INTRODUCTION MICROORGANISMS (DMB01)

(MSC MICROBIOLOGY)

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LESSON: 1

HISTORICAL DEVELOPMENT OF MICROBIOLOGY

Objective: To know the important mile stones in the development of the Science of Microbiology

Contents:

1.1. Introduction:

Microbiology is the study of organisms that are too small to be perceived clearly with unaided eye. If an object has a diameter of less than 0.1 mm, the human eye cannot perceive or see it at all, and very little detail can be perceived in an object with a diameter of 1 mm or less. Hence, roughly speaking, the organisms with a diameter of 1 mm or less are microorganisms and fall into the broad domain of Microbiology. The majority of microorganisms exist as single cells, some may occur as colonial forms or filamentous forms but with no division of labour between the cells. The organisms that come under the broad domain of microorganisms are bacteria, fungi, microalgae, protozoa, and the viruses which are acellular forms.

Since the microorganisms are very small and cannot be seen with naked eye, their existence is not known up to 17th century, and their observation has to await the development of microscopes.

Ordinary magnifying glasses, which magnify the image of the objects, were known since antiquity. But it is only in the 16th century the microscopes were developed. First, simple microscopes with a single lens were developed which are just better than the ordinary microscopes. In 1590 Hans and Zacharias developed a compound microscope with two lens system. Some early microscopists have used the microscopes to observe the living systems since the beginning of 17th century. However, the

first person to observe and describe the microorganisms accurately was Anton van Leeuwenhoek of Holland, and he is considered as the discoverer of the microbial world.

1.2. Discovery of the Microbial world:

Anton Van Leeuwenhoek (1632-1723) of Delft city of Holland was the first to report his observations with accurate descriptions and drawings. Preparation of lenses was his hobby and he made about 250 lenses that magnify the objects about 50 to 300 times. He mounted the lenses in brass and silver plates (figure 1.1).

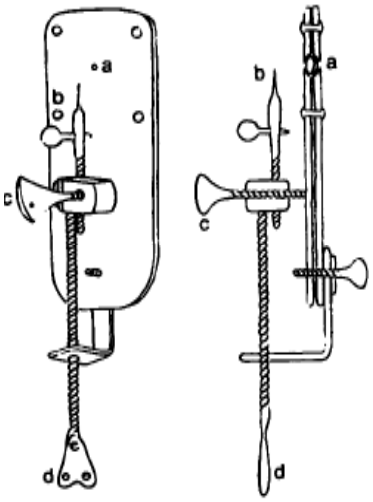

Fig. 1.1 : A drawing to show the construction of one of Leeuwenhoek's microscopes : (a) lens, (b) mounting pin, (c) and (d) focusing screws

Using his lenses he observed microorganisms in various substrata. Leeuwenhoek had realized the importance of recording his observations and he must have had great patience and persistence to continuously observe through specimens and record the drawings of microorganisms. His sketches were elegant in detail and clarity. He communicated his findings to Royal Society of London, which published his letters in the Society's journal. In one of his first letters dated September 7, 1676, he described 'animalcules' (very little animals) which are now recognized as protozoans. In his letter dated September 17, 1983, Leeuwenhoek had given the sketches of animalcules from skum of human mouth. It accurately records the bacteria of different shapes viz. rods, cocci, spirillum and movement of bacteria (Figure 1.2).

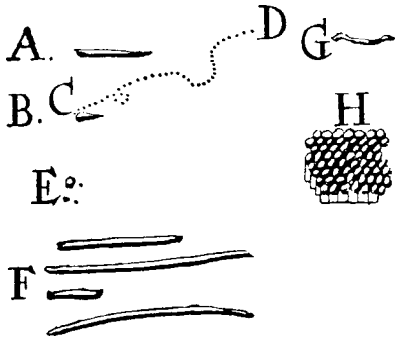

Fig. 1.2 : Leeuwen hock's drawing of bacteria in his letter dated September 17, 1683 (published in 1684). From these drawings we can recognize different types of common bacteria A & B: rod shaped bacteria, C-D : path way of movement of bacterium. E. coccoid bacteria, F. rod shaped bacteria of different sizes G. spiral bacterium H : cocci in clusters.

Leeuwenhoek described his 'animalcules' in great detail, leaving little doubt that he saw bacteria, fungi and many forms of protozoa.

Although Leeuwenhoek's contemporaries marveled at his scientific discoveries, the microscopic exploration of the microbial world, which he so brilliantly begun was not appreciably extended for over a century after his death. The principal reasons for this long delay seem to have been technical ones. Simple microscopes of high magnification are both difficult to prepare and tiring to use. Consequently most of Leeuwenhoek's contemporaries and immediate successors used compound microscopes. Despite the intrinsic superiority of the compound microscopes, the ones available in the seventeenth and eighteenth centuries suffered from serious optical defects, which made them less effective working instruments than Leeuwenhoek's simple microscopes. Thus Leeuwenhoek's English contemporary, Robert Hooke, a very capable and careful observer, could not repeat with his own compound microscope many of the finer observations reported by Leeuwenhoek.

The major optical improvements that eventually to lead to construction of compound microscopes of quality that we use today began about 1820 and extended through the succeeding half century. These improvements closely followed by resumed exploration of microbial world and resulted by the end of 19th century in a detailed knowledge of the microbial groups.

The contemporaries of Leeuwenhoek, though appreciated the discovery of microorganism by Leeuwenhoek, considered that the microorganisms are as 'little oddities of nature' with no special significance. This belief was due to the prevailing dogma of spontaneous generation. It required the entry of Louis Pasteur and his brilliant experiments to disprove the theory of spontaneous generation, for the microorganisms to receive the attention they deserved.

1.3. Controversy over Spontaneous generation theory:

The theory of spontaneous generation stated by Aristotle in 346 B.C. expressed a belief that life could and did appear spontaneously from nonliving or decaying matter. Today the theory of spontaneous generation appears absurd, and certainly it was the product of inadequate observation and faulty deduction. Never the less, it figured prominently in scientific thought, especially in the study of disease and various natural processes such as fermentation.

One of the first to refute the doctrine of spontaneous generation was the Italian Naturalist and physician Francesco Redi. In 1665, he showed that maggots (small animals) did not emerge spontaneously from decaying meat. Redi put meat in three separate containers. One of this is closed with a paper cover, another left uncovered and the 3rd was covered with fine gauze cloth. Naturally, the meet readily putrefied and attracted flies. Redi made the following observations.

1. The paper-covered container showed no evidence of any flies or maggots.

2. Flies laid their eggs on the meet in uncovered container, and with in a short period of time maggots and newly emerging adult files appeared.

3. Although no maggots were present in the meet in the gauze-covered container they did

appear on the covering. Apparently the smell of putrefying meat attracted flies. Unable to reach it, they laid their eggs on gauze.

From the above observations he concluded that maggots and the flies into which they develop came not from meat but from the eggs left on the meat by other flies.

With the discovery of bacteria and other microbes by Leeuwenhoek in the later part of 17th century, the focus shifted to spontaneous generation of bacteria from the spontaneous generation of small animals and plants. However, Leeuwenhoek himself did never entered the controversy over spontaneous generation.

In the early days, bacteria were grown in meat broth or infusion. The broth medium becomes turbid with the growth of bacteria. The proponents of spontaneous generation theory held that bacterial growth occur in meat broth spontaneously making the broth turbid.

In 1749, John Needham a Roman Catholic priest, reported the results of his experiments, which he believed proved that bacteria arose spontaneously where no such living forms existed before. Needham's studies consisted of tightly corking flasks of boiled mutton broth and observing them periodically for cloudiness as an indication of microbial growth. It's contents remained clear at first, but eventually became turbid. Examining a few drops of these cloudy preparations under a microscope, Needham found them to be teeming with microorganisms. Since boiling was known to destroy microorganisms as well as any other living cells, Needham believed that his experiments not only provided a clear demonstration of spontaneous generation but also showed that the organic matter in his flasks possessed a vital or vegetative force that could bestow the properties of life on the non-living elements present.

In 1765, the Abbe Lazzaro Spallanzani, an Italian Naturalist, reinvestigated Needham's findings and conclusions. He questioned the method of the heating procedure used by Needham. Spallanzani found that sealed flasks containing infusions heated for one hour showed no cloudiness after a reasonable period. This experiment was repeated several times, with the same result every time. To counter the Spallanzani's observations, Needham argued that the prolonged boiling procedure destroyed the life rendering "vegetative force". Spallanzani responded to Needham's criticism by breaking the seal on his heated, closed flasks allowing exposure to air. Within a short time, the contents of these flasks became turbid, showing that the long heated organic matter was still capable of supporting life.

The effect of Spallanzani's experiments was short lived. Soon after the discovery of oxygen by Joseph Priestly and the demonstration oxygen's importance to life by Antoine Laurent Lavosier in 1775, the proponents of spontaneous generation theory criticized Spallanzani's findings on the grounds that sufficient oxygen was not present in his sealed flasks to support microbial growth.

In 1836, Theodor Schwann set up two separate flasks, both of which held infusion of same type. Into one flask air was passed through a red-hot tube while the other flasks received unheated air. The second was the experimental control. Soon growth developed in the control, while the flask receiving heated air remained sterile.

Franz Schulze also performed similar experiments in the same year. However, his approach involved a different method of treating the air before entering flasks. Air was allowed to enter nutrient flask only after it had passed through solutions of strong chemicals such as sulfuric acid and sodium hydroxide. Schulze's results were the similar to those of Schwann. No growth developed in the flask receiving the treated air.

Upon learning of the experiments of Schwann and Schulze, the supporters of spontaneous generation insisted that the drastic treatments of air destroyed all possible life rendering power. Heinrich George Fredrich Schroder and Theodor Von Dusch, who introduced the use of cotton plugs for bacteriological culture flasks and tubes, countered the objections of the proponents of spontaneous generation theory in 1854. Using a system similar to that of Schulze and Schwann, these scientists allowed air to enter untreated in any way except by being filtered through cotton wool that had been previously sterilized in an oven. The flasks with the cotton plugs remained sterile while flasks exposed to unfiltered air clearly contained microorganisms.

Although these various experiments might seem to be conclusive, the issue was far from settled. In 1859, French Naturalist Felix Pouchet claimed to have carried out experiments showing clearly that microbial growth could occur without contamination by air, there by providing renewed hope for the supporters of spontaneous generation. About this time, the studies of Pasteur on fermentation were becoming well known, and several other scientists also began to recognize the role of microorganisms in wine and vinegar production (fermentation) and Food spoilage (putrefaction) processes. However, the acceptance of Pasteur's findings on biological functions of microbes was threatened by the claims of Pouchet. Irritated by these arguments, Pasteur set out to disprove spontaneous generation once for all.

Pasteur was convinced that microorganisms existed in the air and when they settle on organic matter they multiply and produce large populations. To demonstrate the presence of microbes in air, he drew large amounts of air through a rubber tube with a plug of nitrocellulose (guncotton). A piece of guncotton was examined under a microscope. It showed a wide variety of spores. He then added a piece of gun cotton to the sterile meat infusion, in which microbial growth appeared. Thus Pasteur proved that air carries large number of microorganisms, and when they enter the broth medium microbial growth occur.

Next, he proceeded to show that quality if air is different from place to place and air of high mountains is almost free from microbial contamination. He prepared broth media, poured in flasks, sterilized and their mouths were sealed by heating. He opened the flasks containing nutrient broth to air of crowded city areas, of countryside and of high mountains. In each succeeding experiment fewer flasks become contaminated with microorganisms. Thus he showed that it was not air itself but the dust in the air that carried the microbes.

Then he began to plan an experiment to let in air into flasks with nutrient broth but not the microbes. When he was experimenting on it, an elderly professor of Chemistry Prof. Balard, who appreciated the work of Pasteur entered his laboratory and suggested to him to prepare flasks, then heat and bend their necks in a long downward s-shape, so that air would be able to pass along it but dust would fall down wards with due force of gravity and would not be able to travel the round bends.

Excited with the idea, Pasteur proceeded to carryout the experiment with swan-neck flasks (figure 1.3), so named because of the S-shaped necks resemble the neck of a swan.

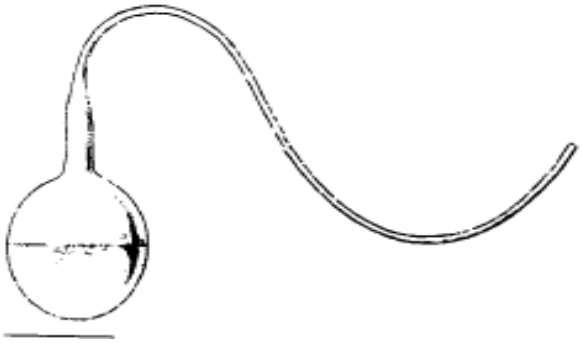

Fig. 1.3 : The swan-necked flask used by Pasteur during his studies on spontaneous generation. The construction of the neck permitted free access of air to the flask contents but prevented entry of microorganisms present in the air

Pasteur boiled the flasks of broth and left them open to air. No microorganisms developed in the broth. When the flasks were tilled so that the liquid medium touch the dust, the microorganisms soon developed in the flasks. When the neck was later cut off microorganisms quickly developed in the broth.

Thus Pasteur silenced all but the most ardent supporters of spontaneous generation and soon became a National celebrity.

Most authorities agree that the final blow to spontaneous generation theory was delivered by a Irish physicist Tyndall. Aware of Pasteur's filter experiments regarding the presence of microorganisms on dust and greater likelihood of microbial contamination in a dusty environment, Tyndall devised a system to determine if air lacking dust particles contained microorganisms. He built a chamber equipped with side windows and curved tubular vents through which bacteria could not enter. The inside surface of this box were coated with glycerol to trap the dust particles that sooner or later would came to settle on the surfaces. This chamber was filled with a rack of test tubes. When the chamber was found to be optically empty of floating matter, simply by shining a beam of light through its windows, the test tubes were filled with a broth, which was then sterilized by immersing the tubes in boiling brine. Tyndall found that the broth remained sterile even through it was in direct contact with air of the chamber. When dust-containing air was introduced, microbial growth appeared after brief time. Thus, with his specialized chambers, Tyndall demonstrated that bacterial life occurred in sterile broth only after it was introduced from outside source.

1.4. Germ theory of disease:

Disease is as old as man, but the knowledge on the cause of disease is very recent. In ancient times many people believed that diseases were sent by Gods as punishment for their sins. This can be referred to as supernatural theory. When man began worshiping and exploring the nature, the disease is attributed to natural causes such as comets, eclipses, floods, earthquakes or major astrological disturbances which charged the air with miasmata or pollution. This is referred to as miasmata theory.

The writings of ancient civilizations of Hindus, Greeks, Romans, Egyptians, Jews and Chinese clearly mentioned that introduction of sick man into healthy population resulted in spread of the disease in population. This is referred to as contagious theory.

Heironymous Fracastorius (1478-1553), an Italian Physician worked on the outbreaks of syphilis, plague, typhoid etc., and in 1546 published a book entitled "De Contagion". According to him, contagion is an imperceptible particle, which he called seminaria, that passes from a patient to healthy person by direct contact, through fomites (inanimate objects used by the patients) or through air. However, his views received little attention because he could not demonstrate the presence of seminaria.

In 1840, Jacob Friedrich Gustav Henle (1809-1885) noted that the nature of all diseases is not same and classified diseases into three categories viz. miasmatic, contagious and miasmatic-contagious diseases, and cited malaria, syphilis and small pox as examples for the three types of diseases respectively. He further theorized that any suspected pathogen must associate with the disease in all cases, pathogen isolated and on inoculation it must produce the disease. These formed the basis for famous Koch postulates enunciated by Robert Koch, a student of Henle.

Robert-Koch provided the definitive proof for germ theory of disease while working with anthrax, a dreaded blood disease of cattle and sheep. The association of a bacterium with anthrax disease was first reported in 1863 by Casmir Joseph Davaine, a French Physician. He had cultivated a bacterium from the diseased animals but could not establish its pathogenicity. Koch began his studies on anthrax in 1875. He injected mice with the blood of diseased sheep and cattle. He then performed meticulous autopsies and noted that the same symptoms appeared regularly. Next, he isolated a few rod shaped bacteria from a mouse's blood by plating the bacteria in the sterile aqueous humor from an ox's eye. Koch watched for hours as the bacteria multiplied and finally transformed into resisting spores. At this point, he took several spores on a silver needle and injected them into a healthy mouse. The symptoms of anthrax appeared with in hours. Koch autopsied the animals and found their blood swarming with rod shaped bacteria. He reisolated the bacteria in sterile aqueous humor.

Koch communicated his findings to Ferdinand Cohn, a famous bacteriologist of that time who discovered the process of multiplication is bacteria and formation of resistant endosperms by some bacteria. In 1876, Cohn invited the 33 years old physician to present his work at the University of Breslau. Koch successfully presented the proof for germ theory of diseases. Koch's procedures were come to known as Koch postulates. They were quickly adopted as a guide for relating specific organisms to specific diseases.

The Koch Postulates are

1. The same microorganism must be identified in all cases of the disease.
2. The microorganism must be isolated and ground in pure culture.
3. The disease must be reproduced in experimental animals inoculated with these pure cultures.
4. The same organism must them be recovered from the experimentally diseased animals.

With the impetus given by the germ theory of disease, the causal agents of a number of human diseases such as tuberculosis, typhoid, cholera, plague, diphtheria, pneumonia, diarrhoea, gonorrhoea,

meningitis, tetanus, botulism, dysentery, syphilis, whooping cough etc. were discovered by the beginning of 20th century, and the last quarter of 19th century is described as "golden age of bacteriology" that laid sound foundations for medical microbiology.

1.5. Germ theory of Fermentation:

Fermentation is a natural process in which alcohol and organic acids such as vinegar citric acid, and lactic and are formed from dissolved sugar in the presence of microorganisms and in the absence of air. The results of fermentation reactions including souring of milk and preparation of alcoholic beverages have been known and used all over the world throughout the history. Two theories viz. non-vital theory (non biological theory) and vital theory (biological theory) were proposed to explain the process of fermentation.

According to non-vital theory, yeasts seen in fermenting materials are products rather than cause of fermentation. During the mid 19th century (1839 to 1869), supporters of non-vital theory of fermentation, including three influential chemists viz. Jacob Berzelius, Justin Von Liebig and Friedrich Wohler, believed that essential, unstable chemical entities called 'ferments' produced the reactions by acting as catalysts or enzymes, simply activating chemical reactions. These unstable ferments were formed by the action of air on sugar containing fluids. The resulting ferments passed their instability to sugar molecules, which in turn decomposed to form the products of fermentation. Liebig used as support for the non-vital theory the absence of any yeasts in acetic and lactic acid fermentations.

The German Physiologist Theodor schwann in 1837 clearly demonstrated the role of yeasts in alcoholic fermentation. He showed that exposing the organisms to heat and chemical agents stopped all fermentation. C.C. Latour and F. Kutzing also independently reported the role of yeasts in alcoholic fermentation. These observations were not readily accepted by the non-vitalists who and argued that it is an entirely chemical process during which breakdown of albumin result in production of alcohol and $C0_{2.}$

Louis Pasteur, who was a professor of Chemistry at Lille University, entered the controversy on the nature of fermentations, mainly at the request of wine merchants of Lille town like Monsieur Bogo and others, to solve the problem of souring of wine. He found that the souring of wine is caused by conversion of sugar to lactic acid. He found rod shaped bacteria in vats showing lactic acid fermentation, while in vats showing alcoholic fermentation the oval cells of yeast were observed.

In a series of classic experiments, Pasteur should that alcohol is produced from grape-yeast mixture. Since chemists of that time argued that wine fermentation is due to break down of protein albumin, he started his experiments with pure albumin. His experimental setups and results are as follows.

1)	Albumin solutions -	Incubations	No Fermentation
2)	Grape juice	Incubations	No Fermentation
3)	Grape juice + Pure yeast	Incubations	Wine +Yeast's
4)	Grape juice + yeast's	Heated and incubated	No wine
5)	Grape juice + Bacteria	Incubation	Sour wine

6)	Grape juice + Yeast's + Bacteria	Heated and incubated	No fermentation
7)	Addition of yeasts for the above setup	Incubation	Good wine.

Thus Pasteur Proved that fermentation in which alcohol is produced is a biological process brought about by yeasts. He further proved that fermentation may yield different end products and a separate organism is involved in each fermentation process. Thus alcohol fermentation is due to yeasts, lactic acid fermentation is due to lactic acid bacteria, butyric acid fermentation is due to butyric acid bacteria, acetic acid fermentation is due to acetic acid bacteria and so on.

Pasteur's studies on fermentation had great effect on food and industrial microbiology. For preventing the souring of wine by acid fermentation, Pasteur developed a method of partial sterilization, which came to be known as pasteurization. It led to the further studies on principles of food spoilage and preservation leading to establishment of food microbiology. With the proof that microorganisms cause fermentation, the microbes are exploited to produce various chemicals industrially and thus industrial microbiology was established.

1.6. Development of vaccines:

Human body possesses immune system that is activated when a pathogen enters the body and produce antibodies to eliminate or neutralize the invading pathogen. A method of stimulating the human immune system with attenuated or killed preparations of pathogens was developed to prevent the outbreak of diseases. The process of was first developed to prevent the outbreak of small pox by using contents of cowpox pustules. Hence, the process was named as vaccination, and the material used is called vaccine. In Latin, Vacca means cow.

Small pox is a dreaded disease and it is also called variola (Latin, vartus means vessel). It is characterized by the formation of fluid filled vesicles all over the body. The vesicles soon become deep pustules that break open and emit pus. In Oriental civilizations, as a preventive measure against small pox, scrapings from boils of the diseased were inoculated to healthy ones, who developed mild disease and then recovered. Since no one was attacked twice, the practice was quite wide spread. In the early 18th century Lady Wortley Montagu, wife of English ambassador to Turkey introduced the technique in England in 1721. It was then called variolation. However, it was never widely used in England despite her vigorous attempts to promote it.

1.6.1. Edward Jenner and Small pox vaccine: Edward Jenner, an English physician, became interested in small pox in 1770s because of its regular incidence during that period. At that time there was a strong belief in the farmers of rural England that people who acquired from cattle a mild disease called cowpox became immune to small pox. A milkmaid who claimed that she could not catch small pox because she had recovered from cowpox stimulated Jenner's interest in developing control measure for the disease. Jenner started systematic studies on small pox in 1778.

Jenner's first immunization recipient was a healthy 8 year old boy not known to have either cowpox or small pox previously. .Jenner inoculated the boy with exudates from cowpox vesicles. As expected, it caused only mild symptoms in the boy. When Jenner inoculated the boy with small pox

virus, the boy showed no symptoms of the disease. Then Jenner began inoculating a large number of his patients with exudates from cowpox vesicles to make them immune against small pox.

Jenner published the results of 23 successful vaccinations in 1798, after 20 years of experimentation. Although Jenner did not understand the nature and cause of small pox, he did manage to successfully protect his patients from the dreaded disease through exposures to cow pox virus. His process is came to be known as vaccination.

The impact of the success of his method of preventing small pox was so great that Napoleon Bonaparte of France had ordered his entire army vaccinated in 1806, and the effort to vaccinate the American population was led by president Thomas Jefferson. For successfully developing a method of immunizing people against a dreaded disease, small pox, Edward Jenner is considered as "Father of Immunology".

1.6.2. Pasteur and development of vaccines: Pasteur recognized the value of attenuated cultures as vaccine against chicken cholera in the same manner as Edward Jenner used cowpox vaccination to provide immunity to small pox, and it was quite successful in preventing chicken cholera.

Then, Pasteur set about developing a vaccine for anthrax. Soon Pasteur and his associates were able to attenuate the pathogen by growing the culture at 42-43^0 C where spores could not develop. Pasteur publicly demonstrated the efficacy of the anthrax vaccine in 1881.

After the success of anthrax vaccine, Pasteur proceeded to develop a vaccine for cure of rabies. Rabies is a terrible disease transmitted by the bite of mad dogs or wolves. The victims invariably die from suffocation or paralysis. Because of the nature of symptoms, Pasteur and his team reasoned that the microbe was probably in the central nervous system and proceeded to experiment with that assumption. They took tissue from the spinal cord of a mad dog, which had died from rabies and injected it into a rabbit. After 15 days the rabbits died and they tried to isolate the pathogen. Since they could not find any microorganism, they worked with the bone marrow of the experimentally killed rabbits. They dehydrated the spinal marrow and chilled at different temperatures for varying Periods to attenuate it. The dried contents of the rabbit spinal cord, when used as vaccine on dogs, it protected the dogs from rabies.

On July 6, 1885, a 9 years old boy Joseph Meister was brought to Pasteur by his parents. He was bitten by a mad dog in his village. Since Rabies vaccine was not ready for human tests, Pasteur asked colleagues from the Academy of Medicine, whether the boy would develop rabies or not. They counted his 14 deep wounds and said he would since the boy would any way destined to develop rabies and die, Pasteur decided to test his vaccine on him. On the evening of July 6, the boy was injected with the extract from the weakened spinal cord of a rabbit which had died of rabies 15 days before. Over the next 10 days, more injections were given, each day with a strong extract. The last inoculation July 16 contained a very virulent material. Miraculously the boy survived, bites healed and he never contracted rabies again. The news of his cure flashed round the word and Pasteur become the only man in the world who could save rabies patients. By October 1886, about 25,000 people have been treated. The

Academy of science decided to found an institute to be called the "Pasteur institute" to organize the treatment of rabies. The word responded and money poured in.

1.6.3. BCG vaccine: Inspired by the work of Louis Pasteur on development of vaccines by attenuation, two French workers Calmette and Guerin developed live attenuated vaccine against tuberculosis, and it is known BCG (Bacillus of Calmette and Guerin) vaccine. They developed the vaccine from a bovine strain of the pathogen by culturing it for 13 years with 239 serial subculturings on bile potato medium. At first it is less attenuated and gave protection in 80% of cases. But now it is thoroughly attenuated and made completely harmless. At present the vaccine is prepared by taking the cultures grown on glycerine-bile-potato medium, for not more than 14 days.

Thus the works of Edward Jenner and Louis Pasteur laid the foundation for development of vaccines for preventing the outbreak of diseases. With development of proper technologies, nowadays, subcellular vaccines, synthetic vaccines and recombinant vaccines are prepared for prevention of a number of viral and bacterial diseases.

1.7. Discovery of antibiotics:

Antibiotics are chemical compounds produced by living microorganisms which in small concentrations inhibit the growth of other microorganisms. Pasteur and Joubert in 1877 for the first time reported that the growth of *Bacillus anthracis* was inhibited by the presence of other bacteria in cultures, and termed the phenomenon as antibiosis. Emmerich and Low (1899) discovered pyocyanase from the growth of *Pseudomonas aeruginosa* and other chemicals now known as pyocyanin having antibiotic properties. However, the credit for the discovery of antibiotic of pharmaceutical importance goes to Alexander Fleming, a Scottish Physician working in St. Mary's Hospital in London. During his work on wound infecting bacteria he identified the first antibiotic, Penicillin. The discovery of penicillin is a celebrated example of chance phenomenon in the history of medical microbiology. In 1928, before going on a brief vacation, he inoculated a number of petriplates with bacteria isolated from wound infections. On his return from vacation, he noticed that among the pile of petri plates on the bench one that had been streaked with a culture of *Staphylococcus aureus* was contaminated by a single colony of a fungus and bacterial colonies immediately surrounding the mould were transparent and apparently undergoing lysis. Instead of throwing away the contaminated plate, he immediately realized the significance of the observation. He reasoned that the mould was excreting into the medium a chemical that caused the surrounding colonies to lyse. He isolated the fungus and identified it as *Penicillium notatum*. The culture filtrates of the fungus were consistently tested positive for their antimicrobial activity, and Fleming named the active principle in the culture filtrate as Penicillin, because it was produced by the fungus *Penicillium*. Penicillin proved to be chemically unstable, and Fleming was unable to purify it. Working with impure preparations, he demonstrated its remarkable effectiveness in inhibiting the growth of many Gram positive bacteria and he even used it with success for the local treatment of human eye infection.

In 1940, Howard Florey and Ernst Chain of Oxford University obtained the culture of *Penicillium* from Alexander Fleming, and successfully isolated penicillin in pure and stable form. At that time England was at war, and hence, industrial development of penicillin was undertaken in United States,

and marketed since 1942. It remains as one of the most effective chemotherapeutic drug for treatment of many bacterial infections even today. For the discovery and production of penicillin, Fleming, Florey and Chain received Nobel prize in 1945. In 1944, Selman Waksman, Professor of Soil Microbiology at Rutjers University in New Jersy, USA, announced that he had discovered an antimicrobial drug Streptomycin produced by an actinomycete, *Streptomyeces griseus*, after a patient screening of about 10,000 strains soil bacteria, actinomycetes and fungi. Streptomycin was found to be a very effective drug in treatment of dreaded disease tuberculosis, and a number of diseases caused Gram negative bacteria. For the discovery of streptomycin Waksman received Nobel Prize in 1952.

In 1945 Prof. Brotzu of Sardinia University, Italy, isolated a fungus *Cephalosporium acremonium* from seawage fall and found that it produces antimicrobial principle. He sent the culture to Howard Florey of penicillin fame in 1948, and by 1955 Florey reported that the fungus produces not one but a group of 7 antibiotics and called them Cephalosporins because they were produced by the fungus *Cephalosporium*. In 1947 John Ehrlich, Paul Burkholder and David Gottlieb discovered a wide spectrum antibiotic, Chloramphenicol, from the culture filtrate of *Streptomyces venezuelae*. Tetracyclines are a group of antibiotics with a common four ring structure having wide spectrum of antibacterial activity, and they were discovered by scientists at Lederle laboratory of Pharmaceutical industry. They screened a multitude of soil microorganisms and isolated chlortetracycline from *Streptomyces aureofaciens*. It was the first antibiotic in tetracycline group. Oxytetracycline, the second in the group was isolated from *Streptomyces rimosus* in 1950.

Rachel Brown and Elizabeth Hazen of Public Health Department in New York, USA, systematically screened a number of isolates of soil microorganisms specifically for an antibiotic that is effective against fungi. In 1948 they isolated an antibiotic they called Nystatin from culture filtrates of *Streptomyces noursei*. It was the first antifungal antibiotic.

Since 1950s thousands of different antibiotics produced by fungi, actinomycetes and eubacteria have been isolated and characterized. However, only a small fraction of these are of therapeutic value, and about 50 are currently produced on a large scale for medical and veterinary use.

1.8. Later developments in Microbiology:

The above described important studies on microorganisms eventually led to the development of different branches of applied microbiology such as medical microbiology, immunology and public health, chemotherapy, food and industrial microbiology. In 1886 German scientists Hellriegal and Wilfarth conclusively proved the biological nitrogen fixation by bacteria (*Rhizobium*) associated with the legume root nodules, and it led to the development of agricultural microbiology.

The interest generated by early discoveries on the importance of microorganisms naturally led to the extensive studies on exploration of microbes in natural environments, and basic branches of microbiology such bacteriology, mycology, phycology, protozoology and virology were established

in 20th century. These studies also established the branch of environmental microbiology.

Bacterial classification required studies on the nutrients that bacteria utilize and the products that they synthesize, and it led to the development of the branches of bacterial physiology and biochemistry.

Charles Griffith in 1928 carriedout inoculation studies on mice with *Streptococcus pneumoniae* and found that avirulent strains of the bacteria become virulent. The interest generated on this discovery led to the identification of DNA as hereditary material and bacterial genetics.

The development of bacterial genetics, physiology and biochemistry during 1950sand 1960s led to an advanced understanding of DNA, RNA and protein synthesis. The field of molecular biology arose to a great extent from these bacterial studies.

Thus the Science of Microbiology was well established in 20th century, and as many scientists believe "21st century is going to be the century of biology", and microbes are going to play a greater role in the future studies in biology. As Louis Pasteur expressed "the role of infinitely small in nature is infinitely large", and it is left to the ingenuity and inventiveness of man to explore and exploit the microbes for the greater benefit of mankind.

1.9. Summary:

Some of the important events that led to the establishment of Microbiology as a field of Science are explained in this lesson. The discovery of microbial world by Leeuwenhoek is the beginning of Microbiological studies. The controversy on the theory of spontaneous generation and the studies of various scientists, including Louis Pasteur and John Tyndall who proved it wrong, are explained. The germ theory of disease, proved by Robert Koch, started the field of Medical Microbiology. The germ theory of fermentation proved by Louis Pasteur had great impact on Industrial Microbiology. The development of vaccines and discovery of antibiotics, which had great impact on public health and chemotherapy, are also explained.

1.10. Model questions:

Essay type Questions

1. What is spontaneous generation ? Explain the controversy on spontaneous generation theory and its impact on biology
2. Discuss the major discoveries that established Microbiology as a field of Science.
3. Explain the germ theory of disease and Koch postulates
4. Give an account of discovery of microorganisms and their significance
5. Discuss the germ theory of fermentation and its significance

Short answer questions

6. Discovery of microbial world
7. Germ theory of disease
8. Germ theory of fermentation
9. Development of vaccines
10. Discovery of antibiotics

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LESSON: 2

HISTORICAL DEVELOPMENT OF MICROBIOLOGY (FOUNDERS OF MICROBIOLOGY)

Objective: To learn about the significant contributions made by the founders of the Science of Microbiology viz. Anton Van Leeuwenhoek, Louis Pasteur and Robert Koch.

Contents:

2.1. Introduction:

In the first lesson, we have studied the important mile stones in the development of the Science of Microbiology. In this lesson, the life and contributions of the three important persons viz. Anton Van Leeuwenhoek, the discoverer of the Microbial world; Louis Pasteur, who laid firm foundations for the development of Microbiology as a separate branch of science; and Robert Koch, who proved the germ theory of disease and contributed to the discovery of the cause of a number of microbial diseases, are explained in detail.

2.2. Anton Van Leeuwenhoek (1632-1723) (Fig. 2.1):

Fig. 2.1

Anton Van Leeuwenhoek is a native of Delft city in Holland. He is the owner of a dry goods shop and enjoyed a comfortable living selling silk, wool and cotton cloths and others. He was head of the city council, inspector of weights and measures, court surveyor and official wine taster. From 1653 onwards he developed a curious hobby of constructing simple (one lens) microscopes and this hobby was perhaps related to the use of magnifying glasses by cloth merchants of that time to examine the defects in fabrics. Even though Leeuwenhoek had no formal education and never attended a University, it is his hobby of construction of microscopes coupled with his unusual degree of curiosity to observe almost every conceivable object that could be looked at through a microscope, that led him to the discovery of microbial world and made him an immortal scientific personality.

2.2.1. Construction of microscopes: Starting from 1653, Leeuwenhoek constructed more than 500 simple, one-lens microscopes. Although he was not the first to build a microscope, his were the finest of that time. During his spare time he ground pieces of glass into fine double concave lenses and made simple microscopes by placing the lenses between two silver or brass plates riveted together. His lenses were, in fact, only refinement of simple magnifying glasses and are capable of magnifying the image around 50 to 300 times. Although compound microscope had already been invented by Hans and Zacharias Jansen in 1590, Leeuwenhoek found his devices more suitable for observing the specimens with transmitted light.

Initially he used his lenses to inspect the quality of cloth, but as his fascination with microscopic objects developed, he examined almost every conceivable object on which he could use his microscopes. He had illuminated the liquid specimens by placing them between two pieces of glass and made light to fall on the object at 45^0 angle so as to provide a form of dark field illumination and made the objects clearly visible. He perfected the technique of observation for 20 years before reporting his findings.

2.2.2 Discovery of microbial life: Leeuwenhoek's position in the development of Microbiology has been firmly established because of his remarkable observations and descriptions of microbial forms of life.

In September 1674, Leeuwenhoek brought a glass with greenish cloudy water from a marshy lake out side the city of Delft, and observed a drop of it under his lens. The water teemed with tiny microorganisms. He called these microbial life forms as 'animalcules' (meaning little animals) because they are showing active movements. His curiosity aroused by his observation of microbial life forms in lake water and soon he located animalcules in rain water, materials, drinks, in the scum of the teeth, faeces, semen and eventually in most of the specimens he observed.

Leeuwenhoek had realized the importance of recording his observations and he must have had great patience and persistence to continuously observe through specimens and record the drawings of microorganisms. His sketches were elegant in detail and clarity. From his descriptions and sketches, it is evident that he had observed

1) *Amoeba*, *Paramecium* and other protozoans.
2) Yeasts and hyphae of filamentous fungi.
3) Various microscopic algae including *Volvox*.

A part from the above, his observations on bacteria are most significant. In his letter to the Royal Society of London, dated September 17, 1683, he provided his illustrations of bacteria in the form of rods, spheres and spirals. It was published in 1684. This sketch of Leeuwenhoek became a classic, and adored the title pages of many of the modern text books of bacteriology and Microbiology (see figure 1.2 in lesson 1).

In addition to the diversity of microbial word, Leeuwenhoek emphasized its incredible abundance also. For example, in one of his papers, describing for the first time the characteristic bacteria of

human mouth, he wrote "I have had several gentle women in my house, who were keen on seeing the little eels in vinegar, but some of them were so disgusted at the spectacle that they vowed they would never use vinegar again. But what if one should tell such people in future that there are more animals living in the scum of teeth in a man's mouth, than there are men in a whole kingdom".

Although not a professional scientist, Leeuwenhoek performed some simple experiments that throw light on the existence of microorganisms. For example, after observing animalcules in rainwater, he decided to test whether microorganisms came from heaven or came from earthly sources. He washed a porcelain bowl in fresh rainwater, set it out in his garden during a rainstorm, collected rainwater and observed the freshly collected rainwater. He could not observe any animalcules in fresh rainwater. After allowing the water to stand for a few days, he once again observed drops of water from the sample and found numerous microorganisms. He concluded that for abundance of microbes in water, multiplication of a few microbes present in rainwater is the main cause. However, he did not enter the controversy on spontaneous generation.

2.2.3. Other contributions:

Leeuwenhoek's discoveries went beyond the world of microbes. He made many other contributions of biological significance.

1. Provided confirming evidence for William Harvey's theory of blood circulation.
2. Constructed an aquatic microscope and observed the flow of red blood cells through capillaries of a fish's tailfin.
3. Observed muscle fibre striations.
4. Observed nuclei of fish blood cells.
5. Discovered the coverings (myelin sheaths) of nerve fibres.
6. Described the optic nerve of a cow.
7. Described the structure of cotton seeds.
8. Described the structure of slices of cork.

2.2.4. Association with Royal Society of London: Leeuwehoek developed intimate association with Royal society of London, the only scientific group of that time. Leeuwenhoek did not directly contact the Royal society, because England and Holland were bitter rivals at that time fighting for colonization of India. Regnier de Graff, a Fellow of Royal Society, who came to know about the discoveries of Leeuwenhoek suggested to the society in 1673 to contact Leeuwenhoek. They did so and Leewenhoek positively responded, and began sending his observations and illustrations in his mother tongue and they were translated to English for publication purpose. Beginning from 1673 up to 1723 till his death, he contributed more than 200 letters to the Royal Society journal over a period of 50 years.

In 1680, Leenwenhoek was elected as the Fellow of the Royal Society (F.R.S), and with Issac Newton and Robert Boyle, became one of the most famous men his times. Peter the Great of Russia, and Queen of England visited his place to peer into his microscopes.

2.2.5. Legacy of Leeuwenhoek: It is interesting to note that Leeuwenhoek had little formal education and knew no language but his native tongue, yet because of his almost child like curiosity and great skills as an objective observer, he discovered some of the greatest secrets of nature.

Because of his almost numerous observations and careful measurements and recording of specimens, Leeuwenhoek is considered as the father of Bacteriology, hematology, Protozoology and other sciences for which microscope is the main investigating tool.

Although Leeuwenhoek's contemporaries marveled at his discoveries, not much importance was given to them, because they considered the animalcules of Leeuwenhoek as nothing more than little oddities of nature. This is due to the prevailing dogma of spontaneous generation. Full realization of the importance of his discoveries had to wait for about 200 years and arrival of Louis Pasteur and Robert Koch.

2.3. Louis Pasteur (1822-1895) (Fig. 2.2)

Fig. 2.2

Louis Pasteur, the French Chemist whose experiments on microorganisms led to the greatest medical break through of all time and became a legend of science in his own life time, was born on December 27, 1822 in a small town of France called Dole, in a middle class family. He graduated from the University of Sorbonne, where he was greatly impressed by the lectures of Chemistry Professor Jean Baptiste Dumas. In 1843, he joined the famous French Institute Ecole Normale Superieure in Paris for research in Organic chemistry and physics. He worked on sterioisomerism of tartaric acid crystals for his thesis. In 1848 he achieved distinction in organic chemistry for his discovery that tartaric acid, a four-carbon compound, forms two types of crystals, with same chemical constitution. He successfully separated the crystals while looking through a microscope. In doing so, he developed a skill that helped him in his later studies on microorganisms. In 1849, he was appointed as lecturer in Chemistry at the University of Strasbourg, Where he continued his studies on crystals. In 1854 he was made Professor of Chemistry and Dean of New faculty of science at the University of Lillie, in the industrial city of Lillie. In 1855, he began studies on the problem of souring of wine, at the request of Monsieur Bijou, a manufacturer of alcohol from beet sugar, which ultimately proved the role of microbes in fermentation, development of pasteurization technique to prevent souring of wine, and discovery of anaerobic life forms. At the end of 1857 he became Director of Scientific studies in his old school, Ecole Normale in France, where he entered the controversy of spontaneous generation and successfully proved that spontaneous generation is wrong, and microorganism in the air, were deposited on organic matter develop putrefaction. Pasteur realized that the disease in animals and humans is also due to microbes and emphatically stated that "it is in the power of man to make parasitic illnesses disappear from the face of the globe, if the doctrine of spontaneous generation is wrong, as I am of sure of it". The significance of Pasteur's proof of germs in the air, was quickly appreciated by Joseph Lister, Professor of Surgery in Edinburgh, who developed the methods of antiseptic surgery.

In 1865, Pasteur took up of the problem of pebrine disease of silk worms, and developed a method of controlling the disease on the assumption that it is caused by a microbe, even though he could not isolate any pathogen.

In 1878, Pasteur began studying a poultry disease that is taking heavy toll of chickens and developed a vaccine for it.

In 1881, Pasteur successfully developed a vaccine for rabies, a dread disease transmitted to humans by the bite of mad dogs.

In 1886 Pasteur Institute was established in the centre of Paris for mass production of Rabies vaccine.

In 1895 on September 28, Pasteur died at the age of 72 with much satisfaction that he is leaving the world in a better position than when he entered it.

2.3.1. Significant contributions of Louis Pasteur: Louis Pasteur, a trained organic chemist, entered the field of Microbiology through his studies on fermentation and successfully proved the germ theory of fermentation (explained in lesson 1). The next major contribution of Louis Pasteur that had a great bearing on realizing the importance of microorganisms is proving that spontaneous generation is wrong (explained in the lesson 1). The other major contributions of Pasteur are described here.

2.3.1.1. Pasteurization: After finding the cause of souring of wine through his studies on fermentation, Pasteur proceeded to solve the problem. He tried to eliminate the unwanted microorganisms by using a variety of antiseptics but the results were not satisfactory . Then after much hesitation, he considered the possibility of using heat as a sterilizing agent. Heating destroy the flavour, the most important quality of wine. Hence, he proceeded cautiously and after much experimentation, came to the conclusion that partial sterilization for a specific period of time at temperatures between 50^0 C to 60^0 Celiminate the unwanted microbes without changing the quality of wine. This process of partial sterilization was soon came to be known as pasteurization. This process was found applicable in production of wine, beer, cider, vinegar, milk and countless other perishable beverages, foods and other organic acids. The name of Pasteur is forever linked in the public mind with the process of pasteurization.

Pasteur also showed that souring of milk is caused by microorganisms. Today milk and other foods are routinely pasteurized by heating at 63^0 C for 30 minutes or at 71^0 C for 15 seconds. These temperatures are adequate to reduce the number of food spoiling organisms and many pathogenic organisms, including *Mycobacterium tuberculosis* transmitted through milk.

It was characteristic of Pasteur that he did not remain satisfied with formulating the theoretical basis of heat sterilization but took active interest in designing of industrial equipment adapted to the heating of fluids in large volumes and at low cost. It is worth noting that though Pasteur took patents to protect the rights to his discovery, he decided to release his patents to the public and he did not derive any financial benefit even from the development and sale of large scale industrial equipment devised for Pasteurization.

2.3.1.2. Discovery of anaerobes: During his studies on butyric acid fermentation, Pasteur discovered a fundamental biological phenomenon, the existence of life forms that can live only in the absence of free oxygen. While examining microscopically the fluids that are undergoing butyric acid fermentation, Pasteur observed that the bacteria at the margin of a flattened drop, in close contact with air, became immobile where as those in the centre of the drop remained motile. This suggested that air had an inhibiting effect on the microorganisms he is observing. To verify the effect of air on butyric acid bacteria, Pasteur passed air through fermenting fluid, which resulted in retarding and sometimes completely arresting the butyric and fermentation. He concluded that some organisms can live only in the absence of oxygen, a gas previously considered essential for maintenance of all life. He introduced the terms aerobes and anaerobes to describe the life forms which cannot live without oxygen and to those which can live only in the absence of oxygen, respectively.

2.3.1.3. **Studies on Pebrine disease of silkworm**: In 1865, Louis Pasteur began to work on pebrine disease of silkworm, mainly at the request of Professor Dumas. Prof. Dumas is a native of Alais village in south of France, known for its silk industry. In that village and surroundings, pebrine disease was killing silk worms in large numbers and it devastated the silk industry.

The disease starts on the surface of the silk worms like a dusting of pepper grains. In south of France it was called Pebrine from pebre the local name for pepper.

Pasteur microscopically observed hundreds of diseased silk worms, their feed and eggs. He discovered that the first sign of disease in a mature moth is appearance of a little globule in the body and it was a microbe. It multiplied and spread through out the body and passed into the eggs and then to the worms. So he suggested to the farmers to check the moth after she had laid eggs for any globules in her body. If globules are present, those eggs would be diseased and must be destroyed. If the moth's body is clear of globules, the eggs would be sound, and healthy worms would emerge.

Thus Pasteur solved the problem of pebrine disease, by assuming the microbial cause of the disease and spread of the pathogen from diseased to healthy and from diseased moth to off spring. Now, we know that the pebrine disease is due to a protozoan *Nosema bombicus* belonging to sarcodina.

2.3.1.4. Studies on Chicken Cholera: In 1878 Pasteur began his studies of chicken cholera, which became a menace of poultry industry. The disease is unrelated to human cholera. After staggering about lethargically hens would fall dead next day. After several attempts Pasteur found a microbe and isolated it. Later it was named *Pasteurella aviseptica*. A small drop of fresh culture could quickly kill a chicken.

One of the most celebrated of chance observations arose from Pasteur's studies on this disease. When some chicken were inoculated with an old culture, they barely sickened and then recovered completely. When inoculated with a fresh virulent culture, these hens were quite resistant to the disease. Soon it was determined that the virulence of the microbe could be attenuated by storing the cultures for variable intervals of time until all virulence was lost. Pasteur recognized the value of attenuated cultures as vaccine against chicken cholera in the same manner as Edward Jenner used cowpox vaccination to provide immunity to small pox.

Jenner's method was based on using a disease known not to be harmful to people, to produce protection against a disease which was dangerous.

But what was happening with chicken cholera was different, weakened microbes of the disease itself had raised the hen's own defences.

It revolutionized the methods for developing vaccines

2.3.1.5. **Development of anthrax vaccine**: Having successfully developed a method of preventing chicken cholera, Pasteur set about doing the same for anthrax. Soon Pasteur and his associates were able to attenuate the pathogen by growing the culture at 42-43^0 C where spores could not develop.

In an example of his gift for dramatis and his readiness to meet opposition in open battle, Pasteur participated in a great public demonstration of his anthrax vaccine in May 1881.

On May 5, 1881, before a great crowd, he inoculated with attenuated strain 24 sheep, 6 cows and 1 goat and the same numbers were kept as controls.

On May 17, test animals were reinoculated with a somewhat more virulent culture.

On May 31, Pasteur inoculated the test animals which received vaccination and also the control animals with virulent strain of the pathogen.

By early June, it become very clear that all vaccinated animals survived while control animals were dead or dieing. Pasteur achieved stunning success.

The impact of the Pasteur's demonstration was so great that by 1884, 3.4 million sheep and 4,40,000 cattle had been vaccinated against anthrax.

2.3.1.6. **Development of rabies vaccine**: Pasteur is remembered more for his cure of rabies than for vaccines against chicken cholera and anthrax. Rabies is a terrible disease transmitted by the bite of mad dogs or wolves. The victims invariably die from suffocation or paralysis. Because of the nature of symptoms, Pasteur and his team reasoned that the microbe was probably in the central nervous system and proceeded to experiment with that assumption. They took tissue from the spinal cord of a mad dog, which had died from rabies and injected it into a rabbit. After 15 days the rabbit died. They tried to attenuate the pathogen. Since they could not find any microorganism, they worked with the bone marrow of the experimentally killed rabbits. They dehydrated the spinal marrow and chilled at different temperatures for varying periods to attenuate it. The dried controls of the rabbit spinal cord, when used as vaccine on dogs, it protected the dogs from rabies.

On July 6, 1885, a 9 year old boy Joseph Meister was brought to Pasteur by his parents. He was bitten by a mad dog in his village. Since Rabies vaccine was not ready for human tests, Pasteur asked colleagues from the Academy of Medicine, whether the boy would develop rabies or not. They counted his 14 deep wounds and said he would. Since the boy would any way destined to develop rabies and die, Pasteur decided to test his vaccine on him. On the evening of July 6, the boy was injected with the

extract from the weakened spinal cord of a rabbit, which died of rabies 15 days before. Over the next 10 days, more injections were given, each day with a strong extract. The last inoculation on July 16 contained a very virulent material. Miraculously the boy survived, bites healed and he never contracted rabies again.

The news of his cure flashed round the world and Pasteur became the only man in the world who could save rabies patients. By October 1886, about 25,000 people have been treated. The Academy of science decided to establish an institute to be called the "Pasteur Institute" to organize the treatment of rabies. The world responded and money poured in.

2.3.2. **Pasteur's legacy**: Pasteur's had trained and inspired young men, who went on to achieve distinctions.

Dr Roux and Dr Yersin developed the treatment for diphtheria
Dr Yersin discovered the pathogen of plague.

Dr Metchnikoff, one of Pasteur's most brilliant assistants discovered macrophages and laid the foundations for the theory of cell mediated immunity.

Dr Charles Chamberland invented porcelain filters that paved the way for discovery of viruses.

The Pasteur institute has become the most famous Centre for study of microbes and microbial diseases. Institutes established for development of rabies vaccine, where ever they are in the world, are named as "Pasteur Institute". The Pasteur institute at Paris continues to break new grounds in microbial research and the AIDS virus was first isolated there in 1983.

As Joseph Lister in his speech in 1892 at the great ceremony held at Sorbonne to recognize Pasteur's achievements, told " he had raised the veil that for all the centuries made infectious illness a dark mystery".

He will be forever remembered by public for his rabies vaccine and Pasteurization.

2.4. Robert Koch (1843 – 1910) (Fig. 2.3)

Fig. 2.3

Robert Koch, a native of East Prussia, now part of Germany, is a physician par excellence and one of the trailblazers of Microbiology. He was a student of Prof. Gustav Henle who outlined the theoretical steps to identify a microorganism as a pathogen. Through his meticulously planned experiments, Koch provided the proof for germ theory of disease in 1876. He identified the bacterial cause of anthrax, tuberculosis and cholera, developed pure culture techniques and established a strong school of microbiologists, because of whose contributions, the last quarter of 19th century is came to be regarded as 'Golden age of Bacteriology'. He was one of the early microbiologists to receive Nobel prize. He was awarded Nobel prize in 1905 in recognition of his contributions to the understanding of tuberculosis in particular, and Microbiology in general.

2.4.1. **Germ theory of disease**: Even though a number of workers had suspected the role of microbes in causation of disease in animals and man, Robert Koch provided the definitive proof for germ theory of disease. The contribution of Koch to germ theory of disease is explained in lesson 1 (1.5).

2.4.2. **Development of pure culture techniques**:

After his successful demonstration of germ theory of disease, Robert Koch received recognition for his work and was appointed as Director of Imperial Health Office. His studies during this period led to the development of pure culture techniques, that sparked further development of Microbiology.

The earlier approaches to isolate bacteria focused on the development of solid natural media such as freshly cut surfaces of potato and carrot, freshly backed bread slices etc. On inoculation, bacteria grew on the surface of the natural substrata as distinct colonies, each consisting of millions of cells of same organism. However, these natural media are not satisfactory for culturing of all types of bacteria. Koch theorized that since bacteria could grow as compact colonies on natural solid substrata, they could also grow on the solidified broth medium also. So, to solidify the broth he added gelatin to the broth, and while this preparation was still warm and liquid it was poured on sterilized glass plates and allowed to cool and solidify. Bacteria could then be introduced by spreading one drop of specimen solution across the surface of gelatin. This process is known as streaking. The inoculated glass plate was covered by a belljar and left to incubate. By using the bacteria grown on solid media for inoculation of test animals, Koch proved that bacteria, not the toxins in the broth, were the cause of the disease.

In 1881, Koch demonstrated his pure culture techniques to the International medical congress meeting at Lister's laboratory in London. Pasteur and several of his coworkers were present as Koch outlined his methods. Several days later Koch received a personal letter of congratulations from Pasteur.

2.4.3 Use of Agar: On one occasion Koch was dismayed to find that gelatin medium was liquefied. It appeared that certain types of bacteria were producing a chemical substance to digest the gelatin. Moreover gelatin liquefied at high incubator temperatures commonly used to cultivate bacteria.

Walter Hesse, an associate of Koch, mentioned the problem to his wife and laboratory Assistant Fanny Hesse. For years, she was using a seaweed powder called agar to solidify her jams and jellies. She learnt the use of agar from her mother, who learnt it from friends living in Java. Fanny Hesse suggested the use of agar as a solidifying agent for bacteriological medium also. Walter Hesse was sufficiently impressed and recommended the use of agar to Koch. Soon Koch was using agar routinely in his culture media and in 1884 he first mentioned agar in his paper on the isolation of tubercle bacillus.

2.4.4. Studies on Tuberculosis: Robert Koch developed an interest to study the cause of tuberculosis, a highly contagious dreaded disease of respiratory tract. In 1882 he isolated *Mycobacterium tuberculosis* and proved its pathogenicity. He reported the formation of characteristic tubercles by the pathogen and described various types of symptoms caused by it.

As Louis Pasteur developed vaccines against anthrax and rabies, Koch also tried to develop a vaccine against tuberculosis. Instead of using attenuated strains as vaccine, he tried to extract a virulent chemical factor from the cultures of the pathogen, that could immunize the people without fear of causing infection. In 1890, he isolated a protein fraction from old cultures of *M. tuberculosis* and called it tuberculin. When tuberculin was injected into the skin of the persons not previously exposed to tuberculosis, there appeared no reaction, but when it was injected into the forearm of exposed persons, small rash (erythema) developed. This was referred to as '**Koch Phenomenon**'. He announced that tuberculin will act as a vaccine against TB. But soon it was found that it was not having the properties of a vaccine. However Koch phenomenon was soon developed into a skin test to identity persons already sensitized by inapparent infection by *M. tuberculosis*.

2.4.5. Discovery of Cholera: In 1883, Koch was appointed as head of the Cholera commission to investigate the epidemic out breaks of the disease in various parts of the world. First he visited Egypt and isolated a bacterium from cholera patients in Alexandria (present name Kairo). He then visited India and also found the similar organism to cause the disease in Calcutta and surroundings. Further, he isolated the organism from various surface waters. In 1884 Berlin conference, he announced the discovery of *Vibrio* as the causative agent of cholera.

2.4.6. Other studies: In 1885 he was appointed to the University of Berlin. In 1891 he became Director of the institute of infectious diseases. At various times, as part of his professional duty, he studied malaria, plague sleeping sickness and other diseases. But it is for the Germ theory of disease that he will be remembered forever, and it is for his studies on tuberculosis that he got Nobel Prize in 1905.

In 1910 he died of a stroke at the age of 66.

2.4.7. Legacy of Robert Koch: The influence of Robert Koch on development of Microbiology and Medicine went beyond his personal contributions. He built a strong school of Microbiology in Germany and trained a dedicated band of scientists who contributed further to the advancement of science. Some of the well known associates of Robert Koch who made a mark with their contributions are

1) Richard Julius Petri who invented petridishes for culturing of microorganisms.

2) Gregor Gaffky who isolated the typhoid bacillus in1884.

3) Friedrich Loeffler (1884) who isolated diphtheria bacillus in 1884

4) Emil von Behring who developed the successful treatment of diphtheria by injecting antitoxin, a blood product (actually a preparation of antibodies) obtained from animals injected with diphtheria toxin. For this contribution, which saved the lives of children from diphtherite infections, he was awarded Nobel prize in 1901, even ahead of Robert Koch.

A part from the above mentioned, a number of young men from various parts of the world and many more were inspired by his work.

Thus, the science of Microbiology was immensely enriched due to the efforts of Robert Koch, who will be forever remembered, along with Leeuweuhoek and Louis Pasteur, as founders of Microbiology.

2.5. Summary: A brief sketch of life and important contributions of three great founding fathers of the science of Microbiology are given in the lesson. Details of construction of simple microscopes, discovery of microbes and other contributions of Leeuwenhoek and his association with Royal Society of London are described. Details of significant contributions of Louis Pasteur, other than his works on fermentation and controversy on spontaneous generation (which are given in lesson 1), such as pasteurization, discovery of anaerobes, studies on pebrine disease of silk worms and chicken cholera, development of vaccines for anthrax and rabies are described. Important contributions of Robert Koch (other than germ theory of disease which is described in lesson 1) such as development of pure culture techniques, use of agar, studies on tuberculosis and cholera are described.

2.6. Model questions:

Essay type Questions

1. Discuss the contributions Leeuwenhoek, Pasteur and Koch in development of Microbiology
2. Discuss the contributions of Leeuwenhoek to the field of Microbiology
3. Discuss the major contributions of Louis Pasteur to the field of Microbiology
4. Discuss the influence of Louis Pasteur on development of Microbiology
5. Discuss the major contributions of Robert Koch to the field of Microbiology

Short answer questions

6. Leeuwenhoek
7. Louis Pasteur
8. Robert Koch
9. Discovery of Microbial world
10. Pasteurization
11. Discovery of anaerobes
12. Legacy of Louis Pasteur
13. Koch’s Phenomenon
14. Discovery of Cholera

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LESSON 3

MICROBIAL DIVERSITY

3.0. Objectives

- The study of microbial diversity or microbial systematics.
- Classification of microorganisms in early classification systems.
- Classification of microorganisms by Ernst Haeckel
- Whittaker's modern classification systems.
- Classification given by Carl Woese based on rRNA sequence
- Position of acellular non living/living microorganisms like Viruses, Viroids and Prions in classification systems.

3.1. INTRODUCTION

Imagine a university library with its many thousands of volumes, without a logical method for arranging these books. Such a disordered library would be practically useless. Books could not be easily found and once found, could not be replaced in locations where they could be found again. There would be no method for systematic addition of new publications to the collection.

Now, consider a living library that contains as many as 5 million different kinds of organisms. The sheer numbers and diversity of organisms including microorganisms make it necessary to have an organized system for classification. The identification of microorganisms become a formidable, if not impossible task without such a system. The systematic categorization of organisms into a coherent scheme is known as Taxonomy. Taxonomy not only to organize plants, animals and microbes into categories, but also can be useful in showing possible evolutionary relationships among similar types of organisms.

Microbiology is the study of microorganisms, a large diverse group of microscopic organisms that exist as single cells or cell clusters. It also includes viruses, which are microscopic but not cellular. Microbial cells are thus distinct from the cells of animals and plants, which are unable to live alone in nature and can exist only as parts of multicellular organisms (Fig 3.1).

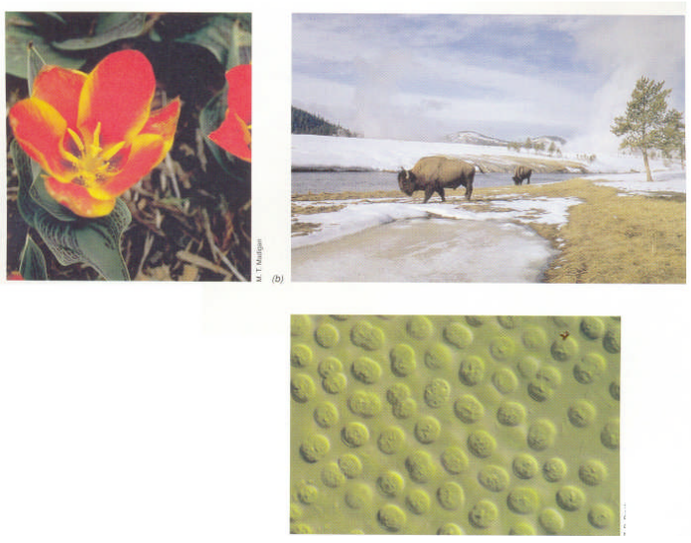


Fig. 3.1. Living organisms are composed of cells.

A single microbial cell is able to carry out its life processes of growth, energy generation and reproduction independently of other cells, either of the same kind or of a different kind.

Life has existed on Earth for about 4.6 million years. For most of that time, life was exclusively microbial Stromatolites, which are domed rocks formed from banded layers of sediment where bacteria have been grown in coastal marine ecosystems, have been discovered that are 3.8 billion years old. Stromatolite formation is brought about when sediments adhere to the polysaccharide capsules and slimes of bacterial cells. As the bacteria move they leave behind banded layers of sediment. The ancient Stromatolites located in western Australia and South Africa indicate that microbial life evolved and began to diversify shortly after the formation of the Earth (Fig. 3.2).

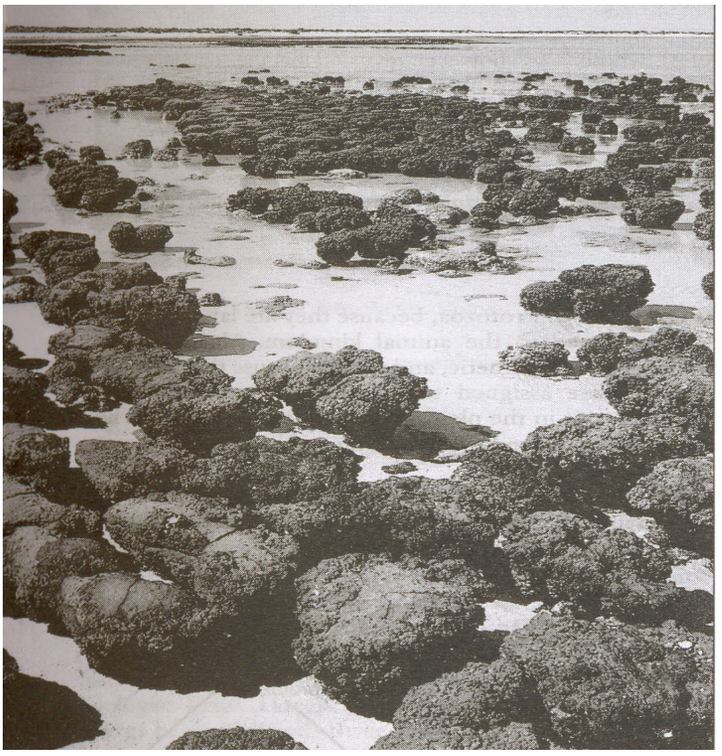


Fig. 3.2. Stromatolites in shallow sea water in western Australia. These are 1000-2000 years old.

