

A TEXT BOOK OF **BOTANY**

SINGH • PANDE • JAIN

FIFTH EDITION

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A TEXT BOOK OF
BOTANY
FIFTH EDITION

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Diversity of Microbes: Introduction

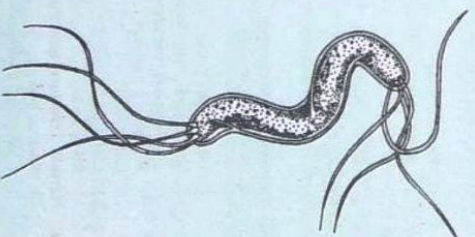
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Microorganisms, or the **microbes** as they are commonly called, are the smallest living organisms. They are so small that we cannot see them without magnifying them about 500 times with a microscope. Microbes live almost every where on earth. Incredible as it may seem, some grow at boiling temperatures in hot springs and at over 110°C in under sea volcanic hydrothermal vents. Others live in ice at temperatures below freezing point. Some live in very acidic environments (pH = 1.0) while others are found in saturated salt solutions. Microbes have also been found even in harsh environment of Antarctica.

Microbes preceded humans on earth by billions of years. Thus we have evolved in their world and, more recently, they in ours. For this reason, it should not seem surprising that microbes live intimately with us, on and in our bodies. Without our permission they inhabit all of our orifices, including our mouth, nose, eyes, ears and anal and genito-urinary tracts. However, most of them are harmless symbionts using our bodies as their home and also protect us from pathogenic species.

The major groups of microbes include bacteria, algae, fungi, viruses and protozoa. Like higher plants and animals, most are also living organisms and consist of one or more cells. Viruses, however, are acellular entities on the borderline between the living and non-living; they behave like living organisms when they gain entry to cells. Microbes range in size from small viruses (20 nm in diameter) to large protozoans (5 mm or more in diameter). Thus largest microbes are as much as 2,50,000 times the size of the smallest ones.



POSITION OF MICROBES IN THE LIVING WORLD

The earliest system of classification categorized living organisms in just two groups—**Animalia** and the **Plantae**. Microbes were placed in either kingdom on the basis of their ability of active movement and photosynthesis. Ernst Haeckel in 1866 proposed a third group **Protista** to include some relatively simple biological forms. Some of the protists lacked nucleus (e.g., bacteria) and some were nucleated organisms (e.g., protozoa, algae and fungi).

With the advent of electron microscopy, the differences in the ultrastructure of cells became apparent. It led the scientists to establish two different cell organization—**prokaryotic** and

eukaryotic. In prokaryotic cells the nuclear material is not surrounded by a nuclear membrane; it includes bacteria and cyanobacteria (blue-green algae). The eukaryotic cell organization is, however, much more complex with each cell organelle having its own limiting membrane (Table 1). All animals and plants (including algae and fungi) have eukaryotic cell organization.

However, a need of natural system of classification, based on ancestral relationships of organisms was always felt. Such a classification was proposed by Robert H. Whittaker in 1969. It is one of the most widely accepted systems. This system is based on the following three criteria:

- (1) **Complexity of cell structure**; i.e., whether the cell is prokaryotic or eukaryotic.
- (2) **Complexity of body organization**; i.e., whether the organism is unicellular and simple or multicellular and complex.

Table 1. Similarities and differences between prokaryotic and eukaryotic cells.

Characteristic	Prokaryotic Cell	Eukaryotic Cell
[I] Extracellular Structures		
1. Cell wall	Peptidoglycan found in most cells	Cellulose, chitin or both are found on plant and fungal cells
2. External layer	Capsule or slime layer	Pellicle or shell in certain protists
3. Flagella	Made of fibrils of flagellin	Consist of complex membrane enclosed structures with '9 + 2' microtubule arrangement
4. Cilia	Absent	Present as structures shorter than, but similar to flagella in some eukaryotic cells
5. Pili	Present as attachment or conjugation pili in some prokaryotic cells	Absent
[II] Intracellular Structures		
6. Plasma membrane	Fluid-mosaic structure lacking sterols	Fluid mosaic structure containing sterols
7. Internal membranes	Only in photosynthetic organisms	Numerous membrane enclosed organelles present
8. Endoplasmic reticulum, chloroplast, mitochondria	Absent	Present
9. Respiratory enzymes	Present in cell membrane	Present in mitochondria
10. Golgi body	Absent	Present
11. Lysosomes	Absent	Present
12. Ribosomes	70S	80S in cytoplasm and on ER, 70S in organelles
[III] Nuclear Structure and Function		
13. Nucleus with bounding membrane	Absent	Present
14. Nucleolus	Absent	Present
15. Histones	Absent	Present
16. Genetic material (DNA)	Found in single chromosome	Typically found in paired chromosomes
17. Extrachromosomal DNA	In plasmids	In organelles such as mitochondria and chloroplasts
18. Mitosis	Absent	Present
19. Reproduction	Only asexual	Sexual or asexual

- (3) **Mode of nutrition**; i.e., the methods used by organisms to obtain nourishment. It may be **autotrophic** (photosynthetic) or **heterotrophic** (take food by ingestion or absorption).

Whittaker divided organisms into following **five kingdoms**. He placed all prokaryotes in the Kingdom Monera and most unicellular simple eukaryotes in the Kingdom Protista. He considered fungi, which acquire nutrition solely by absorption, sufficiently different from plants.

Kingdom-Monera **(Kingdom of Prokaryotes)**

Kingdom Monera includes all prokaryotic organisms—**eubacteria** (true bacteria), **cyanobacteria** and **archaebacteria**. All monerans are unicellular, they **lack true nuclei** and other membrane bound organelles, such as mitochondria, plastids, lysosomes, etc. Their **DNA is without histones** (proteins) and is found in the form of single chromosome. Monerans reproduce chiefly by **binary fission** or may undergo **genetic recombination**.

Monerans are important decomposers and mineralisers in the biosphere.

Kingdom-Protista **(Kingdom of Unicellular Eukaryotes)**

All protists are unicellular eukaryotic organisms. They **possess true nucleus and other membrane bound organelles**, such as mitochondria, chloroplasts, endoplasmic reticulum, etc. Most of the protists possess flagella or cilia at some stage during their life cycle. Protists are extremely complex; their cells show even more diversity than is found among the cells in multicellular kingdoms. They **do not develop from an embryo** as plants and animals do. The Kingdom includes a variety of life forms. For example, among the protists are algae which resemble plants (e.g., diatoms), the protozoa which resemble animals (e.g., *Amoeba*, *Paramecium*) and euglenoids (e.g., *Euglena*) which have both plant and animal characteristics.

Protists are either **autotrophic** (photosynthetic) or **holozoic** which feed on other protists by ingestion. A few protists live on animals as parasites.

Kingdom-Fungi **(Kingdom of Multicellular Decomposers)**

This Kingdom includes unicellular (e.g., yeasts), multicellular (e.g., molds) and macroscopic (e.g., mushrooms) fungi. In multicellular fungi the cells join together to form thin tubes called hyphae. The cell wall of fungi is mostly made of **chitin**.

All fungi lack chlorophyll. They are either **saprophytes** (obtaining food from dead and decaying plant or animal matter) or **parasites** (obtaining food from living organisms).

Although fungi have some characteristics in common with plants but their mode of nutrition and certain reproductive processes are not shared with any other organisms.

Kingdom-Plantae **(Kingdom of Multicellular Producers)**

Kingdom Plantae includes multicellular autotrophic plants, viz., thallophytes and tracheophytes. These organisms are characterized by the presence of a cell wall made of **cellulose**. The main groups included in this kingdom are sea weeds like green, red and brown algae, all mosses, ferns, conifers and flowering plants.

Plants are characterized by the presence of photosynthetic pigment—**chlorophyll** and are the only organisms which have the capacity to synthesize complex organic molecules from carbon dioxide and water utilizing the light energy trapped by the chlorophyll molecules as the source of energy. Thus they are the primary producers on land and along sea shores.

Kingdom-Animalia **(Kingdom of Multicellular Consumers)**

This Kingdom includes multicellular animals (invertebrates and vertebrates). They are **heterotrophic** and obtain energy by ingesting

organic matter. Animal cells **do not possess cell wall**, plastids and central vacuole. All animals are derived from zygote. They show organ-system organization. Very few animals show cellular organization (e.g., sponges) or tissue organization (e.g., *Hydra*). Animals **show locomotion** which is made possible by well developed muscular cells.

Although Whittaker's system is the most widely accepted system of classification, it has certain anomalies. For example, the kingdoms Monera and Protista are still heterogeneous. Both these kingdoms include walled and wall less organisms, photosynthetic and non-photosynthetic organisms, and unicellular and filamentous or mycelial forms. Besides this, in Whittaker's system algae have been divided in three Kingdoms—blue-green algae in kingdom Monera, unicellular algae in kingdom Protista and multicellular algae in kingdom Plantae.

A detailed study of the nucleotide sequence of tRNA in the ribosomes of different types of cells revealed that there are distinctly two different types of cells in prokaryotes. Thus now we know three kinds of cell organization in the organisms—two prokaryotic and one eukaryotic. Accordingly, Carl Woese (1978) proposed a **three Domain system** for the classification of organisms. The rank of the Domain is above kingdom. The three Domains Woese recognized are—**Eubacteria**, **Archaea** (Archaeobacteria) and **Eucarya**. The Eubacteria includes prokaryotes which **contain peptidoglycan in their cell wall** (e.g., gram positive and gram negative bacteria, mycoplasmas).

The Archaea (archaeobacteria) on the other hand have **cell wall without peptidoglycan**; they often live in extreme environments and show unusual metabolic processes.

The domain Archaea includes three kingdoms—the **methanogens**, **extreme halophiles** and **thermoacidophiles**. The **methanogens are strict anaerobes** and have been isolated from divergent anaerobic environments such as waterlogged soil-like sediments, marshes, marine sediments and gastrointestinal tracts of animals including humans. They produce methane from carbon dioxide and hydrogen. The **extreme halophiles** occur in highly saline environments such as salt lakes, salt evaporation ponds and

surfaces of salt preserved food. These are obligate anaerobes. **Thermoacidophiles** grow in hot acidic environments such as hot water springs, geothermally heated marine sediments and submarine hydrothermal vents. They are obligate aerobes, facultative aerobes or obligate anaerobes.

A comparison of the three cell types as proposed by Carl Woese is given in Table 2.

MAIN GROUPS OF MICROORGANISMS

Microorganisms show a great diversity in their cell structure and function. Some broad groups of microorganisms are discussed below.

PRIONS

Prions are small proteinaceous infectious particles that do not contain nucleic acids. They were first identified by American neurobiologist Stanley Prusiner in 1982 in sheep affected by neurological disease. These are the only disease causing organisms which does not have nucleic acids. The major portion of this infectious agent is a protein, called **PrP** (prion protein). The prion protein exists in two configurations. One is a normal cellular form (prp^c) that is found in the brains of all adult mammals and does not cause disease. The second form is a structural variation of the normal form that causes disease and is found only in infected cells. The infected form is given a symbol consisting of PrP with a superscript that indicates the source of prion. For example, prions from sheep infected with prion disease scrapie are PrP^{SC} . Prions are known to cause several diseases in humans and animals.

VIROIDS

Viroids are **subviral pathogenic particles consisting of short strand of naked RNA**. As there is no protective protein coat (capsid) around the nucleic acid, the viroids lack a definite shape. The RNA strand has only

Table 2. A comparison of the characteristics of Eubacteria, Archaea and Eucarya.

Characteristic	Eubacteria	Archaea	Eucarya
1. Cell type	Prokaryotic	Prokaryotic	Eukaryotic
2. Cell wall	Contains peptidoglycan	No peptidoglycan	Mainly contains carbohydrate cellulose
3. Membrane lipids	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage
4. Start signal for protein synthesis	Formyl methionine	Methionine	Methionine
5. Antibiotic sensitivity	Present	Absent	Absent

300-400 nucleotides which are often internally paired. It gives the RNA strand a circular configuration. Absence of protein coat is probably due to intercon type of base sequences in viroids which do not code for polypeptides. Many plant diseases (e.g., Chrysanthemum stunt, Chrysanthemum chlorotic mottle, Cucumber pale fruit) and a few animal diseases are of viroid origin.

VIRUSES

Viruses are sub-microscopic obligate intracellular parasites, i.e., they essentially require living host cells in order to multiply. A simple virus particle, called virion, has a nucleic acid core of genetic material enclosed within a protein coat. They are smaller than bacteria, measuring 20-14,000 nm in length. Viruses contain only a single type of nucleic acid, i.e., either RNA (ribovirus) or DNA (deoxyvirus). They use the machinery of the host cell for their multiplication. Viruses are host specific and infect vertebrates, invertebrates, algae, fungi, protists and bacteria. Many diseases of human beings like hepatitis B, AIDS, cancer, herpes, mumps, polio, rabies, European encephalitis and common cold (influenza) are caused by viruses. In plants viruses cause mosaic diseases, leaf curls, etc.

RICKETTSIAS

Rickettsias are the smallest prokaryotes, 0.3-0.7 μm wide and 1-2 μm long. They are usually rod-shaped (commonly called coccobacilli) but can exist in many alternate forms. Unlike most bacteria, it is difficult to stain rickettsias with ordinary aniline dyes. They can be stained with Giemsa's stain. They have a typical prokaryotic structure. Mucopolysaccharide is the main constituent of their cell wall. Like viruses, rickettsias are also obligate intracellular parasites and grow only within the living host cells. Most of the rickettsias are transmitted to human beings by insects and ticks. In humans rickettsias cause diseases of spotted fever group (e.g., rocky spotted fever, endemic typhus, epidemic typhus, scrub typhus, Q fever, etc.).

MYCOPLASMAS

Mycoplasmas can be defined as prokaryotes without a cell wall, hence highly pleomorphic. Most of them are aerobes or facultative anaerobes. Mycoplasmas have been cultured in the laboratory in organic media containing sterols. They contain both RNA and DNA but it is usually less than half that normally occurs in other prokaryotes. Mycoplasmas are known to cause diseases in animals and plants.

BACTERIA

Bacteria form the largest group of prokaryotes. They vary greatly in their cell shape, cell arrangements, motility, oxygen requirements, nutritional and metabolic properties and reactions to Gram's stain. Their varied physiological activities enable them to develop on a very wide range of organic and inorganic substrates.

Modern techniques in molecular biology and biochemistry have provided sufficient evidences to support the division of bacteria into two groups, viz., **Archaeobacteria** and **Eubacteria**. Archaeobacteria are characterized by (i) the absence of muramic acid and D-amino acid in their cell wall, (ii) absence of fatty acid in their membranes, and (iii) presence of several subunits in the RNA polymerase. Besides these in many archaeobacteria the membrane is monolayered. They are resistant to antibiotic—chloramphenicol. They often live in extreme environments such as high salt concentration or hot acidic streams.

Eubacteria are characterized by the presence of N-acetylmuramic acid and N-acetylglucosamine in their cell wall. Lipids present in their cell membrane are straight chain molecules connected with ester linkages forming bilayered structure. In eubacteria the DNA polymerase is made of four subunits. They are sensitive to chloramphenicol.

CYANOBACTERIA

Cyanobacteria are prokaryotes. They **resemble algae in the presence of chlorophyll *a* and oxygenic photosynthesis** (photosynthesis in eubacteria is anoxygenic). Many bacteria have specialized cells called heterocysts which contain nitrogen fixing enzymes.

METABOLIC DIVERSITY AMONG MICROORGANISMS

Microorganisms basically differ from one another in the substrate that they can utilize as food and in their mechanisms for gaining energy. There are microorganisms in nature that can utilize any

carbon containing constituent as a nutrient source that is a component of living cells. Besides this, many other compounds unrelated to living cells can also be utilized as substrate for growth. Among compounds that microorganisms can actually utilize as carbon/energy source are carbon monoxide, cyanide and methane. Thus it is evident that microorganisms can sustain themselves on inorganic substances by using pathways that are unavailable to either plants or animals. These marked nutritional differences are referred to collectively as representing the microbes 'metabolic diversity'.

On the basis of sources of energy, organisms are either **phototrophs** (use light as primary source of energy) or **chemotrophs** (derive energy by the oxidation-reduction of inorganic or organic compounds). On the basis of their principal carbon source, organisms can be classified into **autotrophs** (use CO_2 as carbon source) and **heterotrophs** (take carbon from an organic source). Combining energy and carbon sources, organisms have been grouped into **photoautotrophs**, **photoheterotrophs**, **chemoautotrophs** and **chemoheterotrophs**.

PHOTOAUTOTROPHS

Photoautotrophs use **light as source of energy and CO_2 as their chief source of carbon**. **Photosynthetic bacteria** (e.g., cyanobacteria) are good examples of photoautotrophs. Like higher plants cyanobacteria use hydrogen atoms of water to reduce carbon dioxide and produce oxygen.

Several other photosynthetic bacteria, such as **green sulphur bacteria** (e.g., *Chlorobium*) use sulphur compounds (such as H_2S) to reduce carbon dioxide, instead of water. As such photosynthetic process in these bacteria does not produce oxygen (anoxygenic photosynthesis).

PHOTOHETEROTROPHS

Photoheterotrophs use light as source of energy but **use organic compounds** (such as alcohol, fatty acids or carbohydrates) **as carbon source instead of carbon dioxide**. **Green nonsulphur bacteria** (e.g., *Chloroflexus*) and **purple sulphur bacteria**

(e.g., *Rhodospseudomonas*) are common examples of photoheterotrophs.

CHEMOAUTOTROPHS

Chemoautotrophs **use electrons from reduced inorganic compounds**, such as hydrogen sulphide, elemental sulphur, ammonia, hydrogen gas, etc., **as a source of energy**. Like photoautotrophs these organisms also use carbon dioxide as their principal source of carbon. Bacteria such as *Beggiatoa*, *Thiobacillus* sp., *Nitrosomonas* and *Nitrobacter* are some common chemoautotrophs.

CHEMOHETEROTROPHS

Chemoheterotrophs usually **use the same organic compounds as the energy source and carbon source**. For example, if a chemoheterotrophic microorganism uses glucose, then carbon in glucose molecule serves as source of carbon and hydrogen atom, and glucose molecule is also used as source of energy. Most of the microbes belong to this category.

ENVIRONMENTAL DIVERSITY AMONG MICROORGANISMS

A high rate of growth and adaptability makes the microbes ubiquitous. They occur wherever life is possible and form a significant percentage of total biomass on the earth.

Microorganisms have the ability to live in the extreme conditions of temperature, acidity and alkalinity. This may be attributed either to their less sensitive cellular mechanisms or to their capacity of controlling the cell wall composition in the presence of extreme environments.

As microorganisms have high metabolic rate, they metabolize common nutrients more rapidly or use nutrients that competing organisms can not metabolize. This makes the microorganisms successful even in a very competitive environment. On the other hand, microorganisms adapted to extreme environments face no competition. For example, a hot spring will exclusively have thermophiles and salt lakes will have only halophiles.

Important Questions

►► Long answer questions

1. Give an outline of the five kingdom system of classification.
2. What are micro-organisms? Write important features of the main groups of micro-organisms.
3. "Micro-organisms show a great diversity in their cell structure and function". Justify the statement by giving suitable examples.
4. "Like other organisms, micro-organisms also vary in their nutritional patterns". Comment upon the statement.
5. Write short notes on :
(i) Prions, (ii) Viroids, (iii) Chemoheterotrophs, (iv) Photoautotrophs.

►► Short answer questions

1. What logic is used to divide living organisms into five kingdoms?
2. "Despite the great heterogeneity in protists, there are features that all members share." Comment.
3. How will you differentiate a photoautotroph with that of chemoautotroph?
4. Differentiate viruses with that of viroids.
5. Write the distinguishing features of Archaeobacteria and Eubacteria.
6. In what respects a prokaryotic cell differs from that of eukaryotic cell.
7. Write a brief note on prions.
8. Name a few diseases of humans caused by rickettsias.
9. "A high rate of growth and adaptability make the microbes ubiquitous." Comment upon the statement.
10. Classify microbes on the basis of energy and carbon sources.

>>> Very short answer questions

1. Name the technical term applied to the organism which does not have nuclear wall.
2. Who postulated the germ theory of disease?
3. Give an example of chemosynthetic bacterium.
4. Name a chemoautotrophic bacterium.
5. Name a photoautotrophic bacterium.
6. What are prions?
7. Name the scientist who first discovered prions.
8. What are viroids?
9. Who proposed a three Domain system for the classification of organisms?
10. Name the three Domains recognized by Woese (1978).
11. Prokaryotic organisms were included in which kingdom by Whittaker?
12. Who proposed five kingdom system of classification of organisms?
13. Tell one distinguishing feature between eukaryotes and prokaryotes.
14. Name the five kingdoms recognized by Robert H. Whittaker in 1969.
15. Write a distinguishing character of eubacteria.

>>> Fill in the blanks

1. Bacteria have been put in the kingdom Monera because they are
2. bacteria are characterized by the presence of N-acetyl muramic acid and N-acetyl glucosamine in their cell wall.
3. Viroids are subviral pathogenic particles consisting of short strand of naked
4. are small proteinaceous infectious particles that do not contain nucleic acids.
5. Archaeobacteria are characterized by the of muramic acid and D-amino acid in their cell walls.
6. proposed a three Domain system for the classification of organisms.
7. are prokaryotes without a cell wall, hence highly pleomorphic.
8. In cyanobacteria, the photosynthesis is oxygenic, whereas in eubacteria, it is
9. use light as a source of energy but use organic compounds as carbon source instead of carbon dioxide.
10. Green non-sulphur bacteria and purple-sulphur bacteria are common examples of heterotrophs.

>>> True and false statements

1. Bacteria and cyanobacteria exhibit prokaryotic cell organization.
2. Carl Woese (1978) recognized three kinds of cell organization in the organisms—two prokaryotic and one eukaryotic.
3. The Archaeobacteria have cell walls without peptidoglycan.
4. Viroids are small proteinaceous infectious particles that do not contain nucleic acids.
5. Stanley Prusiner was the first biologist who identified the prions.
6. Rickettsias are sub-viral pathogenic particles consisting of a short strand of naked RNA.
7. Viruses are sub-microscopic obligate intracellular parasites.
8. In eubacteria the DNA polymerase is made of four sub-units.
9. Photoautotrophs use light as source of energy and CO_2 as their chief source of carbon.
10. *Nitrobacter* is a common example of chemoheterotrophs.

>>> Multiple choice questions

1. Which one of the following is neither a prokaryote nor an eukaryote?
(a) *Nostoc* (b) *Saccharomyces*
(c) *E. coli* (d) T.M.V.
2. Which of the following do prokaryotes possess?
(a) golgi apparatus (b) meiotic mechanisms
(c) mitochondria (d) ribosomes
3. The prokaryotic genetic system contains:
(a) either DNA or histones
(b) neither DNA nor histones
(c) DNA but no histones
(d) DNA and histones
4. Microbiology is the study of:
(a) higher organisms (b) nucleoproteins
(c) micro-organisms (d) macro-organisms
5. True nucleus is absent in:
(a) lichens (b) bacteria
(c) fungi (d) algae

ANSWERS

►► Very short answer questions

1. Prokaryotes, 2. Robert Koch, 3. Sulphur bacterium, 4. Nitrifying bacterium, 5. Green sulphur bacterium, 6. Small proteinaceous infectious particles that do not contain nucleic acids, 7. Stanley Prusiner in 1982, 8. Subviral pathogenic particles consisting of short strand of naked RNA, 9. Carl Woese (1978), 10. Eubacteria, Archaea and Eucarya, 11. Monera, 12. Robert H. Whittaker (1969), 13. Eukaryotes possess a true nucleus, whereas prokaryotes lack a true membrane-bound nucleus, 14. Monera, Protista, Fungi, Plantae and Animalia, 15. Presence of N-acetyl muramic acid and N-acetyl glucosamine in their cell walls.

►► Fill in the blanks

1. prokaryotic, 2. eu, 3. RNA, 4. prions, 5. absence, 6. Carl Woese (1978), 7. mycoplasmas, 8. anoxygenic, 9. photoheterotrophs, 10. photo.

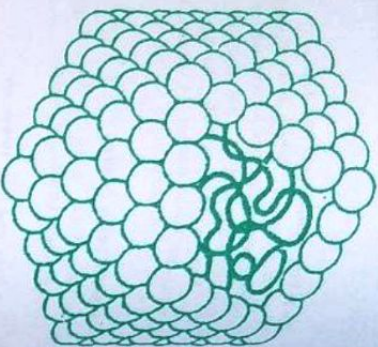
►► True and false statements

1. True, 2. True, 3. True, 4. False, 5. True, 6. False, 7. True, 8. True, 9. True, 10. False.

►► Multiple choice questions

1. (d), 2. (d), 3. (c), 4. (c), 5. (b).

Distribution of Microorganisms in Nature



A large variety of micro-organisms occur in the environment surrounding us. They are found in the soil, air, water, in plants, animals and various food products. They have remarkable ability to adapt themselves according to the environment. These micro-organisms affect our daily life considerably.

SOIL MICROFLORA

Several types of micro-organisms, such as algae, bacteria, fungi, protozoa, actinomycetes, etc. occur in the soil. The physical and chemical properties of the soil determine the nature of the soil upon the number and nature of microbes occur. Soil fertility also depends on the number of microbes occur in the top layer of the soil at a depth of 5-10 cm. But in deeper layers (1.5-5 meters), microbes occur in larger numbers. Their number also depends on the nature of fertilizers used in the soil and faecal contamination. Besides, degree of aeration, temperature and pH, and agricultural practices equally effect the distribution of micro-organisms in the soil.

BACTERIA

Bacteria are an important group of micro-organisms present in the soil, and they constitute approximately half of the total microbial

Distribution of Microorganisms in Nature

13

biomass. One gram fertile soil may contain as many as 10^9 bacteria. Bacteria occur in all types of soil, but their number decreases with the depth of the soil.

Those bacteria which occur in soil are cocci (0.5 μ m), bacilli (0.5-30 μ m) and spiral forms. The bacilli are in the highest number and they swim actively in the soil solution. Some common soil bacteria are the species of *Pseudomonas*, *Arthrobacter*, *Achromobacter*, *Bacillus*, *Clostridium*, *Micrococcus*, *Flavobacterium*, *Chromobacterium* and *Mycobacterium*. Both, autotrophic and heterotrophic bacteria occur in the soil. Chemosynthetic autotrophic bacteria present in the soil are the species of *Thiobacillus*, *Ferrobacillus*, *Nitrosomonas* and *Nitrobacter*.

Escherichia bacteria seldom occur in the soil, but for sewage contamination. In cellulose-rich environment, several cellulolytic bacteria, such as species of *Cytophaga* and *Sporocytophaga* are found in plenty.

ACTINOMYCETES

A large number of actinomycetes are present in dry and warm soil. They are particularly abundant in the soil rich in decomposed organic materials. Species of *Streptomyces*, *Micromonospora* and *Nocardia* are some common actinomycetes which occur in soils. They are responsible for the characteristic musty or earthy smell of a freshly ploughed field. They are capable of degrading many complex chemical substances and thus play an important role.

FUNGI

Several fungi are present in the soil and they play an important role for the improvement of soil nutrients in neutral and alkaline soils. Hundreds of species of molds inhabit the soil. The quality and quantity of organic materials present in the soil have a direct effect on the fungal population of the soil. The development of fungi is especially favoured by soils having an acidic reaction and where the aerobic

condition is likely to be present near the surface. Fungi also occur in plenty in arable soils and their number depends on the soil moisture. Besides, agricultural practices (crop rotation, use of fertilizers and insecticides, etc.) and the depth of the soil also influence the fungal composition. Some important soil fungi are the species of *Aspergillus*, *Borytis*, *Cephalosporium*, *Penicillium*, *Alternaria*, *Monilia*, *Fusarium*, *Verticillium*, *Mucor*, *Rhizopus*, *Pythium*, *Cunninghamella*, *Chaetomium* and *Rhizoctonia*. Yeasts are, however, not very common in the soil except in vineyard and orchard soils. Some fungi, such as species of *Alternaria*, *Aspergillus*, *Cladosporium* and *Dematiun*, are helpful in the preservation of organic materials in the soil.

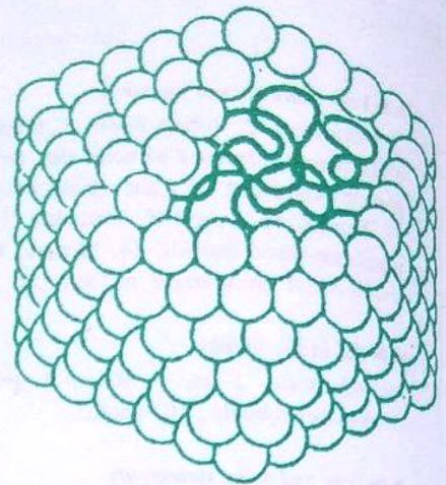
ALGAE

Many algae occur on the surface of moist soils, where sufficient light is available. The growth of algae is beneficial for soil conservation and in improving soil structure. In paddy fields, blue-green algae play a significant role in nitrogen fixation. Species of *Chlorella*, *Chlorococcum*, *Protoisphon*, *Aphanocapsa*, *Anabaena*, *Chroococcus*, *Nostoc* and *Scytonema* are some common algal taxa present in the soil.

PROTOZOA

Protozoans are present in great abundance in the upper layer of the soil and their number has a direct effect on bacterial population, since they ingest bacteria. The use of organic manures in the field is responsible for the increase in protozoan population. Several types of protozoa are found in the soil, but flagellates and amoebae usually outnumber the ciliates. Depending upon the conditions of the soil, protozoans may exist in vegetative or cyst form. They are helpful in maintaining the equilibrium of the microbial flora in the soil. Protozoans present in the soil belong to the class Mastigophora (e.g., species of *Allantion*, *Bodo*, *Cercobodo*, *Cercomonas*, *Einosiphon*, *Heteromita*, *Monas*, *Spiromonas*, and *Spongomonas*); the class

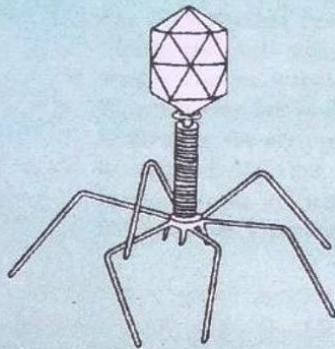
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Sarcodina (*Amoeba*, *Biomyxa*, *Nuclearia* and *Trinema*) and the class Ciliata (e.g., species of *Balantiophorus*, *Colpoda*, *Gastrostyla*, *Oxytricha*, *Pleurotricha* and *Vorticella*).

VIRUSES

Viruses are present in very small number in the soil. Bacteriophages ingest bacteria and actinomycetes and some viruses infect the fungi present in the soil.

WATER MICROFLORA

Approximately three fourth of the earth's surface is covered with water. Water is one of the naturally occurring essential requirements of all forms of life and it serves as the second natural medium for the growth of micro-organisms next to the soil. Micro-organisms widely occur both in fresh water and sea water. The nature and intensity of microbes present in water depend on the available nutrients, environment, physical factors and the presence of other organisms. The vital activities of the micro-organisms present in the aquatic environment directly affect our lives in various ways, influencing the health of human beings and other aquatic life. Besides, micro-organisms play a very significant role in aquatic food chain and in recycling of elements.

In aquatic environment, micro-organisms occur from water surface to greater depths. The various water sources, such as ponds, pools, lakes, streams, rivers and oceans, show a great diversity in their microflora.

MICROORGANISMS IN FRESH WATER

Normally, the water of ponds, pools, lakes and rivers is known as fresh water. In this type of water organic nutrients occur in low amounts. Thus in fresh water the number of bacteria is very limited. Only a few species of *Bacillus*, *Monococcus*, *Micrococcus*, *Pseudomonas* and

Flavobacterium are present in fresh waters. If there is abundance of decayed organic material at the bottom of the water body some anaerobic bacteria like *Clostridium* may also occur.

MICROORGANISMS IN POLLUTED WATER

Due to the presence of sewage, and domestic and industrial wastes, the polluted water contains large amount of organic materials. The microbes present in such waters are usually heterotrophic in nature. Due to incomplete digestion of organic materials by these micro-organisms various types of acids, bases, alcohols and gases are produced. Coliform bacteria and Gram'-ve' non-spore forming bacilli are chief inhabitants of sewage. Besides, several non-coliform bacteria, such as species of *Streptococcus*, *Proteus* and *Pseudomonas*, may also be present. Some soil-inhabiting saprophytic bacteria like *Spirochaetes* also occur in sewage. Several anaerobic bacteria (e.g., *Clostridium*, *Desulphovibrio*) are also present in the mud and ooze of water bottom.

MICROORGANISMS IN SEA WATER

A high concentration of salts is found in sea water. The microbes which occur in sea water are usually halophilic and psychrophilic in nature. Diatoms and dinoflagellates play a significant role in food chain. Majority of the microbes occur in littoral and coastal zones where there is abundance of nutrients. A few occur in benthic and abyssal zones. Normally, the number of microbes increases with the depth of the sea, and their nature is influenced mainly by water temperature, salinity and pressure. A variety of bacteria and some fungi, algae, actinomycetes and protozoans constitutes the microflora of sea water. The bacteria are usually motile Gram '-ve' bacilli, but several photogenic bacteria also occur in sea water. The fungi which occur in sea water include the species of *Chytridium*, *Patersonia* and *Ophiobolus*.

AIR MICROFLORA

Air is not a natural environment for the growth and multiplication of micro-organisms, but it acts as a very good medium for their dispersal from one place to another. Several factors, such as humidity, temperature, sunlight and suspension of organic and inorganic materials affect distribution of microbes in the air. Greater the amount of dust and smoke in the air higher is the number of microbes. Each particle of dust or smoke is able to absorb numerous microbes on its surface. Micro-organisms enter the air from several sources, such as soil, organic wastes of human and animals, oral, nasal and rectal passages of animals and human beings, and lungs through coughs and sneezes.

Diverse species of micro-organisms are present in the air. Spores of *Bacillus* and *Clostridium*, ascospores of yeast, cysts of protozoans, Gram '-ve' rods and non-spore forming bacteria like *Sarcina lutea* and *Micrococcus lutea* are commonly found in the air of over populated regions. Spores of several fungi, such as species of *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus* are present in abundance in the atmosphere over the oceans.

MILK MICROFLORA

Milk and dairy products are very important components of our food, and are excellent culture media for the growth of many microbial species. Milk secreted into the udders of a healthy cow is sterile, but it is frequently contaminated when it is drawn from the cow. It may be further contaminated in subsequent handling and processing. Milking performed under hygienic conditions, following sanitary practices, reduces the chance of entry of micro-organisms into the milk.

The micro-organisms found in milk vary considerably and are dependent on the specific conditions associated with milking. Bacteria, yeasts,

moulds and bacteriophages are commonly encountered. Bacteria are the most common micro-organisms found associated with dairy processing industry. They include Gram '+ve' cocci, Gram '+ve' and Gram '-ve' non-spore forming rods and Gram '-ve' spore forming rods. Yeasts are fairly common in raw cream during hot weather, but they are potential contaminants throughout the year. Moulds often grow on milk and dairy products and appear as a fluffy or fuzzy growth. Bacteriophages are particularly obnoxious in starter cultures used for making cultured milk, butter and cheese.

Various types of microbes present in milk and dairy products bring about many chemical changes; some important ones are as follows.

[A] Lactic Acid Production

Several microbes like lactobacilli, micrococci and *Streptococcus lactis* are associated with lactic acid production. Dairy products, such as curd, cheese, butter milk and butter are obtained by the activity of these lactic acid bacteria.

[B] Gas Production

Species of *Clostridium*, *Torula*, *Torulopsis*, etc., ferment carbohydrates by producing gases and acids. *Clostridium* produces butyl alcohol by fermenting molasses. Similarly, species of *Mycoderma* form vinegar by fermenting sugary solution.

[C] Proteolysis and Lipolysis

Several bacilli and streptococci are responsible for proteolysis of milk, which results in an accumulation of soluble nitrogenous products that impart a bad taste to the milk. Similarly, some bacteria like *Pseudomonas* and *Achromobacter*, yeasts and moulds like *Penicillium* cause lipolysis of milk.

Several species of *Enterobacter*, *Klebsiella* and *Streptococcus* make the milk viscous and it becomes ropy in nature.

[D] Pathogenic Micro-Organisms

Many pathogenic micro-organisms, such as pathogens of tuberculosis, typhoid, dysentery, and diphtheria are transmitted through milk.

(MICROBIOLOGY)

Table 1. Microbes present in the milk and effects produced by them.

Biochemical types	Representative micro-organisms	Sources of micro-organisms	End products
1. Acid products	Lactobacilli	Silage, manure, feed stocks Dairy utensils, silage	Lactic acid
	Streptococci		Fermentation of lactose sugar into lactic acid or acetic acid
	Microbacteria	Manure, dairy utensils, dairy products	Lactic acid
2. Gas producers	Micrococci	Udder, dairy utensils	Lactic acid, fatty acid
	Coliforms	Manure, polluted water, soil and plants	Acid, gases and other neutral products
	Coliforms, <i>Clostridium butyricum</i> , <i>Torula cremoris</i>	Soil, manure, water Feed stocks	CO ₂ , H ₂ , etc.
3. Ropy fermentation	<i>Enterobacter aerogenes</i> , <i>Streptococcus cremoris</i>	Soil water, plants, feed stock	Viscous polysaccharide synthesis
4. Proteolysis	<i>Bacillus</i> , <i>Proteus</i> , <i>Pseudomonas</i>	Water, soil, utensils	Hydrolysis of casein protein into peptides and amino acids
5. Lipolysis	<i>Achromobacter lipolyticum</i> , <i>Candida lipolytica</i> , <i>Flavobacterium</i> , <i>Penicillium</i> , <i>Pseudomonas</i>	Water, soil, utensils	Hydrolysis of milk, fats into glycerol and fatty acids, produces rancidity

Several other microbes also occur in milk; for instance, *Serratia marcescens* is responsible for **red rot** of milk.

Some important microbes present in milk and biochemical changes brought about by them are given in Table 1.

FOOD MICROFLORA

Most of our foods are excellent media for rapid microbial growth. Food materials contain organic substances in plenty and sufficient amount of water, and they are neutral or slightly acidic in nature. They are subjected to natural contamination by many different kinds of micro-organisms, including pathogens. Metabolic activities of microbes alter the condition of food, resulting in its spoilage. The air borne microbes fall on fruits and vegetables and enter through

(MICROBIOLOGY)

the ruptured skin. The micro-organisms present in the soil reach the processing plants through the crops. Several insects are also responsible for the transference of microbes to the food. In general, the keeping quality of food depends on the success of preventing the entry of micro-organisms and of restricting their growth.

The spoilage of food materials by micro-organisms depends on the physical and chemical qualities of food material. Some common micro-organisms responsible for spoilage of food are listed in Table 2.

MICROFLORA OF HUMAN BODY

Thousands of microbes are present around us and many inhabit human body in natural course. Most of the indigenous microbes of the human body are **commensals**, i.e., they do not harm the

Table 2. Micro-organisms spoiling food and food products.

Food	Type of spoilage	Associated micro-organisms
1. Bread	Mouldy	<i>Rhizopus stolonifer</i> , <i>Aspergillus niger</i> , <i>Penicillium</i> , <i>Mucor</i>
2. Fruits and Vegetables	Ropy	<i>Bacillus</i>
	Soft rot	<i>Rhizopus</i> , <i>Erwinia</i>
	Black mold rot	<i>Aspergillus niger</i>
	Grey mold rot	<i>Botrytis</i>
3. Pickles, fresh meat	Putrefaction	<i>Rhodotorula</i> , <i>Clostridium</i> , <i>Pseudomonas</i> <i>Proteus vulgaris</i> , <i>Alcaligenes</i> , <i>Chromobacterium</i>
4. Processed meat	Mouldy	<i>Aspergillus</i> , <i>Rhizopus</i> , <i>Penicillium</i>
	Souring	<i>Micrococcus</i> , <i>Pseudomonas</i> , <i>Bacillus</i>
	Greening	<i>Leuconostoc</i> , <i>Lactobacillus</i> , <i>Pediococci</i> .
	Slimy	<i>Leuconostoc</i>
5. Fish	Putrefaction	<i>Flavobacterium</i> , <i>Micrococcus</i> , <i>Halobacterium</i>
6. Eggs	Green rot	<i>Pseudomonas fluorescens</i>
	Black rot	<i>Proteus</i>
	Red rot	<i>Serratia marcescens</i>
	Off-flavour	<i>Acetobacter</i> , <i>Leuconostoc</i> , <i>Lactobacillus</i>
7. Conc. orange juice	Ropy syrup	<i>Aerobacter aerogenes</i>
8. Sugar products, syrups	yeasty	<i>Saccharomyces</i> , <i>Torula</i> , <i>Zygosaccharomyces</i>
	Pink syrup	<i>Micrococcus roseus</i>
	Green syrup	<i>Pseudomonas fluorescens</i>
	Mouldy	<i>Aspergillus</i> , <i>Penicillium</i>

host. They obtain their nourishment from secretions and excretory wastes of the human body. Some microbes show mutualistic symbiosis. They act as scavengers by ingesting excretory wastes or are beneficial to the host; for instance, certain intestinal bacteria synthesize vitamin B, E and K, whereas others protect the host from the pathogenic microbes.

MICROBES OF THE SKIN

The human skin always remains in contact with bacteria present in the air, but most of them are unable to grow since the skin secretes some bactericidal substances. But many bacteria occur on superficial squamous epithelium of the skin. *Staphylococcus*, *Streptococcus*, diptherioids (aerobic corynebacteria), *Propionibacterium*, moulds, yeasts and some pathogenic bacteria live on the surface of the skin. They receive their nutrition from the secretions of sebaceous and dead cells.

MICROBES OF THE MOUTH CAVITY

Continuous presence of soluble nutrients and abundance of moisture in the mouth cavity provide a suitable environment for the growth of bacteria. Several microbes inhabit in the mouth cavity; some common ones are *Staphylococcus aureus*, *S. epidermidis*, *S. mitis*, peptostreptococci, lactobacilli, *Actinomyces*, *Haemophilus influenzae*, *Bacteroides oralis*, *Fusobacterium nucleatum*, *Candida albicans* and *Treponema denticola*. Certain pathogenic microbes, such as pneumococci, *Entamoeba* and trichomonads also occur on the mucous membrane of the mouth.

MICROBES OF GASTRO-INTESTINAL TRACT

Several micro-organisms, such as *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*,

(MICROBIOLOGY)

α -hemolytic streptococci, *Haemophilus influenzae* and *Neisseria* inhabit the pharynx.

When the stomach functions normally, it is almost devoid of microbes due to the presence of gastric juices. Many Gram '+ve' facultative bacteria (enterococci, lactobacilli) and fungi (*Candida albicans*) are found in the duodenum, whereas some anaerobic Gram '-ve' bacteria inhabit the ileum.

A large number of micro-organisms are found in the large intestine (colon). They include Gram '-ve' bacilli (*Fusobacterium nucleatum*, *F. necrophorum*, *Bacteroides oralis*), Gram '+ve' bacilli (lactobacilli, *Eubacterium limosum*, *Clostridium*), peptostreptococci, enterococci, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus* and *Candida albicans*.

MICROBES IN RESPIRATORY TRACT

We inhale a large number of adsorbed micro-organisms along with dust particles. Most of

them are trapped in the nasal cavity. Some staphylococci, aerobic corynebacteria (diphtheroids), Gram '-ve' cocci (*Branhamella*) and Gram '-ve' rods (*Haemophilus influenzae*) inhabit the nasal cavity.

MICROBES OF THE VAGINA AND OTHER ORGANS

For the first two days after birth, the child's vagina is sterile but after 2-5 days cocci bacteria become fixed in it. These bacteria are replaced by lactobacilli after puberty.

Mycobacterium smegmatis and mycoplasmas are usually found on the external parts of the genitals. Peptostreptococci, Enterobacteriaceae, *Clostridium*, *Staphylococcus*, *Candida albicans* and *Trichomonas vaginalis* are some common micro-organisms associated with urogenital organs.

Staphylococcus albus, *Corynebacterium xerosis* and mycoplasmas are usually associated with the mucous membrane of the eye.

Important Questions

►► Long answer questions

1. Write an essay on distribution of microbes in nature.
2. Describe the normal microflora of the human body.
3. Write short notes on:
 - (i) Soil microflora; (ii) Milk microflora

►► Short answer questions

1. 'Soil fertility also depends upon the number and nature of microbes present in the top layer of the soil'. Comment upon the statement.
2. 'Diverse species of micro-organisms are present in the air'. Are you satisfied with this statement?
3. 'Most of the indigenous microbes of the human body are commensals'. Justify the statement.

►► Very short answer questions

1. Name the micro-organisms responsible for the characteristic musty or earthy smell of a freshly ploughed field.
2. Which micro-organisms are responsible for the production of dairy products?
3. Name two micro-organisms responsible for rosy fermentation of milk.
4. Name the micro-organism responsible for green rot of eggs.
5. Name the micro-organism responsible for red rot of milk.

►► Fill in the blanks

1. Bacteria occur in all types of soil, but their number
2. In paddy fields, algae play a significant role in nitrogen fixation.
3. The microbes which occur in sea water are usually and in nature.
4. Greater the amount of dust and smoke in the air is the number of microbes.
5. *Clostridium* produces by fermenting molasses.

(MICROBIOLOGY)

True and false statements

1. In cultivable lands, microbes occur in larger numbers.
2. *Escherichia* bacteria seldom occur in the soil, but for sewage contamination.
3. Fungi play an important role for the improvement of soil nutrients in neutral and alkaline soils.
4. Protozoans present in soil has a direct effect on bacterial population.
5. The fungi which occur in sea water include the species of *Chytridium*, *Patersonia* and *Ophiobolus*.
6. Air is a rich environment for the growth and multiplication of micro-organisms.
7. Milk secreted into the udders of a healthy cow is sterile.
8. Moulds usually appear as a fluffy or fuzzy growth on milk and dairy products.
9. Species of *Mycoderma* form butyl alcohol by fermenting sugary solution.
10. Mouth cavity does not provide a suitable environment for the growth of bacteria.

Multiple choice questions

1. Which bacteria is predominant in curd?
(a) *Lactobacillus lactis* (b) *Escherichia coli*
(c) *Vibrio cholerae* (d) none of the above
2. Process of making the milk germ free by heating it at 62-8°C for 30 minutes is called:
(a) dehydration (b) immunization
(c) pasteurization (d) sterilization
3. Name a microbe associated with the gastro-intestinal tract:
(a) *Staphylococcus aureus*
(b) *Mycobacterium smegmatis*
(c) *Trichomonas vaginalis*
(d) all the above
4. Name the micro-organism responsible for the ropy fermentation of milk:
(a) *Enterobacter aerogenes*
(b) *Clostridium butyricum*
(c) *Candida lipolytica*
(d) *Pseudomonas fluorescens*
5. The following fungi occur in sea water:
(a) *Chytridium* (b) *Patersonia*
(c) *Ophiobolus*
(d) all the above
6. Which of the following bacteria are in maximum concentration in curd ?
(a) *Clostridium* (b) *Streptococcus lactis*
(c) *Pseudomonas* (d) *Serratia marcescens*
7. *Haemophilus influenzae*, a microorganism associated with the respiratory tract of human beings, is a:
(a) Gram '-ve' cocci
(b) Gram '+ve' bacilli
(c) Gram '+ve' cocci
(d) Gram '-ve' bacilli
8. Which of the following microorganisms causes putrefaction of fresh meat ?
(a) *Botrytis* (b) *Clostridium*
(c) *Serratia* (d) *Aerobacter*
9. Proteolytic bacteria — *Bacillus* and *Pseudomonas*, present in the milk, cause:
(a) hydrolysis of casein (b) hydrolysis of milk fats
(c) viscous polysaccharide synthesis
(d) lactic acid fermentation of lactose sugar
10. A fresh water pond with very low concentration of organic substances, most commonly has:
(a) *Clostridium* (b) *Spirochaetes*
(c) *Micrococcus* (d) *Achromobacter*

ANSWERS

Very short answer questions

1. Actinomycetes, 2. Lactic acid bacteria, 3. *Enterobacter aerogenes*, *Streptococcus cremoris*, 4. *Serratia marcescens*, 5. *Pseudomonas fluorescens*.

Fill in the blanks

1. decreases, 2. blue-green, 3. halophilic, psychrophilic, 4. higher, 5. butyl alcohol.

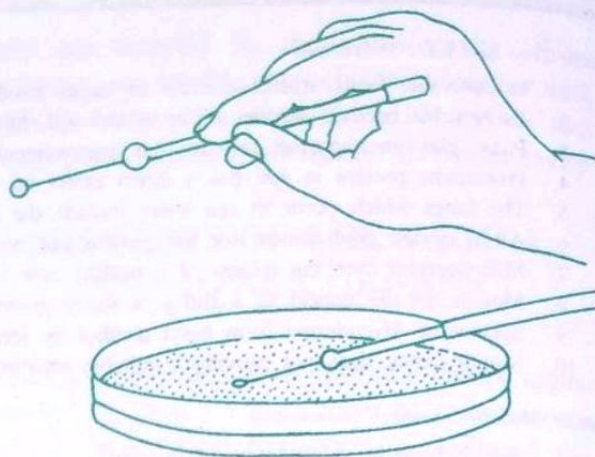
True and false statements

1. True, 2. True, 3. True, 4. True, 5. True, 6. False, 7. True, 8. True, 9. False, 10. False.

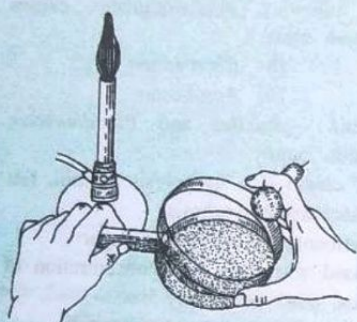
Multiple choice questions

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

3



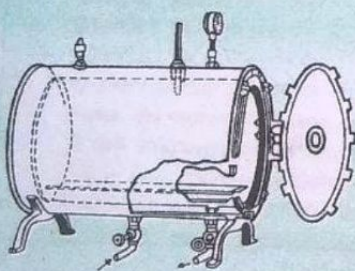
Isolation and Cultivation of Micro-organisms



When we examine micro-organisms as they occur in natural environments, they are contaminated and mixed with several other forms of life. In order to understand more about them we have to separate and study them individually. For this purpose we have to isolate micro-organisms and culture them under artificial conditions.

The growing of micro-organisms in an artificial medium is known as cultivation. Cultivation of microbes in the laboratory is a tedious job and involves a number of steps. When we culture we usually get a **mixed culture**, i.e. a large number of microbes grow together. But by various isolating techniques, we can obtain a culture which contains just one species of micro-organism. This culture is known as **pure culture** and the process of obtaining a pure culture from a mixed culture is known as **isolation** of the micro-organisms.

STERILIZATION



Micro-organisms are present everywhere. These organisms contaminate all the equipments used for the study. In the isolation of micro-organisms for detailed study, one must take utmost care to avoid the contaminants. The sterilization means making free from organisms. A maximum care is taken in sterilizing the media, glasswares and other instruments used in the culture technique to obtain a pure culture of any organism free from contamination. Some important methods of sterilization are as follows :

- (1) Sterilization by heat
 - (i) Dry heat sterilization
 - (ii) Wet heat sterilization

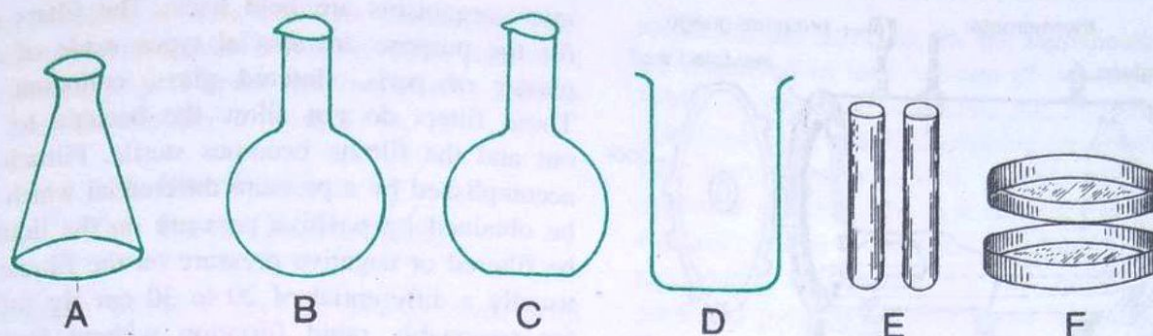


Fig. 1. Some important glasswares used in cultivation of micro-organisms.

- (2) Sterilization by filtration
- (3) Sterilization by chemical action
- (4) Sterilization by ultra-violet rays (radiation)

1. Sterilization by Heat

Heat is most commonly applied in the microbiological laboratories for sterilization.

[A] Dry Heat Sterilization

Direct heating of the instruments in a flame is an easy way of sterilization. Inoculating needles, scissors, forceps, scalpels, etc. are commonly sterilized by direct heat. The neck and mouth of specimen tubes, flasks and culture tubes are also passed through flame till they become sterilised. The process of sterilising the articles with flame is called **flaming**. Another method of dry heat sterilization is to keep thoroughly washed and dried glasswares, such as petri dishes, beakers, flasks, specimen tubes, etc. (Fig. 1) packed within a box provided with a lid, inside thermostatically controlled electric oven (Fig. 2 A). The specimen tubes, flasks and bottles are plugged before keeping them in an oven for sterilization. Complete sterilization of the equipment is accomplished by maintaining a temperature of 160°C for not less than 4 hr. inside the oven. The articles used for inoculation should be sterilized with oven only a few hours before these are used to avoid contamination.

[B] Wet Heat Sterilization

Wet heat (steam) is more efficient in penetrating materials and is preferred in sterilizing the media

used for culturing micro-organisms. The common ways by which wet heat is employed in the laboratory are **boiling**, **pasteurization**, and **autoclaving**. **Boiling** is a common method of sterilization. The instruments such as inoculation needles, scalpels, culture tubes, slides, petri dishes, syringes, etc., are kept in a container filled with distilled water. These are allowed to boil for at least 15 minutes, and by then all the articles become free from the organisms, but not the spores which are resistant to the heat of boiling water. However, prolonged boiling will kill most of the spore forming micro-organisms. If the articles are not to be used immediately these should be stored in a sterile container. In **pasteurization**, the medium is heated with steam for one hour. Sometimes, the material for sterilization is heated with circulating steam at 100°C for 20 minutes on three consecutive days. The intermittent heating helps in completely eliminating even the resistant microbes without much affecting the medium which is sterilized. This method was first used by Tyndall and it is now commonly known as **tyndallization**.

Steam under pressure is more efficient in sterilizing many materials especially the liquid media for microbial cultures. This technique of sterilization is known as **autoclaving**. This is carried out in the laboratory in an autoclave. An autoclave is a cylindrical metallic vessel with double walls. There are various types of autoclaves in use (Fig. 2). In **simple autoclave**, the body is made up of gun metal and it is cylindrical in appearance and closed at one end by hinged door. A gasket seal is provided between the door and

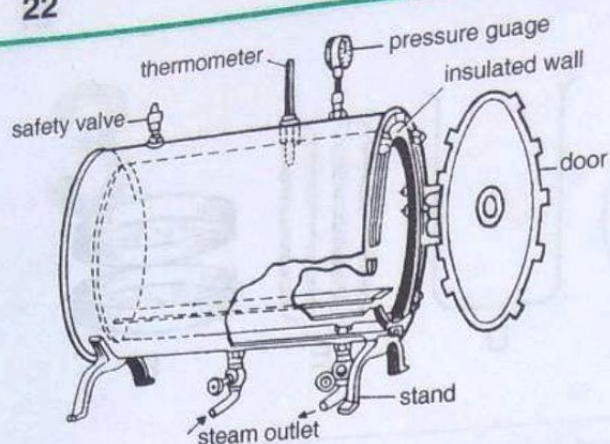


Fig. 2. Autoclave.

cylinder. It can withstand high temperature. A perforated metal tray is provided within the barrel which is used for keeping those articles which are to be sterilised. The water present below the perforated tray is boiled by an electric heater to produce the steam. The **steam jacketed autoclave** is a modified form of simple autoclave. In simple autoclave much of heat is wasted from the surface of barrel. To check this, a steam-jacket is provided around the barrel in large autoclaves. Inside the autoclave steam pressure is increased and the temperature increases proportionately. Usually autoclaving is done at 15 lb. pressure for 15 minutes. The autoclave is used to sterilise most of the solid and liquid media required for microbial cultures. They are added to Erlenmeyer flasks or test tubes and the mouth plugged with cotton wool. While sterilization takes place, the steam enters through the cotton wool and acts on the contents and after the process the plugs act as filters and help in maintaining sterility inside.

2. Sterilization by Filtration

There are some materials which will be destroyed even with little heating. Such thermolabile materials should be handled very carefully and they are usually sterilized by filtration through filters of such fine porosity that most

micro-organisms are held back. The filters used for the purpose are special types made of clay, plaster of paris, sintered glass, collodion, etc. These filters do not allow the bacteria to pass out and the filtrate becomes sterile. Filtration is accomplished by a pressure differential which may be obtained by positive pressure on the liquid to be filtered or negative pressure on the filtrate and usually a differential of 20 to 30 cm Hg suffices for reasonably rapid filtration without foaming. The filtrate should always be stored in a container which was made sterile before hand.

