MSCBOT-501

M.Sc. I Semester

# FUNGI, LICHEN, VIRUSES AND BACTERIA

DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

**MSCBOT-501**

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# CONTENTS

# BLOCK-1- BASICS OF FUNGI AND LICHENS

# UNIT-1 HABIT, GENERAL CHARACTERISTICS, NUTRITION AND REPRODUCTION OF FUNGI

## Contents:

## *1.1 OBJECTIVES*

After reading this unit students will be able-

- To know about the habit and general characteristics of fungi
- To understand about the nutrition of fungi
- To learn about the reproduction and life cycle of fungi.

## *1.2 INTRODUCTION*

The word "Fungi" (singular Fungus) is derived from Latin word FUNGOUR means to flourish, previously used with reference to Mushroom. The fungi are achlorophyllous and heterotrophic thallophytes. They constitute a large and diverse group of plant kingdom. Their resemblance with algae in many respects and therefore, included in the group thallophyta because their plant body consists of a thallus made up of hyphae which together constitute mycelium. In usage meaning of the word has been expanded and includes thallus like, no-green plants such ***as molds, yeasts*** and other similar organisms closely related to mushrooms (Fig. 1.1). Thus, the fungi are a large group of simple thallus like plants, lacking chlorophyll.

At present, about 5100 genera and more than 50,000 species of fungi are known, but this number is constantly increasing because of the new researches throughout the world. The study of fungi is known as Mycology ***(mykes = mushroom***, ***logos= study***), and the scientist concerned with fungi is called Mycologist. It deals with the life- histories, their relationships and evolutionary techniques of fungi alongwith the studies of their taxonomy and classification.

**Habitat of Fungi:** The fungi are most diversified in their habitat. They are found in almost all possible types of habitats in the earth, where organic material either living or dead is present (Fig. 1.2). Thus they are universal or cosmopolitan in distribution. Most of the fungi are terrestrial, occurs in soil, rich in dead and decaying organic materials. The terrestrial fungi thrive best in humus soil. Some other species are aquatic found in the water and are called the aquatic fungi.

In this category most of the Phycomycetes are categorized under aquatic habitats. The aquatic fungi are considered primitive ones. They live on decaying organic matter and living organisms found in fresh water and produced motile flagellated reproductive cells. These swims to new localities. The terrestrial fungi are considered as more advanced than aquatic ones. They produced non- motile reproductive cells which passively dispersed by wind, water or by animals etc. However, many fungi grow on foodstuffs such as fruits, vegetables, jams, pickles, bread etc.

Some fungi are found upon algae and other aquatic plants in epiphytic state. Some fungi are found in drinking water and hence cause many harmful and severe diseases to human beings.

Some species are even subterranean and found under the surface of the earth. They are present all the time in air that we breaths.

The parasitic fungi found upon the hosts, mostly in the vascular plants i.e. ***Pinus, Cycas*** etc., and cause various diseases and do great harm to the host. Even some fungi are found in the alimentary canals of mammals and human beings where they cause the stomach disorder. Some fungi can cause the skin diseases. Mostly they prefer to grow in dim and darkness.

*Fig.1.1: Agaricus species-A common Mushroom*

*Fig.1.2: Fungus showing habit sketch*

## *1.3 HABIT OF FUNGI (SOMATIC OR VEGETATIVE PHASE)*

**Plant Body**: The plant body or vegetative phase of fungi is represented by thallus. It may be unicellular or multicellular filamentous (Fig. 1.3 & 1.4).

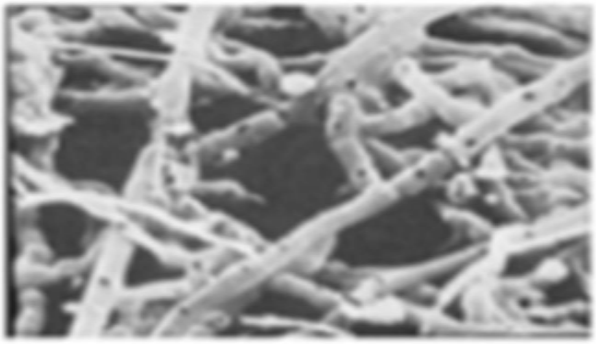

*Fig.1.3: Structure of fungal mycelium*

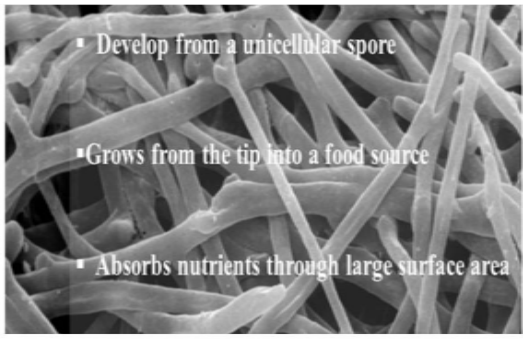


*Fig.1.4: Fungal mycelium network*

**Unicellular Thallus**: In lower fungi especially in unicellular structure more or less spherical in shape. It performs both the phases of life within a single cell. After maturation or completion of vegetative phase it performs the sexual phase such fungi are known as ***holocarpic fungi***, but both the phases of life cycle do not occur together in same thallus (***Synchytrium)***. In this case mycelium is completely absent.

Contrary to this, in Plasmodiophora, a naked multinucleate, amoeboid mass of protoplast represents the vegetative phase and is termed ***plasmodium***. The protoplast of diploid plasmodium divide to form resting spores, these spores further forms vegetative phase. Similarly ***Yeasts*** also have a unicellular thallus but sometimes cells remain attached in chains forming a pseudomycelium and thus related to filamentous forms.

**Filamentous Thallus:** The plant body of most of the fungi is branched and filamentous hyphae or filamentous thallus which forms a net like structure known as mycelium except some unicellular fungi. The mycelium originates through germination of spores. Germination of spores takes place after getting suitable substratum where conditions for life are favourable. When the spore after germination produces only a short, tubular structure of limited growth it forms to a net like interwoven cottony mass of fine branched filaments. The long, fine structures or filaments are called as hyphae.

After some time at the stage of maturity, some of these hyphae extend into the air and bear reproductive bodies while the remaining ones continue the normal growth. Such fungi are categorized under ***eucarpic fungi***. The hyphae form the vegetative body or thallus of the fungi known as mycelium or we can say that mycelium is the structural unit of fungi and the medium upon which the mycelium grows is known as substratum. Mycelium is the most significant part of fungus it procure food, and act similarly as a plant cell and perform all the activities of life-cycles i.e. respiration, absorption, digestion, excretion and growth except photosynthesis ( due to lack of chlorophyll)

The embedded hyphae are usually colorless and constituting the mycelium branch and spread it in all possible directions within and over the substratum and forms a loose and ramify network. While in some cases the aerial hyphae becomes colored. Generally they may be with blue, black, brown, yellow red and orange colored tints. Hyphae grow in length at the tips and termed as apical hyphae.

**Types of Mycelium**: The fungal mycelium is a simple multinucleate structure lacking completely the internal boundaries. The hyphae may be segmented or non- segmented. The segment hyphae possess cross walls in them at regular intervals called septa. The mycelium having septa called septate while the hyphae without cross walls are known as aseptate ones (Fig. 1.5). On the basis of presence or absence of internal septa, the fungal mycelium may be divided into two categories.

**(i) Aseptate Mycelium**: In the lower fungi or algal fungi the Phycomycetes the plant body in the vegetative phase usually lacks internal partitions of any kind, although it bear multinucleus. Thus the hyphae are aseptate and multinucleate. It grows terminally through apical parts of the hyphae and simultaneously accompanied by increase in the number of nuclei by nuclear divisions. This type of mycelium with multinucleus and lacking septa/ or partition internally is

also called as Coenocytic. However, during reproductive phase septa formation takes place either to cut-off reproductive structure (conidia, spores etc.) or to seal off a damaged portion, septa lacks pores and is solid plate only.

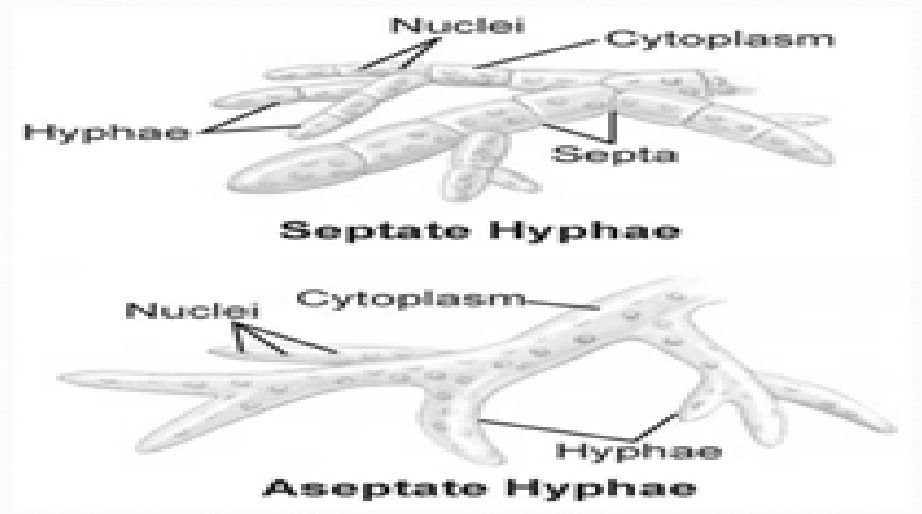


*Fig. 1.5: Types of Fungal mycelium*

**(ii) Septate Mycelium:** Phycomycetes, lacks septa in their hyphae while other higher fungi i.e. Ascomycetes and Basidiomycetes, they develop internal cross walls in the mycelium called septa hence the hyphae divide into segments. These segments nay be uninucleate or multinucleate and appear at regular intervals behind the tip of hyphae. Generally the septa in a septate mycelium are transverse while oblique or longitudinal septa are rarely appeared. The formation of septum is always originated by the division of nucleus. In the binucleate and multinucleate cells there is simultaneous division takes place in all the nuclei before wall formation. Usually each septum bears a pore for the communication of the cytoplasmic organelles and even the nuclei from one cell to another cell. Thus it clears that the septa is not the complete and it is evident that the distinction between aseptate (coenocytic) and septate hyphae is not so intense as it was thought before.

**Origin of septa**: The origin of septa in all fungi, possess septa i.e. Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Imperfect fungi) is always similar. These septa or segmentation (the cross walls) are arise in regular intervals behind the hyphal tip. Based on their nature of origin the septum may be either annular, when septum originate at the periphery as a ring inside of tubular hyphal wall or centripetally, when the annular growth grows slowly inwards (inside) the centre increasing in width and decreasing the pore diameter like an iris diaphragm (Fig. 1.6). Due to this firstly a cross wall or septum. The centripetal (towards the centre) growth of the septum is rapid in fungi.

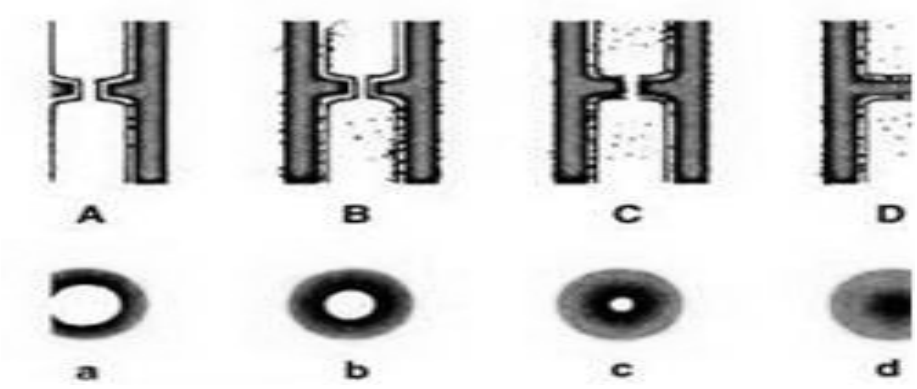


*Fig.1.6: Stages in the development of septa*

**Septal Pore**: Usually each septum bears a small pore in its centre for the protoplasmic continuity from cell to cell. The septa in the septate fungi is a simple pore in the middle of the cross wall of mycelium. It occurs in all the septate fungi except, the Basidiomycetes. In some cases may be slightly swollen where pore formation takes place. While in Basidiomycetes it is more complex due to further elaboration and named as **'dolipore'** ***(Latin- dolium means a large jar*).** In this case, the rim of the central pore is somewhat swollen and thick and form a barrel shaped structure with open ends. These open ends are guarded by cap like covers. Under electron microscope a curved double membrane on each side of the septum was seen. This membrane structure looks like a parenthesis (Fig. 1.7 & 1.8). Thus the central pore cap is therefore, called the ***parenthosome.*** The simple complete pore however, permits the free movement of cytoplasm, mitochondria and nuclei from one cell to another but the parenthesome may close the passage under certain conditions. However, presence of parenthesome is the significant point and characteristic feature of Basidiomycetes.

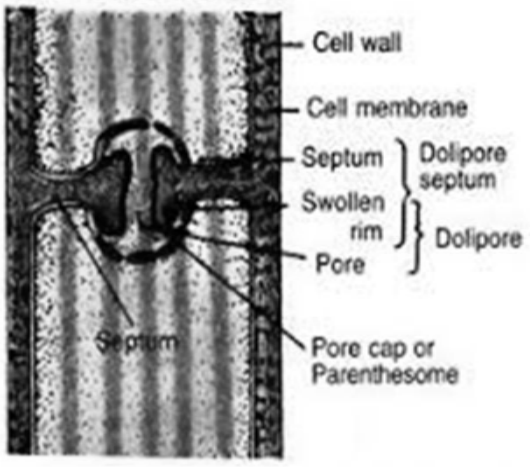


*Fig.1.7 Annular septa of a Basidiomycetes with dolipore septa*

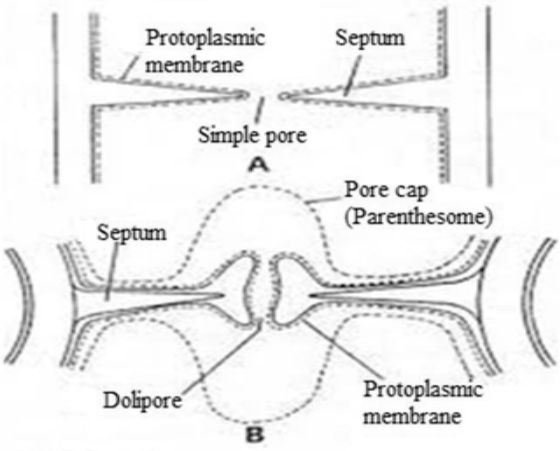


*Fig.1.8 Septal pore in Fungi A- simple pore, B- dolipore in Basidiomycetes*

**Structure of fungal cell**: Usually all the fungal cell consists of a strong, rigid cell wall except the slime molds. A typical fungal cell (Fig. 1.9) possess following structures:

**Cell wall**: The composition of cell wall is not similar in all the fungi. It vary among different group of fungi or even between the different species belongs to same group. The cell wall of fungi in general consists of chemically different substance from normal cellulose called fungus cellulose or a chitin. It is revealed through electron microscopic studies that cellulose and chitin are formed by elongated microfibrillar unit and the suggested molecular formula for the above is $C_{22}H_{52}N_4O_{21,}$these are arranged in layers and forms the basis of the structural rigidity of fungal cell and run parallel to the surface. Non- fibrillar components are also associated with the microfibrillar components.

The fungal cellulose may constitute carbohydrates, cellulose, pectose, callose and related compounds mixed with other substances. The composition of the cell wall is not a constant feature and changes according to the age of mycelium, temperature, composition and $P_H$ of the medium. The cells of the mycelium, as a whole are filled up with colorless cytoplasm. In aseptate (coenocytic) mycelium numerous nuclei are embedded in the cytoplasm beside this many irregular vacuoles also found. The oil droplets and glycogen granules also presents in food reserves in the cytoplasm. Most of the parasitic fungi secrete certain enzymes on living plants which dissolve the host cell wall.

In higher fungi and especially Zygomycetes, the basic structural component of cell wall is chitin, principally a polysaccharide having a nitrogen containing sugar naming glucosamine. The other substances probably closely or loosely associated with chitin in cell wall are as protein, lipid, Pectic materials, cellulose and minerals. However, there is no clear evidence about this association. Based on several studies it is reported that the wall of Ascomycetes and Basidiomycetes is made of the insoluble B- glucon which is the predominant structural material (Burnet, 1968). However, presence of chitin is reported. While, in the yeasts and a few other Hemiascomycetideae chitin is absent, but as per studies presence of lignin is also reported in several fungi by some investigators. Contrary to this in the lower fungi especially in biflagellate Oomycetes they show distinct nature of cellulose of the cell wall from other fungi, while, glucon predominates in their cell walls.

**Protoplast**: Within the cell wall cell contain protoplast, the living material. A typical fungal cell is differentiated into all other usual cell parts as in other living cells i.e. cell membrane or plasma membrane, cell organelles- mitochondria, Golgi apparatus, endoplasmic reticulum, vacuolated cytoplasm and one or more nuclei. Chloroplast is totally absent in fungal cell.

**Cell or plasma membrane**: It is a living membrane, extremely thin, delicate and closely lining with the protoplast. The plasma membrane pressed against the hyphae wall except the invaginational regions. The plasma membrane performs special functions and is the surface layer of the protoplast and is differentially permeable by nature.

**Cytoplasm:** The plasma membrane encloses the colorless material, cytoplasm with sap filled vacuoles. In young hyphae and hyphal tips, the cytoplasm looks rather homogenous comparatively to mature hyphae. In the cytoplasm other organelles and inclusions are emerged. The organelles are living structures and each have a specific role and function. The organelles included mitochondria, ribosomes, endoplsmic reticulum, golgy apparatus and many vacuole, all these are living organelles. In between cell wall and plasma membrane, some specific membranous structure, the lysosomes are common. Pigments, secretory glands and stored food in the form of glycogen and oil drops are present in the form of inclusions.

**Endoplasmic reticulum**: The presence of ribosomes in fungal cytoplasm is invested after the discovery of electron microscope. Endoplasmic reticulum of fungal cell is composed of membranes or micro-tubular structures usually surround with small granules. Comparatively to green plants it is more regular and loose in fungi, but in some fungi it is highly vesicular.

**Mitochondria**: The small usually spherical bodies, the mitochondria also present in the cytoplasm. It is in- folded and form cristae which are in the form of irregular tubules or in the parallel flat plate like structures. Like green plants, the fungal mitochondria also function as the "power house" of the cell,

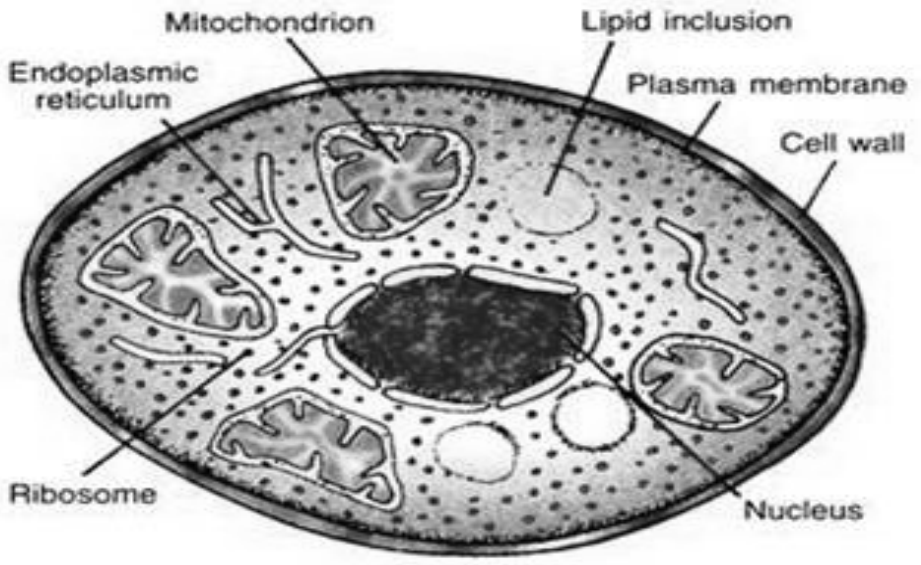


*Fig.1.9: Fine structure of Torula yeast cell based on an electron monograph*

**Golgi apparatus**: In fungal cell, there is exceptional certainity of the presence of golgy apparatus (dictyosomes) like structures except the members of Oomycetes.

**Vacuoles:** Presence of vacuoles in fungal cell is a prominent feature. They appear in the old cells but in young hyphae or cell and in hyphal tips lack vacuoles. They enlarge with age and show

tendency to coalesces and ultimately reduce the cytoplasm to thin living layer immediately within the cell wall.

**Inclusions:** Various types of inclusions are also present in the cytoplasm including stored food materials in the form of carbohydrates, lipids granules, glycogen granules, oil droplets, volutin and some proteinaceous material. The fungi lacks chlorophyll and starch while carotenoids are present either throughout the cytoplasm or concentrated in the form of small lipid granules. Beside this, it also contains some other kinds of enzymes and organic acids.

**Nucleus:** Each individual cell contains one, two or more nuclei in the somatic cell depending upon the condition or species. The shape and size of nuclei is also vary from globose to spherical, ranging from 1-3 u in diameter and cannot be visible without special techniques. A single nucleus consists three clear cut portions i) The outermost definite nuclear membrane ii) Inside this body contains chromatin strands and iii) the central body with a clear area around it. On staining the central body takes heavy iron haematoxylin stain and is usually Feulgen negative in nature. It appears amorphous or granular mass under electron microscope, as well as the nuclear membrane also show three layers structure and consists of inner and outer layers filled with electron dense material while the middle one shows electron transparent substance. On electron microscopic studies it is also reveals that the nuclear membrane at certain points is continuous with the endoplasmic reticulum and also has pores in it.

**Fungal Flagella**: Presence of flagella is the characteristic feature of lower fungi, the Phycomycetes as they have motile cells in their life cycle except in case of Zygomycetes. However, higher fungi, i.e. Ascomycetes, Basidiomycetes and Deuteromycetes, lack the motile cells in their life cycle. In the lower fungi, the zoospores and gametes, represents the motile cells are bearing one or two whip like threads of protoplasmic material, the flagella. These flagella are thin hair like emergenses of the cell cytoplasm and related to locomotion.

**Structure of the Flagellum:** Internally a flagellum has a central or axial filament known as axonema. The axonema is surrounded by a double membrane, composed of cytoplasm and is an extension of plasma membrane of the motile cell. The axosome is composed of minute fibrils arranged in 9+2 arrangement among which two are central and nine are peripheral (Fig.1.10) studied under electron microscope. The nine peripheral fibrils each fibril is composed of two thin fibrils lie side by side and form an axial thread, elastic in nature and may be enclosed by a subsidiary membrane of its own. While both the central single fibrils sometimes surrounded by a subsidiary sheath of its own. The two central or axial fibrils are enclosed by the nine peripheral doublet fibrils. Again the 9+2 set of fibrils is enclosed within a double layered cytoplasmic sheath. This type of 9+2 arrangement of flagellum is to be the basic and significant structure of the flagellum of motile cells of eukaryotic fungi except bacteria.

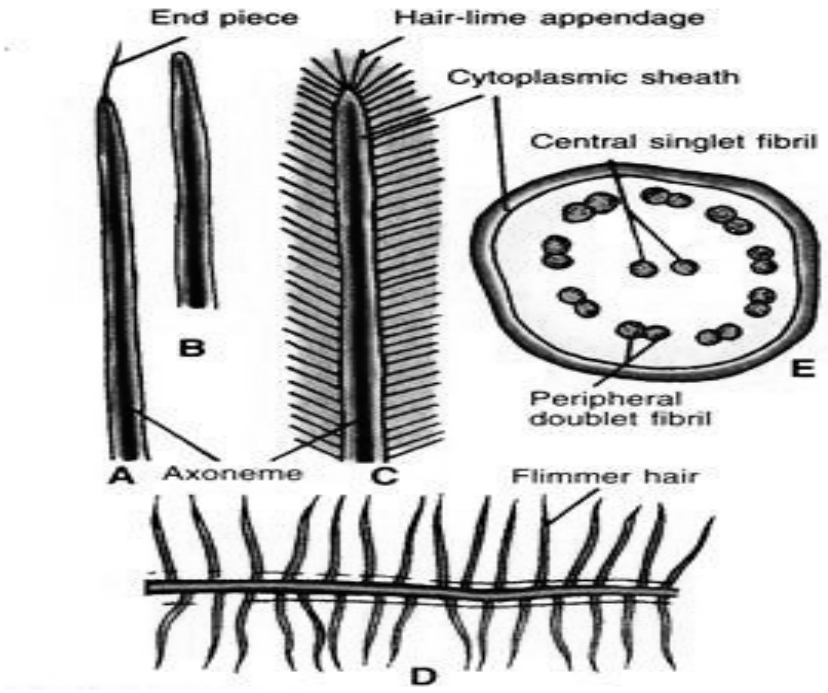


*Fig.1.10: Fungal flagella-A- L.S. upper portion of an acronematic or whiplash flagellum with a drawn out pointed tip or end piece, B- L.S. whiplash flagellum with blunt tip, C- L.S. pentonematic or tinsel flagellum, E- the eukaryotic flagellum showing 9+2 arrangement of fibrils, D- diagrammatic repr*esentation of a part of the tinsel flagellum from a electron micrograph (Based on Manton et. al.).

**Kinds of Flagella:** In fungi three types of flagella are reported among the Whiplash type and tinsel types are common while the whiplash one is again sub divided into two sub categories (Fig. 1.11).

**(i) Whiplash Flagellum**: It resembles morphologically like a "Whiplash" and has a smooth surface and narrows towards the tip to form a distinct end piece. It is supposed that the end piece is a thin auxesome of varying length. On the basis of the structure of end part this flagellum is again categorized into further divisions such as-

a) Whiplash with an end piece and

b) Whiplash flagellum with a blunt tip.

The whiplash flagellum is also known by the names if acrometic or peitchgeisal flagellum.

**(ii) Tinsel flagellum**: It does not have the structure like whiplash flagellum ending with a blunt end but bears small, lateral hairs like appendages known as flimmer hair or mastigoneme. From anxoneme arises the flimmer hairs on the tinsel flagellum are also named as flimmer or filmmergeisel or pantonematic flagellum.

**Flagellation in Lower Fungi**: Detailed studies of flagella in fungi i.e. its number, kind and position on the motile cell is known as flagellation. While in case of classification of lower fungi or especially in Phycomycetes flagellation plays an important role. As perstudies each class of

the lower fungi it is constant and differ from class to class. Thus it is an important character in classifying the lower fungi into different classes. Among lower fungi i.e. Chytridiomycetes the motile cell furnished with a single whiplash type flagellum.

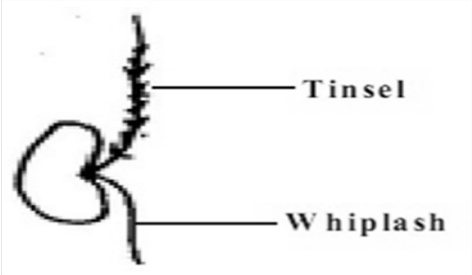


*Fig. 1.11 Structural differences in tinsel and whiplash type flagella*

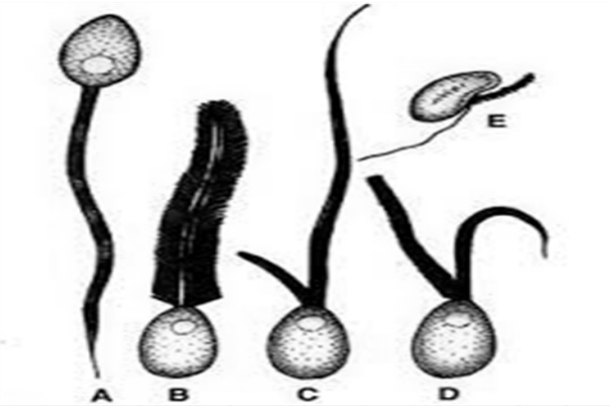


*Fig. 1.13 Flagellation in Lower fungi*

## *1.4 GENERAL CHARACTERISTICS OF FUNGI*

1. Fungi are cosmopolitan in distribution and can occur in any habitat where life is possible. Some fresh or marine water, some terrestrial and still others are air borne. Many species are parasitic infecting animals and plants.
2. Plant body filamentous hyphae which form network structure known as mycelium.
3. Hyphae aseptate and coenocytin in lower forms or septate and uni, bi or multinucleate in higher forms.
4. Septa usually have simple pores but in Basidiomycetes septa with dolipore which is a double membrane structure. A dolipore septum has a pore surrounded by a proteinous membrane called parenthosome (Fig. 1.7).
5. Except slime molds the protoplast remains surrounded by a distinct cell wall. The main component of cell wall is fungal cellulose known as chitin. But in lower fungi (Oomycetes) the cell wall is composed of cellulose and glucon.
6. Fungi are entirely devoid of chlorophyll, but carotenoids are normally present.
7. Hyphae are homo or heterokeryotic or diploid (Diploid phase is usually ephemeral).
8. Higher fungi are immotile i.e. motile cells do not occur in their life at any stage. But lower fungi (gametes and asexual zoospores are uni or biflagellate).
9. Flagella are two types (i) Whiplash type (acronematic) or smooth walled and (ii) Tinsel type (pantonematic) with numerous minute hair like mastigonemes on their surface.
10. Hyphae structure is variously modified into (Fig: 1.12)

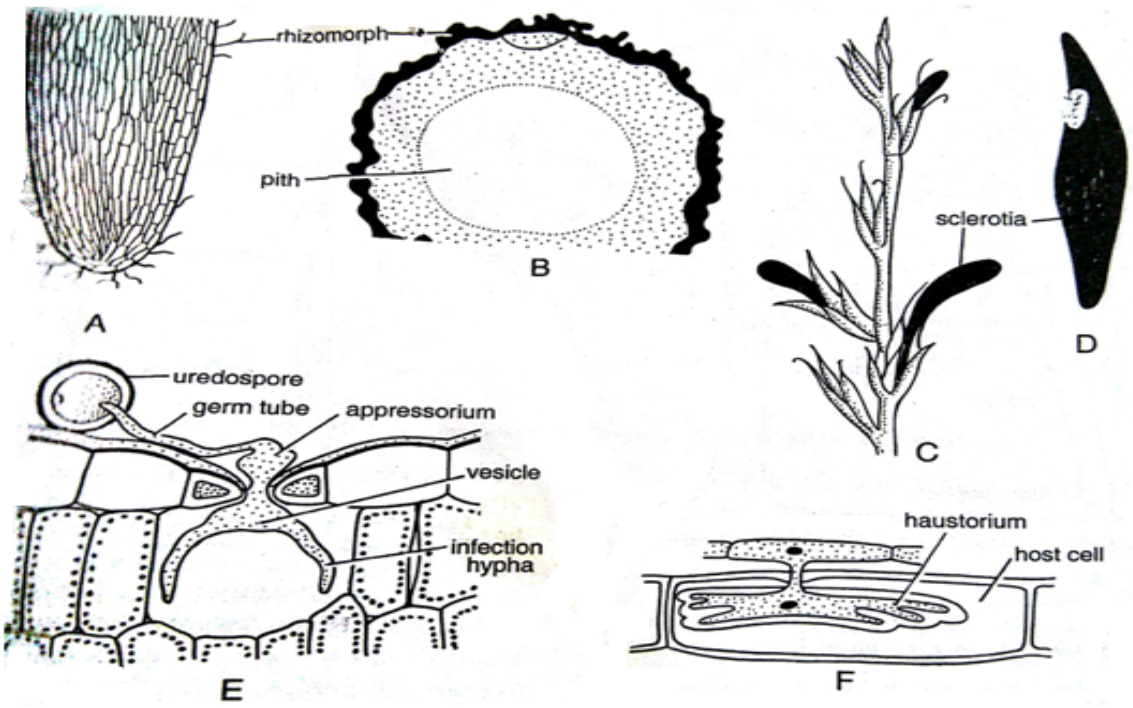


*Fig.1.12: Modifications of hyphal structure: A-B, Rhizomorph; C-D. Sclerotia; E. Appressorium; F. Haustorium*

**I- Prosenchyma-** When the hyphae lie more or less parallel to one-another, they unite to form a rather loosely interwoven structure.

**II- Pseudoparenchyma**- When hyphae become closely intertwined forming a tissue consists of hollow tube running in all direction.

**III- Rhizomorph**- It is a thick strand or root like aggregation of somatic hyphae.

**IV- Sclerotium-** It is a compact globose, structure formed by the aggregation and adhesion of hyphae. May survive for long period showing the resting stage of fungus, help in vegetative reproduction.

**V- Appresorium-** It is a terminal simple or lobed swollen structure of germ tubes or infecting hyphae found in many parasitic fungi e.g., rusts, and powdery mildew helps in penetration.

**VI-Haustoria**- Intercellulary absorbing structures of obligate parasitic fungi, meant for absorbing food material from the help of different shapes.

**VII-Hyphal traps (Snares)-** The predatious fungi develop sticky hyphae or network of hyphal loop known as hyphal traps which helps in capturing nematodes.

**VIII-Stroma-**These are compact somatic structures much like mattresses. Fructifications are usually formed in them.

11. Fungi are heterotrophs, may be-

(A) **Parasites-** (i) Obligate parasites e.g.-*Puccinia*, (ii) Facultative parasites e.g., *Ustilago*

(B) **Saprophytes-** (i) Obligate Saprophytes e.g., *Mucor*, (ii) Facultative Saprophytes e.g., *Fusarium*.

(C) **Symbionts**- e.g., Lichens and Mycorrhiza.

12. Some are eucarpic (only a part forms reproductive units). In unicellular fungi whole cell transferred to reproductive unit i.e. are Holocarpic.
13. Reproduction takes place by all three types-

(A) **Vegetative:** By (i) Fragmentation e.g., *Rhizopus, Mucor*
(ii) Fission e.g., *Yeasts*
(iii) Budding e.g., *Ustilago*

(B) **Asexual:** During favourable conditions by a variety of spores. Spores may be unicellular e.g., *Aspergillus* or multicellular e.g., *Alternari*a, may be endogenous e.g., *Mucor* or exogenous, e.g., *Aspergillus*.

(C) **Sexual:** Process of sexual reproduction is completed in three phases:
**I. Plasmogamy-** Fusion of protoplast by two compatible gametes.
**II. Karyogamy-** Fusion of two opposite nuclei.
**III Meiosis: reductional nuclear division.**

In Phycomycetes lower fungi karyogamy occurs just after plasmogamy. In Ascomycetes karyogamy is delayed while in Basidiomycetes there is a long gap between plasmogamy and karyogamy. Plasmogamy occurs in quite earlier in vegetative phase while karyogamy is delayed (Fig. 1.14).

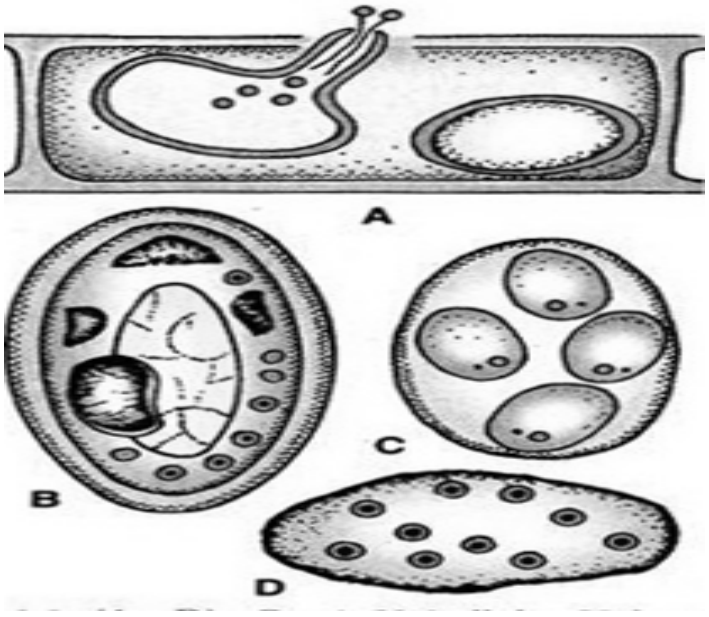


*Fig. 1.14: Fungi Unicellular Holocarpic Thalii A (A to D) A- Olpidium endogenum (holocarpic chytrid), two organisms within the algal host, B- Yeast unicellular thallus, C- Reproductive phase of B, D- Diploid plasmodium of Plasmodiophora*

**Fungal Tissues:** AS mentioned earlier a fungal mycelium is a loosely interwoven mass of hyphae to form a network. In some cases either the whole mycelium or its some part modified to

form a false tissue. Such false tissue is called Plactenchyma. All fungal tissue come under this term which further sub- categorized into two parts-(i) Prosenchyma and (ii) Pseudoparenchyma (Fig. 1.15).

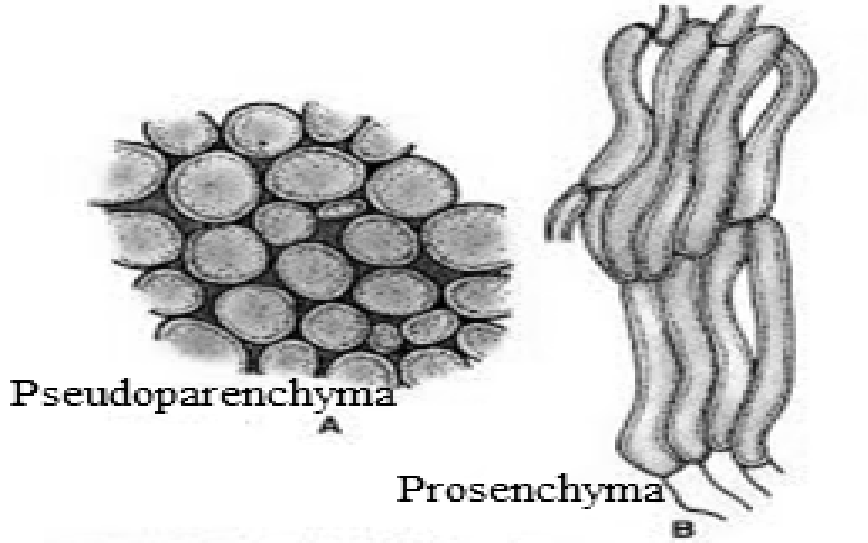


*Fig.1.15-(A-B) : Fungi, kinds of fungal tissues A- Pseudoparenchyma, B- Prosenchyma (Diagrammatic)*

**(i) Prosenchyma-** This tissue is composed of loose interwoven hyphae. These hyphae runs parallel to each other and maintain their identity. The cells are elongated in shape.

**(ii) Pseudoparenchyma**- In higher fungi during reproduction, or fructification the hyphae become interwoven and forms a compact mass of hyphae and the hyphae fused together and lose their identityand transformed into oval or isodimetric cells which resembles the structure of parenchymatous cells of higher plants and hence is known as pseudoparenchyma.

## *1.5 NUTRITION OF FUNGI*

As clear from studies that fungi obtain their food either from dead and decaying materials or they absorb it directly through the cell membrane from the living organisms. They obtain their food material either from the living host (Parasites) or from dead organic matter (Saprophytes) in the form of liquid. They secrete certain enzymes which help in breaking down complex proteins and carbohydrates and other materials of host into simpler ones. On the basis of their mode of nutrition they are grouped into following categories-

**1-Saprophytes**: These fungi obtain their nutrition from dead and decaying organic matter. This type of mode of nutrition is known as saprophytic and the fungi are called as saprophytes. Some saprophytes i.e. ***Mucor mucedo*** can obtain their nutrition only from dead organic materials, and do not have the capacity to infect living plants or animals. They are known as pure

or obligate saprophytes, while contrary to this those saprophytic fungi i.e. ***Fusarium, Pythium*** have the capacity to infect living organisms and so known as facultative parasites.

**(a) Obligate Saprophytes**: These fungi which obtain their nutrition from other non living plants or animals. They can grow only upon suitable non living tissue. They can survive on dead and artificial media.

**(b) Facultative parasites:** The members actually thrive best if they grow on dead organic matters but they have potentiality of infecting the living plants and animals. e.g., Fusarium, Pythium, etc.

**2. Parasites:** The parasitic fungi absorb their food material from the living tissues of the hosts on which they parasitize. Those members which can only grow or live on living host and obtain their food material from them are known as parasites. Such parasitic fungi are quite harmful to their hosts and cause many serious diseases. Many diseases of the important crops are caused by parasitic fungi. The rusts, smuts, bunts, mildews and many other plant diseases are important examples of fungal diseases of crops. Their mode of life is parasitic and the relation of host and parasite is called the parasitism. Parasites which cause diseases are called pathogens. Some parasites can survive and grow only in living cells and are called biotrophs or Obligate Parasites. Others can infect their host and bring about it's death and then live saprotrophically on the remains, they are called necrotrophs or facultative parasites.

**(a) Obligate parasites:** The parasites which survive only on living hosts are called the obligate parasites. Powdery and Downey mildews are the best example of obligate parasites. They do not occur as saprophytes in nature although they can be grown on nutrient media in the living culture, e.g., *Puccinia*, *Peronospora*, *Reuenelia, Melampsora*, etc.

**(b) Facultative saprophytes:** These members are actually parasites but in the absence of living host, they can also thrive or live on dead organic matters in nature. They are termed facultative saprophytes, e.g., smut, like: Ustilago, Sphacelotheca.

**3-Symbionts:** Some fungi grow on other organisms and both are benefitted mutually, such association is known as symbiosis, **Mycorrhiza** and **Lichens** are common. The thallus of Lichen consists of a fungus symbiotically living with an alga.

Mycorrhiza is another example of symbiosis. In this case the fungus associated with the roots of certain higher plants, the forest trees such as ***Pinus*** tree. The fungal hyphae form a weft around the pine roots. The root hairs are absent in this region. Although the actual role of fungal hyphae is not known yet it is suggested that the fungal hyphae perform the work of the root hairs. They absorb water and minerals in solution from soil and transfer to the roots of the tree and on the

otherhand, the tree provides food to the fungus. This type of mutual beneficial relationship or association between a fungus and the roots of higher plants is called **Mycorrhiza.**

On the basis of its mode of fungal hyphae attachment on host it is categorized into two groups- i) when the fungal hyphae live on the surface it is called **ectotrophic** while ii) when they penetrate the root and grow inside the root, it is called **endotrophic**.

**Nutritional requirements**: All eukaryote consists of non-chlorophyllous heterotrophs, the fungi that absorb nutrients from dead and decaying or living organic matter, have cell walls composed of chitin and store excess energy as glycogen. The fungi utilize both organic and inorganic materials as the source of their nutrient supply. Due to absence of chlorophyll the fungi are unable to photosynthesize or use carbon-di-oxide to synthesize the organic materials. Thus in their natural habitats they obtain their nutrition from dead organic materials as saprophytes or from living organisms as parasites.

The constituent elements of the organic and inorganic substances which fungi use of are C,O, H, N, P, K, Mg, S, P, Cu, Mo, Fe and Zn. Calcium is required by some fungi but not all. Thus fungi used a variety of organic substances. Carbon the main source of fungal nutrition is obtained in the form of carbohydrates while in case of Yeast acetates used as source of carbon. Carbohydrates are essential and needed for building up the body as a source of energy.

Being heterotrophs fungi fulfill their needs of carbon and energy by breaking down and assimilating organic matter. Fungi are capable of absorbing and metabolizing a wide variety of carbohydrates including glucose, xylose and fructose. Most difficult carbohydrates are also easily digested such as cellulose and hemicellulose. For all fungi Glucose is suitable. Of the carbohydrates source of carbon most fungi use simple sugar. Some fungi use Mannitol and Maltose as a source of carbon. While for others sugar is also a good source of carbon. Some fungi are able to make growth on fat. Some other fungi utilize organic acid and higher alcohols as a sole source of energy while they grow better on a substance, containing a suitable carbohydrate.

For better growth beside carbon, fungi require nitrogen. To obtain nitrogen they utilize both organic and inorganic materials as the source. Protein, peptide, amino acids are the chief source of protein. Some specific fungi also show their special choice for certain nitrogen sources (e.g., ***Blastocladiales*** and ***Saprolegniales*** etc. grow only with organic nitrogen such as amino acids. Fungi are the good decomposers of proteins and other organic and inorganic materials fulfill their nitrogen requirement. As per studies generally in pure cultures gelatin, casein, egg albumin, amino acids, peptides can serve as sources of organic nitrogen for growth.

Water is the major constituent of fungal mycelium and forms about 85- 90% of the entire weight of the body. Beside these macronutrients or constituents of fungi require micronutrients also in fairly large amounts among them are sulphur, phosphorus, magnesium, potassium. The fungi

obtain their micronutrients from simple inorganic salts as for proper growth in a culture medium these must be supplied. Calcium in some cases required as micronutrients. Some trace elements such as Fe, Cu, Mg, Bo, Co, Mo are required by some fungi in minute trace. These trace elements or micronutrients are considered essential for growth.

The fungi also require some growth factors or vitamins in minute amount for growth, like all other organisms. Some fungi are capable to synthesize their own supply of appropriate growth factor or vitamins from a simple nutrient solution of definite composition. They are called auxo-autotrophic as they are independent or autotrophic for vitamins. While there are others, which are unable to synthesize essential growth substances and depend either in whole or in part for their needs of growth factors and are heterotrophic or dependent for their needs of growth factors and are termed as auxo- trophic. To function properly, the fungus needs a strong enzyme- thimine ($B_1$), rivoflavin ($B_2$), biotin, pyredoxin ($B_6$) etc. Nicotinic acid and pantothemic acid are also required by some fungi. While a vast majority however, require thimine ($B_1$). These growth factors act as catalyst in their action.

Thus in brief the basic nutritional needs of fungi are sum up as:

1. They need a suitable organic material as a chief source of carbon and energy.
2. They also need a suitable source of nitrogen.
3. Some micronutrients are also required for better growth including S, P, K, Mg, in little amount.
4. Some trace elements in the form of inorganic ions of Fe, Zn, Co, Mg and Mo also requires only in minute traces.
5. Certain growth factors, vitamins and enzymes in trace amounts also essential for growth.

Besides, all these above mentioned nutritional requirements growth of fungi is also influenced/ affected by certain other factors such as temperature, moisture, $O_2$ supply, PH value and by-products of metabolism.

**Mode of Nutrition**: As fungi are heterotrophs, they lack chlorophyll and thus unable to synthesize their food. They require their nutrition by absorption. They absorb small organic materials from the surrounding medium. They fulfill their needs of carbon and energy by breaking down the assimilatory organic matter. Thus divided into two categories according to their method of obtaining food namely- the saprophytes or commonly called saprobes and parasites. Saprophytes as defined *(sapro+ phytes* means*rotton material +plants*) or the plants absorbs the required organic nutrients (nutrition) directly through the cell membrane from the substratum which abounds in dead and decaying matter of both plant and animal origin. They cannot ingest solid food material. Saccharomyces (Yeasts) and molds are the best and common example of saprophytes. However, the parasites absorb their food from the living body of plant and animal. They absorb organic material through the cell wall from the tissues of the host (Fig. 1.16). The common examples of parasite are smuts and rusts. Some fungi live in close

association of higher plants where they are mutually beneficial to each other. This type of relationship where both the partners benefitted and the fungi get nutrition from living plant and animal both.

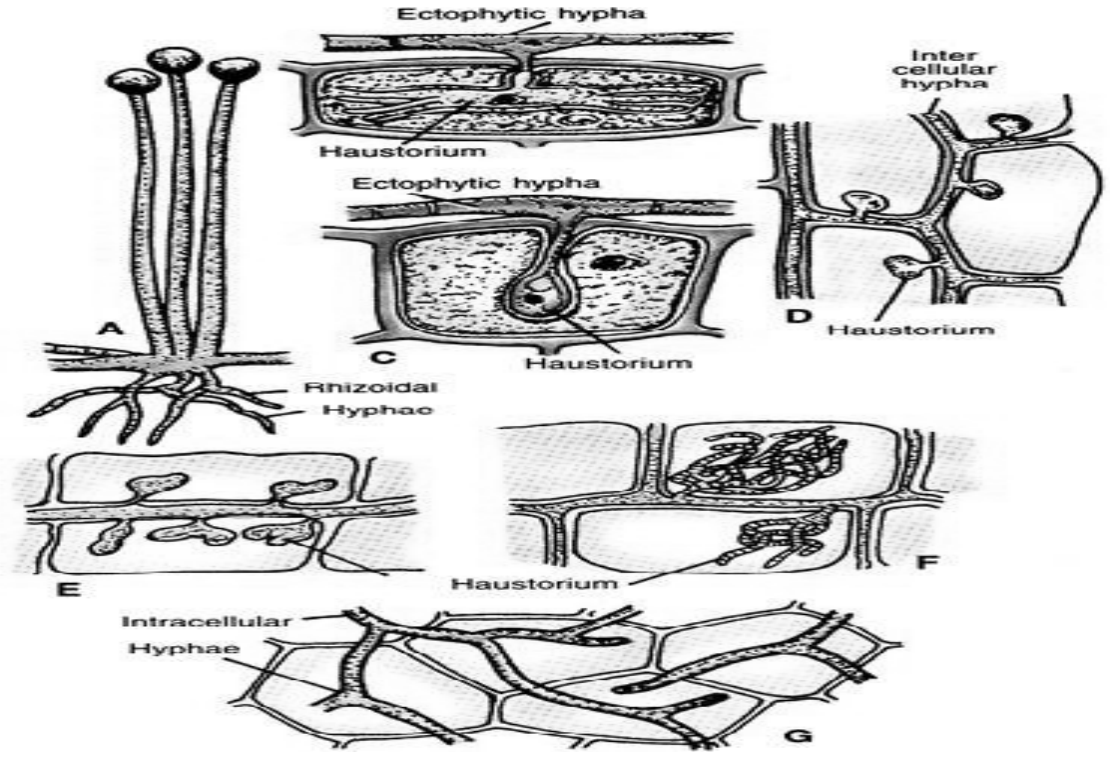


*Fig.1.16: Modes of nutrition in fungi*

This type of mode of nutrition is known as symbiosis and participants the symbionts. The most common example is Lichens and mycorrhiza. As in case of lichen the fungi associated with algae. The algal partner synthesizes the organic food and the fungal partner is responsible for the Absorption of inorganic nutrient and water also helps in reproduction. In another case certain fungi develop in the roots of higher plants (***Cycas, Pinus*** etc.) the mycorrhiza developed. The fungi absorb their food from the roots and in response beneficial to the plants. Based on their position in host cell the mycorrhiza may be external or internal. Thus they are known as ectophytic and endophytic respectively.

## *1.6 REPRODUCTION OF FUNGI*

Fungi reproduce naturally by a variety of means as vegetative, asexual and sexual ones.

**Vegetative Reproduction:** In vegetative reproduction budding, fission, sclerotia, rhizomorph are the common methods. In vegetative reproduction the union of mature sex cells or sex organs does not involve. The common method of vegetative reproduction are-

i. **Fragmentations**- when the hyphae break up into small fragments either accidentally or drying or dehydration. Each fragment develops into a new individual in favorable conditions.
ii. **Fission**- Fission of somatic cells yields two similar daughter cells. In fission of the cell constricts in the centre and divides into two cells.
iii. **Budding**- Budding of somatic cells or spores takes place and each bud is an outgrowth of the parent cell developing into a new individual, common example is *Saccharomyces* (Yeast cell).
iv. **Sclerotia**- The sclerotia are resistant and perennating bodies. They survive for many years. Structurally each sclerotium is cushion- like structure of compact mycelium. They give rise new myceli on favorable conditions.
v. **Rhizomorphs**- The rhizomorphs are the modified mycelium. They are resistant to unfavourable conditions and give rise new mycelia on favourable conditions.

**2-Asexual Reproduction-** The asexual reproduction takes place by means of different kinds of spores. Each spore may develop into a new individual. The main function of asexual spores is disseminate the species. These are produced in large number. In fungi there are many kinds of asexual spores (Fig. 1.17).

i. **Aplanospores-** The non- motile spore while zoospores are motile and their motility being due to the presence of flagella (***Mucor, Rhizopus, Pilobolus***).
ii. **Sporangiophores-** These are single celled spores and formed within sacs known as sporangia (singular sporangium) and formed at the end of the special hyphae.
iii. **Conidiophore** or **Conidia** (singular conidium) are small, single celled spores formed at the tip or side of the hyphae.
iv. **Chlamydospores-** These are thick walled, single spores and formed from cells of the vegetative hyphae. These spores are highly resistant to adverse conditions (***Ustilago).***
v. **Oidia or (oidium** singular) are also single celled spores and formed by disjointing hyphal cell and are also known as arthrospores (***Collybia, Coprinus).***
vi. **Blastospore**- These spores are formed by budding.

**3-Sexual Reproduction-** A large number of fungi reproduces sexually. However, the members of higher fungi Deuteromycetes or "Fungi Imperfecti" lack sexual reproduction. Sexual reproduction is carried out by fusion of compatible nuclei of two parent cell. The process of sexual reproduction begins with the joining of two cells and fusion of their protoplast this process is known as plasmogamy. In the second step, fusion of two nuclei from the two fusing gametes takes place and a diploid zygotic nucleus is formed. This phase is known as Karyogamy. ***(Karyon= nucleus, gamy= union***). After karyogamy reduction division or meiosis takes place which again reduces the number of chromosomes to haploid number and thus haploid stage is re-established.

The sex organelles of fungi, if they are present, are called gametangia (singular gametangium). They may form sex cells or gametes and may contain instead one or more gamete nuclei. If the male and female gametangia are morphologically different then the male gametangium is called antheridium and the female gametangium is called the oogonium. The various methods of sexual reproduction by which the compatible nuclei are brought together by the following methods-

**(i) Planogametic Fusion-** This involves the fusion of two necked motile gametes or planogametes. It is of tree types depending upon the nature and structure of the fusing gametes-

**a) Isogamy**- When the fusing gametes are morphologically similar but physiologically different. They are formed on different hyphae e.g., ***Synchytrium***) (Fig. 1.18)

**b) Anisogamy**- When the fusing gametes are morphologically as well as physiologically dissimilar. Themale gamete is smaller and more active than the female gamete (e. g. ***Allomyces).***

**c) Oogamy-** When the female gamete or ovum or egg is non- motile and male gamete or anthrozoid is motile. They are formed in specialized gametangia known as oogonium and antheridium respectively (Fig. 1.18).

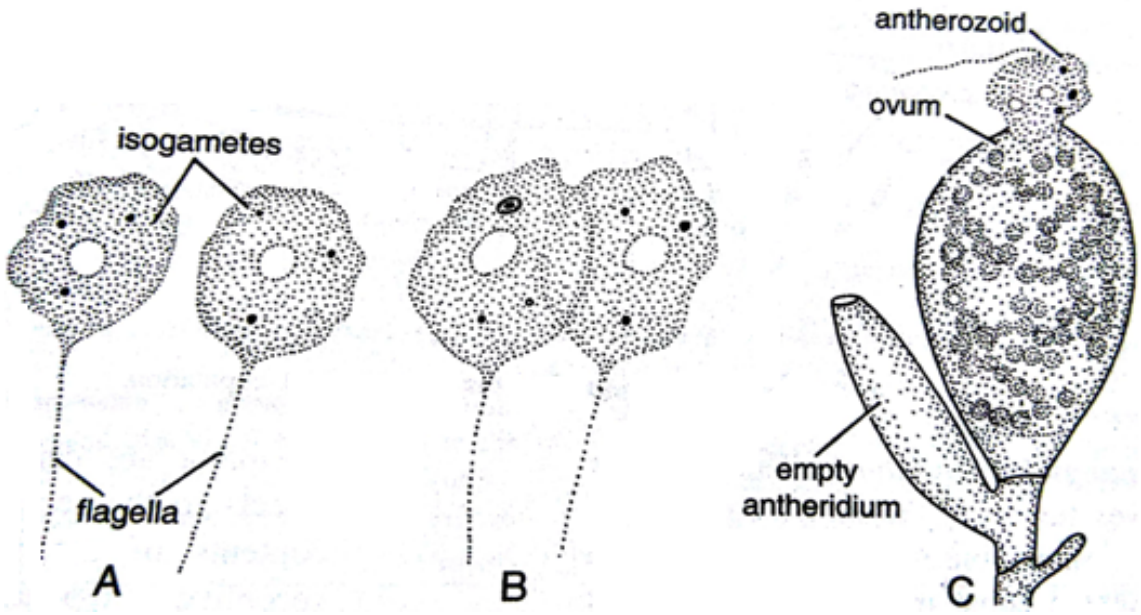


*Fig. 1.18: Planogametic copulation: A. Isogamy; B. Anisogamy; C. Oogamy*

**(ii) Gametangial Contact-** Here the two gametangia come into close contact but do not fuse. The wall nucleus migrates through a pore or fertilization tube into the female gametangia (e. g. ***Albugo, Aspergillus***, ***Phytophthora*** etc.) (Fig. 1.19).

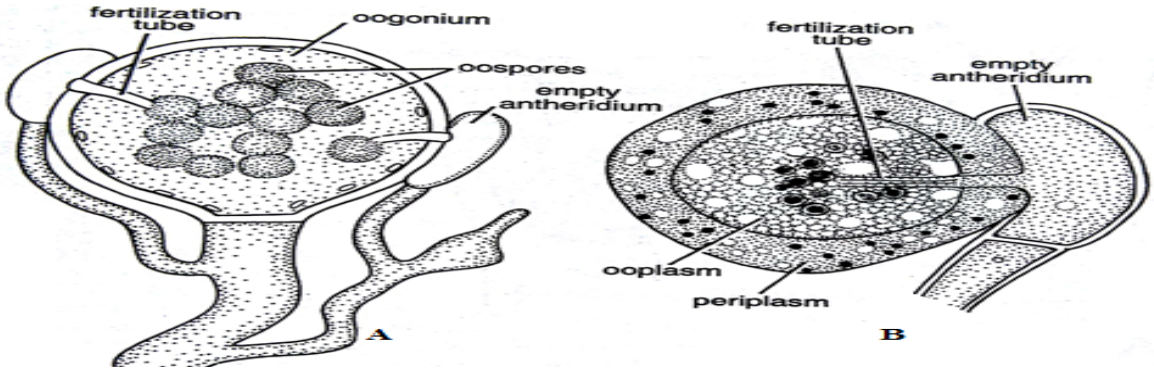


*Fig. 1.19: Sexual reproduction: Gamentangial contact*

**(iii) Gametangial Fusion-** This process involves fusion of the entire content of two compatible gametangia. The two compatible gametangia come in close contact, at the point of contact their walls dissolve and their contents mix. The two gametangia ultimately fuse resulting in karyogamy (e.g., ***Mucor, Rhizopus***). (Fig. 1.20)

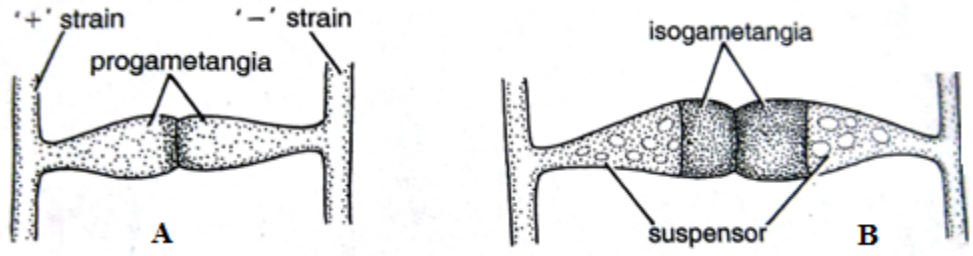


*Fig. 1.20: Sexual reproduction: Gamentangial copulation*

**(iv) Spermatization-** In highly advanced forms of fungi e.g., Puccinia, sex organs are completely absent and the sexual process is accomplished by minute spore like spermatia (the male gamete) and specialized receptive hyphae, Carpogyne (the female gamete). The spermatia are carried to the receptive hyphae by air, water or insects and the content of the spermatium enter the receptive hyphae through a pore. (Fig. 1.21)

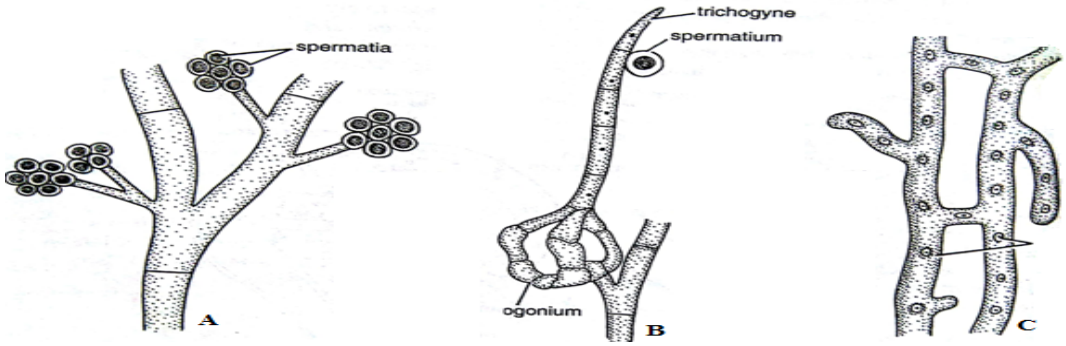


*Fig. 1.21: Sexual reproduction: A-B. Spermatization; Somatogamy*

**(v) Somatogamy**- In higher fungi the sex organs are at all completely absent but fusion of two vegetative hyphae or somatic hyphae takes over the sexual function and fuse together (e. g. ***Morchella, Agaricus, Peziza).***

## *1.7 LIFE CYCLE*

In fungi there are five basic types of life cycles are reported as follows-
(i) Asexual through vegetative hyphae.
(ii) Haploid life cycle
(iii) Diplontic life cycle
(iv) Haplo-diplobiontic life cycle
(v) Haplo-dikaryotic life cycle.

**i) Asexual through vegetative hyphae**. In fungi asexual reproduction takes place by means of fragmentation, budding, conidia, may be endogenous or exogenous depending on conditions, zoospores, Chlamydospores, aplanospores etc.

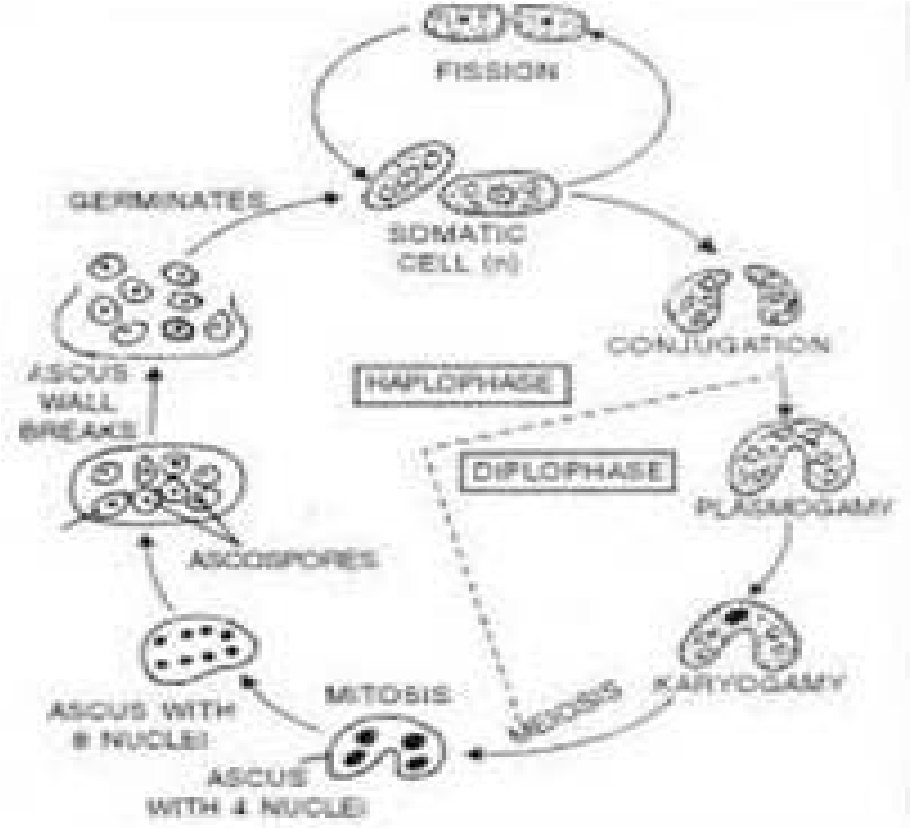


*Fig. 1.22: Asexual Life cycle of Fungi*

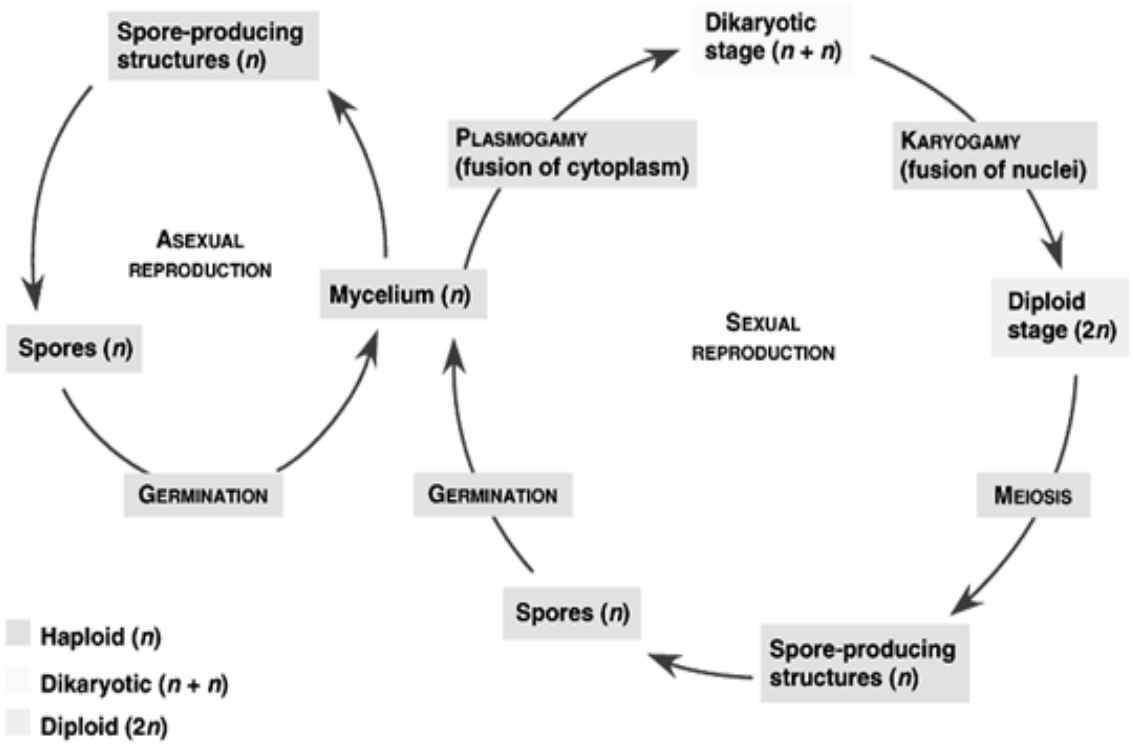


*Fig.1.23: Asexual and sexual life cycle pattern of fungi*

In this type of life cycle the haploid mycelium generally produces haploid reproductive bodies without any gametic union and after maturation these haploid spores germinates and again produces haploid mycelia. About 20% fungi propagated only by asexual means. Asexual reproduction takes place during favorable conditions. Thus, asexual life cycle is haploid dominated (Fig.1.22).

**ii) Haploid life cycle:** Haploid life cycle is found in fungi especially in ***Saccharomyces octosporus***. Here the haploid phase is very elaborate and the diploid phase is very short, confined to the zygote cell only. The haploid somatic cells functions as potential gametangia. Two somatic gametangia meet in pairs and form a proturbance towards the other and the cell wall dissolves at the meeting point and form a common passage called conjugation tube. The nuclei of both gametangia move into this conjugation tube fuse and form zygote. This zygote functions as ascus which after first meiosis and second mitotic division forms 8 haploid ascospores. These ascoppores after liberation behaves like somatic cell. Thus the haploid phase is larger comparatively to diploid phase. This type of life cycle is known as haplontic life- cycle (Fig.1.24).

**iii) Diplontic life cycle:** This type life cycle is commonly reported in another yeast cell ***Saccharomyces ludwigii***. This life cycle is just reverse to haplontic where diploid somatic stage is long and haploid phase is short represented by ascospores. These ascospores behave as

gametangia and pairs by fusion and form zygote cell. It forms small tube. The germ tube became multicellular and functions as a diploid sprout mycelium. The new diploid yeast cell forms buds detached after maturation from the parent mycelium and functions as sprout cell. Thus after the commencement of favorable conditions these cells function as asci. The diploid of each ascus after meiosis produces haploid ascospores (Fig.1.25).

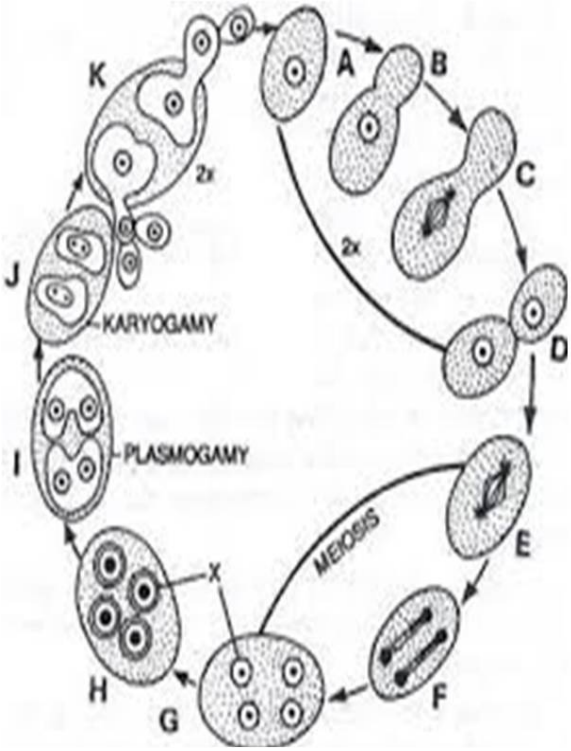


*Fig. 1.24 Haplontic life cycle of Saccharomyces octosporus*

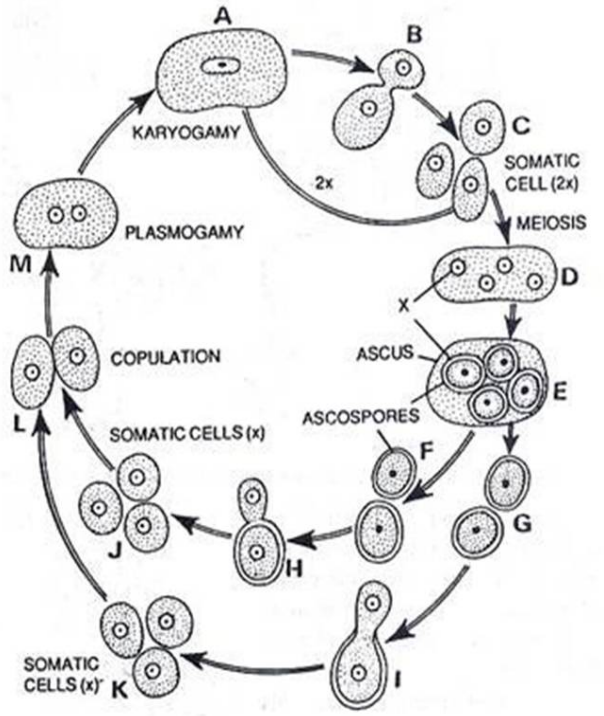


*Fig.1.25 Diplontic life cycle of Saccharomyces ludwigii*

**iv) Haplo-diplobiontic life cycle:** In this type of life cycle both haploid and diploid phases are of equally long. This type of life cycle is reported in ***Saccharomyces cerevisiae***. In this case total ascospores formed. Out of the four, two groups of mating type, each with 2 ascus. Normally two different mating types' haploid cells multiply by budding. In certain environmental conditions two somatic cells of opposite mating types behaves as gametangia and formed diploid zygote by budding and formed numerous diploid cells. During adverse conditions i. e. scarcity of food, the diploid cells functions as ascus. In each ascus 4 ascospores formed by reduction division. These ascospores after liberation form many haploid somatic cells by budding (Fig.1.26).

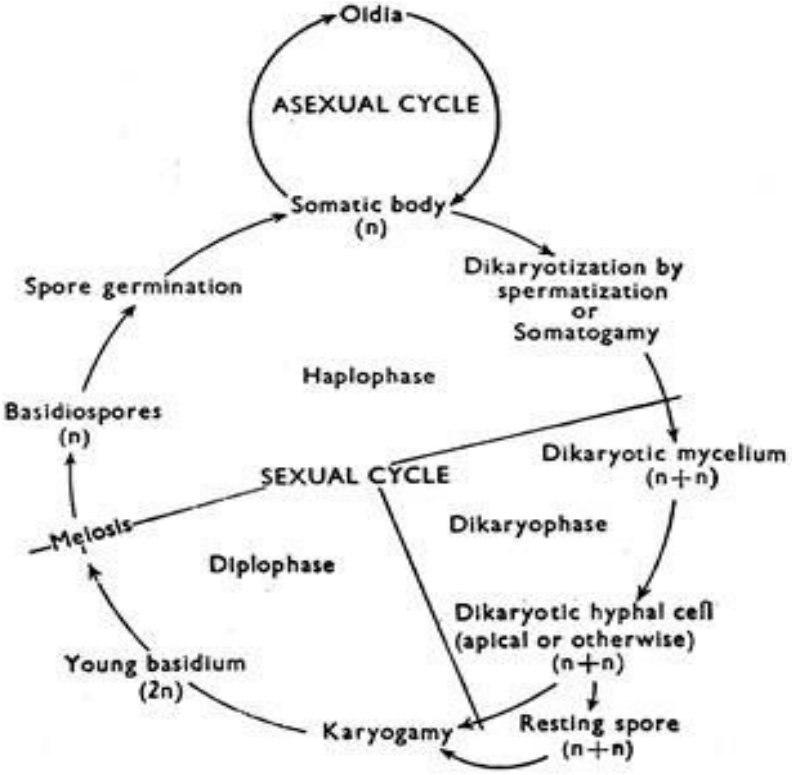


*Fig.1.26: Life cycle pattern in Basidiomycetes*

**v) Haplo-dikaryotic life cycle:** The general life- cycle pattern of the Basidiomycetes has resemblance with that of Ascomycetes. The basidiospores forms germ tube on germination gives rise to the haplophasic somatic body represented by primary mycelium. The primary mycelium often produces Oidia and completed asexual life cycle (Fig.1.27)

The oidia may behave as spores and give rise to primary mycelia or by Spermatization with compatible primary mycelia take part in the formation of dikaryotic secondary mycelium. This may also be formed by somatogamy between two compatible primary mycelia. The dikaryotic apical cell of the hyphae of the secondary mycelium develops into basidium. It may so happen that resting spores may be formed from the dikaryotic hyphal cells. Each resting spore on germination gives rise to a basidium. Karyogamy takes place in the basidium producing diplophasic condition which is followed immediately by meiosis and ultimately haploid basidiospores are produced. The basidiospores germinate to produce haplophasic somatic body thus the life cycle is completed. The life cycle comprises of three phases of haplophase, dikaryophase and diplophase of which dikaryotic phase is very much prolonged and is nutritionally independent of haplophase. The haplophase is also well represented just as in almost all fungi the diplophase is very short and followed by meiosis.

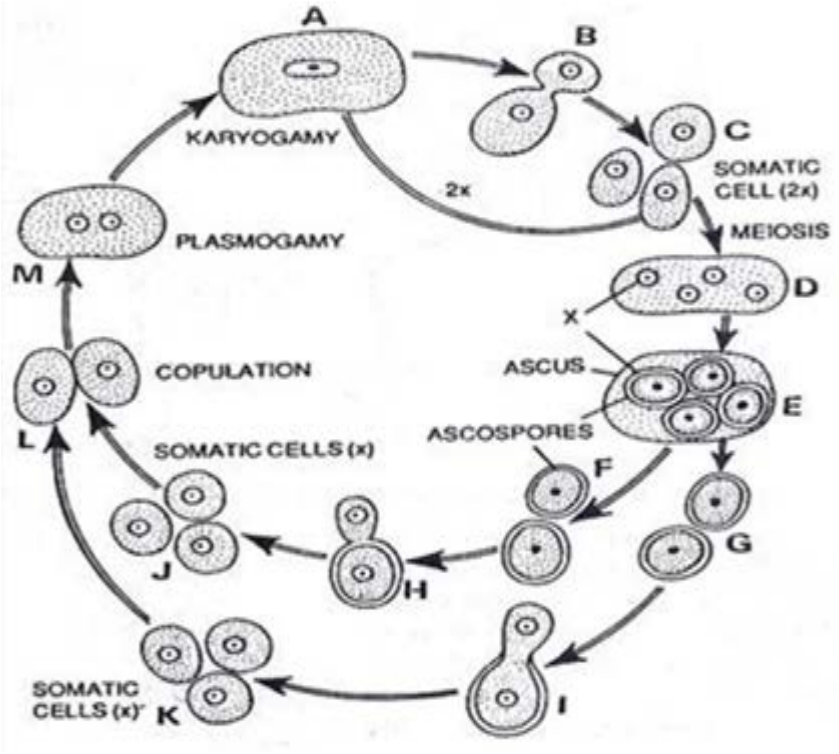


*Fig. 1.27: Haplo-diplontic life cycle of Saccharomyces cerevisiae*

## *1.8 SUMMARY*

A fungus is a kind of living organism, yeasts, molds and mushroom are types of fungi. The fungi are a separate kingdom of living things, different from plants and animals. Their cell wall contains chitin unlike the walls of plants which contain cellulose. The kingdom fungi, includes some of the most important organisms both in terms of their ecological and economic roles by breaking down dead organic materials. They continue the cycle of nutrients through ecosystems. In addition, most vascular plants could not grow without the symbiotic fungi.

## *1.9 GLOSSARY*

**Ascomycetes:** A class of fungi distinguished by the ascus.
**Basidiomycetes**: A class of fungi that form basidiospores,
**Basidiospore:** A sexual spore produced following the union of two nuclei on a specialized club like structure known as basidium.
**Basidium**: A club shaped specialized structure of the Basidiomycetes on which are borne the exogenous basidiospore.

**Budding:** Form of vegetative reproduction in which a new cell is formed as an outgrowth from the parent cell.
**Cell:** The microscopic, functionally and structurally basic unit of all living organisms.
**Cell Wall:** A rigid external covering of the cytoplasmic membrane.
**Chitin:** A polymer of N- acetyl glucosamin present in the covering layer of arthropods and in the cell wall of many fungi.
**Coenocytic**: A term applied to a cell or aseptate hyphae containing numerous nuclei.
**Conidiophore:** A hyphae which bears conidia.
**Conidia:** Any asexual spores which are formed at the tip of the hyphae and which are not enclosed within a sac also called conidia.
**Conjugation**: A reproductive process characterized by the temporary fusion of the reproducing partners and transfer of genes.
**Cytoplasm**: A living matter of a cell between the cell membrane and the nucleus.
**Epidermis**: The outer surface of tissue.
**Imperfect Fungi:** Fungi that do not have a sexual cycle.
**Fission:** An asexual process by which some micro- organisms reproduce.
**Flagella**: A thin filamentous appendage on cells, responsible for swimming motility.
**Fungus**: A micro- organism that lacks chlorophyll and usually filamentous, a mold or yeast.
**Gametangium:** Sex organelle of fungi.
**Glucan:** A polymer of glucose.
**Gram Positive Bacteria**: Bacteria that retain the crystal violet - iodine complex when stained by the Gram technique and thus acquire the color of the dye and thus appear dark blue or violet.
**Gram Negative Bacteria**: Bacteria that do not retain crystal violet- iodine complex when subjected to the Gram technique and thus acquire the color of the dye (usually a red dye) that is used to counterstain the cell.
**Gram Stain:** A differential stain by which the bacteria are classed as Gram-positive and Gram-negative depending whether they retain or loose the primary stain (crystal violet) when subjected to treatment with a decolorizing agent.
**Haustoria**: Root like projections through which a fungus obtains nourishment from the host
**Heterotrophs**: A micro- organism that is unable to use carbon-di-oxide as its sole source of carbon to synthesize food.
**Host**: An organism harboring another organism as a parasite.
**Imperfect Fungi**: Fungi that do not have a sexual cycle.
**Microbiology**: The study of organisms of microscopic size, including their culture, economic importance, pathogenicity etc.
**Microorganisms:** Any organism of microscopic dimensions.
**Mold**: A fungus characterized by a filamentous structure.
**Mycology**: The study of fungi.
**Mycoplasma:** A member of group of bacteria lacking cell wall.
**Mycorrhiza:** A symbiotic association of a fungus with the roots of a higher plant.

**Mycosis**: A disease caused by fungi.
**Mycotoxin:** any toxic substance produced by fungi.
**Pathogen:** An organism capable of producing disease.
**Perfect Fungi**: Fungi those reproduce by asexual and sexual means.
**Yeast:** A kind of fungus that is unicellular and lacks typical mycelia.

## *1.10 SELF-ASSESSMENT QUESTIONS*

### 1.10.1 Multiple Choice Questions:

1. Sexual reproduction takes place in
(i) Ascomycetes (ii) Basidiomycetes
(iii) Phycomycetes (iv) Deuteromycetes

2. Only asexual reproduction is found in
(i) Ascomycetes (ii) Basidiomycetes
(iii) Oomycetes (iv) Deuteromycetes

3. Fungal spores produced asexually at the tip of the hyphae are called
(i) Conidia (ii) Sporangiophores
(iii) Spore (iv) Arthrospore

4. Mycorrhiza is a term to indicate
(i) Fungal association with stem (ii) Bacteria associated with root
(iii) Fungal association with root (iv) Study of fungi

5. The obligate parasitic fungi absorb their nourishment from the host cell through
(i) The surface (ii) Haustoria
(iii) Appressoria (iv) Rhizoids

6. Perfect stage of fungus means
(i) When the fungus is perfectly healthy (ii) When it is reproduces asexually
(iii) When it reproduces sexually (iv) When it forms perfect resting spores

7. Haustoria are produced in the case of mycelium which is-
(i) Both intracellular and endoparasite, (ii) Ectoparasite,
(iii) Both intercellular and endoparasitic (iv) Either endoparasitic or intercellular

8. The fungus which is so important for its use in genetic studies is
(i) *Aspergillus* (ii) *Rhizopus*

(iii) *Penicillium* (iv) *Neurospora*

9. Fungi are always-
(i) Parasitic (ii) Saprophytic
(iii) Autotrophic (iv) Hetrotrophic

10. An organism which is normally saprophyte, but can also a parasite is called:
(i) Facultative saprophyte (ii) Partial saprophyte
(iii) Facultative parasite (iv) Partial parasite

11. Which of the following diseases caused by a fungus:
(i) Cholera (ii) Rust of wheat
(iii) T.B. (iv)Tetanus

12. Mycology is the study of:
(i) Algae (ii) Fungi
(iii) Bryophyte (iv) Pteridophytes

13. The aseptate mycelium is found in:
(i) Lower fungi (ii) Higher fungi
(iii) Fungi Imperfecti (iv) None of above

14. Coprophilous fungi are growing in:
(i) Grasses (ii) Dung
(iii) Animals (iv) Wood

15. The fungus which is so important for its use in genetic studies is:
(i) Aspergillus (ii) Rhizopus
(iii) Penicillium (iv) Neurospore

**1.10.1 Answers Key:** 1-(iv), 2-(iv), 3-(ii), 4-(iii), 5-(ii), 6-(ii), 7-(iv), 8-(iv), 9 (iv), 10 (iii), 11 (ii), 12 (iii), 13 ( i), 14 (ii), 15 (iv).

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## *1.13 TERMINAL QUESTIONS*

1. Describe the salient features of fungi as a group with suitable diagrams?
2. Describe in detail various modes of nutrition in Fungi?
3. Describe the somatic or vegetative phase of life in fungi/
4. Describe the structure of fungal cell, illustrate with suitable diagrams?
5. Describe in detail the structure and kinds of fungal flagella?
6. Describe the structure of fungal cell and how it differs with plant cell?
7. Write short notes on following-
    (i) Septate mycelium
    (ii) Aseptate mycelium
    (iii) Annular pore

(iv) Dolipore

8. Define the following terms
   (i) Flagellation in fungi
   (ii) Pseudoparenchyma
   (iii) Prosenchyma
   (iv) Nutrition in fungi
   (v) Mycorrhiza
9. Describe the structure, reproduction and diagnostic features of fungi.
10. What is mycelium? Give the modifications of fungal mycelium?
11. Describe briefly asexual reproduction in fungi, illustrate with suitable diagrams.
12. Describe different types of sexual reproduction in fungi.
13. Write short notes on following-
   (i) Chlamydospores
   (ii) Oidiospores
   (iii) Conidia
   (iv) Budding
14. Define the following terms-
   (i) Planogemetic fusion
   (ii) Gametangial fusion
   (iii) Spermatization
   (iv) Somatogamy
15. Define the differences between these terms-
   (i) Fission and budding
   (ii) Isogamy and Anisogamy
   (iii) Somatogamy and Karyogamy
   (iv) Planospores and Aplanospores

# UNIT-2 CLASSIFICATION, PHYLOGENY AND ECONOMIC IMPORTANCE OF FUNGI

## Contents:

## 2.1 OBJECTIVES

After reading the unit students will be able to-

- Study different classification systems of fungi.
- Understand the phylogeny and economic importance of fungi.

## 2.2 INTRODUCTION

The fungi have been classically characterized and classified by the appearance of their colony e.g., color, size etc. hyphal organization, and the structure of reproductive spores. Generally fungi are capable of reproducing sexually under appropriate conditions, besides reproducing by various asexual means. The sexual stage of a fungus is known as its perfect stage. But for a good number of fungi, the perfect stage is unknown. Such fungi are designated as imperfect fungi ***(fungi -Imperfecti***).

As fungi are classified mainly on the basis of their sexual stage, the imperfect fungi cannot be placed in any of the major fungal categories. They are therefore, assembled in a special group Deuteromycetes. In case the perfect stage of any member of this group is discovered, it is transformed to its original taxon. While most of the sexual stages found subsequently among members of this class have been of the Ascomycetes type. Sexual stages are reported in Ascomycetes and Basidiomycetes.

## 2.3 CLASSIFICATION OF FUNGI

The classification of fungi is based primarily on the characteristics of the sexual spores and fruiting bodies present during the sexual stages of their life cycles. However, many fungi produce sexual spores and different types of fruiting bodies only under certain environmental conditions. Those fungi, produce spores asexually are known as ***"Imperfect fungi"***, while the others completed their life cycle without gametic or sexual means and these are known as "***perfect fungi***". These fungi must be classified on other characteristics of the morphology of their asexual spores and thalii. Thus imperfect higher fungi are previously placed on a special class called "Class Deuteromycetes".

Probably Bauhia may be the pioneer worker who for the first time tried to classify fungi in his book ***"Pinax Theatri Botanici"*** (1623) and described 100 species of fungi. After that Tournefort (1694) in his book ***"Elements de Botanique"*** divided fungi into six classes. Carolus Linnaeus (1753) placed all fungi in $24^{th}$ class ***"Cryptogamia"*** in his famous book ***"Species Plantarum".*** However, Elias Magnus Fries in his book ***"Systema Mycologium***" for the first time give the

detailed classification of fungi (published in there volumes during 1821/ 1832) . For the first time he divided fungi into four classes-

1) Coniomycetes
2) Hyphomycetes
3) Gastromycetes
4) Hymenomycetes.

In 1882 Saccardo in his famous book "Sylloge Fungorum" divided fungi into six classes and included Bacteria and slime molds in fungi. The six class are-

1) Schizomycetes
2) Myxomycetes
3) Phycomycetes
4) Ascomycetes
5) Basidiomycetes
6) Deuteromycetes

Previously Bacteria were also included with fungi but latter the modern mycologists unanimously exclude the bacteria from the fungi on the basis of lack of true nucleus in the bacteria. However, inclusion of slime molds among the fungi, mycologists favors this.