During the first few million years of life on Earth, evolution had resulted already in various morphologically and physiologically diverse microorganisms. Since that time, evolution has resulted in the extraordinary diversity of microorganisms—bacteria, archaea and eukaryotes—that inhibit the Earth today. Besides microbial life, evolution also produced about 600 million years ago the “higher forms of life”, the plants and animals that represent the canopy of the evolutionary tree. The microbial world is so diverse, given that microorganisms have been evolving for over 3.8 billion years compared to only 0.6 billion years for plants and animals.

Like all living organisms, new species of microorganisms evolved through the interactions of their genomes with the environment. In accordance with Darwinian principles, mutations, genetic recombination and natural selection all played roles in the evolution of new microbial species.

We know a great deal about the evolutionary history or phylogeny (from the Greek ***Phylon*** meaning tribe and ***genesis*** meaning origin), of higher organisms because of the fossil records they left behind. Scientists who study the evolution and phylogeny of animals and plants traditionally ignore microorganisms. They are concerned with fossils and mathematical model of evolution. They play little attention to the fact that bacteria, archaea and the higher plants and animals all evolved from the same progenitors, that most of evolution occurred at the cellular level of microorganisms, and that bacteria are diverse and the basis of mitochondria and chloroplasts of eukaryotic cells. Earlier microbiologist, Martinius Beijerink, recognized that microorganisms represented information and show evolutionary linkages for all living organisms.

It became clear that the origins of organells, mitochondria and chloroplasts of eukaryotic cells were formerly prokaryotic bacterial cells that had been acquired during eukaryotic cellular evolution. Thus the application of molecular analyses has led to the current view that all organisms evolved from a common ancestor along three distinct paths to form the great diversity of microorganisms, plants and animals that exist today.

Based on this philosophy, the modern science of *microbial systematics* developed. Microbial systematics, the study of microbial diversity—relied on observable phenotypic characteristics such as morphology and metabolism, to interfere the phylogeny of microorganisms. Later, systematics turned to molecular analyses to reveal the evolution of the microbial world.

It has been difficult to classify microorganisms based on how they are related to each other in terms of their evolutionary relationships. We used fossils that were presented in the Earth's crust to piece together evolutionary relationships of plants and animals. Microorganisms lack structures like bones and woody tissues that are preserved as fossils. Many microorganisms look exactly alike, we are unable to simply look at a microorganism and identify it. The classification of microorganisms has been based on relationships in morphology, physiology and its evolutionary history as revealed in its genetics among actual living microorganisms.

An understanding of microbial diversity requires an appreciation of the evolutionary roots of cells. Because evolution has shaped all life on Earth, the structural and functional diversity we see in cells' represents successful evolutionary events that have conferred survival value on the microorganisms extant today.

Many microorganisms are unicellular. Each organism is composed of a single cell. There are also some microorganisms that form multicellular groups of associated cells. None of these microorganisms form integrated units called tissues, that would serve different functions. This character distinguishes microorganisms from plants and animals, which are multicellular and form different tissues. This fundamental difference was recognized in 1866 by Ernst Hackel, when he defined microorganisms as Protists, as organisms lacking tissue differentiation.

There are two architecturally different types of cells of living organisms, prokaryotic cells and Eukaryotic cells. Cells with a nucleus occur in all organisms except archaea and bacteria. All cells have some common features irrespective of prokaryote or eukaryote cell.

Cells of all organisms:

1. Are highly organized
2. Are capable of growth and reproduction
3. Contain the same hereditary molecule,DNA that passes hereditary information to offspring cells.

Until a decade ago, the functional, structural differences between eukaryotic and prokaryotic cells led most scientists to believe that there were two primary lines of evolution: one leading to organisms with proteolytic cells, the bacteria and the other organisms. All organisms with prokaryotic cells were considered as to be bacteria. The bacteria and prokaryote were though to be synonymous. This view changed radically in 1980s when molecular biologists, led by Carl Woese, began to analyses the informational molecules that directly reflect the heredity of a cell.

Analyze and comparisions of similarity of RNA molecules of ribosomes revealed that there were three principle times of evolution that formed three separate domains of cellular evolution: bacterial cells, archaeal cells and eukaryotic cells (Fig 3.3).

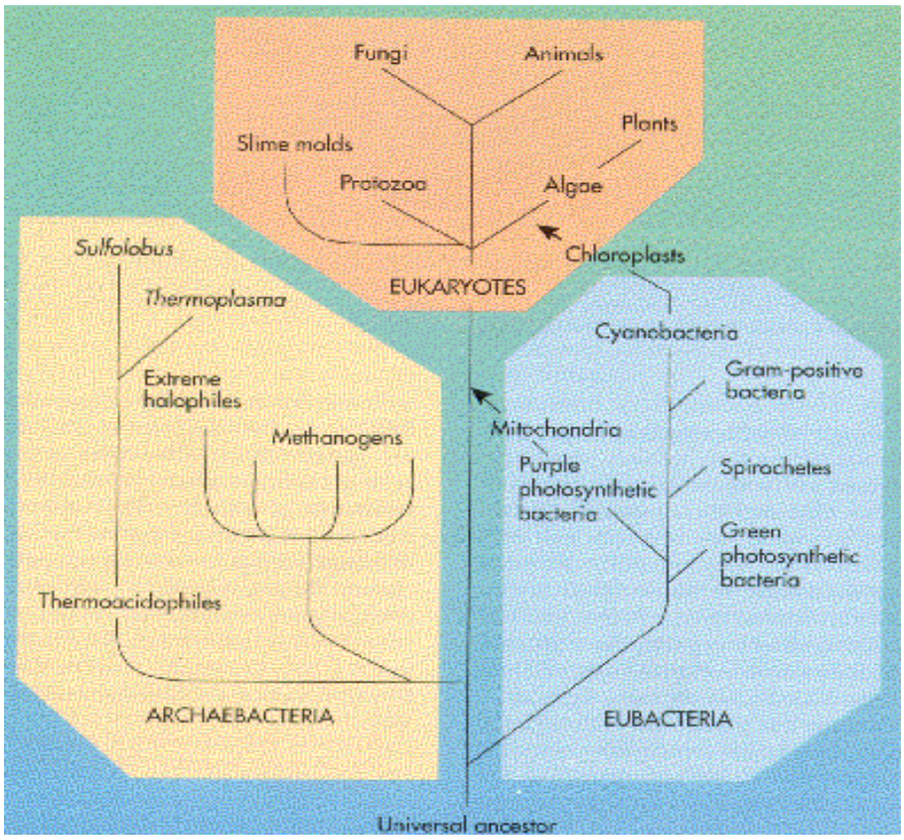


Fig. 3.3. The three-Kingdom classification system proposed by Carl Woese.

The earliest schemes assigned microorganisms to one or the other of the two major categories of living things—plants and animals. Protozoa, because they are largely motile, were assigned to the animal kingdom. As Algae are photosynthetic and as Fungi are non motile, were assigned to the plant kingdom. Bacteria were also put in the plant kingdom as there was no more logical place for them and the Archaea were not known at the time.

3.2. CLASSIFICATION SYSTEMS

Early classification systems : Early classification systems date from the time of Aristotle, before the existence of microorganisms was know (Fig 3.4).

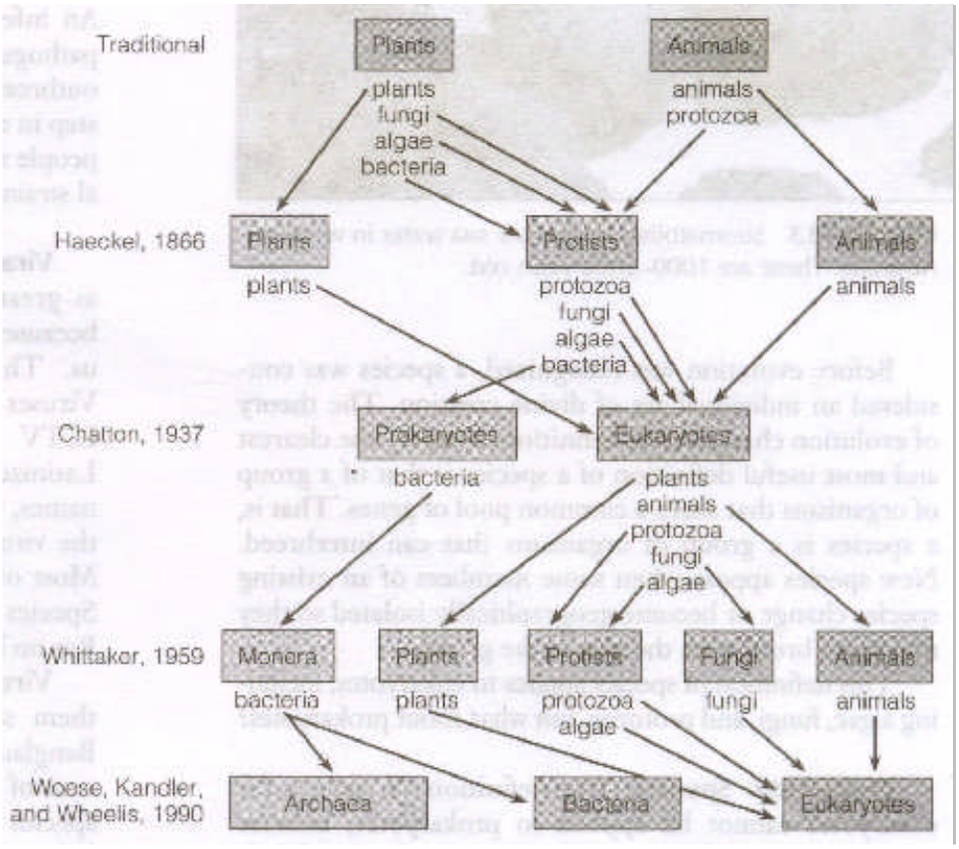


Fig. 3.4. Evolution of the major schemes for classifying organisms.

In the eighteenth century, after the microbial world was discovered, Swedish Botanist, Carl Linnaeus, established the first comprehensive classification systems of all living things. Linnaeus placed all microorganisms into a single genus, *Chaos*, because he could not establish objective criteria for distinguishing among them.

The universal phylogenetic tree shows the relative evolutionary positions of major groups of living organisms. The tree shows that the Eukaryota are not of recent origin but instead are as ancient as either of the prokaryotic lineages. Before endosymbiotic events led to the modern eukaryotic cell, organelle-less Eukarya inhibited Earth and these organisms shared common ancestry with the other two evolutionary lines (Fig 3.5).

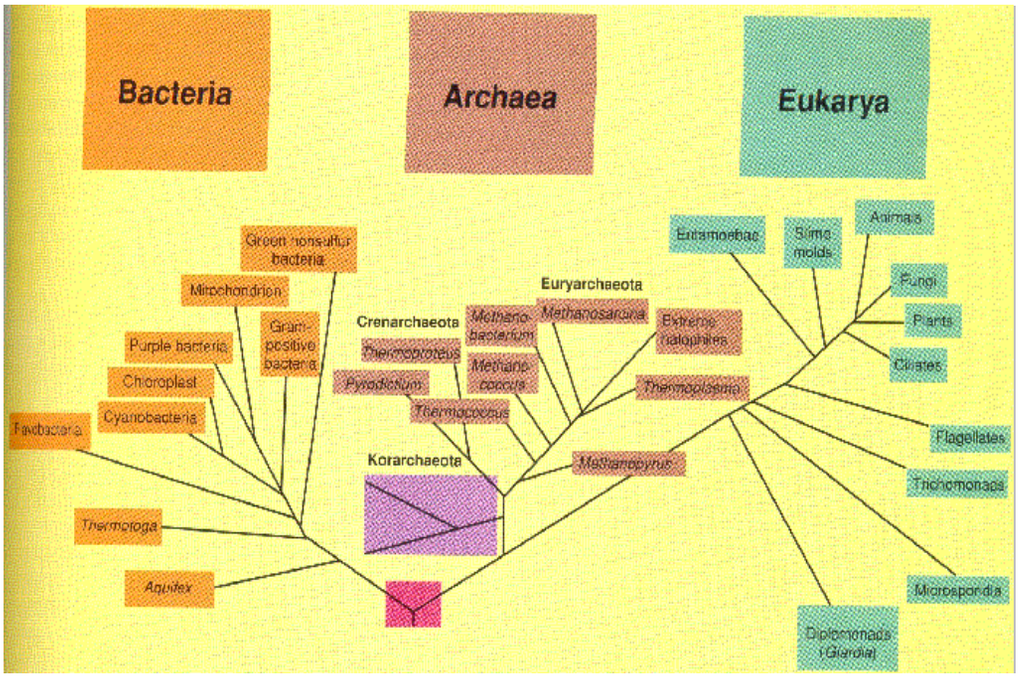


Fig. 3.5. Rooted universal phylogenetic tree as determined from comparative rRNA sequencing.

The root of the universal tree has been determined by rRNA sequencing and by related macromolecular sequencing methods. But it clearly indicates that initial evolution from the universal ancestor was at first in two directions, the Bacteria versus the Archaea - Eukarya line. The Archaea and Eukarya diverged to yield two major lineages, so both are phylogenetically more related to each other.

The figure also shows that Archaea branching off the tree at a point closest to the root. This leads to the conclusion that Archaea remains as the most primitive of the organisms in the three domains. Eukarya, the farthest way from the universal ancestor are the least primitive, that is, the most evolved.

Emphasis given here is that none of the organisms living today are primitive. All extant life forms are modern organisms. Certain of these organisms may be phylogenetically similar to primitive organisms and may represent stems of the evolutionary tree that have changed little for millions if not billions of years. In this respect they are not themselves primitive.The microbial world is extremely diverse. Some organisms have common features that permit grouping into common categories. Scientists use a hierarchial (ranking system) organization structure in which organisms are classified according to their degree of similarity.

For example :

(Domain) Kingdom	Bacteria
(Phylum) Division	Gracilicutes
Class	Scotobacteria
Order	Spirochaetales
Family	Spirochaetaceae
Genus	***Treponema***
Species	***pallidum***
Strain	Nichols

The broadest groups in a classification system are called kingdoms and the smallest are called species. Related species are grouped into genera. For example, ***Mycobacterium leprae*** and ***Mycobacterium tuberculosis*** are both in the genus ***Mycobacterium***.

In 1866, Ernst Heinrich Haeckel proposed a classification system based on inferred evolutionary relationships among species. This was the first attempt to include evolution theory within a classification system. Evolution is the process of change that results from the interaction between the genetic information and the environment of organisms of species. Evolution results in the formation of new organisms that are better adapted to the environment.

Haeckel's sytem contained three kingdoms: Protista, Animalia and Plantae. Haeckel proposed that the microorganisms, bacteria, fungi , algae and protozoa all belonged to one primary kingdom, which he called the ***Protista***. The Protista were simple structural organization, that is, lack of specialized tissues. Hackel believed that the Protista was the first kingdom to evolve and that both the Plantae and Animalia evolved from the Protista. They both had tissues. The Plantae had specialized tissues. The Animalia also had specialized tissue but were heterotrophic. They used organic compounds for energy and growth. Its recognition of microorganisms as a single kingdom distinct from plants and animals as appealing to microbiologists even today.

3.3. Modern classification systems

Robert H.Whittaker in1969 proposed a modification of Haeckel's system that become widely accepted by biologists (Fig.3.6).

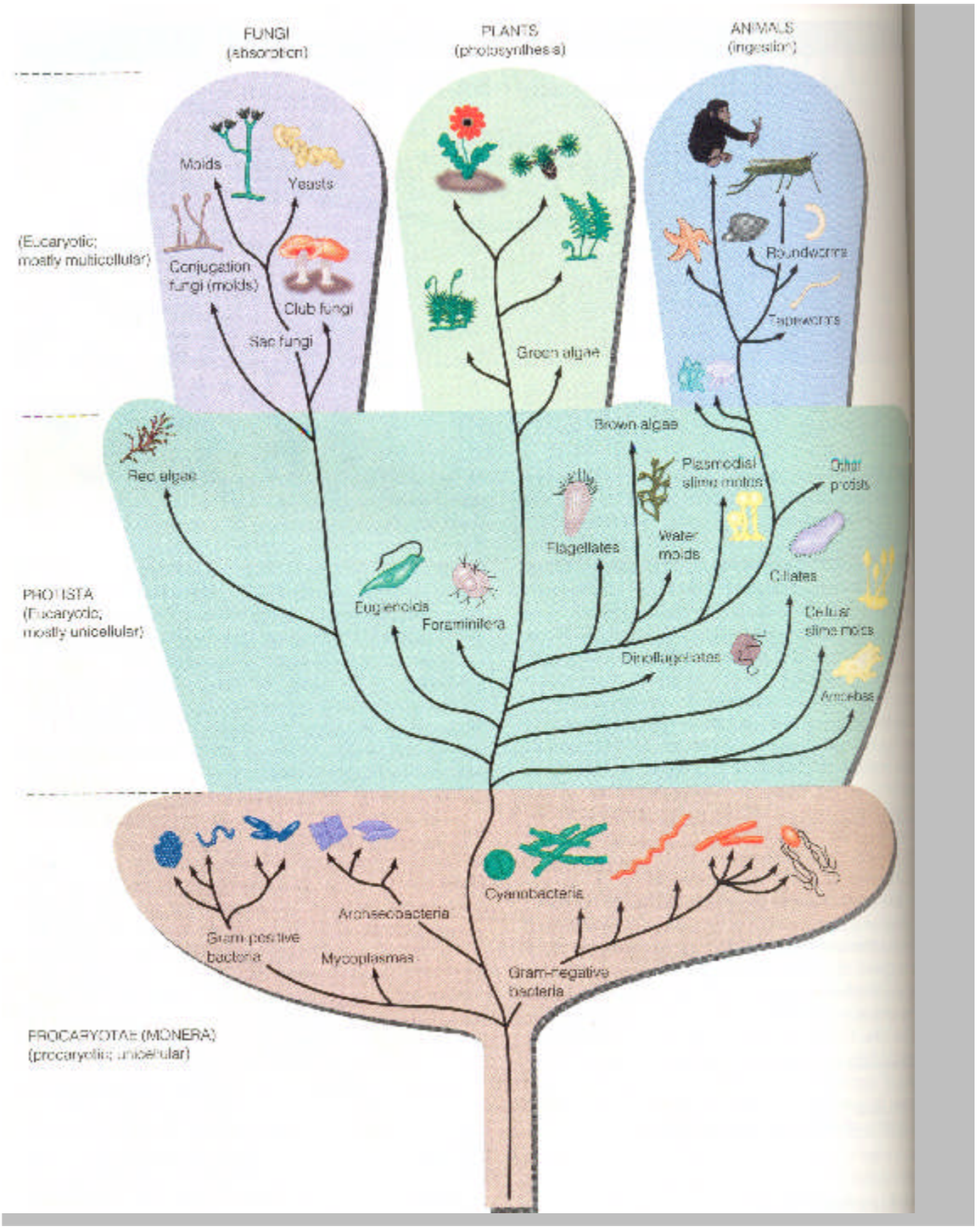


Fig. 3.6. Five-kingdom system. This is a commonly accepted system of classification .

Whittaker tried to organize his classification system along evolutionary lines. He proposed a five-kingdom classification system: Animalia, Plantae, Fungi, Protista and Monera (Table 3.1).The microorganisms constitute three of the kingdoms. The kingdom **Monera** contains the bacteria. These are separated from all other organisms based on their prokaryotic cell structure.

Table.3.1. Major differences among Kingdoms in the Five-Kingdoms scheme of classification.

Major Differences among Kingdoms in the Five-Kingdoms Scheme of Classification

Property	Plantae	Animalia	Protista	Fungi	Monera
Cell type	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Prokaryotic
Cell organization	Mostly multicellular	Mostly multicellular	Mostly unicellular	Multicellular and unicellular	Mostly unicellular
Cell wall	Present	Absent	Present in some; absent in others	Present	Present in most
Nutritional class	Phototrophic	Heterotrophic	Heterotrophic and phototrophic	Heterotrophic	Phototrophic, heterotrophic, or chemoautotrophic
Mode of nutrition	Mostly absorptive	Mostly ingestive	Absorptive or ingestive	Absorptive	Absorptive
Motility	Mostly nonmotile	Mostly motile	Motile or nonmotile	Nonmotile	Motile or nonmotile

The kingdom **Monera** contians the bacteria, which are separated from all other organisms based on their prokaryotic cell structure. The kingdom Protista contains the unicellular algae and protozoa based on the fact that they have eukaryotic cells and are unicellular. The unicellular and multicellular fungi are kept in kingdom Fungi, based on the absence of specialized tissues.

According to Whittaker, the Monera were the first organisms on Earth and the Protista evolved directly from the Monera. Whittaker proposed that fungi, plants and animals evolved from the protists via three separate directions of evolution. These evolutionary lines were based on their nutritional needs. According to Whittaker's hypothesis, the fungi evolved as the most complex multicellular organisms that obtained their nutrients by absorption, that is, by taking up the chemicals that they needed for growth and reproduction. Animals evolved based on the ability to ingest other organisms to meet their nutritional needs. Plants evolved based on their photosynthetic capacity. They synthesize organic compounds from inorganic compounds using light as an energy source. Among the plants he included the multicellular algae like brown and red algae.

In the early 1970s, development in the field of molecular biology provided the first tool for directly examining the evolutionary relationships among microorganisms. Methods were developed that permitted determination of the degree of relatedness of the DNA from two different organisms. DNA provides a molecular records of relatedness that is far more accurate than the fossil record for any organisms. RNA can be used to study relatedness among organisms. RNA is made directly using the information in the DNA.

Carl Woese, in 1970s, analyzed RNA to explore the evolution of microorganisms. Woese reasoned that the RNA of ribosomes was changed only relatively slowly during the evolution of new organisms. Ribosomes are the sites where proteins are made within all cells. So, ribosomal RNA (rRNA) should not have changed much as a result of small evolutionary steps. rRNA should change only as a result of major steps in evolution.

Before Woese's studies, it was accepted that all bacteria were closely related because they all were prokaryotic cells. Woese's analyses of rRNA molecules revealed that the bacteria fell into two distinct and only distantly related groups.

Woese proposed a new and radically different classification system that defines these three groups as three primary Kingdoms for all living things: Archaebacteria (Archaea) Eubacteria and Eukaryotes (Fig.3.3). According to Woese's theory, three separate paths of evolution from a common progenitor cell produced three different types of cells: the archaebacterial cell, the eubacterial cell and the eukaryotic cell. Further studies on the physiological characterstics of the organisms designated by Woese as archaebacteria appear to substantiate his proposal. The chemical composition of the plasma membranes of archaebacteria is totally different from all other cells. Archaebacteria have metabolic capabilities not found in any other organism.

With regard to the evolution of the modern eukaryotic cell, with its multiple organelles, Woese accepted an idea put forth a few years earlier by Lynn Margulis—the endosymbiotic theory of evolution. Margulis proposed that some bacterial cells had begun to live within the predecessor of modern eukaryotic cells in a mutually beneficial (symbiotic) relationship that probably helped each meet its metabolic energy and nutritional requirements (Fig. 3.7).

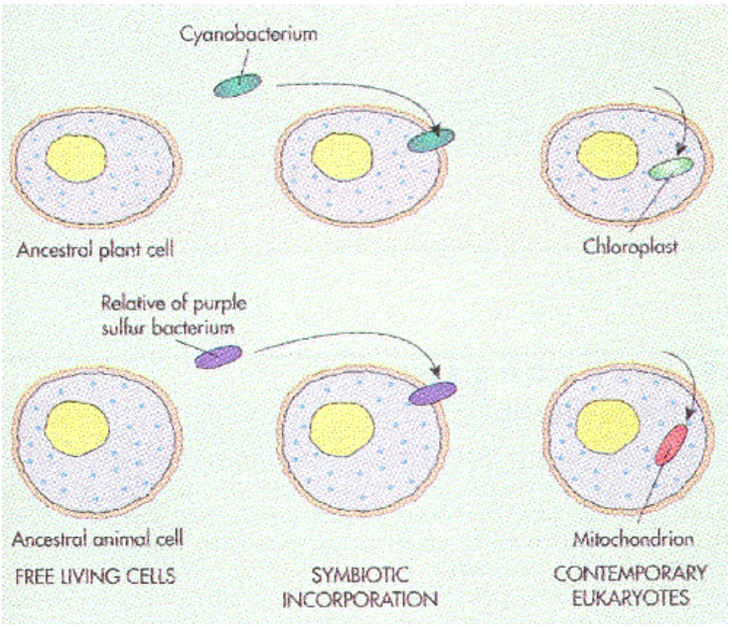


Fig. 3.7. Endosymbiotic theory.

Living together, the prokaryotic and eukaryotic organisms could help each other live independently. The endosymbiotic bacteria lost their capability for independent existence and developed into two of the cellular organells (mitochondria and chloroplasts) involved in energy transformations that are found in eukaryotic cells today.

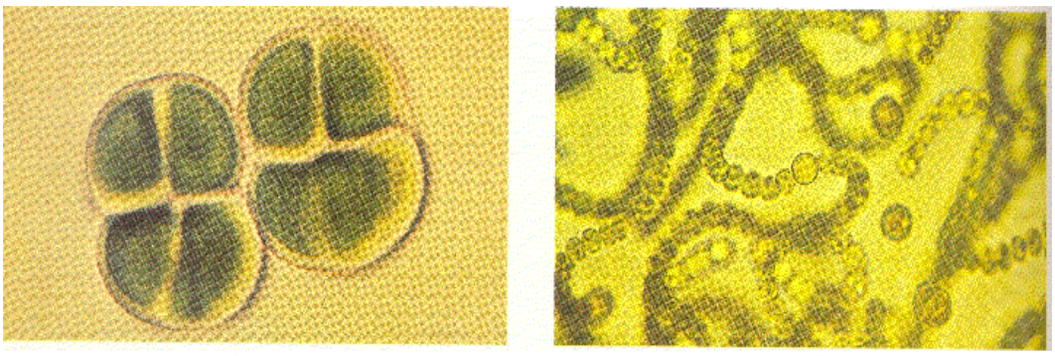
Woese was not concerned with the future evolution of the eukaryotic cell. He believed that the origins of the different types of cells, as revealed by the molecular records of evolution retained within DNA and rRNA molecules, should be used as the primary criteria in defining Kingdoms. Most microbiologists who concentrate their studies on organisms composed of one or relatively few cells readily accept this concept. Many biologists who study higher organisms have difficulty in accepting Woese's system because it places all organisms with eukaryortic cells including fungi, protozoa, algae, plants and animals into a single Kingdom, the eukaryotes. It is not surprising that lumping humans into the same Kingdom as fungi and algae meets some resistance and it is likely to subdivide the eukaryotes.

Classification systems continue to develop based on our abilities to examine the characteristics of organisms. Classification of living organisms has changed from systems based on first observational glimpes at the microbial world to systems based on detailed molecular analyses.

None of these classification systems considers the viruses. The viruese are treated as non living entities. The genetic molecules (DNA or RNA) of viruses indicates that they probably evolved from their respective host cells. They probably did not evolve in a hereditary lineage from one virus to the next. Hence, it is appropriate to classify viruses in relation to their host cells, for example, as a tobacco mosaic virus.

An understanding of microbial diversity requires an appreciation of the evolutionary roots of cells. Because evolution as shaped all life on Earth, the structural and functional diversity we see in cells represents successful evolutionary events that, through the process of natural selection, have conferred survival value on the microrganisms extant today. Microbial diversity can be seen in terms of variations in cell size and morphology, metabolic stratagies, motility, cell division, developmental biology, adoptation to environmetal extremes, and many other structural and functional aspects of the cell.

Several evolutionary branches occur within the **Bacteria**, including pathogens, and most of the bacteria commonly found in soil, water, animal digestive tracts and many other environments. Some of these organisms contain pigments that allow them to use light as an energy source in a process called phototrophy, others rely on organic chemicals as energy source and some can use inorganic chemicals as fuel to drive cellular processes. Most of the prokaryotes encountered in everyday life are phylogenetically Bacteria and some have evolved special structures such as spores for aiding survival. Oxic/O_2 containing environments, as well as anoxic habitats are inhabited by various species of Bacteria (Fig.3.8).



A **B**

Fig. 3.8. Oxygenic photosynthetic bacteria. ***A) Chroococcus turgidus B) Nostoc***

With the prokaryotes called **Archaea** are anarobes. Many also thrive under unusal growth conditions, inhabiting extreme environments, hot springs, extremely salty bodies of water, and highly acidic and alkaline soils and water. Certain species of Archaea currently define the limits of biological tolerence to physiochemical extremes. Certain Archaea also show unusual biochemical features like the methanogenes which produce methane gas.

Among the Eukarya, the microorganisms are the algae, fungi and protozoa. Algae are eukaryotic photosynthetic microorganisms that contain chlorophyll and utilize light energy to generate their chemical energy (Fig.3.9).

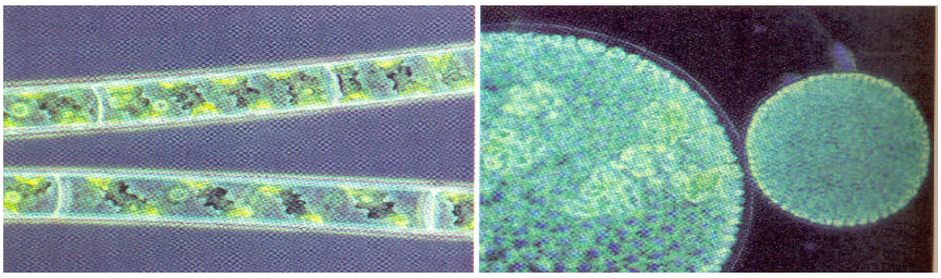


Fig.3.9. A) green algae ***Volvox aureus*** B) ***Spirogyra*** species.

They are the only eukaryotic photosyntheic microorganisms. As such they are the microorganisms most closely related to the plants. They also are able to produce oxygen (O_2) from water. The characteristics used in the classification of algae have been adapted from botanists. They are generally not metabolic characteristics that must be experimentally determined. The major groups of algae are identified by their characteristic colour pigments and cell morphologies. The major groups of algae are green algae, euglenoids, golden and yellow-green algae, cryptomonads and dinoflagellates.

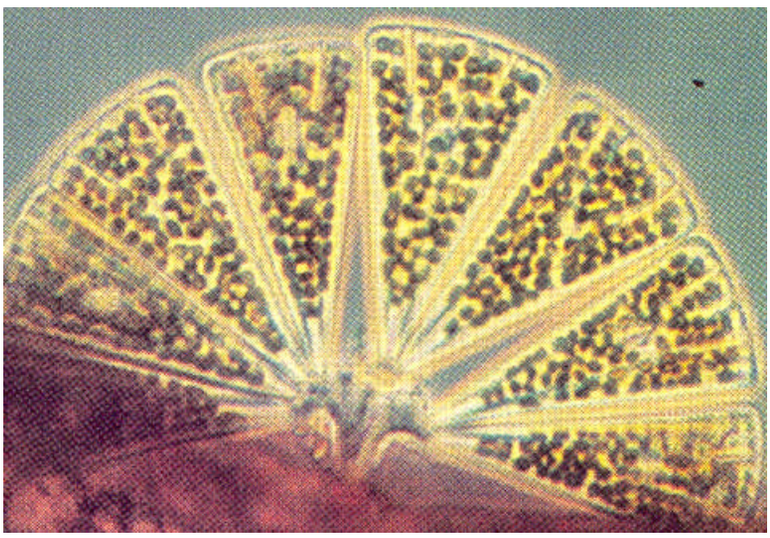


Fig.3.10. Micrograph of the marine diatom ***Licmophora***

The diatoms are unicellular algae that have cell walls conaining silica (Fig.3.10). They are beautiful when seen through the microscope. The euglenoid algae, dinoflagellates and many green algae are also unicellular. Some green algae are multicellular but do not form structures as complex as brown and red algae.

Some microorganisms, namely the red and brown algae, produce complex macroscopic multicellular structures. They are not microorganisms and have been reclassified as plants instead of algae.

The **Protozoa** are unicellular, non photosynthetic eukaryotes. They generally lack a cell wall. Many of the protozoa are motile. Since at one time all organisms that moved were considered as animals. The protozoa have heterotrophic metabolism. They obtain energy from organic substances such as proteins. Protozoa tend to engulf their food source similar to higher animals. Many of the characteristics used in classifying protozoa are analogous to the morphological characteristics used in describing animals.

Like bacteria, the **Fungi** are extremely diverse. Unlike bacteria, fungi are composed of eukaryotic cells. Most fungi for example, yeasts are unicellular (Fig.3.11).

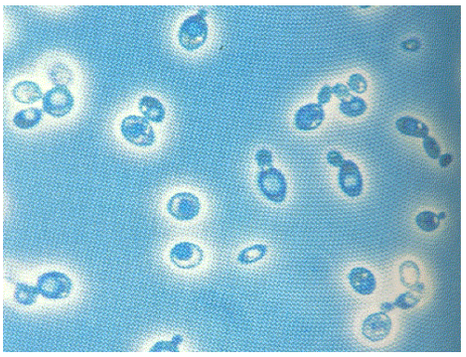


Fig.3.11. Micrograph of ***Saccharomyces cerevisiae***

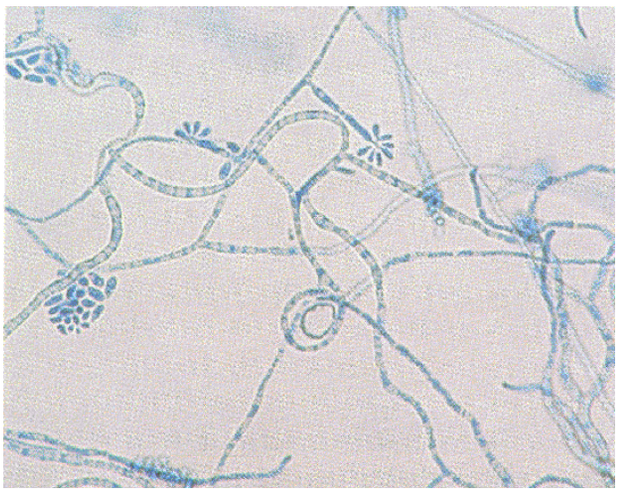


Fig.3.12. Micrograph of the fungus ***Exophiala jeanselmi***

Others, called filamentous fungi or molds form tube-like filaments called hyphae (Fig.3.12).Some hyphae are coenocytic, that is, they lack cross-walls to separate cells. Coenocytic hyphae are multinucleate.

Hyphae which are composed of many cells, can form integrated masses called **mycelia**. Mycelia are the visible structures seen when molds grown on bread and other substances. In some cases, elongation of hyphae occurs without forming separate cells. Long multicellular fungal hyphae develop. Separate cells are formed by branches and cross walls as the hyphae grow. These cross walls are called septa. Cellular materials flow through pores in the septa, even when cross walls form.

Fungi absorb nutrients from their surroundings, often from plant materials. In nature, fungi are very important decomposers. They cause the decay of dead logs. Unfortunately many are also plant pathogens. A few also cause human diseases.

The classification of fungi is based primarily on their sexual reproductive spores (Fig.3.13).

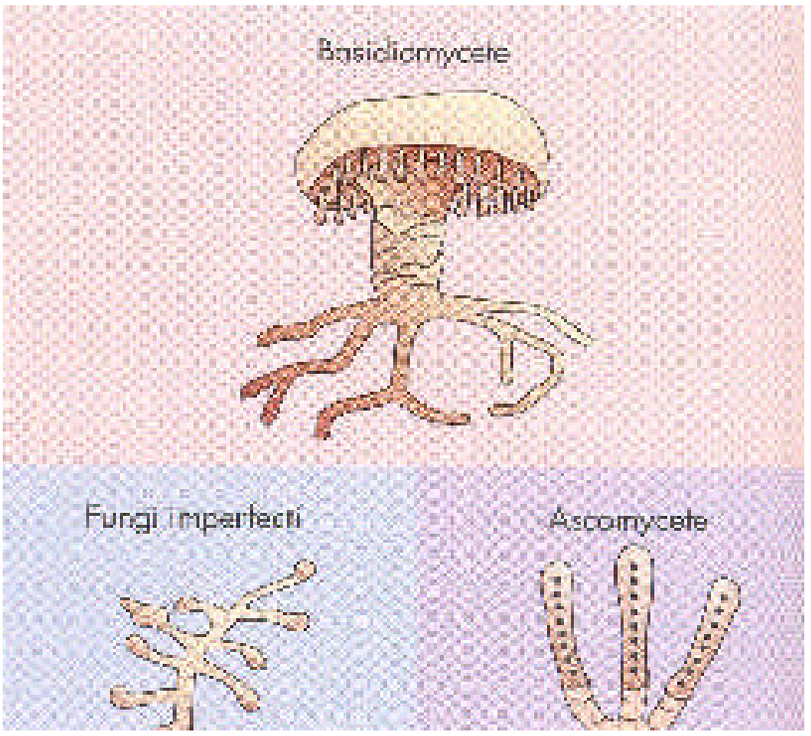


Fig. 3.13. The classification of fungi based on spore formation

Some sexual spores called ascospores are formed within a specialized structure known as the ascus, the fungi that produce them are called ascomycetes. Another fungi, basidiomycetes produce basidiospores within a structure called basidium for example, mushroom. Other fungi, deuteromycetes or fungi imperfectii, have no known sexual reproductive phase. They are restricted to asexual means of reproduction, for example, ***Aspergillus*** and ***Penicillium.***

Acellular non-living microorganisms

Viruses, viroids and prions are acellular/noncellular, nonliving microorganisms that can replicate in a compatable host organism (Table.3.2).

Table. 3.2. Some characteristics of accllular, nonliving microorganisms.

Some Characteristics of Acellular, Nonliving Microorganisms

MICROORGANISM	DESCRIPTION
Virus	Highly structured; contains DNA or RNA as the genetic informational molecule surrounded by a protein coat; may have an additional outer lipid-containing structure called an envelope; replicates within specific hosts (bacteriophage replicate in bacterial cells, plant viruses replicate in plant cells, and animal viruses replicate in animal cells), using the synthetic capabilities of the host cells.
Viroid	Infectious RNA molecule that can be replicated within specific plant cells
Prion	Infectious protein molecule that can be replicated within specific animal cells

This type of replication within a host gives them their “life like character”. Viruses, viroids and prions are obligate intracellular parasites that do not have an independent capacity to carry out life functions. At the time of their replication, they use the living cells’ metabolic functions. So they disrupt normal cellular functions, producing diseases in those organisms.

Virus: A virus is made up of two essential parts: a central genetic nucleic acid molecule and a protein coat called a capsid (Fig.3.14).

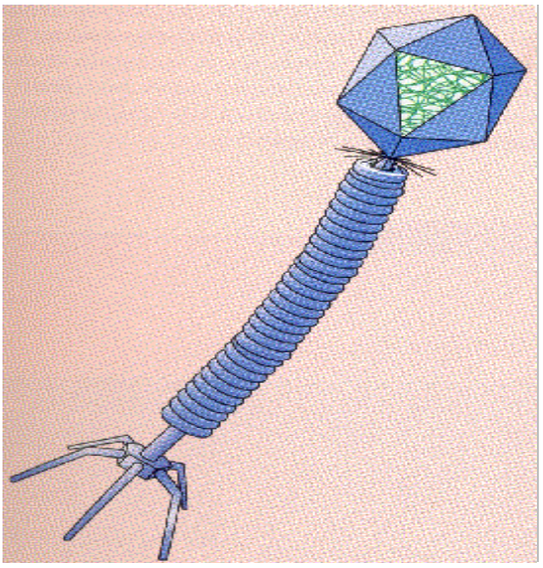


Fig. 3.14. A virus has a protein coat (capsid) surrounding a nucleic acid hereditary molecule (RNA or DNA)

The capsid surrounds, gives shape to the virus and protects the nucleic acid. It also helps to establish the specificity of the virus for a particular host cell. Some viruses have an external viral envelope made up of plasma membrane acquire from host cells within which they replicate. Viruses contain either DNA or RNA as their hereditary molecule.

There are three groups of viruses, Animal viruses, infect and replicate within animal cells, Plant viruses, within plant cells and Bacteriophage or phage within bacterial cells. Where the viruses are classified? Viruses are not classified as part of any of the kingdoms discussed in this chapter, because,

1) Viruses are not composed of cells
2) They use the anabolic machinery within living host cells to multiply
3) A viral genome can direct biosynthesis inside a host cell and some viral genomes can be incorporated into the host genome.

Origin of viruses: Viruses are obligate intracellular parasites. So they must have evolved after a suitable host cell has evolved. There are two hypotheses on the origin of viruses.

1) They arose from independently replicating strands of nucleic acids such as plasmids.
2) They develop from degenerative cells that, through many generations gradually lost the ability to survive independently, but could survive when associated with another cells.

In general, the International Committee on Taxonomy of Viruses (ICTV) has not yet established higher taxa (order through kingdom) for viruses. The two exceptions are

1) complex bacteriophages will probably be placed in their own order
2) enveloped viruses with one negative strand of RNA has been classified in the newly established order Mononegavirales. Viruses are grouped by nucleic acid types, morphology and the presence or absence of an envelope.

ICTV has organized the 2000 known species of viruses into 73 families, each of which shares morphologic characteristics and reproductive stratagies. Family names end in "viridae". Likewise genera of viruses share certain characteristics, the suffix-virus is used for genus name. A viral species is defined as a group of viruses sharing the same genetic information and ecological niche. Specific epithets for viruses have not yet been established, so viral species are designated by descriptive common names, such as HIV, with sub species (in any) designated by a number (HIV-I).

Viroids: Viroids composed of RNA as genetic information. They have no structures surrounding their genetic molecules. Inside a suitable host cell the RNA of a viroid replicates. It manifests disease symptoms in the host organism. They appear to affect only plants.

Prions: The most recently discovered and least understood microoganisms are the prions, proteinaceous infectious particles. They seem to be composed only of proteins. These organisms have no structures. They are only individual protein molecules that contain the information that codes for their replication when they infect a suitable host cell. They are properly called infectious proteins. Prions do not store their genetic material in nucleic acid molecules. This presents a problem in understanding how prions replicate.
Prions do not fit into our current understanding of how genetic information in nucleic acid molecules is replicated and how it can determine the specific structural and functional characteristics of each organism. Scientists do not understand how prions replicate. Prions cause diseases to animals and human.

3.4. SUMMARY

- Microorganisms evolved from abiotically formed micelles.
- As microorganisms evolved they altered their surroundings, permitting new forms of life to develope.
- Early classification systems were based on observable characteristics of microorganisms, such as shape and motility.
- The species is the fundamental unit used in classifying all organisms.

- Modern classification systems attempt to reflect evolutionary relationships
- The contemporary classification system of Woese, which is based on genetic analyses at the molecular level, reorganizes three primary Kingdoms: archaebacteria (primitive specialized prokaryotes), eubacteria (common prokaryotes or “true bacteria”) and eukaryotes (all other cellular organisms, including plants, animals, protozoa, algae and fungi).
- The acellular microorganisms include viruses, viroids and prions.
- Viruses have two parts: a protein coat (the capsid) and a hereditary molecule, which may be DNA or RNA.
- Viroids are composed of only RNA.
- Prions are infectious proteins.
- The eubacteria and archaebacteria are prokaryotic cells.
- Like other organisms, bacteria are named using a binomial system of genus and species.
- The major groups of bacteria are distinguished based on morphological, metabolic and genetic characteristics.
- The fungi are heterotrophic eukaryotic microorganisms. Fungal cells commonly have cell walls. Some fungi form multicellular filamentous growth called mycelia and others are unicellular (yeasts). The fungi are classified into major groups based on their modes of reproduction and in particular, the reproductive spores they produce.
- The Algae are photosynthetic eukaryotic micoorganisms. The major groups of algae are defined on their characteristic pigments and cell morphologies.
- The protozoa are heterotrophic microorganisms. The cells of protozoa lack cell walls. The major groups protozoa are classified based on their means of motility or on specialized morphological characterisitics.

3.5. Model questions

1) Why is hard to classify microorganisms by their evolutionary relationships?
2) Compare and contrast the classification systems of Linnaeus, Haeckel, Whittaker and Woese?
3) How do viroids differ from viruses?
4) What are prions? Why are prions different from other living organisms?
5) What are the main characteristics of organisms in each of the Kingdoms in the five-kingdom system of classification?
6) How are the bacteria named?
7) What are the main features of the endosymbiont theory of evolution?
8) What are the modern methods used to classify organisms?
9) How could life have evolved on Earth?

3.6. Reference books

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Dr.P.KIRANMAYEE

Lesson - 4

MORPHOLOGY AND ULTRA STRUCTURE OF BACTERIAL CELL

4.0 Objective:

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

4.1 Introduction:

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

4.2 Morphology of bacterial cell:

4.2.1 Shape, arrangement and size:

Typically, bacteria display three basic shapes viz., spherical, rodlike and spiral but variations abound. A spherical bacterium is called a coccus. These cocci can exist as individual cells, but also are associated in characteristic arrangements that are frequently useful in bacterial identification. Different shapes of bacteria are given in Fig. 4.1.

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

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

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

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

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

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

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

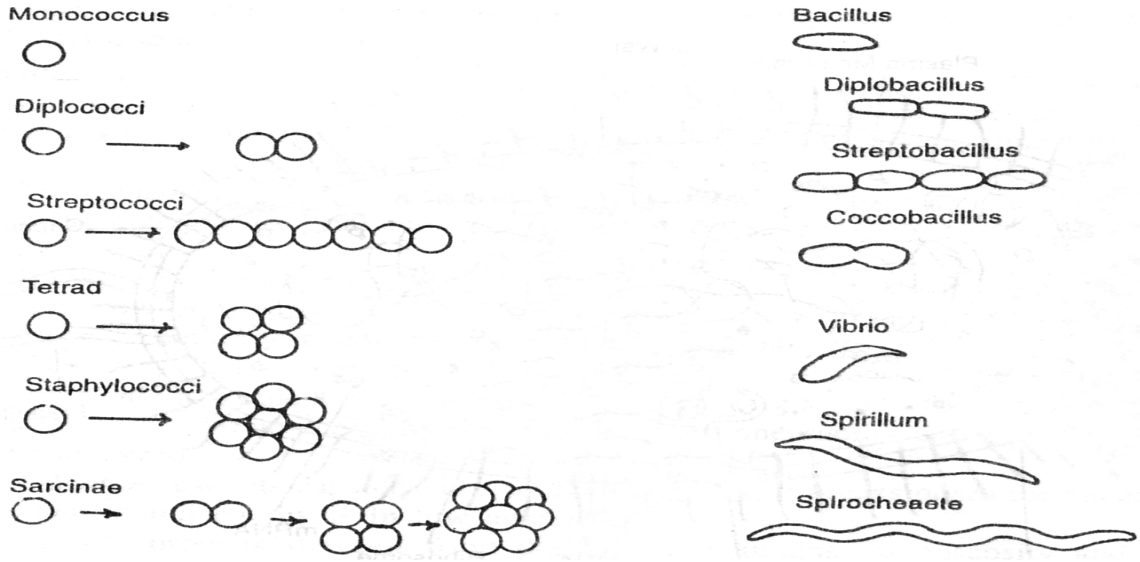

Figure 4.1 – Different shapes of bacteria

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

4.3 Ultra structure of bacterial cell:

Structurally bacterial cells consist the following—

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

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

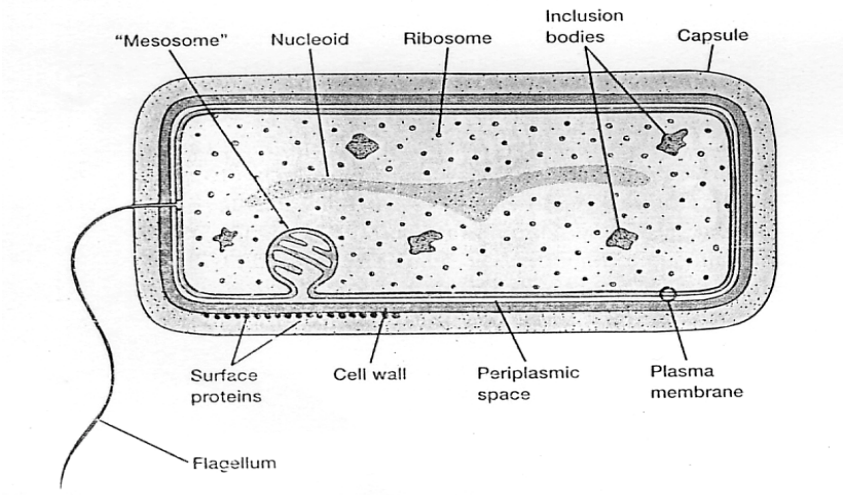

Figure. 4.2 – Structure of a typical bacterial cell

4.3.1 Structures external to the plasma membrane:

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

4.3.1.1.1 Flagella:

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

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

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

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

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

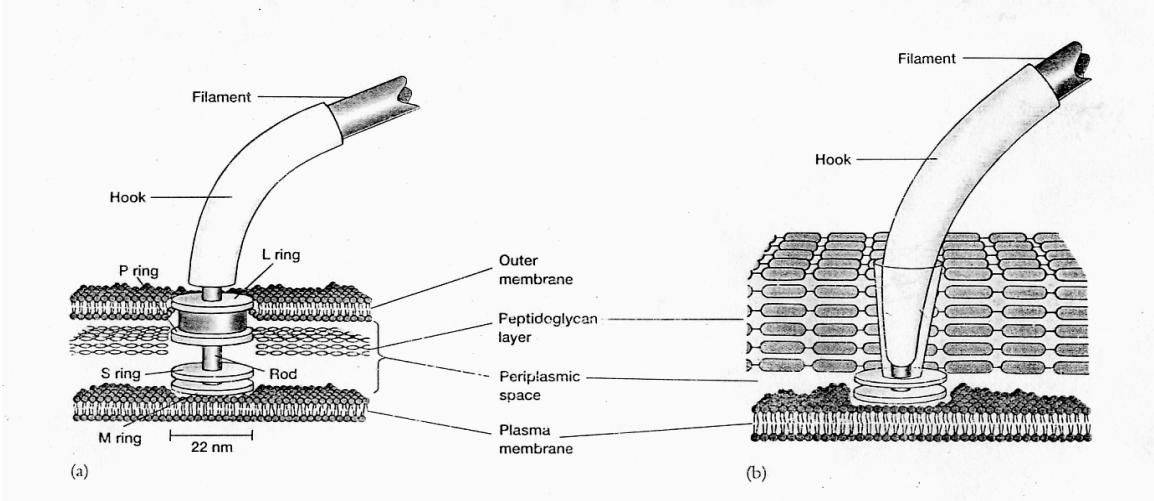

Figure- 4.3. a) Gram-ve bacterial flagellum b) Gram +ve bacterial flagellum

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

rings are absent and only inner pair of rings i.e., S and M rings are found associated with cell membrane and cell wall.

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

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

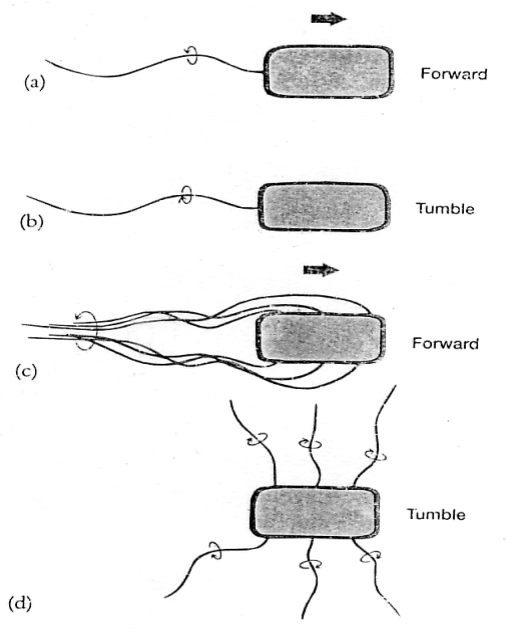

Figure- 4.4. Flagellar motility a & b – motion of monotrichous bacteria

c & d – motion of peritrichous bacteria

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

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

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

4.3.1.2 Glycocalyx: Many bacteria synthesize and secrete large amounts of viscous , organic polymer material that surrounds the bacterial cell. This slimy or gummy material is generally termed as glycocalyx. The glycocalyx may vary in its composition among different organisms but usually composed of glycoproteins and a large number of different polysaccharides. However, an exception is seen in *Bacillus anthracis* where the glycocalyx is made up of poly-D-glutamic acid which is a polypeptide. The glycocalyx may be thick or thin, rigid or flexible depending upon the chemical nature in a specific organism.

If the glycocalyx forms a rigid, condensed, well defined and organized layer that tightly and closely surrounding the cell, the layer is termed as Capsule. If the glycocalyx is disorganized and loosely surround the cell, it is referred as slime layer. The capsule made up of by single kind of sugars is termed as homopolysaccharide capsule (*Streptococcus mutans*) and the capsule with more than one kind of sugars is known as heteropolysaccharide capsule (*Streptococcus pneumoniae*).

Functions of Glycocalyx layer includes—

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

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

4.3.1.3.1 Peptidoglycan:
The single most important component of cell wall is the peptidoglycan layer of murein layer (Fig. 4.5) which is common to both Gram +ve and Gram –ve bacteria. In Gram +ve bacteria as many as 40 sheets of murein forms the peptidoglycan whereas in Gram –ve bacteria the number of murein sheets is usually two. Peptidoglycan is homogenous layer of 20-80 nm thickness in Gram +ve bacteria and accounts to 40-90% of cell wall dry weight but it is only 2-7 nm thickness in Gram –ve bacteria accounting to only 5-20% of total cell wall dry weight. Peptidoglycan is made of two parts, a peptide portion composed of amino acids connected by peptide linkages and a glycan or sugar portion. Structurally the peptidoglycan can be divided into three components viz., backbone, tetrapeptide side chain and peptide interbridge or cross link.

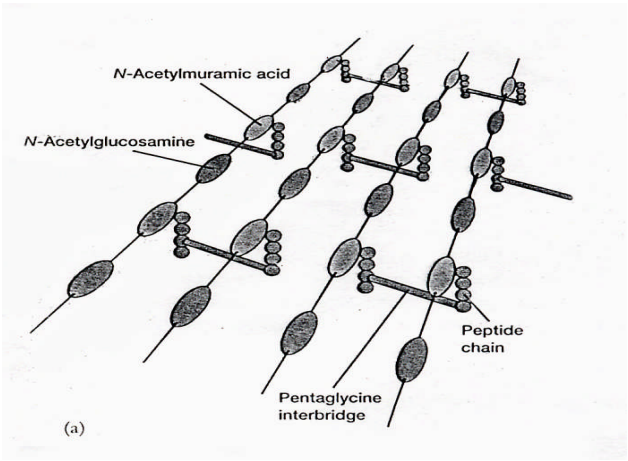

Figure- 4.5 Schematic diagram of peptidogycan structure

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

4.3.1.3.3 Tetrapeptide side chain: This component of peptidoglycan contains four amino acids which includes L-alanine, D-glutamic acid or its derivative, L-lysine or Diaminopimelic acid (DAP) and D-alanine. Diaminopimelic acid is found in all Gram –ve bacteria and in few Gram +ve bacteria. Most Gram +ve cocci have lysine instead of DAP. The tetrapeptide side chain is connected to the carboxyl group of N-acetylmuramic acid but not to N-acetylglucosamine residue (Fig. 4.6).

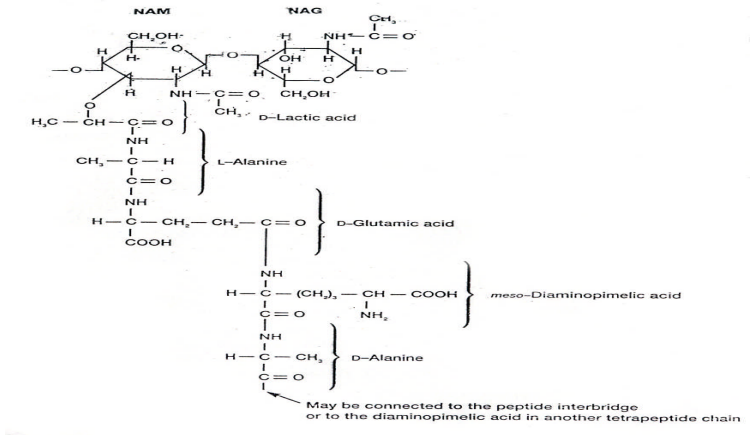

Figure - 4.6 Tetrapeptide side chain

4.3.1.3.4 Peptide interbridge or Cross-link: Two tetrapeptide side chains of two adjacent murein stands or of the same strand are connected by cross-links or inter-bridges. In Gram –ve bacteria the cross-link is direct between amino group of DAP of one tetrapeptide side chain and carboxyl group of terminal D-alanine of other tetrapeptide side chain (Fig. 4.7). In Gram +ve bacteria the cross-link is by a peptide interbridge composed of amino acids. For example, the interbridge in Staphylococcus aureus is composed of five glycine amino acids (Fig. 4.8).

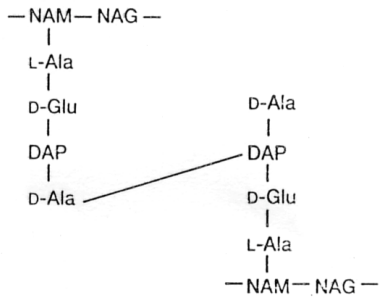

Figure-4.7 Cross linkage in Gram –negative bacteria

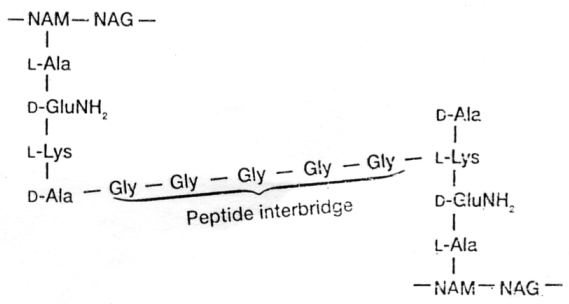

Figure- 4.8 Cross linkage in Gram –positive bacteria

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

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

4.3.1.3.6 Gram-negative cell wall: The Gram –ve cell wall (Fig. 4.9) is far more complex than the Gram +ve cell wall. Outside the thin peptidoglycan in Gram –ve bacteria an outer membrane is present. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan by its protein portion and associate with the hydrophobic portion of the outer membrane by its fatty acid end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein. The outer membrane and cytoplasmic membrane may appear to be in direct contact at many locations or adhesion sites called as Bayer junctions named after the scientist Mannfred Bayer.

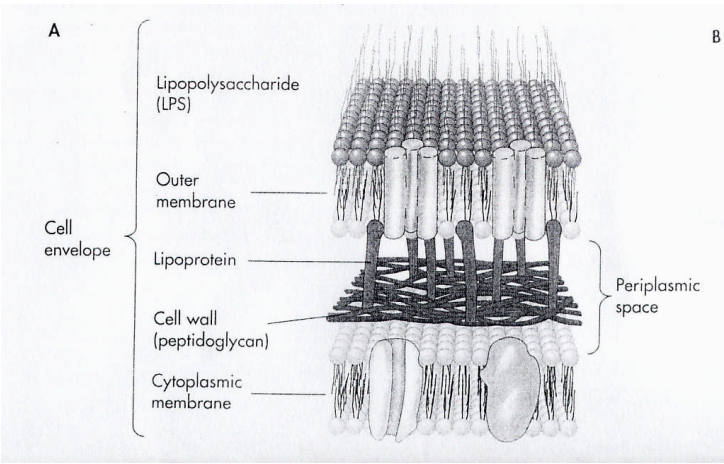

Figure- 4.9 Gram-negative cell wall structure

4.3.1.3.7 Outer membrane: The outer membrane is the exclusive component of Gram –ve cell wall and it is a lipid bilayer containing phospholipids, proteins, lipoproteins and lipopolysaccharides. Unlike the cytoplasmic membrane, outer membrane is relatively permeable to most small molecules. The outer membrane contains lipopolysaccharides (LPS), which are not found in cytoplasmic membranes. LPS is often called as endotoxin as it may result in shock and death in some animals.

LPS is a complex molecule composed of distinct regions (Fig. 4.10). The innermost portion of LPS is a lipid, called Lipid A, that anchors the LPS to the hydrophobic portion of the outer membrane. Lipid A consists of N-acetylglucosamine disaccharide linked via ester and amide bonds to unusual fatty acids such as β-hydroxymyristic acid, caproic acid and lauric acid. The toxic portion of LPS lies in the lipid A. The polysaccharide portion of the LPS, which is external to lipid A, consists of a core polysaccharide and a repeat polysaccharide called the O-antigen or O-polysaccharide. The core polysaccharide is fairly consistent fro most Gram –ve bacteria and contains glucose, galactose, N-acetylglucosamine, and unusual sugars such as the 8-carbon sugar ketodeoxyoctulosonic acid (KDO) and heptoses (7-carbon sugars). The repeat polysaccharide consists of 3-5 sugars whose sequence is repeated up to about 25 times. The O-polysaccharide typically contains glucose, galactose, rhamnose, mannose, and several dideoxy sugars such as abequose, colitose, paratose and tyvelose.

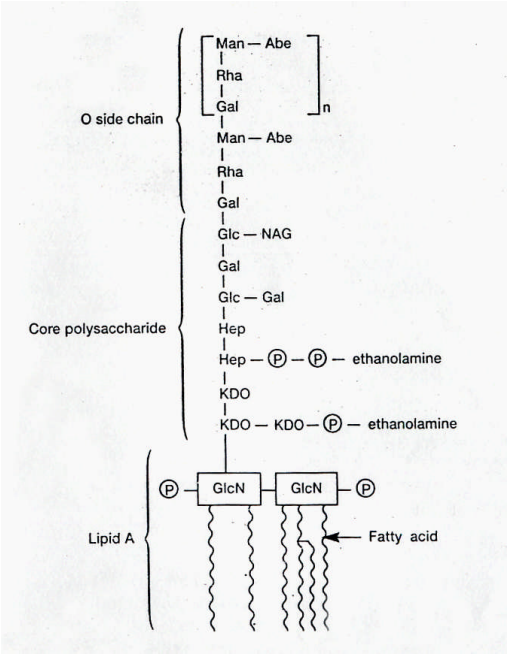

Figure- 4.10 Structure of LPS

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

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

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

4.3.1.3.8 Periplasmic space: The region between the cytoplasmic and outer membranes is known as periplasmic space or periplasmic gel. The substance that occupies the periplasmic space is the periplasm. This is an important region in Gram –ve bacteria where diverse chemical reactions occur, including oxidation-reduction reaction, osmotic regulation, solute transport, protein secretion, and hydrolytic activities. Several proteins such as binding proteins, chemoreceptors, and enzymes (oxidases and dehydrogenases) are found in the periplasmic space. The binding proteins facilitate the transport of substances into the cell by delivering substances to carriers that are bound to the cytoplasmic membrane. The hydrolytic enzymes in the periplasm break down larger molecules to

smaller products for easy transportation across the cytoplasmic membrane. The chemoreceptors by binding with the substances direct the cell's movement toward or away from those substances. Oligosaccharides present in the periplasmic region involves in osmoregulatory function.

