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Programme: M.Sc., Marine Biotechnology

Course Title : Microbiology

Course Code: 21CC1

Unit-I
Bacterial Morphological types & Cell wall characteristics

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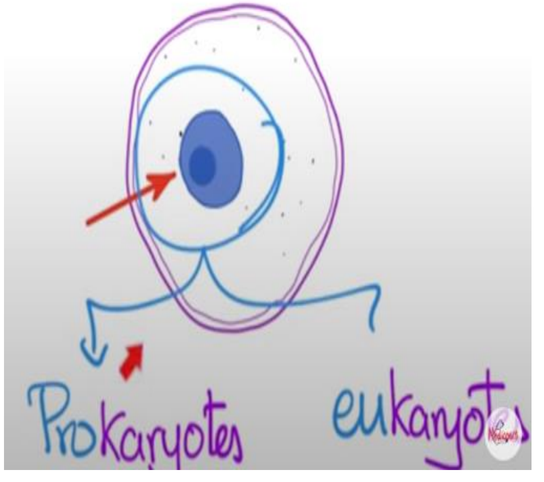
Introduction:

Traditionally, cellular organisms have been divided into two broad categories, based on their cell type. They are either **prokaryotic** or **eukaryotic**. In general, prokaryotes are smaller, simpler, with a lot less stuff, which would make eukaryotes larger, more complex, and more cluttered. The crux of their key difference can be deduced from their names: "karyose" is a Greek word meaning "nut" or "center," a reference to the nucleus of a cell. "Pro" means "before," while "Eu" means "true," indicating that prokaryotes lack a nucleus ("before a nucleus") while eukaryotes have a true nucleus. More recently, microbiologists have been rebelling against the term prokaryote because it lumps both bacteria and the more recently discovered archaea in the same category. Both cells are prokaryotic because they lack a nucleus and other organelles (such as mitochondria, Golgi apparatus, endoplasmic reticulum, etc), but they aren't closely related genetically. So, to honor these differences this text will refer to the groups as the archaea, the bacteria, and the eukaryotes and try to leave the prokaryotic reference out of it.



Cell Components

All cells (bacterial, archaeal, eukaryotic) share four common components:

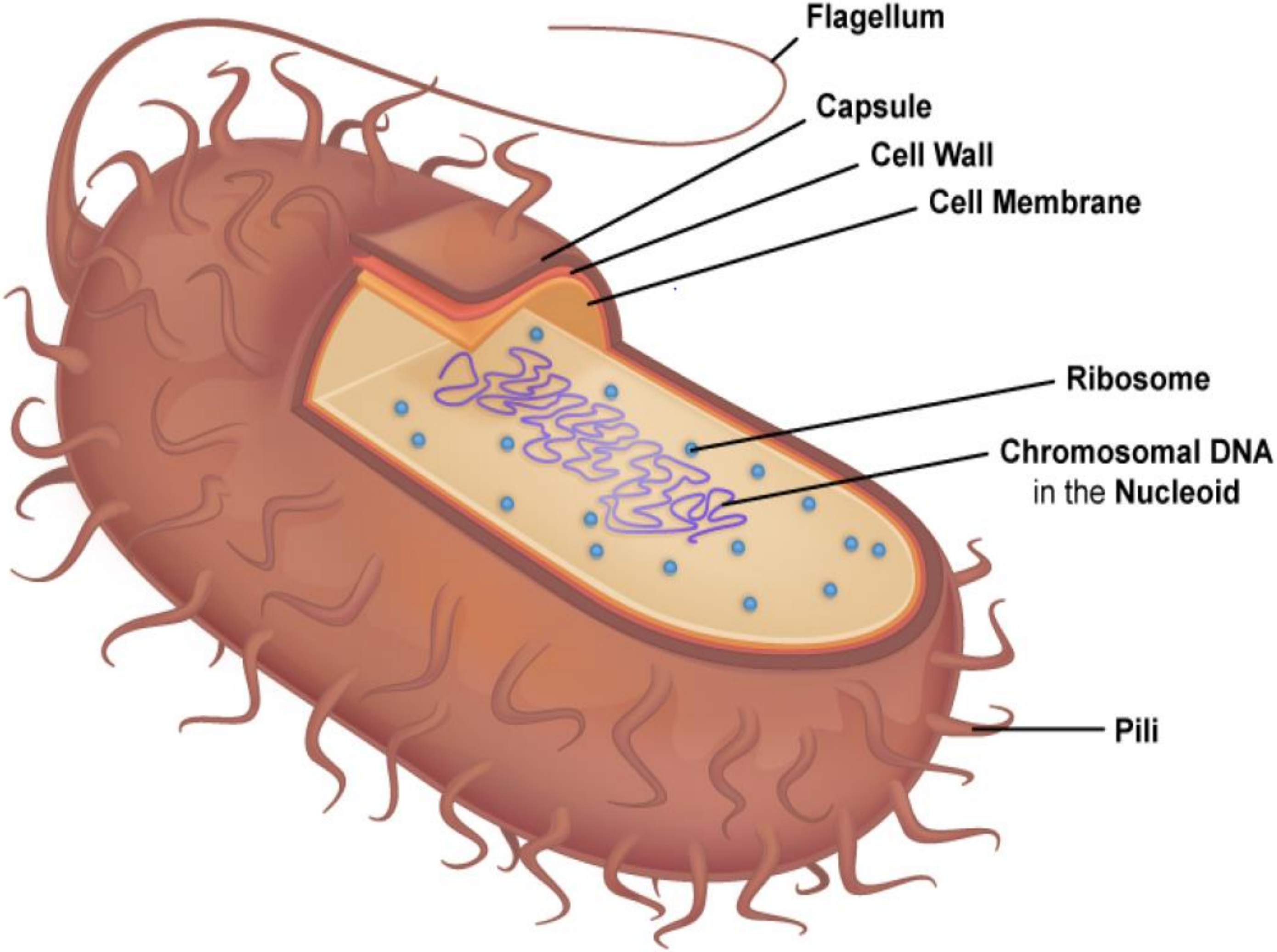
Cytoplasm – **cytoplasm** is the gel-like fluid that fills each cell, providing an aqueous environment for the chemical reactions that take place in a cell. It is composed of mostly water, with some salts and proteins.

DNA – deoxyribonucleic acid or **DNA** is the genetic material of the cell, the instructions for the cell's abilities and characteristics. This complete set of genes, referred to as a **genome**, is localized in an irregularly-shaped region known as the **nucleoid** in bacterial and archaeal cells, and enclosed into a membrane-bound **nucleus** in eukaryotic cells.

