2

Microbiology

Doctor 2018 | Medicine | JU

Sheet Slides

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"In the name of God the compassionate the merciful"

*Please note that this sheet is the second **bacteriology** lecture.

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:

1. Bacteria (prokaryotes).
2. Eukarya: fungi and protozoa .

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

Q: what is the **importance** of studying the structure and morphology of bacteria?

A: It is important to understand the basic structural properties and physiology of micro-organisms to establish our approach to infections.

Q: how were we able to understand and know the morphology and structure of bacteria? what methods did we use?

A: Our understanding of microbial cytology aided by **developments in genetic manipulation** combined with advances in **fluorescence** and **electron microscopy.**

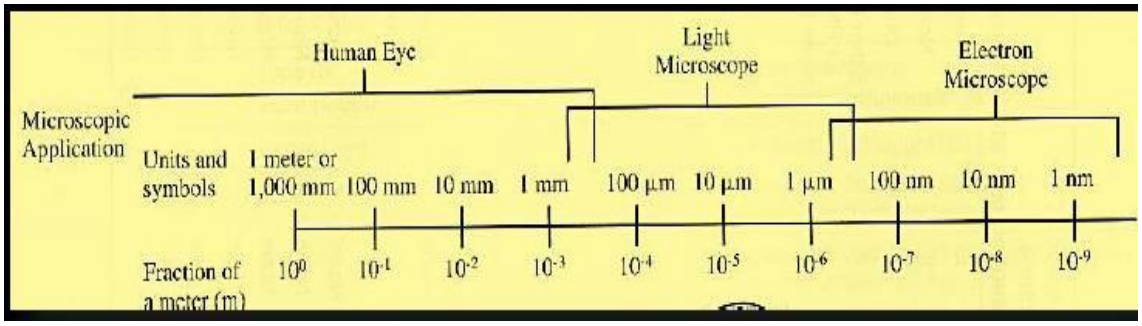
- **Some general information you should know:**

- 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 is a **Prokaryote**, so:

1. No true nucleus
2. No organelles

- Bacteria divide by: binary fission asexual.
- Unit for measurement of bacteria: **Micron** or micrometer, µm: 1µm=10-3 mm

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



- **Essential components of bacteria, such as:**

  1. Cell wall
  2. Cytoplasmic membrane (another name is plasma membrane)
  3. Ribosomes
  4. Nucleoid

- **Accessory components (not every bacterium has):** Capsule, Pilus or fimbria, Flagella, Spores, Plasmid, Transposons.

Bacterial structure: take a general idea

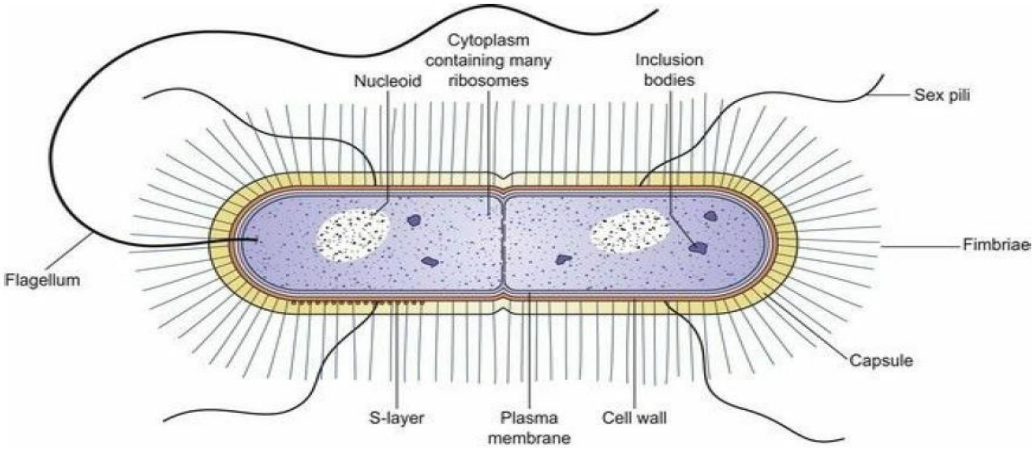


Fig. 2.2 Diagram illustrating the key features of bacterial cells. The S-layer is a variably demonstrated ordered protein layer.

Look to the cytoplasm, nucleoid, pili (hair like structure), flagellum, capsule, cell wall, plasma membrane.