4.3.1.3.9 An outline comparison of Gram +ve and Gram –ve bacterial cell walls:

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

4.3.2 Plasma membrane: The boundary layer that surrounds the cytoplasmic contents of the bacterial cell is known as plasma membrane or cell membrane. This plasma membrane constitutes 8-15% of the cell dry weight. It is distinct from the cell wall by its shrinkage nature under high osmotic pressure. It is a critical barrier that separates the inside of the cell from its environment. Structurally it is a tri-laminar unit membrane with a thickness of 7-8 nm. In electron microscopy, membrane appears as outer and inner electron-dense layers with middle electron-transparent space. The plasma membrane structure is said to be fluid-mosaic model (Fig. 4.11) proposed by S.J. Singer and G. Nicholson.

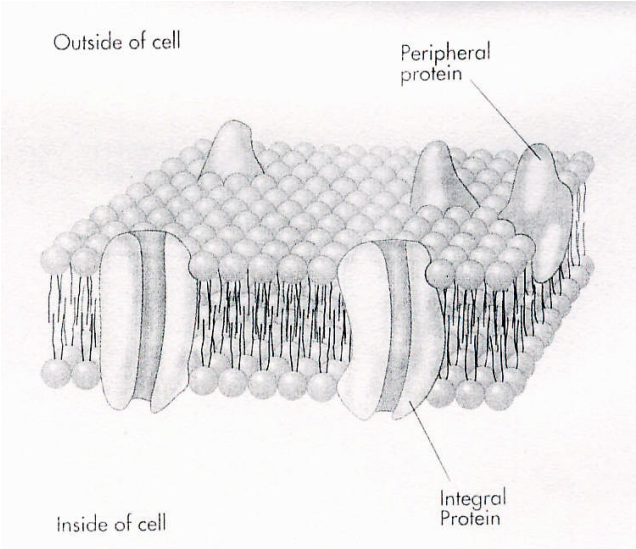

Figure – 4.11 Structure of Plasma membrane

The cell membrane is largely lipoprotein in nature with about 20-30% phospholipids such as phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl choline and about 60-70% proteins. The phospholipids are structurally asymmetric and are amphipathic with polar and non-polar ends. These phospholipids from a lipid bilayer. The lipids contain hydrophobic fatty acid groups directed inward and hydrophilic glycerol groups directed outwards and associate with water. The proteins embedded in fluid matrix of lipid are known as integral or intrinsic proteins which account to 70-80% of total proteins. These integral proteins are insoluble and cannot be removed easily. The proteins that are loosely attached to membrane are called as peripheral or extrinsic proteins which account to 20-30% and can be easily freed from membrane.

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

The major functions of plasma membrane are

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

4.3.3 Structures internal to the plasma membrane:

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

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

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

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

4.3.3.5 Chlorosomes: These chlorosomes are the pigments that are housed in a series of cigar shaped vesicles. These vesicles are arranged in a cortical layer that immediately underlies the cell membrane. Chlorosomes are the part of the photosynthetic apparatus in green photosynthetic bacteria. Chlorosomes are 50 nm wide and 100-150 nm long with an enclosed simple membrane of 3-5 nm thick.

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

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

4.3.3.8 Inclusion bodies:
In prokaryotic cells, a variety of cellular reserve materials or granules or inclusion bodies are seen. The nature of these inclusion bodies may differ in different organisms but almost always function in the storage of energy or serve as structural building blocks. These inclusion bodies are either organic or inorganic in nature and present in cytoplasmic matrix. PHB granules and glycogen granules are important organic forms and phosphate granules and sulphur granules are the important inorganic forms.
4.3.3.8.1 Poly β-hydroxybutyric acid (PHB) granules: PHB are the most common inclusion bodies in prokaryotic cells. PHB is a lipid-like compound containing a number of monomeric β-hydroxybutyric acid units. These monomeric units are linked by ester bonds between the carboxyl and hydroxyl groups of adjacent units to form poly- β-hydroxybutyric acid molecule. These polymers aggregate together to form PHB granules. These granules can be stained with sudan black. PHB granules serve as a storage depot for carbon and energy.

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

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

4.3.3.8.4 Sulphur granules: A variety of bacteria like purple photosynthetic bacteria are capable of oxidizing the reduced sulphur compounds such as H_2S, thiosulphate and accumulate the resulting elemental sulphur in cells in the form of granules. During the reactions of energy metabolism or biosynthesis, elemental sulphur frequently accumulates inside the cell in large readily visible

granules. These granules of elemental sulphur remain in cells as long as the source of reduced sulphur is available. When the reduced sulphur source becomes limiting, the sulphur in granules is oxidized to sulphate and the granules ultimately disappear.

4.4 Bacterial Endospore:

During the unfavourable environmental conditions, some Gram-positive bacteria such as *Bacillus, Clostridium* and few others produce a special resistant, dormant structure called an endospore. These endospores are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, chemical disinfectants and desiccation. They can remain viable for a number of years. Formation of spore from the vegetative cell is known as sporogenesis or sporulation. Spore position in the mother cell frequently differs among species. Spores may be centrally located, close to one end (sub-terminal) or definitely terminal (4.12).

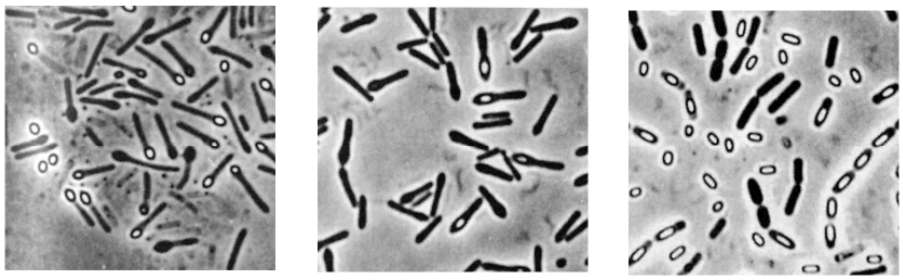

Figure – 4.12 Terminal, Sub-terminal and centrally located spores

The spore (Fig. 4.13) is often surrounded by a thin, delicate covering called the exosporium. A spore coat lies beneath the exosporium, is composed of several protein layers and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The cortex, which may occupy as much as half of spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The spore cell wall or core wall is inside the cortex and surrounds the protoplast or core. The core has the normal cell structures such as ribosomes and a nucleoid. The dipicolinic acid forming complex with calcium ions in the core is believed to responsible for the heat resistance of endospores. When the environmental conditions become more favorable, endospores germinate into vegetative cells.

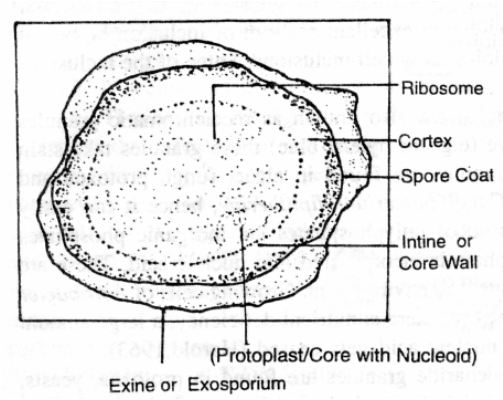

Figure- 4.13 Structure of bacterial endospore

4.5 Comparison between prokaryotic cell and eukaryotic cell:

Character	**Prokaryotic cell**	**Eukaryotic cell**
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
DNA	Single molecule, not complexed with histones	Present in several chromosomes, usually complexed with histones
Division	No mitosis	Mitosis occurs, mitotic apparatus with microtubular spindle
Sexual reproduction	Fragmentory process, no meiosis, only portions of genetic complement usually reassorted	Regular process, meiosis occurs, reassortment of whole chromo-some complement
Endo and exocytosis	Absent	Present
Plasma membrane	Usually lacks sterols	Sterols are usually present
Internal membranes	Relatively simple, comprises mesosomes	Complex , includes endoplasmic reticulum, golgi apparatus etc.
Ribosomes	70S in size	80S size except for mitochondria and chloroplasts whose ribosomes

		are of 70S
Simple membranous organelles	Absent	Present as vacuoles, lysosomes, peroxisomes
Respiratory system	Part of plasma membrane or mesosomes	Mitochondria
Photosynthetic apparatus	In the form of organized internal membranes or vesicles, chloroplasts absent	Chloroplasts
Cell walls	Present in most forms and composed of peptidoglycan. Other polysaccharides, proteins and glycoproteins are seen in some	Present in plants, algae, fungi and absent in animals and protozoa. Usually polysaccharide
Endospores	Present in some forms and are very heat resistant	Absent
Gas vesicles	Present in some	Absent
Flagellar movement	Flagella are sub-microscopic in size, each flagellum is composed of one fiber of molecular dimension, flagella rotate	Flagella or cilia, microscopic in size, composed of microtubular elements arranged in a characteristic pattern of nine outer doublets and two central singlets, do not rotate
Non-flagellar movement	Gliding motility, gas vesicle mediated	Cytoplasmic streaming and amoeboid movement, gliding motility

4.6 Summary:

Bacteria may be spherical, rod-shaped, spiral or filamentous in shape. Some form buds and stalks, and some are pleomorphic without any characteristic shape. Bacterial cells can remain together after division to from pairs, chains, and clusters of various sizes and shapes. All bacteria are prokaryotes and structurally much simpler than eukaryotes. The plasma membrane and most other membranes are composed of a lipid bilayer in which integral proteins are buried. Peripheral proteins are more loosely attached to membranes. The plasma membrane may invaginate to form some simple structures such as membrane systems containing photosynthetic and respiratory assemblies and possibly mesosomes. The cytoplasmic matrix contains inclusion bodies and ribosomes. The genetic material is located in an area called the nucleoid and it is not enclosed by a membrane.

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

4.7 Model questions:

Q-1. Give an account on ultra structure of the bacterial cell.

Q-2. Describe the physical and chemical structure of cell wall in Gram-positive and Gram-negative bacteria.

Q-3. Compare the characters of prokaryotic cell and eukaryotic cell.

Q-4. Write a short notes on

(1) Different shapes of bacteria (2) Peptidoglycan

(3) Teichoic acids (4) LPS layer

(5) Periplasmic space (6) Bacterial endospore

(7) Mesosomes (8) Inclusion bodies

(9) Pasma membrane (10) Outer membrane

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LESSON: 5

PRINCIPLES OF BACTERIAL TAXONOMY

5.0. Objective

To study the classification and taxonomy of bacteria and different approaches used in classification of microorganisms.

5.1. Introduction

Because of the bewildering diversity of living organisms, it is desirable to classify or arrange them into groups based on their mutual similarities. Taxonomy (Greek *taxis,* arrangement or order, and *norms, 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 parts: Classification, Nomenclature, and Identification. Classification is the arrangement of organisms into groups or taxa (s, taxon) based on mutual similarity or evolutionary relatedness. Nomenclature is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules. Identification is the practical side of taxonomy, the process of 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 particular group share many characteristics. Second, taxonomy allows us to make predictions and frame hypotheses for further research based on knowledge of similar organisms. Third, taxonomy places microorganisms in meaningful, useful groups with precise names so that microbiologist can work with them and communicate efficiency. Fourth, taxonomy is essential for accurate identification of microorganisms. Its practical importance in this respect can hardly be overemphasized.

5.2. Classification Systems

Once taxonomically relevant characteristics of microorganisms have been collected, they may be used to construct a classification system. The most desirable classification system, called a NATURAL CLASSIFICATION, arranges organisms into groups, whose members share many characteristics and reflects into groups and reflects as much as possible the biological nature of organisms. There are two general ways in which classification systems can be constructed. Organisms can be group together based on overall similarity to forms a phenetic system or they can be grouped based on probable evolutionary relationships to produce phylogenetic system.

Phenetic Classification

Many taxonomists maintain that the most natural classification is the one with the greatest information content or predictive value. A good classification should bring order to biological diversity and may even clarify the function of a morphological structure. For example, if motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility. When viewed in this way, the best natural classification system may be a Phenetic system, one that groups organisms together based on the mutual similarity of their phenotypic characteristics. Although phenetic studies can reveal possible evolutionary relationships, they are not dependent on phylogenetic analysis. 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. Obviously the best phonetic classification is one constructed by comparing as many attributes as possible. Organisms sharing many characteristic make up a single group or taxon.

Numerical taxonomy:

The development of computers has made possible the quantitative approach known as numerical taxonomy. Peter H.A., Sneath and Robert Sokal have defined numerical taxonomy as “the grouping by numerical methods of taxonomic units into taxa on the basis of their character states”.

The process beings with a determination of the presence or absence of selected characters in the group of organisms under study. A character usually is defined as an attribute about which a single statement can be made. Many characters, at least 50 and preferably several hundred, should be compared for an accurate and reliable classification. It is best to include many different kinds of data: morphological, biochemical and physiological.

After character analysis, as association coefficient, a function that measures the agreement between characters possessed by two organisms is calculated for each pair of organisms in the group. The simple matching coefficient (S_{SM}), the most commonly used coefficient in bacteriology, is the proportion of characters that match regardless of whether the attribute is present or absent (table5.1). Sometimes the Jaccard coefficient (S_J) is calculated by ignoring any characters that both organisms lack (Table 5.1). Both coefficients increase linearly in value from 0.0 (no matches) to 1.0 (100% matches).

Table 5.1 The Calculation of Association Coefficients for Two Organisms

In this example, organisms A and B are compared in terms of the characters they do and do not share. The terms in the association coefficient equations are defined as follows:

		Organism B: 1	Organism B: 0
Organism A	1	a	b
	0	c	d

a = number of characters coded as present (1) for both organism
b and c = numbers of characters differing (1,0 or 0,1) between the two organisms
d = number of characters absent (0) in both organisms
Total number of characters compared = a + b + c + d

The simple matching coefficient $(S_{SM}) = \dfrac{a + b}{a + b + c + d}$

The Jaccard coefficient $(S_J) = \dfrac{a}{a + b + c}$

Organisms with great similarly are grouped together and separated from dissimilar organisms such groups of organisms are called phenons (sometimes called phenoms).

Phylogenetic Classification

Following the publication in 1859 of Drawin's *On the Origin of Species*, biologists began trying to develop phylogenetic or phyletic classification systems. These are systems based on evolutionary relationships rather than general resemblance (the term phylogeny [Greek phylon, tribe or race, and genesis, generation or origin] refers to the evolurationary development of a species) T has proven difficult for prokaryotes and other microorganisms, primarily because of the lack of a good fossil record. The direct comparison of genetic material and gene products such as RNA proteins overcomes many of these problems.

5.3. Major Characteristics Used In Taxonomy

Many characteristics are used in classifying and identifying microorganisms. Characteristics, have been divided in two groups Classical and Molecular

Classical Characteristics

Classical approaches to taxonomy make use of morphological, physiological, biochemical, 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.

Morphological Characteristics

Morphological features are important in microbial taxonomy for many reasons. Morphology is easy to study and analyze, particularly in eucaryotic microorganisms and the more complex prokaryotes. In addition, morphological comparisons are valuable because structural features depend on the normally (at least in eucaryotes) these do not vary greatly with environmental changes. Thus morphological similarly often is a good indication of phylogenetic relatedness.

Many different morphological features are employed in the classification and identification of microorganisms (Table 5.2). Although the light microscope has always been a very important tool, its resolution limit of about 0.2_m reduces its usefulness in viewing smaller microorganisms and structures. The transmission and scanning electron microscopes, with their greater resolution, have immensely aided the study of all microbial groups.

Table 5.2 Some Morphological Features Used in Classification and identification.

Feature	**Microbial Groups**
Cell shape	All major groups[a]
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

[a]Used in classifying and identifying at least some bacteria, algae, fungi and protozoa.

Physiological and metabolic characteristics

Physiological and metabolic characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.(Table 5.3) lists some of the most important of these properties.

Table 5.3 Some Physiological and Metabolic Characteristics Used in Classification and Identification
Carbon and nitrogen sources
Cell wall constituents
Energy sources
Fermentation products
General nutritional type
Growth temperature optimum and range
Luminescence
Mechanisms 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

Ecological Characteristics

Many properties are ecological in nature since they affect the relation of microorganisms to their environment. Often these are taxonomically valuable because even very closely related microorganisms can differ considerably with respect to ecological characteristics. Microorganisms living in various parts of the human body markedly differ from one another and from those growing in freshwater, terrestrial, and marine environments. Some examples of taxonomically important ecological properties are life cycle patterns; the nature of symbiotic relationships; the ability to cause disease in a particular host; and habitat preferences such as requirements for temperature, pH, oxygen, and osmotic concentration. Many growth requirements are also considered physiological characteristics (Table 5.3)

Genetic analysis

Because most eukaryotes are bale to reproduce sexually, genetic analysis has been of considerable usefulness in the classification of these organisms. As mentioned earlier, the species is defined in terms of sexual reproduction where possible. Although prokaryotes do not reproduce sexually, the study of chromosomal gene exchange through transformation and conjugation is sometimes useful in their classification.

Transformation can occur between different prokaryotic species but only rarely between genera. The demonstration of transformation between two strains provides evidence of a close relationship since transformation cannot occur unless the genomes are fairly similar. Transformation studies have been carried out with several genera: *Bacillus, Micrococcus, Haemophilus, Rhizobium,* and others. Despite transformation's usefulness, its results are sometimes hard to interpret because an absence of transformation may result from factors other than major differences in DNA sequence.

Conjugation studies also yield taxonomically useful data, particularly with the enteric bacteria. For example, *Escherichia* can undergo conjugation with the genera *Salmonella* and *Shigella* but not with *Proteus* and *Enterobacter*. These observations fit with other data showing that the first three genera are more closely related to one another than to *Proteus* and *Enterobacter*.

Plasmids are undoubtedly important in taxonomy because they are present in most bacterial genera, and many carry genes coding for phenotypic traits. Because plasmids could have a significant effect on classification if they carried the gene for a trait of major importance in the classification scheme, it is best to base a classification on many characters. When the identification of a group is based on a few characteristics and some of these are coded for by plasmid genes, errors may result. For example, hydrogen sulfide production and lactose fermentation are very important in the taxonomy of the enteric bacteria, yet genes for both traits can be borne on plasmids as well as bacterial chromosomes. One must take care to avoid errors as a result of plasmid-borne traits.

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.

Molecular characteristics

Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids. Because these are either gene products are gene themselves. Comparison of proteins and nucleic acid yield construable information about true related tests. The recent molecular approaches have become important in prokaryotic taxonomy.

Comparison Of Proteins

The amino acid sequences of proteins are direct reflections of mRNA sequences and therefore closely related to the structures of the genes coding fro their synthesis. For this reason, comparisons of proteins from different microorganisms are very useful taxonomically. There are several ways to compare proteins. The most direct approach is to determine the amino acid sequence of proteins with

the same function. The sequence of proteins with dissimilar functions often change at different rates; some sequence change quite rapidly, whereas others are very stable. Nevertheless, if the sequences of proteins with the same function similar, the organisms possessing them are probably closely related. The sequences of cytochromes and other electron transport proteins, histiones, heat-shock proteins, transcription and translation proteins, and a variety of metabolic enzymes have been used in taxonomic studies. Because proteins sequencing is slow and expensive, more indirect methods of comparing proteins frequently have been employed. The electrophoretic mobility of proteins is useful in studying relationships at the species and subspecies levels. Antibodies can discriminate between very similar proteins, and immunologic techniques are used to compare proteins from different microorganisms.

The physical, kinetic and regulatory properties of enzymes have been employed in taxonomic studies. Because enzyme behavior reflects amino acid sequence, this approach is useful in studying some microbial groups, and group specific patterns of regulation have been found.

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 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 :

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

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 performances liquid chromatography (HPLC), physical methods are easier and more often used. The G + C content often is determined from the melting temperature (T_m) 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 increase during strand separation. When a DNA sample is slowly heated, the absorbance increases as hydrogen bonds are broken and reaches a plateau when all the 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 CsCl 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. If two organisms

differ in their G + C content by more than about 10%, their genomes have quite different base sequences. On the other hand, it is not safe to assume that organisms with very similar G + C contents also have similar DNA base sequences because two very different base sequences can be constructed from the same proportions of AT and GC base pairs. Only if two microorganisms also are alike phenotypically does their similar G + C content suggest close relatedness.

G + C content data are taxonomically valuable in at least two ways. First, they can confirm a taxonomic scheme developed using other data. If organisms in the same taxon are too dissimilar in G + C content, the taxon probably should be divided. Second, G + C content appears to be useful in characterizing prokaryotic genera since the variation within a genus is usually less than 10% even though the content may vary greatly between genera. For example, *Staphylococcus* has a G + C content of 30 to 38%, whereas *Micrococcus* DNA has 64 to 75% G + C; yet these two genera of gram-positive cocci have many other features in common.

Nucleic Acid Hybridization

The similarity between genomes can be compared more directly by use of nucleic acid hybridization studies. If a mixture of single stranded DNA formed by heating ds DNA is cooled and held at a temperature about 25^0C below the T_m, strands with complementary base sequences will reassociate to form stable ds DNA, whereas non complementary 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 50^0C below the T_m will allow hybrids of more diverse ssDNAa to form. Incubation at 10 to 15^0C below the T_m permits hybrid formation only with almost identical strands.

In one of the more widely used hybridization techniques, nitrocellulose filters with bound non radioactive DNA strands are incubated at the appropriate temperature with single stranded DNA fragments made radioactive with ^{32}P, ^{3}H, or ^{14}C. After radioactive fragments are allowed to hybridize with the membrane-bound ss DNA, the membrane is washed to remove any non hybridized ss DNA 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 (Table 5.5 provides examples). Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions and less than a 5% difference in T_m often are considered members of the same species.

Table 5.5 Comparison of Neisseria Species by DNA Hybridization Experiments

Membrane – Attached DNA[a]	Percent Homology[b]
Neisseria meningitides	100
N. gonorrhoeae	78
N. sicca	45
N. flava	35

Source : Data from T.E. Staley and R.R. Colwell, "Applications of Molecular Genetics and Numerical Taxonomy tot eh Classification of Bacteria" in Annual Review of Ecology and Systematic, 8:282, 1973.

[a]The experimental membrane attached nonradioactive DNA from each species was incubated with radioactive N. meningitides DNA, and the amount of radioactivity bound to the membrane was measured. The more radioactivity bound, the greater the homology between DNA sequences.

$$b \quad \frac{\text{N. meningitides DNA bound to experimental DNA}}{\text{Amount bound to membrane attached N. meningitides DNA}} \times 100$$

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 are compared by carrying our DNA-RNA hybridization experiments using radioactive ribosomal or transfer RNA. Distant relationships can be detected because rRNA and tRNA genes represent only a small portion of the total DNA genome and have not evolved as rapidly as most other microbial genes. The technique is similar to that employed for DNA-DNA hybridization: membrane-bound DNA is incubated with radioactive rRNA, washed and counted. An even more accurate measurement of homology is obtained by finding the temperature required to dissociate and remove half the radioactive rRNA from the membrane; the higher this temperature, the stronger the rRNA-DNA complex and the more similar the sequences.

Molecular (rRNA) based classification

Although classical numerical taxonomic approaches are useful for classification of Micro Organism at the species and sub species levels, Reliable Phylogenetic classification of Higher level groupings of evolutionary divergent Micro Organisms are not feasible using such taxonomic methods. Classification systems that relay on phenotypic features have resulted in the assignment of bacteria to suprageneric groups, many of which were subsequently shown to be heterogeneous. Only by using molecular analysis could classification systems be developed in which higher order phylogenetic groups were properly classified. Genetic measures such as r RNA analysis and DNA hybridization provide the measures of higher-level taxonomic groupings.

The bacterial systematic, which begin as largely intuitive subject, has become objective with the introduction and application of new molecular methods. This became clear with the recognition that bacteria and archaea represent phylogenetically distinct prokaryotes. This major break through in determining evolution and phylogeny of prokaryotes occurred with the introduction of rRNA sequencing techniques.

The RNA components of Ribosomes(rRNAs) are among the most evolutionarily conserved macromolecules in all living systems. Their functional roles in primitive information processing systems must have been well established in the earliest common ancestors of bacteria archaea and eukaryotes. Ribosomal RNA genes in all contemporarily share the common ancestry and they don't appear to

undergo lateral gene transfer between species. Because of functional constraints large portions of ribosomal RNA genes are well conserved and the sequence can be used to measure phylogenetic distances between even the most distantly related organisms. In essence, changes in RNA nucleotide sequence are indices of evolutionary change.

The comparison of rRNA molecules isolated from different organisms is useful for determining the evolutionary relations ships of all living things. There are many possible nucleotide sequences of RNA molecules. Any similarity in two nucleotide sequence suggest some phylogenetic relationship between these nucleotide sequences and the organisms that contain them. In particular the 16s rRNA of bacteria and archaea is used to determine the phylogenetic relationships among these microorganisms. For Eukaryotes, 18s rRNA is analyzed. The advantages of using 16s rRNA and 18s rRNA's is that they are found in all organisms, are large enough molecules to provide a significant number of nucleotides to compare sequences and yet they are small enough to analyze easily. This is why CARL WOESE who had begun phylogenetic studies with 5s rRNA which has only 120 nucleotides switched his studies to the larges 16s rRNA which has 1500 nucleotides. He argued that 16s and 18s rRNA's make excellent molecular chronometers because they are 1) occur universally in all organisms, 2) Have long highly conserved regions to asses close relation ships, and 3) have sufficient variable reasons to asses close relationships, and 4) are not prone to rapid sequence change due to selection because of their central function in gene expression.

The first complete rRNA gene sequence was determined for E.coli. A comparison of this sequence with the oligonucleotide catalogue data reviled the universally conserved elements(Short sequences that appeared to be conserved in all organisms) are distributed along the entire length of the E.coli rRNA. Similar sequence analysis of rRNa coding regions from *saccharomyces cerevisiae* and *Xenopuslaevis, Dictyostelium, Disoideum, Halobactrium*, and from several mitochondrial and chloroplast genomes confirmed this observation and identified the existence of these conserved elements. At the same time these analysis showed that the rRNa of bacterial archaeal and eukaryal domains were specific to those domains – each has its own characteristics rRNAs with diagnostic sequences and characteristics secondary structures. Analyzing these rRNA forms the bases for the phylogenetic analysis of organisms in all three domains of life.

Several methods can be used to analysis the rRNA molecules(FIG). In the original analytical approach cell were grown in the presence of phosphate containing the radioactive isotope 32_p so that the radio labeled phosphate was incorporated into the nucleic acid including rRNA. The ribosomal RNA was recovered from the cells in high quantities and then digested with T_1 ribonuclease. This enzyme cuts the RNA so that every oligonucleotide is produced and ended with a guanine residue at the 3'prime- OH position. Typically the oliginucleotide produced in this procedure had upto 20 nucleotides, these oligonucloetides were separated by gel electrophoresis and their nucleotide sequence were determined. Oligonuclotides with six or more nucleotides were catalogued for comparison with those obtained from the rRNA' of other microorganisms . The term catalogue is used to mean the listing of specific nucleotide sequences from the organisms rRNA. The nucleotide sequences comprising the rRNA catalogue normally placed into the computer for analysis. Oligonucleotides of six of more nucleotides were chosen because they were likely to occur only once in a 16s rRNA and yet there generally would be about 25 such sequences to provide a sufficient basis for comparison.

Another approach for analyzing rRNA sequence is to extract the rRNA from the cells and to analyze it directly or to use the rRNA as a template for making cDNA and then using the polymerase chain reaction(PCR) to produce sufficient DNA for analysis. Typically if rRNA is to be captured for analysis, cells are ruptured in the presence of Dnase to degrade all DNA. The RNA is then extracted with phenol and water. Large RNA molecules in the aqueous phase. After precipitation of RNA with alcohol and salt, a DNA primer that is complementary to the conserved region of the 16s rRNA is added. Reverse transcriptase can then be used to generated cDNAs .The cDNAs can be amplified using PCR and complete sequences of the nucleotide in the cDNAs determined so that the nucleotide sequence in the rRNAs can be deduced from these analysis.

Comparison of Nucleotide sequences of 16s rRNA allow the calculation evolutionary distances and the construction of phylogenetic trees that show relative evolutionary positions and relationships. The resultant phylogeny based on 16s rRNA analysis revealed the separate domains of bacteria and Archaea and eukaryotes, When Carl Woese first declared the existence of the Archaea and that they represented a third domain of life most microbiologist shunned the proposal, viewing it as heresy to break to prokayote- Eukaryote paradigm.

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 rRNA isolated from the 50S and 30S subunits, respectively, of prokaryotic ribosome. 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 important advantage as distantly related organisms can be studied only sequences that change little with time.

There are several ways to sequence for rRNA. Ribosomal RNAs can be characterized in terms of partial sequences by the oligonucleotide cataloging methods as follows. Purified, radioactive 16S rRNA is treated with the enzymeT1 ribonuclease which cleaves it in to 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 (S_{ab} value) are calculated. Complete rRNA s 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 procaryotic taxonomy.

5.4. Microbial Taxonomy And Classification

Introduction

Classification (ordering of organisms into groups, or taxa) is one aspect of taxonomy, which is the process, based on established procedures and rules, of describing groups of organisms, their interrelationships, and the boundaries between groups of organisms (Fig.5.). In addition to classification, taxonomy is concerned with nomenclature (assign names to the units described in a classification system), and identification (applying the system of classification and nomenclature to assign the proper name to an unknown organism and to place it in its proper position within the classification system).

Classification attempts to differentiate microbial taxa into structured groups so that the members of a group are more closely related to each other than they are to members of any other group. Classification is coherent scheme by which a collection of organisms is arranged to reflect the relationships between individual and groups. The ordering of their similarities. Historically many classification systems used for microorganisms were artificial rather than natural; they are based on observable phenotypic features and not on evolutionary (genetic) relatedness. Taxa based on observed phenotypic characteristics may not accurately reflect genetic similarities and such a classification may not correspond to the evolutionary flow of events. It is possible for genetically dissimilar bacteria produce yellow pigments, and a characteristic scheme based on such a phenotypic characteristic could produce a taxonomic group of genetically unrelated bacteria. In fact, classification systems are filled with errors made by using such phenotypic characteristics. Various groups of bacteria that have been defined on the basis of their apparent phenotypic relationship are now considered to be “groups of uncertain taxonomic affinity” because the taxonomic group may not be homologously similar and therefore may not accurately have genetic similarities.

Taxonomic hierarchies

When classifying organisms, systematizes use a taxonomic hierarchy consisting of different organizational levels (Table 5). Ideally, each defined level of a taxonomic hierarchy represents a coherent degree of homology, that is of genetic and evolutionary similarity. Each taxonomic group should be monophyletic, that is the members of each taxa should have the same evolutionary history. A genus, for example should contain only species that evolved from the same ancestral species that first evolved in that genus. Classification systems based on molecular analyses aimed at directly assessing phylogeny tend to meet this condition. In contrast, taxa defined on phenotypic characteristics often are polyphyletic, that is taxa defined by such systems often include organisms with different evolutionary histories that represent varying degrees of analogous (phenotypic) similarity.

The levels of a taxonomic hierarchy, from the highest to the lowest, are domains or empires, kingdoms, phyla or divisions, classes orders families, genera and species (Fig5). By assuming similarity between species, they may be arranged into genera which may in turn, be fused arranged into genera, which may, in turn, be fused into higher taxa such as families until the whole range of variation is accounted for in the hierarchical system. The hierarchical separation of micro organisms into taxonomic grouping of species, genera, families and so forth can be defined at the molecular level. Separate species of microorganisms are distinguished if their DNA reassociation is less than 70%. Based on 16S rRNA sequence data, a similarity of less than 98% is considered evidence for separate species. At

the genus level, interspecies DNA reassociation values of less than 20% to 30% are considered indicative of separate genera. A similarity of less than about 93% to 05% in 16S rRNA sequence also is considered evidence for separate genera. Different families are distinguished when their 16S sequence similarities are less than 89% to 93%.

While taxonomists agree today that taxonomic hierarchies should be based on phylogeny, there are no formal rules for establishing such hierarchies and hence the groups that are defined in a microbial classification system are based on many subjective decisions. Some taxonomists are "lumpers" tending to place many similar organisms into large taxonomic units. In contrast, other taxonomists are "splitters", favoring small taxonomic groups that emphasize even minor differences between organisms. Tremendous ambiguity exists relative to the higher taxonomic levels of bacteria because of their great diversity. Hence bacteriologists often ignore the phylum, class, and order and focus on genus and species.

Although species are the basic taxonomic units, the genetic variability of microorganisms permits a further division into subspecies or types that describe the specific clone of cells. The subspecies or type may differ physiologically (biovar), morphologically (morphovar), or antigenically (serovar). It is often important to differentiate the subspecies of a given micro organisms. For example one strain of a bacterial species may produce a toxin and be a virulent pathogen, and other strains of the same species may be nonpathogenic. A strain is a population of cells that are descendents of a single cell. The ability to distinguish correctly between such strains and subspecies of a particular microbial species is of obvious importance in medical and industrial microbiology. Pure cultures grown in the microbiology laboratory represent individual strains of a species. After organisms is defined as representing a new species of strain, a culture generally is deposited in an appropriate culture collection as the type culture. That type culture and its description become the foundation for future reference.

An alternate method of describing species today is based on molecular analyses. The nucleotide sequence of informational molecules can be determined even for microorganisms that have yet to be cultured. New taxa can be defined based on finding nucleotide sequences that are sufficiently divergent to merit placing them into new groups. Thus many have never been cultured. Some of these can be observed under a microscope and specially tagged using labeled gene probes so that at least their appearance can be described. In many cases, however little or nothing is known about the physiologies of these newly discovered taxonomic groupings, it to say that there are tens of thousands of diverse microbial species that have yet to be classified and described.

5.5. Classification of Prokaryotes According to the Second Edition of Bergey's Manual of Systematic Bacteriology

Bergey's Manual of Systematic bacteriology

In 1923, David Bergey, professor of bacteriology at University of Pensylvania and four colleagues published a classification of bacteria that could be used for identification of bacterial species, the *Bergey's Manual of Determinative bacteriology.* This manual is now in its ninth edition. The first edition of *Bergeys Manual of Systematic bacteriology,* a more detailed work that contains descriptions of all prokaryotic species currently identified, also is available. The first volume of the second edition

has been published recently. This section briefly describes the current edition of *Bergeys Manual of Systematic.*

The First Edition of Bergey's Manual of Systematic Bacteriology

Because it has been possible in the past to classify prokaryotes satisfactorily based on phylogenetic relationship, the system given in the first edition of *Bergeys Manual of Systematic Bacteriology* is primarily phonetic. Each of the 33 sections in the four volumes contains prokaryotes that share a few easily determined characteristics and bears a title that either describes these properties or provides the vernacular names of the prokaryotes included. The characteristics used to define sections are normally features such as general shape, motility, the presence of endospores, the mode of energy production, and so forth. Prokaryotic group are divided among the four volumes in the following manner: (1) gram-negative bacteria of general, medical or industrial importance; (2) gram-positive bacteria other than actinomycetes; (3) gram-negative bacteria with distinctive properties, cyanobacteria, and archaea; and (4) actinomycetes (gram-positive filamentous bacteria).

That even determine the volume into which a species is placed. There are good reasons for this significance. As noted in chapater 3, Gram staining usually reflects fundamental differences in bacterial wall structure. Gram-staining properties also are correlated with many other properties of bacteria. Typical gram-negative bacteria, gram-positive bacteria, and mycoplasmas (bacterial lacking walls) differ in many characteristics, as can be seen in table 19.9. For these and other reasons, bacteria traditionally have been classified as gram positive or gram negative. This approach is retained to some extent in more phylogentic classifications and is useful way to think about bacterial diversity.

The Second Edition of Bergey's Manual of Systematic Bacteriology

There has been enormous progress in prokaryotic taxonomy since 1984, the year the first volume of Bergey's Manual of Systematic bacteriology was published. In particular, the sequencing of for RNA, DNA, and proteins has made phylogenetic analysis of prokaryotes feasible. As a consequence, the second edition of *Bergey's manual* will be largely phylogenetic rather than phonetic and thus quite different from the first edition.

The second edition will be published in five volumes. It will have more ecological information about individual taxa. The second edition will not group all the clinically important prokaryotes together as the first edition all did. Instead, pathogenic species will be placed phylogenetically and thus scattered throughout the following five 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, Fibrobacteres, bacteroidetes and Fusobacteria (Volume 5 also will contain a section that updates descriptions and phylogenetic arrangements that have been revised since publication of volume 1).

The second edition's five volumes will have a different organization than the first edition. The greatest change in organization of the volumes will be with respect tot eh gram-negative bacteria. The first edition describes all gram-negative bacteria of general, medical or industrial importance; volume describes the Archaes, Cyanobacteria, and remaining gram-negative groups. The second edition describes the gram-negative bacteria in three volumes, with volume 2 reserved for the Proteobacteria. The two editions treat the gram-positive bacteria more similarly. Although volume 2 of the first edition does not have some G+C bacteria, much of its coverage is equivalent to the new volume 3. Volume 4 of the first edition describes the Actinomycetes and is similar to volume 4 of the second edition (high G+C gram-positive bacteria), although the new volume 4 will have broader coverage. For example, *Micrococcus* and *Corynebacterium* are in volume 2 of the first edition and will be in volume 4 of the second edition.

The Second Edition will be published in five volumes it will have more ecological information about individual taxa. The second edition will not group all the clinically important prokaryotes together as the first edition did instead pathogenic species will be placed phylogenetically and thus scattered through out the five following volumes.

Volume 1.

Archaea

The Archaea comprise organisms that evolved as separate domain often retaining specialized phenotypic characteristics. Archaea has several features relative to their cell structure that permit them to live in extreme habitats and to function under conditions considered inhospitable to life

The cytoplasmic membrane of archaea are unique in structure and chemical composition they have high protein content and diverse lipids including are pholipids sulfolipids, glycolipids and a non polar isoprenoid lipid. The structure of membrane of many archaea is a lipid bilayer composed of glycerol diether lipids. But some archaeal membranes are monolayers composed of glycerol tetraether lipids. These monolayers are heat stable The archeal cell wall is distinct in chemical composition then the other organisms ,some Archaea stain gram negative and some gram positive, no archaean has a true bacterial gram negative or gram positive cell wall structure-all archaea lack peptidoglycan in their cell walls.

TheA chromosomes resemble bacterial chromosomes in that it is circular however there is a difference in the organization of Archaeal chromosomes. The chromosome of Archaea is associated with proteins which make is similar someway to eukaryotic chromosomes. Histone like proteins are involved in maintaining the structure of Archaeal chromosomes. Introns occur with the Archaeal chromosomes. Archaeal introns found in the stable RNA genes of thermophilic and halophilic Archaea sliced by Archaeal specific mechanism. The promoter sequences of some Archaea indicates the presence of two conserved regions one conserved region occurs at the sight of transcription. The second sequence occurs 25 base pairs of stream of transcriptional startsight resembles the TATA box of eukaryotic cell genomes The ribosomes of the Archaeal are of 70s type composed of 30s and 50s subunits resembling 70s ribosomes of bacterial cells

Volume 2

Proteobacteria (Purple Bacteria)

The proteobacteria are a coherent deep evolutionary branch on the bacterial tree encompassing a phenotypically very diverse group of Gram-negative bacteria. Even though they have a common evolutionary history, which has been confirmed by multiple measures including DNA-rRNA hybridizations, 16S rRNA cataloging and 16S or 5S sequencing the proteobacteria confront us with a bewildering range of phenotype features, apparently indicating independent and uncoordinated evolutionary modifications. The proteobacteria include photoautrophs, compounds, chemoorganotrophs that use many sites. These bacteria exhibit many physiological trate reduction, and so forth sugar oxidations, nitrate reduction, and so forth that appear to have arisen independently in many genera and species in the proteobacteria. Many live on the surfaces of plants and animals.

Apparently the proteocateria have common underlying biosynthetic and cellular housekeeping functions that reflect their common ancestry metabolic capabilities of the recent evolutionary changes that have increased diversity within this group. This could have occurred as a result of genetic exchange; Gram negative bacteria are sometimes described as promiscuous because of their frequent exchange of plasmids and recombination that crosses species and genus boundaries. It may also be that the phenotypic metabolic diversity is the centers of relatively minor changes in the active centers of the enzymes that have underlying homologous proteins.

The proteobacteira have been divided into five separate lineages based on rRNA sequences; these lineages are designated the alpha, beta, gamma, delta and epsilon subgroups. These subgroups of proteobacteria have characteristic differences. For example, an unusual polyamine-2hydroxyputresceine occurs as a specific component within members of the beta subgroup of the proteobacteria. In the alpha subgroup, a triamine, such as spermidine or symhomospermidine, is found as a characteristic biochemical component. Even these subgroups of proteobacteria contain very diverse genera species. The diversity of phenotypic features brings into question the evolutionary linkage of the proteobacteria and the course of evolution, especially of the processes involved in cellular energy generation.

The occurrence of bacteriochlorophy 11 dependent photosynthesis in three of the subgroups of the proteobacteria (alpha, beta, gamma) suggest that the proteobacteira are all derived from photosynthetic ancestors. The proteobacteria originally were named the purple bacteria to reflect this relationship to the purple bacteria to reflect this relationship to the purple photosynthetic bacteria. Chemoautotrophic bacteria, such as *E.Coli* appear to have lost their photosynthetic capabilities. Other non photosynthetic bacteria in the proteobacteria retain metabolic traces of their photosynthetic past. Bacteriochlorophyll a, for example, neither of which are anaerobic phototrophs.

Volume 3 & 4

Gram – Positive Bacteria

Based on comparisons of 16S rRNA catalogs and sequences, the gram-positive bacteria can be divided into two major evolutionary lines of descent: gram-positive bacteria with a low mole% G + C (clostridial lineage) and gram positive bacteria with a high mole% G + C (actinomycete lineage). The dividing line between these independent lineages is at about mole % G + C 50. Pylogenetic analyses

based on 16S rRNA support the division of the Gram – positive bacteria into these two distinct evolutionary branches.

Volume 3

Low Mole% G + C Gram-positive Bacteria

The low mole% G + C Gram-positive group generally include most Gram-positive bacteria: endospore forming genera, including Lactobacillus, *Listeria, Kurthia*, *Pediococcus, Mycoplasma*, *Heliobacterium* and others. All of the lactic acid bacteria and staphylococci are in this group. Based on oligonucleotide catalogs of 16S rRNA the lactic acid bacteria *Bacillus* and *Streptococcus* form a supercluster within the clostridial lineage. The lactobacillus lineage diverges at about the same similarly coefficient as the *Bacillus* and *Streptococcus* lineages. The divergence from a primitive anaerobic clostridia ancestor may have occurred as long ago as 2 similarly coefficients between species tend to be low. This suggests that eh divisions in the lactobacilli are phylogenetically deep and probably ancient.

Comparisons of 16S rRNA sequences, 5S rRNA sequences, and rRNA homologies have divided the clostridia into three phylogenetic groups. *Clostridium* represent one of the largest genera of all the bacteria because they are defined by relatively few criteria: anaerobic, endospore forming, non sulfate reducing Gram-positive bacteria. There are over 100 species within this group. The members of groups I and II are fairly homogenous in their intragroup relationships, whereas the members of group III show little or no rRNA sequence similarly.

Members of the genus bacillus show a divergence from their clostridia relatives with an S_{AB} of 0.4. Bacillus essentially differs from Clostridium species in that the former are aerobic and the latter are anerobic. This divergence corresponds tot eh appearance of high concentrations of oxygen in the Earth's atmosphere about 700 to 800 million years ago. This hypothesis is further supported by the appearance of other Gram-negative and Gram-positive aerobic lineages at about the same time.

The fact that the low mole% G + C Gram-positive group contains both endospore-forming and nonedospore forming genera leads to some speculation about when endospore formatting has been highly conserved in bacillus and Clostridium, which have been studied most extensively. This conservation suggest that the mechanism of sporulation developed only once during evolutionary history. Since Clostridium are phylogenetically more ancient than Bacillus, the ancestral progenitor of this group was probably an anaerobic spore forming bacterium. Later divergence from this ancestor would have resulted in the loss of the ability to sporulate. It follows that Kurthia, Pediococcus, Lactobacillus and other members of the low G + C gram-positive bacterial group must have lost the ability of differentiate into spores.

Mycoplasma species appear to be descendents of the Bacillus – Lactobacillus – Streptococcus lineatge. Mycoplasmas are unusual bacteria both in terms of their phenotype- they lack cell walls and genotype, which appears to have evolved more rapidly than other bacteria. The more rapid the rate of evolution at the genotypic level one manifestation of which is a lineage on a phylogenetic tree that is abnormally long the more unusual and atypical the resulting phenotypic changes. The most clear examples of such rapid genotypic change and unusual phenotype so far encountered among the bacteria

are the mycoplasmas. Phenotypically, mycoplasmas constitute a separate bacterial class; their uniqueness includes lack of a cell wall and the small size of their genomes. Based on rRNA sequence analysis, which is a measure of genotype, the mycoplasmas represent a typical bacterial group. Thus mycoplasmas represent a relatively superficial branching within bacterial phylogeny. What is unusual about the mycoplasmas by this genotypic measure is that their individual lineages tend top be evolving more rapidly than normal bacteria. Mycoplasmas have the smallest genomes among selfreproducign have the smallest genomes among self reproducing organisms. Genome sizes for members of the Mollicutes appear to fall into two clusters: one composed of Mycoplasma and Ureaplasma species have genomes of about 750 kb, and the other of Acholeplasma, Spiroplasma, Anaeroplasma and Asteroleplasma species have genomes of about twice that size. Given that the mycoplasmas evolved from Gram-positive bacteria with much larger genomes, it appears that their evolution involved an unexpected streamlining of genetic information needed to enhance efficiency for survival and reproduction.

Perhaps as surprising as the revelation that the mycoplasmas are descendants of the low mole% G + C Gram-positive bacteria is the discovery that Epulopiscium fishelsoni the largest of all bacteria is closely related tot eh endospore formers. E. fishelsoni is a descendant of Clostridium lentocellus, a cellulolytic, endospore forming anaerobe. Sicne E. fishelsoni lives in the gut of the herbivorous surgeonfish it is likely that it can grow using cellulose as a substrate. Although E. fishelsoni does not form endospores it exhibits an unusual form of reproduction that resembles the initial stages of sporulation. This bacterium produces multiple daughter cells. Which are released through a slit in the mother cell.

Analyses of 16S rRNA indicate that E.fishelsoni is closely related to Metabacterium polyspora, which forms multiple endospores. There are physical similarities between the appearances of inclusions within cells of E. fishelsoni and M.polyspora, except the inclusions in E. fishelsoni are metabholically active daughter cells and those within M.polyspora are resting endospores. The origin of the live daughter cells involved in the reproduction of Epulopiscium appear to have evolved as a modification of the sporulation process in a predecessor to this most unusual bacterium.

Volume 4

High Mole% G + C Gram-positive Bacteria

Gram-positive bacteria with a high mole% G + C (>55) comprise a morphologically diverse group that is phylogenetically related. This lineage of high mole% G+ C Gram-positive bacteria includes the actinomycetes, actinobacteria (Arthrobacter, Micrococcus, Oerskovia, Brevibacterium and Actinomyces among other genera), corynebacteria, mycobacteria, bifidobacteria and propionibacteria. Within this phenotypically diverse group that branched from the low mole% G + C Gram-positive bacterial lineage, there has been a continuation of cell wall evolution as seen in variations in the biochemical compositions of the peptidoglycan molecules. Several of these bacteria, most notably the mycobacteria, produce mycolic acids that make their cell walls biochemically distinct from other bacteria. There also has been the evolution of branching hyphae as a means of cells growth. Many of the actinobacteria exhibit irregular morphologies but the true actinomycetes from mycelia that bear a variety of spores. Complex life cycles with various spores and the production of growth regulators, many of which are antibiotics, characterize the actinomycetes.

Actinomycetes have been separated into six subgroups based S_{AB} values of oligonucleotide sequences: actinoplanetes. Maduromuycetes, nocardioforms, streptomycetes, thermomonospora and those with multilocular sporangia; the actinobacteria, and propionovbacteria form separate related groups. Based on 16S rRNA analyses, Cornebacterium species are placed into their own family – corynebacteriaceae and Mycobacterium species are grouped together with Nocardia and Rhodococcus species in the family Mycobacteriaceae. Detailed comparisons of 16S rRNA nucleotide sequences indicates that the genus Mycobacterium is most closeluy relatred to Streptomyces lividans (93% similarly) and less so to Bacillus subtilis (74% similarity). Interstingly, phylogenetic classifications of the mycobacteria based on these 16S rRNA analyses support the traditional phonetic separation of fast growing mycobacteria, such as Mycobacterium phlei, and slow growing mycobacteria such as Mycobacterium tuberculosis.

Analyses of 16S rRNA sequences from several cynobacterial strains show that the diversity within the cynobacteria is much less than that seen in other bacterial groups. Although the cynobacteria as a group display extensive morphological and physiological diversity, they are relatively closely related to one another on a phylogenetic level. Also, the diverse branching of the five orders within the cyanobacteria (Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stignomatales) show similar branching depths. The small change in sequence diversity and multiple fanlike branch arrangements within each order make sit difficult to assess the order of branching during evolution. Therefore taxonomic classifications of cyanobacteria based principally on morphology do not necessary reflect phylogenetic relationship.

Cyanobacteria often form symbiotic relationships with other host organisms as external (ectocyanosis) or internal (endocyanosis) symbionts. The endosymbiotic is referred to as a cyanelle. Symbionts have their own life cycle and their own genome. However, in evolutionary time the endosymbiont develops a stronger dependence on its host cell and changes in the morphology and metabolism of the endosymbiont can be seen. For example, the cyanelles of Cyanophora paradoxa exhibit a smaller genome and some of the functional genes of the cyanelle have been moved to the nucleus. Eventually the symbiont loses its potential for independent life and becomes a cyanoplast, which is equivalent to a eukaryotic organelle.

5.6. Summary

Characterization ,Classification and identification are major objective in all branches of the biological sciences. Classification is a means of bringing order to the bewildering variety of organisms in nature . Once we learn the characteristics of an organisms, we can compare it with other organisms to discover similarities and differences , in order to identify and classify microorganisms , we must first their characteristics . It is usually not feasible to study the characteristics of a single microorganism because because of its small size so we have to study the characteristics of a culture _a population of microorganisms so determining the characteristics is important in for classification but is done for other reasons as well . The different characteristics considered for classification of microorganisms include Morphological , Chemical, Cultural, Antigenic , Genetic , Ecological characters. Many classification schemes exist but most cover only one of few bacteria .One classification scheme is important , however because of its broad scope and wide acceptance Bergay”s Manual of determinative Bacteriology this work not only provides description of all established genera and species of bacteria but it also provide the practical arrangement of taxa that is useful for their identification

5.7. Key Words

1) Taxonomy
2) Genetic Analysis
3) Nucleic Acid base Composition
4) Nucleic Acid Hybridization
5) Taxonomic Hierarchies
6) 16 years RNA Sequencing
7) Domains
8) Kingdoms
9) Genera
10) Species
11) Nomenclature
12) Bergey's Manual
13) Archaea
14) Proteobacteria

5.8. Model Questions

Essay Type Questions

1) Define the following terms: taxonomy, classification, taxon and nomenclature.
2) What is numerical taxonomy why are computers so important to this approach.
3) Summarize the advantages of using each major group of characteristics (morphological, physiological, ecological, genetic and molecular) in classification and identification.
4) Brefiely describe the ways which proteins from different organisms can be compared.
5) How are rRNA sequencing studies conducted and why is rRNA so suitable for determining relatedness.
6) Describe nucleic acid hybridization and methodology involving nucleic acid hybridization.
7) What is G + C content of DNA and how can it be determined through melting temperature studies and density gradient centrifugations.
8) How does Woese divide organisms into domains are empires in his universal phylogenetic tree.

Short Answer Type Questions

1) Importance of rRNA in classification.

2) Numerical taxonomy
3) DNA hybridization
4) Kingdoms
5) Domains
6) Ecological classification

5.9. Reference Books

1) Principles of microbiology, Ronald M. Atlas, McGraw Hill, 2nd Edition.
2) Biology of microorganisms, Thomas D. Brock, Michael P. Madigan, Prentice Hall Englewood Cliffs, 5th Edition.
3) Microbiology, Michael J. Pelczar, Jr., E.C.S.Chan, Noel R. Krieg, 5th Edition..
4) Microbiology, Lansing M. Prescott, John P. Harley, Donald A.Klein

P. SUDHAKAR

Lesson - 7

DETAILED STUDY OF THE BACTERIAL GENERA

7.0 Objective

To know the characteristic features of different bacterial genera which is very important and significant in identifying the genera.

7.1 Introduction

Bacteria are prokaryotic organisms and can be distinguished from eukaryotic organisms but in some instances it may be difficult, especially the hyphae fromed by actinomycetes might be confused with the hyphae formed by molds. Some eukaryotic cells are as small as bacteria, and some bacteria are as large as some eukaryotes. Characterizing tests for the identification of various bacterial genera isolated from different sources range from the descriptive through simple biochemical tests for the detection of metabolic products or enzyme action, to highly specialized techniques required for the estimation of the GC percentage of the bacterial DNA. The desirability of careful morphological examination of strains as a first step in identification cannot be overemphasized. Simpler biochemical and physiological tests which are frequently useful in identification of bacteria at the species level include oxides, catalase, urease, nitrate reduction, H_2S production, acid or gas production from sugars, tests of amino acid metabolism, temperature range of growth, response to NaCl and antibiotic sensitivity. In some genera, the nutritional requirements, ability to utilize specific substrates and production of polyhydroxybutyrate are also useful. For a practical point of view, it is important to identify a bacterium by its characters.

7.2 BACILLUS

7.2.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : II
Section No. : 13
Group : Endospore forming Gram-positive rods and cocci
Type species : *Bacillus subtilis*

7.2.2 Characteristics

The genus *Bacillus* was erected by Cohn in 1872 to accommodate a bacterium described by C.G. Ehrenberg in 1835 as *Vibrio subtilis*. Distributed in a wide range of habitats, a few species are pathogenic to vertebrates or invertebrates. Members of the genus *Bacillus* can be easily isolated from soil or air. They are the most common organisms to appear when soils are streaked on agar plates. Morphologically the cells are rod shaped (Fig. 7.1) and straight, with a size range of 0.5-2.5 μm x 1.2 – 10 μm. The cells are often arranged in pairs or chains with rounded or squared ends. Cells stain Gram-positive and are motile by peritrichous flagella.

Members of the genus grow in the temperature range of -5^{o}C to 75^{o}C and in the pH range of 2.0 to 8.0. They tolerate the salt concentration of 2-25% range. Members are aerobic or facultatively anaerobic. They exhibit a great diversity in physiological abilities and biochemical properties. Most of the members are chemoorganotrophs with fermentative or respiratory metabolism. Members usually grow well on synthetic media containing sugars, organic acids, alcohols and so as sole carbon source, and ammonium as the sole nitrogen source. A few isolates require vitamins for their growth. Many bacilli produce extracellular hydrolytic enzymes that can break down the polysaccharides, nucleic acids and lipids permitting the organisms to use these products as carbon sources. Species of this genus produce endospores which are oval or sometimes round or cylindrical in shape. These endospores are highly resistant to many adverse conditions. Sporulation process is not repressed by the exposure to air and not more than one spore is produced per cell. Members of the genus *Bacillus* produce the antibiotics like bacitracin, gramicidin and polymyxin.

7.2.3 Species:

Bacillus subtilis ----- small rods, occur singly, lateral flagella, stains uniformly, produce endospores. Cell size is 0.8 μm x 1.5-1.8 μm. Colonies on agar medium are round or irregular, surface is dull, become thick and opaque, may be wrinkled. May be cream or brown in colour. Species produce 2,3-butanediol and glycerol during glucose fermentation.

Bacillus cereus ------ cells tend to occur in chains. Polyhydroxy butyric acid granules and volutin granules are the reserve food materials. Some strains produce red pigment, some produce yellow green fluorescent pigment, and some produce pinkish brown pigment. On fermentation of glucose, 2,3-butanediol and glycerol are produced by this species. Causes some forms of food poisoning and can infect humans.

Bacillus licheniformis ------ colonies are opaque on agar medium. Hair like outgrowths are common. Many strains form red pigment on media containing sufficient iron. Produce 2,3-butanediol and glycerol on fermentation of glucose.

Bacillus thuringiensis ----- can be used as insecticide as it produces a solid protein crystal called as parasporal body next to their endospores during sporulation. This B.T. parasporal body contains protein toxin that kill over 100 species of moths by dissolving in the alkaline gut contents of caterpillars and destroying their gut epithelium. The solubilized toxin proteins are cleaved by midgut proteases to smaller toxic polypeptides that attack the epithelial cells. The alkaline gut contents escape into the blood causing paralysis and death. This species is pathogenic to larvae of Lepidoptera.

Bacillus sphaericus----- produce parasporal body that contains proteins toxic to larvae of mosquito.

Bacillus anthracis ----- well known for causing anthrax disease in both animals and humans

Bacillus polymyxa ----- multiplication occurs in decomposing vegegation, participlate in retting of flax, produce 2,3-butanediol ethanol and H_2S, spores are widely spreaded, produce the antibiotic, polymyxin.
The other important species of this genus include *B. macerans, B. brevis, B. coagulans, B. stearothermophilus* etc.

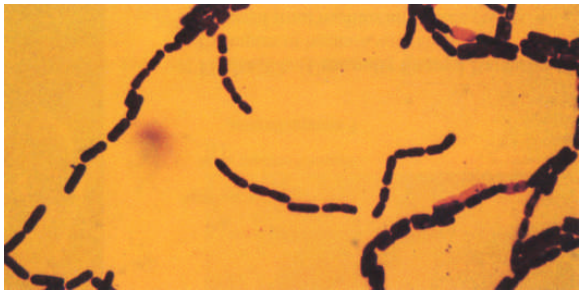

Figure-7.1 Cell structure of *Bacillus megaterium*

7.3 CLOSTRIDIUM

7.3.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : II
Section No. : 13
Group : Endospore forming Gram-positive rods and cocci
Type species : *Clostridium butyricum*

7.3.2 Characteristics

The genus Clostridium was established by Prazmocoski in 1880 to accommodate butyric acid fermenting organism and was named as Clostridium butyricum. More than 60 species were described in this genus. Soil is the main habitat of clostridia where they live primarily in anaerobic pockets

made by facultative organisms acting upon various organic compounds present. They are also found in fresh water and marine waters especially in the anaerobic zones. In addition, a number of clostridia have adopted to the anaerobic environment of the mammalian intestinal tract. Many clostridia cause spoilage of canned foods. Some soil clostridia cause serious diseases in man and animals mainly by their ability to produce toxins. Tetanus of humans, gasgangrene in humans and domestic animals and botulism in sheep and ducks are caused by members of Clostridium genus.

Morphologically, the cells are straight or slightly curved rods. In size, they measure 0.3-2.0 μm x 1.5-8.0 μm. Mostly occur as single cells, may be arranged in pairs or short chains with rounded or sometimes pointed ends. Some are known to be pleomorphic. They are Gram-positive. Most of them are motile by peritrichous flagella, a few are non-motile. Produce highly resistant, heat stable endospores which may be spherical or oval placed in the center or eccentric or distal (Fig. 7.2) or sub-terminal positions.

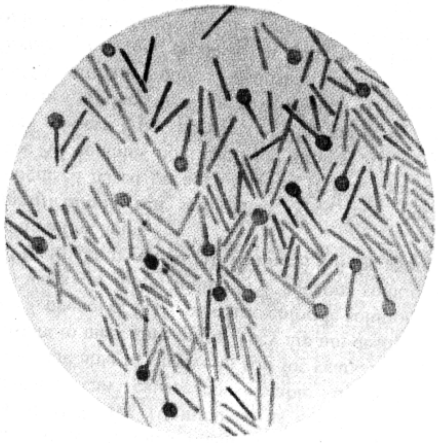

Figure-7.2 Endospore of *Clostridium tetani*

On nutrient agar members show little or no growth, show good growth on glucose agar. Colonies are circular to irregular, spreading type with white to cream colour. In glucose broth, they show diffuse turbid growth and ropy sediment. A red coloured pigment is produced by C. rubrum. Most species are chemo-organotrophic. Members may be saccharolytic, proteolytic, neither or both. Usually they produce mixtures of organic acids and alcohols from carbohydrates or peptones. Species do not carry dissimilatory sulfate reduction. They are catalase negative and obligately anaerobic. If at all growth occurs in air, it is scanty and sporulation is inhibited. Metabolically they are very diverse with optimum temperature of 10-65^{o}C. Biochemically clostridia show much variation and this property is commercially exploited to produce organic acids.

Various species of this genus are known to be capable of fermenting different substrates like cellulose, sugar, starch, pectin, proteins, amino acids, carbohydrates, purines, ethanol to yield different end products like acetic acid, lactic acid, succinic acid, ehanol, H_2, CO_2, acetone, butanol, butyric acid, fatty acids, formate, acetate, isobutyric acid, isovaleric aicds, butyric acid. The

pathogenic species of this genus produce highly potent neurotoxin namely Botulinum toxin and tetanospasmin.

7.3.3 Species:

Clostridium butyricum ------ Straight or slightly curved rods, 0.6-1.2 by 3.0-7.0 μm, with rounded ends; occurring singly, in pairs, in short chains and occasionally long filaments. Motile with peritrichous flagella. Spores are oval and eccentric to subterminal, with no exosporium and no appendages. Gram-positive becoming negative in old cultures. Cell wall contains diaminopimelic acid and glucose is the only cell wall sugar. Little or no growth on nutrient agar and good growth on glucose agar. Surface colonies circular to slightly irregular, 1-3 mm in diameter, slightly raised, white to cream color, glossy to matt surface. Good growth and gas production in broth media with fermentable carbohydrate. Fermentation products include acetic acid, butyric acid and butanol. Casein and gelatin are not hydrolyzed. Milk becomes acid with early coagulation and often with stormy fermentation. Does not require amino acids or vitamins, other than biotin, for growth. Optimum temperature for growth is 25^{o}-37^{o}C. found in soil, animal feces, cheese, naturally soured milk. The G+C content of the DNA is 27-28 moles %.

