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सर्वश्रेष्ठ परीक्षा पथ-प्रदर्शक

B.Sc. First Year (Certificate Course) Semester-I

BOTANY

Microbiology & Plant Pathology

SYLLABUS

UNIT-I **A.** Introduction to Indian ancient, Vedic and heritage Botany and contribution of Indian Botanists (in all branches), in context with the holistic development of modern science and technology, has to be taught, practiced and assessed via class interaction/ assignments / self-study mentioned under Continuous Internal Evaluation (CIE). **B. Microbial Techniques & instrumentation :** Microscopy : Elementary knowledge of Light, phase contrast, electron, scanning and transmission electron microscopy, staining techniques for light microscopy, sample preparation for electron microscopy. Common equipment of microbiology lab and principle of their working : autoclave, oven, laminar air flow, centrifuge. Colorimetry and spectrophotometry, immobilization methods, fermentation and fermenters.

UNIT-II **Microbial world :** Cell structure of Eukaryotic and prokaryotic cells, Gram positive and Gram-negative bacteria, Structure of a bacteria and plasmids; Bacterial Chemotaxis and Quorum sensing, Bacterial Growth curve, factors affecting growth of microbes; measurement of growth; Batch culture, fed batch culture and continuous culture; Synchronous growth of microbes; Sporulation and reproduction and recombination in bacteria. Viruses, general characteristics, viral culture, Structure of viruses,TMV and retro viruses, Bacteriophages, Structure of T4 &, ë-phage; Lytic and Lysogenic cycles, mycophages, viroid, Prions & mycoplasma & phytoplasma, Actinomycetes (Actinobacteria) and their economic uses.

UNIT-III **Phycology :** Range of thallus organization in Algae, Pigments, Flagella, Reserve food, Types of Reproduction, Classification and comparative life cycle of : Nostoc, Chlorella, Volvox, Oedogonium, Chara; Ectocarpus, Sargassum, Polysiphonia. Phycoviruses, Economic importance of algae : Role of algae in soil fertility : biofertilizer : Nitrogen fixation : Symbiosis; Commercialproducts of algae : biofuel, Agar, Diatomite.

UNIT-IV **Mycology :** Comparative study of general characteristics, nutrition, life cycle, Economic importance of Fungi, Classification upto class. Distinguishingcharacters of Myxomycota: General characters of True Fungi (Eumycota): Mastigomycotina Synchytrium: Zygomycotina: Rhizopus, Ascomycotina: Saccharomyces, Penicillium, Peziza. Basidiomycotina: Ustilago, Puccinia, Agaricus; Deuteromycotina: Fusarium,Alternaria. Heterothallism, Physiological specialization, Heterokaryosis & Parasexuality.

UNIT-V **Mushroom Cultivation, Lichenology & Mycorrhiza :** Mushroom cultivation. General account of lichens, reproduction and significance; Mycorrhiza: ectomycorrhiza and endomycorrhiza and their significance.

UNIT-VI **Plant Pathology :** Disease concept, Symptoms, Etiology & causal complex, Primary and secondary inoculum, Infection, Pathogenicity and pathogenesis, Koch's Postulates. Mechanism of infection (Brief idea about Pre-penetration, Penetration and Post- penetration), Disease cycle (monocyclic, polycyclic and polyetic). Defense mechanism with special reference to Phytoalexin, Resistance- Systemic acquired and Induced systemic fungicides- Bordeaux mixture, Lime Sulphur, Tobacco decoction, Neem cake & oil

UNIT-VII **Diseases and Control :** Symptoms, Causal organism, Disease cycle and Control measures of – Early & Late Blight of Potato, Black Stem Rust of Wheat, Alternaria spot' and 'White rust of Crucifers, Red Rot of Sugarcane, Wilting of Arhar, Mosaic diseases on tobacco and cucumber, yellow vein mosaic of bhindi; Citrus Canker. Little leaf of brinjal; Damping off of seedlings, Disease management: Quarantine, Chemical, Biological, Integrated pest disease management.

UNIT-VIII **Applied Microbiology :** Elementary knowledge of Food fermentations and food produced by microbes, Production of amino acids, antibiotics, enzymes, vitamins, alcoholic beverages, organic acid & genetic recombinant vaccines. Mass production of bacterial biofertilizers, blue green algae, Azolla and mycorrhiza. Plant growth promoting rhizobacteria & biopesticides : Trichoderma sp. and Pseudomonas, Single cell proteins (Spirulina), Organic farming inputs, Microbiology of water, Biopolymers, Bioindicators, Biosensors, Bioremediation, Production of biofuels, Biodegradation of pollutants and Biodeterioration of materials & Cultural Property. Microbial Biofactories (E.coli and Yeast) for production of recombinant proteins.

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UNIT-I

History of Botany and Microbial Techniques and Instrumentation

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. Write the names of four Vedas.

Ans. Four Vedas : (i) or peepal (*Ficus religiosa*), (ii) Neeli (*Indigofera tinctoria*), (iii) Palash (*Butea monosperma*), (iv) Durva or Doob (*Cynodon dactylon*)

Q.2. Name the three level of biodiversity.

Ans. The three level of biodiversity are ecological diversity, species diversity and genetic diversity.

Q.3. Who wrote 'Chapters on the History of Botany in India'?

Ans. 'Chapters on the History of Botany in India' was written by Burkill in 1965.

Q.4. Who is father of Biology?

Ans. Aristotle is called father of Biology.

Q.5. In light microscope, lenses are formed of glass, what is found in electron, microscope instead of lenses?

Ans. In electron microscope, electrons magnetic condensor lenses are used instead of glass lenses of light microscopes.

Q.6. Which microscope is used for the study of effect of chemical agents on living chromosomes?

Ans. Phase contrast microscope is used for the study of effect of chemical agents on living chromosomes.

Q.7. Give resolving power of electron microscope.

Ans. $\frac{\lambda}{2} = \frac{0.5\ \text{Å}}{2} = 0.25\ \text{Å}$

Q.8. In TEM and SEM why electrons are used in place of light rays?

Ans. The wavelength of electrons is very short. Therefore, the resolving power of TEM and SEM is much higher than that of light microscope.

Q.9. Name the most widely used method of determining amount of protein or nucleic acid in a solution.

Ans. Spectrophotometry is the most widely used method of determining amount of protein or nucleic acid in a solution.

Q.10. Who discovered electron microscope?

Ans. **Knoll** and **Ruska** (1932) from German, **Martin** (1934) from Belgium and **Prebus** and **Miller** from Canada independently developed electron microscope.

Q.11. Name the instrument that is used to measure the amount of light absorbed by the molecules of a solution.

Ans. Spectrophotometer is used to measure the amount of light absorbed by the molecules of a solution.

Q.12. What is a spectrophotometer?

Ans. A spectrophotometer is a sophisticated type of colorimeter which is used to measure absorption of radiation in the visible and UV regions of spectrum.

Q.13. What is the basis of spectrophotometry?

Ans. Spectrophotometry is based on the 'Beer Lambert Principle.' It can scan the wavelength range over UV and visible regions.

Q.14. 'In spectrophotometry quartz container is used in place of glass.' Why?

Ans. Unlike glass, quartz does not absorb ultraviolet light.

Q.15. Which amino acids absorb maximum light in UV range?

Ans. Amino acids : tyrosine and phenylalanine absorb maximum light in UV range.

Q.16. Why a hydrogen or deuterium lamp is used in spectrophotometer?

Ans. This type of lamp provides light of the wavelength in the UV region of spectrum.

Q.17. What is RCF?

Ans. RCF represents 'relative centrifugal force' which is generated by high speed rotor.

Q.18. Define wavelength.

Ans. Wavelength (λ) is the distance between successive peaks of curves and is measured as nanometer (10^{-9} meter or 10 Å).

Q.19. What is frequency of radiation?

Ans. Frequency of radiation (γ) is the number of successive peaks passing a given point in the one second.

Q.20. What is qualitative spectroscopy?

Ans. Quantitative spectroscopy is the technique of studying frequency of radiation absorbed or emitted by an atom or molecule for obtaining information about its identity.

Q.21. What is Svedberg unit?

Ans. A Svedberg unit (S) is defined as sedimentation coefficient of 1×10^{-13} seconds.

Q.22. Name three industries in which immobilized cell are used.

Ans. Pharmaceutical, environmental and biosensor industries.

Q.23. What are composite carriers?

Ans. Composite carriers are combination of inorganic and organic materials. These two types of materials compliment each other, it shows superiority of composite materials.

Q.24. Name two natural polymers used for encapsulation during cell immobilization.

Ans. Both natural and synthetic polymers have been used for microbial encapsulation. But algae polysaccharide such as agar, agarose, alginate and carrageenan are classified as natural polymers.

Q.25. Name two main substrates used for fermentation.

Ans. Carbohydrates and amino acids are used for fermentation.

Q.26. What is fermenters used for?

Ans. Fermenters are used for the commercial production of certain substances (e.g. alcohol, antibiotics, organic acids, enzymes etc.)

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Write a brief note on botany is vedic literature.

Ans.

Botany in Vedic Literature

The word 'Veda' is derived from Sanskrit word *'vid'* which means 'to know'. Veda consider and visualise this universe as multidimensional reality and explain it in same perspective. In that sense, *Rigveda,* undoubtedly the earliest textual source of science followed by other three vedas—*Atharva Veda, Yajur Veda* and *Sam Veda.* Hence the four vedas are the ultimate repository of knowledge. So far subject area of science in vedic literature is concerned, almost all aspects of modern science and technology is discussed. Proper screening of vedas clearly indicates that there is hardly any aspect of Botany which has not been scientifically described in Vedic literature. For instance, *Rigveda* clearly highlights ecology and environmental science as described in present days. Role of solar radiations and water in supporting ecosystem and various organisms has been described. Several terminology of plant morphology, anatomy, taxonomy and physiology mentioned in vedas are not only scientific but are being used in modern Botany as well. Saints of those days were well aware about the process of photosynthesis. Hydrological cycle has also been described in various hymns of *Rigveda.* Importance of plant diversity and need of their conservation is one important aspect of human duty expressed in vedas. Ethno-botany and medicinal plants have got important place in *Atharva Veda.* In fact, many aspects of modern botany can be traced back to vedas and contemporary literature.

Q.2. Write the role of Modern Era.

Ans.

Modern Era

During the first three decades of the 20th century intensive exploration was done all over the country, mostly by the Botanical Survey of India. This resulted in the publication of a number of provincial floras. Besides, some important works have appeared on forest vegetation. Besides, several floras of small areas have been published from 1900 onwards and several important monographs were published on Indian Botany. Floristic studies in India have received a fresh impetus in recent years.

Curiosity and the human instinct to survive were the main factors that resulted in amassing of biological knowledge over the years. The vast panorama of life is too much complicated to be studied in its entirely by any single investigator. Hence, systems of classification of living organisms that permit the relative isolation of one or another type of organism for organised investigation have been constructed. Besides, various biological disciplines, such as

taxonomy, morphology, cytology, physiology, ecology, molecular biology, biochemistry, biophysics, etc. have been distinguished depending on the nature of study.

1. Distinguished Scientists and Philosophers

(i) Aristotle—Father of Biology
(ii) Charaka—Father of Ayurveda
(iii) Hippocrates—Father of medicine
(iv) Robert Hooke—Father of cytology
(v) Alec Jeffreys—Father of DNA finger printing
(vi) Carl Linnaeus—Father of Taxonomy
(vii) G.J. Mendel—Father of Genetics
(viii) H.A. de Bary—Father of plant pathology
(ix) Leeuwenhock—Father of Bacteriology
(x) Louis Pasteur—Father of Microbiology
(xi) Stephen Hales—Father of plant physiology
(xii) Theophrastus—Father of Botany

2. Famous Indian Botanists

(i) J.C. Bose—Plant physiology
(ii) Birbal Sahni—Father of Indian Palaeobotany
(iii) P. Maheshwari—Embryology
(iv) T.S. Sadasivan—Mycology
(v) S.R. Kashyap—Father of Indian Bryology
(vi) H. Santapau—Taxonomy
(vii) P.N. Mehta—Pteridology
(viii) R. Mishra—Ecology
(ix) B.B. Mundkar—Plant pathology
(x) M.O.P. Iyengar—Father of Indian Algology
(xi) M.S. Swaminathan—Cytogenetics and Plant Breeding
(xii) B.P. Pal—Plant Breeding
(xiii) A.K. Sharma—Cytology
(xiv) V. Puri—Morphology

Q.3. Write a short note on Phase contrast microscope.

Ans.

Phase Contrast Microscope

Because of their very small size, most microbial cells absorb and scatter little of the light that passes through them; thus they are nearly transparent and are very difficult to see with transmitted light. One of the great advances in microscopy in the 20th century was the development of sophisticated optial means of enhancing contrast by manipulating the light inside the microscope. The most common type of contrast instrument is the **phase-contrast microscope.** Microbiologists routinely use phase-contrast microscopes to observe live microbes suspended in water.

Phase contrast microscope was invented by **Zernicke** in 1935. It is used in the study of living cells. The microscope is so designed that light passing through it is controlled by special phase contrast objectives and a condenser assembly, in Fig.

The special optical system of the microscope clearly makes visible materials differing only slightly in their refractive indices. The microscope has arrangements for controlled illumination. The light passing from one material through another of a slightly different density will be bent or refracted from its original path. These wavelength differences are transformed into corresponding variations of brightness. Therefore, structural details of objects varying only slightly in thickness or refractive indices could be easily seen.

The objectives are specially designed to enhance even the small differences. The lateral and central planes of objectives are adjusted to retard or advance wavelengths of light by annular phase plate. The phenomenon is called **phase effect.** In bright or negative contrast the two sets of rays are added and the object appears brighter than the surroundings. In dark or positive contrast, the two sets of rays are subtracted and hence the object appears darker than the surroundings.

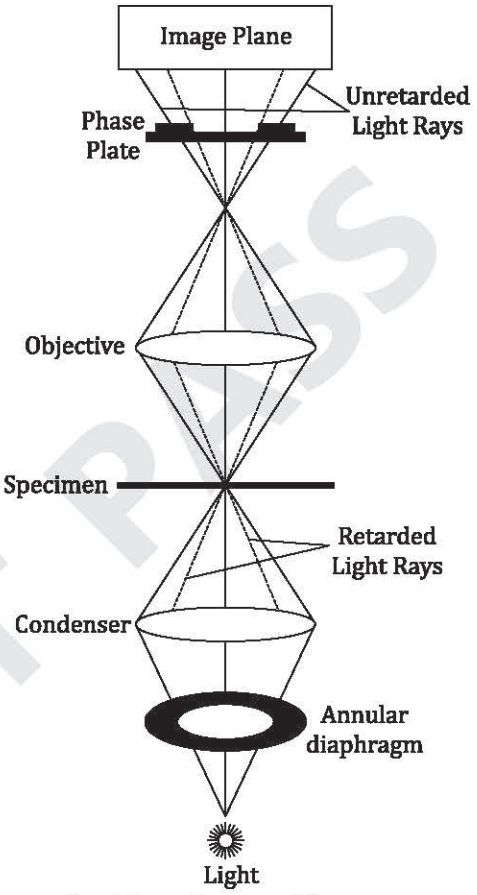


Fig. : Phase Contrast Microscope

Applications : 1. It allows observation of living cells, tissues, microbes, parasites, etc.

2. It is useful in the study of cultured cells to observe various stages of mitosis.

3. Phenomena like pinocytosis, phagocytosis, spermatogenesis, oogenesis, cyclosis, etc. can also be observed directly.

Limitations of Phase-contrast microscope : 1. Plant-contrast condensers and objective lenses add considerable cost to the microscope and so phase contrast is often not used in teaching laboratories, though they are in common use in microbiology laboratories of hospitals and research institutes.

2. To use phase-contrast, the light path must be aligned.

3. Generally, more light is needed for phase contrast than for corresponding bright-field viewing. Since the technique is based on the diminishment of the brightness of most objects.

Q.4. Write short note on fluorescence microscope.

Ans.

Fluorescence Microscope

One of the most widely used forms of microscopy in biological research today is fluorescence microscopy because it can be used to visualize the location of specific molecules in a cell. For example, specific proteins, or specific DNA sequence, can be visualized by this kind of microscopy.

Fluorescence was first reported by **Kohler** (1904). **Coons** (1941), introduced the fluorescent antibody technique. However, the basic technique of fluorescence microscopy was developed by **Price** and **Schwart** (1956). **Haitinger** (1959) and **Holborow** (1970). Certain chemicals appear coloured by absorbing ultraviolet light *e.g.*, vitamin A, thiamine, ribroflavin, calcium and porphyrins. This is called **auto fluorescence.** Some fluorescent dyes, called **fluorochromes,** are also used to stain and detect cell components. Catechol and indoleamines produce fluorescence with aldehydes. Acridine orange stain gives geen fluorescence to DNA and red to RNA. Fluorescence technique can be applied to all kinds of biological materials. Substances below the resolution of light microscope can be observed by fluorescent microscope.

Q.5. Describe the Laminar air flow technique.

Ans.

Laminar Air Flow

Laminar air flow is an equipment having an air blower in the rear side of the chamber which can produce air flow with uniform velocity along parallel flow lines. There is a special filter system-high efficiency particulate air filter (HEPA filter) which can remove particles as small as 0.3 μm. In front of the blower, there is also peculiar mechanism from which the air blown from the blower produces uniform air velocity along parallel flow lines. These are horizontal and vertical types.

1. **Principle :** Laminar flow can produce dust free air current with uniform velocity along parallel flow lines which help in transferring the culture media bacteria free. Air is passed through these special filters into the enclosure and the filters does not allow any kind of microbes to enter into the system. Due to uniform velocity and parallel flow of air current we can perform pouring, plating, slanting, streaking without any kind of contamination.
2. **Precautions :** Following precautions should be taken care of before handling the apparatus. We should put off our shoes before entering to operate the apparatus. We should wash our hands with soap and we should not talk inside the chamber while doing experiment, otherwise there will be a chance for contamination with certain bacteria or microorganisms through air of our mouth.
3. **Procedure :** Dust particles are removed from the surface of the laminar flow with the help of smooth cloth using alcohol. The UV light should be switch on for 30 minutes before performing the experiment and the front covering glass of laminar flow is opened and kept properly. The air blower is set at the desired degree so that the air inside the chamber is to be expelled, because the air which is already inside the chamber may be contaminated. One should sit properly in front of the chamber and wash the hands and stage of the chamber again with alcohol to reduce contamination. All the experiments, *i.e.*, pouring, plating, streaking etc. are to be done with in the flame zone of the sprit lamp. The required materials are to be placed side by side on the stage of laminar flow.
4. **Uses :** Within the chamber of laminar flow, we can transfer any media for culturing bacteria or fungi or any microbe without any contamination. The parallel and smooth air flow blown cot from inside the chamber of the laminar flow, is dust free.

Q.6. Write short note on staining technique.

Ans.

Staining Techniques

Depending upon the number of dyes used to stain a particular specimen, staining techniques can be broadly categorised into following two types :

1. **Simple staining :** A simple stain generally makes all the organisms in a sample appear to be of the same colour, even if the sample contains more than one type of organisms. This technique involves the application of aqueous or alcoholic solution of only a single basic dye. A simple stain generally highlights the entire organism as a result of which the shape and basic structures become clearly visible. The smear on the slide is rinsed to remove excess stain. Occasionally a mordant is added to the solution to intensify the stain. The mordant increases **the affinity of stain for a biological specimens;** it also coats the cellular structures to make them thicker so that they become easily visible under the microscope.
2. **Differential staining :** Bacteria differ from each other in their shape and size, but also in their chemical composition. In contrast to simple staining, differential staining distinguishes organisms based on their interactions with multiple stains. Thus differential staining distinguishes between types of bacteria; this is the principle of differential staining. Some commonly used differential stains are Gram stain and acid-fast stain.

   In a capsulated bacteria, clear halozone appears around bacterial cells against dark background. However, if no capsule is present, you will see no halozone.
3. **Flagella staining :** Flagella are tail-like extra-cellular structures used for locomotion by some bacteria, archae a and eukaryotes. Being very thin structures, flagella cannot be seen under light microscope without a specialized flagella staining technique.

   To stain flagella first applying mordant (generally tannic acid, occasionally potassium alum), which coats the flagella; then the specimen is stained with pararosaniline or basic fuchsin. Staining makes the flagella thick making them easily visible.

Flagella staining is commonly used by microbiologists, since the location and number of flagella can be useful in classification and identification of bacteria in a sample. When staining the flagella, it is important to handle the specimen with great care; flagella are delicate structures that can easily be damaged or pulled off. It may spoil the attempts to accurately locate and count the number of flagella.

Q.7. Differentiate between immobilized treated and immobilized non-treated cells.

Ans.

Types of Immobilized Cells

The immobilized cells can be categorized into following two classes :

1. **Immobilized Treated Cells :** These cells are often utilized in a dead state with appropriate treatment before or after immobilization, although the desired enzymes are in active and stable state.
2. **Immobilized Non-treated (living) Cells :** A variety of reactions can also be catalyzed by immobilized living cells, whether they are resting or growing in gel matrices. Immobilized resting cells are often termed as **immobilized non-treated cells.**

Immobilized cells are often kept in growing state within the gel-matrices by a continuous supply of suitable nutrients, especially if their biological functions are useful, as in case of conventional fermentation procedures. Immobilized growing cells serve as **renewable or self-proliferating biocatalysts.** These are located in a certain defined region of space and are protected against unfavourable circumstances outside their small domain. Physico-chemical interactions between gels and the cells often have favourable effects on the stability of entrapped cells.

However, both immobilized resting cells and immobilized growing cells have some **disadvantages**—(i) yield of products may be lowered by the consumption of substances as carbon and energy sources to maintain the cells in a living and growing state, and (ii) in case of immobilized growing cells, products may be contaminated by cells leaked from gel matrices. Nevertheless, **immobilized living cells are most promising because they retain enzyme activities for a very long time.**

Q.8. Write differences between light and electron microcopes.

Ans. Differences between Light and Electron Microscopes

1. Electron microscope has a cathode as the source of electrons either in the form of a filament or needle. In light microscope light rays are the illuminating agent.
2. For collecting and focussing the electron beam on the object there is an electro-magnetic condenser lens. In light microscope the light condenser is a concave mirror or biconvex lens.
3. The objective lens is also formed of electromagnetic substance, whereas it is simple achromatic lens is light microscope.

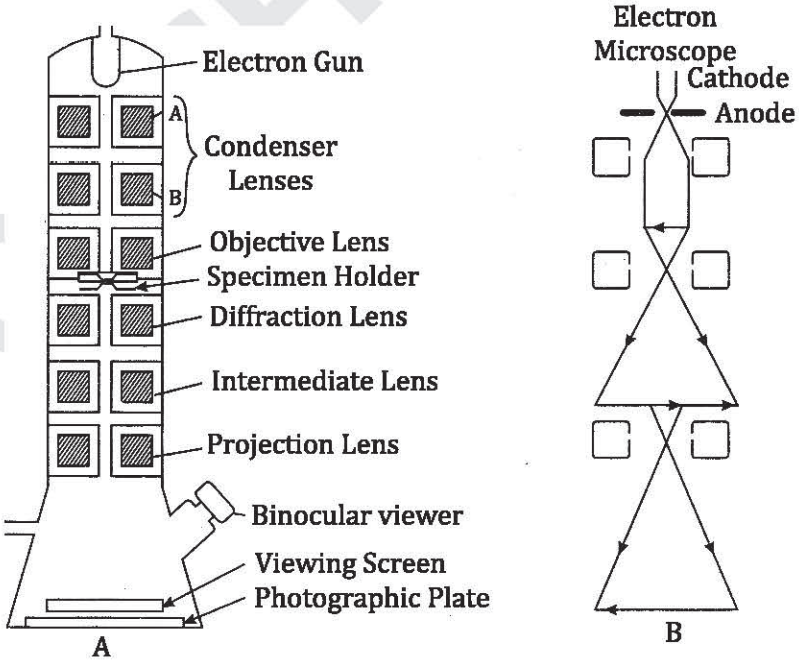


Fig. : Diagram to compare the formation of image in A. light microscope, B. Electron microscope

4. The image is received on a fluorescent viewing screen or photographic plate.
5. The source of illumination in electron microscope is electron beam, whereas it is light in case of light microscope.
6. In electron microscope image formation depends mainly upon the dispersion or scattering (or diffraction) of electrons; in light microscope image formation and contrast is brought about by differential absorption of light by different zones of the object.
7. Image formed by the electron microscope is not in the form as seen in light microscope but it gives position of molecules of the objects which are diffracted in the form of concentric rings or spots.

Q.9. Describe the comparison between immobilized and free cell fermentations.

Ans.

Comparison between Immobilized and Free Cell Fermentations

Character	Immobilized Cell Fermentation	Free Cell Fermentation
Cell growth	Fermentation is faster due to reduction of non-productive growth phase.	Slower fermentation because all growth is required for production.
Production	High substrate concentration.	Low substrate concentration to prevent inhibition.
	High cell productivity.	Low cell productivity.
	High product yield.	Low product yield.
	High final product concentration improved resistance of cells to inhibitory substrates or products.	Product inhibition often results in low final product concentration achieved.
Process operation	No cell was-out in continuous fermen -tation even at high dilution rate.	Difficult to perform continuous process due to cell washout.
	Smaller fermentor size needed due to the high cell density.	Large fermentor required.
	Simplified process design due to the separation of products and cells.	Effective separtion and concentration steps are necessary in downstream processing.
	Reuse of cells for prolonged period of time due to cell regeneration.	Cell cannot be reused.
	Reduced risk of microbial contamination.	More prone to contamination.

Q.10. Discuss Lambert-Beer's law of absorption of light by biomolecules.

Ans.

Lambert-Beer's Law

A wide range of biomolecules absorb light. Different biomolecules absorb light at different wavelengths. For example, amino acids tyrosine and tryptophan absorb light at 280 nm. The amount of light absorbed is measured by spectrophotometer and is used to detect and identify molecules present in the solution and to measure their concentration. Photometry or spectrophotometry is based on **Lambert-Beer's law.**

According to Lambert-Beer law the intensity of transmitted light is less than the intensity of incident light because some light is absorbed by the molecules present in the solution. The **Lambert-Beer's expression** is

$$\log \frac{I_0}{I} = \varepsilon cl, \text{ here}$$

1. ε **(epson)** is the molar extinction coefficient. It is expressed in units of litres per mole-centimeter.
2. *c* is the concentration of molecules of absorbing species. It is expressed in moles per litre.
3. *l* is the path length of the light absorbing sample. It repesents thickness of the absorbing sample and is expressed in cm.

The expression $\log \frac{I_0}{I}$ is called **absorbance** and is designated by ***A***. The absorbance **(A)** depends on the following features :

1. **Wavelength of Light :** Biomolecules absorb light at characteristics wavelengths. According to Lambert-Beer Law, the incident light absorbed is parallel and monochromatic. It is formed by light rays of single wavelength.
2. **Concentration of Absorbing Solute Molecules :** The absorbance of a given solution is directly proportional to the concentration of absorbing solute/molecules in the absorbing layer of fixed path length in the solution.
3. **Thickness of Absorbing Layer :** The amount of incident light absorbed by a given solution at a given wavelength is related to the thickness of absorbing layer. The thickness of absorbing layer is also called **path length.**
4. **Nature of Absorbing Molecules :** The amount of incident light absorbed by a solution at a given wavelength depends on the nature of light absorbing biomolecules.
5. **Absorption spectrum :** Absorption spectrum is a plot of the intensity of light absorbed relative to its wavelength. It is used to identify the molecules present in a solution. By studying absorption spectra of different pigments, it has been established that chlorophylls absorb most strongly in violet-blue and red regions of spectrum, while carotenoids like 8-earotme absorb maximum in green region and phycobilins in the middle band of the spectrum.
6. **Uses of spectrophotometer :** Spectrophotometer is the instrument used to measure the amount of light of specific wavelength that is absorbed by a solution. It is used to determine the nature of molecules and their concentration in a given solution. Spectrophotometry is the most widely used method for determining the amount of protein in nucleic acid present in a given solution.
7. **Different molecules of atoms absorb light of specific wavelengths :** The electrons of atoms or molecules become energised by absorbing photons from incident light. The energised electrons tend to move from inner to the outer orbitals. Since, the number of orbitals in which an electron can exist and the number of electrons that can stay in an orbital are limited, a given atom or molecule can absorb light of specific wavelengths.

Q.11. Write the principle and structure of colorimeter.

Ans.

Colorimeter

Principle of Colorimeter

Colorimeter is an instrument which works by using the interaction of light energy with coloured solutions of certain molecules or the coloured products of their reaction. When light passes through a coloured solution some wavelengths of light are absorbed more than others. The amount of light absorbed is proportional to the intensity of the colour of the solution and hence to the concentration of the compound in the solution. Colorimetry is based upon **'Beer Lambert Law'** which states that (i) absorption of light is related to the concentration of the absorber molecules and (ii) absorption of light is related to the path length or thickness of absorbing medium.

Structure of Colorimeter

The basic components of a colorimeter are :

1. A source of monochromatic light or a simple light source
2. Filter
3. Cuvette or cell to hold absorbing fluid
4. Photoelectric cell
5. A galvanometer to detect changes in the intensity of light signal and convert it into electric signal which can be read on the meter scale.

Components of Colorimeter

If some colometers monochromatic light source is used but in others coloured filters are used. Filters with different colours absorb all but only certain limited range of wavelengths. This limited range is called **band width** of the filter. The filter is placed before the absorption cell. The choice of filter is made to allow maximum transmission of colour absorbed.

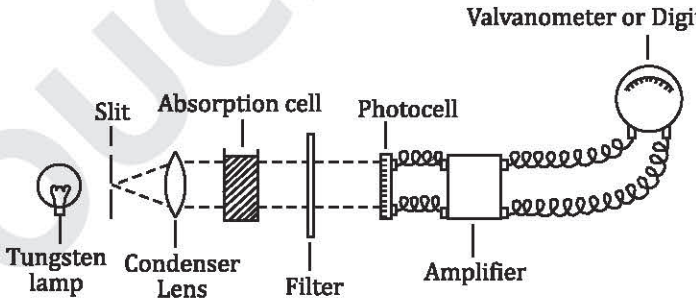


Fig. : Components of a Colorimeter

If a coloured solution is illuminated with monochromatic light of a known wavelength, its **optical density** (O.D.) is proportional to the concentration of coloured molecules multiplied by the depth of solution in the light path. It means the amount of light absorbed by a coloured solution is directly proportional to the logarithm of the thickness of the absorbing medium as shown by Beer Lambert Law :

i.e.,

$$\text{O.D.} = \log 10 \frac{I_0}{I}$$

where I_0 is the intensity of incident light and I is the intensity of transmitted light.

Q.12. Write a short note on stages of fermentation.

Ans.

Stages of Fermentation

Fermentation process has various stages, which primarily depend upon the nature of substrates :

1. Primary Fermentation

This is a brief phase in which microbes begin working rapidly on raw ingredients such as fruits, vegetables or dairy products. Microbes present in the surrounding medium prevent putrefying bacteria from colonizing the food. Instead yeast and other microbes convert carbohydrates (sugars) into other substances, such as alcohols and acids.

2. Secondary Fermentation

This is relatively a longer stage of fermentation which usually lasts for several days or even weeks. During this stage alcohol levels rise, yeasts and other microbes die off and their available food sources (the carbohydrates) become very limited. The pH of the ferment also changes which eventually affects the chemical reactions taking place between the microbes and the remaining substrates. Once the concentration of alcohol in the medium reaches beyond 12-15% it kills yeasts, thereby preventing further fermentation. The final liquid obtained is subjected to distillation to remove water contents. The alcohol thus obtained can be condensed to higher percentage of alcohol.

The length of stages of fermentation varies depending upon the nature of the desired and products.

Q.13. Write a detailed note on fermentation industry.

Ans.

Fermentation Industry

Fermentation industry is the collective term applied to the commercial processes that exploit the capacity of microbes to make useful products. Every microorganism useful in fermentation industry is unique and carefully selected for its ability to perform a desired task. Microbes freshly isolated from the environment or from stock cultures, are equally potential as source of useful products. It requires careful manipulation and selection of strains, it results in the production of profitable quantities of commercial products.

It is not uncommon for a microorganism present in the environment to synthesize metabolites other than those required for its own growth and maintenance. Metabolites such as amino acids, B-complex, vitamins, and nucleic acid bases may be overproduced by a microorganism and excreted into the proximate area in the micro environment. Other microorganism in the environmental niche can then assimitate these compounds. One strain may overproduce one metabolite but may require a metabolite released by another strain for its own growth.

Such organisms can survive when growing together. Such harmonious associations occur widely in nature, and microorganisms isolated from various niches commonly have requirement for one or more simple metabolites. Those microorganisms that tend to overproduce in nature may, when isolated in pure culture and subjected to the rigors of laboratory growth, produce even greater quantities of a given metabolite. Unbalanced growth in the laboratory may also lead to the expression of inherent genetic information and the production of identifiable quantities of metabolites such as antibiotics.

Recycling of organic matter in nature depends on the ability of microbes to synthesize enzymes that disassemble macromolecules. Among these are lipases, amylases and

proteinases. Several of these enzymes that digrupt macromolecules are of commercial interest.

Q.14. Give the detailed short note on continuous stirred tank bioreactor.

Ans. **Continuous Stirred Tank Bioreactor**

In this type of bioreactors, the suspension of microbes in the medium is stirred continuously by impellers (Fig.). During the process fresh nutrients are added and odd/used nutrient is removed from the fermenter. Consequently, the concentration of microorganisms and that of the components of the medium in the fermenter does not vary with time.

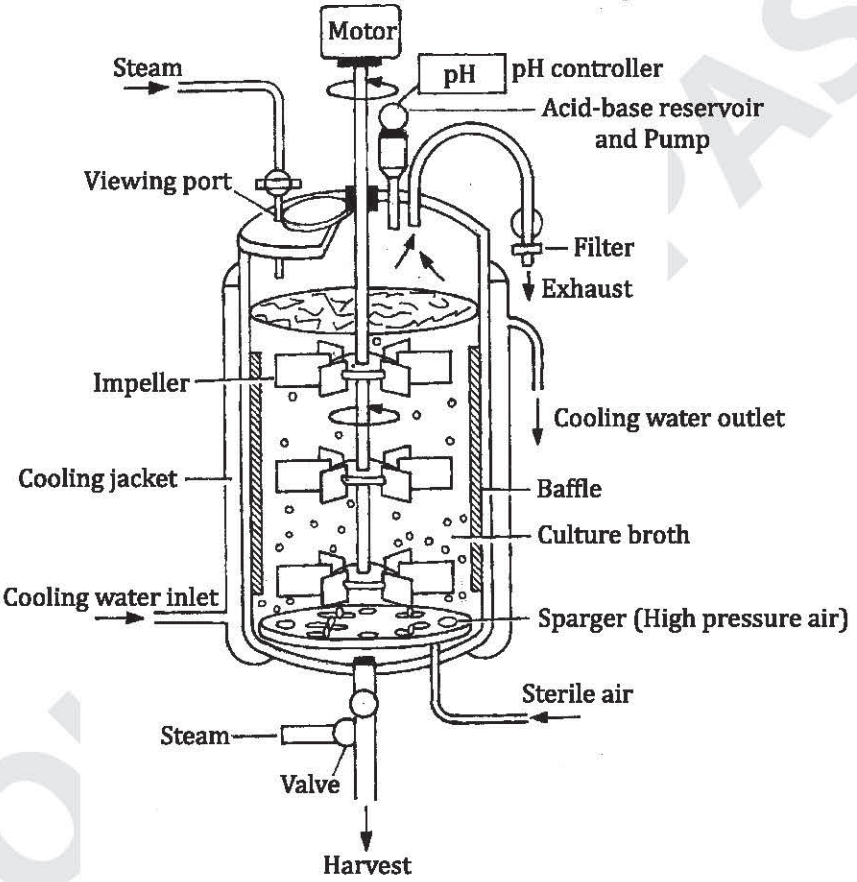


Fig. : Continuous stirred tank bioreactor

The most successful continuous system to data have been those employing yeast and bacteria. The fermenters commonly used in waste water treatment plants are based on this technology. In continuous stirred tank bioreactors several primary and secondary reactions are catalyzed by microorganisms, the rate of reactions depends upon the concentration of microorganisms in the medium. Therefore, while taking the products from the bioreactor, it is essential to retain at least a portion within the bioreactor. If the flow rate from the bioreactor is too high, then all the microorganism will be swept from the bioreactor and the reactions will eventually stop. The phenomenon is known as 'wash out'. If microorganisms are fed to the bioreactor simultaneously with the substrate feed, the problems associated with washout are abated, and the reactions proceed normally.

Q.15. What are the advantage of fermentation?

Ans.

Advantages of Fermentation

Some of the advantages of fermentation are as under :

1. Fermented foods are rich in probiotics. Probiotics have beneficial micro-organisms which help in extracting nutrients from food.
2. Probiotics help in boosting the immune system as the gut produces antibiotic, antiviral antitumor and antifungal substances. Pathogens don't survive in the acidic environment fermented food create.
3. Fermentation helps in neutralizing antinutrients like phytic acid which occurs in grains, nuts, seeds and legumes and can cause mineral deficiencies. Besides, phytales also make certain hard to digest starches, proteins and fats, therefore, neutralizing them is extremely beneficial.
4. Fermentation can increase the vitamins and minerals in food and make them more viable for absorption. Fermentation increases vitamin B and C and enhances folic acid, riboflavin, niacin, thiamin and biotin. The probiotics, enzymes and lactic acid in fermented foods, facilitate the absorption of these vitamins and minerals present in the body. Some major products obtained through the meditation of microbes are listed in table.

Major Commercial Products obtained from Microbes

S.No.	Food Category	Food Product
1.	Foods	(i) Fermented meat, cheese and milk products
		(ii) Edible mushrooms
		(iii) Leavened bread-baker's yeast
		(iv) Coffee
		(v) Pickles, olives
		(vi) Single-cell protein
2.	Flavouring agents and food supplements	(i) Vinegar
		(ii) Nucleotides
		(iii) Amino acids
		(iv) Vitamins
3.	Beverages	(i) Wines
		(ii) Beer
		(iii) Whiskey
4.	Organic acids	(i) Citric acid
		(ii) Itaconic acid
5.	Enzymes and microbial transformations	(i) Commercial enzymes
		(ii) Sterol conversions
6.	Inhibitors	(i) Biocides
		(ii) Antibiotics
7.	Products of genetically engineered microbes	(i) Insulin
		(ii) Human growth factors

Q.16. What is Neuberg's fermentation?

Ans.

Neuberg's Fermentation

Yeasts utilize pyruvate during fermentation resulting in the formation of an intermediary product acetaldehyde. This is trapped by hydrogen sulfite to yield the acetaldehyde in precipitated from and fluid product formation is glycerol as shown below :

$$CH_3CHO + NaHSO_3 \longrightarrow CH_2—CHOH—SO_3Na$$

Now in place of acetaldehyde , dihydroxyacetone phosphate acts as a hydrogen acceptor where is reduced to glycerol-3-phosphate. After removal of phosphate *i.e.,* dephoshorylation, it gives glycerol as given below :

$$C_6H_{12}O_6 + H_2SO_3 \longrightarrow CH_2—CHOH—SO_3Na + Glycerol + CO_2$$

Neuberg's fermentation process is categorized as reward and third fermentation. The first fermentation equation is given below :

$$2\ Glucose + H_2O \longrightarrow C_2H_5OH + acetate + glycerol + 2CO_2$$

Q.17. Write differences between light microscope and phase contrast microscope.

Ans.

Differences between Light Microscope and Phase Contrast Microscope

S.No.	Light Microscope	Phase Contrast Microscope
1.	There is no phase plate.	A glass phase plate is placed above the objective lens to increase the phase difference between the refracted and unrefracted light rays.
2.	Refracted light rays are delayed by one quarter wavelength (0.25 λ).	Refracted light rays are relayed by one half wave length (0.50 λ).
3.	Difference in the wave lengths of refracted and unrefracted light rays is not changed.	Increases the difference to produced maximum contrast in the image.
4.	The whole image looks equally bright.	Brightness corresponds to difference in the refractive indices of different cell organelles.
5.	Used both for living and dead organisms.	Distinction of structures in unstained living cells is greatly improved.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Describe about some famous Indian botanists.

Ans.

Contribution of Some Indian Botanist

1. **S.R. Kashyap (1882-1934) :** Professor S.R. Kashyap is called Father of **Indian Bryology.** He was born in Punjab in 1882. He obtained his M.Sc. degree in Botany from Punjab and went to Cambridge University for further studies. After completing his research degree he joined Govt. College Lahore.

   Professor Kashyap was first secretary of Indian Botanical Society. He was President of Indian Science Congress in 1932. Although he did some work on Pteridophyta also, he is known mainly for the work on Bryophyta. Two of his books are very famous-'Liverworts of Western Himalayas and Punjab Plains' Part I (1929) (S.R. Kashyap) and Part II (1932) (Kashyap and Chopra).

He discovered some new genera and many new species of Bryophyta. His theory of Retrogressive Evolution in Liverworts (Marchantiales) is well accepted by bryologists of the world.

2. **B. Sahani (1891-1949) :** Professor Sahani was born in Punjab in 1891. His field of specialization was Paleobotany. Due to his enormous contribution in this field, he is called **'Father of Indian Paleobotany'.**

   His main contribution is regarding the class Pentoxylae a gymnosperms of Jurassic period. Birbal Sahani Institute of Paleobotany was established in 1946 at Lucknow because of his untiring efforts. This institute is well known throughout the world. Scientists from different parts of the world come to work in this institute. He established Paleobotanical Society in India in the year 1946, a journal (Paleobotanist) which is published from Lucknow. Professor Sahani died in 1949.

3. **P. Maheshwari (1904-1966) :** Late Professor P. Maheshwari was Head of Botany Department at University of Delhi. His research work on the Embryology of plants is well known throughout the botanical world.

   Prof. Maheshwari was born in Jaipur in 1904. He obtained his M.Sc. in 1927 and Ph.D in 1931 from Allahabad University. He joined the staff of Botany Department of Agra College, Agra in 1930 and become Associate Professor in 1935. Afterwards he joined Allahabad University (1937 to 1939), Lucknow University (1939) and became reader and head of Botany Department at University of Dacca in the end of 1939. In 1949 he was appointed as Professor and Head of Botany Department at University of Delhi. He continued to hold this post until his death (1966).

   Prof. Maheshwari was president of Indian Botanical Society in 1951 and became founder and first president of society of plant morphologists in 1951. He was editor of the journal *Phytomorphology.*

   'An introduction to the Embryology of Angiosperms' written by Prof. Maheshwari is a widely recognised text book on the subject. Prof. Maheshwari in 1963 edited and published the book 'Recent Advances in Embryology of Angisoperms'. In recent years he was taking interest in the experimental side of embryology and established a new branch experiment embryology.

   Prof. Maheshwari was second Indian Botanist to be awarded F.R.S. by Royal Society of London in 1965.

4. **K.C. Mehta (1892-1950) :** Professor K.C. Mehta was born in Amritsar in 1892. His field of specialisation was Plant Pathology. He is famous for his research regarding the recurrence of rust in plains in India.

   He obtained his M.Sc. degree in 1914. He was appointed Assistant Professor of Botany at Agra College in 1915. In 1920, he went to Cambridge University where he worked on the Black Rust of Cereals. He was awarded Ph.D. degree by Cambridge University in 1922. In 1923 he became Professor of Botany at Agra College and soon after he was appointed Principal of that College. In 1941 he was awarded D.Sc. degree by Cambridge University.

   On the basis of this researches on recurrence of Black Rust in the plains of India he concluded that the infection (uredospore) spreads from Himalayas in the North and Nilgiri and Puleny Hills in the south. He presided over the session of Indian Botanical Society in 1939.

5. **M.S. Swaminathan :** Born 7 August, 1925; Kumhbakonam (Tamil Nadu), Ph.D. (1952), University of Cambridge, U.K.; D.Sc. (h.c.) from 33 Universities.

   Specialisation : Genetics, Cytogenetics, Plant Breeding, Sustainable Agriculture.

   Research Achievements : Swaminathan early researches (1947-60) included the elucidation of the origin and differentiation of cultivated potato (*Solanum tuberosum Linn.*), cytogenetic interrelationships among *Triticum* spp. induction of mutations for qualitative and polygenic traits in wheat, methodologies for detecting and assessing indirect, effects or radiations with particular reference to the nutritional safety of irradiated foods. His later researches (1960-80) had as their main focus the conservation of biological diversity with particular reference to rice and wheat, modification of plant architecture and growth rhythm for raising yield ceilings in wheat and rice and the development of crop production strategies which can lead to higher yields per unit by land, water, energy and time. Recent researches (1980-83) relate to rice breeding and biotechnology, impact of climate change on crop productivity and the observation of coastal biodiversity with particular reference to mangrove ecosystem.

   Positions : Director General, International Rice Research Institute, Philippines (1982-28); Member, Agriculture and Science, Planning Commission (1980-82); Principal Secretary, Ministry of Agriculture (1979-80); Director General, ICAR and secretary, Department of Agricultural Research and Education (1972-79); Director, Indian Agriculture Research Institute New Delhi (1966-72), President Indian Science Congress 1976 F.R.S.

6. **A.K. Sharma :** Born 1924; Calcutta (West Bengal), D.Sc., (1955), University of Calcutta.

   Specialization : Cytogenetics, Cytochemistry and Cell Biology. Research Achievements : Sharma's contributions include : new techniques for studying the physical and chemical nature of chromosomes, adopted all over the world for plant, animal and human systems, the latest technique being orcein banding for repetitive DNA; repeat DNA analysis as a measure of genetic diversity; a new concept of speciation in asexual organisms; clarification of the chemical nature of plant chromosomes through techniques specially evolved; inducing division in adult nuclei through certain metabolic precursor for studying chromosomes in relation to differentiation; reorientation of angiosperm taxonomy; a new concept of dynamism of structure and behaviour of chromosomes in plant, animal and human systems; using embryo irradiation and in-vitro cultures for generating variability; the concept of dynamic DNA; the tissue culture as a means for gene variability and conservation endangered species. Sharma has shown that the chemical composition of chromosomes varies during organogenesis, differentiation and reproduction, with the basic genetic skeleton being maintained.

   Position : Professor Centre of Advanced Study on Cell and Chromosome Research, Department of Botany, University of Calcutta.

   Learned and Professional Societies : President, Indian Society of Cytologists and Geneticists (1976-78), Indian Botanical Society (1980), President, Indian Science Congress (1981), Padma Bhushan (1983).

7. **R.P. Boy :** Born 1920; Gangapur (Bihar)

   Ph.D. (1953), University of Combridge, UK.

   Specialization : Cytogenetics, Plant Breeding, Tissue Culture, Cytotaxonomy. Roy's work on genomic (chromosomal) analysis of the species of *Aegilops* led to the identification of species involved in the origin and evolution of bread wheats. He detected an excellent sex determination genetic system in cucurbit (*Coccinea indica*). Established the relative role of X and Y chromosomes in the determination and manifestation of sex by raising a hierarchy of polyploid forms, namely triploid, tetraploid, pentaploid and hexaploid and also trisome, tetrasome, double trisome, etc. Located the specific chromosome region carrying the male maturity and fertility genes. Employed tissue culture to raise haploid, aneuploids, and sex-aberrant types to score specific genes and to locate mutant genes in the mutagenic treated materials. Pioneered cytogenetie investigations in an important timber sal (*Shorea robusta*), *S. assamica* and a dozen other allied timber genera. Did genome analysis by raising interspecific hybrids in the fern genus *Adiantum.*

   Positions : Senior Professor of Botany and Dean of Science, Head of Department and Coordinator, UGC centre of Special Assistance in Cytogenetics CSIR, Emeritus Scientist (1982), all at Patna University.

   Awards and Honours : President, Indian Science Congress (1972).

8. **Sir Jagdish Chandra Bose (1858-1937) :** Sir Jagdish Chandra Bose was born on November 13, 1858 in village Rari-khal (Now in Bangladesh). Sir Bose, did his experiments on plants while he was professor of Physics in Presidency college, Calcutta. His main contributions are as follows :

   (i) Like animals, plants are living and have almost similar metabolisms.

   (ii) While working with *Desmodium sp.* he gave a hypothesis that rythmic movements of cortical tissue, present outside the vascular tissue is responsible, for upward movement of water in plants. However, this theory is no longer tenable now.

   (iii) He designed many important instruments on which several discoveries of Botany and Physics are based. Some of these instruments are :

   (a) Resonant recorder,
   (b) Oscillating recorder,
   (c) Magnetic crescograph,
   (d) Photosynthetic recoder,
   (e) Diametric contraction apparatus.

In 1917, he established a research centre at Calcutta which is named after him as "Bose Research Institute, Calcutta". Due to his important contributions, he was awarded by F.R.S. (Fellow of Royal society of London in 1922). He was president of Indian Science Congress in 1927.

Q.2. What is centrifuge? Describe its structural components. Discuss the principle on which it works?

Ans.

Centrifuge

Centrifuge is a device which is used for physical separation of cellular components and macromolecules by using centrifugal force or gravitational pull. The cell organelles or organic

macromolecules fall out of the colloidal suspension and sediment according to their size or molecular weight.

Principle of Centrifugation

Centrifuge machine works on the principle of gravitational pull created by centrifugal force. When substances of sufficient mass are spun in a centrifuge, the centrifugal force creates a gravitational field in which heavier materials are pulled to the periphery of the circle. The centrifugal force applied to the sample is equal to the gravitational force, *i.e.,* the acceleration. It is measured in gravities (G)

$$G = \omega^2 r$$

where ω represents angular velocity and r is the radius of rotation.

The speed of centrifuge is measured as revolutions per minute (rpm).

Components of a Centrifuge

A centrifuge has two main parts : **head** and **rotor.** The head is a revolving disc having four metal cups or four containers for holding the tubes. The sample to be centrifuged is filled in these tubes. The rotor is a high speed motor which rotates the head to create centrifugal pull. The speed of rotation is controlled by a speed control switch.

Different Types of Centrifuges

Various types of centrifuges available in the market are classified into following four categories :

1. **Small Bench Centrifuges :** These are a simple table top models. Their rotation speed ranges rom 4,000 to 6,000 rpm and generates a relative centrifugal force (RCF) of 3,000 to 7,000 g. These are also called laboratory centrifuges. These are used for sedimentation of routine blood samples for separating RBCs.
2. **Continuous Flow Centrifuges :** These centrifuges are without tubes. Instead rotor is tubular. The particles are sedimented against its wall and the supernatant flows out continuously. They are used in large scale harvesting of bacteria.
3. **Ultracentrifuges :** Their speed varies from 60,000 to 1,00,000 rpm. These are very gentle and are used in the sedimentation of cellular and subcellular components. These are of two types :
   (i) **Preparative Ultracentrifuges :** Their speed is 80,000 rpm and centrifugal field upto 6,00,000 g. These are used in isolating and purifying cellular and subcellular components (such as ER, microsome fraction and ribosomes, etc.) and also biological macromolecules. The preparative ultracentrifuge may be of **different type** or **density gradient type.** In differential centrifugation the homogenate is exposed to increasing centrifugal force on after the other. Each time sediment is separated and homogenate is exposed to higher RCF.

   The sediment is called **pellet** and contains a particular fraction. In **density gradient centrifugation,** the cell organelles or cellular macromolecules are separated into layers according to their density and the viscosity of medium.

   (ii) **Analytical Utracentrifuges :** These have a speed of 70,000 rpm and RCF about 50,00,000 g. Their rotor contains a motor as well as optical device and is enclosed in a refrigerated and vacuum chamber. These are used in measuring differences in sedimentation coefficients and diffusion coefficients, in determining relative

molecular mass of the particles, estimation of purity of molecules and conformational changes in the macromolecules.

4. **Large Capacity Refrigerated Centrifuges :** These have the rotational speed of 6,000 rpm, RCF of 6,500 g and capacity of 100 cc. Their rotor chamber is refrigerated to control temperature. These are used to sediment RBCs and large cell organelles (nuclei, mitochondria and chloroplasts) and yeast cells.
5. **High Speed Refrigerated Centrifuges :** These have a speed of 25000 rpm and centrifugal force of 60,000 g. They are refrigerated and are used to sediment smaller micro-organisms, larger cellular organelles and cellular debris.

Working of a Centrifuge : A homogenate of paniculate matter is prepared. It is poured into the centrifugal tubes. The tubes are then placed in the metal cups of the head. The speed of the rotor is according to the requirement. The rotor is allowed to run for a required period. The particulate matter or its constituents get settled at the bottom of the lube.

Q.3. Describe structural components and applications of spectrophotometer.

Ans.

Spectrophotometer

Spectrophotometer is the instrument that measures the absorption of radiation in the visible and UV regions of spectrum. It is a sophisticated type of colorimeter, Spectrophotometer is a combination of two words : Spectro implies the whole range of continuous wavelength that is produced by a light source and photometer is a device for measuring light. It means it can measure the light rays of wavelength ranging over UV as well as visible regions of the spectra and obtain absorption spectra.

Components of Spectrophotometer

The basic components of spectrophotometer are :

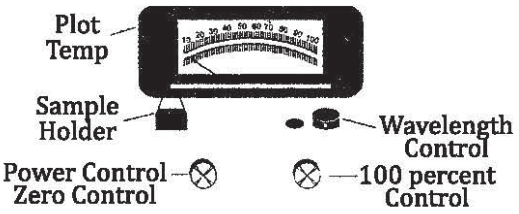


Fig. : Spectrophotometer

1. **Source of Light :** The light source provides electromagnetic radiation in the UV and visible regions of the spectrum. A tungsten lamp is used to obtain light in the visible range. For UV region, a hydrogen lamp or deuterium lamp or Xenon lamp is used. For infra-red rays either a Nernst glower or globar is used. Nernst glower is a hollow rod of yttrium and zirconium oxide. On being heated to about 1450°C, it produces infra-red rays. Globar is a rod of silicon carbide heated to about 1200°C. A small slit allows the passage of a thin light beam to pass through and reach the monochrornator.
2. **Monochromator :** Monochromators produce light only one colour or of one particular wavelength. They are either optic filters or diffraction gratings or both. Glass prisms are used for visible light and quartz prism is used for UV light (glass absorbs radiation below 400 nm). The instrument has a provision for selecting required wavelength by adjusting the knob.

   The slit S_2 allows a thin beam of desired bandwidth to pass through and reach the sample.
3. **An Absorption Cell :** This is the area where sample is placed in a cuvette. The cuvette used for optical light is made up of glass and is optically transparent, but for UV light it

is made of quartz. It has optical path length of 1 cm and contains 2.5-3 ml sample solution.

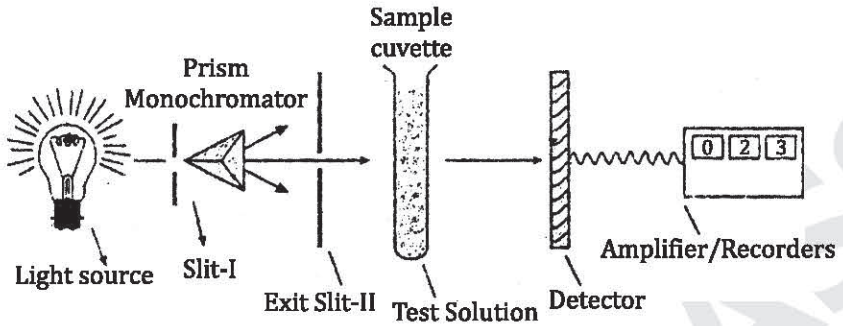


Fig.

4. **A Detector :** It measures absorbance of light rays or UV rays transmitted by the sample solution. It is formed of a **photocell,** which compares the intensity of light transmitted by the pure solvent (I) with that, transmitted by the sample solution (I_0). For the visible and UV light photoelectric detectors or **photocells** are used. For infra-red rays thermal detectors like bolometers are used. In the photocell, photons impinging on the metal surface in vacuum cause emission of electrons. These are attracted by a positive electrode. Thus, a current of electron flows which causes a potential difference across a resistor present in the system.
5. **Potentiometer :** The potential difference is recorded on the potentiometer. It is calibrated to read absorbance and transmittance.

   The value of absorbance $\frac{A}{I} = \log I_0$

   I and of transmittance $I = \frac{I}{I_0}$

   The transmittance is recorded as a percentage and may vary from 0 to 100. The range of absorbance recorded ranges from 0-2.0.
6. **Amplifier :** Photomultiplier tubes are used instead of photocells, because in photomultiplier tubes the emitted electrons are accelerated by high potential and produce secondary electrons by collision with gas molecules. This results in large current. Therefore, even very small changes in electron concentration are measured.

Application of Spectrophotometer

Spectrophotometer is used in :

1. Measuring the growth kinetics.
2. Structural studies of proteins and nucleic acids.
3. Enzyme kinetics and assays.
4. Measure effect of pH, ionic strength, etc.
5. Qualitative and quantitative estimation of biomolecules like proteins nucleic acids, cytochromes and pigments.
6. Testing purity and homogeneity of samples and compounds.
7. Identification of compounds.

Q.4. What is cell immobilization? What are the advantage of this technology?

Ans.

Cell Immobilization

Cell immobilization has been defined as physical confinement or localization of viable microbial cells to a certain defined region of space in such a way as to limit their free migration while retaining their catalytic activities for repeated and continuous use.

In present times, immobilization is applied basically to all types of biocatalysts including enzymes, cellular organelles, animal and plant cells, microorganisms, etc. Currently immobilization have found wide applications not only in the field of biotechnology, but also in pharmaceutical, environment, food energy and biosensor industries. In fact, cell immobilization has emerged as an alternative for enzyme immobilization. Immobilization of cells containing specific enzymes has several advantages such as elimination of long and expensive procedures for enzyme separation and purification. In comparison to immobilized enzymes, immobilized cells provide new possibilities since they can be used as natural enzymes.

Immobilization Techniques

Immobilization of cells requires a **support** or a **carrier** which can hold multiple cells in a limited space. Selection of carrier with good performance is one of the most important issues in the application of immobilized microbial technology. Ideally, the immobilized microbial cell carrier should have good mass transfer, air permeability and transmittance. It should not be easily decomposed by microorganisms, must have good mechanical strength, long service life and lost cost.

Commonly used carriers can be classified into three categories :

1. **Organic carriers :** These include **polymer gel carrier,** such as agar, carrageenan, calcium alginate, etc., and **polymer gel carriers of organic synthesis,** such as polypropylene, ammonium polyacrylate gel, polyvinyl alcohol gel, light hardening resins, polypropylene acid gel, etc.
2. **Inorganic carriers :** Inorganic carrier include porous glass, diatomite, activated carbon, quartz, sand, etc.
3. **Composite carriers :** These are combination of inorganic and organic materials. These two types of materials compliment each other, it shows superiority of composite materials.

Following are some of the common techniques of immobilization :

1. Entrapment 2. Encapsulation 3. Adhesion/Adsorption 4. Polyurethane foam for Immobilization by adsorption.

Applications and Advantages of Immobilized Cells

Cell immobilization is one of the most exciting aspects of biotechnology. It has been used as one of the most effective methods to improve the performance and economics of many fermentation processes. The immobilized cells provide easy separation of biomass in fermentors. It allows for more efficient operation by reducing the non productive growth phase. It is well recognized that the high cell density of immobilized cells improves the product yield.

Besides wide scale application of immobilized cells in fermentation, in recent times immobilized cells are being used in several new areas. For example, these are being used in

bioremediation of hydrocarbons. Entrapment of cells inside a porous matrix is most suitable technique for this purpose. Entrapped cells are more tolerant to pH and temperature changes. In addition, due to easier separation of micro-organisms by these techniques immobilized cells are generally preferred to free cell systems.

Immobilized (biocatalyst) cells are being utilized for the production of biofuels. For example, production of ethanol from glucose and molasses using resting cells of *Saccharomyces cerevisiae* entrapped in calcium alginate and α-carrageenan, respectively. A variety of other compounds, such as L-isoleucine, bacitracin, 2,3-butanediol, α-amylase, protease, vitamin B_{12} etc. have also been obtained using immobilized cells.

Some applications of immobilized treated cells

S.No.	Micro-organism	Immobilization method	Substrate	Product
1.	*Escherichia coli*	Entrapment in polysacchride gel	Ammonium fumarate	L-Aspartate
2.	*E. coli*	Entrapment with α-carrageenan	Ammonium fumarate	L-Aspartate
3.	*Erwinia herbicola*	Entrapment in collagen	Pyruvate + Phenol + NH_3	L-Tyrosine
4.	*Saccharomyces cerevisiae*	Entrapment in polyacrylamide gel	L-Glutamate + L-cysteine + glycine	Glutalhione
5.	*Brevibacterium ammoniagenes*	Entrapment in polyacrylamide gel	Fumarate	L-malate
6.	*Micrococcus luteus*	Immobilization on carbodimide activate carboxymethylcellulose	L-Histidine	Uroconic acid

As you know, microbial cells and cellular organelles contain metabolic systems that catalyze or mediate more complicated reactions for the synthesis and conversion of numerous compounds. The immobilized microbial cells and organelles, in turn, permit immobilization of multistep and cooperative enzyme systems. Furthermore, with immobilization of microbial cells, the procedure for extracting enzymes form the cells is no longer required. This avoids inactivation of enzymes during tedious and time consuming purification procedures. This increases the stability of many membrane associated enzymes. This illustrates the advantages of immobilized microbial cells over immobilized enzymes in various fields.

Q.5. Describe the fermentation and their types.

Ans.

Fermentation

Fermentation is a metabolic process by which molecules such as glucose are broken down anaerobically to provide energy for the growth of micro-organisms. During the process carbon dioxide is released, a characteristic feature of fermentation. Thus fermentation is an ATP generating metabolic process in which one organic compound (the energy source) serves as a donor of electrons and another organic compound is the electron acceptor. The term fermentation was first used by French chemist and microbiologist, **Louis Pasteur** in the 19th century to describe the changes brought about by yeasts and other micro-organisms growing anaerobically. He also recognized that ethyl alcohol and carbon dioxide are the only products of fermentation.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2$$

Later it was discovered that fermentation reactions are not peculiar to the action of yeasts but also occur in many other instances of glucose utilization. For example, during strenuous activity, muscles use ATP faster than oxygen can be supplied to muscle cells. In such circumstances, muscle cells catalyze the formation of lactate from glucose.

The principal substrates for fermentation include carbohydrates, amino acids, purines and pyrimidines. The most studied fermentation processes include alcoholic fermentation by yeasts, lactic acid fermentation in bacteria and anaerobic dissimilation of amino acids by clostridium.

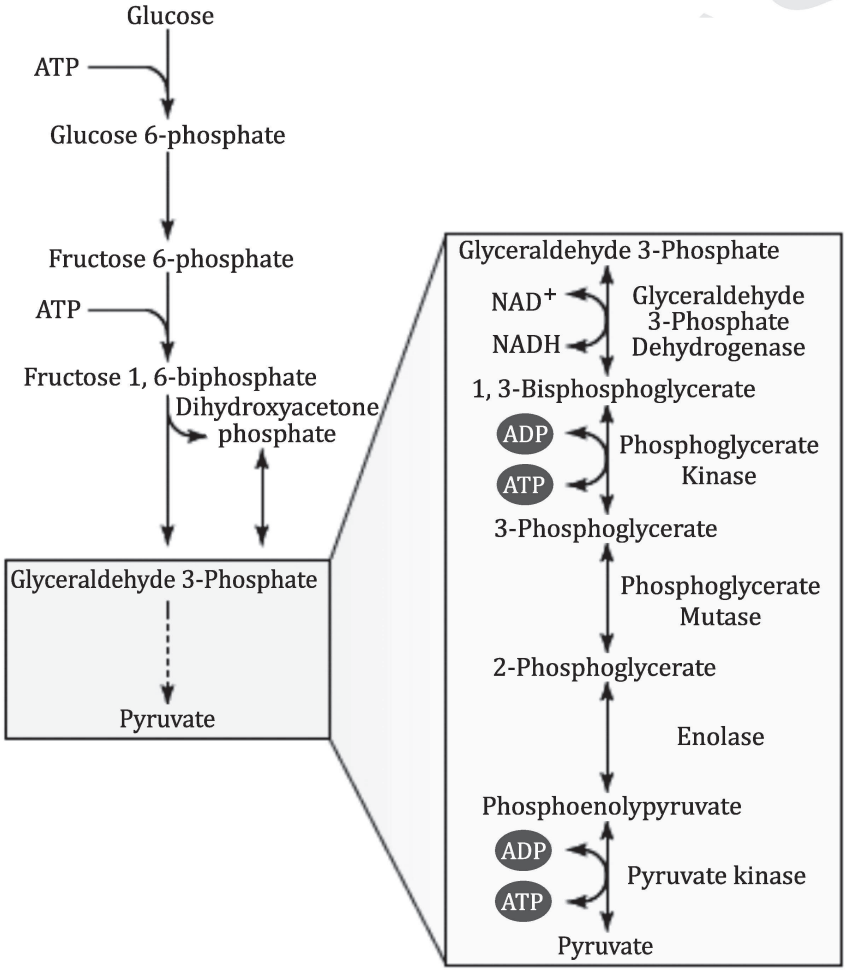


Fig. : Fermentation : Generation of pyruvate through the process of glycolysis is the first step in fermentation

Fermentation is essentially a closed system in which the redox level of substrate and products are internally balanced. This is illustrated by the following reaction :

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \longrightarrow \underset{(6C+12H+6O)}{2CH_3CH_2OH+2CO_2}$$

One good example of balanced fermentation process that occur under strictly anaerobic conditions is **glycolysis.** This pathway, as you know, is also the major route of glucose catabolism in aerobic organisms. Besides, it is also the energy producing system in many lactic acid bacteria and other micro-organisms that utilize sugar anaerobically. The lactic acid fermentation requires two molecules of ATP to form fructose 1,6-diphosphate, which is cleaved to two molecules of glyceraldehyde 3-phosphate. The oxidant NAD along with glyceraldehyde 3-phosphate dehydrogenase generates a high free-energy intermediate. This undergoes a phosphate (Pi) substitution to form energy-rich 1,3-diphosphoglycerate. The end product of the reactions is pyruvate. The reduction of pyruvate to lactate permits the regeneration of the oxidized form of NAD^+. The net reaction is :

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} \rightarrow 2\text{Lactate} + 2\text{ATP}$$

The total amount of energy generated by glycolysis (2ATP) is meager when compared with 38ATP that can result from the complete aerobic oxidation of one molecule of glucose.

Strictly anerobic clostridia have evolved with an array of ATP generating mechanisms that utilize many different substrates. Among the substrates that can be fermented by various strains of the genus *Clostridium* are pyruvic acid, purines, pyrimidines, nicotinic acid, carbohydrates and amino acids. Other energy rich compounds that can be involved in substrate level phosphorylation are propionyl-CoA, butyryl-CoA and butyrylphosphate.

There are several distinct fermentation pathways that bacterial species living under anaerobic conditions use to generate energy. Generally these fermentations that involve glucose and the major products generated are listed in table.

