

## **UNIT:C: BACTERIA AND MOLLI CUTES:**

- **PART:1:** General characteristics,Morphological characters
- **PART:2:** Basic methods of classification and Reproduction

### **PART:1**

**A.Bacteria: Definition:** Bacteria are microscopic unicellular,prokaryotic organisms devoid of chlorophyll,most commonly reproduce by transverse binary fission and the resulting cells are identical in size and morphology(Shape:spherical/rod,spiral or curved spherical arrangements of cell(coccus/micrococcus/diplococcus(paired),tetraacoccus(arranged in 4)).

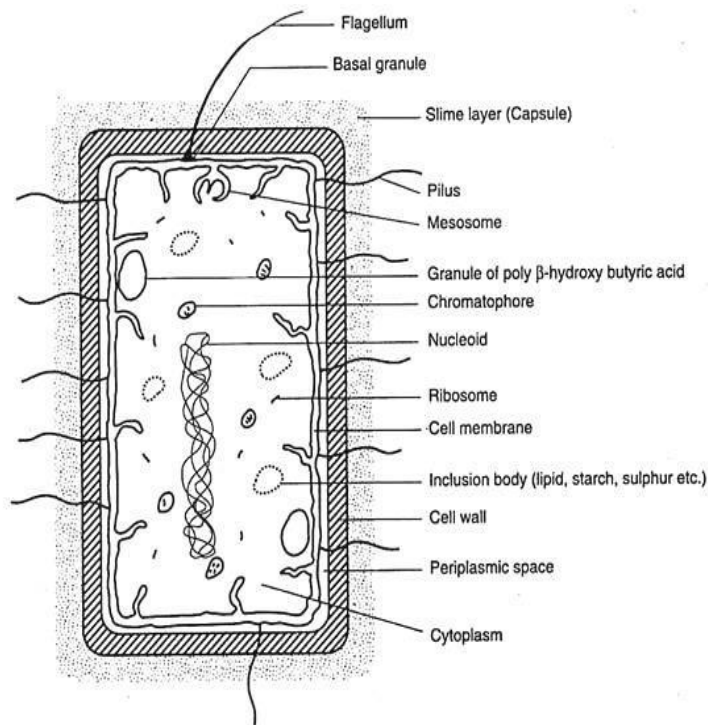
### **B.General characteristics of Phytopathogenic bacteria**

1. Bacteria are small: 0.6-3.5 microns in length, 0.5-1.0 microns in diameter
2. Most plant pathogenic bacteria are facultative saprophytes and can be grown artificially on nutrient media; however, fastidious vascular bacteria are difficult to grow in culture and some of them have yet to be grown in culture.
3. Bacteria may be rod shaped, spherical, spiral, or filamentous (threadlike).
4. Mostly flagellate hence motile.Some bacteria can move through liquid media by means of flagella, whereas others have no flagella and cannot move themselves.
5. Some can transform themselves into spores, and the filamentous bacteria *Streptomyces* can produce spores, called conidia, at the end of the filament. Other bacteria, however, do not produce any spores.
6. The vegetative stages of most types of bacteria reproduce by simple fission.
7. Bacteria multiply with astonishing rapidity they can produce tremendous numbers of cells in a short period of time.
8. Carbohydrate decomposition is mostly aerobic or oxidative (Except, *Erwinia*,which is facultative anaerobe)
9. Mostly gram-ve,rarely gram+ve(gram+ve genera:*Streptomyces*,*Corynebacterium*,*Clavibacter*,*Curtobacterium*)
10. PPB are passive invaders,i.e. enters plants through wounds or natural openings
11. Survive in/on debris and spread by means of water,rain,insects and agricultural implements.
12. Susceptible to phages,none of them cause human or human diseases.
13. Cellwall is rigid
14. Slow growth compared to other saprophytic bacteria.
15. Incubation period: 36-48hrs 25°C
16. Other distinguished features;
  - Their genetic material (DNA) is not enclosed within a membrane.
  - They lack other membrane-bounded organelles

- Their DNA is not associated with histone proteins (special chromosomal proteins found in eukaryotes).
- Their cell walls most of the time contains the complex polysaccharide peptidoglycan.
- They usually divide by binary fission. During this process, the DNA is copied and the cell splits into two cells.

**Detailed description of the bacteria cell**

A bacteria cell is protected by cell envelop which made-up of capsule,a cellwall and plasma membrane.In some cells slime cover instead of capsule,outer plasma membrane between capsule and cellwallA variety of structures are found in prokaryotic cells. The cells are always bounded by a chemically complex cell wall. In between the cell wall and the periplasmic space lies the plasma membrane . Internal membrane bound organelles are absent in prokaryotes and hence the internalmorphologically is very simple.The genetic material is localized ina discrete region called nucleoid and not separated from the surrounding cytoplasm by membrane. Ribosomes and inclusions are found scattered in the cytoplasmic matrix. Bacteria use flagella for locomotion, and in addition, many cells are surrounded by a capsule or slime layer external to the cell wall.



**Fig: Detailed structural composition of bacterial cell**

## □ **The bacterial cell wall**

It's a complex, semi-rigid structure that surrounds the underlying plasma membrane and protects the cell and the internal parts from adverse surrounding environment. Except for Mycoplasmas and some Achaea most bacteria have strong walls that give them shape and protect them from osmotic lysis; wall shape and strength is primarily due to peptidoglycan. The cell walls of pathogenic bacteria have components that contribute to their pathogenicity and protect a cell from toxic substances and are the site of action of several antibiotics. Major functions – prevent cells from rupturing, maintains shape and serves as a point of anchorage for flagella. Christian Gram developed the Gram stain in 1884, and it became evident that bacteria could be divided into two major groups based on their response to staining. Gram positive bacteria stained purple and the gram negative ones colored pink or red by the technique. The gram positive cell wall consists of a single thick layer of 20 to 80 nm of peptidoglycan or murein layer lying outside the cell membrane. In gram negative cells, the peptidoglycan layer is thin of about 2 to 7 nm surrounded by a 7 to 8 nm thick outer membrane. The gram positive cells are stronger than gram negative ones because of the peptidoglycan layer.

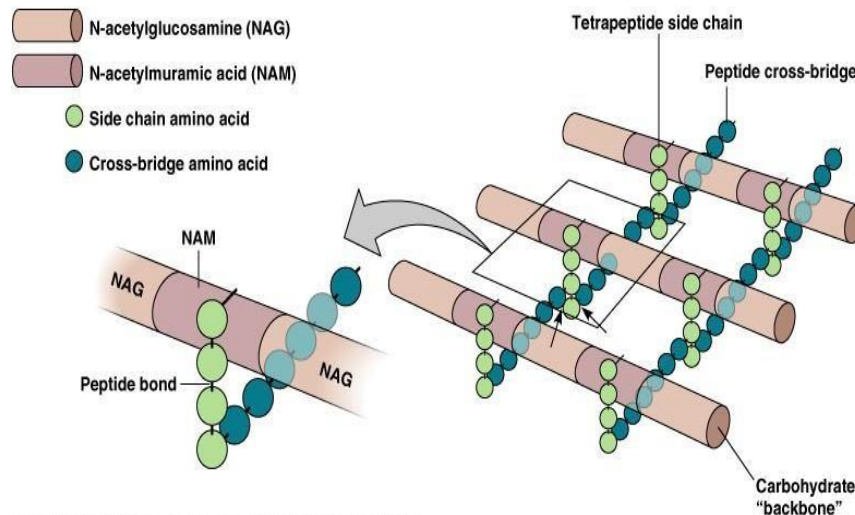
A space is seen between the plasma membrane and the outer membrane in electron micrographs of gram negative bacteria and a similar one but smaller gap may be observed between the plasma membrane and wall in gram positive bacteria. This space is called **periplasmic space**. The substance that occupies the space is the periplasm. The periplasmic space in gram negative bacteria ranges from 1nm to as great as 71 nm. It contains many proteins and participates in nutrient acquisition, hydrolytic enzymes attacking nucleic acids and phosphorylated molecules. The periplasmic space contains enzymes involved in peptidoglycan synthesis and modification of toxic compounds that could harm the cell. Gram positive bacteria may not have a visible periplasmic space but appear to have as many periplasmic proteins and several enzymes that are secreted are often called **exozymes**.

The Archaea differs from other prokaryotes. The walls lack peptidoglycan and are composed of proteins, glycoprotein or polysaccharides.

### **Structure of Peptidoglycan**

The cell wall of bacteria is composed of a macromolecular network of polymer called **peptidoglycan (murein)**. The polymer contains two sugar derivatives, Monosaccharides- **N-acetylglucosamine (NAG)** and **N-acetylmuramicacid (NAM)**. In addition, several different amino acids, three of which – D-Glutamic acid, D-alanine, and mesodiaminopimelic acid – not found in proteins are present. The presence of D-amino acids protects against attack by most peptidases. 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. Many bacteria substitute another diaminoacid, usually L-Lysine, in the third position for meso-diaminopimelic 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.