7.4 STAPHYLOCOCCUS

7.4.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : II
Section No. : 12
Group : Gram-positive cocci
Type species : *Staphylococcus aureus*

7.4.2 Characteristics:

First observed in human pyogenic lesions by Von Recklinghausen in 1871 and the genus Staphylococcus was erected by Rosenbach in 1884. Derived the name as so because of the arrangement of berry like cells in a grape bunch fashion (Fig. 7.3). Most of the strains are pathogenic. Mainly associated with skin, skin glands and mucous membranes of warm-blooded animals. They also serve as hosts for a wide range of bacteriophages.

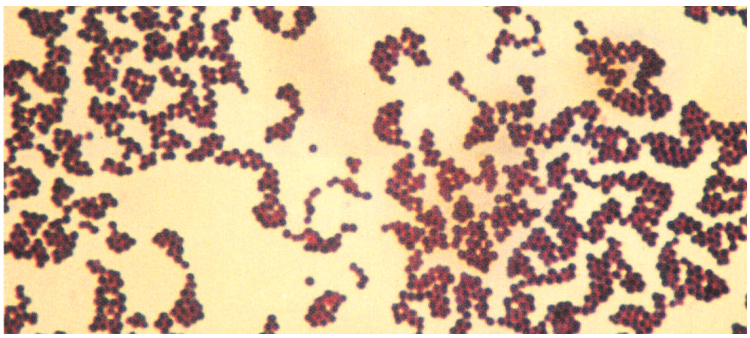

Figure- 7.3 Grape bunch structure of *Staphylococcus aureus*

Cells are spherical with a diameter in the range of 0.5 μm -1.5 μm. They occur singly or in pairs or in characteristic irregular clusters. They are non-motile. Resting stages are not known in the genus. Members are Gram-positive with normal peptidoglycan and teichoic acids in the cell wall. They can grow over a temperature range of 6.5^{o}C – 46^{o}C with optimum temperature between 35^{o}C to 40^{o}C. The range of pH in which the organism grows is 4.2 – 9.3, but the optimum pH is between 7.0 to 7.5. Most strains grow in the presence of 15% NaCl or 40% bile. Members are chemoorganotrophs with respiratory and fementative metabolisms. They show a positive catalase reaction. Menaquinones and Cytochromes a, b, and O form electron transport system. They employ O_2 as the universal terminal electron acceptor. Amino acids and vitamins are required for aerobic growth. Uracil and fermentable carbon source are required for anaerobic growth.

A wide range of carbohydrates may be utilized, particularly in the presence of air with the production of acid with no detectable gas. However, acid is not usually produced from arabinose, cellobiose, inositol, inulin and raffinose. Under anaerobic conditions, the main product of glucose fermentation is lactic acid. Main products of glucose fermentation in aerobic conditions are acetic acid and small amounts of CO_2. Starch is usually not hydrolyzed but a variety of protein and fat-containing substrates are hydrolyzed. Some members of the genus produce extracellular enzymes and toxins. Staphylococcus spp. are usually sensitive to heat and moderately resistant to γ-radiation. They are sensitive to antibiotics like β –lactams, macrolides, tetracyclines, novobiocin and chloramphenical, and also to antibacterials such as phenols and their derivatives, salicylanilides, carbanilides, halogens and their derivatives. But they are resistant to lysozyme, polymyxin and polyenes.

7.4.3 Species:

Staphylococcus aureus ----- Cell walls contain organic phosphorus, ribitol, glucosamine, muramic acid, glycine, lysine, aspartic acid, serine, glutamic acid, alanine and small amounts of threonine, proline, valine and leucine. Cell membranes contain the glycolipids, mono and diglucosyl-diglyceride and the phospholipids, lysyl-phospatidyl-glycerol, phosphatidyl-glycerol and cardiolipin. Colonies are smooth, low-convex, glistening with entire edge. Colonial pigmentation is extremely variable, hence the variety of specific epithets such as *aureus*, *albus* and *citreus* have been applied to this species. Colonies of most strains are orange in color although certain antibiotic resistant strains are commonly yellow pigemented.

Chemoorganotrophs with respiratory and fermentative metabolism. Acid is produced aerobically and anaerobically from glucose, lactose, maltose and mannitol. In air a wider range of carbohydrates are used as carbon and energy sources and hexoses, pentoses, disaccharides and sugar alcohols are metabolized with the production of acid. Acetoin is produced as an end-product of glucose metabolism. Nitrates reduced by nitratases. Ammonia produced from arginine by arginine dihydrolase. Proteases, lipases, phospholipases, lipoprotein lipases, esterases and lyases are produced. Coagulases are produced by virtually all strains. At least three hymolysins are produced (alpha, beta and delta), distinguished by type and range of hemolysis on sheep, rabbit and human erythrocytes.

Facultative anaerobes growing best under aerobic conditions. Most strains grow best at optimum temperature of 30^{o}-37^{o}C and optimum pH of 7.0 – 7.5 and in 15% sodium chloride or 40% bile. The G+C content of the DNA is 30.7-39 moles %. Potential pathogens causing a wide range of infections

and intoxications. Originally isolated from pus in wounds but also found in nasal membranes, hair follicles, skin and perineum of warm-blooded animals.

Other important species in the genus are *Staph. epidermidis, Staph. Hominis, Staph. saprophyticus and Staph. capitus.*

7.5 RHIZOBIUM

7.5.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : I
Section No. : 4
Group : Gram-negative aerobic/microaerophilic rods and cocci
Type species : *Rhizobium leguminosarum*

7.5.2 Characteristics

Rhizobium is placed under the family rhizobiaceae. Cells are rod shaped with a size range of 0.5-0.9 μm width and 1.2-3.0 μm length. Commonly pleomorphic under adverse growth conditions. Cells stain Gram-positive and motility occurs by one polar flagellum or sub-polar flagellum or by 2-6 peritrichous flagella. Few strains possess fimbriae. Usually contain polyhydroxy butyric acid granules which are refractile by phase contrast microscopy.

Colonies are circular, convex, semi-translucent, raised and mucilaginous, 2-4 mm in diameter within 3-5 days on yeast-mannitol-mineral salts agar medium. In agitated broth a pronounced turbidity develops after 2 or 3 days. The optimum temperature for a good growth range from 25^{o}C to 30^{o}C and the optimum pH for growth range from 6.0 to 7.0. Members of the genus are aerobic with respiratory metabolism and use O_2 as terminal electron acceptor. Often grow well under O_2 tensions less than 1.0 kPa.

Mostly chemoorganotrophic and utilize a wide range of carbohydrates and salts of organic acids as carbon source without any gas formation. They are not capable of utilizing cellulose and starch. In mineral salts medium containing mannitol and other carbohydrates, they produce an acidic reaction. On carbohydrate media, growth is usually accompanied by copious extracellular polysaccharide slime. Ammonium salts, nitrite, nitrate and most amino acids can serve as nitrogen sources. Some strains will grow in a simple mineral salts medium with vitamin-free casein hydrolysate as the sole source of both carbon and nitrogen. Peptone is poorly utilized. Casein and agar are not hydrolyzed. Some strains require biotin or other water soluble vitamins for their growth.

Species of *Rhizobium* are able to invade the root hairs of temperate-zone and some tropical-zone leguminous plants and incite production of root nodules (Fig. 7.4). The bacteria are present in root nodules in the form of bacteroids. These bacteroides are pleomorphic, swollen and misshapen rhizobial cells which involves in the fixation of atmospheric nitrogen into ammonia that can be utilized by host plants. This conversion is catalyzed by the enzyme nitrogenase. All strains of the genus that nodulate the plants exhibit host range affinities referred as host specificity.

Figure – 7.4 Root nodule formed by *Rhizobium* species

7.5.3 Species:
Rhizobium leguminosarum ----- Motile by two to six peritrichous flagella. Fimbriae described on a few strains. Some forms are encapsulated. Colonies are circular, covex, semitranslucent, raised and mucilaginous; usually 2-4 mm in diameter within 3-5 days on yeast mannitol mineral salts agar. Pronounced turbidity develops after 2-3 days in agitated broth. Utilize a wide range of carbohydrates, glucose, mannitol or sucrose are usually preferred. Some strains require biotin or other water-soluble vitamins. Generally cause nodule formation on temperate zone leguminous plants. The G+C content of the DNA ranges from 59.1-63.1 moles %.

Important other species of the genus include – *R. loti* and *R. meliloti* among the others.

7.6 AGROBACTERIUM

7.6.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : I
Section No. : 4
Group : Gram-negative aerobic/microaerophilic rods and cocci
Type species : *Agrobacterium tumefaciens*

7.6.2 Characteristics:

The genus *Agrobacterium* was erected by Cohn in 1942 to accommodate three species into it. A fourth species was added in 1947. The genus with four important species was placed in the family Rhizobiaceae. Cells are rods in shape with a size range of 0.6 μm –1.0 μm in width and 1.5 μm - 3.0 μm in length. Cells occur either singly or in pairs. They are Gram-negative and non-spore forming. Motility occurs by 1-6 peritrichous flagella. The optimum temperature for the growth of organism is

between 25^{o}C to 28^{o}C. Colonies are usually convex, circular, smooth and non-pigmented. The growth on carbohydrate containing media is usually accompanied by copious amounts of extracellular polysaccharide slime. Members of the genus are positive to catalase, urease and oxidase activities.

Nutritionally *Agrobacterium* species are chemorganotrophs capable of utilizing a wide range of carbohydrates, salts of organic acids and amino acids as carbon source. Cellulose, starch, and galactose cannot be utilized. Ammonium salts and nitrates can serve as nitrogen sources for strains of some species and biovars, whereas, some species require amino acids and other growth factors.

Except the *A. radiobacter*, other members of this genus invade the crown, roots and stems of a great variety of dicotyledenous and some gymnosperms via wounds causing the transformation of the plant cells into autonomously proliferating tumor cells. The induced plant disease are commonly known as crown gall, hairy root and cane gall. Some strains possess a wide host range and some others possess a very limited host range. The tumor (Fig. 7.5) induction by *Agrobacterium tumefaciens* is due to the presence of a large tumor inducing plasmid called Ti-plasmid in the bacterial cells. Following infection, a part of Ti-plasmid called transfer DNA called T-DNA is integrated into the genome of the plants. This T-DNA carries genetic information for tumor formation and for the production of a number of modified amino acids called opines. Octopine and nopaline are the two most common opines.

Figure- 7.5 Tumour growth formed by *Agrobacterium tumefaciens*

Opines are produced by plant cells transformed by T-DNA and serve as a source of carbon and nitrogen for *Agrobacterium* cells, but not essential for tumor formation. Ti-plasmid also encodes genes for several virulence factors and for two phytoharmones, auxin and cytokinin. The ratio of these harmones affect the final morphology of the tumor. The T-DNA portion of the Ti-plasmid moves from bacterium to plant and integrate into plant genome and subsequently transcribed and translated. *Agrobacterium tumefaciens* has been used as a significant vector to introduce foreign DNA into plants to generate transgenic plants through rDNA technology.

7.6.3 Species:

Agrobacterium tumefaciens----- Exhibit rapid growth on meat extract or yeast extract peptone media. A range of simple carbohydrates, organic acids and amino acids serve as carbon and energy sources. On mannitol nitrate glycerophosphate agar medium, colonies show mucoid growth with halo or browning and often with a white precipitate. Causes galls of plants in more than 40 families. Gall tissues are ill-defined consisting of disorganized masses of hyperplastic and hypertrophic tissues interspersed with badly organized groups of elements resembling trachea.

Agrobacterium rhizogenes ----- Causes hairy root or woolly knot disease whereby masses of intertwined fleshy and fibrous roots are produced on nursery stock.

Agrobacterium rubi ------ Causes the formation of small spherical growths or elongated ridges described as beading, corralling or knotting on black and purple cane raspberries and to a lesser extent on red raspberries.

7.7 ESCHERICHIA

7.7.1 Taxonomic Position

Bergey's manual : first edition of Bergey's manual of Systematic Bacteriology
Volume No. : I
Section No. : 5
Group : Facultatively anaerobic Gram-negative rods
Type species : *Escherichia coli*

7.7.2 Characteristics:

Genus *Escherichia* is placed in the family Enterobacteriaceae. *E.coli* was first isolated by the German Bactriologist Theodar Escherich in 1885 from the intestinal contents and described it as Bacterium coli. In 1919, Castellani and Chalmers erected the genus *Escherichia* in honour to Escherich. *E.coli* is the universal inhabitant of large intestine i.e., colon of humans and warm blooded animals. It occurs in polluted waters, sewage etc. and the presence of it is taken as an indicator of faecal matter contamination. Few strains of *E. blattae* occurs in the hind gut of cockroaches. The pathogenic forms cause intestinal disorders or diarrohoea and urogenital tract diseases.

Cells of *Escherichia* are straight rods which measure between 1.1 μm to 1.5 μm in width and 2.0 μm to 6.0 μm in length. They may occur singly or in pairs. Many strains possess capsules or microcapsules. Cells are motile or non-motile, if motile they move by peritrichous flagella. *Escherichia* possesses frimbriae or pili for attachment. They grow well at an optimum temperature of 37^{o}C and pH of 7.0. Colonies on nutrient agar may be smooth, low convex, moist shiny surface,

gray and easily emulsifiable in saline or colonies may be rough and do not emulsify in saline. In broth medium, growth is shown by a general turbidity and a heavy deposit in S-forms and a clear supernatant with granular deposit in R-form.

Members are facultatively anaerobic and chemoorganotrophs having both respiratory and fermentative types of metabolism. Nutritionally so versatile and readily grow on simple media. Biochemically, organisms are negative for oxidase, Voges-Proskauer and citrate tests and positive for catalase and MR tests. Serologically possess O-antigen or somatic antigen, K-antigen or capsular antigen and H-antigen or flagellar antigen. Some strains produce enterotoxins which are of two types namely heat-stable toxins and heat-labile toxins. Some strains of *E. coli* produce colicins, an antibiotic like substances. The important pathogenic strains of *E. coli* are EPEC, EHEC, ETEC and E1EC strains.
E. coli is undoubtedly the best studied bacterium and experimental organism of choice for various studies because of its rapid growth ability, simple nutritional requirements besides the easiness to work with the organism. The ability of *E. coli* to support the growth of a whole range of bacterial viruses made it possible to study the details of nature and multiplication of viruses.

7.7.3 Species:
Escherichia coli ----- Many strains have capsules or similar less well developed structures. Fimbriae on many strains; subdivided by their direct hemagglutinating capacity or differences in morphology into several fimbrial types; the sex (or F) fimbrial type can be detected by its affinity for special male phages and by its antigenic properties. In broth, growth is shown by a general turbidity and a heavy deposit which disperses completely on shaking (S form); the extreme R form shows a clear supernatantand a granular deposit which does not disintegrate completely on shaking.

Found in the lower part of the intestine of warm blooded animals. Many, if not all, members may show opportunistic pathogenicity like urinary tract infections in man, mastitis in cows etc. A limited number of well defined serotypes is closely associated with certain infectious enteric diseases in human infants and young of other animals. Hemolytic strains are found in high frequency in pigs.

Other species of the genus include *E. blattae, E. fergusorii, E. hermanii* and *E. vulneris*.

7.8 Summary

Different genera of bacteria possess their own characteristic features besides some common characters. Each bacterial genus may sometimes differ with other genus in their ecological and medical significance. Bacillus and Clostridium are the two important genera belonging to Gram-positive endospore forming bacteria. Bacillus species are aerobic or facultatively anaerobic Gram-positive rods that include the etiologic agent of anthrax (Bacillus anthracis). Another species is used as an important biological insecticide (Bacillus thuringiensis). The members of Clostridium are obligate anaerobic Gram-positive rods whose members include the etiologic agent of tetanus (Clostridium tetani), botulism (Clostridium botulinum), and gas gangrene (Clostridium perfringens). These three diseases are among the most significant causes of death in humans.

One of the most important Gram-negative rods in agriculture is the genus Rhizobium. The Rhizobium species are symbiotic, that is, they live on the roots of legume plants and perform

nitrogen fixation. In this process they trap nitrogen from the air and fix it into nitrogen compounds that can be used by plants in their metabolism. Another important Gram-negative rod bacterium in agriculture is the Agrobacterium tumefaciens. This bacterium is a plant pathogen and infects the plants causing tumor-like growth called crown gall. DNA technologists have used the ability of Agrobacterium to insert its genes in the plant and have isolated its plasmids to deliver foreign genes to a plant.

The most familiar bacterium Escherichia coli is a type of enteric bacterium. It is a Gram-negative, facultatively anaerobic bacterium that inhabits the human intestine. Most strains of E. coli live as harmless commensals in the human intestine, but there are certain strains that are considered pathogenic as they invade the tissues and produce toxins. These strains are said to be enteroinvasive and enterotoxic strains, respectively. One strain,
E. coli 0157:H7, has been implicated in food-related outbreaks of intestinal disease in recent years.

One of the Gram-positive cocci important to the humans is the genus Staphylococcus. Members of this genus occur in grape-like clusters and include the organism S. aureus. This organism may be the cause of abscesses, boils, and carbuncles, as well as toxic shock syndrome. Species of Staphylococcus can be aerobic, facultatively anaerobic or anaerobic.

7.9 Model Questions:

Q-1. Describe the characteristic features of the genera *Bacillus* and *Clostridium*
Q-2. Give an account on the salient features of *Rhizobium* and *Agrobacterium*
Q-3. Write an essay on the characters and significance of *Staphylococcus* and *Escherichia*
Q-4. Write short notes on

(1) *Bacillus* (2) *Rhizobium*

(3) *Clostridium* (4) *Agrobacterium*

(5) *Staphylococcus* (6) *Escherichia*

7.10 Reference books:

1. R.E. Buchanan and N.E. Gibbons (Co-editors) -- Bergey's Manual of Determinative Bacteriology (8th edition) 1974 – The Williams & Wilkins Company
2. John G.Holt et. al.-- Bergey's Manual of Determinative Bacteriology (9th edition) 1994 – Williams & Wilkins Company
3. Noel R. Krieg and John G.Holt (editors) – Bergey's Manual of Systematic Bacteriology (1st edition) 1984 – Williams & Wilkins
4. R. Ananthanarayan and C.K. Jayaram Paniker -- Text book of microbiology (5th edition) 1998 – Orient Longman Ltd.

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LESSON: 8

DISCOVERY, MORPHOLOGY AND CHEMISTRY OF VIRUSES

Objective: To know about the discovery of viruses, their morphology and chemistry

Contents

8.1. Introduction:

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

8.2. Discovery of viruses:

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

Martinus Beijerinck, (1896), a Dutch soil microbiologist who collaborated with Adolf Mayer and showed that the sap of infected tobacco plants retained its infectivity after filtration and also he proved that the filtered sap regain its "strength" of infection after dilution. He explained that the pathogen is an organism smaller than bacteria, not observable in the light microscope and able to produce it self only in the living plant tissue, and hence, it cannot be cultured outside the host. Beijerinck

called this filterable agent as a “Contagium vivum fluidum” (contagious living liquid). Loeffler and Frosch (1898) isolated and described the first filterable agent from animals, the foot-and-mouth disease virus (FMDV).

Walter Reed and his Co-workers (1901) recognized the first filterable agent from human, yellow fever virus. Lode and Gruber (1901) reported the virus causing plague in fowls and named it as Fowl Plague virus. In 1903 Remlinger and Riffat-Bay identified a causal agent infecting the dogs known as Rabies virus. Negri (1903) demonstrated that the nerve cells of rabies infected dogs contained prominent crystalline inclusion bodies, and they were later named as “Negri bodies”.

In 1911 Ellermann, Bang and Rous discovered and confirmed the cancer producing capacity of filterable agents in chicken and fowl. Peyton Rous first demonstrated a solid tumor virus of chicken, known as Rous Sarcoma virus, is a filterable agent.

In 1915, Frederick W. Twort noticed that some bacterial colonies underwent a visible change and become “Water looking” (more transparent). He called this phenomenon as glassy transformation and named the clear circular spots as ‘*taches vierges*’ (plaques). d’Herelle developed the plaque assay in 1917 and named the agents infecting bacteria are “Bacteriophages”.

First successful cultivation of vaccinia virus in tissue culture was reported by Parker and Nye in the year 1925. Max Schleisinger (1932) purified the phages and reported that they were composed of protein and DNA in roughly equal proportions.

Emory Ellis and Max Delbruck (1939) designed one-step growth curve experiment, in which an infected bacterium release hundreds of phages synchronously after 90 minutes latent or eclipse period. The first clear pictures of bacteriophases had been obtained by Tom Anderson and Delbruck (1942). The first mutants of bacteriophages were isolated and characterized by Delbruck in the year 1946, and he also reported that mixed phage infection leads to genetic recombination.

Seymour Cohen (1947) examined the effects of phage infection on DNA and RNA levels in infected cells using a colorimetric analysis. The result of Monod and Wollman in 1947 made the clear point that a virus could redirect cellular macromolecular synthetic processes in infected cells.

In 1949 first successful cultivation of poliovirus in Human tissue culture was performed by John Enders. In 1949 Andre Lwoff studied the lysogenic phages of *Bacillus magaterium*. When lysogenic bacteria were lysed from without, no virus was detected.

Hershey and Chase (1952) utilized labeled viral protein with $^{35}SO_4$ and nucleic acids with $^{32}PO_4$ to follow phase attachment to bacteria. They found that the viral DNA was the genetic material not the viral protein coat.

Wyatt and Cohene (1953) identified a new base hydroxymethyl cytosine in T-even phage DNA in the place of cytosine which was present in bacterial DNA. In 1953 Lowoff and Wollman discovered the temperate phages.

Jacob and Wollman (1954) made the important observation that a genetic cross between a lysogenic bacterial strain and a nonlysogenic recipient resulted in the induction of the virus after conjugation. This process was called "Zygotic induction".

In 1956 Takahasi and Frankel–Conrat demonstrated the reconstitution of TMV. The closed circular and superhelical nature of polyoma virus DNA was first elucidated by Dulbecco and Vogt (1963).

The crystallization of TMV in 1935 by Wendell Stanley brought this infectious agent into the world of the chemists. Bawden and Pirie (1936) demonstrated that crystals of TMV contained 0.5% phosphorus and 5% RNA. Kaushe and his coworkers in 1939 had taken the first electron microscopic picture of TMV and it confirmed the rod shape of the virus particles. The ultrastructure of TMV was elucidated by Flanklin, Klug, Holmes, Knight, Harris and Gierer.

Jonas.Salk in1955 succesfully developed a killed vaccine for intravenous use against poliomyelitis, and Albert Sabin in1957 developed an attenuated virus vaccine for oral use against polio virus. Single stranded DNA was discovered in _X 174 Bacteriophages by R.L. Sinsheimer (1959). The ultrastructure of T_2 bacteriophage was repored by S.Brenner, G.Strisinger, R.W. Horne and D. Crowther in the year 1959.

In 1962 Caspar and Klug described the geometric principles of icosahedral structure of TMV. In 1962 Woff, Home and Tournier formulated a unified system of classification of viruses.

The viruses infecting cyanobacteria named as cyanophages were discovered by Shaflerman and Moris (1963). Reverse transcription *i.e.* DNA synthesis from RNA, a unique phenomenon observed in viruses alone, was reported by Howard Temin and David Baltimore in 964.

In 1967 Kornberg and Co-workers made attempts for artificial synthesis of viruses. Cancer causing virus was discovered by Schidolovski, Ahmad and Gallow in 1971.

Sabin, Tam and Dress (1973) reported that the human cancer may also be caused by Herpes simplex virus. In 1976 Sanger made genetic mapping of the bacteriophage _X174. Galibert (1979) elucidated of nucleotide sequence of Hepatities B virus (HBV) genome and Sninsky (1979) cloned HBV genome in E. coli. Robert Gallow (1984) identified AIDS as a viral disease.

8.3. MORPHOLOGY OF VIRUSES

Viruses are in many shapes and sizes. The broadest distinction is enveloped and non-enveloped viruses. Enveloped viruses contain a lipid-bilayer membrane and non-enveloped viruses donot contain the lipid-bilayer membrane. Further categorization of virus structure depends on their molecular organization. The progress made in understanding the viral architecture in atomic detail now allows to study the similarities across various families of viruses.

Electron microscopy is the most useful tool to determine the general morphology of a virus particle. The isolated and purified virus particles from the tissue gives more detailed images in electronmicroscopy. The traditional thin-sections of infected cells are also used to examine the virus

particles and their localization in the cells. Quantitative methods for image analysis, originally developed for studying negatively stained particles, have been applied effectively to such images.

Cryoelectron microscopy is used to study the unstable or relatively impure preparations. Higher resolution picture can be obtained by X-ray diffraction method, if single crystals of the relevant structure can be prepared. In 1930 the simple plant virus such as Tomato bushy stunt virus (TBSV) was crystallized and studied.

Crick and Watson (1956) proposed that virus shells would be highly symmetric objects. Identical subunits with specific interactions in general produce symmetric structures.

Symmetry **:** It refers to the operations that describe
Shape **:** An object refers to the geometry of its outline.
Virion **:** Complete infectious virus particle
Envelope **:** Lipid bilayer carrying viral glycoproteins
Capsid(Coat) **:** Protein shell surrounding the nucleic acid (genome)
Nucleocapsid (Core) **:** Nucleic acid – protein assembly packaged within the Virion.
Capsomere **:** Morphological units present in the capsid.

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

8.3.1. Helical structures

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

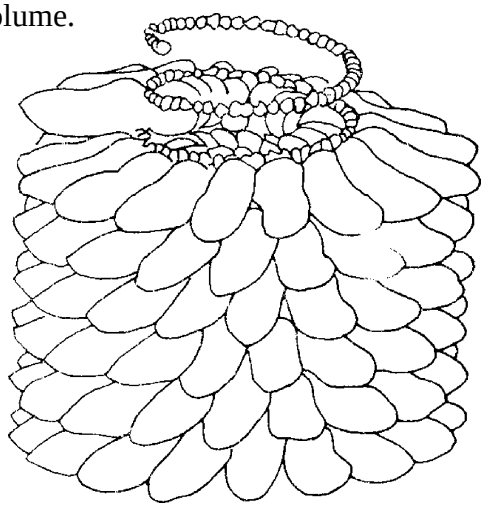

Fig. 8.1 The Helical structure of TMV

In structural point of view, the first identified and understood helical nucleocapsid virus was TMV (Fig. 8.1). The virus particle comprises a single molecule of (+) strand RNA and about 6.4 kb in

length, enclosed within a helical protein coat. The coat is built with a single protein which folds into an extended clog-shaped structure. Due to repetitive interaction the coat protein subunits forms disks and arranged like "*lock washers"*, which in turn assemble as a long, rodlike, right handed helix with 16.3 coat protein molecules per turn. Each coat protein molecule binds with three nucleotides of the RNA genome in the interior of the helix. The coat protein molecules engage in identical, equivalent interactions with one another and with the genome, allowing construction of a large, stable structure from a single protein subunit.

Icosahedral structures :

An Icosahedral structure is a solid with 20 triangular faces and 12 vertices related by two, three and five fold axes of rotational symmetry, thus the viral shows this type of structures are called Icosahedral symmetry (Fig.8.2).

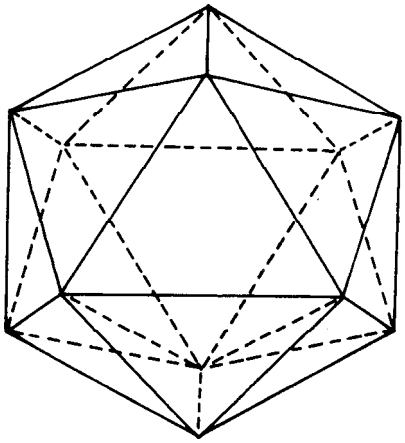

Fig. 8.2. The icosahedral structure of a virus

Icosahedral symmetry allows formation of a closed shell with the smallest number of identical subunits (60). These subunits are related to one another by two, three and five fold rotational axes that define icosahedral symmetry. All subunits interact with their neighbors in an identical or equivalent manner.

In 1962 Caspar and Klug developed a theory for the structural properties of larger particles with icosahedral symmetry. The theory was the proposition that when a capsid contains more than 60 subuntis, each subunit occupies a quasi equivalent position.

The triangulation number was also proposed by Caspar and Klug, the description of the triangular faces of large icosahedral structure in terms of its subdivision into smaller triangles termed facets. The triangulation number and quasi equivalent bonding among subunits describe the structural properties of many simple viruses with icosahedral symmetry.

Quasi-equivalent designs are exemplified by a number of animal and plant viruses such as Norwalk viruses and Tomato bushy stunt virus (TBSV). These consist of 180 genetically and chemically identical subunits that form the capsid. The shell domain (S domain) of these two viruses is about 200 residues and folded structure of the domain is again a Jelly – roll – _-barrel .

The contents of an icosahedral asymmetric unit can be described as A, B and C, which are chemically identical subunits with different conformations. The A and B conformations are nearly identical with discarded arms and similar hinge angles. C confirmation has an ordered arm and a different hinge angle from A and B.

An Icosahedrally symmetric structure is folded–up as a hexagonal net, 12 uniformly spaced six fold vertices are transformed into five fold vertices. The intervening two fold, three fold and six fold symmetry axes of the flat net are transformed either into quasi-two fold, quasi-three fold and quasi-six fold axes of the icosahedral net. A number of the viral architecture designs predicted by Caspar and Klug among various viruses of plant, vertebrates and insect viruses.

Herpes virus capsids which had T=16 structure with 12 pentamers and 150 hexamers of the major capsid protein assemble around a scaffold protein. Adenoviruses exhibit a combination of non-equivalent and quasi-equivalent interactions with T=25 icosahedral lattice. It had 12 pentons on the five fold positions and 240 hexons on the six fold positions. Double stranded RNA viruses (Blue tongue virus) exhibit both non-equivalent and quasi-equivalent interactions in separate protein shells consisting of 120 identical subunits with T=13.

8.3.3. Binal structure of viruses

Bacteriophages are the best examples of binal viruses. They exhibit a morphology of comination of two structures - the head and tail. The head is usually icosahedral and the tail is helical in symmetry. (Fig. 8.3). The head shows all types of traingulation numbers as exhibited by normal icosahedral viruses. The heads of lambda and P22 exhigit the heighest traingulation number of T = 7 with 12 pentamers and 60 hexamers. Other morphological variations observed among phages include phages with much elongated heads - myoviridae, short tails - podoviridae and long tails - siphoviridae.

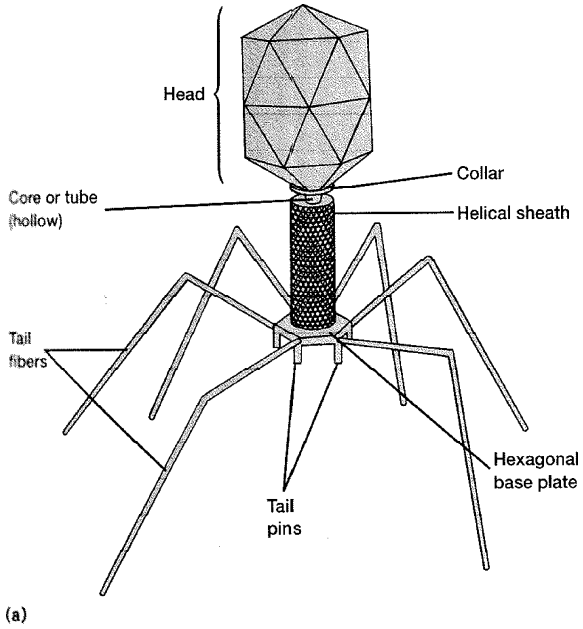

Fig. 8.3. The binal structure of T4 bacteriophage.

8.4. CHEMISTRY OF VIRUSES

Max Schlesinger (1933) partially established the chemistry of viruses through the studies on bacteriophages and concluded these were possibly nucleoproteins. The complete chemical identity was determined in TMV by Stanley (1935) through crystallization. Later, Bawden and Pirie (1937) were established that the nucleoprotein nature of TMV strains as Ribonucleic acid. The field of "Chemical era of Virology" was launched by C.A. Knight (1974).

8.4.1. Chemical Virology :

Chemical composition of various viruses was thoroughly studied for the last four decades. Chemically, viruses are nucleoproteins. Majority of the viruses consist of two components, one is outer proteinaceous covering or sheath (capsid) and the other is internal component, which is a nucleic acid (genome) – either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Some of the viruses contain third component made up of lipids and carbohydrates (envelope). The composition of proteins, lipids, and carbohydrates varies both qualitatively and quantitatively from virus to virus. The composition nucleic acids are also varies from virus to virus – bacterial and animal viruses are mostly DNA viruses whereas plant viruses are in most cases RNA viruses (table-8.1).

Table – 8.1 : Chemical composition of certain viruses

RNA Viruses

	Percent of			
Virus name	**RNA**	**Proteins**	**Lipids**	**Carbohydrate**
Simian virus	1	73	20	6
Tobacco mosaic virus	5	95	-	-
Rous Sarcoma virus	2	62	35	1
Influenza virus	1	74	19	6
Fowl plaque	2	69	26	2
Tomato spotted wilt virus	5	71	19	5
Potato X virus	6	94	-	-
Tomato bushy stunt virus	17	83	-	-
Tobacco necrosis virus	19	81	-	-
Cucumber mosaic	18	82	-	-
Reovirus	21	79	-	-
Poliomyelitis	26	74	-	-
Coliphage	F2	30	70	--

DNA Viruses :

	Percent of			
Virus name	**DNA**	**Proteins**	**Lipids**	**Carbohydrate**
Vaccinia virus	5	88	5	2
Herpes simplex virus	9	67	22	2
Adenoviruses	13	87	-	-
Coliphage M13	12	88	-	-
Polyoma virus	16	84	-	-
Cauliflower mosaic virus	16	84	-	-
Shope papilloma virus	18	82	-	-
Coliphage ϕX174	26	74	-	-

8.4.2. Viral Nucleic acids

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

Genomic diversity is one of the most important characteristic features in viruses. Various types of viral nucleic acids were recorded by different scientists. The straight chain, cyclic, super coiled nucleic acid structures of viruses were reported. Single stranded and double stranded nucleic acid types were identified. Segmented and non-segmented genomes of virus nucleic acid are the special feature for viruses. Recently the sense of the viruses was also noticed on the bases of polarity in single stranded genome (RNA) viruses. Characteristics of viral nucleic acids are given in the table 8.2.

Table – 8.2. Characteristics of some viral nucleic acids

Virus	Nucleic acid type (genome)	Stranded ness	Polarity	Structure	Segmented/ Nonsegmented
Picorna virus	RNA	Single	Positive	Linear	Nonsegmented
Rhabdovirus	RNA	Single	Negative	Linear	Nonsegmented
Orthomyxovirus	RNA	Single	Negative	Linear	Segmented
Reovirus	RNA	Double	-	Linear	Segmented
Herpes virus	DNA	Double	-	Linear	Nonsegmented
Parvovirus	DNA	Single	-	Linear	Nonsegmented
Hepadna virus	DNA	Partially double	-	Circular	Gapped

Primary structure of viral nucleic acids relates to the proportion and arrangement of various nucleotides in a specific manner. The primary structure of viral nucleic acids can be determined into two ways – one is to determine the proportions of purines and pyrimidines and the second one is to determine the sequence arrangement of the nucleotides. The molar the variation in the base ratios of RNA and DNA of different viruses. It clearly reflects in AT/GC ratio of DNA viruses and AU/GC ratio of RNA viruses (table – 8.3).

Table – 8.3 : Nucleic acid base ratios of viruses (moles percent)

Virus	Nucleic acid	A	G	C	T	V	AT/GC	AU/GC
Herpes simplex	DNA	16	34	34	16	-	0.47	-
Adeno virus	DNA	21	29	29	21	-	0.73	-
Coliphage _X174	DNA	24	25	19	32	-	1.27	-
SV 40	DNA	26	24	24	26	-	1.04	-
Coliphage T_4	DNA	33	17	17	33	-	1.92	-
Iridescent virus	DNA	34	16	16	34	-	2.12	-
Tomato bushy stunt virus	RNA	25	28	21	-	26	-	1.04
Tobacco mosaic virus	RNA	28	24	22	-	26	-	1.15
Influenza	RNA	23	20	24	-	33	-	0.8
Foot and RNA mouth disease	RNA	26	24	28	-	22	-	0.93

In double stranded nucleic acid molecules there is direct one to one correspondence between the purine and the pyrimidine bases. Base ratios are almost identical in similar viruses. Eg : Papilloma virus, Polyoma virus and SV40 virus. Some times dissimilar viruses may also have nearer identical base ratios. Eg. Coliphage, T_4 and Iridescent virus. Higher proportion of uracil was estimated than other bases in influenza virus. The base ratio analyses donot allow a close insight into the primary structure of nucleic acid molecules. The nucleotide sequence analysis is the better approach to know about the arrangement of nucleotides in viral nucleic acids. The chain of nucleic acid was cleaved by using different endo, exo and restriction nucleases at specific sites and analysed on the electrophoresis. The first sequence of coliphage MS_2 viral genome was reported by Fiers et al., in 1976 and later _X174 DNA sequence was reported by Sanger et al., in 1977. Coding capacity and some of the other features were revealed by Sanger and his group, these are the capacity of the same stretch of DNA to code for two proteins which are translated in different reading frames, position of certain genes are within the region of another gene and presence of sequences responsible for promotion and termination of gene functions. Two methods were used to study the viral nucleotide sequence analysis – nearest neighbour frequency analysis and homology analysis. The earlier method provides information on the frequency with which various nucleotide pairs or "*doublets*" occur in a particular nucleic acids or poly nucleotide molecule. The later method gives the information about nucleic acid sequences on the

bases of hybridization of nucleic acid molecules. In recent years restriction fragment length analysis an another method is being used to study the more accurate homology analysis between specific viruses. The secondary structure of viral nucleic acid is useful to study the three dimensional configuration and biological function.

8.4.3. Viral proteins

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

The primary structure of a viral protein is its basic structure. The secondary, tertiary and quaternary structures of viral proteins are essentially dependent upon the primary features, which in their turn are derived from the composition of the amino acids and their sequential arrangements. The amino acid composition data reveals that viral coat proteins are constituted of the common protein amino acids. The amino acid composition does not reveal the protein structure. The end group analysis is useful to study the amino group (NH_2) and carboxyl terminal (C) groups of viruses. The enzymatic analysis is being used to achieve this parameter in viruses. Carboxyl peptidases are used as a selective enzymes to cleave the C-terminal amino acid residues from protein units. Harris and Knight (1955) reported that 2320 threonine residues were liberated per mole of TMV when treated with carboxyl peptidase.

Amino acid analysis is being used to understand the primary structure of viral proteins. In this analysis the cleavage of a large polypeptide chain into smaller fragments, determination of sequence of these fragments and determination of the sequence of amino acids in the individual fragments are performed. In 1959 Woody and Knight successfully analysed the TMV coat protein by tryptic digestion method. Other TMV proteins were analysed by using various proteolytic enzymes like chemotrypsin, pepsin and subtlisin. The secondary structures and higher configuration of the viral proteins are being studied by using X-ray crystallography analyses. This method is useful to study the spatial arrangement of various subunits of the coat proteins and their alignment with central nucleic acid.

8.4.4. Viral carbohydrates

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

been revealed that the protein and carbohydrate moieties in glycoproteins are linked by formation of bonds between the carbohydrate chain and aspargine, serine, threonine residues of proteins.

8.4.5. Viral lipids

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

Some of the viruses consist of special components in their structure, These are polyamines (T_2, T_4 phages). The polyamines are putrescine, spermidine and spermine. Polyamines are also reported in Herpes virus and Influenza virus. Traces of polyamines are identified in Turnip yellow mosaic virus and Broad bean mottle virus. Inorganic divalent metal ions were also found in certain viruses. Eg. Ca^{+2}, Mg^{+2} (TMV, Southern Bean Mosaic Virus). Some plant viruses such as Tobacco streak virus (TSV) have a zinc finger binding domain that specifically binds an atom of zinc in a protein involved in nucleic acid binding.

8.5. SUMMARY

The discovery of viruses started in the studies on tobacco mosaic disease. The causal agent of the disease can pass through the bacterial filters. Hence it was first described as “contagium vivum fluidum” and general term virus (meaning poison in Latin) was applied to causal agent of tobacco mosaic disease and causal agents of other diseases that can pass through bacterial filters. Stanley in 1935 crystallised the virus and its chemical nature was determined by a number of workers. The first electron microscopic picture of TMV was taken by Kaushe and his coworkers. Structurally the viruses are three types viz. helical, icosahedral and banal. Details of the structures are described. Chemically viruses are made of nucleic acids, proteins, carbohydrates and lipids.

8.6. MODEL QUESTIONS

Essay type questions

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

Short answer type questions

5. Discovery of viruses
6. Tobacco mosaic virus
7. Bacteriophages

8. viral nucleic acids
9. Helical viruses
10. Icosahedral symmetry

8.7. REFERENCE BOOKS

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LESSON: 9

CLASSIFICATION OF VIRUSES AND SUBVIRAL PARTICLES

Objective: To know about the classification of viruses and about subviral particles

Contents:

9.1. Introduction

Humans have an innate desire to name and to classify. Virologists are interested to name and classify the viruses in the early periods of history. Virus classification is an arrangement of viruses showing similar properties into groups and it does have certain properties - a) It gives a structured arrangement of the organism, b) It helps with communication between the virologists, c) It enables properties of new viruses to be predicted and d) It could reveal possible evolutionary relationships. Earlier workers gave a virus name derived from the host in which it was found together with the most conspicuous disease symptoms, for example, tobacco mosaic virus. In early 1930 three important facts began to be recognized a) Viruses can exist as different strains, which may cause very different symptoms in the same host, b) different viruses may cause very similar symptoms on the same host and c) some diseases may be caused by a mixture of two unrelated viruses. Johnson in 1927 stressed the need for using some criteria other than disease symptoms and host for identifying viruses. He suggested that a virus should be named by adding the word "*virus*" and a number to the common name for the host in which it was first found, Eg. tobacco virus 1 for TMV. In 1935 Johnson and Higgan compiled a descriptive key based on five characters – modes of transmission, natural or differential hosts, *invitro longevity*, thermal death point and distinctive or specific symptoms. In this pattern about 50 viruses were identified and placed in different groups. Smith (1937) proposed a scheme in which the known viruses or virus diseases were divided into 51 groups. Viruses are named and grouped according to the generic name of the host in which they were first found. Eg. TMV was named as Nicotiana virus group. Smith was the first to catalogue the known viruses into groups. In 1932 Holmes published a classification based primarily on host reactions and methods of transmission and he used the Latin binomial-trinomial system of naming. Eg. TMV was named as *Marmor tabaci*, Holmes. He classified the viruses on the bases of diseases rather than the viruses. Between 1940 and 1960 various schemes were proposed for classification of viruses. In 1962 Lwoff, Horne and Tournier advanced a comprehensive scheme for the classification of all viruses under the classical Linnaeus hierarchial system. Virus taxonomy serves an important practical purpose as well, in that the identification of a limited number of biologic characteristics, such as virion morphology, genome

structure, or antigenic properties, quickly provides a focus for identification of an unknown agent for the clinician or epidemiologist and can significantly influence further investigation into treatment or prevention of a virus disease.

9.2. CLASSIFICATION OF VIRUSES

9.2.1. Universal system of virus taxonomy

Gibbs, Harrison, Watson and Wildy (1966) proposed multiple criteria based system and categorized the viruses as "*Cryptograms*". These efforts stimulated to develop the "Universal taxonomy system". International Committee on Taxonomy of viruses (ICTV) was established in 1966 and functions are 1. Classification and naming of viruses, and 2.Coordinating the activities of study groups in the implementation of taxonomic rules. In 1970 the ICTV developed the universal system of virus taxonomy at Mexico city meeting. The significant proportion of all existing viruses of humans, domestic animals and economically important plants were entered into this taxonomic system. The present universal system of virus taxonomy is set arbitrarily at hierarchical levels of order, family, subfamily, genus, species and lower hierarchical levels, such as subspecies, strain and variant.

ICTV has adopted nomenclature for viruses. It specifies the suffixes for the various taxa and rules for written descriptions of viruses. Names for genera, subfamilies, families and orders must be single words ending with suffixes – virus, -virinae, - viridae and –virales respectively.

Eg : Order – *Mononegavirales*
Family – *Paramyxo viridae*
Subfamily – *Pneumovirinae*
Genes – *Pneumovirus*
Species – *Human respiratory syncytial virus*

9.2.1.1. Virus orders: It represents groupings of families of viruses that share common characteristics and are distinct from other orders and families Eg. *Mononegavirales*.

9.2.1.2. Virus families and subfamilies: Virus families represent groupings of genera of viruses that share common characteristics and are distinct from the member viruses of families. Eg. *Paramyxoviridae*. Subfamilies represent the apparent intrinsic complexity of the relationships among member viruses. Eg. *Pneumovirinae*

9.2.1.3. Virus Genera: It represent groupings of species of viruses that share common characteristics and are distinct from the member viruses of other genera. Eg. *Pneumovirus*

9.2.1.4. Virus Species: The species taxon has always been regarded as the most important hierarchical level in classification. "A virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche". Eg. Human respiratory syncytial virus.

9.2.1.5. **Tentative species:** The taxonomic status of the species can't currently be unambiguously determined.

9.2.1.6. Type species: The species used to define the taxon, has been identified for each genus. All taxonomic levels need not be used for a given grouping of viruses, whereas most species are grouped into genera and genera into families, all families not contain subfamilies and only a few families have been grouped into orders. The family is the highest consistently used taxonomic grouping and have distinct virion morphology, genome structure and replication strategy.

9.2.2. Criteria used in virus classification (taxonomy)

9.2.2.1. Virion properties

A. Morphological properties of virions

1. Size
2. Shape
3. Presence or absence of an envelope or peplomers
4. Capsomeric symmetry and structure

B. Physical properties of virions

1. Molecular mass
2. Buoyant density
3. Sedimentation coefficient
4. pH stability
5. Thermal stability
6. Cation (Mg^{2+}, Mn^{2+}, Ca^{2+}) stability
7. Solvent stability
8. Detergent stability
9. Radiation stability

C. Properties of the genome

1. Type of nucleic acid, DNA or RNA
2. Strandedness : single-stranded or double-stranded
3. Linear or circular
4. Sense: positive, negative or ambisense
5. Number of segments
6. Size of genome or genome segments
7. Presence or absence and type of 5’ terminal cap
8. Presence or absence of 5’ terminal covalently-linked polypeptide
9. Presence or absence of 3’ terminal poly(A) tract (or other specific tract)
10. Nucleotide sequence comparisons

D. Properties of proteins

1. Number
2. Size
3. Functional activities (especially Virion transcriptase, Virion reverse transcriptase, Virion hemagglutinin, Virion neuraminidase, Virion fusion protein)
4. Amino acid sequence comparisons

E. Lipids
1. Presence or absence
2. Nature

F. Carbohydrates
1. Presence or absence
2. Nature

9.2.2.2. Genome organization and replication
1. Genome organization
2. Strategy of replication of nucleic acid
3. Characteristics of transcription
4. Characteristics of translation and post-translational processing
5. Sites of accumulation of Virion proteins, site of assembly, site of maturation and release
6. Cytopathology, inclusion body formation

9.2.2.3. Antigenic properties
1. Serological relationships
2. Mapping epitopes

9.2.2.4. Biological properties
1. Host range, natural and experimental
2. Pathogenicity, association with disease
3. Tissue tropisms, pathology, histopathology
4. Mode of transmission in nature
5. Vector relationships
6. Geographic distribution

The criteria used to specify the order of presentation are nature of the viral genome, strandedness of the viral genome, polarity of the viral genome and reverse transcription. In addition, other categories have been created for subviral agents, those are viroids, satellites and prions.

A separate category exists for unassigned viruses. In order of presentation of viruses nine groupings are specified – dsDNA viruses, ssDNA viruses, dsRNA viruses, negative sense ssRNA viruses, positive sense ssRNA viruses, DNA and RNA reverse transcribing viruses, subviral agents – viroids, satellites, prions and unassigned viruses.

ICTV has established the universal database named as ICTVdB and it is accessible on the world wild web at http://life.anu.edu.au/viruses/welcome.htm.

9.2.3. Classification of viruses infecting vertebrates, invertebrates, fungal, bacterial and plants

The classification of viruses approved by International Committee on Taxonomy of Viruses (ICTV) is given in the table 9.1 and the drawings of the viruses belonging to important families of animal viruses, plant viruses and bacteriophages are shown in the figure 9.1a,b,c.

Table – 9.1. Classification of viruses (ICTV system)

Order	Family	Subfamily	Genus	Host category : type species or example
Double-stranded DNA viruses				
	Myoviridae	"Unnamed, the T4-like phages"		Bacteria : coliphage T4
	Siphoviridae	"Unnamed, the λ-like phages"		Bacteria : coliphage λ
	Podoviridae	"Unnamed, the T7-like phages"		Bacteria : coliphage T7
	Tectiviridae		Tectivirus	Bacteria : enterobacteria phage PRD 1
	Corticoviridae		Corticovirus	Bacteira : alteromonas phage PM2
	Plasmaviridae		Plasmavirus	Mycoplasma : Acholeplasma phage L2
	Lipothrixviridae		Lipothrixvirus	Archaebacteria : Thermoproteus phage 1
	Poxviridae	Chordopoxvirinae		
			Orthopoxvirus	Vertebrates : Vaccinia virus
			Parapoxvirus	Vertebrates : orf virus
			Avipoxvirus	Vertebrates : fowlpox virus
			Capripoxvirus	Vertebrates : sheeppox virus
			Leporipoxvirus	Vertebrates : myxoma virus
			Suipoxvirus	Vertebrates : swinepox virus
			Molluscipoxvirus	Vertebrates : molluscum contagiosum virus
			Yatapoxvirus	Vertebrates : Yaba monkeypox virus
		Entomopoxvirinae		
			Entomopoxvirus A	Invertebrates : Melolontha melolontha virus
			Entomopoxvirus B	Invertebrates : Amsacta moorei virus
			Entomopoxvirus C	Invertebrates : Chironomus luridus virus
	"Unnamed, African swine fever-like viruses"	"Unnamed, African swine fever-like viruses"		Vertebrates". African swine fever virus
	Iridoviridae		Iridovirus	Invertebrates . Chilo iridescent virus
			Chloriridovirus	Invertebrates . mosquito iridescent virus
			Ranavirus	Vertebrates : frog virus 3
			Lymphocystivirus	Vertebrates : flounder iridescent virus
			"Unnamed, goldfish virus 1-like viruses"	Vertebrates : goldfish virus 1
	Phycodnaviridae		Phycodnavirus	Green algae : Paramecium bursaria Chlorella virus 1
	Baculoviridae		Nucleopolyhedrovirus	Invertebrates : Autographa californica nuclear polyhedrosis virus
			Granulovirus	Invertebrates : Plodia interpunctella virus

	Herpesviridae	Alphaherpesvirinae	
		Simplexvirus	Vertebrates : human herpesvirus 1 (herpes simplex virus 1)
		Varicellovirus	Vertebrates : human herpesvirus 3 (varicella-zoster virus)
		Betaherpesvirinae Cytomegalovirus	Vertebrates : human herpesvirus 5 (human cytomegalovirus)
		Muromegalovirus	Vertebrates : mouse cytomegalovirus 1
		Roseolovirus	Vertebrates : human herpesvirus 6/3
		Gammaherpesvirinae	
		Lymphocryptovirus	Vertebrates : human herpesvirus 4 (Epstein Barr virus)
		Rhadinovirus	Vertebrates : ateline herpesvirus 2
	Adenoviridae	Mastadenovirus	Vertebrates : human adenovirus 2
		Aviadenovirus	Vertebrates : fowl adenovirus 1
		Rhizidovirus	Fungi : Rhizidiomyces virus
	Papovaviridae	Papillomavirus	Vertebrates : cottontail rabbit papillomavirus (Shope)
		Polyomavirus	Vertebrates : polyomavirus
	Polydnaviridae	Ichnovirus	Invertebrates : Campoletis sonorensis virus
		Bracovirus	Invertebrates : Cotesia melanoscela virus
Single-stranded DNA viruses			
	Inoviridae	Inovirus	Bacteria : coliphage fd
		Plectrovirus	Mycoplasma : Acholeplasma phage L51
	Microviridae	Microvirus	Bacteria : coliphage FX174
		Spiromicrovirus	Spiroplasma : Spiroplasma phage SpV4
		Bdellomicrovirus	Bacteria:Bdellovibrio phage MAC1
		Chlamydiamicrovirus	Chlamydia: Chlamydia phage Chp1
	Geminiviridae	"Unnamed, Subgroup I Viruses"	Plants : maize streak virus
		"Unnamed, Subgroup II viruses"	Plants : beet curly top virus
		"Unnamed, Subgroup III viruses"	Plants : tomato golden mosaic virus
	Circoviridae	Circovirus	Vertebrate : Chicken anemiavirus
	Parvoviridae	Chordoparvovirinae Parvovirus	Vertebrates: minute virus of mice
		Dependovirus	Vertebrates: adeno-associated virus 2
		Erythrovirus	Vertebrates: human parvovirus B 19
		Entomoparvovirinae Densovirus	Invertebrates: Junonia coenia virus
		Iteravirus	Invertebrates: Bombyx mori virus
		Contravirus	Invertebrates: Aedes aegypti virus
DNA and RNA reverse transcribing viruses			
		Badnavirus	Plants: commelina yellow mottle virus
		Caulimovirus	Plant: cauliflower mosaic virus
	Hepadnaviridae	Orthohepadnavirus	Vertebrates: hepatitis B virus
		Avihepadnavirus	Vertebrates: duck hepatitis virus
	Retroviridae	"Unnamed, mammalian type B retroviruses"	Vertebrates : mouse mammary tumor virus

			"Unnamed, mammalian type C retroviruses"	Vertebrates: murine leukemia virus
			"Unnamed, avain type C retroviruses"	Vertebrates: avain leucosis virus
			Unnamed mammalian type D retroviruses"	Vertebrates: Mason-Pfizer monkey virus
			"Unnamed, HTLV/BLV viruses"	Vertebrates: bovine leukemia virus
			Lentivirus	Vertebrates: human immunodeficiency virus1
			Spumavirus	Vertebrates: human foamy virus 1
Double-stranded RNA viruses				
	Cystoviridae		Cystovirus	Bacteria: Pseudomonas phage 16
	Reoviridae		Orthoreovirus	Vertebrates: reovirus 3
			Orbivirus	Vertebrates: bluetongue virus 1
			Coltivirus	Vertebrates: Colorado tick fever virus
			Rotavirus	Vertebrates: simian rotavirus SA11
			Aquareovirus	Vertebrates: golden shiner virus
			Cypovirus	Invertebrates: Bombyx mori cytoplasmic polyhedrosis virus 1
			Phytoreovirus	Plants: wound tumor virus
			Fijivirus	Plants: Fiji disease virus
			Oryzavirus	Plants: rice ragged stunt virus
	Birnaviridae		Aquabirnavirus	Vertebrates: Infectious pancreatic necrosis virus
			Avibirnavirus	Vertebrates: Infectious bursal disease virus
			Entomobirnavirus	Invertebrates: Drosophila X virus
	Totiviridae		Totivirus	Fungi: Saccharomyces cerevisiae virus L-A
			Giardiavirus	Protozoa: Giardia lamblia virus
			Leishmaniavirus	Protozoa: Leishmania brasiliensis virus 1-1
	Partitiviridae		Partitivirus	Fungi: Gaeumannomyces graminis virus 019/6A
			Chrysovirus	Fungi: Penicillium chrysogenum virus
			Alphacryptovirus	Plants: White clover cryptic virus I
			Betacryptovirus	Plants: White clover cryptic virus II
			Hypovirus	Fungi: Cryphonectria parasitica virus 1-EP713
Negative-sense, single-stranded RNA viruses				
Mono negavirales				
	Paramyxoviridae	Paramyxovirinae	Paramyxovirus	Vertebrates: parainfluenza virus 1
			Morbillivirus	Vertebrates: measles virus
			Rubulavirus	Vertebrates: mumps virus
		Pneumovirinae	Pneumovirus	Vertebrates: respiratory syncytial virus
	Rhabdoviridae		Lyssavirus	Vertebrates: rabies virus
			Vesiculovirus	Vertebrates: vesicular stomatitis Indiana virus 1
			Ephemerovirus	Vertebrates: bovine ephemeral fever virus
			Cytorhabdovirus	Plants: lettuce necrotic yellows virus
			Nucleorhabdovirus	Plants: potato yellow dwarf virus

Filoviridae	Filovirus	Vertebrates: Marburg virus
Orthomyxoviridae	Influenzavirus A, B	Vertebrates: Influenza A virus A/PR/8/34(H1N1) Vertebrates:
	Influenzavirus C	Influenza C virus
	"Unnamed, Thogoto-like viruses"	Vertebrates: Thogoto virus
Bunyaviridae	Bunyavirus	Vertebrates: Bunyamwera virus
	Nairovirus	Vertebrates: Nairobi sheep disease virus
	Phlebovirus	Vertebrates: sandfly fever Sicilian virus
	Hantavirus	Vertebrates: Hantaan virus
	Tospovirus	Plants: tomato spotted wilt virus
Arenaviridae	Arenavirus	Vertebrates: lymphocytic choriomeningitis virus
	Tenuivirus	Plants: rice stripe virus
Positive-sense, single-stranded RNA viruses		
Leviviridae	Levivirus	Bacteria: coliphage MS2
	Allolevivirus	Bacteria: coliphage Q.
Picornaviridae	Enterovirus	Vertebrates: poliovirus 1
	Aphthovirus	Vertebrates: foot-and-mouth disease virus O
	Cardiovirus	Vertebrates: encephalomyocarditis virus
	Hepatovirus	Vertebrates: hepatitis A virus
	Rhinovirus	Vertebrates: human rhinovirus 1A
Sequiviridae	Sequivirus	Plants: parsnip yellow fleck virus
	Waikavirus	Plants: rice tungro spherical virus
Comoviridae	Comovirus	Plants: cowpea mosaic virus
	Nepovirus	Plants: tobacco ringspot virus
	Fabavirus	Plants: broad bean wilt virus 1
Potyviridae	Potyvirus	Plants: potato virus Y
	Rymovirus	Plants: barley yellow mosaic virus
	Bymovirus	Plants: ryegrass mosaic virus
Caliciviridae	Calicivirus	Vertebrates: vesicular exanthema of swine virus
Astroviridae	Astrovirus	Vertebrates: human astrovirus 1
Nodaviridae	Nodavirus	Invertebrates: Nodamura virus
Tetraviridae	"Unnamed, Nudaurelia *B capensis*-like viruses"	Invertebrates: Nudaurelia *B capensis* virus
	"Unnamed, Nudaurelia ω capensis like viruses"	Invertebrates: Nudaurelia ω capensis virus
	Sobemovirus	Plants: southern bean mosaic virus
	Luteovirus	Plants: barley yellow dwarf virus
	Enamovirus	Plants: Pea enation mosaic virus
	Umbravirus	Plants: carrot mottle virus
Tombusviridae	Tombusvirus	Plants: tomato bushy stunt virus
	Carmovirus	Plants: carnation mottle virus
	Necrovirus	Plants: tobacco necrosis virus
	Dianthovirus	Plants: carnation ringspot virus
	Machlomovirus	Plants: maize chlorotic mottle virus
Coronaviridae	Coronavirus	Vertebrates: avian infectious bronchitis virus
	Torovirus	Vertebrates: Berne virus
Flaviviridae	Flavivirus	Vertebrates: yellow fever virus

		Pestivirus	Vertebrates: bovine virus diarrhea virus
		"Unnamed, hepatitis C-like viruses"	Vertebrates: hepatitis C virus
	Togaviridae	Alphavirus	Vertebrates: Sindbis virus
		Rubivirus	Vertebrates: rubella virus
		Tobamovirus	Plants: tobacco mosaic virus
		Tobravirus	Plants: tobacco rattle virus
		Hordeivirus	Plants: barley stripe mosaic virus
		Furovirus	Plants: soil-borne wheat mosaic virus
	Bromoviridae	Bromovirus	Plants: brome mosaic virus
		Cucumovirus	Plants: cucumber mosaic virus
		Ilarvirus	Plants: tobacco streak virus
		Alfamovirus	Plants: alfalfa mosaic virus
		Idaeovirus	Plants: rasberry bushy dwarf virus
		Closterovirus	Plants: beet yellows virus
		Capillovirus	Plants: apple stem grooving virus
		Trichovirus	Plants: apple chlorotid leaf spot virus
		Tymovirus	Plants: turnip yellow mosaic virus
		Carlavirus	Plants: carnation latent virus
		Potexvirus	Plants: potato X virus
	Barnaviridae	Barnavirus	Fungi: mushroom bacilliform virus
		Marafivirus	Plants: maize rayado fino virus
Subviral agents: satellites, viroids, and prions			
Taxon: undefined, unnamed		Satellites	Plants: cucumber mosaic virus satellite
Genus		Deltavirus	Vertebrates: hepatitis delta virus
Taxon: undefined, unnamed		Viroids	Plants: potato spindle tuber viroid
Taxon: undefined, unnamed		Prions	Vertebrates: scrapie agent

[a] Quotation marks are used to denote that the taxon has not been named or that the taxon name has not been approved by the ICTV.

[b] Vertebrate arthropod-borne viruses are listed according to their vertebrate hosts.

