Introduction, Bacterial Classification

Classification on the basis of gram stain and Bacterial cell wall

- Of all the different classification systems, the Gram stain has withstood the test of time. Discovered by H.C. Gram in 1884 it remains an important and useful technique to this day.
- It allows a large proportion of clinically important bacteria to be classified as either **Gram positive or negative** based on their morphology and differential staining properties.
- Slides are sequentially stained with crystal violet, iodine, then destained with alcohol and counter-stained with safranin. Gram positive bacteria stain blue-purple and Gram negative bacteria stain red.
- The difference between the two groups is believed to be due to a much larger peptidoglycan (cell wall) in Gram positives. As a result the iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with the Gram negatives where the crystal violet is readily eluted from the bacteria.
- As a result bacteria can be distinguished based on their morphology and staining properties.
- Some bacteria such as mycobacteria are not reliably stained due to the large lipid content of the peptidoglycan. Alternative staining techniques (Kinyoun or acid fast stain) are therefore used that take advantage of the resistance to destaining after lengthier initial staining.

Classification of bacteria on the basis of shape

In the year 1872 scientist Cohn classified bacteria to 4 major types depending on their shapes are as follows:

A) Cocci: These types of bacteria are unicellular, spherical or elliptical shape. Either they may remain as a single cell or may aggregate together for various configurations. They are as follows:

- **Monococcus:**– they are also called micrococcus and represented by single, discrete round Example: *Micrococcus flavus*.
- **Diplococcus:**– the cell of the Diplococcus divides ones in a particular plane and after division, the cells remain attached to each other. Example: *Diplococcus pneumonia*.
- **Streptococcus:** – here the cells divide repeatedly in one plane to form chain of cells. Example: – *Streptococcus pyogenes.*
- **Tetracoccus:** – this consists of four round cells, which defied in two planes at a right angles to one another. Example: – *Gaffkya tetragena. Staphylococcus*: – here the cells divided into three planes forming a structured like bunches of grapes giving and irregular configuration. Example: – *Staphylococcus aureus*.
- **Sarcina:** -in this case the cells divide in three planes but they form a cube like configuration consisting of eight or sixteen cells but they have a regular shape. Example: –*Sarcina lutea*.

B) Bacilli: – These are rod shaped or cylindrical bacteria which either remain singly or in pairs. Example: –

Bacillus cereus.

C) Vibro: – The vibro are the curved, comma shaped bacteria and represented by a single genus. Example: – *Vibro cholerae*.

D) Spirilla: – These type of bacteria are spiral or spring like with multiple curvature and terminal flagella. Example: –*Spirillum volutans*.

Others:-

Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus).

Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

Classification on the basis of Mode of Nutrition

1.Phototrophs:

- Those bacteria which gain energy from light.
- Phototrops are further divided into two groups on the basis of source of electron.
- **Photolithotrophs:** these bacteria gain energy from light and uses reduced inorganic compounds such as H2S as electron source. Eg. *Chromatium okenii*.
- **Photoorganotrophs:** these bacteria gain energy from light and uses organic compounds such as succinate as electron source.

2. **Chemotrophs:**

- Those bacteria gain energy from chemical compounds.
- They cannot carry out photosynthesis.
- Chemotrops are further divided into two groups on the basis of source of electron.
- **Chemolithotrophs:** they gain energy from oxidation of chemical compound and reduces inorganic compounds such as NH3 as electron source. Eg. *Nitrosomonas.*
- **Chemoorganotrophs:** they gain energy from chemical compounds and uses organic compound such as glucose and amino acids as source of electron. eg. *Pseudomonas pseudoflava.*

3. **Autotrophs:**

- Those bacteria which uses carbondioxide as sole source of carbon to prepare its own food.
- Autotrophs are divided into two types on the basis of energy utilized to assimilate carbondioxide. ie. Photoautotrophs and chemoautotrophs.

- **Photoautotrophs:** they utilized light to assimilate CO2. They are further divided into two group on the basis of electron sources. Ie. **Photolithotropic autotrophs** and **Photoorganotropic autotrophs**
- **Chemoautotrophs:** They utilize chemical energy for assimilation of CO2.

4. **Heterotrophs:**

- Those bacteria which uses organic compound as carbon source.
- They lack the ability to fix CO2.
- Most of the human pathogenic bacteria are heterotropic in nature.
- Some heterotrops are simple, because they have simple nutritional requirement. However there are some bacteria that require special nutrients for their growth; known as fastidious heterotrophs.

Classification of bacteria on the basis of temperature Requirement

Bacteria can be classified into the following major types on the basis of their temperatures response as indicated below:

1.Psychrophiles:

- Bacteria that can grow at 0°C or below but the optimum temperature of growth is 15 °C or below and maximum temperature is 20°C are called psychrophiles
- Psychrophiles have polyunsaturated fatty acids in their cell membrane which gives fluid nature to the cell membrane even at lower temperature.
- Examples: *Vibrio psychroerythrus, vibrio marinus, Polaromonas vaculata, Psychroflexus.*

2. **Psychrotrops (facultative psychrophiles):**

- Those bacteria that can grow even at 0°C but optimum temperature for growth is (20-30)°C

3. **Mesophiles:**

- Those bacteria that can grow best between (25-40)^{o} C but optimum temperature for growth is 37C
- Most of the human pathogens are mesophilic in nature.
- Examples: *E. coli, Salmonella, Klebsiella, Staphylococci.*

