

2nd year Medical Students – JU Bacterial Structure and Morphology

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Bacterial structure and morphology

- Medical microbiology is science of studying micro-organisms that are associated with human disease
- Agents of infection include cellular organisms belonging to two of the three recently defined *domains of life*:

Bacteria (prokaryotes)

Eukarya: fungi and protozoa.

- *The subcellular entities viruses, viroids and prions also cause infection but depend on host cells and tissues for propagation.*

- It is important to understand the basic structural properties and physiology of micro-organisms to establish our approach to infections
- Our understanding of microbial cytology aided by developments in genetic manipulation combined with advances in fluorescence and electron microscopy

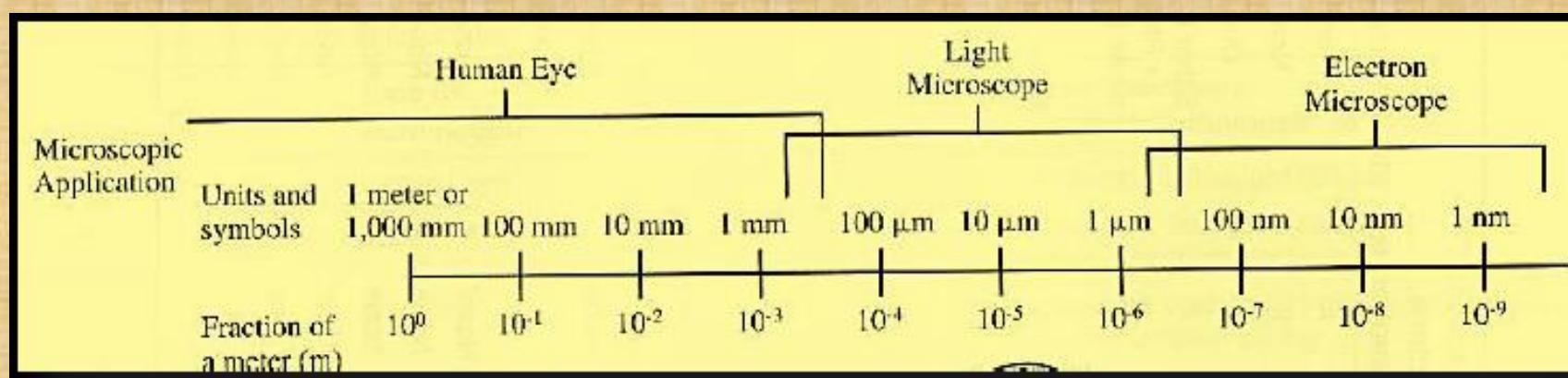
- Micro-organisms are microscopic in size and are usually unicellular.
- The diameter of the smallest body that can be resolved and seen clearly with the naked eye is about 100 μm .
- All medically relevant bacteria are smaller than this and a microscope is therefore necessary to see individual cells

Bacterial cell:

- Prokaryotes
 - No true nucleus
 - No organelles
- Divide-binary fission asexual

- Unit for measurement :Micron or micrometer, μm : $1\mu\text{m}=10^{-3} \text{ mm}$

- Size: Varies with kinds of bacteria, range from 0.2 to 6 μm .



Bacterial structure

- Essential components such as

Cell wall

Cytoplasmic membrane

Ribosome

Nucleoid

- Accessory components (not every bacteria has):

Capsule, Pilus or fimbria, Flagella

Spores, Plasmid, Transposons

Bacterial structure

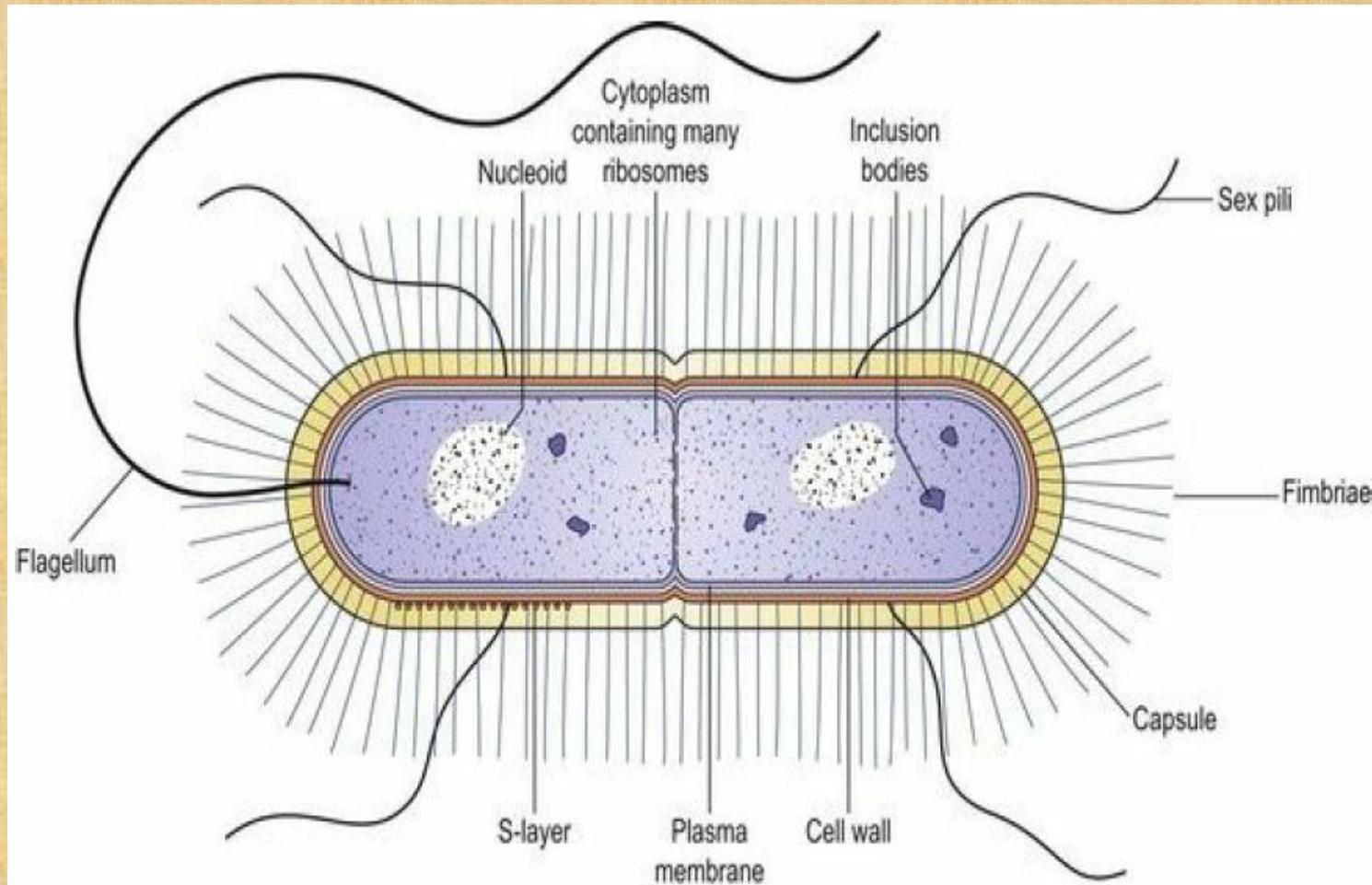


Fig. 2.2 Diagram illustrating the key features of bacterial cells. The S-layer is a variably demonstrated ordered protein layer.

