M.Sc. Previous Year

Botany, MB-03

BIOLOGY AND DIVERSITY OF LOWER PLANTS: CRYPTOGAMS

मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल

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INTRODUCTION

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There are millions of different organisms that inhabit the planet Earth. The most common forms of organisms are those which can be seen without the aid of any magnifying devices. On the other hand, there are some organisms which are smallest of the small and the simplest of the simple. These organisms perform incredible number of activities, which cannot be even achieved by human beings. These minute agents are called *microorganisms* and are studied under the subject '*Microbiology*'. The structure and activities of these microorganisms can be studied only with the help of some specialized techniques until recently, bacteria were the only known type of prokaryotic cells. The discipline of biology related to their study is called bacteriology.

Phycology, also known as algology is the scientific study of algae. It is a branch of life science and is often regarded as a sub-discipline of botany. Algae are considered as important primary producers in aquatic ecosystems. Microscopic algae are the source of much of Earth's oxygen. Algae are also very important ecologically because they are the beginning of the food chain for other animals. Most algae are eukaryotic, photosynthetic organisms that live in a wet environment. Mycology is the branch of biology concerned with the study of fungi, including their genetic and biochemical properties, taxonomy and their use to humans as a source for tinder, medicine, food, and entheogens, as well as their dangers, such as toxicity or infection. A biologist specializing in mycology is called a mycologist. Mycology branches into the field of phytopathology, i.e., the study of plant diseases, and the two disciplines remain closely related because the vast majority of plant pathogens are fungi.

Bryophytes are an informal group consisting of three divisions of non-vascular land plants the liverworts, hornworts and mosses. They are characteristically limited in size and prefer moist habitats although they can survive in drier environments. The bryophytes consist of about 20,000 plant species. Bryophytes produce enclosed reproductive structures, gametangia and sporangia, but they do not produce flowers or seeds and reproduce via spores. Bryophytes are usually considered to be a paraphyletic group and not a monophyletic group, although some studies have produced contrary results. Regardless of their status, the name is convenient and remains in use as an informal collective term. Traditionally, all living land plants without vascular tissues are classified in a single taxonomic group, often a division or phylum.

A pteridophyte is a vascular plant with xylem and phloem that disperses spores. Pteridophytes do not produce flowers or seeds, and are also referred as 'Cryptogams', i.e., means of reproduction is hidden. The pteridophytes include the Ferns, Horsetails, and the Lycophytes, i.e., Club Mosses, Spike Mosses, and Quillworts. These are not a monophyletic group because ferns and horsetails are more closely related to seed plants than to the lycophytes. Therefore, 'Pteridophyta' is no longer a widely accepted taxon, although the term pteridophyte remains in common parlance, as do pteridology and pteridologists as a science and its practitioner, to indicate lycophytes and ferns as an informal grouping, such as the International Association of Pteridologists and the Pteridophyte Phylogeny Group.

This book, *Biology and Diversity of Lower Plants: Cryptogames,* has been written in keeping with the self-instructional mode or the SIM format wherein each Unit begins with an Introduction to the topic, followed by an outline of the Objectives. The detailed content is then presented in a simple and organized manner, interspersed with Check Your Progress questions to test the student's understanding of the topics covered. A Summary along with a list of Key Terms and a set of Questions and Exercises is provided at the end of each Unit for effective recapitulation.

UNIT 1 MICROBIOLOGY: AN INTRODUCTION

NOTES

Structure

1.0 INTRODUCTION

Have you ever wondered about the number of diseases an individual may suffer throughout his life–be it a common cold or something more serious? In some cases, the infection or the disease may also prove to be fatal for an individual. In this fast pace of life, rarely would you try to understand the how's and why's of a disease or the measures to prevent it.

In the process of trying to understand the cause of a simple infection you will be inevitably introduced into the very interesting world of microorganisms which are not visible to the naked eye. Apart from the diseases they cause, these organisms are also beneficial in some way or the other to the human race.

You will find the study of microorganisms very intriguing as it will provide you with all the detailed information regarding numerous microscopic organisms that exist and their relationship—be it beneficial or harmful—with human beings.

In order to fulfill the above objectives, Microbiology as a separate branch of study was established by Louis Pasteur and Robert Koch. The scope of microbiology is broad and includes other branches such as virology, parasitology, mycology, bacteriology and immunology.

In the unit, you will study about the three kingdom concept by Hackle, five kingdom concept by Whittaker, three domain concept by Carl Woese.

1.1 OBJECTIVES

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After going through this unit, you should be able to:

- Explain and define microbiology
- Trace the history of medical microbiology
- Elaborate upon the concept of micrometry
- Discuss Haeckel's three - kingdom classification
- Discuss Whittakers's five-kingom classification
- Discuss Three-domain concept of Carl Woese

1.2 INTRODUCTION TO MICROBIOLOGY

You will find the study of microorganisms very intriguing as it will provide you with all the detailed information regarding numerous microscopic organisms that exist and their relationship—be it beneficial or harmful—with human beings.

In order to fulfill the above objectives, Microbiology as a separate branch of science was established by Louis Pasteur and Robert Koch. The scope of microbiology is broad and includes other branches such as virology, parasitology, mycology, bacteriology and immunology.

Microbiology has been defined in various ways. The following are some of the definitions of microbiology:

(i) Microbiology is the study of living organisms of microscopic size.

(ii) Microbiology is a branch of biology dealing especially with microscopic forms of life.

(iii) Microbiology is the scientific study of microorganisms, and deals with the structure, function and classification of these organisms and the ways in which they can be controlled and used.

(iv) Microbiology is the branch of biology dealing with microorganisms and their effects on other living organisms.

Historical Introduction to Medical Microbiology

Before really getting into the detailed study it is very essential that you have a broad historical overview. In ancient times, epidemic and even endemic diseases were believed to be supernatural in origin, sent by the gods as punishment for the sins of human kind. The concept of contagion was also known because people were aware that certain diseases spread by direct or indirect contact. Another ancient belief was that these diseases were produced by invisible living creatures.

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So in order to probe into the causative agents of such diseases which was a mystery to the human kind, in more recent times a branch of science developed which was known as **Medical Microbiology**. This is the subdivision of microbiology concerned with the finding of the causative agents of infectious diseases of man, the response of the host to infection and various methods of diagnosis, treatment and prevention. The period from 1854 to 1914 is referred to as the Golden Age of Microbiology because in this era microbiologists achieved the following:

- Studied the chemical activities of microorganisms
- Improved the techniques for performing microscopy
- Progressed in the techniques of culturing microorganisms
- Developed vaccines and surgical techniques

Antony von Leeuwenhoek (1632–1723) used a simple microscope to observe and report bacterial organisms for the first time in 1673. He described various types of bacteria accurately and communicated these descriptions to the Royal Society of London. He made the first microscope that consisted of a single biconvex lens that magnified about 200 times, and found microorganisms in materials such as water, mud, saliva and the intestinal contents of healthy subjects. He called them 'animalcules'.

Louis Pasteur (1822–1895) proved conclusively that all forms of life, even microbes, arose only from their like and not *de novo (De novo is a Latin expression meaning "from the beginning," "afresh," "anew," "beginning again.").*

Scope of Microbiology and Microscopy

As a student, it is very important for you to know the scope of a particular branch of study, so that a specific subject or field of study can be conveniently selected for study. This section will provide you with the relevance of the field of microbiology and microscopy.

Microbiology has simplified the process of understanding the biology of microorganisms. However, with this simplification, it has also brought some challenges, which have influenced the society in both positive and negative ways. The most difficult challenges for the basic understanding of microbial cells are the spread of diseases like acquired immunodeficiency syndrome (AIDS) that appear without showing any signs of their presence. The functioning style of cells at the elementary level can be now easily understood by the help of new discoveries in the field. How the microorganisms play a crucial role in the environment can be understood through the microbial ecology.

A biofilm is considered as a powerful form of organization of microbial communities. A huge number of unculturable microbes can be analysed and their characteristic features can be studied with the help of genomic tools.

Microorganisms play a more positive role than simply causing infectious diseases. They can provide humanity with an even better and more healthful existence.

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As mentioned, the scope of microbiology is very wide. Some of the areas of microbiological studies are the following:

- **Bacteriology (study of bacteria):** The smallest, simplest, single-celled prokaryotic microorganisms and archaea–prokaryotic microorganisms that form an ancient group intermediate between the bacteria and eukaryotes.
- **Mycology (study of fungi):** Microscopic eukaryotic forms (molds and yeasts), higher forms (mushrooms, toadstools and puffballs), and slime molds.
- **Virology (study of viruses):** Infectious agents containing either DNA or RNA that require living cells for their replication/ or reproduction) and viral diseases
- **Parasitology:** Study of parasitism and parasites that include pathogenic protozoa, helminth worms and some insects
- **Microbial ecology:** Study of interrelationships between microbes and the environment.
- **Microbial morphology:** Study of detailed structures of microorganisms.
- **Microbial systematics:** Classification, naming and identification of microorganisms and constructions of the phylogenetic tree of life.
- **Microbial physiology:** Study of metabolism of microbes at the cellular and molecular levels.
- **Microbial biochemistry:** Study of the discovery of microbial enzymes and the chemical reactions carried out by them.
- **Molecular microbiology:** Study of genome (i.e., genomics) of microorganisms and construction of phylogenetic tree based on rRNA.
- **Microbial genetics:** Study of heredity and variation in varieties.
- **Molecular biology:** Advanced study of the genetic material (DNA, RNA) and protein synthesis.
- **Immunology:** The immune system that protects against infections and attempts to understand many phenomena responsible for both acquired and innate immunity, in addition to the study of antibody–antigen reactions in the laboratory.
- **Agricultural microbiology:** Study of relationships of microbes and crops with an emphasis on control of plant diseases and improvement of yields.

- **Food Microbiology:** Interaction of microorganisms and food in relation to food bioprocess.
- **Dairy Microbiology:** Study of the production and maintenance of quality dairy products.
- **Industrial Microbiology:** Industrial uses of microbes in the production of alcoholic beverages, vitamins, amino acids, enzymes, antibiotics and other drugs.
- **Marine Microbiology:** Study of microorganisms and their activity concerning human and animal health in fresh, estuarine and marine waters.
- **Air Microbiology:** Role of aerospora in contamination and spoilage of food and dissemination of plant and animal diseases through air.
- **Exomicrobiology:** Exploration for microbial life in outer space.
- **Diagnostic Microbiology:** Explores the fundamental principles and techniques involved in the study of pathogenic organisms as well as their application in the diagnosis of infectious diseases.
- **Epidemiology and Public:** Monitoring, control and spread of diseases in communities.
- **Biotechnology:** The scientific manipulation of living organisms, especially at the molecular and genetic level to produce useful products.

To observe and study the microorganisms the concept of microscopy comes into the picture. It is the technical field of using microscopes to view the disease-causing organisms which cannot be seen with the unaided eye. So microscopy is considered an inherent part of Microbiology.

Concept of Micrometry

The method of measuring a microscopic organism under a microscope with the help of a calibrated scale is known as micrometry. Devices like the eyepiece micrometer and stage graticule are used in the measurement and calibration processes. This fundamental methodology is applied to the measurement of any specimens noticed in the microscope and for performing measurements at high magnifications in compound optical microscopy must be brought into practice.

The most common of these is the application of eyepiece reticles in combination with stage micrometers where measurements made with compound microscopes fall into the size range of 0.2 micrometers to 25 millimeter.

A lot of approaches have been employed for measuring linear, area, and volume specimen dimensions with the microscope called micrometry or morphometrics.

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1.2.1 Different Elements

The science dealing with the study of microscopic organisms (microbes) is known as microbiology. Microorganisms are found in all ecosystems and exist in close relationship with humans and other living beings. They may benefit or harm the host depending on the relationship. The study of the microbes causing disease in man, their diagnosis, prevention and treatment and the response of human body to such infection is called medical microbiology. Medical microbiology deals with all microbes causing disease in man. These microbes may be bacteria, viruses, protozoa or fungi. Bacteria are the smallest and simplest unicellular organisms and the branch of microbiology dealing with bacteria is called bacteriology.

Concept of Infectious Disease

The spread of disease by direct or indirect contact has been speculated since ancient times. The concept of diseases spreading by close contact and touch has been known since biblical times. However, due to lack of scientific evidence other forces like supernatural causes and bad air were blamed for various infectious diseases. Plague known as Black Death was described as early as fifth century BC by the Greek historian Thucydides. In 14^{th} century it had wiped out entire villages in Europe. It killed 25 million people in Europe over 5 years from 1347 to 1352. In 1546 a physician of Verona named Girolamo Fracastoro concluded that the communicable diseases were caused by certain living agents which spread by contact and infected objects. Massive developments were seen in the next 250 years in the field of infectious diseases.

Development of Bacteriology

Bacteria existed long before humans evolved, and bacterial diseases probably co-evolved with each species which involuntarily hosts them. Many bacterial diseases that we see today have been around for a very long time, while few may have developed later. But for a long time we were not aware of them as a cause of infectious diseases. With the beginning of developments in microbiology, bacterial pathogens became apparent. Bacteria have played an important role in the daily lives of humans since ages. They can be harmful or useful depending on their association. Bacteria have been used in the wine industry and bakeries since earliest times. They are being utilized now in pharmaceutical industry for production of vaccine, drugs, chemicals etc.

The involvement of external agencies in causing disease in man has been known since early time but definitive findings were established after the work of many scientists in the 18^{th} and 19^{th} century. Known and newly-discovered bacteria can be blamed for new and old diseases. The most obvious diseases caused by bacteria are infectious diseases.

Various causes of infectious diseases include bacteria, viruses, parasites and fungi.

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Contributions of Leeuwenhoek

Transmission of some disease by contact is known to man since ancient times. As these organisms could not be seen with naked eyes, their identification was possible only after discovery of microscope. Antony von Leeuwenhoek (1632–1723) is credited with discovery of simple microscope. With the help of his primitive microscope he observed many microorganisms. He described various free living and parasitic protozoa, fungi and yeast from feces of humans and animals. However, the importance of his discoveries was realized only after some two centuries later. His observations laid the foundations of microbiology.

Concept of Fermentation

Early nineteenth century saw a lot of interest in fermentation and putrefaction. Changes occurred in organic substances to produce alcohol and acetic acid. Many observers noticed that this was result of activity of some living organism that converts the sugars in the juice to alcohol, and at the same time produces the desired flavours. However this view was challenged by many scientists who believed that that fermentation was purely a chemical change and organisms were formed probably after the reaction. Wine and vinegar making industry wanted to find ways to control this fermentation reaction so as to control the taste and texture of wine. Spoilage of food was noticed to be due to the action of microorganisms. The chemical changes which are brought about in food due to the cation of bacteria is called putrefaction.

The overall process of fermentation involves conversion of glucose or sugar ($C_6H_{12}O_6$) to alcohol (CH_3CH_2OH) and carbon dioxide gas (CO_2). The reactions which occur within the yeast to make this happen are very complex but the overall process can be summarized as follows:

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

$$\underset{\text{(Glucose)}}{\text{Sugar}} \longrightarrow \underset{\text{(Ethyl alcohol)}}{\text{Alcohol}} + \text{Carbon dioxide gas} + \text{Energy}$$

Contributions of Louis Pasteur

The concept of 'germ theory of disease' by Henle laid the foundations of modern day bacteriology. This asserted the fact many diseases were actually caused by multiplication of microorganisms in the body

Modern day microbiology was developed by the extensive contributions of scientists like Louis Pasteur and Robert Koch. (1822–1895). Louis Pasteur is known as the father of microbiology. He was a French chemist interested in studying fermentation of wine and beer. His contributions include:

- Germ theory of disease: This concept had been suggested by many scientists earlier but Louis Pasteur developed it and conducted

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experiments that clearly indicated its correctness and managed to convince most of Europe it was true.

- Discovery of the principles of fermentation in microbes.
- Discovery of origin of life i.e. life originates from life itself and not de novo
- Discovery of basic principles and techniques of sterilization including steam sterilizer, hot air oven and autoclave.
- Discovery of anaerobiosis , i.e., some microorganisms can develop and live without air or oxygen. This process of aerating the broths to decrease fermentation rate was called the Pasteur Effect. This benefitted the wine industry a lot.
- Introduction of concept of live attenuated vaccine to prevent disease.
- Development of vaccine against hydrophobia or rabies and anthrax.
- Studies on silkworm, chicken cholera and anthrax bacilli to develop various methods and techniques in bacteriology. In 1865 large numbers of silkworms were killed by a mysterious disease. Pasteur worked several years proving it was a microbe attacking silkworm eggs which caused the disease, and that eliminating this microbe within silkworm nurseries would eradicate the disease
- Development of technique of pasteurization: His research demonstrated that the growth of micro-organisms was responsible for spoiling beverages, such as beer, wine and milk. So he developed a technique which prevents the activity of microbes to spoil milk. This is used even now extensively to preserve milk.

Contributions of Robert Koch

Heinrich Hermann Robert Koch (1834–1910) was a German general practitioner. He is considered as the father of bacteriology. He was awarded Nobel Prize for his contributions to microbiology in 1905. His contributions include:

- Discovery of causative agents of anthrax, tuberculosis and cholera. He described the bacteria responsible for anthrax in animals.
- He invented methods to purify the bacteria from blood samples and grew it in pure cultures.
- He found that, while it could not survive outside a host for long, anthrax built resistant forms called spores in order to survive for along time.
- He postulated that the disease was caused due to entry of spore in the body. Development of techniques of fixing and staining the bacteria so as to visualize them easily.
- Koch identified the Vibrio bacterium that caused cholera.

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- Development of solid media for cultivation of bacteria in the laboratory was firstly used by Koch. In his quest to grow bacteria outside the body in laboratory he used several ingredients to solidify its media. He used potato, gelatin and finally settled on agar-agar gel, as agar was inert and did not melt at room temperature.
- He discovered the isolation staining and purification techniques of various bacteria in laboratory.
- He discovered Koch's phenomenon. He observed that guinea pigs infected previously with tubercle bacilli responded by an exaggerated inflammatory response to local inoculation of second dose of the bacilli. This hypersensitivity reaction is known as Koch's phenomenon.
- Formulated Koch' postulates. Koch's postulates describe the relationship of the microorganisms to disease. These are as follows:
    - o The organism causing disease must be constantly associated with lesions of the disease.
    - o It should be possible to isolate the organism in pure culture from the lesion.
    - o Inoculation of this organism in suitable laboratory animal must produce the same disease.
    - o It should be possible to isolate the organism again from the lesion/disease produced in the laboratory animal.

These postulates help to establish relationship between the disease and the suspected causative agent. However, it may not always be possible to satisfy all the postulates especially if the organism cannot be grown in cell free media in the laboratory. Koch's pupils found the organisms responsible for diphtheria, typhoid, pneumonia, gonorrhoea, meningitis, leprosy, bubonic plague syphilis and tetanus.

Contributions of Alexander Fleming

The discovery of penicillin by Sir Alexander Fleming started the era of antibiotic research and there has been no looking back with development of newer compounds every day. Fleming's discovered and isolated penicillin in September 1928 from a mould *Penicillium notatum.* This was the start of antibiotics. These were described as substances which were produced by one group of organisms and lethal to another group of microorganisms. Fleming also discovered that bacteria developed resistance to antibiotics whenever too little penicillin was used or when it was used for too short a period. Fleming cautioned not to use penicillin unless there was a properly diagnosed reason for it to be used, and that if it were used, never to use too little, or for too short a period, since these are the circumstances under which bacterial resistance to antibiotics develops. These finding are so true even today. It is a known fact now that antibiotic misuse is responsible for antibiotic resistance amongst bacteria.

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Other Contributors to Microbiology

Joseph Lister (1827–1912) was a British surgeon who applied the principles of Louis Pasteur and introduced aseptic surgical techniques. He used carbolic acid for antisepsis. He is regarded as the father of antiseptic surgery.

The work of Louis Pasteur and Robert Koch had a profound influence on many scientists. Their discoveries had a lot of influence on agriculture and industrial applications. In 1877, upon Pasteur's suggestion Schloesing and Muntz solved the mystery of nitrogen fixation in soil by bacteria. They demonstrated that the formation of nitrates was due to the action of living organisms. Later Winogradsky in 1890 isolated and described the nitrogen fixing bacteria. Later came the concept that it was probably the action of primitive microbial forms that made life on earth possible.

Bacterial causes of diseases of plants and animals were described by many workers. The organism responsible for pear blight and yellowing of hyacinths were identified and described in 1878 and 1883. The role of bacteria in decay of food was established. This led to numerous developments in the field of food preservation techniques.

Later other scientists went on to discover more bacteria as the causative agents of many diseases, e.g., *Lepra bacilli* (Hansen 1874), *Diphtheria* (Klebs and Loeffler, 1884), *Pneumococcus* (Frankel 1886), *Plague bacilli* (Yersin, 1890), etc.

Treatments for infection were described using plants with antimicrobial properties described over 2,500 years ago in Chinese medicine. Ancient Egypt and ancient Greeks used molds to treat infections. The discovery of the natural antibiotics produced by microorganisms emerged from earlier work on the observation of antibiosis between micro-organisms. Synthetic antibiotic chemotherapy as a science and the story of antibiotic development began in Germany with a German medical scientist named Paul Ehrlich in the late 1880s. In 1878, he found that different living cells showed different affinity to various aniline dyes. This led to the search of chemotherapeutic agents. He also noted that upon fuchsin stained tubercle bacilli retained their color even after treatment with mineral acid. This laid foundation of acid fast stain. Christian Gram developed a differential method of staining known as Gram's stain. This classified the organisms into Gram negative and Gram positive. Later the differences were found to be due to differences in the cell walls of bacteria.

All these findings also led to recognition of various factors and mechanisms to protect host. As the role of various bacteria in causing disease became clear, research started on developing compounds which could kill or contain them. The term 'antibiotic' was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is inhibitory or kills the growth of other microorganisms in high dilution. This original definition excluded naturally occurring substances that kill bacteria but are not produced by microorganisms. It also excluded synthetic antibacterial compounds such as the sulphonamides. Research in bacteriology has now reached molecular level with the introduction of nucleic acid detection and polymerase chain reaction. These assays though expensive but accurate are now available for detection of practically all pathogens.

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Table 1.1 lists some Nobel laureates in the field of bacteriology.

Table 1.1 *Some Nobel Laureates in the Field of Bacteriology*

Year	Name	Research
1901	Emil von Behring	Discovery of diphtheria antitoxin
1905	Robert Koch	Discovery of various bacteria
1928	Charles Nicolle	Typhus fever
1939	Gerhardt Domagk	Antibacterial effect of prontosil
1945	Sir Alexander Fleming, Sir Howard Florey, Ernst B Chain	Discovery of penicillin
1952	S. A. Waksman	Discovery of streptomycin
1962	James D Watson, Francis Crick	Discovery of DNA
1993	Kary Mullis	Polymerase chain reaction
2005	Barry J. Marshall, Robin Warren	Helicobacter pylori role in peptic ulcer

Check Your Progress

1. What is immunology.
2. Define biotechnology.
3. Define bacteriology.
4. Define virology.

1.3 HAECKEL'S THREE-KINGDOM CONCEPT

As the knowledge of the properties of various groups of microbial life exploded around the middle of the 19th century, it became apparent that at this level of biological knowledge a division of the living world into two kingdoms was difficult to maintain on a logical and consistent ground.

Although the two kingdom system suited at the level of well-developed advance organisms, it could not satisfy biologists at the level of microbial forms of life.

Many of the microbes possessed both 'plant-like' and 'animal-like' characteristics simultaneously, and many others enjoyed such characteristics which were unique to them and not found in either plants or animals.

Slime moulds, which were considered to be protozoan and grouped under the kingdom Animalia, were found phagotrophic and amoeboid (animal-like) in their vegetative state but they resembled true fungi in their reproductive state (plant-like).

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Motility by means of flagella was found the only animal-like character in flagellate protozoa: many of them possessed cell wall and were phototrophs (plant-like). Bacteria were found having very little in common with either plants or animals.

Many algae and fungi, which were earlier thought to be immotile thus classified as plants, were found either motile or producing motile structures (zoospores, gametes, etc.) during their life.

In view of the foregoing, attempts were made to find a solution and the same was proposed in 1866 by E. Haeckel, a German zoologist and Darwin's disciple.

Haeckel suggested that the inconsistencies of the two kingdom system could be avoided by the recognition of a third kingdom, and he proposed Protista as a new kingdom to accommodate organisms exhibiting characters either common to both plants and animals, or unique to their own.

Thus, a three kingdom system consisting of kingdoms Protista, Plantae, and Animalia (Refer Figure 1.1) came into being. This arrangement by Haeckel was done on the basis of morphological complexities and tissue system, division of labour, and mode of nutrition.

Organisms lacking morphological complexities, tissue system, division of labour, and enjoying diversified type of modes of nutrition were segregated and put under the kingdom Protista (algae, fungi, protozoa and bacteria).

Organisms having diverse tissue-system with well- defined division of labour and maximum morphological complexities in their body remained segregated from protists and were bifurcated into two categories: those enjoying autotrophic mode of nutrition were considered to be plants and put under kingdom Plantae, and those that have entirely holophagic (phagotrophic) mode of nutrition were considered to be animals and put under kingdom Animalia. According to this system, all known microorganisms came to be recognised as protists; neither plants nor animals.

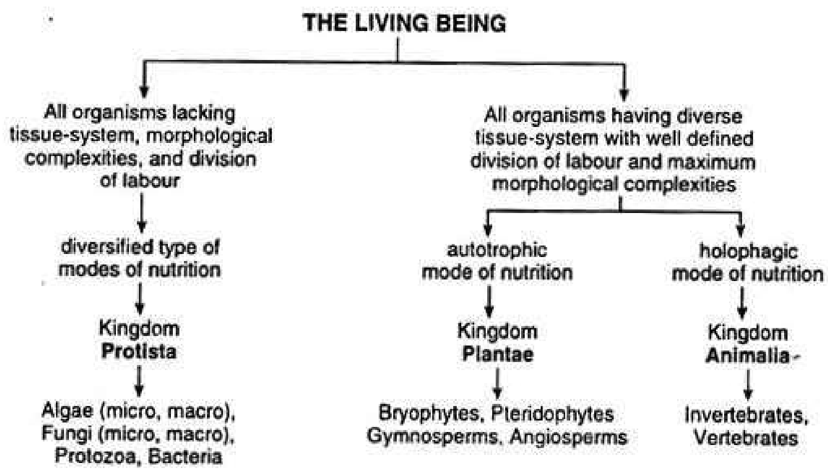

Fig. 1.1 *Three Kingdom System*

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Figure 1.2 explains about the Hackel's three-kingdom classification.

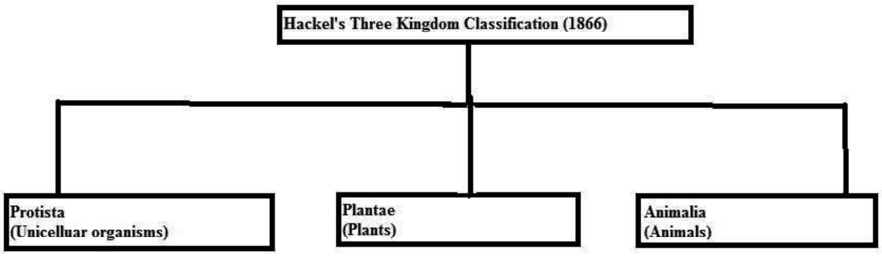

Fig. 1.2 *Haeckel's Three Kingdom Classification*

Check Your Progress

5. What were Haeckel's three kingdom classification?
6. In which year Hackel proposed three kingdom classification?
7. What was put under the kingdom Protista?

1.4 WHITTAKER'S FIVE KINGDOM CONCEPT

Although most of the inconsistencies of protists came to an end with the proposal of two separate kingdoms (Monera and Protoctista) in four kingdom system, the heterogeneity among protoctists were still discernible. All fungi were non-photosynthetic and enjoyed very distinct mode of nutrition (absorptive, osmotrophic).

Similarly, some of the algae have lost their photosynthetic ability (for example, some euglenophycophytes), lacked cell wall, moved freely and thus were considered closely related with protozoa; other algal forms exhibiting more extensive organizational development and totally photosynthetic nature were considered more closely related with plants.

Considering such inconsistencies of protoctistans. R.H. Whittakar (1969) proposed a comprehensive five kingdom system comprising of kingdom — Monera, Protista, Fungi (Mycophyta), Plantae, and Animalia (Refer Figure 1.3).

He retained bacteria and cyanobacteria (blue green algae) under kingdom Monera, retained microalgae (the pliytoplanktonic algal forms considered closely related with protozoa), protozoa, and slime moulds under Kingdom Protista (he adopted term Protista rather than Protoctista of Copeland); separated, fungi completely form protoctists and accommodated them under a new kingdom Fungi (Mycophyta); segregated extensively developed completely photosynthetic algal forms (the macroalgae considered closely related with plants) from protoctists and accommodated them along with plants under kingdom Plantae; and retained invertebrate and vertebrate animals as such under kingdom Animalia. In this way, the microorganisms spread into three kingdoms (Monera, Protista, Fungi) and came to be recognised as monerans, protistans and fungi (mycophytans); neither plants nor animals.

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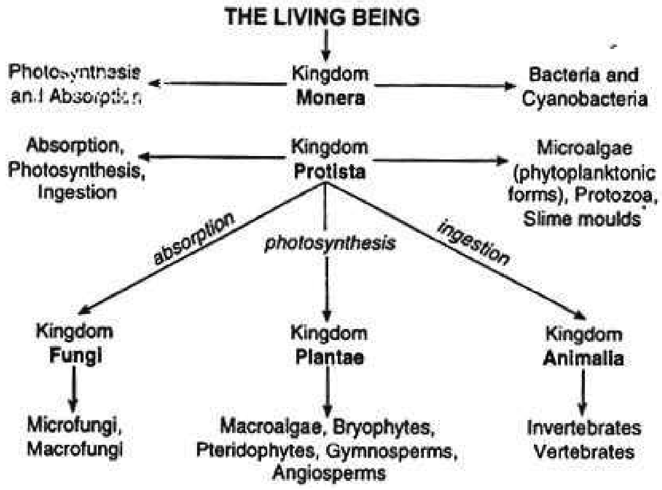

Fig. 1.3 *Five Kingdom System*

Whittaker's system of organisms classification is based on evolutionary relationship of phenotypic (observable) characteristics in which three levels of cellular organization are thought to have evolved along three different lines of nutritional strategies: photosynthesis, absorption, and ingestion. (Refer Figure 1.4).

The phenotypic characteristics taken into account to raise five kingdoms by Whittaker are:

(1) Cell type-prokaryotic or eukaryotic,

(2) Level of organization—solitary and colonial unicellular organization or multicellular, and

(3) Nutritional type.

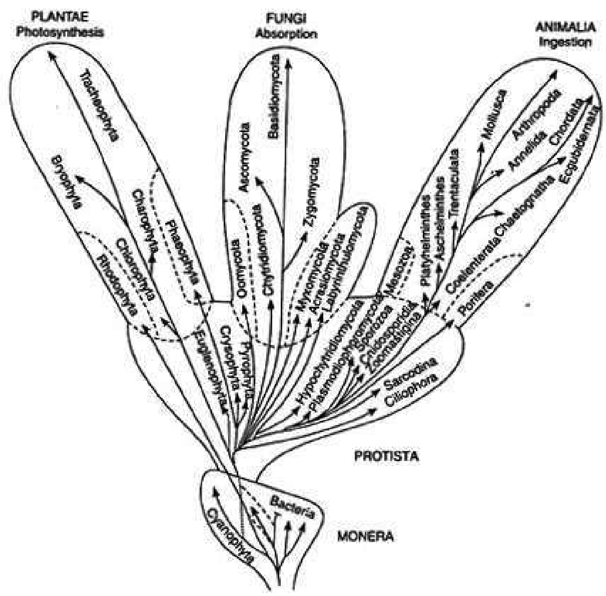

Fig. 1.4 *Five Kingdom System of Classification by R.H. Whittacker's (1969)*

NOTES

General Characteristics of Whittacker's five kingdoms

1. **Kingdom Monera (Archaebacteria, Bacteria, and Cyanobacteria):**
    - The monerans consist of all the prokaryotes, and majority of them are represented by the smallest organisms on earth.
    - The nuclei of the monerans are not organized with nuclear membrane, nucleoplasm, chromatin fibres and nucleoli, and are referred to as 'incipient nuclei'.
    - The moneran cells typically lack in cell organelles such as chloroplasts, mitochondria and other membrane-bound organelles like endoplasmic reticulum, Golgi bodies, etc.
    - The respiratory enzymes, which are mainly the concern of mitochondria in other organisms, however, are present along the infolding's of plasma membrane called 'mesosomes'.
    - Flagella, if present, are 8 stranded lacking 9 + 2 arrangement; each strand is made up of a protein named flagellin.
    - The monerans are also unique in having specific type of ribosomes distributed in cytoplasm having 70 sedimentation co efficient (70S) as against 80 sedimentation coefficient (80S) ribosomes arrayed on membranes in other organisms.
    - Monerans reproduce asexually, they lack true sexual reproduction.
2. **Kingdom Protista (Phytoplanktonic Algae, Protozoa, and Slime Moulds):**
    - Eukaryotic with solitary or colonial unicellular organization without any differentiation into tissues and organs.
    - Mostly aquatic forms called planktons; the planktons may be photosynthetic and cell-walled (phytoplanktons) or may be non-photosynthetic and wall-less (zooplanktons).
    - Nutrition absorptive, photosynthetic, or ingestive. Photosynthetic pigments are chlorophylls present in plastids (chloroplasts).
    - The protistan cells possess cell organelles such as mitochondria, Iysosomes, centrioles and other membrane-bound organelles like endoplasmic reticulum, Golgi bodies, etc.
    - The cellular organization is of two envelop type, i.e., besides plasma membrane, internal membranes occur around certain organelles.
    - Genetic material is organized in the form of a true nucleus. DNA is associated with histone proteins.
3. **Kingdom Fungi (The Fungi)**
    - Fungi are ubiquitous found in any conceivable habitat.

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- They are versatile by virtue of their high degree of adaptability. Anything that can be decomposed to yield energy invites fungi to colonize it.
- One-to-all known fungi are heterotrophic and absorb food from the environment employing extracellular digestion.
- The vegetative body of fungi is usually filamentous; the filament is called a hypha, which is thread-like, extensively branched, and surrounded by cell wall.
- The vegetative body, even when it forms tissues, is never differentiated into root and shoot and, most important of all, has no specialized vessels for internal transport of nutrients.
- The cell wall is characteristically mainly constituted of chitin (commonly called fungal cellulose); recently, cellulose has been found as chief constituent of cell wall (members of Oomycetes).
- The reserve food material is glycogen (animal starch).

4. **Kingdom Plantae (Macroalgae and Plants)**
   - Primarily autotrophic; some heterotrophic, a few saprophytic.
   - Organisms multicellular (except some algae) with walled and frequently vacuolate eukaryotic cells.
   - Simple multicellular to advanced tissue organization.
   - Autotrophism by means of photosynthesis; photosynthetic pigments are chlorophylls present in plastids (chloroplasts).
   - Development of individuals, as a result of sexual reproduction, proceeds from solid embryos except in the algal groups.
   - Food reserve is usually starch and fat.
5. **Kingdom Animalia (Invertebrate and Vertebrate Animals)**
   - Individuals multicellular with wall-less eukaryotic cells.
   - Multi-cellularity accompanied with cellular tissue and organ-system levels of organization with complex cell junctions.
   - Nutrition primarily ingestive with digestion in an internal cavity, but some forms are absorptive and some lack digestive cavity.
   - Reproduction mainly sexual with meiosis forming gametes; haploid stages other than gametes almost lacking above lowest phyla.
   - Zygote develops into an embryo.

Merits of Five Kingdom System

- Placement of prokaryotes separately in the kingdom monera is well appreciable as the prokaryotes differ from all other organisms (eukaryotes) in their cellular, physiological, and reproductive organization.

- Many intermediate or transitional forms occur in unicellular eukaryotes, which had been included both amongst plants and animals. Placement of these unicellular eukaryotes into kingdom protista has removed this anomaly.
- Five kingdom system has tried to bring out phytogenetic relationships even amongst the primitive forms hence appears more natural.

Demerits of Five Kingdom System

- It lacks distinction between archaebacteria and eubacteria.
- The kingdom protista still appears too diverse to be taxonomically useful.
- A system based on phytogenetic relationships amongst organisms cannot be established in real terms until all the distinct evolutionary tendencies are taken into account. Five kingdom system does not satisfy in this respect.

Check Your Progress

8. Who proposed five kingdom classification?
9. In which year was five kingdom classification proposed?
10. What is five kingdom classification?

1.5 THREE-DOMAIN CONCEPT OF CARL WOESE

The Three Domain System, developed by Carl Woese, is a system for classifying biological organisms. Over the years, scientists have developed several systems for the classification of organisms. From the late 1960's, organisms had been classified according to a Five Kingdom system. This classification system model was based on principles developed by Swedish scientist Carolus Linnaeus, whose hierarchical system groups organisms based on common physical characteristics.

The Three Domain System

As scientists learn more about organisms, classification systems change. Genetic sequencing has given researchers a whole new way of analyzing relationships between organisms. The current system, the Three Domain System, groups organisms primarily based on differences in **ribosomal** RNA (rRNA) structure. Ribosomal RNA is a molecular building block for ribosomes. Under this system, organisms are classified into three domains and six kingdoms. The domains are **Archaea, Bacteria,** and **Eukarya.** The kingdoms are **Archaebacteria** (ancient bacteria), **Eubacteria** (true bacteria), **Protista, Fungi, Plantae,** and **Animalia.**

Archaea Domain

This domain contains single-celled organisms known as archaea. Archaea have genes that are similar to both bacteria and eukaryotes. Because they are very similar

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to bacteria in appearance, they were originally mistaken for bacteria. Like bacteria, Archaea are prokaryotic organisms and do not have a membrane-bound nucleus.

They also lack internal cell organelles and many are about the same size as and similar in shape to bacteria. Archaea reproduce by binary fission, have one circular chromosome, and use flagella to move around in their environment as do bacteria. Archaea differ from bacteria in cell wall composition and differ from both bacteria and eukaryotes in membrane composition and rRNA type. These differences are substantial enough to warrant that archaea have a separate domain.

Archaea are extreme organisms that live under some of the most extreme environmental conditions. This includes within hydrothermal vents, acidic springs, and under Arctic ice. Archaea are divided into three main phyla: Crenarchaeota, Euryarchaeota, and Korarchaeota.

- Crenarchaeota include many organisms that are hyperthermophiles and thermoacidophiles. These archaea thrive in environments with great temperature extremes (hyperthermophiles) and in extremely hot and acidic environments (thermoacidophiles).
- Archaea known as methanogens are of the Euryarchaeota phylum. They produce methane as a byproduct of metabolism and require an oxygen-free environment.
- Little is known about *Korarchaeota archaea* as few species have been found living in places such as hot springs, hydrothermal vents, and obsidian pools.

Bacteria Domain

Bacteria are classified under the **Bacteria Domain**. These organisms are generally feared because some are pathogenic and capable of causing disease. However, bacteria are essential to life as some are part of the human microbiota. These bacteria preform vital functions, such as enabling us to properly digest and absorb nutrients from the foods we eat.

Bacteria living on the skin prevents pathogenic microbes from colonizing the area and also help in the activation of the immune system. Bacteria are also crucial for the recycling of nutrients in the global ecosystem as they are primary decomposers.

Bacteria have a unique cell wall composition and rRNA type. They are grouped into five main categories:

Proteobacteria: This phylum contains the largest group of bacteria and includes *E.coli, Salmonella, Heliobacter pylori*, and *Vibrio. bacteria*.

Cyanobacteria: These bacteria are capable of photosynthesis. They are also known as blue-green algae because of their color.

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Firmicutes: These gram-positive bacteria include Clostridium, Bacillus, and mycoplasmas (bacteria without cell walls).

Chlamydiae: These parasitic bacteria reproduce inside their host's cells. Organisms include *Chlamydia trachomatis* (causes chlamydia STD) and *Chlamydophila pneumoniae* (causes pneumonia).

Spirochetes: These corkscrew-shaped bacteria exhibit a unique twisting motion. Examples include *Borrelia burgdorferi* (cause Lyme disease) and *Treponema pallidum* (cause syphilis).

Eukarya Domain

The Eukarya domain includes eukaryotes or organisms that have a membrane-bound nucleus. This domain is further subdivided into the kingdoms Protista, Fungi, Plantae, and Animalia. Eukaryotes have rRNA that is distinct from bacteria and archaeans. Plant and fungi organisms contain cell walls that are different in composition than bacteria. Eukaryotic cells are typically resistant to antibacterial antibiotics. Organisms in this domain include protists, fungi, plants, and animals. Examples include algae, amoeba, fungi, molds, yeast, ferns, mosses, flowering plants, sponges, insects, and mammals.

1.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Immunology is defined as the immune system that protects against infections and attempts to understand many phenomena responsible for both acquired and innate immunity, in addition to the study of antibody–antigen reactions in the laboratory.
2. Biotechnology can be defined as the scientific manipulation of living organisms, especially at the molecular and genetic level to produce useful products.
3. Bacteriology is study of bacteria. The smallest, simplest, single-celled prokaryotic microorganisms and archaea–prokaryotic microorganisms that form an ancient group intermediate between the bacteria and eukaryotes.
4. Virology is study of viruses. Infectious agents containing either DNA or RNA that require living cells for their replication/ or reproduction and viral diseases.
5. Haeckel's three kingdom classification was - Protista, Plantae, Animalia.
6. Haeckel proposed three kingdom classification in the year 1866.
7. Kingdom Protista contains algae, fungi, protozoa, bacteria.
8. R.H. Whittaker proposed five kingom classification.

9. R.H. Whittaker proposed three kingdom classification in the year 1969.
10 Five kingdom classification comprises of Kingdom - Monera, Protista, Fungi, Plantae and Animalia.

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1.7 SUMMARY

- The very existence of microbes was established by Antony van Leeuwenhoek (1677) who saw microbes under the microscope.
- Microbiology is the study of living organisms of microscopic size.
- Medical microbiology is the subdivision of microbiology concerned with the causative agents of infectious disease of man, the response of the host to infection and various methods of diagnosis, treatment and Prevention.
- The very existence of microbes was established by Antony van Leeuwenhoek (1677) who saw microbes under the microscope.
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1.8 KEY TERMS

- **Bacteriology** (study of bacteria): The smallest, simplest, single-celled prokaryotic microorganisms and archaea–prokaryotic microorganisms that form an ancient group intermediate between the bacteria and eukaryotes.
- **Mycology** (study of fungi): Microscopic eukaryotic forms (molds and yeasts), higher forms (mushrooms, toadstools and puffballs), and slime molds.
- **Virology** (study of viruses): Infectious agents containing either DNA or RNA that require living cells for their replication/ or reproduction) and viral diseases.
- **Parasitology**: Study of parasitism and parasites that include pathogenic protozoa, helminth worms and some insects
- **Microbial ecology**: Study of interrelationships between microbes and the environment.
- **Microbial morphology:** Study of detailed structures of microorganisms.
- **Microbial systematics**: Classification, naming and identification of microorganisms and constructions of the phylogenetic tree of life.
- **Microbial physiology:** Study of metabolism of microbes at the cellular and molecular levels.

- **Microbial biochemistry**: Study of the discovery of microbial enzymes and the chemical reactions carried out by them.
- **Molecular microbiology:** Study of genome (i.e., genomics) of microorganisms and construction of phylogenetic tree based on rRNA.
- **Microbial genetics**: Study of heredity and variation in varieties.
- **Molecular biology:** Advanced study of the genetic material (DNA, RNA) and protein synthesis.
- **Micrometry**: Method of measuring a microscopic organism under a microscope with the help of a calibrated scale.

1.9 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Briefly describe the evolution and scope of Microbiology.
2. Describe scope of microbilogy and microscopy.
3. Define the concept of micrometry.
4. What are the five kingdom claasifications proposed by Whittaker? Explain in brief.
5. Discuss the concept of infectious disease.
6. Brief about three domain concept of Carl Woese.

Long-Answer Questions

1. Discuss historical introduction to medical microbiology and the scope of microbiology and microscopy.
2. List the areas of microbiological studies.
3. Discuss Haeckel's three kingdom concept.
4. Discuss Whittaker's five kingdom concept.
5. Discus three-domain concept of Carl Woese.

1.10 FURTHER READING

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UNIT 2 CLASSIFICATION OF BACTERIA

NOTES

Structure

2.0 INTRODUCTION

Considering the bewildering diversity of living organisms, it is logical to classify or arrange them into groups based on their mutual similarities. Physiological as well as morphological differences are necessary to distinguish bacteria accurately.

Taxonomy is defined as the science of biological classification. It includes classification, nomenclature and identification. Classification is the arrangement of organisms into groups or taxa based on mutual similarity or evolutionary relatedness while nomenclature is the branch of taxonomy that deals with the assignment of names to taxonomic groups in agreement with published rules.

In this unit, you will study about the earliest classification of bacteria and their classification based on Bergey's manual.

2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the basis of earliest microbial classification
- Explain the classification scheme of bacteria according to Bergey's manual of determinative bacteriology
- Understand Bergy's classification of determinative bacteriology

2.2 CLASSIFICATION OF BACTERIA ACCORDING TO BERGEY'S MANUAL

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In 1750, *Carl Linnaeus* devised the first classification system, placing plants and animals in separate systems. He placed all microorganisms in one genus which was named *Chaos.* The first attempt to classify microscopic plants and animals was apparently undertaken by Müller in 1773. He placed all of them in a group known as the 'infusoria'. Later classifications were proposed by Ehrenberg (1838), Dujardin (1841), Perty (1852), Cohn (1854), Zopf (1885) and Migula (1897). Cohn believed that bacteria showed more of the characteristics of plants than of animals and suggested that they be classified in the plant kingdom. Nägeli (1857) appeared to be the first to suggest the use of the term 'schizomycetes' (fission fungi) to include all organisms classified as bacteria. Cohn (1872) believed that it was a difficult task to classify bacteria into genera and species. He pointed out that so many species looked alike that a classification based on morphology alone was insufficient. Zopf (1879) did not agree with Cohn's idea of fixity of species; nevertheless he classified bacteria into four groups on the basis of morphology. The classification was as follows:

I. ***Coccaceae*** – Spherical forms.

II. ***Bacteriaceae*** – Spherical, rod and filamentous forms, with no distinction between base and apex.

III. ***Leptotricheae*** – Spherical, rod and filamentous forms, the latter with distinction between base and apex.

IV. ***Cladotricheae*** – Spherical, rod and spirals, and showing dichotomy.

The Three Kingdom System was given by Haeckel, a Swiss naturalist, in 1866. He was the first scientist to create a natural kingdom for the microbes, which had been discovered nearly two centuries before by Antony van Leeuwenhoek. Haeckel placed all unicellular (microscopic) organisms in a new kingdom, called 'Protista', on the level with the existing kingdoms for plants (Plantae) and animals (Animalia), which are multicellular (macroscopic) organisms. This was followed by creation of four kingdom system in 1950s. In this system four kingdoms were created. The cells lacking the intracellular compartment of nucleus were shifted to fourth kingdom called Monera. Protista remained as a kingdom of eurkaryotic microorganisms. Later in 1967 Whittaker, a botanist at the University of California, refined the system into five kingdoms, by identifying the Fungi as a separate multicellular eucaryotic kingdom of organisms, distinguished by their absorptive mode of nutrition.

The developments of various molecular techniques resulted in changes in classification of bacteria as well. Phylogenetic analysis of all forms of cellular life based on comparison of nucleotide sequences of the small subunit ribosomal RNA (ssrRNA) that is contained in all organisms was done by Carl Woese in 1970s.

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He found that rRNA found in all cells, it could be analyzed to determine the exact sequence of nucleotide bases in its makeup. His analysis of RNA molecules from different types of cells revealed a new dichotomy, among the prokaryotes. There exist two types of prokaryotes, as fundamentally unrelated to one another as they are to eukaryotes. Thus, Woese defined three cellular domains of life: Eukaryotes, Eubacteria and Archaebacteria. Whittaker's Plant, Animal and Fungi kingdoms (all of the multicellular eucaryotes) are at branch tips of the Eukaryote Domain, while other eukaryote branches lead to protists (unicellular algae and protozoa). Although the definitive difference between Woese's Archaea and Bacteria is based on fundamental differences in the nucleotide base sequence in the ssrRNA, there are many biochemical and phenotypic differences between the two groups of prokaryotes. Archaea are more closely related to eukaryotes than bacteria.

Thus, on the basis of small subunit ribosomal RNA (rRNA), the cell's membrane lipid structure and its sensitivity to antibiotics the Woese's classification forms a tree of life; this has three branches: Archaea, Bacteria and Eukaryotes. Bacteria and Archaea share the prokaryotic type of configuration while Eukaryotes consists of all eukaryotic cell-types, including protista, fungi, plants and animals.

Basic Concepts of Taxonomy

Taxonomy (Greek *taxis,* arrangement or order, and *nomos*, law, or *nemein*, to distribute or govern) is defined as the science of biological classification. In a broader sense, it consists of three separate but interrelated disciplines –

I. **Classification:** This is the arrangement of organisms into groups or taxa (s., taxon) based on mutual similarity or evolutionary relatedness.

II. **Nomenclature**: This is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.

III. **Identification:** This is the practical side of taxonomy, the process if determining that a particular isolate belongs to a recognized taxon.

Taxonomy is important for several reasons – First, it allows us to organize huge amounts of knowledge about organisms because all members of a particular group share many characteristics. Second, taxonomy allows us to make predictions and frame hypothesis for further research based on knowledge of similar organisms. Third, taxonomy places microorganisms into meaningful, useful groups with precise names so that microbiologists can work with them and communicate efficiently. Fourth, taxonomy is essential for accurate identification of microorganisms.

2.2.1 Classification of Bacteria

There is no 'official' classification of bacteria. The classification scheme which is accepted by most microbiologists is followed. This is according to Bergey's manual of determinative bacteriology which comes closest to providing a concise and accurate system of classifying bacteria. A classification that is of little use to microbiologists will soon be ignored or modified. The classification of bacteria has

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two purposes. One is purely for descriptive reasons while the other is to assess evolutionary descent by phylogenetic analysis. In the descriptive or determinative analysis the organisms are grouped into one common pool or group and to describe the basis on which these groups are made. The second purpose is to provide a natural phylogenetic classification aimed at reflecting the evolutionary descent. Here organisms are grouped into successive levels of ordering called taxons (Refer Table 2.1) in a hierarchical family tree of species, genus, family, tribe and order.

Table 2.1 *Taxonomic Ranks of Bacteria Explained with Example*

Formal rank	Example
Kingdom	Procaryotae
Division	Gracilicutes
Class	Scotobacteria
Order	Spirochaetales
Family	Spirochaetaceae
Genus	Treponema
Species	Treponema pallidum

Methods of Classification

There are two general ways in which classification system can be constructed:

- **Phenetic classification system** – Here, organisms are grouped together based on mutual similarity of their phenotypic characteristics. They compare many traits without assuming that any features are more phylogenetically important than others – that is, unweighted traits are employed in estimating general similarity. Organisms sharing many characteristics make up a single group or taxon. Obviously the best phenetic classification is one constructed by comparing as many attributes as possible. Although phenetic studies can reveal possible evolutionary relationships, they are not dependent on phylogenetic analysis.
- **Phylogenetic classification system** – Came into existence after the publication of *'On the Origin of Species'* by *Charles Darwin* in 1859. Here, organisms are grouped together based on probable evolutionary relationships rather than general resemblance. This has proved difficult for prokaryotes and other microorganisms, primarily because of the lack of good fossil records.

Interspecies classification methods

Many characteristics are used in classifying and identifying microorganisms at the species level. For sake of clarity, characteristics have been divided into two groups: Classical and Molecular.

- **Classical characteristics** – Classical approaches to taxonomy made use of morphological, physiological, ecological, and genetic characteristics. These characteristics have been employed in microbial taxonomy for many years. They are quite useful in routine identification and may provide phylogenetic information as well. This is called phenotypic classification.

- **Molecular characteristics** – Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids. Because these are either direct gene products or the genes themselves, comparisons of proteins and nucleic acids yield considerable information about true relatedness. These more recent molecular approaches have become increasingly important in prokaryotic taxonomy. This is also known as the genotypic classification.

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Phenotypic Classification

In order to identify and unknown organism and relate it to previously described organisms, diagnostic bacteriology has to rely on certain characteristics. Theses must be easy to recognize, i.e., visible features like shape, size, color, staining, motility, capsule and colony morphology; formation of characteristic chemical product/byproduct; nutrition, presence of characteristic macromolecule and habitat. Organisms are identified up to species level based on all factors. Species are a population of organisms that are capable of interbreeding only amongst members of the same group. Carolus Linnaeus (1707-1778) was a Swedish botanist and professor of medicine who gave a binomial system of classification for plants. He is known as the father of modern taxonomy. The same Linnaean scheme is followed in bacteriology. Each species is assigned an official Latin name with the name of genus in capital letters followed by the uncapitalized species designation. These methods of classification are based on phenotypic characters. These are liable to vary. As organisms evolve over time there occurs an accumulation of mutations in the genes. These genes thus differ not only in their products but also in their base sequence. The developments in molecular genetics have in the past few decades revolutionized the taxonomy. This has provided precise and quantitative methods for classification (Refer Table 2.2).

Table 2.2 *Some Morphological Features used in Classification and Identification*

Cell shape	All major groups
Cell size	All major groups
Colonial morphology	All major groups
Ultrastructural characteristics	All major groups
Staining behavior	Bacteria, some fungi
Cilia and flagella	All major groups
Mechanism of motility	Gliding bacteria, spirochetes
Endospore shape and location	Endospore forming bacteria
Spore morphology and location	Bacteria, algae, fungi
Cellular inclusions	All major groups
Color	All major groups

Some physiological and metabolic characteristics used in classification and identification are as follows:

- Carbon and nitrogen sources
- Cell wall constituents

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- Energy sources
- Fermentation products
- General nutritional type
- Growth temperature optimum and range
- Luminescence
- Mechanism of energy conversion
- Motility
- Osmotic tolerance
- Oxygen relationships
- pH optimum and growth range
- Photosynthetic pigments
- Salt requirements and tolerance
- Secondary metabolites formed
- Sensitivity to metabolic inhibitors and antibiotics
- Storage inclusions

Genotypic Classification

Nucleic Acid Base Composition - Microbial genomes can be directly compared, and taxonomic similarity can be estimated in many ways. The first, and possibly the simplest, technique to be employed is the determination of DNA base composition. DNA is composed of nucleic acids guanine, cytosine, adenine and thymine. Adenine pairs with thymine (A-T) while guanine pairs with cytosine (G-C). The pairing between guanine-cytosine is much stronger than adenine thymine. Hence the melting point of DNA, i.e. the temperature at which the two strands separate is determined by the G+C content in the DNA. The composition remains same in vertebrates (40% G+C and 60% A+T). This varies very much in bacteria. G+C content varies between 25-30moles% to 70-80 moles %. So, by measuring the G+C content it is possible to assign bacteria to species. For any particular species the G+C content remains the same. However this system is also not foolproof. The DNA composition can place phenotypically unrelated organism into one group. This can create confusion in routine diagnosis and identification schemes.

DNA contains four purine and pyrimidine bases: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T). In double-stranded DNA, A pairs with T, and G pairs with C. Thus the (G+C)/(A+T) ratio or **G+C content,** the percent of G+C in DNA, reflects the base sequence and varies with sequence changes as follows:

$$\text{Mol\% G + C} = \frac{\text{G + C}}{\text{G+ C + A + T}} \times 100$$

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The base composition of DNA can be determined in several ways. Although the G+C content can be ascertained after hydrolysis of DNA and analysis of its bases with high-performance liquid chromatography (HPLC), physical methods are easier and more often used. The G+C content often is determined from the melting temperature (*Tm*) of DNA. In double-stranded DNA three hydrogen bonds join GC base pairs, and two bonds connect AT base pairs. As a result DNA with a greater G+C content will have more hydrogen bonds, and its strands will separate only at higher temperatures—that is, it will have a higher melting point. DNA melting can be easily followed spectrophotometrically because the absorbance of 260 nm UV light by DNA increases during strand separation. When a DNA sample is slowly heated, the absorbance increases as hydrogen bonds are broken and reaches a plateau when the entire DNA has become single stranded. The midpoint of the rising curve gives the melting temperature, a direct measure of the G+C content. Since the density of DNA also increases linearly with G+C content, the percent G+C can be obtained by centrifuging DNA in a cesium chloride density gradient. The G+C content of many microorganisms has been determined. The G+C content of DNA from animals and higher plants averages around 40% and ranges between 30 and 50%. In contrast, the DNA of both eukaryotic and prokaryotic microorganisms varies greatly in G+C content; prokaryotic G+C content is the most variable, ranging from around 25 to almost 80%. Despite such a wide range of variation, the G+C content of strains within a particular species is constant.

Nucleic acid hybridization - The similarity between genomes can be compared more directly by use of nucleic acid hybridization studies. DNA sequences can change long before DNA composition changes. Even a single mutation can change the sequence. Homology of sequences (DNA-DNA homology) can be measured quantitatively in terms of the ability of the two strands from two different sources to form molecular hybrids in vitro. This is useful in closely related groups of bacteria, but is not applicable in the broad range of variation between two distant organisms. DNA-DNA hybridization is unequivocally the 'gold standard' for proposed new species and for the definitive assignment of a strain with ambiguous properties to the correct taxonomic unit.

Charles Sibley and *Jon Ahlquist* were the first scientists to pioneer this technique. If a mixture of single stranded DNA formed by heating dsDNA is cooled and held at a temperature about 25°C below the *Tm*, strands with complementary base sequences will reassociate to form stable dsDNA, whereas noncomplementary strands will remain single. Because strands with similar, but not identical, sequences associate to form less temperature stable dsDNA hybrids, incubation of the mixture at 30 to 50°C below the *Tm* will allow hybrids of more diverse ssDNAs to form. Incubation at 10 to 15°C below the *Tm* permits hybrid formation only with almost identical strands. In one of the more widely used hybridization techniques, nitrocellulose filters with bound nonradioactive DNA strands are incubated at the appropriate temperature with single-stranded DNA fragments made radioactive

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with 32P, 3H, or 14C. After radioactive fragments are allowed to hybridize with the membrane-bound ss DNA, the membrane is washed to remove any nonhybridized ssDNA and its radioactivity is measured. The quantity of radioactivity bound to the filter reflects the amount of hybridization and thus the similarity of the DNA sequences. The degree of similarity or homology is expressed as the percent of experimental DNA radioactivity retained on the filter compared with the percent of homologous DNA radioactivity bound under the same conditions. Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in *Tm* often are considered members of the same species. If DNA molecules are very different in sequence, they will not form a stable, detectable hybrid. Therefore DNA-DNA hybridization is used to study only closely related microorganisms. More distantly related organisms are compared by carrying out DNA-RNA hybridization experiments using radioactive ribosomal or transfer RNA.

Nucleic acid sequencing - Despite the usefulness of G+C content determination and nucleic acid hybridization studies, genome structures can be directly compared only by sequencing DNA and RNA. Techniques for rapidly sequencing both DNA and RNA are now available; thus far RNA sequencing has been used more extensively in microbial taxonomy. Most attention has been given to sequences of the 5S and 16S rRNAs isolated from the 50S and 30S subunits, respectively, of prokaryotic ribosomes. The rRNAs are almost ideal for studies of microbial evolution and relatedness since they are essential to a critical organelle found in all microorganisms. Their functional role is the same in all ribosomes. Furthermore, their structure changes very slowly with time, presumably because of their constant and critical role. Because rRNA contains variable and stable sequences, both closely related and very distantly related microorganisms can be compared. This is an important advantage as distantly related organisms can be studied only using sequences that change little with time. There are several ways to sequence rRNA. Ribosomal RNAs can be characterized in terms of partial sequences by the oligonucleotide cataloging method as follows. Purified, radioactive 16S rRNA is treated with the enzyme T1 ribonuclease, which cleaves it into fragments. The fragments are separated, and all fragments composed of at least six nucleotides are sequenced. The sequences of corresponding 16S rRNA fragments from different prokaryotes are then aligned and compared using a computer, and association coefficients (*Sab* values) are calculated. Complete rRNAs now are sequenced using procedures like the following. First, RNA is isolated and purified. Then, reverse transcriptase is used to make complementary DNA (cDNA) using primers that are complementary to conserved rRNA sequences. Next, the polymerase chain reaction amplifies the cDNA. Finally, the cDNA is sequenced and the rRNA sequence deduced from the results. Recently complete prokaryotic genomes have been sequenced. Direct comparison of complete genome sequences undoubtedly will become important in prokaryotic taxonomy.

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2.2.2 The Proteobacteria

Proteobacteria constitute the majority of known bacteria of medical, industrial, and agricultural significance.The group is defined primarily in terms of rRNA sequences. All Proteobacteria are Gram negative, with an outer membrane mainly composed of lipopolysaccharides. Most embers move using flagella, but some are non-motile or rely on bacterial gliding. The last include the myxobacteria, a unique group of bacteria that can aggregate to form multicellular fruiting bodies. There is also a wide variety in the types of metabolism. Most members are facultatively or obligately anaerobic and heterotrophic, but there are numerous exceptions. A variety of genera, which are not closely related to each other, convert energy from light through photosynthesis. These are called purple bacteria, referring to their mostly reddish pigmentation. The Proteobacteria are divided into six sections, referred to by the Greek letters alpha through zeta, again based on rRNA sequences. These are often treated as classes. The divisions of the proteobacteria were once regarded as subclasses (for example α-subclass of the *Proteobacteria*), but are now regarded as classes (for example the Alphaproteobacteria). Six sections of Proteobacteria are:

I. Alphaproteobacteria
II. Betaproteobacteria
III. Gammaproteobacteria
IV. Deltaproteobacteria
V. Epsilonproteobacteria
VI. Zetaproteobacteria

I. The Alpha Proteobacteria

A. It include most of the oligotrophic proteobacteria.

B. The Purple Nonsulfur Bacteria

1. Like all purple bacteria, the purple nonsulfur bacteria use anoxygenic photosynthesis, possess bacteriochlorophyls a and b and have their photosystems in membranes that are continuous with the plasma membrane.
2. Flexible in their choice of an energy source; normally they are grown anaerobically as photoorganoheterotrophs, but can grow aerobically.
3. They do not oxidize elemental sulfur to sulfate.
4. Most have not been cultured.
5. They are found in the mud and water of lakes and ponds with abundant organic matter and low sulfide levels.

A. *Rickettsia* and *Coxiella*

Rickettsia is placed in the α-proteobacteria while *Coxiella* will be in the γ-proteobacteria; however, they are discussed together because of similar life styles.

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1. These bacteria are rod-shaped, coccoid or pleomorphic, with typical gram-negative walls and no flagella.
2. Size varies but tends to be small (0.3 - 2.0 µm).
3. All are parasitic or mutualistic.
4. Rickettsias enter the host by phagocytosis; escape phagosome, and then reproduce in the cytoplasm by binary fission; in contrast *Coxiella* remains in the phagosome after fusion with a lysosome and reproduces within the resulting phagolysosome. Eventually the host cell bursts releasing new organisms.
5. Metabolism is different
   a. It lacks the glycolytic pathway, and does not use glucose as an energy source.
   b. Oxidize glutamate and tricarboxylic acid cycle intermediates (for example, succinate).

   Order contains many important pathogens. They also are important pathogens in dogs, horses, sheep, and cattle.
   a. *R. prowazekii* and *R. typhi* - typhus fever
   b. *R. ricketsii* - Rocky Mountain Spotted Fever
   c. *Coxiella burnetii* - Q fever

B. The *Caulobacteraceae* and *Hyphomicrobiaceae*

1. They have one of three distinguishing features:
   a. Prostheca - an extension of the cell, including the plasma membrane, that is narrower than the mature cell.
   b. Stalk - a nonliving appendage produced by the cells and extending from it.
   c. Reproduction by budding - parental cell retains its identity and progeny are much smaller than the parental cell.

1. *Hyphomicrobium* they are chemoheterotrophic, aerobic, budding bacteria that frequently attach to solid objects in freshwater, marine and terrestrial environments.
   a. Mature cell produces a hypha or prostheca that elongates.
   b. The nucleoid divides and a copy moves into the hypha while a bud forms at its end.
   c. The bud matures, produces one to three flagella, and a septum divides the bud from the hypha.
   d. The bud is released as an oval- to pear-shaped swarmer cell.

2. *Caulobacter*
    a. May be polarly flagellated rods or may possess prostheca and holdfast, by which they attach to solid substrata.
    b. Usually found in low-nutrient freshwater and marine habitats, but are also present in soil.
    c. Often adhere to bacteria, algae, and other microorganisms and may absorb nutrients released by their hosts.
    d. Prostheca differs from that of *Hyphomicrobium* in that it lacks cytoplasmic components and is composed almost totally of plasma membrane and cell wall.

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C. Family *Rhizobiaceae*

Contains the genera *Rhizobium* and *Agrobacterium*

1. Rhizobium
    a. Gram-negative, motile rods, often containing poly-hydroxybutyrate granules.
    b. Become pleiomorphic under adverse conditions.
    c. Grow symbiotically within root nodules cells of legumes as nitrogen-fixing bacteroids.

2. Agrobacterium
    a. Not capable of nitrogen fixation.
    b. Transforms infected plant cells into autonomously proliferating tumors.
    c. *A. tumefaciens* (best studied) causes crown gall disease by means of tumor including (Ti) plasmid.

A. Nitrifying Bacteria

1. *Bradyrhizobiaceae* - *Nitrobacter* and *Nitrococcus*
2. *Nitrosomonadaceae* - *Nitrosomonas, Nitrosococcus,* and *Nitrosospira*
3. All are aerobic, gram-negative organisms without endospores and able to oxidize either ammonia or nitrate.
4. May be rod-shaped, ellipsoidal, spherical, spirillar or lobate with either polar or peritrichous flagella.
5. *Nitrobacter* and *Nitrococcus* oxidize nitrite to nitrate.
6. *Nitrosomonas, Nitrosospira* and *Nitrosococcus* oxidize ammonia to nitrate.
7. If two genera such as *Nitrobacter* and *Nitrosomonas* grow together in a niche, ammonia is converted to nitrate (nitrification).
8. Nitrate is readily used by plants but also is easily leached from the soil or denitrified to nitrogen gas.

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II. The Beta Proteobacteria

Overlap alpha-proteobacteria metabolically but tend to use substances that diffuse from organic decomposition in the anaerobic zone of habitats. They exhibit considerable metabolic diversity.

A. Order *Neisseriales*

1. Members of the genus *Neisseria* are nonmotile, aerobic, gram-negative cocci that most often occur in pairs with adjacent sides flattened.
2. May have capsules and fimbriae.
3. Chemoorganotrophic, oxidase-positive, and almost always catalase-positive.
4. *Neisseria gonorrhoeae* - causative agent of gonorrhea.
5. *Neisseria meningitidis* - one of the causative agents of bacterial meningitis.

B. Order *Burkholderiales*

1. The order contains four families, three of them with well-known genera.

1. *Burkholderiaceae* - contains seven genera, formed from the genus Pseudomonas based on rRNA data, including the genus *Burkholderia.*

- Gram-negative, aerobic, nonfermentative, non-spore-forming, mesophilic, straight rods.
- All but one species are motile with a single flagellum or a tuft of polar flagella.
- Catalase-positive, often oxidase-positive.
- Use poly—hydroxybutyrate as their carbon reserve.
- *B. cepacia* is very active in recycling organic materials; is a plant pathogen; can cause disease in hospital patients due to contaminated equipment and medications; a particular problem with cystic fibrosis patients.

1. *Alcaliginaceae* - contains the genus *Bordetella*

- Gram-negative, aerobic coccobacilli.
- Chemoorganotroph, with respiratory metabolism, that requires organic sulfur and nitrogen (in the form of amino acids) for growth.
- Mammalian parasite that multiplies in respiratory epithelial cells.
- *B. pertussis* - non-motile, encapsulated species that is the causative agent for whooping cough.

2. *Comamonadaceae*

- Contains twelve genera with diverse characteristics.
- Some have a sheath, a hollow tubelike structure surrounding a chain of cells. It is helpful in several ways:
- Help bacteria attach to surfaces.

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- Help to obtain nutrients from slowly running water as it flows past.
- Help protect against predators.

3. *Sphaerotilus* forms long chains and prefers slowly running freshwater polluted with sewage or industrial waste; can form tangled masses that interfere with activated sludge tanks used in sewage treatment
4. *Leptothrix* deposits large amounts of iron and manganese oxides in its sheath; this seems to protect it and allows it to grow in the presence of high concentrations of soluble iron compounds.

C. Order *Nitrosomonadaceae*

Includes the nitrifying bacteria, *Nitrosomonas, Nitrosococcus*, and *Nitrosospira, Gallionella* - a stalked chemolithotroph and *Spirillaceae* - *Spirillum* and *Thiobacillus*; the latter is one of the best studied chemolithotrophs and the most prominent of the colorless sulfur bacteria.

III. The Gamma Proteobacteria

1. Largest subgroup of proteobacteria, including several deeply branching groups.
2. The 2nd edition of *Bergey's Manual* divides this group into 13 orders, 19 families, and around 130 genera
3. Divided into two families - Chromatiaceae and Ectothiorhodospiraceae in the order
   A. Family *Ectothiorhodospira* has red, polarly flagellated, spiral-shaped cells that deposit sulfur globules externally; internal photosynthetic membranes are organized as lamellar stacks.
   B. Family *Chromatiaceae* contain twelve genera. They are:
      1. Strict anaerobes and usually photolithoautotrophs; deposit sulfur granules internally.
      2. Hydrogen may serve as an electron donor.
      3. *Thiospirillum, Thiocapsa*, and *Chromatium* are typical and are usually found in anaerobic, sulfide-rich zones of lakes.
   A. Order *Thiotrichales*
   1. Contains some of the colorless sulfur bacteria that are nonphotosynthetic, nonfruiting, gliding bacteria
   2. Two of the best studied gliding genera are *Beggiatoa* and *Leucothrix*
   a. *Beggiatoa*
      a. Microaerophilic and grows in sulfide-rich habitats.
      b. They lack a sheath.
      c. Metabolically versatile; can oxidize hydrogen sulfide to sulfur (deposited internally) and can oxidize sulfur to sulfate; can also grow heterotrophically with acetate as a carbon source and some may incorporate CO_2 autotrophically.

b. *Leucothrix*
   a. Aerobic chemoorganotroph, marine, attached to solid substrates by a holdfast
   b. Complex lifestyle in which dispersal is by formation of gonidia

c. *Thiotrix* is a related genus that forms sheathed filaments and is chemolithotrophic; oxidizes hydrogen sulfide and deposits sulfur granules internally.

B. Order *Methylococcales*

1. Contains rods, vibrios, and cocci that use methane and methanol as their sole carbon and energy source (methylotrophs).
2. Contains five genera including *Methylococcus* (spherical, nonmotile) and *Methylomonas* (straight, curved, or branched rods with a single polar flagellum).
3. Complex arrays of intracellular membranes.
4. Methane is oxidized to methanol and then to formaldehyde which can then be assimilated into cell material.

C. Order *Pseudomonadales*

1. The genus *Pseudomonas* is the most important in this order and contains straight or slightly curved rods; motile by polar flagella, lack a sheath or prosthecae.
   a. Aerobic chemoheterotrophs
   b. Over 70 species in the genus *Pseudomonas*
   c. Around 25 species have recently been removed and at least seven new genera have been formed.
   d. Mineralization of a wide variety of organic compounds; useful in sewage.
2. The genus *Azotobacter* are large ovoid bacteria that are motile by peritrichous flagella; aerobic, catalase positive; fix nitrogen nonsymbiotically; widespread in soil and water.

D. Order *Vibrionales*

1. Contains one family that are gram-negative, straight or curved rods with polar flagella.
2. Oxidase-positive and use D-glucose as their sole or primary carbon and energy source.
3. Aquatic with widespread distribution in freshwater and marine habitats.
4. Five genera: *Vibrio, Photobacterium, Salinivibrio, Listonella*, and *Allomonas*.
5. Pathogens include *V. cholerae* - cholera, *V. parahaemolyticus* - gastroenteritis after eating contaminated seafood, and *V. anguillarum* - a fish pathogen.

6. Some, *V. fischeri* and at least two species of *Photobacterium* are among the few marine bacteria capable of bioluminescence; some bioluminescent species live symbiotically in the luminous organs of fish while others are free-living

E. Order *Enterobacteriales (Refer Table 2.3)*

1. One family containing over 35 genera.
2. Degrade sugars by Embden-Meyerhof pathway.
3. The majority (for example, *Escherichia, Proteus, Salmonella* and *Shigella*) carry out mixed acid fermentation while others (for example, *Enterobacter, Serratia, Erwinia* and *Klebsiella*) carry out butanediol Fermentation.
4. Gram-negative, peritrichously flagellated or nonmotile facultatively anaerobic, straight rods with simple nutritional requirements.
5. Similarity in appearance among enteric bacteria requires the use of biochemical tests for identification.
6. Rapid commercial identification systems (for example, Enterotube, API 20-E) are based on these tests.

Table 2.3 *Biochemical Characteristics of Enterobacteriaceae*

	TSI	ON	GAS	H2S	VP	IND	CIT	PDA	UR	MO	LYS	OR	AR
E coli	A/A	+	+	–	–	+	–	–	–	+	+	+/–	–/+
Shi A-C	Ak/A	–	–	–	–	–/+	–	–	–	–	–	–	–
Shi D	Ak/A	+	–	–	–	–	–	–	–	–	–	+	–
Ed	Ak/A	–	+	+	–	+	–	–	–	+	+	+	–
Sal	Ak/A	–	+	+	–	–	+	–	–	+	+	+	+/–
Cit	A/A Ak/A	+	+	+	–	–	+	–	+/–	+	–	–/+	+/–
Yer	A/A	+	–	–	–	+/–	–	–	+/–	RT (1)	–	+	–
	TSI	ON	GAS	H2S	VP	IND	CIT	PDA	UR	MO	LYS	OR	AR
Kle pne	A/A	+	+	–	+	–	+	–	+	–	+	–	–
Kle oxy	A/A	+	+	–	+	+	+	–	+	–	+	–	–
En aer	A/A	+	+	–	+	–	+	–	–	+	+	+	–
En cloa	A/A	+	+	–	+	–	+	–	+/–	+	–	+	+
Serr (1)	A/A	+	+	–	+	–	+	–	–	+	+	+	–
Haf	Ak/A	+	+	–	+	–	–	–	–	+	+	+	–
Pan	A/A Alk/A	+	–/+	–	+/–	–/+	+/–	–/+	–/+	–	–	–	–
	TSI	ON	GAS	H2S	VP	IND	CIT	PDA	UR	MO	LYS	OR	AR
P. mir	Ak/A	–	+	+	+/–	–	+/–	+	+	+s	–	+	–
P. vulg	A/A	–	+/–	+	–	+	–/+	+	+	+s	–	–	–
Mor	Ak/A	–	+	–	–	+	–	+	+	+	–	+	
Prov	Ak/A	–	–	–	–	+	+	+	+	+	–	–	–

RT = room temperature

Produces DNase, lipase, and gelatinase

S = swarming motility

Key : Shi a-V Shigella group At oC, Shi D: Shigella group D, Ed: Edwardsiella, Sal: Salmonella. Cit: Citrobacter, Yer: Yersinia

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Key: Kel pne: K. pneumaniae, Kle oxy: K. oxytoca, Serr: Serratia, Haf: Hafnia, Pan: Pantoea,

En aer: Enterobacter aergenses, En cloa: E. cloaca

Key: P. mir: P. mirabilis, P. vulg: P. vulgaris, Mor: Morgenella, Prov: Providenitia

Key for reactions: TSI Triple sugar iron agar, ON: ONPG, Ind: Indole, Cit citrate, UR: urease, Mo: motility, Lys: Iysine, Or: Ornithine, Arg: arginine

Order *Pasteurellales*

1. Small, nonmotile, normally oxidase-positive with complex nutritional requirements.
2. Parasitic in vertebrates.
3. Four genera: *Pasteurella, Haemophilus, Actinobacillus* and *Lonepinella*
4. Best known for the diseases they cause:
   a. *P. multilocida* - fowl cholera
   b. *P. haemolytica* - pneumonia in cattle, sheep and goats (e.g., ‘shipping fever’ in cattle)
   c. *H. influenzae* - major human pathogen that causes a variety of diseases, including meningitis in children.