4. **Thermophiles:**

- Those bacteria that can best grow above 45C.
- Thermophiles capable of growing in mesophilic range are called facultative thermophiles.
- True thermophiles are called as Stenothermophiles, they are obligate thermophiles,

- Thermophils contains saturated fattyacids in their cell membrane so their cell membrane does not become too fluid even at higher temperature.
- Examples: *Streptococcus thermophiles, Bacillus stearothermophilus, Thermus aquaticus.*

5. **Hypethermophiles:**

- Those bacteria that have optimum temperature of growth above 80C.
- Mostly Archeobacteria are hyperthermophiles.
- Monolayer cell membrane of Archeobacteria is more resistant to heat and they adopt to grow in higher remperature.
- Examples: *Thermodesulfobacterium, Aquifex, Pyrolobus fumari, Thermotoga.*

Classification of bacteria on the basis of Oxygen Requirement

Obligate Aerobes:
- Require oxygen to live.
- Example: *Pseudomonas*, common nosocomial pathogen.

Facultative Anaerobes:
- Can use oxygen, but can grow in its absence.
- They have complex set of enzymes.
- Examples: *E. coli*, *Staphylococcus*, yeasts, and many intestinal bacteria.

Obligate Anaerobes:
- Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.
- Examples: *Clostridium* bacteria that cause tetanus and botulism.

Aerotolerant Anaerobes:
- Cannot use oxygen, but tolerate its presence.
- Can break down toxic forms of oxygen.
- Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

Microaerophiles:
- Require oxygen, but at low concentrations.
- Sensitive to toxic forms of oxygen.
- Example: *Campylobacter*.

Classification of bacteria on the basis of pH of Growth

Acidophiles:
- These bacteria grow best at an acidic pH.
- The cytoplasm of these bacteria are acidic in nature.
- Some acidopiles are thermophilic in nature, such bacteria are called Thermoacidophiles.
- Examples: *Thiobacillus thioxidans, Thiobacillus, ferroxidans, Thermoplasma, Sulfolobus*

2. **Alkaliphiles:**
- These bacteria grow best at an alkaline pH.
- Example: *Vibrio cholerae* optimum ph of growth is 8.2.

3. **Neutrophiles:**
- These bacteria grow best at neutral pH (6.5-7.5).
- Most of the bacteria grow at neutral pH.
- Example: *E. coli*

Classification of bacteria on the basis of Osmotic Pressure Requirement

Halophiles:

- Require moderate to large salt concentrations.
- Cell membrane of halophilic bacteria is made up of glycoprotein with high content of negatively charged glutamic acid and aspartic acids. So high concentration of Na+ ion concentration is required to shield the –ve charge.
- Ocean water contains 3.5% salt. Most such bacteria are present in the oceans.
- *Archeobacteria, Halobacterium, Halococcus.*

Extreme or Obligate Halophiles:

- Require a very high salt concentrations (20 to 30%).
- Bacteria in Dead Sea, brine vats.

Facultative Halophiles:

- Do not require high salt concentrations for growth, but tolerate upto 2% salt or more.

Classification of bacteria on the basis of Number of Flagella

On the basis of flagella the bacteria can be classified as:

1. **Atrichos: –** These bacteria has no flagella. Example: *Corynebacterium diptherae*.
2. **Monotrichous:** – One flagellum is attached to one end of the bacteria cell. Example: – *Vibro cholerae*.
3. **Lophotrichous: –** Bunch of flagella is attached to one end of the bacteria cell. Example: *Pseudomonas*.
4. **Amphitrichous:** – Bunch of flagella arising from both end of the bacteria cell. Example: *Rhodospirillum rubrum.*
5. **Peritrichous :** – The flagella are evenly distributed surrounding the entire bacterial cell. Example: *Bacillus.*

Classification of bacteria on the basis of Spore formation

1. **Spore forming bacteria:**

- Those bacteria that produce spore during unfavorable condition.
- These are further divided into two groups:

i) Endospore forming bacteria: Spore is produced within the bacterial cell.
Examples. *Bacillus, Clostridium, Sporosarcina* etc
ii) Exospore forming bacteria: Spore is produced outside the cell.
Example. *Methylosinus*

2. **Non sporing bacteria:**

- Those bacteria which do not produce spores.

Eg. *E. coli, Salmonella.*

References

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More notes to refer point wise:-

Different from parasites and fungi (eukaryotic)

- **Prokaryotic organisms**
  - **simple unicellular organisms**
  - **no nuclear membrane**
  - **no mitochondria**
  - **no Golgi bodies**
  - **no endoplastic reticulum**
- **Complex cell wall**
  - **Gram-positive**
  - **Gram-negative**

Microbial Disease

- **The relationship between many organisms and their diseases is not simple.**
- **Most organisms do not cause a single, well- defined disease, although some do *e.g., Treponema pallidum*--syphilis.**
- **More common for infections to result in many manifestation of disease *e.g., S. aureus*-- endocarditis, pneumonia, skin infections, bone infections, sepsis, food poisoning.**