H. A. de Bary (26 January 1831- 19 January 1883) was a German surgeon, botanist, microbiologist and mycologist worked on fungal systematics and physiology). He is considered a ***founding father of plant pathology*** as well as a founder father of modern mycology. His extensive and careful studies on the life history of fungi and his contribution to the understanding of alga and higher plants were landmark of biology. He is known for demonstrating sexual life cycle of fungi and study of plant diseases and coining the word "symbiosis".

Bessey (1950) supported the idea of de Bary (1887) to place the slime molds under the name Mycetozoa. He divided the fungi on two major groups and the basis of their classification is reproductive characters. He divided true fungi into following major groups.

**1. Lower Fungi:** In these fruiting bodies not formed it includes only one **Class-**

**(a) Phycomycetes.**

**2. Higher Fungi:** In these fungi the fruiting bodies are formed and this is again divided into three classes-

**a) Ascomycetes**- Fruiting body is asci, ascospores formed endogenously.

**b) Basidiomycetes**- Fruiting body is basidia and basidiospores formed exogenously

**c) Deuteromycetes**- Sexual stage is totally absent.

This trend of dividing fungi into four classes continued till 1950.

Sparrow (1958) divided Fungi into nine classes emphasizing the importance of flagellation of the zoospores in the classification.

According to him flagellation should be treated as a good phylogenetic and taxonomic criterion-
According to Sparrow (1958) the nine classes are-

1. Chytridiomycetes
2. Hyphochytridiomycetes
3. Plasmodiophoromycetes
4. Oomycetes
5. Zygomycetes
6. Trichomycetes
7. Ascomycetes
8. Basidiomycetes
9. Deuteromycetes

E. A. Bessey (1950) proposed a different system of classification of fungi as follows-

**Fungi: i) Mycetozoa**

**ii) True Fungi**

**1. Lower Fungi**

(a) Phycomycetes)

**2. Higher Fungi (Phylum Carpomycetes**)

a) Ascomycetes

b) Basidiomycetes (Fungi Imperfecti).

After that Martin (1961) on the basis of the septation and spore characters proposed a new system of classification. The outline of his classification is follows--

**Division-Mycota:** The mycologists placed division Mycota under the kingdom Plantae including the non- chlorophyllous, non- green nucleated thallophytes, the fungi having the following-

## Significant Features

a) Based on their mode of nutrition they may be either saprophytes- obtain nutrition from dead and decaying materials or may be parasites- obtain nutrition from living organisms. The lack chlorophyll so unable to synthesize their own food and hence the mode of nutrition is 'Heterotrophic' and absorptive except slimemolds in which it is phagotrophic to engulf the nutrition.
b) Somatic or vegetative phase may be unicellular, multicellular filamentous or simply plasmodium.
c) Plant body is eukaryotic, presence of true nucleus with a nuclear membrane and nucleoli in the cell.
d) Cell wall is made up of chitin but exceptionally some Oomycetes have a cellulose cell wall.

e) Reserve food material accumulated in the form of glycogen and not starch (as in algae).
f) The fungi reproduce both by asexual and sexual means.

The division Mycota divides into two sub- divisions-
1. Myxomycotina and 2. Eumycotina

**1. Sub-Division-Myxomycotina:** It includes the slime molds and possess following characteristic features-

i. The thallus is non-green, multinucleate undifferentiated mass of protoplasm generally known as "plasmodium".
ii. The plasmodium is amoeboid in shape and lacks definite cell wall.
iii. It is holocarpic, free living and diploid in nature.
iv. The reproductive units or spores are meiospores.

All the free- living slime molds are placed in the class Myxomycetes.

**2. Sub-Division-Eumycotina:** Except the slime molds all the other fungi are included in this sub division. This group includes about 80,000 known species of fungi. The significant characteristic of features of the group are-

i. The somatic or vegetative phase is a filamentous structure known as mycelium while exceptionally a few unicellular forms are also reported.
ii. Unit of structure of the plant body/ thallus or mycelium is known as hyphae and not the cell.
iii. The cell/ hyphal wall usually composed of chitin or fungus cellulose, or a mixture of cellulose and chitin or rarely of cellulose.
iv. Most of the members are eucarpic except a few. The cell contains definite nucleus with a nuclear membrane and nucleoli.
v. The septa present between the cells, each have a central pore.
vi. The filamentous forms grow in length by means of apical cell.
vii. Reproduction takes place by means of asexual and sexual means.
viii. Asexual reproduction or spore formation takes place by mitospores which may be motile or non- motile.

There is gradual and progressive simplification and ultimately elimination of the sexual apparatus from the lower to the higher fungi.

Previously in ancient time mycologists divide the sub- division Eumycotina, includes true fungi into four classes as- Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. This scheme of classification of true fungi is still followed in many countries. This classification is referred as four class classification. However, the modern mycologists suggests that the Phycomycetes- includes lower fungi is not a natural group of closely related forms.

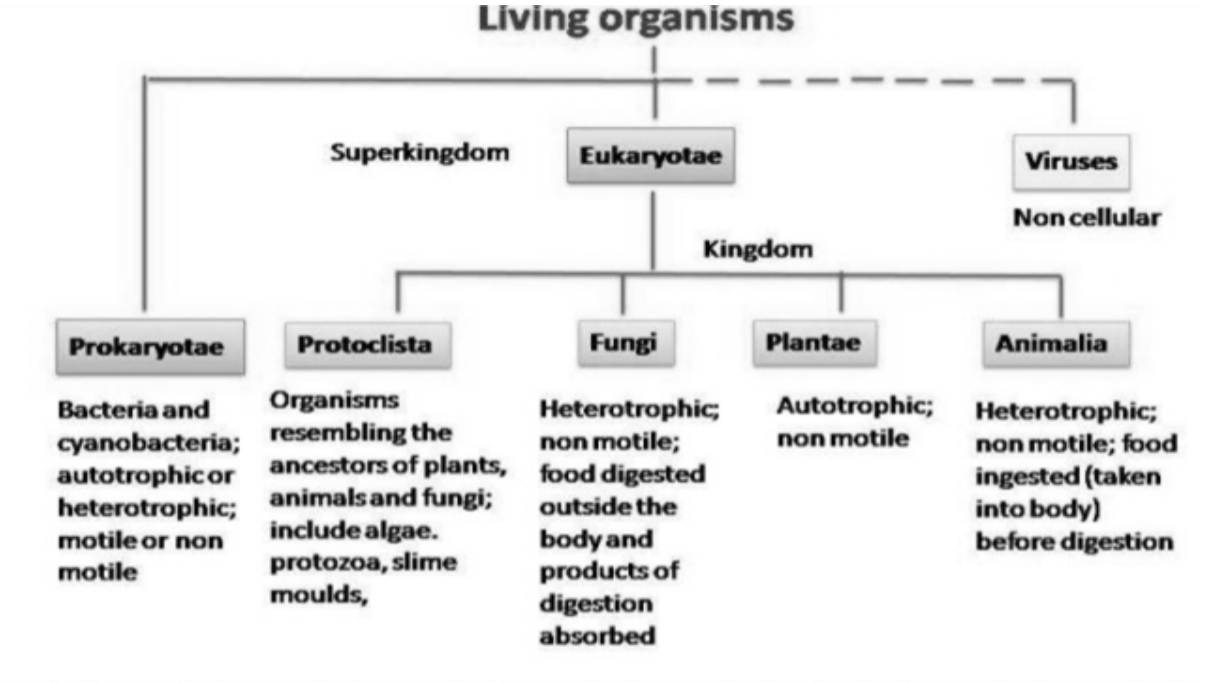


*Fig. 2.1: Five kingdom classification given by Margulis and Schwartz (1998)*

It is Alexopoulos (1962), who took a logical step and split lower fungi included in the Phycomycetes.

A summarized outline scheme of classification of Eumycotina or true fungi followed in this edition of the text is given below-

The true fungi divide customarily into two main groups-
i) The lower fungi
ii) The higher fungi

**i) The lower fungi or Phycomycetes-** They have the characteristic features as follows-
1. The thallus is comparatively simple may be unicellular or filamentous mycelium.
2. In the actively growing vegetative mycelium septa usually remain suppressed.
3. Reproduce asexually generally motile rarely non- motile Sporangiophores or by means of conidia.
Earlier the mycologists placed the lower fungi in a single class Phycomycetes, split again in six classes as following mainly based on the presence and absence of motile spores in the life- cycle including the number, form, and position of flagella on motile cells-

## (A) Lower Fungi-Phycomycetes:

(a) Fungi produced motile cells- divided into four classes.

1. **Class Chytridiomycetes-** This class included lower fungi, motile cells have a single whiplash type flagellum inserted at the posterior end generally known as opisthocont. The members of this class are called the Chytrids.
2. **Class Hypochytridiomycetes-** In this class the motile cells or zoospores possess a single tinsel type flagellum inserted at the anterior end.
3. **Class Plasmodiophoromycetes-** The motile cells or zoospores possess two flagella (biflagellate. Both the flagella are whiplash type with unequal size- one is longer than the other. The longer one has sharply pointed end and the shorter one has blunt end, example of this group are the root organisms.
4. **Class Oomycetes-** This class also includes biflagellate motile cells. Both the flagella are of equal length. One of these points forwards and the other traits behind. The former is of tinsel type while the latter is of whiplash type. Water molds, downey mildews and blights are the common examples of this class.

(b) Fungi produced non- motile cells- divided into two classes-

5. **Class Zygomycetes-** Motile cells are absent. Asexual reproduction takes place by the means of Sporangiophores which are encapsulated, dissemination of spores by means of wind. Pin molds and bread molds are common examples.
6. **Class Trichomycetes-** Motile cells are absent. Asexual reproduction takes place by means of conidia originated exogenously at the tip of the hyphae known as conidiophores. These fungi are often parasitic on arthropods.

**(B) The Higher Fungi:** The somatic or vegetative phase is mostly of a mycelium which is usually septate. The septa have each a central pore and thus are incomplete. In the life- cycle no motile cells are produced. The higher fungi further divided into three following classes-

7. **Class Ascomycetes-** The perfect stage or sexual spores are called ascospores which are endogenous in nature. The ascospores are produced within special sac- like structures known as asci. Fusion of gametangial or somatic hyphae represents plasmogamy. Somatic phase is usually a septate mycelium which mostly asexually multiplies by conidia or rarely it is unicellular (e.g., Yeasts) and is propagated by budding or rarely by fission.
8. **Class Basidiomycetes-** The basidiospore are the characteristic spores of the sexual or perfect stage which are exogenous in origin and borne externally on club- shaped structures known as basidia. The simple or complex mycelium with or without clamp connection represents the somatic/ vegetative phase. Absence of sexual organs, however, plasmogamy takes place by fusion of hyphal fusion.
9. **Class Deuteromycetes or Fungi Imperfecti-** This class includes higher fungi in which sexual or perfect stage is unknown. Somatic/ vegetative phase consists of a septate mycelium multiplies asexually by conidia, produced on conidiophores, which may occur either isolated or may be aggregated to form complex structures such as pycnidia, synnema and acervuli.

The classification of fungi described above has been followed in this text. However, for the guidance of the students a brief account of the classification proposed by Ainsworth (1973) and Alexopoulos (1962) is given below-

Alexopoulos and Mim (1979) placed the fungi in the kingdom Mycetae of sub kingdom Eukaryota including slime molds. It includes four other kingdom mycetae into three divisions- (i) Gymnomycota (ii) Mastigomycota and (iii) Amastigomycota.

Classification proposed by G.M. Smith (1955). GM Smith an American botanist (1885-1959), proposed to include all fungi into seven classes belonging to divisions (Fig. 2.2).

Further they divided division into sub divisions and classes into sub classes and orders.

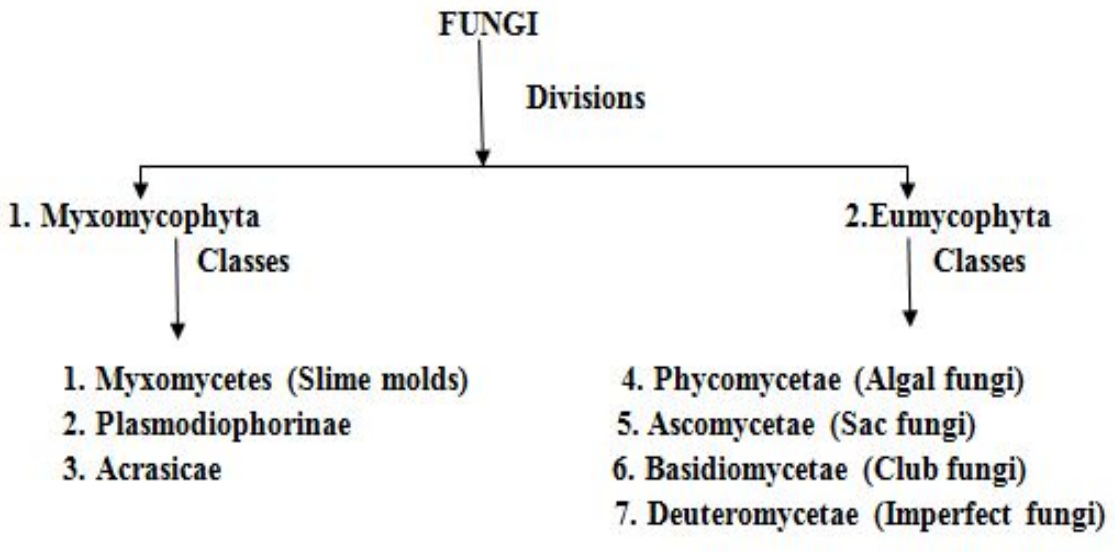


*Fig. 2.2: System of classification of fungiproposed by G. M. Smith (1955)*

A more natural system of classification of fungi was proposed by Ainsworth (1973). This classification has been accepted by many present day mycologists like Webster (1980), Bilgrami (1985) and Dube (1987). Ainsworth placed all the fungi under the Kingdom Mycota as follows-

Although the classification of fungi is still not very clear for which an ideal and unanimously accepted for scheme is yet to be proposed from time to time by mycologists. The important among them are Bessey (1950), Sparrow (1958), Ainsworth (1961), Alexopoulos (1962), Gaumann (1964) and Hawker (1967).

They all are well organized suitable and mostly based on following characteristics-

1) Nature of somatic phase/ or vegetative phase of fungi-

a) It may be unicellular mycelium or multicellular mycelium.

If multicellular- (i) may be septate or (ii) aseptate mycelium.

2) Presence and kinds of asexual spores or mitospores

a) May be conidia or b) Sporangiophores.

If sporangiophores-(i) either motile or (ii) non- motile, again they may be divided on the basis of the form, number and arrangement of flagella in the motile spores.

3) Kinds of sporangia

4) Nature of life-cycle

(a) asexual or

(b) sexual

The life- cycle may further categorized as-

i) Haplontic life- cycle- When haploid stage dominates

ii) Diplontic life- cycle- when diploid stage dominates

iii) Haplo-diplontic life- cycle- when haploid and diploid both the stages shares equally

iv) Haploid dikaryotic life- cycle- when haploid somatic mycelium forms diploid mycelium and these dikaryotic mycelia participated in the life- cycle without union of gametes Basidiomycetes.

## Classification Proposed By Alexopoulos (1962)

**Division Mycota-- Sub- Division- 1- Myxomycotina**

**2. Eumycotina**

**Sub- division- 1. Myxomycotina**

**Class 1- Myxomycetes-** 1. Chytridiomycetes
2. Hyphochytridiomycetes
3. Plasmodiophoromycetes
4. Oomycetes
5. Trichomycetes
6. Zygomycetes

**Form- Class-Deuteromycetes**

7. Ascomycetes
8. Basidiomycetes

**Class- Myxomycetes**- Sub-Class 1. Ceratiomycetidae (spores borne externally on individual stalks)

**Order- 1**. Ceratiomyxales

Sub- Class 2. Myxogastromycetidae (Spores borne internally in fructification)

**Order-** 1. Liceales
2. Echinosteliales
3. Trichiales
4. Stemonitales

5. Physarales

**Class- Chytridiomycetes-**

**Order-1**. Chytridiales (True mycelium not present) In some species rhizomycelium present)

2. Blastocladiales (True mycelium present, Planogametic sexual reproduction, thick walled resistant sporangia are characteristic feature)
3. Monoblepharidales (True mycelium present, sexual reproduction by fusion motile male with non-motile female, no resistant sporangia)

**Class- Hypochytridiomycetes-**

**Single Order-**1. Hypochytriales (Aquatic Fungi, Uniflagellate Motile Cells, Tinsel Anteriorly Inserted Flagella).

**Class- Oomycetes-**

**Order-**1. Saprolegniales

2. Leptomitales
3. Lagenidiales
4. Peronosporales

**Class- Plasmodiophoromycetes-**

**Single Order-1.** Plasmodiophorales

**Single Family-** Plasmodiophoraceae (Always parasitic assimilative phase, Thallus multinucleate remain within host cells generally vascular plants, causes heterotrophy, zoospores with two whiplash type flagella)

**Class-Zygomycetes-**

**Single Order**-1. Mucorales, (Mostly saprobes, others weakly parasitic on plants, sexual reproduction by sporangia containing one to many aplanospores or conidia) Single Family-Mucoraceae.

**Class- Trichomycetes** (Thallus simple or branched and multinucleate; often parasitic on arthropods)

**Class- Ascomycetes-**

**Sub- Class-** 1. Hemiascomycetidae (Asci necked, ascocarp and ascogenous hyphae not present).

**Order-1.** Endomycetales, 2.Taphrinale

**Sub- Class-2**. Euascomycetidae (Asci usually unitunicate borne either in a cleistothecium perithecium or apothecium).

**Series-1. Plactomycetes- Order-** 1. Eurotiales
2. Microascles
3. Onygenales

**Series-2. Pyrenomycetes Order-** 1. Erysiphales
2. Meliolales
3. Chaetomiales
4. Clavicipitales
5. Sphaeriales
6. Diaporthales

7. Hypocreales
8. Coryneliales
9. Coronophorales

**Class- Basidiomycetes-**

**Sub- Class-1**. Hetrobasidiomycetidae (basidium septate)

**Order-** 1.Tremellales
2. Uredinales
3. Ustilaginales

**Sub- Class-** 2. Homobasidiomycetidae (Basidium non-septate).

**Order-1**. Exobasidiales- Series 1. Hymenomycetes

**Order-2**. Polyporales

**Order-3.** Agaricales- Series- Gastromycetes

**Order-4**. Hymenogastrales

**Order-5**. Lycoperdales

**Order-6.** Sclerodermatales

**Order- 7**. Phallales

**Order- 8.** Nidulariales.

**Form- Class- Deuteromycetes- (The Imperfect Fungi)**

**Order-1** Sphaeropsidales (Reproduction by means of conidia, borne in pycnidia)

**Form- Family-** 1. Sphaeropsidaceae
2. Zythiaceae
3. Leptostromataceae
4. Excipulaceae

**Order-2-** Melanconiales (Reproduction by means of conidia, Borne in acervuli)

**Form Family** 1. Malanconiaceae- Genus *Colletorichum*

**Order-3**- Moniliales (Reproduction by means of conidia, borne otherwise by budding or Oidia)

**Form Family**- 1. Moniliaceae
2. Dematiaceae (Genus- *Helminthosporium, Alternaria*, *Cerospora*)
3. Stilbellaceae
4. Tuberculariaceae (Genus *Fusarium*)

**Order-4**- Mycelia Sterllia- Reproduction structures totally absent).

Important Form genera- *Rhizoctonia* and *Sclerotium*.

## Some characteristic features of important fungi (According to Alexopoulos, 1962)

**Division-Mycota** (**True Fungi**): Lacking chlorophyll, plant body ranges from unicellular microscopic to multicellular mycelium, true nuclei with nuclear membrane, nucleoli present , cell walls contains chitin or celluloseor both alongwith other polysaccharides, reproduces sexually or asexually, spores are propagating units, two sub units--1.Myxomycotina and
2. Eumycotina.

**1. Sub- Division-Myxomycotina**- Plant body is amoeba like lacking definite cell wall, Somatic structure, A free living plasmodium (i.e. multinucleate mass of protoplasm, The entire plasmodium without definite cell wall), single class Myxomycetes.

**2. Sub- Division- Eumycotina-** The representatives of true fungi, typically filamentous, a few unicellular, the organisms provided with cell walls only a few exceptions, reproduces sexually and asexually, divided into eight classes and one form class as below-

**Class-1.Chytridiomycetes-** They bears single posteriorly inserted flagella, Motile cells either zoospores or planogametes produced, each with a single posterior whiplash flagellum structure of thalli varies, divided accordingly into three orders 1. Chytridiales, 2. Blastocladiales, 3.Monoblepharidales.

**Class- 2. Hypochytridiomycetes-** Aquatic fungi parasitic on algae and fungi or saprobes on plant and insect debris in the water, motile cells possess a single anterior tinsel flagellum, single order - hypochytridiales.

**Class- 3. Oomycetes-** Fungi with well- developed coenocetic (aseptate) mycelium reproduces asexually by means of biflagellate zoospores each bearing one tinsel and one whiplash flagella, the former directed forward and the latter directed backward, zoospores formed in various types of sporangia, oospore represents perfect spore, divided into 4 orders- 1. Saprolegniales, 2. Leptomitales, 3.Lagenidiales and 4.Peronosporales.

**Class- 4. Plasmodiophoromycetes-** Obligate endoparasitic fungi on algae, fungi and vascular plants non- cellular without cell wall, thalli multinucleate living in the cells of their hosts, motile cells bearing two unequal anterior whiplash- type flagella, no distinct fruiting bodies formed but resting spores produced in masses, single order- Plasmodiophorales.

**Class- 5. Trichomycetes-** Plant body simple or branched filamentous coenocytic thallus, attached to the external cuticle or to the digestive track of the living arthropods, mycelium not immersed in host tissue, divided into 5 orders.

**Class- 6- Zygomycetes-** Saprobes or parasitic fungi, mycelium well developed coenocytic or septate, reproduces sexually resulted in the formation of a resting spore formed by the fusion of two usually equal gametangia, motile cells absent, divided into 3 orders- 1.Mucorales, 2. Entomophthorales, 3. Zoopagales.

**Class- 7. Ascomycetes-** Somatic plant body multicellular consistsof a septate mycelium,insome one celled, motile cells or gametes totally absent, reproduces sexually, produced ascospores formed inside sac- like structures the ascus, divided into 3 sub classes: 1. Hemiascomycetidae, 2.Euascomycetidae and 3. Loculoascomycetidae.

**Class- 8. Basidiomycetes-** Produced highly complicated fruiting bodies the basidiocarps, sexually produced spores, basidiospores, formed exogenously on a specialized organ the basidium after karyogamy and meiosis, divided into 2 sub- classes- 1. Hetrobasidiomycetidae and 2 Homobasidiomycetidae.

**Form- Class- Deuteromycetes-** Also known as Fungi Imperfecti- absence of sexual reproduction a parasexual cycle may be present, divided into 4 orders- 1. Sphaeropsidales, 2. Melanconiales, 3 Moniliales and 4.Mycelia Sterlia.

**Class- Oomycetes- Order 1. Saprolegniales-** Thallus holocarpic eucarpic, zoospores always formed within the sporangium, Aquatic saprophytes except few, diplanetic, and monoplanetic or rarely aplanatic, oogonium not differentiated internally into ooplasm and periplasm, 3 families- 1. Ectogelceae, 2.Thraustochytriaceae, 3.Saprolegniaceae.
**Order-2. Peronosporales-** Thallus eucarpic, terrestrial parasitic on higher plants, oogonium differentiated into ooplasm and periplasm, 3 families- 1.Pythiaceae, 2.Albuginaceae, 3.Peronosporaceae.

**Class- Ascomycetes-** Sub- class- Hemiascomycetidae- Order 1. Endomycetales- Asci developed directly from zygotes each developed from the union of two cells or parthenogenetically from single cells, divided into 4 families- 1. Ascodeaceae, 2.Endomycetaceae, 3. Spermophthoraceae, 4. Saccharomycetaceae.

**Order- 2. Taphrinales-** Asci arising directly from cells of sexually produced dikaryotic thallus, family- Taphrinaceae.

**Sub- Class-Euascomycetidae- Series-1. Plectomycetes- Order- Eurotiales-** Ascocarp sessile and without an ostiole, divided in 3 families- Ascosphaeriaceae, 2.Gymnoascaceae, 3.Eurotiacea.

**Series 2. Pyrenomycetes- Order- Erysiphales-** Mycelium largely superficial, ascocarp closed cleistothecium, typically black or dark colored with wall appendages, single family- Erysiphaceae.

**Series- 3 Discomycetes- Order 3. Pezizales-** Ascocarp an open apothecium or a modified form of it, Apothecium above ground/ epigeous, asci opercululate or sub-operculate, divided into 3 families- 1.Sarcoscyphaceae, 2.Pezizaceae and 3.Halvellaceae.

**Series -4. Clavicipitales-** Asci persistent, thread like ascospores, ascocarp a perithecium with an ostiole, pariphyses present, single family- Clavicipitaceae.

**Series 5. Sphaeriales-** Ascocarps and stromata if present dark, membranous/ caronous, fruiting body perithcia typically white or bright coloured, presence of paraphyses and apical paraphyses, mature asci attached to the inner perithecial wall, divided in to 4 families- 1. Sordariaceae. 2. Phyllachoraceae, 3.Diatrypaceae and 4. Xylariaceae.

**Class Basidiomycetes-**Sub- class- Heterobasidiomycetidae-Order. Uridinales- Plant parasite, basidiocarp absent, promycelium/ basidium arising from a thick walled probasidium, a teleutospore, basidiospores produced on strigmata, discharged forcibly, divided in to 3 families- 1.Pucciniaceae, 2.Malamsporaceae, 3.Coleosporiaceae.

**Order 2.** Ustilaginales-Mostly parasites on vascular plants, basidocarp lacking, basidiospores sessile, forcibly not discharged, teleutospore formed in a manner similar to that of Chlamydospores, divided in to 3 families1.Ustilaginaceae, 2.Tilietiaceae. 3. Graphiolacea

**Sub-class-Homobasidiomycetidae**- **Order- 1.Exobasidiales-** Series 1- Hymenomycetes- Basidiocarp present, hymenium present and exposed before the spores are mature.

**Order- Polyporales-** Basidiocarp and hymenium present, hymenium gymonocarpic texture of not soft and putrescent, divided in to 6 families 1. Laphoraceae, 2. Clavariaceae, 3.Cantarellaceae,
4. Hydnaceae, 5.Meruliacea, and Polyporaceae.
**Order- Agaricales-** Basidiocarp present, Hymenium borne on gills/ lamellae, or if lining the inside of pores then basidiocarp soft and putrescent, divided into 5 families- 1. Boletaceae, 2.Paxillaceae, 3. Russulaceae. 4. Hygrophoraceae and Agaricaceae.

**Series- Gastromycetes**- Hymenium present or absent, basidiocarp remaining closed at least until the spores have been released from the basidia condition known as angiocarpoc.

**Order- Lycoperdales-** Hymenium present in early stages, spores generally light colored, small in size, Gleba powdery, glebal chambers not separating from peridium, divided into 3 families 1. Arachniaceae, 2. Lcoperdaceae and 3.Geastraceae.

**Order-Nidulariales-** Gleba waxy, glebal chambers forming waxy peridioles or entire gleba separating as a unit from the peridium, divided in to 2 families- 1.Sphaerobolaceae, 2.Nidulariaceae.

## System of Classification Given By Ainsworth (1971)

### Kingdom- Fungi -

**Division- 1. Myxomycota (**Somatic body a mass of free living plasmodium without firm wall)
**Single Class- Myxomycetes**
**Division- 2. Eumycota-(**Somatic body unicellular or multicellular and filamentous**).**
**Sub- Division- 1. Mastigomycotina** (Motile cells present (zoospores), perfect spore, oospore.
**Sub- division- 2. Zygomycotina**- Motile cells absent- A) Perfect stage present- i) Perfect spore zygospore.
**Sub- Division- 3. Ascomycotina**- Motile cells absent - B) Perfect stage present- ii) Perfect spore ascospores.
**Sub- Division- 4. Basidiomycotina**- Motile cells absent-C) Perfect stage present-iii) Perfect spores basidiospores.

**Sub- Division- 5. Deuteromycotina**- Motile cells absent- D) perfect stage absent.

**Sub- Division- Mastigomycotina-**

**Class 1. Chytridiomycetes** (Posteriorly uniflagellate zoospore with whiplash type flagellum).

**Order-**1.Hyrpochytriales,
2.Chytridiale,
3.Blastocladiales,
4.Monoblepharidales.

**Class-2.Hypochytridiomycetes-** (Anteriorly uniflagellate zoospore with tinsel type flagellum).

**Order-** 1.Hypochytriales.

**Class- 3. Oomycetes-** ( Zoospores biflagellate, anterior flagellum tinsel type, posterior flagellum whiplash type, cell wall cellulose).

**Order-**1.Saprolegniales
2. Leptomitales
3. Laginidiales
4.Peronosporales

**Class- 4. Plasmodiophoromycetes-** (Biflagellate heterokonean swarmers/ zoospores, whiplash type flagella).

**Order -** 1.Plasmodiophorales.

**Sub- Division Zygomycotina-**

**Class- Zygomycetes-** (Mostly saprophytic, sometimes parasites or mycoparasites, attacking insects also but then developing mycelium inside the inner lining of digestive tract, develops zygospore generally spherical in shape).

Order -1.Mucorales.
2.Entomophthorales.
3.Zoopagales.

**Class- 2. Trichomycetes -** (Mostly commensals with the guts of arthropods, hyphae attached to inner lining of digestive tract, sometimes on external parts of aquatic living arthropods, zygosporesknown bipolar or biconical.

**Order-** 1.Harpellales,
2. Asellariales,
3. Eccrinales,
4. Amoebidales.

**Sub- Division- Ascomycotina-** A) (Thallus mycelial or yeast like, ascocarp and ascogenoushyphae absent, asci naked).

**Class- Hemiascomycetes-**

**Order-** 1. Endomycetales,
2. Protomycetales,
3. Taphrinales.

B) Thallus mycelial, ascocarp and ascogenous hyphae present

**Class-Loculoascomycetes-** i) Ascus bitunicate, ascocarp an ascostroma,

**Order-** 1. Myrianngiales.
2 .Microthyriales.
3. Hysteriales
4. Pleosporales.
5. Dothideales.

B) Ascus unitunicate, if bitunicate then ascocarp and apothecium present.

**Class 3- Plectomycetes-** (Asci scattered at various levels within a cleistothecium, ascospores aseptate.

**Order-** 1. Euritiales,
2. Micro ascoales,
3. Onygenales.

**Class 4- Laboulbenimycetes-** (Asci regularly arranged forming a hymenium at the base or periphery of ascocarp). Ectoparasitic on arthropods, thallus reduced, ascocarp a perithecium, ascus inopercululate.

**Order-** 1. Laboulbeniales

**Class 5- Pyrenomycetes-** (Asci regularly arranged forming a hymenium at the base or periphery of ascocarp, not Ectoparasitic on arthropods, ascocarp a perithecium, ascus inoperculate).

**Order-** 1. Erysiphales
2. Meliolales
3. Coroyophorales
4. Sphaeriales
5. Clavicipitales

**Class 6- Discomycetes**- Ascocarp an apothecium, hypogeal or epigeal, apothecium often massive, ascus inoperculate or operculate.

**Order -**1.Helotiales.
2. Pezizales.
3.Tuberales.

**Sub- division- Basidiomycotina-**

**Class-1.Teliomyetes**- (Parasitic on vascular-plants, basidiocarp absent, basidium arising from thick walled probasidium, teleutosori on host tissue.

**Order-**1.Uredinales,
2.Ustilaginales.

**Class- Hymenomycetes-** (Saprophytic and rarely parasitic,well developed basidiocarp present, basidia arranged in a hymenium, basidiocarp gymnocarpic or angiocarpous, hymenium exposed throughout its development.

**Sub- Class-1. Phragmobasidiomycetidae-** (*Basidium phragmobasidium*),

**Order-** 1. Tremellaels

2. Auriculariales
3. Septobasidiales

**Sub- Class- Holobasidiomycetes**- (*Basidium holobasidium*),

**Order-** 1.Exobasidiales
2. Brachybasidiales
3. Dacrymycetales
4. Tulasnellales (Polyporales)
5. Aphyllophorales
6. Agaricales

**Class- 1.Gastomycetes**- (Basidiocarp angiocarpous, basidium holobasidium.

**Order-** 1.Podoxales
2. Phyllales
3. Hymenogastrales
4. Lycoperdales
5. Geitiriales
6. Tulostematales
7. Nidulariales
8. Melanogastrales
9. Sclerodermatales

**Sub- division- Deuteromycotina-**

**Class- Blastomycetes-** ( pseudomycelium with yeast like budding or without pseudomycelium, true mycelium absent or underdeveloped), Order- nil

**Class- 2. Hyphomycetes-** (Mycelium developed assimilatory budding absent, sterile mycelium present; spores borne on sporophores, sporophores may be grouped together but pycnidia and acervuli absent).

**Order-** 1.Moniliales, 2.Mycelia sterilia.

**Class- 3. Coelomycetes**- (Mycelium developed assimilatory budding cells absent, spores or conidia formed in pycnidia or acervuli).

**Order-** 1. Melanconiales
2. Sphaeropsidal

Many mycologists classify fungi from time to time but still yet all is not settled in mycological classification and that differences of opinion on classification are very numerous. Divergent views arise because of our incomplete knowledge on the structural, developmental and physiological aspects of fungi. Therefore, as our knowledge increases, our classification schemes are bound to change.

Taxonomy of the fungi follows the recommendations of the committee on ***"International Rules of Botanical Nomenclature"***. According to these rules- various taxa have ending as follows- the

divisions of fungi end in **-mycota,** of sub- divisions in "**mycotina",** of classes in '**mycetes**, sub-classes in **- mycetidae**, of orders in- **ales** and of familiesin- **aceae.**

Genera and species have no standard endings. Thebinominal name of an organism is composed of two words. The first designated the genus and the second refers the species. The genus name is always started with capital letter while the specific name started with small letter. Hand written binominals should always be underlined and when printed should be italics e. g. *Solanum nigrum.*


## Recent Development in Fungal Taxonomy

In recent time significant and revolutionary changes have been witnessed in the classification of fungi. In recent past there have been drastic changes witnessed in the taxonomy of fungi with the help of a new approach by the establishment of a new approach by the communication's platform, the deep hyphae, the AFTOL (Assembling Fungal Tree of Life) project which is mainly based on the aspect of molecular studies of the fungus, their gene sequencing, multigen phylogenies and data analysis. On the basis of all these studies Hibbett et. al., 2007, proposed a detailed and modern scheme of fungal classification. That has been a shaped by 67 mycologists. These authors emphasized the broad support and input called on worldwide mycologists to adopt this broadly described and unified system of fungal classification.

Hibbett in his classification represents a set of three diagram and taxa of uncertain position listed as ***"incertae sedis"*** means no clear position in classification. The classification has one kingdom, 0ne sub kingdom, 7 phyla, 10 sub phyla, 12 sub classes and 179 orders.

## Summary of Hibbett's Classification

**Kingdom:** Fungi

**Phylum** 1: Chytridiomycota (Arachemycota)

| | |
|---|---|
| 2. Neocallimastiomycota | Mucoromycotina |
| 3. Blastocladiomycota | Etomophomycotina |
| 4. Microsporidia | Zoopagomycotina |

5. Glomeromycota----------Sub phyla-- Kickxellomycotina

| | |
|---|---|
| **Sub Kingdom: Dikarya** | **i) Taphrinomycota** |
| **Phylum: 1 Ascomycota: ----------** | **ii) Saccharomycotina** |
| | **iii) Pezizomycotina** |
| **Phylum: 2 --------Sub phyla------** | **i) Pucciniomycotina** |
| | **ii) Ustilaginomycotina** |
| | **iii)Agaricomycotina** |

Basidiomycota' ***incertae sedis'*** not placed in any sub- phylum are class- Walleminmycetes and Entorrhizomycetes.

In this system of classification the Basidiomycetes, Uridinimycetes, and Ustilaginomycetes were renamed and elevated to the rank of phylum. The Agaricomycotina Pucciniomycotina and Ustilagimycotina respectively categorized to minimize the confusion between taxon name and informal term. The terms which have traditionally been placed under Zygomycota and Chytridiomycota shows Basal Fungal Lineages. This is the most significant dramatic changes reported in this classification. On the basis of the studies of molecular and cytological analysis of rRNA, tet1 and rbp1 these groups shows polyphyletic origin. Chytridiomycota have been retained as phylum rank, while Blatocladiales, a traditional member of Chytridiomycota upgraded to the rank of phylum Blastocladiomycota, likewise this Neocallimasigales also elevated to phylum level. Mostly mycologists recognized four major groups of true fungi are- Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (Alexopoulos et. al., 1996). Based on recent studies it supports recognition of an additional phyla named Glomeromycota which was traditionally placed under Zygomycota that form an association with roots of most plants and new group Mycrosporidia, of parasitic organisms that live inside the cells of animals are also now considered to fungal kingdom. While Glomenomycota and Dikarya was classified as SYMBIOMYCOTA based on r RNA analysis which is a new clad, but the taxon is not included which in the classification as its placement is not yet clear.

Recently Weber (2009) in his "Recent Development in the Molecular Taxonomy of Fungi" tried to summarized current phylogenic concept and taxonomic placement of fungi as- in three major groups-

i) Non- fungal organisms
ii) The basal fungi and
iii) Dikarya

## *2.4 PHYLOGENY OF FUNGI*

There is no clear idea about the origin and evolutionary relationship of fungi. They are the subject of future research for mycologists. There are two different schools of opinions about their origin. According to the traditional hypothesis algae is regarded as the ancestor of fungi, while some mycologists of modern time consider protozoa as the ancestor of fungi.

According to the ancient/ traditional group supported algae as the ancestor of fungi as they originated by the degeneration of algae which degenerate their chlorophyll and adopted the heterotrophic mode of nutrition. It is supported by the facts that certain flagellates under different conditions develop chlorophyll and live as saprophyte.

Further the followers of algal hypothesis are divided into two groups- some suggests a monophyletic origin and others support polyphyletic origin. They hold that all the fungi namely Phycomycetes, Ascomycetes, Basidiomycetes represents one main evolutionary line of fungi from an algal ancestor except slime molds and some simple fungi. But most of the mycologists not agreed with monophyletic ancestry. They support the polyphyletic origin from the various groups of algae. Based on multiple origins, the fungi are considered a heterogenous aggregation as the Phycomycetes having evolved from one class of algae and Ascomycetes from another class, while the Basidiomycetes have been originated from the Ascomycetes.

The supporters of algal origin of the Phycomycetes further divided into different opinions. Among them one favors that the origin of fungi belongs to siphonous algae originated from green algae, while others supports the Xanthophycean ancestors such as Vaucheria. These studies revealed that there are differences in metabolism and type of flagellation of motile cells in the algae and Phycomycetes. So on the basis of this fact- that the difference in metabolism and type of flagellation in the two groups (algae and fungi) the opponents of the chlorophycean origin of Phycomycetes consider this hypothesis untenable

The others, who supports the Xanthophycean origin of Phycomycetes base on the ground that both have similarities in their structure and evolution between the oogamous Phycomycetes (Oomycetes) and oogamous yellow- green algae (xanthophyceae) such as Vaucheria.

In both -1) the somatic/ vegetative hyphae is a coenocytic, aseptate filamentous thallus.
2) Their life cycle patterns are also similar and oogamous sexual reproduction.
3) There is similarity in the chemical composition of cell wall in both.

De Bary (1881), for the first time propounded this view of phycomycetean origin of fungi from Vaucheria like ancestors. De Bary give hypothesis that the origin of *saprolegian* like Oomycetes from a Vaucheria like ancestor by loss of chlorophyll and thus change in mode of nutrition. Later Chytrids originated from this and the Zygomycetes by retrogression.

A group of mycologists led by Bassey (1942) support the algal origin of fungi from the unicellular coccoid xanthophyceae The basis of this hypothesis is - the similarity in structure,

position of flagella which is generally anterior, nature of reserve food material - is glycogen in both the Phycomycetes and the coccoid ancestor and presence of cellulose in cell wall.

The supporters of this hypothesis believes in two divergent line- one with anteriorly uniflagellate swarmers and other with biflagellate swarmers, but presence of posteriorly uniflagellate swarmers and absence of cellulose in the cell walls of some uniflagellate Phycomycetes is the basis to oppose this hypothesis by the opponent mycologists. They believe that the origin of all uniflagellate and biflagellate Phycomycetes is not from a common ancestor. They are polyphyletic in their origin, some having evolved from a protozoan (flagellate) ancestor and the others from an algal ancestor.

Fischer and Dangeard support the protozoan or flagellate ancestry of the Phycomycetes. According to modern mycologists the hypothesis is more reliable as there is similarity in structure, position of flagella (anteriorly inserted and similar metabolism in both Phycomycetes and protozoans. On the basis of this hypothesis the Phycomycetes are evolved from the flagellates via chytrids. Thus the uniflagellate forms originate from the uniflagellate protozoa and the biflagellate originates from the biflagellate protozoa. Further these non- mycelial forms gave rise to the more advanced mycelial form by further evolution.

Beside this, there is another line of mycologists; Hawker (1967) suggests a polyphyletic origin of Phycomycetes/ lower fungi from the aquatic flagellates along parallel line. According to this hypothesis, the primary Phycomycetes are belonging to chytridiales, Hypocchytridiomycetes and Plasmodiophoromycetes from flagellates. While the Oomycetes originates from filamentous algal ancestors as they resemble in the cell wall composition (cellulose nature), life-cycle, form of sex organs, and similarity in the form of mitochondria and endoplasm. Thus by the loss of chlorophyll they may have evolved from an algal ancestor.

Similarly the Zygomycetes and chytrids resemble at the flagellation. Thus Hawker suggests common ancestry for both groups. This proves that there is no relationship between different groups of fungi because the fungi have an independent or polyphyletic origin.
In that case the classes of fungi should be raised to the status of divisions or phyla, but this view is unable to explain the close similarities between different classes of fungi which is a drawback of this hypothesis (Fig. 2.3 & 2.4).

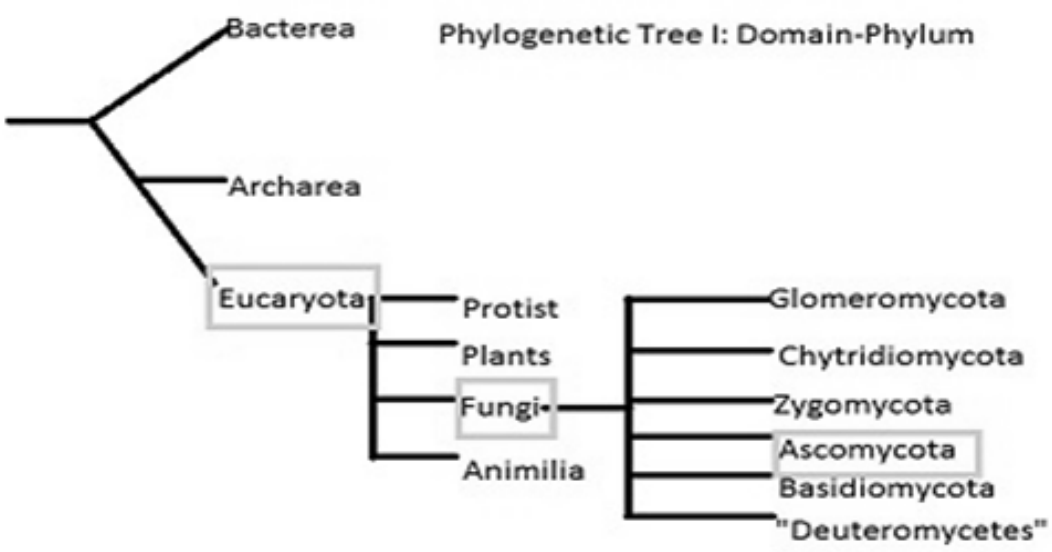


*Fig. 2.3: Phylogenetic Tree of Fungi*

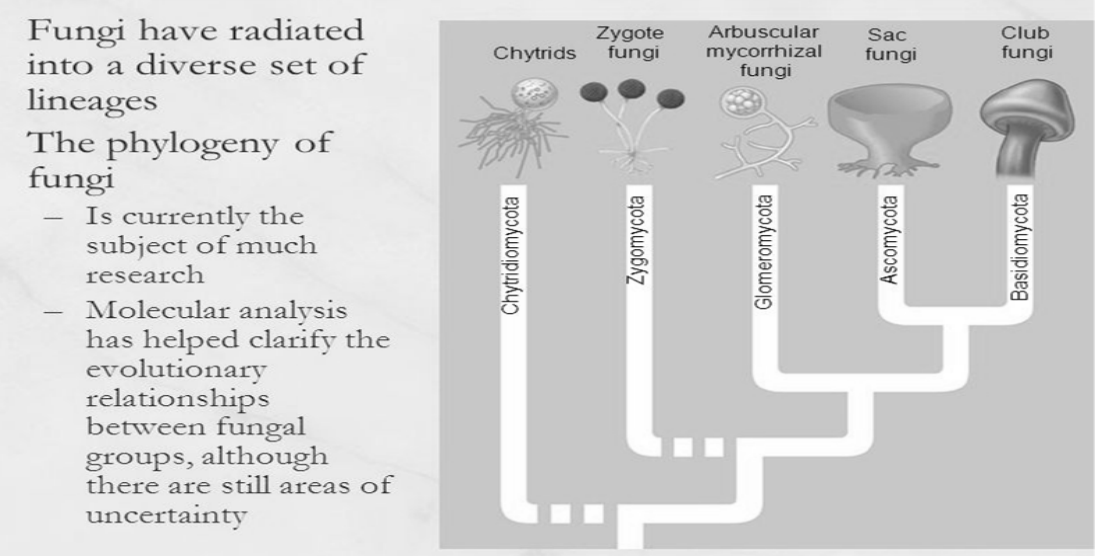


*Fig. 2.4: Phylogenetic classification of Fungi on molecular basis*

## Similarities between different classes of fungi:

1. Similarity of the somatic phase or mycelium, which differs in the magnitude of specializationand differentiation in different groups.
2. Resembles between the antheridia and oogonia of Phycomycetes and the sex organ of Ascomycetes.
3. Similar origin, nature and development of conidia in different groups.
4. Similar origin, physiology and phylogeny of the ascus and basidium.

# *2.5 ECONOMIC IMPORTANCE OF FUNGI*

Fungi play a significant role in our daily life. They are our greater friends as well as foes. Some are highly useful to mankind while others are highly injurious and are responsible for many diseases in plants, animals and human beings.

**(A) Useful Fungi:** Fungi are useful to us in many ways.

**1-Fungi as Food:** Fungi play important role in human food production. Several mushrooms and morels e.g., *Agaricus bisporus*, *Amantia vaginata*, *Boletus edulis, Clavatia gigantean*, *Morchella, Pleurotus, Volvaria*, *Volvariella volvacea*, are used as food. *Agaricus compestris* is cultivated in manyareas of north and south India and *Morchella esculenta* is grown in Punjab and Kashmir. Mushrooms are preferred for food because of their fairly large proteincontents (21-30%). They are also rich in vitamins, carbohydrates, minerals and amino acids.

Yeast is an important source of vitamin B and D, and rich in protein. Similarly a popular food, 'sufu' is produced from species of *Mucor* and *Antimucor.* 'Incaparina' foods developed by the Institute of Nutrition of Central America and Panama consists of corn (26%), cotton seed flour (38%), Sorghum (26%), Yeast (3%) and minerals and vitamins. Northern Utilization Research and Development Division of USDA has developed protein cake by combining wheat, barley, oat, rice and soybean flour cooked and fermented with the help of *Rhizopus oligosporus*. This cake is rich in niacin and riboflavin. Single cell protein (SCP) obtained from yeast, species of *Aspergillus, Penicillium, Fusarium, Neurospora* and Candida is a complete substitute for conventional protein foods.

**2-Fungi as medicines:** Several fungi are used in the production of medicines.
**Ergot:** is prepared from the sclerotia of *Cleviceps purpurea.* It contains some alkaloids which are used to induce uterine contraction for abortion, in the treatment of menstrual disorders and to check haemorrhages.
**Ephedrine:** Synthesized from benzaldehyde by the action of yeast and it used in the treatment of asthma and nasal troubles.
**Steroids:** Steroids are complex organic compounds, effective against rheumatoid arthritis, allergic, dermatologic, and other diseases. Their extraction from biological system is highly expensive. A wide variety of fungi have the capacity of synthesizing many steroids. e.g., cortisone is prepared by the fermentation of plant glycosides by *Rhizopus nigricans* and *Aspergillus niger.*
**Vitamins:** Vitamins are accessory micronutrients, which are required by living organisms for their proper growth. Several yeasts are good source of vitamin B complex. Ergosterol a precursor of vitamin D, is synthesized from some molds and yeasts. *Eremothemium ashbyii* is a rich source of vitamin $B_{12}$, whereas vitamin A is extracted from *Rhodotorula gracilis.*

**Antibiotics:** Alexander Fleming in 1944 for the first time extracted the wonder drug penicillin from *Penicillium notatum*. Since then several fungi are active against human pathogens e.g., spread of *Candida albicans* is prevented by the extract of *Trichoderma sponaceum*. Many edible mushrooms like *Agaricus bisporus* and *Lentinus edodes* have the ability to lower blood cholesterol.

**3- Fungi in production of organic acids**

**(i) Citric Acid**- Produced by fermenting sucrose and molasses by *Aspergillus niger* and *A. wentii.*

**(ii) Itaconic acid-** Produced by fermentation of sugars by *Aspergillus itaconicum* and *A.terreus.*

**(iii) Gluconic Acid-** Produced by fermenting sugars by *Aspergillus niger* and *Penicillium purpurogenum*.

**(iv) Kojic acid-** Produced by fermentation of sugar by *Aspergillus oryzae.*

**(v) Gallic acid-** It is synthesized by the hydrolysis of gallotannin present in gall nuts by *Penicillium glaucum* and *Aspergillus gallomyces.*

**(vi) Fumaric acid:** obtained by fermentation of sugars by *Rhizopus stolonifer*.

Besides this several species of *Mucor* and *Rhizopus* are used in the production of lactic, oxalic and succinic acid.

**4- Fungi in Industry**

**(i) In brewery:** Alcoholic fermentation with the help of fungi is the basis of brewing industries. Wine is produced by fermenting rice with *Aspergillus oryzae, Saccharmyces cerevisiae* is used in production of beer.

**(ii) In baking Industry:** Fermentation of carbohydrates by *Saccharomyces cerevisiae* produces ethyl alcohol and $CO_2$. Carbondioxide liberated in this process is used in the preparation of breads and cakes.

**(iii) In cheese Industry:** Some species of *Penicillium* e.g., *P. candidum, P. roequeforti, P. camembertile* are used for manufacture of cheese. They impart specific flavours to cheese.

**5- Fungi in Enzyme Production**

**(i) Invertase:** Yeast *Saccharomyces cerevisiae* is used for extraction of the enzyme invertase.

**(ii) Zymase:** The enzyme zymase obtained from *Saccharomyces cerevisiae* by fermentation of carbohydrates.

**(iii) Amylase:** *Aspergillus niger* and *A. oryzae* are used in the production of the enzyme amylase.

**(iv) Cellulase:** *Trichoderma reesli* is used in the production of the enzyme Cellulase.

**6- Fungi in agriculture:** Fungi play an important role in agriculture in various ways-

**(i) As scavengers:** The ratio of carbon dioxide in the atmosphere is maintained by decomposition of plants and animals debris by fungi and bacteria. Decomposition is mainly

carried by saprophytic fungi. The enzymes secreted by these fungi decompose complex organic substance into their inorganic components and these increase soil fertility.

**(ii) In biological Control:** Fungi play an important role in biological control of diseases. For instance, *Trichoderma lignorum* suppresses the growth of root fungus. *Pythium* and the growth of *Rhizoctonia solani* can be checked by *Penicillium vermiculatum* and *Rhizoctonia oryzae.* Several fungi are also utilized in controlling soil borne organisms like amoeba and nematodes. For instance, Nematophthora gyrophila is capable to control effectively Heterodua avenae, a cereal cyst nematode.

**(iii) As mycorrhiza:** A symbiotic relationship between fungal hyphae and roots of higher plants is known as mycorrhiza. Several fungi like Rhizectonia, Phoma Trichoderma, Boletus, Phallus, Scleroderma and Amantia form mycorrhizal relationship with higher plants. The fungal partner of mycorrhiza obtains food from the roots of higher plants and in return it supplies mineral elements to the other partner.

**(iv) In Soil aggregation and soil fertility:** Some fungi such as species of *Absidia, Aspergillus, Cladosporium, Chaetomium, Mucor, Penicillum* and *Rhizopus* have soil binding properties. The mucilaginous substances secreted by them are helpful in soil aggregation. In forest ecosystem the natural mushroom flora greatly helps in biodegradation of woody wastes. The ultimate end product in the form of humus is quite useful for the growth of other plants.

**(v) As Growth hormones:** Gibberellin produced by *Gibberella fujikuroi*, is an important plant hormone. It is used to accelerate growth of many crops. The hormone trisporic acid is obtained from *Mucor mucedo* and *Choanephora trispora.*

**(vi) As insecticides:** Many insect pests can be controlled by the use of fungi *Aschersonia aleyroidis, Empusa sepulchralis, Fusarium oxysporum* and *Metarhiziun anisopliae*.

**(vii) Fungi as Test organisms:** Several fungi are used as important research tools for the study of various fundamental biological processes. *Neurospora* has become an ideal material for genetical studies. *Neuropsopra crassa* is used as a test organism to detect the presence and quality of vitamins B in a given sample. *Aspergillus niger* is used for the detected of trace elements like zinc, nickel and copper. Several fungi such as species of *Aspergillus, Absidia, Penicillium, Torulopsis, Endomyces, Rhodotorula* are capable of synthesizing fats and fatty substances.

**(viii) Fungi as Dyes:** Several fungi which possess coloured spores and mycelium are utilized as dyes. A red pigment obtained from *Monascus purpureus* is used for colouring rice and *Cercosporina kikuchii* produces a reddish-violet novel pigment, neocercosporin. A similar maroon pigment is obtained from *Aspergillus fumigatus*. A brown pigment atromentin is obtained from *Paxillus atromentosus* and a yellow pigment citrinin from *Penicillium citrinum.*

## (B) Harmful Fungi

**1- Fungi as Pathogens:** Most of the parasitic fungi cause disease in plants. The common fungal diseases of plants are Rusts, smuts, blights, mildews, roots and wilts. They are responsible for destroying crops worth billions of rupees. Disastrous Irish Potato famine of 1845-49 caused by

*Phytophthora infestans* was responsible for taking lives of approximately one million people. Similarly tobacco yield was reduced by over 60% in North Africa and Middle East in 1962 due to the infection of *Peronospora tobacina*. In England over five million elm trees were destroyed during 1967-77 because of infection of *Ceratocystis ulmi.*

Besides plant diseases several fungal species lives parasitically on/in animals and they cause various diseases. Actinomycosis and blackleg disease in animals are also caused by fungi. Members of Entomophorales are parasites on insects and other animals. Similarly members of Saprolegniales e.g., *Saprolegnia* are parasitic on the fish.

Several fungi infect human beings causing diseases of skin, respiratory tract, lungs, viscera, nasal sinuses, eye, liver and kidney. Wind-borne spores of several fungi like *Alternaria, Aspergillus, Chaetomium, Helminthosporium, Monilia, Phoma, Trichoderma* are allergic and cause distressing symptoms.

**2- Spoilage of Food stuffs:** A large number of food articles, if not properly stored are spoiled by fungi like *Mucor, Rhizopus*, Aspergillus, Penicillium, and yeast. Dairy products are spoiled by the species of *Mucor, Oidium, Torula, Penicillium* and *Cladosporium*. Several species of *Alternaria, Aspergillus* and *Rhizopus* cause past harvest diseases in fruits and vegetables, thus shortening their storage life.

**3- Deterioration of Articles:** Most important paper documents, valuable leather articles, textiles, plastic objects, photographic films, electronic goods, rubber, painted surfaces etc. are destroyed by fungi. *Aspergillus niger, Stachybotrys atra* and species of *Alternaria* and *Cephalosporium* are some common fungi with spoil paper. Moulds are responsible for the damage of Cellulose fibers. The fungi mainly responsible for deterioration of painted surfaces are *Pullularia pullulans, Phoma glomerata* and species of *Alternaria* and *Cladosporium*. Jute articles are destroyed by *Chaetomium globosum, Momnoniella echinata* and *Stachybotrys atra*. Rubber products are spoiled by the species of *Aspergillus* and *Penicillum*.

**4- Destruction of Timbers:** Several fungi like *Polyporus* sp., *Serpula lacrymans, Penicillium divarianum, Fusarium negundi, Lentinus lapidens* are responsible for the destruction of valuable timbers. *Armillaria mellea* causes wood rot diseases in several trees. Wood degradation by fungi is usually of two types- White rots and brown rots. Some fungi grow in sap wood and stain it. *Chlorosplenium aeruginosum* and *Penicillium divaricatum* impart characteristic green and yellow stain to hard wood and *Lasiosphaeria pezizula* imparts grayish alive stain to heat and sap-wood.

**5- Hallucinogenic Fungi:** Some fungi such as *Amanita phalloides, A.verna* and *Boletus santanus* are highly poisonous and if ingested they may prove fatal. Several mushrooms as *Amanita muscaria, Psilocybe maxicana* and *Panaeolus sp*. secrete hallucinogenic substances may destroy brain cells and perception power of human beings.

**6- Mycotoxins:** Many fungi produce mycotoxins which are responsible for food poisoning and other distressing symptoms. They may be-

**(i) Food toxins:** The toxin production can occur in most plant products but cereals and oil seed crops are mostly contaminated. Mainly four groups of toxins are mainly associated with human diseases.

**(a) Aflatoxins:** Produced by *Aspergillus flavus, A. fumigatus, A. parasiticus* and *Penicillium islandicum.*

**(b) Ochratoxin:** Mainly produced by *Aspergillus ochraceus* and *Penicillium viridicatum* when they infest stored maize, pea nuts, beans and mixed animal feeds.

**(c) Zearalenone:** A phenolic resorcyclic acid Lactone is produced by several species of Fusarium growing on maize.

**(d) Trichothecenes:** are produced by several species of *Cephalosporium, Fusarium, Myrothcium* and *Trichoderma.*

**(ii) Ergot Toxins:** The sclerotia of Claviveps purpurea contain poisonous alkaloids like ergotamine, ergometrimine, ergocristine and ergonorin.

**(iii) Mushroom toxins:** Several mushrooms produce mycotoxins which causes diarrhoea and vomiting in early stage but in severe cases, liver damage, kidney failure, and even death may take place *Amanita phalloides* produces about ten toxins. Gyromitrin, a highly fatal toxin is produced by the species of Helmella. Toxin muscarine produced by Inocype and Clitocybe. Species of Coprinus produce the toxin coprine that affects the autonomic nervous system.

## *2.6 SUMMARY*

Fungi include mushrooms, rusts, smuts, puffballs, truffles, morels and molds more than 70,000 species of fungi have been identified. Thus the significance of fungal classification is to distinguish these fungal organisms with their distinguishing taxonomic features. Generally, fungi are capable to reproducing sexually under appropriate conditions, besides reproducing by various asexual means. The sexual stage of a fungus is known as its perfect stage. But for a good number of fungi, the perfect stage is unknown. Such fungi are designated as imperfect fungi (Fungi Imperfecti). Because fungi are classified mainly on the basis of their sexual stage the imperfect fungi cannot be placed in any of major fungal categories. They are therefore, assembled in a special group Deuteromycetes. In case the perfect stage of any member of this group is discovered, it is transformed to its original taxon. A number of mycologists try to classify fungi on the basis of their studies yet there is not a single one to satisfy the universal mycologists. Recently based on genetic, molecular and phylogenetic studies, conducted by modern

mycologists, reveals no lines of fungal classifications among the Hibbett and coworkers opens the new era in this field.

## *2.7 GLOSSARY*

**Aplanospore:** A non- motile spore.
**Ascomycetes**: A class of fungi distinguished by the ascus.
**Ascopore:** A sexual spore, characteristic of the Ascomycetes, produced in a sac like structure (an ascus) after the union of two nuclei.
**Ascus:** A sac like structure, characteristic of the Ascomycetes, in which ascospores are produced.
**Basidiospores:** A sexual spore produced followed the union of two nuclei on a specialized club like structure.
**Basidiomycetes:** A class of fungi that form basidiospores.
**Budding:** A form of sexual reproduction, (in yeast}, in which a new cell is formed as an outgrowth from the parent cell.
**Cell:** The microscopic, functionally and structurally basic unit of all living organisms.
**Cellulose:** A complex polysaccharides consisting of many glucose molecules, the characteristicand structural material of plant cell walls.
**Chitin:** A polymer of N- acetyl glucosamine present in the covering layer of arthropods and in the cell wall of many fungi.
**Coenocytic:** A term applied to a cell or an aseptate hypha containing many nuclei.
**Conidiophore:** A hypha which bears Conidiospores.
**Conidiospores:** Any asexual spore which are formed at the tip of a hypha and which are not enclosed within a sac.
**Conidium:** An asexual spore that may be one cell or many celled and may be many sizes and shapes also called Conidiospores.
**Eukaryotic:** A cell that possesses a definite or true nucleus.
**Exogenous**: Produced or originated without.
**Fission**: An asexual process by which some microorganisms reproduces; transverse cell divisions.
**Flagellates:** Members of one of the sub phyla of phylum Protozoa.
**Flagellum:** A thin, filamentous appendages for swimming mobility.
**Glycogen:** A carbohydrate of the polysaccharide group stored by animals. It yields glucose on hydrolysis.
**Heterotroph:** A microorganism that is unable to use carbon dioxide as its sole source of carbon and require one or more organic compounds
**Microscopic:** Visible only with the aid of a microscope.
**Microorganism:** Any organism of microscopic dimensions.

**Mold:** a fungus characterized by a filamentous structure.
**Mycelium:** A mass of thread filamentous structure composing of a network that constitutes the vegetative structure of a fungus.
**Nucleus:** The structure in a cell that contains the chromosomes.
**Metabolism:** The system of chemical changes by which the nutritional and functional activities of an organism are maintained.
**Oogamy:** The union of an egg cell and a sperm cell.
**Mycology:** The study of fungi.
**Parasite:** An organism that, derive its nourishment from a living plant or animal host.
**Pathogen:** An organism capable of producing disease.
**Perfect fungi**: Fungi with both asexual and sexual life.
**Phylogeny:** The evolutionary or ancestral history of organism.
**Prokaryote:** A type of cell in which the nuclear substance is not enclosed within a membrane. e.g., a bacterium, or cyanobacterium.
**Taxonomy:** The classification, (arrangement), nomenclature (naming), and identification of organism.
**Taxon:** Pl. taxa- a taxonomic group, such as a species, genus or family.
**Tissue:** a collection of cells forming a structure.
**Yeast:** a kind of fungus that is unicellular and lacks typical mycelia.

## *2.8 SELF-ASSESSMENT QUESTIONS*

### 2.8.1 Objective Type Questions:

i) The science of study of fungi is known as----------
ii) The cell wall of the fungi is made up of----------
iii) The classification of fungi is based both on ----------stage and type of----------
iv) The group of fungi which lack sexual reproduction are placed in the class----------
v) Class Phycomycetes is also known as----------
vi) The fruiting bodies of Ascomycetes is----------
vii) Members of Chytridiomycetes produced --------cell having only single ---------- flagella.
viii) Motile cells are absent in ---------- and----------.

**Answer Key**: i) Mycology, ii) Chitin, iii) mycelial stage and reproduction, iv) Deuteromycetes, v) lower fungi, vi) asci, vii) motile, whiplash, viii) Zygomycetes, Trichomycetes

### 2.8.2 Multiple Choice Questions:

1. Imperfect stage of fungi refers to-

(a) Poorly developed mycelium (b) Poorly developed sex organs
(c) Conidial stage (d) Immature fruiting bodies

2. The branch of Botany that studies fungi is known as-
(a) Morphology (b) Mycology
(c) Microbiology (d) Phycology

3. All fungi are-
(a) Autotrophs (b) Heterotrophs
(c)Saprophytes (d) Parasites

4. The formula of chitin is-
(a) $C_{20}\ H_{54}N_2O_{21}$ (b) $(C_{22}\ H_{54}N_4O_2)_n$
(c) $C_{22}H_{24}N_4O_{21}$ (d) $C_{16}H_{28}N_2O_4$

5. Perfect stage of fungus means-
(a) The fungus reproduces asexually (b) The fungus reproduces sexually
(c) The fungus is perfectly healthy (d) It forms resting spores.

6. Deuteromycetes are also known as-
(a) Perfect fungi (b) Imperfect fungi
(c) Parasitic fungi (d) Saprophytic fungi.

**Answer Key**: 1. (b), 2. (b), 3. (b), 4. (d), 5 (b), 6 (b)

## *2.9 REFERENCES*


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## *2.10 SUGGESTED READINGS*

- https:// www.google.co.in.
- Wikipedia
- https://www.Biologyreference.com
- www. Biologydiscussion.com/fungi--
- Your library.com
- The Fungi (1994) 3rd edition. M.J. Carlila, Academic Press, America.


## *2.11 TERMINAL QUESTIONS*

### 2.11.1 Short Answer Questions:

1. Give an outline of any one system of classification of fungi?
2. Write short notes on-
    i) Classification of fungi
    ii) Phylogeny of fungi
    iii) Fungi Imperfecti.

### 2.11.2 Long Answer Questions

1. Describe the classification of fungi as proposed by Alexopoulos?
2. Describe the salient features of important group of fungi?
3. Write in detail the classification of fungi given by Ainsworth?
4. Define the characteristic features of Division Mycota?
5. Describe any recent system of classification of fungi giving its important characteristic features?
6. Describe the salient features of sub- division Myxomycotina and Eumycotina?
7. Write short note on the recent development in fungal taxonomy?
8. Discuss the origin and phylogeny of fungi?
9. Describe the differences in the classification of Alexopoulos and Ainsworth?
10. Write short notes -
    i) Discuss the status of fungi in the five kingdom classification?
    ii) Write the classification of fungi proposed by Sparrow?

# UNIT-3 GENERAL ACCOUNT OF LICHEN

## Contents:

## 3.1 OBJECTIVES

After reading this unit student will be able:

- To know about general parts of the lichen and their occurrence.
- To learn the basic growth forms of lichens
- To understand the methods of reproduction in lichens.
- To study the economic importance of lichens.

## 3.2 INTRODUCTION

The term 'Lichen' was used for the first time by Theophrastus (the father of Botany, 371-284 B.C.) to denote a superficial growth on the tree barks. Lichen has been defined as 'a stable self-supporting association of a phycobiont and mycobiont' in 1989 edition of the 'Dictionary of the Fungi'. Lichen is not a single organism but a small group of curious plants. It is a symbiotic association between a fungus and algae or cyanobacteria. Cyanobacteria are sometimes referred to as 'blue-green algae', though they are quite distinct from the algae. The fungal partner may be referred to as the Mycobiont (Mykes= fungus, bios=life). The non-fungal partner contains chlorophyll and is called the phycobiont (Phykos= alga, bios= life). The lichen symbiosis is thought to be a mutualism, since both the fungi and the photosynthetic partners benefit.

In case of algae, it conducts photosynthesis to produce food for itself and for the fungal partner. The fungal partner, in turn, protects the algae from drying out, providing it with water, and in some cases, minerals. Some hold it to be a typical case of symbiosis whereas others consider it to be parasitism. There's also evidence of parasitism. The algae and bacteria partners can exist on their own in nature, the fungal partner cannot. In the case of fungal/ algae partnership, the fungal partner destroys algae cells in the process of exchanging nutrients with it. The lichen survives this only because algae reproduce cells faster than they are destroyed. The relationship between the two partners is now considered to be a case of 'helotism' a master-slave relationship where algae act as a slave for the fungal master. In 1867 Swiss botanist Simon Schwendener first proposed the theory of the duality of the lichen thallus. According to his theory, alga and fungus share a relationship as helotism where the Alga was slave providing nutrient to fungal master. In 1887 De-Bary used the term Symbiosis for association of lichen. Schwendener's dual theory of lichens has been accepted by every one for which experimental proof has been obtained.

## 3.3 OCCURRENCE

Lichen is a group of tiny plants that looks like moss and grows on the surface of things such as rocks, trees, and walls. Lichens grow relatively slowly. Growth rate depends both on the species and on

the environmental conditions around it. The smaller encrusting lichens may grow as little as 1mm a year. Large forms may grow up to 1 cm per year. Lichens occur from sea level to alpine peaks and from the hot deserts of the world to the cold Arctic and Antarctic. Lichens can grow in locations impossible for most plants, such as bare rock, walls, roofs, sterile soil and sand etc. Based on the substratum on which the lichens are growing, lichens are of following types-

**(a) Muscicolour lichens:** Lichens growing along with mosses. e.g., *Cladonia*.

**(b) Follicolous lichens:** Lichens growing on the surface of leaves. e.g., *Calicium*.

**(c) Terricolous lichens:** Lichens growing on the surface of soil, in hot climate with sufficient rain and dry summer (terrestrial) e.g., *Cladonia, Florekeana, Lecidea, Collema* etc.

**(d) Saxicolous lichens:** Lichens growing on the surface of rocks and stones in cold climate. e.g., *Dermatocarpon, Xanthoria, Verrucaria* etc.

**(e) Corticolous lichens:** Lichens growing on the surface of barks of trees mainly in the sub-tropical and tropical regions. e.g., *Parmelia, Usnea, Grpahis*.

**(f) Lignicolous:** Grow directly on wood. e.g., *Calicium* etc.

**(g) Marine Lichens**: Grow on siliceous rocky shores of Sea e.g., *Verrucaria, Caloplaca* etc.

**(h) Fresh water lichens:** Grow on hard siliceous rocks in fresh water. e.g., *Epheba, Hymenelia* etc.

## *3.4 GENERAL STRUCTURE*

In lichen, the mycobiont produces a thallus, which houses the photobiont. There are three major morphological types of thalli: foliose, fruticose and crustose (Fig. 3.1).

### 3.4.1 Classification of lichen:

**(A) Lichens are classified on the basis of Growth forms:**

**1-Crustose Lichens (Encrusting Lichens):** These lichens occur as thin or thick crust over soil, rocks or tree barks. These are very closely adhered to the substratum on which they are present and it is difficult to remove them from substratum. Fruiting bodies are present on the upper surface, common examples are *Ochrolechia, Graphis scripta, Rhizocarpon,* etc.

**2-Foliose Lichens (Leafy lichens):** These lichens have a flat, expanded, leaf like thallus (generally grayish or brownish in colour) which spread out in a horizontal layer over the surface. They are attached to the substratum by rhizoid like outgrowth called the rhizines and can be easily dismantled without damaging the substrates. Common examples are *Physcia, Parmelia, Gyrophora,* etc.

**3- Fruticose Lichens (Shrubby Lichens):** These are the upright or hanging lichens. These lichens have a thallus that is branched and bushy and can hand from the substrate. It may be erect

or pendant. These are flat, cylindrical, or ribbon like, well branched and resemble with little shrubs. These lichens are attached only at the base by a flat disc and can be removes from the surface by hand. e.g., *Cladonia rangiferina, Usnea barbata* etc.

**There are few intermediate categories of growth forms such as-**

**4- Leprose Lichens:** A leprose lichen is a lichen with a powdery or granular surface. In leprose lichens the thallus surface is composed of granules containing algal cells and fungal hyphae. Leprose lichens lack an outer "skin", or cortex. Leprose lichens have no inner or outer cortex. They sometimes have a weak kind of medulla. e.g., *Leparia incana*.

**5-Squamulose lichens:** Squamulose lichens are a group of lichens that are scale-like. They are somewhere in between the foliose lichens (flat leaf-like) and the fruticose lichens (erect growing). In Squamulose lichens, the thallus is composed of usually small, flat, usually massed, often overlapping scales- 'squamules'. If they are raised from the substrate and appear leafy, the lichen may appear to be foliose lichen, but the underside does not have a "skin" (cortex), as foliose lichens do e.g., *Normandina pulchella.*

**6-Filamentous Lichens:** Filamentous lichen is a lichen that has a growth form like a mass of thin, stringy, non-branching hairs or filaments of the alga (Trentepohlia or trichome-forming cyanobacteria). These lichens are generally darker in colour and unlike most other lichen growth forms, the filaments of fungus do not determine the shape. e.g., Cystocoleus, Ephebe, Coenogonium, Racodium etc.

**7-Gelatinous Lichens:** Gelatinous lichens are lichens in which the phycobiont (the principal symbiont) is a cyanobacterium. In gelatinous lichens the cyanobacteria produce a polysaccharide that absorbs and retains water. They become gelatinous when wet and brittle when dry.

**8-Dimorphic lichens:** In dimorphic lichens single characters of both foliose/ Squamulose and fruticose lichens. The squamulose and fruticose lichens. The squamules are the primary thallus, which bears erect body of fructicose lichen, the secondary thallus.

**9-Placodioid:** A placodioid lichen is a crustose lichen (the thallus is generally crustose) with a growth form that radiates out from a center, sometimes peeling up at the ends of the radial arms to have a leafy form, but without a cortex on the underside, like a foliose lichen. Some placodioid species can be confused with foliose species, e.g., Crustose- Placodioid species of caloplaca, especially *C. flavescens*, can resemble the foliose *Xanthoria elegans*, but the latter has true foliose lobes with a lower cortex.

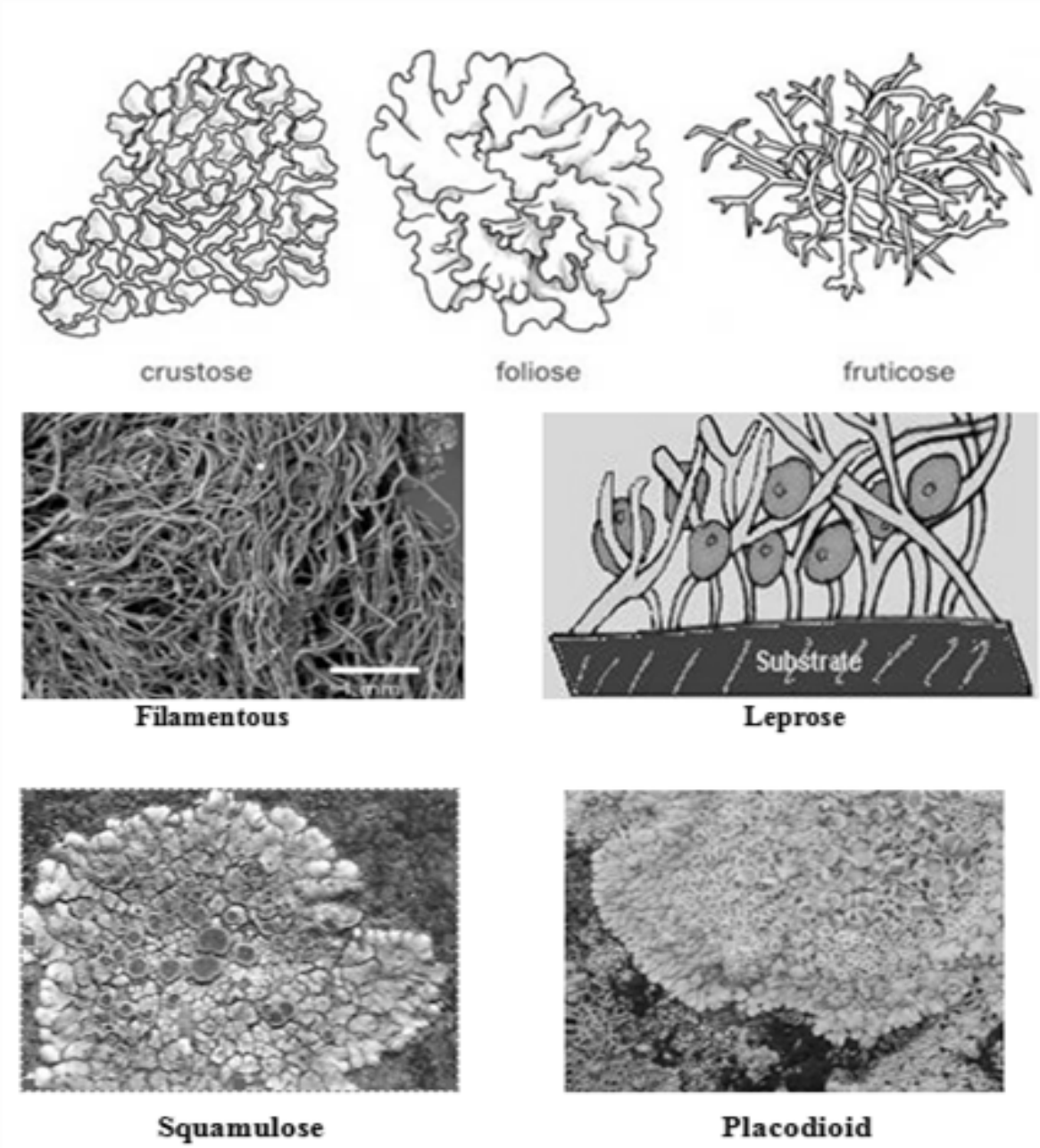


*Fig. 3.1: Different types of Lichens*

**(B) On the basis of nature of fungal componenet:** The fungal partner mainly belongs to ascomycetes apart from basidiomycetes and rarely deuteromycetes. On the basis of the nature of fungal components, lichens are divided to three classes-

**(i) Ascolichens:** In this, the fungal component belongs to Ascomycetes. Sexual reproduction of Ascolichens is similar to those of Ascomycotina. They produce ascus with ascospores after sexual reproduction. Majority of lichens (more than 95% of the lichens) are Ascolichens. Such lichens are further divided into two sub groups:

**(a) Gynocarpeae:** In which fruiting body (i.e. ascocarp) is apothecium. e.g., Parmelia.

**(b) Pyrenocarpeae:** In which the ascocarp is perithicium type. e.g., Dermatocarpon.

**(ii) Basidiolichens:** In this, fungal component belongs to basidiomycetes. e.g., *Dictonema, Corella*. Sexual reproduction is similar to those of Basidiomycotina. They produce Basidia and Basidiospores during sexual reproduction. Only very few lichen (4 genera reported so far) belongs to Basidiolichen.

**(iii) Deuterolichens:** Deuterolichens are also known as lichen imperfectii. The fungal partners belong to Deuteromycotina division of fungi. These lichens lack sexual reproduction or should say that lichens with sterile thalli are constituted by this group. e.g., *Lepraria, Leprocaulo, Crysothrix.*

**3.4.2 Colour:** Lichens show many colours such as green, yellow, orange, white, grey etc. The colouration is due to the pigmentation of algal component in the lichens. In some lichens, a special pigment called usnic acid is present which give lichens a variety of colours. In the absence of special pigments, lichens are generally bright green to olive grey when it is wet and grey or grayish-green to brown when dry. In high moisture surroundings, lichens appear greener because the water absorbed fungal mater become more transparent and as a result the green colour algal pigments get exposed. Colours vary due to genetics, age and on the angle of exposure to light.

**3.4.3 Internal structure of lichen:** Internally the thallus is composed of fungal and algal components. Such type of thallus is called consortium. On the basis of internal structure of thallus, the lichens are divided into two groups, namely, heteromerous and homoiomerous lichens (Fig. 3.2).

**(a) Structure of heteromerous lichen:** Thalli or most foliose and fruticose lichens are differentiated into several layers of tissues, and therefore known a heteromerous. A transverse section of the heteromerous lichen can be divided into following distinct zone-

**(i) Upper cortex:** It forms the upper surface of the thallus. It is thick and protective in nature and consists of fungal hyphae. The compactly interwoven hyphae produce a tissue like layer (Plectenchyma and Pseudoparenchyma) called the upper cortex. The intercellular spaces are absent, if present, they are filled with gelatinous substances. In some species of foliose lichens this layer is interruptions or areas are called breathing pores and serve for aerations. In addition to these certain other structures are also present for gaseous exchange. These are known as cyphellae.

**(ii) Algal zone or gonidial layer:** It is a zone below the upper cortex. This layer consists of loosely interwoven hyphae intermingled with algal cells. This algal zone is the photosynthetic region of the lichen. This layer is also known as gonidial layer because of the earlier concept that these cells are having reproductive function.

**(iii) Medulla:** It is the central core of the thallus and is composed of loosely arranged fungal hyphae with intercellular spaces. The hyphae run in all directions. Usually, the wall of the fungal hyphae is thick and strong.

**(iv) Lower cortex:** The lower cortex is below medulla. It is formed by fungal component and made up of compact hyphae. They may be parallel to perpendicular to the surface to the surface of the thallus. Thje bundle of hyphae (rhizinae) arise from the lower surface and penetrate the substratum functioning as anchoraging and absorbing organs. In some lichens, the lower cortex is absent. e.g., *Lobaria, Pulmonaira* and is replaced with a sheet of hyphae forming hypothallus.

**(b) Homoiomerous lichens:** In some lichens for example, *Collema* and *Leptogium*, the thallus shows a simple structure. It consists of a loosely interwoven mass of fungal hyphae with algal cells equally distributed through a gelatinous matrix. Thalli of such lichens are not differentiated into layers of tissues and therefore, known as homoiomerous.

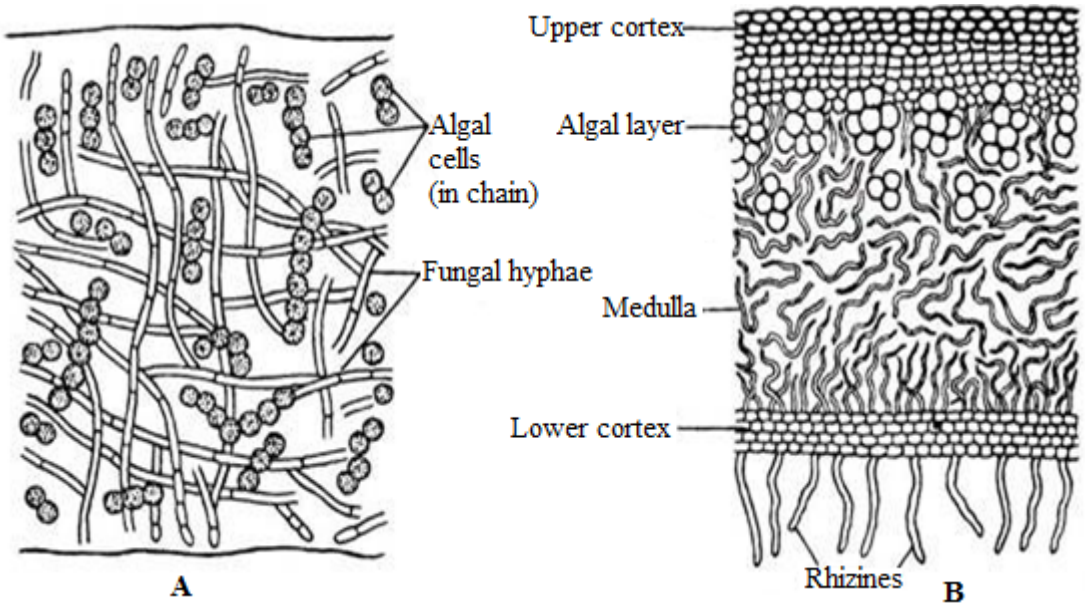


*Fig. 3.2: Internal structure of lichen thallus, A-Homoiomerous thallus, B-Heteromerous thallus*

**3.4.4 Anatomy of the lichen thallus:** The vegetative structures which are associated with the lichen thallus are (Fig. 3.3):

**(i) Breathing pores:** These are localized openings which develop in the upper cortex. In some lichens, e.g., *Parmelia*, the upper cortex is interrupted by some openings, called breathing pores. The breathing pores serve for aeration and helps in respiration.

**(ii) Cyphellae:** They occur on the lower surface of the thallus quite commonly in the genus Stricta. If seen with naked eyes these structures appear as cup-like white spots but under the microscope they appear as small, hollow, circular, white cavities. From these cavities medulla is exposed and hyphae protrude out. If these cavities are of a definite form with a distinct border,

these are called cyphellae. (The function of these structures is to allow free passage of air to the algal cells.(or their function is aeration.)

**(iii) Cephalodia:** Cephalodia are small, dark-coloured, hard, gall-like structures found in some species of lichens that contain cyanobacterial symbionts. Cephalodia can occur within the tissues of the lichen, or on its upper or lower surface e.g., *Peltigera aphthosa, Lobaria, Pulmonaria* etc. They contain fungal hyphae of the same type as the mother thallus, but the algal elements are always different. They probably help in retaining the moisture.

**(iv) Isidia (singular "Isidium"):** Isidium is a vegetative reproductive structure present on the surface of the lichen thallus consisting of both fungal hyphae and algal cells. Isidia are fragile structures and may break off and be distributed by wind, animals, and splashing raindrops. They consist of an external cortical layer and an internal algal layer. In terms of structure, isidia may vary in form in different lichen species as- Cylindrical, warty, cigar shaped, clavate (club-shaped), Scale shaped, coralloid (coral-shaped), rod-shaped etc.

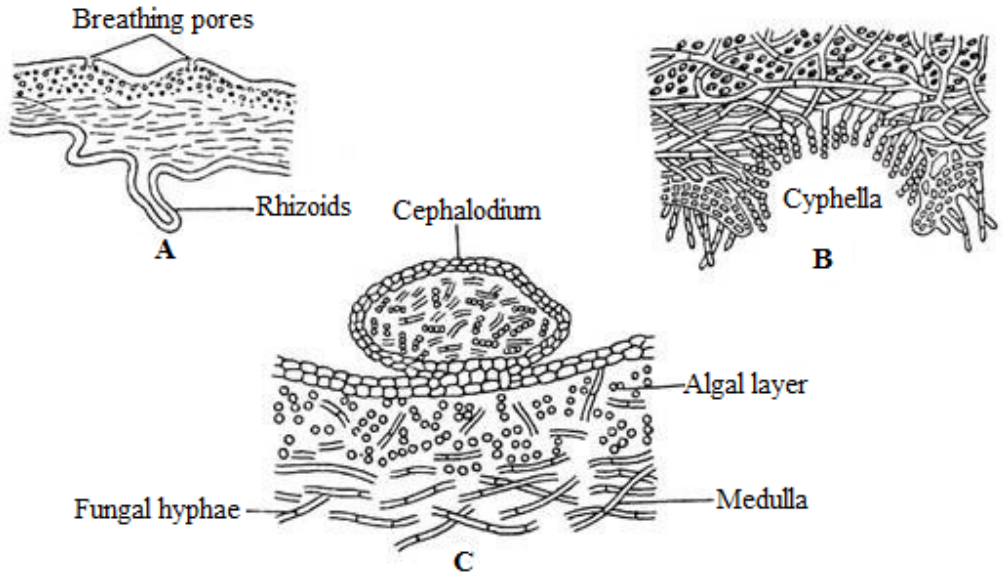


*Fig. 3.3: Structures of lichen thallus: (A)-Breathing pores, B-Cyphella, C-Cephalodium*

## *3.5 NUTRITION*

Just like all living organisms, lichens need nutrients to survive and grow. The main nutrients include oxygen, carbon and nitrogen. Most of the lichens do not have the mechanisms to absorb nutrients from soil and they depend on atmospheric sources for nutrition. Lichens do not have roots, stem or leaves, that absorb water and nutrients as plants do, but like plants, they produce their own food by photosynthesis. The algal component, called the photobiont, makes its own food through photosynthesis. The fungus component is called the mycobiont, uses the hyphae to absorb food from its surroundings. It's attached to its substrate by filaments known as rhizines or

by a single, central extension of the thallus called a holdfast. When lichens grow on plants, they do not live as parasites, but instead use the plants as a substrate. Lichens grow on soil and rock surface are capable of entrapping soluble nutrients from the respective sources. Precipitation, dew, fog and gaseous absorption plays an important role in atmospheric nutrition of lichens. Cyanobacteria can make amino acids directly from the nitrogen gas in the atmosphere.

## *3.6 REPRODUCTION IN LICHEN*

Most lichens reproduce asexually; when conditions are favourable they simply expand across the surface of the rock or tree. In dry conditions they become crumbly and small pieces break off and are dispersed by the wind. The fungal part of many lichens also sometimes reproduces sexually to produce spores. These spores must meet up with an algal partner in order to form a new lichen.

