Microbiology





Morphology & Ultra-Structure of Microorganism

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References

- **1. Essential Microbiology for Dentistry**
- by Lakshman Samaranayake
- 2. Microbiology and Immunology
- by Richard Hunt

Microbiology

- Microbiology: is a great complex of biological sciences about microorganisms, it studies organisms too small to be seen without magnification.
- Micro too small to be seen with the naked eye
- **Bio** life
- **logy** study of
 - Microorganisms are ubiquitous, include:
 - Obacteria
 - **O** viruses
 - Ofungi
 - Parasite {protozoa & helminthes (worms)}
 - Oalgae

History of the Study of Microorganisms

- Robert Hooke (1665)
- 1. little boxes or cells
- 2.Cell Theory

The cell theory states that:

- All living things are made of one or morecells.
- Cells are the basic unit of structure and function in living things.
- All cells come from other cells.

- van Leeuwenhoek (1674)
- He was the first to actually see living microorganisms and named their as "animalcules"
- He constructed first microscope with magnification about 50 to 300 times

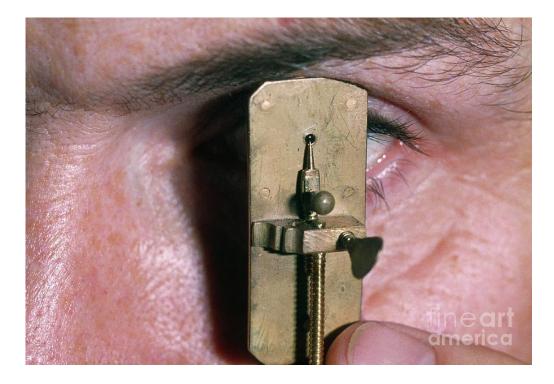


PLATE XXIV

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- Louis Pasteur (1822)
- Established fermentation : was the result of microbial activity
- Introduced techniques of sterilization
- Showed that microorganisms do not arise by spontaneous generation.
- Elaborated methods of pathogenic micro-organisms attenuation
- Developed rabies vaccine.

• Robert Koch (1843)

- Elaborated methods of staining and cultivation of microorganisms.
- Discovered causative agents of anthrax, cholera and tubercle bacillus.
- Elaborated criteria for proving relationship between a microorganism and a specific diseases (Koch's postulates).
 - 1. The microorganism must be present in every case of the disease but absent in healthy organism.
 - 2. The suspected microorganism must be isolated and grown in a pure culture.
 - 3. The same disease must result when the isolated microorganism is inoculated into a healthyhost.
 - 4. The same microorganism must be isolated again from the diseased host.

Cell Types

• Based on the organization of their cellular structures,all living cells can be divided into two categories:

Eukaryotic Cells Cell that have membrane-bound organelles

Animals, plants, fungi, protozoa, and algae

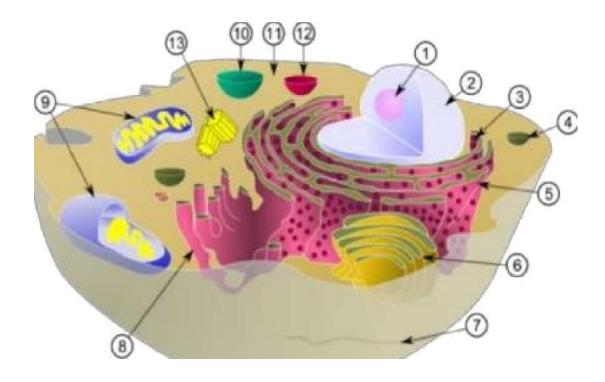
prokaryotic cells
Cells that <u>do not</u> have membrane-bound organelles
Unicellular organisms such as bacteria and blue
green algae



- All cells are surrounded by a *plasma membrane*.
- The semi-fluid substance within the cell is called "cytosol", containing the cell organelles.
- All cells contain chromosomes which have genes in the form of DNA.
- All cells have tiny organelles called "*Ribosomes*" that make proteins.

A major difference between prokaryotic and eukaryotic cells is the location of chromosomes.
1. In an eukaryotic cell, chromosomes are contained in a membrane-enclosed organelle, the *nucleus*.