3. Sterilization by Chemical Action

It is a quick method of sterilizing instruments, glass apparatus or any other article used in culture technique. Several chemicals are known to be bactericidal in their properties, but only those which are most effective in low concentrations and cheap are commonly used. In most laboratories 0.1 per cent mercuric chloride solution is used for sterilizing the surface of work benches, glassware, rubber tubings, etc. The surface of inoculation table is wiped with cotton soaked in cresol solution to make it sterile before use. 3%

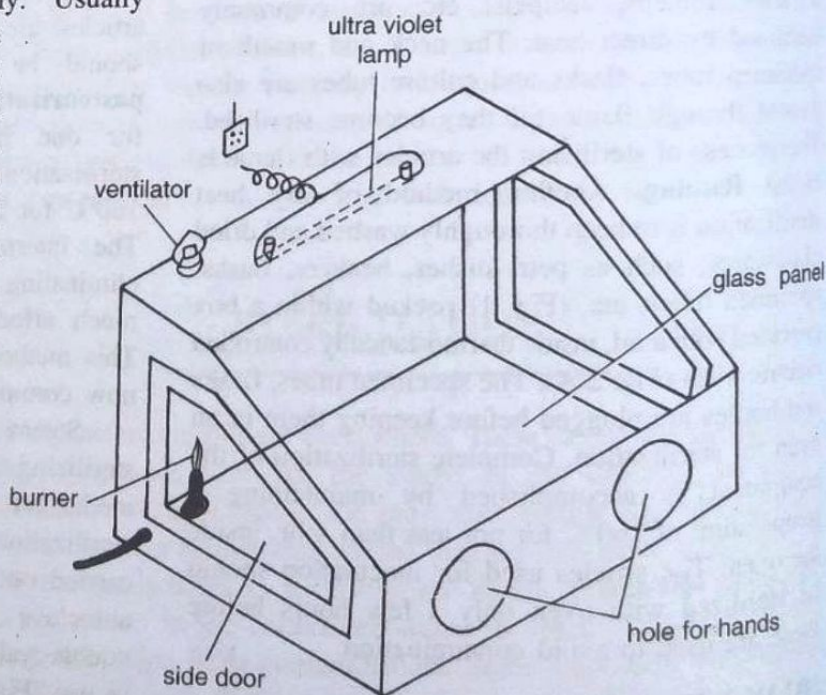


Fig. 3. Inoculation hood box.

cresol solution is also used for sterilizing Petri dishes and instruments. Similarly 90% alcohol is used for dipping scalpels and inoculation needles and later the alcohol is flamed.

4. Sterilization by U-V Rays (Radiations)

Sterilization by U-V rays is usually done with U-V lamp. The U-V rays are especially utilised for sterilizing culture rooms and tables (Fig. 3). The material to be sterilized is taken into special glass tubes which do not absorb U-V rays and are exposed for an hour.

CULTURE MEDIA

The organisms are grown on suitable culture media. A culture medium is the solution of different chemicals which support the growth of micro-organisms. The culture media are usually of two types, viz. (i) **natural culture medium**, and (ii) **synthetic culture medium**.

[A] Natural Culture Medium

Natural media are those used on the basis of experience and not on the basis of exact knowledge; their exact composition is unknown and variable. They include peptones, milk, meat extracts and infusions, gelatin, agar, vegetable juices, etc. For growing fungi on natural media, damp bread, rotten apples, etc. are exposed to atmosphere and growth of *Mucor*, *Rhizopus*, *Aspergillus* and *Penicillium* are marked.

[B] Synthetic Culture Medium

The synthetic media are prepared in the laboratory by adding requisite quantity of chemicals prescribed according to the need of a particular organism. They are of definitely known composition. The synthetic media are further of two types, viz. (a) **liquid media**, and (b) **solid**

media. In the liquid media, the ingredients needed are dissolved in total volume of deionised water. While in the solid medium, the ingredients are dissolved in a vessel containing deionised water and then agar is added as prescribed for a particular medium. The agar solidifies the medium. Besides basal nutrient medium, specified compounds are added in the medium for a particular purpose. In both liquid and solid media, the pH is adjusted by adding acid (hydrochloric acid) and base (sodium hydroxide) and is measured either by pH paper, BDH indicator or by pH meter.

Dispensing the Medium

Either unsterilized or sterilized medium is poured into the sterilized flasks, culture tubes and Petri dishes (Fig. 4). The unsterilized medium is poured into the flasks and culture tubes by semiautomatic syringe, funnel and automatic filler. The liquid medium (broth) is dispensed into test tubes or flasks which are plugged with non-absorbent cotton wool plugs. The pouring of sterilized medium is

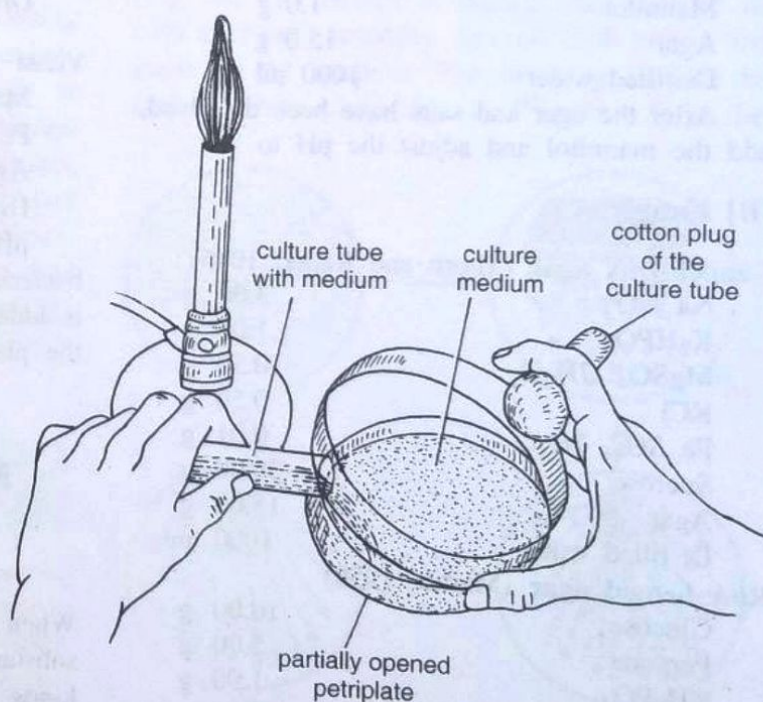


Fig. 4. Pouring of medium.

usually carried out in Petri dishes already sterilized in a special sterilized inoculation chamber.

Some Important Culture Media

Some important media which are generally used for culturing the various micro-organisms are given below.

[A] Bacteria

Nutrient agar

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Distilled water	1000 ml
Heat until agar and peptone dissolve. Adjust pH to 6.6 to 7.0.	

Asparagine mannitol agar (Thornton, 1922)

K ₂ HPO ₄	1.0 g
KNO ₃	0.5 g
MgSO ₄ · 7H ₂ O	0.2 g
CaCl ₂ · 6H ₂ O	0.1 g
NaCl	0.1 g
FeCl ₂ · 6H ₂ O	in traces
Asparagine	0.5 g
Mannitol	1.0 g
Agar	15.0 g
Distilled water	1000 ml

After the agar and salts have been dissolved, add the mannitol and adjust the pH to 7.4.

[B] Fungi

Czapek-Dox agar (Thorn and Raper, 1945)

Na NO ₃	3.00 g
K ₂ HPO ₄	1.00 g
MgSO ₄ · 7H ₂ O	0.50 g
KCl	0.50 g
Fe SO ₄ · 7H ₂ O	0.01 g
Sucrose	30.00 g
Agar	15.00 g
Distilled water	1000 ml

Rose-bengal agar (Martin, 1950)

Glucose	10.00 g
Peptone	5.00 g
KH ₂ PO ₄	1.00 g
MgSO ₄ · 7H ₂ O	0.50 g
Streptomycin Agar	30.00 mg
Rose-bengal	0.035 g
Distilled water	1000 ml

The antibiotic is sterilized separately and added aseptically to the sterilized medium. Aureomycin (35 to 2,000 µg) may be substituted for streptomycin. The medium is especially recommended for the isolation of fungi in the presence of large numbers of bacteria.

Potato-dextrose agar (PDA)

Potatoes, peeled and meshed	200g
D-glucose	20 g
Agar	15 g
Distilled water	1000 ml

Potato slices are first steam cooked in 500 ml water and agar is melted in other 500 ml water. Now melted agar and potato meshes are mixed together, filtered and dextrose is added.

[C] Actinomycetes

Kenknight and Munaier's medium

Dextrose	1.00 g
KH ₂ PO ₄	0.10 g
NaNO ₃	0.10 g
KCl	0.10 g
MgSO ₄ · 7H ₂ O	0.10 g
Agar	15.00 g
Distilled water	1000 ml

Yeast malt extract agar

Malt extract	30.0 g
Peptone	5.0 g
Agar	20.0 g
Distilled water	1000 ml

pH is adjusted to 5.4. In order to inhibit bacterial growth, 10% sterile lactic acid solution is added to the molten medium just before pouring the plates so as to bring down the pH to 3.5.

METHODS OF OBTAINING PURE CULTURES

When fluid culture media are inoculated with substances such as soil, water, or excreta, many kinds of organisms develop simultaneously side by side, and a heterogeneous mixture or mixed culture of microbes results. Any technical procedure for obtaining pure culture is dependent

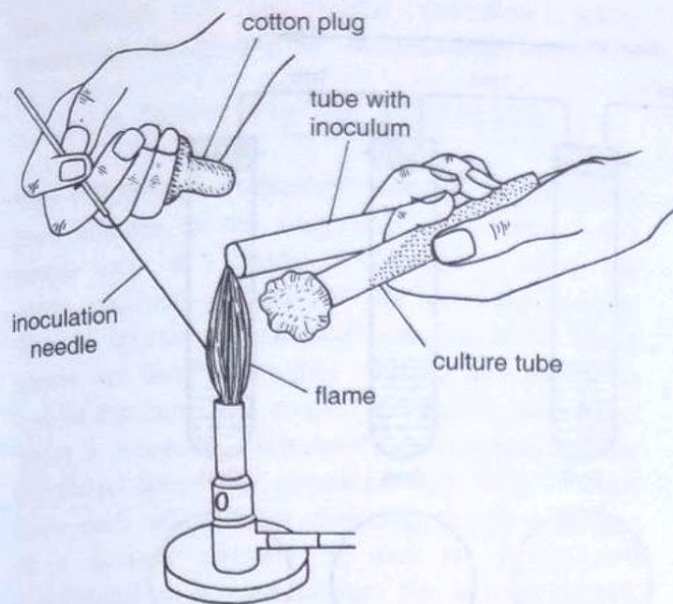


Fig. 5. Technique of inoculation.

upon the isolation of a single viable microbe which is allowed to multiply in a suitable culture medium. The first reliable method of isolation of pure culture was devised by **Robert Koch** in 1881. Since then, various isolating techniques have been evolved with only minor modifications. If nutrient gelatin or agar is inoculated with fluid and is then solidified and kept under favourable temperature conditions, many of the microbes that have been introduced are able to multiply and form distinct colonies. If the colonies are not closely crowded, a pure culture may be obtained by touching a colony with the tip of a sterile needle and inoculating tubes of fresh culture media.

ISOLATION OF MICRO-ORGANISMS

The Petri dishes or flasks with sterilised media are inoculated with an organism (Fig. 5) and are placed in culture chamber for its growth. Before inoculation, both the hand, inoculation instruments, tables, etc., are sterilized by wiping them with cotton wool soaked in alcohol. Some important methods used for obtaining pure cultures are as follows:

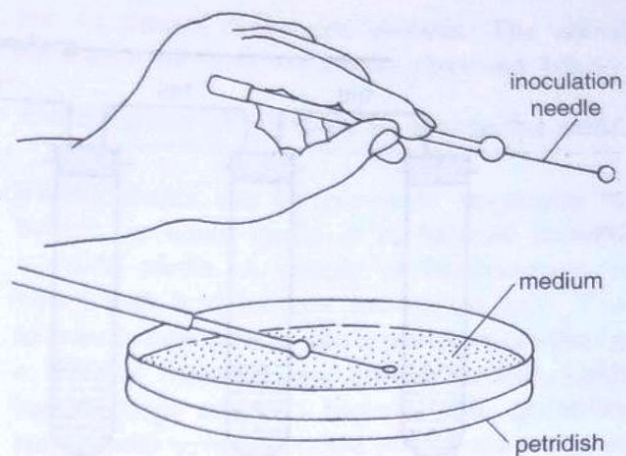


Fig. 6. Way of holding the loop for streaking in an agar medium.

1. Streaking Technique

Streaking is the most widely used method of isolation. This method is most suitable for bacterial and fungal cultures. Bacterial patches are picked up with the help of sterilized loop and is streaked back and forth across the surface of agar medium (Fig. 6). The needle is flamed and allowed to cool after each streaking. Several such streaks are made on the medium. The streaking is done in some definite plan (Fig. 7). The care is to be

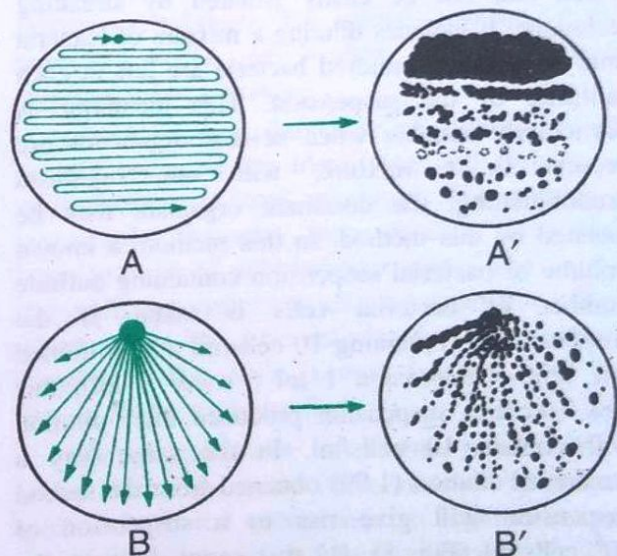


Fig. 7 A-B. Streaking technique: A, B. Methods of inoculation, A', B'. growth after inoculation.

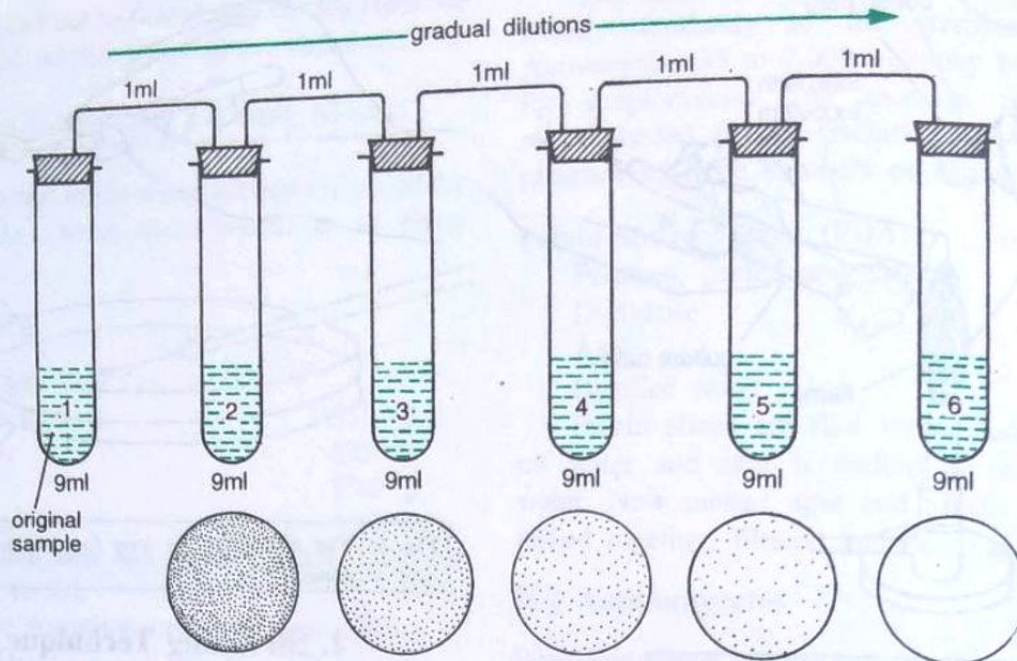


Fig. 8. Dilution plate technique of isolation.

taken not to break the surface of the medium during streaking.

2. Dilution Technique

This technique is most suitable for the bacteria which can not be easily isolated by streaking technique. It includes diluting a mixture of bacteria until only a few hundred bacteria are left in each millilitre of the suspension. This technique is particularly suitable when several organisms are present in a mixture, with one organism predominating, the dominant organism may be isolated by this method. In this method, a known volume of bacterial suspension containing definite number of bacterial cells is taken. If the suspension is containing 10 cells/ml and is diluted one tenth (suspension 1 ml : water 9 ml), this new (second) suspension produced after dilution will contain 10 cells/ml. In the same way a hundredth dilution (1:99) obtained from the second suspension will give rise to a suspension of 10^4 cells/ml (Fig. 8). By this serial dilution, the chances of dominant organism in pure condition in the culture is increased. Ultimately, a little amount of the suspension is pipetted out and

spread over the medium of Petri dish. A luxuriant growth of most of the bacteria is obtained after a period of 24 hours at $25^{\circ}\text{--}30^{\circ}\text{C}$.

3. Single Cell Technique

In this method, a suspension of microbes is placed on the cavity slide. Thereafter, a single cell is removed with the help of sterile micropipette with the aid of a microscope. The cell is then transferred to sterile culture medium (Fig. 9). The progeny obtained, originates from single cell.

4. Selective Enrichment Technique

The technique involves the use of media and conditions of cultivation which favour the growth of desired species. Generally, some specific substances are added in the media which inhibit

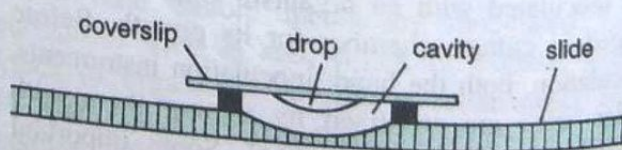


Fig. 9. Single cell technique.

the growth of undesirable microbes, while promoting the growth of the desirable ones.

5. Spore Plating Technique

This technique is particularly suitable for obtaining pure cultures of the fungus. In this method, the sterile loop of a needle is allowed to touch the spore producing organ of the growing fungus. Several spores get adhered with the loop. These spores are then thoroughly washed, and plated on a solid medium. The entire procedure is performed under a binocular microscope. After streaking, the individual spore will germinate at a little distance from each other. After obtaining the pure culture of a desired microbe, it may be grown and maintained as a pure culture for a long period, by various ways. After satisfactory growth, the pure culture may be stored in a refrigerator for sometime. But usually, the culture medium is exhausted. Thus, it becomes essential to transfer the organism to another culture tube. Such transfer of organism from one to another medium is known as **subculturing**. Subculturing should be done by skilled hands to avoid contamination.

CULTIVATION OF VIRUSES

Viruses are obligate intracellular parasites. They have no biosynthetic capacity of their own but can manipulate a viable cell genetically to synthesize viral particles. Thus viruses require living cells to support their replication.

The primary purpose of virus cultivation is :

- (1) to isolate and identify viruses in clinical samples,
- (2) to study virus structure, its replication, genetics and effects on host cell, and
- (3) to prepare viruses for vaccine production.

To cultivate viruses they must be provided with living cells, instead of a fairly simple chemical medium. Previously viruses were cultivated by transfer from plant to plant, animal to animal or bacterial culture to bacterial culture. Presently viruses are cultivated by inoculating them in bacterial cells, living animals, embryonated eggs

and in tissues or in cell cultures. The above cultivation methods are briefly discussed below.

Cultivation of viruses in Bacterial cells

Bacteriophages can be grown in suspension of bacteria in liquid media or in bacterial cultures on solid media. A sample of bacteriophage is mixed with host bacteria and melted agar. This mixture is now poured into a petriplate containing a layer of hardened agar growth medium. Each bacteriophage infects a bacterial cell, multiplies and releases several hundred new phage particles. These newly produced viruses infect other bacterial cells in the vicinity and more new phages are produced. Within a short span of time several multiplication cycles occur and all the bacteria in the area surrounding the original virus are destroyed. At this stage plaques (clear areas) appear in the petriplate. Each plaque in the petriplate corresponds to a single virus in the initial suspension.

Cultivation of Viruses in Living Animals

Certain viruses which are difficult to cultivate in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, rabbits and primates. The animals selected for this purpose should be healthy and free from communicable diseases.

Suckling mice (less than 48 hours old) are usually preferred for identifying and isolating a virus from a clinical specimen. The animal is inoculated through intracerebral, intranasal, intraperitoneal or subcutaneous route. The animal shows disease symptoms after an incubation period which varies for different viruses and in animal inoculated. The virus is now isolated from the disease tissue and purified.

Though cultivation of viruses in animals is useful for the study of immune responses, epidemiology and oncogenesis. It is however, not suitable for the study of human viruses. Besides this, cultivation of viruses in animals is expensive and there are issues related to animal welfare systems.

Cultivation of Viruses in Embryonated Egg

One of the convenient and inexpensive medium for the cultivation of many animal viruses is embryonated egg. A fertilized egg is incubated for 6 to 8 days at a temperature suitable for hatching. At this stage a hole is drilled in the shell of the embryonated egg and a viral

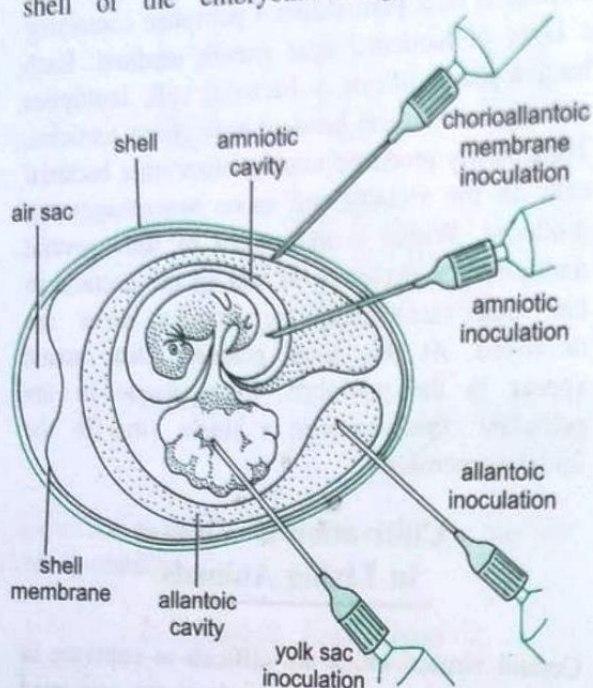


Fig. 10. Cultivation of viruses in embryonated egg.

suspension is inoculated in it. There are several membranes in an egg (Fig. 10) and the virus is injected in the region most suitable for its growth.

Egg inoculation is suitable for diagnostic purposes as the lesions produced in the embryo are characteristic of the given virus. Embryonated eggs are used for production of vaccines.

Cultivation of Viruses in Cell Cultures

Viruses that infect eukaryotic cells are now generally cultivated in monolayers of cells obtained from plant or animal tissues. Cells are removed aseptically from a plant or animal tissue and are placed in a sterile glass or plastic vessel containing suitable culture medium. The cells form monolayer on the surface of the medium. This monolayer is also called **cell culture**.

A virus inoculum is now spread over a cell monolayer. If the cells in the monolayer are susceptible, the virus enters the cells and multiplies. Sometimes repeated multiplication of viruses may result in the deterioration of the cells of the monolayer. Plant viruses can be easily cultivated in monolayers of plant tissue, in cultures of separated cells in suspension and in protoplast cultures.

Bacterial viruses can be cultivated by inoculating a suitable susceptible bacterial culture with a virus.

Important Questions

Long answer questions

1. What are the general principles of methods of isolation and cultivation of micro-organisms?
2. Describe the methods of isolation and inoculation of bacteria in any bacterial disease studied by you.
3. How do the methods used for cultivating viruses differ from those used for cultivating bacteria? Describe some important methods for propagating the plant and animal viruses.

Short answer questions

1. What is pure culture?
2. Describe in brief some important methods of sterilization.
3. Write a note on pasteurization.
4. What is an autoclave? For what purpose is it used?
5. Write the composition of Czapek-Dox agar medium used for cultivation of fungi.
6. How a pure culture is obtained from a mixed culture?

►► Very short answer questions

1. What do you understand by the term 'isolation of the micro-organisms'?
2. Define the term 'flaming'.
3. Name a culture medium which is used for the cultivation of fungi.
4. Enumerate some important methods of isolation of micro-organisms.
5. Which technique is usually used for the cultivation of viruses?

►► Fill in the blanks

1. In pasteurization, the culture medium is heated with for one hour.
2. Nutrient agar medium is generally used for culturing the
3. The first reliable method of isolation of pure culture was devised by in
4. are usually propagated in embryonated eggs, plasma clots, tissue culture, etc.

►► True and false statements

1. The growing of micro-organisms in an artificial medium is known as cultivation.
2. Heat is most commonly applied in the microbiological laboratories for sterilization.
3. The process of sterilizing the articles with flame is called autoclaving.
4. Synthetic culture media are those used on the basis of experience and not on the basis of exact knowledge; their exact composition is unknown.
5. Potato-dextrose-agar (PDA) medium is generally used for the cultivation of fungi.

►► Multiple choice questions

1. Asparagine mannitol agar medium is generally used for culturing:
 - (a) bacteria
 - (b) viruses
 - (c) fungi
 - (d) all the above
2. The growing of micro-organisms in an artificial medium is known as:
 - (a) cultivation
 - (b) isolation
 - (c) sterilization
 - (d) tyndallization
3. The following technique is used for the isolation of micro-organisms:
 - (a) streaking technique
 - (b) dilution technique
 - (c) selective enrichment technique
 - (d) all the above
4. Viruses can be cultured effectively on the following medium:
 - (a) nutrient agar
 - (b) Czapek-Dox agar
 - (c) yeast malt extract agar
 - (d) plasma clot

ANSWERS**►► Very short answer questions**

1. the process of obtaining a pure culture from a mixed culture, 2. the process of sterilizing the articles with flame, 3. Czapek-Dox agar medium, 4. streaking, dilution, single cell, selective enrichment, spore plating techniques, 5. plasma clot technique.

►► Fill in the blanks

1. steam, 2. bacteria, 3. Robert Koch, 1881, 4. animal viruses.

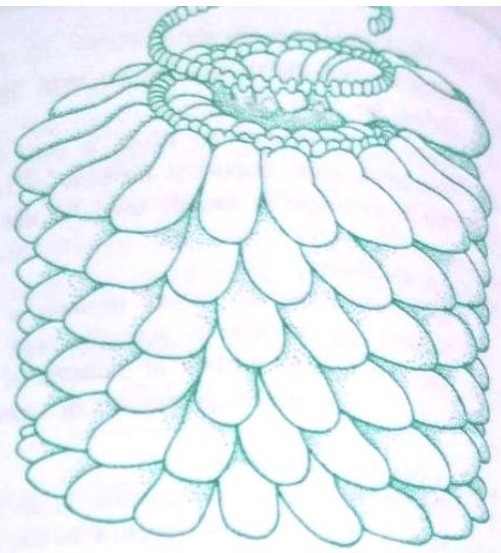
►► True and false statements

1. True, 2. True, 3. False, 4. False, 5. True.

►► Multiple choice questions

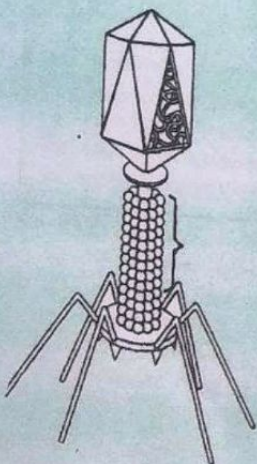
1. (c), 2. (a), 3. (d), 4. (d).

4



Viruses, Viroids and Prions

VIRUSES



The name virus (Latin *virus* = venom of poisonous fluid) was given by Pasteur to the **causative agents of infectious diseases**. Adolph Mayer (1886), a Dutch scientist, and D. J. Ivanowsky (1892), a Russian scientist, recognized certain microbes as causative agent of mosaic disease of tobacco. The basic criterion used to differentiate these agents from other familiar microbial agents of diseases was **their ability to pass through bacteria-proof filters**. These agents were thus designated as **filterable viruses** (now it has been established that many microbes of bacterial nature are also capable of passing through such filters, therefore, the word 'filterable' was subsequently dropped).

M. W. Beijerinck, a Dutch microbiologist, demonstrated in 1898 that viruses differ from other cellular organisms. He discovered that the virus of tobacco mosaic disease could be precipitated from a suspension of alcohol without losing its infectious power and the fluid was capable of diffusing through Chamberland filter- candle. This feature was contrary to that bacteria which were retained by the filter. This led Beijerinck to believe that the fluid itself was living and he put forward the principle of "*Contagium vivum fluidum*" (infectious living fluid). In the same year, Loeffler and Frosch demonstrated that foot-and-mouth disease of cattle is caused by a filtrate apparently free of any bacteria. They concluded that if the agent of this disease is particulate, it must be smaller than the diameter of the smallest known bacteria. Nearly 40 years after these

observations, the structure of virus was studied by Wendell M. Stanley, an organic chemist, in 1935. He showed that the **infectious principle of virus could be crystallized** and that the crystals consisted largely of proteins. For many years it was thought that virus is simply a protein molecule, but later it was discovered that virus contains a small but constant amount of RNA or DNA in addition to protein. The nucleic acid carries genetic information to code for the production of new infectious particles. A virus is, therefore, not simply a protein but a **nucleoprotein** and **its infectious principle is the nucleic acid** rather than protein. Stanley was awarded Nobel prize in 1946 for this discovery.

Subsequently, many already known diseases like mumps, chicken pox and hog cholera, as well as several newly identified diseases, were found to be caused by filterable agents, i.e., viruses. An English scientist, F.W. Twort (1915), and a Canadian, Felix d'Herelle (1917), independently discovered that a virus was capable of dissolving or lysing bacterial cells. This virus was designated as **bacteriophage**, i.e., eater of bacteria.

Luria (1953) described viruses as submicroscopic entities capable of being introduced into specific living cells and reproducing inside such cells only. According to Andre Lwoff (1966), a Nobel Laureate French virologist, the most appropriate definition of viruses is "viruses are viruses". Some biologists think that viruses are descendants of cellular organisms that have become highly specialized as parasites, others consider that viruses were originally fragments of DNA or RNA broken off from the nucleic acids of cellular organisms. However, it is now well established that viruses are obligately parasitic, self-replicating non-cellular organism and essentially composed of a protein covering surrounding a central nucleic acid molecule (either DNA or RNA). Most of the modern virologists prefer not to define viruses by any specific definition but describe them by their various physical, chemical, biological and clinical properties.

Origin of Viruses

The origin of viruses has been a subject of considerable speculation in the scientific community. However, there is general agreement that viruses (phage) probably infected bacteria during the period when Archaea and Eubacteria were the sole life forms on the earth. Later, when eukaryotes evolved, they were also attacked by viruses.

Following three major theories have been proposed for the origin of viruses.

[A] Theory of Coevolution

According to this theory viruses originated in the 'primordial soup' and coevolved with the more complex life forms (e.g., Archaea and Eubacteria). The earliest self replicating genetic system was probably composed of RNA. RNA can promote RNA polymerization and this process was probably accelerated by the proteins present in the 'primordial soup'. The DNA template is much more effective and originated early in evolution. RNA then became the messenger between the DNA template and protein synthesis. Thus the genetic code came into being and permitted orderly replication. Gradually the early replicative forms became complex and became encased in a lipid sac; thus its metabolic machinery became separated from the surrounding environment. Such individual units may have been the ancestor of the Archaea and Eubacteria. At the same time some replicative forms, composed mainly of self-replicating nucleic acid surrounded by protein coat, may have retained simplicity. This entity was the forerunner of the virus. It gradually evolved and acquired the ability to invade and take over the genetic machinery of a host. Thus there was coevolution of the bacterium and virus.

[B] Retrograde Evolution

This theory is based on the assumption that viruses originated from such free-living or parasitic prokaryotes which gradually lost biosynthetic capacity and genetic information. Eventually the

(MICROBIOLOGY)

prokaryotes evolved to simply a group of genes known as virus. It seems possible that intracellular parasitic microorganisms could have become more and more dependent on the host cell for readymade metabolites and in the process lost much of its own biosynthetic capacity. The theory proposes intracellular parasitic bacteria, such as rickettsiae or the chlamydiae as the potential examples that could regress to viral state.

[C] Escaped Gene Theory

The theory proposes that pieces of host-cell RNA or DNA gained independence from cellular control and escaped from the cell. Living organisms make duplicates of their genetic information by initiating replication at a specific site, called the initiation site. The replication cycle ends when a full complement of the genome is synthesized. If initiation of replication begins elsewhere on the genome, this duplication occurs independent of host control. A virus that could recognize nucleotide sequences at sites other than the start site and that carried the proper polymerase could have the capacity to produce RNA or DNA without interference from normal control mechanisms.

The origin of viruses may have been with episomes (plasmids) or transposons. These are circular DNA molecules that replicate in cytoplasm and can be integrated into or excised from various sites in the host chromosome. Plasmids can also move from cell to cell carrying information such as fertility or antibiotic resistance. Transposons are bits of DNA present in both prokaryotic and eukaryotic cells that can move from one site in a chromosome to another site carrying genetic information. The DNA of transposon carries a gene for synthesis of the reverse transcriptase and transposon elements with such properties have some similarity to retroviruses. Analysis of nucleotide sequences in viruses indicates that they are quite equivalent of specific sequences in the host cell. Evidence accumulated so far strongly supports the proposal that viruses originated from 'escaped' host nucleic acid.

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General Characteristics of Viruses

Viruses are sub-microscopic particles which have been studied in detail by electron microscope, using selective techniques. A simple virus particle often designated as **virion**, consists of a nucleic acid core of genetic material (genome) enclosed within protein coat (Fig. 1). The amount of protein in different viruses varies from 60-95 per cent and the rest is nucleic acid.

[A] Size

Earlier the size of viruses was measured by using the technique of filtration through collodion membranes of known porosity. But now techniques like ultracentrifugation and electron microscopy are employed. Viruses are very small in size, varying over a wide range from 20-350 nm. The largest are the orthopoxviruses, measuring about 240 nm × 300 nm, i.e., approximately 1/10 the size of a red blood cell. The complex bacteriophages are about 65 nm × 200 nm. Among the smallest viruses known are the enteroviruses, which are less than 30 nm in diameter. The dimensions of some common viruses are shown in Table 1 and Fig. 2.

[B] Nucleic Acid

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

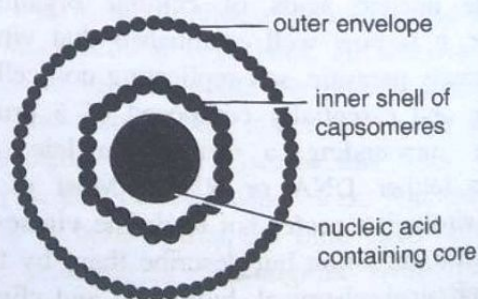


Fig. 1. Virus : General structure.

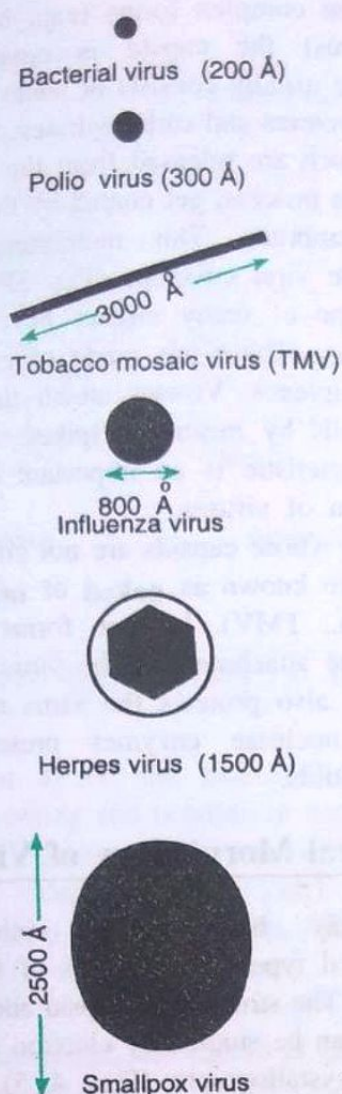


Fig. 2. Virus : Variations in the shape and size.

Table 1. Size of some well known viruses.

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

Table 2. Characteristics of nucleic acids of some viruses.

Virus	Nucleic acid type	Strands	Nucleotide pairs	Molecular weight in Dalton
Polyoma	DNA	Double	4,500	3×10^6
Adenovirus	DNA	Double	35,000	23×10^6
Coliphage T ₄	DNA	Double	200,000	130×10^6
Coliphage T ₂	DNA	Double	60,000	40×10^6
Tobacco mosaic virus	RNA	Single	7,500	2.5×10^6
Tobacco necrosis virus	RNA	Single	1,500	0.5×10^6
Bean mosaic virus	RNA	Single	3,000	1×10^6
Polio virus	RNA	Single	6,000	2×10^6

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

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

Characteristics of nucleic acids of some better known plant and animal viruses are given in Table 2.

[C] The Protein Coat

The nucleic acid core of the virus is protected by a protein coat called the **capsid**. Each capsid consists of several identical protein subunits, known as **capsomeres**. In some viruses the proteins composing the capsomeres are of a single type, while in others several types of proteins may be present. These subunits are usually arranged

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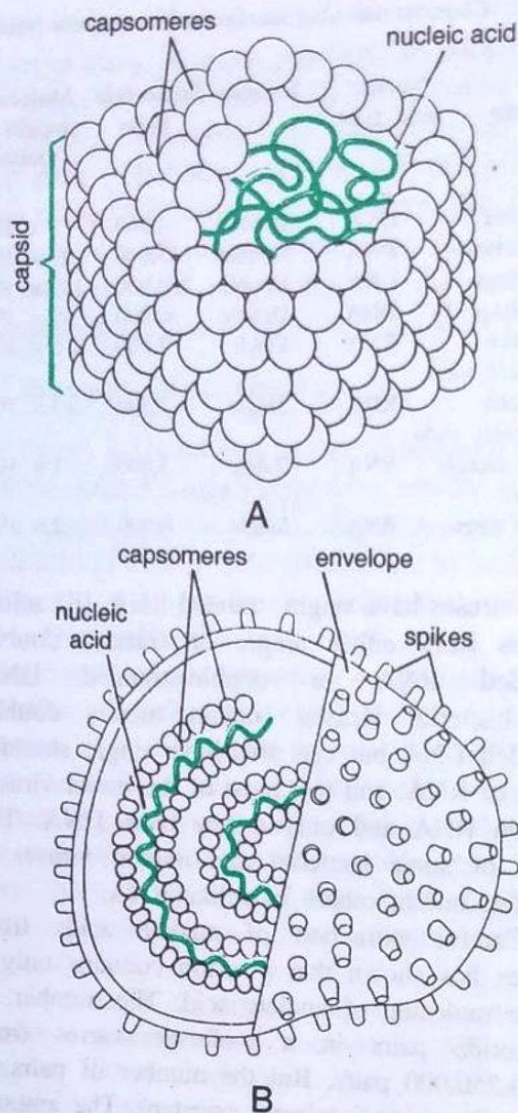


Fig. 3 A-B. Virus morphology : A. Polyhedral virus (without envelope), B. Helical virus (with envelope).

in helical or polyhedral geometric forms. The number of proteins and the arrangement of capsomeres are characteristic of specific viruses and thus can be useful in their identification and classification.

The capsomeres forming the capsid (protein coat) of a virion are of two types—**pentamer**, made of five identical monomers, and **hexamer**, having six monomers. Each monomer is connected with the neighbouring monomers on either side with the help of bonds. Likewise, the capsomeres are also connected with each other, but the bonds between the capsomeres are weak (Fig. 3A).

(MICROBIOLOGY)

In some complex forms (e.g., influenza and herpes virus) the capsid is covered by an **envelope**. It usually consists of some combination of lipids, proteins and carbohydrates. Some animal viruses, which are released from the host cell by an extrusion process, get coated by the host cell's plasma membrane. This membrane eventually becomes the viral envelope (Fig. 3B).

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

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

General Morphology of Viruses

Viruses may be classified into various morphological types on the basis of their capsid architecture. The structure of capsid and individual capsomere can be studied by electron microscopy and X-ray crystallography (Figs. 4, 5). Following are some of the common morphological forms of viruses.

[A] Helical Viruses

These viruses are cylindrical or rod-like in form and the central nucleic acid strand is coiled like a helical spring. The protein subunits (capsomeres) are helically arranged around the helical spring. The common examples of helical viruses are New Castle virus, Mumps virus, Rabies virus and Tobacco mosaic virus (Fig. 4A).

[B] Polyhedral Viruses

In these viruses the nucleic acid is packed in an unknown manner within a hollow polyhedral head. They have been classified further into **tetrahedral**, **octahedral** and **icosahedral** form depending upon the number of faces. Of these, icosahedral form

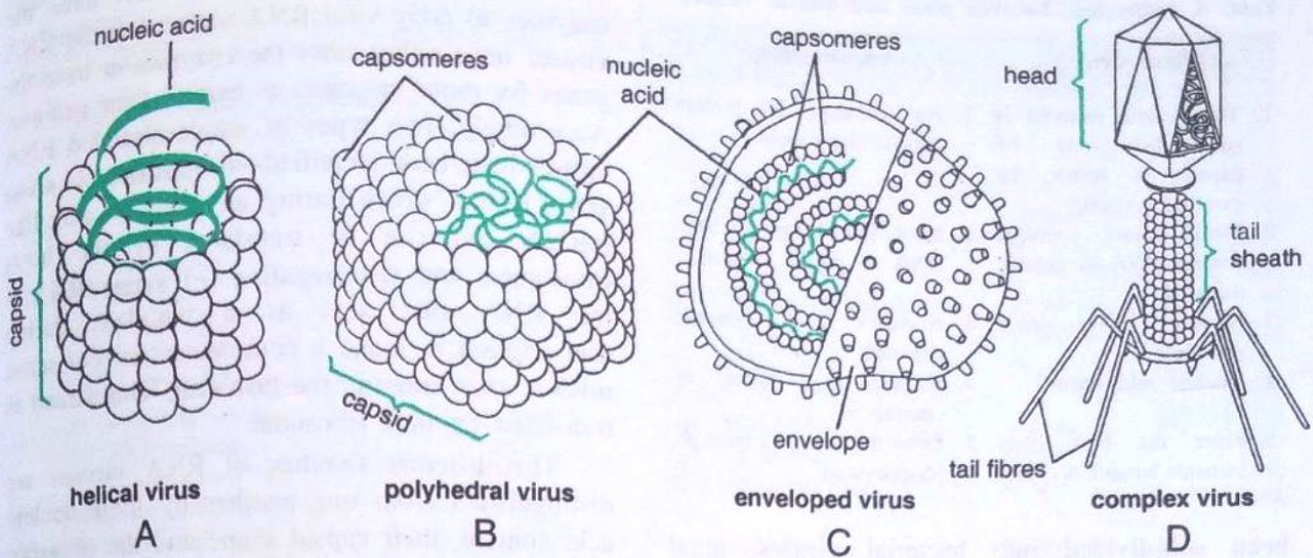


Fig. 4 A-D. Virus morphology : A. Helical virus, B. Polyhedral virus, C. Enveloped virus, D. Complex virus.

is considered to be the most efficient shape because of packing and bending of capsomeres in a near spherical form. An icosahedron has 12 corners, 20 triangular faces and 30 edges (e.g., Adenovirus, Poliovirus; Fig. 4B).

[C] Enveloped Viruses

As mentioned earlier, the capsid of these viruses is covered by an envelope. Such viruses may be spherical, helical or polyhedral in shape (Fig. 4C).

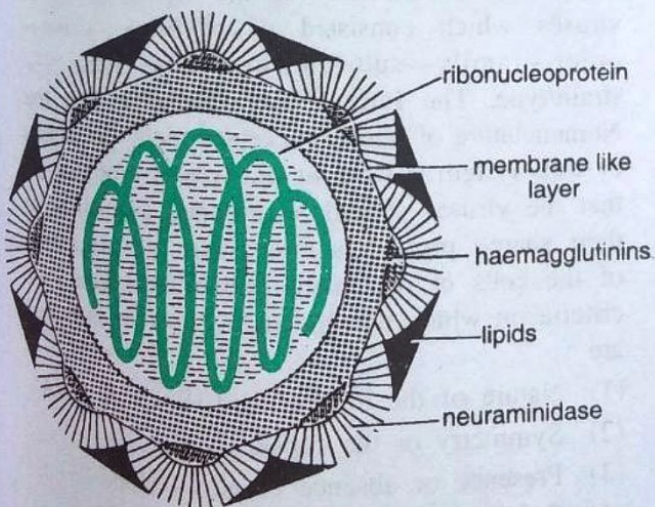


Fig. 5. Influenza virus.

[D] Complex Viruses

Bacterial viruses or bacteriophages have a complex structure, hence called **complex viruses**. For example, T-even bacteriophages have a capsid (head) to which other structures like a helical tail sheath, base plate, tail fibers and pins are attached (Fig. 4 D). Another example of complex viruses are pox viruses in which the nucleic acid is surrounded by several coats.

Classification and Nomenclature of Viruses

[A] Classification

Viruses do not fit into the established biological classification of cellular organisms. This is mainly due to the pseudo-living nature of viruses; They are non-living particles with some chemical characteristics similar to those of life. Initially, viruses were classified into the following four groups on the basis of their host range, and clinical, epidemiological and pathological symptoms.

1. Plant viruses. These viruses infect only plants and depending upon the host they have

Table 3. Differences between plant and animal viruses.

Plant virus	Animal virus
1. The genetic material is surrounded only by capsid, it forms the external boundary.	1. An envelop is present outside the capsid.
2. Most plant viruses contain RNA as genetic material.	2. Most animal viruses have DNA as genetic material.
3. Nucleic acid single stranded.	3. Nucleic acid double stranded.
4. Nucleic acid linear.	4. Nucleic acid linear or circular.
5. Enter the host body through wound or pore.	5. Enter the host cell through phagocytosis.

been sub-divided into bacterial viruses, algal viruses, fungal viruses, etc. (see Table 3).

2. Invertebrate viruses. Viruses infecting invertebrates have been included in this group.

3. Vertebrate viruses. This group includes viruses infecting vertebrate animals.

4. Dual-host viruses. This group includes those viruses which infect two different hosts mentioned above.

Holmes (1948) included all viruses in a single order **Virales** which was divided into three sub-orders.

1. Phagineae. This sub-order includes viruses infecting bacteria, i.e., bacteriophage.

2. Phytophagineae. It includes viruses infecting plants.

3. Zoophagineae. It includes viruses infecting animals.

Since viruses are very different from cellular organisms, it is difficult to classify them into typical taxonomic categories, viz., kingdom, phylum, etc. **International Committee on Taxonomy of Viruses (ICTV)** has suggested 'family' as the highest taxonomic category for viruses.

Major groups of viruses are distinguished first by their nucleic acid content as either RNA or DNA viruses. Subsequent sub-divisions are based largely on several other properties of nucleic acids which are briefly mentioned below.

The **RNA viruses** can be single-stranded (ssRNA) or double-stranded (dsRNA). Most of the RNA viruses are, however, single-stranded. As

most of the eukaryotic cells do not have the enzymes to copy viral RNA molecules, the RNA viruses must either carry the enzymes or have the genes for those enzymes as part of their genome. Accordingly, two types of single stranded RNA viruses have been identified—(i) **positive (+) sense RNA** is that which during an infection acts like mRNA and can be translated by the hosts ribosomes, and (ii) **negative (-) sense RNA** is the RNA that acts as a template during transcription to make a complementary (+) sense mRNA after entering the host cell. This strand is translated by host ribosome.

The different families of RNA viruses are distinguished from one another by their nucleic acid content, their capsid shape and the presence and absence of an envelope. Some important human, animal and plant virus families are given in Table 4.

Like RNA viruses, the **DNA viruses** are grouped into families according to their DNA organization. The double stranded DNA (ds DNA) viruses are further separated on the basis of the shape of their DNA (linear or circular), their capsid shape, and the presence or absence of an envelope. Some important families of DNA viruses are given in Table 5.

Based on information obtained from ultracentrifuge and electron microscopic studies, Lwoff, Horne and Tournier (1962) proposed a comprehensive scheme for the classification of viruses which consisted of phylum—class—order—family—sub-family—genus—species—strain/type. The International Committee on the Nomenclature of Viruses accepted many principles of this system. Lwoff *et al.* system emphasized that the viruses should be grouped according to their shared properties rather than the properties of the cells or organisms they infect. The four criteria on which this hierarchical system is based are :

- (1) Nature of the nucleic acid (RNA or DNA).
- (2) Symmetry or the capsid.
- (3) Presence or absence of an envelope.
- (4) Dimensions of the virion and capsid.

An outline of Lwoff, Horne and Tournier's system of classification is given in Table 6.

Table 4. Classification of major groups of RNA viruses that cause human, animal and plant diseases.

Family	Size (nm)	Example	Infection/diseases
[I] Human and animal virus families			
(+) sense RNA viruses			
1. Picornaviridae	8-30	Enterovirus	Polio
		Rhinovirus	Common cold
		Hepatovirus	Hepatitis A
2. Togaviridae	40-90	Rubella virus	Rubella (German measles)
		Equine encephalitis virus	Equine encephalites
3. Retroviridae	100	HTLV-I	Adult leukemia, tumors
		HIV	AIDS
(-) sense RNA viruses			
4. Paramyxoviridae	150-200	Morbillivirus	Measles
5. Rhabdoviridae	70-180	Lyssavirus	Rabies
6. Orthomyxoviridae	100-200	Influenza virus	Influenza A and B
Double stranded RNA virus			
7. Reoviridae	70	Rotavirus	Respiratory and gastrointestinal infections
(+) sense RNA virus			
8. Coronaviridae	25		Avian bronchitis
[II] Plant virus families			
(+) sense RNA virus			
9. Tobamoviridae	15 × 300	Tobacco mosaic virus	Tobacco mosaic
10. Cucumoviridae	30		Cucumber mosaic
11. Carlaviridae	15 × 650		Carnation latent
(-) sense RNA virus			
12. Tospoviridae	90	Tospovirus	Tomato spotted wilt
13. Rhabdoviridae	70 × 170	Rhabdovirus	Lettuce necrotic yellows
Double stranded RNA virus			
14. Phytoreoviridae	80	Phytoreovirus	Wound tumor

Table 5. Classification of major groups of DNA viruses that cause human, animal and plant diseases.

Family	Size (nm)	Example	Infection/diseases
[I] Human and animal virus families			
Double stranded linear DNA			
1. Poxviridae	250 × 350	Orthopoxvirus	Small pox, cow pox
2. Adenoviridae	75	Human adenovirus	Conjunctivitis
3. Herpesviridae	120-200	Simplexvirus	Oral and genital herpes
Double stranded circular DNA			
4. Papoviridae	45-55	Human papilloma viruses	Warts, cervical and penile cancer
5. Baculoviridae	40 × 400	Baculoviruses	Polyhedrosis
6. Hepadnaviridae	40 × 45	Hepatitis B virus	Hepatitis B
[II] Plant virus families			
Double stranded linear DNA			
7. Caulimoviridae	50	Caulimovirus	Cauliflower mosaic
Single stranded circular DNA			
8. Geminiviridae	18 × 30	Geminivirus	Maize streak

David Baltimore (1971) divided viruses into seven groups on the basis of their **nucleic acid** (DNA or RNA), **strandedness** (single-stranded or double-stranded), **sense** and **method of replication**. The central theme of Baltimore system of virus classification is that all viruses must generate positive sense RNA from their genomes in order to produce proteins. The precise mechanism whereby this is achieved differs in each virus family. These various type of virus genomes can be categorised into seven fundamentally different genomes which obviously require different basic strategies for their replication. Thus classifying viruses according to their genome means that those in a given category will all behave in a similar fashion. The groups, designated by Roman numerals are given in Table 7.

ICTV (2012) recognized **seven orders, 96 families, 22 subfamilies, 420 genera** and **2,618 species** of viruses. The seven orders and the characteristics of their genome are summarised below.

1. **Order Caudovirales** include tailed ds DNA (bacteriophages).
2. **Order Herpesvirales** contain large eukaryotic viruses.
3. **Order Ligamenvirales** include linear, ds DNA archaean viruses.
4. **Order Mononegavirales** include linear, ds DNA archaean viruses.
5. **Order Nidovirales** are composed of (+) strand ss RNA viruses infecting vertebrates.
6. **Order Picornavirales** contain small (+) strand ssRNA viruses that infect a variety of plants, insects and animal hosts.
7. **Order Tymovirales** contain monopartite (+) ss RNA viruses that infect plants.

[B] Nomenclature

As the binomial system of nomenclature is not suitable for viruses, International Committee for Virus Nomenclature put forward a new system

Table. 6. Lwoff, Horne and Tournier's system of classification.

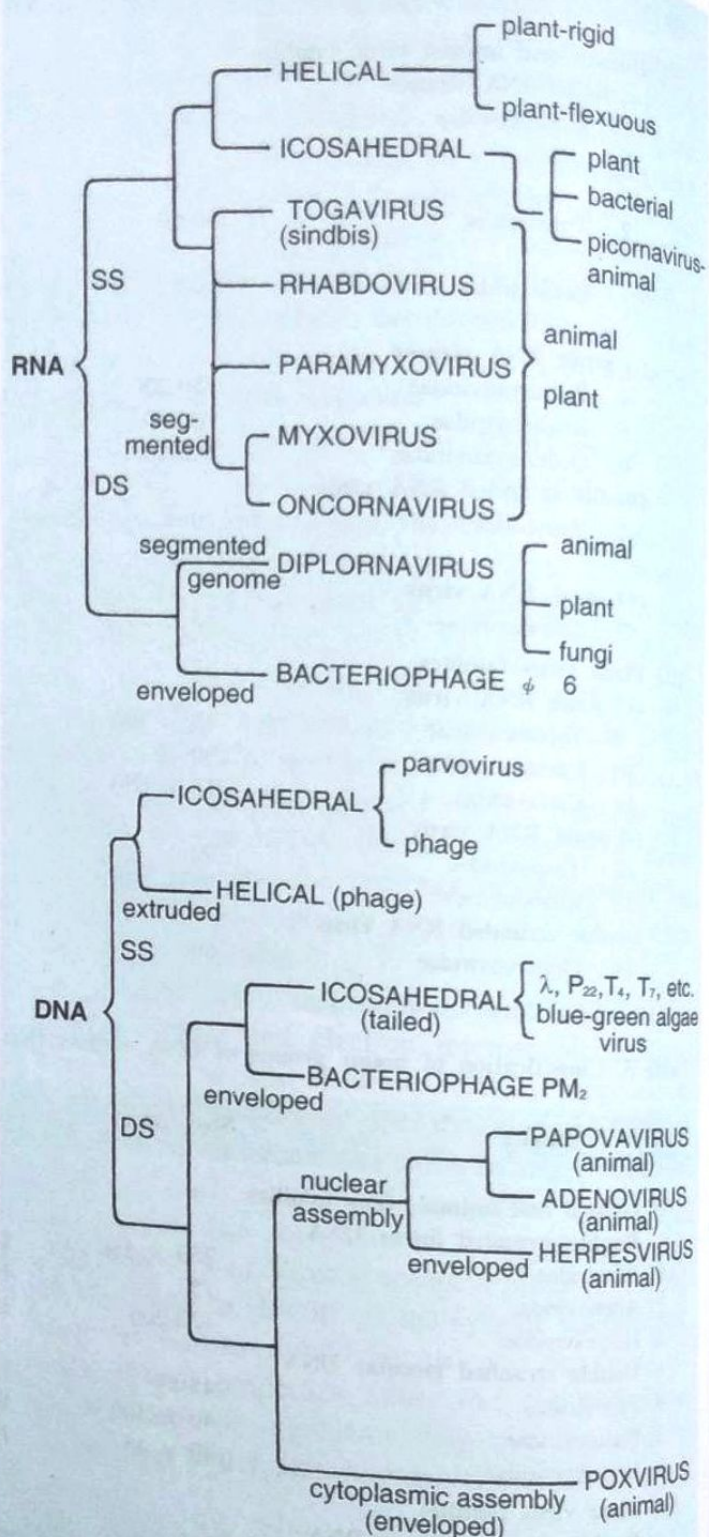


Table 7. Seven groups of viruses recognised by David Baltimore and their characteristics.

Group	Nucleic acid	Examples	Envelope	Genome size (kb)
I.	*ds DNA	Herpes virus	+	120-200
		Pox virus	+	130-375
		Adenovirus	-	30-42
		Papillomavirus	-	5.3-8.0
II.	**ss DNA	Parvovirus	-	5.0
III.	ds RNA	Reovirus	-	18-31
IV.	***+ ssRNA	Tagovirus	+	9.7-11.8
		Poliovirus	-	7.4
		Foot-and-mouth disease virus	-	7.5
		Hepatitis A virus	-	7.5
V.	***-ss RNA	Hepatitis C virus	+	10.5
VI.	(reverse RNA)	Influenza virus	+	12-15
VII.	(reverse DNA)	HIV	+	9.7
		Hepatitis B virus	+	3.1

**ss = single stranded

**ds = double stranded

***(+) or positive sense RNA is the one which can function as mRNA for the synthesis of proteins.

***(-) or negative sense RNA must first be converted into complementary positive sense strand before it is able to synthesize proteins.

for naming viruses. According to this system, the name of a virus consists of two parts, the first part represents the **common name** of the virus and the second part contains the coded information about the virus. The second part is known as **cryptogram**. The common name of the virus is usually not changed, but cryptogram can change as and when new information becomes available about the virus.

A cryptogram has the following **four pairs of coded information**.

