BIOLOGY AND DIVERSITY OF VIRUSES, BACTERIA, ALAGE AND FUNGI

M.Sc. BOTANY

SEMESTER-I, PAPER-III

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M.Sc. BOTANY: BIOLOGY AND DIVERSITY OF VIRUSES, BACTERIA, ALAGE AND FUNGI

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

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M.Sc. BOTANY

SEMESTER-I, PAPER-III

103BO24-BIOLOGY AND DIVERSITY OF VIRUSES, BACTERIA, ALAGE AND FUNGI

SYLLABUS

UNIT-I

1) Brief account of discovery of viruses; general properties.
2) Structure, cultivation, and purification of viruses
3) Transmission of viruses.
4) Brief account of bacteriophages and plant viruses; Economic importance.

UNIT-II

1) Morphology and ultra structure of bacteria.
2) Nutritional types (autotrophs and heterotrophs); Growth of Bacteria;
3) Recombination in bacteria (transformation, transduction and conjugation);
4) General characters of Actinomycetes, Archaebacteria, Mycoplasmas and Cyanobacteria; Economic importance.

UNIT-III

1) Distributionand thallus organization,
2) Classification and economic importance of algae;
3) Brief account of Chlorophyceae, Rhodophyceae, Phaeophyceae, andBacillariophyceae.
4) Algae as primary producers and commercial products. Algae as SCP. Algal blooms and toxins.

UNIT-IV

1) General charactersand Nutrition of Fungi
2) Reproduction of Fungi
3) Classification of Fungi (Ainsworth System);
4) Brief account of Zygomycotina, Ascomycotina, Basidiomycotina andDeteuromycotina.

UNIT-V

1) Ecto and endomycorrhizal associations;
2) Edible and poisonous mushrooms,
3) Mushroom cultivation;
4) Importance of Fungi in Agriculture and industry and Mycotoxins.

REFERENCE BOOKS:

1) An Introduction to Fungi: by Webster, J. (1985). Cambridge Univ. Press.
2) Brock Biology of Microorganisms: by Madigan. Mordinko and Parker (2000). Prentice Hall.
3) Introduction to Plant Viruses: by Mandahar. C.I. (1978). Chand & Co., New Delhi.
4) Introductory Phycology by Kumar, H.D. (1988). Affiliated East-West Press. Ltd., New Delhi.
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ACHARYA NAGARJUNA UNIVERSITY : CENTRE FOR DISTANCE EDUCATION

M.Sc. – Botany - Program code: 01

Program Structure

Program code	Program	Internal assessment	External exams	Max. Marks	credits
SEMISTER 1					
101BO24	Plant Systematics	30	70	100	4
102BO24	Reproductive Biology of Angiosperms	30	70	100	4
103BO24	Biology and Diversity of Viruses, Bacteria, Algae and Fungi	30	70	100	4
104BO24	Outlines of Bryophytes, Pteridophytes, Gymnosperms and Plant Fossils	30	70	100	4
105BO24	Plant Systematics and Reproductive Biology of Angiosperms	30	70	100	4
106BO24	Biology and Diversity of Viruses, Bacteria, Algae, and Fungi and Outlines of Bryophytes, Pteridophytes Gymnosperms and Plant Fossils	30	70	100	4
SEMISTER 2					
201BO24	Plant Ecology and Biodiversity	30	70	100	4
202BO24	Plant Physiology	30	70	100	4
203BO24	Compulsory Foundation – Cell Biology	30	70	100	4
204BO24	Plant Structure and Development	30	70	100	4
205BO24	Plant Ecology and Biodiversity and Plant Physiology	30	70	100	4
206BO24	Cell Biology and Plant Structure and Development	30	70	100	4
SEMISTER 3					
301BO24	Plant Pathology	30	70	100	4
302BO24	Plant Metabolism	30	70	100	4
303BO24	Ethnobotany and Ethnomedicine	30	70	100	4
304BO24	Molecular Biology of Plants	30	70	100	4
305BO24	Plant Pathology and Plant Metabolism	30	70	100	4
306BO24	Ethnobotany and Ethnomedicine and Molecular Biology of Plants	30	70	100	4
SEMISTER 4					
401BO24	Plant cell, Tissue and Organ Culture	30	70	100	4
402BO24	Genetic engineering and Bioinformatics	30	70	100	4
403BO24	Cytogenetics and Plant Breeding	30	70	100	4
404BO24	Horticulture and Landscaping	30	70	100	4
405BO24	Plant cell, Tissue and Organ Culture and Genetic engineering and Bioinformatics	30	70	100	4
406BO24	Cytogenetics and Plant Breeding and Horticulture and Landscaping	30	70	100	4

(103BO24)

M.Sc. DEGREE EXAMINATION, MODEL QUESTION PAPER
M.Sc. BOTANY-FIRST SEMESTER
PAPER-III: BIOLOGY AND DIVERSITY OF VIRUSES, BACTERIA, ALAGE AND FUNGI

Time: Three hours **Maximum: 70 marks**

Answer All Questions **5 × 14 = 70M**
Each Question carries equal marks (5 x 14=70)

UNIT-I

1 a) Give a detailed note on cultivation, purification and transmission of viruses.

OR

b) Give a brief note on bacteriophages and their economic importance.

UNIT-II

2 a) Give a detailed note on morphology and ultra structure of bacteria.

OR

b) Give a brief account on general characters of Actinomycetes, Archaebacteria and their economic importance.

UNIT-III

3 a) Give a detailed note on classification and economic importance of algae.

OR

b) Give a detailed note on Algal blooms and toxins.

UNIT-IV

4 a) Give a brief note on classification of Fungi by Ainsworth system.

OR

b) Give a brief account of Zygomycotina, Ascomycotina.

UNIT-V

5 a) Give an account on what is Mycorrhizae; ecto and endomycorrhizal associations.

OR

b) Give a detailed note on importance of fungi in agriculture and industry.

CONTENTS

LESSON - 1

DISCOVERY, GENERAL PROPERTIES, STRUCTURE AND ECONOMIC IMPORTANCE OF VIRUSES

1.0 OBJECTIVE OF THE LESSON:

Students will know about the discovery of viruses, their morphology and chemistry.

STRUCTURE OF THE LESSON:

1.1 INTRODUCTION:

Several viral diseases can be found in ancient records. The famous Greek poet Homer described the "Rabid dogs" before 1,000 BC. Egyptian Hieroglyphs depicted a man with a withered leg and the "drop foot" syndrome characteristic of poliomyelitis and pustular lesions characteristic of small pox. Small pox was endemic in the Ganges river basin during 5^{th} century BC and subsequently spread to the other parts of Asia and Europe. The other viral diseases such as mumps, measles, influenza, and yellow-fever are known from ancient times. Yellow fever disease has been described since the discovery of Africa by Europeans. Striping patterns of petals known as "colour breaking" in tulips were described in 1579 in Western Europe and were caused by a virus infection.

1.2 DISCOVERY OF VIRUSES:

The first report of a pathogenic agent smaller than any known "bacterium" appeared in 1892. Adolf Mayer, a German scientist named "tobacco mosaic disease" after the dark and light

spots on infected leaves in the year 1876 from Holland. This was the first experimental transmission of tobacco mosaic disease, but he failed to prove the Koch's postulates. Mayer concluded that the mosaic disease "is a bacterial, but that the infectious forms have not yet been isolated, not are their forms and mode of life known". In 1890 Dimitri Iwanowski, a Russian Scientist, passed the infected tobacco leaf sap through the Chamberland filter and reported that "the sap of leaves infected with tobacco mosaic disease retains its infectious properties even after filtration through Chamberland filter candles" on February 12, 1892. The term virus (Slimy liquid or poison in Latin) was applied to the causal agent of tobacco mosaic disease, and also for any infectious filterable agent.

Martinus Beijerinck, (1896), a Dutch soil microbiologist who collaborated with Adolf Mayer and showed that the sap of infected tobacco plants retained its infectivity after filtration and also he proved that the filtered sap regain its "strength" of infection after dilution. He explained that the pathogen is an organism smaller than bacteria, not observable in the light microscope and able to produce itself only in the living plant tissue, and hence, it cannot be cultured outside the host. Beijerinck called this filterable agent as a "Contagium vivum fluidum" (contagious living liquid). Loeffler and Frosch (1898) isolated and described the first filterable agent from animals, the foot-and-mouth disease virus (FMDV).

Walter Reed and his Co-workers (1901) recognized the first filterable agent from human, yellow fever virus. Lode and Gruber (1901) reported the virus causing plague in fowls and named it as Fowl Plague virus. In 1903 Remlinger and Riffat-Bay identified a causal agent infecting the dogs known as Rabies virus. Negri (1903) demonstrated that the nerve cells of rabies infected dogs contained prominent crystalline inclusion bodies, and they were later named as "Negri bodies". In 1911 Ellermann, Bang and Rous discovered and confirmed the cancer producing capacity of filterable agents in chicken and fowl. Peyton Rous first demonstrated a solid tumor virus of chicken, known as Rous Sarcoma virus, is a filterable agent.

In 1915, Frederick W. Twort noticed that some bacterial colonies underwent a visible change and become "Water looking" (more transparent). He called this phenomenon as glassy transformation and named the clear circular spots as '*taches vierges*' (plaques). d'Herelle developed the plaque assay in 1917 and named the agents infecting bacteria are "Bacteriophages". First successful cultivation of vaccinia virus in tissue culture was reported by Parker and Nye in the year 1925. Max Schleisinger (1932) purified the phages and reported that they were composed of protein and DNA in roughly equal proportions.

Emory Ellis and Max Delbruck (1939) designed one-step growth curve experiment, in which an infected bacterium releases hundreds of phages synchronously after 90 minutes latent or eclipse period. The first clear pictures of bacteriophages had been obtained by Tom Anderson

and Delbruck (1942). The first mutants of bacteriophages were isolated and characterized by Delbruck in the year 1946, and he also reported that mixed phage infection leads to genetic recombination. Seymour Cohen (1947) examined the effects of phage infection on DNA and RNA levels in infected cells using a colorimetric analysis. The result of Monod and Wollman in 1947 made the clear point that a virus could redirect cellular macromolecular synthetic processes in infected cells.

In 1949, first successful cultivation of poliovirus in Human tissue culture was performed by John Enders. In 1949, Andre Lwoff studied the lysoogenic phages of *Bacillus magaterium*. When lysogenic bacteria were lysed from without, no virus was detected. Hershey and Chase (1952) utilized labeled viral protein with 35_S and nucleic acids with $32_P$to follow phase attachment to bacteria. They found that the viral DNA was the genetic material not the viral protein coat. In 1953, Lowoff and Wollman discovered the temperate phages. In 1956, Takahasi and Frankel-Conrat demonstrated the reconstitution of TMV. The closed circular and super helical nature of polyoma virus DNA was first elucidated by Dulbecco and Vogt (1963).

The crystallization of TMV in 1935 by Wendell Stanley brought this infectious agent into the world of the chemists. Bawden and Pirie (1936) demonstrated that crystals of TMV contained 0.5% phosphorus and 5% RNA. Kaushe and his coworkers in 1939 had taken the first electron microscopic picture of TMV and it confirmed the rod shape of the virus particles. Jonas Salk (1955) successfully developed a killed vaccine for intravenous use against poliomyelitis, and Albert Sabin in1957 developed an attenuated virus vaccine for oral use against polio virus. Single stranded DNA was discovered in ϕX 174 bacteriophages by R.L. Sinsheimer (1959). The ultrastructure of T_2 bacteriophage was reported by S. Brenner, G. Strisinger, R.W. Horne and D. Crowther in 1959. In 1962, Caspar and Klug described the geometric principles of icosahedral structure of TMV. In 1962, Woff Home and Tournier formulated a unified system of classification of viruses. The viruses infecting cyanobacteria named as cyanophages were discovered by Shaflerman and Moris (1963). Reverse transcription *i.e.* DNA synthesis from RNA, a unique phenomenon observed in viruses alone, was reported by Howard Temin and David Baltimore in 964.

In 1967 Kornberg and Co-workers made attempts for artificial synthesis of viruses. Cancer causing virus was discovered by Schidolovski, Ahmad and Gallow in 1971. Sabin, Tam and Dress (1973) reported that the human cancer may also be caused by Herpes simplex virus. In 1976 Sanger made genetic mapping of the bacteriophage ϕX174. Galibert (1979) elucidated the nucleotide sequence of Hepatitis B virus (HBV) genome and Sninsky (1979) cloned HBV genome in *E. coli*. Robert Gallow (1984) identified AIDS as a viral disease.

1.3 PROPERTIES OF VIRUSES:

Viruses exhibit the following characteristic properties:

1) **No Independent Metabolism:** Viruses cannot multiply outside a living cell. No virus has been cultivated in a cell-free medium. Viruses do not possess an independent metabolism for synthesis of proteins, carbohydrates and nucleic acids.

2) **Nucleocapsid:** Viruses have a very simple structure and are composed of a nucleic acid core surrounded by a protein coat known as nucleocapsid.

3) **Absence of Cellular Structure:** Viruses does not have any cytoplasm, limiting cell membrane and cytoplasmic organelles. The viruses utilize the ribosomes of the host cell for protein synthesis during reproduction.

4) **Nucleic Acids:** Viruses have only one type of nucleic acid, either DNA or RNA. The Rous Sarcoma Virus (RSV) has both RNA and DNA and is therefore called RNA-DNA virus.

5) **Crystallization:** Many viruses can be crystallized, and thus behave like chemicals. The crystallized forms retain their infectivity.

6) **No Growth and Division:** The power of growth and division is absent in viruses. Various components of the virus are formed independently and assembled to form daughter viruses.

7) **Enzyme:** Lysozyme and transcriptase enzymes are present in viruses.

8) **Absence of Respiration:** Viruses does not have any respiratory system.

9) **Transmission:** Viruses require transmission because it cannot pass from one living organism to another without transmission.

10) **Host Specific:** Viruses are host-specific. Each virus can infect only a specific species and requires definite cells within host organisms for reproduction.

11) **Antibiotics have No Effect on Viruses:** Most of the antibiotics usually block a specific reaction in bacterial metabolism. The viruses have no metabolism of their own and utilize the metabolic machinery of the host cell for their reproduction. So, the antibiotics have no effect on viruses.

12) **Mutation:** They show mutation like other living beings.

1.4 STRUCTURE OF VIRUSES:

Viruses are in many shapes and sizes (Fig. 1.1). In majority of the cases, they range from 10-300 nm in diameter. In Filoviruses, the length of the particle can be up to 1400 nm with a diameter of 80 nm. Small pox virus is of the same size as smallest bacterium and Lymphogranuloma virus is larger than the smallest bacteria. Recently, Mimivirus with a diameter of 400 nm have been discovered, which possess the largest viral genome with 1000 genes, whereas some bacteria contain only around 500 genes.

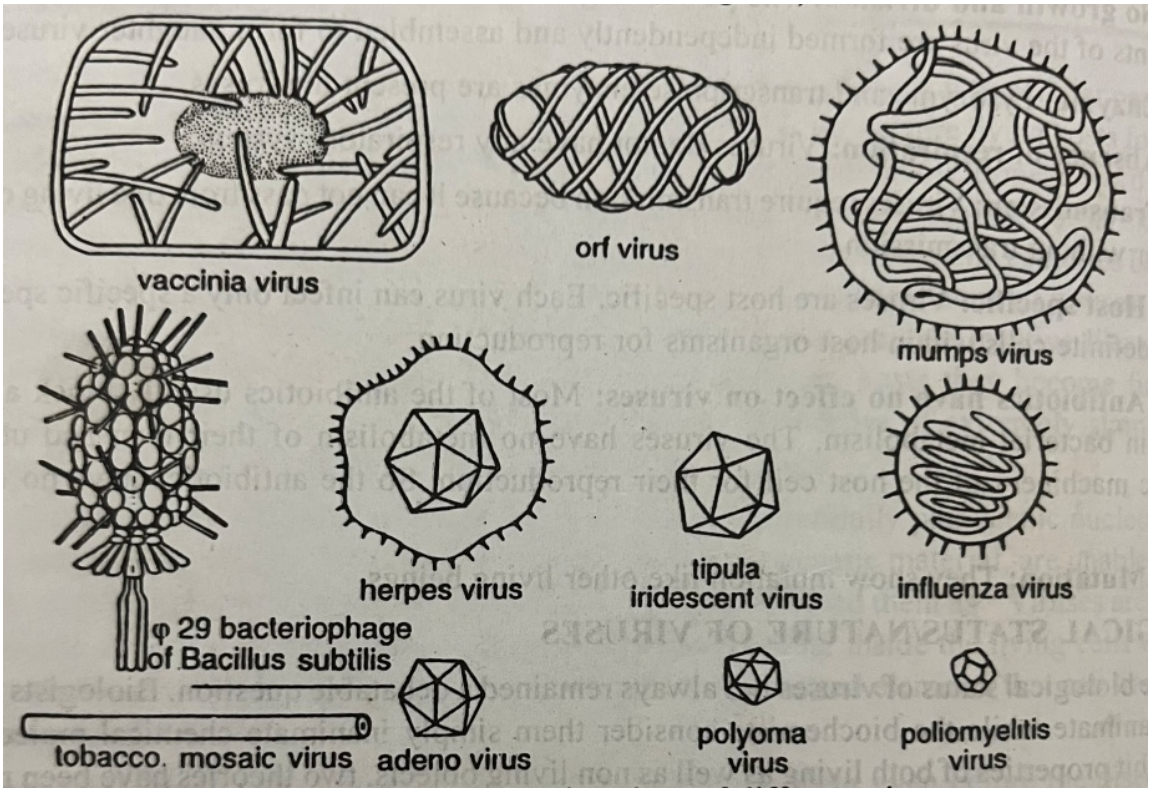

Figure- 1.1: Comparative sizes of different viruses

The broadest distinction is enveloped and non-enveloped viruses. Enveloped viruses contain a lipid-bilayer membrane and non-enveloped viruses do not contain the lipid-bilayer membrane. Further categorization of virus structure depends on their molecular organization. The progress made in understanding the viral architecture in atomic detail now allows studying the similarities across various families of viruses.

Electron microscopy is the most useful tool to determine the general morphology of a virus particle. The isolated and purified virus particles from the tissue gives more detailed images in electron microscopy. The traditional thin sections of infected cells are also used to examine the virus particles and their localization in the cells. Quantitative methods for image analysis, originally developed for studying negatively stained particles, have been applied effectively to such images. Cryoelectron microscopy is used to study the unstable or relatively impure preparations. Higher resolution picture can be obtained by X-ray diffraction method, if single crystals of the relevant structure can be prepared. In 1930, the simple plant virus such as Tomato bushy stunt virus (TBSV) was crystallized and studied. Crick and Watson (1956) proposed that virus shells would be highly symmetric objects. Identical subunits with specific interactions in general produce symmetric structures.

Structurally the viruses are classified as Helical, Icosahedral and Binal viruses.

Helical Structure:

Some of the viruses are rod-like or filamentous structures with helical symmetry. Helical symmetry is described by the number of structural units per turn of the helix. A characteristic feature of a helical structure is that any volume can be enclosed simply by varying the length of the helix, such a structure is said to be "open". In contrast, capsids with icosahedral symmetry are "closed" structures of fixed internal volume.

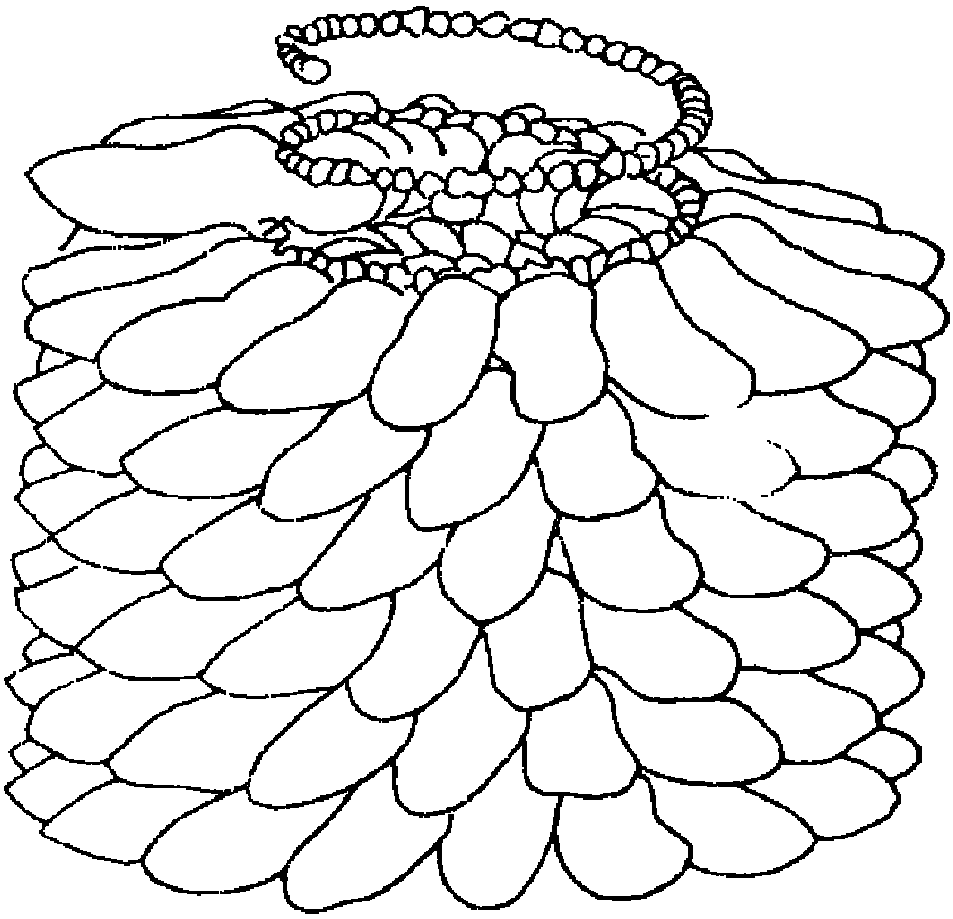

Figure-1.2: The Helical structure of TMV

In structural point of view, the first identified and understood helical nucleocapsid virus was TMV (Fig. 1.2). The virus particle comprises a single molecule of (+) strand RNA and about 6.4 kb in length, enclosed within a helical protein coat. The coat is built with a single protein which folds into an extended clog-shaped structure. Due to repetitive interaction the coat protein subunits forms disks and arranged like *"lock washers"*, which in turn assemble as a long, rod-like, right handed helix with 16.3 coat protein molecules per turn. Each coat protein molecule binds with three nucleotides of the RNA genome in the interior of the helix. The coat protein molecules engage in identical, equivalent interactions with one another and with the genome, allowing construction of a large, stable structure from a single type of protein subunits.

Icosahedral Structure:

An Icosahedral structure is a solid one with 20 triangular faces and 12 vertices. The icosahedral symmetry allows the formation of a closed shell with the smallest number of identical subunits (60). These subunits are related to one another by two, three and five fold rotational axes that define icosahedral symmetry (Fig.1.3). All subunits interact with their neighbors in an identical or equivalent manner.

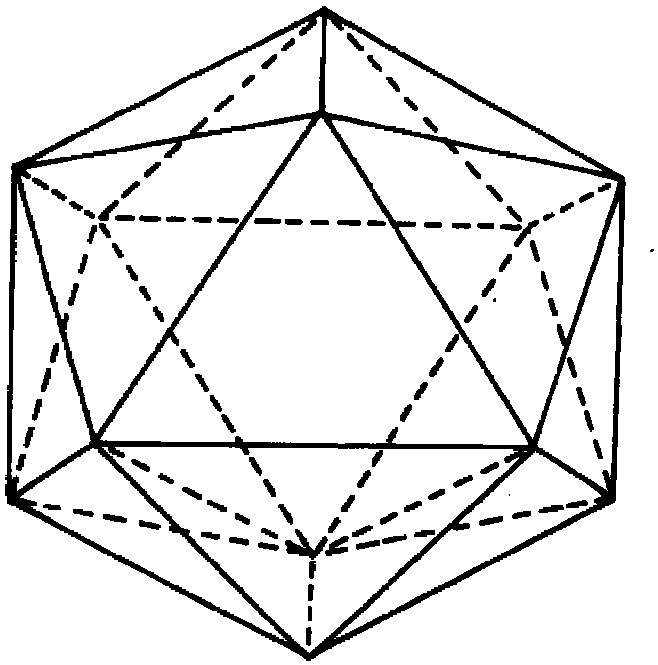

Figure-1.3: The icosahedral structure of a virus

In 1962, Caspar and Klug developed a theory for the structural properties of larger particles with icosahedral symmetry. The theory proposes that when a capsid contains more than 60 subunits, each subunit occupies a quasi-equivalent position. The triangulation number was also proposed by Caspar and Klug, the description of the triangular faces of large icosahedral structure in terms of its subdivision into smaller triangles termed facets. The triangulation number and quasi-equivalent bonding among subunits describe the structural properties of many simple viruses with icosahedral symmetry.

Quasi-equivalent designs are exemplified by a number of animal and plant viruses such as Norwalk viruses and Tomato bushy stunt virus (TBSV). These consist of 180 genetically and chemically identical subunits that form the capsid. The shell domain (S domain) of these two viruses is about 200 residues and folded structure of the domain is again a Jelly – roll – barrel model. The contents of an icosahedral asymmetric unit can be described as A, B and C, which are chemically identical subunits with different conformations. The A and B conformations are nearly identical with discarded arms and similar hinge angles. C confirmation has an ordered arm and a different hinge angle from A and B.

An icosahedral symmetric structure is folded–up as a hexagonal net, 12 uniformly spaced six fold vertices are transformed into five-fold vertices. The intervening two fold, three fold and six fold symmetry axes of the flat net are transformed either into quasi-two fold, quasi-three fold and quasi- six fold axes of the icosahedral net. A number of the viral architecture designs predicted by Caspar and Klug among various viruses of plant, vertebrates and insect viruses. Herpes virus capsids which had T=16 structure with 12 pentamers and 150 hexamers of the major capsid protein assemble around a scaffold protein. Adenoviruses exhibit a combination of non-equivalent and quasi-equivalent interactions with T=25 icosahedral lattice. It had 12 pentons on the five-fold positions and 240 hexons on the six-fold positions. Double stranded RNA viruses (Blue tongue virus) exhibit both non-equivalent and quasi-equivalent interactions in separate protein shells consisting of 120 identical subunits with T=13.

Binal Structure:

Binal structure is a complex one and bacteriophages are the best examples of binal viruses. They exhibit morphology of combination of two structures - the 'head' and 'tail'. The head is usually icosahedral and the tail is helical in symmetry (Fig. 1.4). The head shows all types of triangulation numbers as exhibited by normal icosahedral viruses. The head of lambda exhibit the highest triangulation number of T = 7 with 12 pentamers and 60 hexamers. Other morphological variations observed among phages include phages with much elongated heads - myoviridae, short tails - podoviridae and long tails - siphoviridae.

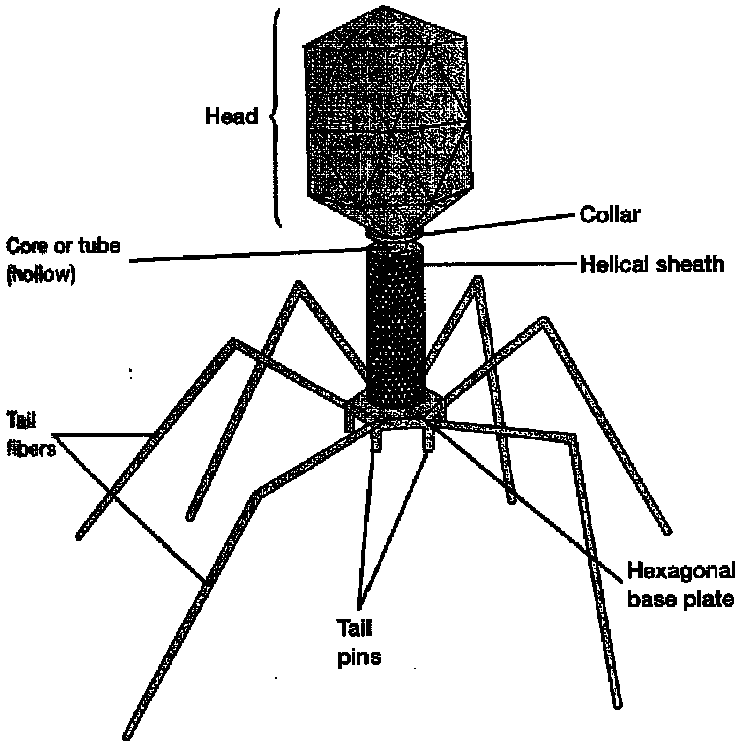

Figure-1.4: The Binal Structure of T4 Bacteriophage.

1.5 CHEMISTRY OF VIRUSES:

Max Schlesinger (1933) partially established the chemistry of viruses through the studies on bacteriophages and concluded as possibly nucleoproteins. The complete chemical identity was determined in TMV by Stanley (1935) through crystallization. Later, Bawden and Pirie (1937) established that the nucleoprotein nature of TMV strains as Ribonucleic acid. The field of "Chemical era of Virology" was launched by C.A. Knight (1974). Chemically, viruses are nucleoproteins and consist of two components, one is outer proteinaceous covering or sheath (capsid) and the other is internal component, which is a nucleic acid (genome) – either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Some of the viruses contain third component made up of lipids and carbohydrates (envelope) in the case of enveloped viruses. The composition of proteins, lipids, and carbohydrates varies both qualitatively and quantitatively from virus to virus. The type or nature of nucleic acids are also varies from virus to virus – bacterial and animal viruses are mostly DNA viruses, whereas plant viruses are in most cases RNA viruses.

Viral Nucleic Acids:

Infectiousness of the viruses depends on nucleic acid moiety only. The type and content of nucleic acid varies from virus to virus. Large size viruses consist of high quantity of nucleic acid. Greater amount of nucleic acid is necessary for the synthesis of complex viruses. The nucleic acid content of a virus can be calculated from the molecular weight of virus and its nucleic acid percentage.

Genomic diversity is one of the most important characteristic features in viruses. Various types of viral nucleic acid structures viz., straight chain, cyclic, super coiled were recorded and reported by different scientists. Single stranded and double stranded nucleic acid types were identified. Segmented and non-segmented genomes of virus nucleic acid are the special feature for viruses.

Recently the sense of the viruses was also noticed on the bases of polarity in single stranded genome (RNA) viruses. Some examples are :

ss DNA – Linear – Parvoviruses

Circular – Gemini viruses, ϕX174 phage

ds DNA – Linear – Adeno virus, Vaccinia virus, λ phage

Circular – SV-40, Polyoma virus, CaMV

ss RNA – Linear, positive sense – Polio virus, TMV

– Linear, negative sense – Rhabdo virus, Paramyxo viruses

Partially ds DNA – Circular – Hepadna virus

Non-segmented genome viruses – Picorna virus, Rhabdo virus, Herpes virus, Parvovirus.

Segmented genome viruses – Influenza viruses, Orthomyxo viruses, Reo viruses

Primary structure of viral nucleic acids relates to the proportion and arrangement of various nucleotides in a specific manner. The primary structure of viral nucleic acids can be determined into two ways – one is to determine the proportions of purines and pyrimidines and the second one is to determine the sequence arrangement of the nucleotides. In double stranded nucleic acid molecules, there is direct one to one correspondence between the purine and the pyrimidine bases. Base ratios are almost identical in similar viruses. Eg., Papilloma virus, Polyoma virus and SV40 virus. Sometimes dissimilar viruses may also have nearer identical base ratios. Eg., Coliphage, T4 and Iridescent virus. Higher proportion of uracil was estimated than other bases in influenza virus. The base ratio analyses do not allow a close insight into the primary structure of nucleic acid molecules. The nucleotide sequence analysis is the better approach to know about the arrangement of nucleotides in viral nucleic acids.

Viral Proteins:

Proteins are the basic biochemical units of the viruses which are wrapped as outer component of the genome as a capsid or coat or sheath. The coat protein gives the characteristic shape to viruses. The protein coats of viruses are not in unitary structure and composed of varying number of identical subunits. Subunits present in the capsid are called as capsomeres. These capsomeres which are assembled and form a structural unit to the virus. These capsomeres are either homopolymers or heteropolymers. In complex viruses, along with coat protein, some of the non-coat proteins are also associated and act as functional enzymes as an internal proteinaceous entities, Eg., Neuraminidase (Influenza virus), lysozyme (Phages).

The primary structure is the basic form of a viral protein. The secondary, tertiary and quaternary structures of viral proteins are essentially dependent upon the primary features, which in turn derived from the composition of the amino acids and their sequential arrangements. Amino acid analysis is being used to understand the primary structure of viral proteins. In this analysis, the cleavage of a large polypeptide chain into smaller fragments, determination of sequence of these fragments and determination of the sequence of amino acids in the individual fragments are performed. In 1959, Woody and Knight successfully analysed the

TMV coat protein by tryptic digestion method. Other TMV proteins were analysed by using various proteolytic enzymes like chemotrypsin, pepsin and subtilisin. The secondary structures and higher configuration of the viral proteins are being studied by using X-ray crystallography analyses. This method is useful to study the spatial arrangement of various subunits of the coat proteins and their alignment with central nucleic acid.

Viral Carbohydrates:

Two types of carbohydrates are associated with viruses. The first type of carbohydrates is associated with viral nucleic acids namely the ribose and deoxyribose sugars, either of them is found in viruses. The second type of carbohydrates is mostly associated with capsid or nucleocapsid proteins (glycoproteins) and lipids (glycolipids). These are simple sugars which are linked with hydroxyl methyl cytosine residue. The analysis of glycoproteins and glycolipid components revealed that the carbohydrate component is mainly made of fructose, galactose, glucosamine and mannose. It has also been revealed that the protein and carbohydrate moieties in glycoproteins are linked by formation of bonds between the carbohydrate chain and asparagine, serine, threonine residues of proteins.

Viral Lipids:

Lipids are found in most of the enveloped viruses. Several kinds of lipids (phospholipids, cholesterol, fatty acids, and glycolipids) are associated with various animal viruses, plant viruses and bacteriophages. These lipids are located in the envelope of the viruses. Lipids play an important role at the time of virus maturation and budding. Recent studies revealed that there is a significant difference in lipid component both qualitatively and quantitatively, in the envelop of viruses. The proteins and polysaccharides are linked loosely and form a lipoprotein and glycolipid complex in the viruses.

Some of the viruses consist of special components in their structure. These are polyamines (T2, T4 phages). The polyamines are putrescine, spermidine and spermine. Polyamines are also reported in Herpes virus and Influenza virus. Traces of polyamines are identified in Turnip yellow mosaic virus and Broad bean mottle virus. Inorganic divalent metal ions are also found in certain viruses. Eg., Ca^{2+}, Mg^{2+} (TMV, Southern Bean Mosaic Virus). Some plant viruses such as Tobacco streak virus (TSV) have a zinc finger binding domain that specifically binds an atom of zinc in a protein involved in nucleic acid binding.

1.6 ECONOMIC IMPORTANCE:

Viruses are known mostly for their harmful activities. They cause many human, animal and plant diseases. In addition to this, they also play a useful role in understanding the various important biological phenomena.

Useful Applications:

The viruses provide simple systems for the study of molecular biology. They form a very convenient and suitable for the understanding of DNA replication, RNA processing, protein transport and immunology. The concept of split genes emerged from the studies of Louise Chow and Richard Roberts and Philip Sharp on adenoviruses, for which they were awarded Nobel Prize in Medicine or Physiology in 1993. More recently, they are being used as vectors to introduce genes into target cells e.g., Cauliflower Mosaic Virus (CaMV). They are useful in the development of various vaccines against viral diseases. A new concept of 'virotherapy' is emerging which holds promising role in the treatment of cancers and also in gene therapy. Massachusetts Institute of Technology has successfully developed nanoscale metallic wires using genetically engineered viruses in 2006. This institute has also developed a battery using viruses, which is for better than any other made up of current materials. In future, the viruses will find a place in liquid crystals, solar and fuel cells and electronics.

Harmful Activities:

Many diseases of humans, animals, and plants are caused by various viruses and some of the diseases are so detrimental.

Human Diseases:

Many deadly diseases and epidemics are caused by several viruses. Some of the viral epidemics such as smallpox have devastated the human populations in Asia, Africa and America. AIDS has claimed more than 25 million lives till the date of early 2006. Many Asian countries were in the grip of SARS (Severe Acute Respiratory Syndrome) in 2003-2005. Angola was attacked by Marbug virus in 2004-05 which caused worst epidemic hemorrhagic fever. The recently out broken COVID 19 (Corona virus disease) is an infectious disease caused by the SARS-CoV-2 virus symptomized by fever, cough, tiredness, loss of taste or smell etc.

Some Examples are:

Disease	System/Organ Affected	Causal Agent	Transmission
AIDS	Immune system	Human Immunodeficiency virus	Blood, Genital secretions
Chicken pox	skin	Varicella-Zoster virus	Contact
Dengue	Lymph nodes	Arbovirus	*Aedes aegypti*
Hepatitis (A-E)	Liver	Hepatitis (A-E) virus	Contaminated food and water for A and E; contaminated blood in B-D
Measles	Skin	Measles virus	Contact
Mumps	Salivary gland	Mumps virus	Respiratory exudates
Poliomyelitis	Brain	Poliovirus	Ingestion
Rabies	Brian	Rabies virus	Rabid animal
Small pox	Skin	Variola virus	Contact

Animal Diseases: Many diseases such as rabies are caused by viruses in animals.

Plant Diseases: Several plant diseases like mosaics, stunting, ring spotting, necrosis, yellowing, vein clearing, blossom breaks etc., are caused by viruses. Some of these diseases account for heavy economic losses to the farmers. Some examples are –

Disease	Causal agent	Transmission
Cauliflower mosaic	Cauliflower mosaic virus	Aphids
Cucumber mosaic	Cucumber mosaic virus	Insect (*Aphis*, *Myzus*)
Leaf curl of tomato	Potato leaf roll virus	Insect (*Bremisia*)
Potato mosaic	Potato virus X	Physical, through cell sap
Rice Tungro	Rice Tungro virus	Aphids and grass hoppers
Tobacco mosaic	Tobacco mosaic virus	Physical, through cell sap
Tomato spotted wilt	Tomato spotted wilt virus	Insect (*Thrips tabaci*)

1.7 SUMMARY:

The discovery of viruses started with the studies on tobacco mosaic disease. The causal agent of the disease can pass through the bacterial filters. Hence, it was first described as "contagium vivum fluidum" and general term virus (meaning poison in Latin) was applied to causal agent of tobacco mosaic disease and causal agents of other diseases that can pass through bacterial filters. Stanley in 1935, crystallized the virus and its chemical nature was determined by a number of workers. The first electron microscopic picture of TMV was taken by Kaushe and his coworkers. Structurally the viruses are of three types viz. helical, icosahedral and binal. Chemically viruses are made of nucleic acids, proteins, carbohydrates and lipids. Viruses have a major role in medicine research and diagnosis. They are useful in vaccine production, gene therapy, cancer therapy, virus based diagnosis, research aspects, as biopesticides.

1.8 TECHNICAL TERMS:

Virion, Bacteriophage, Helical structure, Icosahedral symmetry, Binal structure, capsomeres, Pentamers, Hexamers, Capsid.

1.9 SELF ASSESSMENT QUESTIONS:

1) Describe the properties and morphology of viruses with suitable examples.
2) Discuss in detail the structure of viruses.
3) Explain in detail about the chemical nature of viruses.
4) Discuss the morphology and chemistry of plant and animal viruses.

1.10 SUGGESTED READINGS:

1) Virology - Frankel - Conrat et.al., 3rd Edition, 1994, Prentice Hall Publications.
2) Principles of Virology - S.J. Flint et.al., 2000, ASM Press.
3) Introduction to Modern Virology - Dimmock et.al., 5th edition, 2001, Blackwell Sci. Publications.
4) Plant Virology - R. Hull, 4th edition, 2001, Academic Press.
5) Fundamentals of Virology - D.M. Knipe and P.M. Howley, 4th edition, 2001, Lippincott.
6) Applied Plant Virology - D.G.A. Walkey, 1985, Heinemann Publication.

Dr. J. Madhavi

LESSON - 2

CULTIVATION, PURIFICATION AND TRANSMISSION OF VIRUSES

2.0 OBJECTIVE OF THE LESSON:

Students will have the knowledge about the methods of culturing the viruses, how they can be purified and transmitted from one host to another host.

STRUCTURE OF THE LESSON:

2.1 INTRODUCTION:

For the study of any virus it is essential to understand the nature and properties of individual virus. Cultivation and purification are the initial steps in the study of a virus. Cultivation of viruses can be done only on living cells because all are obligate pathogens, need a living cell for their survival. Different methods, including the whole organism, organ and cell cultures are used for animal viral cultivation and tissue culture and protoplast culture are used for plant viruses. For the study of properties of viruses it is essential to purify the viruses by different methods including precipitation filtration and centrifugation.

2.2 CULTIVATION OF ANIMAL VIRUSES:

Animal Viruses can be isolated from an affected host by harvesting excreted or secreted material, blood, or tissue and testing for induction of the original symptoms in the identical host or for induction of some abnormal pathology in a substitute host or in cell culture. Historically, dogs, cats, rabbits, rats, guinea pigs, hamsters, mice and chickens have all been found to be useful in laboratory investigations although most animal methods have now been replaced by cell culture methods. Once the presence of a virus has been established, it is often desirable to prepare a genetically pure clone, either by limiting serial dilution or by plaque purification.

Laboratory Animals and Embryonated Chicken Eggs:

Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs. Whole animals could include the natural host- laboratory animals such as rabbits, mice, rats and hamsters. In the case of laboratory animals, newborn or suckling rodents often provide the best hosts. Today, laboratory animals are rarely used for routine cultivation of virus but they still play an essential role in studies of viral pathogenesis.

The use of embryonated chicken eggs was introduced by Goodpasture *et.al.* in 1932 and developed subsequently by Beveridge and Burnet. The developing chick embryo, 10 to 14 days after fertilization, provides a variety of differentiated tissues, including the amnion, allantois, chorion, yolk sac, which serve as substrates for growth of a wide variety of viruses, including orthomyxoviruses, paramyxoviruses, rhabdoviruses, togaviruses, herpesviruses and poxviruses (Fig. 2.1). Several viruses from each of the groups cause discrete and characteristic foci – pocks, when introduced onto the chorioallantoic membrane (CAM) of embryonated eggs, thus providing a method for identification of virus types, or for quantifying virus stocks or assessing virus pathogenicity. Although the embryonated eggs have been almost wholly replaced by cell culture techniques, they are still the most convenient method for growing high tier stocks of some viruses and they thus continue to be used both in research laboratories and for vaccine production. In addition, pock formation on the CAM still provides a specialized method for assay of variants of poxviruses - wild type rabbit pox and cowpox viruses cause red hemorrhagic pocks on the CAM, whereas viruses deficient in specific virulence genes cause white pocks as a result of the infiltration of the lesions with inflammatory cells.

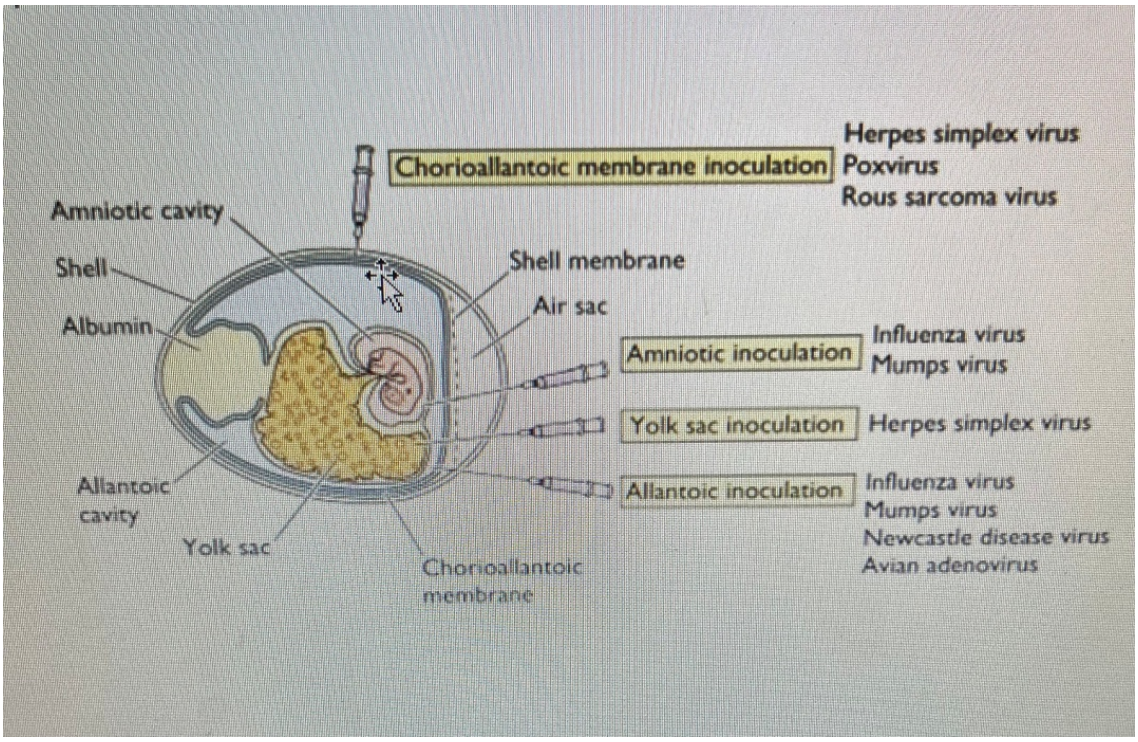

Figure-2.1: Cultivation of viruses in chicken embryonated egg.

Organ Cultures:

Organ cultures use the whole organ for culturing which provides the natural conditions for the virus. They have the advantage of maintaining the differentiated state of the cell. However, there are technical difficulties in their large-scale use, and as a result they have not been widely used. Ciliated cells lining the trachea continue to beat in coordinated waves while the tissue remains healthy. Multiplication of some viruses causes the synchrony to be lost and eventually causes the ciliated cells to death. Virus is also released into fluids surrounding the tissue and can be measured if appropriate assays are available.

Cell Cultures:

In the 1950-60 period more than 400 viruses were isolated and cultured (Golden Age) by the cell cultures. Two discoveries greatly enhanced the usefulness of cell culture for virologists. First, the discovery and use of antibiotics made it possible to prevent bacterial contamination. Second, biologists found that proteolytic enzymes, particularly trypsin, can free animal cells from surrounding tissues without injuring the free cells. Cells in culture are kept in an isotonic solution, consisting of a mixture of salts in their normal physiological proportions supplemented with serum (usually 5-10%), and in such a growth medium most cells rapidly adhere to the surface of suitable glass or plastic vessels. Serum is a complex mixture of proteins and other compounds, without which mitosis does not occur. After cell division the cells form a mono layer in the vessels. Synthetic substitutes are now available but these are expensive and employed mainly for specialized purposes. All components used in cell

culture have to be sterile and handled under aseptic conditions to prevent the growth of bacteria and fungi. Antibiotics have been invaluable in establishing cells in culture, and routine cell culture dates from 1950s when they first appeared on the market.

Cultured cells are either diploid or heteroploid (having more than the diploid number of chromosomes but not simple multiple of it). Diploid cell lines undergo a finite number of divisions, from around 10 to 100 whereas the heteroploid cells will divide forever. The latter are known as continuous cell lines and they originate from naturally occurring tumors or from some spontaneous event that alter the control of division of a diploid cell. Diploid cell lines are most easily obtained from embryos by reducing lungs, kidneys or the whole body to a suspension of single cells. Cell cultures are of three basic types: Primary cell cultures, Cell strains and Cell lines, which may be derived from many animal species, and differ substantially in their characteristics.

Primary Cell Cultures: (Fig. 2.2.)

A primary cell culture is defined as a culture of cells obtained from the original tissue that have been cultivated in vitro for the first time, and that have not been sub cultured. Primary cell cultures can be established from whole animal embryos or from selected tissues from embryos, newborn animals or adult animals of almost any species. The most commonly used cell culture in virology obtained from primates, including humans and monkeys, rodents including hamsters, rats and mice and birds most notably chickens. Cells to be cultured are obtained by mincing tissue and dispersing individual cells by treatment with proteases and/or collagenase to disrupt cell-cell interactions and interactions of cells with the extra-cellular matrix. With the exception of cells from the hemopoietic system, normal vertebrate cells will grow and divide only when attached to a solid surface. Dispersed cells are therefore placed in a plastic flask or dish, the surface of which has been treated to promote cell attachment. The cells are incubated in a buffered nutrient medium in the presence of blood serum, which contains a complex mixture of hormones and factors required for the growth of normal cells. The blood serum may come from a variety or sources, but bovine serum is most commonly used. Under these conditions, cells which attach to the surface of the dish will divide and migrate until the surface of the dish is covered with a single layer of cells , a mono layer, whereupon they will remain viable but cease to divide. If the cell mono layer is wounded by scraping cells from an isolated area, cells on the border of the wound will resume division and migration until the mono layer is reformed, whereupon cell division again ceases. Primary cultures may contain a mixture of cell types and they retain the closet resemblance to the tissue of origin.

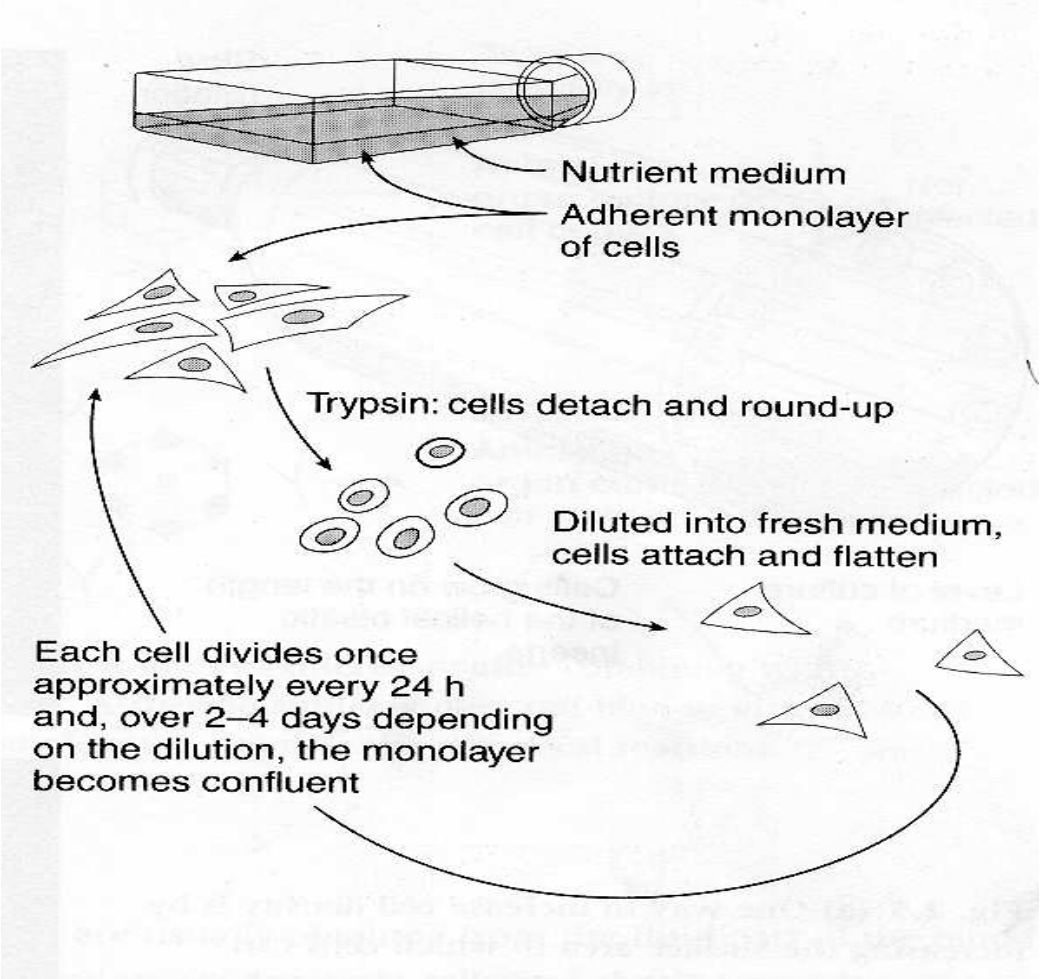

Figure-2.2: Cell Cultures (Source: Dimmock *et al* 2001)

Cell Strains:

Normal vertebrate cells cannot be generated indefinitely in culture. Instead, after a limited number of cell generations, usually 20 to 100 depending on the age and species of the original animal, cultured normal cells cease to divide and they degenerate and die, a phenomenon called crisis or senescence. Primary cell cultures may contain a mixture of cell types but only a few cell types survive after sub culturing and by subsequent generations, after second and third, typically only one cell type remains in the cell strain.

Cell strains are usually composed of one of two basic cell types, fibroblast like or epithelial-like, cells. Fibroblasts have an elongated, spindle shape, whereas epithelial cells have a polygonal shape. Although after only a few generations, only one cell type may remain in a cell strain, continued generations may select for faster growing variants, such that the characteristic of a cell strain may change with increasing generation number. Despite the fact that normal cell strains experience senescence in culture, they may be maintained for many years by expanding the culture to a large number of cells.

Cell Lines:

At any time during the culture of a cell strain, cells in the culture may become transformed. Transformation is a complex phenomenon, in the context of cell culture. The most important characteristic of transformation is that the transformed cells become immortalized. Immortal cell cultures are called cell lines or sometimes continuous cell lines to distinguish them from primary cultures and cell strains. Immortalization can occur spontaneously during passage of a cell strain or it can be induced by treatment with chemical mutagens, infection with tumorogenic viruses or transfection with oncogenes.

In addition, cells cultured from tumor tissue frequently readily establish immortal cell lines in culture. Spontaneous immortalization does not occur in cultured cells from all animal species. Thus immortalization occurs frequently during culture of rodent cells, for example in mouse and hamster cell strains and in monkey kidney cells, but it occurs rarely in chicken or human cells. Like cell strains, cell lines are usually composed of cells that are either fibroblast-like or epithelial-like in morphology. These cell lines play a important role in the present day viral vaccine preparations.

Modern Methods of Cell Culture:

The methods described above are suitable for research and clinical or diagnostic laboratories, but it is difficult to scale up for commercial purposes which need increased cell densities. One of the earliest method to increase the cell density is to grow cells in suspension, and this has been refined, using hybridoma cells (which are immortalize antibody-synthesizing or B cells) that produces monoclonal antibodies (Mabs). As many cells grow only when anchored to a solid surface, the modified modern method is aimed to increase the surface area, by fitting the spiral inserts into the conventional culture bottles (Fig. 2.3.).

The added advantage of this method is by rotating the bottle slowly (5 rev/h) only a small volume of culture medium is enough for culturing. Another method is to grow cells on ‘micro carriers’- tiny particles, on which cells attach and divide. The surface area afforded by 1kg of micro carriers is about 2.5 m^2 and the space take up (a prime consideration in commercial practice) is very economical. This method combines the ease of handling cell suspensions with matrix for the cell to grow on.

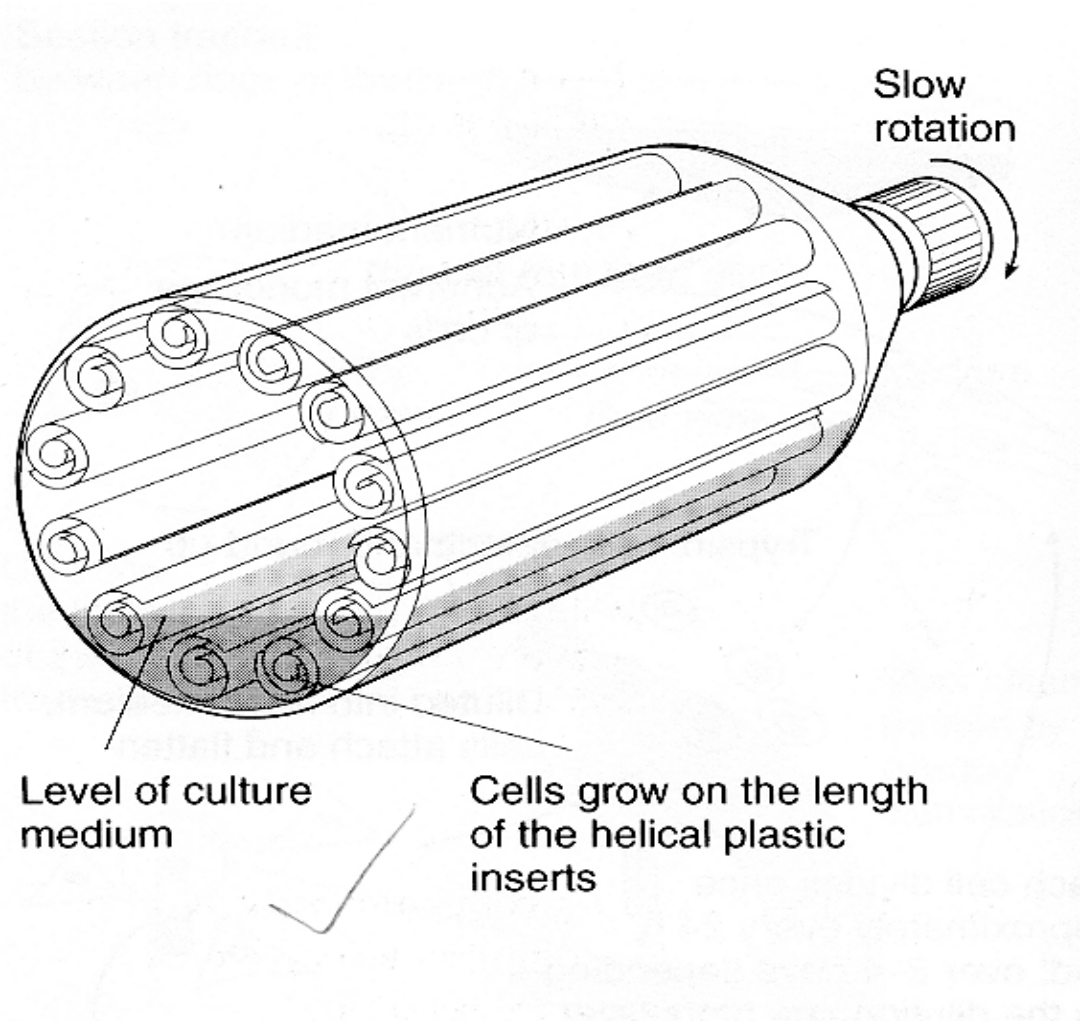

Figure-2.3: Culture bottle lined with spiral plastic coils.
(Source: Dimmock *et al* 2001)

The Plaque Assay:

The plaque assay is the most quantitative and the most useful biologic assay for viruses. Developed originally for the study of bacteriophage by d'Herelle in the early 1990s, the plaque assay was adapted to animal viruses by Dulbecco and Vogt in 1953. This assay was relatively simple and permits a qualitative assay for individual virus variants that differ in growth properties or cytopathology.

The plaque assay is based simply on the ability of a single infectious virus particle to give rise to a macroscopic area of cytopathic effect on a normal monolayer of cultured cells. Specifically, if a single cell in a monolayer is infected with a single virus particle; new viruses resulting from the initial infection can infect surrounding cells, which in turn produce viruses that infect additional surrounding cells. Over a period of days, the exact length of time depends on the particular virus; the initial infection thus gives rise through multiple rounds of infection to an area of infection called a plaque.

The plaque assay can be used to quantify virus in the following manner. A sample of virus of unknown concentration is serially diluted in an appropriate medium, and measured aliquots of each dilution are seeded on to mono layers of cultured cells. Infected cells are overlaid with a semisolid nutrient medium usually consisting of growth medium and agar. The semisolid medium prevents formation of secondary plaques through diffusion of virus from the original site of infection to new sites, ensuring that each plaque that develops in the assay originated from a single infectious particle in the starting inoculum. After an appropriate period of incubation to allow development of plaques, the mono layer is stained so that the plaques can be visualized. The staining technique depends on the cyto-pathology, but vital dyes such as neutral red are common. Neutral red is taken up by living cells but not by dead cells, so that plaques become visible as clear areas on a red mono layer of cells. In cases where the virus cytopathology results in cell lysis or detachment of cells from the dish, plaques exist literally as holder in the monolayer, and a permanent record of the assay can be made by staining the monolayer with a general stain such as crystal violet, prepared in a fixative such as ethanol. The aim of this assay is to identify a dilution of virus that yields 20 to 100 plaques on a single dish that is, a number large enough to be statistically significant. Usually a series of four to six 10-fold dilutions are tested. Dishes inoculated with low dilutions of virus will contain only dead cells or too many plaques to count. Whereas dishes inoculated with high dilutions of virus will contain very few plagues. Dishes containing an appropriate number of plaques are counted, and the concentration of infectious virus in the original sample can then be calculated by taking into account the serial dilution. The resulting value is called a titer, and it is expressed in plaque-forming units per milliliter or pfu/ml, to emphasize specifically that only viruses that are capable of forming plaques have been quantified. In this method an error of up to 100% is always possible because it mainly involves the multiple serial pipetting steps. However, a critical benefit of the plaque assay is that it measures infectivity, but it is important to understand that infectivity does not necessarily correspond exactly to the number of virus particles in a preparation.

2.3 CULTIVATION OF PLANT VIRUSES:

Although viruses cannot be grown in a synthetic medium, the cell, in which they live, can be propagated. This procedure is called tissue culture. Plant viruses can be cultivated either by tissue cultures or by protoplast culture method. In tissue culture method, the ex-plants pieces of tissues are used, while in protoplast culture the cells from the host plants are used. In tissue culture various plant parts- roots, endosperm, pollen, and pieces of stem are commonly used.

Plant Tissue Culture:

White (1934) was the first to examine the possibilities of growing plant viruses in tissue-culture. He investigated the multiplication of tobacco and cucumber mosaic viruses in growing excised tomato root tips. A tomato plant already systematically infected with the viruses was used and the stem was cut up into segments, these were thoroughly washed and were suspended by threads in 3-litre conical flasks containing a little water. The pieces of stem were kept out of contract with the water on the sides of the flask, the flasks were then plugged with cotton – wool and allowed to stand till roots developed. After 11 days, the root tips were removed and placed in 125 ml conical flasks, each flask contain 50 ml of nutrient medium as follows

$Ca(NO_3)_2$	**0.60 millimols**
$MgSO_4$	**0.30 millimols**
KNO_3	**0.80 millimols**
KCl	**0.87 millimols**
KH_2PO_4	**0.09 millimols**
$Fe_2(SO_4)_3$	**0.006 millimols**
Sucrose	**2 % by weight**
Yeast extract	**0.01 %**

At the end of a week, the surviving cultures were cut into pieces of about 10 mm long. After further subculture, a single root tip was selected as parent stock for all subsequent subcultures. It was found that the two viruses continued to multiply actively in growing isolated root tips for at least 25 to 30 weeks. Using the above technique, tobacco mosaic (TMV) and tobacco necrosis (TNV) viruses can be cultured in root tips of tobacco plants also. In addition to root tip cultures, plant viruses can also be cultured in callus tissues. In general, the tissues grew on a wide range of concentrations of the salts tested, but best growth was apparent when concentrations were increased over those of the basal medium (given above). Further it was found that increased phosphate concentrations increase the growth of tobacco-callus tissue. After callus development, infecting the callus with virus is also a difficult task in this method.

Since it is difficult to infect tissue cultures with viruses *de novo*, it is better to start the culture with tissues from systemically infected plants. However it is not always possible to get such type of plants, then it is better to employ alternative methods. One possible way is to use the natural vector of a virus to infect cultured tissue. In case of tobacco necrosis, the virus was

inoculated to tobacco callus tissues by zoospores of the fungus *Olpidium brassicae*. Two strains of tobacco necrosis virus and three isolates of *O. brassicae* were used. One day before inoculation, the callus tissues were transferred to small filter-paper cups pushed into vials containing 5 ml Hoaglands solution (1:20 dilution). The solution just touched the bottom of the paper cup, which was used to prevent the callus cells being lost in the liquid. The method of inoculation was to add to each vial 1ml of Hoagland's solution containing zoospores and 0.5 ml containing purified virus. Four or 5 days after inoculation, the virus in the tissues and in the fluid beneath them was assayed by infectivity tests on French beans, the test plant for TNV. All three isolates of *Olpidium* transmitted both strains of TNV to the tobacco callus tissue.

Similarly it is also possible to propagate the virus in tissue cultures of insect vectors. Tissue cultures derived from the vector insect *Agallia constricta* (Van Duzee) were infected with wound-tumor virus and the infection was detected by staining with fluorescent-conjugated antibody, and by infectivity tests. These experiments demonstrate multiplication of the wound- tumor virus in the inoculated tissues of the leaf-hopper. Many viruses - chilli mosaic virus, sun hemp mosaic virus and ring spot strain of potato virus X and a type strain of TMV have also been successfully cultivated in normal callus tissue obtained from virus-affected White Burley tobacco plants.

Protoplast Culture:

Use of protoplasts and isolated cells in the study of viruses has many advantages over the inoculated leaves. In the latter case only a few cells are initially infected and the virus replication must be studied against an overwhelming background of uninfected cells. Moreover the cells in the inoculated leaf are in varying stages of virus synthesis ranging from uninfected cells to cells in which virus synthesis is completed. Replication of virus within protoplast may be demonstrated in various ways, staining with fluorescent antibodies, infectivity assays, electron microscopy, incorporation of radioactive precursors into viruses and serology.

Certain conditions must be satisfied in order to achieve successful protoplast infection.

1) Protoplasts should be freshly washed with 0.7 M mannitol immediately before adding the inoculum.
2) Poly-l-Ornithine should be used to the inoculum
3) pH and osmolarity during inoculation should be within acceptable limits.
4) After infection takes place the protoplasts should be washed to remove excess virus and inoculation medium and then re-suspended in the incubation medium.

It is necessary to use very large numbers of virus particles to establish infection in inoculated protoplasts. Infection of protoplasts can be 10 times more efficient than is infection in a leaf probably because of the easier accessibility of the protoplast. A simplified method of obtaining tobacco protoplasts for infection with tobacco mosaic virus is - incubating the tobacco leaf tissue, from which the lower epidermis was peeled, overnight with 0.3-0.4 percent Macerozyme and 0.6-1.2 percent cellulase, depending on leaf condition, produced a good yield of protoplasts that were susceptible to infection by TMV. Highest concentration of virus can be attained, when the protoplasts were inoculated as soon as they were washed free from the enzymes. Protoplast culture of viruses is an important tool in plant virus study and offers much scope for progress. The time course of virus replication and its kinetics can be measured, and with protoplasts infected *in vitro* it is possible to get a picture of the generation time of plant viruses.

Abrasive Method:

This is one of the simplest methods for cultivation of plant viruses. The viruses that are sap transmitted are generally cultivated by this method. In this method carborundum was used an abrasive to make injury on the leaf, through which virus can make entry. First, a fine homogenate of the infected leaf was prepared by using K_2HPO_4/Na_2SO_3 solution in the ratio of 1.5 ml solution to 1 g leaf, in a pre-cooled mortar. In a healthy plant, the leaf to be inoculated is marked and dusted with carborundum. Then by using the folded square of muslin cloth, the filtered homogenate of the infected leaf was applied on the surface of the leaf which was dusted with carborundum. The square of muslin dipped in the sap was firmly stroked on the upper surface of the leaf until complete leaf was moistened. These treated plants were kept in a green house at 22- 25^oC. Then plants were observed for the appearance of symptoms from 4 to 5 days after inoculation. By this method viruses like *Cucumber mosaic virus* can be cultured in *Chaenopodium quinoa*, the local lesion host.

2.4 CULTIVATION OF BACTERIOPHAGES:

Bacterial viruses are easily isolated and cultivated in young, actively growing cultures of bacteria in broth or on agar plates. In liquid cultures, lysing of the bacteria may cause a cloudy culture to become clear, whereas in agar-plate cultures, clear zones, or plaques, become visible to the unaided eye. The principal requirement for the isolation and cultivation of phages that optimal conditions for growth of the host organisms be provided. The best and most usual source of bacteriophages is the host habitat. For example, coliphages or other phages pathogenic for other bacteria found in the intestinal tract can be best isolated from sewage or manure. This can be done by centrifugation or filtration of the source material and addition of chloroform to kill the bacterial cells. A small amount (0.1 ml) of this preparation

is mixed with the host organism and spread on an agar medium. Growth of phage is indicated by the appearance of plaques in the otherwise opaque growth of the host bacterium occurs (Fig. 2.4).

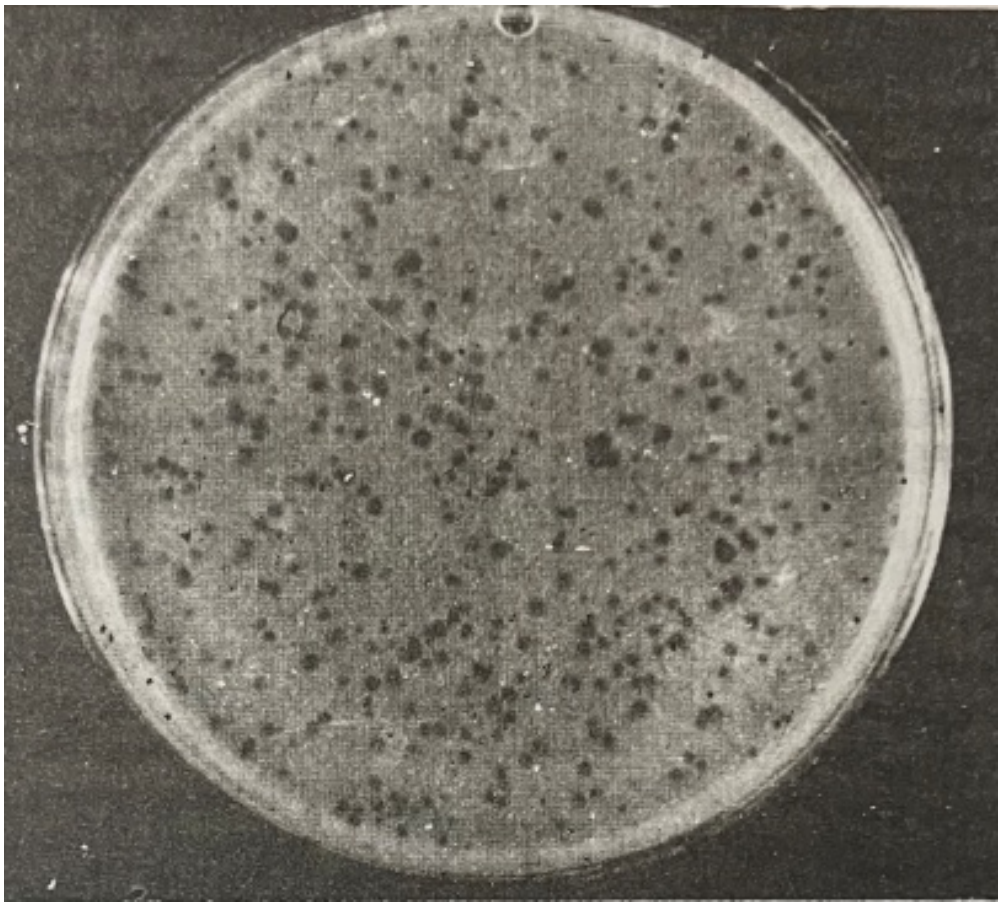

Figure-2.4: Plaque assay of bacteriophages

2.5 PURIFICATION OF VIRUSES:

Purification is essential for the study of structure, replication and other biological aspects of viruses. These procedures are mainly aimed at removal of all contaminants without the loss of viruses. Viruses are basically proteins which are often more stable than normal cell components. Because of these characteristics, many techniques useful for the isolation of proteins and organelles can be employed in virus isolation and purification. Many viruses are purified quite satisfactorily by differential centrifugation or by repeated precipitation. However, more selective separation techniques are necessary, where the contaminant material have similar properties to those of the virus. Preferred procedures for the isolation and subsequent purification of viruses, are – Differential centrifugation; Density gradient centrifugation (Rate zonal and isopycnic density gradient centrifugation), Precipitation with ammonium sulfate or polyethylene glycol and filtration.

Precipitation and Filtration:

Many viruses can be precipitated simply by lowering the pH of the extracts until the virus is precipitated at its isoelectric point. The pH at which viruses can be precipitated is usually in the range of 3.4 –5.5. This is the simplest method for precipitating the viruses, however some viruses are inactivated during this procedure. Another simplest method is precipitation by salts - ammonium sulfate. Only salt tolerant viruses can be precipitated by this method. Most of the viruses are precipitated at saturated ammonium sulfate concentrations between 20% and 40%, however some viruses require about 80% saturation as for broad bean mottle virus. In this method the crude virus sediment is re-dissolved in water or buffer and treated with gradually increasing amounts of water saturated with ammonium sulfate. When a precipitate appears, it is separated by low-speed centrifugation and more ammonium sulfate is added slowly to the supernatant until another precipitate forms, which is centrifuged off. Usually the salt, as saturated solution or as crystals, is added slowly to sap and left for several hours or overnight for better precipitation.

The same principle is used in stepwise precipitation with polyethylene glycol, which can be used, in contrast to ammonium sulfate, with salt-sensitive viruses. Precipitation with PEG is also commonly used as an early purification step to concentrate animal viruses or bacteriophage from large volumes of culture media into which the viruses were released from infected cells. Some plant viruses could be preferentially precipitated in a single-phase polyethylene glycol (PEG) system, although some host DNA may also be precipitated. Since that time, precipitation with PEG has become one of the commonest procedures used in virus isolation. In case of viruses which are stable in organic solvents, precipitation by adding solvents is preferable. Ethanol and methanol have been used to precipitate plant and animal viruses. However, these alcohols are known to denature some viruses especially when the precipitation was performed at unfavorable temperatures. The only advantage in this method is – it minimizes the time and cost, particularly when large samples are processed.

Filtration is another method for purification of viruses where it can be done through filter paper, or through filter paper supporting a pad of Celite is sometimes used to precipitate viruses. If Celite is used it will also act as an adsorbant and under certain conditions as very effective in removing green material, the common contaminant, in plant preparations. This method is a best substitute for slow speed centrifugation.

Differential Centrifugation:

Differential centrifugation consists of alternating cycles of high and low speed centrifugation at 20,000 to 50,000 rpm (e.g. a force of 1,00,000 g) for 1 to 3 hr sediments viruses together with other particulate material and leaves soluble components of the extract of less than 106 molecular weight in the supernatant. The sediment is then dispersed in water or in a suitable buffer; this must often be done by allowing the pellet to soften rather than by vigorous stirring. The extract is then again centrifuged at 8000 to 10,000 g to remove membranous and fibrous cell components, denatured proteins, and so on. Subsequent centrifugation of the supernatant solution at the higher speed again brings down viruses, and two or three repetitions of this procedure, discarding low-speed sediments and high-speed supernatants, yields many plant viruses including TMV in comparatively pure form.

Density Gradient Centrifugation:

One of the most useful procedures for purification, particularly for less stable viruses is density gradient centrifugation. Sucrose is the most commonly used material for making the gradient. It is a relatively mild procedure, although there may be a loss of infectivity with some viruses. It can give some indication of the purity of the preparation. It allows a correlation between particles and infectivity to be made and it frequently reveals the presence of non-infective virus like multi-particle viruses. Gradient centrifugation can be done in two different way, one by considering the size and density of the particles together (rate zonal centrifugation) and the second by considering only the density (isopycnic centrifugation).

Rate Zonal Density Gradient Centrifugation:

This method utilizes centrifugation through increasing concentrations of sucrose, glycerol, cesium chloride and so on. Sucrose gradients (e.g. 25 to 5 percent) are mostly preferred and easily prepared, and they afford good separations of viruses and other particles and molecules of various sizes and densities. The gradient was prepared by pouring the sucrose solution in a test tube such that its concentration smoothly and linearly increase between the top and the bottom of the tube, the highest density of which does not exceed the densest viral particle to be separated. The virus preparation is layered on top of the gradient and centrifuged. The particles are separated based on differences in their sedimentation rates i.e., based on both size and density. The virus was clearly visible in the test tube as a light scattered band, when a light beam is allowed to pass through the test tube. From the test tube the virus is then recovered either by piercing the test tube at the scattered light region or by introducing the

syringe from top of the test tube up to the zone. Pure virus preparation can be obtained by this method. Salt sensitive and low-density viruses are preferentially purified by this method.

Isopycnic Gradient Centrifugation:

A different use of density gradient centrifugation is isopycnic equilibrium centrifugation. This method relies strictly on the different buoyant densities of viruses, proteins, nucleic acids and so on. Each type of molecule will move in an increasing density gradient to the level at which its density equals that of the gradient and stop there. Most commonly, salts of heavy metals (Cesium or Rubidium) at high density (above 1-2 g/ml) are used since these form gradients automatically under the g forces of the ultracentrifuge. Thus, centrifugation of viruses in such a salt solution of appropriate concentration will result in location of the virus as a sharp band at the particular level of the tube where the solution density equals to the buoyant density of virus. This centrifugation requires a long time (36-48h) for equilibrium to be reached and can be used for salt-stable viruses only. Isopycnic density gradient centrifugation is particularly useful in the separation and characterization of nucleic acids, since these have higher density than viruses and other cell components and are not dissociated by salts. Very slight differences in the density of nucleic acids due to different ratios of G-C as compared with A-T base pairs, or the presence of heavy elements (5-bromo-uracil-instead of uracil) or isotopes can easily be detected by this method.

2.6 TRANSMISSION OF VIRUSES:

Viruses are obligate parasites that depend for survival on being able to spread from one susceptible individual to another fairly frequently. The mode of transmission of plant and animal viruses are different in the nature and in the lab condition.

Transmission of Plant Viruses:

Many plant viruses are transmitted from plant to plant by Invertebrate vectors, Nematodes, Fungi, Mechanical, Seed and Pollen and Grafting.

Transmissions by Invertebrates:

Many plant viruses are transmitted from plant to plant in nature by invertebrate vectors. These are Arthropods and Nematodes.

Arthropod Transmission:

These are chewing insects and feed on living green plants as larvae or adults or both and transmit the plant viruses. An important arthropod insects which are transmitting plant viruses namely – Aphids, leaf hoppers, plant hoppers, whiteflies, mealy bugs, beetles, mites, thrips. These vectors are transmitting the plant viruses in different modes – Non-persistent transmission, Semi-persistent transmission and persistent transmission.

Non-Persistent Transmission: In this mode of transmission, the insect vectors acquire the virus within seconds to minutes (Acquisition time) and retain the virus on the stylets only few minutes (Retention time). After acquisition and retention the vectors transmit the virus to healthy susceptible host.

Semi-Persistent Transmission: In this mode of transmission, the vector acquires the virus within minutes to hours and retains the virus in the fore-gut an hour. After retention time the vector successfully transmit the virus to healthy susceptible host.

Persistent Transmission: In this mode of transmission the vector acquire the virus within hours to days and retain the virus in the internal region of body in two ways. Circulative – day to weeks and Propagative – weeks to months. After retention in the body the virus is transmitted through the mouth parts and also transmit through the eggs (transovarial transmission).

Table-2.1: Important examples of Plant Viruses transmitted by insect vectors

Mode of Transmission	Vector	Virus Example
1. Non-persistent	Aphid Leaf hopper Mealy bugs Mites	Potato virus Y Maize chloritic dwarf virus Cocoa-swollen shoot virus Wheat streak mosaic virus
2. Semi-persistent	Aphid White flies	Cauliflower mosaic virus Lettuce chlorosis virus
3. Persistent – circulative	Aphid Leaf hopper White flies	Potato leaf roll virus Beat curly top virus Bean golden mosaic virus
4. Persistent- propagative	Aphid Leaf hopper Thrips	Lettuce necrotic yellows virus Maize rayado fino virus Tomato spotted wilt virus.

Nematode Transmission:

Several important viruses are widely transmitted by soil inhibiting nematodes. Eg. *Longidorus*, *Paralongidorus*, *Xiphenema*, *Paratrichodorus*, *Trichodorus*. Majorly two genera of plant viruses are transmitted by nematodes. Eg: Nepo and Tobra viruses. The nematode transmission of a virus occured by different processes – ingestion, acquisition, adsorption, retention, release, transfer and establishment. Eg. Rice ragged stunt virus, Tobacco rattle virus.

Fungal Transmission:

Several viruses have been transmitted by soil-inhabiting fungi Eg. *Olpidium*, *Polymyxa*, *Spongospora*. The virus survive in the resting spores and zoospores which infect the host. Various degrees of host specificity exist in both the Chytrid and Plasmodiophoral vectors. Zoospores of *Olpidium* species transmit the viruses like Tomato bushy stunt virus, Beet necrotic yellow vein virus.

Mechanical Transmission:

Transmission of plant viruses in the field by natural mechanical damage to the plant tissues is relatively low. It mainly occurs with very stable viruses that multiply to high concentrations in the host plant. Transmits from infected leaves to healthy plant when the leaves rub together by the wind and through root contact. Eg. Potato virus X. Contaminated soil with debris of TMV infected tomato plants, may cause infection in young tomato seedlings. A more common means of mechanical transmission in the field is through normal horticultural practices. TMV may be transmitted in tomato and tobacco crops by contaminated hands, clothing and tools. Many other vi- ruses may be transmitted by unsterlized tools during pruning procedures and when cuttings are taken. Eg. Carnation ringspot virus, Sugarcane mosaic virus.

Seed and Pollen Transmission:

Seed transmission provides a very effective means of introducing virus into a crop at an early stage, giving randomized foci of primary infection throughout the planting. Seed transmission is considerable as economic importance. Viruses may persist in seed for long periods so that commercial distribution of a seed-borne virus over long distances may occur. Two general types of seed transmission can be distinguished – a) Virus persistency on the seed coat b) Virus persistency in the embryo. In the first one, the seeds are externally contaminated with virus and mechanically transmitted to the seedlings. Eg. TMV, Cucumber mosaic virus. In the second and more common type of seed transmission the virus is found with the tissue of embryo. The developing embryo can become infected either before fertilization by infection of the gametes or by direct invasion after fertilization. Eg. Bean common mosaic virus, Pea seed-born mosaic virus.

Pollen Transmission:

Some viruses transmitted from plant to plant via pollen. In addition to embryos becoming infecting as a result of virus infection of the mother plant, female gametes may be also become infected through pollination of the healthy mother plant by infected pollen. The pollen-borne viruses enter the ovule along with the male gamete by passing through the pollen tube as it grows into the embryo sac and the mother plant is infected. Eg. Bean common mosaic virus, Prunus necrotic ring spot virus, Cherry leaf roll virus. A high level of infection in the pollen causes sterility of the pollen and leads to poor fertilization. Eg. Tomato aspermy virus.

Graft Transmission:

Grafting is an ancient horticultural practice in which a union is established between the cut tissues of two different plants. There are many types of grafting procedures established. One of the most common is the union between the shoot portion of one plant, referred to as the *scion* and the root-bearing portion of another called the *stock*. If either scion or stock is infected with virus, the virus passes into the healthy one and establish infection. This method is useful for plant viruses that could not be mechanically sap transmitted and for which no other natural method of transmission was known. Graft transmission of plant viruses is common in horticultural crops, such as Citrus, Apple, Plum etc., Eg. Citrus mosaic virus, Citrus tristeza virus, Apple mosaic virus.

Some of the plant viruses not only transmit by the above procedures but also other minor modes of transmission also- Vegetative propagation: Spread through vegetative propagules such as cuttings, tubers, runners and bulbs. Eg. Banana bunchy top virus, Potato spindle tuber viroid, Sugar cane mosaic virus, Onion yellow dwarf virus; Dodder transmission: *Cuscuta* species are known as Dodder, a vine-like parasitic plant belonging to the family Convolvulaceae are able to transmit plant viruses. The parasite forms consisting of root-like haustoria which penetrates into the infected host tissues to connect with the vascular system. The virus translocates from the infected plant through the haustoria and enters in to the vine and passes through the healthy plant. The virus translocates from the infected tissue along with the nutrients in to the healthy plant. The whole plant is being infected. Eg. Cucumber mosaic virus.

Transmission of Animal Viruses:

Viruses survive in nature only if they are able to be transmitted from one host to another, whether of the same or another species of animals or humans. Transmission cycles require

virus entry into the host body, replication, shedding and subsequent spread to another host. Virus transmission in animals is categorized in to two types – 1) Horizontal transmission and 2) Vertical transmission

Horizontal Transmission:

The transmission of virus between individuals within the popu- lation at risk, and can occur via direct contact, indirect contact, or a common vesicle or air-borne, vector- borne or iatrogenic.

Direct Contact Transmission:

It involved actual physical contact between an infected animal and a susceptible animal by licking, rubbing, biting, coitus. Examples are:

Licking-Measles, Mumps; Rubbing - Sheep pox virus; Biting-Rabies;

Coitus-Sexually transmitted disease- HIV.

Indirect Contact Transmission:

It occurs via fomites, such as shared eating containers, bedding, dander, restraint devices, vehicles, clothing, improperly sterilized surgical equipment, syringes and needles. Fomites-Adenovirus; Clothing-Pox viruses; Surgical equipment, syringes, needles-HIV, HBV.

Common Vehicle Transmission:

The transmission of viruses through fecal contamination of food and water supplies called Feco-oral transmission (Rota viruses) and virus contaminated meat or bone products (Vesicular exanthema, Hog cholera, Pseudorabies, Spongiform encephalopathy).

Airborne Transmission:

Infection of the respiratory tract occurs via droplets and droplet nuclei (aerosols) emitted from infected animals or humans during coughing or sneezing (Influenza virus, Rhino virus, FMDV) or from dander (Marek's disease).

Arthropod-Borne Transmission: It involves the bites of arthropod vectors.

Eg. Mosquitoes – Equine encephalitis virus, Ticks – Tick borne encephalitis virus, Sandfly – Yellow fever virus, Culicoid – Blue tongue virus.

Vertical Transmission: Transmission of virus from infected parent, usually mother to its offspring through embryo, fetus, and newborn. Eg. Mother to Baby – HIV, Colostrum and Milk – Encephalitis virus

Other Modes of Transmission:

Iatrogenic Transmission:

This transmission is caused by the hands of the doctor in course of carrying the patients or animals. This transmission has been important in the spread of virus infection through syringes and needles. Eg. Hepatitis B virus, Cytomegalo virus.

Nosocomial Transmission:

This transmission occurs through hospital or clinic. The respi- ratory virus infections are also often acquired nosocomially. Eg. Influenza virus, Adeno virus.

Zoonotic Transmission:

The term zoonoses used to described infections that are transmis- sible from animals to man and man is the dead-end host. In this transmission the domestic and wild animals are usually playing an important role in the transmission of viral disease by involving close contact with humans. Eg. Rabies, Japanese encephalitis B virus.

2.7 SUMMARY:

Cultivation of viruses can be done different methods depending on the nature of the virus. In the past whole organism was used as media for cultivation. Later, the organ and now it is the cell that is used a s media for cultivation. Obviously virus needs a living system for its multiplication. Animal viruses are mostly cultivated in mono cell culture consisting of either epithelial or fibroblast cells, though the embryonated chicken egg is the method of choice for many viruses. Plant viruses are usually cultivated by tissue culture methods and protoplast cultures. Protoplast cultures are advantageous over tissue cultures is that with protoplasts infected in vitro it is possible to get a picture of the generation time of plant viruses and course of virus replication and its kinetics can be measured. Purification of the viruses was achieved by different methods which include mainly protein purification. Depending on the tolerance towards the precipitating agent, viruses are precipitated using either by ammonium sulfate, polyethylene glycol or solvents. Sometimes they can be isolated by filtration through filter papers with Celite pads. Differential centrifugation is the first option for purification of majority of viruses. In Gradient centrifugation low density and high density viruses are conveniently purified by using sucrose and cesium chloride gradients, respectively.

The mode of transmission of viruses is an important epidemiological aspect. Many of the plant viruses are transmitted by insect vectors, and also by nematodes and fungi. Mechanical transmission, pollen and seed transmission and graft transmission and are other important modes of plant virus transmission. Animal viruses are transmitted by aerosols liberated from infected persons especially with respiratory tract infections, contaminated food and water (intestinal viruses such as poliovirus, Hepatitis A virus) insect vectors (eg. Encephalitis I) infected animals (eg. Rabies) etc.

2.8 TECHNICAL TERMS:

Organ culture, Cell lines, Plaque assay, Abrasion, Centrifugation, Isopycnic, Horizontal transmission, Vertical transmission, Arthropods, Graft transmission, Zoonotic, Airborne.

2.9 SELF ASSESSMENT QUESTIONS

1) Discuss the cultivation methods of plant viruses.

2) Give an account on cultivation of animal viruses.

3) Explain the methods for purification of viruses.

4) Discuss the methods of transmission of plant viruses

5) Discuss the methods of transmission of animal viruses

2.10 SUGGESTED READINGS:

1) Virology - Cornal, F.H and Kimball P.C. 1988 2nd Edition, Prentice Hall, New Jersey.

2) Introduction to Modern virology - Dimmock, N.J; Easton, A.J. and Leppord, K.N. 2001. 5th Edition, Blackwell Sciences.

3) Fundamental Virology - Knipe, D.M, Hoeley M.P. 2001. 4th Edition, Lippincott, Williams Publ.

4) Plant Virology - Mathews, R.C.F. 1981, Academy Press.

5) Plant Viruses - Smith, K.M. 1990. 6th Edition. UBS publ.

6) Applied Plant Virology - Walkey, D.G.A 1988. Heinemann; London.

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LESSON - 3

BRIEF ACCOUNT OF BACTERIOPHAGES AND PLANT VIRUSES

3.0 OBJECTIVE OF THE LESSON:

Students will aware and understand the important features and nature of bacteriophages as well as of plant viruses.

STRUCTURE OF THE LESSON:

3.1 INTRODUCTION:

Viruses (L. virus = poison) are acellular, ultra-microscopic, obligate intracellular parasites of living organisms. They are made up of proteins and nucleic acids and require living hosts for growth and multiplication. Latin word virus may have its roots form a proto-Indo-European word ‘Weis-to melt away or to flow’ used for foul or malodorous fluids. It is also cognate to Sanskrit word ’Vish-poison’. This word came to English many centuries before the discovery of viruses in the last quarter of nineteenth century. The exact nature of viruses, whether living or non-living, is still ambiguous. Outside the living hosts, they are inactive like chemical substances and cannot be considered living but inside the living hosts they become functional pathogens as bacteria, fungi, protozoa etc. and display the properties of extremely simple living organisms.

Lwoff (1957) defined the viruses as ‘Viruses are the infectious, potentially pathogenic nucleoproteins with only one type of nucleic acid which reproduce from their genetic material, are unable to grow and divide and devoid of enzymes’. Luria and Darnell (1968) defined them as ‘Viruses are entities, whole genomes of which are elements of nucleic acid

that replicate inside the living cells using the cellular synthetic machinery and causing the synthesis of specialized elements that can transfer the viral genome to other cells'.

3.2 BACTERIOPHAGES:

Bacteriophages are the viruses which infect and kill the bacteria. They were discovered in 1915 by Twort but described and named by d'Herelle in 1917. Bacteriophages are among the most common and diverse entities in the biosphere. They are ubiquitous viruses that found wherever bacteria exist. They infect specific bacteria by binding to the surface receptor molecules and then enter the cell. Inside the cell, the bacterial polymerase starts translating the viral m-RNA into proteins. These proteins become either new virion or helper proteins or the proteins involved in cell lysis. In T4 bacteriophages of *Escherichia coli*, a colon bacterium, over three hundred phages can be produced in just 20 minutes after injection. Most of the bacteriophages are tadpole in shape and so called as T-bacteriophages or simply T-phages. These T-phages are of two kinds namely – T-even phages (T2, T4, T6 phages) and T-odd phages (T1, T3, T5, T7 phages). The T-even bacteriophages are known for their autonomous virulence, causing cessation of bacterial metabolism upon infection. T-odd phages, in contrast, are dependent virulent phages, relying on continued bacterial metabolism for their lytic cycle.

3.2.1 T-Even Bacteriophages:

The T-even bacteriophages or virulent phages are complex viruses having polyhedral Head and helical Tail. Each phage looks like a minute tadpole larva and is about 200-280nm in length. So, each phage is differentiated into head and tail (Fig. 3.1).

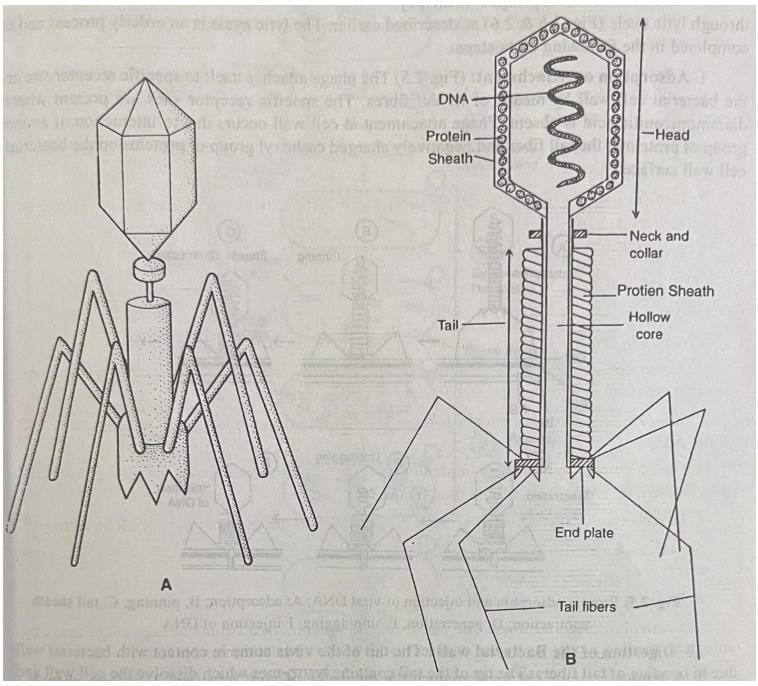


Figure-3.1: Bacteriophage T4, A-External morphology B - Diagramatic L.S.

Head: It is an elongated polyhedral hexagon and about 50 nm in diameter. The capsid consists of about 2000 capsomeres and encloses a central core of circular single thread like double stranded DNA. In place of cytosine, a modified base hydroxymethyl cytosine (HMC) is present.

Tail: It is an elongated helical structure attached to the polyhedral head through a disc-like structure known as 'Collar'. It has a central tube of about 8 nm diameter size and enclosed by a protein sheath. The sheath is composed of 144 subunits arranged in 24 rings with 6 subunits in each ring. The distal end of the sheath is attached to a hexagonal base plate with one tail pin or spike at each corner. Additionally, six long tail fibres are also present on the hexagonal plate with on fibre at each.

Replication or Multiplication:

T-even bacteriophages multiply through lytic cycle (Fig. 3.2), which is an orderly process involving eight steps viz., 1) Adsorption, 2) Digestion of bacterial cell wall, 3) Injection of DNA, 4) Synthesis of Early proteins, 5) Replication of viral nucleic acid, 6) Synthesis of Late proteins, 7) Assembly and maturation of virus particles, 8) Release of viruses.

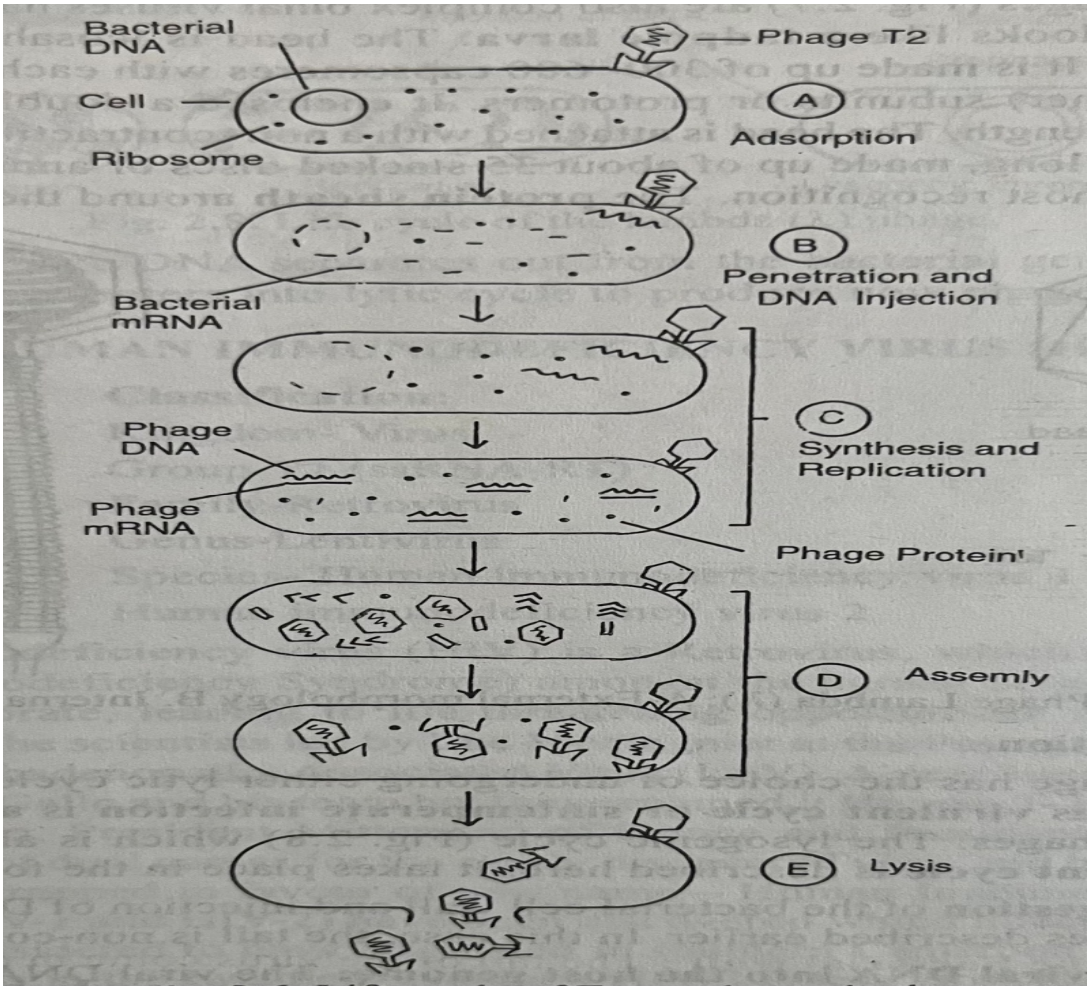


Figure-3.2: Lytic life cycle of T-even bacteriophage

(Source: Diversity of Microbes and Cryptograms – A.K. Thakur and S.L. Bassi).

1) **Adsorption or Attachment:** The phage attaches itself to specific receptor site on the bacterial cell wall by means of its tali fibres. The phage attachment to receptors at cell wall occurs due to interaction of amino group of protein in the tail fibre and negatively charged carboxyl group of proteins on the bacteria.

2) **Digestion of the Bacterial Wall:** The tail of the virus comes in contact with bacterial wall due to bending of tail fibres. The tip of the tail contains lysozyme which dissolves the cell wall and weakens it at the point of contact. The lysozyme forms a hole in the mucopeptide layer of the cell wall. The attachment of a number of phages may cause lysis of the bacterial cell without infection, as many holes are drilled.

3) **Injection of DNA:** The tail sheath contracts to half of its original size. The contraction of tail sheath forces the core tube to penetrate the cell wall of the host like a needle through the hole. The phage DNA passes into the host through tube. The protein coat of the head and the tail remains outside as an empty coat known as 'Ghost cell'.

4) **Synthesis of Early Proteins:** The phage DNA takes the control of cellular activity of the host immediately after the entry. The enzymes needed for phage DNA synthesis are made shortly after infection and are known as 'Early proteins'. The early proteins include DNase and RNase, which break the DNA and RNA of the host. Virus cannot be detected in the host cell and no activity takes place during this period, so this stage is called as 'Eclipse phase'.

5) **Replication of Viral Nucleic Acid:** The phage DNA replication starts after latent period (time lapse between introduction of nucleic acid and cell lysis) of 20 minutes. The nucleotides resulting from the breakdown of bacterial DNA are used up for the replication of the phage DNA. The phage DNA synthesis takes place at the expense of bacterial DNA and RNA. The replication of DNA takes place so rapidly that the bacterial cell soon contains 100-200 phage DNAs.

6) **Synthesis of Late Proteins:** The new proteins synthesized after phage DNA synthesis are known as late proteins. The late m-RNA encodes and produces late proteins. These late proteins include coat proteins, and the phage lysozymes or endolysins. The T4 phage consists about 70-100 genes, of which 50 genes are used in phage morphogenesis. The head, tail and tail fibres are formed independently and they combine later. The formation of the head involves the expression of 18 genes, tail formation requires the encoding of 21 genes and tail fibres requires 6 genes.

7) **Assembly and Maturation of Newly Formed Viruses:** The phage assembly is a sequential process. The late proteins and the viral genome assemble to form a new bacteriophage. The tail components are added later on and tail fibres are attached only

after joining of the head and tail. In this way, inside a single bacterial host cell, several hundreds of new viruses are produced. The process of assembly of complete phages from its individual components is known as maturation.

8) **Release of Viruses from the Host Cell:** The phage lysozymes or endolysins cause weakening of the bacterial cell wall. The complete and matured phages release by sudden bursting of the cell wall of bacterium.

3.2.2 Lambda (λ) Phage:

The lambda (λ) phages are also complex binal viruses having polyhedral head and helical tail. Each phage looks like a 'tadpole larva'. The head is icosahedral in morphology and about 55 nm in diameter. It is made up of about 300-600 capsomeres with each capsomere made up of by 5 (Pentamer) and 6 (Hexamer) subunits or protomers. It encloses a double stranded circular DNA molecule about 17 nm in length. The head is attached with a non-contractile tail through a connector. The tail is about 180 nm long and made up of about35 stacked discs or annuli and possesses a single tail fibre which helps in host recognition. The protein sheath around the tail is absent (Fig. 3.3).