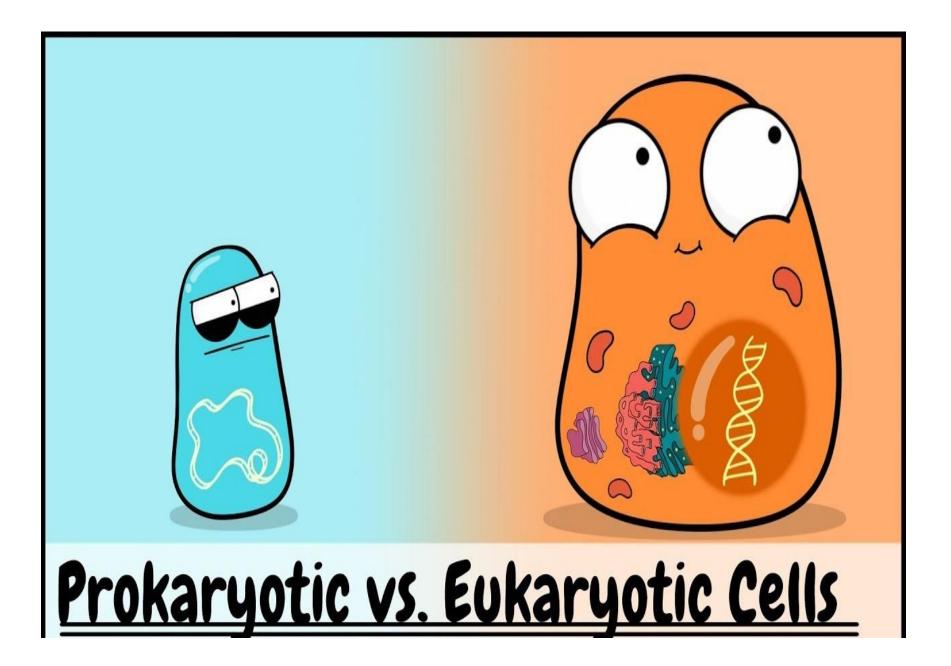
In a prokaryotic cell, the DNA is concentrated in the nucleoid without a membrane separating it from the rest of the cell. 2. Prokaryotic Cells much smaller (microns) and more simple than eukaryotes aod this large surface-to-volume ratio enable in prokaryotes nutrients easily and rapidly reach any part of the cells.



Schematic of typical animal (eukaryotic) cell, showing subcellular components.

Organelles:

(1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough ER
(6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) miachondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrio



Eukaryotic vs. Prokaryotic Cells

Characteristics	Eukaryotic Cells	Prokaryotic Cells
Definition	Any cell that contains a clearly defined nucleus and membrane bound organelles	Any unicellular organism that does not contain a membrane bound nucleus or organelles
Examples	Animal, plant, fungi, and protist cells	Bacteria and Archaea
Nucleus	Present (membrane bound)	Absent (nucleoid region)
Cell Size	Large (10-100 micrometers)	Small (less than a micrometer to 5 micrometers)
DNA Replication	Highly regulated with selective origins and sequences	Replicates entire genome at once
Organism Type	Usually multicellular	Unicellular
Chromosomes	More than one	One long single loop of DNA and plasmids
Ribosomes	Large	Small
Growth Rate/Generation Time	Slower	Faster
Organelles	Present	Absent
Ability to Store Hereditary Information	All eukaryotes have this ability	All prokaryotes have this ability
Cell Wall	Simple: Present in plants and fungi	Complex: Present in all prokaryotes
Plasma Membrane	Present	Present

Size of Bacteria

- Unit of measurement in bacteriology is the micron / micro-meter (µm)
- Bacteria of medical importance
- $0.2 1.5 \ \mu m$ in diameter
- $3-5 \ \mu m$ in length

Shape of Bacteria

- Cocci spherical/ oval shaped (major groups)
- Bacilli rod shaped
- Vibrio comma shaped
- Spirilla rigid spiral forms
- Spirochetes flexible spiral forms
- Actinomycetes branching filamentous bacteria

