes place in ascus mother cell fusing two nuclei and forms diploid nucleus ($2n$). Thus, plasmogamy (in ascogonium) and karyogamy (in ascus mother cell) occur at different places. Meanwhile ascus mother cell elongates and develops into ascus.

9. The diploid nucleus undergoes meiosis resulting in the formation of 4 haploid nuclei.

10. These 4 haploid nuclei further undergo mitosis forming 8 haploid nuclei.

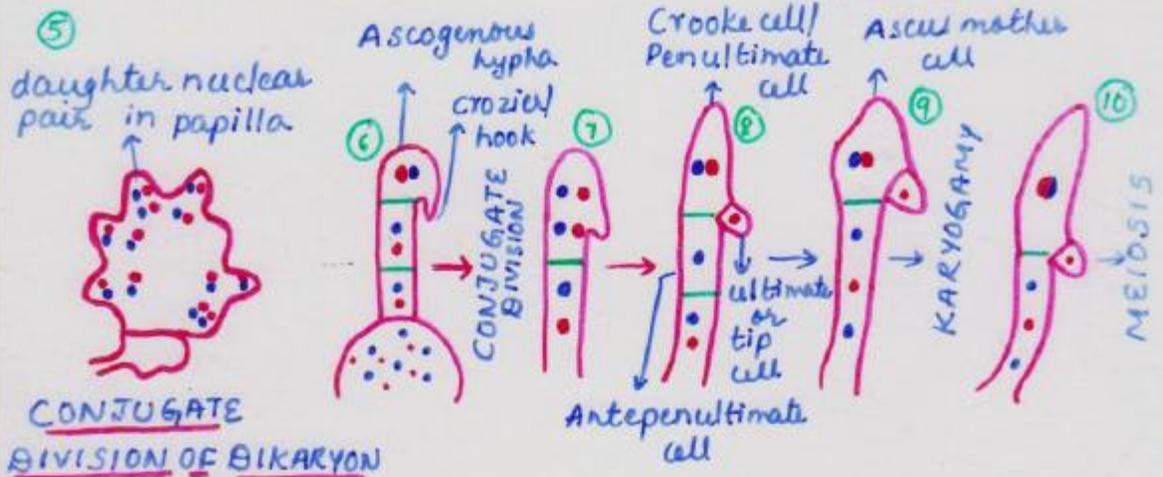
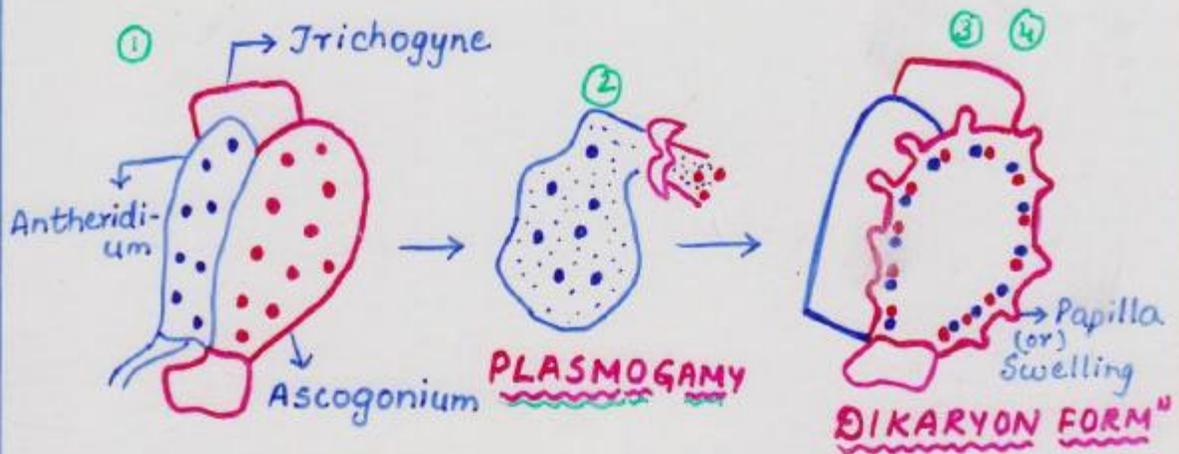
11. The nuclei develop into ascospores by free cell formation. The process of formation of ascospores is called 'Free cell formation' or 'Ascosporogenesis'. The ascospores are formed by aggregation of cytoplasm of the ascus around the nucleus forming definite walls. Epiplasm is the portion of cytoplasm left over, outside the ascospore walls, which supplies nutrients to the developing ascospores.

12. The number of ascospores may be 8, 16, 32, 64, or even more depending on the number of mitotic divisions following meiosis. The ascospores vary in shape, size and some times in color also.

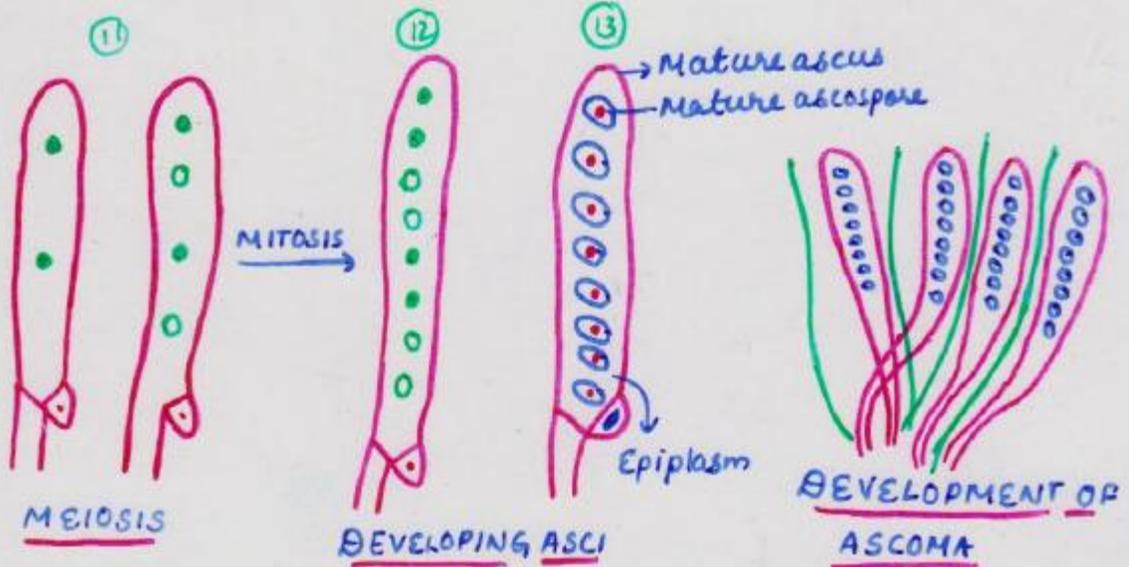
13. Based on compatibility, the members may be homothallic (eg. *Aspergillus*, powdery mildew fungi) or heterothallic (E.g. *Saccharomyces cerevisiae*).

GENERALISED LIFE CYCLE OF ASCOMYCOTINA

Eg: *Pyronema omphalodes*



CONJUGATE DIVISION OF DIKARYON



ASCOSPOROGENESIS = FREE CELL FORMATION

MORPHOLOGY OF ASCI:

Ascus is a sac like structure usually containing a definite number of ascospores (typically eight) formed by a process called free cell formation after karyogamy and meiosis. In majority members of ascomycotina shape of ascus may be elongated, cylindrical, clavate or club shaped, except in some groups where they are globose or ovoid. Asci may be stalked or sessile. Generally ascus represents a single cell but in some ascus may be septate.



IMPORTANT CHARACTERISTICS OF ORDER : ERYSIPTHALES, FAMILY : ERYSIPTHACEAE :

1. Erysiphales is the exceptional order as it produces cleistothecium instead of perithecium. The reason is that the asci are grouped in fascicles or form a basal layer (hymenium) at maturity and ascospores are released violently with force. Cleistothecia are formed on superficial mycelium without formation of stroma.
2. Members cause a disease called powdery mildew because they produce enormous number of conidia on the surface of infected host plants which appear to the naked eye as a white powdery coating.
3. Mycelium is hyaline and mostly ectophytic
4. Members are obligate parasites of plants and nourishment through haustoria.
5. Asci are persistent, globose to pyriform and explodes at the time of release of ascospores.
6. Important plant pathogenic genera are 1. *Erysiphe* 2. *Leveillula* 3. *Phyllactinia*

4. *Uncinula* 5. *Sphaerotheca* 6. *Podosphaera* 7. *Microsphaera*

Somatic characteristics:

Mycelium is well developed, septate, uninucleate, profusely branched entirely superficial (ectophytic) except *Leveillula* (endophytic) and *Phyllactinia* (semi-endophytic), produce haustoria into epidermal cells to absorb nourishment.

Asexual reproduction :

Asexual reproduction is through conidia produced on conidiophores. Conidiophores are long, erect and hyaline.

Three types of conidial stages are recognised in powdery mildews.

1. *Oidium* 2. *Oidiopsis* 3. *Ovulariopsis*

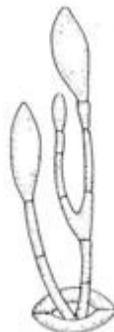
1. ***Oidium (Acrosporium)*** : Mycelium is ectophytic, hyaline. Conidia are developed from a flask shaped mother cell (spore mother cell) formed on a short conidiophore . Conidia are barrel shaped with flat ends and are produced in chains..The conidia are also referred to as meristem arthrospores as these are formed by fragmentation of hyphae. Eg. The perfect stages viz., *Erysiphe*, *Podosphaera*, *Uncinula*, *Sphaerotheca* and *Microsphaera* produce *Oidium* as conidial stage.

2. ***Oidiopsis***: Mycelium is endophytic. Conidiophores may be branched or unbranched, erect, septate, hyaline and emerge through stomata. Conidia are produced singly and cylindrical in shape. Conidia are of two types. a. blunt tip b. pointed tip. Eg. *Leveillula* sp. produce *Oidiopsis* as conidial stage.

3. ***Ovulariopsis***: Mycelium is partly ectophytic and partly endophytic. The conidiophores are hyaline, septate, unbranched, and bear a single conidium. Conidia are rhomboid in shape. In some species, the conidiophores are spiral in shape. Eg. *Phyllactinia subspiralis*. *Phyllactinia* sp .produce ovulariopsis as conidial stage.



Oidium



Oidiopsis



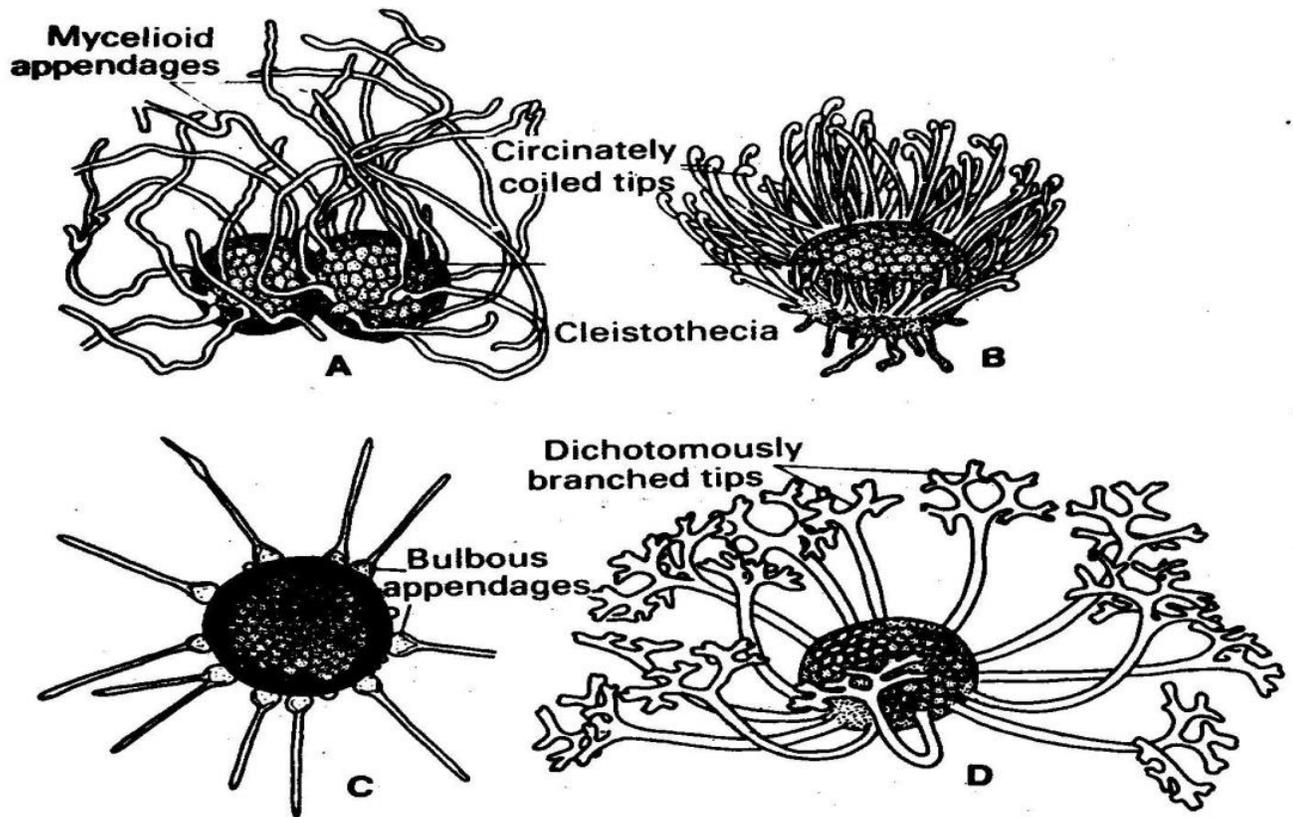
Ovulariopsis

Sexual reproduction:

Some species are homothallic and some are heterothallic. Antheridia and ascogonia are sex organs. Both gametangia are uninucleate. Fruiting body is cleistothecium which is produced on superficial mycelium as a result of gametangial contact. The cleistothecia are first white and finally black in color when mature. The wall is made up of pseudoparenchymatous tissue of several layers called peridium. Overwintering of powdery mildews takes place in cleistothecial stage which are resistant to winter conditions. In perennials, the mycelium may overwinter in the dormant buds of host. In warm weather, many species never form cleistothecia and perpetuate by means of conidia. The cleistothecia are provided with characteristic appendages which vary considerably in length and character.

Types of cleistothecial appendages:

1. **Mycelioid appendages:** These are flexible, flaccid and resemble somatic hyphae. Eg. *Erysiphe*, *Sphaerotheca*, *Leveillula*.
2. **Circinoid / hooked / coiled appendages :** These are rigid with curled or coiled tips. Eg. *Uncinula*.
3. **Dichotomously branched tips:** These are rigid, flattened with dichotomously branched tips. Eg. *Podosphaera*, *Microsphaera*.
4. **Bulbous base with pointed tip:** These are rigid, spear like with bulbous base and pointed tip. Eg. *Phyllactinia*.



Important powdery mildewdiseases:

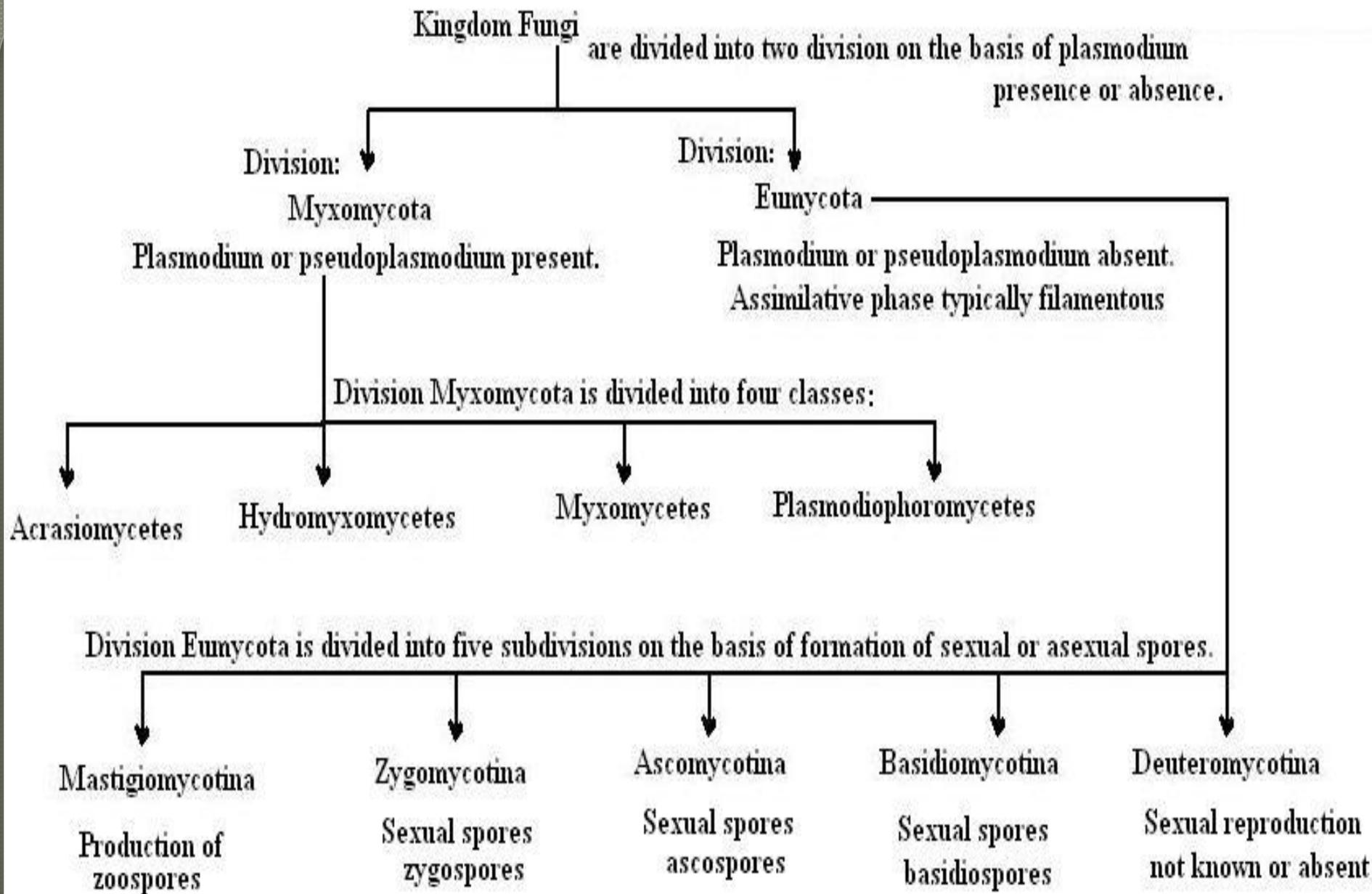
CROP

1. Pea
2. Cucurbits
3. Grasses
4. Mulberry
5. Chillies
6. Apples
7. Roses

PATHOGEN

- Erysiphe polygoni*
E. cichoracearum
E. graminis
Phyllactinia corylea
Leveillula taurica
Podosphaera leucotricha
Sphaerotheca pannosa

CLASSIFICATION OF FUNGI



- **S.D : 1. MASTIGOMYCOTINA**

CLASS 1.CHYTRIDIOMYCETES

ORDER: CHYTRIDIALES

FAMILY: SYNCHYTRIACEAE

Eg. Synchytrium

CLASS 2.OOMYCETES

ORDER: PERONOSPORALES

- FAMILY: PYTHIACEAE

Eg: Pythium, Phytophthora

- FAMILY: ALBUGINACEAE

Eg. Albugo

-FAMILY: PERONOSPORACEAE

Eg: Sclerospora. Peronospora, Peronosclerospora
Plasmopara

S.D :2. ZYGOMYCOTINA

CLASS :ZYGOMYCETES

ORDER: MUCORALES

FAMILY: MUCORACEAE

Eg. Rhizopus, Mucor

S.D 3. ASCOMYCOTINA

CLASS 1. HEMIASCOMYCETES

ORDER: TAPHRINALES

FAMILY: TAPHRINACEAE

Eg. Taphrina

CLASS 2. PLECTOMYCETES

ORDER: EUROTIALES

ERYSIPHALES

FAMILY: EUROTIACEAE

Eg. Eurotium, Talaromyces

ORDER:

FAMILY: ERYSIPHACEAE

Eg. Erysiphe, Leveillula

3. PYRENOMYCETES

ORDER: HYPOCREALES

FAMILY: CLAVICIPITACEAE

Eg. Claviceps

4. DISCOMYCETES

Order: pezizales

family: morchellaceae

eg. Morchella

5. LOCULOASCOMYCETES

Order: pleosporales

Family : venturiaceae

Eg. Venturia

Family : Pleosporaceae

eg. Cochliobolus eg.

Order: dothidiales

Family: dothidiaceae

eg. mycosphaerella

Order: myriangiales

Family: myriangiaceae

Elsinoe

S.D 4. BASIDIOMYCOTINA

Classes 1. Teliomycetes

Order: Uredinales

Family: Pucciniaceae
eg. puccinia, uromyces,

Family: Melampsoraceae
eg. melampsora

Order : Ustilaginales

Family: Ustilaginaceae
eg. Ustilago, Sphaecelotheca

Family: Tilletiaceae
eg. Tilletia, Neovossia

2. HYMENOMYCETES

SUB CLASS: Holobasidiomycetidae

ORDER: Agaricales

FAMILY: Agaricaceae

Eg. Agaricus,

S.D 5 DEUTEROMYCOTINA

CLASSES 1.COELOMYCETES

ORDER: SPHAEROPSIDALES

FAMILY: SPHAEROPSIDACEAE

Eg. Phoma, Phomopsis, Macrophomina,

ORDER: MELANCONIALES

FAMILY: MELANCONIACEAE

Eg. Colletotrichum, Gloeosporium

2. HYPHOMYCETES

ORDER: HYPHOMYCETALES / MONILIALES

FAMILY: MONILIACEAE

Eg. Pyricularia, Botrytis, Verticillium,

FAMILY: DEMATIACEAE

Eg. Alternaria, Bipolaris, Cercospora, Phaeosariopsis

ORDER: STILBELLALES

FAMILY : TUBERCULARIACEAE

Eg. Fusarium, Myrothecium

ORDER: AGONOMYCETALES

FAMILY: AGONOMYCETACEAE

Eg. Sclerotium, Rhizoctonia

Classification of plant diseases

Type of infection

- a. Localized diseases:** These diseases are limited to a definite area of an organ or part(s) of a plant. e.g. leaf spots and anthracnoses caused by different fungi.
- b. Systemic diseases:** In these diseases the pathogen spreads from a single infection point so as to infect all or most of the host tissues. e.g. Downy mildews caused by fungi and mosaics and leaf curls caused by viruses.

Type of perpetuation and spread

a. Soil-borne diseases: The causal agents perpetuate and spread through soil. e.g. Damping off caused by fungi like *Pythium sp.* and root rot caused by *Rhizoctonia spp.*

b. Seed-borne diseases: Seed or seed materials help in the perpetuation and spread of this disease. The disease causing agents may be internally seed-borne or externally seed-borne e.g. Loose smut of wheat caused by *Ustilago nuda tritici* (internally seedborne) and blast of rice caused by *Pyricularia oryzae* (externally seed-borne).

c. Air-borne diseases: In these type of diseases the causal agents are spread by wind (air). e.g. Early leaf spot and late leaf spot of groundnut caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* respectively.

Extent of occurrence and geographic distribution

a. Endemic diseases: It is also known as **enphytotic disease**. When a disease is more or less constantly occurring year after year in a moderate to severe form in a country or locality then it is called as an endemic disease. e.g. **Wart disease of potato caused by *Synchytrium endobioticum* is endemic in Darjeeling**, citrus canker caused by *Xanthomonas campestris pv. citri* is endemic in Asia and sorghum rust caused by *Puccinia purpurea* is endemic in India.

b. Epidemic or epiphytotic diseases: An epidemic or epiphytotic refers to sudden outbreak of a disease periodically over a widespread area in a devastatingly severe form causing extensive losses or complete destruction. Epidemic disease may be present constantly in the locality but assumes a severe form only on occasions. This is because of the occurrence of favourable environment responsible for the rapid development of the disease. But the pathogen may be irregular in appearance or there may be lack of sufficient inoculum to cause the disease. e.g. wheat stem rust (*Puccinia graminis tritici*) and powdery mildew (*Erysiphe graminis var. tritici*), late blight of potato (*Phytophthora infestans*).

c. Sporadic diseases: Sporadic diseases are those, which occur at irregular intervals over limited areas or locations. They occur in relatively few instances. e.g. Fusarium wilt of cotton (*Fusarium oxysporum f.sp. vasinfectum*), grain smut of sorghum (Sporisorium = *Sphacelotheca cruenta*) and wheat loose smut (*Ustilago nuda tritici*).

d. Pandemic diseases: A disease is said to be pandemic when it is prevalent throughout the country, continent or world involving mass mortality. e.g. Late blight of potato and wheat stem rust.

Multiplication of inoculum

- a. In **simple interest disease (monocyclic epidemics)** the disease increase is just like simple interest in money. Here inoculum comes from a reservoir and hence amount of inoculum for a given season's crop is fixed. So there is no repetition of the disease cycle within the crop season. Hence the disease spread will be slow. e.g. Soil inhabiting pathogens like *Pythium sp.*, *Rhizoctonia sp.* and *Sclerotium sp.*
- b. In **compound interest disease (polycyclic epidemics)** the disease increase is just like compound interest in money. Inoculum is multiplied several times (every 7 to 15 days for wheat rust) during crop growth in a season. So the disease spread will be fast. e.g. Wheat stem rust, rice blast, powdery mildew diseases of different crops.

On the basis of symptom

1. Powdery mildew:

White to dirty white powdery mass of conidia, conidiophores and mycelium present on the host surfaces in patches or covering the entire surface.

E.g Powdery mildew of pea (*Erisiphae polygoni*)

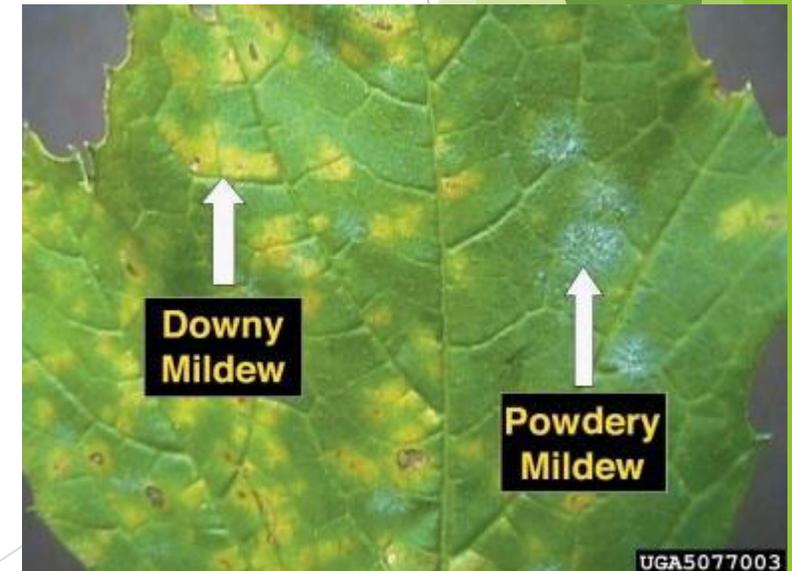


Powdery mildew of pea
(*Erisiphae polygoni*)

1. Downy mildew:

Dirty white with slight pinkish tinged cottony fungal growth consists of sporangia, sporangiophores and mycelium present on lower surface of leaves whereas light yellow or light brown patches appear on upper surface of leaves.

E.g Downy mildew of grapes (*Plasmopara viticola*)



1. Rust:

It gives rust like appearance of infected plant parts. The small pustule of spore of various colours, usually breaking through the host epidermis.

E.g Black rust of Wheat (*Puccinia graminis tritici*), Pea rust (*Uromyces fabae*)



Fig. 7.2. Black rust of Wheat

1. Smut:

Smut means charcoallike powder. The affected plant parts show black or black-purplish powdery mass of spores. Smut usually appear on floral parts but may also found on stem and leaves.

E.g Loose smut of Wheat (*Ustilago nuda tritici*), Whip smut of Sugarcane (*Ustilago scitaminea*) and Leaf smut of Paddy (*Entyloma oryzae*)



Fig. 7.3. Loose smut of Wheat

1. Leaf Spot

Spot is a definite, localized, round to regular lesion, often with a border of a different colour, characterized as to location (leaf spot, fruit spot) and colour (brown spot, black spot); if numerous or if spots enlarge and merge, a large irregular blotch or blight may develop such as gray leaf spot of tomato; black spot of rose; Angular leaf spot of beans, Leaf spot with yellow halo, Fruit spot e.t.c.



Leaf spot

1. Dieback

Dieback progressive browning and death of shoots, branches, and roots starting at the tips to downward e.g. die back of chilli/ citrus

2. Blights:

The invasion by the bacterium leads to very rapid and extensive necrosis of the affected plant parts resulting in scorched appearance of the host.

E.g Bacterial blight of paddy caused by *Xanthomonas campestris pv. oryzae*.



Fig. 7.6. Bacterial blight of paddy

Soft Rots:

The soft rot symptoms are found mainly on fleshy parts. The major effect is softening of the tissues due to disintegration of cells and dissolution of middle lamella, as a result of enzyme action. Very often a dirty liquid oozes out of the affected parts. In many causes, the disintegration is preceded by change of colour.

E.g Black leg and soft rot of potato caused by *Erwinia carotovora* sub species *carotovora* and *Erwinia carotovora* sub species atroseption.



Soft rot

Scabs and Cankers:

Scabs and cankers are corky outgrowth which is formed on leaves, twigs and all other plant parts above the ground. These outgrowths are the result of the reactions of the host tissues to the pathogen. Such reactions are mostly localised and often confined to the parenchymatous tissues of the host plant. Scab is formed by epidermal infection and is not deep seated. Cankers on the other hand, are deep seated and involve the cambium layer.

- E.g 1. Citrus canker caused by *Xanthomonas Campestris* pv. *citri*.
2. Common scab of potato caused by *Streptomyces scabies*.



Wilt:

**Important plant
pathogenic organisms**

1.Fungi

2.Bacteria

3.Fastidious vascular bacteria (RLO's)

4.Mollicutes (phytoplasma and spiroplasma)

5.viruses

6.viroids

7.Algae

8.Flagellated protozoans.

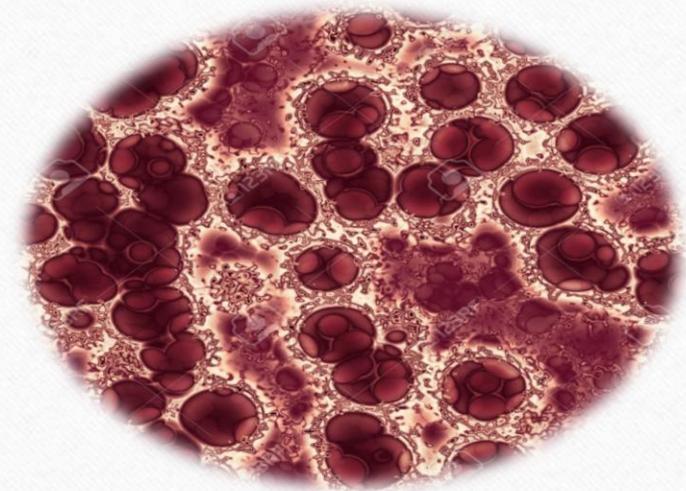
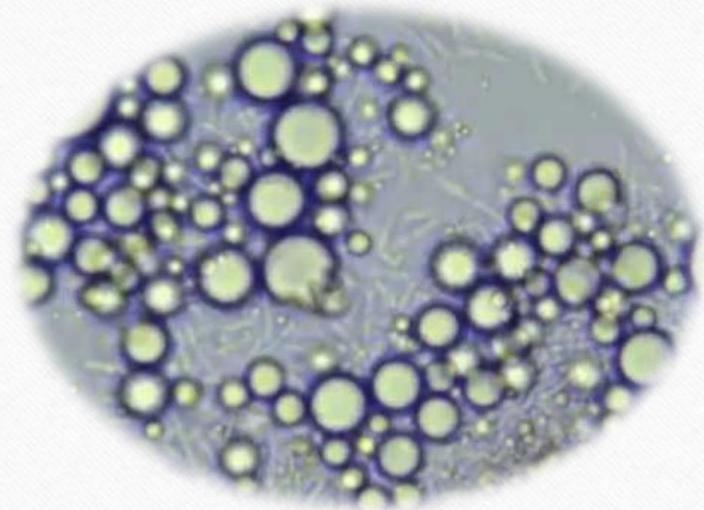
Fungi

Fungi are eukaryotic, spore bearing, achlorophyllous organisms that generally reproduce sexually and asexually and whose filamentous, branched somatic structures are typically surrounded by cell walls consisting chitin or cellulose or both with many organic molecules.



Bacteria

- ❑ Bacteria are extremely minute, rigid, essentially unicellular organisms free of true chlorophyll and generally devoid of any photosynthetic pigment, most commonly multiplying asexually by simple transverse fission, the resulting cell, being of equal or nearly equal in size.
- ❑ These microorganisms are with a primitive nucleus lacking a clearly defined membrane.
- ❑ The bacteria are smaller than fungi and measure about 0.5 to 1.0 x 2.0 to 5.0 μm in size.



Fastidious vascular bacteria (RLO's)/ (Rickettsia-like bacteria – RLB)

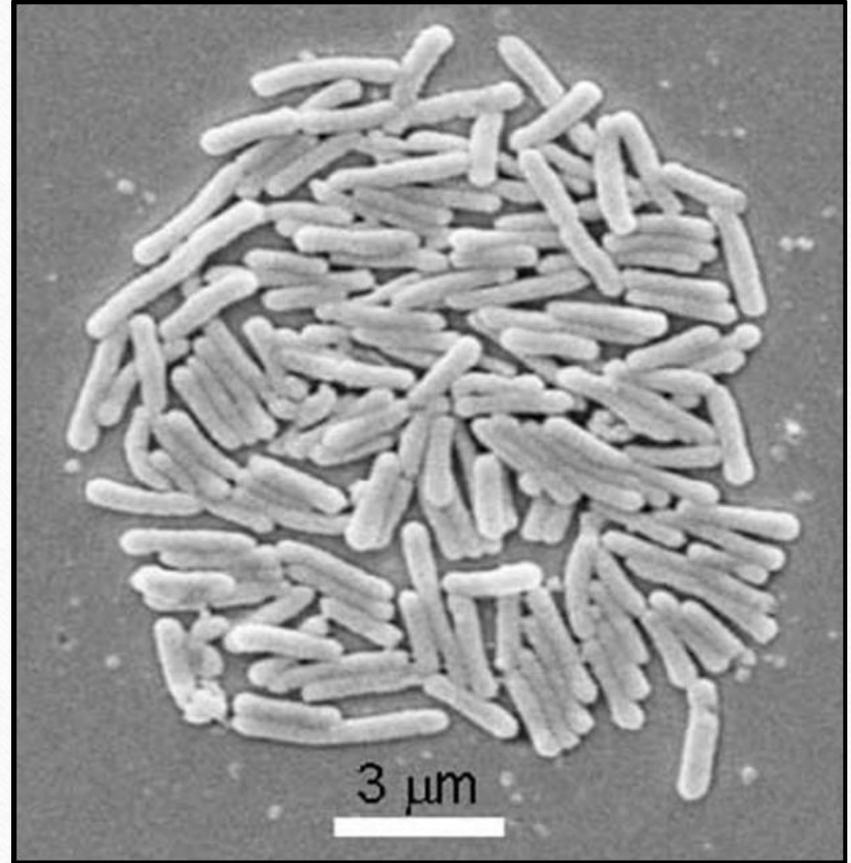
- ❑ Fastidious vascular bacteria are otherwise called Rickettsia - Like bacteria, Rickettsia like organisms (RLO), or fastidious prokaryotes or rickettsia -like walled bacteria.
- ❑ They are small bacteria with a cellular ultrastructure of typical gram-negative bacteria.
- ❑ They are very exacting in their nutritional requirements, refusing to grow on routine bacteriological media.
- ❑ They have a cell wall unlike MLO and spiroplasma.
- ❑ MLO is restricted to phloem tissues where as RLB are restricted mostly to xylem or phloem.

- ❑ A common habit for both is the **insect body fluid** (haemolymph).
- ❑ Both the groups are dependent on **insect vectors for transmission**. Nematode (*Xiphinema index*) also helps in transmission of RLB (yellow disease of grapevine).
- ❑ They reproduce by **binary fission**.
- ❑ They produce **phytoalexins**, which induce characteristic symptoms of the disease.
- ❑ They are **cultured in artificial media** e.g., Pierce's disease of grapevine, almond leaf scorch, phony disease of peach and plum leaf scald.

1 Xylem-limited RLB

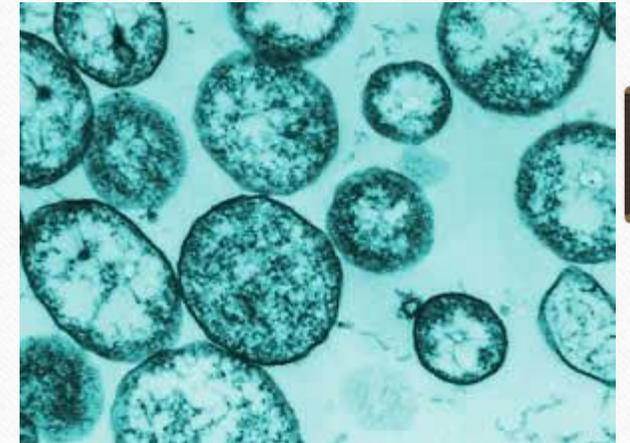
2 Phloem-limited RLB and

3 Non-tissue restricted RLB

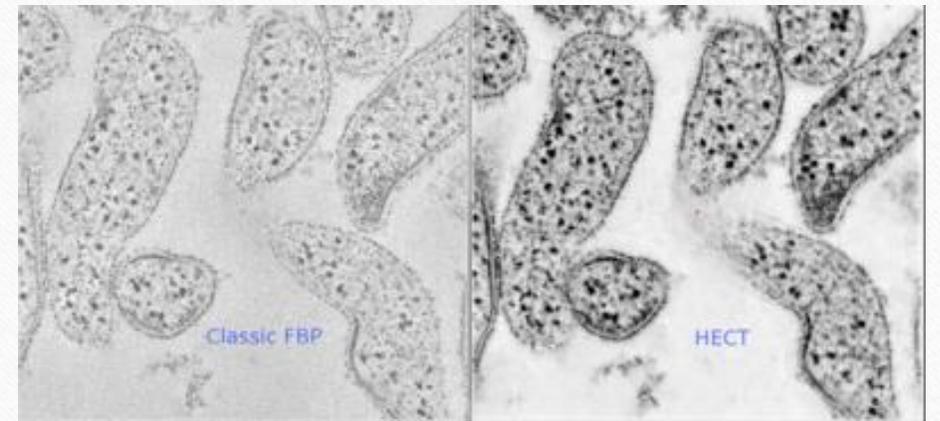


Mollicutes (phytoplasma and spiroplasma)

Phytoplasma: Phytoplasma lack cell wall and are bounded by a unit membrane. They are pleomorphic. They lack cell wall. They have fried egg appearance of colony. They are filterable through 450 nm membrane. They have both DNA and RNA. They cannot be grown on artificial media. They produce symptoms like little leaf, phyllody, spike, yellows, stunting, witches' broom etc. They are mostly transmitted by leafhoppers. They are insensitive to penicillin and sensitive to tetracycline. e.g. phyllody of sesame, little leaf of brinjal.