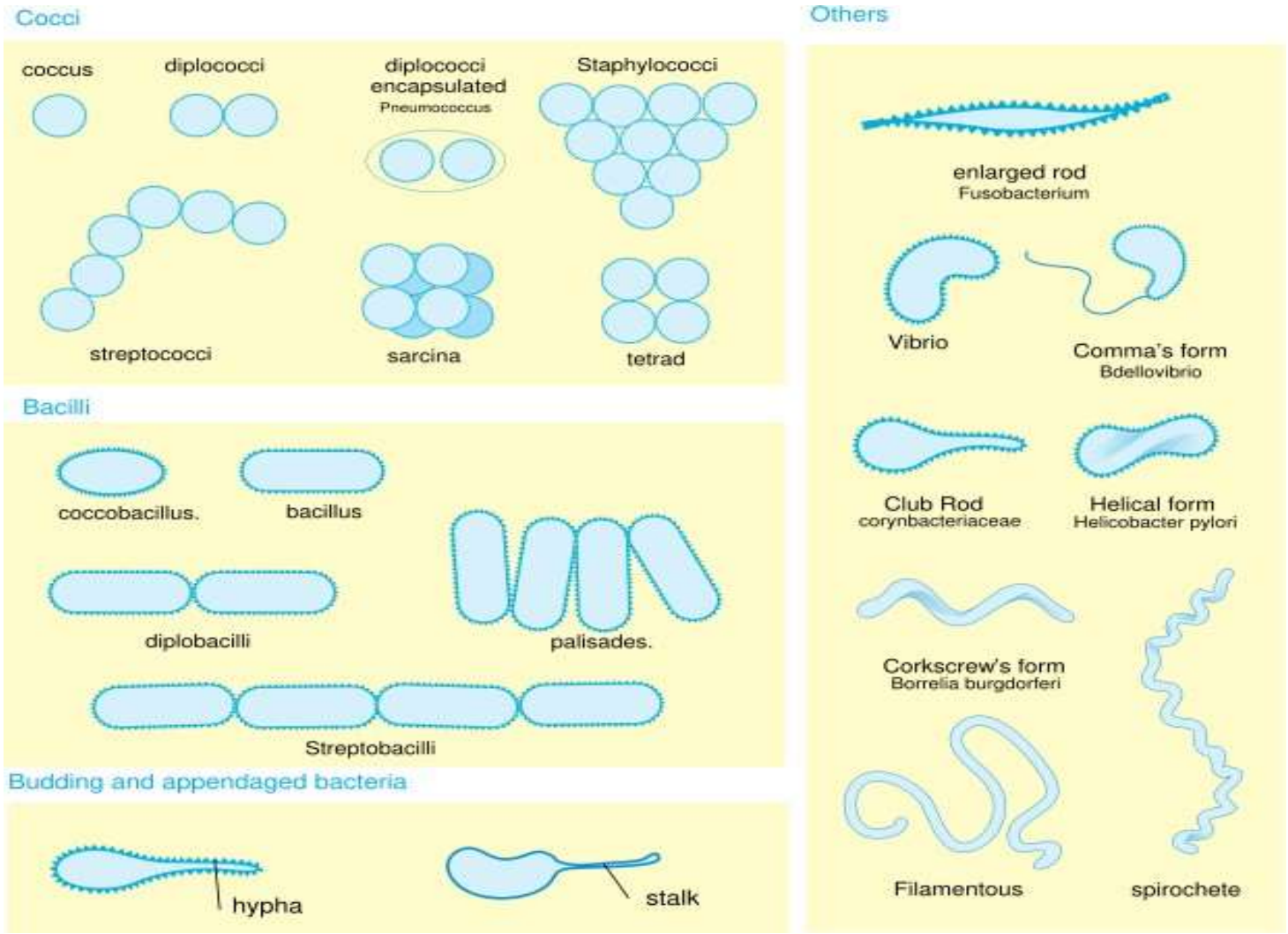
Bacterial Classification

- **Phenotypic**
- **Analytic**
- **Genotypic**

Phenotypic Classification

- Microscopic morphology
  - Gram stain, shape *i.e.*, rods (bacillus), spheres (cocci), curved or spiral, size
- Macroscopic
  - Hemolytic properties on agar containing blood, pigmentation of the colonies, size and shape of colonies, smell and color.
- Serotyping
  - Antibody reactivity to specific antigens
- Antibiogram patterns
  - Susceptibility to antibiotics
- Phage typing
  - Susceptibility to viruses that infect bacteria-- bacteriophages

Bacterial Morphologies


Cocci
coccus
diplococci
diplococci encapsulated
Pneumococcus
Staphylococci
streptococci
sarcina
tetrad
Bacilli
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bacillus
diplobacilli
palisades.
Streptobacilli
Budding and appendaged bacteria
hypha
stalk
Others
enlarged rod
Fusobacterium
Vibrio
Comma's form
Bdellovibrio
Club Rod
corynbacteriaceae
Helical form
Helicobacter pylori
Corkscrew's form
Borrelia burgdorferi
Filamentous
spirochete