IV. The Delta Proteobacteria

1. Small collection of genera but with diverse morphological and physiological characteristics.
2. Chemoorganotrophs placed in two orders and ten families.
   A. Order *Desulfovibrionales*
1. Gram-negative, dissimilatory sulfate- or sulfur-reducing bacteria; strict anaerobes; use elemental sulfur or sulfur compounds as electron acceptors during anaerobic respiration.
2. *Desulfovibrio* - sulfate reducing.
3. *Desulfuromonas* - uses only elemental sulfur.
4. Important in sulfur cycling.
5. Thrive in muds, polluted lake sediments, methane digesters.
6. Negative impact on industry because of their primary role in the anaerobic corrosion of iron in pipelines, heating systems, and other structures.
7. *Bdellovibrio* - Gram-negative curved rods with polar flagella.

B. Order *Myxococcales*

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1. The myxobacteria are gram-negative, aerobic soil bacteria with gliding motility.
2. Form fruiting bodies and dormant myxospores
3. Divided into four families.
4. Micropredators or scavengers that lyse bacteria and yeasts by secretion of an array of digestive enzymes.
5. Use the released peptides and amino acids as primary carbon, nitrogen, and energy source.
6. Found in neutral soils, decaying plant material, and animal dung.
7. Most abundant in warm areas but will grow in the arctic tundra.

V. The Epsilon Proteobacteria

A. Smallest of the proteobacteria groups.

B. Slender, gram-negative rods that can be straight, curved, or helical.

C. One order with two families and two important genera.

1. *Campylobacter* - contains both pathogenic and nonpathogenic species.

a. *C. fetus* - reproductive disease and abortions in cattle and sheep; can cause septicemia and enteritis in humans.

b. *C. jejuni* - causes abortions in sheep and enteritis diarrhea in humans

2. *Helicobacter* Isolated from stomachs and upper intestines of humans, dogs, cats and other mammals. *H. pylori* - cause of gastritis and peptic ulcer disease; produces large quantities of urease and urea hydrolysis appears to be associated with their virulence.

Table 2.4 shows some examples of bacterial classification according to G+C content.

Table 2.4 *Some Examples of Bacterial Classification According to G+C Content*

G+C moles %	*Examples of organisms included*
30-32	Clostridium tetani, c. perfringens.
32-34	C. bifermentans, Leptospira, Staphylococcus aureus.
34-36	Bacillus anthracis, other bacilli, Treponema pallidum
38-40	Haemophilus influenza, Streptococcus pneumonia, S. pyogenes, other Streptococci, Proteus vulgaris, Sporosarcina ureae
40-42	Neisseria catarrhalis
42-44	Bacillus subtilis, B. streathermophilus, Coxiella burnetti,
46-48	Vibrio cholerae, Yersinia pestis
50-52	Escherichia coli, Salmonella spp, Shigella spp, Neisseria meningitidis
52-54	Corynebacterium diphtheriae, enterobacter, Erwinia
54-56	Brucella abortus, Alcaligenes faecalis
56-58	Lactobacillus, corynebacterium spp.

NOTES

Check Your Progress

1. State three physiological and metabolic characteristics used in classification and identification of microbes.
2. When is the DNA-DNA hybridization used?
3. On what basis is the proteobacteria divided?
4. State two characteristics of Caulobactor.
5. Which is the smallest proteobacteria in the proteobacteria group?

2.3 BERGEY'S CLASSIFICATION OF DETERMINATIVE BACTERIOLOGY

In 1923, *David Bergey*, professor of bacteriology at the University of Pennsylvania, and four colleagues published a classification of bacteria that could be used for identification of bacterial species, the *Bergey's Manual of Determinative Bacteriology.* The first edition of Bergey's manual was initiated in 1923. Since then it has provided an outline of systematic bacteriology. Over the years it has been widely used an international reference book for bacterial taxonomy. It is based on a system of classifying bacteria by Gram reaction, metabolism, and morphology. The goal of the manual is to assist in the identification of bacteria and also to indicate the relationships which exist between various kinds of bacteria.

This manual is now in its ninth edition. In 1984, a more detailed work entitled *Bergey's Manual of Determinative Bacteriology* was published, still primarily phenotypic in its classification. Publication of the second edition of *Bergey's Manual of Systematic Bacteriology* was begun in 2001. The first volume of the second edition has been published recently. When it is completed, it will consist of 5 volumes –

- **Volume 1** – *The Archaea, and the Deeply Branching and Phototrophic bacteria*
- **Volume 2** – *The Proteobacteria*
- **Volume 3** – *The Low G + C Gram-Positive Bacteria*
- **Volume 4** – *The High G + C Gram-Positive Bacteria*
- **Volume 5** – *The Planctomycetes, Spirochaetes*

The classification in *Bergey's Manual* is accepted by most microbiologists as the best consensus for prokaryotic taxonomy. Here the classification based on phenotypic characteristics is given based on *Bergey's Manual of Systematic Bacteriology*. 1st edition. 4 vols. (1984). Kingdom prokaryote is divided into five divisions as shown in Table 2.5.

Table 2.5 *Classification of Bacteria According to Bergey's Manual*

Division/Class	*Name*	*Characteristics*	*Important members*
Division I	Gracilicutes	Procaryotes with thin cell walls, implying a gram-negative type cell wall	
Class I	Scotobacteria	Nonphotosynthetic Gram negative aerobic and anaerobic cocci and bacilli	Family: Spirochaetaceae, Spirosomaceae, Pseudomonadaceae, Enterobacteriaceae, Vibrionaceae, Bacteroidaceae, Neisseriaceae, Legionellaceae, Pasteurellaceae, Orders Rickettsiales and Chlamydiales.
Class II	Anoxyphoto-bacteria	Photosynthetic Gram negative bacteria that does not release oxygen	Rhizobiales, Rhodobacterales, Rhodospirillales
Class III	Oxyphoto-bacteria	Photosynthetic Gram negative bacteria	Cyanobacteria, blue green algae, algae such as Nostoc, Oscillatoria
Division II	Firmicutes	Procaryotes with thick and strong skin, indicating a gram-positive type cell wall	
Class I	Firmibacteria	Gram positive bacilli, non-branching, include spore forming members	Micrococcaceae, Streptococcaceae, Peptococcaceae, Bacillaceae, and Lactobacillaceae.
Class II	Thallobacteria	Branching Gram positive bacilli	Actinomyces, mycobacterium, Streptomyces
Division III	Tenericutes	Procaryotes of a pliable and soft nature, indicating the lack of a rigid cell wall	
Class I	Mollicutes	No cell wall, parasites	Mycoplasma, spiroplasma, ureaplasma
Division IV	Mendosicutes	Procaryotes that lack conventional peptidoglycan (archaeobacteria)	
Class I	Archaeobacteria	Unusual cell walls, no cellular organelles, membrane lipids, ribosomes, RNA sequences	Halobacteria, methanobacteria, pyrococcus

Ref: Bergey's Manual of Systematic Bacteriology. 1st ed. 4 vols. (1984)

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Division I Gracilicutes: The members have complex Gram negative type of cell wall consisting of an outer membrane and an inner thin peptidoglycan layer. This contains muramic acid but some organisms may have lost this portion of cell wall. Cells may be of various shapes including spheres, oval, straight or curved rods, helices or filaments. Usually reproduction is by binary fission but some groups may show budding or multiple fission (Pleurocapsules). Endospores are not formed and some may be sheathed or capsulated. Swimming, gliding or non-motility is common. It includes photosynthetic bacteria which are capable of synthesizing their own food. Some members may be obligate intracellular parasites. The division is further subdivided into three classes: Scotobacteria, Anoxyphotobacteria and Oxyphotobacteria.

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Class I Scotobacteria: It includes members of medically important bacteria of family Spirochaetaceae, Spirosomaceae, Pseudomonadaceae, Enterobacteriaceae, Vibrionaceae, Bacteroidaceae, Neisseriaceae, Legionellaceae, Pasteurellaceae and of orders Rickettsiales and Chlamydiales.

Class II Thallobacteria: These are Branching Gram positive bacilli which includes genus Actinomyces, mycobacterium, Streptomyces

Division II Firmicutes: Cells may or may not stain with gram stain. However, the cell structure is that of Gram positive bacteria. It has techoic acid and the amount of peptidoglycan layer is more. Cells may be rounding, straight or filamentous. These filaments may or may not show branching. Reproduction is by binary fission. Some bacteria may produce spores or resting forms or hyphae. All members are nonphotosynthetic. The members may be aerobic, anaerobic and facultative anaerobic bacteria.

Division III Tenericutes: These include members which lack cell wall and do not synthesize the precursors of peptidoglycan. The cells are enclosed by a unit membrane and are highly pleomorphic, small filterable elements. Reproduction is by budding, binary fission or fragmentation. They require complex media to grow and stain Gram negative. These are saprophytes or parasites of man and animals.

Division IV Mendosicutes: Most but not all members possess some form of cell wall, but they lack muramic acid and have no conventional peptidoglycan. Cell walls of some members may be made up of protein macromolecules and heteropolysacchrides. Morphological forms include cocci, bacilli, filaments and irregular forms. Gram stain is variable. Many are strict anaerobes. This group is ecologically most diverse including members which exist at extremes of temperatures. Methanogens, strict halophiles and thermoacidophiles are included in this group.

2.3.1 The Salient Features of Various Bacteria According to *Bergey's Manual of Determinative Bacteriology*

The manual is divided into varoius sections.

The Spirochaetes of Order I are shown in Table 2.6

Table 2.6 *Order I Spirochaetales*

Order I	***Spirochaetales***	
Family	Spirochaetaceae	Leptospiraceae
Genera	Spirochaeta	Leptospira
	Cristispira	Leptonema
	Treponema	
	Borrelia	
	Brachyspira	

General features of Order Spirochaetales: Helical shaped motile bacteria, size varies from 5 to 500 μm long, thin and Gram negative. The ends of

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the cells are not usually hooked. The cells are elongated motile flexible twisted spirally along the long axis. The bacteria are motile with the help of endoflagella. Endoflagella are polar flagella which are located between outer membrane and the cell wall. The spiral shape of these bacteria is also due to endoflagella. The outermost structure of the bacteria is a multilayered membrane called the outer sheath. The endoflagella are covered with this outer sheath as well. Their number can range from 2 to more than 100 per cell. One end of this flagellum is inserted near the pole of the protoplasmic cylinder and the other end is free. The ultra structure of the endoflagella is similar to the bacterial flagella. Spirochetes show three kinds of movements in liquid environment: flexion-extension, corkscrew shaped rotator movement and translator movements. The DNA G+C % mol content ranges from 25 to 65.

Family Spirochaetaceae: Members are helical, thin cells 0.1-3µm wide to 5-250µm long. The ends are usually not hooked. Cells are motile due to endoflagella. They are anaerobic, facultative anaerobic or microaerophillic in their habitat. They utilize carbohydrates and amino acids as energy sources and do not utilize long chain fatty acids. The members are free living in marine and freshwater bodies. Few members are parasites. The family is divided into four genera.

Genus I Spirochete: This includes free living species which are obligatory anaerobic or facultatively anaerobic. Spirochetes are chemo heterotrophic in nature, with lengths between 5 and 250 µm and diameters around 0.1-0.6 µm. There are 2 endoflagella present per cell. They are capable of migrating through agar media. *S. plicatilis* is taken as type species.

Genus II Cristispira: The name of this genus is due to the peculiar shape of crested coil formed due to the flagella. Typical cells of this genus measure about 0.5-3µm in diameter and 30-180µm in length. The cells are helical or undulate showing around 2-10 complete helical turns with rounded/blunt/tapered ends. Ovoid inclusions may be seen sometimes. Cell divides by transverse fission. The endoflagella are high in number around 100 per cell and they are twisted together to form a protoplasmic cylinder. This cylinder can distend the outer sheath to form a ridge or crest (aka crista). The members of this genus are found in helsthy molluscs as part of their normal flora. They have not been isolated in pure culture and are nonpathogenic. *Cristispira pectinis* is the type species.

Genus III Treponema: The name trepos meansto turn and nema means thread. This genus has members known to be pathogenic to man since a long time. The helical rods are short and slender around 0.5-20µm long and 0.1-0.4µm wide having fine spirals and pointed ends. They can from spherical cells under unfavorable environmental conditions. They have one or number of endoflagella and show all types of movements. Species grown in pure culture are obligate anaerobes. They utilize carbohydrates and amino acids as substrates. They can be found in oral cavity, intestinal tract or genital areas of animals and humans. Some species are pathogenic. All members are sensitive to penicillin.

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Genus IV Borrelia: The cells are 0.2-0.5μm in diameter and 3-10μm in length. The coarse, irregular and loosely coiled cells possess around 15-20 endoflagella which wind about the protoplasmic cylinder to overlap in the middle of the cells. The cells are actively motile. They stain well with Geimsa stain. The members grow in microaerophillic conditions are chemoorganotrophic and have complex growth requirements. Only few species have been cultivated in-vitro. They require rabbit serum, bovine serum albumin, peptones and pyruvate for growth in a culture medium. They are pathogenic to man birds and some animals. *Borrelia anserina* is the type species. Other species are *B.afzeli, B. burgdorferi, B. garinii, B. hermsii, B. recurrentis, B.vincentii* etc. More than 36 species of *Borrelia* are known, 12 of these species are known to cause Lyme disease or borreliosis which is a tick borne relapsing fever. *Borrelia recurrentis* is the main species causing relapsing fever in humans. It is transmitted by the human body louse or tick. It can cause epidemic louse borne and endemic tick borne relapsing fever. Humans are the only reservoir of epidemic relapsing fever. No other animal reservoir of *B. recurrentis* is known. Lice that feed on infected humans acquire the organisms which then multiply in the gut of the louse. When an infected louse feeds on an uninfected human, the organism can gain entry in a new host.

Genus V Brachyspira: This includes only one species *Brachyspira aalborgi*. It is parasitic to man. The cells of Brachyspira are very thin, fine about 0.2-1.7 to 6 μm in size. It is chemo-organotrophic and can be isolated in anaerobic conditions. It can be grown in laboratory on tryptose soy agar with 5% calf blood.

Genus VI Serpulina: It was earlier known as Brachyspira. It was created by Stanton as a separate genus in1991. The cells are Gram-negative helical cells with loose coils. Morphological and cultural features are similar to Brachyspira. *S. hyodysentriae* is species pathogenic to pigs.

Family II Leptospiraceae: The cells are helical, flexible 0.1μm in diameter and 6-12μm long. One or both ends if the cells are characteristically hooked. Each cell possesses two endoflagella which rarely overlap in the central region. The cells are motile. The organisms are chemo-organotrophs and can be grown aerobically. They do not utilize carbohydrates or amino acids as energy sources. The members are free living or in association with animals or human hosts. It includes two genera: Leptospira and Leptonema.

Genus I Leptospira: The cells are flexible, helical rods 0.1μm in diameter and 6-20μm in length. They are motile and their ends are typically hooked. Each cell has two endoflagella and 18 or more coils. The morphological features are otherwise similar to those seen in order spirochaetales. Unstained cells are not seen or stain very faintly with aniline dyes. They can be visualized by darkfield illumination and phase contrast microscopy. They are Gram negative cells having a cell envelope consisting of a cytoplasmic and outer membrane. The peptidoglycan layer is associated with the cytoplasmic rather than outer membrane. They grow optimally at 28-30°C. They can exist in vast diverse environments. The nutritional requirements are very simple utilizing fatty acids as their source of energy. *Leptospira interrogans* is the type species. Members of *Leptospira* are grouped into serovars according to their antigenic relatedness.

NOTES

Genus II Leptonema: Morphological features are similar to leptospira except a few minor differences in ultrastructure of flagella and intracytoplasmic tubules. It grows easily in ordinary media. Only one species is known *Leptonema illini*. It is nonpathogenic.

Aerobic/Microaerophillic: Motile, Helical/Vibrioid Gram-Negative Bacteria

Members are Gram-negative helical or vibrioid cells, motile by polar flagella. They swim in straight line with typical corkscrew like motion. May be aerobic or microaerophillic. Growth is poor in anaerobic conditions. Most members fail to utilize carbohydrates. They are chemoorganotrophs which are oxidase positive, and do not produce indole. Members exist in freshwater, soil, plant roots, oral cavity of man and animals or marine environment. Some members found in soil are capable of fixing atmospheric nitrogen. Some members are pathogenic to man and animals. Three genera Micavibrio, Bdellovibrio and Vampirovibrio are predatory on other microorganisms. Many genera are included in this section.

Genus Alteromones: These are Gram-negative, curved bacilli 0.7-1.5µm to 1.8-3µm in size. They are motile with the help of single polar flagella. They do not form endopores or microcysts. They grow optimally at 20°C and not at 35°C. They grow at 4°C as well. They survive in sea water and require a sea water base for laboratory cultivation also. They do not reduce nitrates to nitrites. It liquefies gelatin and has lipase and chitinase activity. Type species is *A. undina.*

Genus Aquaspirillum: These are Gram negative, straight, helical or vibrioid cells which are 0.2-1.4 in diameter. The members are generally motile with the help of polar flagella which are usually present at both poles in the form of tufts. All species are aerobic. They grow optimally at 30-32°C. They occur in stagnant fresh water environments and do not tolerate more than 3% sodium chloride. Carbohydrates are not catabolized. Few species can denitrify. Amino acids or salts of organic acids are often utilized as source of energy. They are oxidase, catalase and phosphatase positive. *Aquaspirillium serpens* is the type species. Other species included in this genus are *A. gracile, A. delicatum, A. polymorphum* etc.

Genus Azospirillum: The cells are large about 1µm in diameter, plump, slightly curved and straight bacilli, often having pointed ends. They are Gram variable on staining and are motile by single polar flagella. They grow optimally at 35-37°C. They grow easily on ordinary media such as potato agar often producing pink colored colonies. Some strains require biotin for growth. They exist free living in soil or in roots of various plants. *A. lipoferum* is the type species. Other species include *A. brasilense*, A. rugosum *and A. picis*. They are capable of fixing atmospheric nitrogen. *A. lipoferum* is used as a biofertilizer as it not only serves to fix nitrogen but also provide essential growth factors for the development of plants.

NOTES

Genus Bdellovibrio: This is a unique group which includes predatory members. The cells are curved rods 0.2-0.5µm in diameter. They are highly motile. They move with the help of single sheathed polar flagella. Members parasitize other Gram-negative bacteria by entering into their periplasmic spaces and feeding on the biopolymers. A unique biphasic life cycle is seen in Bdellovibrio. The highly motile cells attack their prey. It rapidly penetrates the cells and looses its flagella. It soon suspends all metabolic activity of the affected cell and uses the cell as substrate for its own development. The developing Bedellovibrio elongates into a snake like structure which fragments to form more of itself. It fragments to produce small, motile curved cells which leave the prey to star their own cycle. Prey independent strains grow to large structure before fragmenting into smaller forms. They live in soil, sewage freshwater and marine environments acting as natural cleansers. Type species is *B. bacteriovorus*.

Genus Campylobacter: The cells are slender curved thin cells 0.2-0.5µm wide and 0.5-5µm long. They are non-spore forming and may have one or more spirals. They are motile with corkscrew type motion with the help of long polar flagella at one or both ends of the bacterium. They do not ferment or oxidize carbohydrates. They are microaerophillic requiring an oxygen tension of 3-5%. They grow well at 35-37°C. Few strains can grow in aerobic conditions also. It does not require any enrichment in the form of blood or serum. They are oxidase positive, urease negative and reduce nitrates. The members are found in reproductive organs, intestinal tract and oral cavity of man and animals. Some species are pathogenic to man. It produces inflammatory, self-limiting bloody diarrhoea including cramps, fever and pain. *C jejuni* and *C. coli* are commonly involved. Type species is *Campylobacter fetus*. *C fetus* can cause spontaneous abortions in cattle and sheep. It can also cause opportunistic infection in man.

Genus Cellvibrio: Curved cells are 0.2-0.5µm in diameter and 1-1.3µm long. The cells are Gram negative and motile. Each cell possess one polar flagella and 11 lateral flagella. The species are aerobic growing well at 25-35°C. It is oxidase positive, oxidizes glucose and degrades cellulose. It exists in soil and salty waters. *C.mixtus* is the type species. They are not known to cause any disease in man or animals.

Genus Halovibrio: This includes obligatory aerobic organisms which require a high level of salt for their growth. At least 7% sodium chloride is required for the growth of halovibrios. They grow between 15-35°C. The cells are small, thin curved rods which are motile by polar flagella. Type species is *H. variabilis* which is found in Great Salt Lake of North America.

Genus Helicobacter: The cells are thin curved/straight bacilli about0.5-1µm in diameter and 2.5-5µm in length. They show rapid and typical darting motility by means of sheathed polar flagella. The flagella may be unipolar or bipolar and lateral with terminal beads. The organisms are microaerophillic and grow best at 10% carbon dioxide. The strains are slow growing in laboratory on brain heart infusion broth and chocolate agar. It can take up to 5-8 days to grow. They grow best at 37°C but do not show any growth at 25°C. They are strongly catalase and oxidase positive. Their ability to utilize urease at a very rapid rate is often utilized for their detection in clinical samples.

Genus Herbaspirillum: It includes only one species called *H. serpedicae*. It is Gram-negative, Motile, curved rod. It possesses 1-3 polar flagella. It grows in microaerobic environment at 34°C between pH of 5.3 to 8. It is a nitrogen fixer and is found in soil or in root nodules.

Genus Marinomonas: It has only one species *M. communis*. It is Gram negative, curved non-sporeforming motile rod. Motility is due to polar flagella. It requires sea water for its growth. It is found in sea water and coastal areas.

Genus Micavibrio: *M. admirandus* is the only species. It is curved rod motile by means of single unsheathed flagella. It is predatory on the cells of *Xanthomonas maltophilla*. It is an exoparasite which develops on the outside of the cells. It attaches to the cell but does not invade, causing lysis of the cells. It cannot survive in the absence of its host.

Genus Oceanospirallum: The members have been isolated from coastal sea water, decaying seaweed and murine mussels. They are motile with the help of single or tuft of flagella to poles. *O. linum* is the type species. It requires sea water for its growth.

Genus Spirillum: The cells are helical, motile by a tuft of bipolar flagella. They are found in stagnant freshwater and are microaerophillic. *S. volutans* is the type species.

Genus Sporospirillum: Huge helical cells 1.8-4.8µm in diameter and 40-100µm long. These are found in the intenstinal tract of tadpoles. They have never been cultivated.

Genus Vampirovibrio: The species are predatory on eukaryotic algae *Chlorella*. They are motile cells which do not penetrate their host cells.

Non-motile (or Rarely Motile), Gram-Negative Curved Bacteria

Members follow strictly respiratory type of metabolism. The cells are curved. Sometimes coils or helical shapes may also be seen. Overlapping of cells can give rise to ring formation. Most members are non-motile. None of the members are capable of causing disease in man.

Genus Ancylobacter: Curved cells, characterized by formation of rings prior to separation. These are Gram-negative cells which are generally non-motile. Some strains produce gas vacuoles. These are obligatory aerobes growing best at22-37°C. They are oxidase positive forming pellicle in liquid media. They are found in soil and fresh water sources. Type species is *A. aquaticus*.

NOTES

Genus Brachyarcus: The cells are non-motile, bow shaped with gas vacuoles. These are rod-shaped organisms that are bent in an arc by asymmetric, polar cell growth and that frequently assume the shape of a pretzel. Cell division in the center and separation result in two arcs that face each other forming a clover leaf pattern. They may contain sulfur granules. They can attach to surfaces by means of mucoid substance. They grow anaerobically. They are found in ponds and lakes where sulfide is present and oxygen is absent. Type species is *B. thiophilus*.

Genus Cyclobacterium: These are similar to other genera morphologically. They tend to grow in marine environment and require sea water for growth. They do not ferment sugars and are grown aerobically. *C. marinum* is the type species.

Genus Flectobacillus: Rigid straight or curved ring forming bacilli.They are non-motile and obligate aerobes. They have been isolated from fresh and marine waters. *F. major* is the type species.

Genus Meniscus: Gram-negative encapsulated, non-motile, curved or straight cells are generally present. But sometimes pairs, tightly coiled spirals, S shaped and doughnut shaped cells are seen in culture. They are anaerobic but can be aerotolerant. They are known to exist in nature in anaerobic digester sludge. *M. glaucopis* is the type species.

Genus Pelosigma: The cells are S shaped, slender filaments arranged in aggregates of four or multiples of four. The cells are held together by mucoid substance and the whole aggregate may be motile by means of a tuft of flagella. They occur in mud in fresh and brackish waters where sulfide is present. *P. cohenii* is the type species. They have not been isolated in pure form.

Genus Runella: The cells may appear ring shaped due to overlapping of ends. They are nonmotile, obligate aerobe. Colonies produce pale pink colored water soluble pigment. They have been isolated from fresh water. Type species is *R. slithyformis*.

Genus Spirosoma: The cells are rigid straight to curved rods which are nonmotile. Rings may be formed by overlapping of cells. Coils may also be present. They are obligate aerobes. They have been isolated from soil and fresh water. *S linguale* is the type species.

2.3.2 Gram-Negative Aerobic Rods and Cocci

This is one of the largest groups divided into many families and more than 80 genera. It includes organisms causing disease in man, animals and in plants. They are chemoporganotrophic and aerobic. They are nonfermentative, oxidase positive bacteria which are motile by polar or peritrichous flagella. They are mostly saprophytes existing in soil, water and in other environments. Table 2.7 shows various families of section 4.

Table 2.7 *Various Families of Section 4*

Family I	Pseudomonadaceae
Family II	Azotobacteraceae
Family III	Rhizobiaceae
Family IV	Methylococcaceae
Family V	Halobacteriaceae
Family VI	Acetobacteraceae
Family VII	Legionellaceae
Family VIII	Neisseriaceae
Family IX	Xanthomonadaceae
Family X	Moraxellaceae

NOTES

Family I Pseudomonadaceae: The members are oxidase positive due to the presence of cytochrome c oxidase enzyme, non-fermentative and motile by means of polar flagella. The cells are straight or slightly curved Gram-negative rods. Most members metabolize glucose by the Entner Doudoroff pathway mediated by the enzymes 6 phosphoglyceraldehyde dehydrogenase and aldolase. They produce pigments like pyoverdin, pyorubin and pyomelanin. Growth occurs between 4°C and 43°C.

Salient features of few genera that are included in Section 4 are given below:

Genus Azomonas, Azomonotrichon and Azotobacter: The cells are round to coccobacilli occurring singly, in pairs or in clumps. They show extensive pleomorphism. They do not form endospores but are capable of forming cysts. They are motile by means of polar or peritrichous flagella. They strains are aerobic growing in soil and water. They produce water soluble and florescent pigment. *A. agilis* is the type species. They are capable of fixing nitrogen in the soil. One species occurs in association with plant roots.

Genus Mesophilobacter: The name *Mesophilobacter* includes 11 strains of aerobic, mesophilic, nonmotile, oxidase-positive, penicillin-resistant, moderately halophilic, gram-negative coccobacilli that were isolated from seawater. The genus *Mesophilobacter* is closely related to the genera *Acinetobacter, MoraxeZZu,* and *Psychrobacter,* but the levels of deoxyribonucleic acid relatedness to members of these genera are low. The mesophilobacters have optimal growth temperatures of 33 to 37°C and can grow in broth containing 7% sodium chloride. *Mesophilobacter marinus* is the type species.

Genus Pseudomonas: These are found world wide in nature. They are found in soil, water, vegetations, plants and animals. They are found in hospital environment and are responsible for causing many hospital acquired infections. They are capable of developing resistant to antibiotics. Also they are resistant to commonly used disinfectants like cetrimide in the hospitals. The cells are straight or slightly curved Gram negative motile by means of polar flagella. They are non-sporing noncapsultaed, rarely mucoid strains produce slime. When grown in laboratory the strains of *P. aeruginosa* produce musty, mawkish, earthy smell. This is due to a chemical named 2-aminoacetophenone produced by these bacteria.

They are strict aerobe, nonfastidious, grow at 5-42°C. The optimum temperature is 37°C. They form surface pellicle in broth and give rise to iridescent patches with metallic sheen on nutrient agar. Some strains are hemolytic in blood agar. They produce various kinds of pigment produced on solid media.

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Family II Azotobacteraceae: The cells are large ovoid, may or may not be motile, pleomorphic, motile, aerocillay growing. They occur in soil and water. It includes two genera Azotobacter and Azomonas.

Family II Rhizobiaceae: Cells are nonsporing bacilli and motile by means of polar or subpolar flagellum. Star shaped clusters may be seen. They poduce hypertrophies on plants and result in nodule formation in roots of leguminous plants. They show symbiotic relationship fixing atmospheric nitrogen .They include four genera: *Rhizobium* which are fast growing, *Bradyrhizobium* which are slow growing, *Agrobacterium* which do not cause root nodule formation and *Phyllobacterium*. Nitrogen fixing abilities of Phyllobactium are controversial and they cause nodule formation on leaves of ceratin plants.

Genus Rhizobacter: The cells are Gram-negative, capsulated, straight or curved rod with polar flagella or lateral flagella or both and a cell diameter of 0.9 to 1.3 pm. The bacterial cells accumulate poly-P-hydroxybutyrate granules. Glucose is metabolized oxidatively. The bacteria are positive for oxidase and catalase reactions and are susceptible to vibriostatic agent 0129 phosphate. Colonies grown on dilute potato-peptone-glucose agar medium are white, tough, and highly plicated; when grown on dilute yeast extract-peptone-glucose agar medium, they are yellowish white. No water-soluble pigment is produced. In liquid media the bacterium grows as abundant, primarily globular flocs. *R. daucus* can use various kinds of carbon sources, including sugars, polysaccharides, and sugar alcohols, but not benzene derivatives. It is known to cause overgrowths on storage roots has been observed in Japan. They exist in soil and are capable of fixing nitrogen.

Family IV Methylococcaceae: These are now given their own order. These groups of bacteria obtain their carbon and energy from methane. They belong among the gamma subdivision of the Proteobacteria in the new classification. They are Gram negative pleomorphic rods, cocci and vibrios. It includes two genera Methlomonas and Methylococcus.They have internal membranes in the form of flattened discs which are perpendicular to the cell wall. Methane is oxidized to give formaldehyde, which is fixed by a process called the RuMP cycle. Here formaldehyde is combined with the sugar which in turn is broken down to produce glyceraldehydes. This is then used to produce new ribulose and other organic compounds. Catabolism does not involve a complete citric acid cycle.

Family V Halobacteriaceae

Genus Halobacterium: Cells are regular, pleomorphic, rods or discs may be motile or nonmotile. The organisms are responsible for red heat in slated tides and pink in saltwater fish. *H. salinarium* is the type species.

Genus Halococcus: Cells are nonmotile cocci occurring in pairs, tetrads or irregular clusters. They occur in salty lakes and ponds. Type species is *H. morrhuae*.

Family VI Acetobacteriaceae: Cells are ellipsoidal Gram negative to variable occurring single on in pairs, motile by peritrichous flagella or 1-8 polar flagella. It occurs in slighly. It include two genera, Acetobacter and Gluconobacter

Family VII Legionellaceae: The cells are non-sporing, not capsulated, non acid fast, Gram negative bacilli. They are motile by means of two or more polar or lateral flagella. It includes only one genus *Legionella. Legionella* is common in many environments, with more than 50 species and 70 serogroups. They have been isolated from surface water, mud, lakes and ponds. Common sources of *Legionella* are commonly found in cooling towers, large central air conditioning systems, domestic hot water systems, fountains and swimming pools. *L. pheumophilla* is the type species. All species are pathogenic to man. *L. pheumophilla* causes pneumonia in humans. *Legionella* acquired its name after an outbreak of a then-unknown 'mystery disease' in July1976 which sickened 221 persons, causing 34 deaths. The outbreak was first noticed among people attending a convention of the American legion in Philadelphia.

Family VIII Neisseriaceae

Genus Neisseria: The family Neisseriaceae consists of Gram-negative aerobic bacteria from fourteen genera including *Neisseria, Chromobacterium, Eikenella,* and *Kingella.* The *Neisseria* are a large family of commensal bacteria that colonize the mucosal surfaces of many animals. The cells are round 0.6-1µm in diameter occurring singly or often in pairs with adjacent sides flattened. *N. elongata* is the only exception which exists as short rods. No motility is seen and there are no flagella. The strains are aerobic. Some species are fastidious requiring blood for growth. Isolation needs an environment of 3-10% carbon dioxide. All are catalase and oxidase positive except *N. elongata*. They usually inhabit the mucous membranes of humans and animals.

Family IX Xanthomonadaceae

Genus Burkholderia: It includes 4 species which were previously included in Pseudomonas namely *B. cepacia, B. mallei, B. pseudomallei and B. gladioli. B. cepacia* is now named *B. xenovorans*. The cells are straight or slightly curved, non-sporing, non-capsultaed, Gram negative rods which are motile by means of polar flagella. They are small 2.5´0.5mm Gram-negative bacilli, nonmotile, beaded. They are ubiquitous motile, obligately aerobic rod-shaped bacteria including both animal/human and plant pathogens as well as some environmentally-important species. They are aerobe and facultative anerobe and non fastidious. They form small translucent colonies that turn yellow and opaque on aging. They are non fermenters fermenting only glucose with acid and no gas.

Genus Stenotrophomonas: This is included in family Xanthomonadaceae. They are small Gram-negative bacilli that are motile by means of polar flagella. It is catalase and oxidase positive except for S. maltophila which is oxidase negative. They are ubiquitous in aqueous environments, soil and plants, including water, urineor in respiratory secretions. It has also been used in many biotechnology applications. In immunocompromised patients, *S. maltophilia* can lead to nosocomial infections. It is an oppurtunistic pathogen causing urinary tract infection, wound infection & septicemia.

Family X Moraxellaceae

Genus Acinetobacter: It is a member of family Moraxellaceae. Acinetobacter is a compound word from scientific Greek, meaning 'nonmotile rod'. They are Gram-negative, non-motile, oxidase negtaive bacilli. The genus comprises of 17 validly named and 14 unnamed (genomic) species. These are widely distributed in nature. They are able to survive on various surfaces (both moist and dry), on medical equipments and even on healthy human skin in the hospital environment thereby being a major source of infection in debilitated patients. Several species persist in hospital environments and cause severe, life-threatening infections in immunocompromised patients. They have found numerous biotechnological applications as these strains are often ubiquitous, exhibit metabolic versatility, are robust and some provide convenient systems for modern molecular genetic manipulation and subsequent product engineering.

Genus Moraxella: The cells are Gram negative cocci, coccobacilli and bacilli which are oxidase positive and member of the family Moraxellaceae. All members are rods except *M. catarrhalis* which are cocci. The members are widely distributed in humans and animals. The genus is under constant revision. *M. bovis* and *M catarrhalis* can cause infections in humans. Clinically, species of this genus are known to cause ear infections, respiratory infections, septecemias, bacteremias and joint infections. The species are known to exist in cats, dogs, sheep and horses beside other animals, humans and in fresh water.

Genus Zoogloea: Bacteria of the genus *Zoogloea* have historically been considered members of the family Pseudomonadaceae but have been differentiated from other obligately aerobic, Gram-negative, nonsporeforming, rod-shaped bacteria that grow in aquatic habitats on the basis of their production of a characteristic gelatinous matrix. The 'zoogloeal matrix' surrounds clumps of cells found in natural aquatic habitats. They have been isolated from sewage.

2.2.3 Facultatively Anaerobic Gram-Negative Rods

This includes three families *Enterobacteriaceae, Vibrionaceae and Pasteurellaceae* and few genera of lesser importance. Except *Vibrionaceae* all are straight rods. Members of *Vibrionaceae* are curved rods. Cells are gram negative aerobic bacilli found free living or in association with animals or humans.

Subgroup 1 Family Enterobacteriaceae

It is one the most widespread group including many bacteria which are of medical importance. Bacteria are 0.3-1.8μm long, stain gram-negative, may or may not be motile. When motile it is by means of peritrichous flagella, except fro *Tatumella* which has lateral flagella. They are facultatively aerobic growing best at 37°C, though many members can grow at 25°C. They are non-halophylic and chemoorganotrophs having either oxidative or fermentative type of metabolism.They will grow on most simple bacteriological media. Although they show certain biochemical properties many of these are variable and are used to differentiate between them. Glucose and other sugars are metabolised to produce acid or acid and gas. They are catalase positive (except *shigella dysentriae type 1* and *Xenorhabdus* species) and oxidase negative. They reduce nitrates except *Arsenophorus,* some species of *Erwinia,* most *Xenorhabdus, Yersinia, some strains of Klebsiella pneumonia subsp. Ozaenae* and *Pantoea.* They are present worldwide in soil, water, fruits, vegetables, grains, flowering plants, animals and humans. They are present as commensal flora in animals and humans. However, many species are capable of causing life threatening infections. Agents causing diarrhoea, dysnentry, urinary infections, typhoid etcl., belong to this group. There are more than 30 genera in this family. Type genus is *Escherichia*. Table 2.8 shows genera included in this family.

Table 2.8 *Genera included in This Family*

Aishewanella	Brenneria	Edwardsiella	Klebsiella	Photorhabdus	Raoultella	Trabulsiella
Alterococcus	Budvicia	Enterobacter	Kluyera	Poodoom-aamaana	Salmonella	Wigglesworthia
Aquamonas	Buttiauxella	Erwinia	Leclercia	Plesiomonas	Samsonia	Xenorhabdus
Aranicola	Cedecea	Escherichia	Leminorella	Pragia	Serratia	Yersinia
Arsenophonas	Citrobacter	Ewingella	Morgenella	Proteus	Shigella	Yokenella
Azotivirga	Cronobacter	Grimontella	Obesumbacterium	Providencia	Sodalis	
Blochmannia	Dickeya	Hafnia	Pantoea	Rahnella	Tatumella	

These can be differentiated on the basis of various biochemical tests in the laboratory. Salient features of common genera are given below:

Genus Escherichia: Members of this genus are inhabitants of the gastrointestinal tract of warm blooded animals and provide a portion of the vitamin K to their host. While many *Escherichia* are harmless commensals, particular strains of some species are human pathogens and are known as the most common cause of hospital acquired infections, urinary tract infections, gastrointestinal disease, ranging from simple diarrhoea to fatal hemolytic ureaemic syndrome. The cells are motile. Biochemically they are citrate negative, Utilize glucose and lactose producing acid, show negative decoxylation reactions, and are positive for malonate, indole production. Occur in intestinal tract of humans and animal. *E. blattae* is in found in gut of cockroaches. *E. coli* is the type species. It is found in humans as part of normal flora and is also pathogenic. The bacteria can also be grown easily and its genetics is comparatively simple and easy to manipulate or duplicate making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology.

Genus Shigella**:** The strains are morphologically and biochemically similar to *Escherichia*, barring few exceptions. They are non-motile and do not ferment sugars with gas. Also, they are lactose non-fermenters and negative for citrate, H_2S production, urease and VP reaction. *S. dysentriae* is the type species. Four species are known to cause infections in man: subgroup A *S. dysentriae* (12 Serotypes), subgroup B *S. flexneri* (6 serotypes), subgroup C *S. boydii* (23 serotypes) and subgroup D *S. sonnei* (1 serotype). They are pathogens to humans and animals causing shigellosis characterized by bacillary dysentriae. *S. flexneri* is the most frequently isolated species worldwide and accounts for 60% of cases in the developing world; *S. sonnei* causes 77% of cases in the developed world, compared to only 15% of cases in the developing world; and *S. dysenteriae* is usually the cause of epidemics of dysentery.

Genus Salmonella: The genus *Salmonella* was named after Daniel E Salmon and not after salmon fish. It includes the causative agent of typhoid in man. They can also cause fever, gastroenteritis and septecemia. The cells are motile. The organism ferment glucose with acid or acid and gas, are non lactose fermenting, indole and urease negative. Hydogen sulphide is usually produced and citrate is utilized. Phenyalanine (PPA) and tryptophan are not deaminated. *Salmonella* is closely related to the Genus Escherichia. It is found worldwide in cold- and warm-blooded animals (including humans), and in the environment. *Salmonella* infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. The bacteria can survive for several weeks in a dry environment and for several months in water. Hence, they are frequently found in polluted water which is, contaminated from the excreta of carrier animals including humans. Aquatic vertebrates, birds and reptiles can carry salmonella. Poultry, cattle, and sheep frequently being agents of contamination, salmonella can be found in food, particularly meats, eggs and even in dairy products.

Genus Klebsiella: It includes non-motile, Gram-negative, oxidase negative, bacilli which have a prominent polysaccharide capsule. It is a common human pathogen implicated in urinary tract infections, septicemias, pneumonias and soft tissue infections. Presence of capsule and polysaccharide slime is responsible for its virulence. *Klebsiella* species are ubiquitous in nature. Some strains can fix nitrogen. Biochemically they ferment glucose and lactose producing acid and gas, are negative for indole, H_2S, motility and ornithine decarboxylase. They utilize citrate and produce urease enzyme. *K. pneumoniae* is the type species.

Genus Enterobacter: This genus in motile, Gram negative, oxidase negative, bacilli which utilize citrate and do not produce urease enzyme or give positive indole test. They ferment glucose and lactose producing acid and no gas. They do not produce H_2S, lipase, DNase and Tween 80 esterase. They are widely present in nature in humans and animals. Few strains of these bacteria are pathogenic causing oppurtunistic infections in immunocompromised and hospitalized patients. Most commonly they cause infections of the urinary tract and respiratory tract. It is also a fecal coliform.

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Genus Erwinia: It is a motile gram negative rod-shaped bacterium. It utilizes acetate, fumarate, gluconate, malate and succinate.The members of this genus are mostly plant pathogens. It produces fireblight on apple, pear, and other Rosaceous crops. *Erwinia carotovora* causes diseases in many plants. It is a common cause of decay in stored fruits and vegetables. These species produce enzymes that hydrolyze pectin between individual plant cells causing the cells to separate, resulting in plant rot. The host range is quite wide (carrot, potato, tomato, leafy greens, squash and other cucurbits, onion, green peppers, *etc*.) and is able to cause disease in almost any plant tissue it invades. One species is found to be associated with human disease as well.

Genus Serratia: The cells are motile gram negative bacilli which form red/ pink/white colonies. ONPG is hydrolyzed by most strains. The strains may not demonstrate capsule although mucoid. The organism occurs in natural environment in water and plant surfaces or as oppurtunistic pathogen in humans. *Serratia marscens* is the type species. It produces red colored non-diffusible pigment. This pigment is prodigiosin. Another pink colored pigment produced is pyrimine.

Genus Hafnia: It is a motile gram-negative rod-shaped bacterium. Most strains utilize citrate, acetate and malonate. H_2S is not produced. Found in feces of humans, animals and birds, in sewage, soil, water and dairy products. Type species is *Hafnia alvei*. It is not pathogenic normally, but may cause disease in immunocompromised patients. It is often resistant to multiple antibiotics

Genus Edwardsiella: It is a motile gram negative bacillus which requires vitamins and growth factors for growth. It is free living in the environment especially fresh water. However, it can cause disease in eels, catfish and other animals. It can rarely cause disease in man. *E. tarda* is the type species.

Genus Proteus, Providencia and Morganella: *Proteus* species do not ferment lactose. They produce H_2S, ureease enzyme and deaminate phenylalanine. The characteristic feature of *Proteus* is its swarming motility. The organism is highly motile showing typical periodic cycles of migration in concentric circles. It can be seen on soft agar and on media containing blood. It is inhibited by the action of choral hydrate, bile salts and other surface active agents in the medium. It is found in intestines of man and animals, in soil and in polluted waters. One species has been isolated from gypsy moth larvae. *P. vulgaris,* the type species and *P. mirabilis* which is indole negative have been known to cause infections in man. It can cause nosocomial infections and infections of the urinary tract. Urinary tract infections are difficult to treat as urea produced by the organisms tends to change the pH of urine. Strains of *Providencia* and *Morganella* do not show swarming, produce H_2S or hydrolyze gelatin. These characters can be used to differentiate it from Proteus.

Genus Yersinia: The cells are non motile at 37°C but are motile below 30°C., except for *Y. pestis* which is always non-motile. Other phenotypic characters are also temperature dependent. An interesting feature peculiar to some of the *Yersinia* bacteria is the ability to not only survive but actively proliferate at temperatures as low as 1-4 degrees. This creates problems in the food processing industry. Some members of *Yersinia* are pathogenic in humans especially *Y. pestis* which is the causative agent of the plague, *Y. enterocolitica* which causes enteric infections. Rodents are the natural reservoirs of *Yersinia* and rat flea transmits the infection to man.

Subgroup 2 Vibrionaceae

The bacteria are Gram-negative curved or straight bacilli which are motile by means of polar flagella. They are non-sporing, chemoorganotrophs and facultative anaerobes. Most are oxidase positive; utilize D-glucose as primary source of carbon. Most species grow at 37°C except *Photobacterium* which grows at 25°C. *Photobacterium* and most *Vibrio* species require 2-3% salt for growth. The members are primary aquatic inhabitants, growing in sea water and fresh water. They have also been isolated in association with aquatic animals. Several species are pathogenic to man., fishes and other animals. Members of genus *Vibrio, Aaeromonas* and *Plesiomonas* cause infection in humans. *Vibrio* is the type genus. It includes genera *Vibrio, Aeromonas, Plesiomonas, Enhydrobacter and Photobacterium*. It has been proposed now to remove *Aeromonas* to a separate Family *Aermonadaceae* and *Plesiomonas* to family *Enterobacteriaceae.*

Genus Vibrio: The cells Gram-negative curved or straight rods which are motile by means of polar flagellum which is enclosed in a sheath. Lateral flagella may also be synthesized by bacteria on soild media. It is non-sporing, chemoorganotrophs, facultative anaerobes capable of growing on ordinary media. Most species are oxidase positive and require salt for growth. Members are found in sea water, fresh water and in association with aquatic invertebrates. *V. cholerae*, the causative agent of cholera is the type species. Other species such as *V. parahemolyticus, V. mimicus,* etc., can also cause disease in humans.

Genus Aeromonas: Cells are straight coccobacilli to bacilli in shape, occurring singly or in pairs, Gram-negative, generally motile by single polar flagella. Only one species is non-motile. He members are catlase and oxidase positive. They are resistant to vibriostatic agent O/129. They occur in freshwater and sewage. Some species are pathogenic to frogs and fish. *A. hydrophilla* is the type species. It can cause wound infections and septicemia in humans.

Genus Plesiomonas: The cells are straight rod shaped, occurring singly motile by polar lophotrichous flagella. The members are facultatively anaerobic, chemoorganotrophs showing positive reaction for catalase and oxidase. It decarboxylates lysine, arginine and ornithine. The strains occur in fish and other aquatic animals. *P. shigelloides* is the type species. It cans cause diarrhea in man.

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Genus Photobacterium: The cells are plump, thick, Gram-negative straight rods. They require sodium ions for growth. They are commonly found in marine environments and on surfaces and in intestine of marine animals. Some species occur as symbiotic species in luminous organs of marine fishes. *P. phosphoreum* is the type species.

Genus Enhydrobacter: This was included in this family in 1987. The cells are Gram-negative rods which are non-motile forming gas vacuoles under special conditions. It grows poorly on routine laboratory media. Only one species is included in this genus named as *E. aerosaccus.* It has been isolated from freshwater lake in USA.

Subgroup 3 Pasteurellaceae

The cells are straight, coccobacilli to bacilli in shape, showing pleomorphism with cell swelling and filamentous forms. They are aerobic, microaerophillic and facultatively anaerobic. They are catalase and oxidase positive and may require complex media for growth. Most members are parasitic for vertebrates. Many members are commensal in humas and are capable of causing diseases in man as well. This subgroup includes genera: Pasteurella, Haemophilus and Actinobacillus.

Genus Pasteurella: It is a genus of Gram neagtive, faculattively anaerobic organisms which are non-motile and pleomorphic. Most species are catalase and oxidase positive. Many species are zoonotic pathogens, and humans can acquire an infection from domestic animals. They are parasitic on mucous membranes of upper respiratory tract and digestive tract of humans, animals and birds. *P. multicoida* is the type species. It is the most frequent causative agent in human *Pasteurella* infection. *P. multocida* is also known cause of disease in rabbits, and the predominant syndrome is upper respiratory disease. *P. multocida* can be endemic among rabbit colonies and is often transmitted through nasal secretions.

Genus Haemophilus: The cells are Gram-negative, minute to small coccobacilli or rods showing extensive pleomorphism. They are commensal organisms along with some significant pathogenic species such as all members are either aerobic or facultatively anaerobic. Members of the *Haemophilus* genus require at least one of the following blood factors for growth: hemin (factor X) and/or nicotinamide adenine dinucleotide (factor V). This is accomplished easily by lysed blood agar or chocolate agar. Alternatively, *Haemophilus* is sometimes cultured using the 'Staph streak' technique: both *Staphylococcus* and *Haemophilus* organisms are cultured together on a single blood agar plate. In this case, *Haemophilus* colonies will frequently grow in small 'satellite' colonies around the larger *Staphylococcus* colonies. This happens bacause the hemolysis produced by *Staphylococcus* produces the necessary blood factor by-products required for *Haemophilus* growth. *H. influenzae* is the type species it causes sepsis and bacterial meningitis in young children. They occur as obligatory parasites on mucous membranesof man and animals. Other pathogenic species include *H. ducreyi* which causes chacroid.

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Genus Actinobacillus: It is a genus of Gram-negative, non-motile and nonspore-forming, oval to rod-shaped bacteria. Rod shaped cells are often interspersed with coccal elements resulting in Morse code appearance. The bacteria are facultatively aerobic, capable of fermenting carbohydrates (without production of gas) and of reducing nitrates. The genomic DNA contains between 40 and 47 mol % guanine plus cytosine. They occur as parasites or pathogens in mammals, birds, and reptiles. *Actinobacillus lignieresii* is the type species. *A. ureae* and *A. hominis* occur in the respiratory tract of healthy humans and may be involved in the pathogenesis of respiratory tract infections.

Subgroup 4 : Other genera

This includes genera such as *Calymmatobacterium, Gardnerella, Streptobacillus* and *Cardiobacterium*. Most genera have common features.

Anaerobic Gram-Negative Straight, Curved and Helical Rods

This includes family Bacteroidaceae which has several genera they are obligatory anaerobes, Gram-negative, non-spore forming, straight, curved or helical rods. These may or may not be motile. They are chemoporganotrophic, most species producing small amounts of organic acid. They catabolize carbohydrates, peptones or metabolic intermediates. It includes many genera only few common genera are given below.

Genus Bacteroides: The cells are Gram-negative, non-sporing, non-motile or motile by means of peritrichous flagella. Sphingolipids are present in cell membranes which is unusual in bacterial organisms. They also contain meso-diaminopimelic acid in their peptidoglycan layer.

Bacteroides are normally commensals in gastrintestinal tract of humans and animals. They play a fundamental role in processing of complex molecules to simpler ones in the host intestine. Their main source of energy is polysacchride from plant sources, but they can use simple sugars when available. *B. fragilis* is the type species.

Genus Fusobacterium: The cells are Gram-negative, filamentousnon-sporing, non-motile or motile by means of peritrichous flagella. *Fusobacterium* contribute to several human diseases, including periodontal diseases nad topical skin ulcers. Fusobacteria have a potent lipopolysacchride.Current consensus is that *Fusobacterium* should always be treated as a pathogen althogh it is commonly present in oropharynx.

Genus Leptotrichia: The cells are Gram-negative, straight or slightly curved rods which are non-sporing, and non-motile. Although they are anaerobic on first isolation, it can grow later on in aerobic conditions. It resides in oral cavity or in periurethral region of females. *L. buccalis*, the type species is associated with human disease. Almost every case of severe infection with Leptotrichia buccalis reported in medical literature occurred in patients with neutropenia.

Genus Butyrivibrio: Curved rods motile by monotrichous, lophotrichous or polar flagella. They occur in rumen of ruminants and have been isolated from feces of humans, rabbit and horses.

Anaerobic Gram-Negative Cocci

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This group includes family Veillonellaceae. The coccal cells are Gram negative, small arranged in pairs, chains or masses. Adjacent sides of cocci in a pair may be flattened. No endospores are formed and cells are non-motile. They grow under anaerobic conditions and are chemoporganotrophic. They are catalase and oxidase negative. Some species may contain pseudocatalase (a non heme containing compound) which decomposes hydrogen peroxide. The embers are parasites of homothermic living beings such as man, rodents, and pigs. They reside in alimentary tract. The genera included in this family are Veillonella, Acidaminococcus and Megasphaera.

Genus Veillonella: Organisms are arranged in pairs, in masses or in chains. They are catalase and oxidase negative. Lactate is utilized producing acetate, propionate, carbon dioxide and hydrogen. The organisms are parasitic in the oral cavity and intestine of man and other animals. They are non pathogenic under normal conditions, but can invade blood stream after oral surgery. *V. parvula* is the type species.

Genus Acidaminococcus: Cells are oval or kidney shaped, nonsporulating and nonmotile. They utilize aminoacids primarily for growth. They have been isolated from gut of pigs and man. They do not have any pathogenic potential. Type species is *A. ferementans*.

Genus Megasphaera: Small cells arranged in pairs or in short chains. They are nonmotile, catalase negative. Lactate is fermented. They are found in rumen of cattle and sheep and in the gut of man. They do not cause any disease. *M. elsdenii* is the type species.

Gliding bacteria: They do not have flagella, but can propel themselves when in contact with surfaces. The precise mechanisms of gliding motility have not been defined, but may involve rotating structures near the cell surface or the secretion of chemical surfactants.

2.3.4 Gram-Positive Cocci

This section included Class I (Firmibacteria) of the earlier classification. This includes Gram-positive bacilli, which are non-branching. Some members may form spore. It includes genera Micrococcaceae, Streptococcaceae, Peptococcaceae, bacillaceae and Lactobacillaceae. The members are associated with humans and animals. It includes many species implicated in diseases of humans.

Family Micrococcaceae: The cells are spherical, usually nonmotile, occurring single, in pairs or in packets of four, eight or in groups. They divide in one or more planes to form regular or irregular clusters, or packets. They are catalase positive aerobic or facultative anaerobic and grow easily on common media. They are chemoorganotrophs with respiratory or fermentative metabolism. Their nutritional requirements vary but all strains grow in the presence of 5 percent salt. Many members produce pigments. The members are free living, saprophytic or parasites of man and animals. It includes following genera:

Genus Micrococcus: The cells are non sporing Gram-positive cocci about 0.5 to 3 μm in diameter. They are arranged typically appear in tetrads. It has a substantial cell wall, which may comprise as much as 50% of the cell mass. The cells are catalase positive, coagulase negative, oxidize glucose and are susceptible to lysostaphin. Micrococci have been isolated from human skin, animal and dairy products, and beer. They are saprophytes existing in water, dust, and soil. They are usually commensal, non pathogenic organisms, but can act as oppurtunistic pathogen. *M. luteus* on human skin transforms compounds in sweat into compounds with an unpleasant odor. They can grow well in environments with little water or high salt concentrations. Most members are mesophiles. However, few members can exist as psychrophiles such as *Micrococcus antarcticus* which has been isolated from Antarctica. They are known survive for a long time. Strains from soil have been revived after storage in a refrigerator ever after ten years. They play a imporatnt role in synthesis of various chemicals such as long-chain aliphatic hydrocarbons for lubricating oils. They are also involved in detoxification or biodegradation of many other environmental pollutants.

Genus Staphylococcus: The cells are non sporing, Gram-positive cocci 1μm in diameter arranged in irregular clusters, single, pairs, chains, tetrads, non-motile, un capsulated, non-spore formers, facultative anaerobes, grow most rapidly at 37ºC. The cells are arranged in grape like clusters. They grow readily on many types of media and active metabolically, fermenting carbohydrates and produce pigment that range from white to deep yellow. All staphylococci are catalase positive. The genus *Staphylococcus* has at least 30 species. The three main species of clinical importance are: *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. They are known to cause pyogenic or pus producing infections of skin, soft tissues, bones etc. They can also cause food poisoning and fatal septicemia.

Genus Stomatococcus: The cells are large encapsulated, mucoid, Gram-positive cocci. They show a weak catalase and coagulase reaction. They grow as white mucoid colonies, are salt intolerant. It has only one species *S. mucilinaginosis*. It can cause infections in immunosupressed patients.

Genus Planococcus: The cells are Gram positive coccii which are motile with1-3 flagella. Two species namely *P. citreus* and *P. kocuri* have been commonly isolated. The cells are free living, have been isolated primarily from marine waters. They are asaccharolytic, salt tolerant (1-15%) producing yellow-orange pigment on commonly used media. It has not been implicated in human disease till now.

Genus Marinococcus: The cells are pleomorphic, Gram-positive, rods ot cocci. The cells are arranged single, in pairs and in irregular clusters. The cells are aerobic extremely halophilic. The colonies grow on common media, producing red-orange pigment. They are extremely salt tolerant, can tolerate upto 25% salt. They are found in salt lakes and sea water.

Family Streptococcaceae: The family Streptococcaceae has bacteria which are spherical or ovoid, in pairs or chains of varying length or in tetrads. The cells are non-motile or rarely motile. They have complex nutritional requirement. They may be aerobic or aerotolerent or facultatively anaerobic. They are catalase negative.

Genus Streptococcus : The cells are Gram-positive, oxidase, catalase negative cocci which divide along along a single axis thereby appearing in chains. They are faculattive anaerobe.

Streptococcus species are classified based on their hemolytic properties. Alpha hemolysis is caused by a oxidation of iron in hemoglobin, giving it a greenish color on blood agar. Beta hemolysis is complete rupture of red blood cells, giving distinct, wide, clear areas around bacterial colonies on blood agar. Other streptococci are labeled as gamma hemolytic as no hemolysis takes place.

In the medical setting, the most important members from the alpha-hemolytic streptococci are *S. pneumoniae* and Viridans-group of streptococci.

Beta-hemolytic streptococci are further classified by the Lancefield serogrouping. It is based on specific carbohydrates in the bacterial cell wall. These are named Lancefield groups A to V (except I and J). Of these, group A and B are most important in pathogenesis of various diseases.

Genus Enterococcus : These are Gram positive cocci to elongated cells that often occur in pairsor diplococci or in short chains. They are difficult to distinguish from streptococci on physical characteristics alone. Members of the genus *Enterococcus* were classified as *Group D streptococcus* until 1984 when genomic DNA analysis indicated that a separate genus classification would be appropriate. The cells are facultative anaerobic, tolerent to a wide range of environmental conditions such as extreme temperature (10-45°C), pH from 4.5 to10.0 and high salt concentrations. They can grow in presence of high concentration of bile and can be non hemolytic, beta hemolytic or alpha hemolytic on blood agar.

Genus Aerococcus : The cells are catalase negative Gram-positive cocci that resemble staphylococci by Gram stain, but have biochemical and growth characteristics of streptococci and enterococci. The members are often isolated as a common airborne organism in hospital environments and as a marine organism causing a fatal disease in lobster. In human, *aerococci* can be found in a very small number as indigenous inhabitants in the upper respiratory tract and on the skin of normal persons. This organism is generally saprophytic and rarely has been encountered as a human pathogen. It is generally considered as a contaminant in

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clinical cultures, but occasional reports have noted clinically significant roles for this organism in systemic infections such as bacteremia and endocarditis. Aerococci appear to be of low virulence and may be normally pathogenic only in patients with vulnerable conditions. It has been reported as the causative agent of meningitis, endocarditis, bacteremia, or of other infections such as urinary tract infection, septic arthritis, and wound infection.

Genus Gemella : The cells are catalase negative, oxidase negative, Gram -positive cocci which are arranged in pairs, tetrads, small irregular clusters or chains. Elongated rod shaped cells may also be seen. The cells are decolorised easily and may appear Gram-negative. The cells are non motile and non sporing. They are facultative anaerobes. The emmers are commensal flora of mucous membranes of humans and other animals. They have been isolated fronm dental plaques. They can act as oppurtunistic pathogens causing generalized and localised infections.

Genus Leuconostoc : These are Gram-positive ovoid cocci often forming chains. Leuconostoc species are intrinsically resistant to vancomycin and are catalase-negative. All species within this genus are heterofermentative and are able to produce dextran from sucrose. They are generally slime-forming.

They are responsible for causing the 'stink' when creating a sourdough starter; some species are also capable of causing human infection. They are responsible for the fermentation of food item sand bring about the change in taste in picked food products.

Genus Pediococcus : The cells are catalse negative, oxidase negative, Gram-positive cocci. The cells are ararnged singly in pirs or in tetrads, but never in chains or groups. They grow under facultative anaerobic to microaerophillic conditions. They produce lactic acid from glucose. They are responsible for a type of spoilage of beer called 'sarcina sickness' which is characterized by turbidity, a low pH, and by a characteristic 'sarcina odor'. The odor has been shown to be due to the production of diacetyl by the organism. Certain strains also have been shown to cause ropiness in beer due to a heavy capsule formation. In the 6th edition of Bergey's Manual the genus Pediococcus was included in the family Micrococcaceae as an appendix. It is now generally agreed that the genus should be included in the family Lactobacteriaceae.

Genus Lactococcus: It is a genus of pactic acid bacteria that were formerly included in the genus *Streptococcus Group N1.* They are known as homofermentors meaning that they produce a single product, lactic acid from glucose. Their homofermentative character can be altered by adjusting cultural conditions like pH, glucose concentration, and nutrient limitation. They are Gram-positive, catalase negative, non-motile cocci that are found singly, in pairs, or in chains. The genus contains strains known to grow at or below 7°C. These organisms are commonly used in the dairy industry in the manufacture of fermented dairy products like cheeses. They can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as Lactobacillus and

Streptoccoccus. Special interest is placed on the study of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* as they are the strains used as starter cultures in industrial dairy fermentations. Their main purpose in dairy production is the rapid acidification of milk; this causes a drop in the pH of the fermented product which prevents the growth of spoilage bacteria. The bacteria also play a role in the flavor of the final product. *Lactococci* are currently being used in the biotechnology industry. They are easily grown at industrial scale up on cheap whey based media. As food grade bacteria they are used in the production of foreign proteins that are applied to the food industry.

Genus Vagococcus: The cells are Gram-positiove motile catalase negative cocci. They are present singly or in short chains. They have been isolated from river water and chikn faeces. They are occasionally involved in human infections.

Family Peptococcaceae: The family Peptococcaceac has cells varying in diameter from 0.5-2.5 µm. the members are anaerobic, chemoorganotrophic with complex nutritional requirements. They are anaerobic catalase negative cocci. They are found in mouth, the intestine and the respirator tract of man and other animals. They are also found frequently is normal and pathological human female urogenital tracts. Some members have been isolated from soil and from the surface of cereal grains. This family has four genera namely Peptococcus, Peptostreptococcus, Ruminococcus and Sarcina.

Genus Peptococcus : The cells are Gram-positive, catalase negative, anaerobic cocci which have variable size. They are chemoorganotrophic with variable nutritional requirements.The emmebers are non sacchrolytic, In studies of the bacterial flora of normal human skin, *Peptococcus saccharolyticus* was one of the more important organisms we encountered. It was present on the forehead andarm (antecubital fossa) of ca. 20% of all subjects.When present, it was often more numerous than all other organisms combined.

Genus Peptostreptococcus : The genus Peptostreptococcus consists of a group of obligately anaerobic Gram-positive cocci which are phenotypically very diverse. In addition to considerable metabolic diversity, members of the genus have been shown by nucleic acid analysis to be phylogenetically heterogeneous. In particular *Peptostreptococcus anaerobius*, the type species of the genus Peptostreptococcus, is phylogenetically far removed from other peptostreptococci and exhibits a close affinity with certain clostridial species. Currently the genus comprises of 13 species, but unnamed groups of strains which may constitute additional species havebeen described (15, 19); the species Peptostreptococcus asaccharolyticus and Peptostreptococcus prevotii are recognized as genetically heterogeneous taxa.

Genus Ruminococcus: Members of the genus require anaerobic conditions, a carbohydrate energy source such as cellulose, cellobiose, or xylan, and some require carbon dioxide even in complex media. It was early recognized that unknown and apparently unusual growth factors were required by some strains.

Genus Sarcina : The cells are Gram positive cocci arragnged in cuboidal froms. It synthesizes microbial cellulos.Various members of the genus are part of human commensal flora and may be found in skin or intestine. *S. ventriculi* is the type species. It is found on the surface of cereal seeds, in soil, mud, and in the stomachs of humans, rabbits and guinea pigs.

2.3.5 Class II Thallobacteria

Branching Gram positive rods

This group includes Nocardioforms, or mycolata or mycolaic acid containing Actinomycetes.The cells are short, truly branched filaments, frequently occurring in small colonies of tangled filaments. Sometimes septa may be visible. There are no sulphur storage granules, but other storage granules might be present. The cells are usually Gram-positive, but Gram variable cells can also be seen. They are also partially acid fast. Some species are non pathogenic, while few are pathogenuic. They are obligatory aerobes. The colonies are smooth, rough or folded, sometimes compact and leathery in nature. Some members do not form any aerila mycelium, few produce limited mycelium which can fragment into spores.They are found worldwide in soil that is rich with organic matter. In addition, they are also part of oral microflora found in healthy gingiva as well as periodontal pockets. This group in divide into four subgroups:

Subgroup 1: Mycolic acid-containing bacteria (Genus *Gordona, Nocardia, Rhodococcus* and *sukamurella*)

Subgroup 2: *Pseudonocardia* and related genera (Genus *Actinobispora, Actinokineospora, Actinopolyspora, Amycolata, Pseudoamycolata, kibdelosporangium, Saccharomonospora* and *Saccharopolyspora*).

Subgroup 3: *Nocardioides* and *Terrabacter*

Subgroup 4: *Puomicromonospora* and related genera (Genus *Jonesia and Oerskovia*)

Features of common genera are given below.

Genus Nocardia: Most *Nocardia* infections are acquired by inhalation of the bacteria or through traumatic introduction. Most members have aerial hyphae when viewed with a dissecting microscope, particularly when they have been grown on nutritionally-limiting media. Members grow slowly on non-selective culture media, and are strict aerobes with the ability to grow in a wide temperature range. Some species are partially acid fast due to the presence of intermediate-length mycolic acids in their cell wall. They are catalase positive and can grow easily on the most commonly used media with colonies becoming evident in 3–5 days. However sometimes prolonged incubation periods (2–3 weeks) are needed. The genus includes at least 30 different species with ten of them isolated from humans. Species of Nocardia can cause infections of respiratory tract, skin and oral cavity.

Genus Rhodococcus: The cells are cocci which germinate into short rods and form filament with extensive branching. Fragmentation of filaments produces rods, cocci or filaments.

Genus Pseudonocardia: Members of this genus have been found living mutualistically in the metapleural glands of the laef cutter ants.

Genus Oerskovia: These are nocardia-like bacteria found in the soil in many parts of the world. The cells are non-motile. Sometimes the branching filaments of the cell fragment into motile rods. They lack aerial mycelium and have a distinctive cell wall containing large amounts of galactose. They are now known to cause opportunistic pathogen.

Multilocular sporangia

Bergey's Manual of Systematic Bacteriology grouped the genera *Geodermatophilus*, *Dermatophilus* and *Frankia* in the section 'Actinomycetes with multilocular sporangia'. These three genera share the remarkable morphological feature of the formation of sporangia of up to 100 µm in diameter that divide in all three planes. Family *Frankiaceae* was created within the Actinomycetales. Mulitlocular sporangia are also produced in culture by all *Frankia* strains. Sporangia develop by hyphal thickening, then segmentation by septa originating from the inner layer of a double-layered sporangial cell wall. As a result the older more loosely packed spores are distal to younger spores. The intersporal matrix appears to originate from the inner layer of the cell wall. Younger spores are more irregularly shaped, and older spores are spherical to ovoid, and measure about 1-5 mm in size. These sporangiospores are nonmotile, unlike those found in the multilocular sporangia from *Geodermatophilus* or *Dermatophilus*. Sporangia contain a few to several hundred spores depending on the age, and nutrition of particular strains. Members of genus *Frankia* live in root nodules on shrubs and trees. These root nodules, or 'actinorhizal root nodules', are the major nitrogen fixing symbioses in broad areas of the world.

Sporangia develop as terminal or intercalary structures on hyphae. Segmentation within the enlarging sporangia produces a multilocular sporangium containing many spores. Electron microscopy shows the developing spores with electron translucent nucleoid regions with dispersed fibrils and numerous lipid droplets. Like the hyphae, the external walls often show the residue of a laminate envelope. The mature spores show evenly dispersed cytoplasm but tubules are not present in the developed spores.

Actinoplanets

This group comprises of the spore forming actinomycetes realted to the genus Actinoplanes. The members form stable filaments, with little or no aerial growth producing actively motile spores in sporangia. The group contains genus Actinoplanes, Ampullariella, Dactylosporangium, Pilimelia which form motile spores and Micromonospora, Glycomyces and Catellatospora which form nonmotile spores. Cell wall contains meso-Diaminopimelic acid and glycine. Sugars such as arabinose and xylose are found in whole cell hydrolysates. They are present in soil, are mesophillic and aerbic ingrowth requirements. Meny species are source of synthesis of antibiotics.

Streptomyces and related genera

The cells are Gram-positive rods which produce vegetative mycelium. The myceliums do not fragment into bacillary or coccoid bodies. Conidia are borne on sporophores. They are primarily found in soil sometimes as thermophiles in rioting manure. Few species are pathogenic. It is a heterogenous group in which all members have cell wall containing levo-Diaminopimelic acid and glycine. Stable filaments are formed and may produce extensive aerial growth with long spore chains (*Streptomyces, Streptoverticillium*). Other genera (*Intrasporangium, Kineospora, and Sporichthya*) produce little or no aerial growth and have a variety of spore forms. The cells are Gram-positive producing aerial hyphae in chains. Growth occurs in form of branched mycelium with typical aerial hyphae producing conidia. Spiral formation may be seen in hyphae. Some spore baearing hyphae may be arranged in clusters or in short compact spirals.They are aerobic and are found as saprophytes in the soil and decaying vegetation. Streptomycetes are characterised by a complex secondary meatbolism. They are noted for their distinct 'earthy' odor which results from production of a volatile compound. Less commonly they are parasitic on plants and animals. Streptomycetes are infrequent pathogens causing infections in human such as mycetoma caused by *S. somaliensis.* In plants disease can be caused by *S. scabies* or *S. caviscabies.*

Maduramycetes

The cells are aerobic Gram-positive, nonacid-alcohol fast, chemoporganotrophic actinomycetes that form a branched non fragmenting substrate mycelium. The cells are stable filaments that produce spore bearing aerial growth. Short chains of non-motile arthrospores are produced by *Microbispora* (two spores), *Microtetraspora* (four spores), and *Actinomadura* (varying number). Other genera produce spores in sporangia which are motile (*Planobispora, Planomonospora, Spinillospora*) or non-motile (*Streptosporangium*). The cell wall contains meso-Diaminopimelic acid and cell hydrolysates contain madurose. Cell wall do not contain mycolic acids nad are not acid fast. In tissues the growth is in the form of granules consisiting of radiating actinomycosis. Strains have been isolated from cases of Madura foot or mycetoma. Small pink colored colonies are formed on agar surface.

The group is divided into two subgroups which includes flowing genera.

Subgroup 1: *Streptosporangium* and related group (Genus Microbispora, *Microtetraspora, Planobispora, Planomonospora, Spirillospora, Streptosporangium*).

Subgroup 2: *Actinomadura*

Thermospora and related genera

The cells produce stable filaments and produce aerial growth bearing spores. The spores may be single as in *Thermomonospora*, in chains as in *Actinosynnema and Nocardiopsis* or in sporangia-like structures

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As seen in *Streptoalloteichus*.The cell wall contains meso- Diaminopimelic acid, but not characteristic amino acids or sugars in whole cell hydrolysates. It includes four genera *Actinosynnema, Nocardiopsis, Streptoalloteichus and, Thermomonospora.* Member secrete a variety of heat stable enzymes which allow them to survive in overheated substances such as baggase, compost, fodder and manure.They are capabe of degrading celluclose and hence are useful in waste treatment facilities.

Thermoactinomycetes

The stable filaments produce aerial growth. Single spores (which are endospores) are formed on both aerial and vegetative filaments. All species are thermophilic. The cell wall contains meso-Diaminopimelic acid but not characteristic amino acids or sugars.The cells are well developed, fine nonseptate hyphae that grow well into the substrate. It forms single conidia on the tips of simple or branching conidiophores that may be too short. It forms well developed aerial hyphae. It includes only one genus *Thermoactinomyces.* The member are found in soil, human and animal excreta, high temperature compost, self heated hay and manure.

Other genera

Unclassified members are included in this group. The genera included are G*lycomyces, Kibdelosporangium, Kitasatosporia, and Saccarothrix*. They all produce aerial growth bearing chains of run is Spores. The vegetative mycelia are branching; aerial mycelium is produced on certain growth media The mycelium shows fragmentation of the vegetative hyphae but chains of squareended conidia are produced on the aerial hyphae. The cells are Gram-positive, lysozyme sensitive, catalase positive and aerobic. Their cell wall contains meso-diaminopimelic acid and glycine.