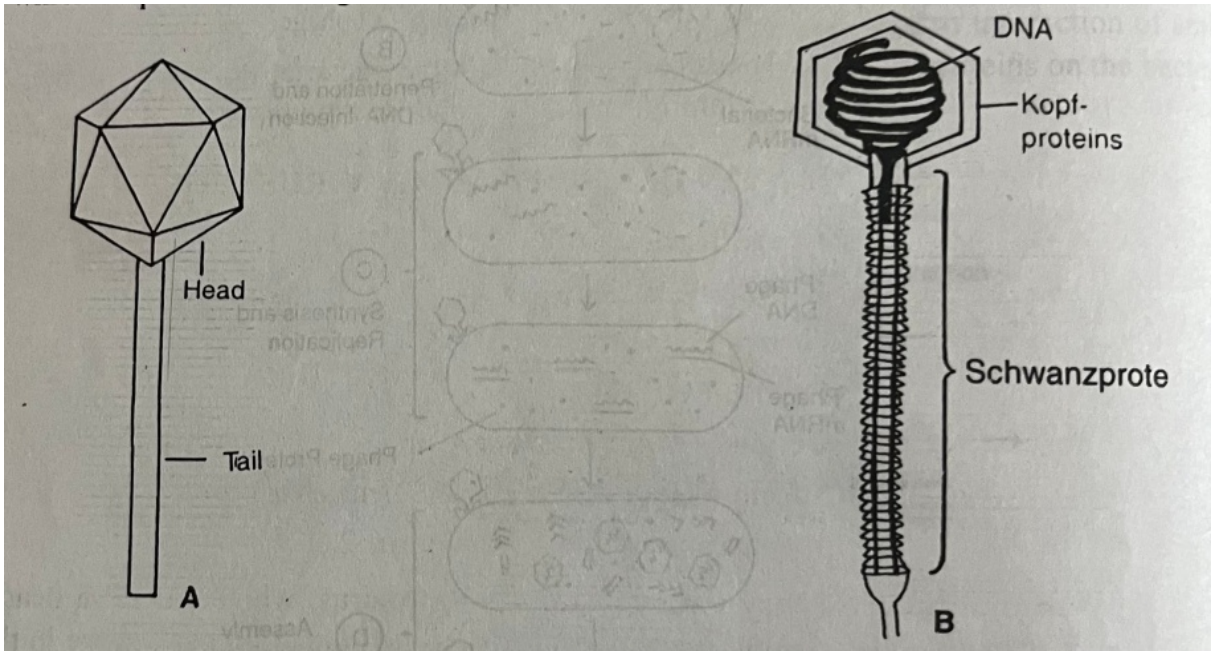


Figure-3.3: Lambda (λ) phage : A. External morphology B. Internal structure

Replication or Multiplication:

The lambda (λ) phage has the choice of undergoing either lytic cycle or lysogenic cycle. The lytic cycle is also known as 'virulent cycle or intemperate infection' is almost similar to the one performed by T even phages. Whereas, the lysogenic cycle is also called as 'temperate infection or non-virulent cycle' which occurs in different sequential steps (Fig. 3.4).

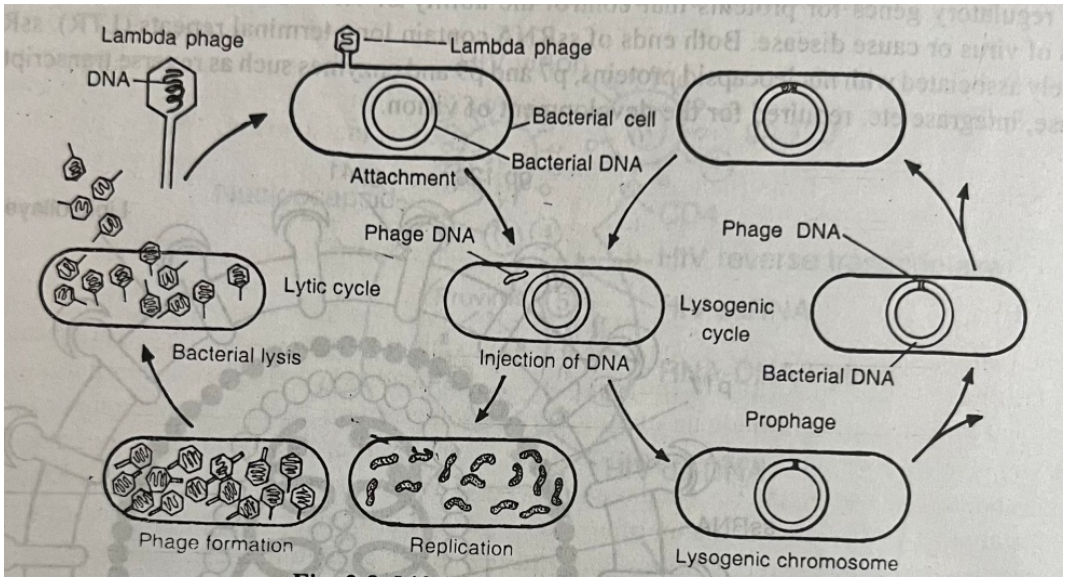


Figure-3.4: Life cycle of the lambda (λ) phage

1) Adsorption, digestion of the bacterial cell wall and injection of DNA into the host cell are almost similar to that of T phages, except the contraction of the tail as the tail is non-contractile one in this virus.

2) Integration of viral DNA into the host genome into the host genome with the aid of an enzyme integrase. The viral genome inserted in bacterial genome is referred to as 'prophage'. Being an integral part, prophage replicates along with the bacterial genome and is passed on to the daughter cells of bacteria and remains within the bacterial genome for several generations. The bacteria having the prophage are called as 'lysogenic bacteria' or lysogen and the viruses having the ability to integrate by themselves into bacterial genome are called lysogenic viruses.

3) Induction occurs when certain inherent factors induce the prophage to change into a virulent form.

4) Excision of prophage from the bacterial genome by the help of an enzyme, namely excisionase. Then, the excised viral genome enters into lytic cycle to produce new phages.

3.3 PLANT VIRUSES:

Plant viruses, though recognized long back that can infect plants, but have not been intensely studied due to some technical difficulties. Most of the plant viruses are RNA viruses. Insects are the most important transmission agents for plant viruses, and some plant viruses are even multiply in insect tissues before being inoculated into another plant. Most plant viruses have an RNA genome and may be either helical or icosahedral in structure. Depending on the virus, the RNA genome may be replicated by either a host RNA-dependent RNA polymerase

or a virus-specific RNA replicase. Plant viruses are transmitted in a variety of ways. Some enter through lesions in plant tissues, while others are transmitted by contaminated seeds, tubers, or pollen. Most of the plant viruses are probably carried and inoculated by plant feeding insects. The Tobacco Mosaic Virus (TMV) nucleocapsid forms spontaneously by self-assembly when discs of coat protein protomers complex with the RNA.

3.3.1 Tobacco Mosaic Virus (TMV):

Tobacco mosaic virus is one of the most extensively studied of all the viruses. It was the first virus to be described and contributed greatly in the development of the science of virology. It has become a model for teaching and understanding virus structure and functions. It is a type member of the group Tobamovirus. TMV is a serious pathogen, which causes mosaic disease on tobacco leaves and many other plants. It is reported to infect more than 199 individual species belonging to 30 families of angiosperms. The most important plants infected by TMV are *Nicotiana*, *Chenopodium*, *Phaseolus*, *Lycopersicon* etc., and a number of ornamental flowers. Adolf Mayer (1883) was the first person to observe the disease in tobacco, which was threating the tobacco crop at that time. He reported that the disease can be transferred between plants similar to bacterial infections. Martinus Beijerinck (1889 showed that a filtered bacteria free culture medium still contained the infectious agent. However, the first concrete evidence for the existence of a viral particle was given by Dmitri Ivanovski (1892). W.M. Stanley was the first person to crystallize TMV and reported that it remains even after crystallization. He was awarded noble prize in chemistry for his discovery in 1946. In 1955, Heinz Fraenkel Conrat and Robley Williams reported that the purified TMV RNA and its capsid proteins can assemble themselves into functional viruses spontaneously.

Structure of TMV:

Tobacco mosaic virus is a rod shaped and rigid RNA virus containing a single stranded RNA molecule of sense polarity. It is approximately 300 nm in length and 18 nm in diameter. The molecular weight of each TMV particle is around $39X10^6$ Daltons. It is composed of an outer protein coat known as capsid and inner, a single strand of helically coiled RNA molecule (Fig.3.5).

A) Capsid:

It is made up of identical protein subunits called capsomeres. It constitutes about 95% of the total weight of the viral particle. The capsomeres are helically arranged around a central cavity, 4 mm in diameter. Each capsid has about 2134 molecules of coat protein or capsomeres. There are 16.3 capsomeres per helix turn and have a pitch of approximately 2.3 nm. Each capsomere has 158 amino acids of 16 different types that are assembled into four

main alpha-helices, which are joined with each other. Each capsomere is about 17,500 Daltons in molecular weight.

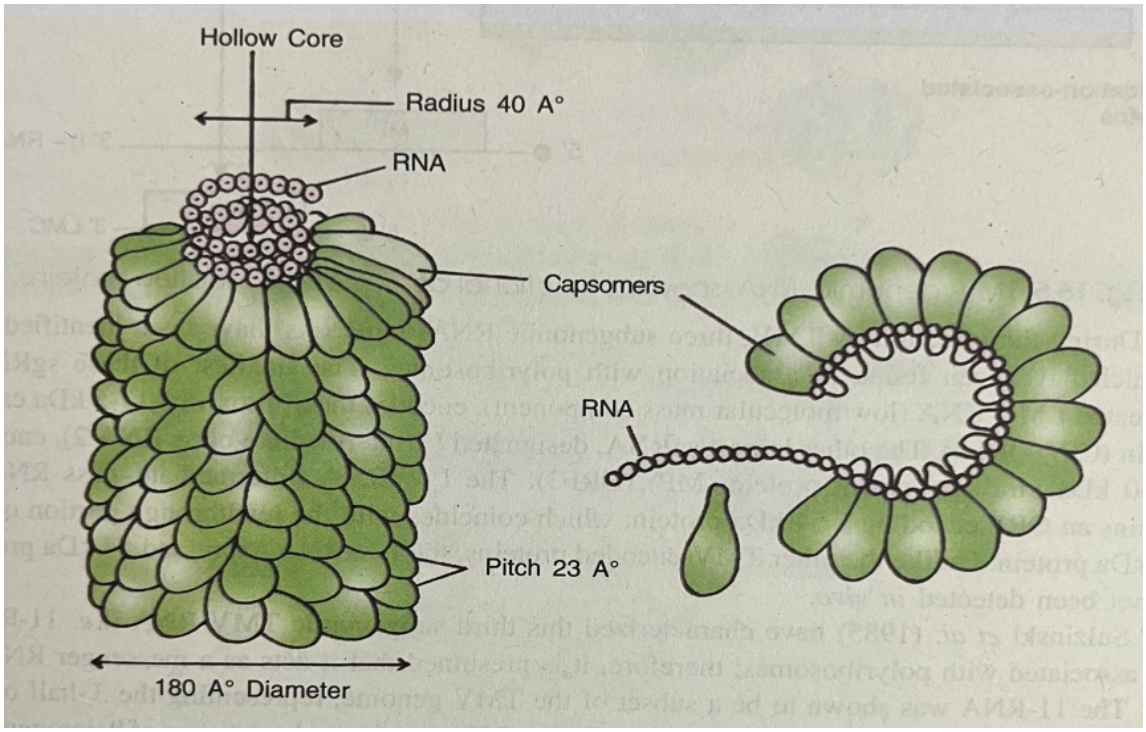


Figure-3.5: TMV Structure

B) Nucleic Acid:

The nuclear material is a single stranded linear molecule of RNA, which is helically coiled around a central cavity. It constitutes about 5% of the total weight of the virion. It consists of approximately 6500 nucleotides. The RNA is located at a radius of approximately 6 nm and is protected from the hydrolytic action of cellular enzymes by the coat proteins. There are 49 nucleotides per turn of RNA helix or 3 nucleotides per protein monomer.

Protein Synthesis:

Takeba (1975) has studied protein synthesis by viral RNA in the isolated protoplasts of mesophyll cells of tobacco. After the entry of viral particles inside the cells, the RNA starts un-coating by removing the capsomeres from the capsid one by one using host enzymes. The viral RNA acts as a template for the synthesis of complementary RNA as well as act as m-RNA for the synthesis of viral proteins. It utilizes host amino acids, nucleotides, t-RNAs and ribosomes for the synthesis of viral proteins and RNA.

Replication/Multiplication:

Virus needs to enter the vascular system for successful colonization of an entire plant. The systemic transport of TMV normally occurs through the phloem sieve elements where viruses move passively with the flow of photosynthates. After rapid systemic spread of the virus in

the phloem, it moves from the phloem to surrounding cells where it reproduces and spreads by cell-to-cell movement. The time between initial infection of one or a few cells and systemic infection of the plant varies from a few days to few weeks depending on the virus, host plant, and environmental conditions.

After penetration, TMV enters the host cells, and replicates in cytoplasm of the infected cells. Inside the cell, the protein coat dissociates and viral nucleic acid becomes free in the cell cytoplasm. After becoming free in the cell cytoplasm, the viral-RNA moves into the nucleus. The viral-RNA first induces the formation of specific enzymes called 'RNA polymerases', in the presence of which 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 strands of which are the copies of the parental viral-RNA. The new viral RNAs are released from the nucleus into the cytoplasm and serve as mRNAs. In cooperation with ribosomes and tRNA of the host cell, each mRNA directs the synthesis of protein subunits (Capsomeres). The new viral RNA organize the protein subunits around it resulting in the formation of complete virions. Unlike the virulent bacteriophages, no lysis of the host cell takes place. The host cells remain alive and viruses move from one cell to the other causing systemic infection (Fig. 3.6).

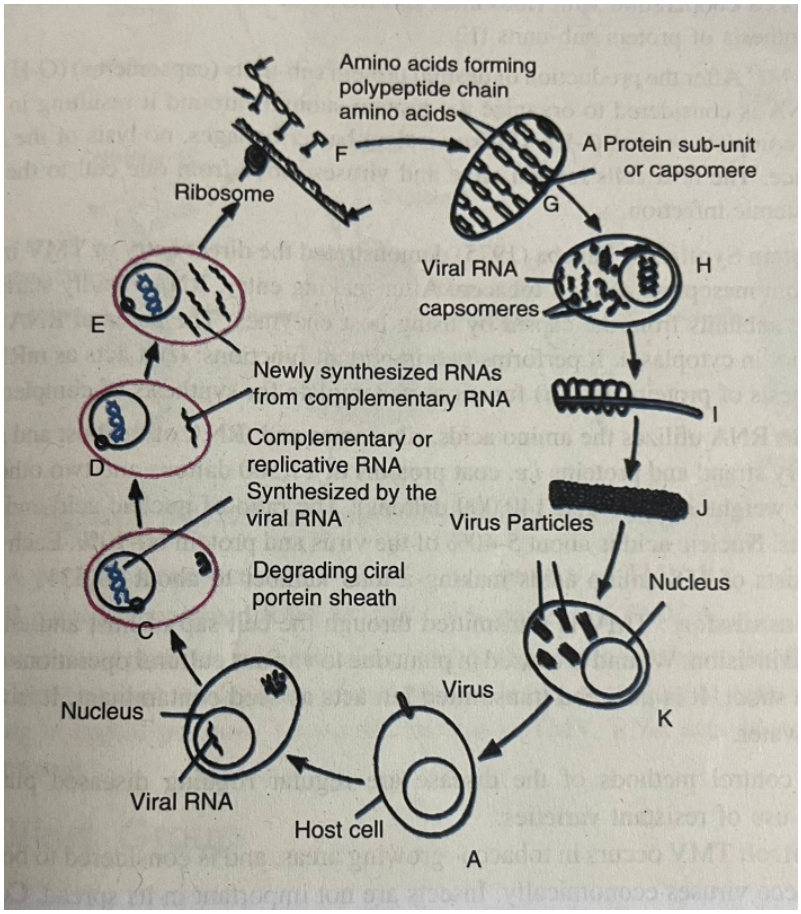


Figure-3.7: Life cycle of TMV

Transmission:

The TMV is transmitted by different modes viz.,

1) **Sap Transmission:** The viruses present in the sap of infected plants can be transmitted to healthy plants through their wounds.
2) **Mechanical transmission:** TMV can also be transmitted artificially through rubbing of infected leaves or their sap on the healthy leaves. It is also transmitted through agricultural tools from infected to healthy plants.
3) **Vector Transmission:** TMV has no true vectors but there are reports of incidental transmission by chewing insects, most probably by mechanical means.
4) **Dodder Transmission:** TMV has also been reported to be transmitted through dodder (*Cuscuta* sp.) in tobacco. However, it does not replicate inside the dodder.
5) **Seed Transmission:** Actually it is not transmitted through seeds or pollens but it may be present in the seed coat. It may infect the young seedlings through wounds developed during germination as in the case of tomato.

Disease Symptoms:

TMV mostly infects the members of the Solanaceae family and elicits a classical mosaic pattern. It may also cause vein banding or necrotic symptom known as 'mosaic burn'. The first symptom starts appearing in the form of light discoloration along the veins of younger leaves. Later, light green and dark green mosaic pattern develops on the infected leaves. The mosaic pattern is often accompanied by distortion and blistering due to uneven growth in the different areas of leaf. The plants which are infected in the early stages of growth, remain stunted while those infected in the later stages show little effects. In severely infected plants, the leaves become highly puckered and remain narrow. Sometimes, the whole plants become malformed beyond recognition. The necrotic lesions remain localized below 28^{o} C while these become systemic above 28^{o} C.

Disease Control:

Tobacco mosaic disease is worldwide in occurrence and is evidenced in every tobacco growing area of India and causes severe crop losses and economically losses to the farmer. The disease can be controlled by different means –

1) The diseased plants should be removed from the fields and be burnt (Roughing).
2) Field sanitation should be maintained.
3) Can be controlled by crop rotation.
4) Agricultural workers should clean their hands and tools properly after removing infected plants.

5) The disease can be effectively controlled by developing and growing disease resistant varieties.

6) The resistance to disease may also be developed in the plants by using coat protein or replicase as mediators.

3.4 SUMMARY:

Bacteriophages are the viruses that infect the bacteria and lyse them. The lytic cycle of virulent bacteriophages is a life cycle that ends with host cell lysis and virion release. The life cycle of T4 bacteriophage includes different steps namely adsorption, penetration, injection of viral genome into bacterial cell, synthesis of virus nucleic acid and capsid proteins, assembly of complete virions and release of phage particles from the host. Temperate phages like lambda phage, unlike virulent phages, often reproduce in synchrony with the host genome to yield a clone of virus-infected cells. The latent form of the phage genome within the lysogen (bacterium with viral genome inserted into its genome) is the prophage. Lysogeny is reversible, and the prophage can be induced to become active again and lyse its host.

Plant viruses are those viruses that infect various plant species and cause several diseases. Most of the plant viruses contain RNA as their genome and structurally either helical or icosahedral. The plant viruses are generally transmitted in a variety of ways, mainly of sap transmission, mechanical transmission and seed transmission. Tobacco mosaic virus is one of the best studied plant RNA viruses. The nucleocapsid of TMV form spontaneously by self-assembly of coat protein protomers and then complex with RNA genetic material.

3.5 TECHNICAL TERMS:

Bacteriophages, Phages, T-even phages, T-odd phages, Lytic cycle, Lysogenic cycle, Capsomeres, Pentamers, Helical symmetry, Binal structure.

3.6 SELF ASSESSMENT QUESTIONS:

1) Give an account on bacteriophages.

2) Compare the lytic and lysogenic life cyles of phages.

3) Write an account on Lambda phage.

4) Give a brief account on TMV.

3.7 SUGGESTED READINGS:

1) Microbiology – Prescott et. al.

2) Microbiology – Pelczar et. al.

3) Introduction to Modern Virology – Dimmock et. al.

4) A Textbook of Microbiology – Dubey and Maheshwari.

5) Diversity of Microbes and Cryptogams – Thakur and Susheel.

Dr. J. Madhavi

LESSON - 4

ULTRA STRUCTURE OF TYPICAL BACTERIAL CELL

4.0 OBJECTIVE OF THE LESSON:

To understand the different structural components of a typical bacterial cell and their functional aspects to survive in different environmental habitats.

STRUCTURE OF THE LESSON:

4.1 INTRODUCTION:

The ultrastructure of bacteria is a typical example for the prokaryotic cell or organism that lacks the membrane bound nucleus and other complex structures. Bacteria are small and simple in structure when compared with eukaryotes, yet they often have characteristic shapes and sizes. Bacteria are one of the most important microbial groups by any criterion like numbers of organisms, general ecological importance and practical importance for humans. Indeed much of the understanding of phenomena in biochemistry and molecular biology comes from research on bacteria.

4.2 MORPHOLOGY OF BACTERIAL CELL:

Typically, bacteria display three basic shapes viz., spherical, rod like and spiral but variations abound. A spherical bacterium is called a coccus. These cocci can exist as individual cells,

but also are associated in characteristic arrangements that are frequently useful in bacterial identification. Different shapes of bacteria are given in Fig. 4.1.

Diplococci : arise when cocci divide and remain together to form pairs. Eg: *Neisseria*

Streptococci: long chains of cocci result when cells adhere after repeated divisions in one plane. Eg: *Streptococcus, Enterococcus, Lactococcus*

Staphylococci: form when cocci divide in random planes to generate irregular grape-like clumps. Eg: *Staphylococcus*

Tetrads : cocci divide in two planes to form square groups of four cells. Eg: *Micrococcus*

Sarcinae : cocci divide in three planes producing cubical packets of eight cells. Eg: *Sarcina*

A rodlike bacterium is called a bacillus and the typical example of this shape is *Bacillus megaterium*. Bacilli differ considerably in their length-to-width ratio and the coccobacilli (Eg: *Brucella*) are the short rods intermediate in shape between cocci and bacilli. The shape of the rod's end often varies between species and may be flat, rounded, cigar-shaped or bifurcated. Although many rods do occur singly, they may remain together after division to form pairs or chains.

A few rod shaped bacteria are curved to form distinctive comma shaped bacteria called as vibrios. Many bacteria are shaped like long rods twisted into spirals or helices. If helices are rigid then called as spirilla and if helices are flexible called as spirochetes. Some bacteria may show some rare shapes like oval-to-pear shape, square shape and star shape. Bacteria that are variable in shape and lack a single characteristic form are called as pleomorphic (Eg: *Corynebacterium*) forms. The actinomycete group of bacteria are filamentous in nature.

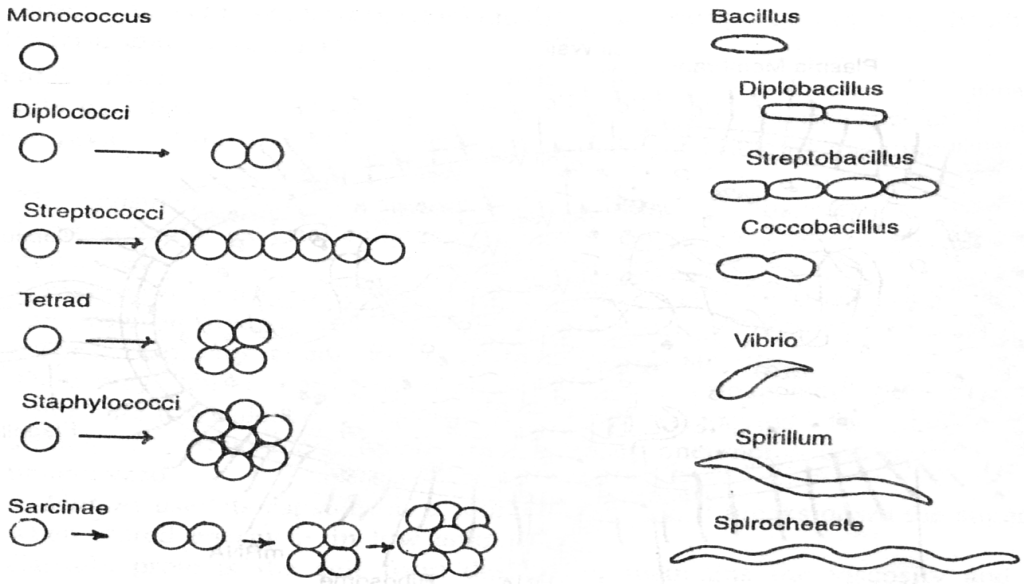


Figure-4.1: Different Shapes of Bacteria

Bacteria vary in size as much as in shape. The smallest (Eg: some members of *Mycoplasmas*) are about 0.3 μm in diameter, approximately the size of the largest viruses. However, the recently discovered nanobacteria or ultramicrobacteria appear to range from around 0.2 μm to less than 0.05 μm in diameter. The model bacillus bacterium, *Escherichia coli,* is of about average size of 1.1 – 1.5 μm wide by 2.0 – 6.0 μm long. A few bacteria are fairly large. For example, some spirochetes occasionally reach 500 μm in length, and the cyanobacterium *Oscillatoria* is about 7 μm in diameter. Very recently, a huge bacterium namely *Epulopiscium fishelsoni* has been discovered in the intestine of the brown surgeonfish, *Acanthurus nigrofuscus*. This bacterium gorws as large as 600 by 80 μm.

4.3 ULTRA STRUCTURE OF BACTERIAL CELL:

Structurally bacterial cells consist the following:

- Components external to the cytoplasmic membrane which include surface appendages (flagella, pili, fimbriae), glycocalyx layer and cell wall.
- Cell membrane or plasma membrane.
- Components internal to the cytoplasmic membrane.

The structure of typical bacterial cell is given in Fig. 4.2 below.

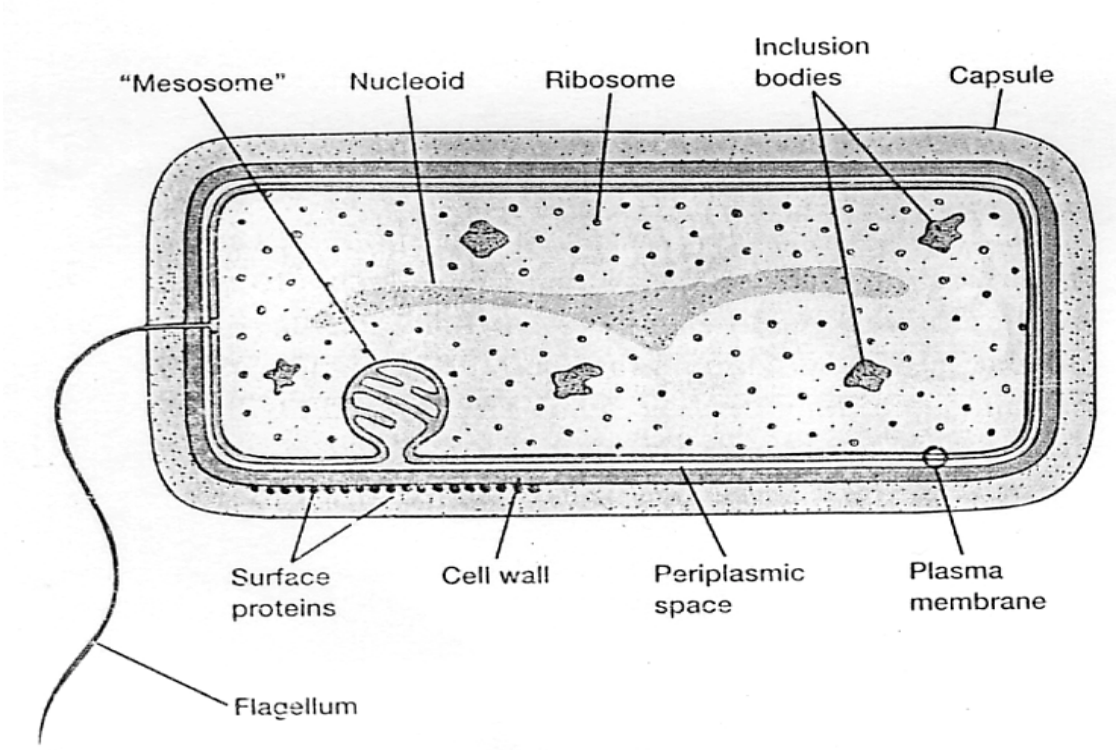


Figure-4.2: Structure of a typical bacterial cell

(Source: Microbiology – Prescott et al.)

4.3.1 Structures External to the Plasma Membrane:

Surface Appendages: The surface appendages that extend from the cell membrane through the cell wall and to the outer surface of the cell. These appendages are of two main types – appendages involve in locomotion (flagella) and appendages do not involve in locomotion (pili and fimbriae).

Flagella:

About half of all known bacteria are motile and move by the use of flagella. The flagella are the long, thin, thread-like, helical, slender and rigid locomotor appendages with about 20 nm diameter and up to 15 to 20 μm length. The diameter of the bacterial flagellum is about one-tenth that of a eukaryote's flagellum. Flagella are so thin they cannot be observed directly with a bright-field microscope, but must be stained with special techniques designed to increase their thickness. The detailed structure of a flagellum (Fig. 4.3) can only be seen in the electron microscope. Bacterial species often differ distinctively in their number and pattern of distribution of flagella.

Monotrichous Bacteria: bacteria with a single polar flagellum located at one end or pole Eg: *Pseudomonas*

Amphitrichous Bacteria: bacteria with two flagella, one at each end Eg: *Spirillum*

Lophotrichous Bacteria: bacteria with a cluster of tuft of flagella at one end or both ends Eg: *Spirillum*

Peritrichous bacteria: bacteria with many flagella spread fairly evenly all over the surface Eg: *Proteus, Salmonella*

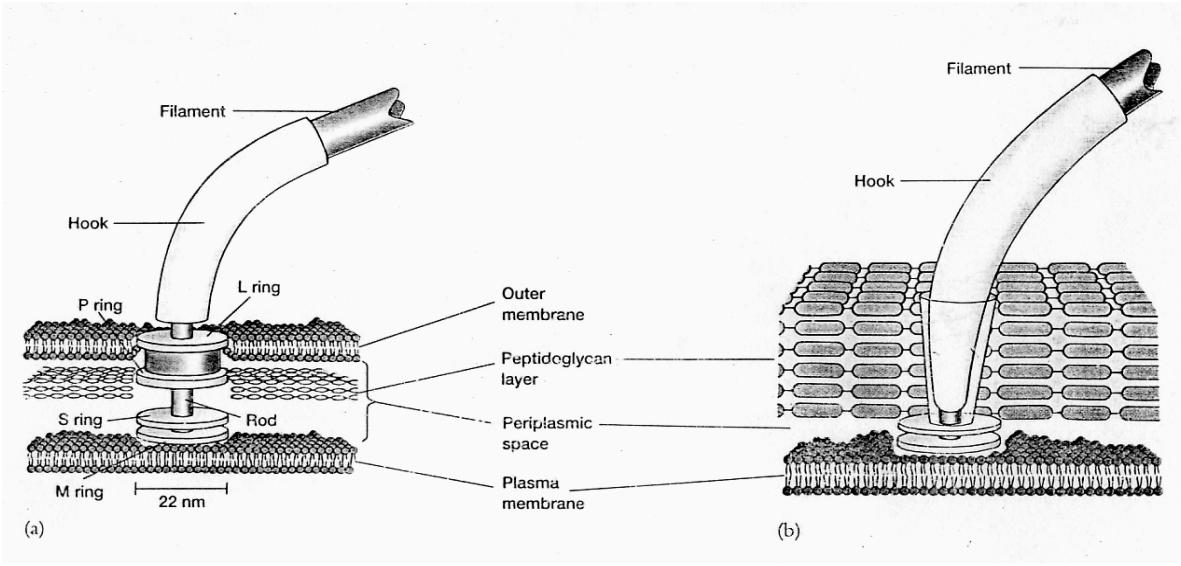


Figure-4.3: a) Gram-ve bacterial flagellum b) Gram +ve bacterial flagellum
(Source: Microbiology – Prescott et al.)

The bacterial flagellum is composed of three main parts namely (i) **Filament** – the longest and most obvious portion of the flagellum that extends from the cell surface to the tip, (ii) **Basal body** – portion of the flagellum that is embedded within the cell, and (iii) **Hook** – a short, curved segment that links the filament to its basal body and acts as a flexible coupling. The filament is a hollow, rigid cylinder constructed of a single protein called flagellin which ranges in molecular weight from 30,000 to 60,000 daltons. The hook and basal body are quite different from the filament. The hook is slightly wider than the filament and is made of different protein subunits. The basal body is the most complex part of a flagellum and consists of a central rod or shaft surrounded by a set of rings. Gram –ve bacteria have two pairs of rings named as outer pair and inner pair. The inner pair of rings (S and M rings) embedded in the cell membrane and outer pair of rings (L and P rings) associated with the peptidoglycan and lipopolysaccharide layers of the cell wall. In Gram +ve bacteria, the outer pair of rings is absent and only inner pair of rings (S and M rings) is found associated with cell membrane and cell wall.

The structure of the bacterial flagellum allows it to spin like a propeller, with the basal body acting like a motor to rotate the flagellum, and thereby to propel the bacterial cell. Rotation of the flagellum requires energy which is supplied by the proton gradient across the cytoplasmic membrane. Approximately 2506 protons must cross the cytoplasmic membrane to power a single rotation of the flagellum. The flagellum can rotate at speeds of up to 1,200 revolutions per minute, thus enabling bacterial cells to move at speeds of 100 μm /second.

The direction of flagellar rotation determines the nature of bacterial movement. Monotrichous, polar flagella rotate counterclockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. The rotating helical flagellar filament thrusts the cell forward with the flagellum trailing behind. Monotrichous bacteria stop and tumble randomly by reversing the direction of flagellar rotation. Peritrichously flagellated bacteria operate in a somewhat similar way. To move forward, the flagella rotate counterclockwise and during this they bend at their hooks to form a rotating bundle that propels them forward and the bacteria run or move in a straight line. When flagella rotate clockwise, the flagellar bundle disrupts and the cell tumbles or twiddle. Both the runs and twiddles are generally random movements. Runs last an average of 1 second during which the bacteria swim about 10-20 times of its body length. Twiddles last about 0.1 second and no forward progress is made. The flagellar motion of monotrichous and peritrichous bacteria are given in Fig. 4.4.

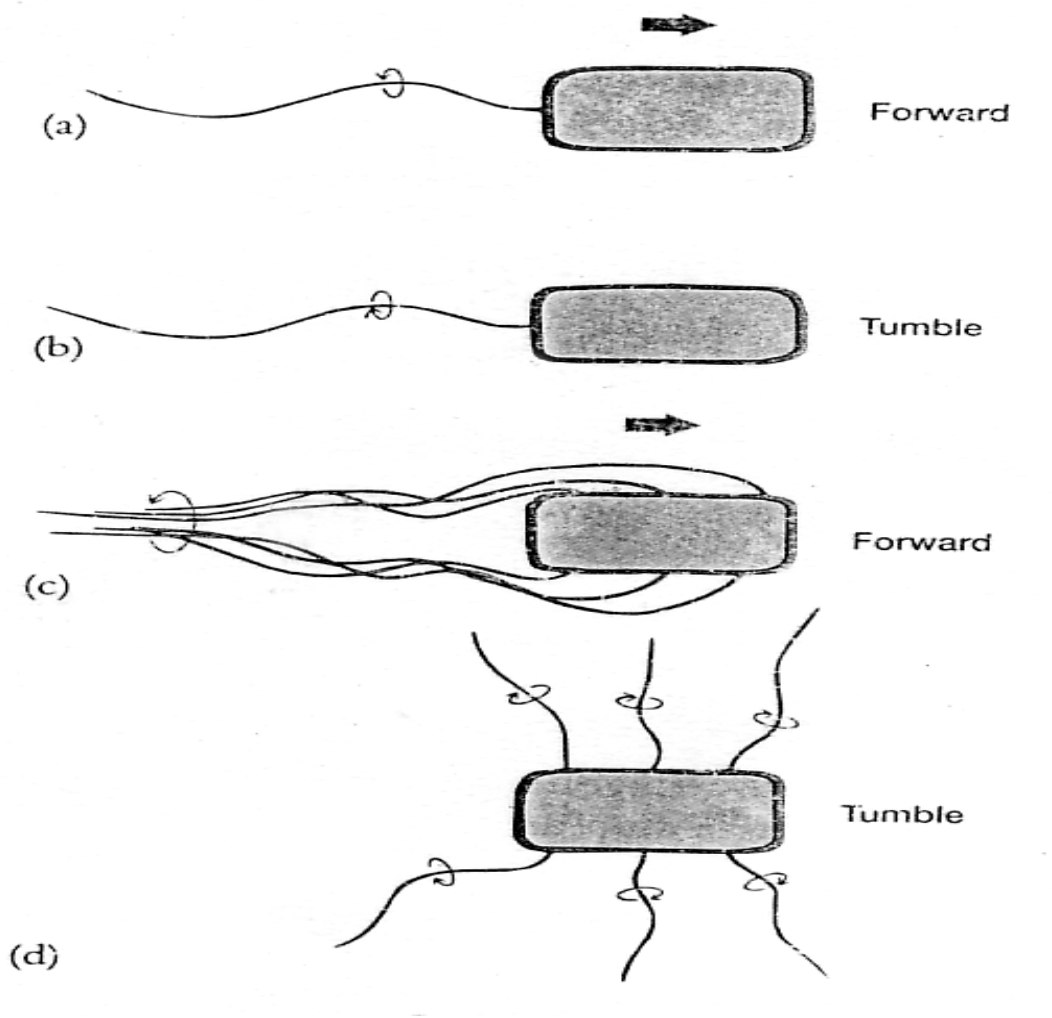


Figure- 4.4: Flagellar motility a & b – motion of monotrichous bacteria
c & d – motion of peritrichous bacteria
(Source: Microbiology – Prescott et al.)

Chemotaxis:

The movement of the bacteria toward a chemical attractant or away from a chemical repellent is known as chemotaxis. This behavior is of obvious advantage to bacteria. The movement towards the attractant is referred as positive chemotaxis and the movement away from the repellent is named as negative chemotaxis. This chemotaxis behavior of bacteria is mediated by some membrane bound chemosensor proteins called as Methyl-accepting chemotaxis proteins (MCPs). The MCPs are transmembrane proteins that interact with the chemorepellents and chemoattractants on the outside the cytoplasmic membrane or indirectly with receptors in the periplasm. The MCPs alternate with methylation and demethylation events and result in the tumbling and runs, respectively.

Pili and Fimbriae:

Many Gram –ve bacteria have short, fine, hair-like appendages that are thinner than flagella and not involved in motility. These are usually called fimbriae or attachment pili. These attachment pili or fimbirae help the bacteria adhere to surfaces such as cell surfaces and in the interface of water and air. They contribute to the pathogenicity of certain bacteria by enhancing the colonization on the surfaces of the cells of other organisms. These fimbriae are also responsible for the formation of pellicles or scums on the surface of the broth medium.

Sex pili or conjugation pili are the similar appendages found only in certain groups of bacteria. These pili are often larger than fimbriae with a diameter around 9 to 10 nm. These pili are made up of by specific protein subunits or monomers called as pilins. These sex pili involve in the transfer of genetic material from one bacterium to the other. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

Glycocalyx:

Many bacteria synthesize and secrete large amounts of viscous, organic polymer material that surrounds the bacterial cell. This slimy or gummy material is generally termed as glycocalyx. The glycocalyx may vary in its composition among different organisms but usually composed of glycoproteins and a large number of different polysaccharides. However, an exception is seen in *Bacillus anthracis* where the glycocalyx is made up of poly-D-glutamic acid which is a polypeptide. The glycocalyx may be thick or thin, rigid or flexible depending upon the chemical nature in a specific organism. If the glycocalyx forms a rigid, condensed, well defined and organized layer that tightly and closely surrounding the cell, the layer is termed as Capsule. If the glycocalyx is disorganized and loosely surround the cell, it is referred as slime layer. The capsule made up of by single kind of sugars is termed as homopolysaccharide capsule (*Streptococcus mutans*) and the capsule with more than one kind of sugars is known as heteropolysaccharide capsule (*Streptococcus pneumoniae*).

Functions of Glycocalyx layer includes:

- Helps in attachment of certain pathogenic bacteria to their hosts
- Gives protection to bacteria from phagocytosis
- Provides resistance to bacteria against desiccation
- During emergency serves as a nutrient source

Cell Wall:

The cell wall is an external structure or layer that surrounds the cell membrane in all bacteria except for the mycoplasmas and some archaeobacteria. Cell wall is firm, rigid in nature and gives protection to the cell from osmotic lysis, provide solid support for flagella and maintain the characteristic shape of the organism. Cell wall accounts to 20-40% of the dry weight of the bacterial cell. The cell walls of many pathogens have components that contribute to their pathogenicity. Basing on the response to the Gram stain developed by Christian Gram in 1884, bacteria could be divided into two major groups namely Gram +ve and Gram –ve bacteria. The nature of the cell wall also contributes to this differential response to Gram stain by bacteria.

Peptidoglycan:

The most important component of cell wall is the peptidoglycan of murein layer which is common to both Gram +ve and Gram –ve bacteria. In Gram +ve bacteria as many as 40 sheets of murein forms the peptidoglycan whereas in Gram –ve bacteria the number of murein sheets is usually two. Peptidoglycan is homogenous layer of 20-80 nm thickness in Gram +ve bacteria and accounts to 40-90% of cell wall dry weight but it is only 2-7 nm thickness in Gram –ve bacteria accounting to only 5-20% of total cell wall dry weight. Peptidoglycan is made of two parts, a peptide portion composed of amino acids connected by peptide linkages and a glycan or sugar portion. Structurally the peptidoglycan can be divided into three components viz., backbone, tetrapeptide side chain and peptide interbridge or cross link.

Backbone:

The glycan portion of the peptidoglycan polymer forms the backbone. This backbone is composed of alternately repeating units of the amino sugars N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) linked to each other by β 1-4 glycosidic bonds. Each strand contains 10-65 disaccharide units.

Tetrapeptide Side Chain:

This component of peptidoglycan contains four amino acids which includes L-alanine, D-glutamic acid or its derivative, L-lysine or Diaminopimelic acid (DAP) and D-alanine. Diaminopimelic acid is found in all Gram –ve bacteria and in few Gram +ve bacteria. Most Gram +ve cocci have lysine instead of DAP. The tetrapeptide side chain is connected to the carboxyl group of N-acetylmuramic acid but not to N-acetylglucosamine residue (Fig. 4.5).

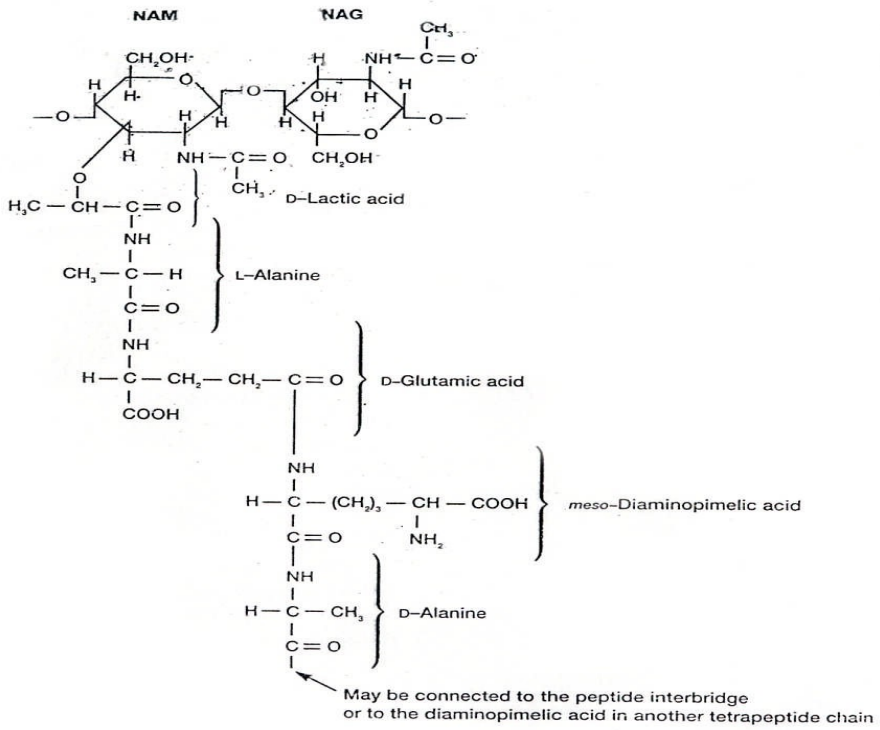


Figure-4.5: Tetrapeptide side chain
(Source: Microbiology – Prescott et al.)

Peptide Interbridge or Cross-Link:

Two tetrapeptide side chains of two adjacent murein stands or of the same strand are connected by cross-links or inter-bridges. In Gram –ve bacteria the cross-link is direct between amino group of DAP of one tetrapeptide side chain and carboxyl group of terminal D-alanine of other tetrapeptide side chain (Fig. 4.6). In Gram +ve bacteria the cross-link is by a peptide interbridge composed of amino acids. For example, the interbridge in *Staphylococcus aureus* is composed of five glycine amino acids (Fig. 4.7).

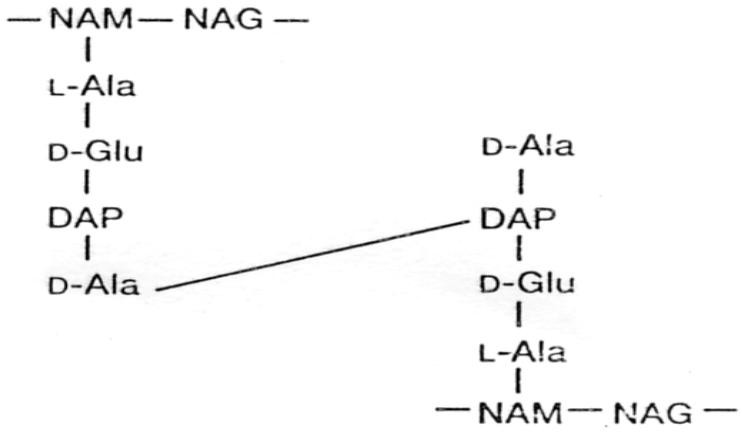


Figure-4.6: Cross linkage in Gram –negative bacteria
(Source: Microbiology – Prescott et al.)

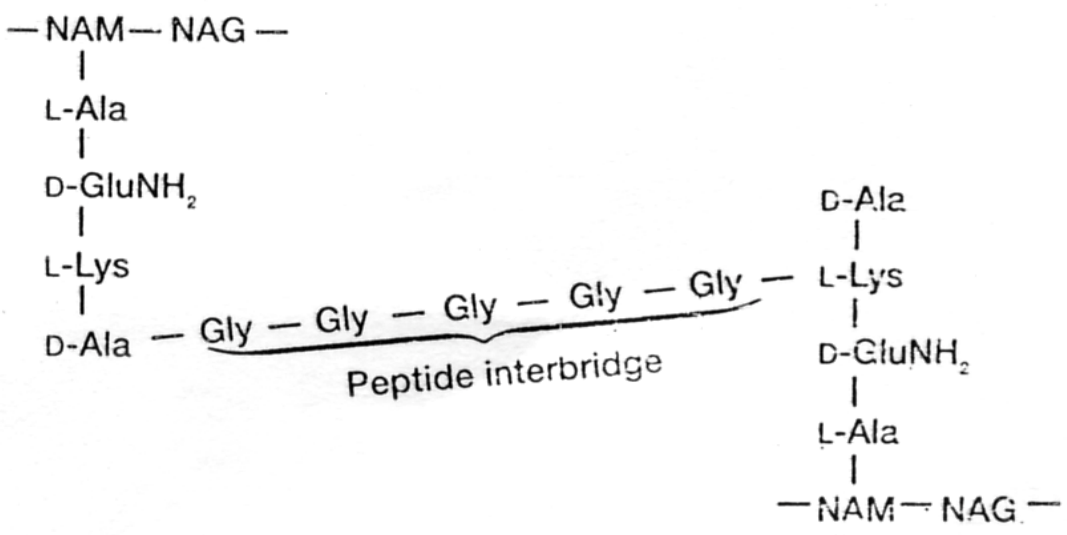


Figure-4.7: Cross linkage in Gram –positive bacteria
(Source: Microbiology – Prescott et al.)

The shape of cell depends on the lengths of the peptidoglycan chains and manner and extent of cross- linking the chains. True peptidoglycan, NAM and DAP are found exclusively in bacteria. The greatest variation in the chemical composition of the peptidoglycan occurs due to the variation in cross-linkages.

Gram-Positive Cell Wall:

The cell wall of the Gram +ve bacteria is thick, homogenous and composed primarily of peptidoglycan, which often contains a peptide interbridge. The cell walls of most Gram +ve bacteria also have teichoic acids, polymers of glycerol or ribitol joined by phosphate groups. Amino acids such as D-alanine or sugars like glucose are attached to the glycerol and ribitol groups. The teichoic acids are connected usually to the peptidoglycan itself by a covalent bond with the six hydroxyl of N-acetylmuramic acid. These teichoic acids are called as lipoteichoic acids when they are attached to lipids of plasma membrane. Teichoic acids are exclusive to Gram +ve bacteria and absent in Gram –ve bacteria. Functionally teichoic acids can bind to protons thereby maintain the cell wall at a relatively low pH which may prevent the autolysins from degrading the cell wall. Teichoic acids also bind cations such as Ca^{2+} and Mg^{2+} and act as receptor sites for some viruses. When phosphate concentrations are low, Gram +ve bacteria replace the phosphate-rich teichoic acids of the cell wall with teichuronic acids. This enables them to conserve phosphate that is essential for ATP, DNA, and other cellular components. Teichuronic acids are polysaccharide chains of uronic acids and N-acetylglucosamine, which fulfill the cell's requirement for an acidic, anionic polysaccharide in the cell wall.

Gram-Negative Cell Wall:

The Gram –ve cell wall (Fig. 4.8) is far more complex than the Gram +ve cell wall. Outside the thin peptidoglycan in Gram –ve bacteria an outer membrane is present. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan by its protein portion and associate with the hydrophobic portion of the outer membrane by its fatty acid end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein.

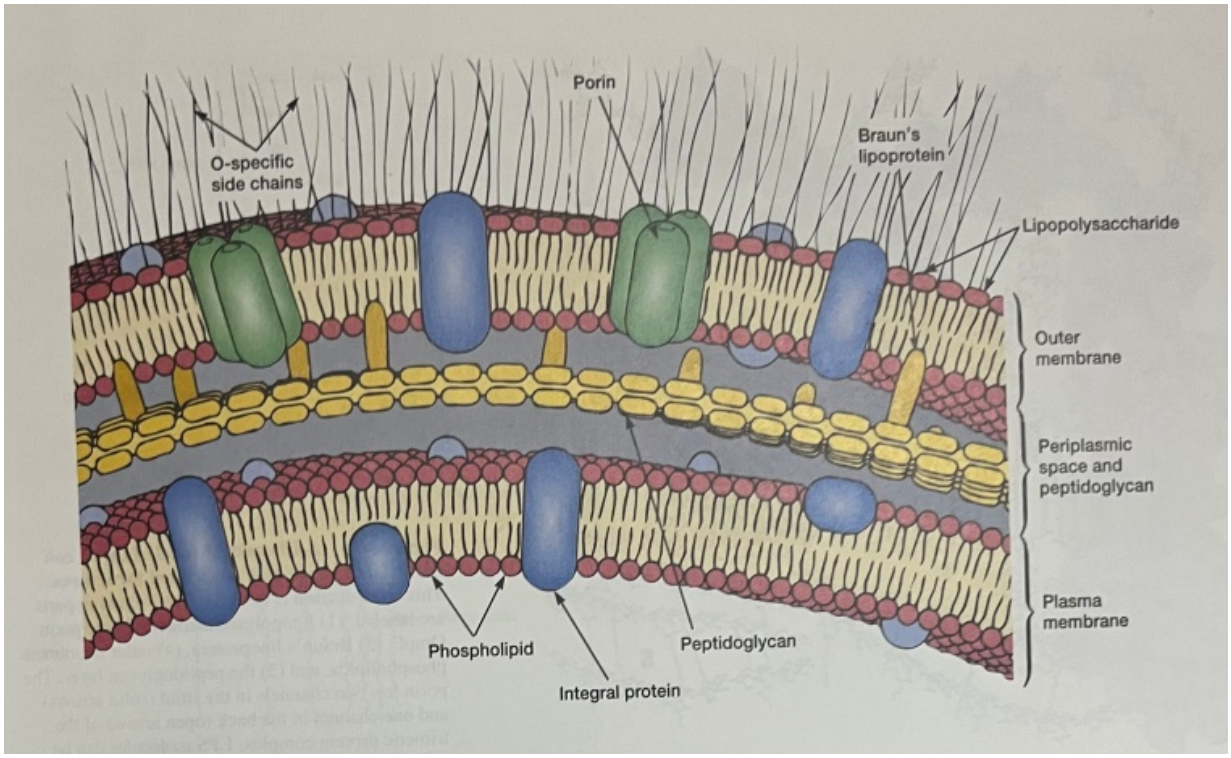


Figure-4.8: Gram-negative envelope
(Source: Microbiology – Prescott et al.)

Outer Membrane:

The outer membrane is the exclusive component of Gram –ve cell wall and it is a lipid bilayer containing phospholipids, proteins, lipoproteins and lipopolysaccharides. Unlike the cytoplasmic membrane, outer membrane is relatively permeable to most small molecules. The outer membrane contains lipopolysaccharides (LPS), which are not found in cytoplasmic membranes. LPS is often called as endotoxin as it may result in shock and death in some animals. LPS is a complex molecule composed of distinct regions (Fig. 4.9). The innermost portion of LPS is a lipid, called Lipid A, that anchors the LPS to the hydrophobic portion of the outer membrane. Lipid A consists of N-acetylglucosamine disaccharide linked via ester and amide bonds to unusual fatty acids such as β-hydroxymyristic acid, caproic acid and lauric acid. The toxic portion of LPS lies in the lipid A. The polysaccharide portion of the

LPS, which is external to lipid A, consists of a core polysaccharide and a repeat polysaccharide called the O-antigen or O-polysaccharide. The core polysaccharide is fairly consistent fro most Gram –ve bacteria and contains glucose, galactose, N- acetylglucosamine, and unusual sugars such as the 8-carbon sugar ketodeoxyoctulosonic acid (KDO) and heptoses (7-carbon sugars). The repeat polysaccharide consists of 3-5 sugars whose sequence is repeated up to about 25 times. The O-polysaccharide typically contains glucose, galactose, rhamnose, mannose, and several dideoxy sugars such as abequose, colitose, paratose and tyvelose.

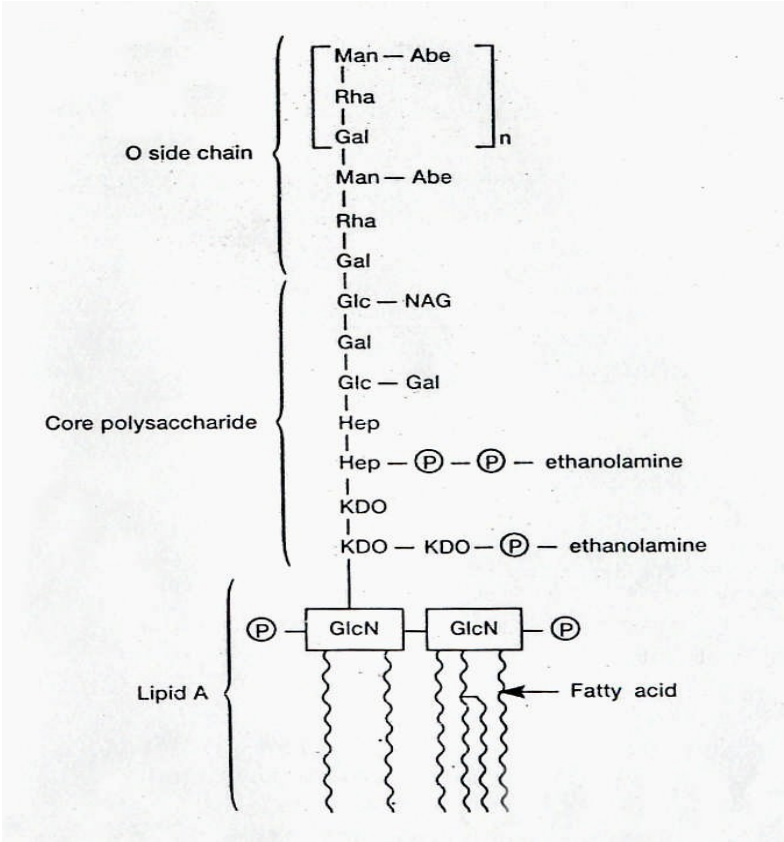


Figure-4.9: Structure of LPS
(Source: Microbiology – Prescott et al.)

The composition of the sugars and their arrangement varies from one Gram –ve bacterium to another or even from one subspecies to another. The important functions of the outer membrane are

- Acts as permeability barrier to toxic lysozyme, betalysin, leukocyte proteins
- Prevents leakage of periplasmic proteins
- Protect enteric bacteria from bile salts
- Shows –ve charge and so evade phagocytosis

The outer membrane contains some proteins which can be categorized into Porin proteins and Non-porin proteins. Porin proteins are the aggregates of three porin molecules that form cross-membrane channels through which some low molecular weight molecules can diffuse. These porin proteins may be specific, for example the maltoprotein that allows only maltose and maltodextrans and or non- specific. The non-porin proteins include the Omp A protein for anchoring and minor proteins that function in vit B_{12} transportation.

Periplasmic Space:

The region between the cytoplasmic and outer membranes is known as periplasmic space or periplasmic gel. The substance that occupies the periplasmic space is the periplasm. This is an important region in Gram –ve bacteria where diverse chemical reactions occur, including oxidation-reduction reaction, osmotic regulation, solute transport, protein secretion, and hydrolytic activities. Several proteins such as binding proteins, chemoreceptors, and enzymes (oxidases and dehydrogenases) are found in the periplasmic space. The binding proteins facilitate the transport of substances into the cell by delivering substances to carriers that are bound to the cytoplasmic membrane. The hydrolytic enzymes in the periplasm break down larger molecules to smaller products for easy transportation across the cytoplasmic membrane. The chemoreceptors by binding with the substances direct the cell's movement toward or away from those substances. Oligosaccharides present in the periplasmic region involves in osmoregulatory function.

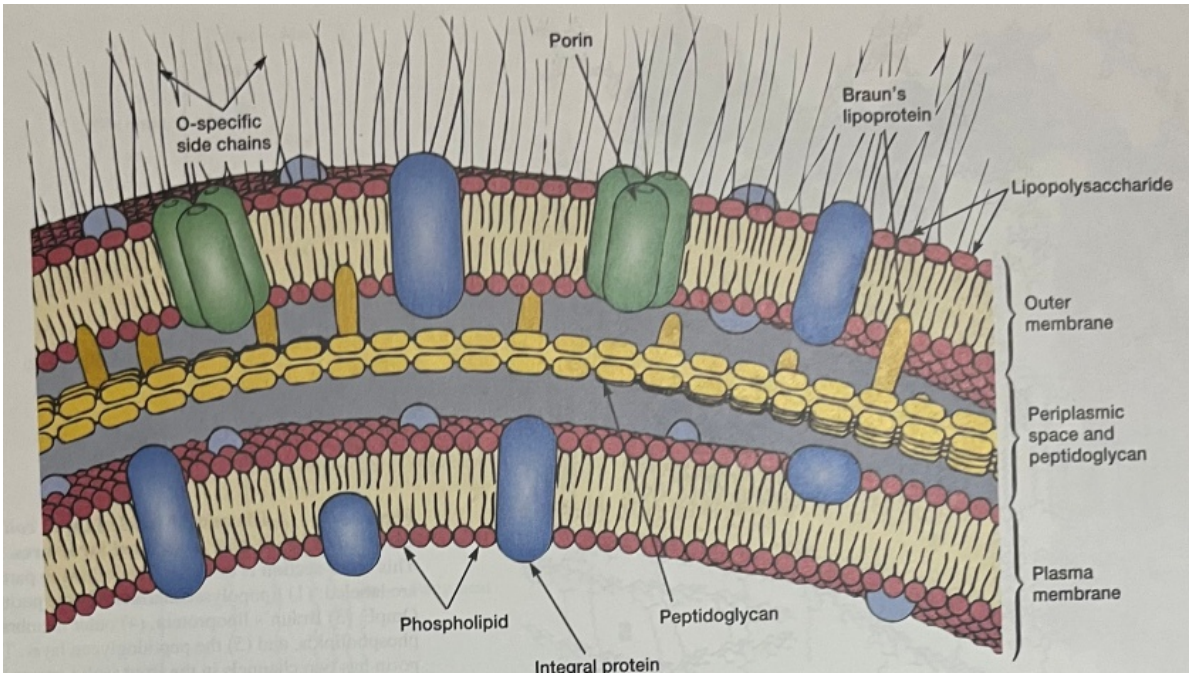
An Outline Comparison of Gram +ve and Gram –ve Bacterial Cell Walls:

Component	**Gram-positive cell wall**	**Gram-negative cell wall**
Peptidoglycan	Always present; occurs as a thick layer	Always present; occurs as a thin layer
Peptidoglycan Tetrapeptide	Most contain lysine in 3rd amino acid position	All contain diaminopimelic acid in 3rd amino acid position
Peptidoglycan cross linkage	Generally pentapeptide, for example, entirely glycine	Direct bonding of diamino-pimelic acid of one chain to the terminal D-alanine of another chain
Teichoic acid	Present	Absent
Teichuronic acid	Present	Absent

Lipoproteins	Absent	Present
Lipopolysaccharide (LPS)	Absent	Present
Outer membrane	Absent	Present
Periplasmic space	Absent	Present

4.3.2 Plasma Membrane:

The boundary layer that surrounds the cytoplasmic contents of the bacterial cell is known as plasma membrane or cell membrane. This plasma membrane constitutes 8- 15% of the cell dry weight. It is distinct from the cell wall by its shrinkage nature under high osmotic pressure. It is a critical barrier that separates the inside of the cell from its environment. Structurally it is a tri-laminar unit membrane with a thickness of 7-8 nm. In electron microscopy, membrane appears as outer and inner electron-dense layers with middle electron-transparent space. The plasma membrane structure is said to be fluid-mosaic model (Fig. 4.10) proposed by S.J. Singer and G. Nicholson.



Figure–4.10: Structure of Plasma membrane
(Source: Microbiology – Prescott et al.)

The cell membrane is largely lipoprotein in nature with about 20-30% phospholipids such as phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl choline and about 60-70% proteins. The phospholipids are structurally asymmetric and are amphipathic with polar and

non- polar ends. These phospholipids form a lipid bilayer. The lipids contain hydrophobic fatty acid groups directed inward and hydrophilic glycerol groups directed outwards and associate with water. The proteins embedded in fluid matrix of lipid are known as integral or intrinsic proteins which account to 70-80% of total proteins. These integral proteins are insoluble and cannot be removed easily. The proteins that are loosely attached to membrane are called as peripheral or extrinsic proteins which account to 20-30% and can be easily freed from membrane.

Some of the functional roles of these membrane proteins are i) Energy transformation ii) Transport of molecules iii) Protein export iv) Association of DNA with membrane v) Chemotaxis vi) Electron and proton transport vii) Penicillin-binding proteins and viii) Flagellar activity. In the absence of sterols, stability to the plasma membrane in bacteria is provided by the presence of some penta-cyclic sterol-like molecules called hopanoids. The plasma membrane invaginate inwardly here and there to form mesosomes.

The major functions of plasma membrane are:

- Selective permeability and transport of solutes
- Electron transport and oxidative phosphorylation in aerobic species
- Excretion of hydrolytic exoenzymes
- Involves in biosynthesis of DNA, cell wall polymers and membrane lipids as contain the required enzymes and carrier molecules
- Involves in sensory transduction systems

4.3.3 Structures Internal to the Plasma Membrane:

Mesosomes:

A simple membrane system present in the bacterial cell comprise these mesosomes. Plasma membrane of bacteria invaginate to form vesicle or tubular or lamellar structures called mesosomes. These invaginations are present in both Gram +ve bacteria and Gram –ve bacteria, but more prominent in Gram +ve bacteria. Mesosomes often found adjacent to septa or cross walls in dividing bacteria and sometimes attached to the bacterial chromosome. In some photosynthetic bacteria such as purple bacteria or nitrifying bacteria that exhibit high respiratory activity, the mesosome system is extensive and complex. The main function of this membrane system is to provide a larger membrane surface for greater metabolic activity.

Genetic Material:

Bacterial cells lack a membrane delimited nucleus. A single, closed, circular, double stranded DNA material is located in an irregularly shaped region called nucleiod or nuclear body or nuclear region. Nucleiod is composed of about 60% DNA, some RNA and a small amount of protein. The nucleiod can be stained with Feulgen stain. In addition to this main chromosomal DNA material, many bacteria possess extra-chromosomal material referred as plasmids. These plasmids are circular, double stranded DNA that can exist and replicate independently or integrated with chromosome. Plasmids are not necessary for growth and reproduction of the bacteria but they confer special characteristic features like drug resistant to the bacteria and thereby give new metabolic activities to their hosts.

Ribosomes:

Cytoplasmic matrix of the cell is often packed with ribosomes in bacteria. Ribosomes also found loosely attached to plasma membrane. The number of ribosomes per cell may be 10,000 or more and they are the sites of protein synthesis. Mg^{2+} ions and chemical energy are required for the function of ribosomes. The ribosomes of bacteria are commonly referred as 70S and are smaller than eukaryotic ribosomes. The ribosomes are complex made up of both protein and ribonucleic acid. The 70S ribosome of bacteria is composed of two components namely 30S and 50S subunits. The smaller 30S subunit is composed of 16S rRNA of 1540 ntd length and 21 proteins. Whereas, the larger 50S subunit is composed of 23S rRNA of 2900 ntd length, 5S rRNA of 120 ntd length and 34 proteins.

Gas vacuoles:

The prokaryotic organisms like cyanobacteria, purple and green photosynthetic bacteria and few aquatic forms (*Halobacterium, Thiothrix*) that exhibit a floating existence in lakes and sea produce these gas vacuoles for buoyancy. Due to the presence of these gas vesicles, organisms come to the surface waters against the gravitational pull referred to as buoyancy phenomenon. Gas vacuoles are the aggregates of gas vesicles. Each vesicle is spindle shaped, hollow but rigid with a constant diameter of 70 nm and varying lengths of 200-1000 nm. Gas vesicle is bounded by a protein layer of 2 nm thick and gives rigidity to the structure. Gas vesicles are impermeable to water and solutes, but permeable to gases. Gas vesicles lose their buoyancy by collapsing due to high hydrostatic pressure.

Chlorosomes:

These chlorosomes are the pigments that are housed in a series of cigar shaped vesicles. These vesicles are arranged in a cortical layer that immediately underlies the cell membrane. Chlorosomes are the part of the photosynthetic apparatus in green photosynthetic bacteria.

Chlorosomes are 50 nm wide and 100-150 nm long with an enclosed simple membrane of 3-5 nm thick.

Polyhedral Bodies:

Members of cyanobacteria, certain purple bacteria and chemoautotrophic bacteria possess some structures known as polyhedral bodies with granular content. These bodies are also called carboxysomes as they contain carboxydismutase, a key enzyme in CO_2 fixation process.

Magnetosomes:

R.P.Blackmore, in 1975, described a remarkable group of bacteria that possess magnetotactic nature. The organelles responsible for this property are termed as magnetosomes. When the organisms are placed in a magnetic field as weak as 0.2 guass, they orient and swim towards one or another of the magnetic poles due to the presence of these magnetic power sensing organelles. The magnetosomes are uniformly shaped, enveloped with magnetite (Fe_3O_4) crystals. These magnetosomes are best seen in *Aquaspirillum magnetotacticum.*

Inclusion Bodies:

In prokaryotic cells, a variety of cellular reserve materials or granules or inclusion bodies are seen. The nature of these inclusion bodies may differ in different organisms but almost always function in the storage of energy or serve as structural building blocks. These inclusion bodies are either organic or inorganic in nature and present in cytoplasmic matrix. PHB granules and glycogen granules are important organic forms and phosphate granules and sulphur granules are the important inorganic forms.

Poly β-hydroxybutyric Acid (PHB) Granules:

PHB are the most common inclusion bodies in prokaryotic cells. PHB is a lipid-like compound containing a number of monomeric β- hydroxybutyric acid units. These monomeric units are linked by ester bonds between the carboxyl and hydroxyl groups of adjacent units to form poly- β-hydroxybutyric acid molecule. These polymers aggregate together to form PHB granules. These granules can be stained with sudan black. PHB granules serve as a storage depot for carbon and energy.

Glycogen Granules:

These are starch-like polymer granules with glucose subunits. Long chains of this polymer is formed by the α-(1-4) glycosidic bonds between adjacent glucose units and the branched chains connect to long chains by α-(1-6) glycosidic bonds. These polymeric molecules aggregate together to form the glycogen granules. These granules are smaller than PHB granules. Glycogen granules are evenly dispersed throughout the cytoplasmic matrix and can be stained to reddish-brown colour with iodine. Glycogen granules serve as carbon storage reservoirs.

Polyphosphate Granules:

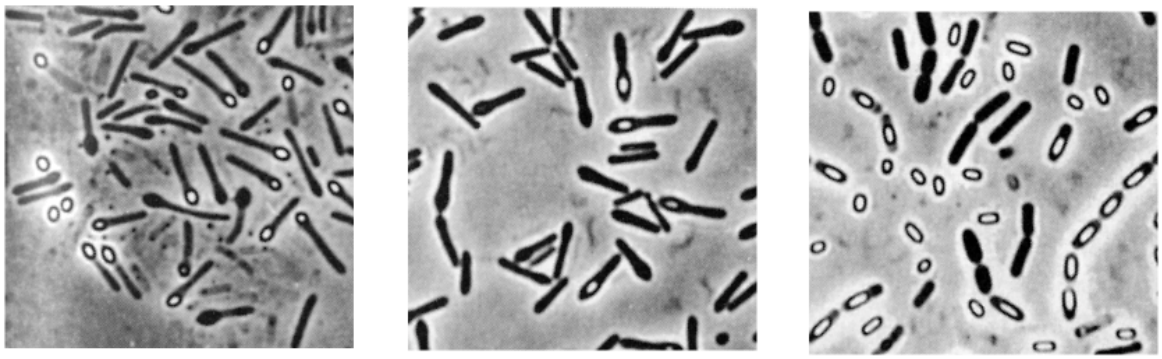
Many bacteria that grow in phosphate rich environments accumulate phosphate as polyphosphate granules. Polyphosphates are linear polymers of orthophosphates joined by ester bonds. Aggregation of these polymers form the polyphosphate granules which function as storage reservoirs for phosphate, an important component of cell constituents such as nucleic acids. Volutin granules is the other name for these polyphosphate granules. They can be stained with either toulidine blue or methylene blue. During the staining, as they exhibit metachromatic effect (color change effect) they are also be called as metachromatic granules.

Sulphur Granules:

A variety of bacteria like purple photosynthetic bacteria are capable of oxidizing the reduced sulphur compounds such as H_2S, thiosulphate and accumulate the resulting elemental sulphur in cells in the form of granules. During the reactions of energy metabolism or biosynthesis, elemental sulphur frequently accumulates inside the cell in large readily visible granules. These granules of elemental sulphur remain in cells as long as the source of reduced sulphur is available. When the reduced sulphur source becomes limiting, the sulphur in granules is oxidized to sulphate and the granules ultimately disappear.

4.4 SPORULATION IN BACTERIA:

During the unfavourable environmental conditions, some Gram-positive bacteria such as *Bacillus, Clostridium* and few others produce a special resistant, dormant structure called an endospore. These endospores are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation. They can remain viable for a number of years. Formation of spore from the vegetative cell is known as sporogenesis or sporulation. Spore position in the mother cell frequently differs among species. Spores may be centrally located, close to one end (sub- terminal) or definitely terminal (Fig. 4.11).



Figure–4.11: Terminal, Sub-terminal and centrally located spores

(Source: Microbiology – Prescott et al.)

The spore (Fig. 4.12) is often surrounded by a thin, delicate covering called the exosporium. A spore coat lies beneath the exosporium, is composed of several protein layers and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The cortex, which may occupy as much as half of spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The spore cell wall or core wall is inside the cortex and surrounds the protoplast or core. The core has the normal cell structures such as ribosomes and a nucleoid. The dipicolinic acid forming complex with calcium ions in the core is believed to responsible for the heat resistance of endospores. When the environmental conditions become more favorable, endospores germinate into vegetative cells.

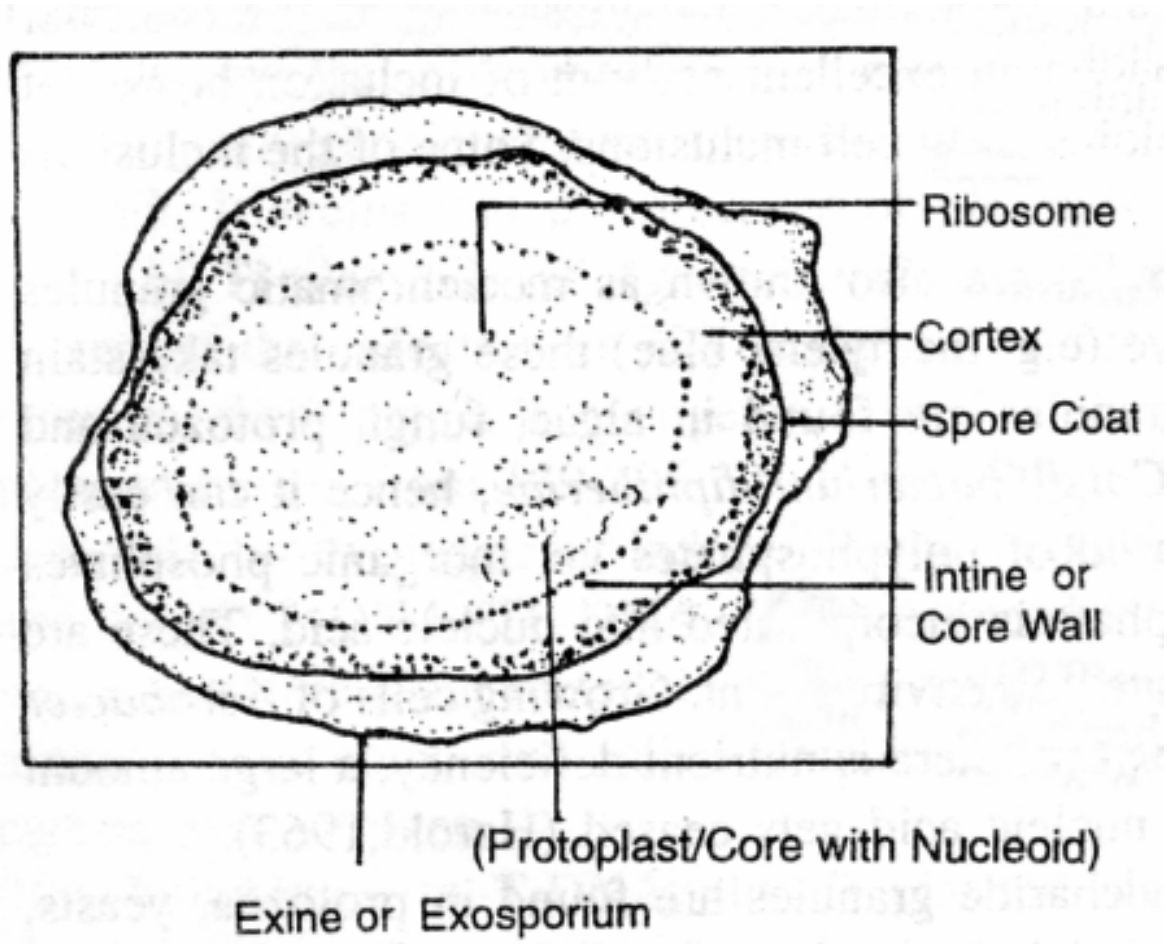


Figure-4.12: Structure of bacterial endospore
(Source: Microbiology – Prescott et al.)

4.5 SUMMARY:

Bacteria may be spherical, rod-shaped, spiral or filamentous in shape. Some form buds and stalks, and some are pleomorphic without any characteristic shape. Bacterial cells can remain together after division to from pairs, chains, and clusters of various sizes and

shapes. All bacteria are prokaryotes and structurally much simpler than eukaryotes. The plasma membrane and most other membranes are composed of a lipid bilayer in which integral proteins are buried. Peripheral proteins are more loosely attached to membranes. The plasma membrane may invaginate to form some simple structures such as membrane systems containing photosynthetic and respiratory assemblies and possibly mesosomes. The cytoplasmic matrix contains inclusion bodies and ribosomes. The genetic material is located in an area called the nucleoid and it is not enclosed by a membrane.

Most bacteria have a cell wall outside the plasma membrane to give them shape and protect from osmotic lysis. Bacterial walls are chemically complex and usually contain peptidoglycan or murein. Bacteria often are classified as either Gram-positive or Gram-negative based on differences in cell wall structure and their response to Gram staining. Gram +ve walls have thick, homogenous layers of peptidoglycan and teichoic acids. Gram –ve bacteria have a thin peptidoglycan layer surrounded by a complex outer membrane containing lipopolysaccharides and other components. Structures such as capsules, fimbriae, and sex pili are found outside the cell wall. Many bacteria are motile, usually by means of threadlike locomotory organelles called flagella. Bacterial species differ in the number and distribution of their flagella. The flagellar filament is a rigid helix and rotates like a propeller to push the bacterium through the water. Motile bacteria can respond to gradients of attractants and repellents by a phenomenon known as chemotaxis. Some bacteria survive adverse environmental conditions by forming endospores, dormant structures resistant to heat, desiccation and many chemicals.

4.6 TECHNICAL TERMS:

Bacterial cell, Gram +ve, Gram –ve, Flagella, Cell wall, Peptidoglycan, Cell membrane, Genetic material, Ribosomes, Chlorosomes, Gas vacuoles, Magnetosomes, Inclusion granules.

4.7 SELF ASSESSMENT QUESTIONS:

1) Give an account on ultra structure of the bacterial cell.
2) Describe the physical and chemical structure of cell wall in Gram-positive and Gram-negative bacteria.
3) Compare the characters of prokaryotic cell and eukaryotic cell.

4.8 SUGGESTED READINGS:

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2) Brock Biology of Microorganisms - Michael T. Madigan, John M. Martinko and Jack Parker (8^{th} edition) 1997 – Prentice Hall International, Inc.

3) Principles of Microbiology - Ronald M. Atlas (2^{nd} edition) 1997 – WCB McGraw-Hill Publishers

4) The Physiology and Biochemistry of Prokaryotes - David White 1995 – Oxford Univ. Press

5) Microbiology-Principles and Explorations - Jacquelyn G. Black (4^{th} edition) 1999 – Prentice Hall International, Inc.

6) Microbiology - M.J. Pelczar Jr., E.C.S. Chan and Noel R. Krieg (5^{th} edition) 1995 – Tata McGraw Hill Publishing Company Ltd.

Prof. V. Umamaheswara Rao

LESSON - 5

BACTERIAL NUTRITION AND GROWTH

5.0 OBJECTIVE OF THE LESSON:

To understand the nutritional types of bacteria based on their nutritional requirements, the various nutrient elements required by them and growth patterns of bacteria.

STRUCTURE OF THE LESSON:

5.1 INTRODUCTION:

Bacteria need a variety of chemical substances to obtain energy and to construct the new cellular components. The basic elements or constituents of a cell come from the natural environment and are transformed by the cell into characteristic constituents of the cell. The substances used in biosynthesis and energy production by the cell are called nutrients and

therefore required for growth. The process of building of cell constituents by the cell from the nutrients obtained from its environment is called Anabolism or Biosynthesis. Anabolism is an energy-required process and the cell acquires this energy either directly from the light or from the breakdown of organic compounds or inorganic compounds into simpler substances. This process of breakdown of organic or inorganic chemicals into simpler constituents is known Catabolism.

Growth in a biological system is defined as 'an increase in mass or size accompanied by the synthesis of macromolecules, leading to the production of new organized structure'. In the case of multinucleate, coenocytic microorganisms the nuclear divisions are not accompanied by cell divisions, so growth results in an increase in cell size but not in cell number. But in the case of many unicellular microorganisms, like bacteria, which multiply or divide by binary fission, 'growth' leads to a rise in cell number. So, in microbiology the growth is defined as an increase in the number of cells. Growth is an essential component of microbial function because of the finite life span of microbes in nature. Because of their small size, it is usually not convenient to investigate the growth and reproduction of individual microorganisms. Therefore, usual practice of investigation of growth is to follow the changes in the total population number from time to time.

5.2 NUTRITIONAL CLASSES OF BACTERIA:

Besides the nutrient elements, bacteria also require sources of energy, hydrogen and electrons for their growth. Basing on the sources from which these requirements are available, bacteria can be grouped into different nutritional classes.

Energy Sources

1) Light ---------- Phototrophs

2) Organic or Inorganic compounds ---------- Chemotrophs

Hydrogen or Electron Sources

1) Reduced inorganic substances ----------Lithotrophs

2) Organic molecules----------Organotrophs

Principal Carbon Source

1) Carbon di-oxide ---------- Autotrophs

2) Reduced, preformed, organic molecules---- Heterotrophs

Bacteria exhibit a great diversity in metabolism. Basing on the primary sources of energy, hydrogen and/or electrons and carbon used, bacteria are categorized into four main nutritional classes viz., Photolithotrophic autotrophs, Photoorganotrophic heterotrophs, Chemolithotrophic autotrophs and Chemoorganotrophic heterotrophs. Of these, photolithotrophic autotrophs and chemoorganotrophic heterotrophs include large majority of the organisms that are studied well. The other two classes include fewer organisms but are ecologically very important. In response to environmental changes, a particular species belonging to a particular nutritional class may alter its nutritional or metabolic pattern.

5.2.1 Photolithotrophic Autotrophs:

Also called simply as Photoautotrophs. Organisms use the light as energy source and CO_2 as carbon source. Inorganic molecules like hydrogen, hydrogen sulfide and elemental sulfur are used as electron donors. E.g.: Purple and green sulfur bacteria and Cyanobacteria.

5.2.2 Photoorganotrophic Heterotrophs:

Organisms use light as energy source and organic matter as electron donor as well as carbon source. These organisms are common inhabitants of polluted lakes and streams. E.g.: Purple non-sulfur bacteria and Green non-sulfur bacteria.

5.2.3 Chemolithotrophic Autotrophs:

Organisms derive both energy and electrons for biosynthesis from reduced inorganic compounds such as iron, nitrogen and sulfur molecules. The carbon source is CO_2. These chemolithotrophs greatly contribute to the chemical transformations of elements that continually occur in ecosystem. The best known examples of this class are Sulfur- oxidizing bacteria, Hydrogen bacteria, Nitrifying bacteria and Iron bacteria.

5.2.4 Chemoorganotrophic Heterotrophs:

This class is also referred as chemoheterotrophs and sometimes even as heterotrophs. They use the organic compounds as sources of energy, hydrogen, electron and carbon for biosynthesis. In most of the cases, the same organic nutrient will satisfy all these requirements. This group includes most non-photosynthetic bacteria.

5.3 NUTRITIONAL ELEMENTS:

The approximate elementary composition of the bacterial cell is 50% carbon, 20% oxygen, 14% nitrogen, 8% hydrogen, 3% phosphorus, 1% sulfur, 1% potassium, 1% sodium, 0.5% calcium, 0.5% magnesium, 0.5% chlorine, 0.2% iron and others account to 0.3%. This cell composition shows that over 95% of cell dry weight is made up of a few major elements. Basing on the amounts in which required, the elements are grouped into two main categories viz., Macroelements and Microelements.

Macroelements:

Also be called major elements or macronutrients. This category includes elements such as carbon, oxygen, nitrogen, hydrogen, sulfur, phosphorus, potassium, magnesium, calcium and iron and contributes to over 95% of the cell dry weight. The first six (C, O, H, N, S, and P) are components of carbohydrates, lipids, proteins, and nucleic acids. The remaining four macroelements exist in the cell as cations and play a variety of roles.

Element	Usual form in nature	Chemical form in culture media	Functions
Carbon	CO_2; Organic compounds	Glucose, malate, acetate, pyruvate etc; complex mixtures (yeast/peptone)	Backbone for all cell organic molecules
Hydrogen	H_2O; Organic compounds	H_2O; Organic compounds	pH maintenance, Hydrogen bonds in macromolecules, prime force in oxidation-reduction reactions
Oxygen	H_2O; O_2; Organic compounds	H_2O; O_2; Organic compounds	Major component in carbohydrates, lipids, proteins
Nitrogen	NH_3, NO_3^-, N_2, Organic Nitrogen compounds	Inorganic: NH_4Cl, $(NH_4)_2SO_4$, KNO_3, N_2 Organic: Amino acids, nitrogen bases, N-containing organic compounds	Major constituents of proteins and nucleic acids; present in peptidoglycan of cell wall
Phosphorus	PO_4^{3-}(inorganic), Organic phosphates	KH_2PO_4, Na_2HPO_4	Present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell component

Sulphur	H_2S, SO_4^{2-}, Organic S compounds, Metal sulphides	Na_2SO_4, $Na_2S_2O_3$, Na_2S, Cysteine, other organic sulphur compounds	Play a structural role in cysteine and methionine amino acids; present in a number of vitamins like thiamine, biotin, lipoic acid
Potassium	K^+ solution /various K salts	KCl, KH_2PO_4	Important in enzyme action and maintain osmotic potential and electrical potential within the cell
Magnesium	Mg^{2+}solution/various Mg salts	$MgCl_2$, $MgSO_4$	For stabilization of ribosomes, cell membranes and nucleic acids; for the activity of many enzymes that involve in phosphate transfer; integral part of chlorophyll molecule
Calcium	Ca^{2+}solution/$CaSO_4$/other Ca salts	$CaCl_2$	Actually not essential for growth of many bacteria, in certain higher bacteria it forms deposits of calcium carbonate and calcium oxalate; gives stability to some extracellular enzymes and to cell
Iron	Fe^{2+}/Fe^{3+}solution /FeS, $Fe(OH)_3$/other Fe salts.	$FeCl_3$, $FeSO_4$, various chelated iron solutions	Plays a major role in cellular respiration; key component of cytochromes, ferridoxins and iron-sulphur proteins that involves in electron transport

Microelements:

These are also called micronutrients or trace elements. These trace elements are normally a part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure. These microelements such as manganese, zinc, cobalt, molybdenum, nickel and copper are needed by most of the cells.

Element	Function
Zinc	Plays a structural role in many enzymes including carbonic anhydrase, alcohol dehydrogenase, RNA and DNA polymerases, alkaline phosphatase, aldolase; in DNA binding proteins; holds the protein subunits together in proper configuration for enzyme activity
Manganese	Activator of many enzymes; component in certain superoxide dismutases; component in water-splitting enzyme of photosystem II in oxygenic phototrophs
Molybdenum	Present in certain enzymes viz., molybdoflavoproteins; in nitrogenases; in formate dehydrogenases
Copper	Play a role in certain enzymes involved in respiration; present in cytochrome oxidase and oxygenases; in some superoxide dismutases; involves in synthesis of melanin
Cobalt	Constituent of B_{12}
Nickel	Present in urease and hydrogenases; part of coenzyme F_{430} of methanogens; required for autotrophic growth of hydrogen-oxidizing bacteria

Growth Factors:

Many photoautotrophic microorganisms often grow well and reproduce when minerals and sources of energy, carbon, nitrogen, phosphorus and sulfur are supplied. These organisms have the enzymes and pathways necessary to synthesize all cell components. But many microorganisms lack one or more essential enzymes to build up their organic cell constituents as they cannot synthesize them. Any organic compound, other than the carbon and energy source, required essentially but cannot be synthesized by organisms is called growth factor. So, these substances must be provided as nutrients.

There are three major classes of growth factors viz., (1) amino acids, (2) purines and pyrimidines, and (3) vitamins. The amino acids are required for protein synthesis whereas purines and pyrimidines are needed for nucleic acid synthesis. Vitamins are the small organic molecules that usually make up all or part of enzyme cofactors, and very small amounts sustain growth. Some microorganisms like *Enterococcus faecalis*, a lactic acid bacterium, require as many as eight different vitamins for growth. Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth response assays for a variety of substances.

Vitamin	Functions	Example
Biotin	Carboxylation (CO_2 fixation); Carboxyl transfer; Fatty acid biosynthesis	*Leuconostoc mesenteroides*
Cobalamin (B_{12})	Molecular rearrangements; reduction and transfer of single carbon fragments; synthesis of deoxyribose	*Lactobacillus* spp.
Folic acid	One-carbon metabolism; methyl group transfer	*Enterococcus faecalis*
Thiamine (B_1)	Pyruvate decarboxylation; α-keto acid oxidation	*Bacillus anthracis*
Riboflavin (B_2)	Precursor of FMN, FAD in flavoproteins involved in electron transport	*Caulobacter vibrioides*
Pyridoxine (B_6)	Amino acid and keto acid transformations	*Lactobacillus* spp.
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives; pyruvate oxidation; fatty acid metabolism	*Proteus morganii*
Nicotinic acid (Niacin)	Precursor of NAD and NADP; electron transfer in oxidation-reduction reactions; and Dehydrogenations	*Brucella abortus*
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate	*Lactobacillus casei*

5.4 BACTERIAL GROWTH:

5.4.1 Growth Curve

The bacterial population generally shows a characteristic growth pattern when grown in a batch culture system or closed system. In this system, cells are incubated in a closed culture vessel with a single batch of the medium without the addition of fresh medium into vessel. In this set up, nutrient concentrations decline and concentrations of wastes increase. The growth pattern of bacteria reproducing by binary fission in the culture system follows a typical curve when a graph is plotted between the logarithm of cell number versus incubation time. The resulting curve is called bacterial growth curve which consists four distinct phases namely Lag phase, Exponential phase, Stationary phase and Death phase (Fig. 5.1).

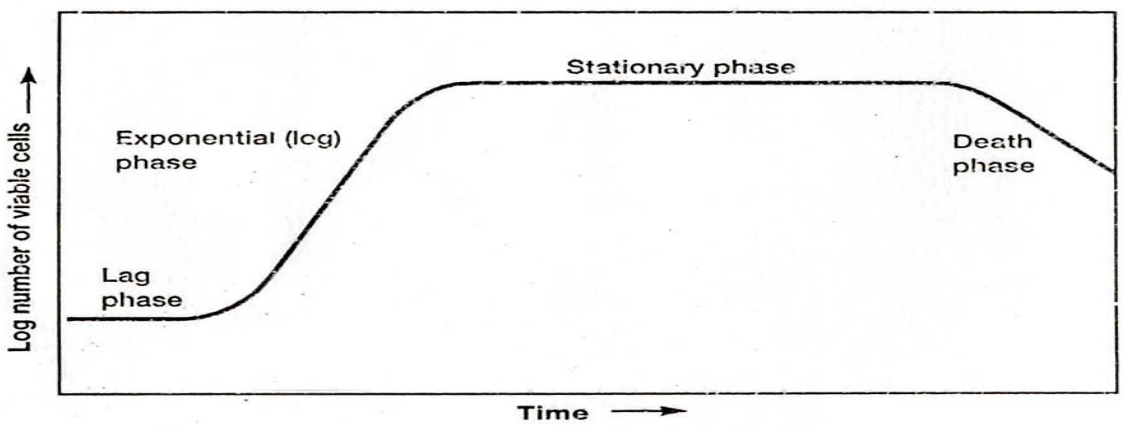


Figure-5.1: Bacterial Growth Curve

Lag Phase:

When bacterial population is inoculated into fresh liquid culture medium, usually no immediate growth will occur. This period is called as lag phase and the increase in cell number of bacteria takes place only after this phase. During this phase, cells adjust to the new environment and undergo the synthesis of new components like essential cofactors, various enzymes which are required for the growth. This lag phase is an essential phase for a population prior to its cell division. The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

Log Phase or Exponential Phase:

This phase is also called Log phase. During this phase, bacteria grow and divide at the

maximum possible rate and the number of cells increases exponentially in a geometrical progression. The growth rate in this phase is dependent on the genetic potential of the organism, nature and composition of the medium and conditions of culturing. The exponential growth rate of one organism differ with that of the another organism. For a given organism, the growth rate during this exponential phase is constant as the cells divide and double in number at regular intervals. The growth curve rises smoothly rather than in discrete jumps in this phase. The population is most uniform in terms of chemical and physiological properties during this phase, therefore exponential phase cultures are usually used in biochemical and physiological studies.

Mathematical Expression of Growth:

During the exponential phase each cell divides at constant intervals and thus population will double in number during a specific length of time called the generation time or doubling time. When culturing is started with the inoculation of one cell, increase in population occurs in a simple geometric progression of the number 2 as the cell number doubles for every generation. This increase can be expressed as a geometric progression as the following

$$\mathbf{1 - 2^1 - 2^2 - 2^3 - 2^4 - 2^5 \text{----------} 2^n}$$

The exponent 'n' is the number of generations

Let N_0 = the initial population number

N_t = the final population number at the time 't'

n = the number of generations in the time 't'

So, $N_t = N_0 \times 2^n$

Solving for the number of generations 'n' by converting the cell number into logarithms of base 10

$$\text{Log}_{10} N_t = \text{Log}_{10} N_0 + n.\log_{10}2$$

$$n = \frac{\log_{10}N_t - \log_{10}N_0}{\log_{10}2}$$

$$n = \frac{\log_{10}N_t - \log_{10}N_0}{0.301} \quad \ldots \text{as the value of } \log_{10}2 \text{ is } 0.301$$

$$n = 3.3\ (\log_{10}N_t - \log_{10}N_0)$$

By using this formula the number of generations can be calculated if initial and final populations are known.

Generation Time: The time required for completion of one generation is called the generation time. Also be called doubling time as the cell number becomes double in one generation. It is denoted by letter 'g' and g = t/n where, t is the time of incubation and 'n' is the number of generations occurred during the incubation time of 't'.

Growth Rate is the change in cell number or mass per unit time and denoted by 'R'. During exponential growth phase the growth rate is reciprocal to the generation time.

So, R = 1/g = n/t

Stationary Phase:

In a batch culture the exponential growth cannot continue for a long period or cannot occur indefinitely. At some point, the growth of population ceases and the growth curve comes horizontal. This phase is called as stationary phase where the total number of viable cells remains constant without any net increase or decrease in the cell number due to the balance between cell division and cell death, but cells remain metabolically active. The stationary phase for bacteria is attained when the population level reaches to a level of 10^9 cells per ml of broth. Bacterial populations enter into the stationary phase for several reasons like (i) limitation and depletion of an essential nutrient in the culture medium (ii) accumulation of some toxic waste products to an inhibitory level.

Death Phase:

This phase is also called as decline phase. When the culturing is continued after the stationary phase, cells may die due to the occurrence of detrimental environmental changes like nutrient deprivation and the buildup of toxic wastes. The death of a microbial, like its growth during exponential phase, is usually logarithmic.

5.4.2 Continuous Culturing:

Exponential growth of organisms occurs for only a few generations and soon reaches the stationary phase in the batch culture system. The growth of bacterial population at a particular rate in the exponential phase can be maintained through continuous culturing system in which the constant environmental conditions are maintained through continual provision of nutrients and removal of wastes. There are two major types of continuous culture systems in common use – 1) The Chemostat and 2) The Turbidostat.

Chemostat:

A chemostat is constructed so that the sterile medium is fed into the culture vessel at the same rate as the medium containing microorganisms is removed. The culture medium for a

chemostat possesses an essential nutrient like an amino acid in limiting quantities. Because of the presence of a limiting nutrient, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrient. So, the concentration of the limiting nutrient in substrate of the culture vessel controls the growth rate. The concentration of the substrate is in turn controlled by the dilution rate.

$$\text{The dilution rate 'D'} = \frac{\text{flow rate (f)}}{\text{vessel volume (v)}}$$

Both the microbial population level and the generation time are related to the dilution rate. As the dilution rate increases the generation time decreases. If the dilution rate rises too high, the microorganisms can actually be washed out of the culture vessel before reproducing because the dilution rate is greater than the maximum growth rate. The limiting nutrient concentration raises at higher dilution rates because fewer microorganisms are present to use it. By adjusting the flow rate, growth rate can be controlled. The chemostat apparatus is shown in Fig.5.2.

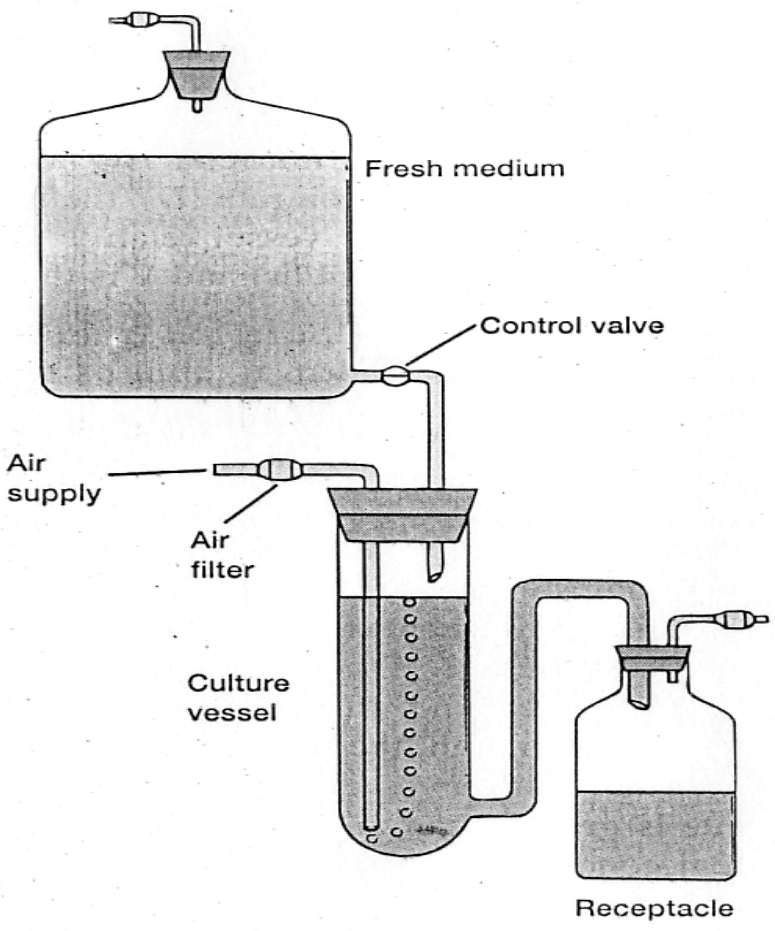


Figure-5.2: Chemostat Apparatus

Turbidostat:

Turbidostat is the second type of continuous culture system. This device (Fig. 5.3) has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity of cell density. The turbidostat differs from the chemostat in several ways. The dilution rate in turbidostat varies rather than remaining constant, and its culture medium lacks a limiting nutrient. The turbidostat operates best at high dilution rates whereas the chemostat is most stable and effective at lower dilution rates.

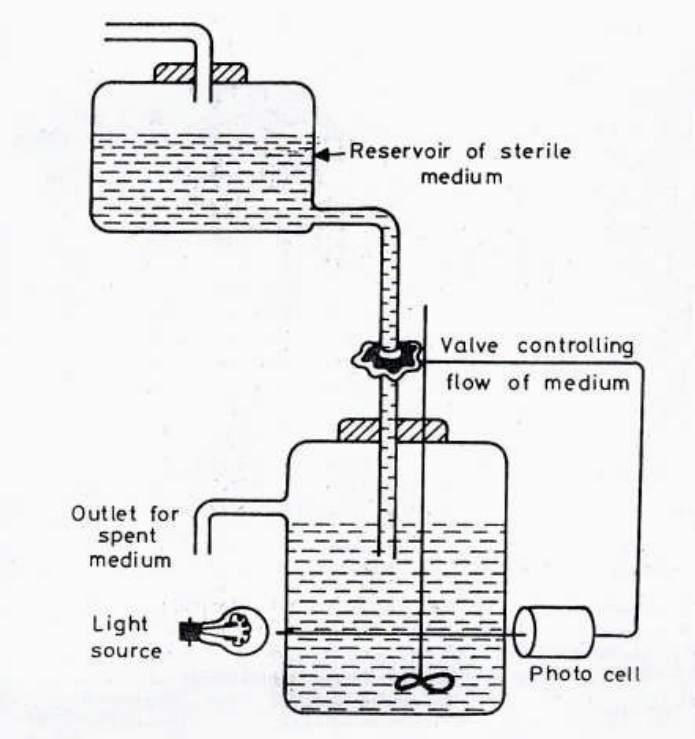


Figure-5.3: Turbidostat apparatus

5.4.3 Synchronous Culturing:

The bacterial population, at any given instant during log phase consists of cells in various stages of division viz., about-to-divide cells, just-divided cells, and cells in the different physiological stages of preparation to division. So, the population is said to be heterogeneous in behavior. To eliminate this heterogeneity synchronous culturing is used in which all the cells provoked to divide at the same time. In this synchronous culture since all the cells are physiologically identical, cell division occurs periodically at constant intervals (Fig. 10.4). This can be achieved by the methods that provoke the entire log-phase culture into the division process simultaneously. Such methods involve the arrest of cell division but not of cytoplasmic growth by chemical or physical means for a period followed by the sudden relief of this inhibition. With this sudden relief, a marked degree of synchronous division of an entire culture is obtained.

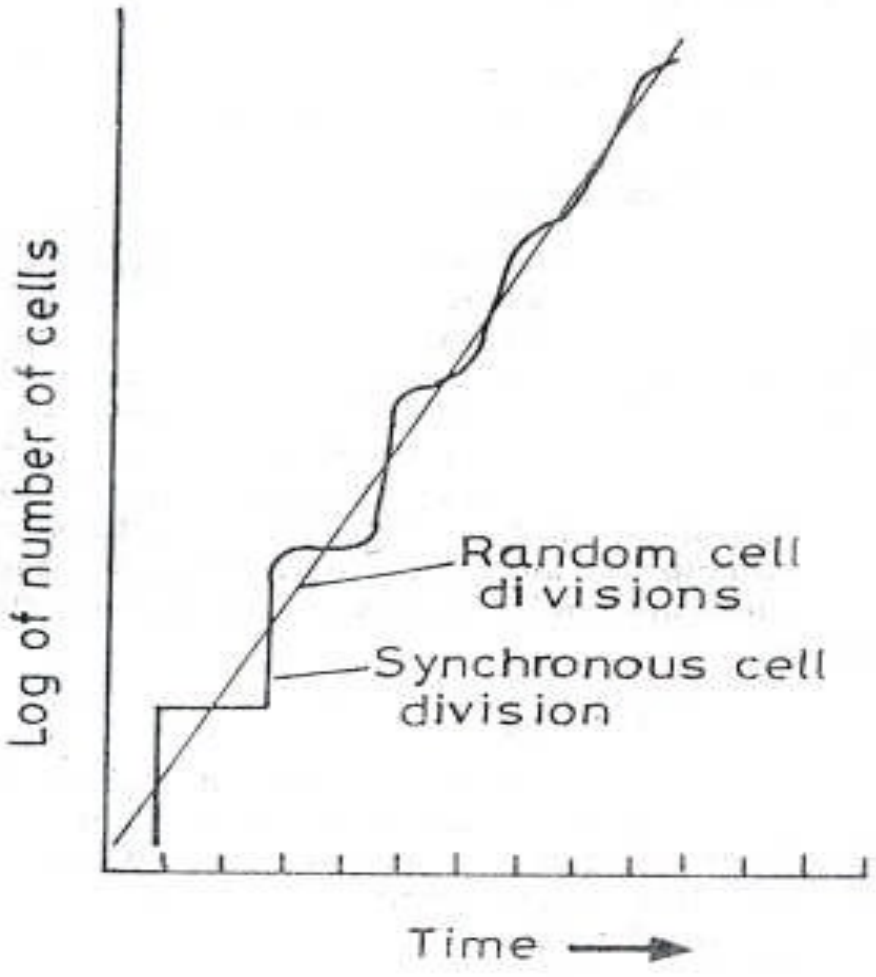


Figure-5.4: Synchronous growth curve

A population can be synchronized by manipulating the physical environment or the chemical composition of the medium. For example, when cells inoculated into a medium at sub-optimal temperature and maintained the same condition for some time, they will metabolize slowly but not divide. When the temperature is subsequently raised, the cells will undergo a synchronized division. Similarly, *E. coli* growth can be synchronized by changing the chemical nature of the medium. When a thymine requiring mutant is starved for thymine by placing it in a thymine deficient medium, it is incapable to grow. Then the addition of thymine in the culture medium causes the surviving of cells to undergo several synchronous divisions.