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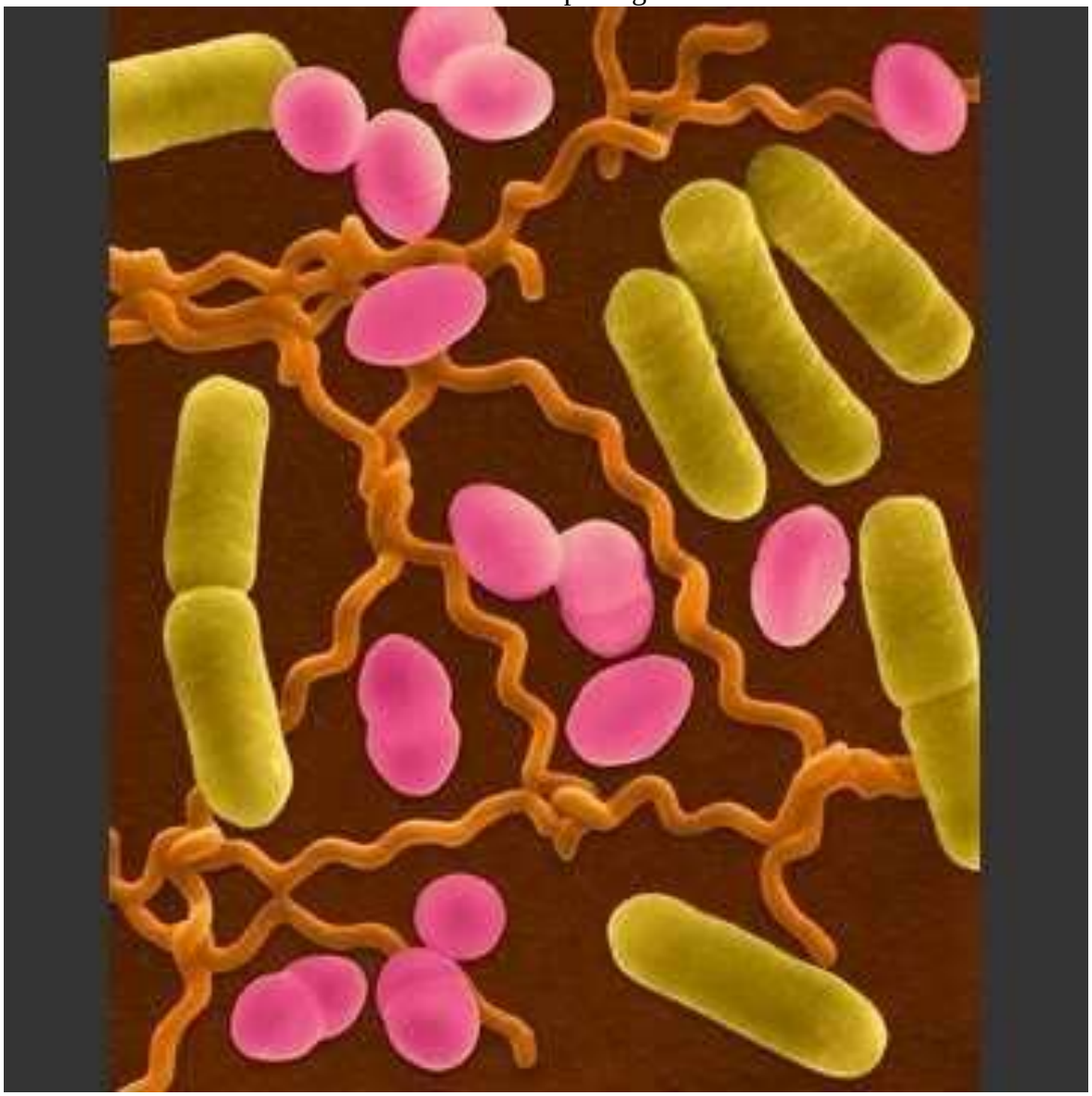