*s-layer is NOT important for us now, and we will not talk about.

Alright, let's start with each structure:

Cytoplasm

- Cytoplasm is bounded peripherally by a very thin, elastic and semi-permeable cytoplasmic (or plasma) membrane (phospholipid bilayer, seen under the electron microscope).
- Outside, and closely covering the plasma membrane, lies the **cell wall**, which is rigid, porous and relatively permeable.
- (Cell wall + Cytoplasmic membrane) =called collectively the **cell envelope.**
- Is a predominantly aqueous environment ,contains nucleoid, ribosomes and numerous other protein and nucleotide–protein complexes.
- Bacterial cytoplasm has cytoskeletal structures (filamentous proteins and filament systems)
- The **importance** of these cytoskeletal structures:
  1. determining cell shape.
  2. division .
  3. spore formation.
  4. 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** (small subunit) and a **50S** (large subunit)."Remember it is 80s in eukaryotes".

Inclusion bodies :

- Food and energy storage granules e.g glycogen and starch (Energy is important for movement, growth, etc.).

Cytoplasmic (plasma membrane):

- Thin, permeable and elastic membrane ,composed of: phospholipids, mesosomes & proteins.

- **Functions** of plasma membrane:

1. Synthesis of precursors of cell wall polymers and membrane lipids.
2. Selective permeability and active transport of molecules into cells.
3. Energy generation by oxidative phosphorylation.
4. Excretion of enzymes and toxins.

Cell wall

Importance:

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

A Danish physician, Hans Christian **Gram**, devised a staining procedure that we now know distinguishes bacteria with a thick (Gram-positive) and a thin (Gram-negative) peptidoglycan layer.

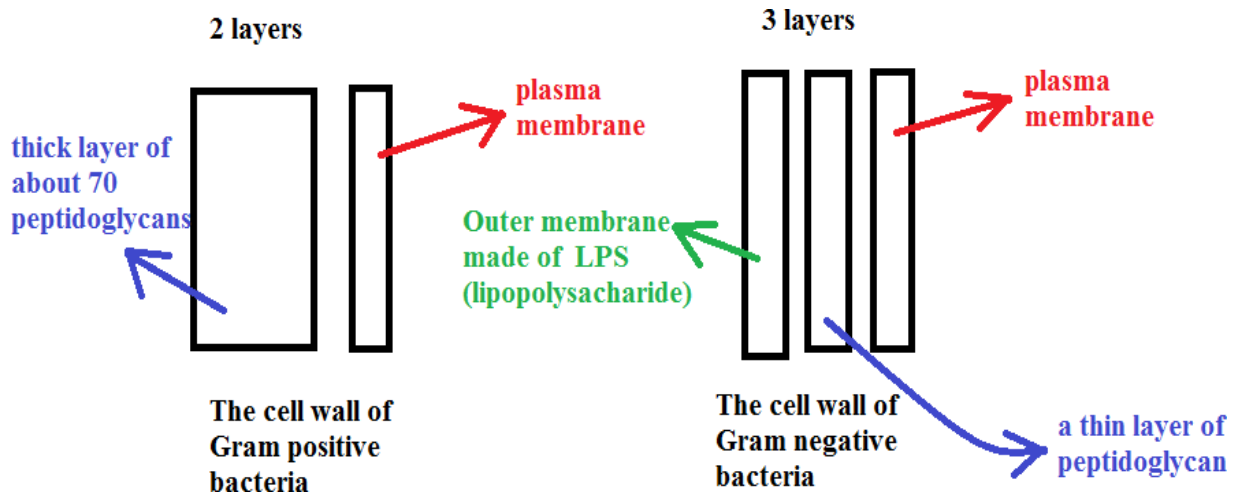
He invented a stain known as **Gram stain**.

In bacteriology, according to Gram, bacteria can either be: Gram-positive bacteria **or** Gram-negative bacteria.