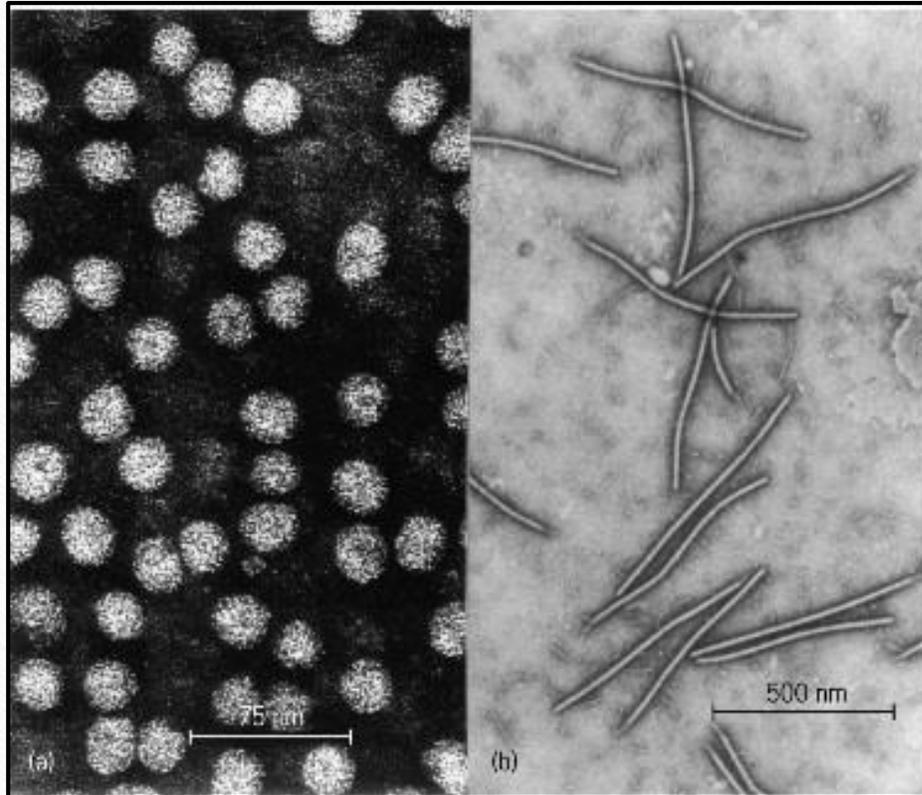


Spiroplasma: Spiroplasmas are helical, wall less prokaryotic micro organisms that are present in phloem of diseased plants and are thought to be a kind of mycoplasma and can be cultured on artificial medium. They require cholesterol for growth and cause diseases in plants, insects and rats. They are insensitive to penicillin and sensitive to erythrocin and tetracycline. e.g. corn stunt, citrus stubborn.



VIRUS

- Viruses are **ultramicroscopic, nucleoprotein entities**, which are infectious agents and **obligately parasitic pathogens**, which are less than 200 m μ in size. They are **devoid of enzymes and depend on the host protein synthesis machinery** (ribosomes). They have **only one type of nucleic acid** viz., RNA or DNA. Most of the plant virus is having RNA. e.g. TMV. Few viruses contain DNA. e.g. Cauliflower mosaic virus, banana bunchy top virus, maize streak virus and sugar beet curly top virus.
- passive transmission – mechanically, vegetative propagation (cuttings) or seed
- active transmission requires vector
 - ✓ plant-feeding arthropods, especially aphids and whiteflies
 - ✓ nematodes
 - ✓ plant-parasitic fungi



range from 30 nm diameter (spherical viruses) to 2 μm (filamentous viruses)

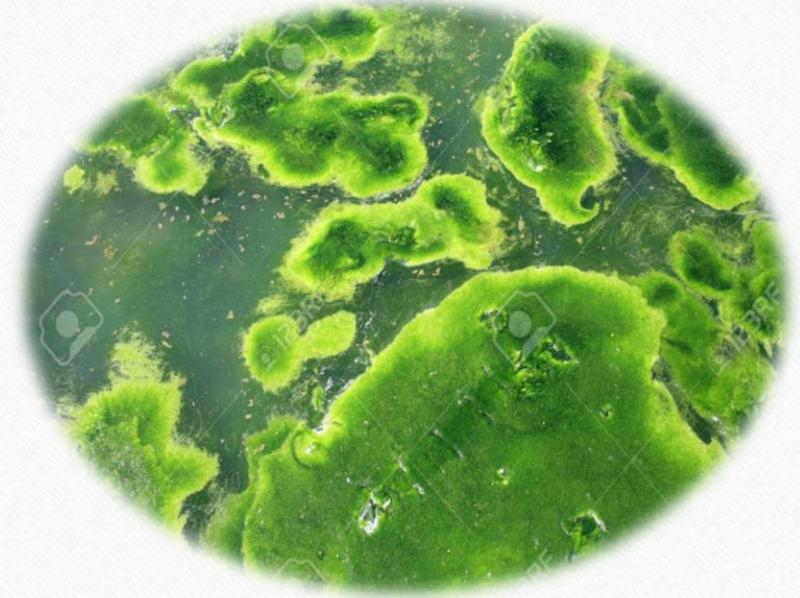
VIROIDS

- They are also called as **mini viruses**.
- naked, infectious RNA – **no protein coat**
- genomes between 246-375 nucleotides
- do not produce any proteins when they infect a plant cell
- use the host cell RNA polymerase to reproduce their RNA and move into other plant cells
- spread through vegetative propagation, mechanical contamination, pollen and seed; vectors not necessary
- They may be small, but the diseases can still be devastating! **Cadang-Cadang** is a viroid disease of coconut palms that has destroyed over 30 million palms in the Philippines
- **Potato spindle tuber viroid** is model pathogen



ALGAE

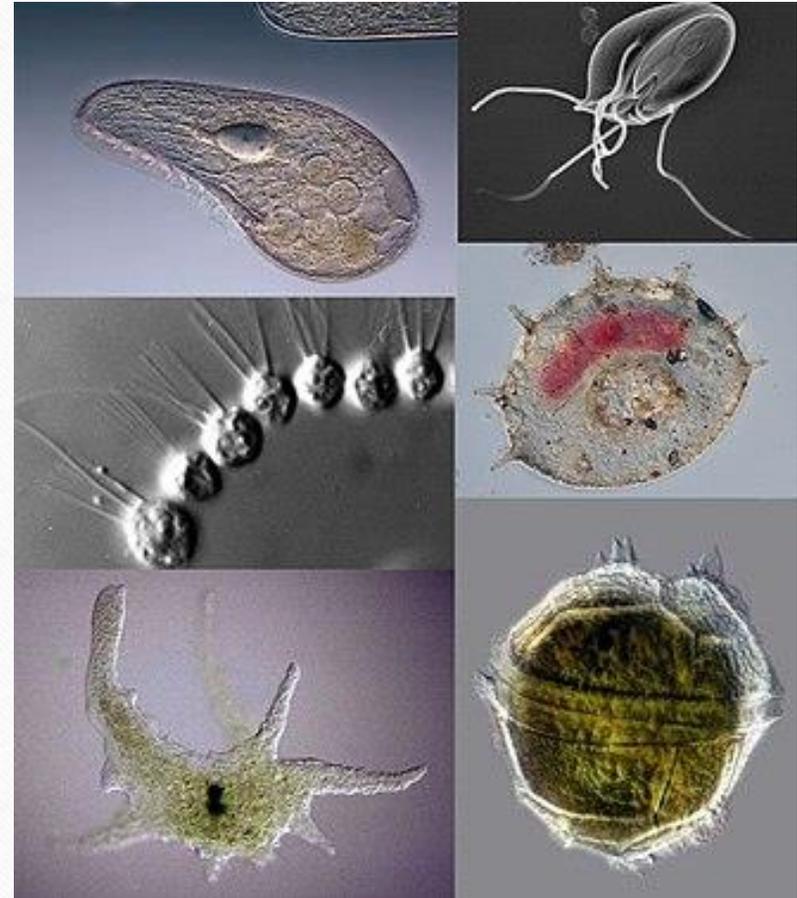
- ❑ Algae are **eukaryotic**, unicellular or multicellular organisms and mostly occur in aquatic environments.
- ❑ Many algae thrive as terrestrial or subterranean algae.
- ❑ The size of algae ranges from 1.0mm to many centimetres in length.
- ❑ They **contain chlorophyll** and are photosynthetic.
- ❑ They reproduce by **asexual and sexual** processes.
- ❑ The study of algae is called **phycology or algology**.



PROTOZOA

- ❑ Protozoa (trypanosomatid flagellates) belonging to the class Mastigophora, order Kinetoplastida and family Trypanosomatidae have been known to parasitize plants.
- ❑ The Mastigophora, or flagellates, are characterized by **one or more long slender flagella** at some or all stages of their life cycle.
- ❑ The flagella are used **for locomotion and food capture**. They are also used as **sense organs**.
- ❑ The body of the flagellates has a definite **long, oval or spherical form**, which is maintained by a thin, flexible membrane cover. In some groups it may be armoured.

- ❑ Flagellates reproduce by **longitudinal fission**.
- ❑ Flagellates apparently cause the phloem necrosis disease of coffee, the heart rot disease of coconut palm and the Marchitez suppressive (sudden wilt or wither) disease of oil palm,
- ❑ Marchitez suppressive is one of the important diseases in oil palm. *Phytomonas staheli* was described from sieve tubes of coconut and oil palm.



PHANEROGAMIC PARASITES

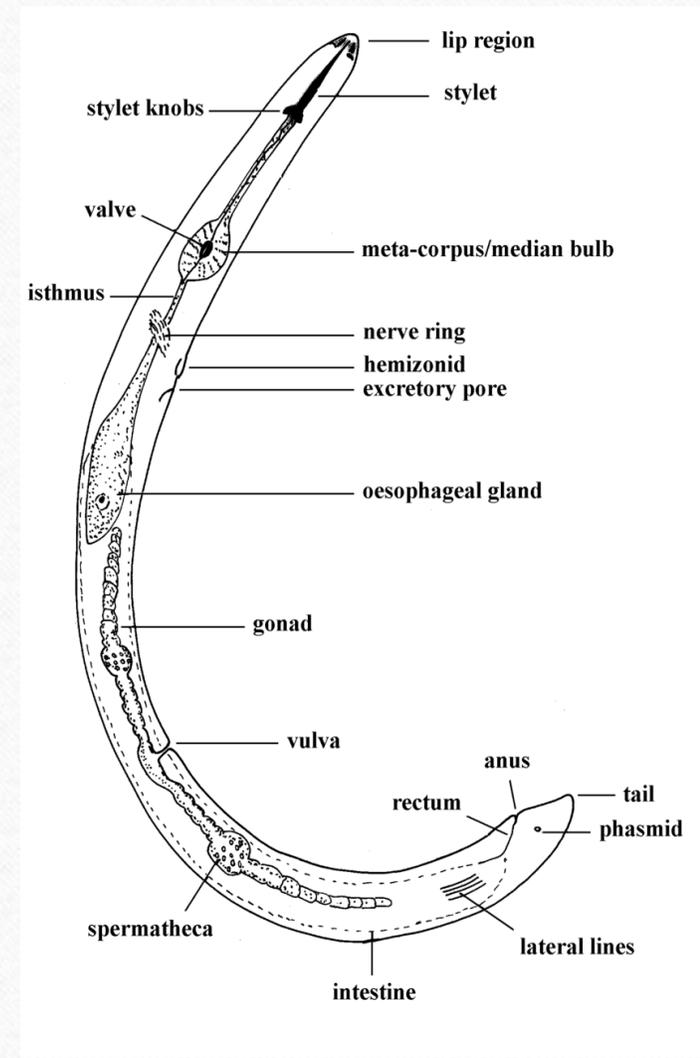
- ❑ Phanerogamic parasites are **flowering plants or seed plants**, which lead a parasitic life on other living plants. They parasitize a great number of economic plants and cause considerable loss in yield.
- ❑ The phanerogamic parasites invade stem or root of the host plants.
- ❑ Nearly 2,500 species of phanerogamic parasites in 11 families have been recorded throughout the world. Among them Orobanchaceae, Scrophulariaceae, Loranthaceae, Convolvulaceae and Lauraceae are important.

- ❑ Some of the parasites, which do not have chlorophyll, depend entirely on the host plants for their food materials. They are called **holo or total parasites**.
- ❑ Some of these parasites possess chlorophyll, which manufacture carbohydrates to a limited extent and depend on the host for mineral, salts and water. These are generally called as **semi or partial parasites**.



Nematodes

- are roundworms (multicellular animals)
 - ✓ have a hollow mouth spear called a stylet
 - ✓ stylet connected to pharynx, which is connected to the intestine
 - ✓ stylet used to puncture plant cells, withdraw food, and secrete protein and metabolites that aid the nematode in parasitizing the plant
- most are free-living (40%) – feed on bacteria, fungi, protozoans and other nematodes. but, many are parasites of animals (44%) and plants (15%)
- need water (even if minimal)
- *Caenorhabditis elegans* – bacterial-feeding nematode (not plant parasitic)



Thank you..

Q.1 Give a detailed note on principles and methods of plant disease management.

Ans.: It conveys a concept of continuous process which is based not only on the principle of eradication of the pathogen but mainly on the principle of minimizing the damage or loss below economic injury level.

Importance: Plant diseases are important because of the losses (qualitative and quantitative) they cause. Loss may occur at any time between sowing of the crop and consumption of the produce. Measures taken to prevent the incidence of the disease, reduce the amount of inoculum that initiates and spreads the disease and finally minimize the loss caused by the disease are called as management practices.

Essential considerations in plant disease Management:

1. Benefit-cost ratio
2. Procedures for disease control should fit into general schedule of operations of crop production
3. Control measures should be adopted on a **co-operative basis** over large adjoining areas. This reduces frequency of applications, cost of control and increases chances of success of control measures
4. Knowledge aspects of disease development is essential for effective economical control. Information is needed on the following aspects
 - a. Cause of a disease
 - b. Mode of survival and dissemination of the pathogen
 - c. Host parasite relationship
 - d. Effect of environment on pathogenesis in the plant or spread in plant population
5. Prevention of disease depends on management of primary inoculum
6. Integration of different approaches of disease management is always recommended

General principles of plant disease management

1. **Avoidance:** Avoiding disease by planting at times when, or in areas where, inoculum is ineffective due to environmental conditions, or is rare or absent
2. **Exclusion of inoculum:** Preventing the inoculum from entering or establishing in the field or area where it does not exist
3. **Eradication:** Reducing, inactivating, eliminating or destroying inoculum at the source, either from a region or from an individual plant in which it is already established
4. **Protection:** Preventing infection by creating a chemical toxic barrier between the plant surface and the pathogen
5. **Disease resistance (Immunization):** Preventing infection or reducing effect of infection by managing the host through improvement of resistance in it by genetic manipulation or by chemical therapy.

I. Avoidance of the pathogen: These methods aim at avoiding the contact between the pathogen and susceptible stage of the crop. This is achieved by

- a. Proper selection of geographical area b.
Proper selection of the field
- c. Adjusting time of sowing d.
Disease escaping varieties
- e. Proper selection of seed and planting material

a) Proper selection of geographical area: Many fungal and bacterial diseases are more severe in wet areas than in dry areas. Cultivation of bajra in wet areas is not profitable due to the diseases, smut (*Tolyposporium penicillariae*) and ergot (*Claviceps microcephala*).

b) Proper selection of the field: Proper selection of field will help in the management of many diseases, especially the soil borne diseases. Raising of a particular crop year after year in the same field makes the soil sick, where disease incidence and severity may be more.

Ex: Wilt of redgram, late blight of potato (*Phytophthora infestans*), green ear of bajra (*Sclerospora graminicola*), etc.

c) Time of sowing: Generally pathogens are able to infect the susceptible plants under certain environmental conditions. Alteration of date of sowing can help in avoidance of favourable conditions for pathogen.

Ex: *Rhizoctonia* root rot of redgram is more severe in the crop sown immediately after the rains. Delayed sowing will help in reducing the incidence of disease.

Ex: Infection of black stem rust of wheat (*Puccinia graminis tritici*) is more in late sowing, hence, early sowing helps in reduction of stem rust incidence.

d) Disease escaping varieties: Certain varieties of crops escape the disease damage because of their growth characteristics. Ex: Early maturing varieties of wheat or pea escape the damage due to *Puccinia graminis tritici* and *Erysiphe polygoni*, respectively.

e) Proper selection of seed and planting material: Selection of seed and seedling material from healthy sources will effectively manage the diseases such as loose smut of wheat (*Ustilago nuda tritici*), bunchy top of banana (*Banana virus-1*), Panama wilt of banana (*Fusarium oxysporum f.sp. cubense*) and whip smut of sugarcane (*Ustilago scitaminae*). Potato seed certification or tuber indexing is followed for obtaining virus free seed tubers. Citrus bud wood certification programme will help in obtaining virus free planting material.

II. Exclusion of the pathogen: These measures aim at preventing the inoculum from entering or establishing in the field or area where it does not exist. Different methods of exclusion are seed treatment, seed inspection & certification, and plant quarantine regulation.

a) Seed inspection and certification: Crops grown for seed purpose are inspected periodically for the presence of diseases that are disseminated by seed. Necessary precautions are to be taken to remove the diseased plants in early stages, and then the crop is certified as disease free. This practice will help in the prevention of inter and intra regional spread of seed borne diseases.

b) Plant quarantine regulation: Plant quarantine is defined as “ a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delaying the spread of the plant pests and diseases in uninfected areas”.

Plant quarantine laws were first enacted in **France** (1660), followed by Denmark (1903) and USA (1912). These rules were aimed at the rapid destruction or eradication of barbery bush which is an alternate host of *Puccinia graminis tritici*.

In India, plant quarantine rules and regulations were issued under **Destructive Insects and Pests Act (DIPA)** in 1914. In India, 16 plant quarantine stations are in operation by the “Directorate of plant protection and quarantine” under the ministry of food and agriculture, government of India.

Plant quarantine measures are of 3 types.

1. Domestic quarantine: Rules and regulations issued prohibiting the movement of insects and diseases and their hosts from one state to another state in India is called domestic quarantine. Domestic quarantine in India exists for two pests (Rooted scale and Sanjose scale) and three diseases (Bunchy top of banana, banana mosaic and wart of potato).

Bunchy top of banana: It is present in Kerala, Assam, Bihar, West Bengal and Orissa. Transport of any part of *Musa* species excluding the fruit is prohibited from these states to other states in India.

Banana mosaic: It is present in Maharashtra and Gujarat. Transport of any part of *Musa* species excluding the fruit is prohibited from these states to other states in India.

Wart of potato: It is endemic in Darjeeling area of West Bengal, therefore seed tubers are not to be imported from West Bengal to other states.

2. Foreign quarantine: Rules and regulations issued prohibiting the import of plants, plant materials, insects and fungi into India from foreign countries by air, sea and land. Foreign quarantine rules may be general or specific. General rules aim at prevention of introduction of pests and diseases into a country, where as the specific rules aim at specific diseases and insect pests. The plant materials are to be imported only through the prescribed ports of entry.

1. **Airports:** Bombay (Santacruz), Calcutta (Dum Dum), Madras (Meenambakam), New delhi (Palam, Safdarjung) and Tiruchurapally.

2. **Sea ports:** Bombay, Calcutta, Vishakapatnam, Trivandrum, Madras, Tuticorin, Cochin and Dhanushkoti.

3. **Land frontiers:** Hussainiwala (Ferozpur district of Punjab), Kharla (Amritsar district of Punjab) and Sukhiapokri (Darjeeling district of West Bengal)

3. **Total embargoes:** Total restriction on import and export of agricultural commodities.

Phytosanitary certificate: It is an official certificate from the country of origin, which should accompany the consignment without which the material may be refused from entry.

Plant diseases introduced into India before/after enforcement of plant quarantine laws:

S.No.	Disease	Year	Introduced into	From
1	Late blight of potato	1883	India	Europe
2	Coffee rust	1879	India	Srilanka
3	Flag smut of wheat	1906	India	Australia
4	Downy mildew of grapes	1910	India	Europe
5	Bacterial blight of rice	1964	India	Phillippines
6	Rice blast	1918	India (Madras)	South East Asia
7	Downy mildew of maize	1912	India (Madras)	Java
8	Ergot of bajra	1957	India (Bombay)	Africa
9	Panama wilt of banana	1920	India	Panama canal
10	Bunchy top of banana	1940	India	Srilanka
11	Wart of potato	1953	India	Netherlands
12	Golden cyst nematode of potato	1961	India	Europe

Diseases not entered into India: Swollen shoot of cocoa, leaf blight of rubber and many viral diseases.

III. Eradication: These methods aim at breaking the infection chain by removing the foci of infection and starvation of the pathogen (i.e., elimination of the pathogen from the area by destruction of sources of primary and secondary inoculum). It is achieved by

a) Rouging: Removal of diseased plants or their affected organs from field, which prevent the dissemination of plant pathogens.

Ex: Loose smut of wheat and barley, whip smut of sugarcane, red rot of sugarcane, ergot of bajra, yellow vein mosaic of bhendi, khatte disease of cardamom, etc. During 1927-

1935, to eradicate citrus canker bacterium in USA, 3 million trees were cut down and burnt.

b) Eradication of alternate and collateral hosts: Eradication of alternate hosts will help in management of many plant diseases.

Ex: Barbery eradication programme in France and USA reduced the severity of black stem rust of wheat

Ex: Eradication of *Thalictrum* species in USA to manage leaf rust of wheat caused by *Puccinia recondita*.

Eradication of collateral hosts, such as *Panicum repens*, *Digitaria marginata* will help in the management of rice blast disease (*Pyricularia oryzae*)

c) Crop rotation: Continuous cultivation of the same crop in the same field helps in the perpetuation of the pathogen in the soil. Soils which are saturated by the pathogen are often referred as **sick soils**. To reduce the incidence and severity of many soil borne diseases, crop rotation is adopted. Crop rotation is applicable to only root inhabitants and facultative saprophytes, and may not work with soil inhabitants.

Ex: Panama wilt of banana (long crop rotation), wheat soil borne mosaic (6 yrs) and club root of cabbage (6-10 yrs), etc.

d) Crop sanitation: Collection and destruction of plant debris from soil will help in the management of soil borne facultative saprophytes as most of these survive in plant debris. Collection and destruction of plant debris is an important method to reduce the primary inoculum.

e) Manures and fertilizers: The deficiency or excess of a nutrient may predispose a plant to some diseases. Excessive nitrogen application aggravates diseases like stem rot, bacterial leaf blight and blast of rice. Nitrate form of nitrogen increases many diseases, whereas, phosphorous and potash application increases the resistance of the host. Addition of farm yard manure or organic manures such as green manure, 60-100 t/ha, helps to manage the diseases like cotton wilt, Ganoderma root rot of citrus, coconut, etc.

f) Mixed cropping: Root rot of cotton (*Phymatotrichum omnivorum*) is reduced when cotton is grown along with sorghum. Intercropping sorghum in cluster bean reduces the incidence of root rot and wilt (*Rhizoctonia solani*)

g) Summer ploughing: Ploughing the soil during summer months expose soil to hot weather which will eradicate heat sensitive soil borne pathogens.

h) Soil amendments: Application of organic amendments like saw dust, straw, oil cake, etc., will effectively manage the diseases caused by *Pythium*, *Phytophthora*, *Verticillium*, *Macrophomina*, *Phymatotrichum* and *Aphanomyces*. Beneficial micro-organisms increases in soil and helps in suppression of pathogenic microbes.

Ex: Application of lime (2500 Kg/ha) reduces the club root of cabbage by increasing soil pH to 8.5

Ex: Application of Sulphur (900 Kg/ha) to soil brings the soil pH to 5.2 and reduces the incidence of common scab of potato (*Streptomyces scabies*).

ij) Changing time of sowing: Pathogens are able to infect susceptible plants under certain environmental conditions. Alternation in date of sowing can help avoidance of favourable conditions for the pathogens.

Ex: Rice blast can be managed by changing planting season from June to September/October.

j) Seed rate and plant density: Close spacing raises atmospheric humidity and favours sporulation by many pathogenic fungi. A spacing of 8'X8' instead of 7'X7' reduces sigatoka disease of banana due to better ventilation and reduced humidity. High density planting in chillies leads to high incidence of damping off in nurseries.

k) Irrigation and drainage: The amount, frequency and method of irrigation may affect the dissemination of certain plant pathogens. Many pathogens, including, *Pseudomonas solanacearum*, *X. campestris pv. oryzae* and *Colletotrichum falcatum* are readily disseminated through irrigation water. High soil moisture favours root knot and other nematodes and the root rots caused by species of *Sclerotium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Phymatotrichum*, etc.

PHYSICAL METHODS: Physical methods include soil solarization and hot water treatments.

i. Soil solarization: Soil solarization or slow soil pasteurization is the hydro/thermal soil heating accomplished by covering moist soil with polyethylene sheets as soil mulch during summer months for 4-6 weeks. Soil solarization was developed for the first time in Israel (Egley and Katan) for the management of plant pathogenic pests, diseases and weeds.

ii. Soil sterilization: Soil can be sterilized in green houses and sometimes in seed beds by aerated steam or hot water. At about 50⁰C, nematodes, some oomycetous fungi and other water molds are killed. At about 60 and 72⁰C, most of the plant pathogenic fungi and bacteria are killed. At about 82⁰C, most weeds, plant pathogenic bacteria and insects are killed. Heat tolerant weed seeds and some plant viruses, such as TMV are killed at or near the boiling point (95-100⁰C).

iii. Hot water or Hot air treatment: Hot water treatment or hot air treatment will prevent the seed borne and sett borne infectious diseases. Hot water treatment of certain seeds, bulbs and nursery stock is done to kill many pathogens present in or on the seed and other propagating materials. Hot water treatment is used for controlling sett borne diseases of sugarcane [whip smut, grassy shoot and red rot of sugarcane (52⁰C for 30 min)] and loose smut of wheat (52⁰C for 10 min).

Biological methods:

Def: Biological control of plant disease is a condition or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except human beings), with the result that there is reduction in incidence of the disease caused by the pathogen (Garett, 1965).

Def: Biological control is the reduction of inoculum density or disease producing activity of a pathogen or a parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment of host or antagonist by mass introduction of one or more antagonists (Baker and Cook, 1974)

Mechanisms of biological control

1. Competition: Most of the biocontrol agents are fast growing and they compete with plant pathogens for space, organic nutrients and minerals. Most aerobic and facultative anaerobic micro-organisms respond to low iron stress by producing extracellular, low molecular weight (500-1000 daltons) iron transport agents, designated as **Siderophores**, which selectively make complex with iron (Fe³⁺) with very high affinity. Siderophore producing strains are able to utilize Fe³⁺ -

Siderophore complex and restrict the growth of deleterious micro-organisms mostly at the plant roots. Iron starvation prevents the germination of spores of fungal pathogens in rhizosphere as well as rhizoplane. Siderophores produced by

Pseudomonas fluorescens (known as **pseudobactins** or **pyoveridins**) helps in the control of soft rot bacterium, *Erwinia caratovora*.

2. Antibiosis: Antagonism mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances is known as antibiosis.

a. **Antibiotics:** Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. At low concentrations, antibiotics are deleterious to the growth or metabolic activities of other micro-organisms.

Ex: *Gliocladium virens* produces **gliotoxin** that was responsible for the death of *Rhizoctonia solani* on potato tubers.

Ex: Colonization of pea seeds by *Trichoderma viride* resulted in the accumulation of significant amount of the antibiotic **viridin** in the seeds, thus controlling *Pythium ultimum*.

Ex: Some strains of *Pseudomonas fluorescens* produce a range of compounds, viz., 2,4- diacetyl phloroglucinol (DAPG), phenazines, pyocyanin, which have broad spectrum activity against many plant pathogenic bacteria and fungi

b. **Bacteriocins:** These are antibiotic like compounds with bactericidal specificity closely related to the bacteriocin producer. Ex: The control of crown gall (caused by *Agrobacterium tumefaciens*) by the related *Agrobacterium radiobacter* strain K 84 is by the production of bacteriocin, **Agrocin K84**.

c. **Volatile compounds:** Antibiosis mediated by volatile compounds has been observed in the management of soil borne pathogens, viz., *Pythium ultimum*, *Rhizoctonia solani* and *Verticillium dahlia*, by *Enterobacter cloacae*. The volatile fraction responsible for inhibition was identified as ammonia.

3. Hyperparasitism: Direct parasitism or lysis and death of the pathogen by another micro-organism when the pathogen is in parasitic phase is known as hyperparasitism.

Ex: *T. harzianum* parasitize and lyse the mycelia of *Rhizoctonia* and *Sclerotium*.

Biocontrol agents for the management of plant pathogens

Biocontrol agent	Pathogen/disease
1. <i>Ampelomyces quisqualis</i>	Powdery mildew fungi
2. <i>Darluca filum</i> , <i>Verticillium lecanii</i>	Rust fungi
3. <i>Pichia gulliermondii</i>	<i>Botrytis</i> , <i>Penicillium</i>

Biocontrol agent

1. *Pasteuria penetrans* (Bacteria)
2. *Paecilomyces lilacinus* (Fungus)

Nematode

- Juvenile parasite of root knot nematode
- Egg parasite of *Meloidogyne incognita*

Important fungal biocontrol agents:

Most of the species of Trichoderma, viz., *T. harzianum*, *T. viride*, *T. virens* (*Gliocladium virens*) are used as biocontrol agents against soil borne diseases, such as, root rots, seedling rots, collar rots, damping off and wilts caused by the species of Pythium, Fusarium, Rhizoctonia, Macrophomina, Sclerotium, Verticillium, etc.

Formulations of biocontrol agents available: *T. viride* (**Ecofit**, **Bioderma** in India), *G. virens* (**GlioGard** in USA), *T. harzianum* (**F-Stop** in USA) and *T. polysporum* (**BINAB- T**)

Important bacterial biocontrol agents:

1. *Pseudomonas fluorescens* (**Dagger-G** against damping off of cotton seedlings in USA)
2. *Bacillus subtilis* (**Kodiak** against damping off and soft rot in USA)
3. *Agrobacterium radiobacter* K-84 (**Gallex** or **Galltrol** against crown gall of stone fruits caused by *Agrobacterium tumefaciens*)

Plant growth promoting Rhizobacteria (PGPR):

Rhizosphere bacteria that favourably affect plant growth and yield of commercially important crops are designated as plant growth promoting rhizobacteria. The growth promoting ability of PGPR is due to their ability to produce phytohormones, Siderophores, Hydrogen cyanide (HCN), chitinases, volatile compounds or antibiotics which will reduce infection of host through phyto-pathogenic micro-organisms.

Many bacterial species, viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, etc., are usually used for the management of plant pathogenic microbes. *Bacillus* has ecological advantages as it produces endospores that are tolerant to extreme environmental conditions. *Pseudomonas fluorescens* have been extensively used to manage soil borne plant pathogenic fungi due to their ability to use many carbon sources that exude from the roots and to compete with microflora by the production of antibiotics, HCN and Siderophores that suppress plant root pathogens.

Q. 2 Give a detailed description of Integrated Plant Disease Management (IPDM)

Ans - Integrated plant disease management can be defined as a decision-based process involving coordinated use of multiple tactics for optimizing the control of pathogen in an ecologically and economically. The implications are:

- ✓ Simultaneous management of multiple pathogens
- ✓ Regular monitoring of pathogen effects, and their natural enemies and antagonists as well
- ✓ Use of economic or treatment thresholds when applying chemicals
- ✓ Integrated use of multiple, suppressive tactics.

Principles of Plant Disease Control

1. **Avoidance**—prevents disease by selecting a time of the year or a site where there is no inoculum or where the environment is not favorable for infection.
2. **Exclusion**—prevents the introduction of inoculum.
3. **Eradication**—eliminates, destroy, or inactivate the inoculum.
4. **Protection**—prevents infection by means of a toxicant or some other barrier to infection.
5. **Resistance**—utilizes cultivars that are resistant to or tolerant of infection.
6. **Therapy**—cure plants that are already infected

Factors affecting occurrences

Factors which affect Plant diseases are micro-organisms, including fungi, bacteria, viruses, mycoplasmas, etc. or may be incited by physiological causes including high or low temperatures, lack or excess of soil moisture and aeration, deficiency or excess of plant nutrients, soil acidity or alkalinity, etc. Factors that limit the rate of disease development are the relatively low amounts of inoculum in the lag stage and the paucity of healthy plants available to the inoculum in the stationary stage.

The causative agents of disease in green plants number in a tens of thousands and include almost every form of life. But primary agents of disease may also be inanimate. Thus nonliving (abiotic) agents of disease include mineral deficiencies and excesses, air pollutants, biologically produced toxicants, improperly used pesticidal chemicals, and such other environmental factors as wind, water, temperature, and sunlight. Nonliving things certainly qualify as primary agents of disease; they continuously irritate plant cells and tissues; they are harmful to the physiological processes of the plant; and they evoke pathological responses that manifest as the symptoms characteristic of the several diseases. But the abiotic agents of disease in plants. The abiotic

agents of plant disease are termed noninfectious, and the diseases they cause are termed noninfectious diseases.

Micro-organisms

The micro-organisms obtain their food either by breaking down dead plant and animal remains (saprophytes) or by attacking living plants and animals (parasites). In order to obtain nutrients, the parasitic organisms excrete enzymes or toxins and kill the cells of the tissues of the host plant, as a result of which either the whole plant or a part of it is damaged or killed, or considerable disturbance takes place in its normal metabolic processes.

Parasites

One of the factors causing plant diseases is parasites, those living organisms that can colonize the tissues of their host-plant victims and can be transmitted from plant to plant. These biotic agents are, therefore, infectious, and the diseases they cause are termed infectious diseases. The infectious agents of plant diseases are treated in the standard textbooks on plant pathology. **Ability to produce an inoculum**

The parasitic pest must produce an inoculum, some structure that is adapted for transmission to a healthy plant and this can either parasitize the host directly or develop another structure that can establish a parasitic relationship with the host. For example, inocula for viruses are the viral particles (virions); for bacteria, the bacterial cells; for fungi, various kinds of spores or the hyphal threads of mold; for nematodes, eggs or second-stage larvae.

Agents/ Media for transportation of inoculum

The inoculum must be transported from its source to a part of a host plant that can be infected. This dispersal of inoculum to susceptible tissue is termed inoculation. Agents of inoculation may be insects (for most viruses and mycoplasmalike organisms and for some bacteria and fungi), wind (for many fungi), and splashing rain (for many fungi).

Wounds, Natural openings

The parasite must enter the host plant, which it can do (depending on the organism) in one or more of three ways; through wounds, through natural openings, or by growing directly through the unbroken protecting surface of the host. Viruses are literally injected into the plant as the homopterous insect carrier probes and feeds within its host. Bacteria depend on wounds or natural openings (for example, stomates, hydathodes, and lenticels) for entrance, but many

fungi can penetrate plant parts by growing directly through plant surfaces, exerting enormous mechanical pressure and possibly softening host surfaces by enzymatic action.

Availability of food

For occurrence of disease one of the factor affecting is, availability of nourishment to grow within its host. This act of colonizations is termed infection. Certainly the parasite damages the cytoplasmic membranes of the host cells, making those membranes freely permeable to solutes that would nourish the parasite. And parasitism certainly results from enzymatic attacks by the parasite upon carbohydrates, proteins, and lipids inside the host cell. The breakdown products of such complex molecules would diffuse across the damaged host-cell membranes and be absorbed by the parasite in the form of sugars, amino acids, and the like. Air-borne parasites of foliage, flower, and fruit.

Preventive and control measures

A. PREVENTIVE MEASURES

Cultural practices

Cultural practices usually influence the development of disease in plants by affecting the environment. Such practices are intended to make the atmospheric, edaphic, or biological surroundings favorable to the crop plant, unfavorable to its parasites. Cultural practices that leads to disease control have little effect on the climate of a region but can exert significant influence on the microclimate of the crop plants in a field. Three stages of parasite's life cycle namely, Survival between crops, production of inoculum for the primary cycle and inoculation can be control by following preventive measures.

Survival between Crops

Organisms that survive in the soil can often be controlled by crop rotations with unsusceptible species. Depending on the system, either of two effects results. Catch crops have been used to control certain nematodes and other soil-borne pathogens. Soil-borne plant pathogens can be controlled by biological methods. Plant parasites may be controlled by antagonistic organisms that can be encouraged to grow luxuriantly by such cultural practices as green manuring and the use of appropriate soil additives. The soil-invading parasite thus becomes an amensal in association with its antagonist. Soil-borne plant parasites may also be killed during their over-seasoning stages by such cultural practices as deep ploughing (as for the pathogen of southern leaf blight of corn), flooding (as for the cottony-rot pathogen and some nematodes), and frequent cultivation and fallow (as for the control of weeds that harbor plant viruses). Plant diseases caused by organisms that survive as parasites within perennial hosts or within the seed of annual plants may be controlled therapeutically. Therapeutic treatments of

heat and surgery are applicable here; those involving the use of chemicals will be mentioned later. Removal of cankered limbs (surgery) helps control fire blight of pears, and the hot-water treatment of cabbage seed controls the bacterial disease known as black rot. Heat therapy is also used to rid perennial hosts of plant-parasitic nematodes.

Production of Inoculum for the Primary Cycle

Environmental factors (particularly temperature, water, and organic and inorganic nutrients) significantly affect Inoculum production. Warm temperature usually breaks dormancy of overseasoning structures; rain may leach growth inhibitors from the soil and permit germination of resting spores; and special nutrients may stimulate the growth of overseasoning structures that produce inoculum.

Dispersal of inoculum and inoculation

Cultural practices that exemplify avoidance are sometimes used to prevent effective dissemination. A second hierarchy of regulatory disease control is plant quarantine, the legally enforced stoppage of plant pathogens at points of entry into political subdivisions. The Plant Quarantine Act of the United States governs importation of plant materials into the country and requires the state govt. to enforce particular measures. Also, states make regulations concerning the movement of plant materials into them or within them. E.g., Florida imposes quarantine against the citrus-canker bacterium, which was eliminated from the state earlier by means of cooperative efforts led by the Florida Department of Agriculture.

Sample inspection

One of the preventive measures to control the diseases is the use of sample inspection method. Laboratory evaluation of the representative sample drawn by the certification agency for the determination of germination, moisture content, weed seed content, admixture, purity, seed-borne pathogens.

B. Control Measures

Chemical Control

The pesticidal chemicals that control plant diseases may be used in very different ways, depending on the parasite to be controlled and on the circumstances it requires for parasitic activities. E.g., a water-soluble eradicated spray is applied once to dormant peach trees to rid them of the overwintering spores of the fungus of peach-leaf curl, whereas relatively insoluble protective fungicides are applied repeatedly to the green leaves of potato plants to safeguard them from penetration by the fungus of late blight. Also, systemic fungicidal chemicals may be used therapeutically.

The oxathiin derivatives that kill the smut fungi that infect embryos are therapeutic, as is benomyl (which has systemic action against powdery mildews and other leaf infecting fungi). Volatile fungicides are often useful as soil-fumigating chemicals that have eradicated action. The chemical control of plant diseases is classified in three categories: seed treatments, soil treatments, and protective sprays and dusts.

Seed Treatments

Chemical treatments of seed may be effective in controlling plant pathogens in, on, and around planted seed. Seed treatment is therapeutic when it kills bacteria or fungi that infect embryos, cotyledons, or endosperms under the seed coat, eradicated when it kills spores of fungi that contaminate seed surfaces, and protective when it prevents penetration of soil-borne fungi into seedling stems. Certified seed is usually given treatment necessary for the control of certain diseases. Seed treatment is of two types; viz., physical and chemical. Physical treatments include hot-water treatment, solar-heat treatment (loose smut of wheat), and the like. Chemical treatments include use of fungicides and bactericides. These fungicides are applied to seed by different methods. In one method, the seed in small lots is treated in simple seed-treaters. The seed-dip method involves preparing fungicide suspension in water, often at field rates, and then dipping the seed in it for a specified time.

