I/IV B.Tech (Regular) I Semester (DEC 2018) DEGREE EXAMINATION

BIOLOGY FOR ENGINEERS (18CE002)

SCHEME OF EVALUATION

1. Answer all questions **(1X10=10 Marks)**

a) Biology - Definition

b) Importance of central vacuole

c) Autotrophs - Definition

d) Binary fission – Role & Definition

e) m-RNA – Role & Definition

f) Name two types of secondary structural elements of proteins

g) Role of Aloe vera in primary health care

h) Importance of phenolic compounds in sterilization

i) List the bacterial growth phases

j) Name the cell wall component of plant cells

UNIT I

2. a) Write an essay on bacterial cell structure

Bacteria cell diagram – 2M

Description of any four cell organelles - Each carries two marks 2X4 = 8M

3. a) Write about the three kingdom classification of microorganisms

Each kingdom carries 1X3=3M

Merits and demerits 2M

3. b) Chloroplasts - Diagram 1M

Description 4M

UNIT II

4. Define medium and explain different types of media used for growth of bacteria

Medium definition - 2M

Any four media each carrying 2marks 2X4=8M

5. a. Write about the differences between mitosis and meiosis

Any Five differences each carrying 1 marks 1X5=5M

b. Write short note on endospore formation

Endospore definition - 1M

Formation of endospore 4M

UNIT III

6. a) Write in detail about the structure of t-RNA

t- RNA structure - 1M

t- RNA description – 4M

b) Write about the different types of proteins

Any ten different types of proteins, each carrying half mark 10X0.5 = 5M

7. Explain in detail about the B model of DNA and its important functions

Watson & Crick model of DNA - Diagram 2M

Structure – 4M

Function – 4M

UNIT IV

8. Define sterilization and explain in detail about different sterilization methods

Sterilization definition – 1M

Any four physical methods each carries 1 marks 1X4= 4M

Any four chemical methods each carries 1 marks 1X4= 4M

9. Write an essay on economic importance of bacteria

Description 2M

Beneficial role 6M

Harmful effect 2M

I/IV B.Tech (Regular) I Semester (DEC 2018) DEGREE EXAMINATION

BIOLOGY FOR ENGINEERS (18CE002)

Answers

1. Answer all questions **(1X10=10 Marks)**

a) Define biology

The study of living organisms, divided into many specialized fields that cover their morphology, physiology, anatomy, behaviour, origin, and distribution.

b) Importance of central vacuole

Each plant cells have a large, central vacuole that occupies most of the cell. The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. In plant cells, the liquid inside the central vacuole provides turgor pressure, which is the outward pressure caused by the fluid inside the cell.

c) Autotrophs

Autotrophs can use CO_2 as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source.

d) Binary fission

Bacterial binary fission is the process that bacteria use to carry out cell division. Bacterial cell division isn't just a means of making more cells for the body. Instead, it's actually how bacteria reproduce, or add more bacteria to the population. Prokaryotic cells, such as bacteria and Archaea, are smaller, simpler and have only one option for reproduction, binary fission

e) m-RNA

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is "turned on" and the mRNA is synthesized through the process of transcription**.**

f) Name two types of secondary structural elements of proteins

α - helix

β - sheets

g) Role of Aloe vera in primary health care

Boosts Immune System, Halts Cancerous Growth, Heals Side Effects of Radiotherapy Treatments, Cures Dermatitis

h) Importance of phenolic compounds in sterilization

Phenol and phenolics (Derivatives of phenol, called phenolics, contain a molecule of phenol that has been chemically altered to reduce its irritating qualities or increase its antibacterial activity in combination with a soap or detergent) such as **cresols, xylenols**, and **orthophenylphenol** are used as disinfectants in

laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics. Phenolics act by denaturing proteins and disrupting cell membranes.

i) List the bacterial growth phases

Lag

Log or exponential

Stationary

Death or decline

j) Name the cell wall component of plant cells

Cellulose

UNIT I

2. a) Write an essay on bacterial cell structure

Prokaryotic cells almost always are bounded by a chemically complex cell wall. Inside this wall, and separated from it by a periplasmic space, lies the plasma membrane. This membrane can be invaginated to form simple internal membranous structures. Since the prokaryotic cell does not contain internal membrane-bound organelles, its interior appears morphologically simple. The genetic material is localized in a discrete region, the nucleoid, and is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusion bodies are scattered about in the cytoplasmic matrix. Both gram-positive and gram-negative cells can use flagella for locomotion. In addition, many cells are surrounded by a capsule or slime layer external to the cell wall.

I. Shape and Arrangement

Prokaryotes would be uniform in shape and size. Many prokaryotes are similar in morphology, there is a remarkable amount of variation due to differences in genetics and ecology. Most commonly encountered bacteria have one of two shapes cocci or bacilli.

Cocci are roughly spherical cells. They can exist as individual cells.

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

Long chains of cocci result when cells adhere after repeated divisions in one plane; Ex: *Streptococcus, Enterococcus, Lactococcus*.

Staphylococcus divides in random planes to generate irregular grapelike clumps.

Divisions in two or three planes can produce symmetrical clusters of cocci.

Micrococcus often divide in two planes to form square groups of four cells called tetrads.

Sarcina, cocci divide in three planes producing cubical packets of eight cells.

The other common bacterial shape is that of a **rod,** often called a **bacillus**. Ex: *Bacillus megaterium*

The shape of the rod's end often varies between species and may be flat, rounded, cigar shaped, or bifurcated.

Although many rods do occur singly, they may remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

vibrios, are curved to form distinctive commas or incomplete spirals.

Actinomycetes characteristically form long multinucleate filaments or hyphae that may branch to produce a network called a **mycelium**.

Many bacteria are shaped like long rods twisted into spirals or helices; they are called **spirilla** if rigid and **spirochetes** when flexible.

The oval to pear shaped *Hyphomicrobium,* produces a bud at the end of a long hypha.

pleomorphic, bacteria are variable in shape and lack a single, characteristic form. Ex: *Corynebacterium.*

Size

Bacteria are extremely small and visible only with the aid of microscope, the smallest possible cell is about 0.14 to 0.2 µm in diameter, but nanobacteria or ultramicrobacteria appear to range from 0.2 to less than 0.05 µm in diameter. The smallest (e.g., some members of the genus *Mycoplasma*) are about 0.3 µm in diameter. A few bacteria become fairly large; some spirochetes occasionally reach 500µm in length, and the cyanobacterium *Oscillatoria* is about 7 µm in diameter. A few bacteria are much larger than the average eucaryotic cell (typical plant and animal cells are around 10–50 µm in diameter.

Functions of Prokaryotic Structures

Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	Buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake
Cell wall	Gives bacteria shape and protection from lysis in dilute solutions
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial mating Flagella Movement
Endospore	Survival under harsh environmental conditions

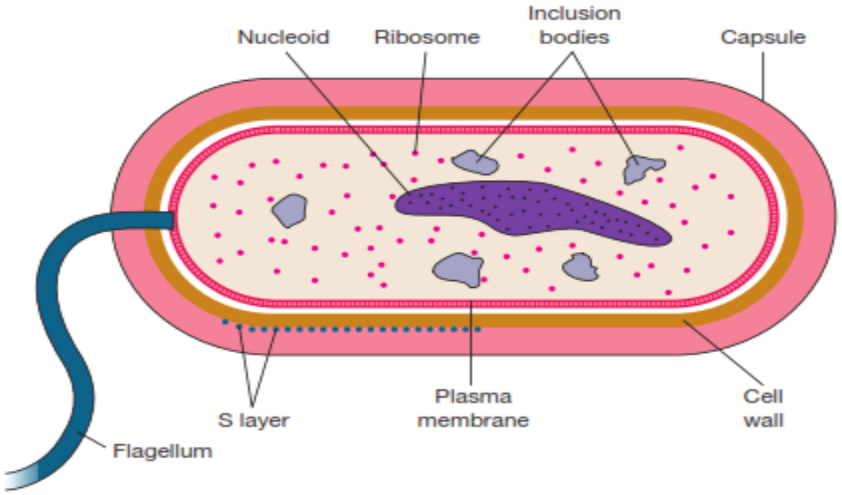


Fig: Morphology of Gram positive bacteria

II. Plasma Membrane

Membranes contain both proteins and lipids, although the exact proportions of protein and lipid vary widely. Most membrane associated lipids are structurally asymmetric with polar and nonpolar ends and are called amphipathic. The polar ends interact with water and are hydrophilic; the nonpolar hydrophobic ends are insoluble in water and tend to associate with one another. This property of lipids enables them to form a bilayer in membranes. The outer surfaces are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water. Many of these amphipathic lipids are phospholipids. Cell membranes are very thin structures, about 5 to 10nm thick. The most widely accepted current model for membrane structure is the fluid mosaic model. They distinguish between two types of membrane proteins. Peripheral proteins are loosely connected to the membrane and can be easily removed. They are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are integral proteins. These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Often carbohydrates are attached to the outer surface of plasma membrane proteins. The plasma membrane is essential to the survival of microorganisms.