The cell wall structure differs between Gram +ve & Gram -ve bacteria as following:

Comparison	Gram **+ve** bacteria	Gram **-ve** bacteria
Number of layers of bacterial cell envelope	2	3
Thickness of peptidoglycan in the cell wall	***Thick layer***, composed of about 70 peptidoglycans	***Thin*** layer of peptidoglycan
Presence of an outer membrane composed of **LPS** (lipopolysaccharide)	NO	Yes

For better understanding, look at the figure below, which shows the differences in cell wall structure between Gram +ve and Gram -ve bacteria:



- Cell walls are present in almost all bacteria <u>except</u> **Mycoplasma**.
- Many antibiotics (penicillins, and cephalosporins) stop bacterial infections by interfering with cell wall synthesis, and thus, stop the peptidoglycan synthesis.
- Antibiotics have no effects on human cells (because there is no cell wall, only a cell membrane).

Cell wall components

- The main component of cell wall in bacteria is **peptidoglycans.**
- Peptidoglycan (can be also named "synonyms": **mucopeptide** or **murein**): is an important component of the cell wall of almost all bacteria.
- Peptidoglycan is composed of :
  N-acetylglucosamine (NAG) and **N-acetylmuramic acid** (NAMA) molecules <u>linked alternately</u> in a chain (see the figures below).

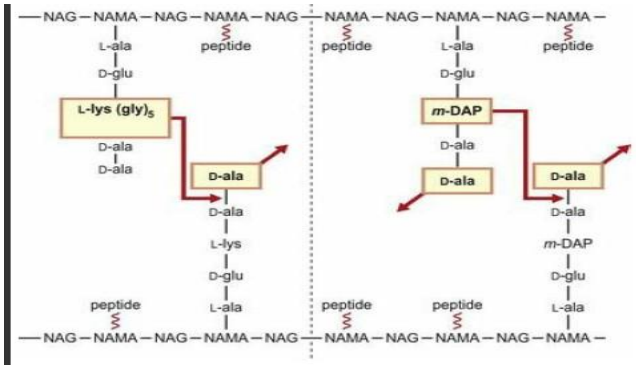
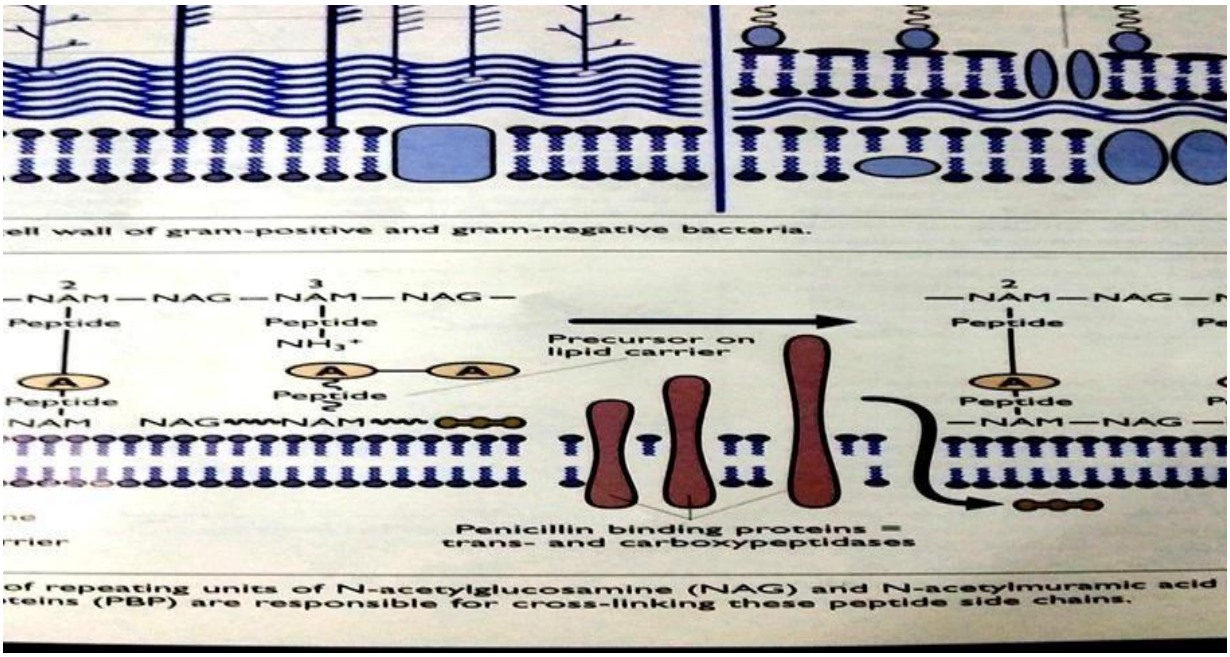