Mycoplasma cell wall less forms

Mycoplasma is the smallest free living self-replicating organisms with the smallest genomes. It is cell wall deficient bacteria which are highly pleomorphic in appearance. The cells measure around 150-250nm. They exist as granules and filaments of various sizes. The granules may be coccoid, balloon, disc, ring or star form. Slender filaments of varying lengths showing true branching may be seen. They even lack cell wall precursors such as muramic acid or diaminopimelic acid. Absence of cell wall makes them resistant to antibacterial agents that target cell walls Cytoplasmic membrane contains sterols and provides elasticity resulting in pleomorphism. *Mycoplasma* species differ from viruses as they grow on cell-free media in vitro, contain both RNA and DNA and show both intracellular and extracellular parasitism in vivo. *Mycoplasma* is generally facultative anaerobes. Growth is enhanced in presence of air. They can be cultivated in laboratory in liquid as well as solid media. Optimum temperature for growth is 22-41°C. Mycoplasmas that are parasitic show host specificity. They are nearly ubiquitous in both the plant and animal kingdoms as colonizers and pathogens. They act primarily as extracellular parasites. Mycoplasmas are being increasingly implicated in pathogenesis of autoimmune diseases.

It contains the following families and genera. Type species is *Mycoplasma mycoides.*

- **Family Mycoplasmataceae:** These essentially require cholesterol and sterol for their growth. This includes pathogenic mycoplasmas for man. It has two genera:
  - o Genus Mycoplasma
  - o Genus Ureaplasma
- **Family Acholeplasmataceae:** This contains one genus Acholeplasma. It includes mostly saprophytic members. They do not require sterols for their growth.
- **Family Spiroplasmataceae:** The members are parasites of arthropods and plants. These are helical shaped and require sterol for their growth. It includes only one genus *Spiroplasma.*
- **Family Anaeroplasmataceae:** It includes genus *Anaeroplasma.* These are strict anaerobes. The members are found in the rumen of cattle and sheep.

Methanogens

Methanogens are microorganisms that produce methane as a metabolic by product in low oxygen tension conditions. They are classified as Archaea, a group quite distinct from bacteria. The cells are usually coccoid (spherical) or bacilli (rod shaped). Methanogens lack peptidoglycan, a polymer that is found in the cell walls of the bacteria but is not seen in Archaea. Some methanogens have a cell wall that is composed of pseudopeptidoglycan aslo known as pseudomurein.

They grow under anaerobic conditions. Although methanogens cannot function under aerobic conditions they can sustain oxygen stresses for a prolonged time. They can reproduce at temperatures of 15 to 100 degrees Celsius. They are known to be the most common archaebacteria in deep subteranean habitats. They are commonly found in areas which have high moisture content and in the guts of animals such as ruminants and humans. In humans and animals they are are responsible for the methane content of belching and flaulence in humans.They are also found in marine sediments, in high temperature areas such as hot springs and in hypothermal vents. They have been found in the 'solid' rock of the Earth's crust, kilometers below the surface, in anaerobic sewage digestors and anoxic freshwater sediments. They are very important as sources of methane or natural gas.

They play the vital ecological role in anaerobic environments of removing excess hydrogen and fermentation products that have been produced by other forms of anaerobic respiration. In deep rocks they obtain their hydrogen from the thermal and radioactive breakdown of water. These are also key agents of remineralization of organic carbon in continental margin sediments and other aquatic sediments with high rates of sedimentation and high sediment organic matter. Under the correct conditions of pressure and temperature, biogenic methane can accumulate in massive deposits of methane clathrates, which account for a significant fraction of organic carbon in continental margin sediments and represent a key reservoir of a potent greenhouse gas. There are over 50 described species of methanogens. The group is divided into as follows:

Subgroup 1: Cell walls with pseudomurein

Order Methanobacteriales

Family Methanobacteriaceae

Genus Methanobacterium

Genus Methanobrevibacter

Family Methanothermaceae

Genus Methanothermus

Subgroup 2: Cell walls without pseudomurein

Order Methanococcales

Family Methanococcaceae

Genus Methanococcus

Family Methanosarcinaceae

Genus Methanolacinia

Genus Methanococcoides

Genus Methanolobus

Genus Methanothrix

Order Methanomicrobiales

Family Methanomicrobiaceae

Genus Methanomicrobium

Genus Methanogenium

Genus Methanospirillum

Family Methanoplanaceae

Genus Methanoplanus

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Subgroup 3: Other Genera

Methanocorpusculum

Methanoculleus

Methanohalobium

Methanohalophilus

Methanosarcina

Methanosphaera

Archeal Sulfate Reducers

This group includes only one genus *Archaeoglobus.* Four archaeal sulfate-reducers have been isolated yet, all from marine hydrothermal areas. These species, with a lower temperature limit of 60°C and optimum growth temperatures above 80°C are members of the genus Archaeaoglobus. Except for A. profundis, all Archaeoglobus strains are facultative chemolithoautotrophs. They survive at high temperature, requiring around 2-4% salt. The cells are Gram-negative, regular to irregular cocci, sometimes triangular forms may be seen, arranged singly or in pairs. They form greenish black colonies on gar. They form blue green fluorescence at 420nm. Cell envelope does not cantain pseudomurein. They have a single layer of glycoprotein subunit with no rigid structures. S-layer may be arranged in hexagonal lattice. The natural habitat of Archeoglobus is anaerobic submarine hydrothermal areas.These thermophilic anerobes can extract electrons from hydrogen, lactate, glucose, etc. and reduce sulfate, sulfite, or thiosulfate to sulfide via anaerobic respiration, but are also weakly methanogenic.

Extremely Halpohillic, Aerobic Archeobacteria

Aerobic archeobacteria are extreme halophiles. The cells are Gram-negative rods (0.6-1 x 1-6 µm) with glycoprotein walls (*Halobacterium*, motile via lophotrichous flagella; *Natronobacterium*) , cocci (0.6-1.5 µm) with sulfated polysaccharide walls (*Halococcus*, *Natronococcus*), flattened disks or cup-shapes (*Haloferax*), irregular disks, triangles, rectangles (*Haloarcula*) , many have yellow-to-red carotenoid pigments that protect them against sunlight. These are organotrophic aerobes that require very high salt and require complex nutrients (vitamins, etc.) for growth. Hypersaline aquatic environments with alkaline pH such as Great Salt Lake, Dead Sea, solar salt evaporating ponds (salterns) and salted fish, are the normal habitat for these Archaea. The Pink halophiles contain a pigment very similar to the rhodopsin in the human retina. They use this visual pigment for a type of photosynthesis that does not produce oxygen.

Cell Wall less Archeae

This includes order Thermoplasmatales with two genera Thermoplasma and Picrophilacea. All isolates are monotrichously flagellated motile cocci and lack

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any cell wall. Crosslinking of the carbohydrate chain of membrane glycoproteins provide what little cell rigidity and osmotic tolerance they have. These organisms are thermoacidophilic heterotrophs. They are facultatively anaerobic where oxygen or sulfur can serve as terminal electron acceptors. They are acidophilic, most isolates growing best at pH2, but some grow as low as pH 0.8. They are also moderately thermophilic, preferring around 60°C for growth. Thermoplasma has been isolated exclusively from smouldering coal refuse piles, and it is presumed that subterranean coal deposits are their natural habitat. These are Archaebacteria from hot springs and other high temperature environments. Some can grow above the boiling temperature of water. A gene taq polymerase isolated from Thermus aquaticusis used in polymerase chain reaction. It codes for heat-stable enzymes that may be of great value in industry and medicine. It is invaluable to medicine, biotechnology, and biological research. Annual sales of taq polymerase an enzyme obtained from the same is a business for roughly half a billion dollars.

Extremely Thermophillic and Hyperthermophile Sulfur Metabolizers

Extremely thermophilic archaebacteria are known to be metabolizers of elemental sulfur and the methanogens. Three subgroups are present in this group. The most studied genera include *Thermoproteus* and *Sulfolobus*. The characteristics of some of the members from each of the order are given below:

Order *Igneococcales*: Most isolates have been cultured from ocean floor volcanoes (hydrothermal vents). All are strict anaerobes. Some species are organotrophs and some are lithotrophs.

Genus *Pyrodictium:* P. occultum is the most thermophilic (hyperthermophile) organisms known to date which grows at optimum temperature of 105ºC.

Genus Desulfurococcus: The cells are cocci shaped and have been isolated from terrestrial and marine volcanic environments. Some members are motile and some are non-motile. They are strict anaerobes that reduce sulfur.

Order Sulfolobales

Genus Sulfolobales: The cells are Gram-negative, aerobes and have an irregular spherical lobed shape. The optimum temperature for growth is between 70 to 80°C with a optimum pH between 2 to 3. *Sulfolobus* is sometimes referred to as a thermoacidophile. The cell walls lack peptdioglycan but contain lipoprotein and carbohydrates. Oxygen is the normal electron acceptor but ferric iron can also be used. Some strains are microaerophilic.

Order Thermoproteales

Genus Thermoproteus: These are members of the order *Thermoproteales*. Cells are long and thin. Occasional branching of the cells and golf ball-like terminal

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structures can be seen. Cell walls are made of glycoprotein subunits. They are strict anaerobes which grow between 70 to 90ºC. In addition, they can also grow organotrophically by oxidising glucose, amino acids, alcohols and organic acids by anaerobic sulfate respiration. They have been isolated from neutral volcanic terrestrial and marine hot springs rich in sulfur.

Genus Thermofilum: The cells are extremely filamentous and very thin. Most of these anaerobic heterotrophs utilize only complex peptide mixtures such as yeast and meat extracts as carbon and nitrogen sources.

Check Your Progress

6. Give the names of Class I Scotobacteria.
7. What is the main characteristic of Genus II Leptonema?
8. Which genus includes the causative agent of anthrax?
9. Which is the most thermophilic (hyperthermophile) organism known to date, as it grows at the optimum temperature of 105ºC?

2.4 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Three physiological and metabolic characteristics used in classification and identification of microbes are as follows:
   - Energy sources
   - Motility
   - Carbon and nitrogen sources
2. The DNA-DNA hybridization is used to study only closely related microorganisms.
3. Proteobacteria are divided on the basis of rRNA sequences.
4. The two characteristics of Caulobactor are as follows:
   i. They may be polarly flagellated rods or may possess prostheca and holdfast, by which they attach to solid substrata.
   ii. They are usually found in low-nutrient freshwater and marine habitats, but are also present in soil.
5. The *Epsilon proteobacteria* is the smallest proteobacteria in the proteobacteria group.
6. Class I Scotobacteria includes members of medically important bacteria of family Spirochaetaceae, Spirosomaceae, Pseudomonadaceae,

Enterobacteriaceae, Vibrionaceae, Bacteroidaceae, Neisseriaceae, Legionellaceae, Pasteurellaceae and of orders Rickettsiales and Chlamydiales.

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7. The morphological features of Genus II Leptonema are similar to leptospira except a few minor differences in the ultrastructure of flagella and intracytoplasmic tubules.

   The members of Genus Escherichia are inhabitants of the gastrointestinal tract of warm-blooded animals and provide a portion of the vitamin K to their host.

   Nitrobacter, Nitrococcus, Nitrospina and Nitrospira are the genera associated with nitrate-oxidizing bacteria.

8. Genus bacillus includes the causative agent of anthrax.
9. *P. occultum* is the most thermophilic (hyperthermophile) organism known to date, as it grows at the optimum temperature of 105ºC.

2.5 SUMMARY

- The first attempt to classify microscopic plants and animals was apparently undertaken by Müller in 1773.
- Woese defined three cellular domains of life: Eukaryotes, Eubacteria and Archaebacteria.
- In Phenetic classification system, organisms are grouped together based on mutual similarity of their phenotypic characteristics.
- Carolus Linnaeus (1707-1778) was a Swedish botanist and professor of medicine who gave a binomial system of classification for plants. He is known as the father of modern taxonomy.
- The G+C content of DNA from animals and higher plants averages around 40 per cent and ranges between 30 and 50 per cent. In contrast, the DNA of both eukaryotic and prokaryotic micro-organisms varies greatly in G+C content; prokaryotic G+C content is the most variable, ranging from around 25 to almost 80 per cent.
- Bergey's Manual of Systematic Bacteriology grouped the genera Geodermatophilus, Dermatophilus and Frankia in the section 'Actinomycetes with multilocular sporangia'. These three genera share the remarkable morphological feature of the formation of sporangia of up to 100 ìm in diameter that divide in all three planes.
- Mycoplasma is the smallest free living self-replicating organisms with the smallest genomes. It is cell wall deficient bacteria, which are highly pleomorphic in appearance.

2.6 KEY TERMS

NOTES

- **Taxonomy:** It is the science of biological classification.
- **Proteobacteria:** These are Gram-negative bacteria with an outer membrane mainly composed of lipopolysaccharides.
- **Gracilicutes:** These are the prokaryotes having thin cell walls, i.e., a Gram-negative type cell wall.

2.7 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Differentiate between phonetic and phylogenetic classification methods.
2. Write a short note on-
   (a) Interspecies classification methods
   (b) Phenotypic classification
   (c) Genotypic classification
   (d) Enterobacteriacae
3. State the characteristics of rickettsia, chlamydia, spirochetes and irregular and nonsporing Gram-positive rods.
4. State what features are associated with facultatively anaerobic gram-negative rodes.
5. How are Genus Gemella and Genus Listeria different from each other?

Long-Answer Questions

1. Discuss the earliest classification of bacteria.
2. Explain the basic features depending on which Bergey classified the microbes.
3. Analyse the microbes that fall in Subgroup 2 Vibrionaceae.
4. Define proteobecteria and its sections.
5. Explain anaerobic Gram-negative cocci in detail.
6. Explain in detail about class II Thallobacteria.

2.8 FURTHER READING

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UNIT 3 VIRUSES

NOTES

Structure

3.0 INTRODUCTION

Viruses (toxin or poison) are obligate intracellular parasites which contain either DNAor RNA. They lack metabolic machinery and depend on the host cell to carry out vital functions. Viruses cannot multiply outside the host because they do not possess enzyme system and protein synthesis machinery. Viruses do not have any cytoplasm and thus cytoplasmic organelles like mitochondria, Golgi, lysosome, ribosome etc. are absent.

The origins of viruses in the evolutionary history of life are unclear; some may have evolved from plasmids, while others may have evolved from bacteria... Viruses infection can spread in many ways, they can get transmitted through plants, insects and animals. Viral infections in animals provoke an immune response that usually eliminates the infecting virus. These immune responses can also be produced by vaccines, which give immunity to specific viral infections. However, some viruses including HIV and those causing viral hepatitis evade these immune responses and cause chronic infections.

In this unit, we will discuss about the structure of viruses and their classifications.

3.1 OBJECTIVES

After going through this unit, you will be able to:

- Define Virus
- Learn about the structure of viruses
- Learn about various classes of viruses

3.2 NOMENCLATURE AND CLASSIFICATION OF VIRUSES

NOTES

In 1935, Schelsinger determined the composition of a virus for the first time. He also showed that a bacteriophage consists of only protein and DNA. Same year, Wendell Stanley introduced the concept of chemical nature of viruses and Twort-d- Herelle coined the term bacteiophage (viruses that infect bacteria). In 1983 LUC Montagnier discovered the HIV virus which is shown in Figure 3.1.

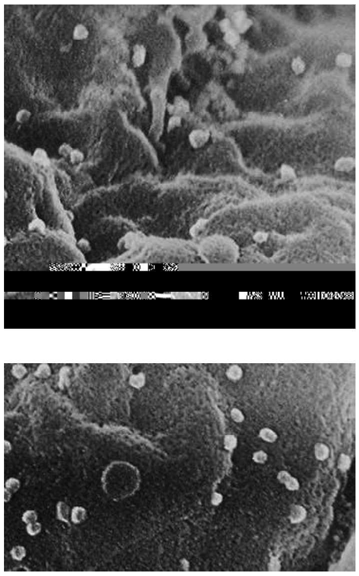

Fig. 3.1 *HIV-1 viruses*

In 1957, L woff defined virus as "strictly intracellular and potentially pathogenic entities with an infectious phase, and (1) possessing only one type of nucleic acid, (2) multiplying in the form of their genetic material, (3) unable to grow and to undergo binary fission, (4) devoid of a Lipmann system.".

In 1968, Luria and Darnell alternatively defined viruses as "elements of genetic material that can determine in the cells in which they reproduce, the biosynthesis of a specific apparatus for their own transfer into other cells."

The famous Russian botanist, Dmitri Iwanowski presented a paper on viruses to the St. Petersburg Academy of Science in 1892. In this, he showed that extracts from tobacco plants infected with mosaic virus (Refer Figure 3.2) could transmit disease to other plants after passing through ceramic filters which can easily filter bacteria. This is generally recognized as the beginning of Virology.

Virology is the study of viruses and virus-like agents; their structure, classification and evolution, their ways to infect and exploit cells for virus reproduction, the diseases they cause, the techniques to isolate and culture them, and their use in research and therapy. Virology is often considered a part of microbiology or of pathology.

NOTES

1. Shape of capsid
2. Mode of transmission
3. Type of vector
4. Symptoms on host after infection
5. Nature of accessory particles

In 1975, Casjens and King classified the viruses on the basis of nucleic acid types, symmetry, absence or presence of the envelope and site of assembly of the envelope. They were classified as:

1. ssRNA , for example, helical, icosahedral, envelope
2. ds RNA, for example, envelope, segmented
3. ssDNA, for example, icosahedral, helical
4. dsDNA, for example, icosahedral, enveloped

In 2001, Dimmock classified viruses into six sections on the basis of their host preference, such as, infecting animals, plants, fungi, bacteria, and satellite viruses and viriods.

Structure of Viruses

Viruses are of different shapes such as filamentous, pleomorphic, bullet shape and their sizes vary from 20 nm–300 nm, in diameter. Most viruses cannot be viewed even though light microscope, so scanning and transmission electron microscopes are used to visualize them.

Viruses contain either DNA or RNA which is surrounded by a protein coat that protects the genetic material. A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called capsid.

Morphological Categories

Morphologically, viruses can be classified into four categories. They are given below:

1. Helical (cylindrical)
2. Icosahedral
3. Envelope
4. Complex

Let us discuss them one by one.

1. **Helical (cylindrical) viruses:** These viruses consist of a single type of capsomer stacked around a central axis to form a helical structure. This

type of arrangement may be present in rod-shaped or filamentous virions. The helical viruses can be short or long, rigid or flexible. The genetic material present in the virus is, generally RNA, which is surrounded by a protective layer called capsid.. The capsid layer itself is enclosed within a membranous layer called envelope, which is made up of a viral protein, the host cell component (lipid) and carbohydrate.

Helical capsids may be naked, for example, tobacco mosaic virus or enveloped, for example, influenza or mumps virus. Tobacco mosaic virus is rod shaped and it consists of a protein tube with a lumen of a single strand helical coiled RNA. The function of the protein is to protect the RNA helix which contains about 49 nucleotides. Some viruses are surrounded by membranous envelopes which are covered with spikes which consist of protein, lipids, and carbohydrate.

2. **Icosahedral (Polyhedral) viruses:** Polyhedral structure is of three types, Icosahedral, Octahedral and Tetrahedral. Out of these, Icosahedral symmetry is the most popular one, as it provides high-level packaging and bonding of subunits. An Icosahedral is a regular polyhedral with 20 triangular faces and 12 corners. In this structure, some capsids are enclosed within envelopes. Turnip yellow mosaic virus has an elongated capsomere which is a cluster of 5 to 6 protein molecules. It surrounds the RNA which is present is in the form of a single chain. The bacteriophage, phi ×174 contains 12 capsomeres which are made up of 60 identical units.
3. **Envelope_viruses:** These viruses contain modified lipid bi-layer cell membranes Most of them depend on the envelope for the infective spike.
4. **Complex viruses:** These viruses possess unique structures such as protein tails or complex outer walls. They do not have definite capsids. However, some of them may contain both capsids and tails. The bacteriophages T2, T4, T6 are examples of complex viruses. Generally, the structure of bacteriophage is tadpole shaped, with distinct head and tail regions. The head capsid is in the form of a prolate icosahedrons and it consists of two ten-faceted equatorial bands, with a pyramidal vertex at either end. The tail has a helical symmetry and it consists of a core tube through which the DNA can pass. The base plate is hexagonal with pins at each corner. With the help of the tail, the virus gets attached to the bacterial host and injects the viral genome into the cell.

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Figure 3.3 shows the external structure of a virus.

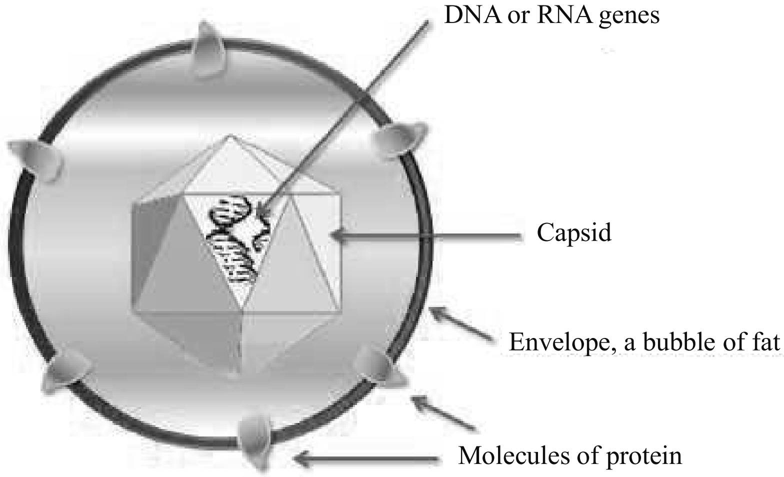

Fig. 3.3 *External Structure of a Virus*

Genetic Material

Capsid

The protein layer which protects the genetic material (DNA, RNA) is known as capsid. A single subunit capsid is known as capsomere. In case of some viruses, the capsid is enclosed by a lipid layer called viral envelope. Capsid is broadly classified according to the structure into two categories; Helical and Icosahedron. The structure of helical is cylindrical while the structure of icosahedron is sphere. When a virus infects the cell, using the protein synthesis method, a new capsid layer is formed according to the genetic material containing inside the virus. The viruses originate from cell membranes and then enter the host cell. The lipid bilayer envelope of these viruses is relatively sensitive to desiccation, heat and detergents, and so these viruses are easier to sterilize than non-enveloped viruses. The envelope contains some projection like structure called spikes. These spikes are clearly visible on some viruses, such as the influenza viruses. On other enveloped viruses, the spikes are extremely difficult to see. The spikes help the virus to penetrate the host cell. Generally, it is composed of hemagglutinin protein that allows the virus particle to enter the cell and also contains neuraminidase that helps the newly formed virus particles to bud out from the host cell membrane.

Nucleic acid

The genetic material which is present inside the capsid is called nucleic acid. Three different types of viruses which contain different types of nucleic acid are, DNA viruses, RNA viruses, and DNA-RNA viruses.

- **DNA viruses:** These viruses contain DNA as the genetic material. Common examples include pox viruses, the coliphages T2,T4,T6,T3 and lamda, papilloma viruses. Adeno viruses consist of double stranded DNA. The

DNA molecule may be cyclic, as in case of SV-40 and papilloma viruses and in case of coilphage lambda it is of linear shape. The T-evev (T2,T4,T6) coilphages contain an unusual base,5-hydroxymethylcytosine in place of cytosine. In case of bacteriophage phi × 174, M–13, the DNA is in circular.

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- **RNA viruses:** The viruses contain RNA as the genetic material. Double stranded RNA is found in the reovirus and wound tumour virus. Single stranded RNA is found in tobacco mosaic virus, influenza virus, poliomyelitis virus, and leukemia virus. Plant virus contains only RNA. The RNA of several viruses fold upon themselves to form helices in some regions.
- **DNA-RNA viruses:** These viruses contain alternatively both RNA and DNA.

An animal virus, as shown in Figure 3.4, contains either DNA or RNA or rarely RNA-DNA.

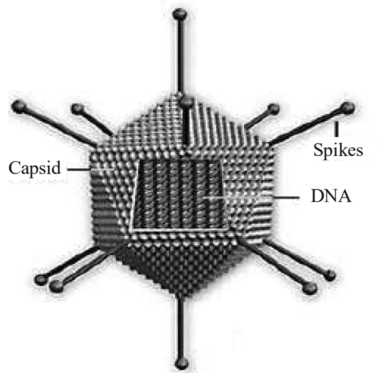

Fig. 3.4 *An Animal Virus*

Table 3.1 summarizes the structures of nucleic acid.

Table 3.1 *Structure of Nucleic Acid*

Type	Structure	Examples
DNA		
Single strand	Linear	parvovirus, M 13,fd phages
Double strand	Circular	Herpes virus, lamda phages ,adeno
RNA		
Single strand	Linear	picoma viruse,paramyxoviruses orthomyxoviruses,Retroviruses
Double strand	Linear	Reoviruses, wound tumor viruses, phage Φ

Check Your Progress

1. Name four categories into which virus can be morphologically classified.
2. Name the three sub-types of polyhedral structure.
3. Define envelope viruses.
4. In a virus cell, what is capsid?
5. What is nucleic acid?

NOTES

3.3 ANSWERS TO 'CHECK YOUR PROGRESS'

1. The four categories into which viruses can be classified are: helical (cylindrical), Icosahedral, Envelope and Complex .
2. The three sub-order of polyhedral structure are: icosahedral, octahedral and tetrahedral.
3. Envelope viruses are those viruses that contain modified lipid bi-layer cell membranes.
4. In a virus cell, the protein layer which protects the genetic material (DNA and RNA) is known as capsid.
5. The genetic material which is present inside the capsid is called nucleic acid.

3.4 SUMMARY

- Virology is the study of viruses and virus-like agents; their structure, classification and evolution; their ways to infect and exploit cells for virus reproduction; the diseases they cause, the techniques to isolate and culture them and their use in research and therapy.
- According to the Linnean system of binomial nomenclature, viruses have three suborder types: viruses attacking on bacteria known as phaginae, viruses attacking on plants known as phytophagine and viruses attacking on animals known as Zoophaginae
- ICTV has developed a five-order system, according to which viruses were classified as caudovirales, herpesvirales, mononegavirales, nidovirales and Picornavirales.
- Viruses are of different shapes, such as filamentous, pleomorphic, bullet shape. Their sizes vary from 20 nm–300 nm in diameter.
- Morphologically, viruses can be classified as helical (cylindrical), icosahedral, envelope and complex.
- The genetic material which is present inside the capsid is called nucleic acid. Three different types of viruses which contain different types of nucleic acid are: DNA viruses, RNA viruses and DNA-RNA viruses.

3.5 KEY TERMS

- **Virus**: It refers to an obligate intracellular parasite which contains either DNA or RNA.

- **Capsid**: It refers to the protein layer which protects the genetic material of the virus.
- **Nucleic acid**: It refers to the genetic material which is present inside the capsid of virus.
- **Virology**: It refers to the study of viruses and virus-like agents.

3.6 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Write a note on the genetic materials of viruses.
2. What are the different diseases caused by viruses?
3. What do you understand by virology?
4. Describe structure of viruses.

Long-Answer Questions

1. Explain the morphological classifications of viruses.
2. Explain nomenclature of viruses.
3. Explain in detail about the classification of viruses.

3.7 FURTHER READING

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UNIT 4 PHYCOLOGY

Structure

4.0 INTRODUCTION

Algae are a diverse group of aquatic organisms that have the ability to conduct photosynthesis. Certain algae are familiar to most people; for instance, seaweeds (such as, kelp or phytoplankton), pond scum or the algal blooms in lakes. However, there exists a vast and varied world of algae that are not only helpful to us, but are critical to our existence.

Algae are simple plants that can range from the microscopic (microalgae), to large seaweeds (macroalgae), such as giant kelp more than one hundred feet in length. Microalgae include both cyanobacteria, similar to bacteria, and formerly called 'blue-green algae', as well as green, brown and red algae. There are more varieties of microalgae, but these are the main ones. Algae can be grown using water resources, such as brackish, sea, and waste water unsuitable for cultivating agricultural crops. When using waste water, such as municipal, animal and even some industrial runoff, they can help in its treatment and purification, while benefiting from using the nutrients present.

Most microalgae grow through photosynthesis, i.e., by converting sunlight, CO_2 and a few nutrients, including nitrogen and phosphorous, into material known as biomass. This is called 'autotrophic' growth. Other algae can grow in the dark using sugar or starch called 'heterotrophic' growth, or even combine both growth modes called 'mixotrophic' growth. Algae are very diverse and found almost everywhere on the planet. They play an important role in many ecosystems, including providing the foundation for the aquatic food chains supporting all fisheries in the oceans and inland, as well as producing about 70 percent of all the air we breathe.

In this unit, you will study about algae in diverse habitats like, freshwater, terrestrial and marine of Indian coasts, criteria in algal classification, outline classification of algae as proposed by Fritsch (1935) and Smith (1955), economic uses of algae in detail.

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4.1 OBJECTIVES

After going through this unit, you will be able to:

- Discuss about algae in diverse habitats like, freshwater, terrestrial and marine of Indian coasts
- Explain the criteria in algal classification
- Outline the classification of algae proposed by Fritsch (1935) and Smith (1955)
- Understand the economic importance of algae

4.2 ALGAE IN DIVERSE HABITATS (FRESHWATER, TERRESTRIAL AND MARINE OF INDIAN COASTS)

Algae can be defined as eukaryotic (nucleus-bearing) organisms that conduct photosynthesize but do not have specialized multicellular reproductive structures of plants, that contains fertile gamete, producing cells surrounded by sterile cells. Algae do not have true roots, stems, and leaves, i.e., the features they share with the vascular lower plants, for example, mosses, liverworts, and hornworts. They are distributed worldwide in the sea, in freshwater and in waste water. Most are microscopic, but some are quite large, for example, some marine seaweeds that can exceed 50 m in length.

The unicellular forms are known as microalgae whereas the multicellular forms comprise macroalgae (Refer Figure 4.1).

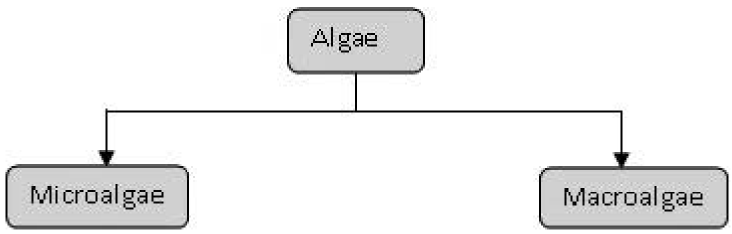

Fig. 4.1 *Classification of Algae*

Microalgae comprise a vast group of photosynthetic, heterotrophic organisms which have an extraordinary potential for cultivation as energy crops, which can be cultivated under difficult agro-climatic conditions and are able to produce a wide range of commercially interesting by products, such as, fats, oils, sugars and functional bioactive compounds. Seaweed is a loose colloquial term encompassing macroscopic, multicellular, benthic marine algae. It includes some members of the red, brown and

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green algae which are photosynthetic, like plants, and 'simple' because they lack the many distinct organs found in land plants. For that reason they are currently excluded from being considered plants. Female sex organ in algae are unicellular, and there is no embryo formation in algae. However, the reproductive structures of some groups of algae, for example, Chlorophyceae are apparently multicellular and the sterile tissue is generally considered as vegetative. Bryophytes onwards in the scale of evolution have the uniform multicellular sex organs, the archegonia, which are not found in algae. For that reason bryophytes are usually called archegoniate plants.

According to Fritsch (1935) algae includes all holophytic organisms that fail to reach the higher level of differentiation characteristic of higher plants. According to R.N Singh, algae are large simple plants displaying a wide range of photosynthetic pigments and evolve oxygen during photosynthesis. Sharma (1987) defines algae as an assemblage of chlorophyll bearing autotrophic thallophytes bounded by a cell wall made up of pure or mixed carbohydrates.

Though the algae have been used for various purposes since long but the orderly and systematized knowledge of algae started with the invention of microscope in the middle of 17th century. **Linnaeus (1754)** coined the term algae while giving classification of Plant Kingdom. Algae are a very old group of plants. The study of algae is known as algalogy or phycology. In the present form the algae were delimited from the rest of plants by A.L. de Jussieu though the term algae was coined by Linnaeus (1753). **Endlicher (1836)** placed them in thallophytes along with fungi. Algae do not constitute any homogeneous natural assemblage of plants. There are over 35000 species of algae which live in all sorts of habitats and show a high degree of morphological and biochemical plasticity. Algae are chlorophyllous thalloid and their achlorophyllous relatives were the gametangia and sporangia are either unicellular or have all the cells fertile and in which the zygote do not develop into an embryo or parasitic sporophyte. The maintenance of group name of algae is justified because no one can expect a single line of evolutionary tendency in these forms which were formed at a time when innumerable types of metabolites were being created and tested by the organisms for their survival in the struggle for existence. The algae are aquatic and are found in fresh water or salt water. Fresh water forms occur abundantly in ponds, lakes, slow flowing streams and water reservoirs.

Generally they are free swimming, free floating or attached to bottom in the shallow water. Some are terrestrial and grow in wet situations such as walls, damp soil, damp shaded sides of trees and have adapted themselves to a life in the air. Some of the algae are endophytic whereas few live in symbiotic relationship with the higher plants. Most of the marine forms are Seaweeds. They inhabit vast area of the ocean. Some of the marine forms are free floating and few are attached. The free floating and free swimming minute algae together with similar other organizations constitute the plankton of the oceans and lakes. The algae which grow attached to the bottom in shallow water along the edges of seas and lakes form the benthos.

According to the habitat, the algae may be classified as follows:

- Terrestrial Algae

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- Aquatic Algae
- Aerophytes
- Thermophytes
- Cryptophytes
- Algae of Unusual Habitats

Terrestrial Algae: Many genera of algae are found on or beneath the moist soil surface and are called terrestrial algae. The algal forms present on the surface of soil, for example, few species of *Vaucheria, Botrydium* (Refer Figure 4.2)*, Ulocladium* are called saprophytes while algal genera having subterranean habitat, for example, few species of *Nostoc* (Refer Figure 4.3)*, Anabaena* and *Euglena* are known as Cryophytes.

Fig. 4.2 *Botrydium*

Fig. 4.3 *Nostoc*

Aquatic Algae: Most of the algal genera are aquatic and are found either completely submerged or free floating on the surface of water. Aquatic algae occurs in pond, pool, tank, streams or in slow running rivers called fresh water forms. Marine algae are found in sea and macroscopic large thalli of brown algae are known as seaweeds. Fresh water forms like *Chlamydomonas, Volvox, Hydrodictyon* are found in stagnant waters, whereas *Cladophora, Oedogonium, Ulothrix* occur in slow running water bodies. Majority of members of Phaeophyceae and Rhodophyceae are present in sea either floating on the surface of sea water or attached with rocks or any surface. The free floating and free swimming microscopic algal forms together with other similar organisms constitute the Planktons of water bodies. Planktons forming algae may either be free floating and are never attached, for example, *Microcystis, Chlamydomonas* (Refer Figure 4.4), or in the beginning may be attached but later on they get detached and become free floating, for example, *Oedogonium, Cladophora.*

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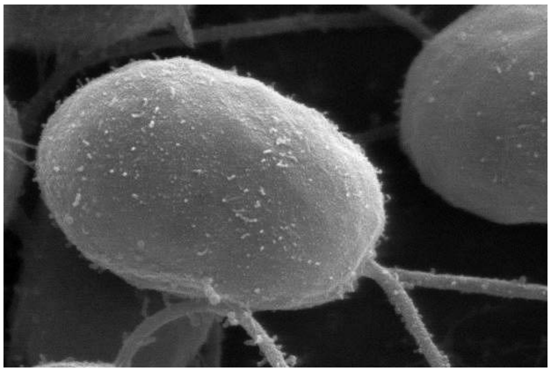

Fig. 4.4 *Chlamydomonas*

Aerophytes: Such algal forms are adapted for aerial mode of life and occur on the tree trunks, moist walls, flower pots, rocks, fencing wires and get their water and carbon dioxide requirements directly from atmosphere are called *xerophytes*. *Trentepohlia* is found on the bark of trees in moist and humid climatic conditions while *Scytonema* have been observed to grow on bark of trees along with Bryophytes.

Thermophytes: The genera of algae occurring in hot springs at quite high temperature are called thermophytes. There are certain algae which are known to tolerate the temperature up to 85 dergee Celsius, for example, few genera belonging to family Chroococcaceae and Oscillatoriaceae. Oscillatoria, *Haplosiphon lignosum* are common examples of thermophytes which can survive up to a temperature of 70 degree Celsius at which generally plant life is not possible.

Cryptohytes: These algae are found on the mountain peaks covered with snow and impart attractive colours to the mountains. *Haematococcus nivalis* gives red colour to Arctic and Alpine regions while *Chlamydomonas yellowstonensis* is responsible for green colour of the snow of the mountains of European countries mainly in arctic region. *Alaska* cryptophytes have been studied by Kol (1941) and following groups are recognized:

- Those algae which are found on snow and not on ice, for example, some species of *Chlamydomonas, Scotiella.*
- Those algae which can grow only on ice and form ice bloom, for example, *Cyclonema.*
- Those algae which grow on both ice and snow, for example, *Cylindrocystis*.
- Those algae which are not true cryptophytes and have their temporary growth on ice or snow, for example, *Gloeocapsa.*

Algae of Unusual Habitat: Many algae are found at various interesting places and according to their habitat may be of following types:

- **Halophytic Algae:** These algae are found in saline water containing high percentage of salts, for example, *Chlamydomonas chrenbergii, Stephanoptera.*
- **Lithophytic Algae:** The members of Cyanophyceae grow on moist rocks, wet walls and other rocky surfaces. Blue green algae *Rivularia* and *Gloeocapsa* occur on exposed rocks, whereas *Nostoc* is found growing in damp shady habitats. Several marine algae belonging to Rhodophyceae and Phaeophyceae are lithophytic in habit and grow on the submerged rocks, for example, *Ectocarpus, Polysiphonia,* etc.
- **Epiphytic Algae:** Such algal forms which grow on the other aquatic plants are called epiphytic algae, for example, *Oedogonium, Phanerochaete.* The colonies of *Rivularia* are observed to grow on Angiospermic plant Scirpus, whereas *Chaetophora* is found on the leaves of *Nelumbo* and *Valliseria.*
- **Epizoic Algae:** Many algae grow on the shells of mollusks, turtles and fins of fishes and are known as epizoic algae, *Cladophora* is found on snails and shells of bivalves, while *protoderma* and *Basicladia* have been observed growing on the back of turtles.
- **Parasitic Algae:** *Cephaleuros virescens* which cause red rust of tea is example of parasitic algae and causes heavy damage to tea foliage in Assam and nearby states. *Chlorochytrium* and *Phyllosiphon* are other examples of parasitic alage. *Polysiphonia festigata* a member of Rhodophyceae is reported as semiparasite on *Ascophyllum nodosum*.
- **Symbiotic Alage:** Several members of Cyanophyceae grow in association with other plants and lichens exhibit good example of it. Almost all the plant

groups are known to have symbiotic association with blue green algae, for example, *Nostoc* is found within the thalli of *Anthoceros and Notothylas*, *Anabaena* is reported in the coralloid roots of Cycas, *Anabaena azollae* occurs in Azolla, etc.

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- **Endozoic Algae:** Contrary to epizoic algal forms endozoic algae are found inside the body of aquatic animals, for example, *Zoochlorella* is found inside *Hydra viridis* while *Zooxanthellae* is known to occur inside the freshwater sponges. According to Langeron (1923) about 15 species of blue green algae belonging to family *Oscillatoria Ceae* are known to occur in the digestive and respiratory tract of vertebrates.

I. Organisation of the Thallus

The algae exhibit a great diversity in the organization of the plant body. The simplest forms are motile or non-motile cells. In many species, the cells are grouped into aggregation called colonies, for example, *Volvox, Pediastrum*. These assume various forms and may be hollow sphere, a flat plate or a filament. The filamentous forms are usually multicellular and the filament may be simple, for example, *Ulothrix*. In some multicellular forms the cells may perform both functions, vegetative and reproductive as in oedogonium, while in others special reproductive cells or organs may be developed, for example, *Chara, Sargassum*, etc. The most highly differentiated marine algae exhibit differentiation and considerable size. Macrocystis, Sargassum are good examples of it. Some of them possess a plant body consisting of parts that bear a superficial resemblance to the roots, leaves and stems of higher plants. The length of the main axis equals or surpasses the height of the tallest tree, for example, thalli of macrocystic may attain a length of more than 60 metres. Why do algae with massive thalli are marine only? Obviously the marine water can provide minerals at the rate at which a huge thallus (Refer Figure 4.5) demands whereas the inland or fresh water cant. Secondly, marine water is relatively more permanent in geological time as compared to inland water.

Fig. 4.5 Thallus

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II. Structure of Algal Cell

The cells constituting the algal thalli are basically of two kinds, prokaryotic and eukaryotic. The prokaryotic cells which constitute thalli of cyanophytes have a cell wall which contains a specific strengthening component not found in the cell walls of other algae, which is mucopeptide. The DNA material representing the nuclear body consists of fibrils which may extend throughout the cell or are concentrated in the central part. The mitotic figures are also lacking. The chlorophyll pigment is bound to photosynthetic lamellae or thylakoids which may be arranged in parallel layers in the periphery of the cytoplasm or form a network extending throughout the cell cytoplasm. They are not organized into grana. The chloroplasts are thus absent and so are the mitochondria Golgi body and endoplasmic reticulum. The ribosomes are present. The nuclear division do not take place by mitosis and no cell plate is formed. Instead there is a ring like extension of the cell wall. It extends inwards like a diaphragm with decreasing aperture and divides the cells into daughter cells. Such simple cells of blue green algae which lack a nuclear membrane, mitochondria, plastids and do not divide by mitosis are called prokaryotic.

The cells constituting the thalli of all other algae excepting the blue greens are called eukaryotic. The eukaryotic cell has the same structure as is typical of the higher plants. The nucleus is separated from the cytoplasm by a distinct of nuclear membrane. The cytoplasm contains membrane bound chloroplasts or chromatophores, mitochondria, Golgi bodies and endoplasmic reticulum.

III Algal Pigments

The colour of algal thallus which varies in different classes of algae is due to presence of definite chemical compounds in their cells. These are called pigments. Each pigment has its own characteristic colour. The particular colour that a thallus has is due to the predominance of one pigment in a combination of several others, for example, brown algae have predominance of Fucoxanthin and phycophein while red and blue green algae have excess of phycoerythrin and phycocyanin pigments respectively. Each group of algae has its own particular combination of pigments and a characteristic colour which is not found in the other algal groups. In fact the various algal groups show striking differences of colour. Thus the nature of the pigments present in the algal cells forms a quick guide to primary classification of algae into divisions. The photosynthetic pigments in algae are of three kinds namely, chlorophylls, carotenoids and phycobilins or biliproteins.

The algal chlorophylls are characterized by green colour and in solution show the phenomenon of fluorescence and emit red light. Chlorophyll pigments are fat soluble compounds and are of five different types, i.e., chlorophyll a, b, c, d and e. Out of these chlorophyll a is universally present in all the groups of algae whereas chlorophyll b, c, d and e have restricted distribution. Carotenoids are fat soluble yellow coloured pigments and are subdivided into carotene, xanthophylls and carotenoid acids. Carotenes are unsaturated hydrocarbons which are fat soluble and yellow in colour whereas xanthophylls are oxygen derivatives of carotenes and have similar properties like carotenes. Carotenoid acid resembles very much with carotenes and xanthophylls and are hydrocarbons consisting a chain of carbon atoms. Phycobilins are water soluble blue and red coloured pigments and are present in members of Cyanophyceae and Rhodophyceae.

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IV. Algal Flagella

The motile cells of algae are provided with fine, protoplasmic, whip like threads, the flagella. They are extremely fine and hyaline emergence of the cytoplasm. In cells possessing firm cell walls, the flagella are connected with the inner cytoplasm through small pores in the cell wall. There is either a single anterior flagellum or the flagella occur in pairs, rarely in great numbers on the cell. The flagella on the cell may be equal (isokont) or unequal (heterokont) in length. When the flagella are inserted laterally on is directed forwards in motion and the other backwards. They function as the locomotory or propelling structures of the cell. Usually there is a single granule at the base of each flagellum known as blepharoplasty.

Most of the cells of the thallus normally contain chlorophyll. The green cells can manufacture their own carbohydrate food from carbon dioxide and water through the agency of sunlight. The aquatic forms obtain water and carbon dioxide by osmosis and diffusion respectively from the medium in which they grow. The aerial forms obtain water from the damp substratum and carbon dioxide from the air. The algae are also able to synthesize oil and proteins from the carbohydrates which they manufacture and soluble forms of nitrogen and other minerals available in solution in the water in which they grow.

V. Food Reserves

Since the early steps in photosynthesis in all the algal groups are practically same it is but natural that the primary products of this process must also be the same. The food materials which accumulate as food reserves in the form of polysaccharides vary from group to group and thus provide useful data for preliminary classification of algae. True starch is typical of only two algal divisions namely chlorophyta and charophyta. The two other kinds of characteristic starches are the cyanophycean starch and floridean starch. The former is characteristic of division cyanophyta and latter of division Rhodophyta. The three other important polysaccharides which accumulate as reserve food are laminarin found in brown algae, paramylon characteristic of euglenoids and leucosin peculiar to the Xanthophyta, Bacillariophyta and Chrysophyta. A proteinaceous compound cyanophycin is found only in cells of blue green algae. Mannitol which was found to be unique to the brown algae has been reported to occur in few red algae. Fats occur as reserve food in appreciable amounts in the cells of Xanthophyta, Bacillariophyta and Chrysophyta. The environmental factors which favour growth of the algae are favourable temperature, suitable light, proper supply of oxygen, carbon dioxide and essential elements.

4.2.1 Reproduction

All kinds of reproduction – vegetative, asexual and sexual reproduction are found in algae. However in the members of cyanophyceae sexual reproduction is completely absent.

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I. **Vegetative Reproduction:** The common methods of vegetative reproduction are by simple cell division, fragmentation, hormogonia formation, primary and secondary protonema, tubers, bulbils, amylum starch or by formation of specialized adventitious branches or thalli called propagule. Algae reproduce by means of fragmentation which is accomplished by the separation of groups of living cells from the parent plant. The detached portion the fragment develops into a new plant. The fission is another common method of vegetative reproduction. It is affected by cell division.

II. **Asexual Reproduction:** Under favourable conditions, algae reproduce asexually. Asexual reproduction in algae takes place by different types of spores formed in favourable and unfavourable conditions by division of the protoplast. Various types of asexual spores formed are zoospores, aplanospores, hypnospores, akinetes, autospores, endospores. Zoospores are usually formed in favourable conditions whereas thick walled hypnospores are formed during adverse conditions. Formation of daughter colonies in *Volvox*, palmella stage in *Chlamydomonas* and gongrosira stage in *Vaucheria* are some specialized methods of asexual reproduction in unfavourable conditions.

III. **Sexual Reproduction:** When the conditions are unfavourable, algae reproduce sexually. During this process, the reproductive units called gametes (male and female gametes) are formed in the gametangia. The plant may be homothallic. The sexual reproduction can be categorized into two types, i.e., Isogamous and Heterogamous. Higher forms reproduce sexually which is advanced method of reproduction in which fusion of two specialized cells known as sex cells or gametes takes place. Sexual reproduction may be **isogamous** in which two identical gametes fuse to form zygote or **heterogamous** in which the gametic union takes place between two dissimilar gametes having different size and behavior. Heterogamy is divided into anisogamy and oogamy.

- **Isogamous:** It is of common occurrence among unicellular algae. In such forms both male and female gametes are morphologically similar but they differ physiologically as in *Chlamydomonas* and *Ulothrix*.
- **Heterogamous:** It is bit advanced type of sexual reproduction where two gametes differ both morphologically and physiologically. Male gametes are smaller in size. This kind of reproduction may be of two types - **Anisogamous** and **Oogamous.**
- **Anisogamous:** In Anisogamous both types of gametes are motile but male gametes are smaller in size and more active, for example, *Chlamydomonas braunii*.
- **Oogamous:** Being an advanced type of sexual reproduction, it is common among the higher algae. The male gametes are motile and are formed in large numbers whereas female gametes are non-motile and bigger in size and produced fewer in number. Male gametes are produced in the antheridium whereas female gamete an egg is produced in oogonium. Some complex type of reproductive bodies found in rhodophyceae are

called spermatozoa and carpogonium, the male and female reproductive bodies respectively.

Fertilization: In isogamous and anisogamous types of sexual reproduction both gametes are first released from their respective gametangia and the fertilization takes place outside gametangia, the process being called external fertilization. In oogamy, the male gametes are liberated from male gametangium which swims to the oogonium. Fertilization takes place inside the oogonium.

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Zygote: The two gametes after fusion form the zygote. Zygote being diploid cell represents the sporophytic phase of the life cycle. It may undergo a period of rest or may germinate immediately after fertilization. In higher algae, the diploid generation is the dominant phase of the life cycle and the gametophytic generation lasts for a short time. In majority of algae, the gametophyte phase is the dominant phase of the life cycle.

Germination of Zygote

The germination of zygote may be direct or indirect type as follows:

- **Direct Type:** In such type of germination, the zygote after meiotic division develops directly into a new plant without producing any intermediate spore like structure, for example, Spirogyra.
- **Indirect Type:** It may be of two types, as follows:
  - Zygote divides meiotically and forms motile or non-motile haploid spores of which each develops into a new plant, for example, Chlamydomonas (Refer Figure 4.6).
  - Zygote divides mitotically and forms a diploid plant. Later, plant reproduces by meiotic division and produces spores. Each spore develops into a new plant, for example, Ectocarpus.

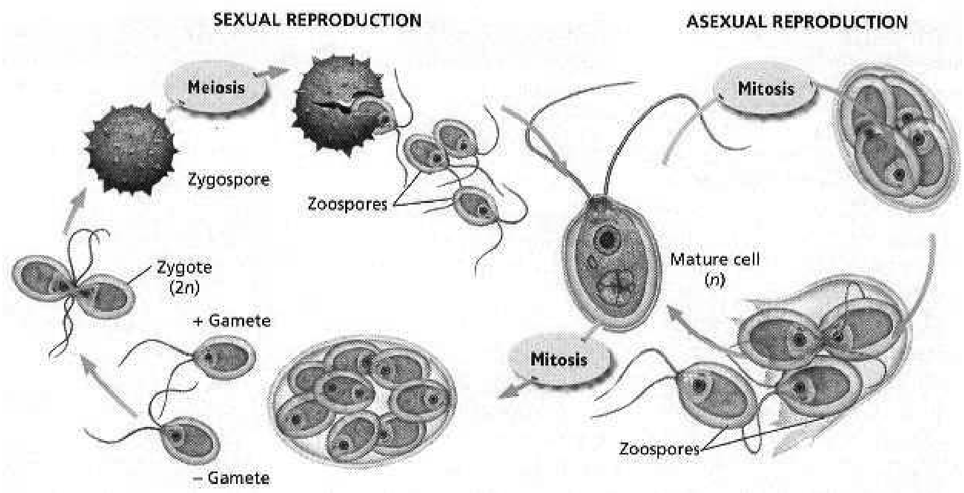

Fig. 4.6 Reproduction in Chlamydomonas

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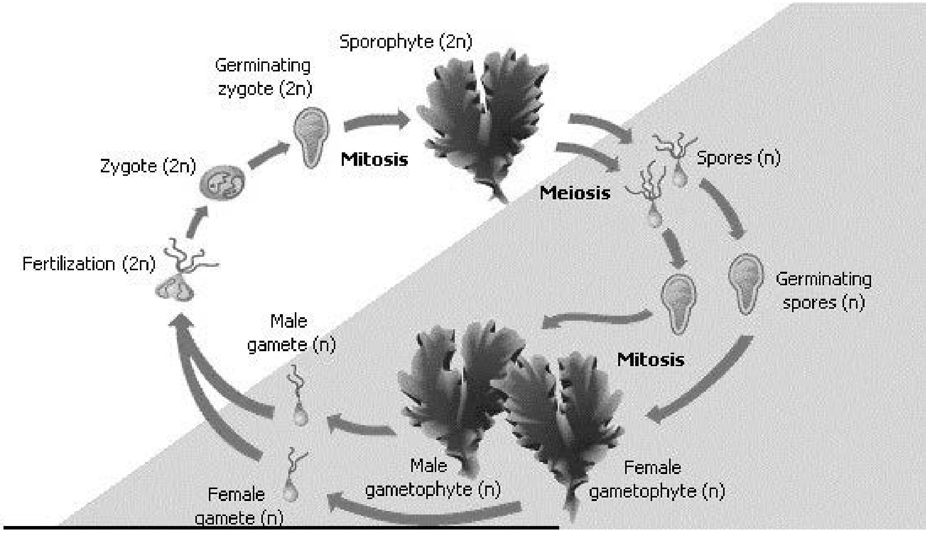

Fig. 4.7 *Reproduction in Algae*

Check Your Progress

1. Define the term algae.
2. In how many types can algae be classified on the basis of habitat?
3. Where are aerophytes algal found?

4.3 CRITERIA IN ALGAL CLASSIFICATION, OUTLINE CLASSIFICATION OF ALGAE AS PROPOSED BY FRITSCH (1935) AND SMITH(1955)

Algal Classification: The committee on the International Code of Botanical Nomenclature has recommended certain suffixes for use in the classification of Algae. These are phyta for division, phyceae for class, phycideae for subclass, ales for order, inales for suborder, aceae for family, oidae for subfamily, Greek name for genus and Latin name for a species.

The primary classification of algae is based on certain morphological and physiological features. The chief among these are as follows:

- Pigment constitution of the cell.
- Chemical nature of stored food materials.
- Kind, number, point of insertion and relative length of the flagella on the motile cell.
- Chemical composition of cell wall.
- Presence or absence of a definitely organized nucleus in the cell or any other significant detail of cell structure.

West (1916) on the basis of the works of phycologists divided algae into 5 categories which are as follows:

- Chlorophyceae
- Isokontae
- Akontae
- Stephanokontae
- Heterokontae

Pascher (1914-15) proposed a new scheme of classification on the basis of phylogeny and interrelationships of the various groups and elevated the classes to the rank of divisions. He divided the algae into 8 divisions which were subdivided into classes. The scheme proposed by him is summarized below:

Division 1: Chrysophyta

- Chrysophyceae
- Diatomeae
- Heterokontae

Division 2: Phaeophyta

- Phaeophyceae

Division 3: Pyrrophyta

- Cryptophyceae
- Desmokontae
- Dinophyceae

Division 4: Euglenophyta

- Euglenophyceae

Division 5: Chlorophyta

- Chlorophyceae
- Conjugatae

Division 6: Charophyta

- Characeae

Division 7: Rhodophyta

- Bangineae
- Floridineae

Division 8: Cyanophyta

- Myxophyceae

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Tilden (1933) recognized the importance of reserve food materials, flagellation and pigments in the plastids in algal classification and divided algae as follows:

- Chlorophyceae
- Myxophyceae
- Rhodophyceae
- Phaeophyceae
- Chrysophyceae

Fritsch (1935) in his book entitled '***The Structure and Reproduction of the Algae***' has classified algae considering phylogeny, affinities and interrelationships of various forms. Characters like structure of plant body, nature of the pigments and reserve food materials, method of reproduction, occurrence, etc., are few basic considerations in his classification. He considered algae as one group equivalent to a division divided living forms of this group into 11 classes. Each class with distinguishing characters:

I. Chlorophyceae (Grass Green)

- The chief pigments are chlorophyll a and b and two yellow pigments. Thus chromatophores are grass green in colour.
- Reserve food material is starch.
- The plants are unicellular motile to heterotrichous filaments. Cell wall consists of cellulose. Pyrenoids are surrounded by starch sheath. Motile cells have equal flagella (2 or 4) oriented similarity.
- Sexual reproduction ranges from isogamous to advanced oogamous type.
- Most forms are fresh water and few marine. There is a marked tendency towards the terrestrial habitat.

II. Xanthophyceae (Yellow Green)

- Chromatophores are yellow green. Yellow xanthophylls is found abundantly.
- Reserve food material is oil.
- Plants are unicellular motile to simple filamentous. Cell wall is rich in pectic compounds and composed of two equal pieces overlapping at their edges. Motile cells have two very unequal flagella. Pyrenoids are absent.
- Sexual reproduction is rare.
- Most forms are fresh water but few are marine also.

III. Chrysophyceae (Brown or Orange)

- Chromatophores are brown or orange coloured. Phycochrysin serves as chief accessory pigment.
- Fat and leucosin are reserve food.
- Plants are unicellular motile to branched filamentous. Flagella are unequal attached at the front end. Cells contain one or two parietal chromatophores.

- Sexual reproduction seldom occurs but is of isogamous type.
- Most forms occur in cold fresh water but few are marine.

IV. Bacillariophyceae (Yellow or Golden Brown)

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- Chromatophores are yellow or golden brown. Nature of accessory pigments is not very definite.
- Reserve food material is fat and volutin.
- All the members are unicellular or colonial. Cell wall is partly composed of silica and partly of pectic compounds. It consists of two halves and each has two or more species. Cell wall is richly ornamented.
- Forms are diploid. Sexual reproduction is of special type which occurs by fusion of protoplasts of ordinary individuals.
- Plants occur widely and make their appearance in all kinds of fresh water, sea, soil and terrestrial habitats.

V. Cryptophyceae (Nearly Brown)

- Chromatophores show diverse pigmentation. It may be some shade of brown. Chromatophores are usually parietal.
- Solid carbohydrates or in some cases starch is present.
- Plants are represented by motile cells and most advanced forms are coccoid. Flagella are slightly unequal.
- It is of isogamous type in the reported cases.
- Plants are equally distributed both in marine and fresh water.

VI. Dinophyceae (Dark Yellow or Brown)

- Chromatophores are dark yellow, brown and contain a number of special pigments. Chromatophores are numerous and discoid.
- Reserve food is starch and oil.
- Plants are unicellular motile to branched filamentous.
- Sexual reproduction is of isogamous type. It is rare and not very definite.
- Plants occur widely as sea water planktons. A few may be fresh water forms

VII. Chloromonadineae (Bright Green)

- Chromatophores are bright green in colour and contains excess of xanthophylls.
- Reserve food is oil.
- The plants are motile flagellate with two almost equal flagella.
- All plants are fresh water forms.

VIII. Euglenineae (Pure Green)

- Chromatophores are pure green. Each cell has several chromatophores.

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- Polysaccharide and paramylon is the reserve food.
- The plants are motile flagellates. Flagella may be one or two arising from the base of canal like invagination at the front end. Plants have complex vacuolar system and a large and prominent nucleus.
- Sexual reproduction is not known. It is of isogamous type.
- Only fresh water forms are known.

IX. Phaeophyceae (Brown)

- Chromatophores are brown containing yellow fucoxanthin.
- Alcohol, polysaccharide and fats are the reserve food.
- The plants may be simple filamentous to bulky parnechymatous forms. Several plants attain a giant size with external or internal differentiation.
- Sexual reproduction ranges from isogamous to oogamous. Motile gametes have two laterally attached flagella. Life cycle shows varied types of alteration of generation.
- All plants but few are marine.

X. Rhodophyceae (Red)

- Chromatophores are red blue containing pigments like red phycoerythrin and blue phycocyanin.
- Floridean starch is the reserve food material.
- The plants are simple filamentous to attaining complexity of structure. Motile structures are not known.
- Sexual reproduction is advanced oogamous type. The male organ produces non-motile gametes and the female organ has a long receptive neck. After sexual reproduction special spores are produced.
- Few forms are fresh water and others are marine.

XI. Myxophyceae (Cyanobacteria)

- The pigments of common occurrence are chlorophyll,carotenoids, xanthophylls , phycocyanin and phycoerythrin.
- Sugars and glycogen are reserve food material.
- The cell has a very rudimentary nucleus, no proper chromatophores, the photosynthetic pigments being diffused throughout the peripheral cytoplasm.
- There is no sexual reproduction.
- Forms are found in sea and fresh water.

G.M. Smith (1955) modified the classification of algae proposed by **Pascher (1914-1931)** and proposed his own system of classification with minor modifications. He divided algae into seven divisions which were subdivided into classes.

- **Division1:** Chlorophyta includes about 5700 forms out of which 90% are fresh water and the remaining 10% are marine. Dominant pigments are chlorophyll a and b, the reserve food starch. Divided into two classes – (i) Chlorophyceae (green algae), for example, *Volvox*, *Ulothrix*, and (ii) Charophyceae (stoneworts), for example, *Chara*.

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- **Division 2:** Euglenophyta includes 450 fresh water or terrestrial forms. Dominant pigments are chlorophyll and beta carotene and reserve foods are paramylum and fats. Has been divided into a single class Euglenophyceae (the euglenoids), for example, *Euglena*.
- **Division 3:** Pyrrophyta include 1000 species mainly unicellular rarely colonial. Pigments are chlorophyll a and c, carotene and xanthophylls. Reserve food is starch or oil. Divided into two classes, i.e.,
  - o Desmophyceae, for example, *Desmarestia*.
  - o Dinophyta (Dinoflagellates), for example, *Dinophysis*.
- **Division 4:** Chrysophyta is represented by 6000 species of which 75% are freshwater and the remaining 25% are marine. Dominant pigments are carotene and xanthophylls and reserve food is leucosin and oil. Divided into three classes:
  - o Chrysophyceae (golden brown algae), for example, *Chromulina*.
  - o Xanthophyceae (Yellow green algae), for example, *Botrydium*.
  - o Bacillariophyceae, for example, *Pinnularia*.
- **Division 5:** Phaeophyceae (Brown algae) is represented by 1000 mostly marine forms, dominant pigments are phycocyanin and fucoxanthin and reserve foods are laminarin and mannitol. Divided into three classes:
  - o Isogeneratae, for example, *Ectocarpus*.
  - o Heterogeneratae, for example, *Myrionema*.
  - o Cyclosporae, for example, *Sargassum*.
- **Division 6:** Cyanophyta (Blue green algae) is represented by 1500 mostly freshwater species. Pigments are chlorophyll a and b, c-phycocyanin and c-phycoerythrin and the reserve food is cyanophycean starch. Motile cells absent. Divided into a single class Myxophyceae, for example, *Nostoc*.
- **Division 7:** Rhodophyta (Red Algae) include 2500 species mostly marine, in which predominant pigments are r-phycoerythrin. Reserve food is floridean starch. Division contains only one class Rhodophyceae, for example, *Polysiphonia, Gracilaria*.

Check Your Progress

4. What are the primary classification of algae is based on?
5. Name the 5 categories of algae that West (1916) classified on the basis of the works of phycologists.
6. List any two features of Chlorophyceae (Grass Green).

4.4 ECONOMIC USES OF ALGAE

Following are the economic importance of algae:

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- **Role in Industry**: The algae are useful as the source of many commercial products. The four major products derived commercially from them are agar agar, alginic acid carrageenan and diatomite. The industrial utilization of algae mainly sea weeds dates back to many hundreds of years.
- **Agar-Agar:** It is mucilage produced by certain red algae and stored along with cellulose in the cell walls. The main source of agar-agar in japan are the thalli of *Gelidium, Gracilaria* and *Gigartina*. The other red algae used for this purpose are *Camphylaephora, Hypnea* and *Furcellaria*. Agar is gelatinous, clear, nitrogen free extract from the genera of red algae. The extract is a gel containing galactose and a sulphate. Its melting point is between 90 and 100 F. At lower temperature it changes into a solid. Agar agar is of great value in the preparation of foodstuffs and us used in the articles of diet. It is insoluble in cold but soluble in hot water. It is almost a necessity to research as it is used as a base for culture media for bacteria, fungi, algae and other tissues. It is used in the preparation of medicines, cosmetics, leather and textile industries. It is used as a laxative, culture medium, baked foods, meat industry and as an emulsifier in dairy products.
- **Carrageenan:** The chief source of carrageenan is a red alga *Chondrus crispus* (Irish moss) and to lesser extent *Gigartina*. It is cell wall polysaccharide and is a mucilaginous extract. Carrageenan is used in food, textile, pharmaceutical, leather and brewing industries. It is used to stabilize emulsions and as remedy for cough. It is used as a component of toothpastes, deodorants, cosmetics and paints is no less important.
- **Alginic Acid:** The chief source of alginic acid are *Ascophyllum, Laminaria, Macrocystis*. Algin is a carbohydrate which occurs in the middle lamellae and primary wall of these sea weeds. It is a colloidal material. The soluble calcium salt of alginic acid is known as algin. The insoluble extract is alginic acid. The salts of alginic acid found in the cell wall of sea weeds are called alginates. The alginates are used as thickeners in food industry, cosmetic and in textile industry as printing pastes. They are of great use in production of plastic and artificial fibres. They are used in rubber industry and in latex production. The alginates are used as emulsifiers, as gelling agents in confectinery. Algin is used in about 80 different commercial products. Due to its wide use, the Kelp beds are harvested at regular intervals by cutting and collecting machines.
- **Diatomite:** Diatomite are the rock like deposit formed from the indestructible, siliceous frustules (cell walls) of the past diatoms that had collected over millions of years on the floor of seas to form oceanic sediments. It extends hundreds and thousands of feet in depth in some areas. These diatomaceous deposits are mined in several parts of the world to obtain diatomaceous earth which is put to commercial uses. It is whitish substance, firm but soft and

light. It is porous and insoluble. It has abrasive qualities and is chemically inert. It is fire proof and highly absorbent. It is used in insulation of refrigerators, boilers, hot and cold pipes, sound proof rooms, packing corrosive chemical liquids, in polishes for metals, insulation for pipes and furnaces, in manufacture of dynamite. It is used as base on automobile and silver polishes. Its use in wine and paper industries is also important.

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- **Role in Agriculture**: The algae is used as fertilizer mainly as sea weed. The agricultural utilization of sea weeds as manure is confined to the farm land near the coastal regions. Large brown and red algae are used as organic fertilizers. They are rich in potassium but poor in nitrogen and phosphorous than the farm manure. In Japan, they are used in rice fields whereas in China for groundnuts and sweet potatoes. In Rajasthan, *Anabaena* and *Spirulina* which are produced in enormous quantities in the Sambhar salt lake, are employed as green manure by localities. The sea weeds are used as compost. In some countries, there is a practice to grind up *Lithothamnion and Lithophyllum* and use it in place of lime. Similar use is made of species of *Chara* which are encrusted with calcium carbonate. The algae such as chlorella also help to aerate water by removing carbon dioxide and restoring supply of oxygen in photosynthesis. This is important to the fish. Their growth is encouraged in the sewage disposal plants. They take up nitrates and phosphates for their metabolism and liberate oxygen in photosynthesis which help the aerobic bacteria to decompose raw sewage.
- **Soil is a Living Mass:** Apart from the soil particles it harbours bacteria, fungi, algae and other microbes. The algae occupy a volume of 3 times that of bacteria. Of the soil alga the blue greens are the most important. They are capable of fixing atmospheric nitrogen in their bodies. Upon liberation, nitrogen in usable form increases soil fertility and improves the growth of crop plants. Blue green algae is the chief agent for nitrogen fixation in rice fields. Some of the important nitrogen fixing blue green algae are *Oscillatoria, Spirulina, Nostoc* and *Cylindrospermum.* The practical application of these algae as fertilizers is the seeding of rice fields with nitrogen fixing species to increase the nitrogen content of the soil.
- **Role of Algae as Space Travel**: The biologists construct a system in which there would be a complete recycling of the biological materials. This biological system will be used during a space flight trip lasting over 30 days. The spaceman will need a device to get rid of carbon dioxide and other body wastes and also require source of oxygen and food. It is considered beneficial to use microscopic, unicellular algae, such as *Chlorella pyrenoidosa* and *Synechococcus* as a food source in space flight. It multiplies rapidly and thus will synthesizes a rich harvest of food utilizing carbon dioxide and liberating oxygen as a byproduct. As a source of nitrogen for protein synthesis it will assist in the decomposition of wastes, such as urine or faeces.
- **Role of Algae as Food**: Algae are important source of food for fishes, aquatic amphibian, mammals and other animals. Some biologists suggest that algae be raised by man as a source of food. Miller (1972) believed that the

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algae can play an important role in the production of protein for human food. In the Pacific islands and orient the red, brown, green algae form regular portion of diet. The colonies of Nostoc are boiled and used as food in Brazil. In the Pacific Islands raw algae are chopped and added to other dishes. The laminarin food from stipes are called Kombu and from Alaria as sarumen in Japan. *Durvillea* and *Ulva* are dried, salted and sold. *Ulva lactuca* was used in salad and soups in Scotland. *Porphyra* is a tasteful dish in England and is common item of diet in Korea, Japan and China. The prolific users of sea weeds as food are the coastal people in China and Japan. *Caulerpa rocemosa* is cultivated in Phillipines as a source of food. In Japan many of them are cultivated in bamboo frames to meet their demand. The algae are considered rich in proteins, fats and Vitamins A, B, C and E. The vitamins A and D which are commercially obtained from the livers of shark and fish come from synthesis by the plankton algae which form the food of the fish. Diatom *Nitzschia* is rich in vitamin A. Vitamin B is found in *Ulva, Laminaria, Porphyra* and *Chondrus crispus. Ulva and Undaria* also contain Vitamin C. *Fucoids* and *Porphyra* are richer.

- **Role of Algae as Fodder:** The sea weeds are used as fodder for animals with beneficial effects. A red algae *Rhodymenia* is used as a cattle fodder in France. The milk yielding capacity of the cattle is enhanced when dried sea weeds forms an ingredient in cow feed. Similarly increased butter and fat content of milk is reported from cattle whose diet is supplemented with sea weed meal. In China, Sargassum is used as fodder. Egg fowls fed on chopped sea weeds have increased iodine and carotene content. The egg laying capacity of the poultry increases. *Microcystis* sp is used being rich in vitamins A and E.
- **Role of Algae as Medicine:** A patient suffering from prolapsed stomach is fed on a diet containing dried agar agar and asked to drink a lot of water. The stomach gets distended and regains its normal condition. A few algae are source of antibiotics which inhibit the growth of other bacteria. Agar agar is an important algal product used in manufacture of pills and ointments. Carrageenan extract, which is another product of algal origin act as blood coagulant. An effective vermifuge is obtained from the extracts of *Digenea*, *Codium* and *Durvillea*. Many algae are used in the treatment of lung, kidney and bladder ailments.
- **Role of Algae in the Origin of Petroleum and Fuel Gas**: The fuels, such as petroleum and natural gas have their origin in the organic matter in the marine environment. Minute algae constituting the plankton trapped the suns energy during photosynthesis. It was transferred to the marine animals as they fed upon them. The organic compounds derived from the dead bodies of plants and animals constitute the plankton which gradually accumulate at the bottom of ocean and are buried by sedentary action. The natural gas associated with oil is methane which is produced by action of methane producing bacteria.
- **Algae and Sewage Disposal**: Sewage is the foul domestic and industrial waste which is deficient in oxygen but bounds in dissolved and suspended

organic and inorganic materials. It harbours microbes of decay and decomposition. The use of small green algae such as Chlorella, Euglena in large shallow tanks of sewage oxidation ponds has proved a rapid, cheap and effective means of converting dangerous and expensive waste into odourless and valuable fertilizer. These tanks promote growth of the algae which flourish at the expense of the mineral nutrients present in the sewage. The rapid photosynthetic activity of the algae produces abundant oxygen which is used by microbes responsible for decomposition of organic matter in the sewage.

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- **Algae and Water Supplies:** In the rainy season and spring, the blue green algae, green algae, golden brown algae and diatoms become so abundant that the water in the ponds, lakes and reservoirs becomes cloudy and assume yellowish tinge. A floating yellowish green scum may develop on the surface of water, also known as manifestations as water blooms. Such concentrations of algae impart unpleasant odour, oily and fishy taste to the drinking water. Some blue green alage produce toxic protein products which are poisonous to fish, cattle, sheep and other domesticated animals.
- **Algae as Biofertilizers:** The idea of biofertilizers originated due to the high cost of chemical fertilizers and high level of industrialization. Manufacture of nitrogenous fertilizers consume high energy generated from fossil fuels like oil and coal which are depleting in countries like India. In addition indiscriminate use of chemical fertilizers has not only put an additional burden on conventional sources of energy but also disturbed the aquatic as well as soil ecosystems.

  Since most of the nutrient supply of the soil is dependent upon the activity of the microbes and plenty of nitrogen present in the atmosphere cannot be used by plants directly. The same is true with sulphur and phosphorus, present in soil in complex forms which have to be mineralized by microbes in order to make them available to the plants for utilization. Cyanobacteria and bacteria are used as biofertilizers as they are known to fix atmospheric nitrogen.

  o **Cyanobacteria Biofertilizer:** Several blue green algae, for example, *Anabaena, Nostoc, Aulosira, Cylindrospermum. Tolypothrix* are good nitrogen fixers and their role in fixation of atmospheric nitrogen in the paddy fields has been found. Unlike Nitrogen fixing bacteria, cyanobacteria can photosynthesize and do not need external supply of carbon compounds. The nitrogenous compounds released in the growing medium include amino acids, proteins and growth promoting substances.

  o **Bacterial Biofertilizer:** Several free living and symbiotic bacteria, for example, *Clostridium, Rhodospirillum, Azotobacter, Bacillus*, etc., are known to fix atmospheric Nitrogen and add to fertility of soil. *Rhizobium* occurs as free living microbe in the soil but does not fix nitrogen unless it establishes symbiotic relationship with legumes. Bacteria are known to secrete growth substance and secondary metabolites which help in seed germination and plant growth.

  o **Mycorrhiza as Biofertilizer:** Mycorrhizae are group of fungi which include types based on the different structures formed inside or outside

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the roots of plants. The fungal symbiont gets shelter and food from the plant while the plant gets a variety of benefits like better uptake of phosphorus, salinity and drought tolerance, heavy metal toxicity and increased plant growth and development.

- **Algae as Water Pollution:** Rapid industrialization and urbanization associated with population explosion has increased the extent of water pollution many fold. Water bodies have been used as a sink for disposal of sewage and industrial wastes since long. This has caused increase in extent of water pollution and decrease in regeneration capacity of water bodies. Unwise use of agrochemicals specially nitrogen and phosphate fertilizers has enriched the permanent water bodies with nutrients which facilitates the growth of diverse aquatic plants specially algae. Sometimes massive growth of bloom forming algae take place due to increased nutrient status of permanent water bodies which deteriorates the water quality of these bodies. Certain algae flourish well in organically polluted water and cause eutrophication of ponds, pools and lakes as a result of continuous increase of organic matter. Besides some algal genera serve as indicators of water pollution. These have been used as biological markers of the extent of water pollution. The composition of algal communities of rivers in relation with its extent of pollution and identified three main zones of polluted river which favour the growth of diverse species of algae. These zones are:
    - o **Polysaprobic Zone:** Polysaprobic zone is rich in the compounds of high molecular weight and is biodegradable in nature. It is oxygen deficient zone and favours the growth of *Euglena, Oscillatoria.*
    - o **Mesosaprobic Zone:** Mesosaprobic zone lies in between polysaprobic zone and oligosaprobic zone and is characterized by having less decomposition of organic matter.
    - o **Polysaprobic Zone:** Polysaprobic zone is not much oxygen deficient and there is moderate availability of oxygen. Few species of *Oscillatoria*, *Ultothrix* have better growth in this zone.
    - o **Oligosaprobic Zone:** Oligosaprobic zone have little amount of biodegradable organic matter, is rich in dissolved oxygen and favours the growth of number of algal genera, such as *Cladophora, Spirogyra,* etc.
- Algal species as indicators of industrial wastes:
    - o **Indicators of Paper Mill Wastes:** *Ulothrix zonata, Scenedesmus bijuga, Pandorina morum, Oscillatoria splendid.*
    - o **Indicators of Oil Wastes***: Diatoma vulgares, Navicula radiosa, Synedra acus, Amphora ovalis.*
    - o **Indicators of Iron as Wastes:** *Surirella linearis and Trachelomonas hispida.*
- Spirogyra is used in the preparation of lens paper by some Japanese manufacturers.