**1-Vegetative and Asexual reproduction:** It takes place by following methods (Fig. 3.4):

**(i) By Fragmentation:** It takes place by death and decay of older parts of the thallus produce smaller pieces which give rise to new thallus. This occurs more frequently in pendant thallus e.g., *Ramalina reticulata.* The new thallus being genetically identical to the thallus from which the fragement came.

**(ii) Isidia:** Isidia are tiny, simple, branched, spiny, elongated out growth from the thallus and contains both photobiont and mycobiont cells covered by the cortical layer of thallus. Each detached isidium may develop into a new thallus under favorable conditions. Common example is *Peltigera* sp.

**(iii) Soredia:** These are small, minute, powdery granules or bud-like out growth present usually over the upper surface or edges of the thalli of many species of lichens. Each soredium consists of few algal cells surrounded by fungal hyphae Soredia detaches from the thallus and are carried away by wind. Falling on suitable substrate, it germinates and gives rise to new thallus. e.g., *Parmedia.*

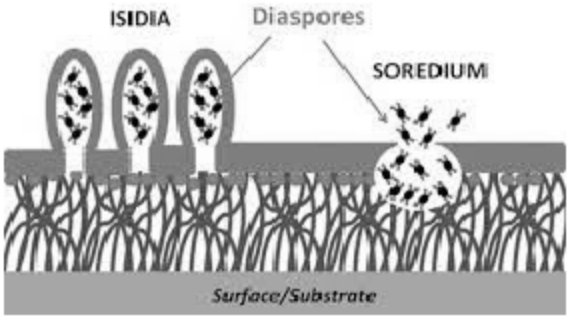


*Fig. 3.4: Asexual reproduction in Lichen*

**2-Sexual Reproduction:** In lichens only the fungal partner may reproduce sexually (Fig. 3.5). The sexually reproducing lichens are either ascomycetes or basidiomycetes. Ascomycetes produce their sexual propagules (called ascospores) within microscopic organs called asci and basidiomycetes produce their sexual propagules (called basidiospores) on microscopic organs called basidia. Often ascospores or basidiospores are simply called spores. A very small number of lichens have the fungal part which belongs to the basidiomycetes.

The fungal component of most of the lichens belongs to the class Ascomycetes, which produce spores in a sac-shaped container, the ascus. The male reproductive organ is called spermogonium and the female is called as carpogonium or ascogonium.

**The Male Sex organs:** The male sex organ is known as spermogonia. In some species of lichens, the pycnidia-like structures function as spermogonia. The spermogonia develop in flask-shaped cavities on the upper surface of the thallus. It opens to the exterior by small pore, an ostiole. A number of hyphae develop from the walls of the cavity. Few of them are sterile and others are fertile. The fertile ones produce the non-motile male cells called spermatia. These non-motile cells develop continuously from the tips of the fertile branches. The spermtaia are set free in a slimy mass through ostiole.

**The female sex organs:** The female sex organs are known as carpogonium. The carpogonium develops fron hyphae deep in the algal layer. It consists of two portions, the upper straight portion is called trichogyne and the lower coiled portion is called ascogonium (oogonium). The ascogonium lies deep in the medullary region of the thallus. The terminal portion of the trichogyne ends in a long cell, which projects beyond the surface of the thallus and has a gelatinous cell wall. It is multicellular and the cells are uninucleate or multinucleate in some species. The basal cell of the ascogonium is fertile.

**Fertilization:** A spore called conidium is released from a pycnidia structure. Pycinidia are flask-like structures embedded in the thallus of the lichen. Conidia can act as “spermatia” in sexual reproduction of the lichen. The spermatia are functional male gametes. The spermatium spore finds its way to a tiny thread (trichogyne) on a surface of lichen and attaches itself. The conidia and the trichogyne both are haploid. The growing trichogyne comes in contact with spermatia. The intervening walls between the spermatium and the trichogyne dissolve at the point of contact. The male nucleus gradually passes downward to the oogonium, where it fuses with the female nucleus. The actual migration of the male nuclei down the trichogyne has not yet been observed, but it is assumed. Fused cell produces ascogenous hyphae within which develop 8 ascospores and asci. The hymenium is made up of Asci and Paraphysis. The fruiting body may be either apothecia e.g., *Parmelia* and *Physcia* or Perithecia e.g., *Peltigera.*

Sexual reproduction results in the formation of apothecia or perithecia. In lichens, fruiting bodies are of following two types:

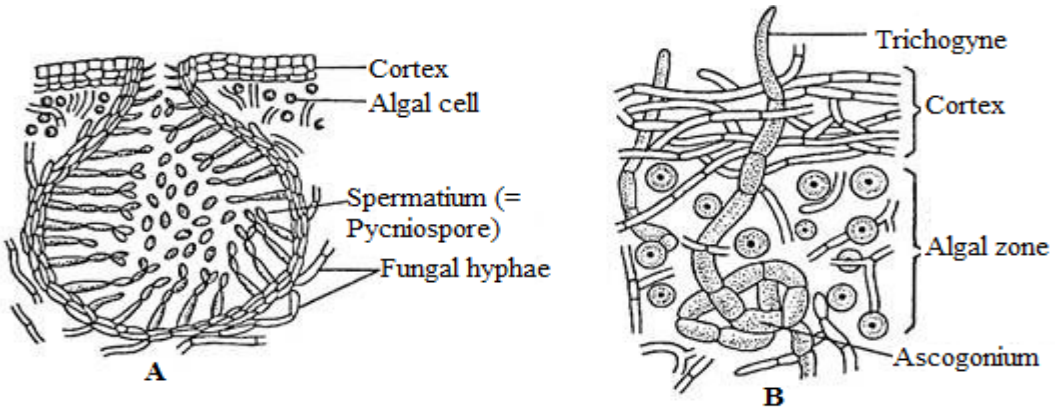


*Fig. 3.5: Sexual reproductive structures, A-Spermogonium (Pycnidium), B-Carpogonium*

**(i) Apothecia:** The most commonly seen sexual reproduction structures are apothecia. These are typically circular and disc-like or cup-like though there are also species in which the apothecial surface bulges outward. They may be of the same colour as the thallus or strikingly different and vary in diameter from under a millimetre to over two centimetres, depending on species. The structure of the apothecium chiefly consists of three parts: hymenium, Hypothecium, and excipulum. The apothecium has a layer of exposed spore-producing cells called asci. The asci are present in the hymenium layer. The hymenium, composed of sac-like asci and sterile, hair-like fungal hyphae known as paraphyses. Asci and paraphyses form a thin inner lining, which is called as hymenial layer. Each ascus contains eight ascospores. The asci are freely exposed at maturity (Fig. 3.6).

**(ii) Perithecia:** Perithecia are generally flask-shaped frutiting bodies in certain ascomycetous fungi that contain the ascospores. Depending on the species perithecia may develop totally on the lichen thallus or embedded in the thallus. It looks like a small black dots on the surface of lichen. At maturity a small opening at the top, called an "ostiole", allows the ascospores to escape (Fig. 3.6).

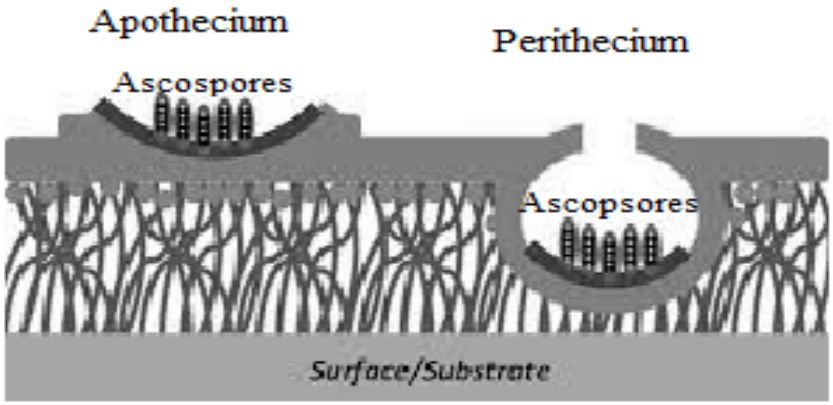


*Fig. 3.6: Fruiting bodies in Lichen*

## *3.7 ECONOMIC AND ECOLOGICAL IMPORTANCE*

**Economic importance:** Lichens are very important economically. Some are given below-

**1-As a source of food:** Certain species of lichens are valuable sources of food. The edible lichens are harvested and dried for human consumption or as fodder for animals. They are rich in polysaccharides. Some vitamins, and certain enzymes. *Cetraria islandica* (Iceland moss) is taken as food in Norway, Sweden, Iceland, Scandinavian countries etc. Species of parmelia or rock flower (known as "rathapu" in telugu and "Kallu huvu" in Kannada) is used in curry preparation and is famous for its delicacy. *Evernia prunastri* was used by Egyptians as baking powder. In France the lichens are used in confectionary for making chocolates and pastries. A species of Parmelia is prized as food in Southern India. *Lecanora esculenta*, commonly called Manna lichen is used in desert tribes of Asia minor.

**2- As a Source:** Certain species of lichens are important. In tundra, Fruticose lichen *Cladonia rangiferina* (Reindeer Moss) and other tundra lichens serve as food for musk ox, Caribou, reindeer and other wild animals. *Cetraria islandica* is used as fodder for horses. The other common used species for animals as fodder are *Stereocaulon, Evernia, Parmelia* and *Lecanora* etc.

**3-Medicinal Use:** Since very early time a few species of lichens have been used in folk medicines to cure fever, jaundice, skin diseases, epilepsy etc. A yellow substance usnic acid secreted from *Usnea barbata*, along with Streptomycin is effective in tuberculosis. It is also used in preparation of ointment for wounds and burns. A mucilaginous substance obtained from *Cetraria islandica* (Iceland moss) is used as a laxative. Protolochesterinic acid, a compound obtained from some lichens, is used in preparation of anti-cancer drugs. *Rocella montagnei*, is used to cure angina. The supposed medicinal benefits of lichens are now known to have no scientific basis. Researches are yet to be conducted on many other medicinal benefits of lichens.

**4- Chemical Uses:** Some species of lichen is useful in the brewing, distilling, tanning, dyeing etc. Orchil, a blue dye obtained from *Roccella* and *Leconara*, is used to dye woolen articles and silk fabrics. The lung wart lichen is used in tanning, brewing and as a substitute for hopes in brewing. In Sweden and Russia alcohol is produced from some species of lichens for example, *Cetraria islandica* which contain carbohydrates in the form of lichenin. Orcein, a biological stain, is obtained from *Rocella tinctoria.* The fungal components of some species of lichens produce coloured pigments that have been used for centuries as dyes in colouring fabrics and paints. One of these is orchill. Litmus is widely used dye in chemical laboratories as an acid-base indicator. It is obtained from *Rocella mountaignel.* Some lichens species synthesize and yield specific organic compounds commonly known as lichen acids. These are useful in identification and classification of lichens.

**5-Perfumes:** Sweet-scented thalli of certain lichens are useful in making scents, perfumes, dhup, hawan samagris etc. Scented incense is obtained from species of *Ramalina* and *Evernia. Evernia,*

*Ramalina* and *Pseudorina* are reported to have perfumed volatile oils, which is used in manufacture of cosmetics. A lichen which is popularly known as an oak moss is used in perfumes as a fixative in Southern Europe. Delicate perfume is got from *Lobularia pulmonaria* and *Evernia prunastri.*

## Ecological Significance of Lichens:

**1-Pollution indicators:** Lichens are present in those areas where the pollution is less, Infact least. Hence they are called as "Pollution indicators". Some lichens are very tolerant to pollutants such is sulphur and nitrogen compounds, while others are very senstitive to the presence of one or both of these chemicals. Just as lichens readily absorb water and nutrients through their surface, so they also readily absorb a wide variety of potentially toxic compounds. One of the ways lichens directly benefit humans is through their ability to absorb everything in their atmosphere, especially pollutants. Any heavy metals or carbon or sulfur or other pollutants in the atmosphere are absorbed into the lichen thallus. Scientists can extract those toxins and determine the levels of air pollution that are present in a given area.

**2- Lichen fix nitrogen:** Various studies have shown that lichens can be a significant source of nitrogen for plants. Due to their association with algae, lichens are able to convert nitrogen in the air into nitrates. When it rains, nitrates are leached from lichens. Fixing nitrogen is the process of changing unusable nitrogen into a usable form of nitrogen. Lichens use cyanobacteria to fix nitrogen from the air.

**3- Useful in soil formation:** When lichens die they contribute organic matter to the soil, improving the soil so that other plants can grow there.

**4- Erosion control:** Lichen enriches the soil by trapping water, dust and slit. Commonly lichens have root-like structures (but not true roots) to anchor themselves to the soil. During downpours much of the raindrops; force is absorbed by the crust and this greatly lessens the erosive potential of intense downpours. Lichens are most noticeable on the tundra, where lichens, mosses, and liverworts constitute the majority of ground cover. This cover helps to insulate the ground, and may provide forage for grazing animals.

**5-Habitat for other organisms:** Lichens provide habitat for many organisms such as insects, arthropods, and other small invertebrates. Birds, for example, use different species of lichens (usually the fruticose types) to build their nests.

**6- Pioneers of vegetation:** Lichens are the pioneer organisms in a new terrain which colonise bare rocks, cliffs and mountains. They secrete organic acids such as carbonic acids which gradually dissolve and disintegrate the rocks. Lichens erode the rocks and accumulate a certain amount of minerals and organic matter. The plants like grasses and mosses appear later in

succession, utilizing the first soil formed by lichens. Lichens thus, can convert a barren region into one that can support vegetation.

### Harmful effects of Lichens:

1- Lichens may have adverse effects on plants. Small fruit trees, Sandal wood trees, small shrubs densely covered with lichens could be damaged. Many epiphytic lichens can have harmful effects on the host plant.
2- A very few lichens are poisonous due to presence of various substances in them. These lichens are known to contain vulpinic acid and usnic acid, e.g., *Vulpicida* and *Letharia*. These lichens are yellow due to high concentrations of bright yellow toxin vulpinic acid. The wolf lichen (*Letharia vulpine*) got its name because it was used in Europe to poison wolves. Many lichen which are yellow in colour may have possibility to be poisonous.
3- In dry season sometimes long threads of pendant lichens as *Usnea barbata* help in spreading of forest fire.
4- Lichens can cause some damage to buildings and man-made structures, it is very slow process and does not endanger those substrates.
5- Some lichens act as allergens.

## *3.8 SUMMARY*

Lichen is not a single organism. It is a symbiotic association between a fungus and algae or cyanobacteria. Algae conducts photosynthesis to produce food for itself and for the fungal partner and in turn, the fungal partner protects the algae from drying out, providing it with water. Lichens come in many colour, sizes and forms. They can grow in anywhere from sea level to Alpine peaks and from the hot deserts to the cold Arctic and Antarctic. The lichen thalli are generally of three types- Fructicose, Foliose and Crustose. There are few intermediate categories of growth forms such as Leprose, squamulose, Filamentous, Gelatinous, Dimorphic, placodioid. The fungal partner mainly belongs to Ascomycetes apart from Basidiomycetes and rarely Deuteromycetes. Lichens reproduce by three methods- sexual, asexual and vegetative reproduction. Most of the Lichens depend on atmospheric sources for nutrition. Lichens are very important economically and ecologically. They are useful in erosion control, pollution indicators, nitrogen fixation, Habitat for other organisms etc, and as a source of food, medicine, fodder, perfumes etc.

## *3.9 GLOSSARY*

**Apothecium (plural apothecia):** One type of fruiting structure produced by the fungal component of the lichen. An apothecium is cup- or disc-shaped (compare with perithecium) and contains the spores, which allow for sexual reproduction.
**Ascocarp:** mature fruiting body of an ascomycetous fungus.
**Ascospore:** A meiospore borne in an ascus.
**Ascus:** The sac or bag-like structure in which ascospores are formed.
**Cilia**: Linear or thread-like appendages projecting from the thallus or apothecia margins, Cilia are the black, hair-like appendages pictured here along the margins of powder-edged ruffle lichen (*Parmotrema stuppeum*) thallus.
**Conidium:** An asexual fungal spore.
**Cortex:** The protective outer wall of the thallus, composed entirely of fungal tissue. Lichens may have two cortices (upper and lower), a single cortex or no cortex at all, depending on growth form. Below the cortex is the photobiont.
**Crustose:** A lichen growth form distinguished by the thallus being tightly adhered to the substrate at all points. Crustose lichens do not have a lower cortex, exposing the hyphae to the substrate. It is impossible to remove crustose lichen from its substrate without impacting the substrate in some way.
**Cyphella (plural cyphellae):** Small depressions or pits in the thallus cortex that are lined with cells (compare with pseudocyphella).
**Cynobacteria:** blue-green algae.
**Filamentous:** Stringy or matted hair like.
**Foliose:** A lichen growth form distinguished by a relatively flat, leaf-like thallus. Foliose lichens have an upper and lower cortex, making it easy to identify an upper and lower thallus surface.
**Fruticose:** A lichen growth form distinguished by a tufted, hanging or stalked thallus. Fruticose lichens have a single, continuous cortex that wraps around the thallus branches, making it difficult to discern an upper and lower surface.
**Gelatinous:** Jelly like.
**Hyphae:** Fungal filaments collectively called hyphae which form a thallus.
**Isidium (plural isidia):** A structure that projects from the thallus and contains both fungal and algal components. An isidia can detach from thallus and therefore serves in vegetative reproduction.
**Leprose:** Powdery.
**Mycobiant:** The fungal partner in lichen.
**Perithecium (plural perithecia):** One type of fruiting structure produced by the fungal component of the lichen. A perithecium is flask-shaped (compare with apothecium) and often embedded the thallus, making it somewhat inconspicuous. A small hole at the top of the perithecium releases spores, which allow for sexual reproduction.
**Pseudocyphella (plural pseudocyphellae):** Small depressions or pits in the thallus associated with cracks in the cortex. The cracks in the cortex are not lined with cells, distinguishing these features from cyphellae.

**Phycobiont:** algal partner in lichen.
**Photobiont:** The photosynthetic organisms are called Photobiont.
**Pycnidia:** Flask-shaped structures which produce conidia.
**Rhizines:** root-like fungal structures is termed rhizines or rhizinae which bind the thallus to its Substrate.
**Soredia:** A powdery or granular structure released from cracks in the thallus cortex. A soredia is essentially the photobiont (algal component) wrapped in fungal hyphae and therefore serves in vegetative, or asexual, reproduction.
**Squamulose:** A lichen growth form, distinguished by small, overlapping thallus units or scales. Squamulose lichens are not as tightly appressed to the substrate as crustose lichens but are more appressed than foliose lichens. These lichens have an upper cortex but may or may not have a lower cortex.
**Symbiotic:** Where both the partners get the mutual benefit by living together.
**Thallus:** The lichen body, which contains both a fungal and algal (photobiont) component.

## *3.10 SELF ASSESSMENT QUESTIONS*

### 3.10.1: Multiple choices Questions-

1. Many scientists consider algal-fungal relationship in lichen as helotism is a:
(a) A kind of symbiotic association (b) A kind of mutualism
(c) Master-slave relationship (d) Master- master relationship

2- More than 95% of the lichens, the fungal partner belong to the class:
(a) Ascomycetes (b) Basidiomycetes
(c) Zygomycetes (d) Mastigomycetes

3. The benefit of algae in this association is:
(a) Food (b) Vitamins
(c) Growth substances (d) protection

4. Graphis is a:
(a) Foliose lichen (b) Fruticose lichen
(c) Crustose lichen (d) Filamentous lichen

5. Terrestrial species that grow in soil are called:
(a) Lignicolous (b) Terricolous
(c) Saxicolous (d) Corticolous

6. If both algal cells and fungal hyphae are uniformly distributed in lichen formation, then called as-

(a) Homoisomerous lichen (b) Heteroisomerous lichen
(c) Homo heteroisomerous lichen (d) Hemiisomerous lichen

7. The fertile layer of ascocarp where ascus is located is called:

(a) Paraphysis (b) Hymenium
(c) Peridium (d) Sub hymenium

8. Leprose lichens are:

(a) Crust like (b) Leaf like
(c) Pendulous (d) Scale like

9. The major group of algae involved in lichen formation is:

(a) Red algae (b) Brown algae
(c) Blue green algae (d) All

10. The male reproductive organ is called:

(a) Carpogonium (b) Tricogyne
(c) Hymenium (d) Spermagonium

**Answers Key:** 1- (c), 2-(a), 3-(d), 4-(c), 5-(b), 6-(a), 7-(b), 8-(d), 9-(c), 10-(d)

### 3.10.2 True or False

1. The symbiotic association between algae and fungi is called Mutualism.
2. Lichens are the major pollution indicators of $SO_2$.
3. Each ascus contains generally 4 ascospores.
4. The female reproductive organ is called Carpogonium.
5. Lichens that are rock dwellers with xerophytic adaptations are called Corticolous.

**Answers Key:** 1- False, 2-True, 3-False, 4-True, 5-False

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## *3.13 TERMINAL QUESTIONS*

1. Define Lichen. Discuss general characteristics of lichen.
2. Give a detailed note on classification of lichen.
3. Describe types of lichens and its nutrition.
4. Explain internal structure of lichen with diagram.
5. Give a detailed note on reproduction of lichen.
6. Discuss about the anatomy of lichen thallus.
7. Write short notes on-
    (a) Economic importance of lichen
    (b) Lichen as a pollution indicator
    (c) Harmful effects of lichen

# BLOCK-2- FUNGI: LIFE CYCLE

# UNIT-4 MASTIGOMYCOTINA AND ZYGOMYCOTINA

## Contents:

# *4.1 OBJECTIVES*

After reading this unit, students will be able:

- To understand the general characteristics of Mastigomycotina and Zygomycotina
- To know the taxonomic status of *Synchytrium, Allomyces* and *Phytophthora.*
- To know the taxonomic status of *Mucor, Rhizopus* and *Pilobolus*.
- To understand the life cycle of *Synchytrium, Allomyces, Phytophthora.*
- To understand the life cycle of *Mucor, Rhizopus, Pilobolus*.

# *4.2 INTRODUCTION*

In the previous unit you have studied various characteristic features of Ascomycotina and Basidiomycotina. This chapter provides the complete information about one of the important sub division of fungi Mastigomycotina. It is also referred as "Zoosporic fungi". They occur mainly in waterlogged soil as saprophytes whereas some of the species occur as parasites.

# *4.3 MASTIGOMYCOTINA*

Different kind of morphology occurs in their members varying from unicellular plant body to filamentous coenocytic mycelium. One of the distinctive features of Mastigomycotina is presence of flagellated cells throughout their life cycle. The chief constituents of the cell wall comprised of chitin and glucan.

Asexual mode of reproduction takes place by means of zoospores whereas sexual reproduction takes place by gametangial copulation. Owing to the presence of haustoria in majority of mastigomycotina, the mode of nutrition is absorptive.

## 4.3.1 *Synchytrium*

### Systematic position

**Kingdom** Mycota
**Division** Eumycota
**Sub-division** Mastigomycotina
**Class** Chytridiomycetes
**Order** Chytridiales
**Family** Synchytriaceae

The genus *Synchytrium* which occur as obligate parasites on fern, algae, flowering plants and mosses is reported to include more than 200 species while in India, the genus is denoted by approximately 80 species. One of the well-known species of *Synchytrium* is *S. endobioticum*which cause massive loss to the potato crop throughout the world by causing serious disease of potato "black wart disease". The symptoms of the disease usually develop below ground portion exhibiting cauliflower like black warty outgrowth (simple or highly branched) on tubers, stolons and stem bases. Sometimes, this kind of outgrowth also appears on aerial parts as green leafy structure on shoots (Fig. 4.1).

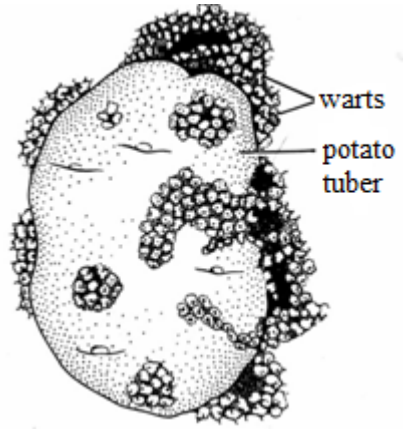


*Fig. 4.1: Synchytrium endobioticum: Infection on potato tuber*

## Vegetative structure

The vegetative body of *Synchytrium* consists of endobiotic thallus which occurs as parasite in the epidermal cells of host forming mass of uninucleate amoeboid protoplast.

## Reproduction

*Synchytrium* reproduces by asexual and sexual mode. During reproductive phase, entire vegetative structure transforms into a reproductive unit which is termed as holocarpic. Vegetative mode of reproduction is absent in *Synchytrium.*

### (a) Asexual reproduction

Under favorable condition (during spring season), it is the most common method of multiplication when several minute, uniflagellate and uninucleate zoospores are liberated from the infected tubers. These zoospores have the potential to swim in film of water for approximately 2-3 hours. Once they get in contact with the host cell, they come to rest and retract their flagella. As the protoplast of the zoospore pierce the host epidermis by amoeboid movement, a minute pore is created in the epidermis of host. By the absorption of food from the host epidermal cell, zoospore increase in size and acquire spherical shape. The host cell also enlarges. Meanwhile the cells surrounding the infected cells also enlarges and starts swelling

(hypertrophy) and show abnormal cell division (hyperplasia) leading to the formation of gall or tumor like structure. The infected cells die finally. The pathogen along with its nucleus increases largely, encyst and develop two layered wall (thick golden brown exospore; thin endospore). This structure is referred as "summer spore" comprising of lipid body and semicircular nucleus which germinates within the host cell.

Before germination, the nucleus enlarges and inner wall projected through a pore in the outer wall forming a vesicle towards the upper portion of the host cell. The contents of the summer spore including nucleus are migrated to the vesicle. The nucleus then undergoes recurrent mitotic divisions to form 32 nuclei. This multinucleated vesicle is called as "prosorus" which split into 4 - 9 multinucleate chambers surrounded by thin hyaline walls. This structure is known as summer sporangium or zoosporangium and this aggregated mass of prosorus is collectively referred as "sorus". The nuclei of each zoosporangium continuously divide to form 200-300 nuclei. The protoplast then divides into several uninucleate fragments as the no. of nuclei.Towards the maturity, sporangium swells by absorbing water and set pressure on the host cell to burst. After eruption, zoospores are released, which then swim actively in water for some time and then infect the new host (Fig. 4.2 A, B).

## (b) Sexual reproduction

During unfavorable condition (winter season), multinucleate segments of prosorus instead of behaving as zoosporangia act as gametangia giving rise to gametes which are smaller in size than zoospores. Two planogametes united to form diploid biflagellate zygote which swim actively for some time and encyst on the surface of host epidermis and pierce the host cell in the same way like zoospores. The surrounding host cell then undergoes repeated cell division and infected cell is suppressed within the host tissue.

During this progressive phase, diploid zygote expand and become encircled by 2-3 layered wall (thick) which is known as resting sporangium or winter sporangium as it remains quiescent throughout the winters. By the decay of the host tissue, these resting or winter sporangia are liberated into the soil. At the onset of spring season (favorable conditions), the resting or winter sporangia become functional and its nucleus undergo repeated divisions of which first is meiotic followed by mitotic divisions giving rise to several haploid nuclei within the sporangium. The protoplast of the resting sporangium differentiates to form many uninucleate daughter protoplasts which then transformed into uniflagellate zoospores. After water absorption, the wall of sporangium rupture and zoopsores are released which in contact with suitable host cause infection leading to the reappearance of cycle (Fig 4.2 and 4.3).

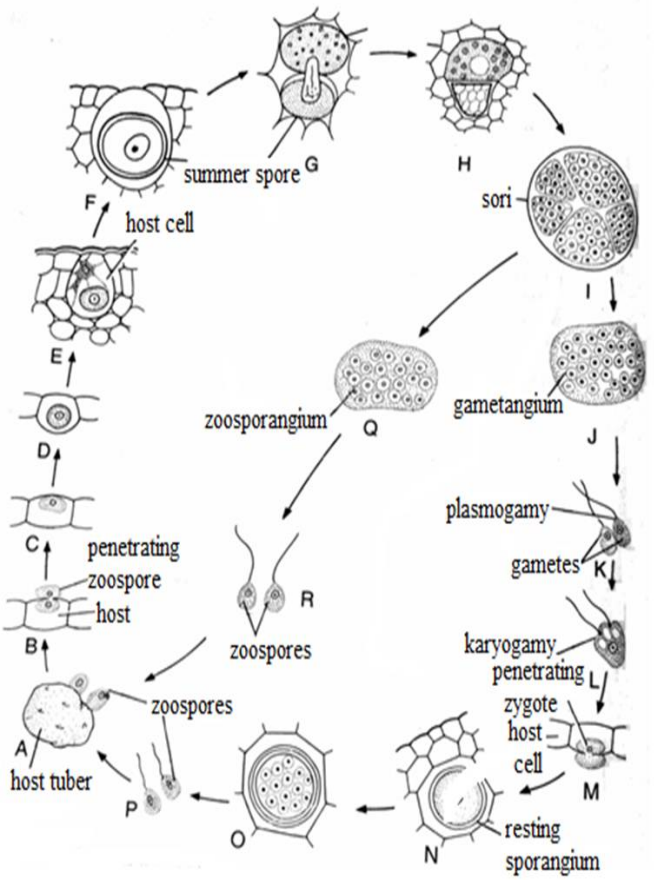


*Fig. 4.2 Diagrammatic life cycle of Synchytrium*

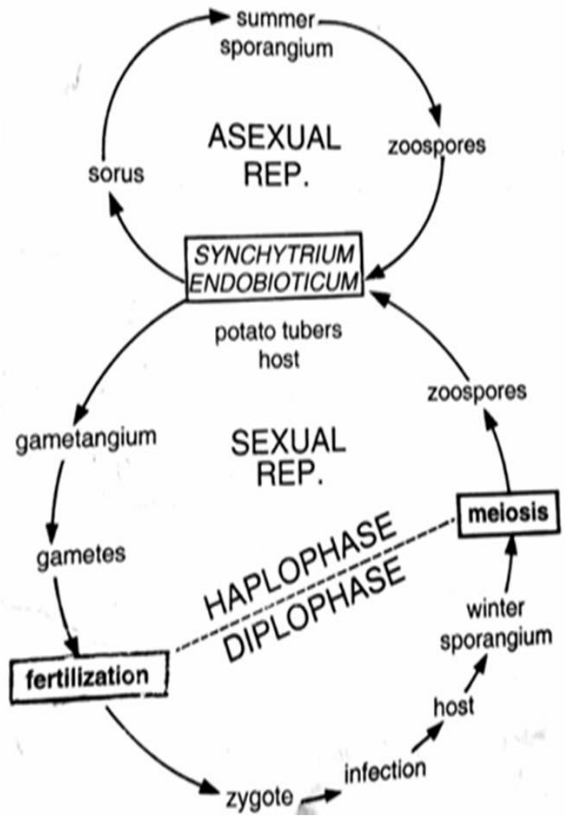


*Fig. 4.3 Graphic life cycle pattern of Synchytrium*

## 4.3.2 *Allomyces*

### Systematic position

**Kingdom-** Mycota
**Division-** Eumycota
**Sub-division-** Mastigomycotina
**Class-** Chytridiomycetes
**Order-** Blastocladiales
**Family-** Blastocladiaceae

The genus *Allomyces* is found to be distributed throughout the world. In India, the genus is reported to include approximately eight species. Among these species, *A. javanicus, A.arbusculus* and *A. macrogynous* are most common. All the species of *Allomyces* are saprophytic, and soil inhabiting. Most commonly its species occur in the places which are seasonally moistened and dessicated. This genus was first reported by E. J. Butler in 1911.

## Vegetative structure

The mycelium is filamentous and attached to the substratum by a clump of fine branched rhizoidal hyphae comprising the rhizoidal system. From the latter arises slender hypha forming trunk like fraction which successively go through dichotomous branching to give rise to major part of mycelium ultimately forming reproductive organs. Cell wall comprised of chitin with considerable amount of β- glucan, proteins and ash.

## Reproduction

*Allomyces* exhibits excessive deviation in life cycle patterns *i.e.* distinct alternation of generation gametothalli (haploid) and sporothalli (diploid), reflecting its capacity of both asexual and sexual reproduction (Fig 4.4).

## (a) Asexual reproduction

It proceeds through sporothalli. At maturity, sporothallus produce two types of sporangia: Colourless thin walled zoosporangia (also called as mitosporangia) and thick walled, reddish brown, resistant sporangia (also called as meiosporangia). Both are carried on the same thallus either singly or in chains at the tip of dichotomies.

The zoosporangia are multinucleate structure containing nuclei. The cytoplasmic contents of the zoosporangia then separated by gradual cleavage into uninucleate daughter protoplast which transform into a diploid, colourless zoospore "mitospores" having whiplash type of flagella at the posterior end. After releasing from the zoosporangia, the zoospore swims about for a while and then encysts. The encysted zoospore then instantly germinates to form new generation of diploid asexual plant similar to parents.

The mature meiosporangia are thick walled and rough structures which are reddish brown in colour. The multinucleate diploid sporangial matter undergoes latent period of 2-6 weeks or even earlier to the cleavage of protoplast into zoospores. Reduction division within them ends with the development of haploid meiospores. Approximately 48 uniflagellate zoospores are formed in sporangium. Towards maturity, the sporangial wall collapse and zoospores discharged in water, swim for a concise interval, encyst and propagate to give rise to gametothalli*i.e*. sexual plant.

## (b) Sexual reproduction

The gametothalli is concerned with sexual reproduction which takes place by planogametic copulation. The gametothalli are homothallic. At maturity, it bears gametangia which are developed in pairs, one above the other.

The male gametangia are orange red in colour, more active and comparatively smaller in size. Contrary to this, the female gametangia is a hyaline structure and much bigger in size. Both the

male and female gametes are motile, uninucleate and posteriorly uniflagellate. The female gametes secrete a sex hormone "sirenin" which serves as chemoattractant leading movement of male gamete toward female gamete resulting in the formation of diploid motile zygote by the fusion of these two gametes. The zygote swims for sometimes, encyst and propagate to give rise to sporothalli.

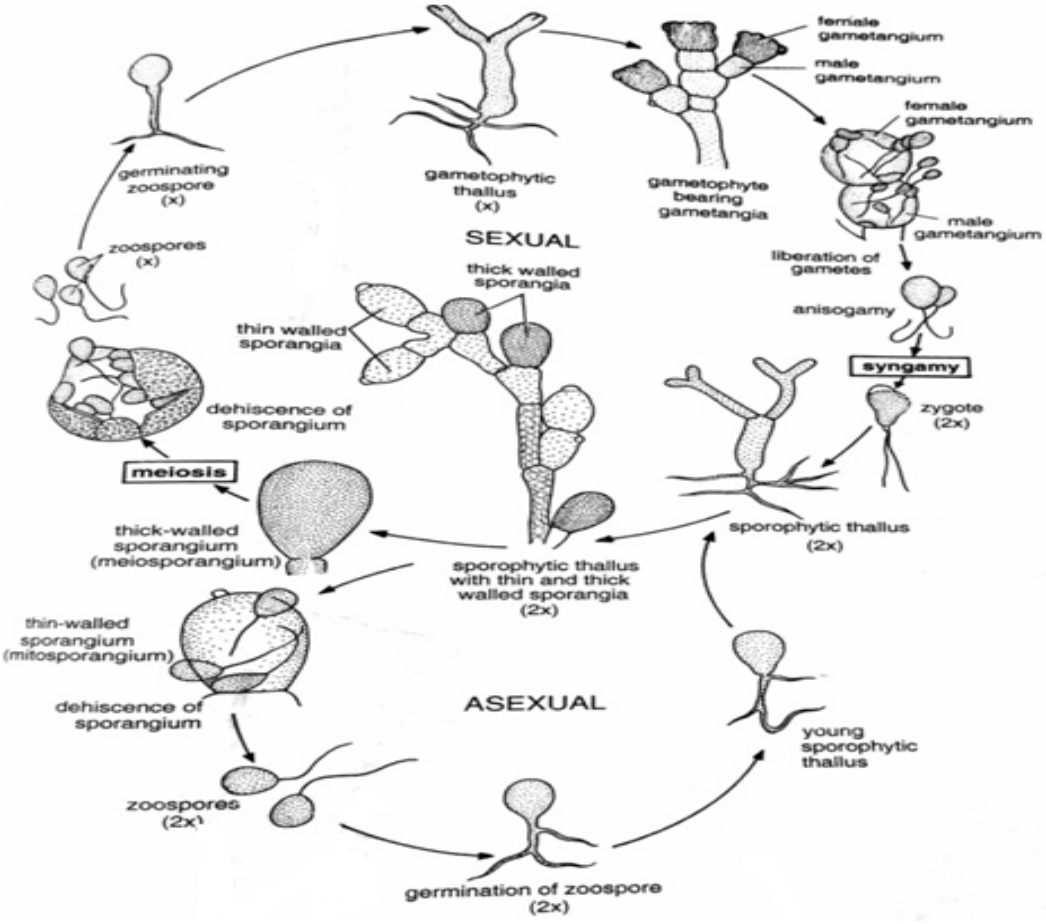


*Fig. 4.4: Diagrammatic life cycle of Allomyces*

## 4.3.3 *Phytophthora*

### Systematic position

**Kingdom-** Mycota
**Division-** Eumycota
**Sub-division-** Mastigomycotina
**Class-** Oomycetes
**Order-** Peronosporales
**Family-** Pythiaceae

The genus *Phytophthora* (Greek: *phyton*: plant; *phthora*; destruction) is one of the most devastating genus represented by approximately 40 species which are distributed throughout the world. Majority of the species are parasitic while few occur as facultative saprophytes. One of the most common species of *Phytophthora* is *P. infastance* causing late blight disease of potato. Low temperature and moist environment support the spread of the disease.

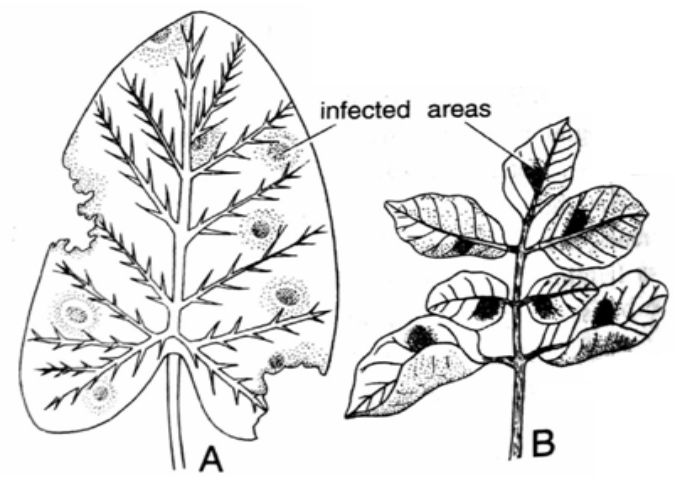


*Fig. 4.5 (A-B): Phytophthora symptoms on Colocasia and Solanum tuberosum leaf*

The symptoms of the disease caused by *Phytophthora* infection can be investigated on any part of the plant. On leaves, they appear in the form of olive green or dark brown, oval or irregular water logged areas. Initially, the symptoms occur at the tips or the margins of older leaves (Fig 4.5 A, B). As the infection spread strongly, they appear in form of blight. On the ventral surface of leaf, white cottony structures are formed due to the growth of conidiophores. In acute case, all part of the host plant become brown and disintegrate.

## Vegetative structure

The mycelium is freely branched comprising of hyaline, aseptate and coenocytic hyphae. The fungal hyphae proliferate in the intercellular spaces between the cells of host tissue. The mycelium gives rise to haustoria (simple or branched) which pierces the host cell wall and absorb food material. The hyphal wall consists of glucan and is 0.1 µm thick.

## Reproduction

*Phytophthora* reproduces by both asexual and sexual means.

## Asexual reproduction

With the onset of favourable conditions (warm and humid weather), a clump of slender diverged hyphae arise from the intercellular mycelium which emerge through the stomata or by penetrating epidermal cells which are present on the lower surface of leaf. The hyphal branches

are freely branched, hyaline functioning as conidiophores or sporangiophores bearing sporangium at the tip which is a thin walled, pear shaped or oval structure with a beak like protrusion measuring 22-25 µm in length and 16- 25 µm in diameter.

The mature sporangia are detached form the sporangiophores represented by nodular swelling and are disseminated by wind, rain drops, or by association with other leaves. They may fall over the ground and get dispersed into the soil and remain viable for few hours and propagate on a proper medium. Temperature and moisture are the key factors for germination of sporangia. The sporangia of *P. infestans* may germinate by indirect and direct mode (Fig. 4.5, Fig 4.6).

Indirect germination: During low temperature (less than 15°C) and moist weather, the sporangium behaves as zoosporangium. The multinucleate protoplast of the sporangium split into 5-10 uninucleate daughter protoplasts which then transform into biflagellate zoospores. Out of the two flagella, one is whiplash and other is tinsel type. The zoospores are liberated by bursting of the apical papilla, swim in a film of water and subsequently set on a substratum, retracting the flagella and germinate. During the period of germination, the zoospore puts out a short hypha called "appressorium" through which infection thread drive its way into the host leaf.

Direct germination: During high temperature (20-23°C) and low relative humidity, the sporangium behaves as conidium. It germinates by generating a germ tube or a short hypha, which penetrate the host leaf.

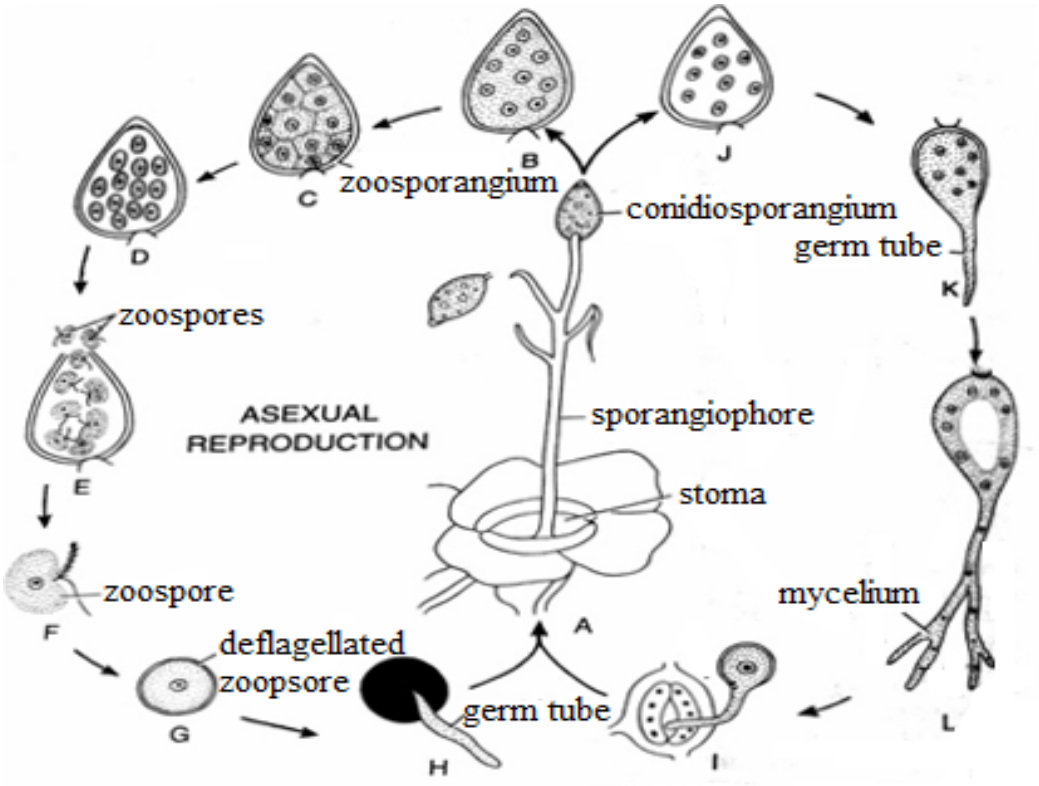


*Fig. 4.6: Asexual reproduction in Phytophthora infestans*

## Sexual reproduction

It is of oogamous type involving the participation of both male and female reproductive structures (Fig 4.6 A). The male and female sex organs are antheridia and oogonia respectively which arise at the tips of lateral branches as antheridial and oogonial initials. Most of the species of *Phytophthora* are heterothallic (*P. infestans, P. palmivora*) while few are homothallic (*P. himalayensis*).

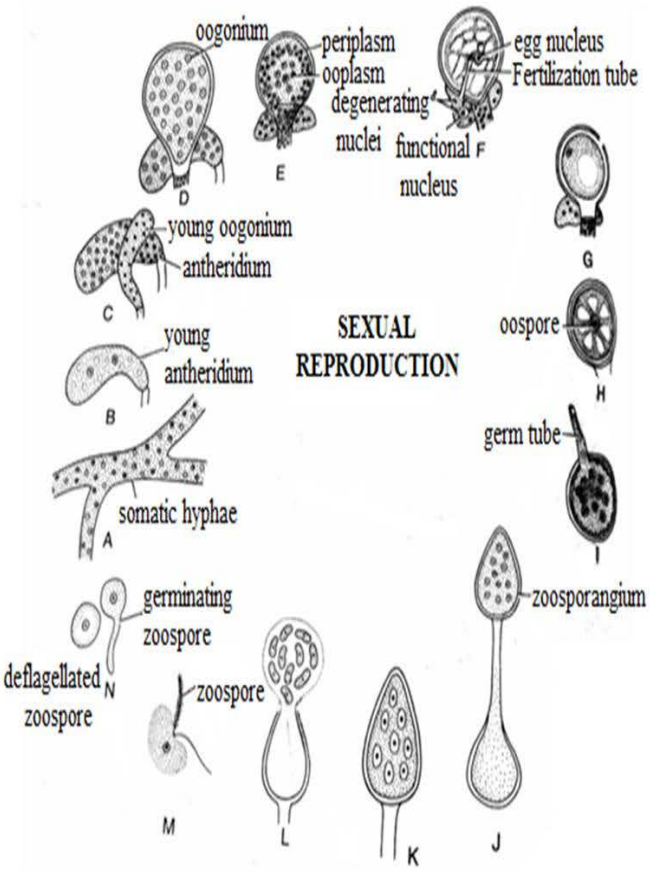


*Fig. 4.7 Sexual reproduction in Phytophthora infestans*

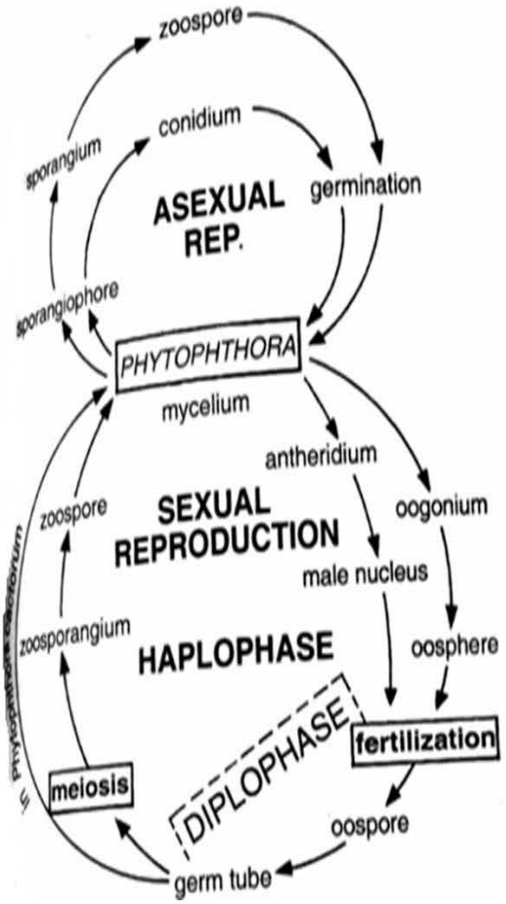


*Fig. 4.8 Phytophthora infestans: Graphical life cycle pattern*

The antheridium which is a club shaped structure formed at the tip or on the lateral side of hypha with one or two nuclei. Later the nuclei divide and produce about 8-12 nuclei. Out of these, only one remains functional and rest disintegrate. The oogonium develops on adjacent hypha of the antheridial branch. Its tip swell to form globose shaped structure containing dense cytoplasm and several nuclei (approximately 40). The oogonial protoplast becomes separated into peripheral cytoplasm and central ooplasm. Prior to fertilization, the central nucleus cleaved into two and

one of them vanishes. The persisting nucleus behaves as egg nucleus while the periplasm nuclei later on collapse.

At the time of fertilization, the common wall at the point of contact of antheridium and oogonium dissipate and male nucleus from antheridium enters into the oogonium (antheridium penetrate oogonium by fertilization tube) resulting in the formation of diploid oospore which is surrounded by double layered wall (Exospore: thin, comprising of pectic substances; endospore: thicker, comprising of cellulose, protein and reserve food material. This oospore undergoes a period of maturation from several weeks to months. With the onset of favorable condition, oospore germinates after the degeneration of host tissue. A germ tube develops from the oospore which immediately develops into the mycelium or the oospore may exhibit terminal sporangia where zoospores are produced which after liberation, progress into new mycelia.

## *4.4 ZYGOMYCOTINA*

In the previous unit you have studied the characteristic features of Mastigomycotina. This chapter provides the complete information about one of the important subdivision of fungi Zygomycotina which derives its name from the thick walled resting spores called as "Zygospores" that are produced by the fusion of two gametangia. One of the distinctive features of Zygomycotina is the absence of motile cells (zoospore). Mostly zygomycetes have coenocytic hyphae. Economically, they have gained enormous importance as they are capable of synthesizing important industrial product. Apart from that, they are ecologically important as they are being increasingly used in biological control of insect pest of crop.

### 4.4.1 *Mucor*

### Systematic position

**Kingdom- Mycota**
**Division- Eumycota**
**Sub-division- Zygomycotina**
**Class- Zygomycetes**
**Order- Mucorales**
**Family- Mucoraceae**

The genus *Mucor* commonly known as pin mould or black mould is a saprophytic fungus that develops on soil, decaying fruits and vegetables is represented by more than 100 species. Few of the species of *Mucor* are coprophilous *i.e.* inhabiting dung. Some of the species of *Mucor* are also air contaminant e.g., *M. mucedo* and *M. racemosus* whereas other species are the causative agent of mucoromycosis in human beings and domestic animals which severely affect lungs, brain and eventually leading to the death.

## Vegetative structure

It appears as white, grey or brownish cottony mycelium which is much branched and coenocytic. The hyphal wall is microfibrillar and complex consisting of chitin-chitosan, polysaccharides, purine, protein, lipids, calcium and magnesium.

## Reproduction

It reproduces by vegetative, asexual (Fig. 4.9) and sexual modes.

**(a) Vegetative reproduction:** It takes place by means of fragmentation. Here the vegetative hyphae may split into small units and they are capable of progressing into new mycelium.

**(b) Asexual reproduction**: It occurs by the formation of aplanospores or chlamydospores or oidia.

### (i) Formation of aplanospores

Under favorable conditions, multinucleate non motile spores (aplanospores) are formed inside the sporangia occurring singly at the tip of sporangiophores. During the formation of sporangium, the tip of sporangiophore swells and cytoplasm along with nuclei pass inside it.

The contents of the tip distinguished into sporoplasm (containing dense cytoplasm) and columellaplasm (containing vacuolated cytoplasm) which finally form columella. Both the zones are differentiated by a layer which fuses laterally and develop into dome- shaped septum referred as “columella”. Meanwhile, cytoplasmic sporoplasm divides into several 2-3 nucleate spores (rarely uninucleate e.g., *M. hiemalis*) which round up and develop into non flagellate spores “sporangiospores” with thin smooth wall. By bursting of this thin smooth wall due to the pressure in columella exerted by the absorption of water in sporangium and columella, spores get dispersed (Fig 4.9). On getting suitable substratum, they germinate by producing germ tube which develops into new mycelium.

### (ii) Formation of chlamydospores

Under unfavorable conditions, mycelium become septate and protoplast of each cell form a thick wall rounded structure (chlamydospore). These perennating bodies germinate and form new mycelium.

### (iii) Oidia

The emerging mycelium in sugary medium split into small pieces called “oidia” which get apart from each other and germinate to give rise to new mycelium. They enlarge by budding and this phase is called “torula”.

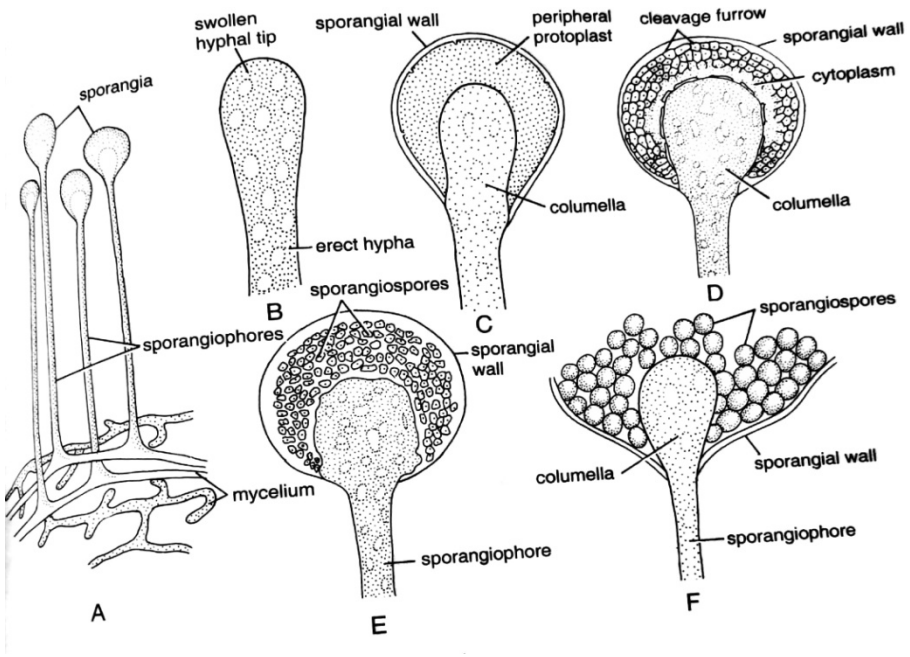


*Fig. 4.9: Asexual reproduction in Mucor*

## (c) Sexual reproduction

It takes place by the development of two multinucleate gametangia which looks alike but is physiologically dissimilar. Mostly the species of *Mucor ga*are heterothallic but some are homothallic. In heterothallic species, zygospores are produced when the mycelia of compatible strains meet whereas in homothallic species, zygospore develops by mycelia evolved from a single spore.

During sexual reproduction, two mycelia of opposite strains (+) and (-) strains come close to each other and develop small outgrowth "progametangia" whose apical ends are swollen and filled with protoplasm come near and a septum is laid down differentiating the apical portion (gametangium) from the basal part (suspensor).As the gametangia mature, the common wall at the point of contact disappear and mixing of contents takes place by nuclear pairing and fusion of (+) and (-) strains giving rise to diploid nuclei (2n) which undergo reduction division. Soon, the young zygospore enlarges and secretes 5 layered structures (2 in exosporium; 3 in endosporium), (Fig 4.10), which undergoes resting period. After long resting period, zygospore germinates.

During germination, exosporium crack and endosporium produces a germ sporangiophores or promycelium which develop a germ sporangium at the tip with large no. of spores. Each spore after liberation germinates to give rise to mycelium.

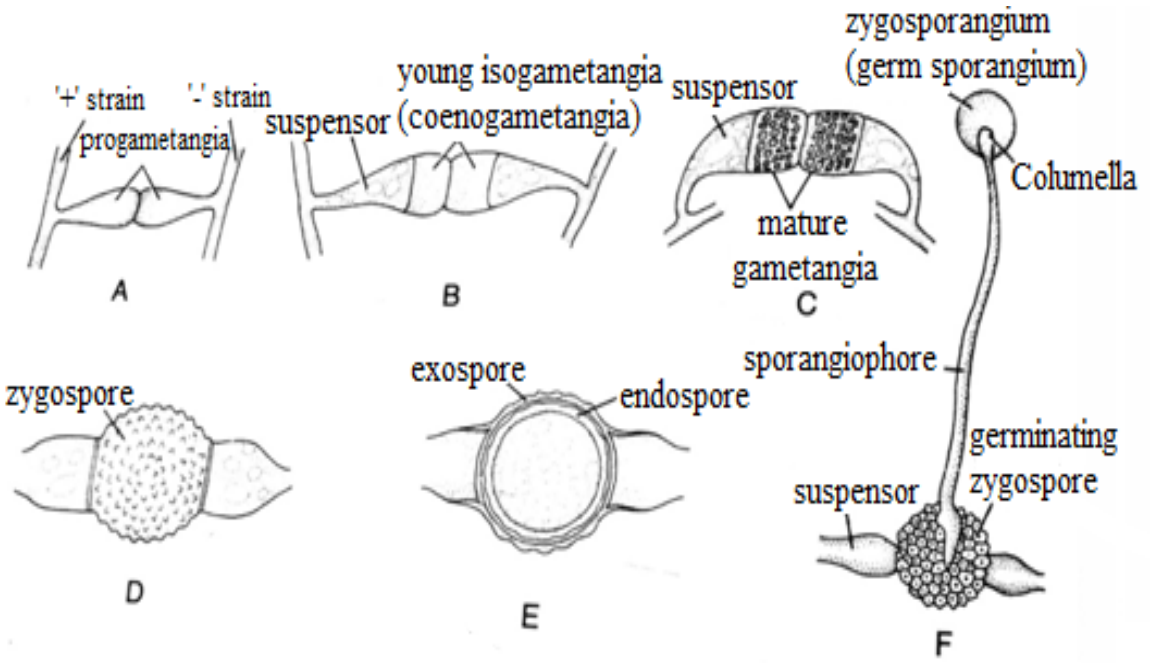


*Fig. 4.10: Sexual reproduction in Mucor*

## 4.4.2 *Rhizopus*

### Systematic position

**Kingdom-** Mycota
**Division-** Eumycota
**Sub-division-** Zygomycotina
**Class-** Zygomycetes
**Order-** Mucorales
**Family-** Mucoraceae

The genus *Rhizopus* is a saprophytic fungus that develop on dead organic stuff is represented by 50 species. Few of the species of *Rhizopus* are weak parasites *e.g*. *R. arrhizus* causes fruit rot of apples; *R. artocarpi* causes fruit drop of jackfruit whereas other species have the potential of synthesizing industrial products *e.g. R. oryzae* in alcoholic fermentation; *R. stolonifer* in production of fumaric acid and *R. nodosus* in lactic acid fermentation. Most widely known species of *Rhizopus* is *R. stolonifer* which is commonly known as “bread mould”.

### Vegetative structure

It appears as white cottony mycelium which is much branched and coenocytic. Three kind of hyphae are reported in the mycelium namely- stonoliferous hyphae; rhizoidal hyphae and sporangiophores respectively (Fig 4.11).

**(i) Stoloniferous hyphae**: It is slightly branched which rise horizontally above the substratum and progess to form clump of rhizoids at some point of link with the substratum.

**(ii) Rhizoidal hyphae**: These are frequently branched hyphae that connect the growing body of organism to a substratum that is able to absorb water and nutrients.

**(iii) Sporangiophores**: These are aerial, unbranched and erect hyphae that grow upward in groups from the stolons. They are also reproductive in function as they possess sporangia.

The hyphae *(i.e.* a tubular structure) are branched, aseptate and coenocytic containing several nuclei, oil droplets, glycogen bodies and vacuoles in cytoplasm. The hyphal wall is microfibrillar and complex consisting of chitin-chitosan, polysaccharides, purine, protein, lipids, calcium and magnesium.

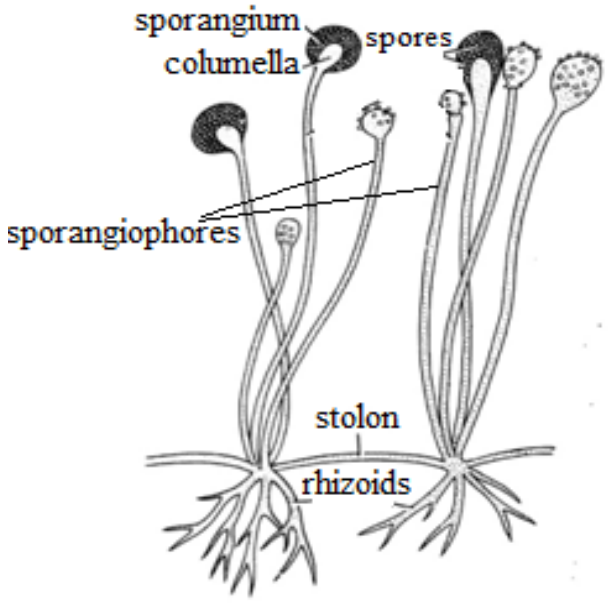


*Fig. 4.11: Vegetative structure of Rhizopus stonolifer*

## Reproduction

It reproduces by vegetative, asexual and sexual modes.

**(a) Vegetative reproduction:** It takes place by means of fragmentation. Here the vegetative hyphae may split into small units and they are capable of progressing into new mycelium.

**(b) Asexual reproduction:** It occurs by the formation of aplanospores or chlamydospores (Fig. 4.11, 4.12)

**(i) Formation of aplanospores:** Under favorable conditions, multinucleate non motile spores (aplanospores) are formed inside the sporangia occurring singly at the tip of sporangiophores.

During the formation of sporangium, the tip of sporangiophore swells and nuclei divide continuously.

The contents of the tip distinguished into central zone (containing vacuolated cytoplasm) and peripheral zone (containing dense cytoplasm with several nuclei). Both the zones are differentiated by a layer which fuses laterally and develop into dome- shaped septum referred as "columella". Meanwhile, cytoplasm divides into several portions which round up and develop into non flagellate spores "sporangiospores" with thin smooth wall. By bursting of this thin smooth wall due to the pressure in columella exerted by the absorption of water in sporangium and columella, spores get dispersed. On getting suitable substratum, they germinate producing germ tube which develops into new mycelium (Fig. 4.12).

**(ii) Formation of chlamydospores:** Under unfavorable conditions, mycelium become septate and protoplast of each cell form a thick wall rounded structure (chlamydospore). These perennating bodies germinate and form new mycelium (Fig. 4.13).

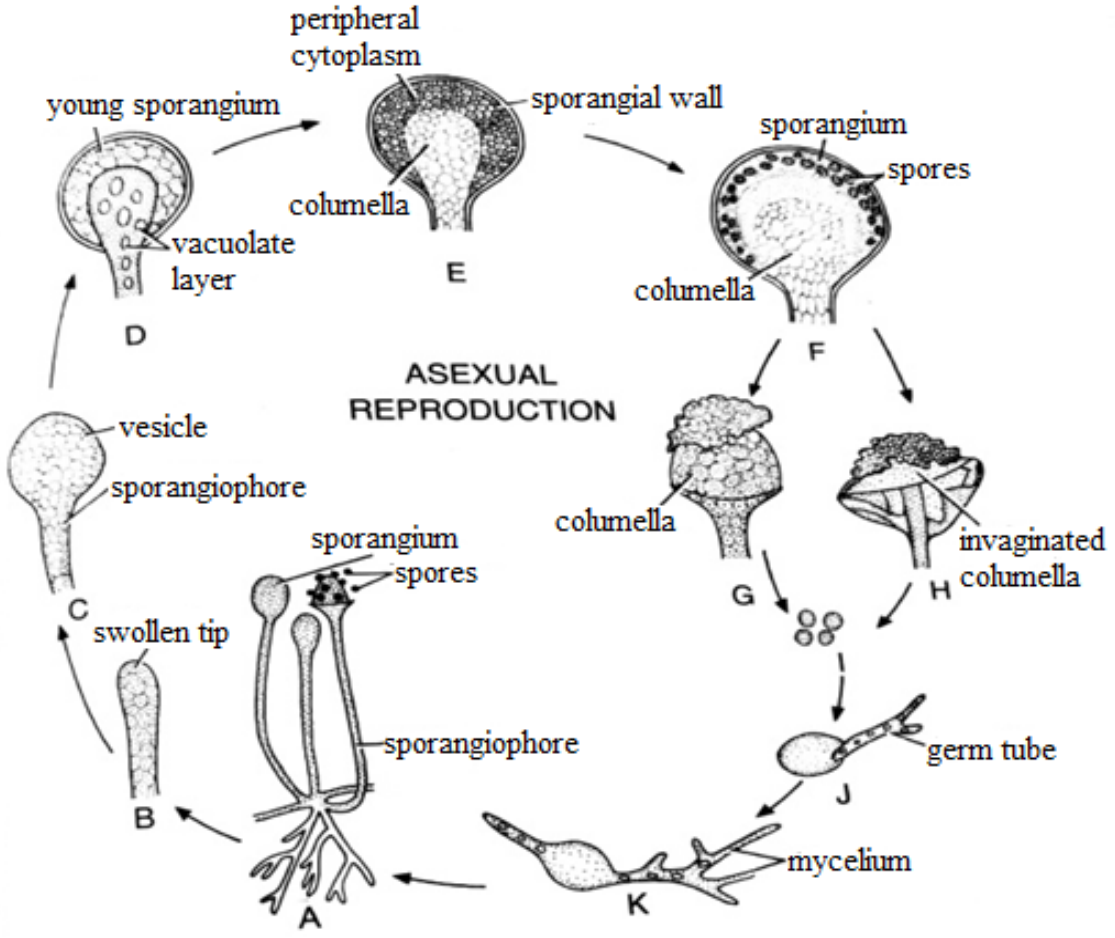


*Fig. 4.12: Asexual reproduction (aplanospore formation) in Rhizopus stonolifer*

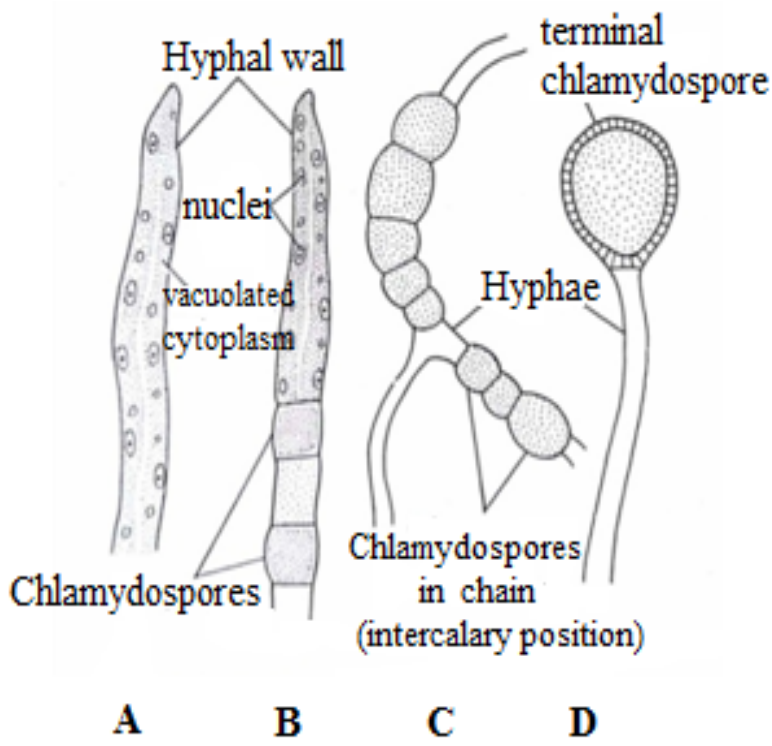


*Fig. 4.13: Chlamydospore formation in Rhizopus stonolifer*

## Sexual reproduction

It takes place by the development of two multinucleate gametangia which looks alike but is physiologically dissimilar. Mostly the species of *Rhizopus* are heterothallic but some are homothallic. In heterothallic species, zygospores are produced when the mycelia of compatible strains meet whereas in homothallic species, zygospore develops by mycelia evolved from a single spore.

During sexual reproduction, two mycelia of opposite strains (+) and (-) strains come close to each other and develop small outgrowth "progametangia" whose apical ends are swollen and filled with protoplasm come near and a septum is laid down differentiating the apical portion (gametangium) from the basal part (suspensor). As the gametangia mature, the common wall at the point of contact disappear and mixing of contents takes place by nuclear pairing and fusion of (+) and (-) strains giving rise to diploid nuclei (2n) and other nuclei which fails to fuse degenerate (Fig. 4.14). + Soon, the young zygospore enlarges and secretes 5 layered structures (2 in exosporium; 3 in endosporium) which undergoes resting period. After long resting period, zygospore germinates. During germination, exosporium crack and endosporium produces a germ sporangiophores or promycelium which develop a germ sporangium at the tip with large no. of spores. Reduction division occurs at the time of germination of zygospore. Each spore after liberation germinates to give rise to mycelium.

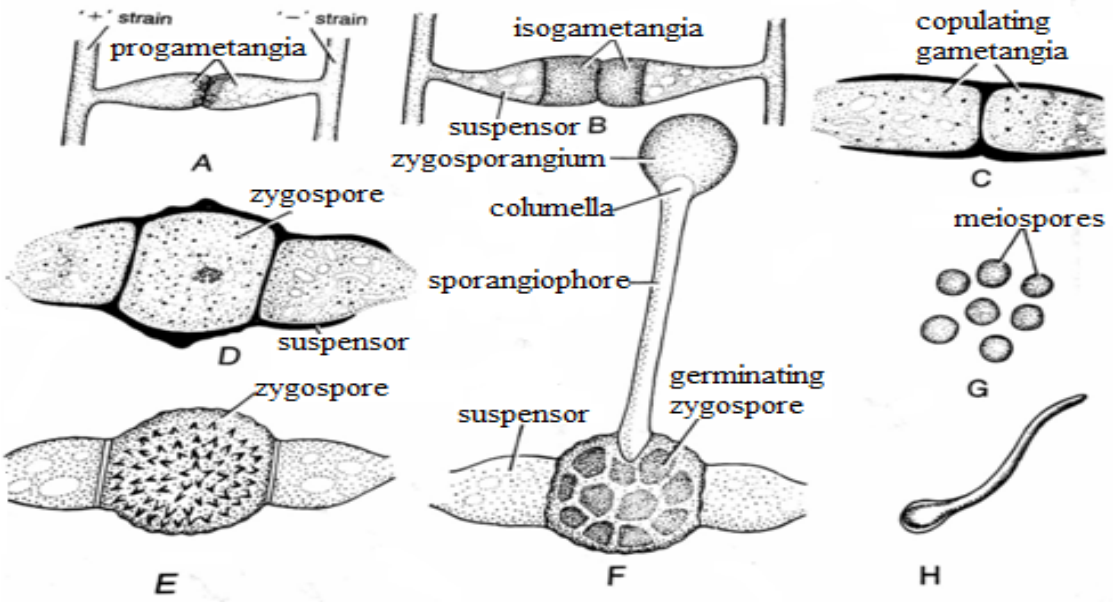


*Fig.4.14: Sexual reproduction in Rhizopus stonolifer*

## 4.4.3 *Pilobolus*

### Systematic position

**Kingdom-** Mycota
**Division-** Eumycota
**Sub-division-** Zygomycotina
**Class-** Zygomycetes
**Order-** Mucorales
**Family-** Pilobolaceae

*Pilobolus* is categorized as a member of zygomycotina which grow on dung of herbivores. It is characterized by positive phototropism and these coprophilous (inhabiting dung) fungi play significant role in ecosystem by participating in recycling of nutrients in faeces.

### Vegetative structure

The mycelium is subaerial, coenocytic which initially grow underneath the surface of dung substratum forming trophocyst (ovoid to globose in shape) which are separated from rest of the hyphae by means of cross walls.

### Reproduction

Life cycle of *Pilobolus* begins with the spores developing on animal dung and disseminating through coenocytic hyphae throughout faeces. Within few days, trophocyst (ovoid to globose shape) form along the hyphae. From the trophocyst, a solid hypha "sporangipohore" emerges whose tip swells to form bulbous subsporangial vesicle and sporangia which contain spores. The sporangium has a light sensitive retina at its base causing sporangia to be aimed toward the source of light and pressure is generated inside subsporangial vesicle by the ions in the cell sap.

Due to this elevated pressure, spores get dispersed by ballistic discharge away from the parent fungus. Owing to the presence of mucilaginous substance on sporangium, make it possible to stick on the substratum wherever they fall on *e.g.,* flora of the atmosphere. A herbivore will utilize the substrate and later on excrete to initiate the new cycle.

## *4.5 SUMMARY*

1. *Synchytrium* occur as obligate parasite on fern, algae, flowering plants and mosses.
2. It reproduces by asexual and sexual mode. During reproductive phase, entire vegetative structure transform into reproductive unit called as "holocarpic".
3. The vegetative body of *Synchytrium* comprises of endobioticthallus.
4. Species of *Allomyces* are saprophytic and soil inhabiting fungi.
5. *Allomyces* exhibit distinct alternation of generation reflecting its potential of both asexual and sexual mode of reproduction.
6. In *Allomyces,* the female gamete secretes a sex hormone "sirenin" which serves as chemo attractant directing male gamete towards female gamete.
7. *Phytophthora* is one of the devastating genus occurring as parasite, few of the species occur as facultative saprophyte.
8. One of the most common species of *Phytophthora* is *P. infastance* causing late blight of potato.
9. *Mucor* and *Rhizopus* are saprophytic fungus commonly referred as Pin mould or black mould and bread mould respectively.
10. Species of *Mucor* are causative agent of Mucoromycosis.
11. In *Mucor,* when the hyphae split into several pieces and enlarge by budding, this phase is called torula stage.
12. Sexual reproduction in *Mucor* is isogamous.
13. Three types of hyphae are reported in the mycelium of *Rhizopus viz.* stonoliferous hyphae, rhizoidal hyphae and sporangiophores
14. The most widely known species of *Rhizopus* is *R. stonolifer*.
15. Some of the species of *Rhizopus* are proficient of synthesizing industrial products *e.g., R. stonolifer* produce fumaric acid; *R. oryzae* used in alcoholic fermentation.

16. In *Rhizopus*, food material is absorbed by rhizoids whereas in *Mucor*, it is absorbed by entire mycelial surface.
17. In *Rhizopus*, reduction division takes place during zygospore germination after resting period wheras in *Mucor*, it takes place after karyogamy, *i.e*. before resting phase of zygospore.
18. *Pilobolus* is a coprophilous fungus and is positively phototropic.
19. *Pilobolus* is characterized by forcibly discharged sporangia.

## *4.6 GLOSSARY*

**Hyphae:** Long filamentous branches of fungi.
**Mycelium:** Vegetative part of fungi comprising web of fine filaments.
**Haustoria:** It is the portion of parasitic fungus that drains nutrients from host tissue.
**Endobiotic:** Living within the tissue of host.
**Antheridia:** Male reproductive body.
**Oogonia:** Female reproductive body.
**Stomata:** Pores which are present in the epidermal surface of leaf.
**Facultative saprophyte:** Those which are normally parasitic but are capable of being saprophytic.
**Coenocytic:** Multinucleate protoplasmic mass.
**Appresorium:** Flattened tip of hyphal branch that enable penetration of host plant.
**Planogametic copulation:** Fusion of naked motile gametes**.**
**Meiosis:** A kind of cell division that results in four daughter cells each with half the number of genetic material of the parent cell.
**Mitosis:** A kind of cell division resulting in formation of two daughter cells each having same no. of chromosomes as the parent nucleus.
**Parasite:** Those which live and draw nourishment from living organism.
**Homothallic:** Presence of reproductive structures (male and female) on the same thallus.
**Heterothallic:** Presence of reproductive structures (male and female) on separate thallus.

## *4.7 SELF ASSESSMENT QUESTIONS*

### 4.7.1. Multiple choice questions:

1. Mastigomycotina commonly known as

(a) Zoosporic fungi (b) Moral fungi
(c) Mushroom (d) Sac fungi

2. *Synchytrium endobioticum* causes

(a) Black wart disease of Potato (b) Late blight of Potato
(c) Early blight of Potato (d) All of the above

3. In *Synchytrium*, resting sporangia is also referred as

(a) Winter spores (b) Summer spores
(c) Both A and B (d) None of the above

4. In *Allomyces*, the vegetative plant body (gametothalli) is

(a) Haploid (b) Diploid
(c) Both of the above (d) None of the above

5. In *Allomyces*, thin walled sporangia are referred as

(a) Zoosporangia (b) Mitosporangia
(c) Both of the above (d) None of the above

6. In *Allomyces*, the cell wall consists of

(a) Chitin (b) β- glucan
(c) Proteins (d) All of the above

7. Late blight of Potato is caused by

(a) *Phytophthora infestans* (b) *Alternaria solani*
(c) *Puccinia* sp. (d) *Albugo candida*

8. Majority of the species of *Phytophthora* are

(a) Parasitic (b) Saprophytic
(c) Both of the above (d) None of the above

9. In *Phytophthora*, the cell wall mainly comprised of

(a) Chitin (b) Glucan
(c) Both of the above (d) None of the above

10. The term “conjugating fungi” is the term assigned to the members of

(a) Ascomycetes (b) Basidiomycetes
(c) Zygomycetes (d) Deuteromycetes

11. Mycelium is the term given to the plant body of

(a) Algae (b) Fungi
(c) Both of the above (d) None of the above

12. *Mucor*/ *Rhizopus*/*Pilobolus* belongs to which class of fungi

(a) Zygomycetes (b) Ascomycetes
(c) Basidiomyctes (d) Deuteromycetes

13. In *Rhizopus*, zygospore develop into

(a) Promycelium (b) Progametangium
(c) Gametangium (d) Zygosporangium

14. *Mucor* is

(a) Saprophytic fungus (b) Parasitic fungus
(c) Both of the above (d) None of the above

15. In *Rhizopus*, the multinucleate filament is

(a) Coenocytic (b) Conidia
(c) Heterothallic (d) Homothallic

16. *Rhizopus stonolifer* is commonly known as

(a) Pin mould (b) Bread mould
(c) Both of the above (d) None of the above

17. In *Mucor* and *Rhizopus*, asexual reproduction takes place by

(a) Chlamydospores (b) Aplanospores
(c) Both of the above (d) None of the above

18. Sporangiospores of *Mucor* are

(a) Haploid (b) Diploid
(c) Both of the above (d) None of the above

**Answer Key:** 1-(a), 2-(a), 3-(a), 4-(a), 5-(c), 6-(d), 7-(a), 8-(a), 9-(b), 10-(c), 11-(b), 12- (a), 13-(a), 14-(a), 15-(a), 16-(b), 17-(c), 18-(a).

## 4.7.2. Fill in the blanks:

1. In *Synchytrium*, when whole vegetative body transform into reproductive unit, then it is referred as ……..
2. The vegetative body of *Synchytrium* consists of ……

3. Sexual reproduction in *Allomyces* takes place by……
4. In *Allomyces*, mitospores on germination give rise to……
5. Early symptoms of *Phytophthora* appear on …….
6. *Mucor* exhibit…….
7. In *Rhizopus*/ *Mucor* species, isogamy occurs between……
8. The haploid structure of *Rhizopus* is……
9. *Pilobolus* fungi characterized by …….

**Answer Key:** Holocarpicmonocentric, 2.Endobioticthallus, 3.Planogametic copulation, 4.Sporophyticthalli, 5.Tip and margins of older leaves, 6.Isogamy, 7.Opposite strains (+) and (-), 8.Mycelia, sporangia, 9.Early development of trophocyst from hyphae.

## 4.7.3. True and False:

1. In *Allomyces*, male gametes are smaller in size and more active than female gametes.
2. In *Allomyces*, the two gametangia (male and female) are born on the same thallus.
3. In *Synchytrium*, the aggregated mass of prosorus is called sorus.
4. In *Phytophthora*, direct germination of sporangia is favored by low temperature and humid weather.
5. The asexual spores of *Mucor* are motile.
6. *Rhizopus* is a parasitic fungus.
7. Mycelium of *Rhizopus* is branched and coenocytic.
8. The mature zygospore in *Rhizopus* is 5 –layered.
9. Species of *Rhizopus / Mucor* are mostly heterothallic, rarely homothallic.

**Answer Key:** 1. True, 2.True, 3.True, 4.False, 5.False, 6.True, 7.True, 8.True, 9. True.

## 4.7.4. Very short answer type questions:

1. Define role of sirenin hormone in *Allomyces.*
2. What favors rapid spread of *Phytophthorainfestans?*
3. How many type of thalli are present in the life cycle of *Allomyces?*
4. Define mucoromycosis?
5. Define torula stage?
6. Define sporangiophores?

**Answer Key:** 1. It acts as chemoattractant leading movement of male gametes toward female gamete, 2. Low temperature and humid environment, 3.Two, 4. It is the disease of lungs, brain in animals and human beings caused by *Mucor* species leading to death, 5. The emerging mycelium in sugary medium split into small pieces called “oidia” which get apart from each other and germinate to give rise to new mycelium. They enlarge by budding *(i.e.* yeast) and this phase is called “torula”, 6. Aerial, unbranched and erect hyphae bearing sporangia.

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## *4.9 TERMINAL QUESTIONS*

### 4.9.1 Short answer type questions:

1. Explain indirect mode of germination of sporangia in *Phytophthora.*
2. Describe salient features of Zygomycotina.
3. Describe salient features of Mastigomycotina.
4. Explain the life cycle of *Piloblous.*
5. Differentiate between *Mucor* and *Rhizopus.*

### 4.9.2. Long answer type questions:

1. Describe the life cycle of *Rhizopus.*
2. Describe the vegetative structure and reproduction in *Mucor.*
3. Explain the life cycle pattern of *Allomyces.*
4. Describe reproduction in *Synchytrium.*

# UNIT-5 ASCOMYCOTINA

## Contents:

## 5.1 OBJECTIVES

After reading this unit student will be able-

- To understand the general characteristic of Ascomycotina
- To understand the life cycle pattern of *Saccharomyce*s
- To know about the *Taphrina*
- To understand the life cycle of *Aspergillus*
- To understand the life cycle of *Penicillium*

## 5.2 INTRODUCTION

In the previous unit you have read the various characteristic features of Mastigomycotina and Zygomycotina. This chapter provides the detailed and informative concept about one of an important class of fungi the Ascomycotina. It is the largest subdivision of fungi and members shows enormous diversity of structure and ecology from the unicellular form like yeast to higher elaborate fruiting structure *Morchella*. The group has existed for many millions of year there is some evidence that they took their origin from Zygomyteous forms. It is a higher class of fungi and not produces zygospore.

Unlike Zygomycotina a peculiar type of asexual reproduction occur in most of the members of this class which accountable by conidia which are arranged basipetaly on conidiophores.

Sexual stage produce ascospores in ascus, this is a fundamental feature of this class and so called the “sac fungi” characterized by sac like structure of ascus and different types of fruiting body called ascocarp produce in this group, according to morphological feature of ascorcarp ascomycotina divided in many subdivisions. Sexual degeneration is also reported in some members of this class.

This group is of particular relevance to human as source for medicinally important compounds such as antibiotics, and making bread, beverage organic compounds. Members of this group are saprophytes or parasites, some plant disease produced by these are powdery mildew of grape, dutch elm disease, curl leaf disease of peach. Most of the members of this class have great economic value as *Neurospora* (Drosophila of plant kingdom) a genus of wide spread species extensively used in genetic and biochemical investigation. The genus *Cordyceps*, commonly known as caterpillar fungi, is used as an important constituent in many drugs. Species of *Penicillium* are the sources of ‘wonder drug of the world’ penicillin which was the first antibiotic.

## *5.3 ASCOMYCOTINA*

Various types of morphology appear in their members, as unicellular form like yeast and multicellular profusely branched and septated mycelium. This group is distinguished from other fungi by the development of ascospores and also by conidial stage. Vegetative reproduction takes place by fission, fragmentation, and budding and it is the most common methods in unicellular form, members reproduce asexually by the non motile spores such as oidia, chlamydospores and conidia. Conidia developed in conidiophores when the spores are borne free i.e. not contained within membrane the sporophore is referred to as conidiophores.

They are homothallic i.e. male and female reproductive organs on same thallus or heterothallic male and female reproductive organ on different thallus. In some heterothallic species, though male (antheridium) and female (ascogonium) sex organ develop on the same thallus but shows self incompatibility means male gamete of one mating type fertilizes ascogonium of other mating type this process is known as physiological heterothallism.

Sexual reproduction takes place by gametangial copulation (e.g., yeast); by gametangial contact (e.g. *Aspergillus, Penicillium*); by somatogamy (e.g., *Morchella*) or by spermatization (e.g., *Polystigma*). Male and female reproductive organs called as antheridia and ascogonia respectively.

After the Gametangial union plasmogamy takes place. Ultimately asci are formed, in which ascospores are produced by karyogamy and meiosis. Dikarophase is also occurring in between the haplophase and diplophase which may be short or long in duration, depending on the organism. The asci may or may not be enclosed in an (fruiting body) ascocarp.

Four types of fruiting body (ascocarp) are found, which are as following-

i. **Apothecium** – It is a plate like structure in which asci develop on the surface of the hymenium and at maturity they remain exposed e.g., *Peziza*.

ii. **Perithecium** – It is a flask shaped fruiting body in which asci develop from the inner surface of the fruiting body. Hair like sterile structure known as paraphysis is present on the neck and mouth of the fruiting body. In the apical portion of the perithecium apical pore is present known as ostiole. e.g., – *Claviceps*.

iii. **Cleistothecium** - It is globose and completely closed fruiting body in which asci are scattered in it. e.g., *Aspergillus, Penicillium***.**

iv. **Ascostroma-** It is a pseudoparenchymatous structure composed of somatic hyphae. The fruiting body lacks a distinct wall and asci are usually bitunicate.

On the basis of the presence or absence of fruiting body this class can be divided into the following subclasses:

**1. Hemiascomycetes:** in this ascocarps are not formed and asci are naked. This sub class may be divided into three orders protomycetales, endomycetales and taphrinales.

**2. Euascomycetes:** asci developed in the fruiting bodies the sub- class divided into following five series- Plaectomycetes, Pyrenomycetes, Loculascomycetes, Laboulbenimycetes, Discomycetes.

## 5.3.1 *Saccharomyces* (Yeast)

### Systematic position

**Kingdom -** Mycota
**Division-** Eumycota
**Class -** Ascomycotina
**Sub-class -** Hemiacomycotina
**Order-** Endomycetales
**Family-** Saccharomycetaceae

The genus *Saccharomyces* (Gr. *Saccharon*, sugar; *mykes*, fungus) is represented by many saprophytic species and found ubiquitously. They are mostly found media rich in sugar or organic matter of vegetable origin. *S. cerevisiae*, commonly known as Brewer yeast or Backer's yeast, is used widely in wine and baking industry.

It produces two types of enzymes: an extracellular invertase and an intracellular zymase. The invertase hydrolyses cane sugar to dextrose or invert sugar and zymase breaks invert sugar into ethyl alcohol and carbon dioxide. They easily destroy soft cheese and similar foods, for they impart repulsive yeasty flavor.

## Vegetative structure

The thalloid plant body is unicellular, but some time formation of pseudo- mycelium during rapid multiplication by budding and cells remain attached in chain. The cells may be globose, elliptical, oval to even rectangular in shape and size ranges about 5-6 x 6-8 µm.

*Saccharomyces cerevisiae* show that the cells are surrounded by a distinct cell wall with three layers. The outermost layer mainly consists of protein-mannan and some chitin; the middle layer mainly of glucan and the innermost layer consist of peptidoglycan. Cellulose is absent in the cell wall. Inner layer next to the cell wall is plasma membrane (plasmalemma) which has series of shallow, elongated pits or invaginations. A large vacuole, limited by a single membrane, the tonoplast, occupied in the center which contains a watery substance, granules of

polymetaphosphate and lipid. A minute double unit membrane nucleus present near one side of the vacuoles (Fig. 5.1).