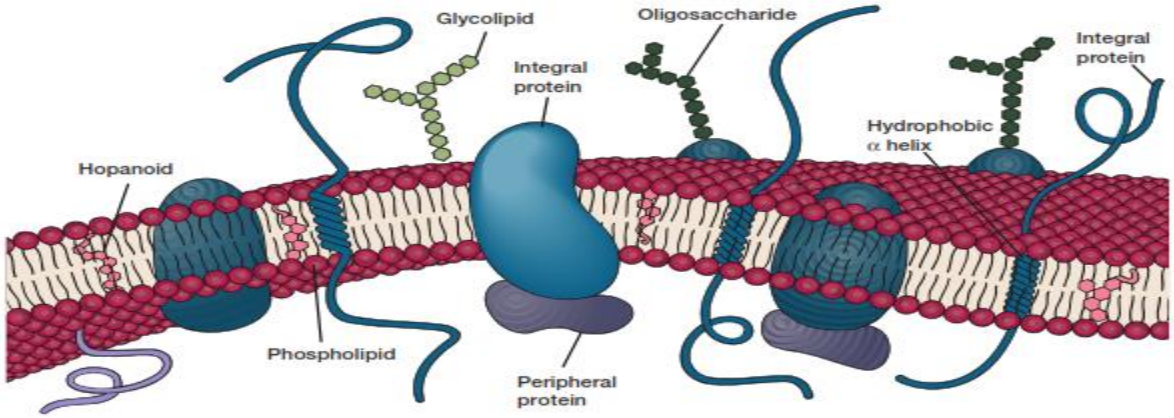


Fig: Structure of a Plasma Membrane

Internal Membrane Systems

Mesosomes are invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae. Their function may be to provide a larger membrane surface for greater metabolic activity. Mesosomes often are found next to septa or cross-walls in dividing bacteria and sometimes seem attached to the bacterial chromosome. Thus they may be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells.

III. Cytoplasmic Matrix

The cytoplasmic matrix is the substance lying between the plasma membrane and the nucleoid. The matrix is largely water (about 70% of bacterial mass is water). It is featureless in electron micrographs but often is packed with ribosomes and highly organized. The plasma membrane and everything within is called the protoplast; thus the cytoplasmic matrix is a major part of the protoplast.

Inclusion bodies

A variety of inclusion bodies, granules of organic or inorganic material that often are clearly visible in a light microscope, is present in the cytoplasmic matrix. Inclusion body membranes vary in composition. Some are protein in nature, whereas others contain lipid. Organic inclusion bodies usually contain either glycogen or

poly-β hydroxybutyrate. Glycogen and PHB inclusion bodies are carbon storage reservoirs providing material for energy and biosynthesis. The inorganic inclusion bodies are **polyphosphate granules** or **volutin granules.**

The volutin granules function as storage reservoirs for phosphate, an important component of cell constituents such as nucleic acids. In some cells they act as an energy reserve.

A most remarkable organic inclusion body is the **gas vacuole.** Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles**. Gas vesicle walls do not contain lipid and are composed entirely of a single small protein. These protein subunits assemble to form a rigid enclosed cylinder that is hollow and impermeable to water but freely permeable to atmospheric gases. Bacteria with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels.

Ribosomes

The cytoplasmic matrix often is packed with ribosomes; they also may be loosely attached to the plasma

membrane. Ribosomes are actually very complex objects made of both protein and ribonucleic acid (RNA). They are the site of protein synthesis; matrix ribosomes synthesize proteins destined to remain within the cell, whereas the plasma membrane ribosomes make proteins for transport to the outside. Prokaryotic ribosomes are smaller, commonly 70S ribosomes, and are constructed of a 50S and a 30S subunit. The S in 70S and similar values stands for Sved berg unit. Ribosomes are similarly composed of a large and a small subunit.

IV. Nucleoid

The prokaryotic chromosome is located in an irregularly shaped region called the nucleoid. Usually prokaryotes contain a single circle of double-stranded deoxyribonucleic acid (DNA), but some have a linear DNA chromosome.

Plasmids

Plasmids are double-stranded DNA molecules, usually circular, that can exist and replicate independently of the chromosome or may be integrated with it; in either case they normally are inherited or passed on to the progeny.

V. Prokaryotic Cell Wall

The cell wall is the layer, usually fairly rigid, that lies just outside the plasma membrane. The gram-positive cell wall consists of a single 20 to 80 nm thick homogeneous peptidoglycan or murein layer lying outside the plasma membrane. In contrast, the gram-negative cell wall is quite complex. It has a 2 to 7 nm peptidoglycan layer surrounded by a 7 to 8 nm thick outer membrane. Because of the thicker peptidoglycan layer, the walls of gram-positive cells are stronger than those of gram-negative bacteria. Frequently a space is seen between the plasma membrane and the outer membrane in electron micrographs of gram-negative bacteria, and sometimes a similar but smaller gap may be observed between the plasma membrane and wall in grampositive bacteria. This space is called the periplasmic space, is more a gel than a fluid-filled space. The substance that occupies the periplasmic space is the periplasm. Gram-positive cells may have periplasm even if they lack a discrete, obvious periplasmic space. Size estimates of the periplasmic space in gram-negative bacteria range from 1 nm to as great as 71 nm. The periplasmic space also contains enzymes involved in peptidoglycan synthesis and the modification of toxic compounds that could harm the cell. Grampositive bacteria may not have a visible periplasmic space and do not appear to have as many periplasmic proteins; rather, they secrete several enzymes that ordinarily would be periplasmic in gram-negative bacteria. Such secreted enzymes are often called exoenzymes. Some enzymes remain in the periplasm and are attached to the plasma membrane.

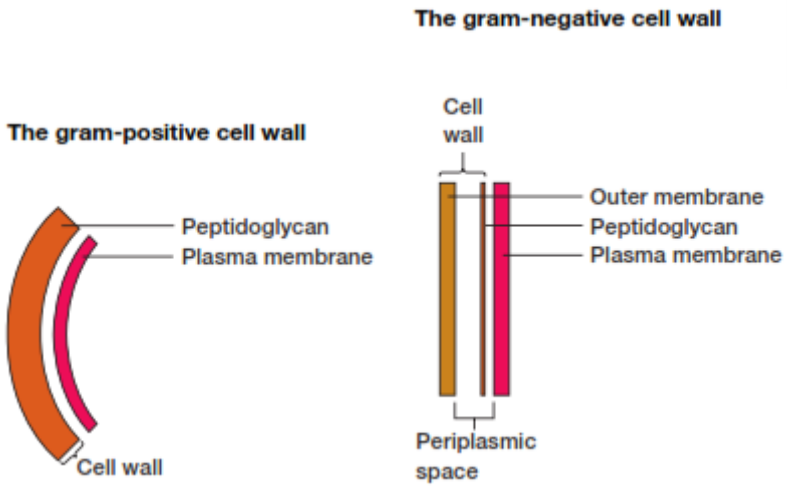


Fig: Gram - positive and Gram - negative cell wall

Peptidoglycan Structure

Peptidoglycan or murein is an enormous polymer composed of many identical subunits. The polymer contains two sugar derivatives, *N*-acetylglucosamine and *N*-acetylmuramic acid, and several different amino

acids, three of which D-glutamic acid, D-alanine, and *meso*-diaminopimelic acid. The backbone of this polymer is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. A peptide chain of four alternating D- and L-amino acids is connected to the carboxyl group of *N*-acetylmuramic acid. Chains of linked peptidoglycan subunits are joined by cross- links between the peptides. Often the carboxyl group of the terminal

D-alanine is connected directly to the amino group of diaminopimelic acid, but a **peptide interbridge** may be used instead. Most gram-negative cell wall peptidoglycan lacks the peptide interbridge. This cross-linking results in an enormous peptidoglycan sac that is actually one dense, interconnected network. These sacs have been isolated from gram-positive bacteria and are strong enough to retain their shape and integrity (**figure 3.20**), yet they are elastic and somewhat stretchable, unlike cellulose. They also must be porous, as molecules can penetrate them.

Gram-Positive Cell Walls

The thick, homogeneous cell wall of gram-positive bacteria is composed primarily of peptidoglycan, which often contains a peptide interbridge. However gram-positive cell walls usually also contain large amounts of **teichoic acids,** polymers of glycerol or ribitol joined by phosphate groups. Amino acids such as D-alanine or

sugars like glucose are attached to the glycerol and ribitol groups. The teichoic acids are connected to either the peptidoglycan itself by a covalent bond with the six hydroxyl of *N*-acetylmuramic acid or to plasma membrane lipids; in the latter case they are called lipoteichoic acids. Teichoic acids appear to extend to the surface of the peptidoglycan, and, because they are negatively charged, help give the gram-positive cell wall its negative charge. Teichoic acids are not present in gram-negative bacteria.

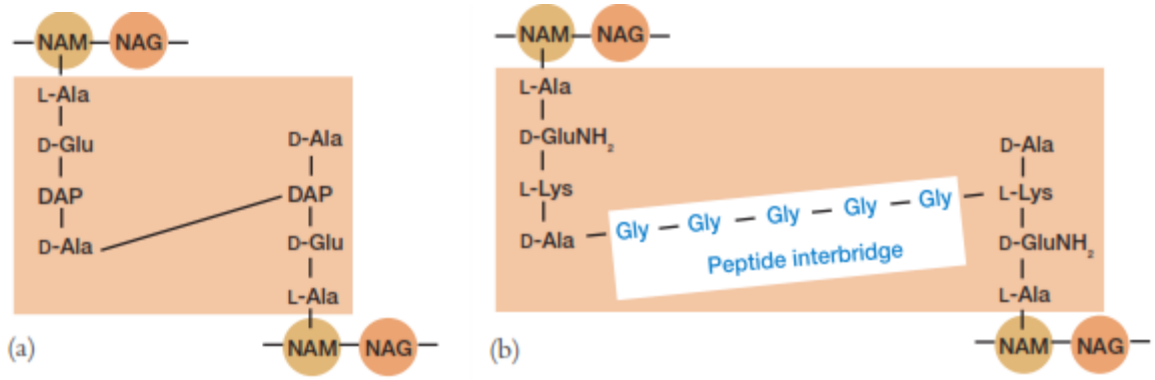


Fig: Peptidoglycan and peptide interbridge of a. gram-negative bacteria b. gram-positive bacteria

Gram-Negative Cell Walls

Gram-negative cell walls are much more complex than gram-positive walls. The outer membrane lies outside the thin peptidoglycan layer. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic

end. The most unusual constituents of the outer membrane are its lipopolysaccharides (LPSs). These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain.