Some chemicals commonly used to control plant diseases

Chemical and use	Relative toxicity	
	Oral	Dermal
Seed treatments (all fungicides)		
Chloraneb	Low	Low
Dichlone	Low	High
Thiram	Moderate	High
Carboxin (systemic and therapeutic)	Low	Low
Soil treatments		
Methyl bromide ^b (general pesticide)	Very high	Very high
PCNB (fungicide)	Low	Moderate
SMDC [vapam] (fungicide, nematicide)	Moderate	Moderate
MIT ["Vorlex"] (fungicide, nematicide)	Moderate	Moderate
D-D mixture (nematicide)	Moderate	Low
Plant-protective treatments		
Copper compounds (fungicides, bactericides)	Moderate	Low
Sulfur (fungicide)	Low	Moderate
Maneb (fungicide)	Very low	Low
Zineb (fungicide)	Very low	Low
Captan (fungicide)	Very low	Very low
Dinocap (fungicide for powdery mildews)	Low	Low
Streptomycin (bactericidal antibiotic)	Very low	Low
Cyclohexamide ^b (fungicidal antibiotic)	Very high	Very high
Benomyl (protective and therapeutic fungicide)	Very low	Very low

The oxathiins (carboxin, DMOC) used to kill embryo infecting smuts of cereal grains have little effect on other organisms, most eradicated and protective chemicals have a wide range of fungicidal activity; they are effective against most seed-infesting and seedling-blight fungi. But specific seed-treatment chemicals often work best to control a given disease of a

single crop-plant species. Moreover, the toxicity of chemicals to seeds varies, and farmers should use only the compounds recommended by the Cooperative Extension Service of their country and state.

Copper and mercury-containing compounds were first used as seed-treating chemicals. But copper is toxic to most seeds and seedlings, and mercury has been banned from use in seed treatments because of the danger it poses to humans and animals. Organic compounds now widely used as protective and eradicated seed treatments include thiram, chloraneb, dichlone, dexon, and captan.

Soil Treatments

Soil-borne plant pathogens greatly increase their populations as soils are cropped continuously, and finally reach such levels that contaminated soils are unfit for crop production. Chemical treatments of soil that eradicate the plant pathogens therein offer the opportunity of rapid reclamation of infested soils for agricultural uses. Preplanting chemical treatment of field soils for the control of nematode-induced diseases, and fumigation of seedbed and greenhouse soils (with methyl bromide, for example) is commonly practiced to eradicate weeds, insects, and plant pathogens. Field applications of soil-treatment chemicals for fungus control are usually restricted to treatments of furrows. Formaldehyde or captan applied is effective against sclerotia-producing fungi that cause seedling blights, stem rots, and root rots of many field crops. Other soil-treatment fungicides are vapam and "Vorlex." Soil treatments made at the time of planting are most effective against parasitic attacks that come early in the growing season.

Protective sprays and dust

Protective fungicides prevent germination, growth, and penetration. In order to use protective fungicides effectively, the farmer must not only select the right fungicide for the job, but also apply it in the right amount, at the right times, and in the right way. Too little fungicide fails to control disease; too much may be toxic to the plants to be protected. The farmer and applicator, therefore, must always follow use instructions to the letter. Timing of applications is also critical.

Advantages

Integrated approach integrates preventive and corrective measures to keep pathogen from causing significant problems, with minimum risk or hazard to human and desirable components of their environment.

Some of the benefits of an integrated approach are as follows:

- Promotes sound structures and healthy plants
- Promotes the sustainable bio based disease management alternatives.
- Reduces the environmental risk associated with management by encouraging the adoption of more ecologically benign control tactics
- Reduces the potential for air and ground water contamination
- Protects the non-target species through reduced impact of plant disease management activities.
- Reduces the need for pesticides and fungicides by using several management methods
- Reduces or eliminates issues related to pesticide residue
- Reduces or eliminates re-entry interval restrictions
- Decreases workers, tenants and public exposure to chemicals
- Alleviates concern of the public about pest & pesticide related practices.
- Maintains or increases the cost-effectiveness of disease management programs

Nature, chemical combination, classification, mode of action and formulation of fungicides.

Use of chemicals for the control of plant diseases is generally referred to as protection or therapy.

Protection: The prevention of the pathogen from entering the host or checking the further development in already infected plants by the application of chemicals is called protection and the chemicals used are called protectants.

Therapy means cure of a disease, in which fungicide is applied after the pathogen is in contact with the host. Chemicals used are called therapeutants.

Fungicide: Any agent (chemical) that kills the fungus

Fungistat: Some chemicals which do not kill fungi, but simply inhibit the fungus growth temporarily.

Antisporulant: The chemical which inhibits spore production without affecting vegetative growth of the fungus.

Fungicides are classified into three categories: Protectants, eradicants and therapeutants.

1. Protectants: These are the chemicals which are effective only when used before infection (prophylactic in behavior). Contact fungicides which kill the pathogen present on the host surface when it comes in contact with the host are called protectants. These are applied to seeds, plant surfaces or soil. These are non-systemic in action (i.e, they cannot penetrate plant tissues). Ex: Zineb,sulphur, captan, Thiram, etc.

2. Eradicants: Those chemicals which eradicate the dormant or active pathogen from the host. They can remain on/in the host for some time. Ex: Lime sulphur, Dodine.

3. Therapeutants: These are the agents that inhibit the development of a disease syndrome in a plant when applied after infection by a pathogen. Therapy can be by physical means (solar and hot water treatment) and chemical means (by use of systemic fungicides, i.e., chemotherapy).

CLASSIFICATION OF FUNGICIDES BASED ON CHEMICAL NATURE

Many fungicides have been developed for purpose of managing crop diseases which may be used as sprays, dusts, paints, pastes, fumigants, etc. The discovery of Bordeaux mixture in 1882 by Professor Millardet, University of Bordeaux, France led to the development of fungicides. Major group of fungicides used include salts of toxic metals and organic acids, organic compounds of sulphur and mercury, quinones and heterocyclic nitrogenous compounds. Copper, mercury, zinc, tin and nickel are some of the metals used as base for inorganic and organic fungicides. The non metal substances include, sulphur, chlorine, phosphorous etc. The fungicides have been classified based on their chemical nature as follows

COPPER FUNGICIDES: Copper fungicides can be classified as preparatory and proprietary copper compounds.

PREPARATORY COPPER FUNGICIDES

Common name	Chemical composition	Diseases managed
1. Bordeaux mixture	It is prepared by suspending 5 Kg of copper sulphate and 5 Kg of lime in 500 liters of water (1%)	Downy mildew of grapes, Coffee rust, Tikka leaf spot of groundnut, citrus canker, citrus scab, etc.
2. Bordeaux paste	It is prepared by mixing 1 Kg of copper sulphate and 1 Kg of lime in 10 liters of water	It is a wound dressing fungicide and can be applied to the pruned parts of the host plants such as fruit crops and ornamentals. Ex: Citrus gummosis, Stem bleeding of coconut, Bud rot of coconut, etc.
3. Burgundy mixture	Sodium carbonate is used in place of lime. It is prepared by mixing 1 Kg of copper sulphate and 1 Kg of sodium carbonate in 100 liters of water	Downy mildew of grapes, Coffee rust, Tikka leaf spot of groundnut, citrus canker, citrus scab
4. Cheshunt compound	It is a compound prepared by mixing 2 parts of copper sulphate and 11 parts of ammonium carbonate	It is used for soil drenching only. Sclerotial wilt diseases of chilli, tomato and groundnut. Fusarial wilt diseases. Damping-off diseases of solanaceous crops.
5. Chaubattia paste	It is a compound prepared by mixing 800g of copper sulphate and 800g of red lead in 1 liter of lanolin or linseed oil	Pink disease of citrus, stem canker and collar rot of apple and pears

Proprietary copper fungicides or Fixed or insoluble copper fungicides: In the fixed or insoluble copper compounds, the copper ion is less soluble than in Bordeaux mixture. So, these are less phytotoxic than Bordeaux mixture but are effective as fungicides.

Common name	Trade name	Dosage	Disease managed
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1. Copper oxy chloride	Blitox-50, Blue copper-50, Cupramar-50	0.3 to 0.5% for foliar application, 25 to 35 Kg/ha for dusting	Anthracnose of grapevine, Tikka leaf spot of groundnut, Sigatoka leaf spot of banana, citrus canker, black arm of cotton
2. Cuprous oxide	Fungimar and Perenox	0.3% for foliar spray	Anthracnose of grapevine, Tikka leaf spot of groundnut, Sigatoka leaf spot of banana, citrus canker, black arm of cotton
3. Copper hydroxide	Kocide	0.3% for foliar spray	Blister blight of tea, False smut of rice, Tikka leaf spot of groundnut

SULPHUR FUNGICIDES

Sulphur is probably the oldest chemical used in plant disease management for the control of powdery mildews and can be classified as inorganic sulphur and organic sulphur. Inorganic sulphur fungicides include lime sulphur and elemental sulphur fungicides. Organic sulphur fungicides, also called as carbamate fungicides, are the derivatives of dithiocarbamic acid.

INORGANIC SULPHUR FUNGICIDES

Common name	Trade name	Dosage	Disease managed
Preparatory sulphur compounds			
1. Lime sulphur	It is prepared by mixing 20 Kg of rock lime and 15 Kg of sulphur in	10-15 liters in 500 liters of water	Powdery mildew of apple, Apple scab, bean rust
2. Sulphur dust	Kolo dust, Mico-999	4-5g/Kg seed for ST, 10-30 Kg/ha for dusting on crops, 100 Kg /ha for soil application in tobacco, 500	Common scab of potato, Grain smut of jowar, Powdery mildew of tobacco, chilli, rose, mango, grapes, etc.
3. Wettable sulphur	Sulfex, Thiovit,	0.2-0.4 % for foliar	Powdery mildews of

ORGANIC SULPHUR COMPOUNDS

Organic sulphur compounds are derived from dithiocarbamic acid and are widely used as spray fungicides. In 1931, Tisdale and Williams were the first to describe the fungicidal nature of Dithiocarbamates. Dithiocarbamates can be categorized into two groups, viz., dialkyl dithiocarbamates (ziram, ferbam and thiram) and monoalkyl dithiocarbamates (nabam, zineb, vapam and maneb).

Common name	Trade name	Dosage	Diseases managed
Dialkyl Dithiocarbamates			
1. Ziram	Ziride, Hexazir, Milbam, Zerlate	0.15 to 0.25% for foliar spray	Anthracnose of pulses, tomato, beans, tobacco, etc., bean rust

2. Ferbam	Coromet, Ferbam, Fermate, Fermocide, Hexaferb, Karbam Black	0.15 to 0.25% for foliar spray	Fungal pathogens of fruits and vegetables, leaf curl of peaches, apple scab, downy mildew of tobacco
3. Thiram	Arasan, Hexathir, Tersan, Thiram, Thiride	0.15 to 0.2% as foliar spray, 0.2-0.3% as dry seed treatment, 15-25Kg/ha as soil application	Soil borne diseases caused by <i>Pythium</i> , <i>Rhizoctonia solani</i> , <i>Fusarium</i> , etc. Rust of ornamental crops, Scab on pears and <i>Botrytis</i> spp. on lettuce
Monoalkyl dithiocarbamates			
1. Nabam	Chembam, Dithane D-14, Dithane A-40 and Parzate liquid	0.2% as foliar spray	Used as foliar spray against leaf spot diseases of fruits and vegetables. Also used against soil borne pathogens, <i>Fusarium</i> , <i>Pythium</i> and <i>Phytophthora</i>
2. Zineb	Dithane Z-78, Hexathana, Lanocol and Parzate	0.1 to 0.3% for foliar application	Chilli die-back and fruit rot, Apple scab, Maize leaf blight, early blight of potato
3. Vapam or Metham sodium	Chem-vape, vapam, vitafume, VPM	1.5 to 2.5 liters per 10 m ² area	Fungicide with fungicidal, nematicidal and insecticidal properties. Soil fungal pathogens like <i>Fusarium</i> , <i>Puthium</i> , <i>Sclerotium</i> and <i>Rhizoctonia</i> .
4. Maneb	Dithane M22, Manzate and MEB. Mancozeb (78% Maneb + 2% zinc ion): Dithane M 45, Indofil M 45	0.2% to 0.3% as foliar application	Early and late blight of potato and tomato, rust diseases of field and fruit crops

HETROCYCLIC NITROGENOUS COMPOUNDS

Common	Trade name	Dosage	Diseases managed
1. Captan (Kittleson's killer)	Captan 50W, Captan 75 W, Esso fungicide, Orthocide 406, Hexacap, Vancide 89	0.2 to 0.3% for dry seed treatment, 0.2 to 0.3% for foliar spray, 25 to 30 Kg/ha for furrow application	Onion smut, Chilli die-back and fruit rot, Damping off of beans, chilli and tomato, seed rots and seedling blights of maize
2. Folpet	Phaltan	0.1 to 0.2% for spraying	Apple scab, tobacco brown spot, rose

3. Captafol	Difosan, Difolaton, Sanspor, Foltaf	0.15 to 0.2% for spraying, 0.25% for seed treatment, 0.15% for soil drenching	Sorghum anthracnose, cotton seedling diseases, seed rot and seedling diseases of rice, downy mildew of crucifers, apple scab
4. Iprodione	Rovral, Glycophene	0.1 to 0.2% for foliar application	Diseases caused by Botrytis, Monilinia, Alternaria, Sclerotinia, Helminthosporium and Rhizoctonia
5. Vinclozolin	Ornalin, Ronilan, Vorlan	0.1 to 0.2% for foliar application	Effective against sclerotia forming fungi like Botrytis, Monilinia and Sclerotinia

The group of heterogeneous fungicides includes some of the best fungicides like captan, folpet, captafol, vinclozoline and Iprodione. Captan, folpet and captafol belong to dicarboximides and are known as phthalamide fungicides. The new members of dicarboximide group are Iprodione, vinclozolin, etc.

MISCELLANEOUS FUNGICIDES

Common	Trade name	Dosage	Diseases managed
1. Chlorothalonil	Bravo, Daconil, Kavach, Thermil, Exotherm, Safeguard	0.2 to 0.3% for foliar application	A broad spectrum contact fungicide often used in greenhouses for control of <i>Botrytis</i> on ornamentals and for several molds and blights of tomato. Also used for the control of sigatoka leaf spot of banana, onion purple blotch, tikka leaf spot and rust of groundnut
2. Dinocap	Karathane, Arathane, Capryl, Mildex, Mildont and crotothane	0.1 to 0.2% for spraying	It is a good acaricide and contact fungicide and it controls powdery mildews of fruits and ornamentals effectively. This can be safely used on sulphur sensitive crops like cucurbits and apple varieties for control of powdery mildews
3. Dodine	Cyprex, Melprex, Guanidol and Syllit	0.075% for spraying	Apple scab, black spot of roses and cherry leaf spot

SYSTEMIC FUNGICIDES

The systemic fungicides were first introduced by Von Schelming and Marshall Kulka in 1966. The discovery of Oxathiin fungicides was soon followed by confirmation of systemic activity of pyrimidines and benzimidazoles. A systemic fungicide is capable of managing a pathogen remote from the point of application. On the basis of chemical nature these fungicides are classified as follows

Common name	Trade name	Dosage	Diseases managed
ACYLALANINES			

1. Metalaxyl	Ridomil 25 % WP, Apron 35 SD, Subdue, Ridomil MZ-72WP	3-6 g/Kg seed for seed treatment, 1 to 1.5 Kg a.i/ha for soil application, 0.1 to 0.2% for foliar spray	It is highly effective against <i>Pythium</i> , <i>Phytophthora</i> and many downy mildew fungi
2. Benalaxyl	Galben 25% WP and 5% G	0.1 to 0.2% for foliar spray, 1 to 1.5 Kg a.i/ha for soil application	Blue mold of tobacco, late blight of potato and tomato, downy mildew of grapevine
AROMATIC HYDROCARBONS			
1. Chloroneb	Demosan	0.2% for seed treatment	Seedling diseases of cotton, peanut, peas and cucurbits caused by species of <i>Pythium</i> , <i>Phytophthora</i> , <i>Rhizoctonia</i> and <i>Sclerotium</i>
BENZIMIDAZOLES			
1. Carbendazim	Bavistin 50WP, MBC, Derosol 60WP, Agrozim, Zoom	0.1% for foliar spray, 0.1% for soil drench, 0.25% for ST, 500-1000ppm for post-harvest dip of fruits	Effectively controls anthracnose, powdery mildews and rusts caused by various fungi. It is also used as a soil drench against wilt diseases and for post harvest treatment of fruits
2. Benomyl	Benlate 50WP	0.1 to 0.2% for ST, 50-60g/100 L for foliar spray, 50-200ppm for soil drenching, 12-45 Kg a.i/ha for soil broadcast, 100-500 ppm for post harvest fruit dip	Effective against powdery mildews of cucurbits, cereals and legumes. It is highly effective against diseases caused by the species of <i>Rhizoctonia</i> , <i>Theilaviopsis</i> and <i>Cephalosporium</i> . Benomyl has no effect against Oomycetes and some dark coloured fungi such as <i>Alternaria</i> and <i>Helminthosporium</i>
3. Thiabendazole	Mertect 60WP, Mycozol, Arbotect, Tecto and Storite	0.2 to 0.3% for spraying, 1000 ppm for fruit dip	Blue and green molds of citrus, loose smut of wheat, Tikka leaf spot of groundnut

ALIPHATICS			
1. Prothiocarb	Previcur, Dynone	5.6 Kg a.i/ha for soil application	Highly active against soil borne Oomycetes like <i>Pythium</i> and <i>Phytophthora</i>
2. Propamocarb	Previcur-N, Dynone-N, Prevex, Benol	3.4 and 4.8 Kg a.i/ha for soil application	Effective against soil borne Oomycetes like <i>Pythium</i> and <i>Phytophthora</i>
OXATHINS or CARBOXIMIDES			

1. Carboxin	Vitavax 75WP, Vitaflow	0.15 to 0.2% for seed treatment, 0.5% for spraying	Highly effective against smut diseases. Commonly used for the control of loose smut of wheat, onion smut, grain smut of sorghum. As a soil drench it is used for the control of diseases caused by <i>Rhizoctonia solani</i> and <i>Macrophomina phaseolina</i> .
2. Oxycarboxin	Plantavax 75 WP, Plantavax 20EC, Plantavax 5% liquid	0.1 to 0.2% for foliar spray, 0.2 to 0.5% for ST	Highly effective against rust diseases. Commonly used for the control of rusts of wheat, sorghum, safflower, legumes, etc.
IMIDAZOLES			
1. Imazalil	Fungaflor, Bromazil and Nuzone	0.1 % as post harvest dip	Blue and green molds of citrus
2. Fanapanil	Sistane 25 EC	0.05% foliar spray	Spot blotch of barley, loose and covered smut of barley
MORPHOLINES			
1. Tridemorph	Calixin 75EC, Bardew, Beacon	0.1% for foliar spray	Powdery mildew of cereals, vegetables and ornamentals. Rusts of pulses, groundnut and coffee, Sigatoka leaf spot of banana, pink disease of rubber, Ganoderma root rot & wilt
ORGANOPHOSPHATES			
1. Iprobenphos	Kitazin 48EC, Kitazin 17G, Kitazin 2% D	30-45 Kg of granules/ha, 1 to 1.5 liters of 48% EC in 1000 ml of water for foliar spray	Fungicide with insecticidal properties. Highly specific against rice blast, stem rot and sheath blight of rice
2. Ediphenphos	Hinosan 30 and 50% EC, Hinosan 2%D	400 to 500 ppm for spraying, 30 to 40 Kg/ha	Highly specific against rice blast, stem rot and sheath blight of rice
ALKYL PHOSPHONATES			
1. Fosetyl-Al or Aluminium Tris	Aliette 80WP	0.15% for foliar spray, 0.2% for soil drench	Ambimobile fungicide. Specific against Oomycetes fungi

PYRIMIDINES			
1. Fenarimol	Rubigan 50% WP, 12%EC	2g/Kg seed as ST, 20 to 40 ml/100 liters of water for spraying	Powdery mildew of cucurbits, apple, mango, roses, grapes and ornamental crops
THIOPHANATES			
1. Thiophanate	Topsin 50WP, Cercobin 50WP	0.1 to 0.2% for spraying	Powdery mildew of cucurbits and apple, club root of crucifers, rice blast

2.Thiophanate methyl	Topsin M 70WP, Cercobin M 70WP	0.1% spraying for	Blast and sheath blight of rice, sigatoka leaf spot of banana, powdery mildew of beans, chilli, peas and cucurbits
TRIAZOLES			
1. Triadimefon	Bayleton, Amiral	0.1 to 0.2% for spraying, 0.1% for seed treatment	Highly effective against powdery mildews and rusts of several crops. Effective against diseases caused by species of <i>Erysiphe</i> , <i>Sphaerotheca</i> , <i>Puccinia</i> , <i>Uromyces</i> , <i>Phakopsora</i> , <i>Hemileia</i> and <i>Gymnosporangium</i>
2. Tricyclazole	Beam 75WP, Baan 75WP, Trooper 75WP	2g/Kg seed for ST, 0.06% for spraying	Highly effective against blast of rice
3. Bitertanol	Baycor and Sibutol	0.05 to 0.1% for foliar spray	Powdery mildews and rusts of various crops, apple scab, <i>Monilinia</i> on fruit crops, late leaf spot of groundnut and sigatoka leaf spot of banana
4. Hexaconazole	Contaf 5%EC, Anvil	0.2% for spraying	Sheath blight of rice, powdery mildew and rust of apple, rust and tikka leaf spot of groundnut
5. Propiconazole	Tilt, 25% EC, Desmel	0.1% for foliar application	Sheath blight of rice, Sigatoka leaf spot of banana, brown rust of wheat
6. Myclobutanil	Systhane 10WP	0.1 to 0.2% for spraying	Apple scab, cedar apple rust and powdery mildew of apple
STROBILURINS			
1. Azoxystrobin	Amistar, Quadris	0.1% spraying for	Broad spectrum fungicide
2.Kresoxim methyl	Ergon, Discus, Stroby	0.1% spraying for	Commonly used for control of ornamental diseases

CLASSIFICATION OF FUNGICIDES BASED ON METHOD OF APPLICATION

The fungicides can also be classified based on the nature of their use in managing the diseases.

1. Seed protectants: Ex. Captan, thiram, carbendazim, carboxin etc.
2. Soil fungicides (preplant): Ex. Bordeaux mixture, copper oxy chloride, Chloropicrin, Formaldehyde, Vapam, etc.
3. Soil fungicides: Ex. Bordeaux mixture, copper oxy chloride, Captan, PCNB, thiram etc.
4. Foliage and blossom: Ex. Capton, ferbam, zineb, mancozeb, chlorothalonil etc.
5. Fruit protectants: Eg. Captan, maneb, carbendazim, mancozeb etc.
6. Eradicants: EX. Lime sulphur
7. Tree wound dressers: Ex. Boreaux paste, chaubattia paste, etc.
8. General purpose sprays and dust formulations.

Disease Resistance

HOST PLANT RESISTANCE (IMMUNIZATION)

Disease resistance: It is the ability of a plant to overcome completely or in some degree the effect of a pathogen or damaging factor.

Susceptibility: The inability of a plant to resist the effect of a pathogen or other damaging factor.

Advantages of resistant varieties:

1. Resistant varieties can be the most simple, practical, effective and economical method of plant disease management.
2. They not only ensure protection against plant diseases but also save the time, energy and money spent on other measures of control
3. Resistant varieties, if evolved can be the only practical method of control of diseases such as wilts, viral diseases, rusts, etc.
4. They are non-toxic to human beings, animals and wild life and do not pollute the environment
5. They are effective only against the target organisms, whereas, chemical methods are not only effective against target organisms but also effective against non-target organisms.
6. The resistance gene, once introduced, is inherited and therefore permanent at no extra cost.

Disadvantages:

1. Breeding of resistant varieties is a slow and expensive process
2. Resistance of the cultivar may be broken down with the evolution of the pathogen

Types of resistance:

1. Vertical resistance: When a variety is more resistant to some races of the pathogen than others, the resistance is called vertical resistance (race-specific resistance, qualitative resistance, discriminatory resistance). Vertical resistance is usually governed by single gene and is unstable.

2. Horizontal resistance: When the resistance is uniformly spread against all the races of a pathogen, then it is called horizontal/generalized/non-specific/field/qualitative resistance. Horizontal resistance is usually governed by several genes and is more stable.

3. Monogenic resistance: When the defense mechanism is controlled by a single gene pair, it is called monogenic resistance.

4. Oligogenic resistance: when the defense mechanism is governed by a few gene pairs, it is called oligogenic resistance.

5. Polygenic resistance: When the defense mechanism is controlled by many genes or more groups of supplementary genes, it is called polygenic resistance.

Cross protection: The phenomenon in which plant tissues infected with mild strain of a virus are protected from infection by other severe strains of the same virus. This strategy is used in the management of severe strains of Citrus Tristeza virus.

Antibiotics.

The term antibiotic was coined from the word „antibiosis“ which literally means „against life“. In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms (Russell, 2004). As a result of this notion, an antibiotic was originally, broadly defined as a substance, produced by one microorganism (Denyer et al., 2004), or of biological origin (Schlegel, 2003) which at low concentrations can inhibit the growth of, or are lethal to other microorganisms (Russell, 2004). However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly

through synthetic means. Whilst some antibiotics are able to completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic (Walsh, 2003). Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterials, antifungals and antivirals to reflect the group of microorganisms they antagonize (Brooks et al., 2004; Russell, 2004).

Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus *Penicillium notatum* but its discovery was first reported in 1929 (Aminov, 2010), and clinical trials first conducted on humans in 1940 (Russell, 2004; Schlegel, 2003).

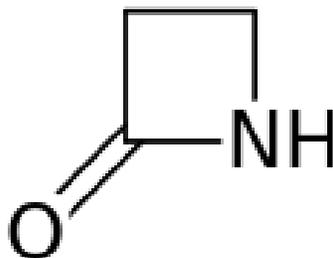


Figure 1. Chemical structure of a beta-lactam ring (Tidwell, 2008).

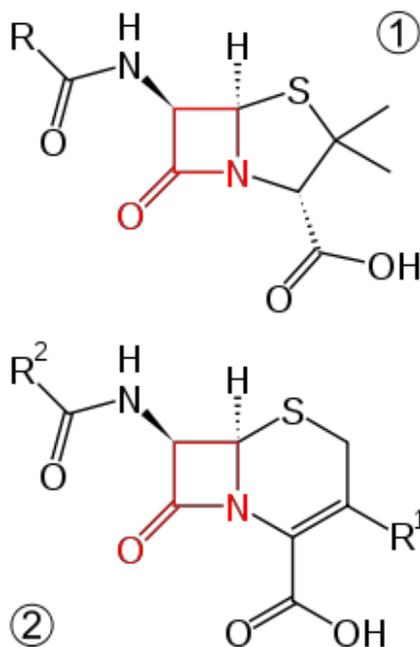


Figure 2. Chemical structure of beta- lactam structure. Core structure of [penicillins](#) (top) and cephalosporins (bottom) (Holten and Onusko, 2000).

The discovery and development of the first significant antibiotic “penicillin” in 1920’s, and subsequent introduction into man’s health care system in the 1940’s has continued to transform the management and fight against bacterial infections (White and Cox, 2013). However, antibiotics are not totally selective in their antibacterial activity. Whilst antagonizing disease causing bacteria, they also antagonize the normal and useful microbiota that we all have and need in our systems as those in the gastrointestinal tract (Walsh, 2003). Prescription and administration of any given antibiotics is

therefore predicated on the overall intended benefit, taking into consideration the attendant side effects. For this reason, it is pertinent to understand the mechanism of action of every identified antibiotic before introduction into our health care delivery system, and recent molecular biological approaches have played very significant roles to elucidate our understanding in this regard.

Hence this paper aimed to review the classification of antibiotics and their mode of action with emphasis on molecular perspectives.

CLASSIFICATION OF ANTIBIOTICS

There are several ways of classifying antibiotics but the most common classification schemes are based on their molecular structures, mode of action and spectrum of activity (Calderon and Sabundayo, 2007). Others include route of administration (injectable, oral and topical). Antibiotics within the same structural class will generally show similar pattern of effectiveness, toxicity and allergic- potential side effects.

Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (van Hoek et al., 2011; Frank and Tacconelli, 2012; Adzitey, 2015).

Beta-lactams

Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive (Figures 1 and 2). They interfere with proteins essential for synthesis of bacterial cell wall, and in the process either kills or inhibits their growth. More succinctly, certain bacterial enzymes termed penicillin-binding protein (PBP) are responsible for cross linking peptide units during synthesis of peptidoglycan. Members of beta-lactam antibiotics are able to bind themselves to these PBP enzymes, and in the process, they interfere with the synthesis of peptidoglycan resulting to lysis and cell death (Heesemann, 1993). The most prominent representatives of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems.

Penicillins

The first antibiotic, penicillin, which was first discovered and reported in 1929 by Alexander Fleming was later found to be among several other antibiotic compounds called the penicillins. (McGeer et al., 2001). Penicillins

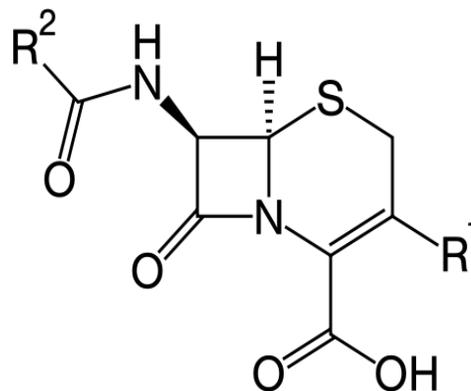


Figure 3. Structure of Cephalosporins (Pegler and Healy, 2007).

are involved in a class of diverse group of compounds, most of which end in the suffix -cillin. They are beta-lactam compounds containing a nucleus of 6-aminopenicillanic acid (lactam plus thiazolidine) ring and other ring side chains (Zahner and Maas, 1972).

Members of Penicillin class include Penicillin G, Penicillin V, Oxacillin (dicloxacillin), Methicillin, Nafcillin, Ampicillin, Amoxicillin, Carbenicillin, Piperacillin, Mezlocillin and Ticarcillin (Boundless, 2016). Penicillin G was the first to be produced amongst this group of antibiotics, and in fact of all antibiotics. Although penicillin G was discovered by Alexander Fleming in the 1920's, it took the efforts of several other workers such as Ernst Chain, Edward Abraham, Norman Heatley and Howard Florey in 1945 to understand the cultural requirements of the fungus and its clinical effectiveness. Furthermore, although Penicillin G was originally discovered and isolated from the fungus *P. notatum* by Alexander Flemming, a close relative *Penicillium chrysogenum* is the preferred choice of source. Also, producing the antibiotics through biochemical microbial fermentation more cost effective as compared to synthesizing it from raw materials (Talaro and Chess, 2008). There is no gainsaying that the discovery of this drug heralded the introduction of antibiotics into our health care delivery system. Sadly, however, Penicillin G has a narrow spectrum; only Gram positive bacteria (streptococci) and some Gram-negative bacteria such as *Treponema pallidum* causative agent for syphilis, and meningococci are sensitive to it (Talaro and Chess, 2008).

As with every biological interaction systems where living systems seek to protect itself from attack, certain bacteria are able to counter the activity of antibiotics by encoding enzymes. In view of this, some antibiotics such as ampicillin, carbenicillin and amoxicillin have been developed semi-synthetically with different side-chains. These side chains confer on the antibiotics the ability to evade the degradative capacity of certain enzymes produced by certain bacterial strains as well as facilitating the movement of antibiotics across the outer membrane of such bacterial cell walls. This double-pronged capability increases their spectrum of activity against Gram-negative bacteria. In particular, some penicillins such as Augmentin are produced in combination with non-antibiotic compound that are able to inhibit the activity of bacterial penicillinase enzyme. Augmentin is actually a drug comprising amoxicillin (antibiotic) and clavulanic acid a non-antibiotic compound. Clavulanic acid is able to inhibit beta-lactamase enzyme thereby prolonging the antibacterial activity of the amoxicillin component of Augmentin even amongst penicillinase producing bacteria (Poirel et al., 2005).

Cephalosporin

Members of this group of antibiotics are similar to penicillin in their structure and mode of action. They form part of the most commonly prescribed and administered antibiotics; more succinctly, they account for one-third of all antibiotics prescribed and administered by the National Health Scheme in the United Kingdom (Talaro and Chess, 2008). The first known member of this group of antibiotics was first isolated by Guiseppe Brotzu in 1945 from the fungus *Cephalosporium acremonium*. Although the drug was first isolated by Guiseppe Brotzu, it was Edward Abraham who got the credit to patent it having been able to extract the compound. Cephalosporins contain 7-aminocephalosporanic acid nucleus and side chain containing 3,6-dihydro-2 H-1,3- thiazane rings (Figure 3). Cephalosporins are used in the treatment of bacterial infections and diseases arising from Penicillinase-producing, Methicillin-susceptible Staphylococci and Streptococci, *Proteus mirabilis*, some *Escherichia coli*, *Klebsiella pneumonia*, *Haemophilus influenza*, *Enterobacter aerogenes* and some *Neisseria* (Pegler and Healy, 2007).

They are subdivided into generations (1st-5th) in accordance to their target organism but later versions are increasingly more effective against Gram-negative pathogens. Cephalosporins have a variety of side chains that enable them get attach to different penicillin-binding proteins (PBPs), to circumvent blood brain barrier, resist breakdown by penicillinase producing bacterial strains and ionize to facilitate entry into Gram-negative bacterial cells (Abraham, 1987).

Monobactams

The discovery of this class of antibiotics was first reported by Skyes and co-workers. The antibiotic was obtained from the bacterium *Chromobacterium violaceum*. They are part of beta-lactam compounds but unlike most other

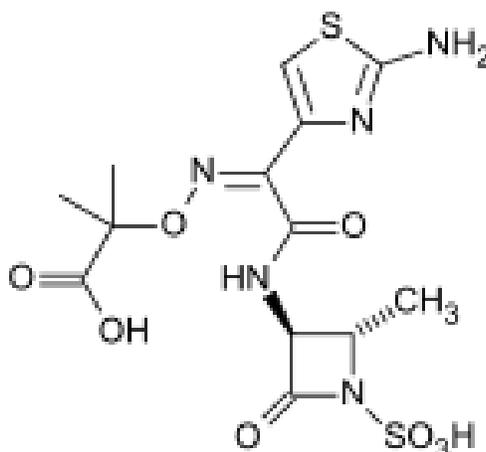


Figure 4. Structure of Monobactam (Bonner and Sykes, 1984).

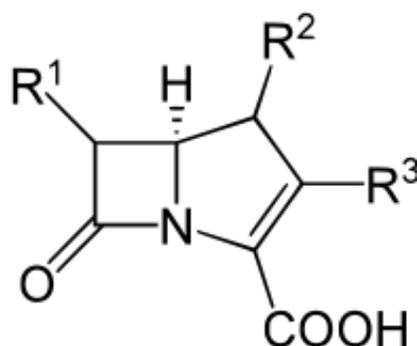


Figure 5. Structure of Carbapenem (Papp-Wallace et al., 2011).

beta-lactams, the beta-lactam ring of monobactams stand alone and is not fused to another ring (Figure 4) (Bonner and Sykes, 1984; Sykes and Bonner, 1985). Aztreonam is the only commercially available monobactam antibiotic, with a narrow spectrum of activity. Aztreonam is active only against aerobic Gram-negative bacteria such as *Neisseria* and *Pseudomonas*; used for treating pneumonia, septicemia and urinary tract infections caused by these groups of bacteria. The monobactams are not effective against Gram-positive bacteria or anaerobes. They are used as injectables and inhalers (Sykes et al., 1981).

Carbapenems

This class of antibiotics, represented in Figure 5, was discovered out of necessity in 1976. Prior to this time in the late 1960's the effectiveness of penicillin was greatly threatened owing to the emergence of beta-lactamase in bacteria. Bacterial beta-lactamases conferred resistance on bacteria against penicillin (Papp-Wallace et al., 2011). This seemingly ugly scenario led scientists to embark on a massive search for beta-lactamase inhibitors. Their efforts yielded result in 1976 when olivanic acids, produced by a Gram-positive bacterium *Streptomyces clavuligerus*, was noted to inhibit beta-lactamase (Brown et al., 1976; Butterworth et al., 1979). Unfortunately, these acids were chemically unstable and could not easily penetrate the bacterial cell. These setbacks slowed down further works on the olivanic acids (Reading and Farmer, 1984), but interestingly, shortly afterwards, two superior beta-lactamase inhibitors were discovered. These were clavulanic acid obtained also from *S. clavuligerus* (Brown et al., 1976), and thienamycin isolated from *Streptomyces cattleya* (Kropp et al., 1976). Thienamycin is reportedly considered to be the first "carbapenem" and serves as a standard for every other carbapenem (Papp-Wallace et al., 2011). A good number of other carbapenems have also been identified (Cassidy et al, 1981; Kobayashi et al., 1982).

Carbapenems occupy a very important place in our fight against bacterial infections. This is because they are able to resist the hydrolytic action of beta-lactamase enzyme. Among the several hundreds of known beta-lactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often called "antibiotics of last resort" and are administered when patients with infections become gravely ill or are suspected of harboring resistant bacteria (Torres et al., 2007). Examples of carbapenem are:

- i.** Imipenem – a broad spectrum effective against aerobic and anaerobic pathogens, usually taken orally and active in low concentrations, with minimal allergy side effects;
- ii.** Meropenem – a broad spectrum effective against non-fermentative Gram-negative bacilli particularly against acquired infections;

iii. Ertapenem – a broad spectrum with limited activity against non-fermentative Gram-negative bacilli (Brink et al., 2004).

Sadly, emergence of bacterial pathogens resistant to this life saving class of antibiotics has been reported. More worrisome is the fact that bacterial resistance to carbapenems is on the increase globally (Livermore et al., 2011; Patel and Bonomo, 2011) and is fast becoming an international concern (Papp-Wallace et al., 2011).

Macrolides

The first antibiotic belonging to this class was first discovered and isolated in 1952 by J. M. McGuire as a

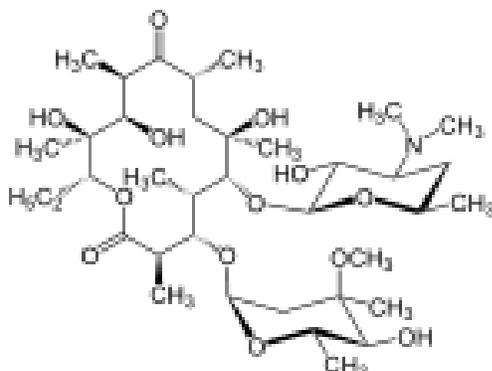


Figure 6. Structure of Macrolide (Hamilton-Miller, 1973).