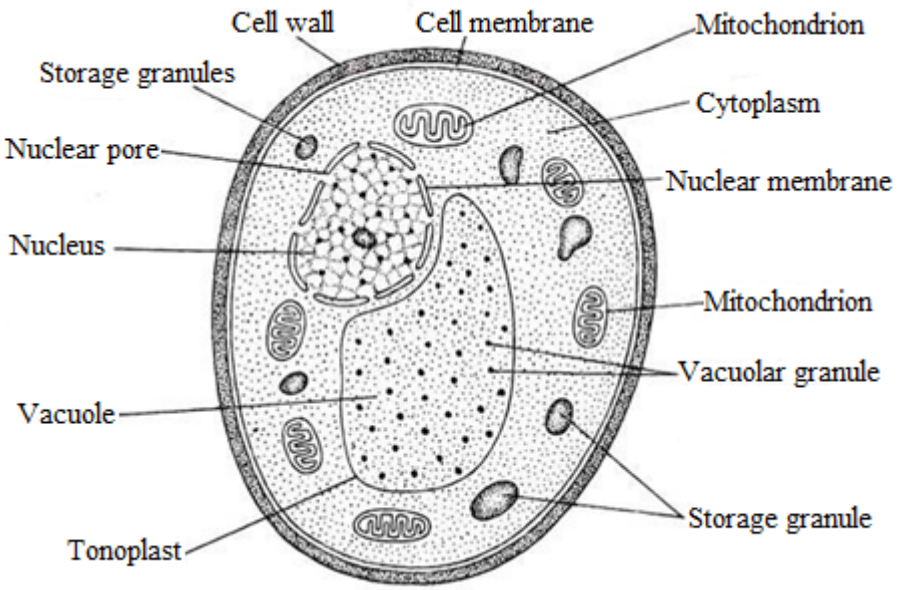


*Fig. 5.1: Cell structure of Saccharomyces cerevisiae under electron microscope*

## Reproduction

*Saccharomyces* reproduces by vegetative, asexual and sexual means.

## Vegetative Reproduction:

Vegetative reproduction takes place by fission and budding.

**(i) Fission:** In a favorable condition a constriction and transverse wall developed in the cell and cell split in to two daughter cells this is called fission. During fission, a constriction appears in the middle of the cell and simultaneously nucleus undergoes mitotic division than two equal sized uninucleated daughter cells are formed (Fig. 5.2).

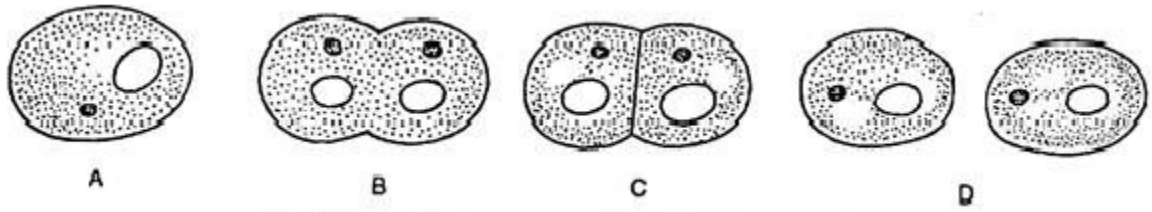


*Fig. 5.2: Fission states of S.cerevisiae*

**(ii) Budding:** It also occurs in favorable condition; in this process a small outgrowth protrude out from the vegetative cell of yeast, protoplast of cell transferred to this small swollen part (Fig.

5.3). After mitotic division two nuclei formed, one transferred to the outgrowth and one remain in the mother .The size of the bud is always smaller than the mother cell. After maturation, these bud separate from the mother and leave a convex scar on the surface, called bud scar. Similar scar with concave surface remains on the wall of the bud, called birth scar.

Sometimes due to rapid division, large numbers of buds develop without being detached from one another and persist in the form of branched or unbranched chain, and give the appearance of false mycelium called pseudo-mycelium Finally the cells get detached and grow individually (Fig.5.4).

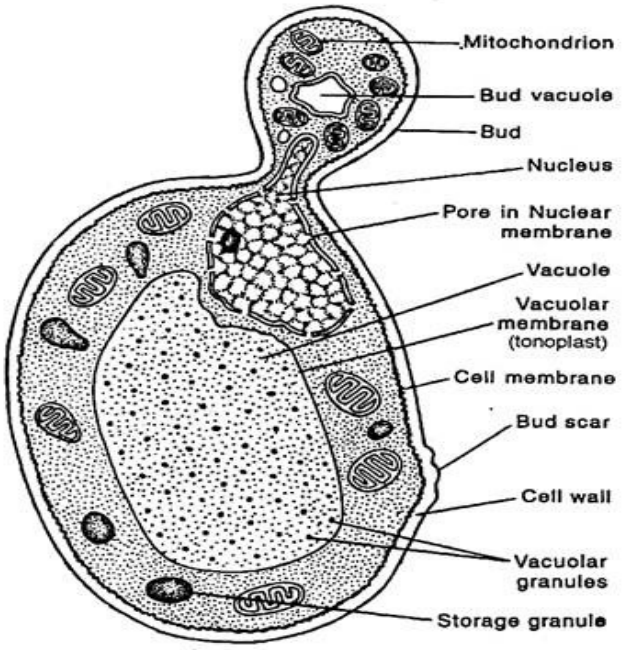


*Fig. 5.3: structure of the budding cell of Sacharomyces cerevisiae under electron microscopic*

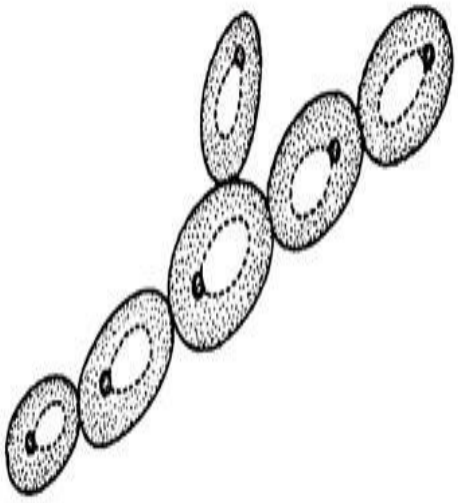

*Fig 5.4: Pseudomycelium of S. cerevisiae*

## Asexual reproduction

**Endospore:** In this process the thick walled spores are formed called endospore. It happens in the unfavourable conditions. During this process four nuclei are form by the mitotic division. The protoplast divides into four units, each with one nucleus and forms four endospores. On coming favourable condition, endospore germinates by budding and buds grow individually (Fig. 5.5).

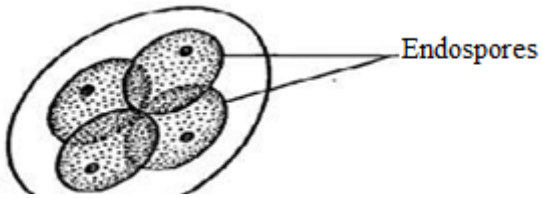


*Fig 5.5: Endospores in a cell*

## Sexual reproduction

It also takes place in unfavourable conditions. In this genus it is very simple process because of entirely absent of sex organs and two somatic cells or two ascospores are involved which assume the function of copulating gametangia. Two such cells come close up and develop beak-like outgrowth towards each other. Both the outgrowths come in contact and dissolve the wall of intervening.

The nuclei of both the gametangia come to the fused outgrowth (conjugation tube) and diploid zygote produced after their fusion. The zygote behaves as an ascus. The diploid nucleus of zygote undergoes reduction division and forming 8 (after mitosis) ascospores. The ascospores are liberated by breaking the ascus wall and behave as somatic cell (Fig 5.6).

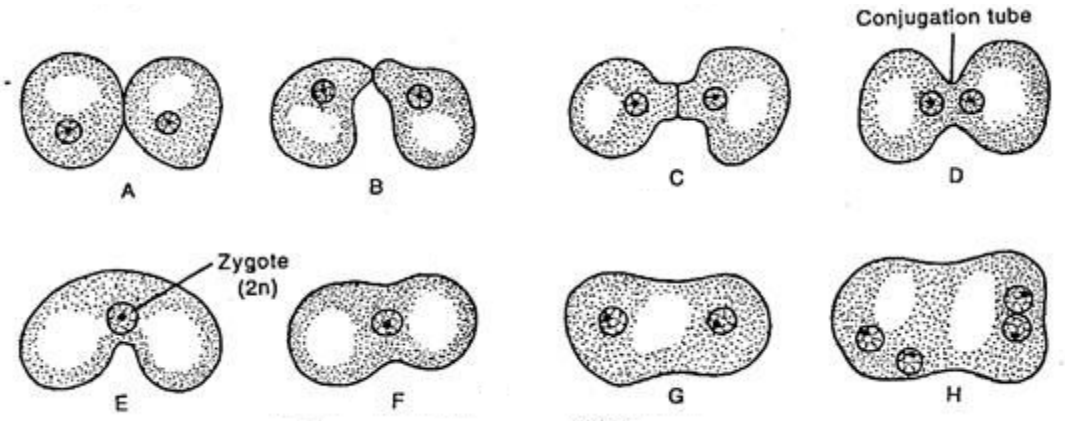


*Fig. 5.6: Different states of sexual reproduction of Saccaromyces cerevisiae*

## Life cycle:

Three type of life cycle have found in yeast.

**1-Haplobiontic life cycle:** In this type of life cycle zygote is only diploid phase and others are in haploid phase. The vegetative cells are haploid and behave as gametangia.

Two such gametangia fuse together and form a diploid cell. The diploid cell behaves as an ascus whose nucleus divides first meiotically, then mitotically; results in the formation of eight ascospores. After maturation, the ascospores liberate by bursting the ascus wall. The ascospores then behave as vegetative cell and continue multiplication through budding. This type of life cycle is found in *Schizosaccharomyces octosporus* (Fig.5.7A).

**2-Diplobiontic life cycle:** This type of life cycle is characterised by more involved diploid phase than the haploid phase, the haploid phase is represented only by ascospore, with short duration. The ascospores behave as gametangia and, without liberating from ascus, they unite in pair. The diploid zygote produced by the fusion of paired gametangia

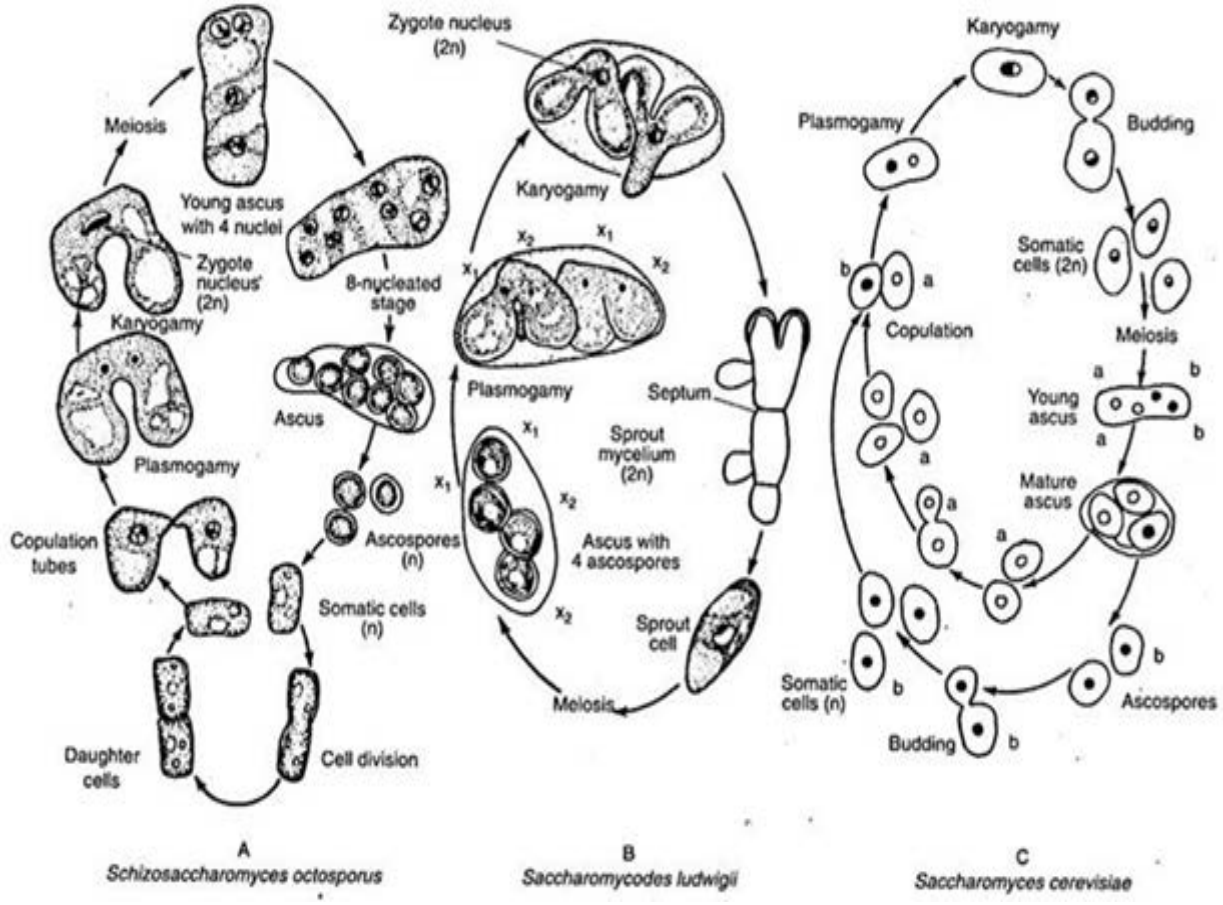


*Fig. 5.7: Life cycle of yeast A. Haplobiontic (Schizosaccharomyces octosporous), B. Diplobiontic (Saccharomyces ludwigi) C. Haplodiplobiontic (Saccharomyces cerevisiae)*

The zygote then germinates by producing germ tube which comes out through the ascus wall. The diploid sprouts develop by budding from multicellular germ tube. After detachment from the mother, the diploid sprouts function as asci and produce four ascospores by reduction division. *Saccharomycodes ludwigii* represent diplobiontic lifecycle (Fig. 5.7B)

**3-Haplo-Diplobiontic Type:** This type of life cycle both type haploid and diploid phases comes more or less equal duration. Haploid cells of opposite mating type normally multiply by budding. Two such cells of opposite mating behave as gametangia and undergo fusion. The fused gametangia develop a diploid zygote.

Budding developed from the diploid zygote like haploid cells and forms many diploid cells. With the insufficiency of food, the diploid cell behaves as an ascus and by meiosis it forms four haploid ascospores. After liberating from the mother wall, the ascospores undergo budding and form many haploid somatic cells. This type of life cycle is found in *Saccharomyces cerevisiae* (Fig.5.7C).

## 5.3.2 Taphrina

### Systematic position

**Kingdom -** Mycota
**Division-** Eumycota
**Class-** Ascomycotina
**Sub-class-** Hemiacomycotina
**Order-** Taphrinales
**Family-** Taphrinaceae

*Taphrina* is a pathogenic fungus causes leaf curl disease and witches' brooms of certain flowering plants. Most important is leaf curl disease of peach the fungus is not limited only to peach (*Prunus persica*) and rather attacks the other *Prunus* species and plum. It occurs all over the world. Infection is favored by low temperature and high humidity.

*Taphrina* is a member of a primitive group within the Ascomycota, it does not contain any fruiting body or ascocarp like other higher member of ascomycota. The fungus is represented by its dimorphic growth stages. The unicellular yeast stage is saprophytic in nature whereas the filamentous stage is the pathogenic stage. Only the yeast stage can be grown in the culture media.

The mycelium is septate, intercellular in nature, hyline and branched and present only in reddened diseased area of the leaf. Mycelial cells of fungus in host plant contain two nuclei. The mycelium in most species of *Taphrina* is annual, but in some species it is perennial.

## Life Cycle

Asexual reproduction takes place by conidia which are uninucleate, thin-walled spores. The conidia are developed from the ascospores by budding. The conidia latter may produce again more conidia or may germinate to produce mycelium. Conidia germinate by germ tubes which

penetrate through cuticle of young leaf and cause infection in the host tissue. On germination the conidial nucleus divides and the two nuclei move into the germ tube. As the mycelium grows, both nuclei divide and produce binucleate cells of the mycelium.

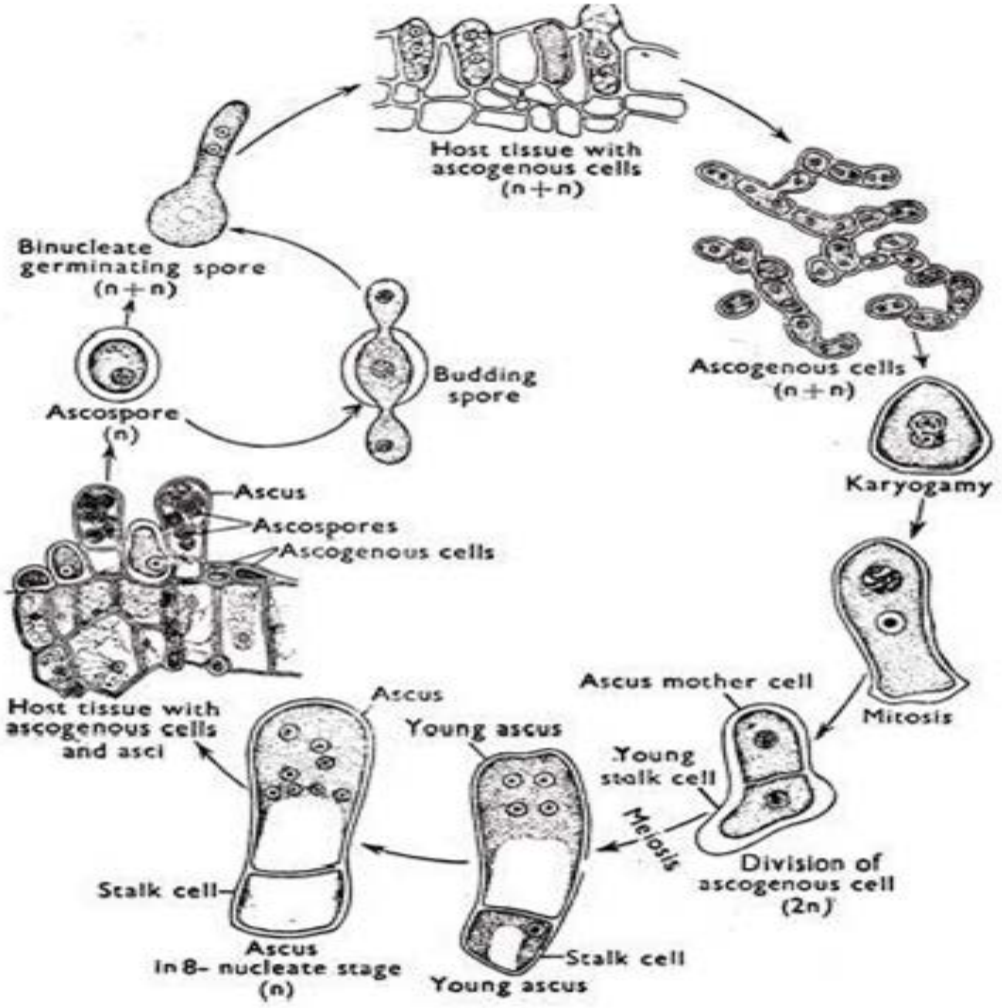


*Fig. 5.8: Life cycle pattern of Taphrina deformans*

At this time, the leaf's cells to elongate, causing twisting and blistering of the leaf. Eventually certain cells of the mycelium begin enlarging, growing to such an extent that they cause the leaf's cuticle, or skin, to burst.

On the plant surface dikaryotic cells separated from each other and form the palisade-like layer of rectangular asci and this is the phase of sexual reproduction. The identifying character of this fungus is that they do not form the ascocarp. The asci are naked.

These cells are ascogenous cells. The ascogenous cells are ovoid, pyriform, or dome-shaped. During the development of an ascus the ascogenous cell elongates perpendicularly to the host

surface. Its nuclei fuse forming a diploid nucleus. Two daughter nuclei are produced from mitotic division of diploid nucleus, of which one moves to the distal end of the elongated ascogenous cell and the other remains at the base.

Now the ascus mother cell starts to develop into an ascus. The protoplasmic contents of the ascus mother cell crowd the tip where the diploid nucleus divides meiotically into daughter nuclei which again divide mitotically to form eight haploid nuclei. Eventually eight ascospores are formed and are spherical or ovoid in shape and measure 3-7 µm in diameter.

Mature asci are exposed by the rupture of the cuticle or epidermis of the host tissue, when club shaped asci become visible. The ascus wall at the apex ruptures causing the ascospores release in the atmosphere. The ascospores, soon after produce small, round or ovoid uninucleate blastospores (also known as conidia) by budding, while they are already in the ascus. Budding is common in ascospore resulting in numerous blastospores. Blastospores continue the budding process even after release.

Dikaryotic condition may be established by copulation of conidia. The ascospores with adhering conidia forming spore balls are ejected forcibly from the asci. They may be carried by wind or splashed in raindrops. On reaching host surface, the dikaryotic conidia germinate by germ tubes which infect the host and produce hyphae with dikaryotic cells (Fig.5.8).

Leaf curl disease are controlled easily by single fungicide spray, preferably in late fall after the leaves have fallen or in early spring before leaf buds swell. Fungicide used most commonly is the Bordeaux mixture.

**Some Indian species of genus Taphrina:** *Taphrina deformans* (Berk.) Tul., *T. maculans* Butler, *T. purni* Tul., *T. rhomboidalis* Syd., P. Syd. & Butler; *T. tubiforme* (Rabenh.) Lagerh.

## 5.3.3 *Aspergillus*

### Systematic position

**Kingdom -** Mycota
**Division-** Eumycota
**Class -** Ascomycotina
**Sub-class-** Plactomycetes
**Order-** Aspergillales
**Family-** Aspergillaceae

*Aspergillus* is a saprophytic fungus, represented by 132 species occurring over a wide range of habitats. Some species of aspergillus are found as parasites on plants, causing crown rot of

groundnut and boll rot of cotton. *A. flavous* contaminates groundnut and other dry food stuffs. It produces some toxic substances called aflatoxins, which are carcinogenic.

### Vegetative structure

The plant body is mycelial. The mycelium is well developed consists of slender, tubular, pale coloured, thin walled, extensively branched hyphae. Some hyphae ramify superficially upon the substratum while some penetrate into the substratum to absorb the food material.

Each cell is multinucleate and is filled with granular cytoplasm, mitochondria, endoplasmic reticulum, ribosomes and vacuoles. Cytoplasms of the adjacent cells remain continuous through simple pore in the cross wall present between the cells. Reserve food material is in the form of oil globules. The species of *Aspergillus* mostly have some characteristic pigments in hyphae, conidiophores and conidia (Fig. 5.9).

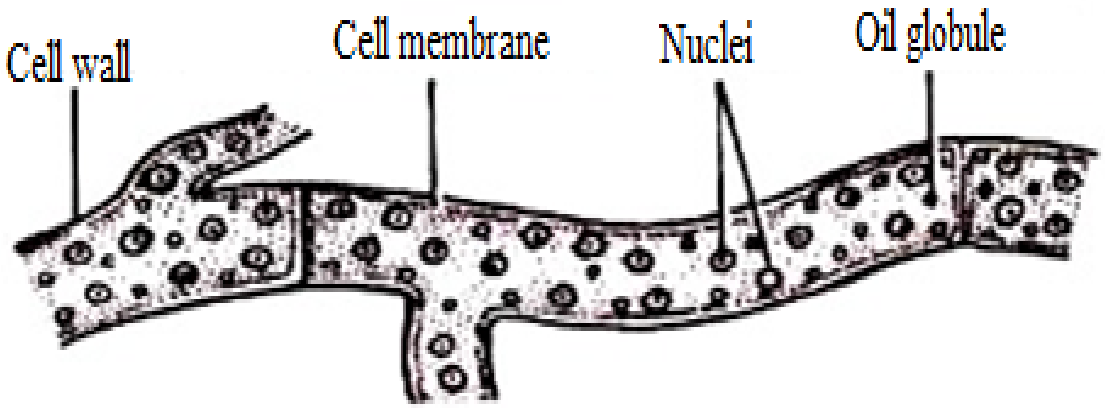


*Fig.5.9: Vegetative structure of Aspergillus*

## Reproduction

It reproduces by vegetative, asexual and sexual means.

**1-Vegetative reproduction:** Under favorable conditions the vegetative mycelium breaks up into small fragments and each fragment grows independently into a new thallus.

**2-Asexual reproduction:** Asexual reproduction takes place by the conidia produce conidiophores. Some cells of the hyphae grow more rapidly and develop a rigid wall and are known as foot cells. Each foot cell produces a special erect branch as an outgrowth known as conidiophore. Conidiophores are usually unbranched and aseptate (Fig 5.10)
The tip of the conidiophore swells up into on elliptical or globular multinucleate head called vesicle. Many radially arranged tubular outgrowths called sterigmata or phialides arises from

these vesicles. In some species primary sterigmata (uniseriate) bear secondary sterigmata. (bi-seriate)

Conidia (sing. = conidium), arise exogenously from the sterigmata or phialides therefore, conidia are also called phialospores or phialaconidia. They are arranged in basipetal succession (i.e., the oldest at the top and the youngest conidium is at its base). The sterigmata elongate at the tip to form a tube and inside this conidia are formed. The sterigmata are uninucleate. At the time of formation of conidia mitotic division takes place in a single nucleus of the phialide and divides into two daughter nuclei, one of the daughter nuclei passes into the tube and forms the first conidium. After the formation of first conidium the upper broken wall of the phialide serves as a cap around it. The second conidium is formed by phialide just below the first .The cytoplasm of both the conidia is confluent through a narrow cellular link called isthmus .The inner conidial wall is formed and continuity of the cytoplasm become stopped. Now isthmus becomes empty and called as connective. A long chain of conidia formed in this fashion (Fig.5.11).

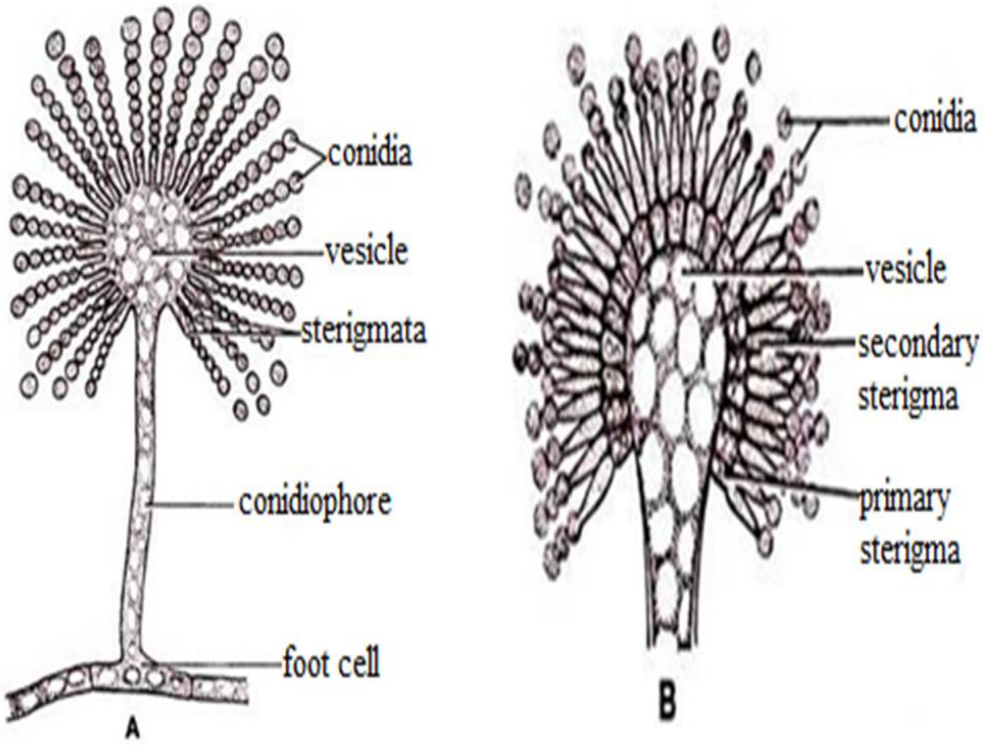


*Fig. 5.10: Asexual reproduction of Aspergillus A. conidiophores with primary sterigmata B. primary and secondary sterigmata*

Conidia are small, globose, unicellular, uninucleate, black, brown or yellow green in colour due to the presence of various pigments. They have two layered wall. Epispore is the outer wall layer which is thick spiny, pigmented whereas the inner one is delicate, thin and is called endospore. Conidia are dispersed by wind. They germinate on suitable substratum by a germ tube. The germ tube becomes septate, branched and forms a new mycelium (Fig. 5.12).

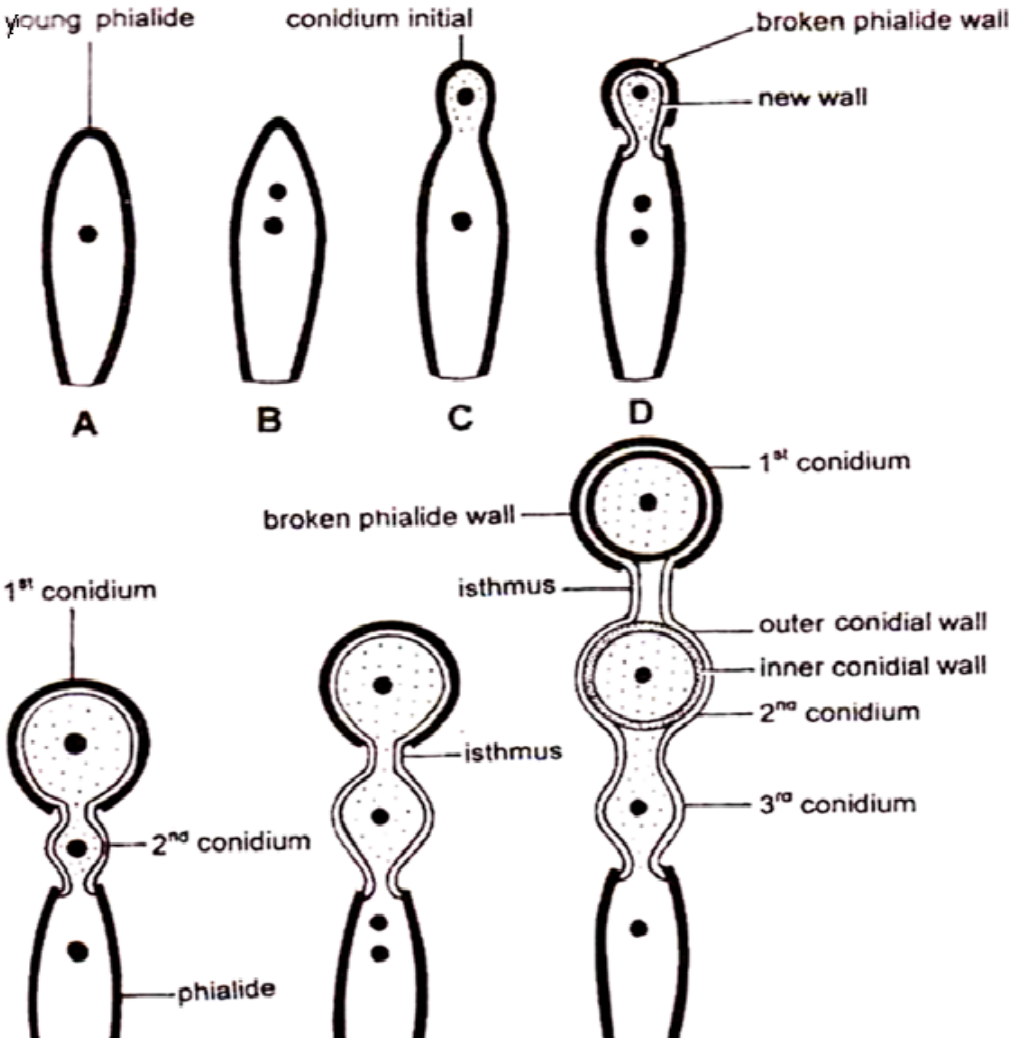


*Fig. 5.11: Asexual reproduction formation of conidia*

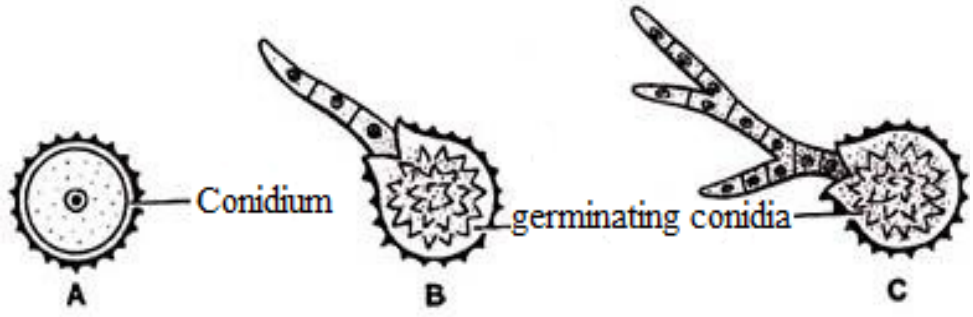


*Fig.5.12: Germination of conidia of Aspergillus*

## 3-Sexual reproduction:

Sexual reproduction is rare in *Aspergillus*. Variation in their sexual behavior occurs in different species of this genus. The antheridium is absent in some species or if present it is nonfunctional. However the female sex organ (ascogonium) develops the asci. In others it is well developed and functional. *Aspergillus* is homothallic. Heterothallism also occur in few species (*A.fischeri, A. heterothallics*).

The male and female sex organs known as antheridia and ascogonia, respectively. In the homothallic species, sex organs are developed close together on the same hypha or on separate nearby hyphae of the same mycelium. Both are elongate, multinucleate and generally coil around each other (Fig 5.13).

### Development of ascogonium

The mycelium which forms ascogonium soon becomes septate and loosely coiled. As ascogonium develops, the coil becomes dense and looks as a spring like structure known as archicarps. The young archicarp can be differentiated into three following parts.

1. Terminal unicellular, multinucleate receptive organ called trichogyne.
2. Middle part is also unicellular, multinucleate ascogonium (gametangium).
3. The basal part is multicellular, multinucleate and forms the stalk.

### Development of Antheridium

The antheridium development initiate just before or during the separation of the ascogonial hypha. The antheridial branch, also known as pollinodium, becomes 2-celled by the formation of septum. The upper cell forms the antheridium proper and lower one, the stalk. Both are multinucleated and unicellular.

### Fertilization

The tip of the archegonium coiled over the trichogyne and fuses with it. The wall at the point of contact dissolves, and contents of the antheridium pass into the ascogonium. The pairing of male and female nuclei takes place in ascogonium this is known as dikayon.

In some species the antheridium is very well developed but the male contents do not transfer and fuse with the contents of the ascogonium (e.g., *A.repens*). In some other species the antheridium may be completely absent (e.g., *A. flavus, A. fisheri*). There is pairing between ascogonial nuclei itself and fruiting body is formed. It shows the degeneration of sex organs in Ascomycetes.

## Development of Ascus

After the pairing of the nuclei, the ascogonium becomes septate. Each segment consists of one male and one female nucleus (dikaryon). From these dikaryotic segments arise ascogenous hyphae. The terminal cell of ascogeneus hypha curves to form a hook like structure known as crozier. The two nuclei of the dikaryon divide by a conjugate division and thus forms four daughter nuclei. Now septa are formed in the crozier in such a way that the penultimate cell has two daughter nuclei and the terminal and basal cells have one daughter nucleus each. The two nuclei present in the penultimate cell fuse to form a diploid nucleus. The cell now functions as ascus mother cell and elongates to form an ascus. Diploid nucleus first undergoes meiotic division and then mitotic division and form eight hapoid nucleus each of these ultimately transferred in to ascospore.

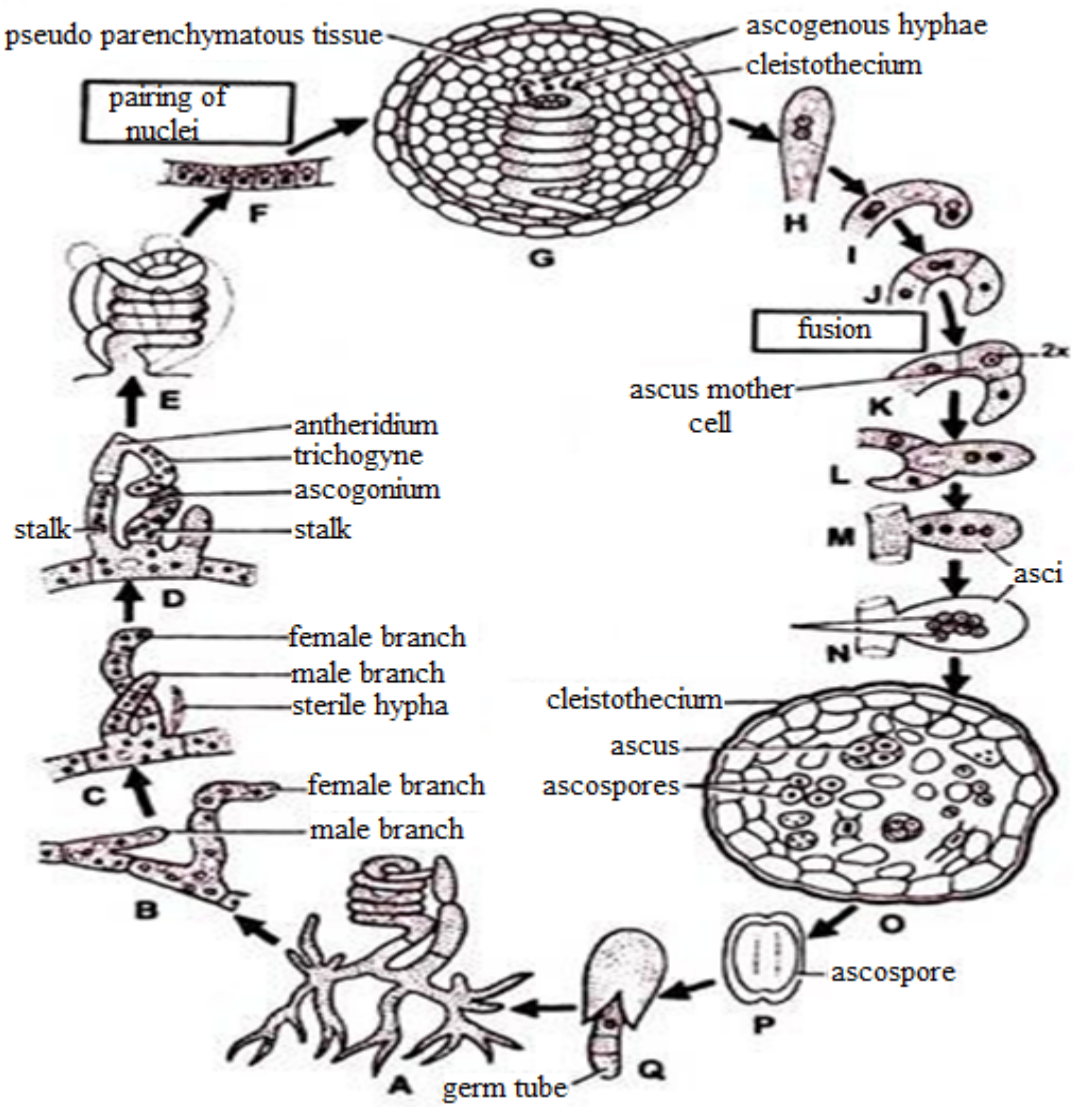


*Fig 5.13: Sexual reproduction of Aspergillus*

## Development of Ascocarp

Simultaneously with the development of ascogenous hyphae, many sterile hyphal branches also develop from the cells lying below the ascogonium. These branches form peridium which is a psudoparenchymatous, two layered structure encloses ascogenous hyphae. Outer layer of the peridium forms a protective covering whereas the inner layer is consumed by the developing asci. The entire structure is known as fruiting body or ascocarp which appears like a hollow ball of the pinhead size. This is a clestothecium type fruiting body (completely closed fruiting body). Ascospores are released by the breakdown of the cleistothecium wall (peridium) and produce new mycelia on suitable substratum after germination.

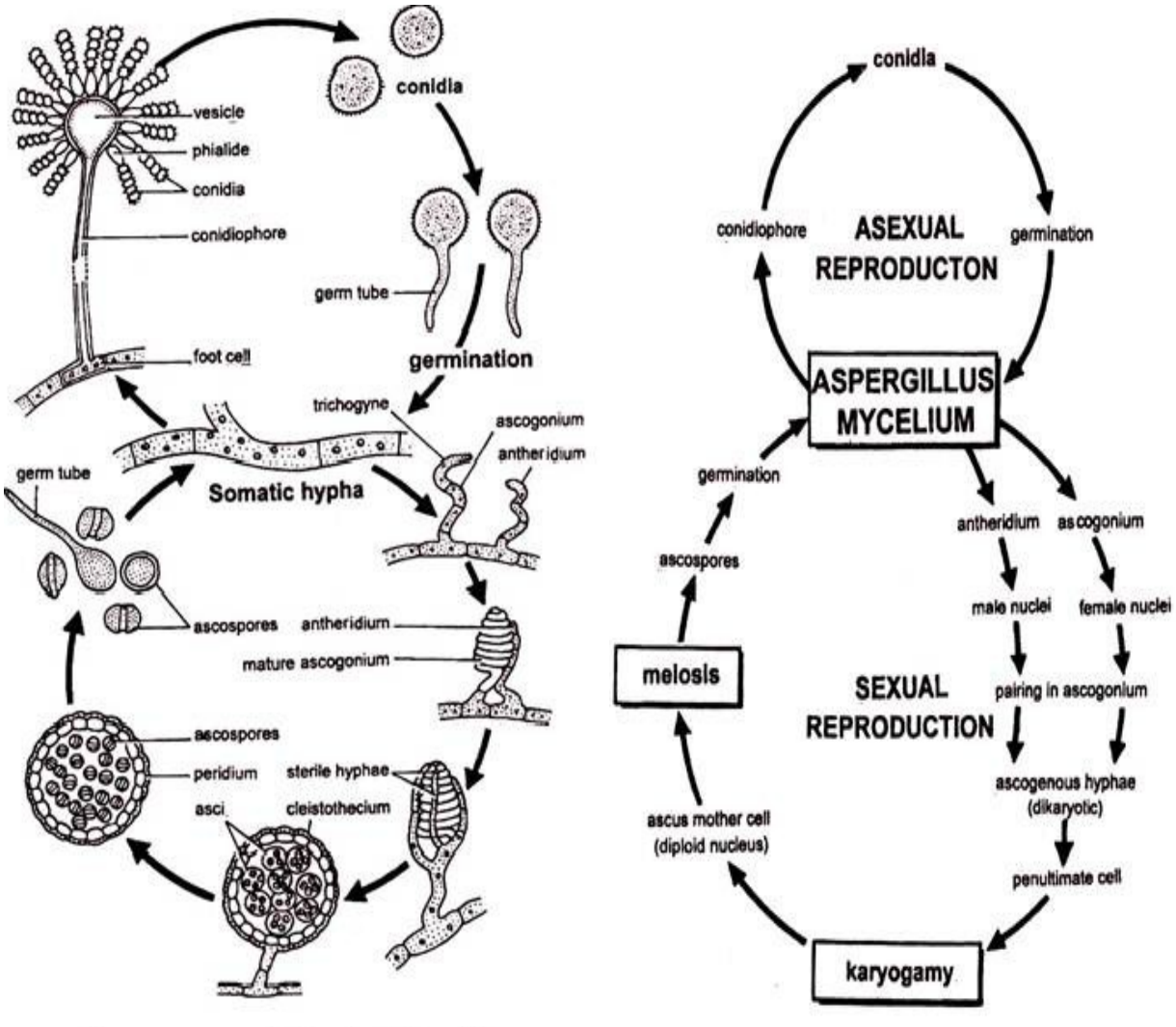


*Fig. 5.14: A. Diagrammatic Life cycle of Aspergillus B. Graphical life cycle pattern of Aspergillus*

## 5.3.4 *Penicillium*

### Systematic position

**Kingdom -** Mycota
**Division-** Eumycota
**Class -** Ascomycotina
**Sub-class-** Plactomycetes
**Order-** Aspergillales/ eurotiales
**Family-** Aspergillaceae

*Penicillium* is commonly known as blue or green mold. It is a saprophytic fungus, which is distributed all over the world. The name penicillium derives from the latin word penicillus, means artist's brush as the structure of its conidiophore resembles to that of the artist's brush. This is the most economic genus and used for produces many important products such as *P. chrysogenum* is the source of the antibiotic penicillin. *P. rocqueforti* and *P. camembertile* are used in the hydrolysis fats and for flavoring of cheese. *P. vitale* is used as a source of the enzyme glucose oxidase which is used in ferment technology. Most of the species of *Penicillium* are harmfull destroys the wood, paper and textile e.g., (*P. divaricatum*).

### Vegetative structure

The mycelium is well developed and profusely branched. It is composed of thin walled colourless, slender, tubular, branched and septate hyphae with a central pore. The hyphae extend in all directions on the substratum and become knotted with one another to form a loose network of hyphae constituting the mycelium. Some of the hyphae may even grow into the interior of the substratum to absorb food material by secrete enzyme known as haustorial hyphae and the rest grow superficially. The aerial hyphae produce reproductive structure and food material provided by the Haustorial hyphae. According to Baker (1944) in some species heterokaryotic mycelium also developed by anastomosing between hyphae of two mycelia. The mycelium in a few species may develop into a pseudoparenchymatous structure, known as sclerotia. The reserve food material is in the form of oil globules (Fig 5.15).

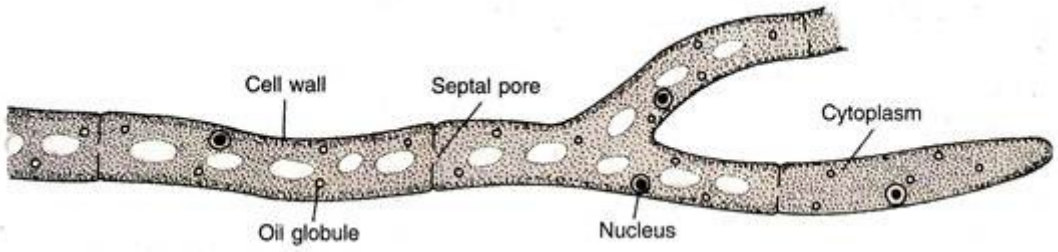


*Fig. 5.15: Vegetative structure of Penicillium*

## Reproduction

*Penicillium* reproduces by vegetative, asexual and sexual method.

### Vegetative Reproduction:

In this genus fragmentation is the most common method of vegetative reproduction. The hyphae break up into small segments. Each segment or fragment grows by repeated division into a developed mycelium.

Sclerotia a compact resting body also found in some species. Sclerotia enable the species to survive on unfavorable period. On the onset of conditions suitable for growth each sclerotium germinates to form a new mycelium. The sclerotia thus serve primarily as a means of perennation rather than multiplication.

### Asexual reproduction:

Normally it takes place by the formation of non-motile, asexual spores, known as conidia which are produced exogenously in long basipetal chains at the tips of long, erect special septate branches called the conidiophores. Unlike Aspergillus a conidiophore arises as an erect, tubular hyphal outgrowth from any cell of the mycelium. They are negatively geotropic and arise singly from any cell of the mycelium. Reaching a certain height branches arise from septate conidiophores, once or twice or even more times. These are termed as primary, secondary or tertiary branches, respectively. In *Penicillium thomii* the conidiophores are unbranched.

In the species with branched conidiophores, the ultimate branches which bear tufts of flask-shaped sterigmata or the phialides are called the metulae. The lower branches which support the metulae are called the rami(singular= ramus) but when conidiophores are unbranched sterigmata developed at the tip of the conidiophores. Baker (1944) reported that the phialides and the upper cells of the conidiophore are uninucleate. The apical portion of the conidiophore with its branches (metulae), sterigmata and chains of conidia looks like a small artist's brush. In *P. claviforme,* many conidiophores aggregated to form a compound club shaped fructification, called coremium. The conidia formed on the coremium are known as coremiospores.

### Development of Conidia

The conidia are formed within the slim tips of the flask-shaped phialides. The conidium initial is formed by the swelling of the tubular tip of the phialide. The phialide nucleus undergoes mitosis and one nucleus migrates into the swollen tip anther daughter nucleus remains in the phialide. The conidium initial protoplast is separate from the phialide protoplast by a thin perforated septum. In this way uninucleate conidium is formed. The tip of phialide below the first conidium

again elongates and swells and a second conidium is formed than a chains formed by repeating this process.

The conidia in the chain are arranged in a basipetal manner. The youngest conidium lies next to the tip of the sterigma and the oldest away from it. The basipatel arrangement of conidia serves two useful purposes. The mature conidia can be easily dispersed from the tips. Secondly it provides the proper nourishment of younger conidia which are close to the tips of the sterigmata.

As the conidial chain increases in length, the connectives between the older conidia break down resulting in the separation of mature conidia. The conidia are thus shed continuously. Being small, light and dry they are dispersed by air currents.

**Germination of Conidia**

The conidium is a tiny spore-like structure globose to ovoid in form. The pigmented spore wall is differentiated into two layers, outer exine which is thick, smooth or spiny and inner thin intine. Majority of the species of *Penicillium* are known only in the conidial stage. Some (about 20 species) are now known to produce cleistothecia. Conidial stage in *Penicillium* is more dominant than in *Aspergillus*.

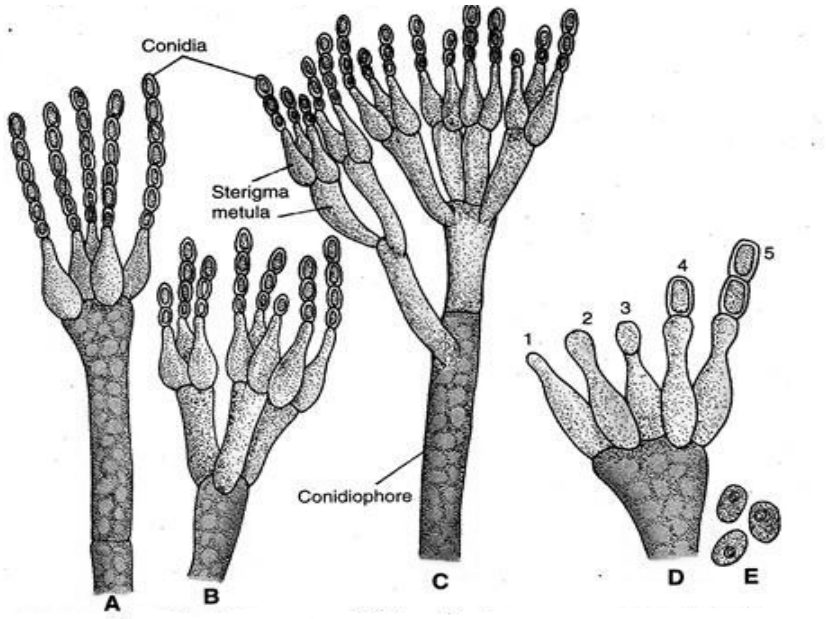


*Fig. 5.16: Different kinds of Conidiophores of Penicilliuma A. unbranched conidiophores; B-C. Branched conidiophore; D.abstriction of conidia; E. mature conidia*

At first when the connection between the two stages was not fully established the conidial or imperfect stage was given the name form or genus *Penicillium* and the sexual or perfect stage as

Talaromyces. The discovery of sexual or perfect stage in these form- species places them in the true Ascomycete genus Talaromyces.

They are dispersed by wind and germinated on suitable substratum the nucleus of the conidium divided rapidly and then a germ tube arises then all nuclei migrate on the germ tube and formation of septa take place. New mycelium is formed by this way.

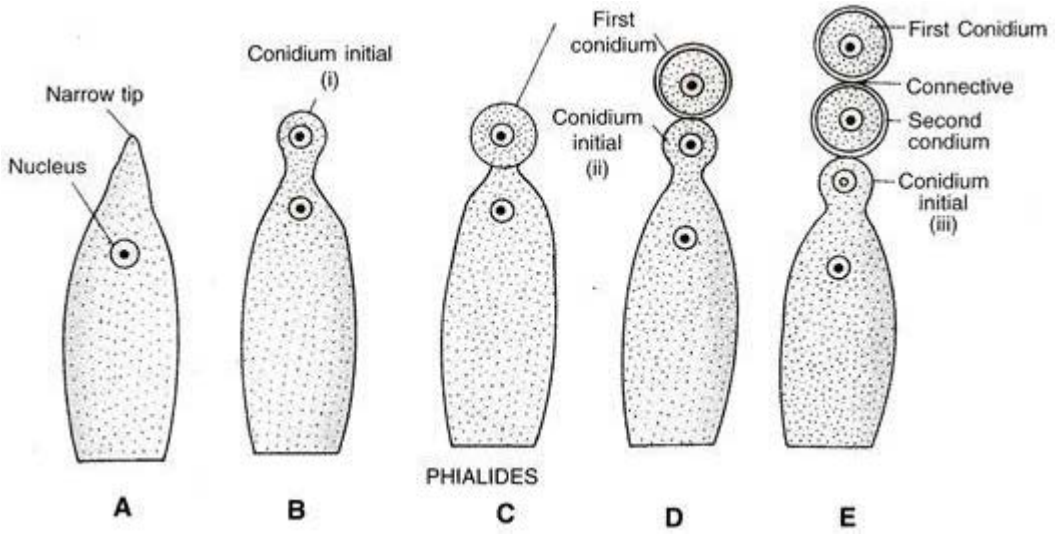


*Fig. 5.17: Development of conidia in chain*

## Sexual reproduction

It has been studied only in some species of Penicillium. They show a gradual reduction in sexuality. The female and male sex organs are functional in most of the species, but in some species *(P. vermiculatum*) even both the sex organs are present, the antheridium does not take part in sexual process. In others *(P. brefeldianum, P. stivitatum*) sex organs do not develop at all and these species show the phenomenon of somatogamy.

Sexual process in *Penicillium* has been studied in a few species such as *P. vermiculatum* (=*Talaromyces vermiculatus*), *P. glaucum, P. brefeldianum* and a few others. All of them are reported to be homothailic. Some species (*P. luteum*) are heterothallic. The male and female organs are known as antheridia and ascogonia respectively (Fig 5.18).

**(a) Ascogonium:** The mature ascogonium is a erect, long multinucleate, aseptate, tubular structure. At its upper end it may be curved like the handle of an umbrella. The vegetative mycelium produces ascogonium as a lateral outgrowth. When young the ascogonium is uninucleate. As it elongates the single nucleus within it divides and re divides to give rise to a definite number of daughter nuclei which is either 32 or 64 (Fig 5.18).

**(b) Antheridium:** After the initiation of ascogonium, an antheridial branch also develops from the same hypha. It grows up and coils spirally around the ascogonium making several turns about it. The antheridium is a short, terminal, club-shaped, uninucleate structure (Fig 5.18).

## Fertilization:

The tip of the antheridial branch touches the ascogonium and the middle wall at the point o contact dissolve. Plasmogamy thus takes place by gametangial contact. Dikaryons are established in the ascogonium. According to Dangeard, the migration of the male nucleus into the ascogonium, does not take place and antheridium plays no role. The female nuclei in the ascogonium, however, arrange themselves in pairs. This is followed as usual by the septation of the ascogonium into binucleate segments. The pairing of the female nuclei in the ascogonium is called autogamy. In some other species of *Penicillium*, both antheridium and ascogonia are claimed to be functional.

The terminal cell of ascogenous hyphae function as ascus mother cells. The two nuclei of the dikaryon present in the ascus mother cell fuse to form a diploid nucleus .After this meiotic and mitotic division takes place this result formation of eight haploid nuclei, each nucleus surrounded by cytoplasm and develops into an ascospore. The outer wall of the ascospore may be smooth echinulate or banded (Fig. 5.19).

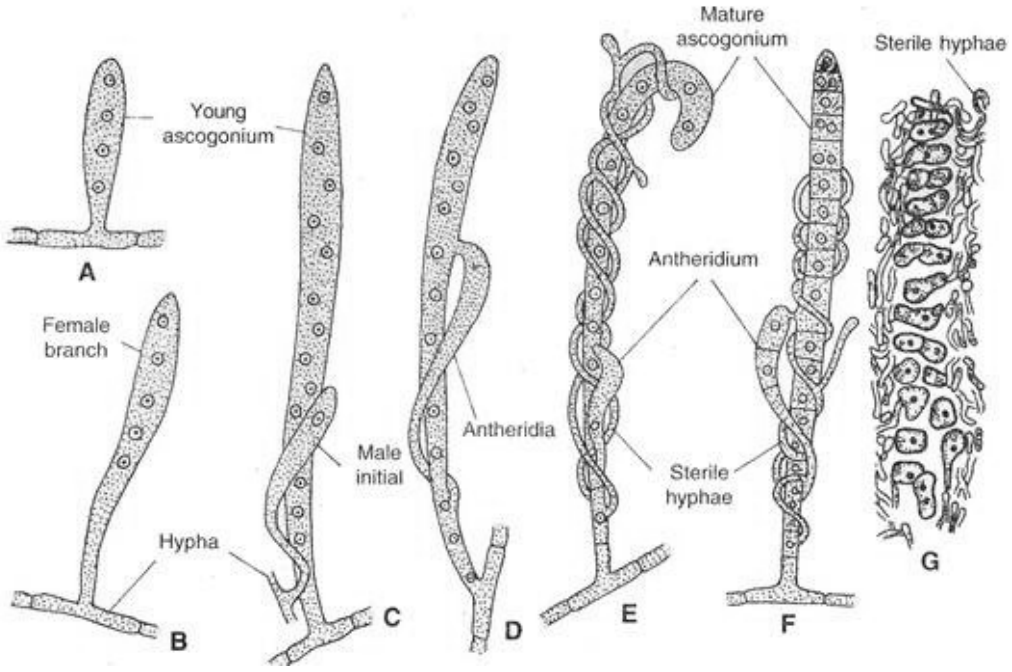


*Fig.5.18: Penicillium sp. showing the stages of sexual reproduction*

## Development of ascocarp

With simultaneous development of ascogonium, some sterile hyphae also developed around the ascogonium and form a multilayered protective covering, the peridium. The ascogenous hyphae surrounded by the peridium form a fruiting body also known as ascocarp. It is a completely closed structure of cleistothecium type. Ascospores are librated by the decay of cleistothecial wall and germinate on a suitable substratum and form new mycelia (Fig. 5.20).

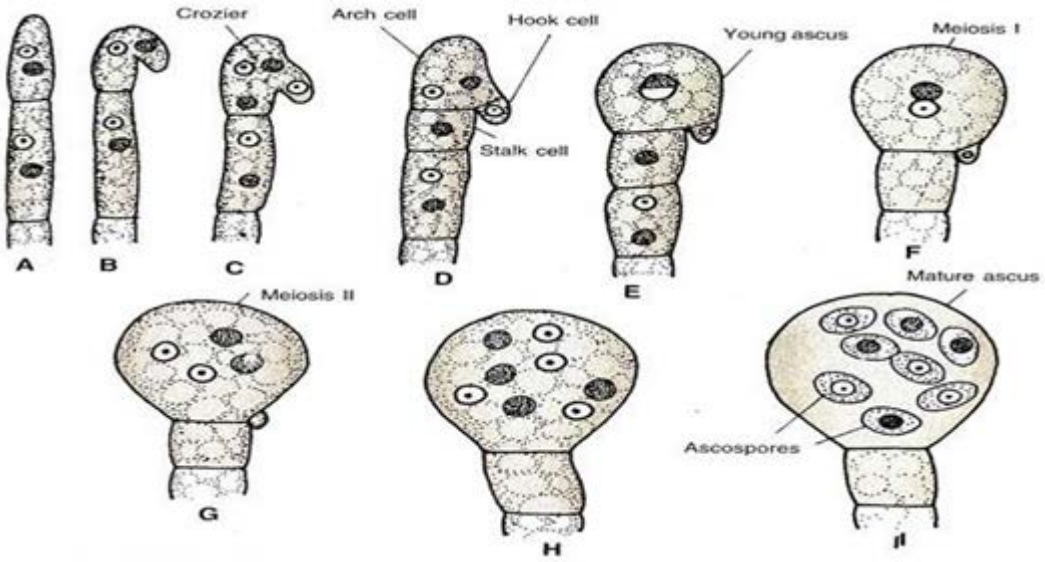


*Fig.5.19: stages of ascus development in Penicillium sp.*

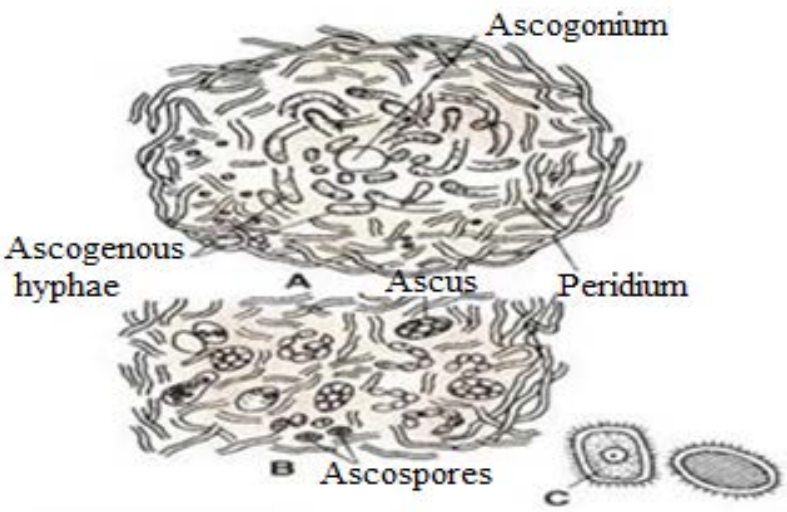


*Fig. 5.20: A. T.S of young cleistothicium B. portion of mature cleistothecium C. ascospores*

## 5.4 SUMMARY

1. *Sacchromyces* is saprophytic, unicellular, holocarpic fungus widely used in brewing and baking industries, fungus reproduce asexually by budding and fission and sexual reproduction take place by the gametangial copulation Sex organs are entirely absent.
2. *Saccharomyces* show three types of life cycle pattern, haplobiontic, diplobiontic and haplodiplobiontic.
3. Mycelial state of Taphrina is dikaryotic and parasitic to many flowering plants. Peach leaf curl is a most important disease caused by this fungus.
4. In most of the member Asexual reproduction takes place by conidia which developed in conidiophores in basipetal chains. . e.g *Aspergillus, Penicillium*
5. The members of aspergillaceae are called blue – green or black mould.
6. *Penicillium* and *Aspergillus* are seprohytic , multicellular fungus hyphae produce a well developed sex organs male sex organ known as antheridia and female sex organ is archegonia. Ascospores developed in asci which are enclosed in a cleistothicium type fruiting body.
7. Ascomycetes produce sexual spore that are called ascospores because they form within a sac known as ascus.
8. Dikaryotic phase also occur in most of the members in between the haplo and diplophase
9. The asci in some ascomycetes e.g., yeast and leaf curl fungi (*Taphrina*) are naked, in all other member of this group asci are produced, singly or in groups in fruiting bodies called ascocarp. The ascus contains eight ascospores which are release by the decay of the ascus and fruiting body.
10. Alexander Fleming in 1944 for the first time extracted the antibiotics penicillin from *Penicillium notatum.*
11. The fungus *Aspergillus* is source of many important commercial product such as citric acid, gluconic acid, gallic acid and two enzymes Invertase and zymase are obtained from *saccharomyces cervisiae.*
12. A toxic aflatoxins which produced by *Aspergillus* is carcinogenic in nature

## 5.5 GLOSSARY

**Mycelium**: It is a vegetative part of a fungus, consisting of a network of fine white filaments
**Unicellular**: In unicellular body consist of only a single cell
**Saprophytic:** An organism that lives on and gets its nourishment from dead and decaying organic material
**Parasitic**: An organism that lives on and gets its nourishment from other living organism.
**Carcinogenic**: cancer causing agents
**Conidiophores:** conidia bearing hyphae

**Homothalic:** Presence of male and female reproductive structures on the same thallus
**Heterothallic:** Male and female reproductive structure on different thallus.
***Ascogonium:*** A female reproductive body in some ascomycetous fungi
**Anthridium**: Male reproductive body
**Gametangial copulation:** Fusion of the entire contents of two contacting gametangia.
**Meiosis:** A type of cell division that results in four daughter cells each with half the number of genetic material of the parent cell.
**Mitotis:** a type of cell division that results in two daughter cells each having the same number of chromosomes as the parent nucleus.
**Ascogenous hyphae**: The ascogenous hyphae are made up of binucleate cells containing one nucleus derived from the male antheridium and the other from the female ascogonium. This condition is represented as n + n, rather than 2n, as the cells are not true diploid cells.
**Dikaryon:** The two nuclei of remain pair in a cell without fusion
**Karyogamy:** Karyogamy is the fusion of two nuclei
**Cleistothecium:** Completely closed fruiting body
**Peridium :** Wall of Cleistothecium
**Anastomosing** : it is a connection or opening between two things especially cavities or passages.
**Basipetal :** Growth downwards towards the base or point of attachment.(younger at the base and older away from it)

## *5.6 SELF ASSESSMENT QUESTIONS*

### 5.6.1 Multiple choice questions:

1. Fruiting body of *Aspergillus* commonly known as
(a) Apothecium (b) Perithecium
(c) Cleistothecium (d) Stroma

2. In ascomycotina karyogamy occur within the
(a) Ascogonium (b) Antheridium
(c) Ascus (d) Ascogenous hypha

3. In which group crozier formation takes place during reproduction
(a) Phycomycotina (b) Ascomycotina
(c) Basidiomycotina (d) Deuteromycotina

4. Ascus is not enclosed in ascocarp in-
(a) *Aspergillus* (b) *Penicillium*
(c) *Taphrina* (d) None of the other

5. Zymase enzyme produced from which of the fungus
(a) *Aspergillus* (b) *Penicillium*
(c) *Saccharomyces* (d) *Taphrina*

6. Ascomycotina commonly called as
(a) Sac fungi (b) Moral fungi
(c) Mushroom (d) Yeast

7. Artist‘s brush commonly called as
(a) *Penicillium* (b) *Aspergillus*
(c) *Yeast* (d) *Taphrina*

8. Taphrina is a responsible for-
(a) Peach leaf curl (b) Late blight
(c) Early blight (d) Wart disease

9. Plate like fruiting body is called
(a) Cleistothecium (b) Perithecium
(c) Apothecium (d) All of the above

10. In *Aspergillus* conidia are arranged in conidiophores
(a) Basipetaly (b) Acropetaly
(c) Centripetaly (d) None of the above

**Answer Key:** 1.(c), 2.(d), 3. (b), 4.(c), 5.(c), 6.(a), 7.(a), 8.(a), 9.(b), 10.(a)

## 5.6.2 Fill in the blanks:

1. The cells of aspergillus hyphae are ……..
2. The two nuclei of different strain present in a cell forms…….
3. When the daughter cell in the budding yeast cells fail to separate, they give rise to a ……
4. Peach leaf curl caused by……
5. The life cycle pattern in *saccharomyces cerevisiae* is……..

**Answer Key:** 1.Multinucleate, 2. Dikaryon, 3. Pseudomycelium, 4.Taphrina, 5.Haplodiplobiontic

## 5.6.3 True and false:

1. Higher member of ascomycotina show gradual reduction of sex organs.
2. Member of ascomycetes commonly known as sac fungi
3. *Neurospora* is known as drosophila of plant kingdom
4. *Aspergillus* is commonly known as artist’s brush

5. In *Penicillium* the conidia show acropetal arrangement

**Answer Key:** 1.True 2.True, 3.True, 4.False, 5. False

### 5.6.4 Very short answer type questions:

1. What type of ascocarp produced in green mold?
2. Which fungus produces aflatoxin?
3. Name the branch which produces conidia in *Penicillium.*
4. What is the name of female sex organ in ascomycotina?
5. What is a cleistothecium?

**Answer Key:** 1.Clestothicium, 2. Aspergillus, 3.Conidiophores, 4.Ascogonium, 5.Completely closed fruiting body

## *5.7 REFERENCES*


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- George N. Agrios, Plant pathology fifth edition, 2014 Elsevier, ISBN 978-81-312-0639-3
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## *5.8 SUGGESTED READINGS*


- Singh, Pande, Jain, fifth edition 2017-2018. A text book of Botany, fifth edition, rastogi publication, ISBN 978-935078-129-6.
- George N. Agrios, Plant pathology fifth edition, 2014 Elsevier, ISBN 978-81-312-0639-3
- A text-book of Mycology and plant pathology Author: John W. Harshberger Publisher: World Public Library Association

# *5.9 TERMINAL QUESTIONS*

## 5.9.1 Short answer type questions:

1. Several member of the Ascomycotina are believed to be losing their ability to reproduce sexually. Comment upon the statement
2. Explain the sex organs of Ascomycotina
3. Explain the life cycle pattern of *Taphrina*.
4. Comment upon the following: budding, fission.
5. Explain the conidia formation in *Aspergillus.*

## 5.9.2 Long answer type questions

1. Explain the different life cycle pattern of *Saccharomyces*
2. Write the sexual reproduction of *Aspergillus*
3. Write the life cycle of *Taphrina*
4. Write the detail about *Penicillium*
5. Elaborate the general characteristics of ascomycotina

# UNIT-6 BASIDIOMYCOTINA AND DEUTEROMYCOTINA

## Contents:

# 6.1 OBJECTIVES

After reading this unit students will be able-

- To understand the general characteristic of Basidiomycotina
- To know the life cycle pattern of *Puccinia*
- To learn about the *Ustilago*
- To understand the lifecycle of *Agaricus*
- To know the general characteristic of Deuteromycotina
- To know about the *Alternaria*
- To understand the lifecycle of *Fusarium*
- To know about *colletotrichum*

# 6.2 INTRODUCTION

Members of Basidiomycotina and Deuteromycotina considered as most advanced fungi, these groups are placed after the Ascomycotina. They are mostly parasite but some are saprophytes. As we have read features about Ascomycotina which produce ascus and ascospores in their developmental stages, basidiomycotina show many different types of charterers and produce basidiospores. Unlike ascomycotina in this group there is lack of specialized sex organs. Dikaryon stage is a characteristic of Basidiomycota is also found in sexually reproducing Ascomycota but in basidiomycota it is long lived. In the Deutromycotina sexual reproduction is completely absent; fungi propagate only by the asexual reproduction through conidia formation.

The two parasitic taxa of basidiomycotina such as *Puccinia* and *Ustilago* are responsible for destruction of cereals and other grasses and causes enormous economic losses. Common name of some saprophytic members of this group are mushrooms, puff balls, toad stools, pore fungi, stinkhorn, bird's nest fungi, bracket fungi, etc. The most conspicuous and familiar Basidiomycota are mushrooms which are edible and a rich source of protein. Member of Deuteromycotina are mostly the facultative parasite cause many disease such as early blight, tikka disease, wilt in plants and athlelete's foot, several skin disease and pulmonary infection in animal.

# 6.3 BASIDIOMYCOTINA

## Vegetative structure

Basidiomycotina is represented by about 550 genera and 25,000 species. Members of basidiomycota are unicellular or multicellular, mycelium is well developed, branched and

septate. Septa may be simple or dolipore. Some features are unique in this group and make it peculiar from another group of fungi. The most indicative feature is the production of **basidia** (sing. basidium), on which sexual spores are produced, and from which the group takes its name. The another characteristic feature of this group is the presence of the long-lived **dikaryon**, in which each cell in the thallus contains two haploid nuclei and the **clamp connection** is also a unique feature of Basidiomycota it is a kind of hyphal outgrowth (Fig.6.1).
In the life cycle of these fungi, the mycelium is found in two or three different stages.

1. **Primary mycelium:** It is a monokaryotic and formed by the germination of basidiospores. It has a haploid nucleus in each cell.

2. **Secondary mycelium:** It is a dikaryotic and dikaryotization is formed by the fusion of two cells of primary mycelia having different stains.

3. **Tertiary mycelium:** It is also a dikaryotic mycelium and form a specialized fruiting body known as basidiocarp.

## Reproduction

Members of basidiomycotina reproduce by vegetative asexual and sexual methods. Vegetative reproduction takes place by fragmation and budding asexual reproduction takes place by oidia, conidia or chlamydospore. Higher taxa of this group lack asexual reproduction. Conjugation of two nuclei of two different strains is method of sexual reproduction and no specialized sex organs develop in this group.

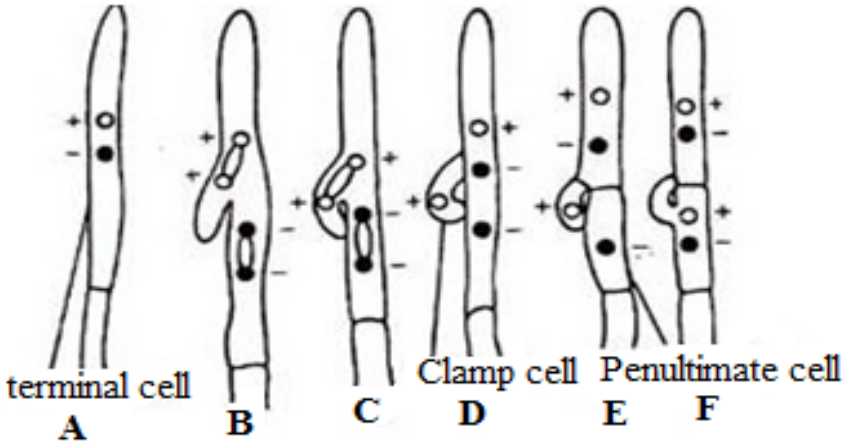



*Fig. 6.1 (A-F): Various stage of clamp connection*

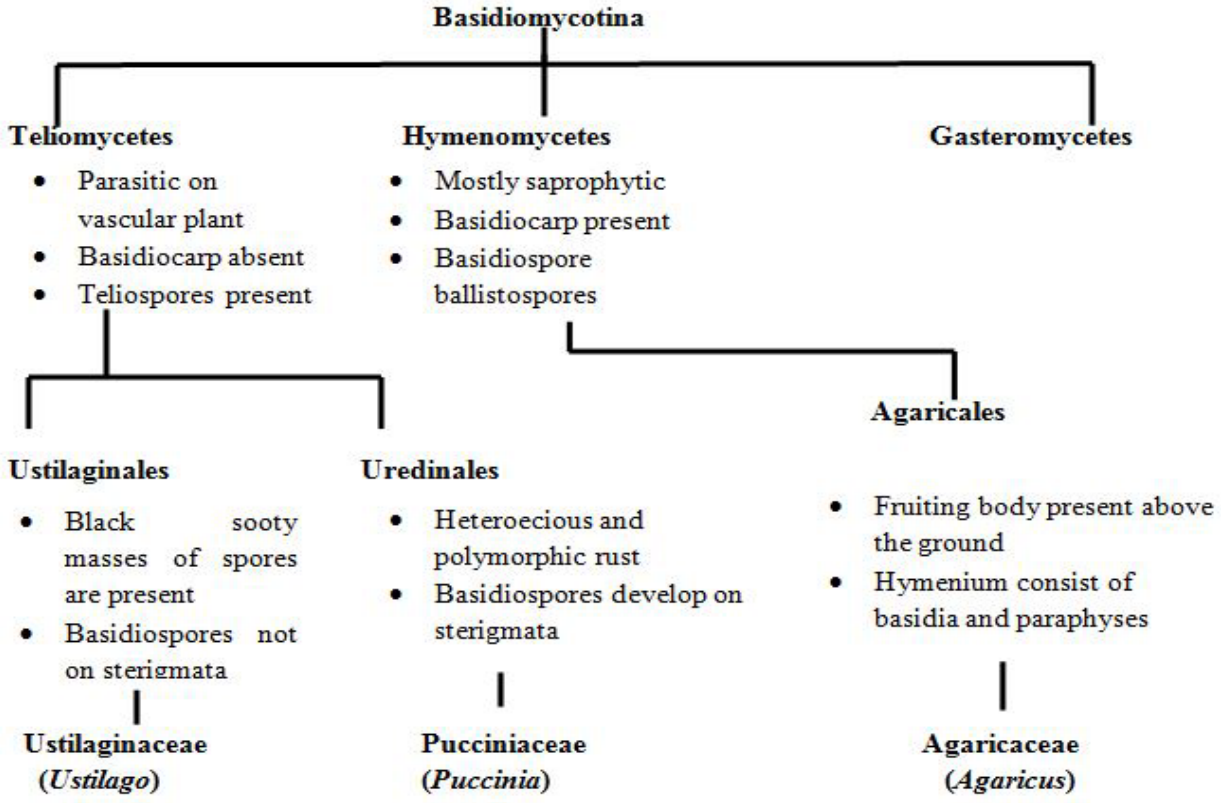
The **basidium** is the cell in which karyogamy (nuclear fusion) and meiosis occur, karyogamy occur after some times of the plasmogamy not immediately and this result in the formation of diploid synkaryon. The diplophase in short lived. After the meiosis haploid basidiospores are

formed exogenously on the basidia. Typically, four spores are produced on each basidium, at the tips of minute stalks called sterigmata. Basidia are not produced by asexual reproduction of basidiomycota. Certain higher taxa of Basidiomycota contain multicellular fruiting bodies known as basidiocarp on which basidia are produce (e.g., mushrooms), but basidia can also be formed directly from a single cells. Generally the basidia are of two types:

**(a) Holobasidium**- aseptate cylindrical structure which produces four sterigmata at its distal end a spore formed at the tip of each sterigma.

**(b) Phragmobasidium**- a transverse or longitudinal septa produce in the basidium. A lateral sterigma is present in each cell which produces a single haploid basidiospore

Dikaryotic secondary mycelium grow by producing clamp connections, this is the unique feature of basidiomycotina. **Clamp connections** are hyphal outgrowths that form when cells in dikaryotic hyphae divide. The cell division is usually confined to apical cell. At the time of division, a short pouch like backwardly directed lateral outgrowth arises from the cell known as clamp connection. Out of two nuclei of the dikaryotic cell one migrate in clamp other remain within cell, Septa are formed across each of the mitotic spindles. The apex of the backward-growing monokaryotic clamp cell fuses with the sub apical cell, reestablishing the dikaryotic condition (Fig.6.1). Anisworth (1973) divided basidiomycotina into teliomycetes hymenomycetes and gasteromycetes.



## 6.3.1 *Puccinia*

Kingdom : Mycota
Division : Eumycotina
Subdivision: Basidiomycetes
Class : Teliomycetes
Order : Uredinales
Family : Pucciniaceae
Genus : ***Puccinia***

*Puccinia* is also known as rust fungus because of rusty appearance of its spores on host surface. It is a large genus of about 1800 species and distributed in all parts of the world. In India the genus is represented by more than 147 species, which causes serious rust diseases in cereal crops like wheat barley oats and maize. The species of *Puccinia* are internal obligate parasite and only spores are seen on the host surface.

Species of puccinia either autoecious i.e., they complete their life cycle on a single host (*P.asparagi, P.butleri*,) or heteroecious i.e., they complete their life cycle in two different host (e.g., *P.graminis, P. coronata*).

In India wheat crop is infected by the following three species of *puccinia* which cause considerable destruction of crop:

- *P. graminis* – black rust or stem rust
- *P. striformis (=P.glumarum)* yellow rust or strip rust
- *P. recondita (= P. tritici)* brown rust or leaf rust

## *Puccinia graminis*

*P. graminis* is an internal obligate parasite, macro cyclic, polymorphic and heteroecious fungus. It affects wide range of hosts including wheat, and other cereals. This disease is also known as stem rust disease. *P. graminis tritici* involves in its life cycle two distinct alternate host plants i.e., wheat (*Triticum vulgare* fam. Poaceae) and Barberry (*Berberis vulgaris* fam. Berberidaceae) so it is a heteroecious. Its life cycle is completed only when both hosts are present but it can survive in absence of alternate host.

The two types of mycelia dikaryotic and monokaryotic occur in the life cycle of this fungus. The dikaryotic mycelium or secondary hyphae (each cell of the mycelium bears two nuclei) on primary host (wheat) and monokaryotic mycelium also called haplophase or primary hyphae (each cell of the mycelium bears only one nucleus) on the secondary or alternate host (Barbery). The monokaryotic mycelium is also called haplomycelium or primary hyphae and the dikaryotic

mycelium is called secondary hyphae. Mycelium form spherical haustoria to absorb the nourishment from the host cell

## Life cycle

*Puccinia* is polymorphic fungus as in the life cycle of *Puccinia* five types of spores are produced which are uredospores, teleutospores, basidiospores, pycnidiospores and aceiospores. These spores developed in different host in a definite sequence. uredospores and teleutospores in primary host, pycniospores and aeciospores stage produced in alternate host and basidiospore stage develop in between the primary and secondary host stage on soil.

### 1-Uredospore stage

The dikaryotic mycelium is formed by the infection of the aeciospores on germination on wheat plant brought from the infected barberry plants or by the uredospore's themselves coming from the neighbouring infected wheat plants.

On reaching the stomata a vesicle like structure known as appresorium developed from the swollen tip of germ tube. The protoplasm of the germ tube migrates into the appressorium. Septa develop between the germ tube and apprisorium. Narrow hyphal threads develop from the apprisorium and enter inside the sub-stomatal chamber through stoma. The dikaryotic mycelium develops in intercellular space in the tissue of leaf, stem and glumes. Many dikaryotic hyphae near sub epidermal region grow erect at right angle to the surface and its tip develops a binucleated uredospore. These spores developed in a group and called uredosori. Uredospore produce reddish brown pustules or streaks on the host surface and these symptoms usually appear in late spring. Uredospores are golden brown binucleate, stalked unicellular and oval ellipsoidal in structure and they are double walled possess four equatorially arranged germ pores. Uredospore can spread disease on re-infect wheat plant. It cannot infect the alternate host (Fig. 6.2.).

### Germination of Uredospores

After coming contact with new wheat plant it germinate within few hours by one or two germ tubes and produce a dikaryotic mycelium from the appressorium. Within 10-12 days of germination mycelium is capable of producing again uredospores. In favourable condition it can cause successive reinfection and spread the disease from field to field.

### 2-Teleutospore stage

At the end of the wheat season, the uredosori also start to produce another kind of spores called teleutospore, in addition to uredospores. As the crop matures ultimately uredosori convert into teleutosori and exclusively produce teletospores. The teleutospores are dark brown or black in

colour, bicelled and spindle shaped structure constricted slightly at saptum. This stage in also called black stage, teleutosori appear as black raised streaks along leaf and stem hence the name black rust is given to the disease. Teleutospore is binucleate structure as the spore mature the two nuclei in a cell fuse a diploid nucleus covered with thick and smooth spore wall. Teleutospore exposed by rupturing the host epidermis. It acts as resting spores and may survive in most unfavourable periods. (Fig. 6.3)

Teleutospore cannot infect wheat plant. They do not require any host for their germination. Under favourable condition of low temperature and high atmospheric humidity they germinate in soil.

## 3-Basidiospore stage

Teleutospore germinate to a germ tube form each cell has a limited growth known as promycelium or epibasidium. After migration of diploid nuclei into the promycelium the meiotic division takes place and thus four haploid nuclei (two are of + strains and two are of – strains) are formed. Septa are developed between the nuclei and promycelium divided it into four transverse chambers.

Each haploid cell of the promycelium produces a slender, short, lateral, tube-like structure known as sterigma. The end swollen part of sterigma forms a spore like cell. The haploid nucleus from each promycelium cell migrates into this developing spore cell through its respective sterigma. Thus, at the tip of each sterigma, a minute spore is formed which is known as basidiospores two are + strains and two are – strains.

Basidiospore is a small, unicellular, thin walled haploid structure they are disseminated by wind and can only germinate on the leaves of alternate host the barberry bushes. Basidiospores can survive only for few days. They perish soon if the alternate host is not available (Fig. 6.4).

## 4-Pycniospore stage

On coming favourable condition basidiospore germinate on the Barberis leaves. A germ tube of the each spore penetrates through the leaf epidermis and grows and produces monokaryotic mycelium of + and – strain in the intercellular spaces of the host tissue. Strains of mycelia depend on the strain of the basidiospore (Fig. 6.7).

On upper and lower epidermis haplomycelium aggregate and forms dense mats. The mycelial mats beneath the upper epidermis are called as primordium of spermogonium while the mats beneath the lower epidermis are known as primordium of aecidium or protoaecidium.

After 7 to 10 days of infection a small flask shaped structure called pycinidium or spermogonium developed from primordium of spermogonia. The pycnidia emerge as minute yellowish specks

on the upper surface of the leaf. The pycinidium opens on the upper surface of the leaf by a minute pore called ostiole. Its wall is lined by 3 types of structure which are follows-

**(i) Periphysis:** The ostiole is surrounded at the periphery by the long, delicate, tapering orange coloured sterile hyphae known as periphysis. They develop near the ostiole from the pycinidial wall and project from the ostiole.

**(ii) Flexuous or Receptive Hyphae:** They also occur from the lateral wall of the spermogonium. They are slender, delicate, cylindrical, septate, simple, branched or un-branched with blunt ends. They are much project beyond the periphysis and sometimes it is difficult to distinguish between the two.

**(iii) Spermatiophores or Pycnidiophores:** The wall of the pycinidium is also lined internally with a palisade – like layer .These are slender, short, vertical, uninucleate hyphae which arise from the base of the spermognium. By the abstraction method at its tip of each spermatiophore (or sporophore) produces several small uninucleate spermatia or pycnidiospores. The spermatia are unicellular, small, oval to spherical, hyaline and smooth walled may be + and - strains structures. They fill the spermogonial cavity and are exuded from the ostiole in a droplet of sticky, sweet liquid. The spermatia neither infect primary host nor secondary host they function as male cell while receptive or flexuous hyphae represent the female sex organs. Their sexual nature depend upon the mycelium, produced by the basidiospores '+' or '-'. The insects are attracted by this sticky liquid.

The spermatia are dispersed from one spermogonium to another spermogonium on the same leaf or another leaf. As a result, the spermatia of one strain are transferred to receptive hyphae of opposite strain this process is known as spermatization. Dikaryotic mycelium formed as a result of spermatization. In which intervening wall at the one stain of contact between these two dissolves and the spermatium nucleus passes downwards through septal pores and form a bi-nucleate cell. This pair of nuclei of opposite strains is called a dikaryon and this process is called dikaryotization. During the course of spermogonial formation some hyphae of each mating type form protoaecidium on reaching the lower surface of leaf if there is no spermatization than their growth become prevents but if there is spermatization the protoaecidium develops into an aecidium (Fig. 6.7).

## 5-Aeciospores stage

On the lower surface of barberry leaf a cup shaped structure developed known as aecidia. They developed from the same mycelium which forms pycnidia on the upper surface. All the cells of primary mycelium are dikaryotized by successive mitotic division of male nucleus and pass through the septal pore. The dikaryotic basal cells of the protoaecidium arrange themselves vertically beneath the lower epidermis and are called as sporophores or aecidiophore. On the side

towards the lower epidermis each bi-nucleate basal cell then cuts off a chain of bi-nucleate cells in basipetal succession (Fig. 6.7).

These cells are the aecidiospore mother cells. These cells further divide transversely to form a large cell and a small cell. The large cell develops into aecidiospore while the small cell remains sterile and is known as disjunctor or intercalary cell. Intercalary cell dissolves and sets free the aeciospores. Simultaneously, with the development of aeciospores the peripheral cells of aecidium divide to form a thick protective covering known as peridium. The aeciospores are liberated by rupturing the wall of peridium on exerting a pressure on it. They are unicellular, polyhedral, thin walled, bi-nucleate and orange yellow coloured.

They are disseminated by wind. They are capable of immediate germination but cannot infect barberry plants. Falling on suitable host germ tube is produced. The further development of the germ tube the dikaryotic mycelium is produced. This is the mycelium which produces the uredospore's and later the teleutospores on wheat. In this way, the life cycle of *Puccinia graminis* is completed.