- Cytoplasm is bounded peripherally by a very thin, elastic and semi-permeable cytoplasmic (or plasma) membrane (phospholipid bilayer).
- Outside, and closely covering this, lies the *cell wall, which is rigid, porous and relatively permeable*
- Cell wall and cytoplasmic membrane called collectively the cell envelope

Cytoplasm:

- Is a predominantly aqueous environment
- Contains nucleoid, ribosomes and numerous other protein and nucleotide–protein complexes
- Bacterial cytoplasm have cytoskeletal structures (filamentous proteins and filament systems)
- The importance of these cytoskeletal structures: determining cell shape, **division** and spore formation antimicrobials targeting.

Nucleoid:

- Area of cytoplasm where bacterial DNA is located
- Bacterial chromosome is double stranded circular and supercoiled
- No nuclear membrane as in eukaryotes

Ribosomes:

- Sites of protein synthesis
- They have a sedimentation coefficient of 70S, being composed of a 30S and a 50S subunit (80s in eukaryotes)

Inclusion bodies:

- Food and energy storage granules e.g glycogen and starch

Cytoplasmic (plasma membrane)

- Thin, permeable and elastic membrane
- Composed of phospholipids , **mesosomes**& proteins
- **Functions:**
 - Synthesis of precursors of cell wall polymers and membrane lipids.
 - Selective permeability and active transport of molecules into cells.
 - **Energy generation** by oxidative phosphorylation.
 - Excretion of enzymes and toxins.

Cell wall

Importance:

- Bacterial rigidity and shape
- protection against osmotic changes
- Porous to allow nutrients passage.
- Structure differs in gram positive & negative bacteria.

Cell wall

- In almost all bacteria except Mycoplasma..
 - Many antibiotics (penicillins, and cephalosporins) stop bacterial infections by interfering with cell wall synthesis
 - Has no effects on human cells (no cell wall only a cell membrane).

Cell wall components

Peptidoglycan (syn. mucopeptide or murein):

- *Is an important component of the cell wall of almost all bacteria.*
- Peptidoglycan is composed of:
N-acetylglucosamine and N-acetylmuramic acid molecules linked alternately in a chain (Fig).

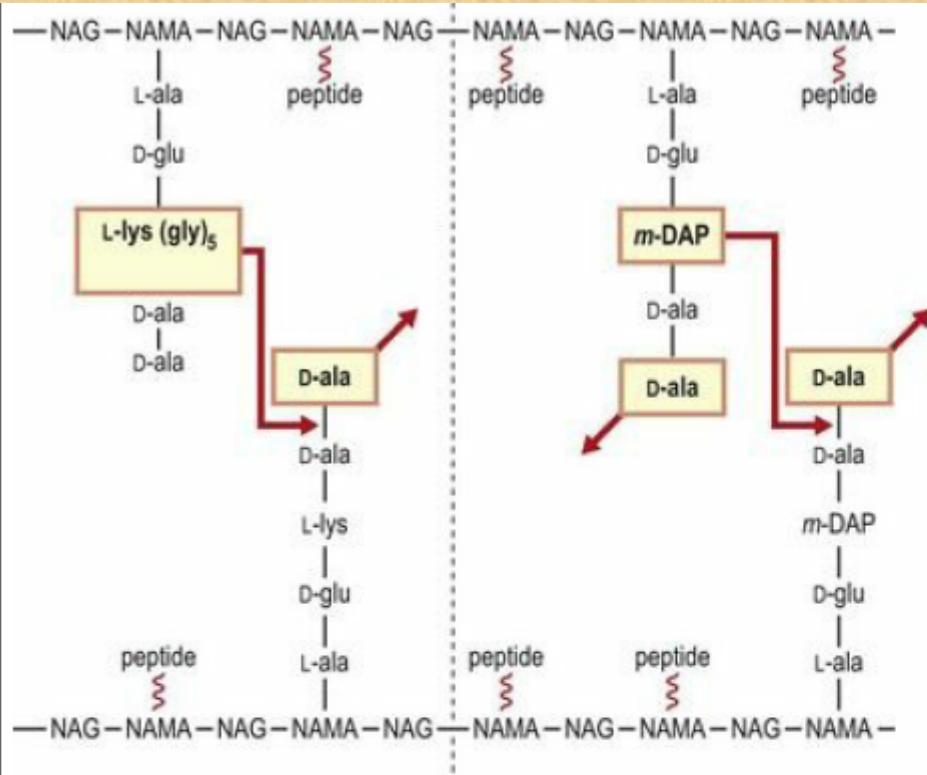
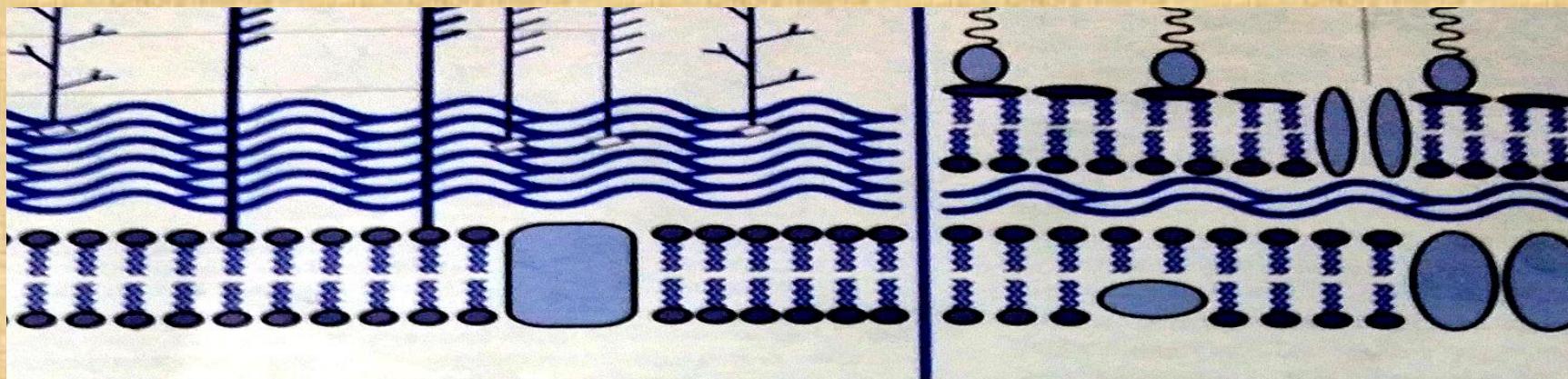
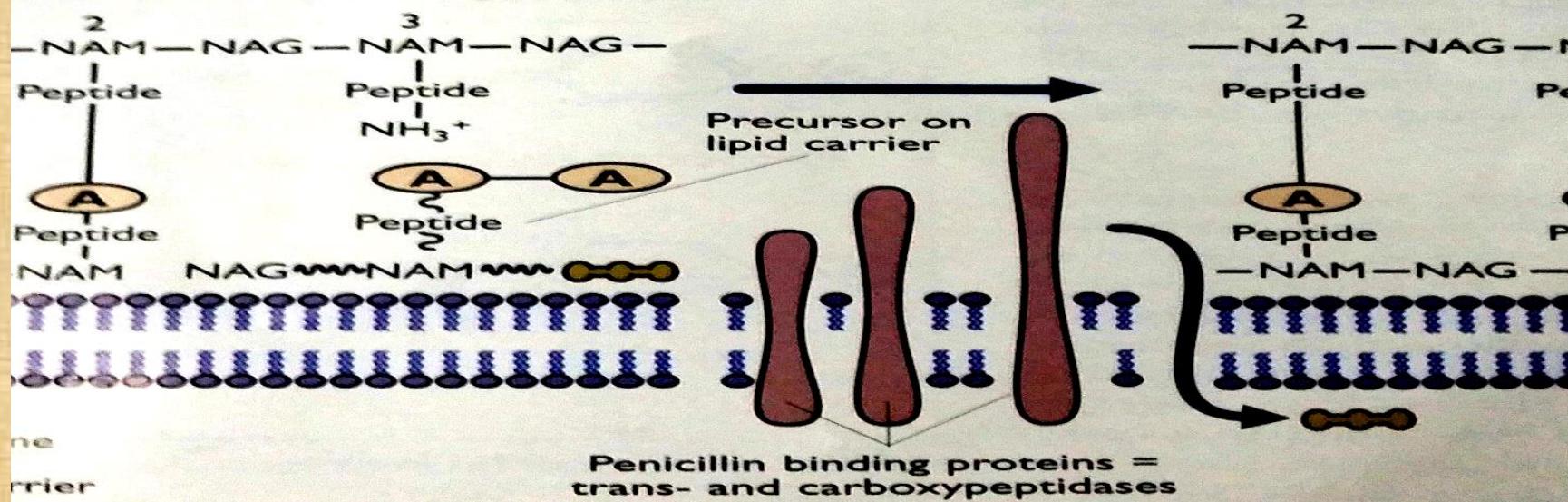