- (1) First pair — represents type of nucleic acid/number of strands in nucleic acid.
- (2) Second pair — represents molecular weight of nucleic acid/percentage amount of nucleic acid in the virus.
- (3) Third pair — denotes the size of virus/size of nucleoprotein.
- (4) Fourth pair — denotes the type of host/carrier used in transmission of virus.

The cryptograms of two viruses are explained below.

1. Cryptogram of tobacco mosaic virus (TMV). The cryptogram of TMV is R/1 : 2/5 : E/E : S/A.

Explanation

- First pair — Nucleic acid RNA = (R)/
Nucleic acid single stranded = 1
- Second pair — Molecular weight of nucleic acid = 2 (in hundred thousands)/Amount of nucleic acid 5%
- Third pair — Virus elongated = E/
Nucleoprotein elongated = E
- Fourth pair — Most seeded plants (Spermatophytes) = S/Transmission of virus by air = A

2. Influenza virus. The cryptogram of this virus is R/1 : (2-3)/10 : S/E : V/A.

Explanation

- First pair — Nucleic acid RNA = (R)/
Nucleic acid single stranded = 1
- Second pair — Molecular weight of nucleic acid = 2 or 3 (hundred thousands)/Amount of nucleic acid = 10%

- Third pair — Virus spherical = S/
Nucleoproteins elongated
= E
- Fourth pair — Host vertebrate = V/Trans-
mission of virus by air = A

TOBACCO MOSAIC VIRUS (TMV)

More than 100 types of plant viruses are known which cause various diseases in plants. Of these, tobacco mosaic virus has been studied most extensively, both in the field as well as in the laboratory. This virus was discovered by D. Iwanowski in 1892, but its isolation from infected plants and crystallization was done by W.M. Stanley in 1935.

[A] Structure

TMV particles appear as bundle of rods or needles under electron microscope. Each rod is approximately 3000 λ in length and 170 λ in

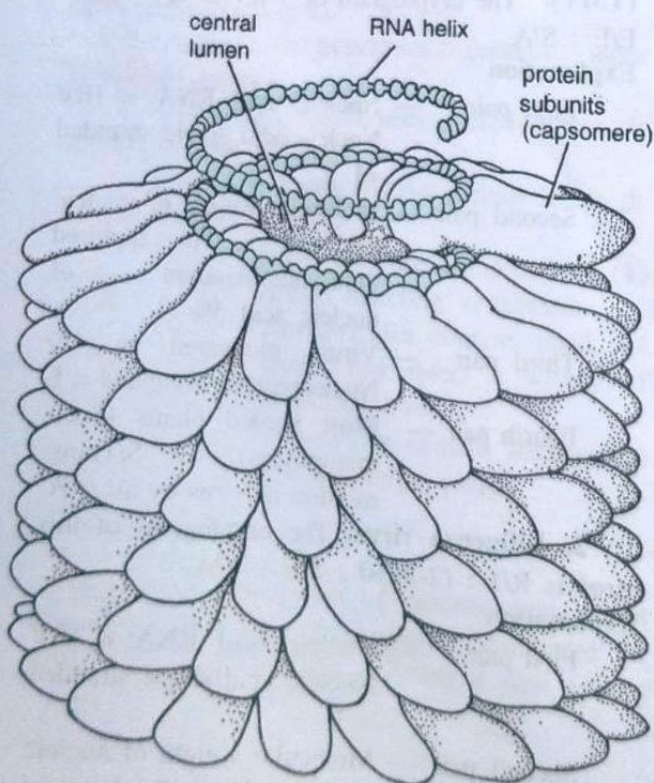


Fig. 6. Tobacco mosaic virus : Structure.

diameter and has a molecular weight of approximately 400,000. The electron microscopic and X-ray crystallographic investigations have revealed that these particles have two constituents — **protein coat** and **nucleic acid** (Fig. 6). The protein coat (capsid) is made up of approximately 2130 identical protein sub-units called **capsomeres**. Each capsomere consists of a long chain of 158 amino acids and its molecular weight is 18,000. The capsomeres are helically arranged around a central single stranded RNA molecule (Fig. 7). The latter consists of some 6,000 nucleotide pairs. The protein and nucleic acid ratio in these particles is 94.4 : 5.6.

The total length of each rod has about 130 helicals and in each helix there are approximately 16.5 protein sub-units. In each helix of RNA there are 49 nucleotides and the helix has a pitch of

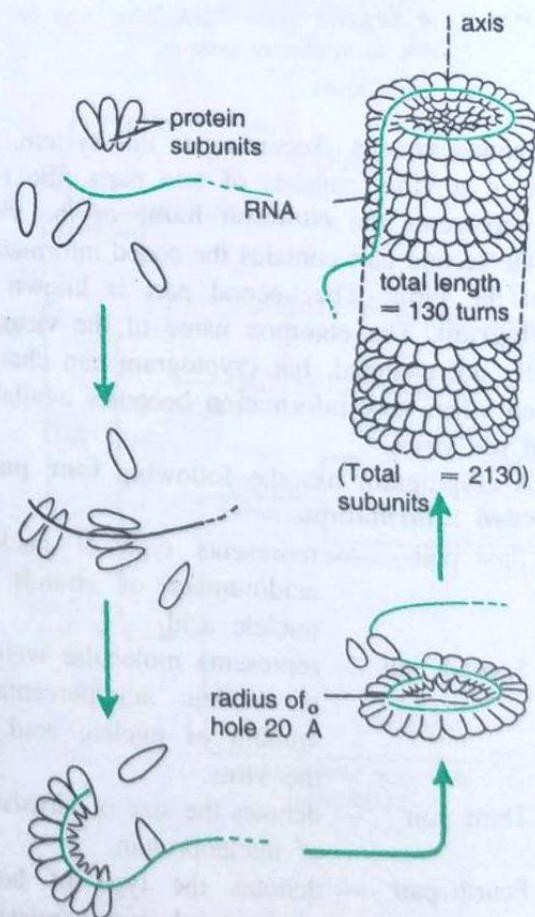


Fig. 7. Tobacco mosaic virus : Structure and its synthesis from RNA and protein subunits.

23λ. The genetic information necessary for the formation of a complete TMV particle is contained in its RNA. This information determines the replication of RNA and sequence of amino acids in the protein sub-units of the capsid. As all sub-units are identical, only one coded gene is required for the formation of all capsomeres. It is possible to remove the protein coat from RNA. The naked RNA is capable of infecting tobacco plant; once inside the host cell, the virus RNA directs the protein synthesizing apparatus of the host cell to synthesize its own proteins. Thus RNA has two functions : (i) **self replication**, and (ii) **synthesis of virus specific proteins** for which it takes the raw material from the host cell.

Although the naked RNA is capable of infecting the host cell, the efficiency of infection is usually one thousandth to one millionth that of the intact virion. At the time of infection, usually the entire virion (i.e., protein coat and nucleic acid) enters into the host cell. Immediately after entering the host cell, the protein coat of virion degenerates, whereas RNA starts the synthesis of messenger RNA which eventually synthesizes protein sub-units.

[B] Sources of TMV Infection

Debris of infected plants lying in the soil is the chief source of infection of tobacco mosaic virus. The infection also passes from one host to another by mechanical means, such as agriculture implements and rubbing of infected leaves over the surface of healthy leaves. The virus is highly contagious and stable and can retain infectivity even after the leaves have been processed into smoking tobacco. According to Johanson (1937), 67% cigar, 11% cigarettes and 62% pipe tobacco is virus infected. A person who has handled a cigarette containing infected tobacco leaves may transmit the virus to healthy plants. Besides, many aphids (e.g., *Myzus persicae*, *M. pseudosolani* and *Microsiphon solanifolii*) are vectors of the virus.

[C] Symptoms of TMV on the Host

The first visible symptoms of TMV infection in tobacco plant appear in the form of downward curling and distortion of young apical leaves.

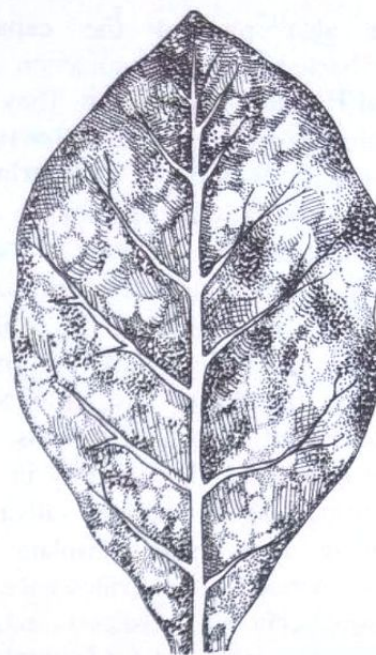


Fig. 8. Tobacco mosaic virus : Symptoms on leaf.

Large spots of dark green colour appear on the upper surface of the leaf which eventually develop into irregular blistered areas (Fig. 8). Other areas of the leaf show typical yellow and green mottling or mosaic symptoms. The plant becomes stunted.

BACTERIOPHAGE

Viruses which infect bacterial cells, are known as bacteriophage or viruses of bacteria. There is hardly any species of bacteria which does not serve as host to one or more viruses. Bacteriophage was discovered by Frederick W. Twort (1915) and Felix d'Herelle (1917) independently while investigating certain types of soil bacteria. They observed that if few drops of highly concentrated bacterial viruses are introduced into a dish with nutrient medium seeded with a culture, then there was no growth of bacteria at the point of the introduction of the virus. This interaction was not restricted only to the solid medium. They observed that when bacteriophage is added in a suspension of *Staphylococcus albus* the bacterial cell wall breaks open (lysis). This

suspension also retained the capability of destroying bacteria. The phenomenon is said to be **Twort-d'Herelle phenomenon**. They called the viruses which destroy bacteria as **bacteriophage**, literally means '**eaters of bacteria**' (Greek *phagein* = to eat). They are also known as **phages**.

Bacteriophages are **obligate parasites** and are found in all such habitats where bacteria can survive. They are abundant in soil, sewage water, fruits, vegetables, milk and nodules of legumes. Specific phages have also been found in the intestine of birds and animals. In human beings phages can be found in intestinal contents, urine, blood, sputum, saliva, pus and nasal exudate. It is easy to isolate the phage during the period of convalescence. Besides, some viruses occur as parasites in actinomycetes (**Actinophages**), yeast cells (**Zymophages**) and blue-green algae (**Cyanophages**). The discovery of bacterial viruses led to great excitement, for it was hoped that they would serve as an effective and simple way to combat bacterial diseases. But due to introduction of antibiotics, phage therapy has only limited use in prophylaxis of infectious diseases.

Although phages are known to occur in all bacteria, most of the work has been done on the phages that attack *Escherichia coli*.

Types of Phages

Bacteriophages have been classified into many arbitrary groups. Some of the broad groups are as under.

[A] T-Phages (T₁-T₇; T standing for 'type')

These phages are characterized by the presence of a tail. They form the largest group of phages (T₁-T₇) and have been studied more extensively than any other group. They contain a double stranded DNA. T-phages have been divided into the following three sub-groups:

[I] T-even phages (T₂, T₄, T₆)

These phages are genetically as well as serologically closely related. They have an **angular head and a contractile tail**. Their multiplication in the bacterial cell is independent of the bacterial DNA, the latter is rapidly destroyed following

Table 8. Important characteristics of some bacteriophages.

Phage	Head	Tail	Nucleic acid type
T-even	Polygonal	Contractile	Double stranded DNA
T ₃ , T ₇	Hexagonal	Short non-contractile	Double stranded DNA
T ₁ , T ₅	Hexagonal	Short non-contractile	Double stranded DNA
fdm, M ₁₃	Filamentous	No differentiation into head & tail	Single stranded DNA
φ × 174	Hexagonal	Tail absent	Single stranded DNA
φ ₂ , R ₁₇ , φ _r	Hexagonal	Tail absent	Single stranded RNA

infection. The DNA of T-even phages contains a unique base, **5-hydroxymethyl cytosine**, in place of cytosine.

[II] T-odd phages (T₁, T₃, T₇)

These phages have different genetic and serological features. They have an **angular head and a short non-contractile tail**. The DNA of these phages is characterized by the presence of **cytosine**.

[III] T₅ phages

These phages contain an **angular head and a long non-contractile tail**. Like T-odd phages, these phages also have cytosine in their DNA.

[B] Virulent and Temperate Phages

Depending on the interaction of phages with the bacterial cell, they have been distinguished into **virulent** or **lytic** and **avirulent** or **temperate** phages. According to Lwoff (1953), bacteriophages exist in three clear-cut states, namely **extracellular virion**, **vegetative phage** and **prophage**. Extracellular virions are complete phage particles with both nucleic acid core and protein coat. They infect bacterial cell. Vegetative phage and prophage, on the other hand, are intracellular, represented by nucleic acid only. As a vegetative phage, the nucleic acid is capable of independent replication, while as a prophage it never replicates independently and is first inserted in the bacterial (host) DNA and then replicates along with bacterial DNA. Bacteria containing prophages are called

lysogenic bacteria, and those viruses whose nucleic acid can become prophage (i.e., gets incorporated in bacterial DNA) are known as **lysogenic**, **temperate** or **avirulent phages** (e.g., F_2 , M_{12}). On the other hand, those viruses which always multiply when they enter the host cell are called **virulent** or **lytic phages** (e.g., T_2 , T_4 phages).

Important characteristics of some bacteriophages are listed in Table 8.

Structure of Bacteriophage

[A] Morphology

Bacteriophages are very small particles and they cannot be separated even by bacterial filters. The structural details of bacteriophages are known through electron microscopic studies of some larger particles of T-even group which infect the bacterium *Escherichia coli*. In their appearance the phage resembles a tadpole or spermatozoid; it is differentiated into a **head** and a **tail** (Fig. 9A). In most phages (T , T_2 , T_6) the head is prismoid but in T_3 and T_7 phages it is hexagonal. There are some phages (e.g., ϕ , ϕ , ϕ , M_{13}) which are filamentous and do not show differentiation into head and tail. The size of the head of T_2 phages is approximately $950 \text{ \AA} \times 650 \text{ \AA}$. The extended part between the head and the tail is called **collar**. The tail is almost equal to the length of the head (950 \AA) and has a diameter of 80 \AA . At the proximal end of the tail a hexagonal **tail plate** or **end plate** is present. The end plate is approximately 200 \AA thick. It has six **tail pins** or **fibres** on its under surface, each about 1500 \AA long (Fig. 9B). The tail pins have two main functions: (i) they help in the adsorption of phage particle on the surface of the bacterium, and (ii) the enzymes secreted by these pins are helpful in the lysis of bacterial cell wall.

[B] Chemical Composition

The phage particles are made up of **protein** (about 50-60%) and **nucleic acids** (40-50%). They also contain a small proportion of lipids in the form of neutral fats. The wall of the head is composed of some 2,000 similar sub-units of proteins. The nucleic acid of phages is either double-stranded

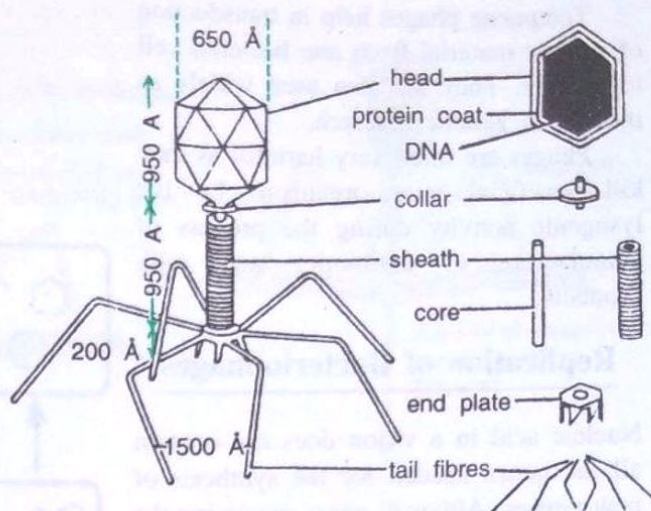


Fig. 9 A-B. T_2 bacteriophage : A. Electron micrograph (schematic representation), B. Components of bacteriophage.

DNA, single stranded DNA or single stranded RNA (DNA and RNA are never present together). Except for coliphage, $\phi \times 174$ phage and few others, most phages **have double stranded DNA**. The phage DNA differs from bacterial DNA chemically. The molecular weight of phage DNA is 2,500,000, and in each phage particle the amount of nucleic acid is approximately 6×10^{-3} mg. DNA is the genetic material of the phage particle; it carries infection and induces the host cell to synthesize more and more phage particles.

Economic Importance of Phage Particles

Phages have been used in prophylaxis and treatment against dysentery, enteric fever, cholera, plague, and many other pathogenic bacterial diseases. However currently, due to introduction of antibiotics, phage therapy and prophylaxis of infectious diseases are used only to a limited extent. Phages are also used in the diagnosis of certain infections. The causative agents of plague, cholera, etc., can be determined with the help of special phages. Phages are also helpful in the lysis of bacteria present in the polluted water. Hence, they can also be used as **scavengers**.

In space microbiology lysogenic cultures are used as radiation detectors. They were used by Russians in the space ship—Vostok-2.

Temperate phages help in transduction of genetic material from one bacterial cell to another. They are also used widely as models in genetic research.

Phages are often very harmful as they kill beneficial micro-organisms by the lysogenic activity during the process of manufacture of antibiotics and milk products.

Replication of Bacteriophages

Nucleic acid in a virion does not contain all the genes needed for the synthesis of new viruses. Although genes governing the synthesis of virion's structural components are present in its nucleic acid, the enzymes needed for protein synthesis, ribosomes, tRNA and for energy production are supplied by the host cell. These are used for synthesizing viral proteins including viral enzymes. Thus **for a virus to multiply, it must invade a host cell and take over the host's metabolic machinery.** Once inside the host cell, even a single virus can give rise to thousands of viruses.

Although the basic mechanism of penetration and multiplication is similar in all the viruses, the process is best studied in bacteriophages. Phages can multiply by two alternate methods—(i) **lytic cycle**, and (ii) **lysogenic cycle**. The lytic cycle ends with the death or lysis of the host cell, whereas the host cell remains alive in the lysogenic cycle. Both the cycles are described in the following pages.

[A] Lytic Cycle

Multiplication of T-even bacteriophages in their host cell (*E. coli*) is an example of the lytic cycle. The process of multiplication involves the following four steps (Fig. 10).

- (1) Infection,
- (2) Synthesis of phage components in the host cells,

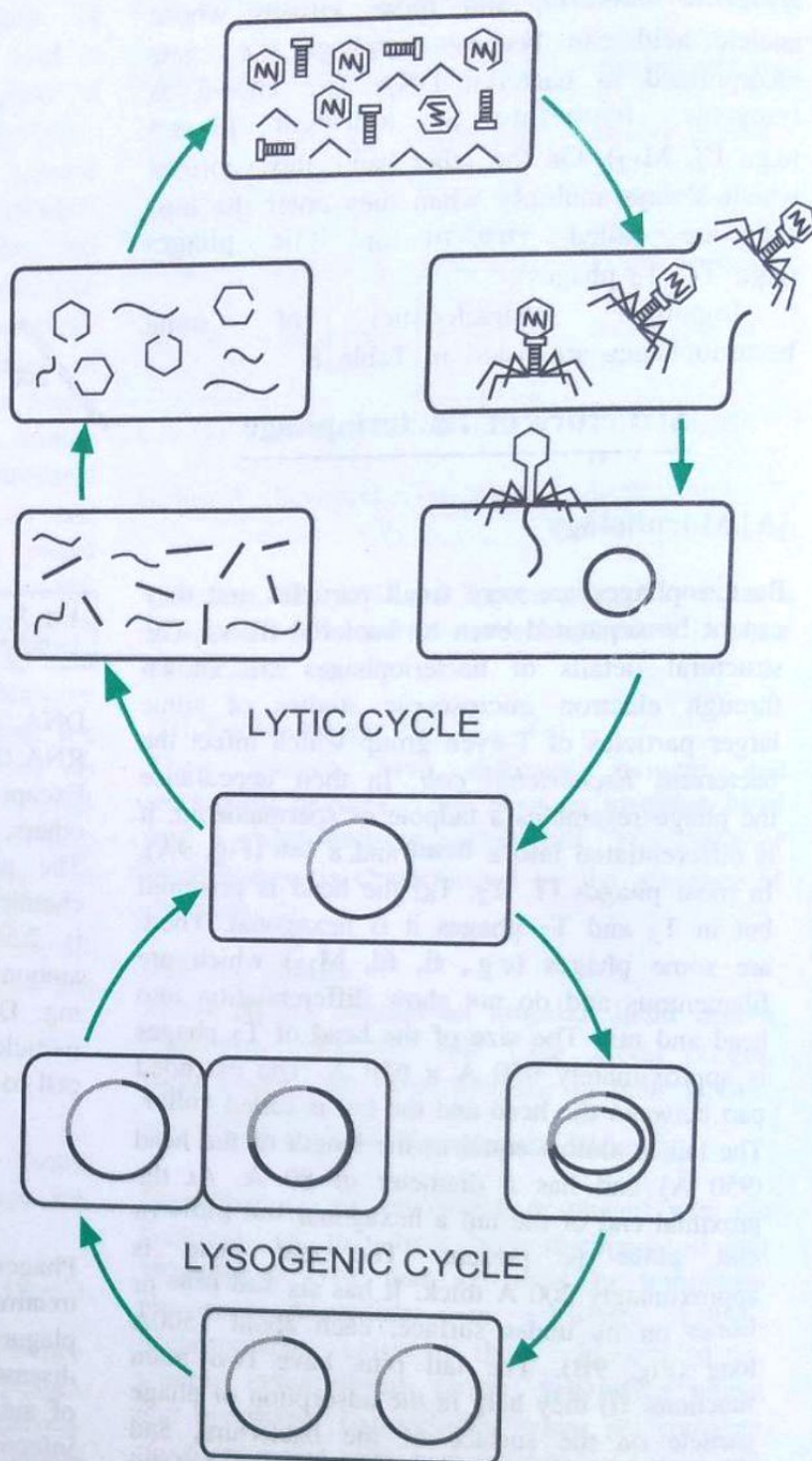


Fig. 10. Virus multiplication : Lytic and lysogenic cycle.

- (3) Assembly of new phage particle,
- (4) Liberation of phage particles from the host cells.

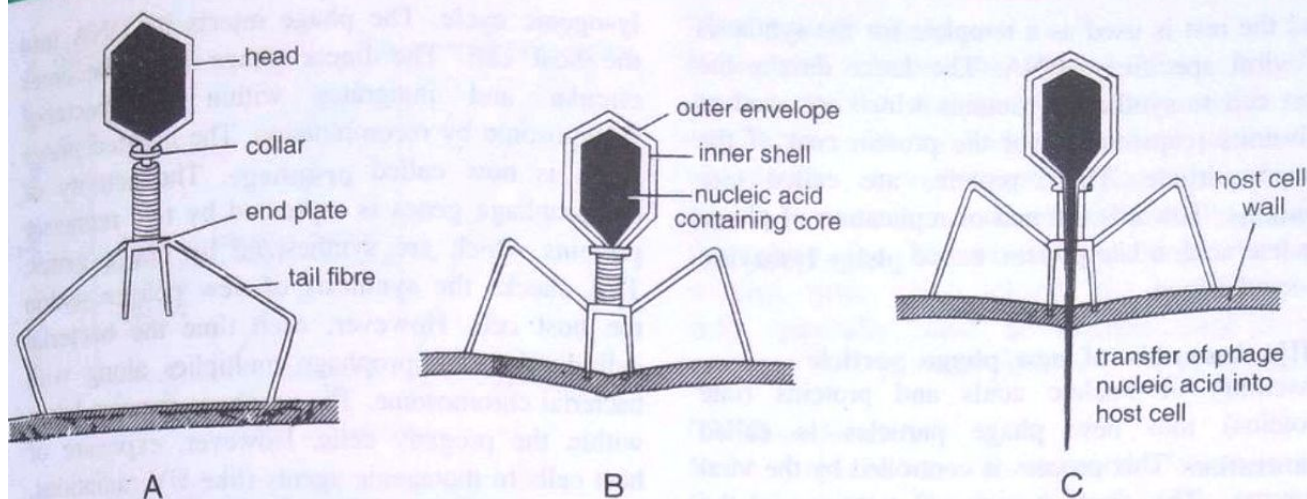


Fig. 11 A-C. Bacteriophage : Mechanism of infection; A. Adsorption of phage on the host surface with the help of tail fibres, B. Contact of end plate with the host surface, C. Transfer of phage nucleic acid into the cell.

[I] Infection

The two important events of this step are adsorption of the phage on the host bacterium by means of its tail fibres, and transfer of phage nucleic acid into the host cell. Adsorption of phage on the host bacterium depends on the mutual affinity of the phage and bacterium. **Only a specific phage can infect a particular bacterium.** Some of the coliphages have so much specificity that they are adsorbed only at a specific site on the host surface. These sites are known as **receptor sites**. In addition, factors such as composition and viscosity of the medium and temperature also affect adsorption.

Adsorption of phage on the surface of host is facilitated by interaction of amino groups of proteins present at the margins of the phage tail, and negatively charged carboxyl groups on the surface of the host. **Thus the host specificity of the phage particle is due to its protein and not the nucleic acid.** As such the host range of free viral nucleic acid is much wider than that of the intact virion. After adsorption, the phage particle secretes a special enzyme of lysozyme type which hydrolyses muramic acid-peptide complex of the bacterial cell wall. Consequently, a minute pore is formed through which the nucleic acid of the phage enters into the host cell. The infection is accomplished through the following steps:

- (1) Immediately after landing on the host surface the tail fibres bent, bringing the end plate of

the phage in contact with the bacterial cell wall (Fig. 11 A-B).

- (2) The tail sheath then contracts, pushing the central tubular part of the tail into the host cell wall just like an injection needle (Fig. 11 C).
- (3) The nucleic acid of the phage flows into the host cell through the hollow centre of the tubular needle.

The protein shell of phage remains attached to the host cell even after the transfer of nucleic acid. Such empty protein shells are called **ghosts**. A host cell, once infected by a phage particle, becomes immune against the infection of the phage of the same type. A phage particle loses its ability of infection after its nucleic acid has been released into the host cell.

[II] Synthesis of phage components in the host cells

The phage nucleic acid, once inside the bacterial cell, takes over the protein synthesis machinery of the cell. It suppresses the synthesis of bacterial protein and directs the metabolism of the cell to synthesize the proteins of the phage particle. This is accomplished by the synthesis of **viral specific m-RNA** (using the host RNA polymerase).

The replication of phage DNA follows the semi-conservative mechanism. Most of the phage DNA serves as a template in its own synthesis

and the rest is used as a template for the synthesis of viral specific m-RNA. The latter directs the host cell to synthesize proteins which are used as sub-units (capsomeres) of the protein coat of the phage particle. These proteins are called **late proteins**. Towards the end of replication of phage nucleic acid, a late protein, called **phage lysozyme** is synthesized.

[III] Assembly of new phage particle

Assembly of nucleic acids and proteins (late proteins) into new phage particles is called **maturation**. This process is controlled by the viral genome. The first step in maturation is the condensation of nucleic acid molecule in crystalline form. The protein sub-units then aggregate around DNA to form the head of the phage. Meanwhile assembly of the tail starts. It is initiated with the attachment of core tube with the tail plate. The sheath around the core tube is formed afterwards. At this stage the tail gets attached to the base of the head. The tail fibres are attached to the end plate in the last.

[IV] Liberation of phage particles from the host cells

The entire cycle of phage development is completed in 30-90 minutes. In an infected bacterium 7-8 phage particles are formed per minute and a total of about 200 phages are formed in a bacterium. Lysis of the host cell wall is essential for the liberation of phage particles. It is facilitated by the lysozyme secreted by the phage DNA in the host cell. The host cell ruptures as a result of lysis and the phage particles are liberated.

[B] Lysogenic Cycle

Some phages do not cause death (lysis) of the host cell when they multiply. In the lysogenic cycle the phage DNA gets incorporated into the host cell's DNA and the host cell multiplies indefinitely along with phage nucleic acid (Fig. 10). Phages multiplying by this method are known as **lysogenic phages** or **temperate phages** and the participating host cells are called **lysogenic cells**.

The life cycle of bacteriophage λ (lambda) is being described here as an example of the

lysogenic cycle. The phage injects its DNA into the host cell. The linear phage DNA becomes circular and integrates within the bacterial chromosome by recombination. The inserted phage DNA is now called **prophage**. The activity of the prophage genes is repressed by two repressor proteins which are synthesized by phage genes. This checks the synthesis of new phages within the host cell. However, each time the bacterial cell divides, the prophage multiplies along with bacterial chromosome. The prophage remains latent within the progeny cells. However, exposure of host cells to mutagenic agents (like UV radiations, certain chemicals, etc.) may lead to the excision of phage DNA and initiation of the lytic cycle.

Phage Growth and Estimation of Phage Number

Phage growth can be detected by observing a phage infected bacterial cell in the laboratory. This growth can be described by a **replication curve** (Fig. 12). The replication curve, besides viral yield, also represents an eclipse period and a latent period. The **eclipse period** spans from penetration of bacterial cell till the biosynthesis of new phage particles begins. During eclipse period no mature virions can be detected in host cells. The period from penetration up to the point of release of new phage particles from the host cells is referred to as **latent period**. Thus latent period is longer and also includes the eclipse period.

Phage particles can not be observed under light microscope and it is also not possible to count their number with an electron microscope. Hence virologists use a special viral assay technique called **plaque assay** to determine phage number in a suspension. In this technique serial dilutions of viral suspension are prepared. A sample of each dilution is inoculated onto a plate containing a layer of susceptible bacteria; this layer is called **bacterial lawn**. An ideal dilution is one in which a single phage infects one bacterial cell. After latent period is over (during which new phage particles are synthesized), the inoculated bacterial cell undergoes lysis and new phage particles are released. These phages then infect surrounding susceptible cells and lyse them. After

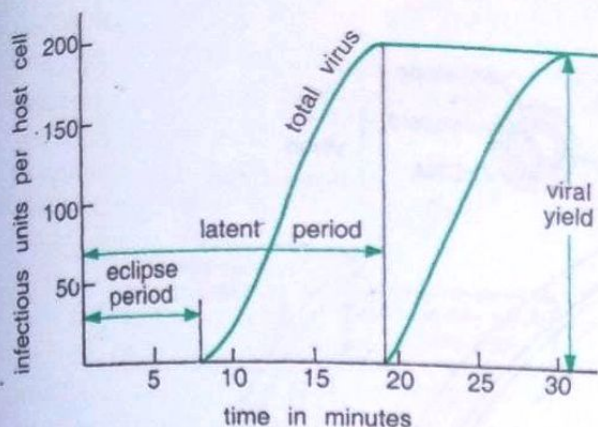


Fig. 12. Growth curve for a phage.

incubation and several rounds of lysis, the bacterial lawn shows clear areas called **plaques**. The plaques represent the areas where viruses have lysed host cells. The uninfected bacterial cells of the (bacterial) lawn also multiply simultaneously and produce a turbid growth layer.

Since each plaque on the petriplate represents the progeny from one infectious phage, therefore, by counting the number of plaques and multiplying that number by the dilution factor, virologists can estimate the number of phages in a milliliter of suspension.

TYPES OF VIRAL INFECTIONS

Viral infections can be categorized into following broad categories.

[I] Lytic infection

Such infections result in the destruction of the host cells. Polio virus, pox virus and the common cold virus cause lytic infections.

[II] Persistent infection

In such infection the virus replicates actively but the host cell retains viability, and viral production continues for an extended period of time. In persistent infection the mature viruses leave the host cell by budding without disrupting the integrity of the plasma membrane. AIDS virus (HIV), rubella virus (*Rubivirus*) and measles virus (*Morbillivirus*) cause persistent infection. Besides these, all plant viruses cause persistent infections.

[III] Latent infection

In such an infection the virus does not actively replicate within the host cell and remains in equilibrium with the host cell without producing any disease often for several years. All of the human herpes viruses can remain in the host cells throughout the life of an individual. Herpes simplex virus, which inhabits the human nerve cells, generally cause no damage until it is activated by a stimulus such as fever or sunburn.

[IV] Transformation

Some viral infections can cause transformation of the host cells. The transformed cells acquire properties that are distinct from the properties of uninfected cells. A transformed cell may change into a cancer cell with fewer growth factor requirements than for the normal cell. As a result the transformed cell reproduces more rapidly, resulting in a mass of cells called a **tumor**. Some tumors are self-limiting; they do not spread and are called **benign**. Other type of tumors, called **malignant**, spread and grow in other tissues and organs causing dysfunction or death of that tissue. All **oncoviruses** (viruses capable of inducing tumors in animals) induce transformation in host cells.

MECHANISM OF REPLICATION OF ANIMAL VIRUSES

As in bacteriophages, replication of animal viruses also involve the processes of adsorption, penetration, synthesis, maturation and release. However, animal viruses perform these processes in ways that differ from those employed by bacteriophages. The steps of the replication of animal are summarized below.

[A] Adsorption

Unlike bacteriophages, animal viruses do not have specialized attachment organs, i.e., tail fibres. However, naked animal viruses possess certain attachment sites on the surface of their capsids, which are made of special type of proteins. These sites are specific and bind with corresponding sites on the host surface. Some other animal viruses

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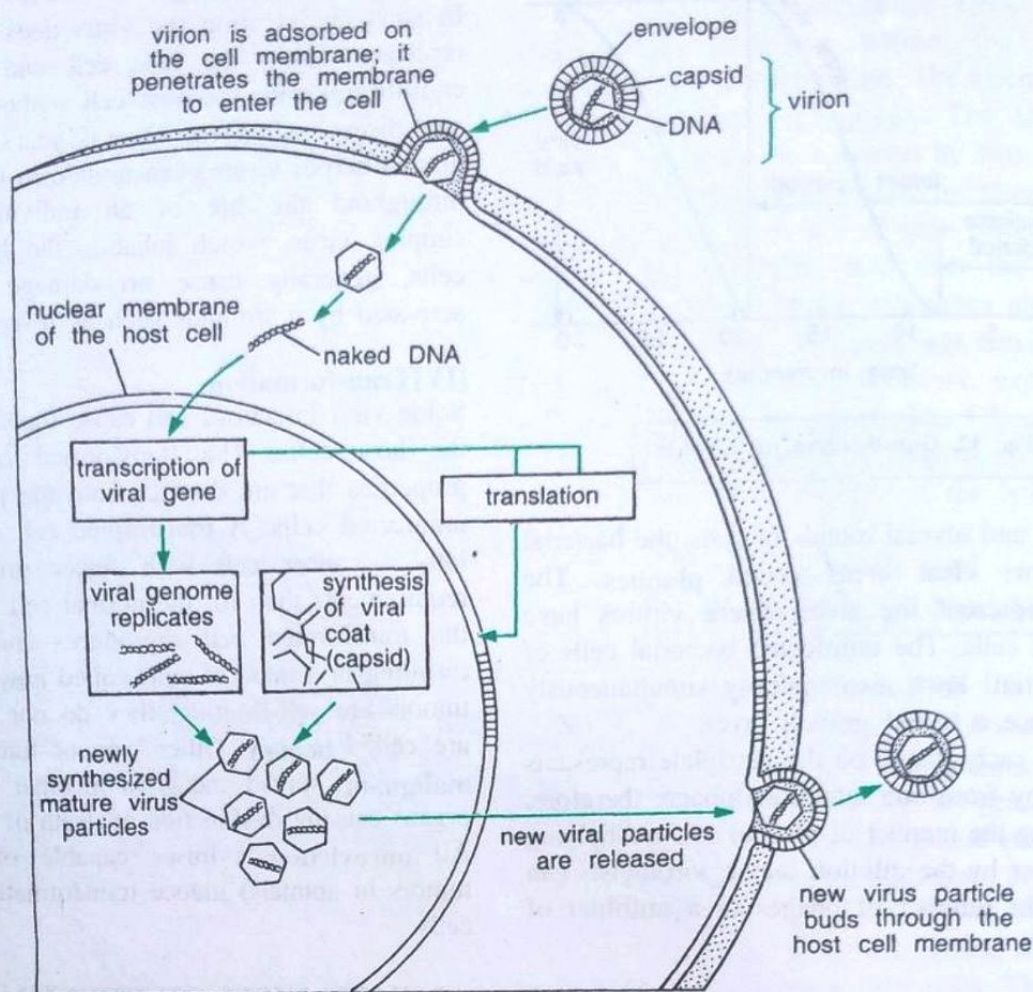


Fig. 13. Plan showing the replication of an enveloped ds DNA animal virus.

(e.g., HIV) have spikes that recognize a membrane protein receptor on the surface of certain specific immune defense cells.

[B] Penetration

Penetration follows soon after the attachment of virion on the host cell membrane. Unlike bacteriophages, animal viruses do not have a mechanism for injecting their nucleic acid into the host cell. In these viruses both, the nucleic acid and the capsid penetrate the host cells usually by endocytosis. Once inside the animal cell, the viral genome is separated from its protein coat, a process called **uncoating**. This is facilitated by proteolytic enzymes secreted by viruses themselves or by the enzymes present in the host cell.

(MICROBIOLOGY)

[C] Synthesis

Synthesis of new genetic material and protein depends on the nature of the infecting viruses. The biosynthesis of DNA animal viruses differs from that of RNA animal viruses.

[I] Synthesis of DNA animal viruses

Most DNA viruses replicate their DNA in the nucleus of the host cell using viral enzymes. They synthesize their capsid and other proteins in the cytoplasm by using the host cell enzymes. The new viral proteins move to the nucleus, where they combine with the new viral DNA to form virions (Fig. 13). Herpesviruses, adenoviruses, papovaviruses and hepadnaviruses show the above pattern of synthesis.

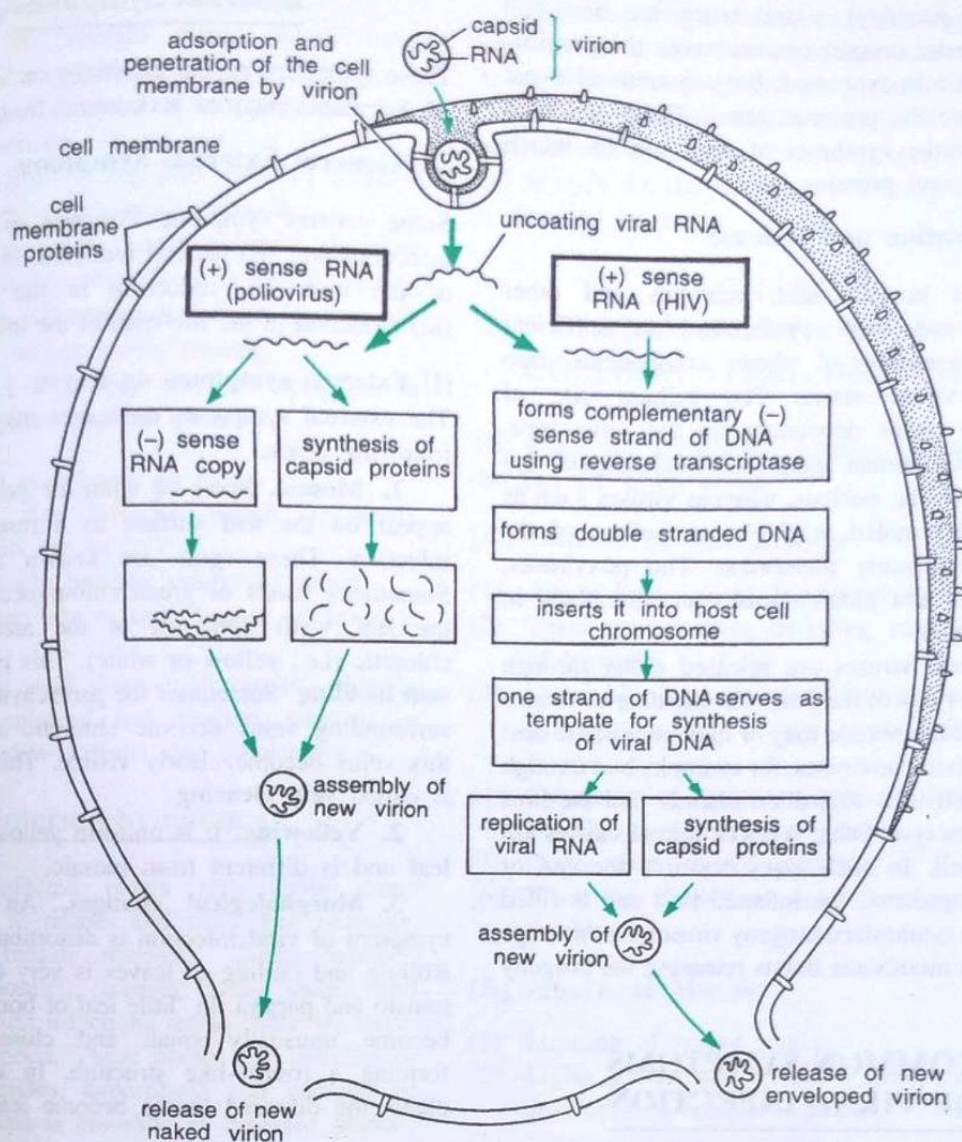


Fig. 14. Replication mechanism of RNA animal virus.

[III] Synthesis of RNA viruses

Different groups of RNA viruses show much variation in the mechanism of mRNA synthesis. In RNA viruses such as picornaviruses, the (+) sense RNA acts as mRNA. Immediately after penetration and uncoating, viral proteins are made which also have important roles in the synthesis of these viruses. For example, one protein inhibits synthetic activities of the host cell. The nucleus of the host cell is not involved.

In retroviruses, such as HIV, the two copies of (+) sense RNA do not act as mRNA; instead they are transcribed into single-stranded (ss) DNA with the help of reverse transcriptase (Fig. 14). Subsequently, the ssDNA is replicated through complementary base pairing to make double stranded (ds) DNA molecule. Once in the cell nucleus, this molecule inserts itself as a provirus into a host cell chromosome and it can remain there for an indefinite period of time. It is passed

(MICROBIOLOGY)

on to the progeny as and when the host cell divides. Under normal circumstances the provirus genes remain unexpressed, but any unusual event may activate the provirus genes. These genes, in turn, direct the synthesis of viral mRNA which forms the viral proteins.

[D] Maturation and Release

Once viral nucleic acid, enzymes and other proteins have been synthesized in sufficient amount, assembly of these components into complete virion starts. The cellular site of maturation varies depending on the virus type. For example, human adenovirus nucleocapsids are assembled in the nucleus, whereas viruses such as HIV are assembled at the inner surface of the host cell's plasma membrane. The poxviruses, polioviruses and picornaviruses are assembled in the cytoplasm.

The new viruses are released either through budding or lyses of the host cell. Budding of virions through the membrane may or may not kill the host cell. Human adenoviruses, for example, bud through the host cell in a controlled manner and the host cell does not lyse. Other types of animal viruses kill the host cell. In such cases, towards the end of replication process, the infected host cell is filled with newly synthesized progeny viruses. At this stage the plasma membrane bursts releasing the progeny of viruses.

COMMON SYMPTOMS OF VIRAL INFECTION

Viral infection produces various symptoms on the host which are widespread (systemic) or are localized to a particular part. In most of the plants these symptoms are highly specific and diagnostic, but sometimes they are non-specific. Occasionally, the symptoms of viral infection are latent and in such cases the presence of virus can be determined by electron microscopic examinations.

Symptoms of viral infection can be grouped into the following three categories:

- (1) External symptoms
- (2) Internal symptoms
- (3) Physiological symptoms

(MICROBIOLOGY)

External Symptoms

These symptoms appear externally on various parts of the plant, such as leaf, stem, fruit, etc.

[A] General External Symptoms

Some general symptoms common to most viral infections are: (i) general reduction in the growth of the host, (ii) reduction in the yield, and (iii) reduction in the life-span of the infected plant.

[I] External symptoms on leaves

The external symptoms on leaves may be of the following types:

1. **Mosaic.** Spots of white or yellow colour appear on the leaf surface as a result of viral infection. These spots are known as **mosaic**. Sometimes bands of green colour occur only on the leaf veins and rest of the area becomes chlorotic (i.e., yellow or white). This is known as **vein banding**. Sometimes the parenchymatous cells surrounding veins become chlorotic and due to this veins become clearly visible. This symptom is called **vein clearing**.

2. **Yellowing.** It is uniform yellowing of the leaf and is different from mosaic.

3. **Morphological changes.** An important symptom of viral infection is distortion of leaves. Rolling and curling of leaves is very common in tomato and papaya. In 'little leaf of brinjal', leaves become unusually small and closely placed, forming a rosette-like structure. In some other plants the diseased leaves become leathery.

[II] External symptoms on stems

The stem of infected plants shows many abnormalities. These include :

- (1) Shortening of internodes and proliferation of lateral buds. It results in the formation of densely packed broom-like structure. This symptom is known as **witches broom**.
- (2) In some cases the stem swells at certain points due to excessive growth of xylem in localized areas.
- (3) In infected plants of *Citrus* the stem becomes pitted.
- (4) Galls are formed on the stem of infected sugarcane.

[III] External symptoms on flowers

The flowers of infected plants develop the following symptoms.

- (1) They become distorted or variegated and their size is reduced.
- (2) The infection of 'big bud virus of tomato' leads to the enlargement of buds.
- (3) Sometimes, as in 'peach yellows' diseased flowers bloom early. There is also formation of pseudo-inflorescences where flowers are sterile or wither before fruiting.
- (4) Virescence is a very common symptom in which flowers become green.

[IV] External symptoms on fruits

Symptoms of viral infection which commonly appear on fruits are as follow:

- (1) Fruits usually become small and pitted.
- (2) The colour, shape and flavour of fruits is changed.
- (3) In 'bushy stunt disease of tomato', flowers become enlarged and mottled, whereas in 'peach yellows' fruits ripe quite early.

Internal Symptoms

The virus infected plants also show many abnormalities in tissues and cells. For example, in 'tobacco mosaic disease', 'dahlia mosaic', 'sugarcane mosaic', 'tomato bushy stunt', and many other viral infections intracellular inclusion bodies are formed. These bodies are lamellar or amoeboid. Besides, hyperplasia and necrosis of cells and tissues is common in diseased plants.

Physiological Symptoms

The various physiological processes of plants become affected directly or indirectly due to virus infection. It leads to the accumulation of many metabolic products in cells. The concentration of oxidised phenolic compounds in cells is responsible for the development of necrotic areas. Virus infected cells possibly synthesize certain new proteins which affect their permeability and enzyme system.

*An important difference between plant and animal virus infections is that an overwhelming majority of plant virus diseases are persistent infections, whereas animal virus diseases are not persistent. This striking difference is presumably due to the absence of an immunological system in plants.

The chlorophyll contents of diseased leaves are low hence the photosynthetic rate of such leaves is decreased. It ultimately results in lower yield. Viruses accelerate the activity of oxidising enzymes like cytochrome oxidase, peroxidase, etc., and as such the rate of respiration is usually high in diseased leaves.

DIAGNOSTIC SYMPTOMS OF SOME COMMON VIRAL DISEASES*

The diagnostic symptoms of some common virus diseases are given below.

[A] Tobacco Mosaic Disease

- (1) Apical leaves show downward curling.
- (2) There is distortion, dwarfing and blistering of leaves.
- (3) Unusually large mottled light-green yellow patches develop on the upper surface of leaves.
- (4) Cytoplasm of epidermal cells and hairs show lamellar or amoeboid bodies.
- (5) Plants show stunted growth.
- (6) There is distortion and discoloration of flowers.

[B] Sugarcane Mosaic

- (1) Mottling of young leaves.
- (2) Light green and yellow areas (mosaic) develop in alternate sequence.
- (3) Stunting of young plants.

[C] Bunchy Top of Banana

- (1) In infected plants leaves usually donot come out of the pseudo-stem.
- (2) Leaves form dense rosette and remain stunted.
- (3) Flowering is inhibited.

[D] Yellow Vein Mosaic of Bhindi

- (1) Stunted growth of leaves.
- (2) Yellowing and vein clearing of leaves. In case of severe infection, chlorosis may involve

interveinal areas, resulting in yellowing of the entire leaf.

- (3) Flowering is reduced and fruits become malformed, distorted and yellow green in colour.

[E] Clump Disease of Groundnut

- (1) Infected plants become bushy and sterile.
- (2) Leaves become smaller and show mosaic character.
- (3) *Petunia* and *Catharanthus* are alternate hosts of this virus.

[F] Leaf Curl of Papaya

- (1) Curling of young leaves.
- (2) Vein clearing and mottling of leaves.
- (3) Flowers become sterile.
- (4) Reduced growth of the plant.

[G] Leaf Mosaic of Cucurbits

- (1) Mottling, distortion and wrinkling of young leaves.
- (2) Downward curling of leaf margins.
- (3) Leaves form a rosette-like clump near the ground, which gives bushy appearance to the plant.
- (4) Fruits develop mosaic symptoms.

TRANSMISSION OF VIRUS

Viruses may spread vertically (from mother to child) or horizontally (from person to person). A virus's ability to spread depends on the make up of the virus.

Some viruses can spread by simple contact, exchange of saliva, coughing or sneezing. Some require sexual contact while others go through the oral-fecal route via contaminated food or water. Still other viruses require an insect like mosquito to carry the viruses from person to person.

Some common modes of transmission of plant viruses are given below.

[A] Transmission by Vegetative Propagation

Plants, which propagate vegetatively, once infected with a virus disease, transmit the pathogen from

one generation to the next. Eventually, the entire population of a given clonal variety may become infected with the same pathogen. Viruses may be transmitted by tubers, corms, bulbs, grafting and cutting. In grafting operations, there is contact between stock and scion and if either of these carries a virus, it may be transmitted to the other host. Transmission of 'potato spindle tuber viroid' (PSTV) takes place through tubers and that of 'Katte disease' through bulbs.

[B] Transmission by Friction and Rubbing

In nature, when leaves of closely growing infected and healthy plants rub together, the healthy plants get infected. Even a gentle rubbing with no obvious damage is enough for transmission of the virus. Viruses are also transmitted by contact of infected leaves with clothes of men and bodies of animals. Potato virus X (PVX), tobacco mosaic virus (TMV) and many others are transmitted by this method.

[C] Transmission Through Alternate Hosts

Viruses causing 'yellow vein mosaic of bhindi' and 'cucurbit mosaic' are transmitted through alternate or collateral hosts.

[D] Transmission Through Soil and Seeds

Viruses which spread through underground natural methods are called soil-borne viruses. The tobacco mosaic virus remains in the field after harvest on debris and it infects the crop in the next season. The virus enters the host through roots by mechanical or biological methods. Although more than 100 seed borne viruses are known, all virus infected seeds are not equally efficient in transmitting the disease. Bean mosaic virus, barley stripe mosaic virus, curly top virus of beet sugar and ring spot virus of soyabean are transmitted through seeds.

Besides, soil-borne nematodes are also known to transmit viruses. The viruses get attached to the stylet or to the gut of the nematodes when they feed on an infected plant and get transferred to the healthy plant when the nematodes feed on them. Tobacco ring-spot virus and tobacco rattle virus are known to be

transmitted by nematodes. However, there is no evidence to suggest that viruses replicate in the nematodes.

[E] Transmission Through Pollen Grains

If flowers of healthy plants are pollinated with the pollens from infected plants, they produce seeds which have virus infection. Such seeds, on germination, give rise to infected plants. 'Stone fruit ring spot virus' and 'bean mosaic virus' are commonly transmitted through pollens.

[F] Transmission Through Fungi

Tobacco necrosis virus is transmitted by a root infecting fungus, *Olpidium brassicae*. Besides, *Synchytrium brassicae* and *Polymyxa graminis* also act as agents for transmission of potato virus.

[G] Transmission Through Insects

Insects are the most common and potential agents of virus transmission. The insects transmitting virus diseases are called **vectors**. Insects like white fly, leaf hopper, plant hopper, aphids, mealy bugs, scale insects and flea-beetles are the potent vectors. 'Yellow vein mosaic of bhindi', 'leaf curl of chilli', 'yellow mosaic of mung', and many other viral diseases are transmitted by white flies, such as *Bemisia* and *Aleyrodes*. Aphids such as *Rhopalosiphum*, *Myzus* and *Brachycaudus* are vectors for 'chirke virus', 'maize mosaic virus' and 'maize dwarf mosaic virus', respectively. When insects suck the juice from the infected plants, viruses enter into their proboscis and salivary glands. Such insects transmit the virus to healthy plants when they visit the latter.

[H] Transmission by Infested Agricultural Tools

It is a common method of virus transmission. Viruses usually get stuck to implements used for cutting, pruning and weeding of infected crops. When virus infested tools are used for healthy plants, the virus is transmitted to the latter. Potato virus X (PVX), 'tobacco mosaic virus' and 'leaf mosaic of cucurbits' are some common viruses which are transmitted by this method.

NATURE OF VIRUSES

The evolution and nature of viruses have been the subjects of numerous investigations and theoretical discussions. Some virologists consider viruses to be acellular forms of a live parasitic system that are functionally closely related with the host cell but develop independently and are genetically free from it. Others consider them cellular genetical factors capable of synthesizing the protein cell wall which protects them from the ill-effects of environment and allows them to penetrate into host cells. Still others consider viruses as non-living transmissible nucleoproteins which have pathogenic properties that constantly arise from cellular substances.

Stanley (1935) successfully crystallized tobacco mosaic virus (TMV) and the crystals retained the property to induce mosaic disease in tobacco. Frankel Conart and Takahasi (1956) and Cochrane *et al.* (1963) were able to reconstitute a typical tobacco mosaic virus from biologically inactive components. It produced a characteristic disease in plants. Later, it was confirmed that infective properties of viruses are due to nucleic acid and the cell wall does not take part in this process.

Thus, despite the simplicity in physical and chemical composition, all attributes of life, such as self-reproduction, variation and transmission of genetic information are inherent in viruses as in other living systems. The main characteristics of viruses are listed below.

[A] Characters Specific to Viruses

- (1) They are granular and can be resolved with electron microscope only.
- (2) Most viruses can not be separated by bacterial filters.
- (3) They can not be grown in artificial medium.
- (4) They produce characteristic symptoms of specific hosts.
- (5) They can be inactivated by chemotherapy and thermotherapy.
- (6) They show response to change in temperature and humidity.

- (7) They are obligate intracellular parasites. In host tissue they show characters of living organisms.
- (8) They lack functional autonomy, but develop independently and are genetically free from the host cell.

[B] Differences Between Viruses and other Organisms

Viruses differ from other organisms in the following characters.

- (1) Viruses possess only one kind of nucleic acid (either DNA or RNA), whereas other organisms have both the nucleic acids.
 - (2) Viruses are devoid of polysaccharides.
 - (3) In viruses there is no cell division. They show replication of genetic material only (for which they are dependent upon the machinery of host cell).
 - (4) Viruses lack cellular organelles like ribosomes, mitochondria, etc. They make use of the ribosomes of host cells.
- Viruses possess both living and non-living properties.

[C] Living Properties of Viruses

- (1) The genetic material of viruses replicates.
- (2) Mutant forms of certain viruses are known, and mutation is essentially a characteristic of living organisms.
- (3) They show sensitivity to many stimulants, such as temperature, chemical substances and radiation.
- (4) They possess antigenic property.
- (5) They multiply in living host cells.
- (6) They show host specificity.

[D] Inanimate Properties of Viruses

- (1) They can be crystallized.
- (2) They are inert outside their specific host cells.
- (3) They are autocatalytic and do not have functional autonomy.

- (4) They are devoid of cell membrane and cell wall.
- (5) They do not respire.

VIROIDS

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

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

Structure of Viroids

A viroid consists of extremely small strand of RNA without any protective protein coat. Electron microscopic studies of purified 'potato spindle tuber viroid' (PSTV) revealed that it has a single stranded RNA molecule containing

*Recently a disease causing agent even smaller than a viroid has been discovered. The 'organism' appears to consist only of protein and has been tentatively named as **prion**.

250-350 nucleotides. The adenine : uracil (A : U = 21.7 : 20.9) and guanine: cytosine (G : C = 28.9 : 28.3) ratios are close to unity.

Viroids do not possess capsid (protein coat) around the RNA molecule.

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

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

[I] RNA-directed replication

According to this view, in uninfected plants there exists a replicase enzyme which accepts a wide variety of RNA species as templates. The presence of RNA-directed RNA polymerase has been shown in healthy plants of chinese cabbage and tobacco.

[II] DNA-directed replication

This hypothesis suggests that viroids might be replicated on DNA templates which are either already present in repressed form in uninfected hosts or are synthesized as a result of viroid infection.

Viroids and Plant Diseases

Viroid diseases are persistent infections, i.e., there is no recovery of infected plants and viroids can be isolated from the diseased plants so long as it is alive. They do not differ significantly from the viral diseases in their symptomatology. The symptoms observed in viral diseases also occur with viroid diseases. These include stunting, epinasty, veinal discoloration, leaf distortion, vein

clearing, localized chlorotic or necrotic spots, mottling of leaves and death of the whole plant. Precise mode of viroid interference with their host metabolism to produce characteristic symptoms is not known. In some viroid infected plants certain host proteins occur in larger amounts than in healthy plants. It is presumably due to viroid-induced metabolic aberrations in the host protein synthesis.

All known viroids are transmitted from one plant to another (i.e., horizontally) by mechanical means. Besides, vertical transmission through the seed and pollen of the infected plants has been shown at least in PSTV. Some viroids are transmitted by aphids.

PRIONS

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

Prions are **made of only proteins; they have no nucleic acids**. The proteins constituting the prions are designated as **PrP** (prion proteins). They have a mass of 27,000-30,000 daltons. These proteins consist of about 250 amino acids and are about 1/100 the size of a small virus. The genes that code for these proteins are found in the normal host DNA. In human beings the PrP genes are located on chromosome 20. It is believed that prions are normal proteins that become folded incorrectly, possibly as a result of mutation (Fig. 15 A, B). How these defective infectious prion particles are transmitted to an uninfected individual and initiate the disease process is not definitely known. Prions can survive on instruments sterilized by formaldehyde or inadequately autoclaved. They also survive for several years on buried animals.