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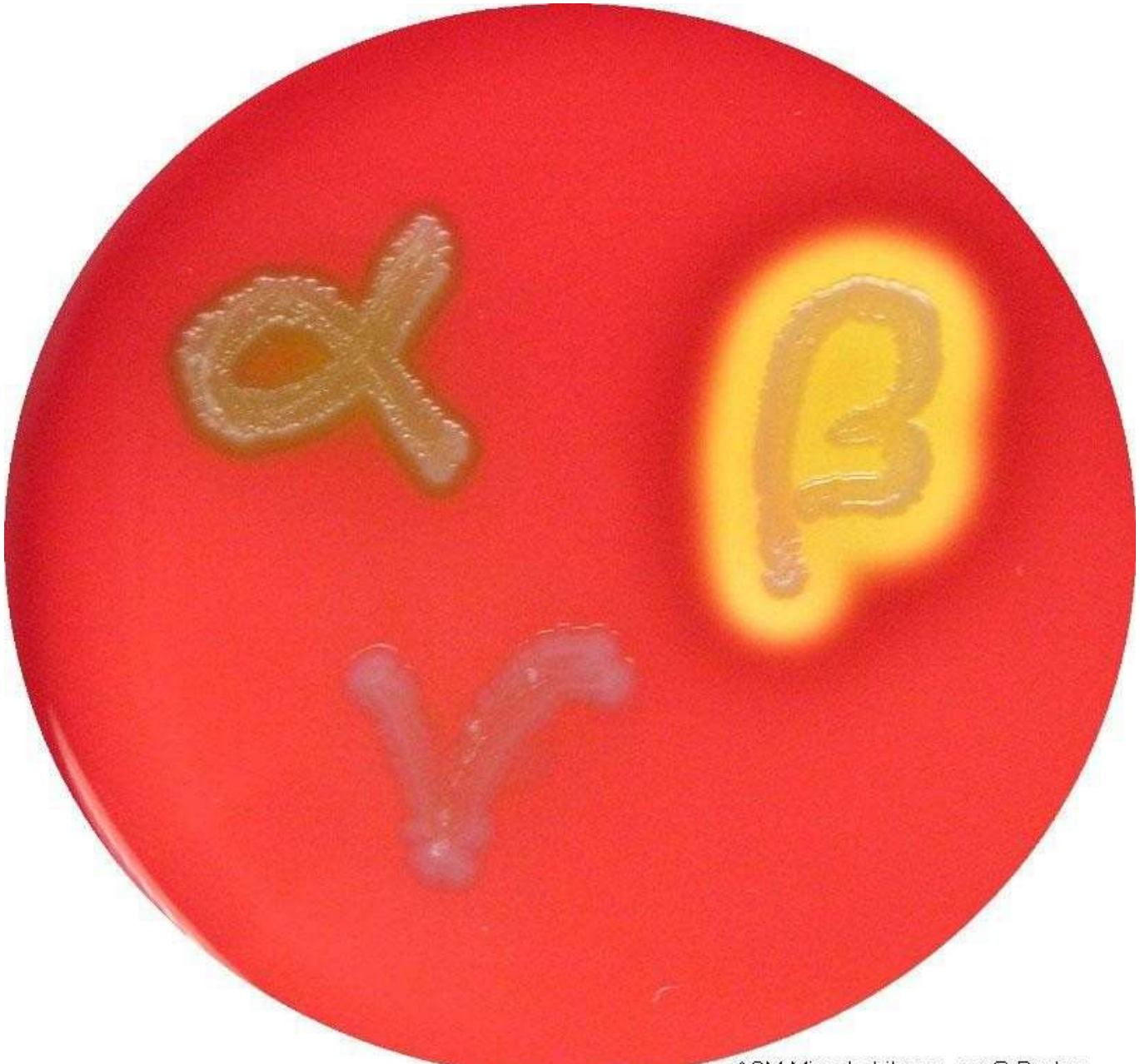
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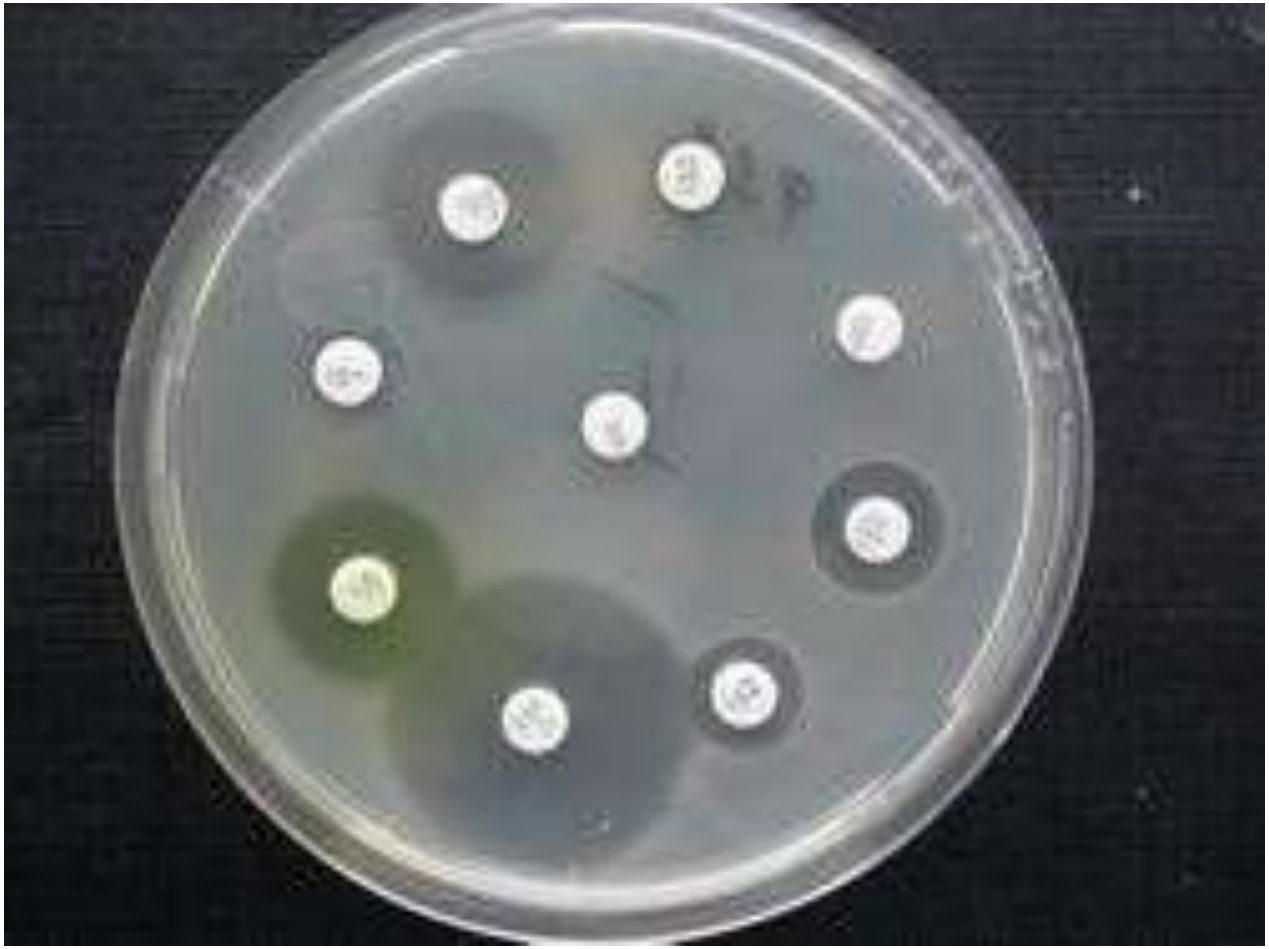
Antibiogram patterns