Examples of Products Generated during Fermentation of Glucose and the Organism Involved.

S.No.	Type	Product	Organism
1.	Mixed acid	Ethanol + acetate + lactate	*Escherichia coli*
2.	Butanediol (neutral)	2,3-butanediol + ethanol	*Entrobacter aerogenes*
3.	Alcoholic	Ethanol	*Zymomonas mobilis*
4.	Homolactic	Lactate	*Lactobacillus acidophilus*
5.	Heterolactic	Lactate + ethanol	*Lactobacillus brevis*
6.	Butanol/acetone	Butanol + acetone	*Clostridium butyricum*

Small amounts of other compounds may be produced during sugar fermentation such as alcohols, oragnic acids, CO_2 and molecular hydrogen (H_2). Other fermentations, not involving sugars, are listed in table. One necessity is a oxidation/reduction state of the products is equivalent to that of the substrate.

Bacterial Fermentation that Utilize Substrates other than Sugars

S.No.	Type	Product	Bacteria
1.	Homoacetic acid	$4H_2 + CO_2 \rightarrow CH_3COOH$	*Clostridium aceticum*
2.	Propionic acid	Lactate $\rightarrow$ Propionate + acetate	*C. propionicum*
3.	Acetylene	Acetylene + $H_2O \rightarrow$ Ethanol + acetate	*Pelobacter acetylenicum*
4.	Oxalate	Oxalate $\rightarrow$ Formate + CO_2	*Oxalobacter formigenes*
5.	Malonate	Malonate $\rightarrow$ Acetate + CO_2	*Malonomas rubra*

Types of Fermentation

Fermentation is generally categorized into following three types :

1. Lactic Acid Fermentation

Certain strains of yeast and bacteria convert starches or sugars into **lactic acid,** requiring no heat during the process. In these anaerobic chemical reactions, pyruvic acid uses nicotinamide adenine dinucleotide and hydrogen (NADH) to form lactic acid and NAD^+. Lactic acid fermentation also occurs in human muscle cells, as mentioned earlier in this chapter.

Glucose → 2 Lactic acid

Lactic acid bacteria are vital to productivity and preserving inexpensive wholesome foods, which is especially important in feeding impoverished populations.

2. Alcoholic Fermentation

This kind of fermentation produces ethanol (an alcohol), hence also known as **ethanol fermentation.** This process involves two main reactions :

(i) The first reaction is catalyzed by pyruvate decarboxylase, a cytoplasmic enzyme. It removes a carboxyl group (COOH) from pyruvic acid, releasing CO_2 as gas. The loss of CO_2 reduces the size of the molecule by one carbon, making acetaldehyde.

(ii) The second reaction is catalyzed by alcohol dehydrogenase to oxidize NADH and NAD^+ and reduces beverages. Ethanol tolerance of yeast is variable, ranging from about 5% to 21% depending on the yeast strain and environmental conditions.

$$\text{Glucose} \xrightarrow[2ADP + 2Pi \quad 2ATP]{\text{Pyruvate decarboxylase}} 2 \text{ Pyruvate}$$

$$2 \text{ Pyruvate} \longrightarrow \text{Acetaldehyde} + 2CO_2$$

$$2 \text{ Acetaldehyde} \xrightarrow[2NAD + 2NAD^+]{\text{Alcohol dehydrogenase}} 2 \text{ Ethanol}$$

3. Acetic Acid Fermentation

Starches and sugars from grains and fruits ferment into sour tasting **vinegar.** These include apple cider vinegar, wine vinegar, etc.

The process involves two-stage fermentation. The first step is **anaerobic fermentation** (it is alcoholic fermentation of sugars into ethanol by yeasts). The second step is an **aerobic fermentation** in which ethanol is oxidized into acetic acid.

Acetic acid is widely used in pickled vegetables, sauce, salads as a raw material for spice.

❑

UNIT-II

Microbial World

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. Who discovered phenomenon of transduction in bacteria?

Ans. In 1952 Zinder and Lederberg discovered phenomenon of transduction in bacteria.

Q.2. Who described conjugation in bacteria?

Ans. Wollman, Jacob and Hayes described conjugation in bacteria.

Q.3. What do you understand by F^+, F^- and Hfr?

Ans. **F^+ cell :** Bacterial cells with fertility factor or F-factor.

F^- cell : Bacterial cells with no fertility factor or no sex factor.

Hfr cell : Donor bacterial cells having high frequency of recombination in which plasmid with F-factor has become integral part of bacterial chromosome.

Q.4. What is sexduation?

Ans. During conjugation when a copy of F-factor from donor (male) cell is transferred to recipient cell and changes the F^- or recipient cell into male or donor cell. This is called **sexduction.**

Q.5. Write two name of *Bacillus* bacteria.

Ans. *B. larvae* and *B. Thuringiensis*

Q.6. What is the main component of cell wall of bacteria?

Ans. Peptidoglycan or murein is the main component of cell wall of Bacteria.

Q.7. Why viruses are also called 'nucleoproteins'?

Ans. Viruses are called nucleoproteins because their body is made up of only nucleic acid and protein. The nucleic acid (either DNA or RNA) is centrally placed and surrounded by a protein coat or covering called capsid.

Q.8. What is a bacteriophage?

Ans. Bacteriophages are the bacteria-eater viruses because they attack bacteria colonies and destroy (lyse) it. The normally are tadpole-like having a hexagonal head and a cylindrical tall. They were discovered by Twort and D'Herelle (1915-17).

Q.9. What is a prophage?

Ans. During lysogenic life cycle the genome of the bacteriophage injected into the host, bacterial cell normally sporoporates or integrates into the bacterial chromosome and remains silent for generations. This state of phage-genome is called prophage.

Q.10. What is a capsid?

Ans. Capsid represents the protein-coat or protein covering around the nucleic acid of a virus. Capsids are made up of many units called capsomeres.

Q.11. What is cyanophage?

Ans. These viruses are parasites on blue-green algae (Cyanophyceae). These were of all discovered in 1963 by **Shafferman** and **Moris.** The structure of cyanophages is similar to the bacteriophages and contain double stranded DNA.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Write the differences between prokaryotic and eukaryotic cell.

Ans. **Differences between Prokaryotic and Eukaryotic Cell**

S.No.	Character	Prokaryotic cell	Eukaryotic cell
1.	Nuclear body	Incipient nucleus. No nuclear membrane	True nucleus, Nuclear membrane present
2.	Mitosis	No Mitosis	Mitosis found
3.	DNA arrangement	One or more molecules but of one type, not in chromosome (histones absent)	In several or many Chromosome (histone present in Chromosome)
4.	Respiratory system	Part of plasma membrane, mitochondria absent	in mitochondria
5.	Photosynthetic apparatus	In internal membranes, chloroplasts absent	In chloroplasts
6.	Golgibodies, Endoplasmic reticulum, Lysosome	Absent	Present
7.	Ribosomes	70S types	80S types
8.	Cell wall	Thin	Thick, chemically different
9.	Flagella	Submicroscopic	Microscopic size, each flagellum composed of fibrils in a distinct 9 + 2 pattern.
10.	Cytoplasmic movements	Cytoplasmic streaming rare or absent	Cytoplasmic streaming often occurs.
11.	Vacuoles	Absent	Present
12.	Lysosome	Absent	Present in animals
13.	Capsule	May be present	Always absent

Q.2. Write the differentiate between Gram negative and Gram positive bacteria.

Ans. **Differences between Gram-negative and Gram-positive Bacteria**

S.No.	Characteristics	Gram-positive bacteria	Gram-negative bacteria
1.	Cell wall structure	Cell wall single layered and 150-200 Å thick.	Cell wall triple layered and 75-120 Å thick.

2.	Chemical composition	Peptidoglycans account for about 80% of the cell wall and the rest are polysaccharides Teichoic acid present. Low in lipids (1-4%) Highly responsive to triphenylmethane Resistant to alkalies and insoluble in 1% KOH solution.	Peptidoglycans account for only about 3-12% of the cell wall. It is mainly composed of lipoproteins and lipid polysaccharides Teichoic acid absent High in lipids (11-22%) Show little response to triphenyl methane Show sensitivity to alkalies and soluble in 1% KOH solution
3.	Rigidity of cell wall	Cell wall is very rigid due to high proportions of peptidoglycans	Cell wall is elastic due to plastic nature of liporprotein -polysaccharide mixture
4.	Susceptibility to penicillin	High susceptibility	Low susceptibility
5.	Nutritional requirements	Relatively complex in many species	Relatively simple

Q.3. What is photosynthetic bacteria?

Ans.

Photosynthetic Bacteria

These are also known as **photoautotrophic** or **photolithotrophic** bacteria. Like higher plants, they are capable of converting radiant energy into chemical energy. The generalized reaction of photosynthesis is :

$$CO_2 + 2H_2A \longrightarrow (CH_2O) + H_2O + 2A$$

Where H_2A is an oxidizable compound and A is the corresponding oxidation product. In higher plants oxygen substitutes A, thus the hydrogen donor is water and the process produces free oxygen.

$$CO_2 + H_2O \longrightarrow (CH_2O) + O_2$$

In bacterial photosynthesis, however, various substances may substitute A, but never oxygen. Oxygen is not released during photosynthesis and as such these bacteria are anaerobic.

Photoautotrophic bacteria are of the following types :

1. **Green sulphur bacteria :** These are small nonmotile, rod-shaped bacteria which are strictly anaerobic photoautotrophs. Their photosynthetic pigment is **chlorobium chlorophyll,** located in the invaginations of cytoplasmic membrane. These bacteria use H_2S or other reduced inorganic sulphur compounds (sulphite or sulphide) as electron donor. Elemental sulphur formed as a by product in this process is deposited extracellularly. *Chlorobium, Prosthecochloris, Pelodictyon,* and *Clathrochloris* are four well known examples of green sulphur bacteria.

$$6CO_2 + 12H_2S \longrightarrow C_6H_{12}O_6 + 6H_2O + 12S$$

2. **Purple sulphur bacteria :** These are also autotrophs and their photosynthetic pigments are **bacteriochlorophyll *a*** and / or ***b.*** The reducing power is provided by H_2S which is oxidized anaerobically, via elemental sulphur, to sulphate. The sulphur is deposited in them intracellularly (extracellularly in *Ectothiorhodospira*). The overall reaction can be represented schematically as :

$$2CO_2 + H_2S + 2H_2O \xrightarrow[\text{bacteriochlorophyll}]{\text{light}} 2(CH_2O) + H_2SO_4$$

Some purple sulphur bacteria can use other reduced sulphur compounds (thiosulphate, sulphite, etc.) in place of H_2S as exogenous reductants.

$$6CO_2 + 9H_2O + 2Na_2S_2O_3 \xrightarrow[\text{bacteriochlorophyll}]{\text{light}} C_6H_{12}O_6 + 6NaHSO_4$$

Thiospirillum, Chromatium, Thiocystis, Thiocapsa, Lamprocystis and *Amoebobacter* are some common examples of purple sulphur bacteria.

3. **Non-sulphur purple bacteria :** These are motile bacteria which do not produce gas vacuoles and never accumulate sulphur within the cell. Their photosynthetic pigment is also bacteriochlorophyll. In their metabolism some organic compounds replace sulphur and the extent of CO_2 reduction depends upon the organic substrate. When the substrate is reduced more than the cell material (CH_2O). *i.e.,* the ratio of hydrogen to oxygen is greater than two to one, the excess hydrogen is used to reduce CO_2. On the other hand, if there is greater oxidation of the substrate, CO_2 is given off just as in respiration.

   For example, if propionic acid is the substrate, CO_2 is reduced :

$$\underset{\text{(Propionic acid)}}{2C_3H_6O_2} + H_2O + CO_2 \longrightarrow 7(CH_2O)$$

   However, if malic acid is the substrate, CO_2 is released :

$$\underset{\text{(Malic acid)}}{C_4H_6O_5} \longrightarrow 3(CH_2O) + CO_2$$

   Non-sulphur purple bacteria can grow in the presence of oxygen unlike purple sulphur bacteria.

Q.4. Describe briefly the batch culture.

Ans.

Batch Culture

Batch culture is generally used for the cultivation of bacteria, yeast and other filamentous fungi in the laboratory. The system consists of a flask or fermenter which is filled with sterilized nutrient solution. It is inoculated with the desired microorganisms. The inoculated flask is maintained at an appropriate temperature for the required period of time.

During the course of entire fermentation nothing is added except oxygen (in case of aerobic microorganisms), an antifoam agent and acid or base to control pH.

As the microbes grow, the nutrients are gradually consumed and by-products accumulate; this affects the growth of the microorganisms.

Following six typical phases of growth can be distinguished :

1. **Lag phase :** This is the initial phase with no apparent growth. However, actual biochemical analyses show metabolic turnover, indicating the cells are in the process of adapting to the environmental conditions and that new growth will eventually begin.
2. **Transient acceleration phase :** This phase marks the beginning of growth in the inoculum cells.

3. **Exponential phase :** In this phase microbial growth proceeds at the maximum possible rate. The nutrients are available in sufficient quantity and the environmental parameters are also optimum. At this stage these are no growth inhibitors in the medium.
4. **Deceleration phase :** Bacteria growing in batch culture go through an aging process. As a result the cells in the later stages of growth would be physiologically different than the cells in the middle stages of growth; their growth actually slows down. This phase is known as deceleration phase. Since during exponential phase the nutrients get exhausted due to rapid growth of microorganisms, hence the deceleration phase follows.
5. **Stationary phase :** Towards the end of the deceleration phase the microbial, growth stops completely due to nutrient exhaustion. This is known as stationary phase.
6. **Death phase :** This is the final stage of the cycle in which growth stops completely. Most biotechnical batch processes are stopped before the death phase as metabolic activity completely stops and cell lysis sets in.

Advantages of Batch Culture

1. The growth cycle in batch culture is of short duration, hence, there is reduced risk of contamination.
2. As compared to continuous culture, batch culture has lower capital investment.
3. Batch culture is easy to operate. The process is often used to optimize conditions in the early stages of experimental design.

Disadvantages of Batch Culture

1. The time intervals between batches are long as the entire process—cleaning, sterilization, preparation and filling of nutrients, and inoculation has to be repeated after each batch.
2. Product obtained also has reagents (used in the process), cell debris; and toxins.

Q.5. Write the differentiate between plant and animal virus.

Ans.

Differences between Plant and Animal Viruses

S.No.	Plant virus	Animal virus
1.	The genetic material is surrounded only by capsid, it forms the external boundary.	An envelop is present outside the capsid.
2.	Most plant viruses contain RNA as genetic material.	Most animal viruses have DNA as genetic material.
3.	Nucleic acid single stranded.	Nucleic acid double stranded.
4.	Nucleic acid linear.	Nucleic acid linear or circular.
5.	Enter the host body through wound or pore.	Enter the host cell through phagocytosis.

Q.6. Write short note on structure of TMV.

Ans.

Structure of Tobacco Mosaic Virus (TMV) (RNA Virus)

Tobacco mosaic virus (TMV) is a positive-sense single-stranded RNA virus species in the genus Tobamovirus that infects a wide range of plants, especially tobacco and other members

of the family Solanaceae. The infection causes characteristic patterns, such as "mosaic"-like mottling and discoloration on the leaves (hence the name). TMV was the first virus to be discovered. Although it was known from the late 19th century that a non-bacterial infectious disease was damaging tobacco crops, it was not until 1930 that the infectious agent was determined to be a virus. It is the first pathogen identified as a virus.

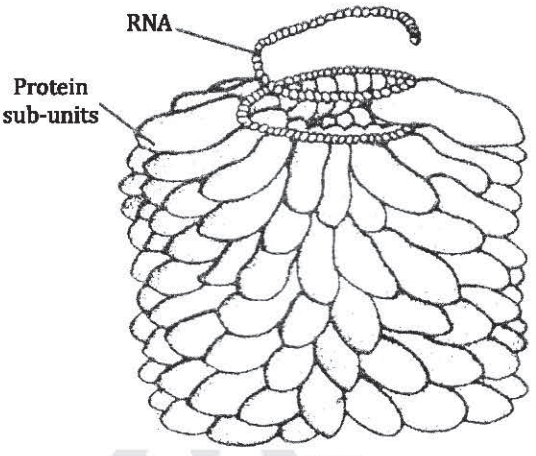


Fig. : Helical virus-TMV

Schematic model of TMV : (i) nucleic acid (RNA), (ii) capsomere protein (Protomer), (iii) capsid

Tobacco mosaic virus has a rod-like appearance. Its capsid is made from 2130 molecules of coat protein and one molecule of genomic single strand RNA, 6400 bases long. The coat protein self-assembles into the rod-like helical structure (16.3 proteins per helix turn) around the RNA, which forms a hairpin loop structure. The protein monomer consists of 158 amino acids which are assembled into four main alpha-helices, which are joined by a prominent loop proximal to the axis of the virion. Virions are ~300 nm in length and ~18 nm in diameter. Negatively stained electron microphotographs show a distinct inner channel of radius ~2 nm. The RNA is located at a radius of ~ 4 nm and is protected from the action of cellular enzymes by the coat protein. X-ray fiber diffraction structure of the intact virus was studied based on an electron density map at 3.6 Å resolution. Inside the capsid helix, near the core, is the coiled RNA molecule, which is made up of 6,395 ± 10 nucleotides.

Genome of Tobacco Mosaic Virus

The TMV genome consists of a 6.3-6.5 kbp single-stranded (ss) RNA. The 3′-terminus has a tRNA-like structure and the 5′ terminus has a methylated nucleotide cap. (m7G5′pppG). The genome encodes 4 open reading frames (ORFs), two of which produce a single protein due to ribosomal readthrough of a leaky UAG stop codon. The 4 genes encode a replicase (with methyltransferase [MT] and RNA helicase [Hel] domains), an RNA-dependent RNA polymerase, a so-called movement protein (MP) and a capsid protein (CP).

TMV is a thermostable virus. On a dried leaf, it can withstand up to 50°C (120 degree Fahrenheit) for 30 minutes. TMV has an index of refraction of about 1.57.

Q.7. Write the note on types of viral infections.

Ans.

Types of Viral Infections

Viral infections can be categorized into following broad categories :

1. **Lytic infection :** Such infections result in the destruction of the host cells. Polio virus, pox virus and the common cold virus cause lytic infections.
2. **Persistent infection :** In such infection the virus replicates actively but the host cell retains viability, and viral production continues for an extended period of time. In persistent infection the mature viruses leave the host cell by budding without disrupting the integrity of the plasma membrane. AIDS virus (HIV), rubella virus

{*Rubivirus*} and measles virus (*Morbillivirus*) cause persistent infection. Besides these, all plant viruses cause persistent infections.

3. **Latent infection :** In such an infection the virus does not actively replicate within the host cell and remains in equilibrium with the host cell without producing any disease often (or several years. All of the human herpes viruses can remain in the host cells throughout the life of an individual. Herpes simplex virus, which inhabits the human nerve cells, generally cause no damage until it is activated by a stimulus such as fever or sunburn.
4. **Transformation :** Some viral infections can cause transformation of the host cells. The transformed cells acquire properties that are distinct from the properties of uninfected cells. A transformed cell may change into a cancer cell with fewer growth factor requirements than for the normal cell. As a result the transformed cell reproduces more rapidly, resulting in a mass of cells called a **tumor.** Some tumors are self-limiting; they do not spread and are called **benign.** Other type of tumors, called **malignant,** spread and grow in other tissues and organs causing dysfunction or death of that tissue. All **oncoviruses** (viruses capable of inducing tumors in animals) induce transformation in host cells.

Q.8. Give a brief account of the structure of viroids.

Ans.

Viroids

Viruses are no longer considered the simplest form of life. In 1971, **T.O. Diener** discovered new infectious agents which were even smaller than viruses. He introduced the term **viroid** for these subviral pathogens. The first viroid came to light in attempts to isolate and characterize the agent of the potato spindle tuber disease (PSTVd) which was assumed to be caused by a virus. The infectious agent of this disease was found to be a RNA strand devoid of nucleoprotein coat. This infectious RNA has a very low molecular weight unlike conventional viruses. Since then many plant diseases (*e.g.,* citrus exocortis, chrysanthemum stunt, cucumber pale fruit and chrysanthemum chlorotic mottle) are known to be caused by viroids. However, the search for viroids of animal disease has been relatively slow and we know only few animal diseases (*e.g.,* scrapie disease of sheep and Alzheimer's disease of human) of viroid origin. Presumably, certain infectious diseases of obscure etiology are caused by agents resembling viroids (*e.g.,* hepatitis D).

Thus viroids can be defined as infectious agents composed exclusively of a single piece of circular single stranded RNA which has some double stranded regions.

Structure of Viroids

A viroid consists of extremely small strand of RNA without any protective protein coat. Electron microscopic studies of purified 'potato spindle tuber viroid' (PSTV) revealed that it has a single stranded RNA molecule containing 250-350 nucleotides. The adenine : uracil (A : U = 21.7 : 20.9) and guanine: cytosine (G : C = 28.9 : 28.3) ratios are close to unity. **Viroids do not possess capsid** (protein coat) around the RNA molecule.

Besides linear RNA, some circular molecules of PSTV have also been reported. The RNA fingerprinting, however, has shown that the circular and linear molecules of PSTV structures are probably not two distinct RNA species. The two structures, more likely, represent two stages of maturity of PSTV. The molecular weight of viroids is in the range of 11,500 – 130,000 daltons.

Viroids do not code for any protein Their replication mechanism uses a host-cell enzyme- RNA polymerase II. Some viroids are ribozymes, having catalytic properties which allow self-cleavage and ligation of unit-size genomes from larger replication intermediates. Although the precise mechanism of viroid replication is not known as yet, the following two hypothesis have been put forward :

1. **RNA-directed replication :** According to this view, in uninfected plants there exists a replicase enzyme which accepts a wide variety of RNA species as templates. The presence of RNA-directed RNA polymerase has been shown in healthy plants of Chinese cabbage and tobacco.
2. **DNA-directed replication :** This hypothesis suggests that viroids might be replicated on DNA templates which are either already present in repressed form in uninfected hosts or are synthesized as a result of viroid infection.

Q.9. Write short note on mycophages.

Ans.

Mycophages (Mycobacterium Phage)

The acid-fast **mycobacteria** are important disease agents (*e.g.,* tuberculosis) but nonpathogenic mycobacteria are commonly found as soil **saprobes.** Mycophage (the **phage** of mycobacteria) are important for their role as molecular tools useful in the study of mycobacteria, especially their genetics. As an offshoot of those efforts, **mycobacteriophage** serve as a phage cohort that is employed in the study of comparative phage genomics, the study of entire phage genomes. An interesting clinical role for mycophage is in phage-based bacterial detection schemes including ones that assess antibiotic susceptibility.

Q.10. Write the symptoms of phytoplasma infection.

Ans.

Symptoms of Phytoplasma Infection

Phytoplasmas are associated with diseases in several hundreds of cultivated herbaceous and woody plants. Their impact on agriculture and periodical outbreak of epidemics make the knowledge of agronomical features and disease progression that influence symptoms important. For better diagnosis, the observer should be able to recognize and distinguish the symptoms of other biotic and abiotic diseases.

One common symptom of phytoplasma infection is **phyllody**—the floral organs become large and fleshy like leaves. Petals are usually green due to loss of pigmentation and plants bearing such flowers remain sterile. Translocation of carbohydrates through phloem is affected in plants infected with phytoplasma. Phytoplasmas inhibit biosynthesis of chlorophyll and trigger its early breakdown. This results in **early yellowing of leaves.**

The other most common and representative symptoms are **stunting, yellowing** and **witches broom** (proliferation of axillary buds due to which plants become bushy).

Several phytoplasmas produce some special proteins called **inducers** or **virulence factors.** These proteins are transported within the plant through phloem and are responsible for producing dwarfism. First such inducer was identified as **TENGU** which caused yellowing of onions. Since then several genes have been identified in the genome of Aster Yellow phytoplasma strain.

Q.11. Describe the structure of reproduction of actinomycetes.

Ans. Actinomycetes are unicellular organisms placed in the order Actinomycetales or the class Schizomycetes. They occur abundantly in soil water, mud, manure, milk and other food

products. Most of the actinomycetes are saprophytes but some are **parasites.** The latter cause some serious diseases in plants as well as animals.

Structure

Actinomycetes (*actis* = **ray,** *mykes* = fungus) are fungus like bacteria with cylindrical cells which are usually united to form filaments resembling the mycelium of a true fungus. The mycelium is branched, non septate and thin (0.2-1.2 um in width). In some species the mycelium breaks up into small bacteria-like cells. In young mycelium the cytoplasm is homogeneous, but at maturity many vacuoles, fat droplets, granules and few rod-shaped bodies develop in the cytoplasm. In actinomycetes, as in true bacteria, there are no well differentiated nuclei but many chromatin granules are present. At maturity, the cell wall of the mycelium becomes fragile and breaks up easily.

Most of the actinomycetes are Gram-positive. They are non-motile but in **Actinoplanes** small flagella are present.

Reproduction

They generally multiply by means of **fragmentation.** The mycelium breaks up into small hyphae and each hypha grows into a new mycelium. Some species of actinomycetes also reproduce by means of **spores** and **conidia,** which develop on sporophores and conidiophores, singly or in long chains.

Q.12. Discuss antibiotic resistant gene in plasmid.

Ans. **Antibiotic Resistant Gene in Plasmid**

The antibiotic resistant gene is one of the main , components of plasmids. These genes play an important role in drug resistance (to one or more antibiotics) thus making treatment of some diseases more challenging.

Plasmids are today known for their ability to transfer from one species of bacteria to another through a process known as conjugation (contact between cells that is followed by transfer of DNA content). In the process, plasmids are capable of conferring antibiotic resistance properties to other species of bacteria.

Some Other Components of Plasmids

Besides the main components mentioned above, plasmids have some other components. These are :

1. **A promoter region :** This component of plasmid is involved in recruiting transcriptional machinery.
2. **Primary binding site :** This is a short sequence of DNA on a single strand that is typically used for the purposes of PCR amplification or DNA sequencing.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. What are prions? Give their characteristics. Which diseases are caused due to prions?

Ans. **Prions**

Several diseases of humans and other animals are caused by **proteinaceous infectious particles.** These small infective agents have been named as **prions** by Stanley Prusiner (1982). He received Nobel Prize in 1987 for his work on prions.

Prions are **made of only proteins; they have no nucleic acids.** The proteins constituting the prions are designated as **PrP** (prion proteins). They have a mass o 27,000-30,000 daltons. These proteins consist of about 250 amino acids and are about 1/100 the size of a small virus. The genes that code for these proteins are found in the normal host DNA. In human beings the PrP genes are located on chromosome 20. It is believed that prions are normal proteins that become folded incorrectly, possibly as a result of mutation (Fig. A. B). How these defective infectious prion particles are transmitted to an uninfected individual and initiate the disease process is not definitely known. Prions can survive on instruments sterilized by formaldehyde or inadequately autoclaved. They also survive for several years, on burried animals.

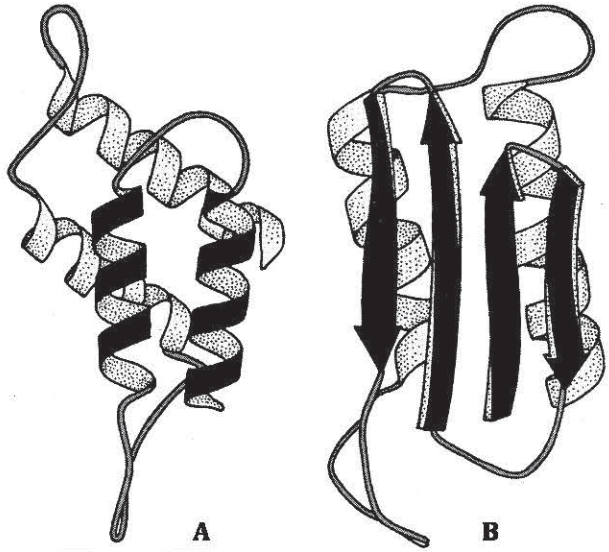


Fig. : A-B. Two forms of prion proteins : A. Harmless form, B. Harmless form (the harmful nature of the protein is because of improper folding of normal prion protein).

Characteristics of Prions

Prions show following characteristics :

1. Prions are not inactivated by temperature up to 90°C (viruses are normally inactivated at this temperature).
2. Prion infection is not sensitive to radiation treatment that damages virus genomes.
3. Enzymes destroying digesting DNA or RNA do not affect the activity of prions.
4. Protein denaturating agents (*e.g.*, phenol, urea, etc.) affect prion activity.

Prions have a long incubation period, hence these infective agents were once assigned the name **slow viruses.** Some of the diseases now believed to be caused by prions were once thought to be slow viruses. However, not all slow virus diseases are caused by prions.

Diseases caused due to Prions

Prions cause a progressive degeneration of the central nervous system and the diseases thus caused by prions are collectively known as **transmissable spongiform encephalopathies** because they destroy neurons and the brain tissue gives a sponge-like appearance. Among animals prions are known to cause **scrapie** (in sheep and goat) and **'mad cow' disease** and

encephalopathy (in mink, elk and cats). In 1996, United Kingdom had to kill hundreds of thousands of cattle because of an outbreak of 'mad cow' disease.

In human beings prions cause **kuru** and **Creutzfeldt-Jakob disease** (CJD). Symptoms of kuru may appear 1 to 15 years after infection as severe headache, followed by loss of coordination and inability to walk and swallow. Creutzfeldt-Jakob disease is characterized by mental degeneration, loss of motor function and eventual death.

The pathological and clinical signs of these diseases suggest that they are closely related. In fact they may be variants of the same disorder. All pathological features are confined to the central nervous system. The prion protein accumulates selectively and abnormally in CNS nerve cells during the course of disease.

Q.2. Describe briefly the structure of a prokaryotic cell.

Ans. Structure of Prokaryotic Cell or Bacterium

Structure of prokaryotic cell is discussed as follows :

1. **Capsule and Slime Layer :** In many bacteria, a slimy **capsule** is present outside the cell wall. It is composed of polysaccharides and serves as an additional protective covering. It protects the cell from antibodies and dehydration. The bacteria which form a capsule are called **capsulated bacteria.**

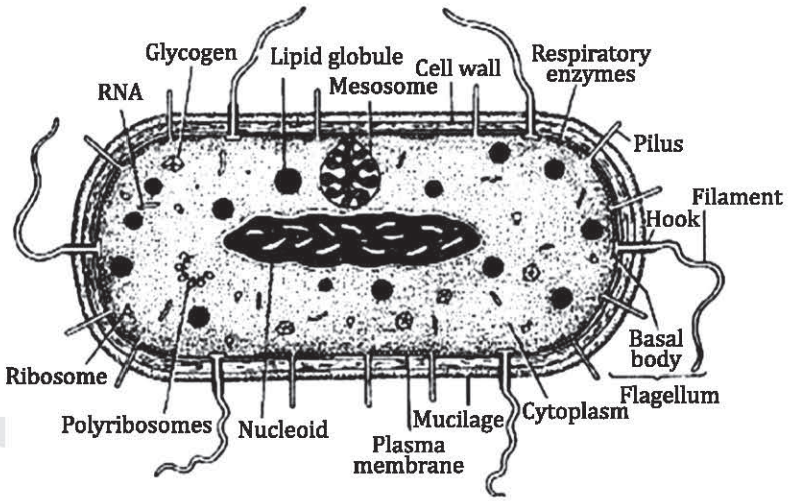


Fig. : Microscopic structure of a bacterium cell (Prokaryotic cell)

2. **Cell Wall :** Bacteria are considered as plants due to the presence of a cell wall. The cell wall is rigid and is 100-140 nm thick. It lies just outside the plasma membrane. It provides a definite shape and protection against mechanical injury, chemicals and pathogens.

   The cell wall is formed of **peptidoglycans** or **mucopeptides** (polysaccharides cross-linked with short chain amino acids), **teichoic acid** (polyphosphate polymer), some **lipids phosphorus, inorganic salts** and a derivative of glucose. It also contains an amino acid, the **diaminopimelic acid.**

3. **Plasma Membrane :** It lies below the cell wall. It is composed of lipo-proteins as in all other organisms. It is trilaminar structure representing unit membrane.

Plasma membrane is differentially permeable as it controls the passage of many solute and solvents. Unlike eukaryotes, bacterial plasma membrane contains enzymes for lipid metabolism, those essential for synthesis of cell wall, enzymes of electron transport (respiratory enzymes) and enzymes for DNA synthesis.

4. **Mesosomes or Chondrioid :** The plasma membrane in many bacteria invaginates to form lamellar or vesicular folds to form mesosomes or chondrioids. The mesosomes surface has enzymes for respiration. Mesosomes also help a separation of two daughter chromosomes (after DNA replication during cell division). They also secrete some extra-cellular substances.

   In photosynthetic bacteria, infoldings of plasma membrane form **carotenoids.** These contain bacterial chlorophyll and other pigments for photosynthesis.

5. **Cytoplasm :** Bacterial cytoplasm contains granules of fats, glycogen and proteins; and volutin granules. Cytoplasm does not show streaming movement.

   Cytoplasm contains 70S type of ribosomes. These occur scattered or may form a chain called **polysome.**

   Also scattered in the cytoplasm are photosynthetic **lamellae.** These contain bacterial chlorophyll and other pigments. Such bacteria are able to make their own food and are called **autotrophic bacteria.**

6. **Nucleoid or Genophore :** Bacteria lack nuclear membrane, nucleolus and nucleoplasm. Nuclear material consists of a ring-shaped or circular DNA or chromosome located in the centre of the cell. Such a structure is called nucleloid or genophore. It lies freely in the cytoplasm and is not enclosed in a nuclear membrane. Its DNA is highly twisted and has several supercoiled loops.

7. **Plasmids :** In *E. coli* and some other bacteria, bacterial cytoplasm also contains small, double-standard DNA molecules. These are called **plasmids.**

Q.3. Describe the detail of the bacterial growth curve.

Ans.

Bacterial Growth Curve

Earlier in this chapter you have read that bacteria can be easily grown in the laboratory. When a culture medium is inoculated with a small bacterial inoculum, population size of the bacteria increases in a predictable pattern. In a culture growth shows changes in the size of the bacterial population over time. You can obtain a distinct curve by plotting log of cell numbers versus time; this is referred to as bacterial growth curve.

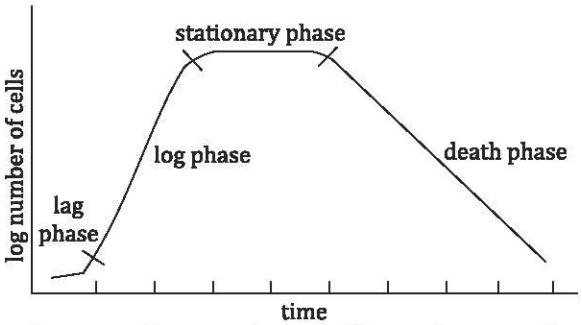


Fig. : Growth curve of a typical bacterium growth in batch culture illustrating the four phases

The growth curve can be used to describe the phases of growth cycle. It shows four distinct phases—(i) the lag phase, (ii) the exponential or log phase, (iii) the stationary phase and the (iv) death or decline phase.

Additionally, the growth curve can yield **generation time** for a particular organism. It is the time interval for the formation of two daughter cells from one or it is the doubling time. It can also be described as the amount of times it takes for a population to double.

Features of the growth curve such as the number of cells, length of each phase, rapidness of growth or death, etc. vary from organism to organism or even with different conditions for the same organism. However, the pattern of four distinct phases of growth remain the same.

The important characteristics of the four phases of growth are described in the following pages.

1. **Lag Phase :** The lag phase is an adaptation period where the bacteria adjust themselves to their new environment. During this phase cellular metabolism is accelerated, resulting in rapid biosynthesis of cellular macromolecules, mainly enzymes. Although **cells increase in size, there is no cell division,** and therefore, no increase in cell numbers. During this phase cells undertake necessary repair work.

   The **duration of the lag phase** varies with the bacterial species, nature of culture medium, incubation temperature, etc. Actively growing cells transferred from one type of medium into the same type of medium have a short lag period. Damaged cells have a long lag period, since they must repair themselves before they can engage in reproduction.

2. **Exponential or Log Phase :** Once the cells have accumulated all the metabolites needed for growth, the physiologically robust cells reproduce rapidly by binary fission. This phase of growth is marked by predictable doubling of population, *i.e.,* 1, 2, 4, 8 and so on. Due to this exponential growth of cells, the growth curve shows a steep rise. However, if the conditions are less than optimal, the growth is relatively slower.

   Since the cells divide very rapidly, **bacteria have smallest size in this phase.**

   Generation time is shortest during log phase and is strongly dependent upon growth factors present in the medium. This phase lasts for several hours depending on the type of organism, conditions of growth and density of organisms on an average the log phase is estimated to last from 6 to 12 hrs.

3. **Stationary Phase :** The rapidly growing bacterial population, at some point of time, runs out of essential nutrients or its growth is inhibited by its own waste products. In stationary phase bacterial population remains constant as there is a balance between cell divisions and cell death. Thus during this phase the culture has highest population density. In a growth curve this phase appears as a flat curve.

   Physiologically, the cells become quite different at this stage as they try to adapt to their new starvation conditions. Many bacterial cells start producing exotoxins. A few new cells that are produced are smaller in size, with bacilli becoming almost spherical in shape. Their plasma membrane becomes less fluid and permeable. These cells show more adhesion and aggregation.

4. **Death or Decline Phase :** This is the last phase of the growth curve in which the number of viable cells decreases in an exponential fashion. The steepness of the slope in the growth curve shows as to how fast cells are losing viability. It is presumed that during decline phase, the culture conditions deteriorate very fast and the cells are

irreparably harmed. Though total count of bacteria may remain constant but the viable count decreases.

Death phase is brought about by various factors such as depletion of nutrients and accumulation of toxic wastes. Not all bacteria die at the same time, some die faster and some are more resistant and remain viable for longer time (such as endospore forming bacteria).

It has been observed that 100% cell death is very unlikely during death phase. Some cells may mutate and adapt themselves to even harsh environments.

5. **Significance of Bacterial Growth Curve :**
   (i) Knowledge of bacterial growth curve is important when they are being used to enhance plant growth, to increase biodegradation of toxic organics, to produce antibiotics and other natural products on industrial scale.
   (ii) To assess whether particular strains of bacteria are adapted to mobilize certain substrates, such as industrial waster or oil pollution knowledge of growth curve is essential.

Q.4. Give an illustrated account of the chemical structure of viruses.

Ans.

Chemical Structure of Viruses

Viruses are the ultramicroscopic fillerable and non-cultivable microorganisms. D. Iwaniwsky, a Russian in 1892 was the first who discovered a viral disease in plants.

A simple virus particle often designated as **virion,** consists of a nucleic acid core of genetic material (genome) enclosed within protein coat. The amount of protein in different viruses varies from 60-95 per cent and the rest is nucleic acid.

1. **Size :** Earlier the size of viruses was measured by using the technique of filtration through collodion membranes of known porosity. But now techniques like ultracentrifugation and electron microscopy are employed. Viruses are very small in size, varying over a wide range from 20-350 nm. The largest are the orthopoxviruses, measuring about 240 nm × 300 nm, *i.e.,* approximately 1/10 the size of a red blood cell. The complex bacteriophages are about 65 nm × 200 nm. Among the smallest viruses known are the enteroviruses, which are less than 30 nm in diameter. The dimensions of some common viruses are shown in table and Fig.

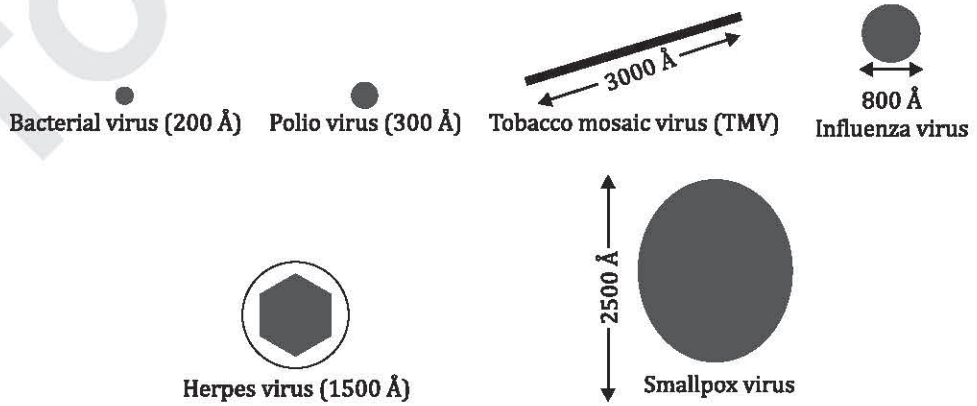


Fig. 1 : Virus : Variations in the shape and size

Size of some well known viruses

Virus	Size (nm)
Turnip yellow mosaic virus	28
ϕ X-174 virus	22
Tomato bushy stunt virus	30
Polio virus	27-30
Alpha virus	35-80
Adeno virus	60-90
Influenza virus	80-120
Herpes virus	180-200
Tobacco mosaic virus	17.5 X 300
Beet yellow virus	10 X 1250

2. **Nucleic Acid :** Viruses differ fundamentally from cellular organisms in that they contain only one type of nucleic acid which may be either DNA or RNA. The viruses containing DNA are called **Deoxyviruses,** whereas those having RNA are known as **Riboviruses.** Viruses vary considerably in the structure of nucleic acids.

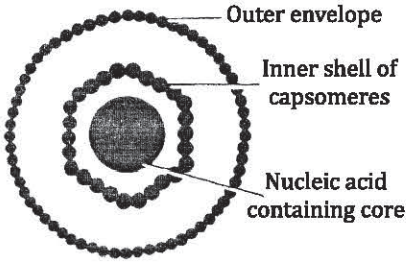


Fig. 2 : Virus : General structure

In general (i) all plant viruses have single stranded RNA, (ii) animal viruses have either single or (rarely) double-stranded RNA or double-stranded DNA, (iii) bacterial viruses contain mostly double-stranded DNA but can also have single stranded DNA or RNA, and (iv) most of the insect viruses contain RNA and only a few have DNA. The DNA of some bacteria and animal viruses is circular, but in others it is like RNA.

Careful extraction of nucleic acids from viruses has shown that a virion contains only a single molecule of nucleic acid. The number of nucleotide pairs in a molecule varies from 1,000-250,000 pairs. But the number of pairs in a specific virion is always constant. The amount of nucleic acid depends on the size of virion; usually larger the size of virion, greater is the amount of nucleic acid.

Characteristics of nucleic acids of some better known plant and animal viruses are given in table.

Characteristics of Nucleic Acids of Some Viruses

Virus	Nucleic acid type	Strands	Nucleotide pairs	Molecular weight in Dalton
Polyoma	DNA	Double	4,500	3×10^6
Adenovirus	DNA	Double	35,000	23×10^6
Coliphage T_1	DNA	Double	200,000	130×10^6

Coliphage T_2	DNA	Double	60,000	40×10^6
Tobacco mosaic virus	RNA	Single	7,500	25×10^6
Tobacco necrosis virus	RNA	Single	1,500	0.5×10^6
Bean mosaic virus	RNA	Single	3,000	1×10^6
Polio virus	RNA	Single	6,000	2×10^6

3. **The Protein Coat :** The nucleic acid core of the virus is protected by a protein coat called the **capsid.** Each capsid consists of several identical protein subunits, known as **capsomeres.** In some viruses the proteins composing the capsomeres are of a single type, while in others several types of protein may be present. These subunits are usually arranged in helical or polyhedral geometric forms. The number of proteins and the arrangement of capsomeres are characteristic of specific viruses and thus can be useful in their identification and classification.

   The capsomeres forming the capsid (protein coat) of a virion are of two types—**pentamer,** made of five identical monomers, and **hexamer,** having six monomers. Each monomer is connected with the neighbouring monomers on either side with the help of bonds. Likewise, the capsomeres are also connected with each other, but the bonds between the capsomeres are weak.

   In some complex forms (*e.g.,* influenza and herpes virus) the capsid is covered by an **envelope.** It usually consists of some combination of lipids, proteins and carbohydrates. Some animal viruses, which are released from the host cell by an extrusion process, get coated by the host cell's plasma membrane. This membrane eventually becomes the viral envelope.

   Envelope of many viruses have projections called **spikes.** These are made of carbohydrate-protein complexes. Viruses attach themselves to the host cells by means of spikes. Besides, the spike characteristic is an important tool for the identification of viruses.

Viruses whose capsids are not covered by an envelope, are known as **naked** or **nonenveloped viruses** (*e.g.,* TMV). In such forms the capsid facilitates the attachment of the virus to the host surface and also protects the virus nucleic acid from the nuclease enzymes present in the biological fluids.

Q.5. Describe about the morphology of viruses in detail.

Ans.

Morphology of Viruses

Viruses may be classified into various morphological types on the basis of their capsid architecture. The structure of capsid and individual capsomere can be studied by electron microscopy and X-ray crystallography (Fig.). Following are some of the common morphological forms of viruses.

1. **Helical Viruses :** These viruses are cylindrical or rod-like in from and the central nucleic acid strand is coiled like a helical spring. The protein subunits (capsomeres) are helically arranged around the helical spring. The common examples of helical viruses are New Castle virus, Mumps virus, Rabies virus and Tobacco mosaic virus (Fig. A).

2. **Polyhedral Viruses :** In these viruses the nucleic acid is packed in an unknown manner within a hollow polyhedral head. They have been classified further into **tetrahedral, octahedral** and **icosahedral** form depending upon the number of faces. Of these, icosahedral form is considered to be the most efficient shape because of packing and bending of capsomeres in a near spherical form. An icosahedron has 12 corners, 20 triangular faces and 30 edges (*e.g.,* Adenovirus, Poliovirus; Fig. B).
3. **Enveloped Viruses :** As mentioned earlier, the capsid of these viruses is covered by an envelope. Such viruses may be spherical, helical or polyhedral in shape (Fig. C).
4. **Complex Viruses :** Bacterial viruses or bacteriophages have a complex structure, hence called **complex viruses.** For example, T-even bacteriophages have a capsid (head) to which other structures like a helical tail sheath, base plate, tail fibers and pins are attached (Fig. D). Another example of complex viruses are pox viruses in which the nucleic acid is surrounded by several coats.

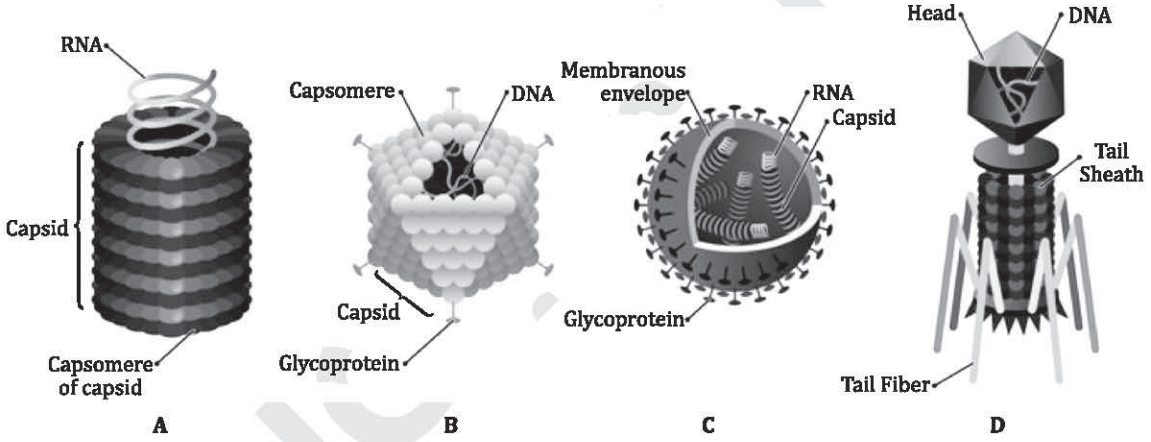


Fig. : A-D. Virus morphology : A. Helical virus, B. Polyhedral virus, C. Enveloped virus, D. Complex virus

Q.6. What are bacteriophages? Describe their structure and multiplication with the help of suitable diagrams.

Ans.

Bacteriophages

The phages that attack the bacterial cells are called **bacteriophages.** They were first described by **Twort** (1918) and **D'Herelle** in 1917.

Structure and Multiplication of Bacteriophage

Viruses, which infect bacterial cells, are known as **Bacteriophages.** It was discovered by **Frederic W. Twort** (1915) and **Felix-d' Herelle** (1917) independently. Bacteriophage are obligate parasite. They are present in all type of bacteria.

Bacteriophage are of following types :

1. **T-phages** (T_1-T_7; T = type) : They are characterised by the presence of a tail. They contain double stranded DNA. They are sub-divided into three sub-groups :

(i) **T-even phages** (T_2, T_4, T_6): They are closely related genetically and serologically. They have an head and tail. In DNA a unusual base is present, *i.e.,* **5-hydroxymethyl cytosine,** in place of cytosine.

(ii) **T-odd phages** (T_1, T_3, T_7): They are different in genetic and serology. The DNA of these phage have cytosine.

(iii) **T_5-phages :** It is composed of angular head and a long non-contractile tail. Cytosine is present in DNA.

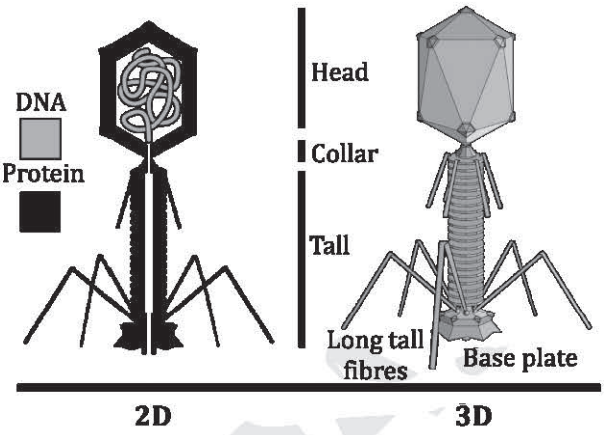


Fig. 1 : Structure of Bacteriophage

2. **Virulent and Temperate Phages :** Bacteria containing prophages are called **Lysogenic bacteria :** The virus whose nucleic acid can become prophage, *i.e.,* gets incorporated in bacterial DNA, are known as **Lysogonic, temperate** or **avirulent phages** (*e.g.,* F_2, M_{12}). The virus which always multiply when they enter the host cells are called **virulent** or **lytic phages** (*e.g.,* T_2, T_4 phages).

Structure of Bacteriophage

A T_4 bacteriophage has a tadpole like structure with a polyhedral **head** and a **helical tail.**

Head : The head consist of a nucleic acid surrounded by a protein coat or **capsid** made up of **capsomeres.** The nucleic acid is double stranded DNA with a molecular weight of 120×10^6 daltons.

Tail : The tail is a tube like structure made up of protein. Its core is surrounded by a thick **shealth.** The sheath contains 24 annular ring. At one end, the tube is jointed to the head by a thin collar. At the other end a hexagonal plate present called **end plate** (basal plate). From the end plate six tail fibre come out. These help in attachment of phase to the host cell.

Reproduction of Bacteriophage

The life-cycle of bacteriophage is of two types :

(I) Virulent or Lytic Life-cycle

In this case the virus (bacteriophage kill the bacteria). It is completed in the following steps.

1. **Infection stage (Adsorption or penetration phase).** This stage is completed into two steps :

(i) **Attachment of the phage to the bacterial surface :** Each bacterium has specific site on the surface which are known as **receptor site,** only a specific phage can infect a particular bacterium. The phage can be attached only on the specific site of bacterium with tail fibre.

(ii) **Penetration into bacterial cell :** The phage particle secrete **Lysozyme** enzyme, which dissolve the bacterial cell. This enzyme can dissolve the muramic acid peptide complex of bacterial cell wall.

The protein coat of phage remain attached to the host cell. Such shells are called **ghost.** A phage particle loses its ability to infection after its nucleic acid has been released into the host cell.

Now the tail sheath contract, pushing the central part of the tail into the host cell wall just like an injection needle. The nucleic acid of the phage flows into the host cell through hollow center.

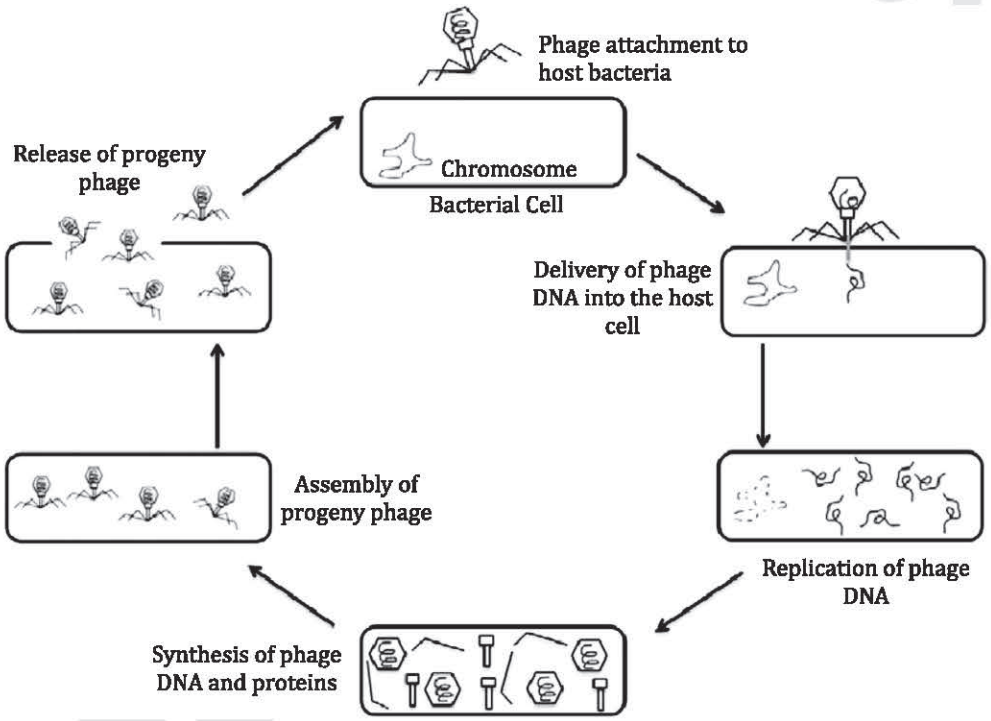


Fig. 2 : Stages in the replication of Bacteriophage (Lytic Life Cycle)

2. **Latent Stage (Period of Eclipse) :** The phage DNA give rise **nuclease enzyme** which attack the bacterial DNA and disintegrate it. The phage DNA remain protected because it has modified cytosine residue and enzyme fail to degrade such altered DNA.

   The phage nucleic acid takeover the protein synthesis machinery of the bacterium. Now the synthesis of protein of phage particle starts. The replication of phage DNA follows the semi conservative mechanism.

3. **Vegetative Stage (Maturation phase) :** Now the assembly of the nucleic acid and protein is started. It is called maturation. This process is controlled by viral genome.

   The condensation of nucleic acid molecule in crystalline form starts. The protein sub-unit then aggregate around DNA to form the head of the phage. Meanwhile assembly of the tail starts the tail gets attached to the base of the head. At last the tail fibres are attached to the end plate.

4. **Lysis stage (Liberation) :** The entire cycle of phage development is completed in 30-90 minute. In an infected bacterium 7-8 phage particles are formed per minute and a total of about 200 phage are formed in a bacterium.

   At last cell of bacterium burst with **Lysozyme** enzyme and phage particles are liberated.

(II) Lysogenic Life-cycle of Phage

Some bacteriophage behave differently. They are called **temperate virus.** They behave in two ways. In free state in the host body they kill the host DNA like the virulent phase but in another state they does not destroyed the bacterial DNA but get attached with DNA of host.

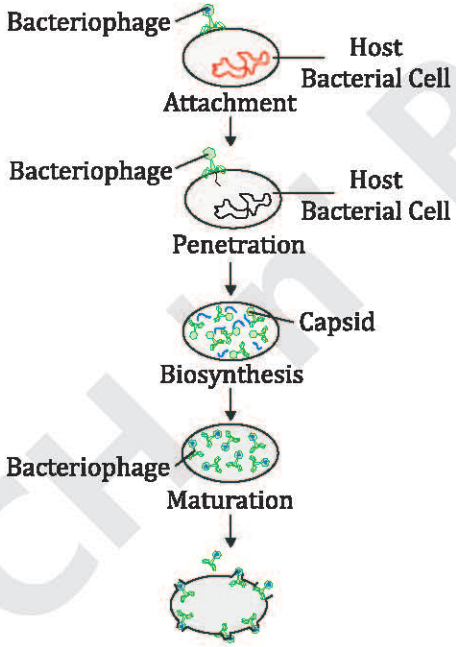


Fig. 3 : Lysogenic life cycle in temperature phage

Now it is called **prophage** or **provirus.** The bacterial host containing prophage are called **Lysogenic bacteria.** Here phage DNA is **symbiont** rather than parasite. It is transmitted to the progeny of the bacteria.

It was disovered by **Jacob, Lowff, Simonovitch** and **Wollman** (1953). The particle like the temperature phages, which remain in the integrated as well as in the free state with in a cell and are dispensable element are called **Episome.**

Q.7. Describe the structure of *Mycoplasma.*

Ans. **Structure of *Mycoplasma***

Among prokaryotic there are cells that naturally have no walls or have very little wall material. These includes members of genus *Mycoplasma.*

Mycoplasma are the smallest known bacteria that can grow and reproduce out side living host cells. The cells are very small, ranging in size from 0.1 to 0.25 μm.

They can pass through bacterial filters and their plasma membranes have lipids called sterols, which are through to help protect them from osmotic lysis.

Mycoplasma species are mostly aerobesor facultative anaerobes. Because they lack cell all, they are highly **pleomorphic.** They can produce filaments that resemble fungi; hence their name (*myco* means fungus).

The most significant human pathogen is *M. pneumonia,* the the causative agent of primary atypical **pneumonia** (commonly called "walking pneumonia").

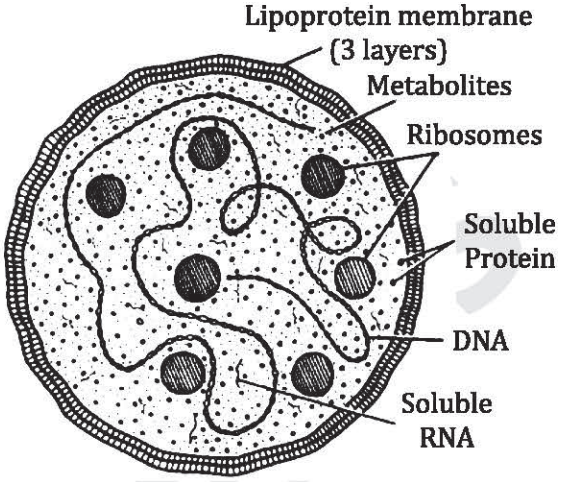


Fig. : *Mycoplasma*

Mycoplasma does not possess a cell wall and are surrounded by cell membrane. These organisms are plemorphic ranging from spherical bodies (100 nm diameter) also called as elementary bodies to large, spherical irregular bodies of 1 μ in diameter of filaments. Usually four types of structures have been reported.

Elementary bodies : These are spherical and surrounded by unit membrane. Their size varies from 80-200 nm. Their contents are indistinct and their role is debatable.

Large cells : Their diameter 500-700 nm and are enclosed by plasma membrane, contains cytoplasm, nuclear area and ribosomes.

Nuclear area consists of DNA strands which lie free in cell. **There is no nuclear membrane.**

Very large cells : These are degenerate and nonviable cells. They may be empty or may contain variable number of vesicles with or without bounding membrane. The nature of these vesicles is not clear.

Filamentous structures : Their length is variable and may be form 60-75 nm in diameter. These are surrounded by the triple layered unit membrane. They contain ribosomes like granules and DNA strands containing nuclear area (Fig.).

Q.8. What are plasmids? Describe their various types.

Ans.

Plasmids

During 1950s, working on conjugation process it was found that maleness in bacteria is determined by a transmissible genetic element. When male and female bacteria conjugate, every female is converted into a male. This inherited property of male is called the F (fertility) factor which is transmitted by cell to cell contact. Therefore, F is a separate genetic element. In 1952, J. Lederberg coined the term plasmid as genetic name for this element. Hence, the plasmids may be defined as a small circular, self replicating and double stranded DNA molecule present in bacterial cell, in addition bacterial chromosome. It replicates independently during cell divisions and inherited by both of daughter cells. Therefore, its function is not governed by the bacterial chromosome.

The number of plasmids ranges from one to hundreds or more per bacterial cell. A plasmid contains 5-100 genes that determine several biological functions. Under certain circumstances they provide special characteristics to the bacterial cell and help them in survivability. They may even lose without harming the bacterial cell.

Plasmids are the circular DNA molecule but in resting stage helix twists in right hand direction at every 400-600 base pairs and forms super coils. The twisted form is called covalently closed circular DNA. After cleaving the twists this form converted into an open circular form of double stranded DNA molecule.

Types of Plasmids

On the basis of function plasmids are divided into several types :

1. **Sex factor or fertility (F) factor :** The plasmids of male cells that confer on their host cells the capability to transmit chromosomal markers but not the other properties are called sex factor or F factor. For the first time the F factor was discovered in *E. coli.* The term sex factor is used in two ways : (a) as genetic name for all plasmids which determines host conjugation and their own transfer irrespective of the transfer of bacterial chromosome, and (b) to describe the set of genes on plasmids whose products mediate the conjugation process. Some times the F factor transfers a nonconjugative plasmid also which is present with it in a cell.
2. **R (resistance) plasmids :** During 1946 several out breaks of dysentry by *Shigella* occured in Japan and resistance against antibiotic drugs developed. In 1955, in Japan a strain of *S. dysenteriae* causing dysentry was isolated that was resistant to four drugs *viz.,* sulfanilamide, streptomycin, chloramphenicol and tetracycline. By 1964, over 50% all *Shigella* hospital isolates in Japan became multiple drug resistant. Similar strains were also isolated from hospital in London in 1962, and that of *Salmonella* in England in 1965.
3. **Col plasmids :** There are many bacterial strains that produce proteinaceous toxins known as bacteriocin which are lethal to other strains of the same genus. Toxins secreted by the strains *E. coli* are called colicins. It kills the sensitive cells. Synthesis of colicins is specified by the plasmids present in *E. coli* cells but not by bacterial chromosome. These plasmids associated with colicin production are called colicinogeny (Col) factor. There are several Col plasmids such as Col B, Col E, Col I, Col V which produce different types of colicins.
4. **Heavy-metal resistance plasmids :** There are several bacterial strains that contain genetic determinant of resistance to heavy metals viz., Hg^{2+}, Ag^{+}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Ni^{2+}, Pb^{3+}, Zn^{2+}, etc. (Bopp *et. al,* 1983). These determinants for resistance are often found on plasmids and transposons (Summers, 1982).
5. **Penicillinase plasmid or *Staphylococcus* aureus :** *Staphylococcus aureus* is a Gram-positive bacterial pathogen causing infection of skin and wounds of humans. After treatment with penicillin antibiotic, several penicillin-resistant *Staphylococci* developed by 1950 throughout the world. High level resistance to penicillin was possible due to secretion of an enzyme, penicillinase which degrade penicillin by hydrolysing its β-lactum ring. During 1970s R.P. Novick isolated the genes (Pt) from plasmid encoding pencillinase.
6. **Degradative plasmids :** Much work has been done on degradative plasmid of *Pseudomonas.* The *pseudomonas* have been found to catalyse a number of unusual complex organic compounds through the special metabolic pathways. Anand Mohan Chakrabarty, an India-borne American scientist, has isolated plasmids complex organic chemicals such as 2,4-D salicylate, 3-cholrobenzene, biphenyls, etc. (Chatterjee *et al.,* 1981).

Q.9. Explain the chemotaxis process in bacteria in detail.

Ans.

Chemotaxis

Chemotaxis is the movement of bacteria towards chemical attraction and away from chemical repellants. Bacteria are attracted towards the nutrients such as sugars and amino acids, and are repelled by harmful substances and bacterial wastes. They also respond to other environmental fluctuations such as temperature, light, gravity, etc.

1. **Demonstration of Chemotaxis :** Chemotaxis can be demonstrated in laboratory by observing them in chemical gradients. A thin capillary tube is filled with an attractant and put into bacterial suspension by lowering down the capillary tube. From the end of capillary attractant is diffused into the suspension and chemical gradient is estblished. Bacteria assemble at the end of capillary tube and swim up the tube. The rate of chemotaxis can be measured by the number of bacteria within the capillary after a given time. It has been found that bacteria also respond to even low concentration (10 - 8M) of some sugars. They increase the responses with inceasing the concentration of sugar. If both the attractants and repellants are present together, bacteria will respond chemicals with the most effective concentration.

2. **Chemotactic Behaviour of Bacteria :** By using the tracking microscope (a microscope with automatic moving stage that keeps an individual bacterial cell in view chemotactic behaviour of bacteria can be studied

   E. coli and other bacteria move randomly in absence of chemical gradient. Bacteria run in a straight direction or slightly curved line for a few second. Then it stops and tumbles for a short while (Fig.). The tumble is followed by a run in different directions. If the attractant concentration is higher, tumbling behaviour is checked and they run for a long time. The opposite response occurs when a repellant is present. The frequency of tumbling decreases when bacteria moves down the gradient away from the repellent. When a bacterium is provided with attractant gradient they tumble less frequently *i.e.,* has long runs while travelling the gradient. Bacteria compare its current environment with that present a few moment ago (Adler, 1976).

3. **Molecular Mechanism of Chemotaxis :** The special proteins called chemoreceptors are supposed to be present in the periplasmic space of plasma membrane that detect the attractants and repellents. These proteins bind to chemicals and transmit signals to the other components of chemosensing system. So far about 20 chemoreceptors for attractants and 10 for repellents have been discovered, a few of them take part in the begining during sugar transport into the cell.

   Parkinson (1993) has studied signal transduction schemes of bacteria, *E.coli* consists of four different chemoreceptors which are often called 'methyl accepting chemotaxis proteins' (MCPs). These four MCPs are localised in patches often at the end of rod-shaped cells. The MCPs act through a series of proteins. The whole responses triggered within 200 mili second. The mechanism of chemotaxis is shown in fig.

   The MCPs are embedded in plasma membrane in such a way that their major parts are exposed on the both side *i.e.* periplasmic side and cytoplasmic side. The periplasmic side consists of a binding site for attachment and repellents. The attractants either directly bind to MCPs molecules usually contain about 4-5 methylation sites containing special glutamic acid residues. Methyls can be added to these gultamic acid carboxyl

group by using S-adenosine methionine as the methylating agent. The chemoreceptor proteins associated with chemotactic responses are CheW, CheA, CheB, CheY and CheZ.

The cytoplasmic side ol MCP binds with two chemoreceptor proteins. First it binds to two molecules of CheW proteins which attach to a CheA protein resulting in formation of a full complex (an MCP dimer, two CheW monomer and a CheA dimer).