Arrangement of bacteria: Cocci



Cocci in pair – Diplococcus

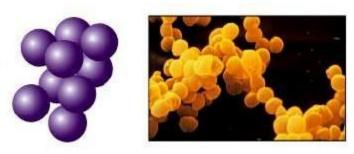


Coccus

Tetrad -

groups of four

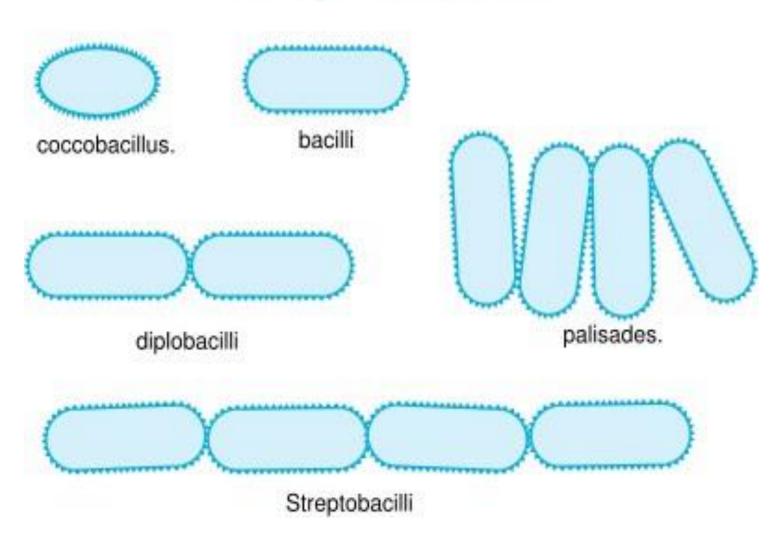
Cocci in chain - Streptococci



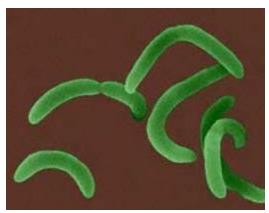
Cocci in cluster - Staphylococci

Arrangement of bacteria: Bacilli

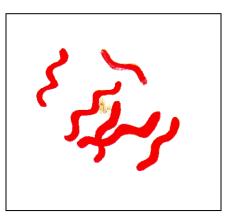
Arrangements of Bacilli



Other shapes of bacteria



Comma shaped



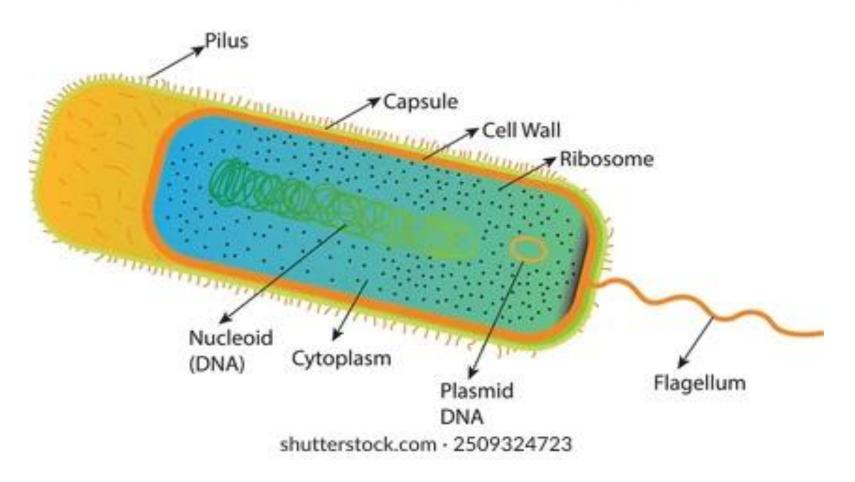
Spirilla



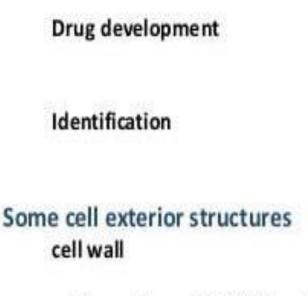
Spirochetes

Anatomy of Bacterial Cell

PROKARYOTIC CELL STRUCTURE



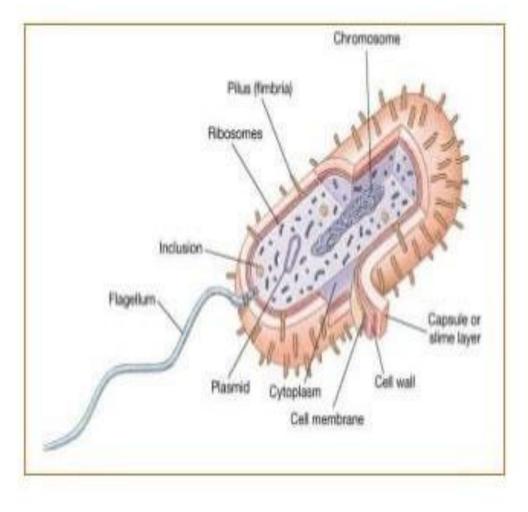
Why study Bacterial Cell Structure? Mechanisms of virulence



cell membrane (lipid bilayer)

capsule

flagellum



pilli

Anatomy of Bacterial Cell

A. Outer layer - two components:

- 1. Rigid cell wall
- 2. Cell membrane or Plasma membrane

B. Cytoplasm – gel like substance contains:

- cytoplasmic inclusions
- ribosomes
- mesosomes
- nucleoid
- C. Additional structures
 - Plasmid
 - slime layer
 - capsule