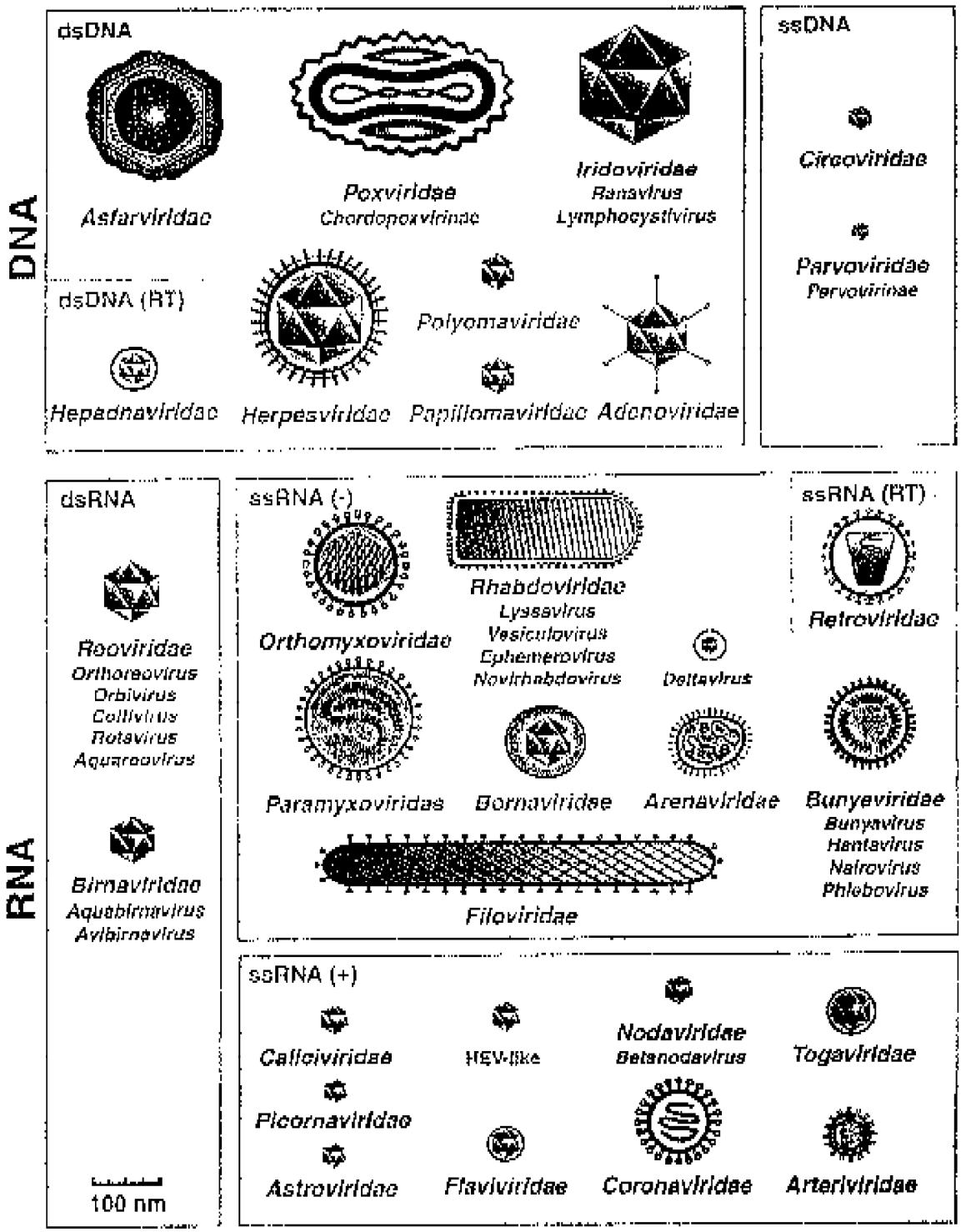

Fig. 9.1 a) Families and Genera of Viruses Infecting Vertebrates

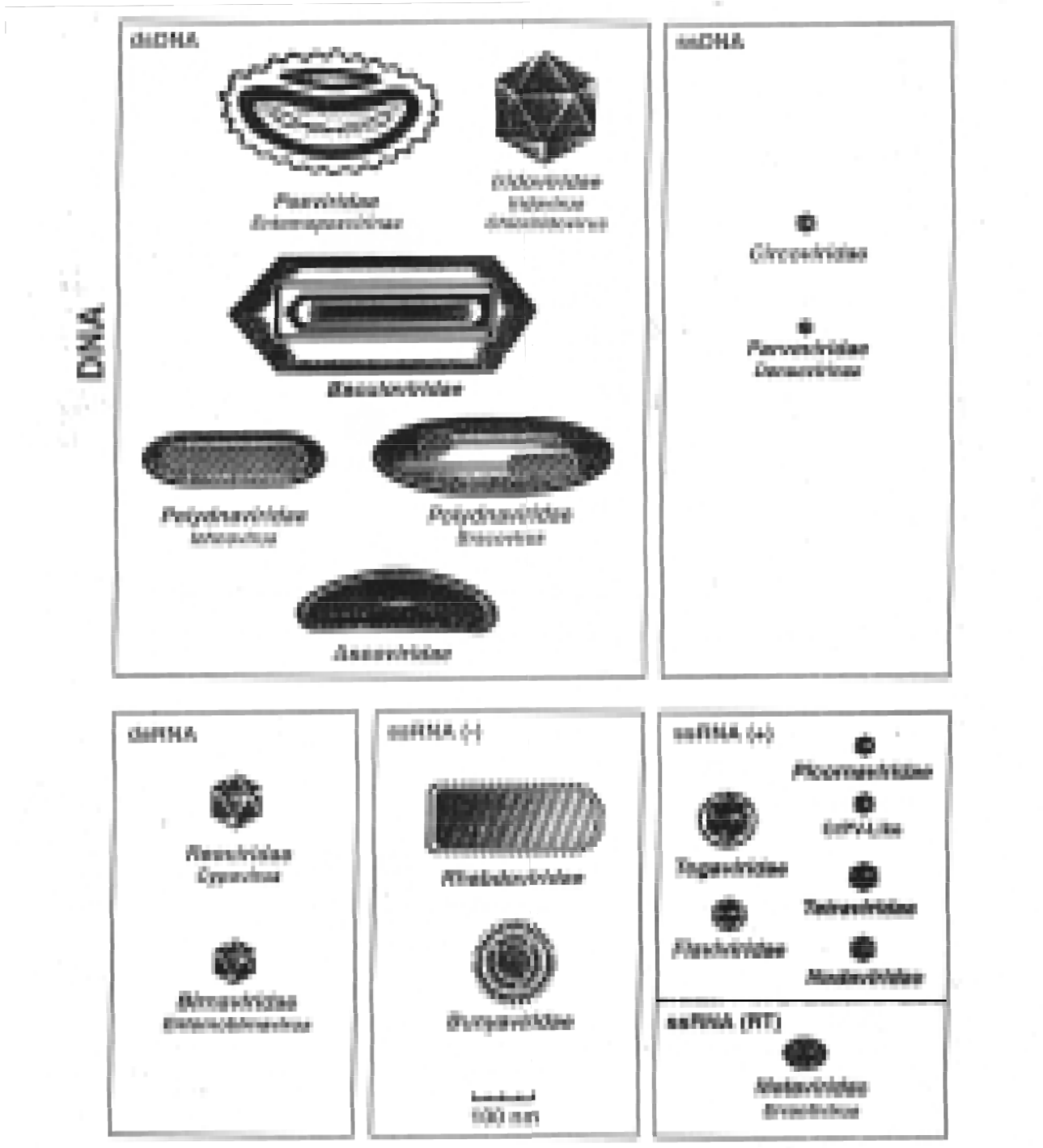

Fig. 9.1 b) Families and Genera of Viruses Infecting Invertebrates

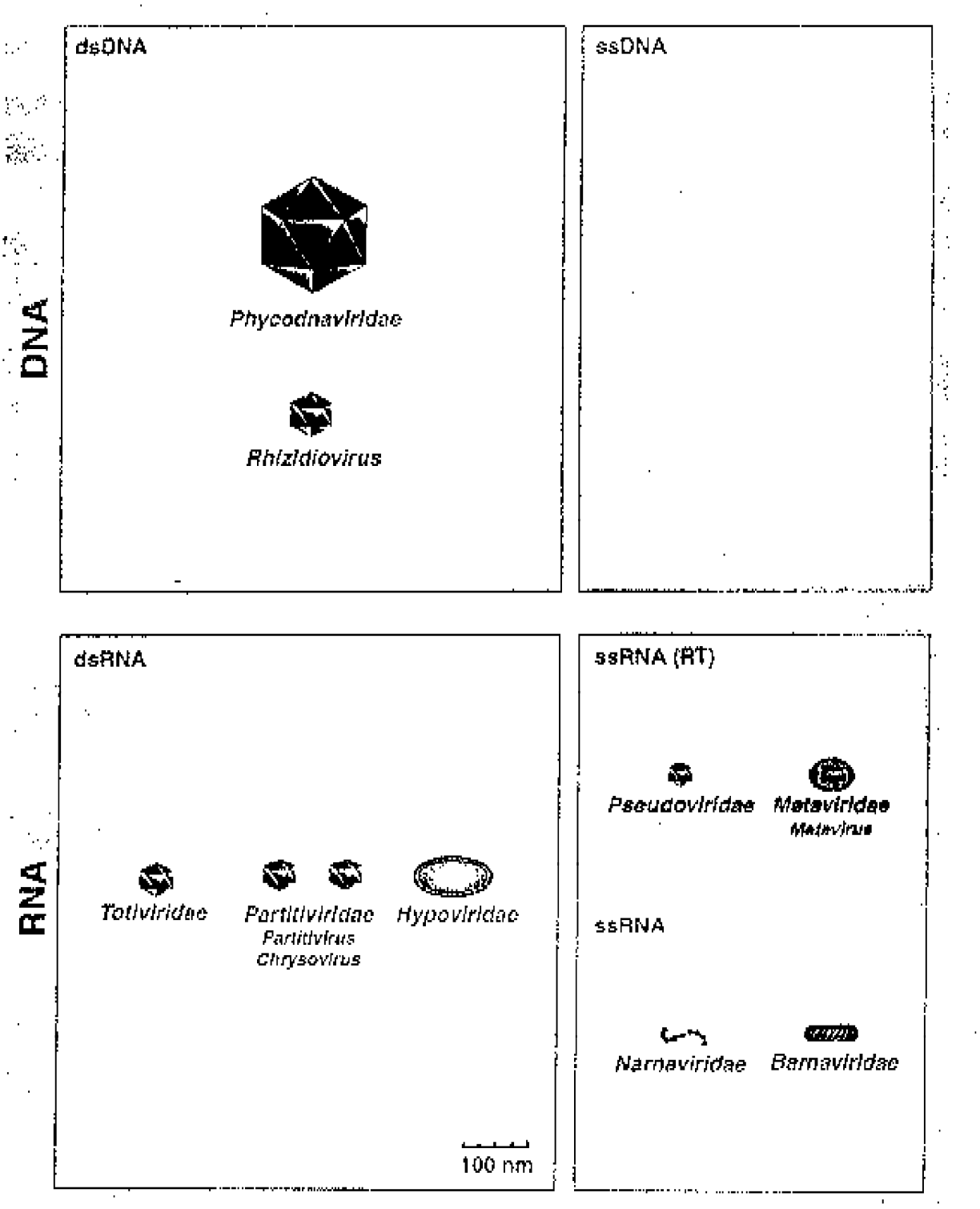

Fig. 9.1 c) Families of Viruses Infecting Algae, Fungi, Yeast And Protozoa

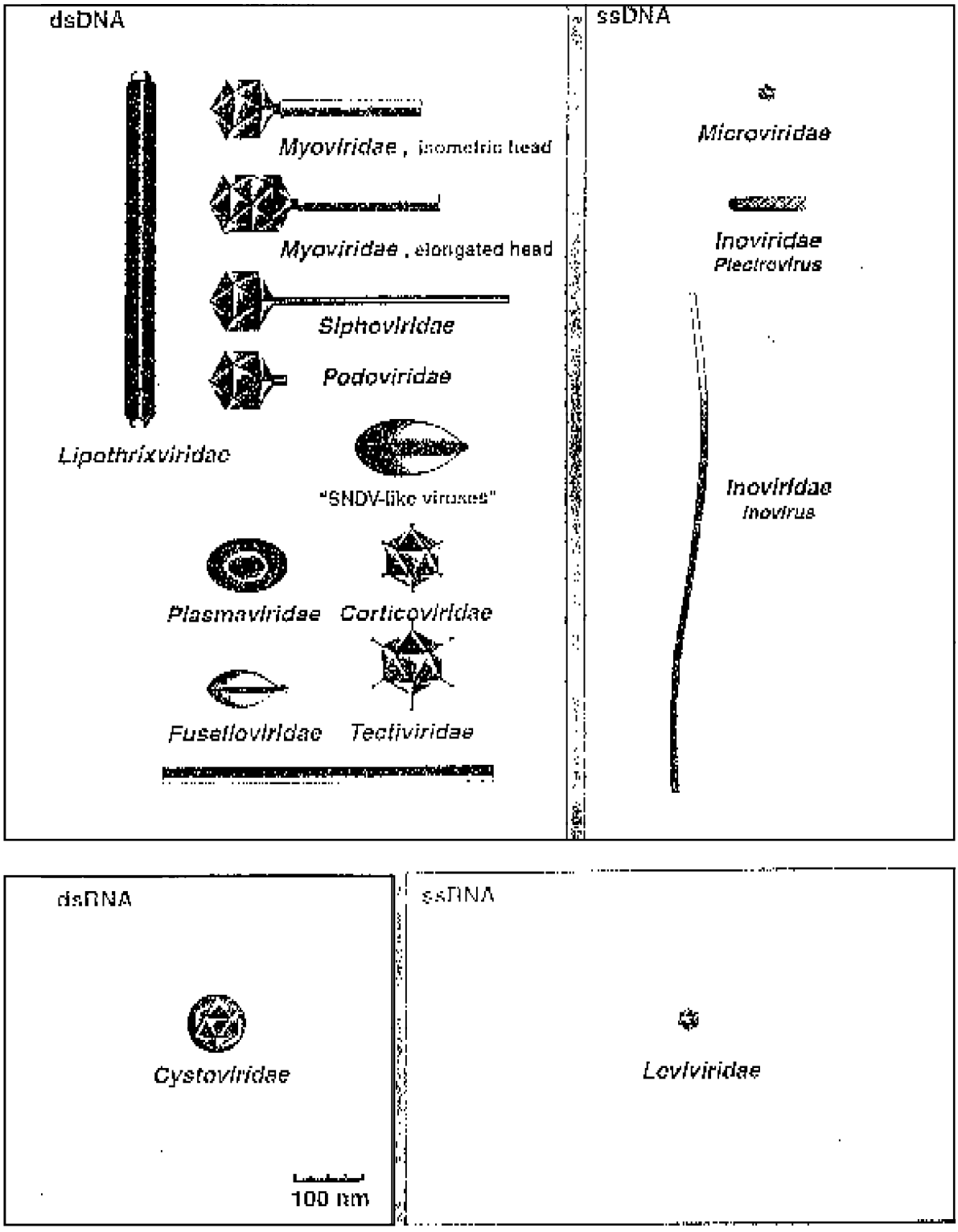

Fig. 9.1 d) Families and Genera of Viruses Infecting Bacteria

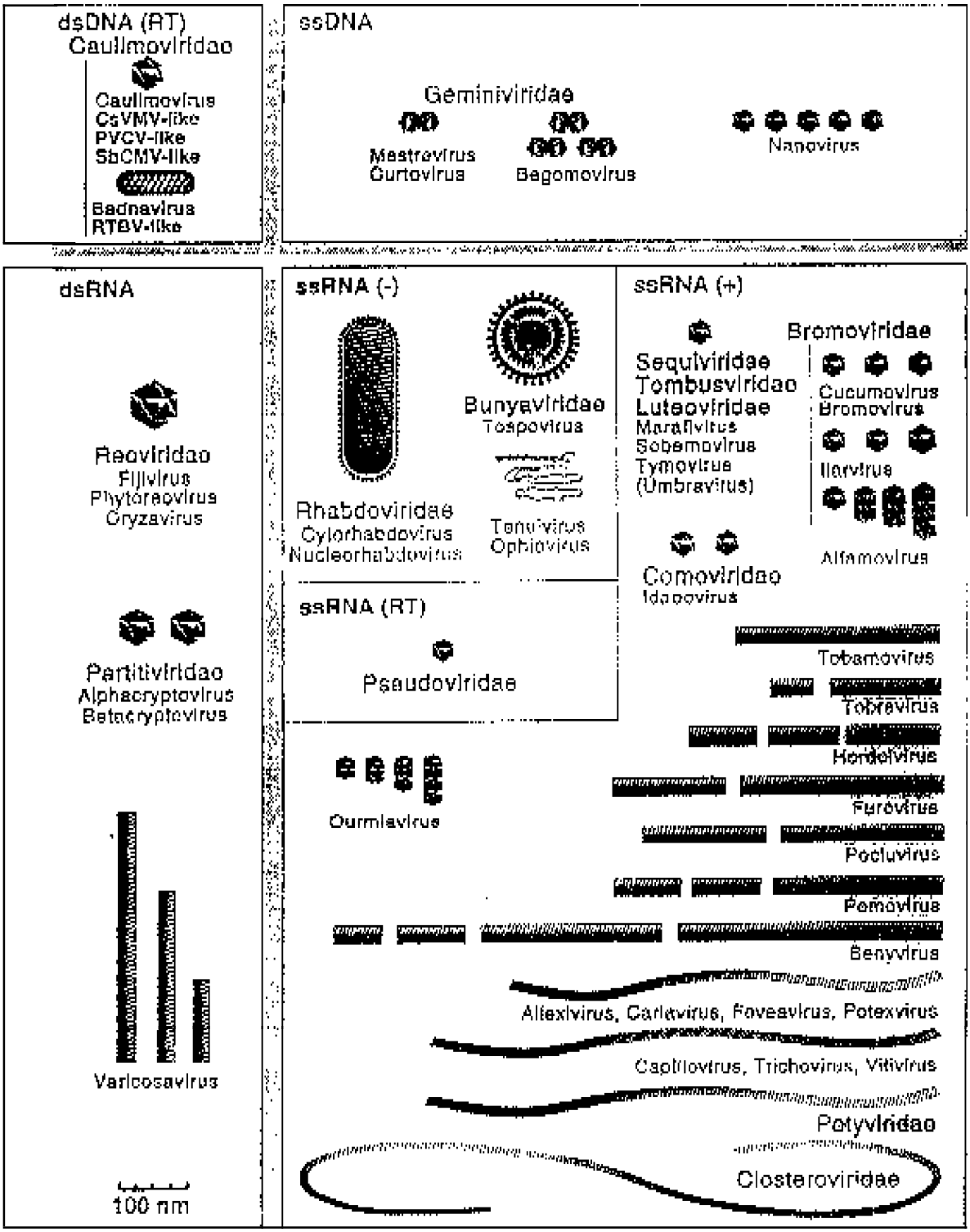

Fig. 9.1 e) Families and Genera of Viruses infecting Plants

9.3. Subviral particles

Viroids, satellite viruses, satellite RNA, defective interfering particles, spongiform encephalopathies – prions are included under this subviral particles. These subviral particles are included under unconventional viruses group of undefined, unnamed taxons.

9.3.1. VIROIDS :

Viroids are small, circular, single-stranded, infectious RNAs (246-370 nt) which are never encapsidated and have no helper virus (and so are distinguished from circular RNA satellites), and many are serious plant pathogens. They have extensive internal base pairing, so that the RNAs resemble double-stranded rods rather than circles. There is no ORF in either sense and hence they encode no protein and are not classified under the Baltimore scheme. Some viroids are replicated in the nucleus by the host's RNA polymerase II. Others are found in chloroplasts. Transmitted through vegetative propagation of the host, by seed, by aphids or through mechanical damage, and so overcome the problem of there being no receptor for naked RNA infected plants.

Diener discovered these plant viroids in the year 1971 in the potato crop which causes spindle tuber disease. Viroids are small circular molecules, a few hundred nucleotides long, with a high degree of secondary structure. They are unable to code any polypeptides and replicate independently of any associated plant virus. The most studied viroid is potato spindle tuber viroid (PSTVd). Based on the sequence and predicted structures of their RNAs, viroids are classified into two families, the pospiviroidae and the Avsunviroidae.

9.3.1.1. Family : ***Pospiviroidae :***

Have a central conserved sequence, e.g. potato spindle tuber viroid, coconut cadang cadang viroid.

9.3.1.2. Family : ***Avsunviroidae :***

No central conserved sequence but undergo self-cleavage, e.g. avocado sunblotch viroid.

Viroids infect both dicot and monocot plants. The disease symptoms are stunting, mottling, leaf distortion and necrosis of plants. Viroid infection appears to cause no gross changes in host nucleic acid metabolism. Significant changes in the composition of cell walls have been found in viroid-infected tissues. Long distance movement of viroids is almost certainly through the phloem. Various cytopathic effects of viroid infection on cellular structures – pronounced corrugations and irregular thickness in cell walls degenerative abnormalities in the chloroplast of the leaves. The relative resistance of viroid RNA to nuclease attack probably facilitates their long-distance movement. Viroids are readily transmitted by mechanical means in most of their hosts through the contaminated tools and implements. PSTVd is transmitted by vegetative propagules, pollens and true seeds, and also low frequency transmission by aphids.

The structure of viroids is unique. It is circular but appear as small rods (fig. 9.2) with an axial ratio of about 20:1. The average length of about 37 nm and the molecules can be seen to be covalently closed circles of about 100 nm contour length. The nucleotide sequences of about 27 members of the viroid group and the range in size from 246-375 nucleotides. All viroids have some degree of se-

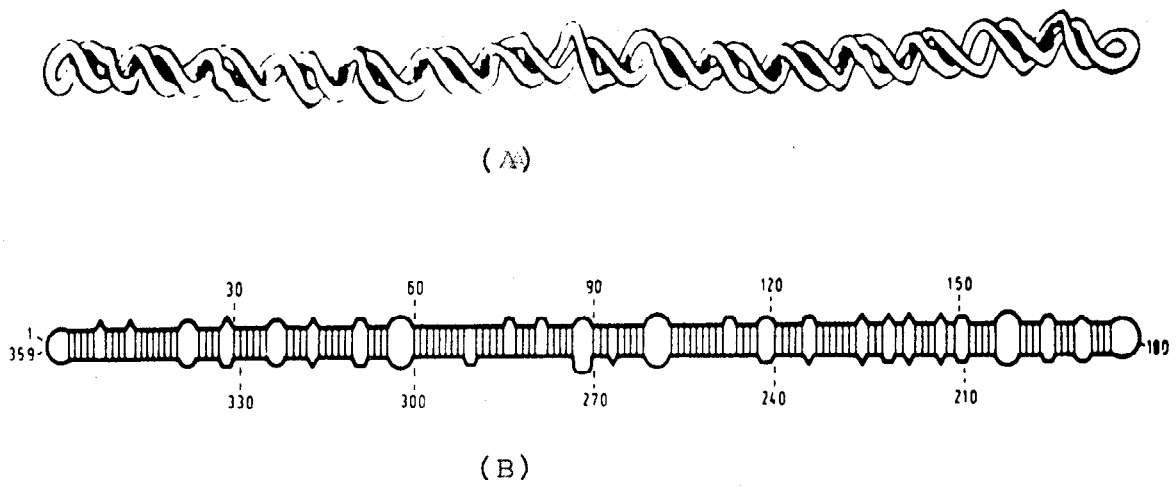

Fig. 9.2 Structure of Viroid (Pstvd) - A) Three dimensional representation of viroid molecule B) Secondary structure of viroid.

quence similarity. Most viroids have a relatively high G+C content (53-60.%). The RNAs of members of the pospiviridae are predicted to form rod-like molecules with base-paired regions interspersed with unpaired loops. Viroids show secondary and tertiary structure which are thought to be important in interactions with the host proteins.

The terminal conserved sequences (TCR) are similar to all viroids. The P domain (Pathogenesis) contains an adenine dominated purine – rich sequence in one strand and an oligo (U) sequence in the opposite strand. The V domain (Variable) is the most variable region in the molecule and show less than 50% homology between closely related viroids.

Viroids replicate in the nucleus of the host cells especially associated with chloroplasts. Entry of viruses into nuclei and plastids is usually controlled by a signal often on a protein associated with the viral genome. RNA strands complementary to viroids RNA were first described by Grill and Semancik (1978) in infected tissue. Viroids replicate via an RNA template. The existence of closed circular monomers complexed to long linear multimers makes it highly probable that a rolling circle type of replication gives rise to progeny viroid RNA. Viroids replicate via a negative strand RNA template. Three enzymatic activities are required for viroid replication by the rolling circle mechanism – RNA polymerase, RNA cleavage and RNA ligase.

Diagnostic procedures for viroids are biological tests, gel electrophoresis, nucleic acid hybridization and Reverse Transcription Polymerase Chain Reaction(RT-PCR). Immunological methods cannot be used for the diagnosis of disease caused by viroids because they do not produce any specific proteins.

9.3.2. PRIONS (SUBACUTE SPONGIFORM ENCEPHALOPATHIES)

The causative agents of the subacute spongiform encephalopathies are prions (Proteinaceous Infectious particles). These are composed of a protein designated as prion protein (PrP).

Prions are highly resistant to inactivation by physical and chemical agents, they are nonimmunogenic and devoid of nucleic acid. These produce slow infections (latent) with long incubation period (years) leads to progressive disease. Later death occurs due to degenaration of the brain tissue (spongiform appearance).

Prototype group of prion diseases :

Kuru – Human,
Scrapie – sheep, goat
Creutzbeldt – Jakob Disease (CJD) - Human, Cattle
Bovien spongiform encephalopathy (Madcow disease) – Bovines
Mink encephalopathy – Mink
Feline encephalopathy – Cats
Wasting disease – Deer, Elk
Gerstmann – Straussler-Scheinker Syndrome (GSS) – Human
Fatal familial insomnia – Human

A protein about 27 K was isolated from the amyloid fibrils and identified as a macromolecule which was associated with the infectivity in the sheep disease scrapie and human disease CJD. Extensive studies had failed to identify the nucleic acids associated with infectious materials. Prions do not contain RNA or DNA. Classification of the prions is essentially based on similarities in epidemiological and pathological findings. These include pathological effects of nervous system, prolonged incubation period and progressive clinical symptoms. Prions are associated with membranous fractions during purification from infected brains. These are sensitive to proteases and resistant to nuclease. No virus like particles have been observed in infectious samples. The infectivity of prions has been found highly resistant to radiation and many other chemical inactivating agents. The relative molecular mass of prions is about 30,000 and revealed that the PrP is a sialogly coprotein 27-30. Recently PrP has been purified and sequenced. A single copy of a gene for this mRNA has been found in the DNAs of infected and normal cells. The role of PrP in the infectivity or pathogenesis has yet to be proved and morphologically similar structures have been observed in noninfectious neuropathies such as Alzheimer's disease.

9.3.2.1. KURU

Kuru (trembling) was the first identified slow infectious disease of humans, in 1966. The disease has been found only in a small region of the Eastern Highland Province of Papua New Guinea, chiefly in a tribe of people called the Fore. The incidence of disease in the Fore has dropped radically since their ritualistic practice of cannibalism and contact with infected tissue was abandoned. It appears that the infectious agent of the disease was transmitted from generation to generation by consumption of the brains of deceased elders or rubbing the body with diseased tissue, a custom that was thought to instill wisdom. The neurological symptoms of kuru in humans are similar to those in sheep with scrapie, but since kuru can be transmitted only to apes, research on the agent has been limited.

9.3.2.2. SCRAPIE

Scrapie, which was described in sheep and goats over 50 years ago, is still found in most parts of the world, despite attempts to eradicate the agent by destroying infected flocks. The disease is characterized by incoordination and unsteady gait, which becomes progressively worse until death.

The infectious agent, in the form of extracts of infected brains, has been passed experimentally to mice, hamsters, ferrets, mink, and monkeys, but apparently is not infectious for chimpanzees or rabbits. The disease induced by the scrapie agent in mink is analogous to that in sheep and also indistinguishable from a transmissible mink encephalopathy (TME) found naturally in mink, which is transmitted by an agent of similarly poorly defined physical properties as the scrapie agent. Likewise, chronic wasting disease (CWD) of mule deer and elk is similar both in pathology and known physical properties to the scrapie agent.

There are no biochemical or cell culture assay systems for the scrapie agent or any other of this group of agents. Transmission of the disease to mice allows endpoint titration of biological activity to be performed, albeit slowly and laboriously. With the finding that the interval from inoculation to onset of disease is inversely proportional to the dose of scrapie agent injected intracerebrally into hamsters, it has become possible to assay samples with the use of four animals in 60 to 70 days, if the titers of scrapie agent in the samples are high. This assay has been used to purify the infectious principle and investigate its structure on the molecular level.

Variants of the scrapie agent have been isolated from standard stocks by passage in mice at limiting dilution. Some of these exhibit different neuropathological effects, including markedly different incubation times at low dosage. These “strains” seem to be stable and do not revert or interconvert with detectable frequency. The existence of such stable variants is most easily explained by the operation of a genetic mechanism based on the presence of a nucleic acid genome in the infectious agent, although no such genome has been found.

9.3.2.3. CJD

Creutzfeldt-Jakob disease (CJD), which occurs, though rarely, throughout the world, typically presents as a dementia in either men or women at least 60 years old. Some cases are clustered in certain families with an evident predisposition. The fact that some of these families also have an apparently higher incidence of Alzheimer’s disease has led to the supposition that the two diseases may be related. However, only CJD is readily transmissible to apes and, occasionally to rodents. It also appears that CJD is transmissible to humans, as indicated by a recent autopsy report of a young man who died of CJD that is believed to have been transmitted in preparations of human growth hormone with which he was treated for retarded growth during his childhood. So convinced were the officials at the U.S. National Institute of Health that the infection came from the hormone, which was prepared from extracts of human pituitaries, that they banned further use of this material for clinical applications. This action served to accelerate government approval of human growth hormone produced by genetic engineering in *E. coli*, which had already undergone unusually protracted clinical trials.

Other reports of transmission of CJD have been linked to corneal transplants taken from individuals dying from this disease; young recipients later developed neurological symptoms of CJD. This agent, like scrapie, has been found to be extremely stable. Brains stored in formalin for over 10 years that were obtained from individuals who died from CJD have been shown to contain the infectious agent when inoculated as extracts into rodents. Another human disease, which progresses more rapidly than CJD and is also transmissible to apes, is the Gerstmann-Straussler syndrome (GSS).

9.3.2.4. GSS

Transmissible spongiform encephalopathies (TSEs) are a unique group of degenerative brain diseases that can be transmitted between individuals by inoculation or ingestion of diseased nervous system tissues. Naturally occurring TSE diseases include GSS also apart from Scrapie, Kuru, and CJD. Typical disease symptoms are dementia and ataxia with progressive loss of brain function, always resulting in death. Pathology consists of prominent astrocytosis and neuronal loss, which appears as a spongiform degeneration in tissue. Certain cases of familial Transmissible cerebral amyloidoses (TCA) present clinically as a progressive cerebellar ataxia, often with pseudobulbar signs, and regularly show deposits of multicentric amyloid plaques in the human brains. It progresses to incapacitation and advanced dementia more slowly than do typical CJD cases and their duration is twice as long as that of famililial CJD (4-5 years). GSS has been transmitted to susceptible subhuman primates on passage of brain suspensions from patients belonging to familial CJD families, presumably without the specific mutation copied in the infectious polypeptide of the Scrapie Associated Firbirls (SAFs) in these infected subhuman primates. The affected primates do not develop the typical multincentric plaques of GSS.

9.4. SUMMARY

The International Committee on Taxonomy of Viruses (ICTV) takes up the classification of viruses. The major characters considered for classification of viruses are 1. properties of virions, 2. genomic organization and replication, 3. antigenic properties and 4. biological properties. Basing on these characters, ICTV has identified a number of families, subfamilies, genera and species in six major groups viz. 1. Double stranded DNA viruses, 2. Single stranded DNA viruses, 3. DNA and RNA reverse transcribing viruses, 4. Double stranded RNA viruses, 5. Negative sense single stranded RNA viruses and 6. Positive sense single stranded RNA viruses.

The presence of living particles smaller than viruses was also identified, and they mainly include viroids, satellite RNAs and Prions. Viroids are associated with plant diseases and are characterized by presence of only infectious nucleic acid particle. Satellite RNAs are those that are dependent upon helper viruses for their replication and hence are mainly associated with other viruses. Prions are infectious protein particles and are associated with slow infectious diseases of humans and animals such as kuru, scrapie, CJ, BSE (mad cow disease), GSS etc. The properties of the subviral particles are explained

9.5. Model questions

Essay type questions

1. Discuss the criteria used for classification of viruses
2. Give an account of outline classification of viruses according to ICTV, and add a note on the criteria used for classification of viruses
3. Give an account of subviral particles
4. Discuss the properties of prions and diseases caused by them

Short answer type questions

5. I.C.T.V. classification of viruses
6. Viroids
7. Prions
8. Spongiform encephalopathies
9. Mad cow disease

9.6. Reference Books

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5. **Medical Virology** by D.O. White and F.J. Fenner, 4th edition,1994, Academic Press
6. **Plant Virology** by R. Hull, 4th edition, 2001, Academic Press
7. **Fundamentals of Virology** by D.M. Knipe and P.M. Howley, 4th edition, 2001, Lippincott.
8. **Fields Virology** by B.N. Fields, D.N. Knipe, P.M. Howley, 3rd edition, 1996, Lippincott.
9. **Encyclopedia of Virology** by R.G. Webster and A. Granoff, 9th edition, 1994, Vol I,II, and III
10. **Applied plant Virology** by D.G.A. Walkey, 1985, Heinemann Publication.

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LESSON: 10

ULTRASTRUCTURE AND REPLICATION OF TMV, HIV AND T4

Objective: To study the ultrastructure and replication of important representatives of Viruses viz. TMV, HIV and T4

Contents:

10.1. Introduction

In a series of experiments Frankel Conrat and Williams (1955) demonstrated that tobacco mosaic virus spontaneously formed when mixtures of purified coat protein and its genomic RNA were incubated together, i.e. the structure that TMV adopts is self-ordered and corresponds to a free energy minimum. This was and remains a remarkable discovery. Despite the great variability shown in virus properties, at a structural level all are based on a few basic designs. Many viruses are enveloped, some are roughly spherical having a symmetry based on an icosahedron. eg.HIV-1.

10.2. Replication strategies:

For a virus to multiply it must obviously infect a cell. Viruses usually have a restricted host range. All must make proteins with three sets of functions- a) ensure replication of the genome, b) package the genome into virus particles and c) alter the metabolism of the infected cell so that viruses are produced. Unraveling the complexities of viral replication is central focus of experimental virology. In 1940s the studies with bacteriophages provided the first in sights on viral replication. Progress has been such that the basic mechanisms of transcripition, translation and nucleic acid replication have now been characterized for all the major families of the viruses and also attention has turned to the strategy of gene expression and regulation. The over view of the DNA and RNA viral replication cycles are shown in the figures 10.1 and 10.2. In general the over all replication cycle of the viruses consisting of ten steps- 1) Attachment 2) Penetration 3) Uncoating 4) Transcription of early mRNA 5) Translation of early proteins 6) Replication of viral DNA 7) Transcription of late mRNA 8) Translation of late proteins 9) Assembly of virions and 10) Release.

In Attachment the Virion cause infection and the virus particles must able to bind to the host cells. Viral attachment proteins bind to receptors on the plasma membrane of the host cell. Penetration

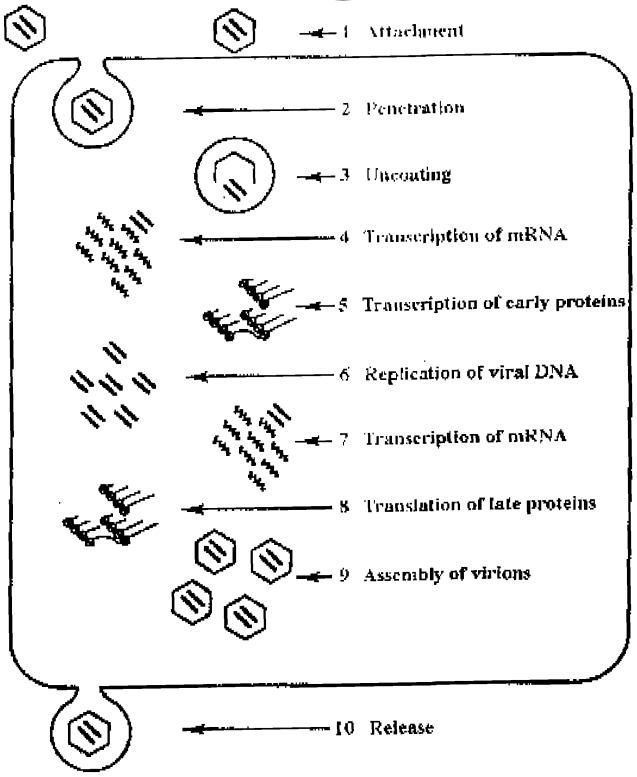

Fig. 10.1. General features of the viral replication cycle - A nonenveloped icosahedral DNA virus as a model.

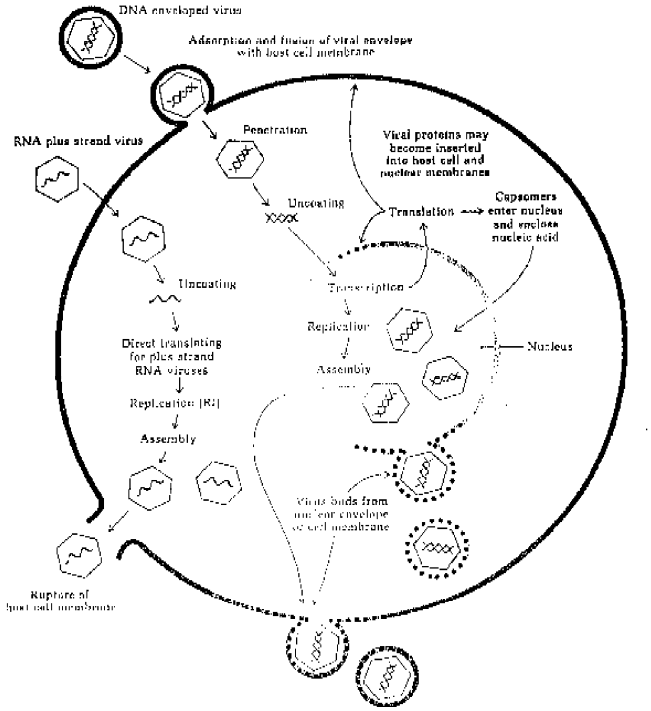

Fig. 10.2. Replication of an enveloped DNA virus and +ssRNA virus

is the following step after attachment. In the penetration Virion enters in the cell by one of two main mechanisms, Endocytosis or Fusion. Endocytosis is the major penetration mechanism which continuously engaged in receptor-mediated for the uptake of macromolecules via specific receptors. The fusion (F) glycoprotein causes the envelope of these viruses to fuse directly with the plasma membrane of the cell. This mechanism may allow the nucleocapsid to be released into the cytoplasm. In the next step uncoating, the viral nucleocapsid is discharged directly into the cytoplasm and transcription commences from nucleic acid still associated with this structure. Certain early viral genes are transcribed into RNA which may then be processed in a number of ways, including splicing. The early gene products translated from this messenger RNA (mRNA) are of three main types: proteins that shut down cellular nucleic acid and protein synthesis, proteins that regulate the expression of the viral genome and enzymes required for the replication of viral nucleic acid. Following viral nucleic acid replication, late viral genes are transcribed. The late proteins are principally viral structural proteins for assembly into new virions, some of these are subject to posttranslational modifications. Each infected cell yields thousands of new virions, which spread to infect other cells. Most families of DNA viruses, transcription and DNA replication take place in the cell nucleus. Some viruses use the cellular RNA polymerase II and other cellular enzymes, but most have their own genes for a range of other enzymes. Some carry “transforming genes” which induce cellular DNA synthesis, to increase the concentration of cellular enzymes and deoxynucleotides to the levels found only during the S phase of the mitotic cycle. RNA viruses have the advantage that ribonucleoside triphosphates are available throughout the cell cycle. However, these viruses must encode their own RNA polymerase(s), since cell lack the capacity to copy RNA from an RNA template. Most RNA viruses replicate in the cytoplasm.

The replication strategy of any virus depends on the nature of its genetic material, and in this respect, viruses can be divided into seven groups. David Baltimore (1971) proposed this scheme.

ClassI: Double-stranded DNA. Pavovaviridae, Adenoviridae, Herpesviridae, Poxviridae.
ClassII: Single-stranded (+) sense DNA. Parovoviridae.
ClassIII: Double-stranded RNA. Reoviridae
ClassIV: Single-stranded RNA.Picornaviridae, Calciviridae, Togaviridae, Flaviviridae.
Class V: Single-stranded (-)RNA. Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Bunyaviridae.
Class VI: Single-stranded (+) sense RNA with DNA intermediate. Retroviridae.
ClassVII: Double-stranded DNA with RNA intermediate. Hepadnaviridae, Caulimovirus.

Viruses with RNA genomes in particular, genome replication and the expression determines the overall course of a virus infection (acute, chronic, persistent or latent) and such is the emphasis placed on gene expression by molecular biologists.

10.3. TOBACCO MOSAIC VIRUS (TMV):

10.3.1. Ultrastructure:

Tobacco mosaic virus is a rigid helical rod, 300 nm long and 18 nm in diameter. The composition of the particle is approximately 95% protein and 5% RNA. The stability of naked TMV RNA is no greater than that of any other ssRNA. The stability of the virus with respect to infectivity is a

consequence of the interactions between neighboring protein subunits and between the protein and the RNA. X-ray diffraction analyses give a detailed picture of the arrangements of the protein subunits and the RNA in the virus rod. The particle comprises approximately 2130 subunits that are closely packed in a helical array. The pitch of the helix is 2.3 nm and the RNA chain is compactly coiled in a helix following that of the protein subunits. Three nucleotides of RNA associated with each protein subunit and there are 49 nucleotides and 16 $^{1/3}$ protein subunits per turn. The phosphates of the RNA are at about 4nm from the rod axis. Negatively stained TMV particles in the electron beam revealed that the basic helix is right – handed and one end of the rod is concave and other end is convex. The 3$^{'}$ end of the RNA is at the convex end and 5$^{'}$ at the concave end. A central canal with a radius of about 2nm (Fig.10.3).

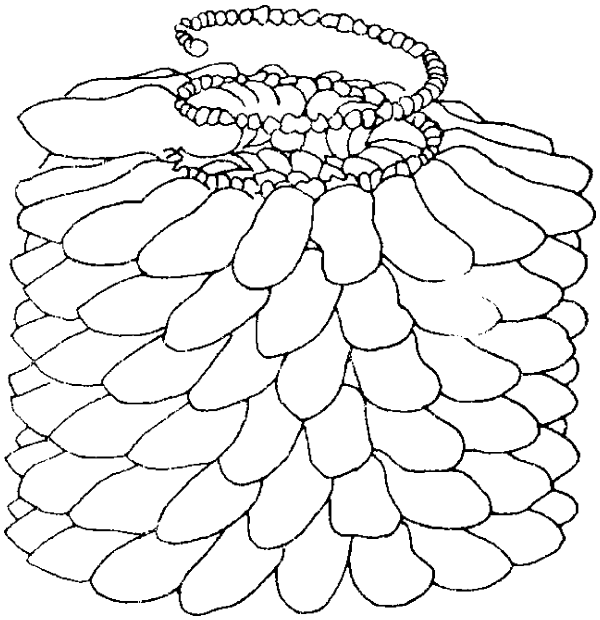

Fig. 10.3. Structure of TMV

10.3.1.1. Coat protein :

The coat protein comprises of 158 amino acids with a molecular weight of 17-18 kDa. Fibre diffraction studies determined the structure of TMV at 2.9 ^{0}A resolution. The protein has a high proportion of secondary structure with 50% of the residue forming four _ – helices and 10% of the residues in _ – structures, in addition to numerous reverse turns. Four closely parallel or anti-parallel _ – helices make up the core of the subunit and the distal ends of the four helices are connected transversely by a narrow and twisted strip of _-sheet. The N and C termini of the protein are to the outside of the particle. One of the reassembly products of TMV protein subunits is a double disk containing two rings of 17 protein subunits. Under appropriate conditions, the disks form true three-dimensional crystal.

10.3.1.2. Nucleic acid :

The RNA binding site is in two parts, being formed by the top of one subunit and the bottom of the next. The three bases associated with each protein subunit form a claw that grips the left radial helix of the top subunit. The electrostatic interactions between protein and RNA are best considered as complementarity between the electrostatic surfaces of protein and RNA.

10.3.2. Replication of TMV :

10.3.2.1. RNA in infected plants

Unencapsidated forms of (-) and (+) strands of TMV RNA are found in infected plants and protoplasts both in RFs and in RIs. In protoplasts, the synthesis of (-) strands ceases 6-8 hours after inoculation, whereas (+) strand synthesis continues for a further 10 hours. It is considered likely that later in the infection cycle most of the (+) strands become encapsidated into virions (Fig.10.4).

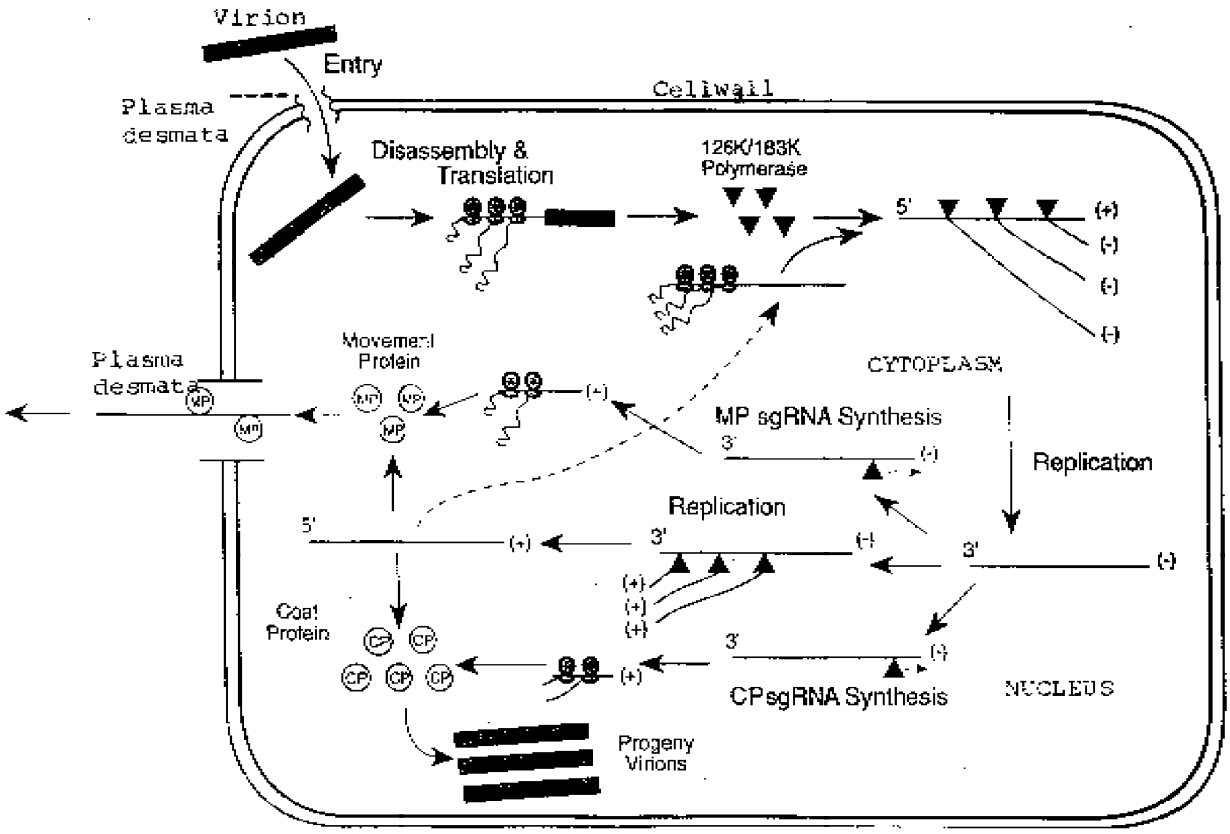

Fig. 10.4. Tobacco mosaic virus replication cycle

10.3.2.2. Replication proteins

The 5' ORF of TMV encodes a 126-kDa protein, the stop codon of which is read through to give a 183-kDa protein; thus, both proteins have the same N-terminal sequence, the 183-kDa protein having a unique C-terminal sequence. Several lines of evidence, including the following, point to both of these proteins being involved in the replication of the viral RNA :

1. Both proteins have the methyl transferase and helicase motifs and the 183-kDa protein also contains the RdRp motif.

2. Both proteins are found in *in vitro* replication complexes isolated from infected plants. In these complexes, the 126-and 183- kDa proteins are in a 1:1 ratio.

3. Antibodies to the 126- and 183-kDa proteins inhibited replication in the *in vitro* system.

4. A mutant engineered to produce the 126-kDa protein, but not the 183-kDa protein, could not replicate.

5. A mutant in which the UAG amber stop codon was mutated to a UAU tyrosine codon, thus producing only the 183-kDa protein, replicated in protoplasts to only 20% the level of wild-type virus. In plants, the mutation reverted to a UAA ochre stop codon, the virus then replicating more efficiently.

6. The helicase domain of the 126-kDa protein interacts with the intervening region of the 183-kDa protein.

To examine the functions of the 126-KDa and 183-kDa proteins, Lewandowski and Dawson developed a bipartite system to express the two proteins from separate RNAs. The 183- KDa protein had all the functions expected from its motifs and it recognized promoters for (-)- and (+) –strand synthesis, transcribed sgRNAs, capped RNAs and replicated defective RNAs; it or its mRNA also moved from cell to cell within the plant. Addition of the 126-kDa protein increased the rate of replication approximately 10-fold; it functioned primarily in *cis.* Thus, efficient replication requires the 183-kDa protein to form a heterodimer with the 126-kDa protein which is probably bound to the template RNA. However, there may be still other functions for the 126-kDa protein and it is produced in about ten times the amount of the read-through product, the 183-kDa protein, yet the two proteins form a 1:1 heterodimer.

The 3' UTR of TMV RNA can be folded into three structural domains, a 3' domain mimicking a tRNA acceptor branch, and an upstream domain comprising three pseudoknots each containing two double-helical segments. Mutational analysis indicated that one of these double-helical segments is essential for replication, the secondary structure rather than the primary structure being the important feature. Four domains were identified in the 3' untranslated region, D1 equivalent to tRNA acceptor arm, D2 similar to a tRNA anticodon arm, an upstream domain D3 and a central core C which connects D1, D2 and D3 domains. These four domains were required for promotion of (-) strand RNA synthesis and that the D2 and C domains bound the RNA polymerase with highest efficiency.

The tRNA-like structures of most TMV strains can be aminoacylated by the host histidyl-tRNA synthetase, but that of the cowpea strain resembles that of TYMV in being aminoacylated with valine. Chimeras in which the 3' UTR of TMV-L is replaced with that of TMV-OM can replicate in protoplasts and plants, though TMV-L replicate to a lower level. When the 3' UTR of TMV-L is replaced with that of Brome Mosaic Virus (BMV) the chimera can replicate but to a much lower efficiency than TMV-L itself. This indicated that the TMV polymerase has some flexibility in its recognition site – which is in contrast to that of BMV which does not recognize BMV RNA3 with the 3' UTR replaced by TMV-L 3' UTR.

Sequences at the 5'- end of TMV RNA are important for replication. Large deletions in the 5' region and deletion of nucleotides 2-8 abolished replication but other small deletions in the 5' UTR did not. This suggests that the 5' replicase binding site may be complex.

Internal sequences in the TMV genome have been recognized to inhibit replication in *trans.* Deletion of the region between nucleotides 3420 and 4902 (sequences encoding the RdRp domain of the 183-kDa protein) created a replication defective RNA that could be replicated *in trans* by TMV.

The satellite TMV (STMV) requires the helper virus for its replication, indicating that it has the necessary *trans*-acting sequences. Although STMV and various subgenomic replicons use TMV replicase in *trans,* the synthesis of (-) strands of genomic RNA prefers interactions in *cis*.

10.4. HUMAN IMMUNODEFICIENCY VIRUS (HIV):

10.4.1. Ultrastructure:

HIV differs from that of other retroviruses because it contains dense core appears cone-shaped and contains some additional minor proteins. This virus (fig. 10.5) is an enveloped virus. 80-100 nm diameter with a complex structure and an usual enzyme, reverse transcriptase (RT). The genome unique among viruses, is diploid consisting of an inverted dimer of positive sense single stranded RNA(+ ss RNA), 7-10 kb in size. It contains 72 peplomers projecting from the envelope are oligomers of a glycoprotein (gp 160), which has been cleaved into two noncovalently linked components, gp 120 and gp 41. The receptor-binding ligand and the most important antigenic domains, notably the V3 loop are present in the gp 120. The gp 120 is the most extensively glycosylated viral protein, it is presumed that this "sugar coating" is a protective device to impede acess of neutralizing antibodies. The hydrophobic membrane anchor is provided by gp 141, which is responsible for viral entry into the host cell by membrane fusion. The viral envelope also contains some cellular proteins, notably class I and class II MHC antigens. The inner surface of the envelope is lined by a myristylated matrix protein, p17, a cleavage product of the 55 KDa gag gene product. The phosphoprotein p24 is the another most abundant viral protein of gag gene product of which the icosahedral nucleocapsid is constructed. The other two proteins are p9 and p7, which are closely associated proteins with the genome of the core Virion. Three viral enzymes are closely associated with genomes are Reverse Transcriptase, Integrase and Protease. Six additional genes vif, vpr, vpu, nef, tat, rev which have regulatory functions. The genomic organiszation of HIV-1 is shown in the figure 10.6. Two types of HIV are recognized – HIV1 and HIV2. HIV2 displays only about 40% nucleotide sequence similarity with HIV 1 and contains a unique gene vpx in lieu of the vpv of HIV-1. Structurally HIV2 is closely

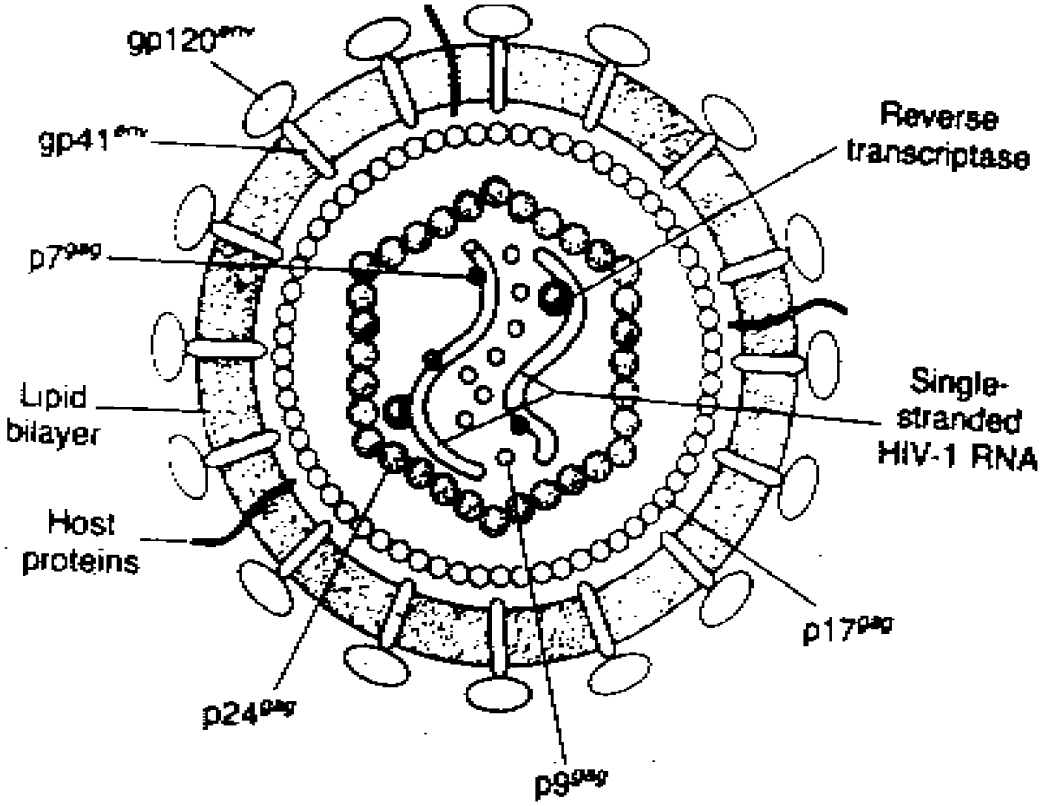

Fig. 10.5. Diagramatic representation of HIV-1 virion

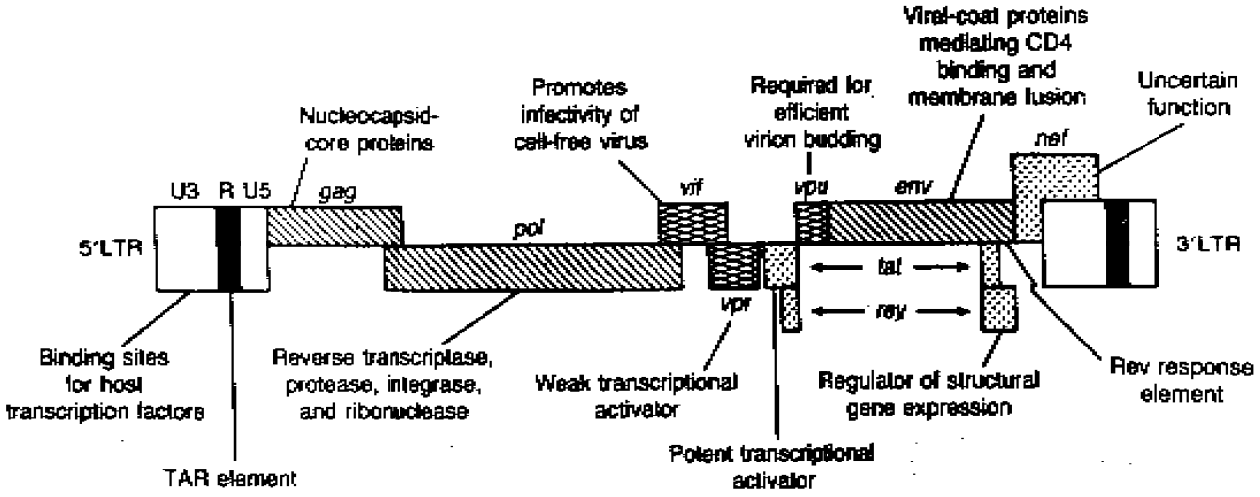

Fig. 10.6. Genomic organization of HIV-1 and functions of gene products

related to the African sooty mangabey simian immunodeficiency virus. HIV 2 is less virulent than HIV1. Five major genotypic subtypes of HIV1, differing by 30-35% in their env and gag sequences. HIV-1 strains can differ by upto 20% in nucleotide sequence.

10.4.2. Overview of the replication cycle of HIV-1 in a T cell:

Infection begins with attachment of the Virion via gp120 to its receptor, CD4. Following entry by gp41-mediated fusion, the genome becomes available for reverse transcription to produce a dsDNA

with long terminal repeats (LTRs) composed of sequences duplicated from the 3' (U3) and 5' (U5) ends of the viral RNA. Complete reverse transcription and intergration of the DNA provirus into a chromosome occur efficiently only in activated and proliferating T lymphocytes. Transcriptional activity of the HIV provirus is regulated by constitutive host cell transcription factors (Spl and the TATA-binding factors) and by activation-inducible members of the NF-kB family of host transcription factors (p50 and p65), both of which bind to specific sequences in the proviral regulatory region, LTR. The virus-coded regulatory protein, Tat, is one of the early gene products; Tat binds to the TAR region in the LTR and greatly amplifies transcription of all the viral genes. Following synthesis of a full-length RNA transcript, a complex array of alternatively spliced viral mRNAs can be produced. The differential expression of distinct species of viral mRNAs is controlled by a second HIV regulatory protein Rev. Early in infection, when the level of Rev is low, only the multiply spliced mRNAs for the regulatory proteins Tat, Rev, and Nef are exported to the cytoplasm for translation. Once a sufficient level of Rev accumulates, the unspliced and singly spliced mRNAs that provide new viral genomes and also encode the structural proteins (Gag and Env), the enzymes (Pol), and the remaining regulatory proteins (Vif, Vpr, and Vpu) are exported to the cytoplasm and translated. Encapsidation of the viral genome is followed by budding from the plasma membrane. The replication cycle of HIV in a T4 cell is shown in the figure 10.7.

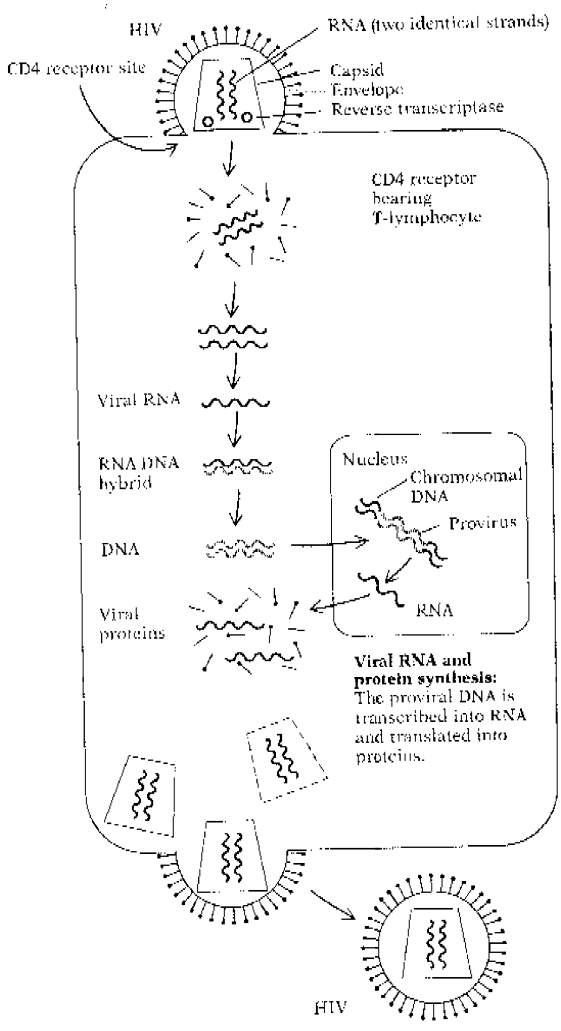

Fig. 10.7. Replication cycle of the Human Immunodeficiency virus

10.5. T4 PHAGES :

10. 5.1. Ultrastructure:

The T4 phage shows banal symmetry (Figure 10.8). The head of phage is an icosahedron with an elongated tail. It has one or two extra bands of hexamers of 85 x 110 nm, but somewhat variable under different conditions. Its shell consists of 5-nm-diameter capsomeres composed mainly of three proteins (gp+ 23, soc gp of 46, and 10 K, 960 molecules per particle and hoc gp of 40 K, 160 molecules), as well as lesser amounts of several other proteins. Each capsomere may consist of six molecules each of gp 23 and gp soc, and one of gp hoc. The contractile tail (25 x 110 nm) is an extraordinarily complex organ, composed of a tube, a sheath, a connecting neck with a collar and whiskers, and a complex base plate with pins and carrying long jointed fibers. The neck consists of six molecules each of four proteins and 18 of another, all about 33 K. The whiskers are a single protein, 38 molecules of 53 K. The tail tube is made of 144 molecules of a 19 K protein, and the sheath of the same number of a glycoprotein of about 70 K. The sheath protein molecules are arranged in 24 rings; as a consequence of a conformational change of that protein, the rings become larger and their number is reduced to 12, leading to the shortened, “contracted” form. This event plays a critical role in the infection mechanism. The base plate plus spikes requires 14 proteins, ranging from 15 to 140 K, most of which are six in number (a few are multiples of six). The six fibers consist of two proteins of 145 and 115 K forming dimmers and two small proteins, one dimeric and the other single. The structural proteins of the virion thus amount to over 30 proteins.