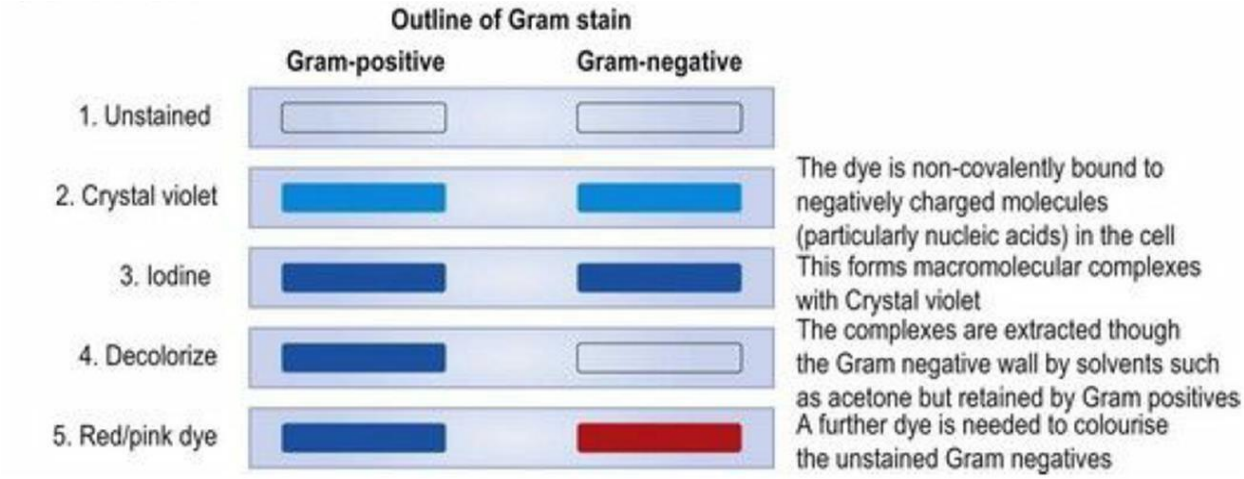
Fig. 2.8 Schematic representation of the peptidoglycan of a representative Gram-positive organism (*Staphylococcus aureus*) and a representative Gram-negative organism (*Escherichia coli*). Note that in the Gram-positive bacterium cross-linking occurs through a peptide bridge (pentaglycine in *Staph. 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.



- The thickness of the peptidoglycan is of great practical importance in differentiating medically significant bacteria. (remember that: +ve Gram bacteria have a thicker layer of peptidoglycan, compared to -ve G).
- Bacterial Shape (morphology) is determined by **cell wall** and **cytoplasmic cytoskeleton**.
- **Following staining**; Bacteria is described by gram stain and shape eg. 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.

- The traditional classification of bacteria is basically relying on this method of staining (see the fig. below).

Gram stain



- Hans Gram used **4 different types of dyes** in order to stain bacteria and to distinguish between the two types (G +ve & G -ve), which are:
    1. Crystal violet (primary stain).
    2. Iodine stain (مثبت لون mordant).
    3. 95% of alcohol (for decolorization).
    4. Counterstain (Safranin stain).
- Depending on the **final color** of bacteria after staining, we can distinguish between G +ve bacteria & G -ve bacteria.
- Now, if the final color was:
    1. **purple** (or **blue**), it is considered to be Gram positive bacteria.
    2. **Pink** (or **reddish in color**), it is Gram negative bacteria.

➔**Procedure of Gram staining:** just for understanding, not for memorizing.

- After doing a bacterial pure culture for 2 different types of unknown bacteria (one is G +ve & the other is G -ve), and putting them in an incubator, we take the petri dishes of the 2 types out, and we pick a bacterial colony of each one. Then:
    1. We take 2 slides and put on each one some drops of distal water.
    2. We pick the first bacterial colony from the first plate and put it on the first slide, then we spread it on this slide. Also, we pick the second colony from the other plate and do the same thing done on the first slide but now on the second slide.
    3. We let them have an air-drying.
    4. We pass each sample quickly on a burner; for fixation. (because the slides will be exposed to a lot of dyes, and after each step we will wash them; so, we need the bacteria sample to be fixed on the slides).
    5. We put Crystal violet (or methylene blue stain) on each slide, and keep it for about 30 seconds.
    6. Washing both slides with water.
       Note: After these steps the color of both samples above are violet (purple).
    7. Then, we put iodine (mordant) on both samples, and we get (crystal violet-iodine) complex.
    8. Washing again with water.
       After these steps the color of both samples above are still violet (purple).
    9. Put 95% of alcohol on both samples; for decolorizing. (It is a **critical step**). Then, we wash it again with water to stop the action of alcohol).