- Several algae are able to metabolise chemicals poured in water reservoirs. Some algae store and concentrate radioactive chemicals as well as heavy metals, for example, *Chlorella, Scenedesmus*.
- Sewage consists of organic and inorganic substances suspended or dissolved in water. It contains a number of faecal and other bacteria. The organic matter is completely decayed in sewage oxidation tanks. In the presence of oxygen the organic matter is completely decomposed and the minerals are released. The mineral rich water is filtered and supplied to agriculture.
- Petroleum reserves are largely organic in nature. Some diatoms play a major role in the formation of petroleum. They have a high rate of photosynthesis. Algae are the major primary producers of food in the marine habitats. Most of the organic matter is derived from them. The dead bodies of organisms settle down at the bottom of the sea where decay is little due to extreme pressure and absence of aerobic conditions. The accumulated organic matter is covered with inorganic sediments. It is slowly changed into petroleum.
- Blue green algae often invade salt pans used for the manufacture of table salt. Their growth imparts foul smell and red rust colour to the salt. Salt crystallization is reduced.
- During rainy seasons, blue green algae can grow on all exposed surfaces of the buildings. They get entrenched there permanently, for example, *Chlorococcum, Scytonema*. Algal growth produces corroding chemicals which damages the building.
- Along with some crustaceans and molluscs, several algae grow on the hulls of ships and boats, for example, *Cladophora, Enteromorpha*. They cause corrosion and shorten the life of vessels. The thick growth of algae produces resistance in the movement of vessels and their speed is reduced.
- The only parasitic algae which caused serious economic loss is *Cephaleuros*. *C. virescens* attacks leaves of tea plants and causes disease known as red rot. It attacks piper plantation of India. Two important parasitic species of this genus are *C. parasitica* and *C. coffeae*.
- In pisciculture the fish larvae are reared in special tanks having flagellate and other smaller algae. A number of adult fishes are also herbivorous and feed on all types of algae belonging to Cyanophyceae, Chlorophyceae and Bacillariophyceae. Besides functioning as article of food, the algae also keep the water habitable for fish by releasing oxygen and consuming carbon dioxide during their photosynthesis.

Check Your Progress

7. What is the main source of agar agar in Japan?
8. Where is Carrageenan used?
9. Define the term algin.
10. What are diatomite?

4.5 ANSWERS TO 'CHECK YOUR PROGRESS'

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1. Algae can be defined as eukaryotic (nucleus-bearing) organisms that conduct photosynthesize but do not have specialized multicellular reproductive structures of plants, that contains fertile gamete, producing cells surrounded by sterile cells.
2. According to the habitat, the algae may be classified as follows:
   - Terrestrial Algae
   - Aquatic Algae
   - Aerophytes
   - Thermophytes
   - Cryptophytes
   - Algae of Unusual Habitats
3. Aerophytes algal forms are adapted for aerial mode of life and occur on the tree trunks, moist walls, flower pots, rocks, fencing wires and get their water and carbon dioxide requirements directly from atmosphere are called *xerophytes*.
4. The primary classification of algae is based on certain morphological and physiological features. The chief among these are as follows:
   - Pigment constitution of the cell.
   - Chemical nature of stored food materials
   - Kind, number, point of insertion and relative length of the flagella on the motile cell.
   - Chemical composition of cell wall.
   - Presence or absence of a definitely organized nucleus in the cell or any other significant detail of cell structure.
5. West (1916) on the basis of the works of phycologists divided algae into 5 categories which are as follows:
   - Chlorophyceae
   - Isokontae
   - Akontae
   - Stephanokontae
   - Heterokontae
6. Chlorophyceae (Grass Green) have the following features:
   - The chief pigments are chlorophyll a and b and two yellow pigments. Thus chromatophores are grass green in colour.
   - Reserve food material is starch.

- The plants are unicellular motile to heterotrichous filaments. Cell wall consists of cellulose. Pyrenoids are surrounded by starch sheath. Motile cells have equal flagella (2 or 4) oriented similarity.

7. The main source of agar agar in Japan are the thalli of *Gelidium, Gracilaria* and *Gigartina*.
8. Carrageenan is used in food, textile, pharmaceutical, leather and brewing industries. It is used to stabilize emulsions and as remedy for cough. Its use as a component of toothpastes, deodorants, cosmetics and paints is no less important.
9. Algin is a carbohydrate which occurs in the middle lamellae and primary wall of these sea weeds.
10. Diatomite are the rock like deposit formed from the indestructible, siliceous frustules (cell walls) of the past diatoms that had collected over millions of years on the floor of seas to form oceanic sediments.

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4.6 SUMMARY

- Algae can be defined as eukaryotic (nucleus-bearing) organisms that conduct photosynthesize but do not have specialized multicellular reproductive structures of plants, that contains fertile gamete, producing cells surrounded by sterile cells.
- Algae do not have true roots, stems, and leaves, i.e., the features they share with the vascular lower plants, for example, mosses, liverworts, and hornworts.
- Microalgae comprise a vast group of photosynthetic, heterotrophic organisms which have an extraordinary potential for cultivation as energy crops, which can be cultivated under difficult agro-climatic conditions and are able to produce a wide range of commercially interesting by products, such as, fats, oils, sugars and functional bioactive compounds.
- Seaweed is a loose colloquial term encompassing macroscopic, multicellular, benthic marine algae. It includes some members of the red, brown and green algae which are photosynthetic, like plants, and 'simple' because they lack the many distinct organs found in land plants.
- According to Fritsch (1935) algae includes all holophytic organisms that fail to reach the higher level of differentiation characteristic of higher plants.
- According to R.N Singh, algae are large simple plants displaying a wide range of photosynthetic pigments and evolve oxygen during photosynthesis.
- Sharma (1987) defines algae as an assemblage of chlorophyll bearing autotrophic thallophytes bounded by a cell wall made up of pure or mixed carbohydrates.
- The study of algae is known as algalogy or phycology.

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- There are over 35000 species of algae which live in all sorts of habitats and show a high degree of morphological and biochemical plasticity.
- Algae are chlorophyllous thalloid and their achlorophyllous relatives were the gametangia and sporangia are either unicellular or have all the cells fertile and in which the zygote do not develop into an embryo or parasitic sporophyte.
- Many genera of algae are found on or beneath the moist soil surface and are called terrestrial algae.
- The algal forms present on the surface of soil, for example, few species of *Vaucheria, Botrydium, Ulocladium* are called saprophytes while algal genera having subterranean habitat, for example, few species of *Nostoc, Anabaena* and *Euglena* are known as Cryophytes.
- Most of the algal genera are aquatic and are found either completely submerged or free floating on the surface of water.
- Aquatic algae occurs in pond, pool, tank, streams or in slow running rivers called fresh water forms.
- Marine algae are found in sea and macroscopic large thalli of brown algae are known as sea weeds.
- Fresh water forms like *Chlamydomonas, Volvox, Hydrodictyon* are found in stagnant waters, whereas *Cladophora, Oedogonium, Ulothrix* occur in slow running water bodies.
- Planktons forming algae may either be free floating and are never attached, for example, *Microcystis, Chlamydomonas*, or in the beginning may be attached but later on they get detached and become free floating, for example, *Oedogonium, Cladophora.*
- The algae exhibit a great diversity in the organization of the plant body. The simplest forms are motile or non-motile cells. In many species, the cells are grouped into aggregation called colonies, for example, Volvox, Pediastrum.
- The cells constituting the algal thalli are basically of two kinds, prokaryotic and eukaryotic.
- The prokaryotic cells which constitute thalli of cyanophytes have a cell wall which contains a specific strengthening component not found in the cell walls of other algae, which is mucopeptide.
- The cells constituting the thalli of all other algae excepting the blue greens are called eukaryotic. The eukaryotic cell has the same structure as is typical of the higher plants.
- The nucleus is separated from the cytoplasm by a distinct of nuclear membrane. The cytoplasm contains membrane bound chloroplasts or chromatophores, mitochondria, Golgi bodies and endoplasmic reticulum.
- The common methods of vegetative reproduction are simple cell division, fragmentation, hormogonia formation, primary and secondary protonema, tubers, bulbils, amylum starch or by formation of specialized adventitious branches or thalli called propagule.

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- In isogamous and anisogamous types of sexual reproduction both gametes are first released from their respective gametangia and the fertilization takes place outside gametangia, the process being called external fertilization.
- In oogamy, the male gametes are liberated from male gametangium which swims to the oogonium. Fertilization takes place inside the oogonium.
- The two gametes after fusion form the zygote. Zygote being diploid cell represents the sporophytic phase of the life cycle. It may undergo a period of rest or may germinate immediately after fertilization.
- In higher algae, the diploid generation is the dominant phase of the life cycle and the gametophytic generation lasts for a short time.
- Pascher (1914-15) proposed a new scheme of classification on the basis of phylogeny and interrelationships of the various groups and elevated the classes to the rank of divisions.
- Fritsch (1935) in his book entitled '*The Structure and Reproduction of the Algae*' has classified algae considering phylogeny, affinities and interrelationships of various forms.
- Characters like structure of plant body, nature of the pigments and reserve food materials, method of reproduction, occurrence, etc., are few basic considerations in Fritsch's classification.
- G.M. Smith (1955) modified the classification of algae proposed by Pascher (1914-1931) and proposed his own system of classification with minor modifications. He divided algae into seven divisions which were subdivided into classes.
- Chlorophyta includes about 5700 forms out of which 90% are fresh water and the remaining 10% are marine.
- Euglenophyta includes 450 fresh water or terrestrial forms.
- Pyrrophyta include 1000 species mainly unicellular rarely colonial and the pigments present are chlorophyll a and c, carotene and xanthophylls.
- Chrysophyta is represented by 6000 species of which 75% are freshwater and the remaining 25% are marine.

  Phaeophyceae (Brown algae) is represented by 1000 mostly marine forms, dominant pigments are phycocyanin and fucoxanthin and reserve foods are laminarin and mannitol.
- Cyanophyta (Blue green algae) is represented by 1500 mostly freshwater species.
- Rhodophyta (Red algae) include 2500 species mostly marine, in which predominant pigments are r-phycoerythrin.
- The algae are useful as the source of many commercial products. The four major products derived commercially from them are agar agar, alginic acid carrageenan and diatomite.

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- The chief source of alginic acid are *Ascophyllum, Laminaria, Macrocystis*.
- Algin is a carbohydrate which occurs in the middle lamellae and primary wall of these sea weeds.
- Diatomite are the rock like deposit formed from the indestructible, siliceous frustules (cell walls) of the past diatoms that had collected over millions of years on the floor of seas to form oceanic sediments. It extends hundreds and thousands of feet in depth in some areas.
- Algae are important source of food of fishes, aquatic amphibian, mammals and other animals.
- In the rainy season and spring, the blue green algae, green algae, golden brown algae and diatoms become so abundant that the water in the ponds, lakes and reservoirs becomes cloudy and assume yellowish tinge.
- Several blue green algae, for example, *Anabaena, Nostoc, Aulosira, Cylindrospermum. Tolypothrix* are good nitrogen fixers and their role in fixation of atmospheric nitrogen in the paddy fields has been found.
- Polysaprobic zone is rich in the compounds of high molecular weight and is biodegradable in nature. It is oxygen deficient zone and favours the growth of *Euglena, Oscillatoria.*
- Mesosaprobic zone lies in between polysaprobic zone and oligosaprobic zone and is characterized by having less decomposition of organic matter as compared to
- Polysaprobic zone is not much oxygen deficient and there is moderate availability of oxygen. Few species of Oscillatoria, Ultothrix have better growth in this zone.
- Oligosaprobic zone have little amount of biodegradable organic matter, is rich in dissolved oxygen and favours the growth of number of algal genera, such as *Cladophora, Spirogyra,* etc.
- Blue green algae often invade salt pans used for the manufacture of table salt. Their growth imparts foul smell and red rust colour to the salt. Salt crystallization is reduced.
- The only parasitic algae which caused serious economic loss is Cephaleuros.
- In pisciculture the fish larvae are reared in special tanks having flagellate and other smaller algae.

4.7 KEY TERMS

- **Phycobilins:** Group of red or blue photosynthetic pigments present in some algae.
- **Isokont:** Flagella of same length or size is known as isokont.

- **Heterokont:** Flagella of different length or size are known as heterokont.
- **Flagella:** Lash like appendage that protrudes from the cell body of certain bacteria and eukaryotic cell.
- **Thallus:** Plant body not differentiated into stem and leaves and lack true roots and a vascular system.
- **Algin:** Algin is a carbohydrate which occurs in the middle lamellae and primary wall of these sea weeds.

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4.8 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Brief a note on algae.
2. What are aquatic algae?
3. Brief a note on organisation of thallus.
4. What is the structure of algal cell?
5. Write short note on algal pigments.
6. Distinguish between the following:
   (i) Isogamous and Heterogamous
   (ii) Anisogamy and Oogamy
7. Write short note on Chlorophyceae.

Long-Answer Questions

1. Write a detailed note on the classification of algae based on their habitat.
2. Explain the types of algae based on unusual habitat giving appropriate examples.
3. Discuss about the various types of reproduction in algae.
4. Write a detailed note on algal classification given by different scientists.
5. Give an account of algae classification given by Fritsch. Write characters of various classes of algae also in detail.
6. Explain the economic importance of algae.

4.9 FURTHER READING

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UNIT 5 MYCOLOGY

NOTES

Structure

5.0 INTRODUCTION

A fungus is any member of the group of eukaryotic organisms that includes microorganisms, such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, fungi, which is separate from the other eukaryotic life kingdoms of plants and animals.

A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is the presence of chitin in their cell walls. Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesis. Growth is their means of mobility, except for spores, i.e., a few of which are flagellated, which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the Eumycota, i.e., a true fungi or Eumycetes, which share a common ancestor, i.e., form a monophyletic group, an interpretation that is also strongly supported by molecular phylogenetics. This fungal group is distinct from the structurally similar Myxomycetes, i.e., slime molds and Oomycetes, i.e., water molds. The discipline of biology devoted to the study of fungi is known as mycology. In the past, mycology was regarded as a branch of botany, although it is now considered that fungi are genetically more closely related to animals than to plants.

In this unit, you will study about fungi and its general characteristics, different types of reproduction in fungi, i.e., asexual, sexual and parasexual in detail.

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5.1 OBJECTIVES

After going through this unit, you will be able:

- Understand the habit, habitat and characteristics of fungi
- Compare fungi from other organisms
- Discuss the range of morphological forms and ultrastructure of the fungal cell
- Explain the mode of nutrition, ecological forms and reproduction in fungi
- Understand the different types of reproduction in fungi, namely asexual, sexual and parasexual

5.2 GENERAL CHARACTERISTICS OF FUNGI

Fungi constitute a large and diverse group of plant kingdom. The Latin word '***fungus***' means **mushroom**. Fungi are achlorophyllous and heterotrophic thallophytes. These resemble algae in various aspects thus have been placed under group thallophyta. The study of fungi is known as **mycology** and the scientists concerned with the study of fungi are known as **mycologist**. Worldwide there are about 50,000 to 100,000 known species of fungi. At present there are about 5100 genera and 50,000 species of fungi. This number is constantly increasing because of the ongoing research throughout the world.

Various large species of fungi like mushroom, morels, smuts, rots and rusts were known to man since time immemorial. However, their nature, origin and development were not known at that time. They grow in variety of habitats and show high diversity in their form, structure of plant body (Refer Figure 5.1), physiological functions and means of reproduction. The plant body is mainly made up of hyphae which together form the mycelium; except for a few cases where mycelium is either completely absent (*Synchytrium* species) or plant body is unicellular (*Saccharomyces* species). Their cell wall is not made up of true cellulose. It is either made up of chitin or fungal cellulose. Since, the fungi cannot synthesize their food from carbon dioxide and water in the presence of sunlight their mode of nutrition is saprophytic, parasitic and symbiotic. As a saprobe they are responsible for decay of organic material, as a parasite they attack living protoplasm and cause disease of plants, animals and human beings. The chief reserve food is glycogen and oils. They lack chlorophyll so starch is not synthesized. Reproduction takes place by means of vegetative, asexual and sexual reproduction.

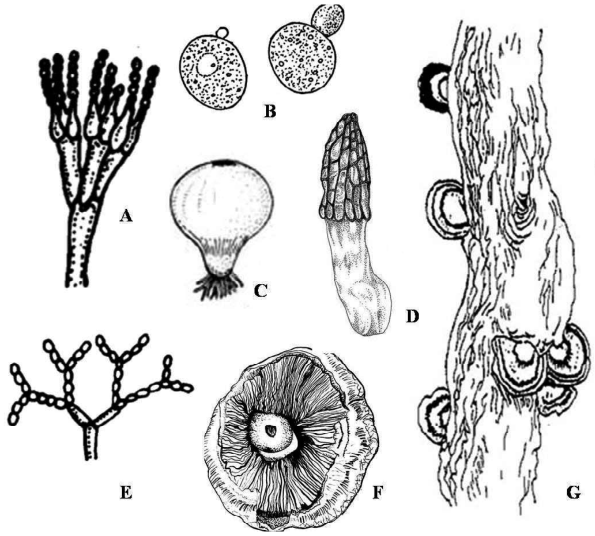

Fig. 5.1 *Various Fungi and their Fruiting Bodies*

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The above Figure 5.1 illustrates the various fungi and their fruiting bodies. A: *Penicillium*, B: *Saccharomyces cerevisiae* (Yeast), C: *Lycoperdon* (Puff Ball), D: *Morchella* (Morel), E: *Neurospora*, F: *Agaricus* (Mushroom), G: Bracket Fungi on Tree Trunk.

5.2.1 Occurrence, Distribution and Characteristics of Fungi

Fungi are cosmopolitan and most diversified in nature. They are found in almost all habitats where life is possible. Some fungi are terrestrial; some are airborne while some occur in fresh as well as marine water. Members of Phycomycetes are found in water and are known as **aquatic fungi**. Fungal species are also found in epiphytic state on algae and some other aquatic plants. Some of the fungal species grow on dead organic material present in water. Some species are found under the surface of earth and are subterranean in nature. A few fungi are also found as endophytes in leaves and stem of healthy plants. These fungi can be categorized between beneficial and harmful species. Some produce secondary metabolite which protect the host from insect and grazing animals while others can be toxic and reduce the crop yield.

Fungi are microscopic as well as macroscopic. Macroscopic fungi such as mushrooms, puff balls, bracket fungi, cup fungi and morels, etc., are recognizable with naked eye even from a distance. However, the microscopic fungi can only be identified with the help of compound microscope by subject experts.

Various species of fungi are parasitic also and infect a variety of plants, animals and human beings. The parasitic fungi which grow on vascular plants causes various diseases in them, for example brown patch, canker, downy mildew, club root, damping off, anthracnose bottom rot, crown wart, etc., and are responsible for economic loss. Alimentary canal of human beings and mammals are also occupied with fungal species where they cause stomach disorders. Some of the fungal species cause skin diseases, such as athlete's foot, mycosis, ringworm, onychomycosis, candidiasis, etc. In this way fungus are diversified and accustomed to unusual habitats.

Characteristics of Fungi that are Different from Other Kingdoms

There are a number of characteristics that makes fungal kingdom different from other plants and animal kingdoms. Some of them are as follows:

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- Fungal body is unicellular and is more often composed of microscopic threads called hyphae.
- They are non-vascular due to the absence of any specialized transport tissue.
- They are heterotrophic and feed on preformed organic material.
- Like animals their stored food material is glycogen whereas plants store food as starch.
- Unlike animals, fungi first digests the food with the help of co-enzymes and then ingests it.
- Majority of fungi reproduces by means of spores that are wind-dispersed.
- Depending upon the environmental conditions, both asexual (mitotic) and sexual (meiotic) spores are produced.
- In the life cycle of fungi only a few stages are motile, such as zoospores.
- Structurally cell wall of fungi is similar to plants but its chemical composition is different. Fungal cell wall are made up of chitin that are β-1,4 linkages of *N*-acetylglucosamine in contrast to plant cell wall which are composed β-1,4 linkages of glucose.
- Fungal cell membrane have unique sterol and ergosterol in contrast to animals which have cholesterol.
- Ultra-structure of cytoplasm is quite similar to plant cells but there are differences in types and structure of organelles.
- Nuclei of fungi is very small and it has only a few chromosomes.
- Meiosis and mitosis occurs inside the nuclear envelope.

5.2.2 Range of Thallus

Following are the different ranges of thallus types in fungi.

I. Unicellular Forms

Yeast: The fungi are eukaryotic organism which are unicellular and can be multicellular or acellular also. Yeast is of wide occurrence, it is found on sugary surface of ripened fruits and can be easily grown in any sugar solution. Individual cells remain attached to each other forming a chain. Structure of a yeast cell resembles with that of a eukaryotic cell (Refer Figure 5.2). The cell has a well-defined nucleus, endoplasmic reticulum, mitochondria and other organelles along with a large area of the cell occupied with a vacuole. Cell wall is of 2-3 layers thickness comprising of glucans and mannans. Variable amount of proteins and lipids are accumulated in the cell depending upon the stage of development. They have a particular ability of utilizing carbohydrate and hence the same *Saccharomyces* is applied to this group.

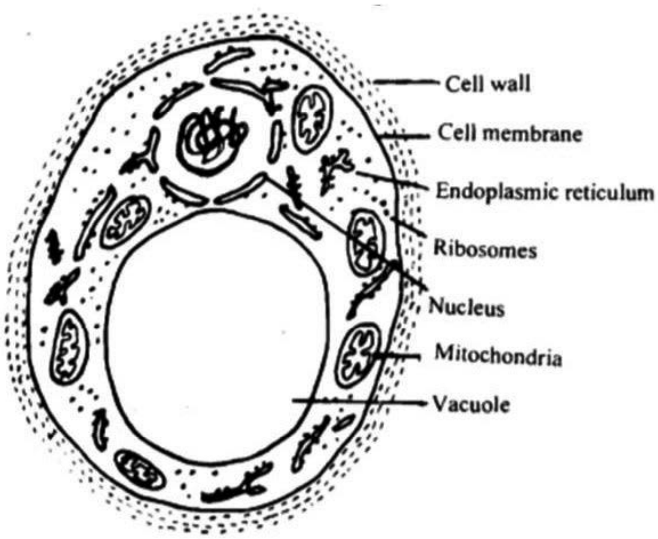

Fig. 5.2 *Structure of a Saccharomyces cerevisiae (Yeast) Cell*

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Slime Moulds: During a certain stage of the life cycle of slime moulds unicellular (multinucleate) forms are also seen. However, these are not considered true fungi since their characters resembles to both protozoa and fungi and it becomes very difficult to characterize them easily. During the course of their life cycle these organisms show protozoan-like (unicellular and multinucleate) or fungus like stages (multicellular). Slime moulds are of two types:

- **Cellular Type:** During the vegetative phase *Dictyostelium discoideum*, a small independent uninucleate cell known as myxamoebae. It feeds on bacteria by phagocytosis and cell multiplication occurs by binary fission. During the later stages of development, large number of myxamoebae comes together to form a single multinucleate slug, but the individual cell retain their cell membranes intact. The resulting structure is known as **pseudoplasmodium**. Like a true fungi, during reproductive phase spores are produced in sporangia. Each spore upon germination gives rise to an amoeba like structure (Refer Figure 5.3 (A)).
- **Plasmodial Type:** During vegetative phase in *Echinostelium minutum*, there is formation of a large mass of multinucleate amoeboid cytoplasm with diploid nuclei (Refer Figure 5.3 (B)). The individual cells are however not delimited by cell membrane. It feeds on bacteria and encysted myxamoeba. They do not have a definite size or shape and thus may be globose or spreading like a sheet over a large area in form of a thin network. It alters its shape depending upon the substratum and engulfs food on its way. During reproductive phase entire plasmodium takes part in formation of frutification which bears spores. Spores germinate to form flagellated cells which later on develop into plasmodium.

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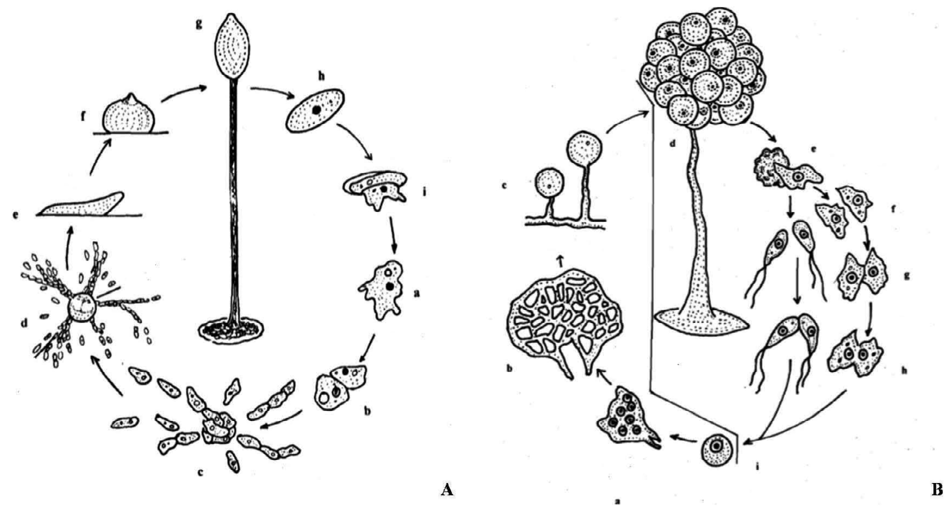

Fig. 5.3 Diagrammatic Representation of Life Cycle of Slime Mould

The above Figure 5.3 shows the diagrammatic representation of life cycle of Slime Mould. In which, A: Cellular Slime Mould *Dictyostelium discoideum* [Amoeba-Like Stage (a); Aggregating Amoebae (b and c); Cellular Plasmodium and Aggregating Amoebae (d); Migration Stage or Slug Stage (e and f); Fruiting Body (g); Spore (h); Spore Germination (i)], B: Plasmodial Slime Mould *Echinostelium minutum* [Young Multinucleate Plasmodium (a); Mature Plasmodium (b); Fructification (c); Formation of Sporangium on Fruiting Body by Meiosis (d); Germinating Spore (e); Myxamoeba or Gamete Formation (f); Fusion of Gametes and Plasmogamy in Myxamoeba (g and h); Zygote Formation (i); Diplophase (i, a, b and c); Haplophase (d, e, f, g and h)].

Slime Mould Plasmodium is brilliantly coloured. Protoplasm of the plasmodium, such as *Physarum polycephalum*, is structure-less and has granules, vacuoles and other bodies embedded in it. Additionally, one of the most fascinating processes about plasmodium is their protoplasm streaming under microscope. This streaming is related to the presence of a contractile protein known as **myxomyosin**.

II. Filamentous Forms

Most of the fungi are filamentous and each filament is known as **hypha**, basic unit of fungal body. Mass of interwoven hyphae is known as **mycelium** which has a large surface-to-volume ratio close to food source, adaptation for absorptive mode of nutrition. Filamentous forms and its various hyphal aggregations have also been discussed in detail in this unit.

III. Pseudoparenchymatous Forms

As it has been already mentioned above that fungus mycelium forms a network of loosely interwoven hyphae. In some specific groups of fungi, the entire mycelium or some part of the fungal mycelium undergoes various modifications. The hyphal mass in cross section appears to be a continuous structure as the walls of hyphae fuses and they lose their individuality. This structure looks like the parenchymatous tissue of higher plants but it is not a true parenchyma. Such a tissue is known as **plectenchyma.**

5.2.3 Organization of Thallus or Vegetative Structure

Fungi show great diversity in thallus organization. Their thallus ranges from single celled structures to massive multinucleate structures. Except the unicellular forms, most of the fungi are made up of threadlike filamentous structures called **hyphae** (singular **hypha**). These hyphae branch profusely and form a network called **mycelium** (plural **mycelia**). Unicellular thalli often give rise to **pseudomycelium**, which is formed when cells produce buds in succession fail to separate from the parent cell and remain attached to each other in an easily detachable chain. However, in some fungi pseudomycelium is formed in response to high sugar concentration, such as Mucorales.

Some fungi may show **dimorphism** in their thallus organization as they may either exist in unicellular or filamentous forms. The change in thallus organization from filamentous to unicellular form is brought about by the alteration in environmental conditions. Some of the **zoopathogenic fungi** are mycelial in culture but become unicellular and yeast like in the host. On the other hand, certain **phytopathogenic fungi** (smut fungus and leaf curl fungus) become yeast like in thallus culture and are mycelial in host cells.

Fungal thallus grows centrifugally as the younger and more active parts are present towards the periphery and older thallus is found towards the center. At the end all thalli form reproductive structures. In unicellular fungi whole thallus may get converted to reproductive structures; such thalli are known as **holocarpic**. However, in most of the fungi thallus is differentiated into vegetative and reproductive part. This type of thallus is known as **eucarpic** thallus.

I. Hypha

Fungal hyphae is microscopic structures which are bound by a rigid cell wall, contains protoplasm, and may be hyaline (uncoloured) or dark with brown and yellow pigment. It grows indeterminately only at its tip and has a fairly constant diameter of approximately 2-20 ìm (depending upon the species). Thallus of higher fungi has the capability to **anastomose**. During this process, neighbouring hyphae in the thallus put out short branches which make contact at the tip and with time may establish contact with each other. With time wall of the hyphae get dissolved at the point of contact and form a continuous tube by joining the two. This process helps in induces efficient cytoplasmic growth, exchange of nuclei, creates a strong mycelial network and coordinated growth of tissues.

Branching is **monopodial** as the apex of the leading hyphae is not suppressed and it grows along with the most active leading branch. **Lateral** branches are usually singly formed but may be formed in pair or opposite also. Sometimes these lateral branches may arise in whorls of three or more and are known as **verticillate**.

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In the members of Zygomycetes and Oomycetes (lower fungi), hyphae are long, multinucleate and comprises of continuous cells called as **coenocytes**. These hyphae are **non-septate** or **aseptate** (Refer Figure 5.4 (A)). Even in these cases septa are formed to delimit aged hyphae or when there is formation of reproductive structures. In Ascomycetes, Deuteromycetes and Basidiomycetes or the higher fungi, hyphal protoplasm is interrupted at regular intervals by cross walls or **septa** (singular **septum**). Hypha in this case is known as **septate** hypha (Refer Figure 5.4 (B)). But the protoplasm is still continuous throughout the fungal hyphae because of the perforations which are present in the cross walls.

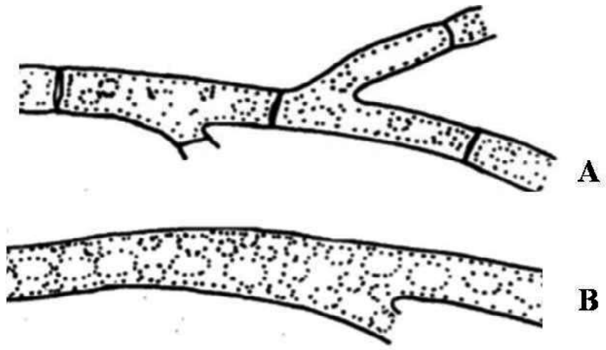


Fig. 5.4 *Diagrammatic Representation of Fungal Hypha. A: Septate. B: Non-Septate or Aseptate*

Septa are of two types: primary and adventitious. All types of septa are formed by centripetal growth from the hyphal wall towards inward. Primary septa is formed as result of nuclear division between daughter nuclei; however the formation of adventitious septa is independent of nuclear division and is formed because of the changes in cytoplasm concentration as it moves from one part of the hyphae to another. In Ascomycetes, cytoplasmic continuity is maintained by a small simple pore in the center of the septa (Refer Figure 5.5 (A)). Perforated septa are also found in Basidiomycetes (except rusts and smuts); however with a slight modification which involves the presence of a barrel shaped inflation with a hemispherical perforated membrane on each side of the opening. This is known as **dolipore septum** (Refer Figure 5.5 (B)). Fungal mycelium is called as **homokaryotic** when individual cells of the septate hyphae have genetically identical nuclei. On the other hand, some fungal mycelium contains nuclei of different genotype which arises as result of mutation or anastomosis of hyphae; these hyphae are known as **heterokaryotic**. Members of Basidiomycetes may have two genetically different nuclei (**dikaryotic**) or may have single, haploid, genetically identical nuclei in each segment (**monokaryotic**).

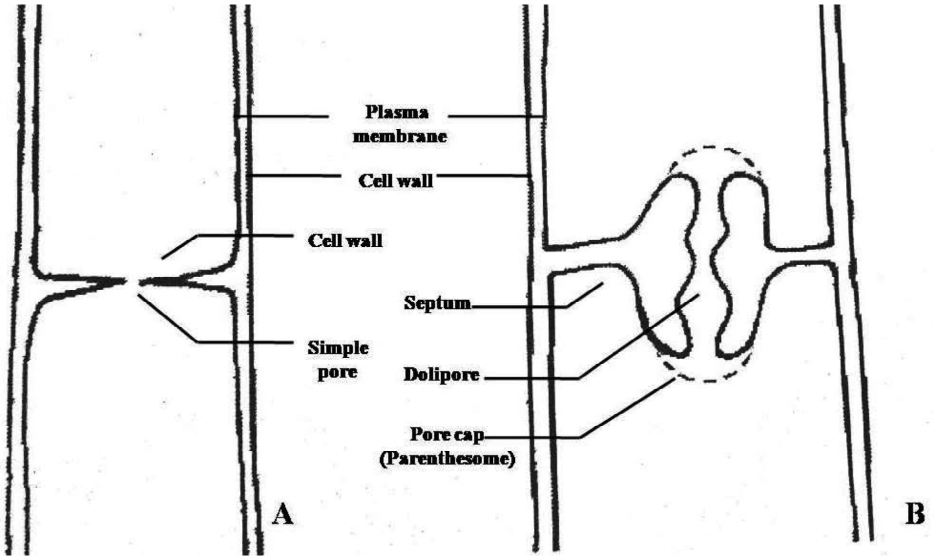
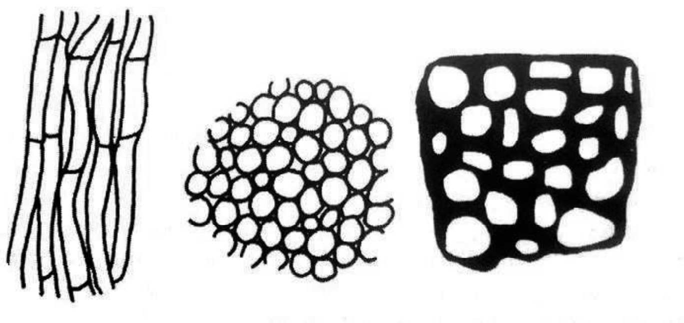


Fig. 5.5 *Types of Primary Septa. A: Simple Septa, B: Dolipore Septum with a Parenthesome*

II. Aggregations of Fungal Hyphae

At certain stages in life history of all fungi they show various degrees of hyphal aggregation ranging from loosely to compactly woven tissues. All such organized fungal tissues are known as **plectenchyma**. The three general types of plectenchyma (Refer Figure 5.6) are as follows:

- **Prosenchyma:** It is a loosely interwoven tissue in which component hyphae lie more or less parallel to one another and their elongated cells are generally distinguishable from each other.
- **Pseudoparenchyma:** It consists of closely packed, isodiametric or oval, thin-walled cells and has a close resemblance with the parenchyma of vascular plants.
- **Pseudosclerenchyma:** It consists of closely packed, thick-walled and dark cells.



A B C

Fig. 5.6 *Types of Plectenchyma. A: Prosenchyma, B: Pseudoparenchyma, C: Pseudosclerenchyma*

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Plectenchyma forms various types of vegetative and reproductive structures, such as mycelial strands, mycorrhiza, rhizomorph, stroma, sclerotia and sporophore.

- **Mycelial Strands** are found in Basidiomycetes and some members of Deuteromycetes. These are the aggregates of parallel and comparatively undifferentiated hyphae. Mycelial strand is formed around one or more leader hyphae; these leader hypha grow out of the thallus margin, become surrounded by their own interweaving and anastomosing branches to form a cord (1-2 mm thick and a few centimeters long). Mycelial strands are involved in translocation of material and these are the means by which fungus is capable of extending itself to a new substratum from an established food base.
- **Mycorrhiza** is the symbiotic association between fungal hyphae (mainly Agaricales) and plant roots. Root tips of coniferous and deciduous plants are often made up of several layers of fungal cells. Fungal mycelium extends into the layer of the soil and then inwards in the cortical cells of the root to form 'Hartig Network'. The extended fungal mycelium replaces the function of the roots and is thus involved in better absorption of minerals from the soil. The roots with mycorrhizal network outperform roots without mycorrhizal associations.
- **Rhizomorph** are highly differentiated root like hyphal aggregations which have a well-developed apical meristem, a central core (thin-walled, elongated cells) and a rind (smaller, thick-walled highly pigmented cells). These are produced by *Armillaria mellea*, a tree and shrub parasite. Rhizomorphs helps the fungus in spreading from one root system to another.
- **Stroma** is a compact hyphal aggregation like a cushion or mattress in which fructifications are formed. These are reported in members of Deuteromycetes, Basidiomycetes and Ascomycetes in the form of various types of ascocarp, pycnidia, basidiocarp, acervuli, synnemata, pycnidia and sporodochia.
- **Sclerotium** (plural **Sclerotia)** is a hard resting body formed by aggregation of somatic hyphae. They may be round, elongated or flattened mass and are the characteristic feature of a particular species in size, shape and colour. **Microsclerotia** (*Macrophomina phaseoli*) may be less than 100 micrometer in diameter and majority of sclerotia do not exceed beyond a diameter of 2 cm. In *Polyporus myllittae* sclerotia are more than 25 cm in diameter and weigh around several kilograms. These are meant for storage and help the fungus in survival during unfavourable environmental conditions by acting as a propagule.
- **Sporophores** are the spore bearing structures which are usually erect and aerial, branched (*Peronospora*) or unbranched (*Albugo*) and bear sporangia (*Albugo*) or conidia (*Peronospora*) on them. The sporophore bearing sporangia are called sporangiophore and those bear conidia are known as conidiophores. Sporophores may often be present in groups and form pycnia, hymenia, sporodochia and acervuli.

5.2.4 Ultra-Structure and Composition of Fungal Cells

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Unicellular yeast as well as the filamentous fungi has a typical eukaryotic cellular organization. As other non-photosynthetic organisms, fungi contain all major organelles except chloroplast. There are a wide range of unusual membrane bound organelles (sites of special function) and other organelles which are the sites of special function and fulfills specific requirement or helps in adapting suitably to a constraint growth habitat. Cells of vegetative hyphae are thin-walled (< 0.2 μm) and rigid. It serves a number of following important functions:

- Provides mechanical strength and helps the fungi in assuming a variety of forms, such as penetrative, ramifying and proliferating cells.
- Acts as molecular sieve and regulates the movement of large molecules through wall pores.
- Determines the characteristic shape and size of fungal spores.
- Provides protection to the cell from osmotic lysis.
- Wall is permeable to nutrients, gases and enzymes.
- Protection to the protoplasm from various environmental stresses.
- Specific components, such as melanin gives protection against the damage of UV radiation.
- Since it is the primary interface between the cell and its environment thus it contains antigens and agglutinins for mating and adhesion to substrate.
- Acts as a binding site for some enzymes.

Fungal hyphae invariably contains a large number of nuclei. In aseptate forms, nuclei may be distributed randomly throughout the cytoplasm. However in septate forms individual cells, depending upon the phase of the life cycle, routinely contain one, two or many nuclei. In most of the fungi, nuclei are quite small. Generally they are spherical to ovoid in shape but they are extremely plastic structure. This property makes them:

- Capable to squeeze through septal pores.
- Easy passage into the narrow structures at the tip of hyphae which forms various spores.
- Become elongated while moving into the germ tube of the germinating spores.

In most of the true fungi there are nucleus-associated organelles commonly known as **Spindle Pole Bodies (SPB)**. These are small, electron dense cytoplasmic structures which lies adjacent to the nuclear envelope. Their function is to act as microtubule organizing centers during mitosis and meiosis. Morphology of the SPB varies depending upon the fungal species and the phase of nuclear division. During interphase, SPB can be flat, bar-shaped, globular to bi-globular mass, or a multilayered disc. During prophase, there is duplication of SPB and in some fungi it appears as a large structure. In later stages of division these duplicated SPB separates and moves to different poles of the dividing nucleus. SPBs can remain outside the nuclear envelope or it can be present inside the envelope. SPB emanate microtubules which forms the spindle apparatus. During mitosis and meiosis, function of SPBs resemble to the role of centriole. SPB are lacking from those 'Fungi' which produce flagellates cells and have a centriole in them, thus SPBs are lacking. In these fungi role of SPBs is taken over by the centriole.

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Nuclear division in fungi is **intranuclear** as majority of the nuclear envelope remains intact till late telophase; during which it breaks in inter zonal region and is again reformed around the daughter nuclei. There is a centrally-positioned (often) prominent **nucleolus** in typical fungal cells (Refer Figure 5.7). Nucleolus can have three fates during cell division depending upon the species:

- Persistent and ultimately undergoes fission.
- Gets dispersed and is no longer visible in dividing nucleus.
- Discarded in the cytoplasm of the cell as an intact entity.

Size of fungal chromosome is quite small owing to which it becomes very difficult to visualize them in squashed and stained preparations. Because of which literature is filled with conflicting reports regarding the number of chromosomes in fungi. However, advance studies, such as Pulsed-Field Gel Electrophoresis (PFGE) and ultra-structure derived reconstruction of serially sectioned synaptonemal complexes form pachytene nuclei are being developed.

Apart from nuclei the other most conspicuous fungal organelle that is present in sub-apical region of growing hyphae is the **mitochondrion** (Refer Figure 5.7). They are numerous in number and appear like tiny thread or rod-like when viewed under light microscope. However, they are electron-dense at ultra-structure level and are oriented more or less parallel to the long axis of the hyphae. Mitochondria in fungi can be lobed, branched or extremely large, thread like form. Cristae of the mitochondria can be flattened (plate like) or tubular (Oomycota).

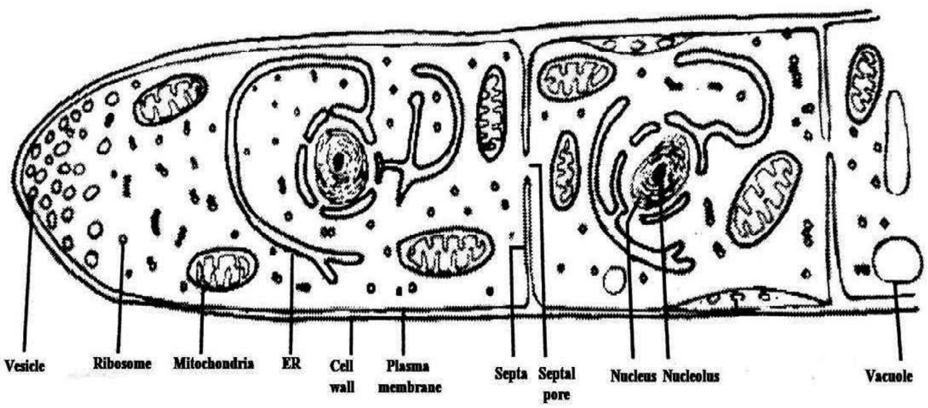

Fig. 5.7 *Diagrammatic Representation of the Apex and Apical Region of Actively Growing Fungal Hyphae (Modified from Deacon 1980)*

Other cytoplasmic components of the fungal hyphae are vacuoles, ribosomes, lipid bodies, endoplasmic reticulum strands, micro bodies, filasomes, Golgi bodies, glycogen storage particles and MultiVesicular Bodies (MVBs). In certain type of fungi **Woronin bodies** are also present which are associated with septal pores. Golgi bodies of true fungi are morphologically very simple and comprises of single cisternal element. Because of the simplicity these organelles are referred as Golgi equivalents. However, they perform similar function as that of morphologically complex Golgi bodies. Relatively little is known about filasomes and MVBs. The former are numerous in number and appears as tiny vesicles coated with dense filamentous material. They are found in close association with plasma membrane, developing septa and contain actin. MVBs are small vacuole like bodies and contain small vesicles. They are usually uniformly distributed but at the time of nuclear division are found in close association with SPBs of dividing nuclei.

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One of the most common components of fungal hyphae is the vacuoles (Refer Figure 5.7). They are morphologically different in different parts of the hyphae. In the sub-apical zone where fungal hypha is actively growing these are small and variously shaped with moderate granular content. While the older part of hyphae has large, centrally located vacuole that almost completely fills the hyphae. In this zone, other cell organelles are found forming a thin layer around the vacuole and are present towards the periphery of the hypha.

I. Hyphal Tip Growth

Growth of fungal hyphae is quite unique and shows similarity with a few other organisms. Hyphal growth is apical with a continuous increase and extension of cell membrane and cell wall at the tip of the hypha. Growth of hypha is directional and polarized and there are several factors responsible for this, such as turgor pressure, cytoskeletal organization and ion concentration gradient. In higher groups of fungi at the tip of growing hyphae a special organization appears which is known as 'spitzenkorper'. Cell wall is composed of micro-fibrillar component (skeleton) on the inner side of the wall and is embedded in an amorphous matrix (extended to the outer side of the wall too). Skeletal component is made up of highly crystalline and water soluble material â-linked glucans (polysaccharides which is made solely or partly of glucose molecule which are joined through α-1,3, α-1,6, β-1,3 or β-1,6 linkages; most abundant are the β-glucans that has variable proportion of β-1,3 and β-1,6 linkage) and chitin (β-1,4 linked homopolymer of *N*-acetylglucosamine). While the matrix consists of polysaccharides (α-glucans and glycoproteins) which are mostly water soluble. Cellulose is generally thought to be absent from cytoskeleton of true fungi also scattered reports indicates its presence. It is the characteristic component of the cell walls of Oomycota and Dictyostelid Slime Moulds.

Two basic and contrasting hypotheses have been proposed to explain hyphal tip growth. The hypotheses are explained below:

- This explanation is referred as 'Steady-State Hypothesis'. According to this, hyphal tip is highly viscoelastic and expandable and the newly formed wall at the hyphal tip is made up of chitin and β-glucans. Because of the subsequent cross-linking of wall polymers, the viscoelastic wall gradually attains rigidity.

- According to the second hypothesis, wall is inherently rigid and for the growth to occur two processes should occur in balance, i.e., lysis of wall followed by formation of wall polymers and pushing out and mending of the wall components.

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In both of these hypotheses, enzymes and wall precursors for the growing hyphal tip is provided by sub-apical region of the hypha. Raw materials required at the hyphal tip are transported with the help of membrane-bound vesicles. Vesicles involved in hyphal tip growth are of two types: micro-vesicles (smaller than 100 nm in diameter) and macro-vesicles (greater than 100 nm in diameter). A unique, pleomorphic and dynamic structure, **Spitzenkörper**, is formed by tight clustering of these vesicles and other structures. Its function is to act as the supply center for vesicles involved in hyphal tip growth. Macro-vesicles are the secretory structures containing enzymes and preformed polymers which form the amorphous matrix of the cell wall. Vesicles are released from Golgi cisternae and transported to the hyphal apex where they fuse with the plasma membrane. Plasma membrane gets elongated as the membrane of the vesicle fuses with it and their contents are released outside where they are used for the wall formation (Refer Figure 5.8).

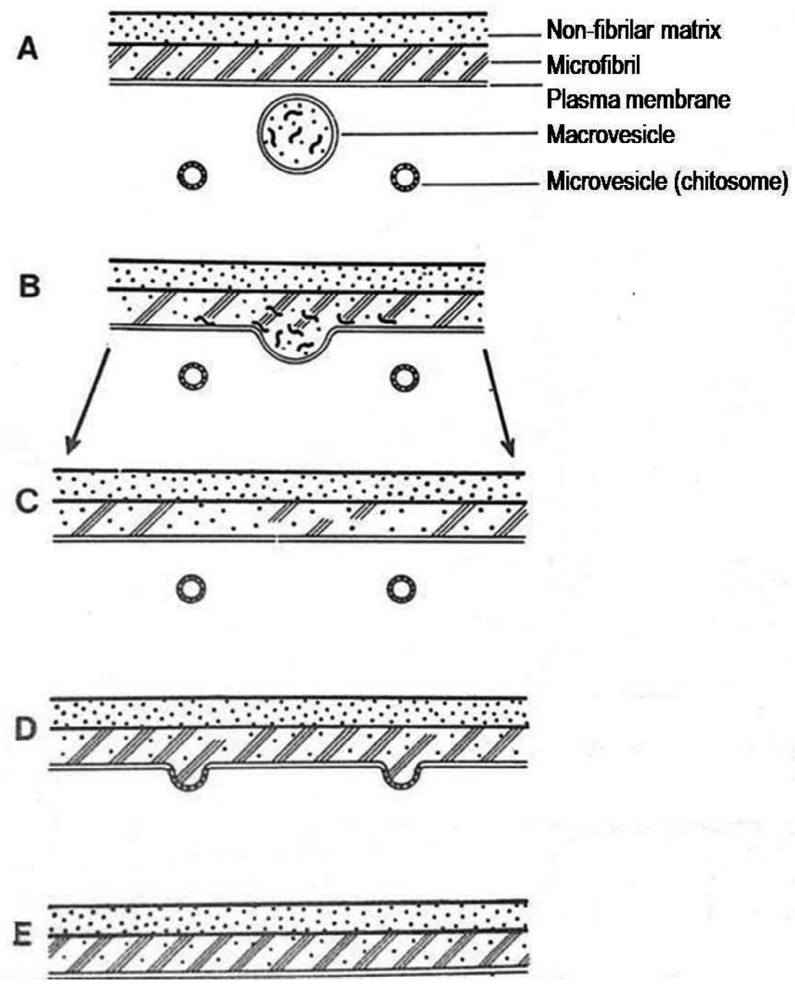

Fig. 5.8 *Events Involved in the Synthesis of Fungal Wall Growth*

The above Figure 5.8 shows the events involved in the synthesis of fungal wall growth representing the roles of macro and chitosome vesicles. Although, for the sake of clarity these events are shown in consecutive steps but these occur simultaneously, i.e., the discharge of non-fibrillar matrix and plasticizer (B); surface area of plasticized wall increases by one unit (C); microfibril deposition (D); cell wall extended by one unit growth (E).

Exact role of micro-vesicles is less clear. Some believe that these tiny vesicles help in movement of chitin synthase (enzyme) at the hyphal tip through the plasma membrane. This enzyme is involved in formation of micro fibrils of chitin skeleton. These vesicles correspond to the **chitosomes** found in fungal cell homogenates. Chitosomes are the small, membrane-bound structures which contain inactive or zymogenic form of chitin synthase, which is activated by proteases after fusion of chitosome membrane with the plasma membrane. Involvement of macro and chitosome vesicles in the formation of fungal cell wall has been shown schematically.

5.2.5 Nutrition

Unlike green plants, fungi are non-chlorophyllous and cannot synthesize their own food. Their structure is so simple that they even cannot obtain their inorganic food directly from the soil. Additionally, a universal character of fungi is that they lack plastids and is completely heterotrophic. Therefore, they must obtain their nutrition from environment, or from living, dead and dying organism. On this basis of this fungi are of below mentioned types.

- **Biotrophs:** These are the group of fungi which obtain their food directly from living host tissues only. Biotrophs secrete chemicals which increases the permeability of membranes to sugars and amino acids. The host cell starts leaking sugars and amino acids which is absorbed by the fungi. During this type of interaction, fungal cells remain confined to the intercellular spaces and obtain nourishment through **haustoria** (plural **haustorium**). Haustoria are the outgrowth of vegetative hyphae which increase the absorptive surface area of the parasitic fungus. They penetrate the host cell and with a miniature pore and come in contact with the plasma membrane of the host cell. Haustoria can be knob like (*Albugo*), elongated or branched (*Peronospora*) and highly branched (*Erysiphe*), (Refer Figure 5.9). A special group of fungi are **biotrophic mycoparasites** which have the ability to parasitize mycelia of other fungi, but do not cause any damage to the host cell, as they are not able to grow on dead organic substrata. Some of the common examples are *Piptocephalis virginiana* (Zygomycetes) that can parasitize *Mucor* hypha.

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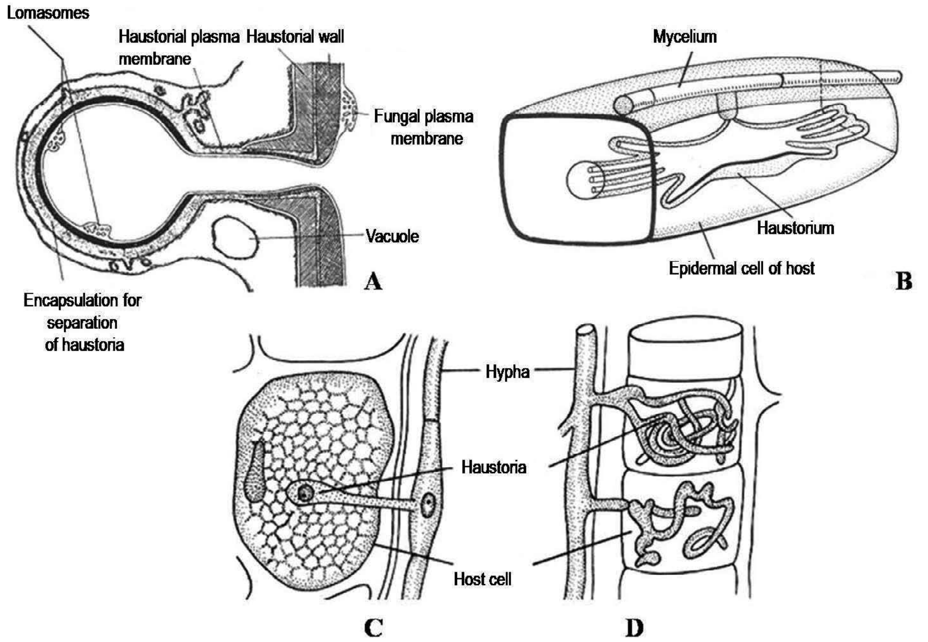

Fig. 5.9 *Types of Haustoria. (A): Knob-Like in Albugo, (B): Highly Branched in Erysiphe, (C): Elongated Capitate in Peronospora, (D): Elongated Digitate in Peronospora*

This interaction is also known as parasitic interaction since one partner is benefitted (fungi) while the other is in loss. The parasites which survive only on the living host are known as **obligate parasite**; for example *Melampsora*, *Peronospora*, *Puccinia*. These cannot be grown on dead organic culture media. However, the parasites which usually thrive on living material but depending upon the need can adopt saprophytic mode of life for a short period of time are known as **facultative saprophytes**; for example *Taphrina deformans* and other smuts. While there are some fungal species which usually pass saprophytic mode of life cycle but under certain environmental conditions these parasitize some suitable host and are called **facultative parasites**; for example *Fusarium*, *Pythium,* etc. Different parasites absorb nutrition in their respective hosts in different ways. Those fungi which have mycelium outside the substratum or host are called **ectoparasite** (*Erysiphe*); however, fungus having their mycelium embedded in host tissue are known as **endoparasite**. Both these type of fungus develop haustoria for better nutrient absorption.

A special category of plant pathogenic species are **hemibiotrophs**. These are those fungi which initially require living cells but cause the death of the host as their hyphae advances. A few carefully documented examples of hemibiotrophs are *Colletotrichum lindemuthianum* (Anthracnose Fungus) and *Cercosporidium personatum* (Peanut Leaf Spot Pathogen).

- **Necrotrophs or Perthotrops:** These are those fungi which attack living cells so virulently that they kill those cells in early course of parasitism and thereafter feed on dead tissue. They may also secrete toxins which can damage plasma membrane of the host cell, causing nutrients to leak out of the cell and be willingly available to the fungus. **Necrotrophic mycoparasites** can parasitize other fungi and kill them. For example, *Trichoderma viride* can coil around the living hypha of various other fungi, such as *Rhizoctonia solani* and kills them (Refer Figure 5.10).

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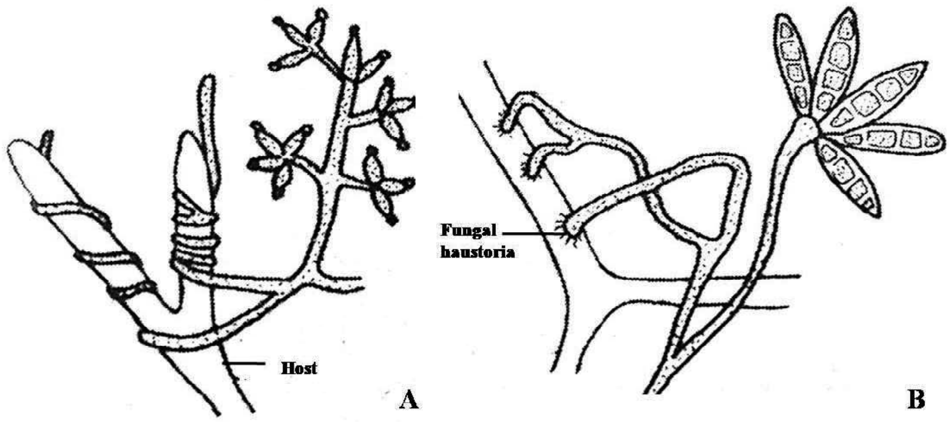

Fig. 5.10 *Diagrammatic Representation of Necrotrophs Feeding on Other Fungi*

The above Figure 5.10 shows the diagrammatic representation of Necrotrophs feeding on other fungi. (A): *Trichoderma viridae* coiled around hyphae of *Rhizoctonia solani*, and (B): Biotrophic mycoparasite has formed haustoria on hyphae of *Mucor* [modified from Deacon 1980].

There are certain groups of fungi which use eelworms, rotifers or protozoa and have developed special mechanisms for capturing these organisms. One of the most interesting mechanisms involves formation of a rapidly constricting ring around a nematode. This ring keeps the nematode captive till the hypha forms haustoria in the body of the nematode. The haustorium absorbs food from the body of the victim and ultimately victim dies. Fungi of the genus *Arthrobotrys*, *Dactylella* and *Dactylaria* employ this method.

- **Saprotrophs:** These fungi secrete digestive/extracellular enzymes through their wall on the dead organic material. These enzymes act on the substrate polymers and convert them into monomers (monosaccharides, amino acid and sugars) which can be easily absorbed by the vegetative hyphae of the Saprotrophs by penetrating deep into the substratum. Some of the good examples of saprophytes are *Aspergillus*, *Agaricus*, *Morchella*, *Penicillium*, *Rhizopus* and *Saprolegnia*. In case of saprophytic fungi, the mycelium can be **ectophytic** (rhizoid is on the substratum; *Rhizopus*) or **endophytic** (rhizoid remain embedded in the substratum).
- **Mutualistic Symbionts:** Mutualistic symbiosis is a positive interaction and is beneficial for both the partners. Some of the fungal species are involved in mutualistic partnership with other organisms and get benefitted nutritionally in the relationship, for example **lichen** and **mycorrhiza**. Lichen is the result of symbiotic association between algae and fungi. Algal partner

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synthesizes organic food and the fungal partner helps in absorption of inorganic nutrients and water. Some of the fungal species develop in the roots of higher plants and mycorrhiza formation occurs. A fungus helps in better absorption of nutrients from the soil. Mycorrhiza may be external or internal. **External mycorrhiza** or **ectophytic mycorrhiza** is confined to outer region of roots. However, internal mycorrhiza has its mycelium embedded deeply in the root cells.

5.2.6 Ecological Groups of Fungi

- **Aquatic Fungi:** Several fungal species are water inhabiting and are known as aquatic fungi. Some of the common examples are Lagenidiales (Parasite on Algae, Water Mould and Small Animals), Chytridiales (on Algae and Water Moulds), Blastocladiales (Water Mould) and Monoblepharidales (Aquatic). Though aquatic fungi are found in almost all fungal groups but most of them are the members of lower fungi.
- **Cellulose Decomposing Fungi:** Some of the fungal species are found growing on cellulose rich material, such as paper and cotton and cause economic losses to the textile industry. Some of the important cellulose decomposers are *Chaetomium globosum* and *Stachybotrys atra*.
- **Coprophilous Fungi:** These are those groups of fungi which grow on dung of certain animals. One of the most common examples of this type of fungi is *Pilobolus* whose growth is associated with the presence of a factor called **coprogen**. Excreta of herbivores, such as pigs, horses, camels, rabbits, mice, elephants, etc., harbor a large number of fungi but only a few of them are exclusively Coprophilous. The first one to appear on moist dung is the members of Mucorales (Lower Fungi); these are succeeded by fructifications of Ascomycetes and later on by Basidiomycetes (Higher Fungi).
- **Dermatophytes:** These are groups of fungi belonging mainly to ***Imperfecti*** (Deuteromycotina) and cause disease of skin of man and animals. The disease caused by these fungi are termed as **dermatomycoses** which is commonly known as Athlete's Foot, Ring Warm, etc. Some of the important diseases causing genera are *Keratinomyces*, *Microsporum*, *Trichophyton* and *Epidermophyton*.
- **Entomogenous Fungi:** These are the group of fungi which are associated with insects. One of the most common examples of this type is the genera found on house fly, *Entomophthora muscae*. The fly appears in moist weather conditions and are surrounded with a while halo (about 2 cm diameter) which is formed by discharged conidia.
- **Lignicolous Fungi:** Though up to a great extent lignin is resistant to fungal attack, but certain specific groups of higher fungi are responsible for decay of wood. For example, wood decay in the logs of *Shorea robusta* is brought about by *Polystictus sanguineus*.
- **Soil Fungi:** Several fungi are also associated with microbiological flora of the soil. When a plant grows in the soil the greatest activity occurs on the

surface of the soil or the **rhizoplane**; whereas the soil nearer to the root is known as **rhizosphere**. Fungal micro flora in the aforementioned two zones plays an important role in growth and disease formation in fungi. Additionally, roots of some of the plant species produce hydrocyanic acid which provides them resistance against disease causing fungi. However, this chemical is not produced in susceptible varieties.

Check Your Progress

1. What is the aggregation of thick strands of fungal hyphae that appears root like are known as?
2. What are the chief characteristics of plasmodial slime moulds?
3. What is dolipore septum?
4. What is pseudoparenchyma?
5. Fungi of which class have septate mycelium?

5.3 REPRODUCTION IN FUNGI

Reproduction means formation of a new individual having all the characteristics which are typical to a particular species. Generally, most of the fungi reproduce through either asexual or sexual mode of reproduction. **Asexual** reproduction is also known as somatic reproduction as it does not involve karyogamy that is the fusion of nuclei and meiosis. Additionally, specialized sex cells or organs are not involved. This is the most important type of reproduction in fungi as it is meant for the propagation of fungal species, repeated several times during the life cycle of a fungus and produces various exact copies of the parent. Many ecologist prefer to club vegetative and asexual mode of reproduction into asexual mode of reproduction because of the fact that both of these do not involve the fusion of nuclei or sex cells or sex organs. **Sexual** reproduction, on the contrary, is characterized by union of two compatible nuclei followed by meiosis. This type of reproduction usually takes place only once in the life cycle of the organism and may or may not involve specialized sex cells or organs. The significance of sexual reproduction is that it results into formation of new genotypes owing to very high incidences of recombination. This helps them in adapting themselves to different environmental conditions. Some fungal species do not go through true sexual reproduction and instead follow **parasexuality**, which offers an important source of genetic variation in the progeny. Fungi that exhibit sexual reproduction can also show parasexuality as these cycles of reproduction are not mutually exclusive.

On the basis of involvement of the thallus in the formation of asexual and sexual reproductive organs fungi are categorized as holocarpic and eucarpic. In some fungi for the formation of reproductive structures or organs the entire thallus gets converted into one or more reproductive structures. This ensures that somatic and reproductive phases do not occur together in the same individual. Those fungi which follow this type of pattern are known as **holocarpic** fungi. However, in majority

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of the fungi the somatic and reproductive phases occur together in the thallus as only a portion of the thallus gets converted into reproductive structure. The remaining portion of the thallus continues with normal somatic activities. Fungal species which show this type of pattern are called **eucarpic** fungi. The eucarpic forms are more differentiated than the holocarpic fungi.

Usually, fungi reproduces both asexually and sexually but not at the same time in their life cycle. Asexual reproduction is more important for colonization of a species as it is repeated several times in the life cycle of a species and results in production of large number of individuals, whereas in most of the fungi the sexual stage is produced only once a year. Because of the pleomorphic nature of the fungi a new terminology was proposed by Hennebert and Weresub (1977) which was widely accepted. According to this, sexual stage of the fungus is described by the term **teleomorph** while asexual stage is described by the term **anamorph**. While whole fungus with all its forms, facets, either latent or expressed even if reproduces by one method is known as **holomorph**. More recently, these terms are replaced by **meiosporic** fungus (teleomorph) and **mitosporic** fungus (anamorph).

5.3.1 Asexual Reproduction

Asexual reproduction takes place when progeny is formed by a single parent without any nuclear contribution from the second parent. Therefore, genetically the progeny is exact copy of the parent cell or thallus. Asexual reproduction is often defined as nonsexual production of specialized reproductive cells, such as spores. Asexual spore is delimited from the thallus and in contrast to the vegetative mycelium they have minimal metabolic turnover, low water content and lack of cytoplasmic movement. Asexual methods of reproduction commonly found in fungi are summarized below.

- **Fragmentation:** Fragmentation results from accidental severing of the mycelium into several small bits or fragments or my mechanical injuries or otherwise breaks into segments of hyphae. Under favourable condition each fragment by further division of cells and apical growth develops into a new mycelium. This method occurs frequently in nature and often employed by ecologist for growth of fungal culture in the laboratory.
- **Thallospores:** In some fungal species, one of the normal means of propagation is the fragmentation of parent hyphae into spores called thallospores (asexual spore). Thallospores are of two types – Arthrospore and Chlamydospore.
    - o **Arthrospore or Oidia:** For the formation of arthrospore distal end of hyphae breaks up into component cells by close septation and each component cell behaves as an individual spore. They are always formed in basipetal succession that is septation starts from the apex of the hyphae and proceeds towards the base. The cells give appearance of beads on a rosary as they become oval or rounded in shape. Each oidium or arthrospore develops into a new mycelium (Refer Figure 5.11 (A)).
    - o **Chlamydospore:** In some fungi, such as *Mucor* and *Fusarium*, some intercalary or terminal segments of the hyphae accumulate large amount

of food reserves and develop thick resistant walls. These thick walled spores are called chlamydospore (Refer Figure 5.11 (B)). These cells function as perennating bodies and help in surviving unfavourable environmental conditions and are released after the intervening hyphae are degenerated. As the favourable conditions return each chlamydospore develops into a new mycelium.

- **Fission of Unicellular Thalli:** This method is typical of some types of yeast, such as *Schizosaccharomyces*. During this process, somatic cells elongate and divide transversely into two daughter cells of similar size and shape (Refer Figure 5.11). First the nucleus divides followed by division of the cytoplasm by wall formation dividing parent cell into two. The two daughter cells separate and can lead independent lives.
- **Budding of Somatic Cells:** In the yeast, *Saccharomyces cerevisiae*, cell reproduces by the means of budding which involves production of an outgrowth or bud from a parent cell or spore (Refer Figure 5.11 (D)). This bud gradually enlarges, constricts and finally forms a new individual by getting separated the parent cell by formation of a cross wall. Sometimes this newly formed bud before getting separated from the parent cell produces a new bud which remains attached for a while to form a chain of buds known as **pseudomycelium**.

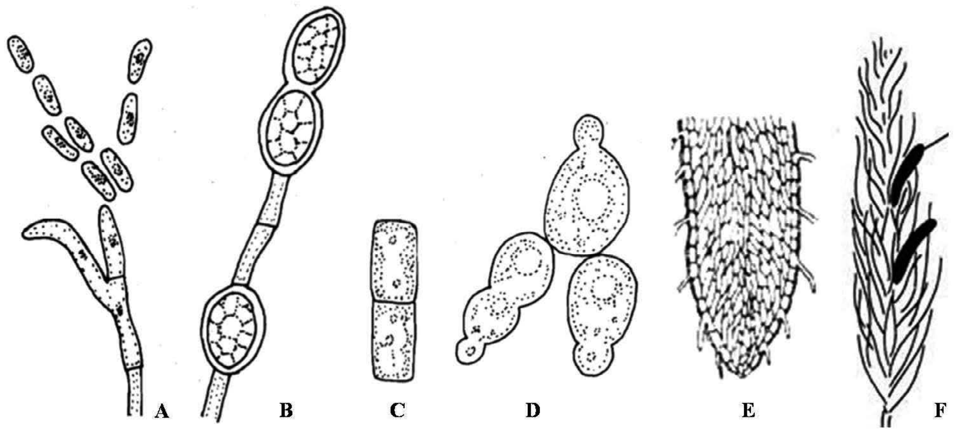

Fig. 5.11 *Structures for Asexual Reproduction in Fungi. (A): Fission, (B): Budding, (C): Rhizomorph, (D): Chlamydospore, (E): Oidia, (F): Sclerotia*

- **Rhizomorphs:** In many higher fungi, such as *Agaricus*, hyphae aggregate to form a cord like structures (Refer Figure 5.11 (E)). These dark brown coloured fine root-like strands or hyphae are known as rhizomorphs. These serve as perennating bodies which remain dormant under unfavourable conditions and give rise to new fruiting bodies.
- **Sclerotia:** Sclerotia, produced by *Claviceps purpurea* (Ergot), are the modification of mycelium (Refer Figure 5.11 (F)). They are rounded or cylindrical or irregular or cushion-shaped structures with a dense mass of thick walled hyphae. The hypha serves as the organs of perrenation and is

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meant for vegetative propagation. With the return of favourable conditions sclerotia germinate to form a new mycelium.

- **Production of Asexual Spores**: This is the most common method of asexual reproduction in fungi wherein special reproductive cells are formed which are known as spores. The process of formation of spores in fungi is called sporulation. These spores vary in shape (globose, oval, oblong, needle-shaped to helical), size (2-150 micron), septation, unicellular or multicellular, mobility (motile or non-motile), wall thickness (thin or thick-walled), pigmentation (hyaline or coloured, such as green, yellow, orange, red, brown to black) and the way they are borne. Some fungus produces only one type of spore while some can produce more than 2-3 types of spores. These spores help in perrenation, propagation and dispersal.

  Fungal spores are usually unicellular. However, they can be uninucleate or multinucleate. Fungi like *Alternaria* and *Curvularia* produce multicellular spores. Mitospores can be categorized into two main types depending upon their method of formation – Sporangiospore and Conidiospore (true conidia).

  o **Sporangiospores:** Numerous minute, uninucleate sporangiospores are produced by cleavage of the cellular content of a sac-like structure called sporangium. Each sporangium is born on hyphae called sporangiophore. Sporangiospore can be non-motile (**aplanospore**) (Refer Figure 5.12 (A)) or motile (**zoospore**) (Refer Figure 5.12 (B) and (C)). Aplanospore are characteristics of terrestrial species, such as *Rhizopus* and *Mucor*. While in aquatic fungi (*Pythium*, Division Oomycota) motile biflagellate zoospores are produced on structures called as zoosporangium. Aplanospores develop a rigid cell wall prior to their release from sporangium. However, the motile zoospores are initially naked. They secrete a cell wall only after the swarming period is over and germinate by forming a germ tube. Aplanospores have a definite spore wall, in contrast to zoospores, and are dispersed by the means of wind and insects. Zoospores may have one or more than one flagella. Basically, the fungal species have two types of flagella – tinsel (feathery structure having a long rachis and have lateral hair projections called mastigonemes or flimmers) and whiplash (long basal portion and a short and flexible upper portion). Depending upon the presence and absence of these two types of flagellum zoospores are of following four types:

    (i) Zoospore with single posterior whiplash flagellum found in Chytridiomycetes.

    (ii) Zoospore with single anterior tinsel flagellum found in Hypochytridiomycetes.

    (iii) Biflagellate zoospore with two anterior whiplash flagella reported in Plasmodiophoromycetes.

    (iv) Biflagellate zoospore with one tinsel and one whiplash flagella as in Oomycetes.

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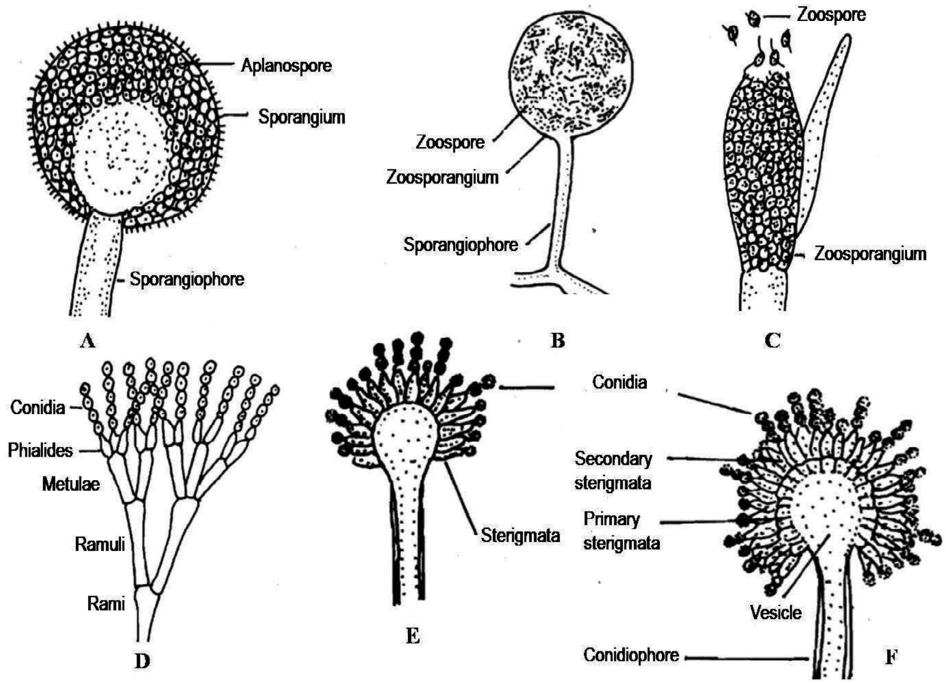

Fig. 5.12 *Sporangiopore and Conidiopore*

The above Figure 5.12 shows the sporangiopore and conidiopore. (A): Sporangia Containing Aplanospore, (B) and (C): Sporangia Containing Flagellated Zoospore, (D): Conidiophores Showing Conidia on Branches in *Penicillium*, (E) and (F): Conidiophores Bearing Conidial Chains on Branches in *Aspergillus*.