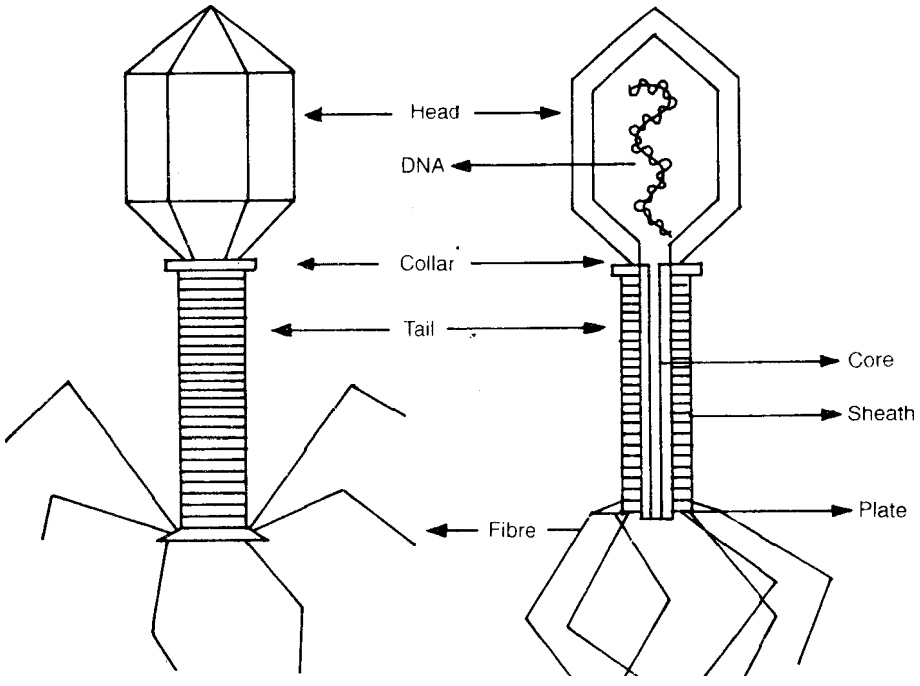

Fig. 10.8. The structure of T4 bacteriophage

The DNA of the T4 phage is linear, 52 nm long. It occupies about half of the head's capacity. It is associated with one major protein (18 K) and lesser amounts of several smaller proteins and polypeptides, as well as considerable amounts of the basic compounds, spermidine and putrescine acting as counter ions to the phosphate groups.

The T4 phages have HMC instead of C. The resultant – OH group is glucosylated, that is, bound to glucose in either of two isomeric forms or to glucosyl-glucose isomers. T2, T4, and T6 phages differ in the extent and nature of glucose substitutions (100 percent total for T2 and T4, which, however, differ in the type of glucose substitution, and 72 percent for T6). The glucose residues play an important role in protecting the phages against restriction endonucleases. Apparently, many *E. coli* strains had learned to break through the first line of defense designed by these phages for this purpose; HMC and glucosylation evolved to strengthen the phage's defenses.

Like most, if not all, linear phage DNAs and many animal virus DNAs, the T4 DNAs are terminally redundant, a sequence of 3000 bases occurring twice. But they have an additional peculiarity termed circular permutation. The mystery that the T4 phage genomes, although clearly linear, behaved genetically as if they were cyclic, with genes from the two ends acting as if they were neighbours, is clearly explained by this phenomenon. Also, the mechanism that produces circular permutation with terminal redundancy is no longer difficult to understand.

10.5.2. Replication Cycle of T4 Phages :

The physical appearance of the tail with appendages, and the number of different proteins that build it, make it evident that this must be an organ of considerable functional sophistication. The tail fibers are normally held near the phage head by the whiskers. Their distal ends represent the site of chemical affinity to cell wall lipoproteins at the outer membrane of the bacterium. Contact by any one fiber may actually suffice for primary attachment. At this stage, environmental changes can still cause release of the phage and abort infection. Subsequently, the other tail fibers finish the job of settling the phage neatly in perpendicular manner on the surface, and a change in the angle of the two parts of the fiber brings the base plate into contact with the cell surface. At that stage the infection process can no longer be aborted. The attachment then leads to considerable conformational changes of the proteins of both the sheath and the base plate. This in turn causes the contraction of the tail. The changes in the base plate enlarge a central hole so that the tail tube can pass through it and pierce the cell wall. Its end then becomes unplugged by the phospholipids of the cell wall. The injection of the head's contents, mostly DNA, into the cell is the end result, although it is not really clear what causes the evacuation of the head that is not changing in shape.

The DNA of these phages may contain as many as 160 genes, of which over 120 have been identified. These are usually presented on a circular map, because the permuted state of the DNA makes it biologically circular, even though physically it appears linear. A few take-home points upon quick inspection of the map : (1) Genes of similar numbers (e.g., 20, 22, 23, 24; and 5-11), related in function, are located near one another on the map. This phenomenon we have encountered repeatedly when considering simpler viruses. (2) Much of the genome, actually almost half of the ring (from half-past seven to half-past two) is needed for nonstructural proteins, such as enzymes, enzyme modifying agents, regulatory proteins, and so on. That this phage forces the cell to convert all its cytosine

to the hydroxymethyl derivative, and to get that compound to the triphosphate stage needed for DNA synthesis, represents one of many functions calling for the existence of many genes. Selective glycosylation, which occurs at the macromolecular level, not at the nucleotide level, is another complex gene function, and so are many other functions not known. It must be noted that not only are the genes of such functional groups close to one another, but the cooperating enzymes also tend to become associated with one another in particulate complexes. Not withstanding their great number of genes, these phages are by no means free agents, independent of their host's collaborative attitude. DNA carries information, but it cannot *do* anything with it until transcribed and translated. And surprisingly, even these most sophisticated of all phages do not carry the needed transcriptase in the Virion – as do the large animal cytoplasmic DNA viruses. Thus, the early phase of T4 phage development, the first few minutes are devoted to letting *E. coli* RNA polymerase go to work and make phage mRNAs. The *E. coli* translation apparatus, ribosomes, and so on, are needed throughout. However, astonishingly, quickly upon infection the phage does stop host DNA transcription, making sure that all the mRNAs translated in the cell are from them on its own.

As with all more complex viruses, genes are transcribed in a definite and advantageous order or programme. Thus, the replication cycle of these phages, only 20 to 30 min long, is usually described as immediate-early, delayed-early, quasi-late, and true-late. Among the very early functions is the production of a phage-specific DNA polymerase. Enzymes are also made early that degrade the host's DNA and bring the resultant deoxynucleotides to the triphosphate level. The formation of HMC and its triphosphate goes hand in hand with the deamination of any excess deoxycytidylic acid. The phage DNA polymerase of 112 K, like *E. coli* polymerase I, also has 3' exonuclease activity and can repair and correct errors. It certainly works by means of Okazaki fragments and requires the product of phage gene 32, a single-strand DNA binding (or helix-destabilizing) protein. These two proteins require and are associated in a complex with gene products 41, 61, 44, 45, and 62, two of which (41, 61) act as a primase. Thus, a complex of seven proteins is needed for rapid DNA synthesis. This complex is also able to replicate T4 DNA *in vitro.*

As far as the RNA polymerase is concerned, the phage continues to use most of the five-chain host enzyme but modifies these peptide chains in various ways at various times to make them more selective for the job at hand. Phage-specified DNA and RNA ligases are produced de novo, as well as many other enzymes acting on nucleic acids. It is believed that it is the need for very rapid synthetic activities that has led such phages to carry genes for so many activities that are normally performed by uninfected *E. coli.* As stated, the most of the translation machinery is the host's, but the phage also produces some particular tRNAs. These are not essential for infection.

10.5.3. Assembly of Large Phage Virions :

The assembly of phage particles has been intensely studied in several instances. These studies have been greatly facilitated by the availability of many mutants, defective in different components of the assembly process of the phage. As a result of mutation, extracts may contain an accumulation of an assembly intermediate. By mixing extracts of *E. coli* infected with different mutant phages and observing the process of *in vitro* phage assembly and its arrest at various stages by electron microscopy and other techniques, a remarkably clear picture of this complex process has been obtained for the T4 and certain other phages. It is the property of each proteinaceous component, be it ultimately

structural or playing an intermediate enzymatic role, to have an affinity to its neighbor or substrate molecule, and to interact with it as soon as there are enough such molecules present to favor this process. As previously observed with simple viruses, the amino acid sequence of each protein leads to a preferred molecular conformation which enables the protein to play its role of aggregating to larger structures with other like or unlike protein molecules, or to express enzymatic functions. In many instances, the same viral protein is active both structurally and enzymatically. The intermediate steps in T4 assembly are, in order :

1. Assembly of the base plate
2. Assembly of the tail tube and sheath
3. A separately initiated assembly of the head
4. An attachment of tail to head
5. An attachment to that particle of the separately assembled tail fibers.

The prohead is formed largely from the ultimate capsid protein gp 23 and the assembly core protein gp 22; the soc and hoc proteins have more recently been discovered, and their role in T-even assembly has not yet been studied in detail. The main internal protein ipIII also enters the prohead during the early stage of assembly. Gp 23 is then cleaved to gp 23 losing the N-terminal 20 percent ; gp 22 is degraded completely; at least four additional proteins play a role in the maturation of the phage head.

The attachment of the tail to the head appears to be a spontaneous event, requiring no enzyme action. The attachment of the fibers, however, requires the presumably enzymatic action of gp 63. Since this occurs only after the head-to-tail attachment, it appears that the whiskers which are part of the head must play a role in positioning the tail fiber properly. The assembly of these fibers requires the interaction of four proteins.

The incorporation of the DNA into the phage head is the least understood step in the T4 phage assembly, and this is true for all phages, and actually for all viruses containing much nucleic acid. Considerable conformational change is required for any nucleic acid molecule to overcome much ionic repulsion in packaging it into the limited space. In most viruses, but particularly in the large phages, most of the nucleic acid becomes an orderly folded coil in the course of this process. This process in the case of the phages is usually accompanied by an enlargement of the prohead and a change from its more spherical to the more icosahedral appearance of the typical phage head. In most phages the prohead contains one to three proteins, which leave the head or become proteolytically altered or degraded as the DNA enters. Also, proteolytic processing of the ultimate capsid proteins frequently accompanies this maturation step.

In the case of T4 phage maturation the cleavage of one of the capsid proteins precedes and possibly initiates the change in head structure associated with phage maturation. The protein that first fills the prohead is reused as such in the maturation of further proheads and has been termed a scaf-folding protein.

It is as unclear what forces make the DNA enter the head of the T4 phages as it is what forces make it later leave the head upon becoming transferred to the host cell. It has been suggested that the expansion of the prohead triggered by entry of the DNA may create a vacuum "that can pull the rest of the DNA in." There is, however, no evidence that the head shrinks as the DNA becomes injected into the host cell.

10.5.4. Lysis :

The T4 viruses carry a gene that codes for a lytic enzyme, lysozyme. The function of this enzyme is to attack a sort of glycoprotein called peptidoglycan that represents a layer in the bacterial cell wall. Several other genes are involved in properly timing this event and consequent lysis of the cell. Lysozyme action is actually not essential for phage development, since mutants lacking the lysozyme function are also viable. Such mutant lysozymes represented the material in which the nature of *frame-shift mutation* was first clearly demonstrated in protein-chemical terms: a deletion of one nucleotide in the coding sequence giving a wrong amino acid sequence – out of phase – which was repaired 15 nucleotides downstream by insertion of one nucleotide. The sequence of the peptides, wild-type and mutant, differing by five amino acids, was established long before the complete amino acid sequence of T4 lysozyme was worked out.

The life cycle of the T-4 phages is illustrated schematically in oversimplified form in Fig.10.9.

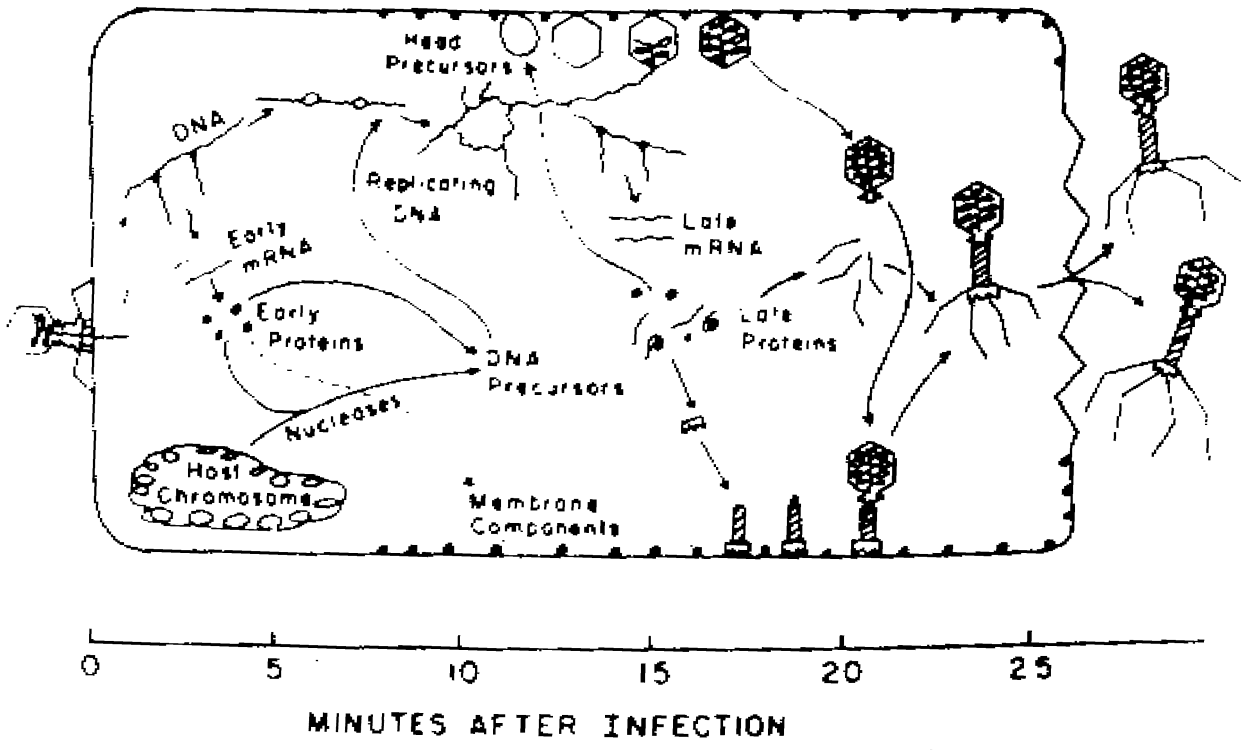

Fig.10.9. Replication cycle of T4 Bacteriophage

10.6. Summary:

The general replication strategies of enveloped and nonenveloped viruses is described. The ultrastructure and replication of TMV, the most important plant virus; HIV – important human virus, and T4 – the bacteriophage are described.

10.7. Model questions:

Essay type questions

1. Discuss the important replication strategies of plant and animal viruses
2. Discuss the ultrastructure and replication cycle of TMV
3. Discuss the ultrastructure and replication cycle of HIV
4. Discuss the ultrastructure and replication cycle of T4 Phage

Short answer type questions

5. TMV
6. HIV
7. Genomic organization of HIV
8. T4 phage
9. Replication cycle of T4 phage
10. Replication cycle of HIV

10.8. Reference books

1. **Virology** by Frankel-Conrat et al., 3rd Edition, 1994, Prentice Hall publications
2. **Principles of Virology** by S.J. Flint et al., 2000, ASM Press
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LESSON: 11

SYMPTOMS OF VIRAL DISEASES AND METHODS OF TRANSMISSION OF VIRUSES

Objective: To study the symptoms caused by plant and animal viruses, and important methods of transmission of viruses

Contents

11.1. Introduction:

Study of virus symptoms is known as symptomatology, it is particularly important in the early days of virus research. In the early period the names and identification of the viruses were given depending on the disease symptoms. Eg. Tobacco mosaic virus, Foot and Mouth disease virus. Recently the dependency on disease symptoms for identification and classification led to much confusion, because many factors can have a marked effect on the disease produced by a given virus. Most virus names in common use include terms that describe an important symptom in a major host or the host from which the virus was first described. Some of viruses may infect the hosts without producing any obvious signs of disease (Latent viruses), some may lead to rapid death of host (Necrosis) and some causes programmed cell death (Apoptosis). Between these extremes, a wide variety of diseases can be produced.

11.2. Symptoms of plant viral diseases:

11.2.1. Macroscopic symptoms:

Most of the plant viruses shows visual symptoms which are known as macroscopic symptoms. Depending on the distribution of the disease these symptoms are classified as local symptoms and systemic symptoms. Symptoms of some of the common viral diseases of plants are shown Figure 11.1.

11.2.1.1 Local symptoms :

Chlorotic spots: Localized lesions those are developed near the site of entry of virus on the leaves. These are not having any economic significance but are important for biological assay. Infected cells may lose chlorophyll and other pigments, giving rise to chlorotic local lesions. Eg. Bean yellow mosaic virus infecting *Chenopodium quinoa*.

Fig: 13.2. a. Yellow vein mosaic of bhendi

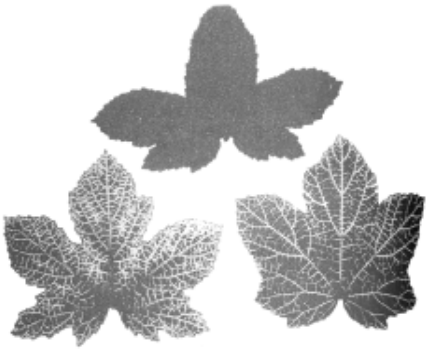
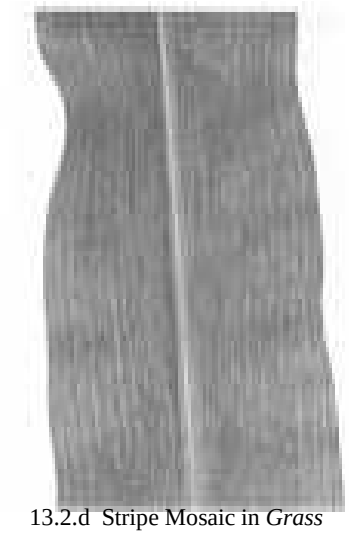


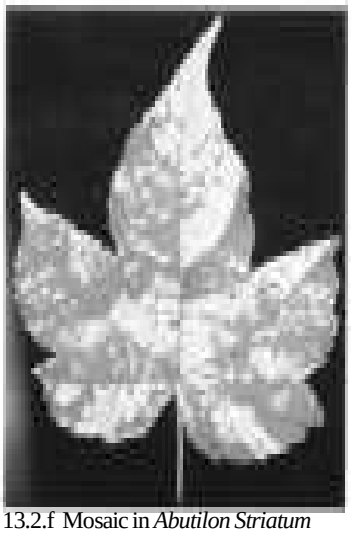
Fig: 13.2 b. Potato leaf showing venal necrosis

13.2.c Tulip Flowers with colour breaking due to tulip breaking virus.



13.2.d Stripe Mosaic in *Grass*

13.2.e Leaf curl and formation of leaf enations in tomato



13.2.f Mosaic in *Abutilon Striatum*

Figs. 11.1. Diseases Symptoms caused by Viruses

Necrotic lesions : Many host virus combinations, the infected cells die, giving rise to necrotic lesions. These are vary from small pinpoint areas to large irregular spreading necrotic patches. Eg. TMV infected *Nicotiana tabacum* NN.

Ringspot lesions **:** These consists of a central group of dead cells. They develop one more superficial concentric rings of dead cells with normal green tissue between them.

Eg. Tomato ring spot virus infected *Nicotiana tabacum*.

11.2.1.2. Systemic symptoms :

Systemic invasion of viruses produce systemic symptoms in the plants. The pattern of disease development for a particular host-virus combination often involves a sequential development of different kinds of symptoms.

Mosaic pattern of symptoms : The most common virus infection is the development of a pattern of light and dark green areas giving a mosaic effect in infected leaves. In dicotyledons the mosaic are irregular in outline and two shades of color involved – dark green and pale or yellow green. Eg. TMV infected tobacco.

Variegation in the mosaic pattern was observed in Turnip yellow mosaic virus (TYMV) infected Chinese cabbage. In monocotyledons, the virus infected leaves shows stripes or streaks of tissue lighter in color than the rest of the leaf. The shades of color vary from pale green to yellow or white and more or less angular streaks or stripes run parallel to the length of the leaf. A variegation or 'breaking' in the color of petals commonly accompanies mosaic or streak symptoms in leaves. The breaking usually consists of flecks, streaks or sectors tissue with color different from normal. Eg. Tulip breaking virus infecting Tulip mosaic disease is also observed on the fruits of Zucchinis infected with CMV.

Yellow disease : Virus infected younger leaves shows clearing or yellowing of the veins. Yellowing is largely confined to the margins of the leaf but yellows disease may lead to a total loss of the crop. Eg. Beet yellows virus infected sugar beet.

Leaf rolling : Virus infection cause upward rolling of leaf or occasionally downward rolling. Epinasty of leaf petioles may sometimes prominent in plants due to viral infection. Eg. Bean roll leaf virus on beans.

Ringspot disease : Concentric rings or irregular lines on the leaves are observed in many plant virus disease and sometimes also observed on the fruits. Eg. Tobacco ring spot virus on brinjal.

Necrotic disease : Death of tissues or organs or whole plant is the main feature of the plant virus disease. Necrotic pattern may follow the veins on the leaves and death of the leaves occurs. Eg. Potato virus X and Potato virus Y in potatoes, Peanut bud necrosis virus , Peanut stem necrosis virus.

11.3. Symptoms of Animal viral diseases :

In animal viral diseases the establishment of infection is either locally or systemically. The localized infections produce disease in the respiratory tract or intestinal tract or genital tract. This causes an acute disease. The systemic infections establish and spread in the body and cause either recovery or death of individuals. This causes chronic disease, in which virus persists for months or for life and causes continuing, often subtle, pathologic effects.

11.3.1. Acute viral infections : Four main categories of acute infections are identified in viral pathogenesis of animals and humans. (1) Respiratory (2) Intestinal (3) Central nervous system and (4) Generalized.

11.3.1.1. Respiratory tract infections : Acute respiratory infections are influenza, viral-bacterial synergistic respiratory infections (Shipping fever). Influenza virus particles in aerosolized droplets are inhaled and alight on the film of mucus that covers the epithelium of the upper respiratory tract. Influenza virus and other respiratory viruses that initial invasion and destruction of just a few epithe-

lial cells can initiate a lesion which can progressively damage the protective layer of mucus and lay bare more and more epithelial cells. Where as influenza infection in mammals is generally restricted to the cells of the respiratory tract, in birds influenza viruses often cause an in apparent infection of digestive tract. Viremia and spread to other organs including the brain occur with virulent strains of avian influenza virus

11.3.1.2. Intestinal infections: Virus characteristically infect different parts of the villi of the intestinal tract. Eg: Rotaviruses, Corona viruses. Infection occurs by ingestion of virus. Infection of the intestinal tract is part of a systemic infection, the incubation period is very short. In viral infections fluid loss is mainly a loss of extracellular fluid due to impaired absorption and osmotic loss. Hypoglycemia due to decreased intestinal absorption, inhibited glyconeogenesis and increased glycolysis follows, completing a complex of pathophysiologic changes that if not promptly corrected results in death of the animal.

11.3.1.3. Neurological infections : The pathogenesis of Rabies, the rabid animal usually results in deposition of rabies infected saliva deep in the striated muscles. Movement along the nerves eventually delivers virus to the central nervous system, usually the spinal cord. Virus reaches the limic system where it replicates extensively and the release of contractile control of behaviour leads to “furious” rabies. Spread within the central nervous system continues, and occurs the clinical charges to “Dumb rabies”. Depression, coma and death from respiratory arrest follows.

11.3.2. Persistent infections : The viral infection that persists for a prolonged period after the primary infection in humans or animals or cells. These persistent viral infections are important for four reasons. (1) The carriers serve as a source of infection, there by enabling the virus to persist in the population even if its infectivity is low (2) They may be reactivated and cause recurrent acute disease (3) They may lead to immunopathologic disease (4) They are some times associated with neoplasms.

Persistent infection is divided into three categories.

1. Latent infections – Infectious virus is not demonstrable except when reactivation occurs, episodes that are sometimes but not always associated with recurrent disease. Eg : Cytomegalo virus, Bovine herpes virus 1

2. Chronic infections – Infectious virus is always demonstrable and often shed and disease may be absent, chronic or may develop late, often with an immunopathological basis. Eg : FMDV, Lymphocytic choriomeningitis virus

3. Slow infections – Infectious virus gradually increase during a very long preclinical phase, leading to a slowly progressive lethal disease.

Eg : Lentiviruses infection, Subacute spongiform encephalopathies

11.4. Methods of transmission of viruses

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

11.4.1. Transmission of plant viruses :

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

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

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

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

11.4.1.4. Semi – persistent transmission : In this mode of transmission the vector acquire the virus within minutes to hours and retain the virus in the fore-gut an hour. After retention time the vector successfully transmit the virus to healthy susceptible host.

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

Table 11.1. Important examples of Plant-Viruses transmitted by insect vectors

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

11.4.1.6. Nematode transmission : Several important viruses are widely transmitted by soil inhibiting nematodes.Eg.Longidorus, Paralongidorus, Xiphenema, Paratrichodorus, Trichodorus. Majorly two genera of plant viruses are transmitted by nematodes. Eg: Nepo and Tobra viruses. The nematode transmission of a virus occured by different processes – ingestion, acquisition, adsorption, retention, release, transfer and establishment.
Eg .Rice ragged stunt virus, Tobacco rattle virus.
11.4.1.7. Fungal transmission : Several viruses have been transmitted by soil-inhabiting fungi Eg. Olpidium, Polymyxa, Spongospora. The virus survive in the resting spores and zoospores which infect the host. Various degrees of host specificity exist in both the Chytrid and Plasmodiophoral vectors. Zoospores of Olpidium species transmits the viruses like Tomato bushy stunt virus, Beet necrotic yellow vein virus.

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

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

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

11.4.1.11. Graft transmission: Grafting is an ancient horticultural practice in which a union is established between the cut tissues of two different plants. There are many types of grafting procedures established. One of the most common is the union between the shoot portion of one plant, refereed to as the *scion* and the root-bearing portion of another called the *stock* . If either scion or stock is infected with virus, the virus pass in to the healthy one and establish infection. This method is useful for plant viruses that could not be mechanically sap transmitted and for which no other natural method

of transmission was known. Graft transmission of plant viruses is common in horticultural crops, such as Citrus, Apple, Plum etc., Eg. Citrus mosaic virus, Citrus tristeza virus, Apple mosaic virus.

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

11.4.2. Transmission of animal viruses :

Viruses survive in nature only if they are able to be transmitted from one host to another, whether of the same or another species of animals or humans. Transmission cycles require virus entry into the host body, replication, shedding and subsequent spread to another host. Virus transmission in animals is categorized in to two types – 1) Horizontal transmission and 2) Vertical transmission

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

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

Licking	-	Measles, Mumps
Rubbing	-	Sheep pox virus
Biting	-	Rabies
Coitus	-	Sexually transmitted disease- HIV.

11.4.2.1.2. Indirect contact transmission : It occurs via fomites, such as shared eating containers, bedding, dander, restraint devices, vehicles, clothing, improperly sterilized surgical equipment, syringes and needles.

Fomites	-	Adeno virus
Clothing	-	Pox viruses

Surgical equipments, syringes, needles –HIV, HBV.

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

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

11.4.2.1.5. Arthropod – borne transmission : It involves the bites of arthropod vectors.
Eg. Mosquitoes – Equine encephalitis virus , Ticks – Tick borne encephalitis virus , Sandfly – Yellow fever virus, Culicoid – Blue tongue virus.

11.4.2.2. Vertical transmission :

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

11.4.3. Other modes of transmission :

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

11.4.3.2. Nosocomial transmission : This transmission occurs through hospital or clinic. The respiratory virus infections are also often acquired nosocomially. Eg. Influenza virus, Adeno virus.

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

Table 11.2. Common Modes of Transmission of Viruses of Animals:

Mode of transmission	**Virus example**
Fecal-oral, respiratory, contact, transplacental	Feline parvovirus
Direct contact, skin abrasions	Papillomaviruses
Sexual	Equine coital exanthema virus
Respiratory	Infectious bovine rhinotracheitis virus
Transplacental	Pseudorabies
Contact	Orf, cowpox
Arthropod, Mechanical	Myxoma virus
Respiratory	Equine rhinoviruses
Ingestion of garbage	Foot-and-mouth disease viruses to pigs
Arthropod	Venezuelan equine encephalitis virus
Arthoropod	Japanese encephalitis virus
Respiratory, fecal-oral, transplacental	Bovine virus diarrhea virus
Arthropod and contact	Vesicular stomatitis
Animal bite	Rabies virus
Arthropod	Rift Valley fever virus
Fecal-oral	Calf rotavirus
Arthropod	Bluetongue viruses
Contact, in ovo	Germ line

Table 11.3. Nonarthropod – Borne Viral Diseases of Animals

Mode of transmission to humans	Virus	Reservoir host
Animal bite	Herpes B virus	Monkey
Contact, through skin abrasions	Cowpox virus	Rodents, cattle, cats
Contact, through oral and skin abrasions	Monkeypox virus	Squirrels, monkeys
Contact, through skin abrasions	Pseudocowpox virus	Cattle
Contact, through akin abrasions	Orf virus	Sheep, goats
Animal bite, scratch	Rabies virus	Terrestrial mammals &bats
Contact with oral secretions or vesicular fluids	Vesicular stomatitis virus	Cattle
Contact, iatrogenic (injection) human-to-human spread	Monkeys	Ebola, Marburg viruses
Respiratory	Influenza A virus	Swine, birds
Contact with rodent urine	Hantaan virus	Rodents
Contact with rodent urine	Lymphocytic chorio-meningitis virus Lassa virus	Rodents

11.5. SUMMARY:

Viruses are obligately intracellular pathogens, and virus infection usually cause visible symptoms which vary with virus – host combination. Some viral infections may be symptom less. The symptoms of virus infections in plants and animals are given much importance in virus classification in the past. In plants virus infections mainly cause two types of symptoms viz. local symptoms eg. Nectrotic or chlorotic spots, and systemic symptoms eg. Mosaic tellowing etc. The virus infections of humans and animals cause some important diseases of respiratory tract (influenza), intestinal tract (rotaviruses), nervous system (rabies). Some virus infections in animals are persistent type which include latent infections (cytomegalovirus) chronic infections (lymphocytic choriomeningitis virus) and slow infections(lentiviruses).

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

11.6. Model questions

Essay type questions

1. Discuss the symptoms caused by virus infection of plants
2. Give an account of symptoms caused by animal viruses
3. Discuss the methods of transmission of plant viruses
4. Discuss the methods of transmission of animal viruses

Short answer type questions

5. Local symptoms
6. Systemic symptoms
7. Acute viral infections
8. Persistant infections
9. Vector transmission of plant viruses
10. Seed and Pollen transmission of plant viruses
11. Graft transmission of viruses
12. Vehicle transmission

11.7. Reference books

1. **Virology** by Frankel-Conrat et al., 3rd Edition, 1994, Prentice Hall publications
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LESSON: 12

MICRO ALGAE – GENERAL ACCOUNT AND ECONOMIC IMPORTANCE

CONTENTS:

12.1.OBJECTIVES

After going through this unit you will be able to:

- Describe the general characters of algae
- Differentiate various types of thalli in algae
- Recognize the position of algae in the plant kingdom
- Describe the various types of reproduction and life cycles in algae and
- Describe the economic importance of algae in industry, agriculture, medicine, oil and gas, sewage treatment, water supplies etc.

INTRODUCTION:

The algae are commonly known as pond scums, frogspittle, water mosses, seaweeds etc. they comprise of a large heterogenous and polyphylitic assemblage of relatively simple plants or thallophytes which lack roots, stems and leaves. As in higher plants, photosynthesis in algae is accompanied by

oxygen evolution. Fritsch defined algae as " all those holophytic forms that fail to reach the level of differentiation that is characteristic to the archegoniate plants". There fore the algae can be distinguished by the following features.

1. The absence of archegonium like structure (the development of archegonium starts from bryophytes onwards in the plant kingdom upto gymnosperms).

2. The reproductive bodies, either sporangia or gametangia are unicellular or multicellular and in both all cells are fertile without any sterile covering or jacket of vegetative cells around their reproductive organs (except *Chara* in which the antheridium develops a sterile jacket around it).

3. The algae differ from the other group of thallophyta, viz, the fungi in having chlorophyll.

12.3 GENERAL ACCOUNT- VEGETATIVE STRUCTURE

12.3.1 DISTRIBUTION

Algae are universal occurrence and they are found in a variety of habitats, such as fresh water, sea water, on snow, on rocks and on or within the plant and animal bodies. They adopt themselves well in all types of habitats. However, large majority of forms occurs in aquatic habitat. Aquatic algae may be floating or suspended i.e., Planktonic or attached and living in the bottom i.e., benthic. Benthic algae grow attached to various substrates and may be classified as epilithic which grow on stones, epipelic which are attached to mud or sand, epiphytic attached to plants and epizoic attached to animals like protozoans, coelenterates, molluscs etc. Some forms may also occur as endophytic (with in the cells of other algae). Very few are reported as parasitic on the leaves of angiosperms, for e.g., *Cephaleuros* occurs on the leaves of *Anacardium* and also on tea leaves.

Some algae especially the members of cyanobacteria (blue green algae) and dinoflagellates occur in the form of blooms. They secrete a number of substances and some of them are toxic to various animals.

The algae are transported from one place to another through the agency of tides, currents and agitation by wind and also by birds animals and ships. They are transported either in vegetative state or in the form of different reproducing stages.

Among algae, some especially the blue green algae thrive on long persistent snows and some inhabit in hot springs at temperatures ranging between 50 – 75^{o}C.

12.3.2. POSITION OF ALGAE IN PLANT KINGDOM

In the plant kingdom, the algae are usually studied first because of several reasons. First the fossil record indicates that the most ancient organisms that contain chlorophyll – a, were probably cyanobacteria (blue green algae). The second reason is the relative simplicity of plant bodies when compared to other groups and lastly the sexual reproduction is more elegant, with great clarity, but in other plants it is complicated by secondary characteristics. Therefore, majority of botanists view the algae as likely progenitors for the remaining members of the plant kingdom because of similarity in pigmentation (chlorophylls- a and b) and in the nature of their photosynthetic product or storage product or reserve food as starch.

12.3.3. RANGE OF PLANT BODY

The vegetative structure of algae shows a wide variety and it ranges in form from unicellular to complex multicelluar thalli. Their size ranges from one micron to several meters. On the basis of thallus organization, algae are divided into the following groups:

(i) Unicellular forms

(ii) Multicellular forms

Unicellular forms are found in all groups of algae except Charophyceae and phaeophyceae. These forms are some times referred to as acelluar, since they function as complete living unit without any cellular differentiation. Unicellular forms are of the following types.

(a) Rhizopodial unicells
(b) Flagellated unicells
(c) Spiral filamentous unicells
(d) Non-motile unicells

(a). Rhizopodial unicells:

These forms lack a rigid cell wall. They posses cytoplasmic projections which help them in amoeboid movement. In the absence of rigid cell wall, these forms are periplastic. Examples: *Rhizochrysis, Rhizochloris, Chrysamoeba.*

(b). Flagellated unicells:

These are flagellated vegetative cells. Such cells show similarity with motile gametes and zoospores. They vary in number and type of flagella. Flagellated unicells may be periplastic without cellwall (*Euglena)* or with a distinct cell wall (*Chlamydomonas*). In *Phacotus* there is a thick calcarious covering (capsule) around the cell wall.(Fig: 12.1.A-E)

(C) Spiral filamentous unicells:

Some unicellular algae form spiral or coiled filamentous structures, e.g., *Spirulina.*(Fig:12.2.A)

(a) Non motile unicells:

There are non-motile coccoidal algae, which do not posses flagella, eyespot, etc., meant for locomotion. The simplest non-motile forms are found in cyanophyceae, where they do not posses well-organized nucleus and plastids (prokaryotic) e.g., *Chroococcus*. The non motile unicells of Bacillariophyceae such as diatoms are made up of two halves or theca, joined by a girdle band (Fig:12.2.B).

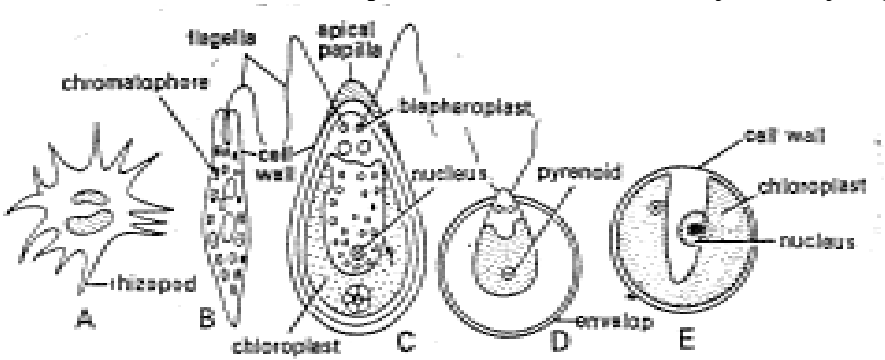


Fig: 12.1.A-E. Unicellular algae: A.*Chrysamoeba,* B. *Euglena,* C. *Chlamydomonas,* D. *Phacotus,* E. *Chlorella*.

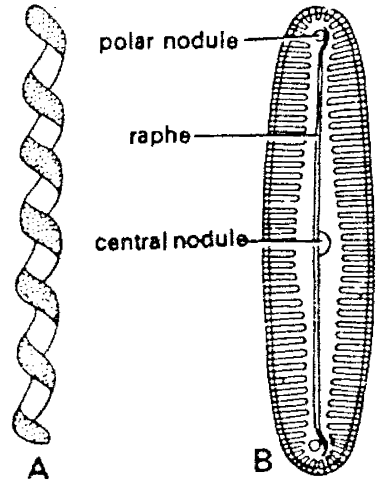


Fig: 12..2 Unicellular algae:A-b: A- *Spirulina*, B- *Pinnularia*

II. Multicellular forms:

Multicellular forms are of four types, 1.**Colonial forms** 2. **Filamentous forms** 3. **Siphonaceuos forms** 4. **Parenchymatous forms**.

The multicellular forms have been derived by repeated divisions of the unicellular forms. The colonial forms are developed by the aggregation of the products of cell division with in a mucilage mass (*Pandorina, Eudorina, Volvox, Hydrodictyon*, Fig:12.3.A-D). The filamentous forms are formed by repeated transverse divisions of cells without separation of daughter cells (*Spirogyra, Oscillatoria, Nostoc* etc, Fig:12.4.A&B). Repeated nuclear divisions without cross wall formation give rise to siphonaceous forms (*Vaucheria, Botrydium*, Fig:12.5A&B). The parenchymatous thalli are formed by the division of cells of a filament in two or more planes (*Ulva, Sargassum*).

Species of *Micromonas* (*M. pusilla* 1.0 X 1.5 μm) are in the range of bacterial size, and where as some of the sea weeds may attain a length over 30 meters (*Macrocystis pyrifer*). Unicelluar (*Chlamydomonas*), heterotrichous (*Coleochaete*), membranous or foliose(*Ulva*) and tubular (*Enteromorpha*) types of plant body occur in algae. Growth of various algae may be diffuse or generalized or it may be localized.

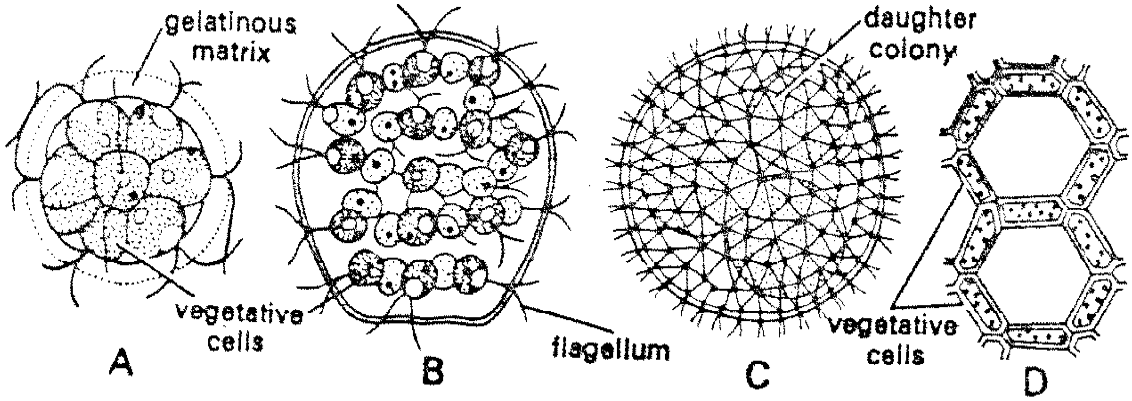
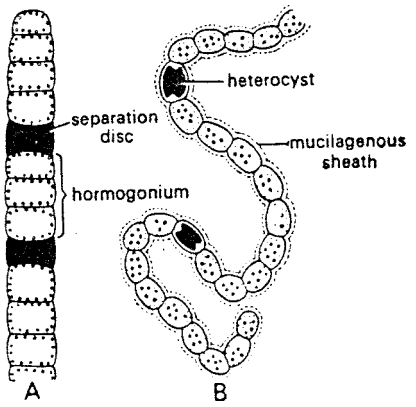


Fig: 12.3.A-D, Colonial algae, A-*Pandorina*, B-*Eudorina*, C-*Volvox*, D-*Hydrodictyon*



Fig;12.4. A-B.Filamentous algae.A.*Oscillatoria,* B. *Nostoc.*

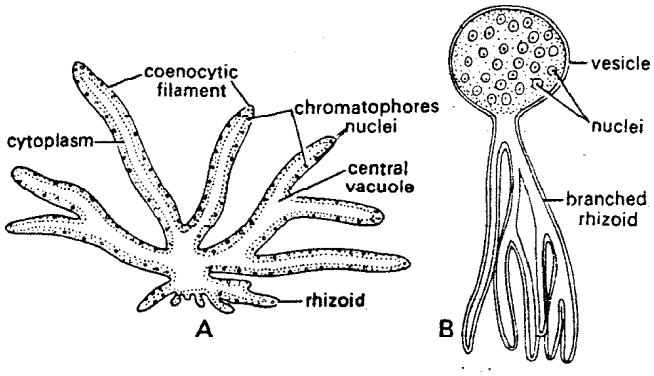


Fig: 12.5.Siphonaceous algae.A. *Vaucheria,* B-*Botrydium.*

12.3.4. CELL STRUCTURE IN ALGAE

On the basis of their organization the algal cells may be differentiated into prokaryotic, mesokaryotic and Eukaryotic types. The prokaryotic cell organization is found in cyanophyceae, which is characterized by 1. The presence of incipient nucleus 2. The absence of membrane bound organells like plastids, mitochondria and Golgibodies. Majority of algae shows this type of cell organization. An intermediate type of cell organization, i.e., mesokaryotic is found in the members of Dinophyceae, where although the nucleus has a nuclear membrane and chromosomes (eukaryotic characters), basic proteins are absent (prokaryotic character)(Fig:12.6.a &b)

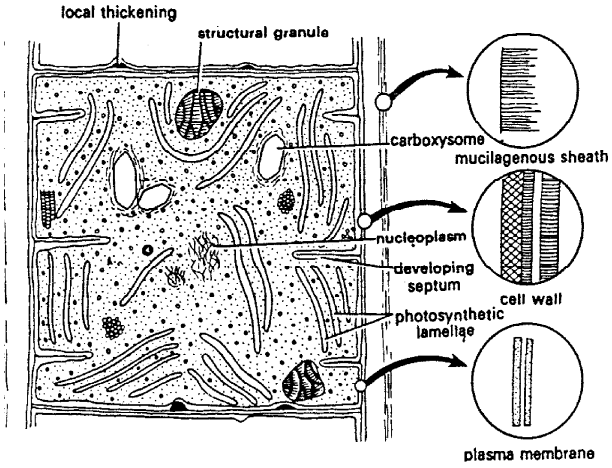


Fig: 12.6.A. Diagrammatic representation of a prokaryotic cell.

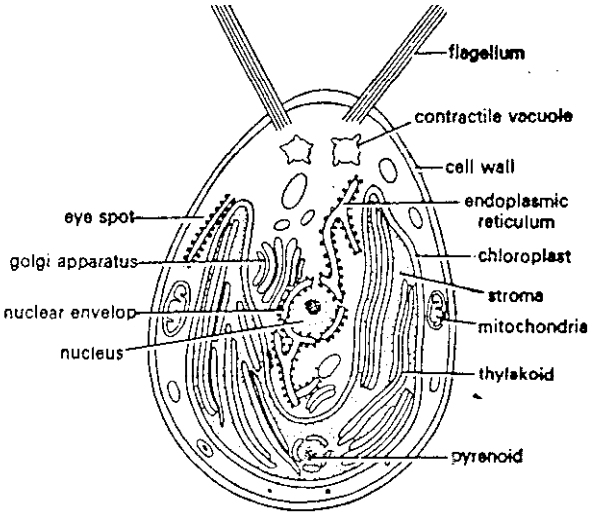


Fig: 12.6.b. Digrammatic representation of a Eukaryotic cell.

12.3.5. PIGMENTS IN ALGAE

Algal cells have a characteristic colour due to the presence of a combination of pigments, specific to each class. Except cyanophyceae, all classes posses these pigments in membrane bound organelles, known as plastids. In blue greens, the pigments are concentrated in peripheral cytoplasm, known as chromoplasm. Plastids are of the following two types:

1. Leucoplasts: these are colourless plastids
2. Chromoplasts: these are colored plastids, those containing both chlorophyll-a, chlorophyll- b are called chloroplasts and those lack chlorophyll- b as chromatophores.

The various types of pigments found in the algal cell are

(a) Chlorophyll: There are five types of chlorophyll, viz., chl a, b, c, d and e. Of these, chlorophyll a is present in all groups of algae, chlorophyll b only in chlorophyceae and euglenophyceae, chlorophyll c largely in algae of marine habitats (phaeophyceae, cryptophyceae, bacillariophyceae and chrysophyceae), chlorophyll d in some red algae only as a trace constituent and chlorophyll e in certain xanthophyceae, such as *Vaucheria hamata* and *Tribonema bombycinum.*

(b) Xanthophyll: More than 20 types of xanthophylls are known. They are formed by the incorporation of molecular oxygen into carotene molecule. Many xanthophylls, common in higher plants (leutin, violaxanthin, and neoxanthin), are found in the members of chlorophyceae and phaeophyceae. Fucoxanthin is the main xanthophyll pigment of phaeophyceae and diatoms.

(c) Carotenes: These are oxygen free alicyclic componds, composed of isoprene units. The 5 types of carotenes occur in algae are α- carotene in chlorophyceae, cryptophyceae and Rhodophyceae, β- carotene in all algal groups, except cryptophyceae, γ- carotene in chlorophyceae, E- carotene in bacillariophyceae, cryptophyceae, phaeophyceae and cyanophyceae and flavacene in members of cyanophyceae.

(d) Phycobilins: These are water-soluble complexes of protein and bile pigments, present in the photosynthetic tissue of plants. Phycobilins are red (phycoerythrin) and blue (phycocyanin)

pigments, which are confined to Rhodophyceae and cyanophyceae respectively. They act as light harvesting pigments in photosynthesis and the light absorbed by them is transferred to chlorophyll a. Thus, like carotenoids, phycobilins are also accessory pigments.

12.3.6. FLAGELLA:

Cyanophyceae and Rhodophyceae their motility is due to small filiform (thread like) protoplasmic appendages, called flagella. The number of flagella varies from one to four. They are mainly of the following two types;

a. **Whiplash or acronematic** flagella, such flagella have a smooth surface(Fig.12.7.1).

b. **Tinsel or pleuronematic** flagella. The surface of these flagella is covered with fine filamentous appendages, known as mastigonemes or flimmers (Fig.12.7.2.A). They are further divided into three categories on the basis of arrangement of mastigonemes.

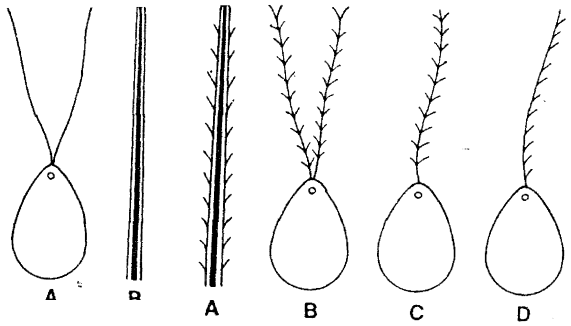
i. **Pantonematic**. In this type the mastigonemes are arranged in two opposite rows or show radial arrangement (Fig.12.7.2.B).

ii. **Pantocronematic.** A pantonematic flagellum with a terminal fibril is known as pantocronematic (Fig12.7.2.C)

iii. **Stichonematic**.Here the mastigonemes develop only on one side of the flagelllum (Fig12.7.2.D).

A motile cell may have either one or both the above mentioned types of flagella. It is a specific character. If all flagella of a cell are similar, it is known as isokont and where dissimilar it is called heterokont.

A transverse section of flagellum consists of nine peripheral and two central fibrils. The nine peripheral fibrils are in pairs where as the central or axial pair of fibrils is in singlet. All fibrils are enclosed with in a common covering, formed by the extension of the plasma membrane, but the two central fibrils have an additional covering of their own. The peripheral as well as central fibrils extend almost through out the length of the flagellum. The nine peripheral fibrils at the proximal end are attached to a hallow basal body(which is separated from the flagellum by a diaphragm). The two central fibrils terminate just short to the diaphragm.(Fig:12.8.)



Fig;12.7.1.A-B. acronematic flagella. A. An algal cell with acronematic flagella,
B. A single acronematic flagellum.

FIG: 12.7.2.A-Pleuronematic flagellum showing mastigonemes.B- A cell with two pantonematic flagella.C. A cell with pantocronematic flagellum, D- A cell with Stichonematic flagellum.

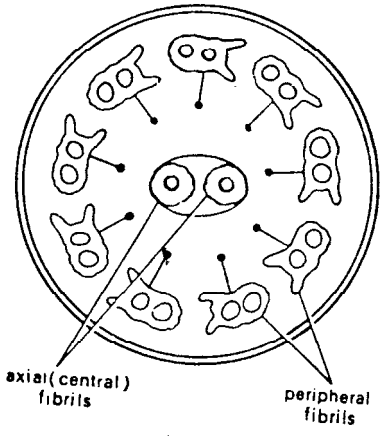


Fig: 12.8 A transection of a flagellum.

12.3.7. RESERVE FOOD:

The primary product of photosynthesis in algae is starch. But due to accumulation of it over a large period, many insoluble products are formed which varies in different algal groups. They are as follows:

Algal group	**Storage food**
Chlorophyceae	Starch
Euglenophyceae	Paramylum starch
Chrysophyceae	Leucosin
Bacillariophyceae	**Chryso laminarin**
Xanthophyceae	Chryso laminarin
Phaeophyceae	Laminarin and mannitol
Rhodophyceae	Floridian starch
Cyanophyceae	Cyanophycean starch

12.4. Reproduction

Reproduction is a process by which a living organism propagates and multiplies the number of its individuals. In algae reproduction generally takes place in three ways.

12.4.1 Vegatative reproduction.

12.4.2. Asexual reproduction.

12.4.3. Sexual reproduction.

12.4.1. Vegetative reproduction:

It is the most common method of reproduction in algae and takes place by the following means:

a. Cell division or Fission:

It is the simplest method of propagation and is commonly found in unicellular algae, Desmids and Diatoms.In this process, the unicelluar algal cell divides mitotically to form two daughter cells, and each eventually grows into an independent organism.

b. Fragmentation:

In filamentous forms like *Ulothrix, Spirogyra* and *Zygnema* the thallus often breakes into small fragments. Each fragment has the capability to grow independently and forms a new thallus. The fragmentation of filaments may be due to mechanical pressure, dissolution of transverse walls between adjoining cells. In colonial blue green algae, vegetative propagation takes place by fragmentation of larger colonies.

c. Hormogonia:

It is a characteristic method of reproduction in blue green algae. Under unfavorable conditions the trichome breaks into segments of varying length called hormogonia. The fragmentation of parent filament into hormogonia may be due to the formation of intercalary heterocysts, specialized separation discs are necredia are due to death and decay of intercalary cells of the trichome. Hormogonia are commonly found in *Nostoc, Oscillatoria* and *Cylindrospermum.*

d. Budding:

In some algae (e.g., *Protosiphon*) vegetative propagation takes place by budding. Bud like structures are formed due to proliferation of vesicles. They eventually get separated from the parent plant by a septum, and have the capacity to form new plants.

12.4.2. Asexual reproduction:

Asexual reproduction takes place by a variety of motile or non-motile spores (cells that germinate without fusing and form new individuals). These spores may be differentiated into the following broad categories on the basis of their structure:

a. Hypnospores:

Aplanospores of some algae like *Pediastrum* and *Sphaerella* secrete thick walls to overcome prolonged period of desiccation. Such thick walled aplanospores are called **hypnospores.** Under favourable conditions, hypnospores germinate and grow into new individuals or their protoplast may form Zoospores. The hypnospores of *Chlamydomonas nivalis* are red in colour due to the deposition of a pigment, haematochrome, in their walls.

b. Autospores:

The aplanospores with a structure similar to the parent cell are called **autospores.** In *Scenedesmus* and many members of the order chlorococcales (e.g., *Chlorella*) the aplanospores aquire all the distinctive features of the parent cell before their liberation from the sporangium. The so formed autospores are infact replica of the parent cell, the only difference is that they are smaller in size.

c. Akinetes:

In some algae vegetative cells develop into thick walled sporelike structures with abundant food reserves. These are called **akinetes.** Unlike aplanospores, akinetes always have additional wall layers around the protoplast, which are fused with the parent wall. They are resistant to unfavorable environmental conditions. The formation of akinetes, besides other factors, is effected by the availability of carbohydrates and light. They are found in many blue green and green algae.

d. Exospores and endospores:

In many blue green algae the protoplast divides to form special type of aplanospores, known as **exospores** and endospores**.** The cell that produces endospores become somewhat enlarged and its contents divide successively in three planes, forming four to many endospores (e.g., *Dermocarpa*). The exospores are formed externally; the protoplast of the cell comes out through a terminal pore and successively cuts spherical spores (e.g., *Chamaesiphon).*

12.4.3. Sexual reproduction:

All groups of algae, except Cyanophyceae, reproduce sexually when gametes fuse to form zygote. On the basis of the structure and physiological behavior of sex organs and their complexity, the following types of sexual reproduction are recognized in different groups of algae.

a. Autogamy:

When two gametes of the same mother cell fuse to form a diploid nucleus, it is called autogamy. In this process there is only karyogamy (fusion of two gametic nuclei). The autogamy lacks incorporation of external genes. Hence, the plants developing as a result of autogamy do not show new characters. Diatoms are the common example of autogamy.

b. Hologamy:

In some unicellular forms (e.g., *Chlamydomonas, Dunaliella)* the vegetative cells of different strains (+) and (-) (female and male) behave as gametes and fuse to form the zygote. From evolutionary point of view hologamy is more advanced than autogamy, as it involves fusion of two cells having different genetic constitution. But hologamy is an inefficient process from the point of view of propagation, as two vegetative cells fuse to form only one zygote.

c. Isogamy:

In isogamy the two gametes, which fuse to form the zygote, are morphologically and physiologically similar. Such gametes are called isogametes, as they are indistinguishable into plus and minus strains. They are usually motile and flagellate. Isogametes are found in *Ulothrix*, *Chlamydomonas* etc.

d. Anisogamy:

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

e. Oogamy:

This is the most advanced type of sexual reproduction. In this process a large non-motile egg or ovum fuses with a small motile sperm or antherozoid (in Rhodophyceae, the sperms are nonmotile). The egg is formed with in the oogonium and the sperms within the
Antheridium.

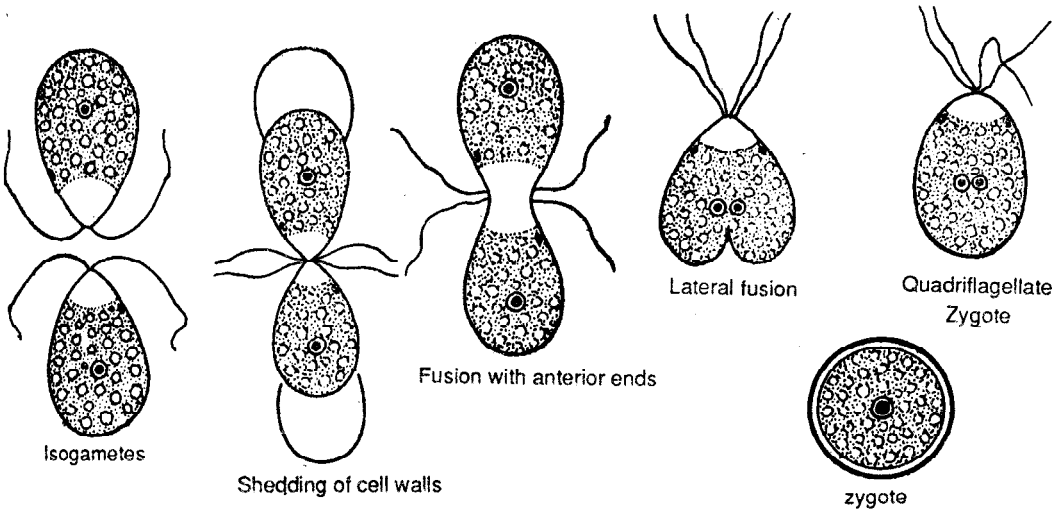


Fig: 12.9 Isogamous Fusion.

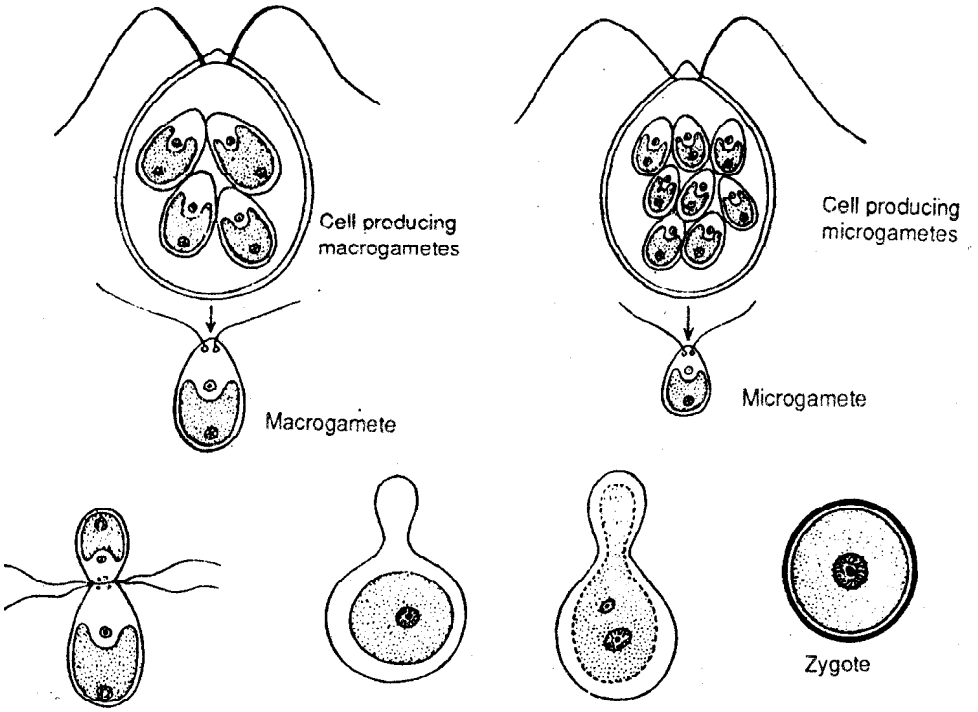


Fig: 12.10 Anisogamous fusion.

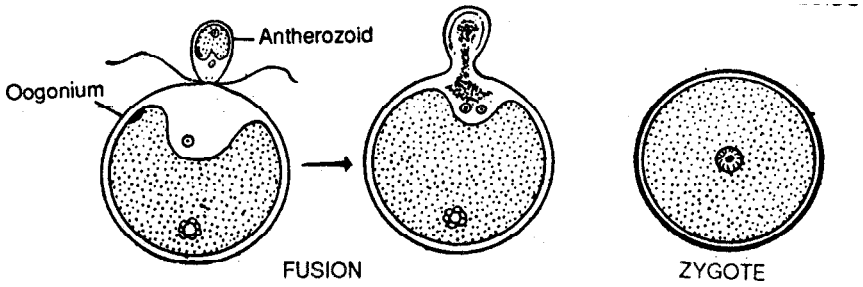


Fig: 12.11 Oogamy.

12.5. LIFE CYCLES:

Different types of life cycles have been recognised in algae. They are haplontic, diplontic, diplohaplontic or haplodiplontic, haplobiontic and diplobiontic life cycles Figs. 12.12.

a. Haplontic life cycle:

In this the parent is haploid and it is dominant phase in the life cycle and the zygote represents the diploid phase with reduction division occurring at the time of germination of zygote (e. g. *Vovox, Oedogonium, Chlamydomonas* .

b. **Diplontic life cycle:**

In this the parent is diploid and it is the dominant phase in the life cycle and the gametes constitute the haploid phase. Reduction division takes place at the time of gamete formation in the diploid phase (e. g. Diatoms, *Sargassum*).

C. Diplo haplontic or haplo diplontic life cycle

In this type of life cycle there will be an alternation of diploid sporophyte with the haploid gametophyte. Both the phases are dominant in the life cycle but two phases are morphologically similar. Hence it is also called as isomorphic life cycle(Eg., *Ectocarpus)*Reduction division takes place at the time of formation of spores by the sporophyte .

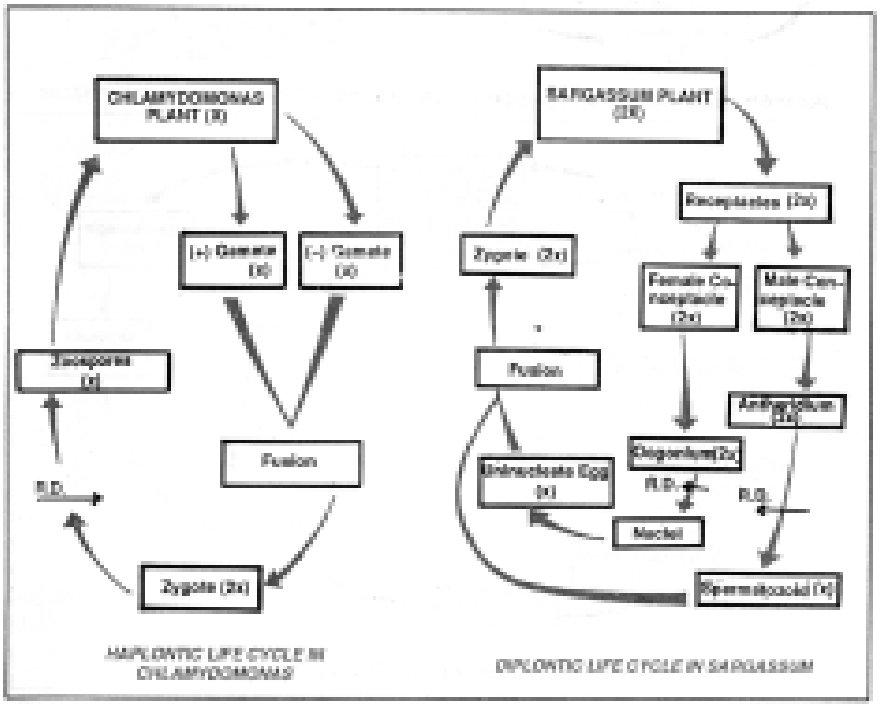
d. Haplobiontic life cycle:

In this, two haploid generations (free living gametophyte and carposporophyte) alternate with a diploid one represented by the zygote (e. g. *Batrachospermum)*. In this there is another gametophytic stage developed by the germination of haploid carpospores, which is a protonema like stag known as **Chantransia** stage and it is morphologically different from the free living gametophytes. Since the life cycle of *Batrachospermum* involves two haploid and one diploid stage, the life cycle is said to be haplobiontic, and since there is difference in the morphology of two gametophytic stages, it is called heteromorphic. Because of the involvement of three phases and three generations viz.,

1. free living gametophytic phase,
2. carposporophytic phase and
3. chantrantia state, the life cycle is said to be tri phase and tri genic. On the whole the the life cycle is described as haplobiontic, heteromorphic, triphase, trigenic alternation of generation.