5.4.4 Biphasic Growth Curve:

A biphasic growth curve reflects the preferential utilization of substrates and this is referred as diauxie phenomenon. A combination of catabolite repression and operon control mechanisms results in a biphasic growth curve (Fig.5.5). In this diauxie phenomenon, when two carbon sources are present in the medium the organism preferentially utilizes one source completely before the use of other. For example, cultures of *E. coli* exhibit the biphasic growth curve when inoculated into a medium containing both glucose and lactose as substrate. While

growing on glucose *E. coli* exhibits the normal lag, log and stationary phases of growth. Rather than exhibiting a prolonged stationary phase, *E. coli* enters a second lag phase when the glucose is no longer readily available in concentrations that suppress disaccharide utilization by catabolite repression. During this second lag phase, allolactose acts as an inducer to derepress the lac operon system. The enzymes that are necessary for lactose metabolism are synthesized, and the bacteria begin to grow exponentially by using the lactose substrate. When the lactose is also utilized, the bacterium enters into the secondary stationary phase.

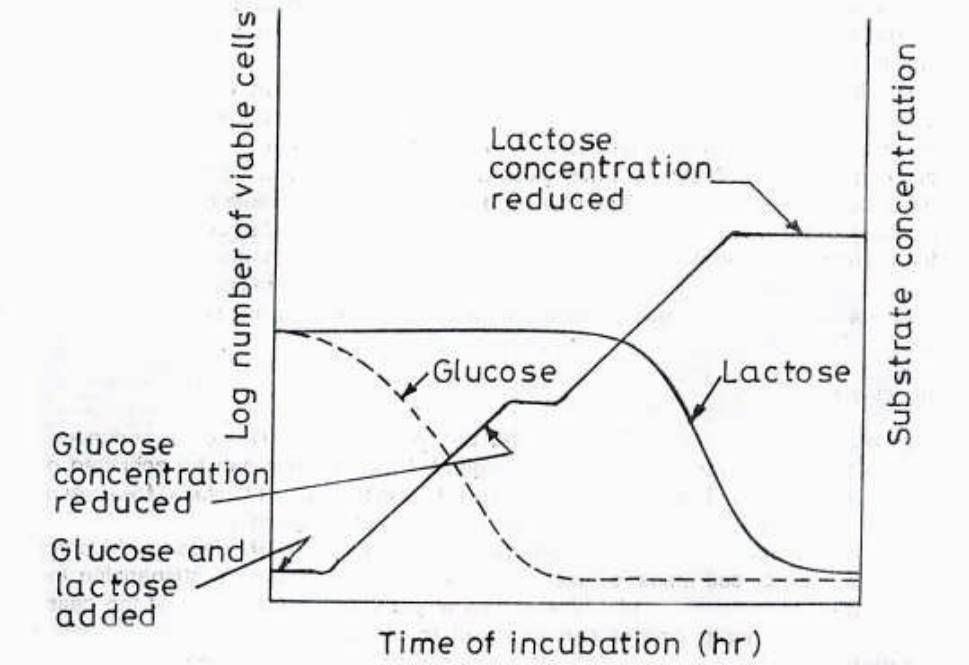


Figure-5.5: Biphasic growth curve

5.5 SUMMARY:

Bacteria require nutrient materials that are used in biosynthesis and energy production. These materials are drawn by the bacteria from their surrounding environments. Bacteria can be categorized into nutritional classes based on the sources from which they acquire the carbon, energy and electrons. Accordingly the nutritional types of bacteria are photolithotrophic autotrophs, photoorganotrophic heterotrophs, chemolithotrophic autotrophs and chemoorganotrophic heterotrophs. Several elements require by bacteria in varying quantities as nutrients. Basing on the amounts or quantities in which they are required the elements are Macroelements (needed in larger quantities) and Microelements (needed in smaller quantities). Various amino acids are also required by some bacteria as essential growth factors. The nutritional requirements of a bacterium are determined by the kind and number of its enzymes. So, the nutritional complexity reflects a deficiency in biosynthetic enzymes.

Growth is an increase in cellular constituents and results in an increase in cell size, cell number of both. When bacteria are grown in a closed system or batch culture, the resulting growth curve usually has four phases viz., lag phase, exponential or log phase, stationary phase and death phase. In lag phase, the cells adapt to the new environment and prepare itself to undergo multiplication. During the exponential phase, the population doubles at a constant interval called the doubling or generation time. The mean growth rate constant is the reciprocal of the generation time. In stationary phase, the cell number remains constant due to the depletion of nutrients. During death or decline phase of growth, bacterial cells die due to the accumulation of toxic substances to lethal levels. Bacteria can be grown in an open system in which nutrients are constantly provided and wastes are removed. A continuous culture system is an open system that can maintain a bacterial population in the log phase for desired period. There are two types of these systems namely chemostat and turbidostat.

5.6 TECHNICAL TERMS:

Photolithotrophic autotrophs, Photoorganotrophic heterotrophs, Chemolithotrophic autotrophs, Chemoorganotrophic heterotrophs, Major elements, Minor elements, Growth factors, Bacterial growth curve, Lag phase, log phase, Stationary phase, Death phase, Chemostat, Turbidostat, Synchronous culturing, Biphasic growth.

5.7 SELF ASSESSMENT QUESTIONS:

1) Define nutrition and explain the types and functions of elements required by bacteria as nutrients.
2) Write a note on different nutritional classes of bacteria.
3) Explain the bacterial growth curve.
4) Describe the continuous and synchronous culturing of bacteria.

5.8 SUGGESTED READINGS:

1) Microbiology - Prescott, L.M., Harley, J.P., and Klein, D.A.
2) Biology of Microorganisms - Brock, T.D. and Madigan, M.T
3) Principles of Microbiology - Atlas, R.M
4) The Physiology and Biochemistry of Prokaryotes - White, D
5) Bacterial Metabolism - Gottschalk, G

Prof. V. Umamaheswara Rao

LESSON – 6

GENETIC RECOMBINATION AND ECONOMIC IMPORTANCE OF BACTERIA

6.0 OBJECTIVE OF THE LESSON:

Students will aware of the types of genetic recombination and the significance of bacteria in various aspects.

STRUCTURE OF THE LESSON:

6.1 INTRODUCTION:

During sexual reproduction, in eukaryotes, either produce gametes or there is the fusion of the protoplasts in the process of conjugation. The genes from each parent are recombined resulting into new combinations of genes together in a single cell is called genetic recombination; consequently the new individual possesses the genotype not possessed by either of the parents. As a result of conjugation between two eukaryotic cells, a zygote is formed after fusion of cells. This type of true fusion of cells or gametes has not been in prokaryotes nevertheless, a portion of DNA from the donor bacterial cell may get transferred to the recipient bacterial cell to form a partial zygote known as merozygote.

The fragment of DNA from the donor cell is known as exogenote which may join the DNA of the recipient cell or endogenote. This happens by the breakage and reunion of the two fragments of DNA i.e., exogenote and endogenote DNA. The new combination of genes enables the bacterium or the microbe to survive under the changed adverse environmental conditions.

6.2 GENETIC RECOMBINATION IN BACTERIA:

In absence of sexual reproduction, some bacteria achieve the benefits of it through three different methods namely 1) Transformation, 2) Conjugation, and 3) Transduction that results in genetic recombination.

6.2.1 Transformation:

Genetic transformation in bacteria is a natural process where bacteria take up free DNA from their environment, integrating it into their genome and acquiring new traits (Fig.6.1). This process is crucial for horizontal gene transfer and can contribute to the spread of antibiotic resistance among bacteria. In other words it can be defined as the introduction of genetic material from dead bacteria in culture or suspension to living bacteria in the same medium. The phenomenon of transformation was discovered in 1928 by Frederick Griffith, a British bacteriologist, in *Diplococcus pneumonia*, a causative agent of pneumonia disease. This bacterium has two strains – (i) 'S' strain – smooth, virulent and capsulated type with serotypes SI, SII and SIII; (ii) 'R' strain – rough, non-virulent and non-capsulated type with serotypes RI, RII and RIII.

Griffith conducted the experiment (Fig. 6.2) by selecting virulent SIII strain, avirulent RII stain and four groups of mice. He injected virulent bacteria into one mice group and all the mice died of pneumonia. He injected avirulent bacteria into another group of mice, and all mice survived. To another mice group, he injected heat killed virulent strain and these mice are also survived. To the last group of mice, he injected a mixture of heat killed virulent strain and live avirulent bacteria. This group of mice died of pneumonia and he could isolate virulent SIII bacteria from the dead mice. He concluded that the avirulent RII was modified into virulent form by a stable and heritable transforming factor released by heat killed SIII strain bacteria and called this phenomenon as 'transformation' (Fig. 6.2).

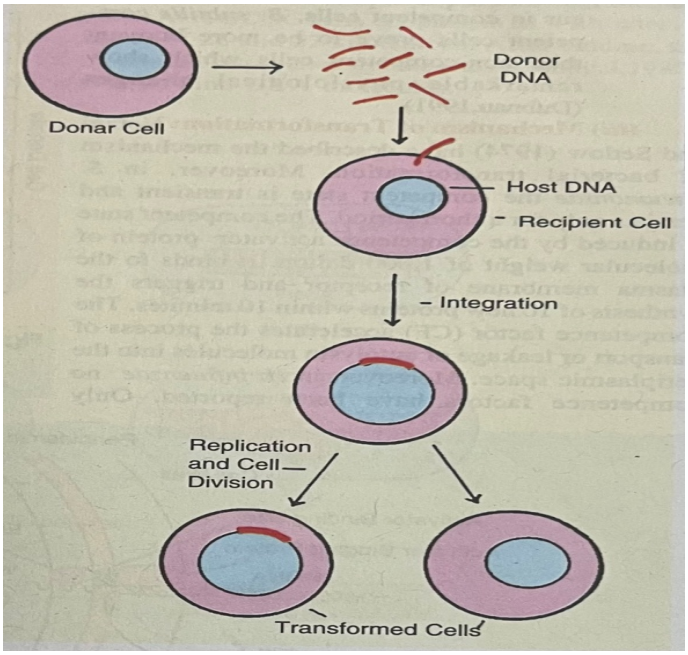


Figure-6.1: Genetic transformation in bacteria

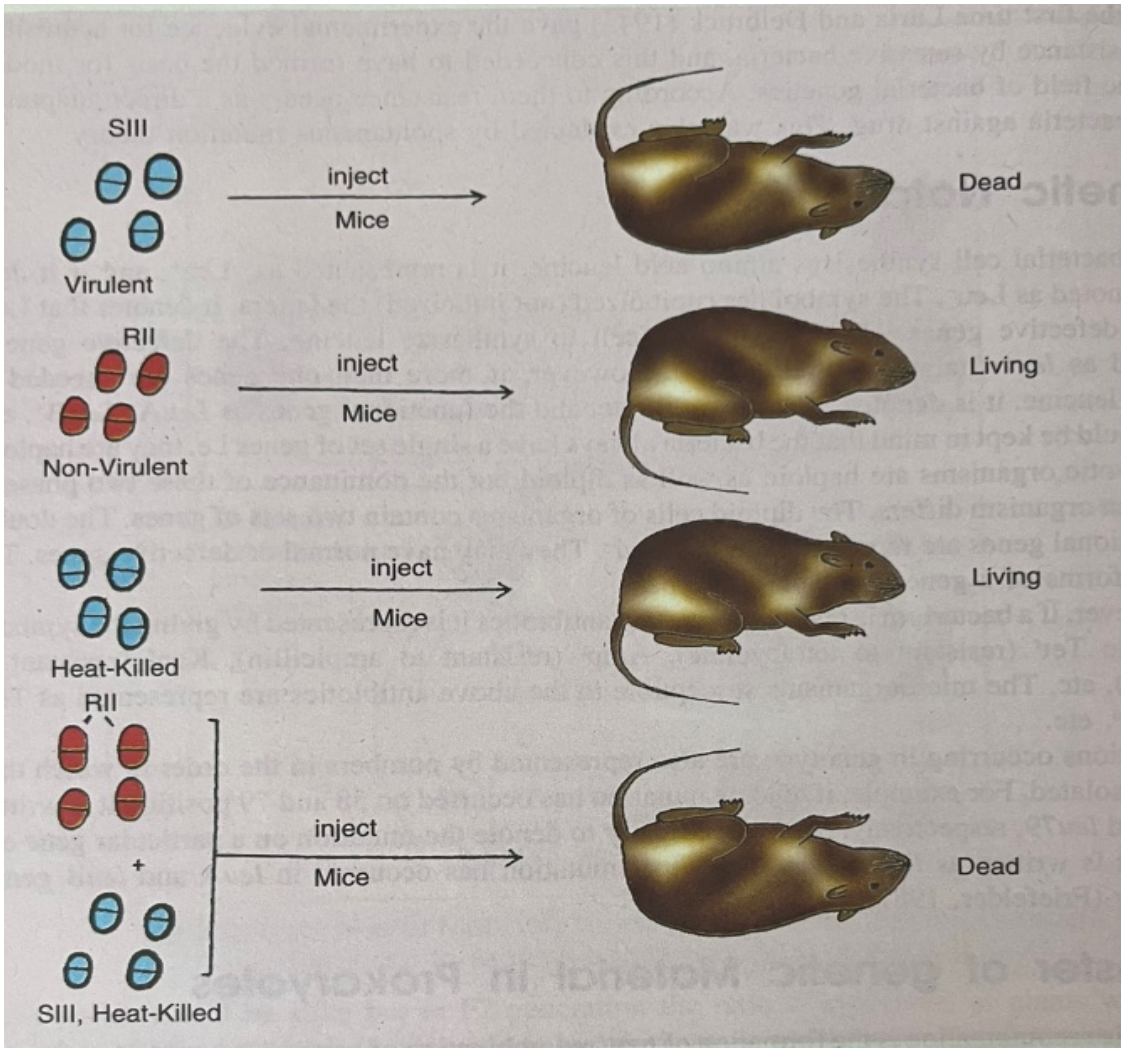


Figure-6.2: Griffith's experiment

Later in 1994, Avery, McCarty and Mc Leod confirmed his findings and proved that the transforming factor was a DNA fragment. They proved that the addition of DNA form capsule producing SIII stains into the cultures of non-capsulated RII strain, transforming the RII strain into a capsulated SIII strain. In these bacteria, capsule is responsible for pathogenesis. When they used DNase along withDNA of SIII strain in the cultures, RII strain was not transformed, so proving the transforming principle. Now, transformation has been reported in many bacteria such as *Streptococcus, Haemophilus, Neisseria, E. coli, Rhizobium* and *Bacillus.*

The mechanism of transformation depends on the ability of receptive recipient bacteria to accept external DNA and undergo transformation is known as 'Competence'. The bacteria having this competence said to be competent. The competence phenomenon is a non-permanent feature and occurs only at certain times in the life cycle. Generally, competence takes just before the stationary phase and towards end of the exponential phase of bacterial growth. The extracellular 'Competence Factor (CF)' of the competent bacteria can induce

competence in non-competent bacterial cells. Generally, the frequency of transformation is very low and usually not exceeding 1%.

6.2.2 Conjugation:

Conjugation is the transfer of genetic material from one bacterial cell to another by direct contact through a tubular outgrowth known as conjugation tube. Two American scientists, Joshua Lederberg and Edward L. Tautum, presented the evidence for the occurrence of conjugation in *E. coli* K12 strain in 1946. In 1950, Davis proved through his 'U-tube' experiment that contact between the two conjugating parent cells is essential for recombination to occur. Some other scientists evidenced that in conjugation process, a part of genetic material from donor bacterial cell transferred to recipient bacterial cell. Later studies proved that the transferred genetic material was a plasmid. Conjugation process has been reported in *Vibrio*, *Salmonella*, *Pseudomonas* and many other bacteria.

In bacterial conjugation, the transfer of genetic material takes place by direct contact between two sexually different strains of bacterial cells. Of the two different strains, one acts as a donor, male or fertile (F^+) cell and the other as a recipient or female (F^-) cell. When two cells of opposite strains come into contact with each other, the sex pilus of the donor cell develops into a conjugation tube, which attaches to a receptor site on the recipient cells and forms a bridge between the donor and recipient cells. Consequently, F^+ plasmid replicates in the donor cell and the newly formed F-factor is transferred to the recipient cell through the conjugation tube which results in the conversion of F^- cell to F^+ cell.

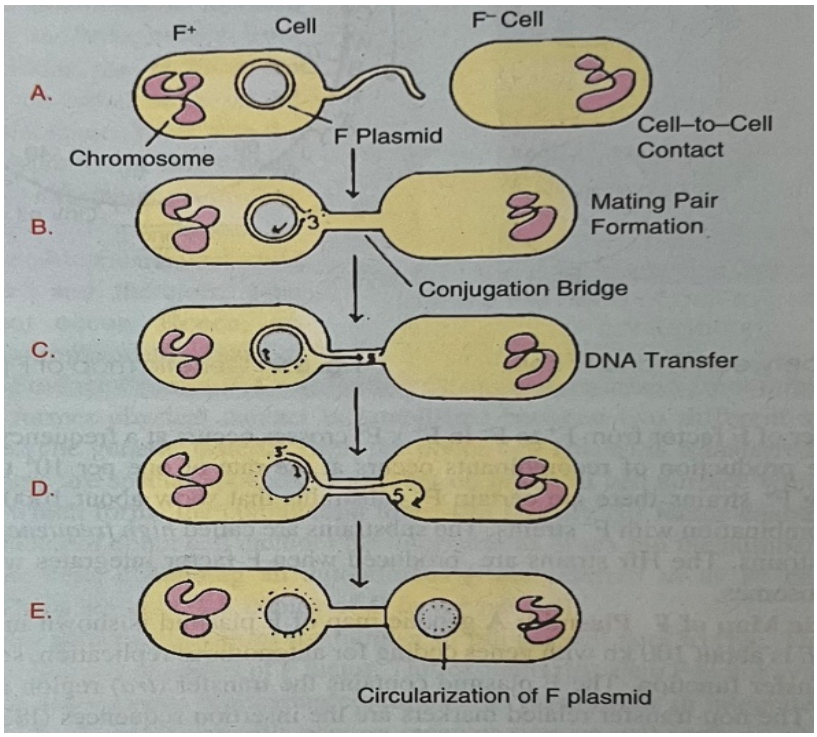


Figure-6.3: Bacterial conjugation

6.2.3 Transduction:

The transfer of genetic material from one bacterium to another one through bacteriophages is known as 'transduction'. Transduction was a chance discovery by Joshua Lederberg and Norton Zinder while studying the conjugation in *Salmonella typhimurium*. Since then, transduction has been discovered in many bacteria such as *E. coli*, *Shigella*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, and *Proteus* etc. In transduction, lysogenic or temperate bacteriophages attack the host bacteria and inject their DNA into them. The injected viral DNA either integrates itself into the bacterial genome and replicates along with it as prophage or remain in the cytoplasm and replicates along with host genome lysis. In both the cases, some of the host DNA may go along with the viral DNA into the genomes of newly synthesized virus particle. The bacteriophages, whose DNA contains a segment of host DNA are known as 'defective phages'. When released from the invaded bacterial cells, the defective transducing phages infect the new host recipient bacterial cells. In this process, they incorporate the bacterial DNA brought form the previously infected bacterial host, into the new host resulting in the formation of a bacterial cell with recombinant DNA or genome. Basing on the genetic material transferred, the transduction is of two types –

(i) Generalized (Non-Specific) Transduction:

The phage mediated transfer of any segment of DNA from any portion of the bacterial genome is known as generalized transduction. In this case, viral DNA do not integrates itself in bacterial genome but remains in cytoplasm and replicates. During the replication, bacterial genome is fragmented and some fragments may get a chance entry into the viral DNA. This hybrid DNA is later packaged into new virus particles. When such defective bacteriophages with hybrid DNA will invade new bacteria, viral DNA and bacterial DNA of the previous host will be transferred to the new host. This transduction was originally discovered in *Salmonella typhimurium* with P22 phage and later in *E. coli* with P1 phage. If this newly introduced bacterial DNA (exogenote) integrates itself into the bacterial genome (endogenote), the process is referred as complete transduction or stable gene transfer. If the exogenote fails to integrate into endogenote but able to expresses itself and survives in the cytoplasm, the process is known as unsuccessful gene transfer. And if the exogenote remains in cytoplasm and unable to integrate as well as express itself, the process is known as abortive transduction.

(ii) Specialized (Restricted) Transduction:

The temperate phage mediated transfer of specific bacterial genes, present adjacent to the prophage, to a new bacterium is known as specialized transduction. For the first time discovered in *E. coli* galactose gene transduction by Lambda (λ) phage. The temperate or lysogenic phages integrate themselves into the bacterial cells genome and remains there ad

prophages. They behave as integral part of the bacterial genome and replicate along with them for many generations. In this way prophages are passed on to many daughter cells. Certain factors make the dormant prophages to come out of the bacterial genome along with adjoining bacterial DNA segments. The prophages multiply multiply in the cytoplasm, assemble into virus particles and escape after host cell lysis. They infect new host bacterial cells and transfer the DNA of the previously invaded bacterial cells into it, so bacterial recombination occurs through transduction. If the newly brought DNA segment has some common genes, pairing and crossing-over can also occur.

6.3 ECONOMIC IMPORTANCE OF BACTERIA:

In the universe, bacteria are ubiquitous and most abundant microorganisms and are variously associated with our lives. They influence our lives in many direct and indirect ways. They are helpful in the production of many useful products and in many processes on one hand and cause diseases of humans, animals and plants on the other. Their activities can be mentioned in four heads viz., A) Useful activities, B) Bacteria in biological research, C) Mutualistic activities, and D) Harmful activities.

A) Useful Activities:

1. Bacteria in Agriculture:

Bacteria increase the soil fertility in one hand and can be used as biopesticide on the other.

(i) Increase in Soil fertility: Many bacteria increase the soil fertility by solubilizing the minerals present in the soil and also by fixing the atmospheric nitrogen. These processes increase the soil fertility.

Ammonification – Due to death of living organisms, organic matter is continuously added to the soil. Some saprophytic bacteria (*Bacillus vulgaris, B. ramosus, B. subtilis, B. mycoides*) act upon the proteins present in this organic matter and convert them into ammonia, a process known as 'ammonification'. Ammonia is further converted into ammonium carbonate, which can be used as a source of nitrogen by some plants. This decomposition of proteins with offensive smell is known as 'putrefaction'. Urea present in animal excreta is also converted into ammonia by *Bacillus pasteuri* and *Sarcina ureae*.

Nitrification – Ammonia liberated through ammonification is oxidized to nitrous acid by Nitosomonas, which in turn combines with the bases present in the soil to become nitrite salt. Nitrobacter oxidizes the nitrite salt into nitrates. Nitrates can be utilized by some plants as a source of nitrogen. This process of conversion of ammonia into nitrates is called as 'nitrification'.

Nitrogen fixation – Nitrogen is a major macronutrient required by the plants in large and atmosphere contains about 78% nitrogen in the form of inert gas which cannot be directly utilized by the plants. Some bacteria are capable of converting this gaseous atmospheric nitrogen into easily metabolizable form. Of these bacteria, some are free living anaerobes (*Clostridium* and photosynthetic green and purple bacteria such as *Rhodospirillum*, *Chromatium*, *Rhodomicrobium* and *Chloropseudomonas*) or aerobes (*Azotobacter* spp.), while some others are symbiotic bacteria. All the prokaryotic organisms that can fix nitrogen are known as 'diazotrophs'. The symbiotic bacteria form associations with roots (Rhizobium and Bradyrhizobium in legume plants), stems (Azorhizobium in Sesbania rostrata) or leaves (Burkholderia in Pavetta indica) of leguminous plants and induce them to develop nodules in which they live and fix atmospheric nitrogen. Nitrogenase enzyme present in the leguminous root nodules helps in this process along with an oxygen carrier and iron containing pigment molecule namely 'leghaemoglobin'. In all the cases, atmospheric N_2 is converted into ammonia which is either incorporated into amino acids by the plants or it leaches out into the surrounding soil, from where it can be taken up by other plants.

Humus:

Humus is a dark brown or black coloured colloidal mass of partially decomposed organic matter which improves the fertility, aeration and water holding capacity of the soil. Certain saprophytic bacteria help in the conversion of organic matter present in the dead organisms or excreta into humus or compost.

The prominent examples of decomposer bacteria are –

Cellulose decomposers – *Bacillus, Pseudomonas, Cellulomonas, Clostridium, Cytophaga*

Hemicellulose decomposers – *Bacillus, Pseudomonas, Erwinia, Cytophaga*

Lignin decomposers – *Micrococcus, Arthrobacter, Xanthomonas, Pesudomonas*

(ii) Bacteria as Biopesticide:

Different chemical pesticides, besides causing environmental pollution, are also harmful to humans, wildlife and pollinators. *Bacillus thuringiensis*, a Gram negative bacterium, can be used in biological pest control against lepidopteran insect pests without any effect on other organisms. It is available in the market under the trade name, 'Thuricide or Dipel'. Recently, the gene for Bt toxin from *B. thuringiensis* was isolated and transferred to cotton plants to protect them against insect pests without any chemical pesticide input.

2. Bacteria in Industry

Many of the food, chemical and pharmaceutical industries are dependent on several microorganisms for the production of different products.

Manufacture of vinegar – Aerobic bacteria, *Acetobacter aceti*, *A. orientalis* and *Closstridium acetobutylicum* are used to convert ethyl alcohol into vinegar. This vinegar has its use in pickles, as preservative, in salads and other products.

In Dairy Industry – Bacteria like *Lactobacillus acidophilus, L. bulgaricus, Streptococcus thermophilus* convert the milk sugar lactose into lactic acid which acidifies the milk and coagulates the milk protein, casein and cause curdling of milk. In Swiss cheese ripening, propionic acid bacteria convert the lactic acid into propionic acid that imparts characteristic flavor to the cheese. The characteristic flavor of American cheese is imparted by release of proteolytic and fat splitting enzymes by some bacteria.

Amino Acid Production – Many amino acids find applications in many industries. Glutamic acid is used in the production of flavouring agent, Monosodium glutamate (MSG). *Arthrobacter acetogenes, Micrococcus glutamicum* and *Corynebacterium glutamicum* are used in manufacture of glutamic acid, which is converted into MSG by *Brevibacterium* sp.

Retting of Fibres – Retting is a process of separation cellulosic fibres from the stems of flax, jute and hemp. The stems are immersed in water, where the soft tissues and pectins are decomposed by the bacterial activity. This loosens the fibres from the secondary xylem. *Clostridium butylicum* and *C. felsineum* are employed in this retting process.

Acetone-Butanol Production – Many sugars are converted into acetic acid, butyric acid, CO_2 and hydrogen by some bacteria. *Clostridium butylicum* converts acetic acid into acetone and butyric acid into butanol, which find many applications in industries.

3. Role in Medicine:

Many of the medicines are produced by using bacteria directly or indirectly. The *Leuconostoc* bacterium converts sucrose to dextrans, which are now used as a partial substitute for blood plasma. Vitamin C is synthesized by using *Acetobacter* spp. And vitamin B_{12} is produced by using *Bacillus megaterium.* Mutant of *Corynebacterium glutamicum* produces large amount of lysine, an essential amino acid for human health. *Corynebacterium* is also used in transformation of plant steroids into Prednisolone, cortisone derivative, which is widely used for asthma. Many bacteria are most important in the production of antibiotics, probiotics, sera and vaccines.

Antibiotics: Antibiotics are the organic substances of microbial origin and inhibit the growth or kill the other microorganisms. First discovered by Sir Alexander Fleming from a fungus, *Penicillium notatum* in 1929. The term antibiotic coined by Selman Walksman in 1942. Some of the examples include –

Antibiotic	**Bacteria**
Bacitracin	– *Bacillus subtilis*
Chloramphenicol	– *Streptomyces venezuelae*
Erythromycin	– *Streptomyces erythreus*
Gentamycin	– *Micromonospora purpurea*
Neomycin	– *Streptomyces fradiae*
Streptomycin	– *Streptomyces griseus*
Tetracycline	– *Streptomyces aureofaciens*

Probiotics: Probiotics are defined as "live microorganisms administered in adequate amounts which confer beneficial health effect on the host". Some common probiotics include various species of *Bifidobacterium* (*B. longum, B. breve, B. bifidum*); *Lactobacillus* (*L. acidophilus, L. plantarum, L. rhamnosus*); *Streptococcus faecalis*; *Clostridium butyricum* and *Bacillus mesentericus* etc. These probiotics benefit the human health in several aspects – 1) increase lactose tolerance, 2) prevent colon cancers, 3) lower the cholesterol level and blood pressure, 4) improve immunity, 5) protect against pathogens, 6) reduce inflammation, 7) useful in irritable bowel syndrome, and 8) reduce ulcerative colitis.

4. Role in Sewage Disposal and Pollution Abatement:

Many anaerobic bacteria such as *Escherichia coli*, *Achromobacter* sp., *Streptococcus* sp., *Clostridium* sp., and *Micrococcus* sp., *Proteus* sp., etc. can be used in sewage disposal. They are the major microflora of septic tanks where they decompose the organic matter into methane and hydrogen gases and undigested matter remains as activated sludge. Many of the bacterial species utilized in bioremediation of organic pollutants. For example – DDT is partially decomposed by *Aerobacter aerogenes*; Paraquat (weedicide) is completely degraded by *Corynebacterium* and *Clostridium* species. Many other pesticides and herbicides are decomposed by many species of *Achromobacter*, *Corynebacterium* and *Pseudomonas*, which otherwise pollute soil and water.

Dr. Anand M. Chakrabarty, an US microbiologist of Indian origin has developed a strain of *Pseudomonas* which can degrade camphor, naphthalene and many other petroleum hydrocarbons. *P. putida* has the potential of clearing oil spills on sea waters, which otherwise harmful to marine flora and fauna. Some bacteria have been discovered which can remove heavy metals like cadmium, mercury, nickel, copper and zinc from contaminated soils and water bodies.

B. Bacteria in Biological Research:

Major discoveries in molecular biology and genetics have taken place through research on bacteria. Most of the work on DNA and genes was carried out on them. Gene concept, regulation of gene activity and many other major discoveries in biological sciences came from studies on bacteria.

C. Mutualistic Activities of Bacteria:

Mutualism is a type of reciprocal relationship in which both the organisms are mutually benefited. Many bacteria form mutualistic associations with humans, cattle and many plants. In the digestive tract of normal human beings, more than 1000 bacterial species are identified that contribute to gut immunity, synthesis of vitamin K, biotin and folic acid and also help in digestion of complex carbohydrates. *Lactobacillus* sp. in intestine inhibits the growth of potential pathogenic bacteria through competitive exclusion. Cattle, sheep, goat and camels harbor many symbiotic bacteria and protozoa in their rumen which help in digestion of cellulose and other complex materials. *Rhizobium* bacterium forms symbiotic associations with roots of leguminous plants that mutually benefit each other.

D. Harmful Activities:

Some bacteria are harmful and cause various diseases in humans, animals and plants. Additionally, they also cause poisoning and spoilage of various foods and detrimental to human health.

Human Diseases: To list, some of the diseases include

Disease	**Causative agent**
Anthrax	– *Bacillus anthracis*
Bacillary dysentery	– *Shigella* sp.
Cholera	– *Vibrio cholera*
Diphtheria	– *Corynebacterium diphtheria*
Gastroenteritis	– *Clostridium perfringens*
Leprosy	– *Mycobacterium leprae*
Plague	– *Yersinia pestis*
Syphilis	– *Treponema pallidum*
Tetanus	– *Clostridium tetani*
Tuberculosis	– *Mycobacterium tuberculosis*
Typhoid	– *Salmonella typhi*

Spoilage of Food Products:

Various fresh and canned food products are spoiled by bacteria and are rendered unpalatable for human consumption by changing the colour, flavor, and also cause souring and putrefaction of the food products. The canned foods are mostly destroyed by thermophilic and acidophilic bacteria such as *Bacillus stearothermophilus* and *Clostridium*. In non-vegetarian foods, pork is the most susceptible for decay due to higher vitamin B content. Meat and fish are spoiled mostly by *Pseudomonas*, *Micrococcus*, *Streptococcus*, *Leuconostoc*, *Proteus*, *Lactobacillus*, *Bacillus*, *Clostridium*, and *Enterobacter*.

Food Poisoning:

Some saprophytic bacteria contaminate the food products and release some powerful toxins (exotoxins, endotoxins) in it. When such contaminated foods are consumed by human beings, these toxins cause food poisoning in the form of serious illness or sometimes even death. Eg. *Clostridium botulinum* causes botulism by releasing botulinum toxin that affects nervous system and causes vomiting followed by paralysis and death; *Staphylococcus aureus* causes food poisoning by producing enterotoxin with the symptoms of nausea, vomiting and diarrhea.

Plant Diseases:

Bacteria causes many plant diseases and their role as causative agents in diseases was pointed for the first time by T.J.Burill in 1878. More than 170 species of non-sporulating and rod shaped bacteria have been reported to cause mostly wilt, crown gall, hairy root, and blight diseases in plants. Some important examples include –

Disease	**Causative agent**
Bacterial blight of rice	– *Xanthomonas oryzae*
Brown rot of potato	– *Pseudomonas solanacearum*
Citrus canker	– *Xanthomonas citri*
Crown gall of many plants	– *Agrobacterium tumefaciens*
Fire blight of apple and pear	– *Erwinia amylovora*
Hairy root disease of many plants	– *Agrobacterium rhizogenes*
Leaf spot of lady finger	– *Xanthomonas esculenti*
Red stripe of sugarcane	– *Pseudomonas rubrilineans*
Scab of potato	– *Streptomyces scabies*
Soft rot of many vegetables	– *Erwinia carotovora*

Loss of Soil Fertility:

Some denitrifying bacteria such as *Bacillus subtilis, B.denitrificans, Pseudomonas denitrificans, P. fluorescence* and *Thiobacillus denitrificans* convert the soil nitrates and ammonia to free nitrogen gas that escapes into the environment thereby reducing the soil fertility.

6.4 SUMMARY:

Genetic recombination in bacteria occurs through 3 different ways namely Transformation, Conjugation and Transduction. The transformation is a process in which a fragment of naked DNA released by the live or dead donor cells is received in the recipient cell. This phenomenon was first observed by F. Griffith in 1928 in *Diplococcus pneumoniae* (*Streptococcus pneumoniae*). The bacteria that are genetically capable to receive the naked DNA are called the competent cells. The process in which two cells come in contact with each other by means of a conjugation tube i.e., by direct contact to transfer genes from one cell to another cell is called as conjugation. The genes that are responsible to control the process of conjugation are located in an extra chromosomal DNA is called as plasmid (fertility factor). The bacteria having the F-factor (F^+ cell) and able to donate is referred as donor cell. The bacteria that devoid of F-factor (F^- cell) and able to receive the DNA is called recipient cell. The transduction is the process of transfer of chromosomal DNA from one cell to another mediated by a bacteriophage. The phenomenon of transduction plays a very useful role in bacterial or microbial recombination of genes.

6.5 TECHNICAL TERMS:

Griffith's experiment, Genetic Recombination, Transformation, Conjugation, Transduction, Ammonification, Nitrification, Nitrogen fixation, Biopesticide.

6.6 SELF ASSESSMENT QUESTIONS:

1) Explain the different modes of genetic recombination in bacteria.
2) Narrate the economic importance of bacteria.

6.7 SUGGESTED READINGS:

1) Microbiology - Prescott, L.M., Harley, J.P., and Klein, D.A.
2) Biology of Microorganisms - Brock, T.D. and Madigan, M.T
3) Principles of Microbiology - Atlas, R.M

Prof. V. Umamaheswara Rao

LESSON - 7

GENERAL CHARACTERS OF ACTINOMYCETES, ARCHAEBACTERIA, MYCOPLASMAS AND CYANOBACTERIA

7.0 OBJECTIVE OF THE LESSON:

Students will be able to understand the general characteristic features of Actinomycetes, Archaebacteria, Mycoplasmas and Cyanobacteria along with their significance.

STRUCTURE OF THE LESSON:

7.1 INTRODUCTION:

Actinomycetes are the prokaryotic filamentous Gram positive bacteria that resembles with mycelium of the fungus and are also called as 'Ray fungi'. They are thin, aseptate and shows branching. In some species, filaments break and appear as small coccoid cells, which are non-motile. Young filaments show homogenous cytoplasm, but after maturity cytoplasm shows vacuoles, fat droplets, granules, etc. They play a crucial role in soil ecology and are significant in the production of antibiotics and other bioactive compounds. These actinobacteria are significant in medicine, veterinary sciences, ecology and biotechnology as they are genetically very diverse.

The group Archaea or archaeobacteria [Greek archaios, ancient, and bakterion, a small rod] are quite diverse in morphology, physiology, reproduction and ecology. Archaebacteria were placed in their own domain, Archaea, in the Three Domain Classification, which was first put forth by Carl Woese in 1977. They can stain either Gram positive or Gram negative and show a great variation in their shape. They can be aerobic, facultatively anaerobic, or strictly

anaerobic. They can grow in anaerobic, hypersaline, high temperature, and also cold environments. They constitute up to 34% of the prokaryotic biomass in coastal Antarctic surface waters. The cell wall composition, membrane lipids, tRNA, ribosomes, elongation factors, metabolism of archaea differ from other eubacteria and eukaryotes.

Cyanobacteria are also called as Blue-green algae. These are prokaryotic autotrophs which are unicellular, filamentous as well as colonial forms that are pigmented and generally live in aquatic habitats. In blue green algae, there are both motile and non-motile forms, which are both aquatic as well as terrestrial forms. In aquatic environments, Blue green algae are the main cause of blooms and scums, which have negative effect on water quality (due to the release of algal toxins) in terms of taste, odour and also cause foaming. These organisms can be free living or attached to the substratum. These are one of the primitive organisms to possess two types of photosystems which use photosynthesis to synthesize food and release oxygen into the environment.

7.2 ACTINOMYETES:

General Characters:

Though all actinobacteria are Gram positive, they exhibit a unique morphology that distinguishes them from other bacteria. Long filamentous structures with branching are formed which are called hyphae and an aggregate of such hyphae forms mycelium. The cell wall of actinomycetes is composed of Peptidoglycan as in Gram positive bacteria, but it also contains mycolic acids which help them resist certain antibiotics. In most of the actinomycetes, spores (conidia) are produced. These spores can withstand stress in the environment around them, thus supports them in their survival and the dispersal of spores.

Physiology and Metabolism:

The metabolic abilities of actinomycetes are very diverse. This ability is the key for them to survive in a variety of conditions. Actinomycetes are generally aerobes, which need oxygen for growth, but there are some anaerobic actinomycetes too. **They are able to degrade** like chitin, cellulose and other complex organic chemical compounds. Thus, they have great role in decomposition of complex substances in the soil. Actinomycetes can withstand and grow in a range of temperatures like from 20^{o} C - 60° C. The Hydrogen ion concentration (pH) levels actinomycetes can grow at range from pH4 - pH10, this feature enables them to colonize and live in varied habitats.

Cell Structure:

The cell structure in actinomycetes is very diverse. They appear in many forms, which include cocci, small rods, long rods, and some exhibit filament or hyphae like cell structures (Fig. 7.1). Their cell organelles are very similar to that of bacteria, but have certain distinct features in their cell wall, cell membrane and other fungi like special structures called as sporangia, that bear spores until they mature and disperse.

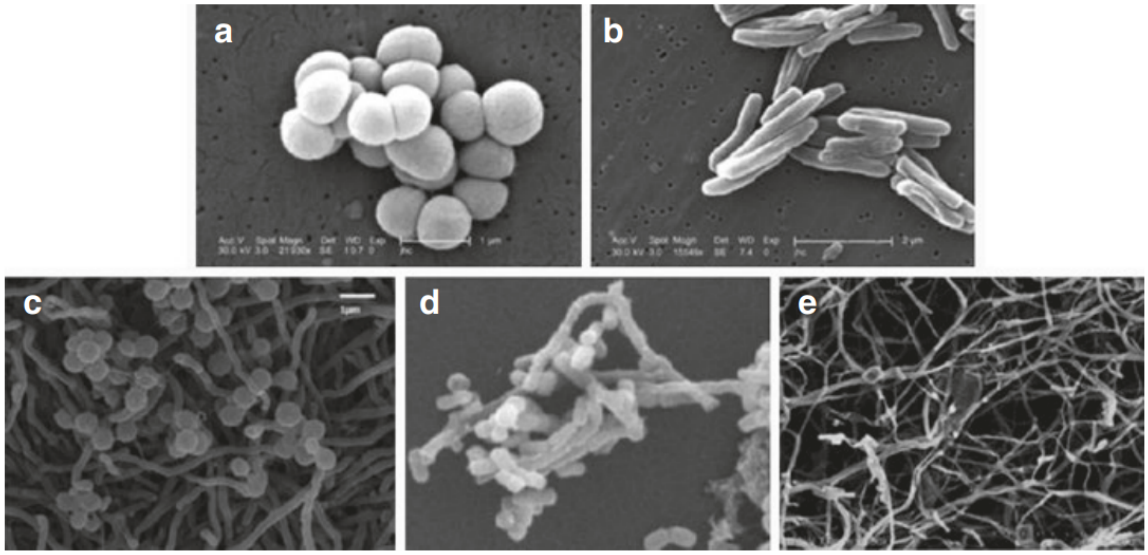


Figure-7.1: Scanning Electron Microscope images of some actinomycetes

(a) cocci of *Micrococcus luteus* (b) rods of *Mycobacterium tuberculosis* (c) branched hyphae of *Micromonospora schwarzwaldensis* (d) fragmenting mycelia of *Nocardia asteroides* and (e) branched aerial hyphae of *Streptomyces mangrovisoli*.

The actinomycetes exhibit similarity with bacteria in terms of cell organelles; on the other hand, they show some specialized structures like spores which have some similarity with fungi. However, they exhibit some unique characters that are not seen in eubacteria as well as in fungi.

Components Present Outside Cytoplasm:

The differentiating features of cell wall and cell membrane of *Streptomyces, Corynebacterium* and *Mycobacterium* are given in Fig. 7.2. In *Streptomyces*, as in other Gram-positive bacteria, the cell wall consists of a peptidoglycan layer that covers the cytoplasmic membrane. *Corynebacterium* and *Mycobacterium* contain more complex cell walls including an additional layer of arabinogalactan and an outer membrane, which is composed of mycolic acids, trehalose monomycolate, trehalose dimycolate and free lipids. The mycobacterial cell wall has a high proportion of covalently attached mycolic acid residues, which constitute a permeability barrier contributing to antibiotic resistance and

pathogenicity. Phosphatidylinositol-mannosides are also present. In other actinobacterial genera, wall teichoic acids are covalently attached to peptidoglycan. Although, lipoteichoic acid is suggested to be available merely in *Firmicutes*, they are reported in two actinobacterial genera, namely, *Agromyces* and *Streptomyces*, as well as in *Thermobifida fusca* which was suggested to have roles in the maintaining the homeostasis associated with cell envelope. Another major compartment of actinobacterial cell wall is teichuronic acids, the heteropolymeric polysaccharides composed of an uronic acid along with amino sugars and neutral monosaccharides which are linked to a polymer of either amino acids or glycerol phosphates. These are of common cell wall components of actinobacteria such as *Propionibacterium, Corynebacterium, Catellatospora, Actinoplanes, Streptomyces,* and *Kribbella*.

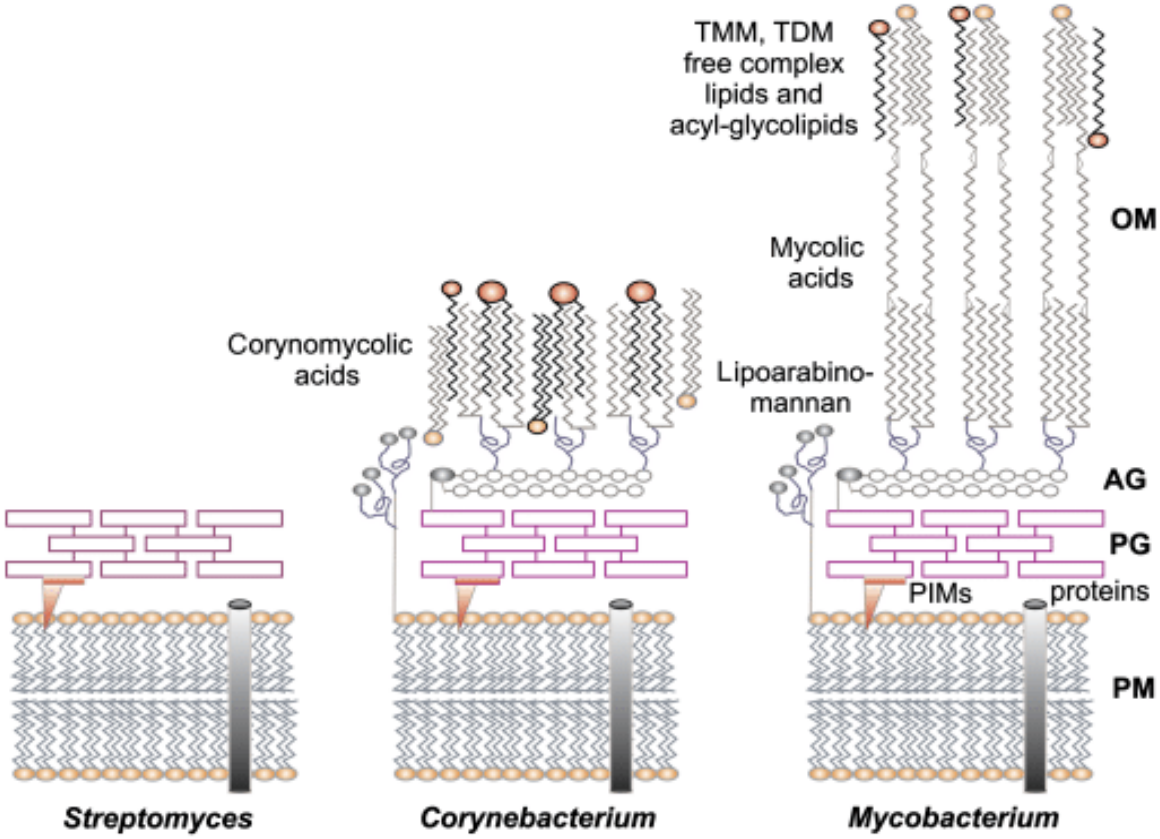


Figure-7.2: Structures outside the cytoplasm of *Streptomyces, Corynebacterium* and *Mycobacterium*: PG- Peptidoglycan, AG – arabinogalactan, OM- Outer membrane, PM- Plasma membrane, PIMs- phosphatidylinositol-mannosides, TMM- trehalose monomycolate, TDM- trehalose dimycolate

Spores:

One of the morphological features that distinguish the actinomycetes from eubacteria is sporulation, forming powdery texture and different coloured spores. They exhibit great diversity in terms of their size, shape and colours. Generally, the shape of actinobacterial

spores is often spherical, although other shapes may be observed such as cuboid in *Chainia*, oval in *Actinomadura*, or claviform spores of *Dactylosporangium*. Moreover, mature spores usually show a variety of colours such as white, pink, grey, blue, and so on. Sporangia, the bag-like structures for the development and release of spores, also vary vastly on the basis of shape and size.

Sporangia:

These are the bag-like structures for the development and release of spores, also vary vastly on the basis of shape and size. They are formed whether on substrate or aerial mycelium and can be globose (*Spirillospora*, *Streptosporangium*), cylindrical (*Planomonospora*, *Planobispora*), claviform (*Dactylosporangium*), and in other shapes while they are 2–50 μm with most of them being 10 μm in size. Just like the spores, sporangia have different types based on the number of spores. Sporangia with few spores may be called oligosporous sporangia while polysporous ones contain numerous spores as the name denotes. Most of the actinobacterial members forming sporangium produce planospores although exceptions exist as for *Stretosporangium* and *Kutzneria*. Morphological sporangia of different actinomycetes are given in figure 7.3.

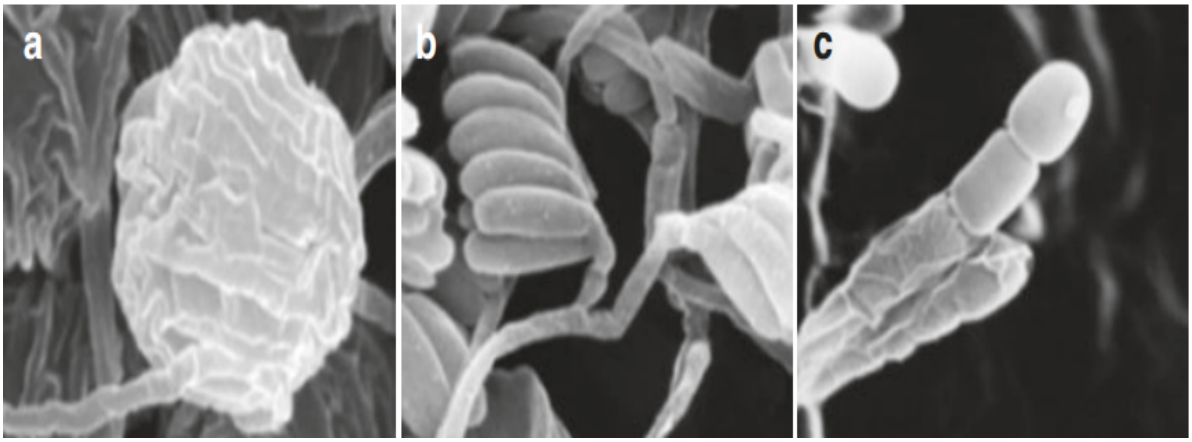


Figure-7.3: Morphology of Sporangia in some actinobacteria.

(a) *Spirillospora albida*

(b) *Planomonospora parontospora*

(c) *Dactylosporangium fulvum*

Reproduction:

Reproduction in actinomycetes is carried out by asexual mode. Sexual reproduction is not seen in them.

Asexual Methods:

Fragmentation: The mycelium can fragment into smaller pieces, each capable of growing into a new organism.

Spore formation: Aerial hyphae produce spores that can be dispersed by wind or water, leading to the establishment of new colonies.

Classification:

The classification of actinomycetes is detailed in Bergey's Manual of Determinative Bacteriology, which provides a systematic approach to identifying and categorizing these microorganisms. The classification of actinomycetes is essential for understanding their ecological roles, potential applications in biotechnology, and their significance in medicine. All the actinomycetes are categorized into one class, which were divided into 5 subclasses. Further they are divided into 6 orders, which are Actinomycetales, Rifidobacteriales, Acidinderobiales, Cariobactenales, Sphaerobacterales and Rabrobacterales. They have their suborders as well. Altogether, they were divided into 44 families and their respective genera.

Within these families, actinomycetes can be further classified into various genera and species, based on their morphological, physiological, and biochemical characteristics. Some key features used for classification include filamentous growth patterns, spore formation, cell wall composition, metabolic capabilities and antibiotic production. Some of the important genera include, *Actinomyces, Streptomyces, Corynebacterium, Micrococcus, Propionibacterium,* etc. The largest genus among all actinobacteria is *Streptomyces*, which include about 150 species.

Significance of Actinobacteria:

Soil Health and Fertility: They contribute to soil fertility by decomposing dead and decaying organic matter, which helps in recycling of nutrients. This makes nutrients available for the plants.

Plant Growth Promotion: Some species form symbiotic relationships with plants, enhancing nutrient uptake and growth, as they also can produce different secondary metabolites that protect the plants from different fungal and bacterial infections.

Antibiotic Production: Actinomycetes, particularly the genus *Streptomyces*, are known for the production of a wide range of antibiotics, including streptomycin and tetracycline, which are crucial in medicine.

Pharmaceuticals: They are a primary source not only for natural antibiotics, but also for anticancer compounds and other bioactive compounds that are used in medicine.

Biotechnology: Actinomycetes are utilized in the production of enzymes, biofuels, bioactive metabolites, and other bioproducts.

Thus, actinomycetes are a diverse and ecologically important group of bacteria with unique morphological and physiological characteristics. Their ability to decompose organic matter, produce antibiotics, and form beneficial relationships with plants highlights their significance in both natural ecosystems and human applications. Understanding their biology and ecology is essential for harnessing their potential in medicine, agriculture, and biotechnology.

7.3 ARCHAEBACTERIA:

General Characters:

Habitat:

Initially, most of the Archaea were isolated from extreme conditions like acidic, hypersaline and thermophilic environments, so they were called as extremophiles (organisms that love to grow in extreme conditions). Archaea inhabit a wide range of environments, including extreme conditions that were once thought to be sterile. These conditions often feature high temperatures exceeding 100°C, such as those found in geothermal springs, hydrothermal vents on the ocean floor, black smokers, oil wells, and extremely acidic or basic lakes, as well as hypersaline areas like the Dead Sea. Hyperthermophilic archaea, such as *Methanopyrus kandleri*, thrive at temperatures of 122º C, marking the highest tolerance for any known living organism. Some archaea inhabit highly acidic conditions, like *Picrophilus torridus*, which exists at nearly pH 0, while others are found in cold oceanic environments, including polar seas. Now-a-days, they are found everywhere like in soil, water surface, on humans, etc.

Cell Structure:

The cells of archaea may be spherical, rod-shaped, spiral, lobed, plate-shaped, and irregularly shaped or pleomorphic. Some are single cells, whereas others form filaments or aggregates. They range in diameter from 0.1 to over 15μm, and some filaments can grow up to 200 μm in length. Generally, the rod shaped archaea measures about 1 to 2μm width by 1 to 5μm length; cocci measure about 1 to 3μm in diameter. Some archaea show chain like arrangement of cells, some cocci exhibit clusters (Fig. 7.4a), some rod shaped cells are curved, some are spiral shaped and some show branching also (Fig. 7.4b). Pleomorphism is also observed in Archaea. It is to be noted that there are extremely small and extremely large archaea. Symbiotic nanoarchaeotes (0.2μm in diameter) are smaller and live as symbionts that require host cells for nutrition. On the other hand, there exists a giant archaeon that is long and made of filaments up to 30 mm in length. The shapes of archaea differ widely from

cocci, rods, to branched rods. The importance of shapes in archaea is to increase the surface area to volume ratio, thereby increasing the nutrient uptake efficiently by easy diffusion of molecules.

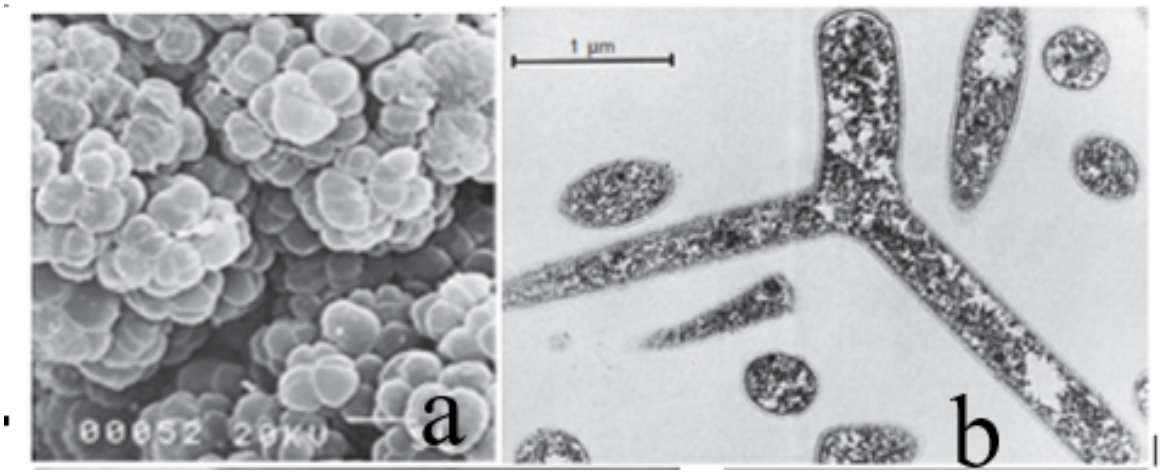


Figure-7.4: Morphological features of some archaea

a) Clusters form of *Methanosarcina mazei*, b) Branched form of *Thermoproteus tenax*,
(Courtesy: Prescott's Microbiology 12th Edition)

Pili (sing. Pilus): It was observed that archaeal pili were made of modified form of pilin proteins, which are present in bacteria.

Flagella (sing. Flagellum): Both the bacterial as well as archaeal flagellum help in locomotion of the cell, but there is a marked difference in the mechanism of the motility. Proton motive force (PMF) is used by bacterial flagella, on the other hand, Adenosine tri-phosphate (ATP) is utilized by archaeal flagella, due to which, the term 'Archaellum' was proposed for archaeal flagellum. Moreover, the proteins that are composed in archaeal flagellum are not exactly similar to the flagellin proteins of eubacteria.

Cell Wall:

Although archaea can stain either Gram positive or Gram negative, their cell wall structure and chemistry differ from that of eubacteria. There is a considerable variation in archaeobacterial cell wall structure. Many Gram positive archaeobacteria have a wall with a single thick homogenous layer like Gram positive eubacteria. Gram negative archaeobacteria lack the outer membrane and complex peptidoglycan network or saccules of Gram negative eubacteria. Instead, they usually have a surface layer of protein or glycoprotein subunits. The cell wall chemistry of archaea is also quite different from that of the eubacteria. The muramic acid and D-amino acids, characteristic of eubactrial peptidoglycan, are absent in archaea. But all the archaea resist the attack by lysozyme and β-lactam antibiotics such as pencillin. Gram positive arachaea can have a variety of complex polymers in their walls.

Methanobacterium and some other methanogens have walls containing 'pseudomurein' (Fig. 7.5), a peptidoglycan-like polymer that has L-amino acids in its cross-links, N-acetyltalosaminuronic acid instead of N-acetylmuramic acid, and β-(1-3) glycosidic bonds instead of β-(1-4) glycosidic bonds. *Methanosarcina* and *Halococcus* contain complex polysaccharides similar to the chondroitin sulphate of animal connective tissue.

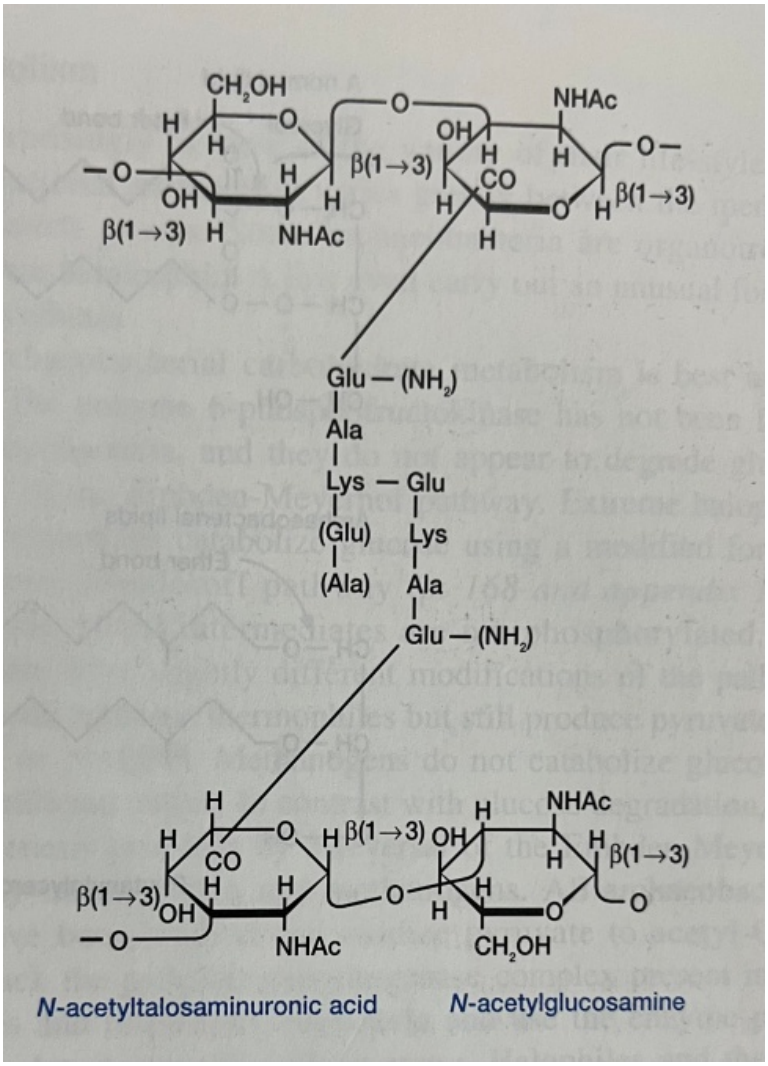


Figure-7.5: Structure of Pseudomurein
(Source: Microbiology - Prescott et. al.)

Gram negative archaea have a layer of protein or glycoprotein outside their plasma membrane. The layer may be as thick as 20 to 40 nm. Sometimes there are two layers, a sheath surrounding an electron-dense layer. Some methanogens (*Methanolobus*), *Halobacterium*, and several other extreme thermophiles (*Sulfolobus*, *Thermoproteus*, and *Pyrodictium*) have glycoproteins in their walls. In contrast, other methanogens (*Methanococcus*, *Methanomicrobium* and *Methanogenium*) and the extreme thermophile, *Desulfurococcus* have protein walls.

Cell Membrane:

There are some distinct features of the plasma membrane which set archaea apart from other domains. An example of this is the chirality of the bond that connects the side chain and the glycerol part of the phospholipid head. Eukaryotes and eubacteria have the D-isomeric form, while archaea have the L-isomeric form. Another distinction is that there is a side chain-glycerol bond that is ether-linked, which is contrary to the ester-linked lipids in eubacteria and eukaryotes. Sometimes two glycerol groups are linked to form an extremely long tetraether. Usually, diether side chains are 20 carbons in size and the tetraether chains are of 40 carbos. Due to ether-linkage, the membrane exhibits more chemical stability. The other differences are related to the side chains which are unbranched fatty acids in bacteria and eukaryotes, while in archaea they are isoprenoid chains which may have some branching at the side chains. Polar lipids are also present in archaea membranes such as phospholipids, sulfolipids, and glycolipids. About 7 to 30% of the membrane lipids are nonpolar lipids, which are usually the derivatives of squalene. Of course, the archaeal membranes may contain a mix of diethers, tetraethers and other lipids. The detailed structure of archaeal phospholipid and comparison of membrane lipids of archaeal, eubacterial and eukaryotic cells are given in figures 7.6 and 7.7, respectively.

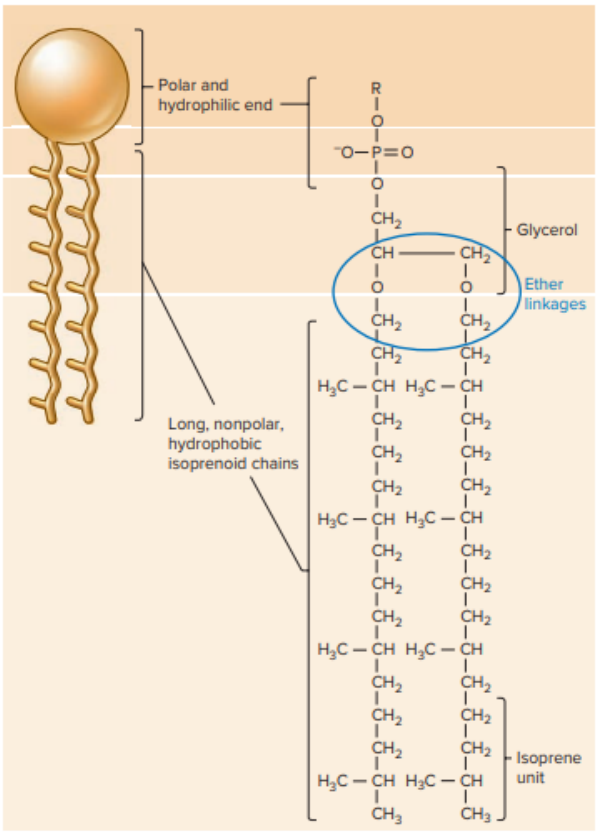


Figure-7.6: Detailed Structure Archaeal Phospholipid

(Source: Microbiology – Prescott et.al.)

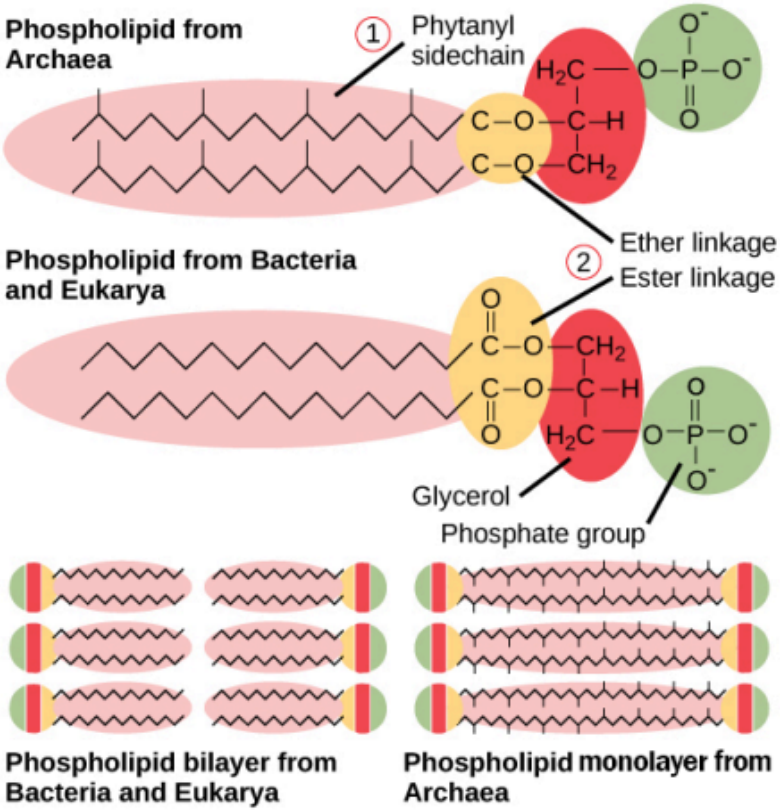


Figure-7.7: Comparison of membrane lipids of Archaea with Bacteria and Eukarya (Source: General Microbiology - Linda Bruslind)

Ribosomes:

Even though there are 70s ribosomes, the same as those of the bacteria, scientists were able to show that archaea must have a separate domain due to differences in rRNA nucleotides. In addition, the shape of archaeal ribosomes is different from that of bacterial ribosomes, and they possess proteins which are different to that of archaea. As a result, they are immune to the drugs like Chloramphenicol that act by blocking the action of bacterial ribosomes.

Genetics:

Some features of archaeobacterial genes are similar to those in eubacteria. Their chromosome is a single, closed and circular DNA. The genomes of some archaea are significantly smaller than the normal eubacterium. The variation in G + C content is great, from about 21 to 68 mol%, is another sign of archaeobacterial diversity. Archaeobacterial mRNA appears similar to that of eubacteria rather than to eukaryotic mRNA. Polygenic mRNA was found and there is no evidence of mRNA splicing. Unlike of eubacteria and eukaryotes, the TψC arm of archaeobacterial tRNA lacks thymine and contains pseudouridine or 1-methylpseudouridine. Some archaea, such as many methanogens in the kingdom Euryarchaeota , differ from other prokaryotes in having histone proteins that associate with DNA to form nucleosome-like

structures. The archaeobacterial DNA-dependent RNA polymerases resemble the eukaryotic enzymes but not that of eubacterial RNA polymerase.There are some unique genes in archaea that code for proteins which are found in them alone. The t-RNA and r-RNA gene sequences in archaea are very different from bacteria and other organisms. It was observed that few archaeal DNA has introns, which is a feature of eukaryotes. Apart from asexual reproduction, horizontal genetic transfer among archaea may occur. Moreover, the RNA polymerase in archaea is very similar to eukaryotes.

Metabolism:

In view of their variety of life-styles, archaeal metabolism varies greatly between the members of different groups. Archaeal species are efficient in methanogenesis (production of methane gas) which is unique to archaea which are called as methanogens. In addition to this, they are chemolithotrophs (use chemicals and grow on rocks), photoautotrophs (use sun light and carbondioxide to synthesize food), and hyperthermophiles (high temperature loving organisms). Because of the absence of the enzyme 6-phosphofructokinase in archaea, they do not degrade glucose by the way of Embden-Meyerhof pathway. Extreme halophiles and thermophiles catabolize the glucose by a modified form of Entner-Doudoroff pathway. Halophiles and the extreme thermophile (*Thermoplasma*) have a functional tricarboxylic acid cycle. The synthetic pathways for amino acids, purines and pyrimidines are similar to those in other organisms. Some methanogens can fix atmospheric dinitrogen. Autotrophy is widespread among the methanogens and extreme thermophiles, and CO_2 fixation occurs in more than one way. *Thermoproteus* and possibly *Sulfolobus* incorporate CO_2 by reductive tricarboxylic acid cycle and methanogenic bacteria and most extreme thermophiles incorporate CO_2 by reductive acetyl-CoA pathway.

Reproduction:

Reproduction in archaea is carried out asexually by various methods. They include: fragmentation, binary fission and budding, but there are no reports on formation of endospores as seen in bacteria. Mitosis is never found in archaea as there is no nuclear membrane; rather they reproduce by binary fission. In the process, archaeal DNA replicates by pulling apart of the both strands while cell grows in size. In few cases, the number of daughter chromosomes formed is more than two and pulled apart, this process is called as multiple fission. Unlike, bacterial origin of replication, multiple origins of replication is formed in archaeal chromosomes. In addition to this, DNA polymerases used by archaea are similar to counterpart enzymes of eukaryotes. On the other hand, FtsZ proteins that help in

cell division forms contractile ring around the cell. The septum constructed in the middle of the cell and its components resemble that of bacteria.

Classification:

Archaea are classified into different phyla namely Crenarchaeota, Euryarchaeota, Korarchaeota.

Crenarchaeota:

The designation Crenarchaeota signifies "scalloped archaea." These organisms frequently exhibit irregular morphological structures. All crenarchaeotes are characterized by the ability to synthesize an unique tetraether lipid, identified as crenarchaeol. Initially, this group included thermophilic and hyperthermophilic archaea, acidophiles and sulphur dependent. The sulphur may be used either as an electron acceptor in anaerobic respiration or as an electron source by lithotrophs. Almost all are strict anaerobes and grow in geothermally heated water of soils that contain elemental sulphur which are referred to as sulphur-rich hot springs or solfatara. This phylum consists three orders namely Igneococcales, Thermoproteales and Sulfolobales and at least 12 genera. The best studied genera of this crenarchaeota are *Thermoproteus* and *Sulfolobus*. Recent investigations have revealed that newly identified Crenarchaeota are inhibited by sulfur and exhibit growth at reduced temperatures. These organisms exhibit a Gram-negative staining characteristic and display considerable morphological diversity, including rod-shaped, coccoid, filamentous, and irregularly shaped cells. A well notable representative of the Crenarchaeota phylum is *Sulfolobus solfataricus*, which has been isolated from geothermally heated sulphuric springs located in Italy and exhibits optimal growth at a temperature of 80° C and a pH range of 2-4. Other examples include *Pyrolobus fumarii* and *Sulfolobus acidocaldarius*.

Euryarchaeota:

It is a well-recognized and the largest phylum of archaea containing diverse genera that can survive in extremely alkaline, extremely saline, and extreme thermophilic conditions. Several methanogens are also grouped under this phylum. Though initially Euryarchaeota were thought to be extremophiles only, several genera were isolated from a normal environment like water springs, soil, water, rhizosphere, intestines, etc. This phylum exhibits considerable diversity, encompassing seven distinct classes: Methanococci, Methanobacteria, Halobacteria, Thermoplasmata, Thermococci, Archaeglobi, and Methanopyri. This phylum comprises nine orders and fifteen families. Based on the habitat, these organisms can be categorized into the following groups: methanogens, extreme halophiles, sulfate reducers, and numerous extreme thermophiles characterized by sulfur-dependent metabolic processes.

Halophiles:

Halo signifies salt and "phil" denotes loving. Organisms classified as halophiles necessitate a saline environment for their sustenance. They inhabit salt lakes and regions where the evaporation of seawater occurs, such as the Great Salt Lake in the United States and the Dead Sea. These organisms are capable of thriving in aquatic environments with salinity levels surpassing 15%, whereas the ocean's salinity is approximately 4%. For example, *Halobacterium* is one of the halophilic archaea, which includes several species, found in salt lakes and high saline ocean environments. *Halobacterium salinarum*, *H. denitrificans* and *H. halobium* are found in The Great Salt Lake in Utah.

Methanogens:

These are the single-celled microorganisms that generate methane as by-product in environments with no presence of oxygen. They are strictly anaerobic and oxygen-sensitive organisms, which die when exposed to oxygen. They produce methane gas in oxygen-poor environments like swamps and marshes, by reducing carbon dioxide using hydrogen. Methanogens usually occur in oxygen-free conditions such as mud at the bottom of lakes and swamps, some animal intestines like cows and humans, and dead and decaying organic matter, etc. Methanogens are added to biogas reactors to produce methane gas for cooking and sewage treatment plants. Examples of methanogens include *Methanofollis aquaemaris*, *M. ethanolicus, M. formosanus* and *M. liminatans.*

Sulphur Reducing and Thermophilic Archaea:

In general, all the thermophilic organisms need heat to grow. In addition, some archaea need calcium or sulphur and few love to live in environments with alkaline pH.

Thermoacidophiles:

These are the archaea, that include both aerobic and anaerobic species. They love to thrive in both acidic and hot environments.

Hyperthermophilic Archaea:

These are the unique archaea, which loves to grow in extreme high temperatures. Eg: *Pyrolobus fumarii*, which holds the record of growing in the highest temperature of about 113^{o}C.

Korarcheota (also called as Xenarchaeota):

The greek work ‘Kore’ or ‘Koros’ means ‘young man’ or ‘young woman’, which was used to name this group of archaea. In addition, the greek adjective ‘archaios’ means ancient.

These are thermophilic, acidophilic and alkanophilic. This group of archaea have only found in hydrothermal environments ranging from terrestrial, including hot springs to marine, including shallow hydrothermal vents and deep-sea hydrothermal vents. However, the greater diversity of Korarchaea found in terrestrial hot springs compared to marine environments. They were found in high temperature hydrothermal environments which can survive up to 128^{o}C. *Korarchaeum cryptofilum* is the organism of this group that was isolated from Yellowstone National Park, which has pH of about 5.7 to 7.0. This group of archaea can tolerate alkalinity up to 11pH. Korarchaeota organism is an obligate anaerobe and grows heterotrophically using peptide and amino acid degradation pathways. The genome of Korarchaeota contains genes for dissimilatory sulphur metabolism in addition to anaerobic methane metabolism.

Significance:

They are microorganisms that are very diverse, found everywhere on earth, especially withstanding extreme environments, play very important roles in the nature.

Methanogenesis:

Production of methane gas is one of the significant things that was explored for the welfare of humans these days. In general, these archaea are the only cause of natural production of methane gas in the nature.

Nutrient Recycling:

Due to the ability of archaeal cells to feed on chemicals or rocks, synthesize food using light and carbondioxide, they are able to survive in many harsh environments or extreme climatic conditions. Moreover, as they have diverse and more complex biochemistry and metabolism, they can decompose (breakdown) and utilise diverse forms of inorganic and organic compounds, which helps the nature in nutrient recycling and in establishing of an ecosystem with diversity.

Biogeochemical Cycling:

Biogeochemical cycles are very important in the nature for the sustenance of living organisms. As archaea are found everywhere, even in the extreme conditions, they take part actively in all biogeochemical cycles like Carbon, Sulphur, Nitrogen and other cycles.

Symbiotic Relationship:

Archaea are symbiotically associated with plant roots for nutrition and space and return some nutrients to plants. They are also associated symbiotically with coral reefs. Some archaea are found to be associated with the gut of animals.

Marine Ecosystem:

Twenty percent of marine microbes are archaea and play a significant role in the production of organic matter, decomposition, nutrient recycling and maintenance of marine ecosystems.

7.4 MYCOPLASMAS:

Mycoplasmas (Gk. Myces = fungus; plasma = form) are one of the smallest, simplest, pleomorphic, cell wall less, and Gram negative bacteria. Previously, this group of bacteria is called as Pleuroneumonis-like Organisms (PPLOs). Now, a new term 'Mollicutes' was introduced and also called as Mollicute-like Organisms (MLOs). Most species require sterols and fatty acids for their growth. Mostly these are facultatively anaerobic, but few are obligate anaerobes. Under favourable conditions, most species form colonies that show a characteristic 'fried egg' appearance on solid medium. They are parasites, commensals or saprophytes, but also pathogenic to humans, animals, plants and insects. Historically, all the wall less bacteria are placed in a single genus, *Mycoplasma*. Later on, class Mollicutes (L. mollis = soft; cutes = skin) was established for all the bacteria which are unable to synthesize peptidoglycan cell wall around them. The latest taxonomy of Mollicutes is –

Domain – Bacteria

Phylum – Mycoplasmatota (previously Tenericutes)

Class – Mollicutes

Orders – 1) Mycoplasmatales

Family – Mycoplasmataceae

Genera: *Mycoplasma*, *Ureaplasma*

2) Acholeplasmatales

Family – Acholeplamataceae

Genus: *Acholeplasma*

3) Entomoplasmatales

Family – Spiroplamataceae

Genus: *Spiroplasma*

4) Anaeroplamatales

Family – Anaeroplasmataceae

Genera: *Anaeroplasma*, *Thermoplasma*

Characteristic Features:

Structurally, the *Mycoplasma* species are pleomorphic, mostly coccoid or oval but can also form elongated, or branched filamentous mycelioid structures. *Mycoplasma* is a Gram negative bacterium devoid of peptidoglycan cell wall. Measures about 0.3 µm in diameter in coccoid forms and up to 100 µm long and 0.4 µm thick in filamentous forms. A triple layered unit membrane with 7.5 – 11.0 nm thickness surrounds the cytoplasm. The cell contains proteins, lipids, carbohydrates, RNA, DNA, 70S ribosomes, and a very small genome (0.6 to 1.35 mega base pairs and around 600 genes, low G+C content of 18-40 mol%). The proteins, glycolipids and lipoglycans exposed on the cell surface function as major antigenic determinants.

The *Mycoplasma* cells exhibit distinct and differential terminal organelles that functions in adherence to the host respiratory epithelium, gliding motility and cell division. *M. pneumonia* and *M. genitalium* with such organelles exhibit gliding movement on liquid covered surfaces. The duplication of this terminal structure is thought to precede cell division. However, the movement is spinning type in spherical forms. *Mycoplasma* lack many enzymatic activities and metabolic pathways due to the minimal genome and proteome. Their nutritional requirements are very complex and usually dependent on obligate parasitic mode of life. They are difficult to cultivate in laboratories, and their complex media requires serum which provides fatty acids and cholesterol for their membrane synthesis, and they grow very slowly in cultures. *Mycoplasma* colonies often exhibit a 'fried egg' appearance on culture plates, characterized by a central, dense, and sunken zone embedded in the agar, surrounded by a lighter, more translucent peripheral zone on the surface, which stands as a key feature in identification of *Mycoplasma* species.

Metabolism:

All the *Mycoplasma* species can synthesize DNA, RNA, proteins and lipids, but doubtful in amino acid synthesis. The sterol requiring species can incorporate them, particularly cholesterol, up to 65% in their membranes. The parasitic *Mycoplasma* possesses a truncated flavin-terminated respiratory system lacking quinones and cytochromes. Oxidative phosphorylation, as an ATP generating mechanism is not operational and the ATPs are generated through the breakdown of arginine by arginine dihydrolase pathway based on substrate level phosphorylation. All the fermentative *Mycoplasma* utilizes glucose and other carbohydrates as energy source through Embden-Meyerhof-Parnas glycolytic path way.

Reproduction:

The information on the types and mechanism of reproduction in *Mycoplasma* is very little, due to its pleomorphic nature. However, *Mycoplasma* is known to reproduce by three different methods, through elementary bodies, budding and binary fission.

Elementary Bodies:

Mycoplasma cell elongates to form filaments and divide unevenly into very minute bodies known as 'Elementary bodies'. These elementary bodies are produced in chains like that of conidia in fungi and are usually formed inside of larger bodies and are dispersed after the rupture of the membrane. The elementary bodies are nearly spherical and 80-200 nm in diameter. Depending upon culture conditions and the species, their generation time ranges from 1-3 hours.

Budding:

In many electron micrographs, bud like structures have been reported on the *Mycoplasma* cells, but there is no conclusive evidence for this mode of reproduction.

Binary Fission:

This type of reproduction is best studied in *M. gallicepticum* and *M. orale*. The binary fission starts with the duplication of the terminal organelle and then the replication of the DNA. The replication bubble separates the DNA double strands. Each strand acts as template for the synthesis of a daughter strand by semi-conservative replication, until the entire DNA is duplicated and cytokinesis takes place later.

Mycoplasma Diseases:

Mycoplasma species cause many diseases in humans and animals. Most of the Mycoplasma species are resistant to the antibiotics such as penicillin and sulphonamide that acts on peptidoglycan cell wall. They are sensitive to tetracycline, azithromycin, and roxithromycin which interfere with metabolic pathways. Some important diseases caused by Mycoplasma species include: Genital infections (*M. genitalium*), Atypical pneumonia (*M. pneumonia*), Rheumatoid arthritis (*M. fermentans*), Still birth and spontaneous abortions (*M. hominis*). The animal diseases caused by *Mycoplasma* include: Chronic respiratory disease of chicken (*Mycoplasma* sp.), Contagious agalactia of sheep and goat (*M. agalactias*), Enzootic pneumonia of pig (*M. suipneumoniae*), and Contagious bovine pleuropneumonia (*M. mycoides* sub. sp. *mycoides*).

The class Mollicutes has five orders and six families. The best studied genera are found in the orders Mycoplasmatales *(Mycoplasma, Ureaplasma),* Entomoplasmatales *(Entomoplasma, Mesoplasma, Spiroplasma),* Acholeplasmatales *(Acholeplasma),* and Anaeroplasmatales *(Anaeroplasma, Asteroleplasma).*

Members of the class Mollicutes are commonly called mycoplasmas. Although they evolved from ancestors with Gram positive cell walls, they now lack cell walls and cannot synthesize peptidoglycan precursors. Thus they are penicillin resistant but susceptible to lysis by

osmotic shock and detergent treatment. Because they are bounded only by a plasma membrane, these prokaryotes are pleomorphic and vary in shape from spherical or pear-shaped organisms, about 0.3 to 0.8 µm in diameter, to branched or helical filaments. Some mycoplasmas (e.g., *M. genitalium*) have a specialized terminal structure that projects from the cell and gives them a flask or pear shape. This structure aids in attachment to eukaryotic cells. They are among the smallest bacteria capable of self-reproduction. Although most are non-motile, some can glide along liquid-covered surfaces. Most species differ from the vast majority of bacteria in requiring sterols for growth, which are incorporated into the plasma membrane. Here sterols may facilitate osmotic stability. Most are facultative anaerobes, but a few are obligate anaerobes. When growing on agar, most species form colonies with a "fried-egg" appearance because they grow into the agar surface at the center while spreading outward on the surface at the colony edges (Figure-7.8). Their genomes are among the smallest found in prokaryotes, ranging from 0.7 to 1.7 Mb; the G-C content ranges from 23 to 41%. The complete genomes of the human pathogens *Mycoplasma genitalium, M. pneumoniae,* and *Ureaplasma urealyticum* have been sequenced. These genomes are characteristically small with less than 1,000 genes; it seems that not many genes are required to sustain a free-living existence. Mycoplasmas can be saprophytes, commensals, or parasites, and many are pathogens of plants, animals, or insects.

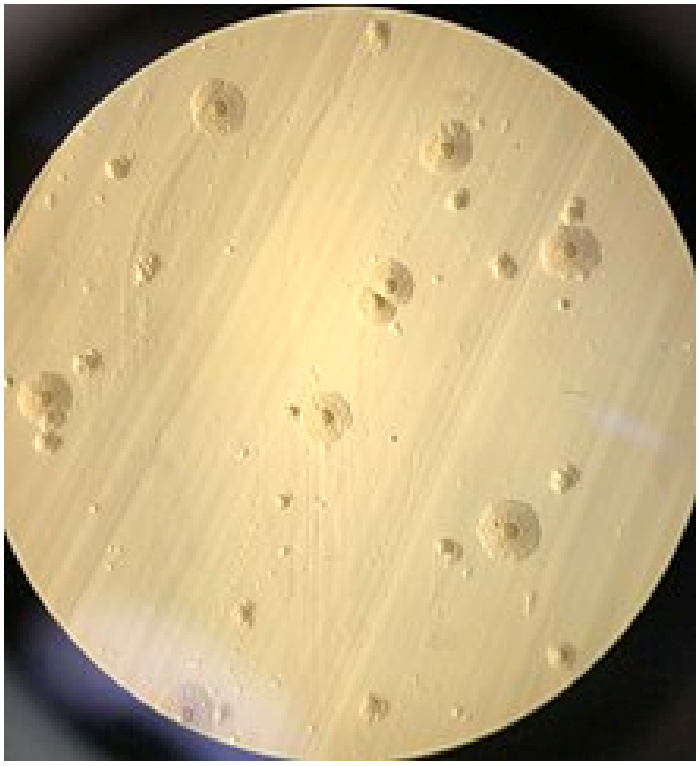


Figure-7.8: Fried egg appearance of *Mycoplasma* colonies on Hay flick agar medium

Due to the lack of a rigid cell wall, *Mycoplasma* species can contort into a broad range of shapes, from round to oblong. So, they are identified as pleomorphic organisms and therefore cannot be identified as rods, cocci or spirals. The plasma membrane forms the outer boundary layer of the cell. They also lack the membrane bound nucleus and other membrane bound organelles. The genetic material is a single duplex DNA which is naked and the ribosomes are of 70S type. The molecular structure of the *Mycoplasma* cell is given in Fig. 7.9. Majority of the *Mycoplasma* species are parasitic in nature due to their inability to synthesize the required growth factors. Over 100 species have been included in the genus *Mycoplasma* are parasites or commensals of humans, animals, and plants. *Mycoplasma* species are among the smallest free-living organisms (about 0.2 - 0.3 μm in diameter). They have been found in the pleural cavities of cattle suffering from pleuropneumonia. These organisms are often called MLOs (mycoplasma-like organisms) or, formerly, PPLOs (pleuropneumonia-like organisms).

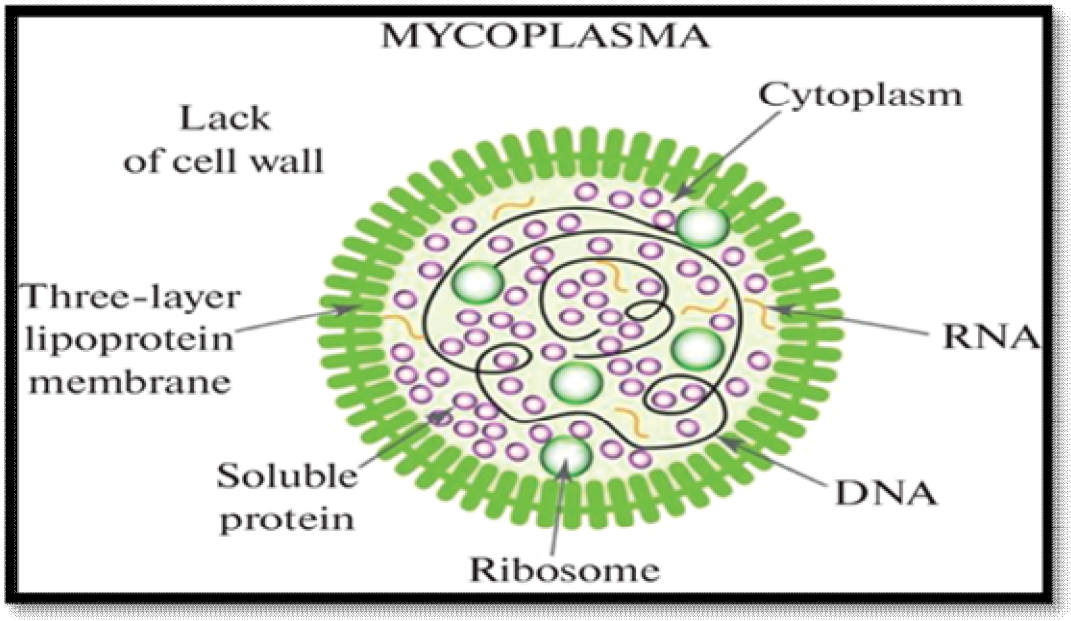


Figure-7.9: Molecular structure of *Mycoplasma*

Metabolically, the mycoplasmas are incapable of synthesizing a number of macromolecules. In addition to requiring sterols, they also need fatty acids, vitamins, amino acids, purines, and pyrimidines. Some may produce ATP by the Embden-Meyerhof pathway and lactic acid fermentation. Others catabolize arginine or urea to generate ATP. The pentose phosphate pathway seems to be functional in at least some mycoplasmas; none appear to have the complete tricarboxylic acid cycle.

Mycoplasmas are remarkably widespread and can be isolated from animals, plants, the soil, and even compost piles. Although their complex growth requirements can make their growth in pure (axenic) cultures difficult, about 10% of the mammalian cell cultures in use are

probably contaminated with mycoplasmas. This seriously interferes with tissue culture experiments. In animals, mycoplasmas colonize mucous membranes and joints and often are associated with diseases of the respiratory and urogenital tracts. Mycoplasmas cause several major diseases in livestock, for example, contagious bovine pleuropneumonia in cattle (*M. mycoides*), chronic respiratory disease in chickens (*M. gallisepticum*), and pneumonia in swine (*M. hyopneumoniae*). *M. pneumonia* causes primary atypical pneumonia in humans. *Ureaplasma urealyticum* is commonly found in the human urogenital tract. It is now known to be associated with premature delivery of newborns, as well as neonatal meningitis and pneumonia. Spiroplasmas have been isolated from insects, ticks, and a variety of plants. They cause disease in citrus plants, cabbage, broccoli, corn, honey bees, and other hosts. Arthropods may often act as vectors and carry the spiroplasmas between plants. It is likely that more pathogenic mollicutes will be discovered with improvement in techniques for their isolation and detection.

7.5 CYANOBACTERIA:

General Characters:

Cyanobacteria are cosmopolitan, which can grow everywhere. Their habitat ranges from water bodies to volcanoes. Some members grow on moist rocks, soil and in deserts also. Most of the members of cyanobacteria grow along with algae in aquatic environments. They include both marine and fresh water cyanobacteria. They are found in all water bodies like seas, rivers, ponds, lakes and even in water storage tanks. Also they grow on snow or hot springs. Some members grow in acidic or alkaline environments also. They contribute about 40 percent of global primary food production along with algae. Furthermore, they grow as endophytes and also in endosymbiotic association with animals. They also live as constituents of lichens.

Thallus Organization:

The Blue green algae don’t have leaves, roots, stems or branches. Instead, they have a structure called ‘Thallus’ (Pl. thalli). These organisms have a range of diversity from unicellular to multicellular and filamentous forms (Fig.7.10). Unicellular forms can either be single cells or colonies. These single cells can either be free living or attached. Multi cellular or filamentous forms can either be uniseriate trichome or multiseriate trichome. Some blue-green algae show true branching, while others show false branching. The cell protoplast is clearly visible as two parts. The peripheral pigmented region called as chromoplasm and a central colourless region, called as centroplasm. Non-filamentous forms are mainly coccoid forms, with spherical, cylindrical or fusiform shapes. Colonies are made by many cells due to repeated divisions and the colonies are enveloped by mucilaginous sheath. Filamentous forms

are formed by long series of cells present one over the other forming a long trichome. Trichome secretes mucilaginous sheath with different consistency, either firm or flexible sheath. Trichome can be either straight, or somewhat bent. In some species, whole trichome is spirally coiled. Cells in trichomes are either uniform, called as homocystous or interrupted at some intervals by heterocysts (special thick walled cells). Morphological structures of some cyanobacteria are given in Fig. 7.11.

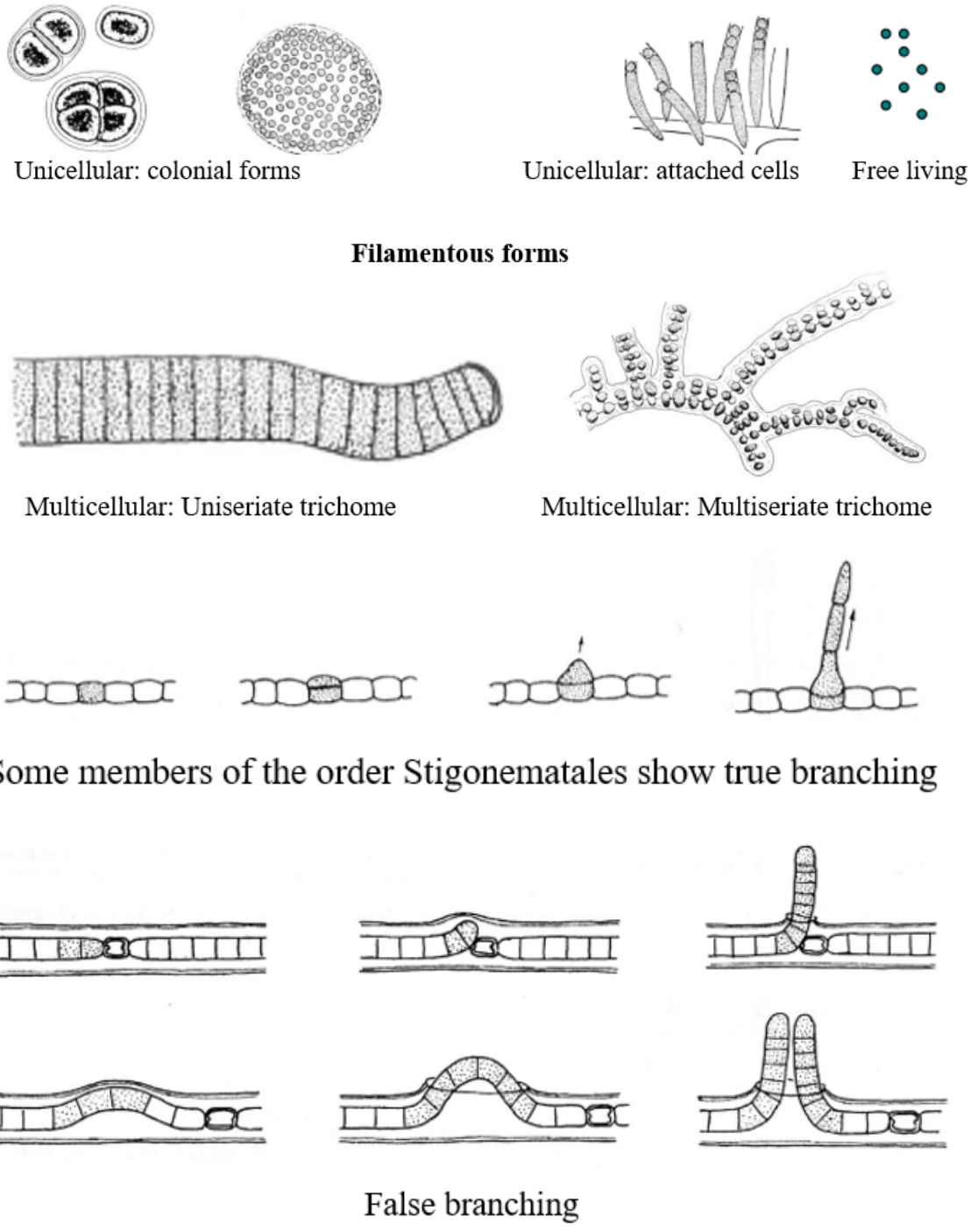


Figure-7.10: Structural diversity in cyanobacteria

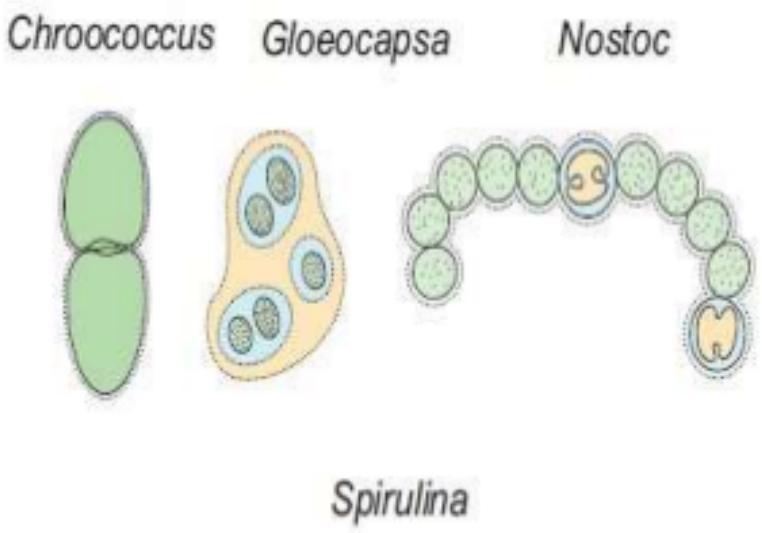


Figure-7.11: Morphological structures of some cyanobateria

Cell Structure:

As prokaryotes, blue-green algae, lack organelles like Nucleus, Mitochondria, Chloroplasts, Golgi apparatus, and Endoplasmic reticulum. Glycogen granules, Phycobilisomes, Cyanophycin, Nucleoid, Cell wall, Plasma membrane, Gas vesicles, Ribosomes, Carboxysomes, Lipid granules, Polyphosphate granules and Thylakoids are present (Fig.7.12).

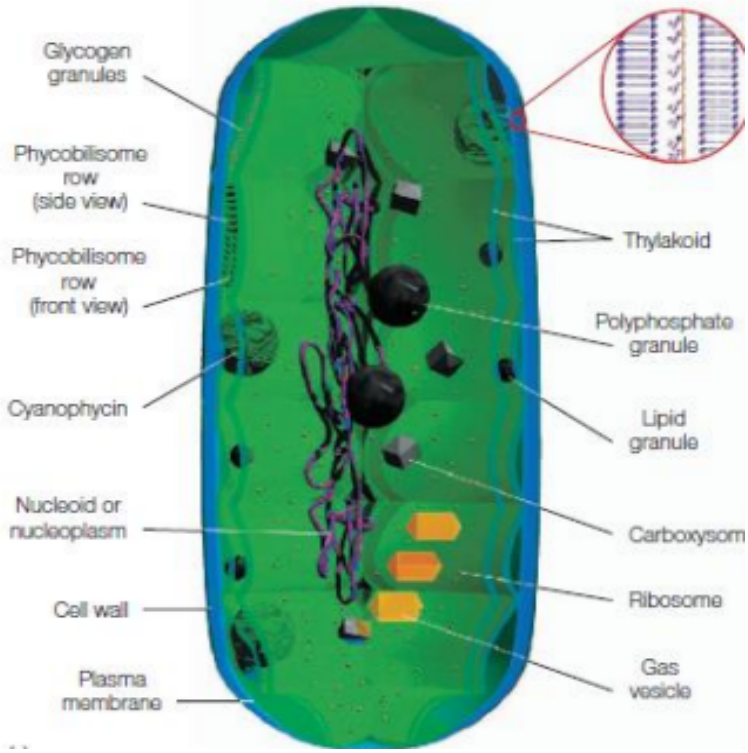


Figure-7.12: Cyanobacterial Cell Structure
(Source: Hartwell T. Crim 1998.)

Cell Wall: The cell wall is Gram negative and consists of murein (peptidoglycan). The cells are embedded in sheath of mucilage. Polypeptides and polysaccharides are present as a thick layer outside the cell wall, which form a brownish pigmented sheath called as 'scytonemin'. This sheath helps the cell in absorbing ultraviolet radiation.

Plasma Membrane: The plasma membrane or cell membrane is selectively permeable as in most living organisms and encloses the cytoplasm. It is composed of lipids and proteins.

Cytoplasm: Cytoplasm lies just beneath the plasma membrane which has some structures with different functions. At the periphery, some structures are located, called as lamellae that have pigments, which are not organised as plastids. These membranes or lamellae are plasma membrane derived, which contain pigments like Xanthophylls, Chlorophylls, c-phycocyanin, c-erythrocyanin and Carotenoids. Presence of c-phycocyanin and c-erythrocyanin is the characteristic feature of cyanobacterial members only. Cytoplasm also shows some membrane bound vesicles, sometimes are present in layers which are stacked vertically. Ribosomes are also found in the cytoplasm, which are scattered everywhere.

Nucleoplasm (Nuclear Material):

The DNA containing region is called as nucleoid, as in bacteria. There is no distinct nuclear membrane, but nuclear material is located at the centre of the cell. Nucleolus is absent and no spindle fibres are formed during cell division, which also makes them different from eukaryotes.

Heterocyst:

Heterocyst is a thick-walled cell that is larger than the adjacent cells, which help in fixing atmospheric nitrogen. It is also called as diazotroph. Heterocysts are photosynthetically inactive as they lack photosystem II, but they are related to nitrogen fixation. These cells are larger, but they are found to be empty under the microscope (akinetes are differentiated with storage products). Heterocysts create anoxygenic environment for the organism which is necessary for the enzyme nitrogenase. This structure is surrounded by glycolipid cell wall that is thick and laminated, which helps in not allowing atmospheric gases into the heterocyst.

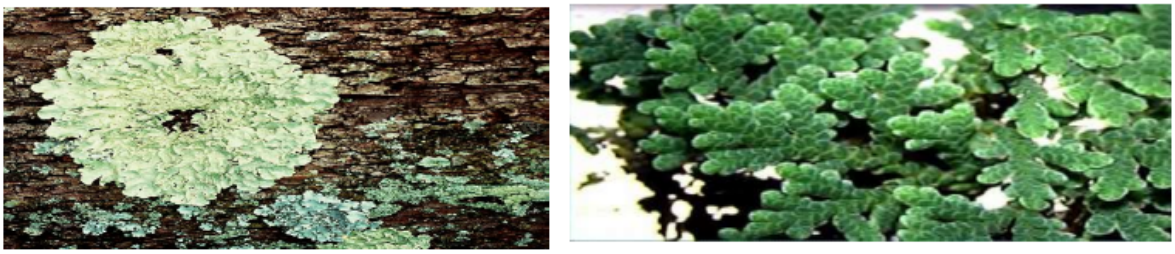
Symbiotic Association:

Lichens: It is the symbiotic association between algal members and fungi. But there are also some cyanobacteria that occur in about 8% of all the species of lichens.