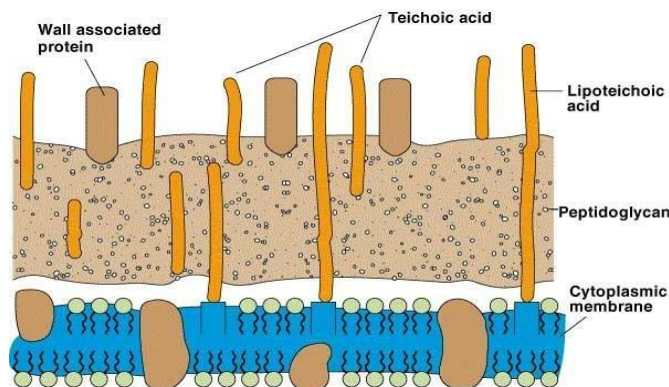
(a) Structure of peptidoglycan in gram-positive bacteria

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**Fig:Peptidoglycan structure in gram-positive cell**

### Gram positive cell walls

The cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure, which contains a peptide interbridge. Gram positive cell walls also contain **teichoic acids**, polymers of glycerol and ribitol joined by phosphate groups. To the glycerol and ribitol groups, the amino acids such as D-alanine or sugars like glucose are attached (Fig. 7). There are two classes to



**Fig: Cell wall of Gram positive bacteria**

teichoic acids; lipoteichoic acid (if they are attached to the lipid of the plasma membrane) and wall teichoic acid (which extend to the surface of the peptidoglycan and are negatively charged). Teichoic acids are not found in gram-negative bacteria.

Some bacteria like *Staphylococci* and most other gram-positive bacteria have a layer of proteins on the surface of their cell wall peptidoglycan. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids and other receptors and these proteins are involved in the interactions of the cell with its environment. Ex. S-layer proteins. Other surface proteins are covalently attached to the peptidoglycan and in gram positive pathogens these have roles such as aiding in adhesion to host tissues, preventing opsonization and blocking phagocytosis.

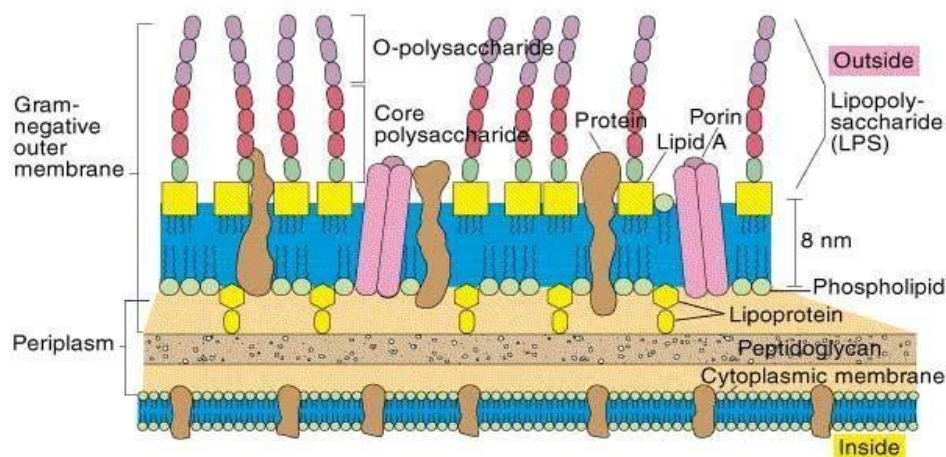
### **Gram negative cell walls**

The cell wall contains only a thin layer of peptidoglycan. In *E. coli*, it is about 2- 3nm thick and contains only one or two layers of sheets of peptidoglycan. The peptidoglycan is bounded to lipoproteins in the outer membrane and is embedded in a soft material, the **periplasmic gel**. Gram negative cell walls do not contain teichoic acids. They are more susceptible to mechanical breakage because of small amount of peptidoglycan.

❖ **Outer membrane** - found primarily in gram-negative cell consists of lipoproteins, liposaccharides and phospholipids and 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 outer membrane and plasma membrane appear to be in direct contact at many locations in the cell wall. The adhesion sites may be regions of direct contact or possibly true membrane fusions. It is proposed that substances can move into the cell through these adhesions sites rather than travelling through the periplasm.

The most unusual constituents of the outer membrane are its lipopolysaccharides. (LPSs). These contain both lipid and carbohydrate and consist of three parts: 1. Lipid A, 2. the core polysaccharide, and 3. the O side chain. The lipid A region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. This region is buried in the outer membrane and the remaining portion projects from the surface. The second region or the core polysaccharide is joined to lipid A and the third region or the O side chain which is a polysaccharide chain extends outward from the core. The O side chain is readily recognized by host antibodies, gram negative bacteria may thwart host defenses by readily changing the nature of their O side chains to avoid detection. The core polysaccharide contains charged sugars and phosphate, LPS

contributes to the negative charge on the bacterial surface (Fig. 8). Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize the membrane structure. Lipid A is often toxic and as a result LPS can act as an endotoxin and cause some symptoms that arise in gram-negative bacterial infections. The function of the outer membrane is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. Provides a barrier to certain antibiotics (like penicillin), lysozyme detergents, heavy metals, bile salts and digestive. **Porins** are proteins that permit small molecules to pass through the outer membrane (about 800 MW). **Specific channel proteins** – allow specific substances like vitamin B12, iron, nucleotides and maltose.



**Fig: Cell wall of gram negative bacteria**

### Atypical cell walls:

- *Mycoplasma* – is a bacterial genus that naturally lacks cell walls. Their plasma membranes have lipids called sterols, which protect them from osmotic lysis.
- Archaeobacteria – have pseudomurein (N-acetylalosaminuronic acid) but no peptidoglycan.
- L forms - are mutant bacteria with defective cell walls.

### **Structures internal to the cell wall**

In this lecture we shall be dealing with the structures internal to the cell wall of a bacterial cell. They include plasma membrane, organelles in the cytoplasm like nuclear area, ribosomes, inclusion bodies and endospores.

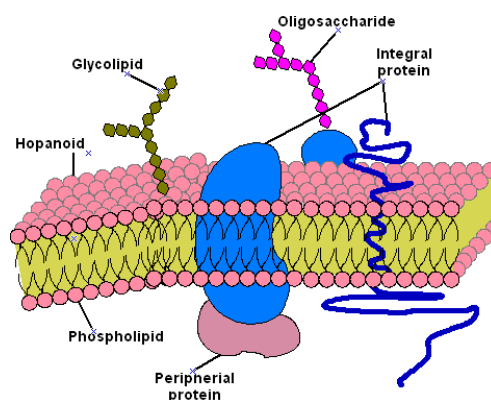
### **Plasma (cytoplasmic) membrane**

Membranes are absolute requirement of all living organisms. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. Plasma membrane – encloses the cytoplasm and consists of phospholipids and proteins (**fluid**

**mosaic model**). Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends. The polar ends interact with water and are hydrophilic and the nonpolar hydrophobic ends are insoluble in water. The lipid composition of bacterial membranes varies with environmental temperature in such a way that the membrane remains fluid during growth. Bacterial membranes usually differ from eukaryotic membranes in lacking sterols such as cholesterol and they contain pentacyclic sterol-like molecules called hopanoids and these are said to stabilize the bacterial membranes. Cell membranes are very thin structures about 5 to 10 nm thick and can be seen only with electron microscope. Plasma membranes have a complex internal structure; the small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer.

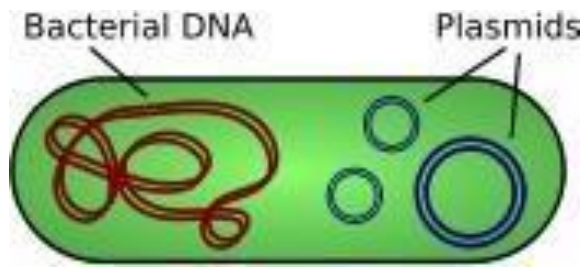
The most widely accepted current model for membrane structure is the fluid mosaic model of S. Jonathan Singer and Garth Nicholson. Two types of membrane proteins are seen, **Peripheral proteins** - which are loosely connected to the membrane and can be easily removed and are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These cannot be easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings. Plasma membranes serve as a selectively permeable barrier; it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The plasma membrane also is the location of a variety of crucial metabolic processes; respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation.

The bacterial plasma membrane can be destroyed by alcohols and polymyxins which cause leakage of intracellular contents and subsequent cell death of the organism.