VI. Components External to the Cell Wall

Capsules, Slime Layers, and S-Layers

Some bacteria have a layer of material lying outside the cell wall. When the layer is well organized and not easily washed off, it is called a capsule. A slime layer is a zone of diffuse, unorganized material that is removed easily. A glycocalyx is a network of polysaccharides extending from the surface of bacteria and other cells. Capsules and slime layers usually are composed of polysaccharides. Capsules contain a great deal of water and can protect bacteria against desiccation. They exclude bacterial viruses and most hydrophobic toxic materials

such as detergents.

S-layer

Many gram-positive and gram-negative bacteria have a regularly structured layer called an S-layer on their surface. The S-layer is composed of protein or glycoprotein. In gram-negative bacteria the S-layer adheres Directly to the outer membrane; it is associated with the peptidoglycan surface in gram-positive bacteria. It may protect the cell against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium . The S-layer also helps maintain the shape and envelope rigidity of at least some bacterial cells. It can promote cell adhesion to surfaces. Finally, the layer seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

Pili and Fimbriae

Many gram-negative bacteria have short, fine, hairlike appendages that are thinner than flagella and not involved in motility. These are usually called fimbriae. Although a cell may be covered with up to 1,000 fimbriae, they are only visible in an electron microscope due to their small size.

Flagella and Motility

Most motile bacteria move by use of flagella, threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20 μm long.

Bacterial species often differ distinctively in their patterns of flagella distribution.

Monotrichous bacteria (*trichous* means hair) have one flagellum; if it is located at an end, it is said to be a polar flagellum. Amphitrichous bacteria, have a single flagellum at each pole. In contrast, lophotrichous bacteria have a cluster of flagella at one or both ends. Flagella are spread fairly evenly over the whole surface of peritrichous, bacteria. Flagellation patterns are very useful in identifying bacteria.

Flagellar Ultrastructure

The bacterial flagellum is composed of three parts. (1) The longest and most obvious portion is the filament, which extends from the cell surface to the tip. (2) A basal body is embedded in the cell; and (3) a short, curved segment, the hook, links the filament to its basal body and acts as a flexible coupling.

The filament is a hollow, rigid cylinder constructed of a single protein called flagellin, which ranges in molecular weight from 30,000 to 60,000. The filament ends with a capping protein. Some bacteria have sheaths

surrounding their flagella. The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. In most gram-negative bacteria, the body has four rings connected to a central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers, respectively. The inner M ring contacts the plasma membrane. Gram- positive bacteria have only two basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan.

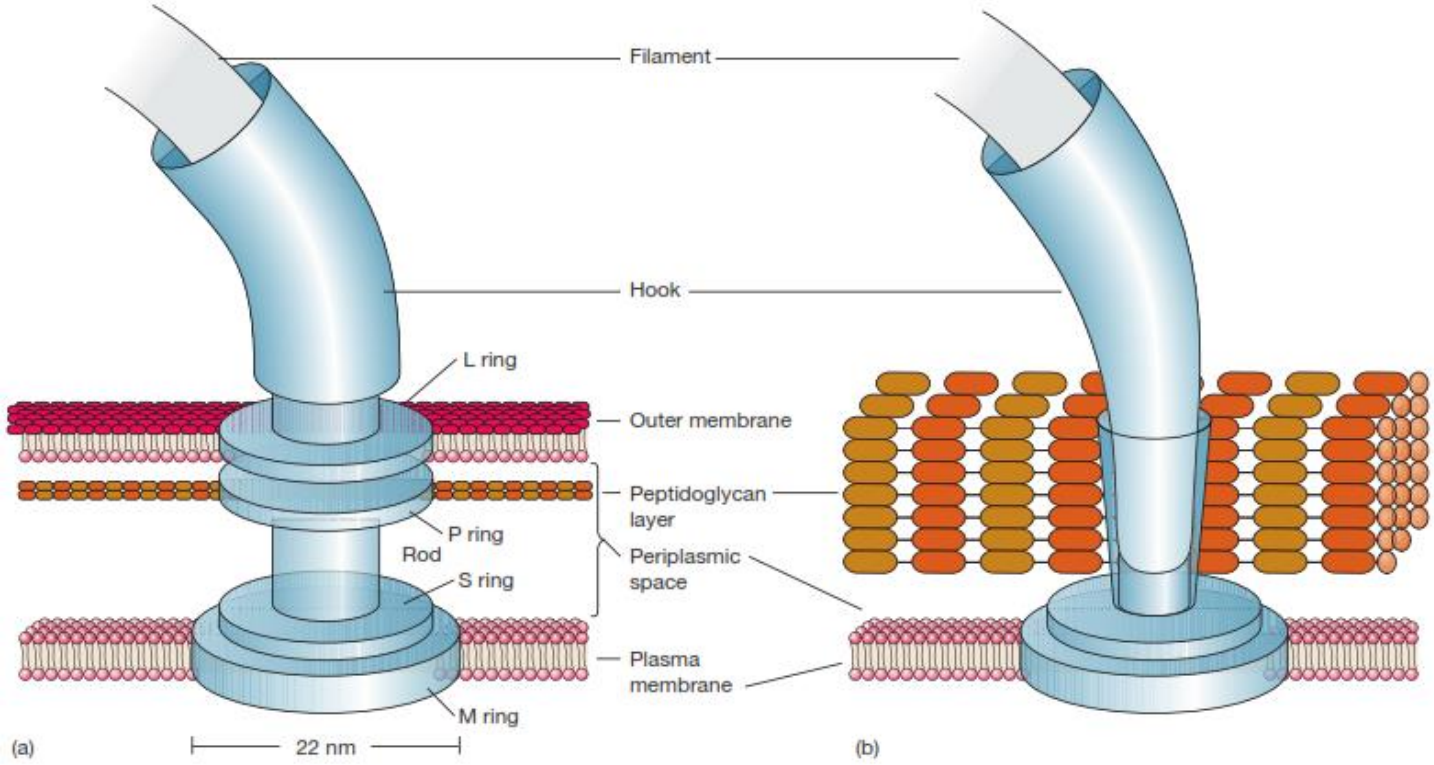


Figure: Ultra structure of bacterial flagella a.gram-negative bacteria b. gram-positive bacteria

3. a) Write about the three kingdom classification of microorganisms

Three kingdom classification was given by Haeckel, a German biologist and philosopher in 1866. The three kingdoms are **Protista, Plantae, Animalia**. In three kingdom system, kingdom protista was included along with kingdom plantae and kingdom animalia. All unicellular organisms and some multicellular organisms are placed in protista (Unicellular- bacteria, protozoans and acellular algae, Multicellular organisms without tissue- Fungi, algae, and slime moulds were included). Thus he proposed three kingdoms namely Protista, Plantae, Animalia..

1. The protists are believed to have evolved from prokaryotic monerans and the precursors from which higher eukaryotic kingdoms - Plantae, Fungi and Animalia —have evolved. The protists exhibit following features:

(a). These have a typical eukaryotic cell organization and possess nucleus, mitochondria, endoplasmic reticulum, Golgi bodies and in some organisms, plastids also.

(b). Locomotion takes place with the help of pseudopodia, cilia or flagella. The flagella or cilia have 9 + 2 internal microtubular structure.

This system was not accepted because it includes both prokaryotic and eukaryotic, chlorophyllous and non-chlorophyllous organisms together.

3. b) Chloroplasts

Chloroplasts also have their own DNA and ribosomes. Chloroplasts function in photosynthesis and can be found in eukaryotic cells such as plants. In photosynthesis, carbon dioxide, water, and light energy are used to make glucose and oxygen.

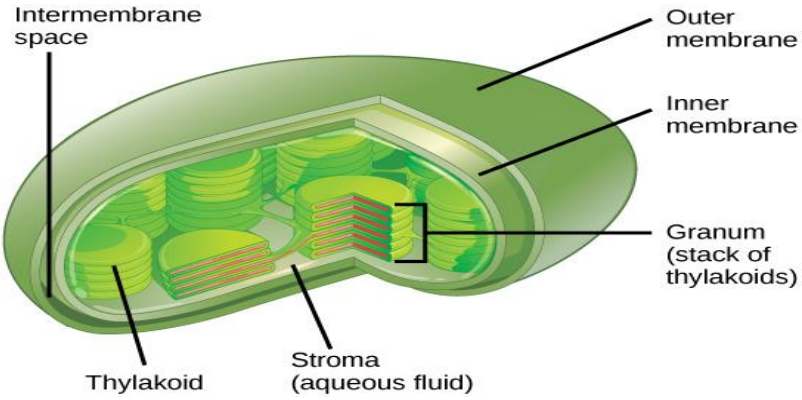


Figure. Chloroplast

Chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked, fluid-filled membrane sacs called thylakoids (Figure 11). Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane and surrounding the grana is called the stroma. The chloroplasts contain a green pigment called chlorophyll, which captures the energy of sunlight for photosynthesis.