- **NOTE:** After this step (adding 95% of alcohol and washing it quickly with water): The Gram-positive bacteria remain purple. BUT Gram-negative bacteria become colorless (**why?**)
- Remember the difference in cell wall structure between the 2 types of bacteria.

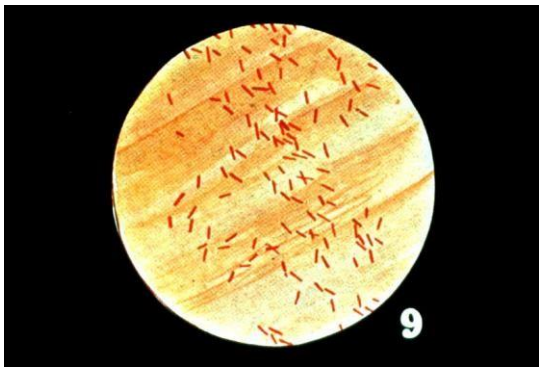
  The Gram -ve have an outer membrane composed of LPS , and this layer will dissolve in alcohol (remember lipids dissolve in alcohol), and the next layer is a very thin layer of peptidoglycan(so, alcohol easily cross through it), and the color is taken out (decolorization happened), so the this sample becomes colorless.

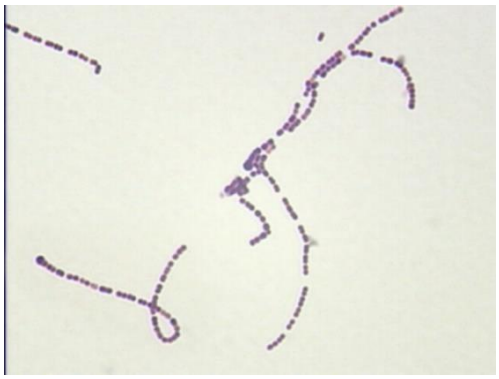
  Whereas, the Gram +ve bacteria, have a very thick layer of peptidoglycan, so alcohol can NOT cross through it, and color remains purple.
- It is a critical step because if we did not wash the slides quickly with water and alcohol remains for a long time, then both samples will become colorless; because alcohol will then also cross the Gram +ve bacteria. (and that is what we don`t want to happen).

10. We put counterstain (Safranin: a reddish or pinkish stain) on both samples, keep it for a short period of time, then wash it with water.

    After adding Safranin, the Gram -ve bacteria will be stained and become red or pink (the color of Safranin). Whereas, the Gram +ve bacteria will stay purple in color (**why?**) because pink cannot mask purple, so pink does not appear (purple is dominant).