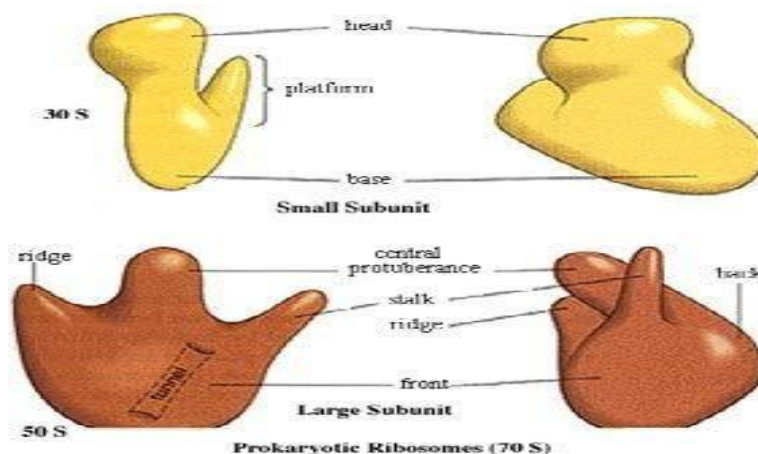
**Fig: Plasma membrane structure**





**Fig: Bacterial DNA and Plasmid**

❖ **Ribosomes:** The cytoplasmic matrix is also packaged with ribosomes, they also may be loosely attached to the plasma membrane. They look like small, featureless particles at low magnification electron microscope. They are made up of both protein and ribonucleic acid (RNA). Ribosome's function as sites 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. The shape of each protein is determined by its amino acids sequence and the special proteins called molecular chaperones or chaperones aid the polypeptide in folding to its proper shape. Prokaryotic ribosomes are smaller than eukaryotic ribosomes (Fig. 11). Ribosomes are composed of two subunits, each subunit being composed of protein and a type of RNA called ribosomal RNA (rRNA). They are comm. Only 70S: 30S subunit (1 molecule of rRNA) and 50S subunit (2 molecules of rRNA) and have dimensions of about 14 to 15 nm, a molecular weight of approximately 2.7 million. The S in 70S stands for Svedberg value or sedimentation coefficient. It is the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater is its Svedberg value. The Sedimentation coefficient is a function of a particle's molecular weight, volume and shape. Several antibiotics, such as streptomycin, neomycin and tetracyclines, exert their antimicrobial effects by inhibiting protein synthesis on ribosomes.



**Fig: Ribosomes in bacteria**



## ❖ **Inclusion bodies:**

Inclusion bodies can be divided into two types:

- Inclusion bodies not bounded by a membrane and lie free in the cytoplasm. Ex. Polyphosphate granules, cyanophycin granules and some glycogen granules.
- Inclusion bodies enclosed by a membrane about 2-4nm thick. Eg. Poly-β-hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes and gas vacuoles.

### □ **Organic inclusion bodies: Glycogen:**

Polymer of glucose units composed of long chains formed by alpha (1-4) glycosidic bonds and branching chains connected to them by alpha (1-6) glycosidic bonds. Eg. glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells (glycogen granules appear reddish brown and starch granules appear blue).

**Poly β-hydroxybutyrate:** Contains beta-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Appear in various species of *Mycobacterium*, *Bacillus*, *Azotobacter*, *Spirillum* and other genera. Lipid inclusions are revealed by use of fat-soluble dyes, such as Sudan dyes.

### □ **Carboxysomes:**

These are polyhedral and hexagonal inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. Bacteria that use carbon dioxide as their sole source of carbon require this enzyme for carbon dioxide fixation during photosynthesis (Ex. nitrifying bacteria, cyanobacteria, and *Thiobacilli*).

### □ **Gas vacuoles:**

These are hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria. Each vacuole consists of rows of several individual gas vesicles, which are hollow cylinders covered by protein. Their function is to maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light and nutrients. They are impermeable to water and permeable to atmospheric gases.

### □ **Inorganic inclusion bodies:**

#### **Polyphosphate granules or Metachromatic granules:**

Linear polymer of organo phosphates joined by ester bonds. Reservoirs for phosphate, an important component of cell nucleic acids and also energy reserves. Represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. Stain red with certain blue

dyes, such as methylene blue, and are collectively known as volutin. Found in algae, fungi and protozoans, as well as bacteria.

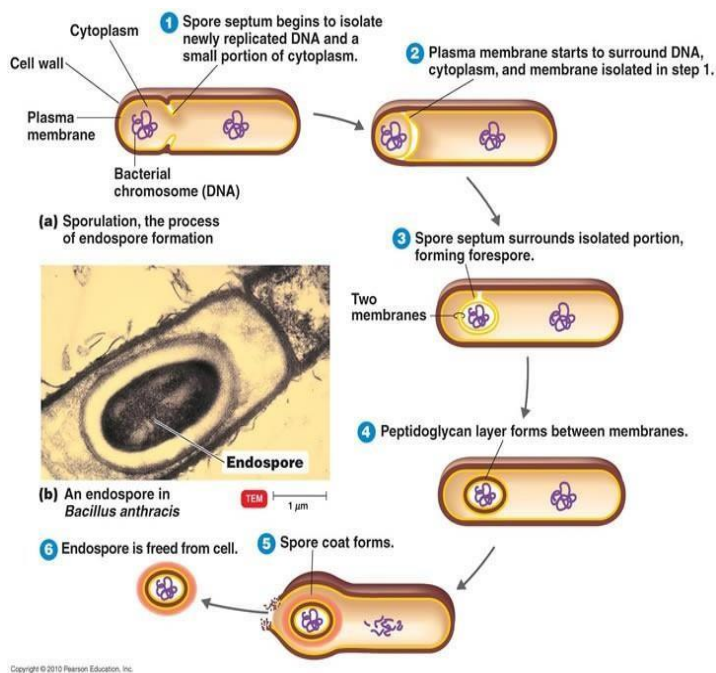
These granules are quite large and are characteristic of *Corynebacterium diphtheriae*, the causative agent of diphtheria, thus they have diagnostic significance.

### Sulphur granules:

Sulphur bacteria, which belong to the genus *Thiobacillus*, derive energy by oxidizing sulfur and sulfur containing compounds. These bacteria may deposit sulfur granules in the cell, where they serve as an energy reserve. Purple photosynthetic bacteria use H<sub>2</sub>S as electron donor and accumulate resulting sulfur in either the periplasmic space or in special cytoplasmic globules.

### ❖ Endospores “An escape pod for DNA”

Endospores are a survival mechanism: they are triggered to form during adverse environmental conditions. They are NOT reproductive structures as only one cell gives rise to one spore and endospores can be identified with special stains and differentiated from the vegetative cell (Fig. 14). Endospores are resistant to: heat: withstand boiling for over one hour; desiccation, UV radiation and chemical disinfectants. The resistance of these spores has serious consequences and some very pathogenic bacteria have the ability to produce such spores



**Fig: Formation of endospore**

## □ Structures external to the cell wall

the structures external to the cell wall of bacterial cells includes glycocalyx, fimbriae, pili, flagella, axial filaments

### **Glycocalyx (Capsules, Slime layers and S-layers)**

It is a viscous (sticky), gelatinous polymer composed of polysaccharide, polypeptide or both. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule** (negative staining). If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer**. Capsules protect pathogenic bacteria from phagocytosis (process by which certain white blood cells engulf and destroy microbes) and contribute to virulence. Capsules are clearly visible in the light microscope by using stains or special capsule stains.

A regularly structured layer called S-layer is usually seen in many gram positive and gram negative bacteria. It consists of proteins or glycoproteins and resembles a pattern something similar to floor tiles. The S-layer adheres directly to the outer membrane in case of gram negative bacteria and with the peptidoglycan surface in gram positive bacteria. These protect the bacteria against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio*. The S layer also helps maintain the shape and envelope rigidity of at least bacterial cells and also promotes cell adhesion to surfaces. Sometimes, the layer also seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

### □ **Fimbriae and Pili:**

Many gram negative bacteria have hairlike appendages that are shorter, straighter and thinner than flagella and are used for attachment rather than for motility. They are usually called fimbriae. These structures contain a protein called pilin. **Fimbriae** - occur at the poles of the bacterial cell, or they can be evenly distributed over the entire surface of the cell. Fimbriae of *Neisseria gonorrhoeae* the causative agent of gonorrhoea help the microbe to colonize mucous membranes to cause the disease. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

❖ **Pili or sex pili or pilus**- usually longer than fimbriae and number only one to ten per cell. Pili function to join bacterial cells prior to the transfer of DNA from one cell to another (sometimes called sex pili). They are genetically determined by sex factors or conjugative plasmids and are required for bacterial mating. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

## □ **Flagella:**

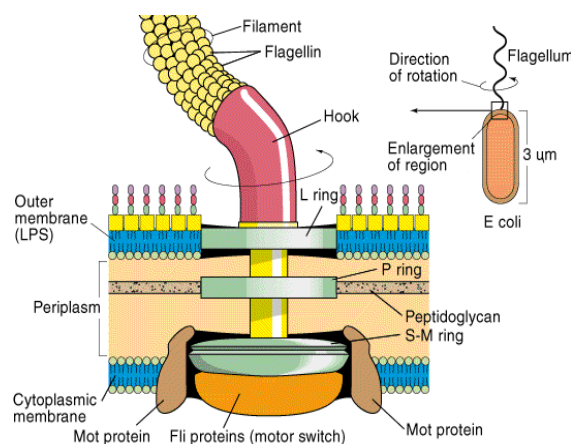
Motile bacteria move by use of flagella, threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20  $\mu\text{m}$  long. Bacterial species often differ distinctively in their patterns of flagella distribution.