Anabena-Azolla: This is one of the remarkable symbiotic relationships that occur between *Anabena* (cyanobacteria) and water fern (*Azolla*), which grows widely in all water logged paddy fields. It is a natural fertilizer for paddy fields that provide rich nitrogen source, as

Anabena has nitrogen fixing ability. It is well documented that the yields of rice greatly increase when farmers use *Azolla* as a natural fertilizer in paddy fields.

Other Symbiotic Relationships: Cyanobacteria are also known to have symbiotic relationships with other organisms like protozoa, amoeba, green algae, diatoms, liverworts, vascular plants, as well as with water molds.



Anabena-Azolla Symbiosis

Production of Toxins:

Cyanobacteria are well known to produce and release some toxins into their habitat, especially in the water bodies, which cause illness to the animals or humans when they consume the water. They include neurotoxins and hepatotoxins. Toxin production distinguishes some cyanobacterial members easily from green algae, as none of the members of green algae were reported to have produced toxins.

Neurotoxins: These toxic substances are alkaloids in nature which target the nervous system in animals and humans. They include saxitoxin and anatoxin, which cause symptoms like gasping, twitching of muscles, convulsions and staggering. The cyanobacterial members that produce these toxins include *Aphanizomenon*, *Oscillatoria* and *Anabena*.

Hepatotoxins: These substances are large compounds which are confronted by liver and causes liver damage due to their remarkable size. The symptoms include diarrhoea, vomiting and weakness. The cyanobacterial members that are responsible for the production of these toxins are *Nostoc*, *Oscillatoria*, *Nodularia*, *Anabena* and *Microcystis*.

Classification:

Phylum Cyanophyta has a class Cyanophyceae, which is divided into 5 orders. They include: Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales.

Order Chroococcales: Unicellular or colonial forms, but never trichome, no base or apex is seen, no exospores are formed.

Family Chroococcaceae: Unicellular or colonial forms are present. Eg. *Microcystis, Gleocapsa*.

Family Entophysalidaceae: Pseudofilamentous forms are seen. Eg. *Entophysalis, Placoma*.

Order Chamaesiphonales: Unicellular cells, these are organized with base/apex, endo/ exospore is present.

A. Family Cylindiaceae: The cells are spherical Eg. *Chroococcidiopsis*

B. Family Chamesiphonaceae: Unicellular cyanobacteria, attached with base/apex, Exospores are present Eg. *Chamaesiphon*

C. Family Dermocarpaceae: Unicellular, attached with base/apex, endospores are present. Eg. *Dermocarpa*

Order Pleurocapsales: These are distinctly filamentous, attached, no hormogones or Heterocysts are present.

Family Pleurocapsaceae: They have firm gelatinous membrane, filamentous in nature, endospores are formed Eg. *Myxosarcina*

Family Hyellaceae: Filaments without hormogones, di/tetrachotomous, endospores. Eg. *Hyella*

Order Nostocales: They are filamentous, homogonalen are seen, akinetes, heterocysts,exospores or endospores are formed. This order shows no true branching.

A. Family Oscillatoriaceae - Trichome shows single row of uniform broad cells, sometimes tapering, unbranched with firm mucilage sheath. Heterocyst or spores are absent, trichome may be spirally coiled Eg. *Lyngbya*, *Oscillatoria*, *Spirulina*, *Phormidium*, *Trichodesmium*.

B. Family Nostocaceae - Filaments are either in single or in a definite colony, heterocyst is present which is positioned terminally or intercalary. Heterocysts can be in single or more than one. Eg. *Anabaena*, *Nostoc*, *Nodularia.*

C. Family Scytonemataceae - Filamentous forms are present with thick firm sheath, lamellated, showing false branching. Heterocyst is intercalary; many trichomes are present in a sheath. Eg. *Plectonema*, *Scytonema*, *Tolypothrix*.

D. Family Microchaetaceae - Trichome with differentiation of base and apex is seen, these are unbranched, sheath can be seen with single trichome and a heterocyst is present. Eg. *Microchaete*.

E. Family Rivulariaceae - Trichome with tapering apex is seen, which is unbranched possessing basal heterocyst. Hormogones are also present. Eg. *Calotrix*, *Homoeothrix*, *Dichothrix*, *Rivularia*, *Gloeotrichia*.

Order Stigonematales:

Filamentous cells with hormogonalen, the structures like heterocysts, akinetes, exo/endospores, with true branching and dichotomy is seen, with prostrate and erect arrangements in this order.

A. **Family Capsosiraceae -** Thallus is attached and hemispherical, free irregular branches are present, filament with series of one or two cells are seen, heterocyst can either be present or absent. Eg. *Stauromatonema*

B. **Family Nostochopsidaceae -** Thallus is made up of erect, but many bent filaments which are branched with two types of branching. Some are long and some with limited growth. Terminal heterocyst are present. Eg. *Nostochopsis*

C. **Family Mastigocladaceae -** Trichome with reverse V shaped branching is seen, intercalary heterocyst is present and endospores are formed. Eg. *Brachytrichia*, and *Mastigocladus.*

D. **Family Mastogocladopsidaceae -** Trichome with V shaped and lateral branching is seen, Heterocyst is lateral, terminal and intercalary Eg. *Mastigocladopsis*

E. **Family Stigonemataceae -** Thallus with free bent filaments are present, which are irregularly branched, often prostrate and erect, there might be a lateral or an intercalary heterocyst, Eg. *Hapalosiphon* and *Stigonema*.

Reproduction:

Reproduction in cyanobacteria is carried out by vegetative and asexual methods. Sexual reproduction is not observed in Cyanobacteria.

Vegetative Reproduction:

The vegetative reproduction is reported through the following methods (Fig.7.13):

Hormogonia or Pseudohormogones:

Short sections of a trichome gets detached and forms a new thallus.

Fragmentation:

The cyanobacteria filament breaks into 2 parts by any injury or by mechanical stress. Both the separated fragments form new thalli.

Endospores and Exospores:

The internal division of the protoplast results in a mass of spores which can be exospores or endospores.

Nanocytes:

These are very small cells formed due to the environment that has very less nutrients.

Planococci or Akinetes:

These are resting spores; these are formed by the cells that are resistant to unfavorable conditions like heat or desiccation.

Asexual Reproduction:

The asexual reproduction is reported through the method called as binary fission.

Binary Fission:

It is the division of an individual single cell to two new individual single cells (Fig. 7.14), this is similar to that of bacterial binary fission.

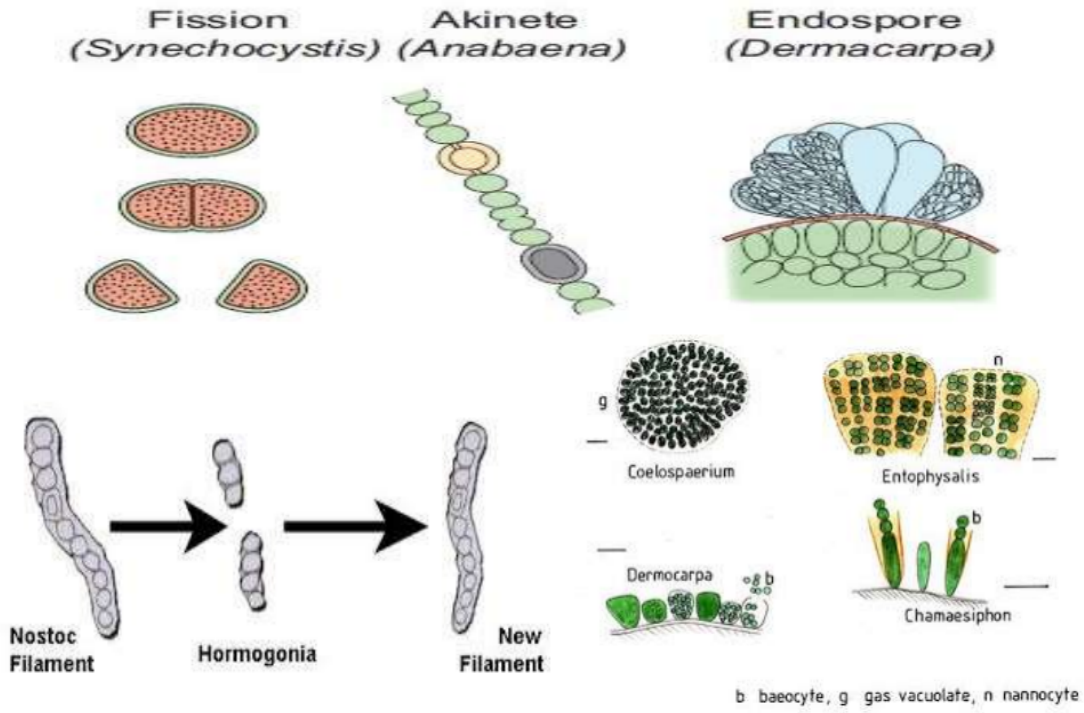


Figure-7.13: Vegetative reproduction in cyanobacteria

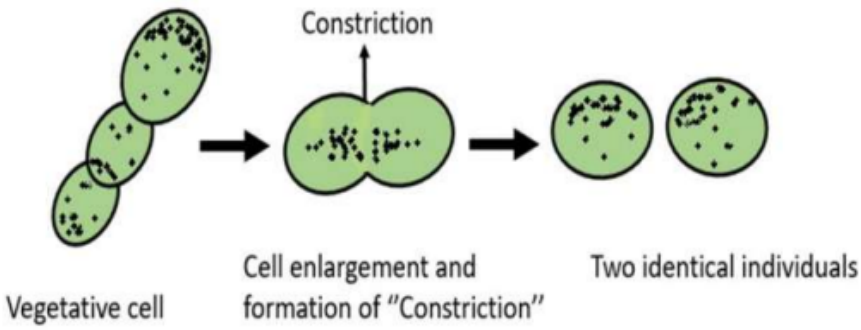


Figure-7.14: Asexual reproduction by binary fission in Cyanobacteria

Significance:

Cyanobacteria have numerous roles in almost all aspects of human life as well as in nature. It ranges from ecology, extending to food industry, medicine, agriculture, food chain, biotechnology, etc. The applications of Cyanobacteria are numerous and they are increasing as research is carried out more on Cyanobacteria. Some of the applications of Blue green algae are discussed below.

Ecological Importance:

Cyanobacteria are one of the very important organisms that help in ecological balance and maintenance of ecosystems. These colonize soil and rocks on their surface, which lead to the formation of mats that help in reduction of soil erosion. Moreover, they help in the formation of coral reefs on the sea bed. Also they precipitate limestone ($CaCO_3$) from waters and helps in the formation of rocks in tropical waters. In addition, one of the very important functions in the environment is carbon sequestering from the atmospheric carbondioxide. This is performed by cyanobacteria that help in the decrease of global warming, which is a great threat to the planet earth these days.

Importance in Agriculture:

As cyanobacterial members are efficient in nitrogen fixation, these can be used as bio fertilizers that help the crop plants crow without chemical contamination. If chemical fertilizers are used, they pollute soil and underground water, so cyanobacteria can be used in the crop fields, which help increase the soil fertility of the field without causing chemical pollution. Moreover, these bio fertilizers grow in the field, making it cost effective for the farmers. Eg: *Anabena-Azolla* symbiosis in paddy fields.

Importance in Food Chain:

Cyanobacteria are very important in the food chain as they contribute a great portion along with algae as primary producers. They grow as planktonic forms like phytoplankton and zooplankton in marine and fresh water habitats, which are consumed by secondary producers. This feature in cyanobacteria is also utilized by cattle breeders, bird keepers, breeders of molluscs, fish and shrimp who provide cyanobacteria derived feed and fodder to the cattle for providing better protein content and vitamins. Generally, *Nostoc*, *Anabena*, *Calothrix* and *Spirulina* are used as food for these animals. In India, *Phormidium valderianum* is used as feed for fish due to its non-toxic nature.

Importance in Food Industry:

The fact that cyanobacteria can be consumed as food has revolutionized the food industry these days. In fact, this practice is followed from long time. People in Japan, Thailand, China

and other countries consume *Nostoc* as food, due to its rich composition of fiber proteins and other nutrients, which have good role in human diet. Members include: *N. commune*, *N.elude*, *N. pruniforme*, etc. In Oregon, USA, *Aphanizomenon* sp. is used as healthy food, which is collected from natural blooms in Lake Klamath. *Spirulina platensis* and other species are consumed by people in Kanembu near Lake Chad, located in North-central Africa from many centuries. *Spirulina* has vitamins and varying protein contents of about 50-70%, which is one of the remarkable sources of protein. In addition, *Spirulina* contains good amount of fats, carbohydrates, minerals and many vitamins like thiamine, riboflavin, β-carotene (Beta carotene) and richest source of vitamin B12.

Importance in Medicine:

Antibiotics and other potential drugs: *Nostoc* was used to treat diseases like fistula, gout and cancers of different types. Natural products from cyanobacteria are very useful to treat many diseases without side effects as in chemically synthesized drugs. Some examples include: Anti-HIV, anticancer, antifungal, antimalarial and antimicrobial agents against, *E. coli*, *Bacillus* sp. and *Staphylococcus* sp. A natural product named Norharmane is produced by *Nodularia harveyana* which has anti-cyanobacterial activity which can be used in controlling harmful Cyanobacterial toxins. Antifungal activity of *Nostoc* sp. is found to be effective against many fungi like Cryptococcus sp. a cause of secondary fungal infections in AIDS patients. Cyanobacteria are found to produce antiviral natural products that are effective against many types of viruses. Some natural products are isolated from *Nostoc* and *Cyanothece* which show inhibitory activity against HIV-1, HIV-2, simian immunodeficiency virus (SIV), feline immunodeficiency virus, and measles virus. Also, they were found to inhibit Influenza and Ebola viruses. In addition to this, Cyanobacteria are known to produce antimalarial drugs like Symplocamide (obtained from *Symploca* sp.) was found to be active against *Plasmodium falciparum*.

Apart from all the above useful applications, cyanobacteria are also significant in having harmful effects. They kill great number of marine fish every year by forming dense concentrations of blooms in the marine as well as in fresh water resources. By releasing toxins into the waters in reservoirs, they cause gastro-intestinal tract illnesses in cattle and in humans.

7.6 SUMMARY:

Actinomycetes, called as actinobacteria are one of the unique and very diverse and one of the very successful microbes on our planet. They share both the characteristics of bacteria and fungi and possess uniqueness in their cell wall and cell membrane. Moreover, they have special structures called spores, for their survival in harsh conditions. They exhibit a variety

of pigmentation on their spores, which makes them identify by their texture. They reproduce asexually, by fragmentation and by the formation of spores. Sexual reproduction is not seen in Actinomycetes. There are also some special reproductive structures like synnemata, reported in Actinomycetes.

Archaea are single celled, prokaryotic cells that are given a separate domain 'Archaea', due to their distinctive and special characters from bacteria and eukaryotes. Most of the archaea are extremophiles that live in extreme conditions like thermophilic or halophilic conditions. Some measures about 400 nm while others measure 3cms in length. There is great diversity of size and shape in archaea. Moreover, archaea differ in their cell wall and cell membrane from bacteria. There is a marked difference in archaea in terms of their genetics, metabolism and in their cell organelles like ribosomes. The archaea are classified into three important phyla viz., Crenarchaeota, Euryarchaeota, and Korarchaeota. Their mode of reproduction is also distinct, from eukaryotes as they do not show sexual reproduction as well as mitosis, because they lack nuclear membrane. As most of them are extremophiles, they have great roles in nutrient recycling, biogeochemical cycles like carbon, sulphur and nitrogen cycles, methanogenesis, maintenance of marine ecosystems, nutrient recycling, and decomposition of complex organic matter and deterioration of toxic chemicals. They are also used in the production of food, industrial enzymes, chemicals like detergents, mining of metals, sewage treatment, as well as in biogas production for cooking.

Mycoplasmas cause several major diseases in livestock, for example, contagious bovine pleuropneumonia in cattle (*M. mycoides*), chronic respiratory disease in chickens (*M. gallisepticum*), and pneumonia in swine (*M. hyopneumoniae*). *M. pneumonia* causes primary atypical pneumonia in humans. *Ureaplasma urealyticum* is commonly found in the human urogenital tract. It is now known to be associated with premature delivery of newborns, as well as neonatal meningitis and pneumonia. Spiroplasmas have been isolated from insects, ticks, and a variety of plants. They cause disease in citrus plants, cabbage, broccoli, corn, honey bees, and other hosts.

Blue green algae or cyanobacteria are photosynthetic prokaryotes, which are the only prokaryotes apart from bacteria. They have a variety of photosynthetic pigments like Chlorophyll-a, Phycobilins, Xanthophylls, Carotenoids and Phycobilins. They are cosmopolitan in nature, but most of the members live in aquatic habitats, in all types of water bodies. They can grow in a variety of environments like hyperthermophilic, marine, fresh water, terrestrial, desert environments and even in volcanic eruptions. They live in symbiotic relationship with a variety of living organisms like fungi, algae and animals. Along with algae, they account for some portion of Lichens. These organisms have a range of diversity from unicellular to multicellular and filamentous forms, which show branching as well as

false branching. Cyanobacteria lack organelles like Nucleus, Mitochondria, Chloroplasts, Golgi apparatus, and Endoplasmic reticulum. Glycogen granules, Phycobilisomes, Cyanophycin, Nucleoid, Cell wall, Plasma membrane, Gas vesicles, Ribosomes, Carboxysomes, Lipid granules, Polyphosphate granules and Thylakoids are present. Cell wall contains of murein (peptidoglycan). Some members form a brownish pigmented sheath called as Scytonemin, which helps the organism in shielding them from Ultraviolet radiation. Some unique features in Cyanobacteria include: formation of heterocysts, having symbiotic relationships and production of toxins in the blooms. Based on different characters, all the cyanobacterial members are classified into five orders. They include: Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales.

Cyanobacteria reproduce in a variety of ways. They include vegetative mode that comprises of hormogonia, fragmentation, spore formation, nanocytes and planococci or akinetes. These organisms also reproduce asexually by binary fission. They play very important role in ecology, by preventing soil erosion, limestone formation, formation of coral reefs and decreasing greenhouse effect by carbon sequestering. Blue green algae like *Anabena* are very important in agriculture as they are the best biofertilizers, which can fix atmospheric nitrogen. Moreover, they have great prominence in human health as they are the source of almost all types of essential drugs like antimicrobials, anti-inflammatory, anticancer, antimalarial, antiviral drugs. Not only good, but there are some members of cyanobacteria that produce toxins in blooms, which cause illness to animals and humans.

7.7 TECHNICAL TERMS:

Actinomycetes, Geosmin, Ray fungi, Mycolic acids, Synnemata, Domain, Extremophiles, Pleomorphism, Hyperthermophiles, Halophiles, Methanogens, Crenarchaeota, Euryarchaeota, Korarchaeota, Fried-egg appearance, Phycobilins, Carotenoids, Lichen, Trichome, Heterocyst, Thylakoid, Nitrogenase, Hormogonia, Akinetes, Plankton, *Spirulina*, *Nostoc*, Biofuels.

7.8 SELF ASSESSMENT QUESTIONS:

1) What are actinobacteria? Discuss their general characters and significance.
2) Give an account on Archaebacteria and their significance.
3) Describe the general features of Mycoplasmas.
4) Discuss the general characters of Cyanobacteria with special focus on its cell structure
5) Explain in detail about the significance of Blue-green algae.

7.9 SUGGESTED READINGS:

1) Microbiology – Prescott et.al. 12th edition.

2) General Microbiology - Linda Bruslind.

3) Brock Biology of Microorganisms – Madigan et.al.

4) Biology and Biotechnology of Actinobacteria – Wink et.al. (10.1007/978-3-319-60339-1, Springer publication).

5) Blue green algae and its application (http://www.phytojournal.com)

Dr. J. Madhavi

LESSON - 8

INTRODUCTION AND THALLUS ORGANIZATION OF ALGAE

8.0 OBJECTIVE OF THE LESSON:

Students will understand the diversity occurring in the thallus organization of algae that helps in their easy identification.

STRUCTURE OF THE LESSON:

8.1 INTRODUCTION:

The algae comprise a heterogeneous group of chlorophyll bearing lower plants with enormous diversity of for, structure, reproduction and life history. The plants may be microscopic as well as macroscopic inhabiting the fresh water and aquatic habitats. The science that deals the study of algae is called Phycology (or Algology). The term 'Phycology' was derived from the Greek word Phykos means sea weeds. These plants are known to occur every place where there is light and moisture. It is true that they grow richly in tile seas and in the freshwater reservoirs such as rivers. Streams, lakes, ponds, damp soil, moist wall, wet tree trunks etc. and also in the extreme habitats such as deserts, snow-clad Polar Regions and hot water.

As in higher green plants, photosynthesis in algae is accompanied by oxygen evolution. However, unlike bryophytes and other higher plants, algae lack a covering or jacket of vegetative cells around their reproductive organs. An exception is the antheridium of the Charophyceae which has a sterile jacket around it. In the Phaeophyceae (Brown algae), some reproductive organs are multicellular and all the cells of such an organ are fertile. With the exception of the blue-green algae (Cyanophyta) and Prochloron all algal members are eukaryotic in nature.

Among the Indian Phycologists, Ghose (1919-1932) was pioneer who made significant contribution on blue-green algae of Punjab. His student, Randhawa (1932-1959) published a series of papers on Zygnemaceae and Oedogoniales of Uttar Pradesh and Punjab. Prof. M.O.P. Iyengar established a strong school of algae at the University of Madras and discovered *Fritschiella tuberosa* (1932) and studied many aspects of Chlorophyceae. Randhawa (1959) rightly called him the 'Father of Modem Algology of India'. Desikachary (1959), a student of Iyengar, had written a monograph on 'Cyanophyta'. Another strong school of algae which has international reputation was built by Bharadwaja, a student of Prof. F.E. Fritsch, at Banaras Hindu University (BHU), Varanasi. Y.S.R.K. Sarma (1960, 1974) worked on nuclear cytology of green algae at B.H.U. At Allahabad, Mitra (a student of Fritsch) contributed to the morphology, taxonomy and life histories of many green and blue-green algal species. Prasad (at Lucknow) worked with Prof. M.B.F. Godward of Queen Mary College (London) on 'Cytogenetics of green algae'. R.J. Patel (Vallabh Vidyanagar), another student of Prof. Godward, made a significant contribution towards the cytology of the Chlorophyceae and, algal flora of Gujarat and Maharashtra.

8.2 THALLUS ORGANIZATION:

Algae show a wide range of variation in thallus organization. Basically, thallus organization is of two types, the unicellular and the multicellular. The wide range of forms that algae exhibit arises from a modification or elaboration of these types.

8.2.1 Unicellular Algae:

Large number of unicellular forms are found in major groups of algae such as Cyanophyta, Chlorophyta, Bacillariophyta and Rhodophyta. However, unicellular forms may be motile or non-motile.

(A) Unicellular Motile:

The motile forms are of two types, the flagellated type, moving by means of flagella (found in all phyla except the Cyanophyceae, Pro chlorophyceae and Rhodophyceae) and, the rhizopodia kind, having the fine protoplasmic projections (rhizopodia) and showing an amaeboid movement (Tribaphyceae). The flagellated cells may have a rigid cell wall or periplastic as in *Euglena*. The flagella may be one in *Chromulina* or two and equal as in *Chlamydomonas* (Fig.8.1A), or two and unequal, e.g., *Cryptomonas*. In some flagellates, external to the periplast, there is a calcareous envelope and they are called encapsulated forms, e.g. *Chrysococcus*.

(B) Unicellular Non-Motile:

Unicellular non-motile algae are coccoid genera which lack both flagella and pseudopodia. However, these forms usually bear a thick cell wall as in *Chlorella* (Fig.8.1B) and *Chroococcus* (Cyanophyta). Unicellular non-motile forms also found in Bacillariophyceae, Xanthophyceae (*Chariciopsis*) and Rhodophyceae (*Porphyridium*). The smallest known eukaryotic algae is *Micromonas pusilla*.

8.2.2 Multicellular Algae:

Depending on the manner in which cells are produced and arranged during vegetative phase, three principal types are recognized. These are colonial, filamentous and siphonous.

A. Colonial Forms:

A colony is a group of separate cells generally similar in structure and function and aggregated by a mucilaginous envelope. There are four main types of colonial organizations - Coenobial, Palmelloid and Dendroid.

Coenobial Type - A coenobium is known to possess a definite shape of the colony and a constant number of cells arranged in a specific manner. The coenobium may be motile or nonmotile. A number of divisions of cells occur during vegetative phase of the colony and the cells are embedded in the mucilaginous matrix. In the motile forms, the cells are flagellated, e.g., *Volvox* (Fig. 8.1C). In the non-motile, the cell are coenocytic, e.g., *Hydrodictyon* (Fig. 8.1D).

Palmelloid Type - Contrary to the coenobium type, in palmelloid forms, neither the number of cells nor the shape and size of the constituent cells, are constant. All the cells are held together in the mucilaginous matrix and give the irregular outline of the thallus, e.g: *Tetraspora* (Fig. 8.1E), *Aphanocapsa* (Cyanophyta).

Dendroid Type - In the dendroid forms, cells are united in a branching manner by the localized production of mucilage at the base of each cell. The whole colony looks like a tree in habit, e.g., *Ecballocystis* (Fig. 8.1F) and *Ecballocystopsis*, both belong to Chlorophyta.

B. Filamentous Forms:

A uniseriate row of cells joined end to end in a transverse plane through middle lamellae constitute a trichome. When this trichome surrounded by sheath, is referred to as a filament.

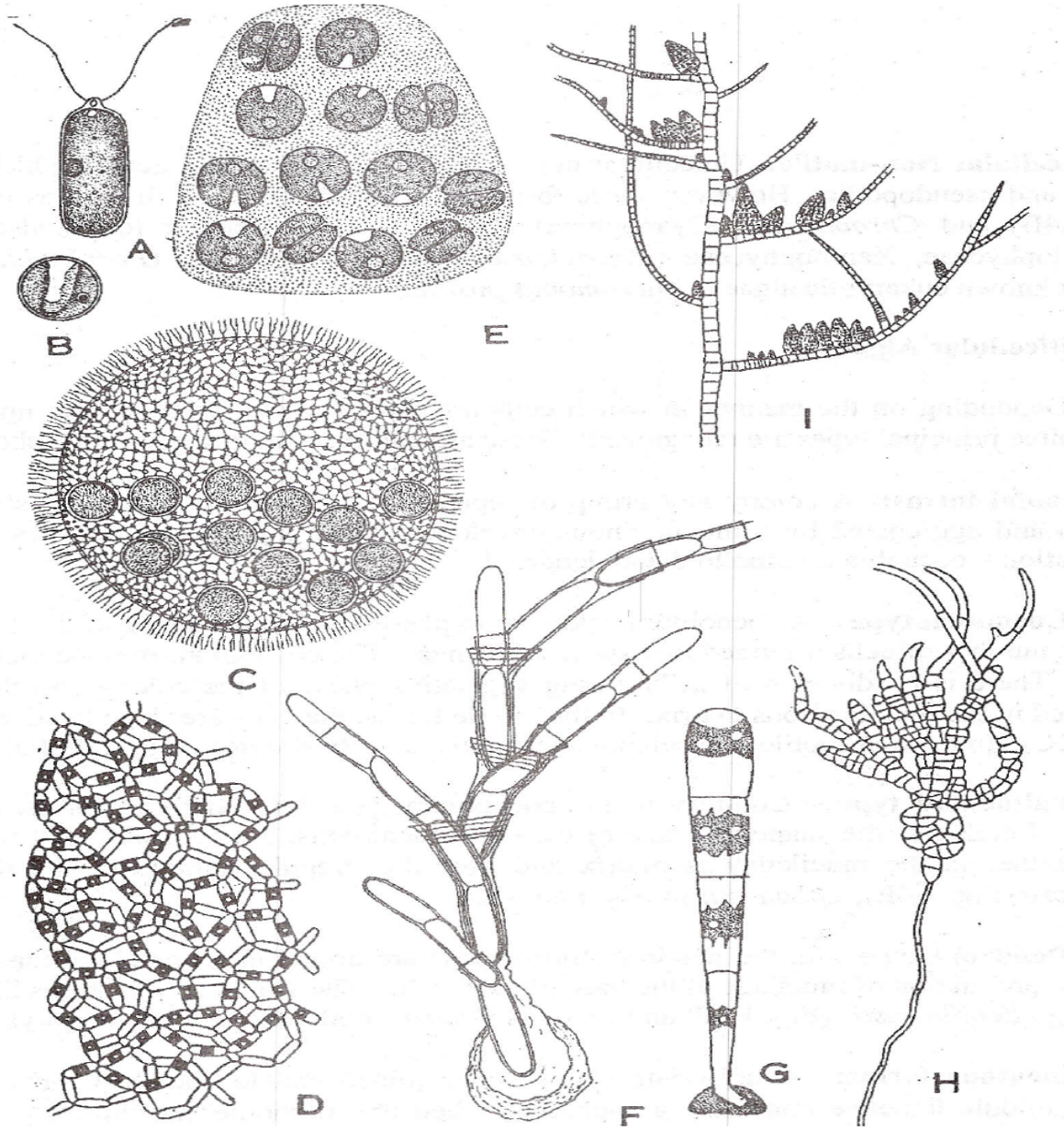


Figure-8.1: Range of Thallus Structure in Algae

A. *Chlamydomonas*, unicellular flagellated motile cell; B. *Chlorella*, unicellular, non-motile coccoid cell; C. *Volvox*, multicellular flagellated motile coenobium; D. *Hydrodictyon*, multicellular, non-motile coenobium; E. *Tetraspora*, palmelloid colony; F. *Ecbellocystopsis*, dendroid colony; G. *Ulothrix*, unbranched filament; H. *Fritschiella*, branched heterotrichous filament; I. *Ectocarpus*, branched filament. (Based on Fritsch, 1935),

Unbranched Filaments: A filament may be unbranched, e.g., *Anabaena*, *Nostoc*, *Oscillatoria* of Cyanophyta and *Oedogonium*, *Ulothrix* (Fig.8.1G) of Chlorophyta. Some filamentous taxa exhibit distinct polarity with the trichomes tapering towards the tip, e.g. *Rivularia*.

Branched Filaments: The branching of the filaments is of two kinds - false and true. In false branching, which occurs in Scytonemataceae (Cyanophyta), the trichome generally fragments due to the degeneration of an intercalary cell (or by the formation of biconcave separation discs), after which one or both of its ends adjacent to the dead cell grow out of the parent sheath, giving the resemblance of branching, e.g. *Scytonema*. True branching results from repeated transverse divisions of the lateral outgrowth produced on the main filament. The truly branched thalli are of following types:

1) **Simple Branched Filament:** e.g. *Cladophora*
2) **Heterotrichous**: In which the thallus is differentiated into an erect and prostrate system of branched filaments, e.g., *Coleochaete, Fritschiella* (Fig.8.1H), and *Draparnaldiopsis* of Chlorophyta, *Ectocarpus* of Phaeophyta, *Batrochospermum* of Rhodophyceae and *Stigonema* of Cyanophyta. In *Draparnaldiopsis*, the prostrate system is well developed. Fritsch opined that the first land plants might have arisen from the algae exhibiting heterotrichous habit.
3) **Pseudoparenchymatous: I**n which the thalli show uniaxial or multiaxial construction, e.g., *Batrachospermum* (uniaxial), *Polysiphonia* (multi axial) (Fig.8.2B) etc. In multi axial pseudoparenchyma, the branches of many axial filaments aggregate in juxtaposition, e.g. *Nemalion.* The central filaments give rise to lateral branches. The branches become compact and is called cortex, e.g., *Codium, Polysiphonia*.

C. Siphonous Forms:

The filamentous habit without occurrence of septa and presence of coenocytic condition constitute the siphonous structure of the thallus. Such condition results into larger thalli in some genera. Many workers considered these genera as acellular or unicellular forms, e.g., *Dichotomosiphon* of Chlorophyta (Fig.8.2A) and *Vaucheria* of Xanthophyta (Fig.8.2D).

D. Parenchymatous Forms:

Parenchymatous condition of a thallus results when uniseriate filaments show potentiality of cell division in more than one plane. In these forms, growth takes place in four ways - diffuse, intercalary, trichothallic and apical. In simple filamentous forms such as *Ulothrix* and *Nostoc*, the growth of thallus is diffuse because each vegetative cell is potentially capable of growth and division. In trichothalic growth, the cells at the base of a hairlike branch are meristematic, e.g., *Rivularia*. A good example of intercalary growth is *Laminaria* in which growth of the thallus is brought about by the meristem located at the junction of the stipe and the blade. The thalli of the Charophyceae, Dictyotales and certain other algae grow by the activity of a single or a group of apical cells.

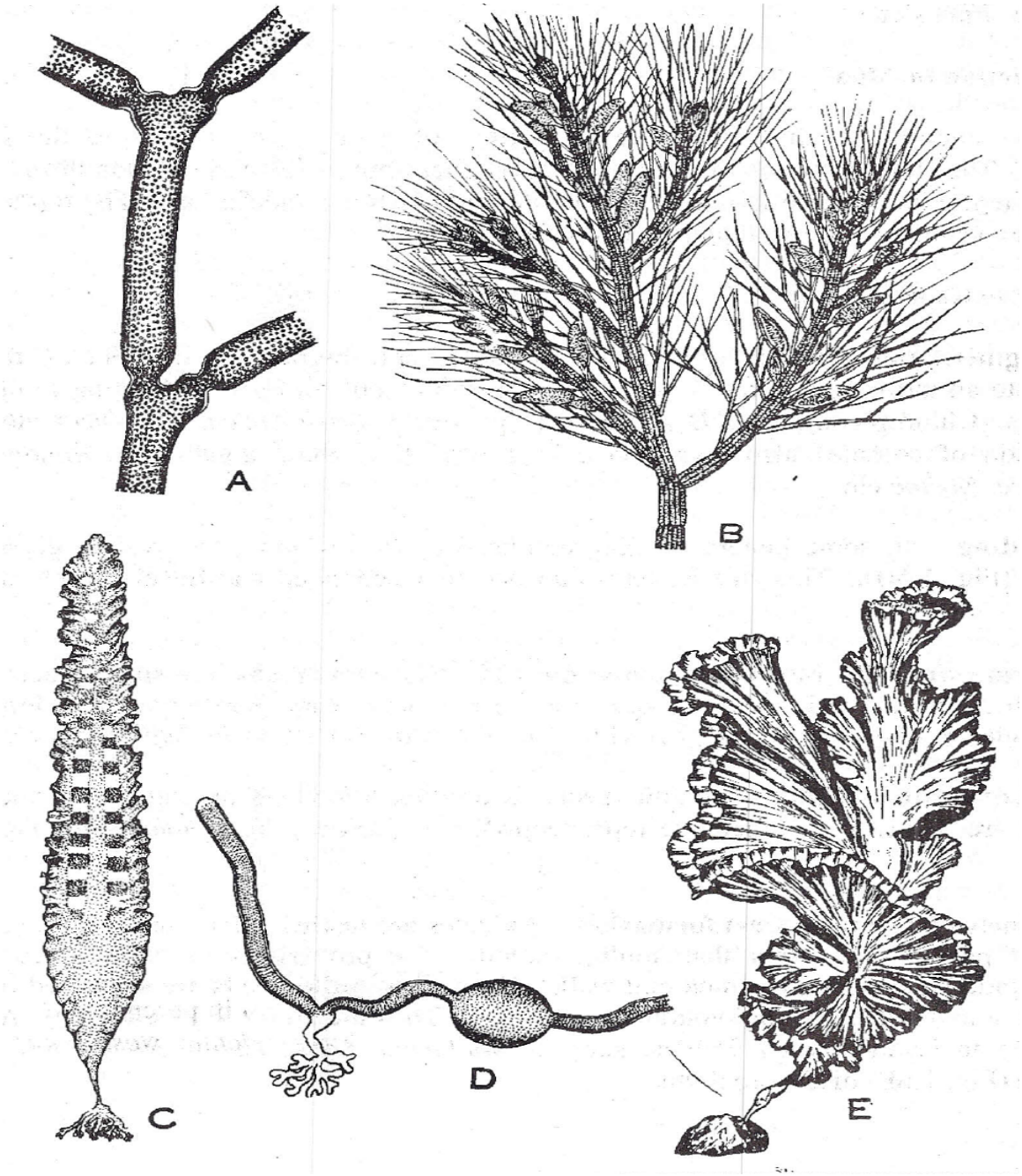


Figure-8.2: A. Dichotomosiphon, B. Polysiphonia, C. Laminaria, D. Vaucheria, E. Ulva

The thallus in these forms may be foliage and flat (*UIva, Porphyra*) or tubular (*Enteromorpha*). In brown algae, parenchymatous habit is well-developed. The thallus is differentiated into central columella, middle cortex and outer meristoderm. Other examples are *Macrocystis, Fucus* etc.

8.3 SUMMARY:

Thallus in algae refers to the plant body which lacks differentiated tissues like roots, stems, and leaves, and can range from a single cell to complex, multicellular structures like

filaments or plates, depending on the algal species; essentially, the thallus organization describes how the algal body is structured, with variations including unicellular, colonial, filamentous (branched or unbranched), and sometimes even resembling a more complex plant-like structure with differentiated parts depending on the alga type. Algal thallus organization constitutes 1. **Simple forms:** Unicellular algae like *Chlamydomonas* or *Chlorella* represent the simplest thallus organization, where the entire organism is a single cell. 2. **Colonial forms:** Groups of unicellular algae can form colonies, like in *Volvox* or *Pediastrum*, where cells are loosely associated together. 3. **Filamentous forms:** Many algae have a filamentous thallus, consisting of chains of cells connected end-to-end, which can be unbranched (*Spirogyra*) or branched (*Cladophora*) depending on the species. 4. **Complexity in structure:** Some larger algae can exhibit a more complex thallus with differentiated parts that resemble roots, stems, and leaves, although they are not true roots, stems, or leaves. Unlike higher plants, algal thalli lack specialized vascular tissue for water transport.

8.4 TECHNICAL TERMS:

Thallus, Unicellular, Multicellular, Colonial, Pamelloid, Parenchymatous, Siphonous, Columella.

8.5 SELF-ASSESSMENT QUESTIONS:

1) Describe thallus organization in algae Thallus organization.
2) Give a brief account on Unicellular algae.
3) Give a brief account on Multicellular algae.

8.6 SUGGESTED READINGS:

1) Introductory Phycology - Kumar, H.D. (1988). Affiliated East-West Press. Ltd., New Delhi.
2) An Introduction to the Algae - Morris. J. (1986). Cambridge University' Press, U.K

Prof. A. Amrutha Valli

LESSON - 9

CLASSIFICATION AND ECONOMIC IMPORTANCE OF ALGAE

9.0 OBJECTIVE OF THE LESSON:

Students will know how the algae are classified according to different systems proposed by different scientists and also understand the economic importance of algae in our daily life and other aspects.

STRUCTURE OF THE LESSON:

9.1 INTRODUCTION:

The classification of algae has been modified from time to time ever since Linneaus placed them in the class Cryptogamia. Till 20^{th} century, it was customary to recognize four classes of algae namely, Chlorophyceae, Phaeophyceae, Rhodophyceae and Myxophyceae. Diatoms

were placed in Phaeophyceae. All motile unicellular and colonial flagellated organisms with chlorophyll are placed in the class Mastigophora of phylum Protozoa. The classification was based on vegetative structures and reproductive processes. In modern systems of classification, many criteria have been taken into consideration so that it is possible to understand the interrelationships and phylogeny. Some of these criteria taken into account for classification are:

1) Photosynthetic pigments.
2) Nature of storage products.
3) Nature of cell wall components.
4) Details of cell structure.
5) Type of flagella.

9.2 CLASSIFICATION:

9.2.1 Fritch System (1945):

According to F.E. Fritsch (1935, 1944, 1945), the algae have been divided into the following eleven classes.

(A) Chlorophyceae

(B) Chrysophyceae

(C) Xanthophyceae

(D) Bacillariophyceae

(E) Cryptophyceae

(F) Chloromonadineae

(G) Phaeophyceae

(H) Myxophyceae

(I) Dinophyceae

(J) Euglenophyceae

(K) Rhodophyceae

Fritsch classification is mainly based on the pigmentation, the assimilatory food products (metabolic products) and type of flagella.

Some important classes are given below:

A. Chlorophyceae:

The class Chlorophyceae is characterized by following features:

a) The pigments such as chlorophyll a, chlorophyll b, xanthophyll and carotenes, are localized in definite plastids or chromatophores.

b) The reserved food material is starch, rarely oil in very few cases. Usually in chromatophores, pyrenoids are present. A part of pyrenoid converts into starch.

c) The flagellation is isokontae type, i.e., both of the flagella are equal in length.

d) Vegetative body may be one to many-celled.

e) Cellulosic cell wall is present.

f) Sexual reproduction ranges from isogamy to oogamy.

g) The life-cycle is mostly of haplontic type.

h) Most of the species are fresh water and few are marine.

e.g., *Chlamydomonas, Volvox, Ulva, Draparnaldia, Oedogonium, Spirogyra, Chara etc.*

B. Xanthophyceae:

a) The chromatophores are yellow green, containing chlorophyll a, carotenes (p-carotene) and xanthophylls in them.

b) Pyrenoids are absent and starch is not found. The chief food products are oils.

c) The flagellation is of heterokontae type, i.e., one flagellum is short and other one is long.

d) The cell wall consists of pectin, and in majority of cases two overlapping halves are present, e.g., *Tribonema*.

e) The sexual reproduction is rarely found.

f) The walls are silicified.

g) The life-cycle is mostly of haplontic type.

h) Majority of species are fresh water, some are marine.

e.g., *Botrydium, Vaucheria etc.*

C. Bacillariophyceae:

a) Gorden brown or yellow colour of the thallus is due to the presence of a pigment called diatomin. The other pigments are chlorophyll. alpha carotenes and xanthophylls. The members of Bacillariophyceae are known as diatoms.

b) Reserved food materials are fats or volutins.

c) The flagellate bodies are 1 or 2 flagellated.

d) Majority of them are unicellular, some are colonial.

e) The sexual reproduction is of special type resulting in the formation of auxospores.

f) They are diplontic.

g) They are widely distributed in sea and fresh waters.

e.g., *Pinnularia, Navicula, Synedra* etc.

D. Phaeophyceae:

a) Brown or yellowish brown colour of the thallus is due to the abundance of carotenoids, especially fucoxanthin. Other pigments are chlorophyll a, chlorophyll c, carotene, violaxanthin and other xanthophylls.

b) The reserved food materials are laminarin, mannitol and alcohols.

c) The plant body is multicellular but motile reproductive structures are unicellular. These motile reproductive cells are pyriform with two laterally inserted flagella; anterior flagellum is longer and pantonematic while the posterior one is short and acronematic.

d) The sexual reproduction ranges from isogamy to oogamy.

e) The life cycles indicate clear alternation of generations.

f) Most of them are marine.

e.g., *Ectocarpus, Dictyota, Sargassum, Laminaria.*

E. Rhodophyceae:

a) They are red algae due to presence ofy-phycoerythrin and c-phycocyanin pigments. The other pigments are chlorophyll a, carotenes and xanthophylls.

b) Reserved food materials are polysaccharides, floridean starch and a soluble sugar called floridoside.

c) The flagellation is absent.

d) Prominent plasmodesmata are present.

e) An advance type of oogamous sexual reproduction is found.

f) Life-cycles show clear alternation of generations.

g) Most of the species are fresh water and the rest are marine.

e.g., *Batrachospermum, Polysiphonia*.

F. Myxophyceae (Cyanophyceae):

a) Excess amount of phycocyanin gives blue-green colour to the thallus. Definite chromatophores are absent but pigments are localized in the peripheral portion of the protoplast. Other photosynthetic pigments are c-phycoerythrin, carotones and xanthophylls.

b) Reserved food materials are sugars and cyanophycean starch.

c) They are prokaryotic in nature.

d) Sexual reproduction is unknown.

e) Majority of them are fresh water forms. Some are found in sea water.

9.2.2 Round's System (1973):

F.E. Round (1973) classified algae into two major groups, i.e., Prokaryota and Eukaryota.

Group-A : Prokaryota - Phylum Cyanophyta

Group-B : Eukaryota - 12.phyla

a. Chlorophyta

b. Euglenophyta

c. Charophyta

d. Prasinophyta

e. Xanthophyta

f. Haptophyta

g. Dinophyta

h. Bacillariophyta

i. Chrysophyta

j. Phaeophyta

k. Rhodophyta

l. Cryptophyta

9.2.3 Whittaker and Margulis System (1978):

Whittaker and Margulis (1978) opined that most of the algal groups may have closer affinities with other protist groups than with each other. On the basis of this idea, they classified all prokaryotes under kingdom Monera, and all algae under super kingdom Eukaryota and

Kingdom Protista or Proctista	**Monera (Prokaryotic Cells)**
Kingdom	**Photomonera (Photosynthetic Prokaryotes)**
Superphylum	**Photobacteria**
Phylum	**Prochlorophyta (Green Oxygenic Prokaryotes)**
Phylum	**Cyanophylta or Cyanobacteria (Blue-Green Algae)**
Phylum	**Eukaryota**
Super Kingdom:	**Protista or Proctista**
Kingdom	

There are 3 super phyla - Chromophyta (8 phyla - yellow and brown algae), Chlorophyta (6 phyla - green algae) and Rhodophyta (red algae).

9.2.4 Larkum and Barret system (1983):

Larkum and Barret (l983) adopted the views of Whittaker and Margulis with some modifications:

Kingdom: Monera (Prokaryotic cells)

Superphylum: Photomonera (Photosynthetic prokaryotes)

Phylum: Eukaryota

Phylum: Protista or Protoctista [eukaryotic cells with solitary and colonial unicellular organization (Protista) or also including simpler multicellular form Protoctista)].

Superkingdom: Chromophyta (yellow and brown flagellate algae)

Phylum: Chrysophyta (golden algae, including Prymnesiophyta and Chlorornonandophyta)

Phylum: Bacillariophyta (Diatoms)

Phylum: Xanthopbyta (yellow-green algae)

Phylum: Haptophyta

Phylum: Eustigmatophyta

Phylum: Dinaflagellata

Phylum: Cryptophyta

Phylum: Phaeophyta (Brown algae)

Superphylum: Chlorophyta (Green algae)

Phylum: Chlorophyta (Grass-green algae)

Phylum: Siphonophyta (Siphonaceous green algae)

Phylum: Prasinophyta

Phylum: Zygnematophyta !conjugating green algae)

Phylum: Charophyte (Seaworts)

Phylum: Euglenophyta

Superphylum: Rhodophyta

9.2.5 Corliss system (1987):

Corliss (1987) classified the algal protists in the following 6 series.

Series I - Chlorophyta

This series comprises both motile (flagellated) and non-motile groups of green algae including unicellular, coenobial, filamentous and multicellular forms. This series contains 5 phyla, these are: (1) Chlorophyta, (2) Prasinophyta, (3) Conjugatophyta, (4) Charophyta and (5) Ulvophyta.

This series is characterized by:

a) The presence of chlorophyll a and chlorophyll b.

b) Flattened mitochondrial cristae.

c) Cellulosic cell walls.

d) Motile cells.

e) Arrangement of thylakoids in several layered grana.

f) Chloroplast bounded by double membrane.

g) Most of these are fresh water ones.

h) Except for the Charophyta, the other phyla typically form a phycoplast cytokinesis; in Charophyta, phragmoplast is formed.

Series II – Chromophyte:

This series is larger than the chlorophyte. The motile members are typically biflagellated with one smooth and other hairy flagellum, except diatoms and haptophytes in which flagellated stage is lacking in their life cycle.

The phyla included in this series are: (1) Chrysophyta, (2) Haptophyta, (3) Bacillariophyta, (4) Xanthophyta. (5) Eustigmatophyta, (6) Phaeophyta,(7) Raphidophyta.

The above Phyla have some Common Characters. These are as follows:

a) Presence of Chlorophyll a and Chlorophyll c (Chlorophyll a in Eustigmatophyta only).

b) Tubular mitochondrial cristae.

c) Typically no cell walls (except Phaeophyta) but often scares.

d) Often silicified cysts.

e) Pair of heterokont flagella.

f) Thylakoid grouped in threes.

g) Chrysolaminarin is main storage reserved food material.'

h) Plastids bounded by 3 or 4 membranes.

i) Uncommon sexual reproduction.

j) Predominantly marine in distribution.

Series III – Euglenophyta:

This series is characterised by the following features:

a) Presence of chlorophyll a and chlorophyll b.

b) Unique pellicle underlaid by interlinked microtubules.

c) Single large mitochondrion with discoidal cristae.

d) Nucleus with prominent endosome.

e) Permanently condensed chromosome.

f) Intranuclear spindle.

g) Presence of cytosome and a contractile vacuolar system.

h) Absence of cellulosic cell wall.

i) Storage of starch.

Series IV – Pyrrophyta:

This series includes the dinoflagellates and pyrrophytes. These are characterized by the following features:

a) Presence of distinctive nuclear apparatus.

b) Presence of cortical alveoli (membrane bound vacuoles lacking contractile activity).

c) Presence of chlorophyll a and chlorophyll c.

d) Plastids generally covered by three membranes.

e) Uniquely positioned two heterodynamic flagella.

Red algae are chactarized by the following features:

a) Presence of phycoerythrin and phycocyanin and chlorophyll a.

b) Plastid is bounded by two layers containing single thylakoid.

c) Presence of floridean starch.

d) Mitochondrial crystae are lamellar.

e) Presence of gelatinous and microfibrillar cell walls.

f) Sexuality is common.

g) Distributed both in fresh water and marine habitats.

Series VI – Cryptophyte:

a) Presence of chlorophyll a and chlorophyll c and phycobilins.

b) Biflagellate with both flagella bearing tubular hairs.

c) Presence of lamellar mitochondrial cristae.

d) Cryptomonads have a distinct gullet (invaginated cell surface functioning as a means of ingestion of food materials) and ejectisomes (extrusomes).

e) Mitosis is usually open.

f) Starch is the reserved food material.

9.3 ECONOMIC IMPORTANCE OF ALGAE:

Since algae are the simple photosynthetic plants, they perform 90% of the total photosynthetic activity globally. They are intimately connected with human beings as a source of food, manure and fodder. They have been exploited in the cleaning of the environment (bioremediation of the environment) and also in the urban sewage treatment plants.

9.3.1 Algae as Food:

Algae and their products have been eaten in the most maritime countries. They are rich in proteins, vitamins and minerals. In the Far East and the Pacific Islands, people eat seaweeds such as *Porphyra*, *Laminaria*, *Undaria*, *Gracillaria*, *Alaria* and *Asparagopsis*. In Japan, about 20 species of green, brown, red and blue-green algae are eaten and total sales of *Porphyra tenera* (a red alga) alone amounts to more than 80 million U.S. Dollars per year. Chemical analysis shows that *P. tenera* has 30-35% proteins, 40-45% carbohydrates and a high percentage of vitamins A, B, C and E. Another alga, *Laminaria* growing on stones, cylinders and ropes, yields a product known as 'Kombu' or 'Konbu'. *Monostroma* (a green algae) cultivating in Japan, yields a product known as 'Aonori'. All these algal products can be served as a staple food for human beings. Some brown algae such as *Sargassum* and *Undaria*, and larger balls of the terrestrial species of *Nostoc* have been used as food by the Chinese and the South Americans (Peru).

Among red algae *Porphyra*, *Chondrus*, *Palmeria*, *Gelidiella* and *Gracillaria* are important edible algae in Canada, Japan, Philippines and Korea. They are used in salads, soups and vegetables. The unicellular algae such as *Chlorella*, *Scenedesmus*, *Spirulina* etc., have been mass cultured on large scale in many countries. *Chlorella* and *Scenedesmus* have been exploited in the spaceships and nuclear submarine programmes as oxygen regenerating and, food and water recycling organisms.

9.3.2 Algae as Fodder and Biofertilizer:

Sea weeds are rich in copper, iron, manganese, boron, cobalt, vanadium, and molybdenum. They are used as fodder for livestock and poultry. *Gracillaria* is used as poultry feed. Algae such as *Ascophyllum*, *Laminaria* and *Fucus* are used as livestock feed. *Spirulina* when feed to fishes, poultry and cattle, their productivity is improved. Mass culture of *Spirulina* is gaining importance and this can be used as feed for fish, poultry and cattle.

Blue-green algae grow luxuriantly in the paddy field soils and contribute significantly in enriching the nitrogen content of the soils. More commonly, nitrogen-fixing genera in the Indian rice fields are *Aulosira*, *Anabaena*, *Calothrix*, *Cylindrospetmum*, *Gloeotrichia*, *Nostoc*, *Scytonema*, *Stigonema*, *Tolypothrix* etc. It has been estimated that about 15-48 kg nitrogen per hectare is fixed in the rice fields by the activity of these algae. They are known as biofertilizers, or Nitrogen fixers. These algae are utilized by the crop plants. The soil also becomes rich in amino acids, vitamins and auxin-like compounds. These ingredients serve to improve the growth of the crop plants.

9.3.3 Commercial Products From Algae:

(**A) Alginates:**

Alginic acid (carbohydrate) is extracted from the cell walls of several brown algae including *Ascophyllum, Durvillaea, Echlonia, Fucus, Laminaria, Lessonia, Macrocystis, Nereocystis, Sargassum, Turbinaria* etc. Alginates have been used in the preparation of flame-proof fabrics and in plastic industries. Alginates are extensively used in pharmaceutical industry, especially in the preparation of dental impressions, gauze material in surgical dressing, and also used as an agent to stop the bleeding. Since alginates are non-toxic and possess colloidal properties, they are commonly used in the preparation of creams, jellies, soups, sauces and antibiotic capsules.

(B) Agar Agar:

Agar agar is a non-nitrogenous gel like substance extracted from the red algae, such as *Gelidium, Gracillaria, Gigartina, Chondrus, Ceramium* etc. Agar is used as gelling and solidifying agent in the preparation of culture media for culturing bacteria, fungi and algae. Besides, it is used as stabilizer and emulsifier in food, cosmetics, leather and pharmaceutical industries.

(C) Carrageenan:

This is a polysaccharide extracted from the cell walls of some red algae. Carrageenan forms colloidal solution with water. It is widely used in the bakery and confectioneries and also in preparation of creams, soups, sauces, cheese, fruit juices and also in the clarification of beer.

(D) Diatomite (Kieselgurh):

It is the cell wall material of diatoms, forming extensive deposits and it is called as diatomaceous earth or kieselgurh. It is insoluble, porous and chemically inert material. It is used in insulating the boilers as it can resist very high temperature. Alfred Nobel used diatomite as an absorbent for nitroglycerine in the manufacture of dynamite. It is also used as industrial filter in sugar refining and brewing industries.

9.3.4 Antibiotics and Medicine:

The well-known antibiotic, chlorellin obtained from *Chlorella* is effective against a number of pathogenic bacteria. Extracts from *Cladophora* and *Lingbya* can kill the pathogenic *Pseudomonas* and *Mycobacterium*. Sodium laminarin sulphate extracted from *Laminaria* can be used as blood anti-coagulant. It has been found that the extracts of *Digenea simplex* can be served as anthelmintic.

9.4 SUMMARY:

Algae are classified primarily based on their pigment types, with the main groups being green algae (Chlorophyceae), brown algae (Phaeophyceae), and red algae (Rhodophyceae). Green algae (Chlorophyceae) are primarily freshwater algae with chlorophyll as their main pigment. Brown algae (Phaeophyceae) are mostly marine algae containing brown pigments like fucoxanthin. Red algae (Rhodophyceae) are marine algae with red pigments like phycoerythrin. Their economic importance lies in their role as a food source for humans and animals in coastal communities, providing nutrients like vitamins and minerals, as well as their use in industries like food production (e.g., agar from red algae for jellies), pharmaceuticals, and as a potential source of biofuel due to their high photosynthetic capabilities.

9.5 TECHNICAL TERMS:

Chlorophyceae, Rhodophyceae, Phaeophyceae, Charophyta, Agar-agar, Carrageenan, Diatomite.

9.6 SELF ASSESSMENT QUESTIONS:

1) Give a detailed account on Fritsch's system of algal classification.
2) Give a detailed account on Round's system of algal classification.
3) Give a detailed account on Whittaker and Margulis classification system of algae.
4) Larkum and Barret system of algal classification.
5) Give a detailed account on economic importance of algae.
6) Give an account on algae as food, fodder and biofertilizer.
7) Write an account on commercial products, antibiotics from algae.

9.7 SUGGESTED READINGS:

1) Introductory Phycology - Kumar, H.D. (1988). Affiliated East-West Press. Ltd, New Delhi.
2) An Introduction to the Algae - Morris. J. (1986). Cambridge University Press, U.K

Prof. A. Amrutha Valli

LESSON - 10

CHLOROPHYTA AND BACILLARIOPHYTA

10.0 OBJECTIVE OF THE LESSON:

Students will have knowledge on the general characters and importance of various forms of green algae and diatoms and also get the familiarity for identification of the members of these two groups.

STRUCTURE OF THE LESSON:

10.1 INTRODUCTION:

The class Chlorophyceae of division Chlorophyta is characterized by the presence of eukaryotic cell organization. The green colour of thallus is due to same pigments as in higher plants. The chloroplasts in which these pigments located, are of different shapes. The pigments are chlorophyll a, chlorophyll b, various carotenes and xanthophylls including

lutein, violaxanthin, neoxanthin, astaxanthin and fucoxanthin. Starch is the reserve food material in this group of algae. The cell wall is chiefly composed of cellulose. The motile cells, if present, possess acronematic type of flagella (smooth and whiplash type) of equal length.

The Phylum Bacillariophyta with a class Bacillariophyceae (diatoms) comprises a homogeneous assemblage of unicellular and colonial forms which differ from other algae in possessing highly sculptured and symmetrically ornamented cell walls. They are also called "Jewels of the plant world". The unicellular diatoms are of two types namely pennate diatoms, (order Pennales) and centric diatoms (order Centrales). The pennate diatoms are elongated, boat shaped or needle shaped and are isobilateral symmetry, e.g. *Pinnularia*. The centrales are circular with radial symmetry, e.g. *Cyclotella*.

The main characters that differentiate the Bacillariophyta from other phyla are: (1) diploid nature of vegetative cell, (2) presence of chlorophyll c and chlorophyll a together with fucoxanthin, diatoxanthin, and diadinoxanthin. The usual brown colouration is due to the predominance of carotenoid pigments, (3) silicified nature of cell walls which consist of two highly perforated overlapping pieces, (4) oil and chrysolaminarin as reserved food materials, and (5) the reduction in cell-size occurring during vegetative multiplication.

10.2 CHLOROPHYTA:

10.2.1 Occurrence:

Green (or grass-green) algae occur widely in fresh and marine water habitats. About 90% of Chlorophyceae are fresh water and the remaining 10% are marine. Most of the Ulvaceae and Siphonales grow in sea water. The order Conjugales is exclusively fresh water. The members of Volvocales and Chlorococcales are the components of planktonic flora. The species of *Vaucheria* are commonly attached to rocks in mountain cataracts. *Coleochaete* grows on aquatic plants and grasses. The species of *Cladophora* grow on mollusc shells. The cryo algae are found upon ice and snow. *Chlamydomonas nivalis* is the main cause of red snow in U.S.A. *Chlorella* is an endophytic and grows inside the tissue of *Hydrilla. Cephaleuros virescens* causes 'Tea Rust' on tea plants in Assam and other parts of North-Eastern region. Some of the green algae grow symbiotically in the thalli of lichens along with fungi; these are *Treboxia, Chlorella, Coccomyxa* etc.

10.2.2 Thallus Organization:

The thallus exhibits a great variation in its habit and structure, ranging from a motile or non-motile cell through colonial, filamentous, parenchymatous and siphonous habits to the highly evolved heterotrichous filament. This highest type of specialization seems to be attained in

the genera such as *Draparnaldiopsis* and *Chara* (Charophyta) which have highly differentiated and complicated thallus. In general, the plant body does not show any differentiation into true root, stem and leaves. For this reason, the plant body of the algae is called thallus.

i) **Unicellular Forms:** They may be unicellular motile, e.g., *Chlamydomonas* and unicellular non-motile, e.g., *Chlorella*, *Chlorococcus* etc. Non-motile unicellular forms are called coccoid forms.

ii) **Colonial Forms:** Thallus consists of loose assemblage of cells, mechanically held together in the gelatinous matrix, forming the colony. The colony may be plate like or hollow sphere and it may be motile or non-motile. In motile colonial forms, definite number of cells held together in the mucilaginous sheath and motility is brought about by lashing movement of flagella, e.g. *Gonium sociale* is a 4-celled colony and *G. pectorale* is 16-celled; *Pandorina* is 8-celled, *Eudorina* is 32-celled (Fig.10.1) and *Pleodorina* is 32-128-celled (Fig. 10.2) colonies. *Volvox* is the highest evolved colonial form and consists of 500-50,000 cells. Only a few cells are reproductive and the rest are vegetative colony of definite number of cells arranged in a specific manner and forming an integrated thallus and it is also called as Coenobium.

Figure10.1: *Eudorina elegans*

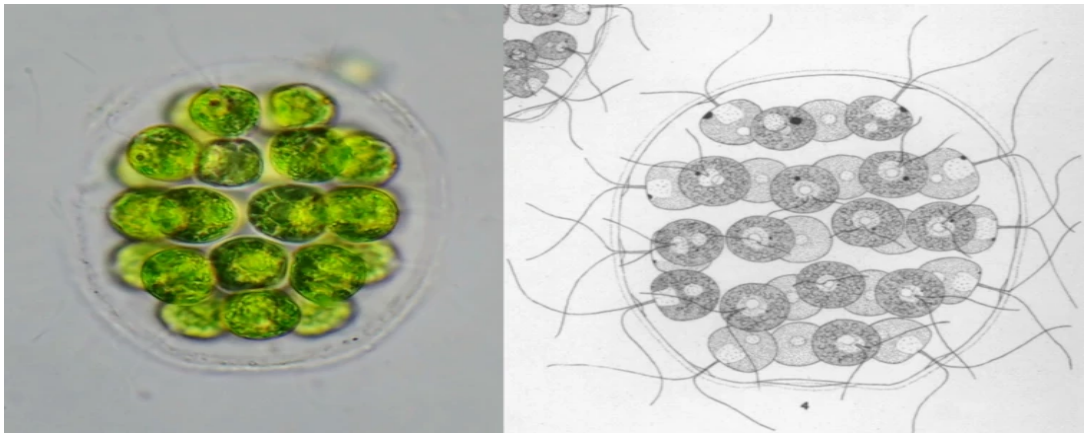


Figure-10.2: *Pleodorina* mature colony

Non-motile colonial forms are: *Hydrodictyon*, *Pediastrum*, *Tetraspora*, *Scenedesmus* etc. They are also called pamelloid forms. In *Chlamydomonas*, the pamelloid phase prevails temporarily.

iii) Siphonous or Coenocytic thallus: In this case, the unicellular thallus is enlarged to form a non-septate multinucleate (coenocytic) sac-like or tubular structure. In *Acetabularia* and *Characium*, single nucleus lies in the base of the stalk; in both the cases as the unicell reaches maturity and enters the reproductive phase, the nucleus undergoes division to form coenocytic condition. It happens for a short time only. Contrary to this, *Protosiphon* is permanently coenocytic.

In *Caulerpa*, the thallus is differentiated into a creeping structure resembles the rhizome and erect leafy shoots (Fig. 10.3). The rhizoids anchor the plant to the substratum. The stolons produce upright fronds which may be leaf-like or cylindrical or in clusters. Both the creeping and upright parts contain mechanical tissues in the form of trabeculae.

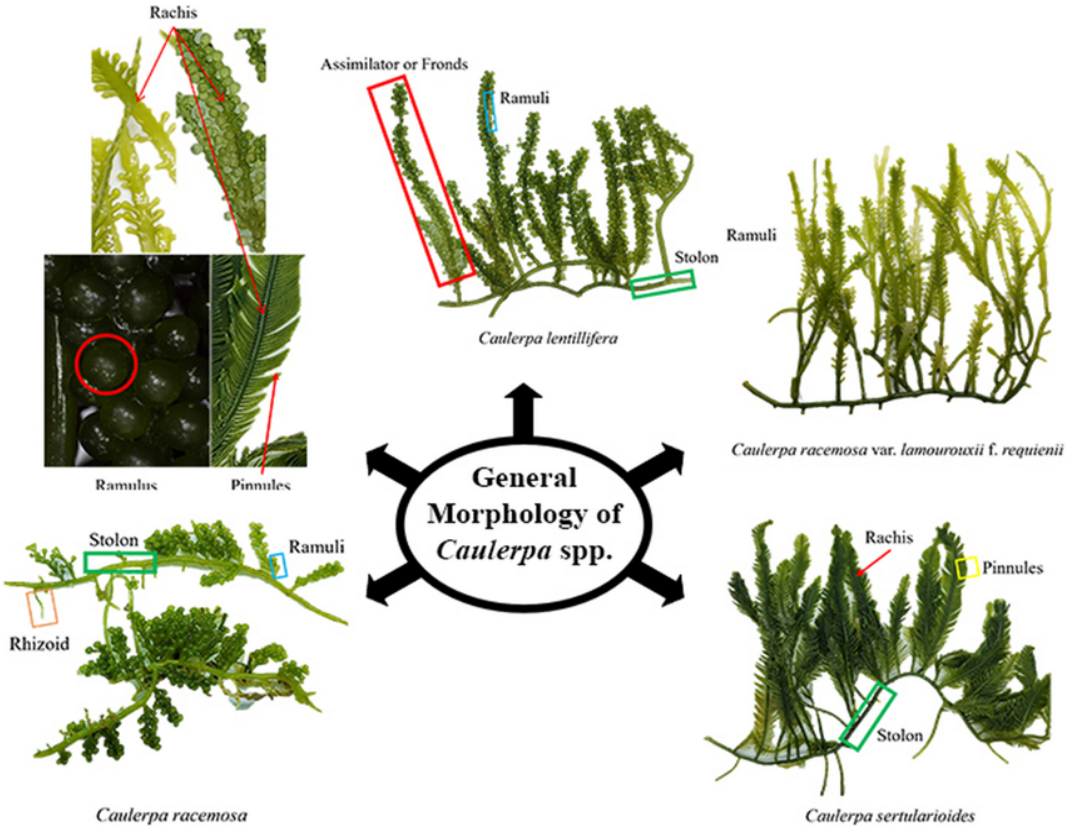


Figure-10.3: *Caulerpa* morphology

iv) Multicellular Filamentous Forms: In these forms, cells are arranged in a linear rows, called the threads or filaments; repeated cell divisions take place in a single plane, resulted into filament. They may be simple unbranched and branched filamentous.

A. Simple unbranched fllamentous forms: They possess a long, thread-like, unbranched filamentous thallus. Like the colonial thallus, all the cells in the filament are alike, self-sufficient and independent-of one another, e.g., *Spirogyra*, *Ulothrix*, *Oedogonium* etc.

The thallus of Ulothrix illustrates a step further in the development of multicellular filamentous forms. As in Spirogyra, the simple filament of *Ulothrix* consists of similar cells but it is attached to the substratum at one end by a rhizoidal cell. Like this, there is a beginning of differentiation of cells accompanied by slight division of labour.

In *Oedogonium*, thallus represents a step still further in the differentiation of cells accompanied by division of labour. It is a simple multicellular filament with a branched or lobed rhizoidal cell. The rhizoidal cell is modified to form a more complex holdfast than the *Ulothrix*. The holdfast is expanded into a flattened disc with outgrowths. Besides, the filament consists of green vegetative cells for nutrition, cap cells for cell division, zoospore formation, and highly specialized reproductive organs, such as antheridia and Oogonia (Fig. 10.4; 10.5).

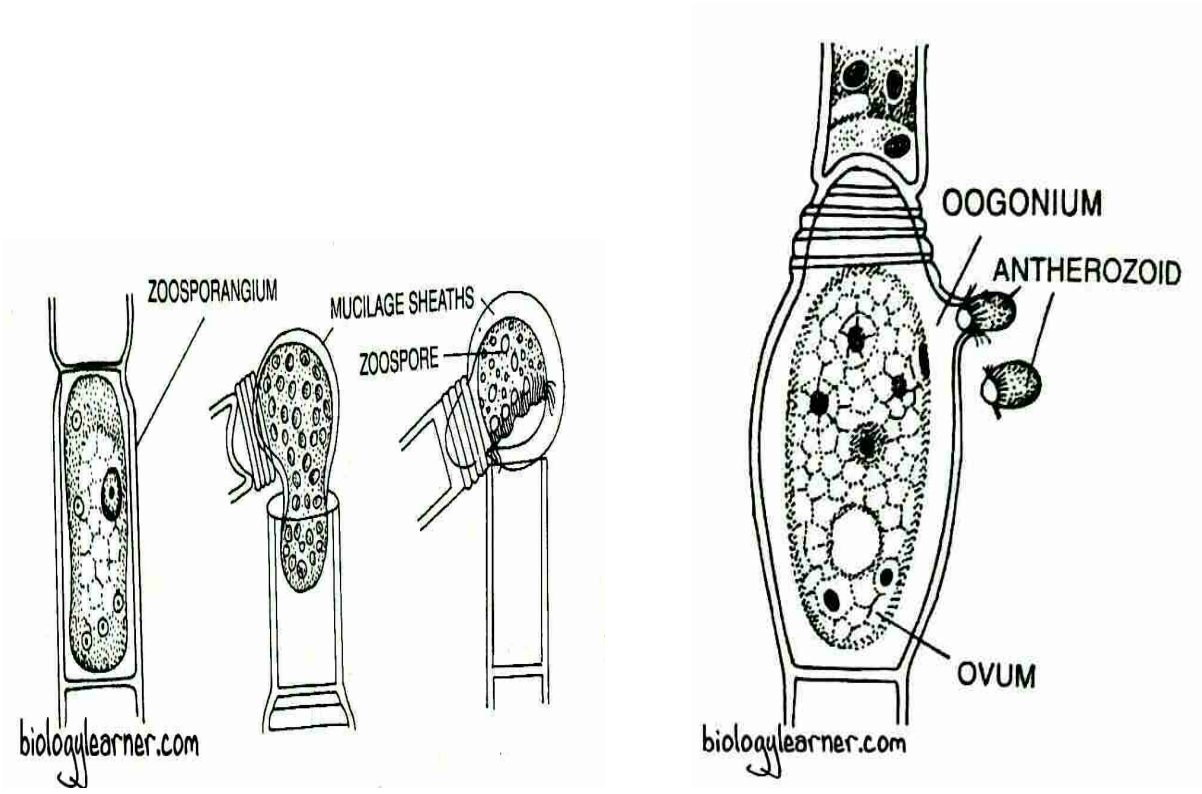


Figure-10.4: *Oedogonium*. A. Zoospore formation; B. Zoospore with crown of flagella; C. Oogamous reproduction in homothallic macrandrous form

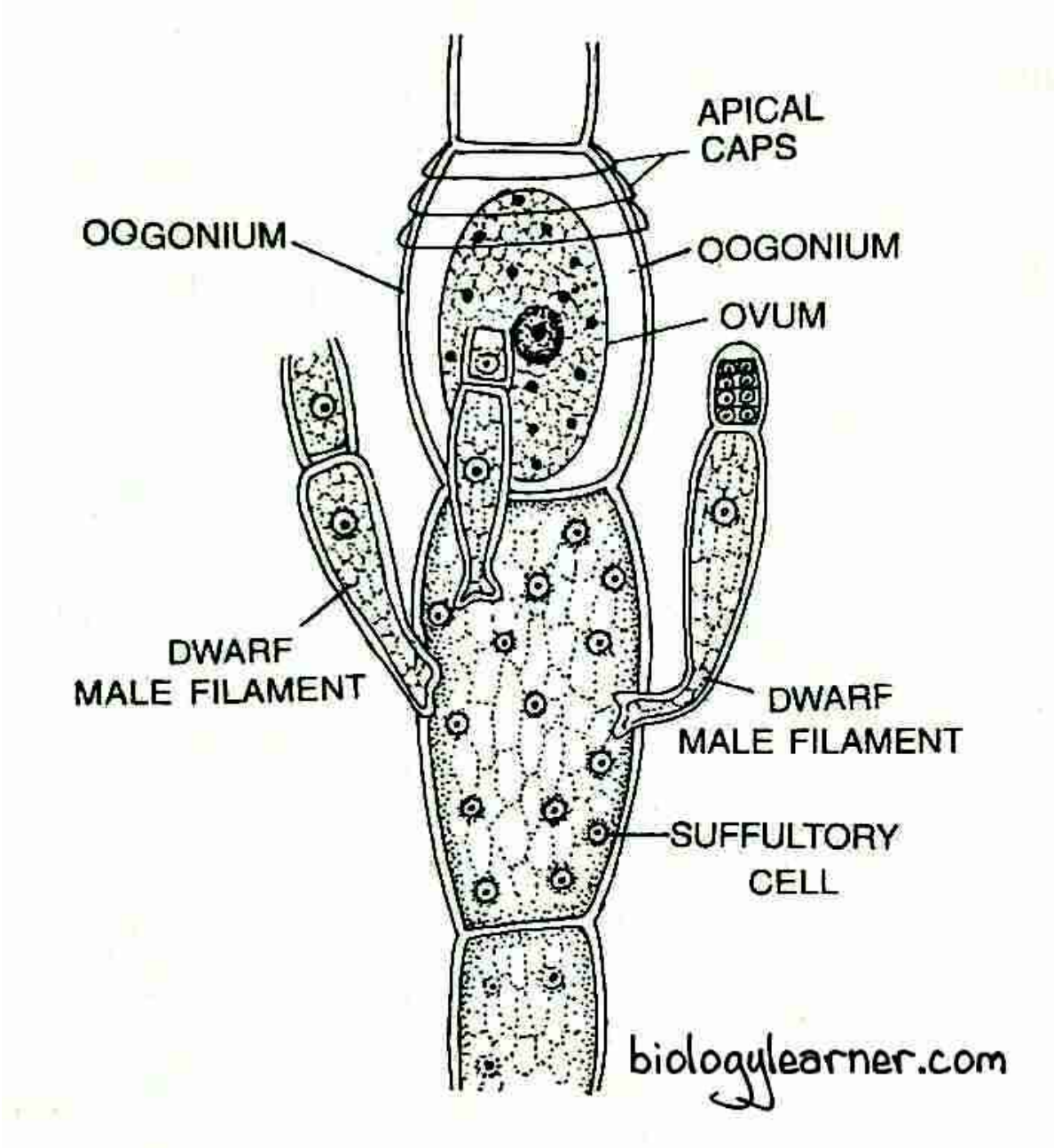


Figure-10.5: Nanandrous species of *Oedogonium*

B. Branched filamentous forms: In these forms, thallus is branched filament e.g., *Bulbochaete*, *Chaetophora*, *Cladophora* etc. It is considered as much more advanced multicellular thallus among green algae. The cells in the main filament undergo divisions and give rise branches. *Cladophora* is profusely branched and attached to the substratum by septate rhizoid growing down from the basal part of the filament.

The cells are large, cylindrical, multinucleate and have an elaborate chloroplast which may form a continuous reticulate network in younger cells but may become parietal in older ones (Fig.10.6).

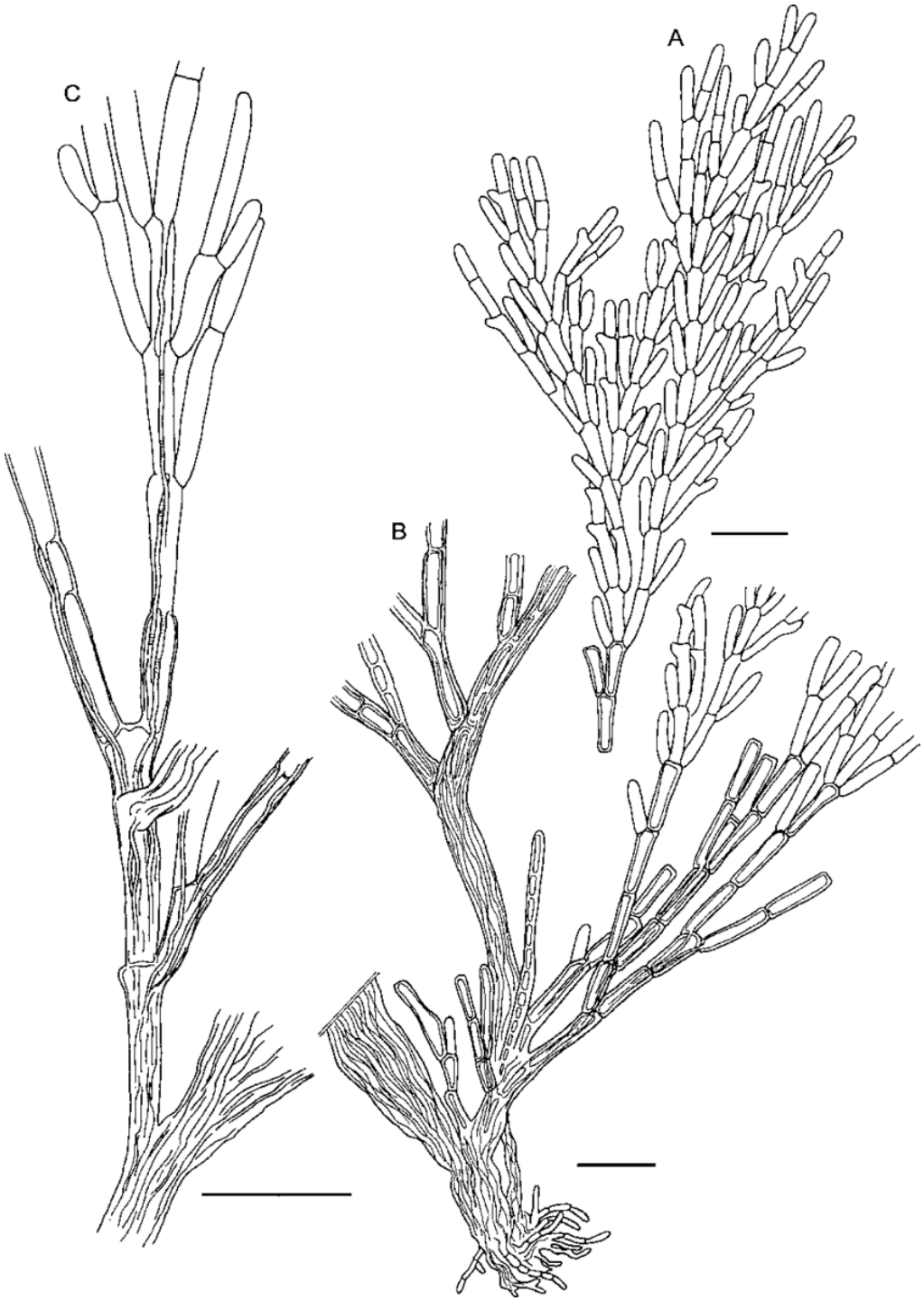


Figure-10.6: Cladophora horii (KZN 356). A. Terminal branch systems; B. Base of thallus with stemlike branches; C. Cells in basal part of the thallus producing rhizoids at their basal poles growing along and into the cell walls of the cells below:

C. Foliaceous Forms: In these forms, the cells divide in more than one plane and give rise thin, flat and plate - like thallus, e.g., *Ulva* (Fig. 10.7 1-2). The thallus is macroscopic and composed of two layers of parenchyma cells. Several multinucleate rhizoids arise from the lower cells of the thallus and attach to the substratum. These rhizoids may penetrate between two layers of the thallus (Fig. 10.7) and finally attach to the substratum.

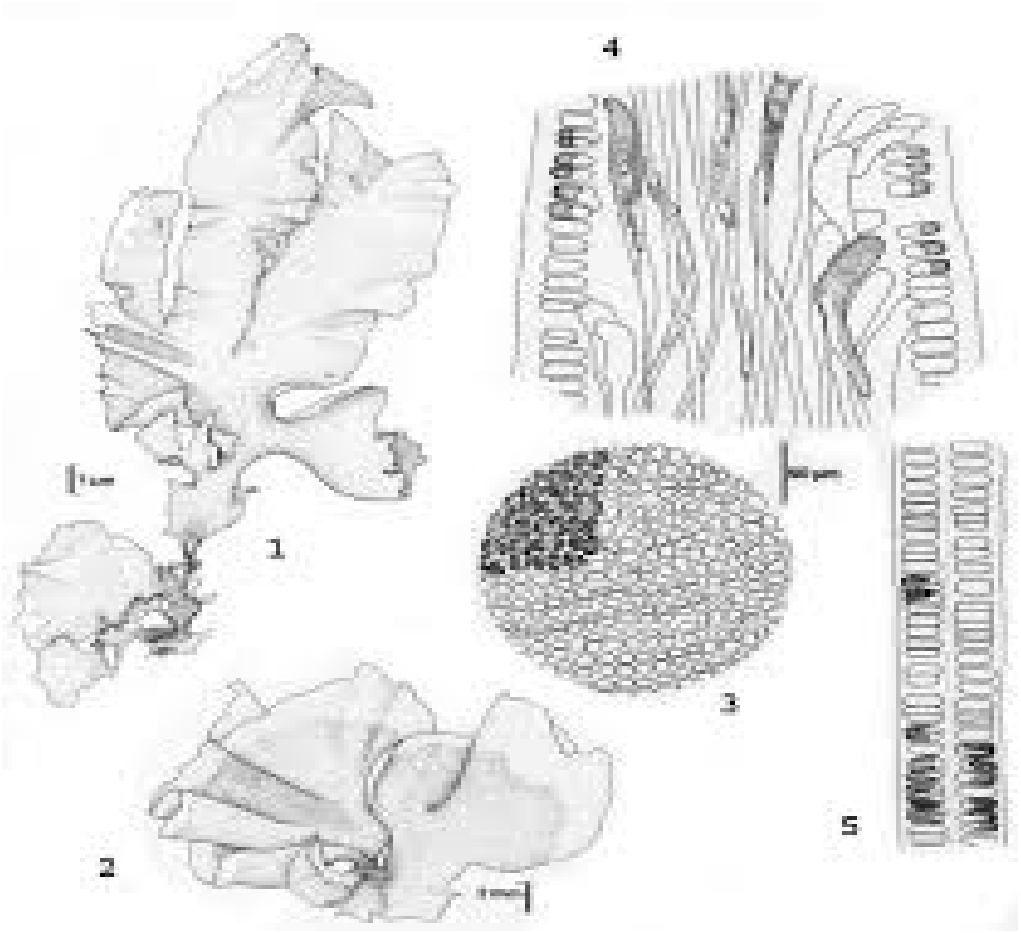


Figure-10.7: *Ulva* 1. Thallus, 2. Longitudinal section

D. Heterotrichous Filamentous Forms: Heterotrichous filamentous thallus is highly evolved habit in Chlorophyta. The members of Chaetophorales possess heterotrichous filamentous thallus, e.g., *Coleochaete*, *Draparnaldia*, *Draparnaldiopsis*, *Stigeoclonium* etc. In the heterotrichous habit, the thallus is distinguishable into a basal prostrate system and an erect system (upright system) composed of branched threads.

In *Stigeoclonium* and *Fritschiella*, both prostrate and erect systems are well developed. In *Stigeoclonium*, the prostrate system is generally formed of short cells (Fig.10.8) and filaments exhibit apical growth. Sometimes prostrate system is profusely branched to form a compact pseudo-parenchymatous disc of one layered stratum. The erect system composed of alternate or opposite, profusely or sparsely branched filaments (Fig. 10.8). The lateral branches alternate and terminate into long, multicellular and hyaline hairs. In *Fritschiella*, the thallus remains differentiated into rhizoidal system, prostrate system, primary projecting and secondary projecting systems.

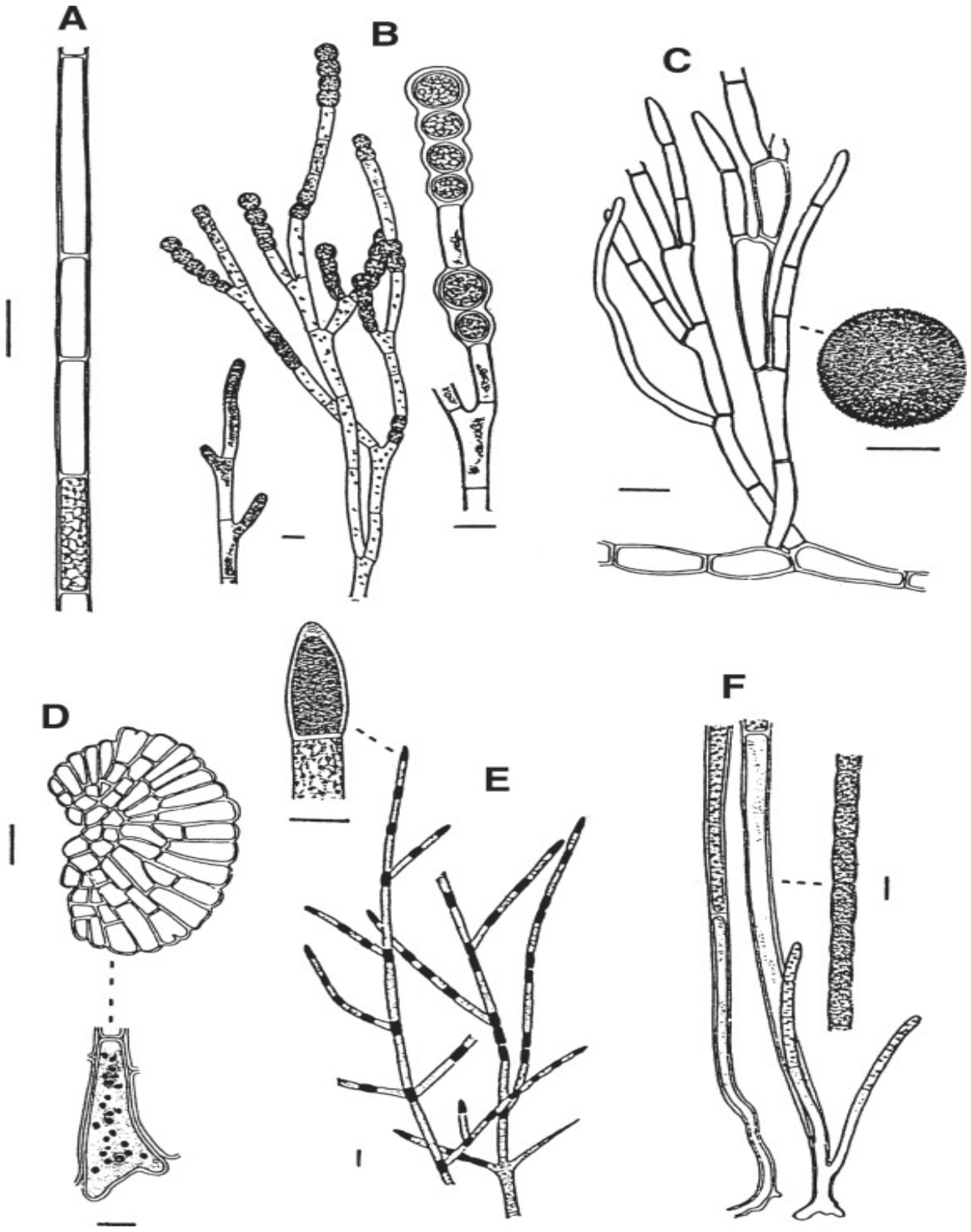


Figure-10.8: *Stigeoclonium*, thallus showing prostrate and erect systems. The heterotrichous habit has undergone a variety of modifications by reduction or elimination of one or other of the system.

In *Coleochaete*, there is a disappearance of erect system resulted in the discoid type of thallus, e.g., *C. orbicularis*, *C. scutata* (Fig. 10.9), *C. soluta* etc. Discoidal thallus consists of

loosely arranged branched filaments distinct from one another. However, in *C. pulvinata*, the thallus is a typical heterotrichous.

In both *Draparnaldia* and *Draparnaldiopsis* there is a complete disappearance of prostrate system and a corresponding elaborate development of the erect (projecting) system. In these genera, the prostrate system is vestigial and represented by a holdfast. *Draparnaldia* is characterized by the (i) presence of a central axis consisting of large barrel-shaped cells, (ii) highly branched whorls of laterals (1imited growth) consisting of much shorter cells, (iii) branches terminating into pointed cells or long, hair-like setae, (iv) parietal, plate-like chloroplast with a single pyrenoid and the soft copious mucilage around the entire thallus.

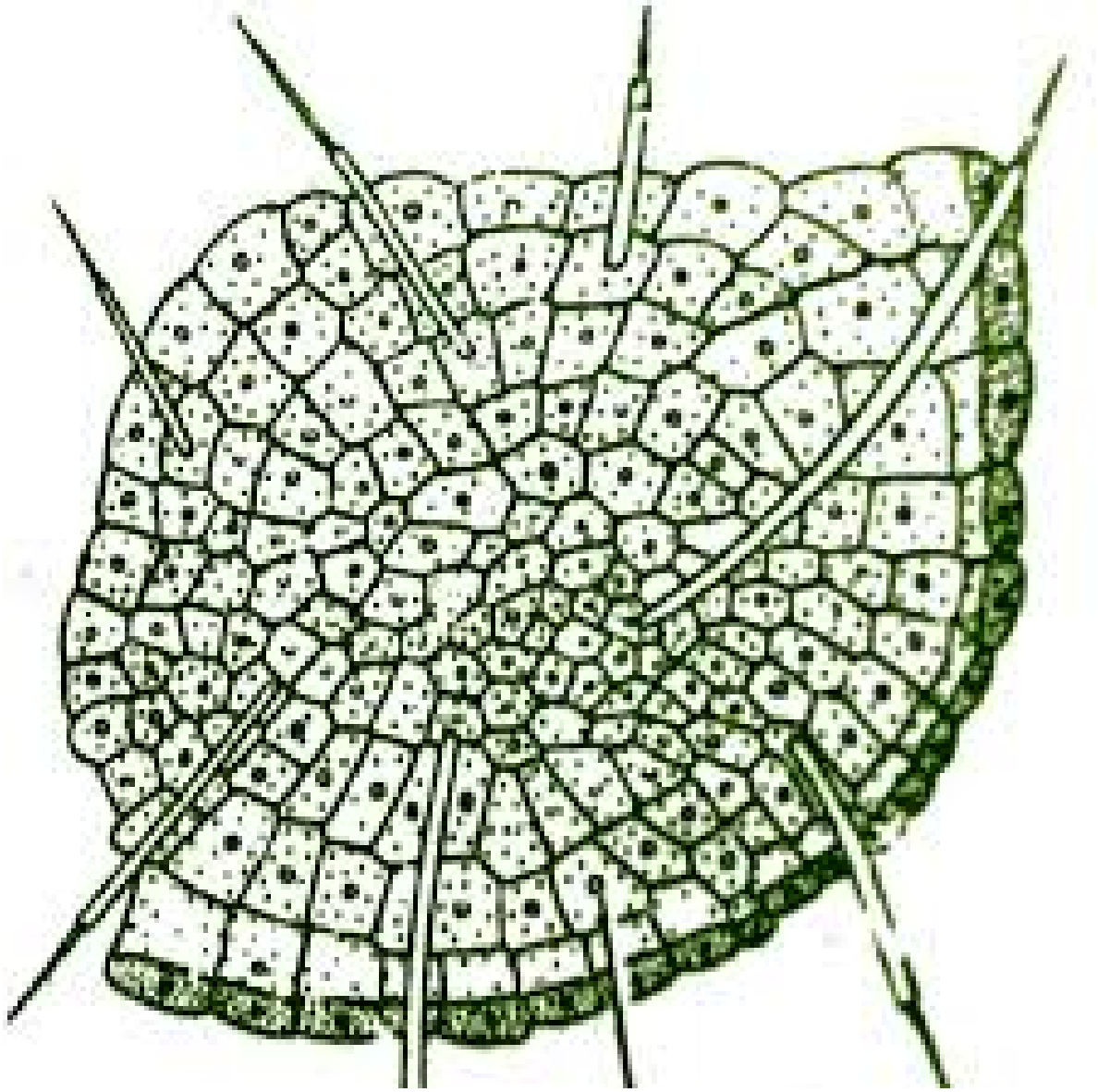


Figure-10.9: *Coleochaete scutata* thallus showing sheathed setae

The thallus of *Draparnaldiopsis* shows further advancement than the *Draparnaldia*. The main filament of *Draparnaldiopsis* is made up of two kinds of cells, long internodal cells and

short nodal cells. Nodal cells alone bear the laterals of limited growth and also lateral of unlimited growth. The cell possesses a single parietal chloroplast with several pyrenoids.

10.2.3 Reproduction

The members of Chlorophyceae are represented by the vegetative, asexual and sexual types of reproduction.

(i) Vegetative Reproduction:

A. Fission: Unicellular organisms produce their progeny through fission, e.g., *Chlamydomonas*, *Pleurococcus* etc.

B. Fragmentation: It is very common in filamentous forms, such as *Oedogonium*, *Ulothrix*, *Sphaeroplea*, *Spirogyra* etc.

C. Akinetes (Resting spores): In some green algae, e.g., *Cladophora*, *Pithophora*, *Ulothrix* etc., the protoplast is rounded-off and covered with very thick cell wall. These cells with abundant reserve food materials are called akinetes. They are capable of withstanding unfavourable environmental conditions. During favourable conditions, akinete germinates liberating a young germling.

(ii) Asexual Reproduction:

a) **Zoospore Formation**: Zoospore production is more common in Chlorophyceae. Zoospore is biflagellate in *Chlamydomonas* and quadriflagellate in *Draparnaldiopsis*, *Fritschiella*, *Ulothrix* etc. In *Oedogonium*, a crown of flagella are present. Zoospores may be produced singly (*Oedogonium*) or in twos, or in still larger number within a vegetative cell or sporangium. Zoospores possess eye spot and chloroplast, and motility is brought about by the lashing movement of flagella. Each zoospore on coming in contact with a suitable substratum and under favourable conditions develops into a new adult individual.

b) **Aplanospore**: In certain cases, aplanospores (non-motile) are formed singly within a cell. Unlike zoospores, protoplasts become rounded, secrete a wall of their own and are non-flagellate and non-motile. Aplanospores occur in *Ulothrix* and also in other genera.

c) **Autospores**: In a few genera, aplanospores have almost similar distinctive shape as the parent cell and are called autos pores. These are commonly found in the order Chlorococcales.

(iii) Sexual Reproduction:

Sexual reproduction is of three types, (A) isogamy, (B) anisogamy, and (C) oogamy.

a) **Isogamy** - In majority of Chlorophyta, sexual reproduction is isogamous in which the two fusing gametes are equal in size and have identical morphology and physiology, e.g., *Chlamydomonas*, *Spirogyra*, *Ulothrix*, *Zygnema* etc. The resultant fusion product is known as zygospore.

b) **Anisogamy** - In this, the fusing gametes are not identical morphologically and physiologically. Active male gametes are of smaller in size as compared to the larger female gametes which are less active, e.g. *Chlamydomonas braunii*.

c) **Oogamy** - Oogamous reproduction involves the fusion between a small flagellate, actively motile male gamete (antherozoid) and, a large, non-motile, passive female gamete, the ovum or oosphere. The ovum is generally produced singly within a specially differentiated and enlarged cell called the oogonium, e.g., *Oedogonium* and *Coleochaete*. The antherozoids are many and produced within an antheridium.

In Oedogonium, there are two forms, viz., (1) macrimdrous, and (2) nanandrous. The macrandrous forms may be monoecious producing antheridia and oogonia on the same plant, or dioecious, producing antheridia and oogonia on different individuals. In the monandrous forms, the sexual plants are diploid; the oogonia are formed on filaments of normal size, whereas the antheridia are produced on filaments known as dwarf males or nanandria. The nanandria are extremely small and ale always found attached to oogonia proper or to the underlying cell. These nanandria are derived from antherozoid-Iike zoospores, known as androspores, which are formed singly within the androsporangia.

A species that bears both oogonia and androsporangia is described as gynandrosporous, whereas, the one that bears them on different filaments are called idioandrosporous, after their liberation and brief swimming, androspores settle on an oogonium or a suffultory cell, and then germinate to give rise nanandria. Each antheridium of the dwarf male generally produces two antherozoids. There has been an evolutionary advancement in sexual reproduction accompanying the reduction in number and an increase in the size of the female gametes. This evolution perhaps occurred independently in different orders of the Chlorophyta. Progressive evolutionary series illustrating the transition from isogamy to oogamy are met within the coenobial Volvocales and in certain Chaetophoraceae, e.g., *Aphanochaete*, *Chaetonema*, *Coleochaete* and *Stigeoclonium*.

10.2.4 Life Cycles:

The following types of life cycles are encountered in Chlorophyceae members.

1) Haplontic Life Cycle:

The Chlorophyceae members, such as *Coleochaete*, *Oedogonium*, *Pandorina* and *Ulothrix* which are haploid, with the zygote representing the only diploid phase in the life cycle (Fig. 10.10). In these, meiosis occurs during the first division of the zygote nucleus.

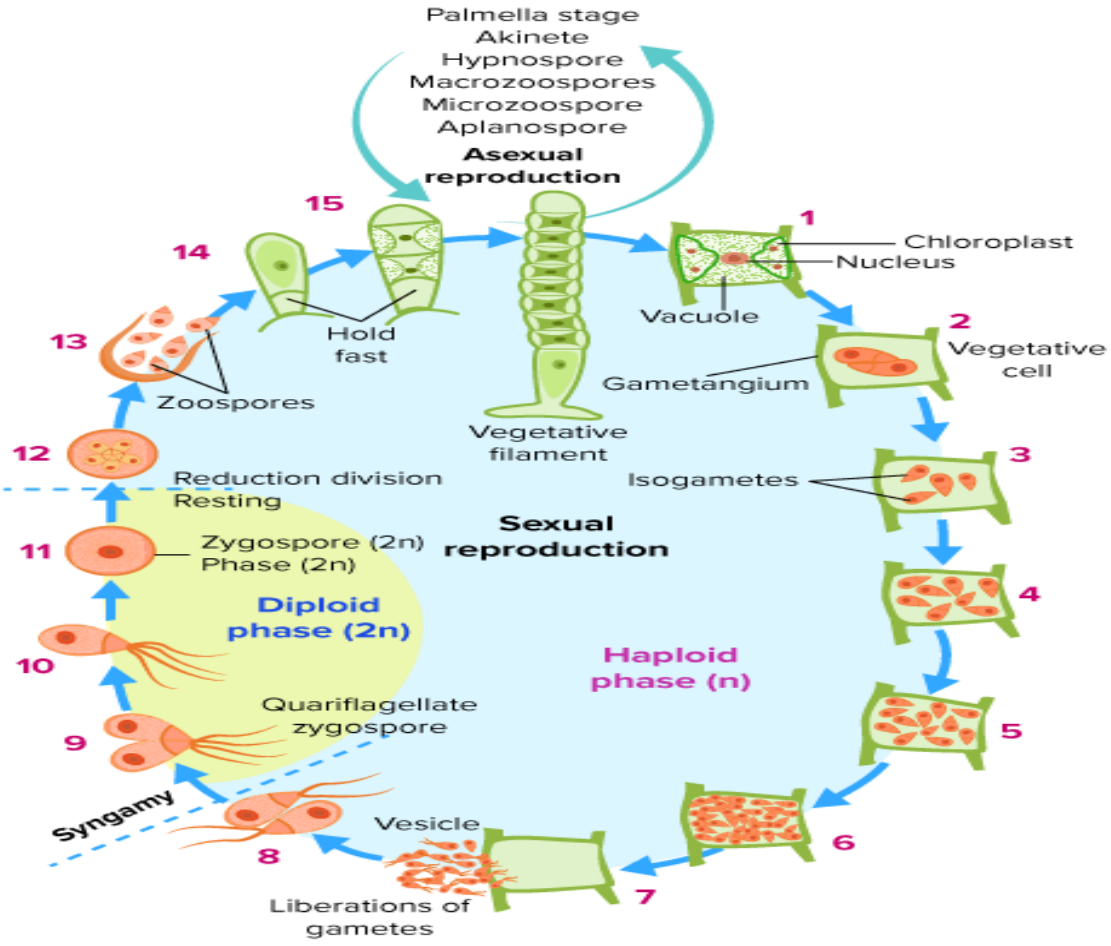


Figure-10.10: Haplontic life cycle of *Ulothrix*

2) Diplontic Life Cycle:

In the diploid forms belonging to Siphonales and Chlorococcales, the vegetative plant is diploid and meiosis occurs during the formation of gametes which represent only haploid stage. The zygote germinates directly into the diploid plant (Fig. 10.11).

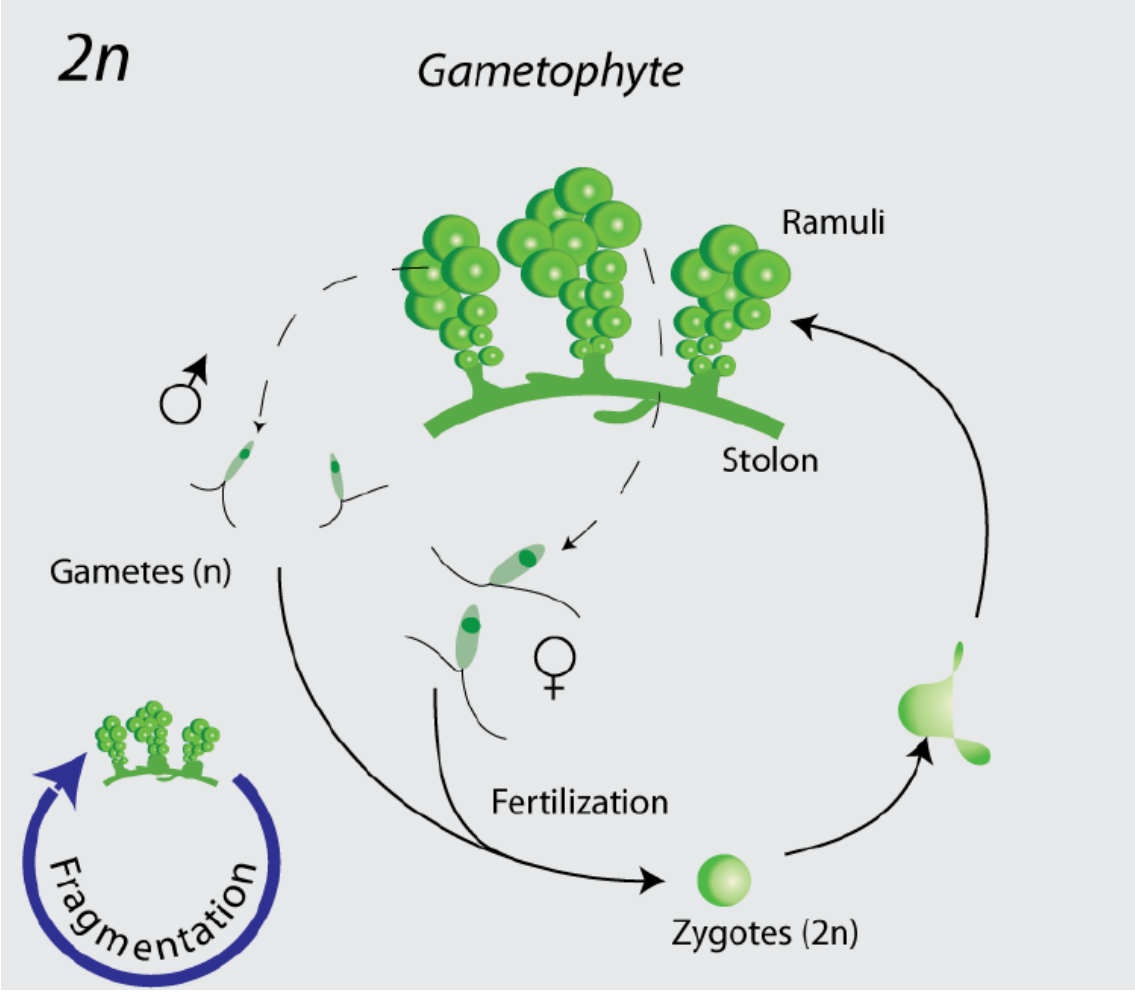


Figure-10.11: Diplontic life cycle of *Caulerpa*

3) Isomorphic Life Cycle:

This involves an alternation between the haploid gametophyte and the diploid sporophyte, both of which are morphologically indistinguishable from each other. The haploid phase produces gametes that fuse to form a zygote which in turn germinates into a diploid sporophytic plant. This sporophyte produces zoospores. Prior to zoospore formation meiosis takes place, e.g., members of Chaetophoraceae, Cladophoraceae (Fig. 10.12) and Ulvaceae.