Prions show following characteristics.

- (1) Prions are not inactivated by temperature up to 90°C (viruses are normally inactivated at this temperature).

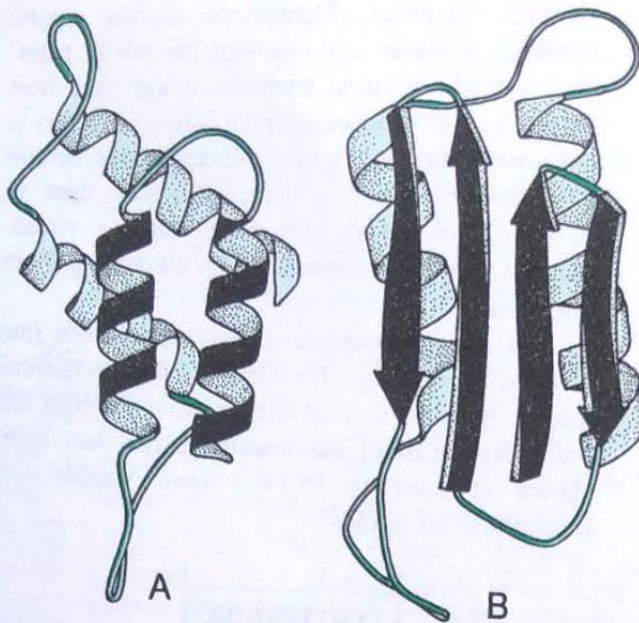


Fig. 15 A-B. Two forms of prion proteins : A. Harmless form, B. Harmful form. (the harmful nature of the protein is because of improper folding of normal prion protein).

- (2) Prion infection is not sensitive to radiation treatment that damages virus genomes.
- (3) Enzymes destroying/digesting DNA or RNA do not affect the activity of prions.
- (4) Protein denaturing agents (e.g., phenol, urea, etc.) affect prion activity.

Prions have a long incubation period, hence these infective agents were once assigned the name

slow viruses. Some of the diseases now believed to be caused by prions were once thought to be slow viruses. However, not all slow virus diseases are caused by prions.

Prions cause a progressive degeneration of the central nervous system and the diseases thus caused by prions are collectively known as **transmissible spongiform encephalopathies** because they destroy neurons and the brain tissue gives a sponge-like appearance. Among animals prions are known to cause **scrapie** (in sheep and goat) and **'mad cow' disease** and **encephalopathy** (in mink, elk and cats). In 1996, United Kingdom had to kill hundreds of thousands of cattle because of an outbreak of 'mad cow' disease.

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

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

Table 9. Comparison of viruses, viroids and prions.

Character	Virus	Viroid	Prion
1. Nucleic acid	Single or double stranded DNA or RNA	Single stranded RNA	Absent
2. Protein envelope or capsid	Present	Absent	Absent
3. Proteins	Present	Absent	Present
4. Study and identification	Electron microscope	Nucleotide sequence identification of nucleic acid	Host cell damage
5. Sensitivity to high temperature	Present	Present	Absent
6. Effect of protein denaturing agent	Proteins denatured	Absent	Proteins denatured
7. Effect of nucleic acid digesting enzymes	Nucleic acid denatured	Nucleic acid denatured	Absent
8. Host	Bacteria, animals and plants	Plants	Mammals

Important Questions

►► Long answer questions

1. Describe the characteristics of viruses. Differentiate between plant and animal viruses.
2. Give an illustrated account of the morphology and chemical structure of viruses.
3. Describe the structure of tobacco mosaic virus.
4. Write an essay on the transmission of viruses.
5. What are bacteriophages? Describe their structure with the help of suitable diagrams.
6. Write short notes on:
 - (i) Yellow vein mosaic of bhindi, (ii) Nature of viruses, (iii) Symptoms of virus infection in plants, (iv) Nomenclature of viruses, (v) Replication of phage particles, (vi) Economic importance of phage particles, (vii) Viroids, (viii) T.M.V., (ix) Bacteriophages, (x) Multiplication in plant viruses, (xi) Viruses are living agents.
7. Give a brief account of modes of transmission in plant viruses.
8. What are the main characteristics of viruses? Give an account of transmission of tobacco mosaic virus.
9. Describe the different replicative cycles seen during the replication of bacteriophages.
10. Draw the labelled diagram of T.M.V. and write an account of its multiplication.
11. Draw labelled diagram of bacteriophage. Describe the method of its reproduction.
12. Give general account of viruses.
13. How the plant viruses are transmitted? Give the control measures for typical plant viral disease.
14. Describe the structure and multiplication of bacteriophages.
15. Describe the structure and means of reproduction in tobacco mosaic virus.
16. Give a general account of morphology of different types of viruses.

►► Short answer questions

1. What are the functions of protein coat and nucleic acid of a virus?
2. Can a virus be classified as a cell? Explain.
3. Differentiate between a plant and an animal virus.
4. Why are viruses not affected by antibiotics?
5. Write any three systemic symptoms in plants infected with viruses.
6. Explain if viruses can be cultured artificially, and if yes, what culture medium will you use for their culture?
7. Write a note on the structure and cryptogram of TMV.
8. Give the structure of virus.
9. Differentiate between the virulent and temperate phages.
10. Distinguish between the lysogenic and lytic phases in the life cycle of a virus.

►► Very short answer questions

1. Name the organism in which RNA serves as a genetic material.
2. Name the type of nucleic acid present in TMV.
3. Who discovered bacteriophages?
4. What name is given to a virus which infects bacteria?
5. What genetic material is found in all plant viruses?
6. Write down the cryptogram of tobacco mosaic virus (TMV).
7. Who coined the term 'viroid'?
8. Name the enzyme which occurs in AIDS virus.
9. Name any two screening tests that are done to confirm whether the person is suffering from AIDS.
10. Define viroids.
11. Name the microorganisms having only one kind of nucleic acid.
12. Who discovered TMV?
13. What is the name of the subunit of the protein coat in viruses?
14. Mention the type of genetic material in most of the animal viruses.
15. What is the name of the protein coat in viruses?
16. Write down the cryptogram of any virus.
17. What kind of nucleic acid is found in the polio virus?
18. Name the viruses which infect the yeast cells.
19. Who discovered viroids?

20. What type of nucleic acid is present in potato spindle tuber viroid (PSTV)?
21. What are prions?

►► Fill in the blanks

1. A virus reproduces by using the metabolic machinery of a
2. A virus recognizes its host by the reaction of specific binding sites on the with those on the or of the host.
3. A viral genome incorporated into the DNA molecule of a bacterium is called
4. Most RNA viruses carry a gene for an enzyme that uses viral RNA as a template in the synthesis of more viral RNA. This enzyme is
5. The protein coat that encloses the viral genes is called a
6. A viroid consists of a very short strand of without any protective coat.
7. The viruses that attack blue-green algae are known as
8. The viruses that attack the fungi are called
9. Bacteriophage T₄ has tail fibres.
10. do not have functional autonomy.

►► True and false statements

1. Viruses have protein coat made up of capsomeres.
2. Viruses can pass through bacterial filters.
3. Yellow vein disease of bhindi is caused by a virus.
4. Viruses can multiply in dead cells.
5. d'Herelle (1917) coined the term 'bacteriophage' for the first time.
6. Shafferman and Morris (1951) are associated with the discovery of cyanophages.
7. Viruses are chemicals in a test tube but living beings inside the host.
8. Viruses are non cellular, obligate intercellular parasites.
9. Viruses have an efficient protein synthesizing system of their own.
10. Double stranded DNA is found in tobacco mosaic viruses.
11. Primitive microbes are known as prions.
12. Prions are made of only proteins.

►► Multiple choice questions

1. The fact which supports the idea that viruses are living is that they :
 - (a) duplicate themselves
 - (b) penetrate plasma membranes
 - (c) can be crystallized
 - (d) are made of protein and DNA
2. Double stranded RNA viruses are called:
 - (a) riboviruses
 - (b) pox viruses
 - (c) reoviruses
 - (d) none of the above
3. Coliphage x174 contains:
 - (a) single-stranded DNA
 - (b) single-stranded RNA
 - (c) double-stranded DNA
 - (d) double-stranded RNA
4. In the lytic cycle of a bacteriophage, the host DNA is:
 - (a) replicated
 - (b) digested into its nucleotides
 - (c) turned off by a protein coat
 - (d) turned on by removal of a protein coat
5. A virus that can reproduce without killing its host is called a:
 - (a) temperate virus
 - (b) lytic virus
 - (c) retroactive virus
 - (d) virion
6. The enzymes involved in viral replication are synthesized:
 - (a) on the viral ribosomes
 - (b) by the host cell
 - (c) on the interior surface of the viral coat
 - (d) on the interior surface of the viral membrane
7. A bacteriophage with a lysogenic cycle must have genes that are:
 - (a) made of RNA
 - (b) made of single stranded DNA
 - (c) made of double stranded DNA
 - (d) within a circular nucleic acid molecule
8. When a virus attacks the bacterium, the material that enters the host is:
 - (a) protein coat
 - (b) nucleic acid
 - (c) both protein coat and nucleic acid
 - (d) none of the above
9. ELISA is a:
 - (a) microscopic technique
 - (b) culture technique
 - (c) serological technique
 - (d) none of the above
10. Localised tissue death is called:
 - (a) mosaic
 - (b) necrosis
 - (c) enation
 - (d) none of the above
11. Who is bacterium eater?
 - (a) coliphage
 - (b) bacteriophage
 - (c) cyanophage
 - (d) TMV
12. Chlorosis is caused by:

- (a) fungi (b) virus
(c) mycoplasma (d) none of the above
13. Outer layer of virus is composed of:
(a) fats (b) proteins
(c) carbohydrates (d) nucleic acid
14. Who discovered TMV?
(a) Bawden (b) Iwanowski
(c) Stanley (d) Twort and d'Herelle
15. Bacteriophages were discovered by:
(a) Griffith (b) Subramanian
(c) Twort (d) none of the above
16. Genetic material in a bacteriophage is:
(a) RNA
(b) DNA
(c) both DNA and RNA
(d) neither DNA nor RNA
17. Leaf curl of papaya is caused by:
(a) fungus (b) mycoplasma
(c) virus (d) bacteria
18. Bacteriophages are:
(a) an organelle of the bacterium
(b) bacterium which infects a higher plant cell
(c) bacterium which infects an animal cell
(d) virus which infects a bacterium
19. Phages which show lysogenic cycle are called:
(a) temperate phages (b) lytic phages
(c) virulent phages (d) none of the above
20. Who isolated the plant virus first?
(a) W. M. Stanley (b) E. C. Stakman
(c) D. Iwanowski (d) K. M. Smith
21. Bacteriophage is made up of following:
(a) protein (b) DNA
(c) nucleoprotein
(d) lipid and protein
22. Name the organism in which RNA serves as genetic material:
(a) Gram (+ve/-ve) bacteria
(b) TMV
(c) *Saprolegnia*
(d) *Agaricus*
23. Viruses synthesize their protein coats:
(a) inside the host cell
(b) outside the host cell
(c) both outside as well as inside the host cell
(d) none of these
24. Which is correct?
(a) a virion is a fully developed virus particle
(b) a virion is a capsid
(c) a virion is a capsomere
(d) none of the above
25. Double stranded DNA (dsDNA) is found in:
(a) herpes virus
(b) TMV
(c) reovirus
(d) coliphage virus

ANSWERS

►► Very short answer questions

1. Tobacco mosaic virus (T.M.V.), 2. RNA, 3. Edward Twort (1915), 4. bacteriophage, 5. Single stranded RNA, 6. R/1 : 2/5 : E/E : S/A, 7. T. O. Diener (1971), 8. Reverse transcriptase, 9. ELISA test, western blot test, 10. A subviral pathogen consisting of a very short strand of RNA without any protective coat, 11. viruses, 12. D. Iwanowski in 1892, 13. Capsomere, 14. Single or (rarely) double-stranded RNA or double-stranded DNA, 15. Capsid, 16. Influenza virus : R/1 : (2-3)/10 : S/E : V/A, 17. Single stranded RNA, 18. Zymophages, 19. T. O. Diener (1971), 20. Single stranded circular RNA molecule, 21. Prions are proteinaceous infectious particles.

►► Fill in the blanks

1. host cell, 2. viral coat, 3. cell membrane, wall, 3. prophage, 4. RNA replicase, 5. capsid, 6. RNA, 7. cyanophage, 8. mycophages, 9. six, 10. viruses.

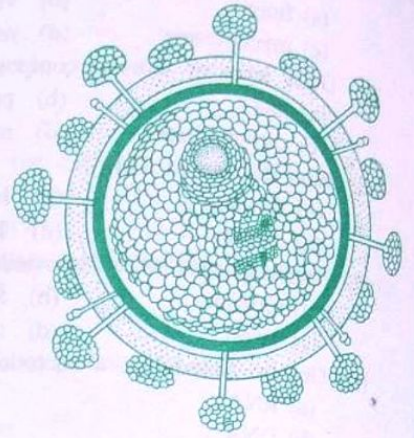
►► True and false statements

1. True, 2. True, 3. True, 4. False, 5. True, 6. True, 7. True, 8. False, 9. False, 10. False, 11. False, 12. False.

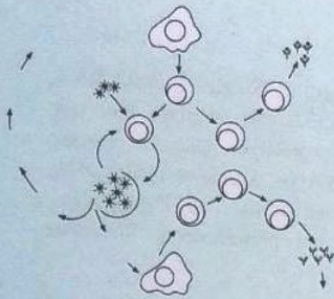
►► Multiple choice questions

1. (a), 2. (c), 3. (a), 4. (b), 5. (a), 6. (b), 7. (c), 8. (b), 9. (c), 10. (b), 11. (b), 12. (b), 13. (b), 14. (b), 15. (c), 16. (b), 17. (c), 18. (d), 19. (a), 20. (c), 21. (c), 22. (b), 23. (a), 24. (a), 25. (a).

5



Acquired Immunodeficiency Syndrome (AIDS)



Acquired immunodeficiency syndrome or AIDS is a fatal disease comprising serious clinical condition of various manifestations characterized by underlying cellular immunodeficiency. The disease probably originated in southern parts of Africa, but it was first detected in June, 1981 at '**Centre for Disease Control**,' Georgia in America. At first when it surfaced in USA, it appeared to be a disease of the male homosexuals and male prostitutes, but nevertheless huge number of others also suffer. AIDS has in recent years grown exponentially into a frightening worldwide epidemic. It is the first great pandemic of 20th century which has claimed thousands of lives throughout the world. WHO estimates that more than 10 million people are currently infected with human immune deficiency virus (HIV). In India, first report of AIDS case came in Jan. 1986 from Chennai and now, in some areas, it is spreading alarmingly.

CAUSES OF AIDS

AIDS is caused by a retrovirus, HIV (human immune deficiency virus). Previously, it was called HTLV-III (Human T-lymphocyte Virus III) by Robert Gallo of National Cancer Institute, USA or LAV (lymphadenopathy associated virus) by Luc. Montagnier of Pasteur Institute, Paris. The virus destroys part of the body's immune system, leaving victims unable to defend themselves against infections and certain cancers.

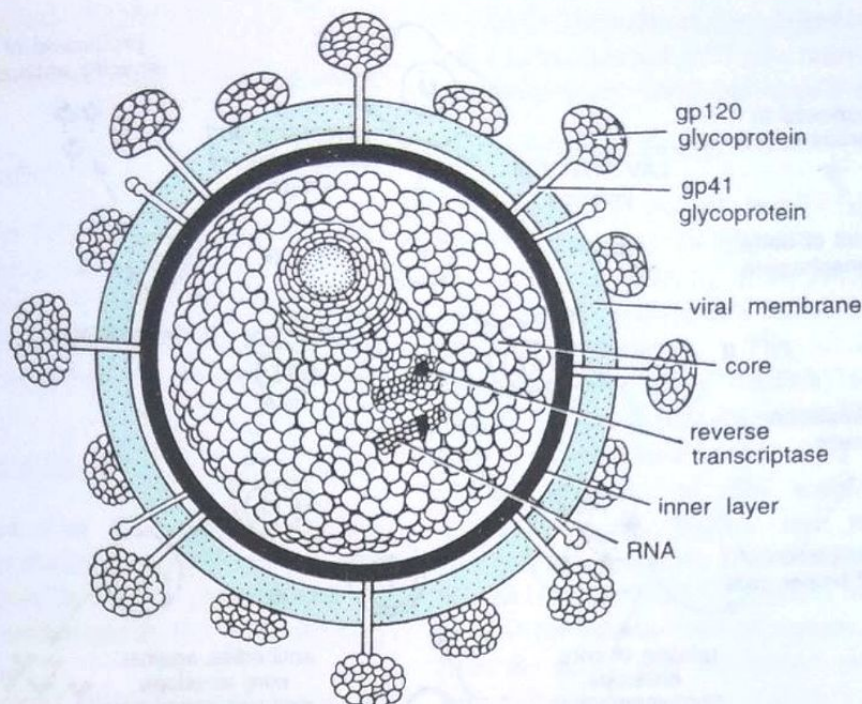


Fig. 1. Structure of AIDS virus.

STRUCTURE OF AIDS VIRUS

The structural model of HIV shows that virus is a sphere containing RNA as genetic material and an enzyme reverse transcriptase. The lentivirus nature is reflected by the capsid which is in the form of an elongated protein core. The major inner protein, called P₂₄ has a molecular weight of 24,000 whereas another protein, called P₁₈ covers the inner surface of the envelope. The envelope is a two-layered lipid or fatty coat. The surface of the virus particle is studded with knob-like structures (Fig. 1). The full genome of HIV codes for its various components. The gene '*Gag*' codes for the RNA containing core; gene '*pol*' is responsible for the production of enzymes reverse transcriptase and endonuclease, whereas gene '*env*' produces the envelope proteins. The newly discovered genes, *tat*, *art*, *sor* and *3'orf* encode small protein that help to regulate gene expression.

MECHANISM OF ACTION

The primary target of HIV is T₄ lymphocytes which play a major role in maintaining the body's response to infection (Fig. 2). Once the virus enters the blood stream, it attacks T₄ lymphocytes and the viral genes are integrated into these cells. There, they can apparently remain dormant for

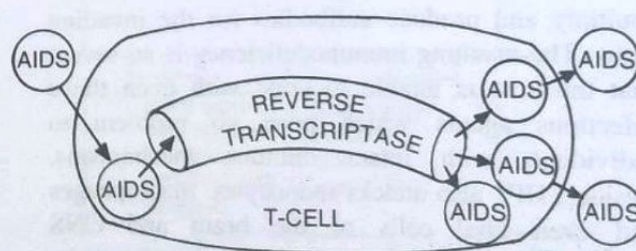


Fig. 2. Invasion and multiplication of HIV in the human body.

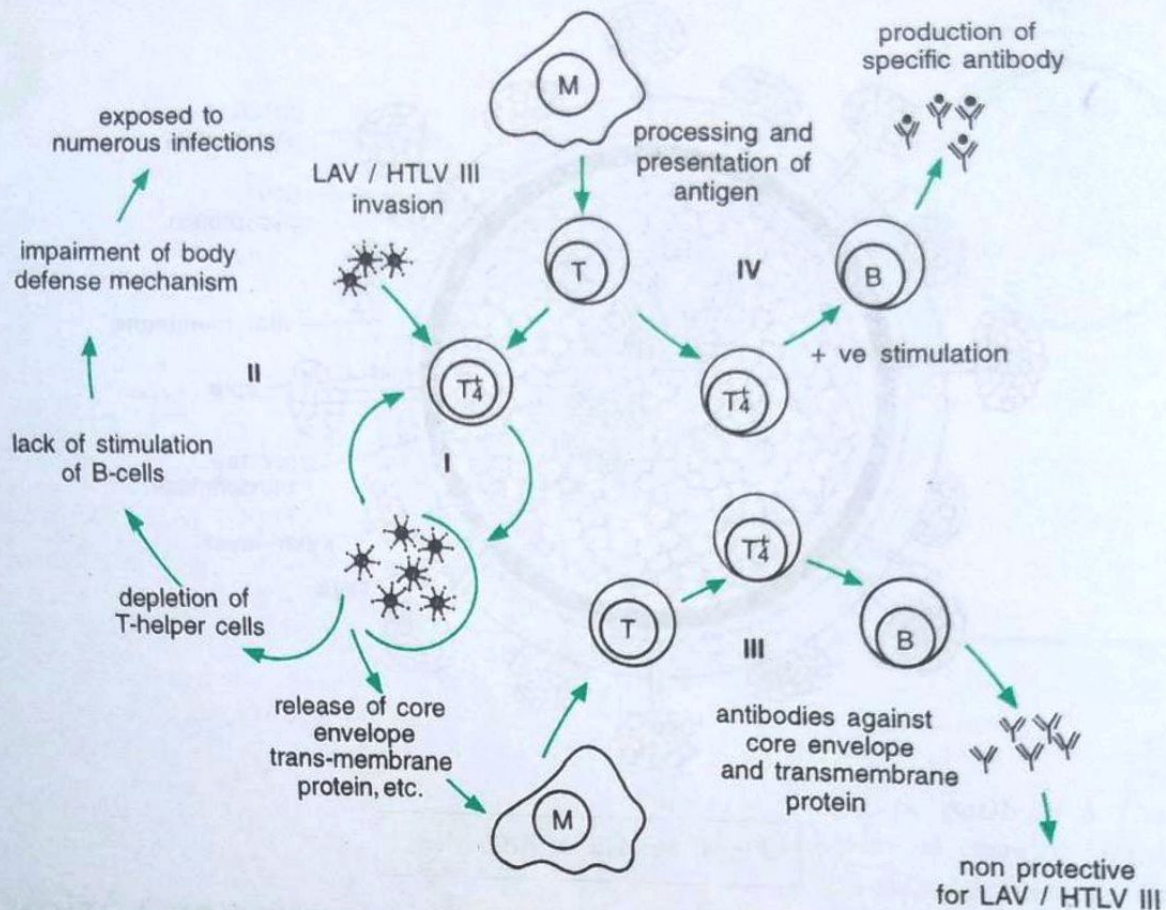


Fig. 3. Mechanism of action of HIV.

indefinite period without causing any ill effects. This is called **incubation period** which ranges from six months to five years or more. The virus converts T-cells from a lymphocyte to HIV factory, thus damaged T-cells are unable to stimulate B-cells. As a result, B-cells could not multiply and produce antibodies for the invading virus. The resulting immunodeficiency is so severe that the host is unable to cope with even those infectious agents which pose no problem to individuals with intact immune mechanisms. Besides, HIV also attacks monocytes, macrophages and even glial cells of the brain and CNS (Fig. 3). Since HIV comprises many variants, its infection may be variable.

SYMPTOMS OF AIDS

Early symptoms of AIDS may be vague and ill-defined. They include weight loss, fever, diarrhoea, oral thrush and enlargement of the lymph glands.

Not all sufferers of early symptoms will go on to develop the full-blown illness. A number of individuals develop a mild condition, known as **ARC** (AIDS-related complex). In severe condition, the patients may develop pneumonia, kaposi's sarcoma (a skin cancer) and lymphoma (cancer of the lymph system). Although AIDS has a long incubation period (possibly five years), once it does develop there is a rapid decline in health. For an individual who develops AIDS, the likelihood of death within a year, is very high.

TRANSMISSION OF HIV

HIV can be transmitted from an infected person to a healthy one by the following means.

[A] Sexual Intercourse

HIV is found in semen and vaginal fluids, thus sexual transmission can occur from man to woman, woman to man or man to man.

[B] Blood and Blood Products

Transfusion of HIV-contaminated blood can infect the recipient.

[III] Shared Needles

Users of intravenous drugs are a major risk group because many of them share needles and syringes without proper cleaning. However, any unsterilised, skin-piercing instrument-including ear-piercing or tattooing needles can spread the disease from one person to another.

[IV] Mother-to-Child

A woman infected with HIV may spread the disease to her child during pregnancy, during birth or shortly after birth. It is also possible that an infected mother could transmit the virus through breast-feeding.

HIV is not spread through casual contact in school, on the job, in the swimming pool, or at the market. It is also not spread by toilet seats, hand shakes, hugs, casual kissing, eating from the same dish, drinking from the same glass, or by food handlers in restaurants. It also does not spread by mosquitoes or other insects.

AIDS DIAGNOSIS

The individuals suffering from HIV are usually diagnosed by the following two methods.

[A] Clinicians Make Diagnosis of AIDS by History and Medical Examination for Symptoms

Doctors usually diagnose AIDS - infected persons from typical history and symptoms shown by the patient. The symptoms of full blown AIDS disease or AIDS-related complex (ARC) are clearly different from the symptoms of other known diseases. Moreover if the patient is of young age group and in high risk group (intravenous drug user, transfusion recipient, hemophilic, or polygamic), one can say with certainty that person is suffering from full blown AIDS or ARC. Besides, the blood sample analysis shows the sign

of anaemia, leukopenia, lymphopenia (low T-helper cell count), reversal of $T_4:T_8$ ratio (0.9), elevated immunoglobulin level and B-cell abnormalities.

[B] Screening Tests

Two types of screening tests - **ELISA test** and **Western blot test** are done to confirm whether the patient is suffering from AIDS (Fig. 4). In these tests the individual's serum is used to detect HIV antibodies. The **enzyme-linked immunosorbent assay** (ELISA) for antibody to human immune deficiency virus (HIV) has proved to be a valuable tool in examining the association between exposure to HIV and AIDS and its related complex. ELISA test is simple and convenient to perform. It is a colour reaction test in which antigen (HIV particles) binds antibodies to HIV (in infected human serum). This complex fixes the added goat antihuman antibody labelled with a chemical enzyme. It gives a colour reaction

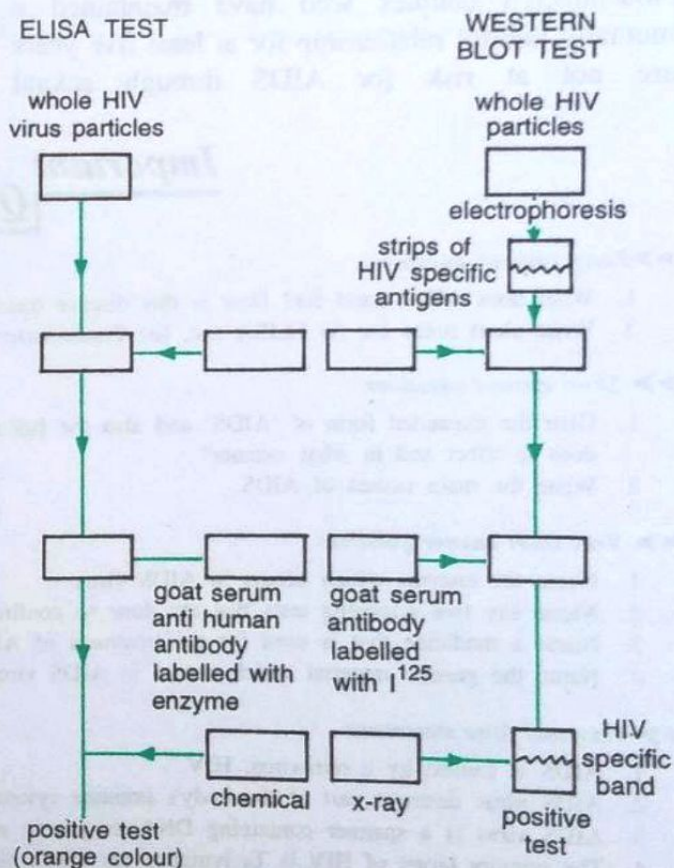


Fig. 4. Screening tests for AIDS.

with a particular chemical, e.g., orange colour with peroxidase labelled goat antihuman antibody. However, ELISA test is not 100% reliable because it is not completely specific for HIV antibodies but also reacts with other related viral antibodies.

The **western blot test** is more specific and confirmatory assay for HIV antibodies. In this test, the prepared HIV particles are transferred to nitrocellulose membrane strips. These strips are then exposed to the infected human serum and to the goat antihuman antibody. Positive test is indicated when these strips display the characteristic band on exposure to X-ray films.

PREVENTION FROM AIDS

Avoidance of drug abuse and promiscuous homosexual intercourse are best preventive methods. Avoid having multiple sexual partners. Non-infected couples who have maintained a mutually faithful relationship for at least five years are not at risk for AIDS through sexual

transmission. Furthermore, one should not share items that could become contaminated with blood, for example, razor, tooth brushes or any skin-piercing instrument.

TREATMENT OF AIDS

The most distressing thing is that at present there is no cure for AIDS patients. So, fatality rate is 100%. There is no effective drug or vaccine available to restore the collapsed immune system of the patient and to control infections permanently and effectively. A recently introduced drug, N-butyl deoxynojirimycin, however, holds some promise. Currently, AZT (azidothymidine), which prevents reverse transcription, is used for the treatment of AIDS. Patients receiving AZT showed increased numbers of circulating helper T-cells, increased immunological capacity, a virostatic effect, weight gain and improved general well being. Ribavirin is another drug which is tried intensively in AIDS patients. The drug stops the progression of infection associated with AIDS.

Important Questions

►► Long answer questions

1. What does AIDS stand for? How is this disease transmitted? Suggest two methods for its prevention.
2. Write short notes on: (i) ELISA test, (ii) Transmission of AIDS, (iii) HIV, (iv) Prevention of AIDS.

►► Short answer questions

1. Give the expanded form of 'AIDS' and also the full name of its causative agent. Which part of the immune system does it affect and in what manner?
2. Write the main causes of AIDS.

►► Very short answer questions

1. Name the enzyme which occurs in AIDS virus.
2. Name any two screening tests that are done to confirm whether the person is suffering from AIDS.
3. Name a medicine that is used for the treatment of AIDS.
4. Name the genetic material which occurs in AIDS virus.

►► True and false statements

1. AIDS is caused by a retrovirus, HIV.
2. AIDS virus destroys part of the body's immune system, leaving victims able to defend themselves against infections.
3. AIDS virus is a sparrow containing DNA as genetic material and an enzyme reverse transcriptase.
4. The primary target of HIV is T₄ lymphocytes which play a major role in maintaining the body's response to infection.

►► Fill in the blanks

1. The.....test is a confirmatory assay for HIV antibodies.
2. HIV contains.....as genetic material and an enzyme.....
3. The surface of HIV is studded with.....structures.
4. Transfusion of HIV-contaminated blood.....infect the recipient.

(MICROBIOLOGY)

►► Multiple choice questions

1. The gene 'Gag' present in the genome of HIV codes for:
(a) RNA containing core
(b) production of enzyme reverse transcriptase
(c) production of envelope proteins
(d) all the above
2. The primary target of HIV is:
(a) RBC
(b) thrombocytes
(c) T₄ lymphocytes
(d) none of the above
3. The following is one of the main symptoms of AIDS:
(a) weight loss
(b) diarrhoea
(c) oral thrush
(d) all the above
4. HIV can be transmitted from an infected person to a healthy one by the following means:
(a) sexual intercourse
(b) blood and blood products
(c) shared needles
(d) all the above

ANSWERS**►► Very short answer questions**

1. reverse transcriptase, 2. ELISA test, western blot test, 3. AZT (azidothymidine), 4. RNA.

►► True and false statements

1. True, 2. False, 3. False, 4. True.

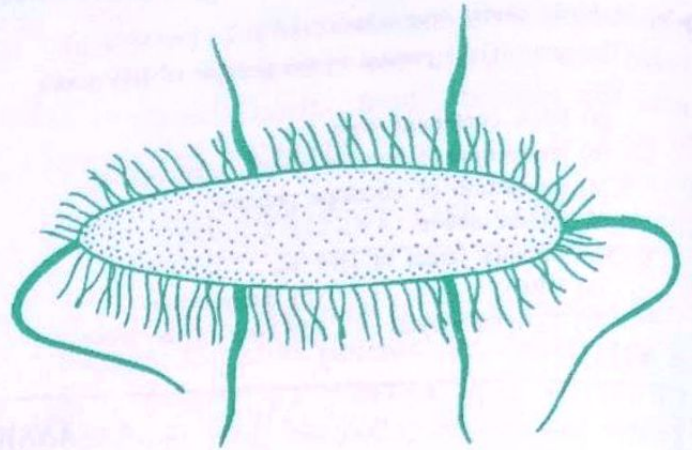
►► Fill in the blanks

1. western blot, 2. RNA, reverse transcriptase, 3. knob-like, 4. can.

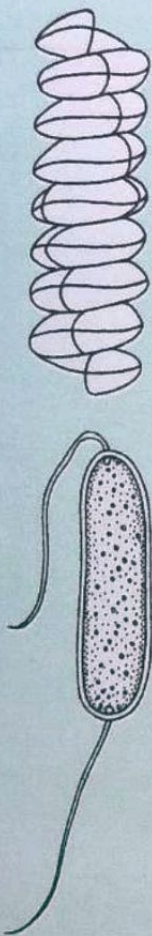
►► Multiple choice questions

1. (a), 2. (c), 3. (d), 4. (d).

6



Bacteria: Structure and Reproduction



The environment around us has a fascinating world of micro-organisms which affect our life directly or indirectly. The existence of microbial world was unknown until the invention of microscope at the beginning of the seventeenth century, which opened the realm of micro-organisms to systematic scientific exploration.

Antony van Leeuwenhoek (1632-1723), a Dutch cloth merchant, was the first to give an illustrated description of micro-organisms. The thousands of microscopic organisms that he saw in a drop of rain water were collectively named as **animalcules**. However, it was not until the development of the compound microscope that biologists became aware of the tremendous numbers and diversity of these organisms in nature. As further information accumulated about these microscopic organisms, it became evident that they did not belong to two familiar kingdoms of living world, i.e., plants and animals and thus shared the characters of both plants and animals. Therefore, they could not be placed judiciously in either of these kingdoms. This necessitated the creation of a new group, and Ernst Haeckel, a German biologist, in 1894, proposed a third kingdom **Protista**. It included all unicellular and microscopic organisms and also many large multicellular forms which do not have any extensive development of tissue. The protists thus were further divided into two large groups, which were called **lower** and **higher protists**. The lower protists are characterized by prokaryotic cellular organization, and they include bacteria and blue-green algae (cyanobacteria). The higher protists, on the other hand, have eukaryotic cell organization and include protozoa, algae and fungi.

(MICROBIOLOGY)

Murray (1968) included all lower prokaryotes (which are characterized by the absence of nuclear membrane and basic proteins in nucleoplasm) in **Procaryotae**. Bergey (1974), in the *Manual of Determinative Bacteriology*, placed bacteria in the kingdom **Prokaryotae**.

GENERAL CHARACTERS OF BACTERIA

Bacteria are microscopic and least differentiated living organisms, believed to be amongst the first primitive organisms on the earth. They show typical prokaryotic characters and have resemblance with both plants and animals. They have been placed in a separate class Schizomycetes under Thallophyta.

Bacteria show considerable variations, but the characters listed below are common to most of the bacteria.

- (1) They are omnipresent, found in all possible habitats one can think of.
- (2) Most of the bacteria have **heterotrophic** mode of nutrition, i.e., they obtain their readymade food directly from external agency, and may be parasitic, saprophytic or symbiotic. Some bacteria are autotrophic; they possess bacteriochlorophyll—a photosynthetic pigment.
- (3) They are unicellular and morphologically least complex of all the living organisms.
- (4) The cell wall of bacteria is rigid and made up of two types of polymers, **amino acid subunits** and **saccharide subunits**. Cellulose, characteristic of the cell wall of higher plants, is absent in bacteria.
- (5) A well organised nucleus, characteristic of eukaryotes, is lacking and discrete chromosomes are also absent. The nuclear material is not surrounded by nuclear membrane.
- (6) Chlorophyll pigments, if present, are located within involuted cytoplasmic membranes; well organised plastids are absent.
- (7) Mitochondria are absent and their function is carried out by complex localized infoldings in the cell membrane, known as **mesosomes**.
- (8) The organelles like endoplasmic reticulum and Golgi apparatus are absent.
- (9) Ribosomes are abundant in bacterial cell but they sediment somewhat more slowly in a centrifuge than do ribosomes of other organisms.
- (10) Binary fission is the most common method of multiplication.
- (11) True sexual reproduction is absent. However, recombination of genetic material occurs by conjugation, transformation and transduction.
- (12) The motile bacteria may possess one or more flagella, composed of eight parallel chains of flagellin (a protein) molecules.
- (13) The gram negative bacteria possess minute hair-like cytoplasmic appendages, known as **pili**, extruding through the cell wall. These appendages are composed of a protein, called **fimbrillin**.

CLASSIFICATION OF BACTERIA

'Bergey's Manual of Determinative Bacteriology' is the standard reference book which deals with the classification and identification of bacteria. The classification of bacteria given in the first edition of the Manual, published in 1923, was mainly based on phenotypic characterization. But as new information poured in as the result of researches at biochemical and molecular levels, the composition and arrangement of higher taxonomic groups—orders, families and tribes have changed substantially in successive editions. In the 8th edition of the Manual, published in 1974, there is a radical departure in the scheme of classification from earlier editions. Bacteria have been distinguished from other micro-organisms on the basis of their typical prokaryotic structure and they have been placed in a separate kingdom **Procaryotae**. Further treatment of this kingdom was based on cellular characteristics rather than

organismal properties. Procaryotae were divided in two divisions—**Cyanobacteria** and **Bacteria**. Bacteria were further subdivided into 19 groups, distinguishable by some readily determined criteria. There were certain **genera of uncertain affiliation** which could not fit perfectly within the set limits of a particular group.

An outline of bacterial classification, as adopted in the latest edition of the Bergey's Manual (1974), is given below.

KINGDOM PROCARYOTAE

Division I. Cyanobacteria

They are photosynthetic prokaryotes and their photosynthetic process is similar to that of higher plants (unlike other photosynthetic bacteria where the process is aerobic and electron donor is water). The photosynthetic pigments are **chlorophyll** and **phycobiliproteins**. These bacteria usually occur as simple or branched chains of cells. They reproduce by binary fission, spores or fragmentation.

Division II. Bacteria

They are unicellular or occasionally show simple arrangements. They are characterized by the presence of a rigid cell wall of **peptidoglycans**. Their photosynthetic process is anaerobic, the electron donor is a substance other than water. Their photosynthetic pigments are **bacteriochlorophylls**.

The division Bacteria has been further divided into 19 groups on the basis of their photosynthetic ability, type of movement, response to Gram stain, etc.

Group 1. Phototrophic Bacteria

These are photosynthetic bacteria, characterized by the presence of a chlorophyll like pigment, **bacteriochlorophyll**.

They occur mostly in aquatic environments.

They are motile or non-motile. This group includes a single order with 3 families and 18 genera.

Group 2. Gliding Bacteria

These bacteria produce slime and show gliding movements. Some forms produce brightly coloured macroscopic fruiting bodies. They occur abundantly in soil, decomposing plant matter and aquatic environment. This group includes 2 orders and 8 families, representing 21 genera. In addition, the group also includes 6 genera of uncertain affiliation.

Group 3. Sheathed Bacteria

They are rod-shaped or filamentous bacteria, surrounded by a sheath of insoluble compounds of iron and manganese. They are motile or non-motile, and Gram-negative and occur in aquatic environments, sludge, etc. This group is represented by 7 genera.

Group 4. Budding and/or Appendage Bacteria

Some bacteria of this group produce a filamentous outgrowth, called **prostheca**, from the body of the cell, and the others have holdfasts. These appendages help them in attachment to some object. They multiply by budding and fission. They occur in soil or in aquatic environment. This group includes 17 genera.

Group 5. Spirochaetes

These bacteria are slender, flexuous and helically coiled, varying in length from 3-500 μm . Some of them are saprophytes and the others are parasites. The parasitic forms are pathogenic to human beings and other animals. They are motile and they multiply by transverse fission. This group includes 5 genera placed in a single family and a single order.

Group 6. Spiral and Curved Bacteria

These bacteria are also helically coiled like spirochaetes but have rigid cell wall. In some species the spiral is not complete and as such they become 'comma' shaped. They are free living, saprophytes or parasites. These bacteria move with the help of flagella and are Gram-negative. This group includes 2 genera, placed in

a single family. The group also has 4 genera of uncertain affiliation.

Group 7. Gram-Negative Aerobic Rods and Cocci

This group includes a large number of Gram-negative aerobic bacteria. Although similar in morphology, they vary in biochemical characteristics, such as metabolism of nitrogen and carbon compounds. They are pathogenic to humans and animals. These bacteria occur in terrestrial as well as aquatic environment. This group includes 5 families represented by 14 genera. There are also 6 genera of uncertain affiliation.

Group 8. Gram-Negative Facultative Anaerobic Rods

Most of the common facultative anaerobes belong to this group. They are morphologically similar but differ in biochemical, physiological and serological characteristics. They are motile (peritrichous) or non-motile. These bacteria are pathogenic to human beings, plants and animals. They occur in all types of environments. This group includes 2 families represented by 17 genera. There are also 9 genera of uncertain affiliation.

Group 9. Gram-Negative Anaerobic Bacteria

They are pleomorphic obligate anaerobes which occur in the oral cavity, intestine and faeces of human beings. The motile forms are peritrichous or monotrichous. Some species are pathogenic to human beings and animals. This group includes 3 genera, placed in a single family. There are also 6 genera of uncertain affiliation.

Group 10. Gram-Negative Cocci and Coccobacilli

They are Gram-negative, non-motile aerobic cocci and coccobacilli which occur in the mucous membranes of humans and other animals. These bacteria have only limited ability to break down carbohydrates and proteins. This group has only one family represented by 4 genera. There are also 2 genera of uncertain affiliation.

Group 11. Gram-Negative Anaerobic Cocci

These are Gram-negative, non-motile, anaerobic bacteria. They are spherical in shape and occur in pairs, masses or in chains. The delimitation of the species within this group is largely on the basis of biochemical characteristics. They occur in the respiratory and intestinal tracts of humans and animals, but are not regarded as pathogenic. This group has one family, represented by 3 genera.

Group 12. Gram-Negative Chemolithotrophic Bacteria

These are autotrophic bacteria which derive energy from the oxidation of inorganic compounds. Their shape is rod-like, spherical or spiral. The motile forms of the group possess flagella. These bacteria are non-pathogenic and occur in soil and aquatic environment. This group includes 2 families, represented by 17 genera.

Group 13. Methane-Producing (Methanogenic) Bacteria

The bacteria of this group are characterised by their ability to produce methane under anaerobic conditions. They occur in terrestrial as well as in aquatic environment. Most of these bacteria are Gram-positive, but some are Gram-negative. This group has 8 genera, placed in a single family.

Group 14. Gram-Positive Cocci

These are Gram-positive, pathogenic or saprophytic cocci, which occur singly, in pairs, in clusters or in chains. They commonly inhabit soil, fresh water and the mucous membranes of warm blooded animals. This group includes 3 families represented by 12 genera.

Group 15. Endospore-Forming Rods and Cocci

These bacteria are characterized by their ability to produce endospores. Most of these bacteria are rod-shaped, and are either aerobic or anaerobic. They are pathogenic and cause infections in humans as well as animals. The group has one

family with 5 genera. One genus of uncertain affiliation is also included in this group.

Group 16. Gram-Positive Asporogenous Rod-shaped Bacteria

These are non-motile, Gram-positive, rod-shaped (bacilli) bacteria. They are anaerobic and mostly occur in milk and milk products (lactobacilli). They ferment milk sugar (lactose) into lactic acid and other acids. They are non-pathogenic and occur in oral cavity, intestine and vagina of humans and animals. This group is represented by a single genus, *Lactobacillus*. There are also 3 genera of uncertain affiliation.

Group 17. Actinomycetes and Related Organisms

These bacteria show pleomorphism and have a tendency to form branched filaments. They are non-motile, Gram-positive and aerobic or anaerobic. Many species are pathogenic to humans and animals. This group includes one order with 8 families, represented by 31 genera. There are also 4 genera of uncertain affiliation.

Group 18. Rickettsias

These are among the smallest bacteria, ranging from 0.3 to 0.7 μm in width and 1.0 to 2.0 μm in length. They are Gram-negative, non-motile, obligate parasites and cause several diseases in humans and animals. This group is represented by 2 orders, 4 families and 18 genera.

Group 19. Mycoplasmas

The organisms of this group lack a true cell wall and the cellular contents are enclosed by a triple-layered non-rigid cell wall. They are Gram-negative, non-motile and facultative anaerobes. They occur in respiratory tract and lower genital tract of mammals and birds. This group has one order, 2 families and 2 genera.

Distribution

Bacteria are cosmopolitan in distribution, occurring in all natural habitats. Their wide distribution is primarily due to the fact that they can withstand great extremes of temperature, moisture, acidity and salinity and are adapted to wide variety of energy sources. They occur in the atmosphere to an height of about six kilometers and on the sea floor five kilometers below the mean sea level. Some are adapted to a normal aerobic environment and others thrive well in the absence of oxygen (i.e., anaerobic). They exist in hot springs and can also survive below freezing point in the Atlantic ice. Similarly, their tolerance to hydrogen ion concentration ranges from pH near 0 to pH 11. Although bacteria can survive high temperature or high acidity, they can not survive both simultaneously; in neutral or alkaline hot springs they can occur up to the boiling point of water, but at a lower pH the upper temperature limit is relatively low. Normally bacteria live at a pressure of one atmosphere but they can tolerate a pressure of 3,000-6,000 atmospheres. The highest pressure in the ocean depth approximates about 1,000 atmospheres, thus pressure has no limiting effect on bacteria. Many species of bacteria are free living, and others are symbiotic, parasitic or saprophytic.

The immense biological success of bacteria is undoubtedly due to their small size, rapid reproductive rate, ability to survive under adverse conditions and metabolic adaptations.

Size

Bacteria are very small microscopic organisms. An average bacterial cell ranges from 0.5 (1 μm = 0.001 mm) to 2.0 μm in diameter. A single drop of water may contain as many as 5×10^6 bacteria. In one gram top soil their number may vary from 1×10^3 to 1×10^9 . Their size also varies with the shape. For example, the radius of spherical or coccus bacteria ranges from

0.5 to 2.5 μm and rod-shaped or bacillus bacteria are larger than cocci and are 0.3 to 15 μm in width. *Thiophysa volutans*, a sulphur bacterium, measuring about 18 μm in diameter, is perhaps the largest amongst all bacteria.

Shape

The bacterial cells show considerable variation in their shape, but all individuals of a species have approximately the same shape. Thus from the point of view of their identification, shape of bacteria is an important characteristic. Although conventional light microscope provides little structural details, it is possible to establish the major morphological groups on this basis. Three cell forms, characteristic of true bacteria, are described below.

[A] Bacillus or Rod-Shaped Bacteria

This is probably the most common form of bacteria. These are rod-shaped, cylindrical or elongate and are motile or non-motile cells (Fig. 1A). The rods may have rounded or blunt ends; some are very short and almost indistinguishable from spherical bacteria, while others are long and narrow. An average bacillus is about 1.5 μm in length and 0.5 μm in diameter.

Examples—*Bacillus anthracis*, *B. fastidious*, *B. polymyxa*, *Lactobacillus*.

The bacillus bacteria are classified into two groups on the basis of their arrangement.

[I] Diplobacillus

When bacillus bacteria occur in pairs, they are called **diplobacillus** (Fig. 1B); e.g., *Corynebacterium diphtheriae*.

[II] Streptobacillus

The bacilli bacteria of this group occur in long chains (Fig. 1C); e.g., *Bacillus tuberculosis*, *B. cereus*.

[B] Coccus or Spherical Bacteria

The spherical or ellipsoidal bacteria are called **cocci** (singular coccus) (Fig. 2 A). They measure 0.5-2.25 μm in diameter. They are non-motile, atriuous and often occur in chains or in clusters

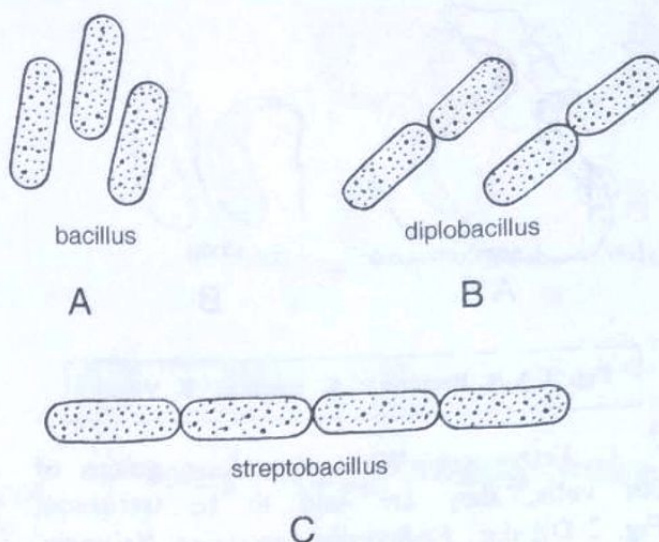


Fig. 1 A-C. Bacteria : Different types of bacillus bacteria.

of variable size and shapes. On the basis of the arrangement and the number of cells in a cluster, the cocci are classified into the following six groups.

1. **Micrococci**. When cocci occur singly, they are known as **micrococci**; e.g., *Micrococcus cerolyticus*, *M. cyrophilus*, *M. luteus*.

2. **Diplococci**. When cocci occur in pairs, they are called **diplococci** (Fig. 2 B); e.g., *Diplococcus pneumoniae*.

3. **Streptococci**. Spherical bacteria when occur in long chains, they are called **streptococci** (Fig. 2 C); e.g., *Streptococcus lactis*, *S. pyogenes*.

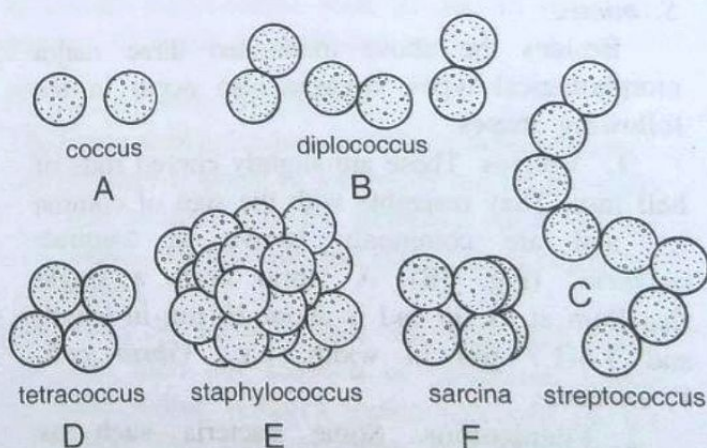


Fig. 2 A-F. Bacteria : Different types of coccus bacteria.

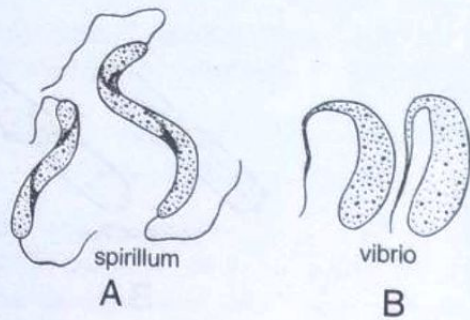


Fig. 3 A-B. Bacteria : A. Spirillum, B. Vibrio.

4. **Tetrads.** When they form groups of four cells, they are said to be **tetrads** (Fig. 2 D); e.g., *Pedococcus cerevisiae*, *Neisseria*.

5. **Staphylococci.** An irregular group of many spherical bacteria is known as **staphylococcus** (Fig. 2 E); e.g., *Staphylococcus albus*, *S. aureus*.

6. **Sarcinae.** When spherical bacteria divide in three planes in a regular pattern producing a cuboidal arrangement of cells, they are said to be **sarcinae** (Fig. 2 F); e.g., *Sarcinae lutea*, *S. verticuli*.

[C] Spiral or Helical Bacteria

These are slightly larger and elongated spiral rods (Fig. 3 A). A spirillum (plural **spirilla**) has more than one turn of a helix. It is about 1.5 μm in diameter and up to 15 μm in length. These bacteria have one or more flagella at each pole. They usually occur singly or in small chains but are seldom found in groups. The common examples are *Spirillum undulatum*, *S. volutans* and *S. minus*.

Besides the above mentioned three major morphological types, bacteria also occur in the following shapes.

1. **Vibrios.** These are slightly curved rods of half turn. They resemble with the sign of comma (,) and are commonly known as '**comma bacteria**' (Fig. 3B). A vibrio bears a single flagellum at its tip and is about 10 μm in length and 1.5-1.7 μm in width; e.g., *Vibrio coli*, *V. cholerae*.

2. **Filamentous.** Some bacteria such as *Beggiatoa* and *Thiothrix* are filamentous.

3. **Pleomorphic.** Some bacteria are capable of changing their shape and size temporarily in

response to changes in the surrounding environment. As such a single bacterium may occur in more than one shape in its life cycle. For example, *Acetobacter* may occur as bacillus (single rods) or streptobacillus (chain of small rods), depending on the environment.

STRUCTURE OF BACTERIAL CELL

The conventional light microscope provides only a vague notion of the internal structure of the bacterial cell. Therefore, the cytological details of these organisms could be possible only after the discovery of electron microscope and development of staining techniques. The bacterial cell shows a typical prokaryotic structure. It is enclosed by three layers, the outermost covering of **slime**, the middle **cell wall** and the innermost **cytoplasmic membrane** (Fig. 4).

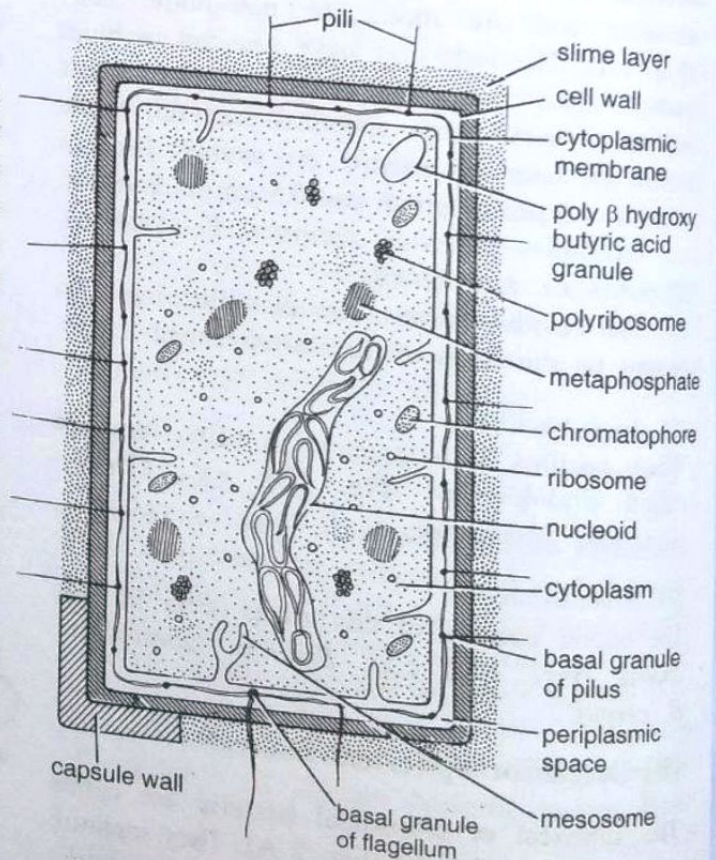


Fig. 4. Bacterial cell (schematic representation).

[A] Slime Layer

It is a gelatinous layer present on the outer surface of the cell wall, usually composed of polysaccharides (e.g., dextran, dextrans, laminan) and/or polypeptide chains of amino acids. When its constituents are only polysaccharides, which form a viscous layer, it is said to be **slime layer**, but when nitrogenous substances (i.e., amino acids) are also present along with polysaccharides, then it is called **capsule**. Various strains of the same species may have capsules of several different strain-specific polysaccharides.

The slime layer/capsule protects the cell from desiccation and antibodies. The capsulated bacteria also remain unaffected by phagocytosis. They are usually **atrichous**.

[B] Cell Wall

The cell wall is characteristic of plant kingdom. In higher plants and algae, it is composed of cellulose and shows a fibrillar structure. In contrast, the bacterial cell wall has a granular structure but is tough and rigid. It varies in thickness from 50 to 100 Å. The three main constituents of cell wall are (i) N-acetylglucosamine (NAG), (ii) N-acetylmuramic acid (NAM), and (iii) a peptide chain of four or five amino acids. These together form a polymer called **peptidoglycan** or **mucopolysaccharide**. The NAG and NAM molecules which are arranged alternately, run in one direction and the peptide chains run crosswise. The latter are also cross-linked to one another (Fig. 5). The rigidity of bacterial cell wall is due to the presence of this polymer. Besides above mentioned three constituents, some other chemicals such as teichoic acid, protein polysaccharides and lipoproteins and lipopolysaccharides are also deposited on the cell wall.

The function of bacterial cell wall appears to be wholly mechanical, giving the cell its shape and rigidity. If the cell wall is removed by laboratory procedures, the naked protoplast becomes spherical. The rigidity of cell wall can be judged by the fact that it can withstand an osmotic pressure of about eight atmospheres per square centimeter.