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Analytic Classification

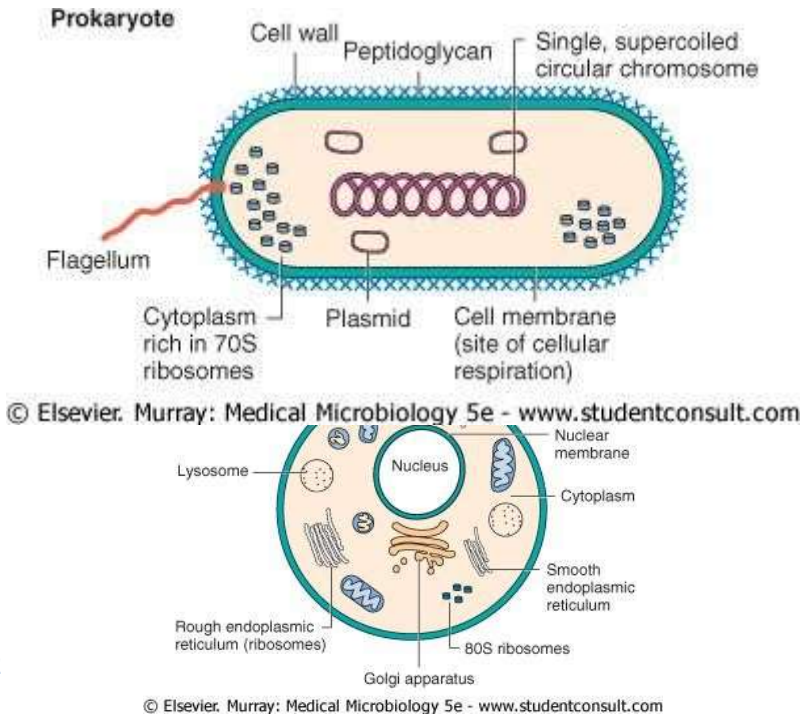
- **Chromatographic pattern of cell wall mycolic acids**
- **Lipid analysis**
- **Proteomic analysis**
  - **These techniques are labor intensive**
  - **Require expensive equipment**
  - **Used primarily in reference laboratories**

Genotypic Analysis

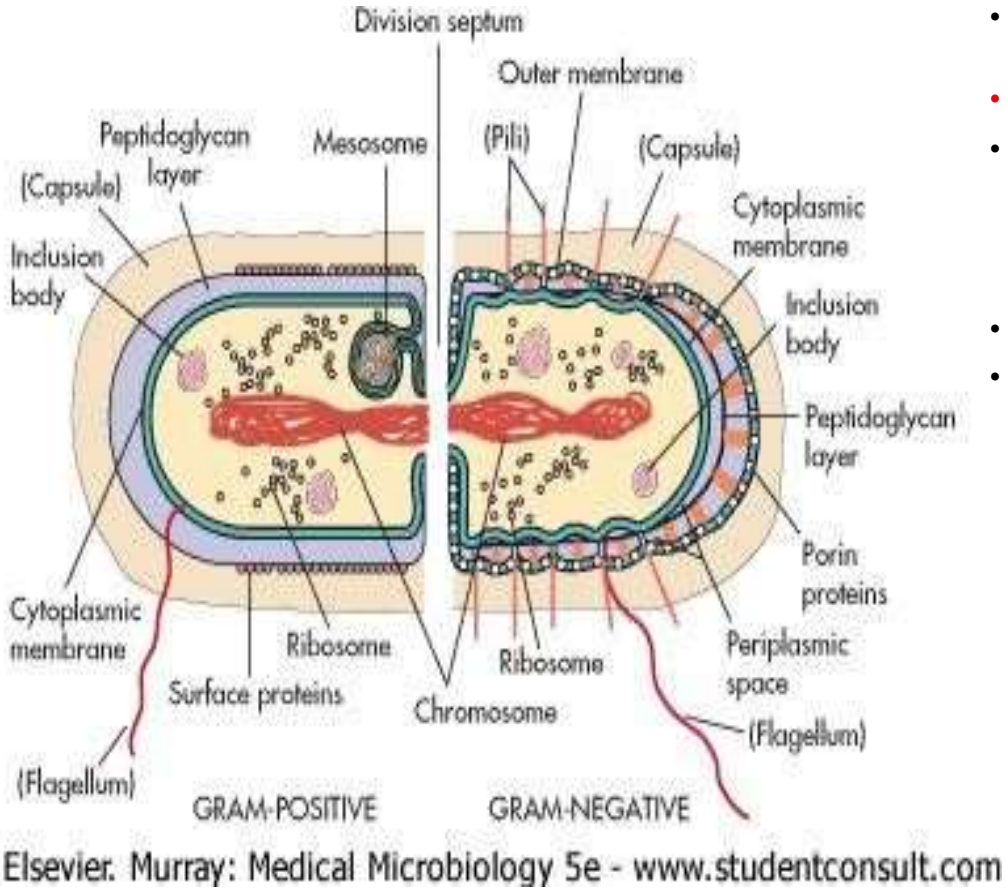
- **Most precise method for bacterial classification.**
  - **Ratio of guanine to cytosine**
  - **DNA hybridization**
  - **Nucleic acid sequence analysis**
    - **PCR**
      - **Chromosomal DNA**
      - **Ribotyping**
      - **Plasmid analysis**

