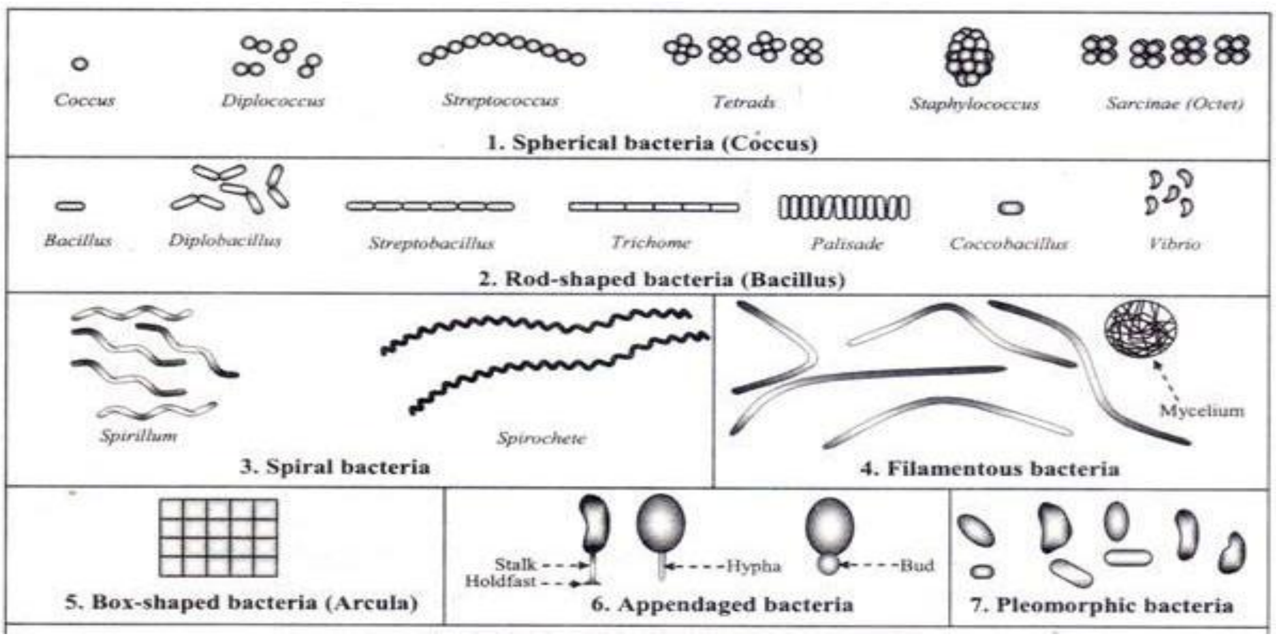
**Lecture 13, 14 and 15: bacterial size, shape and arrangement &**

**Cell structure and components of bacteria and Functional anatomy and reproduction in bacteria**

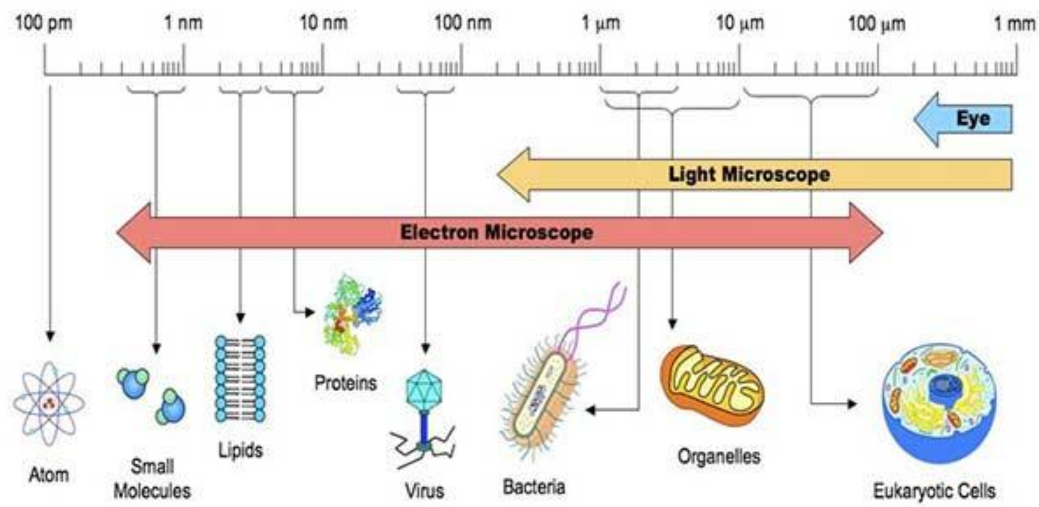
## Bacterial size, shape and arrangement

Bacteria are prokaryotic, unicellular microorganisms, which lack chlorophyll pigments. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles.Due to the presence of a rigid cell wall, bacteria maintain a definite shape, though they vary as shape, size and structure.




When viewed under light microscope, most bacteria appear in variations of three major shapes: the rod (bacillus), the sphere (coccus) and the spiral type (vibrio). In fact, structure of bacteria has two aspects, arrangement and shape. So far as the arrangement is concerned, it may Paired (diplo), Grape-like clusters (staphylo) or Chains (strepto). In shape they may principally be Rods (bacilli), Spheres (cocci), and Spirals (spirillum).

## Size of Bacterial Cell



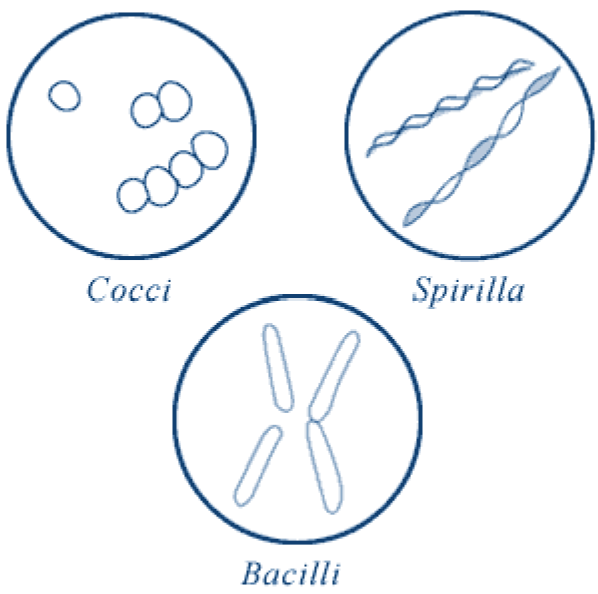

The average diameter of spherical bacteria is 0.5-2.0 µm. For rod-shaped or filamentous bacteria, length is 1-10 µm and diameter is 0.25-1 .0 µm.

- *E. coli* , a bacillus of about average size is 1.1 to 1.5 µm wide by 2.0 to 6.0 µm long.
- Spirochaetes occasionally reach 500 µm in length and the cyanobacterium
- Accepted wisdom is that bacteria are smaller than eukaryotes. But certain cyanobacteria are quite large; *Oscillatoria* cells are 7 micrometers diameter.
- The bacterium, *Epulosiscium fishelsoni* , can be seen with the naked eye (600 mm long by 80 mm in diameter).
- One group of bacteria, called the Mycoplasmas, have individuals with size much smaller than these dimensions. They measure about 0.25 µ and are the smallest cells known so far. They were formerly known as pleuropneumonia-like organisms (PPLO).
- *Mycoplasma gallicepticum, w*ith a size of approximately 200 to 300 nm are thought to be the world smallest bacteria.
- *Thiomargarita namibiensis* is world's largest bacteria, a gram-negative Proteobacterium found in the ocean sediments off the coast of Namibia. Usually it is 0.1—0.3 mm (100—300 µm) across, but bigger cells have been observed up to 0.75 mm (750 µm).

Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 µm in diameter).

**Shape of Bacterial Cell**

The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted), however pleomorphic bacteria can assume several shapes.



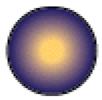

- **Cocci** (or coccus for a single cell) are round cells, sometimes slightly flattened when they are adjacent to one another.
- **Bacilli** (or bacillus for a single cell) are rod-shaped bacteria.
- **Spirilla** (or spirillum for a single cell) are curved bacteria which can range from a gently curved shape to a corkscrew-like spiral. Many spirilla are rigid and capable of movement. A special group of spirilla known as spirochetes are long, slender, and flexible.