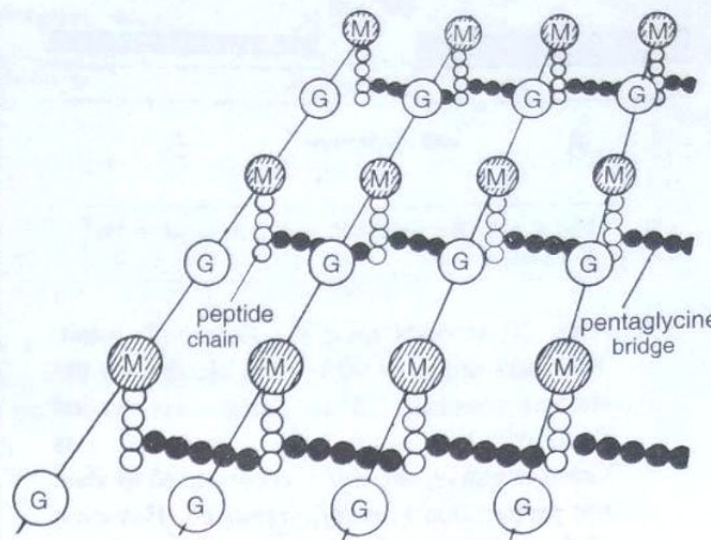


Fig. 5. Structure of peptidoglycan of bacteria : Chains of NAG and NAM are cross linked by short peptide chains.

[C] Staining of Bacteria (Gram's Stain)

The staining techniques have proved very useful in taxonomic grouping of bacteria. One of the important cytological features of bacteria is their reaction to a simple staining procedure discovered by a Danish physician, Christian Gram, in 1884. The procedure involves staining the cells first with crystal violet and then with iodine solution. The group of bacteria which retains the stain even after decolorization with alcohol is called **Gram-positive**, whereas those which lose stain after the treatment with alcohol are called **Gram-negative**. This differential reaction of two types of bacteria to crystal violet-iodine stain is due to different amount of lipids in their cell wall.

[I] Gram stain procedure

The Gram stain procedure includes following steps:

- (1) Prepare a smear of the bacterium on the slide and stain it with the **primary stain**, crystal violet. All bacteria take purple stain of crystal violet.
- (2) After 30 seconds rinse the slide with water and then put solution of potassium iodide and iodine (Gram's iodine solution) on the slide. This solution serves as a **mordant** by complexing with the crystal violet as well as with the cellular material. All bacteria take a deep violet or purple stain.

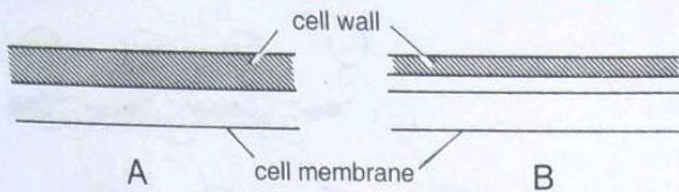


Fig. 6 A-B. Bacterial cell wall : A. Gram + ve, B Gram-ve.

- (3) After 20 seconds rinse the slide with water. Now add drops of 90% ethyl alcohol on the stained smear. This step is called **decolorization**. If the organism is Gram-negative, the dye complex used to stain the preparation is readily removed. However, if the organism is Gram-positive, the dye is not entirely removed; as such the organism retains its purple colour.
- (4) In the last step the slide is **counter stained with safranin**. Gram-negative organisms, which become colourless when washed with alcohol, readily take the stain of safranin and appear red. Since the Gram-positive bacteria are already stained with the intense crystal violet dye, they remain purple in colour.

The cell wall of Gram-positive bacteria has a relatively thick layer (20-80nm) of peptidoglycan. The peptidoglycan layer is closely attached to the outer surface of the cell membrane. Chemically, 60-90% of the cell walls of a Gram-positive bacterium is peptidoglycan. Hence, if peptidoglycan is digested from their cell wall, Gram-positive bacteria have only cell membrane and no cell wall. Thick cell walls of Gram-positive bacteria retain Gram stain (crystal violet-iodine dye) in their cytoplasm and the retention of Gram stain is directly linked to wall thickness. Physiological damage or ageing can make a Gram-positive cell wall leaky due to which the dye escapes. Such organisms can become Gram-variable or even Gram-negative as they age. Therefore, Gram staining is performed on cultures less than 24 hours old.

The cell wall of Gram-negative bacterium is thinner but more complex than that of a

Gram-positive bacterium. It contains only 10-20% peptidoglycan and the remainder are proteins, lipids and various polysaccharides. There is a wide periplasmic space between the inner surface of the cell wall and cell membrane. This space remains filled with sufficient concentration of toxins and enzymes which help in destroying substances that are harmful for the bacterium. These substances, however, do not harm the organisms that produce them. Gram-negative bacteria lose the stain of crystal violet-iodine dye when rinsed with alcohol because of their thin walls and relatively large quantities of lipoproteins and lipopolysaccharides in the walls (alcohol dissolves the lipids which allows the leakage of stain).

The fundamental differences between Gram-positive and Gram-negative bacteria are given in Table 1.

The differential staining property of the cell correlates surprisingly well with many other characteristics. For example, all spore (endospore) forming bacteria are Gram-positive and all polarly flagellated forms are Gram-negative; most of cocci (spherical bacteria) are Gram-positive and bacilli (rod-shaped bacteria) are Gram-negative. The Gram-negative bacteria are, as a rule more resistant to many antibiotics than Gram-positive.

[II] Acid-fast stain

Some bacteria have a covering of waxy or fatty material on their surface which makes their cell wall impermeable to most of the stains. At the same time if once they are stained, it is difficult to destain them. Their decolorization is not possible even with acid alcohol. Such bacteria are called **acid-fast** or **acid-alcohol fast** which refers to their ability to withstand decolorization with acidified ethanol. These organisms can, however, be stained with **Ziehl-Neelson stain**—a solution of basic fuchsin in phenol. The basic fuchsin binds strongly to the cytoplasm. Acid fast bacteria grow slowly because the high lipid contents in their cell wall (60%) impede entry of nutrients into cells. Besides this, the cell also has to expand

Table 1. Comparison between Gram-positive and Gram-negative bacteria.

Characteristics	Gram-positive bacteria	Gram-negative bacteria
1. Cell wall structure	Cell wall single layered and 150-200 Å thick	Cell wall triple layered and 75-120 Å thick
2. Chemical composition	Peptidoglycans account for about 80% of the cell wall and the rest are polysaccharides Teichoic acid present Low in lipids (1-4%) Highly responsive to triphenylmethane Resistant to alkalies and insoluble in 1% KOH solution	Peptidoglycans account for only about 3-12% of the cell wall. It is mainly composed of lipoproteins and lipid polysaccharides Teichoic acid absent High in lipids (11-22%) Show little response to triphenyl methane Show sensitivity to alkalies and soluble in 1% KOH solution
3. Rigidity of cell wall	Cell wall is very rigid due to high proportions of peptidoglycans	Cell wall is elastic due to plastic nature of lipoprotein-polysaccharide mixture
4. Susceptibility to penicillin	High susceptibility	Low susceptibility
5. Nutritional requirements	Relatively complex in many species	Relatively simple

large quantities of energy to synthesize energy. *Mycobacterium tuberculosis* (tuberculosis causing bacteria) and *M. leprae* (causes leprosy) are examples of acid-fast group.

[D] Cytoplasmic Membrane

Inner to cell wall, a semipermeable cytoplasmic membrane is present, which is about 75 Å thick. Chemically, it is composed of a double layer of phospholipid molecules (Fig. 7A, B). Phospholipids are of two types—hydrophobic and hydrophilic. The hydrophilic phospholipid molecules are present towards the outside and the hydrophobic molecules towards the inner side. Proteins are found embedded in the lipid bilayer. Prokaryotic membrane is characterized by the absence of sterols which perhaps account for the enormous resistance of these organisms to antibiotics. The chemical nature of cytoplasmic membrane in Gram-positive and Gram-negative bacteria is basically the same.

Cytoplasmic membrane is the bounding layer of the cytoplasm and it is the center of multifarious activities of the cell. Enzymes of various metabolic pathways, such as synthesis of lipopolysaccharides, phospholipids, teichoic acid, etc., are located in the cytoplasmic membrane. It also controls the entry of organic and inorganic nutrients in the cell.

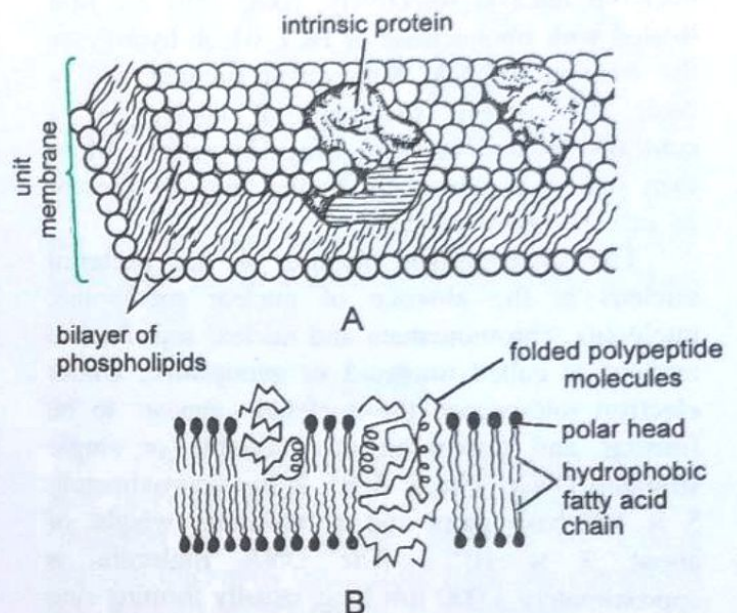


Fig. 7 A-B. Bacteria : Cytoplasmic membrane; A. Fluid mosaic model of cytoplasmic membrane, B. Lipid bilayer.

[E] Cytoplasm and Cytoplasmic Inclusions

The bacterial cytoplasm is a complex mixture of carbohydrates, proteins, lipids, minerals, nucleic acids and water. It stores organic material in the form of glycogen, volutin and poly-β-hydroxybutyrate. Some photosynthetic and non-

photosynthetic bacteria also accumulate sulphur and iron in their cytoplasm.

Besides fluid portion and storage particles, the bacterial cytoplasm also contains a **chromatic** or **nuclear area** and some other inclusions. The bacterial cell is devoid of mitochondria, endoplasmic reticulum, centrosome and Golgi bodies. Although a well organized chloroplast is absent in bacteria, the photosynthetic bacteria have chromatophores in their cytoplasm.

[I] Nuclear material

Basic dyes, which selectively stain chromatin of eukaryotic nucleus, stain most bacterial cells due to the abundance of ribosomes in their cytoplasm. It confers an unusually high nucleic acid stain on the cytoplasmic region. Therefore, to stain the bacterial nucleus selectively, fixed cells are first treated with ribonuclease or HCl, which hydrolyses the ribosomal RNA. Subsequent staining with a basic dye reveals the bacterial nucleus as a centrally located body of irregular outline. More than one such chromatin bodies may be present in actively growing cells.

The characteristic feature of the bacterial nucleus is the absence of nuclear membrane, nucleolus, chromonemata and nuclear sap. Such a nucleus is called **nucleoid** or **genophore**. Under electron microscope the nucleoid appears to be fibrillar and composed of a double or single stranded DNA (Fig. 8 A-B). It has approximately 5×10^9 base pairs and a molecular weight of about 3×10^9 . The DNA molecule is approximately 1,000 μm long, usually forming ring like structure or sometimes remains diffused

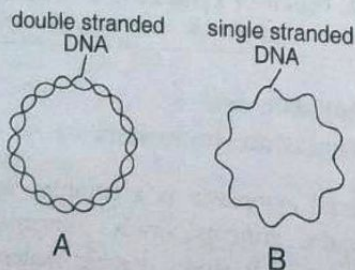


Fig. 8 A-B. Bacterial DNA : A. Double stranded, B. Single stranded.

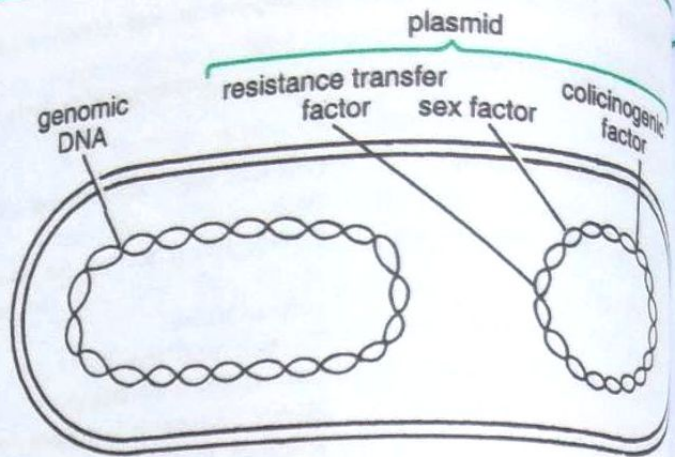


Fig. 9. Bacteria : Genome and plasmid (extrachromosomal factors) of a bacterial cell.

throughout the cytoplasm of the cell. The nucleoid of *Escherichia coli* has a central core of RNA, surrounded by 12-82 supercoilings of DNA. A few protein molecules are also associated with DNA.

The bacterial DNA is devoid of histones hence can not be compared with the chromosomes of eukaryotic cells. However, its double stranded DNA is usually referred to as **bacterial chromosome**.

[II] Plasmids

Bacterial cells also contain some extrachromosomal hereditary determinants which are either independent of bacterial chromosomes or are integrated with them (Fig. 9). Lederberg (1952) coined the term **plasmids** for such extrachromosomal hereditary determinants. They are rings of DNA of various sizes, in the range of 20-100 kbp (the whole bacterial cell is 4,000 kbp).

Each plasmid carries non-essential genes and has no role in viability and growth of bacteria. Hence they are also said to be **dispensable autonomous elements**. One good example of plasmids is the **F - factor** which determines the maleness in bacteria. It is an autonomous element, separate from the bacterial chromosome. It is transmitted from cell to cell by contact or by external agencies. A number of bacterial properties are now known which are determined by plasmid carried genes. Plasmids have been classified on the basis of the host properties which led to their

detection. The following nine type of plasmids are known :

- (i) **F-factor** (for fertility) plasmids,
- (ii) **R-factor** (for resistance) plasmids, (iii) **col-factor** (for colicinogeny) plasmids of the Gram-negative bacteria, (iv) plasmids conferring pathogenicity to mammals, (v) degradative plasmids of *Pseudomonas*, (vi) Mercury-resistance plasmids, (vii) tumor inducing plasmids of *Agrobacterium tumefaciens*, (viii) penicillinase plasmids of *Staphylococcus aureus*, and (ix) cryptic plasmids.

Plasmids are circular double stranded DNA molecules. Their molecular weight ranges between 5×10^7 and 7×10^7 (some cryptic plasmids are even smaller). Each plasmid may contain as many as 100 genes. The general features of plasmid replication are similar to that of chromosome replication, but plasmid seems to operate under its own genetically determined system of replication control. Thus the rate of plasmid replication may not necessarily be equal to that of chromosome replication.

[III] Ribosomes

Ribosomes are the sites of protein synthesis in the bacterial cell as in the eukaryotic cell. In eukaryotes ribosomes are frequently attached to the surface of the endoplasmic reticulum, but in bacteria, which do not have endoplasmic reticulum, ribosomes are free in the cytoplasm. Their number varies from 10,000 to 15,000 in a cell.

Bacterial ribosomes are of 75 S type (eukaryotic ribosomes are of 80 S type) and consist of two subunits. The sedimentation constant of the larger subunit is 50 S and that of the smaller subunit is 30 S. The former has two molecules of RNA and 35 amino acids and the latter has one molecule of RNA and 21 different amino acids.

In a young bacterium ribosomes may occur in groups of 4-6 or more. They are held together by a special RNA molecule, known as messenger RNA. These groups of ribosomes are known as **polyribosomes**.

[IV] Mesosomes

These are complex localized infoldings of the cytoplasmic membrane (Fig. 10). There may be

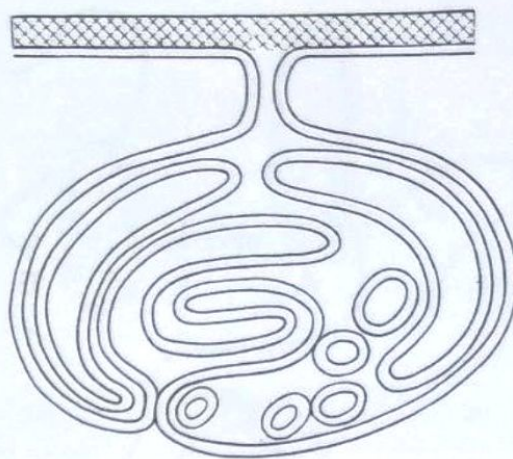


Fig. 10. Bacteria : Mesosome.

2-4 mesosomes in a cell and the number is usually higher in bacteria which show high respiratory activity, such as nitrifying bacteria. It has been suggested that the highly infolded membrane system of mesosomes perhaps serves to accommodate more centers of respiration. But the absence of enzymes like ATPase, dehydrogenase and cytochrome in mesosomes indicates that they are not the sites of respiration. They probably participate in the formation of transverse septum during cell division.

Flagella

The locomotion in bacteria is accomplished by thin hair-like appendages, called **flagella**. Each flagellum is a whip-like structure of almost uniform thickness, originating from the cytoplasm just beneath the cytoplasmic membrane.

Flagella are characteristic of all spiral bacteria and they also usually occur in bacillus bacteria. Coccus bacteria are, however, devoid of flagella and are non-motile. The following categories of bacteria are recognized on the basis of the presence or absence of flagella.

[I] Atrichous

These bacteria are devoid of flagella, hence they are nonmotile (Fig. 11A); e.g., *Lactobacillus*, *Pasteurella*.

[II] Trichous

These bacteria possess flagella, hence they are motile. The flagella are distributed over the surface

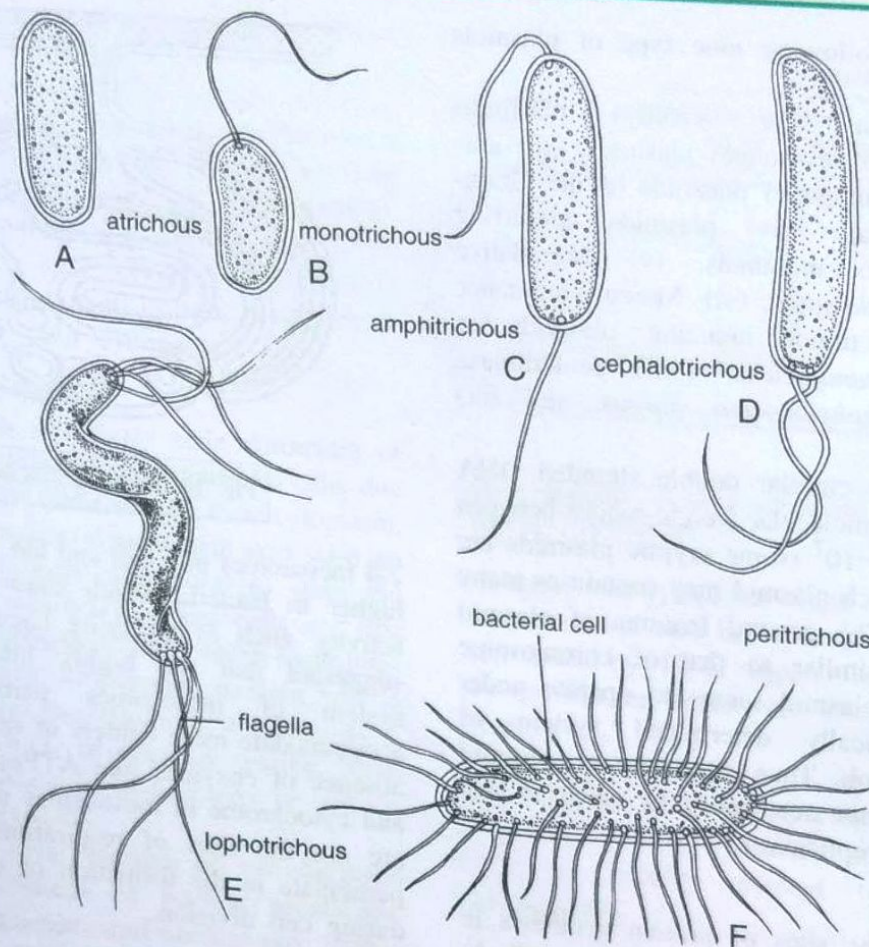


Fig. 11 A-F. Bacteria : Different types of flagellation.

of bacteria in a characteristic fashion. Their number, position and arrangement varies in different species. They may be restricted to the ends of the cell (polar flagella) or uniformly distributed all over the surface of the cell (peritrichous or non-polar flagella). Peritrichous flagellation is of wide occurrence among bacteria, whereas polarly flagellated bacteria form a rather homogeneous assemblage of rods and spirals. Various types of polar and peritrichous flagellation are as follows.

1. Polar flagellation. It is usually found in Gram-negative bacilli and spirilla. The following four types of polar flagellations are recognized.

(a) **Monotrichous.** If only a single flagellum is present at one end of the bacterial cell, it is known as **monotrichous** (Fig. 11B), e.g., *Vibrio cholerae*, *Pseudomonas*.

(b) **Amphitrichous.** When the bacterial cell possesses a single flagellum at each end, it is said to be **amphitrichous** (Fig. 11C). It is a relatively uncommon pattern of flagellation; e.g., *Nitrosomonas*, *Spirillum*.

(c) **Cephalotrichous.** When bacterial cell possesses two or more flagella at one end only, it is called **cephalotrichous** (Fig. 11D); e.g., *Pseudomonas fluorescens*.

(d) **Lophotrichous.** Those bacteria which have tufts of flagella at both the ends are called **lophotrichous**; e.g., *Spirillum volutans* (Fig. 11E).

2. Non-polar flagellation. In non-polar or **peritrichous** flagellation the flagella are evenly distributed throughout the surface of the cell (Fig. 11F); e.g., *Proteus vulgaris*, *Bacillus typhosus*, *Salmonella*, and *Clostridium*.

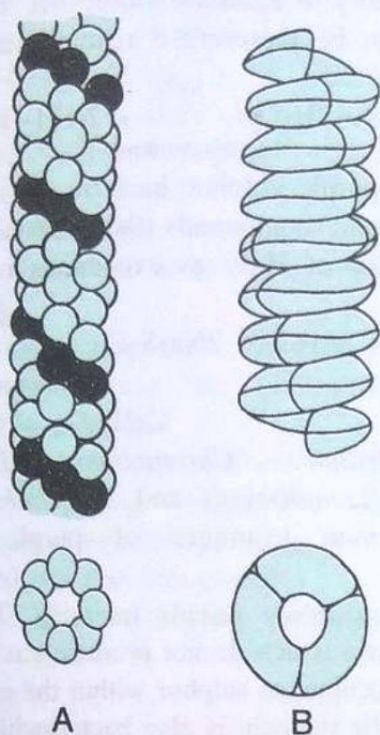


Fig. 12 A-B. Bacterial flagellum : Models showing probable helical arrangement of protein molecules; A. Flagellum, B. Pilus.

[III] Structure of the bacterial flagellum

The bacterial flagella are quite unlike those of other organisms. The flagella and cilia of higher protists and other organisms have an identical $9 + 2$ fibrillar structure. The molecular architecture of bacterial flagellum, derived on the basis of chemical analysis, reveals that it is composed of several chains of **flagellin** (a protein) molecules forming a more or less cylindrical filament (Fig. 12A). The diameter of a flagellin molecule is about 40 \AA and that of a flagellum is about $120\text{-}150 \text{ \AA}$. The orientation of flagellin molecules may vary in different species. The length of flagella ($4\text{-}5 \text{ }\mu\text{m}$ long) is usually more than that of bacterial cell.

The swimming ability of bacteria is due to the presence of flagella. If the cell is mechanically deflagellated, it becomes immotile. The locomotion is brought about by clockwise or anticlockwise spins of flagellum around its own 'axis'. In a liquid medium the speed of movement is more than $20 \text{ }\mu\text{m}$ per second.

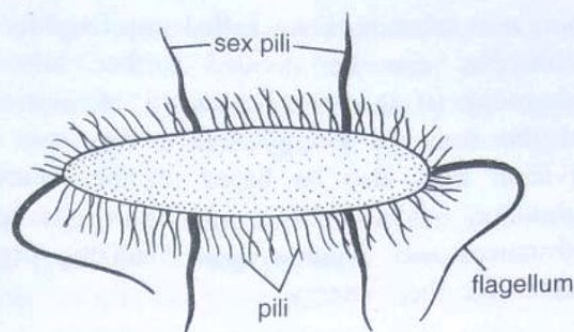


Fig. 13. Bacteria : Polar flagella and pili.

Pili or Fimbriae

Many Gram-negative bacteria possess minute rigid cylindrical appendages extending outwards from the cell wall. These are called **pili** or **fimbriae**. They are smaller than flagella and their length is approximately $2 \text{ }\mu\text{m}$ and diameter $30\text{-}50 \text{ \AA}$. In a bacterial cell there are usually several hundred pili (Fig. 13). Piliation is a variable structural characteristic, readily lost by mutation.

Pili are made up of a special type of protein, called **pilin**. Like flagellin molecules of flagella, pilin molecules are also assembled in helical chains around a central hollow core (Fig. 12B).

Pili are not the organelles of locomotion and their only known general function is to confer the property of adhesiveness. The piliated bacteria tend to stick to one another and produce coherent pellicles on the surface of unagitated liquid cultures. In addition to normal pili, there are some special type of pili, known as **sex pili**. There are usually 1-5 sex pili per cell. They are helpful in the transfer of genetic material, probably by acting as genetic tubes.

NUTRITION OF BACTERIA

On the basis of their mode of nutrition two groups of bacteria recognized are – **autotrophic bacteria**, and **heterotrophic bacteria**.

[A] Autotrophic Bacteria

Those bacteria which are capable of synthesizing their food by themselves from organic and

inorganic substances are called **autotrophic**. The autotrophs can be divided further into two subgroups: (i) **phototrophs**, and (ii) **chemotrophs**, on the basis of energy source they use. The division may also be based on the nature of substances oxidised; **lithotrophs** utilizing inorganic substances and **organotrophs** oxidizing organic matter for their energy.

[I] Photosynthetic bacteria

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



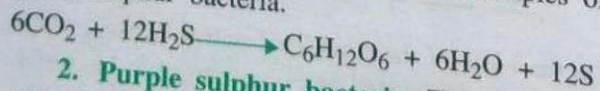
Where H_2A is an oxidizable compound and A is the corresponding oxidation product. In higher plants oxygen substitutes A, thus the hydrogen donor is water and the process produces free oxygen.



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

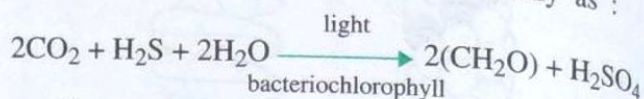
Photoautotrophic bacteria are of the following types:

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

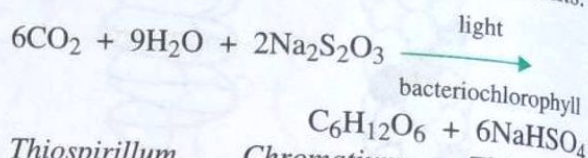


2. Purple sulphur bacteria. These are also autotrophs and their photosynthetic pigments are **bacteriochlorophyll a** and / or **b**. The reducing power is provided by H_2S which is oxidized anaerobically, via elemental sulphur, to sulphate. The sulphur is deposited in them intracellularly

(extracellularly in *Ectothiorhodospira*). The overall reaction can be represented schematically as :



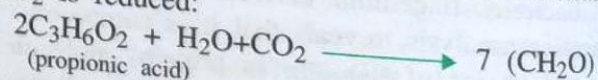
Some purple sulphur bacteria can use other reduced sulphur compounds (thiosulphate, sulphite, etc.) in place of H_2S as exogenous reductants.



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

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

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



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



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

[II] Chemosynthetic bacteria

Photosynthesizing bacteria represent only a small fraction of Schizophyta, but chemosynthetic or chemotrophic bacteria are most abundant and are important geochemical agents. These are non-photosynthetic but autotrophic bacteria. They derive energy from ammonia, nitrate, nitrite,

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ferrous iron, hydrogen sulphide and other inorganic compounds for the synthesis of their food. Thus chemoautotrophs grow in a strictly mineral medium in dark. They are characterized by (i) high specificity with respect to inorganic energy source, and (ii) frequent inability to use organic compounds as energy and carbon sources. Their growth is sometimes adversely affected by organic compounds.

On the basis of substrate specificity, chemoautotrophs can be classified into the following four groups.

1. Sulphur bacteria. These bacteria occur in sulphur containing terrestrial and aquatic environments. They derive energy by the oxidation of reduced sulphur compounds.

The sulphur bacterium, *Beggiatoa*, oxidizes hydrogen sulphide to elemental sulphur:

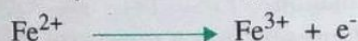


The elemental sulphur is deposited in the filaments as minute granules.

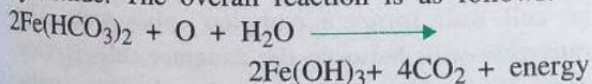
Another sulphur bacterium, *Thiobacillus thiooxidans*, utilizes free sulphur and produces sulphuric acid:



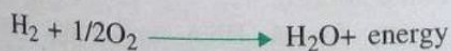
2. Iron bacteria. These bacteria (e.g., *Sphaerotilus*, *Gallionella*, *Ferrobacillus* and *Leptothrix*) form natural colonies in fresh water ponds and springs with high contents of reduced sulphur salts. They oxidize ferrous compounds into ferric forms and the energy released in this process is utilized in the synthesis of organic compounds.



The ferric iron is deposited as insoluble ferric hydroxide. The overall reaction is as follows:



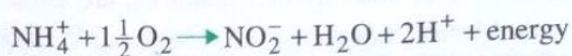
3. Hydrogen bacteria. Many species of chemoautotrophic bacteria have the ability to grow with molecular hydrogen. They oxidize molecular hydrogen and in this process water and energy are obtained.



The common H_2 -oxidising bacteria are *Pseudomonas saccharophila*, *P. facilis*, *Alcaligenes* (MICROBIOLOGY)

eutrophs, *A. paradoxus*, *Nocardia opaca* and *Paracoccus denitrificans* (previously all these bacteria were placed in a separate genus *Hydrogenomonas*, but now they are included in a series of genera).

4. Nitrifying bacteria. These are soil-borne obligate autotrophs incapable of growing in the absence of specific inorganic energy source. They oxidize ammonia to nitrate and help greatly in the economy of nitrogen in nature. The nitrification process is carried out in two steps, each step by a very specialized groups of bacteria. The first step involves oxidation of ammonia or ammonium ions to nitrate with the help of *Nitrosomonas*.



This oxidation is the sole source of energy for *Nitrosomonas*.

The second step of nitrification involves oxidation of nitrite to nitrate.



Here the oxidation is carried out by *Nitrobacter* and oxidation of nitrite is the only source of energy for the bacterium.

[B] Heterotrophic Bacteria

These bacteria obtain their readymade food from any organic source. They can be distinguished into three major nutritional categories.

[I] Parasitic bacteria

Those bacteria which feed on living organisms are known as **parasitic bacteria**. The organism from which parasitic bacteria obtain their food is known as **host**. Some parasitic bacteria are known to cause diseases in plants (e.g., citrus canker) and animals (e.g., pneumonia, typhoid), and such bacteria are called **pathogenic bacteria**.

[II] Saprophytic bacteria

These bacteria grow on dead and decaying organic matter. They obtain their food by decomposing the complex organic molecules into simple inorganic constituents. The decomposition of carbohydrates and proteins by saprophytic bacteria is technically known as **fermentation** and **putrification** respectively.

Breakdown of sugars by yeast is a common example of fermentation. In this process CO_2 is released. Similarly, lactic acid fermentation of milk is carried out by lactic acid bacteria (*Lactobacillus*). In putrefaction reduction of protein to peptone, polypeptide, peptides and amino acids takes place by enzymes secreted by anaerobic bacteria. This process does not require oxygen, but many saprophytic bacteria which breakdown amino acids into methane, CO_2 , NH_3 , H_2 and N_2 do require oxygen.

[III] Symbiotic bacteria

Those bacteria, which grow in close (beneficial) association with other living organisms, are known as **symbiotic bacteria**. In terms of their association with the host, symbiotic bacteria may be **ectosymbionts** (when bacteria live on the surface of the host) or **endosymbionts** (when bacteria live inside the host tissue). The bacteria inhabiting intestine of man and animals are good examples of symbiotic bacteria. The enzymes secreted by these bacteria are helpful in the digestion of cellulose and in return they obtain their food from the host. Similarly, root nodule bacteria (*Rhizobium*), present in the roots of leguminous plants, fix atmospheric nitrogen to augment their nitrogen supply and in return the plants provide them shelter and carbohydrates.

REPRODUCTION

Bacteria reproduce mainly by asexual method and therefore they have a dominant **haploid phase** in their life-cycle. They do not have sex organs or gametes but they definitely show genetic recombination, i.e., exchange of genetic material.

ASEXUAL REPRODUCTION

Asexual reproduction in bacteria takes place by the following methods.

[A] Binary Fission

It is the simplest and most common method of multiplication in bacteria. Under favourable

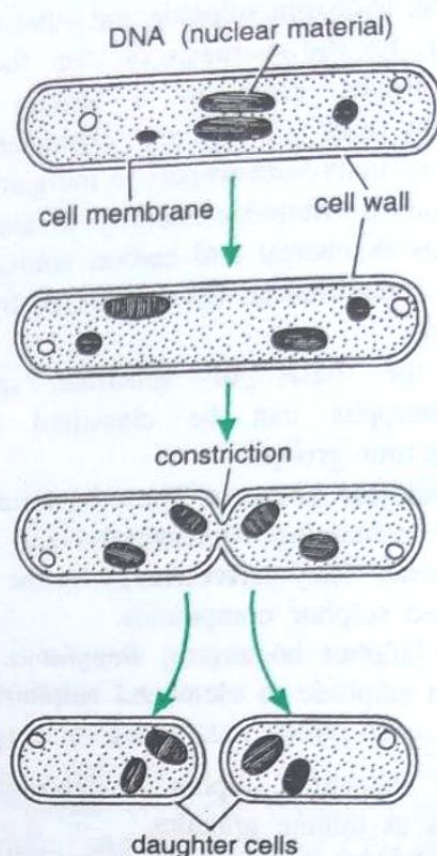


Fig. 14. Bacteria : Binary fission.

conditions the cell divides into two daughter cells by a transverse wall. During fission the cell elongates and there is division of the nuclear material. In Gram-negative bacteria it is followed by the development of a simple median constriction that finally results in complete separation of two daughter cells (without cross wall formation; Fig. 14). In Gram-negative bacteria, however, first a transverse cell membrane is laid down between the two nucleoids of a dividing cell and then the centripetal growth of the cell wall forms a complete cross wall. The transverse wall between the daughter cells is very often incomplete and then the daughter cells remain connected at the transverse membrane. When the planes of the successive divisions are parallel, long chains may be formed and if perpendicular to one another sheets of packets of the cells are formed.

In binary fission, DNA replication precedes septum formation. The two resulting cells are mirror images of one another. Analyses of cell wall components of dividing cells indicate that

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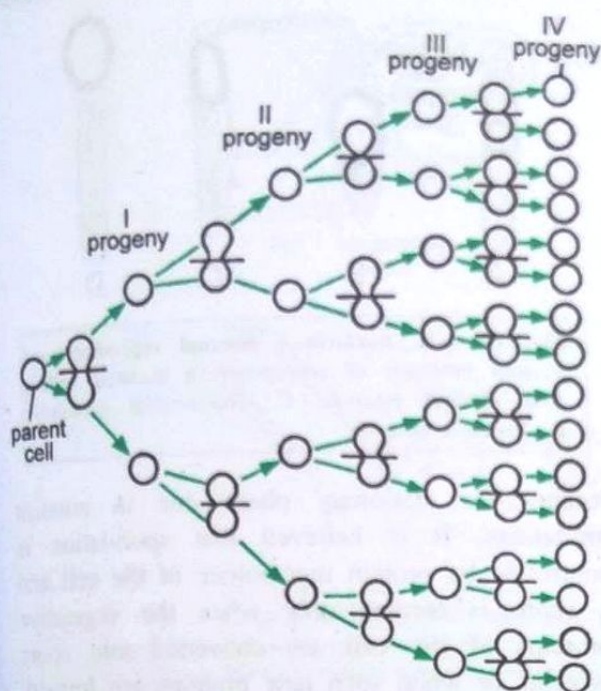


Fig. 15. Bacteria : Rapid multiplication of bacterial cell by binary fission.

the chemical constituents of the original 'mother' cell wall are equally shared in the cell walls of two resulting 'daughter' cells.

Process of binary fission is very rapid (Fig. 15). Bacterial cells many undergo fission every 20 to 30 minutes, and under favourable conditions, within 6 hours, approximately 2,50,000 cells may be formed from a single bacterium. But in nature the essential nutrients are generally a limiting factor and moreover the accumulated waste products of bacteria also restrict their rapid growth. Normally, growth and multiplication is very fast in the early stages, reaching at the optimum and then declining rapidly.

Fission is different from normal mitotic division as it does not involve spindle formation during the division of nuclear material. It is not definitely known whether in fission the genetic material is equally distributed between the daughter cells. Fission is characteristic of all types of bacteria and that is why they have been placed in the class Schizomycetes (*Schizo* = split or cut, *mycetes* = fungi; split, cut or fission fungi).

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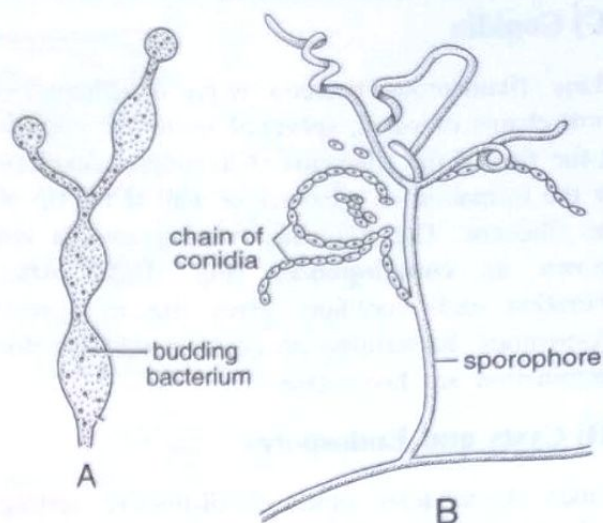


Fig. 16 A-B. Bacteria : Asexual reproduction; A. Budding in *Rhodocyclium*, B. Conidia formation in *Streptomyces*.

[B] Budding

In this type of multiplication the bacterial cell gives out many outgrowths. These outgrowths are filled with cytoplasm and other cell organelles also move into them. These outgrowths, called **buds**, eventually get detached from the parent cell by a constriction and each grows into a new bacterial cell (Fig. 16A).

Budding differs from binary fission in many respects. During binary fission, symmetry of the cell with respect to the longitudinal and transverse axes is maintained throughout the entire process of division. In contrast to binary fission, in budding bacteria most of the new cell wall components are used in the synthesis of the bud instead of being divided equally between the two progeny cells (as in binary fission). In budding the mother cell retains its identity even after the formation of a new progeny. On the contrary in binary fission the mother cell loses its identity after the formation of daughter cells. Consequently, a primitive 'ageing' process occurs in the budding bacteria which is absent in those dividing by binary fission.

Species of *Hyphomicrobium*, *Hyphomonas*, *Pedomicrobium*, *Ancalomicrobium*, *Prosthecomicrobium*, *Labrys* and *Stella* are common examples of budding bacteria.

[C] Conidia

Many filamentous bacteria (e.g., *Streptomyces*) form chains of small, spherical spore-like conidia at the tips of the filaments. A conidium develops by the formation of a transverse wall at the tip of the filament. The filaments bearing conidia are known as **conidiophores** (Fig. 16B). After liberation each conidium gives rise to a new filamentous bacterium, provided conditions for germination are favourable.

[D] Cysts and Endospores

Genus *Azotobacter* produces distinctive resting cells, known as **cysts**. The cysts are formed by the deposition of additional layers around the existing cell wall. Thus the entire contents of the cell are involved in the formation of a cyst. The cysts are resistant to desiccation but not to heat.

Endospores are intracellular resting cells, and they differ from cysts in the sense that in their formation only a part of the original cell is utilized. With a few exceptions, endospore formation is restricted to some large Gram-positive bacilli. Among cocci and spirilla, endospores are formed only in few species, e.g., *Sarcina lutea*, *S. ureae* and *Desulfovibrio desulfuricans*. Typically one endospore is formed in a vegetative cell. The cell producing endospore is termed as sporangium. The shape, size and position of the endospore within the cell may vary in different species. Endospores are oval or spherical and their diameter may be larger or smaller than that of the vegetative cell. Their position within the sporangium may be central, subterminal or terminal (Fig. 17 A-D).

Most of the endospore forming bacteria are soil borne, and few are pathogenic to insects and vertebrates. Pathogenic spore forming bacteria cause disease by toxin production.

Lactobacillus, *Listeria*, *Erysipelothrix*, *Kurthia*, *Renibacterium*, *Arcanobacterium*, *Microbacterium* and *Cellulomonas* are common non-sporing bacteria.

[I] Formation of endospores

The ability to produce endospores is normally never expressed during the active phase of growth. It is expressed only when the vegetative growth

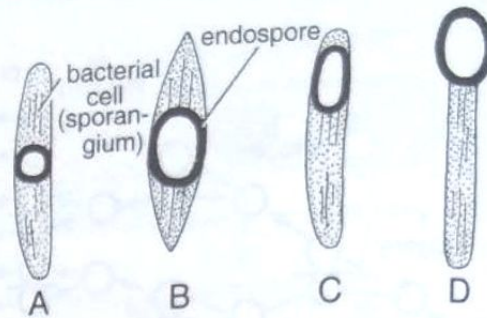


Fig. 17 A-D. Bacteria : Asexual reproduction; Various positions of endospores in bacterial cells. A-B. Central position, C. Sub-terminal position, D. Terminal position.

reaches the stationary phase due to nutrient limitations. It is believed that sporulation is controlled by protein metabolism of the cell and a spore is formed only when the vegetative proteins of the cell are converted into spore proteins or when such new proteins are formed.

At the onset of the sporulation, the two nucleoids present in a cell coalesce to form an axial chromatin thread (Fig. 18 A-B). Then a transverse septum is formed near one pole of the cell, which separates the cytoplasm and DNA of the smaller daughter cell from that of the larger cell (Fig. 18 C-E). The smaller cell, which is destined to form spore, is known as **spore primordium**. The septum formation is not followed by the development of transverse wall (as in normal cell division), instead the membrane of the larger cell rapidly grows around the spore primordium. The primordium gradually increases in size and within few hours of sporulation there is enormous growth in its size and it becomes opaque and highly refractive. The entire process of sporulation is completed in 18-20 hours. After the maturation of endospore, the sporangium (i.e., the bacterial cell in which endospore is formed) remains alive only for a short period. The endospore is liberated due to autolysis of the sporangium (Fig. 18 F-G).

[II] Structure of endospore

The endospore consists of a central protoplast, the core, mainly composed of DNA, tRNAs, ribosomes, enzymes and accessory factors. It is enclosed by a thin membrane, variously called **core membrane**, **germ cell membrane** or **inner membrane**. The multilayered region around the

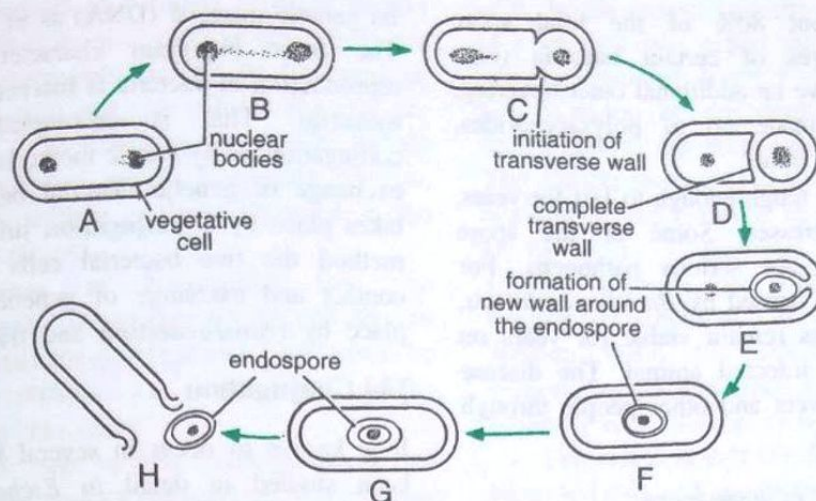


Fig. 18 A-H. Bacteria : Asexual reproduction; Diagrammatic representation of endospore formation.

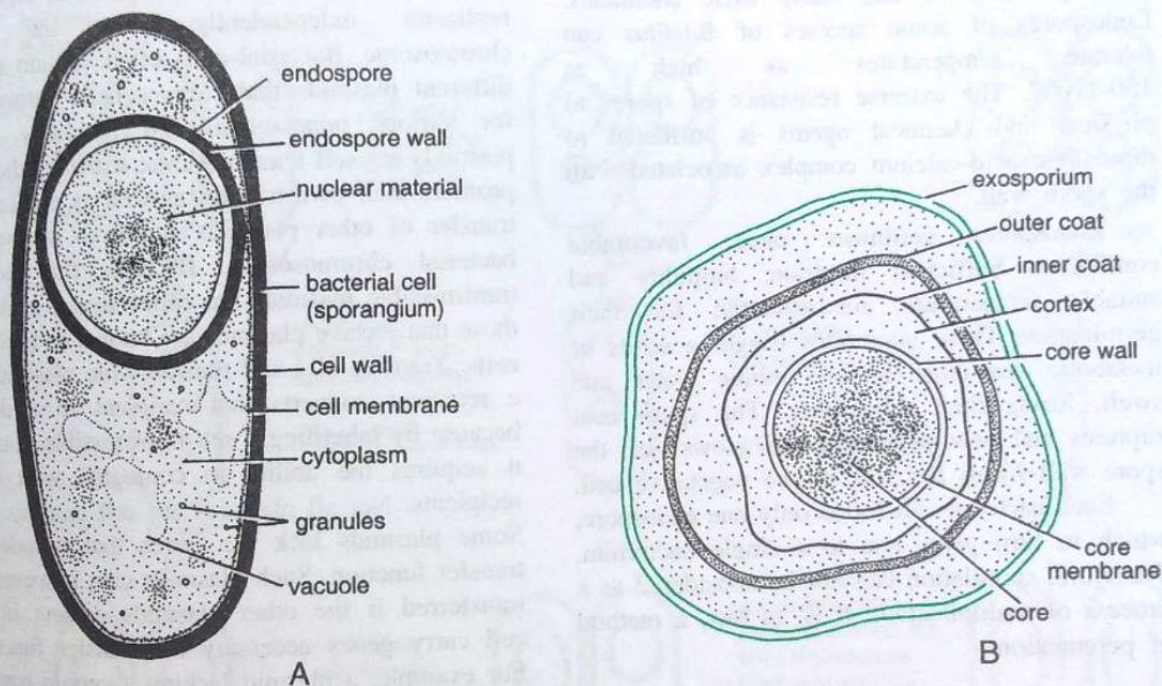


Fig. 19 A-B. Bacteria : Asexual reproduction; A. A bacterial cell with endospore, B. Transverse section of an endospore of *Bacillus* (diagrammatic representation).

core is known as **cortex**, and it is largely composed of calcium—dipicolinic acid—peptidoglycan complex. Outer to the cortex there is an electron dense layer, called **spore coat**,

which is differentiated into an **inner coat** and an **outer coat** (Fig. 19 A, B). The spore coat is mainly composed of proteins and accounts for 30-60 per cent of the dry weight of the spore. It

also contains about 80% of the total spore proteins. Endospores of certain bacteria (e.g., *Bacillus cereus*) have an additional outer covering, the **exosporium**, made up of polysaccharides, proteins and some lipids.

Endospores are tough enough to last for years, enduring many stresses. Some of the spore producing bacteria are serious pathogens. For example, anthrax is caused by *Bacillus anthracis*, and its tough spores remain viable for years on the wool from an infected animal. The disease gets passed to weavers and other people through cuts on their hands.

[III] Germination of endospore

Free endospores do not show any detectable metabolism, but they retain the potential capacity to germinate and develop into vegetative cells. They are highly resistant to heat, ultraviolet and ionizing radiations and many toxic chemicals. Endospores of some species of *Bacillus* can tolerate temperatures as high as 150-170°C. The extreme resistance of spores to physical and chemical agents is attributed to dipicolinic acid-calcium complex associated with the spore wall.

Endospores germinate under favourable conditions. Sufficient nutrition, humidity and suitable temperature are essential for their germination. These conditions trigger a series of metabolic reactions. Spores imbibe water and swell, losing their refractivity. The spore coat ruptures and new vegetative cell grows out; the spore wall forms the wall of the vegetative cell.

Each bacterial cell forms only one endospore, which in turn gives rise to a single bacterium. Therefore, sporulation can not be considered as a process of multiplication. It is, in fact, a method of perennation.

GENETIC RECOMBINATION (SEXUAL REPRODUCTION)

Sexuality in bacteria was first demonstrated by Tatum and Lederberg (1947) in *Escherichia coli*. In bacteria sexual reproduction does not involve fusion of gametes as in other organisms but each heritable character is determined by a portion of

its genetic material (DNA) as in other organisms. The most important characteristic of sexual reproduction in bacteria is **interchange of genetic material**. This is accomplished either by conjugation or by exotic methods. In conjugation, exchange of genetic material between two cells takes place by a **conjugation tube**, but in exotic method the two bacterial cells never come in contact and exchange of genetic material takes place by **transformation** and **transduction**.

[A] Conjugation

It is known to occur in several bacteria but has been studied in detail in *Escherichia coli* by Lederberg (in Wisconsin), Hayes (in London) and Woolman (in Paris).

Conjugation is a mechanism of DNA exchange mediated by plasmids. As mentioned earlier, a plasmid is a circular piece of DNA that replicates independently from the cell's chromosome. Bacterial cells often contain several different plasmids that carry genetic information for various nonessential cell functions. Some plasmids are **self-transmissible**; it means they can promote their own transfer as well as mediate the transfer of other plasmids and even portions of bacterial chromosomes. Bacteria that contain transmissible plasmids are called **donor cells** and those that receive plasmids are known as **recipient cells**. Transfer of a self-transmissible plasmid into a recipient converts that recipient to a donor, because by inheriting a self-transmissible plasmid, it acquires the ability to conjugate with other recipients. **Not all plasmids are self-transmissible**. Some plasmids lack the genes that encode the transfer function. Such plasmids can, however, be transferred if the other plasmids present in the cell carry genes necessary for transfer function. For example, a plasmid lacking a certain transfer function, such as sex pili, can still transfer to a recipient cell, provided, the donor cell has another plasmid that encodes pili. This 'helper' plasmid thus is capable of mobilizing another plasmid for transfer.

Analogous to sexual transfer in higher eukaryotes, donor bacteria are often called **male cells**, while recipient cells are called **female**. Most of the experimental work on conjugation has been

done on the transfer of **F-factor** (fertility factor) plasmid in *Escherichia coli*. Cells in which F-factor is present, is represented by F^+ (it functions as donor or male cell) and cell without F-factor are denoted by F^- (it functions as recipient or female cell). F^- factor has genes that code for the components as well as proteins involved in the biogenesis of sex pili.

The process of conjugation has two characteristics—(i) it requires **direct cell to cell contact**, and (ii) the **conjugating cells must be of opposite strains**, i.e., male and female (F^+ and F^- in this case).

The first step in the conjugation is the establishment of contact between cells of opposite strains. It may be either by direct cell to cell contact or mediated by sex pili. It results in the formation of a channel between the two mating

cells. In the second step the plasmid DNA breaks at a specific site, called the **Origin of transfer**, often abbreviated as **ori T** (Fig. 20A). From this site 5'-region of the double stranded DNA is transferred to the recipient cell via the channel. The transfer process is accompanied by immediate copying of the template strand in the donor cell in the 5' to 3' direction restoring the double stranded molecule (Fig. 20B). In the recipient cell, the transfer strand is also converted to a double stranded DNA (Fig. 20C). At the end of the process the plasmid DNA strand in the donor and recipient cells assume closed circular form.

The recipient cell (F^- cell) after receiving the plasmid DNA or F-factor from donor cell becomes F^+ cell (Fig. 20C). In some cells the F-factor integrates into the bacterial chromosome (Fig. 20 D-E); such cells are called **high-**

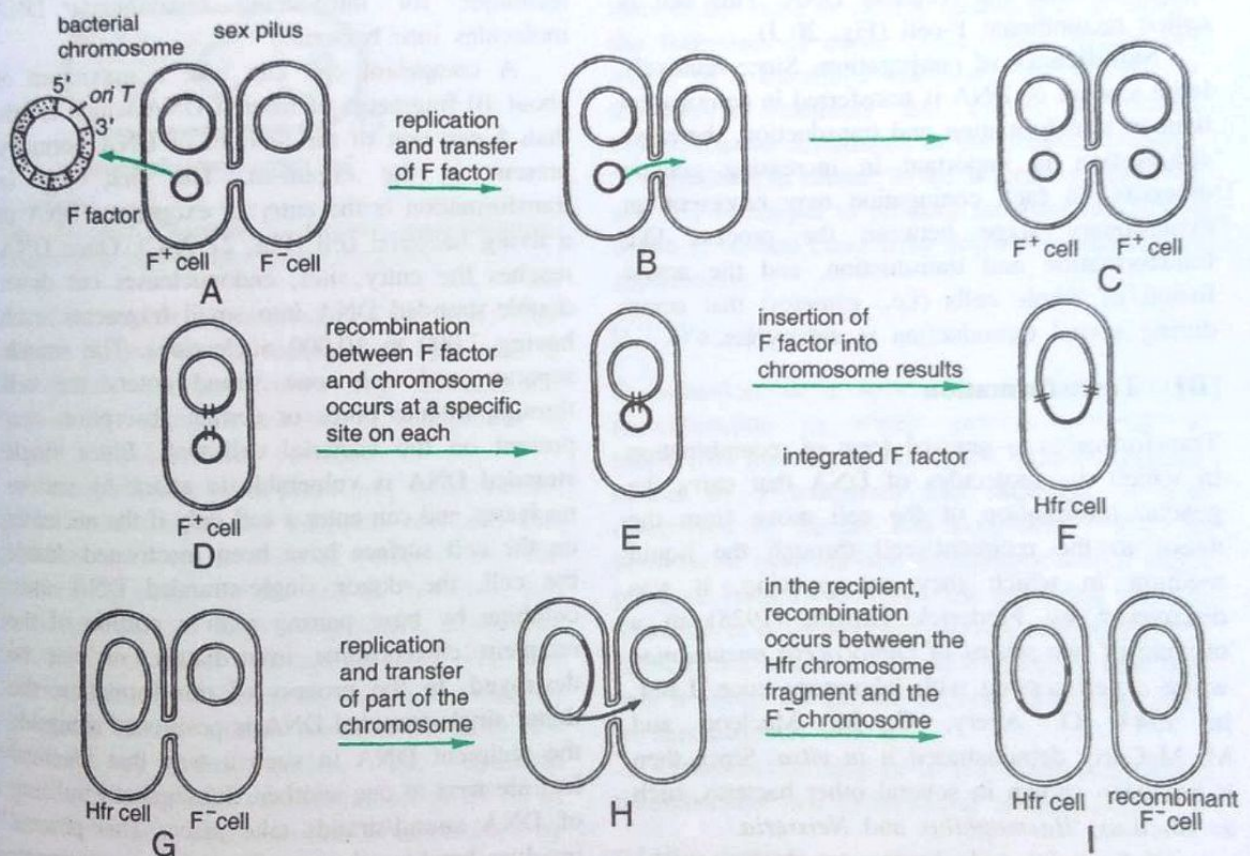


Fig. 20 A-I. Conjugation in bacteria : A-C. Transfer of F-factor from donor (F^+) to recipient (F^-) changes F^- into F^+ . D-F. Formation of Hfr cell. G-I. Formation of recombinant F^- cell when Hfr cell transfers part of its chromosome into F^- cell.

frequency recombinant (Hfr) cells. In the bacterial chromosome there are a few preferred sites where most of the integration can take place. The integration is not absolutely stable; in a population of cells, an equilibrium exists between bacteria with integrated F-plasmid (Hfr) and cells where the F-plasmid is not part of bacterial chromosome (F^- cells).

When conjugation occurs between an Hfr cell and an F^- cell, the Hfr cell's chromosome replicates and a parental strand of the chromosome is transferred to the recipient cell. The replication of the Hfr chromosome begins in the middle of the integrated F-factor and a small piece of F-factor makes its way into the recipient cell (Fig. 20 G-H). Usually the chromosome breaks up before it is completely transferred to the recipient cell. Once within the recipient cell, this piece of donor's chromosome integrates with the recipient DNA. This cell is called recombinant F^- -cell (Fig. 20 I).

Significance of conjugation. Since relatively large amount of DNA is transferred in conjugation than in transformation and transduction, therefore conjugation is important in increasing genetic diversity. In fact, conjugation may represent an evolutionary stage between the process like transformation and transduction, and the actual fusion of whole cells (i.e., gametes) that occur during sexual reproduction in eukaryotes.

[B] Transformation

Transformation is unusual form of recombination in which the molecules of DNA that carry the genetic information of the cell move from the donor to the recipient cell through the liquid medium in which they are growing. It was discovered by Frederick Griffith (1928) in a mixture of two strains of *Diplococcus pneumoniae* while experimenting with laboratory mice. Later, in 1944, O. Avery, C. M. Macleod and M. McCarty demonstrated it *in vitro*. Since then it has been shown in several other bacteria, such as *Bacillus*, *Haemophilus* and *Neisseria*.