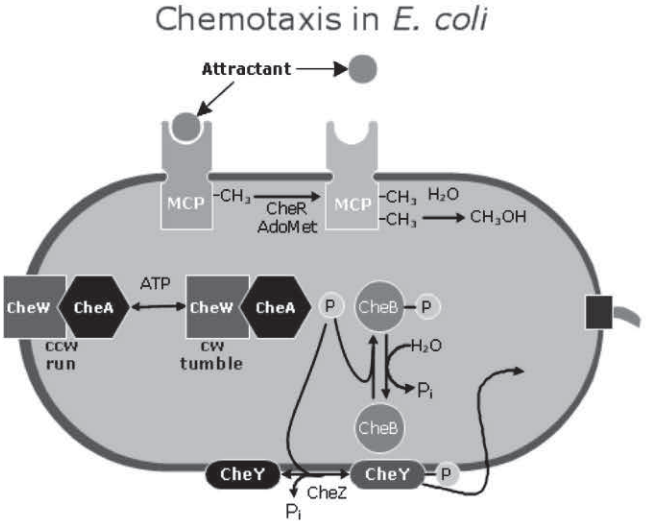


Fig. : The Mechanism of Chemotaxis in *E.coli*

The chemotactic response ariss from a combination of (i) the control of CheA phosphorylation by the concentration of attractants/repellent, (ii) the clockwise rotation promoted by CheY, and (iii) a feed back regulation circuit.

CheA is stimulated by the MCP, when unbound to an attractant, to phosphorylate itself by using ATP through the process of autophosphorylation. Autophosphorylation does not occur when attractant binds to MCP. Phosphorylated CheA provides its phosphate to either CheY of CheB protein receptor. If CheY is phosphorylated it migrates to flagellum, interacts with base protein and results in movement of flagellum clockwise (CW). However, when the concentration of attractant decreases, it results in clockwise rotation and tumbling The CheZ protein removes the phosphate from CheY in about 10 seconds; when the attractant level changes the bacterium cannot remain in tumbling stage for a long time. In the absence of attractant or repellent the system maintains the intermediate concentrations of CheA phosphate and CheY phosphate. Consequently, a normal run-tumble swimming state is maintained.

The responses are shown very quickly. This has a short term memory *i.e.* for a few second of previous attractant/repellent concentration. This adaptation is accomplished by methylation of MCP receptors. Irrespective of the concentration of attractants, methylation reaction is catalysed by the CheR protein. The phosphorylated CheB protein (a methylesterase) hydrolytically removes the methyl group from MCPs. The MCP-attractant complex is a good substrate for CheR protein and a poor substrate for CheB. Due to combining of attractant to MCPs the level of CheY phosphate and CheB phosphate decreases and autophosphorylation of CheA is inhibited.

❑

UNIT-III

Phycology

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. State the two basic type of algal plant body from which enormous range of vegetative structures originate.

Ans. The two basic types of algal plant body are Unicellular and Multicellular.

Q.2. Name the class of algae which does not show vegetative unicellular forms.

Ans. The class of algal is Phaeophyceae. It does not show vegetative unicellular forms.

Q.3. Give any two examples of non-motile unicellular algae.

Ans. The two examples of non-motile unicellular algae are : *Chlorella, Chlorococus, Spirulina.*

Q.4. What is a coenobium?

Ans. A colony with definite number of cells arranged in a particular manner.

Q.5. Name the alga which is a non-motile coenobium that consists of coccoid unicells.

Ans. *Hydrodictyon* consists of coccoid unicells.

Q.6. What are the two basic modifications of filamentous forms?

Ans. The two basic modifications of filamentous forms are : Heterotrichous habit and Pseudoparenchymatous habit.

Q.7. What type of habit is seen in algal forms like *Botrydium, Protosiphon* and *Vaucheria*?

Ans. Siphonous habit is seen in algal forms.

Q.8. Name the advanced parenchymatous thalli in algae where the differentiation of leaf-like, root-like and stem-like structure occur.

Ans. *Sargassum, Laminaria* are the advanced parenchymatous thalli in algae.

Q.9. Which is called heterotrichrous habit?

Ans. A plant having prostrate and errect branches is called heterotrichrous habit.

Q.10. What is Coenobium?

Ans. Coenobium is a colony with definite number of cells.

Q.11. The cells of *Volvox* are connected by what structure?

Ans. The cells of *Volvox* are connected by Cytoplasmic connections.

Q.12. Name the cell in *Volvox* which gives rise to daughter colony.

Ans. Gonidium gives rise to daughter colony.

Q.13. What type of sexual reproduction is found in *Volvox*?
Ans. Oogamous type of sexual reproduction is found in *Volvox*.

Q.14. Differentiate between a Colony and Coenobium.
Ans. Coenobium has definite number of cells, while Colony has indefinite number of cells.

Q.15. What are the fluid cytoplasmic connection in *Volvox* known as?
Ans. The fluid cytoplasmic connection in *Volvox* are known as Plasmodesmata.

Q.16. What is androspore which filament is developed from this structure?
Ans. Dwarf male (Nannandrium).

Q.17. What is the common name of *Chara*?
Ans. Stone wort is the common name of *Chara*.

Q.18. *Chara* usually grows in what type of water?
Ans. *Chara* usually grows in hard water.

Q.19. Name the two types of cortication found in *Chara*.
Ans. Cortication found in *Chara* are ascending and descending.

Q.20. Name the sex organs of *Chara*.
Ans. The sex organs of *Chara* are globule and nucule.

Q.21. Where do the sex organs occur in *Chara*?
Ans. The sex organs occur on primary laterals in *chara*.

Q.22. Why *Chara* is known as stone wort?
Ans. *Chara* is known as stone wort because *Chara* get encrusted with calcium carbonate and become hard brittle and rough.

Q.23. *Polysiphonia* belongs to which class?
Ans. *Polysiphonia* belongs to class Rhodophyceae.

Q.24. What type of chromatophores occur in *Polysiphonia*?
Ans. Disc shaped chromataphores occur in *Polysiphonia*.

Q.25. Where do the male sex organs of *Polysiphonia* develop?
Ans. The male sex organs of *Polysiphonia* develop on male trichoblast.

Q.26. In which member develop the tetrasporophyte?
Ans. The tetrasporophyte develop in *Polysiphonia*.

Q.27. What is the name of female reproductive structure in *Polysiphonia*.
Ans. Carpogonium is the female reproductive structure in *Polysiphonia*.

Q.28. What are the name given to the sex organs of *Polysiphonia*.
Ans. Spermatangium and Carpogonium are the sex organs of *Polysiphonia*.

Q.29. What is procarp?
Ans. Carpogonial filament produced by female *Polysiphonia* is called procarp.

Q.30. Name the type of spores produced in the cystocarp of *Polysiphonia*.
Ans. Diploid carpospore.

Q.31. What is Carposporophyte?

Ans. The complex of placental cell, gonimoblast filaments and the Carposporangia.

Q.32. Tetrasporophytic plant of *Polysiphonia* develops from which type of spore?

Ans. Tetrasporophytic plant of *Polysiphonia* develops from Diploid carpospores.

Q.33. By what name you call the opening of the cystocarp of *Polysiphonia*?

Ans. The opening of the cystocarp of *Polysiphonia* is called Ostiole.

Q.34. Mention the phase to which the tetraspore of *Polysiphonia* belongs?

Ans. The tetraspore of *Polysiphonia* belongs to Haploid phase.

Q.35. How the male gamete of *Polysiphonia* differ from the *Ectocarpus*?

Ans. Male gamete of *Polysiphonia* are non-motile.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Write about the aquatic algae.

Ans.

Aquatic Algae

Aquatic forms are found in fresh water or in saline water of the sea.

1. **Fresh water forms :** These forms occur in fresh water (low salinity water) of ponds, pools, lakes, rivers, etc. Some fresh water forms like *Cladophora, Oedogonium, Ulothrix* and *Chara* are found in slow running water, whereas others like *Chlamydomonas, Volvox, Hydrodictyon* and *Spirogyra* occur in stagnant water.
2. **Marine forms :** These forms occur in saline water of the sea and are represented by the members of Phaeophyceae (e.g., *Ectocarpus, Sargassum, Fucus*) and Rhodophyceae (e.g., *Polysiphonia*).

   The aquatic algae are either free-floating (e.g., *Chlamydomonas, Volvox, Spirogyra*) or are attached to a substratum with the help of an attachment disc, known as **holdfast** (e.g., *Oedogonium, Ulothrix*). Many algae, together with other similar organisms, form free-floating colonies on the surface of water which are called **water blooms** or **phytoplanktons.**

   They may be of two types:

   (i) **Euplanktophytes :** Algae in these phytoplanktons are never attached; they are free- floating from the very beginning. These algae are mostly fresh water in habit (e.g., diatoms, *Cosmarium, Microcystis, Scenedesmus, Pediastrum, Chlamydomonas, Volvox* and some members of the order Chroococcales).

   (ii) **Tychoplanktophytes :** In these phytoplanktons, initially algae are attached, but later they became detached and free-floating (e.g., some species of *Spirogyra, Zygnema, Cladophora, Oedogonium, Microspora, Cylindrospermum, Tetraspora, Rivularia, Nostoc, Gloeotrichia,* etc.).

   Algae like *Dictyosphaerium, Fragilaria, Cosmarium, Volvox, Asterionella* and *Golenkinia* are commonly found in fresh water planktons, whereas *Actinocyclus, Chaetoceros* and *Coscinodiscus* are common in marine planktons.

During night planktons utilize oxygen dissolved in water and as such submerged aquatic organisms do not get sufficient oxygen for respiration. Besides, phytoplanktons also prevent atmospheric oxygen from dissolving in water, thus making the habitat unsuitable for submerged aquatic life.

Many algae are found attached to rocks along the edges of lakes and seas and these forms are called **phytobenthos.**

Q.2. Differentiate between zoospore and aplanospore.

Ans. Differences between zoospore and aplanospore are given below :

Zoospores

These are motile, naked structures with two (e.g., *Chlamydomonas, Ectocarpus*), four (e.g., macrozoospores of *Ulothrix*) or many (e.g., *Oedogonium, Vaucheria*) flagella. The flagella are usually inserted anteriorly, but are lateral in some brown algae. The zoospore has a bit of chloroplast and an eye spot. The cell forming zoospore is known as zoosporangium. The protoplast of zoosporangium may form a single zoospore (e.g., *Oedogonium, Vaucheria*) or undergoes repeated divisions and each segment forms a zoospore (e.g., *Cladophora*). Zoospores are haploid or diploid.

Aplanospores

These are non-motile spores, commonly found in terrestrial algae, but some aquatic algae (e.g., *Ulothrix, Microspora*) also form them during drought conditions. They differ from zoospores in having a distinct wall and in the absence of flagella. Each cell may form a single aplanospore or its protoplast may divide to form many **aplanospores.**

Q.3. Differentiate between anisogamy and oogamy.

Ans.

Anisogamy

In anisogamy fusion takes place between morphologically and physiologically distinct gametes (anisogametes). The male or microgametes are smaller and more active, whereas the female or macrogametes are larger and sluggish. *Chlamydomonas braunii* and *Pandorina* are common examples of anisogamy.

Oogamy

This is the most advanced type of sexual reproduction. In this process a large non-motile egg or ovum fuses with a small motile sperm or antherozoid (in Rhodophyceae, sperms are non-motile). Egg is formed within the oogonium and sperms within the antheridium. *Volvox, Oedogonium, Chara, Vaucheria, Sargassum, Batrachospermum* and *Potysiphonia* are common examples of oogamy.

Q.4. Write a short note on Bacillariophyceae.

Ans.

Class Bacillariophyceae
(Diatoms, Yellow or Golden-Brown Algae)

The members of this class are characterised by the dominance of golden-brown pigments, viz. fucoxanthin, diatoxanthin and diadinoxanthin. The chromatophores have pyrenoids and the photosynthetic products are fat and volutin. The cell wall is pectic and silicified and variously ornamented. Usually it consists of two halves which are radially or bilaterally symmetrical.

The motile cells usually have a single flagellum. The sexual reproduction takes place by fusion and by the formation of gametes or auxospores.

The class includes **two** orders: (i) **Centrales** (e.g., *Cyclotella, Chaetoceras*), and (ii) **Pennales** (e.g., *Grammatophora, Navicula, Pinnularia, Denticula, Epithemia*).

Q.5. What are hormogonia? How are they formed?

Ans.

Hormogonia

The trichome breaks into small segments due to the degeneration of intercalary vegetative cells or because of the presence of intercalary heterocysts. Multicellular fragments so formed are called hormogonia. They come out of the gelatinous sheath of the colony, grow rapidly and form new colonies. Very often, the hormogones fail to come out of the parent colony and divide inside the gelatinous sheath of the parent colony.

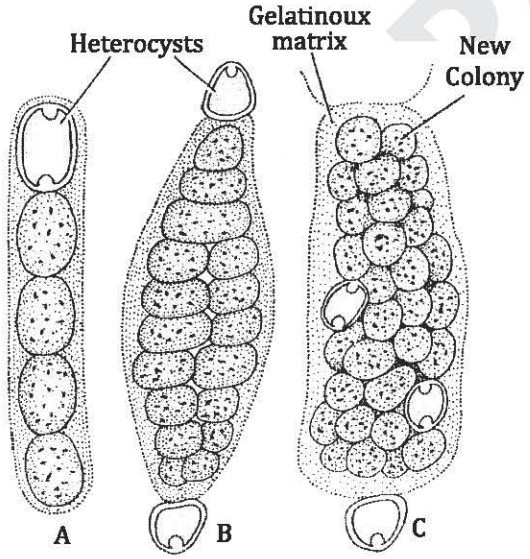


Fig. : A-C, *Nostoc* : Reproduction by hormogonia

This results in a large number of trichomes inside the parent colony.

Q.6. Describe the formation of daughter colony in *Volvox*.

Ans.

Development of Daughter Colony in *Volvox*

As mentioned above, the daughter colony develops from the **gonidium**. The enlarged and spherical gonidium undergoes three longitudinal divisions (with respect to the colony) forming eight cells. These cells arrange themselves in a curved plate and this stage is called **plakea stage**. Each of these eight cells again divides longitudinally forming 16 cells. At this stage the cells form an almost complete sphere except for a small opening, the **phialopore,** directed towards the periphery of the colony. The phialopore eventually becomes the posterior pole of the daughter colony. These 16 cells divide repeatedly and may form as many as 60,000 cells. However, such large number of divisions usually do not occur and divisions stop after a fixed number of cells have been formed.

At this stage the cells are naked and are in close contact with each other. The anterior papillate part of each cell is directed towards the centre of the hollow sphere. The normal orientation of the cells of a colony is achieved by inversion of cells of this sphere. The inversion starts with the formation of a constriction at the posterior end of the sphere (*i.e.,* opposite the phialopore) which pushes the cells of the posterior end towards the phialopore. In the mean time the phialopore becomes broader and the cells of the posterior part are pushed through it. This process is known as **inversion.** At the end of inversion the anterior papillate ends of the cells face towards the periphery of the colony.

After inversion of the colony is completed the cells secrete gelatinous cell wall and develop flagella. In this way a daughter colony is formed inside each gonidium. Many daughter colonies may develop within a parent colony at the same time and they swim freely in the gelatinous matrix of the parent colony. The daughter colonies are released after the rupture or disintegration of the parent colony. The parent colony usually degenerates after the release of daughter colonies, but in *V. africanus* and *V. carteri* two to four generations of daughter colonies can be found within the parent colony.

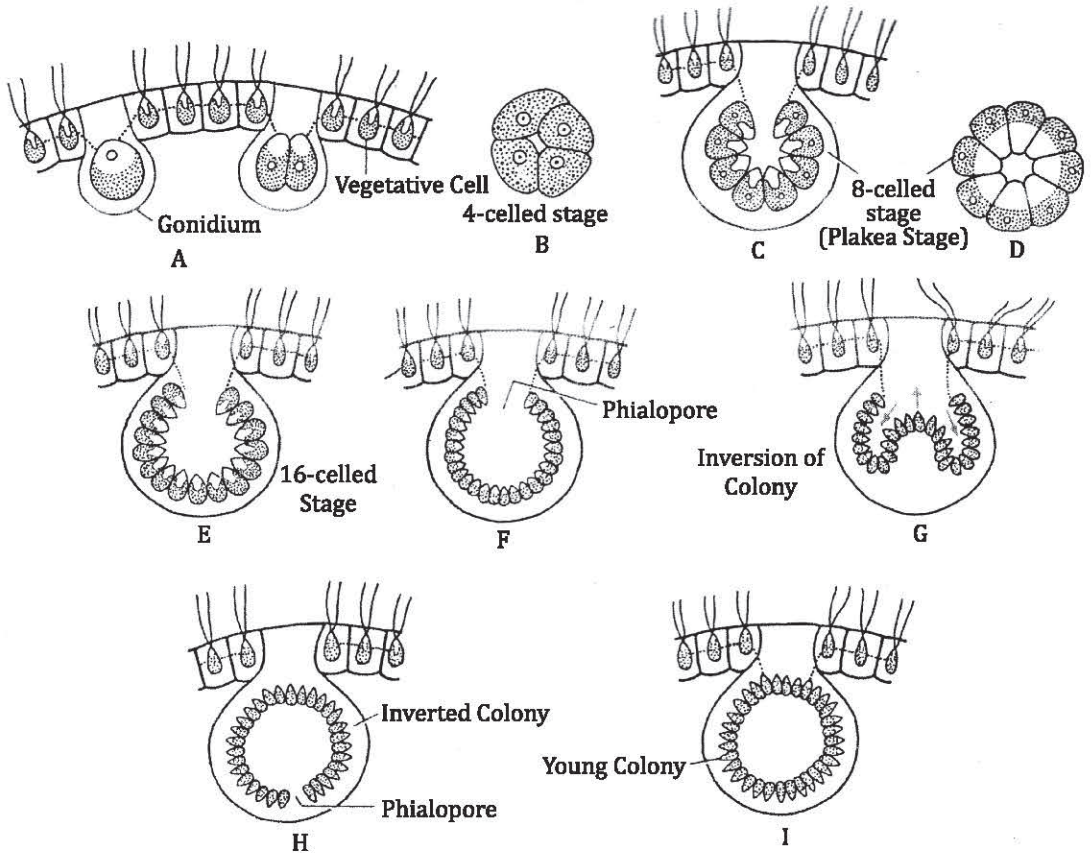


Fig. : A-I. *Volvox* : Asexual reproduction (development of daughter colony); A-D. Formation of plakea stage. E. 16-celled stage, F-H. Stages of inversion, I. Daughter colony

Q.7. Briefly describe the thallus structure of *Hydrodictyon.*

Ans.

Thallus Structure of *Hydrodictyon*

Hydrodictyon is a macroscopic coenobial alga forming free floating pentagonal or hexagonal mesh structure on the surface of quiet ponds of fresh water, hence the name **water net.** The mature coenobium is sausage-shaped and hollow in the centre, usually measuring 20-80 cm in length. The coenobium consists of few hundred to several thousand cells. The long cylindrical pencil-shaped or ovoid cells are joined at the ends forming a design of hexagons and pentagons.

All the cells of a colony have the same general structure. Each cell is surrounded by a firm cell wall of cellulose. Inner to the cell wall, there is a thin layer of cytoplasm which encloses a large central vacuole. The young cell is uninucleate with a parietal chloroplast, but at maturity it becomes multinucleate and has a reticulate chloroplast. There are several pyrenoids in a mature cell.

The number of cells in a coenobium is fixed when it is young. Further growth of the coenobium is entirely due to the increase in cell size and not the number of cells.

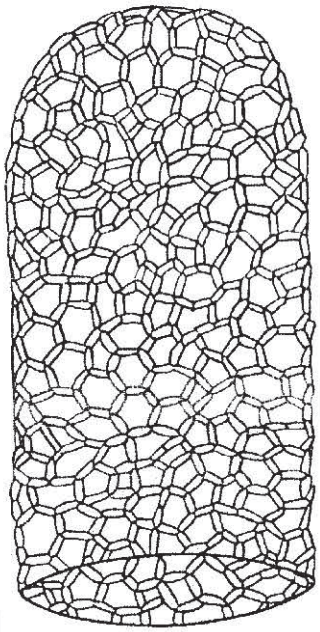


Fig. 1 : *Hydrodictyon* : A part of mature coenobium

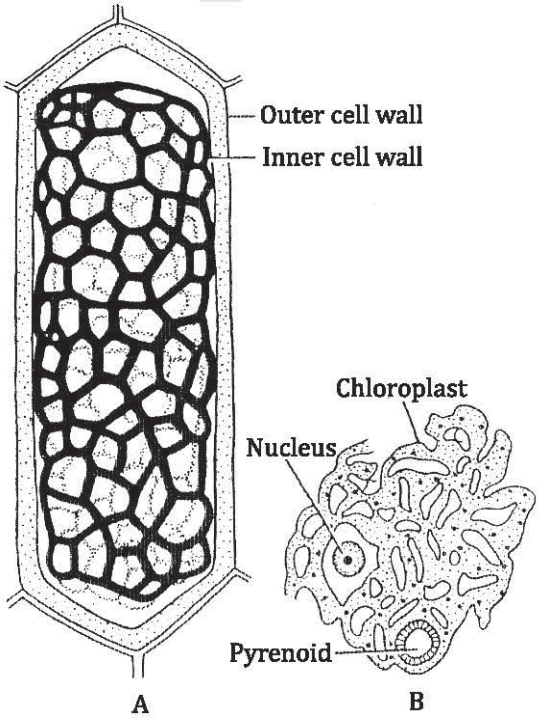


Fig. 2 : A-B. *Hydrodictyon* : A. A single cell, B. A part of chloroplast of mature cell showing pyrenoid (also note the presence of nucleus)

Q.8. Describe the cell structure of *Oedogonium.*

Ans.

Cell Structure of *Oedogonium*

The cell has a fairly thick and rigid cell wall; it is differentiated into an outer **chitin,** a middle **pectin** and an inner **cellulose** layer. Just interior to the cell wall, cell membrane is present

which encloses the protoplast. The centre of the cell is occupied by a large vacuole. The protoplast forms a thin layer in between the central vacuole and the cell membrane. The chloroplast is single, large, **reticulate,** extending in the form of a sheet throughout the protoplast. Most of the strands of the chloroplast are parallel to the long axis of the cell. Many **pyrenoids** are present at the intersections of the chloroplast reticulum. The cells are **uninucleate.** The nucleus lies in the middle region of the cell in between the chloroplast reticulum.

A characteristic feature of *Oedogonium* is the presence of distinctive ring-like scar at the distal end of some cells. The band, formed at the time of cell division, is called **apical cap,** and the cell with apical cap is known as **cap cell.**

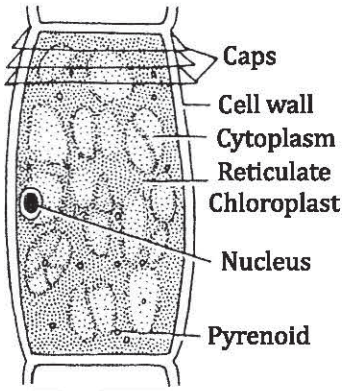


Fig. : *Oedogonium* : Cell structure

Q.9. Why is *Chara* known as stonewort?

Ans. Members of the order—Charales are commonly known as **stoneworts, brittleworts, muskgrass** or **muskworts.** These are common in quiet freshwater habitats such as ponds and streams; a few species are found in brackish water. **Stoneworts** are found in all continents except Antarctica. Charales are the only living stoneworts. These aquatic algae look like regular vascular plants. The thallus is well organized and is differentiated into **rhizoids** and an erect **branched axis.** The axis is divided into **nodes** and **internodes.** At the nodes of the main axis, **branches of unlimited growth** are present in whorls. Growth of the axis occurs at the apex and the plant is anchored in the muddy or sandy bottom of lakes/ponds by translucent rhizoids. The thallus in some stoneworts is encrusted with white lime, giving a crusty texture, hence the name **brittlewort.** Often the thallus has a strong unpleasant smell of hydrogen sulphide and thus the plants are sometimes called **skunweed.**

Chara growing in heavy water become encrusted with calcium and magnesium carbonate and hence the common name **stoneworts** is given to it. These deposits make their body rough and brittle. The metabolic proceses associated with this deposition, often give *Chara* plants a distinctive and unpleasant smell of hydrogen sulphide. *Chara* is of great ecological importance as these plants increase the calcarious contents of the ponds and lakes.

Chara can be undesirable in ponds and lakes because it can carpet the bottom and crowd out other species. An over abundance of *Chara* is brought about by excessive nutrients entering the water, such as fertilizer, agricultural runoff and leaking sewage tanks and pipes.

Q.10. Write a short note on algae as biofertilizers.

Ans.

Algae as Biofertilizers

Terrestrial algae play an important role in soil biology. Blue-green algae act as **nitrogen fixing agent in rice fields.** *Anabaena cylindrica, Tolypothrix tenuis, Aulosira fertilissima, Oscillatoria princeps, Nostoc commune* and many other members of Cyanophyceae have the capability of fixing atmospheric nitrogen. Bluegreen algae and *Azolla* constitute a system which is the main source of algal biofertilizers in south and south-east Asia. By inoculating rice fields with blue-greens, **paddy yield can be increased by 30%.**

Larger brown and red algae are used as organic manure. They are usually rich in potassium but have relatively low nitrogen and phosphorus proportions. The problem of fertilizers in

developing countries can be solved to a great extent if blue-green algae are used in combination with brown and red algae.

Algae also affect soil fertility indirectly. For example, their extracellular products serve as a source of carbon and nitrogen for microorganisms in the soil. These microorganisms in turn help in preserving the nitrogen, sulphur and phosphorus elements present in the soil. Besides, extracellular products of some algae are stored as organic or/and inorganic ions which can be easily utilized by crop plants. Some coralline or lime-depositing algae such as *Lithothamnion* and *Lithophyllum* are used for liming crop fields.

Blue-green algae also **help in reclamation of saline and alkaline soils.** The growth of blue-green algae in saline and alkaline water-logged fields results in decrease in pH and increase in phosphorus, nitrogen and organic matter contents of the field and thus converting it into fertile and cultivable land.

Q.11. Write a short note on alternation of generation in *Sargassum*.

Ans. **Alternation of Generations of *Sargassum***

The life-cycle of *Sargassum* does not show any alternation of generations. The thallus is diploid and sporophytic. The reproductive organs, antheridia and oogonia, are produced within special flask-shaped conceptacles. Both these structures are diploid and produce antherozoids and egg after meiosis. Thus the haploid phase, in the life-cycle of *Sargassum*, is represented only by gametes. The haploid male and female gametes fuse to form diploid zygote. The zygote germinates into a diploid sporophytic thallus.

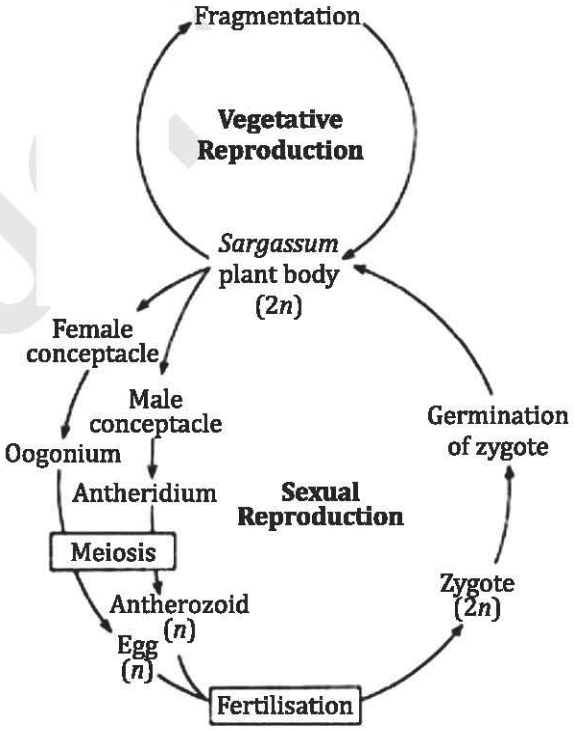


Fig. : *Sargassum* : Schematic representation of life-cycle

Q.12. Describe the economic importance of algae in industry.

Ans.

Algae in Industry

Many products of commercial and pharmaceutical importance have been derived from algae. Agar, carrageenan, alginate and diatomite are some important commercial products of algal origin.

1. **Agar-Agar :** Agar is obtained commercially from species of *Gelidium, Pterocladia, Gracilaria, Acanthopeltis, Ahnfeltia, Chondrus* and *Gigartina.* Japan and South-east Asia are the main production centres of agar. Agarophytes (red algae used in production of agar) are collected by diving and then cleaned and bleached in the sun with several washings in freshwater. The material is boiled for several hours and the extract is acidified. This is then frozen and thawed. The agar is then dried and sold in the form of flakes, cakes or powder. In modern methods agar is decolorized and deodorized using activated charcoal.

   The greatest use of **agar** is in **food, pharmaceutical** and **cosmetic industry.** It is used in the manufacture of processed cheese, puddings, creams and jellies. In preservation and canning of fish and meat it is used as **gelling and thickening** agent. Pharmaceutically, agar is used as it **laxative,** but more frequently it senes as an inert carrier for drugs which require a slow release. It is an important constituent of cosmetics (ointments, lotions, etc.) It is used for almost it century **as stiffening agent in culture media.**

2. **Carrageenan :** Carrageenan is obtained from the cell walls of ***Chondrus crispus*** (Irish moss) and ***Gigartina stellata.*** In Philippines, *Eucheuma* is extensively cultured as a source of carrageenan. For extraction, dried plants are washed with fresh water to reduce salt contents and then boiled with excess of water. The soluble carrageenan is separated from insoluble residue by a centrifuge. The soluble fraction on evaporation under vacuum yields carrageenan. Carragreenan is also an **excellent stabiliser** in milk products, it reacts with the milk protein-caesin.

   Carrageenan is extensively used in **stabilization of emulsions in paints, cosmetics, toothpaste, ice creams and lotions.** In alcohol and sugar industry it is used **as a clearing agent.** In recent years carrageenan has supplanted agar in food and pharmaceutical industry. It is also utilized in the textile, leather and brewing industries.

3. **Alginate :** These are salts of alginic acid which occur in the cell wall (particularly middle lamella and primary wall) of the brown algae belonging to the order Laminariales. Alginate is **extracted from *Laminaria, Ascophyllum, Fucus, Macrocystis, Ecklonia, Lessonia*** and ***Durvillea.*** The alginic acid contents of cell walls vary from 14-40%. They are also affected by seasonal factors, yield being the highest in winter and the lowest in summer.

   Alginates are non-toxic and viscous and readily form gel, hence **useful as thickner** (in preparalion of soup, sauce, cream, printing ink), **emulsifier** (in ice cream, synthetic cream, polish, emulsion paints) and **gelling agent** (in confectionery and dental impression powder). Alginic acid **stops bleeding** effectively. **Flame proof fabrics** are also prepared from alginates. When mixed with resin, alginates are used for **binding panel boards.**

4. **Diatomite :** After the death of diatom cells frustules usually dissolve but under certain environmental conditions they may remain intact and accumulate at the bottom of the water body where they occur. If the conditions are exceptionally favourable such accumulation may reach considerable thickness. These **deposits of diatomaceous earth are known as diatomite.** The most extensive deposits of diatomite are found at Lampoc in California.

   Diatomite is insoluble, chemically inert and shows exceptional physical properties that make it suitable for use in many industries. In oil and chemical industries it is **used as a filter.** It is heat resistance hence **used as insulator in boilers and blast furnaces.** Because of its inert nature, diatomaceous earth is used as **an absorbant of nitroglycerine.** Diatomite is used in the manufacture of car and silver polishing powders, water glass, tooth paste, paints and phonogram records. It is also used as a **filler for battery boxes** and with bakelite for switch and fuse boxes.

5. **Funori :** A type of glue, known as **funori,** is obtained from ***Gloiopeltis furcata.*** It is used in cosmetic industry for hair curling and dyeing prepartions. It is also an important paper and textile sizing agent.

Q.13. Write a short note on algae as antibiotics and in medicines.

Ans. **Algae as Antibiotics and in Medicines**

Many algae such as *Chlorella, Cladophora, Lyngbya, Polysiphonia, Laminaria* and *Halidrys* synthesize antibiotic substances. The first such antibiotic, **chlorellin,** was obtained from *Chlorella.* Extract of *Ascophyllum nodosum* is **effective against both Gram-positive and Gram-negative bacteria.** Water passed through a filter containing ***Nitzschia palea*** showed antibiotic property.

Members of the order Charales have **larvicidal property** hence may be useful in controlling mosquitoes. Extracts from *Alsidium, Corallina, Codium, Durvillea,* etc., are effective vermifuge.

Tse-ko-Tsoi, an antihelmitic drug, is prepared from a red alga, *Digenia simplex,* in South China. Some algae are used in the **treatment of diseases of kidney, urinary bladder and lungs.** Brown algae are used in various **goiter medicines** because of their high iodine contents. *Gelidium* is useful in stomach disorders.

Sea weeds are a good source of a number of vitamins. The diatom *Nitzschia* is fairly **rich in vitamin A,** riboflavin is present in good amount in *Porphyra tenera, Gelidium arnansii* and *Chondrus* spp. *Furcellania fastigata* and *Rhodomela subfusca* are **rich in thiamine,** and **vitamin C** content of *Porphyra lacinata* is higher than that of oranges.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Describe the basic types of cell organization found in algae.

Ans. **Cell Organization in Algae**

On the basis of their organization algal cells may be differentiated into **prokaryotic, mesokaryotic** and **eukaryotic** types. The **prokaryotic cell** organization is found in Cyanophyceae which is characterized by : (i) the presence of incipient nucleus, (ii) the absence of membrane bound organelles like plastids, endoplasmic reticulum, Golgi bodies and mitochondria, (iii) the absence of basic proteins-the histones in DNA, (iv) the presence of mucopeptide in the cell wall, and (v) the absence of mitosis.

A **eukaryotic cell,** on the other hand, is characterized by the presence of a well organized nucleus and membrane bound organelles like plastids, mitochondria and Golgi bodies. Majority of algae shows this type of cell organization. An intermediate type of cell organization, *i.e.,* **mesokaryotic** is found in the members of Dinophyceae, where although the nucleus has a nuclear membrane and chromosomes (eukaryotic characters), basic proteins are absent (prokaryotic character).

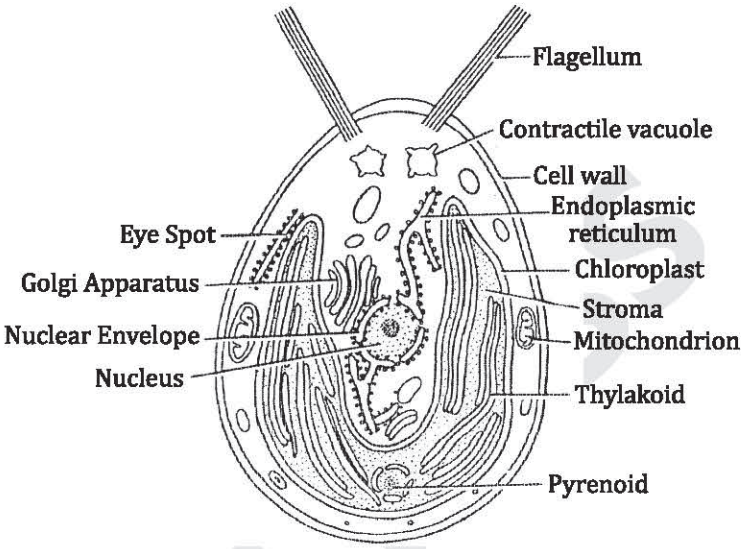


Fig. 1 : Diagrammatic representation of a eukaryotic cell (*Chlamydomonas* cell)

1. **Cell Wall :** Most of the non-motile unicellular and multicellular algae possess a typical cellulosic cell wall of non-living matter outside the cytoplasmic membrane. It is composed of pure or mixed carbohydrates (cellulose, mucilage, pectin etc.), and substances like alginic acid, fucoidin, fucin and hemicelluloses are present in brown algae (Phaeophyceae), pectin in red algae (Rhodophyceae), calcium carbonate in coralline algae (red algae) and silica in diatoms (Bacillariophyceae).

   Electron microscopic studies have revealed that cellulosic cell wall is composed of microfibrils which vary in thickness from 30 to 200 μm. The microfibrils remain embedded in a smooth or granular matrix and are variously oriented. They may be arranged in two layers at right angles to each other, in three layers or in a random manner. The cell wall of diatoms is more complex, having pores with perforated membranes.

   In blue-green algae (Cyanophyceae), the cell wall is mucopolymeric, mainly consisting of glucosamine, amino acids, muramic acid and diaminopimelic acid.

   A true cell wall is, however, absent in some algae like *Euglena, Gymnodinium* and *Pyramimonas.* They simply have a bounding membrane of cytoplasm, known as pellicle.

2. **Flagella :** Motile vegetative or reproductive cells are found in all groups of algae except Cyanophyceae and Rhodophyceae. Their motility is due to small filiform (thread like) protoplasmic appendages, called **flagella.** The number of flagella varies from

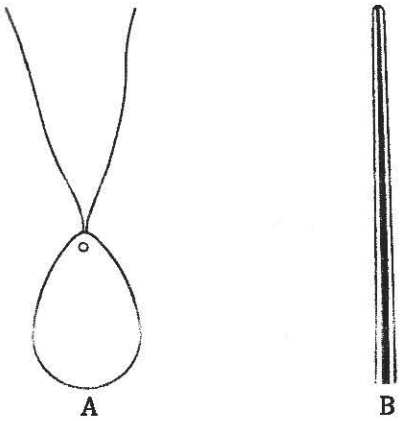


Fig. 2 : A-B. Acronematic flagella : A. An algal cell with acronematic flagella, B. A single acronematic flagellum.

one to four to many *(Oedogonium, Vaucheria)*. They are mainly of the following two types:

(i) **Whiplash or acronematic flagella :** Such flagella have a smooth surface.

(ii) **Tinsel or pleuronematic flagella :** The surface of these flagella is covered with fine filamentous appendages, known as **mastigonemes** or **flimmers**. They are further divided into three categories on the basis of arrangement of mastigonemes.

(a) Pantonematic : In this type mastigonemes are arranged in two opposite rows or show radial arrangement.

(b) Pantocronematic : A pantonematic flagellum with a terminal fibril is known as pantocronematic.

(c) Stichonematic : Here mastigonemes develop only on one side of the flagellum.

A motile cell may have either one or two types of flagella. It is a specific character. If all flagella of a cell are similar, it is known as **isokont** and when dissimilar, it is called **heterokont.**

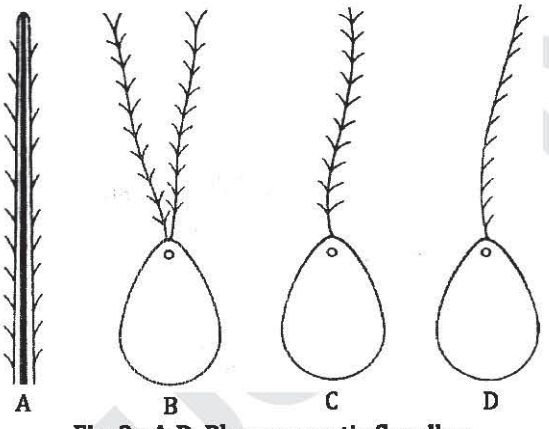


Fig. 3 : A-D. Pleuronematic flagella : A. A pleuronematic flagellum showing mastigonemes, B. A cell with two pantonematic flagella, C. A cell with pantocronematic flagellum, D. A cell with stichonematic flagellum

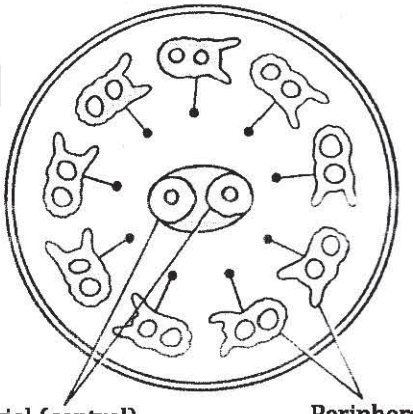


Fig. 4 : Transection of a flagellum

The motile stages of Chlorophyceae possess two or four anteriorly inserted whiplash flagella of equal length, whereas the members of Phaeophyceae and Xanthophyceae have one whiplash and one tinsel flagellum of unequal length.

A transection of flagellum reveals that it consists of nine peripheral doublet and two central singlet fibrils. All fibrils are enclosed within a common covering, formed by the extension of plasma membrane, but the two central fibrils have an additional covering of their own. The peripheral as well as central fibrils extend almost throughout the length of the flagellum. The nine peripheral fibrils at the proximal end are attached to a hollow basal body (which is separated from the flagellum by a diaphragm). The two central fibrils terminate just short to the diaphragm.

3. **Nucleus :** Almost all algae, with the exception of Cyanophyceae, have a well organized eukaryotic nucleus. In Cyanophyceae the genetic material is not found within the membrane bound nucleus and the DNA strands do not combine with histones to form chromosomes. Such a nucleus is called **prokaryotic** or **incipient.** The nucleus in Dinophyceae is also not truely eukaryotic, although it is membrane bound, but well organised chromosomes are not present.

   The nucleus generally lies alongside the cell wall but sometimes it is suspended in the centre of the cell by fine cytoplasmic threads (e.g., *Spirogyra, Zygnema).* The nucleus varies in size from less than 1 μm to 80 μm. The nuclear membrane is two layered, separated by a perinuclear space. The outer membrane is continuous with the membranes of endoplasmic reticulum. Each nucleus contains one or more dark stained nucleoli or endosomes. The four types of nucleolar structures found in algae are: (i) a single nucleolus per nucleus, (ii) two or more distinct nucleoli per nucleus, (iii) a complex nucleolar mass, and (iv) a linear association of large number of small nucleoli.

   There is a great variation in the morphology of chromosomes. They are small and spherical in desmids and elongated and thread-like in *Oedogonium.* Chromosomes may have a localized (median or sub-terminal) or diffused centromere. The size of chromosomes varies from 0.25 μm or less (e.g., Ulotrichales) to more than 12 μm (e.g., Oedogoniales and Charales). The number of chromosomes also shows much variation, the lowest number, n = .2, is found in *Porphyra linearis* (Rhodophyceae) and the highest, n =.592, in *Netrium digitalis* (Chlorophyceae).

4. **Golgi Bodies or Dictyosomes :** Golgi bodies are present in all algal cells except blue-green algae, and can be seen under electron microscope. Golgi bodies are composed of 2-20 flat vesicles which are arranged in stacks. Each stack is called a dictyosome (all dictyosomes collectively form the Golgi apparatus).

   The exact role of Golgi bodies in algae is not known. They are associated with the synthesis of cell metabolites, and have also been shown to contribute to the plasma membrane as in higher plants.

5. **Mitochondria :** Well organised mitochondria are present in all algal cells with the exception of blue-green algae. A mitochondrion is bounded, by a double membrane. The inner membrane is, projected into lumen and forms finger-like projections, called cristae. There are usually few cristae in Chlorophyceae and many in Chrysophyceae. Phaeophyceae and Xanthophyceae. The lumen, surrounded by bounding membranes, is filled with a granular matrix which contains nucleic acids (RNA and DNA). Their shape and number per cell varies in different groups of algae. There are usually more than one mitochondria per cell, but in *Micromonas* (Chlorophyceae) each cell contains a single mitochondrion.

   The respiratory enzymes are located in mitochondria, and hence mitochondria act as respiratory centres of the cell. Mitochondria are also the sites of enzyme action in protein synthesis and amino acid interconversions.

6. **Endoplasmic Reticulum (ER) :** The cytoplasm of the algal cell is traversed by a system of interconnecting tubules, known as **endoplasmic reticulum.** It is connected with the outer membrane of the nuclear envelope, but does not penetrate other cell organelles

like chloroplast or pyrenoid. The surface of ER is studded with ribosomes, the sites of protein synthesis in the cell.

7. **Eye-spot or Stigma :** The motile vegetative and reproductive cells of algae have a pigmented spot, known as **eye-spot** or **stigma.** On the basis of their position and structure, five types of eye-spots are recognised.

   Type A : Eye-spot is located in the chloroplast and it has no association with flagella, *e.g.*, Chlorophyceae and Cryptophyceae.

   Type B : Eye-spot is located in the chloroplast and associated with a swollen flagellum, *e.g.*, members of Chrysophyceae, Phaeophyceae and Xanthophyceae.

   Type C : Eye-spots are independent clusters of osmophilic granules and are situated at the anterior side of the cell, near the flagellar swelling, e.g., Euglenophyceae.

   Type D : Eye-spots having osmophilic granular structure with membranous lamellae and are situated near flagellar bases, as in Dinophyceae.

   Type E : Eye-spot is made up of a lens, retinoid and pigmented cup. These are the largest and most complex type of eye-spots and are found in some Dinophyceae.

   Although the eye spot is considered to be a light sensitive organelle which directs the movements of swimming cells, certain mutants of *Chlamydomonas* lack eye spot and are still phototactic. Some workers consider the eye spot as a shading organelle which possibly modifies the optical quality of transmitted light before it is focussed on the flagellar swelling. According to this view, flagellar swelling, rather than eye spot, is the primary receptor of light.

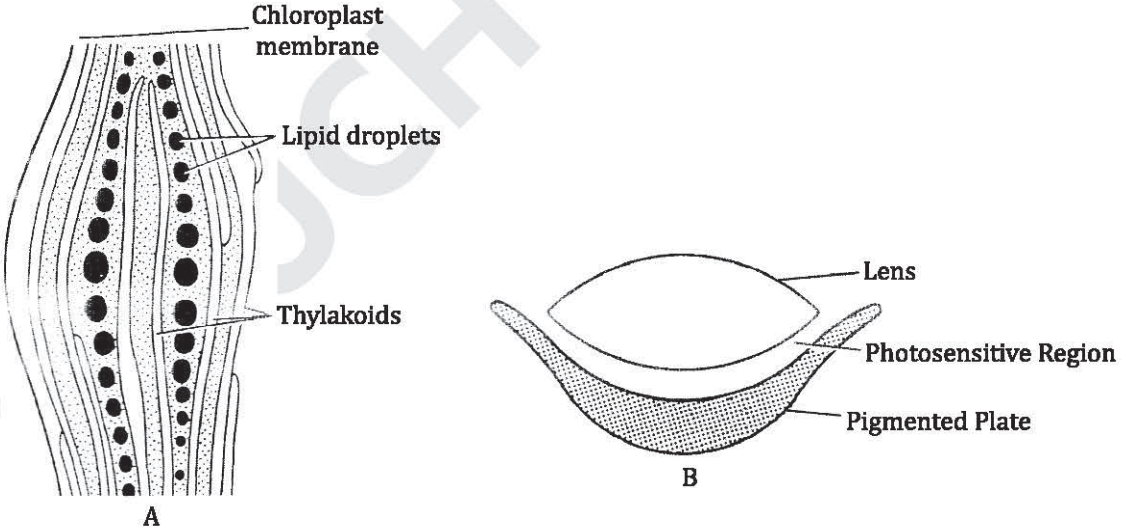


Fig. 5 : A-B. Eye-spots : A. Eye-spot of *Chlamydomonas* cell as seen under electron microscope (note the presence of thylakoids), B. Eye-spot as seen under light microscope

8. **Vacuoles :** Except Cyanophyceae, mature cells of almost all classes of algae possess one or more vacuoles. Each vacuole is bounded by a distinct membrane, called **tonoplast.** Motile algal cells may have the following two types of vacuoles :

(i) **Simple or contractile vacuoles :** Such vacuoles, found in the members of Chlorophyceae, show periodic contractions and throw out the waste products out of the cell.

(ii) **Complex vacuoles :** A complex vacuole consists of: (i) a tube-like **cytopharynx,** (ii) a large reservoir, and (iii) a group of vacuoles of varying sizes. All these parts work in close coordination. These vacuoles are characteristic of Dinophyceae and Euglenophyceae.

Vacuoles act as the main osmoregulatory organ in the cell and help in regulating the absorption of water and solutes. In holozoic forms, the cytopharynx of complex vacuoles serves as gullet. Sometimes, vacuoles also store reserve food materials such as laminarin and chrysolaminarin.

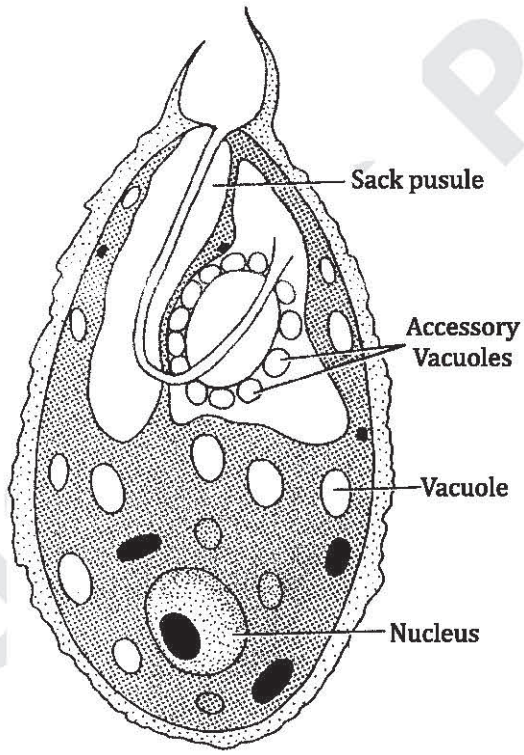


Fig. 6 : Vacuolar apparatus of *Phalacroma* cell

9. **Gas Vacuoles :** These gas containing cavities are characteristic of the mature healthy cells of Cyanophyceae. They occur as stacks of small transparent cylinders of uniform diameter. Their walls are freely permeable to gases. They render buoyancy to planktonic forms. They also act as a screen against intense light.
10. **Pigments :** Algal cells have a characteristic colour due to the presence of a combination of pigments, specific to each class. In all classes, except Cyanophyceae, these pigments are present within membrane bound organelles, known as **plastids.** In blue-greens, the pigments are concentrated in the peripheral cytoplasm, known as **chromoplasm.** Plastids are of the following two types:

(i) **Leucoplast :** These are colourless plastids.

(ii) **Chromoplast :** These are coloured plastids; those containing both chlorophyll *a* and chlorophyll *b* are called **chloroplasts** and those lacking chlorophyll *b* as **chromatophores.**

Different forms of chloroplasts occur in algae. The following are the main types :

(a) **cup-shaped,** *e.g., Chlamydomonas* and *Volvox.*

(b) **discoid,** *e.g., Vaucheria, Chara* and centric diatoms.

(c) **girdle-shaped,** *e.g., Ulothrix.*

(d) **reticulate,** *e.g., Oedogonium, Hydrodictyon* and *Cladophora.*

(e) **spiral,** *e.g., Spirogyra.*

(f) **stellate,** *e.g., Zygnema.*

The various types of pigments found in the algal cells are given below.

A. **Chlorophyll :** There are five types of chlorophylls, viz. chl *a, b, c, d* and *e.* Of these, chlorophyll *a* is present in all groups of algae, chlorophyll *b* only in Chlorophyceae and Euglenophyceae, chlorophyll *c* largely in algae of marine habitats (Phaeophyceae, Cryptophyceae, Bacillariophyceae and Chrysophyceae), chlorophyll *d* in some red algae only as a trace constituent and chlorophyll *e* in certain Xanthophyceae such as *Vaucheria hamata* and *Tribonema bombycinum.*

B. **Xanthophyll :** More than 20 types of xanthophylls are known. They are formed by the incorporation of molecular oxygen into carotene molecule. Many xanthophylls, common in higher plants (lutein, violaxanthin and neoxanthin), are found in the members of Chlorophyceae and Phaeophyceae. Fucoxanthin is the main xanthophyll pigment of Phaeophyceae and diatoms whereas myxoxanthophyll, myxoxanthin and oscilloxanthin are found only in Cyanonhyceae.

C. **Carotenes :** These are oxygen free alicyclic compounds, composed of isoprene units. The five types of carotenes occur in algae are: **α-carotene** in Chlorophyceae; Cryptophyceae and Rhodophyceae; **β-carotene** in all algal groups, except Cryptophyceae, **c-carotene** in Chlorophyceae; **E-carotene** in Bacillariophyceae, Cryptophyceae. Phaeophyceae and Cyanophyceae and **flavacene** in members of Cyanophyceae.

D. **Phycobilins :** These are water soluble complexes of protein and bile pigments, present in the photosynthetic tissue of plants. Phycobilins are red (phycoerythrin) and blue (phycocyanin) pigments which are confined to Rhodophyceae and Cyanophyceae respectively. They act as light harvesting pigments in photosynthesis and the light absorbed by them is transferred to chlorophyll *a.* Thus, like carotenoids, phycobilins are also accessory pigments.

11. **Pyrenoids :** Pyrenoids are proteinaceous bodies present in chromatophores. These organelles are considered to be associated with the synthesis and storage of starch. In

members of Chlorophyceae, pyrenoids are surrounded by starch plates. But in some algae such as diatoms, they accumulate lipids instead of starch.

A chromatophore may have one (e.g., *Chlamydomonas*) or more than one (e.g.. *Oedogonium*) pyrenoids. These pyrenoids are embedded in the chromatophore (e.g., *Chlamydomonas*) or attached on its surface with the help of a stalk (e.g., *Ectocarpus*). The embedded pyrenoids are usually traversed by large number of chromatophore lamellae.

In some algae pyrenoids are transient structures, found only at certain stages. Their presence, in such cases, is associated with the photosynthetic activity of the cell and availability of stored food material.

Q.2. Give an outline classification of algae proposed by Smith.

Ans.

Classification Proposed by Smith

The classification of algae proposed by Smith (1933, 51, 55) is based on the physiological characteristics of vegetative cells and the morphology of motile reproductive cells. He divided algae into **seven divisions** and then related classes were included in each division.

The seven divisions of algae recognised by Smith are as follows:

Division 1. CHLOROPHYTA

This division includes about 6,750 species, of these 90% are fresh water and the remaining 10% are marine. Chlorophyll *a* and *b* are the dominant pigments; the reserve food is starch. Motile reproductive cells are usually bi- or quadriflagellate; flagella are equal, of whiplash type and anteriorly inserted.

The following **two classes** were included in this division :

Class 1. Chlorophyceae (grass-green algae; *e.g., Volvox, Ulothrix, Oedogonium*).

Class 2. Charophyceae (stoneworts; *e.g., Chara*).

Division 2. EUGLENOPHYTA

There are 450 fresh-water or terrestrial species in this division. The dominant pigments of these algae are chlorophyll a, *b* and β-carotene, and their reserve food is paramylum and fats. Motile cells may be uni-, bi-, or triflagellate; flagella anterior, inserted into a narrow gullet. Multiplication takes place usually by cell division This division has only **one class.**

Class Euglenophyceae (euglenoids; *e.g., Euglena*).

Division 3. PYRROPHYTA

There are about 1,030 species, mainly unicellular and rarely colonial forms. The pigments are chlorophyll *a*, chlorophyll *c*, β-carotene and xanthophylls; the food reserve is starch and/or oil. The cell wall is cellulosic. Motile cells are usually with two anteriorly inserted unlike flagella. Sexual reproduction is rarely present.

This division has been divided into the following **two classes.**

Class 1. Desmophyceae (dinophysids; *e.g., Exuviaella*).

Class 2. Dinophyceae (dinoflagelloids; *e.g., Dinophysis, Dinastridium*).

Division 4. CHRYSOPHYTA

This division is represented by more than 6,000 species, about three-fourth of which are fresh-water and one- fourth marine. These algae are characterised by the predominance of carotenes and xanthophylls. The food reserve is **leucosin** and oil. The cell wall is usually

composed of two overlapping silicified halves. Sexual reproduction is iso-, aniso-, or oogamous.

This division includes the **three classes**:

Class 1. Chrysophyceae (golden brown algae; *e.g., Chromulina*).

Class 2. Xanthophyceae (yellow-green algae; *e.g., Botrydium*).

Class 3. Bacillariophyceae (diatoms; *e.g., Pinnularia*).

Division 5. PHAEOPHYTA (Brown Algae)

There are about 1,000 species of brown algae, mostly marine. The dominant pigments are phycophein and fucoxanthin; the assimilatory products are **laminarin** (polysaccharide) and **mannitol** (alcohol). The cell wall is cellulosic with fucinic and alginic acids. Motile reproductive cells are pyriform with two laterally inserted flagella, one of which is of tinsel type. Sexual reproduction is iso-, aniso-, or oogamous type.

This division has been divided into the following **three classes** :

Class 1. Isogeneratae (*e.g., Ectocarpus, Sphacelaria, Dictyota*).

Class 2. Heterogeneratae (*e.g., Myrionema, Laminaria*).

Class 3. Cyclosporeae (*e.g., Sargassum, Fucus*).

Division 6. CYANOPHYTA (Blue-green Algae)

This division is represented by 1,500 species,

mostly fresh water. Some species are free-living, while others grow on larger algae or within the tissue of other plants. The cell is prokaryotic; the nucleus lacks nuclear membrane and nucleolus. In addition to other pigments, they contain a blue (c-phycocyanin) and a red (c-phycoerythrin) pigment. The food is stored in the form of cyanophycean starch. Motile stages are absent but vegetative filaments of some forms show gliding movements. Sexual reproduction is absent; asexual reproduction takes place by hormogonia. fragmentation, akinetes, etc.

The members of this division are placed in a single class, **Myxophyceae** or **Cyanophyceae** (e.g., *Nostoc, Anabaena, Oscillatoria*).

Division 7. RHODOPHYTA (Red Algae)

This division includes about 2,500 species, mostly marine. They have a predominance of r-phycoerythrin which masks the other pigments to give them a distinctive red colour. They store food in the form of **floridean starch.** The thallus is non-motile and complex. Sexual reproduction is oogamous; motile reproductive cells are not found.

This division contains only **one class, Rhodophyceae** (e.g. *Batrachospermum, Polysiphonia, Chondrus, Gracilaria*).

Q.3. Give a brief and illustrated account of the systematic position, occurrence structure and reproduction in *Nostoc*.

Ans. ***Nostoc***

Systematic Position or Classification

Algae

Class — Myxophyceae

Order — Nostocales

Family — Nostocaceae

Genus — *Nostoc*

Occurrence

Nostoc is a filamentous form which occurs in the form of colonies (*Nostoc* colonies). These *Nostoc* colonies are commonly found in fresh water and in moist soil. It forms a solid or hollow gelatinous colony of varying size which is found free floating over the surface of water. *Nostoc* may also be found symbiolically with certain lichens or as endophyte in the thalli of *Anthoceros*, roots of *Cycas*. This algae is of great economic value in paddy fields where its species fix atmospheric nitrogen.

Structure

The *Nostoc* colonies are very much eontroted and are embedded in a mucilaginous envelope. This mucilaginous sheath is more or less firm. Within this sheath innumerable, Nostoc

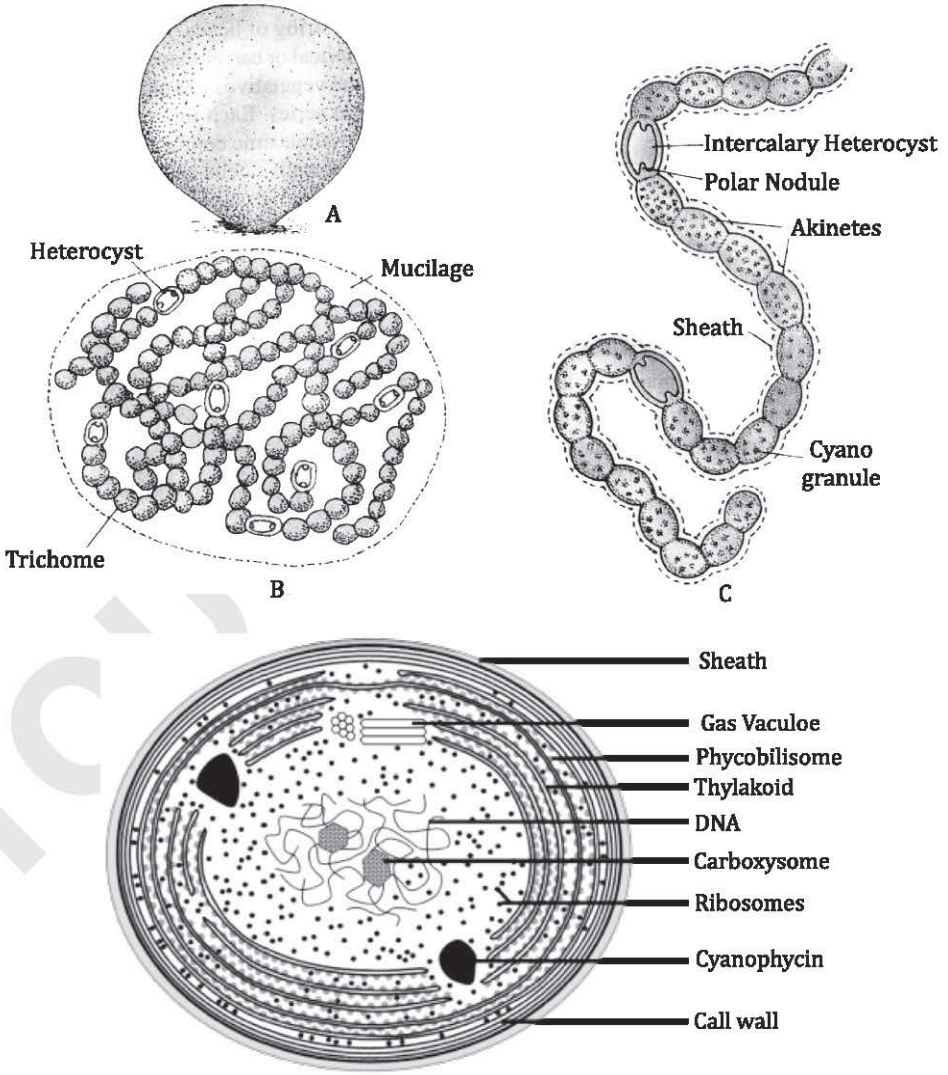


Fig. 1 : ABCD : *Nostoc*-Morphology. A. Portion of colony under low power. B. C. Filament enlarged. D. Cell (as seen under electron microscope)

filaments lying irregularly are present. Each *Nostoc* plant is an unbranched filamentous algae. Each filament contains numerous rounded of oval cell attached one above the other and apparently the filament is of moniliform or looks like a chain of beads. Usually every filament is enveloped by a gelatinous sheath which in some cases may be altogether absent. All the cells of filaments are not alike but at intervals are found slightly longer rounded, colourless, yellowish cells with thickened walls-known as heterocysts and are specially concerned in the multiplication of filaments.

Each cell of *Nostoc* filament is circular or oval in outline and has a two layered cell wall, the outer one of peptic compounds and inner one of cellulose. Inside the cell wall is the peripheral chromoplasm having pseudovacuoles pigments and reserve food in the centre is the centroplasm or incipient nucleus. The plant is autotrophic in its nutrition.

Reproduction

There is no sexual reproduction but it reprouduces asexully or vegetatively by the following methods :

1. **Hormogonia :** During the favourable conditions the trichomes generally break from the ploace where heterocyst is united to a vegetative cell. After breaking up, the homogonia form new filaments.
2. **Heterocysts :** The heterocysts are intercalary in origin and are generally isolated. The reproduction by heterocysts is not very common in this genus. In **N. commune,** the heterocysts have been observed to give rise to new filament, the new filament is liberated by the breaking of the wall of heterocyst.
3. **Akinetes or Arthospore :** These are found when a colony is mature and conditions are unfavourable. They occur in between the two heterocysts. Each akinite is somewhat oval in outline having exospore and endospore. On the approach of favourable conditions they germinate by the rupture of thick exospore. The cytoplasmic contents by repeated division develop into a new filament.

Heterocyst

In majority of filamentous Myxophyceae the button like heterocyst are found. However they are absent in oxcillatorieaceae. Each heterocyst is a spherical oval structure with a thick wall of two layers. The heterocysts may be intercalars (*e.g. Nostoc*) or terminal (*e.g.* ***Rivularia*** and ***Glaeotrichia***) in position.

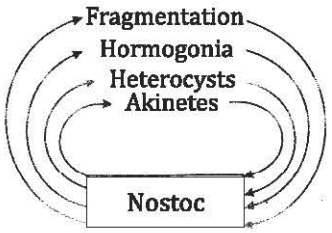


Fig. 2 : Graphic Life Cycle of *Nostoc*

Heterocysts develop by the metamorphosis of the ordinary vegetative cells.

The nature of the heterocyst is also very debatable and controversial. **Kohl** (1930) and **Borzi** (1878) considered them as a device for the multiplication of the filament as in most case the hormogones break as the point or heterocyst. **Hegle** (1901) **Fritsch** (1904) suggested that they are meant for the storage of food reserve. **Gietler** (1921) believes that they are abortive reproductive structure. **Fritsch** (1951) suggested that the terminal heterocyst promote active growth and division. As cells become further removed by growth, intercalary heterocysts are formed.

Fay *et. al.* 1986 reported that heterocyst has role in nitrogen fixation. Recent researches have established that heterocyst is the site of nitrogen fixation in most of blue green algae.

Q.4. Write the classification of *Volvox*. Also explain the reproductive process in *Volvox*.

Ans. ***Volvox***

Systematic Position

Kingdom — Plantal
Division — Chlorophyta
Class — Chlorophyceae
Order — Volvocales
Family — Volvocaceae (According to Smith)
Genus — ***Volvox***

Occurrence

Volvox (Volvere = to roll) includes a dozen or more species and often found as minute green balls, just visible to the naked eyes, swimming in temporary or permanent fresh water ponds. This is very common in rainy seasons.