### **Structure:**

Transmission electron microscopic studies have shown that the bacterial flagellum is composed of three parts. 1) **Filament**– outermost region and contain the globular protein flagellin 2) **Hook**– the filament is attached to hook, which consists of a different protein

3) **Basal body**- which anchors the flagellum to the cell wall and plasma membrane. It consists of a small central rod inserted into it are a series of rings. The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin** (MW from 30,000 to 60,000). Some bacteria have sheaths surrounding their flagella.

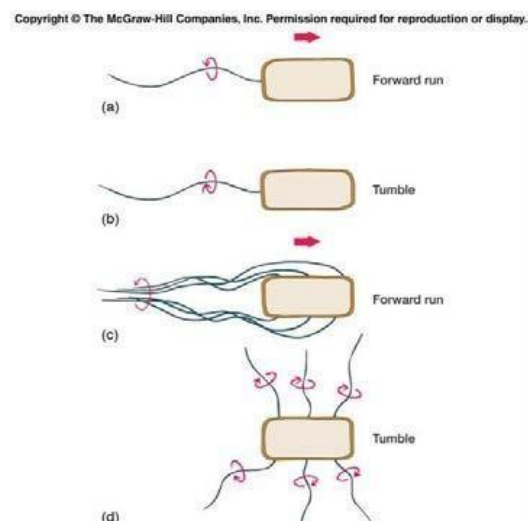
The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex structure of the flagellum. In *E.coli* and Gram negative bacteria, the body has four rings connected to central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers. The inner M ring connects the plasma membrane. Gram positive have only two basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan. The synthesis of flagella is a complex process involving at least 20 to 30 genes. Flagellin subunits are transported through the filament's hollow internal core. When they reach the tip, the subunits spontaneously aggregate under the direction of a special filament cap so that the filament grows at its tip rather than at the base. Filament synthesis is an excellent example of self-assembly.



**Fig:Structure of bacterial flagella (Gram negative)**

## Flagellar movement:

The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial movement. The movement in monotrichous bacteria stop and tumble randomly by reversing the flagellar rotation. The polar flagella, rotate counter clockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. Peritrichous bacteria also operate in a similar way. To move forward, the flagella rotate counter clockwise. As they do so, they bend at their hooks to form a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles. Motility enables the bacterium to move toward a favorable environment or away from a particular stimulus called taxis. Chemotaxis (include chemicals) and phototaxis(include light). Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as **chemotaxis**. The mechanism of chemotaxis in *E.coli* has been studied most. Forward swimming is due to counterclockwise rotation of the flagellum, whereas tumbling results from clockwise rotation. The bacteria must be able to avoid toxic substances and collect in nutrient-rich regions and at the proper oxygen levels. *E.coli* has four different chemoreceptors that recognize serine, aspartate and maltose, ribose and galactose and dipeptides respectively. These chemoreceptors often are called methyl-accepting chemotaxis proteins (MCPs)



**Fig: Bacterial flagellar movement**

(This part for brief note purpose)

### ❖ Prokaryotes – Structure/Function

Prokaryotes are distinguished from eukaryotes by their smaller size (0.2-10µm), their lack of internal organelles (*e.g.*, mitochondria), the presence of a cell wall and their cell division by binary fission rather than mitosis. They lack introns, are not capable of endo/exocytosis and have single-stranded circular DNA rather than multiple discrete chromosomes. Bacteria share a number of common structures that are briefly described below.

**1) Slime (extracellular polysaccharide):** This is extracellular material, loosely associated with the bacteria, that is elaborated by some bacterial species that facilitates colonization of smooth, prosthetic surfaces such as intravascular catheters.

**2) Capsule:** This polysaccharide outer coating of the bacterial surface often plays a role in preventing phagocytosis of bacteria.

**3) Peptidoglycan (cell wall)** Provides bacterial shape and rigidity. The cell wall consists of alternating units of N-acetylglucosamine and N-acetylmuramic acid. The polysaccharide chains are cross-linked by a peptide bridge. It is a primary target of antimicrobial therapy – because it is specific to prokaryotes.

#### ❖ Assembly of the peptidoglycan:

This is a critical step for bacterial survival. The sequence of events is outlined below.

- I. Synthesis begins with formation of a water soluble, nucleotide-linked precursor (N-acetylmuramic acid - NAM) also carrying a pentapeptide in the cytoplasm.
- II. ii. The precursor is then linked to a lipid-like carrier in the cell membrane (bactoprenol) and N-acetyl glucosamine (NAG) is added to the NAM. This complex is mobilized across the cytoplasm
- III. iii. The disaccharide subunit (NAM-NAG) is then added to the end of a glycan strand.
- IV. iv. The final step is the transpeptidation reaction catalyzed by a transpeptidase enzyme (also called penicillin binding proteins) that crosslinks the growing strand with others.

**4) Cytoplasmic membrane:** This is a phospholipid bilayer that assumes many of the functions of eukaryotic organelles such as the biosynthetic processes.

**5) Flagella:** These provide bacteria with the capacity for locomotion. They vary in number and location.

**6) Pili:** These structures project from the cell surface enabling bacteria to adhere to host tissue surfaces. Based on their amino acid structure their affinity for particular host tissue surfaces can be remarkably specific.

**6) Secreted products:** There are a variety of these products including exotoxins that are proteins grouped into A-B toxins, membrane damaging toxins (*e.g.*, hemolysins) and hydrolytic enzymes capable of destroying host tissues and extracellular matrices.

## C. Morphological characteristics

### ❖ Size and Shape of Bacteria

Prokaryotes are among the smallest of all organisms (0.5 to 2.0  $\mu\text{m}$ ). Because of their small size, bacteria have a large surface-to-volume ratio. The smallest member of the genus is about 0.3 $\mu\text{m}$  in diameter. Even smaller cells have been reported like the nanobacteria or ultramicrobacteria appear to range from around 0.2 $\mu\text{m}$  to not less than 0.05 $\mu\text{m}$ . *E. coli*, a bacillus of about average size is 1.1 to  $\mu\text{m}$  wide by 2.0 to 6.0  $\mu\text{m}$  long. Spirochaetes occasionally reach 500  $\mu\text{m}$  in length and the cyanobacterium *Oscillatoria* is about 7  $\mu\text{m}$  in diameter. The bacterium, *Epulosiscium fishelsoni*, can be seen with the naked eye (600  $\mu\text{m}$  long by 80  $\mu\text{m}$  in diameter). Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50  $\mu\text{m}$  in diameter).

### *Shape and arrangement:*

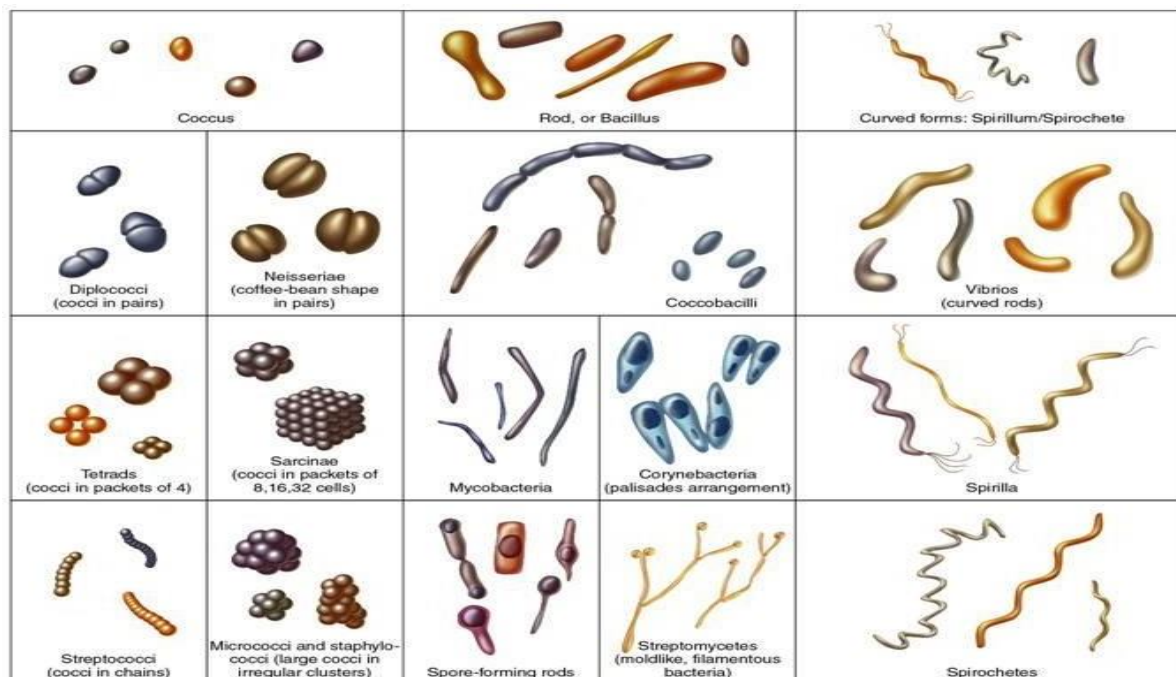
Typically bacteria have three basic shapes – spherical, rod like and spiral.