- **Conidiospores:** These asexual spores are non-motile, deciduous and are formed externally as single separate cells either directly on the mycelium or on morphologically differentiate hyphae called conidiophores. These structures may be simple or branched and septate or aseptate. Conidia may be produced singly as in *Phytophthora* or in chains at the tip of conidiophores as in *Aspergillus* or at the tip of the branches of conidiophores as in *Penicillium* (Refer Figure 5.12 (D), (E) and (F)). Conidiophores often arise singly and are scattered in mycelium. However, sometimes they may also arise in specialized structures called fruiting bodies. Depending upon their appearance they are termed as **synnema**, **sporodochia**, **acervuli** (saucer-shaped), **pycnidia** (flask-shaped) or **pustules** (Refer Figure 5.13).

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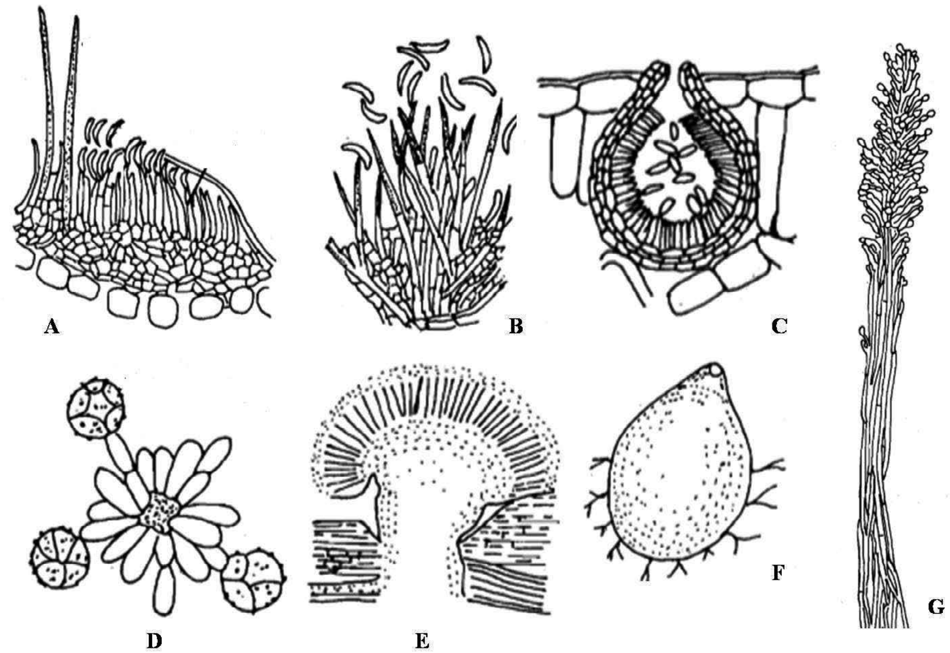

Fig. 5.13 Different Types of Fruiting Bodies in Fungi. (A) and (B): Acervuli, (C): Pustule, (D) and (E): Sporodochium, (F): Pycnidium, (G): Synnema

5.3.2 Sexual Reproduction

This process involves fusion of two compatible sex cells or gametes of opposite strains. Sexual reproduction is also known as perfect state in comparison to asexual reproduction which is considered as imperfect state. Fungi show remarkable diversity in their process of sexual reproduction. Sexual reproduction basically involves three phases (Refer Figure 5.14):

- Plasmogamy
- Karyogamy
- Meiosis

Plasmogamy is the union of protoplast of reproductive cells or hyphae to bring the nuclei of the two parents (one from male and one from female) together in a pair. Although, these nuclei do not fuse with each other and the resulting cell with two nuclei is called dikaryon. This condition is unique for fungi and may continue for several generations as such, as the two nuclei of dikaryon divide simultaneously during each cell division and are passed on to the daughter hyphae.

Karyogamy is the process of fusion of two nuclei. In lower fungi, it may immediately follow plasmogamy. However, in higher fungi it may be delayed for a long time.

Meiosis sooner or later follows karyogamy, eventually occurs in all sexually reproducing fungi and produces four genetically different spores. All the three processes are regulated by a variety of mechanisms and morphological developments.

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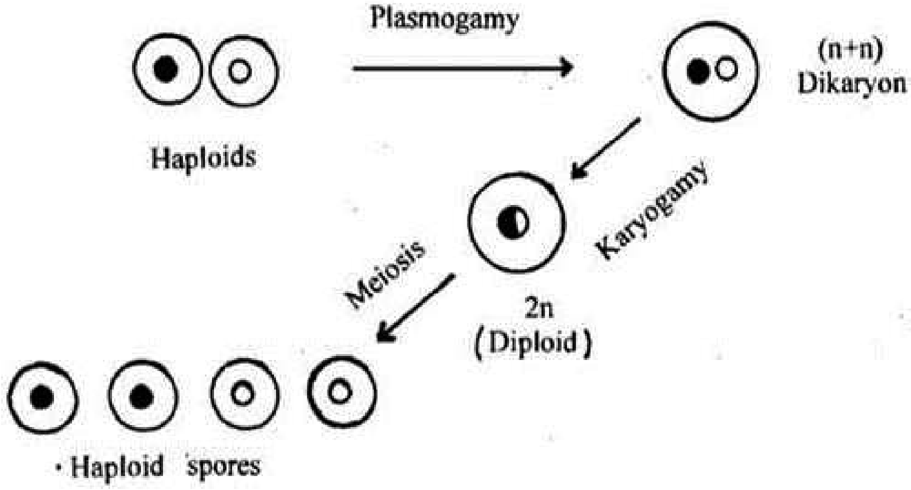

Fig. 5.14 Phases of Sexual Reproduction in Fungi

Types of Plasmogamy

Plasmogamy occurs through a variety of mechanisms in fungi. Some of them are as follows:

- **Planogametic Copulation:** This process involves fusion of two gametes (Refer Figure 5.15). Like algae sexual reproduction in fungi can be isogamous, anisogamous or oogamous type. Isogamy is the simplest type amongst all as it involves fusion of two morphologically and physiologically similar gametes as observed in *Olpidium* and *Catenaria*. Anisogamy and Oogamy are known as heterogamous sexual reproduction due to the involvement of morphologically and physiologically dissimilar gametes. Anisogamy is seen in only one genus *Anomyces*. In Oogamy, motile zoospore enters oogonium, fuses with egg or oosphere and develops into a zygote. Oogamy has been observed in *Albugo* and *Pythium*.

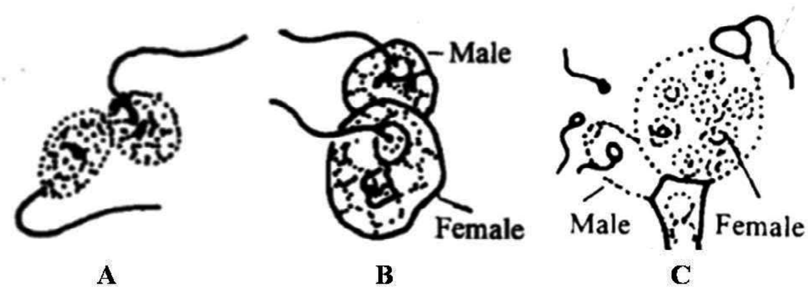

Fig. 5.15 Diagrammatic Representation of Plasmogamy in Fungi by Means of Planogametic Copulation. (A): Isogamy, (B): Anisogamy, (C): Oogamy

- **Gametangial Copulation:** The two gametangia comes into contact and the entire content of the two fuses together and becomes one as seen in case of *Rhizopus* and *Mucor* (Refer Figure 5.16). However, in some fungal species

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entire protoplast of one gamete flows into the other gamete through a pore. The recipient is female and the donor is male.

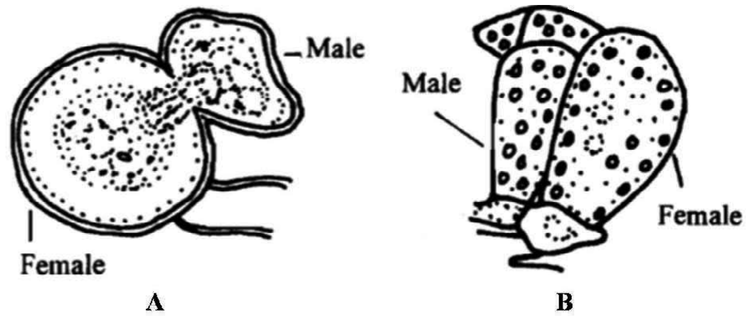

Fig. 5.16 *Diagrammatic Representation of Plasmogamy in Fungi by means of Gametangial Copulation*

- **Gametangial Contact:** The nucleus in the antheridium represents the male gamete. Male gamete here is not a separate entity. The oogonium and the antheridium come in contact through a tube and one of the nuclei from the antheridium migrates into the oogonium (Refer Figure 5.17). Interestingly, the two gametangia do not fuse. It has been observed in *Penicillium*.

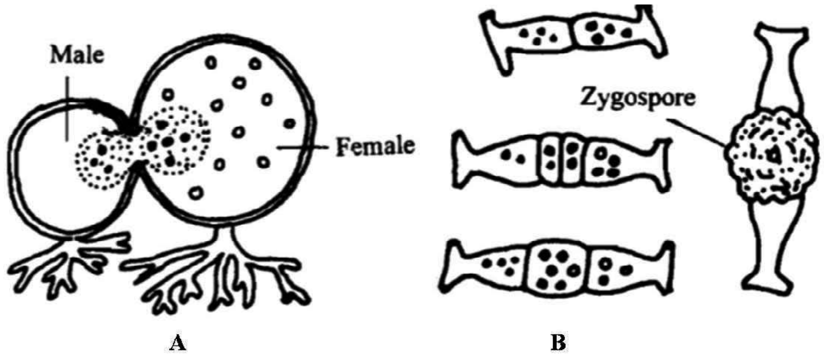

Fig. 5.17 *Diagrammatic Representation of Plasmogamy in Fungi by means of Gametangial Contact*

- **Spermatization:** This is one of the most remarkable modes of plasmogamy. The spermatia which are minute conidia like structures are produced externally on special hyphae called spermatiophore. Spermatia develop inside cavities called as spermatogonia (Refer Figure 5.18). Female cell can be a gametangium, vegetative hyphae or even specialized receptive hyphae.

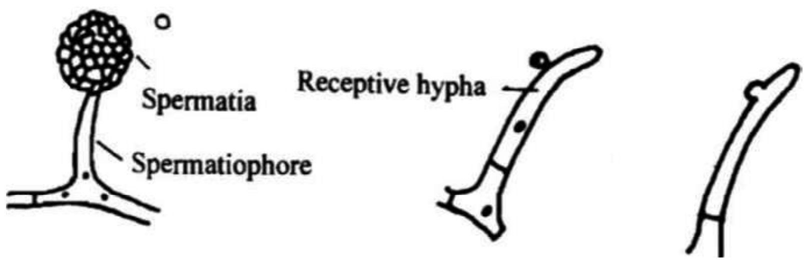

Fig. 5.18 Diagrammatic Representation of Plasmogamy in Fungi by means of Spermatization

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- **Somatogamy:** A progressive degeneration of sexuality has been observed in higher fungi such as Ascomycetes and Basidiomycetes. The entire process gets simplified as there is fusion of two mycelia which belong to opposite strains (Refer Figure 5.19). Post fertilization changes are quite apparent as there is production of fruiting body known as ascocarp and basidiocarp in Ascomycetes and Basidiomycetes, respectively.

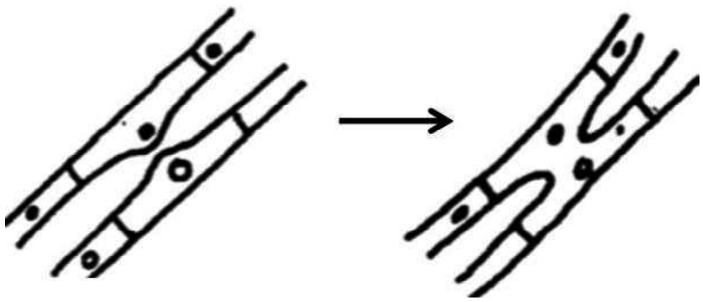

Fig. 5.19 Plasmogamy in Fungi by Means of Somatogamy

Fusion of male and female nuclei leading to the formation of diploid nucleus occurs after the gametangial fusion or aforementioned methods of plasmogamy. Subsequent reduction division takes place and four haploid spores are produced. Different divisions of fungi have their characteristic specialized spore sacs, for example in Ascomycetes the spores or the ascospores are produced inside the ascus which is present inside the fruiting body **ascocarp**. According to their specific features ascocarps are recognized as **cleistothecium** (indehiscent), **apothecium** (cup or saucer shaped), **perithecium** (flask-shaped) and **pseudoperithecium**.

I. Sexual Cycles in Fungi

The three basic processes plasmogamy, karyogamy and meiosis, occur at a specific time and in a regular sequence during the sexual stage of each fungal species. On this basis three types of sexual cycle have been recognized in fungi:

1 **Haplobiontic–Haploid Life Cycle:** The term haplobiontic refers to single generation (Refer Figure 5.20). In this type of life cycle there is only one free living haploid (n) thallus and is found in most of the fungi belonging to Mucorales and Ascomycetes.

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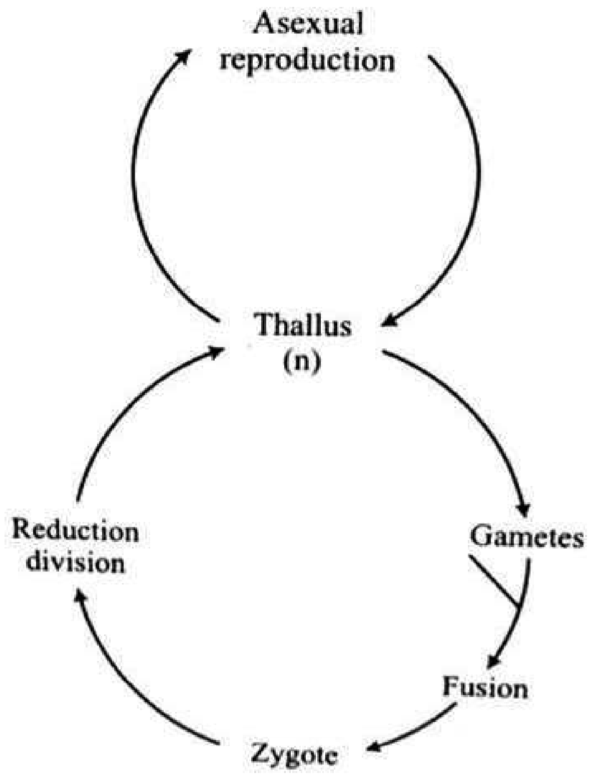

Fig. 5.20 Diagrammatic Representation of Haplobiontic–Haploid Life Cycle of Sexually Reproducing Fungal Species

- **Haplobiontic–Diploid Life Cycle:** The sexual cycle in which there is only one free living diploid (2n) thallus is known as haplobiontic-diploid life cycle (Refer Figure 5.21). Many Oomycetes and the yeast *Saccharomyces cerevisiae* shows this life cycle (Refer Figure 5.21 (A)). However, in many higher Basidiomycetes, this life cycle is slightly modified as two functionally different mycelial states can be distinguished in them (Refer Figure 5.21(B)). The first one is **monokaryon** and the other one is dikaryon. The primary mycelium which is formed by the germination of a haploid (n) sexual spore and contains a nucleus of a single genotype is known as monokaryon. It has the capability of indefinite vegetative growth and is usually sexually sterile but can produce numerous asexual spores. On contrary, the mycelium which contains two nuclei of different genotypes is termed heterokaryon or **dikaryon** or the secondary mycelium. It is formed as a result of anastomosis of homokaryotic hyphae of opposite strains. These haploid nuclei of compatible mating types associate; divide conjugately and at the end get assorted as one pair per cell. In case of Basidiomycetes, dikaryon phase predominates the life cycle, is alone capable of indefinite propagation in nature and is necessary for sexual reproduction to occur. During somatic division the two nuclei of the dikaryon remain discrete and eventually fuse in **basidia** (specialized reproductive cells), produce a diploid nucleus which undergoes meiosis immediately and produces sexual spores or **basidiospores**. In general, four basidiospores develop per basidium and each contains four haploid nuclei produced during meiosis.

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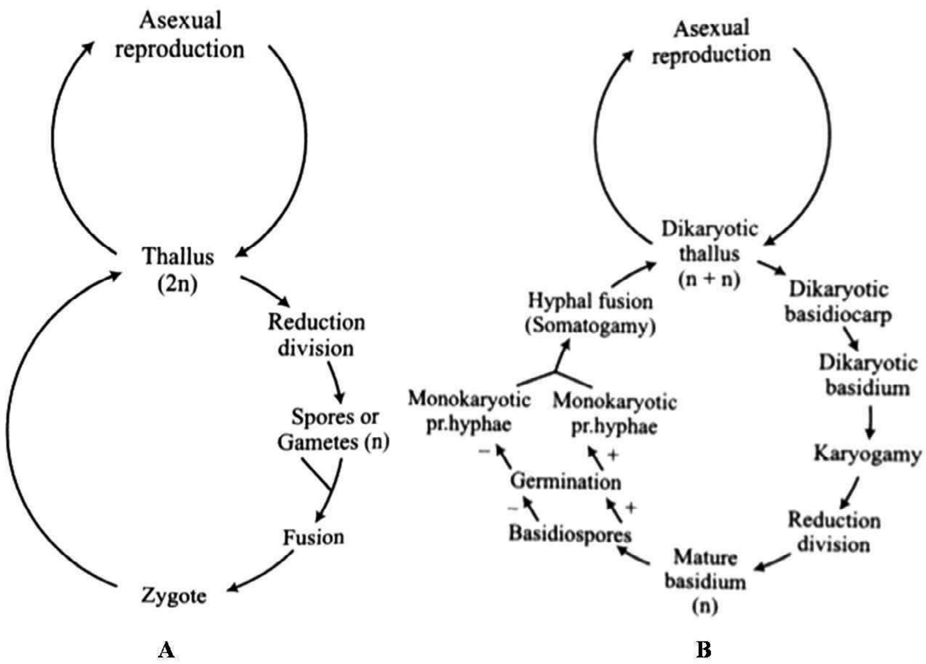

Fig. 5.21 *Diagrammatic Representation of Life Cycle of Sexually Reproducing Fungi. (A): Haplobiontic Diploid Life Cycle, (B): Modified Haplobiontic Diploid Life Cycle*

- **Diplobiontic Life Cycle:** The term diplobiontic refers to two generations. In this sexual cycle there is alternation of generation between haploid and diploid phases (Refer Figure 5.22). Although, this cycle rare in fungal species but have been discovered in certain members of Blastocladiales such as *Allomyces*. Alternation of generation in **Allomyces** is isomorphic as the gametophytic (n) generation is morphologically similar to sporophytic (2n) generation.

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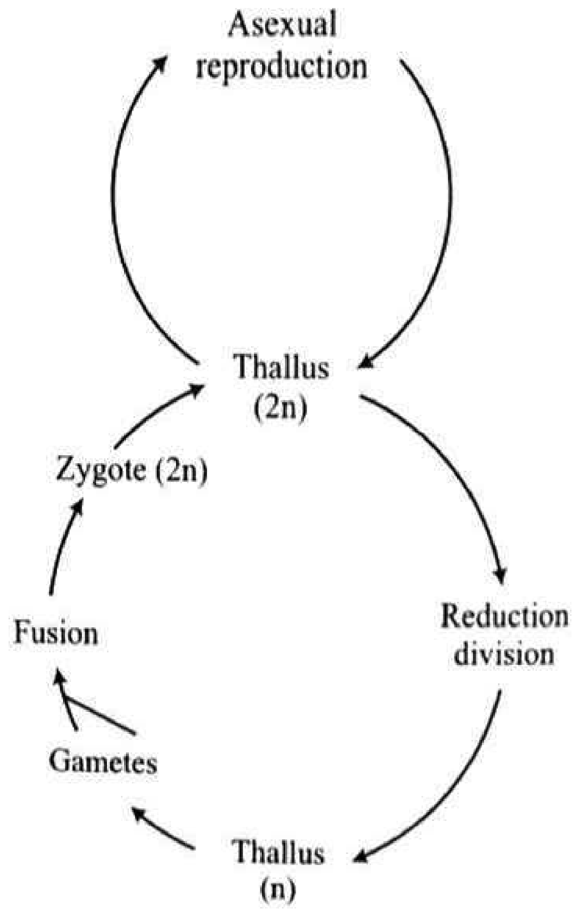

Fig. 5.22 *Diagrammatic Representation of Diplobiontic Life Cycle of Sexually Reproducing Fungi*

II. Sex Organs in Fungi

Sex organs in fungi are known as **gametangia** (singular gametangium). Gametangia may contain differentiated sex cells called gametes or may have only one or more nuclei as gametes. These gametangia or gametes can be morphologically similar also and they might show some morphological dissimilarity also. Morphologically similar gametangia and gametes are known as **isogametangia** and **isogametes**, respectively. However, morphologically distinct ones are known as **heterogametangia** and **heterogametes**, respectively. In the latter case, female gametangium is called **oogonium** (plural **oogonia**) in Oomycetes and **ascogonium** (plural **ascogonia**) in Ascomycetes. The male gametangium is known as **antheridium** (plural **antheridia**).

Most of the fungi can be distinguished into following three categories on the basis of sex:

(i) Monoecious or **Hermaphroditic** or **Bisexual:** Each thallus bears both male and female sex organs which may not be compatible.

(ii) **Dioecious:** Some thallus bears only male sex organs and some thallus bears only female sex organs.

(iii) **Sexually Undifferentiated:** In these fungi, sexual structure are produced but morphologically male and female sexual organs cannot be distinguished.

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III. Homothallism and Heterothallism

These three types have been identified on the basis of compatibility of aforementioned sex categories.

IV. Homothallic Species

Homothallic fungi are self-fertile where a single individual is capable of sexual reproduction even when cultured solely. There is very less control or no control at all over the type of nuclei which will fuse. Thus, self-fertilization takes place as has been observed in *Rhizopus sexualis* (Refer Figure 5.23). In this situation each individual has the ability to develop sexual organs and complete sexual cycle. This condition was terms as **homothallism** by Blakeslee (1904). It is predominantly seen in lower fungi, quite common in Ascomycetes but rarely observed in Basidiomycetes. Since, majority of fungi display homothallism, this seems to be a primitive condition from which heterothallism has evolved later on.

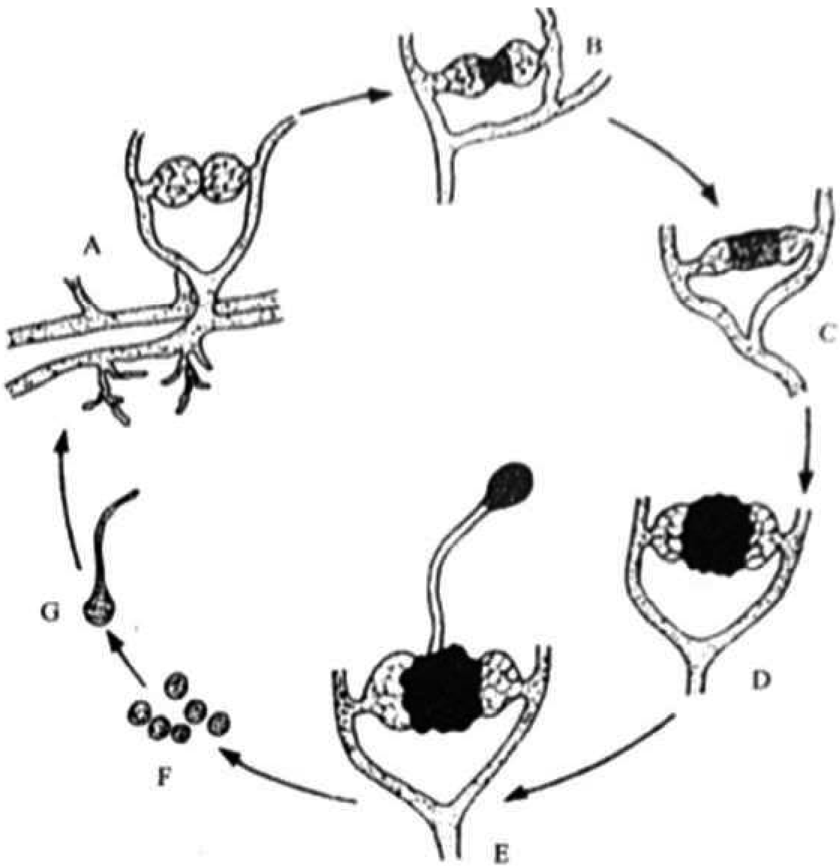

Fig. 5.23 *Sexual Reproduction in Homothallic Rhizopus sexualis*

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The above Figure 5.23 shows the diagrammatic representation of sexual reproduction in homothallic *Rhizopus sexualis* [Progametangia Formation in Hyphae (A), Formation of Gametangia (B), Formation of Prozygosporangium (C), Development of Zygosporangium (D), Zygospore Germination and Germ-Sporangium Formation at the Tip of Sporangiophore (E), Released Sporangiospores (F), Sporangiospore Germinate to Form New Mycelia (G)].

V. Heterothallic Species

These are those fungi, such as *Rhizopus stolonifer*, *Neurospora crassa* and *Mucor mucedo* which require two compatible mating partners for mating to occur. However, these conjugant individuals may or may not develop morphologically distinguishable gametangia. This condition was named as **heterothallism** by Blakeslee (1904). Heterothallism has certain disadvantages over homothallism as it promotes out breeding and increases resultant variability in the progeny. This occurs in all major groups of fungi, such as Hymenomycetes, Mucorales, Uredinales, Saprolegniales, Euascomycetes and Ustilaginales, etc. Whitehouse (1949) distinguished two types of heterothallism:

- **Morphological Heterothallism:** The two interacting thalli have morphologically distinct male and female sex organs or gametes. Haplodioecious term is also used for this type of heterothallism (Refer Figure 5.24).

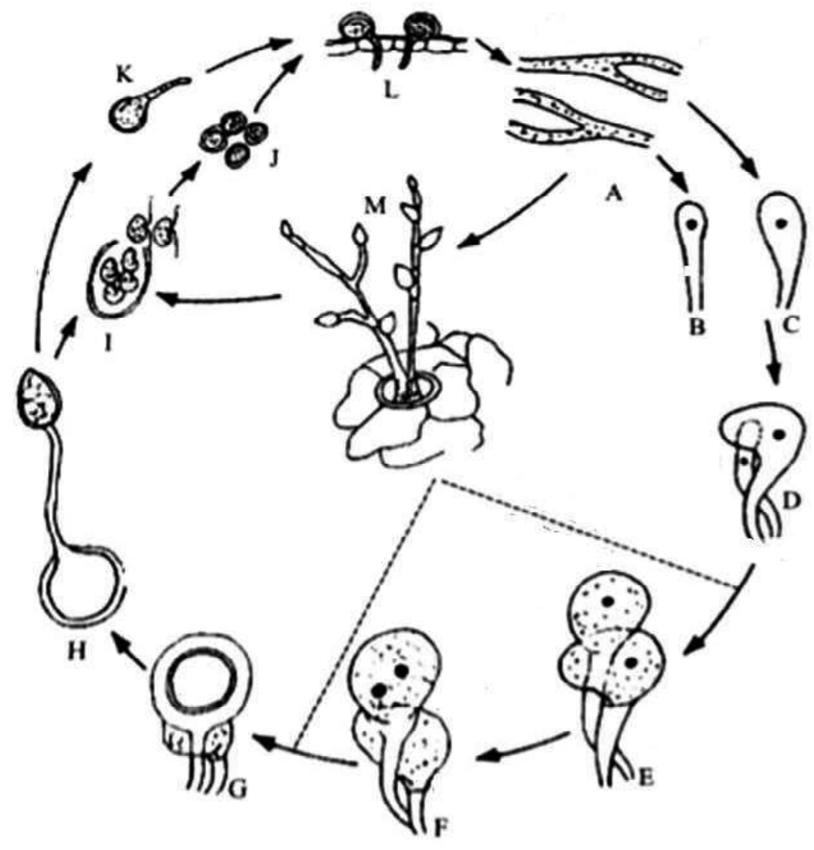

Fig. 5.24 *Life Cycle of Phytopthora infestans*

The above Figure 5.24 shows the diagrammatic representation of life cycle of *Phytopthora infestans* [Vegetative Hyphae of Different Mating Types suppose A_1 and A_2 (A); Oogonium and Antheridium of Opposite Mating Types (B) and (C); Penetration of Antheridium into Oogonium (D); Conversion of Oogonium into a Globose Structure above Antheridium (E); Plasmogamy (F); Diploid Oospore (G); Germinated Oospore (H); Differentiation of Zoospore and its Release (I); Encystment of Zoospore (J); Germination of Zoospore (K); Germination and Host Penetration (L); Sporangia Borne on Sporangiophore (M); Haplophase (F) and (E); Diplophase (A, B, C, D, G, H, I, J K and L)] .

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- **Physiological Heterothallism:** These are those heterothallic species which differ in incompatibility factor or mating type, irrespective of the availability or non-availability of differentiated sex organs and gametes. This involves either two alleles at single locus or multiple alleles at one or two loci (Refer Figure 5.25).

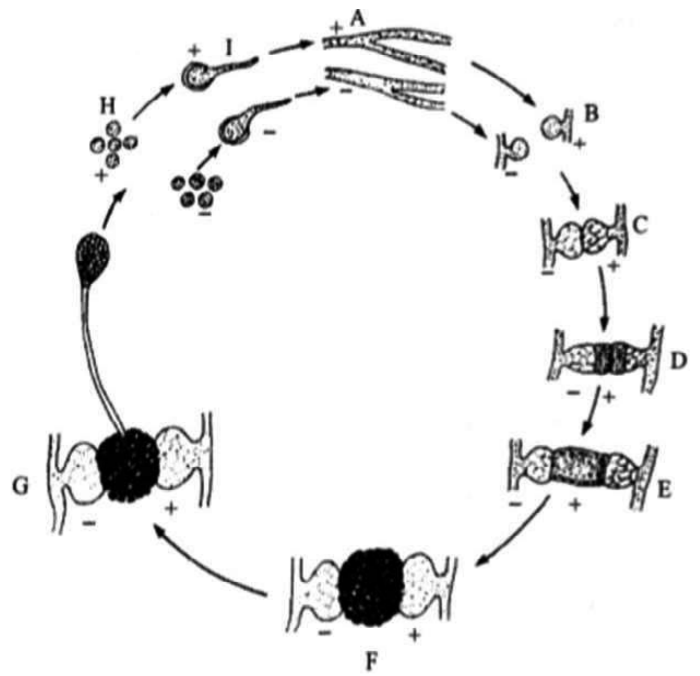

Fig. 5.25 *Sexual Reproduction in Heterothallic Rhizopus stolonifer*

The above Figure 5.25 shows the diagrammatic representation of sexual reproduction in heterothallic *Rhizopus stolonifer*. (A): Vegetative Hyphae, (B): Compatible Zygophores of Opposite Mating Types, (C): Progametangia Formation, (D): Gametangia Formation, (E): Formation of Prozygosporangium, (F): Development of Zygosporangium, (G): Germ-Sporangium, (H): Release of Two Types of Spores, (I): Germination of Spores.

- o **Two-Allele Heterothallism:** Heterothallism is determined by two alleles at a single locus. Fungal species having this type of heterothallism are divided into two strains commonly called as plus (+) and minus (-) strain

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or 'A' and 'a'. Plus (+) and minus (-) strain are essential for mating, whereas the (+) and (+), and (-) and (-) strains are self-sterile or self-incompatible (Refer Figure 5.26). Members of Mucorales (*Rhizopus*, *Mucor*, *Phycomyces*), Pyrenomycete (*Neurospora*), Discomycetes (*Ascobolus*), Rust Fungus (*Puccinia*) and Smut Fungus (*Ustilago*).

	A	a
A	-	+
a	+	-

A = plus strain **a = minus strain**

+ = compatible cross **- = incompatible cross**

Fig. 5.26 *Mating Reactions of Possible Isolates of a Two-Allelic Heterothallic Fungus*

- **Multiple-Allele Heterothallism:** In this type sexual compatibility is controlled by more than two alleles at a genetic locus. It is advantageous over two allelic heterothallism as probability of meeting compatible strains is increased as well as the magnitude of out breeding is also enhanced. Multiple allelomorphs heterothallism can be bipolar, tetrapolar and octapolar.
- **Bipolar or Unifactorial Heterothallism:** In this type of heterothallism, sexual compatibility is controlled by single locus but that single locus can have multiple allele. A cross only occurs when the allele in the mating thalli are different. If locus is designated as 'A' multiple alleles will be A_1, A_2, A_3, A_4, A_5, and so on. The thallus containing A_1 mating type can successfully mate with any thallus except the one which contains A_1 (Refer Figure 5.27). It is commonly found in members of Ascomycetes (*Neurospora*, *Sordaria*, *Ascobolus*, and *Aspergillus*), Myxomycetes and Basidiomycetes. Some Zygomycetes, such as *Coprinus comatus* also have single mating type locus (hence **unifactorial**) but numerous mating type alleles.

	A_1	A_2	A_3	A_4
A_1	-	+	+	+
A_2	+	-	+	+
A_3	+	+	-	+
A_4	+	+	+	-

+ = Compatible cross **- = Incompatible cross**

Fig. 5.27 *Mating Reaction of Four Probable Isolates of Bipolar Heterothallic Fungus*

Compatible mating takes place between any haploid mycelia that differ in mating type. Thus the resulting diploid basidium nucleus is heterozygous for mating type. Meiotic division, further, gives rise to four haploid spores. Two haploid spores of the basidium are of one mating type and other two spores are of the other mating type (Refer Figure 5.28). Such a system is termed **bipolar**. Since the diploid strains yield two mating types thus the inbreeding potential is 50 per cent. However, since a population has large number of mating types thus there is probability that most of the encounters will be with non-sibling strains and out breeding potential can approach 100 per cent.

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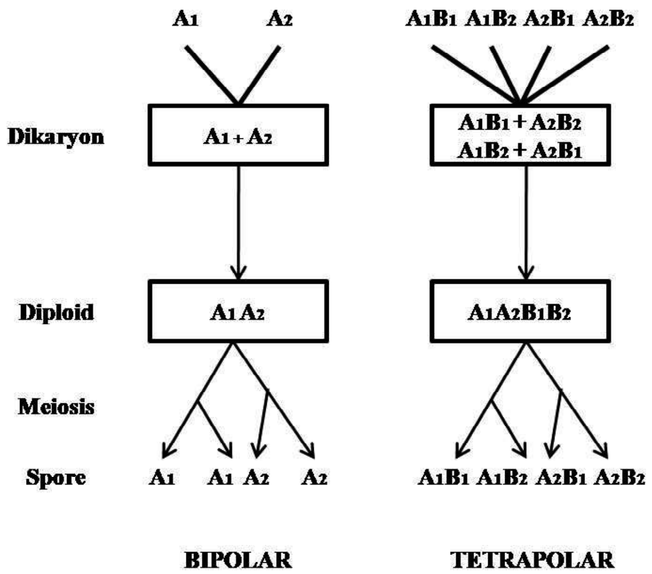

Fig. 5.28 *Segregation of Compatibility Factors and Polarity in Bipolar and Tetrapolar Heterothallism*

- **Tetrapolar or Bifactorial Heterothallism:** This is very similar to bipolar multiple heterothallism except the fact that there are two loci which control the compatibility. Each locus in this case is multi-allelic. According to some estimate there are at least 100 alleles present at each locus. These loci segregate independently at meiosis and since two factors are involved the heterothallism is said to be bifactorial. The two factors are designated as 'A' and 'B' and its two alleles are A_1, A_2 and B_1, B_2, respectively. Thus fungi belonging to this category will have four mating types A_1B_1, A_2B_2, A_1B_2 and A_2B_1. The resulting spores will have four different mating types that is why this system is called as Tetrapolar. This kind of heterothallism has been observed in *Coprinus cinereus* and *Schizophyllum commune* (members of Basidiomycetes). The former contains 164 'A' factor and 79 'B' factors whereas the later contains 450 'A' and 79 'B' factors. Such a high number of factors ensure 100 per cent out breeding potential. A compatible mating takes place only if the 'A' and 'B' alleles differ in both the thalli. For example, if one mycelium has allele A_1B_1, this will not

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mate with mycelium containing alleles A_1B_1 but can successfully mate with mycelium having A_2B_2 alleles as alleles at both the loci are different. Similarly, a cross will not be successful if alleles at any of the loci are same (Refer Figure 5.29). However, in some fungi hyphae bearing common allele at any of the loci often result in heterokaryon formation but fructification rarely occur. If a heterokaryon has common 'A' allele and a different 'B' allele a **flat reaction** occurs and the resulting heterokaryon is depressed, sparse and its older hyphae are irregularly branched. In some fungi, this prevents the appearance of clamp connections, causes morphological or metabolic abnormality in mycelium and disturbs regularity of nuclear division. However, if it has a different 'A' allele and common 'B' allele **barrage reaction** occurs and hyphae of mating colonies approach towards each other but cease near the center and leaves a strip or barrage zone that physically separates the two colonies. Common 'B' allele often prevents nuclear migration, karyogamy, clamp connection and meiosis.

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	-	FL	B	+
A_1B_2	FL	-	+	B
A_2B_1	B	+	-	FL
A_2B_2	+	B	FL	-

+ = Compatible cross **B = Barrage reaction**

- = Incompatible cross **FL = Flat reaction**

Fig. 5.29 *Mating Reaction between Four Probable Isolates of Bifactorial Heterothallic Fungus*

- **Octapolar or Trifactorial Heterothallism:** In this type the incompatibility is controlled by three factors 'A', 'B' and 'C' and these are inherited independently. Since three factors are involved, thus it is known as trifactorial heterothallism. For nuclear migration and mature fruit body formation all the three factors needs to be in heterozygous condition. This heterothallism is reported in basidiomycetous fungus *Psathyrella coprobia*.

VI. Secondarily Homothallic Species

In some of the heterothallic fungi a slight modification has been seen in the way the nuclei are incorporated in individual spores. In these species, during spore formation two nuclei of opposite mating types are incorporated regularly into each spore (or at least in some spores). Germlings of these spores are self-fertile and behave as homothallic but actually they are heterothallic. This condition is also known as

pseudohomothallism. It is reported in 10-15 % of the fungal species, for example four-spored *Neurospora crassa* and *Podospora anserina* (Refer Figure 5.30).

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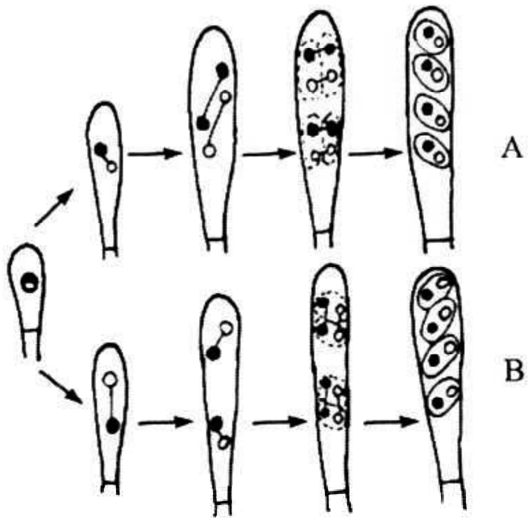

Fig. 5.30 *Diagrammatic Representation of Mechanism of Secondary Homothallism. (A): Neurospora species, (B): Podospora anserina*

VII. Hormonal Control of Mating

Differentiation of gametes, their movement towards each other and their fusion for the formation of zygote all are very complex processes and they considerable variation in their form and duration. Different mating types secret different diffusible substances called '**hormones**' or '**pheromones**' which regulate these processes. 'Hormones' act on the same individual who produce them however, 'pheromones' produced by some other organism and act on some other individuals. Fungal sex hormones are produce in very low quantities, sometimes by induction of some other hormone and sometime constitutively. The first evidence about involvement of hormones in regulating sexual reproduction was demonstrated in Mucorales (Zygomycetes) by Burgeff (1924). In this fungus trisporic acid was involved in induction of zygophores. Trispotic acids are C18 terpenoids which induce the complete switch over from asexual to sexual differentiation in (+) and (-) strains of mycelia. This was demonstrated by the ability of zygophores formation even after physical separation of the two mating types by a membrane or an air gap. This hormone is universal for all Mucorales, such as *Mucor*, *Blakeslea* and *Phycomyces,* etc.

Simplest of all hormonal system has been observed in *Allomyces* (Chytridiomycetes) which produces a potent attractant for smaller bright-orange male gametes. This chemical attractant is called as **sirenin**. Out of all the swimming cells produced by fungi namely male and female gametes, zygote, diploid and haploid zoospores only male gamete respond to this.

Hormone system in *Achyla* (Oomycetes) is much more complex. Sexual differentiation in *Achyla ambisexualis* and *Achyla bisexualis* is controlled by complementary hormone which diffuses from one hypha to another. Male cells do

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not produce any hormone of their own. Female cells produces hormone 'A' continuously and induces the vegetatively growing male cells to produce numerous antheridial branches. In response to this, male cells releases hormone 'B' which diffuses back to female cells and induces them to produce oogonial initials from vegetative phase. It has been estimated that there are a total of nine compounds which are responsible for these sequence of events. These compounds include four 'Sequentially-Acting Hormones' and five 'Modifying Substances'. The female hormone 'A' is steroidal in nature and is known as **Antheridiol**. The complementary hormone 'B' produced by the male cells in response is also steroidal in nature and called as **Oogonial**.

Members of Oomycetes order *Peronosporales*, *Pythium* and *Phytophthora* also shows hormonal regulation of sexual reproduction. It has been seen in several species of *Phytopthora* that both mating types 'A_1'and 'A_2' from oospores when paired with the opposite mating type of same or different species on opposite side of polycarbonate membrane. Successful formation of oospores on both the sides of the polycarbonate membranes indicated stimulation of sexual reproduction by the hormones produced by the strains present on opposite sides of the membrane. The sexual hormone (α_1) produced by 'A_1' isolate induces sexual reproduction in 'A_2' isolate but not in 'A_1' isolate. Similarly, sexual hormone (α_2) produced by isolate 'A_2' induces sexual reproduction in isolate 'A_1' but not in 'A_2' isolate. Some homothallic species of Phytopthora also have the ability to produce á hormone and induce oospore formation in 'A_1' and 'A_2' isolates like their heterothallic counterparts.

Mating in *Saccharomyces cerevisiae* is also reported to be regulated by diffusible sex hormones. There are two mating types in *Saccharomyces cerevisiae* 'α' and 'a' which produces 'á-factor' and 'a-factor', respectively. When these mating types are brought together their cells fuse to form diploid zygote. Diploid cells neither secrete nor respond to mating factors as in this phase most of the genes involved in pheromone production and response are inactivated. The 'α-factor' is a mixture of four peptides whereas the 'a-factor' is an undecapeptide. Additionally, the 'α-factor' is not species specific as the one produced by *S. cerevisiae* and *S. kluyveri* is equally effective on 'a' cells of both the species. In case of *Schizosaccharomyces pombe* the cells are of Plus (P) and Minus (M) mating types. Mating between the opposite types occurs only under nitrogen starvation condition and is mediated by pheromones. This pheromone not only induces the conjugation tube formation and cell fusion but is known to serve a continued response pathway which is necessary for the cell to pass through meiosis. The pheromone 'M-Factor' secreted by 'M' cells is a 'Farnesylated' and 'Carboxymethylated Nonapeptide' processed from 42- and 44-amino acid precursors encoded by two genes, *mfm* 1 and *mfm* 2. In member of Ascomycetes *Neurospora crassa* there are two different mating types 'A' and 'a'. Female cells (**trichogynes**) and Male cells (**microconidia**) are formed when the fungus enters the sexual cycle. But the specific recognition of opposite mating type is mediated by the sexual hormones only.

Similarly, in the case of cellular Acrasiomycetes (Slime Moulds) the sexual cycle is controlled by hormones. These produce large dormant structures, the **macrocysts**, whose formation is induced by sex hormones. In some strains of

Dictyostelium discoideum produces a volatile sex hormone which is necessary for the induction of macrocyst production.

In Basidiomycetes, *Tremella mesenterica* is heterobasidiomycetes jelly-fungus which grows vegetatively as budding yeast. The cells of mating types 'A' and 'a' produces diffusible sex factors that ceases budding in opposite mating types and induces the formation of conjugation tubes. These were characterized to be two peptides 'Tremerogen A-10' and 'Tremerogen a-13' isolated from type 'A' and type 'a' cells, respectively. Formation of conjugation tube continues as long as the tremerogen are added, but cells cease to form conjugation tube and resumes budding when tremerogen is removed.

5.3.3 Parasexuality

This is the process in which plasmogamy, karyogamy and meiosis occurs in a fungal species but not at specific time in the life cycle and in the thallus. Therefore, there are various fungi with reap the benefits of sexuality through the parasexual process. However, the true sexual cycle and the parasexual cycle are not mutually exclusive. There are some Deuteromycetes (Fungi imperfecti or imperfect fungi) which do not show true sexual cycle but are found in a variety of habitats either as saprophyte and parasites. This adaptation seems to be possible because of accumulation of genetic variation through the special mechanism called parasexuality. Some Ascomycetes and Basidiomycetes species also exhibit parasexuality in addition to true sexual cycle. Discovery of parasexuality made it realize that sexual reproduction is not the only way of transfer of genetic material.

Parasexuality was first described in *Aspergillus nidulans* by Pontecorvo and Roper (1952). In this filamentous fungus parasexuality coexists with normal sexual cycle. The sequence of heterokaryosis, fusion of genetically dissimilar nuclei, followed by recombination and segregation is termed as parasexual cycle. The essential sequences of events are as follows:

Heterokaryosis: Heterokaryosis means the inclusion of genetically different nuclei in the same cytoplasm coming from mycelium of two different strains. There are several ways in which heterokaryosis can be achieved. However, the most common way is by the anastomosis of somatic hyphae of different genetic constitution. If the hyphae have two genetically different nuclei, the resulting colony that is formed is heterokaryon. **Heterokaryon** has two nuclei of different genotype in a common cytoplasm. Additionally, if the two somatic hyphae differ with respect to cytoplasmic content (Mitochondrial and Plasmid DNA), the resulting colony is **heteroplasmy**. Another method by which mokaryotic mycelium changes into heterokaryon is by means of mutation as seen in some Ascomycetes wherein heterokaryon arises by mutation in one or more nuclei. In *Podospora anserina* and *Neurospora tetrasperma* there is inclusion of genetically different nuclei in a single spore following meiosis.

Fusion and Formation of Diploids: After conversion of a mycelium into a heterokaryotic one, nuclear fusion takes place between haploid nuclei of different genotypes. Roper (1952) was the first to discover nuclear fusion in heterokaryotic vegetative cells of fungus. The nuclear fusion may be of following two types:

(i) Fusion between like nuclei.

(ii) Fusion between unlike nuclei.

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Nuclear fusion leads to the formation of homozygous and heterozygous diploid nucleus, respectively. If the genotype of heterokaryotic mycelium is A and B, then five types of nuclei can be formed after their fusion:

(i) Two Type of Haploid Nuclei (A and B).

(ii) Two Types of Homozygous Diploid Nuclei (AA and BB).

(iii) One Type of Heterozygous Diploid Nuclei (AB).

Heterozygous diploids are relatively less stable at mitosis and their colonies mainly have diploid conidia of parental type. All the aforementioned nuclei multiply at the same rate but the diploid nuclei are present in much smaller number than the haploid nuclei. Interestingly, haploid conidia are relatively small as compared to diploid conidia.

Mitotic Segregation: Meiotic crossing over is an extremely important step in parasexual cycle and it takes place during multiplication of diploid nuclei. The segregants in parasexual cycle arise by mitotic crossing over. It is one of the rarest events and occurs at four-strand stage of mitosis. Chiasmata formation is confined to single chromosome pair out of whole complete sets of chromosome. The recombinations that took place provide the fungus some advantages of sexuality within the parasexual cycle. In this cycle, crossing over occurs once in every 50 mitotic divisions. Mitotic crossing over can occur either in the G1 phase between homologous chromosomes or even after DNA replication in the G2 phase between non-sister chromatids of homologous chromosomes. However, it predominantly occurs in G2 phase (Refer Figure 5.31).

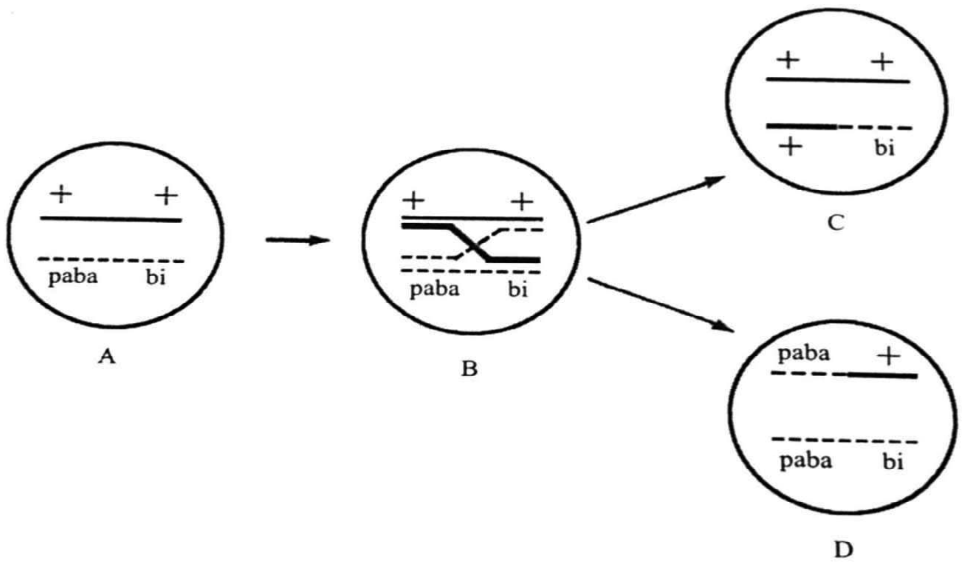

Fig. 5.31 *Mitotic Crossing-Over between a Single Pair of Homologous Chromosomes*

Figure 5.31 shows the diagrammatic representation of mitotic crossing-over between a single pair of homologous chromosomes. (A): Diploid Nucleus Heterozygous for the Genes *paba* and *bi*, (B): Crossing-Over at Chromosome Replication, (C) and (D): Genetically Different Nuclei after Chromosome Segregation.

Haploidization: During successive mitotic divisions, there is gradual loss of chromosomes and diploid nuclei gives rise to haploid nuclei. This is called haploidization. During mitotic division of a diploid nucleus, there is non-disjunction of chromatids of one of the chromosome pair because of which one of the daughter nuclei has 2n + 1 chromosome and the other has 2n - 1 chromosome. The nuclei with incomplete multiples of the haploid number are called as aneuploid. These haploids are genetically unstable and tend to lose more and more chromosomes till a perfect haploid condition is restored. Therefore, 2n + 1 (**Hyper Diploid Nucleus**) will ultimately return to 2n condition, whereas the 2n - 1 (**Hypo Diploid Nucleus**) will revert to n condition. In accordance with this, *Aspergillus nidulans* (n = 8) nuclei are found with 17 (2n + 1), 16 (2n), 15 (2n - 1), 12, 11, 10, 9 chromosomes. Non-disjunction occurs once in every 50 mitotic divisions (Refer Figure 5.32).

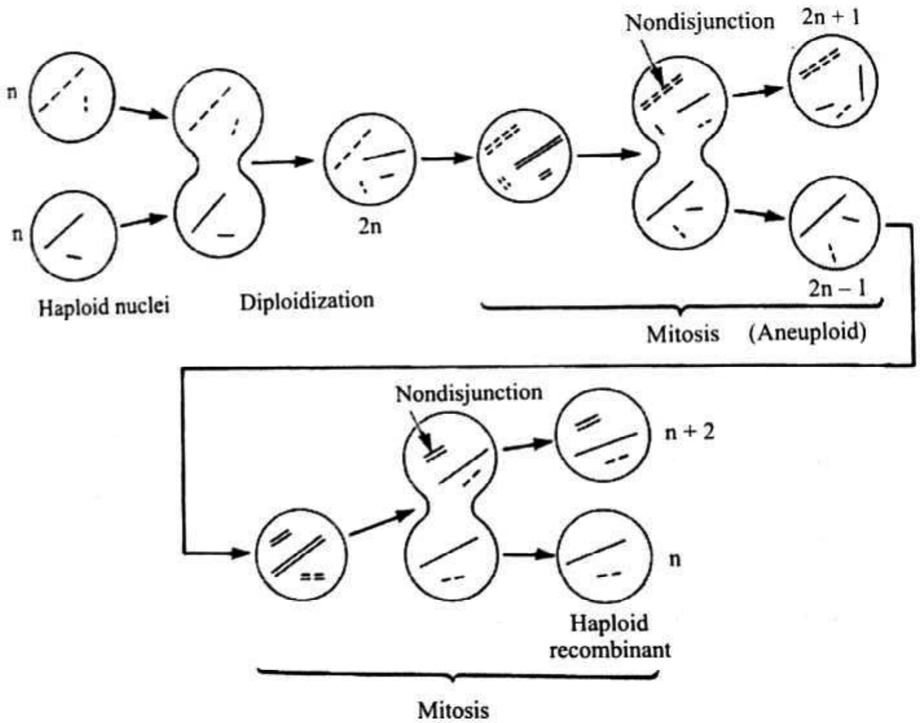

Fig. 5.32 *Diagrammatic Representation of Process of Haplodization*

The above Figure 5.32 shows the diagrammatic representation of process of haplodization, where the process begins with genetically different haploid nuclei in the heterokaryon and terminates with formation of haploid nucleus which is genetically different from the parent nuclei.

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Therefore, parasexual cycle closely resembles sexual cycle as ending result in both the processes are same and give rise to recombinant haploid nuclei. Although, in sexual cycle there is there is extreme uniformity and fine coordination between each and every phases. However, there is no such coordination in parasexual cycle. Many imperfect fungi have been reported to exhibit the parasexual cycle, such as *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus oryzae*, *Aspergillus parasiticus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium italicum*, *Penicillium digitatum*, *Penicillium expansum* and *Cladosporium fulvum*. It has also been detected in certain Ascomycetes and Basidiomycetes (*Ustilago maydis*, *Schizophyllum commune*, *Glomerella cingulata*, etc.) which undergo normal sexual reproduction.

Significance of Parasexual Cycle

It has become a valuable tool for industrial ecologist. Several fungi which belong to fungi imperfecti or Deuteromycetes are used in various industrial processes. New and better strains of these fungi can be produced through parasexual cycle. This cycle provides possibilities for producing strains with desired combination of properties. In the case of plant pathogenic fungi, this process genetic flexibility on the populations. Thus, the process can be successfully employed in genetic control of pathogenicity and altering the host range of several species of *Fusarium*. The process can also be employed for the genetic analysis of those fungi which do not undergo sexual reproduction.

Check Your Progress

6. Enlist various common methods of vegetative reproduction in fungi.
7. What are sclerotia?
8. How many types of sexual reproduction are found in fungi?
9. What is parasexuality?
10. Differentiate between eucarpic and holocarpic fungi?

5.4 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Rhizomorph is the aggregation of thick strands of fungal hyphae that appears root like.
2. There is no definite cell wall. Vegetative structure of the fungus is a free living plasmodium which has multinucleate protoplasm. Nuclei are diploid and entire plasmodium takes part in development of frutification. Flagellated cells develop into myxamoeba.
3. Dolipore septum are the perforated septa which are found in Basidiomycetes (except in rusts and smuts). There is a slight modification than the simple septa which involves the presence of barrel shaped inflation with a hemispherical perforated membrane on each side of the opening. This type of septa is known as dolipore septum.

4. It is false tissue formed by intertwining of the hypha where individual hypha loses their identity. They are made up of elongated cells and run parallel (more or less) to one another. In cross section, the hyphal mass appears as a compact structure of isodiametric or oval cells.
5. Basidiomycetes have septate mycelium.
6. Some of the common methods of reproduction in fungi are fragmentation, fission, budding and chlamydospore, rhizomorphs and oidia formation.
7. Sclerotia are modifications of mycelium which may be rounded, cylindrical, cushion-shaped or irregular in structure with dense mass of thick walled hyphae. These serve as a means of perrenation as seen in *Claviceps* (Ergot).
8. Three types of sexual reproduction are seen in fungi. These are isogamy (fusing gametes are motile and morphologically similar), anisogamy (fusing gametes are motile and morphologically dissimilar) and oogamy (female gamete is larger and non-motile).
9. Parasexuality is the process in which plasmogamy, karyogamy and meiosis occurs in a fungal species but not at specific time in the life cycle and in the thallus. Therefore, there are various fungi with reap the benefits of sexuality through the parasexual process. However, the true sexual cycle and thee parasexual cycle are not mutually exclusive.
10. Eucarpic fungi are those fungi in which a part of thallus participates in the development of reproductive organs, such as in *Aspergillus*. While in holocarpic fungus entire thallus gets converted into reproductive structures as observed in yeast. In the former, somatic and reproductive phases can occur together in the thallus but this is not possible in case of holocarpic fungi.

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5.5 SUMMARY

- Fungi show remarkable diversity in its morphology which ranges from unicellular (yeast and slime moulds) to multicellular and filamentous forms.
- Most of the fungi are multicellular and filamentous. Mycelium is the main part of the body and reproductive structures are born and reproductive hypha.
- Various structures are formed from aggregation of the mycelium, such as stroma, rhizomorphs, sclerotia, etc.
- Ultrastructure of fungal cell shows similarities with eukaryotic cell. In most of the fungi, cell wall is made up of chitin instead of cellulose.
- Nuclei are small and division is intra-nuclear. Fungus grows on a variety of substratum. Interestingly, some members can grow under extreme environmental conditions.
- Fungi live as saprophytes, parasites and symbionts. Saprophytes act as important decomposers in the nature.
- By extracellular secretion they digest the organic substances and absorb part of digested nutrients.

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- Fungi occur as parasites on plants, animals, humans and even on other fungal species and cause several diseases.
- In association with plants roots and algae fungi form symbiotic associations, such as mycorrhiza and lichen.
- Reproduction in fungi occurs by means of vegetative, asexual and sexual methods.
- Fragmentation, fission, budding, oidia, chlamydospore are the common ways of vegetative reproduction.
- A fungus reproduces more frequently asexually than sexually.
- For asexual reproduction spores and conidia are formed on sporangiophore and conidiophores, respectively.
- Depending upon the fungal species, during sexual reproduction, entire thallus or a part of thallus may be participating in formation of gametangia (sex organs).
- Sexual reproduction occurs via three steps plasmogamy, karyogamy and meiosis.
- There are various modes of plasmogamy; it may occur via planogametic and gametangial copulation, gametangial contact, somatogamy and spermatization.
- In different fungal genera, on basis of sequence of the basic processes of sexual reproduction, at different stages of life cycle, three types of sexual cycles has been observed.
- On the basis of sex fungi are identified into three categories: monoecious, dioecious and sexually differentiated forms.
- On the basis of compatibility of the aforementioned categories phenomenon of homothallism, heterothallism and secondarily homothallism are identifies.
- Homothallic species are those species which are self-fertile; whereas the heterothallic species are not self-fertile and require two compatible mating types for mating to occur.
- Heterothallic species are further divided into morphological and physiological heterothallism on the basis of morphology of sexual organs.
- Physiological heterothallism may involve either two alleles at single locus or multiple alleles at one or two loci.
- Secondarily homothallic species are those species in which during spore formation two nuclei of opposite mating types are incorporated in each spore.
- Germlings of these species are self-fertile and behave as homothallic although they are heterothallic.
- Evidences of hormonal control of mating in sexual reproduction and involvement of pheromones are quite prominent in literature.
- Discovery of parasexuality made it realize that sexual reproduction is not the only way of transfer of genetic material.

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- Owing to this process fungus reaps the benefits of sexual reproduction without actually undergoing sexual reproduction.
- Heterokaryosis means the inclusion of genetically different nuclei in the same cytoplasm coming from mycelium of two different strains.
- Meiotic crossing over is an extremely important step in parasexual cycle and it takes place during multiplication of diploid nuclei.
- The segregants in parasexual cycle arise by mitotic crossing over.
- During successive mitotic divisions, there is gradual loss of chromosomes and diploid nuclei gives rise to haploid nuclei. This is called haplodization.
- The nuclei with incomplete multiples of the haploid number are called as aneuploid.
- Parasexuality was first described in *Aspergillus nidulans* by Pontecorvo and Roper (1952). In this filamentous fungus parasexuality coexists with normal sexual cycle.
- The sequence of heterokaryosis, fusion of genetically dissimilar nuclei, followed by recombination and segregation is termed as parasexual cycle.
- Parasexuality has become a popular tool for industrial ecologist for the preparation of new strains of fungus.
- Parasexual cycle closely resembles sexual cycle as ending result in both the processes are same and give rise to recombinant haploid nuclei.
- In sexual cycle there is there is extreme uniformity and fine coordination between each and every phases.
- Many imperfect fungi or fungi imperfecti have been reported to exhibit the parasexual cycle, such as *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus oryzae*, *Aspergillus parasiticus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium italicum*, *Penicillium digitatum*, *Penicillium expansum* and *Cladosporium fulvum*.
- It has also been detected in certain Ascomycetes and Basidiomycetes (*Ustilago maydis*, *Schizophyllum commune*, *Glomerella cingulata*, etc.) which undergo normal sexual reproduction.
- Several fungi which belong to fungi imperfecti or Deuteromycetes are used in various industrial processes.

5.6 KEY TERMS

- **Rhizomorph:** Rhizomorph is the aggregation of thick strands of fungal hyphae that appears root like.
- **Dolipore septum:** Dolipore septum are the perforated septa which are found in Basidiomycetes.

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- **Sclerotia:** Sclerotia are modifications of mycelium which may be rounded, cylindrical, cushion-shaped or irregular in structure with dense mass of thick walled hyphae.
- **Parasexuality:** Parasexuality is the process in which plasmogamy, karyogamy and meiosis occurs in a fungal species but not at specific time in the life cycle and in the thallus.
- **Eucarpic fungi:** Eucarpic fungi are those fungi in which a part of thallus participates in the development of reproductive organs, such as in *Aspergillus*.
- **Haplodization:** During successive mitotic divisions, there is gradual loss of chromosomes and diploid nuclei gives rise to haploid nuclei called as haplodization.
- **Aneuploid:** The nuclei with incomplete multiples of the haploid number are called as aneuploid.

5.7 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Brief a note on fungi.
2. What are the characteristic of fungi that makes them different from other kingdom?
3. What are yeasts?
4. Explain the vegetative structure of thallus.
5. What does the hyphal tip growth of fungi represent?
6. What are saprotrophs?
7. What is planogametic copulation?
8. Explain about the mitotic segregation with the help of examples.
9. What is the significance of parasexual cycle?

Long-Answer Questions

1. Discuss in detail about the occurrence, distribution and general characteristics of fungi.
2. Briefly discuss about the range of thallus with the help of appropriate examples.
3. Explain the organisation of thallus with the help of appropriate examples.
4. What are different fungal aggregations? Explain with the help of suitable examples.
5. Discuss in detail about the ultra-structure and composition of fungal cells.
6. Give the detailed explanation of the types and modes of nutrition in fungi.

7. What are the ecological groups of fungi? Discuss with the help of suitable examples.
8. Explain the types of reproduction in fungi with the help of appropriate examples.
9. Describe the mechanism of parasexuality. What are the various benefits associated with parasexuality for industrial ecologist?

NOTES

5.8 FURTHER READING

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UNIT 6 BRYOPHYTES: ORIGIN, CLASSIFICATION AND VEGETATIVE REPRODUCTION

Structure

6.0 INTRODUCTION

Bryophytes are an informal group consisting of three divisions of non-vascular land plants, the liverworts, hornworts and mosses. They are characteristically limited in size and prefer moist habitats although they can survive in drier environments. The Bryophytes consist of about 20,000 plant species. Bryophytes produce enclosed reproductive structures, gametangia and sporangia, but they do not produce flowers or seeds. They reproduce via spores. Bryophytes are usually considered to be a paraphyletic group and not a monophyletic group, although some studies have produced contrary results. Regardless of their status, the name is convenient and remains in use as an informal collective term.

Bryophytes exist in a wide variety of habitats. They can be found growing in a range of temperatures, cold arctics and in hot deserts, elevations, and sea level to alpine, and moisture, dry deserts to wet rainforests. Bryophytes can grow where vascularized plants cannot because they do not depend on roots for an uptake of nutrients from soil. Bryophytes can survive on rocks and bare soil. In between 510 - 630 million years ago, land plants evolved from aquatic plants, specifically green algae. Molecular phylogenetic studies conclude that Bryophytes are the earliest diverging lineages of the extant land plants. They provide insights into the migration of plants from aquatic environments to land. A number of physical features link Bryophytes to both land plants and aquatic plants.

In this unit, you will study about general features, origin and classification of Bryophytes, mechanism of dehiscence and spore dispersal and asexual reproduction in Bryophytes in detail.

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6.1 OBJECTIVES

After going through this unit, you will be able to:

- Explain general features, origin and classification of Bryophytes
- Describe the mechanism of dehiscence and spore dispersal
- Discuss about asexual reproduction in Bryophytes

6.2 BRYOPHYTES: GENERAL CHARACTERISTICS, ORIGIN AND CLASSIFICATION

Bryophytes are also known as 'Amphibians of Plant Kingdom' or 'Lilliputians of Plant Kingdom'. They are Monosporangiate, i.e., produces one capsule per sporophyte. The term 'Bryophyte' originates from *Greek* and comprise of '*bryon*' means 'Tree-Moss' and '*python*' means 'Plant' and refers to group of plants that swell upon hydration. Previously bryophyta was considered as a different phylum with three subclass, *viz.* Hepaticopsida (Liverworts), Anthocerotopsida (Hornworts) and Bryopsida (Mosses) but now due to the monophyletic origin of all the three distinct groups the classes were raised to phylum, *viz.* Marchantiophyta (Liverworts), Anthocerotophyta (Hornworts) and Bryophyta (Mosses). The present content represents 'Bryophytes' comprising all the three new phylum, i.e., Marchantiophyta, Anthocerotophyta and Bryophyta. Bryophytes contains *ca*. 21925 species out of which *ca.* 9000 species belongs to liverworts; *ca.* 225 species belongs to hornworts and *ca.* 12700 species belongs to mosses. In India there are 2562 taxa of Bryophytes out of which 887 belongs to liverworts, 39 to hornworts and 1636 taxa represents mosses.

The gametophyte of Bryophytes exhibits different habits, such as:

- **Thalloid Form:** The plant body is dorsoventrally flattened and attached to the substratum with the help rhizoids, for example, thalloid liverworts (*Marchantia*, *Riccia*, and *Cryptothallus*) and hornworts.
- **Leafy Forms:** The plant body consists of stem-like cauline structure with microphylls attached to them, for example, leafy liverworts, *Porella*, *Plagiochila* and *Bazzania* and mosses, *Polytrichum*, *Funaria* (Refer Figure 6.1).

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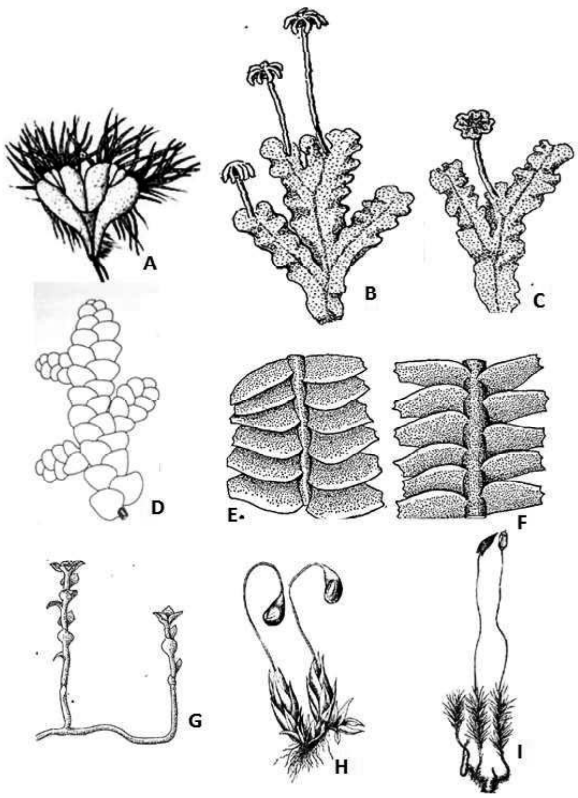

Fig. 6.1 *Thallose Gametophyte of Bryophyte*

The above Figure 6.1 illustrates the Thallose Gametophyte of Bryophyte (A) *Riccia fluitans;* (B) *Marchantia polymorpha* female thallus bearing Archegoniophore; (C) *Marchantia polymorpha* male thallus bearing Antheridiophore. Foliose Gametophyte of Bryophyte; (D) *Porella;* (E) Succubous leaves arrangement in *Plagiochila;* (F) Incubous leaves arrangement *Bazzania;* (G) *Calobryum;* (H) *Funaria* with sporophyte; (I) *Polytrichum* with Sporophyte.

6.2.1 General Characteristics of Bryophytes

Mostly Bryophytes are shade loving land plants (terrestrial) with few aquatic forms (*Riccia fluitans*, *Riella vishwanathai* and *Ricciocarpos natans*). They grow in patches on shaded areas of rocks or ditches or soil and sometimes forms the cushion like mat on spermatophytes bark and leaf. Although there are few xerophytic ones, *viz. Plagiochasma appendiculatum, Riccia discolor* and *Asterella angusta.* They are very small when compared to other plant groups, *viz.,* Pteridophytes, Gymnosperms and Angiosperms. The smallest known bryophyte are *Zoopsis* (*ca.* 5mm) and *Ephemerum* while the largest known are *Fontinalis* and *Dawsonia* (*ca.* 50-70 cm).

The Bryophytes are non-vascular (vascular tissues, i.e., xylem and phloem, are generally absent) and photosynthetic plants but two of the members show saprophytic mode of nutrition *Cryptothallus mirabilis* (Hepaticopsida or Marchantiophyta) and *Buxbaumia aphylla* (Bryopsida or Bryophyta). In lieu of xylem and phloem, hydrome and leptome are present for the conduction of water and nutrition, respectively.

They exhibit alternation of generation, i.e., two distinct life forms are present in their life cycle where gametophyte is the dominant phase while sporophyte is dependent upon the gametophyte for nutrition. The gametophyte is the haploid phase (n) produce gametes and give rise to the sporophyte (2n) through sexual reproduction while sporophyte (2n) produces spore meiotically and give rise to gametophyte (n) through asexual reproduction. The gametophyte give rises to the antheridium (male sex organ) and archegonium (female sex organ). The male and female gametes produced (mitotically) in the antheridium and archegonium fuses and forms the zygote, after fertilization, which ultimately divides and differentiates to produce embryo and sporophyte, i.e., diploid phase (2n) of the life cycle, respectively. Exoscopic embryogeny is present in which, of the two cells resulting from the first division of the egg cell, the outer gives rise to the embryo and the inner to the foot. The sporophyte produces spores after meiotic division and the meiospores give rise to the gametophyte (n). The two phases, i.e., gametophyte and sporophyte have different morphologies, also known as heteromorphic generations.

The dominant phase of life cycle is considered as the plant body, i.e., gametophyte because sporophyte is dependent upon gametophyte. The plant body is dorsoventrally flat and thalloid or differentiated into caulalia (not true stem but functions as stem) and phyllids (not true leaves but function as leaves). Both the forms are attached to the substratum with the help of rhizoids (hair like structure) as true roots are absent.

During fertilization process the male gamete (motile) (spermatozoids) swims to the female gamete (non-motile) (egg) present in the archegonium in presence of water through chemotaxis.

The sporophyte develops after sexual reproduction is dependent upon gametophyte for nutrition and diploid in nature. Sporophytic plant body generally consists of a basal foot, elevating seta connecting foot and capsule and a terminal capsule or sporangium (asexual spore producing part). The spores are produced as a result of meiosis therefore are of haploid nature and dispersed in dry weather only. The Bryophytes are homosporous, i.e., produces same kind of spores except *Macromitrium salakanum* and *Schlotheim grevillea.* The spores germinates to form gametophyte in liverworts and hornworts while there is a distinct phase called protonema exists in mosses. Protonema is a filamentous, complex, thread like structure produced by the germ tube of spore and represents the juvenile gametophyte.

Sexual reproduction in Bryophytes is of oogamous type in which motile antherozoids (male gametes) and non-motile (female gamete) fertilizes in presence of water. The male sex organ is antheridium and female ones are known as archegonium, both are multicellular and surrounded by a layer of sterile jacket of cells. Antheridium consist of androcytes and club or ellipsoidal in shape. Antherozoids (male gametes) are produced in mass from androcytes bear two whiplash flagella and are spirally coiled. Archegonium is flask shaped, present deep into the parenchymatous tissue, sessile, with swollen basal portion known as venter and an elongated neck. The neck consists of four to six neck canal cells and a venter canal cell present in between neck canal cells and egg or oosphere. Antherozoids enters into the neck through the dissolution of cover cells (covering the mouth of archegonium) in presence of water and the passage is given to the antherozoids by dissolution of neck canal cells and venter cell which reaches to the egg and fertilization took place. After fertilization the zygote (2n) (first cell of sporophytic generation) formation took place.

There are three types of sex chromosomes systems in plant kingdom, which are as follows:

- Xx (Female)/ Xy (Male) Chromosome System
- Zz (Male)/ Zy (Female) Chromosome Systems
- U (Female) V (Male) Chromosome System

The first two systems are present in those individuals where diploid phase (sporophyte) is independent of haploid phase (gametophyte) whereas the third one (UV system) is present in Bryophytes where haploid phase (gametophyte) is independent of diploid phase (sporophyte), for example, Bryophytes. *Marchantia polymorpha* and *Physcomitrella patens* are the two model organism in Bryophytes. In 1917, Allen was the first to report sex chromosome in *Sphaerocarpos donnellii* and *S. texanus*. In 1927, Heitz found accessory or microchromosome from *Bazzania trilobata* and called it **m-chromosome** whereas Tatuno (1941) called microchromosomes as **h-chromosome** because of their partial or complete heterochromatic nature.

Some more general characteristics of Bryophytes are as follows:

- Gametophytic and sporophytic phases are present in the life cycle of Bryophytes and both these phases are morphologically distinct (Heteromorphic).
- The gametophytic phase is more conspicuous, independent, long lived, green and freely branched whereas the sporophytic phase is completely dependent on gametophyte.
- In primitive forms for example, Riccia and Marchantia the gametophyte is prostrate and thalloid but in mosses the plant body is erect, differentiated into the stem (axis) and lateral appendages (leaves).
- True roots are absent and the functions of anchorage is performed by filamentous structure, known as Rhizoids.
- The plant body is composed of parenchymatous cells only and lacks fully differentiated xylem and phloem characteristics of true vascular plants.