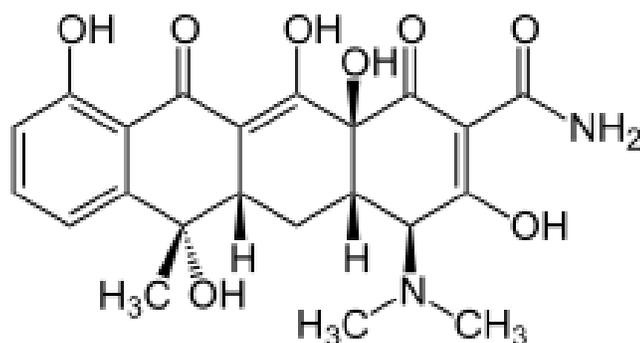


Figure 7. Structure of Tetracycline (Chopra and Roberts, 2001).

metabolic product of a soil inhabiting fungus *Saccharopolyspora erythraea*. This fungus was formerly known as *Streptomyces erythraeus* belonging to the genus *Saccharopolyspora* of actinomycete bacteria (Moore, 2015).

Macrolides are characterized by 14-, 15-, or 16- membered macrocyclic lactone rings with unusual deoxy sugars L-cladinose and D-desosamine attached (Figure 6). They have a wider spectrum of antibiotic activity than Penicillins and are often administered to patients allergic to penicillin (Moore, 2015).

Macrolides either kill or inhibit microorganisms by effectively inhibiting bacterial protein synthesis. They do so by binding to bacterial ribosome, and in the process, prevent the addition of amino acid to polypeptide chains

during protein synthesis. Macrolides tend to build up in the body because the liver is able to recycle it into the bile. They also have the capacity to cause inflammation.

As a result, clinicians usually recommend administering low doses. Although, Macrolides are generally broad spectrum, some bacterial species such as *Streptococcus pneumoniae* have resistance against the antibiotics. Example of members includes Erythromycin, Azithromycin and Clarithromycin (Hamilton-Miller, 1973).

Tetracyclines

Tetracycline was discovered in 1945 from a soil bacterium of the genus *Streptomyces* by Benjamin Duggar (Sanchez et al., 2004). The first member of this class was chlorotetracycline (Aureomycin). Members of this class have four (4) hydrocarbon rings (Figure 7) and they are known by name with the suffix „-cycline“.

Historically, members of this class of antibiotics are grouped into different generations based on the method of synthesis. Those obtained by biosynthesis are said to be First generation. Members include Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline. Members such as Doxycycline, Lymecycline, Meclon

cycline, Methacycline, Minocycline, and Rolitetracycline are considered Second generation because they are derivatives of semi-synthesis. Those obtained from total synthesis such as Tigecycline are considered to be Third generation (Fuoco, 2012).

Their target of antimicrobial activity in bacteria is the ribosome. They disrupt the addition of amino acids to polypeptide chains during protein synthesis in this

bacterial organelle (Medical News Today, 2015). Patients are advised to take tetracyclines at least two hours before or after meals for better absorption. All tetracyclines are

recommended for patients above eight (8) years because

the drugs have shown to cause teeth discoloration among patients below this age can be used in treating malaria, elephantiasis, amoebic parasites and rickettsia (Sanchez et al., 2004).

In the past, antibiotics belonging to this class were very much the envy of numerous Clinicians owing to their wide antimicrobial spectrum but this is no longer the case because numerous bacteria are now able to resist them (Chopra and Roberts, 2001).

Quinolones

This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs. Nalidixic acid was discovered as an impurity during the development of quinine in the early sixties. They are able to interfere with DNA replication and transcription in bacteria. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones which include cinoxacin, norfloxacin, ofloxacin, ciprofloxacin, temafloxacin,

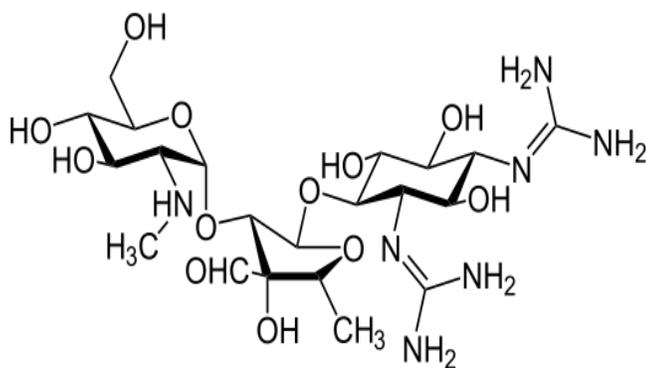


Figure 8. Structure of Aminoglycoside (Streptomycin) (Mingeot-Leclercq et al., 1999).

sparfloxacin, nalidixic acid, enoxacin etc. (Domagala, 1994). Their structure generally consists of two rings but recent generations of quinolones possess an added ring structure which enables them to extend their spectrum of antimicrobial activity to some bacteria, particularly anaerobic bacteria that were hitherto resistant to quinolone.

Since its discovery in the early 1960's, several modifications have been made to its parent structure and this has led to the development and synthesis of many derivatives with tested antibiotic potency. The nomenclature of members of this class of antibiotics is complex (Domagala, 1994) but members are often known by the suffix-oxacin, such as floxacin, ciprofloxacin and levofloxacin.

Modifications in the basic structure of quinolones are reported to have improved their bioavailability and increased both their spectrum of activity and potency; enhancing their performance in the treatment of various forms of illnesses such as urinary, systemic and respiratory tract infections. Notwithstanding these notable feats, there still exist safety concerns with some members of this class of antibiotics which has led to the withdrawal of grepafloxacin, sparfloxacin, temafloxacin, trovafloxacin etc., all belonging to the class quinolones, from the market (Domagala, 1994). Although a good deal of progress is being made in terms of *in vitro* studies and pharmacodynamics, knowledge of the dynamics of toxicity amongst some of this class of antibiotics is yet inconclusive.

Aminoglycosides

The first drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943 (Mahajan and Balachandran, 2012). Streptomycin has been greatly used against *Mycobacterium tuberculosis*, the causal agent of tuberculosis among humans. The aminoglycosides are compounds of usually 3-amino sugars connected by glycosidic bonds (Figure 8). They are obtained from soil Actinomycetes.

Aminoglycoside have a broad spectrum of antibacterial activity. They are able to inhibit the protein synthesis in bacteria by binding to one of the ribosomal subunits (Peterson, 2008), and are effective against aerobic Gram-negative rods and certain Gram-positive bacteria. The oldest known aminoglycoside, as earlier inferred is Streptomycin which has been used severally in treating bubonic plague, tularemia and tuberculosis (Talaro and Chess, 2008). Notwithstanding its effectiveness against a wide array of infections, streptomycin was found to be highly toxic. This unfortunate feature of the drug necessitated the need to search for new members of aminoglycosides that would still be effective against bacteria but less toxic to humans. The search was fruitful with the discoveries of antibiotics such as Gentamicin, Neomycin, Tobramycin and Amikacin. Gentamicin is less toxic and is widely used for infections caused by Gram- negative rods (Escherichia, Pseudomonas, Shigella and Salmonella). Tobramycin, in particular, is used in treating Pseudomonas infections in cystic fibrosis patients (Gilbert, 2000).

Sulphonamides

Sulphonamides are reportedly, the first group of antibiotics used in therapeutic medicine, and they still play very important role in medicine and veterinary practice (Eyssen et al., 1971). Sulphonamides inhibit both Gram-positive and Gram-negative bacteria such as Nocardia, E. coli, Klebsiella, Salmonella, Shigella and Enterobacter, Chlamydia trachomatis and some Protozoa, and are widely used in the treatment of various infections including tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery and some urinary tract infections (Eyssen et al., 1971). Studies have shown that Sulphonamides are also able to impede cancerous cell agents (Stawinski et al., 2013; Xu et al., 2014). The original antibacterial sulphonamide (also spelt sulfonamide by some Workers), are synthetic antimicrobial agents that contain the sulphonamide group (Figure 9) (Henry, 1943).

Sulphonamides are generally thought to be bacteriostatic rather than bactericidal. However, Henry (1943) in his thorough early work opined that sulphonamides may become bactericidal if their concentration is sufficiently high or if the presence of any sulfonamide concentration is accompanied by other environmental conditions unfavourable to bacteria. Such unfavourable conditions would include poor cultural conditions, adverse temperature, antibodies, toxic proteolytic product etc.

Although sulphonamides are adjudged good and effective in treating various diseases and infections, they

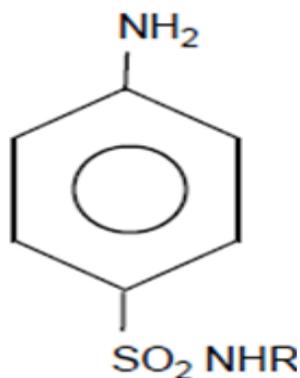


Figure 9. General structure of Sulphamides (Henry, 1943).

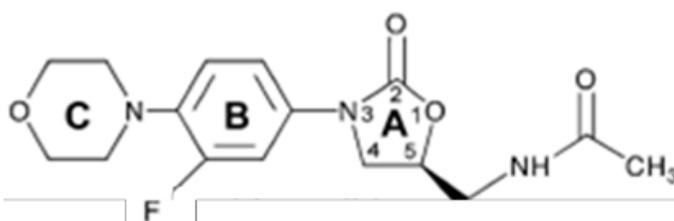


Figure 10. Structure of Linezolid (Leach et al., 2007).

are recommended and administered with caution because of their toxicity and side effects, some of which include urinary tract disorders, haemolytic anaemia, porphyria, and hypersensitivity reactions (Slatore and Tilles, 2004; Choquet-Kastylevsky et al., 2002).

Glycopeptides

Glycopeptide antibiotics generally abbreviated as GPAs were originally obtained as natural products, but the last 20 years witnessed the emergence of semi-synthetic derivatives with improved activity and pharmacokinetic properties (Kahne et al., 2005; Van Bambeke et al., 2004; Van Bambeke, 2004). Naturally, glycopeptides are made of a cyclic peptide of 7 amino acids, to which are bound 2 sugars, hence the name glycopeptides (Kang and Park, 2015). Structures of various forms of glycopeptides are well presented by Yim and Associates (2014). Binding of the antibiotic to its target occurs via the formation of 5 hydrogen bonds with the peptidic backbone of the drug. Sometimes, an additional chlorine and/or sugar is/are attached to the backbone of the drug (as is the case in oritavancin) during synthesis. Drugs with such additional attachments are known to bind more efficiently to the target (Allen and Nikas, 2003; Beauregard et al., 1995). Similarly, a lipophilic side chain antibacterial potency and prolongs half-life of glycopeptides.

Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics approved only recently for use. Linezolid (Figure 10) which represents the first member to be synthesized was approved for clinical application only in the year 2000. Although the mechanism of action of oxazolidinone is not yet fully understood, they are reported to interfere with protein synthesis. Oxazolidinones inhibit protein synthesis by binding to the P site of the ribosomal 50S subunit (Shinabarger et al., 1997; Bozdogan and Appelbaum, 2004). They have a broad spectrum of activity against Gram-positive bacteria including methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant enterococci, penicillin-resistant pneumococci and anaerobes (Bozdogan and Appelbaum, 2004).

Linezolid is used for treatment of respiratory tract and skin infections caused by Gram-positive bacterial pathogens (Moellering, 2003). Oxazolidinones constitute the choice drug in dealing with surgical infections because they easily penetrate and accumulate in the tissue including bone, lung, vegetations (plant-like growth in tissues), haematoma and cerebrospinal fluid (Bozdogan and Appelbaum, 2004). Although adhering to normal standard routines of linezolid administration are usually safe, side effects such as myelosuppression, resulting to anemia and thrombocytopenia are often encountered in cases when treatment is prolonged (Kuter and Tillotson, 2001).

ANTIBIOTICS MODE OF ACTION

The antimicrobial potency of most classes of antibiotic are directed at some unique feature of the bacterial structure or their metabolic processes. The most common targets of antibiotics are illustrated in Figure 11. The mechanism of antibiotic actions are as follows:

- Inhibition of cell wall synthesis
- Breakdown of cell membrane structure or function
- Inhibition of the structure and function of nucleic acids
- Inhibition of protein synthesis
- Blockage of key metabolic pathways

(Talaro and Chess, 2008; Madigan and Martinko, 2006; Wright, 2010)

Inhibition of cell wall synthesis

Most bacterial cells are encased by a rigid layer of peptidoglycan (PG), also called murein in older sources) which both protect the cells in the face of prevailing osmotic pressure consistent

with the often-harsh environment and conditions under which they exist. Peptidoglycan has a degree of cross-linking peptide bonds called β -(1-4) -N- acetyl Hexosamine (Bugg and Walsh, 1992; Holtje, 1998). To stay alive, bacteria must of necessity synthesize peptidoglycan; they do this by the activity of PBPs which are transglycosylases and transpeptidases. These two enzymes play very pivotal roles by adding disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecule and also cross-link strands of immature peptidoglycan units (Park and Uehara, 2008). Drugs like penicillins, carbapenems and cephalosporins are able to block the cross-linking of peptidoglycan units by inhibiting the peptide bond formation catalyzed by PBPs (Josephine et al., 2004).

Most antibiotics belonging to the glycopeptide class of antibiotics (for example, vancomycin) are able to inhibit bacterial growth by inhibiting the synthesis of PG. They inhibit the synthesis of PG by binding themselves to PG units, as well as blocking transglycosylase and transpeptidase activity (Kahne et al., 2005).

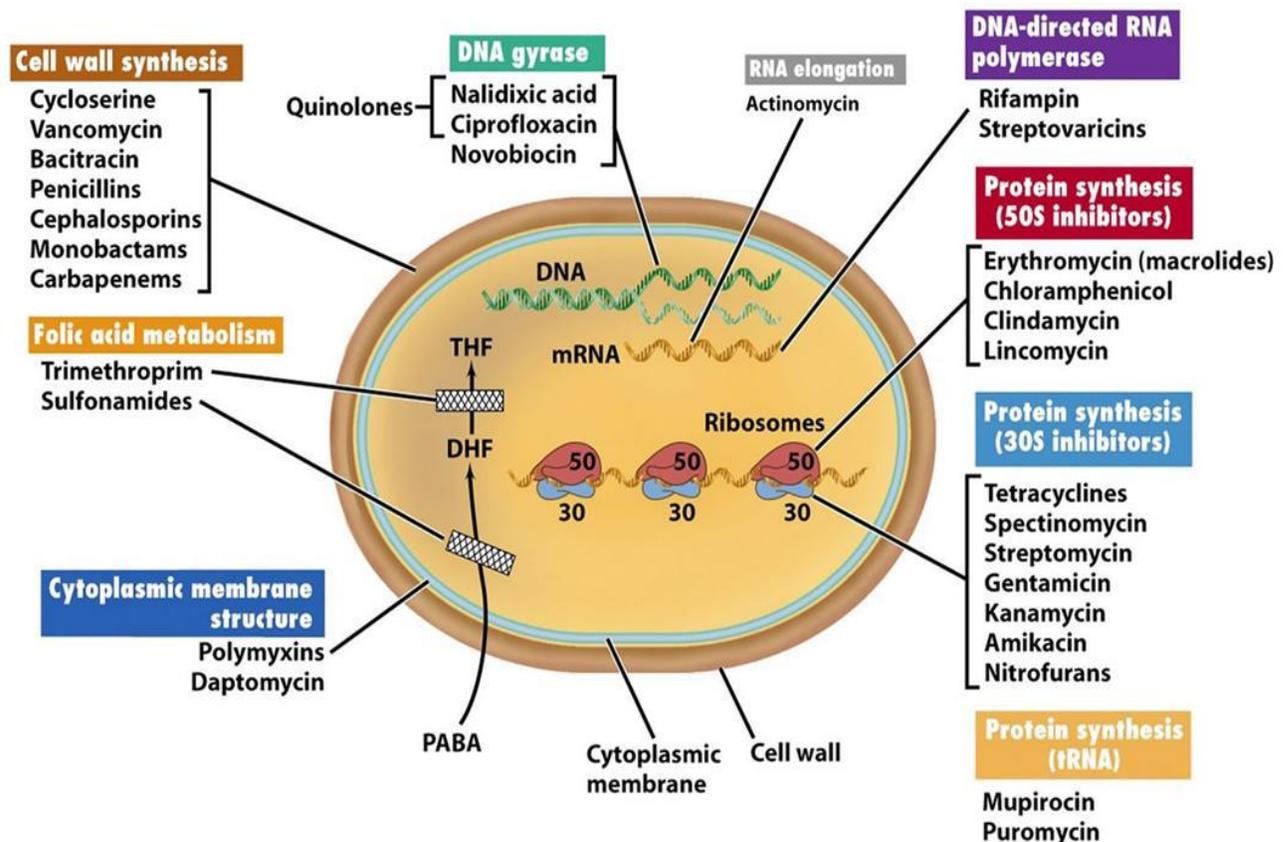


Figure 11. Antibiotic target sites (Madigan and Martinko, 2006).

Breakdown of the cell membrane structure or function

The classes of antibiotics that damage cell membranes of bacteria are specific in each microbial group based on the differences in the types of lipids in their cell membranes. For example, Daptomycin depolarizes calcium-dependent membrane, and that leads to the cessation of macromolecular synthesis and disruption of the cellular membrane in bacteria (Alborn et al., 1991). The polymyxins cause disintegration of bacterial cell membrane by effectively binding to the lipid moiety of the lipopolysaccharide in the bacterial cell (Falagas et al., 2010).

Inhibition of nucleic acid synthesis

The metabolic pathways that result in synthesis of nucleic acids are very essential; disruption of nucleic acid synthesis is inimical to both the survival and posterity of bacterial cells. Antibiotics interfere with nucleic acid synthesis by blocking replication or stopping transcription. DNA replication involves the unwinding of the traditional

double helix structure, a process facilitated by the helicase enzymes (Gale et al., 1981). The quinolones group of antibiotics, for example, do interfere with the functionality of the helicase enzyme thereby disrupts the enzyme from playing its function of unwinding DNA. This antibiotic action of the quinolones ultimately truncates the process of DNA replication and repair amongst susceptible bacteria (Chen et al., 1996). Antibiotics whose mode of action is inhibition of nucleic acid synthesis also target topoisomerase II and topoisomerase IV of bacteria. Disrupting the activities of these enzymes in bacteria adversely affects RNA polymerase which in turn prevents RNA synthesis. Quinolones that inhibit bacterial nucleic acid synthesis in this way do not interact with mammalian RNA polymerase, making them specifically antagonistic to Gram-positive bacteria and some Gram-negative bacteria.

Inhibition of protein synthesis

Living things including bacteria are defined by the amount and type of proteins they are composed of, and continually produce. Proteins are responsible for the structural composition, metabolic and physiological processes, and response to adverse conditions, amongst other roles. However, the type and amount of proteins produced by a bacterium at any given time is dependent on information contained in yet another very important biomolecule – Deoxyribonucleic acid (DNA). DNA determines the type of protein a bacterial cell produces through certain information it harbors within itself. The information is a set of genetic codes called codons, handed down to an identical biomolecule – Ribonucleic acid (RNA), specifically messenger RNA (mRNA). Transfer RNA (tRNA), a similar biomolecule is also formed under the directive of DNA. This biomolecule together with mRNA travels to the ribosomes – the factory for protein synthesis in a living cell. The tRNA then deciphers the codons contained in the mRNA and facilitates the translation of the sequence of codons to a sequence of amino acids which are the building blocks of proteins (Etebu, 2013).

The translation of mRNA into proteins occurs over three sequential phases (initiation, elongation and termination) involving the ribosome and a host of cytoplasmic accessory factors (Gualerzi et al., 2000). Ribosomes are made up of RNA and proteins, and are generally called RIBONUCLEOPROTEINS. The RNA component is what is referred to as Ribosomal RNA (rRNA), and comprises two subunits, one small subunit (SSU) and the other large subunit (LSU). These two subunits are usually described in terms of their sedimentation coefficients (that is, their rate of sedimentation is an ultracentrifuge), and are measured in

Svedberg units (symbols) termed the 30S and 50S, respectively (Nissen et al., 2000). Bacterial possess 5S, 16S and 23S genes on their rRNA (Moore, 2001). The 16S rRNA gene resides as a single RNA gene in their SSU (16S) whilst the other two rRNA genes (23S and 5S) occur on the LSU of the bacterial ribosome (Lafontaine and Tollervey, 2001). There is huge difference between prokaryotic and eukaryotic rRNA, and this feat has greatly enabled Scientists to develop antibiotics that would target rRNA of a wide spectrum of pathogenic bacteria (Hong et al., 2014).

Given the importance of proteins in the metabolic and life processes of all living organisms, whatever disrupts the process of its synthesis in a bacterial cell would ultimately incapacitate the cell; inhibit its growth or even kill it completely. Drugs that inhibit protein synthesis are among the broadest classes of antibiotics and can be divided into two subclasses: the 50S inhibitors and 30S inhibitors.

Antibiotics such as erythromycin, clindamycin, lincomycin, chloramphenicol, linezolid etc. have been shown to be among the 50S ribosome inhibitors (Douthwaite, 1992; Katz and Ashley, 2005). In general terms, antibiotics that inhibit 50S ribosome do so by physically blocking either the initiation phase of protein translation or the elongation phase of protein synthesis where the incoming amino acid is linked up with the growing nascent peptide chain (Patel et al., 2001; Vannuffel and Cocito, 1996; Menninger and Otto, 1982). Examples of antibiotic that block initiation of protein translation are members of Oxazolidinones (Patel et al., 2001) whilst macrolides such as lincosamide and streptogramin block protein synthesis by inhibiting the elongation phase of mRNA translation (Vannuffel and Cocito, 1996; Menninger and Otto, 1982). These latter groups of antibiotics are therefore reportedly ineffective when elongation has progressed beyond a critical length (Tenson et al., 2003).

The 30S ribosome-inhibitors principally work by blocking the access of aminoacyl-tRNAs to the ribosome. Examples of antibiotics that function in this manner include the tetracycline, streptomycin, spectinomycin, etc. (Hong et al., 2014; Chopra and Roberts, 2001). It is worthy to note that some earlier works have shown that tetracycline also inhibits some proteins at the 50S ribosomes (Epe and Woolley, 1984).

Among ribosome inhibitors, the naturally-derived aminoglycoside subclass is the only one that is broadly bactericidal. Macrolides, streptogramins, spectinomycin, tetracyclines and chloramphenicol are typically bacteriostatic. However, some of these ribosome inhibitory antibiotics that are typically bacteriostatic in action could be bactericidal under certain conditions relating to species- or treatment-specific fashion. For example, chloramphenicol known typically to be

Blockage of key metabolic pathways

Some antibiotics like sulphonamides and trimethoprim have been shown to mimic a substrate needed for cellular metabolism of bacteria. This deception cause bacterial enzymes to attach themselves to the antibiotic related glycopeptide antibiotics. Sulphonamides act like tetrahydrofolate which is required for the synthesis of folic acid in bacterial cells (Talaro and Chess, 2008). Folic acid is vital in the metabolism of nucleic acid and amino acids; for this reason, sulphonamides ultimately disrupt the production of nucleic acids (DNA and RNA) and amino acids, as they mimic substrates required for folic acid metabolism (Talaro and chess, 2008).

FUNGAL TISSUES:

Plectenchyma : (plekein=to weave+enchyma=infusion)

Fungal tissues are called plectenchyma i.e., mycelium becomes organized into loosely or compactly woven tissue. This tissue compose various types of vegetative and reproductive structures.

Types of plectenchyma:

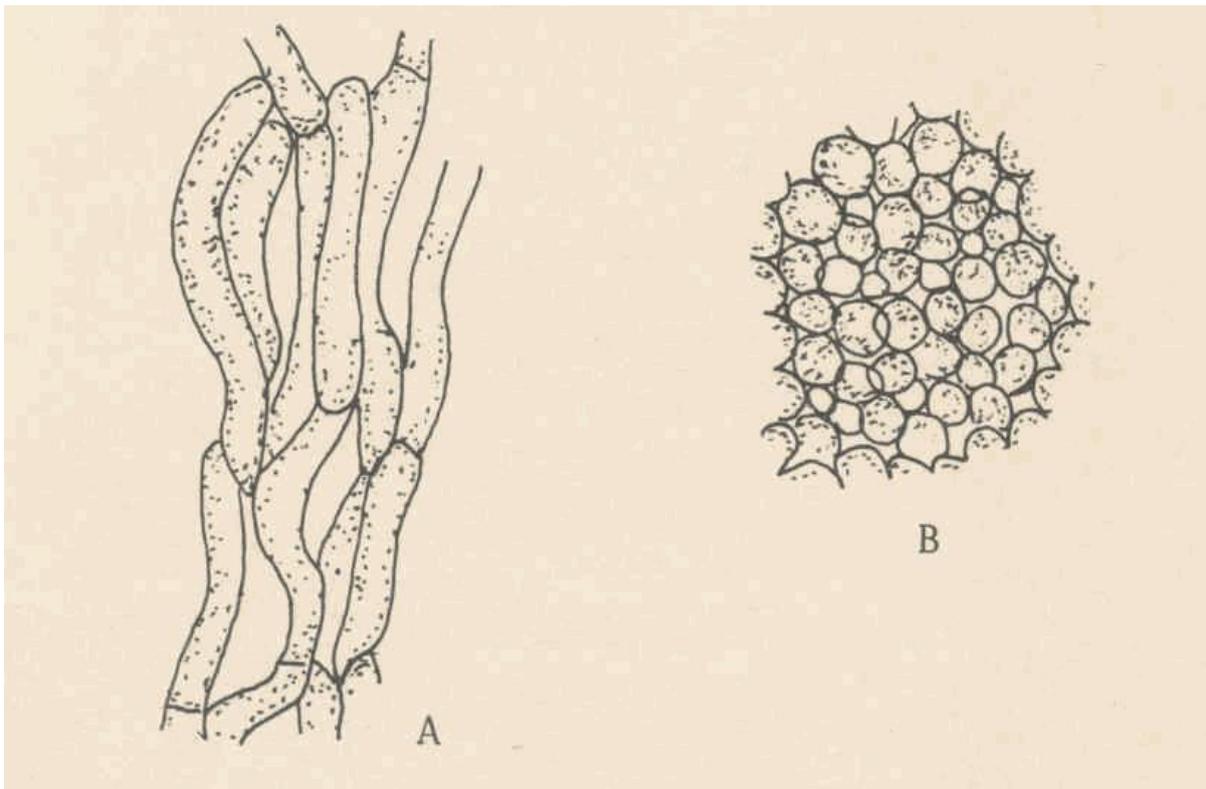
1.Prosenchyma: It is a loosely woven tissue. The component hyphae retain their individuality which can be easily distinguishable as hyphae and lie parallel to one another.

Eg. Trauma in Agaricus.

2. Pseudoparenchyma:It is compactly woven tissue. It consists of closely packed cells which are isodiametric or oval in shape resembling parenchymatous cells of plants and hence the name. The component hyphae lose their individuality and are not distinguishable as hyphae. Eg. Sclerotial bodies of Sclerotium and rhizomorph of Armillariella.

A.Prosenchyma

B.Pseudoparenchyma



3. MODIFICATION OF MYCELIUM/ SPECIALISED SOMATIC STRUCTURES

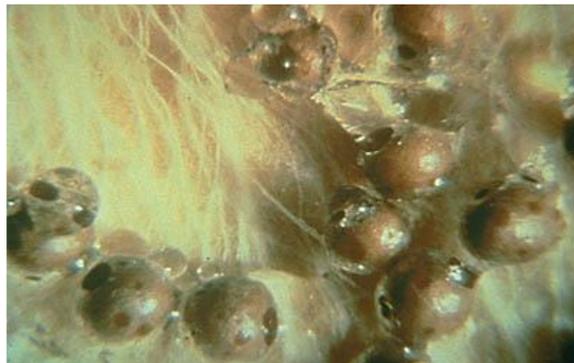
Purpose: 1. to obtain nourishment i. e., for nutrition . 2. to resist or tolerate unfavourable conditions for their survival i.e., over wintering, over summering. 3. for reproduction.

1.Rhizomorphs: (rhiza=root, morph=shape) Thick strands of somatic hyphae in which the hyphae lose their individuality and form complex tissues that are resistant to adverse conditions and remain dormant until favourable conditions return. The structure of growing tip of rhizomorphs resemble that of a root tip, hence the name rhizomorph.

Eg. *Armillariella mellea*.

2. Sclerotium: (skleron=hard) pl.sclerotia: It is a hard, round (looks like mustard seed)/ cylindrical or elongated (*Claviceps*) dark coloured (black or brown)resting body formed due to aggregation of mycelium, the component hyphae lose their individuality , resistant to unfavourable conditions and remain dormant for a longer period of time and germinate on the return of favourable conditions.

Eg. *Sclerotium*, *Rhizoctonia* .



3. Stroma: (stroma=mattress) pl.stromata. It is a compact somatic structure looks like a mattress or a cushion on which or in which fructifications (spores or fruiting bodies) are usually formed.

a. Sub stomatal stroma: cushion like structure formed below epidermis in sub stomatal region from which sporophores are produced. Eg. *Cercospora personata*.

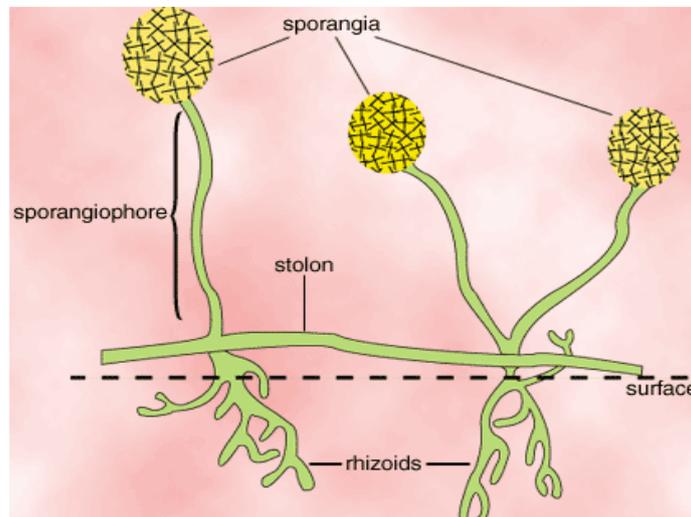
b. Perithecial stroma: When reproductive bodies like perithecia of some fungi are embedded characteristically throughout periphery of stroma, such stroma are called perithecial stroma. Eg.*Claviceps*, *Xylaria*.

4. Haustorium: (hauster=drinker) pl.haustoria.It is a outgrowth of somatic hyphae regarded as special absorbing organ produced on certain hyphae by parasitic fungi for obtaining nourishment by piercing into living cells of host. They may be knob like(*Albugo*), elongated (*Erysiphe*, *Uncinula*), finger like (*Peronospora*).



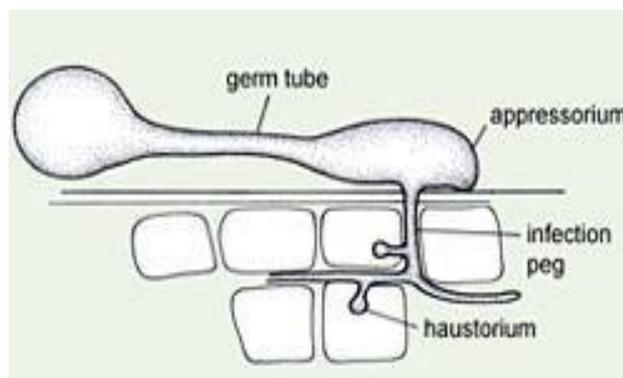
5. Rhizoids: (rhiza=root, oides=like) These are slender root like branched structures found in the substratum produced by some fungi which are useful for anchoring the thallus to substratum and for obtaining nourishment from the substrate.

Eg. *Rhizopus stolonifer*.



6. Appressorium: (apprimere=to press against) pl.appressoria A flattened tip of hyphae or germ tube acting as pressing organ by attaching to the host surface and gives rise to a minute infection peg which usually grows and penetrates the epidermal cells of the host.

Eg. *Puccinia*, *Colletotrichum*, *Erysiphe*.



GENERAL CHARACTERS OF FUNGI

Fungi:

Fungi are eukaryotic, spore bearing, achlorophyllous, heterotrophic organisms that generally reproduce sexually and asexually and whose filamentous, branched somatic structures are typically surrounded by cell walls containing chitin or cellulose or both with many organic molecules and exhibiting absorptive nutrition.

General characters

1. All are eukaryotic - Possess membrane-bound nuclei (containing chromosomes) and a range of membrane-bound cytoplasmic organelles (e.g. mitochondria, vacuoles, endoplasmic reticulum).
2. Most are filamentous - Composed of individual microscopic filaments called hyphae, which exhibit apical growth and which branch to form a network of hyphae called a mycelium.
3. Some are unicellular - e.g. yeasts.
4. Protoplasm of a hypha or cell is surrounded by a rigid wall - Composed primarily of chitin and glucans, although the walls of some species contain cellulose.
5. Many reproduce both sexually and asexually - Both sexual and asexual reproduction often result in the production of spores.
6. Their nuclei are typically haploid and hyphal compartments are often multinucleate - Although the oomycota and some yeast possess diploid nuclei.
7. All are achlorophyllous - They lack chlorophyll pigments and are incapable of photosynthesis.
8. All are chemoheterotrophic (chemo-organotrophic) - They utilise pre-existing organic sources of carbon in their environment and the energy from chemical reactions to synthesize the organic compounds they require for growth and energy.
9. Possess characteristic range of storage compounds - e.g. trehalose, glycogen, sugar alcohols and lipids.
10. May be free-living or may form intimate relationships with other organisms i.e. may be freelifing, parasitic or mutualistic (symbiotic).

SOMATIC STRUCTURES:

Thallus/ Soma

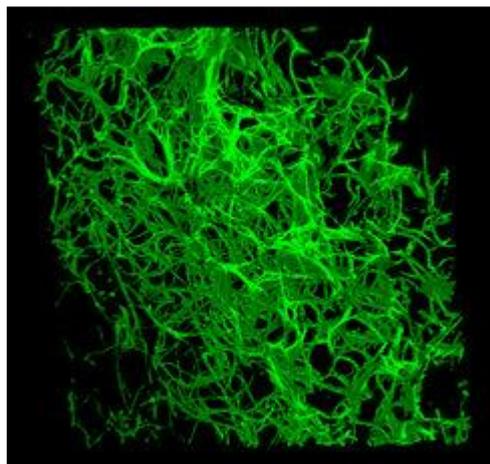
Commonly called as vegetative body or fungal body. A thallus(pl. thalli) is a simple, entire body of the fungus devoid of chlorophyll with no differentiation into stem, roots and leaves lacking vascular system.

Hypha (hypha=web) (pl. hyphae) :

Hypha is a thin, transparent, tubular filament filled with protoplasm. It is the unit of a filamentous thallus and grows by apical elongation.

Mycelium(pl. mycelia):

A net work of hyphae (aggregation of hyphae) constituting the filamentous thallus of a fungus. It may be colourless i.e., hyaline or coloured due to presence of pigments in cell wall. The mycelium may be ectophytic or endophytic.



TYPES OF FUNGAL THALLI:

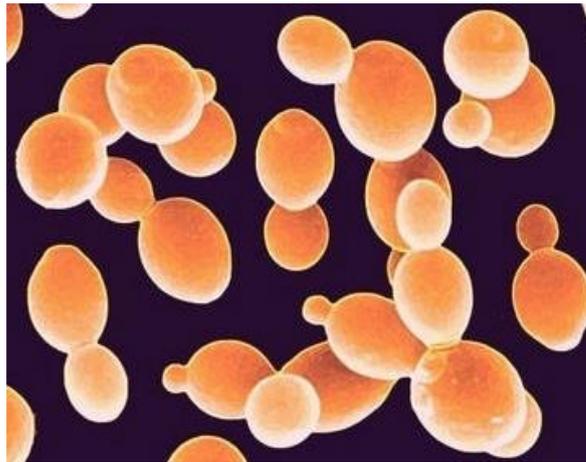
1. Plasmodium (plasma = moulded body): It is a naked, multinucleate mass of protoplasm moving and feeding in amoeboid fashion .

Eg. *Plasmodiophora brassicae*.

***Plasmodiophora brassicae* in host cell**



2.Unicellular thallus: consisting of a single cell. Eg.Chytrids, Synchronium



3.Multi cellular or filamentous thallus: Majority of fungi i.e., a true fungi are filamentous, consisting of a number of branched, thread like filaments called hyphae.Eg.Many fungi,Alternaria.

Fungi based on reproductive structures:

Holocarpic (holos=whole+karpos=fruit): If the thallus is entirely converted into one or more reproductive structures, such thallus is called holocarpic thallus

. Eg.Synchronium

Eucarpic(Eu=good+karpos=fruit):If the thallus is differentiated into a vegetative part which absorbs nutrients and a reproductive part which forms reproductive structures, such thallus is called eucarpic thallus.

Eg.Pythium

Ectophytic fungus:

If the fungal thallus is present on the surface of the host plant, it is called ectophytic

.Eg. Oidium .

Endophytic fungus:

If the fungus penetrates into the host cell / present inside the host, it is called endophytic.

Eg. Puccinia. Endophytic fungus may be intercellular (hypha grows in between the cells), or intra cellular (hypha penetrates into host cell).

Eg.Ustilago, or vascular (xylem vessels) Eg. *Fusarium oxysporum*

Inter cellular hyphae produce special organs called haustoria which penetrate the host cell and absorb food. These are absent in intracellular hyphae.

Endophytic intra cellular mycelium absorb food directly from protoplasm with out any specialized structures. In ectophytic mycelium, haustoria are produced in epidermal cells.

Septation in Fungi :(septum=hedge/partition) (pl.septa)

Some fungal hyphae are provided with partitions or cross walls which divide the fungus into a number of compartments /cells. These cross walls are called septa.

Aseptate hypha/coenocytic hypha: (Koinos=common,kytos=hollow vessel)

A hypha with out septa is called aseptate /non-septate/ coenocytic hypha wherein the nuclei are embedded in cytoplasm.Eg. lower fungi like Oomycetes and Zygomycetes.



Septate hypha:

A hypha with septa or cross walls is called septate hypha.