Reproduction

The reproduction in Volvox takes place by the following methods:

Asexual reproduction

This takes place by formation of **gonidia** or **parthenogonidia.** Any cell from the posterior part of colony functions as gonidial initial cell. It increases in size and the protoplasmic

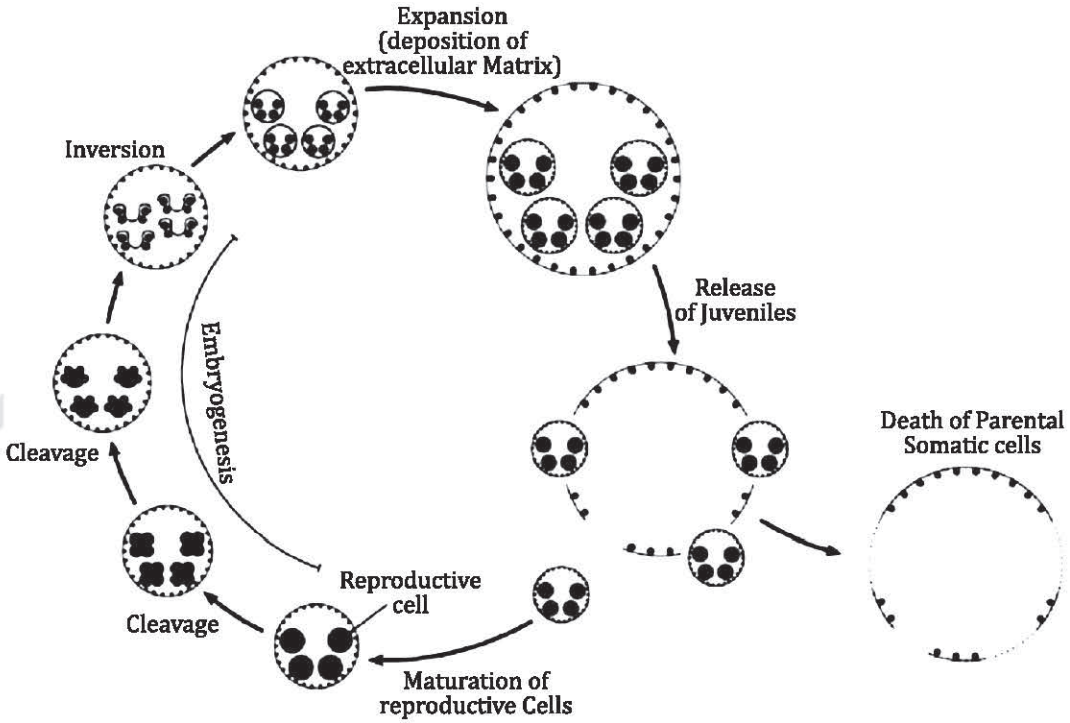
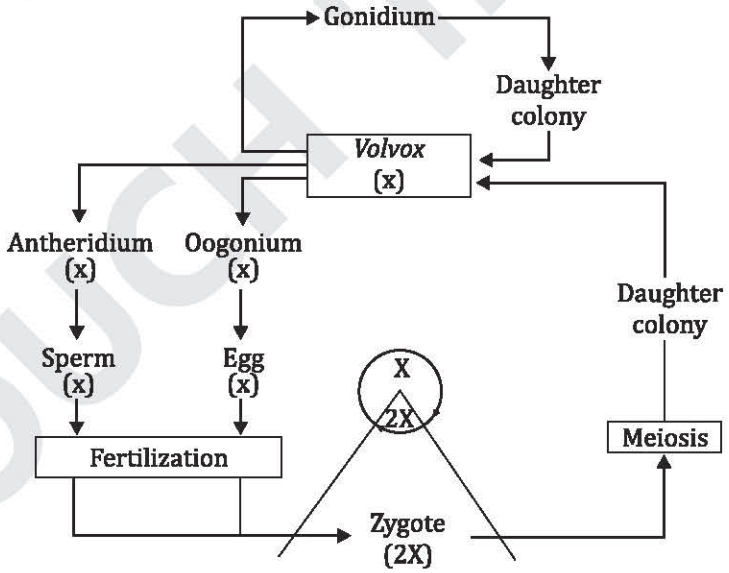


Fig. : Asexual reproduction in *Volvox* : (A-F) formation of daughter individuals. All cells are haploid (*n*)

contents divides longitudinally into two, four, then eight cells (All the four cells divide simultaneously and longitudinally to produce 8 cells plate known as **Plakea stage**) and finally a flat multicellular plate is formed. By subsequent division (16 celled stage) and later a small hollow sphere with a pore, the *Phialopore* is produced. The pointed ends as the cell of this sphere are directed inwards towards the cavity of sphere. Now the cell division cases and young colony curves inwards at the middle like a saucer and then turns itself inside out, by invaginating (inverting) through the phialopore. Inversion get completed in 3-5 hours. Flagella are developed shortly after in inversion on the pointed ends of cells now directed outwards. In this way new daughter colony is produced inside the parent colony enclosed with in the enlarged parent (gonidia) cell wall. The daughter colony slowly revolves within the parent cell which eventually develops into a mucilage membrane surrounding the young colony. It remains there until the parent colony ruptures at the phialopore of adult or any place.

Sexual Reproduction

The sexual reproduction is of **oogamous** type. The colonies of ***Volvox*** may be monoecious (*V. glabator*) or dioecious *(V. aureus*). The sexual reproduction in this case is brought about, with the help of distinct gametes Antherozoids (Spermatozoids) and oogonium. The reproductive cells are posteriorly situated and may be a few dozen to few hundred.



The vegetative cell of ***Volvox*** colony developing into oogonium (Female sex organ) enlarges and acquires the shape of a flask, loses its flagella. These oogonia consist of venter having a egg and a neck.

The vegetative cell of ***Volvox*** colony developing into an **antheridium** (Male reproduction organs) become non-motile, enlarges and the contents divide to form 32 or 64 or more biflagellate antherozoids (Spermatozoids). The plane of division is longitudinal. The antherozoids aggregate in a group and form a packet or bundle of antherozoids. Each

antherozoid is spindle shaped, biflagellate and uninucleate structure. The antherozoids are liberated by the rupture of the antheridial wall.

The fusion of egg and antherozoids results in the formation of a spherical oospore or zygote. It secretes a wall around it. At the time of germination the exospore of zygote ruptures and in time it comes out in the form of a vesicle. The nucleus divides by reduction division and protoplasm migrates into the vesicle. The protoplast may develop into a young colony by mitotic division or it may form a single oospore which later on by longitudinal divisions forms the young colony in the same way as during asexual reproduction *i.e.,* by the process of inversion.

Q.5. Write about the vegetative and asexual reproduction in *Oedogonium* in detail.

Ans. *Oedogonium* is the most common genus of the order Oedogoniales. It is a freshwater filamentous alga with more than 400 species, usually present in permanent water bodies of water, such as ponds, lakes and shallow tanks. The filaments are attached to rocks, logs. etc. or are epiphytic on aquatic plants and other algae.

Reproduction in *Oedogonium*

Oedogonium reproduces by **vegetative, asexual** and **sexual** methods.

I. Vegetative Reproduction

The vegetative multiplication takes place by **fragmentation** and **akinetes.**

1. **Fragmentation :** Like many other algae, small fragments of *Oedogonium* filament have the capability to grow into complete filaments under favourable conditions. Fragmentation takes place by accidental breaking of the filament, dying or dehydration of intercalary cells or conversion of intercalary cells into sporangia.
2. **Akinetes :** These are thick walled reddish or brownish structures, usually formed in small chains during unfavourable conditions. These spores germinate under favourable conditions and form new filaments.

II. Asexual Reproduction

The asexual reproduction is by means of **zoospores.** Under favourable conditions all cells except hold-fast are capable of producing zoospores. The zoospores are formed singly within a cell. Usually the cell with apical cap behaves as zoosporangium. During zoospore formation cell contents contract slightly from the cell wall. A small lens-shaped hyaline area develops on one side of the protoplast which eventually becomes the anterior end of the zoospore. At the base of this hyaline area a ring of basal granules appears, and from each granule a single flagellum arises. The basal granules remain connected with each other by a fibrous strand.

The mature of zoospore is ovoid, spherical or pyriform. It is uninucleate and contains a chloroplast. Sometimes the zoospore also possesses an eye-spot. At maturity of the zoospore, the wall of the zoosporangium splits near the cap region, and the adjacent cell moves apart to make passage for the liberation of the zoospore. It is believed that a mucilagenous substance is secreted at the base of the zoospore in zoosporangium which probably aids in the extrusion of the zoospore after absorbing water. The zoospore comes out of the zoosporangium in a delicate, mucilagenous vesicle which soon gets dissolved and the zoospore is liberated.

Germination of zoospores : After liberation, the zoospore swims for about an hour and then settles on any solid substratum with its anterior end downwards. It retracts its flagella and

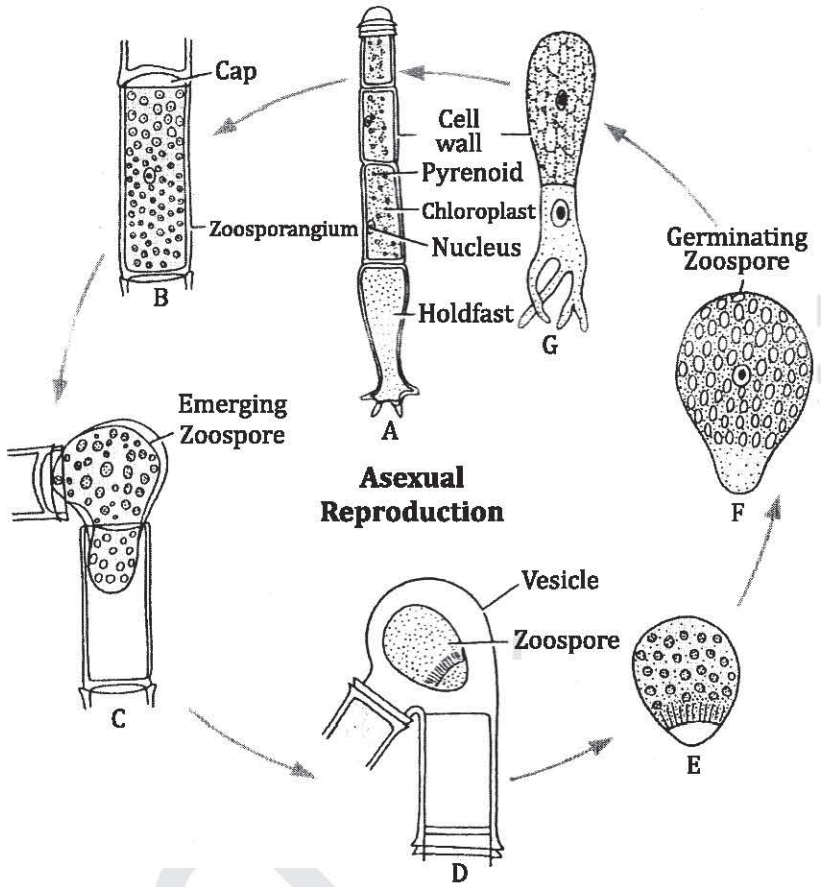


Fig. : A-G. *Oedogonium* : Asexual reproduction; A. A filament, B. Zoosporangium, C-D. Liberation of zoospore, E. A zoospore, F-G. Germination of zoospore

elongates considerably. A transverse septum separates the basal hyaline holdfast from the apical green cell. The apical cell divides repeatedly to form a new filament.

Q.6. Describe the reproduction and life cycle of *Chara.*

Ans. The members of Characeae are cosmopolitan in distribution. They are aquatic or sub-aquatic. Plant body has a horizontal **rhizoidal system** and an **upright system.** The upright system is differentiated into nodes and internodes. From main axis arise **shoots of unlimited growth** which also have nodes and internodes. These shoots bear branches of **limited growth** at their nodes. Members of Characeae are characterized by a crown of five cells on the nucule (oogonium).

Reproduction

Chara reproduces by vegetative and sexual methods. Asexual spores are not found.

I. Vegetative Reproduction

The following structures formed in *Chara* help in vegetative propagation.

1. **Amylum stars :** In some species of Chara (e.g., *C. stelligera*) basal nodal cells develop in a peculiar fashion and form star-shaped aggregates of cells, densely filled with amylum starch. When detached from the parent plant, these bodies develop into new plants.

2. **Root bulbils :** On the rhizoids of *C. aspera* some oval or spherical bulbils develop. On being detached from the parent plant, the bulbil germinates to form a new plant.

   In *C. baltica* bulbils develop on the nodes of the main axis. They also act as perennating organs.

3. **Amorphous bulbils :** In some species of *Chara* (*e.g., C. delicatula*) there is proliferation of the cells of lower nodes. These irregular aggregates of cells are known as **amorphous bulbils.** They help in vegetative propagation.

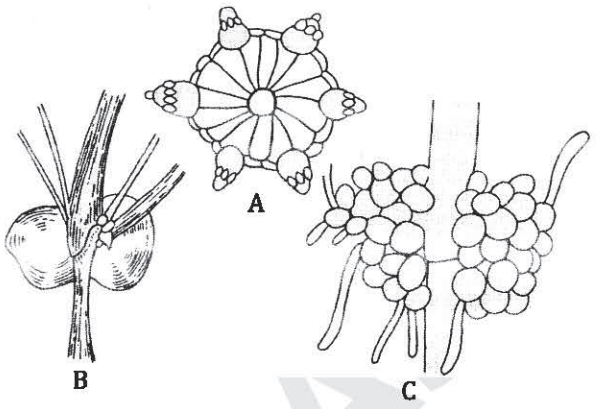


Fig. 1 : A-C. *Chara* : Vegetative reproductive bodies; A. Amylum stars, B. Root bulbils, C. Amorphous bulbils.

4. **Secondary protonema :** These are tubular structures which develop from primary protonema or the basal cell of the rhizoid. Like primary protonema, they also form new plants.

II. Sexual Reproduction

Sexual reproduction in *Chara* is of highly **advanced oogamous** type. Structurally, sex organs of *Chara* are most complex amongst algae. The male sex organ is called **antheridium** or **globule** and the female **oogonium** or **nucule.** Both these organs are macroscopic. Most of the species of *Chara* are homothallic or monoecious and the male and female sex organs are borne on the same branch at juxtaposed position. Some species of *Chara* (e.g., *C. wallichii*) are, however, heterothallic or dioecious, *i.e.,* they bear male and female organs on different plants.

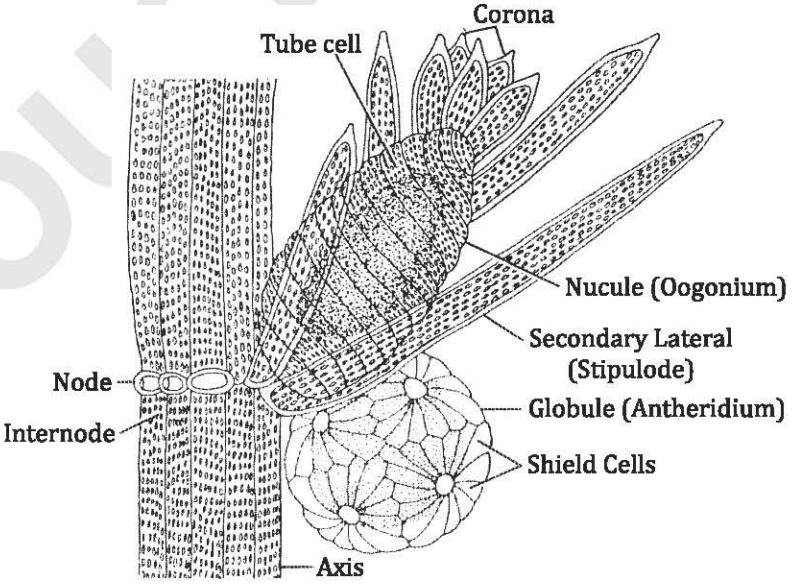


Fig. 2 : *Chara* : A portion of the branch of limited growth with a nucule and a globule.

The sex organs are borne on the branches of limited growth (primary laterals) and the **nucule is located above the globule.** Development of globule and nucule is almost simultaneous, but in some species globule matures before nucule.

1. Antheridium or Globule

(i) **Internal structure of mature antheridium :** Mature antheridium is a large, macroscopic, spherical and conspicuously red or yellow structure. Its wall is made up of eight cells, known as **shield cells.** The outer wall of the shield cells has many infoldings which form various types of ornamentations on the surface. A rod-shaped cylindrical cell arises from the centre of each shield cell which is known as **manubrium.** At the distal end of each manubrium there is one or more small spherical or globose cells which are called **primary capitulum cells.** These cells are in direct contact of manubrium. There is also a second row of capitulum cells, known as **secondary capitula.** Each secondary capitulum cell bears 2-4 long unbranched **antheridial filaments** or **spermatogenous filaments** at its tip. Each filament has 25-250 cells and each cell forms an antherozoid. Thus, each antheridium produces 20,000-50,000 antherozoids.

(ii) **Development of antheridium (globule) :** As mentioned earlier, globules develop at the nodes of the branches of limited growth. A peripheral adaxial cell of the node

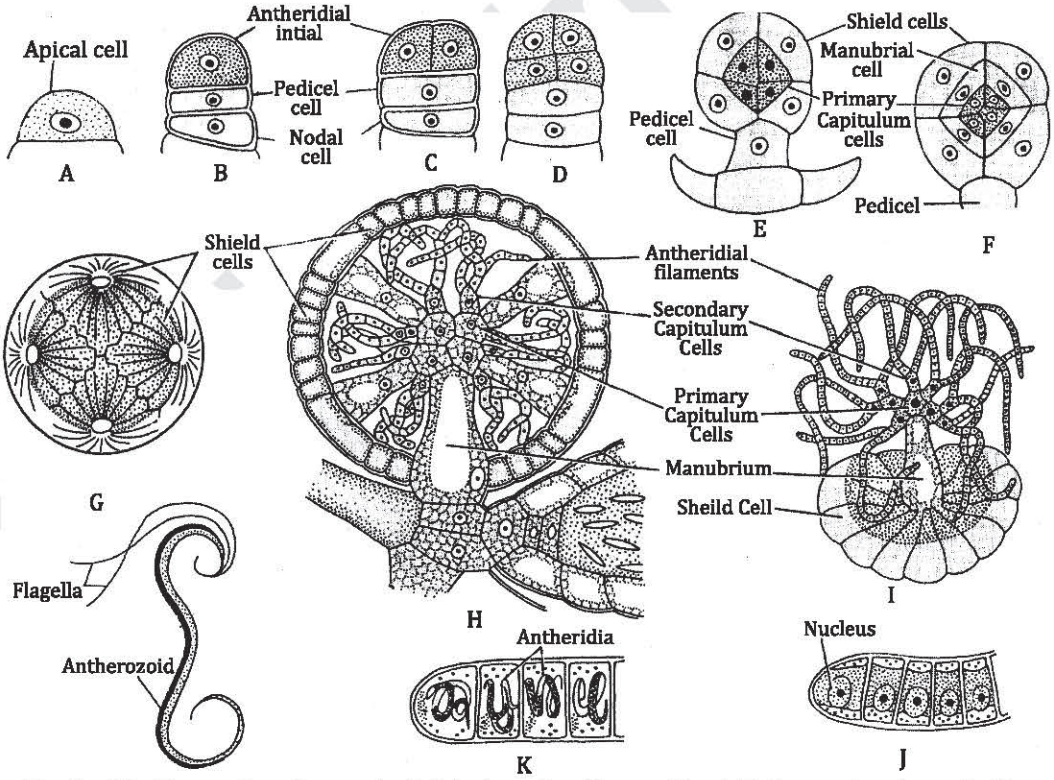


Fig. 3 : A-L. *Chara* : Development of globule and antherozoids; A-F. Successive stages in the development of globule, G. Mature globule, H. Globule in longitudinal section, I. A shield cell with manubrium, primary and secondary capitulum cells and spermatogenous filaments, J-K. Stages in spermatogenesis. L. An antherozoid.

functions as **antheridial initial.** It divides transversely into a basal **pedicel cell** and a terminal antheridial **mother cell.** The pedicel cell does not divide further and forms a small stalk (pedicel) of the antheridium. The antheridial mother cell enlarges in size and then divides by two vertical divisions to form a quadrant. Each of these four cells divides by a transverse wall and thus eight cells are formed. This stage is called **octant stage.** Each cell of the octant divides periclinally as a result of which two layers of eight cells each are formed. The cells of the inner or outer layer again divide periclinally. In this way three radial layers of eight cells each are formed. The cells of the outermost layer enlarge and transform into eight **shield cells.** The cells of the middle layer elongate toward the centre of the antheridium and eventually form eight rod-shaped **manubrial cells.** The cells of the inner layer present at the distal end of the manubrial cells form **primary capitulum cells.** Each primary capitulum cell divides to form six **secondary capitulum cells.** Sometimes secondary capitulum cells may divide again to form **tertiary capitulum cells.** The ultimate capitulum cell divides repeatedly to form 2-4 long, multicellular, thread-like filaments, called **antheridial filaments** or **spermatogenous filaments.** An antheridial filament has up to 250 uninucleate cells.

The cells of the antheridial filament function as **sperm mother cells.** The protoplast of each sperm mother cell gets metamorphosed into a single, spirally coiled biflagellate antherozoid. The process of antherozoid development is known as **spermatogenesis.**

(iii) **Morphology of globule :** According to Hofmeister and Goebel, the globule is a compound structure comprising of a large number of **one-celled antheridia** arranged in uniseriate, branched or unbranched antheridial filaments. They considered antheridia of Charales equivalent to the antheridia of other thallophytes.

The globule is homologous with the branch of limited growth.

(iv) **Liberation of antherozoids :** At maturity, shield cells of the antheridium separate from each other, exposing the antheridial filaments. The sperm mother cells gelatinize in water and the antherozoids are liberated. Liberation of antherozoids usually takes place in the morning.

2. Oogonium or Nucule

(i) **Structure of the mature oogonium :** The nucule of *Chara* is macroscopic oval structure with a short stalk. It is borne at the node of the primary lateral. In homothallic species the nucule lies just above the globule. Five spirally twisted **tube cells** cover the nucule except at the tip, where they form five **corona cells.** In the centre of the nucule there is a single uninucleate egg. The food material is stored in the egg in the form of oil drops and starch grains. At the tip of the egg there is a hyaline area, the **receptive spot.**

Presence of an envelope of sterile cells around the nucule is an advanced character amongst algae. Such a sterile jacket can be compared with the jacket of the archegonium of bryophytes.

(ii) **Development of oogonium (nucule) :** The **oogonium** develops from the upper peripheral cell derived by the division of basal nodal cell, situated at the base of the

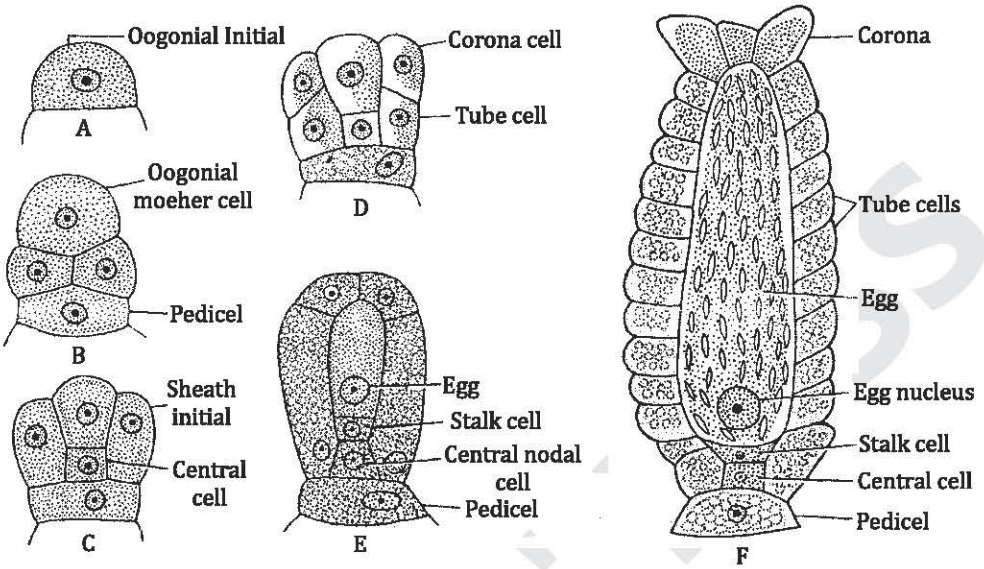


Fig. 4 : A-F : *Chara* : Successive stages in the development of nucule (oogonium).

antheridium. The **oogonial initial** undergoes two transverse divisions to form three cells in a row. The terminal cell functions as the **oogonial mother cell,** the middle as the **sheath initial** and the basal cell elongates to form the **pedicel cell,** supporting the developing nucule.

The oogonial mother cell elongates and then divides transversely into a small basal **stalk cell** and a large distal **oogonium cell.** The latter enlarges considerably, accumulates food and differentiates into an uninucleate egg.

The sheath initial divides vertically to form five **sheath cells.** The latter divide transversely to form two tiers of five cells each. The cells of the upper tier do not elongate much and form the **corona** at the tip of the oogonium. The cells of the lower tier, known as **tube cells,** elongate considerably to form a protective covering around the oogonium.

III. Fertilization

At the maturity of the oogonium, the five tube cells separate from one another and narrow slits are formed in between them. Antherozoids swim through these slits and penetrate gelatinized wall of the oogonium. Many antherozoids may enter into an oogonium but only one of them fuses with the egg to form the **oospore.** Before penetrating the oogonia, antherozoids lose their flagella.

IV. Oospore

The oospore secretes a thick wall. The inner walls of the tube cells also become thickened but the other portions of the sheath wall decay. The oospore thus looks like a thick walled nut. It is yellow, red or brown in colour depending on the species. The oospore, with remains of the

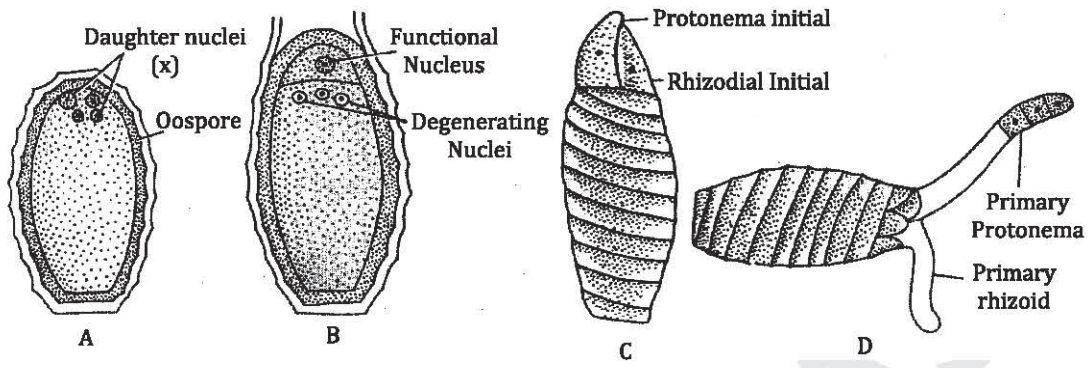


Fig. 5 : A-D. *Chara* : Stages in germination of zygote.

sheath surrounding it, falls to the bottom of the pool. It germinates after a resting period. The duration of resting period varies with environmental conditions.

Germination of oospore : Oospores of *Chara* do not germinate in dark; they require white or red light. When conditions are suitable, the diploid nucleus of oopore migrates to the apical pole and divides meiotically. As a result four haploid nuclei are formed. A transverse wall is now laid down near the anterior end forming two cells. The **upper cell** is small, **lenticular** and uninucleate and the **lower cell** is large and have three haploid nuclei. This cell contains reserve food which is utilized by the developing young gametophyte. The outer wall of the ornamented oospore cracks and the lenticular cell is exposed. It divides vertically to form a **rhizoidal initial** and a **protonemal initial.** Both these cells grow in opposite directions. The rhizoidal initial shows positive geotropism and forms **primary rhizoid,** whereas the protonemal initial shows negative geotropism and forms **primary protonema.** The primary protonema differentiates into nodes and internodes. Rhizoids and secondary protonema arise from the peripheral cells of the basal node. The peripheral cells of the upper nodes give rise to lateral branches.

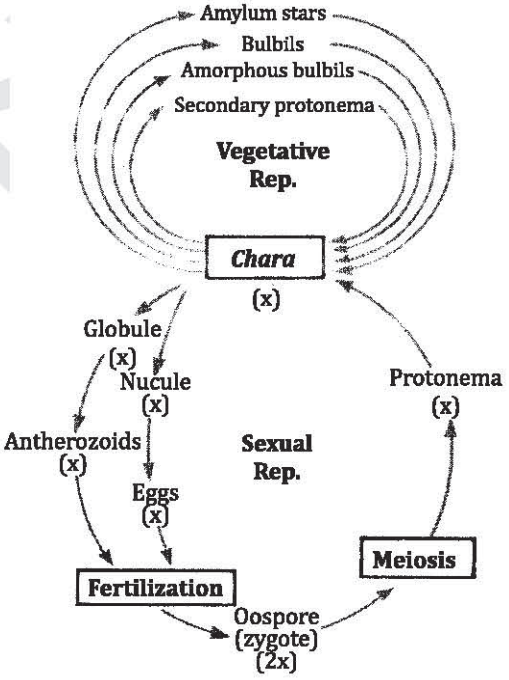


Fig. 6 : *Chara* : Diagrammatic representation of the life-cycle

Alternation of Generations

The plant body of *Chara* is haploid. The diploid condition is found only in oospore. At the time of germination the diploid nucleus of the oospore divides meiotically and thus haploid state is regained. The life-cycle of *Chara* is thus of **haploid predominant** type.

Q.7. Describe the occurrence, structure and method of reproduction of *Ectocarpus.*

Ans. ***Ectocarpus***

Systematic Position

Algae
Phaeophyceae
Ectocarpales
Ectocarpus

Occurrence

Ectocarpus is a widely distributed marine algae which usually remains attached to the substratum by means of rhizoids. Some species may be parasitic or may occur on higher members like of order fucales.

Structure

The plant body is copiously branched and consists of brown filaments. Which are very slender and give the plants a soft fuzy appearance. It shows heterotrichous habit, *i.e.,* the plant body made up of two parts, a creeping portion that serves as hold fast and a number of branches, which arises from it. The erect filaments are usually made up of a single row of cell. Each cell is uninucleate small and rectangular and consists of several chromatophores containing brown pigments. Pyrenoids are also present in the chromatophores. Growth of the filament is intercalary. The plant is autotrophic in nutrition.

A B

Fig. 1 : *Ectocarpus* : Structure (A) Habit sketch, (B) Few filaments under low power.

Reproduction

Ectocarpus reproduces both sexually and asexually.

Asexual reproduction

This may take place in following ways:

(A) This takes place by means of biflagellate **zoospores** produced in **unilocular sporangia** which are borne by the diploid sexual plant. They originate as simple, globular each densely filled with protoplasm. The nucleus undergoes a single reduction division followed by simple division. Around each daughter nucleus protoplasm collects to form zoospore. Each

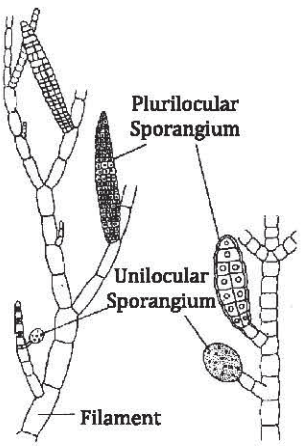


Fig. 2 : A portion of thallus enlarged

zoospore is thus a haploid structure bearing two flagella at its lateral side. On liberation the zoospore swim and settles down and grows into haplid plants.

(B) The asexual reproduction may also take place by formation of diploid biflagellate zoospore, produced in **Plurilocular** or **Neutral sporangia.** These are formed by terminal cells on the short lateral branches. The contents of these undergo repeated division followed

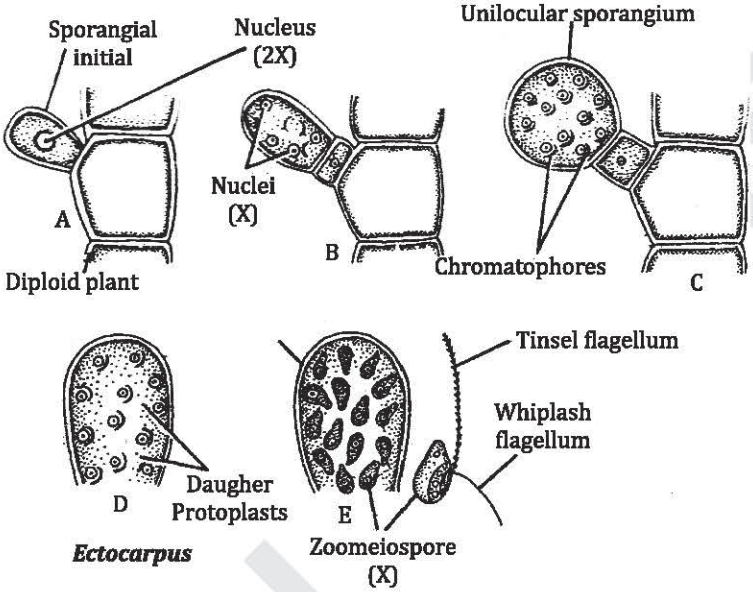


Fig. 3 : A to E : *Ectocarpus* : Zoospore formation in unilocular sporangium.

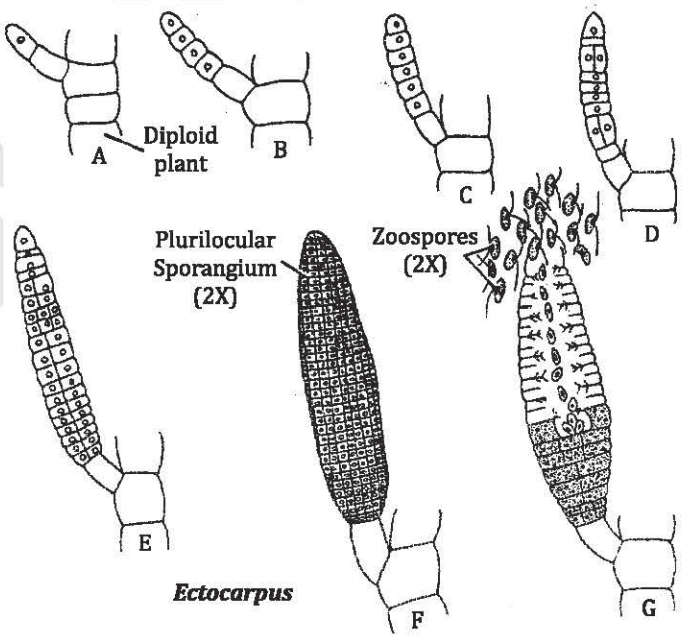


Fig. 4 : A, B : *Ectocarpus* : Zoospore formation in plurilocular sporangia.

by wall formation. As a result a number of cubical chambers of almost equal are formed. Because there is no reduction division at any stage, so the zoospore form diploid plants. On germination, these zoospose form diploid plants.

Sexual Reproduction

This ranges from isogamy to anisogamy. The fusing of motile gametes may be equal size or they may be of unequal size. They are produced inside the plurilocular sporangia borne on

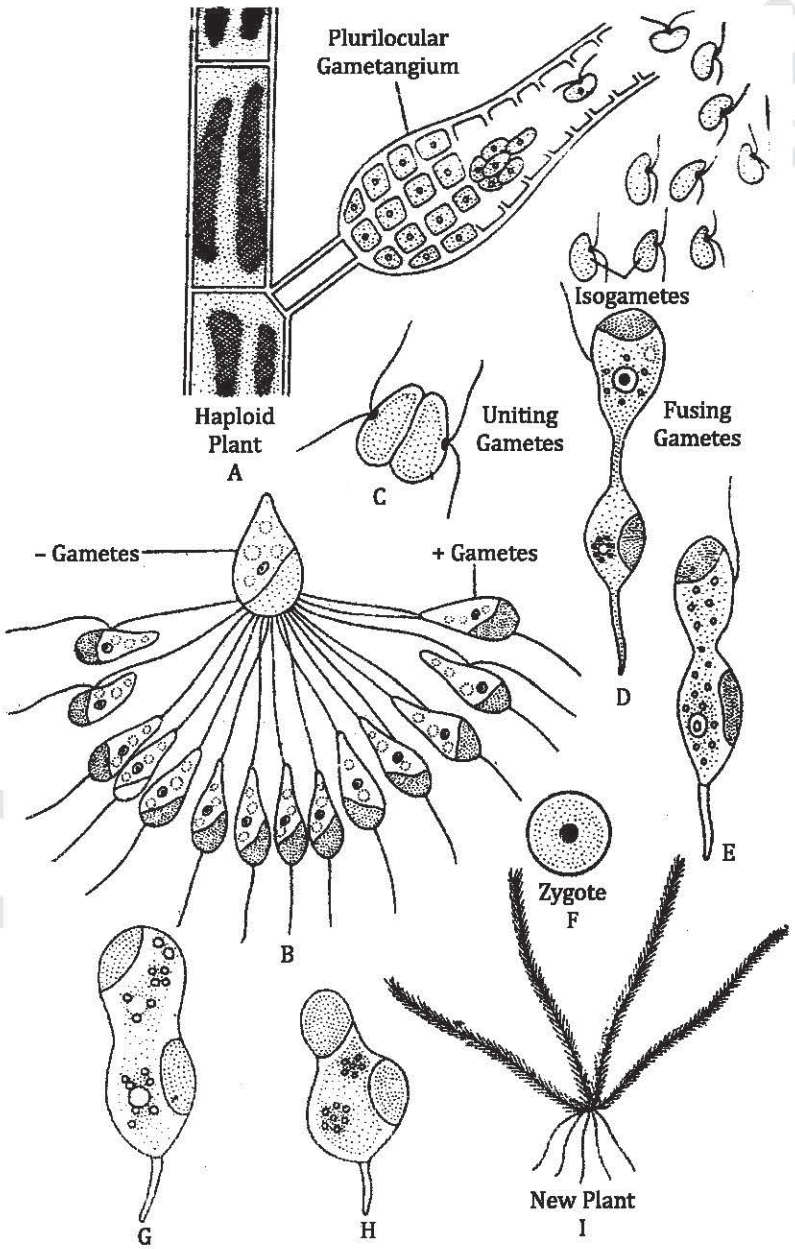


Fig. 5 : A to I : *Ectocarpus*-Sexual Reproduction.

haploid thalli. Such sporangia which are borne on haploid thalli, are known as gametangia. They produce gametes which are haploid in nature plants are usually monocious.

The isogametes or anisogametes on liberation from some different plants unite and form a diploid zygote. This zygote ultimately gives rise to diploid plant which in turn, may bear neutral or unilocular sporangia.

Alternation of Generation

In ***Ectocarpus*** the gametophytes and sporophytes are similar morphological and both are the capable of sexual reproduction by means of zoospores. Some of the plants are haplonts, others diplonts and still others show a regular alternation of generations of gametophytes and sporophytes.

Life Cycle of *Ectocarpus*

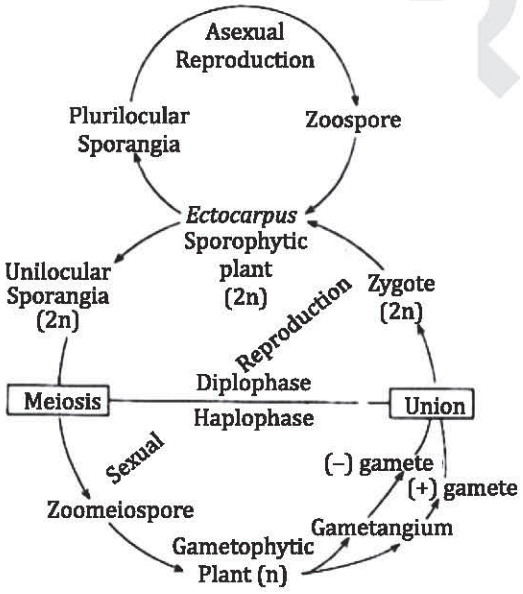


Fig. 6 : *Ectocarpus* Schematic Representation of the Life-cycle

Q.8. Describe the reproduction of *Polysiphonia.*

Ans. The members of this family are **polysiphonous,** and the cells of the central axis are uninucleate. The male and female reproductive structures develop on special branches called **trichoblasts,** present at the apex of the thallus. The trichoblasts, which bear spermatangia, are branched, whereas the female trichoblasts are unbranched.

Reproduction

In the life-cycle of *Polysiphonia* following three types of thalli are found:

1. **Asexual thallus or Tetrasporophyte :** It develops from diploid carpospore and bears **tetrasporangia** in which non-motile tetraspores are formed. Tetrasporophytes do not bear sex organs.

2. **Gametophyte :** It is **haploid thallus** and bears sex organs. Most species of *Polysiphonia* are dioecious, *i.e.,* male and female sex-organs are formed on different thalli. The male gametophyte bears **spermatangia** and the female **carpogonia.**
3. **Carposporophyte :** It develops by mitotic division of zygote and hence **diploid in nature.** The carposporophyte is dependent upon female gametophyte and bears diploid carpospores. *Polysiphonia* reproduces by asexual and sexual methods.

I. Asexual Reproduction

Asexual reproduction takes place by means of non-motile haploid **tetraspores** which are formed in **tetrasporangia,** present on diploid thalli. Tetrasporangia are small spherical stalked structures and develop from pericentral cells. The fertile pericentral cell, which behaves as **tetrasporangial initial,** is smaller than other pericentral cells of the same layer. A vertical wall divides this initial into an outer **cover cell** and an inner **sporangial mother cell.** The cover cell, by further divisions, forms two or more cover cells. The sporangial mother cell divides by a transverse wall into a lower **stalk cell** and an upper **sporangial cell.** The latter undergoes enormous enlargement and functions as **tetrasporangium.** The diploid nucleus of the sporangium divides meiotically forming four haploid nuclei, followed by the division of the protoplast into four haploid uninucleate segments. Each segment develops into a haploid spore, and the four spores of a tetrasporangium are arranged tetrahedrally. Tetraspores are liberated by splitting of the sporangial wall, accompanied by lifting away of the cover cells. Out of the four spores in a sporangium, two produce male gametophytes and two female gametophytes.

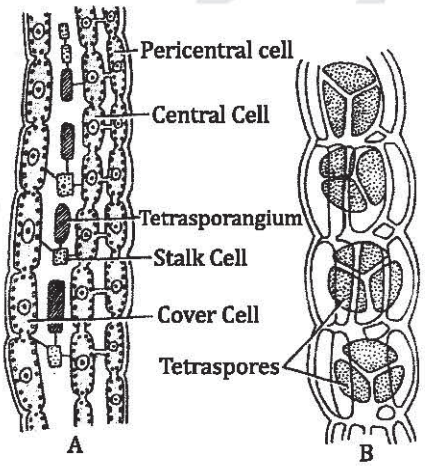


Fig. 1 :A-B. *Polysiphonia* : A. Differentiation of tetrasporangium, B. Tetraspores.

II. Sexual Reproduction

The sexual reproduction in *Polysiphonia* is **oogamous.** The plants are dioecious. The male and female reproductive structures develop on the male and female gametophytes, produced by tetraspores.

1. Male Reproductive Organs

These are called **spermatangia** or **antheridia** and develop on fertile trichoblasts present at the tip of the male gametophyte. A fertile trichoblast, when only 2-3-celled, divides dichotomously. In some species both branches of a dichotomy are fertile, whereas in others

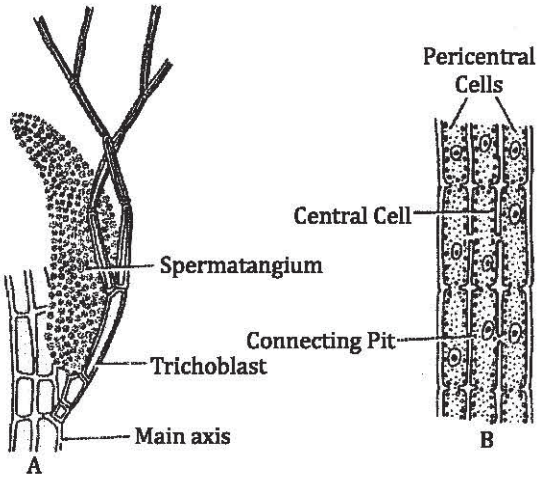


Fig. 2 : A-D. *Polysiphonia* : Development of spermantangium; A. Trichoblast with spermatangium, B. Development of spermantangium.

only one is fertile and the other remains sterile. The latter may divide again forming fertile trichoblasts.

During the development of spermatangium all the cells of the uniaxial fertile trichoblast, except for few basal ones, divide periclinally forming **pericentral cells.** The pericentral cells divide again and each forms one or two spermatangial mother cells on their outer faces.

These cells arrange themselves in a compact layer on the surface of the trichoblast. The spermatangial mother cells in turn divide to produce varying number of spermatangia. Thus, ultimately a compact cone-shaped cluster of spermatangia is formed.

The spermatangium is an oblong or globose unicellular structure. Its cell wall is differentiated into an outer thick, a middle gelatinous and an inner refractive layer. The uninucleate protoplast of the spermatangium forms a single **spermatium.**

The spermatium is liberated through a narrow slit present at the tip of the spermatangium.

2. Female Reproductive Organs

Female reproductive organs of *Polysiphonia* are called **carpogonia.** Like spermatangia, these are also formed on the trichoblast present on the female gametophyte. A flask-shaped

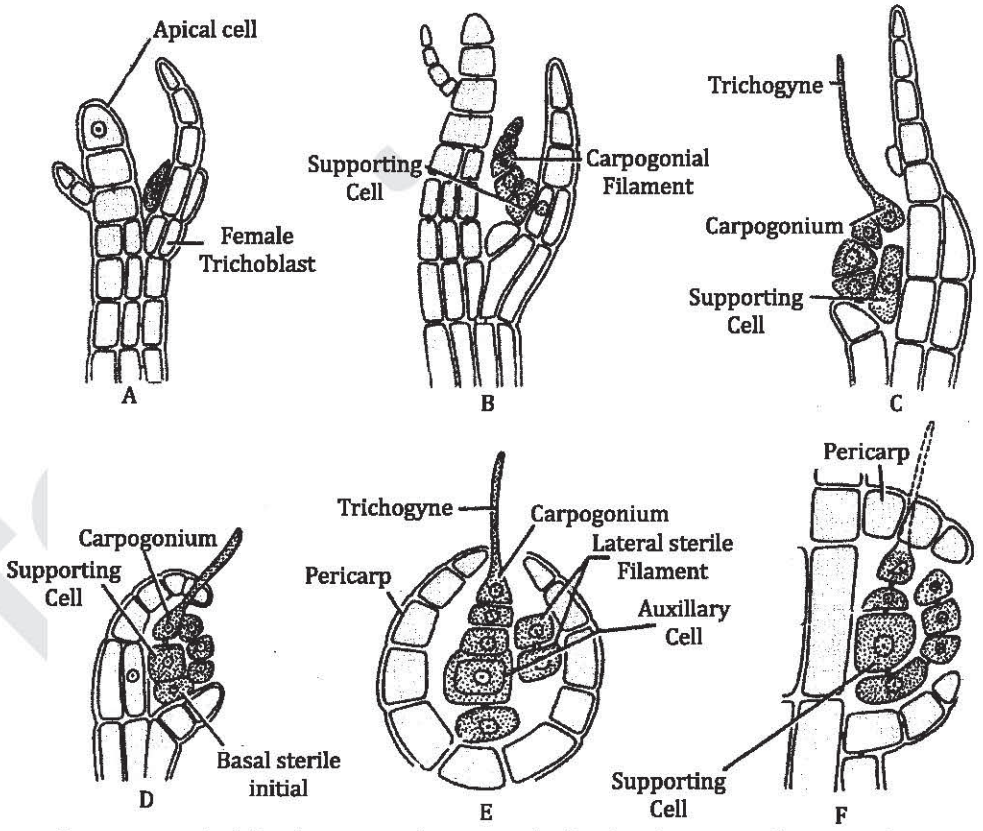


Fig. 3 : A-F. *Polysiphonia* : Successive stages in the development of carpogonium; A. Development of supporting cell, B-C. Development of carpogonial filament and formation of carpogonium, D-E. Formation of basal and lateral sterile cells, F. Differentiation of auxillary cell.

carpogonium develops at the apex of 2-5-celled **carpogonial filament.** The basal swollen part of the carpogonium has an **egg** and the upper narrow and elongated neck forms the **trichogyne.**

A young female trichoblast is uniaxial or unisiphonous, but later becomes multiaxial or polysiphonous by subsequent periclinal divisions. One of the pericentral cells of the adaxial surface of the fertile female trichoblast functions as **supporting cell.** This cell usually lies 3-5 cells below the apex of the trichoblast. The supporting cell divides transversely to form a 4-5 celled **carpogonial filament.** The terminal cell of this filament functions as **carpogonial mother cell** and eventually forms a carpogonium with a relatively long trichogyne.

Meanwhile, the supporting cell cuts off a cell on the basal side which acts as **basal sterile initial** and a cell on the lateral side which functions as **lateral sterile initial.** After fertilization, these initials form basal and lateral sterile filaments, respectively.

III. Fertilization

Male gametes (spermatia) of *Polysiphonia* are non-motile. After their release from spermatangia, they reach the carpogonium with the help of water currents and get attached to the long receptive trichogyne. This is followed by dissolution of the spermatial and trichogynal walls at the point of their contact. The contents of spermatium pass down the trichogyne through this opening and ultimately fuses with the egg nucleus in the swollen base of the carpogonium. Thus zygote is formed.

❑

UNIT-IV

Mycology

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. What are obligate parasites? Also give examples.

Ans. Obligate parasites are those that grow only on a living host, *e.g.,—Albugo, Erysiphae, Puccinia,* etc.

Q.2. Mention one important character that distinguishes fungi from the plant kingdom.

Ans. Complete absence of chlorophyll in fungi.

Q.3. Who is the founder of mycology? Give his one contribution.

Ans. P.A. Micheli. His book is *'Nova Plantarum Genera'* (1729).

Q.4. What is a conidium?

Ans. Conidium is that asexual spore which is produced externally directly on hyphae or hyphal branches (conidiophores) and germinates directly producing germ tube.

Q.5. Who discovered dolipore septum?

Ans. Moore and McAlear (1962).

Q.6. What is mycorrhiza? Give an example.

Ans. Mycorrhizae represent symbiotic (mutualistic) association of fungal hyphae with the roots of higher plants. *e.g.,*—Pinus roots.

Q.7. What is sporangium?

Ans. Sporangium is any sac-like structure which produces spores endogenously (internally).

Q.8. What is a coenocytic hypha?

Ans. Coenocytic hypha is that which lacks septation and its cytoplasm and nuclei freely move.

Q.9. What are rhizomorphs?

Ans. Rhizomorphs are root-like elongated mycelial strands developing underground in some higher fungi especially the basidiomycetes.

Q.10. Write the name of the fungus which has unicellular thallus and produces uniflagellate zoospores.

Ans. *Synchytrium*

Q.11. What is a holocarpic fungus? Give an example.

Ans. The fungus of which the entire vegetative body transforms into one or more reproductive bodies is called a holocarpic fungus *e.g.*, yeast, *Synchytrium*.

Q.12. What is prosorus?

Ans. Prosorus represents the **summer-spore** of *Synchytrium* and, after germination, develops many zoosporangia within it.

Q.13. What is 'wart disease of potato?

Ans. Wart disease of potato is a serious disease of potato tubers caused by *Synchytrium endobioticum*. As a result of this disease spherical outgrowth or warts are evident on potato tubers.

Q.14. What is resting sporangium?

Ans. Resting sporangium occurs in *Synchytrium* and is also called **'winter sporangium'** as it remains dormant throughout the winter. The resting sporangium develops from zygote and, after dormant period germinates producing haploid zoospores that cause new infection.

Q.15. What is mushroom? Give an example.

Ans. Mushrooms are the non-poisonous edible fruiting bodies of some of the Ascomycetous and Basidiomycetous fungi, *e.g., Agaricus.*

Q.16. What is a fairy-ring?

Ans. The fruiting bodies of mushrooms of *Agaricus compestris* develop in a ring above the ground in lawns and forests. Such ring or circle of fruiting bodies is called fairy rings.

Q.17. What are toadstools? Give an example.

Ans. Toadstools are the non-edible poisonous fruiting bodies of certain basidiomycetous fungi. They are often called poisonous mushrooms, *e.g., Amanita.*

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Write short note on nutrition in fungi.

Ans.

Nutrition of Fungi

Fungi are heterotropic organisms. The broad biological categories can be distinguished.

1. **Saprophytes** (Gr. *sapros* = rotton + *bios* = life) : Most fungi live on dead, ecaying plants or animals, or on the soluble organic substances which have been released from living or dead organisms. Some fungi never grow on living organisms and are called as **obligat saprophytes** (*Mucor*).

   Some saprophytes may live on living organism under favourable conditions. These are called as **facultative parasite** (hemisaprophytes) *e.g., Phythium debaryanum.*

2. **Parasites** (Gr. *Parasites* = eatinga beside another) : Some fungi grow on living organism (host) as parasite. Some parasite never grow on dead organic matter and are called as **obligate parasite,** *e.g.,* most rusts and mildews. But some parasitic fungi can live as saprophyte under changed conditions. These are called as **facultative saprophyts** (hemiparasites) e.g., smuts.

   Those parasitic fungi which live on the surface of host are called as **ectoparasite** (*sphaerotheca* sp.) But mostly parasites live inside their hosts and are called as *endoparasities.* In some parasite forms the mycelium may be inter and intracellular, *i.e.,* fungus is partially ectoparasite and partially endoparasite or endophyte such condition is called as **semiendophyte** or **semiendoparasite** (*Phyllactinia*). The

mycelium may be intercellular (lie betwen host cells) or intracellular (within host cells). Parasite organisms often develop haustoria to such nutrients from host cells.

Some of the parasitic species are endobiotic and holocarpic (*Synchytrium*). They usually lack cell wall in early stages of development and live within host cell and some times produced reproductive structures inside host cell. The most advanced forms are epibiotic and eucarpic. In these, reproductive structure are produced outside the host cell and rhizomycelium is embedded in the host tissue.

Predacious fungi capture **eelworms** (smaller nematodes) *e.g., Arthrobotrys oligospora.*

Some fungi parasitise their own kind, *e.g., Piptocephalis sp.* (belong to same order as *Mucor*) parasitise species of *Mucor* and other genera. These are called as **mycoparasites.**

3. **Symbionts :** Two types of symbionts are found.
   (i) **Lichen :** It is a symbiotic association between a fungus and an algae; resulting into a plant body structurally different from either of the two associates. The partners are called as **symbionts.**
   (ii) **Mycorrhiza :** It is an association of roots of higher plants with fungus. The mycorrhiza may be an ecototrophic (mycelium forms a mantle around the growing ascomycetes like *Neurospora* and others. It is similar to the haploid cycle except that paired, conjugate types of nuclei lie in close association in some hyphal segment (dikaryon) which divide synchronously for a greater or lesser period.
4. **Haploid-diploid cycle :** Here haploid and diploid phases alternate regularly. This type of life cycle is usually found in Algae and higher plants and occurs rarely in fungi *e.g., Euallomyces* of genus *Allomyces* and *Ascocybe grovesii* (endomyceteles).
5. **Haploid dikaryotic cycle :** Here, mycelium developed from a meiospore may persist in the haploid condition (as monokaryon). But once a dikaryon is formed, it shows independent growth and may comprise the longest phase of life cycle *e.g.,* members of basidiomycetes except smut fungi.
6. **Dikaryotic cycle :** In the products of meiosis, (ascospores or basidiospores) fuse immediately to form a dikaryon and the fungus remains dikaryotic through out its life cycle *e.g.,* yeast.
7. **Diploid cycle :** Here, haploid phase is confined to the gametes *e.g.,* yeast, slime molds, some member of Blastocladiates, most of oomycetous fungi.

Q.2. Describe Ainsworths classification of Fungi.

Ans. Ainsworth (1966, 71, 73) in his book "*The fungi an advanced treatise*" followed a new scheme of classification :

Kingdom—Mycota (Fungi)

Division-I—Myxomycota

Class 1. Acrasiomycetes
Class 2. Myxomycetes
Class 3. Hydromyxomycetes
Class 4. Plasmodiophoromycetes

Division-II—Eumycota
Sub-division I—Mastigomycotina
Class 1. Chytridiomycetes
Class 2. Hypochyridiomycetes
Class 3. Oomycetes
Sub-division-II-Zygomycotina
Class 1. Zygomycetes
Class 2. Trichomycetes
Sub-division-III-Ascomycotina
Class 1. Hemiascomycetes
Class 2. Plectomycetes
Class 3. Pyrenomycetes
Class 4. Discomycetes
Class 5. Laboulbebenomycetes
Class 6. Loculoascomycetes
Sub-division-IV-Basidiomycotina
Class 1. Teliomycetes
Class 2. Hymenomycetes
Class 3. Gastromycetes
Sub-division-V-Deuteromycotina
Class 1. Coelomycetes
Class 2. Hyphomycetes
Class 3. Agonomycetes

Q.3. Write a brief account of class-Myxomycetes.

Ans.

Class-Myxomycetes

The myxomycetes, commonly known as slime moulds are, peculiar organisms with characteristics of both plants and animals. Their vegetative phase, which consists of a slimy, naked mass of protoplasm with many nuclei, called a *Plasmodium,* is animal like while their reproduction by spores with definite cell walls, is a plant like character. Because they combine both animal and plant characteristics, the Myxomycetes or slime moulds have always been a group of doubtful taxonomic position.

DeBary (1887) considered the slime moulds to be animals and named them Mycetozoa (*mykes* = mushroom + *zoon* = animal). Later, **E.A. Bessey** (1950) and others placed the slime moulds in the phylum Protozoa of the animal kingdom. The American Mycologist **Thomas H. Macbride** (1899) was the first who used the term Myxomycetes. (Gr. *myxa* = slime + *myketes* = mushroom, fungi).

According to **Martin** (1932, 1960), and other most Mycologists, the slime moulds now are regarded as fungi. They, however, differ from true fungi by the lack of a true cell wall and the presence of animal-like amoeboid vegetative bodies.

Most of the slime moulds are saprophytes, growing in damp, shady places, on decaying wood, fallen logs, bark of trees and decaying true fungi. They are frequently found during the rainy season. Some species are parasite on algae, true fungi and higher plants.

They include about 450 species, which are found all over the world. Some common Indian genera are : *Stemonitis (S. allida, S. fusca, S. axifera, S. splendens, S. herbatica), Physarum, Diderma, Didymium, Arcyria, Cribraria, Lycogala, Hemitrichia, Trichia* etc.

Q.4. Write a brief account of Mastigomycotina.

Ans.

Mastigomycotina

1. Mastigomycotina includes true fungi and represented by 200 genera and 800 species.
2. Members may be aquatic, parasites or saprophytes. Parasitic species may grow on other fungi or algae or seed plants or ferns and on some animals particularly insects and fishes. Saprophytes usually occur on damp soil, in water or on food stuffs or on damp soil or dead plants or animals.
3. Thallus is simple and made up of single cell or filaments called **hyphae**. Hyphae are unseptate and coenocytic but sometimes septa may be formed at the time of formation of sex organs or in old mycelium.
4. The lower forms like *Synchytrium* are holocorpic and higher forms are eucarpic.
5. Fungi reproduce by vegetative, asexual and sexual means. Vegetative reproduction is by fragmentation.
6. In lower forms, asexual reproduction takes place by means of spores. These are produced in sporangia borned on sporangiophores. The spores are formed by cleavage of contents of the sporangium into numerous protoplasts. These protoplasts may develop zoospores with one tinsel type flagellum or two whiplash type flagella or non hairy flagella. Sometimes the zoospores may secrete wall and form aplanospores. The whiplash type flagellum is differentiated into a long, rigid basal portion, the **handle** and a short, thin upper portion the **lash.** The tinsel type flagellum is made up of a central axis, bearing a number of short lateral hairs, zoospores are produced by aquatic species and aplanospores are produced *y* terrestrial species. In biflagellate species the anterior flagellum is tinsel type and posterior flagellum is whiplash type.
7. The zoospores are released by aperture of parent wall or sometimes through a terminal pore or may pass on into a special vescile from where they escape later on. In some species zoospores exhibit *diplanetism i.e.,* two periods of activity with an intervening period of rest.
8. In advanced forms like *Albugo,* there is a graual transition, the sporangia themselves taking on the function of conidiophore or conidia. These may be abstricted singly or in chains from the apices of hyphae called **conidiophores**. They are dispersed by wind and may germinate directly. In the presence of sufficient moisture, the conidia or the conidiosporangia may form one to several zoospores.
9. Mastigomycotina has been divided into three classes viz. Chytridiomycetes. Hypochytridiomycetes, *Oomycetes,* chitin in absent in cell wall.
10. On the basis of vegetative and reproductive structures the Chytridiomycetes are classified into three orders : **Chytridiales, Blastocladiales** and **Monoblepharidales.**

Q.5. Explain the general characteristics of Zygomycotina.

Ans.

General Characteristics of Zygomycotina

1. It includes two classes zygomycetes and trichomycetes and is represented by 70 genera and 350 species.
2. All are terrestrial and saprophytic, mostly grow on dead and rotting organic matter in soil or on dung (*i.e., Coprophilous*). Some species are parasitic on fungi, protozoans and insects *Rhizopus sexualis* is a facultative parasite.
3. Mycelium is madeup of branched, aseptate, coenocytic hyphae. Cell wall is usually composed of **chitin.**
4. Absence of motile structures.
5. Equal or unequal gametangia fuse to form a **zygospore.** This type of sexual reproduction is termed as gametangial copulation.
6. Asexual reproduction is by means of non-motile aplanospores produced within sporangia or by means of conidia etc.
7. It has been divided into three orders **Mucorales, Entomophthorales, Zoopagales.** Order Mucorales. (sugar-fungi) includes about 45 genera and 250 species, popularly called as **black moulds.**
8. These are called as sugar-fungi because they assimilate sugars and leave the polysaccharides unutilized.
9. Saprophytic growing on bread, rotting fruit, vegetables, dung, decaying organic matter.
10. Mycelium is aseptate, branched, coenocytic and appears as a greyish-white cottony mass over the substratum, septa may be formed at the time of formation of sex organs, on mature older hyphae.
11. Asexual reproduction by non-motile, non-flagellate spores *i.e.,* sporangiospores or aplanospores. Which are produced in sporangia. The fungi are called **pin mould** due to presence of **pin head** like sporangia. In case of some genera, sporangia produce a large number of spores but in some cases only a few or one spore is produced. Such sporangia are referred to as sporangiolum (Pl. sporangiola), the single spore may fuse with the wall of sporangium and behave like a conidium.
12. Sexual reproduction is isogamous type in *Mucor, Rhizopus* and anisogamous type in *Pilobolus.*
13. Sexual reproduction takes place by means of gametangial copulation. The fusing gametangia contain one coenocytic aplanogamete each. Hyphae bearing gametangia are called as zygophores.
14. The zygospore is the product of sexual fusion. Under favourable conditions the zygospore ruptures and its contents come out in the form of a sporangiophore with terminal zygosporangium or germ-sporangium.

Q.6. Describe the general characteristics of Ascomycotina.

Ans.

General Characteristics of Ascomycotina

1. Ascomycetes *occur* in almost all climatic conditions and in a wide variety of habitats, *i.e.,* in soil, on dung loving fungi (**coprophilous**), in marine as well as freshwater, as

saprophytes of animal and plant remains and also as parasites on plants as well as animals. Mycelia of most parasitic species grow within the host tissue, but powdery mildews grow superficially upon the host showing ectoparasitic nature. Few ascomycetes are entirely **hypogean**, *i.e.* grow and develop only under ground. Marine ascomycetes are either saprobic or parasitic on marine angiosperms or large algae. Recently **Doebbeler** (1985) reported many ascomycetes growing on mosses.

2. The mycelium is well-developed, profusely branched and septate. Each segment of the hypha contains several nuclei. However, yeasts are single celled organisms.
3. In each septum or cross wall of the mycelium there is present a simple central pore. According to **Shatkin** and **Tatum** (1959) and **Moore** (1965) the pore is wide enough to allow mitochondria, nuclei and other cyloplasmic contents to pass from cell to cell.
4. The cell walls contain chitin in the form of microfibrillar skeleton in filamentous members. Mannose, glucose, amino-sugars and proteins, along with many surface enzymes have also been reported in different members. Because of the presence of enzymes the cell wall is not a functionally inert coating in ascomycetes.
5. The chief distinguishing character of all ascomycetes is the presence of a sac-like body, called **ascus** (pl. asci). It contains sexually produced spores called **ascospores**.
6. The ascospores are formed after karyogamy and meiosis. In an ascus the number of ascospores is usually 8. However, in some species their number may vary from 1 to over 1000 in an ascus.
7. The ascospores are always endogenous in origin and are also called **perfect state spores**.
8. The asci are usually grouped to form a definite type of multicellular. fruiting-body called **ascocarp**. The ascocarps remain enveloped in a sheath of sterile hyphae.
9. The ascocarps are either cup or saucer-shaped (apothecites *e.g.,* Discomycetes), flask-shaped (perithecium, *e.g.,* Pyrenomycetes), on spherical and indehiscent (cleistothecium, *e.g.,* many Plectomycetes).
10. Any type of flagellate cell is completely absent in the life cycle of all ascomycetes.
11. Asexual reproduction takes place by the imperfect stage, present in the form of non-motile, exogenously produced spores, called **conidia**. The conidia develop on special reproductive hyphae called **conidiophores**. In some members the asexual reproduction is by means of pycniospores, oidia or chlamydospores.

Q.7. Write short note about economic importance of *Penicillium*.

Ans.

Economic Importance of *Penicillium*

Penicillium is of great economic importance due to their beneficial and harmful activities. As far as its harmful activities are concerned it spoils many food stuff and other goods like furniture and leather goods. Some species causes dangerous diseases in men and animals.

Penicillium is also very useful for human kind. The wonder frug penicillin is extracted from the species. *P. notatum* and *P. crysogenum.* This drug has through a revolutionary change in the science of medicine, some species of *Penicillium* are capable of producing organic acids, such as citric, fumaric, oxalic, gallic acids etc. Some species of *Penicillium* are also used in making cheese.

Q.8. Define the sexuality in *Puccinia.*

Ans.

Sexuality in *Puccinia*

The rust fungus *Puccinia* does not have well defined sex organs. The role of pycnidiospores has been a long time a matter for speculation. Until quite recently they were conisdered to be functionless male cells, which accounts for the order name *i.e.,* spermatia. In 1941, **Meyer** regarded them as male structures but maintained that there is no sexuality among rusts. **Craigie** (1972) told that pycniospores are haploid bodies of (+) or (–) sexes. He also told that *Puccinia graminis* is heterothallic and that the four basidiospores arising from germination teleutospores are two (+) plus and two (–) negative sex. He further showed that for *Puccinia graminis* growing on barberry, an infection with a single basidiospore from a germination teleutospore produces a sours within which are produced numerous pycnia and aecial primordia. The developing pycnidia are either (+) or (–) in their sexual tendencies. It has been seen by experiments that if the pycnia are protected from the visit of insectes the aecial primodia never develop to produce aecia and aecidiospores. However, if insects are allowed free access to this sours and other sori as well the aecia will develop in a few days and produce aecidiospores. Thus, it seems probable that in all rusts producing spermogonia the spermatia are functional and have two sexual phases.