Bryophytes	Pteridophytes
Gametophyte is the dominant phase in the Life cycle.	Sporophyte is the dominant phase in the life cycle.
Plant body is thalloid or foliose.	Plant body is divided into root, stems and leaves.
Vascular tissue is absent.	Vascular tissue is present.
Sporophyte is completely dependent upon	Sporophyte is autotrophic and the gametophyte. Independent.

Algal Origin of Bryophytes

Although fossil records do not show any relationship between Bryophytes and algae, Bryophytes do resemble with algae in their amphibious nature, presence of flagellated spermatozoids and necessity of water for fertilization**.** In 1903 Lignier proposed that algae gave rise to a prohepatic and Bryophytes originated from prohepatics (an ancient group). In 1908 Bower proposed that archegoniate (groups producing archegonia) from algae. In *Ectocarpus* there are number of cells present in the gametangium and each give rise to gamete. Subsequently due to the migration from water to land necessitates them to develop protective covering around gametangium to protect gametes from desiccation. Due to the difference in the photosynthetic pigments this view is not so much supported. In 1903, Smith proposed the similarity between the gametangia of *Ectocarpus* is quite alike to gametangia of *Schizomeris* and antheridia of *Chaetonema* (Chaetophorales). Church (1919), in his article *'Thallasiophyte and the subaerial transmigration'* also supported algal origin of land plants, but he considered marine algae as the ancestors of land plants instead of freshwater forms as suggested by Bower (1908).

Bryophytes Resemble with Algae in the following Characteristics

- Presence of thalloid and gametophytic plant body.
- Autrophic nature of plants.
- In both the groups chloroplasts contain chlorophyll a chlorophyll b, á- carotene and â-carotene, lutein, violaxanthin and zeaxanthin.
- Starch as reserve food material and absence of vascular tissue.
- Presence of motile and flagellate antherozoids and whiplash type of flagella and water is essential for fertilization in both.

Similarities and Dissimilarities with Algae

Bryophytes are more complex than algae in terms of differentiation of tissues. Bryophytes and algae, chlorophyceae are similar in dominant photosynthetic pigment, chlorophyll**,** requirement of water for fertilization, cell wall composition and reserve food material, starch. However Bryophytes and algae are dissimilar in reproductive organs. Bryophytes produce multicellular and jacketed sex organs while algae, except

Chara in general produces unicellular and non-jacketed sex organs. Embryo formation does not take place in algae.

Similarities and Dissimilarities with Pteridophytes

Pteridophytes are more complex in terms of differentiation of tissues than Bryophytes and considered as more evolved group than Bryophytes. Bryophytes resembles pteridophytes in having heteromorphic alternation of generation, jacketed and multicellular sex organs, i.e., antheridia and archegonia, presence of embryo in both the groups. The dissimilarity is mainly due to presence of vascular tissue and independent sporophyte. Pteridophytes have well developed vascular tissue, i.e., xylem and phloem and sporophyte is independent of gametophyte at maturity and represents the dominant phase in the life cycle of pteridophytes. As discussed earlier the gametophyte represents the dominant and independent phase in Bryophytes. In pteridophytes the sporophyte is well differentiated into root, stem and shoot while in Bryophytes the sporophyte is differentiated into foot, seta and capsule.

Significance of Bryophytes

During World War I, *Sphagnum* was used for dressing wounds. There are many advantages of use of *Sphagnum* over cotton as it is softer, retards bacterial growth and absorbs three times much liquid than cottons. *Marchantia polymorpha* and *M. palmata* were used in Indian Himalayan region for the treatment of abscesses while *M. polymorpha* is used for the treatment of liver ailments. Antimicrobial activity were exhibited by *Riccia fluitans*, *Sphagnum portoricense*, *Conocephalum conicum*, *Dumortiera hirsuta*, *Pallavicinia*, *Reboulia*, *Atrichum undulatum*, *Dicranum scoparium*, *Barbula* and *Timmiella*. *Sphagnum* and *Camptothecium* possess antiviral activity. Antitumor activity were reported in *Porella japonica*, *Diplophyllum albicans*, *D. taxifolium*, *Wiesnerella denundata*, *Conocephalum supradecompositum*, *Frullania monocera*, *F. tamarisci*, *Marchantia polymorpha* and *Polytrichum* juniperinum.

There are several other uses of Bryophytes in day to day and scientific field. Out of which some are listed in this section. Traditionally, mosses were used in construction as insulators in walls highlands and cold countries. In ecology, the Bryophytes are used as indicator species for Copper (*Mielichhoferia elongata, Mielichhoferia mielichhoferi* and *Scopelophila*), Iron (*Brachythecium rivulare*, *Fontinalis* sp., *Jungermannia vulcanicola*, *Sphagnum* and *Polytrichum*), high nitrogen content in Soil (*Ceratodon purpureus*), low nitrogen in soil (*Aulacomnium palustre, Pleurozium schreberi, Pogonatum alpinum and Pogonatum urnigerum*), acidic soil indicator (*Pleurozium schreberi*, *Polytrichum* and *Leucobryum*), prediction of paleoclimatic conditions (*Sphagnum* communities determines the level of precipitation and evaporation while that *Tortella flavovirens* (Xeric species) communities used to determine the dry conditions of past), pioneer community on soils devoid of topsoil (*Barbula*, *Bryum* and *Weissia*), preventing erosion of soil on banks (*Atrichum*, *Pogonatum*, *Pohila*, *Trematodon*, *Blasia* and *Nardia*), nitrogen fixation through Cyanobacteria present in *Anthoceros* thallus, heavy metal monitoring through *Hylocomium splendens*, Sulphur Dioxide indicator (*Grimmia pulvinata,* *Entodon compressus*, *Hypnum yokohamae* and *Glyphomitrium humillium*), Bioaccumulators (*Marchantia polymorpha* and *Calymperes delessertii* accumulates Lead; *Hypnum cupressiforme*, *Pottia truncata*, *Dicranella heteromalla*, *Polytrichum ohioense* and *Bryum argenteum* accumulates Cadmium, Zinc and Copper), monitoring of Ozone Layer over Antarctica (*Bryum argenteum*), waste water treatment (*Sphagnum* - Peat Moss), weed control and moisture retention in horticultural practices (*Sphagnum*). Anti-Herbivory and Anti-Pesticides activities were also reported from Bryophytes, for example, Liverwort *Plagiochila* contains a highly poisonous Sesquiterpene (*Hemiacetal Plagioclase*) for mice, slugs avoids shoots of *Brachythecium rutabulum* and *Mnium hornum* due to the presence of Ferulic and Coumaric Acid in the leafy shoots. Several terpenes and phenolic compounds have also been reported from Bryophytes by Asakawa and Campbell.

Sphagnum (Peat Moss) is an excellent source of clean fuel in countries, such as Canada, Ireland, Finland, Poland, Sweden, etc. Peat Moss can be used to produce other fuels, such as ethylene, methanol, hydrogen and natural gas. Due to low harvesting cost peat have potential to become a future fuel entity. Other mosses that are harvested by humans are *Antitrichia*, *Brachythecium*, *Hypnum*, *Isothecium* and *Thudium*. Mosses (*Pleurozium*, *Isothecium*, *Hylocomium*, *Sphagnum*, *Fontinalis*, *Plagiochila* and *Scapania*) were used as chinking materials in wood cabins, insulators and sound barriers in the roofs and house walls of Scotland, Sweden, Alaska, Himalaya, Sweden and Finland. Since very long mosses (*Sphagnum*, *Hypnum*, *Aerobyopsis*, *Trachypodopsis*, *Neckera*, *Cryoptoleptodon* and *Thuidium*) were used for wrapping commodities.

6.2.2 Origin of Bryophytes

Bryophytes are considered as least evolved group of embryophytes. Embryophytes exhibited peculiar characteristics, such as sporopollenin impregnated spore walls and mieotic production of spores in tetrads.

The evidence of the origin of any plant group comes from the fossil records. One of the oldest Bryophyte-like fossil is *Parafunaria sinensis* or *P. sinensis* reported from Kaili formation in Guizhou Province, China of Early-Middle Cambrian (please note bryophyte-like). *P. sinensis* is considered as one of the ancestral form of Bryophytes. The earliest known fossil of Liverworts is *Riccardiothallus devonicus* reported from Yunan, China of Late Devonian and *Metzgeriothallus sharonae* reported from Givetian Shales and Siltstones from New York of Upper Middle Devonian. The earliest known fossil of Hornworts is *Dendroceros victoriensis* reported from the Koonwarra Fossil Bed in Australia of Lower Cretaceous. The earliest fossil record of mosses is *Muscites plumatus* reported from the Drybrook Sandstone of the Forest of Dean, Gloucestershire, England of Lower Carboniferous. The best known fossil of Bryophytes was discovered by Harris (1938) from upper Triassic rocks of Warwickshire and Worcestershire, England is *Naiadita lanceolata*. It is represented by erect axis with spirally arranged a feature similar to Calobryales but based on the presence of rhizoids, spores, archegonia and capsules characters it is believed to be similar to the extant Sphaerocarpales. There is no fossil record of

Liverwort older than Devonian Period, hornwort older than Cretaceous and mosses older than carboniferous period, till date. While the fossil evidence of land plants is dated back to Silurian period, i.e., *Cooksonia hemisphaerica*, a homosporous pteridophyte. Although scientists and researchers believe that the land plant and Bryophytes originated way back than the established fossils stated above due to evidence of presence of dispersed spores and ancient Bryophytes-like fossil from Cambrian-Devonian.

Earlier it was thought that due to the lack of lignified tissue and delicate structure of thallus in the Bryophytes the potential of fossil preservation is low as compared to the tracheophytes. But due the similarity between cell wall composition and presence of chemicals in the bryophyte thallus and vegetative parts of tracheophytes it is evident that there might be some other reason for the low frequency of fossil Bryophytes. The reason behind the low frequency of fossil records of Bryophytes is not the delicate structure of the plant body but lies in the taphonomy of Bryophytes. Taphonomy describes the process of fossilization and how these processes affect the fossils.

Three phyla of Bryophytes are the first to evolve as Embryophytes. The monophyletic origin of each group, i.e., liverworts, hornworts and mosses is now well established. Three groups share some common features that is unique to Bryophytes, such as lack vascular tissue, dominant gametophyte, dependent and short lived sporophyte. All the three groups lacks structural barrier to prevent them from desiccation in dry conditions but they can rehydrate in wet conditions as they possess physiological tolerance. The Bryophytes were placed in between algae and tracheophytes.

Views Regarding the Origin of Bryophytes

There are two views regarding the origin of Bryophytes which are as follows:

The Bryophytes Originated from Progressive Evolution of Algae

Many bryologists supports this view that Bryophytes originated from algae (Green Algae) as they share common features, such as water requirement for fertilization, presence of ciliated antherozoids and photosynthetic pigments. In 1903 Lignier proposed that algae gave rise to a prohepatics and Bryophytes originated from prohepatics (an ancient group). In 1908 Bower proposed that archegoniate (groups producing archegonia) from algae. In *Ectocarpus* there are number of cells present in the gametangium and each give rise to gamete. Subsequently due to the migration from water to land necessitates them to develop protectective covering around gametangium to protect gametes from desiccation. Due to the difference in the photosynthetic pigments this view is not so much supported. Most of bryologist believe that Bryophytes originates from chlorophyceae. In 1903, Smith proposed the similarity between the Gametangia of *Ectocarpus* is quite alike to Gametangia of *Schizomeris* and Antheridia of *Chaetonema* (Chaetophorales). There are protective layer of cells present in other members of Chaetophorales (*Chara* and *Nitella*) around the Gametangia. In 1955, Smith proposed that Bryophytes evolved from

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Chaetophorales (Heterotrichous Green Algae), for example, *Fritschiella*, *Coleochaete* and *Draparnaldiopsis*. But the authentic connecting link in the form of fossil evidence of ancestral hepatics is absent.

The Bryophytes Originated from Retrogressive Evolution of Pteridophytes (*Rhyniophyta*): This thought does not found the general support but several bryologists have supported this view till date. They believe that Bryophytes were originated from primitive pteridophytes through the process of reduction. In Lang, Kidston, Scott, Halle, Haskel, Christensen and Kashyap supported this view. In 1919, Kashyap compared the Sporangia *Rhynia, Horneophyton* and *Sporozoites* (Psilophytales) with the capsules of Anthocerotales, *Andreaea* and *Sphagnum* in the support of pteridophyta lean origin of Bryophytes.

Recently, it has been proposed on the basis of phylogenetic studies that the embryophytes evolved from streptophyte green algae. The land plants, i.e., 'Viridiplantae' represents monophyletic group and was divided into green algae (Chlorophyta and Streptophyta) and Embryophytes (Bryophytes, Pteridophytes, Gymnosperms and Angiosperms). Approximately 725–1200 million year ago Viridiplantae diverged into two lineages of algae, i.e., Chlorophyta and Streptophyta. Streptophyte algae comprised of six different taxa based on morphological characteristics, i.e., Mesostigmatales, Chlorokybales, Klebsormidium, Zygnematales, Charales (Stoneworts) and Coleochaetales. A new division is formed comprised of streptophyte algae and embryophytes known as Streptophyta. Currently, it is believed that the land plants evolved from streptophyte algae. Many cellular characters, such as phragmoplast, plasmodesmata, hexameric cellulose synthase, structure of flagellated cells, oogamous sexual reproduction with zygote retention and physiological characters such as type of photorespiration, phytochrome system originated within streptophyte algae. At present it is believed that terrestrial micro algae are not capable of leaving the substratum and marine macroalgae never invaded the terrestrial habitat. At present Charales are believed to be the sister group of embryophytes.

Amongst the divergence of three groups represents the first divergence lineages of land plants but the divergence sequence of the three groups is remain unresolved. In 2002, based on phylogenetic studies, Samigullin *et al*., speculated that hornworts are the closest relatives of tracheophytes (Vascular plants). Several authors *viz.,* Hedderson *et al*., Nishiyama and Kato, Nickrent *et al*. and Renzaglia *et al*., proposed that Mosses and Liverworts comprised of single clade.

6.2.3 Classification of Bryophytes

The term Bryophyta was first introduced by Braun (1864), however, he included Algae, Fungi, Lichens and Mosses in this group. Later, Algae, Fungi and Lichens were placed in a separate division Thallophyta and Liverworts, Mosses in division Bryophyta. The rank of division Bryophyta to this well-defined group of plants was first given by Schimper (1879).

Eichler (1883) the First to Divide Bryophyta into Two Groups

Group I. Hepaticae

Group II. Musci

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Engler (1892) recognized Hepaticae and Musci as two classes and divided each class into the following three orders

Division. Bryophyta

Class I. Hepaticae Divided into Three Orders

Order 1. Marchantiales

Order 2. Jungermanniales

Order 3. Anthocerotales

Class II. Musci Divided into Three Orders

Order 1. Sphagnales

Order 2. Andreaeales

Order 3. Bryales

Due to isolated characters of *Anthoceros* and related genera, Howe (1899) raised the order Anthocerotales to the rank of a class and divided division Bryophyta into following three classes:

Class I. Hepaticae

Class II. Anthoceros

Class III. Musci

This system of classification was followed by Smith (1938, 1955), Takhtajan (1953), Wardlaw (1955) and Schutser (1958) but preferred to call class Anthocerotes as Anthocerotae. International Code of Botanical Nomenclature (ICBN) suggested in 1956-that the suffix – 'Opsida' should be used for the classes and such usage had already been proposed by Rothmaler (1951) for the classes of Bryophytes.

He changed the class names as:

Class I. Hepaticae as Hepaticopsida

Class II. Anthocerotae as Anthoceropsida

Class III. Musci as Bryopsida

Proskauer (1957) suggested that the class name Anthoceropsida should be changed Anthocerotopsida. Parihar (1965) and Holmes (1986) followed Proskauer's system of classification and divided Bryophyta into three classes:

Class I. Hepaticopsida

Class II. Anthocerotopsida

Class III. Bryopsida

In recent times the former classes has been raised to phylum due to monophyletic origin of each group and represented as:

MARCHANTIOPHYTA (HeNygren *et al*., 2006) formerly Hepaticopsida

BRYOPHYTA (Goffinet and Buck, 2004) formerly Bryopsida

ANTHOCEROTOPHYTA (Duff *et al.,* 2004) formerly Anthocerotopsida

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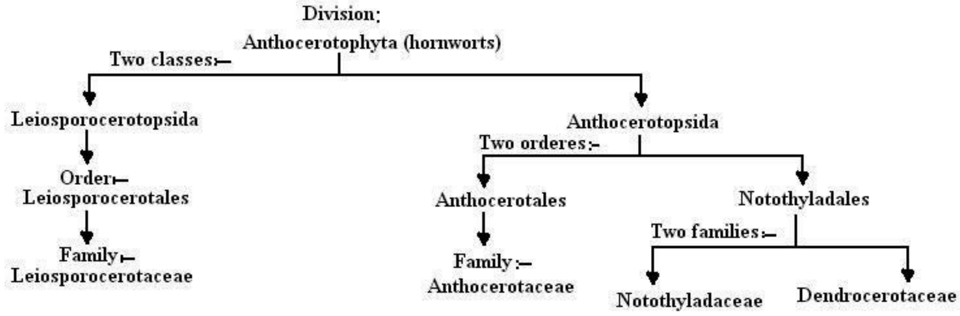

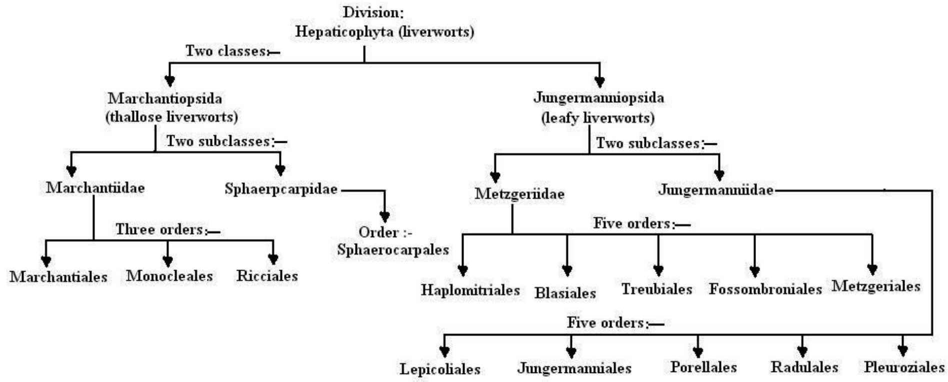

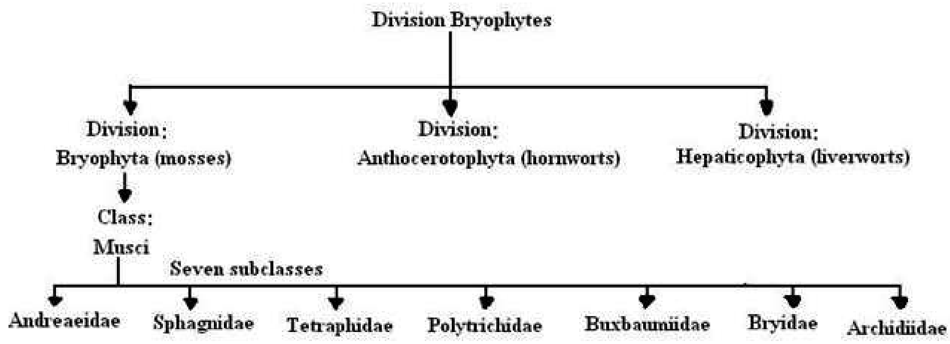

Economic Importance

The economic importance of Bryophytes are as follows:

- During World War I, *Sphagnum* was used for dressing wounds. There are many advantages of use of *Sphagnum* over cotton as it is softer, retards bacterial growth and absorbs three times much liquid than cottons.
- *Marchantia polymorpha* and *Marchantia palmata* were used in Indian Himalayan region for the treatment of abscesses while *Marchantia polymorpha* is used for the treatment of liver ailments.
- Antimicrobial activity were exhibited by *Riccia fluitans*, *Sphagnum portoricense*, *Conocephalum conicum*, *Dumortiera hirsuta*, *Pallavicinia*, *Reboulia*, *Atrichum undulatum*, *Dicranum scoparium*, *Barbula* and *Timmiella*. *Sphagnum* and *Camptothecium* possess antiviral activity.

- Antitumor activity were reported in *Porella japonica*, *Diplophyllum albicans*, *Diplophyllum taxifolium*, *Wiesnerella denundata*, *Conocephalum supradecompositum*, *Frullania monocera*, *Frullania tamarisci*, *Marchantia polymorpha* and *Polytrichum juniperinum*.
- There are several other uses of Bryophytes in day to day and scientific field. Traditionally, mosses were used in construction as insulators in walls highlands and cold countries.
- In ecology, the Bryophytes are used as indicator species for Copper (*Mielichhoferia elongata, Mielichhoferia mielichhoferi* and *Scopelophila*), Iron (*Brachythecium rivulare*, *Fontinalis* sp., *Jungermannia vulcanicola*, *Sphagnum* and *Polytrichum*).
- *Sphagnum* (Peat Moss) is an excellent source of clean fuel in countries such as Canada, Ireland, Finland, Poland, Sweden, etc.
- Peat Moss can be used to produce other fuels, such as ethylene, methanol, hydrogen and natural gas.
- *Antitrichia*, *Brachythecium*, *Hypnum*, *Isothecium* and *Thudium*. Mosses (*Pleurozium*, *Isothecium*, *Hylocomium*, *Sphagnum*, *Fontinalis*, *Plagiochila* and *Scapania*) were used as chinking materials in wood cabins, insulators and sound barriers in the roofs and house walls of Scotland, Sweden, Alaska and Himalaya.
- Anti-herbivory and anti-pesticides activities were also reported from Bryophytes, for example, Liverwort *Plagiochila* contains a highly poisonous Sesquiterpene (*Hemiacetal plagiochila*) for mice, slugs avoids shoots of *Brachythecium rutabulum* and *Mnium hornum* due to the presence of ferulic and coumaric acid in the leafy shoots.

Check Your Progress

1. What is fertilization?
2. Define the term protonema.
3. Give some economic importance of bryophytes.

6.3 MECHANISM OF DEHISCENCE AND SPORE DISPERSAL

Dehiscence is the splitting along a built-in line of weakness in a plant structure in order to release its contents, and is common among fruits, anthers and sporangia. Sometimes this involves the complete detachment of a part. Structures that open in this way are said to be **dehiscent**. Structures that do not open in this way are called **indehiscent**, and rely on other mechanisms such as decay or predation to release the contents.

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A similar process to dehiscence occurs in some flower buds, for example, *Platycodon*, *Fuchsia*, but this is rarely referred to as dehiscence unless circumscissile dehiscence is involved; anthesis is the usual term for the opening of flowers. Dehiscence may or may not involve the loss of a structure through the process of abscission. The lost structures are said to be **caducous**.

Association with Crop Breeding: Manipulation of dehiscence can improve crop yield since a trait that causes seed dispersal is a disadvantage for farmers whose goal is to collect the seed. Many of the agronomically important plants have been bred for reduced shattering.

Mechanisms of Dehiscence

Explosive Dehiscence: Explosive dehiscence is a ballistic form of dispersal that flings seeds or spores far from the parent plant. This rapid plant movement can achieve limited dispersal without the assistance of animals. A notable example is the sandbox tree (*Hura crepitans*), which can fling seeds 100 meters (300 ft) and has been called the 'Dynamite Tree' due to the loud sound of its explosive dehiscence. Another example is *Impatiens*, whose explosive dehiscence is triggered by being touched, leading it to be called the 'Touch-Me-Not'. *Ecballium elaterium*, the 'Squirting Cucumber', uses explosive dehiscence to disperse its seeds, ejecting them from matured fruit in a stream of mucilaginous liquid. Explosive dehiscence of sporangia is a characteristic of *Sphagnum*.

Septicidal and Loculicidal Dehiscence: Septicidal and loculicidal dehiscence may not be completely distinct; in some cases both the septa and the walls of the locules split.

Types of Dehiscence

Dehiscence occurs through breakage of various parts of the enclosing structure; the mechanisms can be classified in various ways, but intermediate forms also occur.

Poricidal Dehiscence: Dehiscence through a small hole (pore) is referred to as poricidal dehiscence. The pore may have a cover (operculate poricidal dehiscense or operculate dehiscence) that is referred to as an operculum or it may not (inoperculate poricidal dehiscense or inoperculate dehiscence).

Poricidal dehiscence occurs in many unrelated organisms, in fruit, causing the release of seeds, and also in the sporangia of many organisms (Flowering Plants, Ferns, Fungi, Slime Molds). Poricidal anthers of various flowers are associated with buzz pollination by insects.

Circumscissile Dehiscence: Circumscissile dehiscence involves a horizontal opening that causes a lid to separate completely. This type of dehiscence occurs in some fruit and anthers and also in some flower buds.

Anther Dehiscence: Anther dehiscence is the final function of the anther that causes the release of pollen grains. This process is coordinated precisely with pollen differentiation, floral development, and flower opening.

The anther wall breaks at a specific site. Usually this site is observed as an indentation between the locules of each theca and runs the length of the anther, but in species with poricidal anther dehiscence it is instead a small pore. If the pollen is released from the anther through a split on the outer side (relative to the center of the flower), this is extrorse dehiscence, and if the pollen is released from the inner side, this is introrse dehiscence. If the pollen is released through a split that is positioned to the side, towards other anthers, rather than towards the inside or outside of the flower, this is latrorse dehiscence.

The stomium is the region of the anther where dehiscence occurs. The degeneration of the stomium and septum cells is part of a developmentally timed cell-death program. Expansion of the endothecial layer and subsequent drying are also required for dehiscence. The endothecium tissue is responsible for the tensions that lead to splitting of the anther. This tissue is usually one to several layers thick, with cells walls of uneven thickness due to uneven lignification. The cells lose water, and the uneven thickness causes the thinner walls of the cells to stretch to a greater extent. This creates a tension that eventually leads to the anther being split along its line of weakness and releasing pollen grains to the atmosphere.

Flower Buds: Flower buds of Eucalyptus and related genera open with circumscissile dehiscence. A small cap separates from the remainder of the bud along a circular horizontal zone.

Fruit Dehiscence: There are many different types of fruit dehiscence that involve different types of structures. Some fruits are indehiscent, and do not open to disperse the seeds. *Xero chassis* dehiscence that occurs upon drying, and hygrochasy is dehiscence that occurs upon wetting, the fruit being hygroscopic. Dehiscent fruits that are derived from one carpel are follicles or legumes, and those derived from multiple carpels are capsules or siliques.

One example of a dehiscent fruit is the silique. This fruit develops from a gynoecium composed of two fused carpels, which, upon fertilization, grow to become a silique that contains the developing seeds. After seed maturation, dehiscence takes place, and valves detach from the central septum freeing the seeds. This is also known as shattering and can be important as a seed dispersal mechanism. This process is similar to anther dehiscence and the region that breaks (dehiscence zone) runs the entire length of the fruit between the valves (the outer walls of the ovary) and the replum (the persisting septa of the ovary). At maturity, the dehiscence zone is effectively a non-lignified layer between two regions of lignified cells in the valve and the replum. Shattering occurs due to the combination of cell wall loosening in the dehiscence zone and the tensions established by the differential mechanical properties of the drying cells.

Spores Dispersal

Spores are developed by the reduction division (meiosis) in the capsule of bryophyte and gave rise to the gametophytic plant body with an intermediate stage, i.e., protonema (a filamentous stage mostly present in mosses). Sporogenous cells, present in the capsule, divides produce spore mother cells or sporocytes. Spore mother cells after meiotic division gave rise to spores. In some Bryophytes where spores and nurse cells are present some of the spore mother cells become sterile and give rise to nutritive or nurse cells, for example, *Riccia* and *Sphaerocarpos*. Whereas in some Liverworts and Hornworts spores and elaters or pseudoelaters are present. In those taxa, sporogenous cells gave to two types mother cells firstly, spore mother cells and secondly, elater mother cells. Elater mother cells divides further to give rise elaters in Liverworts and pseudoelaters in Hornworts. Each cell of spore mother cell undergoes reduction division and give rise to four spores (tetrad) packed in tetrahedral arrangement.

Capsule Structures in Bryophytes: Most of the Bryophytes rely on wind for the dispersal of spores but dispersal of spores by water, insects and animals were also reported in Bryophytes. Since the spore is contained in the closed capsule so it became necessary to liberate spores from the enclosed capsule for their dispersal. Weather conditions, particularly atmospheric humidity, plays a crucial role in dehiscence of capsules. Based on dehiscence the capsules can be divided into following three types:

Disintegrating Capsules: In these types of capsules the capsule wall disintegrates and thus liberating the spores, for example, Liverworts - *Riccia*, *Fossombronia* and *Targionia*.

In *Riccia*, sporophyte consists of a capsule only and devoid of foot and seta. The capsule is immersed deep into the parenchymatous tissue of gametophyte. The gametophytic plant body continues to grow at the tips. The death and decay of older thallus liberates the capsule from parenchymatous tissue and further disintegration of capsule walls results into the dispersal of spores.

In *Fossombronia*, the sporophyte consists of a foot, a long seta and a capsule. At maturity, capsule walls disintegrates and fall off as small plate lets resulting into the exposed spore mass ready for dispersal.

In *Targionia*, the sporophyte consists of a foot, a long seta and a capsule. The sporophyte begins to develop on dorsal surface but rolled downwards during the process the development. The capsule wall disintegrates at maturity of capsules and further in dry conditions the gametophytic plant body is rolled inwards lifting the sporophyte above from the ground sufficiently so that the dispersal of spores takes place through wind.

Mosses- *Acaulon*, *Archidium*, *Ephemerum*, *Pleurophascum* and *Pleuridium*.

The spores of mosses dispersed through water after the disintegration of capsules or some of capsules (*Pleurophascum ovalifolium*) contains air which provides them buoyancy to float in water and the spores liberated after the disintegration of capsule wall.

Splitting Capsules: In splitting capsules the splitting takes place in longitudinal plane, i.e., from apex of capsule towards the seta. The longitudinal plane represents the dehiscence line of thin layer of cells through which spores are liberated. Splitting of capsules takes place either completely where the capsule opens along the dehiscence line up to the apex, for example, liverworts or midway where the capsule opens along the dehiscence line up to just below the apex thus providing a slit like opening for the liberation of spores, for example, hornworts.

Liverworts: *Pellia*, *Marchantia* and *Cephalozia*.

In *Pellia* and *Marchantia*, sporophyte consists of a foot, a seta and a capsule. The capsule dehisces completely under drying condition through four dehiscence line exposing the entire mass of spores and elaters. Elaters present in the capsule absorbs moisture and the hygroscopic movement caused by the elaters causes the spores to disperse in air. The driving force for the hygroscopic movement of elaters are:

- Cohesion Force (between water molecules).
- Adhesion Force (between water molecules and elaters walls).

This kind of mechanism is known as hygroscopic mechanism. In *Cephalozia*, dehiscence is caused by water rupture mechanism in which elaters twist after absorbing the moisture resulting into the dehiscence of capsule. Spring-ring mechanism is present in *Frullania* in which jacket walls curves outwards rapidly resulting into the splitting of capsule into four valves.

Hornworts: *Anthoceros* and *Notothylas*.

In hornworts the splitting of capsule wall is not complete but incomplete reaches up to just below the apex contrasting to slitting mechanism of liverworts where the capsules splits completely into valves. The pseudoelaters present in the hornworts helps in the dispersal of spores.

Mosses- Graphite Mosses (Peristome teeth are absent), for example, *Andreaea*.

In *Andreaea*, the sporophyte is consists of a foot and a capsule. Seta is absent but in lieu of seta pseudopodium is present. The *Andreaea* has a unique paper-lantern-like dehiscence mechanism. Capsule wall splits incompletely through four dehiscence line and the valve cells are of hygroscopic nature which allows to open the slits in dry conditions and in wet condition the slit is closed.

Capsules With an Opening: These kind of dehiscence mechanism found only in mosses. A mouth like opening is present in the capsule of mosses covered by peristome teeth. In general, at maturity calyptra (remnant of archegonium) falls and operculum (cap present at the mouth of capsule) falls off due to the loss of water in annulus (cells present below the operculum). The exposed peristome teeth (doors for dispersal of spores) are triangular and generally present in two layers. One layer is of hygroscopic nature and other exhibits little affinity towards water. With the shrinking of one layer due to water loss results into the opening of mouth.

Mosses: *Funaria*, *Polytrichum*, *Tortula* and *Sphagnum*.

In *Funaria*, the tapered end of peristome teeth is attached to the central disc. In dry conditions, shrinking of teeth creates gaps to develop for dispersal of spores through wind. In wet conditions, teeth swells up resulting into the closing of gap.

In *Polytrichum*, the peristome teeth are very small and non-hygroscopic in nature and attached to large epiphragm (central disc) from tapered end. Over here

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epiphragm is of hygroscopic nature and in dry conditions epiphragm shrinks creating the gaps between peristome teeth for the dispersal of spores whereas in wet conditions the epiphragm dilates and the gaps were closed known as censor mechanism.

In *Tortula*, long and hair-like peristome teeths are present which shrinks in dry condition and allows spores to go through whereas in wet conditions peristome teeth elongates and wrapped themselves through each other.

In *Sphagnum*, peristome teeths are absent. In dry conditions capsules dries up and capsule wall shrinks compressing the air inside resulting in the blast off of operculum and spores with a popping sound known as air gun mechanism.

Spore Dispersal in Bryophytes

The sporophyte generation is short-lived. It comprises a capsule which produces spores by meiosis and a stalk which holds this aloft the gametophyte. The spores once released are dispersed by air currents and, once they settle somewhere moist, germinate. This recommences the gametophyte generation. The spore first produces a filamentous stage called a protonema. These cells are full of chloroplasts. Figure 6.2 shows the late (left) and earliest stages (right) of a moss protonema.

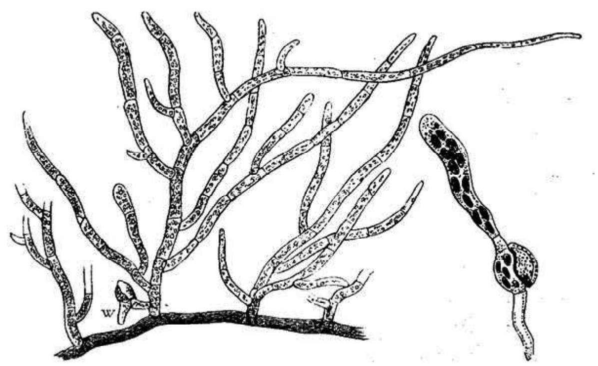

Fig. 6.2 *Late (Left) and Earliest Stages (Right) of a Moss Protonema*

This short-lived phase develops into the familiar gametophyte of the moss or liverwort.

Capsule Structure: The liverwort and moss capsules are very different. In essence, the liverwort capsules is simpler, containing spores and a second cell type called elaters. The moss capsule has several tissues (Refer Figures 6.3 and 6.4).

Fig. 6.3 *L.S. of Liverwort Capsule and Stalk*

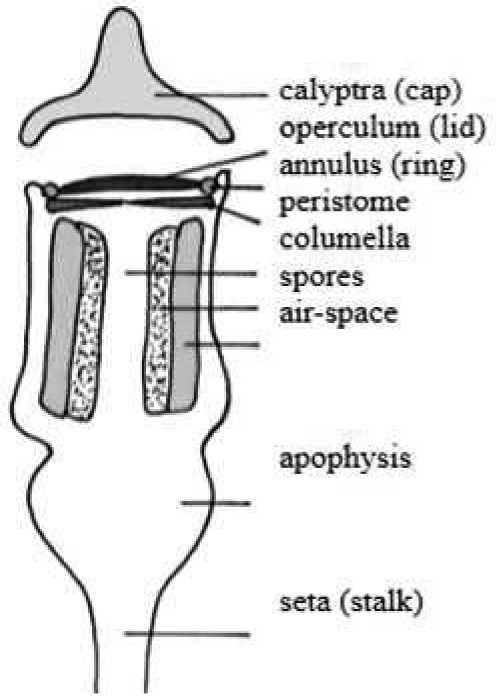

Fig. 6.4 *L.S. of Moss Capsule*

Liverwort Spore Dispersal Mechanisms

Under dry conditions, the liverwort capsule splits into valves or segments, exposing the spores and elaters. Elaters undergo hygroscopic movements causing spores to be flicked into the air, for example, *Pellia* (Refer Figure 6.5).

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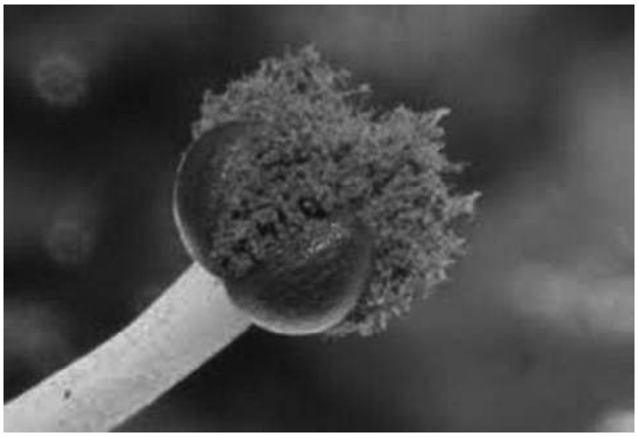

Fig. 6.5 *Exposed Spore/Elater Mass at Centre of Open Valves*

Cephalozia is an extreme example. The elaters are coated in spores and are attached at one end to the capsule wall.

- The capsule splits open.
- Violent hygroscopic movement of elaters detaches them from the capsule wall, flicking them and spores into the air.

Riccia is at the opposite extreme. It is an aquatic, thallose liverwort whose capsule remains embedded in the gametophyte thallus, it has no seta. The spores are few in number and large and are possibly animal-dispersed.

Moss Spore Dispersal Mechanisms

Capsules with an opening kind of dehiscence mechanism is found only in mosses. A mouth like opening is present in the capsule of mosses covered by peristome teeth. In general, at maturity calyptra (remnant of Archegonium) falls and operculum (cap present at the mouth of capsule) falls off due to the loss of water in annulus (cells present below the operculum). The exposed peristome teeth (doors for dispersal of spores) are triangular and generally present in two layers. One layer is of hygroscopic nature and other exhibits little affinity towards water. With the shrinking of one layer due to water loss results into the opening of mouth.

Mosses: *Funaria*, *Polytrichum*, *Tortula* and *Sphagnum*.

With the exception of one group of mosses (Graphite Mosses), all moss capsules have an operculum and peristome.

The general dispersal mechanism under dry conditions is as follows:

- The calyptra (remnant of the archegonium) drops off.
- The operculum is shed as a result of water loss by the annulus.
- The peristome teeth bend outwards.
- Spores fall out of the capsule and are carried by air currents.

The peristome teeth are triangular two-ply structures which operate like trap-doors. One layer tends to readily absorb or lose moisture while the other has little affinity for water. What this means is that as water is lost one side of the peristome teeth shrinks while the other does not. This results in a bending of the teeth outward.

Funaria

Tips of the curved peristome teeth fuse in a central disc (Refer Figure 6.6).

Wet teeth elongate and slits between teeth disappear.

Dry teeth shrink and gaps develop between teeth, allowing spores to sift out.

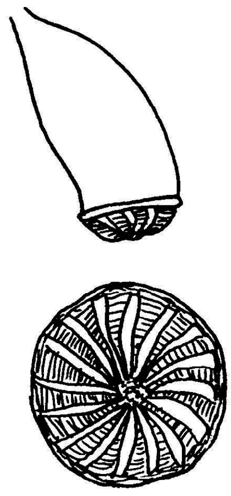

Fig. 6.6 *Funaria*

Polytrichum

The central disc or epiphragm is here very large and the peristome teeth are tiny. This peristome does not respond to moisture (Refer Figure 6.7).

Wet Epiphragm loose, teeth not stretched, gaps between teeth disappear.

Dry Epiphragm taut, teeth stretched, gaps develop between teeth, allowing spores to sift out.

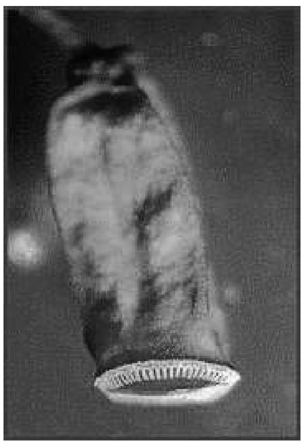

Fig. 6.*7 Polytrichum*

Tortula

Peristome teeth long and hair-like (Refer Figure 6.8).

Wet Teeth elongate and are tightly wrapped around each other.

Dry Teeth shrink, disentangling, allowing spores to sift out.

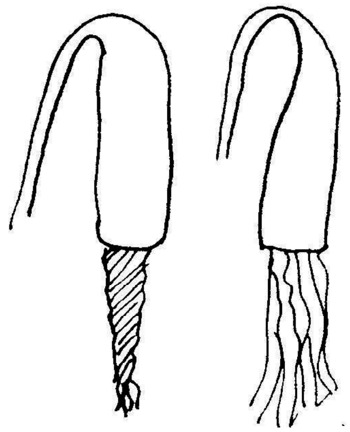

Fig. 6.8 *Tortula*

Sphagnum: Grows in temperate swamps, 'bogs'. Dead plants provide peat moss. Capsule contains air and spores. No peristome. As the capsule dries, it shrinks, compressing the air inside. The operculum is shot off and spores with it. This actually makes a popping noise (Refer Figure 6.9).

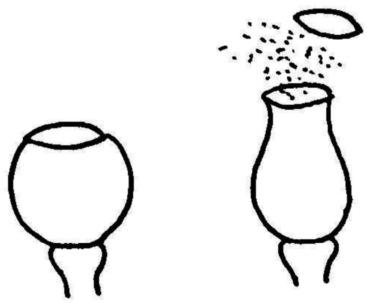

Fig. 6.9 *Sphagnum*

Splachnum: This grows on dung and the capsule with its broad, skirt-like apophysis resembles a flower. The peristome forms a fringe at the top of the capsule. The columella sticks out of the top, covered in sticky spores. Flies are attracted to the capsule by its smell (Refer Figure 6.10).

Fig. 6.10 *Splachnum*

Check Your Progress

4. Define the term dehiscence.
5. What is explosive dehiscence?
6. What is circumscissile dehiscence?
7. What is anther dehiscence?

6.4 VEGETATIVE REPRODUCTION IN BRYOPHYTES

Bryophytes possess a characteristic feature and that is their tendency towards extensive vegetative reproduction. The vegetative reproduction takes place in favourable season for vegetative growth. Majority of the Bryophytes propagate vegetatively and it is brought about in many ways.

Some important methods of vegetative reproduction are as follows:

- Death and Decay of the Older Portion of the Thallus or Fragmentation.
- Persistent Apices.
- Tubers.
- Gemmae.
- Adventitious Branches.
- Regeneration.
- Innovation.
- Primary Protonema.
- Secondary Protonema.
- Bulbils.
- Apospory.
- Cladia.
- Separation of Whole Shoots.
- Separation of Shoot Tips.
- Rhizoidal Tips.

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Death and Decay of the Older Portion of Thallus or by Fragmentation: In Bryophytes the growing point is situated at the tip of the thallus. The basal, posterior or older portion of the thallus starts rotting or disintegrating due to ageing or drought. When this process of disintegration or decay reaches up to the place of dichotomy, the lobes of the thallus get separated. These detached lobes or fragments develop into independent plants by apical growth. This is the most common method of vegetative reproduction in *Riccia, Marchantia, Anthoceros* and some mosses like *Sphagnum*. Figure 6.11 shows the different types of vegetative reproduction in bryophytes.

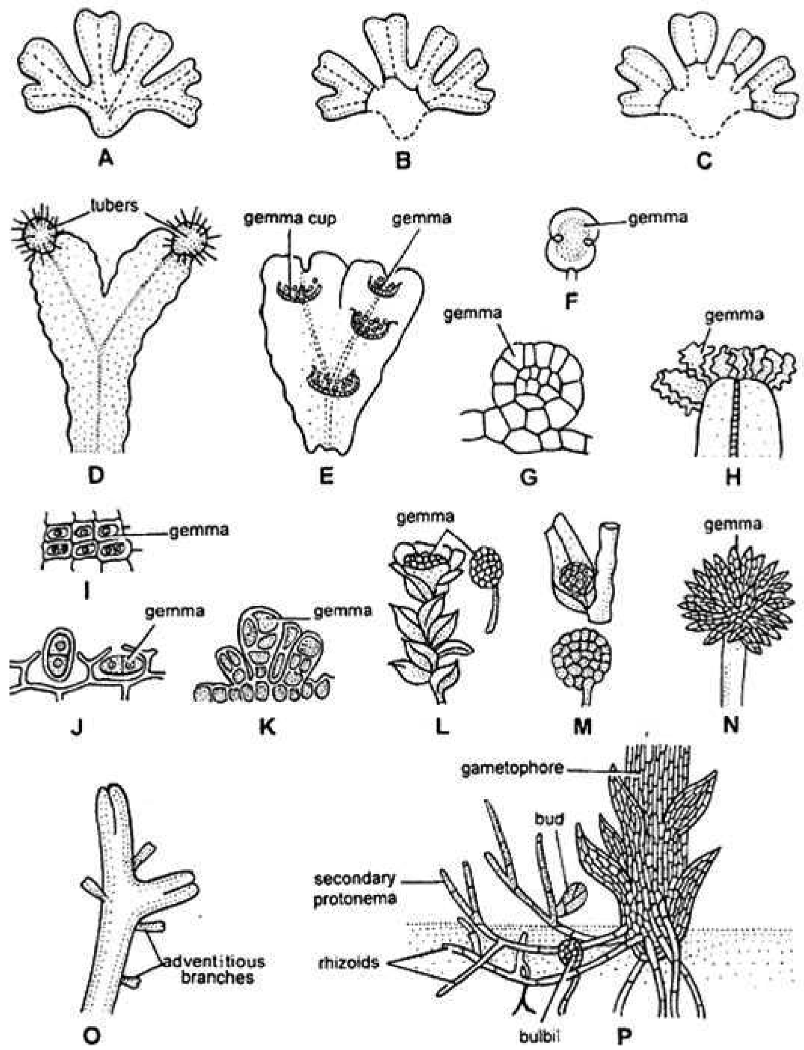

***Fig. 6.11** Different Types of Vegetative Reproduction in Bryophytes*

Persistent Apices: Due to prolonged dry or summer or towards the end of growing season the whole thallus in some Bryophytes , for example, *Riccia, Anthoceros*, *Cyathodium* dries and get destroyed except the growing point. Later, it grows deep into the soil and becomes thick. Under favourable conditions it develops into a new thallus.

Tubers: Tubers are formed in those species which are exposed to desiccation (drying effect of the air). Towards the end of the growing season, the subterranean branches get swollen at their tips to from the underground tubers. On the periphery of a tuber are two to three layers of water proof corky, hyaline cells develop.

These layers surround the inner cells which contain starch, oil globules and albuminous layers. During the unfavourable conditions the thallus dies out but the dormant tubers remain unaffected. On the return of the favourable conditions each tuber germinates to form a new plant for example, *Riccia, Anthoceros, Conocephalum, Conicum, Fossombronia,* etc. Thus, tubers also serve as organ of perennation.

Gemmae: Gemmae are green, multicellular reproductive bodies of various shapes. These are produced in gemma cups, on the surface of the leaves, on stem apex or even inside the cells. They get detached from the parent plant and after falling on a suitable substratum gemmae give rise to a new individual directly for example, Marchantia or indirectly, for example Mosses.

Some Common Forms of Gemmae Produced in Different Bryophytes are:

Class I. Hepaticopsida

Multicellular, Discoid, Gemmae

- Produced in gemma cup on dorsal surface, for example, *Marchantia, Lunularia*.
- Produced on leaves, for example, *Radula*.
- Produced on erect seminiferous branches, for example *Metzgeria nigeria*.

One to Four Celled Gemmae

- One to three celled gemmae on stem apex, for example *Lophozia heterocarpa.*
- One to three celled gemmae on leaves, for example *Marsupella emarginata, Lophozia barbata.*
- Two celled gemmae produced within any external cell of the thallus, for example *Riccardia multifida*.
- Three to four celled gemmae produced in the axils of the leaves, for example *Treubia.*

Sub Spherical Gemmae: Produced in abundance in flask shaped gemma receptacle, for example, *Blasia*.

Star Shaped Gemmae: Produced on the dorsal surface of the thallus, for example, *Blasia*.

Class II. Anthocerotopsida: Multicellular gemmae produced along the margins of the dorsal surface of the thallus, for example *Anthoceros*.

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Class III. Bryopsida

- **Articulated Gemmae:** Produced on the leaves, for example *Ulota phyllantha, Orthotrichum lyelli*, etc.
- **Multicellular Gemmae**
  - o Stalked, green, lenticular gemmae produced at the tip of shoot, for example *Tetraphis pellucida.*
  - o Globular, produced at the base of the stem, for example, *Bryum rubens, B. erythrocarpum.*
  - o Fusiform, produced at the ends of distinct leafless terminal stalk, for example, *Aulacomnium androgynum*.
  - o Produced on the rhizoids of leafy shoots, for example, *Tortula stanfordensis*, *Ditrichum* cylindrical. *Bryum erythrocarpum*, etc.

Adventitious Branches: The adventitious branches develop from the ventral surface of the thallus for example, *Riccia fluitans, Anthoceros*. On being detached from the parent plant these branches develop into new thalli. In *Marchantia, Dumortiera* these branches develop from archegoniophore while in *Pellia* these branches arise from the dorsal surface or margins of the thallus.

Regeneration: The liverworts possess an amazing power of regeneration. Part of the plant or any living cell of the thallus (for example, *rhizoid, scales*).are capable of regenerating the entire plant f for example, *Riccia, Marchantia*, etc.

Innovation: In *Sphagnum* one of the branches in the apical cluster instead of forming drooping branches or divergent branches, develop more vigorously than the others and continues the growth upwards.

This long upright branch has all the characteristics of main axis. It is called innovation. Due to progressive death and decay of the parent plant these innovation become separated from the parent plant and establish themselves as parent plants.

Primary Protonema: Primary protonema is the filament like stage produced by the developing spores of the mosses. It produces the leafy gametophores. It breaks into short filament of cells by the death of cells at intervals. Each detached fragment grows into a new protonema which bears a crown of leafy gametophores for example, *Funaria*.

Secondary Protonema: The protonema formed by other methods than from the germination of spores is called secondary protonema. It may develop from any living cells of the leafy gainetophore, i.e., from leaf, stem, rhizome, injured portion of the leafy gametophore, antheridium, paraphysis or archegonium. From this arise the leafy gametophores or lateral buds in the same manner as in primary protonema, for example, *Funaria, Sphagnum*.

Bulbils: These are small resting buds develop on rhizoids. Bulbils are devoid of chlorophyll but full of starch. On germination bulbils produce a protonema which bears leafy gametophores.

Apospory: The production of diploid gametophyte from the unspecialized sporophyte without meiosis is known as apospory, for example, Anthoceros. In Funaria green protonemal filaments may arise from the unspecialized cells of the various parts of sporogonium. These protonemal filaments bear lateral buds which develop into leafy gametophores.

Claudia: These are the small or broad detachable branches which help in vegetative reproduction. These are of two types:

- **Leaf Cladia:** Arising from the individual cell of the leaf, for example, *Plagiochila*, *Bazzania*, *Frullania fragilifolia*, etc.
- **Stem Cladia**: These cladia arise from the stem and occupy the same position as sexual branches, for example, *Bryopteris*.

Separation of Whole Shoots: A number of catkins like deciduous branches develop over the entire surface of the gametophytic plant. On separation these branches develop into new plant, for example, *Pohlia nutans*.

Separation of Shoot Tips: It occurs in *Campylopus piriformis*. The separated shoot tips develop into new plant.

Rhizoidal Tips: The apical part of the young rhizoids divide and re-divide to form a gemma like mass of cells, for example, *Riccia glauca*. These cells contain chloroplast and are capable to develop into new thallus.

Methods of Perennation

Perennation is the survival from season to season, generally with a period of reduced activity between each season.

The chief methods of perennation are:

- By Persistent Apices
- By Tubers
- By Bulbils

The spore and gemmae can also perennate and in some, instances even the protonema does. The perennial mosses perennate as gametophytes.

Sexual Reproduction in Bryophytes

- Sexual reproduction is highly oogamous.
- Male and female sex organs are known as Antheridia (Singular, Antheridium) and archegonia (Singular, Archegonium), respectively.
- Sex organs are jacketed and multilayered.
- Antheridium is stalked, pear shaped or oblong and has an outer one cell thick jacket which encloses a mass of fertile cells called androcytes. Each androcyte metamorphoses into biflagellate antherozoid.
- Archegonium is stalked, flask shaped structure. It has a basal swollen portion called venter and an elongated neck. The neck is filled with many neck canal cells whereas venter has a large egg cell and a small venter canal cell,

- Antherozoids are attracted towards the neck of the archegonium chemotactically by certain substances (like sugars, malic acid, proteins, inorganic salts of potassium, etc.) present in the mucilaginous substance formed by the degeneration of neck canal cells and venter canal cell.
- Water is essential for fertilization.
- The fertilized egg or zygote is the beginning of the sporophytic phase. It is retained within the venter of the archegonium.

Sporophyte

- Without resting period, the zygote undergoes repeated divisions to form a multicellular structure called the embryo.
- The first division of the zygote is always transverse and the outer cell develops into embryo. Such an embryogeny is called exoscopic.
- Embryo develops into a sporophyte or sporogonium.
- The sporophyte is usually differentiated into foot, seta and capsule. In certain cases it is represented only by capsule (for example, *Riccia*) or by foot and capsule (for example, *Corsinia*).
- Sporophyte is attached to parent gametophytic plant body throughout its life. It partially or completely depends on it for nutrition.
- Foot is basal, bulbous structure. It is embedded in the tissue of parent gametophyte. Its main function is to absorb the food material from the parent gametophyte.
- Seta is present between the foot and capsule. It elongates and pushes the capsule through protective layers. It also conducts the food to the capsule absorbed by foot.
- Capsule is the terminal part of the sporogonium and its function is to produce spores
- All Bryophytes are homosporous, i.e., all spores are similar in shape, size and structure
- Capsule produces sporogenous tissue which develops entirely into spore mother cells it for example, *Riccia* or differentiated into spore mother cells and elater mother cells, for example, *March, Anthoceros*.
- Spore mother cells divide diagonally to produce asexually four haploid spores which are arranged in tetrahedral tetrads.
- Elater mother cells develop into elaters, for example, *Marchantia* or pseudo elaters, for example, *Anthoceros* which are hygroscopic in nature. Elaters are present in liverworts and absent in mosses.
- Venter wall enlarges with the developing sporogonium and forms a protective multicellular layer called calyptra, gametophytic tissue enclosing the sporophyte.

Young Gametophyte

- The meiospore, spore formed after meiosis is the first cell of the gametophytic phase.
- Each spore is unicellular, haploid and germinates into young gametophytic plant, for example, Riccia or Marchantia or first germinates into a filamentous protonema on which buds are produced to give rise to a young gametophytic plant, for example, Funaria.

Asexual Reproduction

Bryophytes (Liverworts, Hornworts and Mosses) exhibits largest diversity of asexual reproductive structures. The key reason behind this kind of diversity of asexual structures is the fact that most of the gametophytes are dioecious in nature and reproduce asexually. The asexual reproductive structures are key to identify some of taxa. Asexual reproduction is comprised of asexual reproduction through specialized structures, fragmentation of parts and plant parts and clonal reproduction.

Asexual reproduction through specialized structures can be further divided into three types:

- Regeneration from Specialized Caducous Organs.
- Protonemal Gemmae and Tubers.
- Asexual Spores.

Regeneration from Specialized Caducous Organs

It is present in mosses but rarely or absent in Liverworts or Hornworts due to presence of thalloid plant body. Different parts, such as leaves, leaf apices, shoots, branches and bulbils behaves as asexual reproductive structures.

By Regeneration of Specialized Organs

Caducous Leaves: In some Bryophytes two types of leaves are present first normal sized vegetative leaves and second specialized diminutive leaves also known as brood leaves. The difference between brood leaves and simple vegetative leaves are presence of an abscission layer for the detachment of from parent shoots, differ in size and shape, development of costa and sometimes they have rhizoids or young plants on borders. Few mosses taxa in which brood leaves are present are *Dicranum montanum*, *Dicranodontium longiristre*, *Campulopus fragilis*, *Syntrichia laevilpila* and *Aulacomnium androgynum*. In few leafy liverworts, *viz., Bazzania nudicaulis*, *Ceratolejeunea caducifolia*, *Cholejeunea decidua*, *Drepano lejeunea propagulifera* and *Plagiochila corniculata* new gametophyte originates either from secondary protonema arises from leaf cells or directly from the costa.

Caducous Leaf Apices: In this type of asexual reproduction leaf tip regularly breaks off due to the presence of thin cell walls in the upper part of leaves. Few examples of mosses are *Tortella fragilis*, *Dicranum viride* and *Campylopus fragilis* whereas few examples of leafy liverworts are *Acrobeles ciliatus, Plagiochila caduciloba* and *Frullania microphylla*.

Caducous Shoot Apices: These are modified shoot tips with presence of abscission line which detaches from the mother plant and grow into whole plant. The detached shoot tips give rise to several shoots and under favourable conditions with the development of rhizoids from their basal parts development into whole plant. Few examples of mosses are *Campylopus flexuosus* and *Bryum argenteum*.

Caducous Branches and Branchlets: These are caducous small branches present in the leaf axils with the presence of abscission layer and reduced leaves. Few example of mosses are *Leucodon sciuroides*, *Platygyrium repens* and *Pseudoleskeella nervosa*. Few example of leafy liverworts are *Herbertus aduncus* subsp. *Hutchinsiae* and *Lejeunea laetevirens*. Few example of thalloid liverworts are *Pellia endiviifolia*, thallus outgrowths and *Conocephalum conicum,* winter buds.

Cladia: These are modified brood branchlets that develop holdfast, derived from rhizoids and bear reduced under leaves. Few example of leafy liverworts are *Drepano lejeunea*, *Odonto lejeunea* and *Lepto lejeunea*.

Caducous Flagelliform Shoots: These are thin branches located in leaf axils with presence of vestigial leaves. They appear like flagella with mastigonemes and thus name flagelliform shoot, for example, *Pseudotaxiphyllum elegans*.

Caducous Bulbils and Perianth: Bud-like structure axillary in position, highly condensed, i.e., small in size with one to many leaves and highly reduced leaf primordia, for example, *Bryum*, *Pohlia* and *Leptobryum pyriforme*. Caducous perianths, for example, *Chonocolea ruwenzorensis* (Liverwort) and *Gymnocolea inflata* (Liverwort).

Specialized Propagules

Gemma (*Plural* Gemmae): These are globose to filamentous structure having one to many-celled asexual propagules. Mostly found in liverworts and mosses. Gamma lacks an apical cell as compared to caducous shoot or buds where apical cells are present. They are borne on dorsal surface of thalli, on stems, etc. Based on the structure on which gemma is borne they can be classified into following groups:

- **Laminal Gemma:** They are one to many celled, spherical, discoidal, filamentous, lamelliform or clavate in in shape and arises from the border of apices of leaf (microphylls) laminal cells. Few taxa in which laminal gemma is found are *Syntrichia latifolia*, *Orthotrichum tenellum* and *Grimmia trichophylla*. In *Oedopodiella australis* gemma arises from the costa cells of leaf. In foliose liverworts they are found in *Lophozia ventricosa* and *Frullania parvistipula*.
- **Costal Gemma:** They are present either on dorsal or ventral surface of leaves, shape (filamentous, clavate or spherical) and arises directly from the costa cells either at base, middle or apical part of leaves. Few mosses in which costal gemma is reported are *Ulota phyllantha*, *Syrrhopodon* and *Syntrichia papillosa*.
- **Cauline/Axillary Gemma:** They are present on stem surface or in the axils of leaves and originates from cortical cells of stems. They are filamentous,

clavate or tuberous in shape. Few mosses in which cauline/axillary gemma is reported are *Pterigynandrum filiforme*, *Isopterygiopsis muelleriana*, *Encalypta streptocarpa*, *Didymodon rigidulus*, *Dicranoloma billardieri*, *Plagiothecium nemorale* and *Anomobryum julaceum*.

- **Endogenous Gemma:** They are ovoid or ellipsoidal in shape and originates endogenously from cell initial. Few taxa in which endogenous gemma is reported are *Jungermannia caespitica*, *Riccardia*, *Grimmia torquata*, *Grimmia trichophylla*, *Bazzania kokowana* and *Racomitrium*.
- **Gametangial Gemma:** Gametangial gemmae are present along together with antheridia and are stalked, multicellular and ovoid or discoid in shape, for example, *Oedipodium griffithianum*.
- **Rhizoidal Gemma:** Rhizoidal gemmae are subterranean, many-celled, club or spherical in shape, originates from rhizoids. They produce secondary protonema from which leafy shoots germinates and have better survival chances. Examples include *Bryoerythrophyllum ferruginascens*, *Leptobryum pyriforme*, *Dicranella staphylina* and *Bryum radiculosum*.
- **Gemma of Liverworts:** Gemmae in liverworts are multicellular, discoid, cup or lunate shaped having many propagules, for example, *Marchantia*, *Lunularia*, *Cavivularia densa* and *Metzgeria*.

Protonemal Brood Cells: Protonemal brood cells are not well known structure and proposed to be derived from protonemal gemmae and the absence of abscission layer, for example, *Diphyscium foliosum*.

Tubers: These are subterranean borne on rhizoids and differ from rhizoidal gemma in sense that an apical cell is present in the tubers. They are thick walled, multicellular, dark brown in colour upon maturity and are of spherical, pyriforme or ellipsoidal in shape. The new gametophyte originates in two different ways first, leafy shoots directly develop from the apical cell. Second, a secondary protonema is produced from which leafy shoots originates. Few examples are *Riccia gougetiana*, *Phaeoceros laevis*, *Phaeoceros bulbiculosus*, *Geothallus tuberosus*, *Fossombronia*, *Sewardiella, Petalophyllum*, *Grimmia pulvinata*, *Bryum rubens* and *Bryum capillare*.

Asexual Spores: Asexual spores are genetically identical to the asexual diaspores or propagules but helps in the alternation of generation. Asexual spores are produced by three means first, genetically identical zygotes formation on the same monoecious thallus resulting in the production of sporophyte known as selfing. Second, when two individual thallus of same germination event become separated and the sporophyte and spore production took place in those two different thallus known as asexual cloning selfing. Third, when a capsule arises due to the process of selfing and several spores are produced in the capsule with the same genetically identity known as spore clone selfing.

Fragmentation: Fragmentation is a process of development of unspecialized fragments capable of functioning as new individual through breaking or death and decay of older gametophyte plant body. Nearly all gametophyte cells are capable of re-growth and have capacity to develop into new gametophytes. In leafy forms fragmentation process is more common than the thalloid forms. New individuals are formed through fragmented parts *via* an intermediate secondary protonema. New gametophyte originates through fragmented leaves (*Bazzania pectinata*, *B. spiralis*, *Plagiochila cuneata*, *P. caduciloba*, *Trichostomum brachydontium*, *Syntrichia fragilis*, *Dicranum viride* and *Dicranoloma billardieri*), fragments of shoots (*Philonotis rigida*, *Leucodon sciuroides*, *Dicranum scoparium*, *Andreaea crassinervia*, *Pseudoleskea catenulata*, *Dicranoloma billardieri*, *Lejeunea laetevirens* and *Plagiochasma appendiculatum*).

Clonal Reproduction: Cloning is a process of producing several individual parts capable of living independently and reproduce after separating them from a single genetic individual. It is similar to angiosperm plants in which stem touches the ground and a new plant arises from the nodes. This kind of propagation spreads the single plant (genet) into a large area giving rise to several individuals (remet) capable of growing independently when the intermediate spacers or stolon or connecting stem is cut down. Similar kind of process took place in *Plectranthus saccatus*, *Polytrichum juniperinum*, *Syntrichia fragilis*, *Fissidens taxifolius* and *Thudium laeviuscula*. In these mosses the spacers decays giving rise to many genetically identical individuals. Depending upon taxa the spacers can be a protonema (with several buds), axes of gametophytic plant body, aerial shoots (stoloniferous axes) or subterranean axes (rhizome).

Check Your Progress

8. What is Cladia?
9. Define the term Gemma.
10. What is Clonal propagation?

6.5 ANSWERS TO 'CHECK YOUR PROGRESS'

1. During fertilization process the male gamete (motile) (spermatozoids) swims to the female gamete (non-motile) (egg) present in the archegonium in presence of water through chemotaxis.
2. Protonema is a filamentous, complex, thread like structure produced by the germ tube of spore and represents the juvenile gametophyte.
3. The economic importance of Bryophytes are as follows:
    - During World War I, *Sphagnum* was used for dressing wounds. There are many advantages of use of *Sphagnum* over cotton as it is softer, retards bacterial growth and absorbs three times much liquid than cottons.
    - *Marchantia polymorpha* and *M. palmata* were used in Indian Himalayan region for the treatment of abscesses while *M. polymorpha* is used for the treatment of liver ailments.

- Antimicrobial activity were exhibited by *Riccia fluitans*, *Sphagnum portoricense*, *Conocephalum conicum*, *Dumortiera hirsuta*, *Pallavicinia*, *Reboulia*, *Atrichum undulatum*, *Dicranum scoparium*, *Barbula* and *Timmiella*. *Sphagnum* and *Camptothecium* possess antiviral activity.
- Antitumor activity were reported in *Porella japonica*, *Diplophyllum albicans*, *D. taxifolium*, *Wiesnerella denundata*, *Conocephalum supradecompositum*, *Frullania monocera*, *F. tamarisci*, *Marchantia polymorpha* and *Polytrichum juniperinum*.

4. Dehiscence is the splitting along a built-in line of weakness in a plant structure in order to release its contents, and is common among fruits, anthers and sporangia.
5. Explosive dehiscence is a ballistic form of dispersal that flings seeds or spores far from the parent plant.
6. Circumscissile dehiscence involves a horizontal opening that causes a lid to separate completely. This type of dehiscence occurs in some fruit and anthers and also in some flower buds.
7. Anther dehiscence is the final function of the anther that causes the release of pollen grains. This process is coordinated precisely with pollen differentiation, floral development, and flower opening.
8. Cladia are modified brood branchlets that develop holdfast (derived from rhizoids) and bear reduced under leaves. Few example of leafy liverworts are *Drepanolejeunea*, *Odontolejeunea* and *Leptolejeunea*.
9. Gemma are globose to filamentous structure having one to many-celled asexual propagules. Mostly found in liverworts and mosses. Gamma lacks an apical cell as compared to caducous shoot or buds where apical cells are present. They are borne on dorsal surface of thalli, on stems, etc.
10. Cloning is a process of producing several individual parts capable of living independently and reproduce after separating them from a single genetic individual. It is similar to angiosperm plants in which stem touches the ground and a new plant arises from the nodes. This kind of propagation spreads the single plant (genet) into a large area giving rise to several individuals (remet) capable of growing independently when the intermediate spacers or stolon or connecting stem is cut down. Similar kind of process took place in *Tylimanthus saccatus*, *Polytrichum juniperinum*, *Syntrichia fragilis*, *Fissidens taxifolius*.

6.6 SUMMARY

- Bryophytes are also known as 'Amphibians of Plant Kingdom' or Lilliputians of Plant Kingdom'. They are Monosporangiate, i.e., produces One Capsule Per Sporophyte.

- The term 'Bryophyte' originates from *Greek* and comprise of '*bryon'* means 'Tree-Moss' and '*python'* means 'Plant' and refers to group of plants that swell upon hydration.
- Previously bryophyta was considered as a different phylum with three subclass, *viz.* Hepaticopsida (Liverworts),
- Mostly Bryophytes are shade loving land plants (terrestrial) with few aquatic forms (*Riccia fluitans*, *Riella vishwanathai* and *Ricciocarpos natans*).
- The Bryophytes are non-vascular (vascular tissues, i.e., xylem and phloem, are generally absent) and photosynthetic plants but two of the members show saprophytic mode of nutrition *Cryptothallus mirabilis* (Hepaticopsida or Marchantiophyta) and *Buxbaumia aphylla* (Bryopsida or Bryophyta).
- The gametophyte is the haploid phase (n) produce gametes and give rise to the sporophyte (2n) through sexual reproduction while sporophyte (2n) produces spore meiotically and give rise to gametophyte (n) through asexual reproduction.
- The gametophyte give rises to the Antheridium (male sex organ) and Archegonium (female sex organ).
- The male and female gametes produced (mitotically) in the antheridium and archegonium fuses and forms the zygote, after fertilization, which ultimately divides and differentiates to produce embryo and sporophyte, i.e., diploid phase (2n) of the life cycle, respectively.
- Exoscopic embryogeny is present in which, of the two cells resulting from the first division of the egg cell, the outer gives rise to the embryo and the inner to the foot.
- Sexual reproduction in Bryophytes is of oogamous type in which motile antherozoids (male gametes) and non-motile (female gamete) fertilizes in presence of water.
- The male sex organ is antheridium and female ones are known as archegonium, both are multicellular and surrounded by a layer of sterile jacket of cells.
- Antherozoids (male gametes) are produced in mass from androcytes bear two whiplash flagella and are spirally coiled.
- Archegonium is flask shaped, present deep into the parenchymatous tissue, sessile, with swollen basal portion known as venter and an elongated neck.
- Antherozoids enters into the neck through the dissolution of cover cells (covering the mouth of archegonium) in presence of water and the passage is given to the antherozoids by dissolution of neck canal cells and venter cell which reaches to the egg and fertilization took place.
- Gametophytic and sporophytic phases are present in the life-cycle of Bryophytes and both these phases are morphologically distinct (Heteromorphic).

- The gametophytic phase is more conspicuous, independent, long lived, green and freely branched whereas the sporophytic phase is completely dependent on gametophyte.
- True roots are absent and the functions of anchorage is performed by filamentous structure, known as Rhizoids.
- The plant body is composed of parenchymatous cells only and lacks fully differentiated xylem and phloem characteristics of true vascular plants.
- Bryophytes are more complex than algae in terms of differentiation of tissues. Bryophytes and algae, chlorophyceae are similar in dominant photosynthetic pigment, chlorophyll, requirement of water for fertilization, cell wall composition and reserve food material, starch.
- Bryophytes produce multicellular and jacketed sex organs while algae, except *Chara* in general produces unicellular and non-jacketed sex organs.
- *Sphagnum* (Peat Moss) is an excellent source of clean fuel in countries, such as Canada, Ireland, Finland, Poland, Sweden, etc. Peat Moss can be used to produce other fuels,such as ethylene, methanol, hydrogen and natural gas.
- Bryophytes are considered as least evolved group of embryophytes. Embryophytes exhibited peculiar characteristics, such as sporopollenin impregnated spore walls and mieotic production of spores in tetrads.
- The earliest known fossil of hornworts is *Dendroceros victoriensis* reported from the Koonwarra Fossil Bed in Australia of Lower Cretaceous.
- The earliest fossil record of mosses is *Muscites plumatus* reported from the Drybrook sandstone of the Forest of Dean, Gloucestershire, England of Lower Carboniferous.
- The best known fossil of Bryophytes was discovered by Harris (1938) from upper Triassic rocks of Warwickshire and Worcestershire, England is *Naiadita lanceolata*.
- Earlier it was thought that due to the lack of lignified tissue and delicate structure of thallus in the Bryophytes the potential of fossil preservation is low as compared to the tracheophytes.
- Dehiscence is the splitting along a built-in line of weakness in a plant structure in order to release its contents, and is common among fruits, anthers and sporangia.
- Dehiscence may or may not involve the loss of a structure through the process of abscission. The lost structures are said to be caducous.
- Manipulation of dehiscence can improve crop yield since a trait that causes seed dispersal is a disadvantage for farmers whose goal is to collect the seed.
- Circumscissile dehiscence involves a horizontal opening that causes a lid to separate completely. This type of dehiscence occurs in some fruit and anthers and also in some flower buds.

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- Anther dehiscence is the final function of the anther that causes the release of pollen grains. This process is coordinated precisely with pollen differentiation, floral development, and flower opening.
- Flower buds of Eucalyptus and related genera open with circumscissile dehiscence. A small cap separates from the remainder of the bud along a circular horizontal zone.
- Fruit dehiscence are many different types of fruit dehiscence that involve different types of structures. Some fruits are indehiscent, and do not open to disperse the seeds.
- In *Fossombronia*, the sporophyte consists of a foot, a long seta and a capsule. At maturity, capsule walls disintegrates and fall off as small plate lets resulting into the exposed spore mass ready for dispersal.
- In *Targionia*, the sporophyte consists of a foot, a long seta and a capsule. The sporophyte begins to develop on dorsal surface but rolled downwards during the process the development.
- In *Funaria*, the tapered end of peristome teeth is attached to the central disc. In dry conditions, shrinking of teeth creates gaps to develop for dispersal of spores through wind. In wet conditions, teeth swells up resulting into the closing of gap.
- In *Polytrichum*, the peristome teeth are very small and non-hygroscopic in nature and attached to large epiphragm (central disc) from tapered end. In *Tortula*, long and hair-like peristome teeths are present which shrinks in dry condition and allows spores to go through whereas in wet conditions peristome teeth elongates and wrapped themselves through each other.
- Tubers are formed in those species which are exposed to desiccation (drying effect of the air).
- Gemmae are green, multicellular reproductive bodies of various shapes. These are produced in gemma cups, on the surface of the leaves, on stem apex or even inside the cells.
- The adventitious branches develop from the ventral surface the thallus for example, *Riccia fluitans*, Anthoceros. On being detached from the parent plant these branches develop into new thalli.
- In some Bryophytes two types of leaves are present first normal sized vegetative leaves and second specialized diminutive leaves also known as brood leaves.
- The difference between brood leaves and simple vegetative leaves are presence of an abscission layer for the detachment of from parent shoots, differ in size and shape, development of costa and sometimes they have rhizoids or young plants on borders.
- Rhizoidal gemmae are subterranean, many-celled, club or spherical in shape, originates from rhizoids. They produce secondary protonema from which leafy shoots germinates and have better survival chances.

- Protonemal brood cells are not well known structure and proposed to be derived from protonemal gemmae and the absence of abscission layer, for example, *Diphyscium foliosum*.
- Fragmentation is a process of development of unspecialized fragments capable of functioning as new individual through breaking or death and decay of older gametophyte plant body.