Eg.common in higher fungi like Ascomycotina, Basidiomycotina and Deuteromycotina



General types of septa:

1. Based on formation:

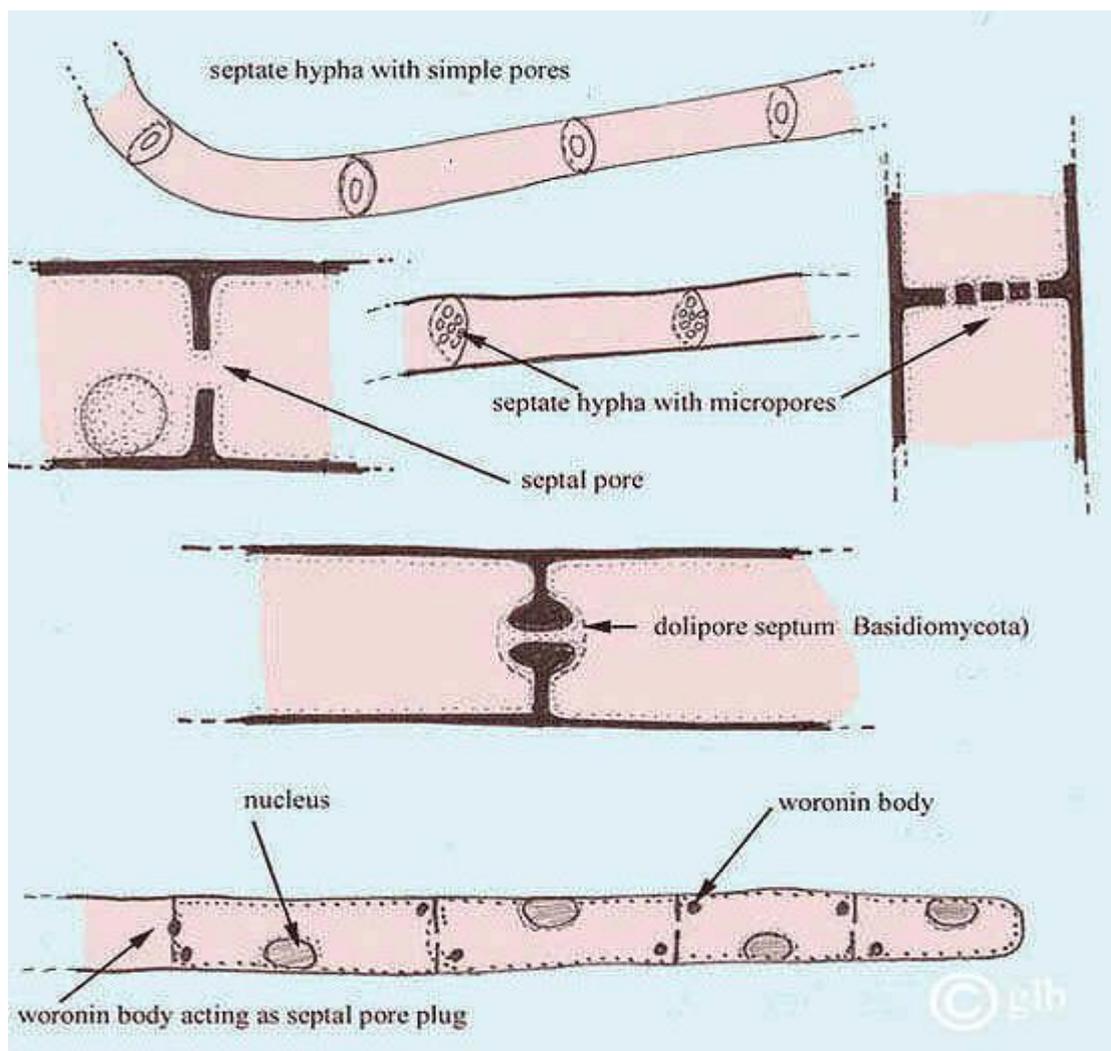
a) Primary septa: These are formed in direct association with nuclear division (mitotic or meiotic) and are laid down between daughter nuclei separating the nuclei /cells. Eg. Higher fungi like Ascomycotina and Basidiomycotina.

b) Adventitious septa: These are formed independent of nuclear division and these are produced to delimit the reproductive structures. Eg. lower fungi like Oomycetes and Zygomycetes in which septa are produced below gametangia (sex organs) which separate them from rest of the cells.

2. Based on construction:

a) Simple septa: It is most common which is a plate like, with or without perforation.

b) Complex septa: A septum with a central pore surrounded by a barrel shaped swelling of the septal wall and covered on both sides by a perforated membrane termed the septal pore cap or parenthosome. Eg. Dolipore septum in Basidiomycotina.



3. Based on perforation:

a) Complete septa: A Septum is a solid plate without any pore or perforations. Eg. Adventitious septa in lower fungi.

b) Incomplete septa: A septum with a central pore

History of Plant --- Pathology



Ancient period

A literature of European and vedic eras will give us some information on the plant diseases and their control measures. Greek philosopher **Theophrastus** recorded some observations on the plant diseases in his book enquiry into plants. His experiences were mostly based on imagination and observation but not on experimentation. He had mentioned that plants of different groups have different diseases which were autonomous or **spontaneous**, i.e., no external cause was associated.

In India, the information on plant diseases is available in ancient literature such as **rigveda**, **atharveda (1500-500BC)**, **arthasashtra of Kautilya (321-186 BC)**, **Sushruta samhita (200-500AD)**, **Vishnupuran (500AD)**, **Agnipurana (500-700AD)**, **Vishnu dharmottar (500-700AD)**, etc. In Rigveda, not only the classification of plant diseases has been given but the germ theory of disease was also advocated.

Vriksha ayurveda by Surpal in ancient India is the first book in which lot of information on plant diseases is available. In this book, plant diseases were categorized into two groups, **internal** (probably physiological diseases) and **external** (probably infectious diseases). External diseases were supposed to be **due to attack of microorganisms and insects**. In this book, a mention of treatments for different diseases caused by different agencies was prescribed which were based on superstition as well as scientific observation. Hygiene, tree surgery, protective covering with pastes and special culture of plants are practices which are still recommended. In chemical treatments, use of honey, ghee, milk, barley flour, pastes made from herbs, plant extracts, etc., were recommended. For the control of root diseases, oilcakes of mahuva, mustard, sesame, castor, etc., were used.

Symptoms of plant diseases such as rust, downy mildew, powdery mildew and blight are often mentioned in the **bible**, **Shakespeare's poems and dramas** of other Christian literature. In **Jataka of Buddhism**, **Raghuvansh of Kalidas** there was also a mention about different symptoms of plant diseases. In Europe and other western countries, after the time of Theophrastus (about 286 BC) no definite opinion could be formed about plant disease for the next 2000 years. In ancient period, the plant diseases were attributed to many causes which include divine power, religious belief, superstition, effect of stars and moon, bad wind and wrath of god, etc.

PRE-MODERN PERIOD

PIER ANTONIO MICHELLI (Italian)

- ❑ He was the **founder and father of Mycology**.
- ❑ He was the first person who observed fungal spores for the first time and conducted many spore germination studies (by growing fungus organisms on freshly cut pieces of melons and pears).
- ❑ In 1729 he published a book “**Nova Plantarum Genera**” in which he gave descriptions about 1900 species in Latin out of which 900 were fungi.



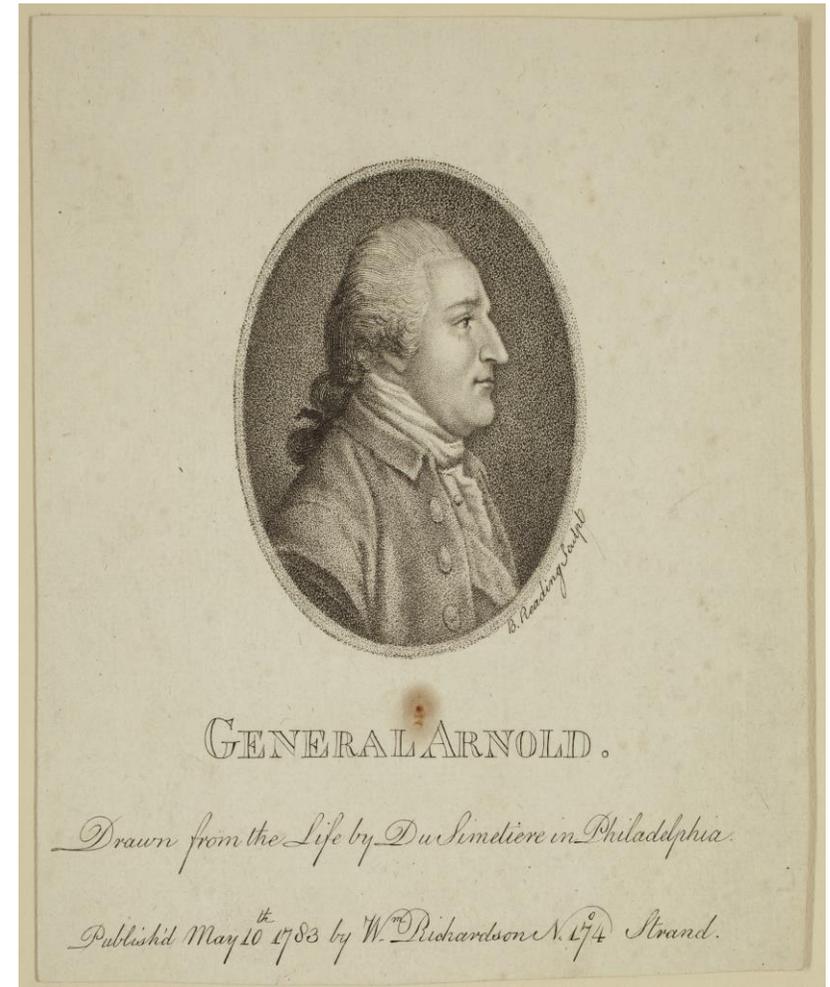
TILLET (French)

- ❑ In 1755, he published a **paper on bunt or stinking smut** of wheat.
- ❑ By well planned experiments he proved that wheat seeds that contained black powder on their surface produced more diseased plants than clean seeds.
- ❑ He emphasized that bunt was an infectious disease and it was closely related with fungus. However, he believed that the disease was caused by some toxin produced by the black powder. He did not know that the black powder contained the spore mass of the fungus.
- ❑ He reported that the chemical treatment of seeds with common salt and lime inhibited the contagious activity.

Modern period

BENEDICT PREVOST (French)

- ❑ He proved that diseases are caused by micro-organisms .
- ❑ He studied wheat bunt disease for about 10 years and in 1807, he published his findings in the paper “**memoir on the immediate cause of bunt or smut of wheat and of several other diseases of plants and on preventives of bunt**”.
- ❑ He proved that the bunt of wheat was caused by the fungus *Tilletia caries*.
- ❑ Discovered the life cycle of bunt fungus
- ❑ He showed that the solution containing copper sulphate prevented the germination of bunt spores and can be used for control of bunt diseases.



CHRISTIAN HENDRICK PERSOON (1761-1831)

- ❑ Persoon first published **observations Mycologicae**.
- ❑ In 1801, he published “**Synopsis methodica fungorum**” for nomenclature of *Ustilaginales, Uredinales and Gasteromycetes*.
- ❑ He also published **Mycologica Europica** in 1822.
- ❑ He gave the name to rust pathogen of wheat as *Puccinia graminis*.

ELIAS MAGNUS FRIES (1821)

- ❑ He published three volumes of “**Systema Mycologium**” for nomenclature of hymenomycetes.
- ❑ Person and fries first time introduced binomial system of nomenclature to classify the fungal organisms.

ANTON DE BARY (Germany)

- ❑ He was the **father and founder of modern Mycology**.
- ❑ In 1863, he studied the epidemics of late blight and renamed the casual organism as *Phytophthora infestans*.
- ❑ He discovered heteroecious nature of rust fungi (1865).
- ❑ He gave detailed account on life cycles of downy mildew genera.
- ❑ He studied about vegetable rotting fungi and damping off fungi.
- ❑ He wrote a book named **“Morphology and Physiology of fungi, lichens and Myxomycetes”** (1866).
- ❑ He reported the role of enzymes and toxins in tissue disintegration caused by *Sclerotinia sclerotiorum*



E. J. Butler (Edwin John Butler)

- ❑ He was **the father of modern plant pathology and father of Indian Mycology.**
- ❑ Monograph: Pythiaceae and allied fungi.
- ❑ Books: a) Fungi and Disease in Plants (1918)
b) Fungi in India (with B.R.Bisby) and
c) Plant Pathology (with S.G.Jones).



E.C. STAKMAN

- ❑ He studies the variability in rust fungus. Contributed valuable information on physiological races of pathogen
- ❑ He concluded that due to continuous evolution of races and biotypes in the species of the rust fungus its pathogenic capability goes on changing and as a result the resistant capability of the host also changes.

T. J. BURRUILL (USA)

- ❑ He proved for the first time that fire blight of apple and pear was caused by a bacterium (now known as *Erwinia amylovora*)

E.F.SMITH (U.S.A)

- ❑ He gave the final proof of the fact that bacteria could be incitants of plant diseases.
- ❑ He also worked on the bacterial wilt of cucurbits and crown gall disease.
- ❑ He is also called as "Father of Phytobacteriology".
- ❑ In 1981, he demonstrated for the first time that budding or grafting could be another method of transmission of plant viruses.
- ❑ He showed the contagious nature of peach yellows.

DOI AND ISHIE (JAPANESE)

- ❑ They found that mycoplasma like organisms (MLO) could be responsible for the disease of the yellows type.
- ❑ Doi observed that MLO's are constantly present in phloem while Ishie observed MLO's temporarily disappeared when the plants are treated with tetracycline antibodies.

BEIJERINCK (Dutch)

- ❑ **Founder of virology.**
- ❑ He proved that the virus inciting tobacco mosaic is not a living microorganism.

W.H.STANLEY

- ❑ In 1935, he proved that viruses can be crystallised. He got Nobel Prize.
- ❑ He treated the sap from diseased leaves of tobacco with ammonium sulphate and obtained a crystalline protein which, when placed on healthy tobacco leaves, could reproduce the disease.
- ❑ He finally proved that viruses are not living microorganisms because no living form can be chemically treated and crystallized and still remain viable.



INDIAN SCIENTISTS

1) B.B MUNDKUR: He worked on the **control of cotton wilt** diseases. He is responsible for the identification and classification of large number of Indian smut fungi He started Indian Phytopathological Society in 1948 and published a journal Indian Phytopathology. His book – Fungi and Plant diseases.

2) J.F.DASTUR: First Indian plant pathologist who was credited for his detailed studies on fungi and plant diseases. He studied the characters of *Phytophthora* and *Phytophthora* diseases of potato and castor. He established *Phytophthora parasitica* from castor.

3) K.C. MEHTA – Life cycle of cereal rusts in India

4) T.S. SADASIVAN - Started the studies on bio-chemistry of host-parasite relationship at University of Madras .Contributed to the concept of vivotoxins .Studied on mechanism of wilting in cotton by *Fusarium vasinfectum*. The production of fusaric acid by this fungus outside the host was demonstrated.

5)M.K PATEL: started plant bacterial research at pune. – 1948. Reported about 40 bacterial plant diseases. Introduced post of “ plant bacteriologist “ at IARI, in 1955

6)N.J. Nayulu- rice viral disease

7)Srinibasan- reported on BLB for the first time in India

8)Y.Y.Neni- book fungicide in plant disease control

9)G.Rangaswami- worked on bacteriology and mycology and book “Disease of crop plant in India”

Socio-economic significant of plant disease

Late blight of potato

Cereal rust

Bengal famine

Coffee rust

Thank you..

INTRODUCTION TO PLANT PATHOLOGY

Plant Pathology/Phytopathology (Phyton= plant, Pathos=suffering, ailment, Logos=study / discourse/knowledge) ie., the study of nature, development and management of plant diseases.

Definition: A branch of Agricultural science which deals with cause, etiology, resulting losses and management of plant diseases.

Importance of plant pathogens:

- 1.Plant diseases caused by micro organisms are of paramount importance to humans because they damage plants and plant products on which human depend for food, clothing, furniture and housing.
2. Millions of people all over the world still depend on their own plant produce for their survival.
3. Plant diseases reduce the quality and quantity of plant produce. Eg.Wheat bunt caused by *Tilletia* sp.
4. Results in increased prices of products to consumer.
5. Results in severe pathological effects on humans and animals that eat plant products.
6. Destroy beauty of environment by damaging plants around home, park, streets and forests.
7. The pesticides used to control disease, pollute the water and environment.
8. Reduce crop yields.
9. Cause financial loss ie., the money spent for plant protection chemicals.
- 10.Changes agricultural pattern.
- 11.Influences the industries ie.,lack of raw material .
- 12.Some plant diseases even change food habits of human population.

Examples of serious diseases that lead to famines:

- Irish famine (1845) – late blight of potato by *Phytophthora infestans* destroyed million hectares of potato fields thus people switched over to other food crops.
- Bengal famine - *Bipolaris oryzae* (1942), West Bengal, India
- Coffee rust - *Hemileia vastatrix* (1868), Srilanka
- Wheat rust - *Puccinia graminis f.sp.tritici* (1940) U.S.A
- Southern corn leaf blight - *Helminthosporium maydis*, U.S.A

Definitions:

1. **Disease** is a malfunctioning process that is caused by continuous irritation which results in some suffering producing symptoms (American Phytopathological society & British Mycological society).

2. **Disease** is an alteration in one or more of the ordered sequential series of physiological processes culminating in a loss of coordination of energy utilization in a plant as a result of continuous irritation from the presence or absence of some agent or factor.

3. **Disease:** Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

Pathogens bring about these irritating processes through different but inter-related pathways

1. by utilizing the host cell contents,
2. by causing death of cells or by interfering with their metabolic activities through their enzymes, toxins and growth regulators,
3. by weakening of tissues due to continuous loss of nutrients, and 4. by interfering with translocation of food, minerals and water.

OBJECTIVES OF PLANT PATHOLOGY: The science of plant pathology has four main objectives:

1. to study the living, non-living and environmental causes of plant diseases,
2. to study the mechanisms of disease development by pathogens,
3. to study the interactions between the plants and the pathogen, and
4. to develop the methods of controlling the diseases and reducing the losses caused by them.

TAXONOMY AND NOMENCLATURE OF FUNGI

Taxonomy : The science of classification. It is concerned with principles of classification.

Classification: Grouping of organisms into classes, orders, families, genera, species etc.

Nomenclature : Art of naming living organisms.

Importance of taxonomy and nomenclature

; 1. for study of fungi

2. for scientific communication between mycologists and plant pathologists throughout the world.

Binomial system of nomenclature was originally introduced by Carl Linnaeus for higher plants. Later, this classification was adopted to fungi by his students C.H. Persoon and E.M. Fries.

Some important rules of nomenclature :

1. According to International code of Botanical Nomenclature, the names of organisms should be binomial i.e., 2 parts. The first part is noun designating genus and the first letter of the genus name should be in capital. The second name is often an adjective, describing the noun which denotes the species, and the first letter should be in small letter. Eg. *Puccinia graminis*.

2. Binomials are usually derived from Greek or Latin.

3. Binomials when hand written should be underlined and when printed italicised. Eg.. Puccinia graminis (hand written) *Puccinia graminis* (printed)

4. Citation of authors name: The full name or abbreviation name of scientist who described fungus first, follows the species name. Eg. *Puccinia graminis* Persoon or Pers.

5. Citation of two authors names: If name of species is transferred to another genus from original (*Botrytis infestans*), the name of first author who first described species must be kept in parenthesis followed by name of second author who gave present status of species. i.e., *Phytophthora infestans*. Eg. *Phytophthora infestans* (Mont.) de Bary.

6. The taxa (groups) used in classification are Kingdom, Division, Class, Order, Family, Genus and Species. Each category may be sub divided into sub groups like Sub- Division, Sub- Class, Sub- Order.

7. Species is the unit of classification or basic taxonomic category (taxon).

8. Species some times broken into variety / *formae speciales* (f.sp.) and varieties into races and races into biotypes.

Standard endings of TAXA :

Division ends with mycota

Sub- Division ends with mycotina

Class with mycetes

Sub- class with mycetidae

Order with ales

Family with aceae

No special ending for genus and species.

TAXA:

Kingdom

Division

Sub-division

Class

Sub-class

Order

Sub-order

Family

Genera

Species

Eg. *Puccinia graminis tritici* race 1

Kingdom :Fungi

Division : Eumycota

Sub-division : Basidiomycotina

Class : Teliomycetes

Order : Uredinales

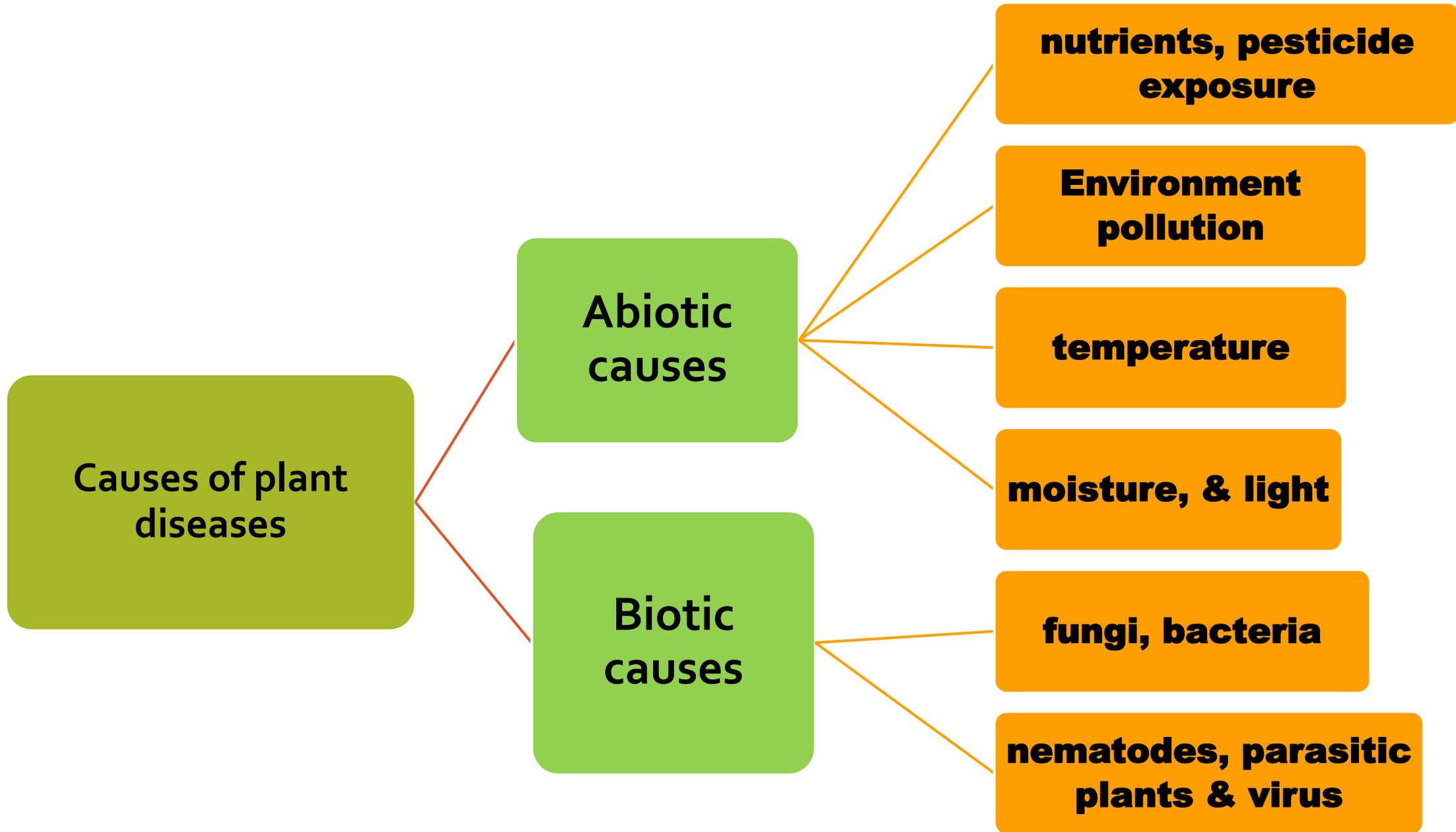
Family : Pucciniaceae

Genus : Puccinia

Species : graminis

Variety : tritici

Causes of plant diseases

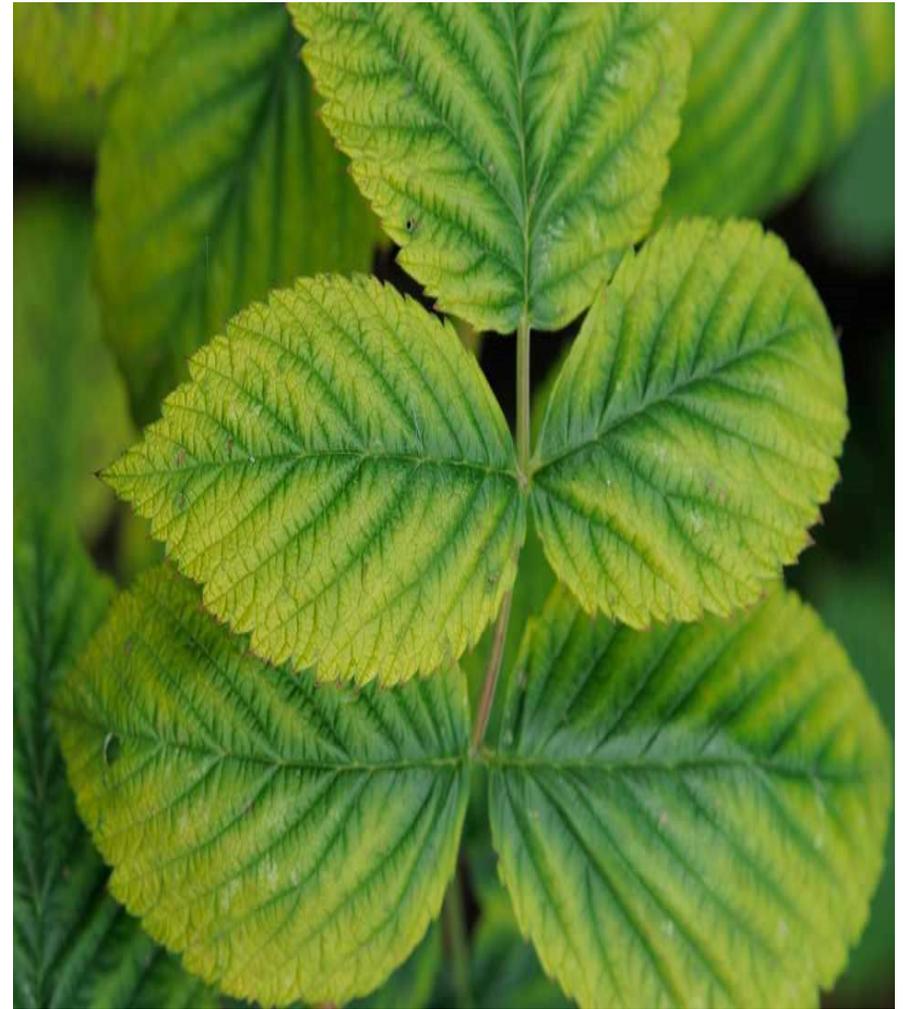


1) Nutritional abnormalities

- Often nutrient abnormalities show up as discoloration of foliage
- Common discoloration symptoms include:
 - a) Yellowing
 - b) Chlorosis
- Two types
 - a) Nutrient deficiencies
 - b) Mineral toxicity

a) Nutrient deficiencies

- ❑ Plants require several major (N,P,Ca,Mg) and minor (iron,copper,Zn) elements for normal growth.
- ❑ Deficiency or lack of any of these essential nutrients results in disease symptoms in the plant.
- ❑ The symptoms in this photo occur due to a lack of molybdenum (minor plant nutrient)



b) Mineral Toxicity

- Presence of excessive available amounts of certain minerals in the soil can lead to mineral toxicity to the plants.
- The extent of injury depends on the mineral, its concentration & species of the plant.
- The plant in this photo has received excess manganese, which has proved toxic and led to yellowing.



2) Pesticide Exposure

- ❑ Some pesticides , if improperly used can cause serious damage to plants.
- ❑ However, the most common type of chemical injury to plants is due to soil residue or spray drift of herbicides.
- ❑ Examples of pesticide toxicity are:
2,4-D damage to beans and tomatoes
- ❑ Glyphose (Roundup) damage to fruit trees
The common symptoms of herbicide exposure are curling and cupping.
- ❑ This plant was exposed to the herbicide 2,4-D



**Curling and cupping
of leaves**

3) Environmental Pollutants

- High level of fluoride in water or fluorine gas in the air can cause symptoms like this.
- The damage is concentrated towards the margins of the leaf where fluorine tends to accumulate.

E.g. Air pollution

Air pollution

- Certain chemicals such as O₃, SO₂ and NO₂ are released into the air from factories, power plants and automobile exhausts.
- These chemicals can accumulate in the atmosphere insufficient concentration to cause damage to plants.
- Ozone damage appears in the form of chlorosis, spots and bleaching of young leaves.
- This common in certain regions of the country where there is a high ozone concentration in smog
- Some of the air pollutants responsible for acid rain cause damage to vegetation in certain regions.





- Ozone damage on morning glory



- Ozone damage or marijuana leaves



4) Extreme Weather Conditions

- Extreme of weather can also lead to plant injury.

Cold injury

- Low temperature, like frost or freeze can damage the exposed or sensitive organs (buds, flowers, young fruits) or may kill the entire plant.
- As the new branch tips began to expand, a period of cold was severe enough to kill the growing tips.



Heat injury

- In this case the temperature was high enough and damage the plant tissue
- When plants or plant parts are exposed to high temperature for prolonged periods, symptoms of scorching or scalding may develop.



5) High/low soil moisture

- Due to excessive watering poor drainage or flooding may cause plants to turn yellow and be stunted.
- Potted indoor plants may show poor development or root rots.
- At the other extreme, low moisture or drought conditions can lead to poor development, wilting and death of plants.

6) high/low intensity

- High light intensity is usually not a problem but low light conditions, especially for indoor plants, lead to etiolation (tissue are yellowish)
- A normal and etioled shoot under sun and artificial lighting.

Thank you . . .

Pathogenesis

Chain of more or less distinct event occurring in succession and leading to development and perpetuation of a disease and pathogen is called pathogenesis or disease cycle.

The disease cycle is close to the life cycle of pathogen, but it refers primarily to the appearance, development and perpetuation of diseases as a function of pathogen rather than to the pathogen itself.

The disease cycle involves changes and its symptom in the plant as well as changes in the pathogen and spans within a growing season and from one growing season to another.

The disease cycle or infection chain can be of two types:

- i) *Continuous infection chain*: In this type of disease cycle, the pathogen remains continuously active form from one host to another. Most viral and some fungal diseases come under this category.
- ii) *Intermittent infection chain*: In this type disease cycle, pathogen survives in dormant state or as active saprophyte to maintain the continuity of chain after the harvest of crop. Most of fungal, bacterial and nematodes diseases fall under this category

Diseases with intermittent infection chain are further of two types:

- a) *Monocyclic diseases*: In such diseases only one cycle of disease is completed in one season and there is no repetition of disease cycle within a season. Such diseases are also called simple interest diseases. Eg. Loose smut of wheat and covered smut of barley etc.
- b) *Polycyclic diseases*: In such diseases many cycle of a disease are repeated within the same crop season on host through spore-infection-spore chain. Such pathogens are responsible for epidemics if the environmental condition favors the disease. Such disease are called compound interests disease. Eg. Rust, powdery mildew, late blight of potato, apple scab etc.

Depending upon the host plants involved, infection chains can be:

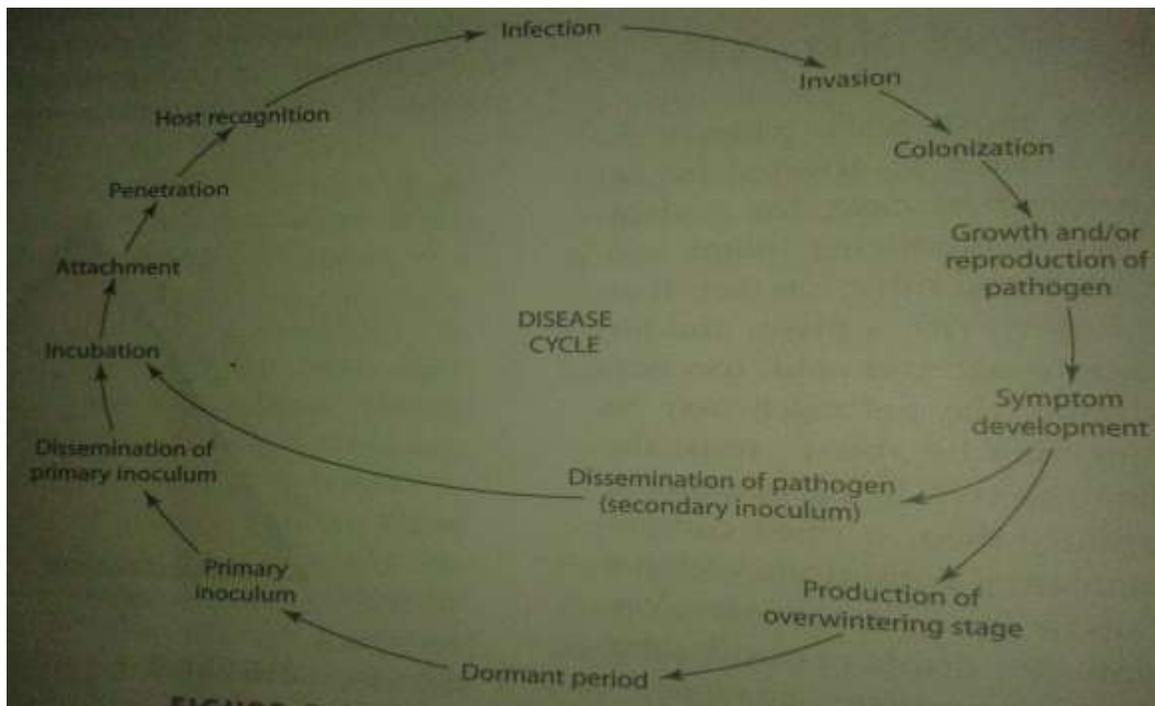
- a) *Homogenous*: when the pathogen survive only one genus of plants.
- b) *Heterogeneous*: when many plant species are involved in disease cycle

Disease developments in plants

For successful development of disease in plants it requires three components i.e. virulent pathogen, susceptible host and favorable environment. If any one of the components is absent there is no disease development.

Main events in disease cycle are

Inoculation, penetration, infection, invasion, colonization, growth and reproduction, dissemination of pathogen, survival of pathogens.



Inoculation

Inoculation is the initial contact of a pathogen with a site of plant where infection is possible.

The pathogen that lands on the host plant or is otherwise brought into contact with the plant is called inoculum.

In fungi, the inoculum may be spore, sclerotia, or fragment of the mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, the inoculum is always a whole individual of the respective organisms. In parasitic higher plants, the inoculum may be plant fragments or seed.

One unit of any pathogen is called a propagule.

Types of inoculum:

- *primary inoculum*: An inoculum that survive in winter or summer and cause original infection in spring or autumn on any is called the primary inoculum and the infection is called primary infections. Eg. Primary inoculum (Sclerotia, mycelium, oospore)

Generally, more the primary inoculum , more severe is the disease.

- *secondary inoculum*: An inoculum produced from primary infections is called secondary inoculum and the infection it causes are called secondary infections. Eg. Secondary inoculum (conidia, urdospores, zoospores).

Source of inoculum

- Fungi, bacteria, parasitic higher plants and nematodes generally produce their inoculum on the infected plants or their inoculum reaches the plant surface when the infected tissue break.
- Viruses, viroids, mollecutes , fastidious bacteria and protozoa produce their inoculum within the plants and the inoculum never reaches the surface naturally. So in case, the inoculum may transmitted from one plant to another through some kind of vector
- Soil: Bacteria, Rhizoctonia, Sclerotinia,
- Diseased debris: Alternaria, Phytophthora
- Alternate hosts: Rusts- wheat rust: *Barberis vulgaris*
- collateral hosts: Viruses: BCMV, PVY, Powdery mildews etc.

Landing and arrival of inoculum

- The inoculum of most of pathogen is carried to host plant passively by wind water and insects.
- Airborne inoculum reaches the host sometime by gravity or by washing with the rains. Some zoospore and nematodes may attracted toward the suger or amino acids diffusing out from the host.

Pre penetration phenomenon

Attachment of pathogen

- Mollecutes protozoa and most of virus are placed directly into the cell of plant by their vector and they are immediately surrounded by cytoplasm, cytoplasmic memberanes and cell wall.

- Fungi, bacteria and parasitic higher plants are first brought into contact or are attached with external surface of the plant before they colonize the host .
- The propagule of pathogen have mucilage substances and consisting of water soluble polysaccharides glycoproteins, lipid and febrile materials on their surface which get moistened become sticky and pathogen to adhere on plant surface.

Spore germination / seed germination and perception of host surface

- Spore germinates by producing a typical mycelium that infects and grows into host plants or they produce a short germ tube that produce aspecialized distinct form of mycelium called haustorium.

Formation of and maturation of appresorium or infection peg

- Appresorium is the swelling produced at the tip of germ tube which contain lipids, polysacchrides, proteins, and enormous glycerol.
- Apresria in certain cases produce a narrow penetration hypha also called infection peg or penetration peg from the base of appresorium and use primarily physical forces to puncture the plant cuticle with that penetration peg.

Recognition between host and pathogen

- It is assumed that when pathogen comes in contact with the host cells, an early events takes place that trigger a fairly rapid reponse in each organisms which either allows or impedes further growth of pathogen and development of disease.

Penetration

Fungi may penetrate in either indirect penetration or direct penetration; bacteria mostly enter through wounds and some time by natural openings and Viruses, viroid's, phytoplasma, RLOs etc. by mechanical means (wounds) and by vectors.

Direct penetration

- Through Appresorium (e.g. involved in direct penetration (eg. *Colletotrichum* spp. and *M. grisea*)
- Germtube e.g. (Apple scab- *V. inequalis*)
- Appresorium give rise to penetration peg

- Haustoria e.g. powdery mildews
- Infection cushion e.g. Fusarium, Sclerotinia – Includes fungi imperfecti & ascomycetes

Indirect Penetration

Penetration through natural openings

- Entry through Stomata e.g. Bean Rust – *Uromyces appendiculatus*
- Entry through hydathodes :Bacteria e.g. *Xanthomonas campestris* spv. *Oryzae*
- Nectothodes: *Erwinia amylovora* causing fire blight of apple & pear.
- Lenticels: *E. amylovora*

Infection

- It is the process by which pathogen establish contact with susceptible cell or tissue of the host and procure nutrients from them
- Successful infection results in the appearance of symptom, on the host plants. Some infection remain latent i.e don't produce symptom right away but at a later when the environmental conditions or the stage of maturity of plants become more favorable
- Symptom may be appear as soon 2-4 days after inoculation or as late 2-3 year after inoculation.
- killing the cells or at least not for long time, and still others kill cells and disorganize surrounding tissue.

Infection process is affected by various factors

- Resistance & susceptibility of the host
- Aggressiveness & virulence of the pathogen
- Environmental factors
- Host nutrition & pH
- Incubation period (depends upon host- pathogen combination, stage of host and environment etc.

Invasion

Invasion is the spread of the pathogen in the host. Most fungi spread into all the tissues of the plant organ (leaves, stem and roots) they infect, either by growing directly through cell as intracellular mycelium or by growing between the cell as an intercellular mycelium.