All bacterial cells having an ability to take up DNA from the environment are said to be **competent**. Transformation competence is a state

of bacterial cell during which the usually rigid cell wall can transport a relatively large DNA macromolecule. This is a highly unusual process for bacteria because they normally lack the ability to transport large macromolecules across the cell wall and through the cytoplasmic membrane. In some bacteria (e.g., *Haemophilus*, *Streptococcus*, *Neisseria*, etc.) competence is expressed during a certain stage of cell division during which the reforming cell wall can allow passage of DNA. The above bacterial genera possess **natural competence** because their cells do not require any special treatment to increase their ability to take up DNA. Not all bacteria, however, are naturally competent to take up DNA; treatment of such cells with calcium or rubidium chloride brings about changes in their cell wall and they become competent. This form of competence is known as **artificial competence** and is a widely used technique for introducing recombinant DNA molecules into bacteria.

A competent cell can take a maximum of about 10 fragments of foreign DNA which is less than 5 per cent of the amount of DNA normally present in the organism. The first step in transformation is the entry of exogenous DNA in a living bacterial cell (Fig. 21 A-C). Once DNA reaches the entry sites, endonucleases cut down double-stranded DNA into small fragments, each having 7,000 to 10,000 nucleotides. The strands separate and only one strand enters the cell through minute pores or certain absorption sites present on the bacterial cell wall. Since single stranded DNA is vulnerable to attack by various nucleases and can enter a cell only if the nucleases on the cell surface have been inactivated. Inside the cell, the donor single-stranded DNA must combine by base pairing with a portion of the recipient chromosome immediately, or else be destroyed. In the process of transformation, the donor single-stranded DNA is positioned alongside the recipient DNA in such a way that identical loci are next to one another. Subsequently splicing of DNA strand/strands take place. This process involves breaking the strand, removing a segment, inserting a new segment and attaching the ends. This DNA segment (donor DNA) now becomes a

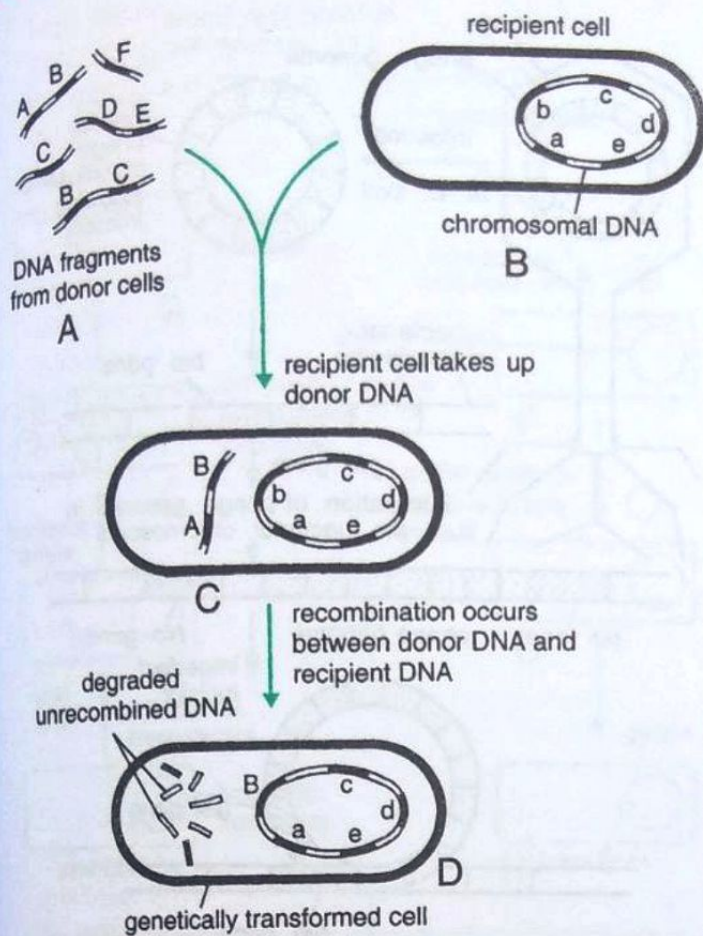


Fig. 21 A-D. Bacteria : Genetic recombination; Diagrammatic representation of transformation.

part of the recipient chromosome (Fig. 21D). The leftover portion of the recipient chromosome is subsequently broken down by intracellular enzymes. As such the number of nucleotides in the cell's DNA remains constant.

Transformation takes place only under certain special condition of the bacterial cell and this condition is known as **state of competence**. The transforming ability of the cell is, however, lost in the presence of high temperature, ultraviolet radiations, chemical mutagens, etc. On the contrary, increase in the concentration of inorganic phosphates in the culture medium increases the frequency of transformation. The optimum temperature range for transformation is 29-32°C. The effectiveness of the process is determined by many factors such as temperature, composition of the nutrient medium and physiological state of the recipient cell. In nature the frequency of

transformation in different bacteria varies from 0.047 to 0.0004%.

The basic differences between transformation and conjugation are as follows.

- (1) In transformation exchange of genetic material takes place between the members of the same species, whereas conjugation occurs between the members of two different genera; e.g., *Escherichia* and *Shigella*, *Salmonella* and *Serratia*, *Salmonella* and *Escherichia*.
- (2) Genetic recombinants obtained through conjugation are temporary, whereas genetic characters incorporated in a transformed cell are permanent.

Significance of transformation. Transformation has been studied only in the laboratory. In nature it may occur in certain environments where bacteria live in large numbers. In such environments some bacteria die and lyse and live ones of the same or closely related species absorb the fragments of donor DNA which escape the action of extracellular nucleases. As such, it is difficult to estimate the degree to which transformation contributes to the genetic diversity of organisms in nature. In the laboratory scientists use this technique to produce recombinant DNA, which in several cases have important commercial applications.

[C] Transduction

Transduction is a special method of genetic recombination in which genetic material is transferred from the donor to the recipient cell by means of a temperate bacteriophage. It was discovered by Zinder and Lederberg (1952) in *Salmonella typhimurium*. In transduction, a small piece of bacterial chromosome (carrying specific character x) is incorporated into an attacking phage particle, and when this particle infects a new host cell, it injects the genetic material (with new piece of chromosome) into it.

Transduction may be of two types—**specialized transduction** and **generalized transduction**.

[I] Specialized transduction

Specialized transduction requires incorporation of the viral DNA into the bacterial chromosome, and

it is therefore carried out only by lysogenic viruses. It occurs following formation of prophage, whereby the viral DNA integrates at a specific site in the bacterial chromosome. For example, lambda (λ) phage inserts into the *E. coli* chromosome between the *gal* gene (which controls galactose use) and the *bio* gene (which controls biotin synthesis). Although the prophage replicates for many generations with the bacterial chromosome, however, it can excise and initiate a virulent cycle. In most cases, the new phage particles released contain only phage genes. Occasionally, excision of the prophage is imprecise, resulting in removal of some DNA on either left, right or both sides of the site of integration. This excised DNA with some host genes (called transduced genes) is now part of the viral genome (Fig. 22). It replicates and is packaged into every viral particle and when such a phage infects a new host cell, the transduced genes become a part of the genome of the host cell. Thus specialized transduction is phage mediated transfer of genes that are near the chromosomal attachment site of the lysogenic phage. The main steps of specialized transduction are given below.

- (1) The bacteriophage gets attached to the bacterial cell on the receptor site and the nucleic acid of phage particle is transferred to the cytoplasm of the bacterial cell.
- (2) The nucleic acid of the phage particle is coded for the synthesis of certain specific proteins in the bacterial cell. These proteins are known as **repressor proteins**, and their function is to check the synthesis of phage particles in the bacterial cell. The phage DNA occurs in the bacterial cell in the form of small fragments, known as **prophage**. These fragments are either free in the cytoplasm or are attached to the chromosome. A bacterial cell with prophage is **lysogenic** (Fig. 23 A), and it may remain lysogenic for several generations. During this period the phage DNA keeps on dividing along with bacterial chromosome. A stage comes when synthesis of the repressor proteins stops in the bacterial cell and synthesis of phage components starts.

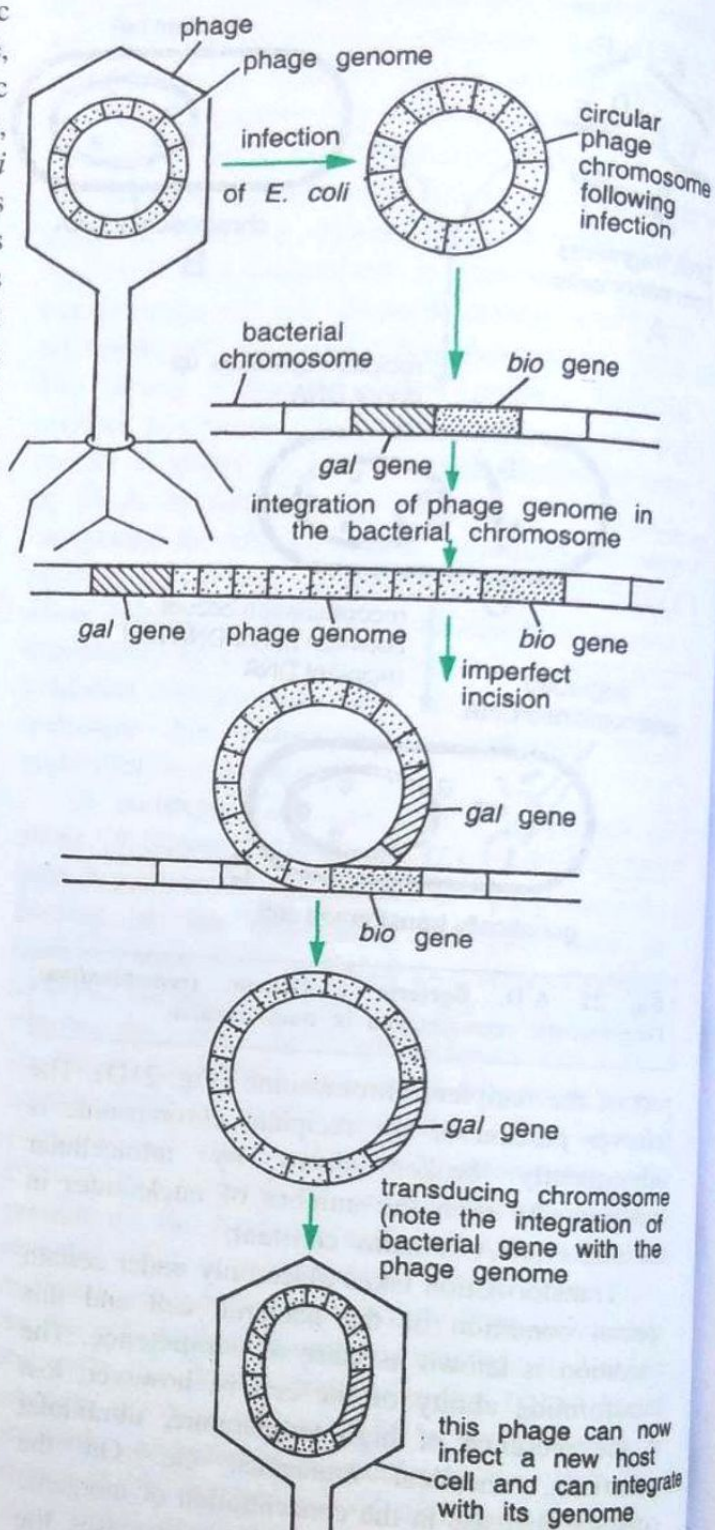


Fig. 22. Bacteria : Diagram showing imperfect incision of phage genome in specialized transduction.

- (3) Under such conditions the phage DNA, which was so far attached to the bacterial chromosome, separates and starts synthesizing phage proteins (Fig. 23 B, C).

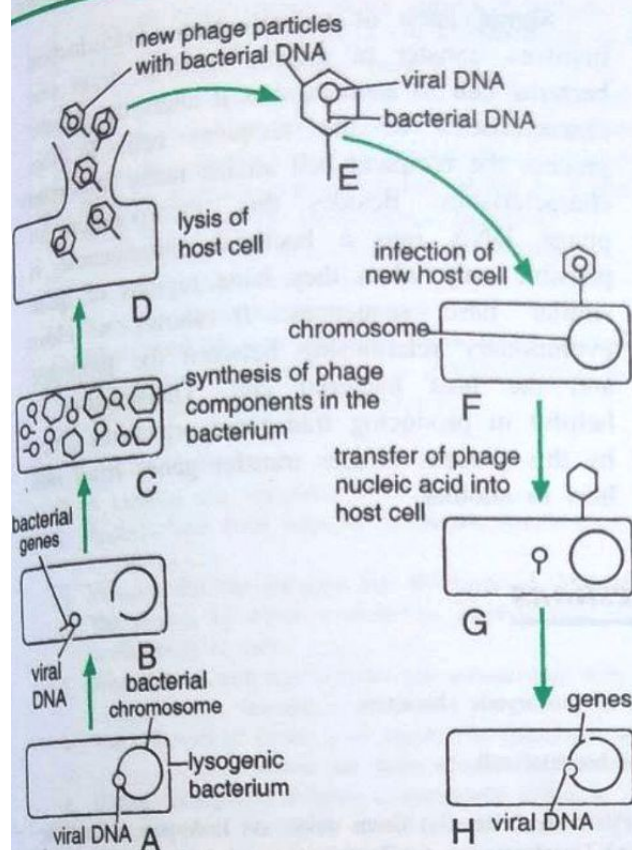


Fig. 23 A-H. Bacteria : Genetic recombination; Diagrammatic representation of specialized transduction.

- (4) When phage DNA breaks off the bacterial chromosome, few genes of the bacterium remain attached to it. These genes keep on replicating along with the phage DNA and become a part and parcel of the phage particle (Fig. 23 D-E). Such phage particles when infect any other bacterial cell, then the bacterial genes present in phage particles are incorporated in the chromosome of the new bacterial cell (i.e., recombinant cell, Fig. 23 F-H). Thus the recombinant cell, besides its own chromosome, also contains few genes of the parent bacterial cell.

[III] Generalized transduction

It is a more common process than the specialized transduction. It involves only those prophage particles which are present in the cytoplasm of

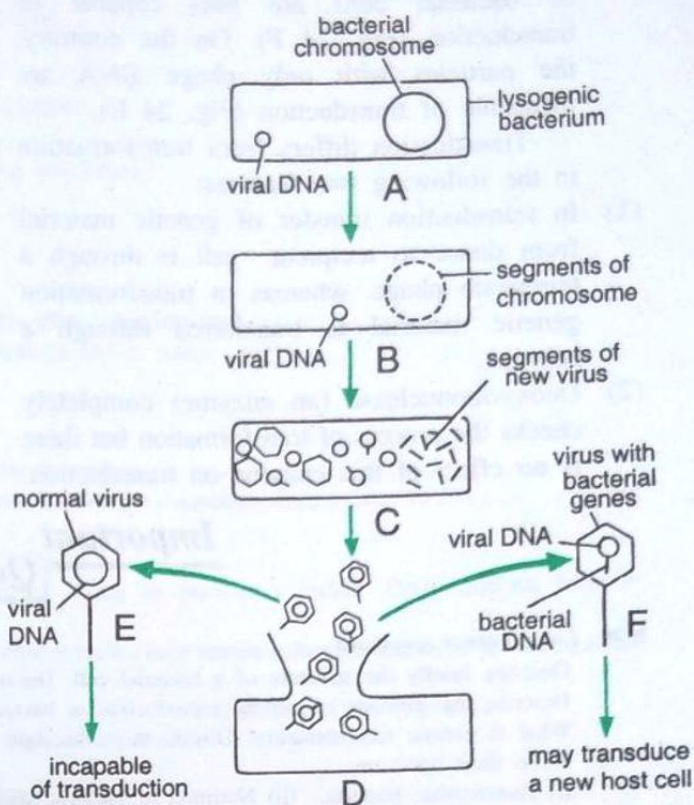


Fig. 24 A-F. Bacteria : Genetic recombination; Diagrammatic representation of generalized transduction.

the infected cell (and not in its chromosome). The main steps of the generalized transduction are as follows:

- (1) The phage DNA, present in the lysogenic bacterial cell, starts synthesizing new phage components. In this process the chromosomes of some bacterial cells get fragmented. Eventually these segments are incorporated in some new phage particles (Fig. 24 A-C). Thus some of the phage particles present in the lysogenic cell have segments of bacterial chromosome incorporated in them, while others have only phage DNA (Fig. 24 D-E).
- (2) If a phage particle with segment of bacterial chromosome in its DNA attacks a bacterium of any other strain the genes of the parent bacterium are transferred to the new cell. Such phage particles (with genes

of bacterial cell) are thus capable of transduction (Fig. 24 F). On the contrary, the particles with only phage DNA are incapable of transduction (Fig. 24 E).

Transduction differs from transformation in the following two features:

- (1) In transduction transfer of genetic material from donor to recipient cell is through a temperate phage, whereas in transformation genetic material is transferred through a solution.
- (2) Deoxyribonuclease (an enzyme) completely checks the process of transformation but there is no effect of this enzyme on transduction.

Significance of transduction. Transduction involves transfer of genetic material from one bacterial cell to another, thus it alters the genetic characteristics of the recipient cell. In this process the recipient cell attains many important characteristics. Besides this, incorporation of phage DNA into a bacterial chromosome is possible only when they have regions of quite similar base sequences. It shows a close evolutionary relationship between the prophage and the host bacterial cell. Transduction is helpful in producing transgenic organisms since by this process viruses transfer genes from one host to another.

Important Questions

➤➤ Long answer questions

1. Describe briefly the structure of a bacterial cell. Discuss its prokaryotic characters.
2. Describe the methods of asexual reproduction in bacteria.
3. What is genetic recombination? Discuss it in the light of bacterial cell.
4. Write short notes on:
 - (i) Auxotrophic bacteria, (ii) Nutrition of bacteria, (iii) Pili or fimbriae, (iv) Gram stain, (v) Endospore in bacteria, (vi) Acid-fast bacteria, (vii) Plasmid, (viii) Conjugation, (ix) Transformation, (x) Transduction, (xi) Morphological forms of bacteria, (xii) Gram '+ve' and Gram '-ve' bacteria, (xiii) Bacterial chromosome, (xiv) Nitrogen fixing bacteria, (xv) Flagella in bacteria.
5. Describe in detail the modes of sexual reproduction in bacteria.
6. Describe the structure and modes of reproduction in bacteria.
7. Describe various forms of bacteria and explain their mode of nutrition.
8. What is genetic recombination? Explain transformation in bacteria.
9. Describe in detail about modern system of bacterial classification.
10. Describe structure and mode of nutrition in bacteria.

➤➤ Short answer questions

1. How does a bacterial flagellum differ from eukaryotic flagellum?
2. You are given a group of bacteria. How would you proceed to determine Gram '+ve' and Gram '-ve' bacteria?
3. How will you differentiate a Gram '+ve' bacterium from the Gram '-ve' bacterium on the basis of staining?
4. What is Gram stain technique? Explain.
5. Write a note on genetic recombination in bacteria.
6. What functions are associated with the following cell structures in a bacterial cell? Capsule, cell wall, cytoplasmic membrane, mesosome, endospore, flagellum and pili.
7. Distinguish between photoautotrophs and chemoautotrophs.
8. Identify all structures of a bacterium external to the cell wall.
9. In terms of cell wall structure and composition, explain the difference between Gram '-ve' and Gram '+ve' bacteria.
10. How the process of fission is different from normal mitotic division?

➤➤ Very short answer questions

1. Name the three modes of genetic transfer between bacterial cells.
2. Who discovered the phenomenon of conjugation in bacterial cells?
3. Name the first bacteriologist.
4. Name the two microbiologists who discovered transduction.
5. Name a rod shaped bacterium which has the capacity to produce endospores.
6. Name the scientist who discovered sexual reproduction in bacteria.

7. Name the protein present in the flagella of bacteria.
8. Name two asexual methods of reproduction found in bacteria.
9. Give two names of *Bacillus* bacteria.
10. What is the main component of cell wall of bacteria?
11. In which bacterium 'transformation' was discovered by Griffith?
12. Name the organelle meant for photosynthesis in bacteria.
13. In which bacterium conjugation was studied by Lederberg and Tatum?
14. Write an example of chemosynthetic bacterium.
15. Name a Gram '-ve' and a Gram '+ve' bacterium.
16. Name a symbiotic bacterium.
17. Name the largest bacterium species.
18. Who coined the term 'plasmid' for extra-chromosomal hereditary determinants?
19. Who discovered the phenomenon of transformation in bacteria and in which year?
20. Name a photoautotrophic bacterium.

►► Fill in the blanks

1. A bacterial cell reproduces by rather than by mitosis.
2. Bacteria have three ways of transferring genes from one individual to another and
3. Plasmids that can integrate into the bacterial DNA are called
4. The process by which a bacterium acquires new genes by taking up parts of a 'naked' DNA molecule from its surroundings is called
5. When treated with crystal violet and subsequently with iodine some bacteria remain colourless and these are called Gram bacteria.
6. The cell wall of Gram '-ve' bacteria is comparatively less than Gram '+ve' bacteria.
7. bacteria are those which use chemical energy for reduction of CO_2 .
8. During conjugation, F-factor is commonly called as
9. are short, curved comma-shaped flagellated bacteria.
10. In bacterial nucleoid, spindles are during cell division.
11. In bacteria, are extensions of plasma membrane which initiate RNA replication and septum formation during cell division.
12. Certain Gram rods have developed a powerful, specialized mechanism for survival through hard times by the formation of
13. A nitrite oxidizing bacterium is
14. Photosynthetic sulphur bacteria differ from higher plants in that they do not have the pigment and they do not use as electron donor in photosynthesis.
15. Unlike plant cell walls which are made of, most bacterial cell wall contains
16. Bacteria with helically coiled shapes are called
17. Transfer of genetic material from one bacterium to another by a virus is known as
18. The change in DNA of a bacterium by incorporation of DNA fragment released by another bacterium is called
19. Cocci which divide only in one plane to form long chain like colonies are known as
20. Bacterial flagella are made up of protein called
21. Bacterial cocci arranged in cubes of eight cocci are called
22. The prokaryotic are 70s.
23. Conjugation in bacteria was discovered by first in
24. The most common method of reproduction in bacteria is
25. The bacteria which have a group of flagella at one end only, are called
26. The bacterial cell wall is made up of
27. When the bacterial cell possesses many flagella distributed throughout its surface, it is known as flagellation.
28. Mycoplasmas are Gram '-ve', non-motile and facultative
29. *Thiophysa volutans*, a bacterium, is perhaps the amongst all bacteria.
30. Plasmids are stranded DNA molecules.

►► True and false statements

1. Transduction is a phage-mediated genetic transfer.
2. Bacteria which are capable of synthesizing food from inorganic materials using chemical energy are called photosynthetic bacteria.

3. The bacterial flagella are similar to eukaryotic flagella in having 9 + 2 structure.
4. In bacteria, nucleoid represents the area in which all the genetic material (DNA) of the cell is concentrated.
5. When van Leeuwenhoek examined scrapings of his teeth under a microscope after drinking hot coffee, he saw living germs in them.
6. Gram stain which is extracted from gram pods is used to differentiate Gram '-ve' and Gram '+ve' bacteria.
7. Bacteria are cosmopolitan in distribution because of having excellent power of tolerating the environmental extremes.
8. Transduction in bacteria was studied by Zinder and Lederberg.
9. Fimbriae are the locomotory organs of bacterial cell.
10. Bacterial cell wall is made up of peptidoglycan polymers or mucopeptides.

►► Multiple choice questions

1. Bacteria were first discovered by:
 - (a) Robert Koch
 - (b) A. V. Leeuwenhoek
 - (c) N. D. Zinder
 - (d) Robert Hooke
2. Bacteria having a tuft of flagella at one end are called:
 - (a) monotrichous
 - (b) lophotrichous
 - (c) amphitrichous
 - (d) peritrichous
3. A bacterial cell divides once every minute. It takes one hour to fill a cup. How much time will it take to fill half the cup?
 - (a) 29 minutes
 - (b) 30 minutes
 - (c) 59 minutes
 - (d) 60 minutes
4. When DNA is exchanged, via cytoplasmic bridges, between two bacteria, the process is called:
 - (a) general transduction
 - (b) restricted transduction
 - (c) transformation
 - (d) conjugation
5. One of the following bacterial characters is a plant character:
 - (a) heterotrophic nutrition
 - (b) prokaryotic organization
 - (c) presence of flagella
 - (d) rigid cell wall
6. During bacterial conjugation, there is:
 - (a) a partial but mutual exchange of genetic material between the conjugants
 - (b) only a partial transfer of genetic material from one conjugant to the other
 - (c) a complete transfer of genetic material from one conjugant to the other
 - (d) a mutual and complete exchange of genetic material between two conjugants
7. Surface appendages used by bacteria to attack one another and to host organism are called:
 - (a) pili
 - (b) spirilla
 - (c) mesosomes
 - (d) thylakoids
8. Compared to Gram '-ve' bacteria, Gram '+ve' bacteria:
 - (a) have less peptidoglycan
 - (b) retain the crystal violet dye
 - (c) have more complex cell walls
 - (d) are more resistant to antibiotics
9. Solutions of crystal violet, iodine and alcohol are used in a staining procedure known as:
 - (a) Gram stain
 - (b) Acid-fast stain
 - (c) Giemsa's stain
 - (d) Wright's stain
10. Plasmids are:
 - (a) viruses
 - (b) a type of cyanobacteria
 - (c) essential bacterial genetic elements
 - (d) extra-chromosomal genetic elements
11. Genetic element in bacteria that can replicate in the cytoplasm or can integrate into the bacterial chromosome and replicate with the host chromosome is:
 - (a) retrovirus
 - (b) plasmid
 - (c) episome
 - (d) plastome
12. Which of the following waters is free from bacteria?
 - (a) deep well water
 - (b) sea water
 - (c) water of hot springs
 - (d) rain water as it falls down
13. The bacterial cell wall is composed of:
 - (a) peptidoglycan
 - (b) lignin
 - (c) suberin
 - (d) cellulose
14. Bacteria lack:
 - (a) cytoplasm
 - (b) cell membrane
 - (c) endoplasmic reticulum
 - (d) DNA
15. Bacterium that must have organic molecules both for energy and as a source of carbon is called:
 - (a) chemoheterotrophs
 - (b) chemoautotrophs
 - (c) photoheterotrophs
 - (d) photoautotrophs
16. Bacteria those get their energy by fermentation and oxygen is lethal for them are called:
 - (a) obligate aerobes
 - (b) facultative aerobes
 - (c) obligate anaerobes
 - (d) facultative anaerobes
17. When bacteria are rod-shaped, they are called:
 - (a) cocci
 - (b) bacilli
 - (c) spirilla
 - (d) vibrio
18. Which one of the following are archaebacteria?
 - (a) blue-green
 - (b) green sulphur
 - (c) rickettsias
 - (d) methanogens
19. 9 + 2 fibrillar arrangement is present in:
 - (a) eukaryotic flagella
 - (b) bacterial flagella
 - (c) bacterial fimbriae
 - (d) T₄ bacteriophage
20. Gram '+ve' bacteria are stained by:
 - (a) crystal violet
 - (b) safranin
 - (c) malachite
 - (d) fast green
21. Who is regarded as first bacteriologist?
 - (a) Pasteur
 - (b) Koch
 - (c) Jenner
 - (d) Leeuwenhoek
22. Who studied conjugation in bacteria?

- (a) Tatum and Lederberg
 - (b) Zinder and Lederberg
 - (c) Frederick Griffith
 - (d) Pasteur
23. Transformation was discovered by:
- (a) Lederberg and Tatum
 - (b) Watson and Crick
 - (c) Griffith
 - (d) Zinder and Lederberg
24. *Escherichia coli* is:
- (a) Gram '+ve' (b) Gram '-ve'
 - (c) virus
 - (d) both Gram '+ve' and Gram '-ve'
25. Plasmids are present in:
- (a) *E. coli* (b) TMV
 - (c) bacteriophage (d) lichens
26. Gram stain is:
- (a) a chemical for differentiation of bacteria
 - (b) a stain produced out of gram seed
 - (c) a staining technique developed by Christian Gram
 - (d) a trade mark
27. Curd bacteria is:
- (a) Gram '+ve'
 - (b) Gram '-ve'
 - (c) *Pseudomonas*
 - (d) none of these
28. Recent studies reveal that:
- (a) mesosomes are artifacts and not true structures
 - (b) mesosomes are true structures
 - (c) mesosomes are in glycocalyx
 - (d) none of the above
29. Who discovered little animalcules?
- (a) Anton Van Leeuwenhoek
 - (b) Louis Pasteur
 - (c) Robert Koch
 - (d) Robert Hooke
30. There is 3 to 4 times more peptidoglycan in:
- (a) Gram '+ve' bacteria in comparison to Gram '-ve' bacteria
 - (b) Gram '-ve' bacteria
 - (c) cyanobacteria
 - (d) plant cells

ANSWERS

>> Very short answer questions

1. Transformation, transduction, conjugation, 2. Tatum and Lederberg (1947), 3. A. V. Leeuwenhoek, 4. Zinder and Lederberg (1952), 5. *Bacillus cereus*, 6. Tatum and Lederberg, 7. Flagellin, 8. Binary fission, budding, 9. *Lactobacillus*, *Corynebacterium*, 10. peptidoglycan, 11. *Diplococcus pneumoniae*, 12. thylakoids, 13. *Escherichia coli*, 14. sulphur bacterium, 15. *Escherichia coli* and *Lactobacillus* respectively, 16. *Rhizobium*, 17. *Thiophysa volutans* (a sulphur bacterium), 18. Lederberg (1952), 19. Frederick Griffith in 1928, 20. Green sulphur bacterium.

>> Fill in the blanks

1. binary fission, 2. transformation, transduction, conjugation, 3. episomes, 4. transformation, 5. negative, 6. rigid, 7. chemosynthetic, 8. episome, 9. vibrios, 10. not formed, 11. mesosomes, 12. +ve, endospores, 13. *Nitrobacter*, 14. chlorophyll *a*, water, 15. cellulose, peptidoglycan, 16. spirilla, 17. transduction, 18. transformation, 19. streptococcus, 20. flagellin, 21. sarcinae, 22. ribosomes, 23. Lederberg and Tatum, *E. coli*, 24. fission, 25. lophotrichous, 26. peptidoglycan, 27. peritrichous, 28. anaerobes, 29. sulphur, largest, 30. circular, double.

>> True and false statements

1. True, 2. False, 3. False, 4. True, 5. False, 6. False, 7. True, 8. True, 9. False, 10. True.

>> Multiple choice questions

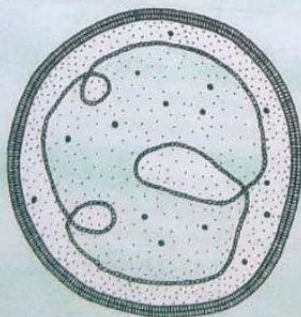
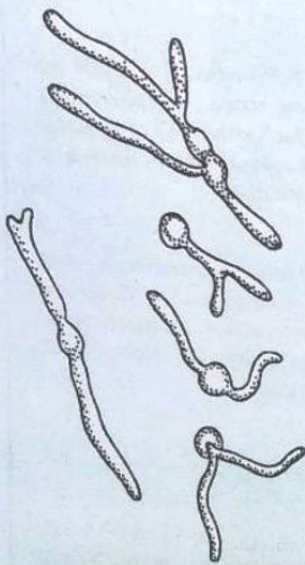
1. (b), 2. (b), 3. (c), 4. (d), 5. (d), 6. (d), 7. (a), 8. (b), 9. (a), 10. (d), 11. (b), 12. (d), 13. (a), 14. (c), 15. (a), 16. (c), 17. (b), 18. (d), 19. (a), 20. (a), 21. (d), 22. (a), 23. (c), 24. (a), 25. (a), 26. (c), 27. (b), 28. (a), 29. (a), 30. (a).

7



Cyanobacteria, Actinomycetes, Mycoplasmas and Rickettsias

CYANOBACTERIA



(MICROBIOLOGY)

Cyanobacteria, also known as blue-green algae, comprise about 1,500 species of photosynthetic prokaryotes that live in a great variety of habitats. They occur in soil, in fresh and sea water and even in the extreme conditions like glacier ice and hot springs. One reason for their wide distribution is their great independence. Their photosynthetic pigments are chlorophyll *a* and phycobiliproteins. They differ from two other major groups of photosynthetic bacteria — purple and green bacteria — in the nature of their photosynthetic pigments and in their capacity to perform oxygenic photosynthesis. Thus cyanobacteria show physiological resemblances to aerobic photoautotrophs.

Since their recognition as a biological group early in the 19th century, cyanobacteria have been treated as a class or division of algae, commonly known as blue-green algae or Cyanophyta. This taxonomic treatment, primarily based on their plant-like photosynthetic activities, became untenable around 1960 when fundamental differences in cellular organization of prokaryotes and eukaryotes were fully elucidated. It became evident that the cells of blue-green algae, long known to be unlike those of any other algal group, are typically prokaryotic.

Many phycologists (Fritsch, 1945; Prescott, 1969; Morris, 1973; Round, 1973), however, consider that recognition of the prokaryotic organisms as bacteria is an intrusion of bacteriologists into their traditional field. Phycologists consider that an organism with chlorophyll *a* and the plant body (thallus) not differentiated into

roots, stems and leaves is an alga. However, the blue-green algae share the following characters with bacteria.

- (1) Both, the blue-green algae and bacteria have a prokaryotic cell structure.
- (2) Nuclear membrane, nucleolus and membrane bound plastids are absent in both the groups.
- (3) The blue-green algae have a mucilagenous sheath outside the cell wall as in bacteria.
- (4) Structures like hormogonia (a characteristic of blue-green algae) are present in some bacteria like *Thiothrix*.
- (5) *Beggiotoa*, a sulphur bacterium and *Oscillatoria* show resemblance in shape and movement.
- (6) The blue-green algae are sensitive to antibiotics like bacteria.
- (7) The blue-green algae resemble with bacteria in many biochemical processes, such as sulphur and nitrogen metabolism.
- (8) As in bacteria, genetic recombination is also known to occur in many blue-green algae.

CELL STRUCTURE

Cells of cyanobacteria may occur singly, enclosed within a mucilagenous envelope (e.g., *Gleocapsa*, *Microcystis*) or form chains called trichomes (e.g., *Oscillatoria*, *Gloeotrichia*) or as a sheet (e.g., *Dermocarpa*). The filament consists of one or more trichomes enveloped in a gelatinous sheath.

The cells show a typical prokaryotic structure. There are no membrane-limited organelles within the cells although a variety of vesicles and inclusions may be present. The cytoplasm is surrounded by a plasma membrane, followed by a firm cell wall and a mucilage sheath (Fig. 1).

[A] Mucilage Sheath

The cell wall of cyanobacteria is surrounded by a thin (e.g., *Anacystis montana*) or thick (e.g., *Anabaena*) mucilage sheath, also known as **slime layer** or **capsule**. In unicellular forms, each individual has a distinct sheath of its own, whereas in filamentous forms, there is a common gelatinous

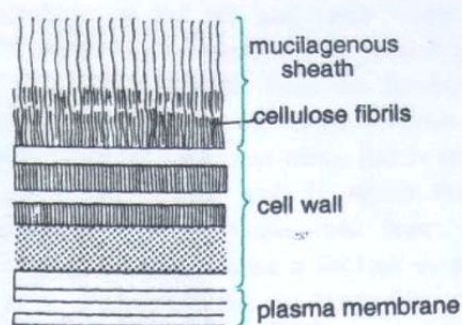


Fig. 1. Cyanobacteria : Cell wall structure.

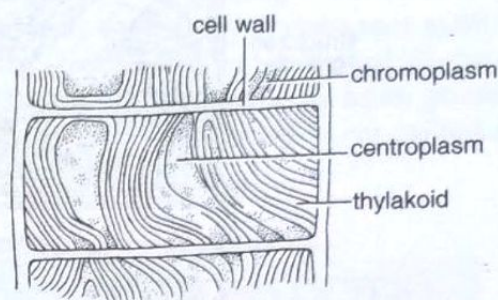


Fig. 2. Cyanobacteria : Cell structure.

sheath. The sheath is composed of a complex mixture of **mucopolysaccharides** and **glucuronic acid**. It is made up of fibres, held together in the mucilage. The fibres arise from the cell wall, but are not firmly attached to it and as such the trichome may turn within the sheath. The orientation of fibres varies in different species. The mucilage sheath probably helps in protecting the cells from desiccation, particularly in those species which do not produce resistant spores. It also helps in gliding movements.

[B] Cell Wall

The cell wall of cyanobacteria is thin and firm and is basically similar to that of Gram-negative bacteria. It is differentiated into four layers which are designated as L_I, L_{II}, L_{III} and L_{IV}; L_I being the inner most lying next to plasma membrane and L_{IV} the outer most. The cell wall is made up of peptidoglycan together with carbohydrates, amino acid and fatty acids.

(MICROBIOLOGY)

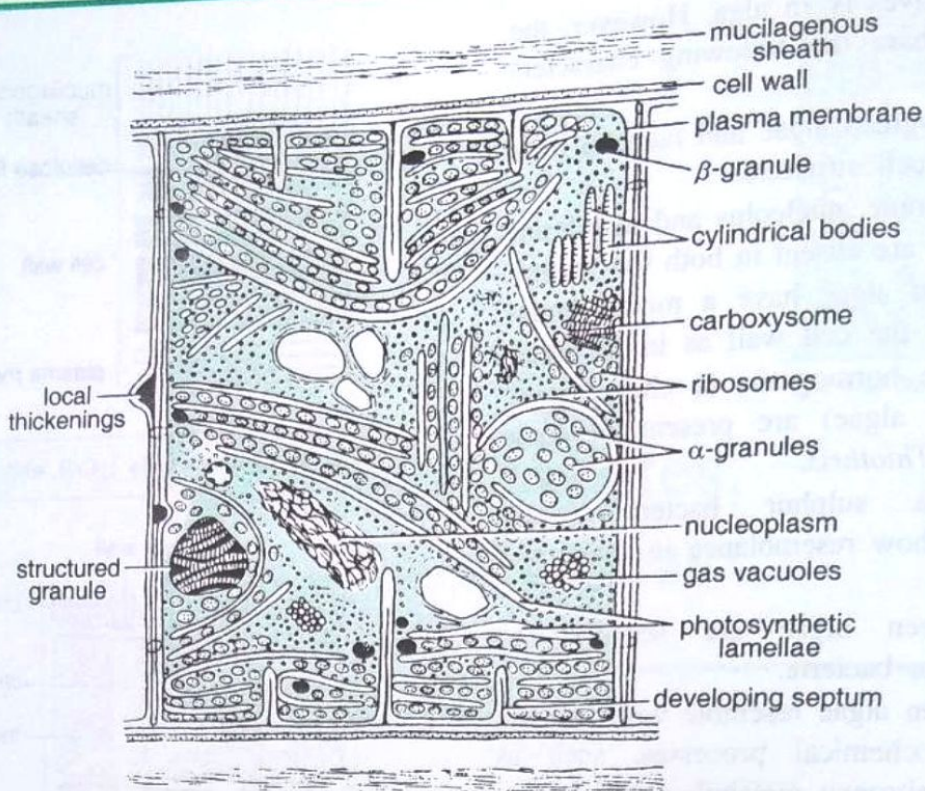


Fig. 3. Cyanobacteria : Schematic representation of electron micrograph.

[C] Cytoplasmic Membrane

It forms the limiting membrane of the cytoplasm and is made up of protein-lipid-protein layers. It has many infoldings which penetrate the cytoplasm. It has been suggested that these infoldings give rise to new pigment containing thylakoids.

[D] Chromatoplasm

It is the peripheral pigmented portion of the protoplast and has a reticulate, alveolar or homogeneous structure. The chromatoplasm is characterized by the presence of flattened vesicular structures, called **thylakoids** or **photosynthetic lamellae** (Figs. 2, 3). A lamella is composed of two membranes joined at the ends and enclosing an intrathylakoid space of 70- 80Å width. The lamellae thus form closed flattened sacs which contain chlorophyll *a*, carotenoids and three phycobiliproteins: c-phyococyanin, allophycocyanin and c- phycoerythrin. The thylakoids are arranged in parallel rows close to the periphery of the cell or they are distributed irregularly throughout the

cell. Thus the pigments are not contained in true membrane-limited plastids.

The photosynthetic lamellae of cyanobacteria are also considered as the sites of cellular respiration and as such they are preferably called **photosynthetic respiratory membranes**. The cells of cyanobacteria are devoid of Golgi apparatus, mitochondria, endoplasmic reticulum and other membrane bound organelles.

In addition to thylakoids, chromatoplasm contains protein granules and gas and oil vesicles.

Cyanophycin granules, also known as **structured granules**, are irregular or polyhedral in shape and they store protein in the form of polypeptides.

Polyhedral bodies are angular in shape and are usually associated with the DNA in the central part of the cell. They are considered to be the carboxysomes which store the carbon dioxide-fixing enzyme ribulose-1,5-bisphosphate carboxylase.

Polyphosphate bodies, also known as **volutin granules**, are small spherical structures, located

near the centre of the cell. They store phosphate and are found abundantly in mature cells grown in the medium with high phosphate contents.

Gas vesicles are small, tubular structures, composed of protein ribs or spirals exclusively. These vesicles which contain gas have quite rigid wall but they are collapsed when pressure is applied. The gas vacuoles shield light and also provide buoyancy.

[E] Centrioplasm

The central colourless region of the cell is known as **centrioplasm** or **nucleoplasm**. It consists of several naked DNA which are not associated with histones. However, in some cyanobacteria a histone-like protein binds nonspecifically with DNA. The nuclear material is not surrounded by a nuclear membrane and nucleoli are also absent. The nucleoplasm is not sharply differentiated and usually intrudes into the peripheral chromatoplasm. Although this region stains like that of the nucleus of eukaryotes, it cannot be regarded as true nucleus and is called **incipient nucleus**.

REPRODUCTION

Gametes or flagellated zoospores are not found in cyanobacteria. The most common method of multiplication is binary fission. The rate of cell division is very high and in certain coccoid forms (e.g., *Anacystis nidulans*) there are as many as 12 doublings in a day. Such a rapid growth results in the formation of dense **water blooms**. Many bloom-forming species are buoyant and may rise to the surface, forming a **scum** or **mat**.

Multiplication also takes place by some specialized structures, such as **hormogonia**, **akinetes**, **endospores** and **heterocysts**.

ECOLOGICAL IMPORTANCE OF CYANOBACTERIA

Cyanobacteria occur in almost every moist environment, in the sea, in fresh water, and on the land. As all cyanobacteria are photosynthetic, they live near the surface, where they can obtain light. They form a considerable part of the

phytoplankton on the sea and fresh water. They produce oxygen as a by-product of photosynthesis, and some species provide food for heterotrophs.

The unpleasant aspect of cyanobacteria is that they cause water pollution as many forms are toxic and slimy. They thrive well in water that gets industrial and sewage waste and form smelly blooms. These blooms cause a decline in the fish population. Cyanobacteria, as they die, provide food for an enormous array of aerobic bacteria, which use all oxygen in the water and suffocate the fish. Fish cannot control populations of cyanobacteria by consuming them because toxins and gelatinous sheaths of cyanobacteria make them inedible. Pretreatment and controlled discharge of industrial and sewage waste in lakes, ponds and rivers can help in reducing water pollution by cyanobacteria.

ACTINOMYCETES

Actinomycetes are unicellular organisms placed in the order Actinomycetales or the class Schizomycetes. They occur abundantly in soil water, mud, manure, milk and other food products. Most of the actinomycetes are **saprophytes** but some are **parasites**. The latter cause some serious diseases in plants as well as animals.

STRUCTURE

Actinomycetes (*actis* = ray, *mykes* = fungus) are fungus like bacteria with cylindrical cells which are usually united to form filaments resembling the mycelium of a true fungus (Fig. 4 A). The mycelium is branched, non-septate and thin (0.2-1.2 μm in width). In some species the mycelium breaks up into small bacteria-like cells. In young mycelium the cytoplasm is homogeneous, but at maturity many vacuoles, fat droplets, granules and few rod-shaped bodies develop in the cytoplasm. In actinomycetes, as in true bacteria, there are no well differentiated nuclei but many chromatin granules are present. At maturity, the cell wall of the mycelium becomes fragile and breaks up easily.

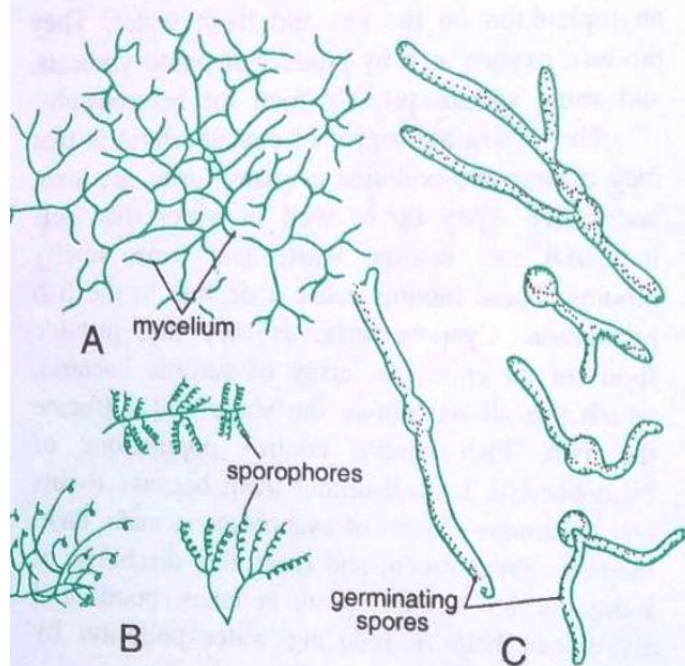


Fig. 4 A-C. Actinomycetes : A. Mycelium, B. Sporophores, C. Spore.

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

REPRODUCTION

They generally multiply by means of **fragmentation**. The mycelium breaks up into small hyphae and each hypha grows into a new mycelium. Some species of actinomycetes also reproduce by means of **spores** and **conidia** which develop on sporophores and conidiophores, singly or in long chains (Fig. 4B, C).

CLASSIFICATION OF ACTINOMYCETES

The order Actinomycetales consists of the following four families.

[A] Actinomycetaceae

The members of this family have a tendency to form branched filaments which are very slender and nonseptate and about a micron in

diameter. They form spores and conidia in long chains.

Example : *Actinomyces* (commonly known as 'ray fungus').

[B] Mycobacteriaceae

These actinomycetes form a rudimentary mycelium, which is unstable and is fragmented into immotile rods. These bacteria are acid-fast.

Examples : *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Mycoplan*.

[C] Micromonosporaceae

The vegetative mycelium of these fungi does not fragment in rod-shaped or cocci forms. It produces a compact colony with a moist and smooth surface. The terminal hyphae of loose aerial mycelia, which arise over the surface of the colony, bear spores either in chains (e.g., *Streptomyces*) or singly (e.g., *Micromonospora*).

Examples : *Micromonospora*, *Streptomyces*.

[D] Proactinomycetaceae

In these forms the mycelial development is transitory and often limited. They do not produce specialized spores and reproduce by fragment of mycelium into short rod-shaped cells.

Examples : *Proactinomyces*, *Connistreptothrix*.

ECONOMIC IMPORTANCE OF ACTINOMYCETES

[A] Useful Activities

Economically, the members of the family Micromonosporaceae of the Actinomycetes are very useful as they are capable of synthesizing antibiotic substances. Some important antibiotics synthesised by them are listed in Table 1.

[B] Harmful Activities

Many actinomycetes cause serious diseases in plants as well as in animals and human beings. Some common diseases caused by actinomycetes are listed in table 2.

Table 1. Some important antibiotics and their source, range and mode of action.

Antibiotic	Source	Range of action	Mode of action
Streptomycin	<i>Streptomyces griseus</i>	Gram '+' ve and Gram '-' ve bacteria, tuberculosis bacteria	Induce abnormal protein synthesis by inhibiting 30s ribosome function
Neomycin	<i>Streptomyces fradiae</i>	Gram '-' ve bacteria inhibits (most of the intestinal bacteria)	Induces abnormal protein synthesis by inhibiting 30s ribosome function
Viomycin	<i>Streptomyces puniceus</i>	Gram '-' ve bacteria, including tuberculosis bacteria	Interferes with protein synthesis
Chlorotetracycline or Aureomycin	<i>Streptomyces aureofaciens</i>	Gram '+' ve and Gram '-' ve bacteria, rickettsia	Interferes with protein synthesis
Oxytetracycline or Terramycin	<i>Streptomyces rimosus</i>	Gram '+' ve and Gram '-' ve bacteria, rickettsia	Interferes with protein synthesis
Novobiocin	<i>Streptomyces niveus</i>	<i>Proteus</i> and <i>Diplococcus</i>	Interferes with protein synthesis
Chloramphenicol or Chloromycetin	<i>Streptomyces venezuelae</i>	Gram '+' ve, Gram '-' ve bacteria, rickettsia	Inhibits protein synthesis by interfering with 50s ribosome function
Erythromycin	<i>Streptomyces erythreus</i>	Gram '+' ve bacteria	Inhibits protein synthesis
Lincomycin	<i>Streptomyces lincolnesis</i>	Gram '+' ve bacteria	Interferes with protein synthesis
Rifamycin	<i>Streptomyces mediterranei</i>	Active against tuberculosis bacteria	Interferes with protein synthesis
Nystatin	<i>Streptomyces noursei</i>	Fungal infections due to <i>Candida</i>	Damages cell membrane

Table 2. Some common diseases caused by actinomycetes.

1. Human diseases	Pathogen
Tuberculosis	<i>Mycobacterium tuberculosis</i>
Leprosy	<i>Mycobacterium leprae</i>
Diphtheria	<i>Corynebacterium diphtheriae</i>
Actinomycosis	<i>Actinomyces israeli</i>
Madura foot	<i>Nocardia madurae</i>
2. Animal diseases	
Actinomycosis or Lumpy jaw	<i>Actinomyces bovis</i>
Tuberculosis of cattle	<i>Mycobacterium bovis</i>
Tuberculosis of domestic fowls and other birds	<i>Mycobacterium avium</i>
3. Plant diseases	
Tundu of wheat	<i>Corynebacterium tritici</i>
Scab of potato	<i>Actinomyces scabies</i>

MYCOPLASMAS

Mycoplasmas are the smallest known aerobic prokaryotes without a cell wall. These microorganisms were first discovered by Pasteur

(1843) while studying the causative agent of pleuropneumonia in cattle (bovine pleuropneumonia). They were designated as PPLO (pleuropneumonia-like organisms). However, Pasteur could not isolate them in pure cultures on standard nutrient media or observe them under the light microscope. In 1898, two French microbiologists, Nocard and Roux, were successful in obtaining pure cultures of these microorganisms in media containing serum. They observed that these organisms could produce disease when inoculated in healthy cattle.

Mycoplasmas are frequent contaminants in tissue cultures rich in organic matter. They have also been found in hot-water springs and other thermal environments. They occur in soil, sewage water, different substrates and in humans, animals and plants. These pleuromorphic microorganisms were named as *Asterococcus mycoides* by Borrel *et al.* (1910). Nowak (1929) placed these organisms in the genus *Mycoplasma*, which belongs to the class Mollicutes of the order Mycoplasmatales.

CLASSIFICATION OF MYCOPLASMAS

The following three genera of mycoplasmas have been recognised on the basis of their nutritional requirements.

[A] Mycoplasma

These mycoplasmas require cholesterol for their growth which is incorporated in their cell membrane. They are parasitic and infect mucous membranes and joints of humans and animals.

[B] Acholeplasma

These mycoplasmas do not require cholesterol for their growth, but will incorporate it into the membrane if it is furnished in the medium. They are found in vertebrates and sometimes in plants, and also occur in soil and sewage water.

[C] Thermoplasma

These mycoplasmas also do not require cholesterol for their growth. They are strictly aerobic, acid loving microorganisms, growing well at pH 0.96-3.0. The optimum temperature for their growth is 59°C.

STRUCTURE OF MYCOPLASMAS

The absence of a true cell wall makes these organisms highly plastic and readily deformable, hence mycoplasmas are irregular and variable in shape. The cells may be coccoid, granular, pear-shaped, cluster-like, ring-like or filamentous (Fig. 5 A-E). The filaments may be branched or unbranched. There is so much variability in shape that no two forms are alike. The cells are small, ranging in diameter between 0.3 and 0.9 μm . Since mycoplasmas pass through many filters (due to their plastic nature) and grow on media which do not contain live tissue, they are considered to be intermediate between bacteria and viruses.

These organisms are covered with a unit lipoprotein cytoplasmic membrane, 7.5-10 μm thick. The cytoplasm contains ribosomes and nucleoplasm-like structure (Fig. 6). Though genetic material is composed of both DNA and RNA, it is less than

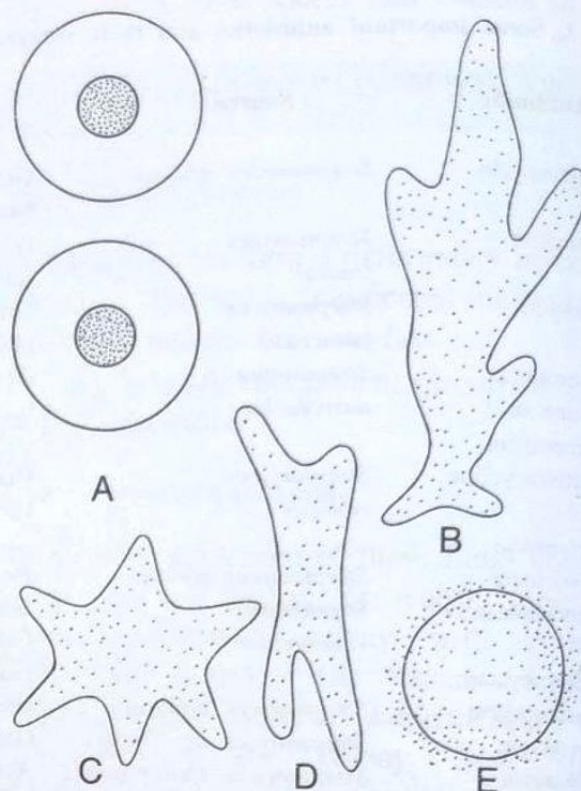


Fig. 5 A-E. Mycoplasma : Morphology of colony and cell shape; A. colony, B-E. Cells of different shapes.

half that usually occurs in other prokaryotes, and is perhaps the lowest limit required for a cellular organism. The amount of DNA is up to 4 per cent and RNA about 8 per cent. The G + C content in DNA ranges between 23 and 40 per cent; Mycoplasmas may be the simplest form of life capable of independent growth and metabolism.

Mycoplasmas are Gram-negative and stain slowly on long exposure to dyes. They are usually non-motile; some forms, however, show gliding movements. They reproduce by budding or binary fission.

They are sensitive to oxytetracycline, streptomycin, erythromycin and chloramphenicol.

They do not have cell wall with peptidoglycans (like that of bacteria) and are insensitive to penicillin and some other antibiotics such as ampicillin and methicillin, which specifically affect peptidoglycan synthesis.

NATURE OF MYCOPLASMAS

Mycoplasmas can grow in a medium which contains no living tissues. They can also pass

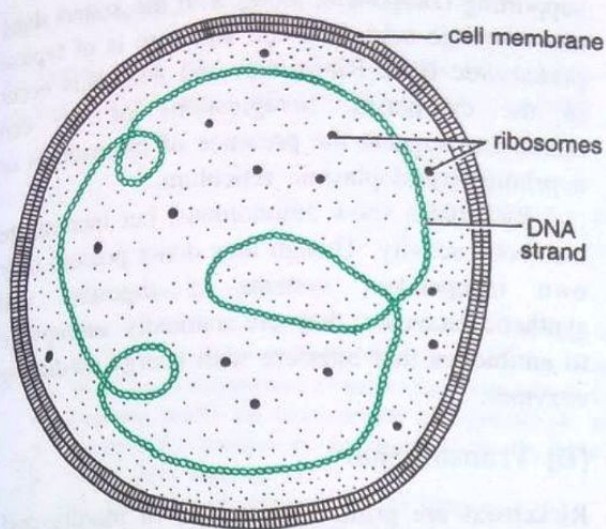


Fig. 6. Mycoplasma : Electron micrograph of a cell.

through many filters which cannot pass bacteria. Because of these two characteristics, mycoplasmas are considered to be intermediate between bacteria and viruses. Chemically, they are more close to bacteria than viruses.

CULTIVATION OF PATHOGENIC MYCOPLASMAS

Pathogenic mycoplasmas can be cultivated on medium containing proteins, sterols (cholesterol), phospholipids, mucins and nucleic acids (DNA and RNA). The growth of some obligate anaerobic mycoplasmas is stimulated by adding yeast extract and cholesterol to the medium. The optimal temperature for their growth is 36-37°C. On a solid medium, the colonies of mycoplasmas have a characteristic round form with a thickened centre and a delicate periphery, like a 'fried egg'. Aerobic mycoplasmas grow on a medium supplemented with 20 per cent horse serum.

SOME IMPORTANT MYCOPLASMAL DISEASES

Mycoplasmas cause serious diseases in human beings, animals and plants. Some of these are described below.

[A] Pathogenesis and Diseases in Human Beings

Pathogenic mycoplasmas affect the respiratory organs, central nervous system, cardiovascular system and urogenital system in human beings. *Mycoplasma hominis*, a species studied most extensively, causes pleuropneumonia, inflammation of genitals, non-specific urethritis, prostatitis, endocarditis and other diseases. Mycoplasmas generally produce diseases in man when his body resistance is significantly low.