6.7 KEY TERMS

- **Protonema:** Protonema is a filamentous, complex, thread like structure produced by the germ tube of spore and represents the juvenile gametophyte.
- **Dehiscence:** Dehiscence is the splitting along a built-in line of weakness in a plant structure in order to release its contents, and is common among fruits, anthers and sporangia.
- **Explosive dehiscence:** Explosive dehiscence is a ballistic form of dispersal that flings seeds or spores far from the parent plant.
- **Anther dehiscence:** Anther dehiscence is the final function of the anther that causes the release of pollen grains.
- **Cladia:** Cladia are modified brood branchlets that develop holdfast (derived from rhizoids) and bear reduced under leaves.
- **Gemma:** Gemma are globose to filamentous structure having one to many-celled asexual propagules.
- **Elaters:** An elater is a cell or structure attached to a cell that is hygroscopic, and therefore changes its shape in response to changes in moisture in the environment.

6.8 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Write down the general characteristics of bryophytes.
2. Differentiate between the following:
   i. Algae and Bryophytes
   ii. Bryophytes and Pteridophytes
   iii. Elaters and Pseudoelaters
   iv. Elaters and Elatophores
3. Write the affinities of Bryophytes with Algae.
4. Write about the algal origin of Bryophytes.
5. Write down the difference between Bryophytes and Pteridophytes.

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6. Write about the economic importance of Bryophytes.
7. Describe briefly the methods of spore dispersal in Bryophytes.
8. Write short notes on the following:
   i. Elaters
   ii. Dehiscence of Spores
   iii. Peristomial Teeth
   iv. Capsule
   v. Tubers
   vi. Cladia
   vii. Gemma
   viii. Asexual Spores
9. Give a brief account on specialized propagules in Bryophytes.

Long-Answer Questions

1. Give an account on the classification of Bryophytes giving appropriate examples.
2. What are the different theories regarding the origin of Bryophytes? Which theory you consider more rationale and why?
3. What are the Bryophytes? What are their features of advancement over algae?
4. Describe in detail the structure of capsule in Bryophytes.
5. Give a detailed account on structure of spore in Bryophytes.
6. Give an illustrated account on the chief methods of vegetative reproduction in Bryophytes.
7. Discuss the methods of vegetative reproduction and perennation in bryophytes you have studied.
8. Give a detailed account on the following:
   i. Fragmentation
   ii. Clonal Reproduction

6.9 FURTHER READING

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UNIT 7 PTERIDOPHYTES: GENERAL CHARACTERISTICS AND REPRODUCTION

Structure

7.0 INTRODUCTION

A pteridophyte is a vascular plant (with xylem and phloem) that disperses spores. Because pteridophytes produce neither flowers nor seeds, they are also referred to as 'cryptogams', meaning that their means of reproduction is hidden. The pteridophytes include the ferns, horsetails, and the lycophytes, i.e., clubmosses, spikemosses, and quillworts. These are not a monophyletic group because ferns and horsetails are more closely related to seed plants than to the lycophytes. Therefore, 'Pteridophyta' is no longer a widely accepted taxon, although the term pteridophyte remains in common parlance, as pteridology and pteridologist as a science and its practitioner, to indicate lycophytes and ferns as an informal grouping, such as the International Association of pteridologists and the Pteridophyte Phylogeny Group.

Pteridophytes display differentiation. The plant body can be divided into a true root, stem, and leaves. A saprophyte is the main plant body here. Some of the species belonging to this division have small leaves called as the microphylls. For example, Selaginella. Megaphylls are the large leaves that some pteridophytes have. For example, Fern plants. The main plant bears the sporangia. These bear some leaf-like appendages called the sporophylls. In a few species such as Selaginella and Equisetum, the sporophylls form compact structures called cones or strobili.

In this unit, you will study about pteridophytes, its general account, reproduction, classification, stelar organisation in detail.

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7.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand what pteridophytes are
- Discuss general characteristics and reproduction of pteridophytes
- Explain the classification of pteridophytes
- Discuss about stelar organisation

7.2 PTERIDOPHYTES: GENERAL ACCOUNT AND REPRODUCTION

In an account of diversity of plant life, on one end are unicellular algae confined to moist habitats and on the other end are higher plants capable of growing on relatively dry land. In between these two extremes of habitats, the plant life is presumed to have originated in water, and in the process of evolution from a free living independent cell- the fundamental unit of structure and function evolved a multicellular organism with a division of labour among its tissue. The next was the origin of sex and its elaboration. One of the manifestations of this elaboration was the postponement of zygotic meiosis leading to the formation of diploid generation; the sporophyte. The sporophyte on reduction division give rise to haploid or gametophytic generation. These generations sexual and asexual alternate with each other and the phenomenon is termed as alternation of generations.

The cyclic alternation between sexual and asexual generation is hypothesized to help in the invasion of land. The land plants have complex internal organization and Pteridophytes occupy a position intermediate between bryophytes and higher land plants. The sexual generation gametophyte of bryophyte and Pteridophytes is dependent on water for fertilization. Bryophytes, the simplest of green land plants, lack root; instead there are rhizoids for absorption. Pteridophytes resemble higher land plants, i.e., gymnosperms and angiosperms in having complex internal organization, vascular elements but differ from them in lacking seed habit. Some of the pteridophytes are heterosporous, they approach seed habit and pteridophytes of the past were seed bearing plants.

The word cryptogams is a synthesis of two Greek terms '*kruptos*' meaning 'Hidden' and '*gamos*' meaning 'Wedded'. This single term encompasses all plants that reproduce by means of spores and do not produce seeds. The algae, fungi, bryophytes and pteridophytes are all cryptogams. The pteridophytes are treated as vascular cryptogams as they have well developed conducting system. The lower cryptogams have no conducting system. The pteron means feather and the name was given to this group because of feather like fronds. Vascular cryptogams are an assemblage of seedless vascular plants that have successfully invaded the land and reproduce by means of spores.

The pteridophytes formed a dominant part of Earths vegetation in the historic past. In the present day flora, they rank only next to the spermatophytes. Although they have been replaced by spermatophytes in modern day flora, yet they occupy an important and crucial position in evolutionary history of plant kingdom. No doubt lesser in number, pteridophytes lend a distinct charm and physiognomy to the landscape. The elegant tree ferns of the warm humid forests of Eastern Himalayas, Pachmarhi and Nilgiri hills, the epiphytic ferns and the hanging club mosses of tropical forest attract ones attention. The lithophytic and terrestrial forms that grow in large number are source of great attraction because of their beautiful foliage. Some of them grow in water and form a luxuriant hydrophytic component of the lakes, ponds and pools (*Azolla* and *Marsilea*). In India, about five hundred species of fern allies have been reported. The pteridophytes have a long fossil history. Their first traces were recognized in the Silurian period of the late Paleozoic age (about 400 million years ago). They flourished well during Devonian, Mississippian and Pennsylvanian periods of the late Paleozoic. Late Paleozoic can be regarded as the 'Age of Pteridophytes'. The tree ferns, giant horsetails and arborescent lycopods dominated the swampy landscape of those ancient ages. The present day lycopods *(Lycopodium, Isoetes, Selaginella, Phylloglossum*) and sphenopsids (*Equisetales*) are the mere relicts of the mighty lycopsid and sphenopsid group. Only the present day ferns have managed to conserve the former stature, diversity and glory of their ancestors. *Psilotum and Tmesipteris*, two surviving remnants of Psilopsida retain the primitive features of the first land plants.

7.2.1 General Characters

- The pteridophytes grow in a large variety of habitats. Mostly they are terrestrial and thrive very well in moist and shady places. While others flourish well in open and dry places like xeric conditions, for example, *Selaginella lepidophylla* and *Sagina pilifera.* A few of them are aquatic, for example. *Azolla*, *Marsilea* and *Salvinia*. Some are epiphytes, for example, *Psilotum flaccidum, Selaginella oregano,* etc.
- The plant body is sporophytic in all the pteridophytes. The sporophyte develops from the diploid zygote which is a diploid cell formed by the process of fertilization of an egg and the sperm. The adult sporophytic plant body is highly variable in form, size and structure. Most of the present day pteridophytes are herbaceous.
- The sporophytic plant body is differentiated into true roots, stem and leaves. In primitive plant groups like psilotales and psilophytales, true roots and well developed leaves may be absent and roots are replaced by rhizoids and the function of leaf that is photosynthesis is performed by green stem axis.
- The sporophytes may be dorsiventral or radial in symmetry.
- Stems may have dichotomous or lateral branching.

- Plants may have microphyllous or megaphyllous leaves. Megaphyllous leaves are large in relation to the stem, for example, ferns while the microphyllous leaves are small in relation to stem, for example, *lycopods and equisetums*.
- The roots are generally adventitious and the primary root is short lived.
- All the vegetative parts of the plant possess vasculature. In different groups of pteridophytes, the vascular system is variable. It may be simple Protostele (*Selaginella*), Siphonostele (*Marsilea*), Dictyostele (Fern Rhizome) or Eustele (*Equisetum*).
- Phloem lacks companion cells. The pteridophytes possess only sieve cells in phloem.
- Primitive vessels are present in some members, for example, *Selaginella* and *Equisetum*.
- The stem, root and the leaves grow by means of a single, two sided or three sided apical cell.
- Secondary growth is absent in majority of the living representatives (except *Isoetes*).
- The sporophytic plant reproduces asexually by means of haploid spores inside the sporangia.
- Sometimes sporangia are also present on the stem. Such sporangia are cauline in origin.
- Leaves which bear spores are called sporophylls.
- The sporophylls may be lying scattered on a plant or may be clustered in definite structures called cones or strobili, for example, *Selaginella, Equisetum*. In *Azolla, Marsilea* and *Salvinia* the sporangia are produced within the specialized structures called sporocarps.
- The plants may be homosporous which produce only one type of spores, for example, *Psilotum, Equisetum, Pteris , Pteridium,* etc., or heterosporous which produces two different types of spores smaller microspores produced within the microsporangium and larger megaspores produced within megasporangium, for example, *Selaginella , Marsilea.*
- According to the mode of development, the sporangia are of two types the eusporangium and leptosporangium. The euspornagium develops from a group of sporangial initials, for example, *Psilotum, Selaginella, Equisetum,* etc., while leptosporangium develops from a single initial and is found in advanced ferns like *Pteris, Pteridium, Marsilea*, etc.
- In higher pteridophytes like *Pteris and Pteridium*, the sporangia are borne in small groups on the sporophyll. Each group is called sorus. Sorus is protected by false indusium in *Pteris* and true indusium in *Pteridium.*
- The spores are haploid and are formed inside the sporangia. The haploid spores germinate to give rise to the gametophytic generation.
- The gametophyte is independent from the sporophyte. It may be autophytic, sporophytic or partly autophytic and partly sporophytic. It is different from sporophyte.

- The homosporous pteridophytes produce monoecious gametophytes while heterosporous ones produce dioecious gametophytes. The microspores produce male gametophytes and megaspores produce female gametophytes
- Monoecious prothalli which are formed from homosporous grow upon the soil and form independent haploid plants, such haploid independent gametophytes are exosporic gametophytes, for example, *Psilotum, Equisetum, Pteris,* etc. Exosporic gametophyte is delicate and thin and is called **prothallus**. They grow exposed to light and remain attached to the ground by means of rhizoids. They manufacture their food by means of chloroplast and live an independent life. The rhizoids help in absorption of water and fixation of prothallus to the ground. Some vascular cryptogams exosporic gametophytes lack chlorophyll and are subterranean in habit, for example, *Psilotum* and *Ophioglossum*. In such plants, prothallus obtains its food by symbiosis through mycorrhiza, which is present in the tissue of prothallus.
- The monoecious prothalli may be protandrous or protogynous.
- The gametophytes are highly reduced in the heterosporous vascular cryptogams. These are called endosporic prothalli, for example, *Selaginella, Isoetes, Marsilea, Azolla, Salvinia*, etc. Such prothalli are represented by a few vegetative cells and the sex organs. They get nourishment from the reserve food stored in the spores.
- The sex organs are antheridia and archegonia. They may be embedded or projecting. They are borne on gametophytes.
- Typically, the gametophytes formed from the homosporous are monoecious, i.e., both antheridia and archegonia are present on the same gametophyte and in large numbers. While the gametophytes which are formed by heterospores are dioecious, that is antheridia and archegonia develop on separate male and female gametophyte.
- The antheridia are either embedded in gametophytic tissue, for example, *Equisetum, Selaginella* or projected above the surface, for example, *Pteris* which is a Leptosporangiate.
- Mature antheridium is globular with an outer sterile wall inside which are found a large number of androcytes. Each androcyte give rise to a single flagellated motile antherozoid.
- The archegonia in pteridophytes resemble closely with those of bryophytes.
- Each archegonium is a flask shaped structure, with a basal swollen, embedded portion, the venter and a short neck. The wall of the venter develops from the prothallus tissue, so the venter lies embedded in the prothallus tissue. The venter encloses an egg and ventral canal cell. Inside neck are found neck canal cells.
- The archegonia have four longitudinal rows of neck cells whose height varies from two to six cells.
- Neck canal cells vary from one to 14.
- At maturity, the apical cells separate, the neck canal cells disintegrate thus forming a passage for the antherozoid to reach the egg cell.

- Fertilization occurs in the presence of water. Water is required for dehiscence of antheridia, liberation of antherozoids, movement of antherozoids to archegonia, maturation of archegonia and syngamy
- The fusion of the antherozoids with an egg restores the diploid chromosome number and results in formation of diploid zygote, which is first cell of sporophytic generation.
- The zygote develops into embryo within the archegonial venter and is surrounded and nourished by the prothallial tissue, until it develops its own root, stem and leaves. The sporophyte is dependent on the gametophyte only during its early stages.
- The development of embryo may be endoscopic which means that the axis of embryo directed inwards away from the archegonial neck, for example, *Selaginella* or exoscopic that is the axis of embryo directed outwards towards archegonial neck, for example, *Psilotum, Equisetum*. But in case of *Marsilea, Pteris* and *Pteridium* it is lateral, i.e., the embryo axis is at right angle to the long axis of archegonium.
- The alternation of generation is heteromorphic in all the pteridophytes

Sporophyte

- The main independent plant body of pteridophytes is sporophyte. It develops from the diploid zygote after fertilization and exhibit a great variation in form, size and structure. Most of the present day pteridophytes are herbaceous except a few woody tree ferns.
- Plants are differentiated into true roots, true leaves and true stems. In case of a few members of Psilophytales and psilotales, the leaves and roots are absent. Their functions are taken up by the other organs, for example, roots are replaced by rhizoids and stem axis becomes photosynthetic.
- The primary root is short lived. It is replaced by adventitious roots which arise endogenously.
- Plants exhibit dorsiventral or radial symmetry. The branching of stem may be dichotomous or monopodial.
- The stem bears leaves, which may be small microphyllous or very large in relation to stem microphyllous.
- All vegetative parts possess vascular tissues organized in definite groups or steles. The stele may be simple protostele, medullated protostele or siphonostele.
- The xylem mainly consists of tracheids and xylem parenchyma. The vessels are generally absent, but primitive type of vessels have been reported in *Selaginella* and *Equisetum*.
- The phloem consists of sieve tube and phloem parenchyma. The companion cells are absent.
- Secondary growth do not occur in most of the living pteridophytes. (Except *Isoetes*).

Reproduction

- The sporophytic plant body produces haploid spores, which serve as means the asexual reproduction.
- The spores are produced inside the sporangia which may be borne on stem or leaves. The leaves bearing sporangia are called sporophylls.
- The development of sporangia may be eusporangiate, i.e., sporangia originates from a group of initial cells or leptosporangiate, i.e., sporangium originates from a single superficial cells.
- The sporophylls may be scattered on the plant or may be restricted to particular region called strobili or cones. In some cases, the sporangia are produced within specialized structures called sporocarps.
- In some higher pteridophytes, the sporangia are borne in small groups on the sporophyll. Each group is called **sorus**. It may be protected by false indusium (*Pteris*) or true indusium.
- The plants may be homosporous, i.e., produce only one type of spores (*Psilotum, Lycopodium, Equisetum, Pteridium, Pteris,* etc.) or heterosporous, i.e., produce two different types of spores- smaller microspores and larger megaspores (*Selaginella, Marsilea).*

Gametophyte

- It is independent from the sporophyte and may be autophytic or saprophytic or partly autophytic and partly saprophytic. The homosporous pteridophytes have Monoecious Prothalli.
- They may be protandrous or protogynous (*Equisetum*). Dioecious prothalli have been reported in Equisetum and some ferns. The pteridophyta includes homosporous as well as heterosporous forms.
- The homosporous forms are characterized by exosporic gametophytes in which the germination of spore and development of gametophyte are under control of external conditions.
- In contrast, heterosporous forms produce endosporic gametophytes which seem to be independent of external control, since in forms like *Marsilea* and *Salvinia,* the germination of spore, development of gametangia and gametes and even embryo development may take place in dark.
- Morphologically, the gametophyte has nothing in common with sporophyte, its form and pattern of development are different. The gametophytic phase is reduced in pteridophytes as compared to allied cryptogams, bryophytes. There is distinct shortening of the life span of gametophyte as we proceed from homosporous to heterosporous forms.
- In homosporous forms the gametophyte is a long lived structure and may require years to attain maturity. In contrast, the gametophytic span in heterosporous forms ranges from few hours to few days. Besides life span, reduction in the form of the gametophyte is found in heterosporous forms.

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The male gametophytes are extremely reduced in *Selaginella* and *Isoetes,* consisting of a single prothallial cell and an antheridium.

- In homosporous forms vegetative propagation by adventitious branching is of frequent occurrence and the gametophytes can be maintained indefinitely in cultures by subculture and prevention of fertilization.

Sex Organs

- The sexual reproduction is oogamous. The male sex organs are antheridia and female sex organs are archegonia. Both the sex organs are borne on gametophytic prothalli.
- Homosporous pteridophytes produce monoecious prothalli, i.e., both antheridia and archegonia are borne on the same prothallus. The heterosporous pteridophytes produce dioecious prothalli, i.e., antheridia are borne on male prothallus and archegonia are borne on female prothallus.
- The antheridia are either embedded in the tissue of gametophyte or projected above the surface. Each antheridium has single layered sterile jacket enclosing a large number of androcytes. The androcytes metamorphosis into flagellated motile antherozoids.
- The archegonia typically resembles those found in bryophytes. Each archegonium is flask shaped, embedded in the tissue of gametophyte. It is differentiated into neck and venter. The neck consists of 4 vertical rows of cells. The axial row consists of neck canal cells, venter canal cell and an egg. The neck canal cells vary from 1 to 14 in number.
- Fertilization occurs in presence of water. It is needed for dehiscence of antheridia, liberation of antherozoids, movement of antherozoids from antheridia to archegonia, maturation of archegonia and syngamy. The haploid antherozoid fuses with the haploid egg and form diploid zygote, which is first cell of sporophytic generation.
- The diploid zygote is retained within archegonial venter. It develops into a young sporophyte which remains attached to the gametophyte and gets nourishment during its early stages from gametophyte.
- The development of embryo may be endoscopic, i.e., the axis of embryo directed inwards away from archegonial neck (*Lycopodium, Selaginella*) or exoscopic, i.e., the axis of embryo directed outwards towards archegonial neck.
- The alternation of generation in all the members of pteridophytes is heterologous type.

Habitat of Pteridophytes

Pteridophytes the first land vascular plants are represented by about 10,000 species distributed throughout the world, both in tropical and temperate climates. Most of the members are terrestrial in nature, prefer to grow in cool and shady places, however aquatic and xerophytic habitat is not uncommon for pteridophytes.

- **Terrestrial Habitat:** Members right from fossil pteridophytes to the most advanced pteridophytes grow in terrestrial habitats. Among *Psilophyta*, terrestrial species are represented by *P. nudum, Osmunda* sp. Most of the ferns are terrestrial in nature mostly grow in hilly regions (*Dryopteris, Pteris, Pteridium, Adiantum*, etc.) preferring moist and shady environment.
- **Aquatic Pteridophytes**: Most of the members of family Marsileaceae and Salviniaceae grow in aquatic and semi aquatic habitats. Plants of these group are known as aquatic ferns. Examples are *M. minuta, M. quadrifolia, S. auriculata, Azolla mexicana.*
- **Xerophytic Pteridophytes**: Most of the xerophytic plants are reported from the genus *Selaginella.* The common Indian xerophytic species are *S. wightii* and *S. repanda.*

7.2.2 Alternation of Generations

Hofmeister (1851) used this term in plants. He observed that in mosses and ferns there are two types of morphologically distinct individuals in the life cycle. Both alternate in a life cycle, i.e., there are some events which lead one generation to produce the other and cause alteration. Strasburger discovered the process of meiosis in plants. The discovery revealed that the reduction in the number of chromosomes leads to formation of a new individual in the life cycle. This individual has haploid number of chromosomes in its nuclei. It bears sex organs and is concerned with sexual reproduction. It was given the name gametophyte and represented gametophytic generation. The haploid gametes unite and establish a diploid nucleus or synkaryon. Fertilization establishes a diploid cell or the zygote, which is pioneer structure of the diploid individual or the sporophyte. It germinates to form embryo, which in turn develops into the sporophyte individual. This generation is termed the sporophytic generation. The sporophyte individual bears sporangia which produces spores as a result of meiosis. These spores are haploid and known as meiospores. The spores are the pioneer structures of the gametophyte generation. They germinate to give rise to the gametophyte individual known as prothallus in vascular cryptogams.

The above events lead us to following conclusions:

- There are two distinct individuals in the life cycle of vascular cryptogams.
- These individuals are produced by the germination of the haploid spores and diploid zygote.
- The one produced by the spores is the gametophytic plant.
- The zygote produces the embryo which give rise to diploid individual called sporophyte. It bears haploid spores after a process called the meiosis.
- The two generation alternate with each other in the life cycle.
- Meiosis and syngamy are the two significant stages that switch on the life cycle from one generation to the other.
- The number of chromosomes is halved during meiosis and is doubled during syngamy or fertilization.

NOTES

In vascular plants the sporophyte individual is a complicated, independent and a dominant generation, whereas the gametophyte is comparatively much reduced. The gametophyte in the homosporous forms are short-lived, is independent and may be surface living, green, subterranean and non-green. They are always exosporic and not enclosed by spore wall. The heterosporous land plants display a reduction in their gametophytes. The gametophytes are reduced and endosporic. They have separate male and female prothalli. The male prothallus is reduced and represented by a single prothallial cell. The female prothallus is well developed because it has to nourish the developing embryo.

Both the homosporous and heterosporous vascular plants exhibit heteromorphic type of alternation of generations because the sporophyte and the gametophyte individuals present marked morphological and anatomical differences.

Abnormalities in the Life Cycle

The normal life cycle of a vascular plant has two alternating generations. These are the diploid sporophyte and the haploid gametophyte. Both alternate regularly in the life cycle and this alternation is brought by two steps known as the fertilization and the meiosis. Figure 7.1 shows the life cycle of *sphenophyta*.

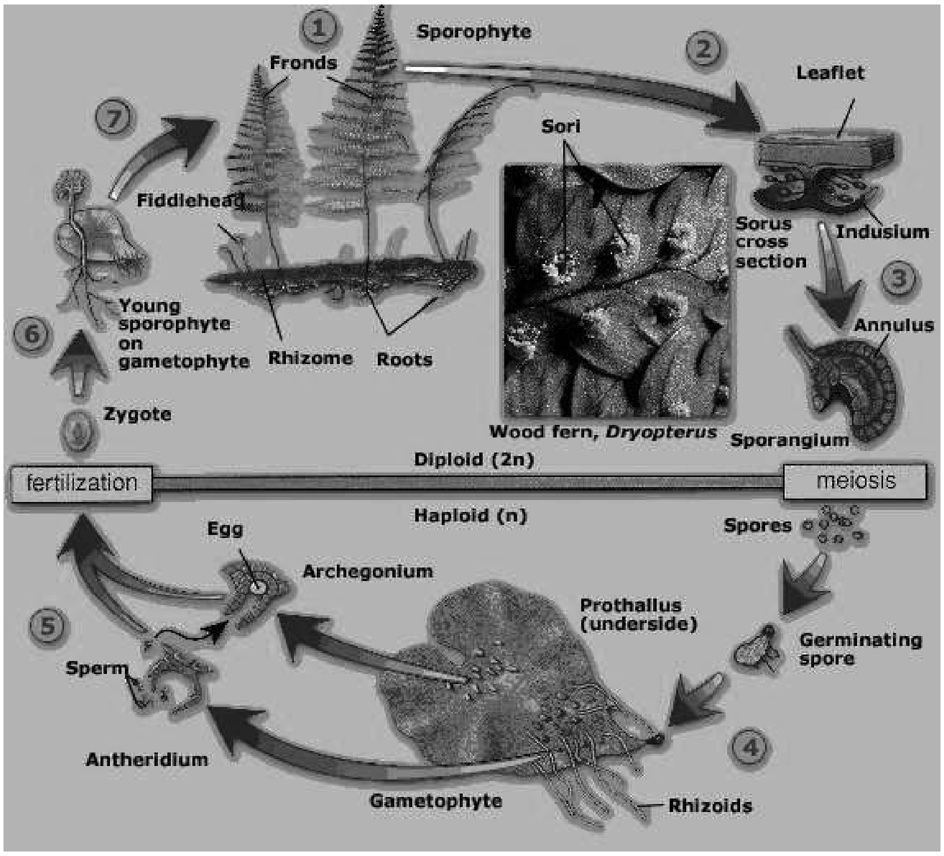

Fig. 7.1 *Life Cycle of Sphenophyta*

Apogamy

It was reported by Farlow (1874) in *Pteris cretica*. Apogamy is defined as the development of a sporophyte directly from the gametophyte without intervention of sex organs and gametes. The sporophytes formed have the same chromosome number as the gametophyte, i.e., haploid number for the species. Apogamy occurs in nature and has been induced under experimental conditions. It is common and a widespread phenomenon in the ferns. Natural apogamy has been reported in more than 50 species of ferns belonging to 20 genera.

In some species of ferns apogamy appears to be a necessity and is a regular process. It is due to inherited constitution of the plant. Natural apogamy is known in *Dryopteris, Pteris, Adiantum, Osmunda, Athyrium, Cheilanthes, Asplenium,* etc. There are many more genera in which apogamy has been induced experimentally. Parthenocarpic development of the egg into the sporophyte has been included under apogamy as apogamy is the development of vegetative tissue of the prothallus into the sporophyte. Apogamy has been induced in Lycopodium and Equisetum. About 12 percent of the cytologically studied hoosporous ferns are found to be apogamous.

Causes of Apogamy

Many workers regard failure of normal fertilization as a cause of apogamous production of sporophytes. Other conditions favouring apogamy are culture in bright light and at higher temperatures, by lowering the vitality of the prothallus by fungal and algal attack, failure of formation of functional sex organs under various unfavourable nutritional conditions. Evans (1965) has reported an interesting case of apogamy in a species of Polypodium. He reported the formation of 32 mitospores in the sporangia. The sporangia have 16 spore mother cells which do not undergo meiosis but divide mitotically into 32 spores. These spores are reniform in shape and occur in diads. According to Williams (1938), in addition to environmental factors, there must be some internal factors such as the nature of inherent susceptibility due to abnormal nuclear composition and behavior that induce apogamy. In some ferns ageing of the prothallus is found to be one of the factors which influence apogamous developments of prothalli of some ferns. Apogamy has been induced in Regnellidium by Loyal and Chopra (1973). Sporophytic buds have been formed on the prothalli of Lycopodium grown in culture medium containing coconut milk and sucrose. This clearly indicate that nutritional factors do effect the induction of apogamy.

Apospory

The phenomenon of apospory was recorded by Druery (1884) in a fern called *Athyrium filix femina var. clarissima*. It is the production of a gametophyte directly from the vegetative cells of the sporophyte without reduction division and spore formation. He observed the development of prothalli from the stalk of sporangium as well as from the spore case of this fern. Apospory was noticed in many ferns and some workers induced it under cultural conditions. During this phenomenon gametophytes or prothalli develop from the vegetative tissue of the sporophyte and not from the spores. Such prothalli are diploid, i.e., they have the same chromosome number as the sporophyte. Normally the haploid spores germinate and give rise to the haploid gametophyte but during apospory spores are not required. Apospory is the development of gametophytes from the vegetative parts of the sporophyte without the intervention of spores. In apospory, a filamentous or heart shaped gametophyte may be formed from one or more cells of any vegetative portion of a young or a mature sporophyte. Apospory is rarely of constant occurrence in any fern but occurs occasionally in nature and can be induced under cultural conditions.

Causes of Apospory

Several factors are known to influence the aposporous development of gametophytes from vegetative tissue of the sporophyte.

- Briston (1962) demonstrated that mineral nutrition is responsible for the formation of prothalli from callus tissue obtained from the sporophytes of Pteris cretica. When he supplied sucrose to such callus tissue, it develops into sporophyte.
- It was found that there is pronounced relation between the stage of development of sporophytic cells and the kind of organs regenerated. They observed that aposporous gametophytes developed on decapitated young sporophytes with one or two leaves whereas in older sporophytes only shoot buds developed.
- In some ferns, the leaves develop prothalli under dim light and sporophytic buds under strong light.
- The work done in Punjab University Botany Laboratories (1969 to 1973) on tissue culture has revealed interesting results. They obtained root callus tissue from the roots of tetraploid *Cyclosorus dentatus* on Knudsons medium +2% sucrose, 2-4 D. The callus was fragile and its cell suspension were obtained in sterile distilled water.

Palta (1973) induced apospory in *Pteris vittata*. Sexually reproduced normal sporophytes produced gametophytes from the juvenile leaves. These gametophytes bare normal sex organs in Knudson medium without sucrose. Palta (1973) also produced apogamous sporophytes in *P. vittata* by using Knudsons medium 2% sucrose. Then this apogamous sporophyte was placed in Knudsons medium without sucrose. After about 21 days, the apical pinnae of the same sporophyte produced aposporous gametophytes and he concluded that by changing the nutritional set up of the environment, apogamy and apospory can be induced at any time.

7.2.3 Affinities of Pteridophytes

The pteridophytes differ from the algae, fungi and bryophytes by the possession of highly differentiated plant bodies with true roots, stems, leaves and vascular tissues. They differ from spermatophytes as the pteridophytes do not produce flowers, fruits or seeds. So pteridophytes occupy an intermediate position between bryophytes and spermatophytes and so have some similarities with bryophytes on one hand and some with spermatophytes on the other.

Bryophytes and Pteridophytes

- Terrestrial habit.
- The sporophytes reproduce asexually by means of haploid spores. The spores are formed from diploid spore mother cells by meiosis.
- The sex organs are identical with respect to structure and ontogeny.
- The sex organs are jacketed and the jacket is sterile.
- Flagellated motile sperms or antherozoids.
- Fertilization takes place in the presence of water.
- The zygote is retained within archegonium and develops into embryo.
- Young embryo is dependent upon the gametophyte.
- Both these groups exhibit distinct and regular alternation of generations.

But pteridophytes are clearly advanced over bryophytes and show some features of advancement over bryophytes.

- In bryophytes the gametophyte is dominant and conspicuous generation and diploid sporophyte bears only spore producing structure and latter is dependent on the gametophyte for food. While in vascular cryptogams the sporophyte continues a large dominant phase in the life cycle, while the gametophyte is always small and inconspicuous.
- In pteridophytes all the vegetative organs of sporophyte have vascular supply with xylem and phloem as vascular tissues.
- The sporophytic plant body of pteridophytes shows differentiation into true roots, stem and leaves which was not there in bryophytes.
- The sporophyte of the pteridophytes is larger than the gametophyte and is independent at maturity.

Similarities between Pteridophytes and Spermatophytes

Pteridophytes resemble spermatophytes (seed bearing plants) in following characteristics:

- The sporophyte is dominant, independent, large and conspicuous phase in the life cycle of both these groups.
- In both the groups the sporophytic plant body is differentiated into true roots, stem and leaves.
- The gametophyte is inconspicuous and small in both these groups.
- All the vegetative parts of sporophyte have vascular supply. The vasculature consists of typical xylem and phloem cells. The xylem consists of xylem tracheids and xylem parenchyma. Phloem consists of sieve tubes and phloem parenchyma (xylem vessels and companion cells are absent in pteridophytes).
- Leaves have stomata and perform the function of photosynthesis.
- The plants in both the groups show distinct alteration of generations.

NOTES

But definitely spermatophytes are highly advanced than pteridophytes which have a long fossil history.

Life cycle of Pteridophytes

The life cycle of a pteridophytes comprises of two distinct phases or generations, each of which produces the other. The sporophyte is the diploid and dominant part of the life cycle and is organized into stem, leaves and roots. It has vascular tissue developed in it. The sporophytic plant develops sporangia (scattered in sori, in strobilus or in sporocarp) within which are present diploid cells called sporocytes or spore mother cells. Each spore mother cell divides by meiotic division. The spores formed by meiosis are haploid in genetic constitution. In members like *Pteridium, Pteris, Dryopteris, Equisetum and Lycopodium* all the spores are alike and the individuals are called homosporous. These spores germinate to produce gametophytic generation. Gametophytic generation is the second phase of life cycle and is haploid. Homosporous produce only one type of gametophyte or prothallus. The gametophytic generation is always small, bear male and female gametes. The antheridium bears male gametes in large number while the archegonium bears one egg. Fertilization takes place in the presence of water. Fusion of sperm with the egg results in formation of diploid zygote. With the accomplishment of fertilization, sporophytic generation starts again and zygote is the first cell of diploid generation and gametes are the last cells of haploid generation.

In heterosporous pteridophytes like *Selaginella* and *Marsilea*, the spores are of two types, the smaller one is called microspore or male spore. It develops within microsporangia and larger spores are called megaspores and formed in megasporangia. Microspores germinate to form male gametophyte and megaspore germinate to form female gametophyte. Male gametophyte has male sex organ which bears many male gametes or sperms and female gametophyte has one egg per archegonium. In heterosporous pteridophyte, fertilization occurs in the presence of water which results in formation of a diploid cell or zygote which develops sporophytic generation. The two generations of life cycle the gametophytic and sporophytic alternate with each other. This is called alternation of generation but both these generation differ in morphological and anatomical characters so in pteridophytes there is heterologous alternation of generations.

From the studies of life cycle of ferns and fern allies, we conclude that the occurrence of normal morphological and cytological alteration of generations in addition to apospory and apogamy in polyploids indicate that haploidy and diploidy alone cannot determine the features of a generations. According to Mehra (1975), the fern system is elastic and can be changed according to will by various experimental techniques and nutritional media along with different concentrations of sucrose. Even certain metabolic and nutritional factors may be responsible for manipulating the activity of genes. This is evident as a result of cytology of apogamy and apospory and the studies of polyploidy, that no relation exists between meiosis and alteration of gametophyte and sporophyte in a life cycle.

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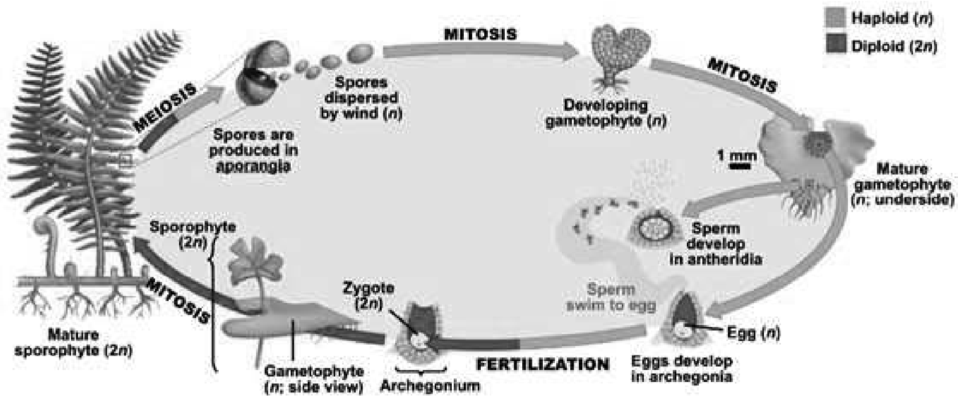

***Fig.** 7.2 Life Cycle of Pteridophyte*

Check Your Progress

1. What are the habitat of pteridophyte?
2. Name some xerophytic pteridophytes?
3. Why pteridophytes are called vascular cryptogams?

7.3 CLASSIFICATION OF PTERIDOPHYTES

Pteridophytes are the most primitive group of land plants with a well-developed vascular system. The vascular system have been divided into pteridophyta and spermatophyte on the basis of reproduction by spores in the former and reproduction by seeds in the latter group. Accordingly, the vascular cryptogams (Psilotum, Equisetum and ferns) were placed in division spermatophyte. The classification of the vascular cryptogams has undergone vast changes in the past. In the new system of classification, all the vascular plants are placed in a distinct group of plant kingdom, the Tracheophyta.

Tracheophyta is characterized by following characters:

- Vascular system is well developed in sporophytic plant body.
- Sporophyte is the dominant generation.
- For most of the part of life cycle, sporophyte is independent of gametophyte.

Historical Background

Linnaeus, 1754: Selected reproductive organs as the main criteria of classification. He divided plants into 24 classes on the basis of flowers. The twenty fourth class: Cryptogamia included plants without visible flowers. This class included Algae, Fungi, Mosses and Ferns.

A.P. de Canadolle, 1819: Divided plants into two main groups: Phanerogams (plants with distinct flowers) and Cryptogams (plants with hidden or unknown flowers) the group cryptogams included pteridophytes.

Endlicher, 1840: Endlicher followed 'de Canadolle' system of classification but subdivided the plant kingdom into Thallophyta and Cormophyta. The later was divided into Acrobrya, Amphibrya and Acyamphibrya. The pteridophytes were included in Acrobrya.

Brongniart, 1843: Divided the plant kingdom into two main groups as, Cryptogamae (which include non-flowering plants) and Phanerogamae (which included flowering plants).

Darwin, 1859: Proposed his theory of organic evolution.

Eichler, 1883: Divided plant kingdom into two sub groups: Cryptogamae and Phanerogamae. The cryptogamae was divided into divisions: Thallophyta (Algae and fungi), Bryophyta and Pteridophyta (included Lycopodiaceae, Equisetinae and Filicineae). Pteridophyta was given a rank of division.

Engler, 1886: Divided the plant kingdom into two subkingdoms i) Thallophyta and ii) Embryophyta. The later included all those plants where zygote develops into multicellular embryo that undergoes its early development within either an archegonium or embryo sac. The embryophyta was divided into divisions: Bryophyta, Pteridophyta and Spermatophyta.

Sinnott, 1935: Introduced a new term 'Tracheophyta' to include all plants having vascular tissue (xylem and phloem).

Eames, 1936: Considered the presence or absence of differentiated vascular system and natural affinities of the plants concerned. Eames divided Plant kingdom into Divisions- Thallophyta, Bryophyta and Tracheophyta.

Division- Tracheophyta was divided into 4 main groups:

- Psilopsida (included *Psilotales* and *Psilophytales*).
- Lycopsida (included *Lycopodiales*, *Selaginellales*, *Isoetales*, *Lepidodendrales* and *Pleuromeia Les*).
- Sphenopsida (included *Hyeniales*, *Sphenophyllales* and *Equisetales*).
- Pteropsida (was divided into classes as - Filicinae, Gymnospermae and Angiospermae).

Though the 4 groups of Tracheophyta were accepted by a majority of botanists, there was no agreement regarding their taxonomic ranks.

Tippo, 1942: Gave Tracheophyta the rank of phylum to include all the vascular plants. Tippo divided phylum, Tracheophyta into four subphyla: Psilopsida, Lycopsida, Sphenopsida and Pteropsida.

The ranks phylum and subphylum were not in accord with International Code of Botanical Nomenclature (ICBN). According to the rule the ending for division is phyta, for subdivision is Phytina, for class Opsida and for subclass Idae.

Reimers, 1954: Gave Pteridophyta a rank of division and divided into following 5 classes:

- Psilophytopsida (included Psilophytales)
- Psilotopsida (included Psilotales)

- Lycopsida (included Protolepidodendrales, Lycopodiales, and Lepidodendrales).
- Sphenopsida (included Hyeniales, Sphenophyllales, Calamitales, and Equisetales).
- Pteropsida (included Primofilices, Eusporangiate, Osmundine, Leptosporangiate)

Wardlaw, 1955: Classified the vascular plants in accordance with the rules of International code of Botanical nomenclature. He followed Eames and included all the vascular plants in the division 'Tracheophyta'. He subdivided it into 4 subdivisions: Psilopsida, Lycopsida, Sphenopsida and Filicopsida (Pteropsida).

The classification of the vascular cryptogams has undergone vast changes. On the basis of the presence or absence of seeds the older taxonomists place the vascular plants in two divisions: Pteridophyta and Spermatophyta. The former includes the primitive vascular plants which bear no seeds. According to **'traditional system of classification'** the pteridophytes are divided into following classes and orders:

Division Pteridophyta

1. Class Psilophytineae

- Order Psilotales. Examples: *Psilotum* and *Tmesipteris*
- Order Psilophytales. Example: *Rhynia*.

2. Class Lycopodiaceae

- Order Lycopodiales. Examples: *Lycopodium*, *Selaginella*
- Order Isoetales. Example: *Isoetes*
- Order Lepidodendrales Example: *Lepidodendron*.

3. Class Equisetineae

- Order Equisetales represented by *Equisetum*
- Order Sphenophyllales represented by *Sphenophyllum*

4. Class Filicinae

- Order Filicales. Examples: *Dryopteris, Marsilea*.
- Order Marattiales represented by *Marattia*.
- Order Ophioglossales represented by *Ophioglossum* and *Botrychium*.
- Order Osmundales represented by *Osmunda*.
- Fossil Orders.

On the basis of origin and development of vascular plants. Sinnott introduced a term Tracheophyta for plants having vascular arrangements including Thallophyta and Bryophyta. So in revised system of classification proposed by Tippo (1942) all the vascular plants are placed in a single division Tracheophyta.

Division Tracheophyta

1. Subdivision Psilopsida

Class Psilophytineae

- Order Psilotales
- Psilophytales

2. Subdivision Lycopsida

Class Lycopodiaceae

- Order Lepidodendrales
- Order Lycopodiales
- Order Pleuromeia Les
- Order Isoetales

3. Subdivision Sphenopsida

Class Equisetineae

- Order Equisetales
- Order Sphenophyllales
- Fossil Orders

4. Subdivision Pteropsida

The term is used in a restrictive sense to cover the Megaphyllous Pteridophytes only.

Class Filicinae

- Order Ophioglossales
- Order Filicales
- Order Osmundales
- Order Marattiales
- Fossil Orders

According to the recommendations of ICBN (1952), the name of division should end in suffix 'Phyta', of subdivision in Phytina and of class in Opsida. On this basis Smith (1955) divides vascular cryptogams into 4 divisions, Psilophyta, Lepidophyta, Calamophyta and Pterophyta.

I. Division Psilophyta

It included the most primitive and oldest known land inhabiting vascular plants which are rootless. The division comprises the following classes:

1. Class Psilophytopsida

Order Psilophytales.

It includes the most primitive fossil vascular cryptogams, for example, *Rhynia, Horneophyton*, etc.

2. Class Psilotopsida

Order Psilotales.

It includes two living genera which are allied to the Psilophytales. These are *Psilotum* and *Tmesipteris.*

II. Division Lycophyta or Lepidophyta

On the basis of the presence or absence of ligules, this division may be divided into following two classes:

1. Class Eligulopsida

Order Lycopodiales**.**

The order includes homosporous, non-ligulate forms, such as *Lycopodium* and *Phylloglossum.*

2. Class Liliopsida

The class includes heterosporous forms with ligulate leaves. The four orders in this class are:

- Order Selaginellales represented by *Selaginella*.
- Order Isoetales represented by *Isoetes*.
- Order Pleuromeia Les, for example, *Pleuromeia*.
- Order Lepidodendrales represented by *Lepidodendron*, *Stigmaria*, *Lepidocarpon*.

III. Division Arthrophyta or Calamophyta or sphenophyta

The division includes two classes:

1. Class Spenophyllopsida: This class includes the well-known extinct genus Sphenophyllum which is placed in following order:

Order Sphenophyllales

2. Class Calamopsida: This class includes both fossil and living forms. The living genus Equisetum is referred to the following order:

Order Equisetales

IV. Division Filicophyta or Pterophyta

The classes included in this division are given below with the important orders:

1. **Class Eusporangiopsida:** The class includes the following two orders:
   - **Order Ophioglossales:** The order is represented by two important genera *Ophioglossum* and *Botrychium.*
   - **Order Marattiales** It includes six genera of which *Marattia* is well known.
2. **Class Protoleptosporangiopsida** The class includes the following order:
   - **Order Osmundales:** It is represented by three living genera of which *Osmunda* is widely distributed.
3. **Class Leptosporangiopsida**: It is divided into three orders:
   - **Order Filicales:** Almost all the members of this order are homosporous. The green thallus like prothallus bear sex organs on the lower surface. The sporangia are borne in groups called the sori on the margin or abaxial face of the sporophyll.
   - **Order Marsileales**: It includes heterosporous forms, for example, *Marsilea regnellidium*.
   - **Order Salviniales**: This order includes the heterosporous, free floating aquatic forms, for example, *Salvinia* and *Azolla*.
4. **Class Primopteropsida:** The class includes the many extinct genera of ferns. It includes the orders:

   Order Cladoxylales

   Order Coenopteridales

These were probably the ancestors of modern ferns and appeared in the mid and survived up to the end of the Palaeozoic.

Reimer's (1954) classified Pteridophytes or the seedless vascular plants as follows:

Pteridophyta- Seedless Vascular Plants

Class A PSILOPHYTOPSIDA

Order Psilophytales (Fossil)

Class B PSILOTOPSIDA

Order Psilotales

Class C LYCOPSIDA

Order 1. Protolepidodendrales (Fossil)

Order 2. Lepidodendrales (Fossil)

Order 3. Lycopodiales

Order 4 Selaginellales

Order 5 Isoetales

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Class D SPHENOPSIDA

Order 1. Hyeniales

Order 2. Sphenophyllales

Order 3. Calamitales

Order 4. Equisetales

Class E PTEROPSIDA

Subclass 1. Primofilices (Fossil)

Order 1. Cladoxylales (Fossil)

Order 2. Coenopteridales (Fossil)

Subclass 2. Eusporangiatae

Order 1. Marattiales

Order 2. Ophioglossales

Subclass 3. Osmundidae

Order Osmundales

Subclass 4. Leptosporangiatae

Order 1. Filicales

Order 2. Marsileales

Order 3 Salviniales

Cronquist, Takhtajan and Zimmermann (1966) classified Pteridophytes into following Five Divisions

1. Division. Rhyniophyta

   Class. Rhyniatae

   Order. Rhyniales

2. Division: Psilotophyta

   Class: Psilotatae

   Order: Psilotales

3. Division: Lycopodiophyta

   Class: Lycopodiatae (Orders. Asteroxylales, Drepanophycales and Lycopodiales)

   Class: Isoetatae (Orders. Lepidodendrales, Selaginellales, Pleuromiales, Isoetales)

4. Division: Equisetophyta

   Class: Sphenophyllatae (Orders- Sphenophyllales, Psudeoborniales)

   Class: Equisetatae (Orders- Calamitales, Equisetales)

5. Division – Polypodiophyta

   Class- Polypodiatae (Subclasses. Protopteridiidae, Ophioglossiidae, Marrattiidae, Polypodiidae, Marsilleidae, Salviniidae)

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Bierhorst (1971) followed the following system of classification:

Division—Tracheophyta

Class—Rhyniopsida

Order—Rhyniales

Class— Zosterophyllopsida

Order—Zosterophyllales

Class- Lycopodiopsida

Orders—Asteroxylales, Lycopodiales, Selaginellales, Lepidodendrales, Isoetales

Class- Cladoxylopsida

Order—Cladoxylales

Class- Equisetopsida

Order- Hyeniales, Spenophyllales, Equisetales, Psuedoborniales

Class- Coenopteridopsida

Order- Coenopteridales

Class- Filicopsida

Order- Filicales, Marsileales, Salviniales

Class- Ophioglossopsida

Order- Ophioglossales

Class- Marattiopsida

Order- Marattiales

Sporne in 1975 suggested a system of classification in which he divided pteridophytes into six classes instead of five.

These classes are Psilopsida, Psilotopsida, Lycopsida, Sphenopsida, Pteropsida, and Progymnospermopsida. Sporne's system of classification is actually a modification of the Reimer's (1954) system.

According to Parihar (1996), the classification system of Pteridophytes has undergone vast changes during last five decades and modern system of classification divides pteridophytes into seven divisions as: Rhyniophyta, Trimerophytes, Zosterophyllophyta, Psilotophyta, Lycopodiophyta, Equisetophyta and Polypodiophyta.

Check Your Progress

4. What characteristics does Tracheophyta possess?
5. What were Sinnott contribution?

7.4 STELAR ORGANIZATION

NOTES

The term '*stele*' is Greek word meaning 'Pillar' was used for the first time by Van-Tieghem and Douliot (1866) to designate the unit of vascular system. It is applicable only to the primary vasculature of shoot and root, particularly of lower plants such as pteridophytes. In case of higher plants, such as gymnosperms and angiosperms the term stele cannot be applied.

The central vascular cylinder of the primary axis of pteridophytes is usually referred to as stele. Besides xylem and phloem, it also includes pith and is bounded by pericycle. The concept of the stele as the fundamental unit of vascular system put forward by Van Tieghem and Douliot (1886) is the basis of a theory known as stelar theory.

According to this theory the primary bodies of the stem and root are basically alike in gross anatomy, as each consists of a central cylinder, the stele, surrounded by cortex. The stele consists of the vascular tissues, the pericycle and the pith. The stele and the cortex are separated from each other by the endodermis. It means endodermis constitutes the innermost layer of the cortex and the pericycle the outermost portion of the stele. The idea of the stellar system was widely accepted by scientists and proved to be useful in the anatomical and phylogenetic studies of the vascular plants.

Stelar theory gives a picture that stele or central cylinder is present in adult stem and stele is a real entity but has not been able to explain the vascular system of higher vascular plants like ferns, gymnosperms and angiosperms, where leaf traces are large in relation to the vascular system of the axis and appears to play major role in the structure of axial vascular system. In such plants, if the large leaf traces are to be considered as units of structure, then the stele of the stem would be a composite structure.

In some of the lower vascular plants, such as *Selaginella* and *Lycopodium* (Microphyllous Plants) the leaves are simple and small and their weak and small leaf traces are connected at the periphery to the well-developed vascular cylinder of the stem. But in higher plants like ferns, gymnosperms and angiosperms the leaf traces are large and the vascular system of the stem is greatly influenced in its form by the development of large leaves.

The stele of the stem in Filicophyta is a composite structure with both cauline and foliar vascular components Wetmore (1953) consider that at least in some ferns the vascular tissue in the stem is foliar in origin. Sterling (1945) consider that in gymnosperms and angiosperms the primary vascular system of the stem is associated with the leaves and is described as a system of interconnected leaf traces. The stele may be defined as the central core of axis which includes primary vascular tissues (xylem and phloem) with or without pith and some surrounding fundamental tissues such as pericycle.

Types of Stele and Evolution of Steler System

The simplest and most primitive type of stele consists of a central solid core of xylem surrounded by phloem followed by pericycle. Such stele is called protostele. The other types of steles have been derived from it by appearance of pith or leaf and branch gaps during the course of evolution. Jeffrey recognized two main types of steles in pteridophytes, i.e., protostele and siphonostele

1. Protostele

It is the simplest and most primitive type of stele found in pteridophytes. It consists of central solid core of xylem surrounded by phloem followed by pericycle. Such a stele is found in the juvenile stage of sporophytes in almost all the pteridophytes. The protostele is found in the adult, sporophytes of many pteridophytes such as species of *Lycopodium, Selaginella,* and *Psilotum,* etc.

Brebner has divided the protostele into two main types- Haplostele and Actinostele.

(i) Haplostele: It is the simplest type of protostele in which the central solid core of xylem is surrounded by a uniform layer of phloem. The pith is absent. The protoxylem usually faces towards periphery. The haplostele may be monarch, diarch or polyarch depending upon the number of protoxylem points. This type of stele is found in primitive fossil forms of psilophyta like *Rhynia, Horneophyton* and number of living pteridophytes.

(ii) Actinostele: It is derived from haplostele. In this case the xylem is angular radiating arms. The protoxylem lies at the tip of each arm. The xylem is surrounded by phloem. The phloem is not continuous, but occurs in patches in the concavities of star shaped xylem.

In few cases the radiating arms of xylem deepen further so that the xylem becomes star shaped in transverse section. The phloem invaginates deeper into the concavities. Such a stele is called stellate actinostele.

In some cases, the xylem becomes dissected into several plate like structures. The xylem in a transverse section, appears in the form of separated parallel plates with phloem occupying the spaces between the two plates. Such a protostele is called plectostele, for example, *Lycopodium clavatum*.

In some cases the xylem becomes dissected into several pieces. In a transverse section it appears as if the xylem strands are embedded in the ground tissue of phloem. Such condition of stele is called mixed protostele, for example, *Lycopodium cernuum.*

2. Siphonostele

A siphon like stele in which the centre is occupied by pith surrounded by xylem, phloem and pericycle is called siphonostele. It is formed by medullation in the protostele. The pith may be parenchymatous, sclerenchymatous or a hollow cavity.

The siphonostele has been classified into two types:

(i) Ectophloic Siphonostele: In this case, the central pith is surrounded by xylem which is followed by phloem, pericycle and endodermis, for example *Osmunda.*

(ii) Amphiphloic Siphonostele: In this case, the ring of xylem is surrounded on both outer and inner sides by phloem, pericycle and endodermis. The arrangement of various tissues in an amphiphloic siphonostele from periphery towards centre is – outer endodermis, outer pericycle, outer phloem, xylem, inner phloem, inner pericycle, inner endodermis and pith.

If siphonostele is not perforated by leaf gap, it is called **cladosiphonic siphonostele**, for example, a few species of *Selaginella*. If it is perforated by a single leaf gap, it is called **phyllo siphonic siphonostele**, for example, *Nephrolepis.*

If it is perforated by a number of leaf gaps, which are scattered but not overlapping, it is called **solenostele**. A solenostele may be ectophloic or amphiphloic. A siphonostele perforated by several overlapping leaf gaps is called dictyostele. The examples are *Pteris, Dryopteris, Pteridium, Adiantum*, etc. In a cross section, the dictyostele shows the presence of several separate strands called meristeles. Each meristeles resembles protostele in its general structure.

A dictyostele consisting of two or more concentric rings of meristeles is called polycyclic dictyostele, for example, *Pteridium aquilinum.*

The vascular system consisting of much dissected siphonostele having its vascular strands separated apart by ground parenchyma or medullary ray is called eustele, for example, internodes of Equisetum. This type of stele is found in highly evolved vascular plants, such as angiosperms.

7.4.1 Origin of Siphonostele from Protostele

There is a general acceptance that the siphonostele has evolved from a protostele. Two theories have been proposed accounting the phylogenetic origin of the pith

- **Intrastelar Theory:** This theory is supported by Boodle (1901), Bower (1911), Petry (1914) and others. According to this theory, the pith originates by metamorphosis of the inner vascular elements into parenchyma. Support for this theory is from a number of plants in which the central region of the protostele consists of both tracheids and parenchyma cells.
- **Extrastellar Theory:** This theory was put forth by Jeffrey (1897, 1902, 1917). According to this theory, the pith is extrastellar in origin and the pith has originated as a result of the invasion of cortical tissues into the stele through the leaf gaps and branch gaps in the course of phylogenetic development of the vascular plants. According to this theory the pith is cortical in nature.

The main arguments in favour of the first hypothesis is derived from ontogenetic and comparative study of living as well as fossil forms. In small stems of Schizaea it is found that at the base the structure is typically protostelic, slightly higher up a mass of parenchyma cells occupies the centre of the xylem. This central parenchymatous mass may be called a pith. During the establishment of the central parenchymatous mass it was also found that the vascular system is completely surrounded by endodermis and there is no possibility of any readjustment of tissues in nature of flow or intrusion of parenchyma from outside the stele. Even isolated tracheids found to be scattered in the parenchymatous pith near the inner boundary of the xylem. Sometimes the tracheids may be scattered throughout the pith, in stems where it consists normally of parenchyma only, for example, in Osmunda regalis. This condition is called mixed pith and found in primitive living ferns, for example, Osmunda. This type of stele with mixed pith is though to be intermediate between a true protostele and a true siphonostele.

The second hypothesis, i.e., extrastellar origin of pith was given by Jeffrey. In genera like *Phylloglossum, Selaginella laevigata*, there is present inner endodermis at the inner face of the vascular tissue and as endodermis is a structure peculiar to cortex, so the pith is cortical in origin. According to Jeffrey (1917) in some genera there is also very close similarity in the structure of the pith and cortex, for example, in *Psilotum and Tmesipteris*, the medullary tissue is composed to a large extent of brown sclerenchymatous tissue resembling similar structure found in external fundamental tissue. On the basis of extrastellar theory it is difficult to interpret the occurrence of mixed pith, as well as to envisage the type of invasion of cortex that would produce such complicated stellar structures as polycyclic siphonostele.

Modifications of Siphonostele

Depending on the presence of non-overlapping or overlapping gaps, the siphonostele is of three types these are

- **Solenostele:** A siphonostele with non-overlapping leaf gaps is called solenostele. The solenostele may be ectophloic and amphiphloic. Usually in the lower part of the stem where leaves are sparsely placed, the stele is solenostele.
- **Dictyostele:** In ferns like Dryopteris, Pteris, Ophioglossum, etc. there is a very small rhizome with crowded leaves and so the leaf gaps overlap with each other. Such a siphonostele with overlapping leaf gaps is called dictyostele. It has many scattered vascular strands. Each strand is called meristele.
- **Polycyclic Stele:** Stelar system of certain pteridophytes is still more complex. They have two or more concentric rings of vascular tissue and are polycyclic stele, for example, there are two rings of vascular tissue in *Pteridium aquilinum*, three rings in *Montania pectinata.*

If in a polycyclic stele the outer cylinder is solenostele, it is called polycyclic solenostele and if outer cylinder is dictyostele, it is known as polycyclic dictyostele.

Perforated Dictyostele

In some pteridophytes, the gaps occur in the stele which are not associated with leaf traces. Such gaps are called perforations. Such type of dictyostele is called perforated dictyostele, for example, leptosporangiate ferns like *Polypodium.*

Acyclostele

In some ferns in addition to the main solenostele or dictyostele, some meristeles are found in the pith, or rarely in the cortex. These meristeles are not arranged in any cycle but are more or less irregularly scattered. Such stele is called **acyclostele**, for example, *Cyathea, Alsophila.*

In the Cladoxylales the stellar system is very complex. It consists of a number of radially elongated meristeles, whose inner ends are free or connected with each other in different manners, such as V or W form.

Eustele

Brebner (1902) reported another modification of siphonostele in which the vascular system consists of a ring of collateral or bicollateral strands including the pericycle and medullary ground tissue with the leaf gaps and the interfascicular areas are not clearly delimited from one another. This is highly evolved type of stele. It is found in gymnosperms and dicots. A condition of secondary specialization is found in Cucurbitaceae and Solanaceae family where vascular bundles are bicollateral, i.e., Phloem is present on both the sides of xylem.

Atactostele

The most complex stele is the atactostele, for example, in Monocotyledons. Brebner (1902) described it as the monostele of typical monocotyledons consisting of a number of scattered strands embedded in the ground tissue.

Leaf and Branch Gaps

In ferns, gymnosperms and angiosperms siphonostelic central cylinder has parenchymatous areas in the tubular cylinder which correspond to traces going to leaves or branches. Where the leaf traces diverges into a leaf it appears as though a section of the vascular cylinder of the stem is deviated to one side and just above the point of departure of the leaf trace, gaps appear in the vascular cylinder of stem. These parenchymatous areas or gaps persist only for a small distance and higher up the vascular tissue is present in direct line above the diverged leaf trace. Such parenchymatous areas in the vascular system of the stem located adaxially around and above the point of departure of the leaf traces and branch traces are known as leaf gaps and branch gaps respectively. These gaps are not the breaks in the continuity of the axial vasculature, because lateral connections occur between the tissues above and below the gaps. Around the edges the endodermis, pericycle and usually the phloem of the inside become continuous with that of the outside and through the gaps the pith and cortex become continuous (Refer Figure 7.3).

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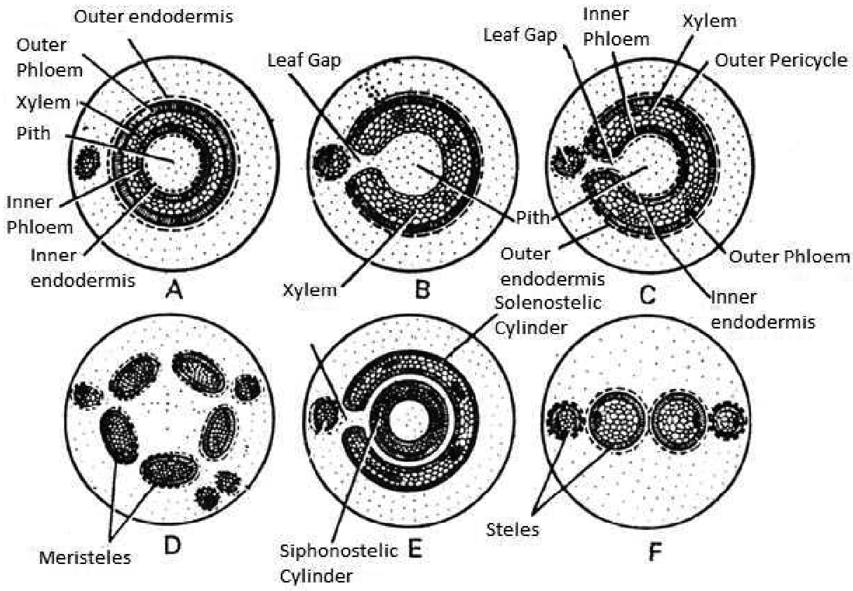

Fig. 7.3 *Leaf and Branch Gaps in Atactostele*

Check Your Progress

6. What is leaf gap?
7. What is solenostele?

7.5 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Pteridophytes can be terrestrial, aquatic or xerophytic in nature.
2. Most of the xerophytic plants are reported from the genus *Selaginella.* The common Indian xerophytic species are *S. wightii* and *S. repanda.*
3. The pteridophytes are treated as vascular cryptogams as they have well developed conducting system. The lower cryptogams have no conducting system.
4. Tracheophyta is characterized by following characters:
   - Vascular system is well developed in sporophytic plant body
   - Sporophyte is the dominant generation.
   - For most of the part of life cycle, sporophyte is independent of gametophyte.
5. Sinnott, in 1935 introduced a new term 'Tracheophyta' to include all plants having vascular tissue (xylem and phloem).
6. Leaf gap is space in the stem of a plant through which the leaf grows.
7. Solenostele is most highly developed type of stele present in Selaginella and arranged as polystele**.**

7.6 SUMMARY

- The vascular cryptogams possess an independent sporophyte with a vascular system.
- All living pteridophytes are herbaceous except few woody ferns.
- Vascular cryptogams are assemblage of seedless vascular plants that have invaded the land and reproduce by means of spores.
- The sporophyte in the vascular cryptogams is no doubt independent but depend upon the gametophyte during its earlier stages of development.
- Both homosporous and heterosporous vascular plants exhibit heteromorphic type of alteration of generation because the sporophyte and the gametophyte individuals present marked morphological and anatomical differences.
- The microspores are produced in large numbers and are smaller in size, they germinate to develop two celled prothallus that bears antheridium which produces antherozoids.
- Megaspores are large in size and produce female prothallus. Chances of self-fertilization is rare and cross fertilization is more. This leads to outbreeding and to variations and rapid evolution.
- Psilophyta is rootless, dichotomously branched with rhizomes, with erect aerial branches, thick walled homosporous sporangia, grow on ground or as epiphytes.
- Sphenophyta contain roots, rhizomatous with erect branches leaves are whorled, thick walled homosporous aggregated in strobilus, generally perennial.
- Lycopodiophyta are creeping, erect or pendulous, spirally arranged leaves, thick walled homosporous, endosporic gametophytes and widely distributed.
- Pterophyta contain thick or thin walled leptosporangiate, heterosporous, free living or endosporic ferns, epiphytic, aquatic or terrestrial in nature.
- The stele contains vascular tissues, pericycle and the pith.
- Stele is present in stem but has not been able to explain the vascular system of higher plants like ferns, gymnosperms and angiosperms where leaf traces are large in relation to vascular system of axis.
- Schmidt suggested two main types of steles in pteridophytes, i.e., Protostele and Siphonostele.
- Protostele are of two types, i.e., Haplostele and actinostele.
- Protostele is not sufficient to meet the requirements of large diameter of stem so modified protostele with central pith is called siphonostele.
- Siphonostele is of two types, i.e., Cladosiphonic and Phyllo Siphonic.
- Solenostele is siphonostele with non-overlapping leaf gaps.

7.7 KEY TERMS

- **Gametophyte:** The gamete producing and usually haploid phase, producing the zygote from which the sporophyte arises.

- **Sporophyte:** The asexual and usually diploid phase producing spores from which gametophyte arises.
- **Meiosis:** Process of reduction division where single cell divides to produce four cells with half the original amount of genetic information.
- **Apospory:** Development of gametophytes without meiosis and spores from vegetative cells of the sporophyte.
- **Apogamy:** Type of reproduction occurring in ferns in which sporophyte develops from the gametophyte.
- **Rhyniophyta:** Subdivision of extinct early vascular plants that are considered to be similar to the genus Rhynia.
- **Lycophyta:** It is extant vascular plants and contains extinct plants.
- **Sphenophyta:** Also known as horsetails, vascular plants with a fossil record present during Devonian period.
- **Pterophyta:** Member of vascular plants that reproduce via spores and have neither seeds nor flowers.
- **Plectostele:** An actinostele where xylem structure is arranged in parallel plates.
- **Actinostele:** A vascular core having xylem and phloem in alternating or radial groups within a pericycle.
- **Siphonostele:** A stele system where well developed pith is present.

7.8 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. Describe in brief about the habitat of pteridophytes.
2. Write few general characteristics of sporophytes.
3. What are gametophytes?
4. What causes apospory?
5. What are the similarities between pteridophytes and spermatophytes?

Long-Answer Questions

1. Explain the general characters of pteridophytes.
2. Detail a note on reproduction in pteridophytes.
3. Explain the life cycle of pteridophytes.
4. Discuss about the classification of pteridophytes.
5. Explain in detail about Reimers system of classification
6. Explain the stellar system in Pteridophytes.

7.9 FURTHER READING

NOTES

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UNIT 8 SALIENT FEATURES OF SOME PTERIDOPHYTES

NOTES

Structure

8.0 INTRODUCTION

Pteridophytes are a phylum of plants. They are the vascular plants, those having xylem and phloem tissues that reproduce by releasing spores rather than seeds, and they include the highly diverse true ferns and other graceful, primarily forest-dwelling plants. There are about eleven thousand different species of pteridophytes, making them the most diverse land plants after the flowering plants, i.e., angiosperms. Pteridophytes may represent the closest living relatives, sister group to the seed plants. Seed plants include the angiosperms, the conifers, and a smaller assortment of other plants.

As in seed plants, the greatest diversity of pteridophytes is found in the tropics, with only about six hundred species adapted for life in temperate climates. Species living today are relics of ancient lineages that once dominated the land surface. There is a rich fossil record showing that pteridophytes have ancestors dating back nearly four hundred million years. Before there were seed plants, there were pteridophytes, such as large, tree like, up to 36.5 meters (120 feet) tall. Lepidodendron, an ancestor of modern club mosses, which are no more than 0.30 meter (1 foot) tall and shrubby *Sphenophyllum*, a forebear of today's horsetails. Some of the ancient predecessors of modern ferns were also preserved, but there are comparatively few fossils for interpreting relationships among the approximately eleven thousand species of true ferns. These relatively young species probably arose along with the other most recent lineages of vascular plants, the angiosperms or flowering plants, another group lacking an extensive fossil record.

In this unit, you will study about the salient features of various orders like Psilophytales, Psilotales, Lepidodendrales, Isoetales, Sphenophyllales, Ophioglossales and Filicales in detail.

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8.1 OBJECTIVES

After going through this unit, you will be able to:

- Discuss about various orders of pteridophytes
- Explain the salient features of the some orders like Psilophytales, Psilotales, Lepidodendrales, Isoetales, Sphenophyllales, Ophioglossales and Filicales

8.2 PTERIDOPHYTES: BASIC INTRODUCTION AND SALIENT FEATURES OF SOME ORDERS

Salient features of the orders Psilophytales, Psilotales, Lepidodendrales, Isoetales, Sphenophyllales, Ophioglossales and Filicales are discussed below.

8.2.1 Psilophytales

The first fossils of vascular plants were described in 1859 by Canadian geologist Dawson from Devonian peninsula of Canada. The compressions were fragmentary, but Dawson's reconstruction revealed slender axes arising from a subterranean rhizome system. The rhizome lacked roots but had rhizoids. The aerial axes were dichotomously branched with scale like appendages and terminated into sporangia**.** Because of its similarity with Psilotum Dawson named the plant *Psilophyton princeps*. In 1917, when Kidston and Lang described similar plants from Lower Devonian red sandstone cherts of village Rhynie, it was realized that plants of such simple organization had existed and the group psilophytales was instituted to include them. Rhynia gwynne-vaughanii was small plant of up to 17 cm in height. The aerial dichotomous axes arose from subterranean horizontal rhizome. The shoot system lacked differentiation into stem and root, the rhizome instead bore rhizoids. The aerial axes terminated into sporangia and lacked leaves. Rhynia major was larger and attained height up to three times than that of Rhynia gwynne-vaughanii. The aerial axes were bare, i.e., lacked leaves as well as projections.

Another member of Rhynie flora is Asteroxylon mackie. The basal portion of the plant was a naked dichotomously branched rhizome lacking rhizoids. Smaller rhizome branches acted as anchoring and absorbing organs. The erect aerial axes branched monopodially, lateral branches were dichotomous. The stem cortex were divisible into three zones, the outer and inner cortical regions consist of compact parenchyma, the middle cortex is trabecular, a wide space traversed by plates of tissue. The vascular cylinder is actinostelic.

Rhyniales are the oldest vascular plants, Leclercq (1954), remarked that Devonian Rhynie in general and Rhynia in particular is by no means a representative

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of the earliest land plants. More complex forms (Asteroxylon) coexisted with Rhynia in Devonian times and more complex forms preceded them in fossil record. Therefore the primitive status of Rhynia is questionable. It is possible that Rhynia attained its present structure and without evolving further has existed in this form for millions of years, in that case it is truly primitive. More significant about Rhynia is its simple nature and it is unquestionable. It lacked differentiation into stem and roots, had uniform dichotomous branching and its appendages cannot be called leaves. Its stele was a slender strand of tracheids. The sporangia were modified branch tips. It is a type which is as simple as one could conceive a vascular plant to be.

External Features of Rhynia

- Plant body was sporophytic.
- *Rhynia major* was bigger and attained a height of 50 cm. with a diameter of 1.5 to 6 mm, while *Rhynia gwynne-vaughanii* (Refer Figure 8.1) had a height of 20 cm. and a diameter of 1 to 3 mm.

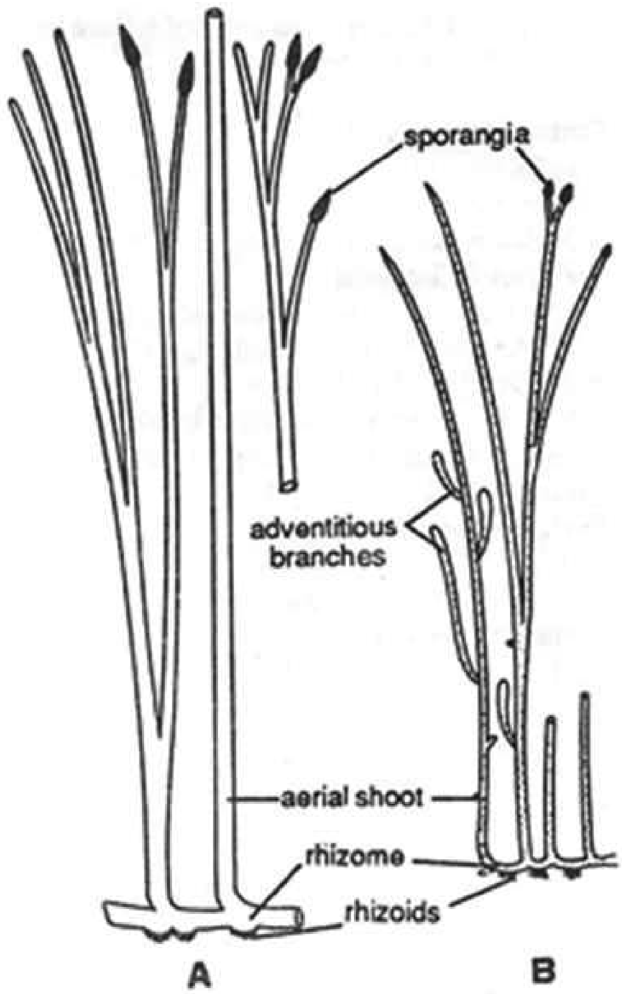

Fig. 8.1 *Rhynia, External Features (A).R Major; (B) R, Gwynne-Vaughanii*

- Plants had a rhizome which was dichotomously branched.
- From rhizome developed many dichotomously branched erect aerial shoots towards the upper side while many rhizoids towards the lower side.
- There were no roots on the plants.
- Aerial shoots of Rhynia major were smooth and devoid of leaf or any other outgrowth, while in case of Rhynia gwynne-vaughanii many adventitious branches were present on the aerial shoots.
- Aerial shoots were either ending into simple vegetative tips or having terminal sporangia.

Anatomy of Rhynia

- Rhizome and aerial shoots of the plant were internally differentiated into epidermis, cortex and stele.
- Stele was of a simple protostele type, i.e., central column of xylem was surrounded by phloem.
- Xylem was made up of only tracheids.
- Phloem was 4 to 5-cells in thickness.
- There was no endodermis in between cortex and stele.
- Cortex was differentiated into outer and inner cortex, and was about 10 times more in thickness than stele.
- The inner cortex was made up of many spherical cells having large intercellular spaces.

8.2.2 Psilotales

It is an interesting group of plants; its representatives are similar in structural organization to that of early land plants. The group is little understood due to restricted distribution of its members. The order comprises two genera, Psilotum and Tmesipteris. Psilotum is a pendulous epiphyte, rare in distribution, restricted to Jamaica, Mexico, Hawaii and few Pacific Islands.

The plant consists of a subterranean rhizome and dichotomously branched aerial axes. The rhizome anchors the plant and is cylindrical, prostrate, dichotomously branched mycorrhizae structure with numerous rhizoids. The plants lack roots and instead rhizoids are 1-3 cells long, serve for absorption. Some branches of rhizome turn upwards and develop into aerial green branches of regular dichotomy**.** On aerial branches, except the base are present small awl shaped leaf like appendages which are without any vascular trace or stomata.