Differences between eukaryotes and prokaryotes

- **Eukaryotes-Greek for true nucleus.**
  - **80S Ribosome**
    - **60S + 40S**
- **Prokaryotes-Greek for primitive nucleus.**
  - **70S Ribosome**
    - **50S + 30S**
- **Peptidoglycan cell wall.**

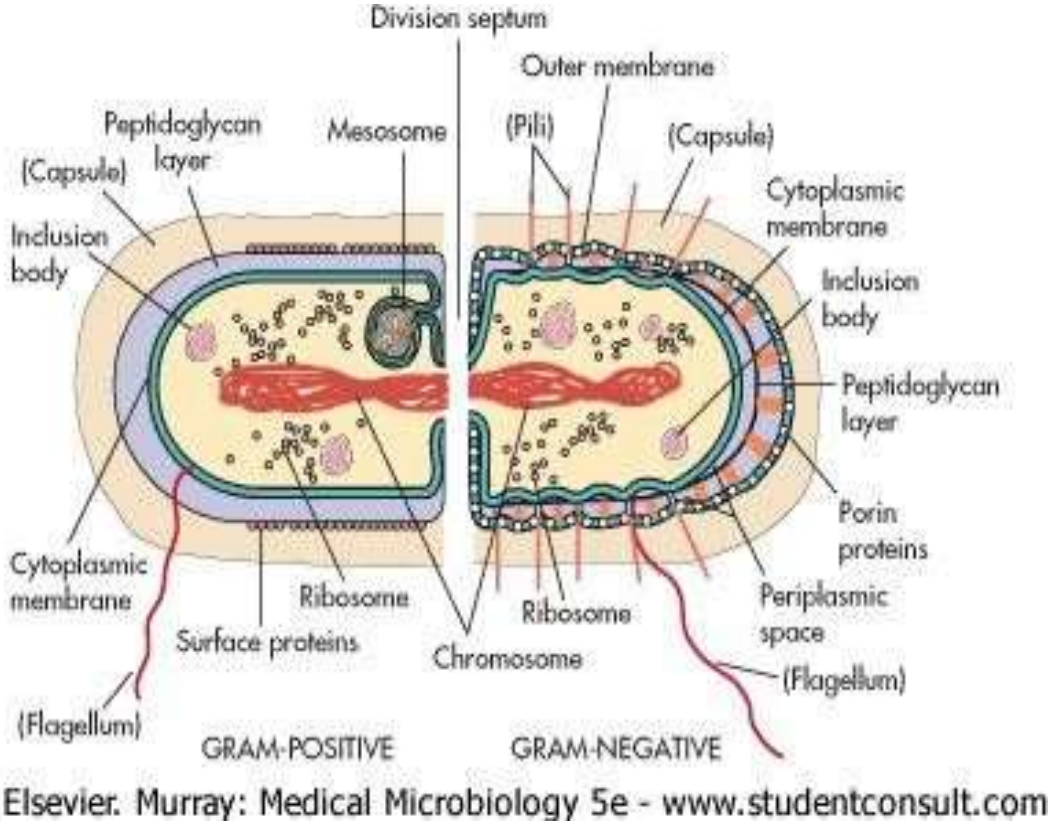


Bacterial Ultrastructure- Cytoplasmic Structures



- **Bacterial chromosome is a single, double-stranded circle--contained in the nucleoid.**
- **RIBOSOMES**
- **Plasmids present in most bacteria.**
  - **confer virulence**
  - **antibiotic resistance**
- **Cytoplasmic membrane**
- **Mesosome**
  - **cytoplasmic membrane**
  - **anchor and pull apart daughter cells**