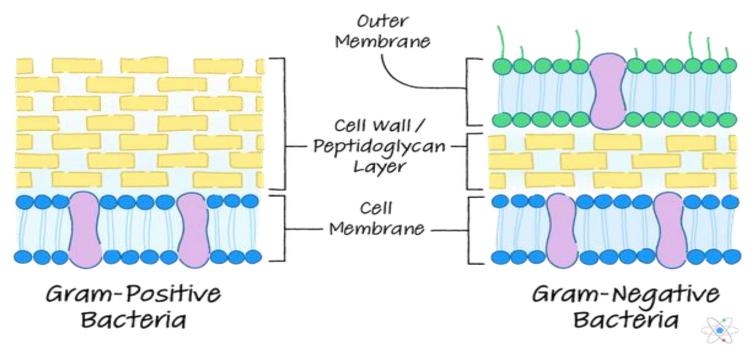
flagella, fimbriae (pili) and spores.

Structure & Function of Cell Components

1. CELL WALL : outermost layer, surrounds cytoplasmic(plasma) membrane

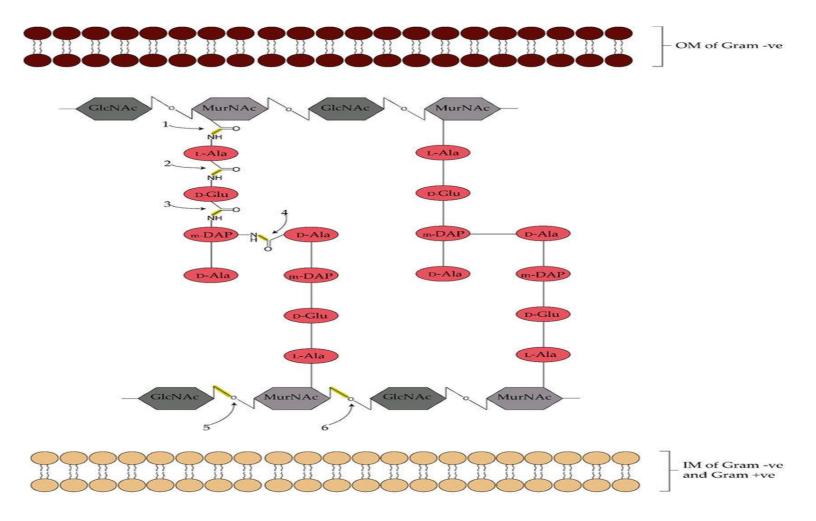
2. Confers shape and rigidity

3. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape.



4. Composed of (peptidoglycan/ murein): formed by N-acetyl glucosamine (NAG) & N-acetyl muramic acid (NAM) alternating

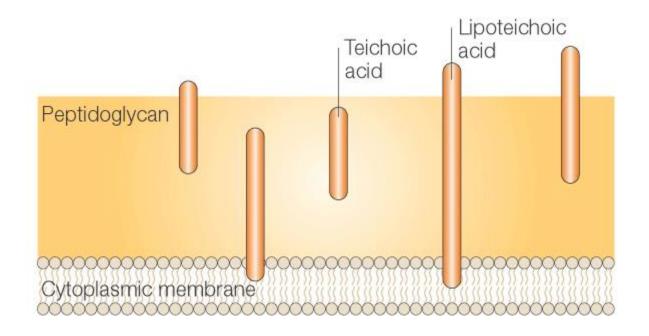
in chains, held by peptide bonds.



- 5. Can not be seen by direct light microscopy and do not stain with simple stains.
- 6. Carries bacterial antigens important in virulence & immunity.
- Chemical nature of the cell wall helps to divide bacteria into two broad groups – Gram positive & Gram negative .
- 8. Gram +ve bacteria have simpler chemical nature than Gram ve bacteria.
- 9. Several antibiotics may interfere with cell wall synthesise.g. Penicillin, Cephalosporins

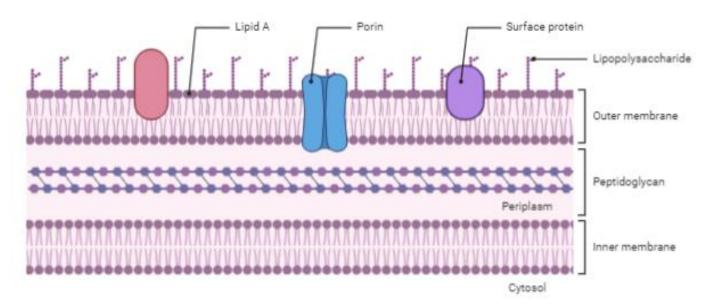
Gram positive cell wall

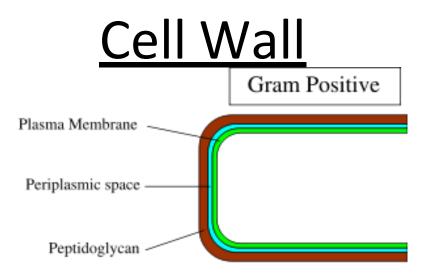
- The Gram-positive cell wall is composed of a **thick**, **multilayered peptidoglycan** sheath outside of the cytoplasmic membrane.
- **Teichoic acids** are linked to and embedded in the peptidoglycan, and **lipoteichoic acids** extend into the cytoplasmic membrane