UNIT II

4. Define medium and explain different types of media used for growth of bacteria

Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture medium.

Classification of culture media used in Microbiology laboratory on the basis of consistency

1. Solid medium

   Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

2. Semisolid media

   They are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

3. Liquid (Broth) medium

   These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth.

Classification of culture media based on the basis of composition

1. Synthetic or chemically defined medium

   A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known.

2. Non synthetic or chemically undefined medium

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Classification of Bacterial Culture Media based on the basis of purpose/ functional use/ application.

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. Basic media

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

Ex: Nutrient Agar

2. Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

Ex: Blood agar

3. Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

a. Selective medium: Differential growth suppression

Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated. Examples of selective media include: Thayer Martin Agar used to recover *Neisseria gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin. Mannitol Salt Agar and Salt Milk Agar used to recover *S.aureus* contains 10% NaCl.

b. Enrichment culture medium

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from fecal specimens.

4. Differential/ indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

Examples of differential media include: Mannitol salts agar (mannitol fermentation = yellow)

5. Transport media:

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Cary Blair transport medium and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected cholera patients.

6. Assay media

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

Other types of medium includes; Media for enumeration of Bacteria.

5. a.Write about the differences between mitosis and meiosis

S.N.	Differences	Mitosis	Meiosis
1	Type of Reproduction	Asexual	Sexual
2	Genetically	Similar	Different
3	Crossing Over	No, crossing over cannot occur.	Yes, mixing of chromosomes can occur.
4	Number of Divisions	One	Two
5	Pairing of Homologs	No	Yes
6	Mother Cells	Can be either haploid or diploid	Always diploid
7	Number of Daughter Cells produced	2 diploid cells	4 haploid cells
8	Chromosome Number	Remains the same.	Reduced by half.
9	Chromosomes Pairing	Does Not Occur	Takes place during zygotene of prophase I and continue upto metaphase I.
10	Creates	Makes everything other than sex cells.	Sex cells only: female egg cells or male sperm cells.
11	Takes Place in	Somatic Cells	Germ Cells
12	Chiasmata	Absent	Observed during prophase I and metaphase I.
13	Spindle Fibres	Disappear completely in telophase.	Do not disappear completely in telophase I.
14	Nucleoli	Reappear at telophase	Do not reappear at telophase I.
15	Steps	Prophase, Metaphase, Anaphase, Telophase.	(Meiosis 1) Prophase I, Metaphase I, Anaphase I, Telophase I; (Meiosis 2) Prophase II, Metaphase II, Anaphase II and Telophase II.

16	Karyokinesis	Occurs in Interphase.	Occurs in Interphase I.
17	Cytokinesis	Occurs in Telophase.	Occurs in Telophase I and in Telophase II.
18	Centromeres Split	The centromeres split during anaphase.	The centromeres do not separate during anaphase I, but during anaphase II.
19	Prophase	Simple	Complicated
20	Prophase	Duration of prophase is short, usually of few hours.	Prophase is comparatively longer and may take days.
21	Synapsis	No Synapsis	Synapsis of Homologous chromosomes takes place during prophase.
22	Exchange of Segments	Two chromatids of a chromosome do not exchange segments during prophase.	Chromatids of two homologous chromosome exchange segments during crossing over.
23	Discovered by	Walther Flemming	Oscar Hertwig
24	Function	Cellular reproduction and general growth and repair of the body.	Genetic diversity through sexual reproduction.
25	Function	Takes part in healing and repair.	Takes part in the formation of gametes and maintenance of chromosome number.

5.b. Write short note on endospore formation

A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore.** Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others.

Spore formation (sporogenesis or sporulation)

It commences when growth ceases due to lack of nutrients. It is a complex process and may be divided into seven stages (**figure 3**).

An axial filament of nuclear material forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature spore in a second membrane (stage III). Next, cortex is laid down in the space between

the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats then are formed around the cortex (stage V), and maturation of the spore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage VII). Sporulation requires only about 10 hours in *Bacillus megaterium.*

Hours from the end of the logarithmic phase of growth: 0.25 h-a typical vegetative cell; 4 h-stage II cell, septation; 5.5 h-stage III cell, engulfment; 6.5 h-stage IV cell, cortex formation; 8 h-stage V cell, coat formation; 10.5 h-stage VI cell, mature spore in sporangium.

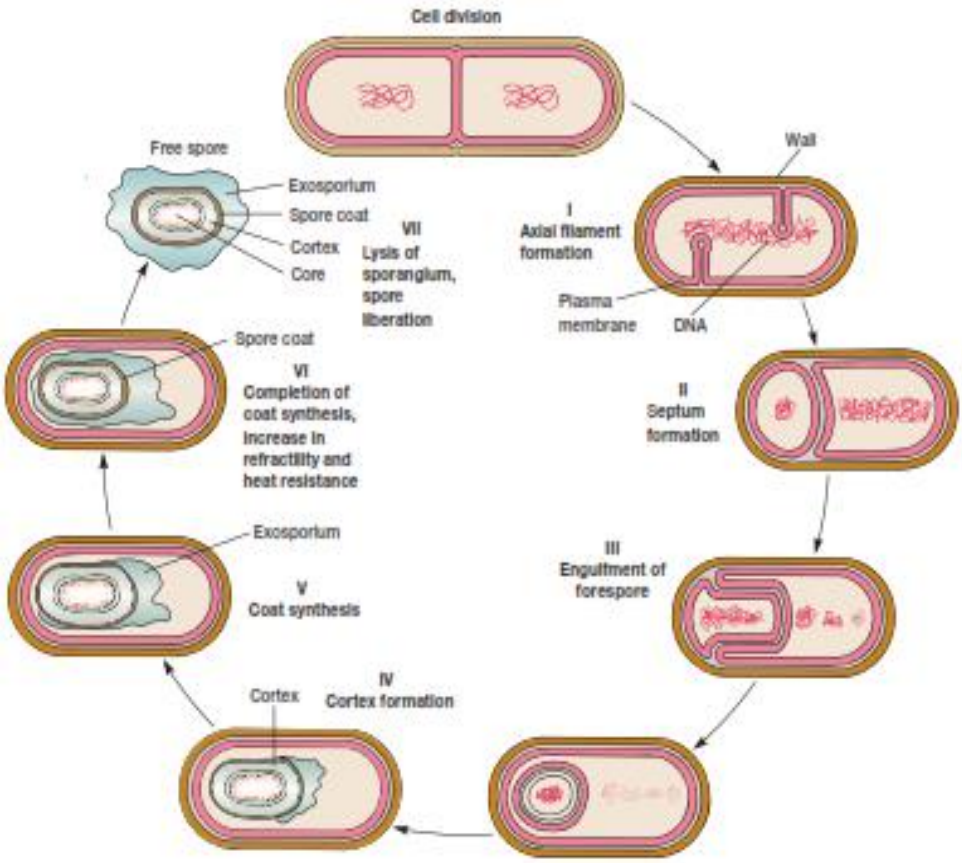


Figure : Endospore Formation: Life cycle of *Bacillus megaterium.*

UNIT III

6. a Write in detail about the structure of t-RNA

Transfer RNA (tRNA)

tRNA is the smallest of the 3 types of RNA having about 75-95 nucleotides. tRNAs are an essential component of translation, where their main function is the transfer of amino acids during protein synthesis. Therefore they are called transfer RNAs. Each of the 20 amino acids has a specific tRNA that binds with it and transfers it to the growing polypeptide chain. tRNAs also act as adapters in the translation of the genetic sequence of mRNA into proteins. Therefore they are also called adaptor molecules.

tRNAs have a clover leaf structure which is stabilized by strong hydrogen bonds between the nucleotides. Apart from the usual 4 bases, they normally contain some unusual bases mostly formed by methylation of the usual bases, for example, methyl guanine and methylcytosine.

The 5′ P end terminates usually into guanine (G), while the 3′ OH end always terminates into a 5′ CCA3′ sequence. Amino acid is carried on the 3′-end, associated with the adenine (A), general structure of clover leaf model is described below (Fig. 4.2).

(1) The 3′-end terminates into 5'CCA3′ sequence that is always unpaired. The terminal A residue is the site at which the amino acid is bound covalently.

(2) Starting from the 3′-end, after the 3′ ACC5′ sequence, the region includes few paired bases.

(3) Then comes the first loop containing 7 unpaired bases. This loop is designated **as "TΨ C loop"** because it always contains a sequence 5′ ribothymidine- pseudouridine- cytidine 3′. This loop is involved in binding to ribosome.

(4) After the "5′ -T Ψ C-3′ loop", in the 5′ direction, there occurs a loop of variable size, called the extra loop or the "lump". The lump may contain 3 to 21 bases.

(5) The third loop contains 7 unpaired bases and it has the "anticodon." Anticodon consists of 3 bases. At the 3′ -end of the anticodon, there is a purine (A or G) while at the 5′ -end, there is always a uracil (U). At the time of protein synthesis, anticodon pairs with its complementary "codon" on mRNA.

(6) The fourth loop is larger than others and contains 8-12 unpaired bases. It is designated as "D-loop" because it is rich in dihydrouridine (UH_2). The enzyme aminoacylsynthetase binds to this loop.