### *Spherical bacteria:*

**Coccus (plural cocci)** in pairs – **diplococci**; in chains – **Streptococci** (*Streptococcus*); in cube like groups of eight – **Sarcinae** (*Sarcina*); in grape like structures – **Staphylococci** (*Staphylococcus*)

### *Rod like bacteria:*

**Bacillus (plural bacilli)** in pairs – **diplococci**; in chains – **streptococci**. Still others look like cocci and are called **coccobacilli**



**Fig: Different morphological characteristics and diversity of bacteria**



## *Spiral bacteria*

**Comma shaped** – *Vibrio (Vibrio)*; **Helical, long and curved** – *Spirilla (Rhodospirillum)*;

**Helical and flexible** – Spirochetes (*Treponema* and *Borrelia*) movement by axial filaments

**In addition there are:** Star shaped cells (genus *Stella*); Square flat cells (halophilic archaeobacteria); Triangular cells (*Haloarcula*); Branching mycelium – like filaments (*Streptomyces*)

□ Genetically most bacteria are monomorphic (single shape), but some have many shapes and are called pleomorphic (*Rhizobium* and *Corynebacterium*)

## PART 2

### Basic methods of classification

#### ❖ Classification of phytopathogenic prokaryotes, according to Bergey's manual of Systematic Bacteriology

##### A. Phenotypic classification

- I. Morphological
- II. Anatomical
- III. Staining
- IV. Cultural characteristics
- V. Nutrition
- VI. Environmental factors
- VII. Biochemical reactions
- VIII. Antigenic structure

##### B. Genotypic classification

- I. DNA-DNA hybridization
- II. G+C content

##### VI. Environmental factors

###### -TEMP

- Psychrophiles (15-20°C)
- Mesophiles (20-40°C)
- Thermophiles (50-60°C)
- Extreme thermophiles (As high as 250°C)

###### -O<sub>2</sub> DEPENDANCE

- Aerobe
- Obligate Aerobe
- Microaerophilic
- Facultative Anaerobe
- Obligate Anaerobe
- Capnophilic
- Aerotolerant

###### -pH

- Acidophiles
- Alkaliphiles
- Neutrophiles

###### -Salt conc.

- Halophiles
- Non halophiles

###### -Atm. pressure

##### A.I. Morphological: Based on shape and size

- Rod shaped (Bacillus)
- Spherical (Coccus)
- Spiral (Spirillum)
- Curved (Vibrio)

##### II. Anatomical: Based on Flagellation

###### -FLAGELLATE

- Monotrichous
- Lophotrichous
- Amphitrichous
- Peritrichous

###### -AFLAGELLATE

###### Spore

- Spore former
- Non spore former

##### V. Based on nutrition:

- Autotroph
- Heterotroph

##### III. Staining:

###### - GRAM STAINING

- Gram positive cocci
- Gram negative cocci
- Gram positive rod
- Gram negative rod

###### - ACID FAST STAINING

- Acid fast bacilli
- Non acid fast bacilli

##### IV. Cultural characteristics

###### -EXTRA GROWTH

###### FACTOR REQUIREMENT

- Fastidious
- Non fastidious

###### -UTILIZATION OF CARBOHYDRATE

- Oxidative
- Fermentative

###### -GROWTH RATE

- Rapid growers
- Slow growers

###### -PIGMENT PRODUCTION

- Pigment producer
- Pigment non producer

❖ Genetic characteristics:

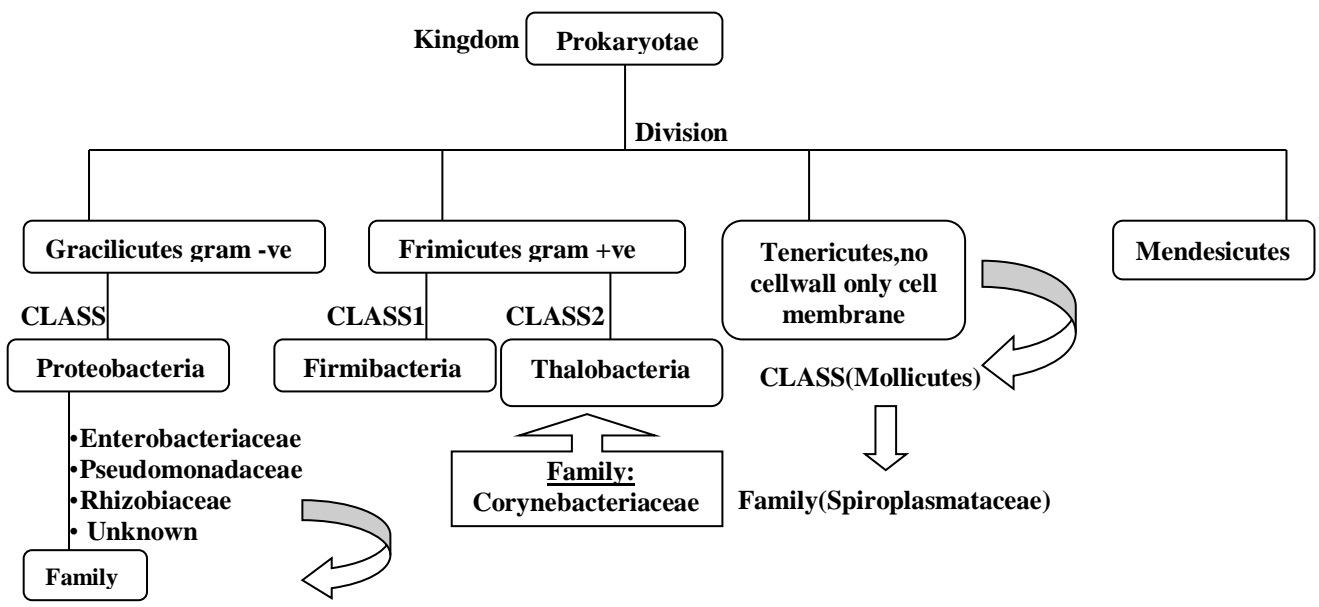
-**DNA Base composition:** DNA molecules made up of base pairs guanine-cytosine and adenine-thymine-base of classification-----

-The sequence of nucleotides base in the sequence of nucleotide is unique for each type of organism most fundamental of all

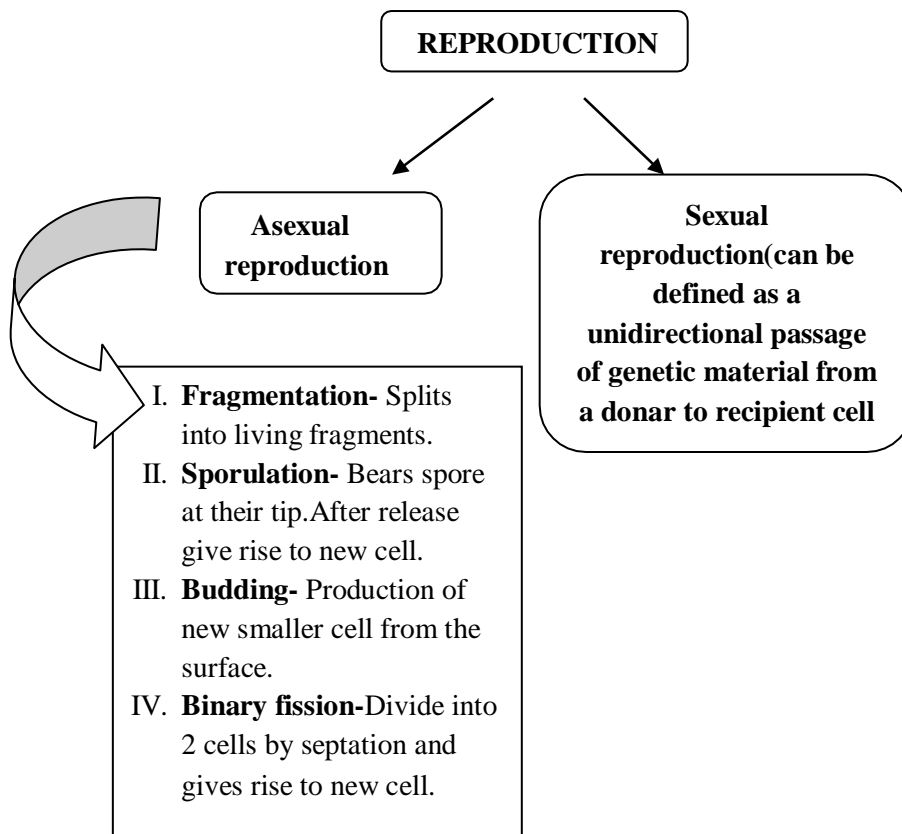
-**Pathogenicity-** Ability to cause disease-Pathogenic to plants and to microorganism.