Fig. 2.8 Schematic representation of the peptidoglycan of a representative Gram-positive organism (*Staphylococcus aureus*) and a representative Gram-negative organism (*Escherichia coli*). Note that in the Gram-positive bacterium cross-linking occurs through a peptide bridge (pentaglycine in *Staph. aureus*), whereas direct cross-linking occurs in *E. coli*. In both cases the terminal D-alanine is lost. Not all peptides are engaged in cross-linking in *E. coli*, and carboxypeptidases remove redundant D-alanine residues. NAG, *N*-acetylglucosamine; NAMA, *N*-acetylmuramic acid; *m*-DAP, *meso*-diaminopimelic acid.



Cell wall of gram-positive and gram-negative bacteria.



of repeating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid. Proteins (PBP) are responsible for cross-linking these peptide side chains.

- The thickness of the peptidoglycan is of great practical importance in differentiating medically significant bacteria.
- A Danish physician, Christian Gram, devised a staining procedure that we now know distinguishes bacteria with a thick (Gram-positive) and a thin (Gram-negative) peptidoglycan layer (see Fig).
- The traditional classification of bacteria is basically relying on this method of staining

- Bacterial Shape (morphology) is determined by cell wall and cytoplasmic cytoskeleton
- Following staining; Bacteria is described by gram stain and shape e.g Gram positive cocci, Gram negative rods or bacilli
- Gram-positive species may sometimes appear Gram-negative under certain conditions of growth, especially in ageing cultures on nutrient agar or after exposure to antibiotics or over decolorization.