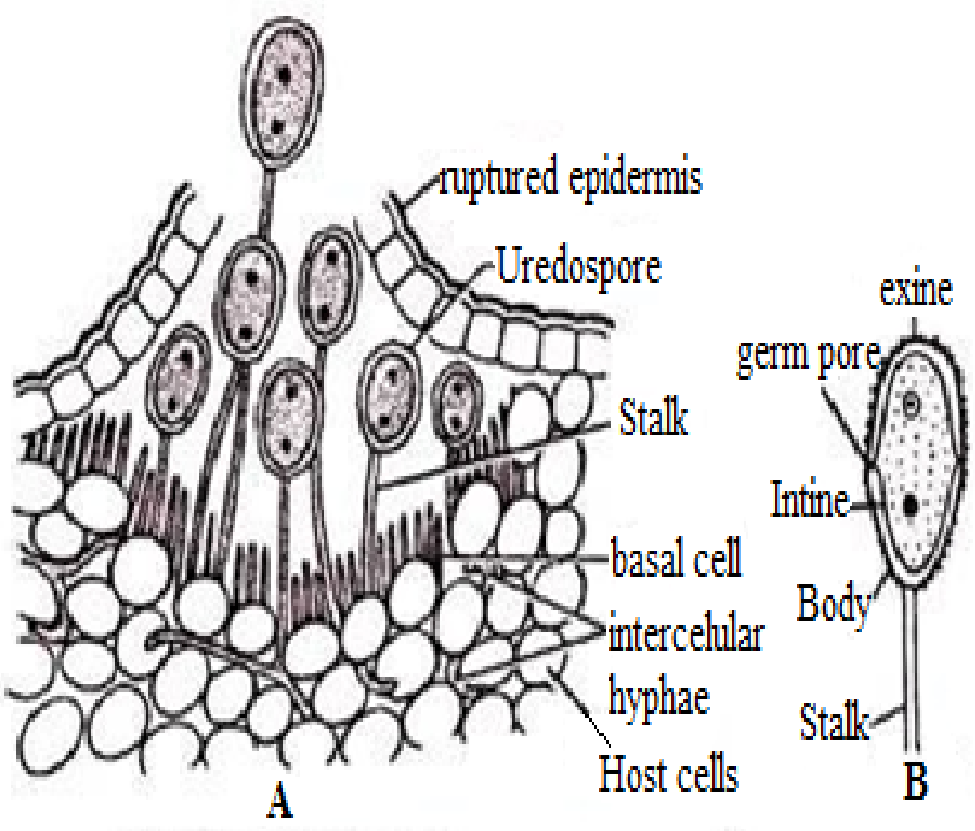



*Fig. 6.2: Puccinia graminis: A. T.S of wheat leaf showing uredosorus; B. A uredospore*

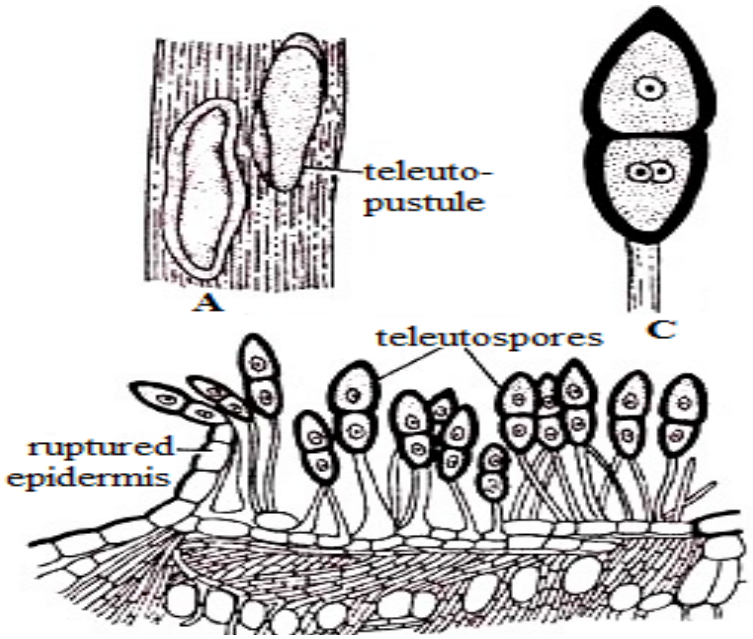



*Fig. 6.3: P. graminis (A-C), A. Teleutopustule on wheat, B. T.S of wheat leaf showing teleutosorus, C. A single teleutospore*

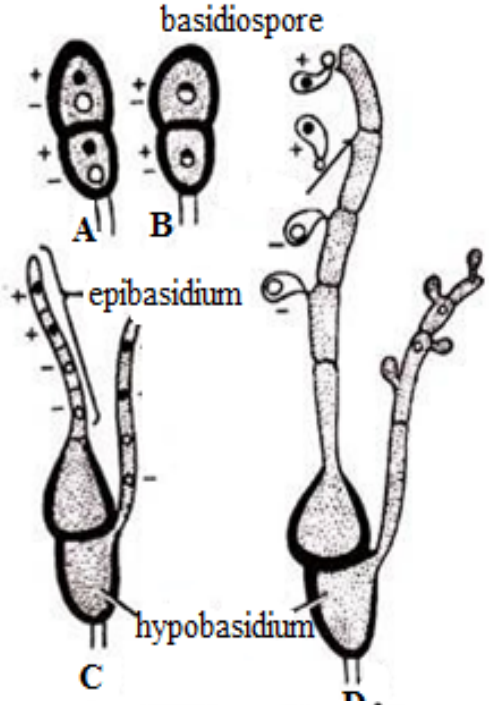



*Fig. 6.4: P. graminis: A. a young teleutospore; B. mature teleutospore, C. Germinating Teleutospore; D. Basidial stage*

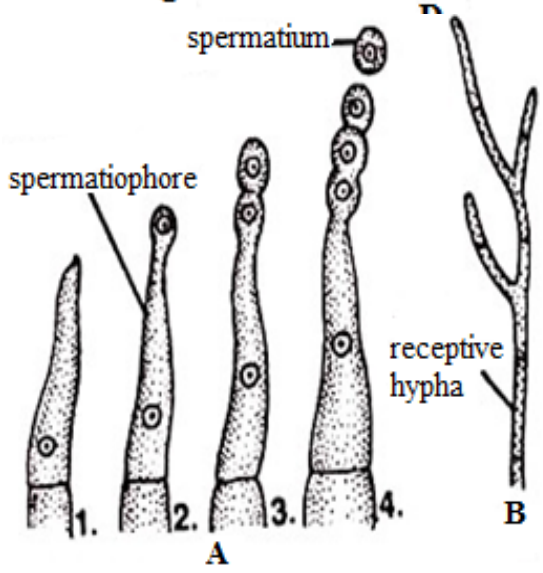



*Fig. 6.5: P. graminis A (1-4) development of spermatium; B. receptive hypha*

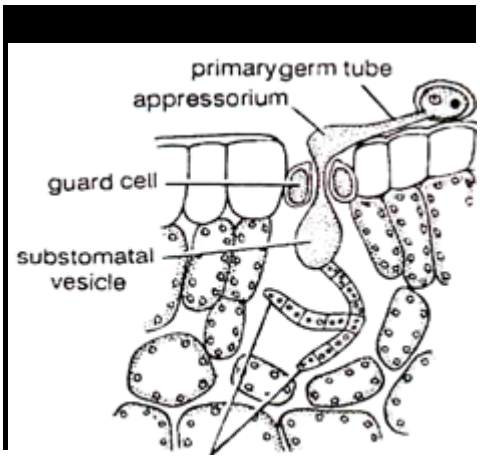



*Fig. 6.6: P. graminis: germinating of uredospore on wheat leaf*

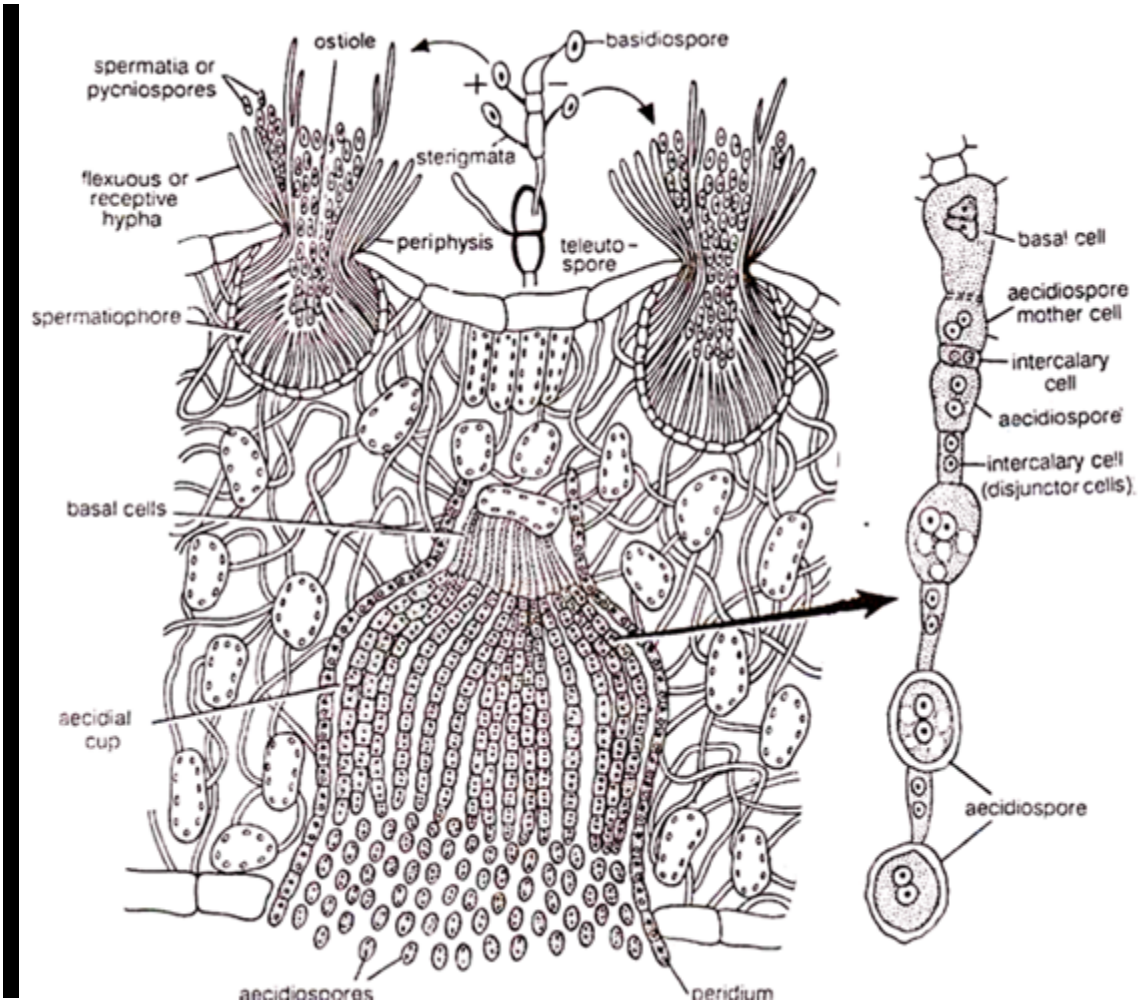



*Fig. 6.7: P. graminis: T.S of barberry showing pycnial and aecial cup*

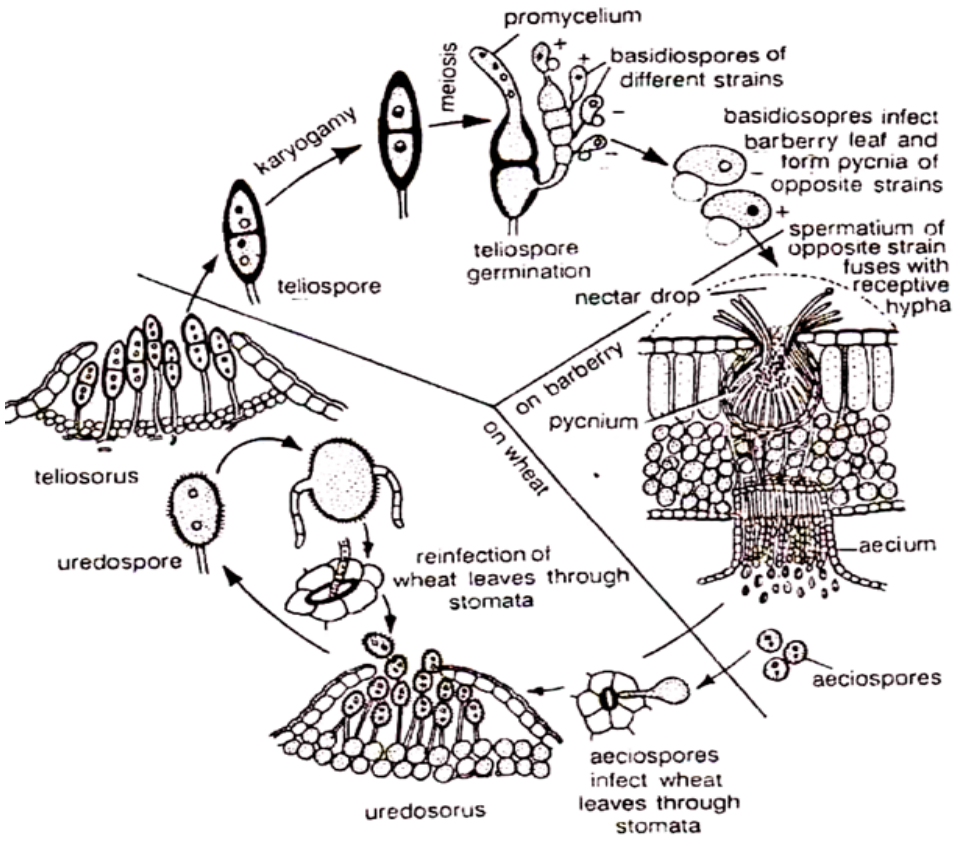



*Fig. 6.8: Diagrammatic life cycle of Puccinia graminis*

## 6.3.2 *Ustilago* (Smut Fungi)

Kingdom : Mycota
Division : Eumycotina
Sub division : Basidiomycotina
Class : Teliomycetes
Order : Ustilagoniales
Family : Ustilaginaceae
Genus : ***Ustilago***

Ustilago is a smut fungus of family ustilaginaceae, the name Ustilago has been derived from a Latin word smut meaning 'burnt' because the members of the genus produce black, sooty powdery mass of spores on the host plant parts imparting them a 'burnt' appearance. This black dusty mass of spores resembles soot or smut, therefore, commonly it is also known as smut fungus. All species of this genus are parasite. This is a autoecious parasite i.e. complete their life cycle in only one host. Most common hosts are the member of cereal plant such as corn, oat, barley, wheat, millet, bajra, and ray. Economically the smut fungi are very destructive genus causing loss of millions of rupees worth annually in our country.

## Symptoms of smut

The symptoms appear only on the floral parts. The floral spikes turn black and remain filled with the smut spores. Smut diseases are of two types- Loose smuts and covered smuts. In the loose smut black sooty mass of spores is exposed at the flowering time and spores can easily blow away by wind which carries them to a healthy inflorescence of host species. In covered smut the spore masses are not exposed remain covered by the wall of the grain and the glumes and the spores are liberated only by the breaking of wall during thrashing (Fig. 6.9).

Some common smut diseases are following:

- Loose smut of oat caused by *U. avenae*
- Loose smut of barley caused by *U. nuda*
- Loose smut of wheat caused by *U. nuda* var. *tritici.*
- Loose smut of doob grass caused by *U. cynodontis*
- Covered smut of Barley caused by *U. hordei*.
- Covered smut of oat caused by *U. kolleri*.
- Whip smut of sugarcane caused by *U. scitaminae*
- Gall smut of corn caused by *U.maydis*

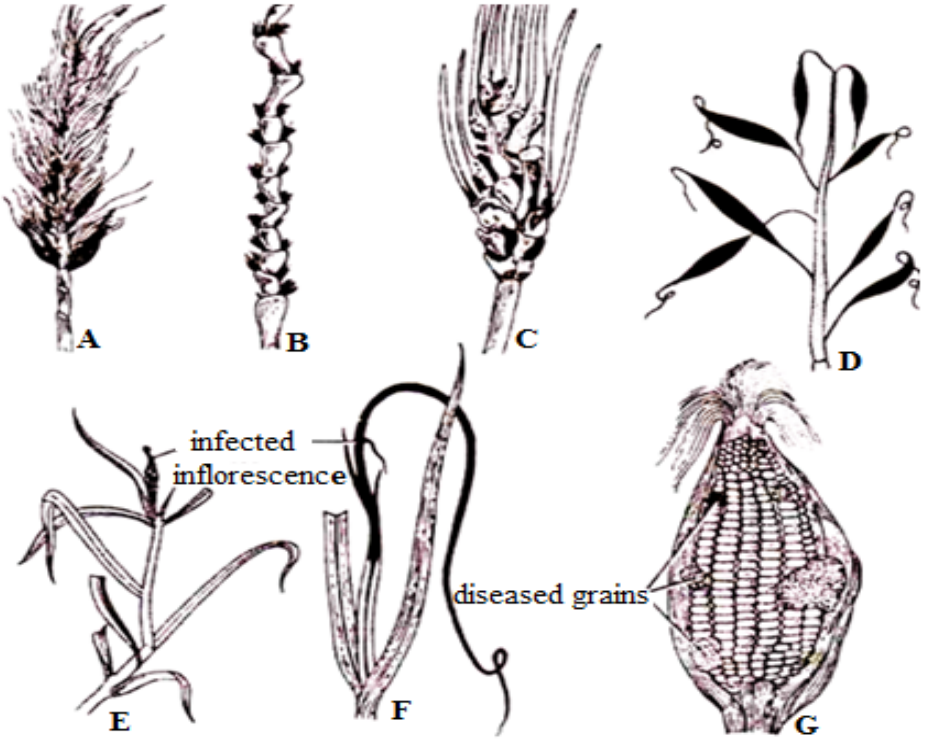



*Fig. 6.9: Ustilago symptoms A. loose smut of wheat caused by U. nuda* var. *tritici., B. bare rachis, C. U. hordei on barley, D. U. kolleri on oat, E. Loose smut of doob grass, F. Whip smut of sugarcane , G. gall smut by U. maydis on maize*

## Vegetative Structure of *Ustilago*

The mycelium is branched, septate, hyaline and intercellular, with or without haustoria. Mycelium of Ustilago passes through two distinct stages of development. These are the primary and secondary mycelia.

**(i) Primary Mycelium:** It is hyaline slender septate hyphae with sinlge haploid nucleus and also called monokaryotic or haplomycelium mycelium (uninucleate) and formed by the germination of basidiospores. It is of very short duration

**(ii) Secondary Mycelium:** It is formed by the dikaryotisation of the primary mycelium and consists of two haploid nuclei in each cell. It extends particularly through the entire life. In fact the secondary mycelium constitutes the most conspicuous and important part of the somatic or vegetative phase of the majority of species of Ustilago. In many species intercellular hyphae may develop distinct haustoria which penetrate the host cell in *U. maydis* mycelium is intracellular the growth of mycelium on host tissue cause little or no effect on vegetative stage of cell.

In most smuts the mycelium is scattered throughout the various parts of the host. It is said to be systemic. However, in some smuts (corn smut) it remains confined to certain parts of the host and is called localized.

## Dikaryotisation of mycelium

The process of dikaryotization initiated by the pairing of two haploid nuclei of opposite strain of a species by this the primary mycelium converted into secondary mycelium. The two nuclei of opposite strain do not fuse in vegetative phase and constitute the dikaryon. By the further growth dikaryotic cell develop a dikaryotic mycelium. The various methods of dikarotization in Ustilago are detailed below (Fig. 6.10):

**1-By fusion between primary hypha (somatogamy)-** This type of dikaryotization occurs in *Ustilago maydis*. On germination of basidiospore it forms primary mycelium on the host surface. This mycelium enters the intercellular space by penetrating the host epidermis. Somatogamy occur between the two opposite strain of hyphae subsequent migration of nuclei into the fusion cells initiate the dikaryotic phase. The dikaryotic cell divides several times by the process of clamp connection and forms secondary mycelium.

**2-Fusion between the germ tubes of two basidiospores**- at the time of basidiospore germination the germ tube of the basidiospores of opposite strains make contact with each other and dissolve the intervening wall at the point of contact. Nucleus of one germ tube migrate into other become binucleate this act establish dikaryophase. The germ tube grows into secondary mycelium. This type of dikaryotization is common in *U. hordei.*

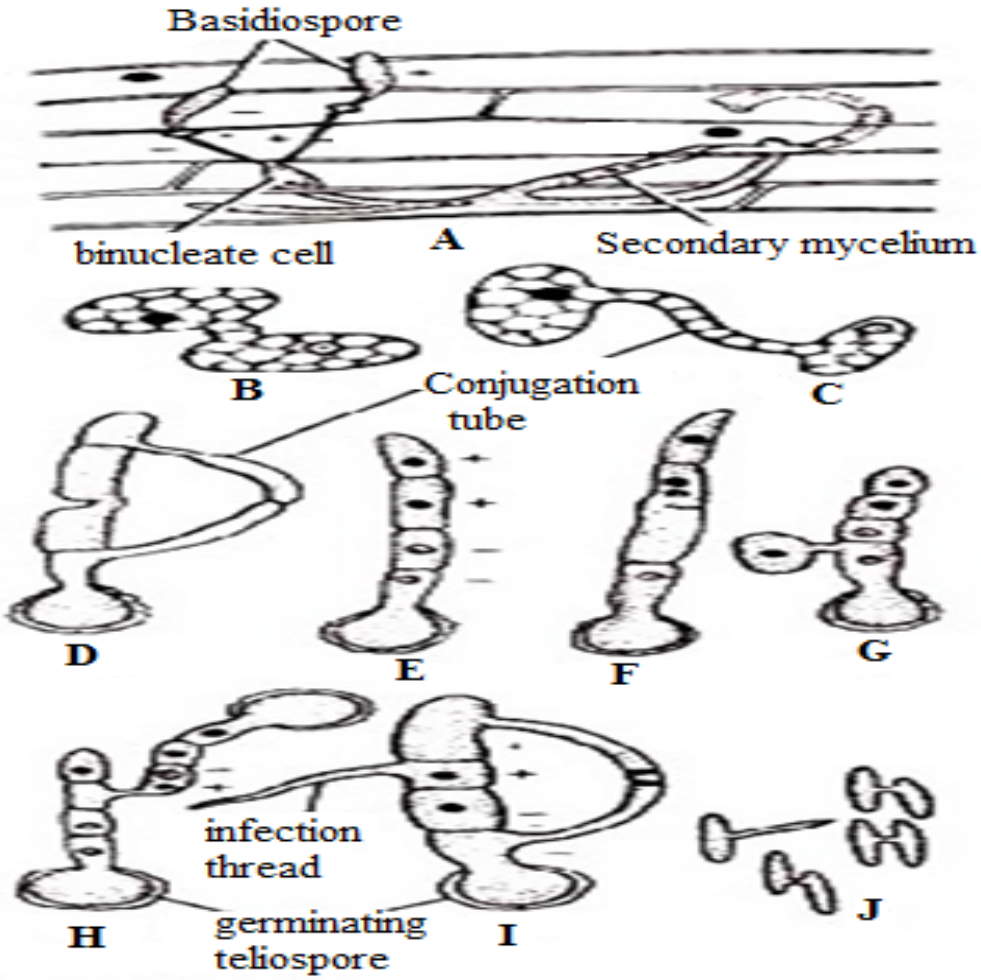



*Fig. 6.10: Various stage of dikaryotization*

**3-By conjugation between the basidiospores –** in some species *(U. violacea* and *U. recepticularum*) the basidiospores multiply by budding to produce secondary spores (sporidia).the common wall between them dissolve at the point of contact or send copulation tube toward each other. The nucleus of one migrates into the other through the connecting link thus dikaryotic spore is formed by the fusion of two sporidia of different strains. Secondary mycelium produces by the germination of dikaryotic spore

**4-By the union of infection threads** -in *U.tritici* the basidium is septate and does not bear basidiospore. Each haploid cell of the basidium produces a minute tubular filament these are called infection threads. Two adjacent infection threads of different strain fuse to form a dikaryotic hypha, which divides to form a dikaryotic mycelium.

**5-By fusion between the two haploid cells of the epibasidium** – in this *(U. hordei, U. carbo*) case fusion takes place between two haploid cells of opposite strains of the same basidium.

## Reproduction in Ustilago

(1) Asexual Reproduction
(2) Sexual Reproduction

**(1) Asexual Reproduction:** It takes place by fragmentation, budding of basidiospores and formation of conidia. However, it is of rare occurrence.

**(2) Sexual Reproduction:** *Ustilago* is autoecious i.e., it completes its life cycle on a single host. Sex organs are completely absent. The three fundamental process plasmogamy, karyogamy and meiosis take places by the fusion of two haploid compatible cells. It produces two kinds of spores during its life cycle i.e., Teliospores or teleutospores and basidiospores.

**(a) Teleutospores:** Teleutospores are produced by the cells of the secondary mycelium (dikaryotic mycelium). These spores are also known as chlamydospores, smut spores or bi-nucleate brand spores. At the flowering time of the host secondary mycelium becomes active and forms a dense mass of hyphae within the host tissues. It is composed of numerous short dikaryotic cells (Fig. 6.2 A-C). These spores are formed in masses known as sori that may developed in various places on the host including flower part such as ovaries and stamen or in a seed. The binucleated protoplasm of each segment cell round off and functions as spore initial. At this stage, the protoplast secretes a thick wall around itself and gelatinized. It results in the formation of a smut spores (Fig. 6.2 D). By the time the spores reach towards maturity, the gelatinous matter disappears and the spores are separated from each other. Each smut spore is bi-nucleate globose, yellow to brown with spiny, reticulate or smooth wall. The outer exine or exosporium is thick it smooth reticulate or spiny the inner intine or endosporium is always thin. The spores are closely appressed in to a hard compact mass called a smut ball or sorus .these are generally resting spores remain dormant under adverse condition. In *U. tritici* the smut spores do not function as resting spores they serve as means of propagating the disease during the growing season.

**(b) Basidiospore:** The smut spores are disseminated by wind may fall on the soil, on the grain and other favorable places. On getting suitable conditions they germinate and infect the healthy plant. Before germinating the two nuclei (one of + strain other of the – strain) of the binucleate teletospore fuse to form a dipoid nucleus called as synkaryon. The thick walled smut spore spore with a synkaryon represents the encysted probasidium or hypobasidium stage. During germination it swells up by absorbing water. The exine burusts and the intine comes out in the form of a short cylindrical hypha called the promycilium it is also called the epibasidium or metabasidium at this stage the diploid nucleus of the teletospore undergo a reduction division resultant four haploid nuclei in the epibasidium. During meiosis two of these nuclei are of plus strain and two of minus strain. Septa are laid down between the four haploid nuclei thus each cell of the basidium has a single haploid nucleus. Latter each haploid nucleus divided mitotically into two daughter nuclei one of these nucleus remains in the basidial cell and other passed into the

developing bud which arise laterally from each epibasidial cell. The haploid uninucleate cell separate as basidiospores also called sporidia.

The remaining nucleus in the epibasidium may undergo repeated division thus each cell of the basidium may form numerous basidiospores (sporidia) the basidiospores of *Ustilago* are not borne on sterigmata consequently the sessile basidiospores of smut are discharged. The basidiospores are uninucleated and thin walled globose or oval structure. They are dispersed by wind may fall on the soil or the host plant

In some species such as *U. maydis* the basidiospore are capable of multiplying by budding like the yeast cell and these new spores are called secondary spores or conidia

In *U. tritici* the basidiospores are lacking. The haploid cells of epibasidium produce short slender infection threads which infect the healthy wheat plant.

## Germination of basidiospores

The haploid basidiospore produce a fine haploid germ tube also called infection tube. Species of *Ustilago* show different modes of infection. In covered smut the teletospore are usually present inside the grain and when such seeds are sown the spore germinate and produce mycelium inside the host tissue such mode of infection is known as seedling infection. In loose smut the infection occurs during flowering when the mycelium enters the young tissue at the base of the ovary, secondary infection may also be caused by chlamydospore or teletospores disseminated by wind. This mode of infection is known as blossom infection

**Control of disease** - Smut disease can be controlled by the crop rotation, seed disinfection and by growing seed of resistant or immune varieties.

**Table No. 1: Compression between rust and smut**

| S.No. | Rust | Smut |
|---|---|---|
| 1. | Rust are autoceious and heteroceious. | Smut are always autoecious. |
| 2. | Three type of spore uredospore teleutospores, aeciospores are formed from the secondary mycelium. | Only one type of spore chlamydospores is formed from the dikaryotic mycelium. |
| 3. | Spores may be unicellular(uredo and aeciospores or bicellular (teleutospores) and stalked or sessile. | Chlamydospores are unicellular and sessile. |
| 4. | Spores are usually formed at the apex of the hyphae. | Chlamydospores are intercalary. |

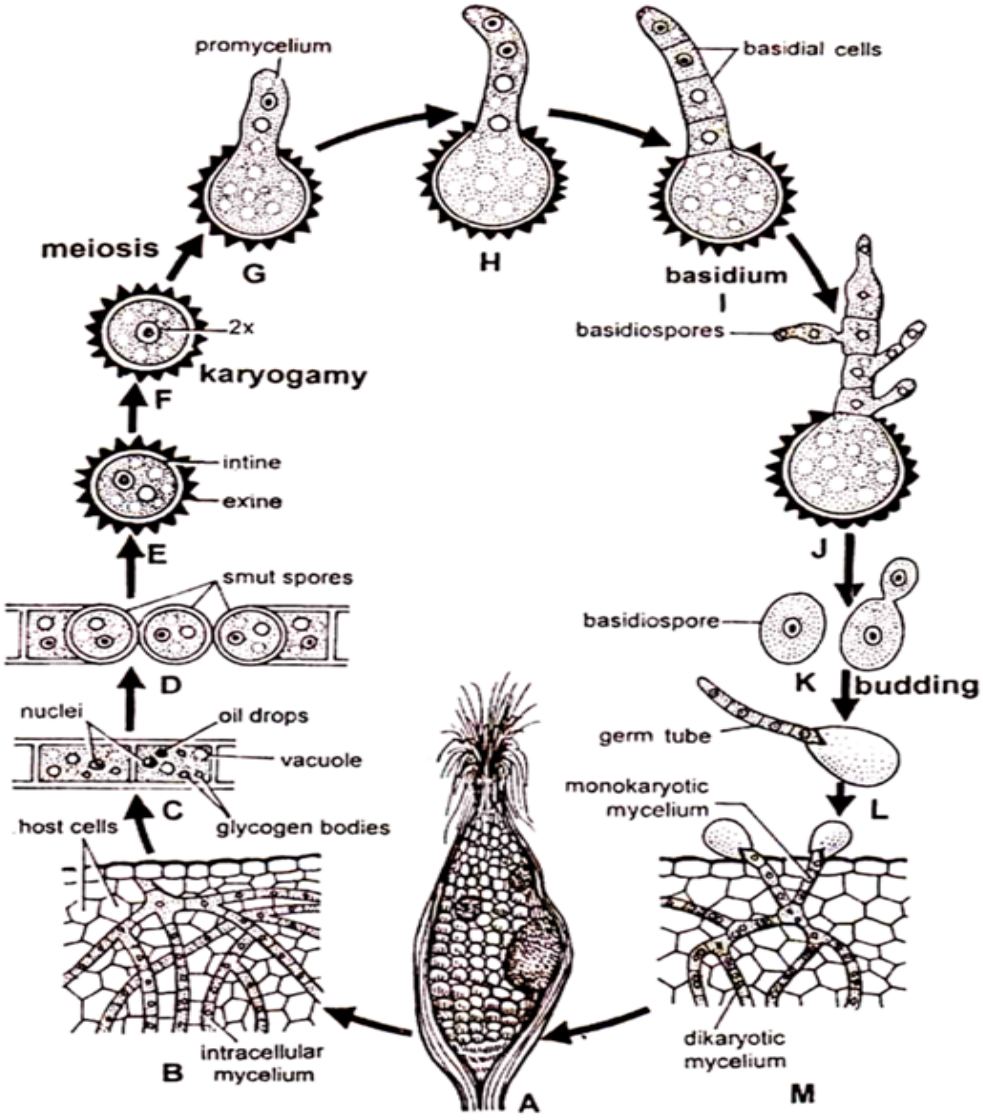



*Fig. 6.11: Diagrammatical life cycle of Ustilago*

## 6.3.3 *Agaricus*

**Systematic position**

Kingdom - Mycota
Division - Eumycotina
Sub division - Basidiomyccotina
Class - Hymenomycetes
Order - Agaricales
Family - Agaricaceae
Genus - ***Agaricus***

## Habit and habitat

*Agaricus* is commonly known as mushroom. It is saprophytic fungi which occur on dead decaying material, in open filed, grass land, and in soil in cellulose and lignin materials. It is an edible gilled fungus which is cosmopolitan in its distribution and grows best in moist shade condition. It is commonly seen in rainy season. There are about 17 species of this genus in India which are known by different names in different parts of country such as kukurmutta, khumb, dhingri. *A. campestris* (the field mushroom and *A.bisporus (=A. brunnescens*) the cultivated mushroom are common edible mushroom commonly cultivated in various part of India. Solan in Himanchal Pradesh is an important centre of mushroom cultivation. *A. silvaticus* and *A. xanthodermus* are highly poisonous species.

## Structure

The structure of *Agaricus* can be studied under the following two heads.

**(i) Vegetative mycelium**

**(ii) Fruiting body (basidiocarp)**

**(i) Vegetative mycelium**- The vegetative mycelium remains underground and is of two types. The primary mycelium arise from a germination of homokaryotic basidiospore, it is septate hyline and monokaryotic it may be + or – strain depending upon strain of basidiospores. The cells contain oil globules vacuoles and are short lived and soon get transformed into secondary mycelium by the fusion of primary mycelium of opposite strains the secondary mycelium is binucleate, septate, perennial, branched ,abounded and long lived. The secondary mycelium of the fungus may or may not possess clamp connections. It contains dolipore septa in which the opening is guarded by both sides with its parenthesomes. The hyphae of the secondary mycelium twist together to form thick white hyphae cords called the rhizomorphs which bear the fruting bodies. The dikaryotic mycelium shows centrifugal growth i.e., hyphae grow outward from a centre in a circle. A circular colony of hyphae is thus formed in the soil at maturity hyphae develop fruiting body in the tip in a more or less circular rings. This process is repeated with further growth of the hyphae so that widening circles of basidiocarp are seen. Thus successive crops of fruiting bodies are produced in larger and larger ring. Based on the ancient belief that they marked the path of dancing fairies, these are called fairy rings (Fig. 6.12).

**(ii) Fruiting body** (basidiocarp) which we ordinarily call the mushroom is not the whole fungus it is simply the fruitification technically called basidiocarp. It is the aerial part of *Agaricus* and develops from the rhizomorph. It is differentiated into a stipe and a umbrella shaped cup, the pileus. The stipe is about 6-9 cm in height thick flashy, cylindrical structure and the pileus is 5 to 10 cm in diameter. There are about 300-600 radial arranged gills on the undersurface of the pileus (Fig. 6.14).

## Reproduction

**1-Vegetative reproduction:** The edible mushrooms are propagated by vegetative reproduction. A small piece of dikaryotic mycelium used as inoculums.

**2-Asexual reproduction**: Mushrooms multiply by the formation of chlamydospore and conidia. The chlamydospore germinate in the terminal or intercalary position on the secondary mycelium, they germinate and produce secondary mycelium. In some species oidia are also formed but these are involved mainly in dikaryotization rather than developing new mycelium.

**3-Sexual reproduction:** Majority of the species are heterothallic *(A. bisporus*) but sex organs are completely lacking. Primary Mycelium is formed by the germination of basidiospores. Compatible nuclei are brought together by fusion of hyphae of opposite strain (somatogamy) or the interaction of oidia with hyphae resulted in the formation of secondary mycelium. The secondary mycelium later develops fruiting body known as basidiocarps.

Somatogamy between two primary hypha of opposite strains take place through the following steps-

**(a) Plasmogamy-** Two vegetative hyphae from mycelium of opposite strains or the same mycelium come in contact with each other, wall dissolve at the point of touching and the dikaryon is formed. A dikaryotic mycelium develops by successive division of the dikaryotic cell. At the time of division the two nuclei of the dikaryotic cell divides conjugately into four daughter nuclei two of (+) strain and two (-) strains. This cell develops a clamp connection which ensures that sister nuclei separate into two daughter cells. This process is repeated several times. The dikaryotic mycelium is perennial and subterranean at the suitable temperature and moisture when the mycelium has absorbed and accumulated abundant food supply it bear fructification called the basidiocarps.

**(b) Karyogamy**- In this step the fusion of the two nuclei of the dikaryon takes place and the formation of diploid nucleus, it is considerably delayed and occurs in the young basidium.

**(c) Meiosis-** After the karyogamy meiosis takes place in basidium thus haploid basidiospores are formed.

## Basidiocarp

### Development of basidiocarp

The basidiocarp develops as tiny white apical swellings on the branches of the underground mycelia strands which are known as rhizomorphs. These hyphae knot enlarge gradually and give rise to button stage of the basidiocarp .at this stage the developing basidiocarp is differentiated into a basal bulbous part and upper hemispherical region. The basal part forms the stipe and the

apical hemispherical part the pileus. Some hyphae at the junction of the stipe and pileus are drawn apart and form a ring like chamber, called prelamellar chamber. The inner surface of the roof of the pre- lamellar chamber become extremely concave and it is lined with alternating radial bands of dividing cells. It form gill premordia which develop gill lamellae that hang downward into prelamellar chamber. Margins of pileus and stipe connected by a membrane called velum or inner veil. The buttons are raised on the soil surface due to the elongation of stalk. The upper region of the button grows more rapidly than the stalk due to this velum rupture and the upper hemispherical region finally expands out as an open umbrella–like structure with numerous gills attached to its lower surface. At this stage piece of velum are still attached to the stipe in the form of ring called annulus.

Button do not grow and remain underground in the dry season but in rainy season when soil is moist, they grow rapidly and come out of the soil surface so in rainy season basidiocarp can be seen frequently (Fig. 6.14).

## Structure of mature basidiocarp

The mature basidiocarp is an umbrella shaped structure with a long massive stipe and a broad pileus. The stipe is a thick fleshy cylindrical structure pinkish white in color. It is usually broader and swollen at the base and centrally attached to the pileus. The upper convex surface of the pileus is white light brown yellow in colour. From under side of pileus hang numerous thin vertical strips or plates of tissue, the gills or lamellae. All gills are not of the same length they may be of full half or quarter length. The surface of the gill is enveloped by a fertile layer the hymenium or thecium. Gills turn brown to purplish black on maturity.

## Internal structure of basidiocarp

Basidiocarp is made up of pseudoparenchymatous mass of interlinking tertiary hyphae

**Stipe-** the stipe is composed of numerous longitudinally runs interwined hyphae. In the peripheral region hyphae compactly arranged and form the pseudoparenchymatous mass of tissue. Whereas they are loosely arranged with large intercellular spaces in the central region called medulla.

**Pileus-** They are also differentiating into cortex and medulla as stipe. Stipe extends right up to it. The hyphae of the stipe region extend into extend into the pileus and fan outwards. Some of these pass down into the gills.

## Internal structure of gills

The gill exhibits a complex structure. It is a sheet of interwoven hyphae which are more closely compacted and denser in this region. In a transverse section of gill following three regions can be distinguished

**Trama**-This is the central sterile region. It is made up of extension of the hyphae of the pileus.

Hypae of this region are irregularly interwoven and more or less longitudinally.

**Sub hymenium or hypothecium-** the hyphae constituting the trama give off short lateral branches which form sub hymenium layer. Cell of these branches are more compactly arranged, isodiametric and 2-3 nucleated. The hypothecium is situated on both side of the trama. These are also the sterile zone like the trama.

**Hymenium or thecium-** This is the outer most fertile layer of the gills and composed of hyphae of the sub hymenium layer. It consist of a closely packed palisade like layer of club shaped cells they are called the basidia. Basidia are the terminal elongation cells of the same hyphae which constitute the trama and the subhymenium .These are the aseptate spore producing body. Each basidium produces four basidiospores on apical end. In between the basidia there are presences of sterile more cylindrical hyphae known as paraphyses or cystidia.

The young basidium has a dikaryon and as the basidium mature the two nuclei fuse to form a diploid nucleus. In the mature basidium synkaryon undergo meiosis to form four haploid nuclei two of + strain and two – strain. At the distal end of the basidium four pegs like outgrowth are formed. These outgrowths are known as sterigmata. The tip of each sterigmata swells to become a basidiospore initials which are small and bead like in appearance. At the junction of sterigmata and basidiospore there is presence of eccentrically placed minute projection known as hilar appendices, due to presence of this basidiospore is slightly oblique placed on the sterigma. Young basidiospores are unpigmented but towards maturity the spore wall turns pinkish purple in colour.

**Germination of basidiospore-** on maturity of basidiospore a drop of liquid appears at the hilar appendics which remains surrounded by a limiting membrane. The drop increase in size gradually and attains a size of about one – fifth of the spore. Then the basidiospore is suddenly shot away from the sterigma. The four basidiospores of a basidium are dispersed. On falling on suitable substratum, it germinates by producing a germ tube that grows into a primary monokaryotic mycelium. Depending on the strain of the basidiospore the mycelium may be + or – strain, after the somatogamy they become secondary mycelium.

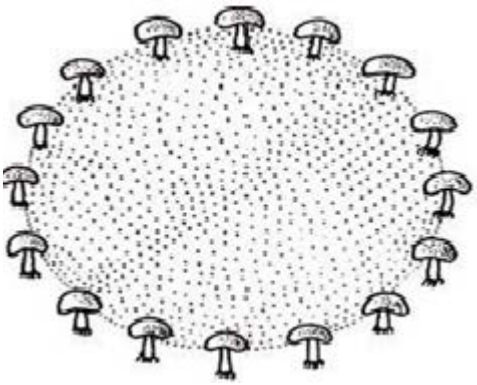


*Fig.6.12. Agaricus fairy rings*

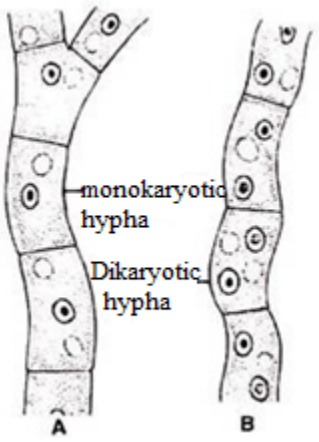



*Fig: 6.13. Agaricus mycelium A. Monokaryotic B. dikaryotic*

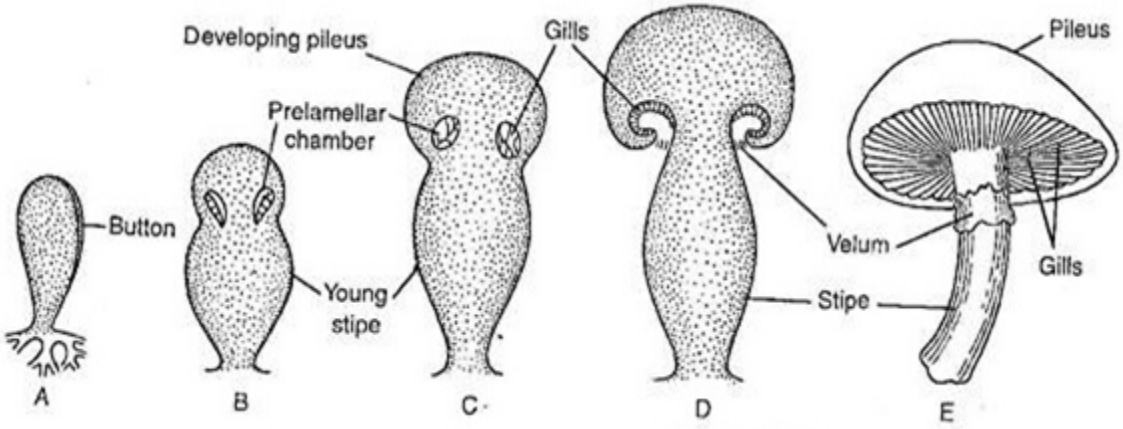



*Fig. 6.14: Agaricus development of basidiocarp*

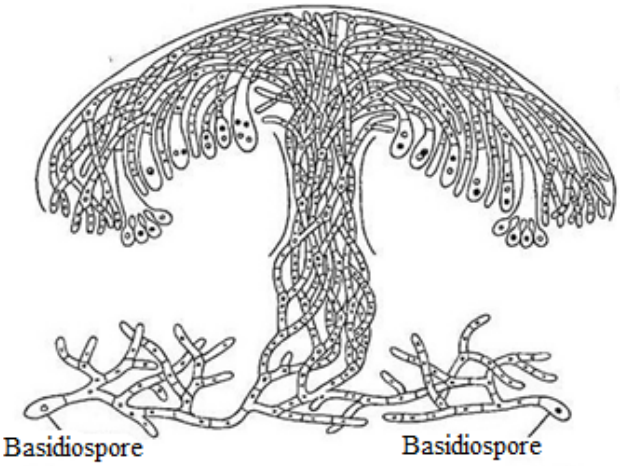



*Fig. 6.15: Agaricus, basidiocarp development by dikaryotic mycelia*

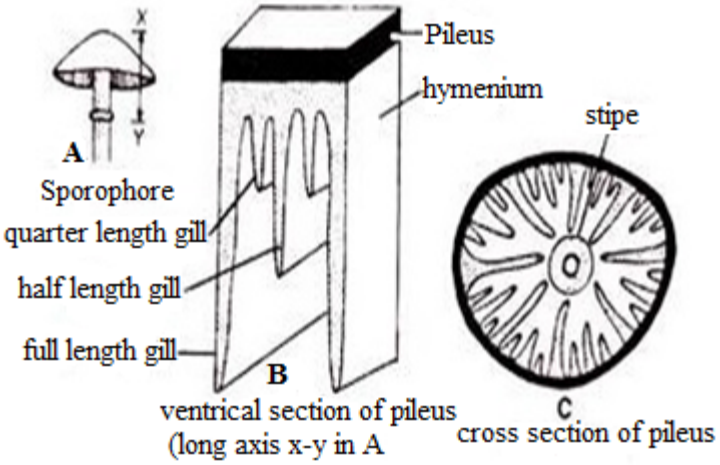



*Fig. 6.16: Agaricus (A, B) vertical section of pileus, C. Cross section of pileus*

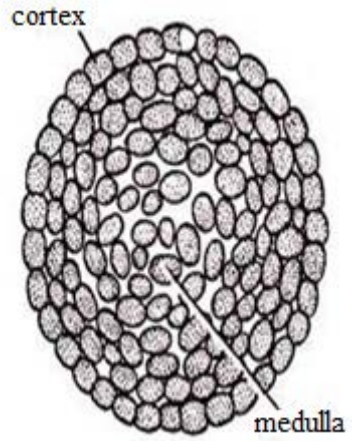



*Fig. 6.17: Agaricus: T.S of stipe*

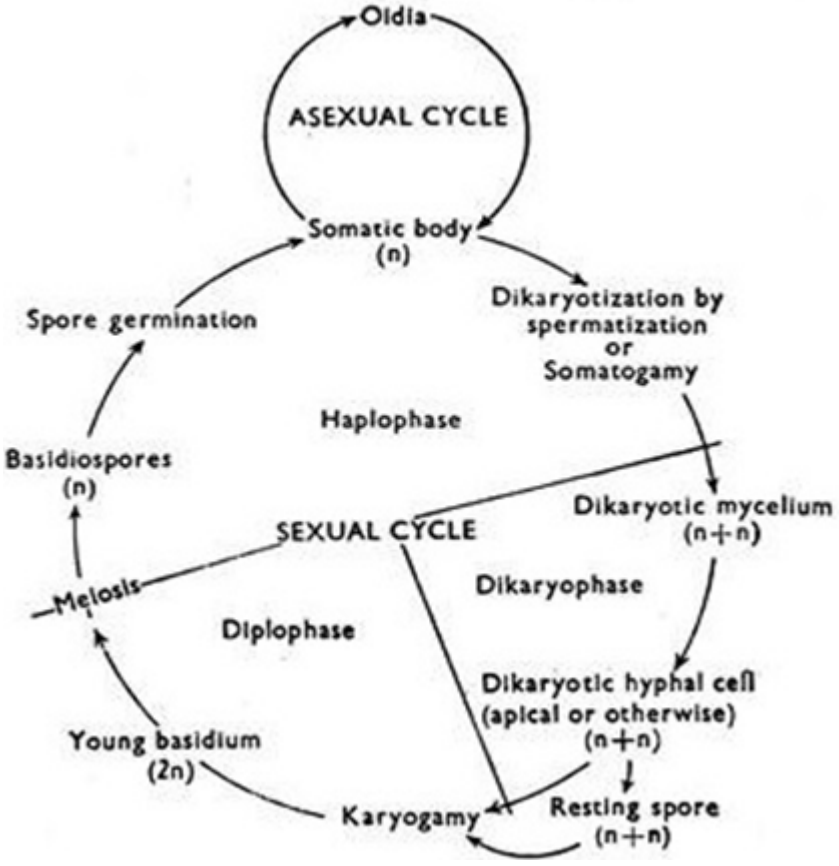



*Fig. 6.18: Graphical life cycle of Agaricus*

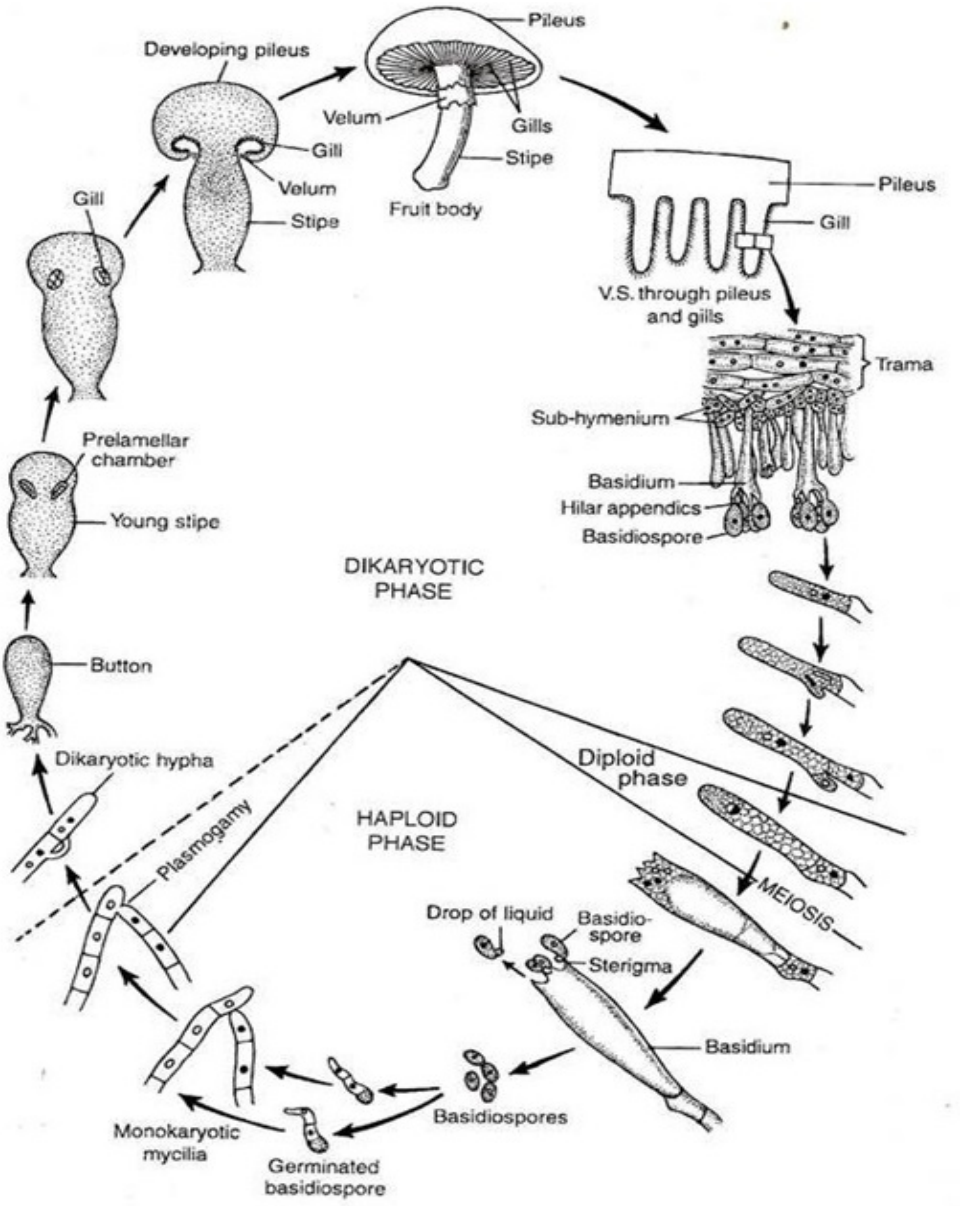



*Fig. 6.19: Diagrammatic life cycle of Agaricus*

## *6.4 DEUTEROMYCOTINA*

This is a special group of fungi in which only asexual stage or imperfect stage is known. The sexual stage also called perfect stage is not yet known. So member of this group also called as fungi imperfecti.

Many of them live as saprophyte and many more as parasite which are the causative agent of disease in plants and animals. These fungi have two names- one on the basis of asexual reproductive structure and other on the basis of sexual stage. Several members who were previously included in deuteromycotina due to absence of sexual stages, later transferred to an

appropriate group when their perfect stages were recorded in nature or in artificial culture media. Thus the sub- division deuteromycotina is a purely artificial and temporary fungal species.

This sub division included about 600 genera and over 20,000 species. The somatic phase in the majority of these fungi consists only of the haploid mycelium with multinucleated cells. Hyphae may be inter or intracellular, It is septate and branched and the reproduction takes place only by the formation of exogeneously developed asexual spores mainly by non motile spore the conidia, oidia and chlamydospores has also been reported.

Conidiophores either free or are formed in some special types of fruiting bodies such as synnemata, acervuli, sporodochia or pycnidia. Some members show parasexuality.

**Classification-** This is the artificial system of classification and mainly based on conidial characters. Various taxa are known as form- order, form- family, and form- genus.

Deuteromycotina divided into the following form orders

**Form- order moniliales-** conidia are usually born freely on the mycelium or on conidiophores free conidiophores are usually free or sometimes they are in groups or form sporodochium. It included both saprophytes and parasites.

**Melanoconiales**- They reproduce by conidia are produced in chain or singly in conidiophores packed closely to form an asexual fructification called an acervulus. It includes plant parasites.

**Sphaeropsidales**- conidia are formed in flask shaped pycnidia or modified form of it. It includes plant parasites.

**Mycelia sterilia-** In these imperfect fungi special reproductive stages including the conidia are totally lacking. They reproduce by sclerotia, rhizomorphs and other vegetative means.

## 6.4.1 Form Genus- *Fusarium*

### Systematic position

Kingdom : Mycota
Division : Eumycotina
Sub division : Deuteromycotina
Form- Class : Hyphomycetes
Form – Order : Moniliales
Form- Family : Tuberculariaceae
Form Genus : ***Fusarium***

Form genus *Fusarium* belongs to form order moniliales and form family tuberculariaceae. This genus includes a large number of species and many forms with in species, many of these are saprophytic some are facultative parasitic and other parasitic some of the species are responsible for many serious disease like damping off of seedling, root rot and wilt diseases.

*Fig. 6.20: Fusarium wilt of Cajanus cajan (arhar)*

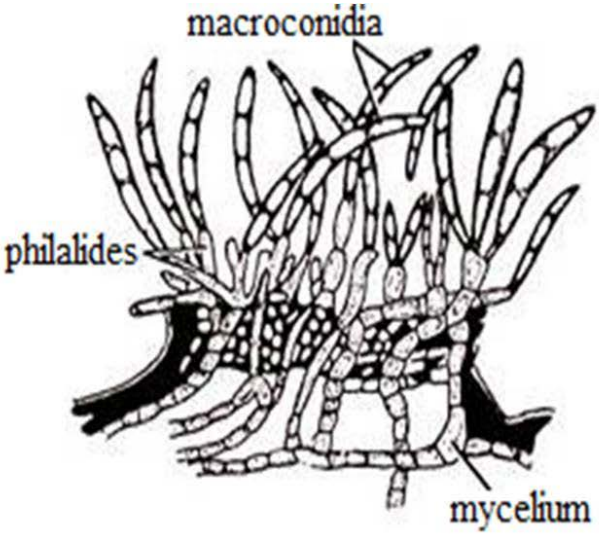



*Fig. 6.21: Fusarium :sporodochium*

Some imporant pathogenic species of fusarium are

- *F. oxysporum* f.*udum* causes wilt of Pigeon pea ( *Cajanus cajan)*
- *F. lini* cause wilt of flax (*Linum ustitatissimum*)
- F. *oxysporum* f. *vasinfectum* causes wilt of cotton *( Gossypium* spp*)*
- *F. solani* causes wilt of potato *( Solanum tuberosum)*
- *F. oxysporum* f. *cubense* cause panama disease of banana.

All the above species are vascular parasite and are thus often referred to as vascular fusaria.

The characteristic symptom is wilting of seedling and mature plants and sudden withering and drying up of leaves, followed by drying of the entire plant. In all the wilts the fungus block the vessels and cause plant to wilt and ultimately death.

**Vegetative structure** – the hyphae are septate and branched may be intercellular or intracellular and uninucleate to multinucleate. When young they may be colourless or with a tinge of pink purple or yellow and become dark coloured at maturity. The dark mycelium produces thick bands which invade xylem tissue and produce toxic secretions and block them completely as a result the plants wilt and die.

**Asexual reproduction**- The asexual reproduction takes place by three kinds of spores, microconidia macroconidia and chlamydospores.

**(a) Microconidia** are small, unicellular or bi-celled, spherical or oval in shape. Their size varies from 5-15 µ x 2−4 µ. They are borne single or in chains on the conidiophores by abstraction method. The conidiophores are distinguishable from the vegetative hyphae.

**(b) Macroconidia-** they are large multicellular sickle shaped or crescent shaped, pointed at the end and broad in the middle and formed at the tips of simple or sparingly branched conidiophores which are assembled to form a sporodochium type of fruitification. The ultimate branches of conidophores which produce conidia are called phialides. The phialides are subulate i.e. owl shaped and have some kind of heel (characteristic of *Fusarium*).

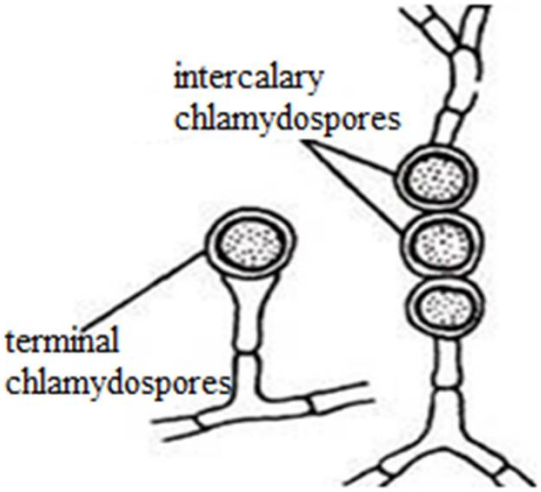



*Fig. 6.22: Fusarium chlamydospores*

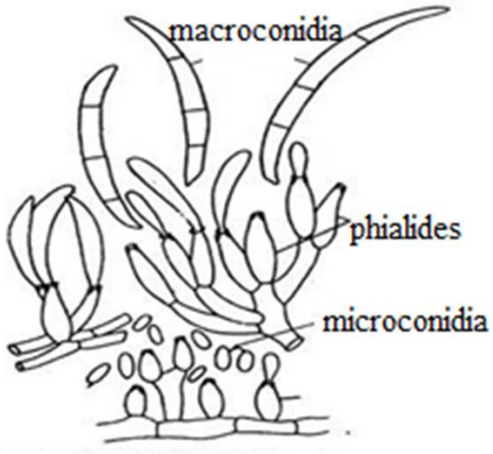



*Fig. 6.23: Fusarium: phlalide, microconidia, macroconidia*

Macro and micro conidia both are disseminated by the wind on falling on a suitable substratum they germinate and produce new infections.

**(c) Chlamydospores:** Under unfavorable condition, the mycelial hyphae produce ovoid or spherical thick walled cells. These are called chlamydospores. They may be formed either single or in chains and may be terminal or intercalary in position. After maturity they get separated from the parent hyphae and act as resting spores, on coming the suitable conditions they germinate by means of germ tubes to form a fresh mycelium.

Sclerotia is an also method of asexual reproduction in this mycelium forms compact thick walled resting body. They function as storage organs and also serve as means of perennation and vegetative reproduction.

The fungus persists as chlamydospores and sclerotia. The conidia and mycelia are short lived. Infection usually takes place through the fibrous root system near the root tip region or through wounds. The Infection usually fevered by the relative high soil temperature.

## 6.4.2 *Alternaria*

### Systematic position

Kingdom - Mycota
Division - Eumycotina
Sub division - Deuteromycotina
Form- class - Hyphomycetes
Form - order - Moniliales
Form- Family - Dematiceae
Form Genus - ***Alternaria***

The fungus is represented by about 50 species. Several form-species are found as saprobes on dead and decaying plant parts and in the soil while some form-species are facultative parasites, infecting a large number of higher plants. The most commonly occurring disease of potato early blight is caused by *Alternaria solani*.

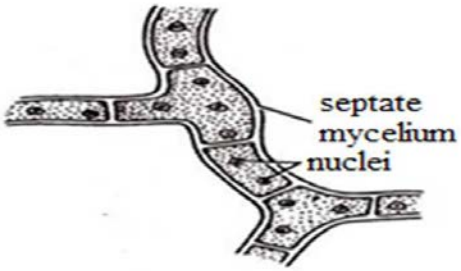



*Fig. 6.24: Alternaria mycelium*


*Fig. 6.25: Alternaria: symptoms of early blight of potato*

### Symptoms of *Alternaria*

*Alternaria* shows the symptoms of blight. Early symptoms appear in the form of yellowish-brown spots on the leaves, which enlarge in size and become round to form the concentric rings. If we study these spots with the hand lens, they appear like the 'target boards' and hence the symptoms are called target board effect. In severe infection entire lamina, petiole, stem and even tubers are badly damaged. Edible parts of the tuber turn brown (Fig. 6.24 & 6.25).

### Vegetative structure

The mycelium is endophytic, profusely branched and septate with multinucleated cell. In parasitic species it is both inter—and intracellular, light brown and without haustoria.

## Reproduction

It has no sexual stage, it reproduces only by conidia which are produced at the tips of conidiophores. The endophytic mycelium grows out as erect and aerial hyphae through the stomata or ruptured epidermis of the infected host tissue. The conidiophores cannot be easily distinguished from the somatic hyphae. The conidia are exogenously produced large dark coloured several celled and muriform structure. Conidia with transverse and longitudinal septa are called 'muriform or dictyospores. The transverse and vertical both type of septation divides the conidia in multicellular component. Usually they are born end to end in chains of two or three. Occasionally they may occur singly at the tip of a hypha. The conidia are readily disseminated by wind.

Each conidium germinates by producing 5-10 germ tubes at a time (Fig. 6.26). In the presence of moisture and suitable temperature, the germ tubes infect the host plant through stomata or, epidermal cells or injuries caused by insects. The perfect stage of *Alternaria* belongs to Loculoascomycets fungus (*Pleaspora infectoria*).

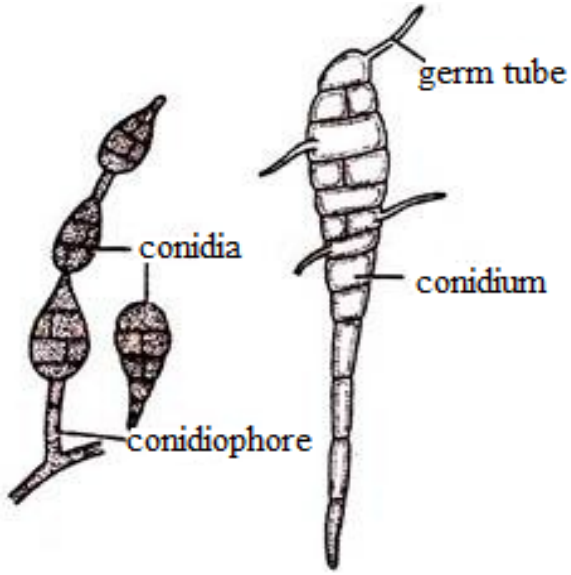



*Fig. 6.26: Alternaria conidia in acropetal chain and germinating conidium*

**Table 2: Some disease caused by the *Alternaria***

| S.NO. | Disease | Species |
|---|---|---|
| 1- | Early blight of potato | *Alternaria solani* |
| 2- | Black point disease of wheat | *A. alternata* (= *A. tenuis*) |
| 3- | Leaf blight of wheat | *A. triticina* |
| 4- | Leaf spot of crucifers | *A. brassicae* |
| 5- | Leaf spot of cucurbits | *A. cucumerina* |
| 6- | Leaf spot of tobacco | *A. longipes* |

## 6.4.3 *Colletotrichum*

Kingdom - Mycota
Division - Eumycotina
Sub division - Deuteromycotina
Form- class - Coelomycetes
Form - order -Melanoconiales
Form- Family - Melanoconiaceae
Form Genus - ***Colletotrichum***

It is a parasitic fungus causing anthracnose disease in plants and develops necrosis and hypoplasia of tissue. The genus is represented by about 21 species some of them are following:

| **Species Name** | **Disease Name** |
|---|---|
| *Collectotrichum falcatum* | Red rot of sugar cane |
| *C. corchorum* | Anthracnose of jute |
| *C. graminicola* | Red leaf spot of sorghum |
| *C. lagenarium* | Anthracnose of cucurbits |
| *C. capsici* | Die back of chillies |
| *C. gloeoporiodes* | Anthracnose of citrus and banana |

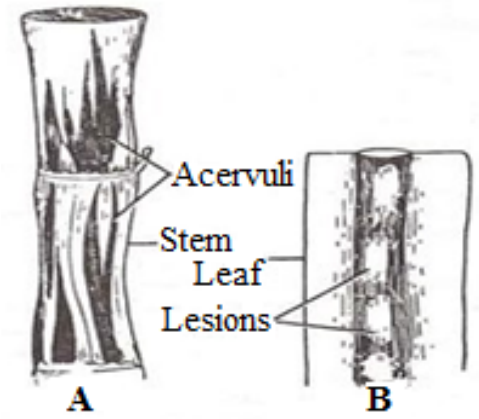



*Fig. 6.27: Colletotrichum falcatum causing red rot of sugarcane: A. Acervuli on stem, B. Acervuli on leaf*

### Vegetative structure

The mycelium is branched intra or intercellular and septate, initially hyphae are hyaline but at maturity develop thick wall and become darker, denser cytoplasm and contain oil droplets. On maturity hyphae closely interwine with each other to form a small black and compact structure under the cuticle or host epidermis.

## Reproduction

Sexual reproduction are lacking in this fungus, it reproduce asexually by development of conidia. The stromata which lie just below the host epidermis produces palisade like layer of dense, simple and hyaline, usually aseptate, unbranched conidiophore and form the plate like fruiting body or acervulus.

Conidiophores are generally short, measuring about 20 µm in length and 8 µm in diameter, in tip of each conidiophore develops a single conidium but occasionally conidia develop in chain acrogenously. After maturation conidia are released by rupturing overlying host epidermis.

The conidia are hyaline crescent or sickle-shaped about 20-30 µm in length and 5-7 µm in breath and al large oil droplet in the centre.

The acervulus is also characterized by the development of protective envelop around the conidiophore which are called setae. The setae are also unbranched like conidiophore but 4-5 times longer and are stiff, pointed, septate and bristle like structure.

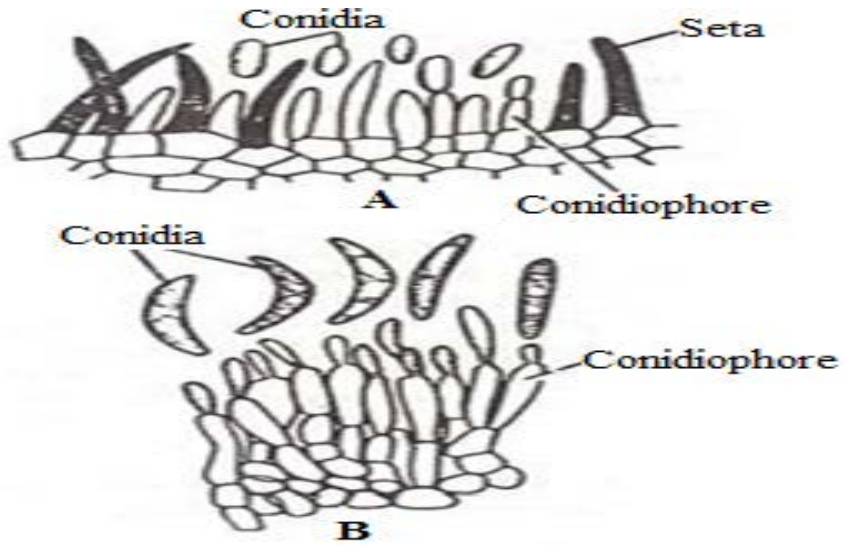



*Fig. 6.28: Colletotricum: A, An acervulus, B, A few conidia*

## Germination of conidia

Conidia germinate only in humid atmosphere and dispersed by rain or by wind. They geminate by the one or more germ tube that grows on the surface of host. Appressorium develop by swelling of germ tube tip. One or more infectious tube develops from each appressorium which penetrate the host epidermis.

On the absent of favourable condition, intercalary and terminal thick walled green black coloured chlamydospores develop by septation of hyphae. These chlamydospores remain viable for long time on coming the favourable condition they germinate to form new mycelium.

## *6.5 SUMMARY*

1. In the basidiomycotina and deuteromycotina the mycelium is well developed branched and septate. The septa are simple in all deuteromycotina and in uredinales and ustilaginales but it is dolipore in Agaricales.
2. No specialize sex organs developed in basidiomycotina and sexual reproduction takes place by conjugation of nuclei of two different strains in certain higher taxa such as *Agaricus* the secondary mycelium is organized to form specialized fruiting body called basidiocarp and differentiated in stipe and pileus.
3. In deuteromycotina sexual reproduction are completely absent so the lack of such type of organized structure, propagation takes place only by the asexual methods.
4. Formation of basidiospores and clamp connection is also a characteristics feature of basidiomycotina.
5. *Ustilago* (Smut fungi) is an autoecious parasite and responsible for two type of smut disease loose smut and covered smut in cereal crops. In the life cycle of *Ustilago* first chlaymydospore are formed and they give rise to basidia and basidiospore which germinate to produce primary mycelium after the somatogamy primary mycelium converted to secondary mycelium.
6. *Puccinia* belongs to uredinales order of basidiomycotina it is a rust fungi because of reddish brown colour of their spores, internal obligate parasite, autoecious or heterocious most rust are polymorphic produce more than one type of spore it may be macrocyclic (e.g *P. graminis* more than one type of dikaryotic spores are formed) or microcyclic (*P. malvacearum* only one type of dikarotic spore is formed)
7. *Puccinia graminis* completes their life cycle in two hosts one is *Triticum valgaris* and other is *Berberis* which is alternate host. It can survive in the absence of alternate host but its life cycle is completed only when both the host are available.
8. Five types of spores are produced in the life cycle of *Puccinia* uredospores and teleutospores develop in primary host (*Triticum valgaris*), pycnidiospore and aeciospores on alternate host (*Berberis vulgaris*). Basidiospores produce from the teletospore and germinate to form pycniospors.
9. *Agaricus* is saprophytic edible fungus commonly known as mushroom it is a very good source of protein. Structure can be divided into vegetative mycelium and umbrella shaped fruiting body (basidiocarp) develop from the rhizomorph.
10. Basidiocarp differentiates into stipe and pileus. Gills are hang down from the pileus, the surface of gills enveloped by a fertile layer the hymenium in which aseptate fertile cells

basidia arranged in palisade like layer and club shaped paraphysis also occur in between the basidia.

11. *Alternaria* is usually a saprophytic fungus but most of the species are parasitic such as *A. solani* cause the early blight of potato produce target board symptom. Mycelium of fungus is multinucleate, well branched, septate, intercellular or intracellular and haustoria are absent.
12. Conidia are present on conidiophores. Each conidium is a multicellular obovoid elliptical or spindle shaped structure and presence of longitudinal and transverse septa. In *A. tenuis* conidia present in chains.
13. *Fusarium* is a facultative parasite cause damping off of seedling, root rot, and wilt disease in many plants. Mycelium of fungus is branched septate inter and intracellular and plug the host vascular lumen completely and produce characteristic symptom of wilting.
14. Sexual reproduction is completely lacking and reproduces only by the two types of asexual spores i.e. microconidia and macroconidia. Chlamydospores are also produced over older mycelium.
15. Member of these two group shows high economic importance,some members are very good source of proteins and vitamins (*Agaricus*) and some cause very destructive disease on various plants (e.*g., Ustilago, Puccinia, Alternaria, Fusarium*)

## *6.6 GLOSSARY*

**Mycelium**: It is a vegetative part of a fungus, consisting of a network of fine filaments.
**Unicellular**: In unicellular body consist of only a single cell.
**Saprophytic:** An organism that lives on and gets its nourishment from dead and decaying organic material.
**Parasitic:** An organism that lives on and gets its nourishment from other living organism.
**Basidiocarp:** fruiting body of basidiomycotina.
**Hymenium:** fertile layer on gills of mushroom in pileus.
**Somatogamy:** fusion of two cells but not their nuclei.
**Conidia:** asexually producing spores on the tip of specialized hyphae.
**Dolipore septa:** A septal structure that is composed of a pore cap surrounding a septal swelling and septal pore.
**Muriform conidia:** Multicelled conidia divided by both vertical and horizontal septa.
**Clamp connection:** A hook – like structure formed by growing hyphal cells.

## *6.7 SELF ASSESSMENT QUESTIONS*

### 6.7.1 Multiple choice questions:

1. Heteroecious fungus is

(a) *Ustilago* (b) *Puccinia*
(c) *Agaricus* (d) *Albugo*

2. Teleutospores are
(a) Motile spore (b) Unicellular spores
(c) Bicelled spores (d) Hexagonal spores

3. Which of the following is an edible fungus?
(a) *Mucor* (b) *Alternaria*
(c) *Agaricus* (d) *Fusarium*

4. Basidia in *Agaricus* produced on
(a) Gill (b) Pileus
(c) Stipe (d) Rhizomorph

5. Somatogamy occur in

(a) *Agaricus* (b) *Rhizopus*
(c) *Fusarium* (d) *Alternaria*

6. Common name of disease caused by the members of uredinales is
(a) Rust (b) Smut
(c) White rust (d) Wart

7. Macrocycle fungus is
(a) *Ustilago* (b) *Puccinia*
(c) *Albugo* (d) *Alternaria*

8. Loose smut of wheat is caused by
(a) *Albugo candida* (b) *Ustilago tritici*
(c) *Puccinia graminis* (d) *Alternaria solani*

9. Whip tail smut disease of sugar cane is caused by
(a) *Ustilago maydis* (b) *Ustilago scitamineae*
(c) *Ustilago hordei* (d) *Ustilago nuda*

10. Macro and micro conidia are produced in
(a) *Ustilago* (b) *Puccinia*
(c) *Alternaria* (d) *Fusarium*

11. Fungi imperfecti are so named because of the absence of
(a) Asexual reproduction (b) Vegetative reproduction
(c) Sexual reproduction (d) None of the above

12. Target board system produce by the
(a) *Fusarium* (b) *Alternaria*
(c) *Puccinia* (d) *Ustilago*

13. Sexual reproduction absent in the member of
(a) Phycomycotina (b) Ascomycotina
(c) Basidiomycotina (d) Deuteromycotina

14. Muriform conidia are formed in
(a) *Fusarium* (b) *Alternaria*
(c) *Puccinia* (d) *Ustilago*

15. Which of the following cause early blight of potato
(a) *Alternaria solani* (b) *Phytophthora infestans*
(c) *Synchyrium endobioticum* (d) *Ustilago nuda*

16. Fairy ring are formed by the
(a) *Rhizopus* (b) *Penicillium*
(c) *Agaricus* (d) *Mucor*

17. Umbrella like cup of mushroom is
(a) Pileus (b) Basidium
(c) Gill (d) Capsid

18. The number of basidiospores produced on each basidium is-
(a) 2 (b) 4
(c) 6 (d) 8

19. Dolipore septum is characteristic feature of
(a) Ascomycotina (b) Basidiomycotina
(c) Deuteromycotina (d) Myxomycotina

20. The dikaryotic mycelium is characterized by the presence in each of its cells of
(a) A single diploid nucleus
(b) A single haploid nucleus
(c) Two haploid nuclei belonging to opposite strains
(d) Two haploid nuclei belonging to similar strains

**Answers Key:** 1-(b), 2-(c), 3-(c), 4-(a), 5-(a), 6-(a), 7-(b), 8-(b), 9-(b),10-(d), 11-(c), 12-(b), 13-(d), 14-(b), 15-(a),16-(c), 17-(a), 18-(b), 19-(b), 20-(c).

### 6.7.2 Fill in the blanks:

(a) The fruiting body of basidiomycotina is known as …….
(b) The process by which primary monokaryotic mycelium becomes secondary dikaryotic mycelium is known as……..
(c) *Ustilago* is commonly known as……
(d) *Puccinia graminis* is a ……rust
(e) Aeciospores of *Puccinia* are produced on the …..surface of ….leaf.

**Answers Key:** (a) Basidiocarp, (b) somatogamy, (c) smut fungi, (d) heteroecious rust, (e) lower surface, barberry

### 6.7.3 True and false

(a) Mushrooms frequently appear in circles on the ground. This phenomenon is known as fairy rings.
(b) Teleutospores are unicellular spores.
(c) In Basidiomycotina well developed sex organs are present.
(d) Target board is a symptom of early blight.
(e) Sexual reproduction is not found in Deuteromycotina**.**

**Answers Key:** (a) True (b) false (c) false (d) true (e) true

### 6.7.4 Very short answer type question:

(a) Name the disease cause by the *Alternaria solani.*
(b) Name the fungus of Deuteromycotin which cause wilt disease in plants.
(c) What is the name of fertile layer present on the surface of the gills?
(d) How many types of spores are formed in *Puccinia graminis*?
(e) Name the causal organism of loose smut of wheat.

**Answers Key:** (a) Early blight of potato (b) *Fusarium* (c) hymenium (d) 5 types of spores ueredospore, teletospore, basidiospore, pycniospore, aceiospore (e) *Ustilago tritici*

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- Singh, Pandey and Jain (2017-2018), Text book of botany, Rastogi publication Fifth edition Wiley, New York ISBN 978-93-5078-129-6
- Vashishta, B.R. Sinha A.K (1962) Fungi, S. Chand publication ISBN 81-219-2826-5


## *6.9 SUGGESTED READING*


- Fungi (1962) by B.R**.** Vashishta and A.K. Sinha, S. Chand publication
- Text book of botany (2017-2018) by Singh, Pandey and Jain, Rastogi publication
- Plant pathology (2013-2014) by P.D.Sharma, Rastogi Publication


## *6.10 TERMINAL QUESTIONS*

1. Write note on smut spores.
2. Write note on clamp connection.
3. Give the structure of different types of spores found in *Puccinia.*
4. Describe the structure of conidia of *Alternaria.*
5. Give the structure of basidiocarp of *Agaricus.*

# BLOCK-3- PLANT VIRUSES AND BACTERIA

# UNIT-7 STRUCTURE, PROPERTIES, CLASSIFICATION AND REPLICATION OF VIRUSES

## Contents:

## 7.1 OBJECTIVES

When we look around us in nature, we find that living organisms are highly variable with respect to size. In comparative and relative terms these are of two major kinds: macro sized and micro sized. The former can be seen with naked eyes while the later only through the microscope. Bacteria, some algae, protozoa, nematode sand some fungi are thus microbes. In addition Viruses and Mycoplasma (e.g., PPLO, MLB) are sometimes also considered to be microbes but they are ultramicroscopic and have features of both living and non-living.

The key objectives of the present unit are:

- To know different kind of virus and their distribution.
- To know their shape and size.
- To understand the properties of viruses
- Classification and nomenclature of viruses
- Replication of viruses

## 7.2 INTRODUCTION

Viruses are simple and acellular infectious agents. The branch of science which deals with the study of viruses is called virology and constitutes a significant part of microbiology. Many of human, animal and plant diseases are caused by these tiny particles. Even the recently appeared AIDS is also caused by virus. The new field of genetic engineering is also based in large part upon discoveries in virology.

The word is from the Latin neuter *vīrus* referring to poison and other noxious liquids, from the same Indo-European base as Sanskrit *viṣa* poison. A virus is a small infectious agent that replicates and show living properties only inside the living cells of other organisms. They can infect all types of life forms, from multicellular organisms to unicellular organisms. In 1892 Dmitri Ivanovsky wrote an article describing a non-bacterial pathogen infecting tobacco plants. Martinus Beijerinck in 1898 discovered tobacco mosaic virus. Viruses are found in almost every ecosystem on Earth and are the most abundant type of biological entity. The study of viruses is known as virology. When viruses are not inside an infected cell or in the process of infecting a cell, it exists in the form of independent particles. These **viral particles**, also known as **virions**, consist of two or three parts: (i) the genetic material made from either DNA or RNA, long molecular structure that carry genetic information; (ii) a protein coat, called the capsid, which surrounds and protects the genetic material; and in some cases (iii) an envelope of lipids that surrounds the protein coat. The shapes of these virus particles range from simple helical and icosahedral forms for some virus species to more complex structures for

others. Most virus species have virions that are too small to be seen with an optical microscope. The average virion is about one-hundredth the size of the average bacterium.

The origins of viruses in the evolutionary history of life are unclear: some may have evolved from plasmids (pieces of DNA that can move between cells) while others may have evolved from bacteria. In evolution, viruses are an important means of horizontal gene transfer, which increases genetic diversity. Viruses are considered by some virologists to be a life form, because they carry genetic material, reproduce, and evolve through natural selection, but lack key characteristics (such as cell structure) that are generally considered necessary to count as life. Because they possess some but not all such qualities, viruses have been described as "organisms at the edge of life" and as replicators. Viruses spread in many ways viruses in plants are often transmitted from plant to plant by insects that feed on plant sap, such as aphids; viruses in animals can be carried by blood-sucking insects. These disease-bearing organisms are known as vectors. Influenza viruses are spread by coughing and sneezing. Norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the faecal-oral route and are passed from person to person by contact, entering the body in food or water. HIV is one of several viruses transmitted through sexual contact and by exposure to infected blood. The range of host cells that a virus can infect is called its "host range". This can be narrow, meaning a virus is capable of infecting few species, or broad, meaning it is capable of infecting many.

## History

The first images of viruses were obtained upon the invention of electron microscopy in 1931 by the German engineers Ernst Ruska and Max Knoll. In 1935 American biochemist and virologist Wendell Meredith Stanley examined the tobacco mosaic virus and found it was mostly made of protein. The tobacco mosaic virus was the first to be crystallized and its structure could therefore be elucidated in detail. The first X-ray diffraction pictures of the crystallized virus were obtained by Bernal and Fankuchen in 1941.

Louis Pasteur was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected using a microscope.In 1884, the French microbiologist Charles Chamberland invented a filter (known today as the Chamberland filter or the Pasteur-Chamberland filter) with pores smaller than bacteria. Thus, he could pass a solution containing bacteria through the filter and completely remove them. In 1892, the Russian biologist Dmitri Ivanovsky used this filter to study what is now known as the tobacco mosaic virus. His experiments showed that crushed leaf extracts from infected tobacco plants remain infectious after filtration. Ivanovsky suggested the infection might be caused by a toxin produced by bacteria, but did not pursue the idea. At the time it was thought that all infectious agents could be retained by filters and grown on a nutrient medium – this was part of the germ theory of disease. In 1898, the Dutch microbiologist Martinus Beijerinck repeated the experiments and became convinced that the filtered solution contained a new form of infectious agent. He

observed that the agent multiplied only in cells that were dividing, but as his experiments did not show that it was made of particles, he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word *virus*. In the early 20th century, the English bacteriologist Frederick Twort discovered a group of viruses that infect bacteria, now called bacteriophages (or commonly *phages*), and the French-Canadian microbiologist Félix d'Herelle described viruses that, when added to bacteria on an agar plate, would produce areas of dead bacteria. By the end of the 19th century, viruses were defined in terms of their infectivity, their ability to be filtered, and their requirement for living hosts. Viruses had been grown only in plants and animals.

The second half of the 20th century was the golden age of virus discovery and most of the over 2,000 recognized species of animal, plant, and bacterial viruses were discovered during these years. In 1957 the cause of Bovine virus diarrhoea (a pesti virus) were discovered. In 1963, the hepatitis B virus was discovered by Baruch Blumberg and in 1965 Howard Temin described the first retrovirus. Reverse transcriptase, the enzyme that retroviruses use to make DNA copies of their RNA, was first described in 1970, independently by Howard Martin Temin and David Baltimore. In 1983 Luc Montagnier's team at the Pasteur Institute in France, first isolated the retrovirus now called HIV. In 1989 Michael Houghton's team at Chiron Corporation discovered Hepatitis C.

## Origin of Virus

Viruses are found wherever there is life and have probably existed since living cells first evolved. The origin of viruses is unclear because they do not form fossils, so molecular techniques have been used to compare the DNA or RNA of viruses and are a useful means of investigating how they arose. In addition, viral genetic material may occasionally integrate into the germ line of the host organisms, by which they can be passed on vertically to the offspring of the host for many generations. There are three main hypotheses that aim to explain the origins of viruses.

**I-Regressive Hypothesis:** Viruses may have once been small cells that parasitized larger cells. Over time, genes not required by their parasitism were lost. The bacteria *Rickettsia* and *Chlamydiae* are living cells that, like viruses, can reproduce only inside host cells. They lend support to this hypothesis, as their dependence on parasitism is likely to have caused the loss of genes that enabled them to survive outside a cell. This is also called the *degeneracy hypothesis* or *reduction hypothesis*.

**II- Escaped gene Theory:** Some viruses may have evolved from bits of DNA or RNA that "escaped" from the genes of a larger organism. The escaped DNA could have come from plasmids (pieces of naked DNA that can move between cells) or transposons (molecules of DNA that replicate and move around to different positions within the genes of the cell). Once called "jumping genes", transposons are examples of mobile genetic elements and could be the

origin of some viruses. They were discovered in maize by Barbara McClintock in 1950.[1] This is sometimes called the *vagrancy hypothesis* or the *escape hypothesis*. Evidence accumulated so far strongly supports the proposed that viruses originated from 'escaped' nucleic acid.

**III-Co-evolution Hypothesis:** This is also called the *virus-first hypothesis* and proposes that viruses may have evolved from complex molecules of protein and nucleic acid at the same time as cells first appeared on Earth and would have been dependent on cellular life for billions of years. Viroids are molecules of RNA that are not classified as viruses because they lack a protein coat. They have characteristics that are common to several viruses and are often called subviral agents. Viroids are important pathogens of plants. They do not code for proteins but interact with the host cell and use the host machinery for their replication.

Viruses are now recognized as ancient and as having origins that pre-date the divergence of life into the three domains. This discovery has led modern virologists to reconsider and re-evaluate these three classical hypotheses. The evidence for an ancestral world of RNA cells and computer analysis of viral and host DNA sequences are giving a better understanding of the evolutionary relationships between different viruses and may help to identify the ancestors of modern viruses. To date, such analyses have not proved which of these hypotheses is correct. It seems unlikely that all currently known viruses have a common ancestor, and viruses have probably arisen numerous times in the past by one or more mechanisms.

Prions are infectious protein molecules that do not contain DNA or RNA. They can cause infections such as Scrapie in sheep, bovine spongiform encephalopathy ("mad cow" disease) in cattle. Although prions are fundamentally different from viruses and viroids, their discovery gives credence to the theory that viruses could have evolved from self-replicating molecules.

## *7.3 STRUCTURE*

Viruses are sub-microscopic acellular, meaning they are biological entities that do not have a cellular structure. Therefore, they lack most of the components of cells, such as organelles, ribosomes, and the plasma membrane. A simple virus particle often designated as virion. A virion consists of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope made of protein and phospholipid membranes derived from the host cell. The capsid is made up of protein subunits called capsomeres. Viruses may also contain additional proteins, such as enzymes.

**General Morphology of Viruses:** Viruses may be classified into various morphological types on the basis of their capsid architecture. The structure of capsid and individual capsomere can be studied by electron microscopy and x-ray crystallography. Following are some of the common morphological forms of viruses (Fig. 7.1).