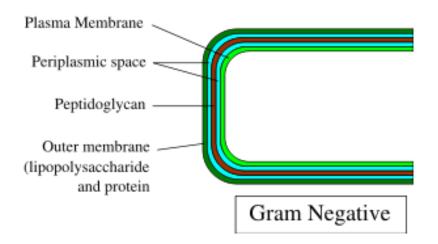


Gram negative cell wall

- The cell wall is composed of an outer membrane linked to thin singlelayered peptidoglycan by lipoproteins.
- The peptidoglycan is located within the periplasmic space that is created between the outer and inner membranes.
- The outer membrane includes *<u>porins</u> allow the passage of small hydrophilic molecules across the membrane and **<u>lipopoly-</u> <u>saccharide</u> molecules that extend into extracellular space.







A comparison between Gram positive and Gram negative cell wall

Gram positive	Gram negative
Thick (multilayered)	Thin (single-layered)
Present	Absent
Absent	present
Virtually none	High
Low	High
	Thick (multilayered) Present Absent Virtually none Low Low Low

G ram reaction

Retain crystal violet dye Can be decolorized

and stain dark violet

to accept

counter

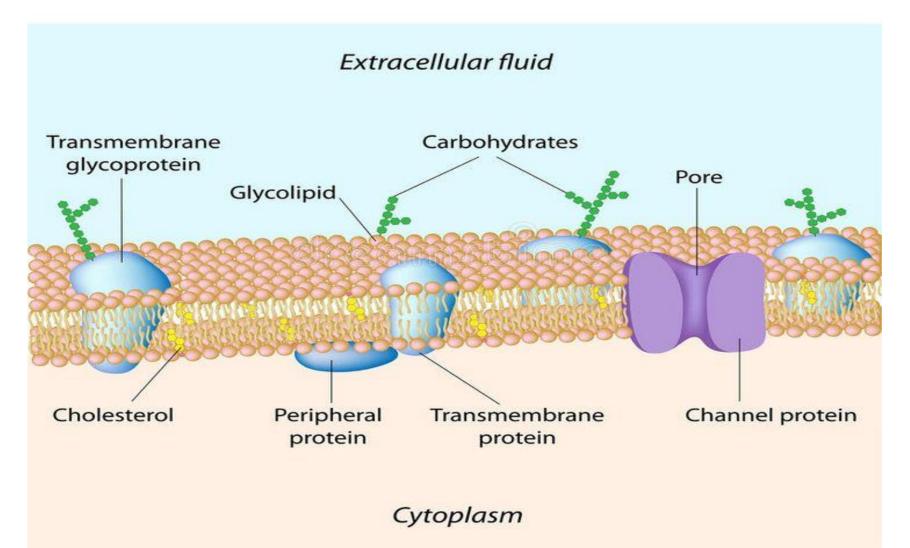
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2. Cytoplasmic (Plasma) membrane

- Thin layer 5-10 nm, separates cell wall from cytoplasm.
- Acts as a semi-permeable membrane controls the inflow and outflow of metabolites.
 - Composed of lipoproteins with small amounts of carbohydrates.

Plasma Membrane Structure

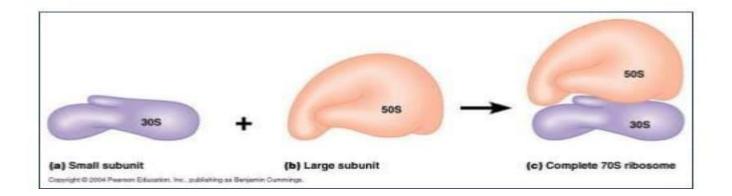


B. Cytoplasm

• Colloidal system of variety of organic & inorganic solutes in viscous watery solution