Gram stain: GO TO LAB

Outline of Gram stain

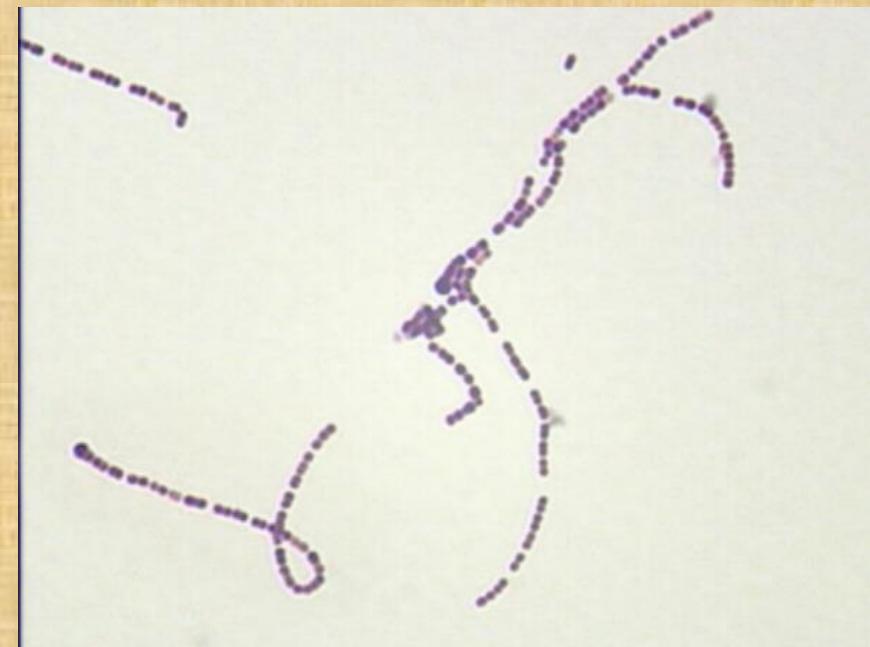
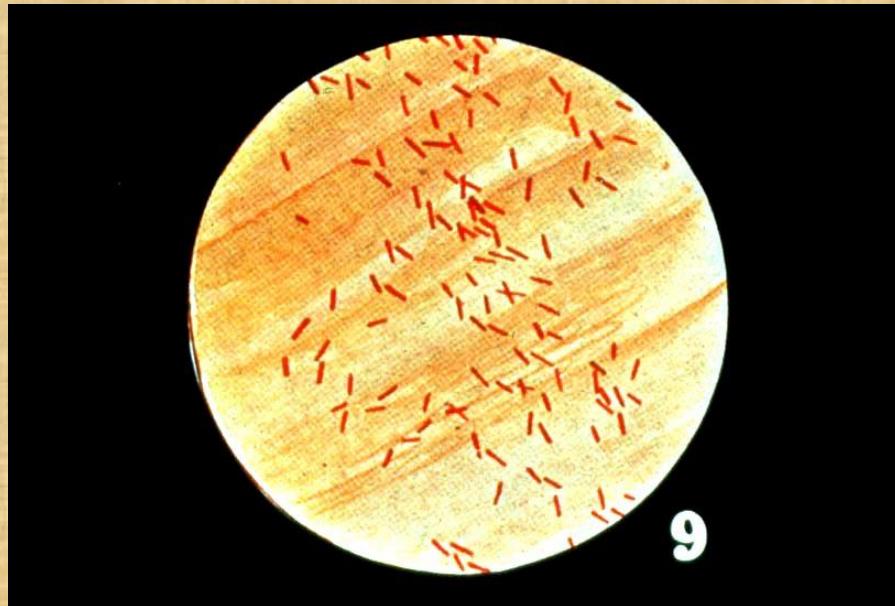
	Gram-positive	Gram-negative
1. Unstained		
2. Crystal violet		
3. Iodine		
4. Decolorize		
5. Red/pink dye		

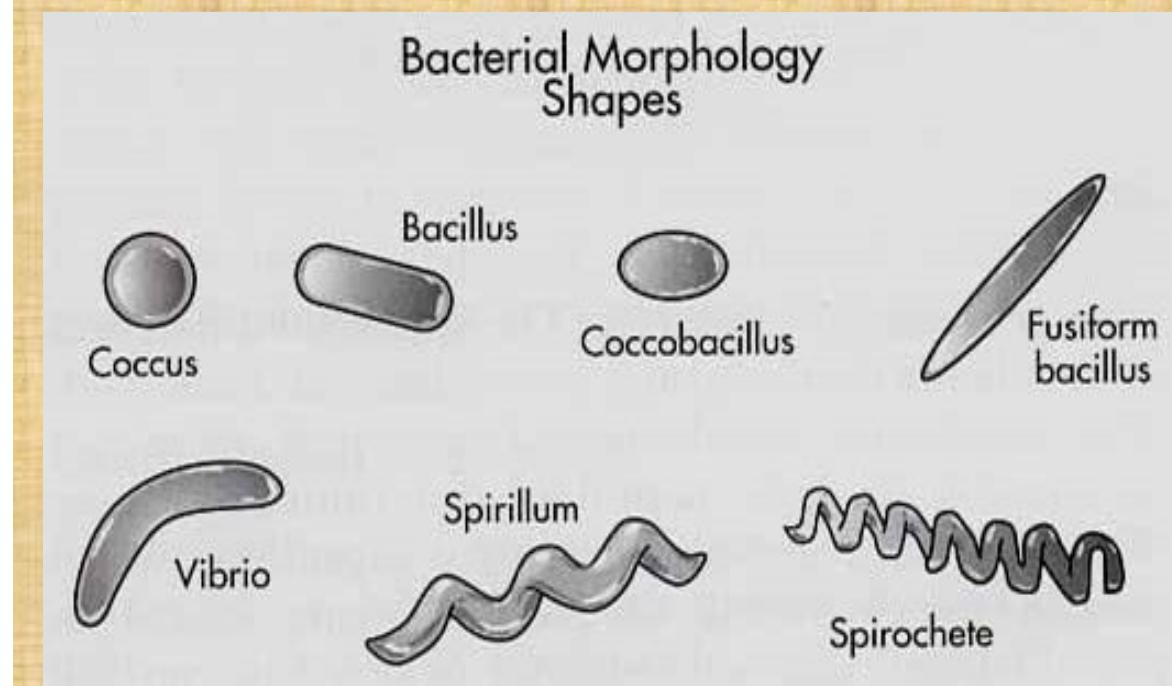
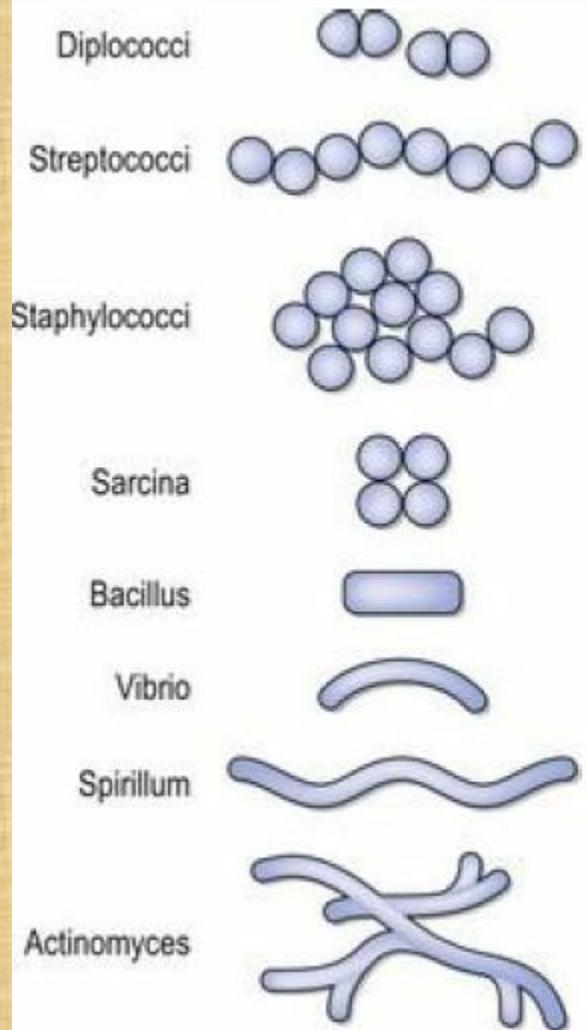
The dye is non-covalently bound to negatively charged molecules (particularly nucleic acids) in the cell. This forms macromolecular complexes with Crystal violet.