Bacterial Ultrastructure- Cell Wall

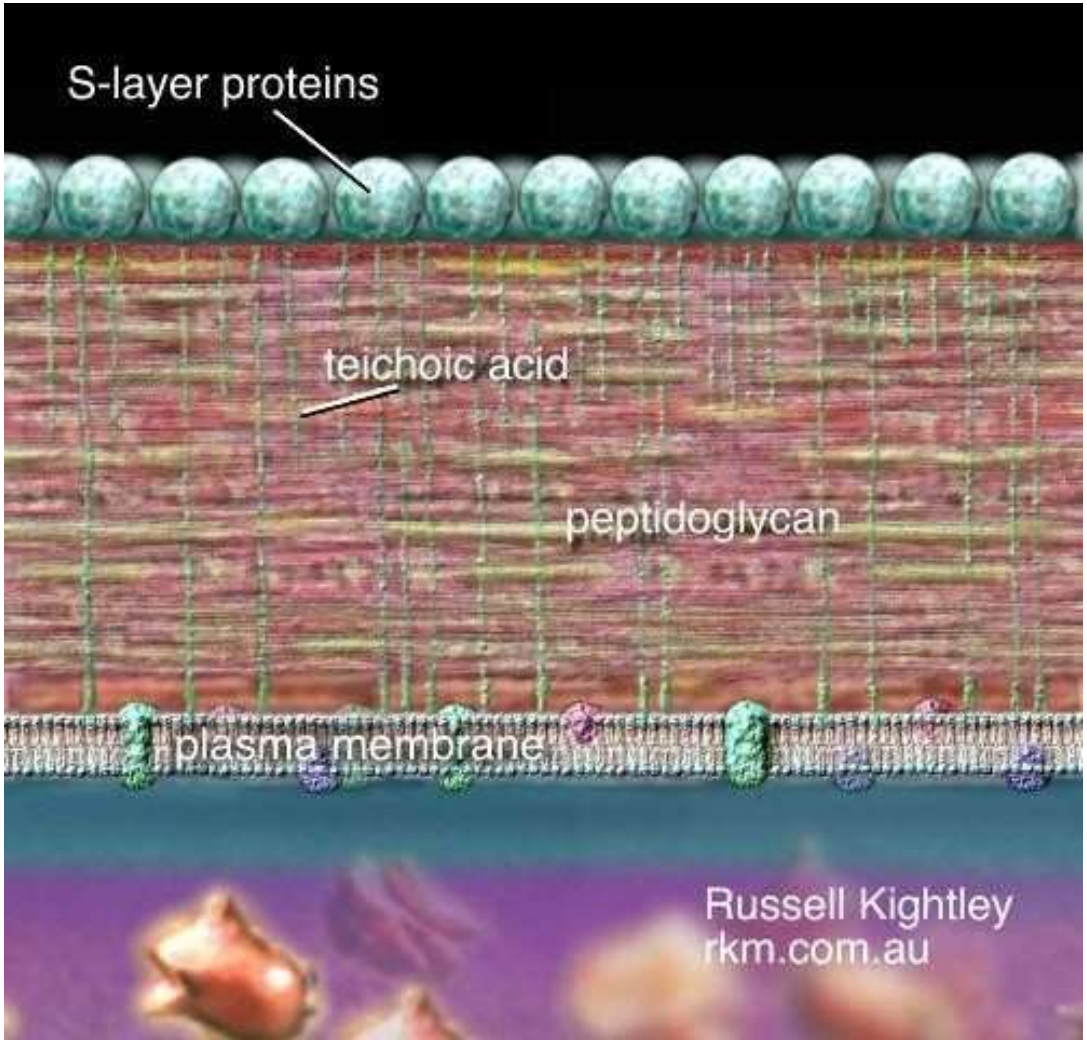


Rigid peptidoglycan layers surround the cytoplasmic membranes of most prokaryotes.

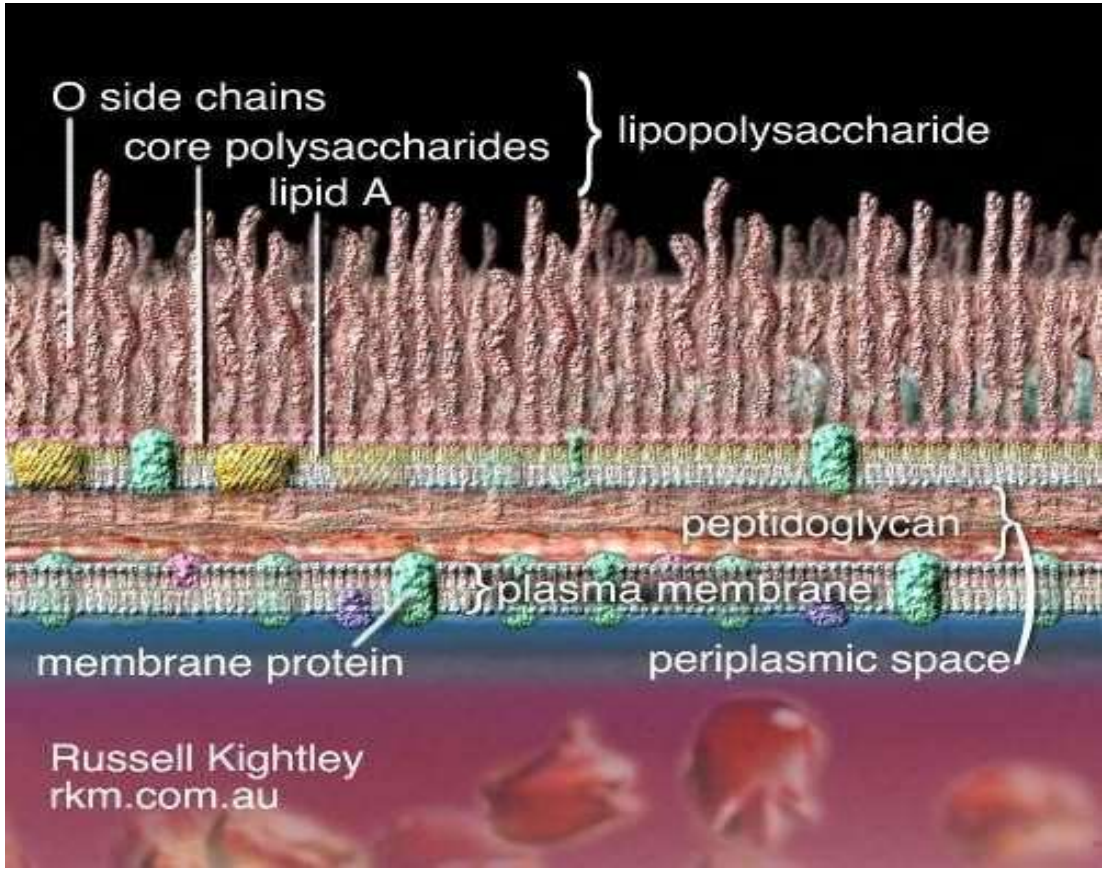
- **Both Gram positive and negative.**

Exceptions are Archaeobacteria organisms and mycoplasmas.

Differences Between Prokaryotes--
The Gram Stain



Gram Positive Cell wall



Gram-Negative Cell wa