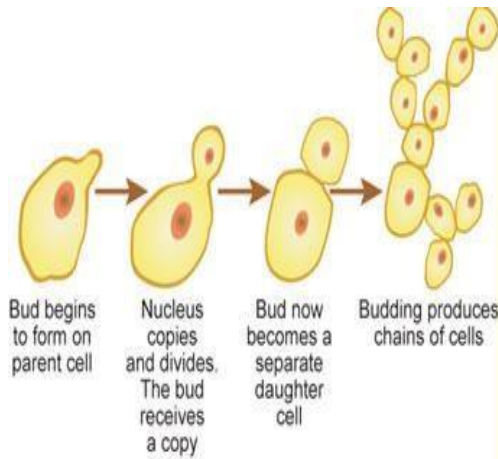
-**Ecological-** Marine microorganisms different from fresh water microorganism

Classification of phytopathogenic prokaryotes, according to Bergey's manual of systematic bacteriology, 1984

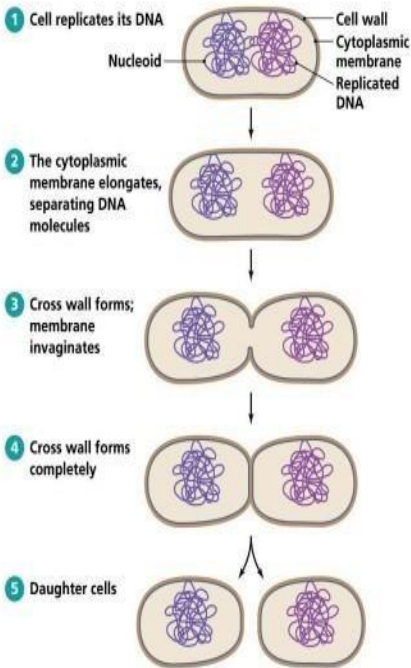


❖ Reproduction of bacteria:





**BUDDING**



**FRAGMENTATION**

**ASEXUAL REPRODUCTION OF BACTERIA**

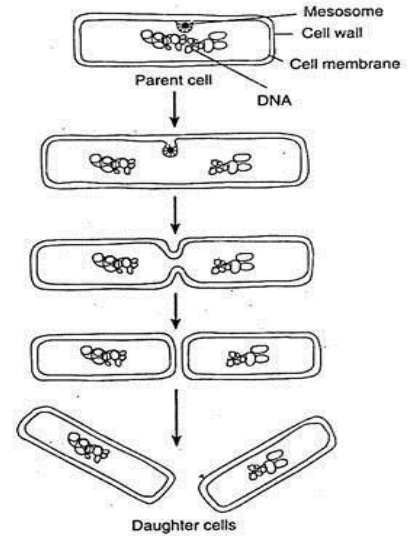
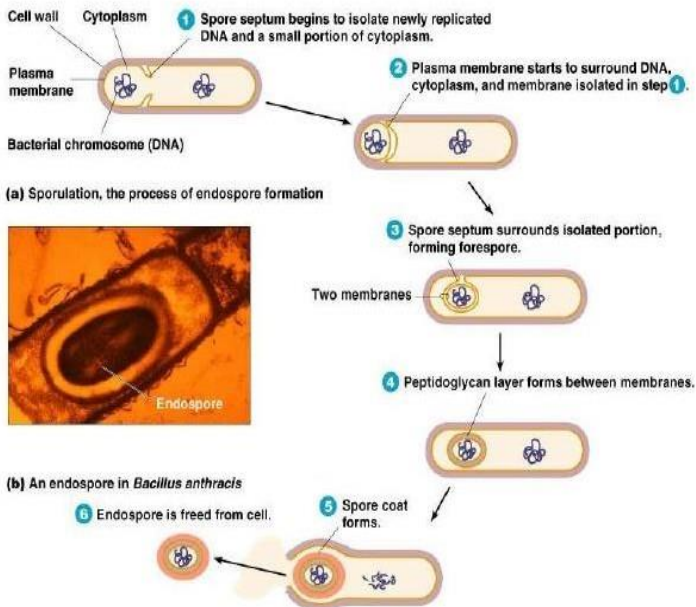


Fig. 2.20 : Binary fission of a bacterium

**BINARY FISSION**

**Sporulation Process**



# Transformation

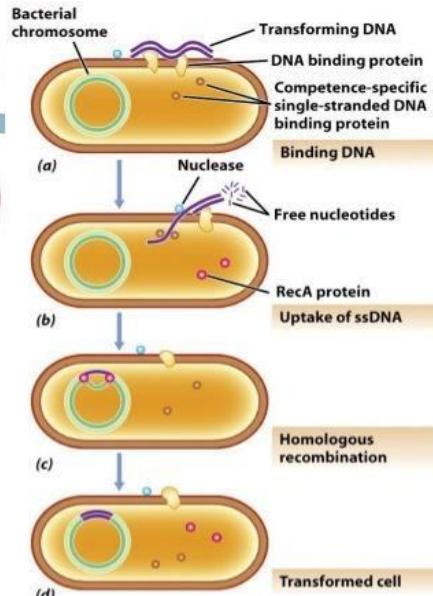
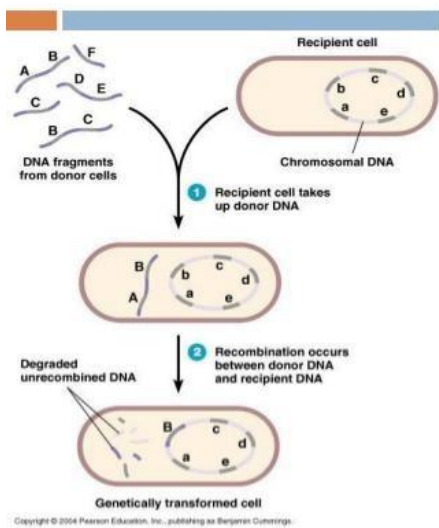
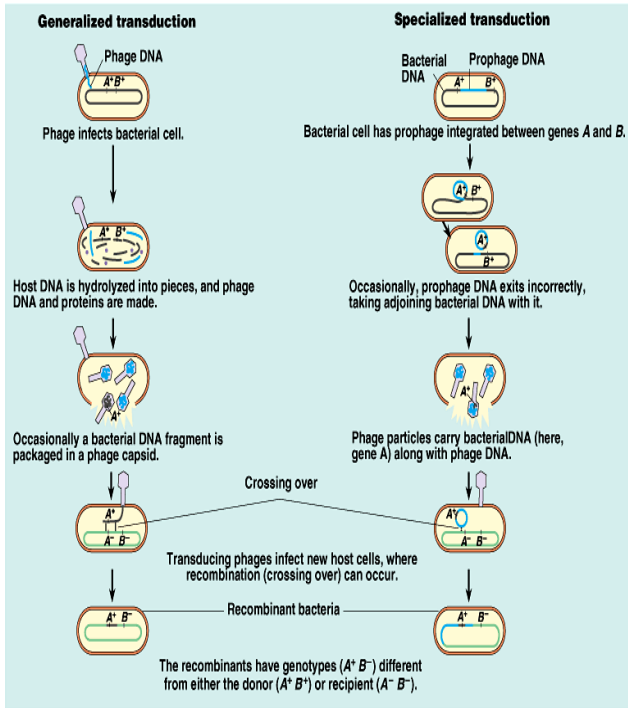
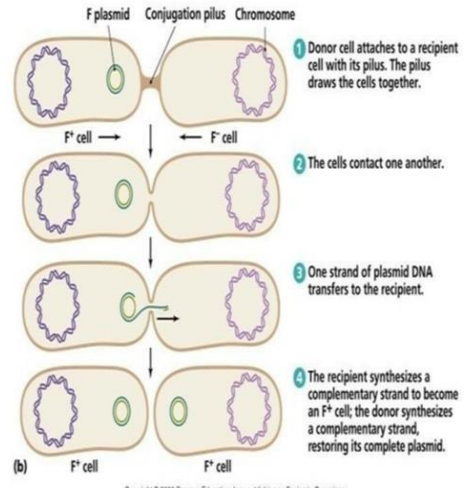