Mycoplasma pneumoniae causes primary atypical pneumonia (PAP), haemorrhagic laryngitis and vesicular inflammation of tympanic membrane. *Mycoplasma hominis* and *M. fermentans* are known to cause infertility in man. *M. orale* and *M. salivarium* also affect human beings.

[B] Pathogenicity for Animals

Mycoplasma mycoides causes pleuropneumonia in cattle. Some strains of this mycoplasma produce pleuropneumonia in goat. *Mycoplasma bovis* causes inflammation of genitals in animals. Another species, *Mycoplasma agalactia*, produces agalactia of sheep and goat.

[C] Mycoplasmal Diseases of Plants

In plants, mycoplasmas produce diseases usually by upsetting the hormonal balance. This imbalance is expressed in the form of symptoms such as dwarfness, loss of apical dominance, enlargement of floral buds and bunched growth of newly formed sprouts. In plants the mycoplasmal diseases are transmitted by insect vectors, belonging to the class Cicadellidae and Psyllidae. Multiplication of mycoplasma within insect body has been demonstrated in many experiments. Insects fed on mycoplasma infected plants become infectious only after a specific latent period. Some common mycoplasmal diseases of plants are : (i) Little leaf disease of brinjal, (ii) Clover dwarf, (iii) Clover phyllody, (iv) Clover virescence, (v) Cotton virescence, (vi) Bunchy top of papaya, (vii) Witches broom of legumes, (viii) Stripe disease of sugar cane, (ix) Big bud of tomato, and (x) Yellow dwarf of tobacco.

RICKETTSIAS

Rickettsias are the smallest prokaryotes, 0.3-0.7 μm wide and 1-2 μm long. They are larger than any of the viruses and can be seen with any ordinary microscope at the magnification around 1000 X. Their shape shows considerable variation; they may be cigarette- or rod-shaped, spherical or ovoid and often pleomorphic. They multiply by transverse binary fission, like bacteria. They form pairs or chains after fission. They are non-motile and do not produce spores.

Unlike most bacteria, it is difficult to stain rickettsias with ordinary basic aniline dyes. They can, however, be stained with Giemsa's stain, and if they take Gram's stain, they are gram '+ve'.

Rickettsias are intracellular obligate parasites which can grow only in the presence of another living cell. In laboratory, they are easily cultivated in 'live chick embryos'. In nature, they exhibit close relationship with blood-sucking arthropods like fleas, lice and ticks.

[A] Structure

Like true bacteria, the cell wall of rickettsias contains mucopolysaccharide as the main

supporting component, along with the amino sugar and muramic acid. The cell structure is of typical prokaryotic type. Ribosomes and nucleoids occur in the cytoplasm. Invaginations of the cell membrane suggest the presence of mesosomes or a primitive endoplasmic reticulum.

Rickettsias show autonomous, but incomplete metabolic activity. Though they do not possess their own independent systems of digestive and synthetic enzymes, they are markedly susceptible to antibiotics that interfere with energy-mediating enzymes.

[B] Transmission

Rickettsias are primarily parasites of insects, and appear only secondarily in man and other animals. They inhabit the cells lining the intestines and other tissues of arthropods, such as ticks, fleas, lice, bed bugs, spiders and mosquitoes. Some species of pathogen rickettsias are found in the salivary glands of biting insects, from where they may be transmitted to man. Other species occur in the intestinal contents of sanguisugent (blood sucking) arthropods and appear in the faeces. Transmission of rickettsias in animal hosts may thus be through bites or by rubbing or scratching the fecal deposits of insect vectors into the skin.

Table 2. Some important pathogenic rickettsias.

Disease	Pathogenic rickettsia	Insect vector	Interrelationship of insect-pathogen-human
1. Rocky mountain spotted fever	<i>Rickettsia rickettsii</i>	Ticks (<i>Dermacentor</i> , <i>Amblyomma</i> , <i>Ornithodoros</i> , etc.)	Pathogen multiplies in wall of tick's midgut; congenitally transferred in tick, humans infected through bite.
2. Scrub typhus	<i>R. trutsugamushi</i>	Red mites (<i>Trombicula</i>)	Pathogen multiplies in gut of mite; congenitally transmitted in mite; humans infected through bite of larval mite.
3. Rickettsiae	<i>R. akari</i>	Mouse mite (<i>Allodermonyssus sanguineus</i>)	Pathogen multiplies in gut of mite; humans infected by bite of mite.
4. Classical typhus fever	<i>R. prowazekii</i>	Body louse (<i>Pediculus humanus</i>)	Pathogen multiplies in epithelium of louse's midgut; humans infected by bite, faeces, or crushing of louse on skin.
5. Trench fever	<i>R. quintana</i>	Body louse (<i>Pediculus humanus</i>)	Pathogen multiplies in midgut of louse; humans infected by faeces or crushing of louse on skin.
6. Murine typhus fever	<i>R. typhi</i>	Fleas (<i>Xenopsylla cheopis</i> , etc.)	Pathogen multiplies in epithelium of midgut of flea; humans infected through bite.

[C] Pathogenic Rickettsias

Rickettsias are the causative agents of several important diseases like typhus fever, rocky

mountain spotted fever, scrub typhus and Q fever. Some important pathogenic rickettsias, their vectors and diseases caused by them are presented in Table 2.

Important Questions

►► Long answer questions

1. Give an illustrated account of the structure of cyanobacteria.
2. What are actinomycetes? Mention their economic importance.
3. What are mycoplasmas? Describe their structure and also comment briefly on their economic importance.
4. Describe briefly the structure and pathogenicity of rickettsias.
5. Describe the structure of mycoplasma infecting plants, and explain its mode of reproduction.
6. Write short notes on:
(i) Symptoms of mycoplasma; (ii) Heterocyst.
7. With the help of the suitable and well-labelled diagrams only describe the ultrastructure of virus, bacteria, bacteriophage and mycoplasma.
8. Compare the cell structure of a blue-green alga with a bacterial cell and justify the name cyanobacteria for blue-green algae.
9. Describe structure and reproduction of mycoplasma.
10. Why bacteria are considered to be related to the cyanobacteria?

►► Short answer questions

1. What are cyanobacteria? Write your opinion about their systematic position.
2. Write a note on mycoplasma.
3. Differentiate between virus and mycoplasma.
4. Give the importance of bacteria and cyanobacteria for the fertility of soil.
5. Give labelled diagram of mycoplasma. Give example of a plant disease caused by it.
6. Write a note on nitrogen fixation by cyanobacteria.
7. Differentiate between virus and mycoplasma.
8. Describe the possibilities of genetic recombination in cyanobacteria.
9. Write the role of heterocysts in nitrogen fixation.
10. Draw well labelled diagram of a cyanophycean cell under electron microscope.
11. 'The cyanobacteria show physiological resemblances to aerobic photoautotrophs.' Comment upon the statement.
12. 'Cyanobacteria cause a decline in the fish population.' Justify the statement.

►► Very short answer questions

1. Name the cell wall material which is present only in cyanobacteria and bacteria.
2. Name the pathogen responsible for pleuropneumonia in cattle.
3. Which pathogen is responsible for little leaf of brinjal?
4. Mention any two features in which blue-green algae resemble bacteria.
5. What is the main component of outer covering of mycoplasma?
6. Name the photosynthetic pigments of cyanobacteria.
7. Name the source of streptomycin.
8. Name the causal organism of tondu of wheat.
9. Which pathogen is responsible for lumpy jaws in animals?
10. What are mycoplasmas?

►► Fill in the blanks

1. Mycoplasmas are the smallest known aerobic prokaryotes a cell wall.
2. Sandal spike is a disease.
3. Filamentous fungi are called
4. Little leaf disease of brinjal is caused by
5. Diphtheria in humans is caused by
6. Erythromycin, obtained from *Streptomyces* is effective against Gram '.....' bacteria.

7. *Mycobacterium leprae* is responsible for in humans.
8. Rickettsias can be stained with
9. causes pleuropneumonia in cattles.
10. Bunchy top of papaya is a disease.

►► True and false statements

1. Mycoplasmas are larger than cyanobacteria.
2. Mycoplasmas are insensitive to penicillins but killed by tetracyclines.
3. Cyanobacteria is the term used for the members of a particular order of bacteria.
4. Big bud of tomato is a mycoplasmal disease.
5. The cyanobacteria have a mucilaginous sheath outside the cell wall as in bacteria.
6. Tuberculosis of domestic fowls is caused by *Mycobacterium bovis*.
7. Actinomycetes are the smallest known aerobic prokaryotes without a cell wall.
8. The absence of a true cell wall makes mycoplasma highly plastic and readily deformable.
9. Mycoplasmas are gram-positive.
10. Species of *Rickettsia* are responsible for typhus fever.

►► Multiple choice questions

1. Cyanobacteria are:
 - (a) photoautotrophs (b) photoheterotrophs
 - (c) chemoautotrophs (d) chemoheterotrophs
2. Cells in some filamentous cyanobacteria that are specialized for nitrogen fixation are called:
 - (a) heterocysts (b) mesosomes
 - (c) volutin (d) phycobilisomes
3. The main symptom caused by a mycoplasma disease in plants is:
 - (a) mosaic (b) little leaf
 - (c) root knot (d) none of the above
4. Mycoplasma cells possess:
 - (a) DNA (b) RNA
 - (c) DNA and RNA (d) none of the above
5. Mycoplasma was discovered by:
 - (a) Nocard and Roux (b) Louis Pasteur
 - (c) Leewenhoek (d) Lederberg and Zinder
6. Which of the following is called 'joker of plant kingdom'?
 - (a) virus (b) bacteria
 - (c) rickettsia (d) mycoplasma
7. The mycoplasmas are:
 - (a) thick-walled bacteria (b) thin-walled bacteria
 - (c) thick-walled cyanobacteria
 - (d) cell wall-less bacteria
8. The heterocyst is present in:
 - (a) *Oscillatoria* (b) *Vaucheria*
 - (c) *Nostoc* (d) none of the above
9. The pigments chlorophyll *a* and phycobiliproteins are found in:
 - (a) fungi (b) bacteria
 - (c) viruses (d) cyanobacteria
10. Who is responsible for pleuropneumonia in cattles?
 - (a) mycoplasma (b) viroids
 - (c) rickettsias (d) actinomycetes

ANSWERS

►► Very short answer questions

1. Peptidoglycan, 2. *Mycoplasma mycoides*, 3. Mycoplasma, 4. In prokaryotic cells and cell wall is made of peptidoglycan, 5. Lipoprotein, 6. Chlorophyll *a* and phycobiliproteins, 7. *Streptomyces griseus*, 8. *Corynebacterium tritici*, 9. *Actinomyces bovis*, 10. Smallest known aerobic prokaryotes without a cell wall.

►► Fill in the blanks

1. without, 2. mycoplasmal, 3. actinomycetes, 4. mycoplasma, 5. *Corynebacterium diphtheriae*, 6. erythreus, '+ve', 7. leprosy, 8. Giemsa's stain, 9. *Mycoplasma mycoides*, 10. mycoplasmal.

►► True and false statements

1. False, 2. True, 3. False, 4. True, 5. True, 6. False, 7. False, 8. True, 9. False, 10. True.

►► Multiple choice questions

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



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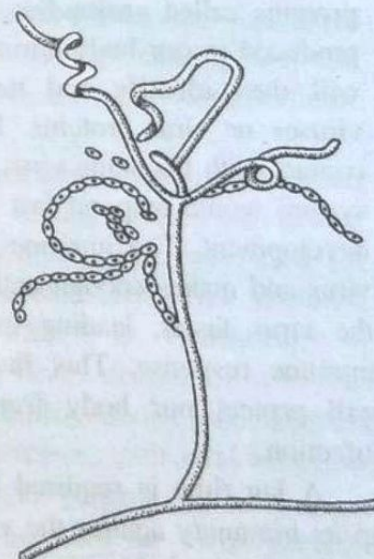
The man lives in an environment that contains innumerable micro-organisms, which are so small to be seen only under high power of a microscope. These organisms have a profound influence, directly or indirectly, on our daily life. Some of these micro-organisms contaminate various food and other useful products, while several others provide many beneficial products by their vital activities. Beneficial activities of microbes are described in the following pages.

ECONOMIC IMPORTANCE OF VIRUSES

MICROBES AND VACCINES

There are several diseases like diphtheria, whooping cough, measles, mumps, yellow fever, small pox, etc., that are unfamiliar to many these days. However, in the 19th and early 20th centuries, these illnesses struck hundreds of thousands of people worldwide and among those most were children. These illnesses killed tens of thousands of people. Today these diseases are not heard of. This change has happened largely because of **vaccines**.

The word vaccine has been derived from the Latin word *vacca* (=cow). It is an immunizing agent which provides specific protection against a given disease. Vaccine is biological preparation that improves immunity to a particular disease.



Vaccines work by mimicking disease agents and stimulate the immune system to build up defenses against them. Before the invention of vaccines, the only way to become immune to a disease was to actually get it and with luck, survive it. This type of immunity against an illness is called **naturally acquired immunity**, wherein the person has to suffer the symptoms of the disease and also risk the complications, which can be quite serious or even deadly. Moreover, if the disease is contagious, it can also be passed on to the family members, friends or even others who come in contact. Vaccines, on the other hand, provide artificially acquired immunity. It is much safer way to become immune to a particular disease. Vaccines can prevent a disease from occurring in the first place and also decrease the risk of complications and its transmission.

When a vaccine is injected into our body, it will trigger an immune response in the same way as our body would respond after an exposure to the virus, but without the person suffering symptoms of the disease. Our body's immune system will detect and recognise the pieces of virus or the killed/weakened virus (also called **antigen**) in the vaccine as a foreign invader and will start an immune response by producing proteins called **antibodies**. The antibody proteins produced in our body during an immune response will then identify and neutralize these foreign viruses or viral proteins. If our body comes in contact with the same virus in future, our immune system would respond fast enough to prevent the development. The immune system recognises the virus and makes enough antibodies to fight against the virus faster, leading to a much more rapid immune response. This faster immune response will protect our body from the potential viral infection.

A lag time is required for the body to build up its immunity against the virus. Hence, a vaccine must be given a few weeks before the individual is likely to be exposed to the virus infection.

Types of Vaccines

Two types of vaccines are available in the market :

[A] Non-Adjuvanted Vaccine

This vaccine has only antigen as its main component. An antigen, as mentioned above, is a chemical substance that will trigger an immune response in the human body and this will cause the body to produce antibodies. Usually virus proteins or weakened viruses are used as vaccine antigens.

[B] Adjuvant Vaccine

This vaccine has two main components — the **antigen** (piece of virus or weakened/killed virus) and an **adjuvant**. An adjuvant is a substance that is added to a vaccine to help in enhancing the immune response of vaccine.

The key advantage of adding an adjuvant is that it reduces the amount of antigen required in the vaccine to create an immune response.

Vaccine Production

Vaccines are produced on commercial scale as they need to be administered to large populations of children and adults to be effective as public health tool.

[A] Generation of Antigen from the Microbe

Vaccine production has several stages, viz. this involves making of an antigen preparation from the microbe. For this, the virus or microbe is grown either in primary cells such as chicken egg-cell lines or cultured human cells. For producing vaccine for a bacterial disease, the concerned bacteria may be grown in bioreactors. The antigen may be a part of the microorganism or it may also be a toxin from the organism. Proteins or parts from the organisms can be generated in yeast, bacteria or cell culture.

[B] Isolation of the Antigens

The antigens are isolated from the cells used to generate it. Weakened or attenuated viruses do not require further purification but recombinant proteins need many operations involving ultrafiltration and/or column chromatography for purification before they are ready for administration.

[C] Addition of Adjuvants of Stabilizers and Preservatives

Once the antigen is developed, the vaccine is formulated by adding adjuvants, stabilizers and preservatives. The role of adjuvants, as mentioned earlier, is to enhance the immune response of the antigen. The stabilizer increases the storage life, and the preservatives allow the use of multidose vials.

Viral vaccines are now available for several viral diseases like polio, measles, hepatitis A, hepatitis C, influenza, German measles, mumps, rabies, etc.

VIRUSES AS RESEARCH TOOLS

We know viruses as pathogens, but they can also be used for the benefit of human. The basic technology of genetic manipulation was developed from studies on bacteria and viruses. Given below are some areas of biological/medical sciences where viruses are being routinely used as model organisms.

Viruses and Gene Therapy

Viruses are widely used in the gene transfer during the production of transgenic plants and animals. In human beings the method has not been attempted to induce modifications in germinal cells due to technical and ethical reasons. However, genetic manipulation of somatic cells of individuals is being attempted for last several years using virus as vectors. In technical terms, this manipulation is known as **gene therapy**.

The objective of gene therapy is to introduce a functional gene into a cell of a human patient to express desired functions or to correct the defective or non-operational genes within these cells. The original idea behind gene therapy was the treatment of individuals with an inherited genetic disorder, but applications in this have been limited. Most of the clinical trials have been made to treat cancer. The common prerequisites for gene therapy are :

- (1) There must be one or very few genes requiring correction for disease control.

- (2) It is essential to identify a suitable therapeutic gene, and
- (3) There must be some method to deliver the gene to the target cell.

Viruses are very good vectors for getting the foreign gene into the desired cells. They have the ability to protect the foreign gene while transporting it to the required area of the body. Several viruses have been used as vectors in clinical trials of gene therapy. Of these adenovirus, retrovirus, vaccinia virus, poxvirus and adeno-associated virus have been most commonly used vectors. A good virus vector is nonpathogenic, integrates into the host genome efficiently and has wide choice of insertion sites.

Viruses in Cancer Control

Though viruses are associated with cancer in human beings, but they also have beneficial applications in the control of cancer. Some viruses are innately able to target and destroy cancer cells. For example, several RNA viruses have innate anti-cancer activity; they produce higher levels of cytotoxicity in cancerous cells. It is therefore, possible that in future some RNA viruses may be found suitable for enhancing cytotoxic activity in targeted cells.

Besides this, prophylactic and therapeutic vaccines are now available for human papilloma virus (associated with cervical cancer) and for hepatitis B virus (associated with hepatocellular carcinoma).

In addition to vaccines, studies have been made to use the cell killing effects of viruses directly. A range of viruses have been used for targeted killing of cancerous cells. This approach of cancer therapy is known as **virotherapy**. It has been observed that virus infection of cancerous cells may enhance both innate and adaptive immune responses. Therefore, such viral infections are beneficial for cancer patients.

Virus may also be used in **virus directed enzyme producing therapy**. In this therapy the virus is used to insert an enzyme into the target cells that can activate an inactive precursor of a cytotoxic drug administered to a patient. Thus the active cytotoxic form of the drug is only produced where the relevant enzyme is present.

Bacteriophage Therapy

As you know bacteriophages are highly specific viruses that invade bacterial cells, disrupt their metabolism and destroy them. They are believed to be the most numerous type of viruses accounting for majority of the 10^{31} viruses present on earth. They occur abundantly in water, with over 10^8 per milliliter recorded from some sources. Majority of bacteriophages are specific to a single host species.

Early experiments on bacteriophages suggested their significant role in disease control. But the early enthusiasm vanished after the discovery of antibiotics. Pharmaceutical companies diverted their research in the area of antibiotics and by 1990s several broad spectrum antibiotics were discovered which were effective against several bacterial fungal infections. But during last decade it has been observed that pathogenic bacteria have developed resistance to several antimicrobial agents, including antibiotics. Moreover, pharmaceutical companies find business of medicines related to cardiac, renal, gastrointestinal and hepatic diseases more lucrative than producing antibiotics. This was the time when phage therapy got revitalized.

In human beings phages can be administered (i) orally in tablet or liquid formulations, (ii) rectally, (iii) locally (skin, eye, ear, nasal mucosa, etc.), (iv) as aerosols, and (v) intravenously. Fortunately there have been no serious complications due to phage therapy in patients. Moreover, phages are extremely common in the environment and are regularly consumed in foods. The treatment uses a single bacteriophage or cocktail of several phages to specifically lyse target pathogenic bacteria.

Bacteriophages are good alternative as therapeutic agents. They remove the pathogen very specifically and leave the other (useful) bacteria intact. This is definitely an advantage over the use of antibiotics which when taken also wipes out several useful bacteria.

Researches have shown the importance of viruses in tracing the travel history of an individual and how the viruses influence the course of a disease. It is known that **Herpes Simplex**

Virus 1 (HSV1) is a common virus that causes cold sores and fever blisters. Most people harbour this virus and the strain (HSV1) is acquired from mothers. Recently scientists identified two distinct strain of this virus inside one person, who passed to strains of HSV-1. One strain of this individual was European/North American and the other was Asian which was likely acquired during the volunteer's military services. Thus in this study HSV-1 strains revealed the person's life-history at molecular level. Using a finger printing technique on HSV-1 could further provide a lot of information that genomes do not provide. Further research could open an altogether new branch — the '**forensic virology**' where viruses could be studied to locate criminals/suspects at a crime scene or trace relatives of unidentified bodies.

Viruses in Pest Control

Use of biological organisms to control damaging pests is known as **biological pest control or biocontrol**. A large variety of organisms are being used to control some important diseases of plants. Biological agents are preferred over chemical agents as their effects are longer lasting and are inherently less toxic than conventional pesticides. However, they account for only a small fragment (approximately 2%) of the total agricultural pesticide usage. It is because of the high specificity and slow effects of biological agents. These agents show less stability in adverse environmental conditions. Moreover, large pesticide companies also do not support their use. Amongst all the microbiological control agents used in agriculture, viral agents account for less than 10 %.

Baculoviruses are a large group of viruses than insects and other arthropods. They are quite specific to the species that they infect. Baculoviruses have thick protein shells around the nucleocapsid that contains viral genome. Therefore, these viruses show high level of environmental stability.

When insects infect the crop, baculoviruses are eaten by insect larvae. They can infect cells of the gut and from there the virus can spread

throughout the body of the insect. Eventually, it results in the death of the insect and a large generation of new viruses is released from the liquified remains of the killed larva.

Viruses have been found to control effectively the larvae of butterflies, moths, rhinoceros beetle, etc.

ECONOMIC IMPORTANCE OF BACTERIA

Bacteria are both our foes as well as our friends. On the one hand, they cause a number of serious diseases of plants and animals, including man. They may threaten the very existence of human race, if there is a bacterial warfare. On the other hand, they run the carbon cycle and the nitrogen cycle thus maintaining the balance of carbon and nitrogen and a number of industries such as dairy, vinegar, tea, tobacco and leather are bound on them. Thus they play an important role in our daily life. Some important useful and harmful activities of bacteria are described below.

USEFUL ACTIVITIES OF BACTERIA

Milk is one of the best complete foods for man and it has high nutritive value. Because of its richness in various nutritional elements, microbes find it a suitable medium for their growth. There are various sources which contaminate milk. The teat openings of cattle are usually contaminated with microbes and the first stream of milk drawn from the udder contains more micro-organisms than the later streams of milk. Subsequent contamination of milk is through air-borne microbes setting in the utensils and milk, through water, drainage, etc.

Various dairy products, such as curd, cheese, butter milk, butter, etc. are obtained by the activity of lactic acid bacteria. These bacteria convert the lactose sugar present in the milk into lactic acid. As a result, the milk becomes sour and the casein protein is separated. Some microbes which ferment

milk are *Lactobacillus acidophilus*, *L. helveticus*, *L. plantarum*, *Streptococcus lactis*, *S. thermophilus* and *Leuconostoc citrovorum*.

Butter is also an important milk product, prepared by churning sour milk. In the process, fat globules are separated and butter milk (skim) is obtained as a by-product. In order to get desired flavour and aroma, a starter culture of *Streptococcus cremoris*, *Leuconostoc citrovorum* or *L. dextranicum* is added to the milk. Mostly pasteurized milk is used for manufacturing butter to ensure that there are no pathogenic micro-organisms.

Cheese making is also an important microbiological process in dairy industry. The first step known as curdling, involves separation of milk protein (casein) from whey. The second step involves ripening of solid curd by the action of bacteria and fungi. Some important microbes associated with cheese manufacturing are *Streptococcus lactis*, *S. cremoris*, *Leuconostoc citrovorum* and *L. dextranicum*. Certain bacteria and yeasts grow on the surface of cheese where they produce enzymes which are responsible for the flavour and aroma of the cheese. For instance, roquefort and camembert cheese are ripened by the blue-moulds, *Penicillium roqueforti* and *P. camemberti* respectively.

Alcoholic Fermentation

Large number of micro-organisms, both prokaryotes (bacteria) and eukaryotes (fungi) are used in alcoholic fermentation. Of these *Saccharomyces cerevisiae* (brewer's yeast) is the most important. Beer, rum, whisky, wine and several other alcoholic beverages are all products of yeast fermentations. The nature of the beverage depends on the material fermented and the strains of yeast used in fermentation. For example, beer is made by yeast fermentation of grains to ethanol and carbon dioxide. Rum is a product of blackstrap molasses fermentation and whisky is a product of corn and rye fermentation. For the production of wine, grapes are fermented by yeast and as such soluble sugars (glucose and fructose) are converted into carbon dioxide and ethyl alcohol. In order to get the desired quality of

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wine, ethyl alcohol is further fermented by using other microbes like *Pediococcus*, *Leuconostoc* and *Lactobacillus*.

Wine is also prepared by fermenting rice grains by *Aspergillus oryzae*.

Butyl alcohol is obtained on fermenting molasses by anaerobic bacteria like *Clostridium acetobutylicum* and *C. butyricum*.

Production of Organic Acids

Several microbes are used in the commercial production of a variety of organic acids.

Citric acid is manufactured by fermenting sucrose and molasses by the species of *Penicillium* and *Aspergillus* (*A. niger*, *A. wentii*).

Itaconic acid is obtained on fermenting glucose or sucrose solution by *Aspergillus itaconicum* and *A. terreus*.

Gluconic acid is prepared by fermenting sugary solution by the species of *Penicillium* like *P. chrysogenum* and *P. perpurogenum*.

Kojic acid is produced when *Aspergillus oryzae* is grown in a medium with sugar plus minerals. The fungus is allowed to grow aerobically at 30-35°C for about 12 days.

Acetic acid is produced by fermenting sugary solutions. It involves : (i) alcoholic fermentation of sugar, and (ii) oxidation of the alcohol to acetic acid. Usually yeast fermentation is used for the production of alcohol. Thereafter, alcohol is exposed to the action of acetic acid bacteria (*Acetobacter* spp).

For the production of lactic acid sugars are fermented by *Lactobacillus* and *Streptococcus lactis*.

Fumaric acid is produced when sugar to which other nutrient salts and calcium carbonate are added is fermented by *Rhizopus stolonifer*.

Production of Enzymes

Several microbes synthesize large quantities of enzymes which can be obtained on a commercial scale. Amylase, zymase, invertase, glucose oxidase, pectinase and protease are some of these enzymes which are synthesized by microbes.

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Amylase is obtained from *Aspergillus oryzae*, *A. niger*, *Rhizopus delamar* and *Bacillus subtilis*. The enzyme is used to hydrolyse starch to dextrin and sugars and in preparing sizes and adhesives, desizing textiles, clarifying fruit juices, manufacturing pharmaceuticals and several other purposes.

Zymase is obtained from *Saccharomyces cerevisiae* and it is used in the production of ethyl alcohol by fermenting carbohydrates.

Invertase is also obtained from *Saccharomyces cerevisiae*. It hydrolyzes sucrose to a mixture of glucose and fructose and is widely used in the production of candy and non-crystallizable syrups from sucrose.

Pectinase is obtained from the species of *Penicillium*. It is used to clarify fruit juices and also to hydrolyse pectins in the stems of flax hemp and jute plants, thus releasing cellulose fibres which are used in the manufacture of linen and burlap, respectively.

Proteases and peptidases are obtained from *Aspergillus oryzae* and *Bacillus subtilis*. Proteases are largely used for baiting and other leather-processing steps, degumming of silks and cleaning in laundries as an adjunct to soap.

Glucose oxidase is produced from *Aspergillus niger* and it is used to remove glucose from egg white and to oxidise glucose to obtain gluconic acid.

Production of Vitamins

Several vitamins are produced by microbes as primary growth products or as bye-products in certain microbial reactions. **Vitamin B₁₂** is commercially produced by *Streptomyces* and *Bacillus* when they are grown in nutritive medium containing sugarcane molasses. **Riboflavin** is produced as a growth product of the mold *Achlya* and *Clostridium acetobutylicum*. Yeast cells are a very good source of **vitamin B** and **D**. **Vitamin A** is produced by *Rhodotorula gracilis*.

Production of Antibiotics and Medicines

Several antibiotics obtained from microbes are mentioned elsewhere in this book.

Micro-organisms are also used in the manufacture of several important medicines. **Ergot**, prepared from a fungus, *Claviceps purpurea*, is used to induce uterine contraction for abortions, menstrual disorders and to check haemorrhages. **e-Ephedrine** is synthesized from benzaldehyde by the activity of yeast, and it is used for the treatment of asthma and nasal troubles. **Cortisones** are prepared by fermentation of glycosides of plants by moulds like *Rhizopus nigricans*, *Aspergillus niger* and species of *Fusarium*, *Cunninghamella* and *Neurospora*. Cortisones are used in the treatment of rheumatoid arthritis.

Besides, a variety of microbiological **antigens** (vaccines) are prepared on commercial scale for the control of infectious diseases through immunization. Development of effective immunizing antigens constitutes major programs in the pharmaceutical industry.

Baking Industry

Saccharomyces cerevisiae is commonly used to **leaven the bread, to produce desired flavour and for conditioning the bread**. Yeast produces CO₂ and alcohol by fermentation of carbohydrates. The carbon dioxide produced during the fermentation is responsible for leavening of dough. The quality of the product depends on the proper selection of yeasts, incubation conditions and choice of raw materials. Some bacteria like *Streptococcus lactis* and *Lactobacillus casei* are used to improve the flavour of dough and quality of the bread.

As Food

A number of micro-organisms are consumed by human beings. *Chlorella*, a **green alga rich in proteins**, is used as food. Several fungi like yeast, *Rhizopus stolonifer*, *Aspergillus oryzae*, *Penicillium notatum* and *Torulopsis utilis* are also rich sources of proteins. **Fats and lipids** are synthesized in large quantities by some microbes and they can be used for industrial manufacture of fat at the time of emergency. *Torulopsis pulcherrima*, *T. lipofera*, *Endomyces vernalis*, *Rhodotorula*

glutinis, *Aspergillus fischeri*, *Penicillium javanicum* and *Geotrichum candidum* are some important fungi which are rich sources of fats. Molasses, waste cellulose material, grain wastes, lactose in whey, etc., can be used as substrates for growing these fungi. A food article, **Indonesian tempeh**, is made by fermenting soaked soybeans with *Rhizopus* spp. Similarly, **shoyu** is prepared by fermenting soybean paste, rice, wheat, etc. by *Aspergillus oryzae*, *Saccharomyces rouxii* and *Lactobacillus*.

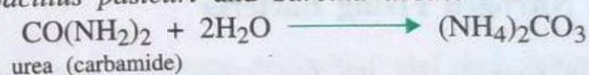
Increase Soil Fertility

Although there is 78% nitrogen in the atmosphere, plants in general do not have the capability of utilizing it directly. However, certain free-living as well as symbiotic bacteria can fix molecular nitrogen into nitrogenous compounds. The nitrogen fixing bacteria can be grouped into the following three categories.

[A] Ammonifying Bacteria

Many saprophytic bacteria hydrolyse the proteins of dead plant and animal matter present in the soil into amino acids and organic nitrogenous bases. The ammonifying bacteria convert amino acids into ammonia (the process is known as **ammonification**). The free ammonia combines with water and carbon dioxide (present in the soil) to form ammonium carbonate. These ammonium compounds are used by many crops as a source of nitrogen. *Bacillus mycoides*, *B. ramosus* and *B. vulgaris* are some important ammonifying bacteria.

Another important source of ammonia in the soil is urea. Animal population of the world excretes more than 2,00,000 tons of urea [CO (NH₂)₂] per day which is decomposed by *Bacillus pasteurii* and *Sarcina ureae*.



[B] Nitrifying Bacteria

Oxidation of ammonia (or ammonium salts) to nitrate is known as nitrification. In nature it is brought about by two highly specialized groups

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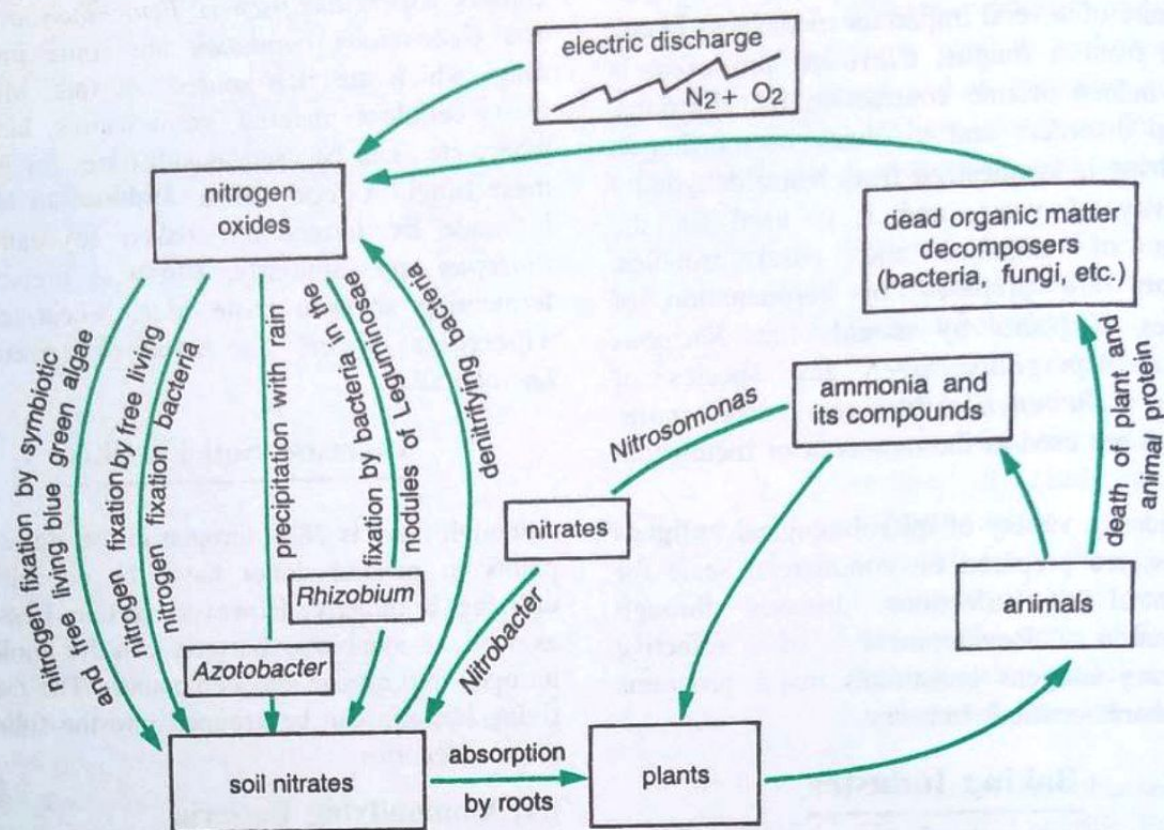
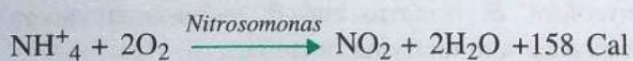


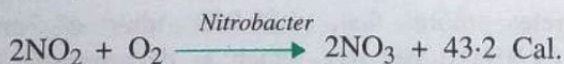
Fig. 1. Nitrogen cycle.

of obligately aerobic chemoautotrophic bacteria. It occurs in two steps.

In the **first step**, ammonia is oxidised to nitrite; the oxidation is brought about by *Nitrosomonas*, *Nitrococcus* or *Nitrospira*.



In the **second step**, nitrite is oxidised to nitrate by *Nitrobacter*.



[C] Nitrogen Fixing Bacteria

These bacteria fix the free nitrogen and are of the following two types.

[I] Symbiotic nitrogen fixers

These bacteria belong to the genus *Rhizobium*. They occur in the root nodules of leguminous

plants. Carbon compounds (i.e., carbohydrate) are supplied to these bacteria by the plant and the bacteria fix nitrogen, producing ammonia and various amino acids. In a field containing legume plants 100-400 kg of nitrogen per hectare can be fixed in a year. Thus leguminous plants increase the utilizable nitrogen in the soil and are therefore, used in crop rotation.

[II] Free-living forms

It is a large and heterogeneous group of nitrogen fixing bacteria which occur free in the soil. Species of *Azotobacter* and *Clostridium* belong to this category. These forms are probably not as important as the symbiotic nitrogen fixers. These are aerobic forms and can also use ammonia as a nitrogen source. Hence they do not fix nitrogen if ammonia is present in the soil. In each hectare of ordinary soil 25-50 kg nitrogen is fixed by these bacteria in a year.

Retting Process

Retting is a controlled microbial decomposition of pectin (the cementing substance which keeps plant tissues together) without simultaneous decomposition of fibres. Thus it results in the separation of fibres. The stems of flax, hemp, etc. are immersed in water for long periods where decomposition sets in. This process is facilitated by anaerobic butyric acid bacteria, such as *Clostridium botulinum*, *C. tetani* and *C. perfringens*. These bacteria primarily decompose the plant pectin, thus freeing fibres. If the process of retting continues for a long period, then cellulose fermenting bacteria develop and destroy the fibres.

Degradation of Petroleum Hydrocarbons

Many bacteria, such as *Pseudomonas*, *Achromobacter*, *Micrococcus* and *Candida* are capable of degrading petroleum hydrocarbons both in marine and fresh water habitats. Amongst these, *Pseudomonas* is the most important genus that can utilize petroleum hydrocarbons. Thus these bacteria can be used to check pollution after oil spillage.

Sewage Disposal

Sewage is the domestic and/or industrial waterborne waste. The domestic sewage includes human excreta and wash water that goes into sewage system. The industrial sewage includes acids, oils, gases and animal and vegetable matter discharged by factories. Sewage contains 99-99.5 per cent water and 0.5-1.2 per cent solids in the form of microscopic particles, colloids, organic compounds and microorganisms. Besides, raw sewage may contain millions of bacteria per milliliter including coliforms, streptococci, anaerobic spore forming bacilli and also types

originating from the intestinal tract of human beings.

The discharge of untreated sewage into rivers and lakes causes : (i) greater dissemination of pathogenic microorganisms, (ii) pollution of drinking water, grasslands and agriculture fields, (iii) increased danger to aquatic life, and (iv) offensive smell. Therefore, it is essential to treat sewage prior to its discharge as this results in the mineralization of the organic components in sewage. A variety of microorganisms (including bacteria, fungi and protozoa), collectively known as sewage fungi, help in the biological degradation of organic matter.

Use of Bacteria in Medicines

Bacteria are also the source of many antibiotics, serums and vaccines. Antibiotics are the chemical substances secreted by certain microorganisms which inhibit the growth and development of other microbes.

The antibacterial property of culture filtrates of *Penicillium notatum* was first observed by Alexander Fleming in 1928 and the active principle of the filtrate, **penicillin**, was isolated by H.W. Florey in 1944. Since then a number of antibiotics have been isolated from bacteria mainly from the species of *Streptomyces* and *Bacillus*.

Antibiotics show the following characteristics.

- (1) They have inhibitory effects only on parasites but donot affect the growth of the host.
- (2) They are effective only when a certain minimum dose is provided.
- (3) They do not interfere with the natural defence mechanism of the host.
- (4) They do not produce undesirable effects (such as allergy, desiccation, etc.) on the host.
- (5) They do not affect the natural microflora of the host.

Some important antibiotics and their source, range of action and mode of action are given in Table 3.

Table 3. Some important antibiotics, their source, range of action and mode of action.

Antibiotics	Source	Range of action	Mode of action
Bacitracin	<i>Bacillus subtilis</i>	Gram + ve bacteria	Inhibits synthesis of bacterial cell walls
Polymyxin G	<i>Bacillus polymyxa</i>	Gram + ve bacteria	Destroys cytoplasmic membranes
Colistin (Polymyxin E)	<i>Bacillus colistinus</i>	Gram + ve bacteria	Destroys cell membrane
Tyrothricin	<i>Bacillus brevis</i>	Gram - ve and cocci	Inhibits formation of bacterial cell wall

Bacteria in Biotechnology

Bacteria are one of the best materials for work on genetic engineering. A gene from a vertebrate animal, including human beings, can be inserted into the DNA of a bacterium. This artificial manipulation of genes within a particular species or between different species is known as **recombinant DNA technology**. In many cases the inserted genes code for commercially useful products.

One of the earliest accomplishment of gene manipulation, involving bacteria, was the production of **human insulin**. This hormone is a small sized protein which consists of two polypeptide chains. In our body this hormone is produced by pancreas and controls the body's uptake of glucose from blood. Shortage of insulin causes diabetes mellitus and the sufferers are forced to use insulin extracted from the pancreas of slaughtered animals. Thus obtaining insulin from animal sources is an expensive process. Moreover, the insulin from animals is not as effective as human insulin.

Human insulin is now produced on commercial scale by recombinant DNA technology. For this synthetic genes coding for the two polypeptide chains of insulin molecule are inserted in the bacterium *E. coli*.

Another human hormone **somatostatin**, which is involved in limiting growth, is now being produced commercially using *E. coli*. Each cell of genetically engineered *E. coli* produces 10,000 molecules of somatostatin. To obtain 5 mg of somatostatin 8 liters of genetically engineered cultures of *E. coli* are required whereas as many as 500,000 sheep brains were needed to get the same amount of hormone from the animal source.

Interferon is an important antiviral human protein produced in response to viral infections.

Transfer of interferon gene into bacteria has been accomplished. Thus it appears possible to use bacteria to manufacture large number of eukaryotic products such as hormones, antibiotics, enzymes, etc., using plasmids as a convenient tool of gene transfer.

Bacteria in Pollution Control

Bacteria are good pollution fighters. They clean up the pollutants and toxic wastes produced by various industrial processes. Bacteria either turn these pollutants into energy sources which they use themselves or produce enzymes that break down toxins into less harmful substances. Use of bacteria to degrade pollutants from soil, underground water, chemical spills, etc., is known as **bioremediation**. Two bacterial genera *Pseudomonas* and *Bacillus* are commonly used bioremedial microbes. Although these bacteria are naturally present in the soil but they degrade the pollutants slowly due to their small number. For example *Pseudomonas* is able to degrade oil for their carbon and energy requirements but the process is too slow to be helpful in cleaning up an oil spill. Scientists have, however, been successful in increasing the population of oil degrading bacteria along the affected beaches by adding nitrogen and phosphorus fertilizers which act as bioenhancers.

Scientists have also successfully developed genetically engineered bacteria which secrete enzymes effective in cleaning drains.

In Production of Bio-gas

Due to oil crisis there is increased interest in use of power alcohol and bio-gas as a supplement to conventional fuels. **Gasohol** (mixture of 90% unleaded gasoline and 10% alcohol) is used in

several countries to combat the energy shortages. However, widespread usage of this fuel mixture depends on the cost of producing alcohol. Large quantities of suitable low-cost raw materials are available for fermentation. Microbial fermentation of sugarcane juice to ethyl alcohol can partially meet energy requirement. **Ethyl alcohol** can also be produced by bioconversion of lignocellulose. Livestock waste can be used for generation of bio-gas (methane) through microbial fermentation. The methane generation is dependent upon microbial conversion of cellulose and other materials to methane under anaerobic conditions. The residue is used as organic fertilizer for crops. Microbes synthesize hydrocarbons and oxygenated compounds, which may serve as liquid fuel. Many marine and fresh water red, brown and green algae, diatoms and phytoplanktons produce heneicosahexane (C_{21}) hydrocarbons. Pentadecane (C_{15}) predominates in brown algae and heptadecane (C_{17}) in red algae.

Hydrogen is a valuable fuel. Several algae such as *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Dunaliella* and *Oscillatoria* have the capability of producing hydrogen from water in sunlight. This process is known as **biophotolysis** and is mediated by the enzyme hydrogenase. However, in cyanobacteria and phototrophic eubacteria, the chief hydrogen-producing enzyme is nitrogenase. This enzyme reduces dinitrogen to ammonia with the use of electrons, protons and ATP. Some of the reduced protons are evolved as hydrogen gas. In cyanobacteria, hydrogen evolution depends upon light which provides electron donors. The pretreatment of *Nostoc muscorum* with sulphide induces a 3- fold stimulation of nitrogenase-catalyzed hydrogen production.

In Petroleum Industry

Micro-organisms are also involved in such diverse areas as petroleum formation, exploration for petroleum, clean-up of oil - spills and deterioration of petroleum products. Many microbes can utilize hydrocarbons as sole sources of carbon and energy and can degrade petroleum hydrocarbons both in marine and fresh water ecosystems. Some important microbes capable of degrading petroleum

hydrocarbons are the species of *Pseudomonas*, *Achromobacter*, *Micrococcus*, *Nocardia*, *Rhodotorula*, *Cunninghamella*, *Penicillium*, *Thermomicrobium*, *Oscillatoria*, *Anabaena*, *Chlorella*, *Dunaliella* and *Porphyridium*. *Clostridium perfringens*, *Vibrio desulphuricans* and *Beggiatoa alba* are some important microbes which are associated with petroleum formation.

In Waste Treatment and Recycling

Microbes play an important role in the **treatment and recycling of sewage, organic and municipal wastes**, and other wastes arising from various industries. Recycled organic wastes can be used as animal feed, fertilizer, fermentable substrates, or soil conditioners. Sewage consists of domestic and industrial water-borne wastes. The application of algal-bacterial systems is of much significance in sewage disposal. The algae release oxygen during photosynthesis and synthesize the bacterial degradation products into new protein-rich algal biomass. Water treatment may be carried out in facultative or integrated ponds. These ponds are suitable for treating municipal sewage and other domestic wastes. Certain algae, such as *Spirulina*, *Scenedesmus* and *Chlorella*, are suitable for this purpose. The mass cultures of these algae in the system are used as animal feeds. Concentrated organic wastes are best treated in anaerobic lagoons which have a diverse variety of micro-organisms that generate methane and carbon dioxide. Besides, some protozoans (e.g., *Opercularia microdiscum*, *Vorticella convallaria*, *Chilodonella uncinata* and *Trachelophyllum pusillum*) play an important role in aerobic sewage treatment. They occur in plenty in both percolating filters and in activated sludge plants.

In Mining

The traditional method of processing ores is a major cause of air pollution. In recent years, micro-organisms have been used in the recovery of minerals from ores without any pollution. Some autotrophic aerobic bacteria like *Thiobacillus ferrooxidans* and *T. thiooxidans* when grown in the presence of copper ores produce acid and

affect oxidation of the ore with subsequent precipitation of the metal. The technique improves the recovery of the metal from the ore and there is no pollution of the atmosphere.

Microbial Immobilization

Immobilized microbial cells of *Escherichia coli*, *Pseudomonas*, *Streptococcus*, *Saccharomyces*, *Aspergillus*, *Micrococcus*, *Streptomyces*, *Enterobacter*, *Nocardia*, *Rhodotorula*, etc. have various applications. They are used to produce amino acids, malate, glutathione, fructose, glucose, ATP, NADP, coenzyme A, vitamin B₁₂ and menthol. *Anabaena*, when adsorbed on glass beads, breaks up water in the presence of light, producing hydrogen gas. Immobilized yeast cells produce ethanol from molasses.

In Bioassay and Biological Warfare

Micro-organisms are found to be useful in several biological studies. Many genetical and biochemical studies are being conducted on micro-organisms. The studies on fine structure of genes and their synthesis were possible only on microbes. Some micro-organisms can be used to assay the potency of chemical substances, such as drugs, vitamins, antibiotics and amino acids. Thus they have practical applications in pharmaceutical industries. Microbiological assays are highly specific and unusually sensitive. For instance, as little as 0.1 nanogram (0.1^{-9} g) per ml of vitamin biotin can be detected by using *Lactobacillus casei*. Similarly, *Neurospora crassa* is proved useful for the detection of vitamin B in a sample. Besides, several microbes like *Aspergillus niger* are used in the detection of traces of zinc, nickel, copper, etc. in a sample. *Azotobacter* is used as test organism to detect the presence of potassium and phosphorus in the soil.

Some microbes act as good pollution indicators. For instance, *Clostridium perfringens* acts as a water quality indicator, especially for recreational water quality and as a monitoring agent for examining chlorinated drinking water in distribution system.

Now-a-days, many harmful and pathogenic microbes (e.g., *Coccidioidomyces*) are used in **biological warfare** to cause disease and death in enemy camp.

In recent times, microbes have found a prominent place in space research. They have been found to be the most useful and the cheapest forerunners of man to explore the space and bring back the required information. Recent studies have demonstrated the capability of bacteria, fungi and viruses to survive fairly long periods of residence in space. Fungi have been sent into space aboard balloons, earth satellites and rockets. Certain yeasts (*Saccharomyces cerevisiae* and *Rhodotorula rubra*) and *Neurospora crassa* showed mutational and physiological damage in space.

HARMFUL ACTIVITIES OF BACTERIA

Pathogenic Activities

Pathogenic ability of bacteria was first recognised by Burrill in as early as 1878. He discovered that fire blight of apple is caused by a bacterium. Today we know that more than 180 diseases of plants are caused by bacteria.

The infection of bacteria to plants gives rise specific symptoms, such as galls, imperfect flowers and fruits, blight, brooming, canker, leaf distortion, leaf spot, dwarfing, rot and wilting. Some important diseases of plants caused by bacteria are listed in Table 1.

There are also many pathogenic bacteria which occur in animals and human beings. They have a characteristic portal of entry and exit into the organism and mode of transmission. They infect almost all systems of the human body and are easily transported to various parts through circulatory system. Some important diseases of human beings caused by bacteria are listed in Table 2.

Some pathogenic bacteria destroy the cells of the host, but most cause diseases because they produce poisonous substances that damage the

Table 1. Some important bacterial diseases of plants.

Diseases	Pathogens
Leaf spot of cherry	<i>Coccomyces hiemates</i>
Ring spot of potato	<i>Xanthomonas solanacearum</i>
Blight of walnut	<i>Xanthomonas juglandis</i>
Blight of paddy	<i>Xanthomonas oryzae</i>
Bacterial spot of peach	<i>Xanthomonas prunii</i>
Citrus canker	<i>Xanthomonas citri</i>
Angular leaf spot of cotton	<i>Xanthomonas malvacearum</i>
Blight of bean	<i>Pseudomonas phaseolicola</i>
Crown gall of sugar beet	<i>Agrobacterium tumefaciens</i>
Wilt of tobacco	<i>Phytophthora solanacearum</i>
Soft rot of mango	<i>Bacterium carotovorus</i>
Tundu of wheat	<i>Corynebacterium tritici</i>

host's metabolism. These substances are called **toxins**. Bacterial toxins may be divided into two classes : **endotoxins** and **exotoxins**. **Endotoxins** (inside toxins) are lipopolysaccharides in the cell walls of Gram '-ve' bacteria; they produce fever and damage to the circulatory system. **Exotoxins** (outside toxins) are much less common than endotoxins; they are proteins and are secreted from the bacterium into the surrounding medium. They

Table 2. Some important bacterial diseases of human beings.

Diseases	Pathogens
Cholera	<i>Vibrio cholerae</i>
Bacterial dysentery	<i>Shigella dysenteriae</i>
Plague	<i>Yersinia pestis</i>
Rheumatic fever	<i>Streptomyces</i> sp.
Tuberculosis	<i>Mycobacterium tuberculosis</i>
Pneumonia	<i>Streptococcus pneumoniae</i>
Typhoid	<i>Salmonella typhi</i>
Jaundice	<i>Leptospira icterohaemorrhagiae</i>
Diphtheria	<i>Corynebacterium diphtheriae</i>
Gastro-enteritis	<i>Escherichia coli</i>
Syphilis	<i>Treponema pallidum</i>
Gonorrhoea	<i>Neisseria gonorrhoeae</i>
Bacterial conjunctivitis	<i>Haemophilus influenzae</i>
Tetanus	<i>Clostridium tetani</i>
Diarrhoea	<i>Bacillus coli</i>

may be carried around the host's body in the blood stream. Bacteria causing diphtheria, tetanus, cholera, dysentery, etc. produce exotoxins.

Food Spoilage

Most of the food materials are excellent media for the growth of bacteria. They grow luxuriantly when temperature and humidity are favourable. Bacteria change the flavour, smell and appearance of food material thus affecting its quality. Carbohydrate fermenting bacteria convert carbohydrate foods into acids, alcohols and gases; lipolytic bacteria convert fatty foods into fatty acids and glycerol and proteolytic bacteria convert protein foods into amino acids, amines, ammonia, etc.

Milk is sterile when it leaves a healthy cow, but it contains several types of bacteria by the time it reaches the table.

Species of *Lactobacillus*, *Streptococcus*, *Micrococcus* and *Proteus* are responsible for spoilage of milk and milk products. The exotoxins produced by these bacteria are the cause of food poisoning. Besides, bacteria causing diseases like tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*) are transmitted through cow's milk. The botulism disease is caused by the exotoxins produced by *Clostridium botulinum*. Swelling of tongue, double vision and respiratory paralysis are main symptoms of this disease.

Many bacteria multiply in water and makes it unpotable. Bacteria causing typhoid fever (*Salmonella typhi*), bacterial dysentery (*Shigella dysenteriae*) and cholera (*Vibrio cholerae*) are generally transmitted through water.

Denitrification

In this process, decomposition of nitrates and nitrites into ammonia and free nitrogen takes place under the influence of denitrifying bacteria, such as *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Escherichia coli*. These bacteria use nitrate as oxidant in respiration. In this process the combined nitrogen of the soil is removed in the form of gas. Thus the soil is depleted of an essential nutrient for plants and thereby decreasing agriculture productivity.

Important Questions

►► Long answer questions

1. Describe some important industrial processes in which microbes play an important role.
2. Write an essay on the usefulness of microbes from industrial point of view.
3. Write an essay on the economic importance of bacteria.
4. All bacteria are not our enemies. Comment upon the statement.
5. Bacteria are our greatest friends and enemies. Illustrate the statement with the help of suitable examples.
6. Write short notes on:
 - (i) Useful activities of bacteria; (ii) Harmful activities of bacteria; (iii) Citrus canker.
7. Microbiology is good friend of mankind. Prove this by giving examples.
8. The beautiful effects of bacteria outweigh the harmful effects they do. Justify.
9. Give an account of application of microbiology.
10. Give the structure of the bacterial cell and describe its harmful activities.
11. What would happen if all bacteria suddenly disappear from the earth? Discuss.
12. 'Bacteria are good pollution fighters'. Comment upon the statement.

►► Short answer questions

1. Write in brief the application of microbiology in dairy industry.
2. 'Micro-organisms are found to be useful in several biological studies'. Comment upon the statement.
3. 'In recent times, microbes have found a prominent place in space research'. Comment upon the statement.
4. 'The nature of the beverage depends on the material fermented and the strains of yeast used in fermentation'. Comment upon the statement.
5. 'Microbes play an important role in agriculture in many ways'. Comment upon the statement.
6. Give the symptoms of citrus canker disease.
7. Write a brief note on the importance of bacteria in industries.
8. Why will food keep longer in cold storage than under ordinary conditions?
9. Our foodstuffs become unfit for human consumption more quickly in summer than in winter. Why?
10. Write a note on the phenomenon of 'bioremediation'.
11. Name five bacterial diseases of plants. Write their causal organisms also.
12. 'Bacteria help in increasing soil fertility.' Comment upon the statement.
13. Write important characteristics of a good antibiotic. Name a few antibiotics obtained from bacteria.
14. 'Bacteria are wonderful for work on genetic engineering.' Justify the statement.
15. Name few species of bacteria responsible for spoilage of milk and milk products.

►► Very short answer questions

1. Name a fungus species which is edible.
2. Name a species of fungus which is a source of antibiotic.
3. Name a fungal genus some species of which are used for the manufacture of special kinds of cheese and other species yield an important antibiotic.
4. Name a fungus whose extract of the sclerotia can be chemically altered to produce a powerful hallucinogenic drug.
5. Yeast cells are the best source of which vitamin.
6. Which fungus is used to bring about alcoholic fermentation?
7. Name two species of microbes which ferment milk.
8. Name the microbe which is commonly used in baking industry.
9. Name a microbe which is the source of microbial insecticide.
10. Name three microbes which are associated with petroleum formation.
11. What is sewage?
12. Name a microbe which is used in biological warfare.
13. Name any two plant diseases caused by bacteria.
14. Name the nitrogen fixing bacterium of root nodule in a leguminous plant.
15. Name a symbiotic bacterium.
16. Name a micro-organism which is usually found associated with the urinogenital organs of human beings.
17. Which micro-organism causes 'crown gall of apple'?
18. Name an insecticidal bacterium.
19. Name two human disease causing bacteria.

20. Name the causal organism of citrus canker disease.
21. Name the bacteria responsible for the following diseases: typhoid, tetanus and cholera.
22. Name a free-living nitrogen fixing bacterium.
23. Write the causal organism of ring spot of potato.
24. Name the bacterium responsible for botulism disease.
25. Name two species of bacteria which are useful in vinegar industry.
26. From which fungus an antibiotic penicillin was first obtained.
27. Name a bacterium which is capable of degrading petroleum hydrocarbons.

►► True and false statements

1. Beer, rum, whisky, wine and several other alcoholic beverages are all products of yeast fermentation.
2. Kojic acid is produced when *Aspergillus oryzae* is grown in a medium with sugar plus minerals.
3. Cortisones are used in the treatment of rheumatoid arthritis.
4. Many genetical and biochemical studies are being conducted on micro-organisms.
5. Some microbes act as good pollution indicators.
6. *Rhizobium* is freely found living in the soil while *Azotobacter* in root nodules.
7. *Azotobacter* is a heterotrophic nitrogen fixing bacterium.
8. Crown gall formation is a viral symptom.
9. Citrus canker is a viral disease.
10. Pathogenic ability of bacteria was first recognized by Burrill in as early as 1878.
11. Blight of paddy is a bacterial disease.
12. Tetanus is caused by *Treponema pallidum*.
13. Species of *Lactobacillus*, *Streptococcus*, *Micrococcus* and *Proteus* are responsible for spoilage of milk and milk products.
14. The ammonifying bacteria convert ammonia into amino acids.
15. Many bacteria secrete crystalline proteins which are highly toxic to the larvae of caterpillars and insects belonging to Lepidoptera.