The complexes are extracted through the Gram negative wall by solvents such as acetone but retained by Gram positives. A further dye is needed to colourise the unstained Gram negatives.

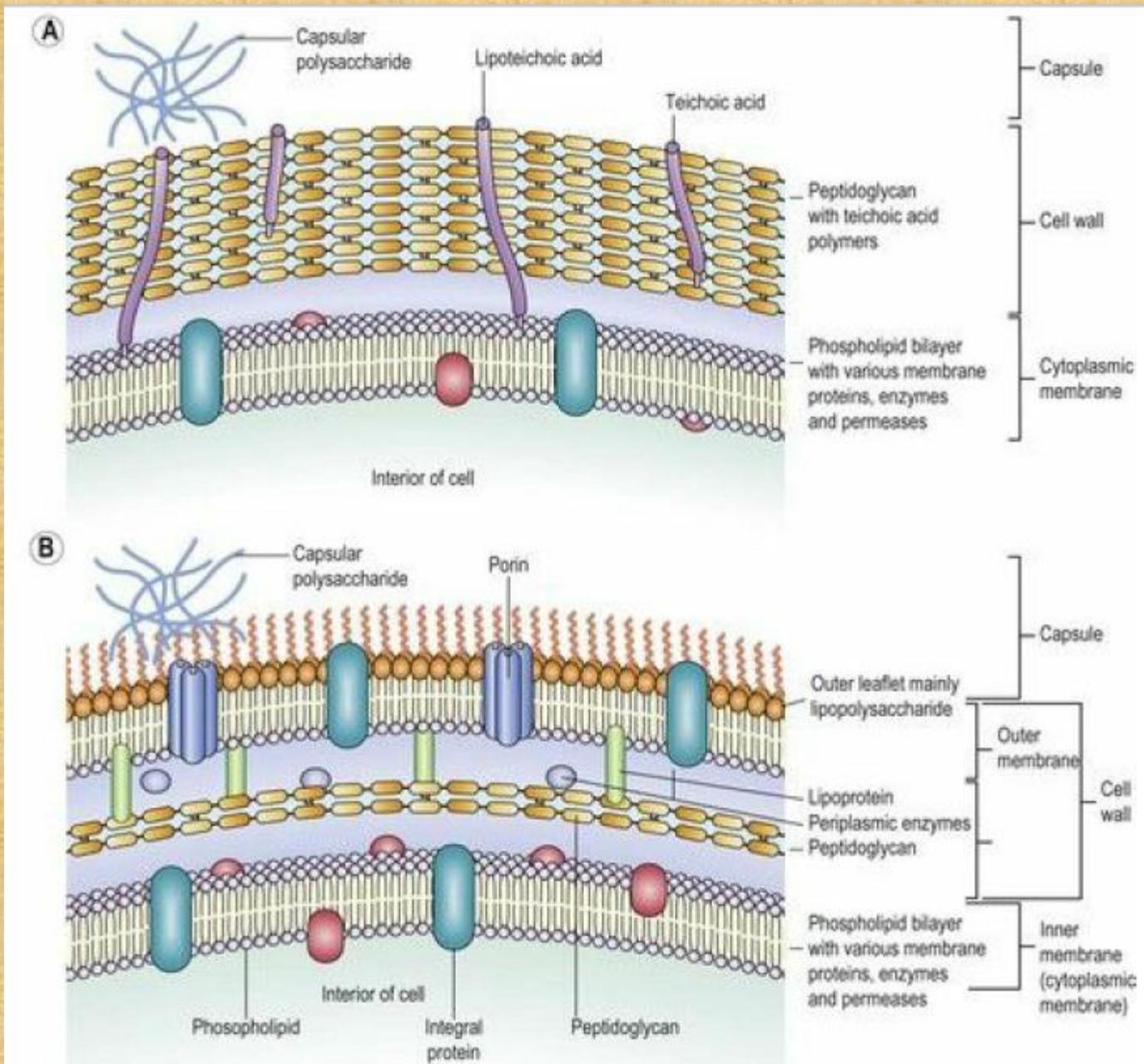
Medical Microbiology

- Gram positive appear violet/blue while gram negative appear pink.
- Importance? Guide your choice of antibiotics.





cell envelop of gram positive (A) and negative bacteria (B)



The gram positive cell wall

- Thick, and the peptidoglycan layer constitutes almost 95% of the cell wall.
- Many Gram-positive bacteria contain relatively large amounts of *teichoic acid* (*a polymer of ribitol or glycerol phosphate complexed with sugar residues*) interspersed with the peptidoglycan.
- Some of this material (*lipoteichoic acid*) is linked to lipids buried in the cell membrane.
- Functions of the Teichoic acids include attachment and antigenic function.

The gram negative cell wall

➤ The peptidoglycan layer is thin constitutes as little as 5-10% of the cell wall

➤ Outer membrane

2 layers of lipids

-Inner layer-phospholipids

-Outer layer-Lipopolysaccharide (endotoxins causing endotoxic shock) consists of 3 regions

1.Lipid A (toxic effect)

2.Core polysaccharide

3.O antigen (antigenic properties)