**Arrangement of Cocci**

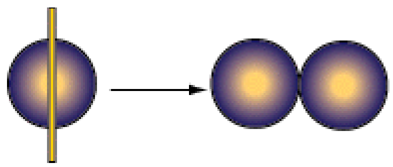
Cocci bacteria can exist singly, in pairs (as diplococci ), in groups of four (as tetrads ), in chains (as streptococci ), in clusters (as stapylococci ), or in cubes consisting of eight cells (as sarcinae). Cocci may be oval, elongated, or flattened on one side. Cocci may remain attached after cell division. These group characteristics are often used to help identify certain cocci.

Cocci appear in several characteristic arrangements, depending on the plane of cellular division and whether the daughter cells stay together or not.

Spherical is called **coccus**.



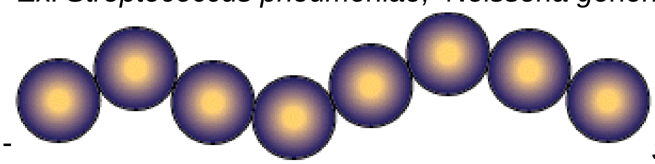
Division along the same plane forms chains; 2 cocci together - **Diplococcus**



**Diplococci**

Ex: *Streptococcus pneumoniae, Neisseria gonorrhoeae,*

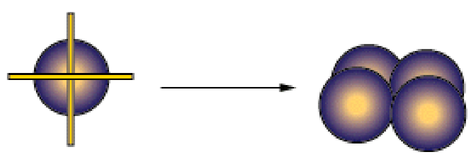
4 - 20 in chains - **Streptococcus**.



***Streptococcus***

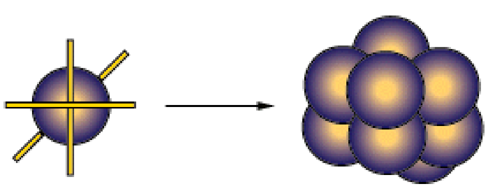
Ex: *Streptococcus pyogenes, Streptococcus agalactiae*

Division along 2 different planes – **Tetrads or Tetracoccus**



*Ex: Aerococcus, Pediococcus and Tetragenococcus*

Division along 3 planes regularly - **Sarcinae**

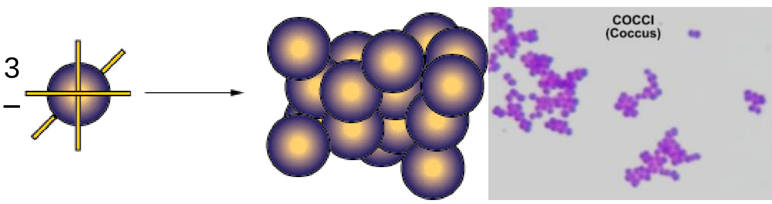


*Sarcina*

The cocci are arranged in a cuboidal manner, as the cells are formed by regular cell divisions in three planes. Cocci that divide in three planes and remain in groups cube like groups of eight.

Ex: *Sarcina ventriculi, Sarcina ureae, etc.*

Division along 3 planes irregularly – **Staphylococcus**




The cocci are arranged in grape-like clusters formed by irregular cell divisions in three plains. Ex.: *Staphylococcus aureus*

**Arrangement of Bacilli**

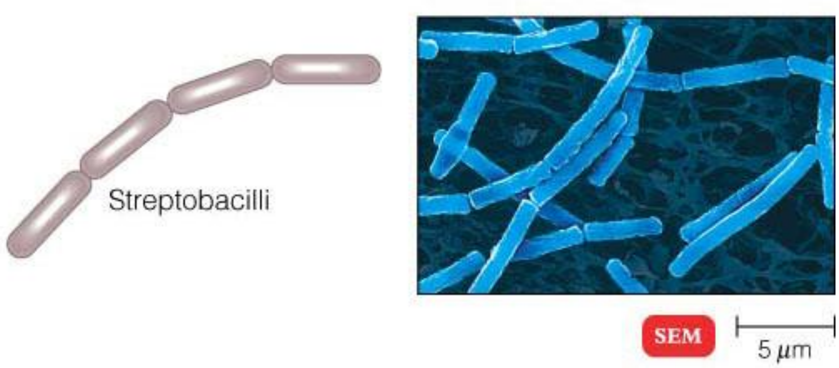
The cylindrical or rod-shaped bacteria are called ‘bacillus’ (plural: bacilli).

**1. Diplobacilli**


Most bacilli appear as single rods. Diplobacilli appear in pairs after division.

Example of Single Rod: *Bacillus cereus*
Examples of Diplobacilli: *Coxiella burnetii, Moraxella bovis, Klebsiella rhinoscleromatis,* etc.

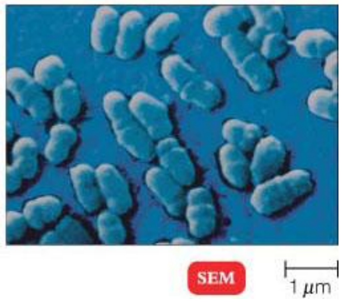
**2. Streptobacilli**




The bacilli are arranged in chains, as the cells divide in one plane.

Examples: *Streptobacillus moniliformis*

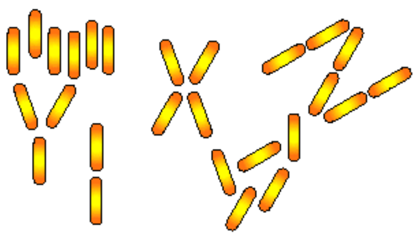
**3. Coccobacilli**


These are so short and stumpy that they appear ovoid. They look like coccus and bacillus.

Examples: *Haemophilus influenzae*, *Gardnerella vaginalis*, and *Chlamydia trachomatis*

**4. Palisades**



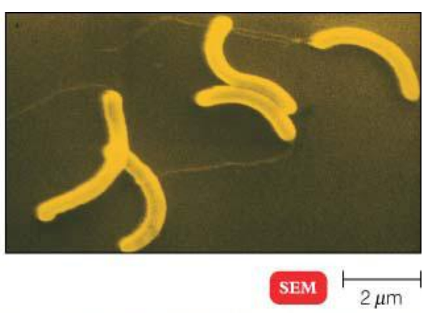
The bacilli bend at the points of division following the cell divisions, resulting in a palisade arrangement resembling a picket fence and angular patterns that look like lmatch sticks or in X, V or Y or Chinese letters

Example: *Corynebacterium diphtheriae*

**Arrangement of Spiral Bacteria**

Spirilla (or spirillum for a single cell) are curved bacteria which can range from a gently curved shape to a corkscrew-like spiral. Many spirilla are rigid and capable of movement. A special group of spirilla known as spirochetes are long, slender, and flexible.

**1. Vibrio**


They are comma-shaped bacteria with less than one complete turn or twist in the cell.

Example: *Vibrio cholerae*

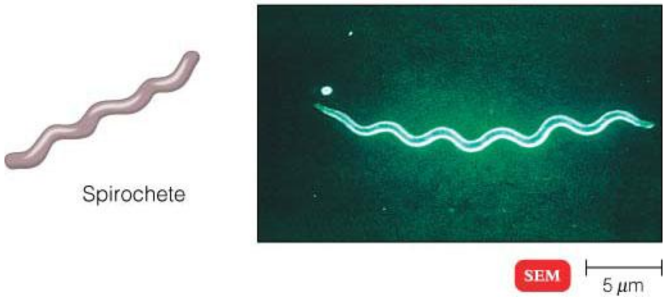
**2. Spirilla**



They have rigid spiral structure. Spirillum with many turns can superficially resemble spirochetes. They do not have outer sheath and endoflagella, but have typical bacterial flagella.

Ex: *Campylobacter jejuni, Helicobacter pylori*, *Spirillum winogradskyi,* etc.

### 3. Spirochetes

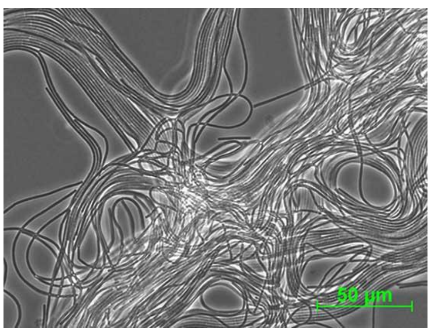



Spirochetes have a helical shape and flexible bodies. Spirochetes move by means of axial filaments, which look like flagella contained beneath a flexible external sheath but lack typical bacterial flagella.

Examples: *Leptospira* species (*Leptospira interrogans*), *Treponema pallidum*, *Borrelia recurrentis*, etc.