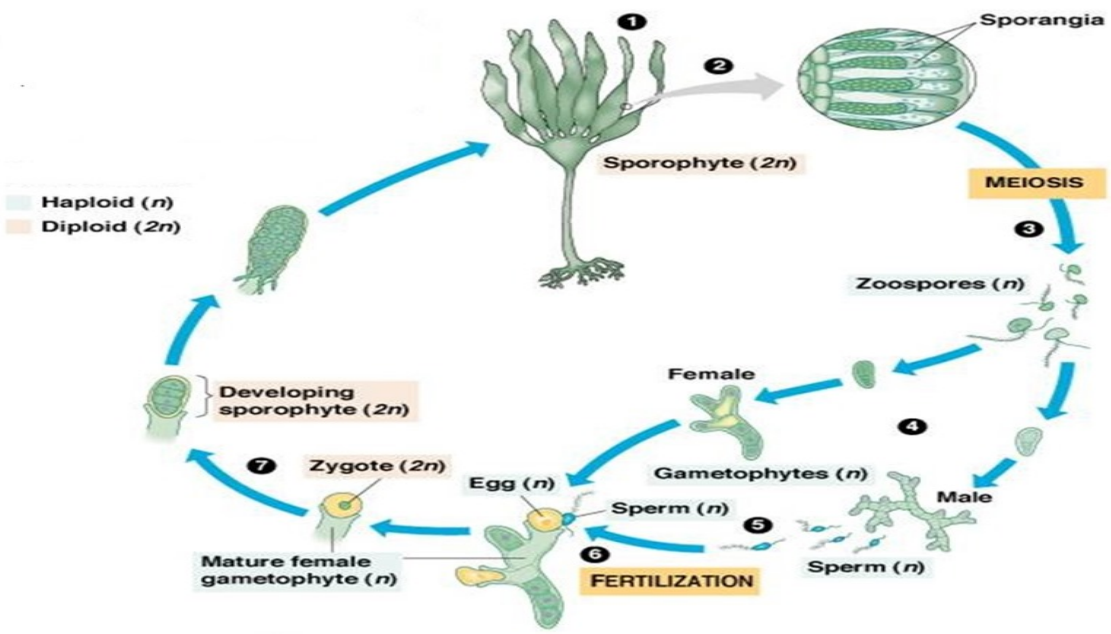


Figure-10.12: Isomorphic life cycle of *Cladophora*

4) Heteromorphic Life Cycle:

This involves an alternation between the morphologically dissimilar haploid gametophyte and diploid sporophyte; e.g., *Urospora*. In *Urospora* gametophyte is dominant and the sporophyte is known as *Codiolum*.

10.3 BACILLARIOPHYTA (DIATOMS):

10.3.1 Occurrence:

Diatoms are cosmopolitan and ubiquitous in distribution. They are the major components of the planktonic vegetation. The most common genera of freshwater habitats are *Asierionella*, *Melosira*, *Navicula*, *Nitzschia* and *Synedra*. Species of *Cocconeis*, *Eunotia* and *Gomphonema* grow epiphytically on other fresh water algae such as *Cladophora* and *Oedogonium*. Species of *Triceratium* and *Hyalodiscus* occur in the littoral and sub-littoral zones as epiphytes on seaweeds. Some marine diatoms are parasitized by the dinoflagellate, *Paulsenella*.

10.3.2 Thallus Organization:

The thalli of diatoms are unicellular, colonial or filamentous. With respect to their shape and valve morphology, the unicellular diatoms have been classified into two orders, Pennales with isobilateral symmetry, e.g., *Pinnularia* (Fig. 10.13A) and Centrales with radial symmetry, e.g., *Cyclotella* (Fig.10.13B). *Triceratium* (Fig.10.13C) has three planes of mirror symmetry.

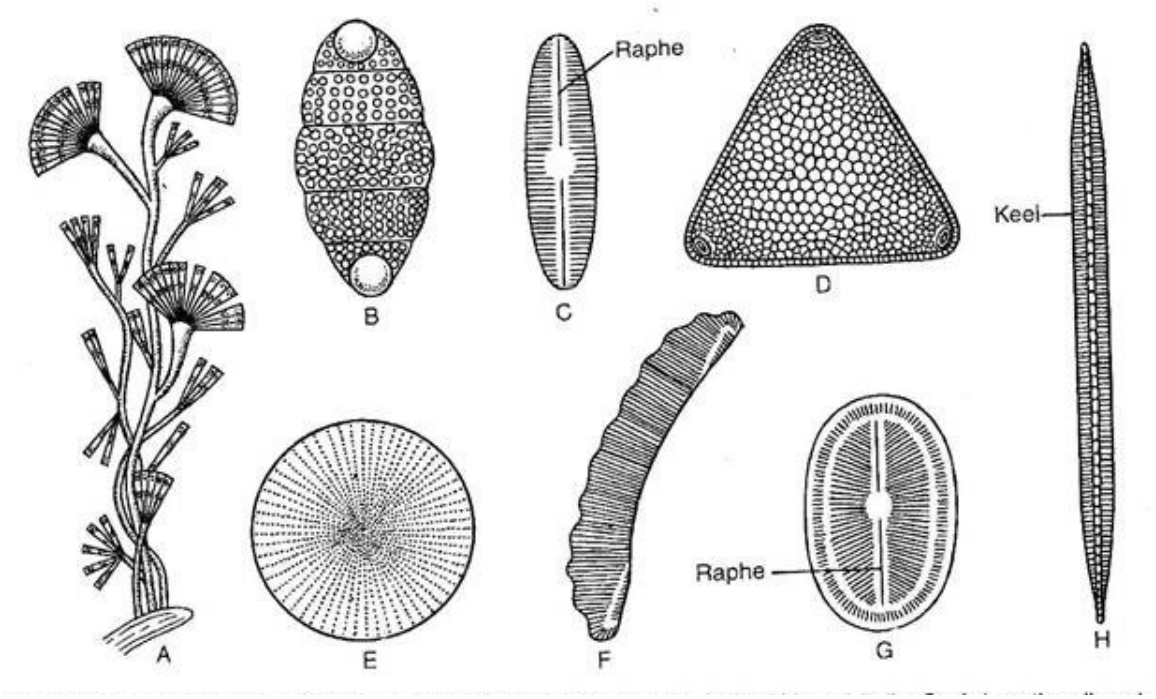


Figure-10.13: Some common diatoms. A. *Pinnularia* B. *Cyclotella* C. *Triceratium*

Further classification of Centrales is based on the presence or absence of the bristles or horns on the cell structure. Pennales are classified according to the presence or absence of raphes (slits) and number and. morphology of raphes on the valves. These raphes run between the median and the apical pores. Colonial diatoms are organized into uniseriate filaments, e.g., *Melosira*. The valve-to-valve connections between the cells of *Navicula confervacea* form filaments maintained by organic material adhering to the centres of the valve faces. Stellate colonies result from the union of cells at their basal ends through localized production of mucilage, as in *Asterionella*.

(l) Valve morphology:

The fine markings found on the surface of the valve vary widely. According to Hendey (1959) four types of secondary structures are present en diatom valves:

a) The punctae are fine perforations arranged in regular rows corresponding to the markings or, striae on the valve surface.

b) The areole, which are cavity-like depressions, coarser and larger than the punctae.

c) The canaliculi, which are tubular canals running through the valve surface.

d) The costae, which are specially thickened regions of the valve, resulting from the heavy accumulation of silica, and represent the valvar ribs. The ribs constitute the backbones of the cell wall.

In Pennate diatoms, the markings are arranged longitudinally, e.g., *Pinnularia, Cymbella* etc., whereas in centric diatoms, these are distributed concentrically, e.g., *Arachnoidiscus.*

(ii) Raphe and Locomotion:

In pennate diatoms, there is a longitudinal slit, known as raphe, which is interrupted in the middle by a central nodule formed by the internal thickenings of the valve. In centric diatoms, raphe is absent, instead, one or more projections, known as labiate processes (merely openings through the valve) are present. The locomotion is brought about by the secretion of mucilage through the labiate processes in centrales. In pennales both locomotion and anchorage involve the secretion of mucopolysaccharide material through raphe.

10.3.3 Cell Structure:

Cell Wall:

The cell wall (frustule) of diatoms consists of two overlapping halves, the upper half is known as epitheca and lower one hypotheca (Fig. 10.14A). Each theca possesses the main surface known as valve and this valve has incurved margins called as connecting band (Cingulum). Overlapping region of epitheca and hypotheca is collectively referred to as girdle. The frustule is enriched with amorphous silica, that may also have small amounts of aluminium, magnesium, iron and titanium. The epitheca and hypothec a can be compared to a petriplate. The lid is corresponding to the epitheca and the main body is compared to hypotheca. The two connecting bands represent the curved sides of the lid and the main body, whereas the valve relates to the top or bottom of the petriplate. Accordingly a cell can be seen from two different views, the girdle view diatom appears rectangular (Fig. 10.14B) and varve view (shape variable).

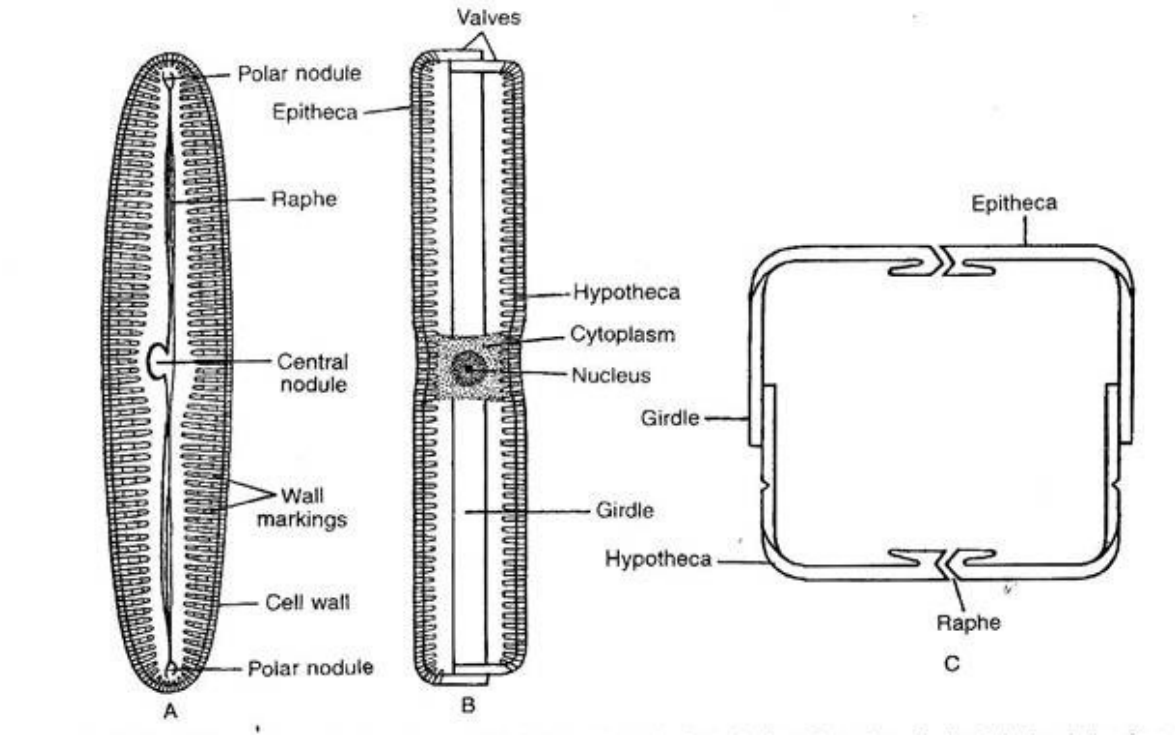


Figure-10.14: *Pinnularia*. A. Transverse section showing relative position of epitheca, hypotheca, connecting band, valve, and raphe; B. Girdle view showing relative position of epitheca and hypotheca.

Protoplast:

Thin layer of cytoplasm surrounding the large vacuole is bounded by plasmamembrane. The cytoplasm is thicker at the region of poles. The cells are uninucleated. In pennales, a single large nucleus is located across the middle of the central vacuole and is connected with the lining layer of the cytoplasm next to the cell wall. In centrales, nucleus occupies the position within the peripheral cytoplasm lining the cell wall. The cytoplasm also includes other cell organelles such as mitochondria, dictyosomes and endoplasmic reticulum. The nuclear division is characteristic of this group in possessing intranuclear and cylindrical spindles with flat ends. In resting condition, the nucleus possesses a number, of small chromatin granules and one to several nucleoli. Further, the chromosomes do not form an equatorial ring during the metaphase.

Chromatophores:

The number and shape of chromatophores varies in this group. In centric diatoms, chromatophores are few and medium sized, and discoidal in shape. In pennales, one or two large parietal chromatophores with irregular lobes are present. Chromatophore is a double membrane organelle consisting of 4-6 lamellae with or without pyrenoids. Chromatophores are olive green to yellowish green in colour, with chlorophyll a and chlorophyllc.

10.3.4 Reproduction:

Both vegetative and sexual reproductions are evidenced in diatoms.

(i) Vegetative multiplication and McDonald-Pfitzer rule. Cell division is the common method of vegetative reproduction. During the process of cell division, the parent cell becomes enlarged, and nucleus divides mitotically. Chromatophores also divide longitudinally and daughter chromatophores come to lie on each' half. After duplication of cell organelles, the cytoplasm cleaves in the middle along the girdle in a plane parallel to the valve faces. This cleavage proceeds centripetally. This resulted into formation of two daughter protoplasts, each one lying in each parental theca. The parental hypotheca serves as the epitheca of one of the two daughter cells whereas the parental epitheca remains as the epitheca of the other daughter cell. Accordingly, the newly formed wall pieces always serve as hypotheca.

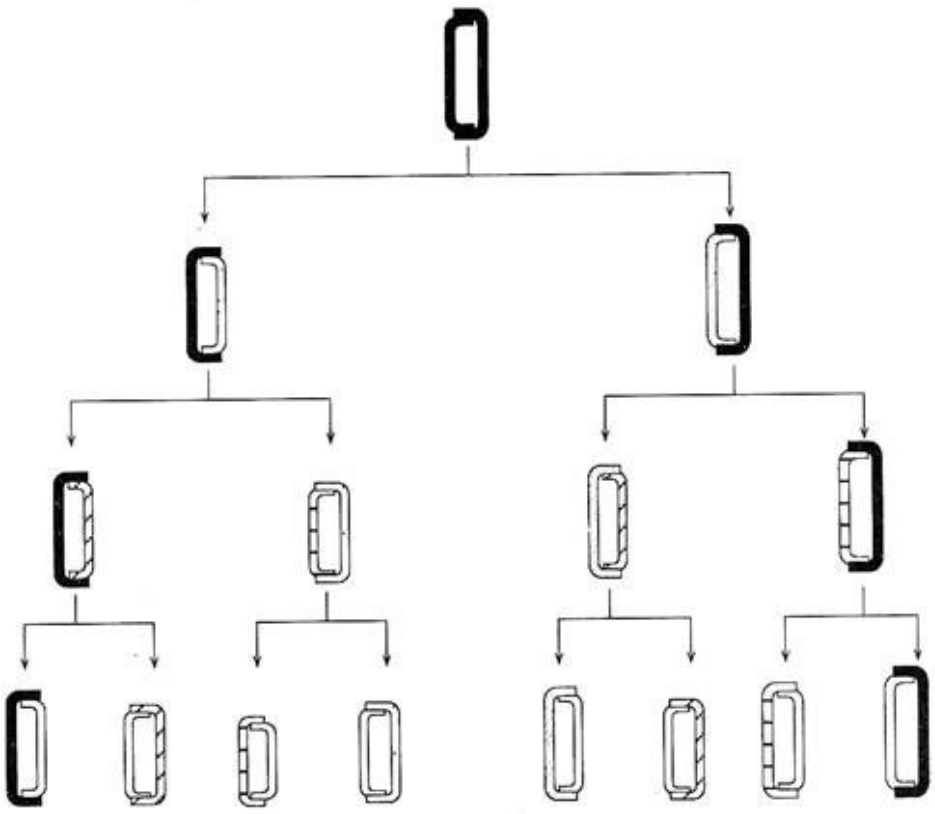


Fig. 3.102 : Cell division in diatoms (diagrammatic) showing reduction in cell size in successive generations except one (extreme right)

Figure-10.15: Dimensions of cell size in successive generation of a diatom (based on Smith, 1955)

McDonald-Pfitzer rule relates to the phenomenon of gradual size reduction in diatom during vegetative cell division. In the above process, two unequal sizes of daughter cells are formed. The one daughter cell which retains the parental epitheca, has the same size as of the parental

cell; whereas the other daughter cell retaining the parental hypotheca which serves as a epitheca, is smaller in its size than parental cell. Like this, the progenies of diatoms become progressively smaller during successive cell divisions.

Table-10.1: Differences between Centrales and Pennales

· Centrales	Pennales .
1 2,500 species spreading over to 100 genera	1 3,000 species spreading over to 70 genera
2 Widely distributed	2 Mostly freshwater forms
3 Cells' circular and radially symmetrical symmetrical	3 Cells elongated and bilaterally"
4 Cell walls with coarse markings	4 Fine markings with punctae
5 Chromatophores many and discoid	5 Chlromatophores 1 or 2 laminate or lobed
6 Nucleus lies in the peripheral cytoplasm connecting the two valves	6 Nucleus in the cytoplasmic bridge
7 Sexual reproduction- oogamous; flagellated spermatozoids amoeboid gametes	7 Sexual reproduction - isogamous;
8 No movement	8 Slow jerky movement
e.g., Cyclotella, Melosira, Cosinodiscus, Biddulphia.	e.g., Navicula, Pinnularia, Cocconies, Svnedra, Surirella, Nitzschia.

(ii) Sexual Reproduction:

The pattern of sexual reproduction differs in both orders - Pennales and Centrales. During this process, auxospore is formed in both the groups. During cell division, those cells become reduced in size, are able to regain their normal size through the formation of auxospore, so it is a “restorative process” rather than multiplication.

Auxospore Formation in Pennales:

It takes place through gametic union, autogamy and parthenogenesis.

These are of the Following Types:

1) Production of one auxospore by two conjugating cells. In this process two uniting cells come very close to each other and become covered by a mucilaginous sheath. The diploid nucleus of each cell undergoes meiosis. Out of four nuclei, three degenerate and only one survives. The surviving nucleus behaves as gamete (n). The gametes come out from the parent frustules and unite together, to form a zygote (2n).

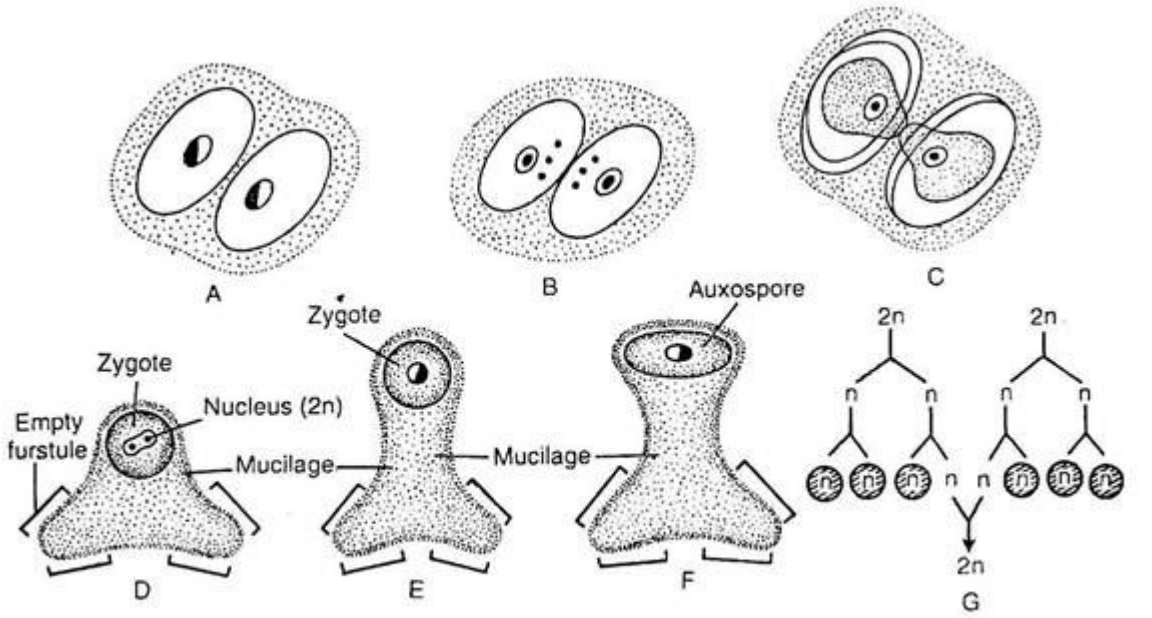


Fig. 3.103 : A-F. Production of one auxospore by two conjugating cells of *Cocconeis placentula*, and G. Nuclear behaviour during reproduction

Fig, 10.16

After a short period of rest the zygote elongates considerably and functions as an auxospore. The auxospore projects out from the parent frustules along with mucilage and elongates in a plane parallel to the long axis of the parent diatom. The auxospore is enclosed in a pectic membrane, the perizonium. The auxospore then develops new frustule inside the perizonium. Thus new diatom cell is formed which regains the normal size. It is found in *Cocconis placentula, Surirella saxonica* etc.

2) Production of two auxospores by two Conjugating Cells:

This is a very common process of auxospore formation. In this process the conjugating cells come very close to each other and get enclosed by mucilage. The nucleus (2n) of each cell undergoes meiotic division and forms four nuclei. Out of four nuclei, two degenerate, the rest two survive. The cytoplasm then divides either equally or unequally and along with one nucleus they behave as gametes. Thus two gametes are formed in each cell.

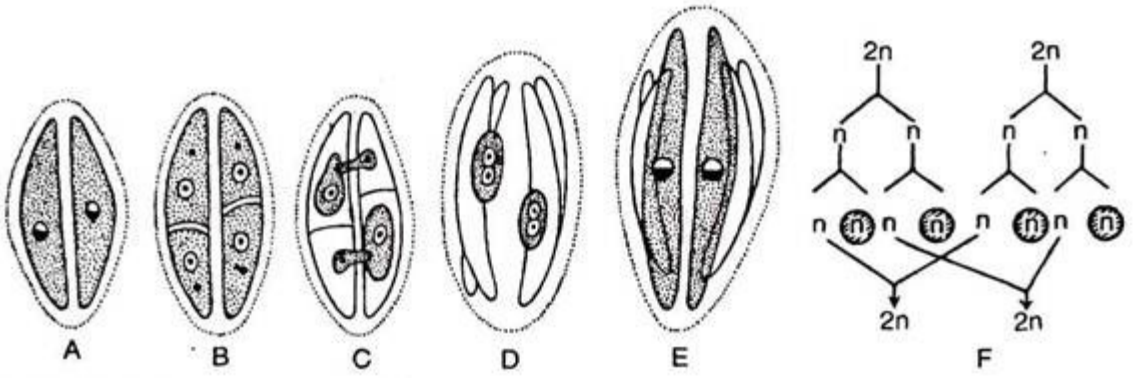


Fig. 3.104 : *Cymbella lanceolata* : A-E. Production of two auxospore by two conjugating individuals, and F. Nuclear behaviour during reproduction

Fig. 10.17

The pattern of union between the gametes varies from species to species. Both the gametes of a cell may be active and fuse with the gametes of other cell, thus two zygotes are produced in a single cell or out of two, one becomes active and fertilizes with the opposite one and thus one zygote is produced in each cell. The zygotes elongate and function as auxospores. The auxospores develop the perizonium around themselves and both of them develop new frustules on their outer sides i.e., inside the perizonium. Thus two diatom cells of normal size are formed. It is found in *Cymbella lanceolata*, *Gomphomema parvulum* etc.

3) Production of One Auxospore by One Cell:

This process of auxospore formation is called Paedogamy (Pedogamy). In this process, the diploid nuclei of a vegetative cell undergo meiosis and form four haploid nuclei. Out of the four nuclei two partially degenerate. Each of the rest two along with the cytoplasm and one partially degenerated nucleus behaves as gamete. Later on, the union between the two sister gametes takes place and forms the zygote. The zygote comes out from the parent frustule and behaves as an auxospore. The auxospore then gets covered by perizonium and develops wall inside the perizonium. Thus one diatom cell of normal size is formed.

4) Production of One Auxospore by Autogamy:

In this process the diploid nucleus undergoes first meiotic division. Thus two haploid nuclei are formed. The two nuclei in the protoplast come side by side, fuse together and form diploid (2n) nucleus. This is called autogamous pairing. The protoplast along with diploid (2n) nucleus comes out from the parent frustule and behaves as an auxospore. The auxospores are then covered by perizonium. New wall develops on the auxospore inner to the perizonium. Thus a new individual of normal size is developed. This is found in *Amphora normani*.

5) Production of Auxospore by Parthenogenesis:

The diatom cells come together and are covered by a common mucilage envelop. The diploid nucleus undergoes two sequential mitotic divisions. Meiotic division does not take place here. One nucleus in each mitotic division degenerates. Thus only one diploid (2n) nucleus along with protoplast remains, and comes out from the mother cell and behaves as an auxospore. The auxospore is then covered by perizonium and secretes new wall around itself. Thus normal size cell is formed.

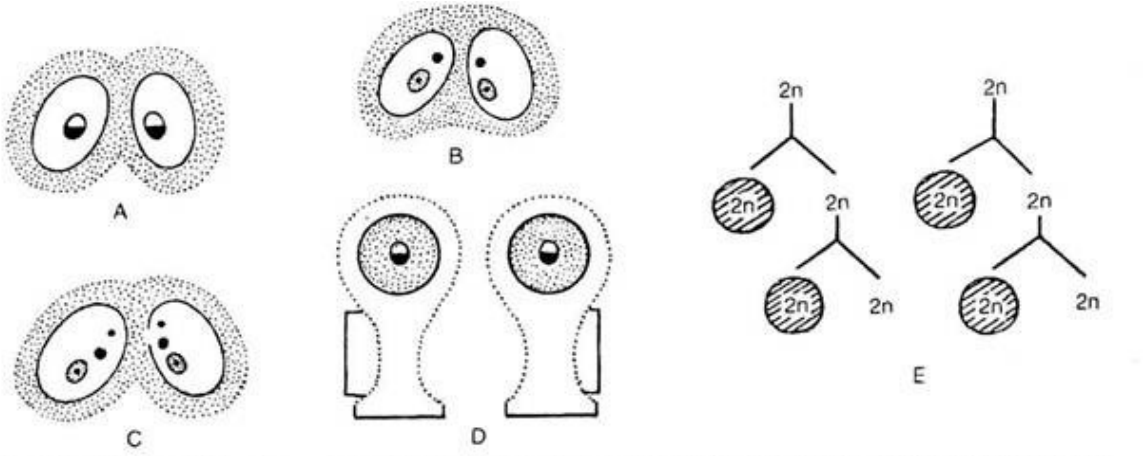


Fig. 3.105 : A-D. Production of auxospore by parthenogenesis in Pennales, and E. Nuclear behaviour during reproduction

Fig. 10.18

6) Production of Auxospore by Oogamy:

In this process the nucleus (2n) of female cell which behaves as oogonium, undergoes meiosis and forms four nuclei. The protoplast is also divided into two unequal parts, each containing two nuclei. The lower half is larger and behaves as functional ovum and the upper smaller one as non-functional ovum. The functional ovum contains one functional nucleus and one non-functional nucleus, which gradually degenerates at maturity.

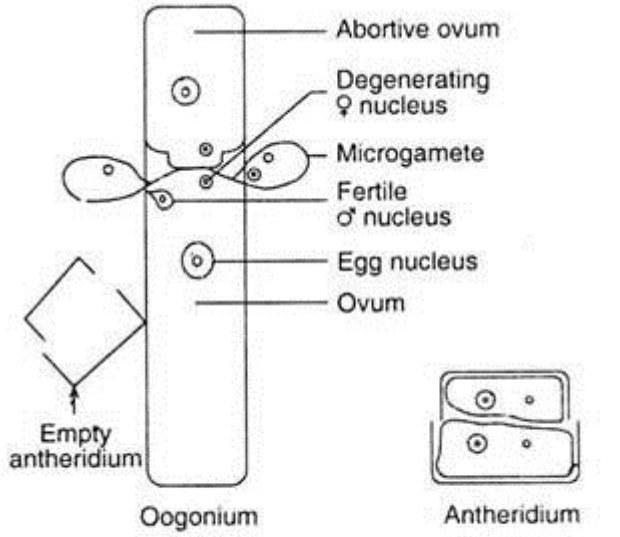


Fig. 3.106 : Production of auxospore by oogamy in Pennales

Fig. 10.19

The male cell (2n) behaves as antheridium, also undergoes meiosis and forms four nuclei. The protoplast also divides into two parts. Thus two microgametes are formed. Each of which contains two nuclei, of which one is functional and other is non-functional. The microgametes are naked, globular and non-flagellate. After coming out, the male gamete fertilizes the egg and forms the zygote (2n). Later it functions as an auxospore and forms new individual of normal size. It is found in *Rhabdonema adriaticum*.

Auxospore Formation in Centrales:

It takes place by autogamy and oogamy.

1) Auxospore Formation by Autogamy:

The protoplast of the vegetative cell secretes mucilage which separates both the theca. The nucleus (2n) then undergoes meiosis and forms four nuclei. Of the four nuclei two degenerate and the other two undergo fusion to form diploid (2n) nucleus again. This is called autogamy. The protoplast with 2n nucleus functions as an auxospore. The auxospore forms fresh frustule inside the perizonium covering and forms cell of normal size. It is found in *Melosira nummuloides.*

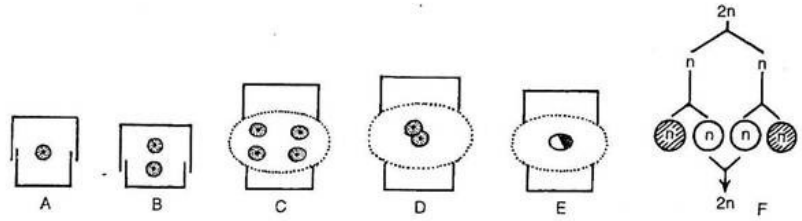


Fig. 3.107 : A-E. Auxospore formation by autogamy in Contrales, and F. Nuclear behaviour during reproduction

Fig. 10.20

2) Auxospore Formation by Oogamy:

Oogamy takes place by the fusion of egg and sperm developed inside the oogonium and antheridium, respectively.

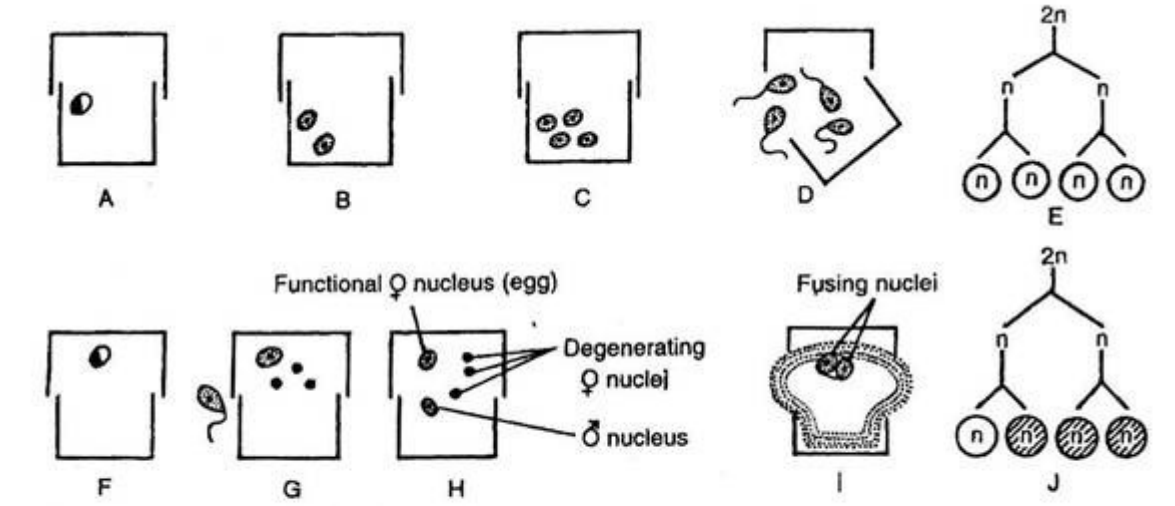


Fig. 3.108 : Auxospore formation by Oogamy in Centrales : A-D. Formation of sperms, E. Nuclear behaviour during sperm formation, F-G. Formation of egg, (F. Single oogonium, G. Sperm approaching oogonium), H. Male nucleus entered inside the oogonium, I. Fusing male and female nuclei and J. Nuclear behaviour during Egg-formation

Fig. 10.21

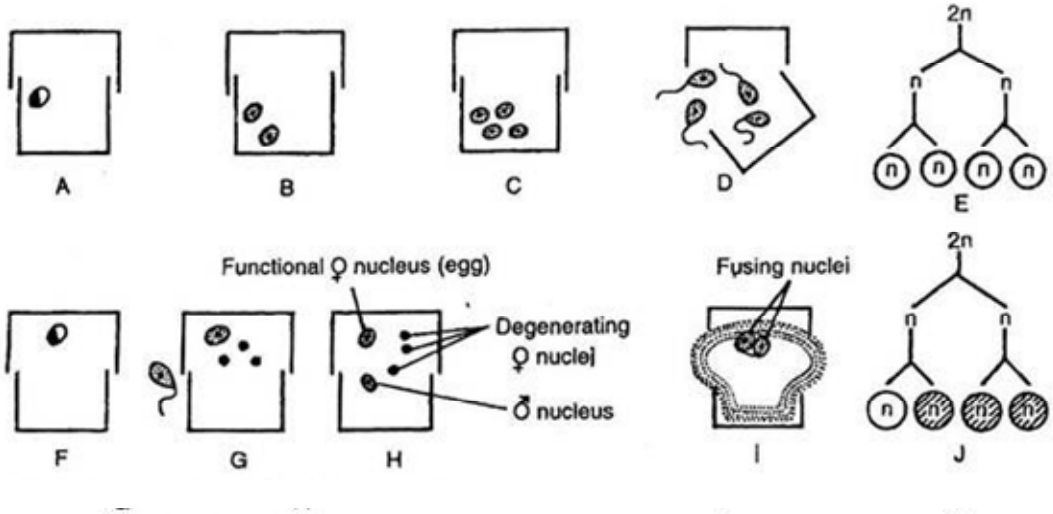
Oogonium: Single vegetative cell behaves as an oogonium. The protoplast of oogonium undergoes meiotic division and forms four nuclei. Of the four nuclei three degenerate and the remaining one functions as an egg.

Antheridium: The pattern of development of sperms varies in different species. In species like *Melosira varians* the protoplast undergoes meiotic division and forms four haploid nuclei. Each haploid nucleus with some protoplast metamorphoses into an uniflagellate (tinsel type) sperm. In others the number of sperms may go up to 8 or even 128.

Fertilisation: After coming out of the antheridium only one sperm enters inside the oogonium and fertilises the egg. The resultant zygote undergoes mitotic division but one nucleus degenerates in each division. The remaining nucleus with its protoplast behaves as an auxospore. The auxospore then develops new wall inside the perizonium covering and forms new cell of normal size like the mother. It is also called firstling cell.

From the above processes of sexual reproduction in both pennales and centrales, it becomes clear that the sexual process in diatom does not lead to multiplication but is to regain the normal size.

3. Resting Spores: These spores are formed during unfavourable conditions. Some members reproduce by the formation of thick-walled resting spores, the cysts or statospores. They are formed in *Melosira*.



Auxospore formation by Oogamy in Centrales : A-D. Formation of sperms, E. Nuclear behaviour during sperm formation, F-G. Formation of egg, (F. Single oogonium, G. Sperm approaching oogonium), H. Male nucleus entered inside the oogonium, I. Fusing male and female nuclei and J. Nuclear behaviour during Egg-formation

Fig. 10.22

10.3.5 Phylogeny:

Due to the siliceous nature of cell walls, they are well preserved in the form of fossils. The fossil evidence shows that centrales are more primitive (reported from jurassic) and pennales might have originated (early tertiary period) from them. The fact that the most of centric diatoms are marine planktonic forms in contrast to the pennales, which are predominantly fresh water, also indicates a centric ancestry for the pennales. The presence of fucoxanthin and chlorophyll a and chlorophyll c links the Bacillariophyta with the Phaeophyta, and the characteristic food reserves (oil and leucosin) relate the diatoms to the Xanthophyta and the Chrysophyta.

10.3.6 Economic Importance:

The siliceous shells of diatoms are accumulated over a longer period of time at the bottom of the aquatic bodies forming extensive deposits called diatomaceous earth or kieselguhr. Large deposits of diatomaceous earth have been found in California, Germany, France, Japan, Spain, Australia and Nicobar Islands. In Lompoc (California), the largest diatomaceous zone in the world, is present which spread to about 30 sq km area with 425 m thick. It is quarried and used commercially as an abrasive (1) in polishes and tooth pastes, (2) in the filtration of

liquids in sugar refineries, (3) used as an inert material filter in paints and plastic industries, (4) as an insulating material for boilers and blast furnaces because it can resist temperature of 1000°F, and (5) also used as an absorbent for nitroglycerine in the manufacture of dynamite.

10.4 SUMMARY:

The phylum Bacillariophyta with a class Bacillariophyceae (diatoms) comprises a homogeneous assemblage of unicellular and colonial forms which differ from other algae in possessing highly sculptured and symmetrically ornamented cell walls. Unicellular diatoms are of two types, viz., Pennales (Pennate diatoms) and Centrales (centric diatoms). Pennales are with isobilateral symmetry, e.g., *Pinnularia* whereas Centrales are with radial symmetry, e.g., *Cyclotella*. Diatoms are the major components of the planktonic vegetation. The cell wall (frustule) of diatom consists of two overlapping halves, the upper half is known as epitheca and lower one hypo theca. Each theca possesses the main surface known as valve and this valve has incurved margins called connecting band (Cingulum). The diatom cells are uninucleated. In Pennales, a single large nucleus is located across the middle of the central vacuole and is connected with the lining layer of the cytoplasm next to the cell wall. In Centrales, nucleus occupies the position within the peripheral cytoplasm lining the cell wall. Chromatophores are olive green to yellowish green in colour, with chlorophyll a and chlorophyll c.

The cell division is the common method in vegetative reproduction. As a result of cell division, two daughter protoplasts are formed, each one lying in the each parental theca. The parental hypotheca serves as the epitheca of one of the two daughter cells whereas the parental epitheca remains as the epitheca of the other daughter cell. McDonald-Pfitzer rule relates to the phenomenon of gradual size reduction in diatom during vegetative cell division: During successive cell divisions, the progenies of a diatom become progressively smaller generation after generation. Sexual reproduction through oogamous type is found in majority of centrales. The auxospore formation takes place by oogamy, e.g., *Melosira varians*, *Cyclotella tenuistriata* and *Biddulphia mobiliensis*. The siliceous shells of diatoms are accumulated over a longer period of time at the bottom of aquatic bodies, forming extensive deposits called diatomaceous earth or Kieselgurh. It has been quarried and used for many industrial purposes.

10.4 TECHNICAL TERMS:

Hypotheca, Epitheca, Oogamus, Pennales, Centrales, Auxospore, Oogonium, Antheridium.

10.5 SELF ASSESSMENT QUESTIONS:

1) Compare and contrast the Centrales and Pennales and add a note on their reproduction.
2) Give an account on thallus structure and reproduction in Bacillariophyta.
3) Economic importance of diatoms.

10.6 SUGGESTED READINGS:

1) Introductory Phycology - Kumar, H.D. 1999. Affiliated East-West Press, New Delhi.
2) Algae - Pandey, D.C. 1981., Kitab Mahal, Allahabad.

Prof. A. Amrutha Valli

LESSON - 11

PHAEOPHYTA AND RHODOPHYTA

11.0 OBJECTIVE OF THE LESSON:

Students will know all the detailed features of Phaeophyta and Rhodophyta members of algae.

STRUCTURE OF THE LESSON:

11.1 INTRODUCTION:

Brown or yellowish brown colour of the thallus of Phaeophyta is due to the presence of abundant carotenoids. The pigment fucoxanthin occurs in sufficiently larger quantity and chiefly responsible for brown colouration to the thallus. Other pigments are chlorophyll a, chlorophyll c, carotene, violaxanthin, diatoxanthin, neoxanthin, flavoxanthin and other xanthophylls. The plant body is multicellular but motile reproductive structure is unicellular. Photosynthetic reserve food is stored in the form of soluble carbohydrate, called laminarin and also mannitol. Cell wall is made up of cellulose, fusinic acid and alginic acid. Zoospores possess laterally inserted two unequal flagella. There are about 1,000 species belonging to 195 genera in the class Phaeophyceae.

The phylum Rhodophyta with a single class Rhodophyceae divided into two sub-classes, viz., Bangiophyceae (or Bangiophycidae) and Florideae (or Florideophycidae). Due to the presence of excess of v-phycoerythrin in their chromatophores, the thalli of various species of Rhodophyta are appeared red in colour. This red pigment masks the colour of other photosynthetic pigments. The Rhodophyta are characterized by the following features:

i) Flagellated motile stages are completely absent.

ii) The sexuality is highly specialized in this group. The non-motile, male gamete, known as

iii) spermatium is passively transported to and lodged on the trichogyne of female carpogonium. Besides, distinct post-fertilization developments are found in this group. Cell wall is made up of polysulphate esters of carbohydrates, in addition to cellulose and pectin.

iv) In multicellular forms, cytoplasmic connections (pit connections) are found between adjacent cells.

v) Characteristic pigments in this group are biliproteins (y-phycoerythrin, y-phycocyanin), taraxanthin and chlorophyll d, in addition to chlorophyll a, and carotene, lutein, zeaxanthin and neoxanthin.

vi) The reserve food materials are floridean starch and galactoside floridosides.

vii) Majority of red algae show the triphasic life cycle, and others biphasic life cycle.

11.2 PHAEOPHYTA:

11.2.1 Occurrence:

Except few (e.g., *Bodanella*, *Heribaudiella*, *Pleurocladia*), most of the Phaeophyceae members are marine. They are abundant in tropics (Sargasso Sea of the Atlantic) and more

prominent in cold waters. They occur in littoral and sublittoral zones of the sea. In sub-littoral zone, thick forest of kelps such as *Laminaria* with sub-flora of *Alaria*, *Cutleria*, *Desmarestia*, *Dictyota* and *Himanthalia* are present. *Laminaria* and *Macrocystis* are known to contribute to a very high rate of primary production.

11.2.2 Thallus Organization:

The structure of thallus of the brown algae ranges from heterotrichous filamentous types through pseudoparenchymatous uniaxial forms to true parenchymatous forms., Phenotypic plasticity is wide spread in this class, unicellular, colonial and unbranched forms are absent. The simplest thallus organization in this phylum is the branched, heterotrichous habit, e.g. *Ectocarpus*. The large sized brown algae are called kelps (*Lamina ria*, *Nereocystis*). Some arecalled rock weeds (*Fucus*). *Postelsia* resembles a palm. *Macrocystis* reaches up to 100 meters.

In general, the vegetative organization of Phaeophyceae is of three following types:

a) **Ectocarpoid Type -** in *Ectocarpus*, heterotrichous type of organization is found. The thallus is profusely branched and cells are joined end to end in a single series.

b) **Pseudoparenchymatous (Corticated) Type** - *Arthrocladia*, *Desmarestia* and *Myronema*, the lateral branches at the lower region of the plant body become rhizoidal and coil around the main axis to form a compact pseudoparenchymatous cortex around the main axis.They are also called haplostichous forms.

c) **Truly Parenchymatous Type** - These are also called Polystichous forms. In these forms, thallus is leaf-like and true parenchymatous, e.g., *Punctaria*. Members of Laminariales and Fucales possess improved type of parenchyma. The other examples for true parenchymatous thallus organization are *Fucus*, *Sargassum*, *Postelsia* etc.

The plant body of *Nereocystis* is 20-25 meters long. It consists of a haptera, a stipe and terminal large gas bladder having many blades on it. In *Laminaria*, the thallus is morphologically distinguishable into holdfast, stipe and blade (Fig. 11.1). The blade which is primarily photosynthetic and spore producing portion, is highly dissected, intercalary meristern is located at the junction of the stipe and the blade (Fig.11.2). Intercalary growth takes place in Laminariales and leads to formation of gigantic plantbody (giant kelp).

Internal Structure:

In *Dictyota*, the strap-like thallus undergoes dichotomous branching repeatedly (Fig.11.3A). The basal portion of the thallus forms a disc-like branched holdfast by which the thallusremains attached to the solid substratum. In transverse section, the, thallus is seen to becomposed of three layers (Fig. 11.3B, C), the central one with large cells and, an upper and lowerepidermis with assimilatory cells from which tufts of mucilage hairs arise. Growth is restricted to the apical region of the branches (apical growth).

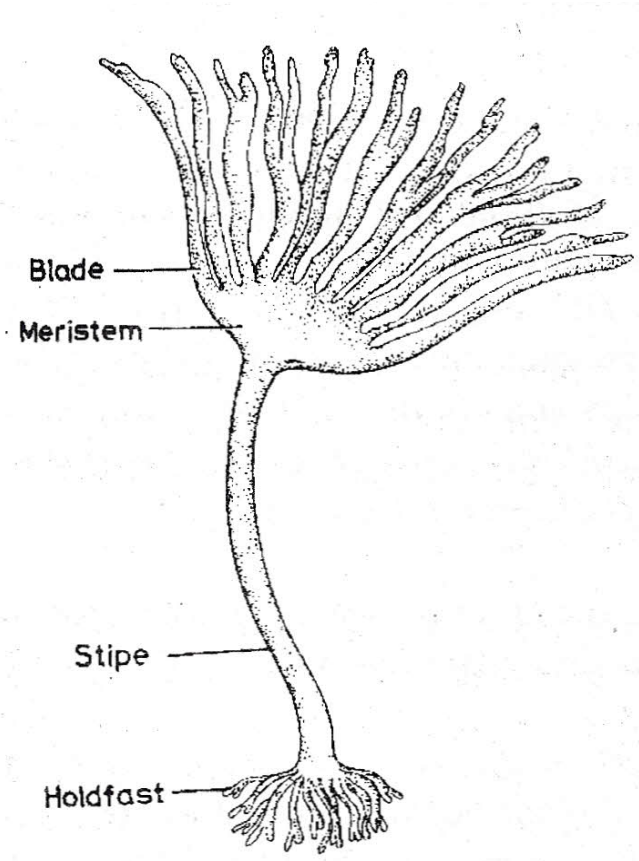


Figure-11.1: *Laminaria hyperborea*, sporophyte structure showing differentiation into holdfast, stipe, and blade.

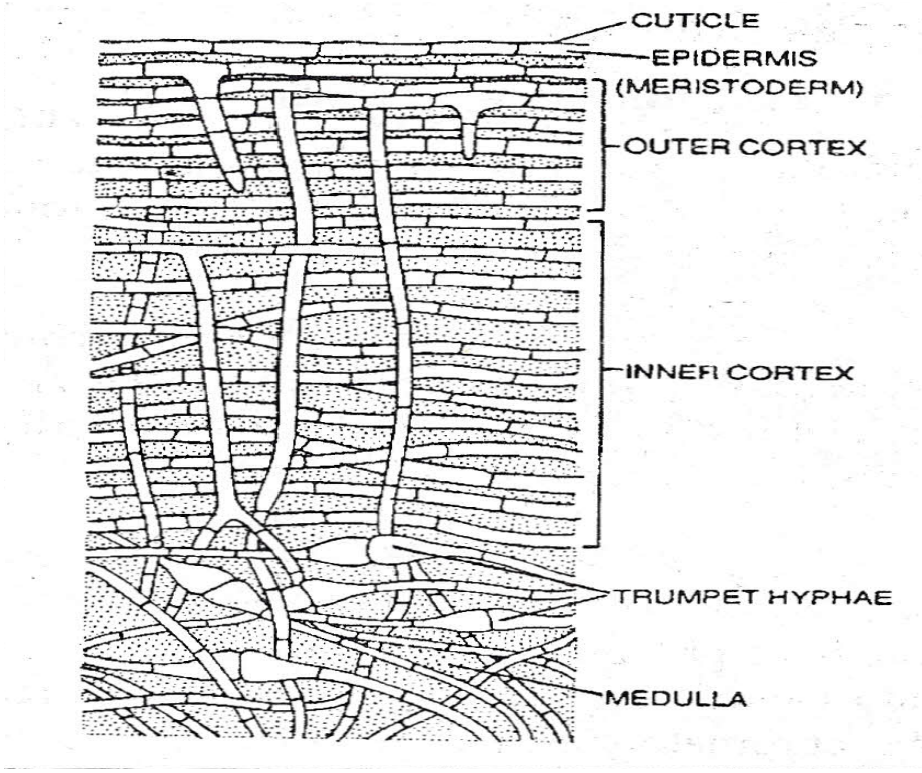


Figure-11.2: *Laminaria* sp. Longitudinal section through medulla of stipe. (adopted from Pandey, 2004).

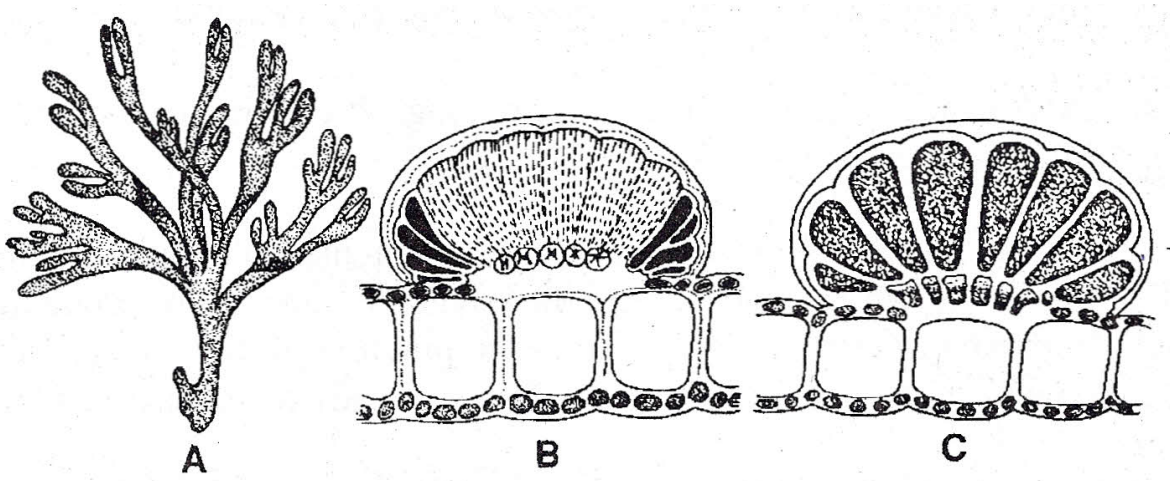


Figure-11.3: *Dictyota dichotoma*. A. Habit; B. Cross-section through antheridial sorus; C. Cross-section through oogonial sorus.

In *Laminaria*, the stipe consists of epidermis, cortex and medulla. Some of the medullary cells produce vertically elongated hyphae, known as trumpet hyphae, lacking chloroplast and involve in water and nutrient conduction.

11.2.3 Cell Structure:

The cell wall is two layered. The outer layer is mucilaginous. The mucilage contains gum-like substances such as algin, fucoidin etc. In Laminariales, special mucilage ducts are found, secreting mucilage. Callose is found in the cell membranes of *Laminaria digitata*. The inner layer is made up of cellulose. In cytoplasm, mitochondria which are closely connected with chromatophores and numerous vacuoles are present. In *Dictyota* and *Fucus* each cell contains a large vacuole. The special bodies called fucosan vesicles are abundantly found in meristematic, photosynthetic and reproduce cells.

Generally, the chromatophores are discoidal and parietal in position. In each cell, there may be one or more chloroplasts. Double membraned chloroplast has got many bands of photosynthetic lamellae. There are 3 or more photosynthetic lamellae (discs) stacked into bands. Generally, chromatophores lack pyrenoids. When present, usually protrudes fromthe chromatophores. These chromatophores contain chlorophyll a, chlorophyll b, xanthophyll and carotenoids as pigments. Fucoxanthin over masks the other pigments, giving a tinge of brown colouration to the plant body. The characteristic reserve food materials of this group are the laminarin, mannitol, fats, hydrolases etc. Iodine is also stored in many algae. In *Laminaria*, iodine concentration is 0.08 - 0.35%. Majority of Phaeophyceae have uninucleate cells. Large nucleus with one or twonucleoli is present in the cell. In Fucales the nucleoli are vacuolated. In Phaeophyceae the basic chromosome number is 8. The vegetative cell structure of a Phaeophyceaen member is given in Figure 11.4.

11.2.4 Reproduction:

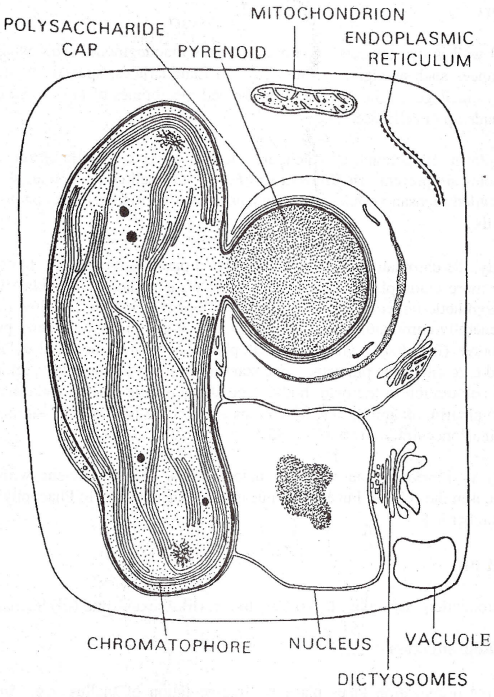
The reproduction takes place by (i) Vegetative, (ii) Asexual, and (iii) Sexual methods.

(i) Vegetative Reproduction:

Vegetative propagation takes place by fragmentation of thallus, e.g., *Sargassum*. In some cases, specialized reproductive branches are formed. They are called Propagules, e.g., *Sphacelaria*. Adventitious buds develop in *Fucus*. They develop by the activity of meristematic cells in young plants. These buds detach from parent plant and develop into a new plant.

(ii) Asexual Reproduction

Asexual reproduction takes place by means of zoospores and also aplanospores formed inside the sporangia.



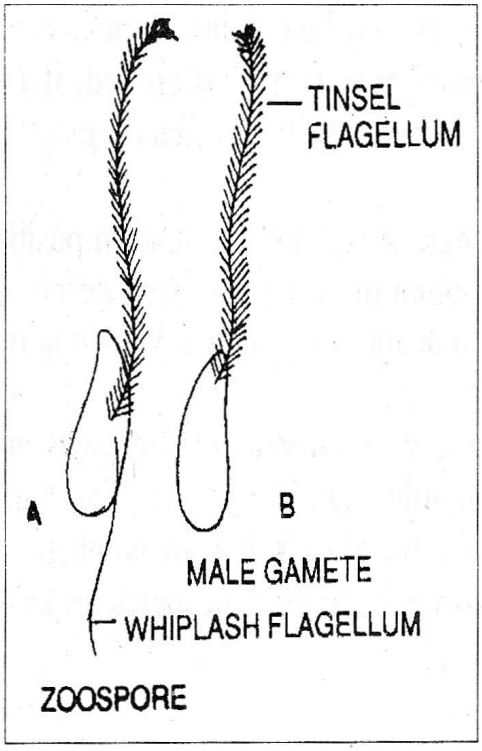


Figure-11.4: Phaeophyceae vegetative cell (Based on Bouck, 1965)

Figure-11.5: Zoospores

Zoospores:

The formation of zoospores is most common in all the members of Phaeophyceae except *Dictyota* and *Fucus*. The zoospores are pyriform and biflagellate. The anterior flagellum is larger than the posterior one (Fig. 11.5) except in Fucales. In Dictyotales, single flagellum isfound on the zoospore. These zoospores are produced in the zoosporangia, .which are of two types, unilocular sporangia and multilocular sporangia (or Plurilocular sporangia) (Fig. 11.6A).

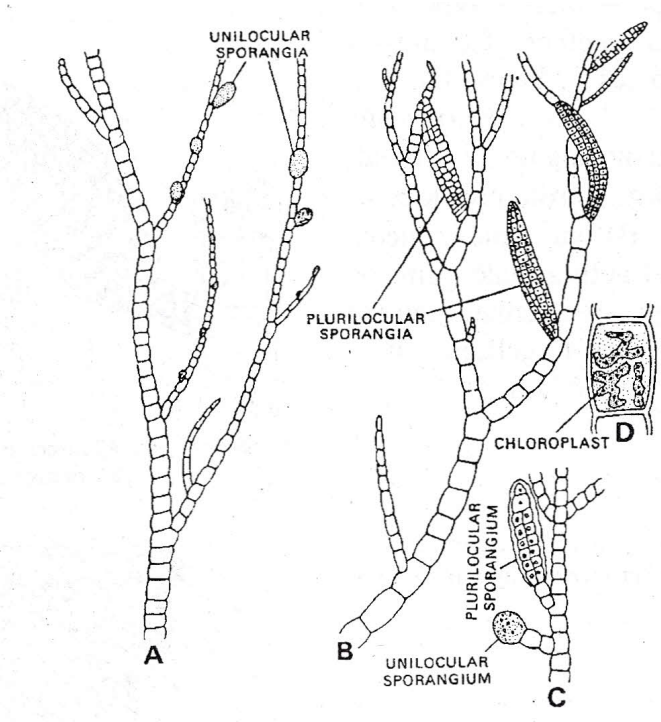


Figure-11.6: *Ectocarpus siliculosus.* A. Part of the plant with unilocular sporangia; B. Part of the plant with plurilocular sporangia; C. Part of the plant of *E. cylindricus* with both uni-and plurilocular sporangia; D. A cell.

The unilocular sporangium may be terminal or intercalary in position. The nucleus in the unilocular sporangium undergoes reduction division and gives rise haploid zoospores. They germinate and gives rise to gametophytic thalli, e.g. *Ectocarpus.* The plurilocular sporangia are always terminal in position. Zoospores formed from the plurilocular sporangium are diploid in nature and, they germinate and give rise to sporophytes, e.g., *Ectocarpus.* The plurilocular sporangia are not known in Fucales and Laminariales.

Aplanospores - In some cases, instead of zoospores unilocular sporangia also produce aplanospores. They are non-motile, e.g., Dictyotales The first division is always reductional. The aplanospores are always less in number. In *Dictyota* and *Zonaria,* each sporangium produces 4 aplanospores and 8 aplanospores, respectively.

(iii) Sexual Reproduction:

Isogamous to oogamous types of sexual reproduction are found in Phaeophyta. Sexual reproduction takes place only in haploid plant (gametophyte).

A. Isogamous type - In this type, two similar gametes are fused together. Isogamy is common in Ectocarpales, Sphacelariales, Dictyosiphonales etc. In *Ectocarpus siliculosus*, physiological anisogamy is found. In this, isogametes are formed from plurilocular gametangium (Fig. 11.7A, B) but some gametes are more active. Several active male gametes cluster around a single passive female gamete with their forwardly directed flagellum. It is called clump formation (Fig. 11.7C).

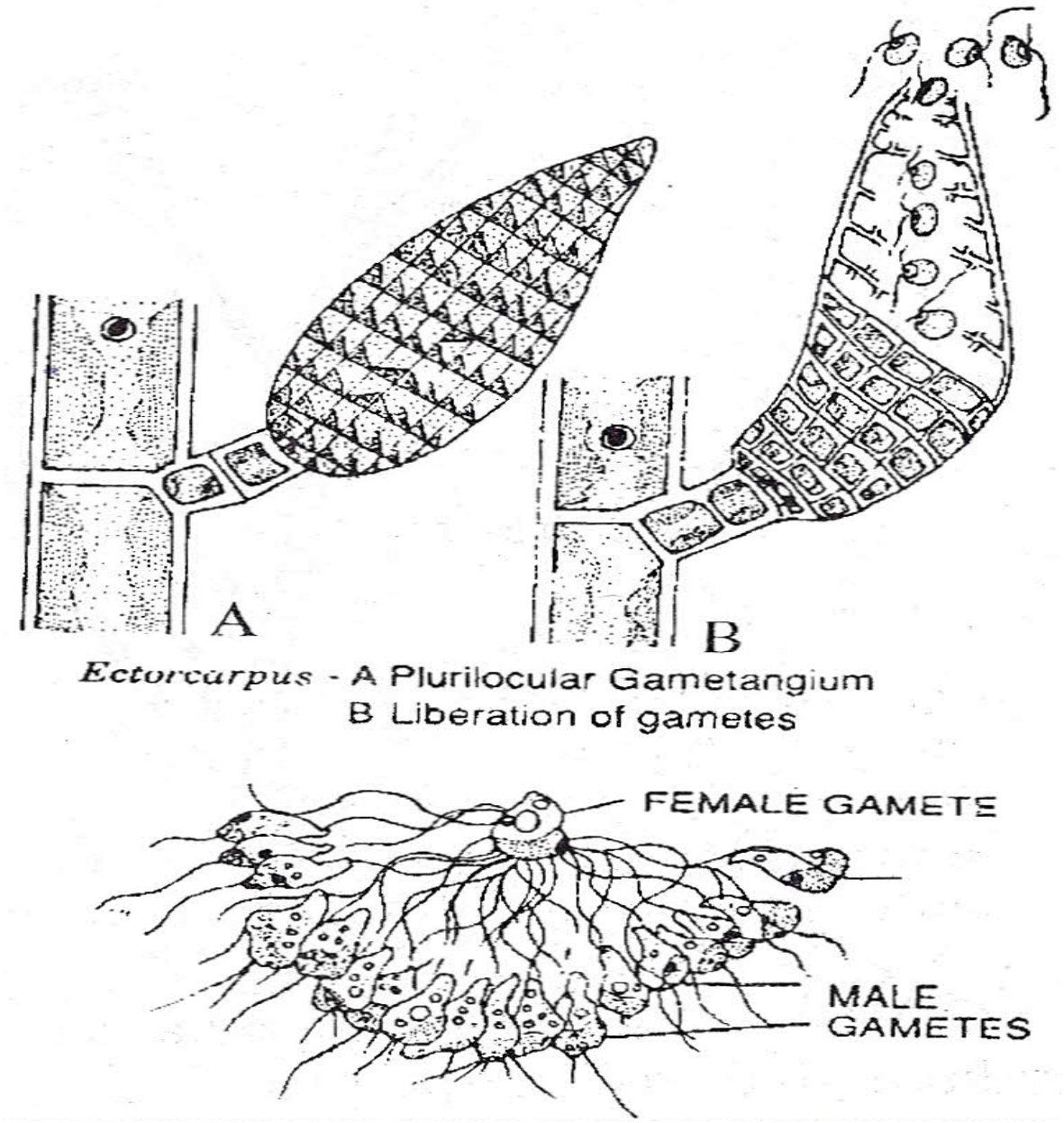


Figure-11.7: *Ectorcarpus* - A. Plurilocular Gametangium, B. Liberation of gametes, C. *Ectocarpus* showing clump formation

B. Anisogamous type - The fusion of two dissimilar games takes place, e.g., *Cutleria*, *Soranthera* and members of Ectocarpales.

C. Oogamous type - Oogamy is quite common in majority of Phaeophyceae. The species may be homothallic or heterothallic.

In Dictyotales the oogonial sorus (Fig.11.8B) and antheridial sorus (Fig. 11.8C, D) occur on different plants whereas in *Fucus* they may occur in the same conceptacles or in separate conceptacles as in *Sargassum*.

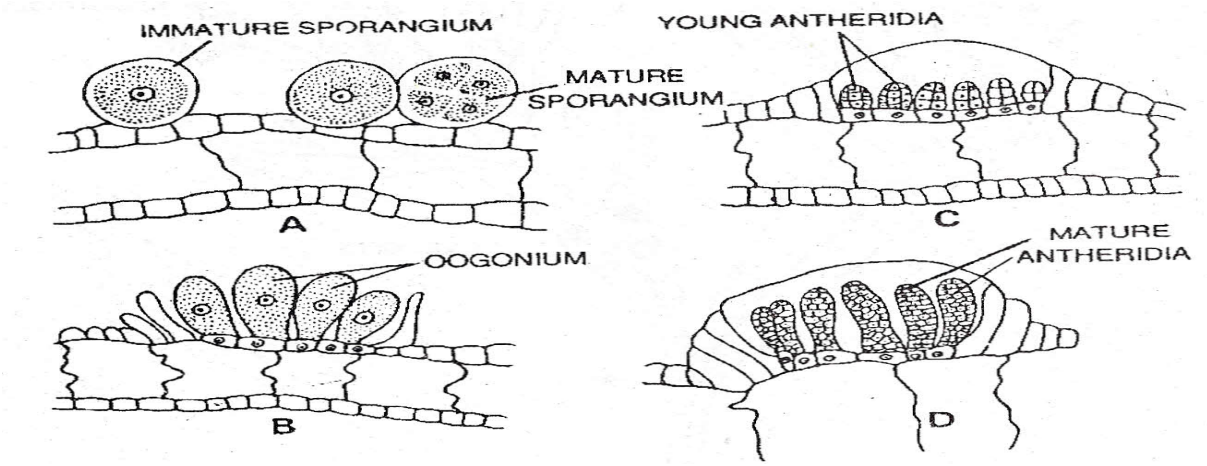


Figure-11.8: *Dictyota dichotoma*. A. Transverse section through a portion of sporangial sorus showing immature and mature sporangia; B. Transverse section through an oogonial sorus showing oogonia; C. Transverse section through antheridial sorus showing young antheridia; D. Transverse section through antheridial sorus showing mature antheridia (adopted from Pandey, 2004).

In Dictyotales, the antheridia are multilocular structures (Fig.11.8C, D). Each cell of antheridium gives rise to spermatozoid. In Desmarestiales and Laminariales, the antheridia are unilocular and each antheridium produces a single spermatozoid. Usually each oogonium produces a single ovum or oosphere (Fig.11.8B). In Fucales, the special reproductive branches bearing receptacles at their tips, are found, e.g., *Sargassum* and *Fucus* (Fig. 11.9). Several fertile conceptacles are scattered over these receptacles.

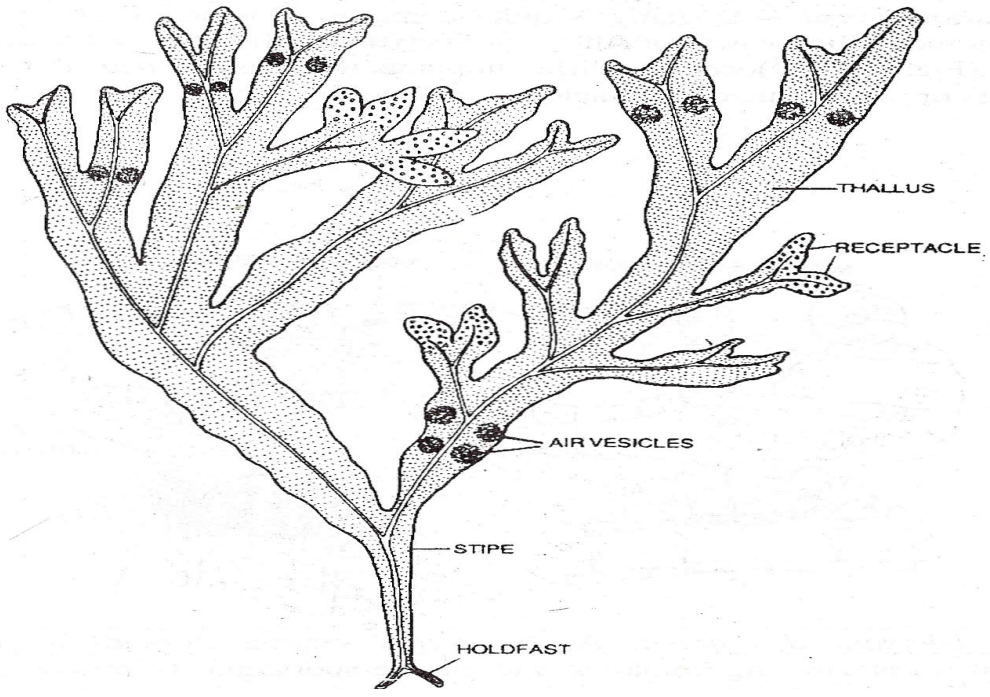


Figure-11.9: *Fucus* sp. complete plant thallus

The development of conceptacle - The conceptacle develops from any superficial cell of the meristoderm usually situated near the apical region of the receptacle. The cell of the meristoderm becomes prominent. The other surrounding cells of the meristoderrn undergo divisions comparatively at a higher rate and bring down the conceptacle initial in the bottom of the flask-like cavity (Fig. 11.10A-E). This initial divides transversely into the tongue cell and basal cell. The tongue cell degenerates but basal cell divides and re-divides anticlinally and gives rise to a fertile layer of cell sheet. This fertile sheet develops the antheridia or oogonia, as the case may be.

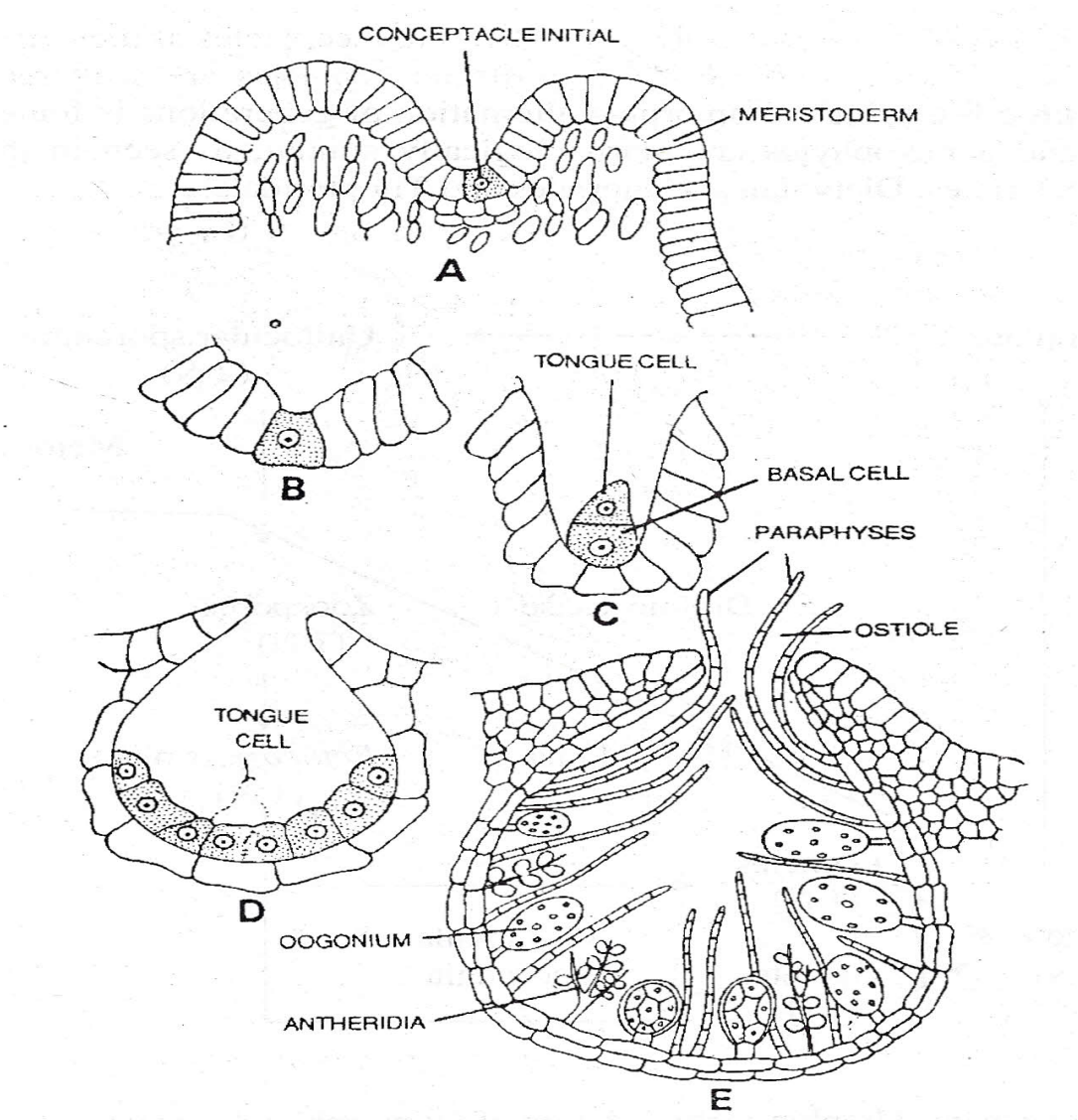


Figure-11.10: *Fucus* sp. Sexual reproduction, development of conceptacle. A-D. Successive stages in the development of conceptacle; E. A conceptacle, with antheridia, oogonia and paraphyses (Adopted from Pandey, 2004)

In *Fucus*, eight eggs are produced in the oogonium. The eggs liberate in the water and fertilization takes place externally.

11.2.5 Life Cycles:

There are three general types of life cycles found in the class Phaeophyceae,

i) **Isomorphic Life Cycle** - Isomorphic alternation of generations is found in this cycle (Fig. 11.11). Both sporophyte and gametophytes are morphologically similar, as seen in the members of Ectocarpales, Sphacelariales, Dictyotales, Cutleriales and Tilopteridales.

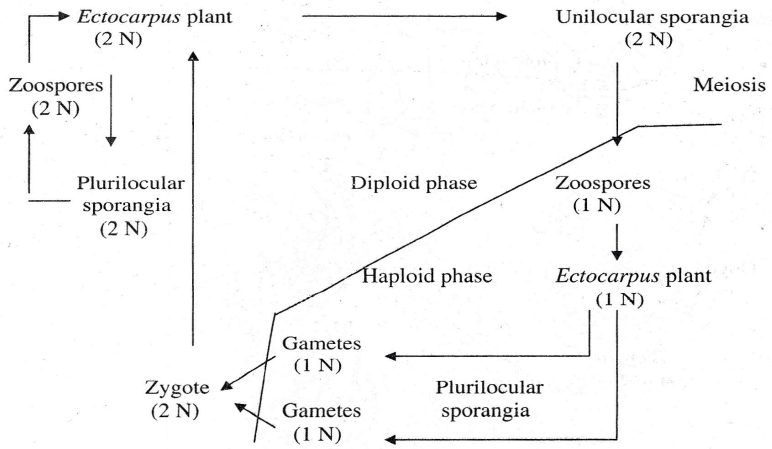


Figure-11.11: *Ectocarpus* - Graphic representation of Isomorphic alternation of generations

ii) **Heteromorphic Life Cycle** - An alternation of morphologically dissimilar (heteromorphic) diploid and haploid generations is found (Fig.11.12) e.g., *Laminaria, Nereocystis*

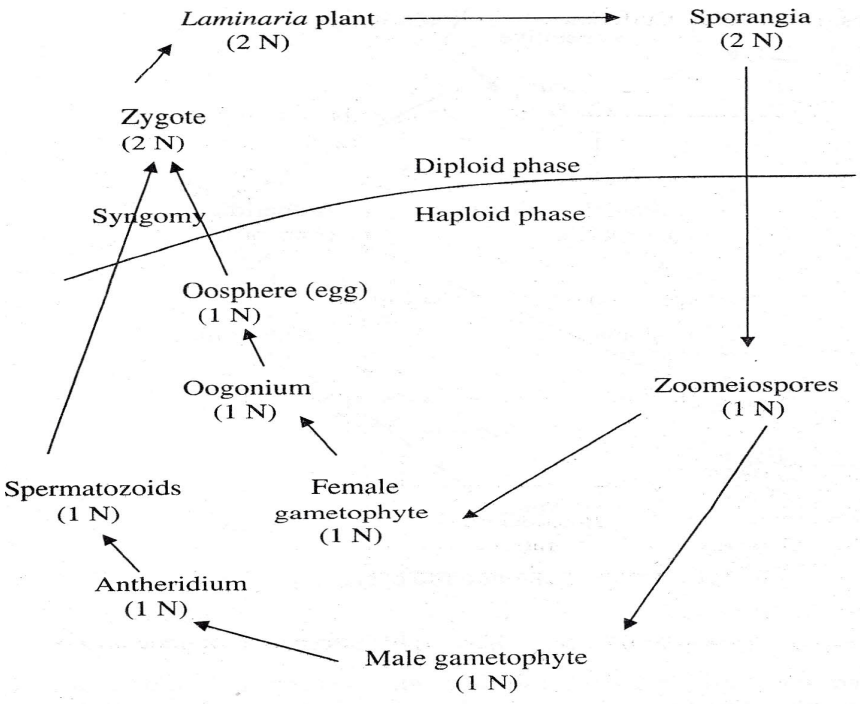


Figure-11.12: *Laminaria* - Heteromorphic alternation of generations

iii) **Diplontic Life Cycle** - In this, there is no alternation of generations and complete suppression of the haploid generation, as seen in *Fucus* (Fig. 11.13). In this genus, gametophytic phase is represented by egg and antherozoids.

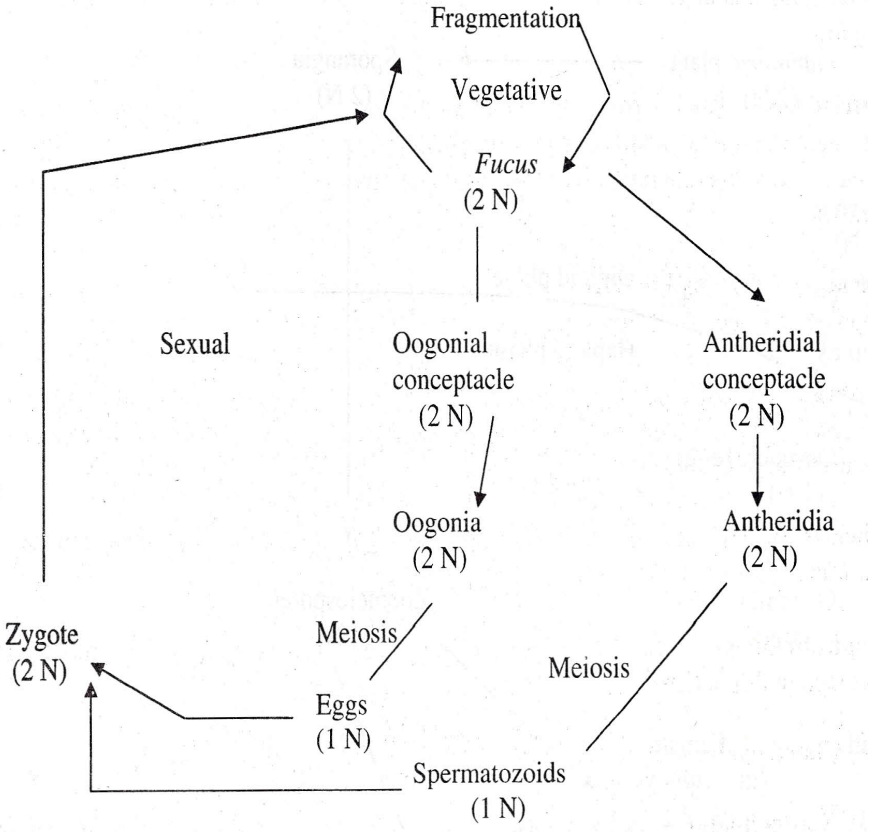


Figure-11.13: *Fucus* - Diplontic life cycle

11.2.6 Classification:

Fritsch classified the class Phaeophyceae into nine orders: (i) Ectocarpales (ii) Tilopteridales (iii) Cutleriales (iv) Sporachnales (v) Desmarestiales (vi) Laminariales (vii) Sphacelariales (viii) Dictyotales (ix) Fucales.

Taylor divided the class Phaeophyceae into 3 sub-classes: (I) Isogeneratae, (II) Heterogeneratae, and (III) Cyclosporae. (i) Subclass Isogeneratae: Those species showing isomorphic alternation of generations are classified under this subclass. This includes 8 orders.

A. Ectocarpales - Branched heterotrichomes plant body; trichothallic growth; reproductive structures terminal or intercalary; the sporophyte produces zoospores; isogamy.

B. Sphacelariales - Growth by a single large apical cell divides lengthwise in regular polysiphonous manner; sporophyte may produce haploid or diploid zoospores.

C. **Tilopteridales** - Thallus freely branched showing trichothallic growth; upper portion of thallus mono siphonous and lower portion polysiphonous. Sporophytes produce unilocularsporangia, each sporangium gives rise a single quadrinucleate aplanospore; gametangia intercalary.

D. **Cutleriales** - Thallus flattened, blade-like or disc-like, dichotomously branched; the sporophyte produces unilocular sporangia only; anisogamy.

E. **Dictyotales** - Flattened, erect, dichotomously branched parenchymatous thallus; growth by single apical cell; sporophyte produces unilocular sporangia, each sporangium contains 4 or 8 aplanospores: Oogamy.

(ii) Subclass: Heterogeneratae: This includes two series, Haplostichineae and Polystichineae.

I. Series Haplostichineae: Thallus consists of one or more filaments; trichothallic growth. Three orders included in this series.

A. **Chordariales** - Branched filamentous sporophyte; isogamy.

B. **Sporochnales** - Each branch of the sporophyte terminates in a tuft of hairs; trichothallic growth; only unilocular sporangia borne terminally in dense clusters; oogamy.

C. **Desmarestiales** - Macroscopic thallus, pseudoparenchymatous cortication; gameophyte microscopic; oogamy.

II. Series Polystichineae: Longitudinal and transverse intercalary cells form a parenchyma.

A. **Punctariales** - Parenchymatous, sporophyte of medium size; sporophyte produces by zoospores; garnetophyre microscopic; isogamy or anisogamy.

B. **Dictyosiphonales** - Profusely branched cylindrical thallus, growth by single apicalcell; unilocular sporangia on sporophyte; gametophyte microscopic, isogamy.

C. **Laminariales** - Sporophyte differentiated into holdfast, stipe and blade, growth byintercalary meristem; internal differentiation of thallus into epidermis, cortex and medulla; sporophyte bears only unilocular sporangia in sori; gametophyte microscopic; oogamy.

(iii) Subclass: Cyclosporae:

Order Fucales: Life cycle is diplontic; parenchymatous thallus; growth by a single apical cell; antheridia and oogonia develop in the conceptacles situated on the receptacles; gametophytic phase represented by eggs and antherozoids; oogamy.

11.2.7 Phylogeny and Interrelationships:

Members of Phaeophyceae show parallel evolution with Chlorophyta and Rhodophyta in having the heterotrichous, uniaxial or multiaxial plant bodies. They also resemble Cryptophyta, Pyrrophyta and Bacillariophyta in possessing chlorophyll c. Further, presence of oil and saturated fats is a common feature of the brown algae as of the Xanthophyceae, Chrysophyceae and Bacillariophyceae. The swarmers (swimming cells) of the Phaeophyta are also very similar to those of Xanthophyceae and Chrysophyceae with reference to the morphology of flagella.Two divergent lines have been established in the evolution of Phaeophyceae. One of these lines has given rise to the groups possessed isomorphic alternation of generations, and the other to the groups of heteromorphic alternation of generations. In both the series there has been a progressive evolution towards the complexity of the thallus and from isogamous to oogamous type of reproduction. The order Fucales may be considered as an advanced one in heteromorphic series.

11.2.8 Economic Importance:

In Japan, people use more than 20 species of brown algae as food. They begin to collect the kelps in July and continue up to October. The acetic acid is extracted from seaweed bymeans of fermentation. In many places, *Nereocystis* is used in the preparation of medicines, poultry feed, and for the extraction of potash salts. About 30 per cent of the dry weight is potassium chloride. *Macrocystis* and *Laminaria* are the chief source for the extraction of algin. Algin is usedin the preparation of paints and varnishes, and also in the preparation of ice creams. *Egregia* (kelp) is used as fertilizer. Seaweeds also contain iodine and other salts.

11.3 RHODOPHYTA:

11.3.1 Occurrence:

Majority of red algae are marine growing in littoral and sublittoral zones. They grow in almost all marine habitats but their greatest concentrations occur in warmer seas. Red algae prefer to grow in deeper waters where they receive only blue-green wavelengths of light. This spectral region is sufficient for the generation of y-phycoerythrin; the dominant red pigment for photosynthesis found in the thalli of this group of .algae. They also exhibit complementary chromatic adaptation, so that the colour of the incident light induces the development-of a particular photosynthetic pigment which has maximum absorption of the incident light: Unicellular red alga *Porphyridium* grows in damp soil. Some are fresh water forms, e.g., *Batrachospermum, Lemanea* etc. Some of the calcareous algae, e.g., *Corallina* and *Lithothamnion* are responsible for the formation of coral reefs. Certain parasitic red algae, e.g., *Gardneriella tuberifera, Ceratocolax hartzii, Choreonema thuretii* etc., lack pigments and penetrate host tissues.

11.3.2 Thallus Organization:

There is a great diversity in the vegetative structure of red algae. The subclass Bangioideae, with a single order Bangiales, comprises unicellular (*Porphyridium*), filamentous (*Goniotrichum*), and parenchymatous (*Porphyra*) forms. The subclass Florideae show more elaborative thalli with two main types of organization, uniaxial and multiaxial. In both the cases pseudoparenchymatous thallus results from the coalescence of filament branches. The uniaxial thalli have a single central or axial filament; which may be corticated, with a number of richly branched laterals organized to form a pseudoparenchymatous structure, e.g., *Batrachospermum* and *Dumontia*.

In *Batrachospermum*, the thallus is uniaxial (monosiphonous). There is a central filament of unlimited growth with a single dome shaped apical cell. This apical cell divides transversely to produce an axial row of large and cylindrical cells.

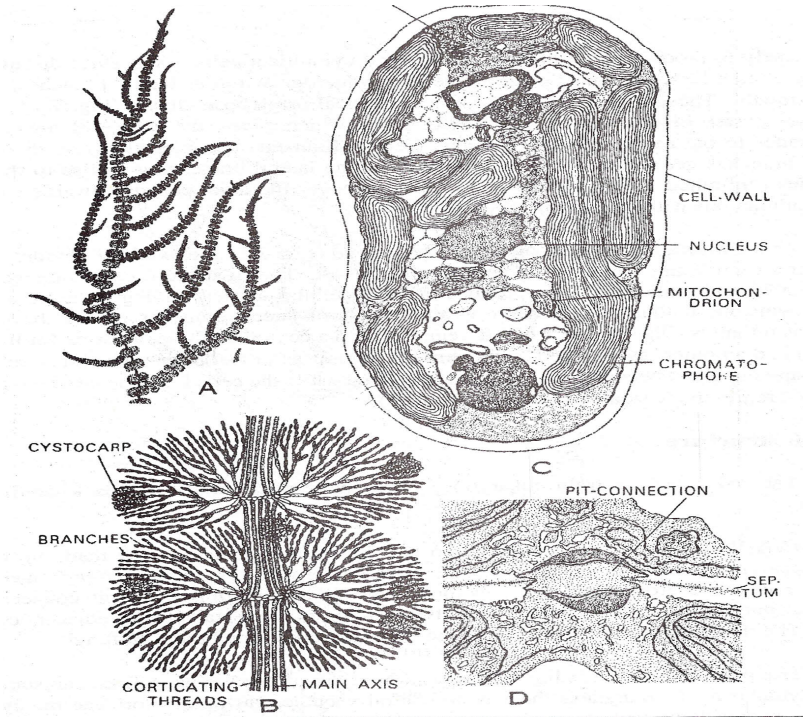


Figure-11.14: *Batrachospermum*, A. Thallus showing habit; B. Part of thallus with glomerules and cystocarps; C. Electron micrograph (diagrammatic) of a vegetative cell, D. Electron micrograph (diagrammatic) at the region of a septum showing pit connection. (C and D - after Brown and Weier, 1970).

At a short distance, below the apical cell, there are four lateral projections, which grow out as lateral branches, limited in their growth. These lateral branches form a spherical or globose cluster (Fig. 11.14A, B). This globose cluster of laterals is called glomerule which gives the whole thallus a beaded appearance to the naked eye. As the axis (central filament) becomes elongate, the whorls of lateral branches get separated. The basal cells of the lateral branches give rise to rhizoid like branches (unlimited growth) which grow out and cover the axial cell

downwards and form a multicellular-axis with cortication. In the multi axial forms, such as *Polysiphonia,* the axial cell divides vertically to form a central and a number of pericentral siphons. The pericentral cells undergo division periclinally and anticlinally to produce a multicellular cortex giving rise to a pseudoparenchymatous thallus or may serve as the initials of lateral branches. Thus the multiaxial cylindrical thallus (Fig. 11.14A, B) is differentiated into a central medulla, a cortex for the storage of food and an outer layer of cells with chromatophores as in *Gelidium*. The inner cells do not divide anymore and become stretched and elongated while the cells from the centre to periphery become progressively smaller (Fig. 12.14, CD, E, F).

11.3.3 Cell Structure:

The red algae are truly eukaryotic. They have microtubules but lack centrioles and flagella.

Cell Wall - It is distinguished into two layers, the outer layer is made up of pectic substances and inner layer with cellulose. In some forms, e.g., *Porphyridium*, mucilaginous envelope is found around the cell and they show gliding motility. The pit connections are lenticular plugs and they are occupied a central position in the septa between adjacent cells (Fig.11.14D). The rhodophycean pit connections are very similar to septal plugs of fungi.

The protoplasm - It is highly viscous. Many scattered grains of floridean starch occur in the cytoplasm. In Bangiales, there is no central vacuole, but in all Florideae the cytoplasm possess a conspicuous central vacuole (tonoplast bound).

The nucleus - The cells are uninucleate in lower Rhodophyceae orders such as Cryptonemiales, Bangiales etc. In higher orders, Ceramiales, Rhodymeniales etc., cells are multinucleate. In some rhodophycean forms, e.g., *Griffthsia,* each cell possesses 3,000-4,000 nuclei. The nuclei are with one or more prominent nucleoli. The nuclei may migrate from one cell to another through a pit connection. There is a well-developed chromatin net work.

The Chromatophores and Pigments - In majority of Rhodophyceae, the number of chromatophores is more than one per cell. They may be band-like in Ceramiales, and irregularly lobed or discoidal in *Polysiphonia*. In lower Rhodophyceae (order Bangiales), there is a single stellate chromatophore in each cell. Chromatophore possesses a centrally placed naked (without starch sheath) pyrenoid. The pigments which are abundantly occurred in red algae are y-phycoerythrin (red water soluble pigments) and y-phycocyanin (blue water soluble pigments). Besides, chlorophyll a, chlorophyll b, xanthophylls and carotenes are present.

Reserve food products - The most important food product is floridean starch. In the form of small grains, it is distributed throughout the cytoplasm. The floridean starch differs from the chlorophycean starch in being devoid of amylose. In some respects, the floridean starch resembles the amylopectin of higher plants. In many Rhodophyceae, a soluble sugar, floridoside is found; Floridoside is a galactoside of glycerol.

Endoplasmic reticulum - In red algae the functions of endoplasmic reticulum are diverse and manifold including (a) septal plug formation, (b) fibrous vacuole formation, (c) spermatial vacuole formation, (d) vesicle formation in spores, (e) mucilage production by fusion cells, and (f) cleavage channel formation and wall secretion during cytokinesis.

Golgi bodies - The golgi-derived vesicles play an important role in cell wall formation and in the production storage food materials. Golgi bodies consist of vesicles of fibrillar, diced or striated.

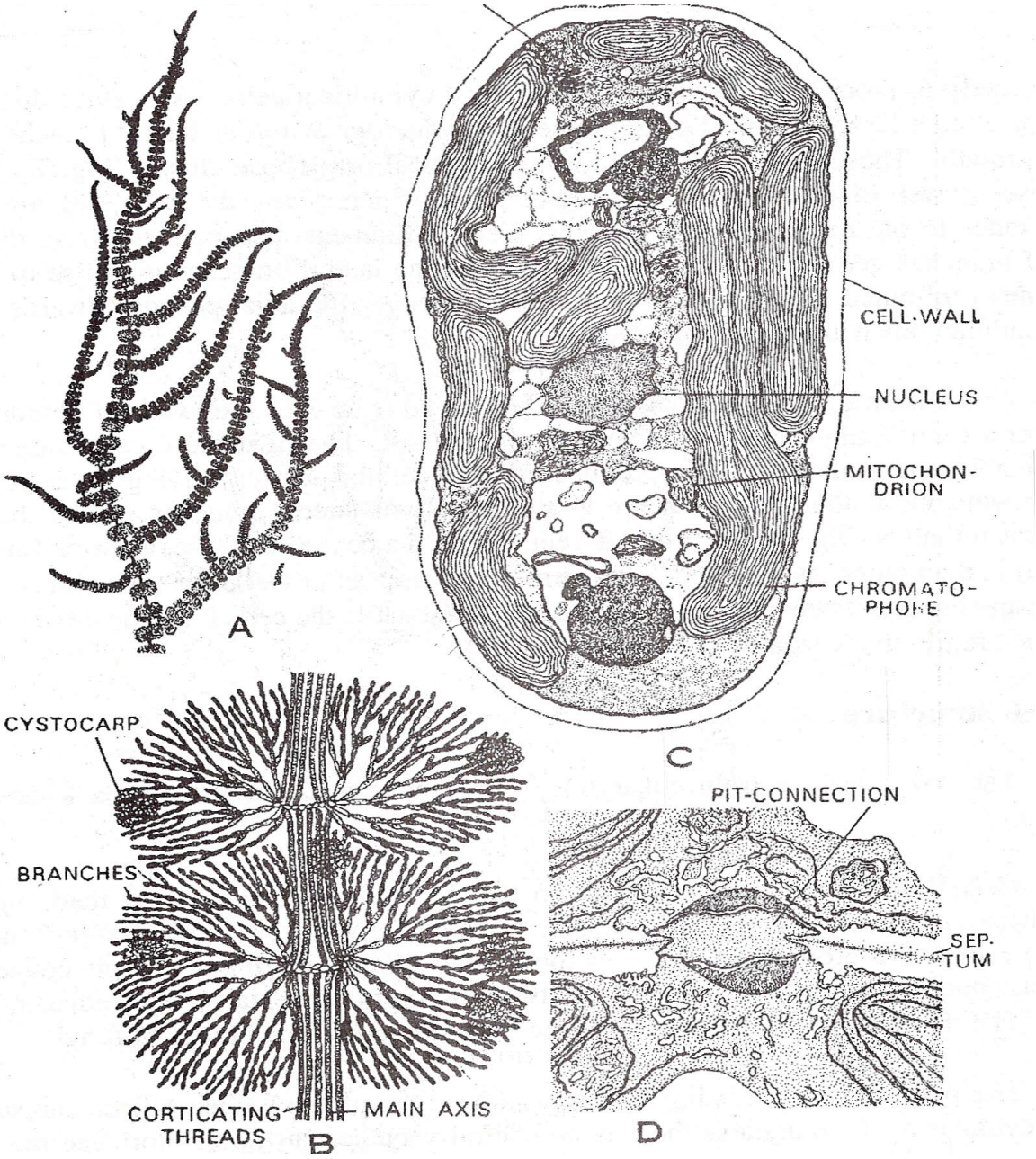


Figure-11.15: *Polysiphonia* sp. A. Habit of the plant; B. Portion of the plant (somewhat enlarged); C-D. Cortical siphons; E. Filament showing central siphons; F. T.S. of siphonous filament (Adopted from Pandey, 2004).

11.3.4 Reproduction:

The reproduction takes place by vegetative, asexual and sexual methods.

(i) Vegetative Reproduction:

Rarely fragmentation of thallus has been reported in fresh water Bangioideae, e.g., *Asterocystis*. The Red Sea-alga, *Centroceros clavatum* produces missile shaped vegetative propagules, carried away by ocean currents and settle on leaves of sea grasses, where they develop into new individual plants.

(ii) Asexual Reproduction:

Motile reproductive structures are completely absent in Rhodophyceae. Asexual reproduction of the gametophyte takes place by monospores, neutral spores, tetraspores and carpospores. Production of monospores in monosporangia (a single monospore in a single monosporangium) is the chief method of propagation in the Chantransia stage of *Batrachospermum*. Asexual reproduction takes place by neutral spores in *Porphyra* and *Bangia*. These spores are produced directly by transformation of vegetative cells into spores. Tetraspores are produced in the tetrasporangium after meiotic division. Tetraspore germinates and gives rise gametophyte, e.g. *Polysiphonia*. Production of carpospores is a common feature in Florideae. In the order Nemalionales, the carpospores are haploid and on germination give rise to sexual gametophytic plant; in the order Florideae, the carpospores are diploid and germinate into a sporophytic plant.

(iii) Sexual Reproduction:

In majority of Rhodophyta, the sexual reproduction is oogamous which is highly elaborative and specialized type. The entire reproductive process in Rhodophyta is unlike that of any other group of the plant-kingdom. Sexual reproduction has not reported in Bangioideae except *Bangia* and *Porphyra*. In oogamy female sex organ known as Carpogonium and male sex organ, spermatangium (antheridium) are involved.

Male Reproductive Structure:

Non-motile male gametes termed as spermatia are brought about by water currents and lodged on the tip of the female reproductive structure. These spermatia are borne singly within a spermatangium. The spermatangia may be disposed in clusters on certain special

branches as in *Polysiphonia* (Fig. 11.16 A, B, C) or in others they may lie in sorias in *Apoglossum*.

Female Reproductive Structure:

The female organ, carpogonium may be sessile in Bangioideae (the most primitive group); but in Florideae, they are borne on a special branch, carpogonial branch or procarp. The procarps are present on greatly reduced fertile trichoblasts of the female gametophyte. The initial of the fertile female trichoblast arises 3-4 cells away from the apex of the thallus. This initial grows into 5-7 cells in length. The three basal cells undergo divisions to give rise peri central cells. One of the peri central cells on the adaxial side (facing axis side) functions as the supporting cell of the future carpogonial filament. This supporting cell by undergoing successive transverse divisions, forms 4-celled; curved carpogonial filament. The terminal cell of the carpogonial filament metamorphoses into a carpogonium with a swollen base and a long, erect trichogyne. The trichogyne is demarcated by a median constriction from the basal, swollen portion of the carpagonium in which egg nucleus is located. Egg cell is rich with protoplasm and reserve food materials.

Fertilization:

Spermatia are lodged on the trichogyne (Fig.11.16 A, B). The tip of the trichogyne becomes mucilaginous and the wall between spermatium and the trichogyne dissolves. The male nucleus enters through the opening, moves down the trichogyne and finally fuses with the egg nucleus. After fertilization trichogyne shrivels away. Post-fertilization changes - Immediately after the fertilization, the zygote divides meiotically to form four haploid nuclei in *Batrachospermum* and other members of the order Nemalionales. (Fig. 11.17). At this time, the fertilized carpogonium develops several small protuberances. Haploid nuclei divide mitotically and migrate into the protuberances. Each protuberance consisting of a single nucleus starts functioning as an initial of a gonimoblast filament. Thus, several branched gonimoblast filaments arise from the base of the carpogoniurn. The terminal cells of the gonimoblast filaments later differentiate into carposporangia within which the haploid carpospores are formed singly.

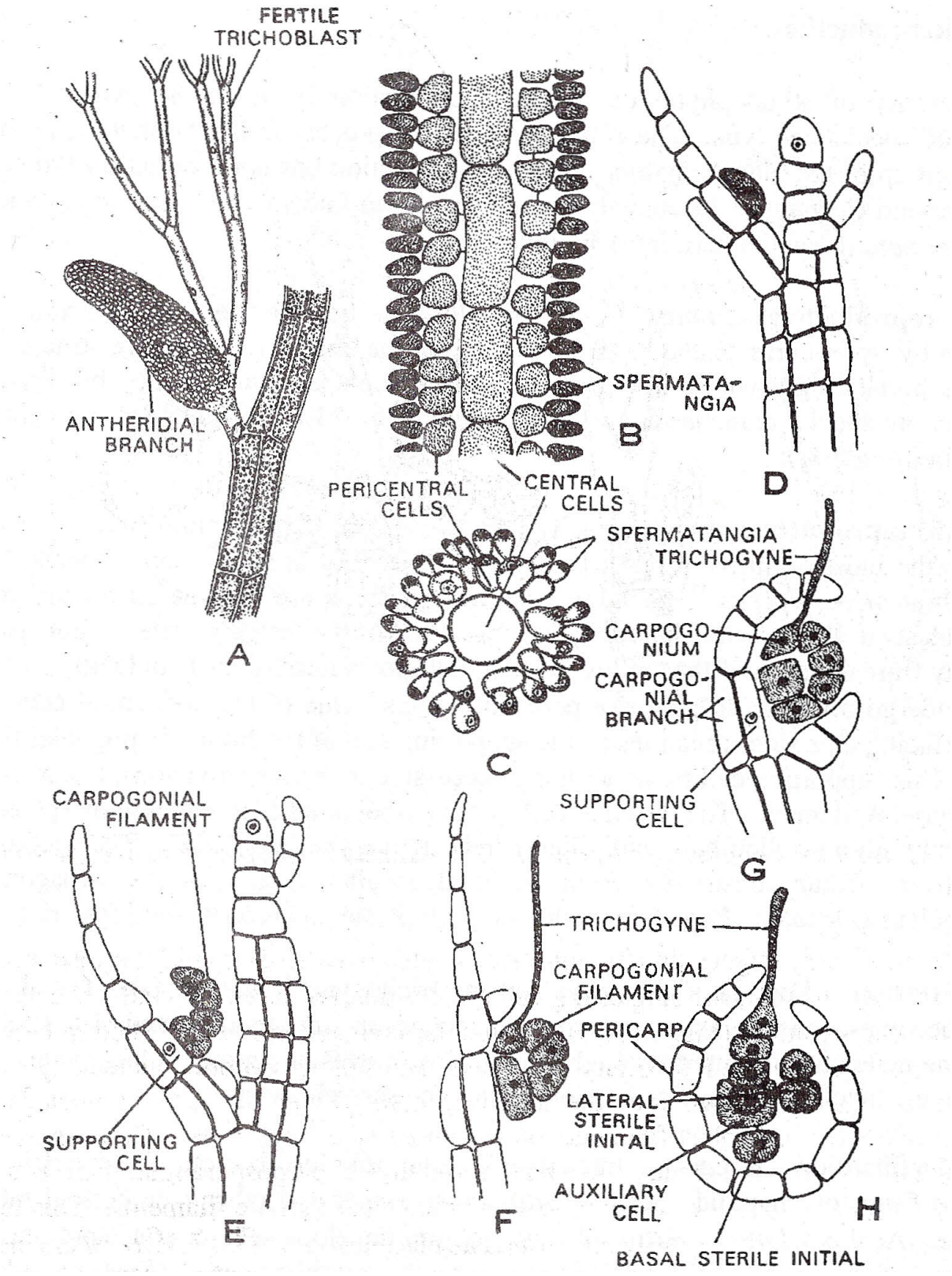


Figure-11.16: *Polysiphonia*, A. Part of a thallus with an antheridial branch; B. Antheridial branch in longitudinal section, C. Transverse section of antheridial branch; D-G. Showing stages in development of carpogonium. (adopted from Pandey, 1981).