Function

Carries the correct amino acid to the site of protein synthesis in the ribosome

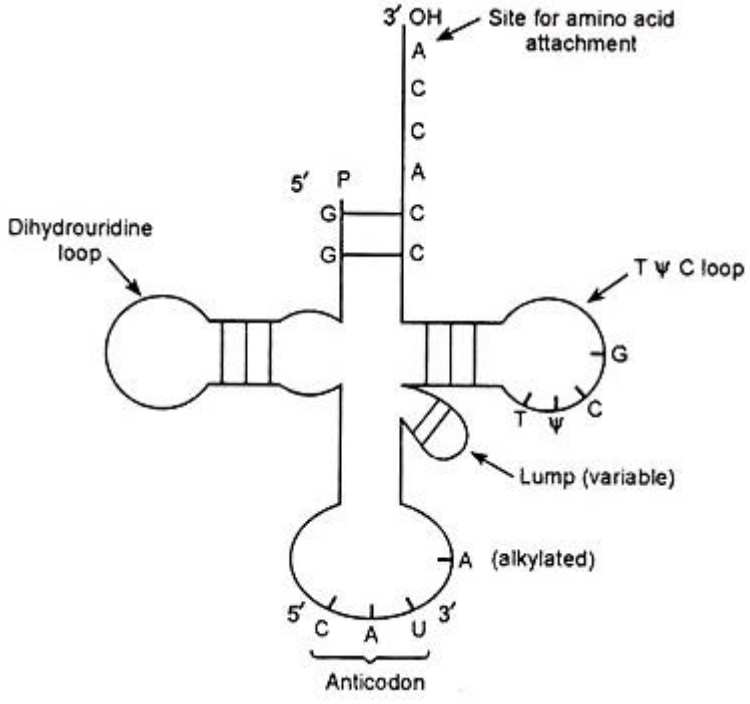


Fig: Clover leaf model of tRNA.

6. b. Write about the different types of proteins

According to classification of proteins, these are again classified into forms, functions and composition.

Classification of Proteins on the Basis of Structure:

Fibrous proteins:

In this classification proteins, The polypeptide chains are elongated and wound about an axis in a helical shape . These are structural proteins. They can be extracellularand will then be insoluble in water and have a protective function: α keratin hair, fibroin silk, elastin of the skin,

collagen tendons.

They can also be intracellular include myosin and tropomyosin muscle cells. careful not to confuse fibrous and filamentary (globular proteins attached to each other).

Globular proteins:

Soluble in water, they are spherical . They have a much more complex than the fibrous protein structure, but they have a much greater variety of biological activities. They can be membrane and then have roles as: Carrier , Receptors , Ion channel , GAP links , Cell adhesion proteins . They may be soluble and be plasma such as albumin, protein hormones such as LH, cytosolic proteins circulating proteins such as Calmodulin .

Classification of proteins based on function:

They may be involved in:

1. the structure and support , is the case of collagen, elastin, glycoproteins membrane
2. the contraction : actin, myosin …
3. the cell adhesion proteins such as GAP junctions and proteins such as cadherin ,
4. the reception signal such membrane insulin receptors or intracellular steroid receptors,
5. the signal transduction , a typical example being formed by the membrane protein G (see course on cellular communication)
6. a signal : they can be informative molecule as growth factors (EGF) and Folicular stimulating hormones (FSH),
7. the immunity , the role of immunoglobulin
8. the transportation such as hemoglobin (O_2) and transferrin (iron)
9. the catalysis : therefore these proteins also play a role in metabolism, replication and DNA transcription, muscle contraction, cell signaling …

Classification of Proteins Based on Composition:

1. There are two main types of proteins. Those containing only amino acids are holoproteins .
2. Those containing a protein moiety (the apoprotein) and a non-protein portion are heteroproteins .
3. Both parts are linked in various ways: covalent bonds , ionic , hydrogen , hydrophobic . This non-protein portion may be a group prosthetic (inducing the emergence of new biological properties setting, as heme in hemoglobin).

If they are carbohydrates that are added in an amount between 5 and 40% of the molecule, the protein is called glycoproteins and glycosylated proteins .If the proportion of carbohydrates to pass more than 90% of

the molecule, one blade of peptidoglycan , they have a passive protection. 4. The element can be added one or more metal cofactors (Cu, Zn, …) that metalloprote

7. Explain in detail about the B model of DNA and its important functions

DNA is the largest biomolecule in the cell and it is negatively charged. **Watson and Crick** in 1953 proposed that **DNA** is made up of two strands that are twisted around each other to form a right-handed helix. 1. The building blocks of nucleic acids are nucleotides. Nucleotides that compose DNA are called **deoxyribonucleotides**. The three components of a deoxyribonucleotide are a five-carbon sugar called **deoxyribose**, a phosphate group, and a **nitrogenous base**, a nitrogen-containing ring structure that is responsible for **complementary base pairing** between nucleic acid strands A **nucleoside** comprises the five-carbon sugar and nitrogenous base. 2. The deoxyribonucleotide is named according to the **nitrogenous bases** (Figure 1). The nitrogenous bases **adenine** (A) and **guanine** (G) are the **purines**; they have a double-ring structure with a six-carbon ring fused to a five-carbon ring. The **pyrimidines**, **cytosine** (C) and **thymine** (T), have only a six-carbon ring structure.

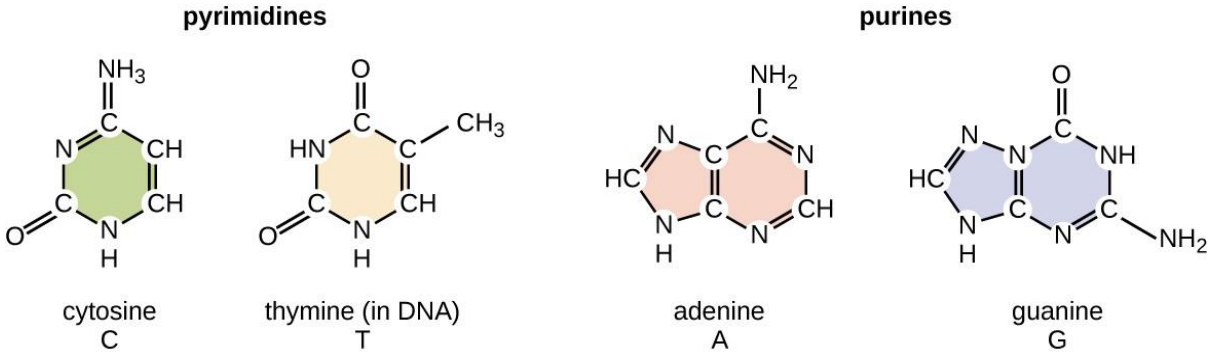


Figure 1. Nitrogenous bases within DNA are categorized into the two-ringed purines adenine and guanine and the single-ringed pyrimidines cytosine and thymine. Thymine is unique to DNA.

3. Individual nucleoside triphosphates combine with each other by covalent bonds known as 5′-3′ **phosphodiester bonds**, or linkages whereby the phosphate group attached to the 5′carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3′ carbon of the sugar of the next nucleotide. Phosphodiester bonding between nucleotides forms the **sugar-phosphate backbone**, the alternating sugar-phosphate structure composing the framework of a nucleic acid strand (Figure 2). To construct the sugar-phosphate backbone, the two terminal phosphates are released from the dNTP as a pyrophosphate. The resulting strand of nucleic acid has a free phosphate group at the 5′ carbon end and a free hydroxyl group at the 3′ carbon end. The phosphodiester linkages betweennucleotides can be cleaved hydrolytically by chemicals or hydrolyzed enzymatically by deoxyribonucleases.