### Others Shapes and Arrangements of Bacteria

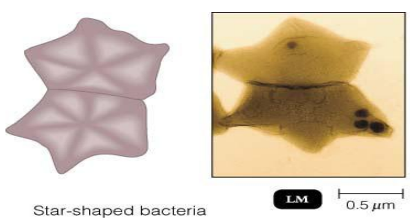
### 1. Filamentous Bacteria




They are very long thin filament-shaped bacteria. Some of them form branching filaments resulting in a network of filaments called 'mycelium'.

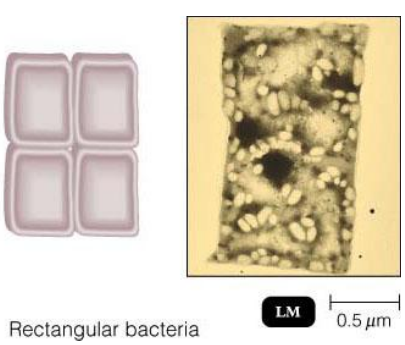
Example: *Candidatus* Savagella

### 2. Star Shaped Bacteria



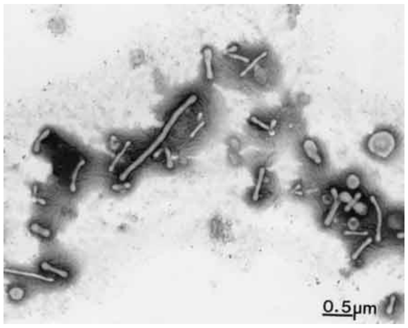

Example: *Stella*

### 3. Rectangular Bacteria




Examples: *Haloarcula* spp

**4. Pleomorphic Bacteria**

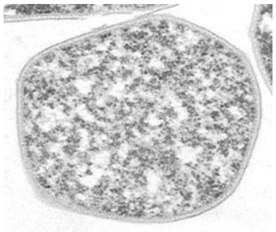



These bacteria do not have any characteristic shape unlike all others described above. They can change their shape. In pure cultures, they can be observed to have different shapes.

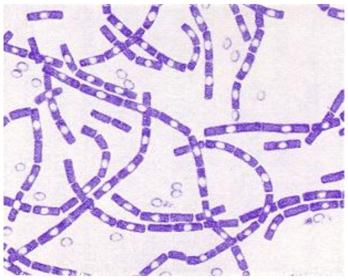
Examples: *Mycoplasma pneumoniae, M. genitalium,* etc.

***5. Pear shaped** – Ex. Pasteuria*

***6. Lobed spheres** - Ex. Sulfolobus*

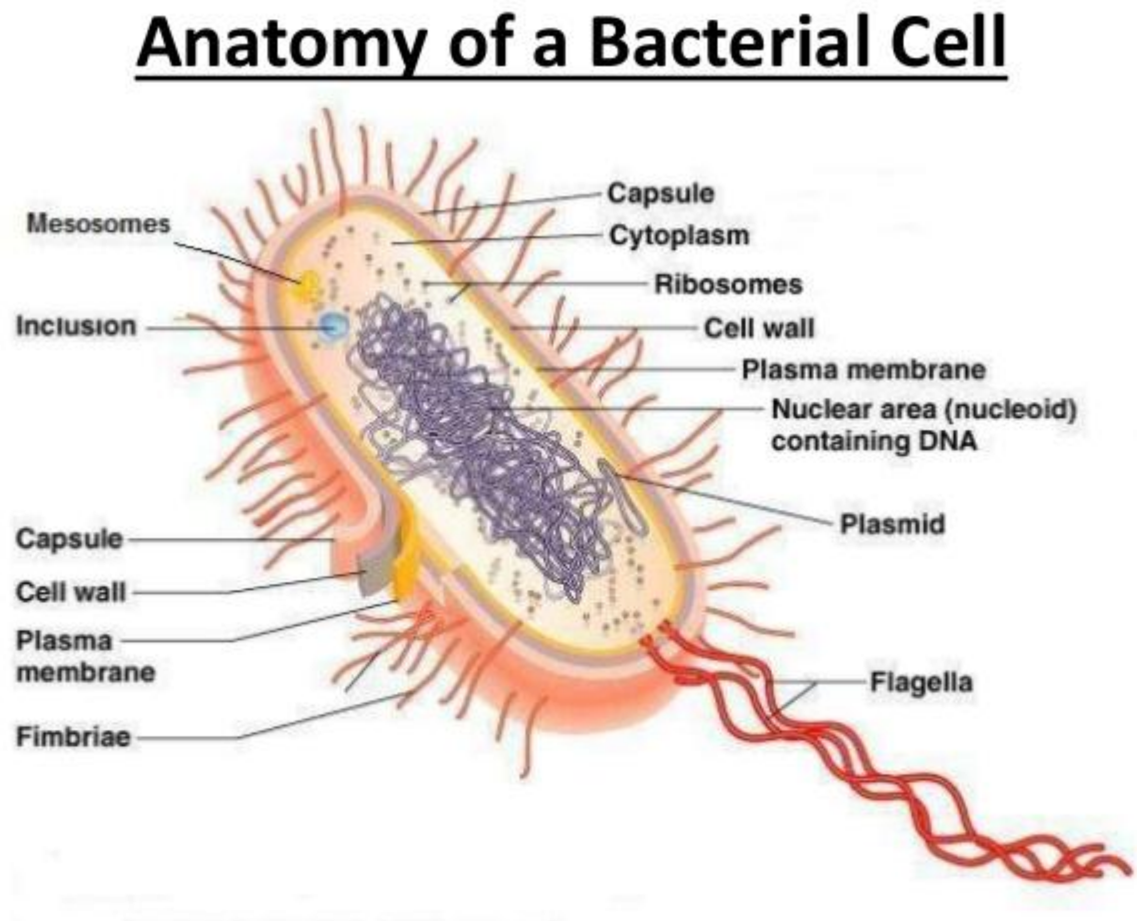


***7. Rod with square end** – Ex. Bacillus anthracis*



**Cell structure and components of bacteria**

**Bacteria - Morphology**




Bacteria are unicellular organisms of relatively simple construction, especially if compared to eukaryotes. Whereas eukaryotic cells have a preponderance of organelles with separate cellular functions. Prokaryotes carry out all cellular functions as individual units.

A prokaryotic cell has five essential structural components: a genome (DNA), ribosomes, cell membrane, cell wall and a surface layer (external structures).

Other than enzymatic reactions, all the cellular reactions incidental to life can be traced back to the activities of these macromolecular structural components. Thus, functional aspects of prokaryotic cells are related directly to the structure and organization of the macromolecules in their cell make-up, i.e., DNA, RNA, phospholipids, proteins and polysaccharides. Diversity within the primary structure of these molecules accounts for the diversity that exists among bacteria.

**I. External structures in bacteria and their function**

This includes flagella, pili, glycocalyx, and other appendages like sheath, prosthecae and stalk

**1. Flagella** (Sin. flagellum)

are hair like, filamentous helical appendage that protrude through cell wall which is responsible for movement of the bacterium. They are very thinner than the eukaryotic flagellum. The diameter is about 20 nm, well below the resolving power of compound microscope. The flagellum is rotated by a motor apparatus in the plasma membrane allowing the cell to swim in the fluid environment.