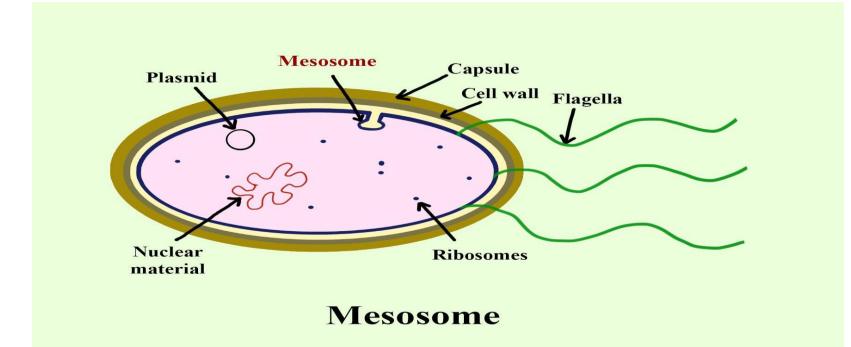
Cytoplasmic Components: • **Ribosomes=RNA**

Ribosome is made up of RNA and protein. It is a factory of protein synthesis



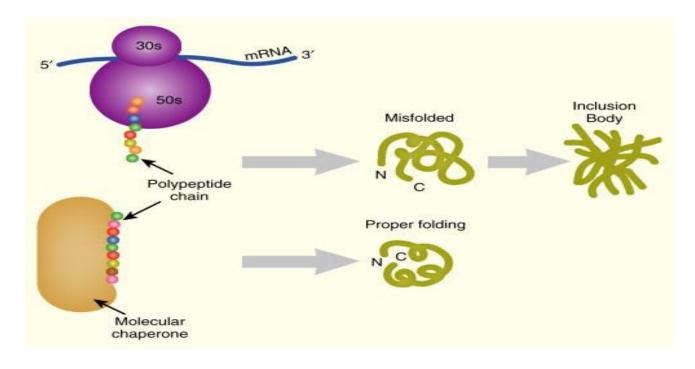
• Mesosomes

- Multi-laminated structures formed as invaginations of plasma membrane.
- Principal sites of respiratory enzymes.
- Coordinate nuclear and cytoplasmic division during binaryfission
- More prominent in Gram +ve bacteria



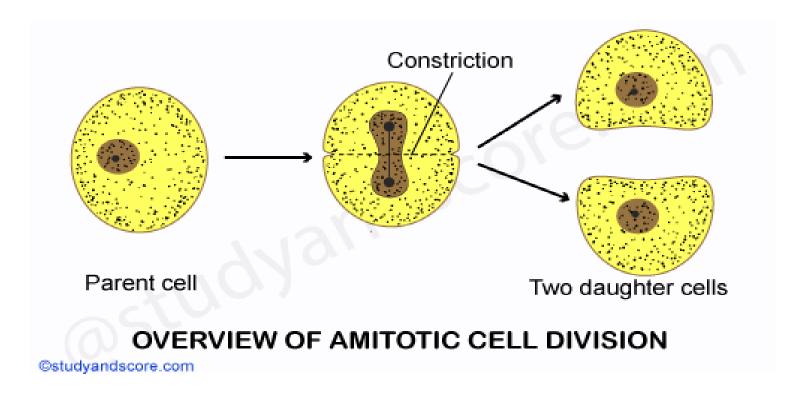
• Intra-cytoplasmic inclusions

- Many species of bacteria produce cytoplasmic inclusion bodies which appears as round granules e.g. metachromatic granules in *(diphtheria bacilli)*.
- Reserve of energy and phosphate for cell metabolism and made up of either glycogen or starch.
- •They appear reddish when stained with polychrome methyleneblue toluidine blue.



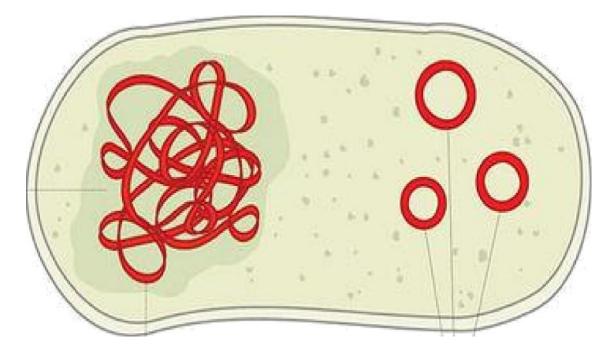
• Nucleus

- No nucleolus and no nuclear membrane.
- Oval or elongated bodies generally 1 per cell.
- Genome single, circular double stranded DNA (one chromosome).
- Divides by **binary fission**



C. Additional Organelles:

- 1. Plasmid
- Extra-nuclear genetic elements consisting of DNA
- Transmitted to daughter cells during binary fission
- May be transferred from one bacterium to another by conjugation
- Not essential for life of the cell
- Confer certain properties e.g. drug resistance because the plasmid carries some genes responsible for drug resistance .