Stout aerial branches bear large conspicuous sporangia. Each sporangium is a 3 lobed structure borne on forked appendage and is synangium; group of fused sporangia with distinct partition walls. At times, the main axis also terminates into clusters of synangia each with variable number of sporangia. It is regarded as bifid sporophyll or a short lateral branch. A synangium on the outside of its loculi is bound by a single layer of large cells, followed by it are few layers of smaller cells. A vascular bundle extends into synangium and subdivides into three parts, corresponding to three sporangia. The sporangium is eusporangiate in development. The primordium of a fertile appendage is nearly of the same size as that of apex. It becomes two lobed. Immediately below the apex of appendage differentiate three groups of sporangial initials which divide periclinally to form wall layers and sporogenous tissue. A part of sporogenous tissue serve as nourishment to the sporocytes. The numerous colourless kidney shaped spores are formed in tetrads are all alike. And disperse through slits in radial walls of the synangium. The spores germinate after four months and form gametophytes. During spore germination no rhizoid is formed and after few divisions an apical cell is established which initiates the axial growth of the gametophyte. The gametophyte are irregularly branched cylindrical structures covered with rhizoids. The gametophyte is a simple parenchymatous structure with a prominent apical cell. It lacks differentiation and quite early in its development comes to have mycorrhizae association. The entire gametophytic surface including rhizoids has a cuticular covering. Sex organs antheridia and archegonia occur in large numbers all over the gametophyte. The archegonium is embedded in gametophyte at its venter, the neck is projecting. The antheridium is large superficial structure with one cell thick jacket of variable cells and encloses numerous spirally coiled multi-flagellate antherozoids. First division of zygote is transverse forming outer epibasal cell and inner hypobasal cell. The latter divides repeated to form a lobed foot and from the former is derived the rhizome. This embryonic pattern in which shoot forming apical cell is directed outwards is called exoscopic and is characteristic of liverworts and mosses and is uncommon in pteridophytes. Figure 8.2 shows Psilotum plant.

***Fig. 8.2** Psilotum*

Salient Features of Psilotum

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- The sporophytes are dichotomously branched with an underground rhizome and upright branches.
- The upright branches are leafless.
- Rhizoids present instead of roots.
- Stem have a relatively simple vascular cylinder.
- The sporangia are borne in groups (trilocular) and form synangia.
- Spores produced are all alike (homosporous).
- The development of gametophyte is exosporic and form monoecious subterranean gametophyte.
- The development of embryo is exoscopic.

Sporophyte

The plant body of Psilotum is sporophytic branched rhizome system and dichotomously branched, slender, upright, green aerial systems that bears small appendages and synangia (Singular: Synangium).

Aerial Stem

Any one of the rhizome tips may turn upward and undergo several dichotomies to give rise to a green aerial shoot. The aerial shoots are slender, generally erect but may be pendent in epiphytes (*P. flaccidum)*. They are perennial and become shrubby by repeated dichotomies and sometimes attain a height up to one meter.

The aerial axis may be cylindrical at base, furrowed in the upper parts (wavy in C.S.), but somewhat flattened with three longitudinal ridges (triangular in C.S.) at the top. The basal part of the aerial axes is smooth but the distal part bears small, scaly appendages and synangia (Refer Figure 8.3).

NOTES

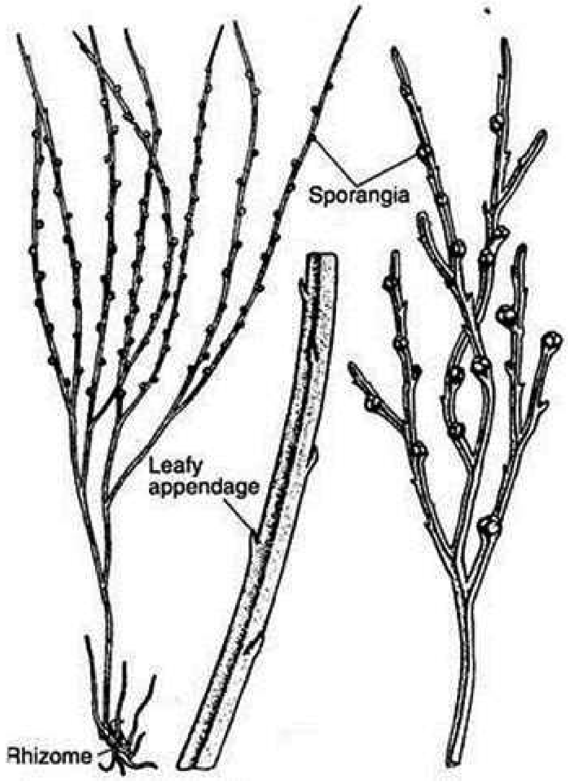

Fig. 8.3 *Psilotum nudum*

The aerial stems are photosynthetic and the general appearance is xerophytic although the plants grow in very moist environment.

In T.S., the aerial system is covered by an epidermis, followed by extensive cortical areas, single-layered endodermis and stele. The stele is siphonostelic in the basal part which becomes actinostelic in the younger branches

The epidermis is single-layered, in which the outer tangential cell walls are heavily cutinised and covered by a layer of cuticle. The epidermis is broken regularly by stomata (Refer Figure 8.4). The stomata of *Psilotum nudum* do not have subsidiary cells.

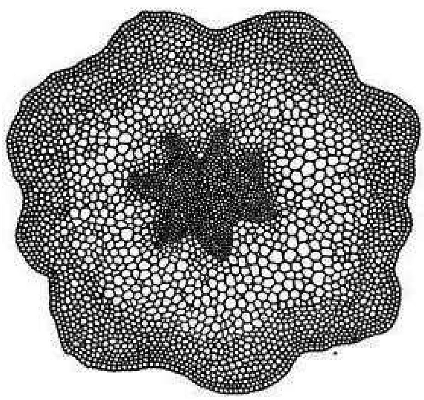

Fig. 8.4 *T.S. of Stem of Psilotum nudum*

NOTES

The cortex is extensive and is divisible into three regions (Refer Figure 8.5). The outer portion (chlorenchymatous) directly beneath the epidermis consists of elongated, lobed chlorophyllous cells with intercellular spaces. This region is two- to five-layered thick and the cells are full of chloroplastida and starch grains.

Internal to this zone, there is a middle cortex of 4 to 5 layers of vertically elongated thick-walled sclerenchymatous cells without intercellular spaces. The walls of these cells apparently become lignified in the lower portion of the aerial stem.

These tissues provide mechanical support to the plant. Further inwards, there is a broader zone of parenchymatous cells (the inner cortex), the cell walls of which become thinner and thinner towards the centre. These cells are without intercellular space but contain more starch grains.

The cortical tissue is bounded inwardly by a single-layered endodermis whose vertically elongated cells have a conspicuous Casparian bands on the radial and end walls.

The centre of the stem is occupied by a ridged or flattened cylinder of primary xylem with protoxylem elements at the tip of each ridge. This cylinder may have as many as ten ridges near the transition region from rhizome to aerial stem. Fewer ridges are present in the more distal parts of the aerial stem.

A single-layered parenchymatous pericycle is present just below the endodermal layer. The phloem is internal to the pericycle and located between the ridges of the xylem.

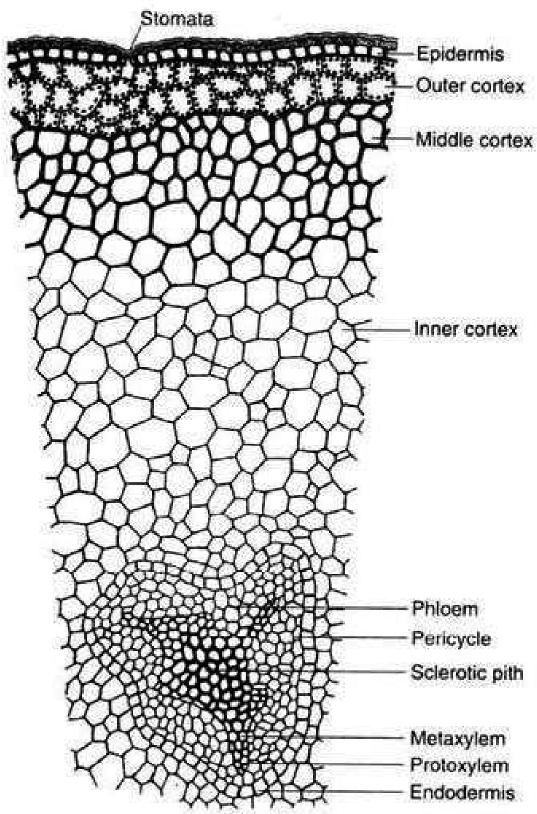

Fig. 8.5 *An Enlarged View of a Segment of T.S. of Psilotum nudum Stem*

At the extreme base, the stem is Protostelic (Actinostelic). In the middle portion the stele is Siphonostelic as the centre of the xylem is occupied by a patch of elongated Sclerenchymatous Cells (Sclerotic Pith).

Rhizome

The basal subterranean branched rhizome is generally hidden beneath the soil or humus. It bears numerous rhizoids, instead of roots, which perform the functions of absorption and anchorage.

In T. S., the rhizome reveals an outermost epidermis, cortex, endodermis, pericycle and stele (Refer Figure 8.6). The epidermis is indistinct and gives rise to 2-celled rhizoid. The cortex is extensive, parenchymatous and differentiated into outer, middle and inner layers.

The outer cortical layer is characterised by the presence of intracellular endophytic mycorrhiza. The cells of middle cortex are large with starch grains, while the cells of the inner cortex are often dark brown in colour because of the presence of phlobaphene (an oxidative product of tannins).

The stele is protostelic (haplostele or actinostele) and is surrounded by a well-defined endodermal layer with conspicuous casparian bands on the radial walls.

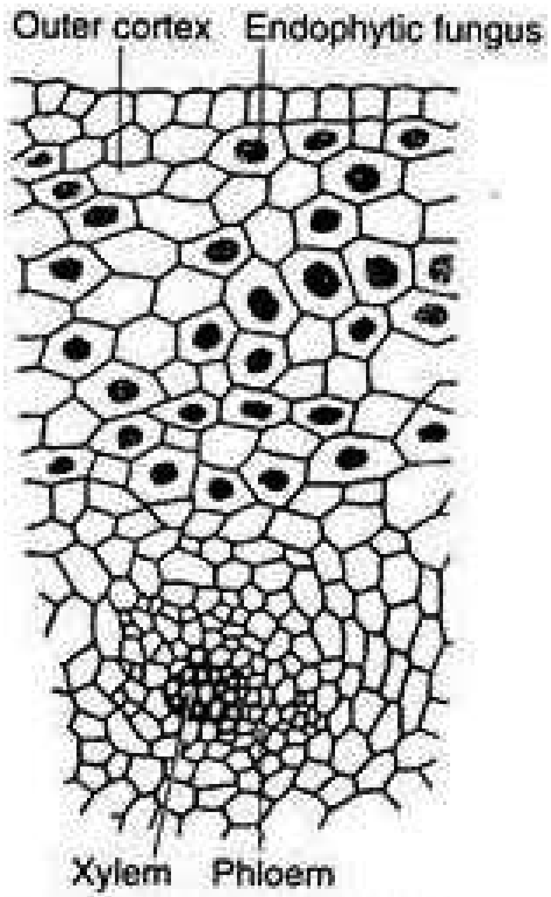

Fig. 8.6 *T.S. of Rhizome of Psilotum nudum*

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Appendages

These are small scale-like structures helically arranged on the upper part of the aerial system. Internally, the appendage is composed of parenchymatous photosynthetic cells, bounded by a single-layered cutinised epidermis. There is no stomata in the appendages. There is no vascular trace in the appendages of *P. nudum*, although minute leaf traces originate from stele in *P. flaccidum* which fade out in cortex.

Reproduction in Psilotum

The Psilotum reproduces vegetatively as well as by spores.

Vegetative Reproduction

The sporophyte as well as gametophyte of Psilotum, for example *P. nudum* propagate vegetatively through the production of Gemmae (Refer Figure 8.7). They are small, multicellular and ovoid structures developing on surface of rhizome (in sporophytic plant body) or prothallus (in the gametophyte).

After detachment from the parent body, gemmae of sporophyte may germinate to form a subterranean shoot, while the gemmae of prothallus, on germination, form a new prothallus.

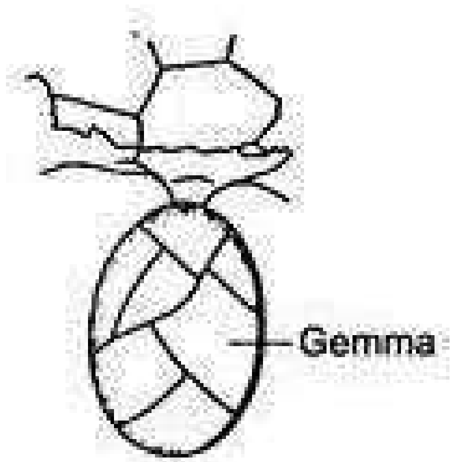

Fig. 8.7 *A Gemma of Psilotum*

Reproduction by Spores

Spore-Producing Structure

At maturity, many of the dichotomously branched aerial shoots become fertile and produce trilocular sporangia known as synangia. The mature synangium is generally a three-lobed structure and each lobe of the synangium corresponds to a sporangium.

The synangia located at the tip of very short axis, measuring 1-2 mm in diameter and closely associated with a forked, foliar appendage. At maturity, the synangium exhibits loculicidal dehiscence.

NOTES

Development of Sporangium

The mode of development of each sporangium of the synangium of Psilotum nudum is of eusporangiate type. Each sporangium develops from a group of superficial initial cells which divide periclinally to produce primary wall initials and primary sporogenous cells.

A sporangial wall of four or five layers is produced through repeated periclinal and anticlinal divisions of the primary wall initials. The sporogenous tissue is produced from repeated divisions of primary sporogenous cells in various planes (Refer Figures 8.8 and 8.9).

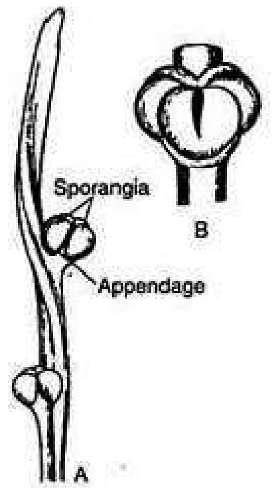

Fig. 8.8 *Psilotum nudum; (A) Fertile Axis Bearing Sporangia with Bifid Appendages; (B) A Trilocular Synangia Showing Dehiscence*

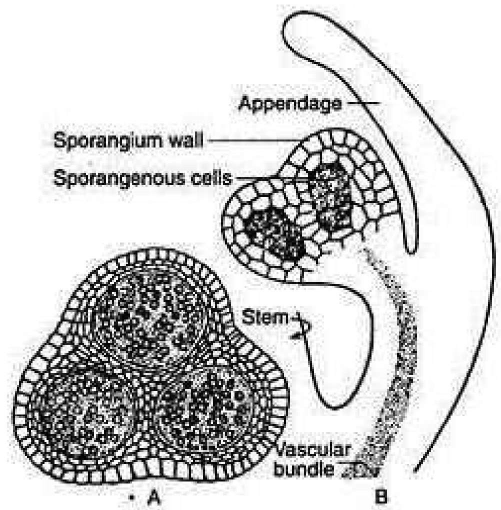

Fig. 8.9 *Psilotum nudum, (A) T.S of Synangium, (B) V.S. Of Fertile Axis through a Synangium*

Further development of sporangenous tissue gives rise to spore mother cells and numerous spores are produced as a result of meiosis of the spore mother cells. The spores are of equal size and shape, i.e., homosporous, bilaterally symmetrical, colourless and kidney-shaped with monolete aperture.

Gametophyte

The mature gametophyte shows a striking similarity with a piece of sporophytic rhizome. It grows as saprophyte with an associated fungus.

Spores germinate exosporically to form the gametophyte. The mature gametophytes are brown, cylindrical, subterranean, radially symmetrical and usually dichotomously branched, but may sometimes become irregular. The surface of the gametophyte is covered by long unicellular, brownish rhizoids. The gametophyte grows by means of apical meristem (Refer Figure 8.10).

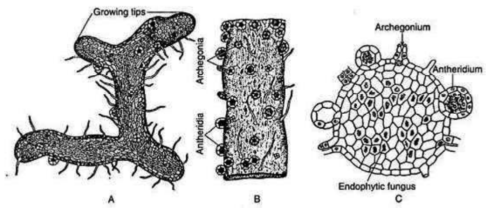

Fig. 8.10 *Psilotum nudum (A) Gametophyte; (B) Enlarged Portion of Gametophyte Showing Sex Organs and Rhizoids; (C) T.S. of Gametophyte*

In T.S., the gametophyte reveals cutinised peripheral cells which encloses many-layered thin-walled parenchymatous cells. Many of the internal parenchyma cells are filled with the hyphy of a symbiotic fungus. It has been observed that in the gametophyte of tetraploid P. nudum, the centre is occupied by xylem with annular, scalariform and reticulate tracheids, surrounded by phloem and an endodermis.

Thus, Psilotum is the only plant in the plant kingdom where the vascular tissues develop in the gametophytic generation. The external resemblance of the sporophytic rhizome and gametophyte, coupled with the presence of vascular tissue in the gametophyte, supported the homologous theory on the origin of alternation of generations.

Sex Organs

The gametophytes of Psilotum are monoecious, i.e., homothallic. Sex organs, i.e., antheridia and archegonia, are superficial and scattered over the surface of the gametophyte. Generally, antheridia are more in number than archegonia.

Antheridium

The antheridium develops from a single superficial cell (antheridial initial) of the prothallus. The periclinal division of the superficial cell produces an outer jacket initial and an inner primary androgonial cell.

The outer jacket initial undergoes repeated anticlinal divisions and forms a single-layered jacket. The inner primary androgonial cell divides in various planes and produces a mass of developing androgonial cells, the last generation of which are the androcytes.

At this stage, the antheridium projects above surface of the prothallus as a minute protuberance. Each androcyte eventually becomes a spirally-coiled, multiflagellate antherozoid and escapes by the disintegration of the opercular cell (Refer Figure 8.11).

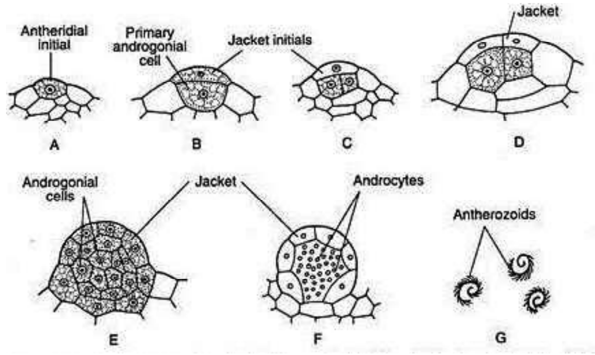

Fig. 8.11 *Psilotum nudum; (A)-(E) Stages in Development of Antheridium; (F), (A) Mature Antheridium; (G) Antherozoids*

Archegonium

The archegonium is also developed from a single superficial cell (archegonial initial) of the prothallus. The initial cell undergoes periclinal division to form an outer primary cover cell and an inner central cell. The anticlinal divisions followed by periclinal divisions of the outer cover cell produces a long projecting neck arranged in four vertical rows of cells with four to six cells in each row.

The central cell divides transversely to form an upper primary neck canal cell and a lower primary venter cell. The nucleus of the primary neck canal cell divides to form two neck canal nuclei and generally this division is not accompanied by wall formation resulting into a binucleate neck canal cell. The primary venter cell divides transversely to produce a large egg and a small ventral canal cell (Refer Figure 8.12)

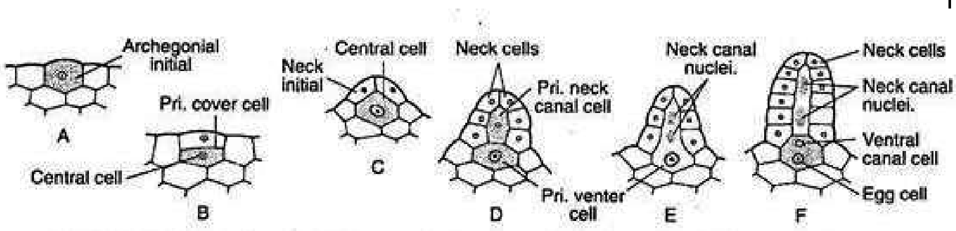

Fig. 8.12 *Psilotum nudum; (A)-(E) Stages in Development of Archegonium; (F), (A) Mature Archegonium*

Fertilisation

At maturity, the cell wall of the lower tier of neck cells becomes thick walled and cutinized. The apical tier, however, breaks away in presence of water and the mucilaginous contents of the neck cells are released. Thus, a free passage is formed for the entry of the antherozoids.

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Fertilisation is accomplished by the union of a multiflagellate sperm and egg, resulting in the formation of a diploid zygote (Refer Figures 8.13 and 8.14).

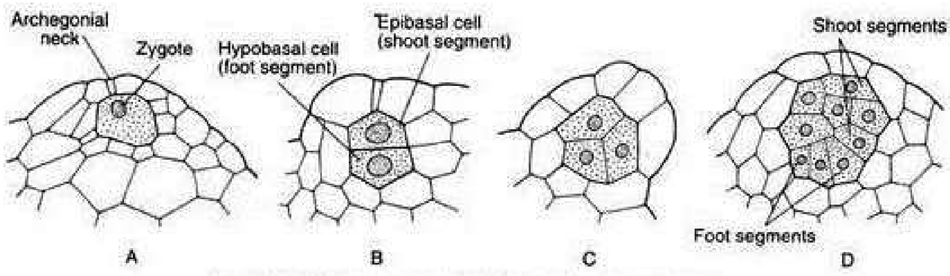

Fig. 8.13 *Psilotum nudum; (A) – (D) Stages in Early Embryogeny*

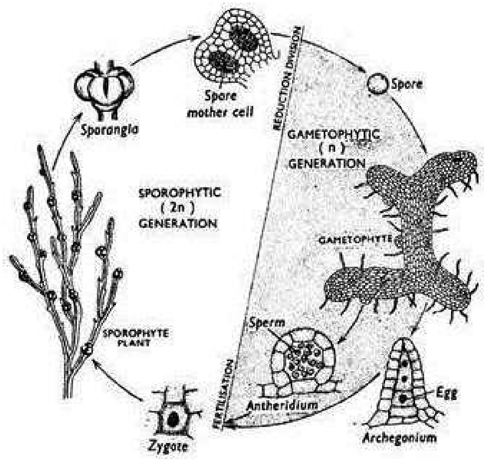

Fig. 8.14 *Life Cycle of Psilotum*

Embryo (New Sporophyte)

The diploid zygote is the mother cell of the sporophytic generation. The first division of the zygote is transverse, forming an outer epibasal cell (directed towards the neck of the archegonium) and an inner hypobasal cell (directed towards the base of the archegonium).

The apical epibasal cell ultimately gives rise to the sporophytic branch system (aerial and underground), while the lower hypobasal cell produces the foot. This type of embryogeny where the shoot forming apical cell is directed outward (towards the neck of the archegonium) is called exoscopic mode of embryo development.

The foot anchors the young sporophyte securely to the gametophyte and absorbs nutrients until the sporophyte becomes physiologically independent.

Phylogeny of Psilotum

Affinity with Ferns

Bierhorst (1971) placed *Psilotum* along with *Tmesipteris* within Filicopsida primarily on the basis of similarities with some ferns like *Gleichenia*, *Stromatolites*, etc., by the following characteristics:

- Axial nature of Gametophytes.
- Superficial position of Antheridia on the Prothallus.
- Exoscopic type of Embryogeny.
- Development of Sporangial Structure.
- Mutiflagellated Spermatozoids.

However, this has been highly criticised by several pteridologists. Except for the above characteristics Psitolates (Psilotum and Tmesipteris) differ from ferns in almost all principal characteristic features.

Moreover, Psilotales are root-less, leaf-less plants (Psilotum) or with microphyllous leaves (Tmesipteris) showing eusporangiate mode of sporangial development, while Gleichenia, Stromatoporoids have well-developed sporophyte with megaphyllous leaves and roots, showing leptosporangiate mode of sporangial development.

Affinity with Rhyniopsida

Psilotum and Tmesipteris resemble the earliest known Silurian-Devonian vascular plants — Rhyniopsida — in the following characteristic features:

- The sporophytes are dichotomously branched with subterranean rhizome and upright branches.
- Absence of roots and sporophytic generation bears rhizoids.
- The branches are leafless, for example Psilotum.
- Sporangia multilayered, in rare instance are terminal.
- Terminal branch shows protostelic configuration.

The above similarities suggest that Psilotales (Psilotum and Tmesipteris) is the most primitive extant group among the vascular plants. Moreover, biochemical characteristics of Psilotum and Tmesipteris have strengthened the above consideration. Tse and Towers (1967) isolated unique phenolic compound, Psilocin, from the two genera which is not found in other groups of pteridophytes.

Hence Psilotum and Tmesipteris constitute a natural group in Pteridophyta and should be placed at the beginning of pteridophyte classification next to early vascular plants and before Lycopsida.

It has been observed through Gel electrophoresis that histone protein profiles of *Psilotum* are more or less identical with the histones of mosses. Thus it suggests the primitiveness of *Psilotum*. Hence *Psilotum* must be treated as a primitive vascular plant.

8.2.3 Lepidodendrales

Lepidodendron were trees and are best known of the fossil lycopods. Material in the form of casts and impressions is abundant in upper carboniferous coal mines**.** Lepidodendron is presumed to be over 30 meters in height and about one meter in girth. The trunks were born on underground system of four axes, the Stigmaria. The trunk was unbranched for some distance and higher up it branched. The entire structure approached a monopodial type, due to successive unequal dichotomies. The ultimate branches were clothed with microphylls which on falling from stem left characteristic scars. The fruiting bodies were similar to those of living lycopods but were much larger.

NOTES

The subterranean system of Lepidodendrales referable to a single monotypic genus Stigmaria ficoides due to closely placed dichotomies occurring at the base of upright trunk and resulting into four main axes. The axes continued to divide and bore spirally arranged lateral appendages. These rootlets are 1 cm in diameter and without root hairs. The major axis has a stele of radially aligned tracheids. A few of tracheids are spiral and rest scalariform indicating that wood was secondary. The lepidodendrales stems are characterized by a pattern of leaf scars and leaf cushions. In lepidodendron, the cushions are vertically elongate with a tapering base. The stems were massive but possessed small amount of vascular tissue. The girth was mainly due to secondary cortex and periderm. Protoxylem poles were exarch. Secondary xylem produced immediately outside protoxylem was restricted and rarely exceed 10 cm without any indication of growth rings. It comprised mainly of radial rows of elongate tracheids and several wood rays. Lepidodendron and Lepidophloios can be separated on the basis of leaf bases.

The leaves have been described under the generic name Lepidophyllum but since the name has a priority in use for a flowering plant it is invalid. In size, the leaves ranged from few millimetres to a meter. The single vascular strand of leaf comprised a group of elongate tracheids enclosed by phloem. Surrounding the bundle is more or less continuous sheath of transfusion tissue. Below the epidermis on both sides is an extensive hypodermal tissue. The stomata are restricted to two longitudinal furrows on underside. Cones are considered to terminate small apical branches in Lepidodendron but in Lepidophyllus the cones terminated short lateral branches. The cones Lepidostrobus vary in length from 5 cm to 40 cm. Some are microsporangiate, others megasporangiate and in others can be seen both micro as well as megasporangia, the former towards the apex and the latter towards the base. The spirally arranged sporophylls with their overlapping apices form compact structures protecting the sporangia. The elongate sporangia are borne on the upper surface of sporophylls and are attached throughout their length to the sporophyll. A ligule is present on the adaxial surface at the point of bending. The micro and megasporangia are similar in structure, the former produced abundant microspores but the number of megaspores varied with the species.

The generic name Lepidocarpon is given to most advanced megasporangiate fructification. The sporangium in Lepidocarpon is elongate sac like structure and differs from Lepidostrobus in being broad at the base and tapering towards the apex. The whole structure was shed like a seed from the parent plant. It is regarded as a step in the evolution of seed.

8.2.4 Isoetales

Ligulate, heterosporous lycopods. The plants are basically hydrophytes. The family isoetaceae was considered to be monotypic (Isoetes) but in 1957 Amstutz added

Stylites from a glacial lake in the Andes mountain range of Peru (Refer Figure 8.15).

Fig. 8.15 *Agave*

NOTES

Isoetes with about 60-70 species is worldwide in distribution, extending from tropics to near arctic region. A large majority of species are hydrophytes that grow on swampy land or submerged. A few species inhabit places which are dry for a portion of the year. The plant resembles a leek and is 5-50 cm tall. Externally it has bulbous stock which on its upper surface arise a tuft of quill like leaves and on the base are roots. The apical meristem lies covered by spirally arranged leaves. The apical meristem consists of several cells with tissue derivation comparable to that of lycopodium. The leaves have an expanded base and an attenuated distal portion. All leaves are potential sporophylls. The sporangium is present on the adaxial surface on the expanded basal region. The leaves are ligulate.

The lower part of the plant axis is divided by a broad basal groove into 2, 3 or rarely 4 lobes. From these lobes arise roots in definite rows radiating across each lobe. The plant axis is reduced and specialized fleshy structure. It has been described as stock, corm, rhizomorph, etc. Its morphological nature has been controversial. Since all the leaves are potential sporophylls, some are tempted to describe the entire plant a single strobilus bearing roots. Isoetes has a definite axis which is highly condensed. On its upper surface are large number of leaves and at its base are adventitious roots.

In a growing season the plant produces megasporangia, it is followed by the production of microsporangia and finally are formed abortive sporangia. The sporangia of Isoetes are the largest, among the present day spore producing plants with highest spore output. The sporangia are eusporangiate. The trabeculae become surrounded by tapetum layer giving it more surface area and making it more effective for the nourishment of developing spores. The microsporangia and megasporangia are identical in early stages of development and can be differentiated only at the time of sporocyte formation. In megasporangium part of the sporogenous tissue and even sporocytes degenerate and are absorbed by growing sporocytes. The microspores are very small elongate structures about 45 microns long and megaspores are large 250 to 900 microns in diameter. The sporangia lack dehiscence mechanism and the spores are set free after decomposition of sporangial wall. Under favourable conditions spores germinate quickly and form endosporic gametophytes. Zygote divides by a wall transverse to archegonial axis.

8.2.5 Spenophyllales

Equisetopsida is referred as sphenopsida. It is after the abundant fossil form, sphenophyllum of the group. Some also called this group as arthropoda because of the jointed nature of their stems. Equisetum is the exclusive living representative of the group. It is a sporophyte with root, stem and leaves. Stems are jointed with whorls of leaves and branches at the nodes. The plant has unique stellar organization. Cones comprise peltate sporangiophores in whorls, bearing reflexed sporangia. The spores are homosporous and antherozoids are multiflagellate.

Equisetum is distributed all over the world except Australia and New Zealand. It is known as horsetail or scouring rushes. The most conspicuous feature of plant are jointed nature of stems, intercalary growth, whorl of branches, small leaves around nodes and sand papery texture. The rough texture of plant is due to deposition of silica in cells on outer surface. Silica helps to protect the plant against predators, pathogens and prevent excessive water loss. Equisetum with an interesting range of growth habits grow in variety of habitats ranging from wet places to damp shaded places along river banks, to exposed xeric conditions. Equisetum is a weed and because of its poisonous nature is of concern to livestock owners. All species have a perennial, subterranean much branched rhizome that helps the plant to spread prolifically. Aerial stems are of two types, fertile and sterile. The former are colourless and unbranched with a strobilus at the apex. These appear before the sterile vegetative green axes with whorls of lateral branches. In some species, the fertile branches are green and may be branched or unbranched. The stems are ridged and differentiated into nodes and internodes. Around each node are small scale leaves. The leaves are jointed at their bases forming a sheath around the node, and their free distal ends give the appearance of a frill (Refer Figure 8.16).

Fig. 8.16 *Equisetum arvense*

The cones are terminal either on the main shoot or on smaller lateral branches. Occasionally, the axis continues to grow and forms indeterminate structure. The cones are made up of compactly arranged whorls of peltate appendages, the spornagiophores. Each sporangiophore has a slender stalk by which it is attached to the strobilus axis and its free distal end is expanded into hexagonal disc. The spornagiophores have been interpreted as sporophylls, stems or other organs. In the cone axis, there is greater development of intermodal xylem with less xylem at the nodes resulting into a netted appearance.

Structure of Isoetes

Sporophyte

The plant resembles a monocot garlic plant in particular (Refer Figure 8.17). Isoetes is commonly called 'Quillwort' due to the quill (a large feather) like structure of the leaves.

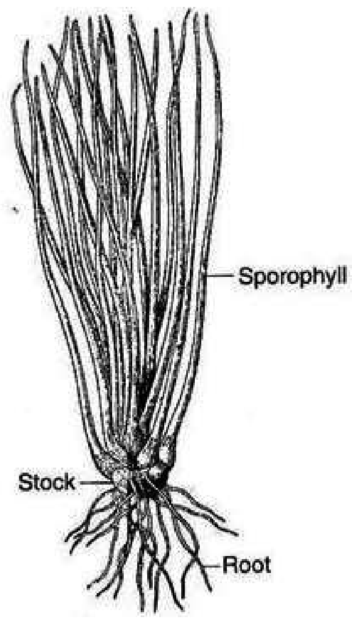

Fig. 8.17 *Isoetes coromandeliana Sporophyte*

The plant body consists of a condensed, lobed corm or axis that bears a tuft of roots at the base and long feathery leaves on the top.

Corm or Axis

The corm or axis is a condensed structure whose morphological nature is debatable. It has been interpreted as an erect rhizome, stock, corm or stem. The upper erect portion is actually the stem axis which gives rise to leaf traces.

The lower horizontal part is called rhizomorph which bears root traces. The axis is covered with dried leaf bases and roots of the previous year. The lower part of the axis is divided by a broad basal groove into 2-3 or rarely 4 lobes. The roots develop in definite rows radiating across each lobe (Refer Figure 8.18). The younger roots are borne nearer the groove, while the older ones are away from it.

NOTES

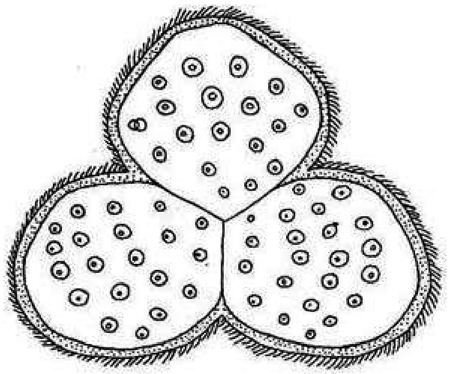

Fig. 8.18 *Isoetes coromandeliana; Basal View of Plants, Showing Lobes and Arrangement of Root Traces*

The apical meristem is covered by spirally arranged leaves and can only be seen after pulling out all the leaves.

The 3-dimensional view of stele indicates that the stele is anchor-shaped and is differentiated into the upper cylindrical stem axis and the lower rhizomorph part which is extended horizontally with upturned lobes (Refer Figures 8.19 and 8.20).

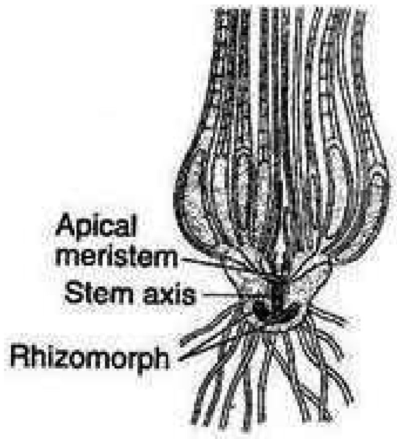

Fig. 8.19 *V.S. Through The Stock of Isoetes*

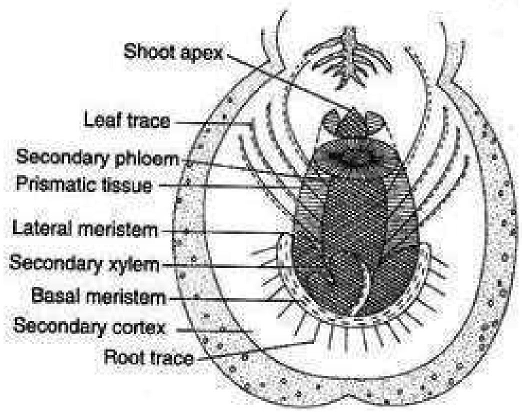

Fig. 8.20 *L.S. of the Axis of Isoetes (3-Dimensinal View)*

The plants with trilobed basal part show four radiating arms in the T.S. of the lower part of stele. Because, one pair of arms diverges widely, while the other two arms come closer to give a trilobed appearance. In rare instances, all the four arms diverge widely to give four-lobed basal part.

The T.S. and L.S. of the upper and the lower parts of the axis shows protostele where xylem is in centre, encircled by phloem that is not delimited from the adjoining cortex due to the absence of endodermis. In mature stem and rhizomorph, a cambium is formed outside the phloem. The xylem is made up of reticulate and spiral tracheids intermixed with parenchyma.

The phloem consists of sieve tubes with sieve plates on the wall. The cortex is made up of starch-filled parenchyma with large intercellular spaces.

The secondary growth is noted in Isoetes. The activity of cambium is bifacial. It produces secondary cortex centrifugally (on the outside). As a result the secondary cortex pushes the primary cortex towards outside and it sloughs off every year as dead tissue

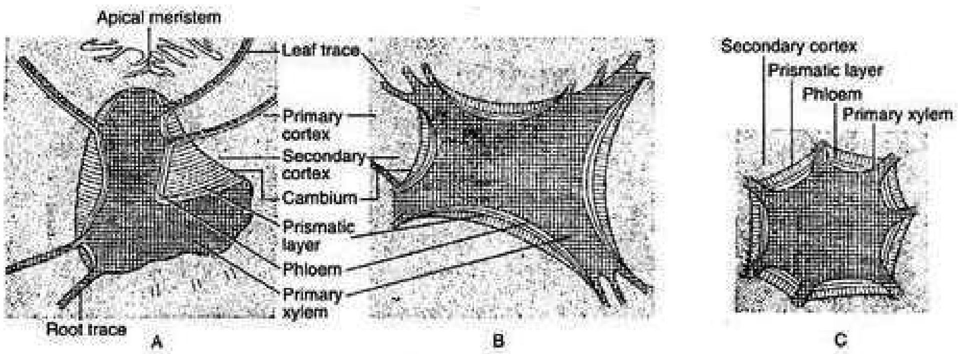

Fig. 8.21 *Isoetes coromandeliana; (A) V.S. of Stock Showing One Rhizomorph Only; (B) T.S. of Stem Axis; (C) T.S. Through Rhizomorph Part*

The cambium produces centripetally (towards Inside) secondary xylem of reticulate tracheids, or secondary phloem or a mixture of secondary xylem, secondary phloem and secondary parenchyma. Due to the inconstant nature of their centripetal secondary tissue, it has been described as 'Prismatic Layer' (Refer Figure 8.21).

Thus, the cambium is abnormal in position (develops outside the phloem) and also abnormal in function (produces secondary cortex outside and secondary phloem inside).

Some authors suggested that the cambium is comprised of two parts: a lateral meristem around erect axis, and a basal meristem around basal part. However, Bierhorst (1971) distinguished the basal meristem along the base of anchor-shaped stele from where only roots develop.

Leaf

The leaves are quill-like, develop from the apical meristem. The leaves are borne in acropetal order overlapping one another in a close spiral phyllotaxy. The leaves are microphyllous, ligulate, sessile with expanded base and an abruptly tapering apex.

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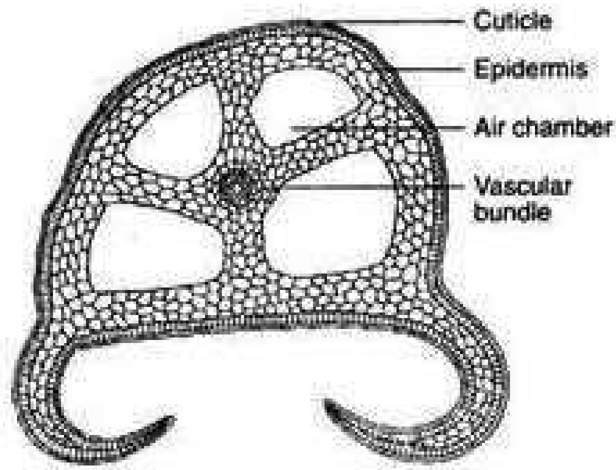

Fig. 8.22 *T.S. of Leaf Isoetes coromandeliana*

In Isoetes all the leaves are potentially fertile, thus leaves are called sporophylls. Each leaf is characterised by having a triangular cordate and colourless ligule on adaxial surface, and a small flap-like velum in-between sporangium and ligule.

Isoetes is heterosporous, thus micro- and megasporophylls are either distributed irregularly on the axis or mega- and micro- sporophylls are arranged successively from periphery to centre.

T.S. of the leaf of Isoetes shows a quadrangular outline with two expanded lateral wings on the lower side. The epidermis is single-layered covered by a thin cuticular layer. There are four longitudinal air chambers running along the whole length of the leaf, separated from each other by transverse diaphragm.

There is a single collateral vascular bundle surrounded by undifferentiated mesophyll. In the basal part, the vascular bundle may be flanked on either side by two mucilage canals. These canals are comparable with the parichnos of the Lepidodendrales.

3. Root

Numerous adventitious roots are developed from the basal part of the stele. The roots are dichotomously branched, bearing numerous root hairs. T.S. of the root of Isoetes shows a single- layered epidermis, followed by 4-8 layered parenchymatous cortex.

There is a central cavity and a single monarch vascular bundle is attached to the inner margin of cortex on one side of the cavity (Refer Figure 8.23). This reminds the Stigmarian rootlets of Lepidodendrales.

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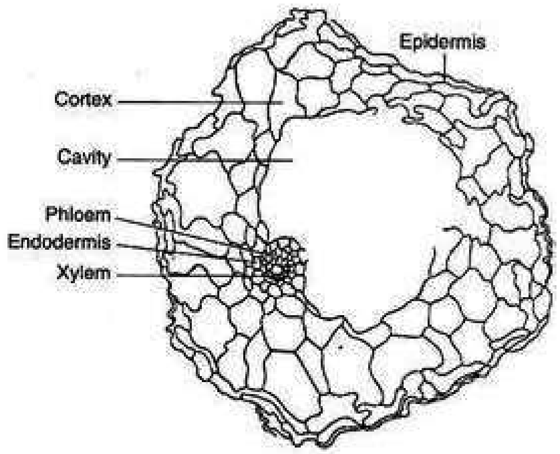

Fig. 8.23 *T.S. of Root of Isoetes coromandeliana*

Reproduction in Isoetes

The sporophyte of Isoetes reproduces mainly by spores. In rare instances, Isoetes propagates vegetatively by buds developing on the stem.

Sporangia

Isoetes is a heterosporous lycopod, hence it produces both the microspore and megaspore. Both the spores are produced in separate sporangia, microspores in microsporangia and megaspores in megasporangia.

Hence there are two types of sporophylls: microsporophyll-bearing microsporangia and megasporophylls bearing megasporangia. A single Isoetes plant bears both the sporophylls. The plant produces megasporangia first, followed by microsporangia and, finally, abortive sporangia.

The sporangia of Isoetes are the largest among the all known extant spore-producing plants. Isoetes also shows highest spore output, about 50 to 300 megaspores per megasporangium and about 150,000 to 1,000,000 microspores per microsporangium. Internally, the sporangium is partially or completely divided by plate-like trabeculae (Refer Figure 8.24).

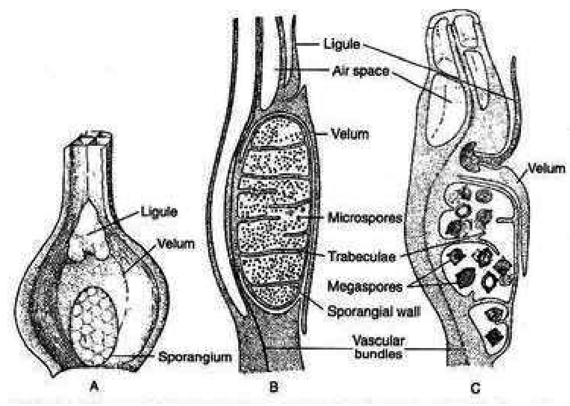

Fig. 8.24 *Isoetes coromandeliana; (A) A Basal Part of A Sporophyll (Adaxial View); (B) Median L.S. of Microsporophyll Base; (C) Median L.S. of Megasporophylls Base*

NOTES

Development of Sporangium

The development of both the microsporangia and megasporangia are similar up to the spore mother cell. The sporangia are of eusporangiate type. A group of sporangial initials divides periclinally to form outer jacket initial and an inner archesporial cell. The jacket initials, by anticlinal and periclinal divisions, produce 2-3- layered jacket of the sporangium.

The archesporial cells divide in all possible planes to produce a mass of sporogenous tissue which differentiates into spore mother cells. At the time of the formation of spore mother cells, some of the sporogenous tissues differentiate into trabeculae.

Some sporogenous tissue bordering the trabeculae and to the sides of sporangium differentiates into a two-layered tapetum. Microsporangia and megasporangia can only be distinguished at the time of sporocyte formation. In microsporangia the sporocytes differentiate after the formation of trabeculae, while in megasporangia the sporocytes differentiate before the formation of trabeculae.

In microsporangia, all the sporogenous cells differentiate and form microspores, whereas in megasporangia most of the sporogenous cells degenerate and only a limited number of surviving sporogenous cells differentiate to produce a limited number of megaspores.

The special mechanism for dehiscence of sporangia is absent, hence spores are released only after the death and decay of the sporangia.

Gametophytes

The microspores and megaspores germinate endoscopically to produce male and female gametophytes, respectively.

Male Gametophyte

Microspores are small (30-40 μm in diam.), triangular with trilete apertures.

Microspores germinate immediately after falling upon a suitable substratum, thus a male gametophyte is formed within a few days.

The first division (1-1) of microspore is asymmetric to give rise to a small prothallial cell and a large antheridial initial. The prothallial cell does not divide, it is adpressed to the base of the spore. The antheridial initial divides (2-2) diagonally to produce two cells, of these the cell close to the prothallial cell becomes the first jacket cell, while the other cell further divides (3-3) at right angle to the previous division.

Out of these two cells thus formed, the cell farthest from the prothallial cell becomes the second jacket cell, whereas the other cell again divides (4-4) in a plane almost parallel to the preceding plane of division to produce two cells. Of the two cells thus formed, the outer cell forms the third jacket cell and the inner one divides (5-5) periclinally to form a fourth jacket cell and a central primary androgonial cell.

The primary androgonial cell by two successive divisions at right angles to each other, forms four androcytes. Thus a mature male gametophyte consists of nine cells: one prothallial cell, four jacket cells and 4 androcytes. Each androcyte metamorphoses into a corkscrew-shaped, multiflagellated (about 15 flagella) antherozoid with a terminal vesicle (Refer Figure 8.25).

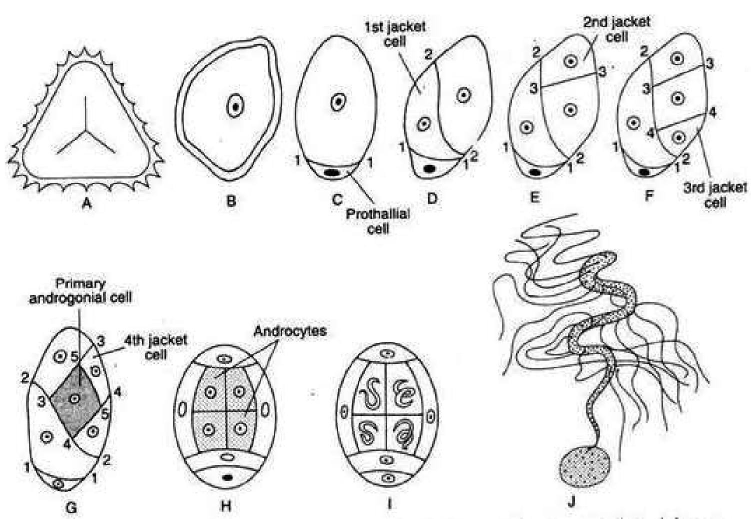

Fig. 8.25 *Isoetes coromandeliana; (A) - (I) Stages in the Development of Male Gametophyte; (J) A Sperm*

The antherozoids are liberated through the expansion of trilete aperture.

Female Gametophyte

The megaspores are comparatively larger (250-900 μm in diameter) than microspores. They are triangular in shape with trilete aperture. The exine is provided with large spinulate projections.

On germination, megaspore begins with a series of free nuclear divisions. About 30-50 nuclei thus formed are dispersed towards the periphery. However, there is no central vacuole as in Selaginella. Later, wall formation starts from the apical part and slowly extends downwards. The wall formation continues even after the formation of new sporophyte.

The megaspore wall breaks open along the trilete aperture to expose the apicaI cellular gametophytic tissue. Generally two to three archegonia develop on this apical exposed tissue. The nature of development of archegonium from the archegonial initial is like that of Selaginella.

A mature archegonium has a neck comprised of four vertical rows of neck cells (4 cells in each row), one neck canal cell, one ventral canal cell and an egg (Refer Figure 8.26).

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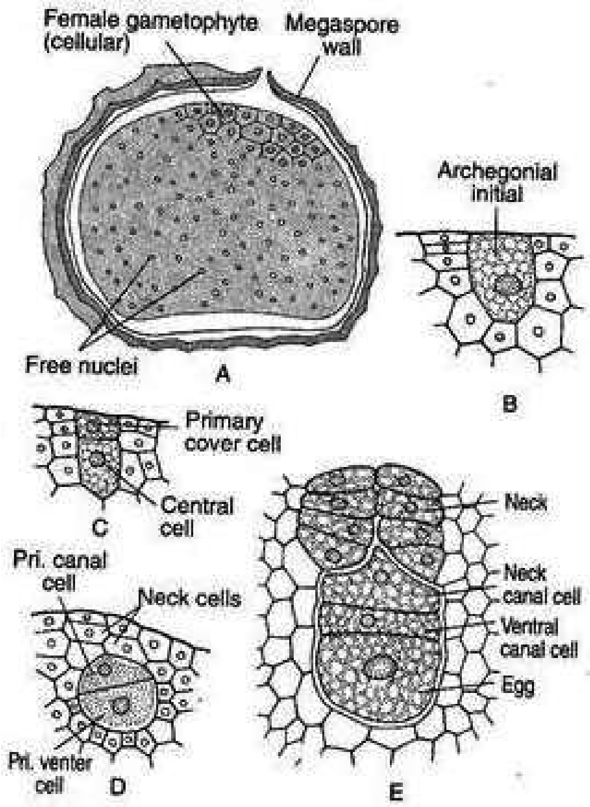

Fig. 8.26 *Isoetes coromandeliana; (A) A Young Female Gametophyte; (B) – (D) Successive Stags in the Development of Archegonium; (E) Mature Archegonium*

Fertilisation

Like other pteridophytes, fertilisation takes place as usual.

The New Sporophyte (Embryo)

The zygote first divides by an oblique wall, followed by a vertical wall, thus four cells are formed in two tiers (Refer Figure 8.27). All the cells contributes to the formation of embryo. Unlike other lycopsids, there is no suspensor in Isoetes embryo. The foot is formed from the two hypobasal cells.

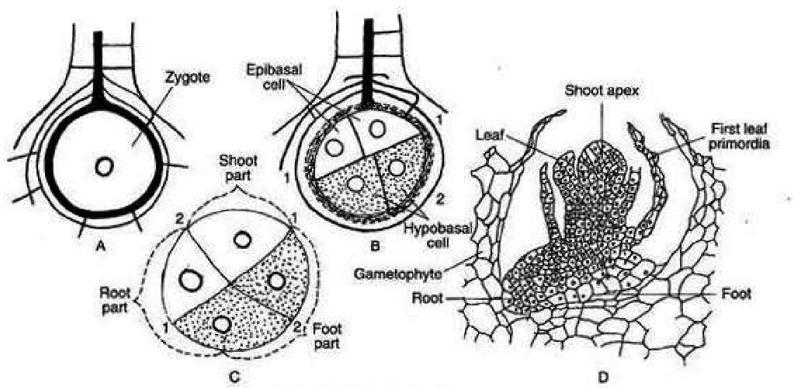

Fig. 8.27 *Isoetes coromandeliana; (A) – (C) the Stages in Development of Embryo; (D) A Female Gametophyte with Mature Embryo*

Out of the two epibasal cells, one contributes to the formation of cotyledon, while root is formed from the other cell. The developmental pattern of embryo is of endoscopic type.

8.2.6 Ophioglossales

NOTES

The ophioglossales are unique in having sporangia borne on a fertile spike that projects from the leaf near the juncture of blade and petiole. They are further characterized by absence of sclerenchyma and each sporangium in receiving its vascular supply. Some of the species of ophioglossales are epiphytes. The stems are subterranean with adventitious roots, and every year produce an aerial complex consisting of a sterile laminar portion and the fertile portion, the spike. In some of the ophioglossum, more than one fertile spike is produced by each complex. Some species of ophioglossales are evergreen, the aerial complex over winters and can be seen along with fresh arrival.

The leaves are simple with reticulate venation, occasionally the leaves are lobed or dichotomously forked. Leaves are pinnate with open dichotomous venation. Young leaves are not circinate coiled. It is in contrast to ferns. The fertile spike, which projects from the leaf near the juncture of blade and petiole, is at variable distance from the stem. The sporangia are marginal in origin and massive. Each receives its independent vascular supply and produces a large number of spores. The sporangia originate from a group of cells. The initial cells undergo periclinal division forming sporogenous tissue and a massive sporangium wall. The outermost wall layer has stomata, innermost wall layer differentiates as tapetum. Figure 8.28 shows sweet scabious.

Fig. 8.28 *Sweet Scabious Plant*

The growth in stem takes place by a single pyramidal apical cell. The stems are soft and fleshy and all parts of plant characteristically lack sclerenchyma. Young plants are protostelic, but a prominent pith is developed. The stele is disrupted by prominent leaf gaps. The gaps are extensive and overlapping forming a dictyostele. The roots are mycorrhizae, thick and fleshy without root hairs and are sparsely branched. In some species, new plants arise from roots by differentiation of a shoot apex.

The gametophytes are subterranean, mycorrhizae and axial structure. Internally, the gametophytes are uniformly parenchymatous structures. The prothallus is a spherical mass of cells with a distinct apical cell. Sex organs are uniformly distributed over the surface of axial gametophytes. Embryogeny in family ophioglossales is highly variable and variations within the same species have been recorded. Variability is expressed in the following features:

- Orientation of first division wall of the zygote.
- Relative size of cells at two celled stage.
- Presence and absence of suspensor.
- Exoscopic or endoscopic embryogeny.
- Organogenesis from the quadrant zones.
- Relative orientation and degree of development of embryonic organs.

The plant group is without any fossil record and is referred to ferns. It is a group showing progress in the evolution of vegetative parts of sporophyte but retaining primitive reproductive features both at gametophyte as well as sporophyte levels.

The anomalous features of ophioglossales are:

- Peculiar plant body, single leaf of fertile and sterile segments.
- Non circinate vernation of leaves with an exception of Botrychium.
- Absence of sclerenchyma in plant body.
- Collateral vascular bundles.
- Secondary growth and its peculiar nature.
- Root with endophytic fungus and absence of root hairs.
- Subterranean axial gametophytes with an associated fungus.

8.2.7 Filicales

Filicales is the largest group of pteridophytes. It comprises over 300 genera and about 9000 species. Many of the plants are of great beauty and have been object of fascination to both professional botanists. The sporangia in Filicales are leptosporangiate in development (sporangium develops from a single initial cell). In homosporous forms the sporangia are marginal or superficial and are grouped together to form sori but don't form synangia. In heterosporous forms the sporangia are borne in specialized structures, sporocarps. Antheridia in homosporous forms are emergent containing small number of antherozoids. During embryogeny the primary organs: root, stem, leaf and foot are referable to an individual cell of the four celled embryo. In past the term filicales was used in a broad sense to include all forms but recent trend is to restrict it for homosporous ferns.

A fern sporophyte most often consists of a horizontal stem, called a rhizome; roots; prominent erect leaves and the fertile parts with sporangia.

- **Roots:** Fern bears the roots or root-like organs for attachment to the substrate and absorption of nutrients and water. They are always fibrous and are structurally very similar to the roots of seed plants. Roots of ferns tend to arise along the stem, commonly near leaf bases.

- **Stems, rhizomes:** The stems of pteridophytes are mainly true rhizomes, i.e., stems that run horizontally at or just beneath the surface of the ground. In some cases the rhizome is erect and tree-like with an apical radial tuft of leaves in which case it is called a trunk. The rhizome may be unbranched or branched. The stem produces leaves or fronds in a more or less regular manner.
- **Leaves:** The megaphyllous leaves are spirally arranged and usually called fronds. These develop in a distinctive manner with the soft growing tip (meristem) rolled up in the center of the crozier (fiddlehead). The crozier is produced by a process of growth and unfolding, and the characteristic pattern thus formed is known as circinate vernation. Leaf development that is initiated at the tip is called acropetal, and this development is characteristic of most modern ferns. Leaves of seed plants, in contrast, tend to develop by overall growth and expansion, i.e., by intercalary differentiation and enlargement. In pendulous or vertical fern fronds, the upper surface is known as adaxial (towards the axis) and lower surfaces of a frond abaxial (away from the axis) surfaces. A fern frond consists of a stipe, the stem supporting the blade, and the blade consists of both a laminar (flattened) photosynthetic tissue and a rachis—that portion of the stem to which the laminar tissue is attached. The blades of fern fronds may vary from being simple (undivided) to being highly dissected, even 'lace-like'. If the leaf tissue is undissected, or the dissections do not reach to the rachis, the frond may be described as lobed or pinnatifid. Otherwise, the blade is compound. Most commonly, the leaflets or secondary rachises are borne alternately (or at most subopposite) on either side of the main rachis. This is the pinnate condition and the leaflets are known as pinnae (singular-pinna). The main vein or mid-rib of a pinna is known as a costa (pl., costae). The arrangement may change from the base of a blade to the tip. Most commonly, the leaflets or secondary rachises are borne alternately (or at most subopposite) on either side of the main rachis. This is the pinnate condition and the leaflets are known as pinnae Many ferns have pinnae that are divided two or more times, and the level of division of the fronds is termed pinnate (or 1-pinnate), or bipinnate (2-pinnate), or tripinnate or even quadripinnata fronds. Each secondary division (division of a pinna) is termed a pinnule, and its mid-vein, a costule. A few species of ferns with divided fronds are not pinnate, but are palmate or bifurcate. Leaves are divided into two types:
    - **Vegetative or Sterile Fronds (Trophophyll):** A leaf that does not produce spores, instead only producing sugars by photosynthesis. Analogous to the typical green leaves of seed plants.
    - **Sporangia-Producing or Fertile Fronds (Sporophyll):** A leaf that produces spores. These leaves are analogous to the scales of pine cones or to stamens and pistil in gymnosperms and angiosperms, respectively.

NOTES

Unlike the seed plants, however, the sporophylls of ferns are typically not very specialized, looking similar to trophophylls and producing sugars by photosynthesis as the trophophylls do.

Separate sporangia-producing (fertile) and vegetative (sterile) fronds are produced in many fern species and this is known as dimorphism.

- **Outgrowths:** Hairs, Scales, and Glands In ferns, outgrowths of the epidermis of stems and leaves are common and varied, and these features are very important in description and identification.
- **Hairs:** Simple outgrowths made up of a single cell or a chain of several cells are generally called hairs or trichomes. Trichomes that have two or three parallel rows of cells at the base and a single file of cells at the tip are called bristles (setae).
- **Scales (Paleae):** Scales are flat plates of epidermal tissue one cell thick and several cells are wide. Scales may be attached basally or centrally on a small stalk. One of the more distinctive features of many hairs and scales is the presence of enlarged and rounded terminal secretory cells.
- **Glandular Hairs:** These are, sometimes called glands, characteristic of particular species.
- Gametophytes of most ferns have distinctive brownish or colorless hairs (rhizoids) that apparently absorption water and to anchor the plant.

Fig. 8.29 *Fern*

Reproduction

- Vegetative reproduction:-Bulbils. Bulbils are vegetative buds usually towards the apex of the frond in some species, but sometimes along the rachis or at the base of pinnae of ferns. These may remain dormant and die with the frond, but under suitable conditions they are capable of producing roots and juvenile fronds and eventually developing into a new plant. The presence of bulbils is taxonomically important, whether it is dormant or active.

- Asexual reproduction by spore formation: Generally sporangia are borne on leaves or modified leaves, but there are few exceptions. Sporangia often organized into clusters called sori (singular, sorus) on abaxial (under) surface of leaves. Developing sori are protected in various ways, such as thin, dry outgrowths of the lamina called indusia or by a reflexed fold of the lamina margin (a false- or pseudo-indusium). Sori with an indusium are called indusiate, those without are exindusiate. Sori may also be protected by paraphyses. The indusium is a special layer of leaf tissue. The Sori and their protective indusia vary in shape and are located on the underside of fertile fronds. Indusium location and shape is used in fern identification. Sporangia in all other vascular plants, and in some ferns arise from multiple layers of cells. These sporangia are called sporangia. In most ferns, the sporangium structure is unique, arising from a single initial. These ferns are called leptosporangiate ferns. When mature and under appropriate conditions the spores are released by dehiscence of lip cells of the annulus (leptosporangiate) and germinate to produce next generation of gametophytes.

Gametophyte: The fern gametophyte, often called a prothallium, or prothallus, is small, always short lived and inconspicuous but independent from the sporophyte and photosynthetic. It is often heart shaped and have an apical Cell in the notch. Gametophytes are without vascular tissue and often only one cell thick for the most part, tapering many cells thick at the centre. They are attached to the substrate by fine multicellular root hairs or rhizoids, and often resemble thallose liverworts.

Sexual Reproduction: The prothallus has a single set of chromosomes (haploid) and is the sexual part of the life cycle. Most ferns are homosporous. On the undersurface of the prothallus, develops the sex organs. The antheridia are near the base Antheridia and archegonia may be on the same gametophyte or on separate gametophytes.

- **Antheridia:** Sperms or antherozoids are produced in antheridia that are either sunken in the gametophyte or protrude from it. The antheridia are formed near the base. These antherozoids have hair-like or whip-like cilia or flagella and are able to swim through water. The antheridia release the sperms through a pore. Release depends on maturity of the sperms and the presence of water.
- **Archegonium:** Near the tip develop archegonia. The female gamete (egg) is formed in archegonium. Archegonium a flask-shaped structure, it consists of a neck, with one or more layers of cells, and a swollen base—the venter—which contains a single non motile female gamete-the egg. Neck-canal cells, located above the egg, disappear as the archegonium matures, thus producing a passage for entry of the sperm. The antherozoids are chemically attracted to the developing archegonia. Sperms swim to the opening at the top of the neck of the archegonium. The neck provides a passageway to the enlarged base (venter), where the egg is located and where fertilization takes place. After fertilization, egg cell or zygote contains a double set of chromosomes (diploid), one from the egg cell, one from the antherozoid. It repeatedly divides mitotically, becoming an embryo, developing in to a sporeling, then into juvenile

plant and eventually into a mature diploid spore-bearing sporophyte. The embryo and sporeling is initially parasitic and dependent on the gametophyte, but rapidly develops roots of its own and becomes an independent photosynthetic sporophytic plant (diploid) as the gametophyte plant dies.

Check Your Progress

1. Give some external features of Rhynia.
2. What are Psilotales?
3. Write about the gametophytes of Psilotum.
4. What is Equisetopsida?
5. What is Filicales?

8.3 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Some of the external features of Rhynia are as follows:
    - Plant body was sporophytic.
    - Rhynia major was bigger and attained a height of 50 cm. with a diameter of 1.5 to 6 mm, while Rhynia gwynne-vaughanii had a height of 20 cm. and a diameter of 1 to 3 mm.
    - Plants had a rhizome which was dichotomously branched.
    - From rhizome developed many dichotomously branched erect aerial shoots towards the upper side while many rhizoids towards the lower side.
    - There were no roots on the plants.
2. Psilotales is an interesting group of plants; its representatives are similar in structural organization to that of early land plants. The group is little understood due to restricted distribution of its members. The order comprises two genera, Psilotum and Tmesipteris. Psilotum is a pendulous epiphyte, rare in distribution, restricted to Jamaica, Mexico, Hawaii and few Pacific Islands.
3. The gametophytes of Psilotum are monoecious, i.e., homothallic. Sex organs, i.e., antheridia and archegonia, are superficial and scattered over the surface of the gametophyte. Generally, antheridia are more in number than archegonia.
4. Equisetopsida is referred as sphenopsida. It is after the abundant fossil form, sphenophyllum of the group. Some also called this group as arthropoda because of the jointed nature of their stems. Equisetum is the exclusive living representative of the group. It is a sporophyte with root, stem and leaves. Stems are jointed with whorls of leaves and branches at the nodes. The plant has unique stellar organization. Cones comprise peltate sporangiophores in whorls, bearing reflexed sporangia. The spores are homosporous and antherozoids are multiflagellate

5. Filicales is the largest group of pteridophytes. It comprises over 300 genera and about 9000 species. Many of the plants are of great beauty and have been object of fascination to both professional botanists. The sporangia in Filicales are leptosporangiate in development (sporangium develops from a single initial cell). In homosporous forms the sporangia are marginal or superficial and are grouped together to form sori but do not form synangia.

8.4 SUMMARY

- The first fossils of vascular plants were described in 1859 by Canadian geologist Dawson from Devonian peninsula of Canada.
- The compressions were fragmentary, but Dawson's reconstruction revealed slender axes arising from a subterranean rhizome system.
- The rhizome lacked roots but had rhizoids.
- The aerial axes were dichotomously branched with scale like appendages and terminated into sporangia**.** Because of its similarity with Psilotum Dawson named the plant *Psilophyton princeps*.
- In 1917, when Kidston and Lang described similar plants from Lower Devonian red sandstone cherts of village Rhynie, it was realized that plants of such simple organization had existed and the group psilophytales was instituted to include them.
- Rhynia gwynne-vaughanii was small plant of up to 17 cm in height. The aerial dichotomous axes arose from subterranean horizontal rhizome.
- The shoot system lacked differentiation into stem and root, the rhizome instead bore rhizoids.
- The aerial axes terminated into sporangia and lacked leaves. *Rhynia major* was larger and attained height up to three times than that of *Rhynia gwynne-vaughanii*.
- Another member of Rhynie flora is Asteroxylon mackie. The basal portion of the plant was a naked dichotomously branched rhizome lacking rhizoids.
- Rhyniales are the oldest vascular plants, Leclercq (1954), remarked that Devonian Rhynie in general and Rhynia in particular is by no means a representative of the earliest land plants.
- More significant about Rhynia is its simple nature and it is unquestionable. It lacked differentiation into stem and roots, had uniform dichotomous branching and its appendages cannot be called leaves.
- The plant consists of a subterranean rhizome and dichotomously branched aerial axes. The rhizome anchors the plant and is cylindrical, prostrate, dichotomously branched mycorrhizae structure with numerous rhizoids.
- The plants lack roots and instead rhizoids are 1-3 cells long, serve for absorption. Some branches of rhizome turn upwards and develop into aerial green branches of regular dichotomy**.**

- Stout aerial branches bear large conspicuous sporangia. Each sporangium is a 3 lobed structure borne on forked appendage and is synangium; group of fused sporangia with distinct partition walls.
- A synangium on the outside of its loculi is bound by a single layer of large cells, followed by it are few layers of smaller cells.
- A vascular bundle extends into synangium and subdivides into three parts, corresponding to three sporangia.
- The sporangium is eusporangiate in development. The primordium of a fertile appendage is nearly of the same size as that of apex. It becomes two lobed.
- Immediately below the apex of appendage differentiate three groups of sporangial initials which divide periclinally to form wall layers and sporogenous tissue.
- A part of sporogenous tissue serve as nourishment to the sporocytes. The numerous colourless kidney shaped spores are formed in tetrads are all alike.
- The gametophyte is a simple parenchymatous structure with a prominent apical cell. It lacks differentiation and quite early in its development comes to have mycorrhizae association.
- The entire gametophytic surface including rhizoids has a cuticular covering. Sex organs antheridia and archegonia occur in large numbers all over the gametophyte.
- The archegonium is embedded in gametophyte at its venter, the neck is projecting.
- The antheridium is large superficial structure with one cell thick jacket of variable cells and encloses numerous spirally coiled multi-flagellate antherozoids.
- The cortex is extensive and is divisible into three regions. The outer portion (chlorenchymatous) directly beneath the epidermis consists of elongated, lobed chlorophyllous cells with intercellular spaces.
- Internal to this zone, there is a middle cortex of 4 to 5 layers of vertically elongated thick-walled sclerenchymatous cells without intercellular spaces.
- The sporophyte as well as gametophyte of *Psilotum*, for example *P. nudum* propagate vegetatively through the production of Gemmae.
- After detachment from the parent body, gemmae of sporophyte may germinate to form a subterranean shoot, while the gemmae of prothallus, on germination, form a new prothallus.
- At maturity, many of the dichotomously branched aerial shoots become fertile and produce trilocular sporangia known as synangia.
- The mature synangium is generally a three-lobed structure and each lobe of the synangium corresponds to a sporangium.
- The synangia located at the tip of very short axis, measuring 1-2 mm in diameter and closely associated with a forked, foliar appendage.

NOTES

- A sporangial wall of four or five layers is produced through repeated periclinal and anticlinal divisions of the primary wall initials.
- The sporogenous tissue is produced from repeated divisions of primary sporogenous cells in various planes.
- *Psilotum* is the only plant in the plant kingdom where the vascular tissues develop in the gametophytic generation.
- The external resemblance of the sporophytic rhizome and gametophyte, coupled with the presence of vascular tissue in the gametophyte, supported the homologous theory on the origin of alternation of generations.
- The foot anchors the young sporophyte securely to the gametophyte and absorbs nutrients until the sporophyte becomes physiologically independent.
- Lepidodendron were trees and are best known of the fossil lycopods. Material in the form of casts and impressions is abundant in upper carboniferous coal mines**.**
- Lepidodendron is presumed to be over 30 meters in height and about one meter in girth. The trunks were born on underground system of four axes, the Stigmaria.
- The trunk was unbranched for some distance and higher up it branched. The entire structure approached a monopodial type, due to successive unequal dichotomies. The ultimate branches were clothed with microphylls which on falling from stem left characteristic scars.
- The subterranean system of Lepidodendrales referable to a single monotypic genus Stigmaria ficoides due to closely placed dichotomies occurring at the base of upright trunk and resulting into four main axes.
- A few of tracheids are spiral and rest scalariform indicating that wood was secondary.
- The lepidodendrales stems are characterized by a pattern of leaf scars and leaf cushions. In lepidodendron, the cushions are vertically elongate with a tapering base.
- Secondary xylem produced immediately outside protoxylem was restricted and rarely exceed 10 cm without any indication of growth rings.
- The leaves have been described under the generic name Lepidophyllum but since the name has a priority in use for a flowering plant it is invalid.
- The single vascular strand of leaf comprised a group of elongate tracheids enclosed by phloem.
- The spirally arranged sporophylls with their overlapping apices form compact structures protecting the sporangia.
- The elongate sporangia are borne on the upper surface of sporophylls and are attached throughout their length to the sporophyll. A ligule is present on the adaxial surface at the point of bending.

NOTES

- The generic name Lepidocarpon is given to most advanced megasporangiate fructification.
- The sporangium in Lepidocarpon is elongate sac like structure and differs from Lepidostrobus in being broad at the base and tapering towards the apex.
- Isoetes with about 60-70 species is worldwide in distribution, extending from tropics to near arctic region.
- A large majority of species are hydrophytes that grow on swampy land or submerged.
- The apical meristem consists of several cells with tissue derivation comparable to that of lycopodium. The leaves have an expanded base and an attenuated distal portion.
- The lower part of the plant axis is divided by a broad basal groove into 2, 3 or rarely 4 lobes. From these lobes arise roots in definite rows radiating across each lobe.
- Equisetopsida is referred as sphenopsida. It is after the abundant fossil form, sphenophyllum of the group.
- Equisetum is the exclusive living representative of the group. It is a sporophyte with root, stem and leaves. Stems are jointed with whorls of leaves and branches at the nodes.
- All species have a perennial, subterranean much branched rhizome that helps the plant to spread prolifically.
- Each sporangiophore has a slender stalk by which it is attached to the strobilus axis and its free distal end is expanded into hexagonal disc.
- The spornagiophores have been interpreted as sporophylls, stems or other organs.
- In the cone axis, there is greater development of intermodal xylem with less xylem at the nodes resulting into a netted appearance.
- The sporangia of Isoetes are the largest among the all known extant spore-producing plants.
- Some sporogenous tissue bordering the trabeculae and to the sides of sporangium differentiates into a two-layered tapetum.
- Microsporangia and megasporangia can only be distinguished at the time of sporocyte formation.
- In microsporangia the sporocytes differentiate after the formation of trabeculae, while in megasporangia the sporocytes differentiate before the formation of trabeculae.
- In microsporangia, all the sporogenous cells differentiate and form microspores, whereas in megasporangia most of the sporogenous cells degenerate and only a limited number of surviving sporogenous cells differentiate to produce a limited number of megaspores.

- The special mechanism for dehiscence of sporangia is absent, hence spores are released only after the death and decay of the sporangia.
- The megaspores are comparatively larger (250-900 μm in diameter) than microspores. They are triangular in shape with trilete aperture. The exine is provided with large spinulate projections.
- The megaspores are comparatively larger (250-900 μm in diameter) than microspores. They are triangular in shape with trilete aperture. The exine is provided with large spinulate projections.
- The megaspore wall breaks open along the trilete aperture to expose the apicaI cellular gametophytic tissue. Generally two to three archegonia develop on this apical exposed tissue.
- The nature of development of archegonium from the archegonial initial is like that of Selaginella.

8.5 KEY TERMS

- **Antheridium:** It is male sex organ of algae, mosses and ferns.
- **Archegonium:** It is female sex organ of ferns.
- **Antherozoid:** The motile male gamete produced in an antheridium.
- **Neck canal cell:** It is present in female gametangia and is haploid in nature.
- **Venter canal cell:** Small cell that is cut off from cell of archegonium just below neck.

8.6 SELF-ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. What are Psilophytales?
2. Give some features of Psilotales.
3. Draw a well-labelled diagram of T.S. of stem of *Psilotum nudum.*
4. Write a short note on Lepidodendrales.
5. What are Isoetales?
6. Write about Sphenophyllales in short.
7. Briefly explain about Ophioglossales**.**
8. Explain the structure and reproduction in *Psilotum*.

Long-Answer Questions

1. Write a note on Psilophytales.
2. Discuss in detail about Psilotales.

NOTES

3. Detail a note on Lepidodendrales.
4. What are Isoetales? Draw well-labelled diagrams to show various stages of reproduction in Isoetales.
5. Describe in detail about Sphenophyllales with help of suitable diagrams.
6. Write a detailed note on Ophioglossales.
7. Describe in detail about reproduction in Filicales.

8.7 FURTHER READING

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