**Structure of flagella**

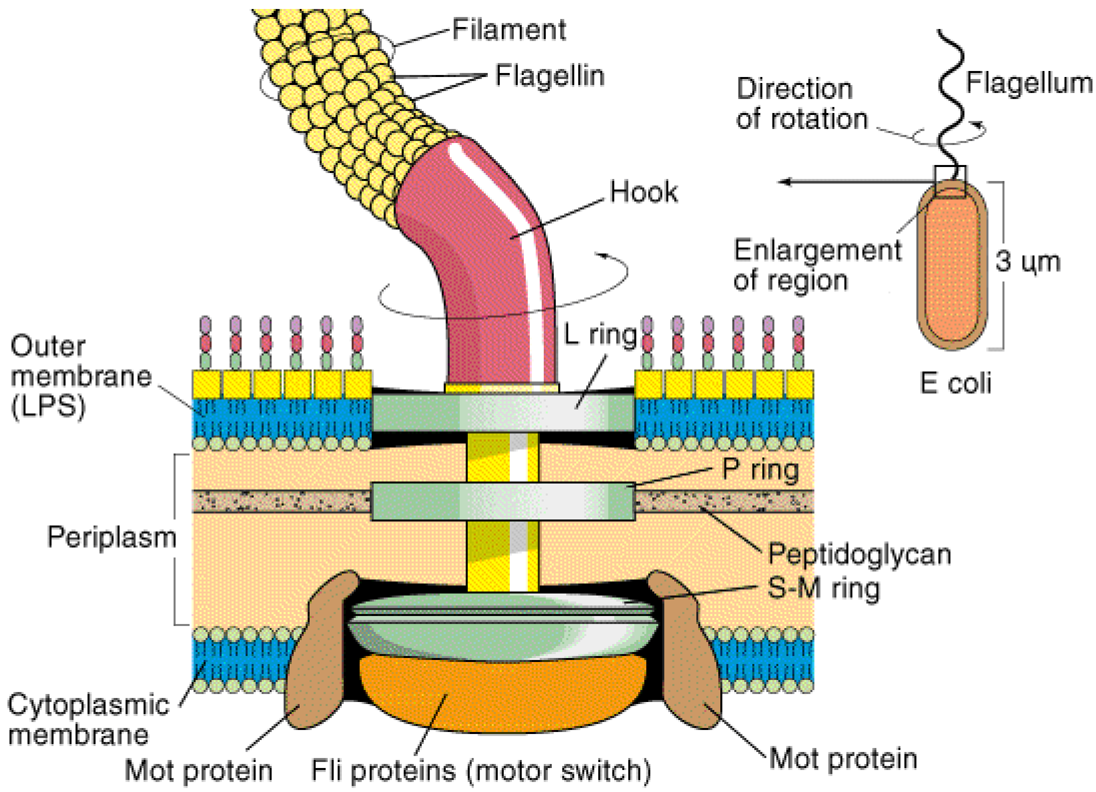
1) **Filament –** outermost region and contain the globular protein flagellin

2) **Hook –** the filament is attached to hook, which consists of a different protein

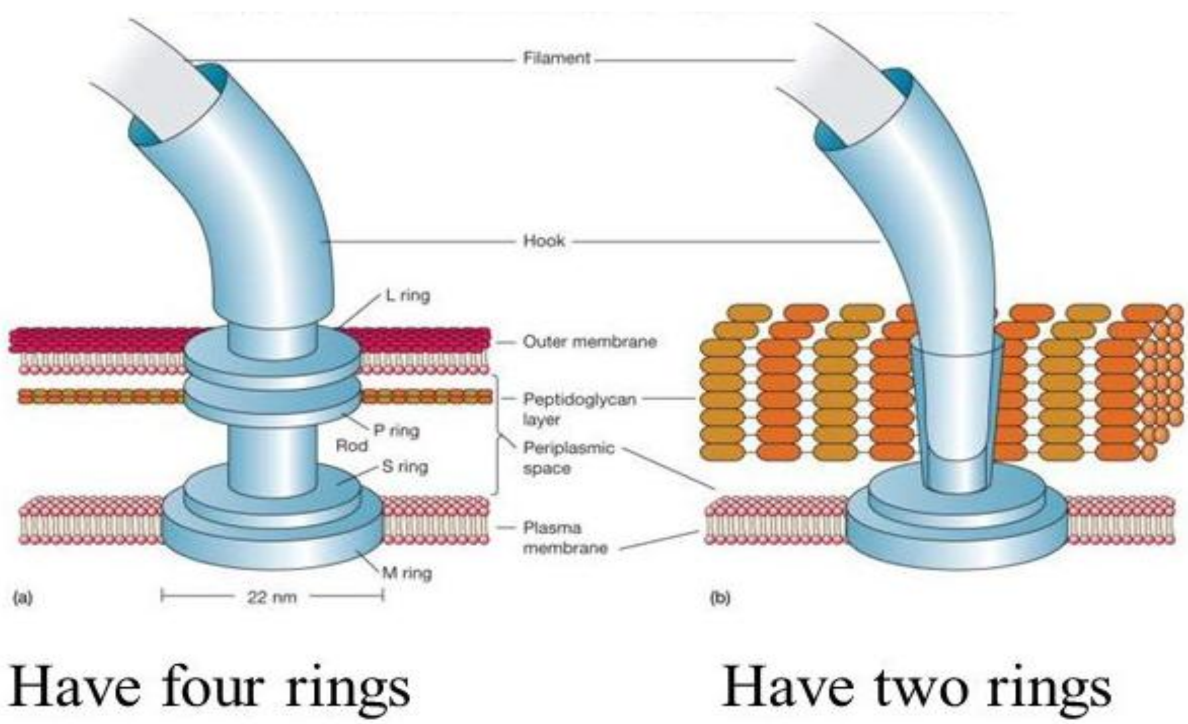
3) **Basal body** - which anchors the flagellum to the cell wall and plasma membrane. It consists of a small central rod inserted into it are a series of rings .

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. For example *Bdellovibrio* has a membranous structure surrounding the filament. *Vibrio cholerae* has a lipopolysaccharide sheath.

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 twp basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan.



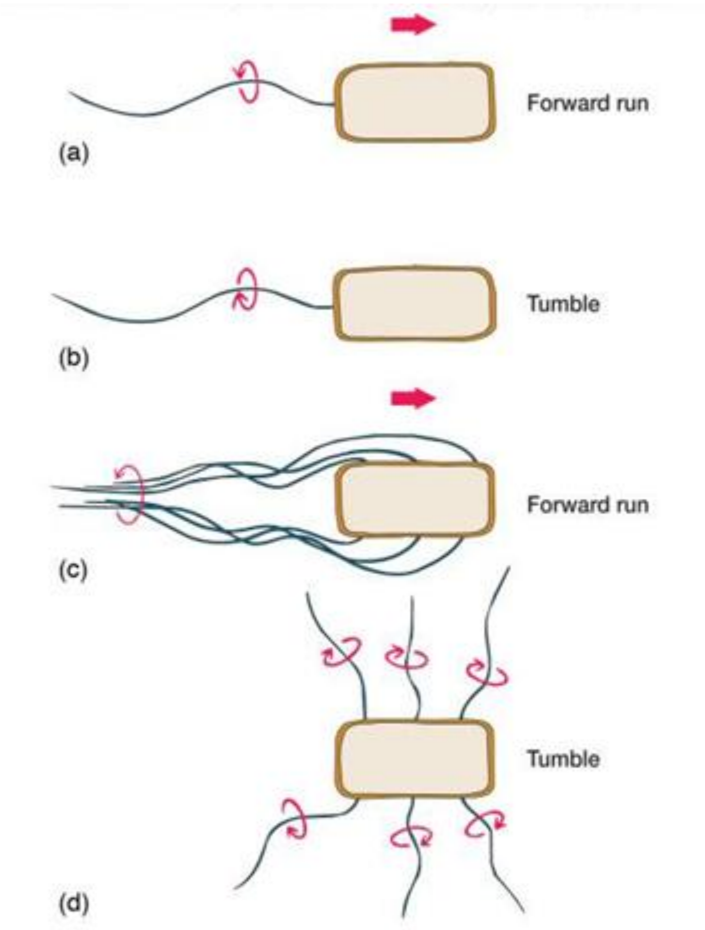

Structure of bacterial flagella (Gram negative)




**Gram negative flagella** **Gram positive flagella**

**Flagellar movement:**

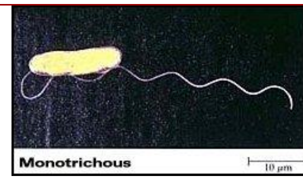
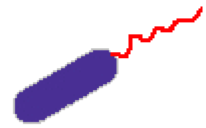
The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial 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 for a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.