Different pathogens invade their host as

- Ectoparasite e.g. powdery mildew
- Endoparasites e.g. wilts, viruses etc
- Sub-cuticular pathogens (Apple scab- *Venturia inaequalis*) • Sub-epidermal pathogens (wheat rust)
- Vascular pathogens (*Pseudomonas solanacearum*) – Ecto-endo parasites e.g. potato canker (*Corticium solani*)

Growth and reproduction of the pathogen and their Colonization

a) Growth

- fungi and parasitic higher plants invade and infect tissues by growing. These pathogens continue to grow and branch out within the infected host indefinitely so that the pathogen spreads into more and more plant tissues until the spread of infection is stopped or plant is dead.
- bacteria, mollecutes, viroids, nematodes and protozoa do not increase in size with time. These pathogens invade and effect new tissues within the plant by reproducing at a rapid rate and increasing their number tremendously in infected tissues.
- Their progenies then are carried passively into new cells and tissues through plasmodesmata (viruses and virioids), phloem (viruses, virioids, mollecutes, some fastidious bacteria and protozoa) or xylem (some bacteria) or they move actively by their own power as in case of protozoa, nematodes and some bacteria

b) Reproduction

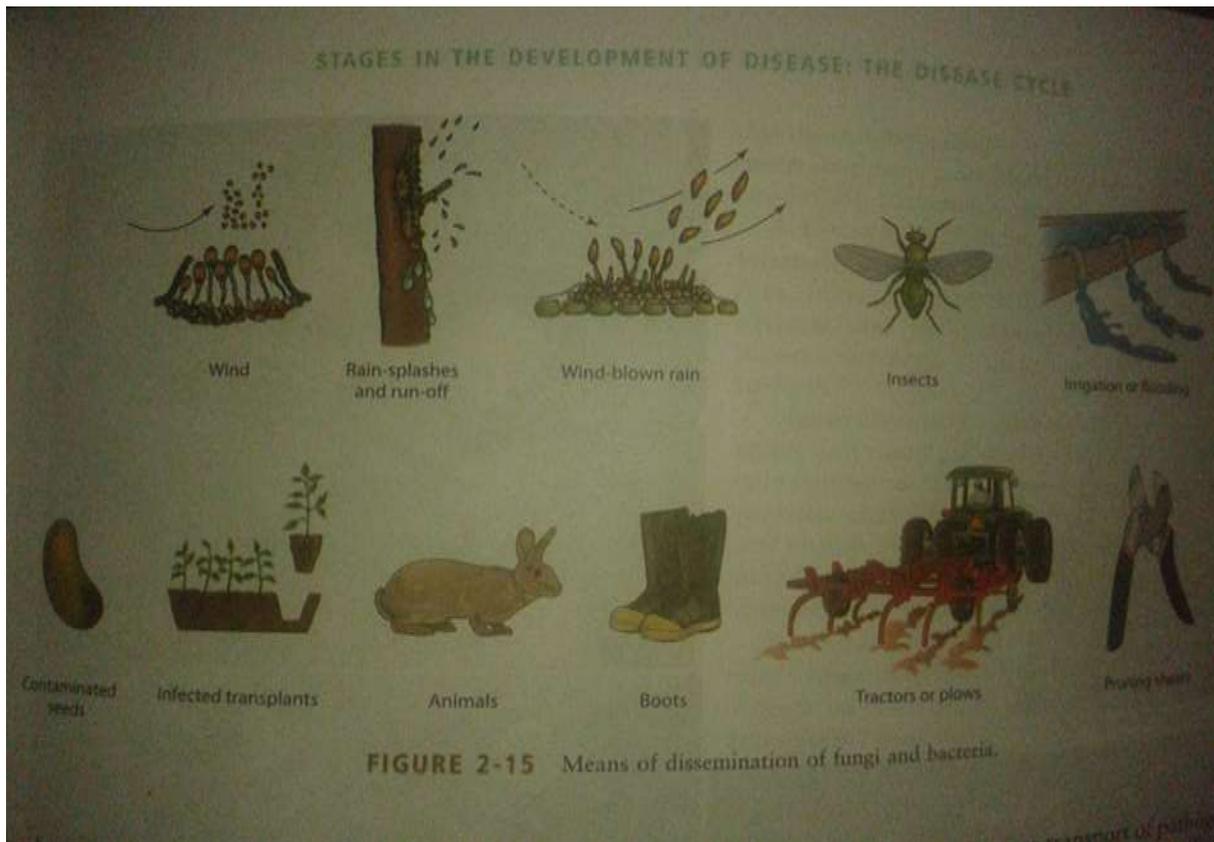
- Fungi reproduce by means of asexual or sexual spores.
- Some fungi produce tremendous spores in one growing season while, others produce them in successive crops. But in any case thousands of spores are produced per square centimetre of infected tissue.
- Parasitic higher plants reproduce by seeds.
- Bacteria and mollecutes reproduce by fission in which one mature individual splits into two equal smaller individuals.
- Under optimum nutritional and environmental conditions, the bacteria divide continuously in the host cells until the nutrients are exhausted..
- Viruses and viroids are replicated by the host cell.
- Nematode female's lay about 300 to 500 eggs and half of them produce females which again will lay the same number of eggs.

c) Colonization

- Majority of plant pathogenic fungi and oomycetes produce a mycelium only within the plants they infect.
- Few fungi and oomycetes produce a mycelium on the surface of their host plants, but most powdery mildew fungi produce a mycelium only on the surface of their hosts..
- Parasitic higher plants produce their seeds on aerial branches.
- Some nematodes lay their eggs at or near the surface of the host plants.
- Bacteria reproduce between the host cells or in xylem or phloem cells of the hosts and come to the surface only through wounds, cracks, stomata etc.
- Viruses, viroids, protozoa and fastidious bacteria reproduce only inside the cells and often do not reach at the surface of the host plant

Dissemination of the pathogen

Dispersal means spread of pathogen propagules from one plant to the another or one area to another area for short as well as long distance.



Dispersal may be: a) Active - move on own power bacteria (some) Pythium (Zoospores)
Fungi- spores expelled forcibly

pathogen disperse with seed- *xanthomonas compestris* pv. *Compestris* black leg of crucifers.

Passive - wind, water, insects, man, animals, machinery

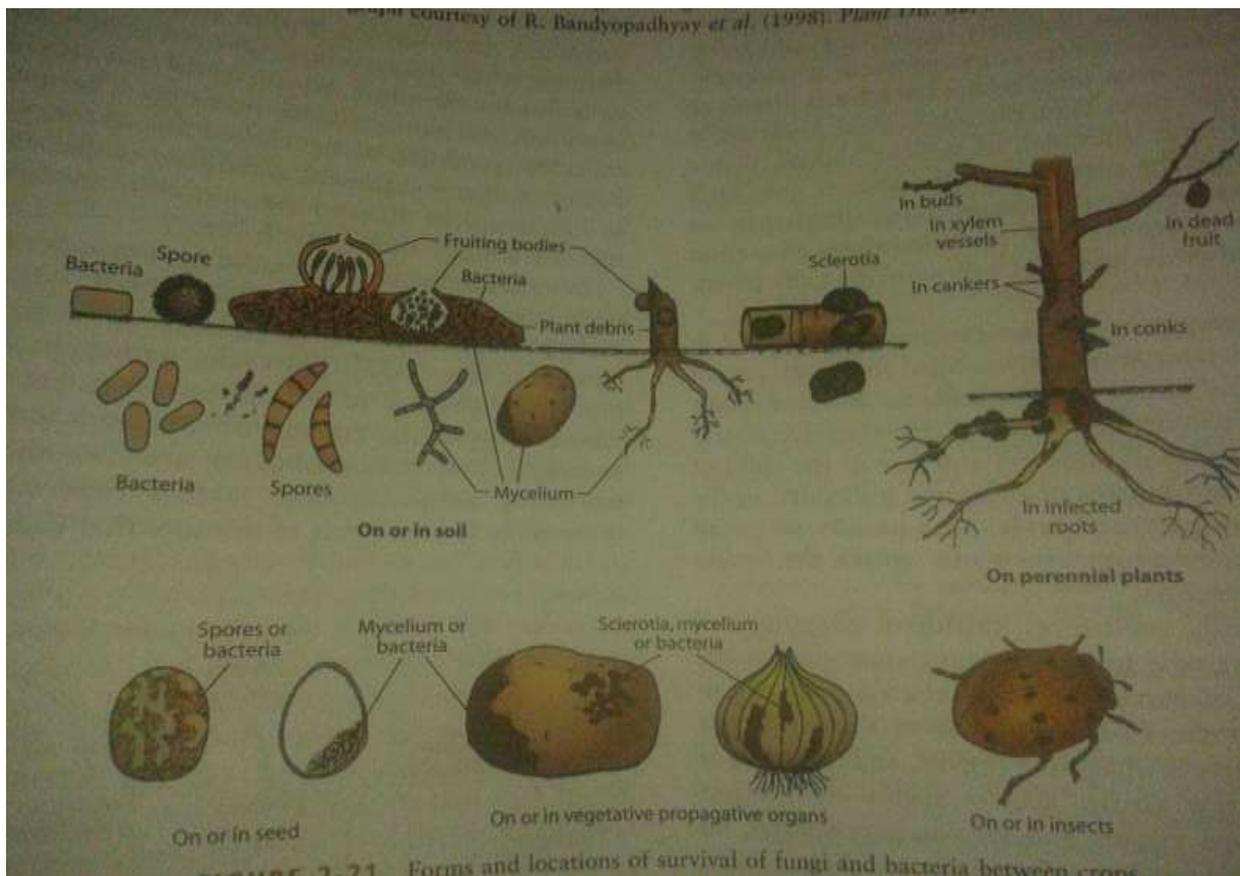
pathogen disperse with seed- *xanthomonas compestris* pv. *Compestris* black leg of crucifers.

Survival of pathogen in absence of host

Plant pathogens can survive in the absence host. They have evolved several mechanism to survive in too cold and dry summer.

In perennial plants on diseased tissue, canker, buds on fallen leaves, infected leaves and fruits.

In annual plants survive the winter or summer as mycelium ininfected plant debris.



- ✓ In infected crop debris : *Claviceps purpurea*
- ✓ In seed: *Ustilago tritici*
- ✓ In soil: *Pythium, Fusarium, Rhizoctonia* etc.
- ✓ On growing plants: *Monillinia*

- ✓ Infected material on host plant: *Erwinia amylovora*, *venturiinaequalis*.
- ✓ Alternate host: *puccinia graminis tritici*
- ✓ As dormant structures e.g. *sclerotia*, *chlamydospores*

References

- ✓ Plant pathology by G N Agriose
- ✓ Modern plant pathology H C Dube.
- ✓ Plant pathology by R S Singh

REPRODUCTION IN FUNGI

Reproduction is the formation of new individuals having all the characteristics of the species.

Types of reproduction:

1. Asexual /non-sexual / vegetative / somatic reproduction
2. Sexual reproduction.

1.Asexual reproduction :

Asexual reproduction stage is also known as imperfect stage and technically called as anamorphic stage. There is no union of nuclei /sex cells/ sex organs. It is repeated several times during the life span of a fungus producing numerous asexual spores. Hence, it is more important for fungi than sexual reproduction. Asexual spores are formed after mitosis ,hence also called mitospores

1. Fragmentation
2. Fission.
3. Budding
4. Sporulation (production of spores)

1. Fragmentation : It is the most common method. Hypha of fungus breaks into small pieces, each broken piece is called a fragment, which function as a propagating unit and grows into a new mycelium.The spores produced by fragmentation are called arthrospores (arthron=joint) (spora=seed) or oidia..

Eg. Oidium, Geotrichum.

Sometimes, the contents of intercalary cells or terminal cells of hypha rounded off and surrounded by thick wall and formed as **chlamydospores** which are thick walled resistant spores produced either singly or in chains.

Eg. Fusarium oxysporum, Ustilago tritici.

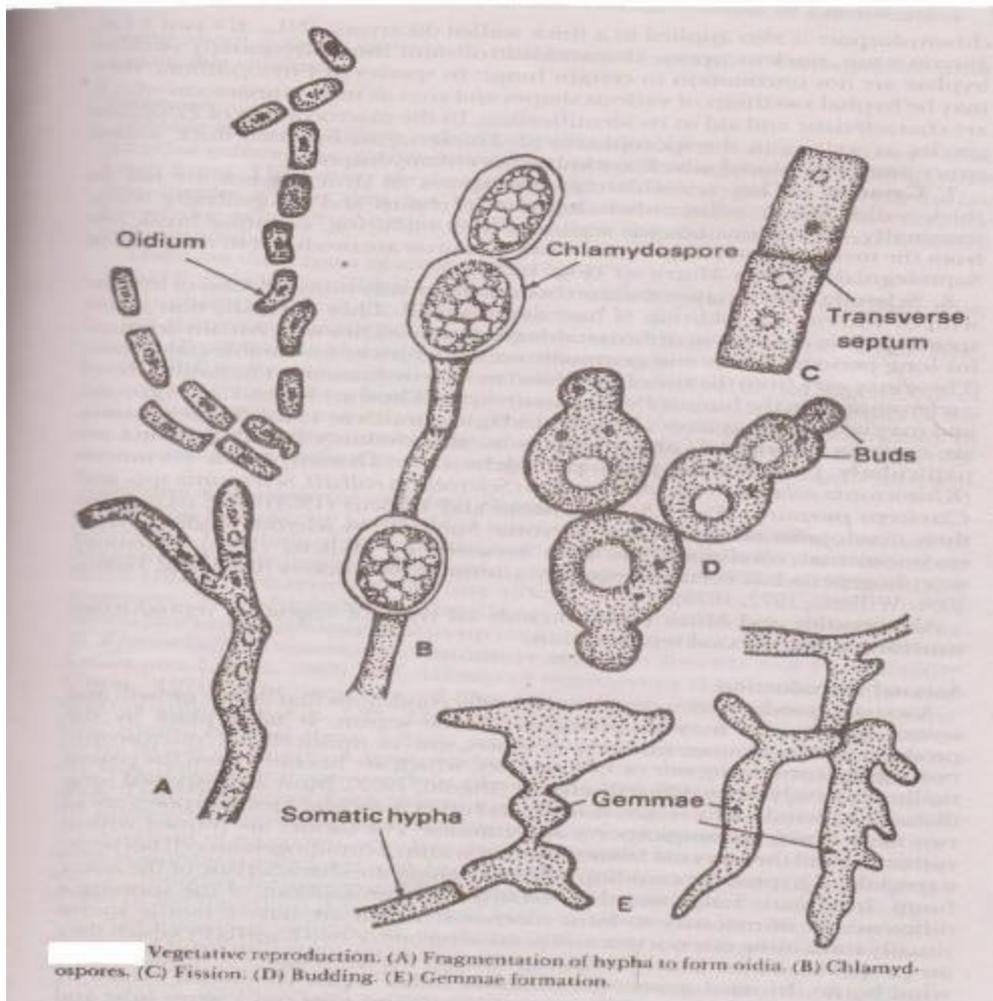
2. Fission / Transverse fission: The parent cell elongates,nucleus under go mitotic division and forms two nuclei, then the contents divide into equal halves by the formation of a transverse septum and separates into two daughter cells.

Eg.Saccharomyces cerevisiae.

3.Budding:

The spores formed through budding are called blastospores. The parent cell puts out initially a small out growth called bud / blastos ie.,sprout or out growth which increases in size and nucleus divides, one daughter nucleus accompanied by a portion of cytoplasm migrates into bud and the other nucleus remains in the parent cell. Later, the bud increases in size and a constriction is formed at the base of bud, cutting off completely from parent cell . Bud, when separated from parent cell, can function as an independent propagating unit.Sometimes multiple buds are also seen i.e., bud over bud and looks like pseudomycelium.

Eg. Saccharomyces cerevisiae.



4. Sporulation (spores):

The process of production of spores is called sporulation.

Spore: It is a minute, simple propagating unit of the fungi, functioning as a seed but differs from it in lacking a preformed embryo that serves in the reproduction of same species. Spores vary in colour, size, number of cells and the way in which they are borne.

There are 2 main types of spores.

1. Sporangiospores
2. Conidia

1.Sporangiospores:

When the asexual spores are produced internally, within the sporangia, such spores are called sporangiospores. The sac like structure which produces sporangiospores is called sporangium. The special hypha bearing sporangium is called sporangiophore which may or may not be distinguishable from hypha. A small sporangium with or without columella

containing a few or single spore is called as **sporangiolum**. Eg. Choanephora trispora. Sporangium which is cylindrical in shape is called as **merosporangium**. Eg. Syncephalastrum racemosum. Sporangium with columella is called as **columellate sporangium**. Eg. Rhizopus stolonifer.

Sporangiospores are of 2 types.

a. Zoospores /planospores

b. Aplanospores a. Zoospores / planospores

a. Zoospores /planospores

sporangiospores which are motile by flagella are called **zoospores**. Also known as **planospores**.

Eg. Pythium, Phytophthora.

b. Aplanospores: Sporangiospores which are non motile without flagella are called aplanospores Eg. Rhizopus stolonifer, Mucor.

Flagellation in fungi:

Flagella : (sing. flagellum) Flagella are thin, hair like delicate structures attached to a basal granule called blepharoplast in cytoplasm and these are the organs of motility in lower fungi and aquatic fungi.

Types of flagella : Flagella of zoospores are of 2 types.

a. Whiplash b. Tinsel

a) Whiplash : A flagellum with long, thick, rigid basal portion and with a short, narrow, flexible, upper portion. It gives a whip like appearance to flagellum.

b) Tinsel : It is a feathery structure consisting of a long rachis with lateral hair like projections called mastigonemes or flimmers on all sides along its entire length.

2. Conidia / Conidiospores: (konis=dust; oides=like)

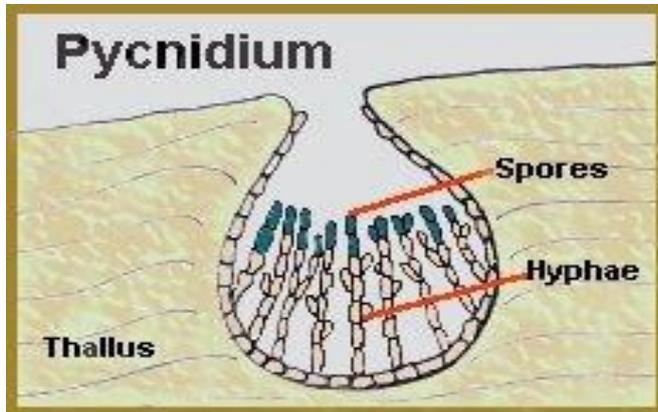
Conidia are non- motile asexual spores which may arise directly from somatic hyphae or from specialized conidiogenous cells (a cell from which conidia are produced) or on conidiophore (hypha which bear conidia). Conidia are produced freely on conidiophore ie., at the tips or sides of conidiophore or may be produced in specialized asexual fruiting bodies viz., pycnidium, acervulus, sporodochium and synnemata.

Asexual fruiting bodies :

(a) **Pycnidium:** (**pl. pycnidia**) It is a globose or flask shaped fruiting body lined inside with conidiophores which produce conidia. It may be completely closed or may have an opening called ostiole.

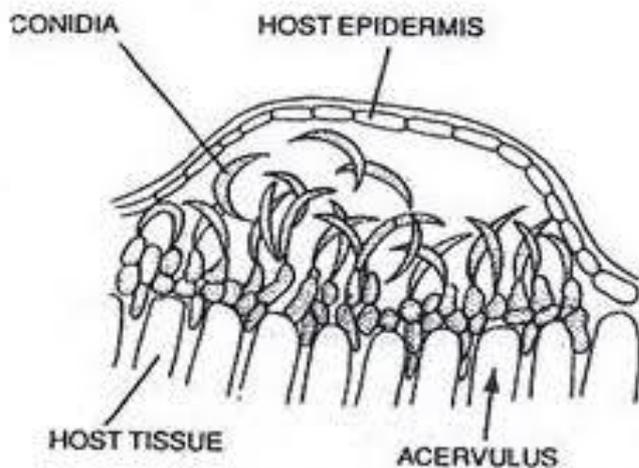
Pycnidium may be provided with small papillum or long neck.

Eg. Phomopsis.



(b) Acervulus: (pl.acervuli) A flat or saucer shaped fruiting body with a stromatic mat of hyphae producing conidia on short conidiophores. An acervulus lacks a definite wall structure and not having an ostiole or definite line of dehiscence. Intermingled with the conidiophores, setae (sing. seta; L. seta = bristle) are found. Setae are long, pointed, dark coloured, sterile structures.

Eg. Colletotrichum, Pestalotiopsis.



(c) Sporodochium : (pl.sporodochia)

A cushion shaped asexual fruiting body. Conidiophores arise from a central stroma and they are woven together on a mass of hyphae and produce conidia.

Eg. Fusarium.

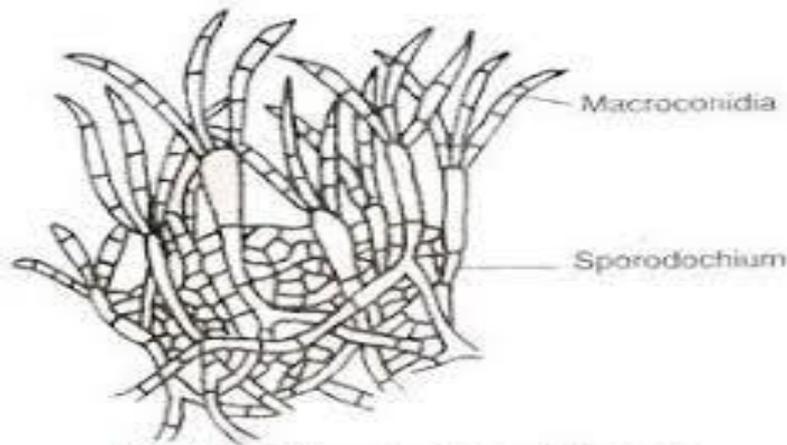
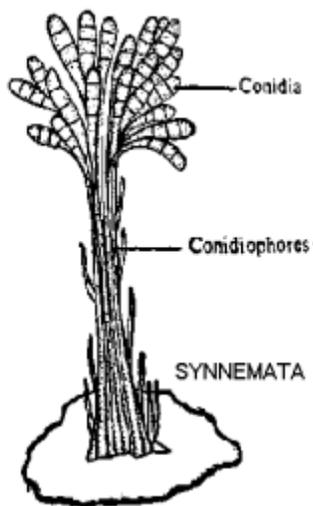


Fig. 22.21. Sporodochium of *Fusarium*

(d) Synnemata: (pl.synnema) A group of conidiophores often united at the base and free at the top. Conidia may be formed at its tip or along the length of synnema, resembling a long handled feather duster.

Eg. Graphium.



4. SEXUAL REPRODUCTION

Sexual reproduction involves union of two compatible nuclei or sex cells or sex organs or somatic cells or somatic hyphae for the formation of new individuals. Sexual stage is perfect stage and technically called as teleomorphic stage.

Sexual spores or sexual structures which contain sexual spores are thick walled, resistant to unfavourable conditions and are viable for longer period and thus these spores help the fungus to perpetuate from one season to another, hence these are called as resting spores. Sexual spores are definite in number.

Sex organs of fungi :

Gametangia: Sex organs of fungi are called gametangia containing gametes or gamete nuclei.

Gametes: Sex cells are called as gametes .

Antheridium: (**pl.antheridia**) Male gametangium is called as antheridium. Male gametangium is small and club shaped.

Oogonium / Ascogonium:(pl.oogonia/ascogonia):

The female gametangium is called Oogonium (oomycetes) or ascogonium (ascomycotina).Female gametangium is large and globose shaped. Male gametes are called antherozoids or sperm or spermatozoids. Female gametes are called egg or oosphere.

Classification of fungi based on sex :

1. Monoecious fungi / hermaphroditic fungi: (mono=single,oikos=home)

The fungi which produce distinguishable male and female sex organs on the same thallus, which may or may not be compatible are called monoecious/ hermaphroditic fungi. Eg. *Pythium aphanidermatum*.

2. Dioecious fungi: (di=two, oikos=home)

The fungi which produce distinguishable male and female sex organs on two different thalli ie., there will be separate male and female thalli . Eg. *Phytophthora infestans*.

Classification of fungi based on compatibility ;

Homothallic fungi: Fungi in which both sexes occur on same thallus, which can reproduce sexually by it self with out the aid of another thallus ie., self compatible / self fertile are called homothallic fungi.

Eg. *Pythium aphanidermatum*.

Heterothallic fungi:

A fungal species consisting of self sterile (self incompatible) thallus requiring the union of two compatible thalli for sexual reproduction, regardless of the possible presence of both male and female organs on the same thallus. Heterothallic fungi are dioecious

Eg. *Phytophthora infestans*..

Phases in Sexual reproduction:.There are 3 phases in sexual reproduction.

1. Plasmogamy: union of two protoplasts takes place.As a result of it the two nuclei come together within the same cell.

2. Karyogamy: union of 2 sexually compatible nuclei brought together by plasmogamy to form a diploid nucleus ($2n$) i.e., zygote.

3. Meiosis: This is reduction division. The number of chromosomes is reduced to haploid (n) i.e., diploid nucleus results into haploid nucleus.

In lower fungi (Phycomycetes-Mastigomycotina and Zygomycotina) plasmogamy, karyogamy and meiosis occurs at regular intervals / sequence i.e., karyogamy follows immediately after plasmogamy. In higher fungi (Ascomycotina, Basidiomycotina), karyogamy is delayed, as a result the nuclei remain in pairs (dikaryotic phase- $n+n$ condition), which may be brief or prolonged. **Dikaryon**: A pair of genetically different nuclei, lying side by side without fusion for a considerable period of time is called dikaryon. A cell containing **dikaryon** is called dikaryotic cell. And the process is known as **dikaryogamy**.

Methods of sexual reproduction :

1. Planogametic copulation. 2. Gametangial contact 3. Gametangial copulation 4. Spermatization 5. Somatogamy.

1. Planogametic copulation (gametogamy): This involves the union of 2 naked gametes one or both of which are motile.

a. Isogamy (Isogamous planogametic copulation) : If both gametes are motile and similar.

Eg. Plasmodiophora brassicae.

b. Anisogamy (Anisogamous planogametic copulation) : If both gametes are motile but dissimilar.

Eg. Allomyces macrogynus

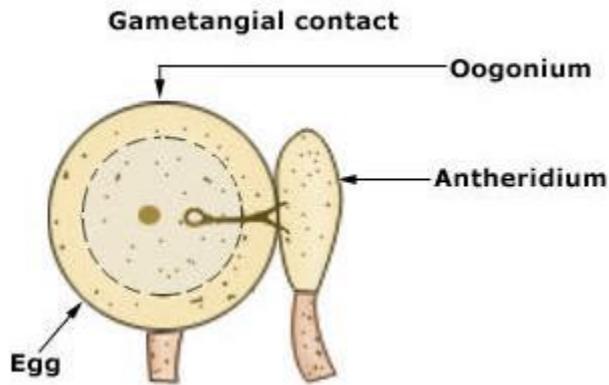
c. Heterogamy: If gametes are dissimilar, one motile, another is non motile.

Eg. Monoblepharis polymorpha.

2. Gametangial contact (gametangy / oogamy):

Male and female gametangia come in contact. At the place of contact, dissolution of wall occurs and a fertilization tube is formed. The contents of male gametangium migrate into female gametangium through a pore or fertilization tube developed at the point of contact. The gametangia do not lose their identity.

Eg. Pythium aphanidermatum.



3. Gametangial copulation (gametangiogamy):

The isogametangia come in contact, their intervening wall dissolves leading to fusion of entire contents of two contacting gametangia to form a single unit. Gametangia lose their identity. The protoplasts fuse and the unit increases in size.

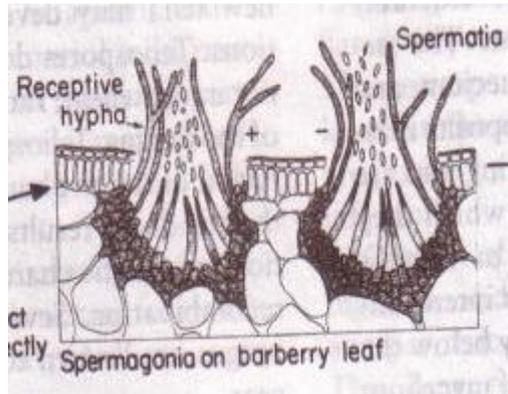
Eg. *Rhizopus stolonifer*.



4. Spermatization:

Minute, uninucleate male cells called as spermatia which are produced on spermatophores in a fruiting body (pycnium) are carried to female reproductive structures called receptive hyphae. Spermatia and receptive hyphae come in contact and contents of male spermatium migrate into female receptive hypha, thus making the cell binucleate. This process is called dikaryotization.

Eg. *Puccinia graminis tritici*



5.Somatogamy: Many higher fungi do not produce sex organs. In such cases somatogamy takes place. It is the union of 2 somatic hyphae or somatic cells representing opposite sexes to form sexual spores.

Eg. *Agaricus campestris*

Parasexual cycle / Parasexuality :

Parasexual cycle is a process in which plasmogamy, karyogamy and haploidisation (non meiotic process) takes place in a sequence but not at specified points in the life cycle of a fungus. It was first discovered in 1952 by Pontecorvo and Roper in *Aspergillus nidulans*, the imperfect stage of *Emericella nidulans*. It is of importance in heterokaryotic fungi (a fungi in which genetically different nuclei are associated in the same protoplast or mycelium). This is one of the methods of producing variability of fungal pathogens. In majority of Deuteromycotina, true sexual cycle is absent but derive many of the benefits of sexuality through this cycle.

Sexual fruiting bodies

a. Pycnium or spermatogonium (pl. pycnia; Gr. pycnos = concentrated) is a fruit body, which is similar to pycnidium and is formed in sexual cycle of rust fungi. Pycnia are produced from primary uninucleate mycelium growing in the tissues of the host. Pycnia may be flask-shaped, conical, flat and sprawling. The flask-shaped type is more typical, The mouth of the flask (called ostiole) is lined by a bunch of unbranched, tapering, pointed, orange coloured hairs called 'periphyses' (sing. periphysis; Gr. peri = around + physis = a being, a growth). The pycnial wall cells send many closely -packed, elongated, tapering, unbranched uninucleate sporogenous cells or spermatiphores (Gr. spermatation = little seed+ phoreus=bearer) in the cavity.

These spermatiphores give rise to a series of uninucleate spermatia (sing. spermatium Gr. spermatation=little seed) or pycnospores in a basipetal fashion. Pycnospore is a non-motile, uninucleate, unicellular spore-like male structure that empties its contents into a receptive female structure during plasmogamy. Pycnospores are variously regarded as gametes or gametangia. e.g. pycnium is produced by *Puccinia graminis tritici* in the alternate host, barberry (*Berberis vulgaris*).

b. Aecium **Aecium** (pl. **Aecia**; Gr. **aikia = injury**) is also formed during sexual cycle. Aecium is a shallow or deep cup-shaped structure produced in a leaf and located in the lower portion and break through the lower epidermis. The wall is known as ‘peridium’. In a young aecium that has not broken the host epidermis, the peridium surrounds the spore chain on all sides, forming a complete dome over them. When the aecium matures, the spore chains push through the roof of the peridium and the aeciospore are released. The torn peridium forms a lip-like structure around the aecial cup.

c. Ascocarps **Ascocarp** (Gr. **askos = sac+ karpos =fruit**) is a fruiting body that contains asci and ascospores. Ascomycetes fungi with few exceptions produce ascocarps. There are four categories ascocarps.

i. Cleistothecium: (pl. cleistothecia; Gr. kleistos = closed + theke = case)

Cleistothecium or cleistocarp is a closed ascocarp and has no ostiole. It is deep brown to black in colour, more or less spherical and often provided with appendages on its body, which serve as organs of anchorage and help in dissemination.

Cleistothecial appendages are of four types viz.

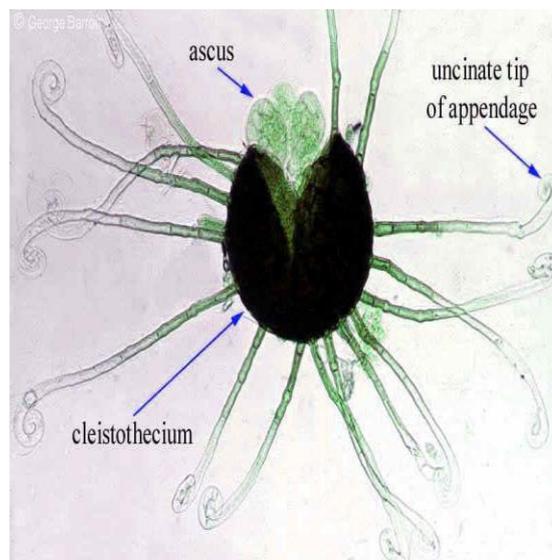
Myceloid appendages: Appendages resemble somatic hyphae in flaccidity and indefinite growth.

e.g. Erysiphe, Sphaerotheca and Leveillula.

Appendages are rigid, spear-like with a bulbous base and pointed tip. e.g. Phyllactinia.

Appendages are rigid with curved tips e.g. Uncinula, Pleochaeta.

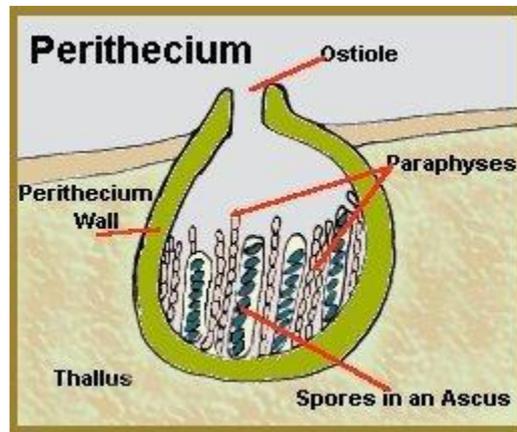
Appendages are rigid with dichotomously branched tips. e.g. Microsphaera, Podosphaera.



ii. Perithecium **Perithecium** (pl. **perithecia**, Gr. **peri = around + theke = a case**) is a flask-shaped ascocarp with a wall of its own. It is provided with a narrow ostiole and may possess

a short or a long neck through which the asci are released at maturity. The asci are intermingled with sterile filaments called paraphyses, which help the asci in with sterile filaments called paraphyses, which help the asci in nutrition and dispersal.

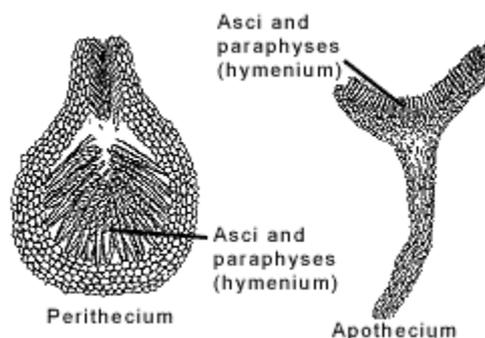
,Eg: Nectria, eratocystis, Podospora, Chaetomium, Xylaria, Ustulina, Rosellinia, Claviceps and Cordyceps.



iii. Apothecium **Apothecium** (pl. **Apothecia**; Gr. **apotheke**=store house) is an open ascocarp. It has a broad opening and is either cup or saucer shaped with asci arranged in a palisade layer within. It is usually fleshy or leathery in nature. An apothecium consists of three parts viz. hymenium, hypothecium and excipulum.

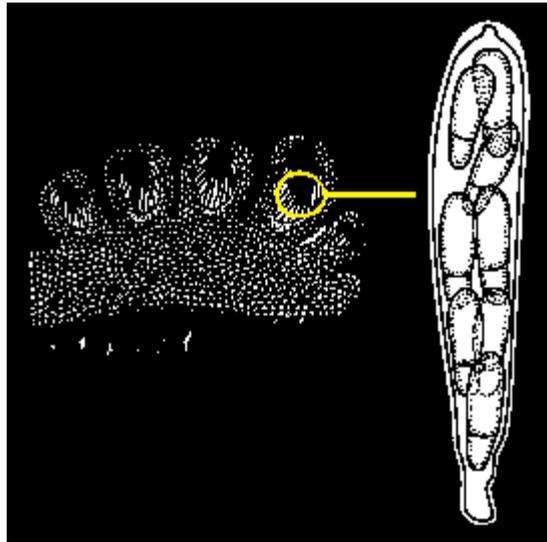
The hymenium is the layer of asci that lines the surface of hollow part of the disc, cup or saddle. The 'hypothecium'(pl. hypothecia; Gr. Hypo=under+ theke =a case) is a thin layer of interwoven hyphae, which is found immediately below the hymenium. The apothecium proper (i.e., the fleshy part of the ascocarp that supports the hypothecium and hymenium) is called excipulum (pl. excipula ; N.L. excipulum=receptacle)

Pyronema, Ascobolus, Peziza, Morchella, Sclerotinia etc



iv. Pseudothecium **Pseudothecium** or **ascostroma** (pl. **ascostromata**; Gr. **askos** = sac + **stroma** = **mattress, cushion**) like perithecium is a flask-shaped ascocarp provided with an ostiole through which the asci are discharged. . In pseudothecium asci are directly formed in a cavity (locule) within the stroma. The stroma itself thus forms the wall of the ascocarp

e.g. Cochliobolus, Pyrenophora, Ophiobolus, Pleospora, Leptosphaeria of the class Loculoascomycetes.



d. Basidiocarps **Basidiocarp** (Gr. **basidion**=small base + **karps** = **fruit**) is a fruiting body, which bears basidia and basidiospores. Basidia are borne on the under surface of fruit body. Basidia bear basidiospores exogenously usually on projections called sterigmata. Basidia are typically formed in definite layers called hymenium (pl. hymenia; Gr. hymen=membrane). Hymenium is composed of basidia and large sterile structures called cystidia (sing. cystidium; Gr. kystis =bladder + -idion = dimin. Suffix). They may vary in size from microscopic to a metre or more in dia. Most fungi in basidiomycotina except smuts (Ustilaginales) and rusts (Uredinales) form basidiocarps. They include mushrooms, (Agaricus, Pleurotus, Volvariella), shelf fungi, coral fungi.

Different types of sexual spores :

Sexual spores are formed after meiosis, hence also called meiospores. 1.Oospores
2.Zygosporos 3.Ascosporos 4.Basidiosporos

1.Oospore: A thick walled sexual resting spore produced by the union of two morphologically different gametangia



Eg. Pythium, Phytophthora, members of class Oomycetes.

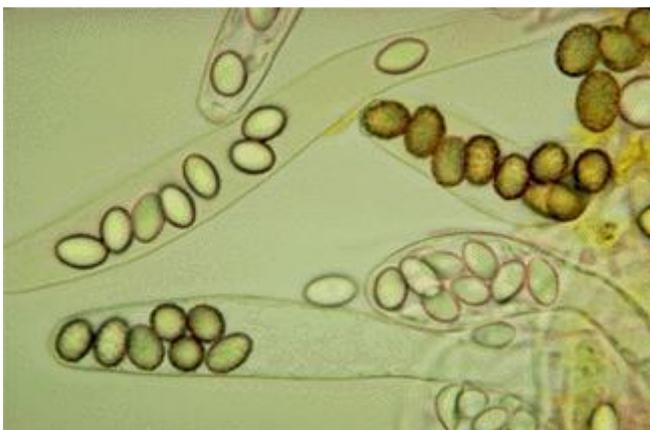
2. Zygospore: A thick walled sexual resting spore produced by the fusion of two morphologically similar gametangia.

Eg. Rhizopus, members of sub-division Zygomycotina



3. Ascospore:

Sexual spore produced in a specialized sac like structure known as ascus. Generally 8 ascospores are formed. Eg. Erysiphe, members of sub-division Ascomycotina.



4. Basidiospore: Sexual spore produced on a club shaped structure known as basidium. Generally 4 basidiospores are formed. Eg. Puccinia, members of sub-division Basidiomycotina.

**TERMS
RELATED TO
PLANT
PATHOLOGY**

Disease and Disorder

Disease: Any malfunctioning of host cells and tissues that result from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms (G.N.Agrios, 1997).

Disorder: Non-infectious plant diseases due to abiotic causes such as adverse soil and environmental conditions are termed disorders. The common characteristic of noninfectious diseases of plants is that they are caused by the lack or excess of something (temperature, soil moisture, soil nutrients, light, air and soil pollutants, air humidity, soil structure and pH) that supports life. Non-infectious plant diseases occur in the absence of pathogens, and cannot, therefore, be transmitted from diseased to healthy plants.

Pathogen and parasite

Pathogen: An entity, usually a micro-organism that can incite disease. In a literal sense a pathogen is any agent that causes pathos (ailment, suffering) or damage. However, the term is generally used to denote living organisms (Fungi, bacteria, MLO's, nematodes etc.,) and viruses but not nutritional deficiencies.

Parasite: Organisms which derive the materials they need for growth from living plants (host or suscept) are called parasites.

Pathogenicity and Pathogenesis

Pathogenicity is the ability of the pathogen to cause disease

Pathogenesis is the chain of events that lead to development of disease in the host (or) sequence of progress in disease development from the initial contact between the pathogen and its host to the completion of the syndrome

Sign vs symptom vs syndrom

Sign: The pathogen or its parts or products seen on a host plant.

Symptom: The external or internal reactions or alterations of a plant as a result of a disease.

Syndrome: The set of varying symptoms characterizing a disease are collectively called a syndrome.

Biotroph vs Hemibiotroph vs Necrotroph

Biotroph: An organism that can live and multiply only on another living organism. They always obtain their food from living tissues on which they complete their life cycle. Ex: Rust, smut and powdery mildew fungi.