- Helical – These viruses are composed of a single type of capsomere stacked around a central axis to form a helical structure, which may have a central cavity, or hollow tube.
- Icosahedral – Most animal viruses are icosahedral or near-spherical with icosahedral symmetry.
- Prolate – This is an isosahedron elongated along one axis and is a common arrangement of the heads of bacteriophages.
- Enveloped viruses – Some species of virus envelope themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as a viral envelope.
- Complex viruses– These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall.

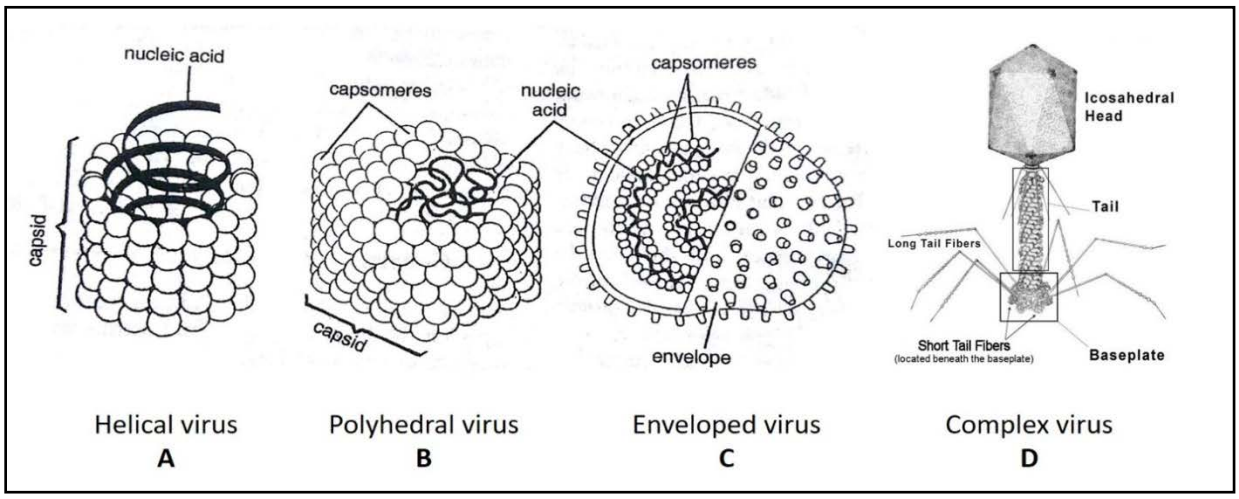



*Fig. 7.1 (A-D): Morphology of viruses, A. Helical virus; B. Polyhedral virus; C. Enveloped virus; D. Complex virus*

## Size

Viruses display a wide diversity of sizes. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 350 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with an optical microscope so scanning and transmission electron microscopes are used to visualize virions. The largest are the orthopoxviruses, measuring about 240 nm x 350 nm. The complex bacteriophages are about 65 nm x 200nm. Among the smallest viruses known are the enteroviruses, which are less than 30 nm. in diameter (Fig. 7.2).

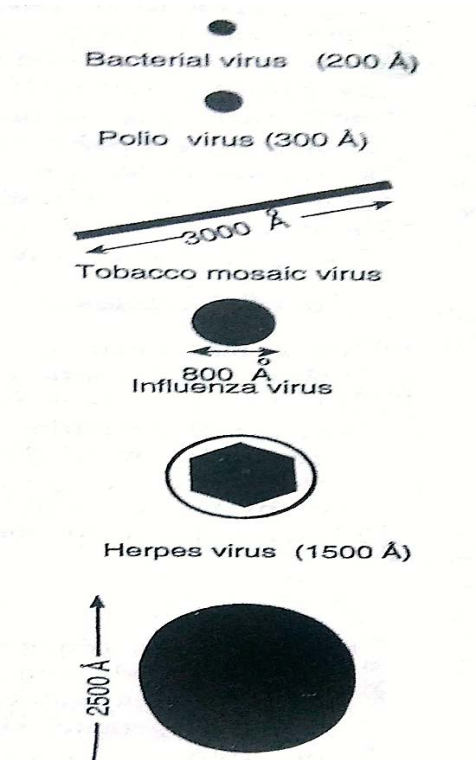



*Fig. 7.2: Variation of size in viruses*

## Shape

Viruses core in many shapes and sizes, but these are consistent and distinct for each viral family. In general, the shapes of viruses are classified into four groups: filamentous, isometric (or icosahedral), enveloped, and head and tail. Filamentous viruses are long and cylindrical. Many plant viruses are filamentous, including TMV (tobacco mosaic virus). Isometric viruses have shapes that are roughly spherical, such as poliovirus or herpesviruses. Enveloped viruses have membranes surrounding capsids. Animal viruses, such as HIV, are frequently enveloped. Head and tail viruses infect bacteria. They have a head that is similar to icosahedral viruses and a tail shape like filamentous viruses.

Viruses can be either complex (Fig. 7.3) in shape or relatively simple. Overall, the shape of the virion and the presence or absence of an envelope tell us little about what disease the virus may cause or what species it might infect, but they are still useful means to begin viral classification (Fig. 7.4).

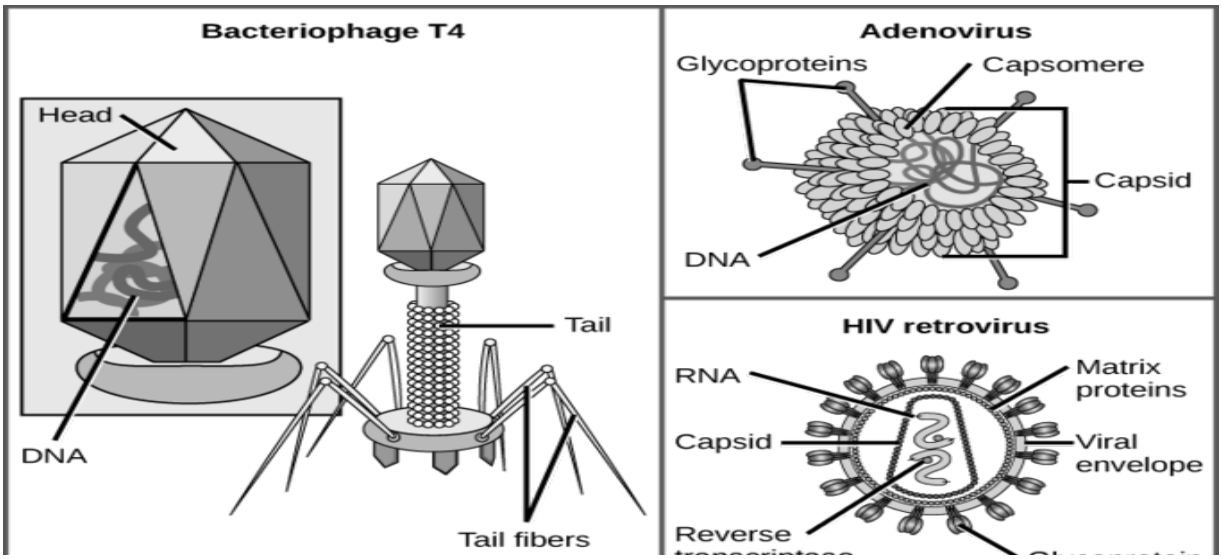
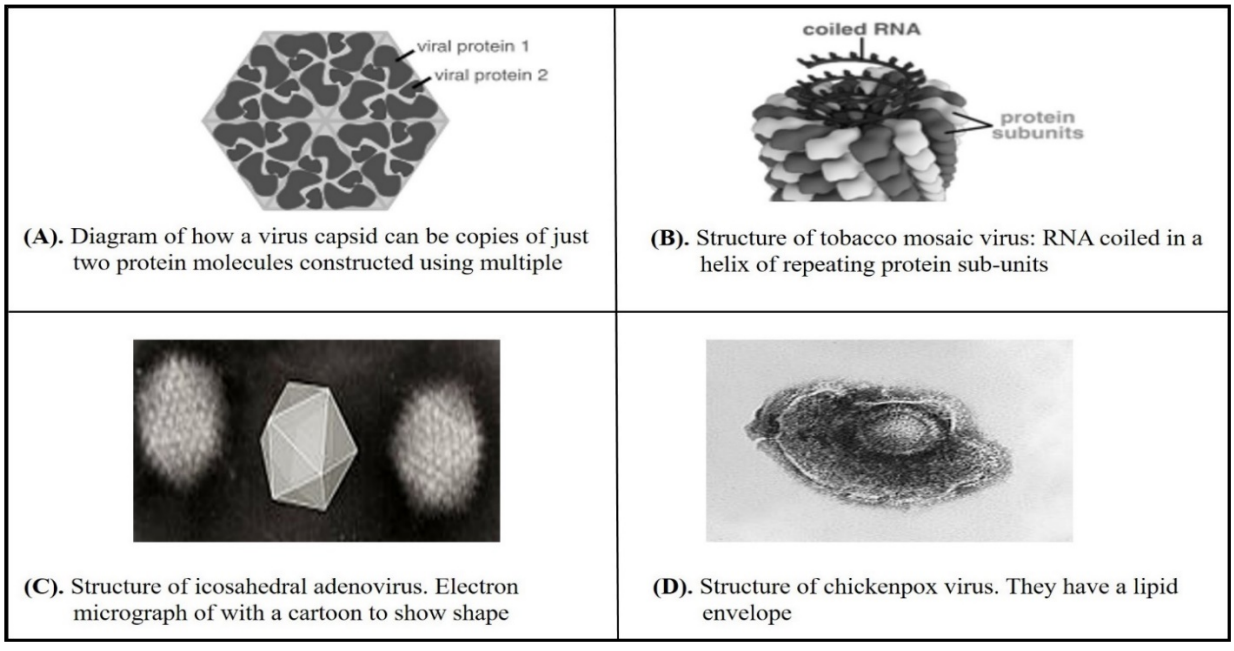



*Fig. 7.3: Diagrammatic representation of relatively-complex viruses*

The bacteriophage T4, with its DNA-containing head group and tail fibers that attach to host cells; adenovirus, which uses spikes from its capsid to bind to host cells; and HIV, which uses glycoproteins embedded in its envelope to bind to host cells.




**(A).** Diagram of how a virus capsid can be copies of just two protein molecules constructed using multiple

**(B).** Structure of tobacco mosaic virus: RNA coiled in a helix of repeating protein sub-units

**(C).** Structure of icosahedral adenovirus. Electron micrograph of with a cartoon to show shape

**(D).** Structure of chickenpox virus. They have a lipid envelope

*Fig. 7.4 (A-D): Different shapes and size of viruses*

Enveloped virions like HIV consist of nucleic acid and capsid proteins surrounded by a phospholipid bilayer envelope and its associated proteins. Glycoproteins embedded in the viral

envelope are used to attach to host cells. Other envelope proteins include the matrix proteins that stabilize the envelope and often play a role in the assembly of progeny virions. Chicken pox, influenza, and mumps are examples of diseases caused by viruses with envelopes. Because of the fragility of the envelope, non-enveloped viruses are more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.

## The Protein Coat

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomeres. Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsomeres forming the capsid of a virion are of two types – Pentamer, made up of five identical monomers and Hexamer, having six monomers. Each monomer is connected with the neighbouring monomers on either side with the help of bonds. Likewise, the capsomeres are also connected with each other, but the bonds between the capsomeres are weak (Fig. 7.4).

In some complex forms (e.g., influenza and herpes virus) the capsid is covered by an envelope. Some animal viruses, which are released from the host cell by an extrusion process, get coated by the host cell's plasma membrane. This membrane eventually becomes the viral envelope. Envelope of many viruses has projections called spikes. Viruses attach themselves to the host cells by means of spikes.

Viruses, whose capsids are not covered by an envelope, are known as naked or non-enveloped viruses (e.g., TMV). In such forms the capsid facilitates the attachment of the viruses to the host surface and also protects the virus nucleic acid from the nuclease enzymes present in the biological fluids.

## Nucleic Acid

Viruses differ fundamentally from cellular organisms in that they contain only one type of nucleic acid which may be either DNA or RNA. The viruses containing DNA are called Deoxyviruses, whereas, those having RNA are known as Riboviruses. In general (i) all plant viruses have single stranded RNA, (ii) animal viruses have either single or (rarely) double-stranded RNA or double-stranded DNA. (iii) bacterial viruses contain mostly doubles stranded DNA but can also have single stranded DNA or RNA (iv) most of the insect viruses contain RNA and only a few have DNA. DNA of some bacterial and animal viruses is circular but in

others it is like RNA. The extraction of nucleic acids from viruses has shown that a virion contains only a single molecule of nucleic acid. The number of nucleotide pair in a molecule varies from 1,000-2,50,000 pairs. But the number of pairs in a specific virion is always constant. The amount of nucleic acid depends on the size of virion, usually larger the size of virion greater is the amount of nucleic acid (Table 7.1).

**Table-7.1: Variations in viral nucleic acids**

| S.No. | Type of nucleic acid | Structure of Nucleic acid | Example |
|---|---|---|---|
| 1- | Single stranded DNA (s-s DNA) | Linear single stranded | Parvovirus |
| | | Circular single stranded | Ø 174, M 13 fd phages |
| 2- | Double stranded DNA (ds DNA) | Linear double stranded | Herpes viruses, adenoviruses T-coliphages, lambda phages and other bacteriophages |
| | | Linear double stranded with breaks in one chain | T5 coliphage |
| | | Double stranded with cross-linked ends | Vaccinia |
| | | Closed circular double stranded | Polyoma, SV-40, PM2 phage, Cauliflower mosaic virus (CMV) |
| 3- | Single stranded RNA (s-s RNA) | Linear, single stranded, positive strand | Polio virus, togaviruses, RNA bacteriophage, TMV, and most plant viruses. |
| | | Linear, single stranded, negative strand | Rhabdoviruses (rabies virus) paramyxoviruses (mumps, measles) |
| | | Linear, single stranded, segmented, positive strand | Brome mosaic virus |
| | | Linear, single stranded, segmented, diploid (Two identical strands), positive strand | Retroviruses (Rous sarcoma virus) |
| | | Linear, single stranded, segmented, negative strand | Paramyxo viruses, orthomyxoviruses (influenza virus) |
| 4- | Double stranded RNA (ds RNA) | Linear, double stranded, segmented | Reovirus, wound-tumor virus of plants, CPV of insects, phage Ø 6, many mycoviruses |

## Types of Nucleic Acid

Unlike nearly all living organisms that use DNA as their genetic material, viruses may use either DNA or RNA. The virus core contains the genome or total genetic content of the virus. Viral genomes tend to be small, containing only those genes that encode proteins that the virus cannot

obtain from the host cell. This genetic material may be single or double-stranded. It may also be linear or circular. While most viruses contain a single nucleic acid, others have genomes that have several, called segments.

In DNA viruses, the viral DNA directs the host cell's replication proteins to synthesize new copies of the viral genome and to transcribe and translate that genome into viral proteins. DNA viruses cause human diseases, such as chickenpox, hepatitis B, and some venereal diseases, like herpes and genital warts.

RNA viruses contain only RNA as their genetic material. To replicate their genomes in the host cell, the RNA viruses encode enzymes that can replicate RNA into DNA, which cannot be done by the host cell. These RNA polymerase enzymes are more likely to make copying errors than DNA polymerases and, therefore, often make mistakes during transcription. For this reason, mutations in RNA viruses occur more frequently than in DNA viruses. This causes them to change and adapt more rapidly to their host. Human diseases caused by RNA viruses include hepatitis C, measles, and rabies.

## *7.4 PROPERTIES OF VIRUSES*

Viruses are a biological enigma. We are not sure as to their true status as a biological entity. Are they living or non-living? They exhibit characters of inanimates (non-living); on the other hand, they show some properties of living as well. The picture, naturally, is quite confusing. If we accept Oparin's hypothesis of origin of life that the inanimate and the animate worlds are merely the parts of the same evolving system, all the controversy would just vanish into air. Therefore, it may be asserted that even if the issue remains unsolved, viruses definitely have a very important place in the biological system of this universe. These could be regarded as the something unique, representing may be, a line of development which did not flourish.

Viruses are unique group of infectious agents that can be differentiated from other pathogens (both prokaryotic and eukaryotic) by the following properties:

1. The viruses are ultramicroscopic particles i.e., they are beyond the resolution of the optical microscope. The size of virus particles ranges from 18 nm to 450 nm.
2. They are not made up of cells. Their structure is very compact and economical.
3. They behave like chemicals and can be crystallized.
4. They do not independently fulfill the characteristics of life.
5. They are inert macromolecules outside the host cell and active only inside the host cells.
6. They are geometrical in shape and form crystal like masses.
7. Their basic structure consists of a protein capsid and nucleic acid. The 'viroids' consist of a single strand of naked nucleic acid without protein coat.

8. Capsid is made up of repeating subunits. It encloses and protects nucleic acid. Additional layers may be very complex and contain carbohydrates, lipids and additional proteins.
9. Nucleic acid may be either DNA or RNA but not both.
10. Nucleic acid may be double stranded DNA or single stranded DNA or single stranded RNA or Double stranded RNA (Fig.7.5).
11. Molecules present on virus surface provide high specificity for host cell.
12. Viruses multiply by assembly line method. They do not divide. The cycle of multiplication include:
    (a) Attachment of virus to host cell.
    (b) Penetration of genetical material.
    (c) Production of virus components by cell.
    (d) Assembly of new viruses, and
    (e) Release from host cell.
13. They lack enzymes for most metabolic processes.
14. They lack machinery for the synthesis of proteins.
15. They are obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants and animals.
16. Many of the viruses have a close biological relationship with an arthropod or other type of vector on which they are dependent for their transmission from one host to other.

**(A) Viruses are like dead things(non-living) in the following respects:-**

1. They can be crystallized like a sugar molecule and thus behave as chemicals.
2. Outside the host, they are inert like a chemical.
3. They are auto catalytic and lack functional autonomy.
4. They lack any type of membranes and cell wall.
5. They can be precipitated by a number of chemicals.
6. They show no sign of respiration, excretion and other metabolic activities.

**(B) Viruses are living molecules because of the following properties:-**

1. They can grow and multiply within the specific hosts.
2. They show mutations.
3. They can be transmitted from the diseased host plant or animal to the healthy ones.
4. They react to stimuli-heat, chemicals and radiations.
5. They have genetic materials either DNA or RNA or very rarely both DNA and RNA (e.g., Rous sarcoma virus, RSV).

**(C) They differ from living organisms in the following properties:-**

1. Unit of structure of a virus is virion which lacks protoplasm and cytoplasmic organelles (c.f., cell)

2. A virion contains only one type of nucleic acid, either DNA or RNA (c.f., living organisms like bacteria, algae, protozoa, which contain both of them).
3. They lack fats, polysaccharides and enzymes (c.f., organisms).
4. Contrary to an organism, a virus reproduces solely from genetic material i.e., (DNA/RNA).
5. They cannot undergo binary fission.
6. They are inert and cannot multiply outside the host cells. They are metabolically inactive outside the host cell because they do not have enzyme systems and protein synthesis machinery.

**(D) Other properties:-**

1. They are smallest biological atoms.
2. They do not have sex organs.
3. They cannot move on their own.
4. They do not have system for the production of energy with high potential.
5. They are obligate parasites (i.e., only multiply in the living cells). Actually they are pathogens because they cause disease but not parasites.
6. They exhibit host specificity (i.e., Physiological specialization).
7. They are ultra filterable and ultra microscopic.
8. They do not have any pigments.
9. The two major components of the virus (a nucleic acid and protein shell) are produced and are assembled in the host cell.
10. They are highly resistant to general poisons, antibiotics, drugs, high temperature, alkalies and salts.
11. They are antigenic. On being introduced into the animal body they stimulate the production of specific antibodies.
12. They utilize the ribosomes of the host cell for protein synthesis during their multiplication.

## *7.5 CLASSIFICATION OF VIRUSES*

Viruses do not fit into the established biological classification of cellular organisms. This is mainly due to pseudo-living nature of viruses; they are non-living particles with some chemical characteristics similar to those of life. Initially, viruses were classified into the following four groups on the basis of their host range, and clinical, epidemiological and pathological symptoms.

**1. Plant viruses** : These viruses infect only plants and depending upon the host they have been sub- divided into bacterial viruses algal viruses, fungal viruses, etc.

**2. Invertebrate viruses** : Viruses infecting invertebrates have been included in this group.

**3. Vertebrate Viruses** : This group includes viruses infecting vertebrate animals.
**4. Dual-host viruses** : This group includes those viruses which infects two different hosts mentioned above.

Holmes (1948) included all viruses in a single order Virales which were divided into three sub-orders.

**1. Phagineae** : This sub-order includes viruses infecting bacteria *i.e*., bacteriophage.
**2. Phytophagineae** : It includes viruses infecting plants.
**3. Zoophagineae** : It includes viruses infecting animals.

**(A).** Based on information obtained from ultracentrifuge and electron microscopic studies, Lwoff, Horne and Tournier (1962) proposed a comprehensive scheme for the classification of viruses which consisted of phylum-class-order-family-sub-family-genus-species-strain/type. The **International Committee on the Nomenclature of Viruses** accepted many principles of this system. Lwoff *et al*. system emphasized that the viruses should be grouped according to their shared properties rather that the properties of the cells or organisms they infect. The four criteria on which this hierarchical system is based are:

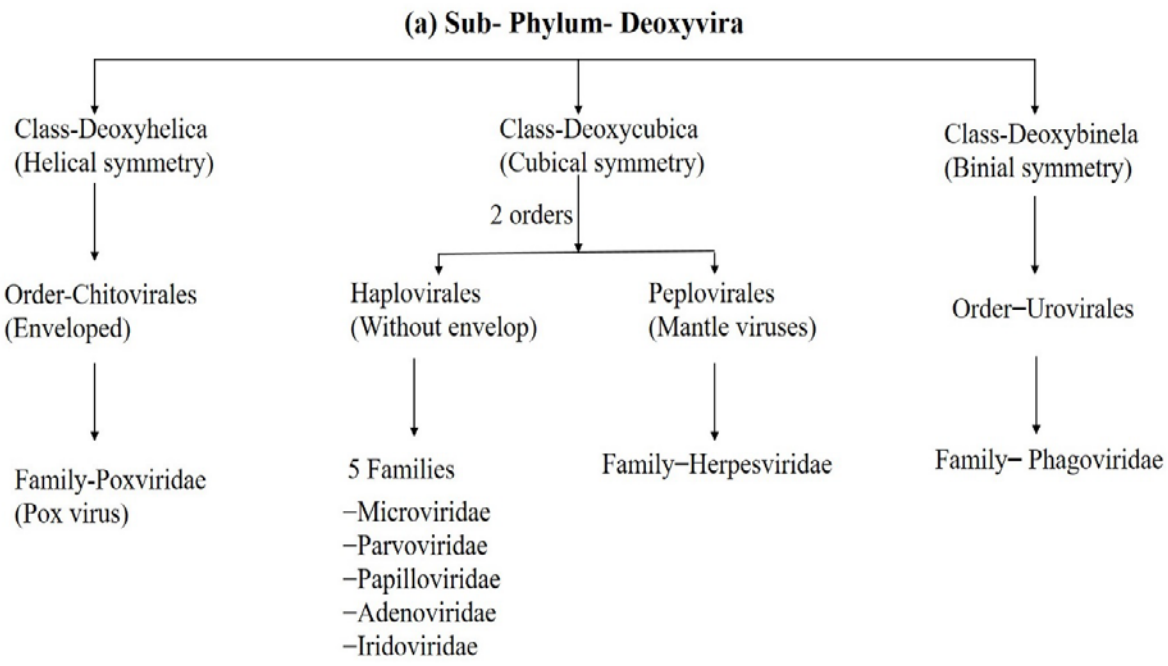
(1) Nature of the nucleic acid (RNA or DNA).
(2) Symmetry or the capsid.
(3) Presence or absence of an envelope.
(4) Dimensions of the virion and capsid.

A brief outline of PCNV (A Provisional Committee on Nomenclature of Viruses) classification is as follows:

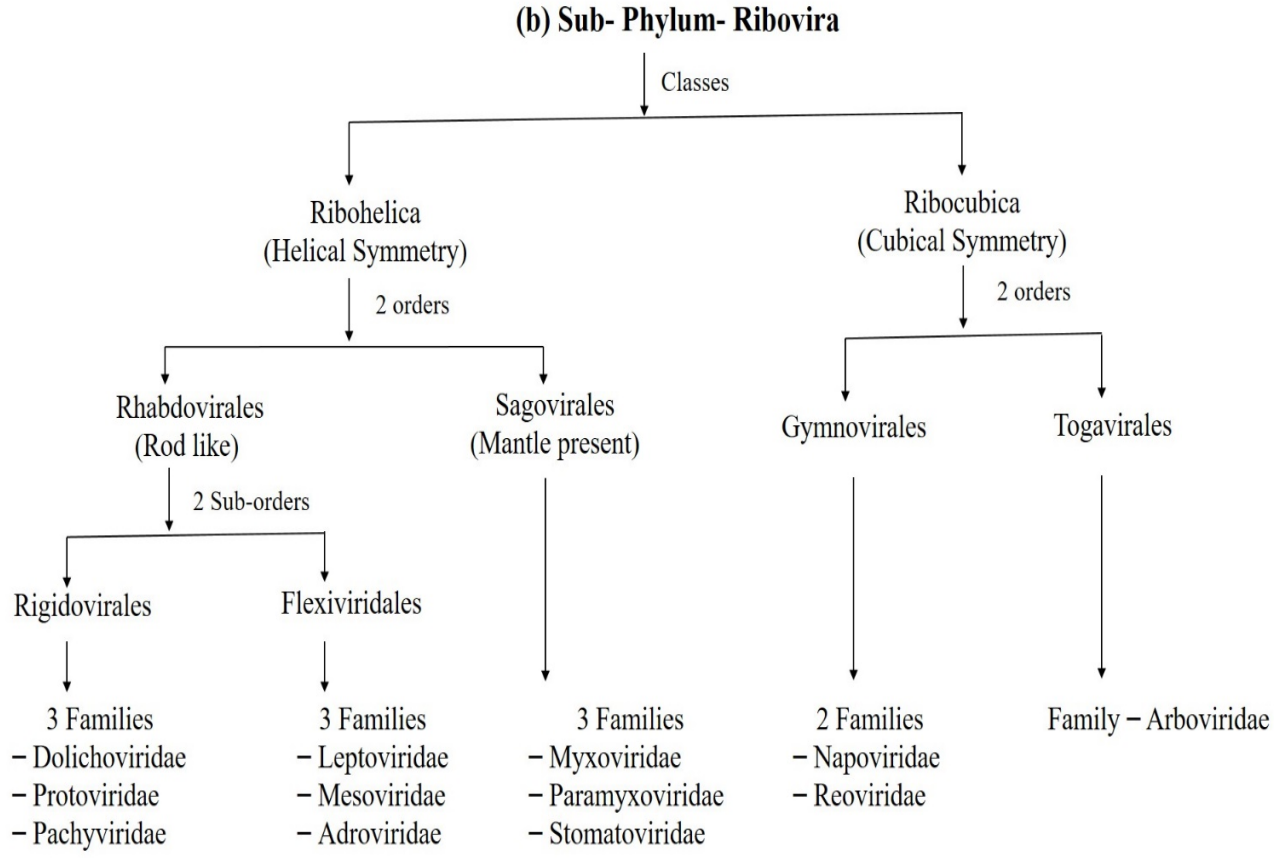
**(PCVN Classification)**

Phylum-vira is divided into two subphyla on the basis of type of nucleic acid present.

**(a) Sub-phylum:** Deoxyvira- These are DNA containing viruses. This subphylum is divided into three classes on the basis of the symmetry.




**(b) Sub-phylum:**Ribovira- This includes RNA containing viruses. These are also further divided on the basis of symmetry as follows:



## (B). David Baltimore classification

Baltimore classification is a classification system which places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), and method of replication.

- **Group I** : double-stranded DNA viruses
- **Group II** : single-stranded DNA viruses
- **Group III** : double-stranded RNA viruses
- **Group IV** : positive-sense single-stranded RNA viruses
- **Group V** : negative-sense single-stranded RNA viruses
- **Group VI** : reverse transcribing Diploid single-stranded RNA viruses
- **Group VII** : reverse transcribing Circular double-stranded DNA viruses

## (C). ICTV Classification

International Committee on Taxonomy of Viruses has suggested 'family' as the highest taxonomic category for viruses. The International Committee on Taxonomy of Viruses devised and implemented several rules on the naming and classification of viruses early in the 1990's. To this day they oversee the naming and placement of viral species into the framework. The system shares many features with the classification system of cellular organisms, such as taxon structure. Viral classification starts at the level of order and follows as thus, with the taxon suffixes given in italics:

**Order** − (*virales*)
**Family** − (*viridae*)
**Subfamily** − (*virinae*)
**Genus** − (*virus*)
**Species** − (*virus*)

However, this system of nomenclature differs from other taxonomic codes on several points. A minor point is that names of orders and families are italicized, as in the *ICBN*. Most notably, species names generally take the form of [*Disease*] *Virus*. The recognition of orders is very recent and has been deliberately slow; to date, only three have been named, and most families remain unplaced. Approximately 80 families and 4000 species of virus are known.

## (D) Classification of virus on the basis of genetic material

The classification of viral on the basis of nucleic acids is given in the Fig. 7.5. According to this classification the virus can be categorized as:

### DNA viruses:

- **Group I**: viruses possess double-stranded DNA and include such virus families as Herpesviridae (examples like HSV1 (oral herpes), HSV2 (genital herpes), VZV (chickenpox), EBV (Epstein-Barr virus), CMV (Cytomegalovirus)), Poxviridae (smallpox) and many tailed bacteriophages. The mimivirus was also placed into this group (Table 7.2).
- **Group II**: viruses possess single-stranded DNA and include such virus families as Parvoviridae and the important bacteriophage M13.

## RNA viruses:

- **Group III**: viruses possess double-stranded RNA genomes, e.g., rotavirus. These genomes are always segmented.
- **Group IV**: viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus.
- **Group V**: viruses possess negative-sense single-stranded RNA genomes. The deadly Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps and rabies.

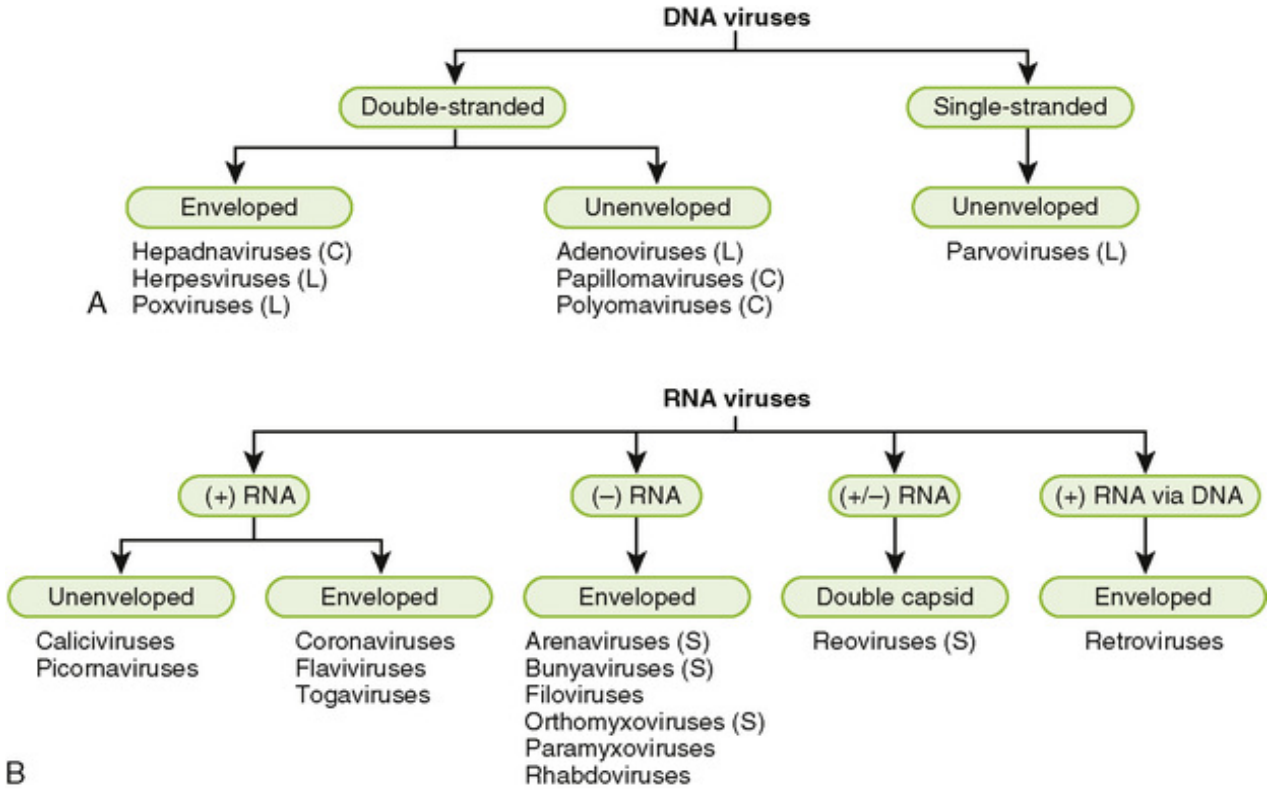



*Fig. 7.5: Viral classification on the basis of nucleic acids*

## Reverse transcribing viruses

- **Group VI**: viruses possess single-stranded RNA genomes and replicate using reverse transcriptase. The retroviruses are included in this group, of which HIV is a member.
- **Group VII**: viruses possess double-stranded DNA genomes and replicate using reverse transcriptase. The hepatitis B virus can be found in this group.

**(E)**.The different families of RNA viruses are distinguished from one another by their nucleic acid content, their capsid shape and the presence and absence of an envelope. Some important human, animal and plant virus families are given in Table 7.3.

**Table 7.2: Classification of major groups of DNA viruses that cause human, animal and plant diseases.**

| | Family | Size (nm) | Example | Infection/diseases |
|---|---|---|---|---|
| **[I]** | **Human and animal virus families** | | | |
| | **Double stranded linear DNA** | | | |
| 1. | Poxviridae | 250×350 | Orthopoxvirus | Small pox, cow pox |
| 2. | Adenoviridae | 75 | Human adenovirus | Conjunctivitis |
| 3. | Herpesviridae | 120-200 | Simplexvirus | Oral and genital herpes |
| | **Double stranded circular DNA** | | | |
| 4. | Papoviridae | 45-55 | Human papilloma viruses | Warts, cervical and Progressive Leukoencephalopathy (PML) |
| 5. | Baculoviridae | 40×400 | Baculoviruses | Polyhedrosis |
| 6. | Hepadnaviridae | 40×45 | Hepatitis B virus | Hepatitis B |
| **[II]** | **Plant virus families** | | | |
| | **Double stranded linear DNA** | | | |
| 7. | Caulimoviridae | 50 | Caulimovirus | Cauliflower mosaic |
| | **Single stranded circular DNA** | | | |
| 8. | Geminiviridae | 18×30 | Geminivirus | Maize streak |

**Table-7.3: Classification of major groups of RNA viruses that cause human, animal and plant diseases.**

| S.N. | Family | Size (nm) | Example | Infection/Diseases |
|---|---|---|---|---|
| **[I]** | **Human and animal virus families** | | | |
| | **(+) sense RNA virus** | | | |
| | 1.Picornaviridae | 8-30 | Entrovirus<br>Rhinovirus | Polio<br>Common cold |

| | | | | |
|---|---|---|---|---|
| | | | Hepatovirus | Hepatitis A |
| | 2.Togaviridae | 40-90 | Rubella virus | Rubella (German measles) |
| | | | Equine encephalitis virus | Equine encephalites |
| | | | HTLV-I | Adult leukemia, tumors, |
| | 3.Retroviridae | 100 | HIV | AIDS |
| | **(-) sense RNA virus** | | | |
| | 4.Paramyxoviridae | 150-200 | Morbillivirus | Measles |
| | 5.Rhabdoviridae | 70-180 | Lyssavirus | Rabies |
| | 6.Orthomyxoviridae | 100-200 | Influenza virus | Influenza A and B |
| | **Double stranded RNA virus** | | | |
| | 7. Reoviridae | 70 | Rotavirus | Respiratory and gastrointestinal infections |
| | **(+) sense RNA virus** | | | |
| | 8.Coronaviridae | 25 | | Avian bronchitis |
| **[II]** | **Plant virus families** | | | |
| | **(+) sense RNA virus** | | | |
| | 9.Tobamoviridae | 15×300 | Tobacco mosaic virus | Tobacco mosaic |
| | 10.Cucumoviridae | 30 | | Cucumber mosaic |
| | 11.Carlaviridae | 15×650 | | Carnation latent |
| | **(-) sense RNA virus** | | | |
| | 12.Tospoviridae | 90 | Tospovirus | Tomato spotted wilt |
| | 13.Rhabdoviridae | 70×170 | Rhabdovirus | Lettuce necrotic yellows |
| | **Double stranded RNA virus** | | | |
| | 14.Phytoreoviridae | 80 | Phytoreovirus | Wound tumor |

Based on information obtained from ultracentrifuge and electron microscopic studies Lwott, Home and Tournier (1962) proposed a system of classification of Viruses (known as LHT system) which was accepted by ICVN (International Committee for virus Nomenclature (1966). Classification is based upon (i) type of nucleic acid, (ii) Molecular weight of virus, (iii) Shape and size of virus, (iv) Symmetry of virus, (v) Number of protein subunits in a capsid, (vi) Diameter of nucleic acid coil, (vii) presence of outer envelope, (viii) Intercellular multiplication, (ix) temperature inactivation of virus, (x) method of viral transmission, (xi) symptoms of virus on the host plant.

## *7.6 REPLICATION OF VIRUS*

Viruses multiply only in living cells. The host cell must provide the energy and synthetic machinery and the low molecular-weight precursors for the synthesis of viral proteins and nucleic acids. **Viral replication** is the formation of biological viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur. From the perspective of the virus, the purpose of viral replication is to allow production

and survival of its kind. By generating abundant copies of its genome and packaging these copies into viruses, the virus is able to continue infecting new hosts. Replication between viruses is greatly varied and depends on the type of genes involved in them. Most DNA viruses assemble in the nucleus while most RNA viruses develop solely in cytoplasm. The virus replication (Fig. 7.6) occurs in seven stages, namely;

1- Adsorption/ Attachment
2- Entry
3- Uncoating
4- Replication
5- Transcription / mRNA production
6-Synthesis of virus components
7- Virion assembly
8- Release (Liberation Stage)

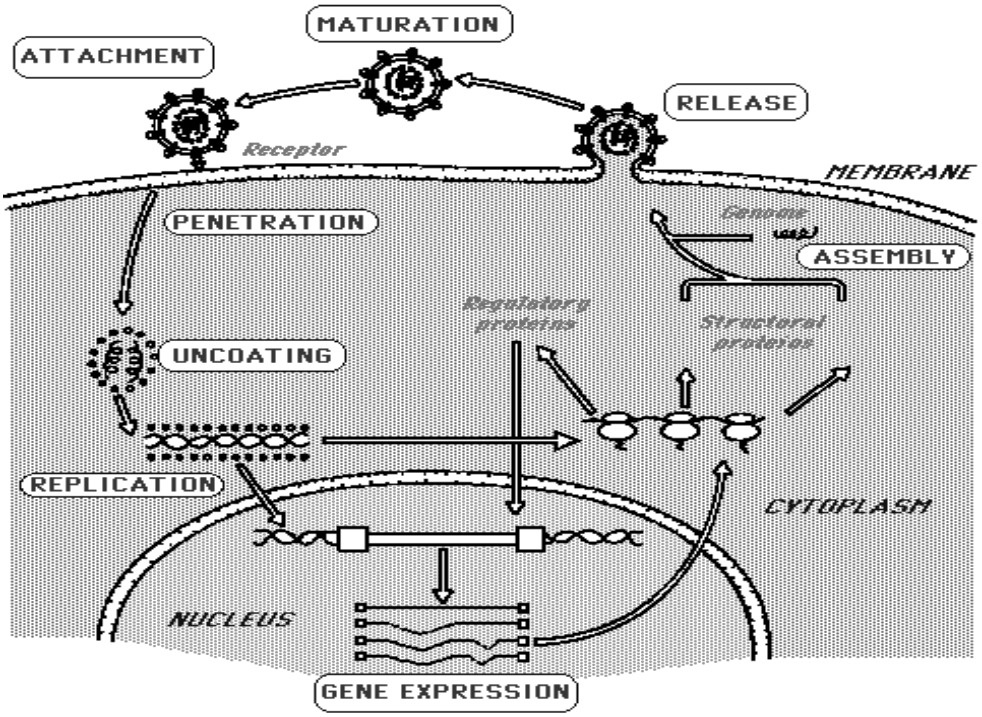



*Fig. 7.6: Generalized replication process in viruses*

**1. Adsorption:** The virus attaches to the cell membrane of the host cell. It then injects its DNA or RNA into the host to initiate infection. ***Attachment*** is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV infects a limited range of human leucocytes. This is because its surface protein, gp120, specifically interacts with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface

of CD4+ T-Cells. This mechanism has evolved to favour those viruses that infect only cells in which they are capable of replication. Attachment to the receptor can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

**2. Entry:** The cell membrane of the host cell invaginates the virus particle, enclosing it in a pinocytotic vacuole. This protects the cell from antibodies, as in the case of the HIV virus.
***Penetration*** follows attachment: Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called viral entry. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. Nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata. Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. Given that bacterial cell walls are much thinner than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

**3. Uncoating:** Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end-result is the releasing of the viral genomic nucleic acid.

**4. Replication:** Replication of viruses involves primarily multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins (Fig. 7.7 and 7.8).

**(a) Genome replication:** The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

**(i) DNA viruses:** The genome replication of most DNA viruses takes place in the cell's nucleus. If the cell has the appropriate receptor on its surface, these viruses enter the cell sometimes by direct fusion with the cell membrane (e.g., herpesviruses) or – more usually – by receptor-mediated endocytosis. Most DNA viruses are entirely dependent on the host cell's DNA, RNA synthesizing machinery and RNA processing machinery. Viruses with larger genomes may encode much of this machinery themselves. In eukaryotes the viral genome must cross the cell's nuclear membrane to access this machinery, while in bacteria it need only enter the cell.

**(ii) RNA viruses:** Replication usually takes place in the cytoplasm. RNA viruses can be placed into four different groups depending on their modes of replication. The polarity (whether or not it can be used directly by ribosomes to make proteins) of single-stranded RNA viruses largely determines the replicative mechanism; the other major criterion is whether the genetic material is single-stranded or double-stranded. All RNA viruses use their own RNA replicase enzymes to create copies of their genomes.

**(iii) Reverse transcribing viruses:** These have ssRNA (*Retroviridae*, *Metaviridae*, *Pseudoviridae*) or dsDNA (*Caulimoviridae*, and *Hepadnaviridae*) in their particles. Reverse transcribing viruses with RNA genomes (retroviruses), use a DNA intermediate to replicate, whereas those with DNA genomes (pararetroviruses) use an RNA intermediate during genome replication. Both types use a reverse transcriptase, or RNA-dependent DNA polymerase enzyme, to carry out the nucleic acid conversion. Retroviruses integrate the DNA produced by reverse transcription into the host genome as a provirus as a part of the replication process; pararetroviruses do not, although integrated genome copies of especially plant pararetroviruses can give rise to infectious virus. They are susceptible to antiviral drugs that inhibit the reverse transcriptase enzyme, e.g., zidovudine and lamivudine. An example of the first type is HIV, which is a retrovirus. Examples of the second type are the *Hepadnaviridae*, which includes Hepatitis B virus.

**5. Transcription / mRNA production:** For some RNA viruses, the infecting RNA produces messenger RNA (mRNA). This is translation of the genome into protein produces. For others with negative stranded RNA and DNA, viruses are produced by transcription then translation. The mRNA is used to instruct the host cell to make virus components. The virus takes advantage of the existing cell structures to replicate itself.

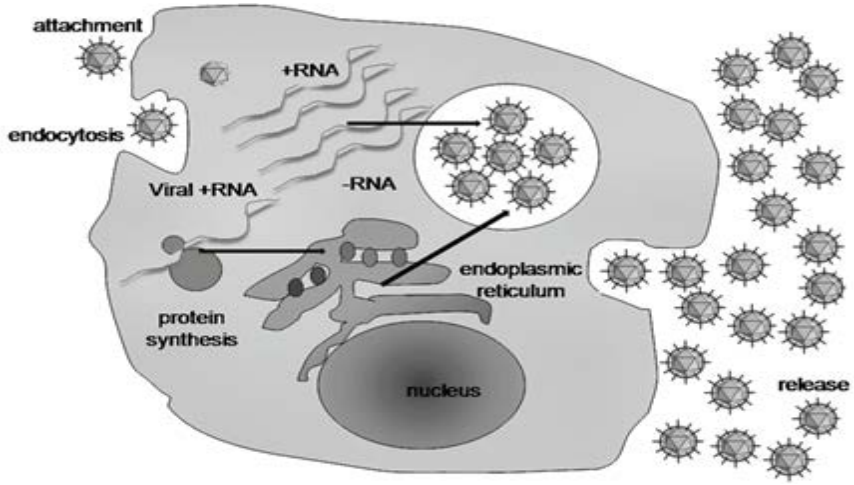



*Fig. 7.7:A typical virus replication cycle*

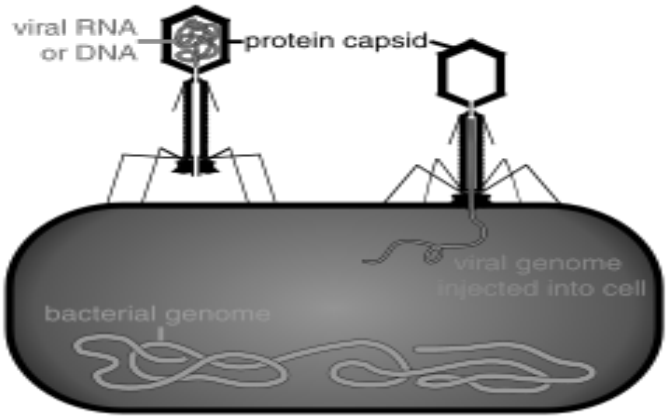



*Fig. 7.8: Replication process in typical bacteriophage*

**6. Synthesis of Virus components:** The following components are manufactured by the virus through the host's existing organelles:

1-Viral protein synthesis: virus mRNA is translated on cell ribosomes into two types of virus protein.
2- Structural: the proteins which make up the virus particle are manufactured and assembled.
3- Non – structural: not found in particle, mainly enzymes for virus genome replication.
4-Viral nucleic acid synthesis (genome replication) new virus genome are synthesized, templates are either the parental genome or with single stranded nucleic acid genomes, newly formed complementary strands. By a virus called polymerate or replicate in some DNA viruses by a cell enzyme. This is done in rapidly dividing cells.

**7. Assembly:** A virion is simply an active or intact virus particle. In this stage, newly synthesized genome (nucleic acid), and proteins are assembled to form new virus particles. This may take place in the cell's nucleus, cytoplasm, or at plasma membrane for most developed viruses. Following the structure-mediated self-*assembly* of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs after the virus has been released from the host cell.

**8. Release (Liberation Stage) –** The viruses, now being mature are released by either sudden rupture of the cell, or gradual extrusion (budding) of enveloped viruses through the cell membrane. The new viruses may invade or attack other cells, or remain dormant in the cell. The viruses can be *released* from the host cell by 'lysis', a process that kills the cell by bursting its membrane and cell wall if present: This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome (Fig. 7.9). The viral genome is then known as a "provirus" or, in the case of bacteriohages a "prophage". Whenever the host divides,

the viral genome is also replicated. The viral genome is mostly silent within the host. At some point, the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.

In the case of bacterial viruses, the release of progeny virions takes place by lysis of the infected bacterium. However, in the case of animal viruses, release usually occurs without cell lysis.

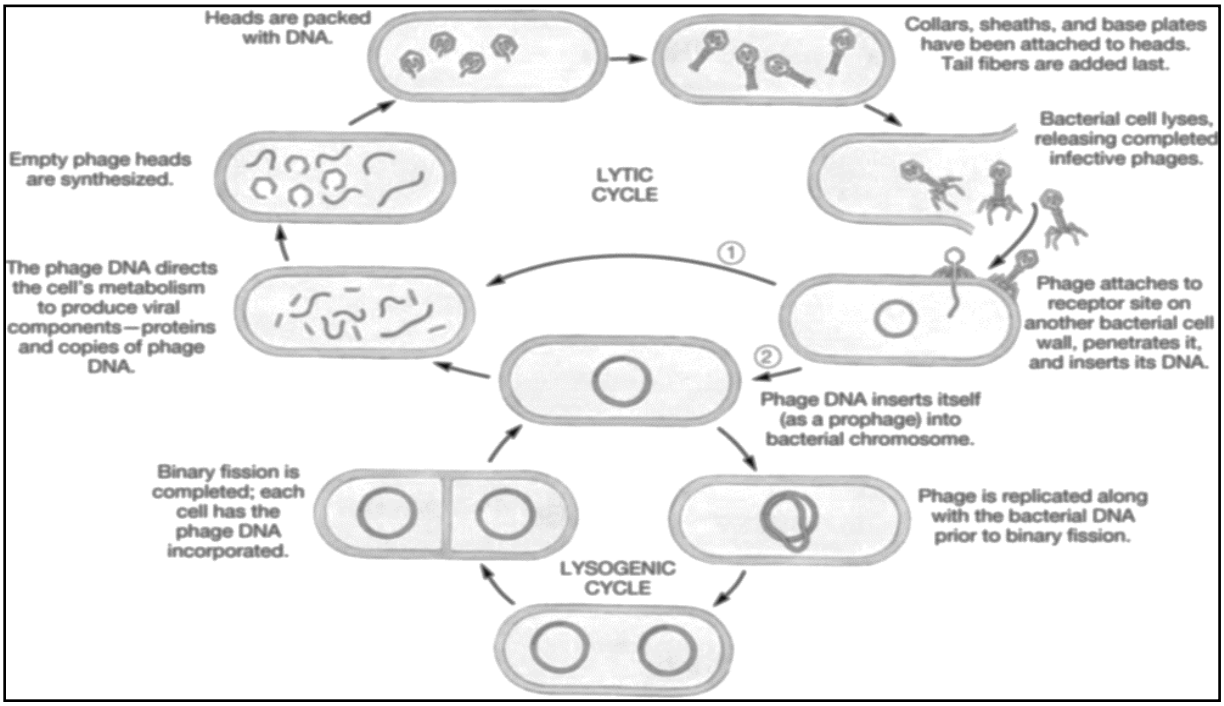



*Fig. 7.9: Lytic and lysogenic cycle in bacteriophage*

## Structure of Tobacco Mosaic Virus (TMV)

TMV is a simple rod-shaped helical virus consisting of centrally located single- stranded RNA (5.6%) enveloped by a protein coat (94.4%). The rod is considered to be 3,000 Å in length and about 180 Å in diameter. The protein coat is technically called ‘capsid’. R. Franklin estimated 2,130 sub-units, namely, capsomeres in a complete helical rod and 49 capsomeres on every three turns of the helix; thus there would be about 130 turns per rod of TMV.

The diameter of RNA helix is about 80 Å and the RNA molecule lies about 50 Å inward from the outer-most surface of the rod. The central core of the rod is about 40 Å in diameter. Each capsomere is a grape like structure containing about 158 amino acids and having a molecular weight of 17,000 dalton as determined by Knight.

The ssRNA is little more in length (about 3300 Å) slightly protruding from one end of the rod. The RNA molecule consists of about 7300 nucleotides; the molecular weight of the RNA molecule being about 25,000 dalton.

## Life-cycle (Replication) of Tobacco Mosaic Virus (TMV)

Plant viruses like TMV penetrate and enter the host cells in toto and their replication completes within such infected host cells (Fig. 7.10). Inside the host cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm.

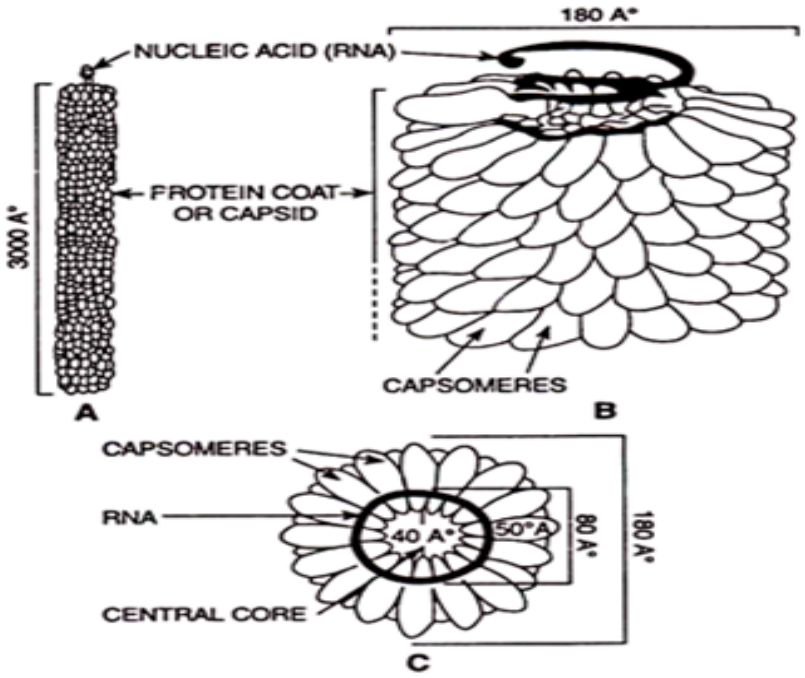



*Fig. 7.10: Tobacco Mosaic Virus (TMV) A. Surface View, B. An enlarged view showing RNA capsomere arrangement, C. View in section*

Although the sites for different steps of the viral multiplication and formation of new viruseshave not yet been determined with absolute certainty, the studies suggest ha alter becoming free in the cell cytoplasm the viral-RNA moves into the nucleus (possibly into the nucleolus).The viral-RNA first induces the formation of specific enzymes called 'RNA polymerases' the single-stranded viral-RNA synthesizes an additional RNA strand called replicative RNA.

This RNA strand is complementary to the viral genome and serves as 'template' for producing new RNA single strand which is the copy of the parental viral-RNA. The new viral-RNAs are released from the nucleus into die cytoplasm and serve as messenger-RNAs (mRNAs). Each mRNA, in cooperation with ribosomes and t-RNA of the host cell directs the synthesis of protein subunits.

After the desired protein sub-units (capsomeres) have been produced, the new viral nucleic acid is considered to organize the protein subunit around it resulting in the formation of complete virus particle, the virion. No 'lysis' of the host cell, as seen in case of virulent bacteriophages, takes place. The host ells remain alive and viruses move from one cell to the other causing systemic infection. When transmitted by some means the viruses infect other healthy plants.

## *7.7 SUMMARY*

A **virus** is a small infectious agent that replicates and show living properties only inside the living cells of other organisms. They can infect all types of life forms, from multicellular organisms to unicellular organisms.

Viruses of all shapes and sizes consist of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope. Viruses are classified into four groups based on shape: filamentous, isometric (or icosahedral), enveloped, and head and tail.

Many viruses attach to their host cells to facilitate penetration of the cell membrane, allowing their replication inside the cell.

Non-enveloped viruses can be more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.

The virus core contains the small single- or double-stranded genome that encodes the proteins that the virus cannot get from the host cell.

Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble in the cell.

## *7.8 GLOSSARY*

**Assembly**: The gathering and replication of viruses within a cell by using the metabolism of the host organism.

**Attachment**: The condition where the capsid proteins of the virus bind to certain receptors of the host organism.

**Capsid**: The protein covering of a virus particle.

**Envelope**: A lipid casing that surrounds the capsid that covers a virus. A viral envelope assists the virus in infiltrating the cells of the host organism.

**Gene Expression**: An activity where information from a gene is made into functional gene material.

**Genome Replication**: The reproduction of genetic material, particularly that in the structure of DNA.

**Latent Infection**: A viral infection that exists in dormancy and does not exhibit symptoms.

**Maturation**: The phase during replication at which a virus becomes infectious.
**mRNA**: A form of ribonucleic acid which carries copied genetic information from DNA to the cell ribosome.
**Neucleocapsid**: The composition of a virus that includes the DNA, RNA, and the capsid protein cover.
**Penetration**: The process of the virus entering the cell of the host organism, causing infection.
**Receptor**: A specific type of molecule found on a cell membrane that a virus is able to attach to.
**Release**: The process of the death of a host cell that discharges a virus.
**Uncoating**: A condition when the protein capsid of the virus is unsheathed due to enzymes of the cells of the host organism.
**Vector**: Insects, such as mosquitoes or ticks, that carries disease from one organism to another.
**Virions**: A virus particle, which invades the cells of a host organism, causing infection.
**Virus Attachment Protein**: A specific protein found on a virus in charge of fixating to the receptor.

## *7.9 SELF ASSESSMENT QUESTIONS*

### 7.9.1: Multiple Choice Questions:

1. Double Stranded RNA viruses are called-
(a) Ribovirus (b) Pox virus
(c) Riovirus (d) None of the above

2. A virus that can reproduce without killing its host-
(a) Temperate virus (b) Lytic virus
(c)Retroactive virus (d) Virion

3. Coliphage x 174 contains
(a) Single stranded DNA (b) Single stranded RNA
(c) Double stranded DNA (d) Double stranded RNA

4. In the lytic cycle of bacteriophage, the host DNA is-
(a) Replicated (b) Digested into its nucleotides
(c) Turned off by a protein coat (d) Turned on by removal of a protein coat

5. The enzymes involved in viral replication are synthesized:
(a) On the viral ribosomes (b) By the host cell
(c) On the interior surface of the viral coat (d) On the interior surface of the viral membrane

6. When a virus attacks the bacterium, the material that enters the host cell is-
(a) Protein coat (b) Nucleic acid

(c) Both Protein coat and Nucleic acid (d) None of the above

7. Who discovered TMV-
(a) Bawden (b) Iwanowski
(c) Stanley (d) Twort and d'Herelle

8. Leaf curl of papaya is caused by-
(a) Fungus (b) Mycoplasma
(c) Virus (d) Bacteria

9. Who isolated plant viruses first-
(a) W. M. Stanley (b) E.C. Stakman
(c) D. Iwanowski (d) K.M. Smith

10. Double Stranded DNA (dsDNA) is found in:
(a) Herpes virus (b) TMV
(c) Reovirus (d) Coliphage virus

**Answer Keys:** 1-(c), 2-(a), 3-(a), 4-(b), 5-(b), 6-(b), 7-(b), 8-(c), 9-(c), 10-(a)

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## *7.12 TERMINAL QUESTIONS*

1. Give an illustrated account of the morphology and chemical structure of the virus.
2. Draw a well labeled diagram of T.M.V. and write an account of its multiplication.
3. Give a general account of morphology of different types of viruses.
4. Give an illustrated account of morphology and chemical structureof virus.
5. Write Short Notes on the following:
    (i) Nomenclature of virus
    (ii) Replication of phage particles
    (iii) Viruses are living agents

# UNIT-8 PATHOGENESIS OF INFECTION, TRANSMISSION OF VIRUSES AND BACTERIOPHAGES

## Contents:

## *8.1 OBJECTIVE*

In the present unit student will be able to-

- Know what is pathogenesis?
- learn the Mechanism of pathogenesis
- understand about the Transmission of virus in plants
- know the General composition and structure of bacteriophage
- learn Lytic and Lysogenic multiplication cycle

## *8.2 INTRODUCTION*

Pathogenesis is the actual mechanism of disease development and this phase involves complex host-pathogen interactions. The causal organism of a disease is pathogen. Pathogenesis is the process of infection or the actual way in which the disease develops in plant body infection is the establishment of pathogenic microorganisms within the host, following entrance. It signifies the sum of biological processes which take place in the host body after penetration of the pathogen, independent of the fact whether the pathogen cause a disease or not. As the result of infection visible or latent diseases are produced in the host plants. Infect, infection is process of inter struggle between the organisms (pathogen and host) living in two different environmental conditions. The potential capacity of infection of any pathogen is called its pathogenicity. The degree of pathogenicity is known as virulence.

In the first section of this unit we will cover pathogenesis and its mechanism emphasizing mainly in animal system, in second section we will be familiar with transmission of the viruses in plant system while in the last section we will cover important aspects of the bacteriophages. Pathogenic mechanisms of viral disease include (1) implantation of virus at the portal of entry, (2) local replication, (3) spread to target organs (disease sites), and (4) spread to sites of shedding of virus into the environment. Most virus types spread among cells extracellularly, but some may also spread intracellularly. Establishment of local infection may lead to localized disease and localized shedding of virus. Virions implant onto living cells mainly via the respiratory, gastrointestinal, skin-penetrating, and genital routes although other routes can be used. The final outcome of infection may be determined by the dose and location of the virus as well as its infectivity and virulence.

Depending on the balance between virus and host defenses, virus multiplication in the target organ may be sufficient to cause disease and death. Accessibility of virus to tissue, cell susceptibility to virus multiplication, and virus susceptibility to host defenses are certain factors that affect pathogenic mechanisms. Since viruses are obligate parasites, these can be transmitted (spread) only by modes which can ensure their carriage in a living condition –mechanically by

rubbing of healthy and diseased leaves through e.g., fools, insects, vegetative propagated bodies etc. In nature the transmission occur most commonly through insects (vectors) rarely by air, water or soil.

Animal viruses are generally transmitted t by water and fodder *e.g.,* foot and mouth disease virus. Ticks, dog and mosquitoes are also known to transport some viral diseases. Plant viruses are chiefly transmitted by Seed, by Vegetative Propagation, by Mechanical Means by Soil, by Insect, by Fungi and by nematode.

Bacteriophages (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria.).There are many similarities between bacteriophages and animal cell viruses. Thus, bacteriophages can be viewed as model systems for animal cell viruses.

## *8.3 PATHOGENESIS OF INFECTION*

Pathogenesis is a process in which an initial infection becomes a disease. **Viral pathogenesis** is the study of how biological viruses cause diseases in their target hosts, usually carried out at the cellular or molecular level. It is a specialized field of study in virology. Viral disease is the sum of the effects on the host caused by the replication of the virus and of the host's subsequent immune response. Viruses are able to initiate infection, disperse throughout the body, and replicate due to virulence characteristics. Mechanisms of pathogenesis of viral diseases include: implantation of the virus at the portal of entry, local replication, spread to target organs, and shedding of the virus into the environment. There are several factors that affect pathogenic mechanisms. Some of these factors include virulence characteristics of the virus that is infecting. In order to cause disease, the virus must overpower several inhibitory effects present in the host. Some of the inhibitory effects include distance, physical barriers, host defense, and conflicting cellular susceptibilities. These inhibitory effects may differ among individuals and different races due to the inhibitory effects being genetically controlled.

There must be sufficient virus available to initiate the infection. Cells at the site of infection must be accessible, susceptible, and allow the virus to enter, and the host anti-viral defense systems must be ineffective or absent. There are several mechanisms in **animal virus** that must occur for a viral disease to develop.

**8.3.1 Implantation at Portal of Entry:** The virus must implant at the entry portal into the body. Implantation is the earliest stage of pathogenesis. Implantation frequency is at its greatest where viruses directly contact living cells. Animal viruses usually implant on cells of respiratory, gastrointestinal, skin and genital tissues. Some viruses are capable of implanting in a fetus through infected germ cells at the time of fertilization. The virulence of the virus and implantation point is a couple of factors that impact the severity of the disease.

**1-Local Replication and Local Spread:** Local replication and spread of the virus follows implantation. Replicated virus from the initially infected cell has the capability to disperse to neighboring extracellular fluids or cells. Spread occurs by the neighboring cell being infected or the virus being released into extracellular fluid.

**2-Replication:** The invading virus must reproduce itself in large numbers. It usually does this intracellularly.

**3-Dissemination in Nerves:** The spread of virus through the nerves is less common than the spread through the bloodstream.

**4-Dispersal:** The replicated viruses must spread to target organs (disease sites) throughout the body. The most common route of spread from the portal of entry is the circulatory system, which the virus reaches via the lymphatic system. Viruses can access target organs from the blood capillaries by multiplying inside endothelial cells, moving through gaps, or by being carried inside the organ on leukocytes. Some viruses, such as Herpes, rabies and polio viruses, can also disseminate via nerves.

**5-Shedding:** The viruses must spread to sites where shedding into the environment can occur. The respiratory, alimentary and urogenital tracts and the blood are the most frequent sites of shedding.

Fortunately for the survival of humans and animals (and hence for the infecting virus), most natural selective pressures favor the dominance of less virulent strains. Because these strains do not cause severe disease or death, their replication and transmission are not impaired by an incapacitated host. Mild or in apparent infections can result from absence of one or more virulence factors. For example, a virus that has all the virulence characteristics except the ability to multiply at elevated temperatures is arrested at the febrile stage of infection and causes a milder disease than its totally virulent counterpart. Live virus vaccines are composed of viruses deficient in one or more virulence factors; they cause only in apparent infections and yet are able to replicate sufficiently to induce immunity.

The occurrence of spontaneous or induced mutations in viral genetic material may alter the pathogenesis of the induced disease, e.g., HIV. These mutations can be of particular importance with the development of drug resistant strains of virus.

Disease does not always follow successful virus replication in the target organ. Disease occurs only if the virus replicates sufficiently to damage essential cells directly, to cause the release of toxic substances from infected tissues, to damage cellular genes or to damage organ function indirectly as a result of the host immune response to the presence of virus antigens.

Other factors that determine whether infection and disease occur are the many virulence characteristics of the infecting virus. To cause disease, the infecting virus must be able to overcome the inhibitory effects of physical barriers, distance, host defence, and differing cellular susceptibilities to infection. The inhibitory effects are genetically controlled and therefore may vary among individuals and races. Virulence characteristics enable the virus to initiate infection, spread in the body, and replicate to large enough numbers to impair the target organ. These factors include the ability to replicate under certain circumstances during inflammation, during the febrile response, in migratory cells, and in the presence of natural body inhibitors and interferon. Extremely virulent strains often occur within virus populations. Occasionally, these strains become dominant as a result of unusual selective pressures. The viral proteins and genes responsible for specific virulence functions are only just beginning to be identified.

## 8.3.2 Cellular Pathogenesis

Pathogenesis at the cellular level can be viewed as a process that occurs in progressive stages leading to cellular disease. As noted above, an essential aspect of viral pathogenesis at the cellular level is the competition between the synthetic needs of the virus and those of the host cell. Since viruses must use the cell's machinery to synthesize their own nucleic acids and proteins, they have evolved various mechanisms to subvert the cell's normal functions to those required for production of viral macromolecules and eventually viral progeny. The function of some of the viral genetic elements associated with virulence may be related to providing conditions in which the synthetic needs of the virus compete effectively for a limited supply of cellular macromolecule components and synthetic machinery, such as ribosomes.

## 8.3.3 Tissue Tropism

Most viruses have an affinity for specific tissues; that is, they display tissue specificity or tropism. This specificity is determined by selective susceptibility of cells, physical barriers, local temperature and pH, and host defences. Many examples of viral tissue tropism (Animal virus) are known. Polioviruses selectively infect and destroy certain nerve cells, which have a higher concentration of surface receptors for polioviruses than do virus-resistant cells. Rhinoviruses multiply exclusively in the upper respiratory tract because they are adapted to multiply best at low temperature and pH and high oxygen tension. Enteroviruses can multiply in the intestine, partly because they resist inactivation by digestive enzymes, bile, and acid. The cell receptors for some viruses have been identified. Rabies virus uses the acetylcholine receptor present on neurons as a receptor, and hepatitis B virus binds to polymerized albumin receptors found on liver cells. Similarly, Epstein-Barr virus uses complement CD21 receptors on B lymphocytes, and human immunodeficiency virus uses the CD4 molecules present on T lymphocytes as specific receptors.

Viral tropism is also dictated in part by the presence of specific cell transcription factors that require enhancer sequences within the viral genome. Recently, enhancer sequences have been

shown to participate in the pathogenesis of certain viral infections. Enhancer sequences within the long terminal repeat (LTR) regions of Moloney murine leukemia retrovirus are active in certain host tissues. In addition, JV papovavirus appears to have an enhancer sequence that is active specifically in oligodendroglia cells, and hepatitis B virus enhancer activity is most active in hepatocytes.

### 8.3.4 Factors affecting Pathogenic mechanisms

**1-Accessiblity the host tissues are to the virus**: The degree to which the tissues of the body and organs are accessible. Accessibility is affected by physical barriers (for example: tissue barriers and mucus). It is also impacted by the distance to be travelled through the body and by the natural defence mechanism.

**2-Susceptibility the host cells are to virus multiplication:** Infection is only capable of occurring if virus replicating cells are present. Cellular susceptibility needs a receptor for the virions and an intracellular environment that allows the virus to replicate and release.

**3-Host defence mechanism:** Host defence may inhibit replication. To be able to cause disease, the virus needs to be able to overcome the preventative effects of physical barriers, host defence, and contradicting cellular susceptibilities to infection.

## *8.4 TRANSMISSION OF VIRUSES*

Since viruses are obligate parasites, these can be transmitted (spread) only by mode s which can ensure their carriage in a living condition –mechanically by rubbing of healthy and diseased leaves through e.g., fools, insects, vegetative propagated bodies etc. In nature the transmission occur most commonly through insects (vectors) rarely by air, water or soil. Animal viruses are generally transmitted by water and fodder *e.g*., foot and mouth disease virus. Ticks, dog and mosquitoes are also known to transport some viral diseases. Plant viruses are chiefly transmitted by following eight methods. These methods are:

**1-Seed Transmission of Virus:** Transmission through the seeds of the host plant was earlier considered to play a minor part in the spread of virus diseases. Recently Bennett (1969) listed 53 viruses which are transmitted by seeds of about 124 plant species.

The seeds are important in the spread of a few viruses of legumes, wild cucumber, tomatoes, and curly top virus of beet sugar. In the latter case the seeds carry a high percentage of the virus. The virus, however, does not enter the embryo. It is carried in a portion of the seed of the diseased plants.

**2-Transmission by Vegetative Propagation:** It is one of the chief methods of transmission of virus diseases especially of Potato, Rose, Sugarcane, Raspberry, Strawberry, Turnips, Bulb plants, fruit trees and many ornamentals. The vegetative parts, the infected plants such as the tubers, bulbs, roots, offshoots, buds and scions which are used for propagation, will contain the virus present in the parent. The new plants raised by the above-mentioned vegetative methods are nearly always infected.

**3-Transmission by Mechanical Means:** Many mosaic viruses are transmitted mechanically from diseased plants to healthy ones by the following methods:

(i) By contact of infected and healthy leaves brought about by wind.
(ii) By rubbing the juice of the diseased plants over the surface of the leaves of healthy plants.
(iii) By grafting infected buds on to healthy plants.
(iv) Agricultural implements also play quite an important part. The knife used for cutting the seed pieces and the pruning shears will spread the disease.
(v) Some viruses spread below ground by contact between the roots of diseased and healthy plants.
(vi) Handling plants at planting time and in cultural operation will also help in the spread of viruses such as Sugar beet. Curly top virus and Cucumber mosaic virus.

**4-Transmission by *Cuscuta*:** In many cases Dodder (*Cuscuta*) serves as a transmitting agent and an effective bridge between the infected host and the healthy plants by establishing intimate biological contact through its haustoria.

**5- Transmission by soil:** Quite a number of viruses are transmitted through the soil. Common examples of soil borne viruses are Potato mosaic virus, Oat mosaic, Wheat mosaic, etc. In all these cases the disease is contracted from the soil.

**6-Insect transmission:** Some plant and animal viruses are spread and complete particles introduced into host cells by arthropod vectors and even by dog-bite as in rabies. Among the arthropods most important agents of spread of virus diseases are the insects.

The insect which carries the disease is called a vector. The insect vectors which play a major role in the dissemination of plant viruses are the Aphids, Leafhoppers, Flee beetles, Scale insects, thrips and White flies.

Most of the insect vectors are sucking insects. Aphids transmit more plant viruses than any other insects. Leafhoppers come next in the list. About three hundred plant virus diseases are known to have insert vectors.

The insert obtains virus through its mouth parts at the time of feeding on the diseased plant. It is then inoculated in the healthy plant by means of the mouth part. Inoculation in many cases must be in a certain tissue or upon young leaves.

The virus may remain active in the body of the vector for many days. Instances are however, known when infectivity is soon lost. There are also cases where a vector cannot infect a healthy plant immediately after it has fed on a diseased plant.

There is delay in the development of infective power within the vector. This period of development of infectivity for the virus within the vector is called the incubation period. The duration of the incubation period varies with different viruses from a few hours to days

There also appears to be some relationship between the plant viruses and the insect vectors which transmit them. The precise nature of this relationship is still unknown. The virus disease of sugar beet known as curly leaf or curly top is spread by the leaf-hopper *Circulifer tenellus.* Other sucking insects which feed on sugar beet are unable to transmit this virus. On the other hand peach aphid is the vector of Sugar beet mosaic virus. The leafhopper does not transmit this virus. Thrips transmit the spotted-wilt virus. All vectors of yellow group of viruses are leafhoppers and of mosaic group are aphids.

**7-Transmission by fungi:** The first proof of the fungus as a vector of plant viruses was found by Gorgon in 1958. Fie found that the diseased lettuce was invariably infected by a soil *chytrid, Olpidium.* Later he discovered that the fungus acts as a reservoir and vector of the big vein virus.
The virus acquired by the fungus remains in the oospore. The latter germinates and produces the zoospores which function as infective agents and penetrate lettuce roots. Similarly tobacco necrosis virus has been reported by Teakle (1960) to enter roots of its host by the zoospores of *O. brassicae.*

**8- Some soil inhabiting viruses have nematode vectors:** Animal viruses may gain access to the higher animals through the mouth and nose from dust or contaminated food. Besides infection from outside, virus may also be transmitted from cell to cell but the internal transmission need not be in the form of virus particles.

## *8.5 BACTERIOPHAGES*

A **bacteriophage** also known informally as a ***phage***, is a virus that infects and replicates within Bacteria and Archaea. Bacteriophage was discovered by Frederick W. Twort (1915) and Felix d Herelle (1917) independently while investigating certain types of soil bacteria. They observed that if few drops of highly concentrated bacterial viruses are introduced into a dish with nutrient

medium seeded with a culture, then there was no growth of bacteria at the point of the introduction of the virus. It observed that when bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes and as many as hundreds of genes. Phages replicate within the bacterium following the injection of their genome into its cytoplasm. Bacteriophages are among the most common and diverse entities in the biosphere. Bacteriophages are ubiquitous viruses, found wherever bacteria exist (Fig. 8.1).

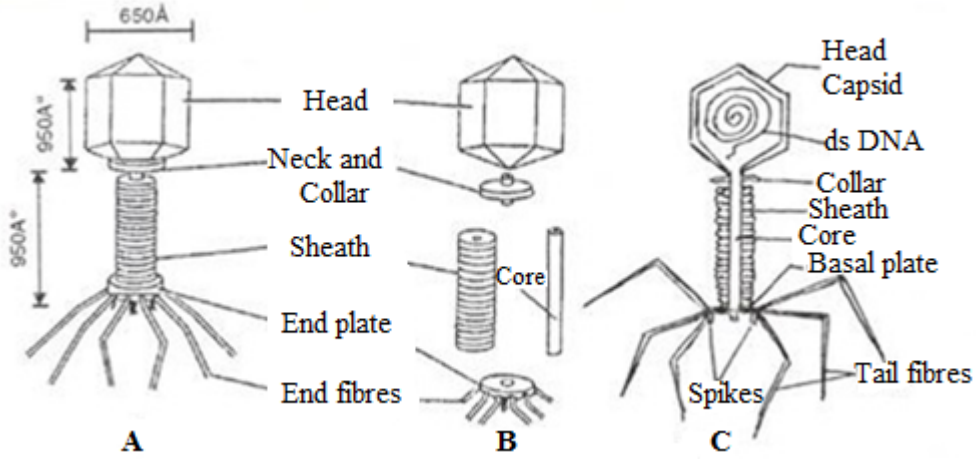


*Fig. 8.1: A $T_4$ Bacteriophage A- External structure B- Parts, C- L.S. of a virion*

Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to $9\times10^8$ virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.

## 8.5.1 Composition of Bacteriophage

Although different bacteriophages may contain different materials they all contain nucleic acid and protein. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acids of phages often contain unusual or modified bases. These modified bases protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. The size of the nucleic acid varies depending upon the phage. The simplest phages only have enough nucleic acid to code for 3-5 average size gene products while the more complex phages may code for over 100 gene products.

The number of different kinds of protein and the amount of each kind of protein in the phage particle will vary depending upon the phage. The simplest phage has many copies of only one or two different proteins while more complex phages may have many different kinds. The proteins function in infection and to protect the nucleic acid from nucleases in the environment.

**Structure:** Bacteriophages have different sizes and shapes. T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length. In their appearance the phage resembles a tadpole or spermatozoid. It is differentiated into a head and tail. In most phages (T1, T2, T6) the head is prismoid but T3 and T7 it is hexagonal.

**Head:** All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid. The size of the head of T2 phages is approximately $950A^0$ X $650A^0$. The extended part between the head and tail is called collar.

**Tail:** Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers or tail pins are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances other structures are involved in binding of the phage particle to the bacterium. Tail fibers or tail pins have to main functions: (i) They help in the adsorption of phage particle on the surface of the bacteria and (ii) The enzymes secreted by the pins are helpful in the lysis of bacterium cell wall.

**Chemical Composition:** These phage particles are made up of protein (about 50-60 %) and nucleic acids (40-50%). They also contain a small proportion of lipids in the form of neutral fats. The wall of the head is composed of some 2,000 similar subunits of proteins. The nucleic acid of phages is either double-stranded DNA, single stranded DNA or single stranded RNA (DNA and RNA are never present together). Except for coliphage, Ø x 174 and few others, most phages have double stranded DNA. The phage DNA differs from bacterial DNA chemically. The molecular weight of phage DNA is 2,500,000, and in each phage particle the amount of nucleic acid is approximately $6X10^{-3}$mg. DNA is the genetic material of the phage particle; it carries infection and induces the host cell to synthesize more and more phage particles.

## 8.5.2 Classification of Bacteriophage

Bacteriophages occur abundantly in the biosphere with different virions, genomes, and lifestyles. Phages are classified by the **International Committee on Taxonomy of Viruses (ICTV)** according to morphology and nucleic acid (Fig. 8.2).

Nineteen families are currently recognized by the ICTV that infect bacteria and archaea. Of these, only two families have RNA genomes, and only five families are enveloped. Of the viral

families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and one (*Tectiviridae*) infects both bacteria and archaea.

**(A) On the basis of presence of single or double strands of genetic material, the bacteriophages are categorized as under:**

**1. The ssDNA Bacteriophages:**

**(i) Icosahedral phages:** φx 174, St-1, φR, BR2, 6SR U3 and G series, e.g., G4, G6, G13, G16. All are like φx 174.

**(ii) Helical (filamentous)**

a) The Ft group: They are F specific phages and absorb to the tip of F type sex pilus e g E. coli phages (fd, fl, M13).
b) If group: They are absorbed to I-type sex pilus specified by R factors, e.g., $If_1$, $IF_2$, etc.
c) The third group is specific to strains carrying $RF_1$ sex factor.

**2. The dsDNA Phages: Following are the examples of dsDNA phages:**

(i) T-odd phage of *E. coli*, e.g., T1, T3, T5, T7.
(ii) T-even phage of *E. coli* e.g., T2, T4, T6.
(iii) The other *E. coli* phages, e.g., P1, P2, Mu, φ80.
(iv) The phages of *Bacillus subtilis*, e.g., PBS 1, PBSX, SPO1, SPO2.
(v) The phage of *Shigella* a, e.g., P2.
(vi) The phage of *Salmonella* e.g., PI, P22.

**3. The ssRNA phages. Examples of the ss RNA bacteriophages are as below:**

**(i) Group I:** E. coli. phages such as f2, MS2, M12, R17, fr, etc.
**(ii) Group II:** The Qβ phages.

**4. The dsRNA phages:** e.g., The φ6 bacteriophage.

**(B) On the basis of Electron Microscopic studies, Bradley (1967) has described the following six morphological types of bacteriophages.**

**Type A:** This type of virus has hexagonal head, a rigid tail with contractile sheath and tail fibers dsRNA, T-even (T2, T4, T6) phages.

**Type B:** This type of phage contains a hexagonal head but lacks contractile sheath. Its tail is flexible and may or may have tail fiber, for example dsDNA phages, *e.g.*, T1, T5 phages.

**Type C:** Type C characterized by a hexagonal head and a tail shorter than head. Tail lacks contractile sheath and may or may not have tail fiber, for example dsDNA phages, *e.g.*, T3, T7.

**Type D:** Type D contains a head which is made up of capsomeres but lacks tail, for example ssDNA phages (e.g., φX174).

**Type E:** This type consists of a head made up of small capsomeres but contains no tail, for example ssRNA phages (e.g., F2, MS2).

**Type F:** Type F is a filamentous phage, for example ssDNA phages (e.g., fd, f1).

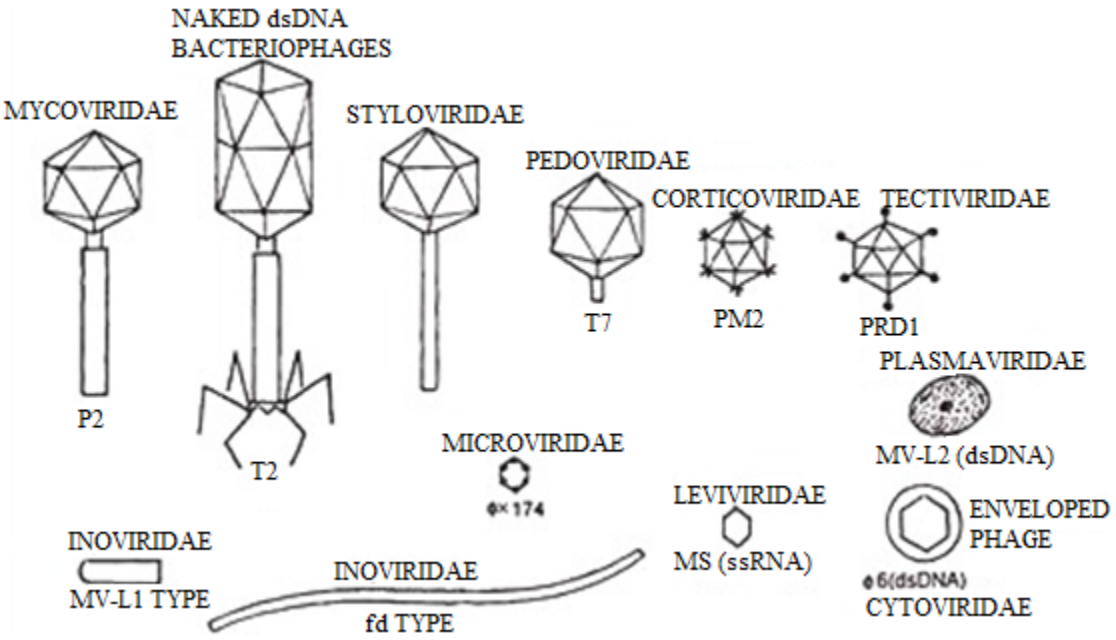


*Fig. 8.2: Families of bacteriophages*

Further a group G is recently discovered which has a lipid containing envelope and has no detectable capsid, for example a dsRNA phage, MV-L2.

## 8.5.3 Replication of Bacteriophage

Nucleic acid in a virion does not contain all the genes needed for the synthesis of new viruses. Although genes governing the synthesis of virion's structural components are present in its nucleic acid, the enzyme needed for protein synthesis, ribosomes, t-RNA and for energy production are supplied by the host cell. These are used for synthesizing viral proteins including viral enzymes. Thus for a virus to multiply, it must invade a host cell and take over the host's metabolic machinery. Once inside the host cell, even a single virus can give rise thousands of viruses.

Although the basic mechanism of penetration and multiplication is similar in all the viruses, the process is best studied in bacteriophages. Phages can be multiplied by two alternate methods-(i)

lytic cycle (Fig. 8.3), and (ii) lysogenic cycle (Fig. 8.6). The lytic cycle ends with the death or lysis of the host cell, whereas the host cell remains alive in the lysogenic cycle.

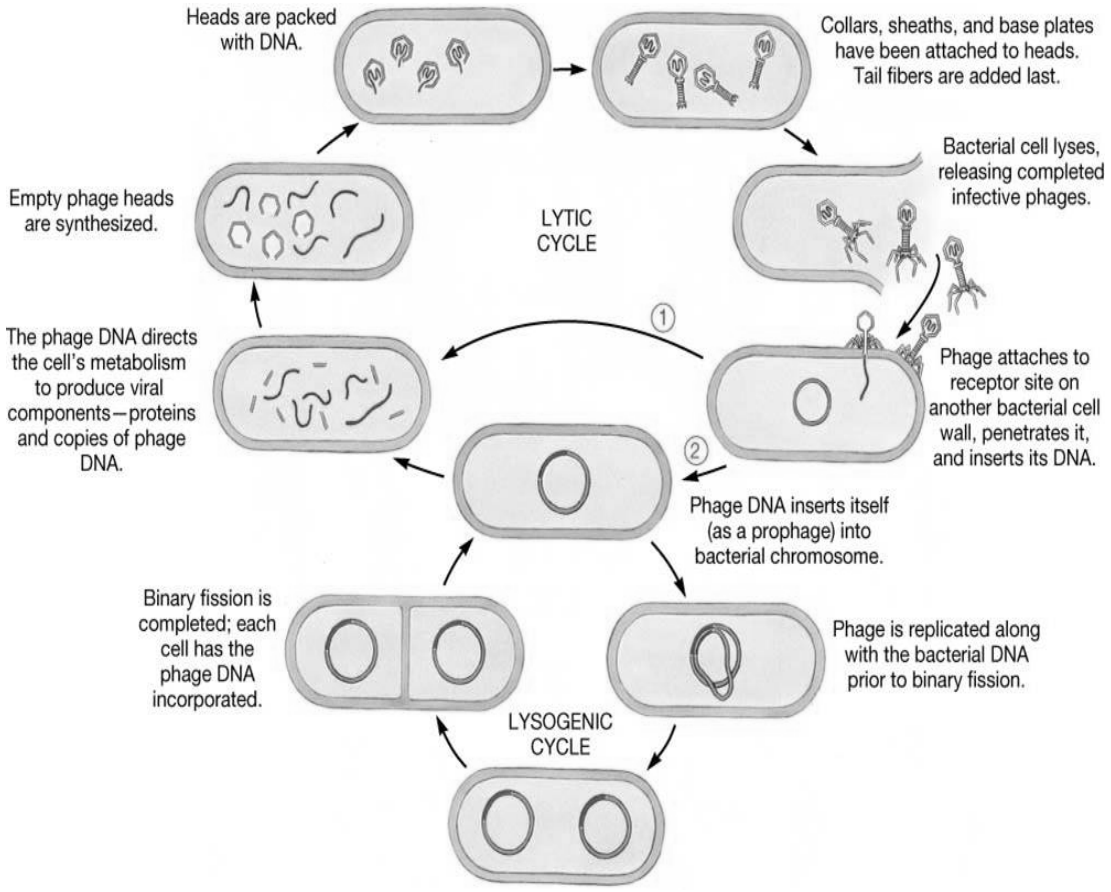


*Fig.8.3: Comparision of lytic and lysogenic cycle*

The life cycle of bacteriophage T even and λ (lambda) are being described here as an example of the lytic and lysogenic cycle respectively.

**(A) Lytic Cycle:** Multiplication of T-even bacteriophage in their host cell (*E. coli*) is an example of the lytic cycle.

**T-Even Phages (dsRNA Virulent Phages):**

The T-even phages ($T_2$, $T_4$ and T6) are homologous and much of our knowledge about bacteriophages is based on them, particularly $T_4$ phage.