Bacterial flagellar movement

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)

The distribution/ arrangement of flagellum in the bacteria vary with different types.

| | | |
|---|---|---|
| a | **Monotrichous flagellum** – single flagellum present in the pole of the bacterium (Ex. *Pseudomonas aeruginosa*) |    |
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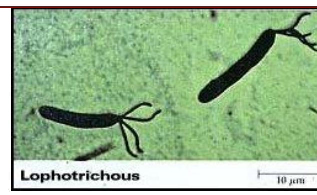
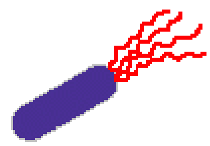
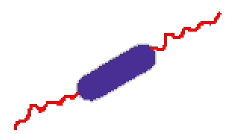
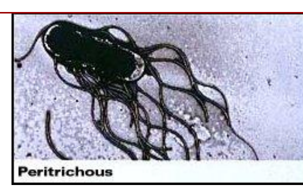
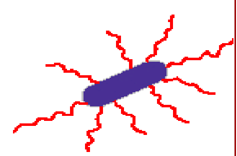
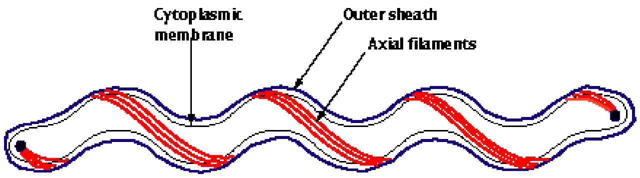
| b | **Lophotrichous flagella** – cluster of flagella present in the pole of the bacterium (*Ex. Pseudomonas fluorescens*) |    |
| | | |
| c | **Amphitrichous flagella** – single or cluster of flagella present in the both pole of bacterium (Ex . *Aquaspirillum serpens*) |   |
| | | |
| d | **Peritrichous flagella** – flagella surrounded through out the body of the bacterium (Ex. *Salmonella typhi*) |    |

**Endoflagella**

The flagella or filaments, present between the outer membrane and inner membrane of the cell wall of the bacterium are called as **endoflagella** or **periplasmic flagella** or **axial filaments**.

They are attached at one end of the cell.  Ex. Spirochetes- group of bacteria; Bacterium : *Spirochete, Leptospira*

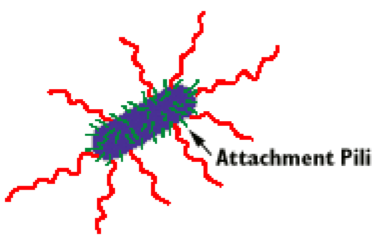



The flagella are responsible for motility of the bacterium.  It rotates like a propeller. Rings in the basal body rotate relative to each other causing the flagella to turn. The energy to drive the basal body is obtained from the proton motive force.  The average speed of bacterial movement is 50 µm/sec which equivalent to its 10 body length.

A very different type of motility, gliding motility, is employed by many bacteria; cyanobacteria, myxobacteria and cytophagas and some mycoplasmas.

**2. Pili** (Sin: pilus)

Pili are hollow, non-helical, filamentous appendage thinner, shorter and more numerous than flagella. They also present in the non-motile bacteria too.  Like flagella, they composed of protein, called pilin. Pili are absent in gram-positive bacteria and present in gram-negative bacteria.Pili are mainly involved in the **adherence or attachment** of bacteria to surfaces, substrates and other cells in nature.




Some pili (**F or sex pili**) are involved in the transfer of DNA from one cell to other, called **conjugation**.  In *E. coli*, both sex pili and common pili are present).

They also involve the virulence determination character of a bacterium.  Ex. *Neisseria gonorrhoeae* causes disease if pili are present.

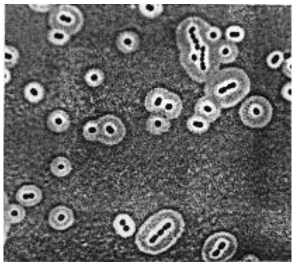
The pili also give resistance to bacterium from phagocytosis (Ex. *Streptococcus pyrogenes*)

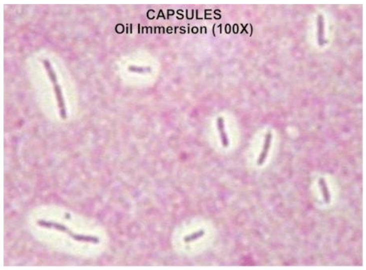
The number of pili varies from few 1-4 to 100 – 200 per bacterium.

**3. Fimbriae**

Fimbriae (sometimes called "attachment pili") are protein tubes that extend out from the outer membrane in many members of the *Proteobacteria*. They are generally short in length and present in high numbers about the entire bacterial cell surface. Fimbriae usually function to facilitate the attachment of a bacterium to a surface (e.g. to form a biofilm) or to other cells (e.g. animal cells during pathogenesis). A few organisms (e.g. *Myxococcus*) use fimbriae for motility to facilitate the assembly of multicellular structures such as fruiting bodies.

**4. Glycocalyx (Capsules, Slime layers and S-layers)**






Some bacterial cells are surrounded by viscous substance forming a covering layer or envelope around the cell wall, are called as capsules.

If it is visible under light microscope using special staining, it is called as **capsule** and if it is so thin, not able to see under light microscope, called as **microcapsules.**

If it is so abundant that many cells are embedded in a common matrix, are called as **slime.**

*Note : The capsule or slime or microcapsule often referred as* ***Glycocalyx***

Most of the capsules are polysaccharide nature, specifically homopolysaccharides. Ex. *Streptococcus mutans* capsule is made up of glucan.

Some bacterial capsule made up of polypeptides. Ex. *Bacillus anthracis* capsule composed of polymers of glutamic acid.

Functions :

- Provide protection against temporary drying
- block the attachment of bacteriophages
- antiphagocytic (Ex. *Streptococcus pneumoniae*)
- Provide virulence to the bacteria (Ex. *S. pnemoniae* causes disease only if capsulated)
- promote attachment of bacteria (ex. *Streptococcus mutans* adheres smooth surface of teeth and causes dental caries)
- Prevent cell aggregation in the suspension by electrical charges
- Important role in biofilm application

## 5. S-layers

An S-layer (surface layer) is a cell surface protein layer found in many different bacteria and in some archaea, where it serves as the cell wall. All S-layers are made up of a two-dimensional array of proteins and have a crystalline appearance, the symmetry of which differs between species. The exact function of S-layers is unknown, but it has been suggested that they act as a partial permeability barrier for large substrates. For example, an S-layer could conceivably keep extracellular proteins near the cell membrane by preventing their diffusion away from the cell. In some pathogenic species, an S-layer may help to facilitate survival within the host by conferring protection against host defence mechanisms.

**6. Other appendages / structures outside the cell wall**

- **Sheath :**

Some species of bacteria, particularly those from fresh water and marine environment form chains or trichomes that are enclosed by hollow tube are called as Sheath**.** Ex. *Sphaerotilus*

- **Prosthecae :**

Some species of bacteria had semirigid extension of cell wall and cell membrane are called as prosthecae**.** This is to increase the surface area of the cell for nutrient absorption. Ex. *Calulobacter.*

- **Stalk :**

The non-living ribbon or tubular appendage that are excreted by the bacterial cell are referred as stalk. Ex. *Gallionella*. They aided for attachment of the bacteria to surface.

II. **Cell wall**

All the bacteria have rigid cell wall. The cell wall is the essential structure that protect the cell from mechanical damage and from osmotic rupture of lysis. Prokaryotes live relatively diluted atmosphere (has lower osmotic pressure than the osmotic pressure of inside the cell), which accumulate high salt concentration inside. The osmotic pressure against the inside of the plasma membrane may be the equivalent to 10—25 atm. Since the plasma membrane is delicate, plastic structure, it must be restrained by an outside wall made of porous, rigid material that has high tensile strength. The cell wall of bacteria deserves special attention for several reasons.

- They are essential for viability
- They are composed of unique compounds no where else in nature
- They are one of the important site for attack by antibiotics
- They provide adherence and receptor sites for drug or virus
- They cause symptoms of disease in animals
- They provide immunological distinction and variation among strains of bacteria

The bacteria can be divided in to two major groups based on their cell wall chemistry, called as gram positive and gram negative bacteria. The gram negative cell wall is a multilayered and quite complex, where as the gram +ve cell wall consists of a single type of molecule and often much thicker.

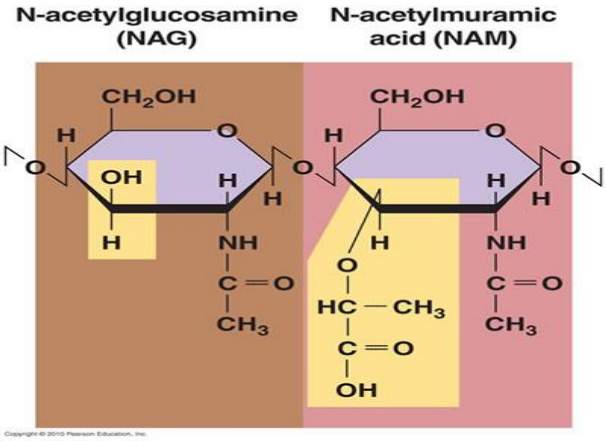
**Composition and Characteristics of cell wall**

The cell wall surrounds the plasma membrane and protects the cell from changes in water pressure.