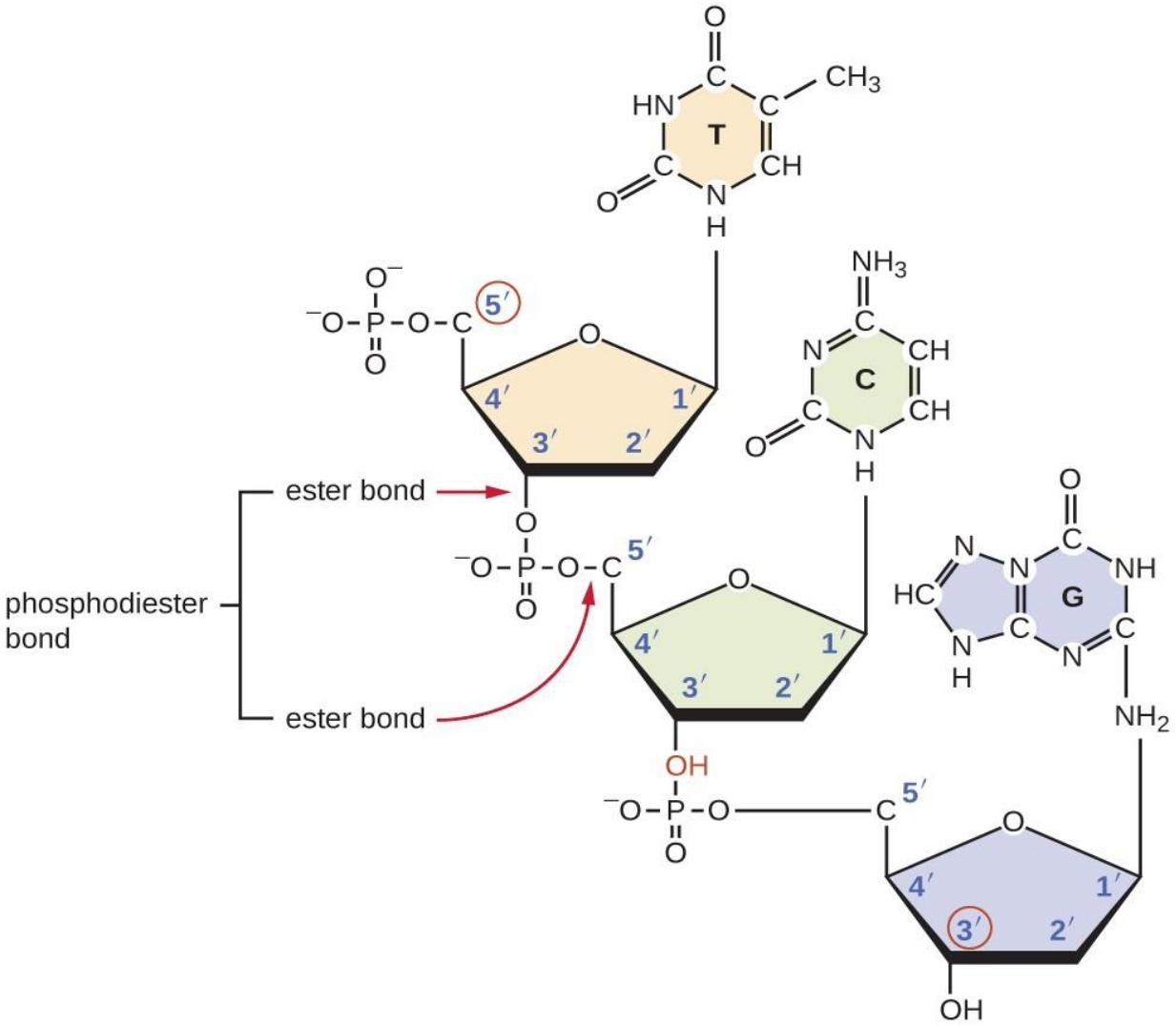


Figure 2. Phosphodiester bonds form between the phosphate group attached to the 5′ carbon of one nucleotide and the hydroxyl group of the 3′ carbon in the next nucleotide, bringing about polymerization of nucleotides in to nucleic acid strands.

4. The two DNA strands are **antiparallel**, such that the 3′ end of one strand faces the 5′ end of the other (Figure 6). The 3′ end of each strand has a free hydroxyl group, while the 5′ end of each strand has a free phosphate group. The sugar and phosphate of the polymerized nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside. These nitrogenous bases on the interior of the molecule interact with each other, base pairing.

5. There are approximately 10 bases per turn in DNA.

6. The asymmetrical spacing of the sugar-phosphate backbones generates major grooves (where the backbone is far apart) and minor grooves (where the backbone is close together) (Figure 6). These grooves are locations where proteins can bind to DNA. The binding of these proteins can alter the structure of DNA, regulate **replication**, or regulate **transcription** of DNA into RNA.

7. Base pairing takes place between a purine and pyrimidine. In DNA, adenine (A) and thymine (T) are **complementary base pairs**, and cytosine (C) and guanine (G) are also complementary base pairs. Chargaff discovered that the amount of **adenine** is approximately equal to the amount of **thymine** in DNA, and that the amount of the **guanine** is approximately equal to **cytosine**. The base pairs are stabilized by hydrogen bonds; adenine and thymine form two hydrogen bonds between them, whereas cytosine and guanine form three hydrogen bonds between them.

8. When DNA is heated, the temperature at which half of the helical structure is lost is defined as melting temperature (Tm). The loss of helical structure in DNA is called denaturation. Cooling or removing chemicals can lead to renaturation of DNA by allowing hydrogen bonds to reform between complementary bases.

9. The pitch of helix is 3.4 nm (34 A^o) with roughly 10 base pairs in each turn. The average distance

between two adjacent base pairs comes to about 0.34 nm..

Functions of DNA:

1. Genetic Information (Genetic Blue Print):

DNA is the genetic material which carries all the hereditary information. The genetic information is coded in the arrangement of its nitrogen bases.

2. Replication:

DNA has unique property of replication or production of carbon copies (Autocatalytic function). This is essential for transfer of genetic information from one cell to its daughters and from one generation to the next.

3. Chromosomes:

DNA occurs inside chromosomes. This is essential for equitable distribution of DNA during cell division.

4. Recombination's:

During meiosis, crossing over gives rise to new combination of genes called recombinations.

5. Mutations:

Changes in sequence of nitrogen bases due to addition, deletion or wrong replication give rise to mutations. Mutations are the fountain head of all variations and evolution.

6. Transcription:

DNA gives rise to RNAs through the process of transcription. It is heterocatalytic activity of DNA.

7. Cellular Metabolism:

It controls the metabolic reactions of the cells through the help of specific RNAs, synthesis of specific proteins, enzymes and hormones.

8. Differentiation:

Due to differential functioning of some specific regions of DNA or genes, different parts of the organisms get differentiated in shape, size and functions.

9. Development:

DNA controls development of an organism through working of an internal genetic clock with or without the help of extrinsic information.

10. DNA Finger Printing:

Hypervariable microsatellite DNA sequences of each individual are distinct. They are used in identification of individuals and deciphering their relationships. The mechanism is called DNA finger printing.

11. Gene Therapy:

Defective heredity can be rectified by incorporating correct genes in place of defective ones.

12. Antisense Therapy:

Excess availability of anti-mRNA or antisense RNAs will not allow the pathogenic genes to express themselves. By this technique failure of angioplasty has been checked. A modification of this technique is RNA interference (RNAi).

UNIT IV

8. a) Define sterilization and explain in detail about different sterilization methods

Sterilization [Latin *sterilis,* unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents.

A sterilizing agent is called a **sterilant.**

The methods that are used in control of microorganisms can be classified as

I. Physical Methods

II. Chemical Methods

I. Physical Methods

Heat and other physical agents are normally used to control microbial growth and sterilize objects. The four most frequently employed physical agents are **heat, low temperatures, filtration, and radiation.**

a. Heat:

Heating is still one of the most popular ways to destroy microorganisms. Either **moist** or **dry heat** may be applied.

Thermal death time (TDT): This is the shortest time needed to kill all organisms in a microbial suspension at a specific temperature and under defined conditions

Moist heat sterilization must be carried out at temperatures above 100°C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure. Steam sterilization is carried out with an **autoclave** (**figure 7.3**), a device somewhat like a fancy pressure cooker. The development of the autoclave by Chamberland in 1884 tremendously stimulated the growth of microbiology. Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed. Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure. At this temperature saturated steam destroys all

vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for about 15 minutes to provide a margin of safety. Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.

Autoclaving must be carried out properly or the processed materials will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121°C even though it may reach a pressure of 15 pounds. The chamber should not be packed too tightly because the steam needs to circulate freely and contact everything in the autoclave. Bacterial endospores will be killed only if they are kept at 121°C for 10 to 12 minutes.

Many substances, such as milk, are treated with controlled heating at temperatures well below boiling, a process known as **pasteurization** in honor of its developer Louis Pasteur. Pasteurization does not sterilize a beverage, but it does kill any pathogens present and drastically slows spoilage by reducing the level of nonpathogenic spoilage microorganisms. Milk can be pasteurized in two ways.

Low temperature long time method (or) Holder method:

The temperature employed is 63°C for 30 minutes.

High temperature short time method or flash pasteurization:

Large quantities of milk are now usually subjected to **flash pasteurization** or high-temperature short-term (HTST) pasteurization, which consists of quick heating to about 72°C for 15 seconds, then rapid cooling.

Ultrahigh-temperature (UHT) sterilization: Milk and milk products are heated at 140 to 150°C for 1 to 3 seconds. UHT-processed milk does not require refrigeration and can be stored at room temperature for about 2 months without flavor changes.

Dry heat sterilization

Many objects are best sterilized in the absence of water by **dry heat sterilization.**

Flaming: Inoculating loops, Points of forceps, spatula, needles etc held in bunsen burner flame till they become red hot inorder to be sterilized. Mouths of culture tubes could be passed a few time through bunsen burner flame without allowing them to become red hot. The bacteria get destroyed.

Incineration: It is an effective way to sterilize and dispose of contaminated paper cups, bags, and dressings. Incineration is destruction of microorganisms by burning.

Hot-air sterilization: The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours. Microbial death apparently results from the oxidation of cell constituents and denaturation of proteins. Hot air is a bad conductor of heat and its penetrating power is low. So it needs longer holding periods than moist heat sterilization.

Uses:

It is used to sterilize glass ware & metal instruments that would corrode if repeatedly exposed to moist heat.

Advantages

1. Dry heat does not corrode glassware and metal instruments
2. It can be used to sterilize powders, oils, and similar items.
3. Most laboratories sterilize glass petri dishes and pipettes with dry heat.