**1. Structure:** The T-even phage is characterized by the presence of a hexagonal head about 900 $Å^0$ wide. It consists of dsDNA molecule protected by a protein coat made up of numerous facets. The DNA molecule, measuring about 52,000 $Å^0$ in length, is coiled and packed inside the head. The head is attached with a cylindrical tail consisting of a hollow core surrounded by protein sheath. The hollow central core measures about 80-100 $Å^0$ in diameter and is considered

continuous from the head to the end of the tail forming a channel through which the nucleic acid moves into invade the host cell being infected. The protein sheath is spirally coiled and is connected to a thin disc-like structure called 'collar' at the base of the head and to a hexagonal 'end plate' at the end of the tail. The protein sheath of the tail is capable of contracting in the longitudinal direction. At the six corners of the hexagonal plate there are small 'spikes' to which very long fibers called 'tail-fibres' are connected. The tail fibres are the organs of attachment to the wall of the bacterial cell (Fig. 8.4 & 8.5).

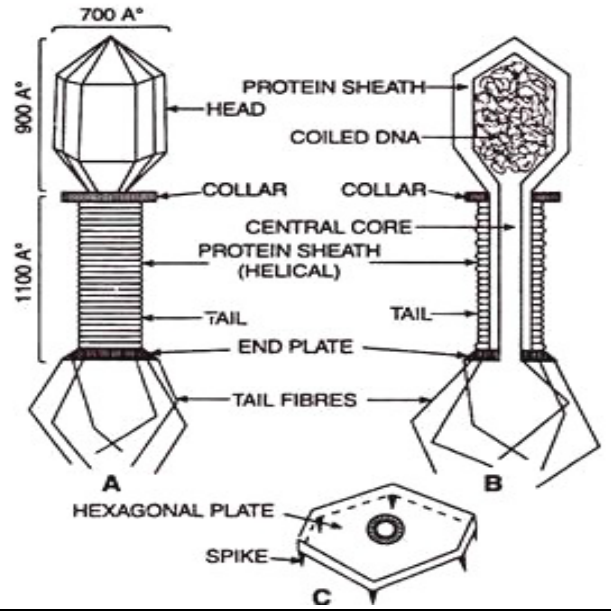


*Fig. 8.4: A. External structure; B. Internal structure; C. end plate*

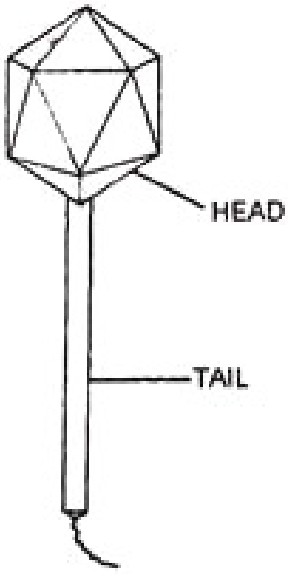


*Fig. 8.5: Structure of lambda ((λ)) bacteriophage*

**2. Life-Cycle (Multiplication or Infection Cycle):** The infection cycle of T-even bacteriophage lasts about 20 minutes, culminating in lysis (bursting-open) of the host cell, *E. coli.*

**The whole process can be classified into:**

**(i) Adsorption or infection:** Attachment of the virus particle onto the surface of the host cell is adsorption or infection. The virus particles possess one or more proteins on the outside that interact with cell surface components called receptors; the receptors are normal surface components of the host (e.g., proteins, carbohydrates, glycoproteins, lipids, lipoproteins, etc.). Infect, these are the receptors that determine which cells will be susceptible-to infection. In the absence of the receptor site, the virus cannot adsorb and hence cannot infect. If the receptor site is altered, the host may become resistant to virus infection.

**(ii) Penetration or Injection:** This process has been studied and beautifully elucidated by B. Kellenberger. After the tail fibres get adsorbed, an 'enzyme-system' is supposed to make a pore' or 'hole' in the cell wall of the host. It is believed that the enzyme-system consists of a 'phage-

lysozyme', which is synthesized during the multiplication of the parent phage inside the host cell and its molecules remain attached to the extreme tip of the tail-fibres of the new progeny phages. This enzyme-system becomes active when the released phage particle infects the new host cell. However, the tail-fibres attached on the surface of the host cell bend to bring the end-plate in contact with the cell wall surface. Now, the protein sheath of the tail longitudinally contracts pushing the central tubular core through the pore inside the wall of the host cell and the phage DNA molecule is released or injected into the cytoplasm. After the DNA is released, the empty protein coat becomes of no use.

**(iii) The Eclipse or the Latent Period:** When the DNA molecule is released in the host cytoplasm, it is not degraded by the nuclease enzymes of the host cell. It has been studied, particularly in $T_4$ phage, that the phage DNA contains glucosylated hydroxymethyl cytosine instead of cytosine, which prevents the nucleases of the bacterium from degrading the phage DNA. The phage DNA, first makes the host cell immune against infection by genetically similar phage particles. Secondly, it immediately takes over the charge of the cell machinery and suppresses all cellular activities such as synthesis of cellular DNA, RNA, proteins, etc. This is the parasitism of a virus at the genetic level. This suppression is short lived and the cell machinery of protein synthesis starts functioning under the control of viral DNA in the place of cellular-DNA.

New messenger-RNA molecules are synthesized very rapidly and a series of new enzymes, namely, 'early proteins' is synthesized. Some of the early proteins are used as enzymes for the viral DNA synthesis. The newly synthesized viral DNA molecules direct the formation of new type of proteins, namely, 'late proteins'. Majority of the late proteins are viral coat proteins, whereas some are phage lysozyme. The viral coat proteins constitute the sheath of the phage and the phage-lysozyme later help in the injection process.

**(iv) Maturation:** Assembly of the various components to constitute a new phage particle within the host cell is called 'maturation'. Head and tail formation start separately; the protein components aggregate around the DNA and form the head of the phage. End-plate is formed first followed by the formation of tubular core. Tail fibres are formed later. Hundreds (about 200) of new phage particles are produced from each bacterium by the time of lysis.

**(v) Lysis or Release:** After the production of new bacteriophages, the host bacterial cell bursts open and the phage particles are released. Bursting open of the host bacterial cell is called 'lysis'.

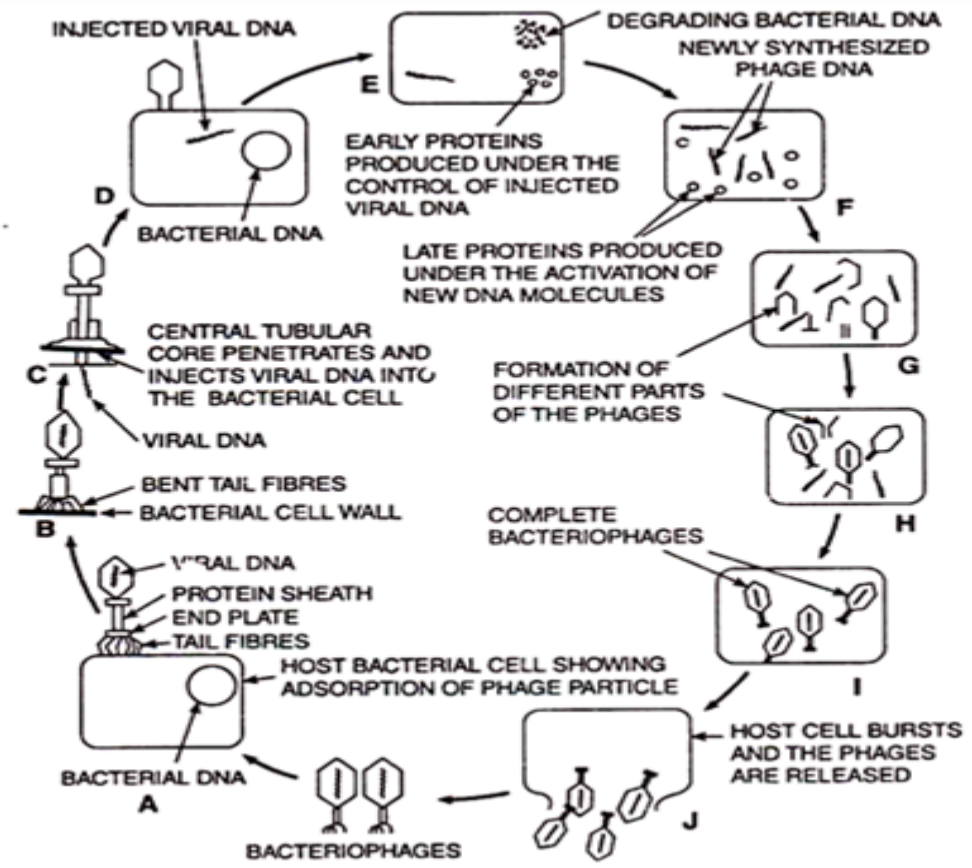


*Fig. 8.6: Lytic –cycle of T-even bacteriophage A. adsorption, B,C,D- penetration or infection, E&F eclipse or latent stage. G,H,I= maturation, J- lysis*

**(B) Lysogenic Cycle:** Some phages do not cause death (lysis) of the host cell when they multiply. In the lysogenic cycle the phage DNA gets incorporated into the host cell's DNA and the host cell multiplies indefinitely along with phage nucleic acid. Phage multiplies by this method are known as lysogenic phages or temperate phages and the participating host cells are called lysogenic cells (Fig. 8.6) .

**Lambda (λ) Phage (dsDNA Temperate Phages):**

**1. Structure:** Morphological structure of phage λ, which infects *E, coli* K12.The phage λ contains double stranded (ds) circular DNA of about 17 µm in length packed in protein head of capsid. The head is icosahedral, 55 nm in diameter consisting of 300-600 capsomeres (subunits) of 37,500 dalton. The capsomeres are arranged in clusters of 5 and 6 subunits i.e., pentamers and hexamers.

The head is joined to a non-contractile 180 µm long tail by a connector. There is a hole in capsid through which passes this narrow neck portion expanding into a knob like structure inside. The

tail possesses a thin tail fibre (25 nm long) at its end which recognises the hosts. Also, the tail consists of about 35 stacked discs or annuli. Unlike T-even phage, it is a simple structure devoid of the tail sheath.

**2. Life-Cycle (Multiplication):** The bacteriophage is first adsorbed on the host wall surface and its DNA is injected into the bacterial cell cytoplasm. The viral DNA, instead of starting lytic cycle, gets inserted into the bacterial DNA and travels through many generations by means of the successive divisions of the cell.

Under certain conditions, the inserted viral DNA may get dissociated from the bacterial DNA, and start functioning as virulent phage culminating in the lysis of the host cell. Such conversion of temperate phage (especially pro-phage) into the virulent phage is referred to as 'induction', which can be artificially achieved by treatment of the bacterial cells with ultraviolet radiation or with hydrogen peroxide.

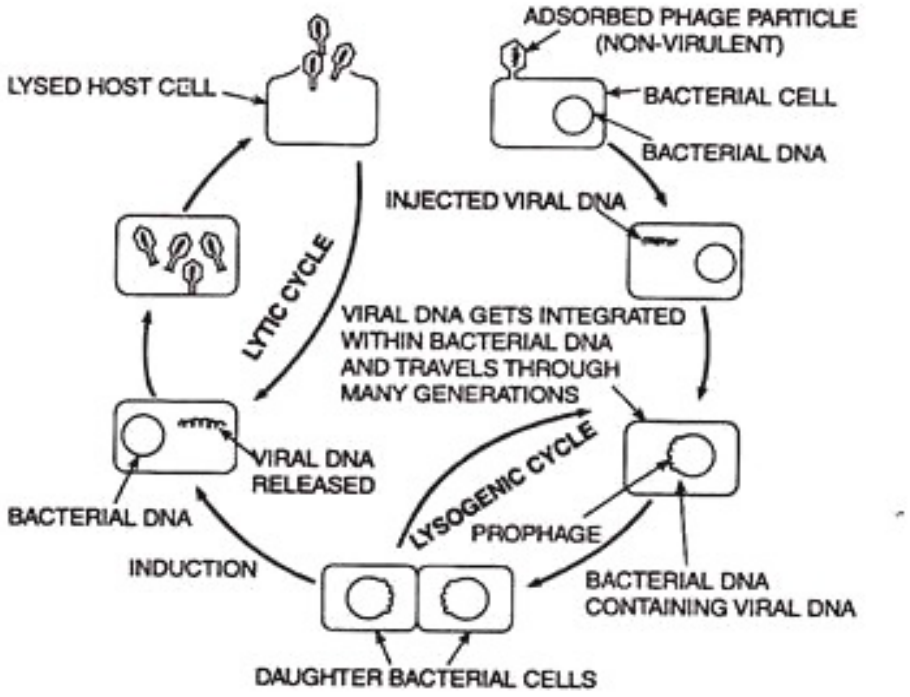


*Fig. 8.7: Life cycle of lysogenic phage (λ) Phage*

## *8.6 SUMMARY*

The term *pathogenesis* refers to the processes related to disease induction; therefore, *viral pathogenesis* often refers to disease induction by a virus rather than the process of infection. It is most useful to consider the pathogenesis of infection independently of whether or not severe or immediate disease is induced. As the pathogenesis of infection is analyzed, the pathogenesis of

disease can be considered as a subset of events that occur *in vivo* during infection. Fundamentally important mechanisms are revealed when one considers how the pathogenesis of infection differs between the host with asymptomatic infection or minimal disease and the host doomed to suffer severe consequences of viral infection.

Viruses are obligate parasites, these can be transmitted (spread) only by modes which can ensure their carriage in a living condition –mechanically by rubbing of healthy and diseased leaves through e.g., fools, insects, vegetative propagated bodies etc. In nature the transmission occur most commonly through insects (vectors) rarely by air, water or soil. Animal viruses are generally transmitted t by water and fodder *e.g*., foot and mouth disease virus. Ticks, dog and mosquitoes are also known to transport some viral diseases.

Plant viruses are chiefly transmitted by eight methods: Transmission by vegetative propagation, Transmission by friction and rubbing; Transmission through alternate host, Through soil and seeds, through pollen grains, through fungi, Through insects, Transmission by infested agricultural tools. A **bacteriophage,** also known informally as a ***phage***, is a virus that infects and replicates within Bacteria and Archaea. The term was derived from "bacteria" and the Greek *phagein* "to devour". Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Although the basic mechanisms of penetration and multiplication are similar in all the viruses, the process is best studied in bacteriophages. Phages can be multiplied by two alternate methods-(i) lytic cycle, and (ii) lysogenic cycle. The lytic cycle ends with the death or lysis of the host cell, whereas the host cell remains alive in the lysogenic cycle.

## *8.7 GLOSSARY*

**Acute Infection**: An active infection from a virus that may have severe symptoms or be occurring over a short period of time.

**Chronic Infection**: An infection from a virus that lasts an extended period of time. Symptoms may vary in severity.

**Envelope**: A lipid casing that surrounds the capsid that covers a virus. A viral envelope assists the virus in infiltrating the cells of the host organism.

**Latent Infection**: A viral infection that exists in dormancy and does not exhibit symptoms.

**Matrix protein**: A type of protein that connects the components of the viral envelope to the nucleus of the virus.

**Maturation**: The phase during replication at which a virus becomes infectious.

**mRNA**: A form of ribonucleic acid which carries copied genetic information from DNA to the cell ribosome.

**Neutralization**: The process of rendering a specific virus ineffective by a particular viral antibody.

**Neucleocapsid**: The composition of a virus that includes the DNA, RNA, and the capsid protein cover.
**Penetration**: The process of the virus entering the cell of the host organism, causing infection.
**Persistent Infection**: A situation where a virus continues to exist within a host. Symptoms may be manifested or in a state of remission, but the virus remains.
**Polyprotein**: A protein that splits to form various polypeptides. Certain viruses produce polyproteins.
**Receptor**: A specific type of molecule found on a cell membrane that a virus is able to attach to.
**Release**: The process of the death of a host cell that discharges a virus.
**Tropism**: The growth or movement of an organism in a specific direction that is provoked by an outside stimulant.
**Uncoating**: A condition when the protein capsid of the virus is unsheathed due to enzymes of the cells of the host organism.
**Vector**: Insects, such as mosquitoes or ticks, that carries disease from one organism to another.
**Virions**: A virus particle, which invades the cells of a host organism, causing infection.
**Virus Attachment Protein**: A specific protein found on a virus in charge of fixating to the receptor.

## *8.8 SELF ASSESSMENT QUESTIONS*

### 8.8.1 Multiple Choice Questions:

1. Who is bacterium eater?
(a) Coliophage (b) Bacteriophage
(c) Cyanophage (d) TMV

2. Bacteriophage was discovered by:
(a) Griffth (b) Subramanian
(c) Twort (d) None of the above

3. Genetic material in a bacterophage is:
(a) RNA (b) DNA
(c) Both DNA and RNA (d) Neither DNA nor RNA

4. Bacteriophages are:
(a) An organelle of the bacterium
(b) Bacterium which infects higher plant cells:
(c) Bacterium which infects an animal cell
(d) Virus which infects bacterium

5. Phage which shows lysogenic cycle are called:

(a) Temperate phage (b) Lytic phage
(c) Virulent phages (d) None of the above

6. Which is correct:
(a) A virion is a fully developed virus particle (b) A virion is a capsid
(c) A virion is a capsomere (d) None of the above

7. Virus synthesizes their protein coat:
(a) Inside the host cell (b) Outside the host cell
(c) Both outside and inside of the host cell (d) None of these

8. How many tail fibres are there in T4 Bacteriophge:
(a) 04 (b) 06
(c) 10 (d) 12

9. Who coined the term viroid:
(a) K.M. Smith (b) E.C. Stakman
(c) D. Iwanowski (d) T.O. Diener

10. Proteinaceous infectious particles are known as:
(a) Bacteria (b) viriods
(c) Prions (d) virus

**8.8.1 Answer Key:** 1-(b), 2-(c), 3-(b), 4-(d), 5-(a), 6-(a), 7-(a), 8-(b), 9-(d), 10-(c)

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## *8.10 SUGGESTED READINGS*


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## *8.11 TERMINAL QUESTIONS*

1- Give an illustrated account of the morphology and chemical structure of the bacteriophage.
2- Draw a well labelled diagram of any lytic phage write an account of its multiplication.
3- Give a general account of morphology of different bacteriophages.
4- Give an illustrated account on mode of transmission of plant viruses
5- Write Short Notes on the following:
   (i) Cellular pathogenesis
   (ii) Tissue tropism
   (iii) Comparision of lytic and lysogenic cycle

# UNIT-9 STRUCTURE, NUTRITION, REPRODUCTION AND ECONOMIC IMPORTANCE OF BACTERIA

## Contents:

## 9.1 OBJECTIVES

After reading this unit you will be able:

- To understand the structure of bacteria.
- To know various modes of nutrition in bacteria.
- To understand various types of reproduction in bacteria.
- To know about various useful and harmful activities of bacteria
- To know about latest major groups of bacteria.

## 9.2 INTRODUCTION

Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometers in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory. The study of bacteria is known as bacteriology.

Bacteria were first observed by the Dutch microscopist Antonie van Leeuwenhoek in 1676. Christian Gottfried Ehrenberg introduced the word "bacterium" in 1828. Louis Pasteur demonstrated in 1859 that the growth of microorganisms causes the fermentation process, and that this growth is not due to spontaneous generation. Robert Koch, a pioneer in medical microbiology, worked on cholera, anthrax and tuberculosis. In his research into tuberculosis Koch finally proved the germ theory, for which he received a Nobel Prize in 1905. Ferdinand Cohn is said to be a founder of bacteriology, studying bacteria from 1870. Cohn was the first to classify bacteria based on their morphology. In 1910, Paul Ehrlich developed the first antibiotic and was awarded Nobel Prize in 1908 for his work on immunology, and pioneered the use of stains to detect and identify bacteria, with his work being the basis of the Gram stain and the Ziehl–Neelsen stain. A major step forward in the study of bacteria came in 1977 when Carl Woese recognised that *Archaea* have a separate line of evolutionary descent from bacteria. This new phylogenetic taxonomy depended on the sequencing of 16S ribosomal RNA, and divided prokaryotes into two evolutionary domains Bacteria and Archaea.

There are typically 40 million bacterial cells in one gram of soil and a million bacterial cells in a millilitre of fresh water. There are approximately $5\times10^{30}$ bacteria on Earth, forming a biomass which exceeds that of all plants and animals. Bacteria are vital in many stages of the nutrient cycle by recycling nutrients such as the fixation of nitrogen from the atmosphere. They also play a vital role in decomposition of dead bodies. In the biological communities surrounding

hydrothermal vents and cold seeps, extremophile bacteria provide the nutrients needed to sustain life by converting dissolved compounds, such as hydrogen sulphide and methane, to energy. The bacteria are also reported from depth of up to 11 kilometres in ocean (deepest known part of the oceans) and even from inside rocks up to 580 metres below the sea floor under 2.6 kilometres of ocean off the coast of the northwestern United States. Thus when we talk about Bacteria habitat "You can find microbes everywhere—they're extremely adaptable to conditions, and survive wherever they are." In a human body the largest number exist in the gut flora, and a large number on the skin. Mostly of them are rendered harmless by the immune system, though many are beneficial. However several species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy, etc. Now a days they are used in sewage treatment and the breakdown of oil spills, production of cheese and yogurt through fermentation, and the recovery of gold, palladium, copper and other metals in the mining sector, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

## *9.3 STRUCTURE OF BACTERIA*

The structure of a typical bacterial cell (Fig. 9.1) can be studied under following headings-

### 1. Shape and Size

The bacterial cell shows considerable variation in their shape, but all individuals of a species have almost same shape. This can be a good method to identify bacteria and thus major morphological groups can be established. Cells are of two basic type coccoid forms and rod shaped (Fig. 9.2).

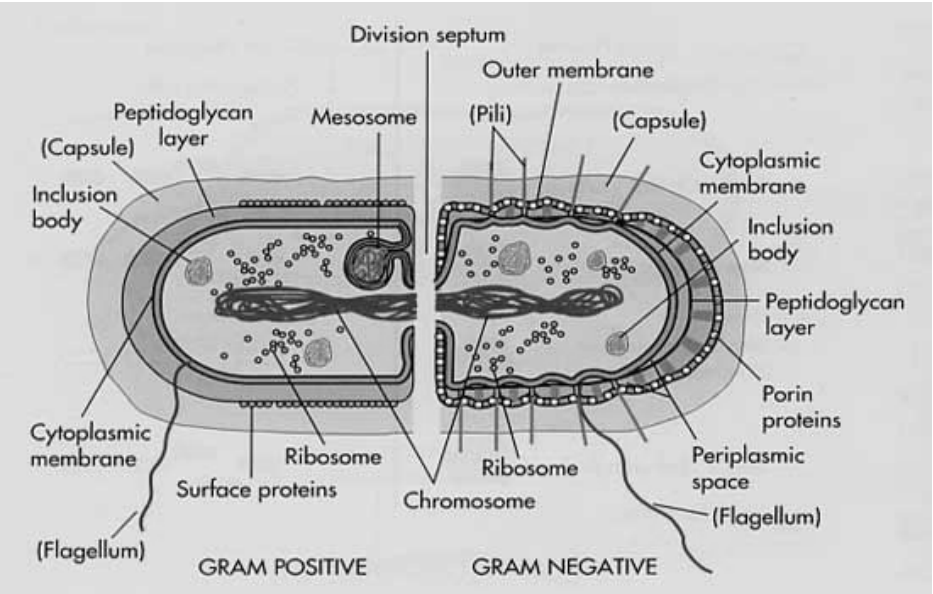



*Fig. 9.1: A typical bacterial cell*

**(A) Coccus or spherical:** The spherical or coccoid bacteria are called cocci (singular coccus). They measures 0.5-2.5 µm in diameter. They are non-motile, atrichous and often occur in chains or in clusters of variable size and shapes. On the basis of the arrangement and number of cells in a cluster the cocci are classified into following six groups:

| | | | |
|---|---|---|---|
| **i** | **Micrococci** | **:** | When cocci occur singly, known as micrococci e.g., *Micrococcus cerolyticus, M. cryophilus, M. luteus*. |
| **ii** | **Diplococci** | **:** | Spherical bacteria in pair, are called as diplococcic. e.g., *Diplococcus pneumonial*. |
| **iii** | **Streptococci** | **:** | Spherical bacteria when occur in long chains are called as streptococci, e.g., *Streptococcus lactis, S. pyogenes.* |
| **iv** | **Tetracocci** | **:** | When they form groups of four cells, called as tetracocci. e.g., *Pedicoccus cerevisiae*, *Neisseria*. |
| **v** | **Staphylococci** | **:** | An irregular group of many spherical bacteria is known as staphylococcus. e.g., *Staphylococcus albus, S.aureus.* |
| **vi** | **Sarcinae** | **:** | When spherical bacteria divide in three planes in a regular pattern producing a cuboidal arrangement of cells, they are said to be sarcinae. e.g., *Sarcinae lutea, S.verticuli.* |

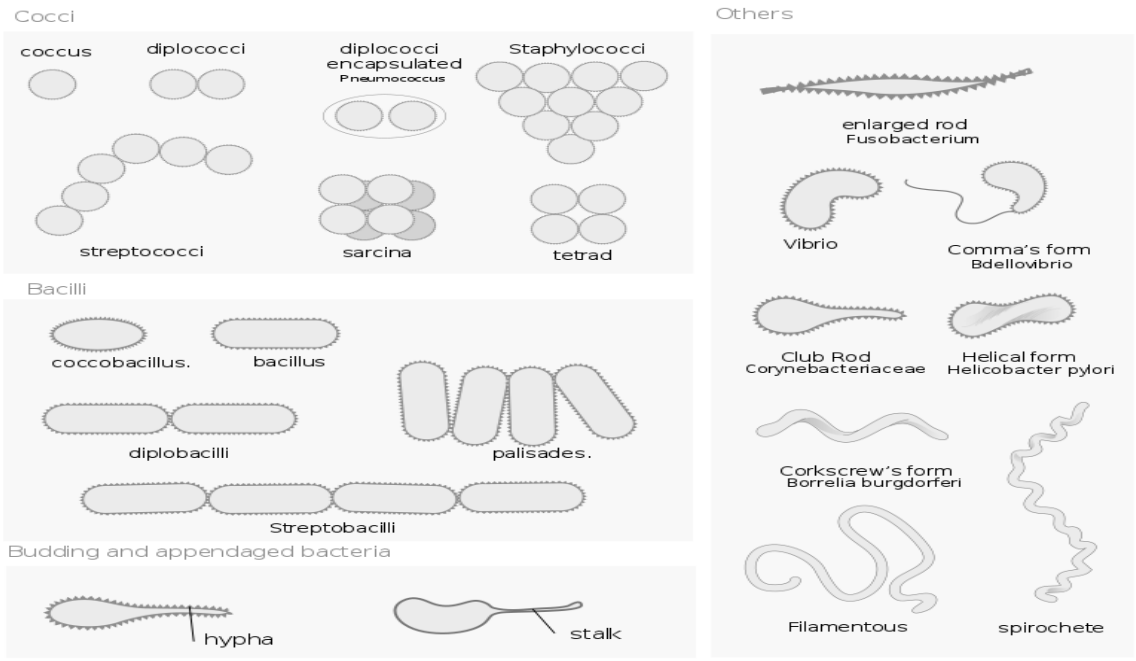



*Fig. 9.2: Diagram showing different shapes of bacteria*

**(B) Bacillus or rod-shaped bacteria:** Bacillus forms are rod shaped cells which are usually elongated. They may be motile or non-motile. Their ends may be rounded or blunt, short or long and narrow. The best example is Lactobacillus, Bacillus megatarium (isolated/in chains) which

are rigid and they may be motile. These rod like structures may be 0.5-3 µ x 100 µ (within range of 10-15 µ). The Bacillus can be grouped into two: (I) Diplobacillus (two), e.g., Corynebacteria and (II) Streptobacillus (chain) e.g., *Bacillus tuberculosis, B. cereus*.

**(C) Spiral or helical bacteria:** Some other forms may be spiral or helix e.g., *Spirillum undulum, S. volutans* has more than one turn of a helix. It is about 1.5 µ in diameter and upto 15 µ in length. They may have one or more flagella at each pole. They usually occur singly or in small chains but are seldom found in groups. Besides the above mentioned three major morphological types, bacteria also occur in the following shapes.

i **Vibrios** **:** These are slightly curved rods of half turn. They resemble with the sign of comma (,) and are commonly known as 'comma bacteria'. A vibrio bears a single flagellum at its tip and about 10 µm in length and 1.5-1.7 µm in width. e.g., *Vibrio coli, V.choleral.*

ii **Filamentous** : Some bacteria such as *Beggiatoa* and *Thiothrix* are filamentous. Some bacteria are capable of changing their shape and size temporarly in response to changes in the surrounding environment.

iii **Pleomorphic** **:** As such a single bacterium may occur in more than one shape in the life cycle. For example, *Acetobacter* may occur in *bacillus,* or *streptobacillus* depending on the environment.

## 2. Slime layer/ Capsule

Slime layer is a gelatinous layer present on the outer surface of cell wall, composed of polysaccharides and polypeptide chain of amino acids. When its constituents are only polysaccharides which form a viscous layer, it is called slime layer, but when nitrogenous substances (i.e. amino acids) are also present along with polysaccharides, then it is called capsule. The capsulated cells are drought resistant. Association of polysaccharides with others makes it antigenetically important (used in serology).

Mucopolysaccharides help bacteria to remain in body without damage. Mucopolysaccharides have virulence (bacteria genetically capable of producing capsule if are pathogenic). If capsule is removed the cells will die. It means that for survival capsule is must. Mucopolysaccharides are sometimes associated with $Ca^{2+}$, $Mg^{2+}$ ions for holding higher amount of water.

The capsule is hard to be removed, even NaCl cannot dissolve it. Thus capsule is removed by chelating polysaccharides like EDTA or EDTA+NaCl in which cells after shaking, shed off capsule. In *Streptococci*, *Staphylococci* mucilage capsule is present only when cells are dividing rapidly. Capsule protects cells from lysozyme activity. Once the capsule is removed the cell is subjected to disruption and wall can be isolated.

## 3. Cell Wall

The cell wall is characteristic of plant kingdom. In higher plants and algae it is composed of cellulose and shows a fibrillar structure. In contrast the bacterial cell wall has a granular structure but it is tough and rigid. It varies in thickness from 50 to 100 $A^0$. The three main constituents of cell wall are: (i) N-acetyl glucosamine (NAG), (ii) N-acetyl muramic acid (NAM), and (iii) a peptide chain of four or five amino acids. These together form a polymer called peptidoglycan or mucopeptide. The NAG and NAM molecules, which are arranged alternatively, run in one direction and the peptide chain run crosswise. The rigidity of bacterial cell wall is due to the presence of this polymer. Besides above mentioned three constituents, some other chemicals such as teichoic acid, protein polysaccharides, lipoproteins. Lipopolysaccharides are also deposited on the cell wall.

The function of bacterial cell wall appears to be wholly mechanical, giving the cell its shape and rigidity. The rigidity of the cell wall can be judged by the fact that it can withstand an osmotic pressure of about eight atmosphere per square centimeter.

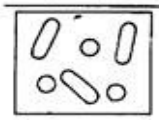
| Colour of Cells | Treatment | Effect |
|---|---|---|
|  | Cells fixed by heating | Cells shrink slightly in size. |
|  | Crystal violet dye applied | All cells stain dark violet or purple. |
|  | Gram's iodine applied | All cells remain dark violet or purple. |
|  | Wash with 95% ethyl alcohol | Gram-positive cells remain dark violet or purple; Gram-negative cells become colourless. |
|  | Safranin (red dye) applied | Gram-positive cells remain dark violet or purple; Gram-negative cells appear red. |
| Purple colour | | |
| Red colour | | |

*Fig. 9.3: Difference between gram positive and gram negative bacteria*

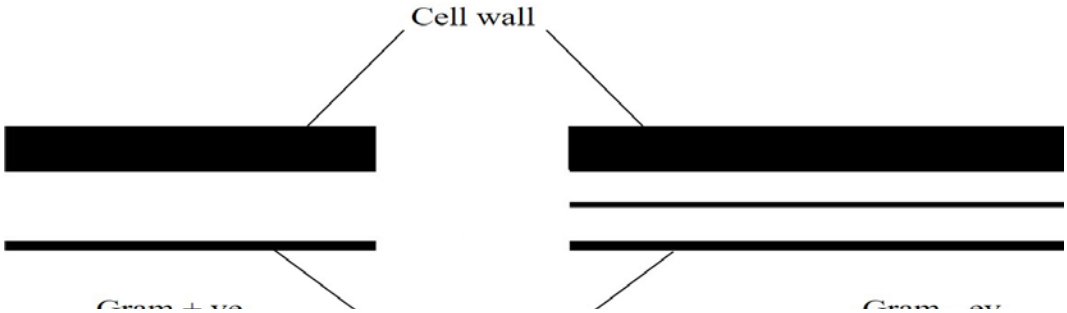



*Fig. 9.4: Bacterial cell wall*

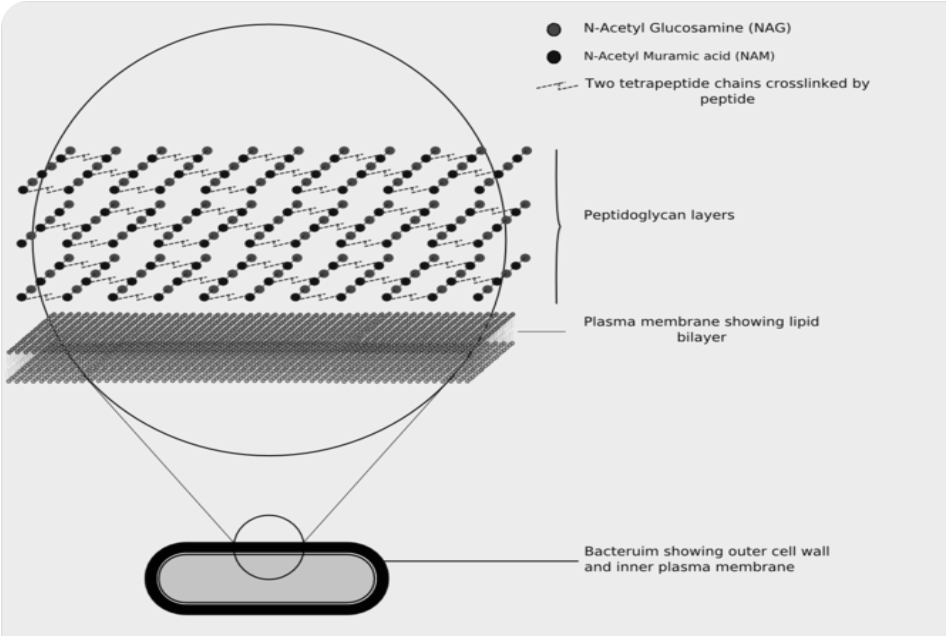



*Fig. 9.5: Cell wall in gram positive bacteria*

The Gram stain, developed in 1884 by Hans Christian Gram, characterizes bacteria based on the structural characteristics of their cell walls (Fig. 9.3 and Fig. 9.4). The thick layers of peptidoglycan in the "gram-positive" cell wall stain purple, while the thin "gram-negative" cell wall appears pink. By combining morphology and Gram-staining, most bacteria can be classified as belonging to one of four groups (gram-positive *cocci*, gram-positive *bacilli*, gram-negative *cocci* and gram-negative *bacilli*).Wall in gram positive differs from gram negative. Generally wall is made up of murein and peptidoglycan (N-acetyl glucosamine NAG and N-aetyl muramic acid NAM) and protein lipids (Fig. 9.5). In gram positive wall is several times thicker. In gram positive there is no layering around cells while in gram negative lamellation of 1-4 layers are present L1, L2, L3 apart from peptidoglycans. Gram positive wall have integrated proteins and lipid layers which is very thin, in gram negative the layer is thick. Integrated proteins are also present but additionally porin proteins (extend from outside to inside) also as channel proteins

are present. When seen under electron microscope outer and inner electron dense layers are found to be separated by electron transparent layer apart from peptidoglycan layer. α- helix protein integrated within peptidoglycan layer. In gram positive the peptidoglycan layer is thick and some light regions are associated with it. Chemical organization in both is same but in gram positive cells there is excessive amount of cross linking by polypeptides (4-6 amino acid residues). In case of gram negative cell cross linking is to a lesser extent. This cross linking is very high, forming a rod/root like structure supported on pillars. Apart from these two, in gram positive techoic acid or lipotechoic acid are also associated with wall. Techoic acid is responsible partly for immunogenic activity of wall. Linking is very important as it makes wall rigid. This cross linking is highly specific and depending of its type, different walls are described in gram positive bacteria Type A, Type B and Type C. A comparison between Gram-positive and Gram-negative bacteria is given in the table 9.1.

**Table 9.1 Comparison between Gram-positive and Gram-negative bacteria**

| S.No. | Characteristics | Gram-positive bacteria | Gram-negative bacteria |
|---|---|---|---|
| 1- | Cell wall structure | Cell wall single layered and 150-200$A^0$ thick | Cell wall triple layered and 75-120$A^0$ thick |
| 2- | Outer membrane | Absent | Present |
| 3- | Periplasmic space | Present in some | Present in all |
| 4- | Chemical composition | Peptidoglycans accounts for about 80% of the cell wall and the rest are polysaccharides, Teichoic acid present Low in lipid (1-4%), Highly responsive to triphenylmethane, Resistant to alkalies and insoluble in 1% KOH solution. | Peptidoglycans accounts only about 3-12 % of the cell wall. It is mainly composed of lipoproteins and lipid polysaccharides. Teichoic acid absent. High in lipid (11-22%). Little response to triphenyl methane, show sensitivity to alkalies and soluble in 1% KOH solution. |
| 5- | Rigidity of cell wall | Cell wall is very rigid due to high proportion of peptidoglycans | Cell wall is elastic due to plastic nature of lipoprotein-polysaccharide mixture. |
| 6- | Susceptibility of Cell wall | High susceptibility | Low susceptibility |
| 7- | Nutritional requirement | Relatively complex in many species | Relatively simple |
| 8- | Permeability to molecules | More penetrable | Less penetrable |

## 4. Cytoplasmic membrane / Plasma Membrane

Inner to cell wall, a semipermeable cytoplasmic membrane is present which is about 75 $A^0$ thick. Chemically it is composed of a double layer of phospholipid molecules. Phospholipids are of two types- hydrophobic and hydrophilic. The hydrophilic phospholipid molecules are present towards the outerside and the hydrophobic molecules towards inner side. Proteins are found embedded in the lipid bilayer. The chemical nature of cytoplasmic membrane in gram (-)ve bacteria is basically the same. Prokaryotic membrane is characterized by the absence of sterols which perhaps account for the enormous resistance of these organisms to antibiotics.

It is a usual bilipid layer different from eukaryotic cell membrane. It is associated with a number of enzymes. Channel enzymes are also present. Special enzymes for pinocytosis and exocytosis are present. The cytoplasmic membrane has many folded structures called mesosomes which are associated with number of activities like seat for protein synthesis, respiratory function, multiplication of chromosomal DNA, and DNA attached by ori. Plasma membrane contains special receptor molecules that help bacteria detect and respond to chemicals in their surroundings. It also controls the entry of organic and inorganic.

## 5. Flagella

The locomotion in bacteria is accomplished by thin-hair like appendages called flagella. Each flagellum is a whiplash like structure of almost uniform thickness, originating from the cytoplasm just beneath the cytoplasmic membrane. Flagella are characteristic of all spiral bacteria and they also usually occur in bacillus bacteria. Coccus bacteria are, however, devoid of flagella and are non motile. The following categories of bacteria are recognized on the basis of the presence or absence of flagella (Fig.9.6).

**1-Atrichous:** These bacteria are devoid of flagella hence are non-motile. e.g., *Lactobacillus, Pasteurella.*

**2-Trichous:** These bacteria possess flagella, hence are motile. The flagella are distributed over the surface of bacteria in a characteristic fashion. The number, position and arrangement vary in different species. They may be restricted to the ends of the cell (polar flagella) or uniformly distributed all over the surface of the cell (non-polar flagella or peritrichous). Peritrichous flagellation is of wide occurrence among bacteria, whereas polarly flagellated bacteria form a homogenous assemblage of rods and spirals. Various types of polar and peritrichous flagellation are as follows:

**(a) Polar flagellation:** It is usually found in Gram-negative bacilli and spirilla. The following four types of polar flagellation are recognized.

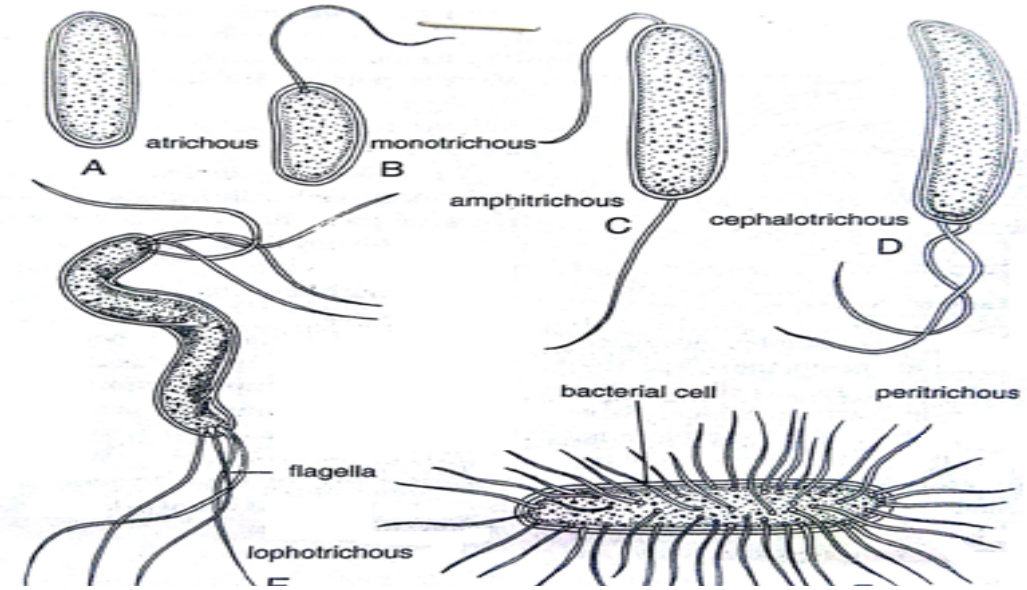



*Fig.9.6: Figure showing different types of flagella*

i. **Monotrichous** : One flagella at one end (Fig. 9.6, A), e.g., *Vibrio chlolerae, Pseudomonas*;
ii. **Amphitrichous** : One flagella at each end (Fig. 9.6, C), e.g., *Nitrosomonas, Spirillum,*
iii. **Cephalotrichous** : Two or more flagella at one end only (Fig. 9.6, B), e.g., *Pseudomonas fluorescens,*
iv **Lophotrichous** : Tufts of flagella at both the ends, e.g., *Spirillum volutans.*

**(b) Non-polar flagellation:** In non-polar or peritrichous flagellation the flagella are evenly distributed throughout the surface of the cell e.g., *Proteus vulgaris, Bacillus typhosus, Salmonella* and *Clostridium*. Flagella are rigid structures about 20nm across and 15-20µm long. Flagella have three parts. 1- The longest portion is the filament which is a hollow rigid cylinder made of the protein called Flagellin. 2- The basal body which is embedded in the cell. 3- Hook a short, curved segment that links the filament to its basal body and acts as a flexible coupling. Flagella are made up of single type of protein and are attached to the outside of the cell. The flagella act as a propeller. It can turn clockwise or anti clockwise to propel the bacteria in different directions. A protein ring in the cell's membrane act as bearing to aid rotation.

Prokaryotic and eukaryotic flagella are very different. Flagella in bacteria has special significance (a) a hook is always present and is never straight, (b) eukaryotic flagella has 9+2 arrangements of microtubules with association to each other have protein attachments called spokes. Laterals of peripheral tubules are made of protein dinein; but in prokaryotes like bacteria flagella organization is simple. Flagellum is made up of contractile protein called flagellin. There

are polymers of this attached laterally/longitudinally by special bondage with the result that there may be 5-6 subunits arranged in spirals creating hollow in centre.

Basal body structure in both eukaryotes and prokaryotes are different. In prokaryotes the basal subunits have only 4 discs. Through hook flagellum passes and whole structure is joined by a flagellin rod. A sort of lever system is provided by these rings. A fixed position of rod L, P, S have a hollow centre through which rod passes and act as bearings of movement of flagella. Rotor moves to 100-300 revolutions/second. Periplasmic space provides the force for rotation by ionic strength and the source of motion is not ATP.

In gram negative bacterium peptidoglycan layer is very small and only 2 rings are available and hook is not as rigid. If bacterium is present in water, the resistance to the cell is very large. Thus for movement very high force is required, usually the movement is anticlockwise. With this movement the cell is pushed forward. This rotation is not constant. After sometime the cell either stops or flagella changes its direction to clockwise movement. Thus the movement of bacteria is zig-zag or Brownian movement. Rotation of flagella is unique. Motion is controlled by ionic balance in periplasmic space.

## 6. Pilli or Fimbriae

These are short tubular extensions which perform specific function usually are of several kinds but made of contractile protein Pilin arranged in 3's varying from 5-30 special hollow in the centre. It is not meant for locomotion but for either clumping, attracts and attach to RBC, for adherence, for transfer of genetic material and form conjugation tubes. They are synthesized because of plasmid (F factor) and are known as sex pilli. It was proved by certain observations that:

(i) In case of plasmid mutants no pilli is synthesized.
(ii) Once striped off cells lose their capacity to transfer DNA.
(iii) The purified Pilli have capacity to bind to recipient cells.
(iv) Phages are highly specific for binding to male cells (F+ cells). These are of two types
    a. Tip binding phase no donor activity
    b. Shaft binding in which donor activity is not affected.

Depending upon studies different pilli are discovered and may be grouped into FI, FII, FIII and FIV. Pillin can cause antigenic effect but flagella cannot.

## 7. Cytoplasm and Cytoplasmic inclusions

Bacterial cytoplasm is a complex mixture of carbohydrates, proteins, lipids, minerals, nucleic acids and water. It stores organic material in the form of glycogen, rolutin and poly-β-hydroxy butyrate. Some photosynthetic and non-photosynthetic bacteria also accumulate sulphur and iron in their cytoplasm.

Besides fluid portion and storage particles, the bacterial cytoplasm also contains chromatic or nuclear area and some other inclusions. The bacterial cell is devoid of mitochondria, endoplasmic reticulum, centrosome and golgi bodies. Although a well organized chloroplast is absent in bacteria, the photosynthetic bacteria have chromatophores in their cytoplasm.

**I. Nuclear material:** The characteristic feature of bacterial nucleus is the absence of nuclear membrane, nucleolus, chromonemata and nuclear sap, such structure is called nucleoid or genophore. Under electron microspore the nucleoid appears to be fibrillar and compared of a double or single stranded DNA. It has approximately $5x10^9$ base pairs and a molecular weight of about $3x10^9$. The DNA molecule is approximately 1,000 µm long, usually forming ring like structure or sometimes remain diffused throughout the cytoplasm of the cell.
*Escherichia coli* has a central core of RNA, surrounded by 12-82 supercoiling of DNA. A few protein molecules are also associated with DNA.

The Bacterial DNA is devoid of histones hence cannot be compared with chromosomes of eukaryotic cells. It is referred as bacterial chromosome.

**II. Plasmids:** Bacterial cells also contain some extrachromosomal heredity determinants which are either independent of bacterial chromosomes or are intergrated with them. Extranuclear materials called as episome are present which may be linear, circular, covalent coiled circular (CCC) = plasmid. Lederberg (1952) gave the term plasmid to those extragenophoral genetic materials. Plasmids may contain about 100 genes having molecular weight ranging from $5X10^7$ to $7X10^7$ or less. The replication of plasmid seems self controlled. They contain different non-essential characters. Based on host properties, the plasmids are classified into different types as:

(i) F-factor for fertility
(ii) Col-factor for colicinogeny
(iii) R-factor for resistance
(iv) Tumor inducing plasmid (*Agrobacterium*)
(v) Degradative plasmid (*Pseudomonas*)
(vi) Pathogenicity to mammals
(vii) Penicillase plasmid (*Staphylococcus*)
(viii) Mercury resistance
(ix) Cryptic plasmids.

Two important genes are associated with plasmids *ori* (origin of replicon) and *tra* (transfer) F. plasmid can occur in low copy number per cell or high copy number per cell (up to 1000).
Plasmid may be of same compatibility or different compatibility groups. Depending upon method of replication these plasmids are of two types:

**(a) Stringent**=multiply usually when cell divides therefore needs protein synthesis for replication (DNA polymerase formation).

**(b) Relaxed**=do not require protein syntheses of cell and DNA polymerase already present is used. In Chloramphenicol presence these continue to multiply but cell number does not increase.

**III. Ribosomes:** Ribosomes are the sites of protein synthesis in bacterial cell as in eukaryotic cell. In eukaryotes ribosomes are frequently attached to the surface of the endoplasmic reticulum but in bacteria which do not have endoplasmic reticulum, ribosomes are free in cytoplasm. Their number varies from 10,000 to 15,000 in a cell. Bacterial ribosomes are 70s type (eukaryotic ribosomes are 80s type) and consists of two subunits. The 70s ribosome is made up of a 50s and 30s subunits. The 50s subunit contains the 23s and 5s rRNA while the 30s subunit contains the 16s rRNA. Prokaryotic cells have three types of rRNA: 16s rRNA, 23s rRNA and 5srRNA. Prokaryotic ribosomes are around 20nm ($200A^0$) in Diameter and are made of 35% ribosomal proteins. Protein is required for numerous cell functions.

In young bacterium ribosomes may occur in groups of 4-6 or more. They are held together by special RNA molecule, known as messenger RNA. These groups of ribosomes are known as polyribosomes.

**IV. Mesosomes:** These are complex localized infoldings of the cytoplasmic membrane (Fig.9.1). There may be 2-4 mesosomes in and the number is usually higher in bacteria which show high respiratory activity, such as nitrifying bacteria. It has been suggested that the highly infolded membrane system of mesosomes perhaps serves to accommodate more centres of respiration. But the absence of enzymes like ATPase, dehydrogenase and cytochrome in mesosome indicates that they are not the sites of respiration. They probably participate in the formation of transverse septum during cell division.

## *9.4 NUTRITION*

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as nutrients or nutritional requirements. Bacteria that are symbionts or obligate intracellular parasites of other cells, usually eukaryotic cells, are (not unexpectedly) difficult to grow outside of their natural host cells. Whether the microbe is a mutualist or parasite, the host cell must ultimately provide the nutritional requirements of its resident. Almost all eukaryotes are either photoautotrophic (e.g., plants and algae) or heterotrophic (e.g., animals, protozoa, fungi). Lithotrophy is unique to prokaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eukaryotic algae. Phototrophy has not been found in the *Archaea*, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

Most of the bacteria do not contain chlorophyll. They are unable to synthesize their own food, but a small group of bacteria are capable of synthesizing their own food. So, nutrition in bacteria is both autotrophic and heterotrophic. At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The table 9.2 shows various nutritional types in bacteria.

**Table 2: Nutrition mode in bacteria**

| Nutritional type | Energy source | Carbon source | Examples |
|---|---|---|---|
| Photoautotrophs | Light | $CO_2$ | Cyanobacteria, some purple and green bacteria |
| Photoheterotrophs | Light | Organic compounds | Some purple and green bacteria |
| Chemoautotrophs or Lithotrophs (Lithoautotrophs) | Inorganic compounds, e.g., $H_2$, $NH_3$, $NO_2$, $H_2S$ | $CO_2$ | A few bacteria and many Archaea |
| Chemoheterotrophs or Heterotrophs | Organic compounds | Organic compounds | Most bacteria, some Archaea |

## 1. Autotrophic

These bacteria can prepare their food by using raw materials and external energy. They are of following types:

**(i) Photoautotrophic:** They can prepare their food by using solar energy in the presence of photosynthetic pigment bacteriochlorophyll and chlorobium chlorophyll. Photosynthesis in bacteria differs from other green plants photosynthesis because there is no release of oxygen in photosynthesis. Such photosynthesis is called anoxygenic photosynthesis.

It is of following types:

**a. Green sulphur bacteria** **:** In this bacterium, the photosynthetic pigment is chlorobium chlorophyll and sulphur is by- product. e.g: *Chlorobium*

**b. Purple sulphur bacteria** **:** In this bacterium, the photosynthetic pigment is bacteriochlorophyll and sulphur is by- product. e.g: *Chromatium*

**c. Non-sulphur bacteria** **:** They have photosynthetic pigment bacteriochlorophyll and sulphur is not a by-product. e.g: *Rhodopseudomonas*

**(ii) Chemoautotrophic*:*** These bacteria prepare their food by using chemical energy in the absence of photosynthetic pigment. This process is called chemosynthesis. It is of following types:

a. Sulphur bacteria: They use chemical energy while there is oxidation of sulphur compound. e.g: *Thiobacillus*

$2H_2S$ or $S_2$+ $O_2$=======➔$2S + 2H_2O$ + energy

b. Iron bacteria: They use chemical energy while there is oxidation compound ($Fe^{2+}$ to $Fe^{3+}$). e.g: *Leptothrix, Ferobacillus, Cladothrix*

$4FeCO_3$+ $O_2$+ $6H_2O$=======➔ $4Fe(OH)_3$+ $4CO_2$+ Energy

c. Hydrogen bacteria: They use chemical energy while there is oxidation of molecular hydrogen. e.g: *Pseudomonas, Hydrogenomonas, Bacillus pectotrophus.*

$H_2$+ ½ $O_2$=====➔ $H_2O$ + Energy

d. Nitrifying bacteria: They use chemical energy while there is oxidation of nitrogen compound. e.g: *Nitrosomonas, Nitrobacter*

$2NO_2$+ $O_2$=========➔ $2NO_3$+ Energy

## 2. Heterotrophic

These bacteria cannot prepare their food themselves. They obtain their food or nutrition from an outside source. It is of following types:

**(i) Parasitic*:*** Parasitic bacteria live on and within other organisms (host) and they obtain their nutrition from the host. If the parasitic bacteria cause diseases and are harmful for their host they are called pathogenic. If the parasitic bacteria cannot cause diseases and are harmless for their host they are called non-pathogenic. e.g.: *Vibrio cholerae*, *Diplococcus pneumoniae*

**(ii) Saprophytic*:*** These bacteria live and grow in dead and decaying organic matters and they obtain their nutrition from dead and decaying organic matters. Firstly they secrete decomposing enzymes which convert complex organic matter into simple and soluble form. These simple and soluble organic matters are absorbed by body surface of saprophytes. e.g.: *Pseudomonas.*

**(iii) Symbiotic***:* Symbiotic bacteria live in close association with other living organisms so that they both are benefited to each other, neither of them is harmed. e.g.: *Rhizobium.*

# *9.5 REPRODUCTION*

The bacteria reproduce by vegetative, asexual and sexual methods of reproduction.

**1. Vegetative reproduction:** The vegetative propagation includes budding, fragmentation and binary fission.

**(a). Binary fission**: The most common way by which the bacteria reproduce itself is the Binary Process. It is a process by which a single bacterial cell simply divides into two in half an hour time (Fig. 9.7). The various events of Binary fission are as follows:

(i). The nucleoid gradually become elongated in size and form dumbel-shaped structure.
(ii). They still remain attached to the plasma membrane with the help of mesosome.
(iii). The duplication of DNA and mesosome takes place and get separate from each other.
(iv). The daughter mesosomes and nucleoids migrate towards the opposite poles.
(v) . The plasma membrane invaginates at the center and the parent cell is divided into two identical cells.

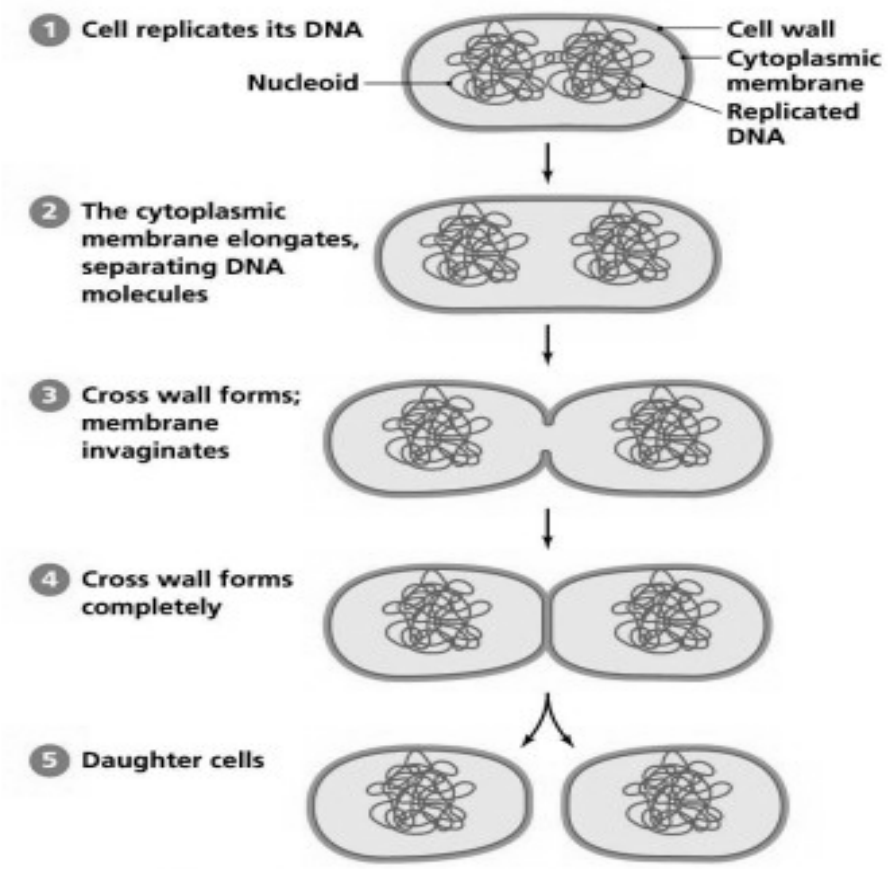



*Fig. 9.7: Diagram showing binary fission*

The daughter cells soon grow to maturity within 20-30 minutes. Under favorable conditions many bacteria divide once in 20-30 minutes.

**(b). Fragmentation**: Mostly during unfavorable conditions, bacterial protoplasm undergoes compartmentalization and subsequent fragmentation, forming minute bodies called gonidia. Under favorable conditions, each gonidium grows to a new bacterium. It becomes apparent that

prior to fragmentation the bacterial genome has to undergo repeated replication so that each fragment gets a copy of it.

**(c). Budding**: In this case, a small protuberance, called bud, develops at one end of the cell. Genome replication follows, and one copy of the genome gets into the bud. Then the bud enlarges, eventually become a daughter cell and finally gets separated from the parent cell. It is comparatively rare process observed in few bacteria like *Rhodopseudomonas, Hyphomicrobium, Pedomicrobium, Hyphomonas* etc.

## 2. Asexual reproduction

**(a) Endospore:** It is not always considered a method of reproduction but perennation in unfavourable conditions (Fig.9.8). These are specialized structures produced for the cells tiding over unfavourable conditions to the extent that they are heat resistant even at $80^oC$ for 10 minutes (during pasteurization). They can withstand draught and can survive for years (200 years). They can survive in radiation also and can withstand acid treatments (conc. $H_2SO_4$).

Their presence is very widely distributed among various groups of bacteria and almost all those bacteria which show endospores are gram positive except one *Archebacterium*. May be rod shaped in *Cornyebacteria* or spiral bacteria. Its structure is very peculiar, spore coat is very thick made up of peptidoglycans rich in diamino pimalic acid, teichoic acid, $Ca^{+2}$ or even dipicolinic acid

Water content of cell is not less than 5%. These spores are formed in both aerobic and anaerobic forms. All these when fixed by heating and stained with carbol fuschin retains the colour which is not washed with acid and thus they are acid fast in character. Formation of endospore is characteristic and is very strategic. Spore formation is observed under conditions of restricted growth starting with accumulation of protein rich content in spore forming region. Numerous metabolic conversions occurs during spore formation sometimes at the expense of PHBA and as well as polysaccharide during anaerobic. During first hour protein of specific nature is formed the reserve food gets depleted. Dipicolenic acid is synthesized which is not usually associated and accumulated. These 2 DPA and $Ca^{2+}$ acts as chelate and makes upto 10-15% of dry weight.

Equal division starts from periphery of the plasma membrane. Two cells are specifically formed, one small and other large. As soon as it occurs, the large cell starts engulfing the smaller one so that the spore becomes embedded in the original cell. It is at this stage that spore coat is laid down. Spore coat becomes double walled structures with DPA accumulated in cortex region. Outer spore envelope is formed by mother cell and is formed of polysaccharide which may remain as such or additionally a exosporium may be laid in *B. cerius* which is also formed of mother cell. This exosporium remains as loose, discrete structure in mature spore. As mature one is getting investing by cortical region much of the water is lost. This state is reached in 7-8 hours which results in completion of endospore formation. Spores are not obligate part of large cell.

It's only in nutrient deficiency conditions as drying, dehydration may induce spore formation. Only drying/dehydration do not cause sporulation. In *B. cerius* if cells are suspended in water without nutrient the cells do not show sporulation even after 5 hours. Once sporulation starts it cannot be checked. Other important criteria are that continuous sub culturing of cells may cause loss of capacity of endospore formation. Since suspension may contain vegetative as well as spore cells therefore the cells can be void to kill the vegetative cells and the spores will continue to remain there. This treatment helps to maintain spore forming culture for long time. Spore on maturity are released by autolysis of vegetative cells. The heat resistance is due to low water content. In *Closteridium botulinum* the spores may even withstand 15 psi for 10-12 minutes. Heat resistance of spore is directly proportional to amount of DPA.

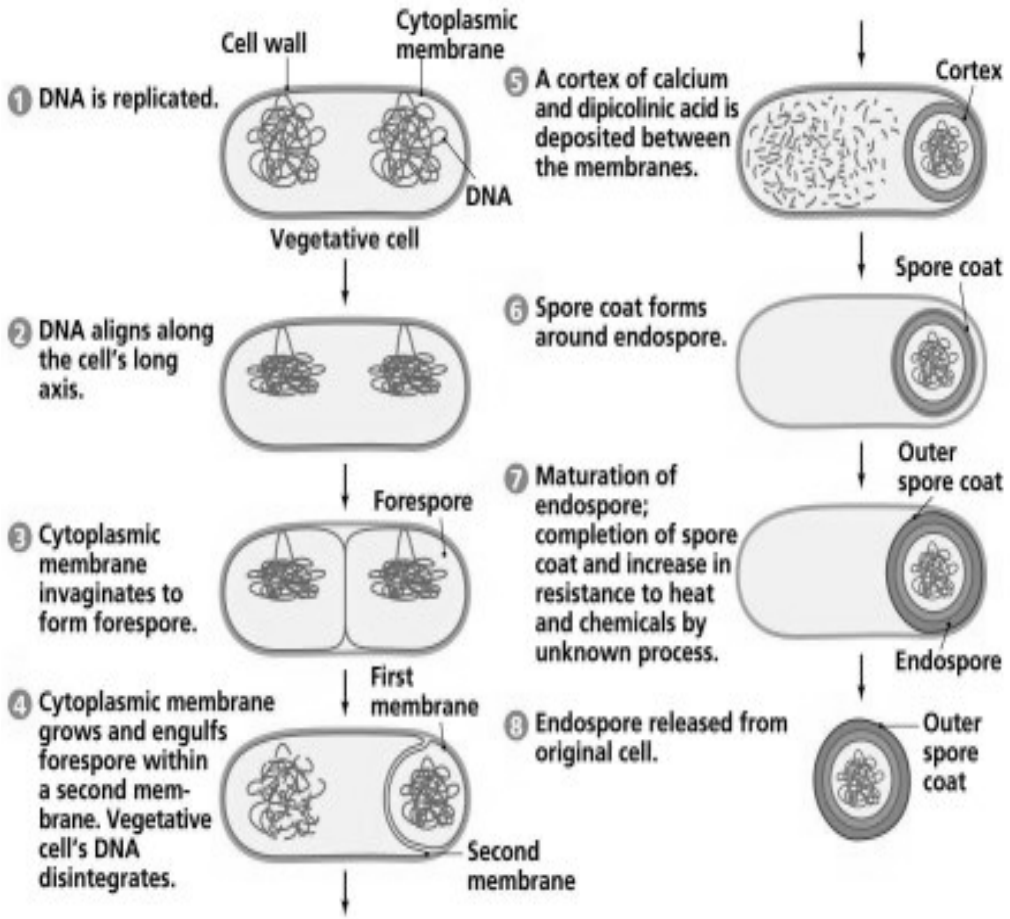



*Fig.9.8: Bacteria: Asexual Reproduction Diagrammatic representation of endospore formation in bacteria*

The radiation resistance is due to S=S bond created in the outer layers of proteins in coat. Higher the S=S bonds greater is the resistance to radiation and it has been observed that spore envelope contains cystine rich protein which is equivalent to keratinin in character. This as well as $Ca^{2+}$, DPA may be responsible for impermeability of spore to various chemicals.

For spore germination conditions required are rich nutrient media but this is only after certain pretreatment e.g., storage of spore/pre exposure to heat. In *B. subtilis* storage of spores up to 7 days and 5 min. heat at $60^{o}$C is minimum requirement for spore germination.

In certain cases boiling for 10 minutes may be a pre requisite for spore to initiate germination provided the heat treatment immediately following by nutrient treatment. In still other cases spore germination determined by glucose, amino acids, nucleotides or certain other substances. During germination permanent physiological alterations takes place. Respiration and other enzymes reactions take place with secretion of DPA and protein molecules. The germination tube is extruded through pore/lateral side and appear to be bound by plasma membrane which may sometimes by enveloped by incomplete cell wall. The survival of these spores under very stringent conditions (50-100 years) *B. coagulans* and *B. circulans* usually in dry soil sample the viability of spore may be lost up to 90% within 50 years but data also suggests that in great amount of soil these may remain viable for more than 1000 years.

**(b) Conidia:** Many filamentous bacteria (e.g., *Streptomyces*) form chains of small, spherical spore-like conidia at the tips of the filaments. A conidium develops by the formation of a transverse wall at the tip of the filament. The filament bearing conidia are known as conidiophores (Fig. 9.9). After liberation each conidium gives rise to a new filamentous bacterium, provided conditions for germination are favourable.

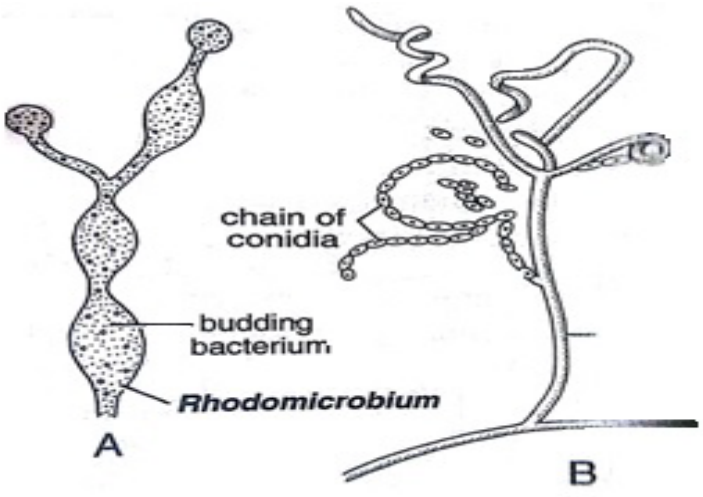



Fig. 9.9: (A-B) Bacterial asexual reproduction; A- Budding in *Rhodomicrobium, B*- Conidia in *Streptomyces*

**(c) Cysts:** Genus *Azotobacter* produces distinctive resting cells known as cysts. The cysts are formed by the deposition of additional layers around the existing cell wall. Thus the entire contents of the cell are involved in the formation of a cyst. The cells are resistant to desiccation but not to heat.

## 3. Sexual reproduction

Typical sexual reproduction is not found in bacteria. It occurs in form of genetic recombination. There are three main methods of genetic recombination: transformation, transduction and conjugation.

**(a) Transformation:** It is unusual form of recombination in which the molecules of DNA that carry the genetic information of cell move from the donor to the recipient cell through the liquid medium in which they are growing. It was discovered by Frederick Griffith (1928) in *Diplococcus pneumonia*e while experimenting with laboratory mice. Later in 1944 O. Avery, C. M. Macleod and M. McCarty demonstrated it *in vitro.* Since then it has been shown in several other bacteria such as *Bacillus haemophilus* and *Neisseria*. All bacterial cells having an ability to take up DNA from the surrounding are said to be competent. It was first observed by Burnett in 1925 who called it as entrainment. This process is found in lab conditions and not in nature (Fig. 9.10 and 9.11).

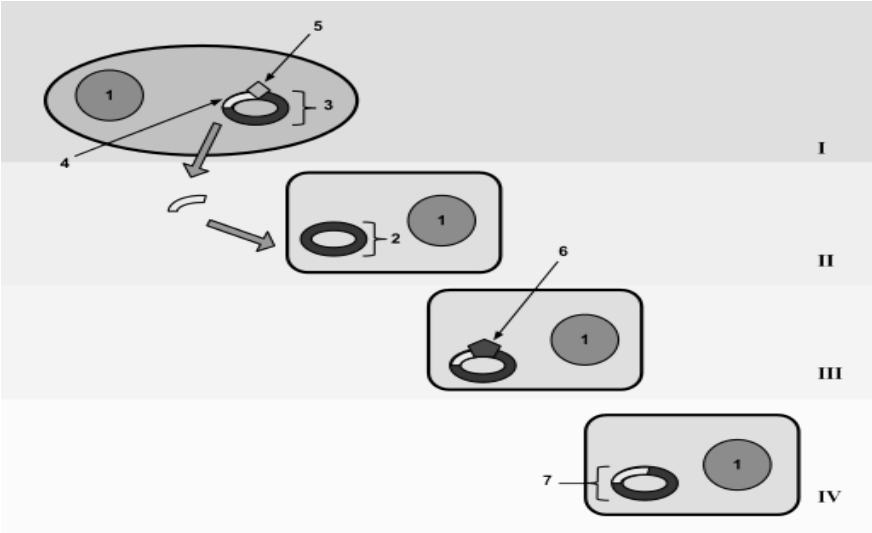



*Fig. 9.10: Diagram showing transformation in Bacterial cell*

Transformation competence is a state of bacterial cell during which the usually rigid cell wall can transport a relatively large DNA macromolecule. This is a highly unusual process for bacteria because they normally lack the ability to transport large macromolecules across the cell wall and through the cytoplasmic membrane. In some bacteria competence is expressed during cell division this is called natural competence (*Haemophilus, Streptococcus*). The competence can be increased by treating cells with calcium or rubidium chloride this is called artificial competence (used in recombinant DNA technology). A competent cell can take a maximum of about 10 fragments of foreign DNA. Transformation takes place in special conditions only known as state of competence. This transforming ability of the cell depends on presence of high temperature, ultraviolet radiations, chemical mutagens, etc. increase in concentration of

inorganic phosphates in the culture medium increases the frequency of transformation. The optimum temperature range is 29-32$^{o}$C.

In nature the frequency of transformation in different bacteria varies from 0.047 to 0.0004%. About 80 species of bacteria are known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria. "Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

## Significance of transformation

Transformation has been studied only in laboratory. In nature it may occur in certain environments where bacteria live in large numbers. In such environments some bacteria die and lyse and live ones of the some or closely related species absorb the fragments of donar DNA which escape the action of extracellular nucleases. As such it is difficult to estimate the degree to which transformation contributes to the genetic diversity of organisms in nature. In the laboratory scientists use the technique to produce recombinant DNA, which in several cases have important commercial applications.

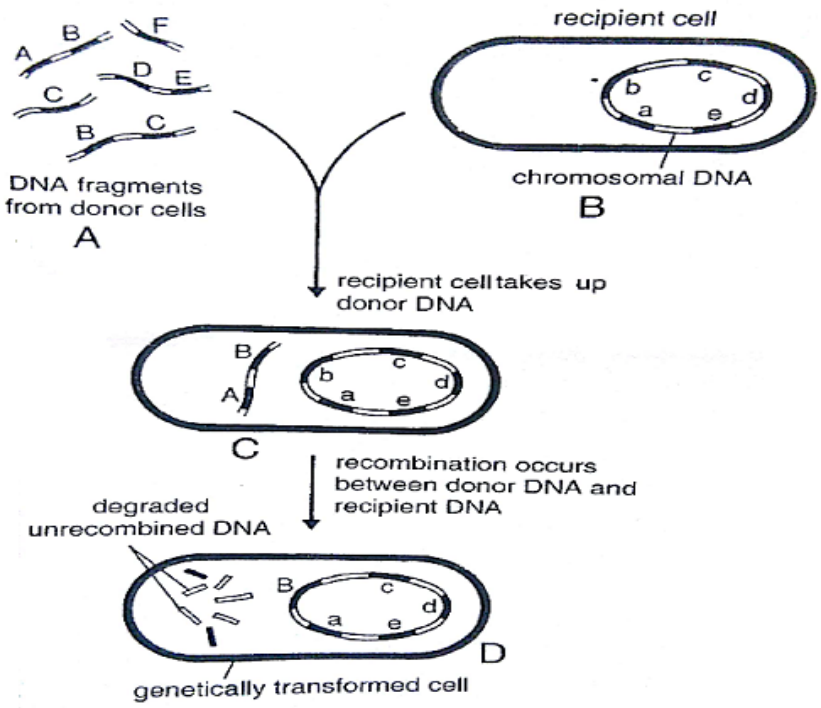



*Fig. 9.11: A-D: Bacteria: Genetic recombination, diagrammatic representation of transformation.*

**(b) Transduction**: In this method, genetic material of one bacterial cell goes to other bacterial cell by agency of bacteriophages or phages (viruses, infecting bacteria). It was first of all reported in *Salmonella typhimurium* by Zinder and Lederberg (1952). The bacteriophage may undergo lytic/lysogenetic life cycle (Fig. 9.12 and 9.13). The transduction can be of two types:

**(i) Generalized transduction**: It involves only those prophage particles which are present in the cytoplasm of the infected cell (and not in the chromosome). It is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage, and very rarely a small number of phages carry the donor (bacterial genome) genome, (1 phage in 10.000 ones carry the donor genome). If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to generalized transduction. The main steps of the generalized transduction are as follows:

(i) The phase DNA present in the lysogenic bacterial cell, starts synthesizing new phage components. In this process the chromosomes of some bacterial cells get fragmented. Eventually these fragments are incorporated in some new phage particles. Thus some of the phage particles present in the lysogenic cell have segments of bacterial chromosome incorporated in them, while others have only phage (Fig. 9.12).

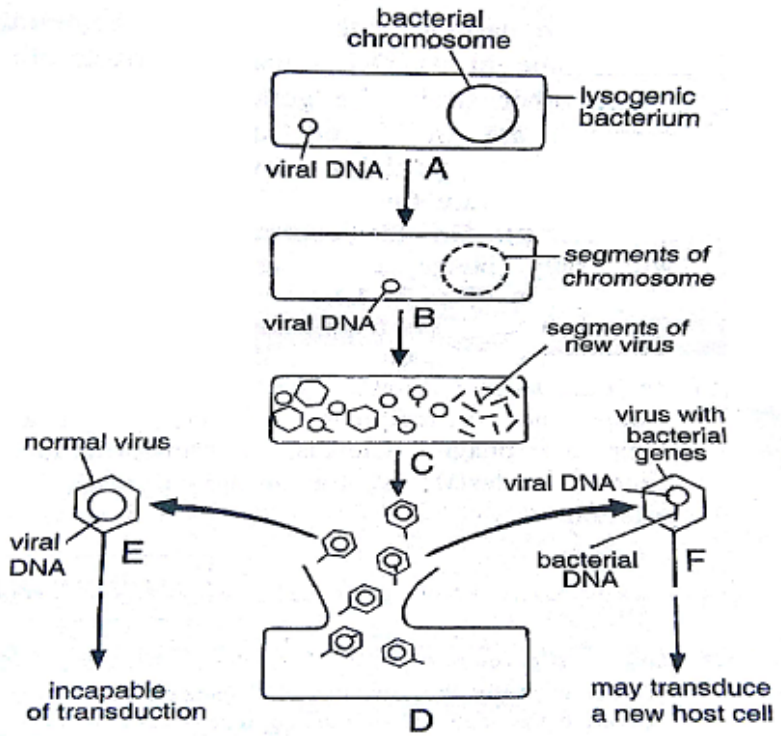



*Fig.9.12 (A-F): Bacteria: Genetic recombination; Diagrammatic representation of generalized transduction*

(ii) If a phage particle with segment of bacterial chromosome in its DNA attacks a bacterium of any other strain, then the present bacterium are transferred to the new cell. Such phage particles (with genes of bacterial cell) are thus capable of transduction. On the contrary, the particles with only phage DNA are incapable of transduction. When the new DNA is inserted into this recipient cell it can fall to one of three fates:

1. The DNA will be absorbed by the cell and be recycled for spare parts.
2. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.
3. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.

**(ii) Specialized transduction:** It is the process by which a restricted set of bacterial genes is transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome. Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA.

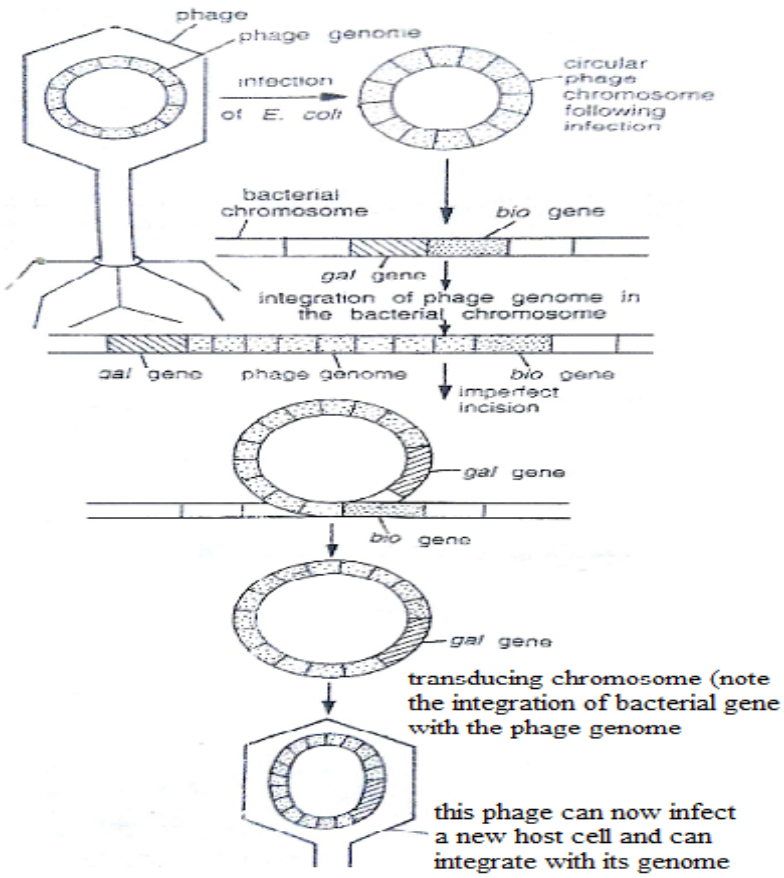



*Fig. 9.13: Bacteria: Diagram showing imperfect incision of phage genome in specialized transduction*

The excised DNA is then packaged into a new virus particle, which then delivers the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage. When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote". An example of specialized transduction (Fig. 9.13 and 9.14) is λ phage in *Escherichia coli.*

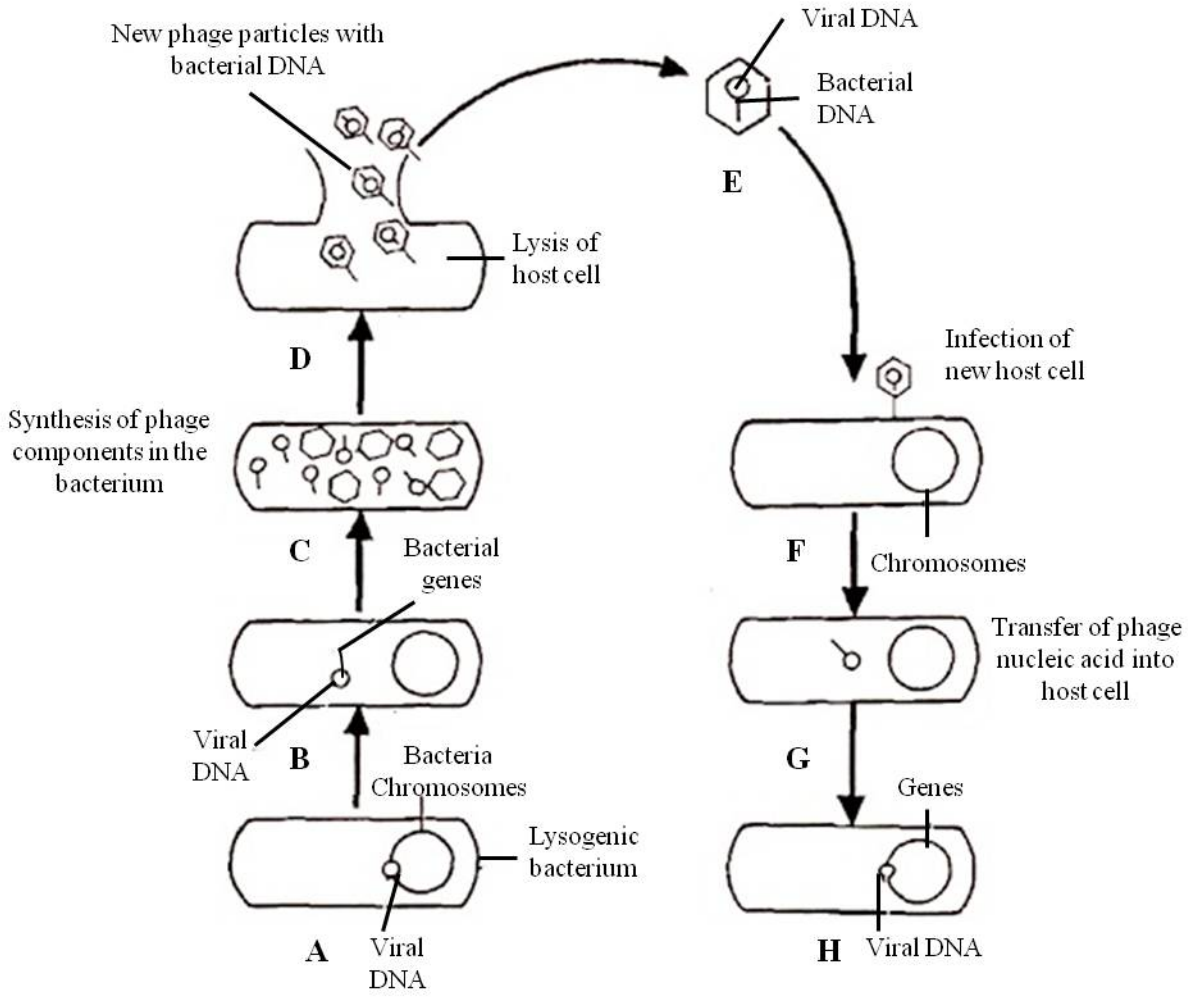



*Fig. 9.14 (A-H): Bacteria A-H: Genetic recombination diagrammatic representation of specialized transduction*

The main steps of specialized transduction are as follows:

(i) The bacteriophage gets attached to the bacterial cell on the receptor site and the nucleic acid of phage particle is transferred to the cytoplasm of the bacterial cell.

(ii) The nucleic acid of the phage particle is coded for the synthesis of certain specific proteins in the bacterial cell. These are known as repressor proteins and their function is to check the syntheses of phage particle in the bacterial cell. The DNA occurs in the bacterial cell in the form

of fragments known as prophage. These fragments are either free in cytoplasm or are attached to chromosome. A bacterial cell with prophage is lysogenic and it may remain lysogenic for several generations. During this period the phage DNA keeps on dividing along with bacterial chromosome. A stage comes when synthesis of the repressor proteins stops in the bacterial cell and synthesis of phage components starts.

(iii) Under such condition to phage DNA, when was so far attached to the bacterial chromosome, separates and starts synthesizing phage proteins.

(iv) When phage DNA breaks off the bacterial chromosomes few genes of the bacterium remain attached to it. These genes keep on replicating along with the phage DNA and become a part and parcel of the phage particle. Such phage particles when infect any other bacterial cell, then the bacterial genes present in phage particles are incorporated in the chromosome of the new bacterial cell (i.e., recombinant cell). Thus recombinant cell, besides it own chromosomes, also contains few genes of the parent bacterial cell.

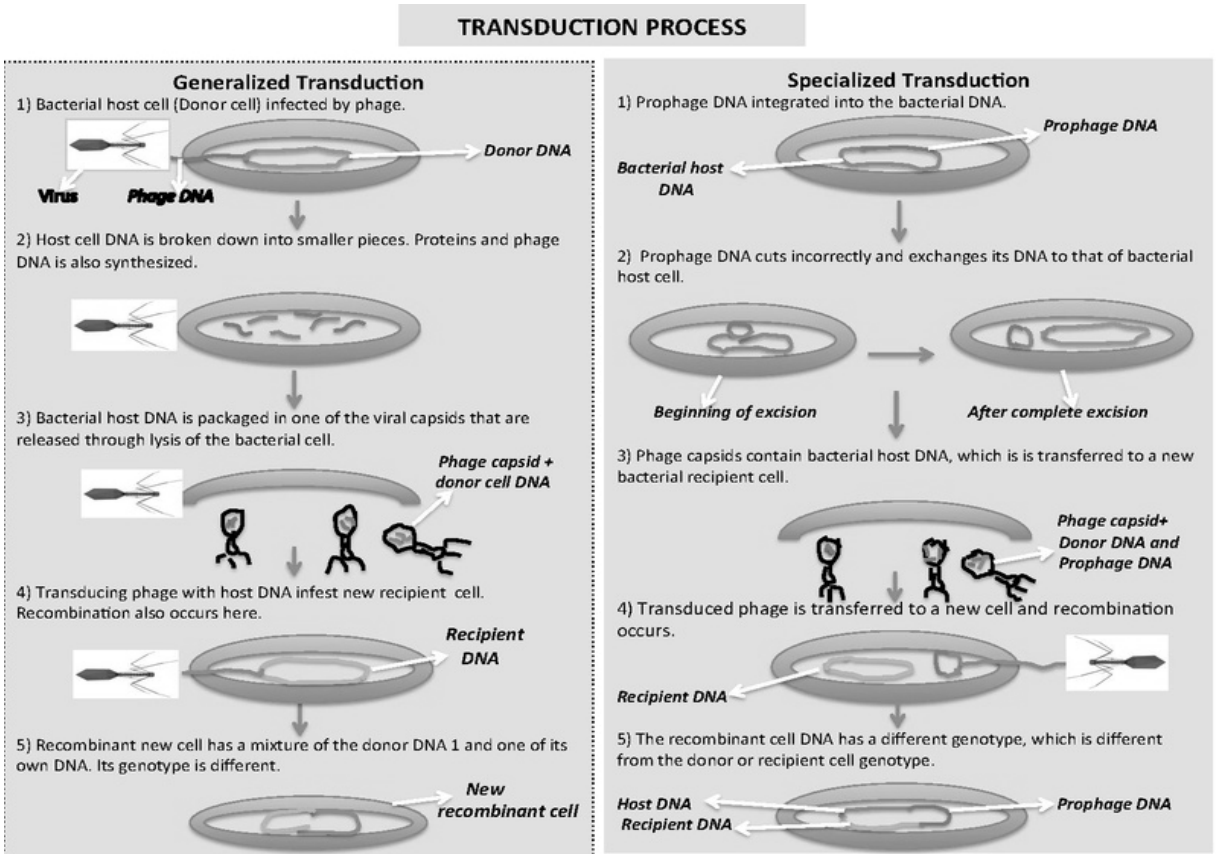



*Fig. 9.15: Difference between generalized and specialized transduction*

The basic difference between generalized and specialized transduction is given in the Fig. 9.15. Transduction differs from transformation in the following ways:

(i) Transduction transfer of genetic material from donar to recipient cell is through a temperate phase whereas, in transformation genetic material is transferred through a solution.

(ii) Deoxyribonuclease (an enzyme) completely checks the process of transformation but there is no effect of this enzyme on transduction.