The bacterial cell wall consists of peptidoglycan (or murein), a polymer consisting of NAG and NAM and short chains of amino acids as L-alanine, D-alanine, D-glutamic acid, and lysine or diamino pimelic acid. .

Penicillin interferes with peptidoglycan synthesis.

**N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) joined as in peptidoglycan**




**Structure of Peptidoglycan in a Cell Wall**

Alternating NAM and NAG molecules form a carbohydrate backbone (the glycan portion).

Rows of NAG and NAM are linked by polypeptides (the peptido- portion).

The structure of the polypeptide cross-bridges may vary but they always have a tetrapeptide sidechain, which consists of 4 amino acids attached to NAMs. The amino acids occur in alternating D and L forms.

While peptidoglycan is present in (most) all bacterial cell walls, there are two basic variations of structure seen in most bacterial cells, one described as Gram-positive and one described as Gram-negative.

**The Gram-positive Cell Wall**

Gram-positive cell walls consist of many layers of peptidoglycan and also contain teichoic acids. In Gram positive bacteria, 90% of the cell wall consists of peptidoglycan layer and few quantities of teicholic acid usually present. Many bacteria has several layers (about 25) of peptidoglycon. In gram negative bacteria, only 10 per cent of wall is peptidoglycon and the major of the wall is consists of outer membrane.

Teichoic acids may:

- bind and regulate movement of cations into and out of the cell
- prevent extensive wall breakdown and possible cell lysis during cell growth
- provide much of the cell wall's antigenicity

**The Gram-negative Cell Wall**

Gram-negative bacteria have a lipopolysaccharide-lipoprotein-phospholipid outer membrane surrounding a thin (sometimes a single) peptidoglycan layer. Only 10 per cent of wall is peptidoglycon and the major of the wall is consists of outer membrane. **Gram-negative cell walls have no teichoic acids.**

The outer membrane protects the cell from phagocytosis and from penicillin, lysozyme, and other chemicals.

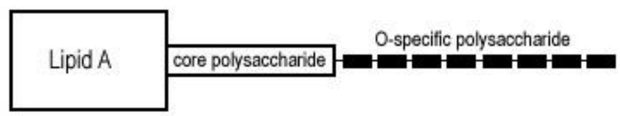
Porins are proteins that permit small molecules to pass through the outer membrane; specific channel proteins allow other molecules to move through the outer membrane.

The lipopolysaccharide component of the outer membrane consists of sugars (O polysaccharides) that function as antigens and lipid A, which is an endotoxin. Endotoxin causes fever and shock.

**Outer membrane :**

Besides peptidoglucan layer, g-ve bacteria contain additional wall layer made of lipo-polysaccharide. The layer is called as lipopolysaccharide layer or LPS layer or outer membrane.

The lipids and polysaccharides are intimately linked and formed this layer. The polysaccharide has two portions namely, o-polysaccharide and core polysaccharide.




O-polysaccharide contains galactose, glucose, rhamnose, mannose, and one or more dideoxy sugar like abequose, colitose, paratose or trylose. These sugars were connected four or five membered sequence which often branched. The core polysaccharide consists of keto deoxyoctonate, glucose, galactose and N-acetyl glucosamine.

The lipid portion of the LPS referred as lipid A, is not a glycerol lipid but instead, the fatty acids are connected by ester amine linkage to disaccharides composed of N-acetyl glucosamine. The fatty acids are caproic, lauric, mysteric, palmitic, stearic acids.

A lipid protein is also found on the inner side of the outer membrane of gram negative bacteria. It acts like an anchor between outer membrane and peptidoglycan layer.

**Endotoxins** :

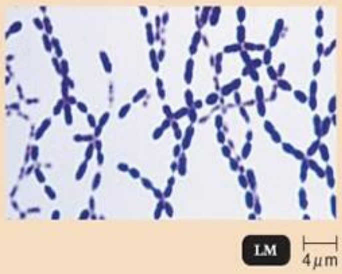
In *Salmonella, Shigella, Escherichia,* the outer membrane causes toxic and pathogenic symptom to human and mammals referred as endotoxins.

**Porins** :

Protein substance present in the outer membrane of g-ve bacteria. They act like channel for entrance and exit of hydrophobic low molecular substances.

**Periplasm :**

The space in between peptidoglycan and outer membrane is referred as periplasm or periplasmic space. In g+ve bacteria, it refers the space between peptidoglucan and cell membrane. This space harbours hydrolytic enzymes, binding proteins, detoxifying enzymes etc.

| Characteristic | Gram-Positive | Gram-Negative |
|---|---|---|
| |  |  |
| Gram Reaction | Retain crystal violet dye and stain blue or purple | Can be decolorized to accept counterstain (safranin) and stain pink or red |
| Peptidoglycan Layer | Thick (multilayered) | Thin (single-layered) |
| Teichoic Acids | Present in many | Absent |
| Periplasmic Space | Absent | Present |
| Outer Membrane | Absent | Present |
| Lipopolysaccharide (LPS) Content | Virtually none | High |
| Lipid and Lipoprotein Content | Low (acid-fast bacteria have lipids linked to peptidoglycan) | High (because of presence of outer membrane) |
| Flagellar Structure | 2 rings in basal body | 4 rings in basal body |
| Toxins Produced | Exotoxins | Endotoxins and exotoxins |
| Resistance to Physical Disruption | High | Low |
| Cell Wall Disruption by Lysozyme | High | Low (requires pretreatment to destabilize outer membrane) |
| Susceptibility to Penicillin and Sulfonamide | High | Low |
| Susceptibility to Streptomycin, Chloramphenicol, and Tetracycline | Low | High |
| Inhibition by Basic Dyes | High | Low |
| Susceptibility to Anionic Detergents | High | Low |
| Resistance to Sodium Azide | High | Low |
| Resistance to Drying | High | Low |



## Cell Walls And The Gram Stain Mechanism

The Gram stain was developed by Christian Gram in 1884 and can differentiate between the two types of cell walls we've just looked at.

The cells are first stained with the **primary stain**, crystal violet.

After about 1 minute of staining excess primary stain is washed off and the **mordant**, Gram's iodine, is applied for another minute.

The iodine forms a complex with the crystal violet and the crystal violet-iodine complex becomes "trapped" inside the peptidoglycan.

At this point cells with either type of cell wall appear purple, due to the presence of the crystal violet-iodine complex.

The next step is to **decolorize** (using acetone-alcohol) the cells for a period of time long enough to dissolve the outer membrane of Gram-negative cells and pull the crystal violet-iodine complex through the thin layer of peptidoglycan.

If done properly, Gram-positive cells retain the crystal violet-iodine complex and appear purple while Gram-negative cells are decolorized (and appear colorless, no?)

Note: decolorizing too long will result in both Gram-positive and Gram-negative cells appearing colorless, while decolorizing for too short a time will result in both cell types retaining the crystal violet-iodine complex and appearing purple.

After decolorization Gram-positive cells (purple) can be differentiated from Gram-negative cells (colorless) but since colorless cells are hard to see the final step in the Gram staining process is to use a **counter-stain**, safranin, to stain the Gram-negative cells pink.

Gram-positive cells will also stain with safranin but it will not be seen on top of the purple crystal violet-iodine remaining in the cells.

**Atypical Cell Walls**

*Mycoplasma* is a bacterial genus that naturally lacks cell walls; the presence of sterols in the plasma membrane protects from osmotic lysis.

*Mycobacterium* is a genus that has mycolic acids in its cell walls, giving it a "waxy" cell wall that is resistant to decolorization with acid-alcohol when stained with carbolfuschin (and so is designated "acid-fast").

Archaea have pseudomurein; they lack peptidoglycan.

**Damage To The Cell Wall**

In the presence of lysozyme, gram-positive cell walls are destroyed, and the remaining cellular contents are referred to as a **protoplast.**

In the presence of lysozyme (after disruption of the outer membrane), gram-negative cell walls are not completely destroyed, and the remaining cellular contents are referred to as **spheroplasts.**

Protoplasts and spheroplast are subject to osmotic lysis.

*Proteus* and some other genera can lose their cell walls spontaneously or in response to penicillin and swell into **L forms** (Lister Institute). L forms can live and divide and/or return to the normal walled state.

Antibiotics such as penicillin interfere with cell wall (peptidoglycan) synthesis.

### III. Cell membrane :

The cell membrane is the most dynamic structure in prokaryotes. They act as selective permeability barrier that regulates the passage of substance in and out of the cell.