Disadvantages

It is slow and not suitable for heat sensitive materials like many plastic and rubber items.

b. Filtration

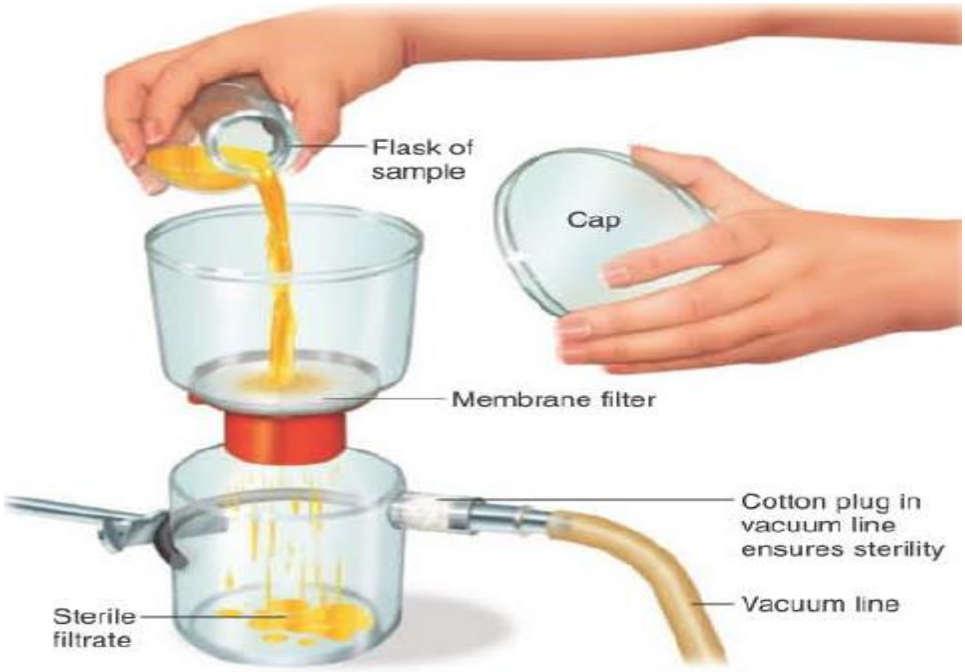
Filtration is the passage of a liquid or gas through a screen like material with pores small enough to retain microorganisms. A vacuum is created in the receiving flask; air pressure then forces the liquid through the filter.

In recent years, **membrane filters**, composed of such substances as cellulose esters or plastic polymers, have become popular for industrial and laboratory use (Fig). These filters are only 0.1 mm thick. The pores of membrane filters include, for example, 0.22µm and 0.45-µm sizes, which are intended for bacteria. Some very flexible bacteria, such as spirochetes, or the wall-less mycoplasma, will sometimes pass through such filters, however. Filters are available with pores as small as 0.01µm, a size that will retain viruses and even some large protein molecules.

Uses:

Filtration is used to sterilize heat-sensitive materials, such as some culture media, enzymes, vaccines, and antibiotic solutions

Fig: Filter sterilization with a disposable, presterilized plastic unit. The sample is placed into the upper chamber and forced through the membrane filter by a vacuum in the lower chamber. Pores in the membrane filter are smaller than the bacteria. So bacteria are retained on the filter. The sterilized sample can then be decanted from the lower chamber. Similar equipment with removable filter disks is used to count bacteria in samples.



Air also can be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out. **Laminar flow biological safety cabinets** employing **high-efficiency particulate air (HEPA) filters,** which remove 99.97% of 0.3 µm particles, are one of the most important air filtration systems. Laminar flow biological safety cabinets force air through

HEPA filters, then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room.

c. Radiation

Radiation has various effects on cells, depending on its wavelength, intensity, and duration. Radiation that kills microorganisms (sterilizing radiation) is of two types: ionizing and nonionizing.

Ionizing radiation- gamma rays, X rays, or high-energy electron beams- has a wavelength shorter than that of nonionizing radiation, less than about 1 nm. Therefore, it carries much more energy. **Gamma rays** are emitted by certain radioactive elements such as cobalt. Gamma rays penetrate deeply but may require hours to sterilize large masses. **Electron beams** are produced by accelerating electrons to high energies in special machines. High-energy electron beams have much lower penetrating power but usually require only a few seconds of exposure. **X rays**, which are produced by machines in a manner similar to the production of electron beams, are similar to gamma rays.

The principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals. These radicals react with organic cellular components, especially DNA & cause sufficient mutations to kill the microbe.

Uses: High-energy electron beams, is used to sterilize pharmaceuticals and disposable dental and medical supplies, such as plastic syringes, surgical gloves, suturing materials, and catheters.

Nonionizing radiation: It has a wavelength longer than that of ionizing radiation, usually greater than about 1 nm. The best example of nonionizing radiation is ultraviolet (UV) light. UV light damages the DNA of exposed cells by causing bonds to form between adjacent pyrimidine bases, usually thymines, in DNA chains. These *thymine dimmers* inhibit correct replication of the DNA during reproduction of the cell. The UV wavelengths most effective for killing microorganisms are about 260 nm; these wavelengths are specifically absorbed by cellular DNA.

Uses:

It is used to control microbes in the air. A UV, or "germicidal," lamp is commonly found in hospital rooms, nurseries, operating rooms, and cafeterias.

UV light is also used to disinfect vaccines and other medical products.

Commercial UV units are used for water treatment. Pathogens and other microorganisms are destroyed when a thin layer of water is passed under the lamps.

Disadvantage:

1. The radiation is not very penetrating, so the organisms to be killed must be directly exposed to the rays. Organisms protected by solids and such coverings as paper, glass, and textiles are not affected.
2. UV light can damage human eyes, and prolonged exposure can cause burns and skin cancer in humans.

Microwaves : Moisture-containing foods are heated by microwave action, and the heat will kill most vegetative pathogens.

II. Chemical Agents

Although objects are sometimes disinfected with physical agents, chemicals are more often employed in disinfection and antisepsis.

Factors influence the effectiveness of chemical disinfectants and antiseptics:

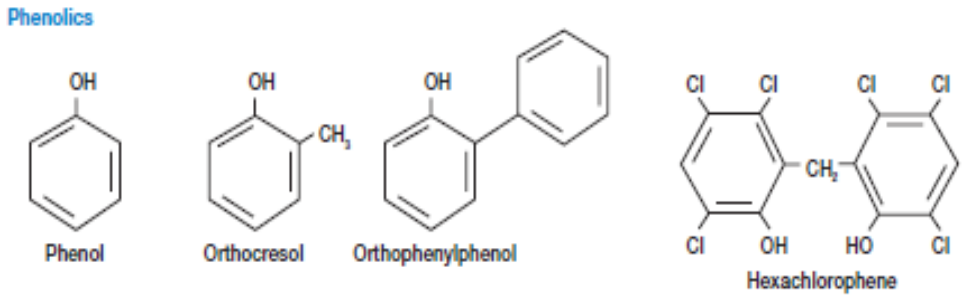
1. The kinds of microorganisms potentially present,
2. The concentration and nature of the disinfectant to be used, and
3. The length of treatment should be considered.

The characteristics of a disinfectant:

1. Ideally the disinfectant must be effective against a wide variety of infectious agents (gram-positive and gram-negative bacteria, acid-fast bacteria, bacterial endospores, fungi, and viruses) at high dilutions and in the presence of organic matter.
2. Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials.
3. The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces.
4. If possible the disinfectant should be relatively inexpensive.

a. Phenolics

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. Today phenol and phenolics (Derivatives of phenol, called phenolics, contain a molecule of phenol that has been chemically altered to reduce its irritating qualities or increase its antibacterial activity in combination with a soap or detergent) such as **cresols, xylenols**, and **orthophenylphenol** are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics. Phenolics act by denaturing proteins and disrupting cell membranes. Advantages of disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they have a disagreeable odor and can cause skin irritation. **Hexachlorophene** has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods. However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

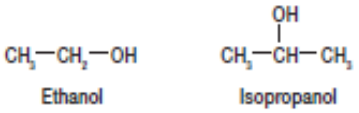


b. Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed. The two most popular alcohol

germicides are ethanol and isopropanol, usually used in about 70 to 80% concentration. They act by denaturing proteins and possibly by dissolving membrane lipids. A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

Alcohols



c. Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table. They exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals.

The halogens iodine and chlorine are important antimicrobial agents. **Iodine** is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores. Iodine often has been applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Iodine is an effective antiseptic

Disadvantages:

Skin may be damaged, a stain is left, and iodine allergies can result.

More recently iodine has been complexed with an organic carrier to form an **iodophor.** Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and laboratories for disinfecting. Some popular brands are Wescodyne for skin and laboratory disinfection and Betadine for wounds.

Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HClO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.

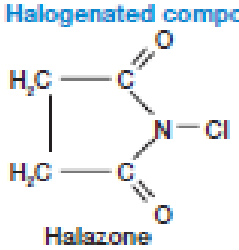
$$Cl_2 + H_2O \rightarrow HCl + HClO$$

$$Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HClO$$

$$HClO \rightarrow HCl + O$$

Death of almost all microorganisms usually occurs within 30 minutes. Since organic material interferes with chlorine action by reacting with chlorine and its products, an excess of chlorine is added to ensure microbial destruction. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water.

Halogenated compound



Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets. Halazone (parasulfone dichloramidobenzoic acid) slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water. Chlorine solutions make very effective laboratory and household disinfectants. An excellent disinfectant-detergent combination can be prepared if a 1/100 dilution of household bleach (e.g., 1.3 floz of Clorox or Purex bleach in 1 gal or 10 ml/liter) is combined with sufficient nonionic detergent (about 1 oz/gal or 7.8 ml/liter) to give a 0.8% detergent concentration. This mixture will remove both dirt and bacteria.

d. Heavy Metals

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides. More recently these have been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal).

Silver nitrate: A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against *Chlamydia* as well as *Neisseria*).

Silver sulfadiazine is used on burns.

Copper sulfate is an effective algicide in lakes and swimming pools.

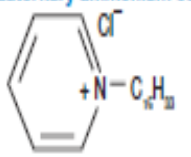
Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

e. Quaternary Ammonium Compounds

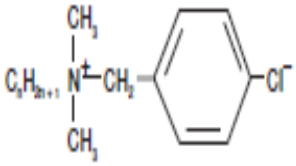
Detergents [Latin *detergere,* to wipe off or away] are organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends. Due to their amphipathic nature, detergents solubilize otherwise insoluble residues and are very effective cleansing agents. Although anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants. The most popular of these disinfectants are quaternary ammonium compounds characterized by a positively charged quaternary nitrogen and a long hydrophobic aliphatic chain. They disrupt microbial membranes and may also denature proteins.