The gram negative cell wall

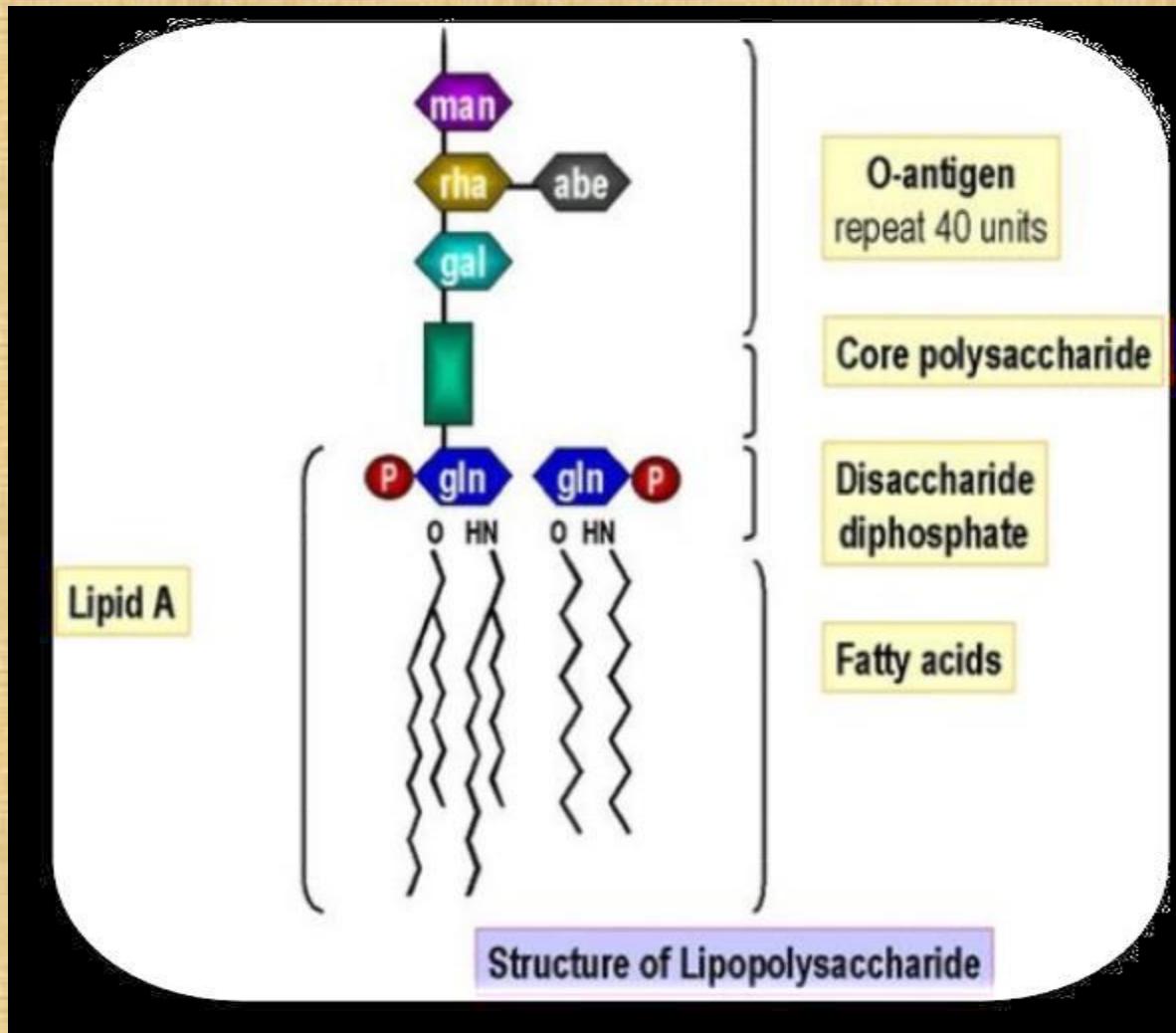
Advantages of outer membrane:

- It protects the peptidoglycan from the effects of lysozyme (a natural body defence substance that cleaves the link between *N-acetylglucosamine* and *N-acetylmuramic acid*).
- It impedes the entry of many antibiotics.

➤ Transmembrane proteins:

- Porins proteins for selective permeability
- Integral proteins that help in attachment

The gram negative cell wall LPS

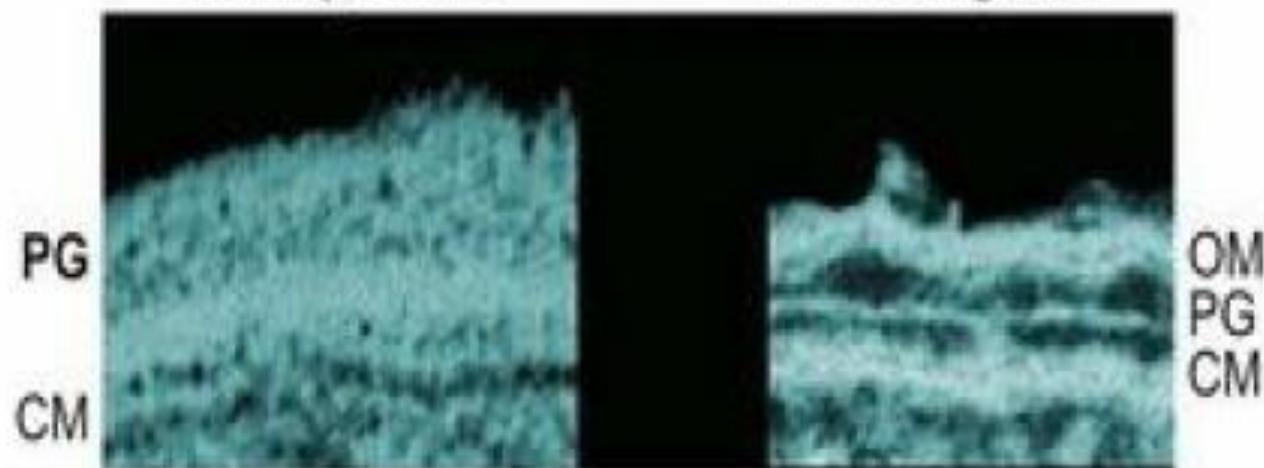


Transmission electron micrograph

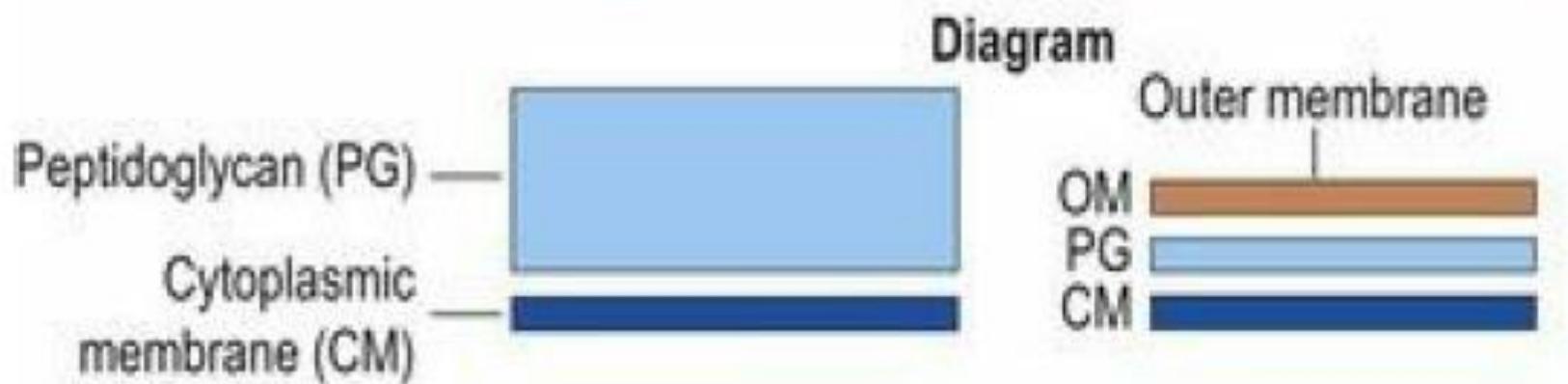
(Colourized negative image)