Hemibiotroph (Facultative Saprophyte): The parasites which attack living tissues in the same way as biotrophs but will continue to grow and reproduce after the tissue is dead called as facultative saprophytes.

Perthotrophs or perthophytes (Necrotroph): A parasite is a necrotroph when it kills the host tissues in advance of penetration and then lives saprophytically Ex: *Sclerotium rolfsii*.

Inoculum, Inoculum potential and Incubation period

Inoculum: It is the part of the pathogen which on contact with susceptible host plant causes infection (or) the infective propagules which on coming in contact with the host plant causes an infection are known as inoculum

Inoculum potential: The energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected (or) The resultant of the action of environment, the vigour of the pathogen to establish an infection, the susceptibility of the host and the amount of inoculum present

Incubation period: The period of time (or time lapse) between penetration of a host by a pathogen and the first appearance of symptoms on the host. It varies with pathogens, hosts and environmental conditions.

Latent period and Dormant stage

Latent period- The period from spore landing until the parasitized tissue produces new spores is called latent period.

Dormant – It is a condition in which pathogen spends long periods during host's life cycle in a quiescent stage during unfavourable condition. This stage is called dormant or quiescent stage.

Predisposition: It is the action of set of environments, prior to penetration and infection, which makes the plant vulnerable to attack by the pathogen. It is related to the effect of environments on the host, not on the pathogen, just before actual penetration occurs .

Colonization of a host results from the establishment, growth and reproduction of the pathogen on or in infected plant.

Infestation refers to the 4 establishment (or “running over”) on the surface of a host by a large number of insects or other animal pests. With infestation there is no implication that infection has occurred.

Hypersensitivity: Excessive sensitivity of plant tissues to certain pathogens. Affected cells are killed quickly, blocking the advance of obligate parasites.

Immune: It denotes that the pathogen can not establish parasitic relationship with the host.

Infection vs Systemic infection

Infection is the establishment of parasitic relationship between two organisms, following entry or penetration (or) the establishment of a parasite within a host plant.

Systemic infection: The growth of pathogen from the point of entry to varying extents without showing adverse effect on tissues through which it passes.

Types of disease

Epidemic or Epiphytotic disease: A disease usually occurs widely but periodically in a destructive form is referred as epidemic or Epiphytotic disease. Ex: Late blight of potato – Irish famine (1845)

Endemic: Constantly present in a moderate to severe form and is confined to a particular country or district. Ex: Club root of cabbage in Nilgiris Black wart of potato – *Synchytrium endobioticum* Onion smut – *Urocystis cepulae*

Sporadic disease: Occur at very irregular intervals and locations and in relatively fewer instances. Ex: Udbatta disease of rice, Angular leaf spot of cucumber – *Pseudomonas lachrymans*

Thank you..

Viruses nature, architecture, multiplication and transmission.

General Concepts

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Morphology

Plant viruses come in different shapes and sizes. Nearly half of them are elongate (rigid rods or flexuous threads), and almost as many are spherical (isometric or polyhedral), with the remaining being cylindrical bacillus-like rods (Figs. 14-4 and 14-5). Some elongated viruses are rigid rods about 15 by 300 nanometers, but most appear as long, thin, flexible threads that are usually 10 to 13 nanometers wide and range in length from 480 to 2,000 nanometers. Rhabdoviruses are short, bacilluslike, cylindrical rods, approximately three to five times as long as they are wide (52–75 by 300–380nm). Most spherical viruses are actually polyhedral, ranging in diameter from about 17 nanometers (tobacco necrosis satellite virus) to 60 nanometers (wound tumor virus). Tomato spotted wilt virus is surrounded by a membrane and has a flexible, spherical shape about 100 nanometers in diameter. Many plant viruses have split genomes, i.e., they consist of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. Thus, some, like tobacco rattle virus, consist of two rods, a long one (195 by 25 nm) and a shorter one (43 by 25 nm), whereas others, like alfalfa mosaic virus, consist of four components of different sizes (Fig. 14-4E). Also, many isometric viruses have two or three different components of the same size but containing nucleic acid strands of different lengths.

In multicomponent viruses, all of the nucleic acid strand components must be present in the plant for the virus to multiply and perform in its usual manner. The surface of viruses consists of a definite number of protein subunits, which are arranged spirally in the elongated viruses and packed on the sides of the polyhedral particles of the spherical viruses (Fig. 14-5). In cross section, the elongated viruses appear as hollow tubes with the protein subunits forming the outer coat and the nucleic acid, also arranged spirally, embedded between the inner ends of two successive spirals of the protein subunits. In spherical viruses the visible shell consists of protein subunits, while the nucleic acid is inside the shell and is arranged in an as yet unknown manner. Rhabdoviruses, and a few spherical viruses, are provided with an outer

lipoprotein envelope or membrane. Inside the membrane is the nucleocapsid, consisting of nucleic acid and protein subunits.

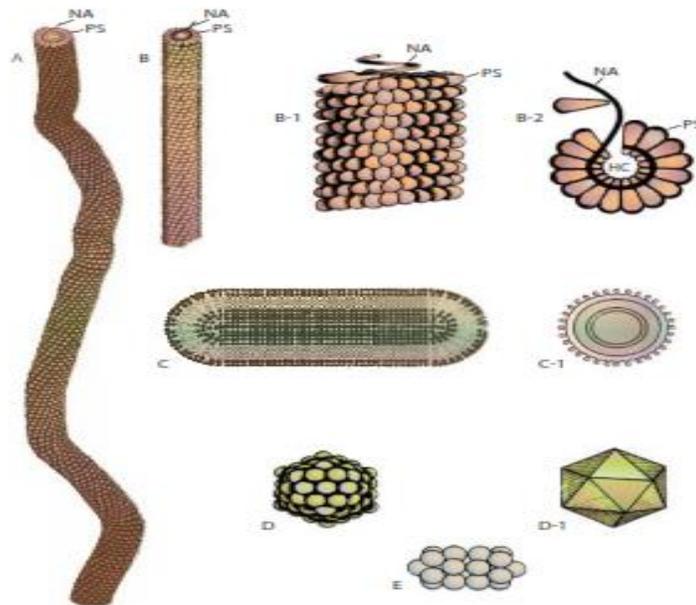


FIGURE 14-5 Relative shapes, sizes, and structures of some representative plant viruses. (A) Flexuous thread-like virus. (B) Rigid rod-shaped virus. (B-1) Side arrangement of protein subunits (PS) and nucleic acid (NA) in viruses A and B. (B-2) Cross-section view of the same viruses. HC, hollow core. (C) Short, bacillus-like virus. (C-1) Cross-section view of such a virus. (D) Isometric polyhedral virus. (D-1) Icosahedron representing the 20-sided symmetry of the protein subunits of the isometric virus. (E) Geminivirus consisting of twin particles.

FIGURE 14-5 Relative shapes, sizes, and structures of some representative plant viruses. (A) Flexuous thread-like virus. (B) Rigid rod-shaped virus. (B-1) Side arrangement of protein subunits (PS) and nucleic acid (NA) in viruses A and B. (B-2) Cross-section view of the same viruses. HC, hollow core. (C) Short, bacillus-like virus. (C-1) Cross-section view of such a virus. (D) Isometric polyhedral virus. (D-1) Icosahedron representing the 20-sided symmetry of the protein subunits of the isometric virus. (E) Geminivirus consisting of twin particles. HC PS

Composition and Structure

Each plant virus consists of at least a nucleic acid and a protein. Some viruses consist of more than one size of nucleic acid and proteins, and some of them contain enzymes or membrane lipids. The nucleic acid makes up 5 to 40% of the virus, protein making up the remaining 60 to 95%. The lower nucleic acid percentages are found in the elongated viruses, whereas the spherical viruses contain higher percentages of nucleic acid. The total mass of the nucleo-

protein of different virus particles varies from 4.6 to 73 million daltons. The weight of the nucleic acid alone, however, ranges only between 1 and 3 million ($1-3 \times 10^6$) daltons per

virus particle for most viruses, although some have up to 6×10^6 daltons and the 12 component wound tumor virus nucleic acid is approximately 16×10^6 daltons. All viral nucleic acid sizes are quite small when compared to 0.5×10^9 daltons for mollicutes and 1.5×10^9 daltons for bacteria.

Composition and Structure of Viral Protein

Viral proteins, like all proteins, consist of amino acids. The sequence of amino acids within a protein, which is dictated by the sequence of nucleotides in the genetic material, determines the nature and properties of the protein. The protein shells of plant viruses are composed of repeating subunits. The amino acid content and sequence for identical protein subunits of a given virus are constant but vary for different viruses and even for different strains of the same virus. Of course, the amino acid content and sequence are different for different proteins of the same virus particle and even more so for different viruses. The content and sequences of amino acids are known for the proteins of many viruses. For example, the protein subunit of tobacco mosaic virus (TMV) consists of 158 amino acids in a constant sequence and has a mass of 17,600 daltons (often written as 17.6 kDa, 17.6 kd, or 17.6 K). In TMV the protein subunits are arranged in a helix containing $16 \frac{1}{3}$ subunits per turn (or 49 subunits per three turns). The central hole of the virus particle down the axis has a diameter of 4 nanometers, whereas the maximum diameter of the particle is 18 nanometers. Each TMV particle consists of approximately 130 helix turns of protein subunits. The nucleic acid is packed tightly in a groove between the helices of protein subunits. In rhabdoviruses the helical nucleoproteins are enveloped in a membrane. In polyhedral plant viruses the protein subunits are packed tightly in arrangements that produce 20 (or some multiple thereof) facets and form a shell. Within this shell the nucleic acid is folded or otherwise organized.

Composition and Structure of Viral Nucleic Acid

The nucleic acid of most plant viruses consists of RNA, but a large number of viruses have been shown to contain DNA. Both RNA and DNA are long, chain-like molecules consisting of hundreds or, more often, thousands of units called nucleotides. Each nucleotide consists of a ring compound called the base attached to a five-carbon sugar [ribose (I) in RNA, deoxyribose (II) in DNA], which in turn is attached to phosphoric acid (Fig. 14-6). The sugar of one nucleotide reacts with the phosphate of another nucleotide, which is repeated many times, thus forming the RNA or DNA strand. In viral RNA, only one of four bases, adenine, guanine, cytosine, and uracil, can be attached to each ribose molecule. The first two, adenine and guanine, are purines and interact with the other two, uracil and cytosine, the pyrimidines. The chemical formulas of the bases and one of their possible relative positions in the RNA chain are shown in Fig. 14-6 (structure III). DNA is similar to RNA with two small, but very important differences: the oxygen of one sugar hydroxyl is missing and the base uracil is replaced by the base methyluracil, better known as thymine (IV). The size of both RNA and DNA is expressed either in daltons or as the number of bases [kilobases (kb) for single-stranded RNA and DNA or kilobase pairs (kbp) for double-stranded RNA and DNA], or as the number of nucleotides or nucleotide pairs. The sequence and the frequency of the bases on the RNA strand vary from one RNA to another, but they are fixed within a given RNA and determine its properties. Healthy cells of plants always contain doublestranded DNA and single-stranded RNA. Of the nearly 1,000 described plant viruses, most (about 800) contain

single-stranded RNA, but 50 contain double-stranded RNA, 40 contain double-stranded DNA, and about 110 contain single-stranded DNA.

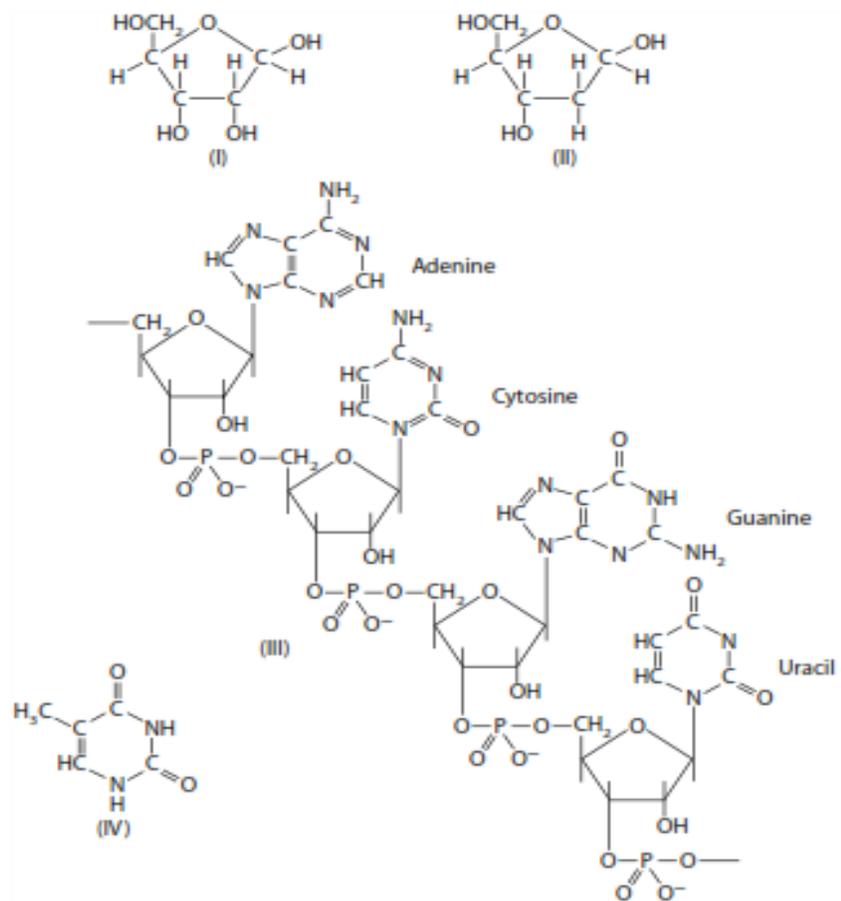


FIGURE 14-6 Chemical formulas of ribose (I), deoxyribose (II), ribonucleic acid or RNA (III), and thymine (IV).

NOMENCLATURE AND CLASSIFICATION OF PLANT VIRUSES

Many plant viruses are named after the most conspicuous symptom they cause on the first host in which they have been studied. Thus, a virus causing a mosaic on tobacco is called tobacco mosaic virus, whereas the disease itself is called tobacco mosaic; another virus causing spotted wilt symptoms on tomato is called tomato spotted wilt virus and the disease is called tomato spotted wilt, and so forth. Considering, however, the variability of symptoms caused by the same virus on the same host plant under different environmental conditions, by different strains of a virus on the same host, or by the same virus on different hosts, it becomes apparent that this system of nomenclature leaves much to be desired. All viruses belong to the kingdom Viruses. Within the kingdom, viruses are distinguished as RNA viruses and DNA viruses, depending on whether the nucleic acid of the virus is RNA or DNA. Viruses are further subdivided depending on whether they possess one or two strands of RNA or DNA of either positive or negative sense, either filamentous or isometric. Within each of these groups there may be viruses replicating via a polymerase enzyme (+RNA or DNA viruses) or via a reverse transcriptase (-RNA or DNA viruses). Most viruses consist of nucleic acid surrounded by coat protein, but some also have a membrane attached to them. Some viruses

have all their genome in one Particle (monopartite viruses), but the genome of other (multipartite) viruses is divided among two, three, or, rarely, four particles. Other characteristics in the classification of viruses include the symmetry of helix in the helical viruses, or number and arrangement of protein subunits in the isometric viruses, size of the virus, and, finally, any other physical, chemical, or biological properties. Figure 14-24 shows diagrammatically the various families and genera of plant viruses. The current nomenclature and classification scheme of plant viruses, along with the type species and the means of transmission of each virus genus, are as follows.

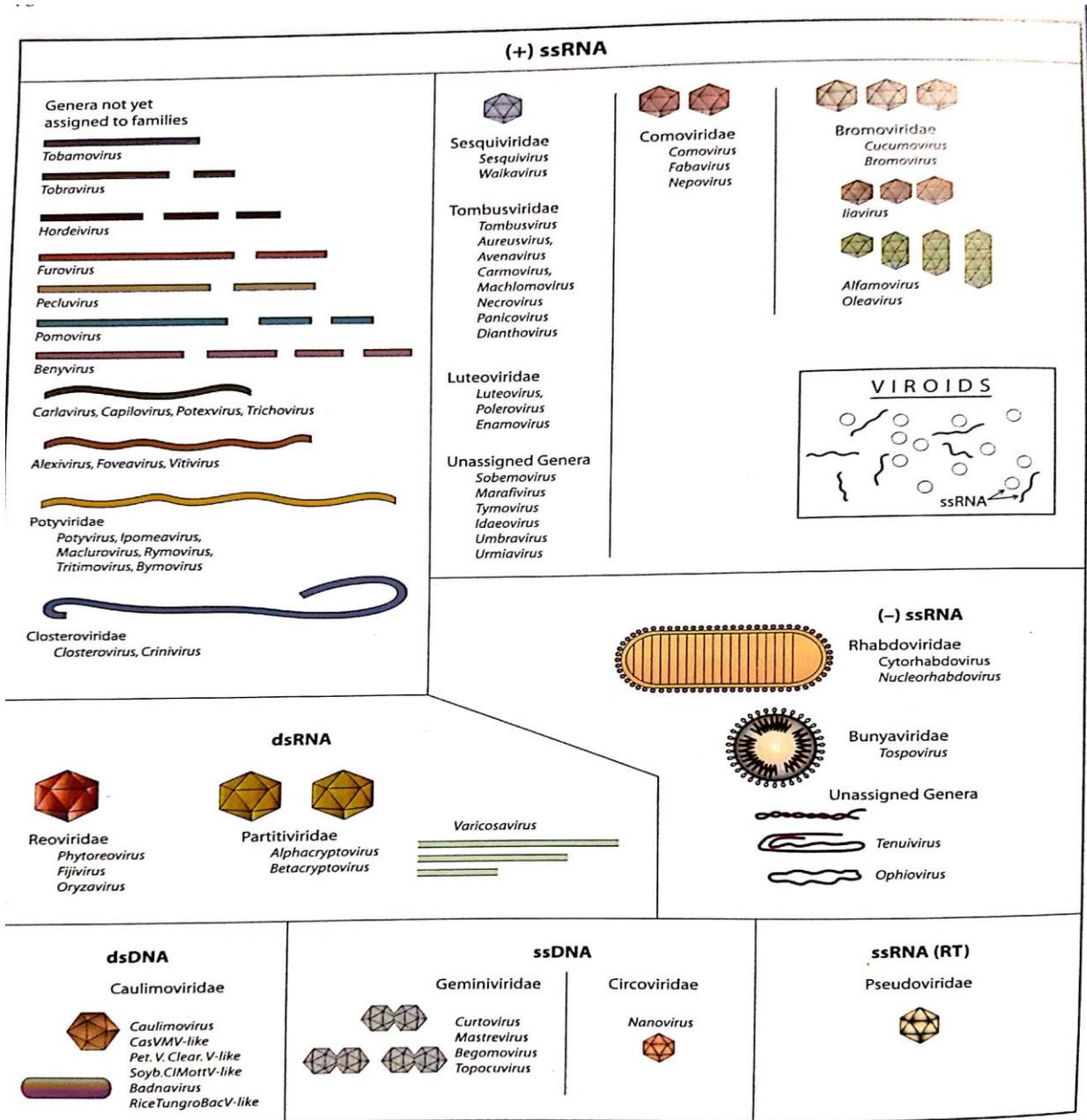


FIGURE 14-24 Schematic diagram of families and genera of viruses and of viroids that infect plants.

Kingdom: Viruses

Virus genera not yet assigned into families
 RNA viruses
 Single-stranded positive RNA [(+) ssRNA]

Rod-shaped particles	Family	Genus	Type species	Remarks
1 ssRNA	—	<i>Tobamovirus</i>	<i>Tobacco mosaic virus</i>	Contact transmission
2 ssRNAs	—	<i>Tobravirus</i>	<i>Tobacco rattle virus</i>	Nematode transmission
3 ssRNAs	—	<i>Hordeivirus</i>	<i>Barley stripe mosaic virus</i>	Seed transmission
2 ssRNAs	—	<i>Furovirus</i>	<i>Soilborne wheat mosaic virus</i>	Fungal transmission
3 ssRNAs	—	<i>Pecluvirus</i>	<i>Peanut clump virus</i>	Fungal and seed transmission
4 ssRNAs	—	<i>Pomovirus</i>	<i>Potato mop-top virus</i>	Fungal transmission, dicots
	—	<i>Benyvirus</i>	<i>Beet necrotic yellow vein virus</i>	Fungal transmission

Filamentous particles

1 ssRNA	—	<i>Allexivirus</i>	<i>Shallot virus X</i>	Eriophyid mite transmission
	—	<i>Carlavirus</i>	<i>Carnation latent virus</i>	No vector
	—	<i>Foveavirus</i>	<i>Apple stem pitting virus</i>	By contact only
	—	<i>Potexvirus</i>	<i>Potato virus X</i>	No vector. Some seed transmission
	—	<i>Capillivirus</i>	<i>Apple stem grooving virus</i>	No vector. Some seed transmission
	—	<i>Trichovirus</i>	<i>Apple chlorotic leafspot virus</i>	No vector. Some seed transmission
	—	<i>Vitivirus</i>	<i>Grapevine virus A</i>	Mealybugs, scale insects, aphids

Isometric particles

1 ssRNA	—	<i>Sobemovirus</i>	<i>Southern bean mosaic virus</i>	Seedborne, beetles, myrids
	—	<i>Marafivirus</i>	<i>Maize rayado fino virus</i>	In Gramineae, leafhoppers
	—	<i>Umbravirus</i>	<i>Carrot mottle virus</i>	Do not code coat proteins
	—	<i>Tymovirus</i>	<i>Turnip yellow mosaic virus</i>	Aphids w/ helper virus
	—			By beetles
2 ssRNAs	—	<i>Idaeovirus</i>	<i>Raspberry bushy dwarf virus</i>	By pollen and seed

Bacilliform particles

3 ssRNAs	—	<i>Ourmiavirus</i>	<i>Ourmia melon virus</i>	No vectors known
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Virus families

Filamentous viruses

1 ssRNA	Potyviridae	<i>Potyvirus</i>	<i>Potato virus Y</i>	Aphids, w/ helper virus
	Potyviridae	<i>Ipomovirus</i>	<i>Sweet pot mild mottle</i>	Whitefly <i>Bemisia tabaci</i>
	Potyviridae	<i>Macluravirus</i>	<i>Maclura mosaic virus</i>	Aphids
	Potyviridae	<i>Rymovirus</i>	<i>Ryegrass mosaic virus</i>	Eriophyid mites
	Potyviridae	<i>Tritimovirus</i>	<i>Wheat streak mosaic virus</i>	Eriophyid mites
	Potyviridae	<i>Bymovirus</i>	<i>Barley yellow mosaic virus</i>	Gramineae, fungal transmission

	1 or 2 ssRNA				
		Closteroviridae	<i>Closterovirus</i>	<i>Beet yellows virus</i>	Aphids, mealybugs, or whiteflies
		Closteroviridae	<i>Crimivirus</i>	<i>Lettuce inf. yellows virus</i>	Whiteflies
	Isometric viruses				
	1 ss(+)RNA				
		Sequiviridae	<i>Sequivirus</i>	<i>Parsnip yellow fleck virus</i>	Aphids
		Sequiviridae	<i>Waikavirus</i>	<i>Rice tungro spherical virus</i>	Leafhoppers or aphids
		Tombusviridae	<i>Tombusvirus</i>	<i>Tomato bushy stunt virus</i>	Soilborne, but vector unknown
		Tombusviridae	<i>Aureusvirus</i>	<i>Pothos latent virus</i>	Soilborne
		Tombusviridae	<i>Arenavirus</i>	<i>Oat chlorotic stunt virus</i>	Soilborne
		Tombusviridae	<i>Carmovirus</i>	<i>Carnation mottle virus</i>	—
		Tombusviridae	<i>Dianthovirus</i>	<i>Carnation ring spot virus</i>	Soilborne, unknown
		Tombusviridae	<i>Machlomovirus</i>	<i>Maize chlorotic mottle virus</i>	Seed, beetles, thrips
		Tombusviridae	<i>Necrovirus</i>	<i>Tobacco necrosis virus</i>	Fungal transmission
		Tombusviridae	<i>Panicovirus</i>	<i>Panicum mosaic virus</i>	Gramineae, mechanical
		Luteoviridae	<i>Luteovirus</i>	<i>Barley yellow dwarf virus</i>	Gramineae, aphids
		Luteoviridae	<i>Polerovirus</i>	<i>Potato leafroll virus</i>	Monocot or dicot plants
		Luteoviridae	<i>Enamovirus</i>	<i>Pea enation mosaic virus</i>	Mechanically, aphids
	2 ss(+)RNAs				
		Comoviridae	<i>Comovirus</i>	<i>Coupea mosaic virus</i>	Chrysomelid beetles
		Comoviridae	<i>Fabavirus</i>	<i>Broad bean wilt virus</i>	Aphids
		Comoviridae	<i>Nepovirus</i>	<i>Tobacco ring spot virus</i>	Nematodes
	3 ss(+)RNAs				
		Bromoviridae	<i>Bromovirus</i>	<i>Brome mosaic virus</i>	Beetles, mechanically
		Bromoviridae	<i>Cucumovirus</i>	<i>Cucumber mosaic virus</i>	Aphids
		Bromoviridae	<i>Alfavirus</i>	<i>Alfalfa mosaic virus</i>	Aphids
		Bromoviridae	<i>Illavirus</i>	<i>Tobacco streak virus</i>	Pollen, seed
		Bromoviridae	<i>Oleavirus</i>	<i>Olive latent virus 2</i>	No vector known
	dsRNA				
		Reoviridae	<i>Phytoreovirus</i>	<i>Wound tumor virus</i>	Leafhoppers
		Reoviridae	<i>Fijivirus</i>	<i>Fiji disease virus</i>	Gramineae, planthoppers
		Reoviridae	<i>Oryzavirus</i>	<i>Rice ragged stunt virus</i>	Planthoppers
		Partitiviridae	<i>Alphacryptovirus</i>	<i>White clover crypto. virus 1</i>	Nonenveloped, latent
		Partitiviridae	<i>Betacryptovirus</i>	<i>White clover crypto. virus 2</i>	Same, seed
		Partitiviridae	<i>Varicosavirus</i>	<i>Lettuce big-vein virus</i>	Fungal transmission
	(-) ssRNA				
	Bacilliform particles				
		Rhabdoviridae	<i>Cytorhabdovirus</i>	<i>Lettuce necrosis yellows virus</i>	Leafhoppers, planthoppers, aphids
		Phabdiviridae	<i>Nucleorhabdovirus</i>	<i>Potato yellow dwarf virus</i>	Same
	Membranous circular particles				
		Bunyaviridae	<i>Tospovirus</i>	<i>Tomato spotted wilt virus</i>	Thrips
	Thin flexuous multipartite viruses				
		—	<i>Tenuivirus</i>	<i>Rice stripe virus</i>	Gramineae, planthoppers
		—	<i>Ophiovirus</i>	<i>Citrus psorosis virus</i>	No vector known
	dsDNA				
	Isometric	Caulimoviridae	<i>Caulimovirus</i>	<i>Cauliflower mosaic virus</i>	Aphids
		Caulimoviridae	<i>Soybean chlorotic mottle virus-like</i>		Aphids
		Caulimoviridae	<i>Cassava vein mosaic virus-like</i>		Aphids
			<i>Petunia vein clearing virus-like</i>		Aphids

WORKING WITH AND MANAGING PLANT VIRUSES

(+)ssDNA

Caulimoviridae	<i>Badnavirus</i>	<i>Commelina yellow mottle virus</i>	Mealybugs
Caulimoviridae	<i>Rice tungro bacilliform virus-like</i>		Leafhoppers
Geminiviridae	<i>Mastrevirus</i>	<i>Maize streak virus</i>	Gramineae, leafhoppers
Geminiviridae	<i>Curtovirus</i>	<i>Beet curly top virus</i>	Dicot, leafhoppers
Geminiviridae	<i>Begomovirus</i>	<i>Bean golden mosaic virus</i>	2 DNAs, whiteflies
Geminiviridae	<i>Topocovirus</i>	<i>Tom. pseudocurly top virus</i>	Treehopper
Circoviridae	<i>Nanovirus</i>	<i>Subteranean clover stunt virus</i>	6 DNAs

ssRNA (RT) Pseudoviridae: retrotransposons

What are Bacteriophages?

Bacteriophages are obligate intracellular parasite on bacteria that uses bacterial machinery system for its own multiplication and development. These are commonly referred as “phage”. Bacteriophages were jointly discovered by Frederick Twort (1915) in England and by Felix d’Herelle (1917) at the Pasteur Institute in France. “Bacteriophage” term was coined by Felix d’Herelle. Some of the examples of bacteriophages are, Spherical phages such as ϕ X174 (ssDNA), Filamentous phages such as M13(ssDNA), T-even phages such as T2, T4 and T6 that infect E.coli, Temperate phages such as λ and μ .

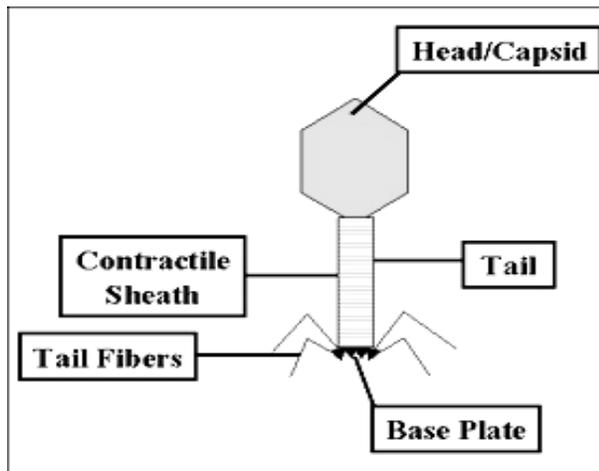


Fig. 8. Basic structure of Bacteriophages

Composition:

All bacteriophages contain nucleic acid as genetic material and protein. Depending upon the phage, the genetic material may be either DNA or RNA. Certain unusual modified bases are present in the genetic material of phages which protect the phage genetic material from nucleases during infection. Protein surrounds the genetic materials and protects to the surrounding environment.

Structure:

The basic structural features of T4 bacteriophages are illustrated in Figure 2. It is approximately 200 nm long and 80-100 nm wide. Size of other phages is of 20 – 200nm in length. All bacteriophages contain head and tail part. Head part is also termed as capsid which is composed of one or different types of proteins. Genetic materials are present inside and protected by capsid. Tails are attached to the capsid in most of the phages. These are hollow tube like structure through which viruses inject their genetic material inside the host during infection. Tail part is more complex structure in phages. In T4 phage, tail part is surrounded by a contractile sheath and basal plate like structure present at the end of tail from which certain tail fibres are attached. Tail fibres help in attachment of phages to bacteria and contractile sheath helps in contraction during infection. Some of the phages do not contain tail fibres at the end. Certain other structures are involved in these phages for binding to the bacterium during infection.

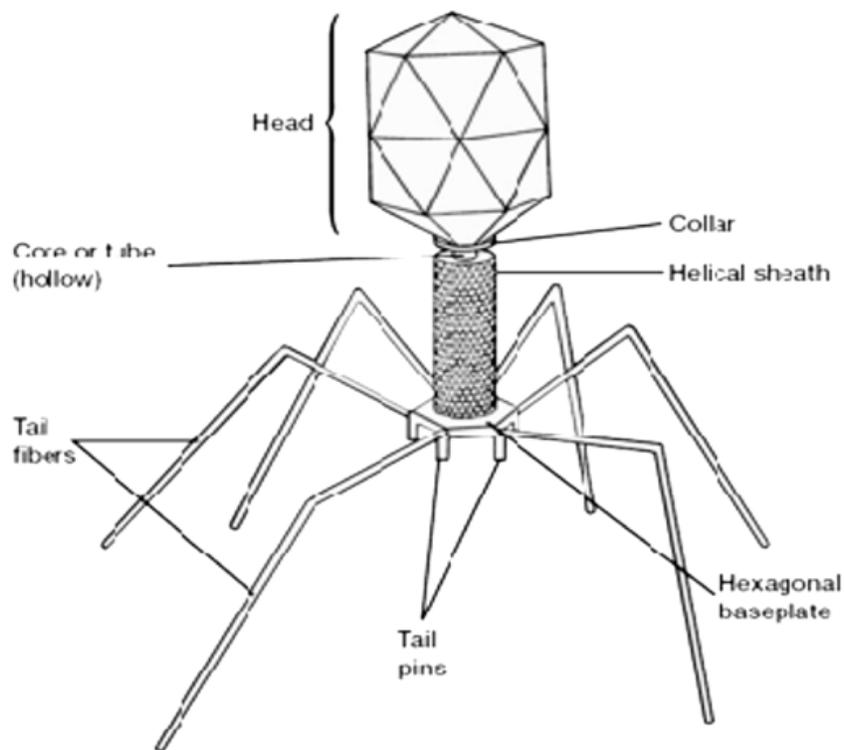


Fig. 9. Structure of T4 Bacteriophage

Multiplication/Infection of Host Cells

The first step in the infections is binding of phage to bacterium which is mediated by tail fibres are some other structures on those phages that lack tail fibres. Binding of phage tail fibre to bacterium is through adsorption process and it is reversible. There are specific receptors are present on bacterial cell surface through which phages bind on it by its tail fibre. These receptors are proteins, lipopolysaccharides, pili and lipoproteins of bacterium. Some phages lacking basal plate and tail fibre bind tightly to bacterial cell surface and it is reversible.

After binding of phage to bacterium, there is contraction in tail by contractile sheath and phages inject their genetic material through hollow tube like tail. Some phages also contain certain enzymes that digest the bacterial cell surface. Phages that don't contain tail fibre and contractile sheath, uses different mechanism for inject its own genetic material inside the host. Only genetic material of phages enters inside the bacterium and the remainder of phages (ex. capsid) remain outside of bacterium.

Life Cycle:

There are two different types of life cycle present in phages: (i) Lytic cycle and (ii) Lysogenic cycle. Lytic (virulent) cycle kill the host cell that they infect, while lysogenic (temperate) cycle establishes a persistent infection without killing the host cell.

(A.) Lytic Cycle:

These are also known as virulent cycle because phages multiply inside the host and lyse the cell at the end of its life cycle. After attachment of tail fibre to host, genetic materials are injected inside the host. The time period between the entry of genetic material inside the host and release of mature phage after end of life cycle is termed as eclipse period. Synthesis of phage components and its packaging into mature phages takes place in this period. After infection, the genetic material of phages uses host biosynthetic machinery for replication, transcription and translation. Structural proteins of phages (capsid, tail etc.) are also synthesized inside the host using host biosynthetic machinery. After synthesis, genetic materials are packed inside the capsid and tail is attached on it. This process is called as maturation of phages. In lytic phage, phages also synthesized lysis protein. Bacterial cells are lysed due to accumulation of phage lysis protein and mature phages are released into the medium. Around 10-1000 phages are released from the bacterial cell. The average yield of phages per infected bacterial cell is known as burst size.

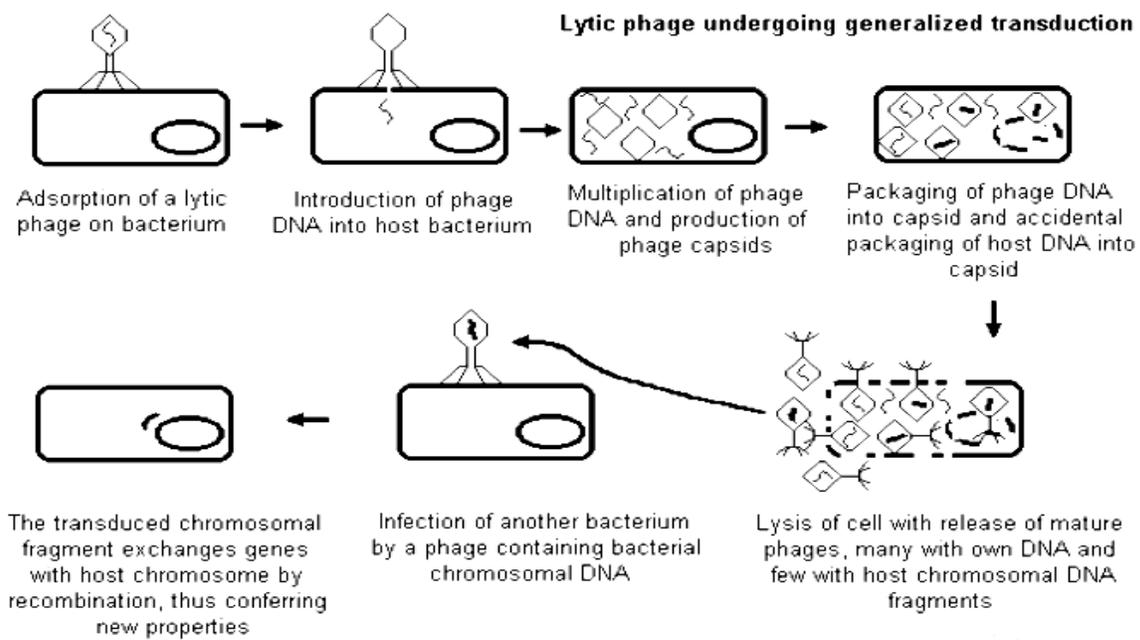


Fig. 10. Lytic cycle of bacteriophage infection

(B.) Lysogenic cycle:

These are also known as temperate phage because phages multiply via the lytic cycle or may enter a dormant state inside the cell. After entry of genetic material into the host cell, the phage DNA integrates into the host chromosome and starts replication along with it and passed to the daughter cells of host. Integration of phage DNA to host chromosome is termed as prophage and bacteria is termed as lysogenic bacteria. Due to integration of phage DNA to host chromosome, extra genes carried by phage get expressed in the host cell and it may change the properties of bacterial cell. This process is termed as lysogenisc or phage conversion.

Due to exposure to UV rays, ionizing radiations, mutagenic chemicals etc, DNA of phage is released from host chromosome and separated phage DNA initiates lytic cycle resulting in the lysis of cell and release of phages into the medium.

Significance of bacteriophages:

- New characteristics are acquired using lysogenic conversion
- Insertional mutation can be induced in bacterial chromosome by random insertion of genes or nucleotides.
- Latent infection in mammalian cells by retroviruses can be studied using Lambda phage as model system.
- In genetic engineering, phages are used extensively where they serve as cloning vectors.
- Phages are used to maintain libraries of genes and monoclonal antibodies
- Natural removal of bacteria from water bodies can be done using bacteriophages

Ultrastructure of TMV

DESIGN AND ASSEMBLY TMV is the classical example of a rod-shaped virus. Its rod shape results from its basic design, namely a regular helical array of identical protein subunits, in which framework is embedded a single molecule of RNA wound as a helix (figure 1b). It is, of course, the RNA that carries the genetic information, i.e. the capacity to instruct the host cell to make many copies of the virus. This general picture was already complete by 1958, when Rosalind Franklin died, and it seemed easy to comprehend how a structure of this type might be built out of identical subunits: the subunits might assemble themselves by repeated identical interactions, like adding steps in a spiral staircase, enclosing the RNA as a corkscrew-like thread as the rod extends. In other words, the assembly might be likened to growth at a screw dislocation in a crystal. We now know that this simple picture of assembly is wrong in all its essentials. The isolated protein can aggregate in different polymorphic forms, and Caspar (1963) suggested that these might play the role of intermediates in the assembly of the helical aggregate. The virus, in fact, assembles in a much more complex way, for what, with hindsight, we can see to be good physical and biological reasons. The story of how my colleagues and I came to suspect that the simple scheme was deficient and how the path of assembly was found has been told elsewhere (Klug 1979). I can only summarize the results here. When the RNA and the coat protein of the virus are taken apart, the protein molecules alone under physiological conditions aggregate, not into a long helix, but into a 20S aggregate. We identified this as a 'disk' (figure 2) — a two-layer cylindrical structure, each layer consisting of a ring of 17 molecules, compared with the $16\frac{1}{3}$ molecules present in each turn of the assembled helix. The disk can be crystallized, but because of the large molecular mass of the disk (600 000 Da) the determination of its exact structure by X-ray methods posed formidable technical and analytical problems.