Cationic detergents like **benzalkonium chloride** and **cetylpyridinium chloride** kill most bacteria but not *M. tuberculosis* or endospores. They do have the advantages of being stable, nontoxic, and bland but they are inactivated by hard water and soap. Cationic detergents are often used as disinfectants for food utensils and small instruments and as skin antiseptics. Zephiran contains benzalkonium chloride and Ceepryn, cetylpyridinium chloride.

Quaternary ammonium compounds



Cetylpyridinium chloride

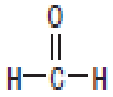


Benzalkonium chloride

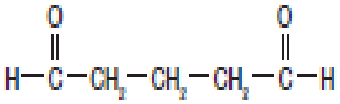
f. Aldehydes

Both of the commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by crosslinking and alkylating molecules. They are sporicidal and can be used as chemical sterilants. **Formaldehyde** is usually dissolved in water or alcohol before use. A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipment. **Glutaraldehyde** usually disinfects objects within about 10 minutes but may require as long as 12 hours to destroy all spores.

Aldehydes



Formaldehyde



Glutaraldehyde

g. Sterilizing Gases

Many heat-sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine components, sutures, and catheters can be sterilized with ethylene oxide gas.

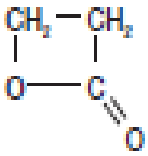
Ethylene oxide (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins. It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps. Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity. Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either CO_2 or dichlorodifluoromethane. The ethylene oxide concentration, humidity, and temperature influence the rate of sterilization. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/liter. Extensive aeration of the sterilized materials is necessary to remove residual EtO because it is so toxic.

Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccines and sera. BPL decomposes to an inactive form after several hours and is therefore not as difficult. It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic. For these reasons, BPL has not been used as extensively as EtO.

Recently **vapor-phase hydrogen peroxide** has been used to decontaminate biological safety cabinets.

Gases

Ethylene oxide



Betapropiolactone

h. Dyes:

Two classes of dye compounds having antimicrobial properties are Triphenyl methane gas, Acridine dyes. These dyes are used extensively as skin & wound antiseptics. Both are bacteriostatic in high dilution but are of low bacteriocidal activity.

Examples of **Triphenyl methane dyes** are Malachite green, Briliant green & Crystal violet. They are more active against gram positive than gram negative organisms.

Examples of dyes derived from **Acridine** are Acriflavin, Proflavine. Acridine dyes are more active against gram positive than gram negative organisms.

9. a) Write an essay on economic importance of bacteria

Bacteria are prokaryote and ultra-microscopic organisms, yet they play an important role in nature. They are of tremendous importance to man. They play an important role in agriculture and medicine and are the basis of many industries. Some are beneficial to man directly or indirectly, others are very harmful as they cause various plant and animal diseases. Economic importance of bacteria refers to the beneficial and harmful effects of bacteria to the nature, humans and environment. Bacteria, have the following economic importance.

1. Beneficial effects of Bacteria

Bacteria play important roles in different fields such as agriculture, industry etc.

A) Role in agriculture

a) **Scavenging Role**: Saprophytic bacteria obtain food from organic remains such as animal excreta, fallen leaves, meat etc. They decompose these substances by action of digestive enzymes aerobically or anaerobically (known as fermentation). Thus they help in sanitation of nature, so also known as scavengers. E.g. *Pseudomonas*

b) **Production of Organic Manure:** Saprophytic bacteria help in breaking of complex organic substance to simpler forms. Thus, in this process, they help to convert farm refuse, dung and other wastes to manure.

c) **Preparation of Ensilage:** Ensilage is preserved cattle fodder prepared by packing fresh chopped fodder sprinkled with molasses. Fermentation activity of bacteria produces lactic acid that acts as preservative in ensilage.

d) **Production of fuel:** Bacteria, while converting animal dung and other organic wastes to manure, help in production of fuel that is a must in gobar gas plant.

e) **Disposal of sewage:** Bacteria help in disposal of sewage by decomposing it and thus, help in environmental sanitation.

f) Fertility of the soil: Bacteria bring about physical and chemical changes in the soil by converting insoluble materials into soluble ones. These bacteria are the ammonifying, nitrifying and the nitrogen fixing Bacteria.

(i) Ammonifying Bacteria

The decay bacteria decompose the proteins into amino acids, which are reduced to ammonia by ammonifying bacteria. The free ammonia combines in the soil to form ammonium salts. This conversion is known as ammonification. Examples are Bacillus ramosus, B. vulgaris etc.

(ii) Nitrifying Bacteria

These bacteria convert ammonium salts into nitrates, which are absorbed by the plants. The nitrifying bacteria are the Nitrobacter and Nitrosomonas. The Nitrosomonas oxidize the ammonium salts into nitrous acid, which forms nitrites in the soil. The Nitrobacter then converts the nitrites into nitrates. This conversion of ammonium salts into available nitrates is called nitrification.

(iii) Nitrogen Fixing Bacteria

These bacteria take up nitrogen from the atmosphere and convert it into organic nitrogen compounds. It is known as nitrogen fixation. The nitrogen-fixing bacteria are of two types. One type includes Azotobacter and Clostridium, which live freely in the soil and fix nitrogen of the air in their bodies in the form of nitrogenous organic compounds. The other types of bacteria are the nodule bacteria, the Bacillus radicicola. Rhizobium lives as symbiotic bacteria in the roots of leguminous plants and forms nodules. These bacteria absorb free nitrogen from the bacterial cell. The leguminous plants thus enrich the fertility of the soil. They are grown for green manuring and rotation of crops.

B) <u>Role in Industry</u>

a) **Dairy Industry:** Bacteria such as *Streptococcus lactis* convert milk sugar lactose into lactic acid that coagulates casein (milk protein). Then, milk is converted into curd, yoghurt, cheese etc needed for the industry.

b) **Production of Organic Compounds:** Fermentation (breakdown of carbohydrate in absence of oxygen) action of various bacteria produces organic compounds like lactic acid (by *Lactobacillus*), acetic acid (by *Acetobacter aceti*), acetone (by *Clostridium acetabutylicum*) etc.

c) **Fibre Retting:** The action of some bacteria like *Clostridium, Pseudomonas* etc. help in fibre retting i.e. separation of stem and leaf fibre of plants from other softer tissue.

d) **Curing:** The leaves of tea and tobacco, beans of coffee and coca are cured off their bitterness with the help of action of certain bacteria such as *Bacillus megatherium.*

e) **Production of Antibiotics:** Number of anti bacterial and anti fungal antibiotics such as Hamycin, Polymyxin, Trichomycin etc are obtained from mycelia bacteria (like *Streptomyces*). Similarly, Bacillus is used for production of antibiotics such as Bacitracin, Gramicidin etc

f) **Production of Vitamins:** Different kinds of vitamins are produced from bacteria like Riboflavin from *Clostridium butylicum,* Vitamin B12 from *Bacillus megatherium* and Vitamin K and B-complex from *Escherichia coli*.

2. Harmful effects of Bacteria:

Though bacteria plays important role in agriculture, industries and natural sanitation etc, it has the following harmful effects:

a) **Food Spoiling:** Saprophytic bacteria always not only help in decomposition of dead matters, but they also cause the rotting of vegetables, fruits, meat, bread etc. Staphylococcus and Clostridium botulinum cause food poisoning when rotten food is eaten,

b) **Food Poisoning:** Bacteria like *Staphylococcus aureus* cause food poisoning and cause people diarrhea and vomiting.

c) **Damaging of domestic articles:** *Spirochete cytophaga* deteriorates cotton, leather and wooden articles.

d) **Denitrification:** Bacteria such as *Thiobacillus* and *Microbacillus*convert nitrate of the soil to the gaseous nitrogen, which decreases the fertility of the soil.

e) **Desulphurication:** Bacteria such as *Desulfovibrio* convert soil sulphates into hydrogen sulphide.

f) **Cause of Diseases:** It is known that over 90% of human diseases and over 10% of plant diseases are caused by bacteria.

(i) Animal Pathogenic Bacteria

There are a large number of parasitic bacteria, which cause various serious diseases in man and domestic animals, sometimes in epidemic form. They are invisible enemies. Some of the common human diseases producing bacteria are Mycobacterium tuberculosis causing tuberculosis, Clostridium tetani causing tetanus, Shigella dysenteriae causing dysentery, Hemophilous influenzea causing influenza, Corynebacteriaum diphtlteriea causing diphtheria, Vibrio cholerae causing cholera, Streptococcus causing blood poisoning, Treponema pallidium causing syphilis, Gonococcus causing gonorrhoea, etc. In domestic animals various diseases are caused by bacteria, e.g., Anthrax, Pneumonia, Tuberculosis, Cholera, Glanders etc.

(ii) Plant Pathogenic Bacteria

Many parasitic bacteria cause serious diseases in cultivated plants, which cause great harm to the crops. Important diseases are Citrus canker, cotton root rot, walnut blight, potato rot, pineapple rot etc. The rot diseases cause black spots on potato, tomato, cabbage, carrot etc.

Bacterial beneficial aspects overweigh their harmful aspects. We can control the harmful activities but their beneficial activities cannot be replaced by artificial processes.