►► Fill in the blanks

1. Wine is prepared by fermenting rice grains by.....
2. Gluconic acid is prepared by fermenting sugary solution by the species of.....
3. Various dairy products are obtained by the activity of.....bacteria.
4. Glucose oxidase is produced by the microbe, named
5. e-Phedrine is synthesized from benzaldehyde by the activity of
6. The causative organism for tetanus is and for tuberculosis
7. Death of cells, tissues or organs of the host due to parasitic infection is known as
8. The resistance of the host towards a pathogenic microbe and its toxin is known as
9. Diseases which occur all over the world and cause mass mortality are known as diseases.
10. The causal organism of angular leaf spot of cotton is
11. The causal organism of citrus canker disease is
12. *Clostridium* produces by fermenting molasses.
13. is a free living whereas is a symbiotic nitrogen fixing bacteria.
14. Bacterium belonging to the genus is associated with dextran production.
15. Two bacterial genera, and are commonly used bioremedial microbes.

►► Multiple choice questions

1. The following species of *Penicillium* is used for the manufacture of camembert cheese:
 - (a) *P. camemberti*
 - (b) *P. roqueforti*
 - (c) *P. notatum*
 - (d) *P. chrysogenum*
2. Butyl alcohol is obtained by fermenting molasses by bacteria like:
 - (a) *Leuconostoc citrovorum*
 - (b) *Clostridium acetobutylicum*
 - (c) *Lactobacillus helveticus*
 - (d) *Thiobacillus thiooxidans*
3. The following microbe is used in biological warfare:
 - (a) *Coccidioidomyces*
 - (b) *Rhodotorula*
 - (c) *Neurospora*
 - (d) *Aspergillus*
4. The following microbe is capable of degrading petroleum hydrocarbons:
 - (a) *Achromobacter*
 - (b) *Nocardia*
 - (c) *Thermomicrobium*
 - (d) all the above

5. Bacteria which, in association with legume roots, fix atmosphere nitrogen are called:
 - (a) *Azotobacter*
 - (b) *Rhizobium*
 - (c) *Pseudomonas*
 - (d) *Escherichia coli*
6. The large distribution of clean quality milk all over the world can be said to be due to the great work of:
 - (a) Blackman
 - (b) Louis Pasteur
 - (c) A. V. Leewenhoek
 - (d) Robert Koch
7. The bacterium that causes 'botulism' belongs to the genus:
 - (a) *Clostridium*
 - (b) *Pseudomonas*
 - (c) *Bacillus*
 - (d) *Staphylococcus*
8. Some bacteria live symbiotically inside root nodules of higher plants. The basic activity of these bacteria is associated with:
 - (a) oxygen cycle
 - (b) carbon cycle
 - (c) nitrogen cycle
 - (d) water cycle
9. A free living bacterium capable of fixing nitrogen is:
 - (a) *Rhizobium*
 - (b) *Azotobacter*
 - (c) *Pseudomonas*
 - (d) *Streptococcus*
10. Biodegradable plastics are made by using which of the following bacterial compounds?
 - (a) proteins
 - (b) lipids
 - (c) alkaloids
 - (d) poly β -hydroxy alconates
11. The micro-organism which fixes atmospheric nitrogen is:
 - (a) *Lactobacillus*
 - (b) *Pseudomonas*
 - (c) *Rhizobium*
 - (d) none of the above
12. Citrus canker is caused by:
 - (a) *Bacillus* sp.
 - (b) *Pseudomonas* sp.
 - (c) *Xanthomonas* sp.
 - (d) none of the above
13. Citrus canker is a:
 - (a) bacterial disease
 - (b) algal disease
 - (c) viral disease
 - (d) fungal disease
14. Bacteria which directly converts atmospheric nitrogen into nitrates are called:
 - (a) nitrifying bacteria
 - (b) nitrogen fixing bacteria
 - (c) denitrifying bacteria
 - (d) purifying bacteria

ANSWERS

►►Very short answer questions

1. *Agaricus campestris*, 2. *Penicillium notatum*, 3. *Penicillium*, 4. *Claviceps purpurea*, 5. vitamin B, 6. *Saccharomyces*, 7. *Lactobacillus acidophilus*, *Streptococcus lactis*, 8. *Saccharomyces cerevisiae*, 9. *Bacillus thuringiensis*, 10. *Clostridium perfringens*, *Vibrio desulphuricans*, *Beggiatoa alba*, 11. domestic and industrial water-borne wastes, 12. *Coccidioidomyces*, 13. Citrus canker caused by *Xanthomonas citri*, blight of paddy caused by *Xanthomonas oryzae*, 14. *Rhizobium* spp., 15. *Rhizobium*, 16. *Trichomonas vaginalis*, 17. *Agrobacterium tumefaciens*, 18. Crystal producing bacilli, 19. *Clostridium tetani* causing tetanus, *Treponema pallidum* causing syphilis, 20. *Xanthomonas citri*, 21. *Salmonella typhi*, *Clostridium tetani*, *Vibrio cholerae*, 22. *Azotobacter*, 23. *Xanthomonas solanacearum*, 24. *Clostridium botulinum*, 25. *Acetobacter aceti*, *Clostridium acetobutylicum*, 26. *Penicillium notatum*, 27. *Pseudomonas*.

►►True and false statements

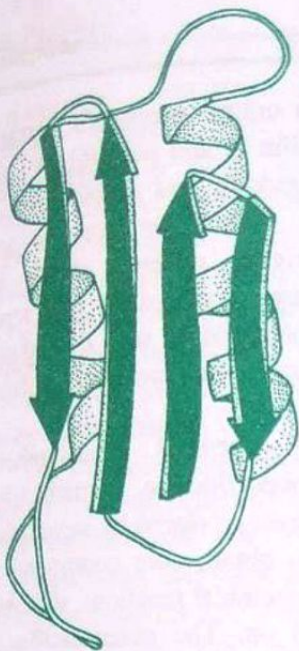
1. True, 2. True, 3. True, 4. True, 5. True, 6. False, 7. True, 8. False, 9. False, 10. True, 11. True, 12. False, 13. True, 14. False, 15. True.

►►Fill in the blanks

1. *Aspergillus oryzae*, 2. *Penicillium*, 3. lactic acid, 4. *Aspergillus niger*, 5. yeast, 6. *Clostridium tetani*, *Mycobacterium tuberculosis*, 7. necrosis, 8. immunity, 9. pandemic, 10. *Xanthomonas malvacearum*, 11. *Xanthomonas citri*, 12. butyric acid, 13. *Azotobacter*, *Rhizobium*, 14. *Leuconostoc*, 15. *Pseudomonas*, *Bacillus*.

►► Multiple choice questions

1. (a), 2. (b), 3. (a), 4. (d), 5. (b), 6. (b), 7. (a), 8. (c), 9. (b), 10. (c), 11. (c), 12. (c), 13. (a), 14. (b).



9

Antibiotics

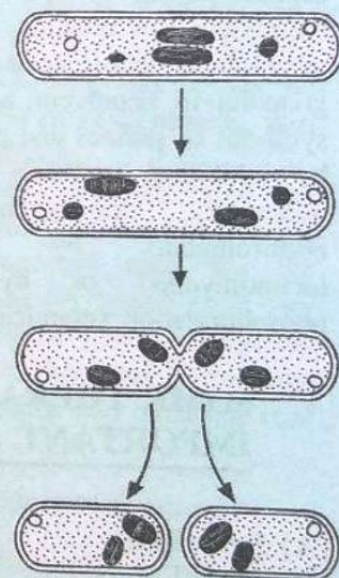
Antibiotics are metabolic products of one organism that is detrimental or inhibitory to other micro-organisms in very small amounts. The study of antibiotics began in 1928, when Alexander Fleming noticed that a fungal colony (*Penicillium notatum*) inhibited the growth of staphylococci. The compound excreted by this fungus could diffuse in the agar and was named **penicillin**. Since then a great many compounds with antibiotic actions have been isolated.

The ability to produce antibiotics has been found mainly in fungi of the group Aspergillales, in actinomycetes, and in a few other bacteria. Streptomycetes are remarkable for the chemical diversity of antibiotics that they produce. More than 2,000 antibiotics have been characterised so far, but only about 50 or so are used therapeutically. But the few effective substances have radically changed the entire medical practice of treating infectious diseases.

QUALITIES OF AN IDEAL ANTIBIOTIC

An ideal antibiotic should possess the following qualities:

- (1) It should have the ability to destroy or inhibit specific pathogenic microorganisms without injuring host cells.
- (2) It should not cause the development of resistant forms of the parasites.
- (3) It should not produce any undesirable side effects in the host, such as allergic reactions, nerve damage, or irritation of the gastro-intestinal tract.
- (4) It should not eliminate the normal microbial flora of the host.
- (5) It should be able to be given orally without inactivation by gastric juices, or by injection without binding to blood proteins.



- (6) It should have a high level of solubility in body fluids.
- (7) It must contact with the parasite by penetrating the cells of the host in effective concentrations.
- (8) It should be a **broad spectrum antibiotic**.

MODE OF ACTION OF ANTIBIOTICS

The action of antibiotics depends mainly on their chemical nature, concentration of the preparation, the particular species of the organism and conditions under which the microorganism functions. The biological mechanism of antibiotics is studied to determine the disorders provoked in the microbial cell by antibiotics, to determine the sites of their main attack in the chain of metabolic reactions, the molecular principles underlying the action of antibiotics, and also the cause of inefficiency of antibiotics against forms of microbes and macroorganisms resistant to it. In general, the mechanism of biological action of the antibiotics are quite specific. They may exert their antimicrobial activity by inhibiting the synthesis of the cell wall (penicillins, bacitracin, cephalosporin), by upsetting the function of the membrane (gramicidins, nystatin), by inhibiting synthesis of nucleic acids selectively (actinomycin, griseofulvin, neomycin, novobiocin), by inhibiting synthesis of purines and pyrimidines (sarcomycin), by inhibiting synthesis of protein (bacitracin, neomycin, tetracyclines, chloramphenicol, erythromycin), by inhibiting respiration (actinomycins) or by inhibiting oxidative phosphorylation (gramicidins).

SOME THERAPEUTICALLY IMPORTANT ANTIBIOTICS

[A] Penicillins

The first of the modern antibiotics, and still among the most useful and widely used are the **penicillins**. Structurally, all penicillins have a common core, a fused β -lactam thiazolidine ring (β -aminopenicillanic acid); different side chains

give each penicillin its unique properties. Natural penicillins (e.g., penicillin G and penicillin V) are produced during the growth and metabolism of certain fungi, namely *Penicillium notatum* and *P. chrysogenum*. Several different kinds of penicillin molecules are produced by the same culture. They can be prepared as salts of sodium, potassium, and other bases and are freely soluble in water.

In recent times, several semisynthetic penicillins (e.g., phenethicillin, methicillin, ampicillin, etc.) have been produced by developing culturing techniques to obtain core compound in quantity and then, by chemical reactions, different side chains are added on. The phenethicillin is more readily absorbed than penicillin V and just as effective as penicillin G. The methicillin is comparatively more resistant to penicillinase, whereas ampicillin is strongly bactericidal and lacks toxicity.

Penicillins mainly act upon actively growing bacteria. They inhibit bacterial cell wall formation by preventing the incorporation of N-acetyl muramic acid, produced within the cell, into the mucopeptide structure that normally constitutes the rigid bacterial cell wall. Penicillin sensitive bacterial cells grown in presence of this antibiotic are abnormally large and have unusual shapes.

Penicillin is the most valuable and powerful means to treat diseases caused by cocci and some anaerobic bacilli. It is most effective against gram '+ve' bacteria, particularly pneumococci, β -hemolytic streptococci, and some staphylococci, some gram '-ve' bacteria (meningococci and gonococci); and the spirochete that causes syphilis. Penicillin is practically non-toxic to humans, except for a small percentage who develop allergic reactions. The high curative properties of penicillin and its extremely low toxicity account for its wide use in medicine. Penicillin is widely used in surgery; it is very helpful in treatment of osteomyelitis, carbuncles, infected wounds, and other diseases. Penicillin is very effective in peritonitis and pneumonia.

[B] Cephalosporins

Cephalosporins are again β -lactam group of antibiotics, derived from strain *Cephalosporium*

accremonium having therapeutic activity against typhoid. In chemical structure, cephalosporin is close to penicillins. As for instance cephalosporin N resembles penicillin G, while cephalosporin C resembles penicillin N. Several semisynthetic cephalosporins (e.g., cephalothin, cephaloridin, cephalexin) are produced by substitution of T aminocephalosporamic acid, obtained from cephalosporin, with various side chains.

The cephalosporins, like the penicillins, exert their antibacterial effect by inhibiting the synthesis of the bacterial cell wall. They are not destroyed by penicillinase, and some are stable at acidic pH.

Cephalosporins are active against many gram '+ve' and gram '-ve' bacteria and are most effective in urinary and respiratory tracts infections.

[C] Griseofulvin

Griseofulvin is commercially produced from a mutant strain of *Penicillium patulum* using stirred aerated fermenters.

Griseofulvin has no antibacterial activity but is highly active against fungi. It interferes with cell wall chitin biosynthesis in growing hyphae. Besides, it also interferes with synthesis of nucleic acid, protein, maleic acid and causes destruction of organelle membrane.

Griseofulvin is used in the treatment of many superficial fungal infections of the skin and body surfaces, such as ring worm. It is also effective in the treatment of some systemic mycoses.

[D] Streptomycin

Streptomycin belongs to the chemical class of antibiotics known as aminoglycosides. It was first isolated by Waksman and his associates (1944) from the culture medium of *Streptomyces griseus*.

Dihydrostreptomycin, spectinomycin, neomycin, kanamycin, gentamicin and tobramycin are some other important antibiotics belonging to this group.

Streptomycin exerts its antimicrobial action through combination with and distortion of

ribosome subunits, thus interfering with protein synthesis.

Streptomycin achieved its clinical success because it was effective against a series of acid-fast and gram '-ve' bacteria that were not sensitive to penicillin. It can give rise to quite marked allergic side reactions in humans. Spectinomycin is more suitable for the treatment of gonorrhoea in persons who are allergic to penicillin. Gentamicin is especially active against some strains of *Pseudomonas*. Neomycin is usually used in the form of lotions and ointments for local application against skin and eye infections. Kanamycin is used to treat tuberculosis; it is also helpful against staphylococcal diseases and to control anthrax, gonorrhoea, and other infections, against which other antibiotics are ineffective. Besides, streptomycin is also used in veterinary medicine and against plant infections.

[E] Tetracyclines

The tetracyclines are excreted by a number of streptomycetes, including *Streptomyces aureofaciens*. The best known are chlorotetracycline (aureomycin), oxytetracycline (terramycin), and tetracycline. They are also closely related chemically, being derived from a naphthacene skeleton.

The tetracyclines act by blocking the binding of RNA (aminoacyl t-RNA) to a specific site on the ribosome during peptide chain elongation, thus inhibiting the protein synthesis.

The tetracyclines are well tolerated by humans. They are regarded as broad-spectrum antibiotics and are effectively used for the treatment of infections caused by many gram '-ve' and some gram '+ve' bacteria. Chlorotetracycline is successfully used to treat bacterial pneumonia, pertussis, scarlet fever, anthrax, and other bacterial diseases.

[F] Chloromycetin (Chloramphenicol)

Chloromycetin was first discovered in cultures of *Streptomyces venezuelae*, but now, it is also produced synthetically. It is very stable and a broad-spectrum antibiotic effective against many

gram '-ve' and gram '+ve' bacteria, spirochaetes, rickettsiae and actinomycetes.

Chloromycetin exerts its antimicrobial action by combining with ribosome subunits, interfering with protein synthesis.

The antibiotic is a specific preparation to treat typhoid and paratyphoid fevers, dysentery, toxic dyspepsia, trachoma, and other diseases.

[G] Erythromycin

Erythromycin was first derived by Selman Waksman (1952) from *Streptomyces erythreus*, isolated from a sample of Philippine soil. It belongs to **macrolide** group of antibiotics. They are characterised by the presence of a macrocyclic lactone ring connected with one or several sugar residues. Erythromycin is active against most gram '+ve' bacteria, some gram '-ve' bacteria (*Neisseria* spp., *Bordetella pertussis*, etc.), and pathogenic spirochaetes.

Erythromycin shows its antimicrobial activity by interacting with ribosome subunits to prevent

the normal sequence of reactions for protein synthesis.

Erythromycin is specially used to treat many infections caused by staphylococci, streptococci, and pneumococci. It is effective against pneumonia, tonsillitis, sepsis, wound infections and diphtheria.

[G] Polymyxin

The antibiotic **polymyxin** is obtained by a bacterium *Bacillus polymyxa*. Chemically, it is a **polypeptide** antibiotic consisting of a ring of seven amino acids, with a side chain attached by a peptide bond. They are active against many gram '-ve' bacteria including *Pseudomonas aeruginosa* frequently involved in infections of the urinary tract or persons with extensive skin burns.

The antibiotic exerts its antibiotic activity by damaging the cell wall structure.

Polymyxins are used to treat meningitis, infections of air passages and the urinary system. Besides, polymyxin ointments are used to treat

Table 1. Some important antibiotics and their source, range of action and mode of action.

Antibiotic	Source	Range of action	Mode of action
A. Antibiotics obtained from actinomycetes			
1. Streptomycin	<i>Streptomyces griseus</i>	Gram '+ve' and Gram '-ve' bacteria tuberculosis bacteria	Induces abnormal protein synthesis by inhibiting 30s ribosome function.
2. Neomycin	<i>Streptomyces fradiae</i>	Gram '-ve' bacteria (inhibits most of the intestinal bacteria)	Induces abnormal protein synthesis by inhibiting 30s ribosome function.
3. Viomycin	<i>Streptomyces puniceus</i>	Gram '-ve' bacteria, including tuberculosis bacteria	Interferes with protein synthesis.
4. Chlorotetracycline (Aureomycin)	<i>Streptomyces aureofaciens</i>	Gram '+ve' and Gram '-ve' bacteria, <i>Treponema</i> , rickettsia	Interferes with protein synthesis.
5. Oxytetracycline (Terramycin)	<i>Streptomyces rimosus</i>	Gram '+ve' and Gram '-ve' bacteria, rickettsia	Interferes with protein synthesis.
6. Novobiocin	<i>Streptomyces niveus</i>	<i>Proteus</i> and <i>Diplococcus</i>	Interferes with protein synthesis.
7. Chloramphenicol (Chloromycetin)	<i>Streptomyces venezuelae</i>	Gram '+ve' and Gram '-ve' bacteria, rickettsia	Inhibits protein synthesis by interfering with the ribosome function.
8. Erythromycin	<i>Streptomyces erythreus</i>	Gram '+ve' bacteria	Inhibits protein synthesis by interfering with 50s ribosome function.
9. Linomycin	<i>Streptomyces</i> spp.	Gram '+ve' bacteria	Interferes with protein synthesis.
10. Rifamycin	<i>Streptomyces mediterranei</i>	Tuberculosis bacteria	Interferes with protein synthesis.

Contd.

11. Nystatin (Mycostatin)	<i>Streptomyces noursei</i>	Fungal infections due to <i>Candida</i>	Damages cell membrane.
12. Kanamycin	<i>Streptomyces kanomyceticus</i>	Most Gram '-ve' bacteria except <i>Pseudomonas</i>	Induces abnormal protein synthesis.
13. Gentamicin	<i>Micromonospora purpurea</i>	Gram '+ ve' and Gram '-ve' bacteria	Induces abnormal protein synthesis.
14. Vancomycin	<i>Streptomyces orientalis</i>	Gram '+ ve' bacteria	Interferes with protein synthesis.
15. Amphotericin	<i>Streptomyces orientalis</i>	Deep-seated mycotic infections	Interferes with membrane function.
B. Antibiotics obtained from bacteria			
16. Bacitracin	<i>Bacillus subtilis</i>	Gram '+ ve' bacteria	Inhibits synthesis of bacterial cell walls.
17. Polymyxin B	<i>Bacillus subtilis</i>	Gram '- ve' bacteria	Destroys cytoplasmic membrane.
18. Colistin (Polymyxin E)	<i>Bacillus colistinus</i>	Gram '- ve' bacteria	Destroys cell membrane.
19. Tyrothricin	<i>Bracillus brevis</i>	Gram '- ve' cocci	Inhibits synthesis of bacterial cell walls.
C. Antibiotics obtained from fungi			
20. Penicillin	<i>Penicillium notatum</i> , <i>P. chrysogenum</i>	Gram '+ ve' bacteria	Inhibits cell wall synthesis.
21. Proliferin	<i>Aspergillus proliferans</i>	Tuberculosis	Inhibits growth.
22. Isopenicillin N	<i>Penicillium chrysogenum</i>	Bacteria	Inhibits growth.
23. L-sarcin	<i>Aspergillus giganteus</i>	Tumour	Inhibits growth.
24. Ramycin	<i>Mucor remannianus</i>	Bacteria	Inhibits growth.
25. Campestrin	<i>Psalliota campestris</i>	Bacteria	Inhibits growth.
26. Ustilagic acid	<i>Ustilago maydis</i>	Fungi	Upsets normal growth.
27. Chaetomin	<i>Chaetomium cochloides</i>		
28. Ergotine	<i>Claviceps purpurea</i>		
29. Citrinin	<i>Penicillium citrinum</i>		
30. Jawaharene	<i>Aspergillus niger</i>	Bacteria, tumours	Inhibits growth.
31. Baccitin A	<i>Gibberella baccata</i>	Bacteria, fungi	Upsets normal growth.
32. Cephalosporin	<i>Cephalosporium</i> spp.	Gram '+ ve' and Gram '- ve' bacteria	Inhibits cell wall synthesis.
33. Fumagillin	<i>Aspergillus fumigatus</i>	Amoebal, staphylococcal bacteriophage	Inhibits growth and multiplication.
34. Griseofulvin	<i>Penicillium nigricans</i> , <i>P. urticae</i> , <i>P. griseofulvum</i>	Fungi	Upsets normal growth.
35. Trichothecin	<i>Trichothecium roseum</i>	Fungi, virus	Arrests growth.
36. Gliotoxin	<i>Trichoderma</i> spp.		
37. Puberulic acid	<i>Penicillium puberulum</i>		

some forms of eczema, furuncles, and other skin diseases.

[I] Bacitracin

Bacitracin is produced by *Bacillus subtilis*. It is also a polypeptide antibiotic, which is highly active against gram '+ve' bacteria and is almost inefficient against gram '-ve' ones. It is effective against many penicillin resistant microbes. In combination with other antibiotics, e.g., with

penicillins, streptomycin, chlorotetracycline, the bacitracins have a synergic effect. It is highly toxic, and hence its use is restricted to external application.

Like polymyxin, it inhibits the synthesis of the bacterial cell wall structure and affects the integrity of the cytoplasmic membrane.

The bacitracins are mostly used for prophylaxis and treatment of surgical infections. Sometimes they are used to treat skin diseases, pneumonia, bacillary dysentery, etc.

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[J] Actinomycin

Actinomycin is one of the first actinomycetes antibiotic that is isolated from *Streptomyces antibioticus* by Waksman and Woodruff (1940) but now is obtained by several other species of *Streptomyces*. It is actually a mixture of several compounds, all of which contain a phenoxazone chromophore, but all are substituted with different polypeptide chains. It is active against gram '+ve' but less active against gram '-ve' bacteria. Some actinomycins have anticancer properties, but their high toxicity is a limiting factor.

Non-Medical Uses of Antibiotics

Antibiotics are now widely used not only in medicine but also in agriculture and in food industry.

[A] Antibiotics in Agriculture

The use of antibiotics for plant disease control has been studied intensely during the past two decades. Diseases of plants are caused by various phytopathogenic agents, such as viruses, bacteria, fungi, protozoa, etc. Antibiotics are used to control various agents and to prevent propagation of infection. The method by which antibiotics are applied depends on the disease, the stage of plant, growth, the plant size, the area of cultivation and the planting method. All techniques are based on the inhibition of growth or destruction of phytopathogenic organisms on the plant surface or inside plant tissues by the antibiotic applied to the surface of the plants or added to the soil. Streptomycin, tetracyclines, griseofulvin, trichothecin, etc. are some important antibiotics used to control plant pathogens.

[B] Antibiotics in Nutrition and Veterinary

Antibiotics are now widely used as growth stimulants in poultry and livestock feeds. Commercially, addition of aureomycin, terramycin or penicillin to poultry feed at the rate of 5 to 20 g/ton of feed increases the rate of young ones by at least 10 per cent.

[C] Antibiotics in Food Preservation

Antibiotics were first reported to be used in the canning industry in 1943. Subtilin, nisin, and some other antibiotics are generally used for this purpose. Besides, antibiotics are also used to preserve fresh foods like meat, etc. For preventing quick spoilage of meat, usually two methods are in use: (i) antemortem feeding of animal with food containing an antibiotic, and (ii) postmortem injection of the antibiotic into the blood system (immediately after slaughtering and releasing the blood). This prolongs the time of safe storage of meat to three days and improves its quality. Similarly, antibiotics are very effective in preventing spoilage of fish. Films and other packaging materials containing antibiotics are sometimes used for packing perishables. This prolongs the time of their storage. Antibiotics are also used for preserving milk and other dairy products. As for example, nisin is used in the manufacture and storage of cheeses.

Despite the apparent advantages of using antibiotics for nutrition of animals and preservation of foods, they should be used with great precautions. Getting inside man with food, antibiotics in small concentrations may stimulate formation of antibiotic-resistant micro-organisms.

Important Questions

►► Long answer questions

1. What are antibiotics? Give an account of antibiotics of bacterial origin.
2. Antibiotics are generally more effective against bacterial infections than against viral infections. What are some of the reasons for this?
3. Enumerate the qualities essential in a good antibiotic. Which genera of micro-organisms produce most of the antibiotics?

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►► Short answer questions

1. Write the qualities of an ideal antibiotic.
2. Name some therapeutically important antibiotics.
3. 'Antibiotics are now widely used not only in medicine but also in agriculture and in food industry'. Comment upon the statement.

►► Very short answer questions

1. Name the first antibiotic discovered.
2. Name the common core present in all penicillins.
3. From which microbe streptomycin is obtained?
4. From which bacterium bacitracin is obtained?

►► True and false statements

1. An ideal antibiotic should be a broad spectrum antibiotic.
2. Different side chains give each penicillin its unique properties.
3. Griseofulvin is produced from a mutant strain of *Streptomyces griseus*.
4. The antibiotic polymyxin is obtained by a bacterium *Bacillus polymyxa*.

►► Fill in the blanks

1. Streptomycin belongs to the chemical class of antibiotics known as
2. Cephalosporins are.....groups of antibiotics, derived from strain.....
3. Chloromycetin was first discovered in cultures of *Streptomyces*.....
4. Actinomycin is one of the first actinomycetes antibiotic that is isolated from.....

►► Multiple choice questions

1. The cephalosporins exert their antibacterial effect by inhibiting the :
(a) synthesis of the bacterial cell wall
(b) protein synthesis
(c) synthesis of nucleic acid
(d) destruction of organelle membrane
2. Streptomycin is obtained from:
(a) *Streptomyces aureofaciens*
(b) *Streptomyces griseus*
(c) *Streptomyces erythreus*
(d) *Streptomyces venezuelae*

ANSWERS

►► Very short answer questions

1. penicillin obtained from *Penicillium notatum* and *P. chrysogenum*, 2. a fused β -lactam thiazolidine ring,
3. *Streptomyces griseus*, 4. *Bacillus subtilis*.

►► True and false statements

1. True, 2. True, 3. False, 4. True.

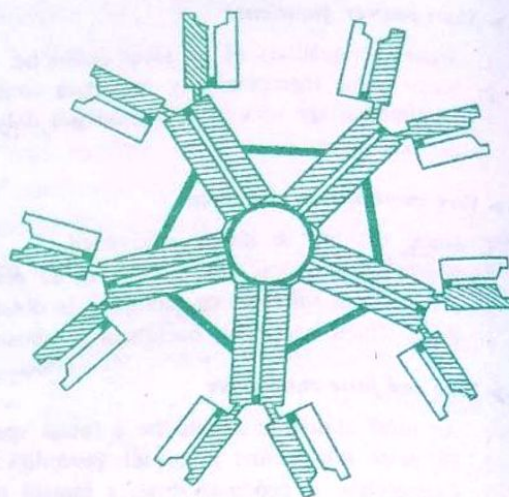
►► Fill in the blanks

1. aminoglycosides, 2. β -lactam, *Cephalosporium acremonium*, 3. *venezuelae*, 4. *Streptomyces antibioticus*.

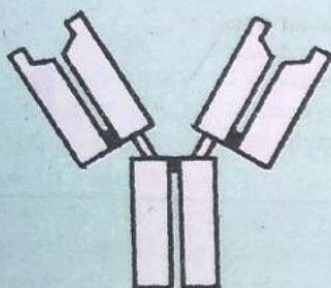
►► Multiple choice questions

1. (a), 2. (b).

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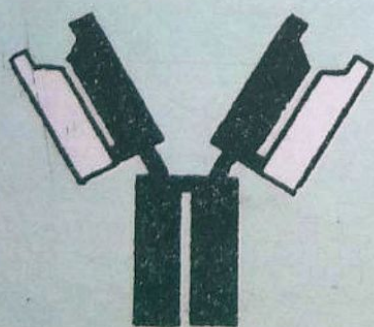


Infection and Immunity



Micro-organisms are responsible for a wide range of diseases in plants and animals. The **disease** is a disorder developed in an organism by harmful physiological changes. The causative agent of a disease is known as **pathogen**, and the biological process of the establishment of a pathogen inside the organism is known as **infection**. The infection results in the development of visible or latent disease in an organism. Broadly speaking, infection is a process of inter-struggle between organisms (pathogen and host) inhabiting two different environmental conditions. The potential capacity of infection of a pathogen is known as its **pathogenicity**. The pathogenicity of a pathogen is its specific character, and it depends on the parasitic adaptation and capacity to life-struggle of the pathogen.

INFECTION



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The term **infection** (Latin, *infectio* = to infect) signifies the sum of biological processes which take place in the body of the host after the penetration of the pathogenic micro-organism, independent of the fact whether the microbe will cause a disease or not. Thus, infection is the establishment of a pathogenic micro-organism within the host following its entrance. The following are the requirements for the origin and development of infection.

[A] Entrance of Pathogen into the Host

The place of entry of the pathogen in the body of the host is important for the occurrence and kind of disease. Certain microbes cause disease

Table 1. Important characteristics of toxins.

Feature	Exotoxins	Endotoxins
1. Bacterial source	Mainly Gram '+ve' bacteria.	Mainly the cell wall of lysed Gram '-ve' bacteria.
2. Chemical nature	Proteinaceous.	Composed of glucoside lipoprotein complexes and polysaccharide specific complexes.
3. Heat tolerance	Inactivated easily by treating at 60-100°C for 30 min.	Withstand autoclaving.
4. Diffusibility	Easily diffuse from the cell into the surrounding medium.	Firmly bound within the bacterial cell.
5. Toxicity	Highly toxic, characterized by selective affection of certain organs and tissues.	Less toxic, selective action poorly expressed.
6. Immunity	Can be converted to toxoids and readily neutralized by antitoxin. Produced highly active antibodies, the antitoxins on parental infection.	Cannot form toxoids and neutralization with antitoxin is not easily possible. Produce precipitins, lysins, opsonins, agglutinins and complements using antibodies on parental infection.
7. Biological effect	Specific for a particular type of cell function.	Show various effects especially the symptoms of hypersensitivity.
8. Lethal dose	Minute amounts.	Comparatively more.

only if they enter the body of the host through a specific path. For instance, when dysentery bacilli enter the body through wound, they do not cause any harm, but if swallowed they might prove to be fatal. Similarly, typhoid bacteria are harmful if they enter the body through mouth. Gonococci and some other microbes like spirochetes enter through abrasions in the skin and set up local infections, such as boils caused by staphylococci.

[B] Growth and Multiplication of Pathogen in the Host

After having gained access to the body of the host successfully, the pathogen secretes several chemical substances like enzymes and toxin. These substances cause physiological, morphological and anatomical abnormalities in the body of the host. Some pathogens also secrete such substances which protect them from the lytic enzymes produced by the host. The enzymes and toxins secreted by the pathogen kill and drive away leucocytes of the host. Thereafter, the pathogen gets an opportunity to grow in the host.

[C] Toxicity of Microbes

The pathogens secrete some poisonous substances which are harmful to the host, but helpful in the disease development. Such substances are called **toxins**. The toxins do not break the structural

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entity of the host but cause a minor change in its metabolism. The potency of the toxins is an important factor in the establishment of the disease. The toxins may be **exotoxins** or **endotoxins**.

Exotoxins are proteinaceous, highly toxic and diffusible substances which microbes secrete in the host tissue, circulatory system, etc. They lose their toxicity when heated or treated chemically and form toxoids. **Endotoxins** are less toxic and consist of glucoside lipo-protein. They are relatively more heat stable and do not form toxoids. They are usually located in the cell wall and are liberated only after the cell disintegrates. They generally play a contributory rather than a primary role in causing disease and are mainly responsible for many of the symptoms. Many pathogens, particularly Gram '-ve' bacteria produce endotoxins.

Some important characteristics of exotoxins and endotoxins are summarized in Table 1.

Besides toxins, virulence of microbes also depends on extracellular enzymes produced by them. Several types of cocci, clostridia and some other bacteria produce the enzyme **hyaluronidase** which helps the pathogen to penetrate the tissues of the host by hydrolyzing hyaluronic acid. **Lecithinase**, produced by *Clostridium perfringens*, destroys various tissue cells and is especially active in the lysis of red blood corpuscles.

Collagenase also produced by *Clostridium perfringens*, destroys collagen tissue found in muscles, bones and cartilage. Some staphylococci and streptococci secrete **leucocidin** enzyme which kills white blood cells. The enzyme **coagulase**, produced by some virulent staphylococci (*Staphylococcus aureus*), clots plasma.

The virulence of pathogenic bacteria also depends on the presence of capsule and pili. The increased virulence of capsulated strains is due to the ability of capsular polysaccharide in preventing phagocytosis by host leucocytes. Presence of pili helps the pathogen to adhere to the surface of host cells and tissues, thus enhancing the virulence of the pathogen (e.g., *Neisseria gonorrhoea*, *Escherichia coli*).

[D] Dosage

This factor in establishing infection is a quantitative one. Under normal conditions, the larger is the dose of the infective microbe the greater the chances of infection.

[E] Defence Mechanisms of Host

All organisms show resistance towards pathogens and as such the host resists the effects of pathogen by active or passive activities. The resistance of the organism towards a specific pathogen depends upon the environmental conditions and genetic constitution of the host. The defensive mechanism of the host determines the extent of infection and severity of the disease.

According to manifestations, infection may be acute or chronic, obvious or latent, mixed or secondary. Acute infections are characterized by a sudden onset and a comparatively short course. Influenza and measles are the excellent examples of acute infections. On the other hand chronic infections have a long span. Malaria and tuberculosis are chronic infection. Typical symptoms do not appear in latent infection. In some cases infection causes a weakening of the body of the host which then becomes susceptible to other diseases. This is known as **secondary infection**. For instance after measles or influenza the body becomes susceptible to pneumonia.

IMMUNITY

The resistance of the host towards a pathogenic microbe and its toxin is known as **immunity** and the study of immunity to infectious diseases is called **immunology**. The degree of resistance depends on the defense mechanism of the host and the virulence and dosage of the pathogen. Immunity can be classified into the following two groups.

[A] Natural, Heritable or Non-Specific Immunity

This type of immunity is hereditary and is passed from one generation to the next. For instance, human beings are immune to cattle plague, and cattle are immune to diseases like cholera and measles. The natural immunity may occur at racial or individual level. Most racial immunities are due to nonspecific factors related to people's way of life. Such immunity may reflect the evolution of resistant humans. For instance, african black people are immune to malaria, whereas majority of the white people are susceptible to this disease.

At individual level, the resistance depends on several factors, such as age, nutrient status, sex, occupation, health, and personal hygiene. Young ones and aged are more susceptible to diseases. Infants are protected from many diseases from birth to six months owing to the presence of maternal antibodies in the blood. In the aged person there is a natural decline in the immune response to infection and that is why they become more susceptible to some diseases such as pneumonia.

[B] Acquired or Specific Immunity

The acquired immunity may be of following two types.

[I] Natural acquired immunity

The host acquires this type of immunity naturally during the course of its life time. The natural acquired immunity may be —

1. Natural active immunity. This is the resistance which is developed as a result of some disease or through recovery from a disease; for instance, the host develops immunity for the diseases like small pox and diphtheria after subjecting them.

2. Natural passive immunity. This type of resistance is found in the newly borne child, acquired from the mother through the placenta. For instance the child borne by mother immune to tetanus also shows resistance towards the disease.

[II] Artificial acquired immunity

The host acquires this type of immunity during the course of life time by artificial means. It is classified into the following two types.

1. Artificial active immunity. This type of immunity is developed by vaccination, made by dead organisms pathogens. The toxin thus entering the body stimulates the production of immunity.

2. Artificial passive immunity. This type of immunity is developed through injection of immune serum present in already immunized organisms.

Antigen

Antigen is a substance that, when introduced into a vertebrate host, provokes an immune response leading to acquired immunity. The immune response results in the formation of specific antibodies. The antibodies are circulated in the blood stream or they stimulate the increase in the number of lymphocytes. The lymphocytes have the capability to destroy other cells. The ability of the antigen to stimulate antibody formation is called its **antigenicity**.

Antigens are organic compounds of high molecular weight. The antigens of proteinaceous nature are known as **complete antigens**. They can produce antibodies. Some antigens which consist of lipids and carbohydrates, and can not produce antibodies on their own are known as **partial antigens**. The partial antigen stimulates antibody formation by combining with proteins. Bacteria, viruses and other microbes, pollen grains, and blood cells of other organisms may act as

antigens. Besides, some important antigens (ABO, MN, S, P, Rh) are also found in red blood cells of human beings. The bacterial antigen is usually secreted in the form of exotoxins and enzymes. Vaccines (suspensions of killed, living or attenuated microbes) are also used as antigens to produce immunity against infection of specific pathogens. **Toxoids** are made by destroying the poisonous portions of toxins. In the process, the properties of antigen is altered. Toxoids are used specifically for protecting individuals against diphtheria, tetanus, etc.

Antibody

Antibodies are specific substances produced in the body of the host on introduction of antigens. All antibody molecules are globulin proteins which have been altered under the influence of antigens. These are also known as **immunoglobulins**. The immunoglobulins are of five types : IgG, IgM, IgA, IgD, IgE. All these types consist of monomeric units, each comprising two light and two heavy polypeptide chains. The chains are joined by disulphide bonds. Each heavy chain has a molecular weight of approximately 55,000 and the light chain of about 25,000. The heavy chains are specific for each type of immunoglobulin. They contain amino acids in a specific sequence which determines the types of immunoglobulin. There are five types of heavy chains (i) γ (gamma), (ii) α (alpha), (iii) δ (delta), (iv) μ (micron), and (v) ϵ (epsilon). The immunoglobulins formed by them are respectively known as Ig G, Ig A, Ig D, Ig M and Ig E. The light chains are mainly of two types : (i) κ (kappa), and (ii) λ (lambda). Both can occur in an immunoglobulin. The terminal portions of both heavy and light chains of each monomeric unit show considerable variation, whereas the remaining portions of the chains are relatively constant in amino acid structure.

Each immunoglobulin is specific in its function. Approximately 70% of normal human serum is IgG. It is the most common type of antibody and it passes from the mother to the foetus through placenta. It provides immunity to the newborn. On the basis of antigenic differences,

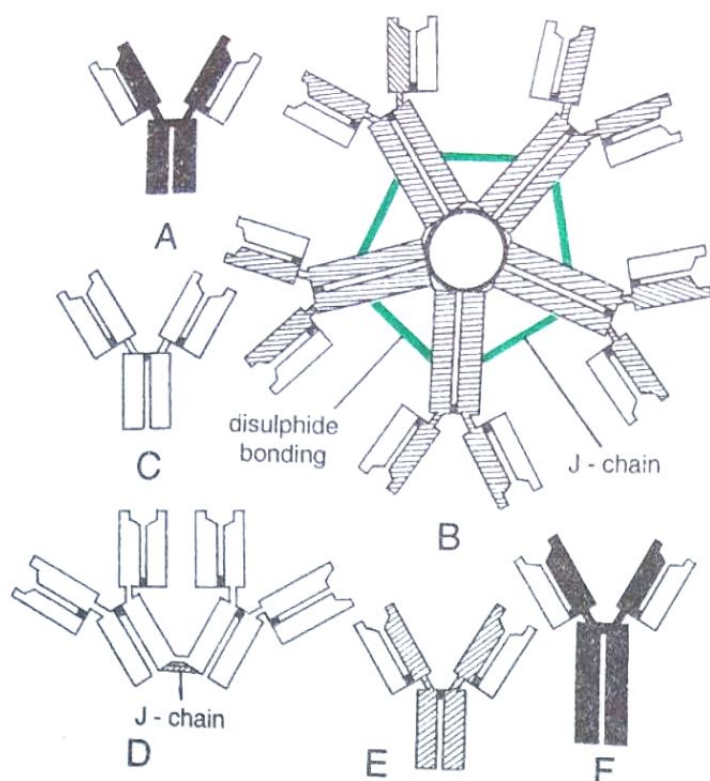


Fig. 1 A-F. Structure of different types of immunoglobulin molecules : A. IgG, B. Ig M, C. Ig A (monomeric), D. Ig A (dimeric), E. Ig D, F. Ig E.

Ig G may be classified into IgG₁, IgG₂, IgG₃, and IgG₄. Each IgM molecule is composed of five monomeric units. There is an additional peptide, called J-chain, attached to one or both the heavy chains. About 6% of the human sera is Ig M. It usually appears following induction by an antigen and is very effective against viral and bacterial antigens. Ig A constitutes about 10% of the total serum. Its basic structure is similar to that of Ig G. Besides blood serum, it also occurs in almost all body secretions like tear, saliva, seminal fluid, urine and colostrum. Ig A is an essential part of defensive mechanism of the host. Only 1% of the serum is Ig D. Little is known about this serum, but it is understood that it regulates the synthesis of other immunoglobulins. About 0.002% of total immunoglobulins present in the serum constitutes Ig E. When combined with antigens, it shows allergic reactions.

Antibodies react against a specific pathogen or its toxic products. Hence, antibodies are

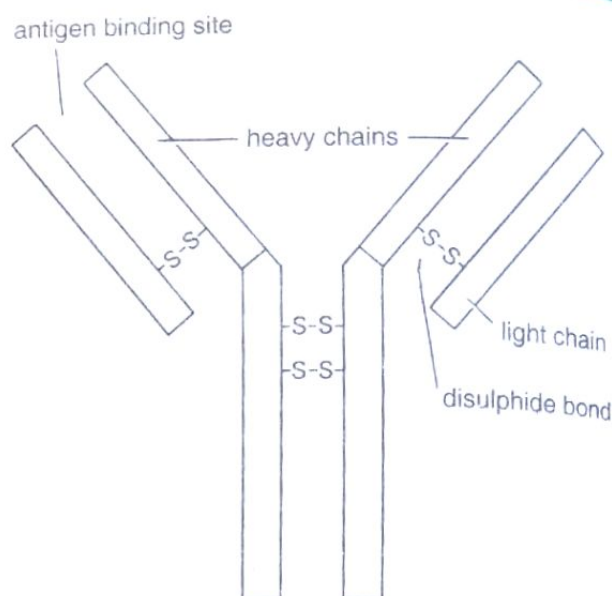


Fig. 2. Monomers of the immunoglobulin molecule joined by two heavy and two light polypeptide chains.

important in the treatment of infections caused by specific micro-organisms.

Serological Reactions

An antibody is an immunoglobulin molecule secreted into the tissue fluids when an organism is exposed to a foreign substance- the antigen. The antibody can combine only with an antigen which is identical or nearly identical to the inducing antigen. In an antigen - antibody reaction, first, union of antigen and antibody occurs, which is usually not visible. The second stage is the visible result of antigen - antibody union, which is exemplified by agglutination, precipitation, opsonization, lysis, etc. Such different manifestations are not necessarily due to various types of antibodies. A particular antibody may be involved in any type of reaction, depending upon the kind and size of antigen, the physical conditions of the suspending fluids, the presence of electrolytes, and several other factors. These reactions can be used to detect antigen using a known antibody, or to detect antibody with a known antigen. Such reactions are thus the basis for several *in vitro* serological tests. Serology is the study of blood serum containing antibodies. Some important serological reactions are as follows.

[A] Agglutination

Agglutination is the visible stage as a result of mixing a particulate or cellular antigen with a homologous antiserum. Agglutinins are antibodies that cause clumping of the bacterial cells for which they are specific. This test is performed by mixing bacterial cells with the serum prepared from animal's blood. When cells interact with the appropriate antibody they clump together and form flocculent masses. This serological reaction is useful in the identification of bacteria. It is easy to perform and simple to interpret. Agglutination tests are classified as **macroscopic** when the test is carried out in small test tubes and **microscopic** when antigen and antiserum are mixed on a slide and examined under microscope. When agglutinated cells are erythrocytes the phenomenon is called **haemagglutination**.

[B] Precipitation

Precipitation occurs when the antigen is soluble instead of cellular. Precipitins are those antibodies that cause precipitation or flocculation of extracts of bacterial cells or other soluble antigens. In precipitin test a reaction takes place between a soluble antigen and its homologous antibody. The reaction is manifested by the formation of a visible precipitate at the interface of the reactants. This

reaction is used in medico-legal work in the identification of blood stains on clothes and weapons used in murder cases.

[C] Opsonization

Opsonins are antibodies that make microorganisms more susceptible to ingestion by phagocytes. The effect is known as **opsonization** (Gr. *opsonin* = to prepare food for). Antigens that have bound antibody are far more easily phagocytized than the antigen alone. Complement-dependent opsonization is important in limiting bacterial infections, particularly in capsulated bacteria.

[D] Lysins

Lysins cause lysis or breakdown of bacterial or other cells which are specifically sensitive to their action. This test is based on the presence of complement-fixing antibodies in the serum. In the presence of complement these antibodies cause lysis of the specific cells. The test determines whether specific antibodies are present in the serum or not. The test is widely used in laboratory diagnosis of many infectious diseases. It is especially useful when the test antigen and antibody combination does not give a visible reaction such as that occurring in agglutination and precipitation.

Important Questions

►► Long answer questions

1. What do you understand by infection and immunity? Discuss briefly.
2. Write short notes on : (i) Antigen, (ii) Antibody, (iii) Acquired immunity, (iv) Serological reactions.

►► Short answer questions

1. What do you understand by the term infection?
2. Differentiate between exotoxins and endotoxins.
3. Write a note on opsonization.

►► Very short answer questions

1. Which type of immunity is heritable-natural or acquired?
2. What is pathogenicity?
3. Which type of toxins—exotoxins or endotoxins—are comparatively more toxic?
4. What are complete antigens?

►► True and false statements

1. The place of entry of the pathogen in the body of the host is important for the occurrence and kind of disease.
2. Endotoxins are proteinaceous, highly toxic and diffusible substances which microbes secrete in the host tissue.

3. The resistance of the host towards a pathogenic microbe and its toxin is known as immunity.
4. The natural immunity is hereditary and is passed from one generation to the next.
5. Antigen is a substance that, when introduced into a vertebrate host provokes an immune response leading to acquired immunity.

►► **Fill in the blanks**

1. Antibodies are specific substances produced in the body of the host on introduction of
2. The biological process of the establishment of a pathogen inside the organism is known as
3. infections are characterized by a sudden onset and a comparatively short course.
4. The study of immunity to infectious diseases is called
5. Artificial immunity is developed through injection of immune serum present in already immunized organisms.

►► **Multiple choice questions**

1. The specific substances produced in the body of the host on introduction of antigens:
 - (a) antibodies
 - (b) toxins
 - (c) pathogen
 - (d) toxoids
2. The following disease is an example of acute infection:
 - (a) influenza
 - (b) malaria
 - (c) tuberculosis
 - (d) all the above
3. The resistance of the host towards a pathogenic microbe and its toxin is known as:
 - (a) infection
 - (b) pathogenicity
 - (c) agglutination
 - (d) immunity

ANSWERS

►► **Very short answer questions**

1. natural, 2. the potential capacity of infection of a pathogen, 3. exotoxins, 4. antigens of proteinaceous nature.

►► **True and false statements**

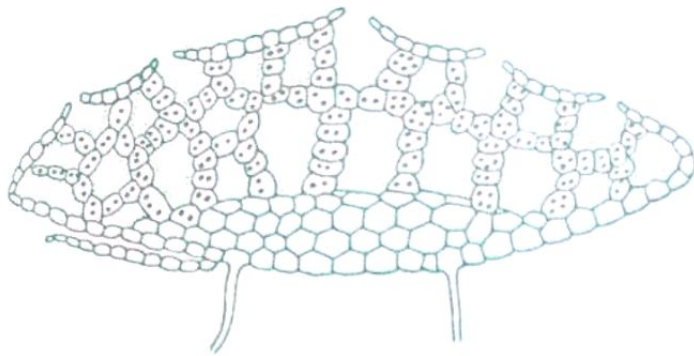
1. True, 2. False, 3. True, 4. True, 5. False.

►► **Fill in the blanks**

1. antigens, 2. infection, 3. acute, 4. immunology, 5. passive.

►► **Multiple choice questions**

1. (a), 2. (a), 3. (d).



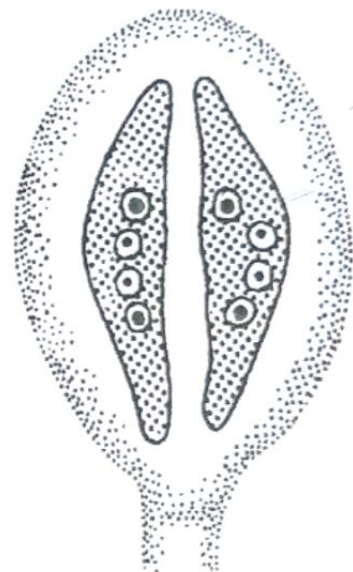
Cryptogams : Introduction

The term **cryptogams** from the Greek *kryptos* (meaning 'hidden') and *gamos* (meaning marriage), was coined by 19th century botanists for plants in which sexual reproduction was not apparent. In contrast, in seed plants the reproductive organs are easily seen. The seed plants have been accordingly termed **phanerogams**, from the Greek *Phaneros*, meaning visible. Some other names such as **thallophytes**, **lower plants** and **spore plants** are also assigned to the cryptogams. **These plants make up around 84% of the world's botanical diversity.** Algae, lichens, mosses and ferns are the best known cryptogams. The group also includes non-photosynthetic organisms traditionally classified as plants, such as fungi and bacteria.

Carolus Linnaeus (1701-1778) recognized cryptogams as a group within the plant kingdom. He divided plants into 25 classes and **Cryptogamia** is one of them. The Cryptogamia was further divided into four orders — **Algae** which included algae, lichens and thallose bryophytes, **Musci** which included mosses and leafy liverworts, **Fungi**, a group of non-photosynthetic thallophytes and **Filices** which included ferns and allies.

Linnaeus' system of classification was wholly artificial. For classification purpose, he considered **structure of flower more fundamental than vegetative characters.** He grouped plants on the basis of number of stamens and carpels, their union and their presence or absence in the flower. This system was easy to follow as whenever any unknown plant was discovered, it could easily be assigned to one of the 25 classes proposed by Linnaeus.

In later systems, called **natural systems**, plants were grouped according to their natural affinities. The first such system was that of A.L. de Jussieu (1789). He divided plants into three major groups—**Acotyledones**, **Monocotyledones** and **Dicotyledones**.

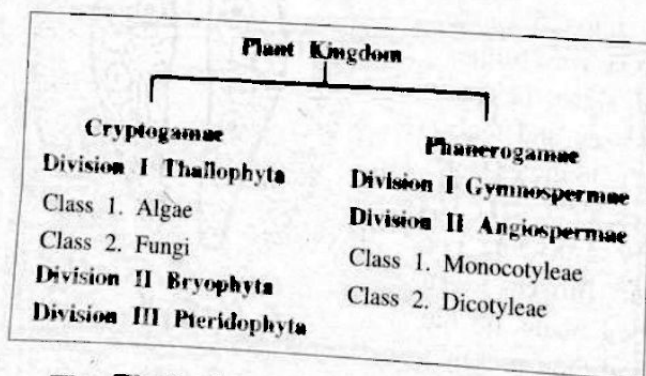


(ALGAE)

His Acotyledones are the approximate equivalent of Linnaeus' Cryptogamia. However, most of the classifications proposed during the first half of the nineteenth century were inadequate so far as the classification of cryptogams was concerned. It was primarily due to insufficient information regarding the life-histories of cryptogams. The scene, however, changed after the publication of German botanist Wilhelm Hofmeister's work in 1851. He studied the life-cycles of a number of different groups of vascular cryptogams and mosses.

Although Hofmeister did not fully understand the processes of gametic union and meiosis, but he knew that sperm stimulates the egg to develop into the new sporophyte. Thus the concept of alternation of gametophytic and sporophytic generations was given by Hofmeister. About the same time phycologists and mycologists brought out the distinctive features of algae and fungi by studying their life-cycles.

The approach to taxonomy changed significantly with the publication of Darwin's theory of evolution in 1859. **The systems of this period are based on phylogeny** in which plants are arranged in an ascending series from the most primitive to the most advanced. Based on the doctrine of evolution, A. W. Eichler (1886) proposed a classification in which he divided entire plant kingdom into—**Cryptogamae** (flowerless plants) and **Phanerogamae**:



The **ThallopHYta** have a plant body that is not clearly differentiated into roots, stem and leaves. They lack water conducting tissue and their sex organs are one-celled, or when multicellular (as in certain brown algae), they do not have the (ALGAE)

gametes surrounded by a layer of sterile cells. Besides above characters, zygote of thallopHYtes never develops into multicellular embryo while still within the female sex organ.

The thallopHYtes include the algae and the fungi. The **algae** have the **chlorophyll** pigment and make their own food by photosynthetic process. Although all algae essentially contain chlorophyll *a* pigment but in some forms the green colour of chlorophyll pigment is masked by other pigments. For example, in Phaeophyceae (brown algae), a brown pigment—**fucoxanthin** and in Rhodophyceae (red algae) a red pigment—**r-phycoerythrin** masks the green colour of the chlorophyll.

Most algae are aquatic ; they are either fresh water or marine (oceanic). Fresh water forms are unicellular, filamentous, colonial or heterotrichous. Some marine algae are large, conspicuous and complex (e.g., *Ulva*, *Laminaria*, *Sargassum*, etc.).

The **Fungi** are achlorophyllous heterotrophic organisms. The group includes molds, mildews, mushrooms and other similar organisms. The thallus of fungi mostly consists of microscopic tubular filaments called **hyphae** and the mass of filaments constituting the thallus is called **mycelium**. Except for a few fungi (such as Myxomycetes), all have a distinct cell wall of chitin and cellulose.

Fungi derive their nutrition from dead or decaying organic matter (called saprophytes) or living host cells (called parasites). A few fungi form mutually beneficial partnership with certain algae (e.g., lichens) or with the roots of certain trees (e.g., mycorrhiza).

Bryophytes are simpler and smaller embryophytes. They lack well developed conducting tissue. The life-cycle of bryophytes have morphologically distinct gametophytic and sporophytic phases. The gametophyte of bryophytes is more conspicuous, long lived, green and independent, whereas the sporophyte is short lived and completely dependent upon the gametophyte. The gametophyte is prostrate and thalloid or erect and differentiated into axis (stem) and lateral appendages (leaves). They lack roots but possess rhizoids which perform the function

of roots. The reproductive structures are multicellular and much more complicated than those of thallophytes.

The **Pteridophytes**, also called **vascular cryptogams**, are those cryptogams that characteristically have a specialized conducting system. As compared to the gametophyte, the sporophyte of pteridophytes is always larger and dominant. It is differentiated into root, stem and leaves. The vascular tissue consists of xylem and phloem. The xylem is without vessels and phloem without sieve tubes.

Robert H. Whittaker (1969) classified organisms on the basis of **complexity of cell structure, body organization** and **mode of nutrition**. He recognized five kingdoms in the biological world, viz., **Monera, Protista, Fungi, Plantae** and **Animalia**. He placed all Prokaryotes (e.g., bacteria, blue-green algae) in the kingdom **Monera**. Unicellular eukaryotes are

included in the kingdom **Protista**. This kingdom includes protistan algae (e.g., dinoflagellates, diatoms, etc.) and euglenoids which show both plant-like and animal-like characters. Fungi (achlorophyllous, spore bearing eukaryotic thallophytes) have been placed in a separate kingdom **Fungi**. All multicellular producers (including green filamentous and parenchymatous algae) are placed in kingdom **Plantae**. The fifth kingdom **Animalia** includes all multicellular consumers.

Though Whittaker's system is more natural and indicates gradual evolution of early plants and animals, but one anomaly of the system is that the algae (which form a single order of Cryptogamae in earlier systems) have been assigned to three kingdoms—blue-green algae in kingdom Monera, eukaryotic unicellular algae in kingdom Protista and multicellular algae in kingdom Plantae.