## Significance of transduction

Transduction involves transfer of genetic material from one bacterial cell to another, thus it alters the genetic characteristics of the recipient cell. In this process the recipient cell attains many important characteristics. Besides this, incorporation of phage DNA into a bacterial chromosome is possible only when they have regions of quite similar base sequences. It shows a close evolutionary relationship between the prophage and the host bacterial cell. Transduction is helpful in producing transgenic organisms since by this process viruses transfer genes from one host to another.

**(c) Conjugation:** It was first reported by Lederberg and Tatum (1946) in *E. coli* bacteria. Cell to cell union occurs between two bacterial cells and genetic material (DNA) of one bacterial cell goes to another cell lengthwise through conjugation tube which is formed by sex pili (Fig. 9.16).

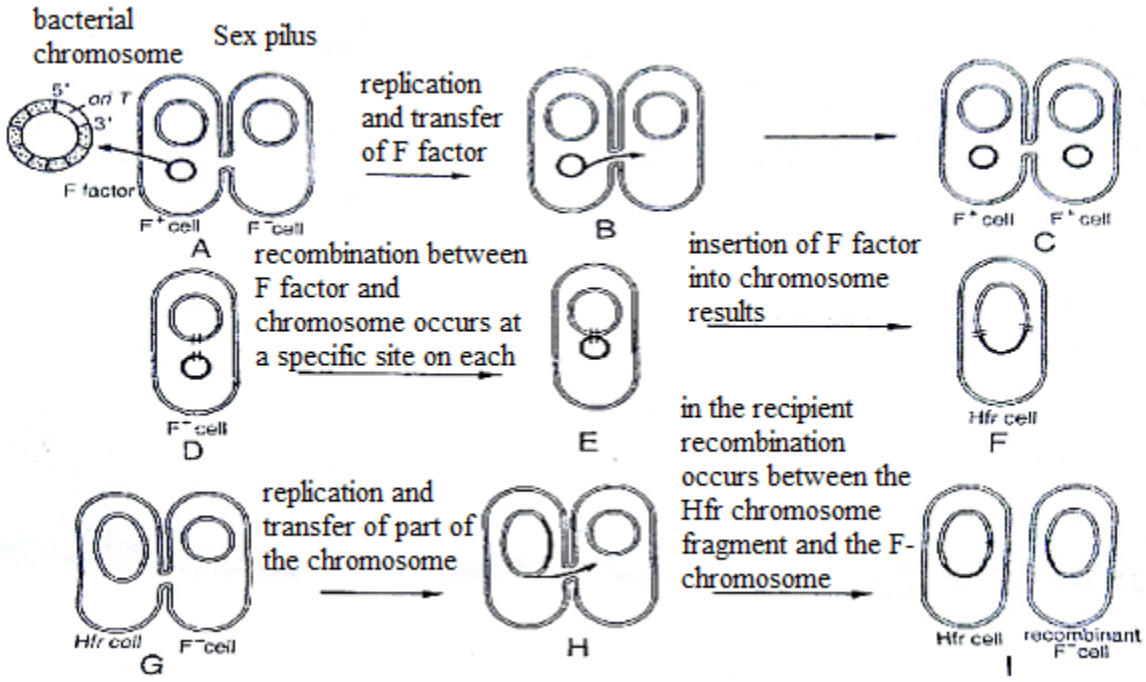



*Fig. 9.16: Diagram showing conjugation in Bacterial cell*

This is a physiological process by which the genetic matter is transferred from one individual bacterium to the other. The bacteria are recognized as F+ (male or donor strain) and F- (female or recipient strain). The F factor is also called fertility or sex factor and is represented by an extra nuclear circular DNA present only in male. The main events in conjugation are

i. Two separate bacterial cells belonging to opposite strains come close to each other.
ii. The conjugation bridge is formed between the two cells using the sex pili or $F^-$ pili.
iii. The F factor replicates in the male.
iv. The F factor slowly migrates into the female through the conjugation tube and an incomplete zygote or merozygote is formed.
v. The presence of F factor in female transforms the $F^-$ strain to $F^+$ strain.
vi. The product of conjugation is just the genetic recombination product of the two strains of cells. The progenies developing from this recombinant product conserve this character of the recombinant DNA that has been transferred during conjugation.

The recipient cell ($F^-$ cell) after receiving plasmid DNA or F factor from donar cell becomes $F^+$ cell. In some cells the $F^-$ factor integrates into the bacterial chromosome, such cells are called high frequency recombination (Hfr) cells. When conjugation occurs between Hfr cell and an $F^-$ cell, the Hfr cells chromosome replicates and a parental strand of the chromosome is transferred to the recipient cell. The replication of the Hfr chromosome begins in the middle of the integrated F-factor and a small piece of F-factor makes its way into the recipient cell. Usually the chromosome breaks up before it is completely transferred to the recipient cell. Once within the recipient cell, this piece of donar's chromosome migrates with the recipient DNA. This cell is called recombinant $F^-$ cell.

## Significance of Conjugation

Since relatively large amount of DNA is transferred in conjugation than in transformation and transduction, therefore conjugation is important in increasing genetic diversity. In fact, conjugation may represent an evolutionary stage between the process, like transformation and transduction and the actual fusion of whole cells (i.e. gametes) that occur during sexual reproduction in eukaryotes.

(i) There are groups of bacteria that use unusual forms or patterns of cell division to reproduce. Some of these bacteria grow to more than twice their starting cell size and then use multiple divisions to produce multiple offspring cells. Some other bacterial lineages reproduce by budding. Still others form internal offspring that develop within the cytoplasm of a larger "mother cell". The following are a few examples of some of these unusual forms of bacterial reproduction. E.g. (i) Baeocyte production in the cyanobacterium *Stanieria. Stanieria* never undergoes binary fission. It starts out as a small, spherical cell approximately 1 to 2 µm in diameter. This cell is referred to as a baeocyte (which literally means "small cell"). The baeocyte begins to grow, eventually forming a vegetative cell up to 30 µm in diameter. As it grows, the cellular DNA is replicated over and over, and the cell produces a thick extracellular matrix. The vegetative cell eventually transitions into a reproductive phase where it undergoes a rapid succession of cytoplasmic fissions to produce dozens or even hundreds of baeocytes.

(ii) Intracellular offspring production by some Firmicutes. *Epulopiscium* spp., *Metabacterium polyspora* and the Segmented Filamentous Bacteria (SFB) form multiple intracellular offspring. For some of these bacteria, this process appears to be the only way to reproduce. Intracellular offspring development in these bacteria shares characteristics with endospore formation in *Bacillus subtilis*. In large *Epulopiscium* spp. this unique reproductive strategy begins with asymmetric cell division, the division takes place near both cell poles. Division forms a large mother cell and two small offspring cells. The smaller cells contain DNA and become fully engulfed by the larger mother cell. The internal offspring grow within the cytoplasm of the mother cell. Once offspring development is complete the mother cell dies and releases the offspring.

The basic difference between transformation and conjugation are as follows:

1. In transformation the exchange of genetic material takes place between the members of the same species, whereas conjugation takes place between members of different species
2. Genetic recombinants obtained by conjugation are temporary where as in transformation they are permanent.

## *9.6 ECONOMIC IMPORTANCE OF BACTERIA*

The bacteria are best known as the causative agent of disease. But bacteria do not always mean disease. Many of them are beneficial while several others are neither harmful nor beneficial.

**(A). Beneficial Activities:** For our life several kinds of bacteria are must. They are necessary for life on earth for plants which in turn are necessary for animals. Here we will discuss their roles in various fields.

### 1. In agriculture

**(a) Soil fertility**- some of the bacteria maintain and increase soil fertility. They increase the nitrogen content of the soil. Plants cannot utilize free nitrogen which is taken as nitrates and $NH_3$. Since the plants are taking these nutrients continuously the soil becomes nitrogen deficient. Bacteria present in nature are responsible for this regular supply. These bacteria act as nature's farmers and belong to three main categories ammonifying bacteria, nitrifying bacteria and nitrogen fixing bacteria. This is part of nitrogen cycle.

**(i) Ammonifying bacteria-** these bacteria work upon the remaining parts of dead plants and animals and breaks them into amino acids by enzymes. The amino acids are then converted into ammonia by ammonifying bacteria. This ammonia may combine with carbon di oxide and water in the soil to make ammonium carbonate. These are used up by some plants like cereals as a source of nitrogen. *Bacillus mycoides*, *B. ramosus*, and *B.vulgaris* are some important

ammonifying bacteria. Another important source of ammonia in the soil is urea. Animal population of world excretes more than 2,00,000 tons of urea [$CO(NH_2)_2$] per day which is decomposed by *Bacillus pasteuri* and *Sarcina ureae*.

$$CO(NH_2)_2 + H_2O \longrightarrow (NH_4)_2\,CO_3$$
$$(NH_4)_2\,CO_3 \longrightarrow 2NH_3 + CO_2 + H_2O$$

**(ii) Nitrifying bacteria-** the ammonia in the soil is converted into ammonium compounds by chemosynthetic autotrophs in the soil like *Nitrosomonas* and *Nitrobacter* and finally into nitrates.

*Nitrosomonas* $NH_4 + 2\,O_2 \text{ ----------} \rightarrow 2H_2O + NO_2 + \text{energy}$

*Nitrobacter* $NO_3^- + \tfrac{1}{2}\,O_2 \text{----------} \rightarrow NO_3^- + \text{energy}$

(iii) **Nitrogen fixing bacteria-** the largest addition of nitrogen is by biological fixation process through the activity of two types of nitrogen-fixing bacteria. Some free living and some in the root nodules of leguminous plants. This is called nitrogen fixation.

**(a) Free living-** *Azotobacter beijerninckia* and clostridium-they live free in the soil. They take gaseous nitrogen present between soil particles which then make organic compounds.

**(b) Symbiotic-** *Rhizobium leguminosarum*. It lives in roots of plants like Pea, Bean, etc. nodules are also found in roots of *Alnus glutinosa, Casuarina,* etc. in *Pavetta indica* nodules are formed on leaves. This is an excellent example of symbiosis. They give nitrogen compounds to plants which in turn gives carbohydrates to them. The leguminous plants provide a rich nitrogen supply even after their death this is called green manuring. Thus cereals are grown in rotation with legumes.

## 2. Role of bacteria in industry

**(a) Dairy Industry:** Bacteria such as *Streptococcus lactis* convert milk sugar lactose into lactic acid that coagulates casein (milk protein). Then, milk is converted into curd, yoghurt, cheese etc. *Lactobacillus bulgaricus, L. acidophilus, L. plantarium, Streptococcus lactis, S. cremoris* and *S. thermophilus* are some common bacteria used in dairy industry.

**(b) Production of Organic Compounds:** Fermentation (breakdown of carbohydrate in absence of oxygen) action of various bacteria produces organic compounds like lactic acid (by *Lactobacillus*), acetic acid (by *Acetobacter aceti*), acetone (by *Clostridium acetabutylicum*) etc.

**(c) Manufacture of indigo and tanning** in leather making and preparing sponges also use bacteria e.g., *E. coli* in indigo and *Bacillus subtitis* in tanning industry.

**(d) Fibre Retting:** The action of some bacteria like *Clostridium, Pseudomonas* etc. help in fiber retting i.e. separation of stem and leaf fibre of plants from other softer tissue.

**(e) Curing:** The leaves of tea and tobacco, beans of coffee and coca are cured off their bitterness with the help of action of certain bacteria such as *Bacillus megatherium.*

**(f) Production of Antibiotics:** Number of anti bacterial and anti fungal antibiotics such as Hamycin, Polymyxin, Trichomycin etc are obtained from mycelia bacteria (like *Streptomyces*). Similarly, *Bacillus* is used for production of antibiotics such as Bacitracin, Gramicidin etc.

**(g) Production of Vitamins:** Different kinds of vitamins are produced from bacteria like Riboflavin from *Clostridium butylicum*, Vitamin B12 from *Bacillus megatherium* and Vitamin K and B-complex from *Escherichia coli*.

**(B). Harmful Activities**: Though bacteria plays important role in agriculture, industries and natural sanitation etc, it has the following harmful effects:

**(a) Food Spoiling**: Saprophytic bacteria always not only help in decomposition of dead matters, but they also cause the rotting of vegetables, fruits, meat, bread etc. Species of *Lactobacillus*, *Streptococcus*, *Micrococcus* and *Proteus* are responsible for spoilage of milk and milk products. The exotoxins produced by these bacteria are the cause of food poisoning. Many bacteria multiply in water and make it unpotable.

**(b) Food Poisoning**: Bacteria like *Staphylococcus aureus* cause food poisoning and cause people diarrhea and vomiting.

**(c) Damaging of domestic articles:** *Spirochete cytophaga* deteriorates cotton, leather and wooden articles.

**(d) Denitrification:** Bacteria such as *Thiobacillus* and *Microbacillus* convert nitrate of the soil to the gaseous nitrogen. In this process, decomposition of nitrates and nitrites into ammonia and free Nitrogen takes place under the influence of bacteria like *Bacillus licheniformis*, *Pseudomonas aeruginasa* and *Escherichia coli.*

**(e) Desulphurication:** Bacteria such as *Desulfovibrio* convert soil sulphates into hydrogen sulphide.

**(f) Cause of Diseases**: It is known that over 90% of human diseases and over 10% of plant diseases are caused by bacteria. Bacteria causing diseases like tuberculosis (*Mycobacterium boris*) and brucellosis (*Brucella abortus*) are transmitted through cow's milk. The *botulism* disease is caused by exotoxins produced by *Clostridium botulinum.* Smelling of tongue, double vision and respiratory paralysis are main symptoms of disease. Besides these Leaf spots, blight, Ring spot, canker, wilt, crown gall, rot are some important diseases caused by bacteria *Coccomyces, Xanthomonas, X. oryzae, X. solanacearum, X. citri, Phycobacterium, Agrobacterium* and *Bacterium curtororus*.

# *9.7 MAJOR GROUPS OF BACTERIA*

Classification, nomenclature, and identification are the three separate but interrelated areas of taxonomy. Classification can be defined as the arrangement of organisms into taxonomic groups (taxa) on the basis of similarities or relationships. Classification of prokaryotic organisms such as bacteria requires a knowledge obtained by experimental as well as observational techniques, because biochemical, physiologic, genetic, and morphologic properties are often necessary for an adequate description of a taxon. Nomenclature is naming an organism by international rules according to its characteristics. Identification refers to the practical use of a classification scheme:

(1) To isolate and distinguish desirable organisms from undesirable ones;
(2) To verify the special properties of a culture in a clinical setting,
(3) To isolate and identify the causative agent of a disease.

## Criteria for Classification of Bacteria

The valuable information can be obtained microscopically by observing cell shape and the presence or absence of specialized structures such as spores or flagella. Staining procedures such as the Gram stain can provide reliable assessment of the nature of cell surfaces. Some bacteria produce characteristic pigments, and others can be differentiated on the basis of their complement of extracellular enzymes; the activity of these proteins often can be detected as zones of clearing surrounding colonies grown in the presence of insoluble substrates.

Bergey's Manual of Systematic Bacteriology was first published in 1923. This Manual is an effort to classify known bacteria and to make this information accessible in the form of a key. A companion volume, *Bergey's Manual of Determinative Bacteriology,* serves as an aid in the identification of those bacteria that have been described and cultured.

## Description of the Major Categories and Groups of Bacteria

There are two different groups of prokaryotic organisms: eubacteria and archaebacteria. Eubacteria contain the more common bacteria. Archaebacteria do not produce peptidoglycan, live in extreme environments (e.g., high temperature, high salt, or low pH) and carry out unusual metabolic reactions, such as the formation of methane. A key to the four major categories of bacteria and the groups of bacteria comprising these categories is presented in following table 9.3. The four major categories are based on the character of the cell wall: gram-negative eubacteria that have cell walls, gram-positive eubacteria that have cell walls, eubacteria lacking cell walls, and the archaebacteria.

**Table 9.3. Major Categories and Groups of Bacteria That Cause Disease in Humans Used As an Identification Scheme in Bergey's Manual of Determinative Bacteriology, 9th Ed.**

| **I. Gram-negative eubacteria that have cell walls** | |
|---|---|
| Group 1: The spirochetes | *Treponema* |
| | *Borrelia* |
| | *Leptospira* |
| Group 2: Aerobic/microaerophilic, motile helical/vibroid gram-negative bacteria | *Campylobacter* |
| | *Helicobacter* |
| | *Spirillum* |
| Group 3: Nonmotile (or rarely motile) curved bacteria | None |
| Group 4: Gram-negative aerobic/microaerophilic rods and cocci | *Alcaligenes* |
| | *Bordetella* |
| | *Brucella* |
| | *Francisella* |
| | *Legionella* |
| | *Moraxella* |
| | *Neisseria* |
| | *Pseudomonas* |
| | *Rochalimaea* |
| | *Bacteroides* (some species) |
| Group 5: Facultatively anaerobic gram-negative rods | *Escherichia* (and related coliform bacteria) |
| | *Klebsiella* |
| | *Proteus* |
| | *Providencia* |
| | *Salmonella* |
| | *Shigella* |
| | *Yersinia* |
| | *Vibrio* |

| | |
|---|---|
| | *Haemophilus* |
| | *Pasteurella* |
| Group 6: Gram-negative, anaerobic, straight, curved, and helical rods | *Bacteroides* |
| | *Fusobacterium* |
| | *Prevotella* |
| Group 7: Dissimilatory sulfate- or sulfur-reducing bacteria | None |
| Group 8: Anaerobic gram-negative cocci | None |
| Group 9: The rickettsiae and chlamydiae | *Rickettsia* |
| | *Coxiella* |
| | *Chlamydia* |
| Group 10: Anoxygenic phototrophic bacteria | None |
| Group 11: Oxygenic phototrophic bacteria | None |
| Group 12: Aerobic chemolithotrophic bacteria and assorted organisms | None |
| Group 13: Budding or appendaged bacteria | None |
| Group 14: Sheathed bacteria | None |
| Group 15: Nonphotosynthetic, nonfruiting gliding bacteria | *Capnocytophaga* |
| Group 16: Fruiting gliding bacteria: the myxobacteria | None |
| **II. Gram-positive bacteria that have cell walls** | |
| Group 17: Gram-positive cocci | *Enterococcus* |
| | *Peptostreptococcus* |
| | *Staphylococcus* |
| | *Streptococcus* |
| Group 18: Endospore-forming gram-positive rods and cocci | *Bacillus* |
| | *Clostridium* |
| Group 19: Regular, nonsporing gram-positive rods | *Erysepelothrix* |
| | *Listeria* |
| Group 20: Irregular, nonsporing gram-positive rods | *Actinomyces* |
| | *Corynebacterium* |

| | |
|---|---|
| | *Mobiluncus* |
| Group 21: The mycobacteria | *Mycobacterium* |
| Groups 22–29: Actinomycetes | *Nocardia* |
| | *Streptomyces* |
| | *Rhodococcus* |
| **III. Cell wall-less eubacteria: The mycoplasmas or mollicutes** | |
| Group 30: Mycoplasmas | *Mycoplasma* |
| | *Ureaplasma* |
| **IV. Archaebacteria** | |
| Group 31: The methanogens | None |
| Group 32: Archaeal sulfate reducers | None |
| Group 33: Extremely halophilic archaebacteria | None |
| Group 34: Cell wall-less archaebacteria | None |
| Group 35: Extremely thermophilic and hyperthermophilic sulfur metabolizers | None |

Bacteria can be classified on the basis of cell structure, cellular metabolism or on differences in cell components, such as DNA, fatty acids, pigments, antigens and quinones. While these schemes allowed the identification and classification of bacterial strains, it was unclear whether these differences represented variation between distinct species or between strains of the same species. This uncertainty was due to the lack of distinctive structures in most bacteria, as well as lateral gene transfer between unrelated species. Due to lateral gene transfer, some closely related bacteria can have very different morphologies and metabolisms. To overcome this uncertainty, modern bacterial classification emphasizes molecular systematics, using genetic techniques such as guanine cytosine ratio determination, genome-genome hybridisation, as well as sequencing genes that have not undergone extensive lateral gene transfer, such as the rRNA gene. Classification of bacteria is determined by publication in the International Journal of Systematic Bacteriology, and Bergey's Manual of Systematic Bacteriology. The International Committee on Systematic Bacteriology (ICSB) maintains international rules for the naming of bacteria and taxonomic categories and for the ranking of them in the International Code of Nomenclature of Bacteria.

The term "bacteria" was traditionally applied to all microscopic, single-cell prokaryotes. However, molecular systematics showed prokaryotic life to consist of two separate domains, originally called *Eubacteria* and *Archaebacteria*, but now called *Bacteria* and *Archaea* that

evolved independently from an ancient common ancestor. The archaea and eukaryotes are more closely related to each other than either is to the bacteria. These two domains, along with Eukarya, are the basis of the three-domain system, which is currently the most widely used classification system in microbiology. However, due to the relatively recent introduction of molecular systematics and a rapid increase in the number of genome sequences that are available, bacterial classification remains a changing and expanding field. The identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human pathogens was a major impetus for the development of techniques to identify bacteria.

As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods. These methods also allow the detection and identification of "viable but nonculturable" cells that are metabolically active but non-dividing. However, even using these improved methods, the total number of bacterial species is not known and cannot even be estimated with any certainty. Following present classification, there are a little less than 9,300 known species of prokaryotes, which includes bacteria and archaea; but attempts to estimate the true number of bacterial diversity have ranged from $10^7$ to $10^9$ total species—and even these diverse estimates may be off by many orders of magnitude (Fig. 9.17).

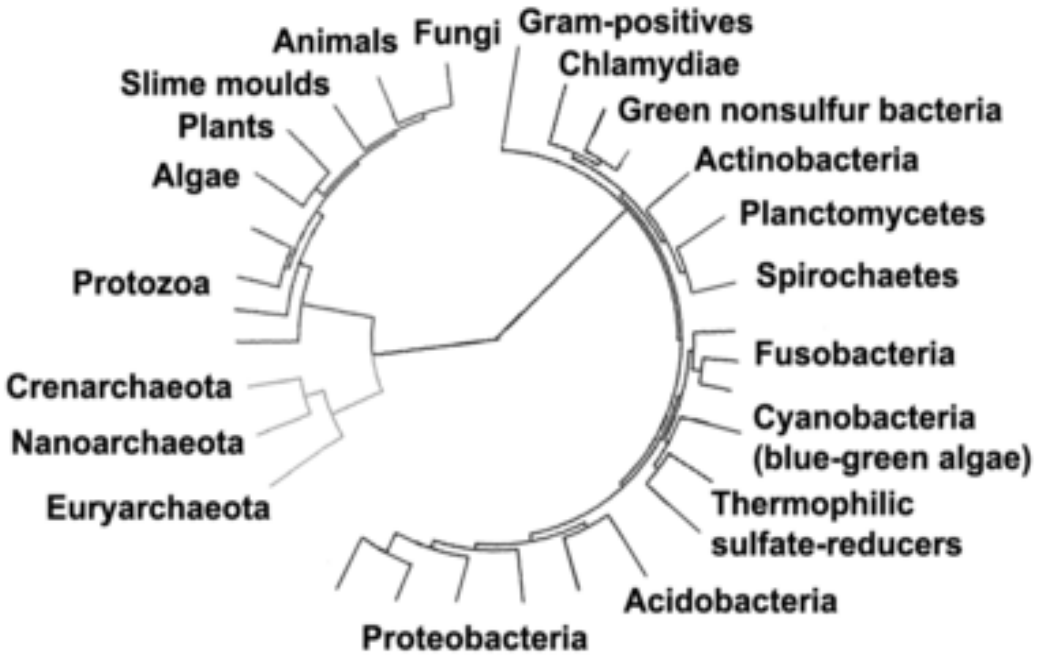



*Fig. 9.17: Diagram showing phylogenetic tree of bacteria*

## *9.8 SUMMARY*

1. Bacteria constitute a large domain of prokaryotic microorganisms.
2. Bacteria vary from a few micrometers in length, bacteria have a number of shapes, ranging from spheres to rods and spirals.
3. Bacteria were among the first life forms to appear on Earth, and are present almost everywhere on Earth.
4. The study of bacteria is known as bacteriology.
5. Bacteria were first observed by the Dutch microscopist A. v. Leeuwenhoek and named by C. G. Ehrenberg in 1828.
6. Cells are of two basic types coccoid forms and rod shaped, other forms may be spiral, helix etc.
7. Bacteria can be non-motile/motile. Motility is due to presence of flagella.
8. Capsule is the outer most, protective covering around bacterial cell, made up of mucopolysaccharides, drought resistant, antigenetically important and help bacteria to remain in body without damage.
9. C. Gram 1884 developed Gram stain to differentiate Gram positive and negative cells.
10. Plasma membrane is a usual bilipid layer different from eukaryotic cell membrane. It is associated with a number of enzymes. The cytoplasmic membrane has many folded structures called mesosomes which are associated with number of activities like seat for protein synthesis, respiratory function, multiplication of chromosomal DNA, etc.
11. On the basis of flagella Bacteria can be Monotrichous, Lophotrichous, Amphitrichous or Peritrichous.
12. Pilli are short tubular extensions which perform specific function usually are of several kinds but made of contractile protein Pilin and help in attracting and attaching to RBC, for adherence, for transfer of genetic material and form conjugation tubes.
13. Single circular DNA molecule of variable length is present in cytoplasm. Extranuclear materials called as episome are present which may be linear, circular, covalent coiled circular (CCC) = plasmid.
14. Lederberg gave term plasmid to those extragenophoral genetic materials.
15. F. plasmid can occur in low copy number per cell or high copy number per cell (up to 1000). Plasmid may be of same compatibility or different compatibility groups.
16. Cytoplasm has Glycogen like bodies PHBA granules (on terminal points) especially in *Rhizobium*. Volatile substances like volatin other crystals apart from photosynthetic pigments as cell inclusions.
17. Bacteria can be of many types on basis of nutrition symbionts or obligate intracellular parasites, parasite, photoautotrophic (e.g., plants and algae) or heterotrophic (e.g., animals, protozoa, fungi), Lithotrophy, Phototrophy, etc.
18. The Bacteria reproduces by Vegetative, asexual and sexual methods.
19. Vegetative reproduction includes budding, fragmentation and binary fission.

20. Asexual reproduction is by means of Endospores and conidia formation.
21. Endospores are formed in unfavourable conditions as they are heat resistant, acid resistant etc.
22. The Sexual Reproduction is in form of genetic recombination. There are three main methods of Genetic Recombination: Transformation, transduction and Conjugation.
23. Transformation where the genetic material of one bacterial cell goes into another bacterial cell by some unknown mechanism and it converts one type of bacterium into another type (non capsulated to capsulated form).
24. In Transduction the genetic material of one bacterial cell goes to other bacterial cell by agency of bacteriophages or phages (viruses, infecting bacteria).
25. In Conjugation Cell to cell union occurs between two bacterial cells and genetic material (DNA) of one bacterial cell goes to another cell lengthwise through conjugation tube which is formed by sex pili.
26. Some of the unusual forms of bacterial reproduction are Baeocyte production in the cyanobacterium *Stanieria.* And Intracellular offspring production by some Firmicutes. *Epulopiscium* spp., *Metabacterium polyspora*
27. Bacteria have both beneficial and harmful activities.
28. Beneficial activities includes their role in agriculture in increasing soil fertility as ammonifying bacteria, nitrifying bacteria and nitrogen fixing bacteria; in industry as dairy, organic acid production, antibiotic production, enzyme production, vitamins etc.
29. The harmful activities of bacteria include food spoiling, food poisoning, denitrification, desulphurication and a number of diseases.
30. There are two different groups of prokaryotic organisms: eubacteria and archaebacteria. Eubacteria contain the more common bacteria.
31. The four major categories are based on the character of the cell wall: gram-negative eubacteria that have cell walls, gram-positive eubacteria that have cell walls, eubacteria lacking cell walls, and the archaebacteria.

## *9.9 GLOSSARY*

**Acetogenic Bacterium-**An aerobic, gram negative bacteria, that is rod-shaped, which is made of non-sporogenous organisms that produce acetic acid as a waste product.

**Aerobic-**This includes organisms that require molecular oxygen to survive (aerobic organisms), an environment that has molecular oxygen, and processes that happen only in the presence of oxygen (aerobic respiration).

**Aerobic Anoxygenic Photosynthesis-**Photosynthetic process which takes place under aerobic conditions, but which does not result in the formation of oxygen.

**Aerotolerant Anaerobes-**Microbes that can survive in both, aerobic and anaerobic conditions, because they obtain their energy by fermentation.

**Alcoholic Fermentation**-A fermentation process that produces alcohol (ethanol) and carbon dioxide from sugars.

**Alpha-proteobacteria**-One of the five sub-groups of proteobacteria, each with distinctive 16S rRNA sequences. Mostly contains oligotrophic proteobacteria, many of which have distinctive morphological features.

**Ammonification-**Liberation of ammonia by micro-organisms acting on organic nitrogenous compounds

**Amphitrichous-**A cell which has a single flagellum at each end

**Amphotericin B-**An antibiotic derived from streptomyces nodosus which is effective against many species of fungi and certain species of leishmania.

**Anaerobic-**Refers to organisms that survive in the absence of oxygen (anearobic organisms), the absence of molecular oxygen, processes occurring in the absence of oxygen like anearobic respiration.

**Anoxygenic Photosynthesis-**A type of photosynthesis where oxygen is not produced. This phenomenon is seen in green and purple bacteria.

**Anthrax-**An often fatal and infectious disease, caused by ingestion or inhalation of spores of Bacillus anthracis, which are normally found in soil. It is acquired by humans through contaminated wool or animal products or by inhalation of airborne spores

**Autotrophic Nitrification-**The combined nitrification action of two autotrophic organisms, one converting ammonium to nitrite and the other oxidizing nitrite to nitrate.

**Auxotroph-**A mutated type of organism that requires specific organic growth factors, in addition to the carbon source present in a minimal medium.

**Axenic-**Pure cultures of micro-organisms, that is, which are not contaminated by any foreign organisms.

**Bacteria-**A domain that contains prokaryotic cells that are not multicellular. Read more on bacteria.

**Bacterial Photosynthesis-**A mode of metabolism, which is light-dependent and where carbon dioxide is reduced to glucose, which is used for energy production and biosynthesis. It is an anaerobic reaction.

**Bacteriochlorophyll-**A light absorbing pigment found in phototrophic bacteria, like green sulfur and purple sulfur bacteria.

**Bacteriocin-**Substances that are produced by bacteria which kill other strains of bacteria by inducing a metabolic block.

**Bacteriorhodopsin-**A protein involved in light mediated ATP synthesis, which contains retinal. It is one of the main characteristics of archaebacteria.

**Bacteriostatic-**An agent that inhibits the growth or multiplication of bacteria, but does not kill them.

**Bacteroid-**A genus of bacteroides, these are Gram negative, rod-shaped, anaerobic bacteria which are normal inhabitants of the oral, respiratory, urogenital and intestinal cavities of animals and humans.

**Baeocytes-**Reproductive cells formed by cyanobacteria through multiple fission. They are small and spherical in shape.

**Balanced Growth-**Microbial growth where all cellular constituents are synthesized at constant rates, in relation to each other.

**Basal Body-**A cylindrical structure that attaches the flagella to the cell body at the base of prokaryotic or eukaryotic organisms.

**Chemoautotroph-**Organisms that obtain their energy from the oxidation of inorganic chemicals and other carbon compounds.

**Chemoheterotroph-**Organisms that obtain energy and carbon from the oxidation of organic compunds.

**Chemolithotroph-**Living organisms that obtain their energy from oxidation of inorganic compunds, which act as electron donors.

**Chemoorganotroph-**Organisms that obtain energy and electrons from the oxidation of organic compounds.

**Colorless Sulfur Bacteria-**A group of nonphotosynthetic bacteria that oxidize sulfur compounds, thus deriving their energy by this process.

**Combinatorial Biology-**The process of transfer of genetic material from one microorganism to another. Mostly used to synthesize products such as antibiotics. It is also used in genetic engineering.

**Conjugants-**Mating partners that participate in conjugation, which is a type of sexual reproduction, seen in protozoans.

**Conjugative Plasmid-** self transmissible plasmid, or a plasmid that can encode all functions required to bring about its conjugation.

**Cyanobacterium-**A photosynthetic, nitrogen fixing bacteria which includes the blue-green bacteria.

**Denitrification-**Reduction of nitrate or nitrite into simpler nitrogenous compounds like molecular nitrogen or nitrogen oxides.

**Diazotroph-**Organism capable of using dinitrogen as its sole nitrogen source.

**Endospore-**A cell which is formed by certain gram-positive bacteria in unfavorable conditions. An endospore is extremely resistant to heat and other harmful agents.

**Enteric Bacteria-**These are bacteria present in the intestinal tract of humans and other animals. They maybe physiologic or pathologic.

**Episome-**An extrachromosomal replicating genetic element found in certain bacteria.

**Eubacteria-**A genus of bacteria belonging to the family Propionibacteriaceae, found as saprophytes in soil and water.

**Gram Stain-**A differential stain that divides bacteria into two groups, as Gram positive and Gram negative, depending on the ability of the organism to retain crystal violet when decolorized with an organic solvent like ethanol.

**Hydrogen Oxidizing Bacterium-**These are bacteria that oxidize hydrogen for energy and synthesize carbohydrates, using carbon dioxide as their source of carbon in the absence of other organic compounds.

**Leghemoglobin**-Red colored pigments rich in iron, which are produced in root nodules during symbiotic association between rhizobia and leguminous plants.

**Lipopolysaccharide** (LPS)-Complex lipid structure containing sugars and fatty acids, which is commonly found in most Gram negative bacteria.

**Lophotrichous-**An organism that has a tuft of flagella that is polar in nature.

**Magnetotactic Bacteria-**Bacteria that orient themselves according to the earth's magnetic field due to the presence of the magnetosomes.

**Microbiology-**The study of micro-organisms, often with the aid of a microscope.

**Microflora-**This includes bacteria, virus, fungi and algae.

**Micrometer-**One millionth of a meter (10-6 meters).

**Micronutrient-**Elements that are required for growth in trace amounts. These include copper, iron, zinc etc.

**Microenvironment**-The immediate physical and chemical surroundings of a microorganism.

**Micro-organism-**An organism that is too small to be seen by the naked eye. Also called microbes, these include bacteria, fungi, protozoans, algae and viruses.

**Nitrate Reduction (biological)-**The process of reduction of nitrate to simpler forms like ammonium by plant and micro-organisms.

**Nitrification-**Biological oxidation of ammonium to nitrite and nitrate.

**Nitrifying Bacteria-**Chemolithotrophs that can carry out the transformation from ammonia to nitrite or nitrate.

**Nitrogen Cycle-**The cycle where nitrogen is used by a living organism, then after the organism dies is restored to soil, followed by its final conversion to its original state of oxidation.

**Nitrogenase-**The enzyme required for biological nitrogen fixation.

**Nodulin-**Proteins produced in root hairs or nodules in response to rhizobial infection.

**Nucleic Acid-**A high molecular weight nucleotide polymer.

**Nucleoid-**The nuclear region of certain organisms like bacteria, which contains chromosomes, but which is not limited by a nuclear membrane.

**Pasteurization-**Process of using heat to kill or reduce the activity of micro-organisms in heat:sensitive materials.

**Pathogen-**An organism that is capable of causing an infection, or harming a host cell.

**Pathogenicity**-The ability of a parasite to infect or inflict damage on a host.

**Peptidoglycan-**Rigid cell wall layer seen in bacteria. It's also called murein.

**Peribacteroid Membrane-**A plant derived membrane which surrounds rhizobia in host cells of legume nodules.

**Periplasmic space-**The area between the cell membrane and cell wall in Gram negative bacteria

**Phosphobacterium-**Bacteria that are good at dissolving insoluble inorganic phosphate that is present in soil.

**Phycobilin-**Water soluble pigment that is seen in cyanobacteria and is the light harvesting pigment for Photosystem II.
**Pilus-**Fimbria like substance present on fertile cells that deals with transfer of DNA during the process of conjugation.
**Polar Flagellation-**The presence of flagella at one or both ends.
**Recombination-**Process by which genetic elements in two separate genomes are brought together in one unit. This is an important step in gene therapy.
**Rhizobacteria-**Bacteria that are found in roots, where they aggressively colonize.
**Rhizobia-**Bacteria capable of living symbiotically in leguminous plant roots, from where they receive energy and commonly fix molecular dinitrogen.
**Rhizosphere-**The zone of soil immediately adjacent to plant roots in which the activity and type of micro-organisms present differ from that in the rest of the soil.
**Rhizosphere Competence-**Ability of an organism to colonize the rhizosphere.
**Slime Layer-**A diffuse layer found immediately outside the cell wall in certain bacteria.
**Sterilization-**The process whereby an object or surface is rendered free of any living micro-organisms.
**Strain-**Population of cells, all of which arise from a single pure isolate.
**Ti plasmid-**A conjugative tumor inducing plasmid that can transfer genes into plants. Seen in the bacterium *Agrobacterium tumefaciens.*
**Transduction-**The process where host genetic information is transferred through an agent like a virus or a bacteriophage.
**Viable but Nonculturable-**Living organisms that cannot be cultured on artificial media.
**Viable Count-**Measurement of the concentration of live cells in a microbial population.
**Vibrio-**Curved, rod-shaped bacteria that cause cholera, belonging to the genus Vibrio.

## *9.10 SELF ASSESSMENT QUESTIONS*

### 9.10.1-Multiple choice questions:

1. Nitrifying bacterium is
(a) *Rhizobium* (b) *Nitrosomonas*
(c) *Nitrobacter* (d) *Bacillus*

2. The bacteria are
(a) Unicellular (b) Bicellular
(c) Multicellular (d) Filamentous

3. The bacterium cell wall is made up of
(a) Chitin (b) Lignin
(c) Peptidoglycan (d) Pectose

4. Bacteria are considered to be plants because
(a) Some of them are green (b) They are present everywhere
(c) Some cannot move (d) They have rigid cell wall

5. Muramic acid is found in the cell wall of
(a) Algae (b) Fungi
(c) Bacteria (d) Viruses

6. Bacteria capable of synthesizing their own food by themselves from organic and inorganic substances are called
(a) Autotrophic (b) Chemoautotrophic
(c) Heterotrophic (d) Nitrifying

7. Citrus canker is caused by
(a) *Rhizobium* (b) TMV
(c) *Xanthomonas* (d) *Aspergillus*

8. Capsule of bacteria is made up of
(a) Polysaccharides (b) Amino sugars
(c) Polypeptides (d) All of the above

9. The branch of science dealing with bacteria is called
(a) Microbiology (b) Phycology
(c) Mycology (d) Bacteriology

10. Bacteria were first observed by
(a) Louis Pasteur (b) Robert Koch
(c) Morgan (d) A. V. Leeuwenhoek

11. Bacterial plasmids are
(a) DNA (b) RNA
(c) Protein (d) Photosynthetic structures

12. Father of medical microbiology is
(a) A.M. Campbell (b) Robert Koch
(c) MK. Allen (d) R. Koch

13. In bacterium F factor is
(a) DNA (b) RNA
(c) DNA and protein (d) RNA and protein

14. Which of the following is a bacterial disease?
(a) Typhoid (b) Cholera

(c) Tuberculosis (d) All of the above

15. The common method of reproduction in bacteria is
(a) Budding (b) Fragmentation
(c) Fission (d) Conjugation

16. Transfer of DNA from donor to recipient through medium is
(a) Translation (b) Transcription
(c) Transduction (d) Transformation

17. Transformation was discovered by
(a) Lederberg & Tatum (b) Griffith
(c) Watson and Crick (d) Zinder & Lederberg

18. The bacteria having no flagella are called
(a) Atrichous (b) Monotrichous
(c) Lophotrichous (d) Amphitrichous

19. Which of the following is not found in bacteria?
(a) Fats (b) Glycogen
(c) Ribosomes (d) Nucleus

20. Process of making milk germ free is called
(a) Dehydration (b) Immunization
(c) Pasteurization (d) Sterilization

**Answer key**: 1-(b), 2-(a), 3-(c), 4-(d), 5-(c), 6-(b), 7-(c), 8-(a), 9-(d), 10-(d), 11-(a), 12-(d), 13-(a), 14-(d), 15-(c), 16-(d), 17-(b), 18-(a), 19-(d), 20-(c)

## 9.10.2-True or False

1. Bacteria are prokaryotes
2. Fission fungi are Schizomycetes
3. Gram stain was given by Griffith
4. Gram positive takes violet colour
5. Mesosomes help in antibiotic resistance
6. Conjugation is by means of viruses
7. Transformation was discovered by Zinder and Lederberg
8. The genetic material of bacteria is DNA
9. Plasmids are also called as episomes
10. Plasmid helps in pilli formation
11. Streptomycin is obtained by *Streptomyces*
12. Bacteria help in nitrogen fixation

13. *Rhizobium* is found in root nodules of leguminous plants
14. Genophore is name of bacterial DNA
15. Peritrichous bacteria lack flagella

**Answer key:** 1- True, 2- True, 3- False, 4- True, 5- False, 6- False, 7- False, 8 -True, 9- True, 10- True, 11- True, 12- True, 13- True, 14-True, 15-False

### 9.10.3-One word or very short answers

1. Write the name of an important book on bacteria?
2. The meaning of Schizomycetes is?
3. Who gave Gram stain?
4. Bacteriology means?
5. Class Schizomycetes has how many orders?
6. The acid in the wall of Gram positive bacteria is?
7. Gram negative bacteria are characterized by presence of?
8. Who discovered transformation?
9. Who discovered transduction?
10. Who discovered conjugation?
11. What are mesosomes?

**Answer key:** (1) Textbook of Microbiology, Prescott, (2). any of numerous microorganisms of the subkingdom (or phylum) Schizophyta, kingdom Monera, comprising the bacteria, (3). Danish bacteriologist Hans Christian Gram, 4). Study of bacteria, (5). 10 orders, (6). Teichoic acid, (7). cell envelopes, which are composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane, (8). 1928 by the British bacteriologist Frederick Griffith, (9). discovered by Norton Zinder and Joshua Lederberg at the University of Wisconsin–Madison in 1952 in Salmonella (10). discovered in 1946 by Joshua Lederberg and Edward Tatum, (11). Mesosomes or chondrioids are folded invaginations in the plasma membrane of bacteria that are produced by the chemical fixation techniques used to prepare samples for electron microscopy.

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## *9.13 TERMINAL QUESTIONS*

### 9.13.1 Short answer questions:

1. Briefly explain about structure of cell wall of Bacteria.
2. Describe about different mechanisms of nutrition in Bacteria?
3. Give suitable well labelled diagram of a typical Bacterial cell.
4. Give various modes of reproduction in Bacteria?

### 9.13.2 Long answer question:

1. Explain in detail about different modes of nutrition in Bacteria.
2. Describe about organization of Bacterial cell?
3. Explain modern classification of Bacteria?
4. Citing suitable examples explain about various useful and harmful activities of Bacteria?

# UNIT-10 A BRIEF ACCOUNT OF MYCOPLASMA, ACTINOMYCETES AND RICKETTSEE

## Contents:

## 10.1 OBJECTIVES

After reading this unit you will be able-

- To know about *Mycoplasma*.
- To learn about *Actinomycetes*.
- To know about *Rickettsee*.
- To understand about the structure and life cycle of *Mycoplasma*, *Actinomycetes* and *Rickettsee*.
- To learn about the useful and harmful aspects of these micro-organisms.

## 10.2 INTRODUCTION

The environment around us has a fascinating world of microorganisms which affect our life directly or indirectly. The existence of microbionts world was unknown until the invention of microscope at the beginning of the 17$^{th}$ century; which opened the realm of microorganisms to systematic scientific exploration. A. v. Leeuwenhoek (1632-1723) a Dutch cloth merchant was the first to give an illustrated description of microorganisms. The thousand of microscopic organisms that he saw in a drop of rain water were collectively named as animalcules. However with the development of the compound microscope biologists became aware of the tremendous numbers and diversities of these organisms in nature. As further information accumulated about these microscopic organisms, it became evident that they did not belong to two familiar kingdoms of living world, i.e. plants and animals and thus shared the characters of both plants and animals therefore; they could not be placed judiciously in either of these kingdoms. These include Bacteria, *Mycoplasma*, *Actinomycetes*, *Rickettsee*, etc. you have learnt about bacteria in previous unit in detail. Some more microbial systems you are going to explore in this unit, i.e. *Mycoplasmas, Actinomycetes* and *Rickettsee*. *Mycoplasmas* are the **"smallest, independently replicating prokaryotes"**. These organisms were first discovered by Pasteur in eighteenth century when he studied the causative agent of the "Bovine pleuropneumonia". They cause diseases in plants and animals both. Actinomycetes are the organisms with characteristics common to both bacteria and fungi. They are numerous and widely distributed in soil and are responsible for earthy smell after rains. The third group *Rickettsee* represents that group of alphaproteobacteria, which include many well-known organisms such as *Acetobacter, Rhodobacter, Rhizobium* and *Agrobacterium.*

## 10.3 A BRIEF ACCOUNT OF MYCOPLASMA

*Mycoplasma* are the smallest, wall less free living prokaryotes belonging to class Mollicutes. These organisms were also named as *Asterococcus mycoides* by Borrel *et al.* (1910). The term *Mycoplasma*, from the Greek *mykes* (fungus) and *plasma* (formed), was first used by Albert

Bernhard Frank in 1889 to describe an altered state of plant cell cytoplasm resulting from infiltration by fungus-like microorganisms. Pasteur (1843) discovered them for the first time when he was studying the causal organism of pleuropneumonia in cattles. He named it Pleuropneumonia like organisms (PPLO) broadly referring to organisms similar in colonial morphology and filterability to the causative agent (a Mycoplasma) of contagious bovine pleuropneumonia. E. Nocard and E.R. Roux (1898) isolated them for the first time from cattle. Julian Nowak later proposed the genus name *Mycoplasma* for certain filamentous microorganisms imagined to have both cellular and acellular stages in their life cycles, which could explain how they were visible with a microscope, but passed through filters impermeable to bacteria.

*Mycoplasmas* are obligate parasites and pathogens of many mammalian and Avian hosts. More than 75 different plant diseases are known to be caused due to Mycoplasma. Now the name of phytopathological *Mycoplasmas* has been changed to *Phytoplasma*.

## Morphology and habit

1. *Mycoplasmas* (Fig. 10.1) are supposed to be between Bacteria and viruses.
2. They are unicellular usually non-motile and gram negative. They can grow in cell free media forming typical “fried egg” shaped colony.
3. The cell is surrounded by soft triple layer lipoprotein membrane, about 10 nm thick.

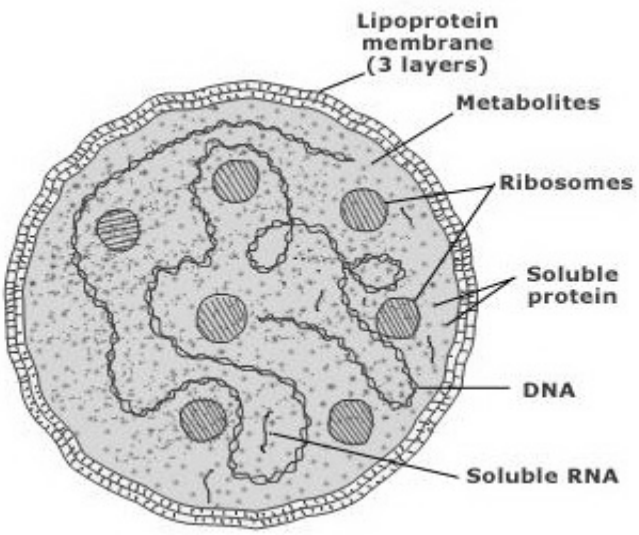



*Fig. 10.1: A typical structure of Mycoplasma*

4. They can be filtered by bacterial filters (ultra filterable). They are highly resistant to penicillium, cephaloridine, vencomycin etc. but inhibited by tetracycline.
5. The cells have both DNA (fine fibrillar double stranded helix) and RNA.

6. The Mycoplasma DNA can be distinguished from bacterial DNA by its low guanine and cytosine content.
7. The DNA is up to four percent and RNA is about eight percent and it is less than half that usually occurs in other protoplasm's. The guanine and cytosine (G and C) contents in DNA range from 23-46 percent.
8. They are inhibited by specific antibody.
9. Within the cytoplasm ribosomes are found scattered in the peripheral zone. These are 14 nm in diameter and resemble with bacteria in sedimentation characteristic of both the nucleoprotein and nucleic acid. The ribosomes are 72S type.
10. Due to the lack of a rigid cell wall, Mycoplasmataceae can contort into a broad range of shapes, from round to oblong. They therefore cannot be classified as rods or cocci. 11. They are highly pleomorphic (depending upon culture media).
11. Some Mycoplasma predominantly assumes spherical shape (300-800 nm in diameter). They may be rod like, ring like, globoid or filamentous. The filaments are of uniform diameter (100-300 nm) and vary in length from 3 nm to 150 nm.
12. In some species e.g., *M. gallisepticum* some polar bodies protrude out from one or the other end of the cell. These are called bleb and are considered to be the site of enzymatic activities and attachment during infection.
13. *Mycoplasma* lack sterols, which is present in the cell membrane of Eukaryotes but they can absorb it from medium. *Mycoplasmas* are common contaminants in cell cultures.
14. *Mycoplasma* cells are physically small – less than 1 µm, so are difficult to detect with a conventional microscope. *Mycoplasma* may induce cellular changes, including chromosome aberrations, changes in metabolism and cell growth. Severe *Mycoplasma* infections may destroy a cell line.
15. Mycoplasma can be detected by techniques like DNA Probe, enzyme immunoassays, PCR, plating on sensitive agar and staining with a DNA stain including DAPI or Hoechst.
16. *Mycoplasmas* are parasitic as well as saprophytic.
17. They are also killed by temperature of 40-55°C in fifteen minutes.

## Taxonomy

1. More than 200 *Mycoplasma* like bodies are found to be associated with sewage, plants, animals, insects, humus, hot water springs and other high temperature environment. They have been found in phloem tissues of diseased plants.
2. In **"Bergey's Manual of Determinative Bacteriology"**. (7th edition, 1957) these groups of organisms have been assigned to a new order Mycoplasma-tales. Edward et al (1967) proposed that these organisms should be placed in a new class mollicutes (Latin, *mollis* = soft, pliable, *cutis* = skin) as the organisms lack defined cell wall.
3. In 1967, a group of Japanese workers, Doi *et al.* observed that yellow disease of some plants is caused by Mycoplasma like organisms. They observed Mycoplasma-like bodies in the phloem of plants infected with several leaf hopper transmitted diseases.

4. Ishiie *et al.* (1967) observed that the Mycoplasma like bodies and symptoms disappeared temporarily when the plants were treated with tetracycline.
5. Over 100 species have been included in the genus *Mycoplasma*. Microbes of the class Mollicutes, to which *Mycoplasma* belongs, are parasites or commensals of humans, animals and plants. The genus *Mycoplasma* uses vertebrate and arthropod hosts.
6. *Mycoplasma* infects animals, *Phytoplasma* infects plants, *Spiroplasmas* infect both plants and insects, *Archeoplasmas* infects animal, plants and insects and *Entomoplasmas* infects insects and plants.
7. At least eleven serologically and biologically distinct Mycoplasmas have been found in man. *M. orale* and *M. salivarium* are found almost in every healthy adult. *M. hominis* is present in a large proportion in sexually active adults. Diseases like primary atypical pneumonia (PAP) in the mouth, pharynx and genito-urinary tract and tonsillitis in humans are caused by *Mycoplasma*.

## Reproduction

Mycoplasma generally grows more slowly than bacteria and do not produce spores. *Mycoplasma* divides by budding and/or by fission and by producing small bodies called elementary bodies or minimal reproductive units. These are formed inside the large bodies or mature cells and their size varies from 330 to 450 nm (smallest known reproductive bodies).

## Transmission

1. *Mycoplasma* like organisms (MLO) or *Phytoplasma* are usually present in phloem of the host plants and are transmitted from host to another host by leaf hoppers but some are transmitted by psyllids, tree hoppers, aphids and mites.

2. Some of the pathogens are known to infect various organs of their leaf hoppers also and multiply in their cells. The vectors cannot transmit the *Phytoplasma* immediately after feeding on the infected plant but after an incubation period of 10 to 45 days.

## Plant diseases caused by *Mycoplasma*

1. *Mycoplasma* causes “Yellows” disease in plant. Some economically important plant diseases caused by *Mycoplasmas* are sandal spike, aster yellows, mulberry dwarf, grassy shoot of sugarcane, citrus greening, seasamum phyllody, little leaf of brinjal, etc.

2. They cause many diseases in human and animals e.g., Pneumonia, Cancer, infertility, infant mortality, sexually transmitted diseases.

# *10.4 A BRIEF ACCOUNT OF ACTINOMYCETES*

The Actinomycetales are an order of Actinobacteria. These are the organisms with characteristics common to both bacteria and fungi but yet possessing distinctive features to delimit them into a distinct category. In the strict taxonomic sense, *Actinomycetes* are clubbed with bacteria the same class of Schizomycetes and confined to the order Actinomycetales. A member of the order is often called an *Actinomycete*. Actinomycetales bacteria can be infected by bacteriophages, which are called actinophages.

Actinomycetes are numerous and widely distributed in soil and are next to bacteria in abundance. They are widely distributed in the soil. *Streptomyces* produce metabolite called geosmins responsible for earthy smell. The plate count estimates give values ranging from $10^4$ to $10^8$ per gram of soil. They are sensitive to acidity / low pH (optimum pH range 6.5 to 8.0) and waterlogged soil conditions. *Streptomyces* prefer alkaline to neutral soils and require versatile nutrients including wide variety of carbon sources such as sugars, alcohol etc.

The population of *Actinomycetes* increases with depth of soil. They are heterotrophic, aerobic and mesophilic (25-30 $^{o}$C) organisms and some species are commonly present in compost and manures are thermophilic growing at 55-65°C temperature (e.g., *Thermoactinomycetes, Streptomyces*). In the order of abundance in soils, the common genera of actinomycetes are *Streptomyces* (nearly 70%), *Nocardia* and *Micromonospora* although *Actinomycetes, Actinoplanes, Micromonospora* and *Streptosporangium* are also generally encountered.

## Morphology

*Actinomycetes* are gram positive and filamentous bacteria (Fig. 10.2 to 10.4). They are unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, like true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi). On culture media unlike slimy distinct colonies of true bacteria which grow quickly, *Actinomycetes* colonies grow slowly, show powdery consistency and stick firmly to agar surface.

One of the members of *Streptomyces* shows branching. The growth is always at the tips of the filaments. The vegetative phase is a complex, tightly woven matrix, resulting in a compact, convoluted mycelium and subsequent colony. As the colony ages, characteristic aerial filaments called sporophore are formed. They project above the colony and give rise to spores distinct from endospores (Fig. 10.5 to 10.6). They produce extracellular enzymes for utilizing starch, cellulose and hemicellulose, proteins and fats even lignin, tannin or even rubber. Sometimes they are acid-fast. The lipid content in cell and cell wall is very high.

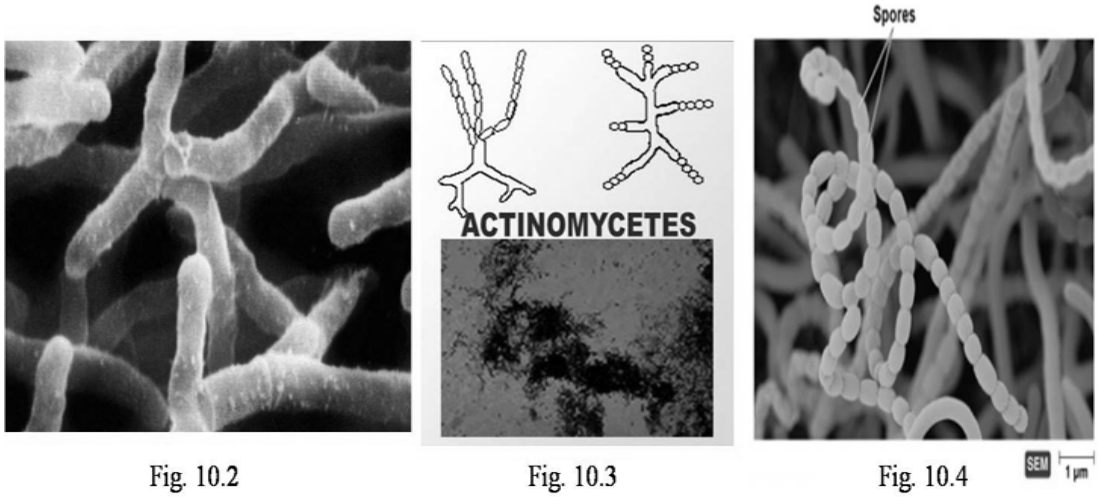



*Fig. 10.2 to 10.4: Thallus of Actinomycetes*

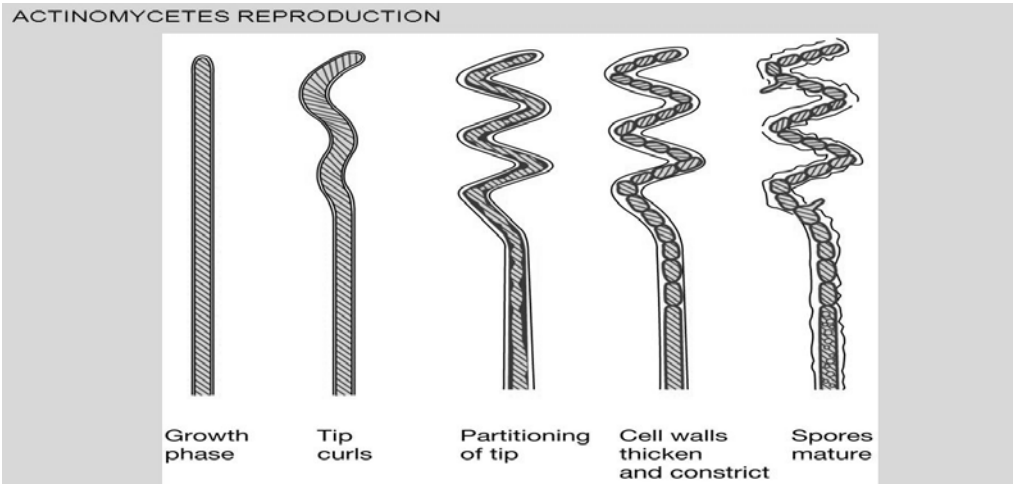



*Fig. 10.5: Spore formation*

## Taxonomy

The *Actinomycetes* are very diverse and contain a variety of subdivisions, as well as yet-unclassified isolates, mainly because some genera are very difficult to classify because of a highly niche-dependent phenotype. For example, *Nocardia* contains several phenotypes first believed to be distinct species before their differences were shown to be entirely dependent on their growth conditions.

Actinomycetales are Gram-positive, but several species have complex cell wall structures that make the Gram staining unsuitable (e.g., *Mycobacteriaceae*).

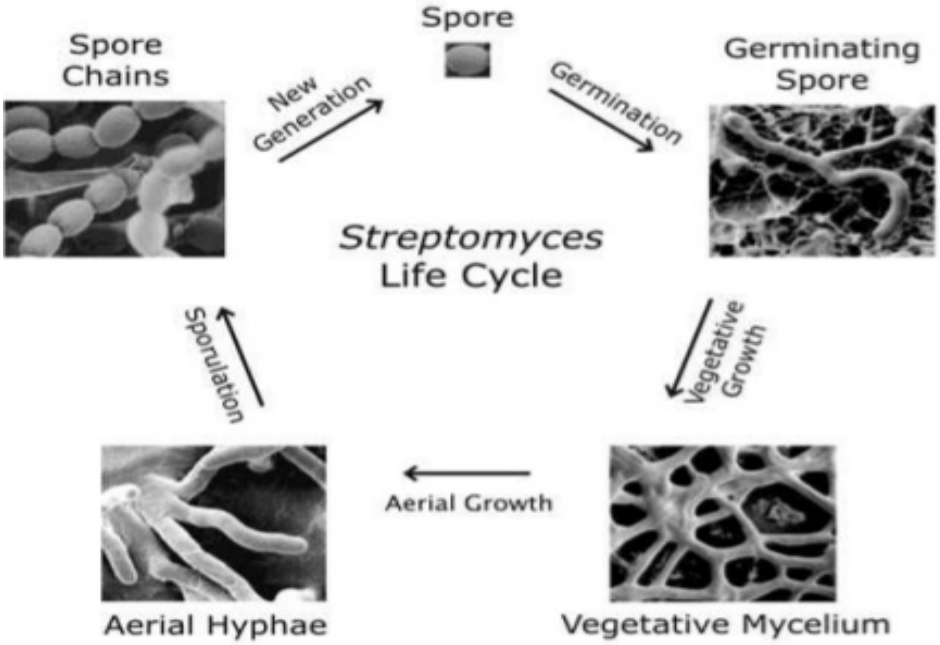



*Fig. 10.6: Typical life cycle of Actinomycetes*

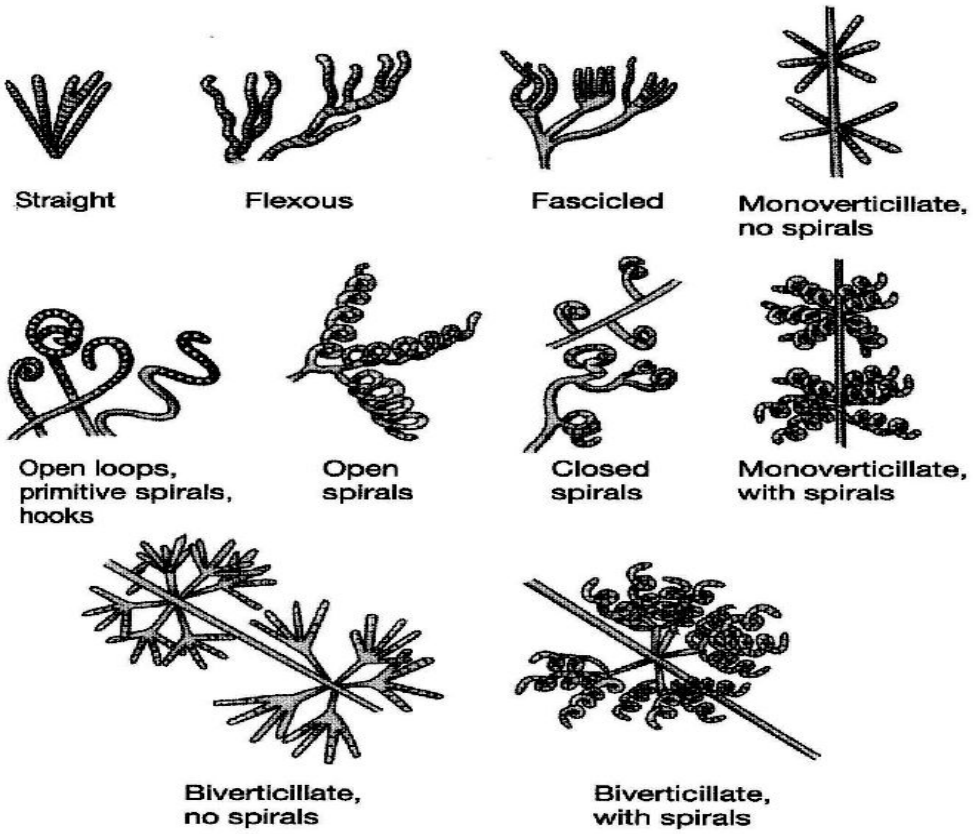



*Fig. 10.7: Morphology of spore bearing structure*

**Reproduction**: They produce hyphae and conidia / sporangia like fungi. Certain *Actinomycetes* whose hyphae undergo segmentation resemble bacteria, both morphologically and physiologically. The morphology of spore bearing structure can be Monoverticillate with no spiral, Monoverticillate with spiral, biverticillate with no spiral, biverticillate with spiral, closed spiral, open spiral and straight flexous (Fig. 10.7)

## Functions / Role of *Actinomycetes*

1. The main role of *Actinomycetes* is to degrade/decompose all sorts of organic substances like cellulose, polysaccharides, protein fats, organic-acids etc. Organic residues / substances added soil are first attacked by bacteria and fungi and later by *Actinomycetes*, because they are slow in activity and growth than bacteria and fungi.
2. They decompose / degrade the more resistant and indecomposable organic substance/matter and produce a number of dark black to brown pigments which contribute to the dark colour of soil humus.
3. They are also responsible for subsequent further decomposition of humus (resistant material) in soil. They are responsible for earthy / musty odor / smell of freshly ploughed soils.
4. Many genera, species and strains (e.g., *Streptomyces*) produce/synthesize number of antibiotics (more than 500) like Streptomycin, Terramycin, Aureomycin etc. Over 60 antibiotics have been found to be useful in human and veterinary medicines, agriculture and industries.
5. The *Frankia* family of Actinobacteria, works in a symbiotic relationship with many non legume plants as nitrogen fixing bacteria. Nitrogen is a critical nutrient for virtually all life forms.
6. We get our nitrogen either directly or indirectly from plants. While nitrogen makes up about 79% of our atmosphere, plants cannot use nitrogen in its gaseous state. It first must be fixed or combined into either ammonia ($NH_3$) or Nitrate ($NO_3$).
7. The natural nitrogen cycle relies on nitrogen fixing bacteria like those found in the *Frankia* family of *actinobacteria*, to supply the fixed nitrogen. Fixed nitrogen is often the limiting factor for growth.
8. The plants that form symbiotic relationships with *Frankia* are called actinorhizal plants. Scientists have found over 160 plants that host these *actinomycetes* including alders, Russian olive, bayberry, sweet fern, bitterbrush and cliff rose. The *Frankia* is able to provide most of the host plant's nitrogen needs. Here is a photo of the very cool looking nitrogen fixing nodules on an alder root (Fig. 10.8).

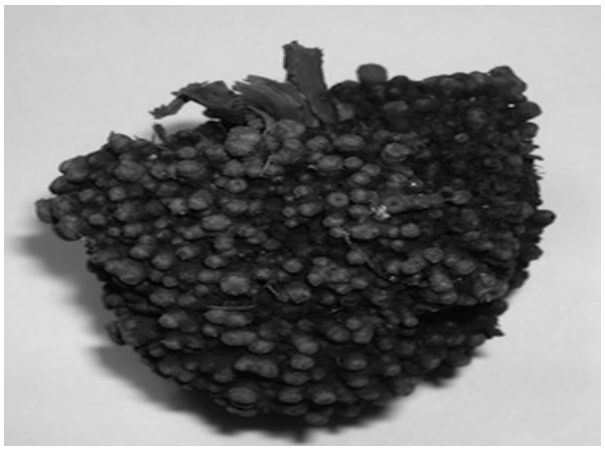


*Fig. 10.8: Frankia on alder root (root nodules with Frankia nitrogen fixing actinobacteria)*

9. These nitrogen fixing bacteria and their host plants are often pioneer species on young nitrogen deficient and disturbed soils such as moraines, volcanic flows and sand dunes. They help to create a reservoir of nitrogen rich soil. Scientists believe that much of the new nitrogen in temperate forests, dry chaparral, sand dunes, moraines, and mine waste tailings is due to the mutualism of *Frankia* and host plants.

### Diseases caused by *Actinomycetes*

1. One of the species *Streptomyces scabies* causes disease "Potato scab" in potato, *Nocardia* causes human nocardial disease which is slowly progressive pneumonia.
2. In about 25–33% of people *Nocardia* infection takes the form of encephalitis and/or brain abscess formation. *Nocardia* may also cause a variety of cutaneous infections such as actinomycetoma (especially *N. brasiliensis*), cellulitis, and subcutaneous abscesses.

## *10.5 RICKETTSEE*

### Morphology

1. ***Rickettsia*** is a genus of small, aerobic, non motile, gram-negative, non spore-forming, highly pleomorphic, non-filterable bacteria (Fig. 10.9 to 10.11).
2. They can be present as cocci (0.1 μm in diameter), rods (1–4 μm long), or thread-like (10 μm long). The typical size of *Rickettsee* varies from 0.2-0.5 μm and 0.3-2 μm).
3. The term *Rickettsia* was named after Howard Taylor Ricketts (1871–1910), who studied Rocky Mountain spotted fever in the Bitterroot Valley of Montana, and eventually died of typhus after studying that disease in Mexico City.

*Fig.10.9: Rickettsiee colony*

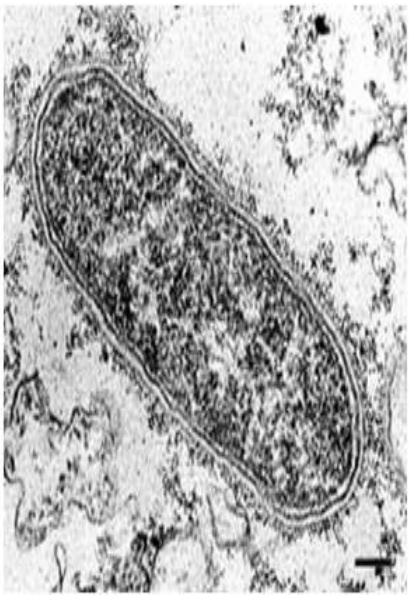


*Fig.10.10: Rickettsiee single cell*

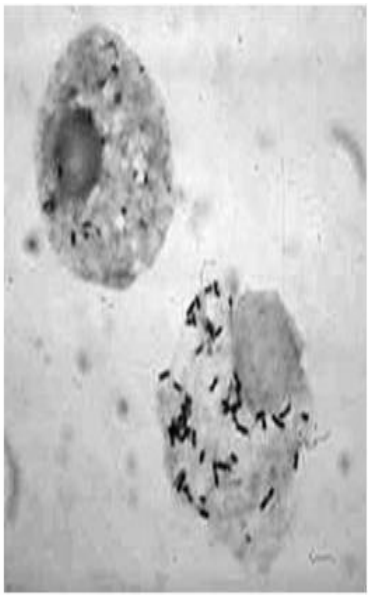
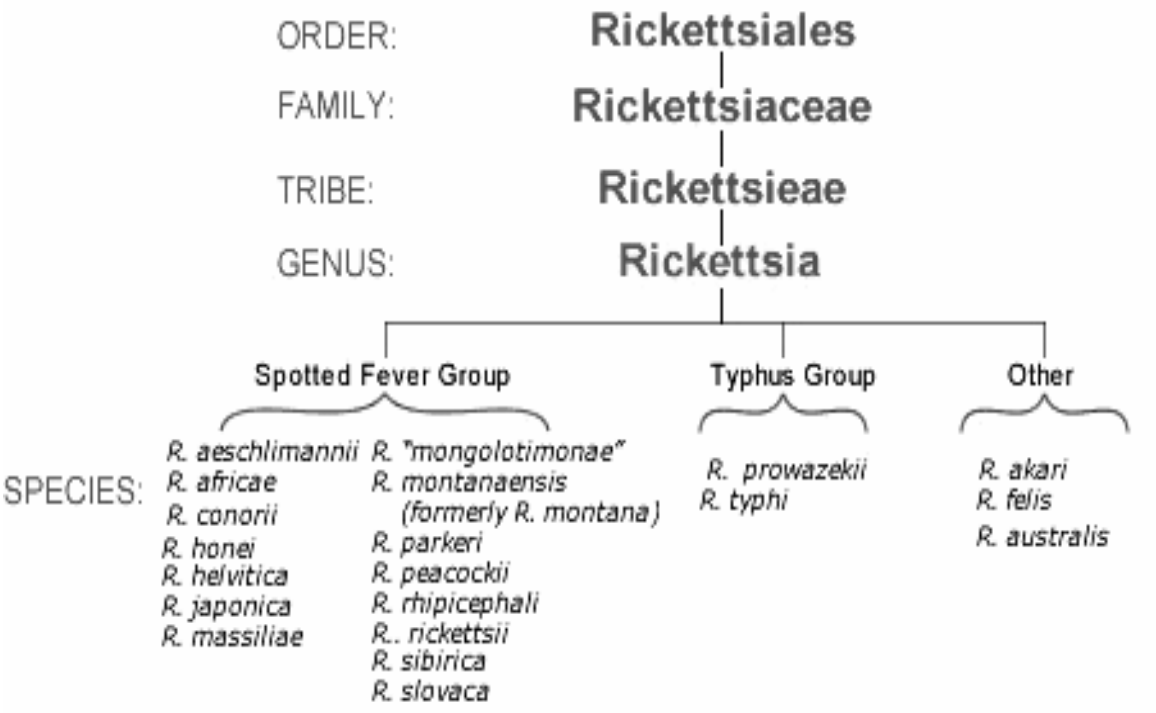


*Fig.10.11: Rickettsiee on host cell*

## Taxonomy

1. *Rickettsee* plural rickettsiae, any member of three genera (*Rickettsia, Coxiella, Rochalimaea*) of bacteria in the family Rickettsiaceae is often used interchangeably for any member of the Rickettsiales.
2. *Rickettsee* are a group of alphaproteobacteria, which include many well-known organisms such as *Acetobacter, Rhodobacter, Rhizobium* and *Agrobacterium.*
3. Very few of the alphaproteobacteria (which can grow in low levels of nutrients and have a long generation than other gram negative bacteria) are pathogens of humans. *Brucella, Bartonella, Rickettsee,* and a related intracellular parasite, *Ehrlichia,* are the main exceptions.
4. The classification of *Rickettsia* into two groups (spotted fever, typhus, and scrub typhus) was initially based on serology (Table-1). This grouping has since been confirmed by DNA sequencing.
5. All three of these contain human pathogens.
6. The scrub typhus group has been reclassified as a related new genus – *Orientia* (but many medical textbooks still list this group under the rickettsial diseases). *Rickettsee* is more widespread than previously believed and are known to be associated with arthropods, leeches, and protists.
7. In March 2010, Swedish researchers reported a case of bacterial meningitis in a woman caused by *Rickettsia helvetica* previously thought to be harmless. They are characterized by acute self-limiting fevers in humans and other animals.

**Table-1 Classification of *Rickettsia***




8. Being obligate intracellular parasites, the *Rickettsia* survival depends on entry, growth, and replication within the cytoplasm/nucleus of eukaryotic host cells (typically endothelial cells of animals).
9. *Rickettsee* cannot live in artificial nutrient environments and is grown either in tissue or embryo cultures; typically, chicken embryos are used (a method developed by Ernest William Goodpasture and his colleagues at Vanderbilt University in the early 1930s).

## Reproduction

1. They divide by binary fission.
2. *Rickettsia* enter host cell by inducing phagocytosis, then immediately escape the phagosome to grow and reproduce within the host cell.
3. The host cells normally lyse and release new organisms. The host cell is also harmed by the toxic effect of the cell wall (CDC).
4. Van-Kirk *et al*. found that actin-based motility (ABM) is a mechanism for intercellular spread. This actin tail aids the pathogen to move through the cytosol and into membrane protrusions of the host cell where it can be engulfed by neighbouring cells and can initiate a new infections cycle.

## Transmission

1. *Rickettsiae* are usually transmitted to humans by a bite from an arthropod carrier.

2. Because certain species can withstand considerable drying, transmission of *Rickettsee* can also occur when arthropod feces are inhaled or enter the skin through abrasion.
3. *Rickettsee* species are transmitted by numerous types of arthropod, including chigger, ticks, fleas, and lice, and are associated with both human and plant disease.
4. Most notably, *Rickettsia* species are the pathogens responsible for typhus, rickettsial pox, Boutonneuse fever, African tick bite fever, Rocky Mountain spotted fever, Flinders Island spotted fever and Queensland tick typhus (Australian tick typhus).
5. Despite the similar name, *Rickettsia* bacteria do not cause rickets, which is a result of vitamin D deficiency.
6. The majority of *Rickettsia* bacteria are susceptible to antibiotics of the tetracycline group.

**Table 2 The following table gives brief information about various species of *Rickettsia*, their habitat and disease caused by them**

| S.No | Species | Location | Disease |
|---|---|---|---|
| **A** | **Spotted fever group** | | |
| **1** | *Rickettsia rickettsii* | (Western Hemisphere | Rocky Mountain spotted fever |
| **2** | *Rickettsia akari* | USA, former Soviet Union | Rickettsial pox |
| **3** | *Rickettsia conorii* | Mediterranean countries, Africa, Southwest Asia, India | Boutonneuse fever |
| **4** | *Rickettsia sibirica* | Siberia, Mongolia, northern China | Siberian tick typhus or North Asian tick typhus |
| **B** | **Typhus group** | | |
| **1** | *Rickettsia prowazekii* | worldwide) | Epidemic typhus, recrudescent typhus, and sporadic typhus |
| **2** | *Rickettsia typhi* | Worldwide | Murine typhus (endemic typhus) |
| **C** | **Scrub typhus group** | | |
| **1** | *R. tsutsugamushi* | worldwide | Flora and fauna pathogenesis<br>• Beet latent rosette RLO<br>• Citrus greening bacterium possibly this citrus greening disease<br>• Clover leaf RLO |

| | | | |
|---|---|---|---|
| | | | • Grapevine infectious necrosis RLO<br>• Grapevine Pierce's RLO<br>• Grapevine yellows RLO<br>• Witch's broom disease on *Larix* spp.<br>• Peach phony RLO<br>• Papaya Bunchy Top Disease |

## Importance of *Rickettsee*

1. *Rickettsee prowazekii* has made science news recently since it has been shown to be the probable origin of eukaryotic mitochondria.
2. Phylogenetic analyses indicate that *R. prowazekii* is more closely related to mitochondria than it is to any bacterium on the Tree of Life. Certain segments of rickettsial genomes resemble those of mitochondria.
3. The deciphered genome of *R. prowazekii* is 1,111,523 bp long and contains 834 genes.
4. Unlike free-living bacteria, it contains no genes for anaerobic glycolysis or genes involved in the biosynthesis and regulation of amino acids and nucleosides. In this regard, it is similar to mitochondrial genomes; in both cases, nuclear (host) resources are used. ATP production in *Rickettsia* is the same as that in mitochondria.
5. In fact, of all the microbes known, the *Rickettsia* is probably the closest relative (in a phylogenetic sense) to the mitochondria.
6. Unlike the latter, the genome of *R. prowazekii*, however, contains a complete set of genes encoding for the tricarboxylic acid cycle and the respiratory chain complex. Still, the genomes of the *Rickettsia*, as well as the mitochondria, are frequently said to be "small, highly derived products of several types of reductive evolution".
7. The recent discovery of another parallel between *Rickettsee* and viruses may become a basis for fighting HIV infection. Human immune response to the scrub typhus pathogen, *Orientia tsutsugamushi*, appears to provide a beneficial effect against HIV infection progress, negatively influencing the virus replication process.
8. A probable reason for this actively studied phenomenon is a certain degree of homology between the rickettsiae and the virus – namely, common epitope(s) due to common genome fragment(s) in both pathogens. Surprisingly, the other infection reported to be likely to provide the same effect (decrease in viral load) is the virus-caused illness dengue fever.
9. Comparative analysis of genomic sequences has also identified five conserved signature indels in important proteins which are uniquely found in members of the genus *Rickettsia*. These indels consist of a four-amino-acid insertion in transcription repair coupling factor Mfd, a 10-amino-acid insertion in ribosomal protein L19, a one-amino-acid insertion in FtsZ,

a one-amino-acid insertion in major sigma factor 70, and a one-amino-acid deletion in exonuclease VII.

10. These indels are all characteristic of the genus and serve as molecular markers for *Rickettsia*. Bacterial small RNAs plays critical roles in virulence and stress/adaptation responses. Although their specific functions have not been discovered in *Rickettsia*, few studies showed the expression of novel sRNA in human microvascular endothelial cells (HMEC) infected with *Rickettsia.*
11. Protective measures against rickettsial disease agents include the control of arthropod carriers when necessary and immunization. Animals that recover from a rickettsiosis exhibit long-lasting immunity. Artificial immunity, as a preventive, is variably effective, typhus and the spotted fevers being among the easiest to immunize against. The most effective treatment of most rickettsioses includes the timely and prolonged administration of large amounts of broad-spectrum antibiotics such as tetracycline or, if tetracycline cannot be used, chloramphenicol.

## *10.6 SUMMARY*

The *Mycoplasma* are extremely small free-living bacteria which lack a cell wall and cytochromes. *Mycoplasmas* are supposed to be between Bacteria and viruses. They are unicellular usually non-motile and gram negative. They can be filtered by bacterial filters. They are highly resistant to penicillium but inhibited by tetracylins. The cells have both DNA and RNA. They are inhibited by specific antibody. Mycoplasma can be cultured on agar media but colonies take up to three weeks to develop. *Mycoplasma* divides by budding and/or by fission and by producing small bodies called elementary bodies or minimal reproductive units.

*Actinomycetes* are one of the most diverse groups of filamentous bacteria capable of surviving in a number of ecological niches due to their bioactive potential. *Actinomycetes* are numerous and widely distributed in soil and are next to bacteria in abundance. They are sensitive to acidity / low pH and waterlogged soil conditions. *Actinomycetes* are gram positive, unicellular like bacteria, but produce a mycelium which is non-septate (coenocytic) and more slender, like true bacteria they do not have distinct cell-wall and their cell wall is without chitin and cellulose (commonly found in the cell wall of fungi). On culture media unlike slimy distinct colonies of true bacteria which grow quickly, *Actinomycetes* colonies grow slowly, show powdery consistency and stick firmly to agar surface. They produce hyphae and conidia / sporangia like fungi.

A large fraction of antibiotics in the market are obtained from *Actinomycetes*. They produce enzyme inhibitors useful for cancer treatment and immunomodifiers that enhance immune response. *Actinomycetes* are also important in plant biotechnology as strains with antagonistic activity against plant pathogens are useful in biocontrol. Their metabolic potential offers a strong

area for research. About 15% of the world's nitrogen fixed naturally is from symbiotic relationships between various species of the *Frankia* family of actinobacteria and their host plants. They are the main nitrogen fixing relationships in large parts of the world and will only become more important as we adjust to climate change.

The *Rickettsia* is extremely small gram-negative rod-shaped, coccoid or pleomorphic bacteria with limited metabolic capabilities. The Family Rickettsiaceae contains three genera *Rickettsia*, *Ehrlichia* and *Coxiella.* All of the members of the Family Rickettsiaceae are obligate intracellular parasites due to a highly permeable cytoplasmic membrane. *Rickettsia* species are transmitted by numerous types of arthropod, including chigger, ticks, fleas, and lice, and are associated with both human and plant disease. Most notably, *Rickettsia* species are the pathogens responsible for typhus, rickettsial pox, Boutonneuse fever, African tick bite fever, etc. *Rickettsia prowazekii* has made science news recently since it has been shown to be the probable origin of eukaryotic mitochondria

## *10.7 GLOSSARY*

**Acellular**: not divided into cells. Consisting of a single complex cell-used especially for protozoa and ciliates.
**Acid fast:** that cannot be easily decolorized with acid alcohol after being stained with dyes such as basic fuchsin.
**Acid fast staining:** a procedure that differentiates between bacteria based on their ability to retain a dye when washed with an acid alcohol solution.
**Actinobacteria**: a group of gram positive bacteria containing the actinomycetes.
**Actinomycete**: an aerobic, gram positive bacterium that forms branching filaments (hyphae) and asexual spores.
**Binary fission**: Asexual reproduction in which a cell or an organism separates into two cells
**Prokaryote**: a microscopic single-celled organism which has neither a distinct nucleus with a membrane nor other specialized organelles, including the bacteria and cyanobacteria.
**Mollicutes**:a class of very small bacteria, some of which have cell walls and others of which have no cell wall but are surrounded by a cell membrane. It includes several genera that cause human diseases including *Erysipelothrix*, *Mycoplasma* and *Ureaplasma*.
**Contagious** (of a disease): spread from one person or organism to another, typically by direct contact.
**Obligate parasites**: A parasite that entirely depends upon a living host for its nourishment, reproduction, habitat, and survival
**Pathogen**: An agent causing disease or illness to its host, such as an organism or infectious particle capable of producing a disease in another organism.
**Pleomorphic**: pertaining to, or exhibiting pleomorphism; polymorphous.

**Chromosome aberrations**: change in chromosome morphology and include duplication, deletion, inversion and translocation.

## *10.8 SELF ASSESSMENT QUESTIONS*

### 10.8.1-Multiple choice questions

1- *Mycoplasma* were discovered by
(a) Nocard and Roux (b) Nowark
(c) Lederberg (d) Pasteur

2- The main function of primary bodies in *Mycoplasma* is
(a) Reproduction (b) Respiration
(c) Secretion (d) Food storage

3-*Mycoplasma* differ from bacteria in not possessing
(a) Cell wall (b) Cell membrane
(c) Ribosome (d) DNA

4- *Actinomycetes* form
(a) Substrate mycelia (b) Aerial mycelia
(c) Both a & b (d) None of the above

5- *Actinomycetes* form spores that are
(a) Asexual (b) Sexual
(c) Both a & b (d) None of the above

6-The *Streptomycetes* represents _____of the viable organisms in the soil
(a) 5% (b) 10%
(c) 1-20% (d) > 20%

7- *Acetobactor*, *Rhizobium* belongs to
(a) *Rickettsia* (b) *Mycoplasma*
(c) *Actinomycetes* (d) Viriods

8-*Rickettsia* is probable ancestors of
(a) Chloroplast (b) Endoplasmic reticulum
(c) Mitochondria (d) Ribosomes

**Answer Key:** 1(d), 2(a), 3(a), 4(c), 5(a), 6(c), 7(a), 8(c)

### 10.8.2-True or false

(1) *Mycoplasma* are also called as *phytoplasma*
(2) *Mycoplasmas* are sensitive to penicillin
(3) Nowak gave the name PPLO to *Mycoplasma*
(4) The population of *Actinomycetes* increases with depth of soil.
(5) *Actinomycetes* have characters common to both bacteria and fungi
(6) the plants which make symbiotic association with *Frankia* are called actinorhizal plants
(7) The *Rickettsia* is named after H.T. Ricketts.
(8) *Rickettsia* in humans is transmitted by bite from arthropod carrier.

**Answer Key:** 1-True, 2-False, 3-False, 4-True, 5-True, 6-True, 7-True, 8-True

### 10.8.3-Fill in the blanks

(1) _____isolated *Mycoplasma* for the first time from cattle.
(2) *Mycoplasmas* are between _____
(3) Sandal spike disease is caused by_____
(4) *Actinomycetes* are usually found in _____habitats
(5) *Rickettsia* belong to group _____
(6) *Acetobacter* belongs to _____
(7) *Rickettsia* is probable ancestors of _____
(8) *Rickettsia* divides by _____

**Answer Key:** 1-Nocard and Roux, (2) Bacteria and viruses, (3) Mycoplasma (4) soil (5) Alphaproteobacteria, (6) *Rickettsia* (7) Mitochondria, (8) Binary fission

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## *10.10 SUGGESTED READINGS*


- Vashishta BR, Sinha AK and Kumar A. Botany for degree students Fungi. S.Chand. New Delhi, 2014.
- *Mycoplasma* at Bac*Dive*- the Bacterial Diversity Metadatabase
- NCBI webpage on Actinomycetales Data extracted from the *"NCBI Taxonomy Browser". National Center for Biotechnology Information. Retrieved 2011-06-05.*


## *10.11 TERMINAL QUESTIONS*

### 10.11.1-Short answer type:

(1) What is *Mycoplasma*? Explain in brief.
(2) Write the name of two diseases caused by *Mycoplasma*.
(3) Describe the structure of *Mycoplasma*.
(4) What are *Actinomycetes*? Explain in brief.
(5) Describe morphology of *Actinomycetes*.
(6) Give important diseases caused by *Actinomycetes*.
(7) Describe the mode of transmission of *Rickettsia* in human.
(8) Discuss the reproduction of *Rickettsia*.
(9) Give some protective measures against *Rickettsia*.

## 10.11.2- Long answer questions:

(1) Write the structure and reproduction of *Mycoplasma*.
(2) What is *Mycoplasma*? How does it differ from bacteria and viruses?
(3) Name five important diseases caused by *Mycoplasma*.
(4) Give structure and reproduction of *Actinomycetes*.
(5) Discuss the role of *Actinomycetes* in ecology.
(6) Give taxonomic details of *Actinomycetes*.
(7) Give classification of *Rickettsia*.
(8) Explain the relationship of *Rickettsia* with mitochondria.
(9) Give some important plant and animal diseases caused by *Rickettsia*.