Figure 10-14 Brock Biology of Microorganisms 11/e  
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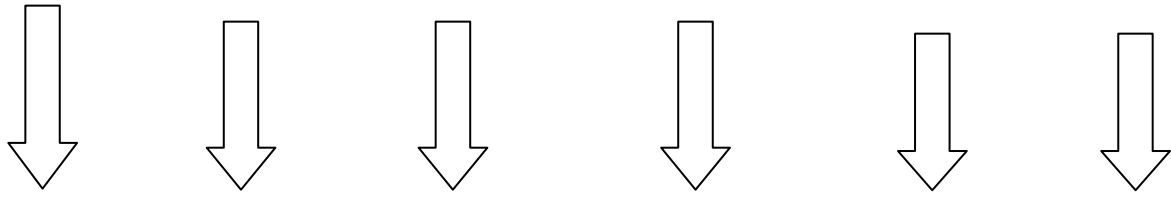
## TRANSDUCTION

# Bacterial Conjugation



## SEXUAL REPRODUCTION IN BACTERIA

## Cultivation of Bacteria



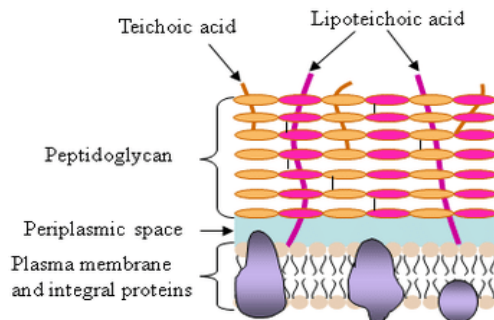
<p><b><u>Autotroph</u></b> are those bacteria which utilize CO<sub>2</sub> as the major source of carbon. <b>Eg: Nitrosomonas</b></p>	<p><b><u>Heterotroph</u></b> are those bacteria which use one/more org. compounds (glucose, amino acid) as their carbon source, they can't utilize CO<sub>2</sub>. <b>Eg. Azospirillum, Staphylococcus, Lactobacillus</b></p>	<p><b><u>Mesophyllus</u></b> can grow best within a temp. range of 25-40°C. All bacteria that are pathogenic are growing at this temp range. <b>Eg: Pseudomonas</b></p>	<p><b><u>Thermophyllus</u></b> can grow best above 45°C. They are facultative thermophilic (can grow in the range of mesophilic). <b>Eg Bacillus thuringiensis</b></p>	<p><b><u>Aerobic</u></b> requires O<sub>2</sub> for their growth and can grow when incubated in an air atmosphere</p>	<p><b><u>Anaerobic</u></b> they don't require O<sub>2</sub> for their growth and can't grow when incubated in air atmosphere</p>
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## Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

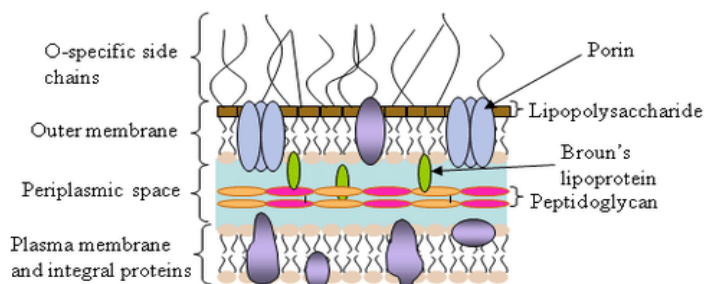
12

Characteristic	Gram-positive	Gram-negative
Gram reaction	Retain crystal violet dye and stain dark violet or purple	Can be decolorized to accept counterstain (safranin); stain red
Peptidoglycan layer	Thick (multilayered)	Thin (single-layered)
Toxins produced	Primarily exotoxins	Primarily endotoxins
Resistance to drying	High	Low
Outer membrane	Absent	Present
Lipid and lipoprotein content	Low	High
Lipopolysaccharide (LPS) content	Virtually none	High

**Gram (+) cell-wall**



**Gram (-) cell-wall**



## ❖ Characteristics of Bacteria Cell Structures

Structure	Functions(s)	Predominant chemical composition
<b>Flagella</b>	Swimming movement	Protein
<b>Pili</b>		
<b>Sex pilus</b>	Stabilizes mating bacteria during DNA transfer by conjugation	Protein
<b>Common pili or fimbriae</b>	Attachment to surfaces; protection against phagotrophic engulfment	Protein
<b>Capsules (includes “slime layers” and glycocalyx)</b>	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; protection against desiccation	Usually polysaccharide; occasionally polypeptide
<b>Cell wall</b>		
<b>Gram-positive bacteria</b>	confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
<b>Gram-negative bacteria</b>	confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-lipopolsaccharide “outer membrane”
<b>Plasma membrane</b>	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
<b>Ribosomes</b>	Sites of translation (protein synthesis)	RNA and protein
<b>Inclusions</b>	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
<b>Chromosome</b>	Genetic material of cell	DNA
<b>Plasmid</b>	Extrachromosomal genetic material	DNA

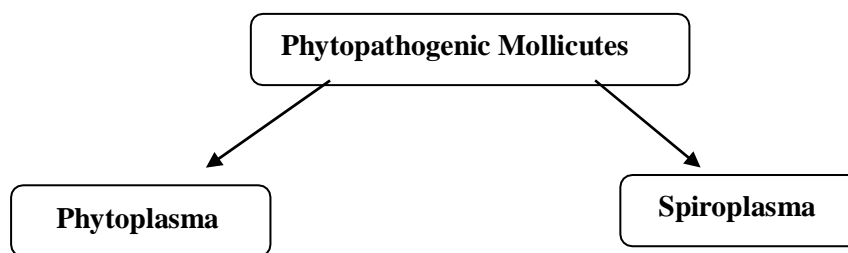


□ **MOLLICUTES:**

**Mollicutes** is a class of bacteria distinguished by the absence of a cell wall. The word "Mollicutes" is derived from the Latin *mollis* (meaning "soft" or "pliable"), and *cutis* (meaning "skin"). Individuals are very small, typically only 0.2–0.3 µm (200-300 nm) in size and have a very small genome size. They vary in form, although most have sterols that make the cell membrane somewhat more rigid. Many are able to move about through gliding, but members of the genus *Spiroplasma* are helical and move by twisting. The best-known genus in the Mollicutes is *Mycoplasma*.

- Mollicutes are parasites of various animals and plants, living on or in the host's cells. Many *Phytoplasma* and *Spiroplasma* are plant pathogens associated with insect vectors. Whereas formerly the trivial name "mycoplasma" has commonly denoted any member of the class Mollicutes, it now refers exclusively to a member of the genus *Mycoplasma*.

- **Subcategory:** Mycoplasmataceae



**A. Phytoplasma: Definition:**

Prokaryotic, pleomorphic, resembles mycoplasma, wall-less, (trilamellar unit membrane) self replicating, pass through filters, sensitive to tetracyclines, transmission by leaf hoppers, found in sieve element.