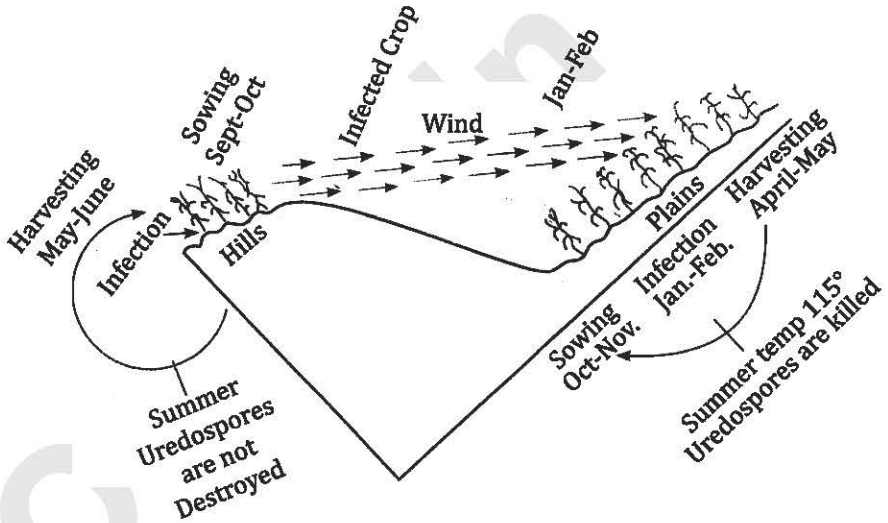


Fig. : Diagrammatic representation of reoccurrence of rust in plains of India

Pool (1940) and **Thirumulachar** (1942) confirmed by their observation that spermatia have an important role in effecting the dikaryotic condition necessary for the development of aecidial primordium **Andrus** (1931-33), **Persoon** (1933) and **Allen** (1934) have also demonstrated that here are certain elongated filaments or receptive (flexous) hyphae comparable to trichogynes. These are produced from the same haploid mycelium as bears the spermatia. They are composed of uninucleate cell and extend out from the uninucleate basel cells of aecial primordia. To these receptive hyphae the spermatia adhere, the nucleus of spermatium enters the receptive hyphae and passes down to the basal nucleus (egg). These two nucleus (+ and –) associate and the cells are diplodize thus initiating the formation of binucleate cells in the aecidal primordium. These binucleate cells divide repeatedly and give rise to chains of uninucleate aecidiospores.

According to the **Buller** and **Lefeldt** "the proces of diplodization consists in the division of the nuclei of the introduced sexual strain and their passage through the septa from cell to the mycelium that it is completely diplodized *i.e.,* each cells possesses nuclei of the both sex "Buller phenomenon".

From the above account it is clear that spermatia which were hitherto regarded as sexual spores on barberry or functionless male cells, are functional sperm cells. They are absolutely essential for the formation of aecidial (+) or (–) strains. The aecia are formed in the event of their function. In any case if due to any reason the fusion fails the spermogonial exudates and also the aecial primordia die off. The spermatia, therefore remain non-functional but effective male cells of the rusts and play an important role in the formation of dikaryotic (binucleate) mycelium and chains of aecidiospores in the aerial primordia.

Q.9. Differentiate between *Rhizopus* and *Mucor.*

Ans.

Differences between *Rhizopus* and *Mucor*

S.No.	*Rhizopus*	*Mucor*
1.	Mycelium is differentiated into three kinds of hyphae, stolons, rhizoids and sporangiophores.	Mycelium is undifferentiated, *i.e.,* it has only one kind of hyphae.
2.	Rhizoids **present.**	Rhizoids **absent.**
3.	Food material is absorbed mainly by **rhizoids.**	Food is absorbed by the entire **mycelial surface.**
4.	Sporangiophores occurs **in tufts** from the stolons opposite the rhizoids.	Sporangiophores usually occur **singly** from any point on the mycelium.
5.	Spores **easily** disseminated by wind.	Spores remain adhered to columella and thus are **not easily** disseminated.
6.	Reduction division occurs during zygospore germination **after** a period of rest.	Reduction division occurs soon after karyogamy **before** the zygospore undergoes a period of rest.

Q.10. Explain the physiological race.

Ans.

Physiologic Race

When a fungus is confined to a particular host the sake of parasitism, it is termed as **physiologic** or **physiological** or **biological specialization.** The following are the different sub-species confined to particular species of hosts.

(i) *Puccinia graminis tritici* on wheat.

(ii) *Puccinia graminis hordeii* on barley.

(iii) *Puccinia graminis avenae* on oat.

The rusts exhibit extreme degree of specialization, as far as their ability to infect plants is concerned. For example, *Puccinia graminis* causes rust disease in several members of the family gramineae *e.g.,* wheat, barley, oat, etc. However, the uredospores of *P. graminis* which infect wheat, fail to infect other hosts say oat or barley. Similarly, the uredospores produced by *P. graminis* on barley, fail to infect wheat or oat. This specialisation shown by the rusts in their parasitism of different hosts.

The phenomenon was first noted by **Shroete** (1879) in *P. graminis.* Eriksson (1894) in Sweden, recognised five varieties of *P. graminis.* To differentiate between these varieties, a third name was added to the binomial *Puccinia graminis* as below :

Puccinia graminis tritici on wheat

P. graminis avenae on oat, *P. graminis secalis* on rye, *P. graminis poae* on Poa sp., *P. graminis agrostidis* on *Agrostis* sp.

He termed these varieties as "Tormae speciales." These are also known as subspecies.

Stakman (1914) and **Levine** (1922) found that even in the subspecies *Puccinia graminis tritici,* the uredospores which infect one variety of wheat, fails to infect another variety. Thus, a further differentiation within the subspecies in required. The term physiological races or biological forms was given to these different forms. The subspecies *Puccinia graminis tritici* is composed of more than 300 physiological races.

Stakman and his associates (1930-1934) have been shown that these specialised forms originated through hybridization on the aecial host. Thus, aecial host provided not only an asylum to the rust fungi, but also an effective ground for hybridization and origin of new races and forms. This explains the occurrence of relatively smaller number of forms of stem rust of wheat in India. The absence of alternate host in India is probably responsible for lesser number of physiological races of the pathogen.

Q.11. Differentiate between Algae and Fungi.

Ans.

Differences between Algae and Fungi

S.No.	Algae	Fungi
1.	They are chlorophyllous autotrophic plants.	They are non-chlorophyllous heterotrophic plants.
2.	The plant body is filamentous or parenchymatous.	The plant body is filamentous or pseudoparenchymatous.
3.	The cell wall is made up of cellulose.	The cell wall is made of chiefly fungal cellulose or chitin.
4.	The reserve food material is usually a carbohydrate.	The reserve food material is in the form of glycogen or fat globules.
5.	They are autotrophic and light is necessary for their growth.	They are heterotrophic and light is not required for their growth and development.
6.	They show progressive complexity in sexual reproduction, *i.e.,* sexual reproduction is simple in the primitive forms and complex in the advanced forms.	They show progressive simplicity in sexual reproduction, *i.e.,* sexual reproduction is complex in the primitive forms and simple in the advanced forms.
7.	They are mostly aquatic, found in fresh and marine waters.	They occur in all types of habitats.

Q.12. Describe the heterokaryosis and parasexuality.

Ans.

Heterokaryosis and Parasexuality

Pontecorve and **Raper** (1952) in *Aspergillus nidulans* saw the genetic recombination which is possible without sexual reproduction and they called it as **parasexual cycle**. In this process, plasmogamy, karyogamy and meiosis do take place, but not at specified time or specified-points in the life cycle of the fungus. The phenomenon of parasexuality is of common occurrence in the members of the class Deuteromycetes. Sexual reproduction is absent in these fungi and hence the parasexual cycle is of much significance. Now several fungi are known where both sexual and parasexual cycles occur.

The following are some important fungi where the phenomenon of parasexuality has been studied : *Aspergillus nidulans, A. niger, A. fumigatus, A. oryzae, Penicillium chrysogenum, P. expansion, P. italicum, Cephalosporium, Cachilobolus sativus, Fusarium Oxysporum, Ustilago maydis, U. hordei, Piricularia oryzae, Coprinus cinereum, Coprinus radiata, Schizophyllum commune* and *Verticillium alboatrum.*

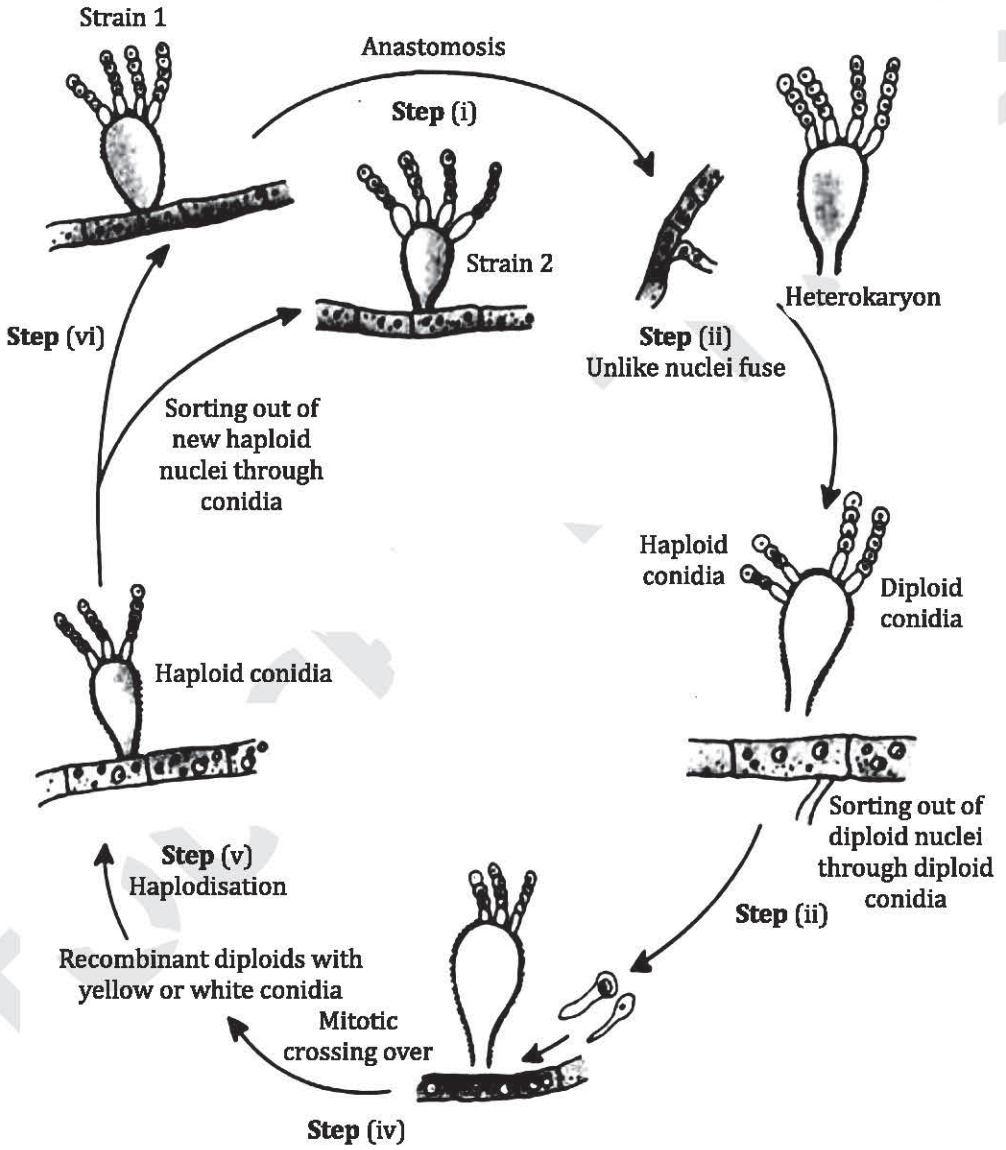


Fig. Step in parasexual cycle as suggested by Pontecover (1958)

The parasexual cycle is completed in a number of steps in *Aspergillus nidulans.* The sequence of these steps is as follows.

(i) Formation of heterokaryotic mycelium

(ii) Nuclear fusion or formation of heterozygous diploid nucleus.

(iii) Multiplication of diploid nuclei
(iv) Occasional mitotic crossing over
(v) Sorting of diploid nuclei
(vi) Occasional haploidiation of diploid nuclei
(vii) Sorting of new haploid strains

After the operation of parasexual cycle in the mycelium for sometime, the following types of nuclei may occur : (i) haploid nuclei in both the mycelia, *i.e.,* the mycelia of the two different genetic constitution which form heterokaryotic mycelium, (ii) some haploid nuclei of new genetic constitution by genetic recombinations, (iii) several types of diploid homozygous nuclei, and (iv) several types of diploid heterozygous nuclei.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Write an essay on economic importance of Fungi.

Ans. **Economic Importance of Fungi**

The group fungi include hundred to species which are of tremendous economic importance to man. In fact our lives are intimately linked with those of fungi. Hardly a day passes when we are not benefited or harmed directly or indirectly by these organisms. A brief account of the beneficial and harmful activities of the fungi is as follows :

Useful Activities

The fungi are useful in many ways. Their usefulness in different aspects of life can be summarized as under :

1. **Destruction of organic waste :** The saprophytic fungi decompose plant and animal remains, thereby acting as natural scavengers. The CO_2 released in the process is used by green plants. By some workers the saprophytic fungi have been designated as "vegetative vultures."
2. **In industry :** (i) Many fungi are employed in the commercial preparation of many organic acids and some vitamin preparation. *Aspergillus niger, A. glaucus, A. clavatus, Citriomyces citrus,* etc. have been recommended for the preparation of citric acid. In the similar way gluconic acid, lactic acid, etc. also prepared from many fungi. Several species of *Aspergillus* and *Fusarium* are the sources of riboflavin a constituent of vitamin-B. Yeasts are also rich in vitamin-B.

(ii) In recent days several fungi have been found to be the basis of entire alcoholic industry. The basis of alcoholic industry is the production of ethyl alcohol by the fermentation of sugars solutions by yeasts. Yeasts are source of complex enzyme **zymase** which is responsible for the process of fermentation.

$$C_6H_{12}O_6 \xrightarrow[\text{Yeasts}]{\text{Zymase}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + 2CO_2$$

The yeasts are thus frequently used in making wines.

(iii) Certain yeasts (e.g., *Saccharomyces cerevisiase*) form the important basis of baking industries.

(iv) Some fungi such as species of *Penicillium* (*P. roqueforti*) used in preparation of certain cheases.

3. **As food :** Many fungi such as mushroom (*Agaricus*), puffballs (*Lycoperdon*), morels (*Morchella*) are edible. They are important as protein sources. These fungi are of great economic value as food. They are regarded as delicates of the table.

   The large scale production of yeasts is used in conversion of carbohydrates and in organic nitrogen salts into edible and nutritional forms. Yeast food supplies a number of vitamins like thiamine, riboflavin, nicotomic acid, pantothenic acid, biotin, etc. Yeast food is hence a supplement of human food requirements.

4. **In medicines or Medicinal value :** Recently many fungi have been found to be responsible for producing certain antibiotic drugs which inhibit the growth of pathogenic microorganisms. Some of the important medicines produced by fungus are as follows :

   (i) **Penicillin** (used to kill bacteria that cause Pneumonia) from *Penicillium notatum*

   (ii) **Streptomycin** from *Streptomyces griseus*

   (iii) **Aureomycin** from *Streptomyces aureofaciens*

   (iv) **Chloromycetin** from *Streptomyces venezuelae*

   (v) **Ergot** from *Claviceps purpurea*

   (vi) **Cheatyomic** from *Chaetomium cochliodes*

5. **In plastic manufacture :** Certain fungi like *Oidium lactis* is widely used in the plastic industry.

6. **Control and insect pest :** Several fungi like Aschersonia *aleyrodis,* Isar a fertnosa. empusa, *Eristalinus repulcharlis* help in controlling the infection of insect pests of the plants.

7. **Nutrition of plant :** Several members of the phycomycetes, Ascomycetes, Basidiomyotes and fungi imperfecti are involved in the formation of my corrhizae which are believed to be fundamental importance in the nutrition of tree like Cycas, Zommia, Finns etc.

8. **Fungi as test organisms :** Some strains of *Aspergillus niger* are highly sensitive to some trace elements. They can detect trace elements like Cu, Zn. These elements when taken up by the fungus may give a particular colour to the conidia.

9. **Phytohormone or Auxins :** Several growth promoting substances like gibberellins are synthesized from the fungi like *Fusarium moniliforme* and *Dematium pullulans,* etc.

10. **Fungi as enzymes :** Some of the fungi help in producing various enzymes on commercial scale as follows :

    (i) **Invertase :** It is obtained from S. cerevisiae and is used in preparation of confectionaries, paper industry, hydrolysis of sugar solution, etc.

    (ii) **Zymase :** It is also obtained from yeast cells and helps in fermentation of carbohydrates to produce ethyl alcohol and carbon dioxide.

(iii) **Amylase :** It is obtained from *Aspergillus sp.* and is used in manufacture of alcohol, etc.

11. **Fungi in research :** Several fungi are being used as tool and raw material of the research problems by cytologists, geneticists and biochemists. Lot of work is being done on the yeast to explain the laws of heredity. The genus *Neurospora* which has been considered as the *Drosophila* of the botanical world because of its early growth and life cycle in the laboratory is being used as a beautiful and informative material in the field of tenticles.

Thus, we see that the role of fungi in human welfare is very much.

Q.2. Describe briefly Alexopoulos classification of Fungi.

Ans.

Alexopoulos Classification of Fungi

C.J. Alexopoulos (1962, 68) placed all fungi (including slime molds) in a separate division **Mycota,** and it was divided into two sub-divisions—**Myxomycotina** and **Eumycotina** on the basis of the absence and presence of cell wall respectively. Slime molds were included in the sub-division Myxomycotina and the true fungi in Eumycotina. His classification is as follows.

Division—Mycota

Thallus microscopic, unicellular or filamentous; nucleus with a distinct nuclear membrane and nucleolus; cell wall chitinous or cellulosic; reproduction by asexual and sexual means.

1. Sub-division—Myxomycotina

Plant body is in the form of naked protoplast, known as **plasmodium.**

Class—Myxomycetes : Vegetative phase is represented by a solitary large multinucleate naked protoplast (plasmodium); reproduction by minute multinucleate walled spores.

2. Sub-division—Eumycotina

Vegetative phase is represented by unicellular or branched siphonaceous mycelium, cell possesses distinct cell wall; hyphae are aseptate and multinucleate or septate; cells are uni, bi, or multinucleate; reproduction by spores or gametes.

The sub-division **Eumycotina** was divided into **eight** classes.

(i) **Class—Chytridiomycetes :** Motile cells with solitary posterior whiplash flagellum.

(ii) **Class—Hyphochytridiomycetes :** Motile cells with solitary anterior tinsel flagellum; includes aquatic fungi.

(iii) **Class—Oomycetes :** Mycelium well developed and multinucleate during vegetative phase; motile cells biflagellate (one flagellum whiplash and the other tinsel type); flagella are arranged in opposite directions.

(iv) **Class—Plasmodiophoromycetes :** Parasitic fungi; cell wall lacking; multinucleate thallus remains inside the host tissue; motile cells with two unequal anterior tinsel flagella.

(v) **Class—Zygomycetes :** Parasitic or saprophytic fungi; mycelium well developed and multinucleate; motile structures absent.

(vi) **Class—Trichomycetes :** Thallus simple or branched and multinucleate; often parasitic on arthropods.

(vii) **Class—Ascomytes :** Hyphae septate; ascopores produced endogenously in specialised sporangium, known as **ascus.**

(viii) **Class—Basidiomycetes :** Hyphae septate; basidiospores produced exogenously on basidium.

Form class—Deuteromyctes : Hyphae septate; reproduces only by asexual spores; sexual phase lacking.

Q.3. Discuss in brief the structure and reproduction of *Stemonitis.*

Ans.

Genus—*Stemonitis*

Systematic Position

Division — Myxomycota (Slime Molds)
Class — Myxomycetes
Family — Stemonitomyceticeae
Order — Stemonitales
Genes — *Stemonitis*

Somatic Phase

The somatic phase is represented by a multinucleate apparently naked acellular slimy protoplasmic mass called the plasmodium. As the plasmodium is the product of syngamy hence it is diploid structure, which is holocarpic, free-living and active. It contains and secrete slime.

The plasmodium is typical **aphanoplasmodium.** The mature plasmodium is branched and network of very fine transparent strands. The protoplasm is not very granular and the veins are not conspicuously differentiated. The protoplasm shows rapid and rhythmically reversible streaming.

Reproduction

Shortly before fruiting, the plasmodium starts becoming flat producing fan-like structures. Under favourable conditions, there start developing a large number of sporangia. Sporangial development is typically epiphypothallic.

The sporangia are cylindrical to fasciculate, stalked and fan-shaped. The stalk extends within the sporangium as columella throughout its length. Capillitium is formed of numerous threads radiating from all parts of the columella and combined into a loose network, the ultimate branches united into a superficial net attached to the evanescent sporangial walls. Lime, if present is never on the capillitium but confined to the hypothallus, stalk, columella and occasionally to the base of the peridium. Spores are violet-brown, liliac or ferruginous by transmitted light. Each spore upon germination produces 1-4 naked swarm cells oryx amoeba. They fuse in pairs to form dipoloid zygotes. As a result of nuclear divisions and growth, zygote develops into plasmodium. Meiosis probably occurs during spore formation in the sporangia.

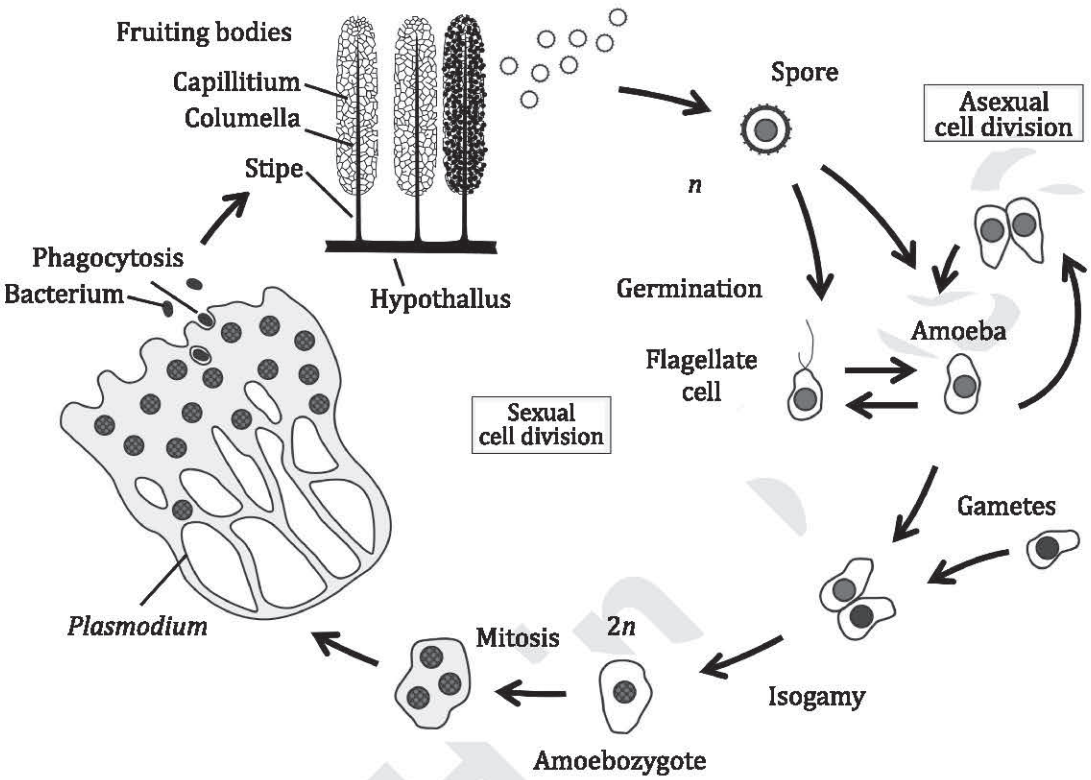


Fig.

Q.4. Describe the reproduction of *Rhizopus* with the help of suitable diagrams.

Ans.

Reproduction in *Rhizopus*

Rhizopus reproduces by **vegetative, asexual** and **sexual** methods.

1. Vegetative Reproduction

It takes place by **fragmentation.** The vegetative hyphae (stolons) may break up into smaller fragments. Each fragment is capable of developing into new mycelium.

2. Asexual Reproduction

It takes place by the formation of aplanospores (sporangiospores) or by chlamydospores.

(i) **Aplanospore formation :** During favourable conditions, it is the most common method of asexual reproduction. The multinucleate non-motile spores, known as **aplanospores** or **sporangiospores,** are formed inside the round black sporangia, which occur singly at the tips of sporangiosphores. Sporangiophores develop in tufts from the stolon opposite the rhizoids. At the time of sporangium formation, the tip of the sporangiophore swells into a knob-like vesicle. The cytoplasm, alongwith many nuclei, flows from the sporangiophore into the swollen vesicle. Ultimately, the swollen tip develops into a large globose structure-the **young sporangium.** The contents of the young sporangium soon differentiate into a peripheral dense multinucleate region and a central region with small flattened vacuoles and fewer nuclei. Thereafter, a cleft is formed between the two regions

by the coalescence of vacuoles. Finally, a dome-shaped septum is laid down, separating the two regions completely. The central vacuolated region is sterile and is known as **columella,** whereas the outer fertile sporangiferous region forms spores. The columella remains in continuity with the protoplast of the sporangiophore. Cleavage in the peripheral sporangiferous zone results in the formation of several multinucleate segments. Each segment ultimately transforms into a globose, multinucleate, non-motile aplanospore.

At maturity, the wall of the sporangium wall dries and the columella collapses like an inverted cup-like dish. Ultimately, the sporangial wall breaks, liberating spores to the atmosphere. The remnants of the sporangium wall can be seen as a frill at the base of the columella.

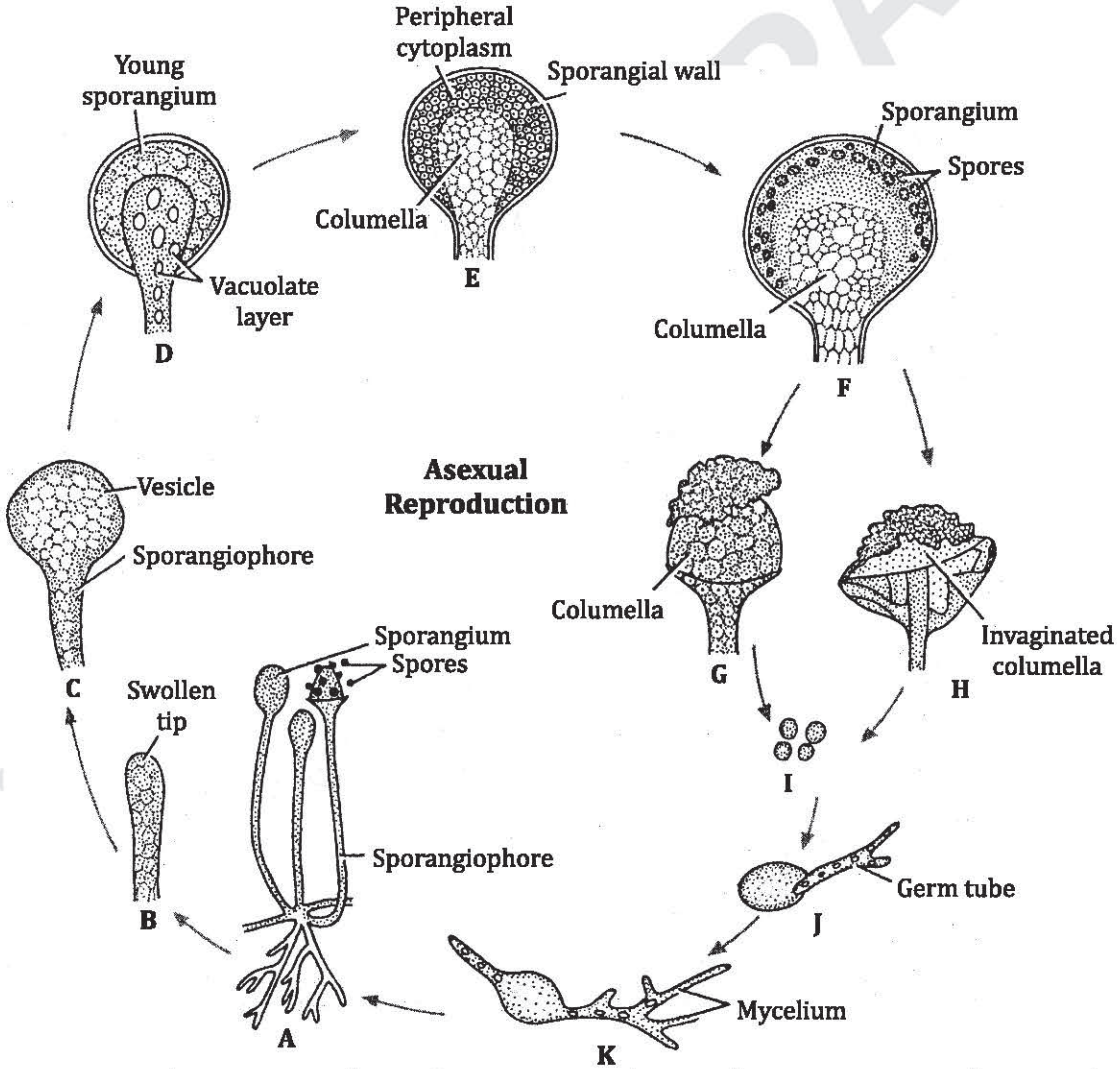


Fig. 1 : A-K. *Rhizopus* : Asexual reproduction; A-F. Develpment of sporangium, G-H. Dehiscence of sporangium, I. Aplansopores, J-K. Germination of spores.

When the spore falls on a suitable substratum, it germinates by producing a germ tube. The germ tube ultimately develops into a fluffy and profusely branched mycelium.

(ii) **Chlamydospore formation** : During unfavourable conditions, asexual reproduction takes place by means of chlamydospores. At the time of chlamydospore formation, the mature mycelium becomes septate and the protoplast of each cell forms a rounded and thick walled structure, known as chlamydospore. The chlamydospores are perennating bodies and they can pass unfavourable conditions. They germinate and form new mycelia.

3. Sexual Reproduction

It takes place by the copulation of two morphologically similar multinucleate gametangia. Most of the species of *Rhizopus* are **heterothallic,** but a few (e.g., *R. sexualis*) are **homothallic.** In heterothallic species, zygospores are formed only when two mycelia of compatible strains come in contact with each other. But in homothallic species, mycelia derived from a single spore may form zygospores.

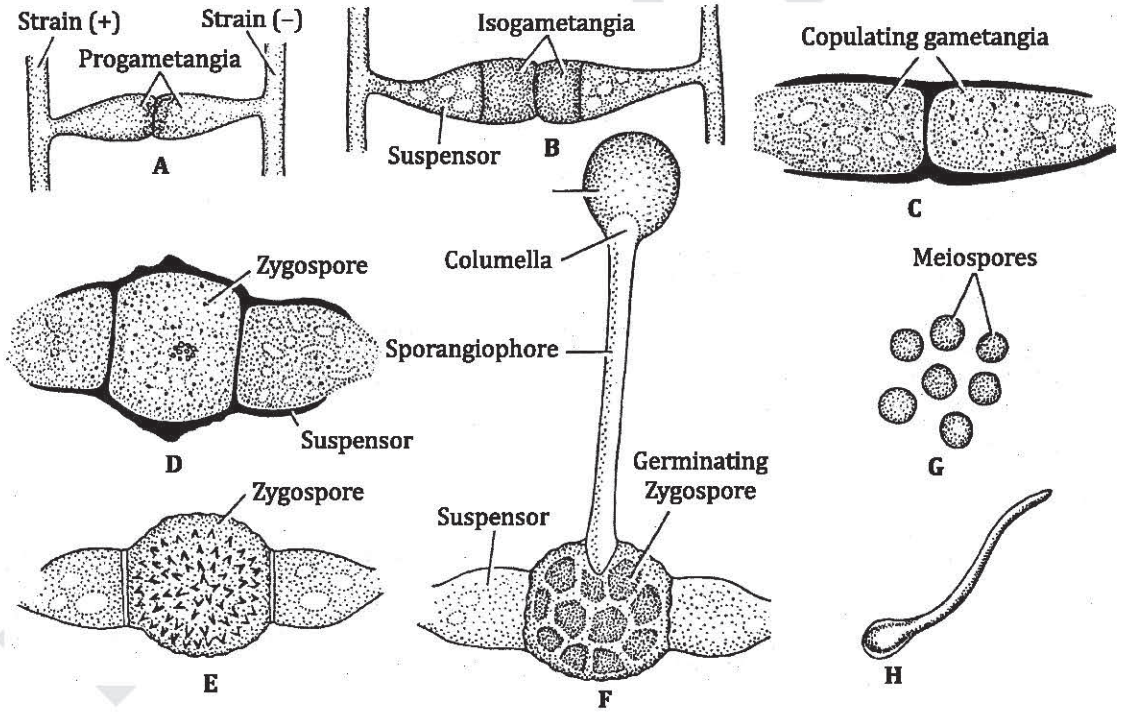


Fig. 2 : A-H. *Rhizopus* : Sexual reproduction; A-B. Formation of gametangia, C-E. Gametangial copulation and formation of zygospore, F. Germination of zygospore, G. Meiospores, H. Germinating meiospore.

During sexual reproduction, gametangia develop as terminal swellings on the tips of two compatible hyphae or hyphal branches. These special hyphae are known as **progametangia.** The progametangia of opposite strains adhere by their tips and enlarge by accumulating cytoplasm. A septum is then formed a little below the tip of the progametangium, separating the terminal gametangium from a proximal suspensor cell. The gametangium has densely granular multinucleate protoplast, whereas the suspensor has a highly vacuolated protoplast.

The walls of the two gametangia at the point of their contact dissolve and the protoplasts of both gametangia unite to form a zygospore. The nuclei of opposite strains pair and fuse to form diploid nuclei in the combined protoplast. The nuclei, which fail to fuse in pairs, ultimately degenerate. Soon, the young zygospore enlarges and secretes several layered thick wall around it.

The zygospore germinates after a long period of rest. During germination, the zygospore cracks open and a germ sporangiophore emerges which develops a germ sporangium at its tip. Reduction division occurs during the formation of germ sporangium and the sporangium contains numerous haploid spores. Each spore, after liberation, germinates to form a new mycelium. Occasionally, copulation does not take place between gametangia and these gametangia get surrounded by a many layered wall and then develop into azygospores.

Q.5. Describe the method of reproduction in *Saccharomyces.*

Ans.

Reproduction in *Saccharomyces*

Saccharomyces (Yeast) were first of all reported by **Leeuwenhoek** (1680). Yeast is the smallest fungus. The reproduction in yeast is vegetative and sexual.

I. Vegetative Reproduction

Yeast reproduce vegetatively either by **fission** or **by budding.** Depending on this character they are grouped as **fission yeasts** (*Schizosaccharomyces*) and **budding yeasts** (*Zygosaccharomyces*).

(i) **By fission :** During reproduction by fission the parent cell elongates, the nucleus divides into two daughter nuclei and gradually a transverse partition wall is laid down somewhat near the middle, starting from periphery to the centre dividing the mother cell into daughter cells. The two daughter cells so formed may remain together for sometime and begin to divide again or they may separate soon and then divide.

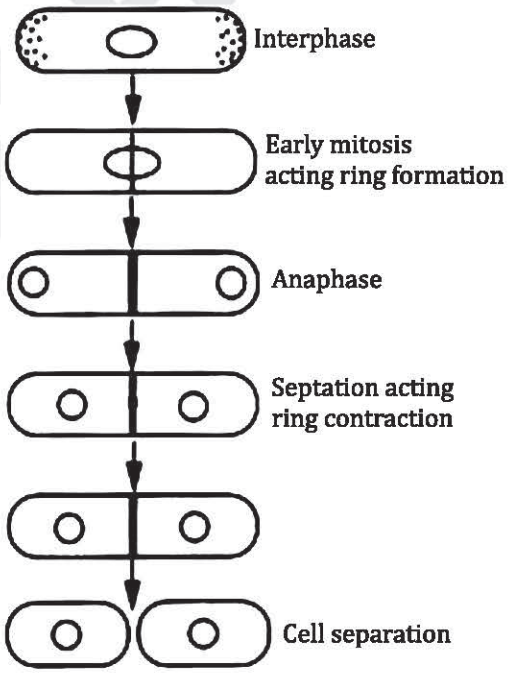


Fig. 1 : Fission in yeast

(ii) **By budding :** Budding yeasts are rather common than the fission yeasts. At the commencement of budding a small portion of the cell wall, usually near the end, softens. The protoplast of the mother cell covered by a thin membrane bulges out in the form of a bud which ultimately develops into a daughter cell. Mean while the nucleus of the other cell divided mitotically, according to some, the division is amitotic. One of the two daughter nuclei migrates into the enlarging bud. The budgrows until it attains the size of the mother cell. The daughter cell then becomes separated from the mother cell and the process may be repeated indefinitely. Quite often the daughter cell also starts producing buds before begin abstracted from the mother cell and the process may be repeated giving rise to chains or groups of yeast cells. In this way a large number of buds are developed without being detached from one another resulting in the formation of branched or unbranched chains of cells constituting the pseudomycelium. The cells in chains of pseudomycelium are loosely joined together. Sooner or later however, the chains break into their constituent cells.

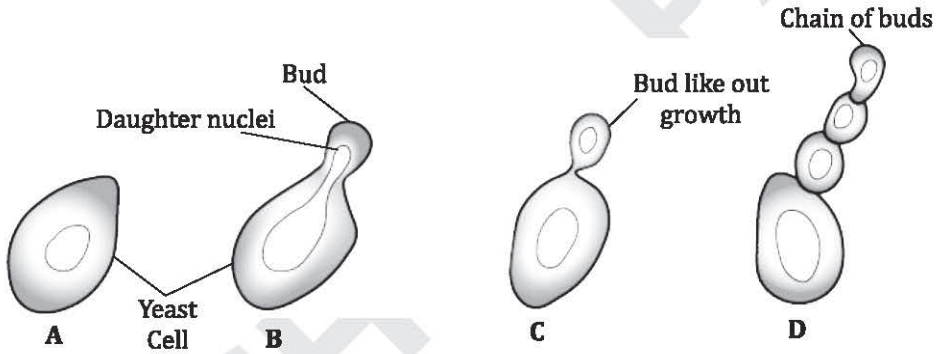


Fig. : Budding in Yeast

II. Sexual Reproduction

It takes place by the union of two cells more often similar in size but sometimes they may be dissimilar in appearance, and by the development of short protuberances which unite to form a conjugation tube. This is followed by the dissolution of intervening walls and nuclear fusion which takes place in the conjugation tube. The subsequent stages are extremely variable and are discussed separately. The copulating pair of cells may be vegetative cells or ascospores. Yeasts may be homothallic or heterothallic.

According to **Guillermond** (1940), three life cycle patterns are distinguishable among yeasts. They are :

1. **Haplobiontic life cycle :** This is exhibited by *Schizosaccharomyces octosporus* which is homothallic. Here, the haploid stage (haplophase) is very elaborate, whereas, the diploid stage (diplophase) is very short being confined to the zygote cell only. Meiosis of the diploid zygote nucleus takes place immediately after karyogamy. The somatic cells are haploid and elongated. They divide by fission forming daughter cells. Any somatic cell is a potential gametangium. During sexual reproduction two cells come in contact. A beak-like protuberance develops from each conjugating cell at the point of contact. A continuous passage is developed by the dissolution of intervening walls at the point of contact where the two nuclei migrate. The passages between the two cells

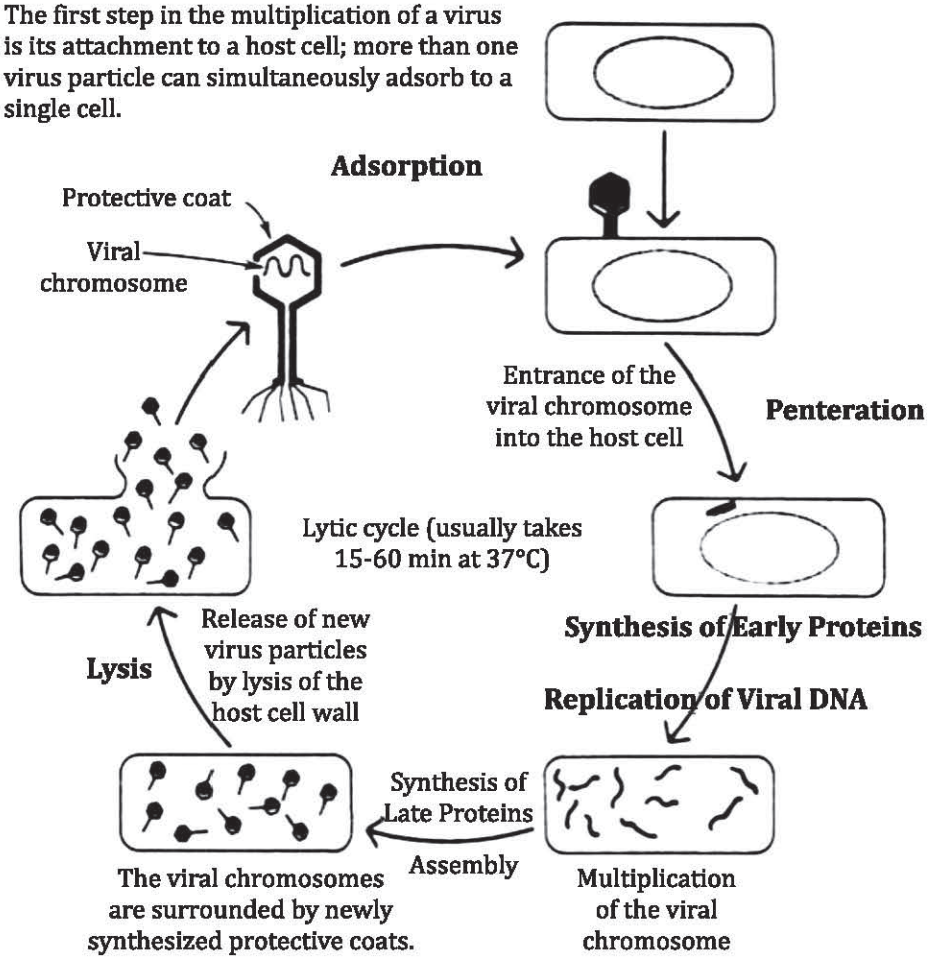


Fig. 3 : Haplobiontic life cycle of *S. octosporus*

enlarge forming a conjugation tube, where karyogamy takes place. Gradually the two cells along with the conjugation tube from the zygote. The zygote ultimately develops into an ascus. The diploid zygotic nucleus undergoes divisions of which the first one is meiotic and other mitotic producing eight haploid nuclei. Each nucleus with cytoplasm develops into an ascospore and the ascus contains eight ascospores. The ascospores liberate by breaking down of ascus wall. They now behave as somatic cells.

2. **Diplobiontic life cycle :** This is exemplified by *Saccharomyces ludwigii.* Here, the diploid somatic stage is long and the haploid stage is very short. The diploid somatic cells produce buds which eventually enlarge to function as asci. The diploid nucleus divides meiotically forming four haploid nuclei around which four ascospores are developed. The ascospores remain confined in the ascus and copulate there forming two diploid cells. Each diploid cell germinates by a germ tube which pushes out through the ascus wall ultimately forming a tubular structure. This tubular structure

behaves as a sprout mycelium from which diploid cells are produced by budding. Thus, the haploid stage is represented by the ascospores only.

3. **Haplo-diplobiontic life cycle :** This is exhibited by *Saccharomyces cerevisiae.* In this type of life cycle both haploid and diploid phases are equally well represented constituting somewhat an alternation of generations. Two haploid cells copulate forming a diploid cell. The diploid cell multiplies by budding producing large number of diploid cells. Eventually each diploid cell behaves as an ascus bearing four ascospores and meiosis takes place during the development of ascospores. The ascospores on being liberated from the ascus multiply by budding producing haploid

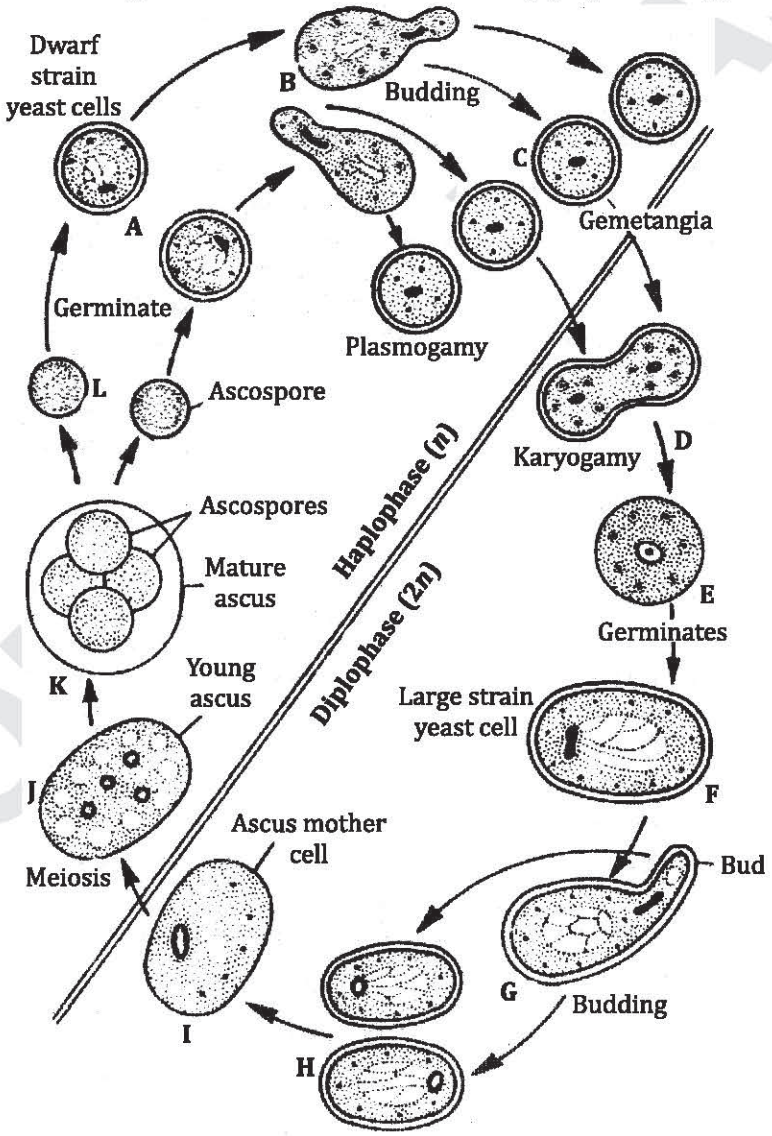


Fig. 4 : Haplo-diplobiontic life cycle of *S. cerevisiae*

cells. It is evident that in this life cycle there are two distinct stages. The diploid stage and the haploid stage which alternate in cyclic order. The vegetative cells may be both haploid and diploid reproducing asexually by budding.

In addition to the above given methods of sexual reproduction the following methods are found :

(i) **Adelphogamy :** Copulation between two adjoining sister cells. This is isogamous and the cells which fuse do not separate after fission but remain united to form short chains.

(ii) **Pedogamy :** Copulation between other and the daughter cell formed by budding. The daughter remains attached to the mother and the nucleus of the bud migrates into the mother. An outgrowth develops at the opposite end of the mother. Both the nuclei move into this and fuse. The fusion nucleus undergoes two divisions one being reduction divisions. Of the four nuclei formed three degenerate and the ascospore is formed around only one. Thus, it is a type of anisogamous type of reproduction and occurs in *Zygosacchromyces chevalieri.*

(iii) **Parthenogamy :** In this asci are formed without actually involving copulation of two cells.

Q.6. Describe the life history of *Penicillium.*

Ans.

Life History of *Penicillium*

Systematic Position

Mycota	Fungi
Ascomycetes	Eumycotina
Plectomycetes	Ascomycetes
Plectoscales	Euascemycetidae
Aspergillaceae	Plectomycetes
Penicillium	Eurotiales
(Old)	Aspergilaceae
	Penicillium
	(Modern)

Occurrence

This is a saprophytic fungus and usually grows upon rotten vegetables, fruits, meat and many other moist and dead organic substrata. This fungus is also known as **green** or **blue mould.** Some species parasitic also *P. notatum* is the most common species.

Structure

This thallus of *Penicillium* consists of such branched pale coloured mycelium and thin walled hyphae. Hyphae are septate and each cell uni or multinucleate.

Reproduction

Penicillium reproduces both sexually and asexually.

1. Asexual Reproduction

It takes place by means of conidia or conidiospores, which are developed on the **conidiosphores.** The conidiospores are mostly branched, septate and consist of multinucleate cell. On the terminal ends of the branches of the conidiospore the bottle like

sterigmata (phialides) are produced. The sterigmata are uninucleate and bear the uninucleate **conidia** in basigeneous chains. Each conidial chain consists of branched or more conidia. Each conidium is uninucleate and green. On liberation the conidia get and on getting suitable media an appropriate condition for germination, they germinate by producing germ tubes which develop into new mycelia.

2. Sexual Reproduction

The sexual reproduction is of rare occurrence the *Penicillium* may be homothallic or heterothallic, *i.e.*, sex organs may arise either from the same or the different mycelia. The male and female sex organs are known as **antheridia** and **ascogonia** respectively.

The ascogonium is an elongated uninucleate structure when young. It arises as a short erect protubearance from an ordinary cell of the mycelium. The protubearance elongates and its nucleus divides repeatedly to from 32 to 64 daughter nuclei the ascogonium at this stage in an elongated, tubular, non-septate and multinucleate structure.

Antheridium also arises as a branch from mycelium called as an **antheridial branch**. It becomes spirally soiled around the ascogonium. Antheridum is uninucleate in the beginning and remains uninucleate always. The apical part of the antheridial branches swells slightly and then cut-off from rest of the hyphae by a cross wall. The club-shaped terminal uninucleate cell thus cut-off and is called the **antheridium.**

Dikaryotization and Plasmogamy

The tip of the antheridium comes in contact with the lateral wall of ascogonium, the wall between the two dissolves and the two protoplast come in contact According to **Dangered** (1907) and may other workers the migration of the male nucleus into the ascogonium does not take place. The ascogonial nuclei arrange.

The meshes in pairs (dikaryons) ascogonium get divided into a number of cells. Each cell contains a pair of nuclei called the **dikaryon**.

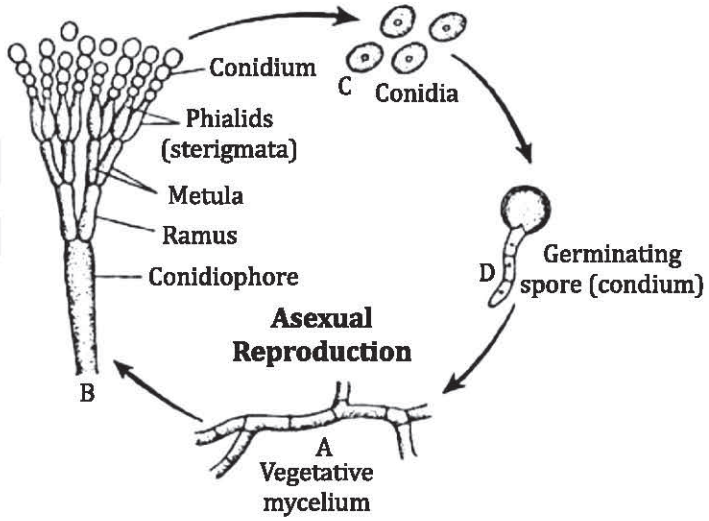


Fig. : *Penicillium* : Asexual phase of life-cycle.

Formation of Ascuss, Ascospores and Ascocarp (Cleistothecium) : Each binucleate cell of the ascogonium is called **ascus** mother cell in which karyogamy takes place. This fusion nucleus undergoes meiosis forming ascospores. The sexual structure become surrounded by vegetative fungal haphae and fruiting body which is known as **ascospores** or **cleistothecium** develops. The pear shaped asci soon dissolve and release the ascospores into the cleistothecium. The ascospores liberated free and germinate to from a new mycelium.

Q.7. Describe in detail about the disease loose smut of wheat and method of its control.

Ans. ***Ustilago tritici* (Loose Smut of Wheat)**

Systematic Position

Mycota
Eumycotina
Basidiomycotina
Teliomycates
Ustilaginales
Ustilaginaceae
Ustilago

General Introduction

Ustilago, is a cosmopolitan genus of group fungi which infects a number of cereals and grasses like wheat, oat, maize, barely, grasses and sugarcane. All species are parasite. The disease caused by *Ustilago* is often referred to as smut diseases. They are so called because of dark coloured, dusty, spore masses which are produced in the infected organs. The spore masses resemble to soot or smut and the parasite. (*Ustilago*) itself known as smut fungi. The disease caused by genus are of much economic importance as they damage the various crops and cause sufficient loss to the country. In Uttar Pradesh and Punjab disease caused by this fungus are known as "Kangiari", "Karanjusa", "Kandua", etc. Some of the important species *Ustilago* are follows:

(i) *U. tritici* → Loose smut of wheat,
(ii) *U. avenae* → Loose smut of oats,
(iii) *U. maydis* →Loose smut of corn (maize),
(iv) *U. hordei* →Covered smut of barley,
(v) *U. nuda* → Loose smut of barley,

Disease Cycle

Most of the species of *Ustilago* are highly specialized and physiological races are common, to the extent that one race of the smut will infect only one variety of cultivated cereal.

The host plant (or cereals like wheat, maize, oat, etc.) get infected at any time during the active life by means of basidiospores produced as a result of germination of conidiospore. Sometimes infection may also take place by conidia which are budded off from basidiospores. The germination may take place in soil or on grains of host or on any vegetative part of host. The mycelia formed by basidiospores are **primary** and uninucleate and represent the **haplophase.** The primary mycelium grows chiefly in intercellular spaces of the host tissue and sends short haustorial branches into host cells.

When the flowering starts in host plant some of the ovaries becomes packed with the mycelium, and as a result become greatly swollen and hypertrophied. Mostly the species of *Ustilago* are heterothallic and the chlamydospores will not be infected by basidiospores of both sexes.

Conjugation between the primary monokaryotic mycelia of two sexes takes place and as a result a **diplophase** (dikaryon) mycelium is formed. The dikaryotic mycelium flourish in the

tissue of host and afterwards on maturity the dikaryon cell of this dikaryotic mycelium from numerous rounded structures called **smut spores** teleutospores, or Chlamydospores which form large powdery black masses. A numerous chlamydospores are arranged in a sorus. The chlamydospores are diploid in nature because in them the function of nuclei of dikaryotic cells take place from which they are formed. The short of chlamydospore produces galls in other parts of hosts like stem, leaves and roots. They completely fill the ovary of the flower and destroy the seed.

A chlamydospore is a thick walled black-brown coloured cell consisting of a two layered (exospore and endospore) cell wall and a diploid nucleus. The chlamydospore on liberation from host germinate on soil under favourable conditions. On germination a tubular outgrowth arises from the chlamydospore called as **epibasidium** or **promycelium.** The diploid nucleus chlamydospore divided reductionally and forms four haploid nuclei. The promycelium becomes four celled and each nucleus passes into each cell. This four celled stage of promycelium is the basidium. From the upper end of each, short sterigma is formed and later at the tip of sterigma basidiospores are formed which are four in number and two are of strain (+) and two. of strains (–). Each basidiospores is haploid and is capable of infecting a new healthy plants.

Fig. 1 : A. Ustilago tritici on wheat (smutted ear), B. Barren rachis.

Symptoms of Smut Disease

When *Ustilago* (smut) infects a host plant then following symptoms are visible on host plant.

(i) The ovary of the flower is completely replaced by chlamydospores.

(ii) Ovary gets filled with dark, sooty powder of smut spores.

(iii) Galls appear on leaves and stem.

(iv) Ears turn black and get shrivelled.

(v) There is no development of grains at all.

Methods of Control of Smut Diseases

In order to prevent the spread of disease the following methods should be adopted.

1. Infected plants and their ears should be destroyed or burnt.
2. We should use disinfectants. For this sulphur dust, formaline agrosone, etc. should be used to make the seeds free from infection before sowing them.
3. **Hot water treatment** : Seeds before sowing should be soaked in water and kept at 20-30°C for 4 or 6 hours. Then these seed should be kept in water as 54°C for 10 minute. This treatment kill the mycelia of smut.
4. By exposing soaked seeds to sunlight during the months of May and June due to which the dormant mycelium inside the seed get killed.

5. **Copper sulphate treatment :** Soak seeds in 50% solution of $CuSO_4$, for 2 hour. This treatment will kill the spores of smut attached outside.
6. We should select healthy seeds for sowing.
7. Breeding with smut resistant varieties.

Life Cycle of *Ustilago*

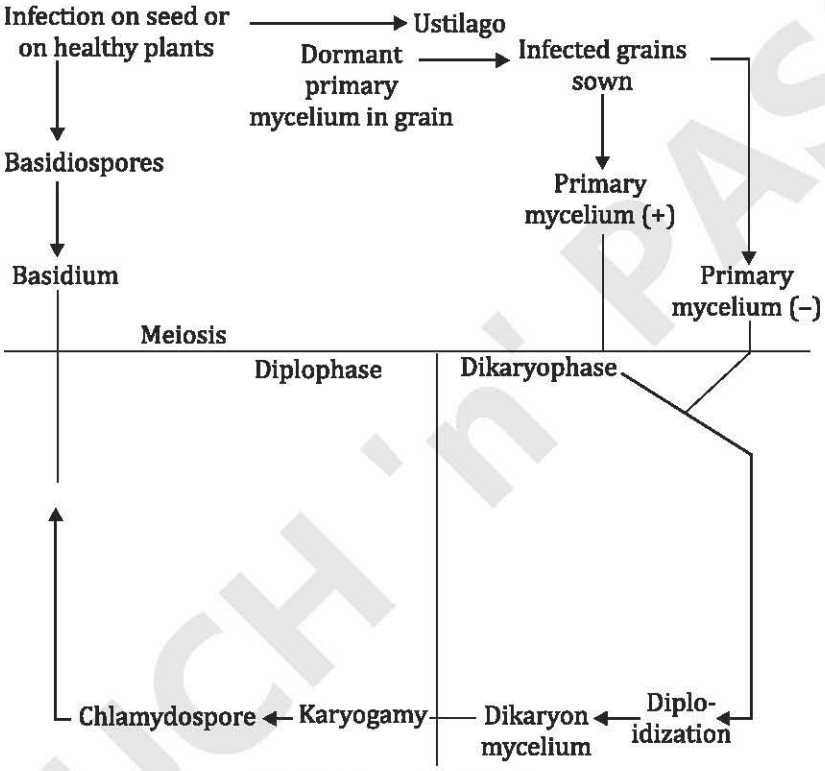


Fig. 2 : Life-cycle of *Ustilago*

Q.8. Write an illustrated account of structure and reproduction of *Agaricus.*

Ans. *Agaricus campestris* or 'snake umbrella' or 'dhingri' is a common gilled fungus (mushroom) and grows in soil rich in humus. The fungus can easily be recognized by the presence of large fruitification or sporophore which looks like umbrella.

Structure and Reproduction

According to somatic structure the plant body of mushroom can be subdivided into main parts:

(i) **Rhizomorph :** This remains hidden in the soil. It is composed of finally divided mycelium and hyphae which form network of threads in the soil. The hyphae are septate and hyaline and contains oil globules, vacuoles and protoplasm. The cells of hyphae are haploid and uninucleate. Numerous mycelia twist together to form a cord like rhizomorph. The mycelia may be of both the strains *i.e.,* (+) and (–). Such different mycelia during the formation of rhizomorph come in contact with, each of their intermediate wall dissolve, migration of nucleus takes place and thus dikaryotization

is brought about. The dikaryotic mycelium branches further and replaces the monokaryotic mycelium. These dikaryotic mycelium produce aerial fruiting bodies year after year.

(ii) **Sporophore or Fruitification :** It is aerial and occurs above the soil. It begins to develop as a tiny dense knot of under ground dikaryotic hyphae forming rhizomorph. In the beginning sporophore appears like an elliptical button hence formed as button stage. Afterwards a construction appears in button which becomes more and more prominent takes the form of cavity or chamber, known as gill chamber or prelamellar cavity. From the roof of this gill cavity are differentiated the gills. The button carried upward by the rapid elongation of the remaining hyphae which form the stem, stalk or stipe. The margin of the buttom is connected with the stalk by a membrane called the **partial** or **innerveil.** By further elongation of the stalk the button projects above the soil and enlarges considerably in size. The growth proceeds more rapidly at upper portion of button and slow at the lower portion: As a result the button opens into an umbrella like cap or the pileus at the same time the inner vein which covers the lower surface of pileus ruptures. Remnants of it remain attached to the stalk or the stipe in the form of a ring or annulus.

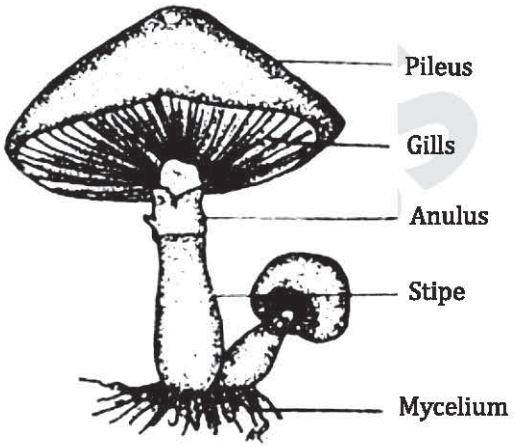


Fig. 1 : *Agricus campestris*-mycelium within substratum and well developed fruiting body

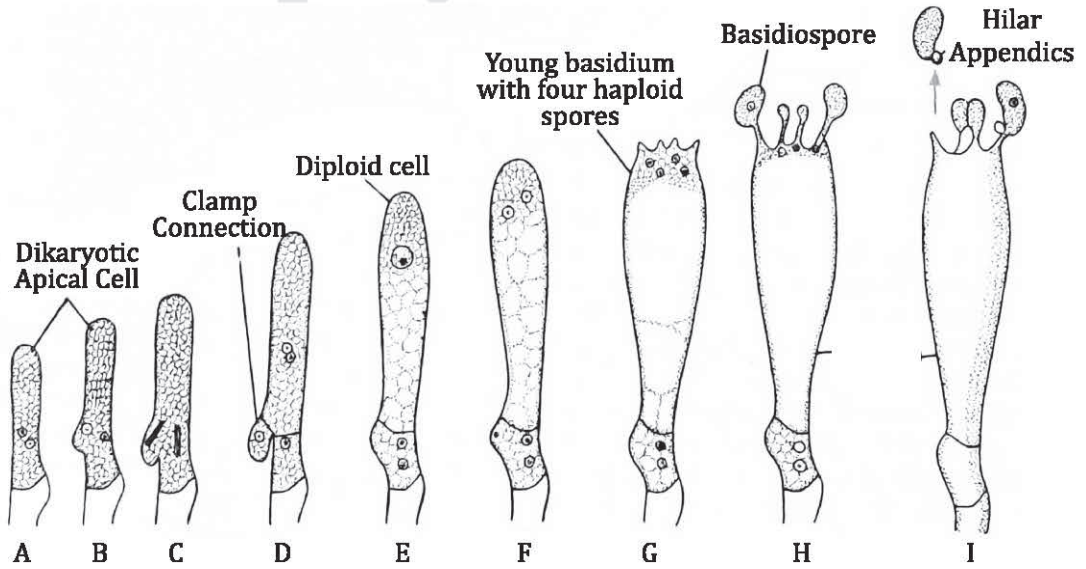


Fig. 2 : A-I : *Agaricus campestris.* Different stages in the development of sporophores (Fruitification)

Thus, the mature fleshy umbrella shaped basidiocarp, sporophore or fruitification of *Agaricus* consists of a thick stalk like-stipe bearing the cap like pileus at the top. From the under surface of pileus long sown numerous thin plates, called **gills** or **lamellae** which radiate from margins of the pileus to the stipe.

Structure of Gills

The gills are situated in chamber. The gills are flesh coloured or pink in young conditions and dark brown in mature conditions. Each gill is lined by a spore bearing layer, the hymenium. If a gill in vertical section is examined microscopically, the following three distinct portions will be seen : trama, subhymenium and hymenium. **Trama** is the central portion of gill consisting of an interwoven mass of slender and long hyphae resulting information of pseudoparenchyma. The hyphae of the trama bend outwards on both the surface of the gill and terminate in a layer of small rounded cells, and it is termed **subhymenium.** Outside to sub-hymenium, on both surface of the gill remain present a compact layer of palisade like cell called **hymenium.** The hymenium region consist basidia which are binucleate (dikaryotic), in between the basidia occur sterile cells called **paraphysis.** At the terminal end of such basidia, two or four sterigmata are developed and a single basidiospores (haploid and uninucleate) is developed on each sterigma.

Basidiospores

The basidia are club-shaped and binucleate. The two nuclei which are on different strains fuse together and a fusion nucleus is formed in each basidium. The fusion nucleus divided by a reduction and a miotic division thus forming four haploid nuclei. Four small tube like structures, the sterigma develop on each basidium. The daughter nuclei pass down into the

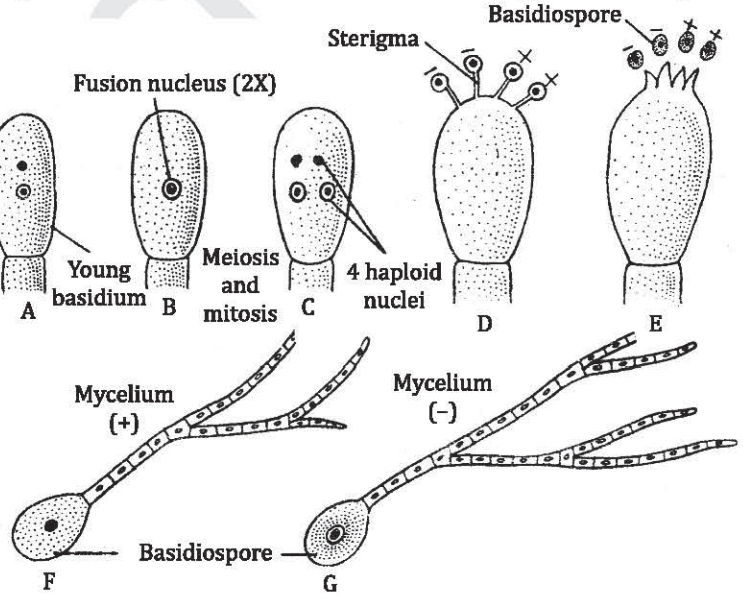


Fig. 3 : *Agaricus compestris* : Development of basidium and basidiopores and germination of basidiospores. A to G.

sterigmata and eventually one basidiospore is formed at the tip of each sterigmata. Out of four basidiospores, two are of strain (+) and two of strains (–).

Each basidiospore after liberation carried by wind to long distance and germinates in monokaryotic haploid mycelium in favourable condition.