Bacterial membrane consists of 40 per cent phospholipids and 60 per cent protein. The phospholipids forms bilayer and the proteins are present scatterly (called integral protein) and some protein present on the surface of the layer (called peripheral protein).

The phospholipids are phosphoglycerides in which straight chain fatty acids are ester linked to glycerol.

Functions of cell membrane :

- Osmotic or permeability barrier
- Location of transport systems for specific solutes (nutrients and ions)
- Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous ATP-synthesizing ATPase
- Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)
- Synthesis of murein (cell wall peptidoglycan)
- Assembly and secretion of extra cytoplasmic proteins
- Coordination of DNA replication and segregation with septum formation and cell division
- Chemotaxis (both motility and sensing functions)

### IV. Mesosomes

The cytoplamic membrane invagination in the form of tubular or vesicle shaped are referred as mesosomes. If the mesosome is present in the centre of the cell referred as central mesosomes and they are involved in the cell division.

The mesosome which is present in the peripheral region (aside) of the cell are referred as peripheral mesosomes and they involved in the transport of extracellular enzymes from cytoplasm to external regions.

### V. Cytoplasm

The cytoplasm of bacterial cell is the place where the functions for cell growth, metabolism, replication carried out. The gel like mat composed of water, protein, enzymes, nutrients, wastes, gases, like non-cellular materials and chromosomal DNA, ribosomes, plasmids like cellular components are present.

### VI. Chromosomal DNA

The bacteria do not contain true membrane enclosed nucleus and chromosomes. They have a long coiled double strand single circular structure often called as chr. DNA or nucleoid or bacterial chromosome or chromatin body.

### VII. Plasmids

A small circular, covalently closed, self replicating extra chromosomal DNA is also present in many bacteria. They are referred as **plasmids**. They are mostly controlling the special characters like pathogenicity, nodulation etc.

### VIII. Ribosomes

The ribosomes are the granular appearance in the cytoplasm involved in the protein synthesis. The size of prokaryotic ribosomes is of 70 S(sedimentation coefficient, Svedberg unit) and are of two sub units of 50S and 30S. The bacterial ribosomes never bound to any organelles in the cytoplasm.

### IX. Cytoplasmic inclusions

The cytoplasm of bacteria often contains inclusion granules referred as cytoplasmic inclusions. Inclusion bodies are usually storage materials of energy, carbon, phosphorus etc. Some aquatic bacteria pocess large sized gas vacuoles which will help to float them.

The following table shows the cytoplasmic inclusions and their role in bacterial cell.

**Some inclusions in bacterial cells**

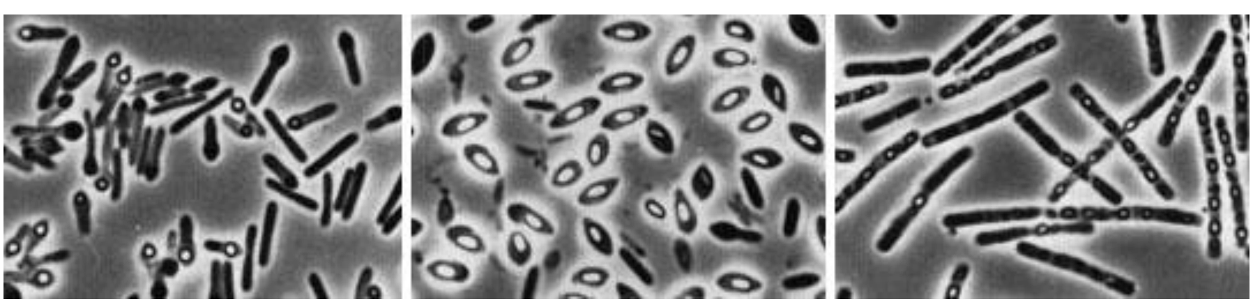
| **Cytoplasmic inclusions** | **Where found** | **Composition** | **Function** |
|---|---|---|---|
| glycogen | many bacteria e.g. *E. coli* | polyglucose | reserve carbon and energy source |
| polybetahydroxyutyric acid (PHB) | many bacteria e.g. *Pseudomonas* | polymerized hydroxy butyrate | reserve carbon and energy source |
| polyphosphate (volutin granules) | many bacteria e.g. *Corynebacterium* | linear or cyclical polymers of $PO_4$ | reserve phosphate; possibly a reserve of high energy phosphate |

| | | | |
|---|---|---|---|
| sulfur globules | phototrophic purple and green sulfur bacteria and lithotrophic colorless sulfur bacteria | elemental sulfur | reserve of electrons (reducing source) in phototrophs; reserve energy source in lithotrophs |
| gas vesicles | aquatic bacteria especially cyanobacteria | protein hulls or shells inflated with gases | buoyancy (floatation) in the vertical water column |
| parasporal crystals | endospore-forming bacilli (genus *Bacillus*) | protein | toxic to certain insects |
| magnetosomes | certain aquatic bacteria | magnetite (iron oxide) $Fe_3O_4$ | orienting and migrating along geo-magnetic field lines |
| carboxysomes | many autotrophic bacteria | enzymes for autotrophic $CO_2$ fixation | site of $CO_2$ fixation |
| phycobilisomes | cyanobacteria | phycobiliproteins | light-harvesting pigments |
| chlorosomes | Green bacteria | lipid and protein and bacteriochlorophyll | light-harvesting pigments and antennae |

**X. Endospores**

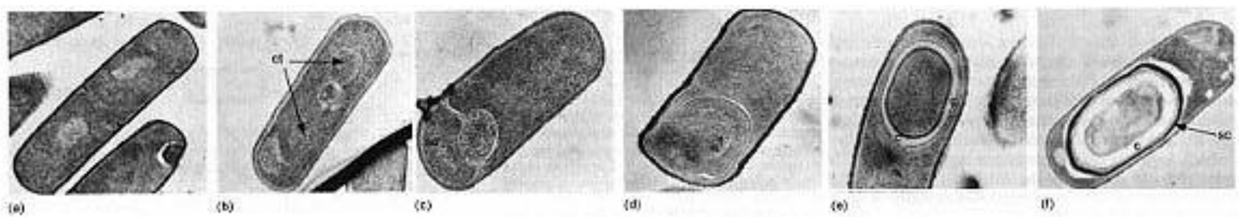
Certain species of bacteria produce spores either within the cells are referred as endospores. They are unique to bacteria and *Bacillus, Clostridium* are common spore forming bacteria.

The endospores are extremely resistance to desiccation, staining, disinfecting chemicals, radiation and heat. The endospore can able to survive in boiling water, uv light and many harmful chemicals. They can survive even 100 years.



Bacterial endospores

First, the DNA fragmented and followed by invagination of cytoplasmic membrane. Then, the portion of the invagination is separated by complete formation of septum (this stage is called forespore). Then formation of cell wall around the forespore and followed by cortex, spore coat and exosporium synthesis occurred. Finally, cell lysed and the spore is released to the external environment.




*Process of endospore formation*

The outer most layer is called as **exosporium**, a thin delicate covering made of protein. The next layer is **spore coat**, composed of spore specific proteins. Below the spore coat is **cortex**, composed of cross linked peptidoglycan. Inside the cortex is spore protoplast which has cell wall (core wall), cell membrane, DNA, ribosome and little cytoplasm.

One chemical substance that is characteristic of endospores not in vegetative cells is **dipicolinic acid** in the core region. They occupy 10% of total dry weight of the spores and responsible for heat resistance.

**Cyst :** Cyst is dormant, thick walled desiccation (drying) resistant form of cell. It can be differentiated from vegetative cells and can germinate under suitable condition. The cyst don't have temperature resistance. Ex. Cyst of *Azotobacter*

**Sporangiospores and Conidiospores :** the filamentous bacteria, actinomycetes produce these kind of spores. The spores were produced at the tip of the hyphae and if the spores are formed in a sac like structure (Sporangium), called as sporangiospores, if not called as conidiospores. These spores do not have heat resistance but can survive long period of drying.

## Summary of characteristics of typical bacterial cell structures

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

## Reproduction in Bacteria - Vegetative, Asexual and Sexual Methods

Bacteria are the simplest, the smallest, and the most successful microorganisms. They were first discovered by **Anton Leeuwenhoek** (1676). In the five kingdom classification, they are placed in Kingdom Monera.

Bacteria reproduce by **Vegetative, asexual and sexual** methods.

**Vegetative reproduction** includes **Budding, Fragmentation** and **Binary fission**

**Budding:**

- In this case, a small protuberance, called bud, develops at one end of the cell. Genome replication follows, and one copy of the genome gets into the bud. Then the bud enlarges, eventually become a daughter cell and finally gets separated from the parent cell.