Gram-positive

Gram-negative



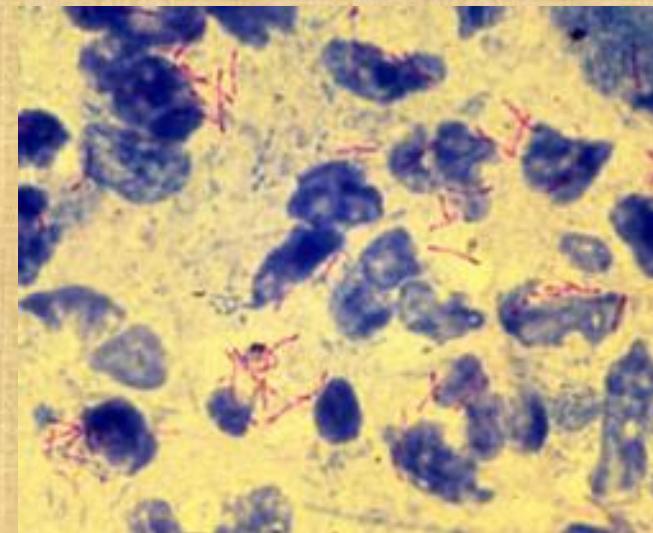
Diagram



- Some bacteria are classified as Gram positive but stain poorly because they have a cell envelop that is rich in hydrophobic lipid mycolic acid

➤ Examples: mycobacterium and corynebacterium

Staining method used: Acid fast stain (Ziehl Neelsen stain)



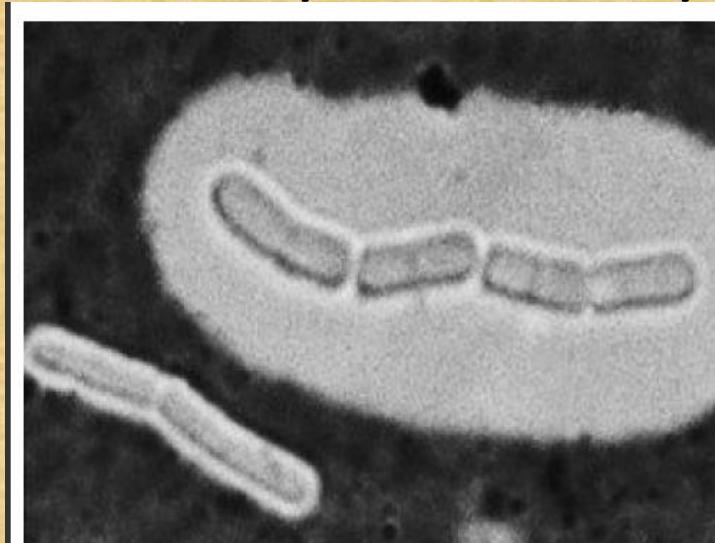
- Some bacteria can not be stained because they are intracellular as *Chlamydiae* & *Rickettsiae*.

Capsule

- Consists largely of water and has only a small content (e.g. 2%) of solids.
- In most bacteria, the solid material is a complex polysaccharide,
- In some bacteria its main constituent is polypeptide e.g *Bacillus*.

Capsule

- Antiphagocytic and protect against lytic action of complement(virulence).
- Adherence (initial step of infection)
- Antigenic (vaccines *Streptococcus pneumoniae*)



- Useful for diagnosis using antiserum against capsular polysaccharide (**quellung reaction**)

Free slime / Glycocalyx

- Polysaccharide coat similar to capsule but secreted extracellularly
- Cover the surfaces like a mucoid film.
- Allow firm adherence to structures e.g.: heart valves, skin, catheters, surface of the teeth(*S. mutans* in dental caries)

Flagella

- A long, filamentous whip-like locomotor appendages
- Originate from cytoplasm and cytoplasmic membrane and protrude via the cell envelop to the surrounding environment
- some bacteria have for movement towards food, cells and other attractants in a process called chemotaxis.
- Flagella consist of a many subunits of protein called flagellin

➤ flagella are important in:

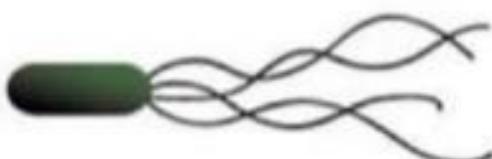
- 1. Identification of Bacteria: specific antibodies against flagellar protein
- 2. Pathogenesis (*E. coli* in *urinary tract infection*)
- 3. Motility of bacteria

fig

Types of flagellar arrangement



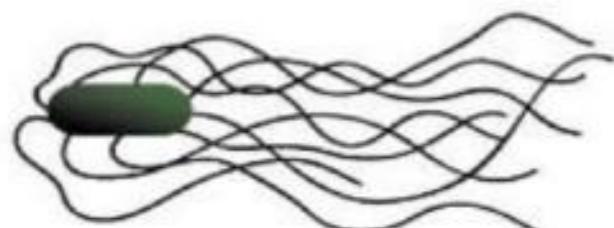
Polar/ Monotrichous – single flagellum at one pole



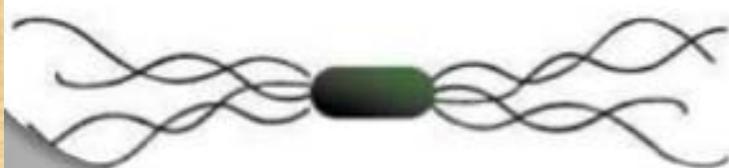
Lophotrichous – tuft of flagella at one pole



Amphitrichous – flagella at both poles



Peritrichous – flagella all over



Amphilophotrichous – tuft of flagella at both ends



Pili

Flagella

Pilli and Fimbriae

- Filamentous appendages made of pilin protein subunit
 - More numerous and straight than flagella
- Fimbriae:
- important in mediating adhesion between the bacterium and host cells
- Pilli:
- Attach specifically to other bacteria that lack these appendages to initiate the process of conjugation (genetic material transfer)
 - also act as receptor sites for certain bacteriophages described as being 'donor specific'