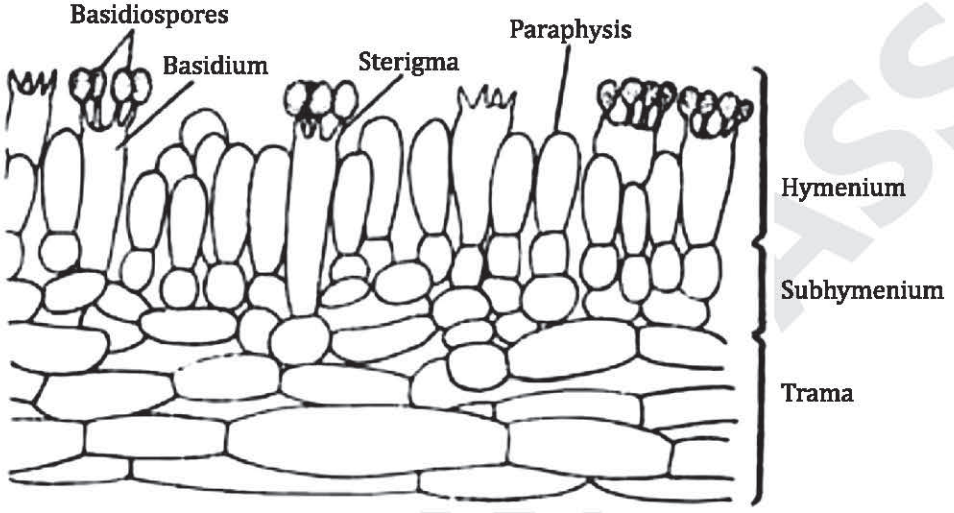


Fig. 4 : T.S. of Lamella showing basidia at various stages of development *Agaricus campestris*

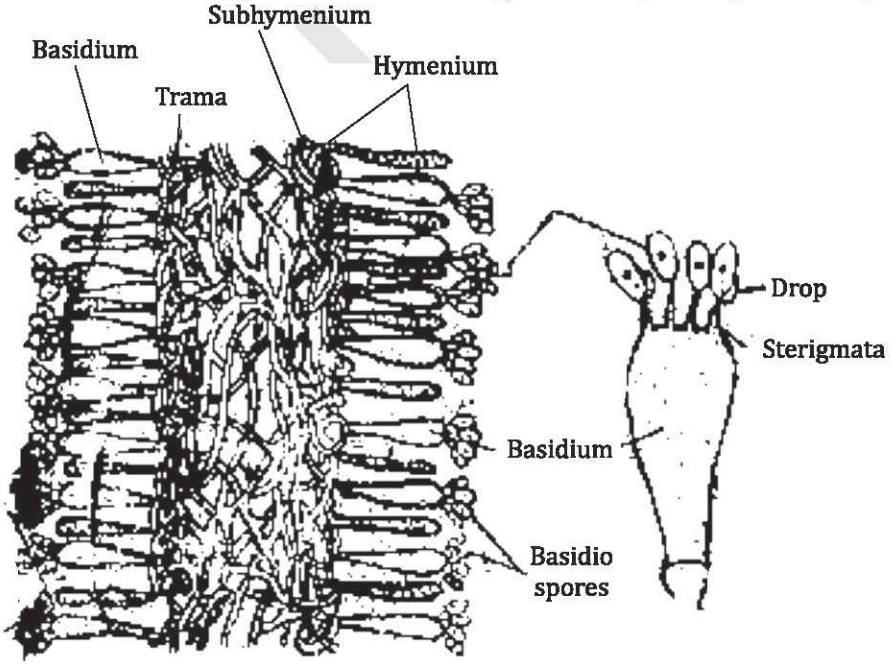


Fig. 5 : *Agaricus campestris* : Structure of a gill. A : V.S. of fruitification through a small portion of pileus and its gills. B. A portion enlarged of gill in V.S.

Life Cycle of *Agaricus*

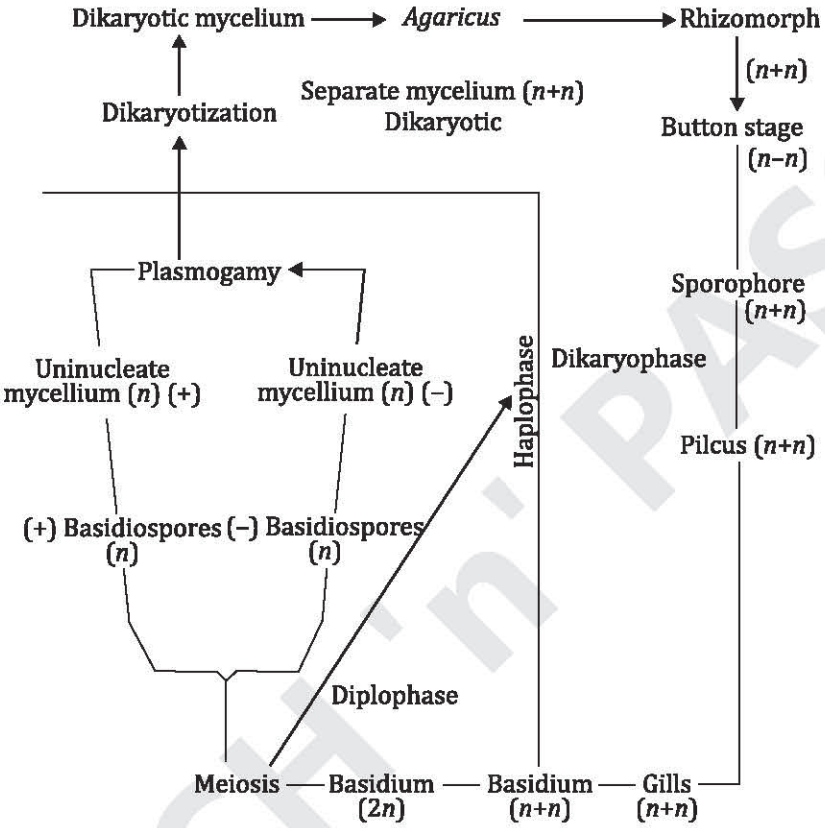


Fig. 6 : Life cycle of *agaricus*

Method of Propagation of *Agaricus*

The edible mushrooms are cultivated on a large scale. They form the bases of a great industry in many foreign countries. In our country they are also cultivated in South India and other places. The beds for cultivation of mushrooms are specially prepared. These contain a substratum of compost rich in nitrogenuous compounds. These beds are then inoculated with crumbs of mushroom spawn.

Fairy Rings of *Agaricus*

There are certain mushroom like *Agaricus praerimorus,* etc. in which the sporophores are produced at the tip of these hyphae and form a sort of ring. This ring is called the fairy ring on account of an only superstition belief that fairies dance in a circle.

Here the mycelium lying within the substratum is perennial and grows from centre with the result that it increases in diameter from year to year. The centrifugal growth of mycelium is followed by death of older hyphae in the centre.

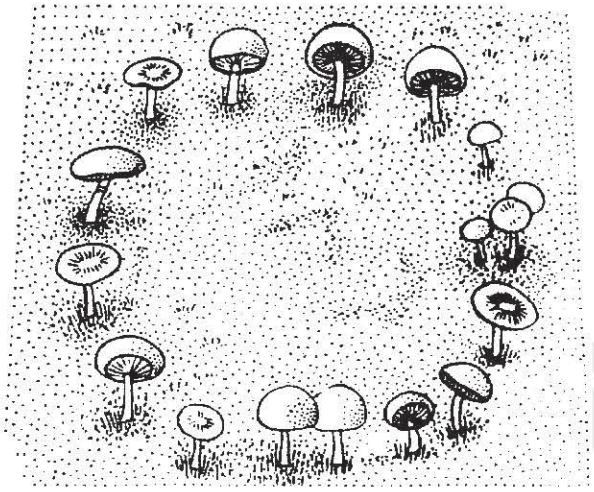


Fig. 7 : Showing fairy ring of mushroom

Q.9. Explain the detail of heterothallism and two allelomorph heterothallism.

Ans.

Heterothallism

The phenomenon of occurrence of two types of mycelia which are morphological alike but physiologically different is called as **heterothallism.**

The phenomenon of heterothallism was first discovered and studied by **Blackeslee** (1904) in mucorales. Heterothallism has been observed in *Mucor mucedo* and *Puccinia graminis.*

In some species of *Mucor* homothallic mycelium is formed, *i.e.,* zygospores are produced by the fusion of hyphae in mycelium produced from one spore, *e.g., Mucor hiemalis,* etc.

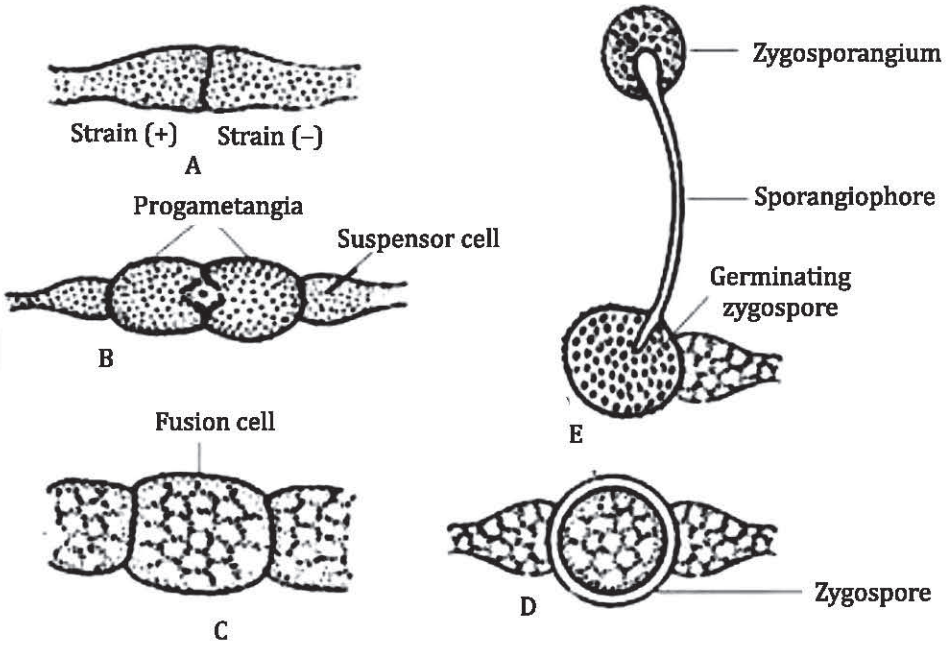


Fig. : Heterothallism in *Mucor*

The physiologically different mycelia of heterothallic species are designated by positive (+) and negative (–) strains. Sexual reproduction occurs only when the hyphae of different strains meet together ; if such hyphae do not occur in the near vicinity no sexual reproduction takes place. Physiologically the mycelia of positive strain show luxuriant growth. Mostly the heterothallic sporangium produces spores of either positive (+) or negative (–) strains. Some mycologists like. **Shear** and **Dodge** named these strains as *A* and *B* while **Tatum** and **Beadle** call them as *A* and *B*.

In *Achlaya bisexualis* there is a peculiar type of heterothallism which is called gynandromiotic type. In this case thalli are usually of the following types:

(i) Purely heterothallic-N Pure Male

Purely heterothallic-N Pure Female

(ii) Partly heterothallic-N Predominantly Male

Partly heterothallic-N Predominantly Female

Two Allelomorph Heterothallism : The two mating sex organs were different in morphological features. **Whitehouse** (1949) recognised the following two types of heterothallism.

1. **Morphological Heterothallism :** When morphologically different male and female sex organs (or gametes) are present in two closely associated mycelia, it is known as morphological heterothallism.

Whitehouse also used the term haplodioecious for morphologically different heterothallic species.

2. **Physiological heterothallism :** In physiological heterothallism, sexual reproduction takes place by two morphologically similar but physiologically different hyphae. The gametangia as well as gametes do not show morphological distinctions. The mating hyphae are different in incompatibility factors. Physiological heterothallism is also known as **haploid incompatibility.** It is of the following two types:

(i) **Two allele heterothallism :** In this type of heterothallism, the nuclei of both mating types are different in genetic characters. The sexual compatibility is controlled by a pair of genetic factors, represented by 'A' and 'a'. Both these factors are present on two different chromatids of a chromosome. Due to the dominance of 'A' over 'a', 'A' and 'a' are also represented by the sign (+) and (–). At the time of meiosis, chromatids with (+) and (–) allele are separated and half of the haploid spores thus formed have (+) and the other half (–) allele. Spores with (+) allele and (–) allele respectively give rise to (+) and (–) mycelia. The mycelia of strains (+) and (+), and (–) and (–) are incompatible. Thus, two complementary mating types (+ and –) are must for sexual reproduction.

The two allele heterothallism has been observed in several fungi, such as *Ascobolus magnificus, Puccinia graminis, Ustilago kolleri, Mucor mucedo* and *Neurospora sitophila.*

(ii) **Multiple allele heterothallism :** In this type of heterothallism, sexual compatibility is determined by more than two alleles. The main advantage of multiple allele heterothallism is that the chances of mating of compatible strains are reased due to larger number of alleles involved.

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UNIT-V

Mushroom Cultivation, Lichenology & Mycorrhiza

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. What is the Reindeer Moss?

Ans. A lichen, namely, *Cladonia rangiferina* is called the Reindeer Moss.

Q.2. Who first discovered lichens?

Ans. Tulsane (1852) first discovered lichens.

Q.3. Who was the pioneer of Lichen studies?

Ans. E. Acharius (1757-1819) was the pioneer of Lichen studies.

Q.4. What are the asexual reproductive bodies in lichens?

Ans. *Soredia, Isidia, Conidia* are the asexual reproductive bodies in lichens.

Q.5. Give name of two crustose lichens.

Ans. Two crustose lichens are : (i) *Graphis* (ii) *Lecanora*.

Q.6. Who was the pioneer scientist who gave the name lichen?

Ans. Theophrastus was the pioneer scientist who gave the name lichen.

Q.7. Write name of two lichens which are important as medicine.

Ans. *Parmelia* and *Peltigera* are the two important lichens used as medicine.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Write a short note on nutritive value of mushrooms.

Ans.

Nutritive Value of Mushrooms

In developed countries, the average consumption of animal protein is about 31 kg per capita per year, whereas it is only 4 kg in India. In India, the plant proteins are more widely used than the animal proteins. Mushrooms are the richest source of vegetable proteins. They contain 21-30% proteins on dry weight basis. Thus the percentage of proteins in mushrooms is much higher than in cereals, pulses, fruits or vegetables. The proteins of mushrooms contain all essential amino acids and their quantity is higher than in the egg. There is also a good amount of lysine amino acid in mushrooms (about 550 mg/g).

Besides, mushrooms contain sufficient quantities of mineral elements, such as Ca, Na, P and K. They also contain folic acid and are as such very good source of iron. They contain vitamins B, C, D and K, which are not distorted during ripening, drying, freezing or canning process. On the other hand, they contain very little fats (0.35-0.65% dry wt) and starches (0.02% dry wt). Thus mushrooms make an excellent food for diabetic and heart patients.

The chemical composition of three important edible mushrooms is given in table.

Chemical composition of three edible mushrooms

Mushroom	Moisture	Ash	Protein	Fat	Carbohydrate	Energy value
		(% on fresh weight basis)				(cal)
Agaricus bisporus	89.50	1.26	3.94	0.16	6.28	34.4
Pleurotus flabellatus	90.95	0.97	2.78	8.65	5.33	24.4
Volvariella diplasia	90.40	1.10	3.90	0.25	5.51	29.2

Q.2. Write a short note on mycotoxins.

Ans.

Mycotoxins

Many fungi produce mycotoxins which are responsible for food poisoning and other distressing symptoms. These mycotoxins may be broadly classified into the following three categories.

1. **Food Toxins :** These toxins are mainly responsible for food poisoning. The toxin production can occur in most plant products, but cereal and oil seed crops are mostly contaminated. The following four groups of food toxins are mainly associated with human diseases.

   (i) **Aflatoxins :** Aflatoxins, the most potent carcinogens, are produced by Aspergillus flavus, A. fumigatus, A. parasiticus and Penicillium islandicum, when they infest dried food and groundnut meal. They are highly oxygenated low molecular weight, heat stable, heterocyclic compounds. Eight forms of aflatoxins, B_1, B_2, G_1, G_2, M_1, M_2, B_{2a} and G_{2a} have been recognised. Of these, aflatoxins B_1, B_2, G_1 and G_2 are the most important. Aflatoxins are derivatives of furanocoumarin. They bind with DNA and prevent its transcription and as such protein synthesis is inhibited. They cause liver cancer in animals and human beings. The clinical symptoms include jaundice, rapidly developing ascites, portal hypertension, etc.

   (ii) **Ochratoxins :** Ochratoxins, closely related derivatives of isocoumarin linked to 1-β-*phenylalanine*, are mainly produced by *Aspergillus ochraceus* and *Penicillium viridicatum*, when they infest stored maize, pea nuts, beans and mixed animal feeds. Of the nine ochratoxins, ochratoxin A is the most important. It causes tubular necrosis of the kidney, mild degeneration of the liver and enteritis of the small intestine in ducklings.

   (iii) **Zearalenone :** Zearalenone, a phenolic resorcyclic acid lactone, is produced by several species of *Fusarium*, such as *F. graminearum* and *F. moniliforme*, growing on maize. It causes esterogenic symptoms in swine.

   (iv) **Trichothecenes :** Trichothecenes are produced by several species of *Cephalosporium, Fusarium, Myrothecium, Stachybotrys* and *Trichoderma*. They possess a tetracyclic 12,13-epoxytrichothecoene skeleton. Of the nearly 30 trichothecenes known, T-2 toxin, nivalenol and deoxynivalenol are most important. They are responsible for severe local irritation, inflammation, sub-epidermal haemorrhage and general necrosis.

2. **Ergot Toxins :** The sclerotia of *Claviceps purpurea* contain some poisonous alkaloids, like ergotamine, ergometrimine, ergocrystinine, ergocristine and ergonovin. Ergot

poisoning in human beings causes diarrhoea, abdominal pain and vomiting. It also effects nerves and results in psychiatric disturbances.

3. **Mushroom Toxins :** Several mushrooms produce mycotoxins which cause diarrhoea and vomiting in earlier stages, but in severe cases, liver damage, kidney failure, complete unconsciousness, and even death may take place.

   Amanita phalloides produces about ten toxins; Of these, phalloidin affects the plasma membrane of liver cells, and α-amanitin causes lesions in stomach and intestine cells. Gyromitrin, a highly fatal toxin is produced by the species of *Helvella*, such as *H. esculenta, H. gigas, H. infula* and *H. underwoodii*. Some species of *Inocybe* and *Clitocybe* produce the toxin muscarine. Species of *Coprinus* produce the toxin coprine, that affects the autonomic nervous system.

Q.3. Discuss the symbiotic relationship in lichens.

Ans.

Symbiotic Relationship

The algal components of lichens belong to Chlorophyceae or Myxophyceae, whereas the fungal components to Basidiomycetes or Ascomycetes. The algal components of about 75% lichens are the members of Chlorophyceae and the fungal components mostly belong to Ascomycetes, except for four genera where they are from Basidiomycetes.

The inter-relationships between the algal and fungal components of lichens are usually considered to be symbiotic in which both algae and fungi are equally benefited. It is believed that the alga synthesizes organic food materials sufficient for both the algal and fungal components. In exchange of food, water and minerals are made available to alga by the fungal component. Besides, the fungal component also protects alga from high temperature and desiccation.

However, some workers believe that the fungal component has a partially or wholly parasitic relationship with the algal component. This view gets support from the facts that (i) haustoria of fungi are seen in algal cells of some lichens, and (ii) on separation, the algal component of lichen can live independently, whereas the fungal component can not survive.

According to yet another view, although the association of both the components of lichens is symbiotic the fungal component shows predominance over the algal component, and the latter is a subordinate partner. Such an association is known as helotism.

Q.4. Write about the classification of lichens.

Ans.

Classification of Lichens

There is no natural system of classification of lichens. According to the rules of the International Code of Botanical Nomenclature, no significance is attached to the algal components for the classification of lichens. Zahlbruckner (1907) classified lichens into the following two groups on the basis of their fungal components.

1. **Ascolichens :** The fungal component of these lichens is a member of the class Ascomycetes. These lichens are divided into two series on the basis of the structure of fruiting body.

   (i) **Gymnocarpeae :** The fruiting body is a disc-like apothecium. These lichens are also known as discolichens (*e.g., Parmelia*).

   (ii) **Pyrenocarpeae :** The fruiting body is a flask shaped perithecium. These lichens are also known as pyrenolichens (*e.g. Dermatocarpon*).

2. **Basidiolichens :** The fungal component of these lichens is a member of the class Basidiomycetes. Genera like *Corella* and *Dictyonema* belong to this group.

However, Alexopoulos and Mims (1979) classified lichens into the following three groups.

(i) **Basidiolichens :** The fungal component is a member of the class Basidiomycetes.

(ii) **Deuterolichens :** The fungal component is a member of the class Deuteromycetes.

(iii) **Ascolichens :** The fungal component is a member of the class Ascomycetes.

Q.5. Discuss briefly the economic importance of lichens as food and fodder.

Ans.

Lichens as Food and Fodder

Lichens are used as food since ancient times. They are important constituents of food in north Polar Tundra and eastern Siberian regions. Species of *Lecanora, Parmelia, Umbillicaria,* and *Cetraria icelandica* (Iceland moss) are some of the lichens which are used as food in many parts of the world. *Umbillicaria esculentus* is a delicacy in Japan, while the species of *Parmelia* are used as curry powder in India.

Lichens contain a polysaccharide- lichenin, but lack true starch and cellulose. *Evernia prunastri* is used by Egyptians for making breads. In France, some lichens are used for making delicious chocolates and pastries. In Japan, *Endocarpon miniatum* is used as a vegetable.

Several lichens (*e.g., Aspicilia calcarea, Lecanora saxicola*) are used as food by mites, snails, caterpillars, slugs, termites, etc.

Lichens, such as *Lobaria pulmonaria, Evernia prunastri, Ramalina fraxinea* and *R. fastigiata* are used as fodder for animals. They possess great nutritive value due to the presence of lichenin. *Cladonia rangifera* (reindeer moss) serves as a common food for animals, in Tundra regions specially reindeer and musk ox. Dried lichens are fed to horses and swans.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Write an essay on cultivation of mushroom.

Ans.

Cultivation of Mushrooms

Mushrooms are heterotrophic as they lack chlorophyll. Hence light is not necessary for their growth. The three major requirements for their cultivation are :

1. Suitable temperature, 2. Good compost, 3. Good quality spawn.

1. **Suitable Temperature :** The most suitable temperature for the growth of mushrooms is between 30° and 37°C. Temperature below 15°C and above 45°C is detrimental for the growth of the mycelium and fruiting bodies. In many parts of India, such as U.P., M.P., Punjab, Haryana, Orissa and Maharashtra, the optimum season for their cultivation is from April to September. In the plains of West Bengal, they are usually grown between March and September. In favourable conditions, the first crop of mushrooms is ready within 30-45 days and thus several crops can be taken in a growing season. Although light has no effect on the growth of mushrooms, the shelf life of mushrooms grown in dark is relatively more.
2. **Good Compost :** Two types of compost—natural and synthetic are used for cultivation of mushrooms.

(i) **Natural compost :** The natural compost is prepared by mixing wheat or barley straw in horse dung. Normally 33 kg straw is mixed with 100 kg of dung. It should be kept in mind that dung of any other animal should not be mixed with horse dung. Stored or rain wet dung is not suitable for compost. A meter high heaps of the fresh and wet dung, obtained from the stable are made in open air. After 3-4 days, when the dung starts producing ammonia due to fermentation, it is turned and stacked again. This process is repeated 4-5 times at the intervals of 5-6 days. During this course, gypsum (25 kg/tonne dung) is added to the dung. Finally, 40 ml nemagon is sprayed on the mixture.

(ii) **Synthetic compost :** The ingredients of the synthetic compost are :

(a) Wheat straw — 300 kg

(b) Wheat bran — 30 kg

(c) Ammonium sulphate or Urea — 4 kg

(d) Potash — 1.5 kg

(e) Gypsum — 30 kg

(f) Calcium ammonium nitrate — 6 kg

Wheat straw (cut to 8-12 cm long pieces) is spread on a clean cement concrete ground and wetted properly with water. Then it is mixed with half of the quantities of the other ingredients, except gypsum. This mixture is stacked into about à meter high heaps and the heaps are covered. After an interval of five days, these stacks are scrapped and the remaining half of the ingredients is thoroughly mixed with it and the whole mixture is piled again. This process is repeated six times. In the third and fourth turning, gypsum is added to the mixture. After the sixth turning, if there is a smell of ammonia, two or more turnings should be given until the smell of ammonia disappears. In the last turning 10 ml malathion (dissolved in 5 litres water) is added to the mixture. In addition 1 litre/kg of lintaff is also added to prevent insect infestation. The final mixture has sufficient quantity of moisture and can fill 20 trays of $100 \times 50 \times 15$ cm.

3. **Good Quality Spawn :** Good quality spawn is essential for better harvest. Different substrates in which mushrooms were already grown are used as inoculum. Mostly the compost left after the harvest of mushroom crop is used as inoculum. Sometimes small pieces of mushroom stipe are used as inoculum. Inoculum of *Agaricus* is usually prepared by adding grains of wheat, rye or millets to the substrate.

Mushrooms are grown in beds of various dimensions ($1 \times 1 \times 1$ m, $3.5 \times 3.5 \times 1$ m, or $0.6 \times 0.6 \times 1$ m). Wooden trays of $100 \times 50 \times 15$ cm dimensions are utilised for the cultivation of *Agaricus*. The wooden trays are filled with the compost and then pressed so that a space of about 3 cm deep is left on the top of the tray. The inoculum is spread over the compost and then it is covered by a thin layer of compost. The trays are finally covered with old newspapers. They are arranged in vertical stacks in such a way that there is sufficient space for aeration between the two trays. There should be a space of about one meter between the roof of the room and the top most trays in the stacks. The trays are sprayed with water at suitable intervals to keep the substrate moist.

In India, usually three types of mushrooms, viz., *Agaricus bisporus* (button mushroom), *Pleurotus* spp. (dhingri) and *Volvariella* spp. (paddy straw mushroom) are cultivated.

The main operations in the cultivation of *Pleurotus sajor-caju* (dhingri) are as follows :

(i) **Substratum :** The substrate is prepared from chopped paddy straw, crushed maize cobs, wheat straw, rye straw, dried and pulvarized grasses, compost, wooden logs, etc.

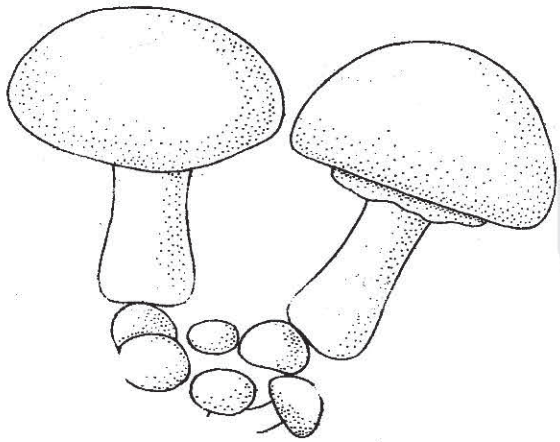


Fig. : *Pleurotus sajor-caju* (dhingri).

(ii) **Method of cultivation :** The substrate is soaked in water in a tank for 8-12 hours. After removal from the tank, it is washed with fresh water and the excess of water is allowed to drain. The substrate is filled in wooden trays of $1 \times 0.50 \times 0.25$ m dimensions. The filling of trays is done in two ways : (i) The trays are first filled up to 9 cm and spawn is sprinkled on the entire surface evenly. Then again the substrate is filled until the final depth of the substrate is 16-18 cm. (ii) The trays are filled with the substrate up to 16-18 cm and then the surface is sprinkled by spawn. After spawning, trays are covered by polythene sheets. They are sprayed with water once or twice a day so that sufficient moisture is maintained. After 10-15 days of spawning, white cottony growth appears on the entire surface and at this stage casing of trays is done. The polythene sheets are removed and casing is done by covering the surface with 2-2.5 cm thick uniform layer of the soil containing 1 part sand, 1 part garden soil and 1 part resin free saw dust. The soil should be sterilized before casing at 20 pound pressure for two hours or by heating it in a drum at about 90°C or by treating it with 5% formaline solution. The cased trays are continued to be irrigated by spraying water. The first flush of mushroom appears after 10-15 days of casing. If casing is not possible, the trays should be left as such and polythene covers be removed after the appearance of the first flush. New flushes of mushrooms continue to appear for 30-45 days after the first flush.

For the healthy growth of mushrooms, maintenance of a temperature of 25 ± 5°C and 85-90% relative humidity are essential. Besides, aeration and spraying of water is also necessary. To control insect pests and diseases, spray of 0.1% malathion and 0.2% diathane Z-78 is required.

(iii) **Harvesting :** Mushrooms are harvested when the pileus is about 8-10 cm in diameter. The harvesting is done by twisting the stalk so that the broken pieces are not left in the trays as they may become the source for spread of diseases. The space formed by the removal of stalks should be filled by sterilized soil.

After harvesting, the lower portion of the stalk with adhering debris is cut with the help of a clean and sharp knife. Mushrooms can be stored in refrigerator for about a week. The fresh mushrooms can be dried either in the sun or in an oven at 55 60°C for 8 hours. The dry mushrooms are packed and sealed before marketing.

Q.2. Give an account of the economic importance of fungi in agriclture.

Ans. Economic Importance of Fungi in Agriculture

Fungi play an important role in agriculture in various ways :

1. **As Scavengers :** The ratio of carbon dioxide in the atmosphere is maintained by decomposition of plants and animals debris by fungi and bacteria. In the absence of these scavengers, the surface of the earth would have covered with the accumulating remains of dead animals and plants.

   In acidic soils the activity of bacteria becomes very slow and under such conditions decomposition is mainly carried by saprophytic fungi. The enzymes secreted by these fungi decompose complex organic substances into their inorganic components and thus increase soil fertility.

2. **In Biological Control :** Fungi play an important role in biological control of diseases; for instance, *Trichoderma lignorum* suppresses the growth of root rot fungus, *Pythium* and the growth of *Rhizoctonia solani* can be checked by *Penicillium vermiculatum* and *Rhizoctonia oryzae. Gliocladium roseum* is used to control *Sclerotinia* diseases. *Trichoderma harzianum* is used to control blight of tomato and peanuts caused by *Sclerotium rolfsii.* Fungal pathogens play an important role in nature in the reduction of weeds. The strategy of using fungal plant pathogens in biological control involves a classic tactic and a bioherbicide tactic. The first approach involves the introduction of a foreign plant pathogen, while the latter envisages the development of endemic pathogens and using them as microbial weed killers (bioherbicides). Pathogens are multiplied *in vitro* and applied as herbicides so that each individual weed plant is heavily inoculated with infectious propagules. For instance, *Septogloeum gillis,* Wallrothiella arecuthdoii and Colletotrichum gloeosporioides have the ability to attack mistletoes (*Arecuthobium* spp.) at all stages of development. Similarly, the growth of the weed- *Eupatorium adenophorum* can be controlled by *Cercospora eupatorii* and Colletotrichum destructive can be used as a biocontrol agent for dodder. Several fungi are also utilized for controlling soil borne organisms like amoeba and nematodes. For instance, *Nematophthora gyrophila* is capable to control effectively *Heterodera avenae,* a cereal cyst nematode.

3. **Importance as Mycorrhiza :** A symbiotic relationship between fungal hyphae and roots of higher plants is known as mycorrhiza. Several fungi, like species of *Rhizoctonia, Phoma, Tricholoma, Boletus, Phallus, Scleroderma* and *Amanita,* form mycorrhizal relationships with higher plants.

4. **As Insecticides :** Many insect pests can be controlled by the use of fungi *Aschersonia aleyroidis, Beauveria bassiana, Cordyceps melonthae, Empusa sepulchralis. Fusarium oxysporum* and *Metarhizium anisopliae.*

   Some soil fungi (*e.g., Trichoderma viride, Fusarium roseum* f. sp. *cerealis, Rhizopus nigricans*) act as 'soil nibblers' since they attack and kill some roots but do not cause excessive damage. Their overall effect is to promote more fibrous root development and to increase total area of root absorption.

5. **In Soil Aggregation and Soil Fertility :** Some fungi, such as species of Absidia, *Aspergillus, Cladosporium, Chaetomium, Mucor, Penicillium,* and *Rhizopus,* have soil-binding properties. The mucilagenous substances secreted by them are helpful in soil aggregation. Yeasts (such as species of *Rhodotorula* and *Saccharomyces*) and several phylloplane fungi have nitrogen fixing capabilities, thus increasing soil fertility. In a forest ecosystem, the natural mushroom flora greatly helps in bio-degradation of woody wastes. The ultimate end product in the form of humus is quite useful for the growth of other plants.

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

Q.3. What are lichens? Describe various modes of their reproduction?

Ans. Lichens are a small group of curious plants. They are made up of algal and fungal components, living together in an intimate symbiotic relationship. The algal component is known as phycobiont (*phykos* = alga, *bios* = life), and the fungal component as mycobiont (*mykes* = fungus, *bios* = life). The plant body of lichens neither resembles algae nor fungi. Thus, lichen is an association of a fungus and an algal photosynthetic symbiont, resulting in a stable thallus of specific structure. Lichenis are biology's extreme survivors - they live in the driest deserts and wettest forests, and on the world's highest summits.

Reproduction in Lichen

Lichens reproduce both by asexual and sexual means :

1. Asexual Reproduction

Asexual reproduction is of common occurrence in lichens. Some common methods of asexual reproduction are as follows.

1. **Fragmentation :** Small fragments of thallus are formed by accidental breaking or by the death or decay of the older parts. Each fragment develops into a new thallus, provided it contains both algal and fungal components.

2. **Seredium :** Some small bud-like outgrowths, known as soredia, develop on the surface of the thallus. Each soredium contains one or few algal cells closely enveloped by a weft of fungal hyphae. The soredia form a granular layer of greyish-white colour on the surface of the thallus. They are detached from the thallus by the impact of wind or rain drops. Sometimes, as in *Parmelia* and *Physcia,* soredia develop in an organised manner in special pustule-like areas. Then they are known as soralia. The soredia germinate on suitable substratum and form new thalli.

3. **Het Cephalodium :** These are small wart-like structures formed on the surface of the thallus. One of the characteristic feature of the cephalodium is that its algal and fungal

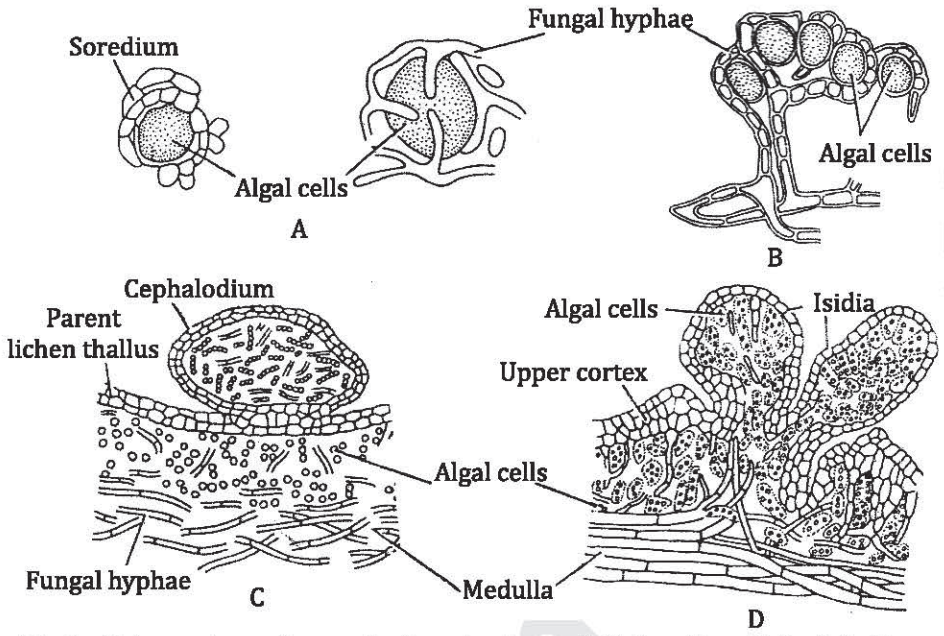


Fig. 1 : Lichens : Asexual reproductive structures; A, B. Soredium, C. Cephalodium, D. Isidium.

components differ from that of the thallus. It is due to the fact that cephalodia develop on the younger parts of the thallus from soredia of some other species. Hence, the cephalodium may be regarded as sterile thallus of some other lichen.

4. **Isidium :** Isidia are small, stalked, greyish-black coral-like outgrowths which develop on the upper surface of the thallus. The isidium has an outer cortical layer enclosing the algal and fungal components. It is usually constricted at the base and is easily detachable from the parent thallus. It germinates under favourable conditions and forms new thallus.

   In addition to propagation, isidia also help in increasing the photosynthetic surface of the thallus. They vary in shape and may be rod-like (*e.g., Parmelia*), coral-like (*e.g., Peltigera*), scale like (*e.g., Collema*) or cigar-like (*e.g., Usnea*).

   Some lichens (*e.g., Physcia, Buellia*) develop flask-shaped *pycnidia* which form pycnidiospores. The latter form fungal hyphae on germination. These hyphae, when come in contact with a suitable alga, develop into a new lichen body. Sometimes fungal hyphae break to form oidiospores.

2. Sexual Reproduction

In lichens the process of sexual reproduction is performed only by the fungal component. The fungal component of most of the lichens belongs to the class Ascomycetes. Hence, the sexual reproduction is similar to that of ascomycetous fungi.

The female sex organs are known as carpogonia. A carpogonium is differentiated into a basal coiled ascogonium and an elongated multicellular trichogyne. The ascogonium remains

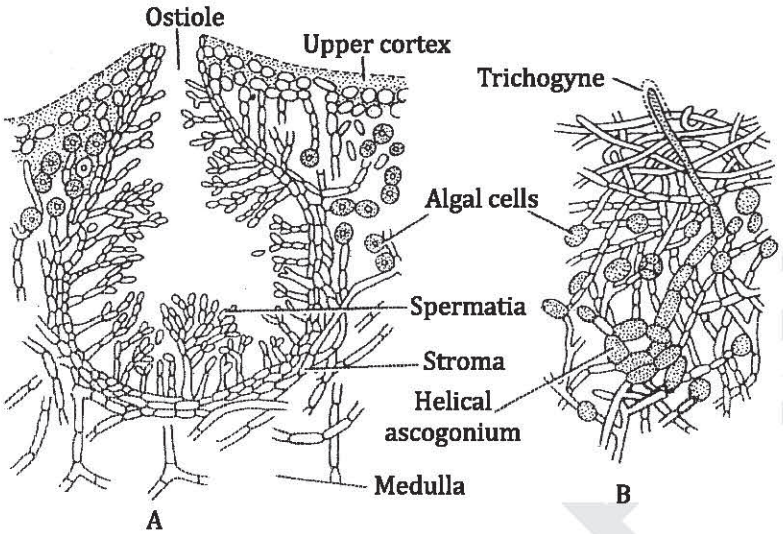


Fig. 2 : Lichen : Reproductive structures; A. Spermogonium, B. Carpogonium

embedded within the algal layer of the thallus, whereas the trichogyne projects over the surface of the thallus.

The male sex organs are flask-shaped spermogonia. They form spermatia which function as male gametes. The spermogonium usually develops close to carpogonium. This enables spermatia to adhere to the projected part of sticky trichogyne. On dissolution of the walls

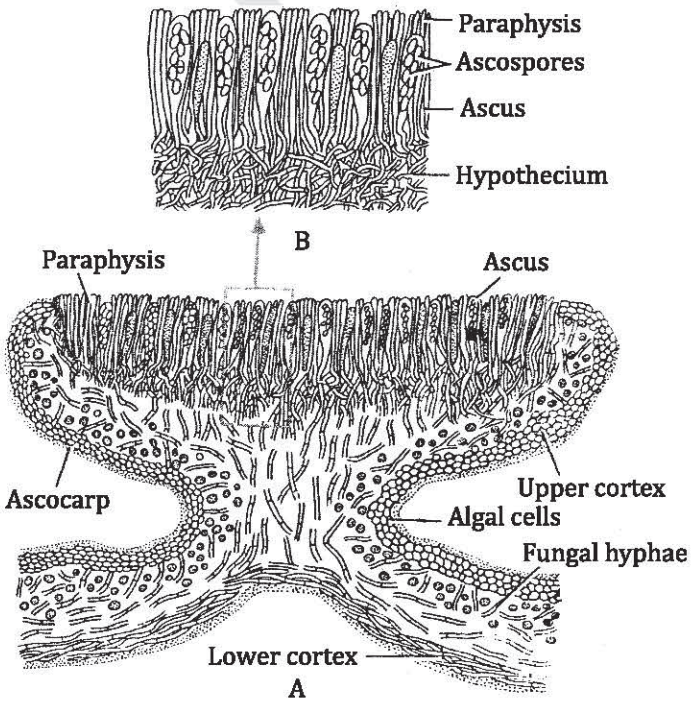


Fig. 3 : Lichen : Structure of fruiting body; A. L.S. of apothecium, B. A part of hymenium showing asci.

between the spermatium and trichogyne, the nucleus of spermatium migrates into the carpogonium through trichogyne. The male nucleus fuses with the female nucleus.

Several branched ascogenous hyphae develop from the base of the fertilized ascogonium. The terminal or penultimate binucleate cell of the ascogenous hypha develops into an ascus. The two nuclei within the ascus fuse to form a diploid nucleus. The diploid nucleus first divides meiotically and then mitotically to form eight haploid daughter nuclei. Each haploid nucleus metamorphoses into an ascospore.

The asci remain enveloped by paraphysis. The somatic tissue surrounding the asci and paraphysis form the fruiting body, which may be apothecium (*e.g., Parmelia, Anaptychia*) or perithecium (*e.g., Dermatocarpon, Verrucaria*).

The ascospores vary considerably in their shape, size, structure and septation. They are hyaline and greenish or brown in colour. They are released gradually from the ascus. The ascospore produces a hypha on germination and this hypha, when comes in contact with a suitable alga, forms a new lichen thallus.

Q.4. What is mycorrhiza? Describe their types and significance for crop plants.

Ans.

Mycorrhiza

It is currently estimated that the world's population will exceed nine billion by 2050. Thus global agricultures will have the task of almost doubling food production. At the same time agriculture will have to reduce the dependence of producers on agro-chemicals in order to safegaurd human and environmental health. Since the forcasted necessary yield increase exceeds the current global capacity to produce food, this highlights the need to implement or revitalize eco-friendly technologies, such AMF-based (Arbuscular mycorrhizal fungus) biofertilizers. Despite its enormous potential, the application of AMF has not been fully adopted by farmers so far. In the following pages you will get some basic information about mycorrhiza.

Mycorrhiza (*pl* = mycorrhizae) is a mutual symbiotic association between a green plant and a fungus. Literally, mycorrhiza means 'fungus root' (*myco* = fungi; *rhiza* = root). This association is markedly different from other common root infecting fungi which cause diseases. Mycorrhizal fungi colonize plant roots and extend far into the soil. Mycorrhizal fungal filaments in the soil are truly extention of the root system and are more effective in nutrient and water absorption than roots themselves.

About 90% of land plants rely on mycorrhizal fungi, especially for mineral nutrients (*e.g.,* phosphorus) and in return the fungus receives nutrients formed by the plant. During winters, when day length is short and exposure to sunlight is reduced, some plants produce only few or no nutrients and thus depend on fungi for sugars, nitrogenous compounds and other nutrients that fungi are able to absorb from waste material in the soil. By sharing the products it absorbs from the soil with its host plant, a fungus can keep its host alive. In some low land forests, the soil contains plenty of mycorrhizal fungi. The mycelial network of these fungi connect the trees together. The trees and their seedlings can use the fungal mycelium to exchange nutrients and chemical messages.

The mycorrhizal interaction between plants and fungi is plant-and-fungus-specific. Not all mycorrhizae forming fungi will always benefit all plants. Several experiments show that association with a wrong fungus actually decreases the health and vigour of the plant. Since there is requirement of specific plant fungus association, mycorrhizae can be important in restablishing native species in areas where they have been lost.

Mycorrhiza forming fungi or their spores are already present in several forest and desert areas, thus need not to be inoculated. If suitable host plants are present in such areas, their yield and vigour improves. However, it is important to note that inoculation with any mycorrhizal fungus may not benefit unless it is specific to plants in the area. There is always requirement for a specific fungus-plant interaction for optimum benefit. It would also be counterproductive to inoculate the soil with a fungus that could strongly benefit a weedy species.

It is important to be careful when using pesticides, particularly fungicides as these chemicals kill all the fungi they encounter. Although these chemicals are effective against plant diseases, they can also destroy beneficial fungi and cause serious problems to plant survival.

Types of Mycorrhizae

There are two main types of mycorrhizal associations, viz., ectomycorrhizae and endomycorrhizae.

1. **Ectomycorrhizae :** These fungi grow as an external sheath (called Hartig net about 40 nm thick) around the root tip. These are present mainly on roots of forest trees, such as conifers and oaks. The fungi penetrate intercellular spaces of the epidermis and cortical regions but not into root cells. The study of relative growth of uninoculated and inoculated pine seedlings revealed extensive growth of roots in inoculated seedlings. On the contrary root growth was very poor in uninoculated seedlings.

   Presumably, over 6000 species of fungi are involved in ectomycorrhizae.

2. **Endomycorrhizae :** In this symbiotic association, there is significant invasion of cortical cells with some fungal hyphae extending outside the roots. This type of fungal plant relationship is present in plants such as orchids. In fact, an orchid cannot grow without these fungal associations.

   Other plants showing endomycorrhizae include cereals, peas, beans, onions, apples, straberry and pasture grasses. In these plants fungi form intricately branched shrub-like structures called arbuscules or bladder-like vesicles within the cell. These associations are often called arbuscular mycorrhizas (AM). These are the most ancient and most abundant type of mycorrhizae. These fungi probably originated between 350 to 450 million years ago and may have played an essential role in the colonization of land by plants. According to an estimate no more than few hundred species of fungi are associated with VA mycorrhizae.

Given table shows a comparison between endomycorrhizae and ectomycorrhizae :

Comparison between Endomycorrhizal Fungi and Ectomycorrhizal Fungi

	Endomycorrhizal Fungi	Ectomycorrhizal Fungi
1.	Form symbiotic relationships with approximately 85% of plant families, mostly commercially produced plants, including green, leafy and fruiting or flowering plants.	Form symbiotic relationships with about 10% of plant families, mostly conifers and American hardwoods.
2.	Penetrate into the root cortex and form nutrient exchange structures within the cells (arbuscules, vesicles etc.). Examples : *Acaulospora, Archaeospora,* Entrophospora, Funneliformis, Gigaspora, Glomus.	Do not penetrate into the root cells, form a sheath around the root called Hartig net. Examples : *Amanita, Boletus, Cortinarius, Dermocybe,* Gliophorus, Humidicutis, Hydrophorus, Laccaria.

Significances of Mycorrhizae

A mycorrhizal relationship is beneficial to the plant in many ways. Some of these are listed below :

1. **Root System Growth :**
   - (i) Mycorrhizal fungi support faster plant establishment.
   - (ii) Mycorrhizal hyphae access water and nutrients beyond the root zone and deliver them to the plant's vascular network.
   - (iii) Increases absorption area by as much as 50 times.
2. **Nutrient Efficiency:**
   - (i) Mycorrhizal hyphae absorb and actively deliver nutrients directly to roots.
   - (ii) Improves utilization of soil nutrients including nitrogen, phosphorus, potassium and micronutrients.
3. **Water Absorption :**
   - (i) Mycorrhizal hyphae absorb and transport soil miosture from beyond the root zone to the plant roots.
   - (ii) The mycorrhizal symbiosis increases the plants effective water utilization capability. This mutualistic symbiosis improves tolerance to stress and greater resistance to draught.

Besides these, the symbiotic relationship with mycorrhizal fungi also provides many additional benefits to plants and their environments. These include improved soil structure, greater transplant success, increased stress tolerance, reduced nutrient runoff and many more.

❑

UNIT-VI

Plant Pathology

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. What is a pathogen?

Ans. A pathogen is that factor (preferrably the living one) that causes a disease.

Q.2. What is primary infection?

Ans. The very first infection caused on the healthy host during the growing season especially from the source of primary inoculum.

Q.3. What are facultative parasites?

Ans. An organism that is usually saprophytic but may grow as a parasite in prevailing conditions. Facultative parasites are also called **necrotrophs** or **perthotrophs**.

Q.4. Define systematic disease.

Ans. The disease in which pathogen spreads throughout the entire plant to varying extents and is associated with almost every stage of plant's life-cycle.

Q.5. Define a 'disease'.

Ans. A disease is the manifestation of a condition wherein any disturbance brought about by living or non-living factor interferring with normal physiological functions of a plant in such a way that the affected plant loses its appearance and/or productivity less than a healthy or normal plant of the same variety.

Q.6. What is disease-symptoms?

Ans. For any disease in a given plant, there is characteristic expression of symptoms normally taking place in a certain sequence during the course of disease-development. Such a series of symptoms is called disease-syndrome.

Q.7. What are downy mildews?

Ans. The downy mildews are internal obligate parasites characterized by superficial 'downy' or cottony growth consisting of sporangiophores and sporangia, e.g., Downy mildew of bajra caused by *Sclerospora graminicola*.

Q.8. What is chlorosis?

Ans. Chlorosis presents an uniform yellowing of leaves due to chlorophyll distortion without any mosaic-pattern.

Q.9. What is vein-clearing?

Ans. Vein-clearing represents a condition, wherein veins and veinlets of young leaves become yellow against the green background of the leaf.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. Explain the term pathogenesis. Name the phase of pathogenesis.

Ans. Pathogenesis is the process of infection or the actual way in which the disease develops in plant body. Infection is the establishment of a pathogenic micro-organism within the host, following entrance. It signifies the sum of biological processes which take place in the host body after penetration of the pathogen, independent of the fact whether the pathogen causes a disease or not. As the result of infection visible or latent diseases are produced in the host plants. In fact, infection is a process of inter-struggle between the organisms (pathogen and host) living in two different environmental conditions. The potential capacity of infection of any pathogen is called its **pathogenicity**. The pathogenicity of every pathogen is its specific feature. This characteristic depends upon the capacity of parasitic adaptation and struggle for existence of the pathogen. In other words, within a pathogenic species different strains have varying degree of disease producing power in one kind of host. The degree of pathogenicity is known as **virulence**. It is, therefore, an index of the quantitative individual nature of the pathogenic micro-organism. The phenomenon of pathogenesis can be understood easily by studying the three phases of the pathogenesis. viz., **pre-penetration, during penetration** and **post-penetration phases**.

Q.2. What are the exception to Koch's postulates?

Ans. **Exceptions to Koch's Postulates**

As discussed above, it has been possible to determine the causative agents of many diseases on the basis of Koch's postulates. However, there are a few exceptions. Some microbes have unique cultural requirements. For example, the virulent strains of *Treponema pallidum*, the causative agent of syphilis, grow only on the synthetic media. Similarly, *Mycobacterium leprae*, the causative agent of leprosy, has never been grown on artificial media. Pathogens like ricketts and viruses, which multiply only within living cells, do not grow in artificial media. These observations have necessitated some modifications in Koch's postulates and use of alternative methods of culturing and detecting certain causative agents. For example. when pathologists were unable to detect the microbes of legionnaries disease (a type of pneumonia caused by *Legionella bacteris*) directly from the diseased individual, they directly inoculated a small part of diseased animals' lung into guinea pigs and they developed pneumonia like symptoms. Symptoms of pneumonia, however, did not develop when pigs were inoculated with tissue from healthy animals. When the diseased tissue samples were cultured in yolk sacs of chick embryos, growth of microbes was observed. These were later identified as the same bacteria which were detected earlier in diseased guinea pigs and human beings. The above observations signify the importance of alternate routes in the detection and identification of pathogens.

Generally pathogens causing diseases are very specific, i.e., a particular disease is caused only by a specific pathogen. But it is not always true as common symptoms are caused by many different classes of microbes. One such disease is nephritis, a kidney disease. As such its becomes difficult to determine which particular microorganism is causing the disease. Contrary to these, there are examples where a single bacterium may be involved in the diseases of lungs, skin, bones, scarlet fever, etc. These can be distinguished from infections of

the same organ by other pathogens only by studying the pathological and clinical conditions together.

Q.3. What is inoculum? Differentiate between primary and secondary inoculum.

Ans.

Inoculum

The portion of the pathogen responsible for infection is called inoculum. It varies in different pathogens. For example, in fungi the inoculum may be spores or fragments of myceluim. But in bacteria, viruses, viroids and protozoa, the entire body behaves as an inoculum. In nematodes, it may be adult nematodes, juveniles or eggs. In parasitic flowering plants, the inoculum may be plant fragments or seeds.

In the preparation / selection of inoculm, the primary objective is usually to achieve a high level of viable biomass in a suitable physiological state for use as an inoculum.

In higher organisms (animals and human beings), the inoculum is introduced into the body to create or increase the body's resistance or immunity against various infectious diseases. You must have heard about several vaccination programmes in which an agent resembling a disease causing microorganism (often made from weakened or killed form of the microbe), its toxins or surface protein is inoculated in the body of susceptible individuals.

Inoculum also has application in industrial microbiology for obtaining products such as antimicrobials, enzymes, beverages, drugs, toxins, vitamins, amino acids, organic acids, food products and recombinant proteins. A proper inoculum must be in an active growth stage and size, free from contamination and have product forming ability. Adequate culture and growth medium are essential for providing the right environment for inoculum.

The inoculum survives either on perennial plants, plant debris or soil, or on the plant parts used for propagation. It is carried to the host plants mostly by water, wind, insects or man. Some types of inoculum present in the soil, such as zoospores and nematodes may be attracted to the host plant by chemical substances like sugars and amino acids diffusing out of the plant roots. This process is known as **chemotaxis**.

In fact, only a tiny fraction of the potential inoculum produced actually lands on the susceptible host plants. The bulk of the inoculum produced lands on the things that cannot become infected.

There are basically two types of inocula :

1. **Primary Inoculum :** The inoculum that remains dormant during winter or summer and brings about original infections in the spring and autumn is called **primary inoculum**, and the infection it causes is known as **primary infection.**

   Generally, the more bundant the primary inoculum and closer it is to the crop, the more severe the disease and the losses that result.

2. **Secondary inoculum :** The inoculum produced from primary infections is called **secondary inoculum**. Like primary inoculum the secondary inoculum causes **secondary infection** and secondary symptoms. The second phase, i.e., the production of secondary inoculum and development of secondary symptoms may repeat several times in a growing season.

Q.4. Write a short note on phytoalexins.

Ans.

Phytoalexins

Phytoalexins are natural products secreted and accumulated temporarily by plants in response to pathogen attack. They have inhibitory activity against bacteria, fungi, nematodes and insects. These are mostly **lipophilic compounds** that have the ability to cross the plasma membrane and act inside the cell. According to Smith (1996), their toxicity in plants occurs as a function of their acidic character.

The term 'phytoalexin' was introduced by the plant pathologists **Karl Otto Muller** and **Hermann Borger**. These were described as a chemically diverse group of broad spectrum antimicrobial compounds synthesized by plants in response to attack from pathogens. Phytoalexins take part in an intricate defense mechanism which enables plants to control invading microorganisms. These broad spectrum inhibitors are chemically diverse with specific characteristics of particular plant species.

According to **Grayer** and **Kokubun** (2001) phytoalexins are secondary metabolites of low molecular weight. They are different from the antifungal proteins and peptides produced by plants which have higher molecular weight. **Sotessl et al.,** (1980) defined phytoalexins as products of metabolism of higher plants, absent in healthy tissues or are present only in insignificant amounts, however, accumulate in significant quantities in response to attack by fungi or bacteria.

Pisatin was the first chemically characterized phytoalexin from pea plants. After this discovery other phytoalexins were isolated from various crops such as beans, rice, barley, banana among others. Several parts of plants such as leaves, flowers, stems, seeds and root tubers can produce phytoalexins.

Phytoalexins have great diversity. More than 300 types of phytoalexins have been characterized which belong to different classes of chemical compounds such as coumarins, diterpenes, flavonoids, phenolic compounds, luteolinidin, apigenidin and apigeninidin, etc. The rate of phytoalexin accumulation is considered a key factor for the establishment of the pathogen infection.

Q.5. Write a short note on biochemical defense mechanism.

Ans.

Biochemical Defense Mechanism

The host also produces some such specific substances whose presence or absence interferes growth and multiplication of the pathogen. These biochemical substances are already present or develop afterwards as the result of the host-pathogen interaction. The preinfectional biochemical defensive mechanism includes the presence of certain chemicals, such as phenols and chlorogenic acid, which are inhibitory to pathogens. For instance, red variety of onion shows resistance toward *Colletotrichum circinans*. The scaly leaves of this variety contain certain phenolic compounds like protocatechuic acid and catechol, which inhibit spore germination of the fungus.

Similarly, the glandular hairs present on the leaves of *Cicer arietimun* secrete malic acid which inhibits spore germination and hyphal growth. The resistance of pears to fire blight caused by *Erwinia amylovora* is due to the presence of a phenolic glucoside, arbutin. The resistance of ripe tomatoes towards *Bacterium vesicatorium* is due to their acidic nature. Unfavourable pH conditions of host tissues are also inhibitory to the growth of pathogens. Some such substances are mentioned below.

Q.6. Describe the events of disease cycle.

Ans.

Disease Cycle

Plant disease cycle represent pathogen biology as a series of interconnected stages of disease development including inoculation, penetration, infection, incubation and reproduction. In order for a disease to develop, a pathogen must be present and successfully invade plant tissues and cells. The chain of events involved in different types of disease cycles are shown in the figure.

In the following pages various events of disease cycle are mentioned briefly.

1. **Inoculation :** This is the introduction of plant pathogen into the host. Different pathogen groups employ different inoculation methods and are equipped with several specialized mechanisms that aid in the inoculation process. For example, some fungal pathogens release spores into the air and the spores are then spread with the help of air currents.

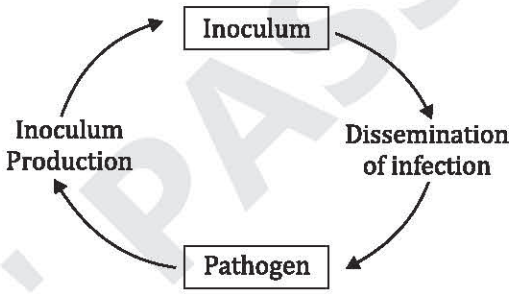


Fig. : The cyclic nature of plant diseases

2. **Penetration :** Wound sites and natural plant openings, such as stomata and hydathodes, facilitate the entrance of some plant pathogens; others have evolved unique mechanisms for direct penetration. Fungi and nematodes are able to actively penetrate host tissues and cells if environmental conditions, such as moisture and temperature, are favourable for the penetration process.
3. **Infection :** This occurs when the pathogen invades the plant tissue and establishes a parasitic relationship between itself and the plant. Viruses, bacteria and phytoplasmas are not able to actively penetrate or enter plant hosts tissues. Therefore, they must rely on other methods to infect plant tissues and cells.

   Associations with insect vectors have been established by these pathogens to help in inoculation and dispersal.
4. **Incubation :** Once inside the plant, pathogens may undergo an incubation period and remain latent for a period of time before initiating disease.
5. **Reproduction :** Plant pathogens can reproduce sexually and asexually. It is dependent on the pathogen.

Plant pathogens have evolved so that they may survive prolonged periods of unfavourable weather conditions. For example, fungal pathogen of brown spot disease produces dark coloured spores which reduce the amount of UV light penetrating the tissue; this prevents cell death, Similarly, soyabean cyst nematodes lay their eggs below the cuticle layer which is very hard; it prevents other microbes and chemicals to penetrate and kill the eggs prior to hatching.

If any step is disturbed in the cycle, the disease will be less severe or fail to develop. Knowing and understanding the disease cycle for a particular disease is very helpful in managing the disease.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Write brief notes on disease symptoms necrosis and blight.

Ans.

Necrosis

Death of cells, tissues or organs of the host due to parasitic infection is known as necrosis. It is the most common and destructive symptom of plant diseases. The necrotic symptoms produced by parasites in different hosts are of the following types :

1. **Spot :** Spots are the most common symptoms produced by fungi. When host cells are killed in a limited area, the necrotic areas appear in the form of spots. The spots may be circular, angular or irregular in shape. Several types of leaf spot disease occur in plants. Tar spots are characteristic leaf spots which appear as raised, black coated bodies.

   They look like a flat drop of tar on the leaf. In advanced stages of disease, the dead tissue of the necrotic areas shrinks and separates from the healthy tissue. More or less angular and elongated spots appearing on the veins on the lower surface of the leaf are known as **anthracnose.**

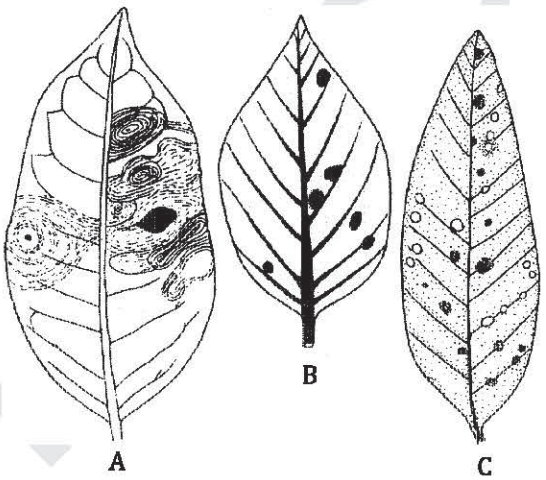


Fig. 1 : Spot symptoms on leaves : A. Circular spots, B. Tar spots, C. Anthracnose.

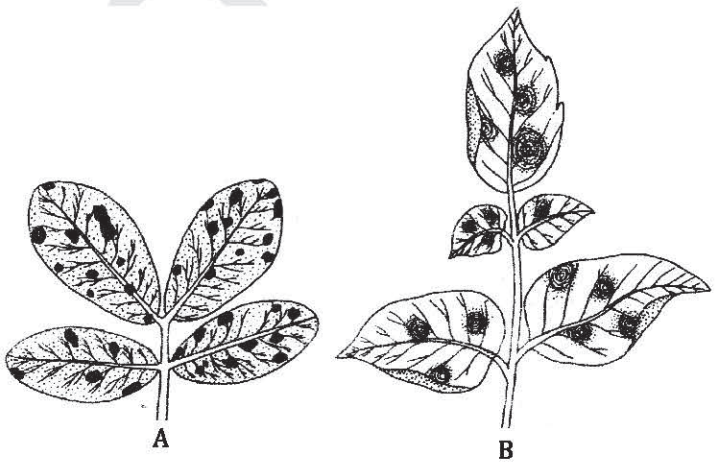


Fig. 2 : Spot symptoms on leaves : A. Groundnut leaves showing symptoms of tikka disease, B. Potato leaves showing concentric rings of early blight.

Leaf spot disease of mango is caused by *Pseudomonas mangiferacindicae*. Concentric rings of brown and black colour are characteristic of several leaf spot diseases caused by the members of Melaconiales and Moniliales.

2. **Stripes or streaks :** When disease symptoms appear in the form of elongated and narrow lesions, they are known as **stripes** or **streaks**. These streaks are usually blackish-brown in colour. Stripe disease of barley is caused by *Helminthosporium*.
3. **Canker :** The dead areas present in the stem bark or cortex of woody trees are known as **cankers**. These are often large and rough areas with definite margins which remain embedded in the tissue. The fruiting bodies of cankerous fungi come outside the host by breaking the canker surface. Citrus canker is caused by *Xanthomonas citri*.
4. **Blight :** In blights the disease symptoms give a burnt appearance. These symptoms develop due to sudden death of various plant parts, such as leaves, branches or flowers. The affected part usually turns brown or black and soon disintegrates. Late blight of potato is caused by *Phytophthora infestans* and leaf blight of wheat by *Alternaria tenuis*.

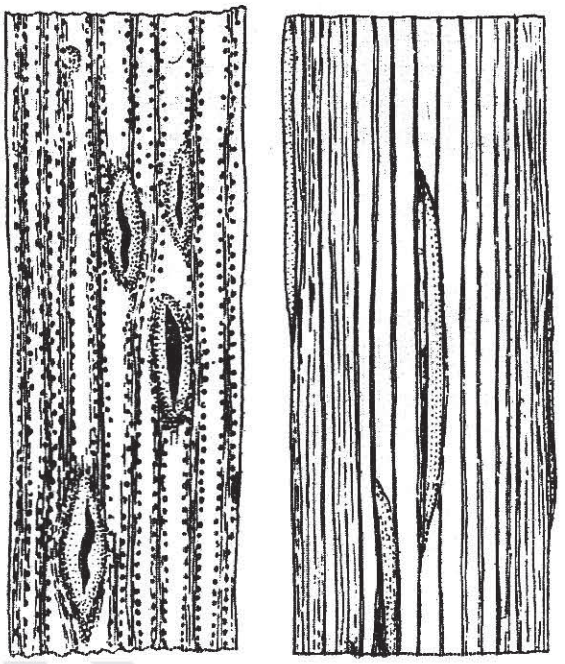


Fig. 3 : Stripes : Symptoms on leaves

5. **Damping off :** In damping off, the pathogen attacks the base of the stem near the soil surface. The tissues become weak in the infected region and ultimately the plant collapses.

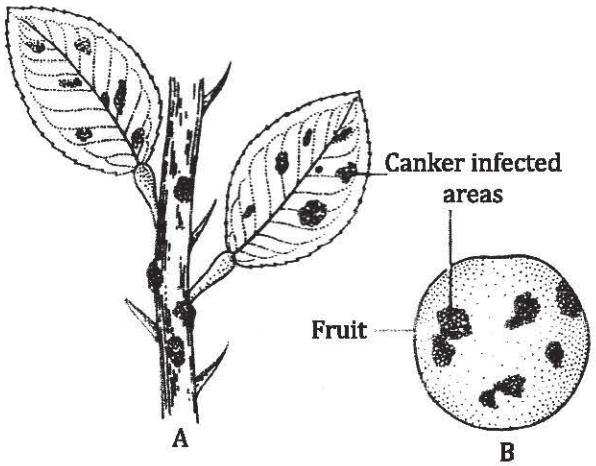


Fig. 4 : Canker : Symptoms on citrus; A. On twig, B. On fruit.

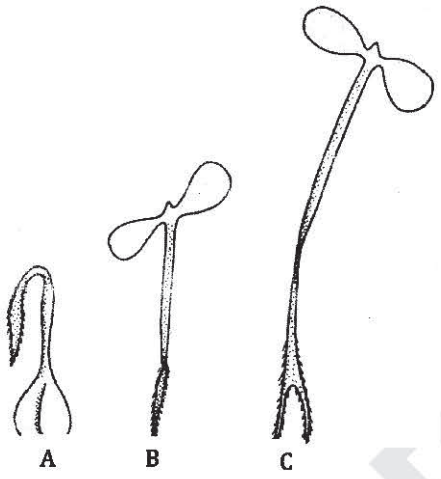


Fig. 5 : Damping off : Symptoms.

Several pathogenic fungi, such as *Pythium, Fusarium* and *Phytophthora* cause damping off disease.

Q.2. What is the difference between hypertrophy and hyperplasia.

Ans.

Hypertrophy and Hyperplasia

Some diseases cause abnormal growth in a particular part of the plant or the whole plant. This growth may be due to the enlargement of the affected cells (hypertrophy) or due to increased cell divisions resulting in a large number of component cells (hyperplasia). The following are some important forms of hypertrophied symptoms.