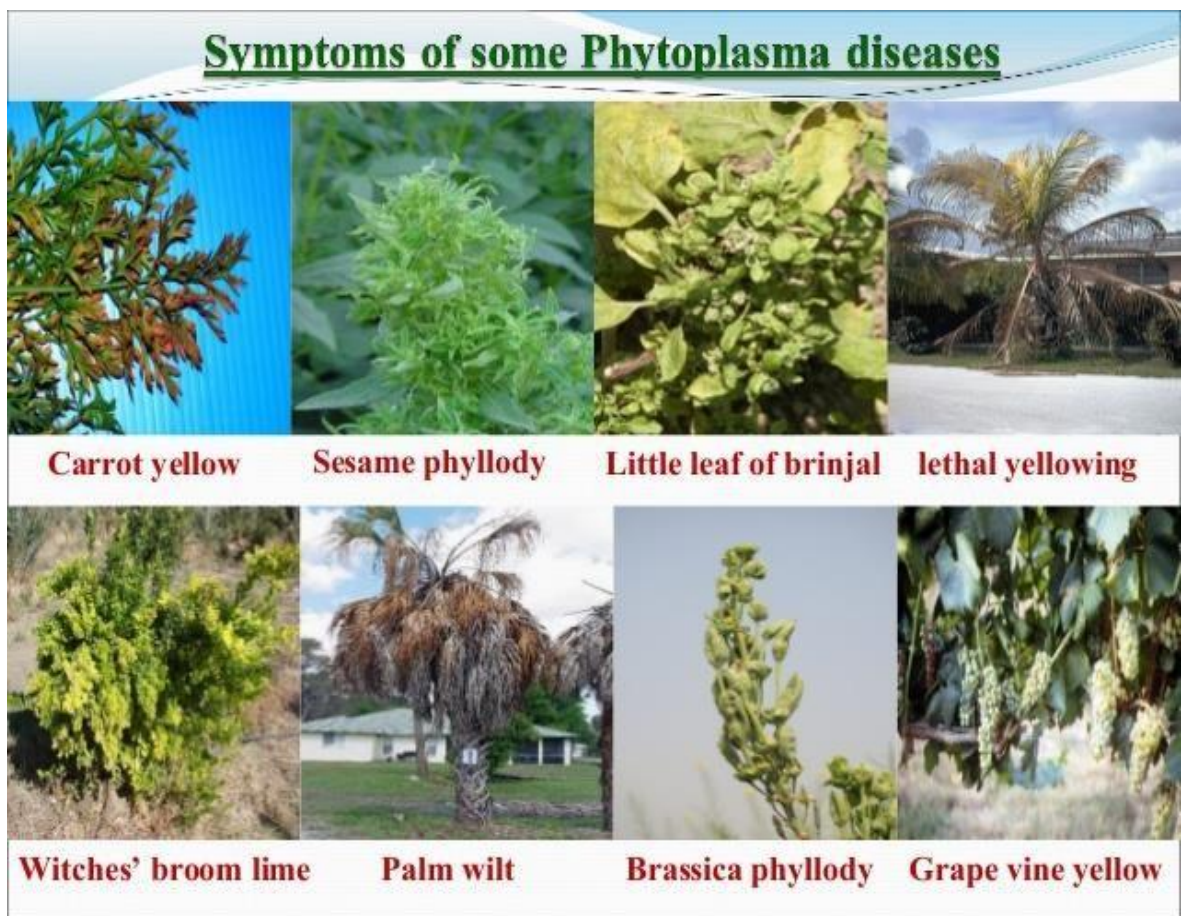
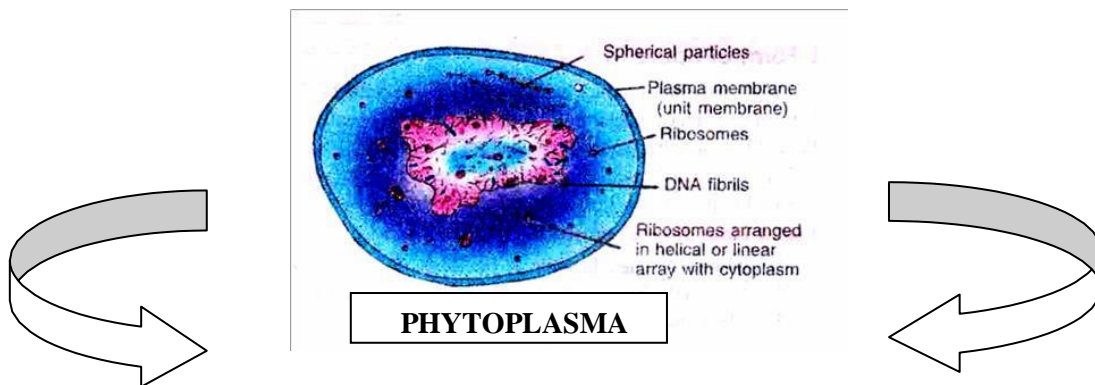
- **Characters of phytoplasma:**
  - a. Size vary from 100 nm-1µm in diameter. obligate parasite.
  - b. Wall less, covered by trilamellar nit membrane with lipoproteins.
  - c. Cells pleomorphic
  - d. Aerobic to facultative anaerobes, nonflagellate, non-spore-formers and gram -ve
  - e. Found intercellularly in phloem vessels in plant.
  - f. Ribosomes of bacteria type.
  - g. Genome is made up of double stranded DNA.
  - h. Osmotically stable.
  - i. Self-replicable by transverse binary fission.
  - j. Resistant to penicillin but sensitive to tetracycline and chloramphenicol.
  - k. Transmission by leaf hoppers and grafting.
  - l. Genetically more relates to acholeplasms than mycoplasmas.
  - m. Can be controlled by cross-protection.

- n. Requires sterol for growth.
- o. Can be controlled by thermotherapy by growing plants at 34-40°C

**Eg. Mulberry dwarf**

**Disease caused by Phytoplasma**

- I. Aster yellows (*Macrosteles fascifrons*)(leaf hopper).
- II. Sesamum phyllody (*Orosius albicinctus, O. orientalis*)(leaf hopper)
- III. Little leaf of brinjal (*Hishimonas phycitis*)(leaf hopper)
- IV. Grassy shoot of sugarcane (*Aphis maydis*) aphid e.t.c



**B. Spiroplasma:Definition:**

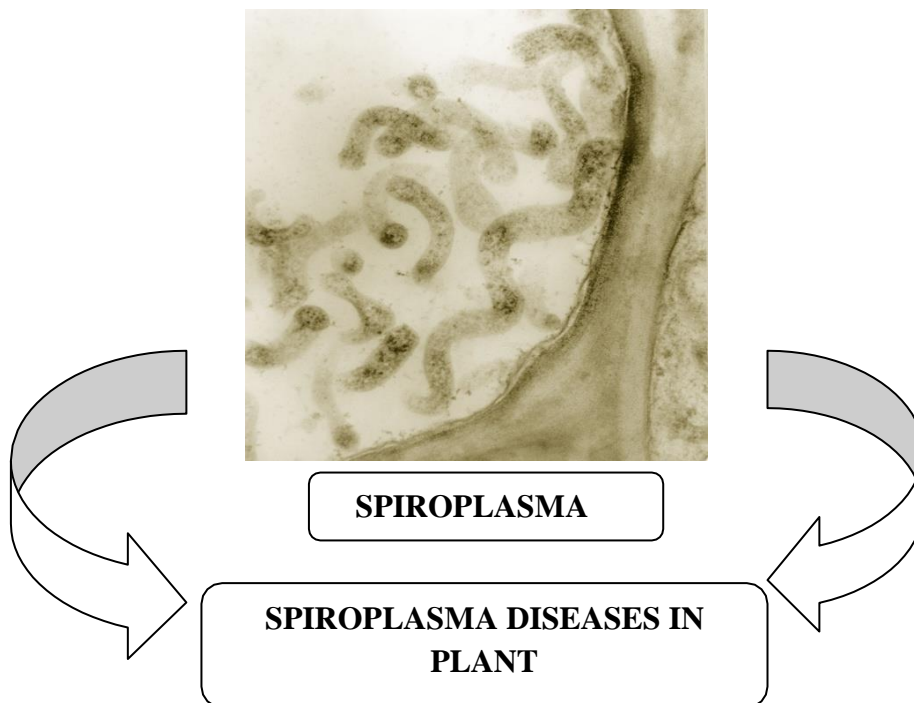
Helical, wall less prokaryotes,phloem of diseased plants,helical in culture,a kind of mycoplasma,but can't be cultured.

**Important Characteristics:**

- a. Helical in liquid media,gram+ve
- b. Size vary from 100-240nm in diameter,no flagella.
- c. Grown on culture medium in laboratory,colony appear like “fried egg” in culture medium.
- d. Multiplication by transverse binary fission.
- e. Resistant to penicillin but sensitive to tetracycline.

**Important disease and vectors:** (leaf hopper)

- I. Corn stunt (*Dalbulus elimatus*)
- II. Citrus stubborn(*Circulifer tenellus*)



**Corn stunt disease**



**Citrus stubborn disease**

**References:**

1. **A Text Book Of Fungi Bacteria And Viruses By H.C Dubey**
2. **Microbiology by R.P Singh**
3. **Plant Pathology by G.N Agrios(5<sup>th</sup> Edition)**
4. **Fundamentals of Plant Bacteriology by Jayashree Jayaraman and Jeevan Prakash Verma**

## QUESTION BANK

### **A. Short Notes: (Each question carries 2marks)**

1. Bacterial cellwall
2. Structure of peptidoglycan
3. Comparison between the Gram+ve and Gram –ve bacterial cellwall
4. Endospore
5. Fimbriae and pilli
6. Flagellar movement
7. Asexual reproduction of bacteria
8. Classification of phytopathogenic prokaryotes, according to Bergey's manual of systemic bacteriology, 1984( with examples)
9. Morphology based classification of bacteria with examples.
10. Difference between prokaryotes and Eukaryotes
11. Inclusion bodies
12. Slime layer

### **B. Broad question:( Each question carries 4marks)**

1. What is bacteria? Explain general characteristics of bacteria.
2. Detailed description of Bacterial cell.along with the functions of each.
3. Distinguish between Gram positive cellwall and Gram negative cellwall with diagrammatic representation.
4. What is flagella? How does it work as the organ of locomotion incase of bacteria?
5. Describe the flagellar structure?
6. Describe the classification based on Morphology?cite examples
7. Describe the classification of bacteria on the basis of flagellation.with examples
8. What are the different modes of reproduction in bacteria?explain with diagram
9. Define mollicutes?Classification of phytopathogenic molliutes?
10. What is Phytoplasma? Briefly discuss about the general characteristics of phyoplasma.give few examples of phytoplasmal dieases in plant.
11. What is Spiroplasma? Briefly discuss about the general characteristics of Spiroplasma.give few examples of Spiroplasmal dieases in plant.