**Fragmentation:**

- Mostly during unfavorable conditions, bacterial protoplasm undergoes compartmentalization and subsequent fragmentation, forming minute bodies called **gonidia.** Under favorable conditions, each gonidium grows to a new bacterium. It becomes apparent that prior to fragmentation the bacterial genome has to undergo repeated replication so that each fragment gets a copy of it.

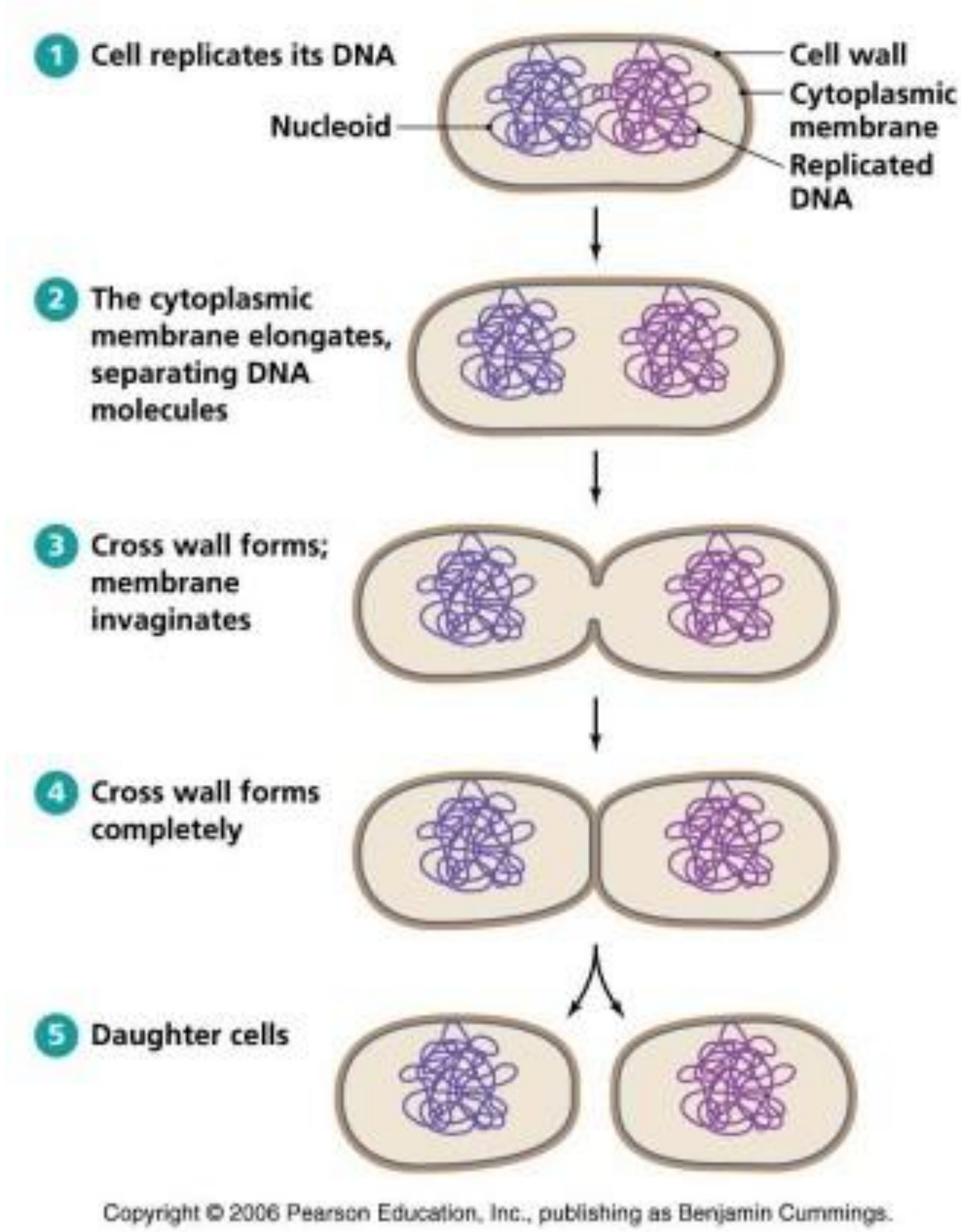
**Binary fission**:

- It is the commonest type of reproduction under favorable conditions in which cell divides into two similar daughter cells. During the process, the bacterial chromosomes get attached to the cell membrane and replicates to the bacterial chromosomes. As the cell enlarges the daughter chromosomes gets separated. A cross wall is formed between the separating daughter chromosomes. It divides the cell into two daughter cells. The daughter cells soon grow to maturity within 20-30 minutes. Under favorable conditions many bacteria divide once in 20-30 minutes.

The process of binary fission is as follows:

1. The cell became elongated from its original size and approximately twice length
2. DNA replication takes place and DNA moves towards  polar region
3. Septum or cross wall (septum) occurs from the mesosome region
4. New cell wall formation occurs
5. After completion, the cells were separated out

Ex. *Bacillus, E. coli, Streptococcus*

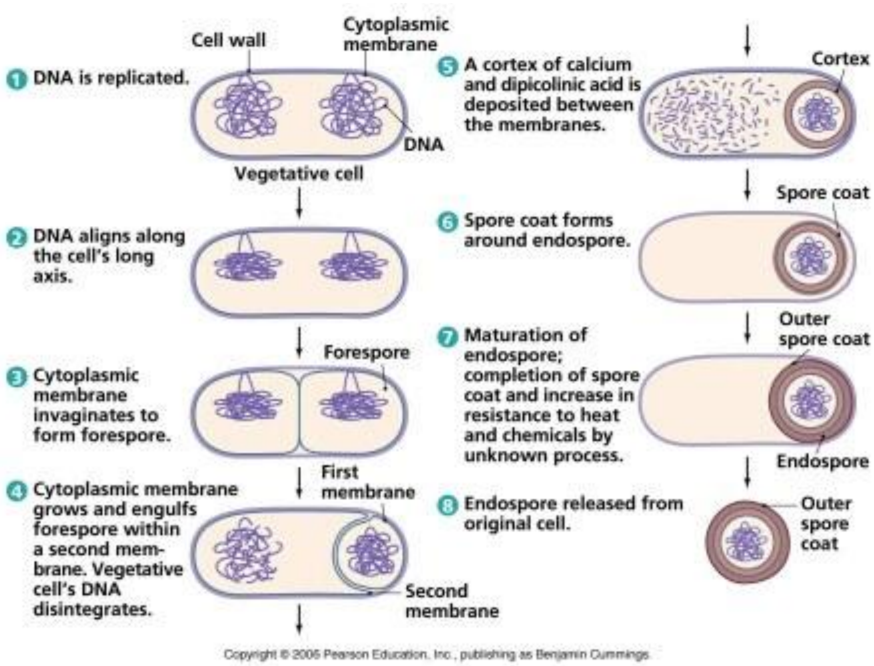



Binary Fission

## Asexual reproduction

**takes place by endospore formation, conidia and zoo spores.**

- **Endospore formation:** Endospore are resting spores formed in some gram positive bacteria (Bacillus and Clostridium) during unfavourable conditions. They are formed within the cells. During this process a part of the protoplast becomes concentrated around the chromosome. A hard resistant wall is secreted around it. The rest of the bacterial cell degenerates; Endospore are very resistant to extreme physical conditions and chemicals. During favourable conditions the spore wall gets ruptured and the protoplasmic mass gives rise to a new bacterium.




Endospore Formation

**Sexual reproduction** occurs in the form of **genetic recombination**. There are three main methods of Genetic Recombination: Transformation, Transduction and Conjugation.

- **Transformation:** Here genetic material of one bacterial cell goes into another bacterial cell by some unknown mechanism and it converts one type of bacterium into another type (non capsulated to capsulated form). This was first studied by Griffith (1928) in *Diplococcus pneumonia.*
- **Transduction:** In this method, genetic material of one bacterial cell goes to other bacterial cell by agency of bacteriophages or phages (viruses, infecting bacteria). It was first of all reported in Salmonella typhimeurium by Zinder and Lederberg (1952).
- **Conjugation:** It was first reported by Lederberg and Tatum (1946) in E.coli bacteria. Cell to cell union occurs between two bacterial cells and genetic material (DNA) of one bacterial cell goes to another cell lengthwise through conjugation tube which is formed by sex pili.