1. **Galls :** These are globose, elongated or irregular malformations or outgrowths developed on the affected parts of the host plant. Small sized galls are known as *tubercle* or *wart*, while large sized galls are called *knot* or *excrescence*. Wart disease of potato is caused by *Synchytrium endobioticum*.
2. **Curl :** Some diseases cause hypertrophy in the cells of some localised areas of the leaf and as such the leaf becomes curled, arched or distorted. These symptoms are known as curl disease. The causal organisms of leaf curl of tomato and leaf curl of papaya are viruses.
3. **Witches broom :** Some pathogens incite branching in the host. These branches develop only in certain limited parts and grow vertically instead of horizontally as the normal branches. These branches may become swollen and bear small hypertrophied leaves.

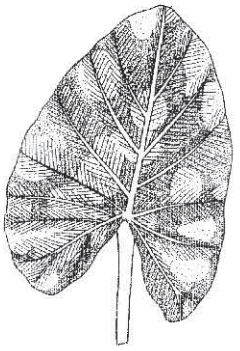


Fig. 1 : Blight : Symptoms on *Colocasia* leaf.

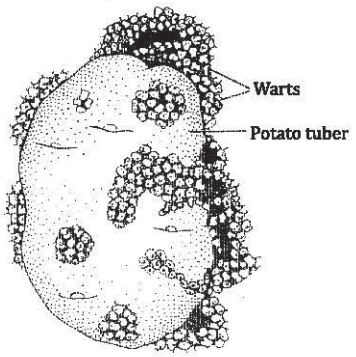


Fig. 2 : Warts : Symptoms on potato

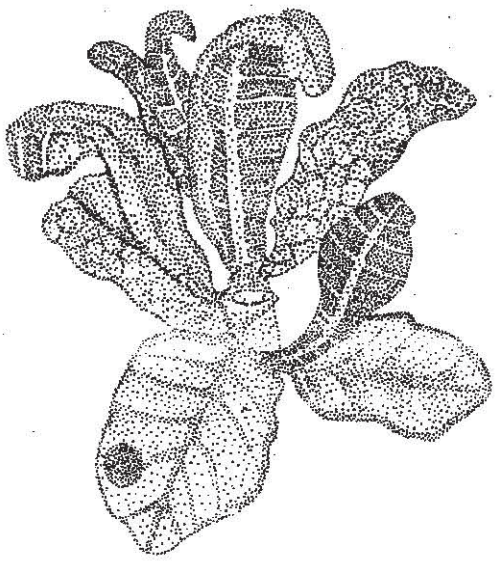


Fig. 3 : Curl : Symptoms on tobacco leaves.

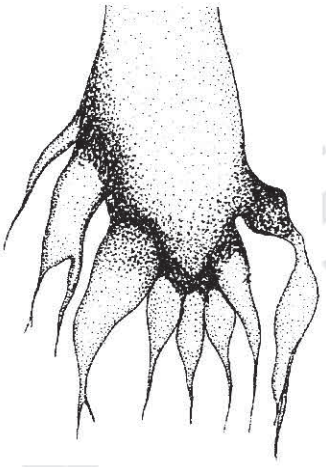


Fig. 4 : Witches broom : Symptoms

4. **Floral abnormalities :** Floral parts of some host plants are enlarged and become fleshy and leaf-like due to the infection of certain fungi, such as *Albugo candida*.

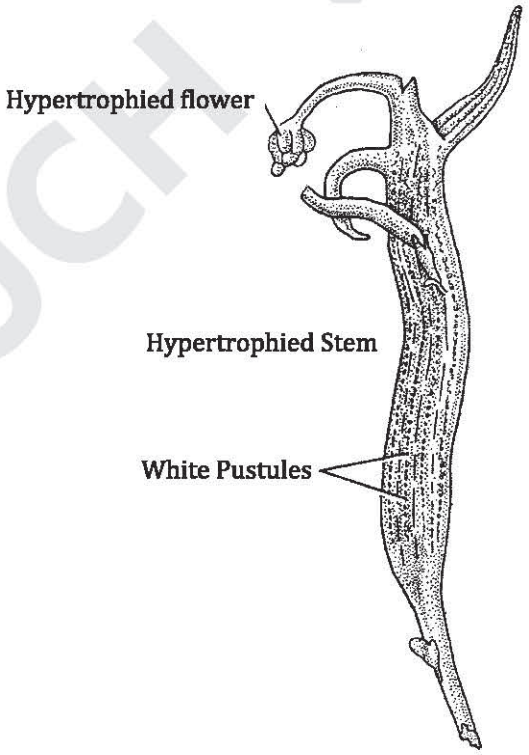


Fig. 5 : Floral abnormalities : Symptoms on *Raphamus* plant.

Q.3. Write Koch postulates. Explain them.

Or Describe the experiment which led to the 'Germ theory of disease'.

Ans. Robert Koch (1843-1910) was a physician and became a country doctor in Wollstein, East Prussia. His wife noticed his restless curiosity for microorganisms and gifted him a microscope. She had no idea that this small gift would go a long way in mitigating the human sufferings. Koch examined many specimens and among these specimens was also the blood of an ox that had succumbed to anthrax, an infectious disease of warm blooded animals. He noted the constant presence of stick-like structures in the blood of diseased animals which were absent in the blood samples of healthy animals.

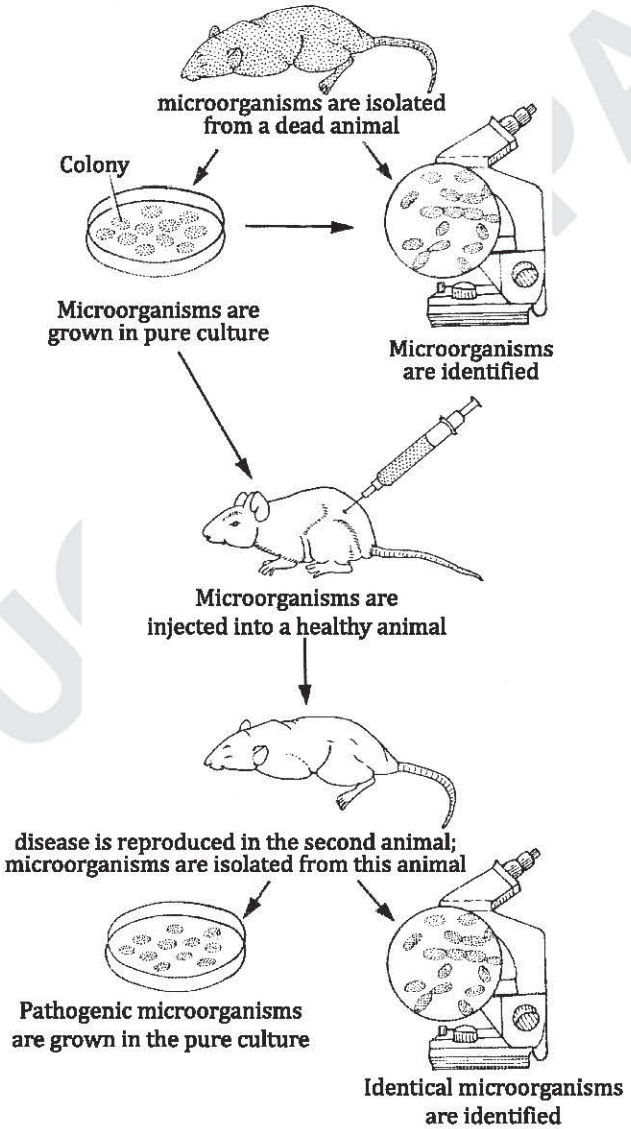


Fig. 1 : Koch's Postulates : Koch's postulates provide a framework for the study of the etiology of any infectious disease.

He observed that the disease symptoms could be transmitted by inoculating a healthy mouse with blood from an animal that had died from anthrax. On the basis of these finding, Koch concluded that the long, cylindrical stick-like bodies (later identified as Bacillus anthracis) might be the viable causative agent of the disease. He cultured these organisms in a fluid obtained from the eye of an ox Koch observed that organisms thus cultured, were able to cause anthrax when injected in mouse. This was the first clear experimental evidence that a bacterium was an agent of disease. Although Leeuwenhoek had first described the microorganisms approximately 350 years ago (in 1673), but it was Koch who proved that they cause diseases. He established in 1882 that tuberculosis in man is caused by tubercle bacillus. On the basis of the results obtained from above experiments, Koch formulated his theory on the relationships between microbes and diseases, commonly referred to as 'Germ theory of diseases' in 1976. The generalizations that he made are now known as **Koch's postulates**. These are :

1. That a specific microorganism is present in all cases of a disease.
2. That the organisms can be obtained in pure culture outside the host.
3. That the organism will, when inoculated into a susceptible host, will develop symptoms similar to those observed in the host from which it was isolated, and
4. That the organism may also be isolated in pure culture from the experimentally infected host.

In 1876, Koch was invited to Breslau, Germany by Ferdinand Cohn to demonstrate his work. Later, he was invited to Berlin to set up a laboratory and devote his time in the study of microorganisms.

Many of the Koch's students successfully grew causal organisms of several diseases. For example, George Gafpy grew typhoid bacillus and Friedrich Loeffler isolated the organisms of diphtheria. In 1890, Emil Behring, an associate of Robert Koch was able to treat diphtheria successfully by injecting patients with antitoxin—the protein synthesized in the body in response to the toxins.

Q.4. Describe systemic acquired and induced systemic resistance in relation to plant disease.

Ans. Resistance : Systemic Acquired and Induced Systemic

During last few decades, crop productivity has been mainly based on the use of high yielding varieties and in the application of high doses of fertilizers and pesticides. Despite crop protection measures, current losses are estimated at 20-40% for the major food crops world-wide. Hence, novel strategies for crop production, with less reliance on chemical products need to be developed.

As plants develop responses to **abiotic stresses** (like mineral deficiency), similarly plants also develop responses to cope with **biotic stresses** provoked by biological agents, like pathogens and insects. Some of these responses are limited to the infested or damaged organs, whereas other responses systematically spread far from the infested organ and affect the whole plant. These latter responses include the systemic acquired **resistance (SAR)** and the **induced systemic resistance (ISR)**. SAR is induced by pathogens and insets while ISR is

mediated by beneficial microbes living in the rhizosphere, such as bacteria and fungi. These root associated mutualistic microbes, besides impacting on plant nutrition and growth, can further boost plant defenses, rendering the entire plant more resistant to pathogens and pests.

Induced systemic resistance and systemic acquired resistance are two different phenomena but both represent active plant defense responses to phytopathogen attack. ISR is similar to hypersensitive response while SAR is alike inherent immunity of plant system. The terms were first coined by **Ross** during his research on interactions between tobacco and its mosaic virus (TMV). Induction of ISR is the function of non-pathogenic plant growth promoting rhizobacteria (PGPR). However, SAR is triggered by infection of a pathogen.

1. Systemic Acquired Resistance (SAR)

It is the resistance which once aquired, remains generalized (systemic) in the plant body. It is inherent resistance capacity of a plant which is activated on exposure to pathogen. It is similar to our immune response followed by Vaccination. Plant's cellular defense system uses recognition receptor proteins to identify microbial pathogen which may have infected plant in the past. Induction of SAR requires pathogen borne tissue necrosis. SAR once induced remains active against broad range of pathogens for prolonged time and it not only resists pathogen attack but also cures disease it caused. SAR develops primarily from tissue necrosis (infection site) caused by pathogen. Necrosis is followed by release and accumulation of **salicylic acid** in phloem tissue that triggers first hypersensitive response and induction of SAR. Salicylic acid is a plant hormone required for the production of PR (pathogen related) proteins. It inhibits production of virulence factor and is also known to control another plant hormone **ethylene** (ET). The proteins are the products of pathogen activated by PR genes.

They are necessary to induce plant's defense. Some of them function as antimicrobials, degrading cell wall of pathogen. Some PRs are specifically antibacterial, antifungal and antiviral. They also possess lytic chitinase, lysozyme and peroxidase enzyme activities which are functional against different pathogens. They also act as messengers to signal pathogen attack. This signalling activates lignin formation and deposition creating efficient barrier to inflicting agents. Biochemical genetics of signalling pathway is still not fully known. Onset of SAR determines plants' defending ability which is found to be very high and elaborated as various plant parts including distal leaves, branches, stems and roots also acquire immunity to pathogens. Every plant part need not to be a site of infection or necrosis or in contact with pathogen.

2. Induced Systemic Resistance (ISR)

It is the generalized (systemic) resistance which is naturally present in plants but is induced or enhanced by plant associated non-pathogenic rhizobacteria or plant promoting rhizobacteria. It is independent of salicylic acid and hence no PR (pathogenesis related) proteins are synthesized. It is plant specific and is dependent on plant's genotype. In induced systemic resistance host specific rhizobacteria have an important role in activating resistance response. The bacteria, in fact, recognize their host plant before setting on ISR. Plant hormones—**Jasmonic acid** and **ethylene** are required in ISR. Jasmonia acid regulates plant's

responces to biotic and abiotic stresses including pathogen attack. Sometimes it is a volatile compound which can reach to plant parts and also to nearby plants to warn off pathogen attack and trigger plant defense responses. Wounding or pathogen attack also stimulates the production of ethylene which then onsets defense responses in favour of plants. Typical ethlene induced visual defense responses include rapid ripening, abscission and senescence of infected tissue.

Besides hormones and volatile gases (ethylene), non-pathogenic rhizobacteria and plant growth promoting rhizobacteria (PGPR), isolated from soil and rhizosphere are potential natural elicitors. A part from rhizobacteria, other microbes, such as fungi, viruses and nematodes also induce ISR. Generally presence of PGPR, either in symbiosis or free-living state in plant's rhizosphere is enough to induce ISR in host plant. Such plants are naturally resistant to their pathogens.

The growth promoting properties of these bacteria are also found to be ISR inducers. The PGPR not only promote plant growth but also act as biocontrol agent. Three plant growth promoting rhizobacteria-*Bacillus, Pseudomonas* and *Rhizobium* are ideal among natural elicitors.

Artificial Elicitation of ISR and SAR

Elicitors or activators of ISR and SAR are in use for successful biocontrol application. Conventional agriculture uses both biological and chemical form of elicitors. They are injected into stems or applied as leaf sprays on plant body. Biological elicitors are formulations (inoculum) of cell extracts of living bacteria, viruses and fungi. Of these, bacteria or precisely rhizobacteria, are routinely utilized to induce ISR. Common chemical elicitors in use to induce SAR, are beta aminobutyric acid, 2-6 dichloroisonicotinic acid, benzothiadiazole, silicon dioxide, paraquat, polyacrylate, Salicylate, etc. These have been used successfully in integrated pest and disease management either solely or in combination with biological elicitors.

❑

UNIT-VII

Diseases and Control

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. Name the organism which causes late blight of potato.

Ans. *Phytophthora infestans* causes late blight of potato.

Q.2. Which organism causes blight of Colocasia?

Ans. *Phytophthora colocasiae* causes blight of Colocasia.

Q.3. In which fungus haustoria are slender and curled?

Ans. Haustoria are slender and curled in *Phytophthora*.

Q.4. Name the cell wall materials of phytophthora.

Ans. Cellulose, glucan are the cell wall materials of phytophthora.

Q.5. What materials are present in the wall of oospore?

Ans. Pectin, cellulose, proteins are materials present in the wall of oospore.

Q.6. What is the method of dikaryotization in *Puccinia*?

Ans. Spermatization is the method of dikaryotization in *Puccinia*.

Q.7. Name the type of basidium present in *Puccinia*.

Ans. Basidium present in *Puccinia* is Phragmobasidium.

Q.8. Which type of spore of *Puccinia* is regarded as conidium?

Ans. Urediniospore is the spore of *Puccinia* regarded as oxidium.

Q.9. Where does reductional division take place in the life cycle of *Puccinia*?

Ans. In the life cycle of *Puccinia*, reductional division takes place in Promycelium produced from telentospore.

Q.10. Write a note on IPM.

Ans. Integrated Pest Management (IPM) : IPM involves use of different pest control methods, which are ecologically sound (*i.e.,* not cause hazard to environment), *e.g.,* biological control methods, better agricultural practices like crop rotation, sanitation etc. starvation method, *i.e.,* growing of target crop away from major crop, ultra low volume spraying method *i.e.,* use of very low and most effective concentration of chemicals, which does not cause pollution, etc.

Q.11. Write the harmful effects of Pesticides.

Ans. Harmful effects (hazards) of pesticides are as follows :

1. As pesticides are non-selective in their mode of action, so there also kill useful organisms along with harmful and thus equilibrium state of ecosystem is distributed.
2. As pesticides are poisonous or toxic, so cause serious health hazards.

3. Excessive and prolonged use of pesticides lead to resistance in pests. Hence more money is to be spend in controlling these resistance pests (this effect is called pesticide (readmill).
4. Due to their non-biodegradable nature, these are biologically magnified in successive trophic levels, which disturb food webs and cause environmental pollution.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. What do you mean of the Alternaria Leaf Spot?

Ans.

Alternaria Leaf Spot

Symptoms and Signs : Alternaria leaf spot appears as fairly large brown spots on leaves, about 0.5 to 0.75 inches (12-18 mm) in diameter. The spots turn black as the fungus produces spores. Leaf spot develops most rapidly in June and July and trees can be almost completely defoliated by early summer when the disease is severe. The disease appears to be most severe where dew forms, humidity is high and air is stagnant.

Comments on the Disease : Alternaria leaf spot can occur on almond trees grown anywhere in the Central Valley, but rarely is it severe enough in the northern San Joaquin Valley to require treatment. It has been most serious on trees in the southern San Joaquin Valley and in the northern Sacramento Valley.

Management :

1. The disease occurs first and is most severe on exposed leaves.
2. Trees trained to an open and spreading canopy usually have more severe Alternaria leaf spot.
3. Trees planted with rows in an east-west direction also have more severe disease than do orchard with rows planted north-south.
4. Varieties that are most susceptible include Carmel, Sonora, Monterey, Winters and Butte.
5. Monitor for signs of the disease in April through June. If monitoring indicates the presence of Alternaria, begin late-spring treatments about mid-April.
6. In orchards with a history of the disease, treat in mid- to late April and again 2 to 3 weeks later.

A disease severity value or DSV model has been developed on tomato and modified for almond for forecasting Alternaria leaf spot. Index values are assigned for specific ranges of average temperatures during leaf wetness periods during a day. Apply fungicide if accumulated index values over a 7-day period reach a value of 10 or higher.

Q.2. Write a short note on red rot of sugarcane.

Ans.

Red Rot of Sugarcane

Red rot of sugarcane is most serious and destructive disease it is very common in very sugarcane growing country of the world. In India, it is specially common in Bihar and eastern Uttar Pradesh and causes a heavy loss to the crop. Serious epiphytotics have occurred in U.P. and Bihar during 1939-40 and 1946-47 seasons.

Symptoms : In early stages, it is somewhat difficult to recognise in the field. All the aerial parts of the plant are infected by the fungus.

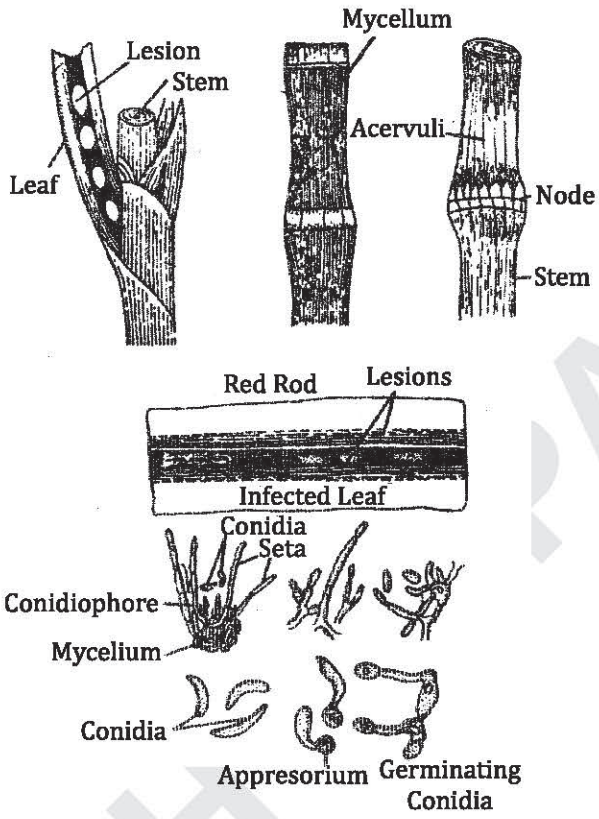


Fig. : *Colletotrichum*

The infected stem becomes somewhat greyish in colour and shrinks at the node. The infection on the stem is interior. He infected leaves become chloretic, withers and droop downwards, to cases of brisk infection the whole stem becomes rotten. On splitting the stem, long red strips are see with white tissue extending cross wise the stem. In the infection occurs in the form of red patches developed on the mid rib. On develop some straw coloured patches and later on acervuli occur in the form of small black dots. Due to this growth of the plant is very much retarded, and sucrose intercane is converted intoalcohol which imparts fowl smell in the field.

Causal Organism : This disease is caused by an imperfect fungus *Colletotrichum falcatum*.

Morphology of the Fungus : The mycelium is profiisely branshed, cylindrical separate and hyaline. It is inter as well as intracellular. The conidia and conidiophores are developed on a saucer shaped flat structure known as acervulus. The acervulus bear septate pointed seta along with conidiophores. The conidia are semilunar in shape uninudeate with a single oil drop.

According to the available evidence, seed sets from diseased canes are the chief means of survival and annual recurrence of the disease. When such sets are planted, shoots are invariably attacked. Once, the fungus establishes, secondary spread occurs by conidia. Ratoon crops may also serve as a source of perennation and inoculum multiplication. Aceruli are also produced on the patches developing on midrib of leaves, High humidity, water-logged conditions, lack of proper cultural operations, continuous cultivation of single variety in a given area, help in appearance of disease and build up of inoculum.

Control : It can be controlled as follows :

1. Field sanitation.
2. Use of healthy sets.
3. Ratooning should be discouraged.
4. Crop rotation.
5. Use of resistant varieties.

A large number of such varieties are evolved. A few among mote recent varieties recommended as resistant to disease are, Co 846, Co 951, Co 975, Co 1007, Co 1148, Co S 109S Go 561.

Q.3. Discuss the disease of citrus canker.

Ans.

Citrus Canker

The disease is widespread in all citrus growing areas of the world, which is said to have originated from China. It is particularly serious in India, China, Japan and Java.

Symptoms : The disease attacks leaves, twigs, thorns, old branches and fruits. On leaves, lesions first appear as small, round, watery translucent spots, which are raised and brown in colour. They first develop on the lower surface and as the disease progresses both surfaces are involved. The spots are white or greyish and finally rupture in the centre giving a rough corky and crater-like appearance. The spots usually become surrounded by a yellow halo. The spots increase in size and coalesce to form elongated lesions on twigs and fruits. On large branches leisons (cankers) are irregular, rougher and much distinct. On fruits, cankers citrus canker disease are similar to those on leaves except that yellow halo is absent and crater-like depression in the centre is more prominent.

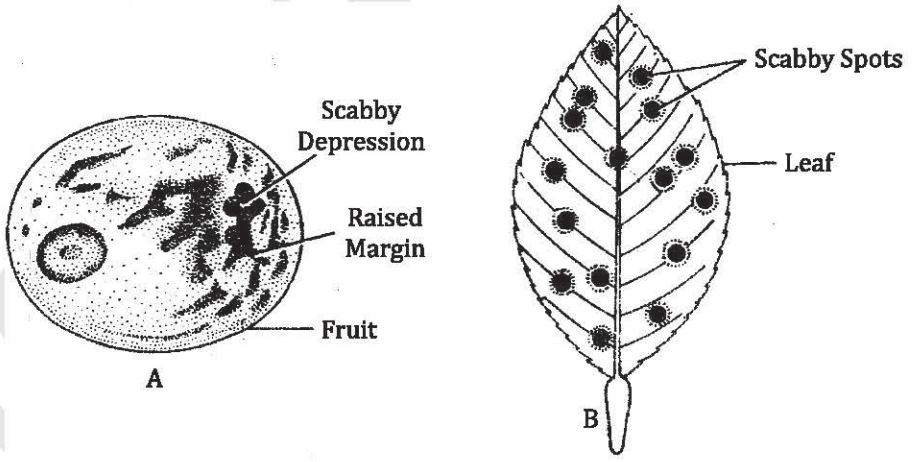


Fig. : Citrus Canker Disease

Causal Organism : The disease is caused by a bactriuirt, *Xanthomonas citri*. The bacterium is a gram-green rod. $1.5 - 2.0 \times 0.5 - 0.75\ \mu m$, forming chains and capsule but no endospores. It is aerobic. Colonies on beef agar are circular, straw yellow to amber yellow. Nitrates are not reduced.

The bacterium enters through stomata and wounds. It multiplies rapidly in intercellular spaces, dissolves the middle lamella and establishes in the cortex. The disease is favoured by

mild temperature and wet weather. Temperature between 20°C and 30°C with good evenly distributed rains are suitable.

Attacked leaves bearing old lesions are the main source of petennation of the pathogen. From the cankers, disease spreads by driving rains and insects. Man is also an important agent of dissemination through infected nursery stock.

Control : It can be controlled by following ways :

1. Complete destruction of diseased plants by burning them.
2. Use of disease free nursery stock for planting.
3. Spraying the plant before planting with 1% Bordeux mixture.
4. Antibiotic spray with streptomycin, phytomycin.

Q.4. Write about the quarantine.

Ans.

Quarantine

Plant quarantine is essential for cultivation of crop and avoid the inoculum from one place to other and from one country to other country. A quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion prevention or delay in the establishment of plant pests and diseases in areas where they are not known to occur. As early as 1946, Central Directorate of Plant Protection quarantive and storage was established under the ministry of Food and Agriculture, Govt. of India Under this Directorate, plant quarantine stations are working at seaports of Bombay (1949), Madras (1950). Cochine (1955), Kolkata (1956), Vishakhapatnam (1957) and Kadla (1961) and at airports of New Delhi, Mumbai, Chennai, Kolkata, Amritsar etc. Besides certain diseases cheifiy viral diseases must be controlled by controlling insect vectors. Thus, exclusion of inoculum may be practiced by following measures.

Plant Quarantine Services

1. Plant Quarantine clearance of the imported plants/plant materials at the port of eatry is mandatory under the provisions of Destructive Insects and Pests Act, 1914 and the Plants, Fruits and Seeds (Regulation of Import into India) (PFS) Order, 1989.
2. There are twenty six Plant Quarantine stations in the country to regulate Import and Export of Plants and Plant materials.
3. India is a Signatory to International Protection Convention, 1951 of FAO. Under the Convention Phytosanitary Certificate (PSC) for export of plants and plant materials at required to be issued by the notified National Authorities.
4. PSC is not necessary in respect of processed plant materials.
5. PSC issued by other than these authorised officers is treated as illegal.
6. Post Entry Quarantine (PEQ) facilities established by the importers are required to be verified by the Designated Inspection Authorities (DIAs).
7. The Import Permits (IPs) for plants/plant materials meant for propagation are issued by the notified officers.

Q.5. What do you understand by pesticides? Explain in brief.

Ans.

Pesticides

Pesticides are the chemical substances which kill pests, *i.e.,* epidemic diseases producing organisms (like insects, fungi, weeds, nematodes, rodents, bacteria etc.) Thus pesticides

include insecticides, fungicides, weedicides, nematicides, rodenticides, bacteriocides, etc. Pesticides may also be called biocides as most of the pests are living.

Pests cause loss of agricultural productivity upto about 30% and thus use of pesticides becomes necessary.

First pesticides to be used commercially is Boradeaux mixture ($CuSO_4 + Ca(OH)_2 + H_2O$), discovered by Millardet (1882).

Types of Pesticides

On the basis of their chemical nature, pesticides are of following types :

1. **Organochlorines :** These are chlorinated organic compounds with many chlorine atoms in a single molecule. Some important organochlorines—are BHC (Benzene hexachloide), DDT (Dichloro diphenyl trichloroethane), Aldrin, Dialdrin, Endosulphan, etc. These are non-biodegradable and hence are biologically magnified.

   DDT is the most important (famous) pesticide of the world and BHC is the most commonly used pesticide in India. Its concentration in flesh of Indian population is 11-30 ppm.

   Organochlorines have much affinity with fatty tissues of the animals (lipophillic) and hence are present in high concentration in fatty tissues of herbivores.

2. **Organophosphates :** These are organic esters of mostly phosphoric and triphosphoric aids. Important organophosphates used are Malathion, Parathion, Fenitrothion, Trithiou, Ethion, TEPP (Tetraacetyl pyrophosphate) etc.

   Malathion is the important and active constituent of flit and is used in large scale control of malaria.

   Organophosphates have strong effect on nervous system of insects and thus kill them by paralysing their nervous system. These interfere with conduction of never impulses in nervous system.

3. **Carbamates :** These are derived from carbonic acid in which hydroxyl group is replaced by $—NH_2$ (amino) group, to form carbamic acid (salts of carbamic acid are carbamates). Important carbamates are ; Carbofuran (furadofo), Aldicarb (Temik), Propoxur (Baygon), Carbaryl etc.

   MIC (Methyl isocyanate) is the raw material for these pesticides.

   The carbamates are structurally similar to acetyl choline and thus have great affinity for enzyme acetyl choline esterase. Carbamates are effective Insecticides and their derivatives like phenyl carbamates are used as herbicides and dithiocarbamates are used as fungicides.

4. **Pyrethoids :** Pyrethrin is an insecticide obtained from floral heads of *Chrysanthemym cinerarifolium* and *C. mafschallii* (fam. Asteraceae). Pyrethoids are the synthetic derivatives of pyretririn.

   Pyrethoids are largest group of insecticides of plant origin, used commercially now a days.

5. **Triazines :** This group of pesticides is derived from urea. Important triazines are Atrazine, Simazine etc. These are being commonly used as herbicides in controlling weeds in tobacco, tea and cotton plants.

Most of the herbicides have effect on photosystem-II of photosynthesis and also disturb translocation of organic food.

Bodeaux mixture is a fungicide (antifungal pesticide), discovered by Millardet. It is having ($CuSO_4$ (40 g), $Ca(OH)_2$ (40 g) and H_2O (5 litres). It is one of the important pesticides used in India.

Q.6. Write a note on Biological Pest Control.

Ans.

Biological Pest Control

Due to harmful effects of pesticides on organisms, some alternative methods of pest control are being used and biological pest control is one of the suitable methods, *i.e.,* use of other organisms to kill the pests constitutes biological pest control and such organisms are called biopesticides (*e.g.,* bioherbicides, bioinsecticides etc.).

Bioherbicides : The first bioherbicide is devine, which is a mycoherbicide, based on fungus *Phytophthorapalmivora*. It is being used since 1981 to control *Morrenia* odorata (milk weed vines) in Citrus orchards. Similarly college is another mycohericide from conidia of *Colletotrichum gloeosporides* fungus.

Bioinsecticides : These include :

1. **Use of parasites, predators and pathogens :** *e.g.,* control of aphids by use of praying mantis or lady bug, *i.e.,* Mantis. Similarly insects affecting maize, cotton, cabbage, sunflowers etc. are controlled by mutant strains of *Bacillus thuringinesis* (*Bt*) bacteria (sporeine).

   By use of lady bugs or praying mantis, aphids (plants bugs or homopterous insects) can be kept under control. Fluted scale insect (*Icerya purchasi*), a pest on *citrus* can be effectively controlled by lady bird beetles. Similarly mosquito larvae can be easily controlled by fish Gambusia and sugar can scale insects are controlled by coccinellid predators.

   Prickly pear cactus (*Opuntia*) in Australia and India was effectively controlled by larvae of *Cactoblastis cactorum* (Cochineal insect).

2. **Use of insect hormones (Sex attractants) :** The insect hormones called pherohormones. (Pheromones) are useful in controlling insects. These pherohormones attract opposite sex insects during breeding season. The natural and synthesic pherohormones are now used to attract the insects towards death traps. The Orient-fruit fly has been eradicated by this method. Similarly, gipsy moth, a pest of conifers can be trapped.

   'Confusion technique' is a variation of this approach and it involves uses of pheromones or sex attractants. In this technique, hydrophobic paper having phenomones or sex attractants is placed over the crop area, due to which characteristic smell is spread over the whole field and thus males are unable to locate the females.

   Use of insect hormones like juveline hormone and molting hormone or eodysone is also made as bioinsecticides. Juveni me hormone should be present in early stages of growth to prevent early maturation, but if the same is given artificially at later stage of growth, the insect is transformed into giant larva (immature adult) which dies quickly. Similarly, periodic shedding of insect cuticle (molting occurs during the process of

growth and ecdysome) hormone is associated with molting. Use of this hormone at inappropriate tome also results in early death of insect.

3. **Sterilization technique :** This is a modern method of biological pest control. In this technique, male insects are sterilized by irradiation, these are released at the time of making and hence their multiplication is checked, *e.g.,* screw worm (*Cochliomyia hominvorax*) and red weavil (a pest on coconut) have been checked by this method.
4. **Use of natural insecticides :** The insecticides of plant or micro-organisms origin are called natural insecticides. These have little toxicity for animals, *e.g.,* Rotanome (form roots of *Derris* sps. and *Lonchocarpus* sps.), different alkaloids like Nicotine (from tobacco), Pyrethnsm and Cinerin (from *Chrysanthemum*); Azadirachtin (from *Margosa* or Neem) are useful natural insecticides.

   Neem or margosa (*Azadirachta indica*) is most useful natural insecticide. It is resistant to about 200 species of insects, nematodes, mites etc.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Describe the life-history, life cycle and control of fungus which caused late blight of potato.

Ans.

Late Blight of Potato Caused by *Phytophthora*

The fungi *Phytophthora* infestens is the most common species which causes the "late blight of potato" all over the world. In india, this disease appear in mild forms in plants, but in hilly tract of Shimla, Kumaon, Darjeeling. Nilgiris, etc. it becomes quite destructive.

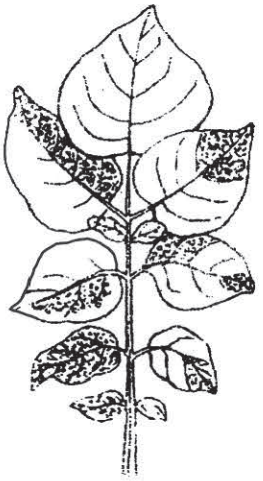


Fig. 1 : *Phytophthora* fungus on Potato leaf

Occurrence

The genus *Phytophthora* including nearly 75 species found all over the world. The species may be either facultative saprophytes or facultative parasites, Nearly 17 species attack, on flowering plants and caues a great damage to important crop plants of economic value such as Potato Kachalu, Arecanuts (Supari), Castor, Cotton, toddy palms, Coconuts, etc. Phytophthora infestens is the common species found on the leaves of the host plant. The conidiosporangia are borne on sympodially branched distinct sporangiophores which come out in small groups through the stomata. The sporangia or conidiosporangia are rounded or lemon shaped. At the interior end of the sporangium there is papilla.

Mode of Infection and Symptoms

The fungus mycelium enters into the leaves of host through stomata. The disease first appears in the form of small brownish necrotic patches at the tips of margins of the leaves. Under favourable environmental conditions the patches radially spreads to overlap the entire leaf surface. The infection soon spreads to petioles and stems, the entire tips of the plant fall over in rotten pulp and the disease becomes epiphytotic. Finally the tubers are affected and they may show dry or wet rot according to the prevailing conditions. In damp soils the rot which

first causes superficial browning or blackening of the tissues, penetrates deeper in the potatoes which turn brown or decay before harvest.

Structure

The mycelium is profusely branched and consists of a septate hyaline, coenocytic hyphae about 4μ-8μ in diameter. The septa may develop at some places at the time of reproductive organs formation. The hyphae lies in the intercellular spaces between the cells of host tissue. They bear the lateral outgrowths, the haustoria.

Disease Cycle

It may be reproductive either by asexual or sexual means.

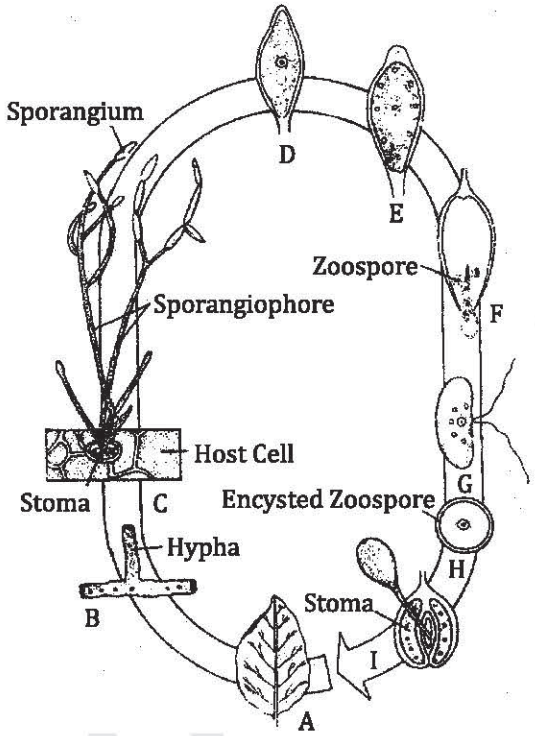


Fig. 2 : A to I Asexual Reproduction in *Phytophthora*

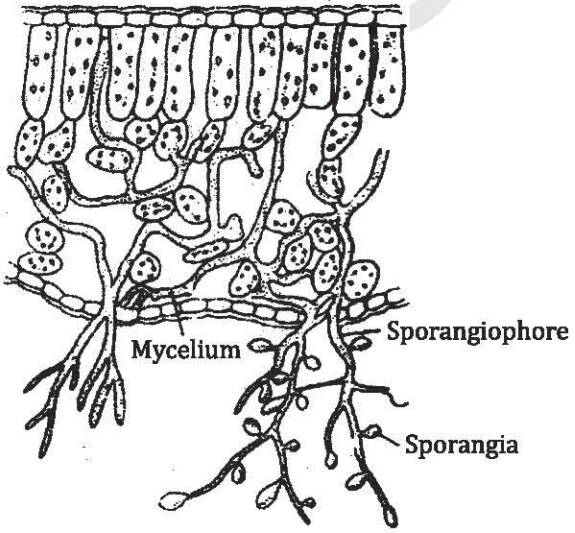


Fig. 3 : T.S. of infected leaf by *Phytophthora* showing Sporangiophore

Asexual Reproduction

This takes place by the formation of detachable conidiosporangia on the leaves of the host plant. The conidiosporangia are borne on sympodially branch distinct sporangiophores which come-out in small groups through the stomata. The sporangia or conidiosporangia are rounded or lemon shaped. At the anterior end of the sporangium there is a papilla. On maturation the protoplasm of the sporangium divides into several uninucleate protoplasts. Each protoplast metamorphoses into a biflagellate, uniform, uninucleate, vacuolate and naked zoospores. The mature sporangium burst at the papilla and the zoospores are liberated in the film of water. The zoospore swims for sometime with he help of their flagella, then come to rest and become, encysted. After sometime in favourable conditions the encysted zoospore germinates by producing a germ tube.

Sexual Reproduction

The sexual reproduction is of oogamous type and takes place with the help of definite male and female reproductive organs known as antheridium and oogonium respectively. The sex organs arise at the tip of short lateral hyphae. The tip of the young oogonial branches punctures the developing antheridium and comes out through it on the otherside where oogonium is cut off. The oognia are pear shaped to spherical smooth, hyaline yellowish or reddish brown in colour. The antheridium which is delimited from its stalk by a septum, is seen to form a funnel shaped collar around the base of oogonium. The young sex organs are multinucleate but at maturity the antheridium as well as oogonium both possess a single nucleus.

At the place of contact of antheridium and oogonium the wall dissolves, making a passage for male nucleus from the antheridium to the egg awaiting fertilization. The, fertilization has not

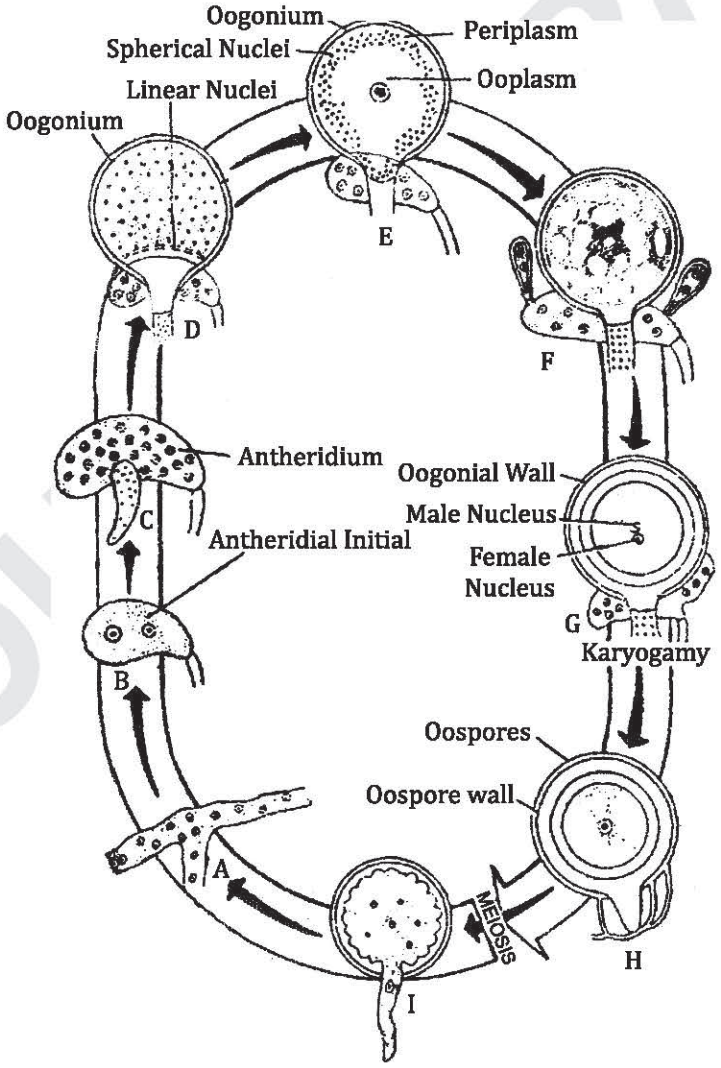


Fig. 4 : A to I. Sexual reproduction in *Phytophthora*

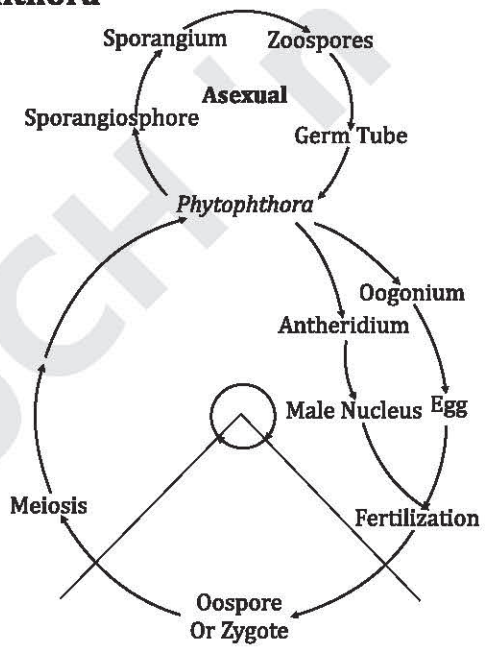
been actually observed in this species (*P. infestans*), but is believed to take place by a fertilization tube.

The thick walles oospore undergoes rest and then germinates probably by a germ tube, through actual gemination has not been seen. Probably the first division of the zygote nucleus is meiotic.

Phytophthora is responsible for number of serious diseases in the plants of great economic importance, as for example :

1.	*P. infestanes*	"Late blight" of potato
2.	*P. arecae*	"Seedling blight" of Areca nuts
3.	*P. colocasiae*	"Seedling blight" of Colocasia
4.	*P. parasitica*	"Seedling blight" of Castor
5.	*P. palmivora*	"Bud rot" of coconut palms
6.	*P. megasperma*	"Blight of cauliflower, tomato and cirtus"
7.	*P. cryptogea*	
8.	*P. crytophthoria*	

Life-Cycle of Phytophthora



Methods to Control the Disease

1. By spraying some fungicides such as bordeaux mixture and diethane.
2. By growing disease resistant varieties.
3. By the selection of health tubers for sowing purposes.
4. By proper manuring to increase resistance.
5. By obtaining the seed tubers from areas where disease does not occur.

6. By soil management through spraying of soil with 10 per cent to 20% H_2SO_4 and 5% $CuSO_4$ solution.
7. By improvement of Harvesting practise.

Q.2. Explain the black stem rust of wheat.

Ans.

Black Stem Rust of Wheat

Disease Black rust of wheat caused by *Puccinia graminis* fungus is present in every country through the world where wheat is grown. This disease is more damaging in moderately moist areas and in moist season in areas with low rainfall.

Symptoms : With the onset of the disease, elongated brown pustules or sori brust through out epidermis of host tissue.

Disease Cycle

Puccinia graminis (Black rust) is a wide spread rust of wild grasses and produces the condition known as 'red rust' or 'black rust'. This is a eufrom macrocyclic or long cycled rust *i.e.,* it completes its life history after passing through five type of spores. *Puccinia* is also heteroecious form of rust because it completes its life history on two hosts which alternates to each other. These hosts are *Triticum vulgare* (Wheat) and *Barbaris vulgaris* (Barberry). In Northern India, the rust does not appear before and thus the damage are less but however, in Southern India it appears as early as November causing enormous loss.

The life history of *Puccinia graminis* is divided into five stages which are based on the nature of the spores at a particular stages. These are follows :

1. **Uredospores :** In plains of India wheat is sown in October or November, but the infection becomes evident only in the month of February or March, when vertically elongate reddish brown or black pustules appear mostly on the stem or leaf. These pustules in reality are Uredosori containing uredospores. Uredosori develop from branched and separate mycelium which is divided into several multicellular hyphae. Each cell of hyphae is divided into several multicellular hyphae arrange themselves in clusters, each hypha is called as sporophore. These are situated below the epidermis. The apical cell of sporophore enlarges and divides into an upper spore initial and lower basal cell. The basal cell form stalk on elongation while spore cell from uredospores. Each spore is surrounded by an outer brownish wall expospore and the inner delicate endospore. It consists of two nuclei and cytoplasm also. On maturation of uredospores

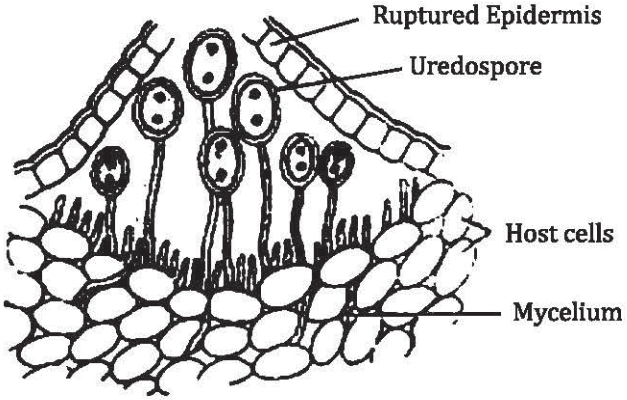
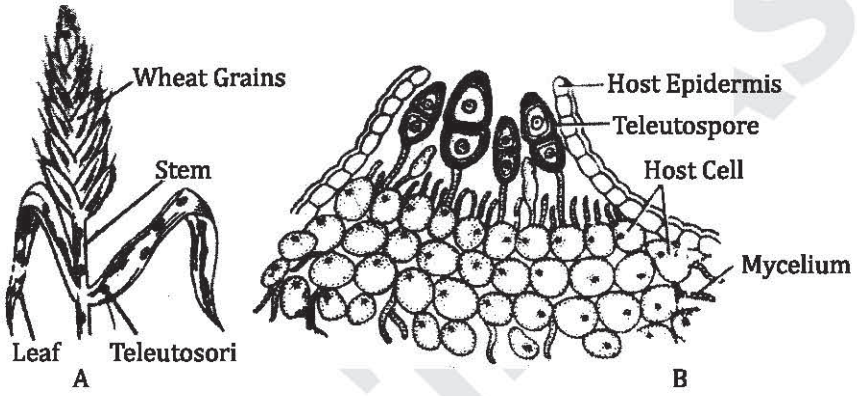


Fig. 1 : *Puccinia graminis* : Uredospore stage.

the epidermis of host bursts and they are liberated in air. Uredospores after dissemination, germinate in favourable conditions and from germ tubes which infect other healthy wheat plants. Thus, the rust disease get spread up through uredospores in the entire field of wheat.

2. **Teleutospores :** Later in the season, in April in the plains of Northern India, when the wheat grains are maturing, the uredia begin to produce a few teleutospores. As the season advances more and more teleutospores are formed. A pustule producing teleutospore is called as teleutospores or telium outwardly these appear as elongated



black coloured streaks on the stem leaf of wheat plants. The stage is called black stage or teleutospore stage of rust. Like uredospores teleutospores also develop from multicellular hyphae possessing dikaryotic cells. The teleutospore is bicelled and spindle shaped. It is covered by exospores and endospore. The dikaryotic nuclei fuse together in the teleutospore and form a dipolid nucleus.

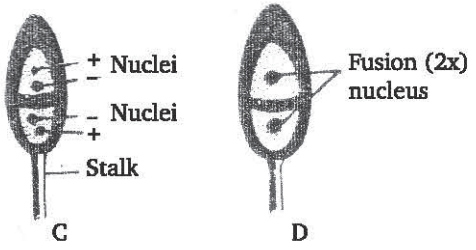


Fig. 2 : A, B, C, D : *Puccinia graminis* : Teleutospore

The teleutospore resist in unfavourable conditions. On maturation the teleutospores are shut out by the rupture of epidermis and are carried to long distance by wind.

3. **Basidiospore :** Teleutospore after liberation fall on soil and in the next spirng season these Teleutospore germinate into a hyphae branch called epibasidium or promycelium. The dipoid nucleus migrated into basidium and divides by a reduction division to form four haploid nuclei. The four haploid nuclei thus produced in each basidium come to lie at more or less equal distance. Septa appear between the nuclei dividing the basidium into four uninucleate haploid cells. From each cell arise a short sterigma, at the apex of which develops a basidiospore, in which passes the haploid nucleus. The basidiospore are small, unicellular, uninucleate haploid and [+] and the other negative (−) strain. The basidiospores are discharged by the water droplet method with force into the air. They are carried by wind to the leaves of the alternate host which is Barberry (*Barberis vulgaris*).

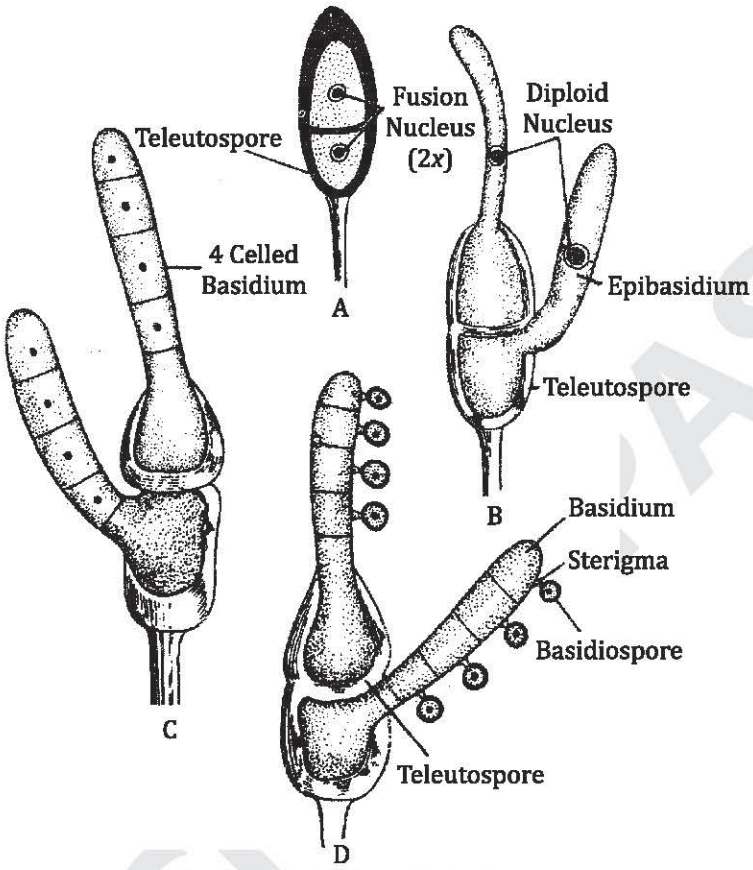


Fig. 3 : Germination of teleutospores

4. **Pycnidiosporess :** The basidiospores, which are unable to infact the wheat plant, after being disseminated fall on the leave of *Barberis vulgaris*. Each basidiospore on germination forms a primary hyhae which penetrates into the leaf cells and ultimately forms specific uninucleate monoploid branched mycelium. Since the fungus is heterothallic the basidiosproe are either of [+] or [–] strain. Few days after infection the monokaryotic mycelium result into the formation of pseudoparenchymatous mass of tissue, which forms the pycnidia or pycnidial or spermogonia. A few slender hyphae develop from this pycnidia which are uninucleate and are called as sporophore. The nucleus of sporophore divides and one daughter nucleus after each division move towards the apex and transforms in a pycnidiospore. Pycnidiospores are formed in chain. Each pycnidiospore is a circular or oval thin walled structure. By the rupture of epidermis the pycindiospores are liberated. The point of rupture is called as ostiole.

   A spermogonium or pycnidium is [+] or [–] according to the mycelium bearing it. It bears spermatophores and spermatia or pycnidiospore. The spermatia or pycnidia or pycnidiospore are the male sex organs and are extruded through the ostiole in a drop of a thick necter like fluid. From the inner side of spermogonial wall arise a large

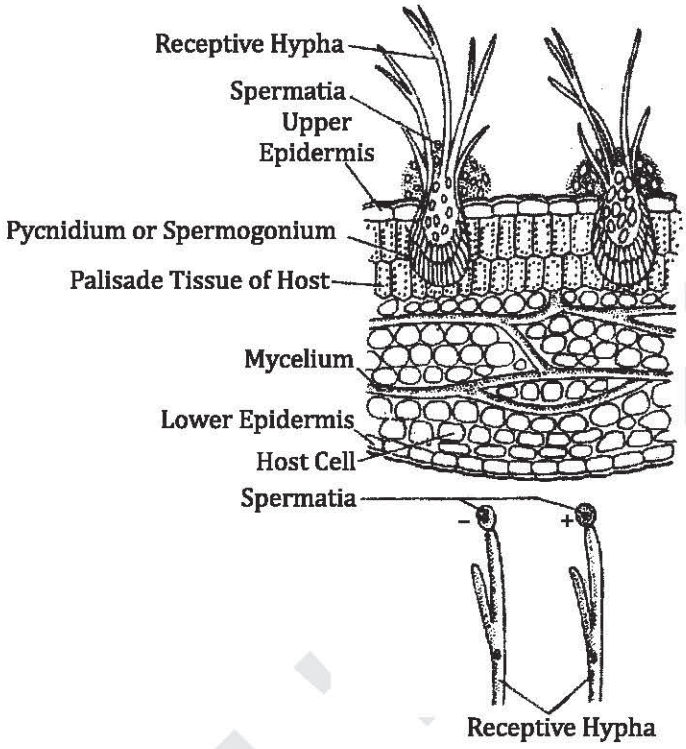


Fig. 4 : T.S. of infected Barberry leaf showing + and – spermogonium

number of hair like, sharp pointed periphysis. These project out through ostiole. A number of longer, cylindrical, and unbranched or slightly branch flexuous hyphae with blunt tips arises just beneath or among the periphysis. These are the ceptive hyphae or flexuous hyphae and represent the female sex organs. They protrude through the ostiole all the spermatia and receptive hyphae produced from a spermogonium bear the same genetic factor, + or – according to the genetic make up of the spermogonium. As mentioned already, since multiple infection by + and – spermogonia are usually found side by side on a barberry leaf. Spermatization takes place if + spermatia are transferred to – receptive hyphae or – spermatia of + receptive hyphae. In nature this takes place by the agency of flies or some other insects which are attracted by the scent and sweetness of the spermogonial exudate.

5. **Aecidiospores :** The monokaryotic mycelia which form pycinidiospores at the upper surface of leaf develop aecidial cups on lower surface. The primary mycelium collects in patches near the lower epidermis of leaf and is known as protoaecidium. Due to the stimulus of sexual act, the protoaecidium develop into a cupshaped aecidium or aecidium from the bottom of which arise closely packed parallel chain of aecidiospore or aeciospores on the side towards the lower epidermis. During the development of an aecium a basal layer of dikaryotic cells is differentiated in a protoacedium. As to now this change from uninucleate to binucleate condition is effected, is not very well understood. Most probably this is due to spermatisation, the spermatial nuclei

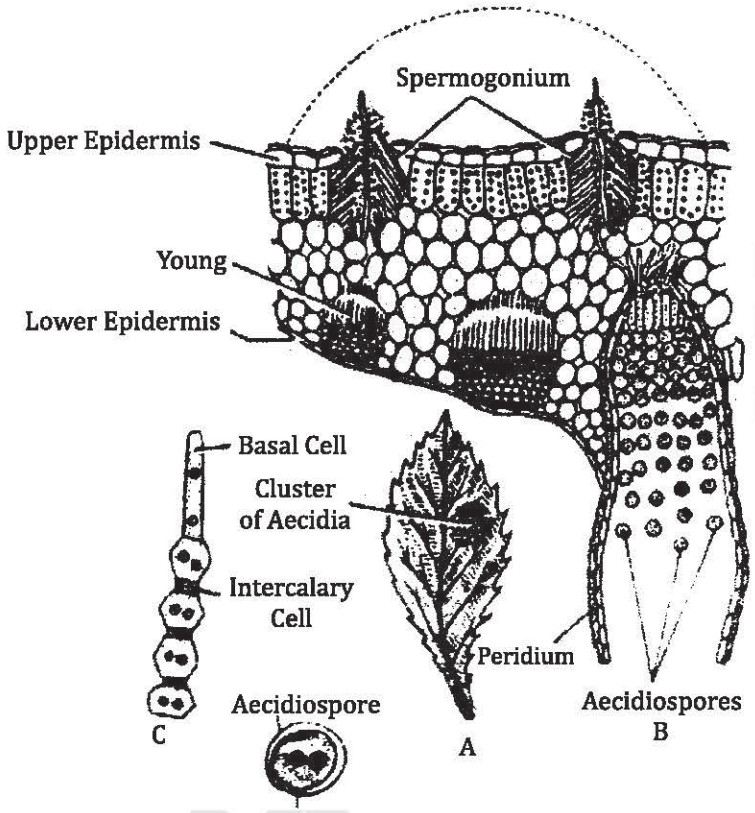
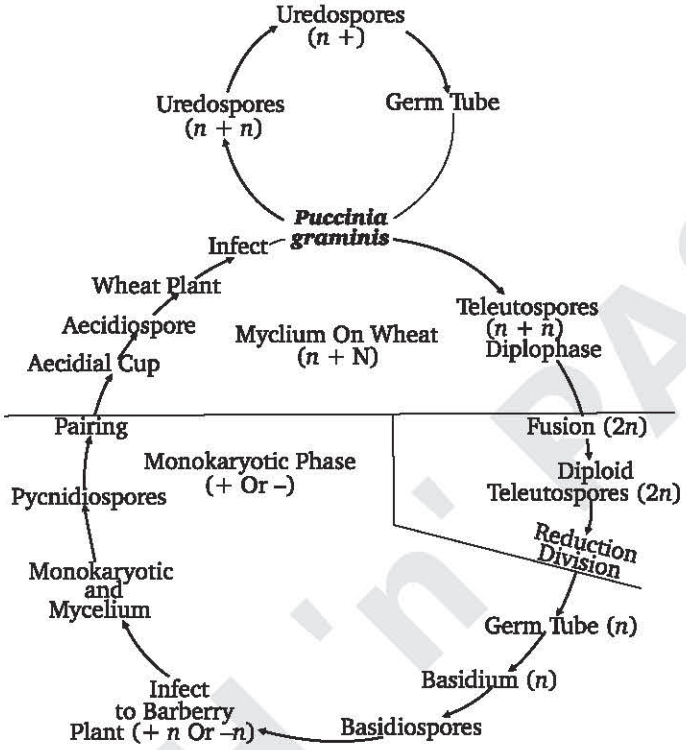


Fig. 5 : *Puccinia graminis* Aecidiospores.
A : Leaf of barberry with Aecidia.
B : Vertical section of barberry leaf showing a spermogonium and an aecidium.
C : A chain of aecidiospores.

travelling down the receptive hyphae migrate from cell to cell of the primary mycelium through spectral perforation, reach the basal cells of the protoaecedium and thus dikaryotise them. These dikaryotic cells multiply and form the hymenium of aecedial cup, while the subhymenium is formed of monokaryotic cells. A few sporophores emerge from dikaryotic hyphae, which form a chain of binucleate or dikaryotic spores called as aecidiospores. Aecidiospores possess sterile disjuncter cells in between them. The wall of aecidial cup is made up of sterile cells called peridium. On maturation of aecium, the spore chains push through the host epidermis and eventually through roof of the peridium; the spores are thus exposed and dispersed by wind. The aecidiospores are binucleate, subglobose to hexagonal, light orange yellow and with six germ pores.

As the aecidiospores cannot reinfect the barberry plant, so they are carried by air to plants and infect the wheat plants. On the wheat plant the germ tube of aecidiospores penetrate through stomata and gives site to dikaryotic myeclium from which uredospores arises. Thus, the life cycle of fungus is completed.

Life Cycle of *Puccinia graminis*



Method to Control Black Rust in India

The following measures may be adopted for controlling rust infection so as to avoid heavy loss of wheat crop :

1. To grow rust resistant varieties like N.P. 97, N.P. 728.
2. What cultivation in hills should be suspended for few years and oats may be cultivated in place of wheat for few years.
3. Destruction of Barberry bushes.
4. Avoid excessive use of nitrogenous manures, which promote excessive growth of rust fungi.
5. Good drainage should be secured.
6. Destruction of infect wheat plants to avoid its spread.
7. Dusting and spraying of sulphur compounds.
8. Early sown and early maturing varieties which suffer less than other should be encouraged.

Q.3. Write an essay of the Mosaic disease on Tobacco.

Ans. **Mosaic Disease on Tobacco**

This is the best known of all virus diseases of plants, and worldwide in distribution. This disease affects more than 150 genera of primarily herbaceous, dicotyledonous plants

including many vegetables (potato, tomato, cucurbits), flowers and weeds. There are serious losses in yield as well as quality of tobacco, tomato and some other crop plants. It is symptomless on apple and grape. TMV affects plants by damage of leaf, flower and fruit and causes stunting of the plant.

Symptoms

The symptoms include various degrees of chlorosis, curling, mottling, dwarfing, distortion, and blistering of leaves, dwarfing of the entire plant, dwarfing, distortion and discoloration of flowers, and in some plants even development of necrotic areas on leaf.

The most common symptom on tobacco is the appearance of mottled dark-green and light-green areas on leaves. The dark green areas are thicker and appear somewhat elevated in a blisterlike manner over the thinner, chlorotic, light, green areas. Stunting of young plants is common, and is accompanied by a slight downward curling and distortion of leaves, that may become narrow and elongated rather than normal oval shape. The petioles may become enlarged (puckered) with enlarged capitate hairs. Old leaves may not show symptoms, young ones develop typical symptoms.

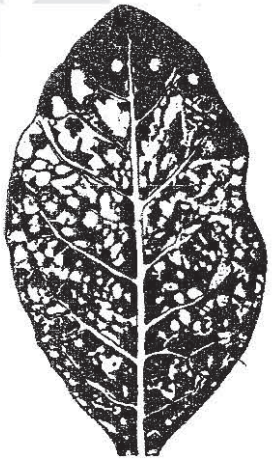


Fig. 1 : Tobacco leaf infected by TMV

Causal Organism

Tobacco Mosaic Virus (TMV) is rod shaped, 300 nm long by 15 nm in diameter. Protein (P) consists of approximately 2130 subunits and each subunit consists of 158 amino acids. The protein subtends are arranged in a helix.

The nucleic acid (HA) is single stranded RNA and consists of about 6400 nucleotides. The RNA strand also forms a helix parallel with that of protein and is located on the protein subunits NAD approx. 20 A out from the inner end of the protein subunits. The molecular weight of each virus particle is between 39 and 40 million molecular weight units.

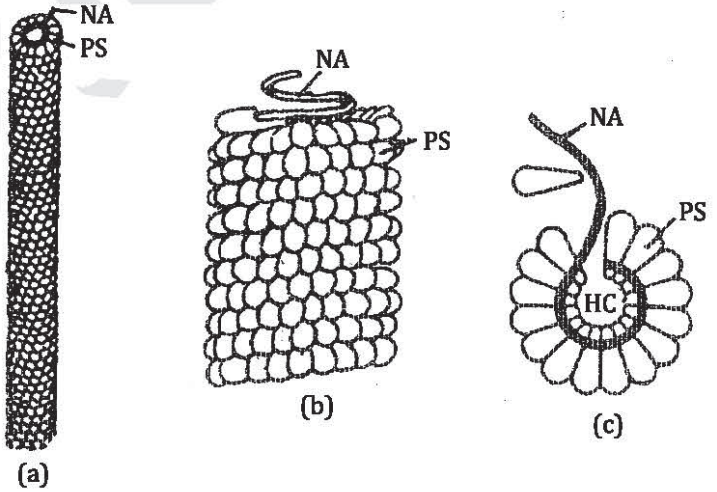


Fig. 2 : Tobacco mosaic virus (TMV) : (a) the rod, (b) side arrangement of protein subunits (PS) and nucleic acid (NA), (c) cross section view of the virus rod, HC-hollow core.

TMV is one of the most thermostable virus known, the thermnal inactivation point of the virus in undiluted plant juice being 93°C. However, in dried infected leaves, the virus retains infectivity even when heated at 120°C for 30 mts. Infected plant may contain up to 4 g of virus per litre of plant juice and the virus retains infectively even at dilutions of 1:1,000,000. In ordinary plant sap, the virus is inactivated in 4-6 weeks, whereas in sterile bacteria free sap the virus may survive for 5 years and in TMV infected leaves kept dry in the laboratory the virus remains infectious for more than 50 years. TMV is transmitted readily through mechanical sap, grafting and dodder. It is not transmitted by insects, except occasionally through their contaminated feet and jaw. The most common method of transmission of TMV in field and greenhouse is through hands of workers handling infected and healthy plants.

Disease Development

TMV survives in infected leaves and stalks in the soil, on surfaces of contaminated seeds, and on contaminated seedbed cloth, and in natural leaf and manufactured tobacco including cigarettes cigars etc. The virus initially infects wounded tissues of tobacco seedlings in seedbed or of transplants in the field. Then it spreads in the field throughout the season. TMV in all plants produces systematic infections, invading all parenchyma cells of plant. The virus moves from cell to cell through phloem. In the cytoplasm of cell TMV appears as crystalline aggregates and as amorphous bodies (x-bodies).