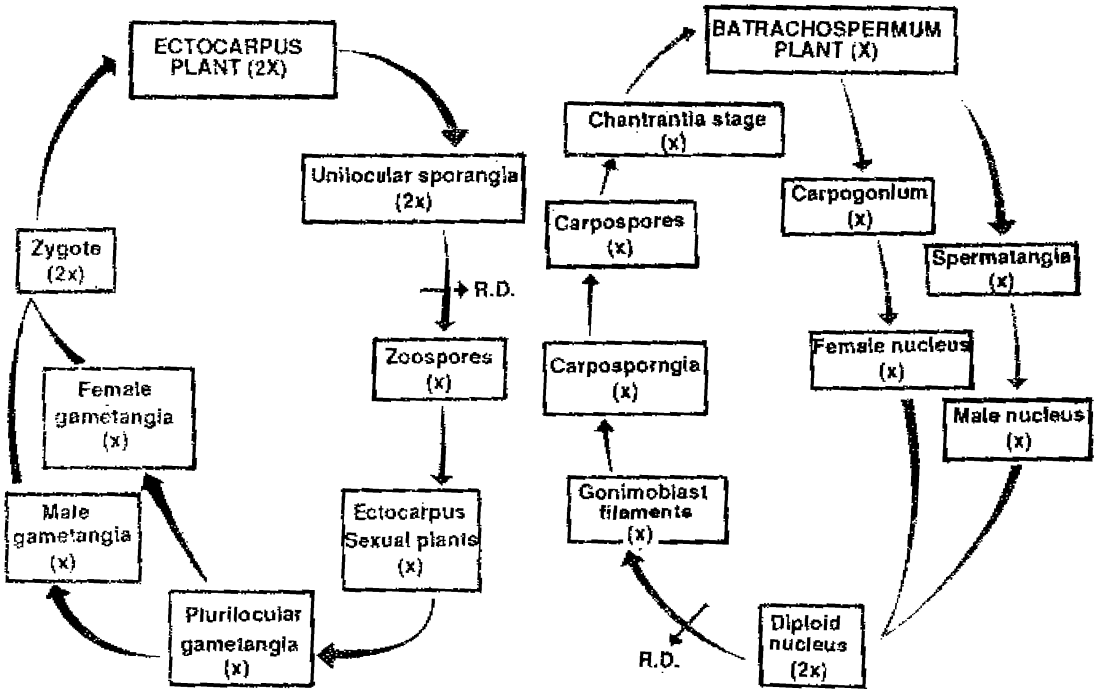
e. Diplobiontic life cycle:

In this, two diploid phases (carposporophytic phase and tetra sporophytic phase) alternate with one haploid phase (gametophytic phase) e.g. *Polysiphonia.* In this, apart from the carposporophyte (diploid), another diploid phase known as tetra sporophytic phase is developed by the germination of diploid carpospores, and this phase is morphologically and anatomically similar with those of gametophytes (male and female plants). Since the life cycle involves two diploid stages (tetra sporophytic phase and carposporophytic phase) and one haploid phase (gametophytic phase), the life cycle is said to be diplobiontic. Because there is no difference in the morphology of different phases, it is said to be isomorphic. Since the life cycle involves triphases, it is also known as triphasic or trigenic. There fore on the whole, the life cycle is said to be diplobiontic, isomorphic, triphasic, trigenic



HAPLONTIC LIFE CYCLE IN CHLAMYDOMONAS

DIPLONTIC LIFE CYCLE IN SARGASSUM



ISOMORPHIC LIFE CYCLE IN ECTOCARPUS

HAPLOBIONTIC LIFE CYCLE IN BATRACHOSPERMUM

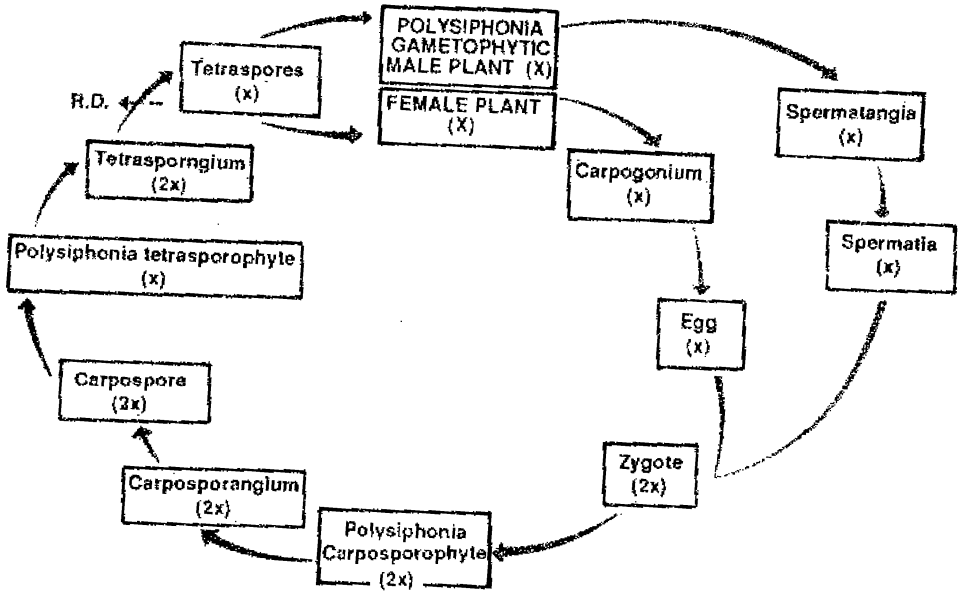


Fig. 12.12. Life Cycles in algae

12.6. ECONOMIC IMPORTANCE OF ALGAE:

During the past few decades, there is phenomenal increase in population and growth, there by the country is particular and the world is generally facing many problems with regard to the quality and quantity of food, feed, drinking water, disposal of sewage, industrial wastes, proper conservation of soil and water, agriculture etc. To tackle these problems, suitable controlled algal growth can provide possible help. Apart from this algae are also important as primary producers in aquatic habitat because of their photosynthetic activity.

12.6.1.Algae as component of ecosystem

Both fresh and marine water contains an enormous variety of algae, which constitute the fundamental or primary link of many diverse food chains. Algae synthesize organic foodstuffs, just as do the plants of the land. As the flesh of land is dependant upon the activities of green leaf, so the fish and other aquatic forms of animal life are dependant, directly or indirectly upon algae and fish and inturn they are important item in daily diet of larger sea animals and man. A number of aquatic algae from the food of fish either directly or indirectly. Diatoms, filamentous forms and some planktonic green algae, are very often found in guts of various species fresh and brackish water fish they appear to be directly utilized as fish food. The reserve food materials in these algae eg.fats and volutin in the diatoms, starch often accompanied by oil in the green algae, sugars and glycogen in the BGA and polysaccharides in *Euglena* are utilized by fish.

12.6.2.Algae in industry:

Many algal forms yield commercial products. They constitute raw materials for certain industries. Agar Agar, alginic acid derivatives, carragenin, funori, diatomite, iodine, potash, bromine are the important commercial products from algae.

Agar Agar:

Agar is a dried or gel like non-nitrogenous substance extract from red algae. It is a complex polysaccharide. It is extracted mostly from *Gelidium* and *Gracillaria.* Species of *Gigartinia chondrus, Ahnfeldita, Phyllophora* are also used for extraction of agar. The extract is a gel containing galactose and sulfate. It melts between 90^0 and 100^0F. It is extensively used as a base for culture media used in culturing bacteria, fungi and other tissues. Hence it is of great value in microbiological labs. It is also used as a stabilizer or emulsifier in food, cosmetics, leather and pharmaceuticals industries. In medicine agar is used as laxative. It is also used in tobacco and fruit cakes to serve a moisture retaining agent, in confectionery for making jelly candies, in drawing tungston wires as a lubricant, in photo films and plates and as coating material for capsules.

Algin and Alginates :

Algin is a derivative of alginic acid which is obtained from brown algae like *Laminaria, Sargassum* and *Fucus.* Alginic acid consists of D-manuronic acid2-Gluconic acid in various proportions. The sodium, potassium and magnesium salts of alginic acid are soluble in water and they give viscous solutions with out gel formation. Calcium alginates and other salts of copper, cobalt, mercury etc are insoluble in water. Algin possesses remarkable water absorbing quality. So, it is useful in various industries in which a thickening, suspending and stabilizing colloid is required. Alginates are the salts of alginic acid found in the cell walls of Phaeophyceae (*Sargassum* and *Turbinaria*). Alginates are used in the preparation of certain plastic articles and fireproof fabrics. Alginic acid can effectively stop bleeding and is employed as gauze in internal operations.

Diatomite:

1. The cell material of Diatoms is called diatomite. The silicious cell walls are insoluble in water and accumulates as sediments in sea and fresh water basins. They contain 88% of silica. It is also used as a basing automobile and silver polishes.
2. The diatomite is used mainly as filtration aid for oils, pains, varnishes, paper products.
3. It is an insulating material for high and low temperature furnaces. It is also employed in sugar refining and brewing industry, insulation of refrigerators, boilers, hollow file bricks for the construction of constant temperature rooms and soundproof rooms.

CARRAGHEENIN:

1. It is mostly extracted from *Chondrus crispus* (Irish moss) and to a lessor proportion from the species of *Gigartia*
2. Organic sulphate content is very high in these compounds and the chemical nature and properties of agaroits are different from agar agar.
3. It is an important component of both tooth paste, deodorants, cosmetics and paints.
4. It is also used as a cleaning agent for liquors beet sugar, juices and in leather finishing industries.

Iodine and other minerals:

Iodine and potash are obtained from brown see weeds like *Laminaria, Fucus* etc. and bromine from the red see weeds like *Rhodomela* and *Poltsiphonia.*

Funori or Glue:

Funori or glue is obtained from the species of *Gloeopeltis* in Japan. It is used as a sizing agent in paper and cloth industry and also used as an adhesive.

12.6.3.FISH FOOD:

1. The algae are fruit fully utilized in fish culture can very well be indicated from the successful culture of *Siamese* fish, *Tilapia mosambica* which is voracious feeder of filamentous algae.
2. A culture of *Scenedesmus* is often exclusively used as a daily dose of fishmeal for the culture *Tilapia mosambica.*
3. Besides serving as food material algae releases sugar in the aquatic medium and remove carbondioxide.
4. *Cladophora, Oedogonium, Spirogyra, Ulothrix, Pithophora* present in fresh water lakes and ponds directly serve as fish food.

12.6.4.ALGAE USED FOR RECREATIONAL PURPOSES:

1. Some algal forms are grown for their aesthetic properties in recreational areas like lakes and streams along with fish.
2. Spirogyra are grown in garden ponds
3. Species of *Hydrodictyon, Volvox, Desmids* and *Diatoms* are wonderful objects of nature when seen by naked eye or with the aid of microscope.

12.6.5. ALGAE USELFUL IN SEAWAGE TREATMENT PLANTS:

Species of *Chlamydomonas, Scenedesmus, Chlorella* and *Euglena* are used in sewage treatment plants for providing (through photosynthesis) the oxygen necessary for rapid decomposition of the sewage by bacteria.

12.6.6. ALGAE AND WATER SUPPLIES:

1. In the summer months the phytoplankton in ponds, lakes and reservoirs may become so abundant and water becomes cloudy and assumes a yellowish or greenish fringe.
2. A floating mat or scum is formed on the surface of water and one called as water blooms.
3. These water blooms are objectionable not only in public water supplies but also in water using for bathing, fishing and other recreational purposes.
4. BGA are mostly involved in the concentrations of water supplies, but the greens flagellated golden brown and diatoms are involved at times.
5. *Prymnesium parvum, Gymnodinium veneficum* and *Microcystis sps.* cause mortality of fish and domestic animals that drink water infested with these algae.
6. The living and the death and decaying algae impart disagreeable oily or fishy odors to the water.
7. The presence of algae in reservoirs as water blooms requires grater concentrations of chlorine for bacterial control and cause difficulties in filtration.

12.6.7.ALGAE AS THE ORIGIN OF PETROLIUM AND GAS:

The origin of oil and gas has been a mater of controversy but it is now generally believed that oil and gas were formed from organic matter in marine environments.

1. Planktons one main source of organic matter in seas. Minute marine algae captured the energy of sunlight, which was inturn transferred to animals that feed upon them.

2. Organic compounds derived from the plankton, both plant and animal accumulated in mud deposits in shallow waters of ocean floor. These materials were buried by sedimentary action, they converted into oil and gas in an oxygen free environment.

3. Natural gas is methane, which can be produced by certain anaerobic bacteria. Gas is associated with oil and can result from the action of methane producing bacteria upon organic matter.

12.6.8.ALGAE AND LIMESTONE FORMATION:

1. Many species of algae, they withdraw calcium from both fresh and marine water. They deposit it in the form of calcium carbonate in their cell walls or gelatinous sheaths. E.g., BGA, certain greens, reds etc.
2. BGA are chiefly important in fresh waters, they are responsible for the formation of extensive lime stones deposits around hot springs and glaciers.
3. Red algae are most important in the construction of coral reefs and islands.
4. They also play a significant role in the production of beds of lime stone rocks, which may be thousand feet thick.

12.6.9. ALGAE USED IN SPACE RESEARCH AND OTHER FUNDAMENTAL STUDIES:

CHLORELLA: Chlorella is being used in space research. It has been used for keeping the air in space vehicles pure on long inter planetary flights. The algae restore oxygen into the space vehicles by its photosynthesis. Species of *chlorella, chlamydomonas* and *Acetabularia* are used as tools for solving fundamental biochemical and genetical problems.

12.6.10. ALGAE USED AS FODDER:

1. Some kinds of algae such as *Rhodymenia palmata* and *Alaria esculenta* are favorable food for goats, cows and sheep.

2. Seaweed meal factories have been operating in U. S. A for several decades, providing supplementary feeds for poultry, cattle and hogs.

12.6.11. ALGAE USED AS FERTILIZERS:

1. Members of brown and red algae have been used in coastal areas as fertilizer both for improving the texture and fertility of rocky soils.
2. The weeds may be allowed to rot in the field and are composted with other organic materials. The resulting manner when added to agricultural fields enriches them in mineral nutrients like potassium, phosphorous, trace elements and growth regulators.

3. BGA like *Nostoc, Anabaena, Tolypothrix* etc., can fix the atmospheric N_2.
4. The N_2 fixing BGA grow luxuriantly in tropical fields (rice fields). The rice fields seeded with BGA increases N_2 content of the soil.
5. Farmers of Rajasthan are using algal mats consisting of *Anabaenopsis* and *Spirulina* as green manure.
6. BGA like *Nostoc, Scytonema and Anabaena* can be employed in the reclamation of alkaline and user lands.

12.6.12.Algae used as Food:

Large number of algae have entered into the diets of human beings from ancient times.

1. *Laminaria* and *Gracillaria* were used as food plants by Chinese several years ago.
2. The ancient inhabitants of Japan ate *Porphyra* as a healthful supplement to their rice diet.
3. Kombu, A Japanese food is prepared from the stipes of species of *Laminaria.*
4. Irish moss or Caragheen (*Chondrus Chrispus*) Which was cooked with milk, seasoned with Vanilla or fruit and made into a highly potable dish known as blankmanges. By jelling quality of Irish moss it is used as food.
5. Algae are rich in carbohydrates, vitamins (A,E, C & D) in organic substances, e.g., iodine (goiter is unknown among the people who eat sea weeds).
6. In Japan powered *Chlorella ellipsoidea* has been used successfully mixing with green tea.

12.6.13. Medicinal uses of algae:

1. Brown algae contain high iodine. Hence it is used in various goiter medicines.
2. By keeping patients on agar agar diet, the prolapsed stomach conditions can be treated.
3. The red alga *Simplex* is used as drug.
4. Physicians in the East use extracts from *Corallina* and *Codium* for the treatment of kidney, bladder and lung diseases.
5. Certain algal members are known to produce antibacterial substances effective against a number of pathogenic bacteria.
6. Chlorellin is obtained from the genus *Chlorella.*

12.7 Summary

The members of algae are cosmopolitan in distribution and occur predominantly in aquatic habitats. In the plant kingdom these members occupy first place, since they are considered to be earliest oxygen evolving photosynthetic organisms. The plant body ranges from a single cell to com-

plex gaint body. Though the thallus is well developed and reach many meters (30 meters) in length in some of the brown algae (e.g. *Macrocystis*),the thallus is not differentiated into root, stem and leaves. The thallus is mainly two types: 1) unicellular and 2) multicellular. Unicellular types may be motile or nonmotile. Multicellular thalli are 1) colonial (motile or nonmotile) 2) aggregations 3) filamentous unbranched 4) filamentous branched 5) heterotrichous 6) flattened leaf like or ribbon like 7) tubular 8) siphonous 9) polysiphonous 10) pseudo parenchymatous and 11) parenchymatous. Photosynthetic pigments differ from group to group, therefore, different groups exhibit different colours. Chlorophyll a is the main photosynthetic pigment in all the algal groups. The reserve food material also differs from group to group. Reproduction takes place mainly by three ways 1) vegetative 2) asexual and 3) sexual

The classification of algae is mainly based on pigmentation, storage products, flagella, cell wall and thallus organization. Different types of life cycles have been recognized. They are haplontic, diplontic, haplodiplontic or diplohaplontic, haplobiontic and diplobiontic.

From algae a number of commercial products like agar- agar, algin (alginates), caragheenin and diatomite are obtained. The blue green algae (cyanobacteria) place an important role in agriculture and serves as biofertilizers. Most of the members of algae are used as food for human beings, fishes and other aquatic animals as they are rich in proteins, fats and vitamins. Marine algae (seaweeds) are used as fodder for cattle and sheep and also used as poultry feed. Because of the antibiotic nature, blood anticoagulant properties, the extracts of algae are being used as medicines and in the preparation of tablets, pills, capsules etc. some green and blue green algae are used in the treatment of sewage and help in sewage disposal. There are some disadvantages also with the algae.

12.8. Key Termnology:

Acronematic: A flagllum which is smooth and tapered at its distal end

Alternation of generations: In a life cycle a haploid, gamete producing phase alternating with a diploid spore producing phase and the spores germinate into a haploid phase.

Aplanospore: An ontogenetically potential zoospore which lost the motility.

Benthos, Benthic: Bottom living, attached to or resting on the substrate.

Coenobium: a colony with a fixed number of cells arranged in a regular fashion at the time of its development and no furthur increase in cell number.

Coenocytic: multinucleate and with or without septa.

Phycobilin: Biliprotein pigments of blue green and red algae.

Thallus: a plant body which is not differentiated into vascularized roots, stems and leaves.

Zoospore: a flagellated asexual reproductive cell.

Zygote: The cellular product of gametic union.

12.9.MODEL QUESTIONS:

1.Describe the chief methods of reproduction in algae

2.Briefly describe the range of vegetative structure in algae.

3.Write essay on economic importance of algae

4.Write **short notes** on

a. pigments in algae

b. reserve foods in algae.

c. Life cycles in algae.

d. Flagella

e. Vegetative reproduction

f. Algae in industry

g. Algae as fertilizers.

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LESSON: 13

FUNGI: STRUCTURE AND REPRODUCTION

CONTENTS

13.1 OBJECTIVES :

By the end of this lesson you will be able to :

(i) understand what are fungi
(ii) describe the vegetative features of fungi and
(iii) describe the various means of asexual and sexual methods of reproduction.

13.2. INTRODUCTION

Fungi (L.fungus = mushroom) are achlorophyllous, heterotrophic, eukaryotic and spore bearing organisms, and whose usually filamentous, branched somatic structures are typically surrounded by cell wall containing cellulose or chitin, or both. The two features of fungi which are characteristic of them are – the absence of chlorophyll and reproduction by spores – asexual as well as sexual.

Fungi differ from other heterotrophic organisms like bacteria and animals in possessing eukaryotic cell structure and absorptive mode of nutrition respectively.

Fungi are a very large and divergent group of organisms, with more than 1,00,000 named species and many unknown species. They include such well known forms as mushrooms, toadstools, puff-balls, shelf fungi, molds, mildews, rusts and smuts. The branch of science which deals with the study of fungi is termed as **mycology** (Gr.mykes = mushroom, logos = discourse).

13.3. HABITAT :

The fungi are cosmopolitan in distribution and grow in diverse habitats. They grow in almost every habitat, wherever moisture and organic materials are available. A great majority of the fungi are terrestrial and a few are aquatic. Most of the fungi are saprophytic and grow on dead, decaying organic matter. A good number of fungi grow on living plants and animals parasitically and cause diseases. Some fungi grow on our food materials such as bread, jams, jellies, pickles, seeds, fruits and vegetables. Still some fungi make a symbiotic association with algae (lichens) or roots of higher plants (mycorrhizae).

Aquatic fungi live as parasites on fresh-water and marine organisms, or as saprophytes on dead bodies and residues of such organisms. The parasitic fungi greatly influence the ecology of aquatic habitats by causing sudden epidemics of phytoplanktonic algae and other populations like fishes. Aquatic fungi mainly belong to Mastigomycotina and Deuteromycotina.

Air does not serve as a habitat. Only, fragments and spores of terrestrial fungi adapted for aerial dispersal, constitute the 'air spora'. The composition of the air spora is governed only by physical factors of the moving air and not by any nutritional factors. Fungi in the atmosphere are mostly present in the 'troposphere'.

13.4.NUTRITION AND GROWTH REQUIREMENTS :

All fungi are devoid of chlorophyll, and hence heterotrophic in their nutrition. In a strict sense, they are **chemoheterotrophs.** They may be saprophytes or parasites. Saprophytic fungi or saprobes obtain their food from decaying and dead organic matter. They are necessary for maintaining the carbon and mineral cycles in nature. Parasites may be **ectoparasites** when they live on the external surface of the host or **endoparasites** when they live inside the host. The endoparasites are either intercellular or intracellular. Ectoparasites and intercellular parasites produce specialised absorbing organs called **haustoria**. Some fungi obtain their nutrients from other living organisms making a symbiotic association with them. These are called symbionts.

Fungi being heterotrophic, need the external supply of organic compounds as a source of energy and as carbon skeleton for the synthesis of various cellular components. In addition, they need elements like C, H, O, S, N, P, K, Mg, Mn, Fe, Cu, B, Mo and Zn. The essentiality of calcium for fungi has not been confirmed as yet. Glucose and nitrogenous compounds are the best sources of C and N respectively. While some fungi can synthesize their own **Vitamin** requirements, others can not do so and absorb them from the substratum. Excess food is usually stored in the form of glycogen or oil.

Fungi elongate by apical growth of the hyphae. The optimum temperature for their growth is 20^{o}-30^{o}C. The fungi thrive better in acidic medium (pH 5 to 6). Light is not essential for the growth of fungi, though it has been found to play some role in the dispersal of spores.

13.5.VEGETATIVE STRUCTURE

Fungi have two kinds of somatic structures. They are accordingly classified into **non-mycelial fungi** and **mycelial fungi.** The former group comprise the unicellular forms while the latter includes filamentous forms.

13.5.1.UNICELLULAR THALLUS

Some of the lower fungi have a very simple and primitive type of thallus. It is made up of a single cell. The entire thallus gets transformed either into a single reproductive structure **(holocarpic monocentric)** or into several reproductive units **(holocarpic polycentric)**. The example of the former is *Olpidium* and that of the latter is *Synchytrium*. In holocarpic forms, therefore, the somatic and reproductive phases do not exist together at a time.

On the other hand, thallus in most other fungi is differentiated into vegetative (rhizoid) and reproductive portions. Such thalli are called **eucarpic**. In Chytridiaceae and Rhizidiaceae, the thallus is evolved into a rhizomycelium which bears one reproductive structure (**eucarpic monocentric**). In Cladochytriaceae the rhizoidal mycelium bears several reproductive units (**eucarpic polycentric).** Holo corpic and eucarpic forms of chytridialean fungi are shown in Figure 13.1.

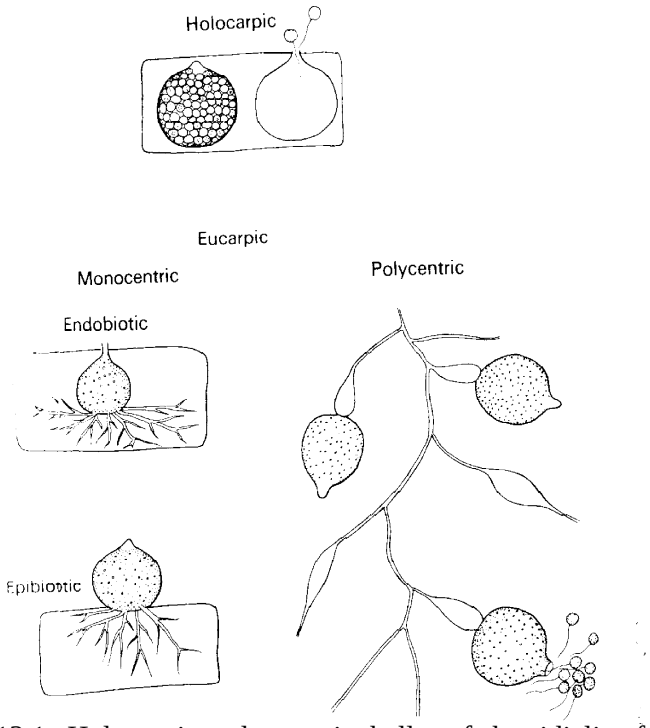

Fig. 13.1. Holocarpic and eucarpic thallus of chytridialian fungi

Generally, the unicellular true fungi have a cell wall. Sometimes the vegetative phase is a free-living, multinucleate, amoeboid mass of protoplasm without wall. Such a structure is called **plasmodium.** E.g. *Coelomyces.*

13.5.2. MYCELIAL THALLUS

The thallus of fungi is generally filamentous in nature. The filamentous and branched thallus is called the **mycelium**, and the tubular filaments are called **hyphae**. The hyphae may be septate or aseptate (Figure 13.2). Septate hyphae have cross walls that divide them into compartments (cells), whereas aseptate hyphae have no cross walls and nuclei lie in a common mass of cytoplasm. Such a condition is called **Coenocytic** and is found in some lower fungi. e.g. *Rhizopus, Mucor*. In septate forms, the cells may contain 1, 2 or more nuclei.

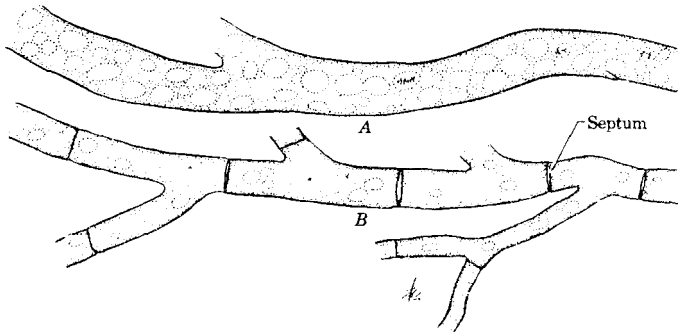

Fig 13.2. A. Coenocytic mycelium; B. Septate mycelium.

The septa may be complete or incomplete. Septa that delimit the reproductive structures are complete and do not contain a pore. Septa of this type are rare in vegetative hyphae. Incomplete septa possess one or more pores in them facilitating the movement of protoplasm from one cell to another. In some fungi, the septum is having a simple central pore. E.g. Ascomycotina and Deuteromycotina. However in Basidiomycotina excluding rusts and smuts, the septum is complex and the pore is surrounded by barrel shaped inflation of the septal membrane. Such a pore is called **dolipore** and the septum is called **dolipore septum** (Figure 13.3).

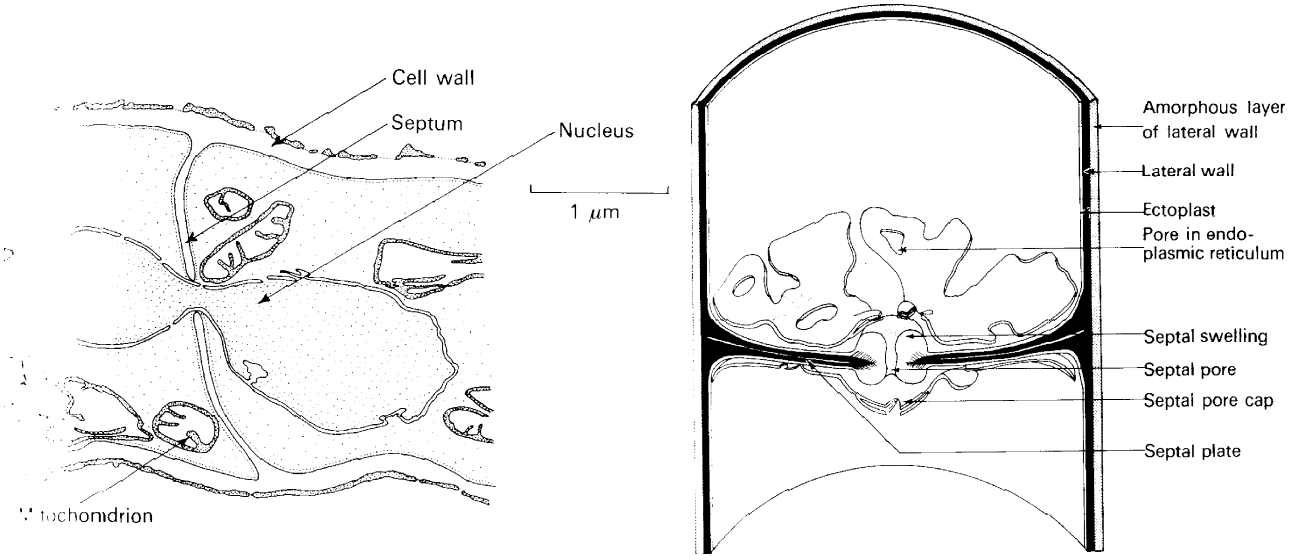

Fig. 13.3. A. Simple pore; B. Dolipore.

13.6.CELL STRUCTURE :

Fungi have eukaryotic cell structure. Except the chloroplasts, the cells possess all the double membrane cell organelles. The cells and hyphae are enclosed by a definite and rigid cell wall composed of various polysaccharides along with lipids, proteins and other substances. In majority of fungi the cell wall is made up of **chitin**, a polymer of N-acetyl glucosamine. But **cellulose** is present in the cell wall of **Oomycetes** along with glucan. In between the cell wall and plasma membrane, some membranous or vesicular structures are present. These are called **lomasomes**, whose function is not known. Cytoplasm contains cell organelles like mitochondria, ribosomes, endoplasmic reticulum, microtubules and vacuoles (Figure 13.4). Well developed dictyosomes are found only in Oomycetes. The cells may possess one or more genetically similar or dissimilar true nuclei. The

mitotic divisions are markedly different from other organisms. In fungi, the nuclear membrane does not disappear during prophase and metaphase. This type of non mitotic nuclear division has been termed as **Karyochorosis**. Meiosis is similar to that of higher organisms.

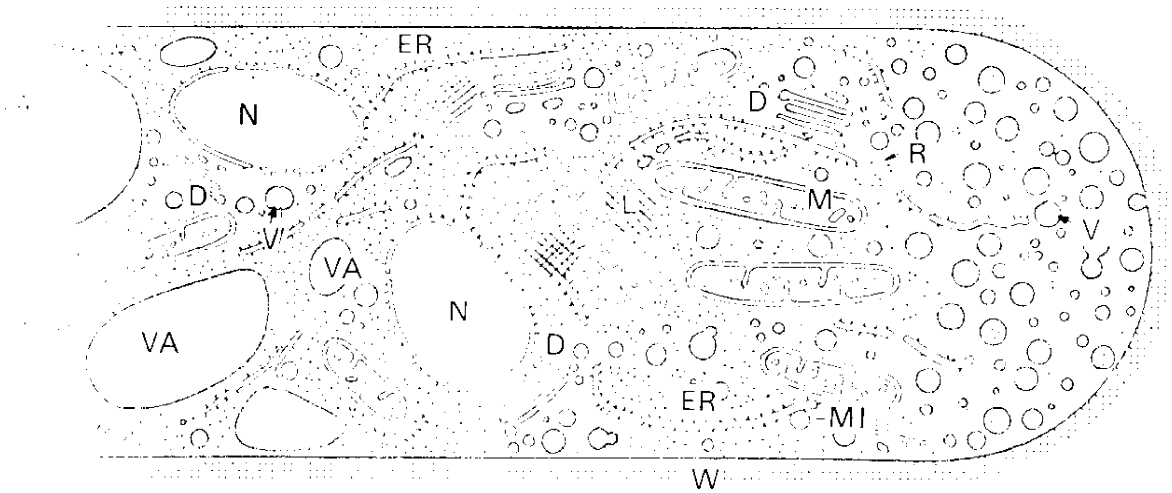

Fig. 13.4. Diagrammatic representation of hyphal apex. D, dictyosome; Er, endoplasmic reticulum; L, lipid body; M, mitochondrion; MI, microbody; N, nucleus; R, ribosome; V, cytoplasmic vesicle; VA, vacuole, W, wall.

13.7. AGGREGATIONS OF HYPHAE INTO TISSUES :

The mycelium of fungi consists of a network of loose hyphae. In many higher fungi, however, the hyphae grow together in groups, intertwine, adhere and from a lose or compact mass of tissue-like structure termed **plectenchyma** (Figure 13.5). Plectenchyma can be of two kinds.

(a) **Prosenchyma** : The hyphae are loosely interwoven and lie more or less parallel to one another.

(b) **Pseudoparenchyma** : The hyphae are very closely packed together,

lose their identity, and appear parenchyma like oval cells. The typical nature of the individual hyphae can not be distinguished.

Prosenchyma and pseudoparenchyma are the constituents of several kinds of somatic and reproductive structures of fungi. They are described below :

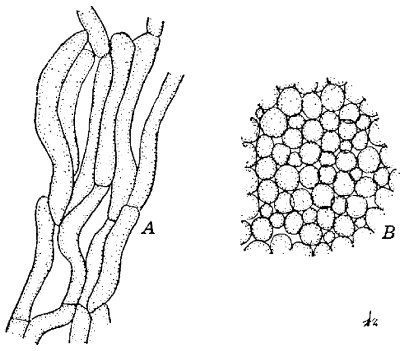

Fig. 13.5. Fungal tissues. A. Prosenchyma; B. Pseudoparenchyma.

(A) **STROMA** (Gl. Stroma = mattress) : The compact pseudoparenchymatous tissue in or on which the fruit bodies (sporophores) are formed is known as stroma. E.g. *Claviceps.*

(B) **SCLEROTIUM** (Gr. Skleros = hard) : In some higher fungi, the mycelium after vigorous active growth forms compact and hard masses of irregularly shaped structures called **sclerotia.** They may be spherical, cylindrical, pin head like, child's head or football like. They are generally black or purple outside and grey-white inside. They have rich amount of food reserve and serve as organs of perennation. They germinate when favourable conditions reappear. E.g.*Claviceps, Rhizoctonia solani, Sclerotium rolfsi.*

(C) **RHIZOMORPHS** (Gr. rhiza = root; morph = shape) : These are string-like or fine root-like elongated structures formed by closely placed parallel hyphae. They mostly develop in wood destroying fungi. Rhizomorphs are very characteristic of *Armillaria mellea,* the Honey Mushroom.

13.8.REPRODUCTION :

Fungi reproduce asexually as well as sexually. In some fungi, a third type of reproduction – parasexual reproduction is present.

13.8.1.ASEXUAL REPRODUCTION

It does not involve the union of nuclei, sex cells or sex organs. It is accomplished by one of the following methods.

(A) FRAGMENTATION : The hyphae break into a number of pieces or fragments either accidentally or through external forces. Each fragment develops into a new individual, if suitable conditions are available. It is a very common method of multiplication in Basidiomycetes and Deuteromycetes.

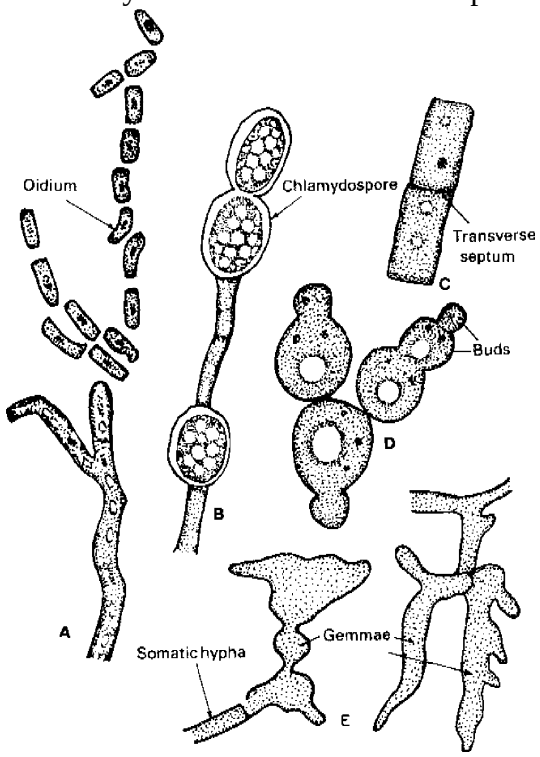

Fig. 13.6. Vegetative reproduction.
(A) Fragmentation of hypha to form oidia.
(B) Chlamydospores.
(C) Fission.
(D) Budding
(E) Gemmae formation.

(B) FISSION : This is the commonest and simplest method of vegetative reproduction especially in some yeasts. This involves the splitting of the cell into two daughter-cells by simple constriction or by a transverse wall.

(C) BUDDING : Budding is common in yeasts. The parent-cell produces small outgrowths, which gradually separate from the parent cell and develop into new individuals. Sometimes chains of buds are formed and appear like short mycelium. Ascospores and basidiospores also show budding.

(D) ARTHROSPORES or **OIDIA** : In some fungi, the hyphae break up into a large number of cells. Each cell rounds off, secretes a wall and behave like a spore. e.g. *Mucor, Geotrichum, Oidium.*

(E) CHLAMYDOSPORES : They are produced like oidia, but they differ from the latter in being thick-walled and coloured black or brown. They are formed from terminal or intercalary cells of a hypha. They take rest during unfavourable conditions and germinate in the presence of favourable conditions. The chalamydospores are characteristic of soil-fungi. e.g. *Phytophthora, Absidia, Mucor, Fusarium.* The term chlamydospore is also used to the thick walled dikaryotic spore, characteristic of smut fungi.

The above five types of reproduction or shown in Figure 13.6.

(F) SPORE FORMATION : Various types of spores, form the most common means of reproduction in majority of fungi. The spores show great variation in their form, and mode of origin.

According to the mode of development, fungal spores are broadly divided into two types : (a) Sporangiospores and (b) Conidia (Fig. 13.7).

(a) **SPORANGIOSPORES :** These spores are produced in a sac like structure called **Sporangium**. The contents of the sporangium are converted through cleavage into many spores. These are commonly produced by lower fungi like Mastigomycotina and Zygomycotina. The spores may be motile or non-motile. The flagellated spores are called **Zoospores** and the non-motile spores are called **aplanospores**. Zoospores may be uni-or biflagellate. In biflagellate zoospores, when one flagellum is of tinsel type and the other is of whiplash type, it is called **heterokontae.** Aplanospores are produced in Mucorales. They are disseminated by wind. e.g. *Rhizopus, Mucor.*

(b) **CONIDIA** : These are non-motile spores produced nakedly (exogenously) by constrictions at the ends of special hyphal branches, called the **conidiophores**. The conidia are produced in fungi belonging to Ascomycotina and Deuteromycotina.

The conidiophore may be unbranched (*Aspergillus)* or branched (*Penicillium*) and may or may not contain a vesicle. At the tip of the vesicle, or branched conidiophore are present small, flask-shaped structures, called **phialides** or **sterigmata**. The tip of each phialide cuts off a number of conidia, in basipetal chains.

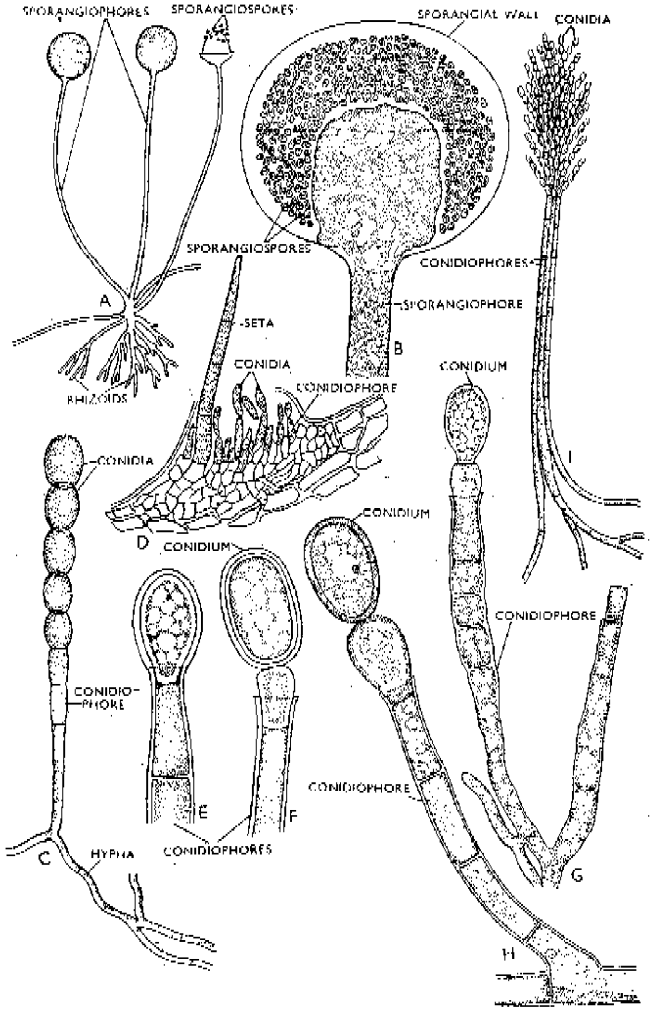

Fig. 13.7. Various types of fungal spores. A. Sporangia, sporangiophores and dispersal of sporangio spores in *Rhizopus stolonifer.* B.Sporangiospores borne in a sporangium of *Rhizopus stolonifer.* C. Conidia in chian borne on a conidiophore in *Erysiphe* sp. D. Section of acervulus of Colletotrichum sp. showing seta and conidia borne on conidiophores. E-H. Development of endoconidium from condidiophore in *Ceratostomella* sp. I. Synnema of *Stysanus* sp.

The conidiophores may be free from each other or they may group together forming a fascicle, called **synnema, acervulus, sporodochium** or **pycnidium** (Figure 13.8).

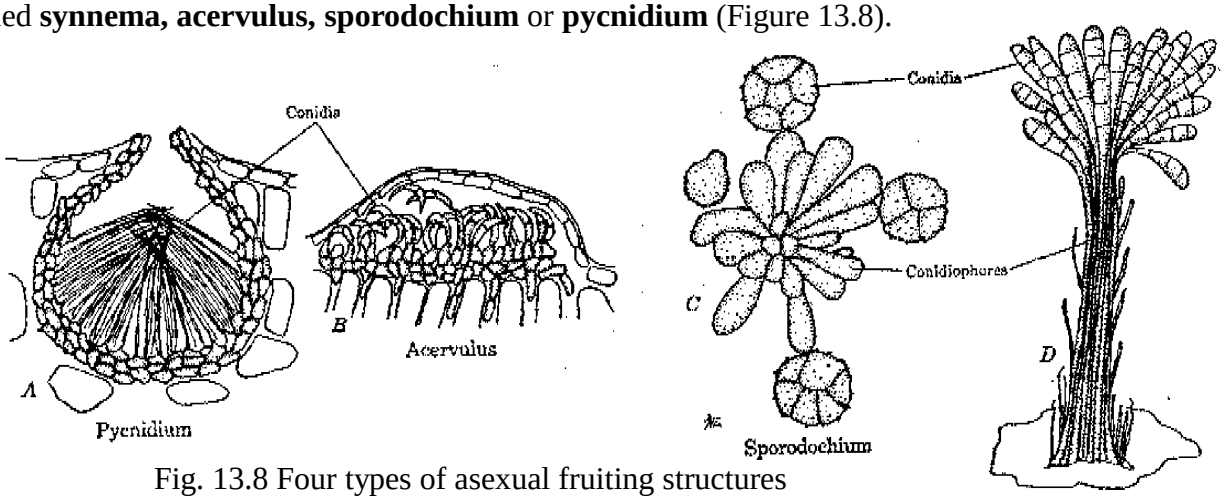

Fig. 13.8 Four types of asexual fruiting structures

13.8.2.SEXUAL REPRODUCTION

The sexual mode of reproduction is found in all groups of fungi except the group popularly known as the Fungi Imperfecti, or the Deuteromycotina. Just as in all other groups of organisms, the sexual reproduction in fungi also, involves three distinct phases - fusion of two protoplasts (plasmogamy), fusion of two compatible nuclei (karyogamy) to form a diploid zygotic nucleus, and the formation of haploid spores by meiosis. Fungal sex organs are called **gametangia**. Morphologically similar gametangia and gametes are called **isogametangia** and **isogametes**, respectively. In heterogametangia, the male gametangium is called **antheridium** and the female gametangium is called **Oogonium** or **ascogonium**.

Plasmogamy is brought about by any one of the following five methods:

A. PLANOGAMETIC COPULATION

This is the fusion between two gametes, in which one or both the gametes are motile. The gametes may be **isogamous** (e.g. *Synchytrium*) or **anisogamous** (e.g. *Allomyces*). Motile antherozoids and nonmotile oogonia are known only in one genus (e.g. *Monoblepharis)*. Planogametic copulation is the characteristic feature of Mastigomycotina.

B. GAMETANGIAL CONTACT

In some fungi (Oomycetes) distinct gametangia – **antheridium** and **Oogonium** are produced. When the gametangia are in contact with each other, the male nucleus migrates into the Oogonium through a short fertilization tube (e.g. *Phytophthora, Pythium, Albugo*) and fertilizes the egg. The fertilised egg is known as Oospore. In ascomycetes, female gametangium is known as ascogonium and male gametangium is called antheridium. When they come in contact with one another, male nuclei migrate from antheridium to ascogonium through a pore formed at the place of contact.(e.g. *Sphaerotheca, Erysiphae).*

C. GAMETANGIAL COPULATION

This is the complete fusion of two gametangia which are similar in all respects. The gametangial wall dissolves and their protoplasts fuse to give rise to a zygote, called **Zygospore**. This type of sexual reproduction is characteristic of fungi belonging to Zygomycotina. E.g. *Rhizopus, Mucor*.

D. SPERMATIZATION

It involves the union of a special minute, uninucleate, spore-like male structure called a **spermatium** (pl. spermatia) with a special female receptive hypha called the **trichogyne**. It occurs in some members of Ascomycotina and Basidiomycotina. The contents of the spermatium is transferred into the receptive hyphae, through a pore which develops at the point of contact between the two. e.g.*Podospora, Puccinia, Neurospora.*

E. SOMATOGAMY

In many of the higher Ascomycetes and Basidiomycetes, the sexual reproduction shows a gradual reduction or abortion of sex organs. Ultimately no sex organs are produced and the sexual act is accomplished by the union of two nuclei from somatic cells. Such a reproduction where somatic cells serve as sexual units is called **somatogamy** or **pseudomixis**. e.g. *Peziza, Agaricus.*

In lower fungi, the fusion of two compatible nuclei (karyogamy) occurs immediately after plasmogamy and a zygote is formed. Meiosis occurs in the diploid zygote at the time of germination. However, karyogamy is delayed in higher fungi, where the result of plasmogamy is a dikaryotic cell. This dikaryotic cell undergoes successive conjugate nuclear divisions and form several dikaryotic hyphae. These dikaryotic cells develop into specialized cells, **asci** (sin. Ascus) in Ascomycetes and **basidia** (Sin. Basidium) in Basidiomycetes, where Karyogamy and meiosis, occurs resulting in the formation of ascospores or basidiospores. This dikaryotic phase in the life cycle of a fungus, which lies between plasmogamy and karyogamy, is a unique feature of the fungi and is not found in other plant or animal groups.

Different types of sexual reproduction in Fungi are shown in Fig. 13.9.

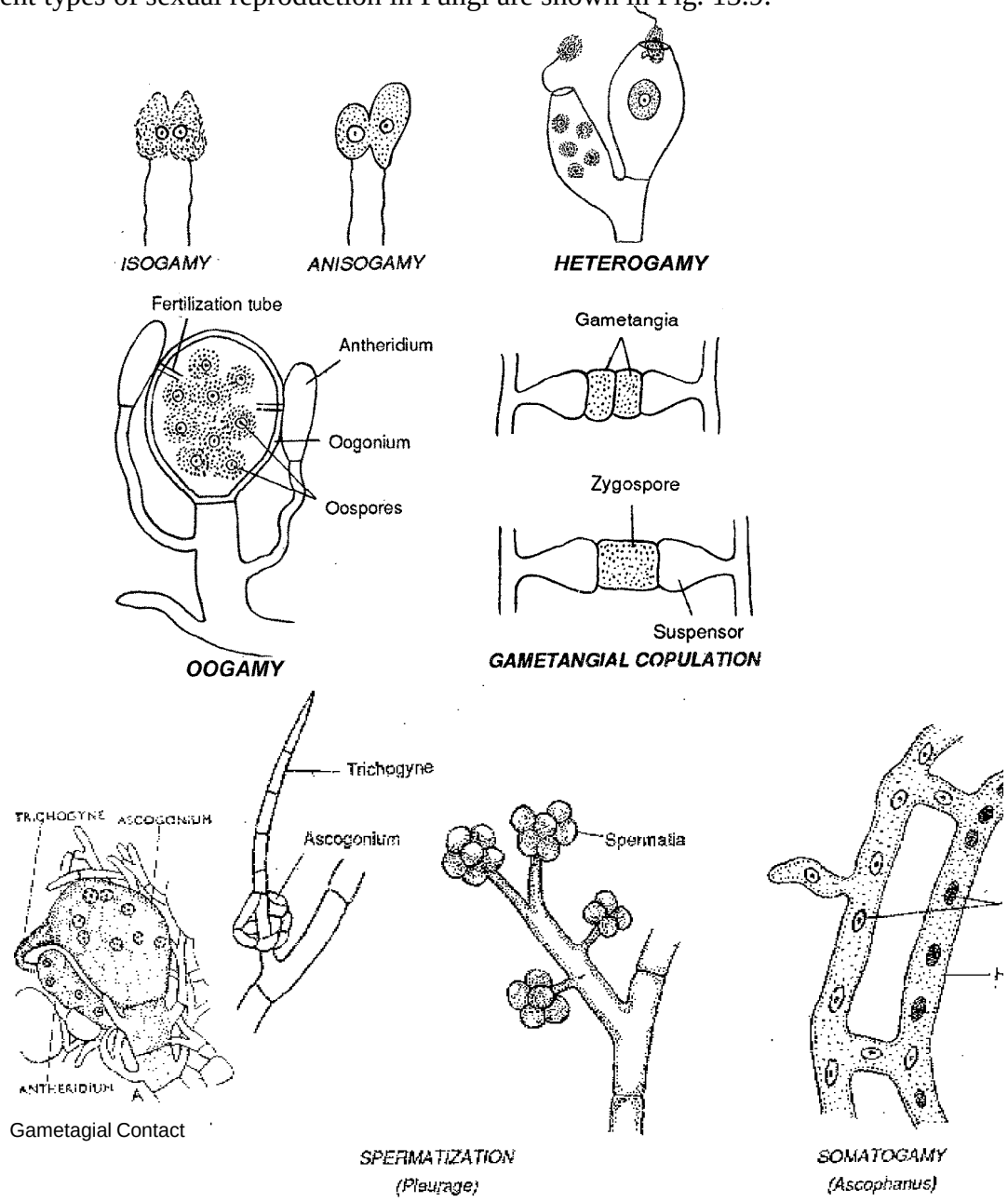

Fig. 13.9. Types of Sexual Reproduction in fungi.

13.9 HETEROTHALLISM

In many species of fungi, sexual reproduction is possible only if two compatible mycelia originating from two separate spores are brought together. Such fungi have been termed **heterothallic** and the phenomenon is called **heterothallism**. The species in which the mycelium from a single spore can give rise to compatible hyphae or sex organs are called **homothallic.**

In heterothallic species, the two interacting thalli are morphologically similar but differ physiologically. So one is often called as plus (+) and the other as minus (-) strain. The difference between male and female thalli depends on genetic factors conferring compatibility or incompatibility. It is determined by either two alleles at one locus (bipolar heterothallism) or by multiple alleles at one or two loci.

(a) **Bipolar (unifactorial) heterothallism**

Fungi in this category consists of two mating types of individuals, and the sexual compatibility is controlled by a pair of genetic factors or alleles 'A' and 'a' located at a single locus on homologous chromosomes. This is therefore called as **bipolar** or **two allele heterothallism**. The spores give rise to two types of thalli (+ and – strains) which must fuse to bring the two alleles together. This form of heterothallism was originally discovered by Blakeslee in Mucorales. Bipolar heterothallism is also present in ascomycetes members. e.g. *Neurospora, Sordaria, Ascobolus.*

(b) **Tetrapolar (bifactorial) heterothallism**

In these species, there are four mating types of thalli. The compatibility is governed by two pairs of factors, Aa and Bb. Thus it involves two compatibility loci each with a pair of alleles A/a and B/b. Only those thalli which combine to give rise to AaBb zygotes are compatible and fertile. e.g. Hymenomycetes and Gasteromycetes.

(c) **Multiple alleles at one locus**

In higher fungi, heterothallism involves a number (more than two) of alleles at either one locus, called **bipolar multiple allelomorph heterothallism**, or at two independent loci called **tetrapolar multiple allelomorph heterothallism**. e.g.Polyporaceae.

13.10. PARASEXUAL CYCLE

Parasexual cycle is a process in which "Plasmogamy, Karyogamy and meiosis take place, but neither in a regular sequence nor at specific stages". The recombination of hereditary properties which occurs during meiotic crossing over in sexual cycle, here in parasexual cycle occurs during the mitotic cycle. Parasexuality was first discovered in 1952 by Ponte Carvo and Roper in *Aspergillus nidulans*, the imperfect state of *Emericella nidulans*. Since then, parasexual phenomena have been identified in several imperfect fungi and in some basidiomycetes and ascomycetes. e.g. *Puccinia graminis, Ustilago maydis, Aspergilli, Fusarium, Penicillium, Pyricularia*.

In fungi, parasexual cycle involves the following stages :

1. The mycelium undergoes heterokaryosis by anastomosis or mutation.

2. Some of the haploid nuclei in the heterokaryon fuse in pairs to form diploid nuclei.

3. These diploid nuclei divide mitotically and during this process, haploidization occurs by a series of atypical and irregularly occurring mitotic divisions.

4. Chromosomes are progressively lost from the diploid nucleus by non-disjunction, to revert to haploid condition.

5. Some of these haploid nuclei may be a recombinant one derived from mitotic crossing-over.

When a mycelium with parasexual cycle produces fructifications the recombinant haploid nuclei are separated into the spores, which are genetically different from the parent mycelium. However, unlike the spores produced in sexual reproduction, where every spore produced (after meiosis) contains a recombinant nucleus; here only a few spores are different genetically.

13.11. SUMMARY

The fungi are cosmopolitan in distribution and grow in diverse habitats. The plant body is mostly an undifferentiated thallus. It may be unicellular or filamentous. The filamentous thallus is known as mycelium. All fungi devoid of chlorophyll and hence heterotrophic in their nutrition. Two general types of reproduction are recognised in fungi – Asexual and sexual.

13.12. SELF ASSESSMENT QUESTIONS

1. Describe briefly the salient features of fungi.
2. Give an account of various modes of asexual reproduction in fungi.
3. How do fungi reproduce sexually? Comment on Heterothallism in fungi.
4. Write short notes on :

(a) Plasmogamy in fungi

(b) Nutrition in fungi.

(c) Parasexual reproduction

(d) Spores in fungi.

SUGGESTED READINGS

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- B.R.C. MURTHY

LESSON – 14
FUNGI : CLASSIFICATION AND ECONOMIC IMPORTANCE

CONTENTS :

14.1. OBJECTIVES:

By the end of this lesson you will be able to:

(i) understand Ainsworth system of classification proposed for fungi. and
(ii) understand the significant role of fungi in nature.

14.2. AINSWORTH CLASSIFICATION

Ainsworth (1973) treated fungi as a separate Kingdom – **Kingdom Fungi.** It is divided into two divisions – **Myxomycota** (plasmodial fungi) and **Eumycota** (non-plasmodial, usually filamentous fungi). The outline classification is given below.

Division – 1	**Division – 2**
MYXOMYCOTA(4 classes) 1. Acrasiomycetes 2. Hydromyxomycetes 3. Myxomycetes 4. Plasmodiophoromycetes	EUMYCOTA (5 sub-divisions) 1. MASTIGOMYCOTINA (3 classes) a. Chytridiomycetes b. Hypochytridiomycetes c. Oomycetes
Division – 1	**Division - 2**
	2. ZYGOMYCOTINA (2 classes) a. Zygomycetes b. Trichomycetes 3. ASCOMYCOTINA (6 classes) a. Hemiascomycetes b. Plectomycetes c. Pyrenomycetes d. Discomycetes e. Loboulbeniomycetes f. Loculoascomycetes 4. BASIDIOMYCOTINA (3 classes) a. Teliomycetes b. Hymenomycetes c. Gasteromycetes 5. DEUTEROMYCOTINA (3 classes) a. Blastomycetes b. Hyphomycetes c. Coelomycetes

14.2.1 **DIVISION : MYXOMYCOTA**

Slime molds and other similar organisms are kept under Myxomycota. The somatic phase resembles an **amoeba** in structure and in the **holozoic** nutrition. They resemble fungi in the production of spores inside special reproductive structures called **sporangia**. Spores are surrounded by rigid cell wall made of cellulose.

The division Myxomycota is divided in to four classes.

1. **Acrasiomycetes** : These are commonly called **cellular slime molds.** Vegetative phase consists of naked amoeboid cells or myxamoebae which aggregate to form a **pseudoplasmodium** called **slug** or grex. Flagellated cells are lacking. Important genera of Acrasiomycetes are – *Dictyostelium, Polysphondylium, Prostelium* and *Acrasis.*

2. **Hydromyxomycetes** : These are commonly **net slime molds** because the thallus is made up of a network of branched tubes called **net plasmodium** or **filoplasmodium.** *Labyrinthula* and *Labyrinthuloides* are the commonly known genera. Two orders – Hydromyxales and Labyrinthulales are included in this class.

3. **Myxomycetes** : These are known as **true slime molds** or **acellular slime molds**. The vegetative phase is a naked, free-living, amoeboid mass of protoplasm called **plasmodium**. The spores develop within a persistent peridium. *Stemonites, Physarum* are the commonly known genera. The class includes 3 sub-classes, 6 orders and 10 families.

4. **Plasmodiophoromycetes** : The members are obligate endoparasites of vascular plants causing hypertrophy and hyperplasia of host cells. *Plasmodiophora brassicae* causes **club rot of crucifers** which is of universal occurrence. The class has a single order with a single family.

14.2.2.**DIVISION : EUMYCOTA**

Most of the fungi belong to the division - **Eumycota** (Gr. Eu = true; good + mykes = fungus). These fungi do not have any plasmodial or pseudo-plasmodial stage during their life-cycle. The division Eumycota was divided into five subdivisions – Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina.

14.2.2.1. **MASTIGOMYCOTINA**

The fungi included under this group are distinguished from other sub-divisions of Eumycota by the presence of flagellate zoospores. On the basis of number, position and type of flagella on the zoospores. This subdivision is divided into three classes:

Class 1. **Chytridiomycetes:** Zoospores are uniflagellate, flagellum posterior and whiplash type. The class includes 4 orders : **Chytridiales,**

Harpochytridiales, Blastocladiales and **Monoblepharidales.** *Synchytrium* and *Olpidium* are the familiar genera.

Class 2. **Hyphochytridiomycetes:** Zoospores are uniflagellate, Flagellum anterior, tinsel type. The class includes one order – **Hyphochytriales**, with three families.

Class 3. **Oomycetes**: Zoospores are biflagellate, with one **tinsel** flagellum and one **whiplash** flagellum. The flagella are inserted apically in **pyriform** and laterally in the **reniform** zoospores. The class includes 4 orders: **Saprolegniales, Leptomiales, Lagenidiales** and **Peronosporales**. *Phytophthora* and *Albugo* are the familiar genera of this class.

14.2.2.2. **ZYGOMYCOTINA**

These fungi are distinguished from other true fungi by the formation of thick walled **Zygosporangium which contains** a **zygospore** during their sexual reproduction. As compared to Mastigomycotina, these fungi reproduce asexually by non-motile spores called the **aplanospores**, produced within the sporangium. The thallus consists of well-developed mycelium that is **coenocylic.**

The Zygomycotina is divided into two classes – Zygomycetes and Trichomycetes.

Class 1. **Zygomycetes** : Majority of the members of this class are saprobic and a few are weak parasites attacking plants and animals. Some Zygomycetes are important **mycorrhizal** fungi. E.g. **Endogone, Glomus**. This class is divided into 3 orders: **Mucorales, Entomophthorales** and **Zoopagales**. **Mucorales** are ubiquitous in soil and dung, mostly as saprophytes, although a few are parasitic on plants and animals. E.g. *Mucor, Rhizopus, Pilobolus.* **Entomophthorales** include a number of insect parasites E.g. *Entomophthora.*

Class 2. **Trichomycetes** : The members of this class are obligate parasites or commensals within the digestive tract of living arthropods. Asexual reproduction takes place through **trichospores** (spores with hair-like appendages), **arthrospores** or **amoeboid cells.** This class is divided into 4 orders, each characterised by a distinctive type of asexual spore. They are : **Harpellales** (Trichospores), **Asellariales** (Arthrospores), **Eccrinales** (Sporangiospores) and **Amoebidiales** (amoeboid cells).