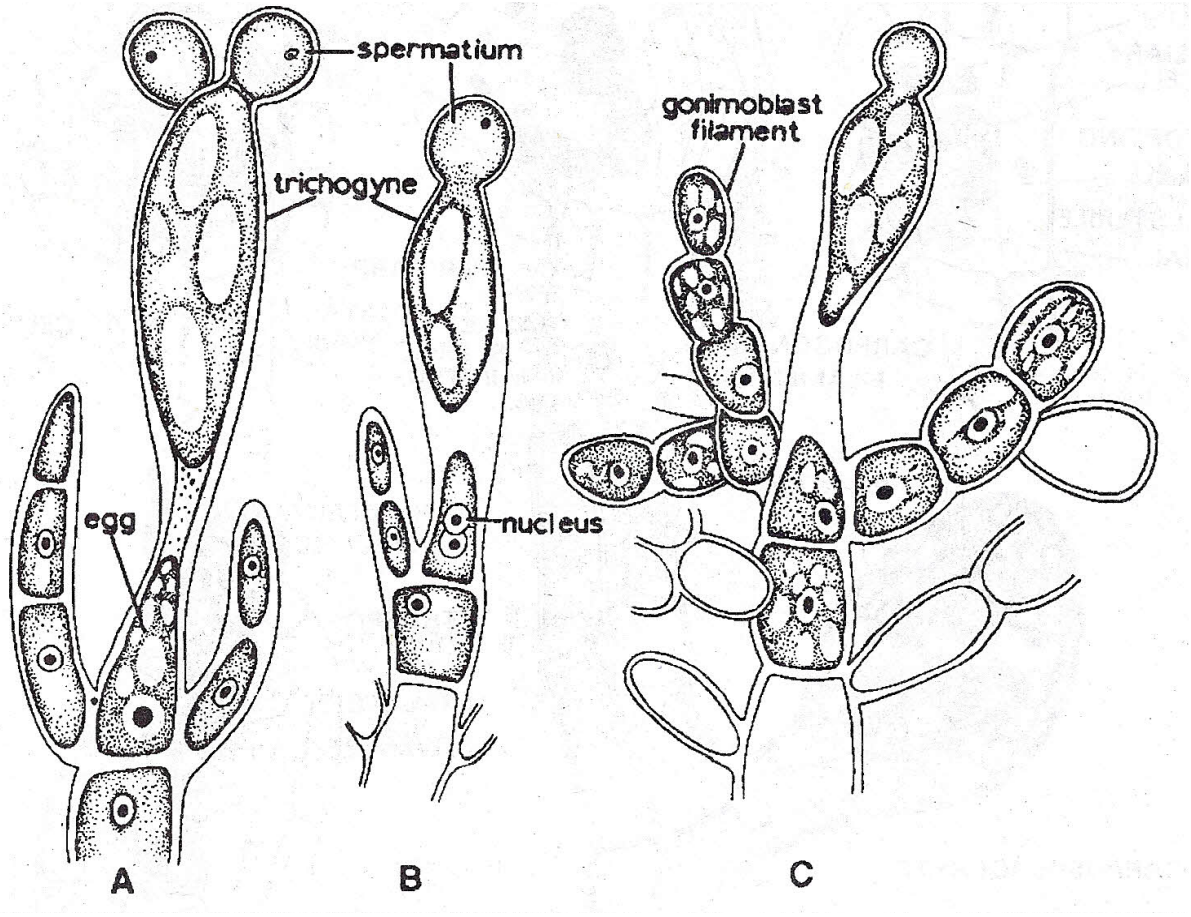


Figure-11.17: *Batrachospermum moniliforme*. A-C, ertilization and post-fertilization stages.

In Polysiphonia, zygote divides mitotically and gives rise to diploid carpospores. After fertilization in *Polysiphonia*, the supporting cell divides and produces an auxiliary cell just near the base of the carpogonium (Fig. 11.18 A, B). A tubular connection is established in between the base of the carpogonium and auxiliary cell, and the diploid zygotic nucleus lying in the carpogonium now passes down into the auxiliary cell. Later the diploid nuc1eus divides mitotically produces gonimoblast filaments on the upper side (Fig.11.18B). Carposporangia and carpospores are diploid in this genus. After the formation of carposporangia, there is a gradual fusion of the supporting cell, the auxiliary cell and the cells of sterile filaments. This leads to a formation of large irregularly shaped cell, termed as placental cell (Fig. 11.18 A,C). In the meantime, fleshy pericarp around the carposporangia is formed by the divisions of pericentral cells near the supporting cell of the female trichoblast. As a result, an urn-shaped fruit body known as cystocarp with an ostiole (opening) is formed (Fig. 11.18D). This is carposporophyte stage with cystocarp in which carposporangia are present.

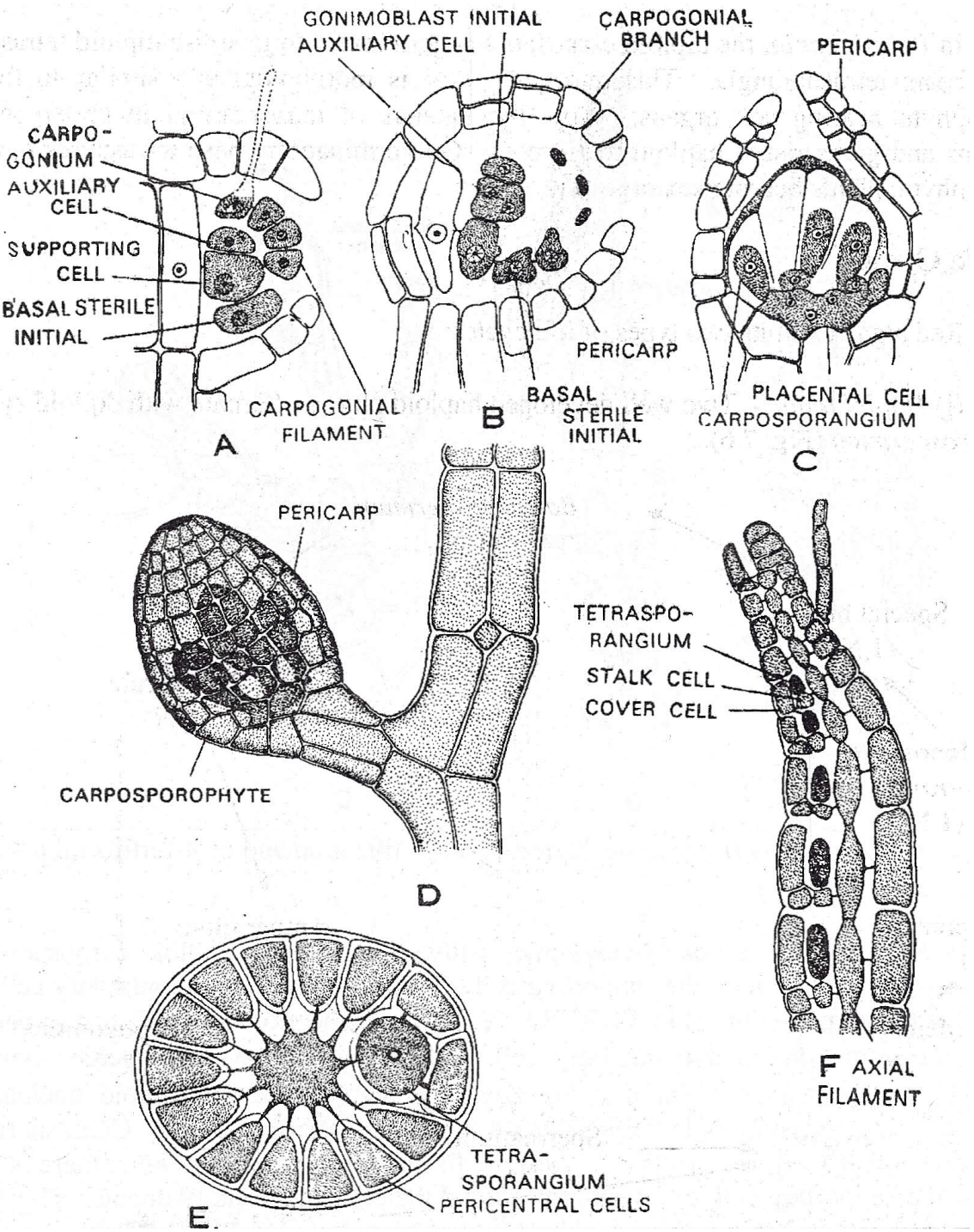


Figure-11.18: *Polysiphonia*. A. Fertilized carpogonium with auxiliary cell; B & C. Stages of development of a carposporophyte; D. Mature cystocarp; E & F. Showing development of a tetrasporophyte in transverse and longitudinal section respectively (Based on Smith, 1955).

In *Polysiphonia*, the diploid carpospore is germinated to give rise diploid tetraspomphyte which bears tetrasporangia. This tetrasporophyte is morphologically similar to the haploid gametophyte bearing sex organs. Now, the nucleus of tetrasporangiurn undergoes meiotic divisions and gives rise to haploid tetraspores. On germination, these tetraspores develop into gametophytic plants bearing sex organs.

11.3.5 Life Cycles:

Red algae exhibits two types of life cycles:

(i) Haplobiontic - Two well developed haploid phases alternate with diploid zygote, e.g., *Batrachospermum* (Fig. 11.19).

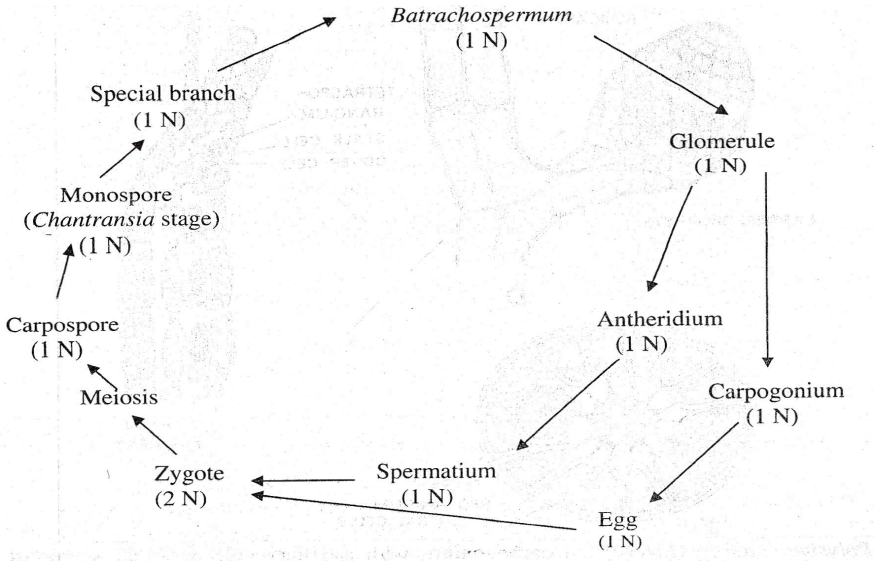


Figure-11.19: Life cycle of *Batrachospermum*

Morphologically, *Batrachospermum* is triphasic (or trigenic) as it involves alternation of three successive, dissimilar haploid somatic generations. So it is also known as HaplohaplohaplonticIife cycle. Cytologically, it is haplobiontic life cycle.

(ii) Diplobiontic - Two diploid phases and one haploid phase occur, e.g., *Polysiphonia* (Fig. 11.20).

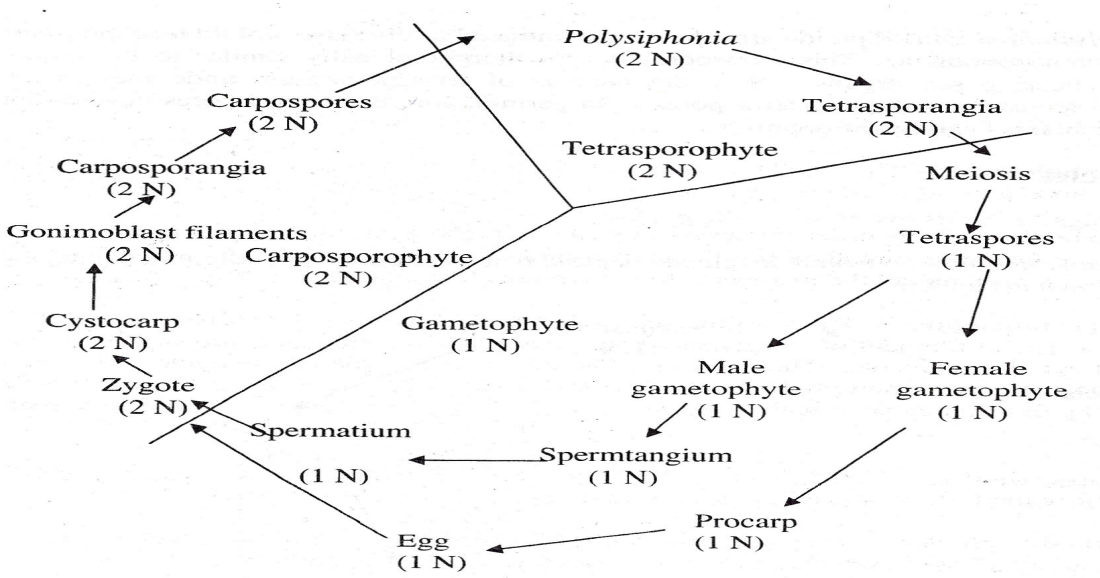


Figure-11.20: Life cycle of *Polysiphonia*

11.3.6 Classification:

The class, Rhodophyceae divided into two subclasses, (i) Bangioideae and (ii) Florideae.

(**A) Bangioideae:**

The subclass Bangioideae is characterized by having the intercalary growth of thallus and diploid carpospores derived directly from the zygote.

There is a single order Bangiales in this subclass.

(B) Florideae

In the subclass Florideae, the growth of the thallus is strictly apical and plasmodesmatal strands are very conspicuous. The carpospores are formed indirectly from the zygote. There are six orders in this subclass, viz., (A) Nemalionales, (B) Gelidiales, (C) Cryptonemiales, (D) Gigartinales, (E) Rhodymeniales, and (F) Ceramiales.

a) Nemalionales - The members of this order are non-tetrasporic, and carposporophytes are derived from zygote which undergoes meiosis, e.g., *Batrachospermum*.

b) Gelidiales - This order possesses only the tetrasporic plants of Florideae where the carposporophyte develops directly from the carpogonium. There is absence of auxiliary cells. The thallus is compact and all the members are uniaxial in construction, e.g., *Gelidium*.

c) Cryptonemiales - They are the only tetra sporic Florideae which bears auxiliary cell borne in a special filament of the gametophyte. Auxiliary cell filaments are supposed to be modified carpogonial filaments. These plants show diplobiontic type of life cycle and possess the most elaborative carposporophytes in the Rhodophyceae. The members of Cryptomoniales have their cell walls impregnated with lime. Some of these calcareous algae develop coral reefs, e.g. *Corallia*.

d) Gigartinales – They are the tetra sporophytic Florideae. The auxiliary cell is developed before the fertilization and it is a vegetative cell of the gametophyte.

e) Rhodymeniales – They are also tetra sporic Florideae. Auxiliary cell is formed before fertilization. Closed cystocarp (without an ostiole) is present in this order.

f) Ceramiales - They are tetra sporic Florideae. The auxiliary cell is formed after fertilization. Diploid carpospores give rise to the diploid sporophyte, e.g., *Polysiphonia*.

11.3.7 Phylogeny and Interrelationships:

Fossil evidences show that the Rhodophyta must have evolved after Cyanophyta during the precambian era. Both groups resemble each other in many morphological and biochemical features. Although the members of Bangioideae are less elaborative than the Florideae but they resemble the Cyanophyta in lacking the sexual reproduction and also in cell structure. The most important resemblances these two groups show are: (1) Presence of biliprotein pigments, (2) total lack of flagellated stages, (3) similarity of rhodophycean and cyanophycean starch, (4) thylakoids arranged singly and widely separated, (5) xylans as chief component in cell wall, (6) sulphated galactose, uronic acid, glucose and xylose as main components in the mucilage of Cyanophyta, Bangiales and Nemalionales of Rhodophyta, and (7) presence of pit connections in many Florideae and Stigonematales of Cyanophyta.

Inspite of these similarities, there are some fundamental differences in the cell structure, pigmentation and reproduction in between these two groups. Rhodophyta and Cyanophyta might have evolved from the common ancestor. Modern phycologists believe that the Rhodophyta is a distinct group of having primitive eukaryotic algae with many similarities with Cyanophyta.

11.3.8 Economic Importance:

The gelling agent, agar used in microbiological media is extracted from red algae. The porphyra is used as an important ingredient in soups and also cooked as a flavouring agent with meat in China and Japan. The alga, *Rhodymenia palmata* is used as a food and also in the preparation of medicines. Another important edible alga, *Chondrus crispus*, commonly known as Irish moss, is utilized in the preparation of various pharmaceuticals in eluding laxatives and cosmetics.

11.4 SUMMARY:

The members of Phaeophyta are brown or yellowish brown in colour due to the presenceof sufficiently larger quantity of fucoxanthin. The plant body is multicellular but motile reproductive structure is unicellular. They are abundant in tropics (Sargasso Sea of the Atlantic) and more prominent in cold waters. The thallus structure of the brown algae ranges from heterotrichous filamentous types through pseudoparenchymatous uniaxial forms to true parenchymatous forms. The simplest thallus organization in this phylum is the branched, heterotrichous habit, e.g., *Ectocarpus*. The large sized brown algae are called kelps (*Lamina ria*, *Nereocystis*). In *Laminaria*, the thallus is morphologically distinguishable into holdfast, stipe and blade. The blade which is primarily photosynthetic and spore, producing portion, is highly dissected. Intercalary growth takes place in Laminariales and leads to formation of gigantic plant body. In *Dictyota*, the thallus is repeatedly branched

dichotomously. In transverse section, thethallus is seen to be composed of three layers, the central one with large cells and, an upper and lower epidermis. The cells are uninucleated and one or more discoidal chromatophores which are parietalin position. The characteristic reserve food materials are the laminarin, mannitol, fats, hydrolases etc. The reproduction takes place by vegetative, asexual and sexual methods. Asexualreproduction takes place by zoospores produced in the unilocular and plurilocular sporangia, e.g., *Ectocarpus*. Isogamous to oogamous types of sexual reproduction are found inPhaeophyta. Oogamy is quite common in majority of Phaeophyceae. In Dictyotales the oogonial sorus and antheridial sorus occur on different plants; whereas in *Fucus* they may occurin the same conceptacles or in separate conceptacles as in *Sargassum*. Two divergent lines have been established in the evolution of Phaeophyceae. One of these lines has given rise to the groups possessed isomorphic alternation of generations, and the other to the groups of heteromorphic alternation of generations. In both the series, there has been a progressive evolution towards the complexity of the thallus and from isogamous to oogamous type of reproduction.

The phylum Rhodophyta is characterized by the pigment, y-phycoerythrin which gives red colour to the thallus. There is a great diversity in the vegetative structure of red algae. The subclass Bangioideae, with a single order Bangiales, comprises unicellular (*Porphyridium*). filamentous *Goniotrichum* and Parenchymatous (*Porphyra*) forms. The red algae are truly eukaryotic. They have microtubules but lack centrioles and flagella. The cells are uninucleate in lower Rhodophyceae orders such as Cryptomoniales and Bangiales. In higher orders, Ceramiales, Rhodymeniales etc., cells are multinucleate. The most important food product is floridean starch. The reproduction takes place by vegetative, asexual and sexual methods. The sexual reproduction is oogamous, which is highly elaborate and specialized type. The female sex organ is known as carpogonium and the male sex organ is spermatangium. During post-fertilization, an urn-shaped fruit body, known as cystocarp with an ostiole (opening) is formed. This is a carposporophyte stage with cystocarp in which carposporangia are present. In *Batrachospermum*, haploid carpospore (present in carposporangia) germinates and gives rise to Chantransia stage which in turn differentiates into adult plant of *Batrachospennum*. In *Polysiphonia*, the diploid carpospore germinates to give rise to the diploid tetrasporophyte that bears tetrasporangia. Now the nucleus of tetrasporangium undergoes meiotic divisions and it gives rise to 4 haploid tetrasproes. On germination, these tetraspores develop into gametophytic plants bearing sex organs.

11.5 TECHNICAL TERMS:

Polysiphonia, *Batrachospermum*, Carpospore, Tetraspores, Chantransia, Gametophye, Sporophyte, Haplobiontic, Diplobiontic.

11.6 SELF ASSESSMENT QUESTIONS:

1) Describe the thallus organization and ultrastructure of cell in Phaeophyceae.
2) Give an account on reproduction and alternation of generations in Phaeophyceae.
3) Give an account on characteristic features and reproduction in Rhodophyta.
4) Discuss characteristic features and post-fertilization changes in Polysiphonia.

11.7 SUGGESTED READINGS:

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Prof. A. Amrutha Valli

LESSON - 12

GENERAL CHARACTERS AND NUTRITION OF FUNGI

12.0 OBJECTIVE OF THE LESSON:

Students are able to know the rich diversity among fungal general characteristics and mode of nutrition in fungi.

STRUCTURE OF THE LESSON:

12.1 INTRODUCTION:

Fungi represent a vast and varied category within the plant kingdom. The term 'fungus' is derived from Latin, meaning mushroom. Fungi are characterized as achlorophyllous and heterotrophic thallophytes. Due to their similarities with algae in several respects, they are classified under the group Thallophyta. The scientific discipline dedicated to the study of fungi is referred to as mycology (from Greek, mykes - mushroom and logos - study), and those who specialize in this field are known as mycologists. Globally, it is estimated that there are between 50,000 and 100,000 recognized species of fungi. Currently, approximately 5,100 genera and 50,000 species of fungi have been identified, a figure that continues to grow as research progresses worldwide. These fungi thrive in a wide range of environments and exhibit significant diversity in their forms, structures, physiological functions, and reproduction.

The primary structure of these organisms consists of hyphae, which collectively form the mycelium, with exceptions in certain cases, mycelium is entirely absent Eg: Synchytrium or the organism is unicellular Eg: Saccharomyces species. Their cell walls are not composed of true cellulose; instead, they are made of chitin or fungal cellulose. Fungi are unable to produce their own food and most of them are saprophytic, parasitic, or symbiotic. Their primary storage forms of energy are glycogen. Reproduction occurs through vegetative,

asexual, and sexual means. P. A. Micheli known as Father of Mycology whereas E. J. Butler refers to as father of Indian Mycology.

Fungi obtain nutrients through heterotrophic absorption, meaning they secrete digestive enzymes into their surroundings to break down complex organic matter into simpler molecules, which they then absorb. They exhibit diverse nutritional modes, including saprophytism (decomposing dead organic material), parasitism (extracting nutrients from living hosts, sometimes causing disease), and mutualism (forming symbiotic relationships, such as mycorrhizae with plant roots or lichens with algae). Unlike plants, fungi lack chlorophyll and cannot perform photosynthesis, relying entirely on external organic sources for sustenance. Their ability to decompose complex substances like cellulose and lignin makes them essential for nutrient cycling in ecosystems.

Fungi exhibit a remarkable degree of cosmopolitanism and diversity, thriving in nearly every habitat. They prefer to grow in warm and humid places. They can be classified into various categories, with some existing in terrestrial environments, others in the air, and some inhabiting both freshwater and marine ecosystems. Additionally, certain fungal species can be found in an epiphytic state on algae and various aquatic plants, while others decompose dead organic matter in water. Some fungi reside beneath the earth's surface, displaying a sub-terrestrial lifestyle, and a select few act as endophytes within the leaves and stems of healthy plants. Additionally, numerous fungal species are parasitic, infecting a wide range of plants, animals, and humans.

12.2 THALLUS ORGANIZATION:

The fungal kingdom exhibits several distinct characteristics that differentiate it from the plant, animal, and other biological kingdoms. Except some unicellular forms (e.g. yeasts, *Synchytrium*), the fungal body is a thallus called mycelium. The mycelium is an interwoven mass of thread-like hyphae (Sing, hypha). Hyphae may be septate (with cross wall) and aseptate (without cross wall). Some fungi are dimorphic that found as both unicellular and mycelial forms e.g. *Candida albicans.* The thallus may be two types: 1. Unicellular and 2. Filamentous.

1. Unicellular Thallus:

In some of the lower fungi, thallus is more or less a spherical, single celled structure. At the time of reproduction it becomes a reproductive unit. Such fungi are called holocarpic. In the unicellular holocarpic forms, the mycelium is absent e.g. *Synchytrium*. Some holocarpic fungi (e.g., yeast) producing bud cells in succession and these remain attached to one another in a chain. Such a chain of bud cells is referred to as pseudomycelium. Different unicellular forms of fungi are as follows.

Yeast:

Yeast is of wide occurrence; is found on sugary surface of ripened fruits and can be easily grown in any sugar solution. Individual cells remain attached to each other forming a chain. Fine structure of a yeast cell resembles with that of a eukaryotic cell. The cell has a well-defined nucleus, endoplasmic reticulum, mitochondria and other organelles along with a large area of the cell occupied with a vacuole.

Slime Molds:

Slime moulds are usually multicellular, sometimes unicellular (multinucleate) forms are also seen. However, these are not considered true fungi. During the course of their life cycle these organisms show protozoan-like (unicellular and multinucleate) or fungus-like stages (multicellular) Slime moulds are of two types:

a) **Cellular Type:** *Dictyostelium discoideum* referred to as myxamoeba. It depends on bacteria during vegetative stage and divides via binary fission. Later numerous myxamoeba aggregate to form a single multinucleate structure known as a slug, while the individual cells are maintain their distinct cell membranes. This formation is termed pseudoplasmodium. Similar to true fungi, during the reproductive phase, spores are generated within sporangia. Each spore, upon germination, develops into an amoeba-like structure.

b) **Plasmodial Type:** *Echinostelium minutum* forms a large mass of multinucleate amoeboid cytoplasm with diploid nuclei during vegetative phase. The individual cells are however not delimited by cell membrane. It feeds on bacteria and encysted myxamoeba. They do not have a definite size or shape. It alters its shape depending upon the substratum. During reproductive phase entire plasmodium takes part in formation of frutification which bears spores. Spores germinate to form flagellated cells which later on develop into plasmodium.

2. Filamentous Thallus: In most true fungi, the thallus is filamentous composed of Hyphae. Loosely aggregated hyphae are collectively forms a network known as mycelium. Each hypha may vary in shapes and sizes. Branching of hyphae is dichotomous. On the basis of presence or absence of septa the hyphae of mycelical fungi are of two types a) Non-septate hyphae or Aseptate hyphae b) Septate hyphae

a) **Nonseptate or Aseptate Hyphae:** Mycelium contains numerous nuclei, lying in a common mass of cytoplasm, without cross wall in the hyphae, E.g., oomycetes and zygomycetes. Such a condition is known as coenocytic. However, septa may be laid down at the time of formation of reproductive organs to delimit them from the rest of the vegetative hyphae, therefore called Pseudosepta. E.g., *Allomyces.*

b) Septate Hyphae: Hyphae are septate and hyphal segments may contain one, two or more nuclei. E.g., Ascomycotina, Basidiomycotina and Deuteromycotina. There are two types of septa i) Primary septa ii) Adventious septa

i) Primary Septa: Primary septa are formed in association with mitotic or meiotic nuclear division, and they separate the daughter nuclei. Ascomycotina, Basidiomycotina and their asexual states

ii) Adventitious Septa: Adventitious septa are formed in the absence of mitosis or meiosis and occur especially in association with change in the local concentration of cytoplasm. Eg: lower groups of fungi as mastigomycotina and zygomycotina.

In ascomycetes, the continuity of cytoplasm is preserved through a small, simple pore located at the center of the septa. In basidiomycetes, with the exception of rusts and smuts, perforated septa are also present, albeit with a slight modification characterized by a barrel shaped inflation featuring a hemispherical perforated membrane on either side of the opening, referred to as the dolipore septum. Fungal mycelium is classified as homokaryotic when the individual cells of the septate hyphae possess genetically identical nuclei. Conversely, some fungal mycelium contains nuclei of varying genotypes, which may result from mutations or the anastomosis of hyphae; these are termed heterokaryotic. Members of basidiomycetes can exhibit either two genetically distinct nuclei (dikaryotic) or a single haploid nucleus that is genetically identical within each segment (monokaryotic).

Aggregations of Fungal Hyphae:

At certain stages in life history of all fungi they show various degrees of hyphal aggregation ranging from loosely to compactly woven tissues. All such organized fungal tissues are known as plectenchyma. The three general types of plectenchyma are:

a) Prosenchyma/Prosoplectenchyma: Hyphae are loosely interwoven lying more or less parallel to each other.

b) Pseudoparenchyma/paraplectenchyma: Hyphae are compactly interwoven looking like a parenchyma in cross-section.

c) Pseudosclerenchyma: It consists of closely packed, thick-walled and dark cells

Plectenchyma forms various types of vegetative and reproductive structures such as mycelial strands, mycorrhiza, rhizomorph, stroma, sclerotia and sporophore.

Mycelial Strands: These structures are found in Basidiomycetes and some Deuteromycetes. They consist of parallel and relatively simple hyphae. A mycelial strand forms around one or more leading hyphae, which grow from the edge of the thallus. These leading hyphae become surrounded by their own branching and intertwining parts, creating a cord that is 1-2 mm thick and several centimeters long. Mycelial strands help transport materials and allow the fungus to reach new food sources from an existing base.

Mycorrhiza: It is a symbiotic relationship between fungal threads (Agaricales) and the roots of plants. The tips of roots in both coniferous and deciduous plants often consist of multiple layers of fungal cells. The fungal mycelium grows into the soil and then into the root's cortical cells, creating a structure known as the 'Hartig network.' This extended fungal mycelium takes over some of the root's functions, helping to absorb more minerals from the soil. Roots that have this mycorrhizal network perform better than those that do not.

Rhizomorph (Gr. *rhiza* = root + *morphe* = shape): These are highly differentiated root like hyphal aggregations which have a well developed apical meristem, a central core (thin-walled, elongated cells) and a rind (smaller, thick-walled highly pigmented cells). These are produced by *Armillaria mellea*, a tree and shrub parasite. Rhizomorphs helps the fungus in spreading from one root system to another.

Stroma: This is a small cluster of hyphae that looks like a cushion or mattress where fruiting bodies develop. These structures are found in groups like Deuteromycetes, Basidiomycetes, and Ascomycetes, appearing as different types of ascocarps, pycnidia, basidiocarps, acervuli, synnemata, and sporodochia.

Sclerotium (pl. **Sclerotia, Gr. Skleros=haid):** A tough resting structure is created by the clumping of somatic hyphae. These structures can be round, long, or flat, and their size, shape, and color are unique feature of specific species. Each Sclerotium germinates into a mycelium, on return of favourable condition e.g., *Penicillium.*

Microsclerotia (*Macrophomina phaseoli*): Most sclerotia are less than 100 micrometers wide, with many not exceeding 2 cm. In contrast, Polyporus myllittae has sclerotia that can be more than 25 cm wide and weigh several kilograms. These structures store nutrients and help the fungus endure harsh conditions by functioning as a propagule.

Sporophores: These structures produce spores and are typically upright and above ground. They can be branched, like in Peronospora, or unbranched, like in Albugo. They carry sporangia in Albugo and conidia in Peronospora. Structures that hold sporangia are called sporangiophores, while those that hold conidia are called conidiophores. Sporophores often appear in clusters and can create formations like pycnia, hymenia, sporodochia, and acervuli.

Rhizoids: A rhizoid is a small, root-like branch of the thallus that usually grows in clusters at the bottom. They help with anchoring and absorbing nutrients, as seen in organisms like Rhizophydium and Rhizopus.

Appressoria (Sing. appressorium): It is a terminal, swollen, sticky structure made of infecting hyphae that sticks to the host or other surfaces, aiding in the infection process. This structure is formed by certain parasitic fungi like powdery mildews and rust.

Haustoria (Sing. haustorium): Haustoria are specialized structures that arise from hyphae, primarily serving the purpose of nutrient absorption. These organs are typical of obligate parasitic organisms. Their morphology can differ significantly, exhibiting various froms like button-shaped, elongated, finger-like, or branched. Additionally, haustoria produce specific enzymes that facilitate the hydrolysis of proteins and carbohydrates present in the host plant.

Hyphal traps (Snares): The predacious fungi produce sticky filaments or a network of loops called hyphal traps or snares. They use these to catch nematodes.

Stromata: These are small body structures similar to mattresses. Fruits usually develop on or inside them.

12.3 ULTRA-STRUCTURE AND COMPOSITION OF FUNGAL CELLS:

Unicellular yeast as well as the filamentous fungi has a typical eukaryotic cellular organization (Fig. 12.1). As other non-photosynthetic organisms, fungi contain all major organelles except chloroplast. The detailed structure of fungal cell is given here under.

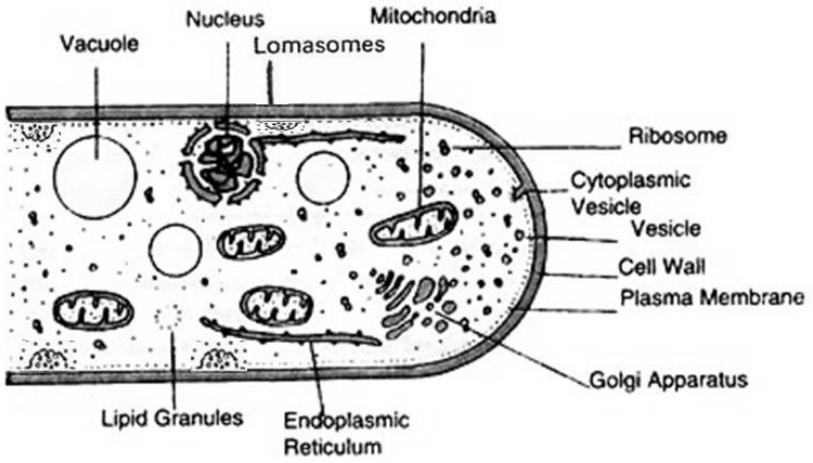

Figure-12.1: Ultra structure of fungal cell (https://tinyurl.com/5fryhz7e)

Cell Wall: Fungal cells, with the exception of slime molds (Myxomycetes), are characterized by a rigid cell wall and various organelles. The composition of the cell wall varies among different fungal taxa. Chemical analyses indicate that the cell wall is primarily composed of 80-90% polysaccharides (glycans), with the remainder consisting of proteins (such as glycoproteins and glycalyx) and lipids (including phospholipids and glycolipids) as illustrated in Fig. 12.2. The cell walls typically contain chitin (a polymer of N-acetyl glucosamine), cellulose (a polymer of D-glucose), or other glucans, which are organized into fibrils that form distinct layers. Most fungi do not possess cellulose in their cell walls, with the notable exception of Oomycetes; however, chitin and cellulose are often found together in certain species, such as *Ceratocystis* and *Rhizidiomyces*, which contain a variant of chitin referred to as fungus cellulose. The structural formulas for the repeating units of cellulose and chitin are presented in Fig.12.3.

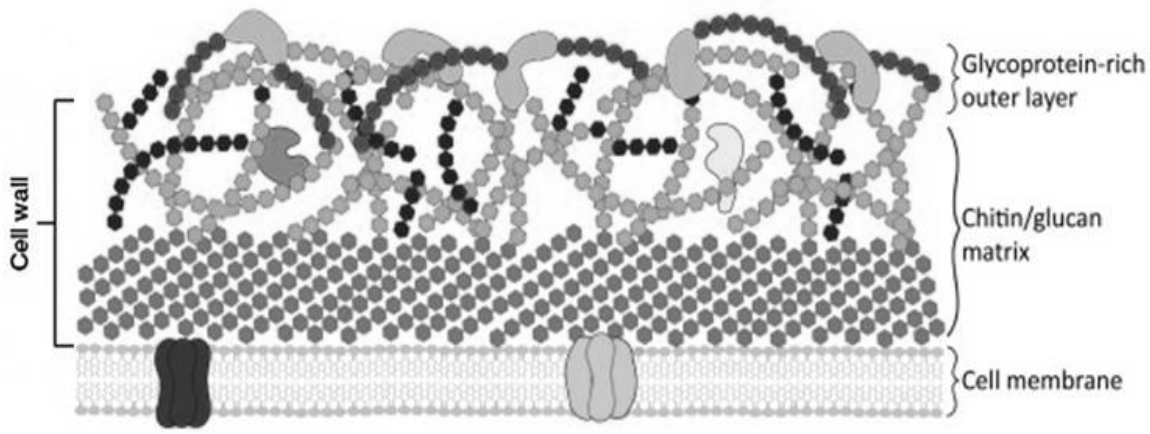

Figure-12.2: Structure of bacterial cell wall (https://tinyurl.com/4kmmatdw)

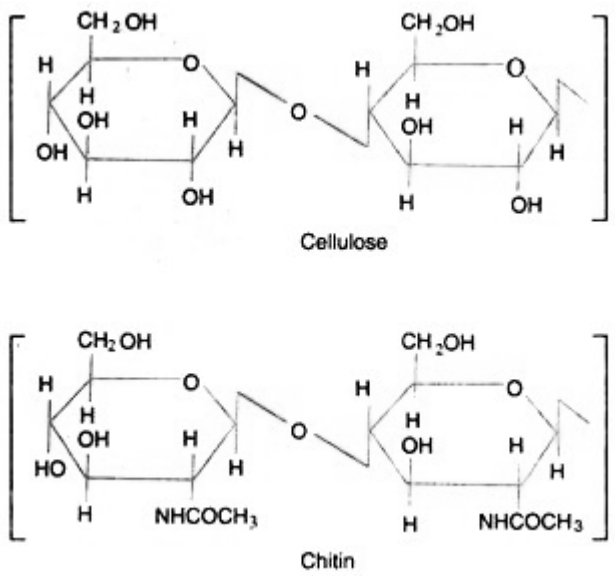

Figure-12.3: Structures of cellulose and chitin (https://tinyurl.com/zawhcjyy)

Chitin constitutes the principal component of cell walls; however, cellulose is also present in Oomycetes, accompanied by glucans. In addition to cellulose, their cell walls incorporate an amino acid and a hydroxy-protein. Furthermore, various proteins and enzymes are associated with these cell walls. In the genera *Peronospora* and *Saprolegnia*, true cellulose is identified, whereas *Phytophthora* and *Pythium* are characterized by the absence of cellulose and a higher concentration of glucans. Certain fungi have been documented to possess chitin within their cell walls. The predominant constituents of cell walls in Zygomycetes, Ascomycetes, and Basidiomycetes are chitin; however, yeasts and some members of Hemiascomycetidae lack chitin, with their cell walls composed of micro-fibrils of mannans and β-glucan (Table 12.1).

Table-12.1: Taxonomy of Fungal Cell Walls

Taxonomic grouping	Fibrillar polymers	Matrix polymers	Perforate septa present or absent
Oomycetes	β(1,3)-, β(1,6)-Glucan Cellulose	Glucan	Absent
Chytridomycetes	Chitin; glucan	Glucan	Absent
Zygomycetes	Chitin; chitosan	Polyglucuronic acid; glucuronomannoproteins	Absent
Basidiomycetes	Chitin; β(1,3)-, β(1,6)-glucans	α(1,3)-Glucan; xylomannoproteins	Present (mostly Dolipore)
Ascomycetes/ Deuteromycetes	Chitin; β(1,3)-, β(1,6)-glucans	α(1,3)-Glucan; galactomannoproteins	Present (mostly simple with large central pore)

Plasma Membrane (Plasma lemma/Cell Membrane): The plasma membrane, which encases the cytoplasm, is situated just beyond the cell wall. This membrane exhibits semipermeable properties and shares structural and functional characteristics with prokaryotic membranes. Notably, specialized organelles are present on the surface of the plasma membrane. It has the capacity to invaginate, forming pouch-like structures that contain granular or vesicular substances. Moore and McAlear (1962) designated these formations as lomasomes. Lomasomes arise from various membrane configurations external to the plasma membrane, known as plasmalemmasomes. These plasmalemmasomes may facilitate the regulation of material transport into and out of the cell and could also be involved in the utilization of bicarbonate during the process of photosynthesis.

Cytoplasm: Cytoplasm is a colorless substance that contains sap-filled vacuoles. The inclusions found within the cytoplasm are typically non-functional, dead, Eg: glycogen, oil droplets, pigments, and secretory granules. With the exception of chloroplasts, most organelles such as the endoplasmic reticulum, mitochondria, ribosomes, Golgi apparatus, microbodies, filasomes, vacuoles, and multivesicular bodies (MVBs) are located within the fungal cytoplasm. Additionally, certain fungi possess Woronin bodies, which are linked to septal pores. Lomasomes can also be found situated between the plasma membrane and the cell wall.

Endoplasmic Reticulum: The structure consists of a network of microtubules interspersed with small granules. In the majority of fungi, it exhibits a highly vesicular nature. When compared to the cells of green plants, it appears loose and irregular. In multinucleate hyphae, the nuclei can be linked by endoplasmic reticulum.

Mitochondria: Mitochondria, which are numerous small structures ranging from spherical to elongated shapes, are found throughout the cytoplasm. These organelles are encased in a double membrane. The inner membrane features infoldings that create parallel flat plates of irregular tubules, referred to as cristae. Mitochondria contain their own mitochondrial DNA (mt-DNA), which exists as circular double helical molecules that lack histones. Additionally, mitochondria possess their own machinery for the transcription and translation of organelle-specific DNA.

Golgi Apparatus/Dictyosomes: In fungal cells, the Golgi apparatus is infrequently found, with the exception of Oomycetes (such as Pythium) and non-fungal eukaryotic cells. In these organisms, the Golgi apparatus is characterized by stacks of folded membranes, play vital role in secretion. Notably, in Saccharomyces cells, one can observe a Golgi apparatus composed of three flattened sacs.

Vacuoles: Vacuoles are present in the mature cells of hyphae, while the tips of young hyphae do not contain vacuoles. As the hyphae age, the vacuoles merge together. These vacuoles are encased by a membrane referred to as the tonoplast.

Septum: A cross wall serves to separate adjacent cells in fungal structures (Figure 4.4). There are primarily three types of septa observed in fungal cells: (a) Complete septa, which are devoid of pores and are infrequently found in vegetative hyphae (b) Perforated septa, which possess a pore allowing the free passage of cytoplasmic organelles, such as mitochondria and nuclei, commonly seen in Ascomycetes and Deuteromycetes, and (c) Dolipore septa, named after the Latin word "dolium," meaning a large jar, which are characteristic of basidiomycetes and exhibit greater complexity. The central pore of these septa is encircled by a curved flange of wall material, often thickened to create a barrel-shaped cylindrical structure. Additionally, these septa are frequently covered by the perforated endoplasmic reticulum, with the central pore cap referred to as the parthenosome.

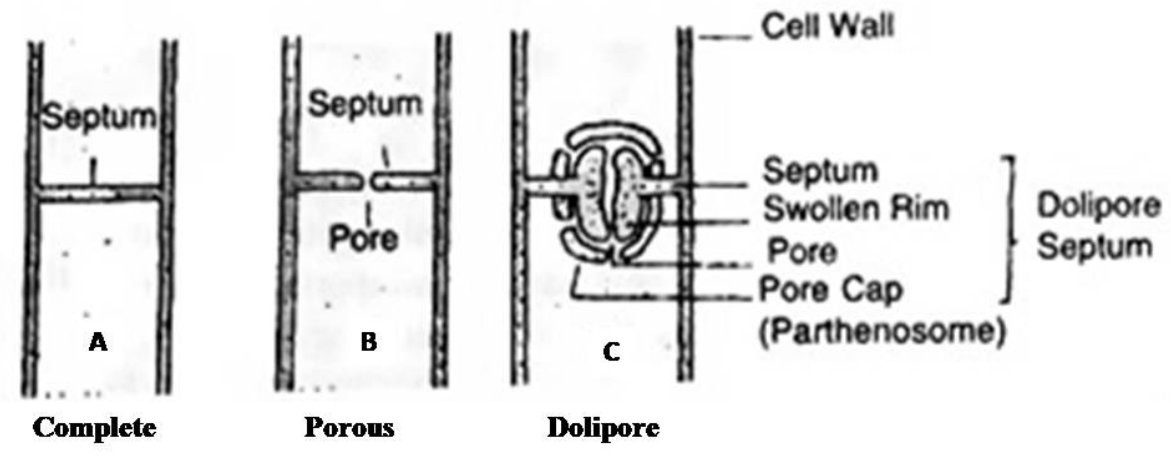

Figure-12.4: Various types of septa in fungi (https://tinyurl.com/5fryhz7e)

Cytoplasmic Inclusions:

The cytoplasm is composed of a range of inclusions, including lipid droplets, glycogen, trehalose, proteinaceous substances, and volutin. Glycogen is stored within the vacuoles. The cytoplasm also secretes various metabolites, including enzymes and organic acids. In fully developed cells, there is a significant presence of lipids and glycogen.

Nucleus:

The cytoplasm may contain one or more spherical nuclei, each measuring approximately 1-3 micrometers in diameter. A nucleus is characterized by a bilayered, porous nuclear envelope that surrounds the chromosomes and the nucleolus. Chromosomes are composed of DNA along with several fundamental proteins known as histones. The DNA content undergoes continuous changes in relation to cell growth. Nuclear pores facilitate the exchange of materials between the cytoplasm and the nucleus.

12.4 FUNGAL NUTRITION:

Unlike green plants, fungi meet their nutritional requirements by assimilating preformed organic matter and carbohydrates are the preferred nutrient source. Fungi are achlorophyllas, heterotrophic eukaryotic thallophytes. Fungi can readily absorb and metabolize a variety of soluble carbohydrates, such as glucose, xylose, sucrose, and fructose, but are also characteristically well equipped to use insoluble carbohydrates like starches, cellulose, hemicelluloses, and lignin.

Fungi Nutritional Requirements:

Fungi are heterotrophic in nutrition. They are chlorophyll deficient organisms, hence cannot manufacture carbohydrates. Fungi mostly dependent on dead or living organic matter for their energy requirements. All fungi are chemoheterotrophic and can synthesize the organic compounds from pre-existing organic sources using chemical reactions. Since their protoplasm is protected by a rigid wall, fungi must obtain their nutrients by the process of absorption. Small molecules (e.g. simple sugars, amino acids) in solution can be absorbed directly across the fungal wall and plasma membrane. Larger, more complex molecules (e.g. polymers such as polysaccharides and proteins) broken down into smaller molecules, and absorbed. This degradation takes place outside the fungal cell or hypha and is achieved by enzymes which are either released through or are bound to the fungal wall. Because these enzymes act outside the cell they are called Extracellular enzymes.

Essential Elements:

Fungi require various elements as food source in extremely micro and macro elements. These elements include C, O, H, N, P, K, Mg, S, P, Mn, Cu, Mo, Fe, Zn and Calcium. The macro elements are body builders and provide energy for metabolic processes.

Sources of Elements:

The organic substances usually utilized by fungi are very varied in nature. The carbohydrates are needed for building up the body and also as a source of energy. In a typical fungus, 50% of the dry weight is carbon of the carbohydrate source of carbon, most fungi use simple sugars. Glucose is suitable for almost all fungi and next in preference are the fructose and Sucrose. The polysaccharides, starch and cellulose are utilized by a fewer fungi which can synthesize the appropriate hydrolytic enzymes. Less commonly used sugars are the hexose sugars and some pentoses. Mannitol is equivalent to glucose for many fungi. Maltose which occurs in nature as a byproduct of starch hydrolysis is utilized by many fungi. Basidiomycetes include most of the lignin-utilizing fungi. Some fungi are able to make good growth on fats as the only source of carbon. Proteins, lipids some organic acids and higher alcohols are utilized by some fungi as a sole energy source of growth, however, is always better on a substance containing a suitable carbohydrate. Besides carbon, fungi require nitrogen. Fungi require nitrogen through both organic and inorganic materials.

In nature, fungi decompose proteins and peptide or an amino acid to obtain their supply of nitrogen. The members of saprolegniaceae and blastocladiales grow only with organic nitrogen such as amino acid. In pure cultures amino acids, peptides, or peptones gelatin, casein and egg albumin can serve as sources of organic nitrogen for building up protoplasm. Urea is also considered as a utilizable nitrogen source for some fungi. Many fungi, however, obtain nitrogen from inorganic sources. A number of fungi are known which use both nitrate and ammonium salts (Example: *Absidia* sp., *Mucor hiemalis*, *Lenzites trabea* and *Marasmius* sp.). Fewer fungi are able to utilize nitrate salts. Organic sources of nitrogen can also serve as sources of carbon. Soil inhabiting *Rhodotorula* and yeast-like *Pullularia pullans* fix atmospheric nitrogen. Hydrogen and oxygen are supplied in the form of water which is the major constituent of fungus mycelium forming about 85- 90% of the entire weight. The chief among the inorganic nutrients which the fungi require in fairly large amounts for their mineral nutrition are sulphur, phosphorus, potassium and macronutrients the fungi obtain from simple inorganic salts or sources such as sulphates for sulphur, and phosphates for phosphorus.

Some fungi are reported to require only minute traces of iron, zinc, copper, manganese and cobalt and molybdenum in anionic forms. Calcium is not known to be needed by the fungi in

general. Some, however, require it as a micronutrient. Some fungi are reported to require only minute traces of iron, zinc, copper, manganese and cobalt and molybdenum. These trace elements or micronutrients are considered essential of growth. The form in which the major and the minor metallic element requirements are utilised is the anion. Fungi store excess food in the form of glycogen or lipids. Fungi utilize the vitamins or growth factors in minute amounts. The important fungal vitamins, which may function in enzyme systems include thiamine (B1), biotin, pyredoxine (B6) and riboflavin (B2). A few fungi also need nicotinic acid and pantothenic acid. The vast majority, however, require thiamine (B1). A few fungi also need nicotinic acid and pantothenic acid.

Mode of Nutrition in Fungi:

On the basis of mode of nutrition fungi are classified into four groups. 1. Saprophytes 2. Parasites 3. Symbionts 4. Predaceous.

1. Saprophytic Fungi: Saprophytic fungi obtain their nutrition from dead organic matter. It may be both animal or plant origin. The vegetative phase of these fungi directly absorb nutrition required for their growth. Some species bear special structures for absorption of nutrition called rhizoids. These fungi mainly produce exo-enzymes for release of simple organic matter. They may be of two types.

i) **Ectophytic saprophytes:** Grow on the surface of organic matter.

ii) **Endophytic saprophytes:** Grow inside the organic matter Ex. Saprolegnia, Mucor, Rhizopus, Aspergillus, Penicillium, Agaricus etc.

2. Parasitic fungi: The parasitic fungi absorb their food material from the living tissues of the hosts on which they parasitize. Such parasitic fungi are quite harmful to their hosts and cause many serious diseases (Fig.12.5). The living organisms on which fungi grow are called host. The growing fungi are harmful to the host as they develop disease conditions in their host. Such relationship is known as parasitism.

The parasitic fungi absorb their food from the hosts in different ways either internal or external. On the basis of location of parasitic fungi in their host they are classified into two groups.

i. Ectoparasitic: Fungi live on outer surface of host (Ex. *Erysiphe*)

ii. Endoparasitic: Fungi grow inside the host tissue (Ex. *Fusarium*)

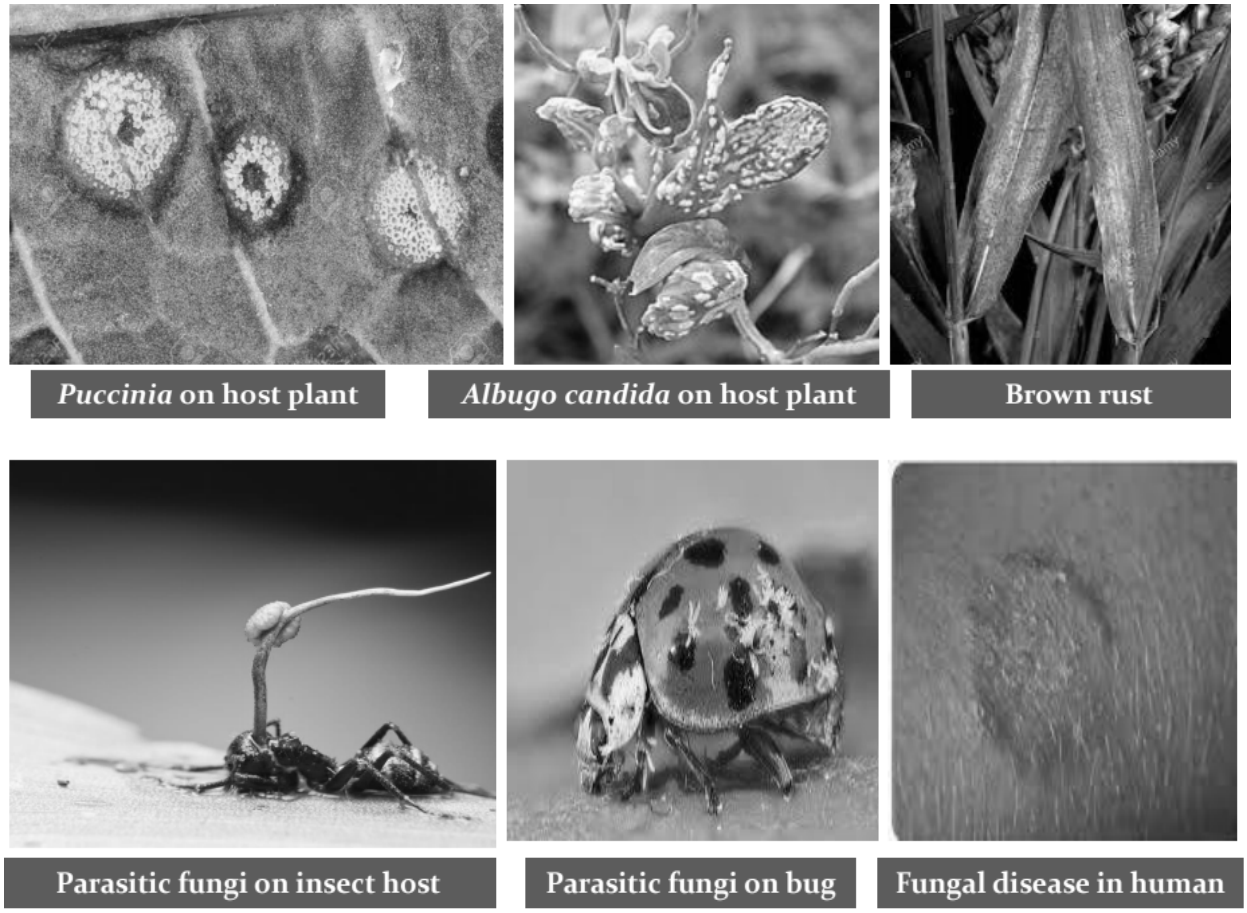

Figure-12.5: Fungal infections on various plants and animals

In ectoparasitic type of fungi cushion-like appressoria develop on the surface of the host and from each appressorium a peg-like structure develops which penetrates the host epidermal cell giving rise to a branched or unbranched absorbing organ called the haustorium (Fig.12.6). Its size and shape varies in different fungal groups. It may be round, knob like, club like or branched Ex. *Erysiphae*, *Phytophthora*, *Albugo*. These fungi cause the great losses to the human beings directly or indirectly. The rusts, smuts, bunts, mildews and many other plant diseases are important examples of fungal diseases of crops. The haustoria may also develop from the mycelium of endoparasites. The haustoria vary in their shapes. They may be small, rounded, and button-like as in *Albugo*, branched and convolute as in *Peronospora* and highly branched as in *Erysiphe*. In the case of rusts and mildews the mycelium remains confined in the pustules and not in the whole body of the plant. This type of fungus is called the localized fungus. When the mycelium prevails in the whole of the plant it is said to be systemic fungus, e.g., smuts. When the mycelium is confined to the intercellular spaces it is called intercellular mycelium and in other cases the mycelium penetrates the host tissue and said to be intracellular.

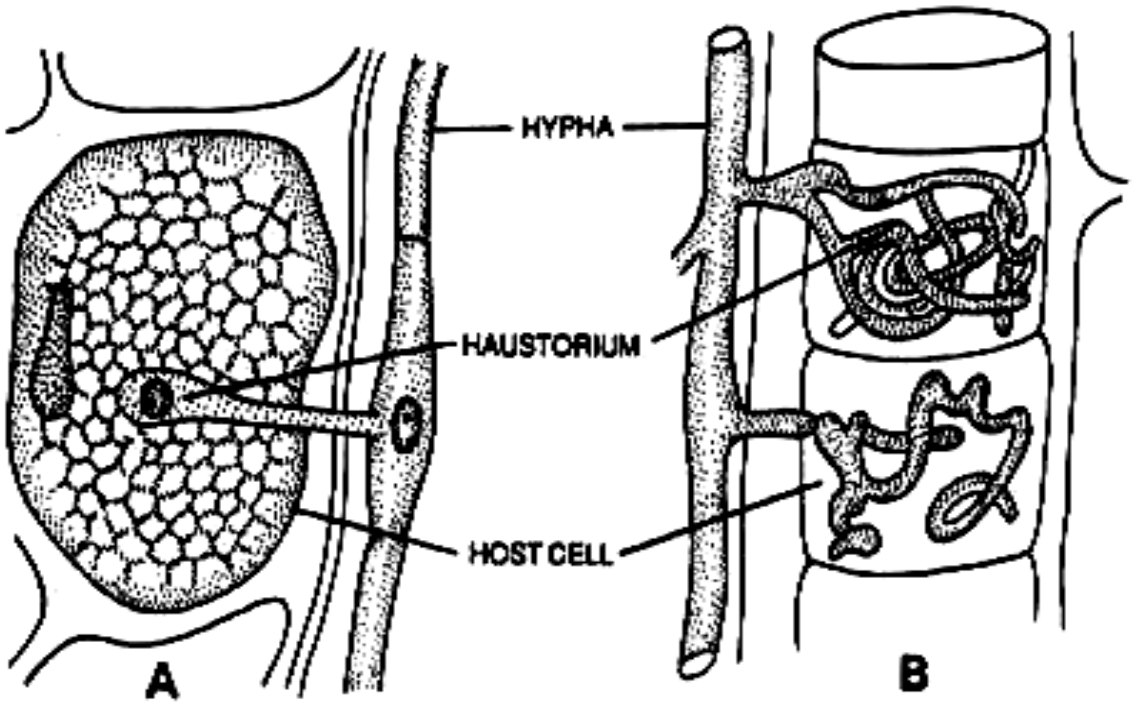

Figure-12.6: (A) Elongated capitates haustorium (B) Branched or digitate haustorium

Based on their mode of infection fungi are of three types.

i) **Obligate parasites:** essentially require living host, not able to live on dead organic matter Ex. *Puccinia*, *Albugo*

ii) **Facultative Saprophytes:** These are parasites but can live on dead organic matter when specific host is not available Ex. *Taphrina*.

iii) **Facultative parasites:** These are usually saprophytes but under certain conditions they parasitized living host Ex. *Fusarium*, *Phythium*

3. Symbionts: These fungi grow on or with living organisms but both of them are mutually benefitted Ex. Lichen and Mycorrhiza. Lichens are symbiotic association of algae and fungi. Mycorrhiza are symbitic association of fungi and roots of higher plants. It may be ecto or endo mycorrization in location.

Lichens Nutrition: Lichens are symbiotic associations between a fungus (mycobiont) and a photosynthetic partner (photobiont) either a green alga or a cyanobacterium. The mechanism of nutrition in lichens is based on a mutualistic relationship, where both partners benefit from each other. Usually a green alga (e.g., *Trebouxia*) or a cyanobacterium (e.g., *Nostoc*) performs photosynthesis and produces organic carbohydrates (e.g., glucose, polyols). These carbohydrates are then transferred to the fungal partner. Carbohydrates produced by the photobiont move to the fungal partner through diffusion or cytoplasmic connections. The fungal hyphae, especially in the medullary layer, absorb these nutrients. Mycobiont partner (ascomycete or basidiomycete) absorbs the photosynthates (sugars) from the photobiont. In

return, the fungus provides inorganic nutrients like nitrate, phosphate, and other minerals by absorbing them from dust, rain, and the substratum. It also provides water, minerals to the photobiont by absorbing moisture and nutrients from the atmosphere and substratum through its hyphae. Apart from this it creates a protective environment by forming a thallus, which protects the photobiont from desiccation and harsh light. There are two types of symbiosis in lichen Nutrition 1. Mutualism: Most common form, where both partners benefit. 2. Controlled Parasitism: The fungus may exert control over the algal partner, taking more food than necessary.

Mycorrhizal Nutrition: Mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant. This relationship is particularly important for plant nutrition and is crucial in nutrient-poor soils. Mycorrhiza is of two types 1. Ectomycorrhiza (EM): Fungi form a sheath around the root surface. Hyphae penetrate between root cortex cells but not into cells. This type of nutrition is very common in trees (e.g., pine, oak). 2. Endomycorrhiza (Arbuscular Mycorrhiza or AM): Fungal hyphae penetrate root cortical cells and forms structures like arbuscules (exchange sites) and vesicles (storage). This type of nutrition is found in most herbaceous plants and crops. During mycorrhizal nutrition fungal spores first forms colonization by germination in the soil and grow toward plant roots. Chemical signals like strigolactones from roots and Myc factors from fungi are guide each other in recognition and colonization. In AM fungi, hyphae enter roots and form arbuscules within cortical cells for nutrient exchange. The arbuscules (in AM) or Hartig net (in EM) serve as interfaces. The interface allows bidirectional nutrient flow without direct cytoplasmic mixing. Fungal hyphae extend far into the soil beyond the root depletion zone. They absorb phosphorus (P) in the form of phosphate ions and nitrogen (N) as ammonium or nitrate. Other minerals such as zinc, copper, calcium, etc. also absorbed by fungal hyphae. Mycorrhiza can also access organic forms of nutrients unavailable to roots. Nutrients absorbed by fungal hyphae are translocated to the root interface. They are released into the plant root cells through specialized transporter proteins at the periarbuscular membrane (in AM). In return, plants supply carbon (as sugars) to the fungus, derived from photosynthesis. Further the symbiosis is regulated by Hormonal signals (e.g., auxins, cytokinins). Mycorrhizal nutrition increased nutrient uptake efficiency, especially in poor soils, enhanced water absorption, disease resistance, stress tolerance and improved soil structure due to hyphal networks.

4. Predacious Fungi: There are many animal trapping fungi which have developed ingenious mechanisms for capturing small animals such as eel worms, rotifers or protozoa. The fungi possess special hyphal traps called snares which capture small animals like amoeba and

nematodes. These fungi usually inhabit in the soil. They possess rapid constructing hyphal traps and penetrating haustoria to fatch nutrition from their prey. Some predacious fungi secrete a sticky substance on the surface of their hyphae to which a passing small animal adheres. Haustorium like hyphae then grows into the body of the animal and absorbs food, finally animals die. Eg: *Arthrobotrys, Dactylaria*

Mechanism of Nutrition: The whole mycelium may have the power to absorb these nutrients or this task may be assigned to special portions of the mycelium. In saprophytic fungi the hyphae (*Mucor mucedo*) or rhizodial hyphae (*Rhizopus stolonifer*) come in intimate contact with nutrients in the substratum and absorb soluble smaller molecules such sugars and amino acids. Insoluble complex substances such as proteins, lipids etc. are first broken into soluble monomers (digested) by secreting extra-cellular enzymes and then absorbed. The mycelium of the parasites is rarely ectophytic but frequently it grows inside the host. The hyphae either ramify in the intercellular space between the host cells or penetrate into the host cells. The intercellular hyphae of some highly specialized (obligate) plant parasites give out slender lateral outgrowths.

12.5 SUMMARY:

Fungi are a diverse group of eukaryotic organisms that include yeasts, molds, and mushrooms. They belong to the kingdom Fungi and play crucial roles in ecosystems as decomposers, symbionts, and even pathogens. Their unique characteristics distinguish them from plants, animals, and bacteria. Fungi are eukaryotic organisms with a well-defined nucleus and membrane-bound organelles. Their cell walls are primarily composed of chitin, which provides structural support, distinguishing them from plants that have cellulose-based cell walls. Fungi are heterotrophic organisms, meaning they obtain their nutrients from external sources. They exhibit absorptive nutrition by secreting digestive enzymes into their environment and absorbing the breakdown products. They can be saprophytic (decomposers), parasitic (feeding on living hosts), or mutualistic (engaging in symbiotic relationships). Fungi reproduce both sexually and asexually. Asexual reproduction occurs through spore formation (e.g., conidia or sporangiospores), budding (as in yeasts), or fragmentation of hyphae. Sexual reproduction involves the fusion of specialized reproductive structures, leading to genetic variation. Most fungi grow as multicellular filamentous structures called hyphae, which form a network called mycelium. Some fungi, such as yeasts, exist as unicellular organisms. The hyphae can be septate (divided by cross-walls) or coenocytic (without cross-walls). Fungi are essential decomposers, breaking down organic matter and recycling nutrients in ecosystems. Some form mutualistic associations, such as mycorrhizae with plant roots and lichens with algae or cyanobacteria. Others act as pathogens, causing diseases in plants, animals, and

humans. Fungi have various industrial and medical applications. They are used in food production (e.g., yeast in baking and fermentation), antibiotics (e.g., *Penicillium* producing penicillin), and biotechnology. However, some fungi cause spoilage and diseases, such as rusts, smuts, and mycoses in humans.

Fungi obtain their nutrients through heterotrophic absorption, meaning they rely on organic substances from their environment rather than producing their own food through photosynthesis. Unlike plants, fungi lack chlorophyll and cannot perform photosynthesis, so they derive energy by breaking down organic matter. They achieve this by secreting digestive enzymes into their surroundings, which break down complex molecules like carbohydrates, proteins, and lipids into smaller, absorbable forms. Fungi exhibit different modes of nutrition based on their ecological roles. Saprophytic fungi play a crucial role in decomposition by breaking down dead organic material, such as fallen leaves, wood, and animal remains, recycling essential nutrients back into the ecosystem. Examples of saprophytic fungi include mushrooms, molds, and many species of yeast. Parasitic fungi, on the other hand, extract nutrients from living organisms, often causing diseases in plants, animals, and even humans. They penetrate host tissues using specialized structures called haustoria, which help them absorb nutrients directly from the host cells. Examples of parasitic fungi include rusts, smuts, and the fungal pathogens responsible for athlete's foot and ringworm. Another category of fungi are mutualistic fungi, which form symbiotic relationships with other organisms for mutual benefit. A well-known example is mycorrhizal fungi, which associate with plant roots, enhancing water and nutrient absorption while receiving carbohydrates from the plant in return. Lichens are another example, where fungi and algae or cyanobacteria live together, with the fungal partner providing structure and moisture while the photosynthetic partner produces food. Fungi use specialized structures such as hyphae and mycelium to maximize nutrient absorption. The extensive network of thread-like hyphae increases surface area, allowing for efficient absorption of nutrients. In some fungi, specialized hyphae called rhizoids anchor them to their substrate, further aiding in nutrient intake.

12.6 TECHNICAL TEERMS:

Ascomycetes, Appressed, Arbuscular Mycorrhiza (AM), Coprophilous, Hypha, Lichen, Saprophyte, Slime Moulds.

12.7 SELF ASSESSMENT QUESTIONS:

1) Describe the general characters of fungi.
2) Explain the modes of nutrition in fungi.
3) Describe the ultra structure of fungal cell.

12.8 SUGGESTED READINGS:

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Dr. Y.R.K.V. Tirupati Rao

LESSON - 13

REPRODUCTION IN FUNGI

13.0 OBJECTIVE OF THE LESSON:

Students are able to learn the various asexual and sexual methods of reproduction in the different fungal species.

STRUCTURE OF THE LESSON:

13.1 INTRODUCTION:

Reproduction is the biological process by which organisms produce offspring, ensuring the continuation of their species. Generally, most of the fungi reproduce through either asexual or sexual mode of reproduction. On the basis of involvement of the thallus in the formation of asexual and sexual reproductive organs fungi are categorized as holocarpic and eucarpic. In some fungi for the formation of reproductive structures the entire thallus gets converted into one or more reproductive structures. This ensures that somatic and reproductive phases do not occur together in the same individual. Those fungi which follow this type of pattern are known as **holocarpic** (Gr. *holos* = whole + *karpos* = fruit) fungi. However, in majority of the fungi the somatic and reproductive phases occur together in the thallus as only a portion of the thallus gets converted into reproductive structure. The remaining portion of the thallus continues with normal somatic activities. Fungal species which show this type of pattern are called **eucarpic** (*eu* = good + *karpos* = fruit) fungi. The eucarpic forms are more differentiated than the holocarpic fungi. Usually, fungi reproduces both asexually and sexually but not at the same time in their life cycle.

Asexual reproduction is more important for colonization of a species as it is repeated several times in the life cycle of a species and results in production of large number of individuals, whereas in most of the fungi the sexual stage is produced only once a year. Because of the pleomorphic nature of the fungi a new terminology was proposed by Hennebert and Weresub (1977) which was widely accepted. According to this, sexual stage of the fungus is described by the term **teleomorph** while asexual stage is described by the term **anamorph**. While

whole fungus with all its forms, facets, either latent or expressed even if reproduces by one method is known as **holomorph**. More recently, these terms are replaced by **meiosporic** fungus (teleomorph) and **mitosporic** fungus (anamorph).

13.2 TYPES OF REPRODUCTION:

Asexual reproduction is also known as somatic reproduction and it does not involve karyogamy (Gr. *karyon* = nut + *gamos* = marriage). This is the most important type of reproduction in fungi. **Sexual** reproduction, on the contrary, is characterized by union of two compatible nuclei followed by meiosis. This type of reproduction usually takes place only once in the life cycle of the organism and may or may not involve specialized sex cells or organs. This reproduction helps them in adapting themselves to different environmental conditions.

Asexual Reproduction:

Asexual reproduction takes place when progeny is formed by a single parent without any nuclear contribution from the second parent. Therefore, genetically the progeny is exact copy of the parent cell or thallus. Asexual reproduction is considered as imperfect state. Asexual reproduction is often defined as nonsexual production of specialized reproductive cells such as spores. Asexual spore is delimited from the thallus and in contrast to the vegetative mycelium they have minimal metabolic turnover, low water content and lack of cytoplasmic movement. The following types of asexual methods of reproduction were observed in fungi.

Fragmentation:

Fragmentation results from accidental severing of the mycelium into several small bits or fragments by mechanical injuries. Under favourable condition each fragment further develops into a new mycelium (Fig.13.1).

Figure-13.1: Fragmentation in fungi (https://tinyurl.com/4ryh2r6p)

Thallospores: In some fungal species, the fragmentation of parent hyphae forms into various spore like structures called thallospores (asexual spore). Thallospores are of two types a) Arthrospore and b) Chlamydospore.

a) Arthrospore or Oidia: The distal end of hyphae breaks up into component cells by close septation and each component cell behaves as an individual spore (Fig.13.2). They are always formed in basipetal succession that is septation starts from the apex of the hyphae and proceeds towards the base. The cells give appearance of beads due to their oval or round shape. Each oidium or arthrospore develops into a new mycelium.

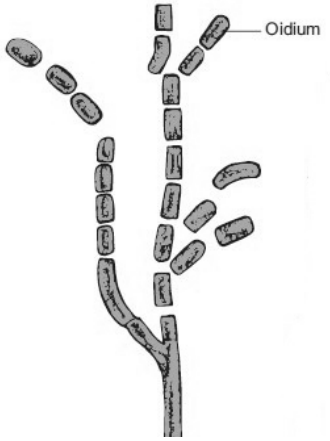

Figure-13.2: Oidia in fungi (https://tinyurl.com/3c453u2w)

b) Chlamydospore:

In some fungi such as *Mucor* and *Fusarium*, some intercalary or terminal segments of the hyphae accumulate large amount of food reserves and develop thick resistant walls. These thick walled spores are called chlamydospore (Fig.13.3). These cells function as perennating bodies and help in surviving unfavourable environmental conditions and are released after the intervening hyphae are degenerated. As the favourable conditions return each chlamydospore develops into a new mycelium.

Figure-13.3: Chlamydospores in fungi (https://tinyurl.com/3c453u2w)

Fission of Unicellular Thalli:

This method is typical of some types of yeast such as *Schizosaccharomyces.* During this process, somatic cells elongate and divide transversely into two daughter cells of similar size and shape. First the nucleus divides followed by division of the cytoplasm by wall formation dividing parent cell into two. The two daughter cells separate and can lead independent lives (Fig.13.4).

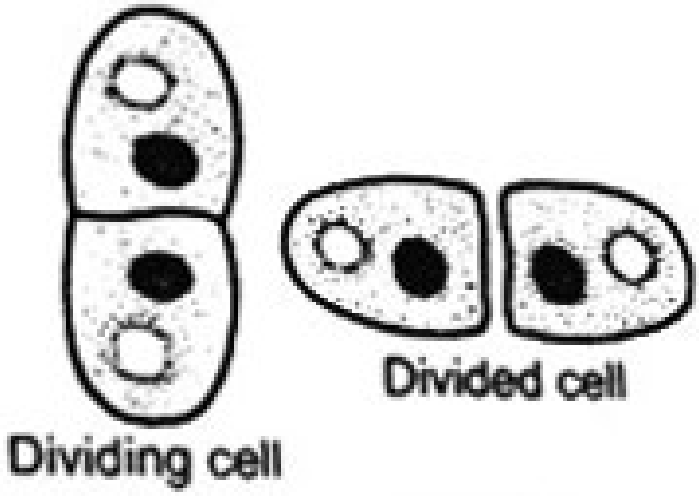

Figure-13.4: Fission in fungi (https://tinyurl.com/4ryh2r6p)

Budding of Somatic Cells:

The yeast, *Saccharomyces cerevisiae,* cell reproduces by the means of budding which involves production of an outgrowth or bud from a parent cell or spore. This bud gradually enlarges, constricts and finally forms a new individual by getting separated the parent cell by formation of a cross wall. Sometimes this newly formed bud before getting separated from the parent cell produces a new bud which remains attached for a while to form a chain of buds known as pseudomycelium (Fig.13.5).

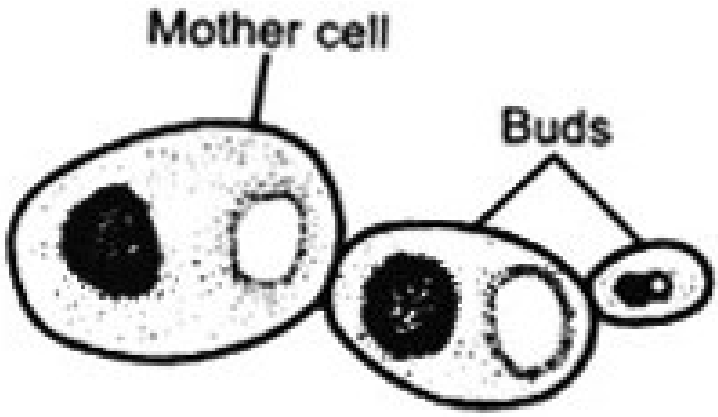

Figure-13.5: Budding in fungi (https://tinyurl.com/4ryh2r6p)

Rhizomorphs:

In many higher fungi such as *Agaricus*, hyphae aggregate to form a cord like structures. These dark brown coloured fine root-like strands or hyphae are known as rhizomorphs (Fig.13.6). These serve as perennating bodies which remain dormant under unfavourable conditions and give rise to new fruiting bodies.

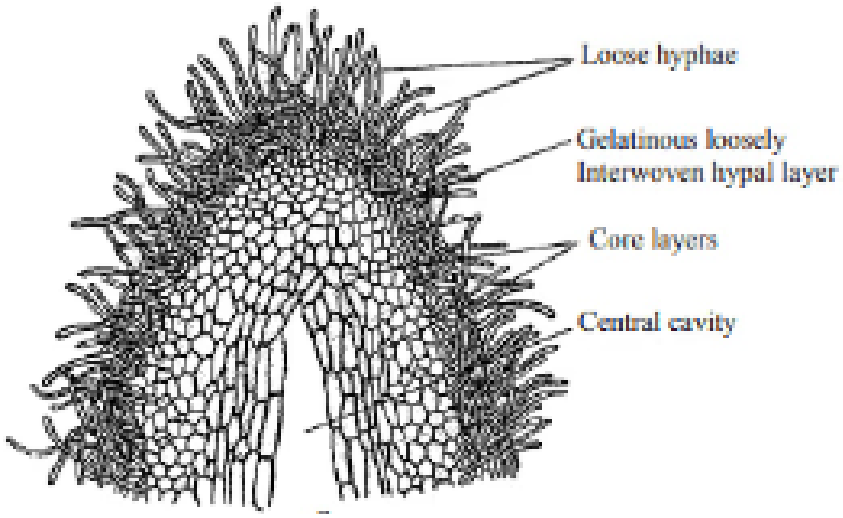

Figure-13.6: Rhizomorphs in fungi (https://tinyurl.com/dzsnr7mu)

Sclerotia:

Sclerotia, produced by *Claviceps purpurea* (ergot), are the modification of mycelium. They are rounded or cylindrical or irregular or cushion-shaped structures with a dense mass of thick walled hyphae. The hypha serves as the organs of perrenation and is meant for vegetative propagation. With the return of favourable conditions sclerotia germinate to form a new mycelium (Fig.13.7).

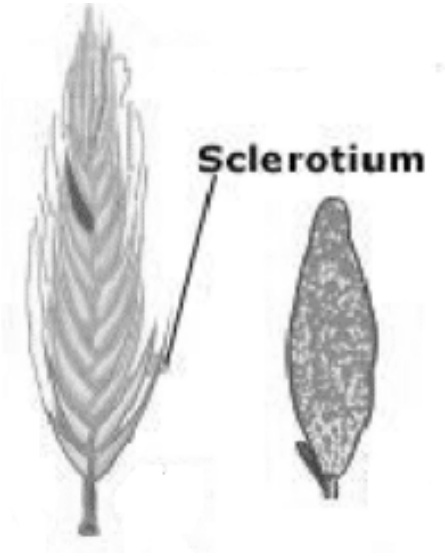

Figure-13.7: Sclerotia in fungi (https://tinyurl.com/3ak4stpa)

Production of Asexual Spores:

This is the most common method of asexual reproduction in fungi wherein special reproductive cells are formed which are known as spores or mitospores. The process of formation of spores in fungi is called sporulation. These spores vary in shape i.e. globose, oval, oblong, needle-shaped to helical with a size ranges from 2-150 micron. These are septate, unicellular or multicellular (*Alternaria* and *Curvularia*), motile or non-motile, may be thin or thick-walled with hyaline or coloured pigments such as green, yellow, orange, red, brown to black. Some fungus produces only one type of spore while some can produce more than 2-3 types of spores. These spores help in perrenation, propagation and dispersal. Mitospores can be categorized into two main types depending upon their method of formation a) Sporangiospore and b) Conidiospore.

a) Sporangiospores: Numerous minute, uninucleate sporangiospores are produced by cleavage of the cellular content of a sac-like structure called sporangium. Each sporangium is born on hyphae called sporangiophore. Sporangiospore can be non-motile (**aplanospore**) or motile (**zoospore**).

Aplanospores: These are characteristics of terrestrial species such as *Rhizopus* and *Mucor*. Aplanospores have a definite spore wall and are dispersed by the means of wind and insects. Aplanospores develop a rigid cell wall prior to their release from sporangium (Fig.13.8).

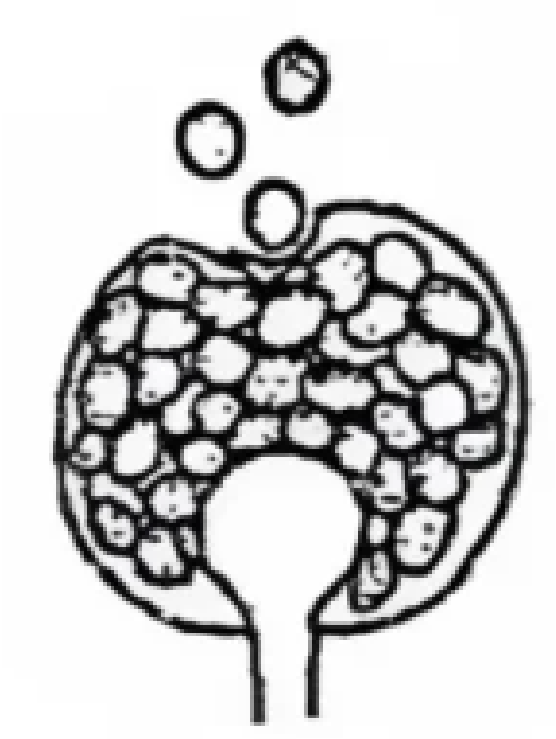

Figure-13.8: Aplanospores in fungi (https://tinyurl.com/bdfyeyu3)

Zoospores: In aquatic fungi (*Pythium*, Division Oomycota) motile biflagellate zoospores are produced on structures called as zoosporangium (Fig. 13.9). However, the motile zoospores are initially naked. They secrete a cell wall only after the swarming period is over and germinate by forming a germ tube. Zoospores may have one or more than one flagella. Basically, the fungal species have two types of flagella 1. Tinsel type - feathery structure having a long rachis and have lateral hair projections called mastigonemes or flimmers and 2. Whiplash type - long basal portion and a short and flexible upper portion. Depending upon the presence and absence of these two types of flagellum zoospores are of four types:

i) Zoospore with single posterior whiplash flagellum Eg: Chytridiomycetes

ii) Zoospore with single anterior tinsel flagellum Eg: Hypochytridiomycetes

iii) Biflagellate zoospore with two anterior whiplash flagella Eg: Plasmodiophoromycetes

iv) Biflagellate zoospore with one tinsel and one whiplash flagella as in Oomycetes.

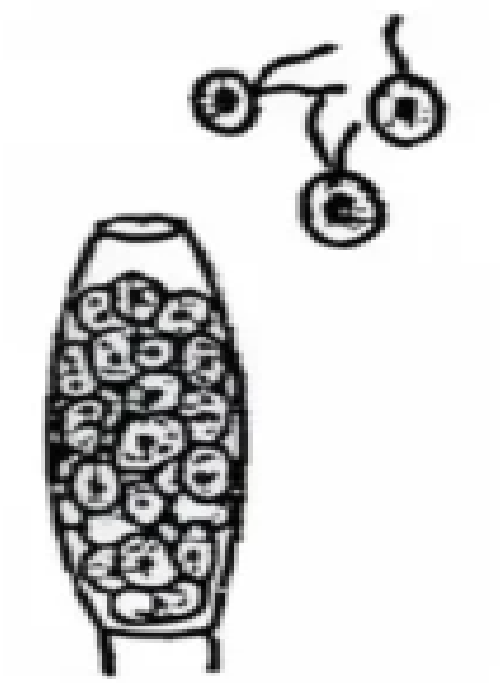

Figure-13.9: Zoospores in fungi (https://tinyurl.com/bdfyeyu3)

Conidiospores: These asexual spores are non-motile, deciduous and are formed externally as single separate cells either directly on the mycelium or on morphologically differentiate hyphae called conidiophores (Fig.13.10). These structures may be simple or branched and septate or aseptate. Conidia may be produced singly as in *Phytophthora* or in chains at the tip of conidiophores as in *Aspergillus* or at the tip of the branches of conidiophores as in *Penicillium*. Conidiophores often arise singly and are scattered in mycelium. However, sometimes they may also arise in specialized structures called fruiting bodies. Depending upon their appearance they are termed as **synnema**, **sporodochia**, **acervuli** (saucer-shaped), **pycnidia** (flask-shaped) or **pustules**.

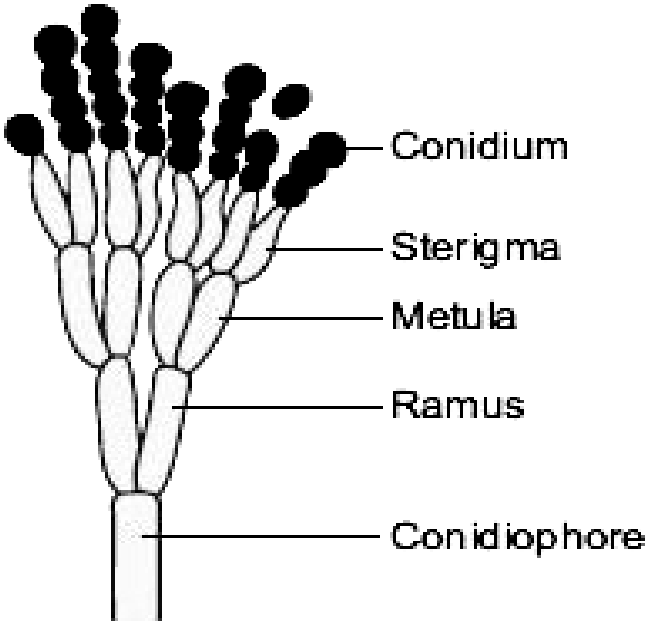

Figure-13.10: Conidiospores in fungi (https://tinyurl.com/3c453u2w)

Significance of Asexual Reproduction in Fungi: Asexual reproduction ensures fungi can thrive, adapt, and persist in diverse ecological niches, making them highly successful organisms in nature. Asexual reproduction in fungi is significant for several reasons:

1) **Rapid Population Growth:** Asexual reproduction allows fungi to produce large numbers of offspring quickly, ensuring the survival and spread of the species.
2) **Survival in Stable Environments:** Since asexual reproduction produces genetically identical offspring (clones), it is advantageous in stable environments where existing traits are already well-suited for survival.
3) **Efficient Spore Dispersal:** Many fungi reproduce asexually through spores (e.g., conidia or sporangiospores), which can be dispersed over long distances by wind, water, or animals.
4) **Colonization of New Habitats:** Asexual reproduction enables fungi to rapidly colonize new environments without the need for a mating partner.
5) **Resilience to Harsh Conditions:** Certain asexual structures, such as chlamydospores, help fungi survive unfavorable conditions and germinate when conditions improve.
6) **Medical and Industrial Importance:** Many fungi that reproduce asexually (e.g., *Penicillium*, *Aspergillus*) are used in medicine (antibiotics, fermentation) and industry (cheese production, enzyme production).
7) **Pathogenicity and Disease Spread:** Many fungal pathogens of plants and animals reproduce asexually, leading to rapid disease outbreaks, as seen in *Candida* infections or plant diseases like rusts and mildews.

1) Sexual Reproduction:

This process involves fusion of two compatible sex cells or gametes of opposite strains. Sexual reproduction is also known as perfect state. Fungi show remarkable diversity in their process of sexual reproduction.

Sex Organs in Fungi:

Sex organs in fungi are known as **gametangia** (sing. gametangium). Gametangia may contain differentiated sex cells called gametes or may have only one or more nuclei as gametes. These gametangia or gametes can be morphologically similar also and they might show some morphological dissimilarity also. Morphologically similar gametangia and gametes are known as **isogametangia** and **isogametes**, respectively. However, morphologically distinct ones are known as **heterogametangia** and **heterogametes**, respectively. In the later case, female gametangium is called **oogonium** (pl. **oogonia**) in Oomycetes and **ascogonium** (pl. **ascogonia**) in ascomycetes. The male gametangium is known as **antheridium** (pl. **antheridia**).

Most of the fungi can be distinguished into three categories on the basis of sex i) Monoecious ii) Dioecious iii) Sexually undifferentiated.

i) **Monoecious:** These are also named as hermaphroditic or bisexual. Each thallus bears both male and female sex organs which may not be compatible;

ii) **Dioecious:** Some thallus bears only male sex organs and some thallus bears only female sex organs.

iii) **Sexually Undifferentiated:** In these fungi, sexual structures are produced but morphologically male and female sexual organs cannot be distinguished.

Sexual reproduction basically involves three phases: (I) plasmogamy (II) karyogamy (III) meiosis.

I) Plasmogamy: It is the union of protoplast of reproductive cells or hyphae to bring the nuclei of the two parents together in a pair. Although, these nuclei do not fuse with each other and the resulting cell with two nuclei is called dikaryon. This condition is unique for fungi and may continue for several generations as such. Plasmogamy occurs through a variety of mechanisms in fungi. Some of them are as follows:

Planogametic Copulation:

This process involves fusion of two gametes. Sexual reproduction in fungi can be divided into i) Isogamous, ii) Heterogamy.

i) Isogamy: Isogamy in fungal sexual reproduction refers to the fusion of gametes that are morphologically similar in size and structure, but differ in mating type, ensuring genetic compatibility (Fig.13.11). Eg: *Olpidium* and *Catenaria*.

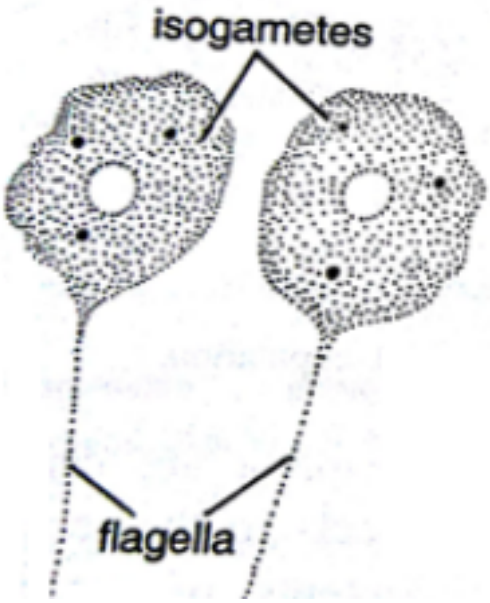

Figure-13.11: Isogamy in fungi (https://tinyurl.com/376t9zrr)

ii) Heterogamy: When the fusing gametes are morphologically as well as physiologically different. Heterogamous reproduction is of two types:

Anisogamy: It consists of the fusion of two motile gametes where the **male gamete is small and more active** than the female gamete (Fig.13.12), e.g., *Allomyces.*

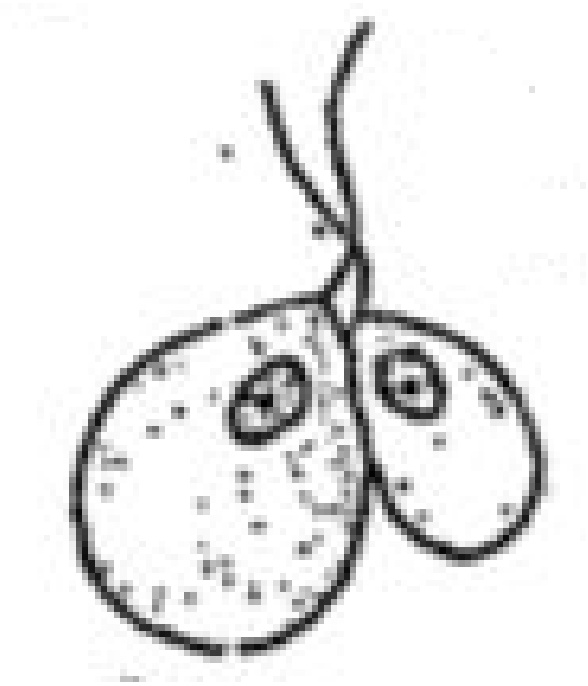

Figure-13.12: Ansiogamy in fungi (https://tinyurl.com/55avfhz7)

Oogamy: In this process the **motile male gamete** antherozooid fuses with the **large, non-motile female gamete e**gg or ovum) (Fig.13.13) e.g., *Synchytrium* etc.

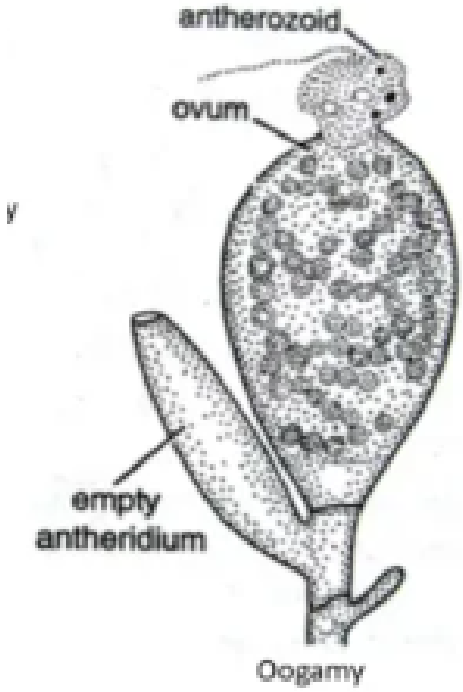

Figure-13.13: Oogamy in fungi (https://tinyurl.com/376t9zrr)

Gametangial Copulation: The two gametangia comes into contact and the entire content of the two parents fuses together and becomes one (Fig.13.14). Eg: *Rhizopus* and *Mucor*. However, in some fungal species entire protoplast of one gamete flows into the other gamete through a pore. The recipient is female and the donor is male.

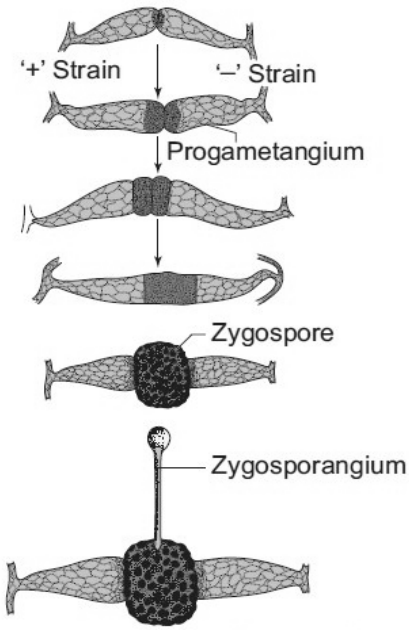

Figure-13.14: Gametangial copulation in fungi (https://tinyurl.com/5c4hsnne)

Gametangial Contact: The nucleus in the antheridium represents the male gamete. Male gamete here is not a separate entity. The oogonium and the antheridium come in contact through a tube and one of the nuclei from the antheridium migrates into the oogonium (Fig.13.15). Interestingly, the two gametangia do not fuse Eg: *Penicillium*, *Phytophthora*, *Albugo*, *Pythium*.

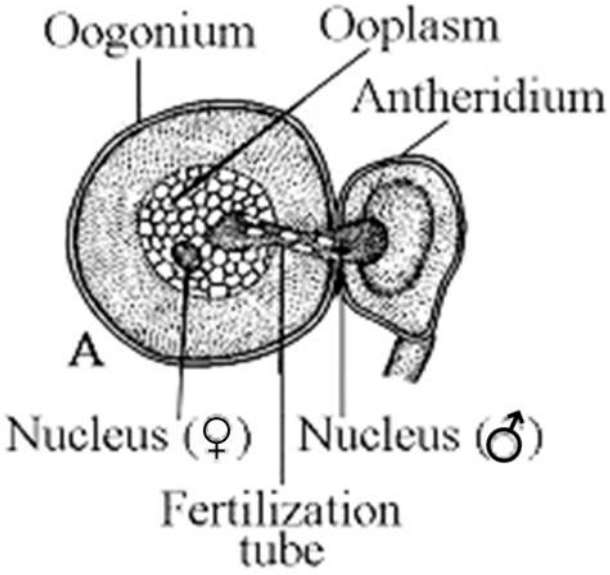

Figure-13.15: Gamentangial contact in fungi (https://tinyurl.com/nhdyrubf)

Spermatization: This is one of the most remarkable modes of plasmogamy. The spermatia which are minute conidia like structures are produced externally on special hyphae called spermatiophore (Fig.13.16). Spermatia develop inside cavities called as spermatogonia. Female cell can be a gametangium, vegetative hyphae or even specialized receptive hyphae. Eg: Puccinales (rust fungi).

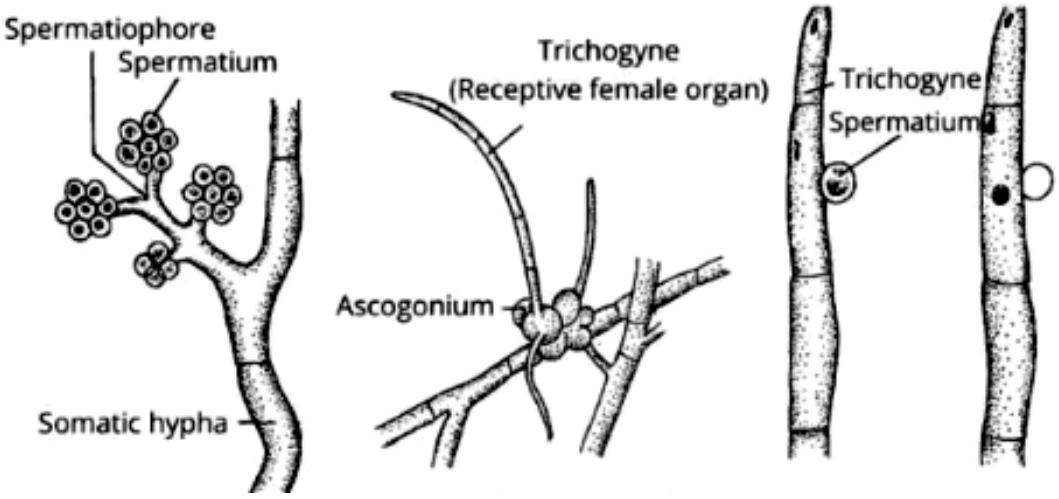

Figure-13.16: Spermatization in fungi (https://tinyurl.com/kfdufft3)

Somatogamy: It involves the merging of somatic hyphae, leading to plasmogamy (cytoplasmic fusion) but not immediate nuclear fusion (karyogamy). Results in a heterokaryotic or dikaryotic stage, where nuclei from both parents coexist in a shared cytoplasm (Fig.13.17) Eg: Basidiomycota and Ascomycota.

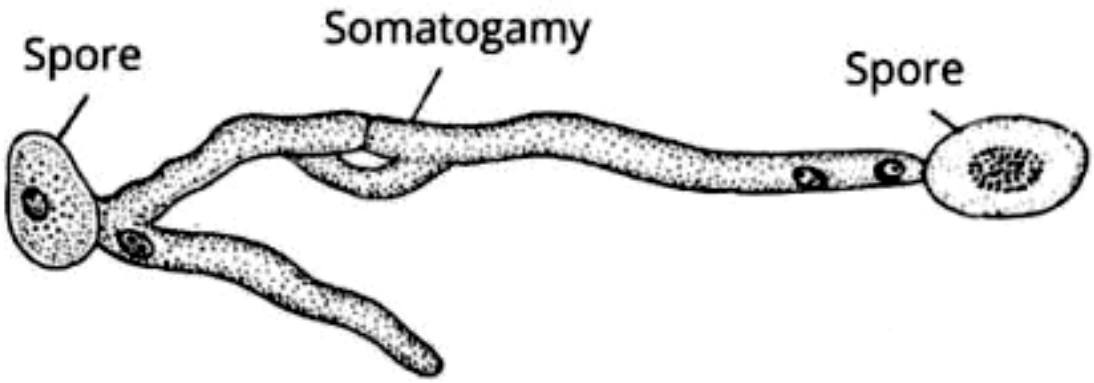

Figure-13.17: Somatogamy in fungi (https://tinyurl.com/kfdufft3)

II) Karyogamy: It is the process of fusion of two nuclei. In lower fungi, it may immediately follow plasmogamy. However, in higher fungi it may be delayed for a long time.

Types of Sexual Reproduction in Fungi Based on Karyogamy:

Fungi show Different Types of Sexual Reproduction based on type of Karyogamy:

a) **Zygomycota (Zygosporic Fungi):** Karyogamy occurs within a **zygosporangium**, a thick-walled structure that later undergoes meiosis. Example: *Rhizopus* (bread mold).

b) **Ascomycota (Sac Fungi):** Karyogamy happens inside an **ascus**, where a diploid nucleus forms and immediately undergoes meiosis, producing ascospores. Example: *Saccharomyces, Aspergillus, Penicillium.*

c) **Basidiomycota (Club Fungi):** Karyogamy takes place in a **basidium**, where it is followed by meiosis to form basidiospores. Example: *Mushrooms, Puccinia* (rust fungi).

d) **Chytridiomycota (Primitive Fungi):** Karyogamy occurs within a **resting sporangium**, followed by meiosis. Example: *Batrachochytrium* (chytrid fungi).

III) Meiosis: Meiosis in fungal sexual reproduction is a crucial process that generates genetic diversity. Fungi exhibit diverse reproductive strategies, but in sexually reproducing fungi, meiosis occurs after the fusion of haploid nuclei (karyogamy) to produce genetically varied spores. This process produces four genetically different spores.

Above all the three processes are regulated by a variety of mechanisms and morphological developments.

Significance of Sexual Reproduction: Sexual reproduction in fungi is significant for several reasons:

1) **Genetic Diversity:** Sexual reproduction promotes genetic variation through recombination, allowing fungi to adapt to changing environments, resist diseases, and develop beneficial traits.
2) **Survival in Harsh Conditions:** Many fungi produce hardy sexual spores (e.g., ascospores, basidiospores, zygospores) that can withstand extreme conditions like drought, heat, or lack of nutrients, ensuring survival and dispersal.
3) **Evolutionary Adaptation:** The mixing of genetic material through meiosis and fusion of gametes (or gamete-like structures) leads to evolutionary advantages, helping fungi develop resistance to antifungal treatments or environmental stressors.
4) **Restoration of Genetic Integrity:** Sexual reproduction can help eliminate harmful mutations by allowing recombination and selection for beneficial traits.
5) **Formation of Specialized Structures:** Sexual reproduction results in complex reproductive structures like mushrooms, asci, and zygospores, which play a key role in fungal classification and lifecycle progression.
6) **Ecological Role:** Many fungi engage in symbiotic relationships (e.g., mycorrhizae with plants) where sexual reproduction helps maintain a stable and diverse population that benefits ecosystems.