Control

1. Sanitation is the main method. Crop should not be grown at least for two years in seedbeds or fields where diseased crop was grown. Removal of diseased plants and of some solanaceous weeds haboring the virus early in the season helps in reduction and elimination of subsequent spread of the virus.
2. Chewing and smoking of tobacco during handling of tobacco and other susceptible plants should be avoided.
3. Workers in the field must wash hands with 3% trisodium phosphate or soap.
4. Equipment and instruments used in plantations must be sterilised.
5. TMV-resistant varieties of tobacco must be grown, though these may be of low quality.

Q.4. Describe the damping off of seedlings in detail.

Ans.

Damping off of Seedlings

Damping off disease of seedlings is widely distributed all over the world. It occurs in valleys and forest soils, in tropical and temperate climates, and in every greenhouse and nursery bed. The disease affects seeds, seedlings and older plants of almost all kinds of vegetables, flowers, cereals and many fruit and forest trees. In all cases maximum damage is done to the seed and seedling roots during germination either before or after emergence. Frequently seedlings in seedbeds are completely destroyed or they die soon after transplantation. Poor seed germination or poor emergence of seedlings is due to damping off infections in preemergence stage. Older plants are not killed, but they develop stem lesions or root rots, thus retarding their growth.

Symptoms

Seeds in infested soils fails to germinate. They become soft and mushy, turn brown, shrink and finally disintegrate (seed rot). This will result into poor stands or poor emergence. Poor stands, however, are also due to infections of seedlings before they emerge above the soil line. This is preemergence killing or preemergence damping off. The initial infection appears as a

slightly darkened, water-soaked spot. Infected area enlarges rapidly, the invoked cells collapse and the seedling is overruin by the fungus and dies shortly.

However, seedlings that have already emerged are usually attacked at the roots and sometimes at or below the soil line. The succulent tissues of seedlings are invaded and cells killed rapidly. The infected area becomes water soaked and discoloured and the cells soon callapse: The basal part of seedling stem is much thinner and softer than the upper part. Due to this invaded basal part can not support the upper part and the seedling falls over the soil. The fallen seedling is also invaded, that quickly withers and dies. This phase is called the post-emergence damping off. Infection of older plants is generally limited to roots which are damaged and killed (root rot). Soft fleshy organs as vegetables as cucurbits, green beans, potatoes, cabbage etc. are also attacked by these fungi during long wet periods in field, in storage and transit. They cause fruit rots.

Causal Organisms

Several fungi have been reported to be responsible for damping off of seedlings. These are species of *Rhizoctonia, Fusarium, Phytophthora* and *Pythium*. Of these, *Phythium* appears to be the most important. Several species of this genus are involved, *e.g., P. debaryanum, P. aphanidermatum, P. ultimum* but the effects of each of them on its hosts is usually similar to that of others.

The mycelium is white, slender, profusely branched with coenocytic hyphae. There are produced terminal and intercalary sporangia, which may be spherical, filamentous or of various shapes. They germinate to develop a vesicle in which there are formed zoospores. Released zoospores swim for sometime, encyst and infect the host tissue by germ tubes.

Sex organs-antheridia and oogonia develop at the ends of short hyphae. After fertilisation (plasmogamy through gametangial contact), thick-walled oospores develop. Oospores are resistant to extremes of temperatures and other adverse factors. They go under a period of rest and germinate in the next season. Each oospore produces a vesicle at the end of germ tube. Inside the vesicle zoospores are formed. Both sporangia and cospores germinate at low temperature of 10 -18°C to form zoospores, but directly into germ tubes at temperature above 18°C. The pathogans remain in waters, soil on dead plant and animal matter as saprophytes. The fungus enter the seeds by direct penetration of moistened, swollen tissues occurs through mechanical pressure and dissolution by enzymes. Pectinolytic enzymes breakdown the protoplast of invaded cells.

Control

1. Soil disinfection with chemicals, like formalin (1 part to 50 parts water); captan, thiram, blitox-50 etc. in a 0.2-0.5% suspension.
2. Soil sterilisation by stem or dry heat.
3. Seed protectants, which include several types of chemicals as phygon, agrosan GN, arasan, semesan, captan, ceresan, blittox-50 and others. These are applied to seed in dry or wet form.
4. Seed treatment followed by spraying of seedlings with ziram, chloranil, captan, soluble coppers etc.
5. Cultural practices, that include chiefly good drainage, improvement of soil aeration, check on excessive use of nitrate forms of fertilizers etc.

Q.5. Write an essay on chemical control of plant disease. Also write about the preparation method of Bardeaux mixture fungicide.

Ans.

Chemotherapy or Chemical Control of Plant Diseases

When the entry of the pathogen to the host plant seems difficult to check and the diseases is likely to appear through wind bone primary or secondary inoculum, the application of suitable chemicals are desirable to destroy the pathogen as early as it establishes on are host. The chemicals which are capable of killing the fungi are known as fungicides. Some fungicides who do not kill the fungi but simply inhibit fungal growth temporarely are called fungistal. Some other fungicides who inhibit spore production without affecting the growth of vegetative hyphae are called antisporulants. Fungicides which are only when applied piror to fungal infection are called protectant and those capable of erdicating the fungi and so curing the disease after infection are called therapeutants. Fungicides which remove pathogenic fungi from an infection count are called eradicants.

Fungicides are used in various forms such as suspensions or slurris, wettable powders, dusts, granules and emulsifiable concentrates etc. Spraying and dusting of fungicides are more common in practice.

There are also fungicides which kill a fungus after it has infected a host plant, sulphur and certain organic sulphur preparations such as Karathane belong to this class. They kill powdery mildews after infection.

Ideal or Good Fungicide

A good fungicide should not only be toxic to the parasite or inhibit the germination of its spore without causing phytotoxicity, but should have following qualities also :

1. High field performance.
2. Inherent fungitoxicity.
3. Availability of the active constituents.
4. Good coverage of host surface.
5. Low phytotoxicity.
6. Stability in storage.
7. Stability after dilution to spray strength.
8. Low toxicity to human beings and cattles.
9. Should stick to the surface after drying (tenacity).
10. Good wetting property.
11. Compatibility with pesticides, nematicides, herbicides, viricides and fertilizers.

Fungicides and their Methods of Applications

According to their chemical nature and formulations the fungicides may be divided into following categories :

1. **Spray materials :** Materials available in solution or emulsified form and wettable fungicides are used as spray which provide a protective covering over the surface.
2. **Dust materials :** Insoluble powders are dusted over the surface.
3. **Seed treatment materials :** Seed treatment with fungicides are done with dry powder dips or slurries. Seed treatment are done by two ways :

(i) **Seed disinfectants :** Which destroy the fungi associated with seed surface Externally seed borne pathogens can be controlled by seed disinfections, ceresan, panogen copper sulphate and copper carbonate arc fungicides used as seed disinfections.

(ii) **Seed prolectants :** Which disinfect the seed and stick to the seed surface and thus temporarely protecting the seedlings during emergence. Most of the seed protectants are organo-mercurial compounds.

4. **Soil treatment fungicides :** Fungicides to control soil borne diseases in certain cases are used as soil treatment. Although complete control of a soil borne disease is often not obtained fir expected by this procedure. Most of the chemicals applied to soil as fungicides are diffused through soil particle to be effective and such treatment can be feasible only in case of high value crop. Soil fungicides are usually applied as soil drenches or in furrows so as to reduce the cost of application.

   There are following methods of soil treatments :

   (i) Drenching of soil with solution or suspension.

   (ii) Soil fumigations such as chloropicrin, methyl bromide, pentachloronitrobenzene (PCNB), etc.

   (iii) Furrow application.

   (iv) Broadcasting of dusts, powders and granules.

5. **Treatment of tubers, bulbs and seed-materials :** Potato scab and black scurf diseases are controlled by treating the seed material in solutions of mercuric chloride, formaldehyde etc. by dip treatment. Tubers are also dip treated with mercury fungicides like aretan, emison etc. Gladiolus bulbs may be treated with Cersam to eradicate *Fusarium* spp. Sugarcane seed sets are dip treated with emisan, aretan, abavit to avoid red-rot disease.

6. **Control of post-harvest diseases of Fruits and Vegetables :** Fruits and vegetables are also affected by various diseased during storage. Storage decay of grapes and citrus may be controlled by use on Nitrogen and sulphur di-oxide. Penicillium decay of citrus are controlled by Borax dip 6-8% at 30.0°C for 4-5 minutes.

7. **Treatment of tree wounds or tree surgery :** Cut-wounds are resulted from pruning or by cutting of diseased part of a plant. Such wounds may require wound dressing so as to avoid infection of cut parts. Therefore cut wounds are sterilized first by swabbing of 0.5 to 1.0 p.c. of sodium hypochloride or 70 p.c. ethyl alchohal or with 1 : 1000 solution of mercuric chloride. Wound parts so sterilized are dressed with mixture of lanolin 10 parts, rosin 2 parts and gum 2 parts. Bordeaux paste or Chaubattia paste are also used as wound dressing.

8. **Disinfection of ware houses :** Floors and walls of warehouses should be disinfected with copper sulphate solution (1 lb in 5 gallon of water or with chloropicrin tear gas @ 1 lb/1000 cubic feet).

9. **Control of insect-vectors :** Most of the virus diseases are transmitted by insects known as vectors. Such insect vectors should be controlled by use of suitable insecticides or through biological control in order to avoid transmission of viral diseases.

Bordeaux Mixture

Bordeaux mixture was first used by Profesor Millardet in 1882 who demonstrated its effectiveness on downy mildew of grapes. He was Professor at University of Bordeaux in France.

Requirements : (1) Copper sulphate or blue stone ($CuSO_4 \cdot 5H_2O$).
(2) Quick lime, (3) Water

Formulae :

1. 4 : 4 : 50 = 08%
   (4 lbs copper sulphate + 4 lbs quick line + 50 gallons of water).
2. 5 : 5 : 50 = 1.0%
   (5 lbs copper sulphate + 5 lbs quick line + 50 gallons of water).
3. 6 : 6 : 50 = 1.2%
   (6 lbs copper sulphate + 6 lbs quick line + 50 gallons of water).

Now a days formula 5 : 5 : 50 are commonly used for disease control.

Method of Preparation of Bordeaux Mixture

Preparation of 5:5:50 Bordeaux Mixture

Copper sulphate and lime are separately added in sufficient quantity of water (25 gallons each). These two solutions are then mixed simultaneously into a third container by agitating thoroughly. The bordeaux mixture is now ready for use. The containers used for its preparation should be such which do not react with copper sulphate. Wooden or earthem vessels are suitable for this purpose. After preparation the finished mixture must be tested for free copper with the help of a clean iron blade. Iron blade is dipped for sometime in the mixture and observed if the copper deposite on its blade. Following chemical reaction takes place in bordeaux mixture.

$$CuSO_4 + Ca(OH)_2 = Cu(OH)_2 = Cu(OH_2) + CaSO_4$$

Prepared bordeaux mixture should be used as early as possible so as to avoid loss in effectiveness. Agromon Limited, Bangalore has presented Instant Bordeaux in the market which can easily be prepared before use. Two kilograms of Inston Bordeaux is dissolve in 400 litres of water to prepare 1 percent (5 : 5 : 50) bordeaux mixture.

Q.6. Describe the concept of integrated disease control.

Ans.

Integrated Disease Control

The concept of integrated disease control was developed by Stern *et. al,* in 1959. Since then the concept has expanded beyond the original vision to embrace not only chemical and biological control but a wide variety of additional approaches to pest suppression. In fact, integrated pest management (IPM) is an effective and environmentally sensitive approach to pest management. It takes the advantage of all appropriate pest management options including judicious use of pesticides. Although some of the principles of integrated pest management are similar to those that apply to organic farming, but the latter limits the use of pesticides to those that are produced from natural sources as opposed to synthetic chemicals.

Farmers practicing IPM, follow a four-tiered approach. The four steps are :

1. **Set Action Thresholds :** Before taking any pest control action, IPM first sets an action threshold, a point at which pest population or environmental conditions indicate that pest control action must be taken. Sighting a single pest does not always mean control

is needed. The level at which pests will become an economic threat is critical to guide future pest control decisions.

2. **Monitor and Identify Pests :** Not all insects, weeds and other living organisms require control. Many organisms are not at all harmful and some are even beneficial. IPM programmes work to monitor for pests and identify them accurately, so that appropriate control decisions can be made in conjunction with action threshold. Such monitoring and identification removes the possibility of using pesticides when they are not really needed or that the wrong kind of pesticides will be used.
3. **Prevention :** As a first line of pest control. IPM programmes work to manage the crop, lawn or indoor space to prevent pests from becoming a threat. In an agricultural crop, this may mean using cultural methods, such as rotating between different crops, selecting pest-resistant varieties, and planting pestfree root stock. These control methods can be very effective and cost-efficient. Prevention presents little or no risk to people or the environment.
4. **Control :** Once action threshold monitoring and identification indicate that pest control measures are required, and preventive methods are no longer effective. IPM programmes then evaluate the proper control method, both for its effectiveness and risk. Effective and relatively less risky pest controls are chosen first. These may include highly targeted chemicals, such as pheromones to disrupt pest mating, or mechanical control, such as trapping or weeding. However, if further monitoring, identifications and action thresholds indicate that less risky controls are not working, then additional pest control methods would be employed. These may include targeted spraying of pesticides. Broadcast spraying of non-specific pesticides is the last resort.

   Thus IPM system includes following elements :

   (i) Identification of pests and their natural enemies.

   (ii) Continuous monitoring sampling and assessment of pests and natural enemy population.

   (iii) Determining the pest population levels that can be tolerated based on aesthetic, economic and health concerns, and setting action threshold where pest populations or environmental condition warrant remedial action.

   (iv) Preventing pest population through improved sanitation, management of waste, addition of physical barriers and modification of habitats that attract or harbor pests.

   (v) Relying on nontoxic, biological, cultural or mechanical pest management methods or on the use of natural control agents, as far as possible.

   (vi) If use of chemical pesticides is inevitable, preference should be given to the products that are the least harmful to human health and the environment.

❑

UNIT-VIII

Applied Microbiology

SECTION-A VERY SHORT ANSWER TYPE QUESTIONS

Q.1. Which organisms are found in the limnetic zone with sufficient oxygen?

Ans. Microorganisms of limnetic zone are pseudomonads, such as *Cytophaga, Caulobacter* and *Hyphomicrobium.*

Q.2. What does the yellow precipitate in streams polluted with coal mining wastes indicate?

Ans. Yellow precipitate indicates formation of iron hydroxides which are formed due to low pH.

Q.3. What does MPN indicate?

Ans. MPN is most probable number of *Coliform* bacteria in a water sample.

Q.4. Which microorganism is used as an indicator for testing the purity of water against contamination of faecal matter?

Ans. *Escherichia coli* is used as an indicator for testing the purity of water.

Q.5. What are the diseases caused by some strains of coliform bacteria?

Ans. Diarrhoea and opportunistic urinary infection are diseases caused by some strains of *coliform bacteria.*

Q.6. What is sherry?

Ans. It is like natural wine with additional surface growth floor at 27°C. The alcohol content is 18-21%. The microorganisms used are *S. cerevisiae, S. beticus and S. bayanus.* It is S. beticus that grows on the surface as a film producing aldehydes from alcohol.

Q.7. What is the raw material and which is the organism used to make wine?

Ans. Wine is prepared from grapes using S. cerevisiae and contains 14% or less alcohol.

Q.8. What is beer? How is it prepared?

Ans. Beer is made using yeast *Saccharomyces carbsbergensis.* The germinated barley seeds are ground to release starches and amylase enzyme. The extract is called malt. The enzymes in malt hydrolyze starch to fermentable sugars. The liquid, called wort, is sterilized. The flowers of Hops are added for flavour. Yeast is then added and incubated from 37°-49°C. In the presence of yeast sugar gets converted into alcohol and CO_2. The alcohol is 4 per cent. The yeast grows on the bottom of the fermenting vessel.

Q.9. Give the basic steps in making red wine.

Ans. The basic steps in making red wine are as follows : (i) Grapes are picked. (ii) Crushed and destemmed. (iii) Sulfide is added to kill undesirable microorganisms (iv) Inoculum

(yeast) is added (v) Fermentation is carried out (vi) The resultant is pressed to separate solids froni wine. (vii) Wine is clarified in settling vats. (viii) Wine is filtered and stored for aging.

Q.10. What is sparkling wine (champagne)?

Ans. It is like natural wine using yeast S, cerevisiae with secondary fermentation in a bottle. 2.5% sugar and yeast are added to bottled wine. It is incubated at 15°C and the bottle is inverted to collect the yeast in the neck of the bottle. In secondary fermentation yeast produces CO_2 and yeast settles quickly.

Q.11. What is rum or Jamaica?

Ans. It is prepared from cane molasses inoculated froni previous fermentations. Wild yeast is used for the purpose. Oak aging adds colour and is finally distilled to concentrate. Alcohol content is 50-55 per cent.

Q.12. What is brandy?

Ans. Fruits are crushed and added to *S. cerevisiae* and distilled for concentration. It contains 40-43 per cent alcohol.

Q.13. What is red wine?

Ans. Forred wine black grapes are used and pressing does not occur until after fermentation. It is prepared like natural wine incubated at 25°C. Aged in Oak for 3-5 years and in bottle for 5-15 years.

Q.14. What is white wine?

Ans. It is almost white to colourless. For white wine either black or white grapes are used, the juice being extracted from the pulp by pressing before fermentation. White wine is incubated at 10-15°C and aged for 2-3 years in bottles.

SECTION-B SHORT ANSWER TYPE QUESTIONS

Q.1. What is the role of microbes in food and dairy industries?

Ans. **Microbes in Food and Dairy Industries**

The role of microbes in food and dairy industries is as follows :

1. **Molds :** Food microbiology not only includes the study of these microbes which provide food due to their high protein value (such as yeast), but on the otherhand, those microbes also which use our food supply as a source of nutrient for their growth and result in deterioration of the food by increasing their numbers, utilizing nutrients, producing enzymatic changes and contributing off flavours by means of break down products. Microorganisms, such as molds (*Mucor, Rhizopus, Botrytis, Aspergillus, Penicillium*, etc.) lead to deterioration of food. Special molds are useful in the manufacture of certain foods or ingredients of foods. Some cheese are mold ripened e.g. blue, Roquefort, camembert, etc, molds are also used in production of oriental foods, e.g. soy sauce, miso, sonti, etc., used as food or feed and are involved in making enzymes such as amylase for bread making or citric acid used is soft drinks. Some molds are harmful (*Aspergillus flavus*) and some molds (*A. parasiticus*) produce toxic metabolites (mycotoxins).

2. **Yeasts :** Yeast refers to those fungi which are generally not filamentous but unicellular and oval or spherical, reproduce by budding or fission and may be useful or harmful in food. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar and surface ripened cheese and yeasts are grown for enzymes and for food. Yeasts are undesirable when spoil fruit juices, syrups, molasses, jam, pickles, wine, beer and other foods. Example of some of the genera are : *Saccharomyces, Schizosaccharomyces, Candida, Kluyveromyces, Zygosaccharomyces, Pichia, Hansenula, Dehammyces, Honseniaspora*, etc.
3. **Bacteria :** Bacteria in a food may be of special significance. Pigmented bacteria cause changes in colour on the surfaces of foods, form film over the surfaces of liquid food, etc. which result in undesirable cloudiness or sediment. Some genera, such as *Acetobacter* oxidises ethyl alcohol to acetic acid, *Aeromonas*, a facultative anaerobe also pathogenic not only to human beings but to fish, frogs and other mammals.

Q.2. Write a short note on riboflavin vitamin.

Ans. **Riboflavin (Vitamin B_2)**

Kuhn, Gyorgy and **Wagner Jauregg** in 1933 isolated riboflavin (also called lactoflavin) from whey of milk where it is present in free riboflavin form. It is also present in other foods (liver, heart, kidney or eggs) as flavoproteins which contain the prosthetic group FMN (flavin mononucleotide or FAD (flavin adenine dinucleotide). Several microorganisms namely, *Clostridium Acetobutylicum, Mycobacterium smegmatis, Myco candida riboflavina, Candida flareri, Eremothecium ashbyii and Ashbya gossypii* are used in commercial production and has got an ability to resist against riboflavin accumulation. Riboflavin is produced also by chemical synthesis but biotransformation of glucose to D-ribose by mutants of Bacillus pumilus and subsequent chemical conversion to riboflavin produced 50% of world wide production.

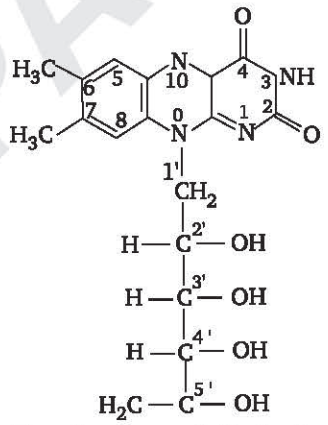


Fig. : Structure of riboflavin

It is an alloxazine derivative which consists of a pteridine ring condensed to a benzene ring. The side chain consists of a C_5-polyhydroxy group, a derivative of ribitol.

Riboflavin is produced in the following steps :

1. **Media preparation and biosynthesis of Riboflavin :** For Riboflavin production, basic medium consists of corn steep liquor 2.25%, commercial peptone 3.5%, soybean oil 4.5% but it can be supplemented further by addition of different peptones, glycine, distiller's solubles, or yeast extract. The glucose and inositol increase the production of riboflavin. The medium should be kept at 26°-28°C at pH 6.8 for 4-5 days incubation. After inoculation the submerged growth of Ashbya gossypi is supported by insufficient air supply. The excess air inhibits mycelial production and reduces the riboflavin yield. The fermentation progresses through three phases.
2. **First phase :** In this phase, rapid growth occurs with small quantity of riboflavin production. The utilization of glucose occurs resulting into decrease in pH due to accumulation of pyruvate. By the end of this phase, the glucose is exhausted and growth ceases.

3. **Second phase :** Sporulation occurs in this phase. The pyruvate decreases in concentration. Ammonia accumulates because of an increase in deaminase activity. The pH reaches towards alkalinity.
4. **Third phase :** There is a rapid synthesis of cell-bound riboflavin (FMN and FAD). This phase is accompanied by rapid increase in catalase activity subsequently cytochromes disappear.

As the fermentation completes, the autolysis takes place which releases free riboflavin into the medium as well as retained in the nucleotide form. It is also observed that certain purines also stimulate riboflavin production without simultaneous growth stimulation.

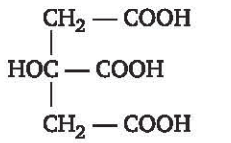
The riboflavin is present both in solution and bound to the mycelium in the fermentation broth. The bound vitamin is released from the cells by heat treatment (1h, 120°C) and the mycelium is separated and discarded. The riboflavin is then further purified. The crystalline riboflavin preparation of high purity have been produced using *Saccharomyces* fermentation with acetate a sole C source.

Uses : It is essential for the growth and reproduction of both humans and animals and thus, it often is recommended as a feed additives for the animal nutrition. The riboflavin deficiency in rats causes stunted growth, dermatifis and eye damage. Ariboflavinosis is a disease in humans caused by riboflavin deficiency.

Q.3. Describe about the production of citric acid.

Ans.

Production of Citric Acid



Citric acid

(2-hydroxy-1, 2, 3 propane tricarboxylic acid)

Citric acid was first produced commercially by **John** and **Edmund Sturage** company in UK in the year 1826. **Scheele** (1789) reported the isolation and crystallization of the four constituents of lemon juice. **Grimoux** and **Adams** (1880) synthesized citric acid from glycerol. **Wehmner** (1893) observed the occurrence of citric acid as a microbial product by using *Penicillium* and *Citromyces*. It was **Millard** (1922) who recorded accumulation of citric acid in culture of Aspergillus niger under condition of nutrition deficiency. Meanwhile, Currie (1917) reported better yield while using *A. niger*. In 1923, **Pfizer** began operating fermentation based process in USA.

It can be synthesized by the process of fermentation which is given as follows :

Fermentation

Aspergillus niger has been the choice for the production of this primary metabolite citric acid for several decades. A large number of other microorganisms (fungi and yeast) such as Aspergillus clavatus, A. wentii, Penicillium luteum, P. citrinum, Mucorpyriforms, Candida lipolytica, C. oleophila, C. guillermondis, Hensenula spp. Torulopsisspp., Pichiaspp., Debaromyces daussenii, etc. have also been used for citric acid production in industries. The advantages of using yeast, rather than A. niger are the possibility of using very high initial sugar concentration together with a much faster fermentations. This combination gives a high productivity run to which must be added the reported insensitivity of the fermentation to variations in the heavy metal content of the crude carbohydrates.

From 1965 onwards, yeasts are used for citric acid production using carbohydrate and *n*-alkanes. In all the processes, a variety of carbohydrates such as beet molasses, cane molasses, sucrose, commercial glucose, starch hydrolysate, etc. used in fermentation medium. The starchy raw material is diluted to obtain 20-25% sugar concentration and mixed with a

nitrogen source (ammonium salts or urea) and other salts. The pH of the medium is kept around 5 when molasses as used and at pH 3 when sucrose used. The fermentation is carried out by any of the processes :

1. **Koji process or solid state fermentation :** It is a Japanese process in which special strains of *Aspergillus niger* are used with the solid substrate such as sweet potato starch.
2. **Liquid surface culture process :** In this case, *A. niger* floats on the surface of a solution.
3. **Submerged fermentation process :** It is the process in which the fungal mycelium grows throughout a solution in a deep tank.

Q.4. Write a short note on production of Mycorrhizal biofertilizer.

Ans.

Production of Mycorrhizal Biofertilizer

Methods of inoculum production of mycorrhizal fungi differ with respects to their nature, depending upon types i.e., ectomycorrhizal or endomycorrhizal.

1. **Ectomycorrhizal Fungi :** In this case, the basidiospores, chopped sporocarps, sclerotia, pure mycelial culture, fragmented mycorrhizal roots or soil from mycorhizosphere region can be used as inoculum. The inoculum is mixed with nursery soil and seeds are sown thereafter.

   Institute for Mycorrhizal Research and Development, USA and Abbot Laboratories, USA have developed a mycelial inoculum of *Pisolithus tinctorius* in a mycelial vermiculite-peat moss substrate with trade name 'MycoRhiz' which is commercially available on large quantities.

2. **VA Mycorrhizal Fungi :** VA mycorrhiza can be produced on a large scale by pot culture technique. This requires the host plant mycorrhizal fungi and natural soil. The host plants which support large scale production of inoculum are sudan grass, strawberry, *Sorghum*, maize, onion, *Citrus*, etc. The starter inoculum of VAM can be isolated from soil by wet sieving and decantation technique (Gerdeman and Nicolson, 1966). VAM spores are surface sterilised and brought to the pot culture. Commonly used pot substrates are sand : soil (1:1, w/w) with a little amount of moisture.

There are two methods of using the inoculum : (a) using a dried spore-root-soil to plants by placing the inoculum several centimetre below the seeds or seedlings, (b) using a mixture of soil-roots and spores in soil pellets and spores are adhered to seed surface with adhesive.

Commercially available pot culture of VA mycorrhizal hosts grown under aseptic conditions can provide effective inoculum. Various types of VAM inocula are currently produced by Native Plants Inc (NPI), Salt Lake City. In India, Tata Energy Research Institute (TERI), New Delhi and Forest Research Institute, Dehradun have established mycorrhizae banks. Inocula of these can be procured as needed and used in horticulture and forestry programmes.

Q.5. What is biopesticide? Explain in brief.

Ans.

Biopesticide-*Pseudomonas*

Pseudomonas aeruginosa is among the most frequently described pathogen causing disease in insects. It is still unknown that certain strains pathogenic to insects by feeding differ from those that are potentially pathogenic to man. *P. aeruginosa* that produces toxic enzymes, has also been used as a model organism to study the mechanism of insect pathogenicity and

immunity. Non-fluorescent pseuctorrionads isolated from insects include *Pseudomonas alcaligens, P. cepacia, P. maltophila and P. acidovorans. Aeromonads* occur in laboratories devoted to the culture of insects in aquatic habitat. Some aeromonads such as *Aeromonas hydrophila* and *A. formicans* are pathogenic to insects.

Pseudomonas cepacia is known to be a versatile bacterium of soil (Sinsabaugh and Howard, 1975), as a plant pathogen (Burkhalder, 1950) and a human pathogen (Ederer and Matsen, 1972) as well as a broad spectrum antagonist to plant pathogens through the production of various types of antibiotics such as pyrrolnitrin (Janisiewicz and Roitman, 1988). *P. cepacia* is a Gram-negative bacterium that has been reported to produce siderophores. This bacterium also acts as plant growth promoting rhizobacteria (PGPR). Suppression of plant diseases may involve secretion of siderophores or antibiotics and/or aggressive root colonization by organisms that displace or exclude deleterious rhizosphere microorganisms. *Pseudomonas fluorescens* is one of the most important biological control agents of many plant diseases causing organisms. These are also common PGPR that secrete siderophores. Seed inoculation with these organisms helps in inducing growth and suppression of diseases. It produces fluorescent siderophores called pyoverdine or pseudobactrin which is characteristic of the fluorescent pseudomonads. The siderophores are low molecular-mass, water soluble, high affinity Fe(III) chelators. Siderophores are secreted under iron-limiting conditions as a means to secure available iron present at low concentration in soil. The ability of certain pseudomonads to utilize a wide range of ferric siderophores as a source of metabolic iron may contribute to their competitiveness and survival in the soil.

Q.6. Write a short note on bacteriological analysis of water.

Ans.

Bacteriological Analysis of Water

There are several methods to detect bacterial contamination of water. The chief objective is to identify coliform organisms as *Escherichia coli*. Their presence indicates that water contains faecal pollutants and is unsafe for consumption. The standard methods used are as follows :

1. **Membrane Filter Technique :** It may be used in the field also. A special collecting bottle is held against the water current and a 100 ml sample is taken. The water is filtered through a membrane filter and the filter pad is then transferred to a plate of bacteriological medium. Bacteria trapped in the filter will form colonies which may be counted.
2. **Standard Plate Count (SPC) :** Water sample is diluted in sterile buffer. Measured amounts are pipetted into Petri dishes. Agar medium is added and plates incubated. Colony counts are made and multiplied by the reciprocal dilution factor to have total bacteria per ml, water. This method is similar to that used for milk.
3. **Most Probable Number (MPN) :** In this procedure, water in 1.0 ml, 1 ml and 0-1 ml amounts is inoculated into lactose broth tubes. The tubes are incubated and coliform organisms may be identified by their production of gas from the lactose. By referring to an MPN table (Table), a statistical range of the number of coliform may be determined by observing how many broth tubes showed gas.

This method does not detect total number of bacteria in the water nor it locates noncoliforms like *Salmonella*. However, it indicates the presence and quantity of coliforms.

Q.7. Define the term 'bioremediation' in brief.

Ans.

Bioremediation

Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives. Biological treatment is a similar approach used to treat wastes including wastewater, industrial waste and solid waste.

Most bioremediation processes involve oxidation reduction reactions where either an electrón acceptor (commonly oxygen) is added to stimulate oxidation of a reduced pollutant (e.g. hydrocarbons) or an electron donor (commonly an organic substrate) is added ten reduce oxidized pollutants (nitrate, perchlorate, oxidized metals, chlorinated solvents, explosives and propellants). In both these approaches, additional nutrients, vitamins, minerals, and pH buffers may be added to optimize conditions for the microorganisms. In some cases, specialized microbial cultures are added (bioaugmentation) to further enhance biodegradation. Some examples of bioremediation related technologies are phytoremediation, mycoremediation, bioventing, bioleaching, landtarrning, bioreactor, composting, bioauqmentation, rhizofiltration and biostimulation.

Most bioremediation processes involve oxidation-reduction (Redox) reactions where a chemical species donates an electron (electron donor) to a different species that accepts the electron (electron acceptor). During this process, the electron donor is said to be oxidized while the electron acceptor is reduced. Common electron acceptors in bioremediation processes include oxygen, nitrate, manganese (III and IV), iron (III), sulfate, carbon dioxide and some pollutants (chlorinated solvents, explosives, oxidized metals and radionuclides). Electron donors include sugars, fats, alcohols, natural organic material, fuel hydrocarbons and a variety of reduced organic pollutants.

Q.8. Write about the biodegradation of pollutant.

Ans.

Biodegradation of Pollutant

Pollution : Pollution is the introduction of contaminants into the natural environment that cause adverse change.

Pollutants, can take the form of chemical substances or energy, such as noise, heat or light. Can be either foreign substances/energies or naturally occurring contaminants.

Air pollution : The release of chemicals and particulates into the atmosphere. Common gaseous pollutants; carbon monoxide, sulfur dioxide, chlorofluorocarbons (CFCs) and nitrogen oxides produced by industry and motor vehicles. Particulate matter, or fine dust is characterized by their micrometre size.

Light pollution : Includes light trespass, over-illumination and astronomical interference.

Littering : The criminal throwing of inappropriate man-made objects, unremoved, onto public and private properties.

Noise pollution : It encompasses roadway noise, aircraft noise, industrial noise as well as high-intensity sonar.

Soil contamination occurs when chemicals are released by spill or underground leakage. Among the most significant soil contaminants are hydrocarbons, heavy metals, herbicides, pesticides, etc.

Radioactive contamination, resulting from 20th century activities in atomic physics, such as nuclear power generation and nuclear weapons research, manufacture and deployment. (See alpha emitters and actinides in the environment.)

Thermal pollution, is a temperature change in natural water bodies caused by human influence, such as use of water as coolant in a power plant.

Visual pollution, refers to the presence of overhead power lines, motorway billboards, scarred landforms (as from strip mining), open storage of trash, municipal solid waste or space debris.

Water pollution, by the discharge of wastewater from commercial and industrial waste; Domestic sewage, and Chemical contaminants, such as chlorine, from treated sewage; Waste and contaminants into surface runoff flowing to surface waters (including urban runoff and agricultural runoff, which may contain chemical fertilizers and pesticides); waste disposal and leaching into groundwater; eutrophication and littering.

1. Wastewater from commercial and industrial waste
2. Domestic sewage
3. Chiorine, from treated sewage
4. Urban runoff
5. Overview of main health effects on humans

Pollutant Degradation

1. Chemically
2. Physically-Evaporate-Temperature Destroy
3. Mechanically-Disperse-Submerged, etc.
4. Biologically-Utilization as nutrient - Utilization as food-Biodegradation.

Biodegradation : Degradation of pollutants by microbes by using the materials as energy sources. Biodegradable simply means to be consumed by microorganisms and return to compounds found in nature.

Bioremediation associated with environmentally friendly products that are capable of decomposing back into natural elements.

Biosurfactant, an extracellular surfactant secreted by micro-organisms, enhances the biodegradation process. Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds.

Including hydrocarbons (e.g., oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides, pesticides, and metals. Decomposition of biodegradable substances may include both biological and abiotic steps.

In situ bioremediation involves treating the contaminated material at the site, while ex-situ involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies are phytoremediation, bioventin Beaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

SECTION-C LONG ANSWER TYPE QUESTIONS

Q.1. Write an essay on the production of antibiotics.

Ans.

Production of Antibiotics

Antibiotics (Fr. *anti* = against, *bios* = life) are chemical substances secreted by some microorganisms which inhibit the growth and development of other microbes. Most of them are produced by actinoycetes, specially the genus *Streptomyces* and filamentous fungi.

The study of antibiotics began by the discovery of penicillin in 1929, when Alexander Fleming proved that the filtrate of a broth culture of Penicillun notarum has antibacterial properties in relation to Gram-positive bacteria. In 1940, E. Chain and H. Florey obtained a relatively stable preparation of penicillin. **Clutterbuck** (1932) studied the chemistry of penicillin and observed that it can be extracted from organic acid solvents from aqueous solution of low pH, and it was heat labile. But its activity was lost during evaporation of solution to dryness. By keeping low temperature during extraction, it was used to demonstrate its curative properties (Abraham et al. 1941). **Florey** and **Heatley** (1941) shifted from (U.K.) to USA during world war aid form the American Govt. By the time American troops entered in France in 1944, sufficient amount of penicillin was available for saving the wounded soldiers.

Penicillins

Penicillins, a group of several penicillins, differ from one another in the side chain attached to its amino group. Most of these penicillins are 6-aminopenicillanic acid derivatives and all have β-lactam ring which is responsible for the antibiotic activity. Pencillin acts against Gram-positive bacteria and inhibit their cell wall synthesis. These are non-toxic to mammals except for certain allergic reactions. More than 100 penicillins have been synthesized so far.

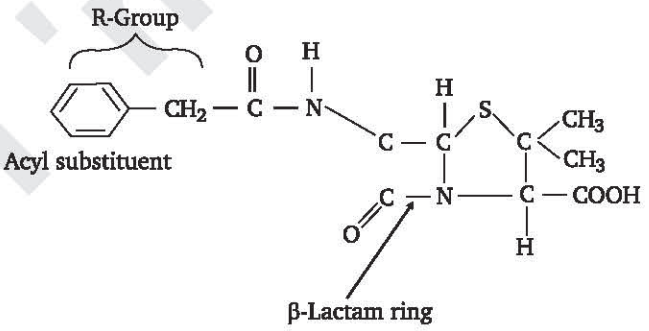


Fig. 1 : Penicillin-G, a natural product

Penicillium species required the production medium which contains lactose (1%), calcium carbonate (1%), cornsteep liquor 8.5%, glucose (1%), sodium hydrogen phosphate (0.4%) and phenyl acetic acid (0.5g). The pH is kept between 5 and 6 and temperature for incubation is 23-25°C. Aeration and agitation are necessary.

Fermentation

Penicillin is produced by fed-batch culture using *Penicillium chrysogenum* Q-176, a fungus that can be grown in stirred fermenters. The inoculum under aerobic condition (seed) can be produced when there is glucose in sufficient amount in the medium. If a particular penicillin is produced, specific precursor (substance added prior or simultaneously with the fermentation which are incorporated without any major change in the molecules) is added in the medium for e.g. phenyl acetic acid or its derivatives such as ethanol amide to get penicillin G. The antifoam agents such as vegetable oil (com or soybean oil) is added to the medium before sterilization.

The spore suspension is inoculated in flasks, each containing 15 g barley seeds, These flasks are vacuum dried, to which sterilized quartz is added.

Streptomycin

It is effective against tuberculosis causing organism, *Mycobacterium tuberculosis* and Gram-negative bacteria. The prolonged use of streptomycin in mass, can produce neurotoxic effects and loss in bearing. Since its discovery by **Schatz, Burgie** and **Waksman** (1944) most of the strains of S. griseus are genetically improved.

Structure of Streptomycin

The commercial available streptomycin is basically hydrochloride of streptomycin ($C_{21}H_{39}N_7O_{12} \cdot 3HCl$) with calcium chloride. During the production of streptomycin, mannosidostreptomycin or hydroxystreptomycin is also produced in the early fermentation. This salt is not economical and is easily converted to streptomycin by the action of *S. griseus*. No precursor is reported to increase the yield.

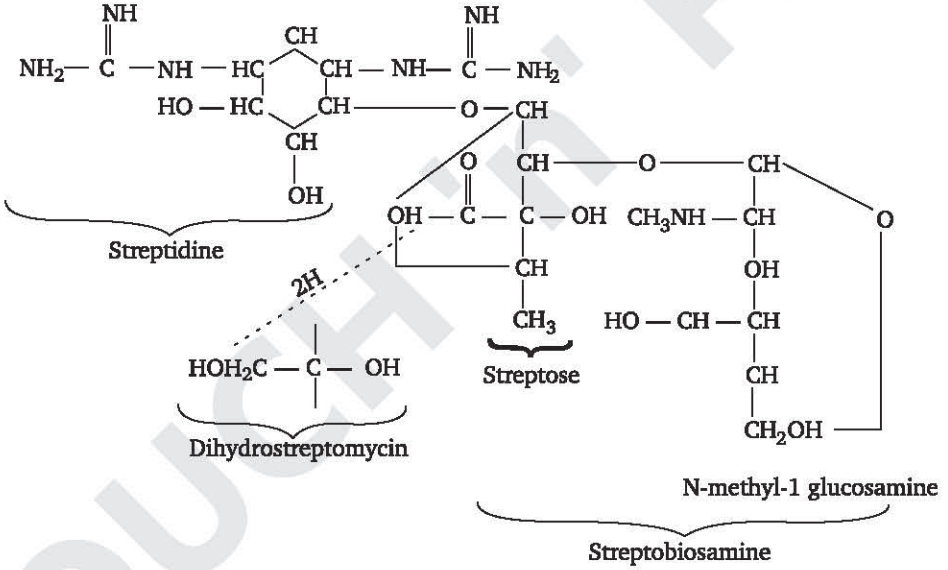


Fig. : Structure of Streptomycin

Q.2. Discuss about the production of alcoholic beverages.

Ans.

Alcoholic Beverages

Yeast is used in the production of alcohol and alcoholic beverages from the time immemorial. The important alcoholic beverages are wine, beer and distilled beverages like **brandy** (or vodka), **whiskey** and **rum**.

1. **Wine :** Wine is produced by the alcoholic fermentation of fruit juices or sugary solutions by the action of yeast. Most wines are made from grapes. Their quality and character depends on the quality of grapes and the duration of preparation. The yeasts involved in wine fermentation are of two types :

   (i) **Wild yeasts :** These are present on the grapes as they are taken from the field and are transferred to juice. It is *Saccharomyces cerevisiae*.

(ii) **Cultivated wine yeast :** It is *Saccharomyces ellipsoideus* which is added to the juice as a starter to begin the fermentation.

In wineries wild yeasts are killed by adding sulfur dioxide at a concentration of 100 ppm. The cultivated wine yeast is resistant to the above concentration of SO_2 : During fermentation temperature is maintained below 29°C between 21-24°C. Fermentation is carried out in vats of various sizes. Lactic acid bacteria are added when wine is made from grapes that are acidic due to high concentration of malic acid Lactobacilli convert malic acid to weaker lactic acid. It gives better tasting wine.

2. **Beer :** Beer is prepared by fermentation of malted grains. The process of making alcoholic beverages from malted grains is called brewing. Malt is prepared from germinated barley seeds. But other grains like corn, rice or wheat may be added to the fermenting liquid, called mash and the process of preparing mash is called mashing. The mixture of grains in the mash is cooked and allowed to steep in a large mash tub at warm temperatures. During heating enzymes from malt cause digestion of starch. The sugars and dextrins liberated by digestion are acted upon by yeast. Preparation of malt is essential because brewing yeasts are unable to digest starch and malt contains enzymes necessary for the hydrolysis of starch. Brewing yeast strains are of following two types :

   (i) **Top Fermenting Yeasts :** They include *Saccharomyces cerevisiae.* They remain uniformly distributed in the fermenting medium and are carried to the top by CO_2 gas generated during fermentation, fermentation occurs at higher temperatures (14-23°C) and is accomplished in a shorter period. These are used in brewing of ales.

   (ii) **Bottom Fermenting yeasts :** They are *Saccharomyces carlsbergensis.* They settle down at the bottom of wort. Fermentation occurs at slightly lower temperature and takes longer.

   Other beverages prepared from malt are ale, porter and stout.

3. **Distilled Alcoholic Beverages :** Distilled alcoholic beverages are prepared by concentrating alcohol from a fermentation by distillation. For whisky, brandy or rum, carbohydrates from cereal grains, potatoes and molasses àre fermented to alcohol. The alcohol is then distilled to make a concentrated alcoholic beverage. The fermented liquid is heated at a high temperature. This causes evaporization of most of the alcohol from fermented liquid. This alcohol is then condensed and collected. The distillation of malt yields whiskey, distillation of fermented molasses yields rum, while distilled wine yields brarjdy. The flavour develops during aging of these beverages that occurs after yeast has been removed. During aging process, complex chemical changes occur, that improve flavour and odour.

Q.3. Explain the genetic recombinant vaccines in detail.

Ans. **Genetic Recombinant Vaccines**

For the production of recombinant vaccines, genes for desired antigens are identified and cloned into suitable vectors. The vectors are introduced into suitable hosts for expression. Production of recombinant vaccine through this method has several advantages. However,

the major problem associated with them is the low level of immunogenicity (of recombinant proteins). Some of the recombinant vaccines are described as follows :

Vaccine for Hepatitis B virus

The characteristics of Hepatitis B virus (HBV) are known. Virus-1, after infection, HBV fails to grow and even in cultured cells it does not grow. This property has been explained to be due to inhibition of its molecular expression and development of vaccines. Plasma of human contained varying amount of antigens. Three types of viral proteins are recognised to be antigenic :

(i) viral surface antigen (HBsAg),

(ii) viral core antigen (HBcAg) and

(iii) the e-antigen (HBeAg).

Recombinant vaccine for HBV was produced by cloning HBsAg gene of the virus in yeast cells. The yeast system has its complex membrane and ability of secreting glycosylate protein. This has made it possible to build an autonomously replicating plasmid containing HBsAg gene near the yeast alcohol dehydrogenase (ADH) R1 promoter. The HBsAg gene contains 6 bp long sequence preceding the AUG that synthesises N-terminal methionine. This is joined to ADH promoter cloned in the yeast vector PMA-56. The recombinant plasmid is inserted into yeast cells. The transformed yeast cells are multiplied in tryptophan-free medium. The transformed

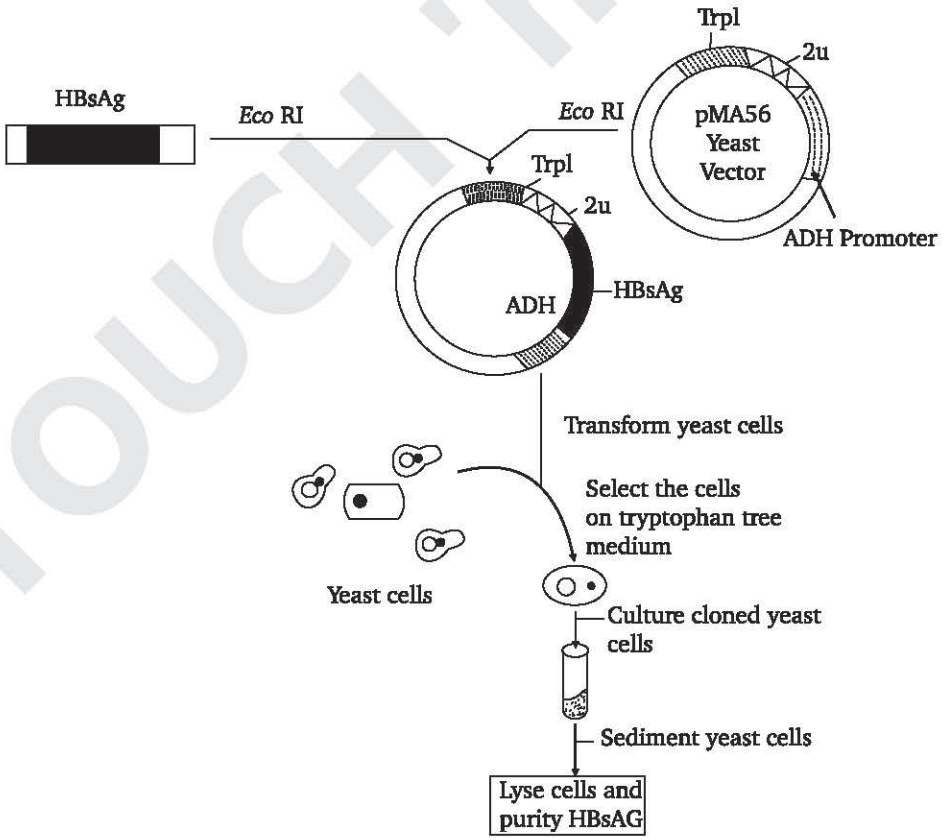


Fig. 1 : Expression of HBsAg gene in yeast

ceils are selected. The cloned yeast cells are cultured for expression of HBsAg gene. This inserted gene sequence expresses and produces particles similar to the 22 μm particle of HBV as these particles are produced in serum of HBV patients. The expressed HBsAg particles have similarily in structure and immunogenicity with those isolated from HBV-infected cells of patients. Its high immunogenicity has made it possible to market the recombinant product as vaccine against HBV infection.

Indigenous Hepatitis-B Vaccine

India's first genetically engineered vaccine (Guni) against HBV developed by a Hyderabad based laboratory (Shantha Biotechnics Pvt. Ltd.) was launched on August 18, 1997. India is the fourth country (after the U.S.A., France and Belgium) to develop this highly advanced vaccine. The indigenous yeast-desired HBV-vaccine is one-third the cost of the imported vaccine. This new vaccine had undergone human clinical trials at Nizam's Institute of Medical Sciences, Hyderabad and K.E.M. Hospital, Mumbai. The clinical trials clearly proved that the seroprotection is about 98%. It was found more effective than the imported vaccine. The Drug Controller General of India has permitted it for commercial manufacture.

2. Vaccine for Foot and Mouth Disease (FMD) Virus

FMD is a very serious disease of animals caused by an RNA virus belonging to the picórna virus group. It consists of ssRNA molecule of 8,000 nucleotides surrounded by a capsid. The capsid is made up of 60 copies of four proteins VPI, VP2, VP3 and VP4. Only VP1 has a little immunogenic activity. The gene coding for VP1 has been identified and cloned on pBR322. The recombinant plasmid was introduced in *E. coli*. About 1,000 molecules of VP1 per bacterial cell were synthesized (Kupper *et al.,* 1981). An outline of making vaccine for FMD virus is as below :

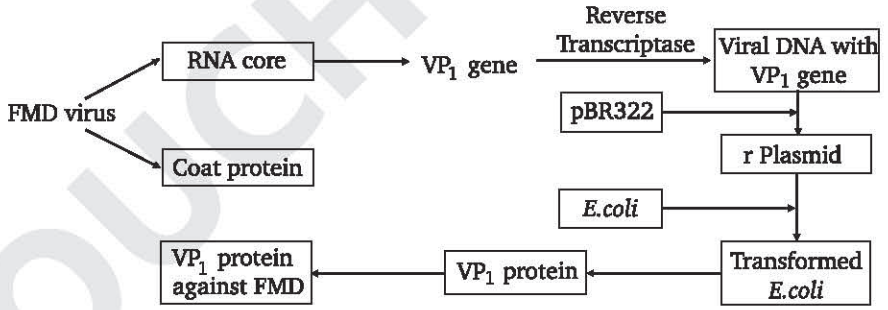


Fig. 2 : Steps for production of FMD vaccine

Q.4. What are single-cell proteins? Explain in detail.

Ans. Single-Cell Proteins (SCP) or Microbial Proteins

Refer to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods and is suitable for human consumption or as animal feeds, industrial agriculture is marked by a high water footprint, high land use, biodiversity destruction, general environmental degradation and contributes to climate change by emission of a third of all greenhouse gases, production of SCP does not necessarily exhibit any of these serious drawbacks. As of today, SCP is commonly grown on agricultural waste products and as such inherits the ecological footprint and water footprint of industrial agriculture. However, SCP

may also be produced entirely independent of agricultural waste products through autotrophic growth. Thanks to the high diversity of microbial metabolism, autotrophic SCP provides several different modes of growth, versatile options of nutrients recycling and a substantially increased efficiency compared to crops.

With the world population reaching 9 billion by 2050, there is strong evidence that agriculture will not be able to meet demand and that there is serious risk of food shortage. Autotrophic SCP represents options of fail-safe mass food-production which can produce food reliably even under harsh climatic conditions.

In 1781, processes for preparing highly concentrated forms of yeast were established. Research on Single Cell Protein Technology started a century ago when **Max Delbruck** and his colleagues found out the high value of surplus brewer's yeast as a feeding supplement for animals. During World War I and World War II, yeast-SCP was employed on a large scale in Germany to counteract food shortages during the war. Inventions for SCP production often represented milestones for biotechnology in general: for example, in 1919, Sak in Denmark and Hayduck in Germany invented a method named, "Zulaufverfahren", (fed-batch) in which sugar solution was fed continuously to an aerated suspension of yeast instead of adding yeast to diluted sugar solution once (batch). In post war period, the Food and Agriculture Organization of the United Nations (FAO) emphasized on hunger and malnutrition problems of the world in 1960 and introduced the concept of protein gap, showing that 25% of the world population had a deficiency of protein intake in their diet. It was also feared that agricultural production would fail to meet the increasing demands of food by humanity. By the mid 60's, almost quarter of a million tons of food yeast were being produced in different parts of the world and Soviet Union alone produced some 900,000 tons by 1970 of food and fodder yeast.

In the 1960s, researchers at British Petroleum developed what they called "proteins-from-oil process": a technology for producing single-cell protein by yeast fed by waxy n-paraffins, a byproduct of oil refineries. Initial research work was done by **Alfred Champagnat** at BP's Lavera Oil Refinery in France; a small pilot plant there started operations in March 1963 and the same construction of the second pilot plant, at Grangemouth Oil Refinery in Britain, was authorized.

The term SCP was coined in 1966 by **Carroll L. Wilson** of MIT.

The "food from oil" idea became quite popular by the 1970s, with Champagnat being awarded the UNESCO Science Prize in 1976 and paraffin-fed yeast facilities being built in a number of countries. The primary use of the product was as poultry and cattle feed.

The Soviets were particularly enthusiastic, opening large "BVK" (belkovo-vitaminny kontsentrat, i.e., "protein-vitamin concentrate") plants next to their oil refineries in Kstovo (1973) and Kirishi (1974). The Soviet Ministry of Microbiological Industry had eight plants of this kind by 1989. However, due to concerns of toxicity of alkanes in SCP and pressured by the environmentalist movements, the government decided to close them down, or convert to some other microbiological processes.

Quorn is a range of vegetarian and vegan meat-substitutes made from Fusarium venenatum mycoprotein, sold in Europe and North America.

Another type of single cell protein-based meat analogue (which does not use fungi however but rather bacteria) is Calysta.

Single-cell proteins develop when microbes ferment waste niaterials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta). With 'electric food' processes the inputs are electricity, CO_2 and trace minerals and chemicals such as fertiliser.

The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation and filtration, or the use of semi-permeable membranes.

The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the cie-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced.

Q.5. Write an essay on bio-indicators in applied botany.

Ans.

Bio-indicators

A bio-indicator is any species (an indicator species) or group of species whose function, population, or status can reveal the qualitative status of the environment. For example, copepods and other small water crustaceans that are present in many water bodies can be monitored for changes (biochemical, physiological, or behavioural) that may indicate a problem within their ecosystem. Bioindicators can tell us about the cumulative effects of different pollutants in the ecosystem and about how long a problem may have been present, which physical and chemical testing cannot.

A biological monitor or biomonitor is an organism that provides quantitative information on the quality of the environment around it. Therefore, a good biomonitor will indicate the presence of the pollutant and can also be used in an attempt to provide additional information about the amount and intensity of the exposure.

A biological indicator is also the name given to a process for assessing the sterility of an environment through the use of resistant microorganism strains (*e.g. Bacillus or Geobacillus*). Biological indicators can be described as the introduction of a highly resistant microorganisms to a given environment before sterilization, tests are conducted to measure the effectiveness of the sterilization processes. As biological indicators use highly resistant microorganisms, any sterilization process that renders them inactive will have also killed off more common, weaker pathogens.

A bio-indicator is an organism or biological response that reveals the presence of pollutants by the occurrence of typical symptoms or measurable responses and is, therefore, more qualitative. These organisms (or communities of organisms) can be used to deliver information on alterations in the environment or the quantity of environmental pollutants by changing in one of the following ways: physiologically, chemically or behaviourally. The information can be deduced through the study of :

1. their content of certain elements or compounds
2. their morphological or cellular structure
3. metabolic biochemical processes

4. behaviour
5. population structure(s).

The importance and relevance of biomonitors, rather than man-made equipment, are justified by the observation that the best indicator of the status of a species or system is itself. Bioindicators can reveal indirect biotic effects of pollutants when many physical or chemical measurements cannot. Through bioindicators, scientists need to observe only the single indicating species to check on the environment rather than monitor the whole community.

The use of a biomonitor is described as biological monitoring and is the use of the properties of an organism to obtain information on certain aspects of the biosphere. Biomonitoring of air pollutants can be passive or active. Experts use passive methods to observe plants growing naturally within the area of interest. Active methods are used to detect the presence of air pollutants by placing test plants of known response and genotype into the study area.

The use of a biomonitor is described as biological monitoring. This refers to the measurement of specific properties of an organism to obtain information on the surrounding physical and chemical environment.

Bioaccumulative indicators are frequently regarded as biomonitors. Depending on the organism selected and their use, there are several types of bioindicators.

Q.6. Explain in detail about the production of biofuels.

Ans.

Production of Biofuels

The word biofuel may refer to the fuels used for the production of electric energy, but in general it refers to liquid fuels used for means of transport.

Tho most common fuels are undoubtedly bioethanol synthetized from carbohydrates and biodiesel (ester) obtained from fats and oils. Although ethanol obtained from starch and sugars, it offers a good contribution from the energy and environment point of view. Later we will examine ethanol produced from cellulose biomass like herbaceous and wooden plants, agricultural and forest residues and large quantities of urban and industrial waste.

In fact, while starch and sugars represent a modest quantity of plant material, cellulose and hemicellulose, which are polymers of sugar molecules, represent most of the biomass. The benefits connected to biofuels derive from the fact that they have a more limited environmental impact than oil derivatives and use waste materials that are usually not employed. Finally, other two biofuels will be analysed, that is methanol and corrected petrol corr pounds.

Bioethanol

Ethanol has always been used for internal combustion engines, as demonstrated by the history of cars. But, although the initial large availability and the low cost of hydrocarbons had not allowed to use them as fuels, after the oil shock of 1973 many other products were studied to replace car fuel (petrol and gas oil). Today, the product that shows a better compromise between price, availability and performance is ethanol.

The synthesis of biomass ethanol is divided into four stages :

1. production of biomass by fixing atmospheric CO_2 into organic carbon
2. conversion of biomass into a food that can be used for fermentation (usually as a sugar), by applying one of the many technological processes available : this conversion

is what mainly differs with the various technological solutions for bioethanol conversion.

3. fermentation of biomass intermediates by using bio-catalizers (micro-organisms like yeast and bacteria) in order to obtain a scarcely concentrated solution of ethanol. This stage can be considered as the oldest biotechnology ever developed by men.
4. by processing the fermentation product the result is : combustible ethanol and by-products that can be used to produce other fuels, chemical compounds, heat and electric energy.

All these last processes, even though they are very different, conclude with the fermentation synthesis. The alcohol fermentation is a process that transforms the glucides contained in vegetal productions into ethanol.

Biodiesel

Recycled vegetable oils, animal fats and kitchen fats can be transformed into biodiesel by using a series of technologies in order to activate those chemical reactions, at low temperatures, that lead to the formation of compounds called esters. Esters can be liquid or solid; they are soluble in organic solvents and have a pleasant smell. Then they are transformed into biodiesel and glycerine. Glycerine is a secondary product that can be used for the production of hand creams, toothpaste and lubricants. Biodiesel can be directly used, since it does not require any type of intervention on the systems that apply it (motors and burners). It is used for motor propulsion (diesel engines) both as pure and mixed with the common gas oil and for heating.

The use of biodiesel reduces the energy dependence on fossil fuels, the greenhouse gases emissions and health risks due to air pollution. It is not toxic and it is biodegradable within 30 days. Diesel mixed with biodiesel has a triple biodegradability.

Biodiesel contains traces of sulphur, that are in line with the new parameters established by EPA (Environmental Protection Agency) and that will be applied from the year 2006.

It is safe to be handled and transported: it can be stored in the same tanks as the diesel and pumped with the same equipment, except when it is cold (it is necessary to use tank heaters or shakers). It can be completely mixed with diesel and for this reason it is a very flexible additive.

Biodiesel, since it is an oxygenated product, helps to complete combustion. The reduction of polluting emissions is proportionate to its concentration in mixtures. One of biodiesel disadvantages is the emission of NOX: studies are being carried out to mitigate this problem.

The performance of engines that use pure biodiesel, however, are 8-15% lower than traditional diesel, due to the different energy contents. In order to solve the above-mentioned problems, a 20% mixture of diesel and biodiesel is used. A mixture of biodiesel, ethanol (up to 15% in volume) and an additive (to help the two substances mix) is called e-diesel.

The mixture is prepared by means of a spray-mixing, a process that does not require any particular equipment nor temperature control. E-diesel largely reduces the emission of particulates as compared to traditional diesel.

Bioproduct

Any compound that can be synthesised from fossil fuels can be similarly produced from biomass. These bioproducts (bioproducts) are therefore produced from renewable energy sources and usually their production needs less energy than their oil-based counterparts.

Researchers demonstrated that the processes to produce biofuel can be combined to obtain antifreeze, plastic materials, glue, artificial sweeteners and toothpaste.

Other reactants to obtain bioproducts are carbon monoxide and hydrogen. They form during biomass heating thanks to the presence of oxygen. This carbon monoxide-hydrogen mixture is known as biosynthesis gas, -which gives life to plastic materials and acids that are essential for the production of photo films, textile and synthetic fibres.

When the biomass is heated without oxygen being present, pyrolysis oil is formed, from which phenol can be extracted, i.e. an intermediate used for the production of wood sticks, plastic moulds and insulating foam.

Methanol

Also known as wood alcohol, methanol is usually produced from natural gas, but it can also be synthesised from biomass. The most common process is biomass gasification, that consists of vaporizing biomass at a high temperature, removing hot gas impurities and making it pass through a catalizer that converts it into methanol.

Corrected petrol compounds deriving from biomass act as fuel additives in order to reduce the emission of pollutants.

The Biomass Dilemma

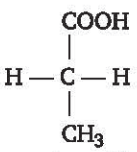
Today there is a growing interest in the use of biomass for producing biofuels, which can be used together with fossil fuels or can even replace them. However, there are some aspects that must be considered and that make the use of biomass on a vast scale controversial.

Q.7. Write about the lactic acid fermentation.

Ans.

Lactic Acid

Lactic acid was first discovered by **Scheele** (1789) from sour milk. Later on, **Pasteur** (1857) identified the microorganism involved in lactic acid production. In the year 1881, first commercial production was started by M/S Clinton Processing Company, Clinton, Iowa (USA). This was based on the fermentation process.



Lactic Acid

Lactic acid production by using chemical process was not economical and recovery and purification were also not upto the mark, hence continuous efforts were made to improve the process. Moreover, the requirement in plastic industry was of very high purity.

1. Fermentation

Lactic acid is produced by several microorganisms which differ in their ability to produce either D (–) lactic acid, L (+) lactic acid or the racemic mixture. The particular acid formed seems to be characteristic of the individual microorganism. The racemic mixture is formed due to the production of an enzyme called 'recemase'. The lactic acid recovered is optically active but becomes inactive due to the action and the enzyme.

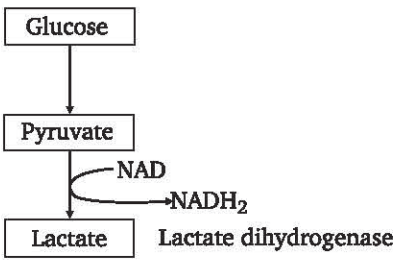


Fig. : Lactic acid production

Various microorganisms are involved in the production of lactic acid. *Rhizopus* organic produces only L(+) lactic acid. However, the production is quite slow and yield is also low. There are mainly two important processes based on end product formation.

(i) **Homofermentative Process :** This process involves certain bacteria namely, *Lactobacillus delbruckii, L. bulgaricus, L. pentosus, L. leichmanii, L. casei, Streptococcus lactis*, etc. These bacteria utilize the BMP pathway to produce pyruvic acid which is then reduced by the lactase dehydrogenase to lactic acid. All the microbes are considered to be anaerobic, although they withstand some oxygen. The end product is lactic acid with traces of others.

(ii) **Heterofermentative Process :** This process involves the action of *Leuconostoc mesenteroides* which produces lactic acid, carbon dioxide, ethanol, acetic acid, water and few other products.

2. Medium and Manufacturing Process

The culture medium contains semirefined sugar (molasses or whey contains semirefined sugar), molasses or whey starch, maltose, lactose, sucrose, calcium carbonate with ammonium hydrogen phosphate. The malt sprouts are mixed and pH is kept between 5.5 and 6.5.

Lactic acid is quite corrosive, hence metals are avoided, consequently wooden fermenters are used. The thermophilic clostridia results in the production of some butanol and butyric acid which are the major contaminants in lactic acid production.

The colonies of *L. delbruckii* are transferred into large culture vessel kept at 45-55°C. Exact stage of culture building requires 16-18/z. A slight excess of calcium carbonate is present in each stage. The inoculum volume is usually 5% and the fermentation is carried out for 5-10 days. The sugar be reduced to 0.11% or less during the fermentation because residual sugar makes the recovery of better quality of lactic acid difficult. Aeration and agitation are required.

3. Uses

Since it is a weak acid with good solvent properties, it polymerises readily for the production of polymers. It provides acidity in foods and beverages and serves as a preservative in food stuff. The delining of hides in leather industries is also carried out by its utilization. Certain other industries such as textile and laundry use lactic acid in fabric treatment. Calcium lactate is employed in baking.

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MODEL PAPER

Microbiology & Plant Pathology

B.Sc.-I (SEM-I) [M.M. : 75

Note : Attempt all the sections as per instructions.

Section-A : Very Short Answer Type Questions

Instruction : Attempt all **FIVE** questions. Each question carries **3 Marks**. Very Short Answer is required, not exceeding 75 words.

1. Name the three level of biodiversity.
2. What are facultative parasites?
3. What is a coenobium?
4. What are obligate parasites? Also give examples.
5. What are the asexual reproductive bodies in lichens?

Section-B : Short Answer Type Questions

Instruction : Attempt all **TWO** questions out of the following 3 questions. Each question carries **7.5 Marks**. Short Answer is required not exceeding 200 words.

6. Write a brief note on botany is vedic literature.

Or Write the differentiate between Gram negative and Gram positive bacteria.

7. Write about the aquatic algae.

Or Write short note on nutrition in fungi.

8. Discuss the symbiotic relationship in lichens.

Or What do you mean of the Alternaria Leaf Spot?

Section-C : Long Answer Type Questions

Instruction : Attempt all **THREE** questions out of the following 5 questions. Each question carries **15 Marks**. Answer is required in detail, between 500-800 words.

9. Describe about some famous Indian botanists.

Or Describe structural components and applications of spectrophotometer.

10. Describe briefly the structure of a prokaryotic cell.

Or Describe the detail of the bacterial growth curve.

11. Give an outline classification of algae proposed by Smith.

Or Write the classification of *Volvox*. Also explain the reproductive process in *Volvox*.

12. Explain the detail of heterothallism and two allelomorph heterothallism.

Or Give an account of the economic importance of fungi in agriclture.

13. Write brief notes on disease symptoms necrosis and blight.

Or Write about the lactic acid fermentation.

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