14.2.2.3. **ASCOMYCOTINA**

The subdivision Ascomycotina is the largest group of fungi, which include a wide range of diverse organisms – yeasts, black molds, green molds, powdery mildews and cup fungi. Most distinctive character of the group is the production, during

sexual reproduction, of special sac-like cell called **ascus**, within which the products of sexual reproduction, the **ascospores**, are formed. Except the Hemiascomycetes, the other members form large conspicuous fruit bodies, the **ascocarps**, that enclose the asci. In most of the Ascomycotina, karyogamy is delayed, which result in a **dikaryotic stage**, interspersed between plasmogamy and karyogamy. No flagellate zoospores are produced and asexual reproductive structures are non-motile.

On the basis of the type of ascocarp, the nature of the ascus wall and the mode of arrangement of asci, this sub division is divided into six classes.

Class 1. **Hemiascomycetes** : These are primitive and morphologically very simple ascomyetes. The mycelium is either poorly developed or totally absent, and a majority of the members are yeast-like. The asci develop singly and directly from the zygote. Ascocarps are totally absent. The class is divided into three orders as follows – **Protomycetales, Endomycetales** and **Taphrinales**. *Saccharomyces, Schizosaccharomyces, Taphrina* are the well known genera of this group.

Class 2. **Plectomycetes** : The asci are irregularly arranged within the ascocarp and do not form a **hymenium**. Ascocarp is typically a **Cleistothecium.** The ascospores are unicellular and are liberated out when the wall of the Cleistothecium breaks or degenerates. The asexual spores (conidia) are the chief means of dispersal of these fungi because the **perfect** or ascus stage is formed only under certain conditions. There is only one order – **Eurotiales** in this class, with 9 families. *Penicillium* and *Aspergillus* are the familiar genera of this class.

Class 3. **Pyrenomycetes**: The class includes the fungi in which ascocarps are surrounded by a peridial wall and contain **unitunicate asci** which are primarily arranged in a hymenial layer. The ascocarps are termed **Perithecia.** They are provided with an opening or **ostiole**, which is lined by **periphyses**. The members of this group grow on a wide range of substrata – soil, dung, decaying plant remains and woody hosts. Some, like **Claviceps** and **Nectria** are important plant pathogens; while some are fungal symbionts of lichens. E.g. *Verrucaria.* Species of *Neurospora* have been widely used in genetical and biochemical studies.

The class is divided into four orders - Erysiphales, Meliolales, Coronophorales and Sphaeriales.

Class 4. **Discomycetes** : The class includes the fungi commonly known as **cup fungi, earth tongue, morels** and **truffles.** These are mostly saprophytes growing on soil, humus, dead logs and dung. Some are important constituents of lichens. E.g. *Tuber, Cladonia*. The fruit body is a cup-shaped or saucer-shaped **Apothecium**. It consists of an open **hymenium**, with asci and intermingled paraphyses. All non-lichen forming

Discomycetes have unitunicate asci. *Peziza, Ascobolus, Morchella* are the familiar genera of this class.

The class includes seven orders. They are: Medeolariales, Cyttariales, Tuberales, Pezizales, Phacidiales, Ostropales and Helotiales.

Class 5. **Laboulbeniomycetes**: The members of this group are minute insect parasites. The fungi have small superficial thallus with perithecia bearing trichogynes and antheridia with non-motile spermatia and do not produce true mycelium. This class is divided into two orders.

Class 6. **Loculoascomycetes**: These are ascomycetes with bitunicate ascus and **septate** ascospores produced in **Pseudothecia**. These members occur as superficial epiphytes, parasites or hyperparasites of fungi and insects. Some of them are serious plant pathogens. E.g. *Venturia inaequalis* and *Mycosphaerella musicola.* This class is divided into five orders and 31 families.

14.2.2.4.**BASIDIOMYCOTINA**

The subdivision Basidiomycotina include true fungi in which the perfect – state spores are called **basidiospores**. The basidiospores are the meiospores and are produced on the outside of a specialised spore-producing structure, the **basidium.** The fruit bodies called **basidiocarps** which are the most attractive and beautiful of all fungi and are called **fungus flowers**. The other distinguishing features of this group include – (i) Highly developed dikaryotic mycelium, (ii) Formation of structures called clamp – connections and (iii) dolipore septa in some of the members. The fungi commonly known as mushrooms, toadstools, puffballs, earthstars, stinkhorns, rusts, smuts, bracket fungi and jelly fungi are included in this group.

Basidiomycotina is divided into three classes – Teliomycetes, Hymenomycetes and Gasteromycetes.

Class 1. **Teliomycetes :** Basidiocarps are absent and are replaced by chalamydospores (smuts) or teleutospores (rusts). Members of this group are important plant pathogens – the **rusts** and the **smuts**. These fungi differ from rest of the Basidiomycotina in the absence of dolipore septa, clamp connections and basidiocarps. The class Teliomycetes includes two orders: **Uredinales** (which include rusts) and **Ustilaginales** (which include smuts).

Class 2. **HYMENOMYCETES**: This is the largest group of Basidiomycetes, and includes many of the well known macro fungi. The basidia are often arranged in a palisade-like fashion to form a **hymenium**, which is fully exposed at maturity. The class is divided into two sub classes, on the basis of basidial structure.

Sub Class: **PHRAGMOBASIDIOMYCETIDAE** : The members of this class are characterised by a divided basidium (phragmo-basidium). The basidiospores are **repetitive** i.e. germinate by producing secondary spores. The basidiocarp is often gelatinous or waxy in nature. The sub class includes three orders – **Tremellales, Auriculariales** and **Septobasidiales**.

Sub Class:**HOLOBASIDIOMYCETIDAE** : The members of this class are characterised by an undivided, cylindrical to club shaped basidium (i.e. **holobasidium**) from which arise four sterigmata, each bearing one basidiospore. The basidiospores are non-repetitive i.e. do not bud off secondary spores. This group contains the most conspicuous fungi such as mushrooms, pore fungi, tooth fungi, boletes and bracket fungi.

This sub-class is divided into six orders – Exobasidiales, Brachybasidiales, Dacrymycetales, Tulsasnellales, Aphyllophorales and Agaricales.

Class 3. **GASTEROMYCETES :** This is an unnatural assemblage of basidiomycetes which share the common character that their basidiospores are passively released (i.e. **Statismospores**). All members of this group are saprobic on rotting wood, dung and on soil. *Rhizopogon* forms sub-terranean fruit bodies, and *Scleroderma* form mycorrhiza with forest trees. *Lycoperdon (pull-ball), Clavatia, Cyathus* (Birds' nest fungus) and *Phallus* (stink horn) are the familiar genera of this group.

This class includes nine orders: (i) Sclerodermatales, (ii) Melanogastrales, (iii) Tulostomatales, (iv) Lycoperdales, (v) Nidulariales, (vi) Phallales, (vii) Gautieriales (viii) Hymenogastrales and (ix) Podaxales.

14.2.2.5. **DEUTEROMYCOTINA**

This is an artificial assemblage of fungi reproducing exclusively by asexual means, usually by **conidia.** Their sexual (perfect) stages either do not exist at all or are yet to be discovered. Since all these fungi apparently lack a sexual phase, these are also called **imperfect fungi**, or **Fungi Imperfect**. All the taxa included in this group are artificial and their status is formal. This is indicated by using the prefix **form** before these taxa – **form family**, **form order**, **form class, form genus, form species** and so on. However, in practice, the prefix "form" is usually not written though implied. The members of this group may be considered as "**the conidial stages of Ascomycotina or – more rarely – Basidiomycotina, whose sexual stages have not been discovered or do not exist.**"

The subdivision Deuteromycotina is divided into three classes: Blastomycetes, Hyphomycetes and Coelomycetes.

Form Class : **BLASTOMYCETES** : True mycelium is absent, or poorly developed; plant body is yeast-like and shows budding. The class includes two families: **Sporobolomycetaceae** (ballistospores formed), **Cryptococcaceae** (ballistospores absent). *Candida, Crytococcus, Torulopsis* are the well known forms.

Form Class : **HYPHOMYCETES** : These members are characterised by well developed mycelium, and the absence of pycnidia or acervuli. Hyphomycetes include four orders – **Agonomycetales** (Conidia absent), **Stilbellales** (Formation of Synnemata), **Tubercularíales** (Formation of Sporodochia) and **Hyphomycetales** (Conidiophores not organised as synnemata or sporodochia). The order Hyphomycetales (= Moniliales) comprises the main body of the Hyphomycetes; and is divided into two families.

i) Form Family: **Moniliaceae** E.g. *Aspergillus, Penicillium, Trichoderma, Verticillium* etc.

ii) Form Family: **Dematiaceae** E.g. *Alternaria, Curvularia, Helminthosporium, Curvularia, Cercospora.*

Form Class: **COELOMYCETES** : The Conidia are formed either in **pycnidia** or **acervuli**. The class includes two orders – **Melanconiales** (fruiting body acervulus) and **Sphaeropsidales** (fruiting body pycnidium).

The order **Melanconiales** contains a single family – **Melanconiaceae**. About 120 genera are included in this family. Most of these are parasitic, causing plant diseases known as **anthracnoses**. Some of the most common genera are – *Colletotrichum, Pestalotia*, *Pestalotiopsis, and Monochaetia.*

The order **Sphaeropsidales** contains four families – **Sphaeropsidaceae, Discellaceae, Nectrioidaceae** and **Leptostromataceae**. The genera included in this order are **leaf-spot** causing fungi. E.g. *Phyllosticta, Phoma, Phomopsis, Diplodia, Botryodiplodia, Septoria* etc.

14.3. ECONOMIC IMPORTANCE OF FUNGI

Fungi are found in every conceivable habitat and bring about a number of changes in the habitat due to their activities. Some of these changes are useful to man, while some of them are harmful.

I. Useful activities :

The history of mycology is intimately associated with understanding of the role of fungi in diseases of crop plants, and for many people the word '**fungus**' implies spoilage. However, there are many aspects of fungal activity which are beneficial to man. Some of the important activities are as follows :

1. **Soil Fertility :**

Along with bacteria, saprophytic fungi decompose huge amounts of plant, animal and human wastes and bring the mineral nutrients back into the soil and atmosphere. Thus they play an important role in bio-geo-chemical cycles.

Fungi like *Absidia, Mucor, Rhizopus, Aspergillus, Cladosporium* and *Penicillium* have soil binding properties by secreting mucilaginous substance. Thus they play an important role in soil aggregation.

Many fungi exist in symbiotic association with the roots of plants, called **mycorrhizae.** Such an association is beneficial for the host as well as the fungus. Mycorrhizae enhance mineral absorption by green plants. A majority of deciduous and evergreen trees have ectomycorrhizae, their roots are surrounded by the hyphae of fungi belonging to the genera like *Amanita, Phallus, Tricholoma* and *Scleroderma.*

2. **Industrial Uses** :

Fungi have two major uses in industry (a) as a source of chemicals and (b) as food or food processing agents.

(a) **Source of Chemicals** :

Fungi produce a wide range of useful compounds like organic acids, alcohol, antibiotics etc. of these, antibiotics are the most familiar. Characteristically, these are produced when the main phase of growth is over, so they are classified as **secondary metabolites**.

The most popular and useful antibiotics produced by fungi are listed below.

Name of the antibiotic	**Producer Organism**	**Active against**
1. Penicillin	*Penicillium notatum* *P.chrysogenum*	Gram positive and gram negative bacteria
2. Cephalosporium	*Emericellopsis minimum* *Acremonium Sp.*	G(+) ve bacteria
3. Griseofulvin	*P.griseofulvum*	Anti fungal
4. Fusidic acid	*Fusidium coccineum* *Mucor ramannianus*	G (+) ve bacteria

Apart from antibiotics, fungi are exploited for the production of five other major chemicals. (i) production of alcohol (ii) production of organic acids (iii) production of glycerol, lipids (iv) modification of steroid compounds and (v) production of extra cellular enzymes. A list of some of these useful compounds obtained from fungi is given below :

Table. Useful compounds obtained from fungi.

Chemical		Source
1. Alcoholic Beverages	-	*Saccharomyces sp.* *Aspergillus oryzae.*
2. **Organic acids**		
Gallic acid	-	*Aspergillus niger, Penicillium glaucum*
Lactic acid	-	*Rhizopus oryzae*
Citric acid	-	*Aspergillus niger*
Itaconic acid	-	*Aspergillus terreus*
Gluconic acid	-	A.*niger*
Kojic acid	-	*A.oryzae, A. flavus.*
3. Glycerol	-	*Yeasts (Zygosaccharomyces)*
	-	*acidifaciens, Sacharromyces*
	-	*rouxii, S.mallis)*
4. Lipids	-	*A.niger, Absidia, Rhizopus, Mortierella*

5. **Enzymes**			**Important application**
Diastase	- *A. oryzae*	-	Manufacture of glucose syrup
Amylase	- *A. oryzae* *A. niger*	-	Digestive aid
Invertase	- *S.cerevisiae*	-	Candy manufacture
Pectinases	- *A. oryzae* *Sclerotina libertina*	-	Clarification of fruit juices
Cellulose	- *Trichoderma koningi*	-	Digestive aid

Vitamins		
Vit. B.complex and Riboflavin	-	*S. cerevisiae*
Riboflavin	-	*Eremothecium ashbyii. (or) Ashbya gossypii*

Alkaloids		
Ergotinine, Ergotamine and ergonovin	-	*Claviceps purpurea* (ergot fungus)
L-ephedrine	-	*Ephedra spp., Yeasts*
Fumigaclarin	-	*Aspergillus fumigatus.*
Steroids		
Hydrocortisone	-	*Cunninghamella blakesleeana*
$\propto$-hydroxyprogestrone	-	*Rhizopus arrhizus.*

(b) **Food and food processing :**

A wide range of food products are obtained from fungi which are relatively more popular in the west. Fungi are also used to make foods palatable, to increase their protein content and also to produce single cell protein (SCP).

(i) **Mushrooms** : Fleshy fruit bodies of *Agaricus, Clavaria, Helvella, Morchella, Volvariella pleurotus* etc., are widely cultivated and is considered as a delicacy.

(ii) **Fermented foods** : A variety of fermented products like Idlies, jalebies, Kanji, Panjabi warries are consumed frequently in Asian countries including India. The fungi responsible for these fermentations are :

Torulopsis candida and Trichosporon pullulans - Idli
Sacchoromyces bayanus - Jalebies
Hansenula anomala - Kaanji
Candida spp. And *S.cerevisiae* -Warries.

Many Asian and oriental foods and drinks are based on fungal fermentation. These include Tempeh, Soy sauce, Tapioca, Tofu and Sufu.

Tempeh is an Indonesian food produced from soybeans. The soyabeans are soaked at 25^{o}C, dried and inoculated with spores of *Rhizopus oligosporus.* The mash is incubated at 32^{o}C for 32 hours, during which mycelial growth occurs. Three other species of *Rhizopus* namely *R. stolonifer*, *R.oryzae* and *R. arrhizus* are also used.

Soy sauce or shoyu, is a brown, salty, tangy sauce produced from a mash consisting of soybeans, wheat and wheat bran. It is produced by the fermentation action of *Aspergillus oryzae, S.rouxii, Zygosaccharomyces soyae* and *Torulopsis* species.

Miso or soy cheese is produced from soybeans by fermentation with *Aspergillus oryzae*. Steam polished rice, placed in shallow trays, is used in the production of starter culture.

Cassava is a tropical crop, which is used in its processed form known as **tapioca.** The tubes are fermented by using a suitable fungus.

(iii) **Cheese** : Various fungi are used in the ripening of different types of cheeses. The unripened cheese is inoculated with fungal spores and incubated in a warm, moist room to promote the growth of filamentous fungi.

Roquefort cheese, Camembert cheese and Brie cheese are produced by using *Penicillium roqueforti, P. camemberti* and *P. candidum* respectively.

(iv) **Single-cell protein (SCP)** : Bacteria and yeasts are more preferred for single cell protein because they can be easily grown on large scale and some use methane and n-alkanes (by – products of the oil industry) as their sole energy sources. Some mycelial fungi also use n-alkanes and they have some advantages over bacteria for SCP production. *Penicillium,*

Fusarium and *Candida* are now used to produce SCP on a pilot scale.

3. **Fungi as Test organisms :**

Some fungi are used to assay potency of drugs and to detect the presence of some chemicals. *Neurospora crassa* is used for Vitamin B-Complex. **Aspergillus niger** and **Scopulariopsis** are used for detection of arsenic and estimation of copper in soil. *Phycomyces blakesleenus* and *Nematospora gossypii* are used for the estimation of *Thiamine*.

4. **Biological Control of Pests** :

Some fungi are antagonistic to and hyperparasitic on other fungi as well as bacteria, nematodes and insects which are serious plant pathogens. These fungi may be used as agents of biological control of diseases. Many yeasts and other phylloplane fungi inhibit pathogenic fungi on aerial parts of plants. Pine stumps are protected from infection of *Heterobasidium annosus (= Fomes annosus*) with the help of *Peniophora gigantea*. The oidia of *p. gigantea* are mixed into the **chain** – saw oil. *Trichoderma harzianum* could effectively control *Sclerotium rolfsii.* A preparation of *Beauveria bassiana* is used for controlling colorado potato beetle. *Coelomomyces* is an aquatic fungus which attacks mosquito larvae and kills them within a short period.

5. **Fungi as Research Tools :**

Many fungi provide excellent material for the study of various fundamental biological processes especially in the field of cytology, genetics, biochemistry and molecular biology. *Neurospora crassa* was used by Beadle and Tatum for elucidating one gene – one enzyme hypothesis. *Physarum polycephalum* is a very good material for the study of DNA synthesis, morphogenesis, mitotic cycle and many other processes.

II. **HARMFUL ACTIVITIES :**

A wide range of fungi are harmful to man, animals and plants.

1. **Destruction of Timber and Timber Products** :

A variety of fungi attack living trees resulting in their weakening or death. The timber and timber products are subject to decay by fungi.

Fungi like *Fomes annosus, Armillaria mellea* and *Fistulina hepatica* cause serious rots in the standing trees. If the cellulose component of wood is destroyed and the brown lignin content is left, it is called **brown rot**. On the other hand, if both cellulose and lignins are attacked, so that wood appears white, it is called **white rot.** Indian wood is chiefly decayed by *Polyporus tomentosus, P. shoreae, P. annosus, P. pini, Fomes annosus* and *Ganoderma* Sp.

Some fungi grow in the sapwood and cause its staining. Such fungi include *Lasiosphaeria pezizula, Penicillium divaricatum* and *Fusarium negundi.*

2. **Spoilage of Paper and Paper goods :**

Extensive decay of pulp wood is caused by *Polystictus hirsutus, P. versicolor, P. abietines, Polyporus adjustus, Stereum purpurem* and many other fungi. *Cladosporium herbarum, Trichoderma lignorum* and *Hormonema pullulans* colonize wood and cause pulp staining and discolourization the pulp. Species of *Aspergillus, Mucor, Penicillium, Cladosporium, Chaetomium, Trichoderma* and *Alternaria* build up slime on the surfaces over which paper pulp moves in paper mills. This results in the formation of translucent spots in the finished paper.

3. **Deterioration of articles :**

Deterioration of leather, textiles, plastics, waxes, rubber, painted surfaces, wool etc., is caused by *Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus, Chaetomium* etc.

4. **Food Spoilage :**

Food products which serve as sources of nutrition for human beings and other animals, also serve as substrates for the growth of microorganisms including fungi. Improper handling of food products may lead to the growth of pathogenic or non-pathogenic fungi. Their presence induce changes in the food such as decreased nutritional content, altered taste, odour, colour and texture i.e. spoil the food and thus render it unsuitable for human consumption. Fruits, Vegetables, milk and milk products and various other food products are subjected to spoilage. Most familiar example of spoilage of food is that of **Rhizopus stolonifer**, the 'bread mold' on bread. Quite a good number of other fungi grow in bread e.g. Aspergillus niger, Penicillium, members of mucorales. Calcium propionate or other mold inhibitors are added in bread to keep it free from molds for a few day. Pickles and sauerkraut are commonly spoiled by film yeasts, pink yeasts i.e. **Rhodotorula** Sp. Spoilage of cured meat is commonly caused by species of *Aspergillus, Penicillium* and *Rhizopus*.

The fungi associated with the grains during storage include the species of *Aspergillus, Penicillium, Phoma, Alternaria, Cladosporium, Fusarium, Curvularia, Drechslera, Mucor* and several others.

Some fungi produce harmful substances called **mycotoxins** in food and cause food poisoning. This is called **mycotoxicosis**. Some of the important mycotoxins of food and feed stuffs and organisms producing these are listed below.

Food/feed stuff	**Mycotoxin**	**Disease**	**Fungi**
1. Groundnut, cotton seed, copra	Aflatoxins	Aflatoxicoses	*Aspergillus flavus A. parasiticus.*

2. Barley	Ochratoxin A+B	Nephrotoxicosis	*A. ochraceous* *Penicillium visidcatum*
3. Apple fruits, Bread & Bakery	Patulin	Neurotoxicosis	*P. patulum* *P. expansum.*
4. Cattle feeds	(a) Fumitremorgin (b) Verruculotoxin	Neurotoxicosis Neurotoxicosis	*Aspergillus fumigatus* *Penicillium Uerraculosum*
5. Cereals	Citrinin	Nephropathy	*Penicillium citrinum.*

5. **Plant Diseases :**

Fungi are amongst the important causal agents of plant diseases. The role of fungi in causing diseases can be judged by the fact that some groups of fungi are known commonly by the diseases they produce. For example, Peronosporaceae, Allbuginaceae, Erysiphaceae, Ustilaginales and Uredinales are commonly known as downy mildews, white rusts, powdery mildews, smuts and rusts respectively. Some of the most commonly known diseases of plants caused by fungi are as follows :

Black wart disease of Potato – *Synchytrium endobioticum*
Late blight of Potato - *Phytophthora infestans*
Early blight of Potato - *Alternaria solani*
White rust of Crucifers - *Peronospora Viticola.*
Club root of Crucifers - *Plasmodiophora brassicae*
Ergot of rye – *Claviceps purpurea*
Loose smut of Wheat – *Ustilago nuda var. tritici*
Loose smut of Maize – *Ustilago maydis.*
Whip smut of Sugarcane – *Ustilago scitaminae*
Black stem rust of Wheat – *Puccinia graministritici*
Brown rust of Wheat – *Puccinia recondita*
Yellow rust of Wheat – *P. striiformis.*
Red rot of Sugarcane – *Colletotrichum falcatum*
Brown spot of rice – *Helminthosporium oryzae.*
Wilt of arhar – *Fusarium udum.*

6. **Diseases of man :**

Fungi cause three types of diseases, collectively called **mycoses,** in man –

(A) **Dermatomycoses :** These are chronic diseases characterised by small, raised patches of skin , which become scaly, or even blisters. There are generally known as ring worm, athlete's foot, barber's itch or dhobi itch. **Epidermophyton floccosum** is quite common in Vishakhaptanam. However, the species of **Trichophyton** especially **T.rubrum** is common in all parts of India.

(B) **Subcutaneous Mycoses**: The fungus invades the wounds and produces subcutaneous nodules. The most common subcutaneous mycoses are – Mycetoma and Sporotrichosis.

Mycetoma also known as **Madura foot** is quite common in India and is caused by **Allescheria boydii, Madurella mycetomi, M.grisea** and **Aspergillus** Spp. They affect the foot principally causing ulceration, granulation and necrosis of bones.

Sporotrichosis, also called **Gardener's disease,** affects primarily the skin of hands, arms and legs; and is caused by **Sporothrix schenckii.**

(C) **Systemic Mycoses :** These are usually acquired by inhaling the spores of free-living fungi, and usually begin as lung infections.
Coccidiomycosis – Coccidiodes immitis
Blastomycosis – Blastomyces dermatitidis.
Histoplasmosis – Histoplasma capsulatum
Cryptococcosis – Cryptococcus neoformans
Aspergillosis – Aspergillus fumigatus, A. niger, A. flavus

14.4 SUMMARY

The fungi characterised by the absence of chlorophyll and possession of thallus like body, are a fascinating group of organisms. They influence human life tremendously either directly or indirectly. They play an important role in medicine, agriculture and in industry. Since the beginning of 20^{th} century, several classifications have been proposed on fungi. However, the classification proposed by Ainsworth (1973) is considered to be the standard one.

14.5. SELF - ASSESSMENT QUESTIONS

1. Briefly discuss the system of classification proposed by Ainsworth
2. Write an essay on the economic importance of Fungi.
3. Write short notes on :
   (a) Edible fungi
   (b) Fermented foods.
   (c) Industrial uses of fungi
   (d) Human fungal diseases.

SUGGESTED READINGS

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- B R C MURTHY

LESSON: 15

PROTOZOA- CLASSSIFICATION, GENERAL ACCOUNT OF STRUCTURE, REPRODUCTION AND SIGNIFICANCE

CONTENTS

15.1 OBJECTIVES:

After going through this chapter you will be able to know

- ❖ Classification of protozoans
- ❖ Size and form, body covering and skeletal layers, locomotor organelles, encystment
- ❖ Mode of reproduction- asexual and sexual methods
- ❖ Significance of protozoa

15.2 . STRUCTURE AND EXPANSION OF THE LESSON

INTRODUCTION:

Protozoans are microscopic and unicellular animalcules, without tissues and organs, having one or more nuclei, but no nucleus ever incharge of a specialized part of a cytoplasm. They exist either single or in colonies which differ from a metazoan in having all the individuals alike except when engaged in reproductive activities.

15.2.1. CLASSIFICATION:

The following classification of protozoa is based on scheme given by committee on taxonomy and taxonomic problems of the society of protozoologist, and mainly proposed by B. N. Honiberg and others (1964)

It divides protozoa first into four sub phyla: (1) Sarcomastigophora (2) Sporozoa (3) Cnidospora (4) Ciliophora

Subphylum 1. Sarcomastigophora

Super class 1. Mastigophora (= Flagellata)

1. Simple, primitive, with firm pellicle.
2. Locomotor organelles flagella.
3. Autotrophic nutrition by phototrophy or heterotrophic nutrition by osmotrophy; some exhibit both.

Class A Phytomastiogophorea (= phytoflagellata)

1. Chlorophyll- bearing chromatophores present in majority.
2. Nutrition mostly holophytic by phototrophy.
3. Reserve food starch or paramylon.
4. Flagella 1 or 2, some times more.

Order 1. Chrysomonadida

1. Small, with a thin pellicle and often amoeboid in form.
2. Gullet absent; stigma often present.
3. Chromatophores 1or 2, yellowish or brownish or yellowish green; discoidal.
4. Starch absent but leucosin and fats may be present.

Examples: *Chrysamoeba, Symura, Ochromonas, Dinobryon.*

Order 2. Cryptomonadida

1. Small, with a rigid pellicle.
2. Anterior gullet reaches upto the middle of the body.
3. Chromatophores 2, yellow to brown or colourless.
4. Reserve food stuff starch, sometimes also oils.

Examples: *Chilomonas, Cryptomonas*

Locomotor organelles pseudopodia or flagella or both.

Order 3. Euglenoidida

1. Large, with a thick and firm pellicle.
2. Anterior end with a gullet or cytopharynx leading into a reservoir.
3. Chromatophores numerous and green, some colourless.
4. Reserve food stuff paramylon and oils

Examples: *Euglena, Peranema, Phacus, Copromonas.*

Order 4. Volvocida (= Phytomonadida)

1. Small, with rigid cellulose covering (theca).
2. No gullet.
3. Chromatophores green and numerous, some colourless.
4. Reserve food stuff starch and oils.

Examples: *Chlamydomonas, Volvox.*

Order 5. Chloromonadida

1. Small, with a delicate and dorso- ventrally flattened pellicle.
2. Gullet present.
3. Chromatophores green and numerous, some colourless.
4. Reserve food stuff oils.

Examples: *Vacularia, Coelomonas.*

Order 6. Dinoflagellida

1. Small and planktonic; naked and amoeboid or with a thick pellicle or cellulose theca.
2. Gullet present or absent.
3. Chromatophores numerous, yellow to brown.
4. Reserve food stuff starch or oils or both.
5. Some are bioluminescent.

Examples: *Noctiluca, Ceratium.*

Class B. Zoomastigophorea (= Zooflagellata)

1. Chlorophyll- bearing chromatophores are absent.
2. Nutrition holozoic or saprozoic.
3. Reserve food glycogen
4. Flagella one to many.

Order 1. Rhizomastigida

1. Small and amoeboid
2. Locomotion both by flagella (1 to 4) and pseudopodia.
3. Chiefly fresh water.

Examples: *Mastigamoeba, Dimorpha.*

Order 2. Kinetoplastida.

1. No gullet. Kinetoplast present.
2. Flagella 1 or 2; no definite pellicle.
3. Fresh water forms, sessile or stalked, solitary or colonial; parasitic forms live in blood.

Examples: *Bodo, Leishmania, Trypanosoma.*

Order 3. Choanoflagellida.

1. A collar rounds the base of a single flagellum.
2. Free- living, colonial.

Example: *Proterospongia.*

Order 4. Diplomonadida.

1. Small, with delicate pellicle and often with a cytostome.
2. Flagella 3 to 8, one often trailing or forming the border often undulating membrane.
3. Mostly intestinal parasites.

Examples: *Hepamita, Giardia.*

Order 5. Hypermastigida.

1. Highly specialized, numerous flagella.
2. Kinetosomes arranged in a circle, plate or longitudinal or spiral rows.
3. Mouth absent, food ingested by pseudopodia.
4. Gut parasites of termites, cockroaches and woodroaches.

Examples: *Lophomonas. Trychonympha.*

Order 6. Trichomonadida.

1. Flagella 4 to 6, one flagellum trailing.
2. Parasites of vertibrates.

Examples: *Trichomonas.*

Superclass II. Opalinata

1. Body covered by flagella.
2. Nuclei many, monomorphic.
3. Reproduction by binary fission or by gametes
4. Parasites in frogs and toads.

Examples: *Opalina*

Superclass III. Sarcodina (= Rhizopoda)

1. Body with out definite pellicle, some with a skeleton of some kind.
2. Locomotion by pseudopodia.
3. Nutrition holozoic or saprozoic.

Class A. Actinopodea

Pseudopodia mainly axopodia with axial filaments, radiating from spherical body.

Subclass (I) Heliozoia

1. Spherical protozoans, called sun-animalcules.
2. Pseudopodia (axopodia) radiating.
3. Naked if skeleton present, of siliceous scales or spines.

Examples: *Actinophrys, Actinosphaerium*

Subclass (ii) Radiolaria.

1. Body with perforated central capsule of tectin in a central capsule membrane, separating the ectoplasm from endoplasm.
2. Pseudopodia as axopodia or filopodia.
3. Skeleton mostly of siliceous spicules are of strontium sulphate

Examples: *Collozoum, Acanthometra.*

Subclass (iii) Proteomyxidia

1. Pseudopodia are filopodia.
2. Mostly parasites on algae.

Examples: *Vampyrella, Pseudospora.*

Class B. Rhizopodea

Pseudopodia as lobopodia, filopodia or reticulopodia, without axial filaments.

Subclass (I) Lobosia

Pseudopodia as lobopodia

Order 1. Amoebida

1. Body amoeboid, without skeleton.
2. Nucleus with honey comb lattice.

Examples: *Amoeba, Entamoeba, Penomyxa.*

Order 2. Arcellinida (= Testacida)

1. Body enclosed in a one chambered shell of pseudochytin with a single opening through which lobopodia protrude

Examples: *Arcella, Difflugia, Euglypha*

Subclass (ii) Filosia

Pseudopodia as filopodia; naked or with a shell with single aperture.

Examples: *Allogromia, Penardia.*

Subclass (iii) Granuloreticulosia

Pseudopodia as reticulopodia

Order Foraminiferida

1. Large sized with uni or multichamber calcareous shell with one or more openings through which reticulopodia emerge.

Examples: *Globigerina, Elphidium (= Polystomella)*

Class C. Piroplasmea

Small parasites in red blood cells of vertibrates.

Examples: *Babesia (* Formerly included with sporozoa, but its species do not produce spores)

Subphylum 2. Sporozoa

Locomotor organelles absent. Exclusively endoparasites

Class A. Telosporea

Spores with out polar capsules or filaments, naked or encysted.

Subclass (I) Gregarinia

1. Mature trophozoites are large and occur in host's gut and body cavities.
2. Male and female gametes merogamous.
3. Sporozoids in sporocysts.
4. Parasites in invertibrates

Examples: *Monocystis, Gregarina*

Subclass (ii) Coccidia

1. Mature trophozoites small and intracellular.
2. Female gamete hologamous.
3. Sporozoites in sporocysts (oocysts).
4. Blood or gut parasites of vertebrates.

Examples: *Eimeria, Isospora, Plasmodium.*

Class B. Toxoplasmea

No formation of spores. Only asexual reproduction.

Examples: *Toxoplasma*

Class C. Haplosporea

Spore cases present

Examples: *Ichthyosporidium*

Subphylum 3. Cnidospora

Spore formation through out life

Class A. Myxosporidea

1. Spores developed from several nuclei.
2. Spores with in 2 or 3 valves

Examples: *Myxidium, Myxobolus, Triactinomyxon*

Class B. Microsporidea

Spores small, with a univalved membrane.

1. With or with out polar capsule, with 1 or 2 filaments.

Examples: *Nosema*

Subphylum 4. Ciliophora

Presence of cilia as locomotor and feeding organelles at some stage in the life cycle.

Class Ciliata (= Infusoria)

1. Locomotor orgalelles numerous hair like cilia, present through out life.
2. Definite mouth (cytostome) and gullet present except in a few parasitic forms. Anal aperture (cytopyge) permanent.
3. One or more contractile vacuoles present even in marine and parasitic types.
4. Mostly two kinds of nuclei- large macro nucleus and smaller micro nucleus.

Subclass (I) Holotricha

1. Body cilia simple and uniform.
2. Buccal cilia mostly absent

Order 1. Gymnostomatida

Examples: *Coleps, Didinium, Nassula*

Order 2. Trichostomatida

Examples: *Bavalantidium, Colpoda.*

Order 3. Chonotrichida
Examples: *Spirochona, Lobochona*

Order 4. Apostomatida
Examples: *Hyalophysa*

Order 5. Astomatida
Examples: *Anoylophyrya, Maupasella*

Order 6. Hymenostomatida
Examples: *Colpidium, Paramecium*

Subclass (ii) Periticha

1. Adult with out body cilia.
2. Apical end with buccal cilia

Order 1. Peritrichida
Examples: *Vorticella, Carchesium*

Subclass (iii) Suctoria

1. Sessile and stalked body.
2. Young with cilia, adult with tentacles.

Order 1. Snctorida
Examples: *Acineta, Ephelota, Podophyra.*

Subclass (iv) Spirotrichia

1. Reduced body cilia.
2. Buccal cilia well marked

Order 1. Heterotrichida
Examples: *Stentor, Bursaria*

Order 2. Oligotrichida
Examples: *Strombidium, Halteria*

Order 3. Hypotrichida
Examples: *Euplotes, Stylonchia.*

15.2.2. STRUCTURE OF PROTOZOANS:

15.2.2.1. SIZE AND FORM:

Most of the protozoa are microscopic. The size ranges from a few thousandth of a millimeter to a millimeter in length. Some of the smallest protozoans are *Babesia*, *Leishmania* and *Plasmodium*. Some protozoa like *Paramecium* are large enough to be seen with the naked eye. Protozoa vary widely in form. Some are asymmetrical (*Amoeba*), radial symmetry is seen mostly in sessile forms (*Verticella*), bilateral symmetry is limited to a few protozoa (*Giardia*) and spherical symmetry (*Radiolaria*).

15.2.2.2. BODY COVERING AND SKELETON:

In Protozoa, the cytoplasm remains separated from the external environment by a cell envelope. This covering protects the body from harmful influences of outer environment, permits a controlled exchange of substances across it, perceives mechanical and chemical stimuli and establishes contact with other cells.It may take the following types.

15.2.2.2.1. PLASMALEMMA:

In some forms the body covering is a thin plasma membrane or plasmalemma. It is provided with longitudinal ridges of mucopolysaccharides which help in the adhesion of organism to the substratum. e.g., *Amoeba* (Fig:15.1.A)

15.2.2.2.2. PELLICLE:

In some protozoans, the body covering is in the form of a differentiated pellicle, which is somewhat thicker and firm. In some forms the pellicle is ridged and sculptured.
Example: *Paramecium, Coleps* (Fig.15.1.B)

15.2.2.2.3. .SKELETAL LAYERS:

Some other protozoa display various secreted layers, often impregnated with foreign bodies. These constitute the protozoa skeleton and include cyst, theca, lorica and test or shell.

(a) Cyst: It is a temporary sheath, and is formed both in free living and parasitic protozoa. Exhaustion of food supply, drought and putrefaction favour encystment.

(b) Theca: It is a coat of closely fitted armour of cellulose layer. In some forms the theca is composed of two valves, while in a majority of dinoflagellates (e.g. *Ceratium, Glenodinium*) differentiated into a number of plates laid out in a definite pattern (fig.15.1.C)

(c) Lorica: It is a covering, which fits less closely to the organism than theca. It is a cup like or vase like structure with an opening through which emerges the anterior part of organism's body or its appendages. In colonial loricated forms, one lorica may be attached to another directly (e.g. *Dinobryon*) or by means of a stalk (e.g. *Peteriodendron*, Fig.15.1. D)

(d) Shell or Test: These are common among protozoa. These coverings are loose contact with the body, provided with one or more openings, through which the animal can protrude itself. In shelled amoebae, like *Arecella* and allied forms, the shell is thin and made up of a chitinous material called pseudochitin (fig.15.1.E)

In *Difflugia*(Fig:15.1.F) shells are formed of sand particles and other foreign substances like pieces of foraminifers are mostly made up of calcium carbonate. In *Euglepha*(Fig:15.1.H) silicious shells are made up of silica.

(e) Radiolarian Skeleton: Continuous internal skeleton is found in radiolarians. The skeleton lies in between the ectoplasm and endoplasm forming the so-called central capsule. The capsule is made up of pseudochitin or silica or strontium sulphate. One or a few or many pores perforate it. In many radiolarians the skeleton consists of a lattice net work, which is variously sculptured and ornamented. (Fig.15.1. I, J, K).

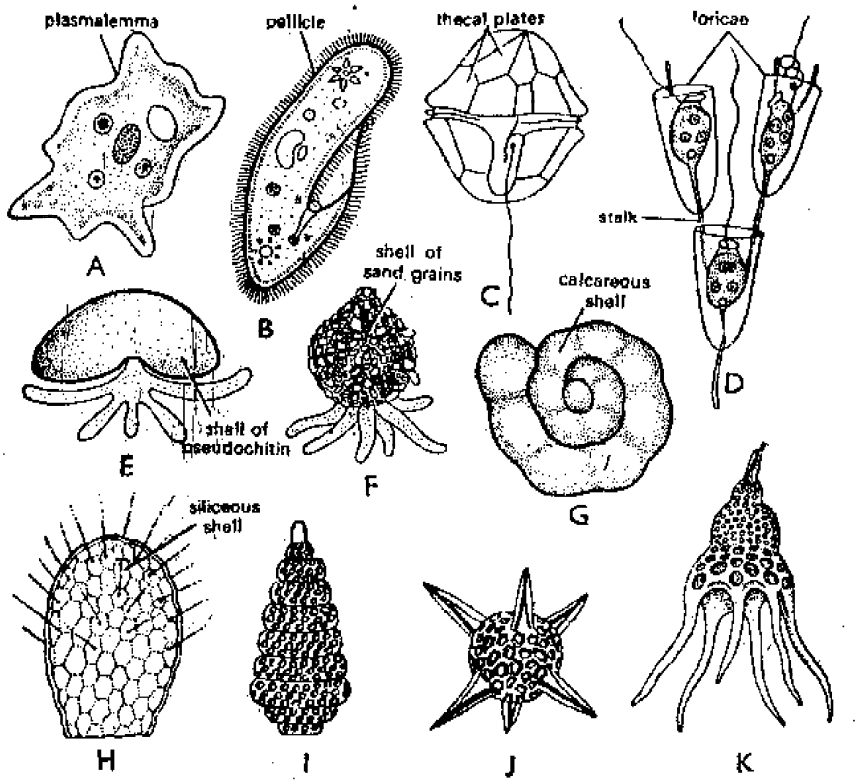


Fig: 15.1. Protozoan skeletons. A. plasmalemma of *Amoeba*, B- Pellcle of *Paramecium*, C- Thecal plates of Glenodinum. D- Lorica of Poterodendron, E- Pseudochitinous shell of Arcella, F- Sand grain shell of *Difflugia*. G- Calcarious shell of *Discorbis*. H- silicious shell of *Euglepha*. I- K – Radiolarion skeletons.

15. 2.2.3. CYTOPLASM:

Cytoplasm consists of the peripheral ectoplasm and central endoplasm. In certain protozoa, the differentiation between these two areas is indistinct. The endoplasm includes endoplasmic reticulum, ribosomes, lysosomes, microtubules, centrioles, plastids, flagella, cilia etc. Other structures like trichocysts, contractile vacuoles, stigmas etc., which are exclusive of protozoa, are seen in certain individuals.

15. 2.2.4. NUCLEUS:

All protozoans possess nuclei. Some have one, others have two or more identical nuclei (E.g. Mastigophora, Sarcodina and Sprorozoa). But some others have two types of nuclei- macronucleus and micronucleus. Micronucleus takes an active part in sexual reproduction and macronucleus is associated with trophic or metabolic activities.

15. 2.2.5. LOCOMOTOR ORAGANELLES:

Locomotor organelles include pseudopodia, flagella, cilia and pellicular contractile structures.

15. 2.2. 5. 1 Pseudopodia:

These are temporary structures formed by the streaming flow of the cytoplasm. On the basis of the form and structure the pseudopodia are of the following types:

(a) Lobopodia: These are lobe- like pseudopodia with broad and rounded ends (*Amoeba*, Fig. 15.2.A).

(b) Filopodia: These are more or less filamentous pseudopodia, usually tapering from base to the pointed tip (*Euglypha*, Fig.15.2. B)

(c) Reticuloppodia: These are branched filamentous and fuse profusely to form a net work (*Globigerina*, Fig.15.2.C)

(d) Axopodia: These are more or less straight and radiating pseudopodia (*Actinophrys*, Fig.15.2. D)

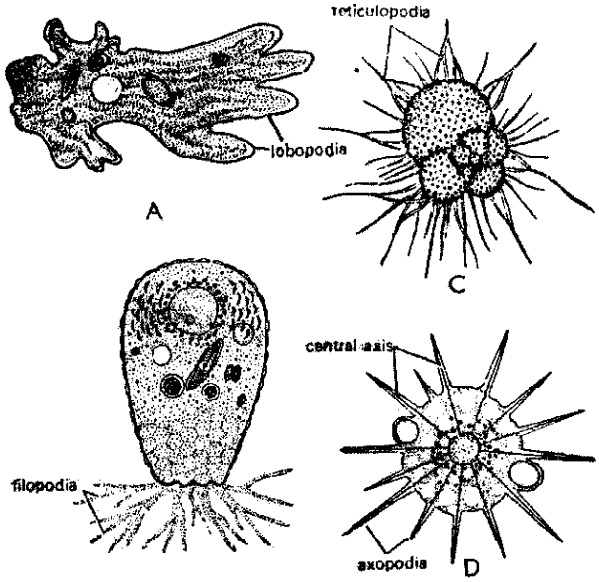


Fig: 15.2. Types of pseudopodia. A- Lobopodia of *Amoeba* B- Filopodia of *Euglepha*. C- Reticulopodia of *Globigerina* D- Axopodia of *Actinophryis* sol.

15.2.2.5.2. Flagella:

These are thread like projections on the cell surface. A typical flagellum consists of an elongate shaft, the axoneme enclosed by an outer sheath. In the axoneme, nine longitudinal peripheral paired fiber form a cylinder, which surrounds the central longitudinal fibres enclosed by a membranous inner sheath. The axoneme ends at the base in a granule, called the blepharoplast.

15.2.2.5.3. Cilia:

Cilia are highly vibratile small ectoplasmic processes. These resemble flagella in their basic structures

15.2.2.5.4 Pellicular Contractile Structure:

In many protozoa in the outer pellicle contractile structures are present called myonemes

15.2.2.6. Regeneration:

Most protozoa can regenerate their lost parts, as normally displayed at fission or encystment. Parasitic protozoa usually have slight regenerative capacity. Nucleus plays an important role in the process.

15.2.2.7. Encystment:

Encystment occurs either during reproduction or during unfavorable periods such as drought, over population, lack of food and oxygen, accumulation of metabolic wastes in the cytoplasm and changes in the temperature and pH. Encystment serves for survival, protection and dispersal. In the encysted state, the animalcule suspends all its physiological activities and lies dormant. When the normal conditions are restored, the cyst takes up water and ruptures and the enclosed animalcule emerges to resume on active life once again.

15.2.3. REPRODUCTION:

Protozoans reproduce in two ways:

15.2.3.1. Asexual Reproduction:

Protozoans exhibit different methods of asexual reproduction. They are:

(a).Binary Fission:

In this a single parent undergo division and produces two daughter individuals. This division is not a mere fragmentation but a complicated process of mitosis. The fission may be either in a transverse plane (e. g. *Paramecium*) or in a longitudinal plane (e.g. *Euglena*) or in an oblique plane (e.g. *Ceratium*) or in any plane (e. g. *Amoeba*). The two daughter organisms produced as a result of binary fission carry all the cytoplasmic organelles of the parent individual. Some organelles like mitochondria, divide at the time of division others like oral apparatus, flagella and contractile vacuoles are formed afresh by one of the daughters (Fig.15.3.A-D).

(a) Plasmotomy:

It is a special type of binary fission concerned with the division of multinucleate protozoa into two or more smaller multinucleate daughter individuals (Fig.15.4.B).

(b) Budding:

Irregular fission resulting in a small daughter individual in the form of a bud. When the bud breaks off it grows to full size. When a parental body produces only one bud it is monotomic (E. g. *Vorticella*). But in some others several buds are formed simultaneously, it is callef7multiple budding (E. g. *Suctoria*).

(c) Multiple Fission:

In this the nuclear division is not followed immediately by the division of the cytoplasm. First the nucleus undergoes a series of divisions and becomes multi nucleate. Later the body cytoplasm divides into as many parts as there are daughter nuclei, which usually arrange themselves at the periphery each getting surrounded by a fragment of cytoplasm. Thus the parent body simultaneously divides into as many daughter individuals as there are nuclei. Multiple fission is quite common in Foraminifera, Radiolaria, Sporozoa and certain Mastigophora.(Fig: 15.4.A)

d. Schizogony:

In this process a series of nuclear divisions results into numerous daughter nuclei. This is followed by the formation of cytoplasmic buds each containing a nucleus. The buds are pinched off to grow into new organisms (E. g. merozoites and sporozoites of *Plasmodium*).

e. Endodyogeny:

This consists in the development of two daughter individuals with in a single parent, which is destroyed in the process (e. g. *Toxoplasmea*)

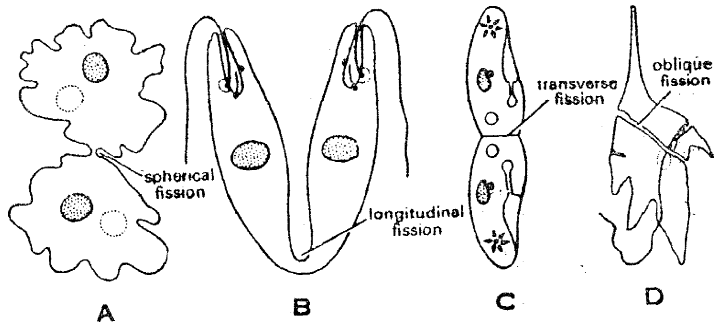


Fig: 15.3.Binary fission in Protozoa. A- *Amoeba*.(irregular) B-*Euglena*(longitudinal). C- *Paramesium* (Transverse) D- *Ceratium*(oblique).(source: Invertebrates— R.S. Kotpal).

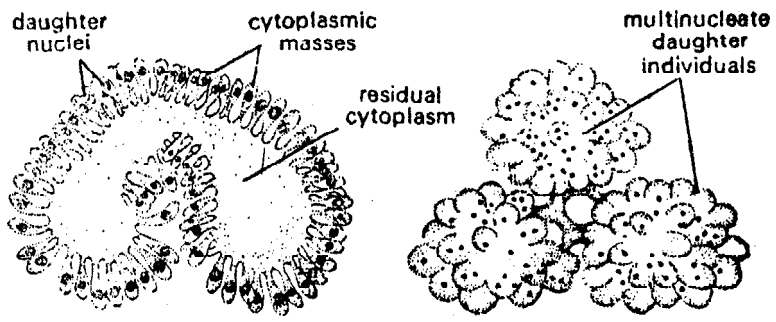


Fig: 15.4 A- Multiple fission B- Plasmotomy.

15.2.3.2. SEXUAL REPRODUCTION:

In protozoa, sexual reproduction occurs by the following processes:

(a) Syngamy:

Syngamy is the complete fusion of two sex cells or the gametes, resulting in the formation of zygote. The zygote is called synkaryon. Depending upon the degree of differentiation displayed by the fusing gametes syngamy is of the following types.

(i) Hologamy:

The two mature protozoan individuals do not form gametes but themselves behave as gametes and fuse together to form zygote. Hologamy occurs in a few Sarcodina and Mastigophora.

(II) Isogamy:

The gametes similar in size and shape but differ in behavior are called isogametes and their union is called isogamy. It is common in Foraminifera, Gregarinia and Phytomonadida.

(iii) Anisogamy:

The fusion occurs between the two dissimilar gametes is called anisogamy. The small and motile gametes are the male or microgametes and the large non-motile ones are the female or macrogametes. It is widely seen in sporozoa.

(iv) Autogamy:

It is the fusion of the gametes derived from the same parent cell as in *Actinophrys* and *Actinospherium*.

B.Conjugation:

This involves fusion of two individuals and is characteristic of ciliates. In conjugation, the two ciliate individuals couple temporarily during meiosis with reciprocal cross- fertilization of their gametic nuclei. Conjugation can only takes place between individuals of the same syngen but belonging to opposite mating types. Several nuclear divisions take place during pre and post fertilization events of the conjugation and the whole phenomenon seems to initiate a new life cycle.

C. Autogamy:

In autogamy only one individual is involved, which displays the same nuclear behaviour as in conjugation. There is self-fertilization of the gametic nuclei. Autogamy ensures a sort of nuclear reorganization (*Paramecium aurelia*).

D. Cytogamy:

This process is in between conjugation and autogamy. The two individuals do pair but the cross-fertilization of their gametic nuclei does not take place. The male gametic nuclei unite with the female gametic nucleus of the same individual.

E. Endomixis:

It is an internal process of nuclear reorganization. It differs from conjugation in that it occurs only with in a single individual and there is no meiosis and fusion of gametic nuclei.

F. Hemixis:

This involves an aberrant behaviour of macronucleus in a single individual. It is supposes to be a sort of purification act on the part of the macronucleus, which under goes degenerative changes independent of binary fission or syngamy.

15.2.4. SIGNIFICANCE:

Several species of protozoa form highly virulent parasites of men and animals causing various dreadful infectious diseases particularly in the tropical countries. The knowledge of these parasites is useful from the medical point of view. The knowledge of portals of entry and the means of transmission of these parasites is of vital importance from the standpoint of preventive medicines. With the discoveries of the alternation of generation, the host, the causes and modes of transmission of malaria, yellow fever, sleeping sickness and other diseases, the unexplored parts of the world have been exploited by the man, countless deaths have been prevented and the food supply has been increased enormously. Thus a general study of the phylum protozoa is most essential to understand the parasitic forms and to fight out their menace to mankind, domestic animals and crops.

Numerous aquatic forms feed upon bacteria and help in the purification of water. Indirectly they form the food of fish, clams and other animals, which are consumed by man. The success of fish culture depends upon a thorough knowledge of protozoa.

15.2.4..1.Useful Protozoa:

(a) Helpful in Sanitation: Numerous holozoic protozoa feed on putrifying bacteria in various bodies of water and thus help indirectly in the purification of water. These protozoa play an important part in the sanitary betterment and the improvement of the modern civilized world in keeping water safe for drinking purpose.

(b) Planktonic Protozoa as Food: Protozoa floating in the plankton of sea provide directly or indirectly the source of food supply to man, fish and other animals. They form one of the first links in the numerous and complicated food chains that exists in the oceans of the world. Clams and young fish feed extensively on aquatic insect larvae, small crustaceans, worms etc. all of which take protozoa as food. Thus protozoa indirectly form the food of fish, clams and other animals, which in their turn are consumed by man.

(c) Symbiotic Protozoa: Some protozoans are found in symbiotic relationship with other organisms. Most outstanding examples of symbionts are several intestinal flagellates (*Trycholympha, Colonympha*) of termites and wood roaches. These flagellates digest cellulose converting it into soluble glycogen substances for their host as well as for themselves.

(d) Oceanic Ooze and Fossil Protozoa: Tiny skeletons of dead pelagic Foraminifera, Radioloaria and Heliozoa sink to the sea bottom forming the soft mud on oceanic ooze. These tiny skeletons are made up of calcium carbonate or silica. Over countless millions of years these skeletons deposited on the floor of ocean became solid and fossilized and converted into some important sedimentary rock strata. These have commercial uses such as filtering agents, abrasives, chalk and building stones.

(e) Protozoa in Study: The protozoa are single celled individuals possessing forms and functions similar to cells of metazoans body. Individual cells from higher animals can be cultured only under carefully controlled, aseptic conditions, but protozoans only require a drop of water on a microscopic slide. Thus they can be used in experiments to illustrate the basic principles of the cell biology and zoology. The specificity of the host among parasitic protozoa provides

clues regarding the phylogenetic relations of the host and also regarding the past geographical state of the earth.

15.2.4..2 Harmful Protozoa:

(a) Soil Protozoa: Several species of protozoa, present in large numbers in soil, feed upon the nitrifying bacteria, and thus decline their activity and consequently tend to decrease the amount of nitrogen given to soil by the nitrifying bacteria.

(b) Water Pollution: Where as some protozoa are helpful in water sanitation, others become responsible for contamination or pollution. The protozoa of fecal origin belong to this later category. Some free living protozoa (e.g. *Uroglenopsis*) also pollute water by producing aromatic and oily secretions with objectionable odours, which render water unfit for human consumption.

Some bioluminescent dinoflagellates, such as *Noctiluca, Gymnodinium, Gonyaulax*, living in sea, some times multiply so extensively as to turn the water red with their bodies. The phenomenon is known, as blooming and is the cause of red tides, often experienced in the sea. Out breaks of this red water often gives a foul and disagreeable odour to the ocean water. Large concentrations of these flagellate protozoans may even lead to destruction of fish and poisoning of edible molluscs, such as clams, oysters and mussels, etc. making then unfit for human consumption

(c) Pathogenic Protozoa: Some protozoa cause diseases in man as well as in animals and these are termed pathogenic protozoa. They occur in all cases of protozoa.

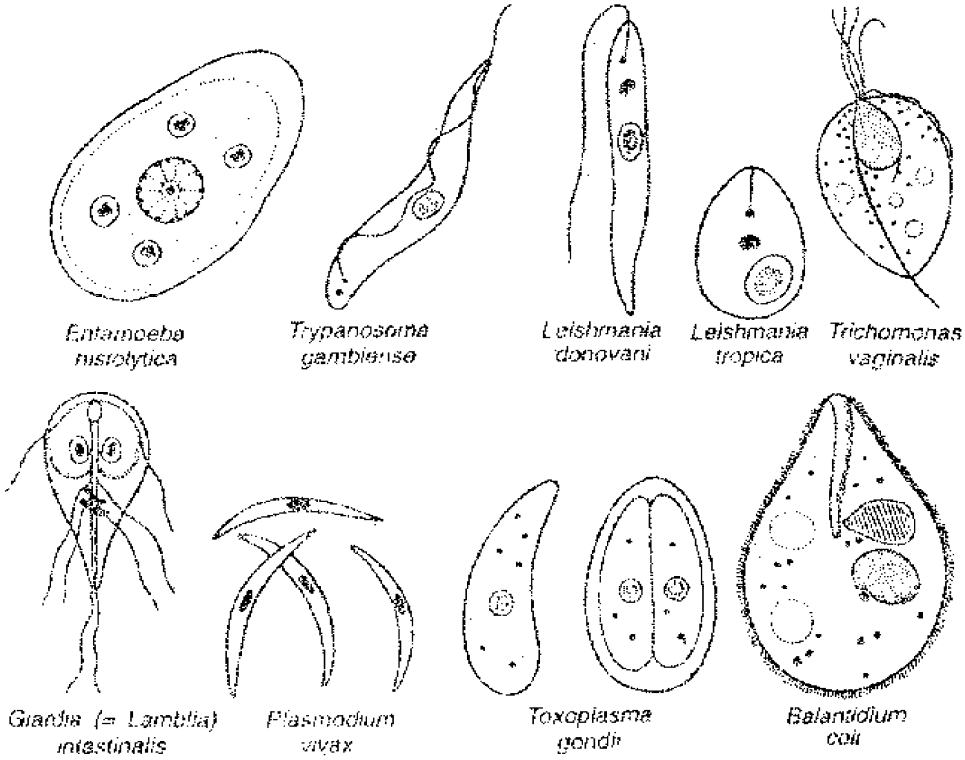
(i) Pathogenic Sarcodines: There are two common genera of parasitic amoebae, entamoeba and endamoeba, which live in the intestine of man and of other animals. Only two species of *Entamoeba: E. histolytica* of man and other mammals and *E. invadens* of reptiles are known to be seriously pathogenic. *E. histolytica* is responsible for amoebic dysentery or amoebiasis in man, which occurs in about 60 to 70% Indian population. *E. invadens*, occurring in the colon of reptiles, causes reptilian amoebiasis.

(ii) Pathogenic Flagellates: Pathogenic species of parasitic flagellates are included in the genera *Leishmania, Trypanosoma, Histomonas, Trichomonas* and *Giardia.* Three pathogenic species of *Leishmania* have been known to cause severe diseases in man. *L. donovani* causes kala- azar, a disease of the spleen and liver, *L. tropica* cause a peculiar type of skin lesion (cutaneous leishmaniasis) and *L. brasiliensis* causes infection of nasopharynx and skin lesion. These are transmitted by sand flies of the genus *Phlebotomus*, Parasitic species of *Trypanosoma* in mammals cause worst diseases. *T. gambiense*, is the causative agent of fatal African sleeping sickness. *T. rhodesiense, T. cruzi, T. equiperdum, T. evansi*, and *T. brucei* are other common pathogenic species.

Histomonas meleagridis is the parasitic mastigamoeba. Of the parasitic species of *Trichomonas*, *T.vaginalis* is the causative organism of vaginal trichomoniasis or vaginitis in human females. *T. foetus* causes trichomoniasis of cattle in U.S and *T. gallinae* is pathogenic in doves, pigeons, turkeys and chickens. Of the numerous species of *Giardia: G. intestinalis (= G. lamblia)* of man causes enterocolitis.

(iii) Pathogenic Sporozoans: Protozoan super class Sporozoa is exclusively of parasitic forms. Though most of sporozoans are harmless, yet some genera like *Plasmodium, Eimeria, Isospora* and *Babesia* include pathogenic species. Species of *Plasmodium* are called malaria parasites as they cause the disease of malaria. Four species of *Plasmodium,* namely *P. vivax, P. malariae, P. ovale* and *P. falciparum* cause malaria in man. Malaria is caused by *P. cyanomolgi* in monkeys, by *P. verghei* in tree rats and by *P. gallinaceum* in jungle fowl of Asia. Pathogenic species of *Eimeria* cause coccidiosis in chickens and rabbits. *E. tenella* and *E. mitis* infect chicken where as *E. mana* and *E. steidae* infect rabbits. *E. canis* in dogs, *E. felina* in cats, *E. bovis* in cattle and *E. intricata* in sheep and goats are also common. *Isospora*, intestinal parasites of man and other animals, include one truly pathogenic species of man, *I. hominis, I.felis, I. bigemina* and *I. riolta* infect cats and dogs and occur in mucous membranes of ileum. Their transmission is by cylindrical oocysts. Species of *Babesia* are intra- erythrocytic parasites of various vertebrates. *Babesia bigemina* of cattle causes the lethal haemoglobinuric fever, red- water fever or Taxas fever. *B. equi* in horses, *B. rohdani* in rodents, *B. felis* in cats, *B. motasi* in goats, etc. cause malignant jaundice, anaemia and fever in their respective hosts.

(iv) Pathogenic Ciliates: *Balantidium coli* is the only important ciliate pathogenic parasite. It is found in the intestine of man and often in frogs.



Fig; 15.5. Some pathogenic protozoan parasites of man.

15.3. SUMMARY:

Protozoa is the earliest and the simplest of unicellular group of animals. Some are free living and some parasitic. Single cell performs all the body functions. Protozoa is divided into five classes on the basis of locomotory organeles. Mastigophora have flagella. Sarcodina possess pseudopodia. No locomotion in Opalinata and sporozoa. Which are parasitic and cilia are present in ciliates. Body naked or covered by a pellicle or plasmalemma or rigid dead cuticle or calcarious or silicious shell. Skeleton usually absent but some forms have internal supporting elements like internal shell. Body consists of a mass of protoplasm, differentiate into one or more nuclei and ecto and endoplasm. The functions of locomotion and feeding are performed by finger like pseudopodia or whip like flagella or hair like cilia. Nutrition is varied in these animals. It may be holozoic, holophytic, saprophytic, parasitic or myxotrophic. Digestion is intra cellular, taking place inside the cell in the food vacuoles. Reproduction is both asexual and sexual. The former takes place by binary or multiple fission and budding while the later takes place by conjugation. several species of protozoa form highly virulent parasites of men and animals causing various dreadful infectious diseases. Numerous aquatic forms feed upon bacteria and help in the purification of water. Indirectly they form the food of fish, clams and other animals, which are consumed by man.

15.4. : TECHNICAL TERMS:

Axopodium: Pseudopodium with an axial filament

Bilateral symmetry: A symmetry with similarity on both sides.

Endo skeleton: A supporting frame work or structure with in the body of an animal.

Parasitism: Relationship between a host and a parasite.

Pathogenic: Disease causing.

Radial symmetry: Pattern of an organism where similar parts are arranged about a common center.

15.5. MODEL QUESTIONS:

1. Mention the chief types of locomotor organelles of protozoa.

2. Write an essay on

a. Protozoa and human diseases

b. Body coverings and skeletons in protozoa.

3. Give a brief account of the modes of reproduction in protozoa.

4. Name some important pathogenic species of protozoa that cause diseases in man.

5. Write short notes on

a. amoebiasis b. holozoic nutrition c. Leishmaniasis d. lobopodia
e. multiple fission f. nucleus in protozoa g. parasitism h. pathogenic protozoa
i. skeleton in protozoa j. symbiotic protozoa k. trypanosomiasis

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