13.5 SUMMARY:

Asexual reproduction in fungi is a prevalent and efficient process that allows rapid propagation without genetic recombination, yielding genetically identical offspring (clones). This method is advantageous in stable environments, enabling quick colonization and resource exploitation. Key mechanisms include: 1. Spore Production: Conidia: Externally borne on specialized hyphae called *conidiophores*; seen in *Penicillium* and *Aspergillus.*

Sporangiospores**:** Formed internally within sac-like *sporangia* on *sporangiophores*; characteristic of zygomycetes like *Rhizopus.* Other spores include chlamydospores (thick-walled, stress-resistant) and arthrospores (from hyphal fragmentation). Budding: Common in yeasts (e.g., *Saccharomyces cerevisiae*), where a daughter cell pinches off from the parent. Fragmentation: Hyphae break into pieces, each growing into a new organism (e.g., molds). Asexual reproduction ensures rapid spread and energy efficiency, bypassing the need for a

mate. However, it limits genetic diversity, potentially reducing adaptability to environmental changes. Many fungi employ both asexual (anamorphic) and sexual (teleomorphic) phases, balancing clonal spread with genetic variation when necessary. This adaptability underscores fungi's ecological success and versatility.

Sexual reproduction in fungi enhances genetic diversity and adaptation, typically occurring under environmental stress or nutrient scarcity. The process involves several key stages: 1. Plasmogamy: Fusion of cytoplasm from two compatible mating types (e.g., + and – strains), mediated by pheromones. This results in a dikaryotic cell (n + n), where two haploid nuclei coexist without immediate fusion. 2. Dikaryotic Stage: The dikaryon forms a mycelium with paired nuclei, which may persist extensively, as in Basidiomycetes. This stage is critical for growth and development in some species. 3. Karyogamy: Nuclear fusion within specialized structures (e.g., ascus, basidium) produces a transient diploid zygote (2n). 4. Meiosis: The diploid nucleus undergoes meiosis, generating haploid spores (e.g., ascospores, basidiospores, zygospores) housed in structures characteristic of each fungal phylum: Ascomycota: Spores develop in sac-like asci. Basidiomycota: Spores form on club-shaped basidia.

Zygomycota: Thick-walled zygospores arise from gametangia fusion. Spore Dispersal: Haploid spores disperse, germinating into new haploid mycelia, completing the cycle. Sexual reproduction promotes genetic variation, aiding fungal adaptation to changing environments. While some fungi rarely exhibit sexual phases (classified as Deuteromycetes), others rely on it for survival. This process underscores the ecological resilience and evolutionary success of fungi across diverse habitats.

13.6 TECHNICAL TERMS:

Budding, Cell Wall, Chitin, Coenocytic, Conidiophore, Conidia, Conjugation, Imperfect Fungi, Fission, Gametangium, Haustoria, Host, Mycorrhiza.

13.7 SELF ASSESSMENT QUESTIONS

1) Describe the types of sexual reproduction in fungi.
2) Explain the various types of asexual reproduction in fungi.
3) Give an account on the significance of sexual and asexual reproduction in fungi.

13.8 SUGGESTED READINGS:

1) Microbiology - Michael J. Pelezer, J.R., E.C.S. Chan, Noel R. Krieg (1993) - Indian Edition (5th Edition), Mc Graw Hill Education (India) Private Limited, 444/1, Sri Ekambara Naicker Industrial Estate, Alapakkam, Povur, Chennai 6oo116, Tamil Nadu, India.

2) Botany for Degree Students Part II FUNGI - B. R. Vashishta, (1990), S. Chand & Company LTD. Ram Nagar, New Delhi, 110055.

3) Botany for Degree Students - B. P. Pandey, (2015). As per UGC Model Curriculum.

4) https://www.biologydiscussion.com/microbiology-2/structure-of-fungal-cell microbiology/64992.

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LESSON - 14

AINSWORTH CLASSIFICATION OF FUNGI

14.0 OBJECTIVE OF THE LESSON:

Students are able to know the latest updates and Ainsworth classification of fungi and their systematic position based on various factors.

STRUCTURE OF THE LESSON:

14.1 INTRODUCTION:

The word "**classification**" may be defined as "**the scientific categorization of organisms in a hierarchical series of group".** In spite of the existence of many varieties, biological strains and physiological or cultural races, the **species is** generally **considered as the smallest group.** More similar species are grouped together into genus, similar genera are grouped into family, families into order, orders into class, similar classes into division, division into kingdom, and kingdom into domain or superkingdom. Fungi are classified on the basis of seven characteristic features like I. Morphological characters, II. Host specialization, III. Physiological Characters, IV. Cytological and genetical characters, V. Serological Characters, VI. Biochemical Character,s VII. Numerical Taxonomy

Recommendations of International Committee:

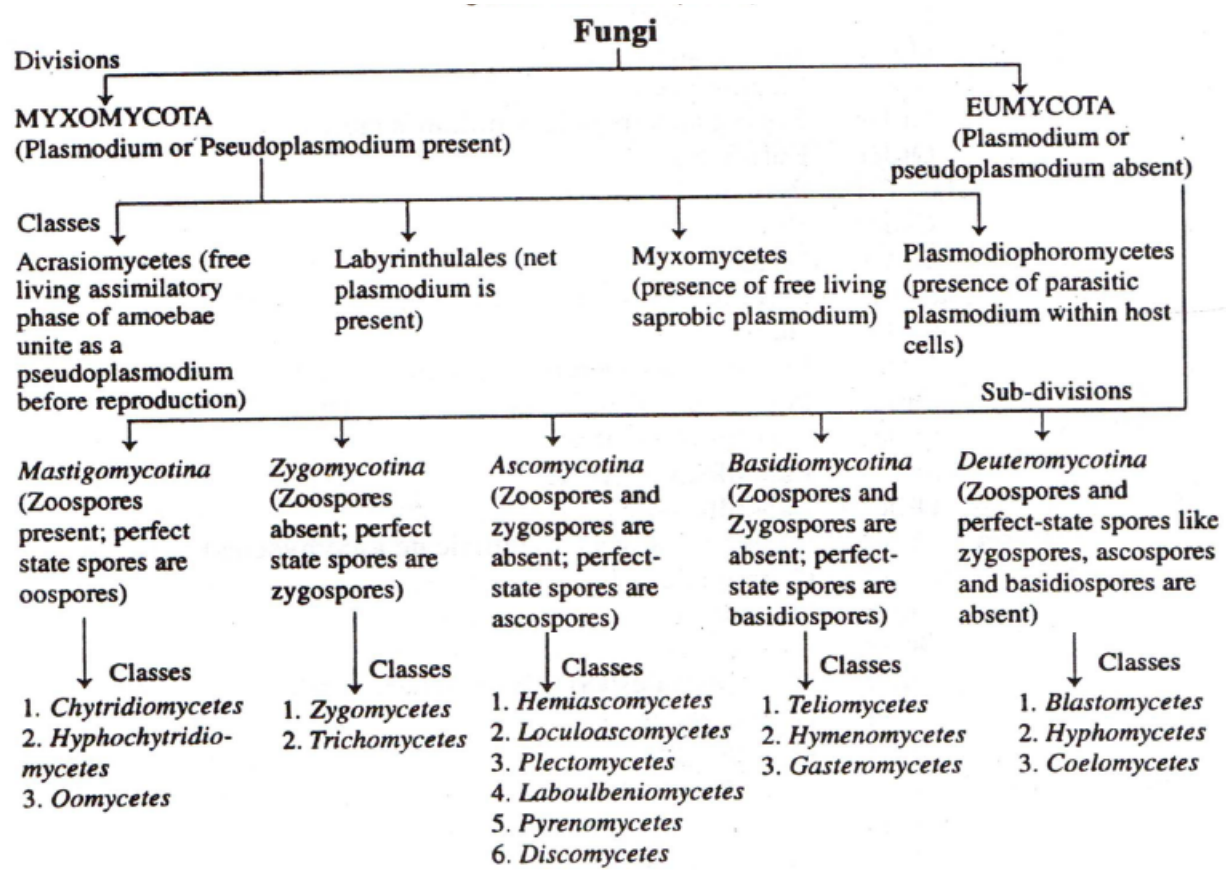
The committee on International Rules of Botanical Nomenclature recommended the use of following "Suffixes" for the division and other major categories of fungi: Division should end in Mycota. Sub-division should end in Mycotina. Classes should end in Mycetes. Sub classes should end in Mycetidae. Order should end in ales. Family should end in aceae. No standard ending have been proposed for genera and species. Species are sometimes broken into varieties, forms and physiological races i.e., Eukaryotic (with true nuclei), Achlorophyllous (without chlorophyll), Unicellular, or multicellular organisms, microscopic or macroscopic in size. Usually all cells are with cell walls and filaments. Fungi typically reproducing by spores produced asexually or sexually. Cell walls contain chitin, cellulose, or

both. Fungi contain about 50,000 living species. Around 500 fossil species are known until now.

The **Ainsworth Classification of Fungi** (1973) is a system for classifying fungi that was developed by **Geoffrey Clough Ainsworth**, a British mycologist. His classification was widely recognized and used for fungal taxonomy, particularly in earlier fungal systematics. Ainsworth classified fungi into major groups based on morphological, reproductive, and physiological characteristics. The classification primarily followed a **hierarchical system**, organizing fungi into different **divisions, classes, orders, families, genera, and species**.

14.2 DIVISION MYXOMYCOTA:

Myxomycota, commonly known as “plasmodial slime molds” or "true slime molds," belong to the class Myxogastria within the phylum Amoebozoa. Historically classified under fungi due to their spore-producing fruiting bodies, modern molecular phylogenetics has reclassified them within the super group Amoebozoa, highlighting their closer relation to amoebae than fungi. This reclassification arose from differences in life cycle, motility, and nutritional strategies. Myxomycota thrive in humid, temperate forests, inhabiting decaying logs, leaf litter, and soil. They favor microhabitats with abundant microbial prey and are globally distributed, from tropical rainforests to Arctic tundras. Some species exhibit niche specificity, while others are cosmopolitan. The vegetative phase is a plasmodium, it is a multinucleate, acellular mass enveloped by a membrane. This coenocytic structure exhibits shuttle streaming (rhythmic cytoplasmic flow) for movement and nutrient distribution.



Unlike cellular slime molds (e.g., *Dictyosteliida*), Myxomycota form a single, large plasmodium without individual cell walls, allowing phagocytosis of bacteria, fungi, and organic debris. The definite cell wall is absent from their amoeba like bodies. Plasmodia navigate via chemotaxis, moving toward food sources or away from hazards, capable of covering several centimeters per hour. Spores are provided with firm walls. Flagellated cell are characteristically produced. Wall-less organisms possess either a Plasmodium (a mass of naked multinucleate protoplasm having amoeboid movement) or a pseudoplasmodium (an aggregation of separate amoeboid cells). Both are of slimy consistency, hence slime molds. The life cycle alternates between asexual and sexual phases which includes I) Haploid and diploid phases: 1. Asexual reproduction: Spores germinate into haploid myxamoebae or flagellated swarm cells. 2. Sexual reproduction: Fusion of haploid cells forms a diploid zygote, developing into the plasmodium. II) Fruiting bodies: Under stress, the plasmodium forms sporangia or intricate fruiting bodies (e.g., *Physarum*), releasing resistant spores.

The division myxomycota again divided into four classes, 1. Class Acrasiomycetes (cellular slime molds) 2. Class Hydromyxomycetes (net slime molds) 3. Class Myxomycetes (true slime molds) 4. Class. Plasmodiophoromycetes (endo-parasitic slime molds).

Class 1: Acrasiomycetes:

Acrasiomycetes is a class of fungi-like protists that belong to the phylum Amoebozoa**.** These organisms are commonly referred to as cellular slime molds and are distinguished by their unique life cycle, which involves both unicellular and multicellular stages**.** They are important for studying cellular communication, differentiation, and aggregation. The vegetative stage consists of individual amoeba-like cells that move and feed independently. Under unfavorable conditions individual amoebae aggregate to form a multicellular structure called a pseudoplasmodium or a slug. Unlike fungi, the vegetative cells lack a rigid cell wall, making them flexible and motile. Generally found in soil, decaying organic matter, and moist environments. The life cycle of Acrasiomycetes alternates between unicellular and multicellular stages, primarily in response to environmental conditions. The life cycle of Acrasiomycetes includes A) Vegetative Phase - Individual cells exist as haploid amoeboid forms. These amoebae move using pseudopodia and feed on bacteria and organic debris via phagocytosis. B) Aggregation Stage - When food becomes scarce, amoebae release signaling molecules (e.g., cyclic AMP). The amoebae chemotactically move toward the signal and aggregate into a slug-like structure (pseudoplasmodium). The slug moves as a coordinated unit toward light and heat. C) Fruiting Body Formation - The slug stops moving and undergoes differentiation. Some cells form a stalk, while others develop into spores. The spores are enclosed in a protective coat and remain dormant until favorable conditions return. D) Spore Germination - When environmental conditions improve, the spores germinate and

release new amoeboid cells, restarting the life cycle. Acrasiomycetes fungi help in breaking down organic material in soil. Serve as food for soil-dwelling microorganisms. Used in studies of cell communication and differentiation. Serve as a model for studying multicellularity and cooperation. May help in understanding cell signaling relevant to cancer research. Potential applications in bioremediation. Eg: 1. *Dictyostelium* - Most studied cellular slime mold and used as a model organism in genetics and cell biology. 2. *Polysphondylium* - similar to *Dictyostelium* but forms symmetrical fruiting bodies. 3. *Acrasis* - Shows unique aggregation behavior.

Class 2: Labyrinthulales:

Labyrinthulales are mostly found in marine and estuarine environments, thriving on decaying organic material, algae, and plant debris. Some species can also be found in freshwater or soil habitats. Unlike *Myxomycota*, which form a plasmodium, *Labyrinthulales* produce an extracellular network of mucilaginous slime tracks. Cells glide along these tracks to facilitate movement and nutrient absorption. They absorb nutrients directly from their environment, a process called osmotrophy. Many are saprotrophs, decomposing organic material, while some are parasites of marine plants, algae, and even mollusks. Unlike true slime molds, *Labyrinthulales* do not form a multinucleate plasmodium. Cells are generally uninucleate and move individually along the slime tracks. Cells are enclosed in a thin wall and can transform into sporangia under suitable conditions. Asexual reproduction occurs via binary fission or zoospore formation. Some species may exhibit sexual reproduction, but it is not well understood. Some members, such as *Labyrinthula*, cause diseases in marine plants. For example, *Labyrinthula zosterae* is responsible for wasting disease in eelgrass (Zostera marina). *Labyrinthulales* are part of the heterokont group, closely related to oomycetes (water molds) and diatoms. They possess biflagellate zoospores in some life stages, a characteristic feature of stramenopiles. The key genera of Labyrinthulales includes *Labyrinthula* (parasitic and saprotrophic species, associated with eelgrass wasting disease); *Thraustochytrium* (important for their production of omega-3 fatty acids and industrial applications); *Aurantiochytrium* (found in marine environments, involved in decomposing organic material).

Class 3: Myxomycetes:

These are unique in the sense that they exhibit characteristics of both fungi and protozoa. There are approximately 1,000 species of Myxomycetes known until now. These species vary widely in terms of the morphology of their fruiting bodies and the types of plasmodial forms they exhibit. The fruiting bodies can vary in shape, size, and color, ranging from simple sporangia to more complex structures with stalks or umbrellas. The color of the sporangium can be yellow, orange, red, purple, or black, depending on the species. They are often found

in temperate forests but can also inhabit other moist habitats around the world. Myxomycetes are commonly found in moist, decaying environments such as forest floors, decaying logs, and leaf litter. They play an important role in nutrient recycling as decomposers, breaking down complex organic material into simpler compounds. Myxomycetes are primarily saprophytic and feed on decaying organic matter. They are also capable of phagocytosis, engulfing bacteria and small organic particles. The plasmodium moves across substrates in search of food, and its ability to digest organic material is a key aspect of its ecological role as decomposers in forest ecosystems. Myxomycetes are often grouped with slime molds that form a complex lifecycle with two distinct phases: one is the plasmodial phase - a multinucleate, non-septate mass of cytoplasm, and the other is the spore-forming stage. The most distinctive feature of Myxomycetes is the formation of a plasmodium. This is a large, amorphous, multinucleate mass of cytoplasm that moves and feeds in a manner similar to an amoeba. The plasmodium does not have cell walls, but it is capable of engulfing and digesting food particles, especially bacteria, fungi, and decaying organic matter. The plasmodium exhibits cytoplasmic streaming, where the cytoplasm flows in an organized way, allowing the plasmodium to move and explore the environment. This movement can be quite rapid. The plasmodium, under favorable conditions, will differentiate and produce fruiting bodies. These fruiting bodies are often spore-bearing and may be in the form of sporangia or similar structures. These fruiting bodies are typically composed of stalked or sessile structures containing spores. Myxomycetes reproduce sexually and asexually. **Asexual reproduction** is achieved through the release of spores from the fruiting bodies, which can disperse and germinate into new myxamoebae. **Sexual reproduction** occurs when two myxamoebae fuse to form a diploid zygote, which then develops into the plasmodium. The plasmodium eventually produces fruiting bodies that release sexual spores. The life cycle of Myxomycetes is highly complex and involves the following stages:

a) **Germination of spores**: The cycle begins when a spore germinates, producing a motile cell called a myxamoeba (a flagellate or amoeboid cell).

b) **Myxamoebae stage**: These cells can feed on bacteria and move via pseudopodia (in amoeboid form) or flagella. In some species, two myxamoebae may fuse to form a zygote, which initiates the next phase of the life cycle.

c) **Plasmodium formation**: When conditions are favorable, the myxamoebae can fuse and merge to form the plasmodium. This is the vegetative or feeding stage of the organism.

d) **Fruiting body formation**: Upon reaching maturity, the plasmodium undergoes a transformation to produce a fruiting body. This fruiting body releases new spores that will begin the cycle anew.

e) **Spore production**: Inside the fruiting body, the spores are produced and are typically released into the environment to germinate into new amoebae or flagellates, completing the cycle.

Myxomycetes are a subject of interest for research in a variety of fields, including **c**ell biology, microbiology, and ecology. The plasmodial stage, with its large, multinucleate structure, provides insights into the regulation of nuclear division and the control of cytoplasmic streaming. Their ability to form large, dynamic plasmodial masses and their complex life cycle offer a unique opportunity to study cellular differentiation, communication, and development. Eg: 1. *Physarum* - A genus that contains species like *Physarum polycephalum*, which is often used in laboratory studies. Its plasmodium can move and solve mazes, making it a subject of interest in research on decision-making and pattern formation. 2. *Fuligo* - Known for producing a bright yellow, spongy fruiting body. 3. *Comatricha* - A species that has distinctive sporangia with stalks. 4. *Amaurochaete* - Another genus with intricate and distinctive fruiting body structures.

Class 4: Plasmodiophoromycetes:

Class Plasmodiophoromycetes belongs to the division Myxomycota within the kingdom Fungi. Plasmodiophoromycetes are known for their significant role in plant pathology, causing various diseases such as clubroot of crucifers (*Plasmodiophora brassicae*) and powdery scab of potatoes (*Spongospora subterranea*). These are intracellular parasites of vascular plants and algae, infecting root tissues. The main vegetative body is a multinucleate plasmodium, similar to slime molds, but confined within host cells. They reproduce by biflagellate zoospores, which serve as the primary means of infection. Unlike true fungi, they lack a filamentous mycelial network. The life cycle is biphasic, consisting of two main stages: 1. Primary Infection (Zoospore Stage) - The resting spores germinate under favorable conditions, releasing primary biflagellate zoospores. Zoospores swim in water films and infect host root hairs. Inside the root cells, the zoospore develops into a primary plasmodium that undergoes nuclear division. 2. Secondary Infection (Resting Spore Formation) - The primary plasmodium cleaves into secondary zoospores, which reinfect other root cells. The secondary infection cycle eventually results in the formation of resting spores, which are released back into the soil as the host tissue decays. These spores can remain dormant in the soil for years, ensuring long-term survival. Plasmodiophoromycetes are responsible for severe plant diseases, leading to crop losses. Resting spores persist in the soil, making disease management difficult. Some species facilitate the transmission of plant viruses. Due to their soilborne nature and long-lived spores, they are difficult to eradicate. Eg: 1. *Plasmodiophora brassicae* - Causal agent of Clubroot Disease. Infects members of the Brassicaceae family (e.g., cabbage, broccoli, mustard). Causes swelling and deformation of roots, leading to poor

nutrient uptake and stunted growth. Disease spreads through contaminated soil and water. 2. *Spongospora subterranea* - Causal agent of Powdery Scab in Potatoes. Affects potato roots and tubers, leading to scab-like lesions. Also acts as a vector for Potato Mop-Top Virus (PMTV). Survives in soil through persistent resting spores. 3. *Polymyxa betae* - Vector of Beet Necrotic Yellow Vein Virus. Infects sugar beet roots and acts as a vector for Beet Necrotic Yellow Vein Virus (BNYVV), which causes rhizomania disease. Unlike *Plasmodiophora brassicae*, it does not cause visible galls but severely affects root function.

14.3 DIVISION EUMYCOTA:

Eumycota, or "true fungi," is a division of the kingdom Fungi that includes organisms with well-defined characteristics. These fungi play important ecological roles as decomposers, symbionts, and pathogens. They have well-organized nuclei within membrane-bound organelles. They lack flagella or cilia, except in some primitive groups. They absorb nutrients from organic matter through extracellular digestion. Can be saprophytic (decomposers), parasitic, or mutualistic (e.g., mycorrhizal fungi). Their cell walls contain chitin, a strong, nitrogenous polysaccharide (unlike plants, which have cellulose). Exist as unicellular (yeasts) or multicellular (molds, mushrooms, etc.). The multicellular form consists of hyphae, which together form a mycelium. Unlike plants, fungi cannot photosynthesize and must obtain organic material for energy. Store carbohydrates as glycogen, similar to animals (unlike plants, which store starch). These break down dead organic matter, recycling nutrients. Form mutualistic relationships (e.g., lichens, mycorrhizae). Cause diseases in plants, animals, and humans (e.g., rusts, athlete’s foot). Can reproduce sexually or asexually through spores. Asexual reproduction often involves spores like conidia or sporangiospores. Sexual reproduction includes zygospores, ascospores, or basidiospores, depending on the fungal group. Division Eumycota is divided into five sub-divisions on the basis of formation of sexual spores or asexual spores i.e. **1. Mastigomycotina 2. Zygomycotina 3. Ascomycotina 4. Basidiomycotina 5. Deuteromycotina**

Sub-Division-1 Mastigomycotina:

Fungi with centrioles present in this sub division. Flagellate cells typically produced during the life cycle. Nutrition mode is typically absorptive. Varying from unicellular that becomes converted into a sporangium, to an extensive, filamentous, coenocytic mycelium. Perfect spores are typically oospores and asexual reproduction occurs typically by zoospores. Sexual reproduction occurs by various means. Sub-division Mastigomycotina divides into 3 classess namely 1. Chitridiomycetes 2. Hypo-chytridiomycetes 3. Oomycetes.

Class 1 Chytridiomycetes:

These are posteriorly uniflagellate, Unicellular or filamentous fungi. These fungi may be holocarpic (having all of the thallus involved in the formation of the fruiting body) or Eucarpic. Motile cells (zoospores or planogametes) are produced characterized by a single, posterior, whiplash flagellum. Mostly aquatic fungi saprobic or parasitic on algae, fungi, or, less often, on flowering plants.

Class 2 Hyphochytridiomycetes:

These are aquatic fungi. Motile cell possess a single anterior tinsel flagellum (i.e., a flagellum with short side branches along the central axis, comb-like). Parasitic on algae and fungi or saprobic on plant and insect debris in water. It includes a single order hypochytriales.

Class 3 Oomycetes:

Fungi with well developed coenocytic mycelium. Aquatic, amphibious, or terrestrial fungi, saprobic, facultatively (occasionally) or obligately (invariably) parasitic on plants, a few on fish. Reproduce Asexually by means of flagellate zoospores each bearing one tensilflagellum directed forward and one whiplash flagellum directed backwards. Zoospores formed in sporangia of various types. Perfect spores are oospores. Sexual reproduction usually by contact of differentiated gametangia (gamete-or sex-cell-producing structures) with nuclei from the male fertilizing differentiated eggs and resulting in thick-walled oospores. Thallus probably diploid with meiosis occurring in the gametangia. This class includes 4 orders 1.Saprolegniales 2. Leptomitales3. Lagnidales 4. Peronosporales

Sub-division-2 Zygomycotina:

Zygomycotina, commonly known as zygote fungi or conjugation fungi, is a subdivision of the fungal division Eumycota. Mostly terrestrial and found in soil, decaying organic matter, and food. Many species are saprophytic, decomposing organic material, while others are parasitic or mutualistic. Saprophytic (e.g., *Rhizopus*), Parasitic (e.g., *Entomophthora* on insects), Mutualistic (e.g., mycorrhizal association in *Glomus*). Filamentous fungi with coenocytic (aseptate) hyphae. Septa appear only in older hyphae or at reproductive structures. These fungi are primarily terrestrial and are characterized by the formation of thick-walled zygospores during sexual reproduction. Asexual reproduction occurs by sporangiospores, which are produced within sporangia borne on sporangiophores. Sexual reproduction takes place via zygosporangia and zygospores formed by the fusion of gametangia from two compatible hyphae. These fungi spoilage food (e.g., *Rhizopus stolonifer* causing bread mold). These fungi have various industrial applications (e.g., enzyme and organic acid production). Acts as biocontrol agents against insect pests. Pathogenic species causing infections like mucormycosis in humans. Zygomycotina is further classified into two major classes: 1. Zygomycetes 2. Trichomycetes.

1. Class: Zygomycetes:

Largest class within Zygomycotina. Mostly saprophytic, decomposing organic matter. Some are opportunistic pathogens. This class divided into three orders i) Order: Mucorales (Common molds) - Fast-growing, saprophytic fungi. Produce sporangiospores in large sporangia. Can cause opportunistic infections (zygomycosis/ mucormycosis). Eg: *Rhizopus stolonifer* – Black bread mold; *Mucor mucedo* – Found in soil and decaying vegetation; *Absidia* – Involved in food spoilage and sometimes infections. ii) Order: Entomophthorales - Insect and animal pathogens. Obligate parasites of insects and animals. Used in biological control of pest insects. Eg: *Entomophthora muscae* – Infects and kills houseflies; *Basidiobolus ranarum* – Causes sub-cutaneous infections in animals and humans. iii) Zoopagales - Mostly parasitic or predatory on microscopic eukaryotes. Found in soil, decaying organic matter, or aquatic environments. Non-motile and reproduce by spores. Their hyphae are often coenocytic. Eg: *Zoopage* sp. – Predatory fungus that traps small nematodes and amoebas; *Piptocephalis* sp. – A mycoparasitic fungus that infects other fungi. *Syncephalis* sp. – Another mycoparasite that attacks Mucorales fungi. *Rhopalomyces* sp. – Parasitic fungus that infects small arthropods.

2. Class: Trichomycetes:

Mostly symbiotic fungi found in the guts of arthropods. Live as commensals or mutualists in aquatic insect larvae. Do not form zygospores but have characteristics similar to Zygomycetes. This class divided into four main orders 1. Order: Harpellales - Found in the guts of aquatic insects. Form branched filaments with specialized attachment structures. Eg: *Harpella sp.* – Found in the gut of aquatic insect larvae. 2. Order: Asellariales - Found in terrestrial isopods. Form intracellular symbiosis. Eg: *Asellaria sp.* – Lives inside crustaceans. iii) Amoebidiales - Amoebidiales are typically ectocommensals or parasites of aquatic arthropods, such as insect larvae and crustaceans. iv) Eccrinales - Eccrinales are commensal organisms found in the digestive tracts of arthropods, including crustaceans (e.g., crabs, shrimp) and insects (e.g., millipedes and cockroaches).

Sub-Division-3 Ascomycotina:

The sub-division Ascomycotina belongs to the division Eumycota, which consists of true fungi. These fungi are characterized by the presence of ascus (plural: asci), a sac-like structure that produces ascospores, typically in groups of eight. Ascomycotina is one of the largest groups of fungi, including both microscopic yeasts and large, complex fungi like morels and truffles. These fungi are saprobic or parasitic on plants, animals, or humans. Some are unicellular but most are filamentous, the hyphae septate with one rarely more, perforations in the septa. Cells are uninucleate or multinucleate. Asexual reproduction takes place by fission, budding, fragmentation, or, more typically, by conidia usually produced on

special sporiferous (spore-producing) hyphae, the conidiophores, which are borne loosely on somatic (main-body) hyphae or variously assembled in asexual fruiting bodies. Sexual reproduction occurs by various means resulting in the production of meiospores (ascospores) formed by free-cell formation in saclike structures (asci), which are produced naked or, more typically, are assembled in characteristic open or closed fruiting bodies (ascocarps). Among these fungi the largest and most commonly known ascomycetes are the morels, cup fungi, saddle fungi, and truffles. This subdivision divided into six classes.

Class 1: Hemiascomycetes (Primitive Ascomycetes):

Simple structure, mostly unicellular or with very simple mycelium. Fungi lack ascocarps (fruiting bodies). Asexual reproduction takes place by budding or fission. Sexual reproduction occurs through the formation of asci without a fruiting body. It has a single order Saccharomycetales (Yeasts). Eg: *Saccharomyces cerevisiae* (Baker's yeast), *Candida albicans* (Pathogenic yeast), *Schizosaccharomyces pombe* (Fission yeast)

Class 2: Plectomycetes (Cleistothecial Ascomycetes):

Produces a cleistothecium (closed fruiting body) where asci are enclosed. Found in soil, decaying organic matter, or as plant pathogens. Asexual reproduction occurs via conidia. This class divides into two main orders i) order: Eurotiales - Common mold fungi. Eg: *Aspergillus niger* (Black mold, industrial enzyme producer), *Penicillium chrysogenum* (Source of penicillin), *Eurotium herbariorum* (Food spoilage fungus). ii) Order: Onygenales - Keratin-decomposing fungi. Eg: *Trichophyton rubrum* (Causes athlete’s foot), *Histoplasma capsulatum* (Causes histoplasmosis)

Class 3: Pyrenomycetes (Perithecial Ascomycetes):

Produces a perithecium, a flask-shaped fruiting body. Asci are produced inside the perithecium with a small opening for spore release. Many are plant pathogens or decomposers. This class includes mainly three orders. i) Order: Hypocreales - Soft-textured fungi, often brightly colored. Eg: *Fusarium oxysporum* (Causes wilt diseases in plants), *Trichoderma harzianum* (Used for biological control). ii) Order: Sordariales – Soil inhabiting fungi, decomposers. Eg: *Neurospora crassa* (Model organism in genetics). iii) Order: Xylariales - Wood-decaying fungi. Eg: *Xylaria polymorpha* (Dead man's fingers, a decomposer)

Class 4: Discomycetes (Apothecial Ascomycetes):

Produce apothecia, an open, cup-shaped fruiting body. Asci are exposed for efficient spore dispersal. Includes edible fungi and plant pathogens. This class includes two main orders i) Order: Pezizales - Cup fungi. Eg: *Morchella esculenta* (Morel mushroom, edible), *Tuber melanosporum* (Black truffle, gourmet delicacy). ii) Order: Helotiales - Leaf-inhabiting fungi. Eg: *Sclerotinia sclerotiorum* (Causes white mold disease in plants).

Class 5: Loculoascomycetes (Bitunicate Ascomycetes):

Produce bitunicate asci, meaning the ascus has two layers. Usually found in decaying wood or as pathogens of plants. Fruiting bodies are often dark-colored, called pseudothecia. This class divides into two main orders. i) Order: Dothideales - Pathogens of plants, forming black fungal spots. Eg: *Venturia inaequalis* (Causes apple scab disease). ii) Order: Pleosporales - Mostly saprophytic or parasitic fungi. Eg: *Alternaria alternata* (Common plant pathogen, causes leaf spots).

Class 6: Laboulbeniomycetes:

This class of fungi includes microscopic, obligate ectoparasites primarily found on arthropods, such as insects and millipedes. These fungi are unique because they do not form traditional mycelia; instead, they develop as small, multicellular thalli that attach to the external cuticle of their hosts. They rely entirely on their hosts for survival but do not usually cause severe harm. They have haustoria-like structures that penetrate the host cuticle for nutrient absorption. They produce ascospores, which are directly transferred between hosts through physical contact. Laboulbeniomycetes is divided into two main orders. Order: i) Laboulbeniales - The largest and most well-known order in Laboulbeniomycetes. This order consists of microscopic, obligate ectoparasites that attach to the exoskeletons of arthropods (mainly insects and millipedes). These fungi form small, complex thalli instead of typical fungal mycelia. These are reproduces via ascospores that spread through direct contact between hosts. Eg: *Laboulbenia*, *Hesperomyces*, *Rickia*. ii) Pyxidiophorales - A less-studied order that includes fungi associated with arthropods but with a different ecological role. Some species are thought to be involved in fungal spore dispersal via insects. Unlike Laboulbeniales, they can form mycelia and have a different method of spore transmission. Eg: *Pyxidiophora*.

Sub-Division 4 Basidiomycotina:

This subdivision includes a vast group of fungi commonly known as club fungi, which produce their sexual spores on a specialized cell called a basidium. Mycelium is usually septate and dikaryotic (each cell contains two nuclei from different parental strains). The hyphae have dolipore septa, which help regulate cytoplasmic flow. Asexual reproduction is usually absent but may occur through conidia, oidia, or chlamydospores. Sexual reproduction involves plasmogamy (fusion of protoplasts), karyogamy (fusion of nuclei), and meiosis, leading to the formation of basidiospores on a structure called the basidium. The dikaryotic phase is dominant in their life cycle. A basidium is a club-shaped structure that bears four haploid basidiospores externally on sterigmata. The spores are forcibly discharged in many species. Many basidiomycetes are saprophytic, decomposing wood, leaf litter, and organic matter. Some are parasitic, causing plant diseases such as rusts and smuts. A few form

mycorrhizal associations with trees, benefiting plant growth. Eg: *Agaricus*, *Amanita* (Mushrooms), *Polyporus* (Bracket fungi), *Puccinia* (Rusts), *Ustilago* (Smuts). This sub-division is further divided into three classes. 1. Teliomycetes 2. Hymenomycetes 3. Gasteromycetes.

Class 1: Teliomycetes:

This class primarily includes rust and smut fungi. These fungi are obligate or facultative plant parasites and are economically significant due to the diseases they cause in crops. Most species are obligate parasites of higher plants, meaning they require a living host to complete their life cycle. They attack cereals, grasses, and other economically important plants. Unlike mushrooms and bracket fungi, Teliomycetes do not produce visible fruiting bodies (basidiocarps). Their spores develop as thick-walled resting spores (teliospores or chlamydospores). The teliospore is the key feature of this class and gives the group its name. Teliospores function as resting spores that later germinate to form basidia. Upon germination, teliospores produce a basidium on which basidiospores are formed. The basidium is often septate (divided into compartments), which differentiates them from other basidiomycetes. Many species have multiple spore stages, including: Urediniospores (repeating stage), Aeciospores (alternate host stage), Teliospores (resting stage), Basidiospores (infection stage). Some rust fungi require two different host plants to complete their life cycle (heteroecious fungi). Cause severe crop diseases, leading to reduced yield. Eg: Wheat rust (*Puccinia graminis*), Corn smut (*Ustilago maydis*). This class divides into two main orders. i) Order: Uredinales (Rust Fungi) - These fungi cause rust diseases in plants. They often have a complex life cycle with multiple hosts. Produce various types of spores, including urediniospores, aeciospores, teliospores, and basidiospores. Eg: *Puccinia graminis* (Wheat rust), *Melampsora lini* (Flax rust). 2. Order: Ustilaginales (Smut Fungi) - Smut fungi attack cereals and grasses, replacing host tissue with black, powdery teliospores. Unlike rust fungi, they generally have a simpler life cycle and do not require an alternate host. Eg: *Ustilago maydis* (Corn smut), *Tilletia indica* (Karnal bunt of wheat).

Class 2: Hymenomycetes:

Hymenomycetes is a traditional class of Basidiomycotina that includes fungi with exposed basidia, forming basidiospores on a hymenium (fertile layer). It comprises mushrooms, bracket fungi, puffballs, coral fungi, and jelly fungi. These fungi are mostly saprophytic or mycorrhizal, with a few parasitic species. Basidia are arranged in a hymenium, a fertile layer covering the spore-bearing surface. The hymenium is often exposed at maturity, facilitating spore dispersal. Most species form large, visible fruiting bodies (basidiocarps), such as mushrooms and shelf fungi. Some have gelatinous or coral-like structures. The mycelium is well-developed, septate, and mostly dikaryotic (contains two genetically distinct nuclei per

cell). The hyphae have dolipore septa, allowing selective cytoplasmic flow. Asexual reproduction is rare, though some species produce conidia. Sexual reproduction occurs via basidiospores, formed externally on club-shaped basidia. The spores are forcibly discharged and dispersed by wind. Many species are saprotrophs, decomposing wood, leaves, and organic matter. Some form mycorrhizal associations with plants, enhancing nutrient absorption. A few are parasitic, such as those causing white rot or root diseases in trees. Eg: Mushrooms: *Agaricus bisporus* (Edible mushroom), *Amanita phalloides* (Death cap); Bracket fungi: *Ganoderma lucidum* (Reishi), *Polyporus*; Jelly fungi: *Tremella*; Coral fungi: *Clavaria*. Hymenomycetes is divided into two major orders based on their basidiocarp structure and basidium type. i) Order: Agaricales (Gilled Fungi or Mushrooms) - Includes most mushrooms, toadstools, and gilled fungi. Basidiocarps are fleshy and umbrella-shaped, with gills (lamellae) on the underside. Eg: *Agaricus bisporus* (Button mushroom), *Amanita* (Poisonous mushrooms). ii) Order: Aphyllophorales (Bracket Fungi, Coral Fungi, and Jelly Fungi) - Includes wood-decaying fungi, coral-like fungi, and jelly fungi. Fruiting bodies vary from leathery, woody, or gelatinous structures. Eg: *Ganoderma lucidum* (Reishi), *Tremella* (Jelly fungus), *Clavaria* (Coral fungus).

Class 3: Gasteromycetes:

Gasteromycetes is a polyphyletic class of Basidiomycotina, historically recognized for fungi that produce their basidiospores internally rather than externally on an exposed hymenium. Unlike Hymenomycetes, where basidiospores develop on an exposed surface, in Gasteromycetes, spores mature inside a closed fruiting body (gasterocarp) and are only released when the structure breaks down or opens. This class includes puffballs, stinkhorns, earthstars, bird’s nest fungi, and false truffles. Unlike other Basidiomycota, spores develop inside a completely enclosed fruiting body. The spores are released only when the fruiting body ruptures, decays, or is mechanically disturbed. Fruiting bodies are of various types i.e. Puffballs (*Lycoperdon*, *Calvatia*): Rounded, closed fruiting bodies that release spores in a puff when ruptured. Stinkhorns (*Phallus*, *Mutinus*): Foul-smelling fungi that attract insects for spore dispersal. Earthstars (*Geastrum*): Puffball-like fungi with outer layers that split open into a star shape. Bird’s Nest Fungi (*Cyathus*, *Crucibulum*): Cup-like structures containing spore-filled "eggs" (peridioles). False Truffles (*Scleroderma*): Underground fruiting bodies similar to true truffles but tougher and inedible. Basidiospore dispersal mechanisms includes Passive dispersal - Many species release spores via wind, rain, or mechanical disturbance (e.g., puffballs). Insect mediated dispersal - Stinkhorns attract flies with their foul smell, which helps in spore distribution. Mycelium is septate and usually dikaryotic. The hyphae often have dolipore septa, like other basidiomycota. Asexual reproduction is rare but can occur through conidia in some species. Sexual reproduction follows the standard basidiomycota life cycle, but basidiospores develop within the fruiting body. These are

mostly saprophytic nature and decomposing wood, leaf litter, and organic matter. Some are mycorrhizal, forming beneficial associations with plant roots. Some species are edible (*Lycoperdon, Calvatia*). Stinkhorns (*Phallus*) are considered medicinal in traditional Chinese medicine. Some species (*Scleroderma*) are toxic and resemble true truffles. This class includes various orders. i) Order: Lycoperdales (Puffballs and Earthstars) - Fruiting bodies are globose or star-shaped. Spores are released through ruptured peridia (outer covering) or an apical pore. Eg: *Lycoperdon perlatum* (Common puffball), *Calvatia gigantea* (Giant puffball), *Geastrum triplex* (Earthstar fungus). ii) Order: Phallales (Stinkhorns) - Foul-smelling fungi that attract insects for spore dispersal. Gleba (spore mass) is slimy and foul-smelling. Example: *Phallus impudicus* (Common stinkhorn), *Mutinus caninus* (Dog stinkhorn). iii) Order: Nidulariales (Bird's Nest Fungi) - Cup-like fruiting bodies resembling a bird's nest. Contains peridioles (egg-like structures) filled with spores. Eg: *Cyathus striatus* (Fluted bird's nest fungus), *Crucibulum laeve* (Smooth bird's nest fungus). iv) Order: Sclerodermatales (False Truffles). Fruiting bodies resemble truffles but are tough and inedible. Some form ectomycorrhizal relationships with trees. Eg: *Scleroderma citrinum* (Common earthball), *Pisolithus tinctorius* (Dye ball fungus, used in mycorrhizal inoculation).

Sub-Division-5 Deuteromycotina:

This sub-division lacks a known sexual reproductive stage. This group is often referred to as Fungi Imperfecti because their sexual reproduction remains unknown, and they are classified based on their asexual (anamorphic) reproductive structures. No known sexual stage (teleomorph), though some may later be linked to ascomycota or basidiomycota. Asexual reproduction occurs mainly by conidia (asexual spores) produced on conidiophores. Hyphal structures are typically septate mycelium (divided by cross-walls), similar to ascomycetes. Nutrition is heterotrophic, generally saprophytic, parasitic, or symbiotic, playing roles in decomposition and diseases. Found in soil, decaying organic matter, and as pathogens of plants, animals, and humans. Includes species causing plant diseases (e.g., *Fusarium* wilt), human infections (e.g., *Candida* spp.), and antibiotic producers (e.g., *Penicillium* for penicillin). Since sexual reproduction is unknown, Deuteromycotina is classified based on conidial morphology and mycelial characteristics into three classes. 1. Blastomycetes 2. Hyphomycetes 3. Coelomycetes.

Class 1. Blastomycetes:

Blastomycetes is an informal grouping within deuteromycotina that consists of unicellular or yeast-like fungi. These fungi reproduce mainly by budding (blastoconidia formation) and lack a known sexual stage. Many species in this group are either saprophytic or pathogenic to humans and animals. Mostly exist as single cells rather than forming mycelium. Asexual

reproduction occurs through the formation of blastoconidia (buds). No known sexual (teleomorphic) stage. Some species may later be reclassified under Ascomycota. Some species exhibit dimorphism, existing as yeasts at one temperature (e.g., 37°C) and as mycelial forms at lower temperatures (e.g., 25°C). Found in soil, decaying organic material, and as opportunistic pathogens in humans and animals. Some species cause diseases like blastomycosis, candidiasis, and cryptococcosis. This class includes three orders. i) Order: Cryptococcales - Yeast-like fungi with thick-walled blastoconidia. Often encapsulated, aiding in pathogenicity. Eg: *Cryptococcus neoformans* - Causes cryptococcosis, primarily in immunocompromised individuals (e.g., AIDS patients). ii) Order: Sporobolomycetales - Yeast like fungi that may produce ballistoconidia (spores forcibly discharged). Found in soil and on plant surfaces. Eg: *Sporobolomyces* spp. - Common saprophytic yeasts. iii) Order: Saccharomycetales (Previously in Deuteromycotina, now in Ascomycota). Includes yeast species that reproduce by budding. Many are economically important in fermentation. Eg: *Candida albicans* - Causes candidiasis, an opportunistic fungal infection, *Saccharomyces cerevisiae* – Used in brewing and baking.

Class 2: Hyphomycetes:

Characterized by the production of conidia on free or exposed conidiophores. Conidiophores may be simple or branched, and conidia may be single or in chains. Many are important plant pathogens or industrially useful fungi. This class includes mainly two orders. i) Order1: Moniliales - Conidia are produced singly or in chains on free conidiophores. The conidiophores are not enclosed in fruiting structures**.** Eg: *Aspergillus* spp. – Includes species used in fermentation and food spoilage, *Penicillium* spp. – Source of the antibiotic penicillin, *Fusarium* spp. – Causes plant diseases such as *Fusarium* wilt, *Trichoderma* spp. – Biocontrol agent against plant pathogens. ii) Order 2: Dematiaceae (Melanconiales) - Produces darkly pigmented (melanized) conidia on conidiophores. This order includes plant pathogens causing leaf spots, cankers, and fruit rots. Eg: *Alternaria* spp. – Causes early blight in tomatoes and potatoes. *Helminthosporium* spp. – Causes leaf spot diseases in cereals.

Class 3: Coelomycetes:

These are primarily asexual fungi that produce conidia (asexual spores) inside specialized fruiting structures known as conidiomata. These structures are either: a) Pycnidia – flask-shaped fruiting bodies that enclose the conidia. b) Acervuli – flat, cushion-like fruiting bodies that erupt from host tissues. Many are plant pathogens, causing diseases in crops and trees. Some are saprophytic, living on decaying organic matter. Eg: *Colletotrichum* – Causes

anthracnose in various plants, *Phoma* – A plant pathogen affecting many crops, *Diplodia* – Causes stem and fruit rots, *Phyllosticta* – Responsible for leaf spot diseases. This includes two main orders. i) Order: Sphaeropsidales - Conidia are formed in pycnidia, which are flask-shaped structures. Eg: *Phoma* spp. – Causes root rot in plants. *Diplodia* spp. – Associated with fruit and stem rot diseases. ii) Order: Melanconiales - Conidia are produced in acervuli, which are cushion-like fruiting bodies. Eg: *Colletotrichum* spp. – Causes anthracnose in many crops. *Gloeosporium* spp. – Pathogens of fruit trees.

14.4 SUMMARY:

The Ainsworth classification of fungi, proposed by Geoffrey Clough Ainsworth, is a hierarchical system that categorizes fungi based on morphology, reproduction, and phylogenetic relationships. Ainsworth's system, which evolved over time, was most notably outlined in *Ainsworth & Bisby's Dictionary of the Fungi*, and it classifies fungi into major groups corresponding to their reproductive structures and life cycles. The classification includes two main divisions **Myxomycota** (slime molds), and Eumycota (True Fungi). Myxomycota exhibit amoeboid and plasmodial stages. it is again divided into 4 classes i.e. Acrasiomycetes, Labyrinthlales, Myxomycetes and Plasmodiophoromycetes. The division Eumycota again devided in to 5 sub-divisions i.e. **Mastigomycotina** (zoosporic fungi), which possess flagellated spores and include groups like Chytridiomycetes; **Zygomycotina** (zygomycetes), characterized by sexual reproduction via zygospores; **Ascomycotina** (ascomycetes), which produce sexual spores in sac-like asci; **Basidiomycotina** (basidiomycetes), forming spores externally on basidia; and **Deuteromycotina** (imperfect fungi), a group of fungi with no known sexual stage.

14.5 TECHNICAL TERMS:

Aplanospore, Ascopore, Ascus, Basidiospores, Basidiomycetes, Cellulose, Chitin, Oogamy, Coenocytic, Perfect fungi, Phylogeny, Taxonomy.

14.6 SELF ASSESSMENT QUESTIONS:

1) Describe the salient features of important group of fungi.
2) Write in detail the classification of fungi given by Ainsworth.
3) Define the characteristic features of Division Mycota.
4) Describe any recent system of classification of fungi giving its important characteristic features.
5) Describe the salient features of sub-divisions Myxomycotina and Eumycotina.

14.7 SUGGESTED READINGS:

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2) Botany for Degree Students Part II FUNGI - B. R. Vashishta, (1990), S. Chand & Company LTD. Ram Nagar, New Delhi 110055.

3) Botany for Degree Students - B. P. Pandey, (2015). As per UGC Model Curriculum,

4) https://www.biologydiscussion.com/microbiology-2/structure-of-fungal-cell-microbiology/64992

Dr. Y.R.K.V. Tirupati Rao

LESSON - 15

ZYGOMYCOTINA, ASCOMYCOTINA, BASIDIOMYCOTINA, DEUTEROMYCOTINA

15.0 OBJECTIVE OF THE LESSON:

By studying this lesson students are able to know the various classes of fungi and their morphological and physiological characteristics of fungi along with methods of reproduction.

STRUCTURE OF THE LESSON:

15.1 INTRODUCTION:

Zygomycotina is a diverse subdivision of fungi characterized by their unique sexual reproduction, which involves the formation of durable zygospores. These primarily saprophytic organisms play a vital role in decomposing organic matter, thereby contributing to nutrient recycling in various ecosystems. Commonly encountered as bread molds such as Rhizopus, zygomycetes also include species with pathogenic properties affecting plants and animals. Despite their relatively simple morphological structure compared to other fungal groups, their evolutionary adaptations provide important insights into the broader development and ecological impact of fungi. Ascomycotina, also known as sac fungi, is a subphylum of the phylum Ascomycota, which includes a diverse group of fungi characterized by the production of spores in sac-like structures called asci. This group comprises yeasts, molds, and larger fungi such as morels and truffles. Ascomycotina plays crucial ecological roles, including decomposing organic matter, forming symbiotic relationships with plants as mycorrhizae, and causing plant diseases. Many species are economically significant, being used in food production (e.g., baking and brewing with *Saccharomyces*), medicine (e.g.,

antibiotic production by *Penicillium*), and biotechnology. Basidiomycotina is a major subdivision of fungi within the phylum Basidiomycota, characterized by the production of spores on specialized structures called basidia. These fungi include mushrooms, puffballs, bracket fungi, rusts, and smuts, many of which play essential ecological roles in decomposition and nutrient cycling. Basidiomycotina species reproduce sexually, typically forming fruiting bodies that release basidiospores for dispersal. Some are beneficial, forming symbiotic relationships with plants as mycorrhizae, while others are pathogenic, causing diseases in crops and trees. Their diversity and significance make them an important group in both natural ecosystems and human industries, such as food production and medicine. Deuteromycotina, also known as the imperfect fungi, is an informal grouping of fungi that reproduce exclusively by asexual means—producing spores called conidia—since their sexual stages have never been observed. This group is polyphyletic, serving as a convenient catch-all for various fungi that lack the diagnostic sexual structures used in traditional fungal classification. Although many deuteromycotina are later reassigned to true fungal phyla such as Ascomycota or Basidiomycota through molecular analyses, they still play significant roles in nature and industry, contributing to processes like food spoilage, the production of antibiotics (for example, penicillin), and diverse biotechnological applications.

15.2 ZYGOMYCOTINA:

Zygomycotina (or Zygomycota) is a group of fungi primarily characterized by their fast-growing, filamentous hyphae and the production of zygospores during sexual reproduction. They are mostly saprophytic but can also be parasitic or symbiotic.

Morphological Characteristics: The hyphae are usually aseptate and lack cross-walls (septa). Septa may form in older hyphae or in reproductive structures (such as zygospores or sporangiophores). Hyphae grow rapidly and spread extensively over substrates like soil, decaying food, and dung. The cell wall is primarily composed of chitin and chitosan (a characteristic that distinguishes them from other fungal groups like Ascomycota and Basidiomycota). The mycelium is well-developed, filamentous, and cottony or fluffy in appearance. Colony color varies from white to greyish or black, depending on the species and age. Sporangiospores are enclosed in sporangia (spore-containing structures). Sporangiophores are stalk-like structures that hold the sporangia above the substrate, aiding spore dispersal. Zygosporangium is a thick-walled, resistant reproductive structure formed by the fusion of two opposite mating types during sexual reproduction. Mostly saprophytic, feeding on decaying organic matter such as bread, fruits, vegetables, and animal dung. Some species are parasitic (e.g., *Entomophthora* attacks insects, and *Basidiobolus* can infect humans and animals).

A few species form symbiotic relationships, such as mycorrhizal associations with plant roots. Generally aerobic, but some species can survive under facultative anaerobic conditions. Capable of breaking down complex organic molecules such as starch, cellulose, and proteins using hydrolytic enzymes. Most grow best at 20–30°C, but some thermophilic species can thrive at higher temperatures (e.g., *Rhizomucor* species). Produce a variety of extracellular enzymes to degrade organic matter, including: Amylases (break down starch), Cellulases (break down cellulose), Lipases (break down lipids) and Proteases (break down proteins). Asexual reproduction is the main mode of reproduction, occurs by sporangiospores (non-motile spores) produced inside sporangia. The sporangia develop at the tips of specialized hyphae called sporangiophores. Upon maturation, the sporangium bursts, releasing spores into the air. These spores land on new substrates and germinate into new mycelia. Sexual reproduction occurs under stressful conditions, it involves heterothallic or homothallic mating types. Gametangial fusion occurs between two opposite mating types (+ and -). Forms a zygosporangium containing a zygospores (thick-walled, resistant spores). The zygospores undergo dormancy and germinate under favorable conditions, forming a new sporangium that releases haploid spores. Species of zygomycotina play a crucial role in breaking down organic matter, recycling nutrients in ecosystems. Some species are used in fermentation (e.g., *Rhizopus oligosporus* in tempeh production). Used for the production of organic acids (e.g., lactic acid, fumaric acid). Enzymes from Zygomycota are used in the food and pharmaceutical industries (e.g., amylase, protease production). Used in bioremediation to break down waste and pollutants.

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.

Morphological Features: Mucor exhibits characteristic morphological features. Physically forming fluffy, cottony, or woolly colonies. Colony colour varies from white to grey, yellowish, or brown as it matures. Mycelium comprised of coenocytic (aseptate) hyphae, which are broad and branched (Fig. 15.1). Lacks true septa except in older mycelium or at reproductive structures. Sporangiophores are unbranched or branched hyphal structures bearing sporangia. Typically erect, long, and arise directly from the substrate. Sporangia are globose (round) or slightly oval structures at the tips of sporangiophores, contain numerous asexual spores. A dome-shaped sterile region present inside the sporangium, separating it from the sporangiophore known as columella. It helps in spore dispersal upon rupture. Some species (e.g., *Mucor circinelloides*) may develop rhizoid-like structures but lack true rhizoids seen in *Rhizopus*.

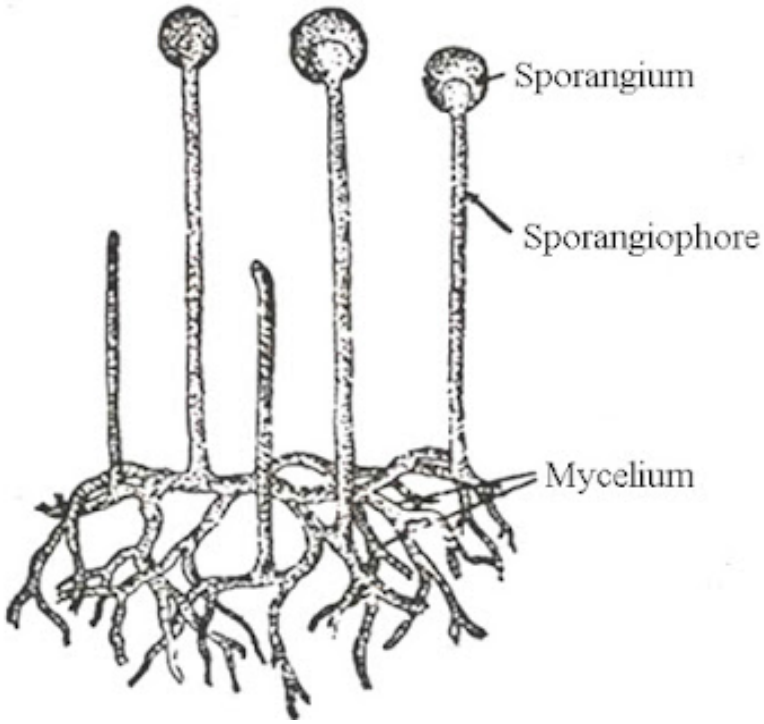

Figure-15.1: External morphology of *Mucor* sps. (https://tinyurl.com/4p3naydw)

Nutritional Features: *Mucor* species are saprophytic fungi, thriving on decaying organic matter, but some species can also act as opportunistic pathogens. Can utilize glucose, sucrose, starch, and cellulose as carbon sources. Some species can ferment sugars under anaerobic conditions. Can use organic (proteins, amino acids) and inorganic (ammonium salts, nitrates) nitrogen sources. Produces amylases, lipases, and proteases to degrade complex organic matter. Can break down lipids, contributing to food spoilage. Thrives in moist environments with abundant organic material. Some species grow in acidic (low pH) or alkaline (high pH) conditions, showing adaptability.

Physiological Features: *Mucor* has diverse physiological characteristics that enable its survival in various environments. Grows optimally between 25–30°C, but some species (e.g., *Mucor indicus*) can tolerate up to 37–40°C. Certain thermophilic species can grow at even higher temperatures. Primarily aerobic, requiring oxygen for growth. Some species can undergo anaerobic fermentation, producing ethanol. Can grow in a wide pH range (4.0–8.0), but prefers neutral to slightly acidic conditions. Some species can tolerate high sugar concentrations, making them common contaminants in food products like bread and fruits.

Reproduction: Fungi reproduce by vegetative, asexual and sexual modes.

Vegetative reproduction: Vegetative reproduction in Zygomycotina occurs without the formation of spores or gametes and involves the propagation of fungal hyphae through various methods. These methods include **fragmentation, budding, fission, and sclerotia formation**.

Modes of Vegetative Reproduction in Zygomycotina

1) **Fragmentation:** This is the most common method of vegetative reproduction in Zygomycotina. The fungal mycelium (network of hyphae) breaks into smaller fragments, and each fragment develops into a new mycelium. The process occurs naturally due to mechanical disturbances, aging, or unfavorable environmental conditions. Example: *Rhizopus stolonifer* (common bread mold) can reproduce through fragmentation when parts of its mycelium break off and establish new colonies.
2) **Budding:** This type of reproduction is relatively rare in Zygomycotina. It involves the formation of a small outgrowth (bud) from a parent cell, which eventually detaches and develops into a new organism. Budding is more commonly observed in yeasts (Ascomycota), but some members of Zygomycotina may exhibit this method under specific conditions.
3) **Fission:** Some unicellular members of Zygomycotina can reproduce by binary fission, where a single cell splits into two identical daughter cells. This process is more typical of bacteria and certain yeasts but has been observed in some primitive fungal cells.
4) **Sclerotia formation:** Under unfavourable conditions, some Zygomycotina form **sclerotia**, which are hardened, dormant structures. Sclerotia contain stored nutrients and can survive extreme conditions like drought or cold. When conditions become favourable, the sclerotia germinate to produce new fungal mycelia. Example: Some *Mucor* species are known to form sclerotia as a survival mechanism.

Significance of vegetative reproduction in zygomycotina: Vegetative reproduction allows fungi to spread quickly in suitable environments. It ensures survival in harsh conditions by forming resistant structures such as sclerotia. Since offspring are clones of the parent, favorable genetic traits are maintained. Mycelial fragmentation allows fungi to take full advantage of available nutrients.

Asexual Reproduction: It occurs by the formation of aplanospores or chlamydospores or oidia.

i) **Formation of aplanospores:** Under favorable conditions, multinucleate non motile spores i.e. aplanospores are formed inside the sporangia occurring singly at the tip of sporangiophores (Fig.15.2). 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 cytoplasmic sporoplasm and vacuolated cytoplasmic columellaplasm. 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. On getting suitable substratum, they germinate by producing germ tube which develops into new mycelium.

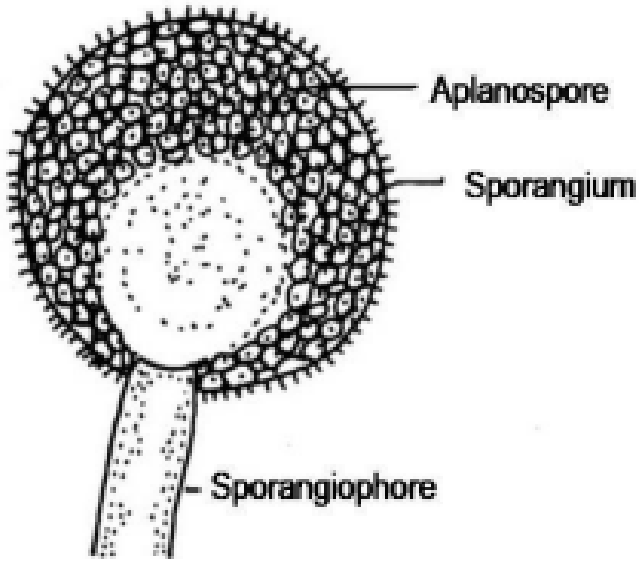

Figure-15.2: Alanospores in *Mucor* (https://tinyurl.com/5e6ucen5)

ii) **Formation of chlamydospores:** Under unfavorable conditions, mycelium become septate and protoplast of each cell form a thick wall rounded structure (chlamydospore) (Fig.15.3). These perennating bodies germinate and form new mycelium.

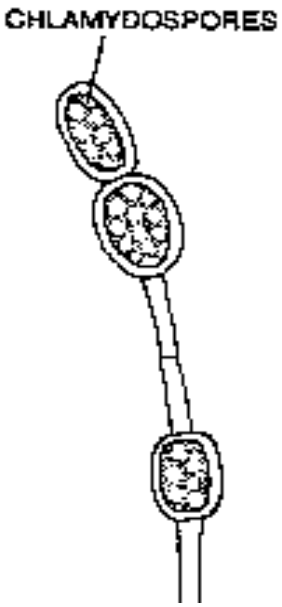

Figure-15.3: Chlamydospores in *Mucor* sps. (https://tinyurl.com/4p3naydw)

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 (Fig.15.4). They enlarge by budding and this phase is called "torula".

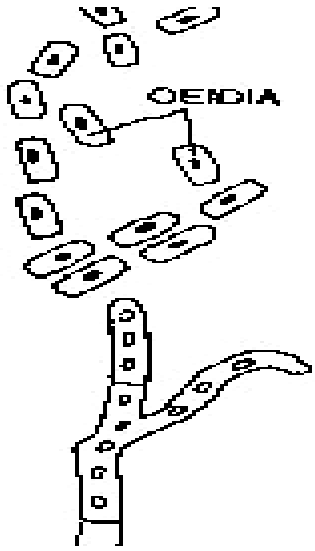

Figure-15.4: Oidia in *Mucor* sps. (https://tinyurl.com/4p3naydw)

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.15.5), 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.

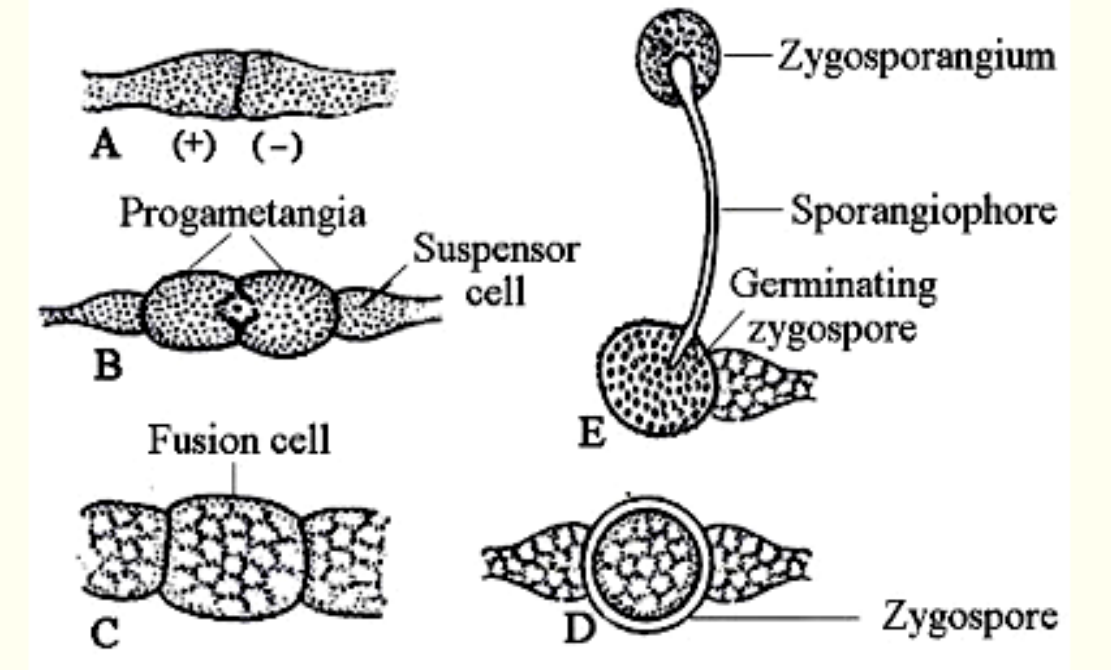

Figure-15.5: Sexual reproduction in *Mucor* sps.(https://tinyurl.com/4p3naydw)

15.3 ASCOMYCOTINA:

Ascomycotina, also known as sac fungi, is a subdivision of fungi characterized by the production of spores in a specialized sac-like structure called an ascus. Morphologically, they exhibit diverse forms, including unicellular yeasts, filamentous molds, and complex fruiting bodies like morels and cup fungi. Their cell walls are primarily composed of chitin, and they may exist as septate hyphae or in yeast form. Physiologically, Ascomycotina are heterotrophic, deriving nutrients through saprophytic, parasitic, or mutualistic lifestyles. They play key roles in decomposition, fermentation, and symbiotic relationships, such as lichens. Reproduction occurs both asexually, through conidia or budding, and sexually, via ascospores formed within asci, typically housed in fruiting bodies called ascocarps.

General Characteristics: Ascomycotina (synonymous with the phylum **Ascomycota** in modern taxonomy) is the largest fungal group, comprising over 64,000 species. Includes

unicellular yeasts (e.g., *Saccharomyces*), filamentous fungi (e.g., *Penicillium*), and macroscopic fungi (e.g., *Morchella*). These play a key role in recycling organic matter (e.g., *Chaetomium*). Some species cause diseases on plants (e.g., powdery mildews, *Cryphonectria parasitica*), animal/human infections (e.g., *Candida albicans*). Few forms of ascomycotina acts as symbionts and form lichens (with algae/cyanobacteria) and mycorrhizae. Species of lichens are ubiquitous, found in soil, freshwater, marine environments. Some are are habituated to hypersaline, arid, or cold environments (e.g., *Debaryomyces hansenii* in salty foods; *Xeromyces* in deserts). Hyphae are septate, with pores allowing cytoplasmic streaming. Cell walls contain chitin and β-glucans. In case of yeasts fungal body is unicellular, reproducing via budding (e.g., *Saccharomyces cerevisiae*). Sclerotia or Stromata are dense hyphal aggregates for survival (e.g., *Claviceps purpurea*).

Species of ascomycotina are having various nutrition types i.e. Saprophytic: Decompose cellulose, lignin, and keratin (e.g., *Chaetomium*). Parasitic: Extract nutrients from hosts (e.g., *Ophiocordyceps* infects insects). Fermentation: Yeasts convert sugars to ethanol/CO_2 (e.g., *Saccharomyces*). Ascomycotina members produce cellulases, ligninases, and proteases for substrate breakdown. Various secondary metabolites are produced by ascomycotina members which includes Antibiotics (e.g., penicillin from *Penicillium*), Toxins (e.g., aflatoxins from *Aspergillus flavus*) and Immunosuppressants (e.g., cyclosporine from *Tolypocladium*).

Members of ascomycotina are having various modes of reproduction includes asexual and sexual. The asexual reproductive sturcutres include Conidiophores - Specialized hyphae producing conidia (e.g., *Aspergillus*) and Synnemata/Sporodochia - Conidiophores aggregated into fruiting bodies are acts as asexual reproductive structures. The sexual reproductive strucutres includes different types of ascocarps Cleistothecia - Closed, globose (e.g., *Eurotium*), Perithecia - Flask-shaped with ostiole (e.g., *Neurospora*), Apothecia - Cup-shaped (e.g., *Morchella*), Chasmothecia - Similar to cleistothecia but with a distinct opening and Asci - Sac-like structures; unitunicate (single wall) or bitunicate (double wall) (e.g., *Peziza*). During asexual reproduction the non-motile spores conidia produced on conidiophores and later dispersed by wind/water. Budding is another mode of asexual reproduction and it is very common in yeasts (e.g., *Candida*). Sexual Reproduction occurs through Plasmogamy - Fusion of compatible hyphae (heterothallic/homothallic), Dikaryon Formation - Results in n+n hyphae, Karyogamy and Meiosis - Occurs in the ascus, producing 4–8 ascospores (often with mitotic divisions) and Ascospores - Variably shaped (spherical, filiform) and ornamented; dispersed via forcible ejection. The mating systems of ascomycotina is of two types Heterothallic - Requires two compatible strains (e.g., *Neurospora crassa*) and Homothallic - Self-fertile (e.g., *Aspergillus nidulans*).

Ascomycotina species are useful in various fields like food production - Yeasts in baking/brewing; truffles/morels as delicacies, Medicine - Antibiotics (penicillin), immunosuppressants (cyclosporine), Biotechnology - Enzymes for industrial use (e.g., pectinases). Members of ascomycotina causes various diseases on plants and humans. The plant diseases includes Ergot of rye (*Claviceps*), Dutch elm disease (*Ophiostoma*). *Aspergillus* (aspergillosis), *Candida* (thrush) causes mycoses in humans. Apart from these ascomycotina members also produce mycotoxins like Aflatoxins (carcinogenic) and ergot alkaloids (hallucinogenic).

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. flavus* contaminates groundnut and other dry food stuffs. 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 (Fig.15.6). 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 specific characteristic pigments in hyphae, conidiophores and conidia.

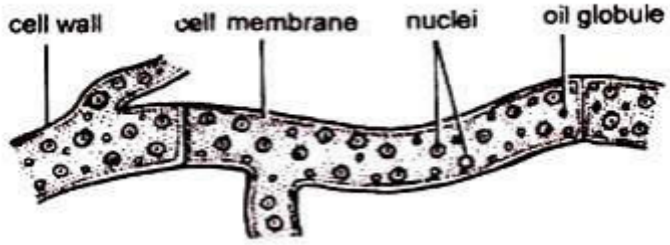

Figure-15.6: Vegetative strucuture of *Aspergillus* (https://tinyurl.com/4dfkx8un)

Reproduction: Aspergillus reproduces by various means includes vegetative, asexual and sexual means.

Vegetative Reproduction: It occurs generally through fragmentation. Under favorable conditions the vegetative mycelium breaks up into small fragments and each fragment grows independently into a new thallus.

Asexual Reproduction: Asexual reproduction occurs through the conidia generated by conidiophores. Certain hyphal cells grow at an accelerated rate, developing a sturdy wall, and are referred to as foot cells. Each foot cell gives rise to a specialized upright branch known as a conidiophore (Fig.15.7 and Fig.15.8). Typically, conidiophores are unbranched and lack septa. The apex of the conidiophore enlarges into an elliptical or globular multinucleate structure called a vesicle. From these vesicles, numerous radially arranged tubular extensions, known as sterigmata or phialides, emerge. In some species, primary sterigmata (uniseriate) can produce secondary sterigmata (bi-seriate). Conidia (singular: conidium) develop externally from the sterigmata or phialides, which is why they are also referred to as phialospores or phialaconidia. They are organized in a basipetal sequence. The sterigmata extend at their tips to form a tube, within which conidia are produced. These sterigmata are uninucleate. During conidia formation, mitotic division occurs within a single nucleus of the phialide, resulting in two daughter nuclei; one of these nuclei enters the tube to form the first conidium. Following the formation of the first conidium, the upper broken wall of the phialide acts as a cap. The second conidium is generated by the phialide just beneath the first. The cytoplasm of both conidia is interconnected through a narrow cellular link known as the isthmus (Fig.15.9). As the inner conidial wall develops, the continuity of the cytoplasm ceases. The isthmus then becomes vacant and is referred to as the connective. This process results in the formation of a long chain of conidia. Conidia are small, globular, unicellular, and uninucleate, exhibiting colors such as black, brown, or yellow-green due to various pigments. They possess a two-layered wall: the outer layer, or epispore, is thick, spiny, and pigmented, while the inner layer, known as the endospore, is delicate and thin. Conidia are dispersed by wind and germinate on a suitable substrate by producing a germ tube. This germ tube becomes septate, branches out, and ultimately forms a new mycelium (Fig.15.10). The complete asexual cycle of *Aspergillus* is given Figure 15.11.

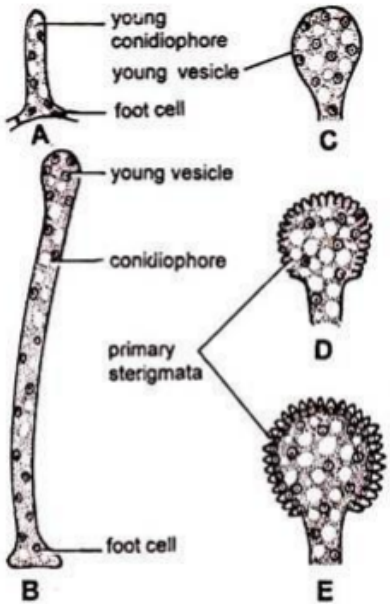

Figure-15.7: Formation of conidia

A. Young condiophore

B-E. Formation of primary sterigmata

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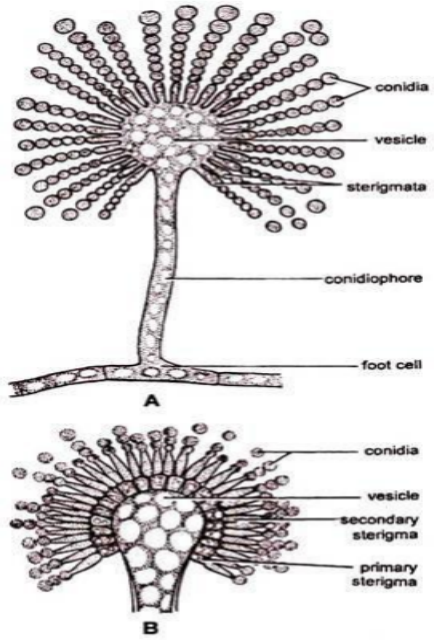

Figure-15.8: *Aspergillus* asexual reproduction

A. Conidipohre with primary sterigmata

B. Primary and secondary sterigmata

(https://tinyurl.com/43srbcsm)

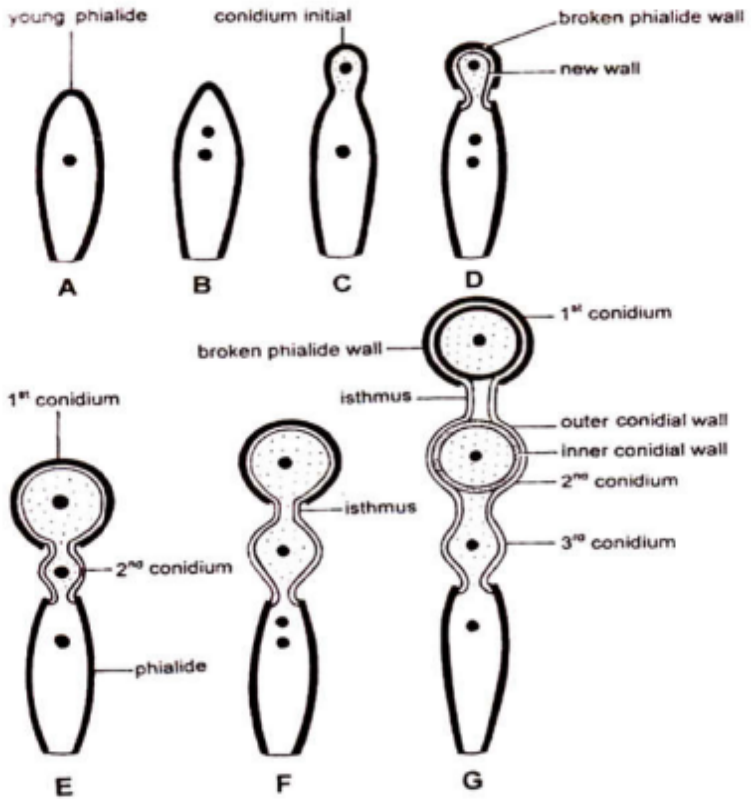

Figure-15.9: Formation of conidia in *Aspergillus* (https://tinyurl.com/mv4nswp2)

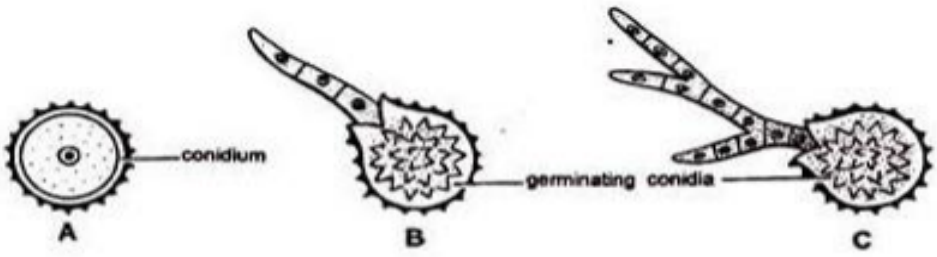

Figure-15.10: Conidia germination in *Aspergillus* (https://tinyurl.com/yhp29fxx)

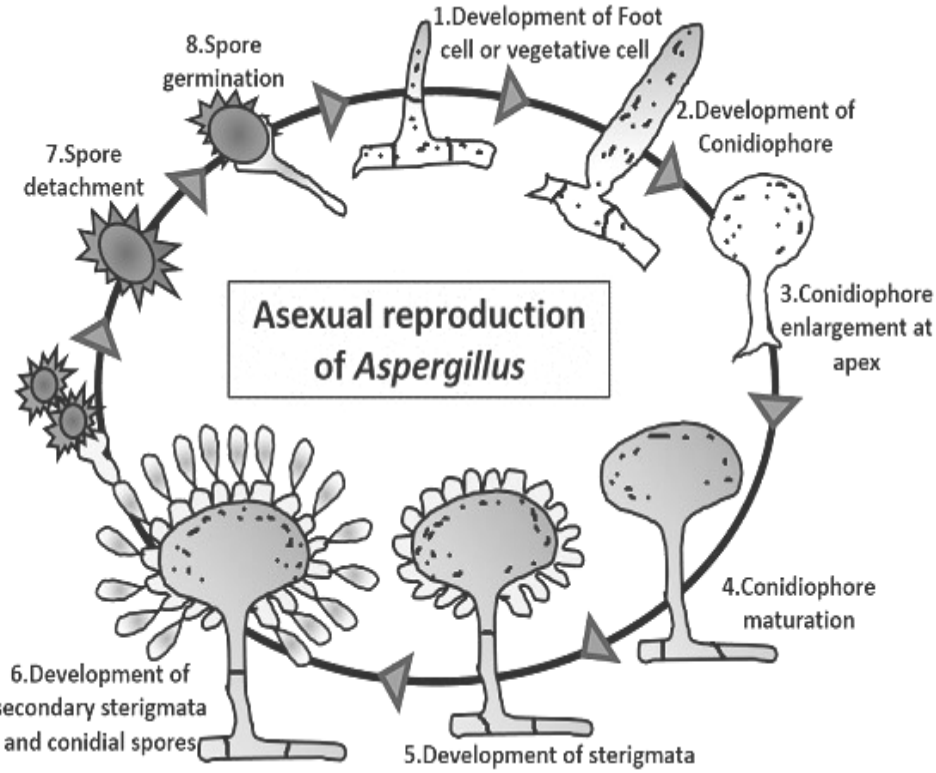

Figure-15.11: Asexual reproduction in *Aspergillus* (https://tinyurl.com/yeyrj58n)

Sexual Reproduction: Sexual reproduction in *Aspergillus* is infrequent, with variations in sexual characteristics observed among different species within this genus. In some species, the antheridium is either absent or nonfunctional, while the female reproductive structure, known as the ascogonium, is responsible for the development of asci. In other species, the ascogonium is well-developed and functional. *Aspergillus* exhibits homothallism, although heterothallism is present in a few species, such as *A. fischeri* and *A. heterothallics*. The male and female reproductive structures are referred to as antheridia and ascogonia, respectively. In homothallic species, these sex organs are located in close proximity on the same hypha or on adjacent hyphae of the same mycelium. Both structures are elongated, multinucleate, and typically entwine around one another. The ascogonial mycelium later becomes septate and loosely coiled. As the ascogonium matures, the coil tightens, forming a spring-like structure known as archicarps. A young archicarp can be divided into three distinct parts: 1. The

terminal portion, a unicellular and multinucleate receptive organ called the trichogyne. 2. The middle section, which is also unicellular and multinucleate, is the ascogonium (gametangium). 3. The basal part, which is multicellular and multinucleate, forms the stalk.

Development of Antheridium: The development of the antheridium begins either just prior to or concurrently with the separation of the ascogonial hypha. The antheridial branch, referred to as the pollinodium, differentiates into two cells through the formation of a septum. The upper cell develops into the antheridium itself, while the lower cell serves as the stalk. Both cells are unicellular and contain multiple nuclei.

Fertilization: The top portion of the archegonium bends over the trichogyne and fuses with it. At this junction, the wall breaks down, permitting the contents of the antheridium to enter the ascogonium. Inside the ascogonium, the male and female nuclei unite, a process known as dikaryon formation. In some species, the antheridium is well-developed, but the male contents do not transfer or combine with those of the ascogonium, as seen in *A. repens*. In contrast, other species may lack an antheridium entirely, such as *A. flavus* and *A. fisheri*. This results in the pairing of ascogonial nuclei, leading to the development of a fruiting body, which signifies the decline of sexual organs in Ascomycetes.

Development of Ascus: Following the fusion of the nuclei, the ascogonium becomes septate. Each segment is composed of one male and one female nucleus, referred to as a dikaryon. From these dikaryotic segments, ascogenous hyphae develop. The terminal cell of the ascogenous hypha bends to create a hook-like structure known as a crozier. The two nuclei within the dikaryon undergo conjugate division, resulting in the formation of four daughter nuclei. Subsequently, septa are established within the crozier, such that the penultimate cell contains two daughter nuclei, while both the terminal and basal cells each contain one daughter nucleus. The two nuclei in the penultimate cell merge to create a diploid nucleus. This cell now acts as the ascus mother cell and elongates to form an ascus. The diploid nucleus first undergoes meiotic division, followed by mitotic division, producing eight haploid nuclei, each of which is ultimately converted into an ascospore.

Development of Ascocarp: Alongside the formation of ascogenous hyphae, numerous sterile hyphal branches emerge from the cells situated beneath the ascogonium. These branches create the peridium, a pseudoparenchymatous structure composed of two layers that encases the ascogenous hyphae. The outer layer serves as a protective shield, while the inner layer is utilized by the developing asci. Collectively, this structure is referred to as the fruiting body or ascocarp, which resembles a hollow sphere approximately the size of a pinhead. This type of fruiting body is classified as a cleistothecium, meaning it is entirely enclosed. The release of ascospores occurs when the wall of the cleistothecium (peridium) breaks down, allowing for the formation of new mycelia on an appropriate substrate following germination. The complete sexual cycle of aspergillus is represented in Fig.15.12.

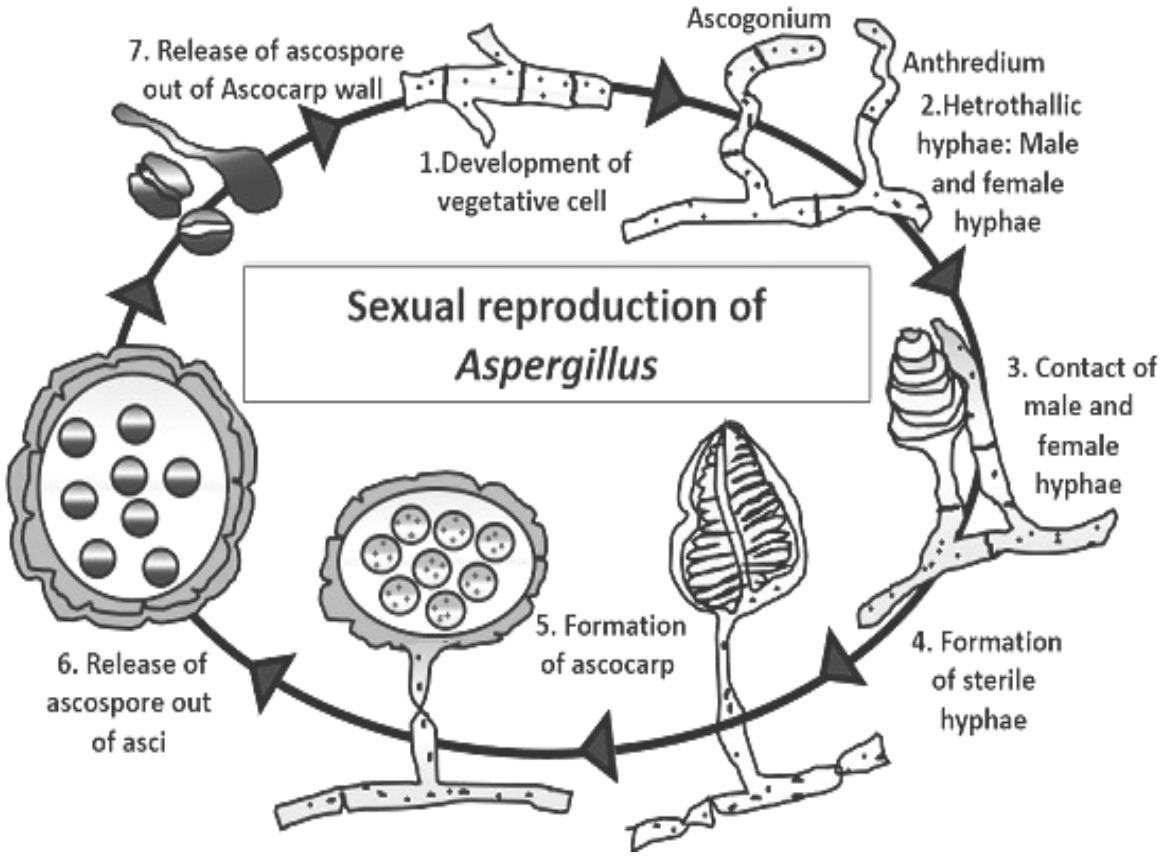

Figure-15.12: Asexual and Sexual reproduction in *Aspergillus* (https://tinyurl.com/3ee5u2tr)

15.4 BASIDIOMYCOTINA:

General characteristics: Basidiomycotina, a major subdivision of fungi, includes mushrooms, puffballs, bracket fungi, rusts, and smuts. They are characterized by the production of basidiospores on a specialized structure called the basidium. This group plays critical ecological roles as decomposers, pathogens, and mutualists. Mycelium is composed of septate hyphae with perforated septa (dolipore septa) regulated by parenthesomes, allowing cytoplasmic continuity while restricting organelle movement. Clamp connections are present in many species to maintain the dikaryotic state post-cell division by ensuring proper nuclear distribution. Cell wall is primarily consists of chitin and glucans, providing structural rigidity.

Members of basidiomycotina are having various types of nutrition which includes Saprophytic - Major decomposers of lignin (e.g., white-rot fungi) and cellulose, critical in nutrient cycling, Parasitic - Rusts (e.g., *Puccinia*) and smuts (e.g., *Ustilago*) infect plants, causing agricultural diseases and Mutualistic - Form ectomycorrhizae with tree roots, enhancing nutrient uptake (e.g., *Amanita*). Ascomycotina species secrete extracellular enzymes (laccases, peroxidases) to degrade complex polymers, apart from this these are also produce secondary metabolites (e.g., antibiotics, toxins like amatoxins in *Amanita*

phalloides). These species can tolerate diverse environments, some thrive in extreme conditions (e.g., *Cryptococcus* spp. as human pathogens).

Reproductive structures of basidiomycotina includes basidiocarp: Macroscopic fruiting body (e.g., mushrooms, puffballs) where spore-producing tissues develop, Hymenium: Fertile layer lining structures like gills, pores, or teeth, containing basidia and basidium: Club-shaped cells where karyogamy and meiosis occur, producing 4 basidiospores on sterigmata. Asexual reproduction occurs through Conidia/Oidia: Asexual spores produced by some species (e.g., rusts), Fragmentation/Budding: Mycelial fragments or yeast-like cells (e.g., *Cryptococcus*). Sexual reproduction occurs through plasmogamy: Fusion of compatible hyphae (somatogamy) forms a dikaryotic (n+n) mycelium, Dikaryotic Growth: Maintained via clamp connections; dominates the life cycle, Basidiocarp Formation: Induced by environmental cues (e.g., humidity, temperature), Karyogamy and Meiosis: In basidium, diploid nucleus undergoes meiosis, yielding 4 haploid basidiospores and Spore Dispersal: Ballistospores are forcibly ejected; germination produces monokaryotic hyphae. Members of basidiomycotina are key decomposers in carbon cycling and mycorrhizal symbionts support forest ecosystems. These impact causes wheat rust, corn smut on plants and cryptococcal meningitis in humans. Ligninolytic enzymes released by ascomycotina species used in bioremediation. Some members are edible mushrooms (e.g., *Agaricus*).

AGARICUS

Systematic position

Kingdom	:	Mycota
Division	:	Eumycotina
Sub division	:	Basidiomyccotina
Class	:	Hymenomycetes
Order	:	Agaricales
Family	:	Agaricaceae
Genus	:	*Agaricus*

Habit and Habitat: *Agaricus*, commonly referred to as mushroom, is a type of saprophytic fungus that thrives on decomposing organic matter found in open fields, grasslands, and soil rich in cellulose and lignin. This edible gilled fungus has a widespread distribution and flourishes in moist, shaded environments, particularly during the rainy season. In India, approximately 17 species of this genus are recognized, each known by various local names such as kukurmutta, khumb, and dhingri. Notable edible varieties include *A. campestris*,

known as the field mushroom, and *A. bisporus* (also referred to as *A. brunnescens*), the cultivated mushroom, both of which are widely grown across different regions of India. However, it is important to note that *A. silvaticus* and *A. xanthodermus* are highly toxic species. The structure of *Agaricus* can be examined under two main categories: (i) vegetative mycelium and (ii) fruiting body (basidiocarp).

(i) Vegetative Mycelium: The vegetative mycelium exists underground and is classified into two types. The primary mycelium develops from the germination of homokaryotic basidiospores, it is septate, hyaline, and monokaryotic, with strains that may be either positive or negative, depending on the basidiospore strain. These cells contain oil globules and vacuoles, are short-lived, and eventually transform into secondary mycelium through the fusion of primary mycelium from opposite strains. The secondary mycelium is binucleate, septate, perennial, branched, abundant, and long-lasting. It may or may not feature clamp connections and contains dolipore septa, with openings protected by parenthesomes on both sides. The hyphae of the secondary mycelium intertwine to create robust white hyphal cords known as rhizomorphs, which support the formation of fruiting bodies. The dikaryotic mycelium exhibits centrifugal growth, meaning that the hyphae expand outward in a circular pattern from a central point. As a result, a circular colony of hyphae develops in the soil, and at maturity, the hyphae produce fruiting bodies at their tips, arranged in roughly circular rings. This process continues with further hyphal growth, leading to the formation of increasingly larger circles of basidiocarps. Consequently, successive generations of fruiting bodies appear in progressively larger rings. These formations are referred to as fairy rings, based on the ancient belief that they indicated the paths of dancing fairies (Fig.15.13).

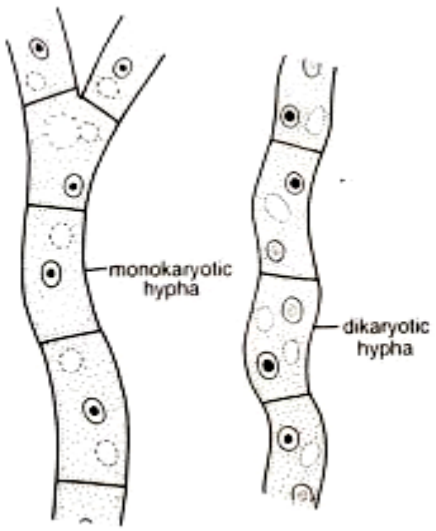

Figure-15.13: *Agaricus* mycelium (https://tinyurl.com/bdzzvc4y)

(ii) Fruiting body: It is commonly known as the mushroom, is not the entirety of the fungus; it is specifically the reproductive structure referred to as the basidiocarp. This aerial component of *Agaricus* develops from the rhizomorph and is divided into a stipe and an umbrella-shaped cap known as the pileus. The stipe typically measures between 6 to 9 cm in height and is a thick, fleshy, cylindrical structure, while the pileus ranges from 5 to 10 cm in diameter. The undersurface of the pileus features approximately 300 to 600 radially arranged gills.

Reproduction:

Vegetative reproduction: *Agaricus* (a genus that includes the common mushroom, *Agaricus bisporus*) primarily reproduces asexually through vegetative methods. The edible mushrooms are propagated by vegetative reproduction. The main vegetative reproduction methods in *Agaricus* include:

1) Fragmentation - The mycelium, which consists of a network of hyphae, can break into fragments. Each fragment can grow into a new mycelium under favourable conditions, leading to the formation of new fungal colonies.
2) Budding (Rarely Seen) - In some cases, small outgrowths may form on the mycelium, which later separate and grow into independent fungal bodies.
3) Fission (In Some Species) - Some yeast-like forms of *Agaricus* may undergo fission, where a cell divides into two daughter cells, though this is not common in *Agaricus bisporus*.
4) Sclerotia Formation (Rare in *Agaricus*) - Under unfavorable conditions, the mycelium may form compact masses called sclerotia. These sclerotia remain dormant and germinate when conditions become suitable. Though *Agaricus* primarily reproduces sexually via basidiospores, vegetative reproduction through mycelial fragmentation is a crucial method for spreading in the environment.

2. Asexual Reproduction: *Agaricus* primarily reproduces through sexual reproduction via basidiospores. However, in certain conditions, it can exhibit asexual reproduction through the following methods: 1. Oidia Formation – Under stressful conditions, some species produce small, thin-walled, asexual spores called oidia, which germinate into new mycelia. 2. Chlamydospore Formation – Thick-walled, resistant spores called chlamydospores may form in the mycelium and help the fungus survive unfavourable conditions. When conditions

improve, they germinate into new mycelia. Though asexual reproduction is rare in *Agaricus*, these mechanisms help in survival and propagation when sexual reproduction is not possible.

3. Sexual Reproduction: Most species are heterothallic, such as *A. bisporus*, yet they completely lack sex organs. Primary mycelium arises from the germination of basidiospores. The fusion of hyphae from opposite strains (somatogamy) or the interaction of oidia with hyphae leads to the combination of compatible nuclei, resulting in the formation of secondary mycelium. This secondary mycelium subsequently develops into fruiting bodies known as basidiocarps. Somatogamy between two primary hypha of opposite strains take place through the following steps (Fig.15.14).

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:** Karyogamy is the process in which two haploid nuclei fuse to form a diploid nucleus. In *Agaricus* (a genus that includes the common mushroom *Agaricus bisporus*), karyogamy is a crucial step in the sexual reproduction cycle. Process of Karyogamy in *Agaricus* is occurs in the following steps: 1. Dikaryotic Stage: In *Agaricus*, plasmogamy (fusion of cytoplasm) occurs between two compatible hyphae, forming a dikaryotic mycelium. Each cell in the dikaryotic hyphae contains two distinct haploid nuclei (n + n), one from each parent. 2. Formation of Basidia: In the mature fruiting body (basidiocarp), dikaryotic cells in the gills develop into basidia. 3. Karyogamy Occurs in Basidia: Inside each young basidium, the two haploid nuclei fuse, forming a single diploid nucleus (2n). This marks the transition from the dikaryotic phase to the diploid phase, though it is very short-lived.

c) **Meiosis and Spore Formation:** Immediately after karyogamy, meiosis occurs, reducing the diploid nucleus into four haploid nuclei. These nuclei migrate into developing basidiospores, which are then released to start a new life cycle.

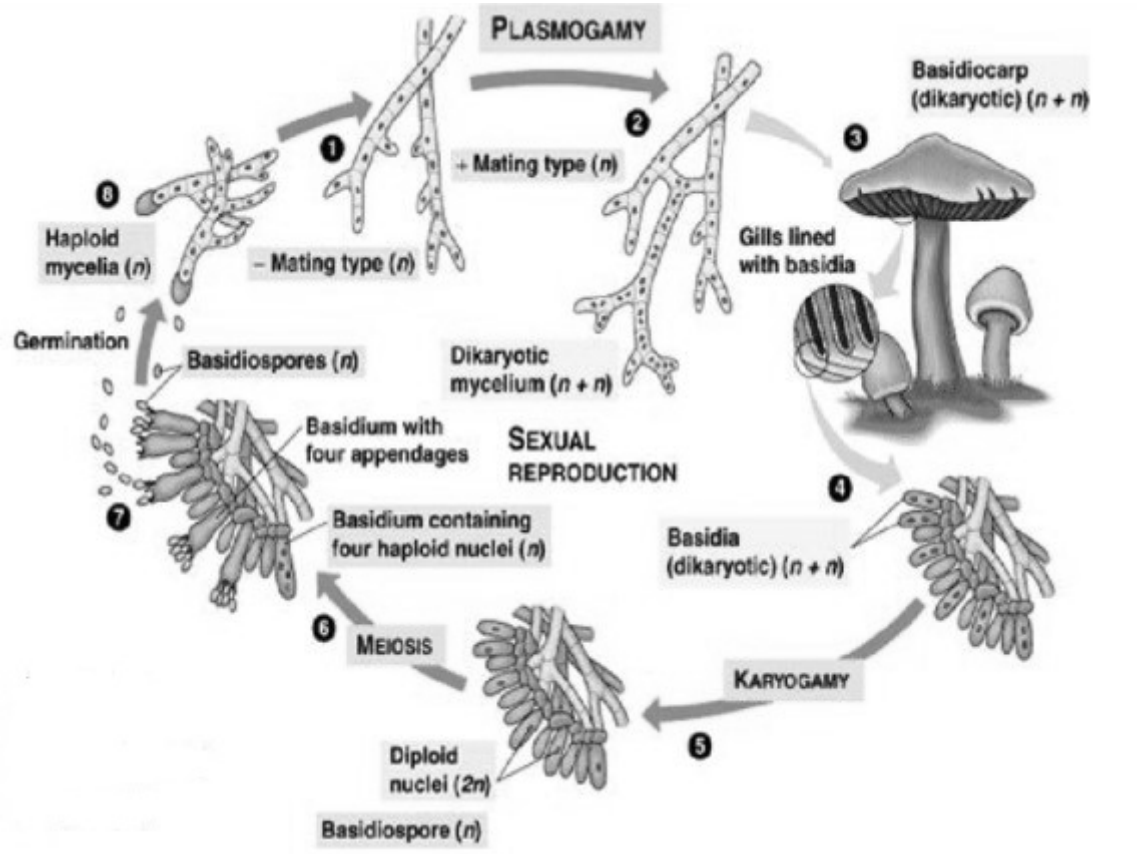

Figure-15.14: Modes of sexual reproduction in *Agaricus* (https://tinyurl.com/3nz5snee)

Basidiocarp

Development of basidiocarp: The basidiocarp initiates as small white protrusions at the ends of the branches of subterranean mycelial strands, known as rhizomorphs. These hyphal clusters gradually enlarge, progressing to the button stage of the basidiocarp. At this stage, the developing structure is characterized by a bulbous base and a rounded upper section. The base forms the stipe, while the upper hemispherical part is referred to as the pileus. Some hyphae at the interface between the stipe and pileus diverge to form a ring-like formation called the prelamellar chamber. The inner surface of this chamber's roof becomes significantly concave and is lined with alternating radial bands of dividing cells, which give rise to gill primordia that develop into gill lamellae extending downward into the prelamellar chamber. A membrane known as the velum or inner veil connects the edges of the pileus and stipe. As the stalk elongates, the buttons rise above the soil, with the upper part of the button growing at a faster rate than the stalk. This rapid growth leads to the rupture of the velum, allowing the upper hemispherical section to expand into an umbrella-like form adorned with numerous gills on its underside. At this point, remnants of the velum remain attached to the stipe, creating a ring known as the annulus. During dry periods, the buttons cease to grow and remain buried, but in the rainy season, when the soil becomes moist, they grow swiftly and emerge from the ground, making the basidiocarp prominently visible during wet weather.

Structure of Mature Basidiocarp: The fully developed basidiocarp exhibits an umbrella-like shape, characterized by a robust, elongated stipe and a wide pileus (Fig.15.15). The stipe is a thick, fleshy, cylindrical structure that appears pinkish-white. Typically, it is wider and swollen at the base, where it connects centrally to the pileus. The upper convex surface of the pileus displays a color range from white to light brown and yellow. Beneath the pileus, numerous slender vertical strips or plates of tissue, known as gills or lamellae, are suspended. These gills vary in length, with some being full, half, or quarter-length. The gill surfaces are covered by a fertile layer called the hymenium or thecium. As the basidiocarp matures, the gills transition to a brown or purplish-black hue. The internal composition of the basidiocarp consists of a pseudoparenchymatous mass formed by interconnecting tertiary hyphae.