Nucleokl

iromosome (circular) Plasmids

• <u>Viscous layer secreted around the cell wall Polysaccharide /</u> <u>polypeptide in nature</u>

2. Capsule

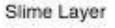
- sharply defined structure, antigenic in nature
- Protects bacteria from lytic enzymes
- Inhibits phagocytosis
- Stained by negative staining using India Ink
- Can be demonstrated by Quellung reaction (capsule swelling reaction)

3.Slime layer – is loosely associated with the bacterium and can be easily washed off, whereas a capsule is attached tightly to thebacterium and has definite boundaries







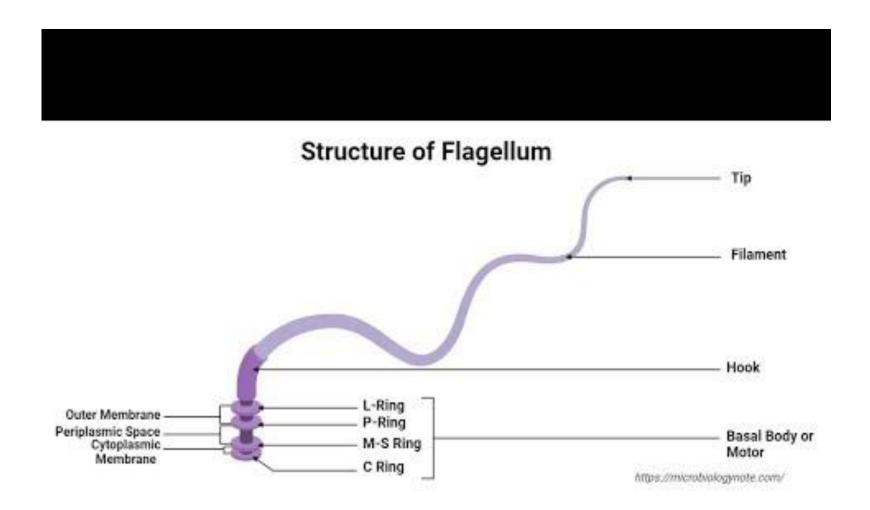


4.Flagella

- Long filamentous surface appendages
- Organs of locomotion
- Composed of proteins called flagellins
- The number and distribution of flagella on the bacterialsurface are characteristic for a given species - hence are useful in identifying and classifying bacteria
- Flagella may serve as antigenic determinants (e.g. the H antigens of flagella in Gram-negative enteric bacteria)
- Presence shown by motility e.g. hanging drop preparation test.

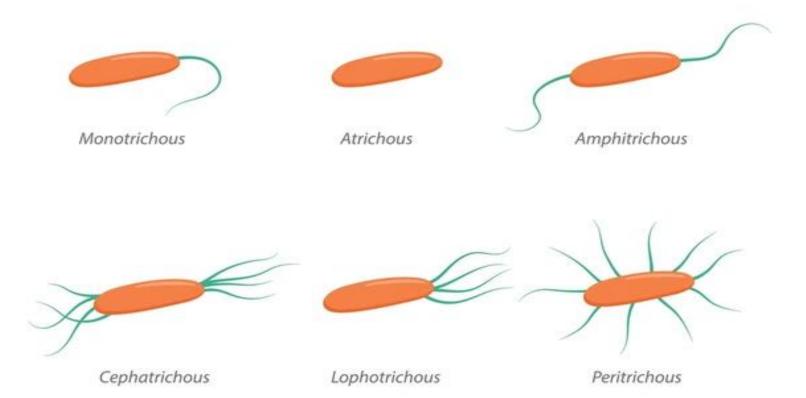
Each flagellum consists of 3 parts:

- 1. Filament
- 2. Hook
- 3. Basal body





Types of flagellar arrangement



4.Fimbriae/ Pili

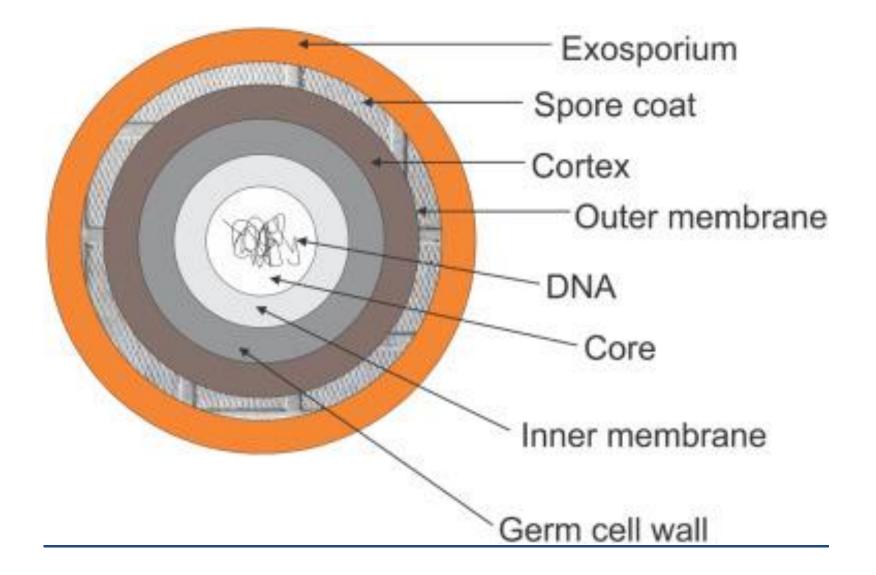
- Thin, hair like appendages on the surface of many Gram negative bacteria
- Acts as organs of adhesion (attachment) -allowing bacteria to colonize environmental surfaces or cells and resist flushing
- Made up of proteins called pilins.
- Pili can be of two types

A.Common pili - short and abundant

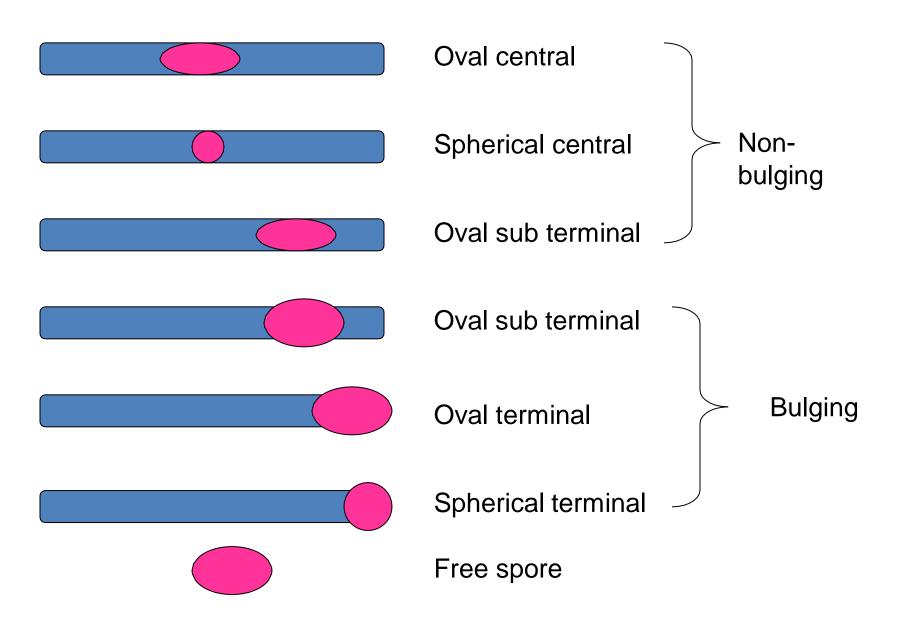
B.Sex pili - very long pili and small number (one to six), helps in conjugation (process of transfer of DNA)

5.Spores :

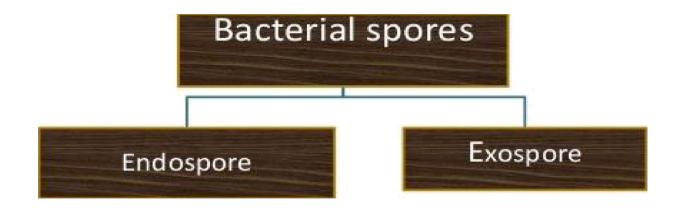
- Highly resistant resting stages formed during adverse environment (depletion of nutrients).
- Formed inside the parent cell, hence called Endospores
- Very resistant to heat, radiation and drying and canremain dormant for hundreds of years.
- Formed by bacteria like *Clostridium* and *Bacillus*



Shape & position of bacterial spore



Œ Bacterial spores undergoes two types of formation —



Endospore;

- Formed inside the parent vegetative cell.
- Endospores are highly durable dehydrated cells, which can survive extreme heat, lack of water, freezing and exposure to many toxic chemicals and radiation.
- Endospores also called as "resting cells".
- Exospore;
- Formed outside the vegetative cell by budding at one end of the cell.
- The Exospore do not contain dipicolinic acid.
- They can resist desiccation and heat.

Dipicolinic acid: is a chemical compound which plays a role in the heat resistance of bacterial endospores