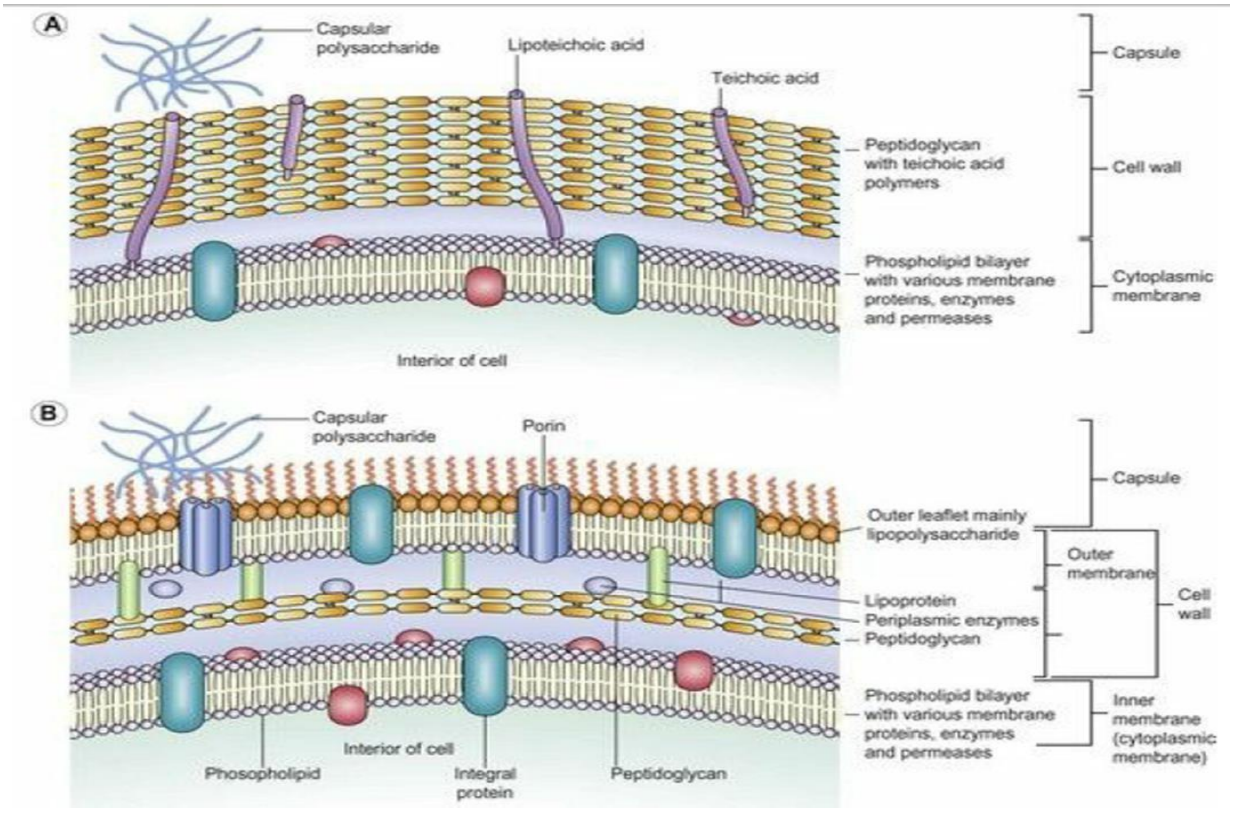
- In the result, Gram positive appear violet/blue (the pic. to the right below) while Gram negative appears pink (the pic. to the left).





- What is the importance of distinguishing between the 2 types?
  It guides your choice of *antibiotics*.
- The **color** and **shape** of bacteria under the microscope both help in identification, so we will be able to know what the cause of the disease is exactly.

cell envelop of gram positive (A) and negative bacteria (B), see the figure below.



More information about Gram positive bacteria cell wall:

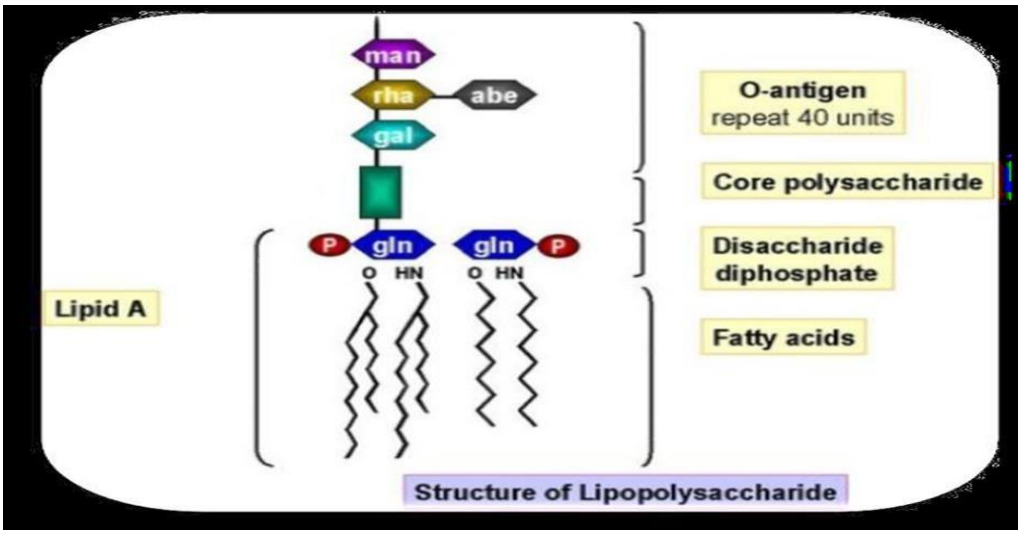
- Thick, and the peptidoglycan layer constitutes almost 95% of the cell wall.
- Many Gram-positive bacteria contain relatively large amounts of **teichoic acid** (a polymer of **ribitol** or **glycerol phosphate** complexed with sugar residues) interspersed with the peptidoglycan.
- Some of this material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