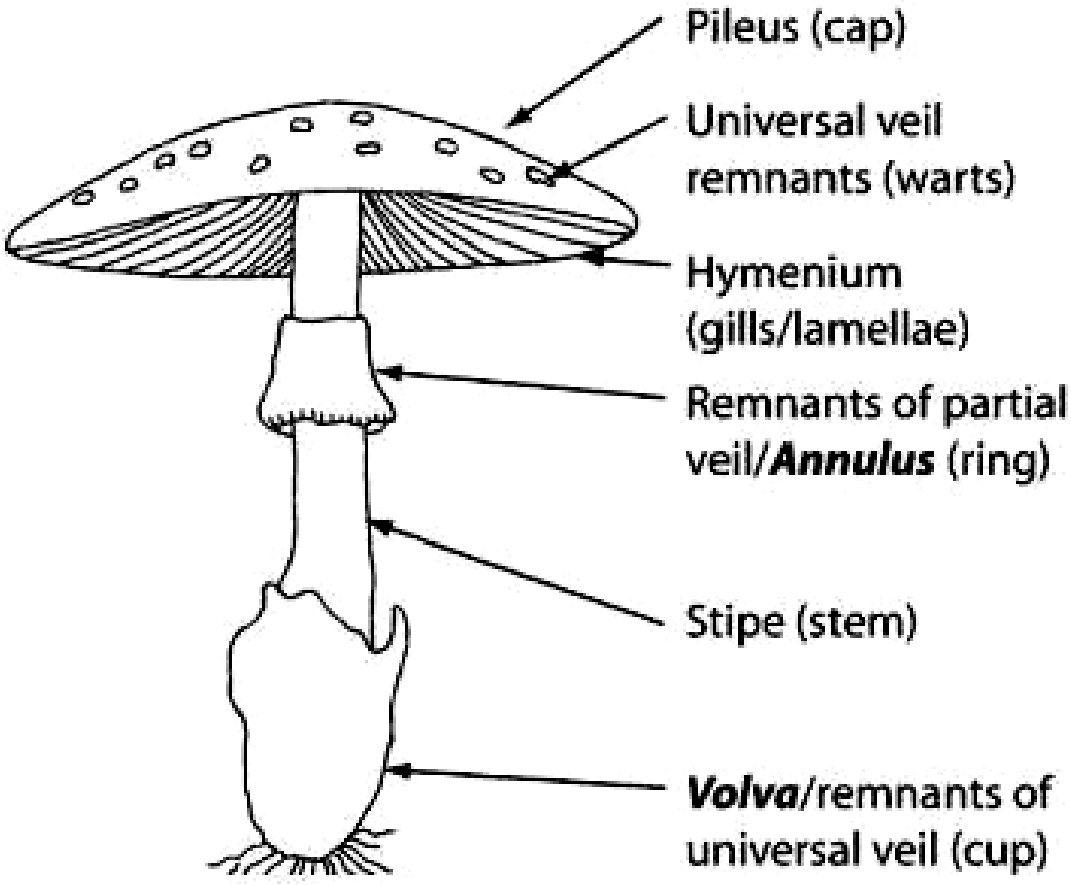

Figure-15.15: Fruiting body of *Agaricus* (https://tinyurl.com/2s3ftm84)

Stipe: The stipe consists of many intertwined hyphae that run longitudinally. In the outer region, the hyphae are densely packed, creating a pseudoparenchymatous tissue mass. In contrast, the central area, known as the medulla, features a looser arrangement of hyphae with significant intercellular spaces.

Pileus: The stipe is also developing into the cortex and medulla. It extends directly to the apex. The hyphae in the stipe area extend into the pileus and spread outward. A portion of these hyphae descends into the gills.

Internal Structure Of Gills: The gill displays a sophisticated structure, consisting of a network of interlaced hyphae that are more tightly packed and denser in this area. A cross-section of the gill reveals three distinct regions.

Trama: This area is known as the central sterile region, consisting of extensions from the hyphae of the pileus. The hyphae in this region are intricately interlaced in an irregular pattern, predominantly oriented longitudinally.

Sub Hymenium or Hypothecium: The hyphae that make up the trama produce short lateral branches that create a subhymenium layer. The cells within these branches are arranged more densely, are isodiametric, and typically contain 2 to 3 nuclei. The hypothecium is located on both sides of the trama and also serves as a sterile zone, similar to the trama itself.

Hymenium or Thecium: This layer represents the outermost fertile region of the gills, consisting of hyphae from the subhymenium layer. It features a densely arranged palisade-like structure made up of club-shaped cells known as basidia. These basidia are the terminal extensions of the same hyphae that form the trama and subhymenium, serving as the aseptate structures responsible for spore production. Each basidium generates four basidiospores at its apical end. Interspersed among the basidia are sterile, more cylindrical hyphae referred to as paraphyses or cystidia. Initially, the young basidium contains a dikaryon, but as it matures, the two nuclei merge to create a diploid nucleus. In the mature basidium, the synkaryon undergoes meiosis, resulting in four haploid nuclei—two of the positive strain and two of the negative strain. At the distal end of the basidium, four peg-like projections, known as sterigmata, develop. The tips of these sterigmata swell to form the initial basidiospores, which appear small and bead-like. At the junction between the sterigmata and the basidiospore, there are minute projections called hilar appendices, which cause the basidiospore to be slightly oblique on the sterigma. Young basidiospores are initially unpigmented, but as they mature, the spore wall takes on a pinkish-purple hue.

Germination of Basidiospore: Upon reaching maturity, a droplet of liquid forms at the hilar appendage of the basidiospore, encased by a limiting membrane. This droplet gradually enlarges, eventually reaching approximately one-fifth the size of the spore. Subsequently, the basidiospore is forcefully ejected from the sterigma, resulting in the dispersal of the four basidiospores produced by a single basidium. When these spores land on a suitable substrate, they germinate by developing a germ tube that evolves into a primary monokaryotic mycelium. Depending on the strain of the basidiospore, the mycelium may exhibit either a positive or negative strain, and following somatogamy, it transitions into secondary mycelium.

15.5 DEUTEROMYCOTINA:

General Characteristics: Deuteromycotina exhibit a diverse range of morphologies, typically forming filamentous mycelia or unicellular structures (yeast-like forms in some species). The vegetative body is primarily mycelial, composed of septate hyphae with cross-walls. Some members, such as *Candida* and *Cryptococcus*, exhibit unicellular, yeast-like growth. Hyphal modifications like chlamydospores (thick-walled survival spores) and sclerotia (hardened mycelial masses) may develop under unfavorable conditions. The cell wall is composed mainly of chitin and glucans. Some members may have melanin deposits, contributing to pathogenicity in host organisms. Many species exhibit pigmentation, with colonies ranging from white, yellow, and green to black due to melanins or carotenoids. Pigmentation is often used for taxonomic classification (e.g., *Penicillium* appears blue-green, *Aspergillus* can be black, yellow, or green). Colonies exhibit diverse textures and colors, forming fluffy, powdery, or slimy textures. Reverse colony color (observed from the underside of the Petri dish) helps in species identification. Deuteromycotina demonstrate remarkable adaptability, allowing them to thrive in varied environments.

These are primarily heterotrophic, absorbing nutrients from organic matter. Most species are saprophytic, decomposing organic material, while others are parasitic (e.g., *Colletotrichum* in plants, *Trichophyton* in humans). Some produce extracellular enzymes like cellulases, proteases, and amylases for nutrient breakdown. Many species are aerobic (e.g., *Penicillium* and *Aspergillus*). Some, like *Candida*, are facultative anaerobes, capable of fermentative metabolism. Most prefer slightly acidic conditions (pH 5-6). Mesophilic (20–40°C), with some thermophilic species surviving at higher temperatures. Many tolerate high sugar and salt concentrations, making them significant contaminants in food industries. Many deuteromycetes produce secondary metabolites, including: Mycotoxins (e.g., aflatoxins from *Aspergillus flavus*). Members of deuteromycotina also produce antibiotics (e.g., penicillin from *Penicillium notatum*).

Since Deuteromycotina lack a known sexual cycle, they primarily reproduce through asexual spores. The predominant mode of reproduction is via conidia, which develop on specialized hyphae called conidiophores. Various types of asexual spores occur in deuteromycotina i.e. Conidia – Non-motile, external spores formed on conidiophores (e.g., *Aspergillus*, *Penicillium*). Chlamydospores – Thick-walled survival spores formed under stress conditions. Blastospores – Budding spores in yeast-like forms and Arthrospores – Fragmented hyphal segments functioning as spores (e.g., *Geotrichum*). The formation of conidia is of two types 1. Thallic: Entire hyphal segments convert into spores. 2. Blastic: Conidia bud out from the parent cell before being cut off by a septum. In some species, sexual stages (telomorphs) have been discovered and linked to Ascomycota or Basidiomycota. The common sexual stages of

deuteromycotina includes Ascospores (in Ascomycota-linked species, e.g., *Neurospora*) and Basidiospores (in Basidiomycota-linked species, e.g., *Filobasidiella* teleomorph of *Cryptococcus*).

ALTERNARIA

Systematic position

Kingdom : Mycota

Division : Eumycotina

Sub division : Deuteromycotina

Class : Hyphomycetes

Order : Moniliales

Family : Dematiceae

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*.

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.

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 into multicellular component (Fig. 15.16). 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. 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*).

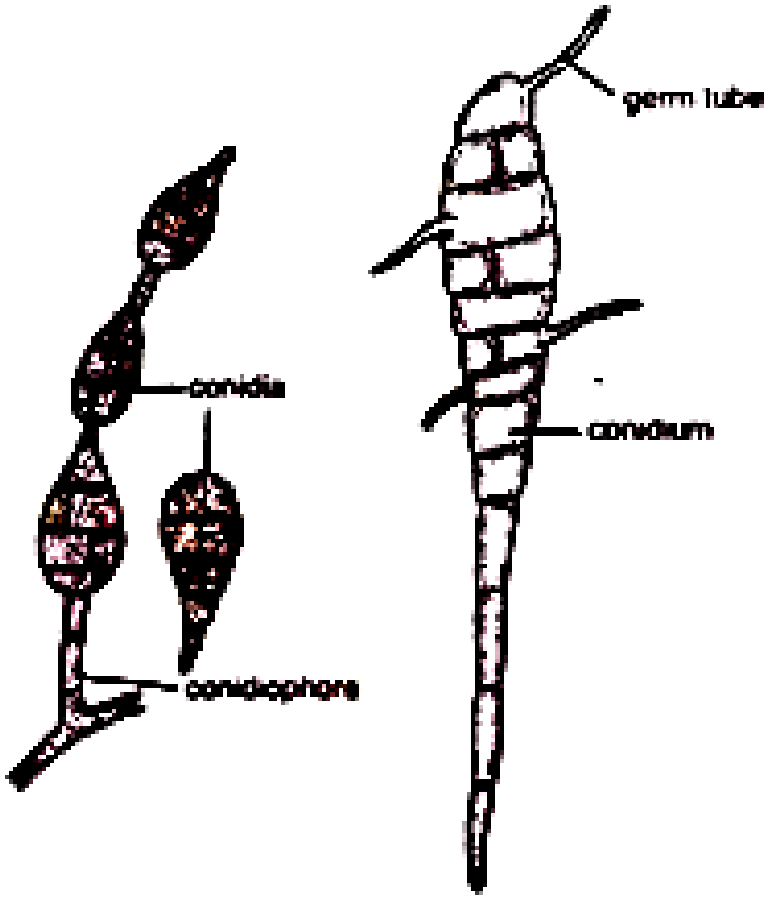

Figure-15.6: Conidia in *Alternaria* (https://tinyurl.com/y9hvpx39)

15.6 SUMMARY:

Zygomycotina is a former subdivision of fungi, now largely classified under the phylum *Mucoromycota* and *Zoopagomycota*. These fungi are primarily saprophytic, decomposing organic matter in soil, plant material, and animal waste, though some are opportunistic pathogens. They reproduce sexually through the formation of zygospores and asexually via sporangiospores. Common genera include *Rhizopus*, *Mucor*, and *Absidia*, some of which can cause infections like mucormycosis in immunocompromised individuals. Their fast-growing nature and ability to thrive in diverse environments make them ecologically significant in nutrient recycling. Ascomycotina, also known as sac fungi, is a subphylum of the

Ascomycota division, comprising the largest group of fungi. They are characterized by the production of sexual spores called ascospores, which are formed within specialized sac-like structures called asci. These fungi exhibit diverse lifestyles, including saprophytic, parasitic, and mutualistic associations, and they play crucial ecological roles in decomposition and nutrient cycling. Ascomycotina includes various species such as yeasts, molds, and filamentous fungi, with notable members like *Saccharomyces cerevisiae* (used in baking and brewing) and *Penicillium* (a source of antibiotics). Many also cause plant diseases, such as *Claviceps purpurea*, which produces toxic alkaloids. Basidiomycotina is a subphylum of fungi within the phylum Basidiomycota, known for producing spores on club-shaped structures called basidia. This group includes mushrooms, puffballs, bracket fungi, rusts, and smuts, playing crucial roles in ecosystems as decomposers, symbionts, and plant pathogens. They reproduce sexually through basidiospores and often form extensive mycelial networks. Many are important in agriculture, forestry, and biotechnology, with some species being edible, medicinal, or used in industrial applications, while others cause significant plant diseases. Deuteromycotina, also known as *Fungi Imperfecti*, is a classification of fungi that lack a known sexual reproductive stage. These fungi reproduce asexually through conidia (asexual spores) and include species from various fungal groups, particularly Ascomycota and Basidiomycota. Deuteromycotina includes important species in medicine (e.g., *Penicillium* for antibiotic production), agriculture (e.g., *Fusarium* as plant pathogens), and food production (e.g., *Aspergillus* in fermentation). Since many deuteromycetes have been reclassified into other fungal groups based on molecular studies, the term "Deuteromycotina" is now considered outdated.

15.7 TECHNICAL TERMS:

Strain, Homothallic, Heterothallic, Pathogen, Conidia, Conidiophores

15.8 SELF ASSESSMENT QUESTIONS:

1) Write an essay on morphological and reproductive features of Mucor.
2) Describe the important characteristics of Ascomycotina.
3) Explain the general characteristics of Zygomycotina.
4) Write an account on Basidiomycotina.
5) Describe the general characters and reproductive features of Aspergillus.

15.9 SUGGESTED READINGS:

1) Microbiology - Michael J. Pelezer, J.R., E.C.S. Chan, Noel R. Krieg (1993) - Indian Edition(5thEdition), Mc Graw Hill Education (India) Private Limited, 444/1, Sri Ekambara Naicker Industrial Estate, Alapakkam, Povur, Chennai 6oo116, Tamil Nadu, India.

2) Botany for Degree Students Part II FUNGI - B. R. Vashishta, (1990), S. Chand & Company LTD. Ram Nagar, New Delhi 110055.

3) Botany for Degree Students - B. P. Pandey, (2015). As per UGC Model Curriculum.

Dr. Y.R.K.V. Tirupati Rao

LESSON - 16

ECTO AND ENDOMYCORHIZAL ASSOCIATIONS

16.0 OBJECTIVE OF THE LESSON:

By studying this lesson students are able to know the various types of fungal associations with plants species and their role in plant growth and development along with nutrient uptake.

STRUCTURE OF THE LESSON:

16.1 INTRODUCTION:

Mycorrhizae are mutualistic symbiotic associations between plant roots and fungi, essential for plant nutrient acquisition and ecosystem stability. The word mycorrhiza is derived from classical Greek word for "mushroom" and "root". In a mycorrhizal association, the underground mycelium are in contact with plant roots, but without causing any harm to the plant. Fossil evidence and DNA sequence analysis suggest that this mutualism appeared 400-460 million years ago. Vesicular arbuscular mycorrhizal fungi belong to the class Zygomycetes, order Endogonales and family Endogonaceae. Fungi may either form extraradical mycelium which grows inside the soil for nourishment or form intraradical mycelium that grow in between and inside the parenchyma cells of plant roots. It also forms structures like vesicles and arbuscules. Mycorrhizal fungi can colonize plants from the sources of inoculums, spores, colonized root fragments, and vegetative hyphae. These inoculants are called “propagules”. Mycorrhizal fungi are responsible in improving growth of host plant species due to increased nutrient uptake, production of growth promoting

substances, tolerance to drought, salinity and synergistic interactions with other beneficial microorganisms. The soil conditions prevalent in sustainable agriculture are likely to be more favourable to mycorhizal fungi than are those under conventional agriculture. Vitadini (1842) was the first to recognise the possible beneficial role of fungal mycelia which mantle the root of higher plants, and this association is named as mycorrhiza (pl. mycorrhizae) i.e., the fungal root, by Frank in 1885.

The AM fungi are widely distributed in natural and agricultural environments and have been found associated with more than 80% of land plants, liverworts, ferns, woody gymnosperms and angiosperms and grasses. Mycorrhizal association is present in 83% Dicots, 79% Monocots, 100% Gymnosperms. This association is not present in families of Amaranthaceae, Brasicaceae, Caryophyllaceae, Chenopodiaceae, Commelinaceae, Lecythidaceae, Portulaceae, Proteaceae, Restionaceae, Sapotaceae, Zygophyllaceae.

Mycorrhizas were traditionally classified into two types ectomycorrhizae (ECM) and endomycorrhizae based on the location of the fungal hyphae in relation to the root tissues of the plant; *ecto* means outside the root, *endo* means inside. The arbuscular mycorrhizae (AM) are the most common type of endomycorrhizae. While ECM predominantly associates with trees in temperate forests, AM occurs in a wide range of plants, including agricultural crops. Peterson and Farquhar (1994) classified the mycorrhizae into three distinct types i.e. (1) Ectomycorrhizae, (2) Endomycorhizae (3) Ectendomycorhizae. The endomycorhizae again devided into a) Vesicular-arbuscularmycorrhizae, (b) Ericoid mycorrhizae, (c) Centianoid mycorrhizae, (d) Orchidoid mycorrhizae, and (e) Monotropoid mycorrhizae.

16.2 ECTOMYCORRHIZAL (ECM) ASSOCIATIONS:

Ectomycorrhizas are the most advanced symbiotic association between higher plants and fungi, involving about 3% of seed plants including the majority of forest trees. It is estimated that over 5,000 fungi species are capable of forming ectomycorrhizal symbiosis. Ectomycorrhizal fungi are mainly *Basidiomycota* and include common woodland mushrooms, such as *Amanita* spp., *Boletus* spp., *Tricholoma* spp. Ectomycorrhizas can be highly specific Eg: *Boletus elegans* with larch and non-specific Eg: *Amanita muscaria* with 20 or more tree species. Ectomycorrhizal fungi depend on the plant host for carbon sources, most being uncompetitive as saprotrophs. With few exceptions (*Tricholoma fumosum* being one), the fungi are unable to utilise cellulose and lignin; but the fungus provides greatly enhanced mineral ion uptake for the plant and the fungus is able to capture nutrients, particularly phosphate and ammonium ions, which the root cannot access. Host plants grow poorly when they lack ectomycorrhizas. This ectomycorrhizal group is reasonably homogenous, but a subgroup, ectendomycorrhizas, has been appended.

Structural Organization:

In this association the plant root system is completely surrounded by a sheath of fungal tissue which can be more than 100 μm thick, though it is usually up to 50 μm thick. Structurally ectomycorhiza devided in to A) Mantle: A thick fungal sheath covering the root surface. B) Hartig Net: A network of hyphae that infiltrates between root cortical cells, enhancing nutrient exchange. C) Extraradical Hyphae: Fungal filaments extending into the soil to acquire nutrients (Fig.16.1).

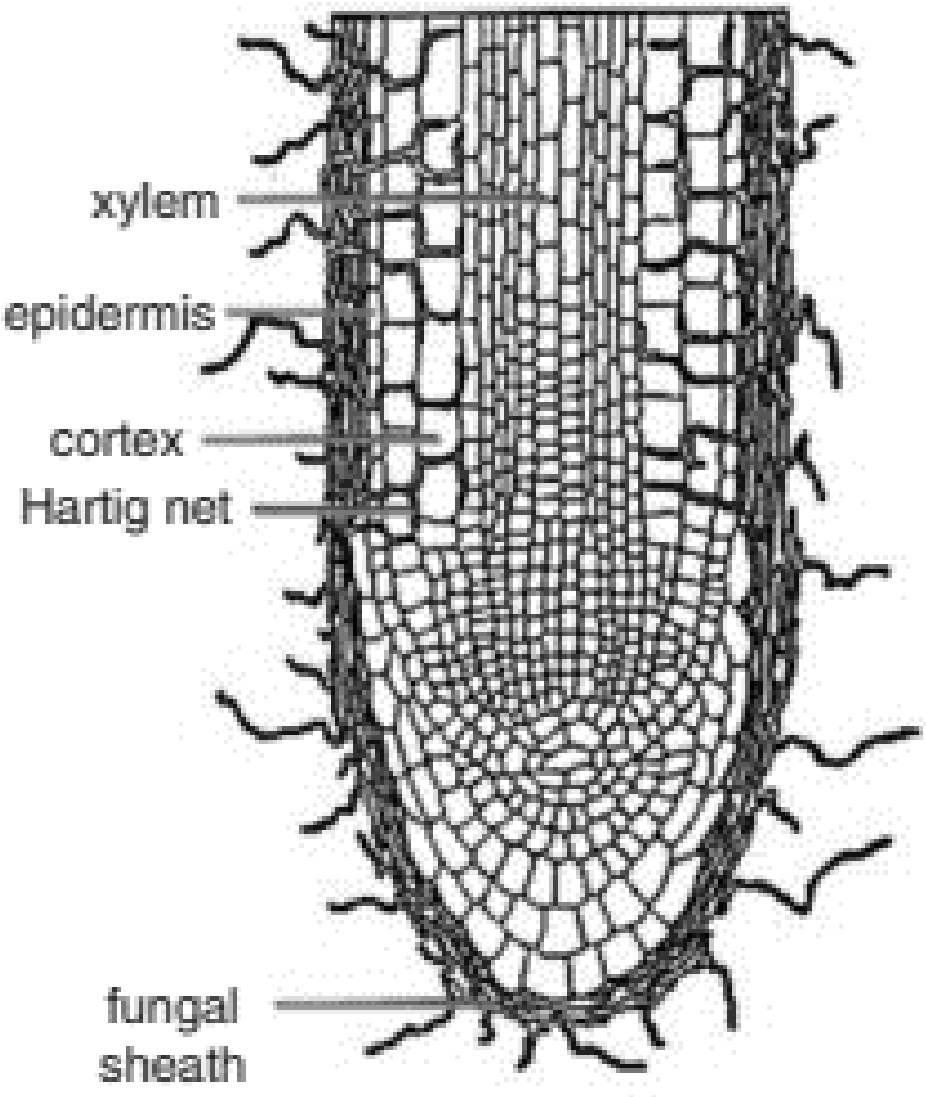

Figure-16.1: Ectomycorhizal association in plants (https://tinyurl.com/5e6ucen5)

Nutrient Exchange Mechanism:

ECM fungi play a key role in nitrogen and phosphorus uptake. They release extracellular enzymes to break down organic matter, making nutrients available for plant uptake. In return, the fungi receive carbohydrates synthesized by the plant via photosynthesis.

Diversity and Host Range:

These symbionts are found in four divisions: Basidiomycota, Ascomycota, Zygomycota and Deuteromycota. ECM fungi associate primarily with trees, including members of Pinaceae, Fagaceae, and Betulaceae families. Notable ECM genera include *Amanita, Boletus, Lactarius,* and *Tuber*.

Ecological Significance:

Ectomycorhiza enhance plant nutrient uptake and drought resistance. Protect against root pathogens. Improve soil structure and carbon sequestration and Facilitate afforestation and reforestation efforts.

16.3 ENDOMYCORRHIZAL (AM) ASSOCIATIONS:

Endomycorrhizae, particularly arbuscular mycorrhizae (AM), are symbiotic associations where fungal hyphae penetrate the root cortical cells. AM fungi belong to the Glomeromycota phylum and are found in over 80% of vascular plant species.

Structural Organization:

Endomycorhiza structurally divided into three parts i) **Arbuscules:** Intracellular tree-like structures facilitating nutrient exchange. ii) **Vesicles:** Lipid storage structures within root cells. iii) **Extraradical Mycelium:** Hyphae extending into the soil to capture nutrients (Fig. 16.2).

Nutrient Exchange Mechanism:

AM fungi improve phosphorus, nitrogen, and micronutrient uptake through extensive mycelial networks. They also contribute to soil aggregation and plant hormone regulation.

Types of Endomycorhizal Associations:

Arbuscular (AM) Endomycorrhizas:

These are the commonest mycorrhizas, and were the first to evolve; the fungi are members of the Glomeromycotina, they are obligate biotrophs, and they are associated with roots of about 80% of plant species, including many crop plants. The AM association is endotrophic, and has previously been referred to as Vesicular-Arbuscular Mycorrhiza (VAM). The AM forms two structures 1. Arbuscules: Highly branched, tree-like structures inside cortical cells for nutrient exchange. 2. Vesicles: Spherical lipid storage structures (not always present). These are the major facilitator of phosphorus uptake with widespread and economically significant type.

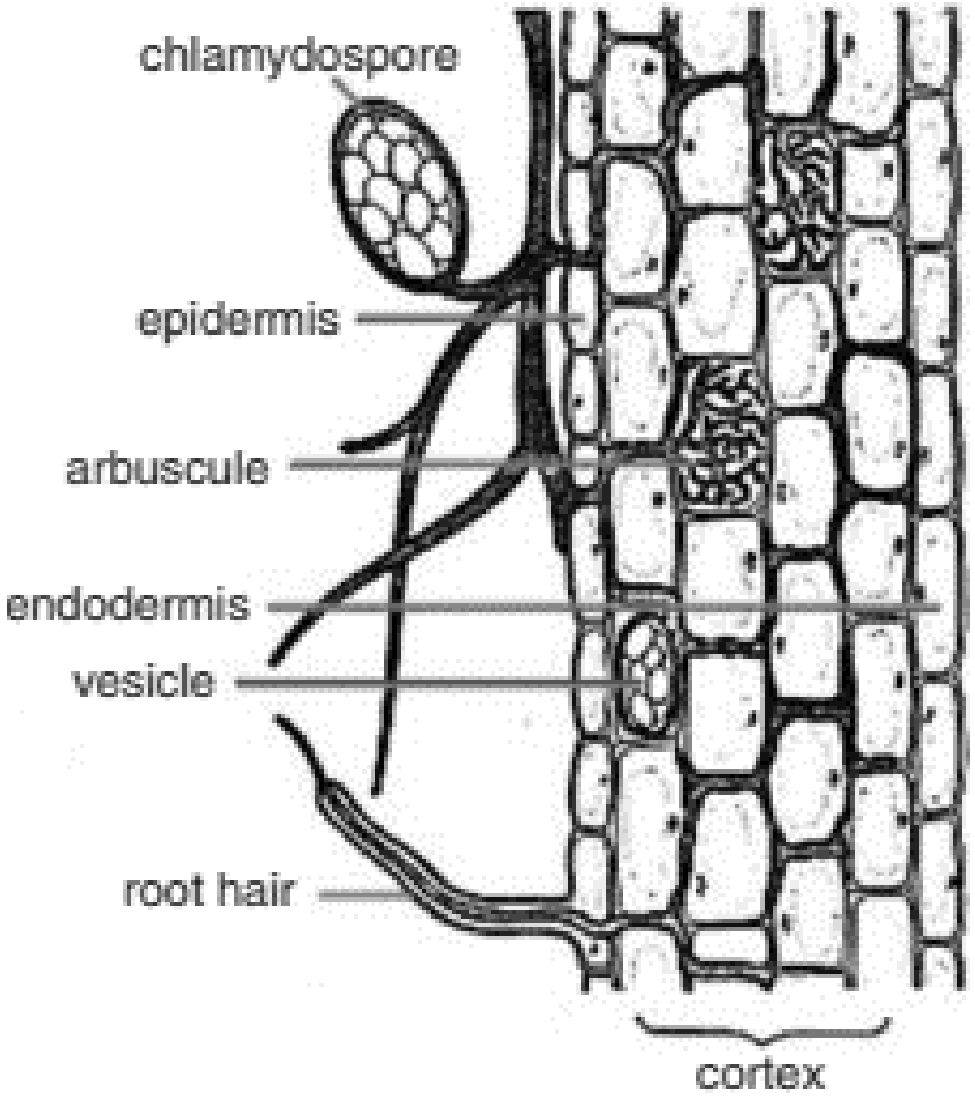

Figure-16.2: Arbuscular Endomycorhiza (https://tinyurl.com/5e6ucen5)

Ericoid Endomycorrhizas:

These are mycorrhizas of *Erica* (heather), *Calluna* (ling) and *Vaccinium* (bilberry), that is, plants that endure moorlands and similar challenging environments. Fungi are members of the Ascomycota (an example is *Hymenos cyphusericae*. The fungi are slow-growing, septate and mostly sterile. They are mostly culturable. During this association the rootlets of the plants are covered by very sparse, loose, dark, septate hyphae that penetrate the cortex forming intercellular coils. After 3-4 weeks the coils degenerate like arbuscles of vesicular-asbuscularmycorrhiza (VAM). Most of the members of Ericaceae grow in acid soil with less amount of P and N nutrition. The fungus gets the photosynthate from the host and improves the mineral uptake and nutrition of the host, especially P and N. Many mycotrophs of Ericaceae show high resistance to metals like Zn and Cu. The mycorrhizal plants also show high tolerance to these metals, which is totally absent in non-infected plants. The fungus

digests polypeptides saprotrophically and passes absorbed nitrogen to the plant host; in extremely harsh conditions the mycorrhiza may even provide the host with carbon sources by metabolising polysaccharides and proteins for their carbon content. Enhances nitrogen and phosphorus uptake, particularly from organic matter. Eg: Ericaceae sps. (Fig. 16.3).

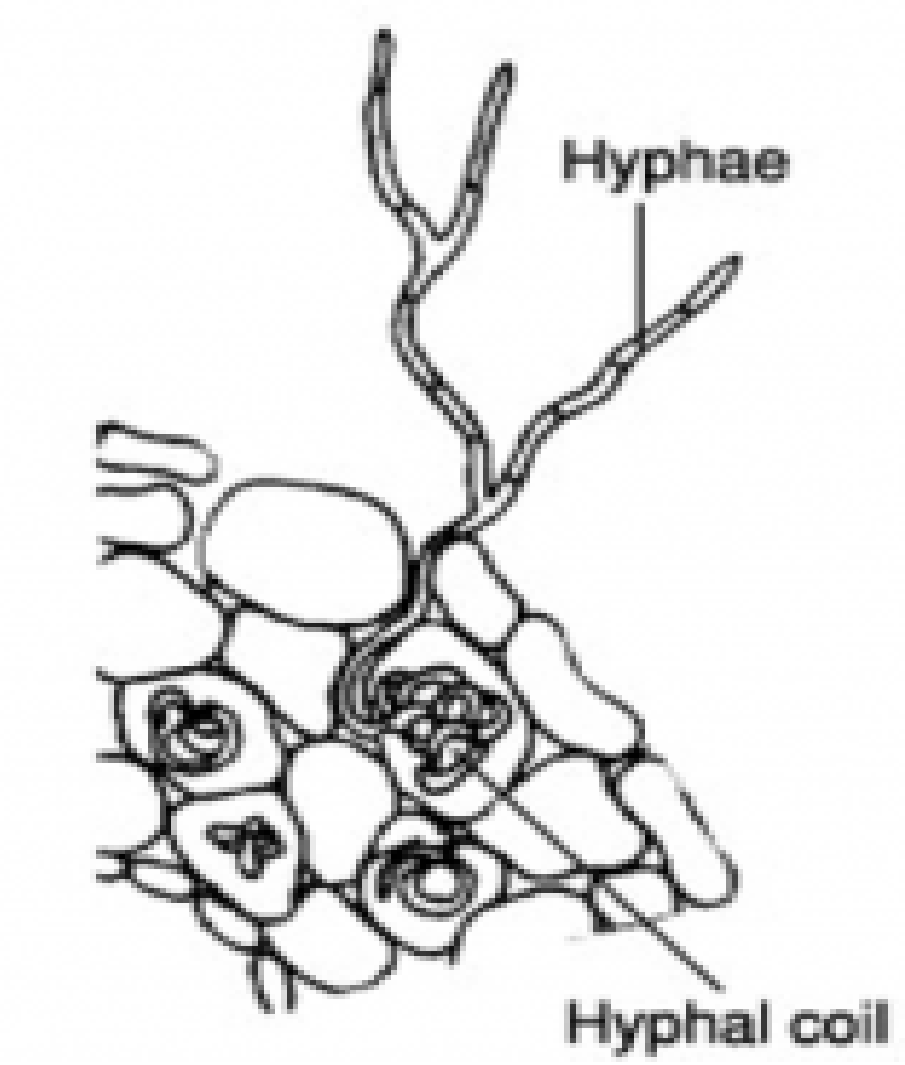

Figure-16.3: Ericoid mycorhiza (https://tinyurl.com/2mvsmf7v)

Centenaroid Mycorrhiza:

These are poorly characterized, but often thought to involve ascomycetous fungi, though basidiomycetes may also play a role. Host plants: Found in gentian relatives (*Centaurium*, *Gentiana*, etc.), often in nutrient-poor alpine or calcareous soils. Intracellular colonization similar to ericoid or orchid mycorrhiza. May involve coiled hyphae or peloton-like structures. These mycorhizae helps plants acquire phosphorus, amino acids, and possibly organic nitrogen in marginal environments. They have the characters of both ericoid and orchid types. These generally habituated to dry, calcareous soils, alpine meadows. Plants with Centenaroid mycorrhiza often show low growth rates with high root-to-shoot ratios.

Orchidaceous Endomycorrhizas:

Orchids produce millions of tiny seeds per capsule, weighing about 0.3-14µg. The embryo of seeds contains 10-100 cells and there is virtually no storage of food. The embryo is encircled in a thin-walled net-like testa that helps in their dispersal. Thus, majority of seeds are unable to germinate without exogenous supply of carbohydrates. Therefore, mycorrhizal association is obligatory for the seeds to germinate. The fungus provides the nutrition to the seeds. Initially the fungus enters the embryo and colonises, being restricted to the cortical cells and provides the nutrition (Fig. 16.4). For non-green orchids, this is obligatory throughout their lives. Apparently, it is a case of parasitism by orchids on the mycorrhizal fungi. Fungi like *Rhizoctonia* (Basidiomycotina), are recognised by hyphal characteristics. *Corticium*, *Ceratobasidium* etc., of Aphylloporales are associated in this type of mycorrhiza

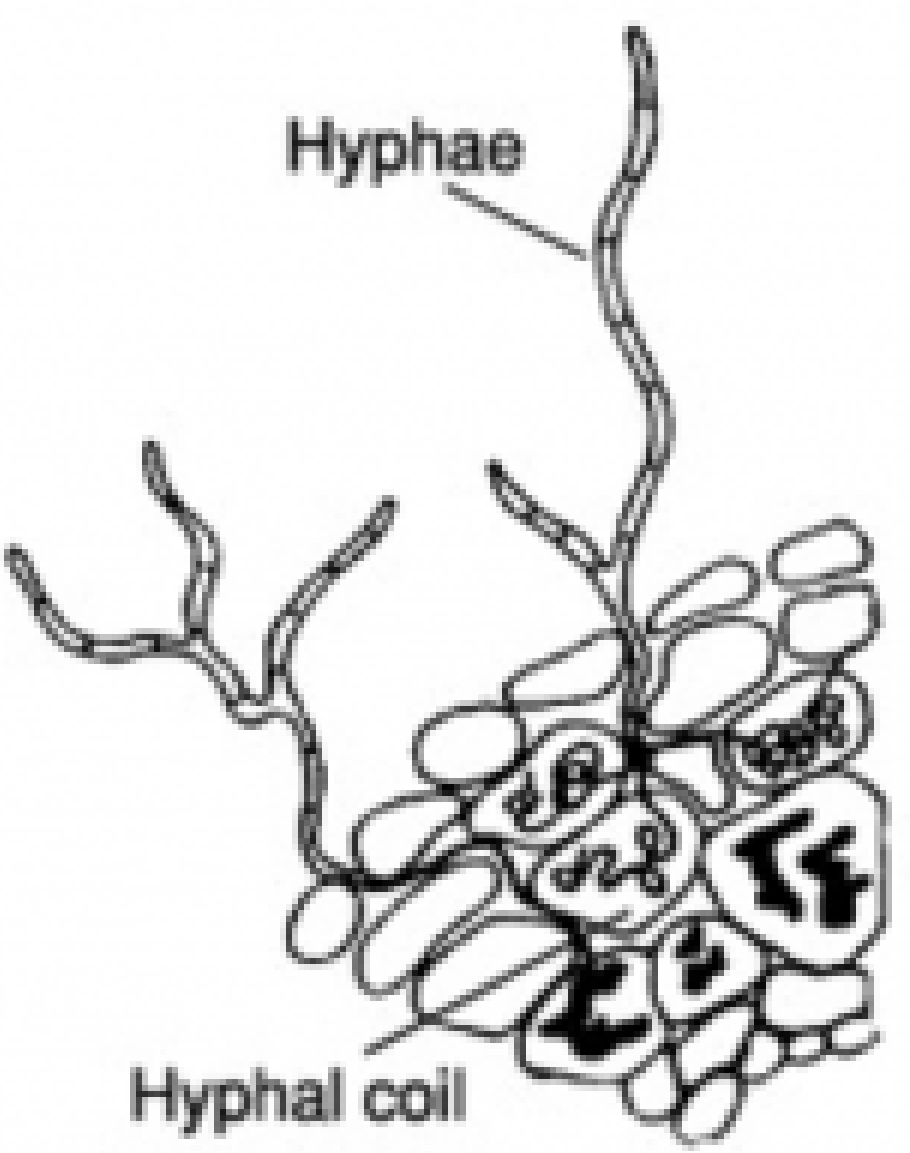

Figure-16.4: Orchid Mycorhiza (https://tinyurl.com/2mvsmf7v)

Monotropoidendo Mycorrhizas:

These association are formed by the achlorophyllous plants of the Montropaceae. *Monotropa hypopitys* is a non-green saprophytic herb. It has short fleshy roots that are invested with a hyphal sheath and often forming hartig net in the cortical zone. Due to absence of chlorophyll, they are unable to synthesise and supply carbohydrate to the fungus. *Boletus* is a mycorrhizal fungus associated with roots of both pine and *Monotropa*.

16.4 ECTENDOMYCORRHIZAL ASSOCIATIONS:

These mycorhiza also named as Arbutoid mycorhiza. These mycorhiza exhibit characteristics of both ectomycorrhizas and endomycorrhizas. Ectendomycorrhizas are essentially restricted to the plant genera *Pinus* (pine), *Picea* (spruce) and, to a lesser extent, *Larix* (Larch). Ectendomycorrhizas have the same characteristics as endomycorrhizas but also show extensive intracellular penetration of the fungal hyphae into living cells of the host root (Fig. 16.5).

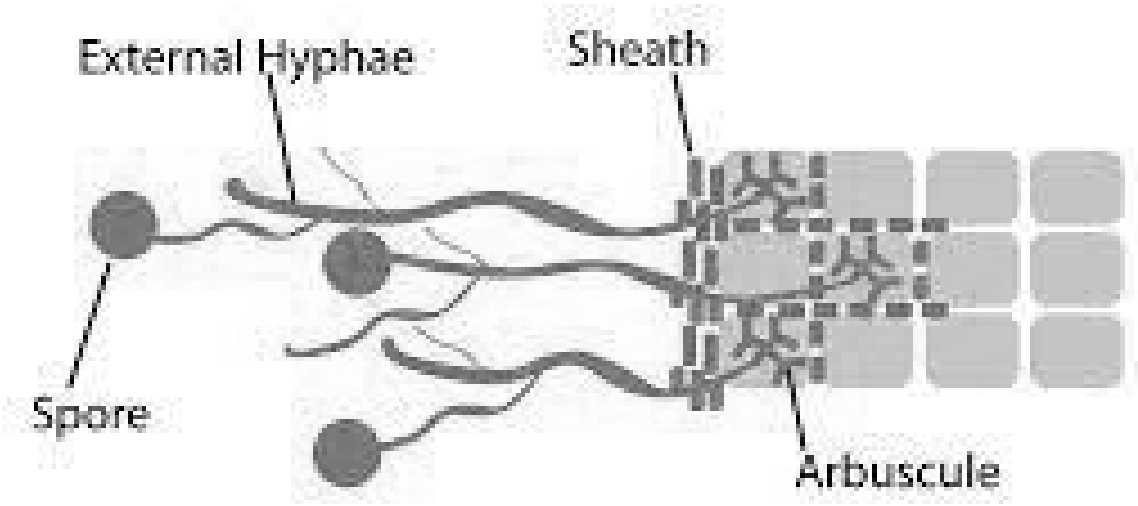

Figure-16.5: Ectendomycorhiza (https://tinyurl.com/3vjpx9td)

Apart from the above mycorhizal associations members of family Gentianaceae showed following type of mycorhizal association.

Gentianoid Mycorrhizae:

Seedlings of some members of Gentianaceae (*Biackstonia perfoliata, Gentianella amarella,* etc.) get infected within 2 weeks of germination. In root, the cortical cells become full of irregular coils of aseptate hyphae. With time the hyphae become lysed. Vesicles are occasionally seen attached to these coils.

Diversity and Host Range:

AM fungi colonize a vast array of plants, from crops to forest trees. Genera such as *Glomus, Rhizophagus,* and *Acaulospora* dominate AM fungal communities.

16.5 MOLECULAR MECHANISMS OF MYCORRHIZAL SYMBIOSIS:

AM fungi are obligate biotrophs, solely dependent on the host plants for their survival. The symbiotic mechanism comprise many steps (Fig. 16.6). The first step is the search for the host root which is an important step in fungal-root-colonization process. The second step is penetration of fungi into the host root for colonization and final establishment of mycorrhizal symbiosis. These steps are described in detail as follows.

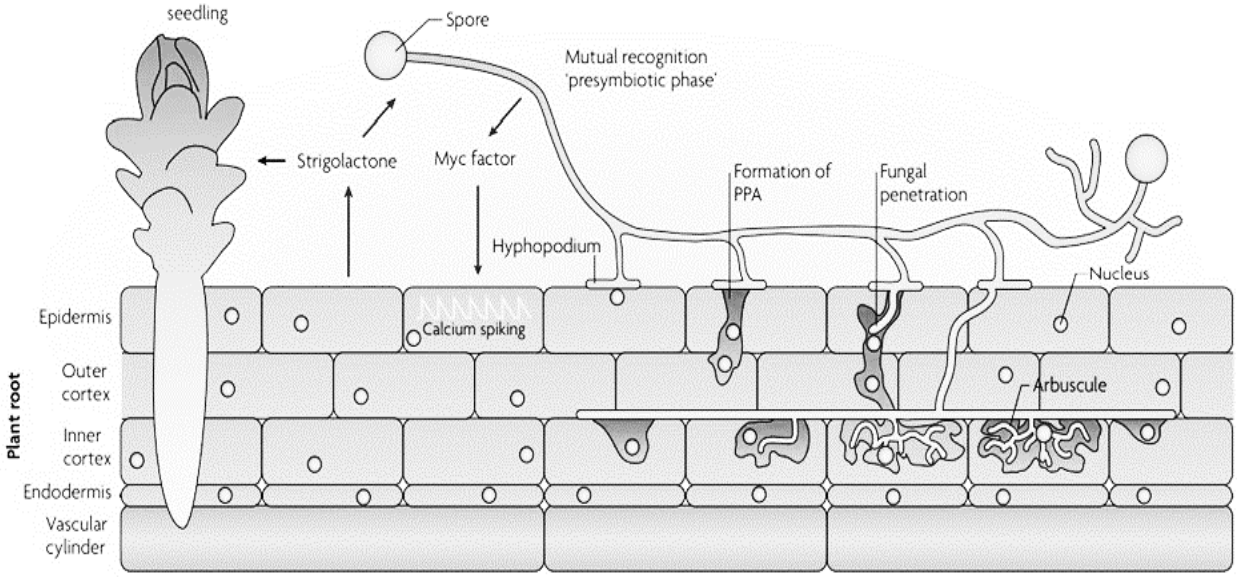

Figure-16.6: Symbiotic mechanism of fungi with root (https://tinyurl.com/2w656vdf)

Initial Recognition of Host Plant Roots by Fungi:

Some bioactive molecules like strigolactones secreted by the roots help fungi identify their host plants. Strigolactones also stimulate AM fungal growth and its branching. The fungi reciprocate to this signal by secreting a set of hypothetical factors known as mycorrhizal factors (Myc). These factors also play a major role in communication between AM fungi and nitrogen-fixing bacteria. The AM interactions are established further with the induction of seven genes (*SYM* genes) (Fig. 16.7). When the host Myc Factor Receptor(s) (MFR) perceive Myc signals, cytosolic calcium secretion is induced in root cells. A second membrane protein (SYMPK) is activated, which codes for a receptor-like kinase with the potential to recognise AM fungal signals directly or indirectly. SYMPK transduce these signals from the cytoplasm to the nucleus by phosphorylating an unknown substrate through its kinase domain. The

localization of all downstream elements present in the cytoplasm, activates rapid signal transduction into the nucleus. Thus, a repeated oscillation of Ca^{2+} concentration occurs in the nucleus and cytoplasm, through the alternate activity of Ca^{2+} channels and transporters. These calcium oscillations are decoded by a calmodulin-dependent protein kinase (CCaMK). CCaMK phosphorylates the product of one of the *SYM* genes (CYCLOPS). This eventually leads to the regulation of other genes and finally root colonization.

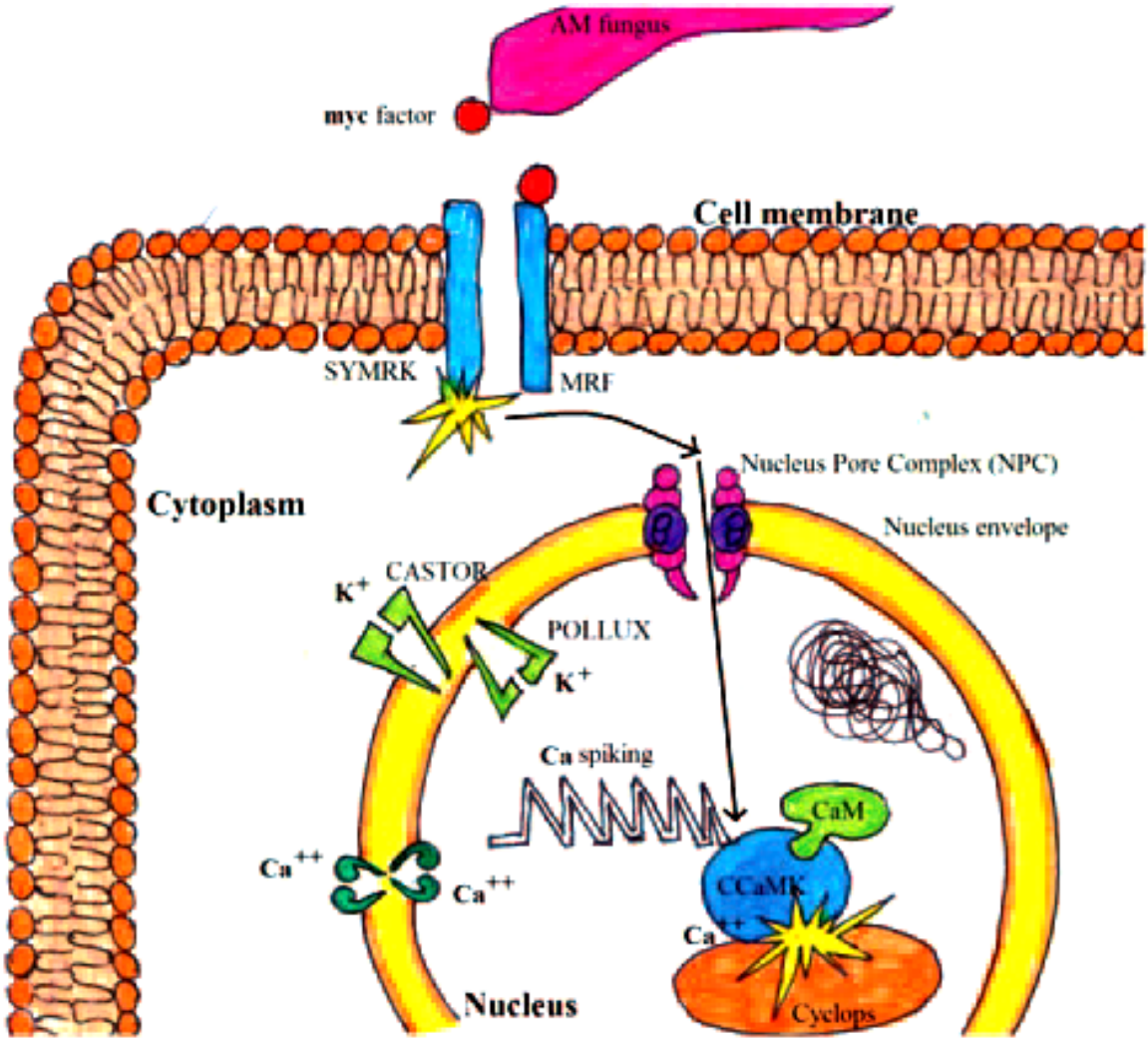

Figure-16.7: Molecular mechanism of mycorhizal association (https://tinyurl.com/3bcdjm74)

Penetration and Establishment of Mycorrhizae:

After chemical acquaintance, the fungal hyphae. Hyphae are long thread-like fungal filaments and mycelium is the intertwined mass of hyphae and the host root interacts with each other, and the hyphae gradually start its propagation into the host root by forming the 'hyphopodium'. Many genes get activated subsequently, owing to hyphopodium formation. This is the primary step of colonization. These are special type of hyphal branch composed of

lobed cells with which the fungi attach to the cell wall of the plant partner. Then a pre-penetration apparatus (PPA), which is indispensable for fungal penetration is developed. This structure allows the fungi to grow inside the plant without breaking the integrity of the cells. The final step of this symbiotic process is the formation of arbuscules. These arbuscules accommodate the fungi into the host cell 8Small tree-like structures. The arbuscular cells function as machines for nutrient transport and acquisition. Numerous genes and proteins are involved in the process of nutrient uptake which finally help in the accomplishment of symbiosis. The molecular mechanism adopted by EM fungi is almost similar but not identical to that of AM fungi. Further research is awaited to unravel the details of the processes underlying EM fungal associations.

Mutual Beneficial Process:

Plant-derived carbon is transported to the fungus through the two membranes at the symbiotic interface (Fig. 16.8). This carbon is first released into the peri-arbuscular space (PAS), probably in the form of sucrose, then cleaved into hexoses and taken up by AM fungi through transport across the fungal plasma membrane. Within the fungal cytoplasm, hexoses are converted into glycogen granules and triacylglycerol (TAG) lipid droplets, which serve as suitable units for long-distance transport through the hyphal network. Nutrients that are acquired by the fungus from the soil and are delivered to the plant cell have to cross the fungal plasma membrane, be transported long distance to the intra-radical hyphae (IRH), including the arbuscules, and subsequently reach the plant cytoplasm across the fungal plasma membrane and the plant periarbuscular membrane (PAM). Phosphate is imported by fungal phosphate transporters that are present in extraradical hyphae (ERH). Phosphate is transported towards the root and IRH in the form of polyphosphate granules, which reside in membrane-enclosed vesicles.

The negative charge of these granules makes them likely transport vehicles for metal ions and arginine. Phosphate is released from polyphosphate granules within IRH. Nitrogen is taken up by ammonium, nitrate or amino-acid transporters in ERH. In AM fungal hyphae, nitrogen is mainly transported as arginine. Within the IRH, nitrogen is released from arginine as urea and either transported to the plant directly or after cleavage to ammonium.

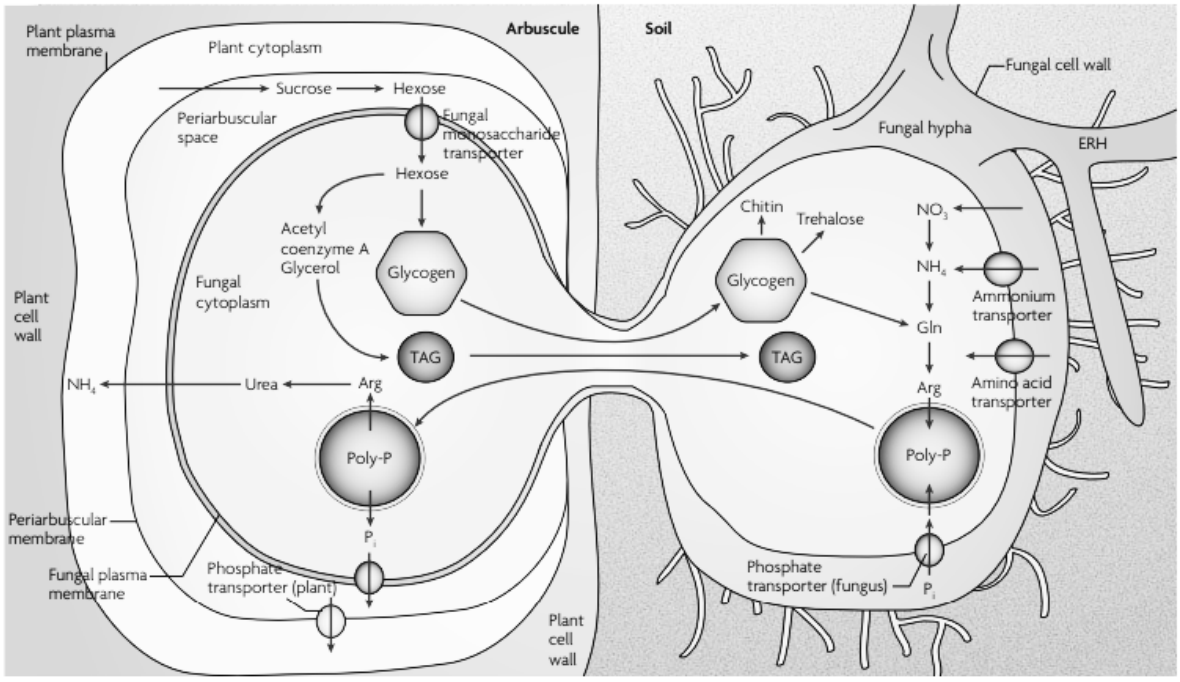

Figure-16.8: Mutual beneficial process of fungi and plant (https://tinyurl.com/2w656vdf)

Threats to Mycorrhizal Associations:

Mycorrhizae are major components of soil ecosystems and thus are essential for the survival of plant species. They also act as indicators of plant health and soil toxicity. Agricultural practices largely impact the activity of mycorrhiza. Soil tillage breaks up AM hyphal networks leading to a significant reduction in colonization of roots and phosphorus absorption from the soil. The classical genetic breeding aproach to improve crop plants in terms of quality or quantity may expedite the loss of AM fungal diversity. Indiscriminate use of fertilizers and pesticides can inhibit the formation and growth of both endo-and ectomycorrhiza. Land and air pollution, mining, deforestation, etc., are some of the non-agricultural activities that have severe impacts on mycorrhizal survivability. The most common industrial air pollutants (SO_2, NO_2, O_3, etc.) emitted into the atmosphere cause severe loss of viability of mycorrhizal propagules resulting in a significant reduction of mycorrhizal colonization in roots. Ozone (O_3) pose an indirect threat to mycorrhizal activity by degrading the photosynthetic pigments which lead to lower photosynthesis rate and hence lower the carbon sources channelled to the fungal partner. Decomposition of excessive ammonia present in the atmosphere cause physiological alterations such as cellular acidosis in plants and mycorrhizal species. Terrestrial pollutants such as polyaromatic hydrocarbons also have an adverse impact on mycorrhizal species.

Ecological and Agricultural Significance:

Enhance plant growth and resistance to environmental stress. Improve soil health and fertility. Reduce dependency on chemical fertilizers and promote sustainable agriculture and land restoration.

Biotechnological and Environmental Applications:

a) **Reforestation and Afforestation:** Both ECM and AM fungi play a crucial role in ecological restoration by enhancing seedling establishment and survival in degraded lands.
b) **Sustainable Agriculture:** AM fungi are increasingly utilized in biofertilizers to reduce reliance on chemical inputs and improve crop resilience.
c) **Climate Change Mitigation:** Mycorrhizal fungi contribute to carbon sequestration by enhancing soil organic matter formation.
d) **Bioremediation:** Mycorrhizal fungi aid in detoxifying contaminated soils by stabilizing heavy metals and breaking down pollutants.

16.6 SUMMARY:

Mycorrhiza is a symbiotic association between certain fungi and the roots of most plants, playing a vital role in plant health and ecosystem functioning. In this relationship, the fungus colonizes the plant's root system, enhancing the plant’s ability to absorb water and essential nutrients such as phosphorus and nitrogen from the soil. In return, the plant provides the fungus with carbohydrates produced through photosynthesis. There are two main types of mycorrhiza: ectomycorrhiza, which forms a sheath around the roots and is common in trees, and endomycorrhiza (also called arbuscular mycorrhiza), which penetrates root cells and is found in most crops and herbaceous plants. Mycorrhizal associations improve plant growth, increase drought and disease resistance, and contribute to better soil structure. Because of these benefits, they are increasingly recognized as important for sustainable agriculture and healthy ecosystems.

Mycorrhizal associations are mutualistic interactions between plant roots and fungi, primarily involving arbuscular mycorrhizal (AM) fungi. At the molecular level, this symbiosis begins with the exchange of signaling molecules: plants secrete strigolactones that activate fungal growth, while fungi release Myc factors that are perceived by plant receptors, triggering the common symbiosis signaling pathway (CSSP). This pathway involves key genes such as DMI1, DMI2, and CCaMK, leading to the formation of arbuscules specialized structures within root cells that facilitate nutrient exchange. Plants benefit from enhanced uptake of nutrients like phosphorus and nitrogen, while fungi receive carbohydrates and lipids. The symbiosis is tightly regulated through specific gene expression and transporters to maintain a balanced and beneficial relationship.

16.7 TECHNICAL TERMS:

Mycorrhiza, Symbiosis, Hearting Net, Nutrients, Hyphae, Ectomycorrhiza, Endomycorrhiza, Ectendomycorrhiza, Arbuscules, Vesicles.

16.8 SELF ASSESSMENT QUESTIONS

1) Give a detailed notes on mycorhirzal associations.
2) Describe the various types of endomycorrhiza.
3) Explain the molecular mechanism of mycorhizal associations.
4) Describe the role of mycorhiza in plant nutrition.

16.9 SUGGESTED READINGS:

1) Microbiology - Michael J. Pelezer, J.R., E.C.S. Chan, Noel R. Krieg (1993) - Indian Edition (5th Edition), Mc Graw Hill Education (India) Private Limited, 444/1, Sri Ekambara Naicker Industrial Estate, Alapakkam, Povur, Chennai 6oo116, Tamil Nadu, India.
2) Botany for Degree Students Part II FUNGI - B. R. Vashishta, (1990), S. Chand & Company LTD. Ram Nagar, New Delhi 110055.
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Dr. K. Babu

LESSON - 17

EDIBLE AND POISONOUS MUSHROOMS AND MUSHROOM CULTIVATION

17.0 OBJECTIVE OF THE LESSON:

Students are able to identify the edible and non-edible mushrooms and know the process of mushroom cultivation.

STRUCTURE OF THE LESSON:

17.1 INTRODUCTION:

Mushrooms represent a fascinating group of fungi with over 14,000 known species globally, of which approximately 700 are edible, and 250 are dangerously toxic. Their ecological roles range from decomposers to symbiotic partners with trees (mycorrhizae), making them vital to forest ecosystems. However, while some mushrooms offer immense nutritional and medicinal benefits, others pose significant risks due to their toxic properties. Mushrooms belong to the fungi kingdom and play crucial roles in ecological systems. They decompose organic matter, recycle nutrients, and contribute to soil health. Edible mushrooms, such as the common button mushroom (*Agaricus bisporus*), provide valuable nutrients like proteins, vitamins, minerals, and antioxidants. Poisonous mushrooms, on the other hand, contain toxic compounds that can lead to severe illness or even death.

17.2 EDIBLE MUSHROOMS:

Habitat and Growth Patterns:

Edible Morels (*Morchella* spp.) grow in spring under hardwoods, with hollow stems and pitted caps. Their toxic counterpart, false morels (*Gyromitra* spp.), have wrinkled,

brain-like caps and cottony interiors. Edible mushrooms are cultivated and harvested worldwide for their culinary and medicinal properties. Some of the most well-known edible mushrooms include (Fig.17.9):

1) **Button Mushroom (*Agaricus bisporus*):** The most commonly consumed mushroom. Available in white and brown forms. Rich in protein, B vitamins, and antioxidants.
2) **Shiitake (*Lentinula edodes*):** Popular in Asian cuisine. Contains compounds that support immune function and cardiovascular health.
3) **Portobello (*Agaricus bisporus*):** A mature version of the button mushroom. Used in grilling and stuffing due to its meaty texture.
4) **Oyster Mushroom (*Pleurotus ostreatus*):** Grows in clusters on trees or logs. Contains compounds with potential cholesterol-lowering effects.
5) **Chanterelle (*Cantharellus cibarius*):** Recognized for its golden color and fruity aroma. A favorite in gourmet dishes.
6) **Morel (*Morchella spp.*):** Highly sought after for its unique texture and flavor. Requires thorough cooking to remove mild toxins.
7) **Lion's Mane (*Hericium erinaceus*):** Known for its potential cognitive benefits. Used in both culinary and medicinal applications.

Nutritional Benefits: Mushrooms are low in calories but rich in protein, fiber, vitamins (B, D), and minerals (selenium, potassium). They contain antioxidants like ergothioneine, linked to reduced inflammation and chronic disease prevention.

17.3 NON-EDIBLE MUSHROOMS:

Habitat and Growth Patterns:

Poisonous Destroying Angels (*Amanita virosa*) thrive in mixed woodlands, often near oaks, and are pure white with a hidden volva. Many toxic mushrooms closely resemble edible varieties, making identification crucial. Some of the most dangerous poisonous mushrooms include (Fig.17.9):

1) **Death Cap (*Amanita phalloides*):** Responsible for most mushroom poisoning fatalities worldwide. Contains amatoxins that cause liver and kidney failure.
2) **Destroying Angel (*Amanita virosa, Amanita bisporigera*):** Resembles edible white mushrooms but is highly toxic. Symptoms appear hours after ingestion and lead to organ failure.
3) **Panther Cap (*Amanita pantherina*):** Contains potent neurotoxins. Causes hallucinations, seizures, and coma.

4) **Fly Agaric (*Amanita muscaria*):** Known for its bright red cap with white spots. Contains psychoactive compounds that can cause nausea, confusion, and delirium.
5) **False Morel (*Gyromitra spp.*):** Resembles true morels but contains gyromitrin, a toxic compound. Can cause severe neurological and liver damage.
6) **Deadly Webcap (*Cortinarius rubellus*):** Causes irreversible kidney damage. Often mistaken for edible mushrooms.
7) **Jack O'Lantern (*Omphalotus olearius*):** Resembles chanterelles but glows in the dark. Causes severe gastrointestinal distress.

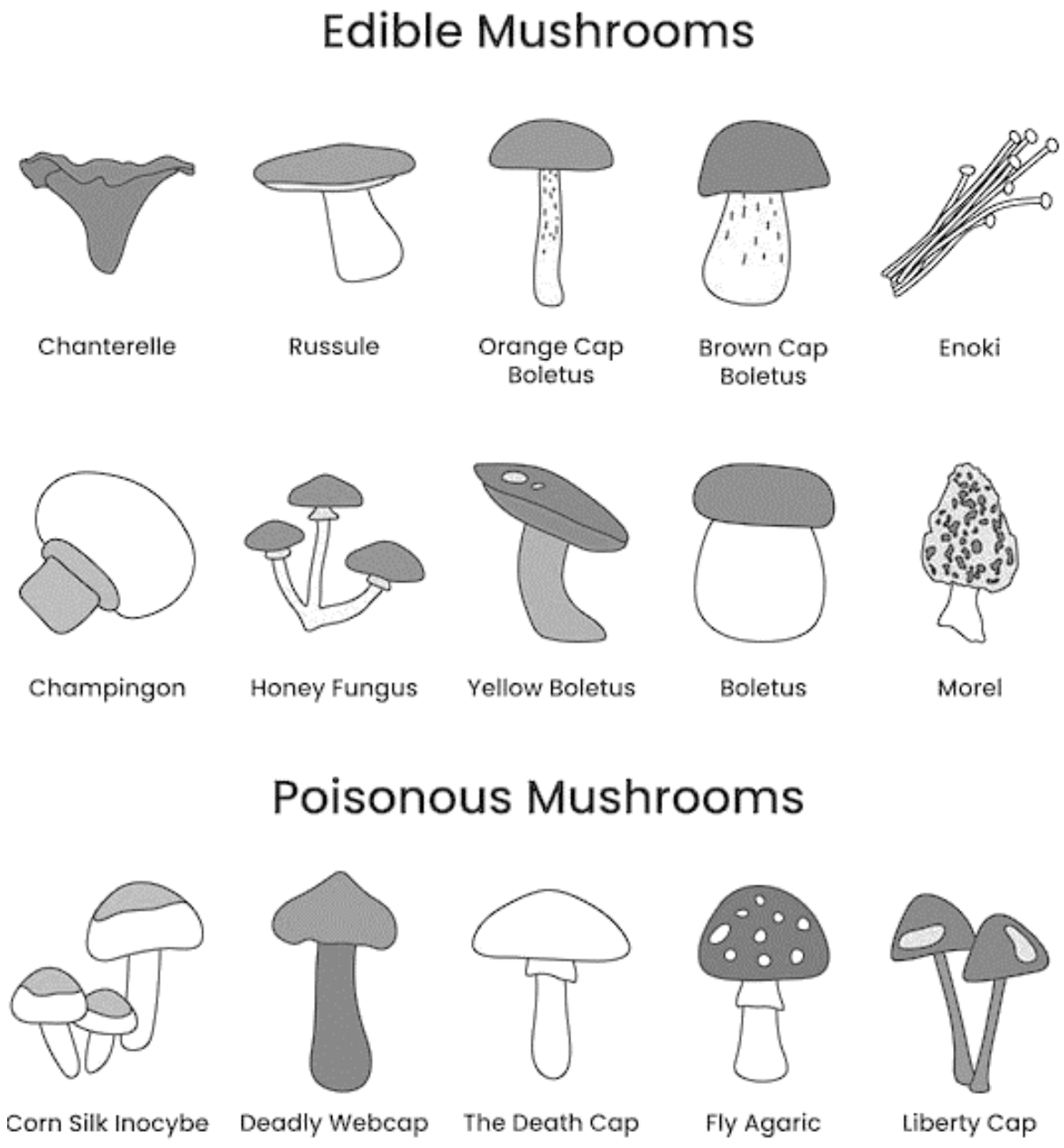

Figure-17.1: Types of edible and non-edible mushrooms (https://tinyurl.com/4xpzbhz4)

Key Differences between Edible and Poisonous Mushrooms:

Morphological Features:

Gills vs. Folds/Pores:

a) **Edible**: Chanterelles (*Cantharellus* spp.) exhibit wrinkled folds instead of true gills and have solid, pale stems. Their poisonous look-alike, the jack-o'-lantern mushroom (*Omphalotus olearius*), has sharp, non-forking gills, orange interior flesh, and grows in dense clusters on decaying wood.
b) **Edible**: Boletes (e.g., *Boletus edulis*) feature sponge-like pores instead of gills. Toxic boletes often stain blue when cut or have red coloration.

Cap and Stem Structures:

Poisonous: Amanitas (e.g., *Amanita phalloides*, "Death Cap") have white gills, a volva (cup-like base), and a ring/skirt on the stem. These traits are absent in edible look-alikes like puffballs (*Calvatia gigantea*), which must be pure white inside without any internal structure.

Toxins and Health Impacts: though mushrooms are found to be potent edible source, they may contain some toxic substances. These substances are as follows.

a) **Amatoxins**: Found in *Amanita* species, these inhibit RNA polymerase II, leading to liver and kidney failure. Symptoms (vomiting, jaundice) appear 6–24 hours post-ingestion, often too late for effective treatment.
b) **Gyromitrin**: Present in false morels, it metabolizes into monomethylhydrazine, causing seizures, hemolysis, and potential carcinogenicity.
c) **Coprine**: In alcohol inky caps (*Coprinopsis atramentaria*), it induces severe nausea when combined with alcohol, even days before or after consumption.
d) **Muscarine**: In *Clitocybe* and *Inocybe* species, it overstimulates the parasympathetic nervous system, resulting in sweating, salivation, and bradycardia.

Identification and Safety Measures: Foragers must take extreme caution when harvesting wild mushrooms. Here are some tips for safe identification:

1) **Consult Experts:** Seek guidance from experienced mycologists or use reliable field guides.
2) **Examine Physical Traits:** Observe color, shape, gill structure, and spore print. Poisonous Amanitas have white gills and a volva (cup-like base).
3) **Avoid Mushrooms with White Gills and Rings:** Many toxic mushrooms, such as the Death Cap, have these features.
4) **Never Rely on Folklore:** Myths like "poisonous mushrooms turn silver black" are misleading.
5) **Test with a Spore Print:** Place the mushroom cap on paper to reveal spore color. Color varies (e.g., white for deadly Amanitas vs. pink for edible Agaricus).
6) **When in Doubt, Leave It Out:** If unsure, do not consume the mushroom.
7) **Habitat:** Morels grow near dying trees, while Galerina thrives on decaying conifers.

Look-Alikes and Pitfalls: Some of the mushrooms are found alike and seems to be edible mushrooms, however these are not real mushrooms.

These mushrooms includes:

1) **False Morels (*Gyromitra*) vs. True Morels**: *Gyromitra* has irregular, brain-like caps vs. honeycombed morels.
2) **Chanterelles vs. Jack-O'-Lanterns (*Omphalotus olearius*)**: The latter glows bioluminescent and grows in clusters.

Effects of Mushroom Poisoning:

Mushroom poisoning symptoms vary based on the type of toxin. Common symptoms include: **Gastrointestinal distress -** Vomiting, diarrhea, and abdominal pain. **Neurotoxicity -** Hallucinations, confusion, and seizures. **Hepatotoxicity -** Liver failure caused by amatoxins. **Nephrotoxicity -** Kidney failure leading to long-term damage.

Treatment of Mushroom Poisoning:

Immediate medical attention is crucial in cases of suspected poisoning. Steps to take include: Do Not Induce Vomiting - Seek emergency medical care immediately. Identify the Mushroom - Provide a sample to medical professionals. Activated Charcoal - May help absorb toxins if taken early. Hospitalization - Severe cases require intensive treatment, including liver transplants.

Safe Foraging Practices:

1) **Positive Identification**: Use multiple field guides and cross-reference features like spore prints (e.g., white for *Amanita*, pink for edible *Agaricus*).
2) **Avoid Risky Groups**: Steer clear of mushrooms with white gills, red caps, or volvas. Novices should focus on "foolproof" species like giant puffballs or chicken of the woods (*Laetiporus sulphureus*).
3) **Cook Thoroughly**: Many edible mushrooms (e.g., morels) are toxic raw but safe when cooked.
4) **Start Small**: Test a tiny portion first to rule out allergies.

Case Studies and Historical Context:

Amanita Poisonings: Account for 90% of fatal mushroom ingestions. In 2023, a family in California mistook *Amanita ocreata* for edible *Agaricus*, resulting in two fatalities.

Cultural Significance: Indigenous groups traditionally use *Psilocybe* mushrooms (containing psilocybin) for rituals, though these can cause hallucinations and disorientation if misused.

17.4 MUSHROOM CULTIVATION:

Mushrooms are the fruiting bodies of fungi. Due to this reason the mushrooms are also called fleshy fungi. Due to absence of chlorophyll, mushrooms are not able to synthesise their own food and have to depend upon outside sources for their nutritional requirements. The mushrooms are fruit bodies or reproductive structures emanating from mycelium, which under natural conditions remain buried under the soil.

History:

The references of mushrooms can be traced back to classical texts of Indian, Greek and roman literature. The wild growing mushrooms were picked for their aroma and palatability. The first cultivation of mushrooms was reported from France during 1650. In Asia, China, South Korea and Taiwan were the first cultivators of mushrooms. At present Taiwan is considered to be the largest contributor of mushrooms to the world market. The first efforts in cultivation of mushrooms in India started way back in 1940 at college of Agriculture, Coimbatore. During 1961, Indian Council of Agricultural Research started a project in collaboration with H.P. Govt. named “Development of Mushroom Cultivation in Himachal Pradesh”. In 1970 H.P. Govt. established a mushroom centre at Solan, in collaboration with United Nations Development Project (UNDP).

Mushrooms are a fleshy fungi (*Basidiomycota, Agaricomycetes*) having a stem, cap and gills underneath the cap. They can be edible, wild and some of them can be toxic too. It contains more than 90% water and less than 1% fat, loaded with Vitamin B, copper and selenium and low in sodium. Mushrooms are rich source of Vitamin D it contains copious amount of precursor of Vitamin Dl “Ergosterol”.

Types of Mushrooms:

Mushrooms are easily cultivable in hilly regions due to abundant moisture but can also be grown in artificial environment with proper temperature and humidity control. Varieties must be identified thoroughly in order to avoid poisonous ones. Some of the major varieties consumed in India are described here under:

Button Mushroom:

Button mushroom (*Agaricus bisporus*) belongs to Class *Basidiomycetes* and Family *Agaricaceae* and is native to Europe and North America. It is of two types white and brown, out of which white button mushroom is commonly grown in India. This variety contributes more than 85% to mushroom production.

Shiitake Mushroom:

Shiitake Mushrooms are native to East Asia and are highly consumed in Asian countries. They readily grow on wood of deciduous and hard wood trees such as Oak, Chestnut, and Maple etc. and require moist and warm climate. These are used in Asian cuisines and traditional medicines.

Oyster Mushroom:

Oyster Mushrooms (*Pleurotus ostreatus*) belongs to *Pleurotus* species. It is known as "Dhingri" in India and has fan or oyster shaped cap. They grow easily on decaying wood or straw.

Paddy Straw Mushroom:

It is usually grown on Rice straw bed and is used extensively in Asian Cuisines. Eg: *Volvariella volvacea* belongs to division *Basidiomycota*.

Cultivation:

The basic requirements for mushroom cultivation are manure/compost, spawns, right temperature and humidity. Favourable growing conditions involve 80%-90% of relative humidity, ample ventilation. A temperature range of 20-28 ºC during spawn run and 12-18 ºC for reproductive growth. Initially for a week temperature must be maintained at 23 ± 20 ºC and then it can be reduced to 16 ± 20 ºC for subsequent weeks. The CO_2 concentration should be 0.08-0.15 %. Under appropriate conditions the pin heads start to appear within few days and progressively mature into button stage. Apart from these insecticides, nutritional supplements like nitrogen, vermiculite, water are also required for a healthy harvest. Based on the method of culturing, mushroom cultivation is divided into log culture, tray culture, stump culture, wall culture, mound culture, bag culture and column culture (Fig. 17.10).

Steps followed during Mushroom Cultivation:

Compost Preparation:

The compost used for mushroom growth usually comprises of wheat straws, horse manure, poultry manure, rice bran, gypsum etc. Utmost care is taken to protect the raw compost against rain or external moisture, in order to control contamination. The chopped wheat straws or rice bran are mixed with horse dung, sprinkled with water and are heaped in a pile to allow fermentation. The fermentation process along with heat development breaks down the chemical compounds in small components. Frequent turnings and watering is done at a specific interval so as to avoid the drying up of compost. Gypsum is sometimes added to the compost to reduce greasiness and allow more aeration. Within 15 to 20 days the compost gets all set to be used as bed, it is then spread onto wooden trays and sowed with spawns.

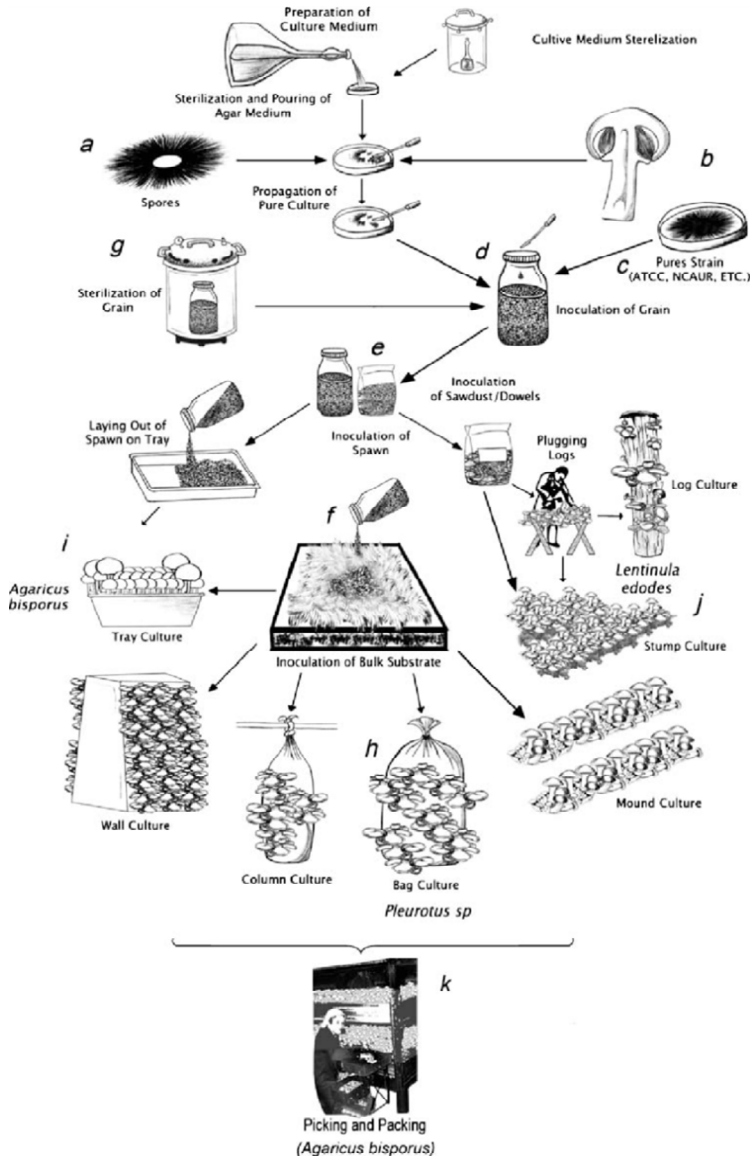

Figure-17.2: Process of mushroom cultivation (https://tinyurl.com/yc45ufmd)

Spawning:

Spawns refers to the mycelium carefully propagated on agars or grains. Spawning is a process of sowing or mixing spawns in compost. The spawns are thoroughly mixed with the compost, are covered with newspaper and is watered sufficiently to maintain the moisture. Throughout the cultivation period humidity is kept high to avoid loss of moisture. Gradually they grow into white cottony mycelium growth.

Casing:

Casing is a kind of sterilized soil or dressing containing cow manure which is spread onto the spawn mixed compost. It is applied when the mycelium growth commences on the compost surface. After 15 to 20 days of its application mushroom head or pins start becoming visible on the surface. They are allowed to mature for a specific time period and are harvested before opening of the cap. Mushrooms with opened cap looks like an umbrella after opening of cap are undesirable and are considered of menial quality.

Harvesting:

Harvesting is done by plucking them from soil using hands or the heads are chopped off using knife. The harvested mushrooms are then subjected to primary processing.

Processing:

Mushroom are very fragile and have a short shelf life, unless consumed fresh. At ambient temperature they lose their freshness within a day and deteriorates rapidly if not processed or refrigerated. They also tend to brown due to presence of compound Tyrosinase. It converts monophenols to diphenols which in turn are oxidized to quinones resulting in the formation of insoluble brown pigment called Melanin. Initial processing involves washing mushrooms to remove adhering soil or compost and blanching them for few minutes to inactivate the enzymes. In order to prevent discoloration they are treated with brine, salt or citric acid prior to canning or packaging.

Common Processing and Preservation Methods:

Drying:

Drying or Dehydration is the oldest and the basic processing method for various food products. Moisture is the most suitable medium for the microbial growth and propagation, hence its removal will cause the microbial activity to cease or become gradual. Mushrooms can be dried either by sun drying or by mechanical drying. Sun drying is the cheapest and popular method but it produces a much darker product. Mechanical drying is rapid and is of various types like Tray drying, Freeze drying, Vacuum drying, Microwave oven drying, Air drying etc. Dried mushrooms can be rehydrated and are used in soups, stews, pickles etc.

Freezing: As mushrooms contain more than 90% water hence freezing is the most suitable method for preservation. They are subjected to various pretreatments to minimize unfavorable effects upon freezing. Colour of *Agaricus bisporus* is better when treated with metabisulphites along with blanching. Blast freezing method is commonly employed at temperature -25 ºC to -30 ºC. Cryogenic freezing extends mushroom's shelf life up to one year when used at - 80 ºC to - 100 ºC for 5-6 minutes.

Sterilization:

Sterilization of mushrooms can be done by using chemicals, steam or by irradiation. The shelf life of mushrooms can be extended by applying a radiation dose of 1 – 3 kGy.

Canning:

Canning involves preservation in brine vinegar, oil or marinades. Freshly harvested mushrooms are utilized for canning purpose. They are cleaned, graded, blanched and then filled into cans along with brine or vinegar followed by lidding. The cans are then exhausted to remove air, heat sterilized, cooled, labelled and packaged for storage or consumption.

Pickling:

Pickling is an age old method which utilizes spice, salt, vinegar and oil as the basic ingredients for food preservation. Pickled mushrooms are made using spices such as turmeric, red chilli, garlic, clove along with salt and oil. Pickling induces fermentation which generates a mild flavour.

17.5 SUMMARY:

Edible mushrooms, such as button mushrooms (*Agaricus bisporus*), chanterelles (*Cantharellus cibarius*), and porcini (*Boletus edulis*), are widely consumed for their rich flavors and nutritional benefits, including vitamins, minerals, and antioxidants. However, some mushrooms are highly toxic and can be fatal if ingested. Poisonous species like the death cap (*Amanita phalloides*), destroying angel (*Amanita virosa*), and the fly agaric (*Amanita muscaria*) contain harmful toxins that can cause severe organ damage or neurological symptoms. Proper identification is crucial, as some toxic mushrooms closely resemble edible varieties, making foraging risky without expert knowledge.

Mushroom cultivation is the process of growing edible fungi under controlled conditions, typically using organic materials like straw, sawdust, or composted manure as a substrate. The cultivation involves selecting suitable mushroom species (such as oyster, button, or shiitake), preparing and sterilizing the growing medium, inoculating it with mushroom spawn, and maintaining optimal temperature, humidity, and ventilation to support growth. It is a sustainable and profitable agricultural practice that requires relatively low investment and space, making it ideal for small-scale farmers and urban growers. Proper hygiene and environmental control are crucial to prevent contamination and ensure a successful yield.

17.6 TECHNICAL TERMS:

Mushroom, poisonous, spawn, sterilization, cultivation

17.8 SELF ASSESSMENT QUESTIONS:

1) Give a note on edible mushrooms and their nutritional properties.
2) Describe the various non-edible mushrooms and make a note on how to identify them.
3) Give a note on commonly cultivated mushrooms.
4) Explain the various steps in mushroom cultivation.

17.9 SUGGESTED READINGS:

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4) https://www.biologydiscussion.com/microbiology-2/structure-of-fungal-cell-microbiology/64992.
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LESSON - 18

IMPORTANCE OF FUNGI IN AGRICULTURE, INDUSTRY AND MYCOTOXINS

18.0 OBJECTIVE OF THE LESSON:

By studying this lesson students came to know the role of fungi in agriculture and industries. The impact of mycotxins on agriculture and on humans will be understood.

STRUCTURE OF THE LESSON:

18.1 INTRODUCTION:

Fungi play a vital role in agriculture, influencing soil health, crop productivity, and disease management. These microorganisms contribute to nutrient cycling, enhance plant growth through symbiotic relationships, and help control pests and diseases. Beneficial fungi, such as mycorrhizal fungi and decomposers, improve soil fertility and support plant development by increasing nutrient and water uptake. Additionally, some fungi act as natural biocontrol agents against plant pathogens and insect pests, reducing the need for chemical pesticides. However, fungi can also be detrimental, causing serious crop diseases, post-harvest spoilage, and mycotoxin contamination, leading to significant economic losses. Understanding and managing the role of fungi in agriculture is essential for sustainable farming practices, ensuring food security, and minimizing environmental impact. By harnessing the benefits of fungi while mitigating their harmful effects, farmers can enhance agricultural productivity in an eco-friendly and cost-effective manner. Fungi play a crucial role in various industries due to their ability to produce valuable enzymes, antibiotics, organic acids, and fermented products. In the food and beverage industry, fungi like *Saccharomyces cerevisiae* are essential for baking, brewing, and winemaking, while *Aspergillus* and *Penicillium* species are used in cheese production and food preservation.

In pharmaceuticals, fungi serve as sources of antibiotics like penicillin and immunosuppressants such as cyclosporine. They are also employed in biotechnology for enzyme production, biofuel generation, and waste management. Additionally, fungi contribute to agriculture by producing biopesticides and biofertilizers, enhancing crop yield and soil health. Their diverse applications make fungi indispensable in modern industries. Yeast is a crucial microorganism with significant roles in food, beverage, and biotechnology industries. It is widely used in baking, where it ferments sugars to produce carbon dioxide, making dough rise. In brewing and winemaking, yeast ferments sugars into alcohol and carbon dioxide, essential for beer, wine, and other alcoholic beverages. Additionally, yeast, particularly *Saccharomyces cerevisiae*, is employed in biotechnology for producing bioethanol, pharmaceuticals, and probiotics. It is also a rich source of vitamins, especially B-complex, and is used in nutritional supplements. Beyond industry, yeast serves as a model organism in scientific research, contributing to genetics, molecular biology, and medicine.

Mycotoxins are toxic secondary metabolites produced by certain fungi, primarily Aspergillus, Penicillium, and Fusarium species, that contaminate food and feed crops. These toxins pose serious health risks to humans and animals, leading to acute poisoning or chronic diseases such as liver damage, immune suppression, and even cancer. Common mycotoxins include aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone. They can contaminate cereals, nuts, spices, fruits, and animal feed, especially under warm and humid conditions. Preventive measures include proper storage, drying, and food safety regulations to minimize contamination and exposure.

18.2 IMPORTANCE OF FUNGI IN AGRICULTURE:

1. Beneficial Roles of Fungi in Agriculture:

Fungi contribute positively to agriculture in several ways, including soil enrichment, plant growth promotion, and biological control.

A. Soil Fertility and Nutrient Cycling:

Saprophytic fungi decompose plant and animal residues, breaking them down into simpler compounds, thus recycling nutrients and improving soil fertility. Fungal decomposition contributes to the formation of humus, which enhances soil structure and water retention. Some fungi, in symbiosis with plants or bacteria, help fix atmospheric nitrogen into a form usable by plants.

B. Mycorrhizal Fungi and Plant Growth Enhancement:

Mycorrhizal fungi, such as arbuscular mycorrhizal (AM) fungi and ectomycorrhizal fungi, form symbiotic relationships with plant roots, improving phosphorus, nitrogen, and water

absorption. Mycorrhizae enhance plant resistance to soil-borne pathogens by competing for nutrients and stimulating plant defense mechanisms. They help plants withstand drought stress by improving root absorption efficiency.

C. Biocontrol of Plant Diseases and Pests:

Some fungi, such as *Trichoderma* species, act as biocontrol agents by suppressing soil-borne pathogens like *Fusarium*, *Rhizoctonia*, and *Pythium* through competition, antibiosis, and mycoparasitism. Entomopathogenic fungi, like *Beauveria bassiana* and *Metarhizium anisopliae*, infect and kill insect pests, reducing reliance on chemical pesticides.

D. Fungal Biofertilizers and Soil Improvement:

Some fungi, such as *Aspergillus* and *Penicillium* species, release organic acids that solubilize phosphate, making it available to plants. Certain fungi enhance the availability of potassium and zinc in the soil, supporting plant growth.

E. Bioremediation and Waste Decomposition:

Fungi help in the breakdown of agricultural pollutants, including pesticides and heavy metals, reducing soil contamination. Fungi accelerate the decomposition of agricultural waste, producing compost that enhances soil health.

2. Detrimental Roles of Fungi in Agriculture:

While fungi offer many benefits, some species can cause significant agricultural problems.

a) **Plant Pathogens and Crop Diseases:** Fungi are responsible for a majority of plant diseases, leading to reduced yields and economic losses. Root and stem rots Caused by fungi like *Fusarium*, *Pythium*, and *Rhizoctonia*. Leaf and fruit diseases such as powdery mildew (*Erysiphe* spp.), downy mildew (*Plasmopara viticola*), and apple scab (*Venturia inaequalis*) are caused due to fungi. The Rust fungi (*Puccinia* spp.) and smut fungi (*Ustilago* spp.) infect cereals like wheat and corn, reducing grain quality. Fungi such as *Aspergillus* and *Penicillium* cause spoilage of stored grains, fruits, and vegetables.

b) **Mycotoxin Contamination:** Certain fungi produce toxic secondary metabolites called mycotoxins, which can contaminate crops and pose health risks to humans and livestock. Aflatoxins: Produced by *Aspergillus flavus* in peanuts, maize, and other crops. Fumonisins: Produced by *Fusarium* species in maize. Ochratoxins: Produced by *Penicillium* and *Aspergillus* in cereals and coffee.

c) **Competition with Crops:** Some fungi form symbiotic relationships with invasive weeds, promoting their growth and reducing crop productivity. Some soil fungi compete with crops for essential nutrients, affecting plant growth.

3. Application of Fungi in Sustainable Agriculture:

Farmers and researchers are harnessing fungi to improve agricultural sustainability and reduce dependence on synthetic chemicals.

a) **Use of Mycorrhizal Inoculants:** Farmers use mycorrhizal fungi inoculants to improve plant growth, especially in degraded soils. Mycorrhizae are widely used in organic agriculture to reduce the need for chemical fertilizers.

b) **Development of Biopesticides and Biofungicides:** *Trichoderma* for disease control used as a biopesticide to combat fungal pathogens. Entomopathogenic fungi applied in integrated pest management (IPM) programs to control insect pests naturally.

c) **Fungal-Based Biofertilizers:** Phosphate-Solubilizing Fungi**:** Used to enhance phosphorus availability in the soil. Fungi like *Pleurotus* spp. are used to accelerate composting processes.

18.3 INDUSTRIAL APPLICATIONS OF FUNGI:

Fungi have a wide range of industrial applications across multiple sectors, including food production, pharmaceuticals, biotechnology, agriculture, and environmental management. Their ability to produce enzymes, antibiotics, organic acids, and other bioactive compounds makes them essential in many industries. Below is a detailed account of their industrial uses:

1. Food and Beverage Industry:

Fungi play a crucial role in food production, particularly in fermentation processes.

a. Fermentation and Food Processing:

Bread-Making: *Saccharomyces cerevisiae* (baker's yeast) is used in bread-making to ferment sugars, producing carbon dioxide that causes dough to rise.

Alcohol Production: Fungi, especially yeasts (*Saccharomyces cerevisiae*), are essential in brewing beer, fermenting wine, and distilling spirits.

Cheese Production: Specific fungal species, such as *Penicillium roqueforti* and *Penicillium camemberti*, are used in ripening blue cheese and Camembert cheese, giving them distinct flavors.

Soy Sauce and Miso Production: *Aspergillus oryzae* is used to ferment soybeans in the production of soy sauce, miso, and sake

b. Edible and Cultivated Mushrooms:

Commercially Grown Mushrooms: Species such as *Agaricus bisporus* (button mushroom), *Lentinula edodes* (shiitake), and *Pleurotus ostreatus* (oyster mushroom) are cultivated for food.

Mycoprotein Production: *Fusarium venenatum* is used to produce Quorn™, a high-protein meat substitute.

2. Pharmaceutical Industry:

Fungi are a major source of antibiotics, immunosuppressants, and other pharmaceutical compounds.

a. Antibiotic Production:

Penicillin: *Penicillium notatum* and *Penicillium chrysogenum* produce penicillin, one of the first and most widely used antibiotics.

Cephalosporins: Produced by *Acremonium* (formerly *Cephalosporium*), these antibiotics are used to treat bacterial infections.

Erythromycin and Tetracycline precursors: Some fungi contribute to the biosynthesis of these antibiotics.

b. Immunosuppressants and Other Drugs:

Cyclosporine: Derived from *Tolypocladium inflatum*, it is used in organ transplant patients to prevent rejection.

Statins: *Aspergillus terreus* produces lovastatin, which is used to lower cholesterol levels.

c. Antifungal and Anticancer Compounds:

Griseofulvin: Produced by *Penicillium* species, it is used to treat fungal infections.

Ergot Alkaloids: *Claviceps purpurea* produces ergot alkaloids, which are used in migraine treatments (e.g., ergotamine) and as precursors for drugs like LSD.

3. Biotechnology and Enzyme Production:

. Fungi are excellent producers of industrial enzymes used in various sectors.

a. Enzyme Production:

Amylases: *Aspergillus oryzae* and *Aspergillus niger* produce amylases, which are used in baking, brewing, and starch processing.

Cellulases: Used in biofuel production, paper processing, and textile industries, these enzymes break down cellulose into glucose.

Proteases: *Aspergillus* species produce proteases used in detergent manufacturing and leather processing.

Lipases: Used in dairy, detergent, and biodiesel industries to break down fats.

b. Biotechnological Applications:

Genetic Engineering: Yeasts like *Saccharomyces cerevisiae* are used as model organisms for recombinant DNA technology.

Bioethanol Production: Fungi help ferment biomass into bioethanol, a renewable fuel.

4. Agriculture and Pest Control:

Fungi are used as biopesticides and soil enhancers.

a. Biopesticides:

Beauveria bassiana: Used as a biological insecticide against pests like aphids and beetles.

Metarhizium anisopliae: Effective against mosquitoes and other insect pests.

Trichoderma spp.: Used as a biocontrol agent against plant pathogens.

b. Soil Improvement and Fertilization:

Mycorrhizal Fungi: Enhance plant growth by improving nutrient absorption.

Composting and Decomposition: Fungi help break down organic matter into nutrient-rich compost.

5. Environmental and Industrial Waste Management:

Fungi contribute to waste management and pollution control.

a. Bioremediation:

Oil Spill Cleanup: *Aspergillus* and *Penicillium* species can break down hydrocarbons in crude oil spills.

Heavy Metal Removal: Fungi absorb and detoxify heavy metals from industrial wastewater.

Plastic Degradation: Some fungi, like *Pestalotiopsis microspora*, can break down polyurethane plastics.

b. Waste Recycling:

Paper Industry: Fungal enzymes help break down lignin in paper pulp processing.

Agricultural Waste Processing: Fungi are used to convert crop residues into valuable biofuels and animal feed.

6. Textile, Leather, and Detergent Industry:

Fungi-derived enzymes contribute to sustainable textile and leather processing.

a. Textile Industry:

Bleaching Agents: Fungal enzymes are used to produce eco-friendly fabric bleaches.

Bio-stoning: Cellulases derived from fungi help in bio-stoning denim fabric.

b. Leather Industry:

Tanning Processes: Fungal proteases help in softening leather.

Eco-friendly Dye Production: Fungi produce natural dyes for textiles.

c. Detergent Industry: Fungal Enzymes used in laundry detergents to break down stains and improve cleaning efficiency.

7. Paper and Pulp Industry:

Fungi assist in pulp processing and paper recycling.

Lignin Degradation: White-rot fungi like *Phanerochaete chrysosporium* degrade lignin, improving pulp quality.

Enzymatic Bleaching: Reduces chemical use in paper bleaching.

8. Cosmetics and Personal Care:

Citric Acid Production: *Aspergillus niger* is used to produce citric acid, which is a common ingredient in cosmetics.

Hyaluronic Acid: Used in skincare products, derived from fungal fermentation.

Pigments and Dyes: Fungi produce natural colors used in cosmetics.

9. Construction and Material Science: Fungi are being explored for sustainable building materials.

Mycelium-Based Materials: Used to make biodegradable packaging, insulation, and even fungal bricks.

Fungal Biomaterials: Used as an alternative to plastic and Styrofoam.

18.4 MYCOTOXINS:

Mycotoxins are toxic secondary metabolites produced by certain species of fungi (molds) that can contaminate food, feed, and agricultural products. They pose significant health risks to humans and animals, causing acute and chronic diseases, including cancer, immune suppression, and organ damage. Mycotoxins are primarily produced by *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps* species, among others.

Types of Mycotoxins: Several mycotoxins have been identified, but the most significant ones in terms of health and economic impact include:

1) **Aflatoxins: These are produced by** *Aspergillus flavus* and *Aspergillus parasiticus.* **Commonly found in** grains (corn, wheat, rice), peanuts, tree nuts, spices, and milk (as aflatoxin M1). Aflotoxins are having Hepatotoxic (liver damage), carcinogenic (causes liver cancer), immunosuppressive, and teratogenic (causes birth defects) effects. Aflatoxin levels are strictly regulated worldwide due to their high toxicity, with the FDA setting limits for food and feed.
2) **Ochratoxins: These toxins produced by** *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Penicillium verrucosum.* **Commonly found in** cereals, coffee, dried fruits, wine, beer, and pork. These are having Nephrotoxic (kidney damage), hepatotoxic, carcinogenic, neurotoxic, and immunosuppressive nature. Ochratoxin A has to be regulated in foodstuffs, particularly in Europe, to limit exposure.
3) **Fumonisins: These are produced by** *Fusarium verticillioides* and *Fusarium proliferatum.* **Commonly found in** corn and corn-based products. These are having neurotoxic, hepatotoxic, nephrotoxic, and associated with esophageal cancer in humans; causes leukoencephalomalacia (brain softening) in horses.
4) **Trichothecenes (e.g., Deoxynivalenol, T-2 Toxin, HT-2 Toxin): Produced by** *Fusarium* species. **Commonly found in c**ereal grains (wheat, barley, oats, rye, and corn). These toxins inhibits protein synthesis, causes vomiting, immune suppression, diarrhea, hemorrhaging, and neurological issues.
5) **Zearalenone: Produced by** *Fusarium* species. **Commonly found in c**orn, wheat, barley, and other cereals. **These are having** Estrogenic effects (mimics estrogen), causing reproductive disorders in animals (infertility, abortion, feminization in males).
6) **Ergot Alkaloids: Produced by** *Claviceps purpurea* (ergot fungus). **Commonly found in** Rye and other cereal grains. These are having t**oxic effects like** Vasoconstriction, gangrene, hallucinations, convulsions (ergotism or "St. Anthony’s Fire").

Factors Influencing Mycotoxin Production:

Mycotoxin production by fungi is influenced by various **environmental, biological, and substrate-related factors**. These factors can determine the type and amount of mycotoxins produced. Key factors include:

1. Environmental Factors:

Temperature: Mycotoxin production varies with temperature. Aflatoxins (produced by *Aspergillus flavus*) thrive in warm conditions (25–35°C). Ochratoxins (produced by *Penicillium* and *Aspergillus* species) are produced at lower temperatures (10–30°C).

Moisture and Water Activity (a_w): High humidity and water activity (>0.7) promote fungal growth and mycotoxin synthesis. Grains stored in moist conditions are more susceptible.

Oxygen Levels: Aerobic conditions favor mycotoxin production. Modified atmosphere storage (low oxygen, high CO_2) can reduce fungal growth.

pH Levels: Most toxigenic fungi prefer slightly acidic to neutral pH (4–7).

2. Biological Factors:

Fungal Strain: Different strains of the same species produce varying amounts of mycotoxins.

Competition with Other Microorganisms: Bacteria, yeasts, and non-toxigenic fungi can inhibit toxin-producing fungi.

Genetic Regulation: Some fungal genes control mycotoxin biosynthesis, which may be activated or suppressed by environmental conditions.

3. Substrate and Nutritional Factors:

Type of Crop or Food Source: Grains (corn, wheat, peanuts) are highly susceptible to aflatoxin contamination. Coffee beans and grapes can contain ochratoxins.

Nutrient Availability: High carbohydrate and nitrogen content favor fungal growth and mycotoxin production.

Presence of Stress Factors: Drought, insect damage, and improper storage conditions increase vulnerability to mycotoxin contamination.

4. Agricultural and Post-Harvest Practices:

Pre-Harvest Conditions: Drought stress and high temperatures increase toxin accumulation.

Storage Conditions: Poor ventilation and high humidity encourage fungal growth.

Processing Methods: Some mycotoxins can survive food processing (e.g., roasting, cooking).

5. Chemical and Physical Stressors:

Pesticides and Fungicides: Some fungicides may inhibit fungal growth but not always prevent mycotoxin production.

UV Light and Radiation: UV exposure can degrade some mycotoxins but may also stress fungi to produce more toxins.

Health Effects of Mycotoxins: Exposure to mycotoxins can cause acute poisoning or chronic diseases, depending on the dose and duration of exposure. Major health effects include: Liver cancer (Aflatoxins), Kidney damage (Ochratoxins), Neurotoxicity (Fumonisins), Hormonal disruption (Zearalenone), Immune suppression (Trichothecenes, Aflatoxins, Ochratoxins). Mycotoxins can enter the human food chain through contaminated plant-based foods, contaminated animal products (meat, eggs, and dairy), and water.

Detection: Fungal toxins can be determined Chromatographic techniques (HPLC, LC-MS, ELISA (Enzyme-Linked Immunosorbent Assay), Biosensors and Spectroscopic methods.

Prevention and Control: We can prevent and control the fungal toxins by using following practices.

1) **Good Agricultural Practices (GAP):** Proper crop rotation, use of resistant varieties, and avoiding drought stress
2) **Good Storage Practices:** Keeping grains dry and cool to prevent mold growth.
3) **Chemical and Biological Detoxification:** Use of mycotoxin binders in animal feed, ozone treatment, and enzymatic degradation
4) **Regulations and Monitoring:** Governments set maximum allowable limits in food and feed, with rigorous testing

18.5 SUMMARY:

Fungi play a crucial role in agriculture by enhancing soil fertility, promoting plant growth, and contributing to disease control. Mycorrhizal fungi form symbiotic relationships with plant roots, improving nutrient and water absorption, which boosts crop yields. Decomposer fungi break down organic matter, recycling essential nutrients into the soil. Some fungi, such as *Beauveria bassiana* and *Trichoderma* species, act as natural biopesticides, helping control harmful pests and plant diseases, reducing the need for chemical pesticides. Additionally, fungi like *Penicillium* are used in post-harvest preservation to prevent spoilage. However, certain pathogenic fungi can also cause significant crop losses, making fungal disease management essential in agriculture.

Fungi play a crucial role in various industrial applications, including pharmaceuticals, food production, biotechnology, and biofuel generation. They are widely used in the production of antibiotics (e.g., Penicillium for penicillin), immunosuppressants, and cholesterol-lowering drugs. In the food industry, fungi contribute to the fermentation of bread, beer, wine, and cheese, with species like *Saccharomyces cerevisiae* being essential for yeast fermentation. Industrial enzymes derived from fungi, such as cellulases and amylases, are used in textiles, detergents, and biofuel production. Additionally, fungi are employed in bioremediation to degrade pollutants and in agriculture as biofertilizers and biopesticides. Their diverse metabolic capabilities make them invaluable across multiple industries.

Mycotoxins are toxic compounds produced by certain fungi that can contaminate food and animal feed, posing serious health risks. These toxins, including aflatoxins, ochratoxins, fumonisins, and trichothecenes, are commonly found in crops like grains, nuts, and coffee when stored under warm, humid conditions. Exposure to mycotoxins can lead to acute poisoning or long-term health effects such as liver damage, immune suppression, and even cancer. While food safety regulations and proper storage methods help minimize contamination, mycotoxins remain a global concern, particularly in regions with inadequate food monitoring and control measures.

18.6 TECHNICAL TERMS:

Mold, Pathogen, Yeast, Mycorhiza, Aflotoxins, Biopesticides, Mycotoxins

18.7 SELF ASSESSMENT QUESTIONS:

1) Write an essay on agricultural importance of fungi.
2) Describe the industrial applications of fungi.
3) Describe the mycotoxins.

18.8 SUGGESTED READINGS:

1) Microbiology - Michael J. Pelezer, J.R., E.C.S. Chan, Noel R. Krieg (1993) - Indian Edition (5th Edition), Mc Graw Hill Education (India) Private Limited, 444/1, Sri Ekambara Naicker Industrial Estate, Alapakkam, Povur, Chennai 6oo116, Tamil Nadu, India.
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