Spores

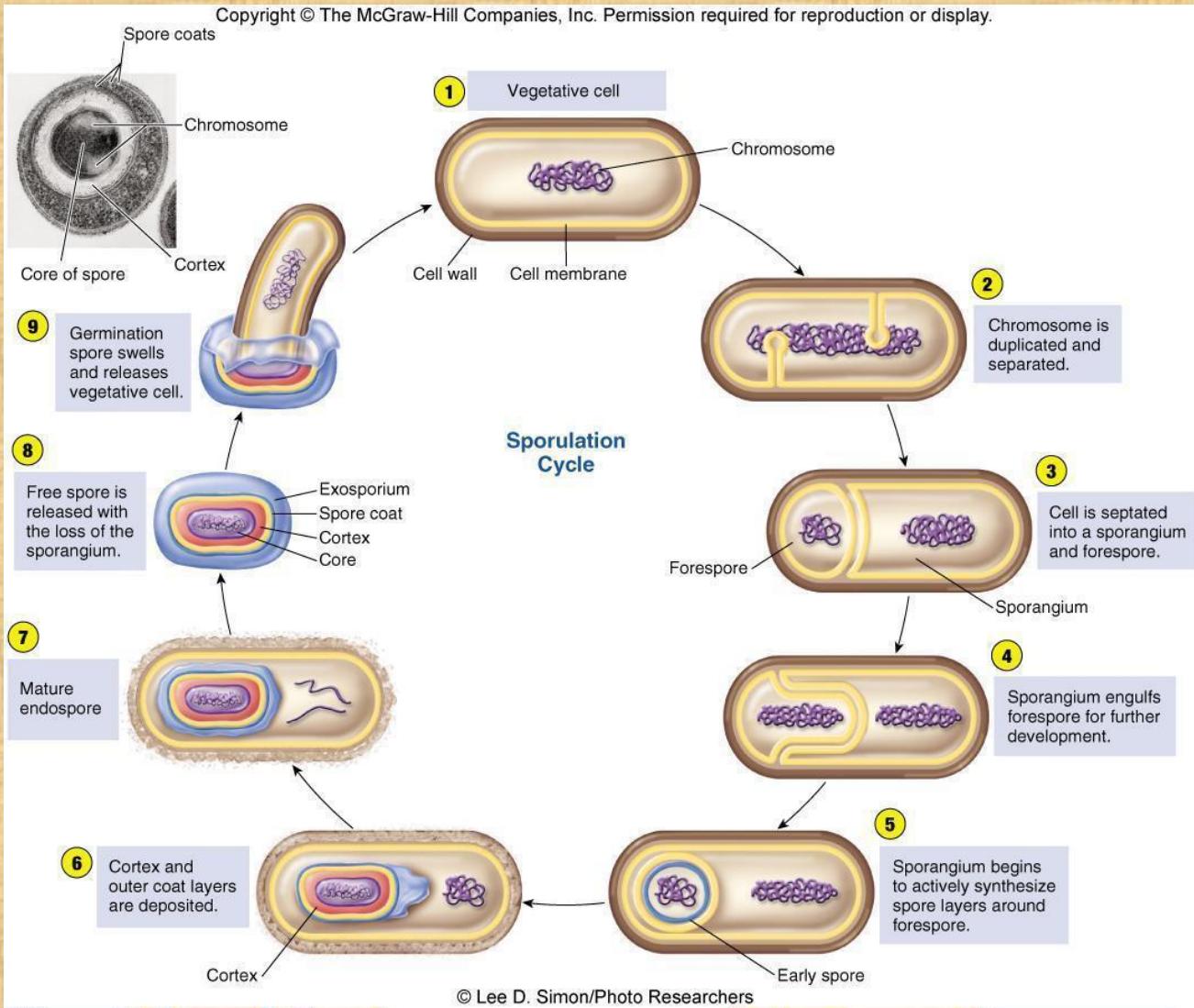
- **Dormant cell**, Highly resistant structures are formed to adverse conditions such as shortage or lack of nutrients.
- John Tyndal

- Resistant to:
 - ✓ Heat, drying irradiation, cold
 - ✓ Boiling >1 hrs till viable

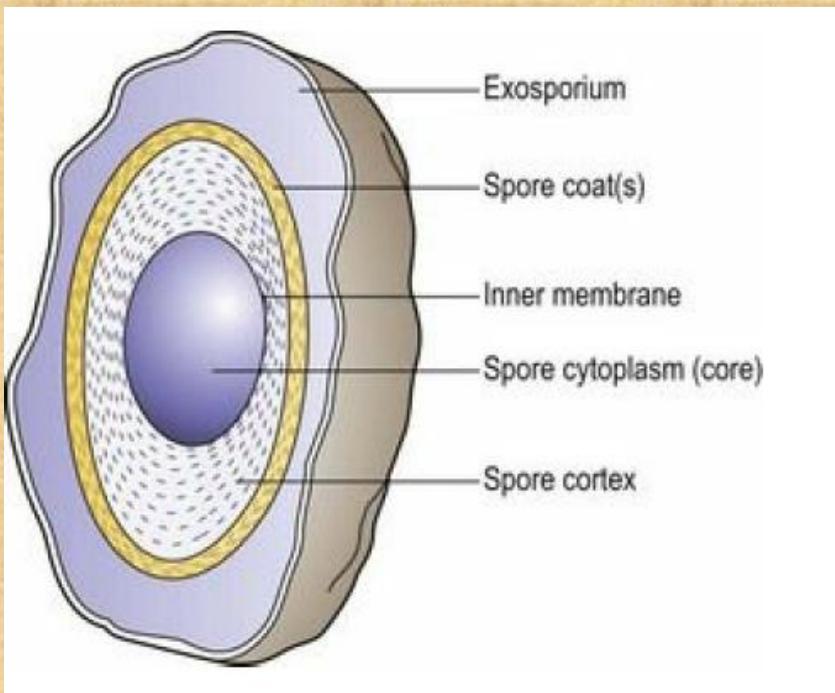
Only moist heat e.g 120 C for 20 minutes will kill them

- Produces by two genera of medical importance: *Bacillus* and *Clostridium*
- Contain calcium dipicolinate and keratin layer
- Stained by different stains e.g **ZN stain, malachite green**
- **Location important in classification**
- Central, Sub-terminal, Terminal
- Sporulation vs germination

Spores



Spores



➤ Plasmid:

- Extra-chromosomal, circular DNA, double-stranded molecule.
- Replicate independent of bacterial chromosome
- Transmissible or non-transmissible plasmids
- contain genes that confer some properties such as antibiotic resistance, virulence factors (exotoxin), genes for pili.
- Plasmids are not essential for cellular survival.

➤ Transposons:

- Pieces of DNA that moves from one site to another either within or between the DNAs of bacteria, plasmids and bacteriophages “Jumping genes”.

The end