More information about the Gram-negative cell wall:

- The peptidoglycan layer is thin constitutes as little as 5-10% of the cell wall.
- ***Outer membrane*** is consisted of 2 layers of lipids:
    1) **Inner layer**: phospholipids
    2) **Outer layer**: Lipopolysaccharide (**endotoxins** causing endotoxic shock), consists of 3 regions:
        a) Lipid A (toxic effect)
        b) Core polysaccharide
        c) O antigen (antigenic properties)

*Please differentiate above between the outer **membrane** & the outer **layer.**

The gram-negative cell wall LPS



Q: which type of bacteria can cause toxic shock syndrome (TSS)?

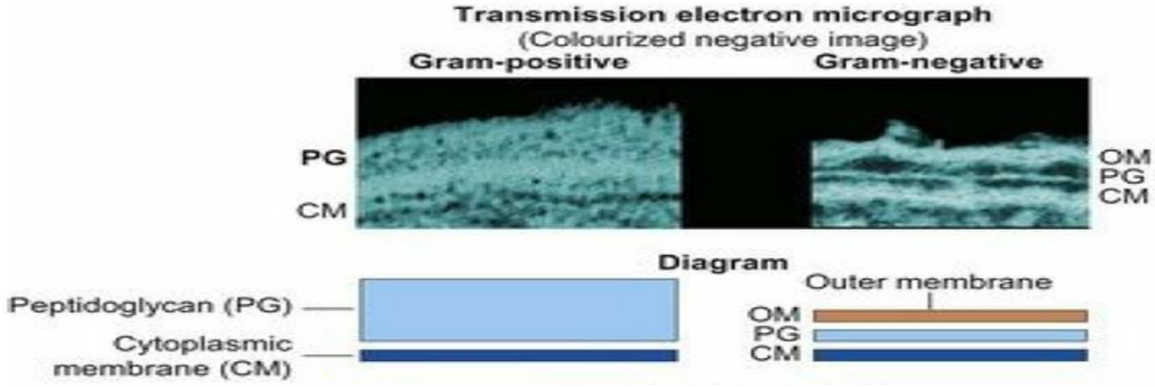
A: gram negative. (why?) because it contains **Lipid A** which has a toxic effect. While it is not present in gram positive bacteria.

Advantages of outer membrane:

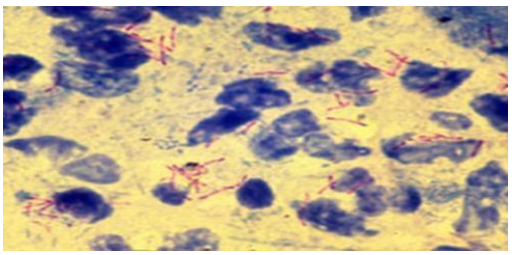
1) It protects the peptidoglycan from the effects of lysozyme (a natural body defense substance that **cleaves** the link between N-acetylglucosamine and N-acetylmuramic acid).
2) It impedes the entry of many antibiotics.

Transmembrane proteins:

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

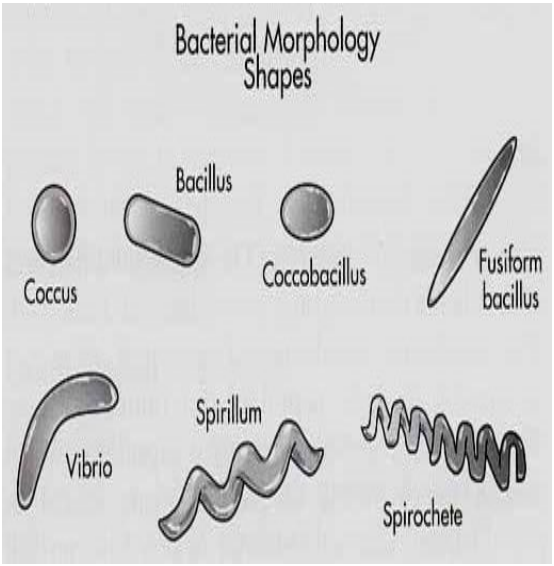


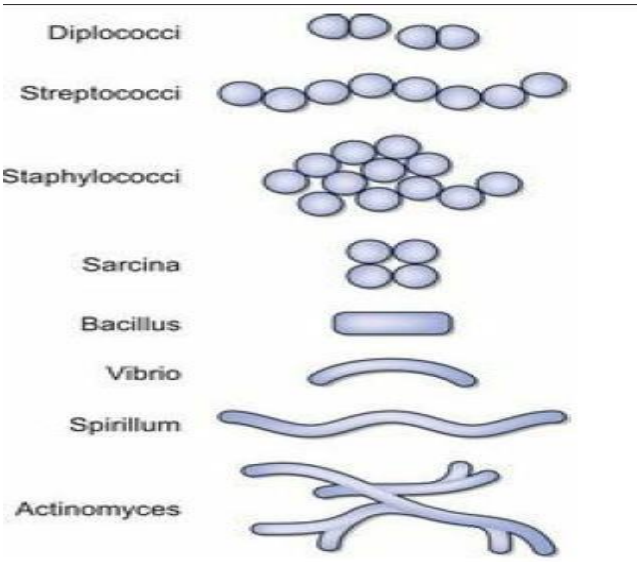
- Some bacteria are classified as Gram positive but stain poorly because they have a cell envelop that is rich in hydrophobic **lipid mycolic acid.**
  So, we can't use Gram stain for it.
- Examples: mycobacterium and Corynebacterium.
- Instead, other staining method used, such as: **Acid fast stain (Ziehl-Neelsen stain).**



- Some bacteria cannot be stained at all because they are <u>intracellular</u> as Chlamydiae & Rickettsiae.

Shapes of bacteria





Coccus: spherical shape.

Bacillus: rods. Vibrio: bent. **Strepto**cocci: **chain** of cocci.

Staphylococci: **cluster** of cocci. Spirillum: spiral structure.

Diplococci: a bacterium that occurs as pairs of cocci (each 2 balls together).

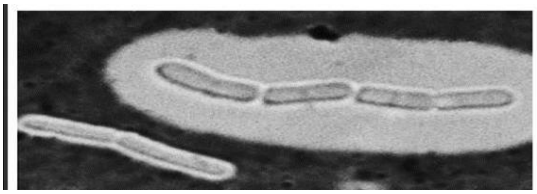
Coccobacillus: has shape in between coccus & bacillus.

ACCESSORY COMPONENTS

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.



Functions and advantages of capsule:

1) Antiphagocytic and protect against lytic action of complement (**virulence**: pathogen's ability to damage or infect a host).
2) Adherence (initial step of infection) (remember that adherence is very important to establish an infection).
3) Antigenic (vaccines Streptococcus pneumoniae) "it can increase our immunity".
4) Useful for diagnosis using antiserum against capsular polysaccharide (quellung reaction).

Free slime/Glycocalyx:

- Free slime is very soft, so it cannot give rigidity or shape, unlike the capsule.
- Polysaccharide coat like capsule but secreted extracellularly.
- Cover the surfaces like a mucoid film.
- Allow firm adherence to structure e.g. heart valves, skin, catheters, surface of the teeth (S. mutans in dental caries).

Flagella:

- A long, filamentous whip-like locomotor(motility) 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 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**.

we should memorize them

Pilli and Fimbriae:

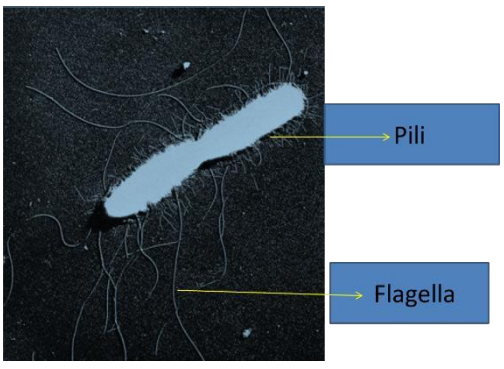
- Filamentous appendages made of pilin protein subunit.
- More numerous and straight than flagella.

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 <u>conjugation</u> (genetic material transfer from one bacterium to another by **sex Pilli**).
- also act as <u>receptor sites</u> for certain bacteriophages described as being 'donor specific'.



GOOD LUCK ^^