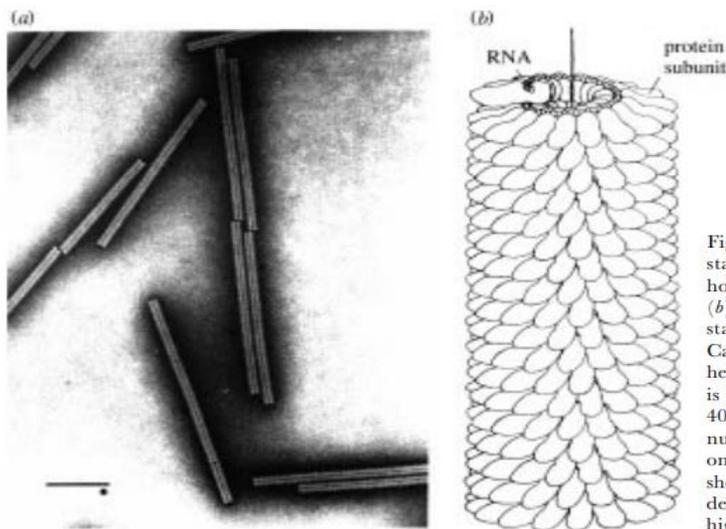


Figure 1. (a) Electron micrograph of negatively stained TMV particles (J. T. Finch). Note the hole down the middle. Scale bar, 100 nm. (b) Diagram summarizing the results of the first stage of structure analysis of TMV (Klug & Caspar 1960). The protein subunits form a tight helical array with $16\frac{1}{3}$ units per turn, and the RNA is packed between the turns at a radius of about 40 Å from the helix axis. There are three nucleotides per protein subunit. Only about one-sixth of the length of a complete particle is shown. The detailed X-ray structure was first determined by Stubbs *et al.* (1977), then carried to higher resolution by Namba & Stubbs (1986) and Namba *et al.* (1989).

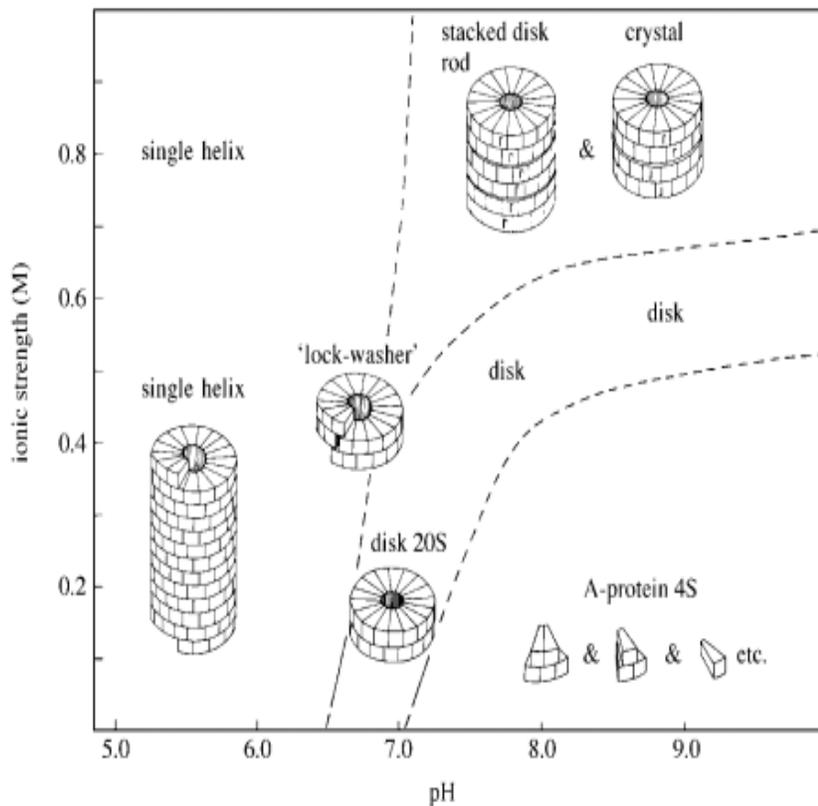


Figure 2. Diagram showing the ranges over which particular species of TMV protein participate significantly in the equilibrium (after Durham *et al.* 1971). This is not a conventional phase diagram: a boundary is drawn where a larger species becomes detectable and does not imply that the smaller species disappears sharply. The 'lock-washer' indicated on the boundary between the 20S disk and the helix is not well defined and represents a metastable transitory state observed when not enough time is allowed for the transition (fig. 1 in Butler, this issue). The boundaries are approximately correct for a protein concentration of 5 mg ml^{-1} at 20°C , but not all species that have been observed are shown. The predominant species at pH 7.0 and $I=0.1$ is the '20S' aggregate (more accurately *ca.* 19.5S), which we identify as a two-layer polar disk. As the pH falls below pH 7, the sedimentation constant increases to values greater than 20S, characteristic of short helical aggregates (Butler *et al.* 1992). The stacked-disk structure is formed by prolonged incubation at alkaline pH, and represents an irreversible half-proteolysed aggregate (Durham 1972; Unwin & Klug 1974).

These were overcome and after a dozen years it was possible to construct an atomic model (Figure 3) showing the detailed structure of the protein subunit and how it interacts with its neighbours (Bloomer *et al.* 1978). This study was pursued to the end, not merely for the sake of knowing the structure of a protein subunit, but because we had shown earlier that the protein disk plays a crucial role in the assembly of the virus from its RNA and protein (Butler & Klug 1971). The disk combines with a specific initiation tract on the single-stranded viral RNA, which is located about 1000 bases from the 3' end (Zimmern & Butler 1977; Zimmern & Wilson 1976), and then dislocates to begin helix growth. Foreign RNAs that do not have this tract are rejected. When the sequence of bases of this initiation tract, or 'origin of assembly' as it is now called, was determined (Zimmern 1977), it was seen that it could be folded so that the initial binding site is exposed at the apex of a stem-loop structure. Now the X-ray studies had shown that the two layers of the protein disk are so arranged as to leave a gap between them, thus in effect extending the central hole of the

disk, rather like a pair of 'jaws', as if waiting to 'bite' the RNA (Figure 3). So we were able to develop the picture of initiation, or nucleation as a physicist would say, of assembly shown in Figure 4 (Butler et al. 1976). The RNA hairpin loop inserts through the central hole of the disk, and the stem of the loop opens up and binds in the 'jaws' formed by the two layers of protein. The disk then dislocates into a helical structure, entrapping the RNA, after which elongation proceeds by the incorporation of further disks, pulling up more RNA through the central hole. There was some controversy surrounding this picture of growth after the initial stages, but there was no doubt about the role of the 20S aggregate (the disk) in initiating assembly, and also in further growth.

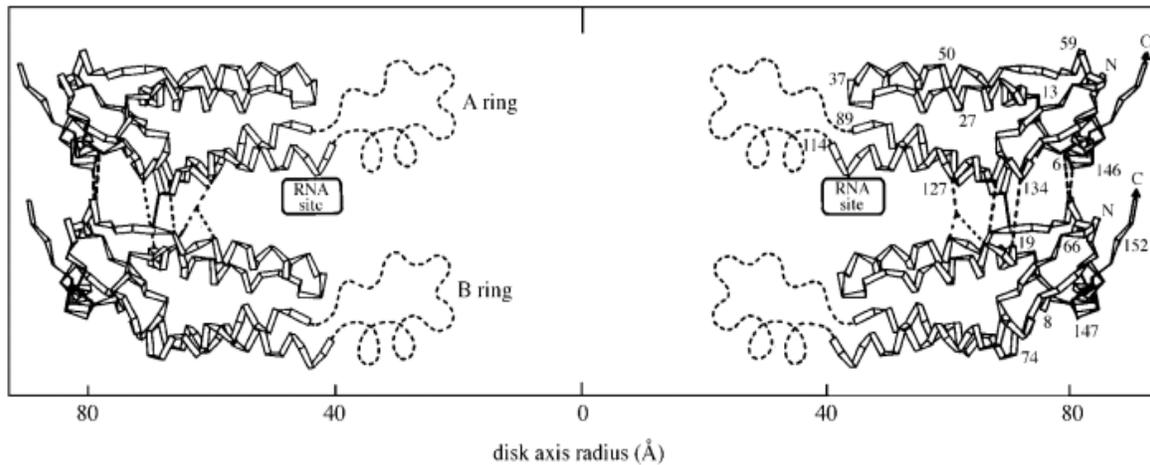


Figure 3. Section through a two-layer disk along its axis, reconstructed from the results of X-ray analysis to a resolution of 2.8 Å (Bloomer *et al.* 1978). The ribbons show the path of the polypeptide chain of the protein subunits. Subunits of the two rings can be seen touching over a small area toward the outside of the disk (where the side-chain contacts that occur between the rings have been drawn in as dashed and solid lines) but opening up into the 'jaws' toward the centre. The dashed lines at low radius indicate schematically the mobile portion of the subunits in the disk, extending in from near the RNA binding sites (between the rings *ca.* 40 Å radius as shown) to the edge of the central hole, which has a radius of 20 Å.

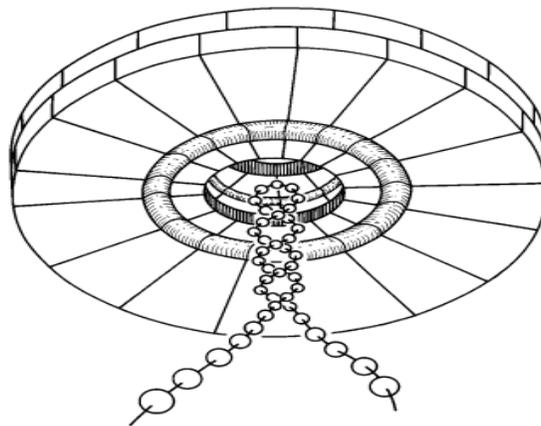


Figure 4. Nucleation of virus assembly is believed to occur by the insertion of a stem-loop hairpin structure of RNA into the central hole of the protein disk and between the two layers of subunits. This loop, formed by the nucleation region of the RNA origin of assembly, binds round the first turn, opening up the base-paired stem as it does so, and causes the disk to dislocate into a short helix. This 'closes the jaws', entrapping the RNA between the turns of protein subunits, and gives a start to the nucleoprotein helix (which can then elongate rapidly to some minimum stable size).

There has also been a controversy about the exact nature of the 20S aggregate, which we identified as a two-layer polar disk. The disk is thus an obligatory intermediate in the assembly of the virus which, simultaneously, fulfills both the physical requirement for nucleating the growth of the helical particle and the biological requirement for specific recognition of the viral RNA. A most intricate structural mechanism has been evolved to give the process an efficiency and purposefulness, whose basis we now understand. TMV is self-assembling, self-nucleating and self-checking. The general conclusion derived from the story of TMV assembly is that one must distinguish between the design of a structure and the construction process used to achieve it. In the TMV structure all 2130 coat protein subunits (except the few at the ends of the particle) make the same non-covalent contacts with each other, and this specific bonding pattern repeated many times leads to a symmetrical final structure. There is nothing in the design of the completed structure which gives a hint that different bonding patterns, and nonequivalent ones at that, are required during the process of assembly. The protein subunit under physiological conditions is designed not to form an endless helix, but a restricted two-layer variant of it—the disk—which is stable and which can be readily converted to the lock-washer, or helical, form. The disk therefore represents an intermediate subassembly by means of which the entropically difficult problem of nucleating helical growth is overcome. At the same time nucleation by the disk subassembly furnishes a mechanism for recognition of the homologous viral RNA (and rejection of foreign RNAs) by providing a long stretch of nucleotide binding sites for interaction with the special sequence of bases on the RNA. In this way specificity for the homologous viral RNA is achieved.

TRANSMISSION OF PLANT VIRUSES

Plant viruses are transmitted from plant to plant in a number of ways. Modes of transmission include vegetative propagation, mechanically through sap, through seed, pollen, dodder, and by specific insects, mites, nematodes, and fungi.

Transmission of Viruses by Vegetative Propagation

Whenever plants are propagated vegetatively by budding or grafting, by cuttings, or by the use of tubers, corms, bulbs, or rhizomes, any viruses present in the mother plant from which these organs are taken will almost always be transmitted to the progeny (Fig. 14-15). Considering that almost all fruit and many ornamental trees and shrubs, many field crops, such as potatoes, and most florist's crops are propagated vegetatively, this means of transmission of viruses is the most important for all these types of crop plants. In cases of propagation by budding, the presence of a virus in the bud or in the root stock may result in an appreciable reduction of successful bud unions with the rootstock and, therefore, in poor stands. The transmission of viruses may also occur through natural root grafts of adjacent plants, particularly trees (Fig. 14-15). For several tree viruses, natural root grafts are the only known means of tree-to-tree spread of the virus within established orchards.

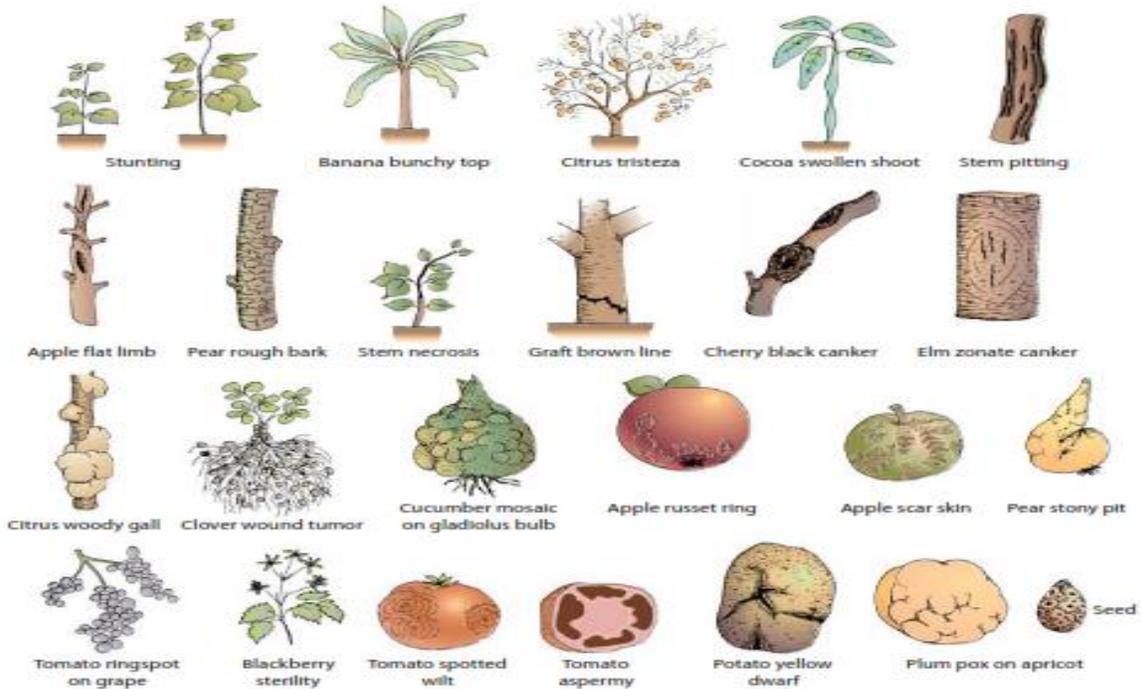


FIGURE 14-14 Types of systemic symptoms caused by viruses and viroids in plants.

Mechanical Transmission of Viruses through Sap

The mechanical transmission of plant viruses in nature by the direct transfer of sap through contact of one plant with another is uncommon and relatively unimportant. Such transmission may take place after a strong wind injures the leaves of adjacent diseased and healthy plants or when plants are wounded during cultural practices by tools, hands, or clothes, or by animals feeding on the plants, and the sap carrying virus is transferred to wounded plants (Figs. 14-16 and 14-17). Of the important plant viruses, *potato virus X*, *tobacco mosaic virus*, and *cucumber mosaic virus* are transmitted through sap in the field and may cause severe losses.

The greatest importance of mechanical transmission of plant viruses stems from its indispensability in studying the viruses that cause plant diseases. For mechanical transmission of a virus, young leaves and flower petals are ground to crush the cells and release the virus in the sap (Fig. 14-16). Often a buffer solution is added to stabilize the virus. The sap may be strained to remove tissue fragments and is then applied to the surface of leaves of young plants dusted previously with an abrasive such as Carborundum to aid in wounding of the cells. The sap is applied by rubbing the leaves gently with a cheesecloth, finger (Fig. 14-13A), glass spatula, or painter's brush dipped in the sap or by using a small sprayer. In successful inoculations, the virus enters the leaf cells through the wounds made by the abrasive or through broken leaf hairs and initiates new infections.

In local-lesion hosts, symptoms usually appear within three to seven days or more, and the number of local lesions is proportional to the concentration of the virus in the sap. In systemically infected hosts, symptoms usually take 10 to 14 days or more to develop. Sometimes the same plants may first develop local lesions and then systemic symptoms. In the mechanical transmission of viruses, the taxonomic relationship of the donor and receiving (indicator) plants is unimportant, as virus from one kind of plant, whether herbaceous or a tree, may be transmitted to dozens of unrelated herbaceous plants (vegetables, flowers, or weeds). Several viruses, however, especially of woody plants, are difficult or, so far, impossible to transmit through sap.

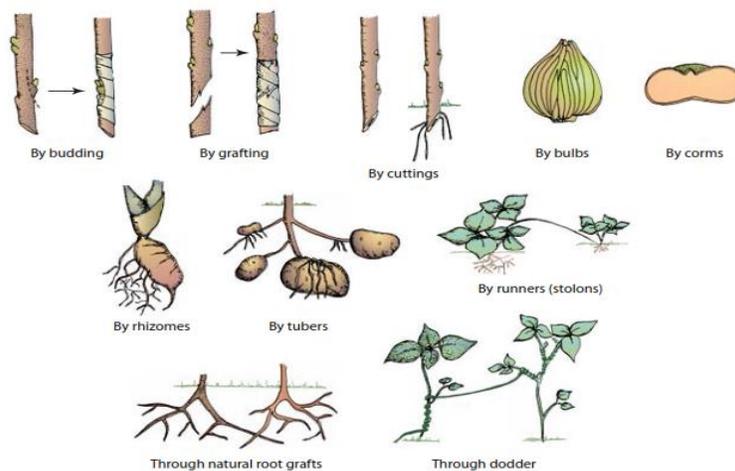


FIGURE 14-15 Transmission of viruses, mollicutes, and other pathogens through vegetative propagation, natural root grafts, and dodder.

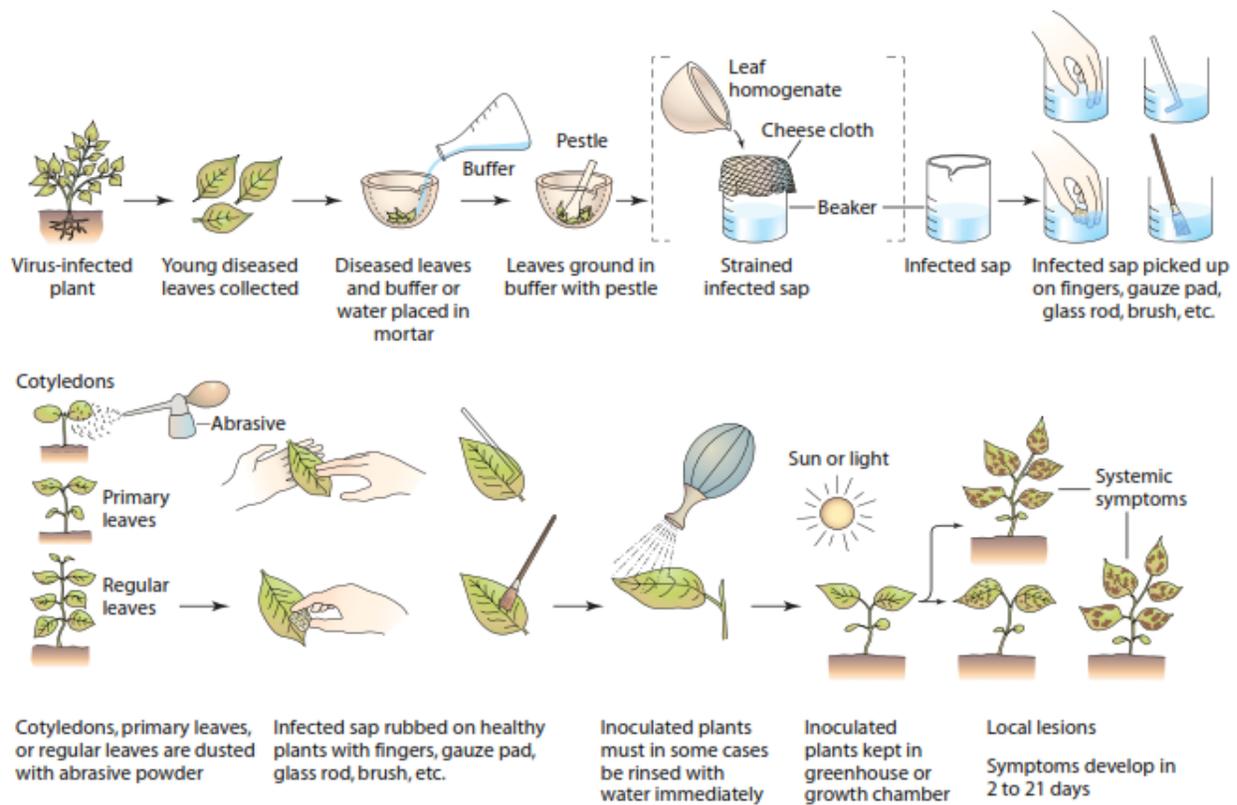


FIGURE 14-16 Typical steps in mechanical or sap transmission of plant viruses.

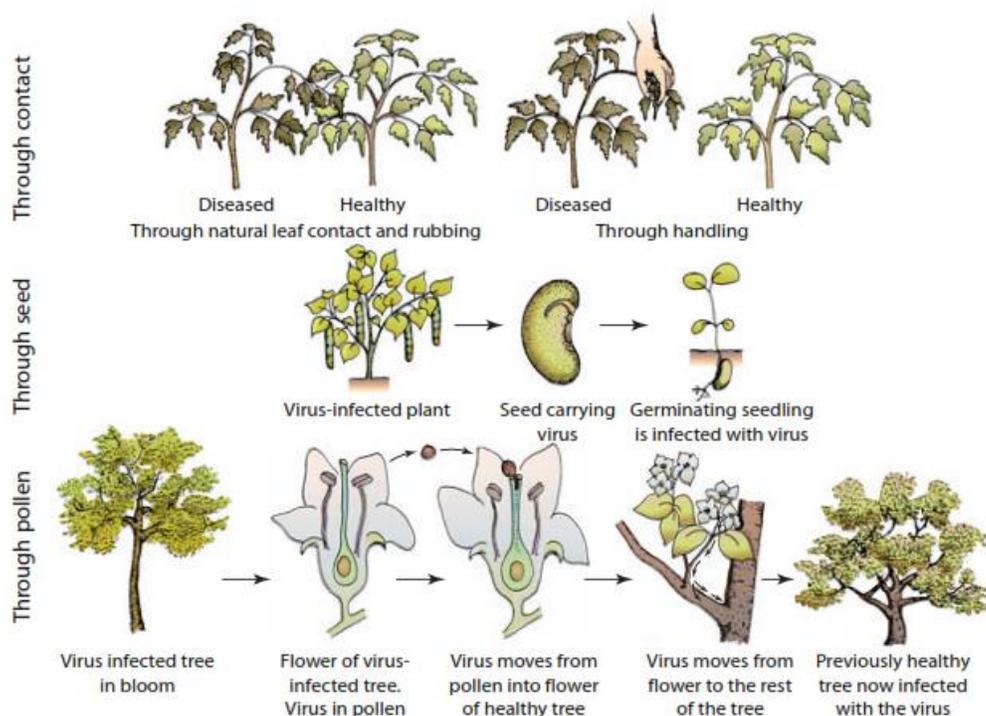


FIGURE 14-17 Plant virus transmission through direct contact of plants, handling, seed, and pollen.

Seed Transmission

More than 100 viruses are transmitted by seed to a smaller or greater extent. As a rule, only a small portion (1–30%) of seeds derived from virus-infected plants of only some hosts of the virus transmit the virus (Fig. 14-17). In some virus–host combinations, however, half or most of the seeds carry the virus, and in a few others 100% of the seeds carry the virus. The frequency of transmission varies with the host–virus combination and with the stage of growth of the mother plant when it becomes infected with the virus. In most seed-transmitted viruses, the virus seems to come primarily from the ovule of infected plants, but several cases are known in which the virus in the seed seems to be just as often derived from the pollen that fertilized the flower. In some host–virus combinations the virus is carried in the integument of the seed and infects seedlings as they are wounded on germination.

Pollen Transmission

Virus transmitted by pollen may result in reduced fruit set, may infect the seed and the seedling that will grow from it, and, in some cases, can spread through the fertilized flower and down into the mother plant, which thus becomes infected with the virus (Fig. 14-17). Such plant-to-plant transmission of virus through pollen is known to occur, for example, in sour cherry infected with *prunus necrotic ring spot virus*.

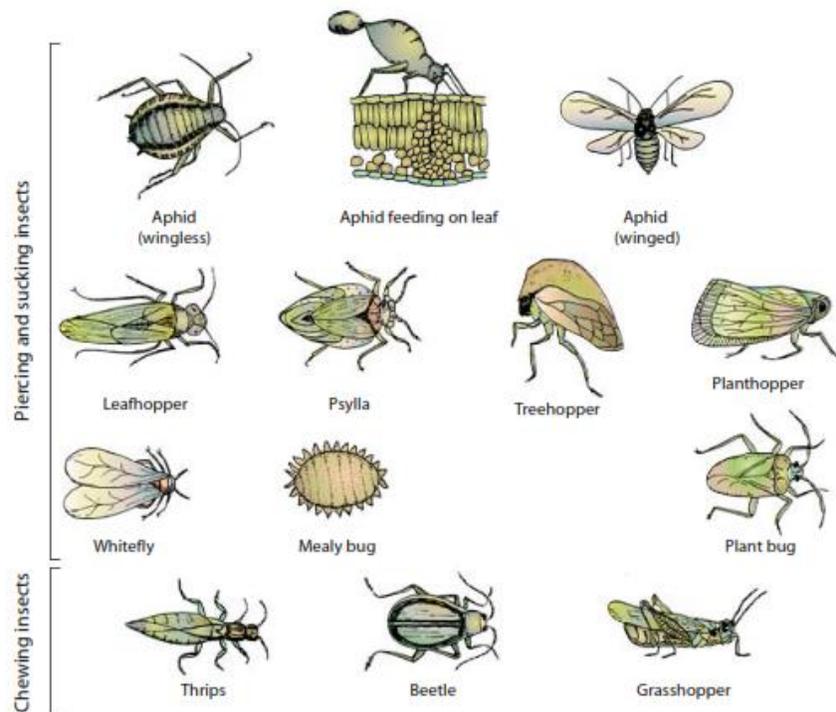


FIGURE 14-18 Insect vectors of plant viruses. Insects in second row from the top also transmit mollicutes and fastidious vascular bacteria.

Insect Transmission

Undoubtedly the most common and economically most important means of transmission of viruses in the field is by insect vectors. Members of relatively few insect groups, however, can transmit plant viruses (Fig. 14-18). The order Homoptera, which includes aphids (Aphididae), leafhoppers (Cicadellidae), and planthoppers (Delphacidae), contains by far the largest number and the most important insect vectors of plant viruses. Other Homoptera that transmit plant viruses are whiteflies (Aleyrodidae), which transmit the usually severe geminiviruses and several other viruses, mealybugs (Coccoidae), and certain treehoppers (Membracidae). A few insect vectors of plant viruses belong to other orders, such as true bugs (Hemiptera), chewing/sucking thrips (Thysanoptera), and beetles (Coleoptera). Grasshoppers (Orthoptera) occasionally seem to carry and transmit a few viruses also. The most important virus vectors are aphids, leafhoppers, whiteflies, and thrips. These and the other groups of Homoptera, as well as true bugs, have piercing and sucking mouthparts. Beetles and grasshoppers have chewing mouthparts. Of these, the beetles are quite effective vectors of certain viruses. Insects with sucking mouthparts carry plant viruses on their stylets —**stylet-borne viruses** — and can acquire and inoculate the virus after short feeding periods of a few seconds to a few minutes. Stylet-borne viruses persist in the vector for only a few to several hours. Therefore, they are also known as **nonpersistent viruses**. With some other viruses, the insect vectors must feed on an infected plant from several minutes or hours to a few days before they accumulate enough virus for transmission. These insects can then transmit the virus after fairly long feeding periods of several minutes to several hours. Such viruses persist in the vector for a few (1 to 4) days and are called **semipersistent viruses**. With still other viruses, the insect vectors accumulate the virus internally and, after passage of the virus through the insect tissues, introduce the virus into plants again through their mouthparts; these viruses are known as **circulative** or **persistent viruses**. Some circulative viruses may multiply in their respective vectors and are then called **propagative viruses**. Viruses transmitted by insects with chewing mouthparts (beetles) may also be circulative or may be carried on the mouthparts. Aphids are the most important insect vectors of plant viruses and transmit the great majority (about 275) of all stylet-borne viruses. As a rule, several aphid species can transmit the same stylet-borne virus, and the same aphid species can transmit several viruses. In many cases, however, the vector–virus relationship is quite specific. Aphids generally acquire the stylet-borne virus after feeding on a diseased plant for only a few seconds (30 seconds or less) and can transmit the virus after transfer to and feeding on a healthy plant for a similarly short time of a few seconds. The length of time aphids remain viruliferous after acquisition of a stylet-borne virus varies from a few minutes to several hours, after which they can no longer transmit the virus. In aphids transmitting stylet-borne viruses, the virus seems to be borne on the tips of the stylets, it is lost easily through the scouring that occurs during the probing of host cells and it does not persist through the molt or egg. Styletborne viruses are said to be transmitted in a nonpersistent manner. In the few cases of aphid transmission of circulative viruses, aphids cannot transmit the virus immediately but must wait several hours after the acquisition feeding; however, once they start to transmit the virus, they continue to do so for many days after the removal of the insects from the virus source (persistent transmission). Approximately 55 plant viruses are transmitted by leafhoppers, planthoppers, and

treehoppers, including viruses with double-stranded RNA, rhabdoviruses, small isometric viruses, and some geminiviruses. Leafhopper and planthopper-transmitted viruses cause disturbances in plants that affect primarily the region of the phloem. All such viruses are circulatory; several are known to multiply in the vector (propagative) and some persist through the molt and are transmitted through the egg stage of the vector. Most leafhopper and planthopper vectors require a feeding period of one to several days before they become viruliferous, but once they have acquired the virus, they may remain viruliferous for the rest of their lives. There is usually an incubation period of 1 to 2 weeks between the time a leafhopper or planthopper acquires a virus and the first time it can transmit it.

Mite Transmission

Primarily mites of the family Eriophyidae have been shown to transmit at least six viruses, including wheat streak mosaic and several other rymoviruses affecting cereals. These mites have piercing and sucking mouthparts (Fig. 14-19). Virus transmission by eriophyid mites seems

to be quite specific, as each of these mites is the only known vector for the virus or viruses it transmits. Another virus, *peach mosaic virus*, is transmitted by mites of the family Tetranychidae.

Nematode Transmission

Approximately 20 plant viruses are transmitted by one or more species of three genera of soil-inhabiting, ectoparasitic nematodes (Fig. 14-19). Nematodes of the genera *Longidorus*, *Paralongidorus*, and *Xiphinema* transmit several polyhedral-shaped viruses known as nepoviruses, such as *grape fanleaf*, *tobacco ring spot*, and other viruses, whereas nematodes of the genera *Trichodorus* and *Paratrichodorus* transmit at least two rod-shaped tobnaviruses, *tobacco rattle* and *pea early browning*. Nematode vectors transmit viruses by feeding on roots of infected plants and then moving on to roots of healthy plants. Juveniles as well as adult nematodes can acquire and transmit viruses; however, the virus is not carried through the juvenile molts or through the eggs, and, after molting, the juveniles or the resulting adults must feed on a virus source before they can transmit again.

Fungus Transmission

Root-infecting fungal-like organisms, the plasmodiophoromycetes *Polymyxa* and *Spongospora*, and the chytridiomycete *Olpidium*, transmit at least 30 plant viruses. Some of these viruses apparently are borne internally in, whereas others are carried externally on the resting spores and the zoospores of the fungi. On infection of new host plants, the fungi introduce the virus and cause symptoms characteristic of the virus they transmit (Fig. 14-19).

Dodder Transmission

Several plant viruses can be transmitted from one plant to another through the bridge formed between two plants by the twining stems of the parasitic plant dodder (*Cuscuta* sp.) (Fig. 14-15). A large number of viruses have been transmitted experimentally this way, frequently between plants belonging to families widely separated taxonomically. The virus is usually transmitted passively through the phloem of the dodder plant from the infected plant to the healthy one.

Q.1 How viruses can be detected by a rapid detection test?

Ans – Virus will be detected by enzyme linked immunosorbent assay (ELISA) as a rapid detection tool.

Q.2. What is viroids?

Ans- Viroids are plant pathogens: small, single-stranded, circular RNA particles that are much simpler than a virus. They do not have a capsid or outer envelope, but, as with viruses, can reproduce only within a host cell. Viroids do not, however, manufacture any proteins. They produce only a single, specific RNA molecule.

Viroids were discovered in 1971 by T.O. Diener, a plant pathologist. It is still not clear how they cause disease, especially since they may cause severe problems in one plant and no particular symptoms in a related species. They encode no proteins. Perhaps they bind to something in the cell and disrupt some regulatory mechanism. The RNA of many viroids contains sections of nucleotide sequence complementary to key regions at the boundaries of RNA introns; maybe that is how they damage cells. They also have nucleotide sequences similar to some seen in transposons and retroviruses.

Q.3 What is prion?

Ans - Prions, so-called because they are proteinaceous, are infectious particles, smaller than viruses, that contain no nucleic acids (neither DNA nor RNA). Historically, the idea of an infectious agent that did not use nucleic acids was considered impossible, but pioneering work by Nobel Prize-winning biologist Stanley Prusiner has convinced the majority of biologists that such agents do indeed exist.

Q.3. What is CRISPR-CAS 9 technique?

Ans - CRISPR-Cas9 is a unique technology that enables researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence.

It is currently the simplest, most versatile and precise method of genetic manipulation and is therefore causing a buzz in the science world.

Q.4 How CRISPR-CAS 9 works?

Ans - The CRISPR-Cas9 system consists of two key molecules that introduce a change into the DNA. These are:

An enzyme - called Cas9. This acts as a pair of ‘molecular scissors’ that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.

A piece of RNA-called guide RNA (gRNA). This consists of a small piece of pre-designed RNA sequence (about 20 bases long) located within a longer RNA scaffold. The scaffold part binds to DNA and the pre-designed sequence ‘guides’ Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome.

The guide RNA was designed to find and bind to a specific sequence in the DNA. The guide RNA has RNA bases that are complementary to those of the target DNA sequence in the genome. This means that, at least in theory, the guide RNA will only bind to the target sequence and no other regions of the genome.

The Cas9 follows the guide RNA to the same location in the DNA sequence and makes a cut across both strands of the DNA.

At this stage the cell recognizes that the DNA is damaged and tries to repair it.

Scientists can use the DNA repair machinery to introduce changes to one or more genes in the genome of a cell of interest.

Study of phanerogamic plant parasites.

PHANEROGAMIC PLANT PARASITES

Most of the diseases are caused by fungi bacteria and viruses. There are few seeds plants called flowering parasites (Phanerogams) which are parasitic on living plants. Some of these attack roots of the host, while some parasites on stem. Some are devoid of chlorophyll and entirely dependent on their host for food supply, while other have chlorophyll and obtain only mineral constituents of food from host by drawing nutrition and water they are called as Holoparasites or complete or total parasite. They have haustoria as absorbing organs, which are sent deep into the vascular bundle of the host to draw nutrients, water and minerals.

Flowering Plant Parasites: There are two types of parasites.

1) Root Parasites:

- i) Striga (Partial root parasite)
- ii) Orobanche (Complete root parasite)

2) Stem Parasites:

- i) Dodder (Cuscuta) (Complete stem parasite)
- ii) Loranthus (Partial Stem parasite)

1. Root Parasites:

1. Total or Complete or Holoparasite: Orobanche(Broomrape/Tokra)

It is annual flashy flowering plant growing to height of about 15-50 cm long, yellow or brownish colour and covered by small thin scaly leaves. Flowers appears in the axil of leaves are white or tubular. Fruits appears in the axil of leaves are white or tubular. Fruits are capsule containing and seeds are very small, black in colour remain viable for several years. The haustoria of parasite

penetrates into the roots of hosts and draw its nourishment. The growth of the plant is retarded, may die some times. It attacks tobacco, tomato, brinjal, cabbage, cauliflower.

2. Hemi Partial or Semi Root Parasite:

Striga (Witch Weed or Turfula or Talop)

Family *Scrophulariaceae*

It is a small plant with bright green leaves grows upto height 20-60 cm leaves bears chlorophylls and developed in clusters of 10-20 % host plant. They are obligate parasites therefore, do not obtain all their nutrient from their host root. Flowers are pink in colour, seed are very minute and produce in grate number 5000 to 100000 seeds plant per years. One flower contain 1200-1500 seeds and remains viable upto 12-40 years. Dissemination takes place with rain water, flood, wind and irrigation water. It cause yellowing and wilting of host leaves. It attacks sugarcane jowar, Maize, cereals and millets.



b. Stem Parasites:

1. Total or Complete or Holoparasite:

Cuscuta or dodder (Amarvel, Lovevine) Family *cuscutaceae*.

Genus – *Cuscuta*

It is non chlorophyllous, leaf less parasitic seed plant.

It is yellow pink or orange in colour and attached to the host. They do not bear leaves but bear minute function less scale leaves is produces flower and fruits. Flower are white, pink or yellowish in colour and found in clusters. Seed are form in capsules. A single plant may be produce 3000 seeds.

The first appearances of parasites is noticed as thread like leaf less stem which devoid of green pigment and twine around the stem or leaves of the host. When stem of parasitic plant comes in contact with host, the minute root like organs. i.e. haustoria penetrates into the host and absorbs. When the relation ship of the host is firmly established, the dodder plant loses the contact from soil.

These affect plant get weakened and yield poorly the seeds spread by animals, water and implements and remain viable when condition are unfavorable.

It attacks berseem alfalfa, clover, flax, onion, potato, ornamental and hedge plants.

2. Partial, Semi or Hemi Stem Parasites: Loranthus

Family-Loranthaceae.

It is a partial parasite of tree trunks and branches with brown stem, dark green leaves but no roots.

1. Stem is thick and flattened of the node, appear in clusters at the point of attack which can be easily spotted on the trees.
2. At the point of attachment with the tree, it shows swellings or tumourous growth where the haustoria are produced. It produces flowers which are long, tubular, greenish, white or red colour and found in clusters. It produces fleshy berries with single seed.
3. The affected host plant becomes stunted in growth and dispersal of seed is mostly through the birds and animals. It attacks mango, citrus, apple, guava.