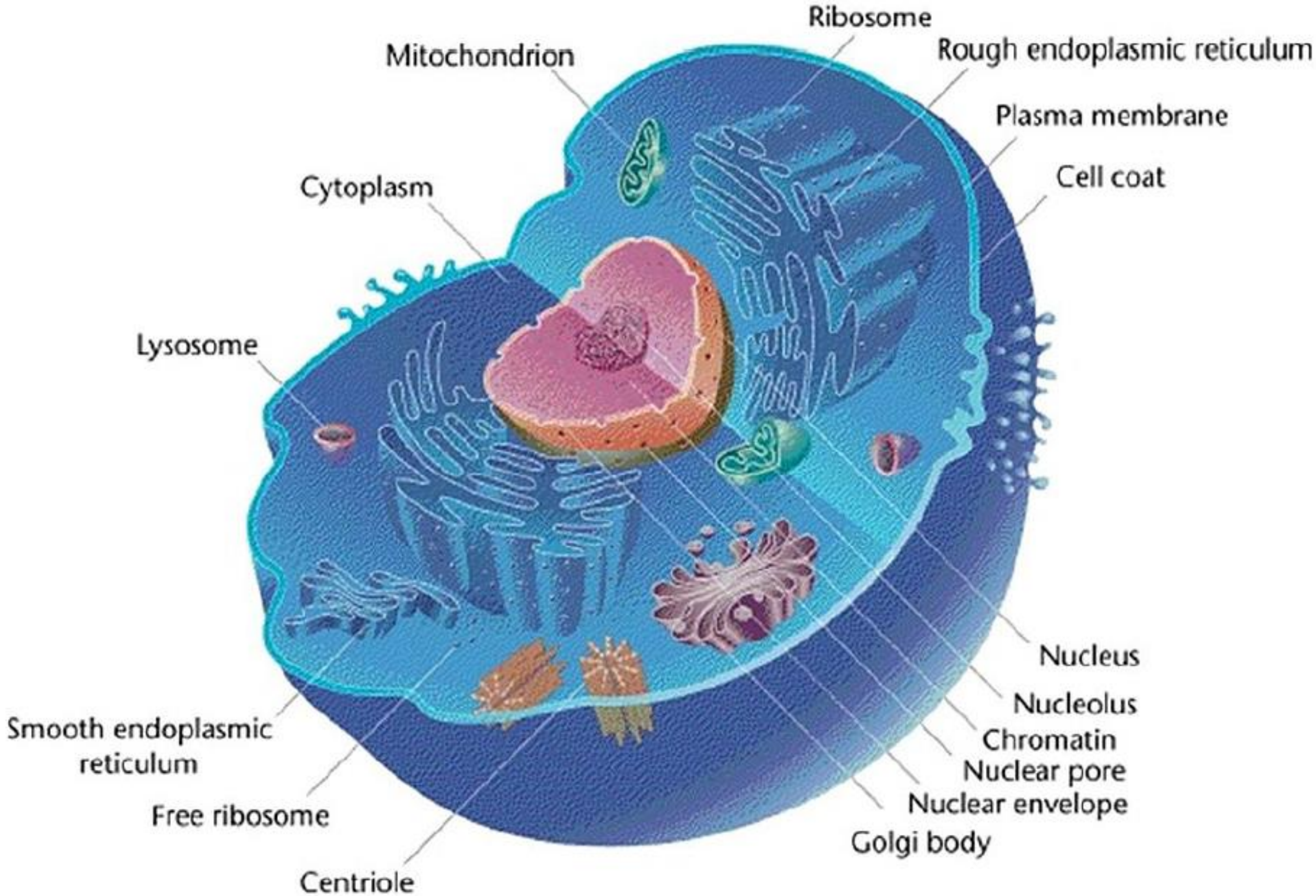
Ribosomes – the protein-making factories of the cell are the **ribosomes**. Composed of both RNA and protein, there are some distinct differences between the ones found in bacteria/archaea and the ones found in eukaryotes, particularly in terms of size and location. The ribosomes of bacteria and archaea are found floating in the cytoplasm, while many of the eukaryotic ribosomes are organized along the endoplasmic reticulum, a eukaryotic organelle. Ribosomes are measured using the **Svedberg unit**, which corresponds to the rate of sedimentation when centrifuged. Bacterial/archaeal ribosomes have a measurement of 70S as a sedimentation value, while eukaryotic ribosomes have a measurement of 80S, an indication of both their larger size and mass.

Plasma Membrane – one of the outer boundaries of every cell is the **plasma membrane** or **cell membrane**. (A plasma membrane can be found elsewhere as well, such as the membrane that bounds the eukaryotic nucleus, while the term cell membrane refers specifically to this boundary of the cell proper). The plasma membrane separates the cell's inner contents from the surrounding environment. While not a strong layer, the plasma membrane participates in several crucial processes for the cell, particularly for bacteria and archaea, which typically only have the one membrane:

- Acts as a semi-permeable barrier to allow for the entrance and exit of select molecules. It functions to let in nutrients, excrete waste products, and possibly keep out dangerous substances such as toxins or antibiotics.
- Performs metabolic processes by participating in the conversion of light or chemical energy into a readily useable form known as ATP. This energy conservation involves the development of a **proton motive force (PMF)**, based on the separation of charges across the membrane, much like a battery.
- "Communicates" with the environment by binding or taking in small molecules that act as signals and provide information important to the cell. The information might relate to nutrients or toxin in the area, as well as information about other organisms.

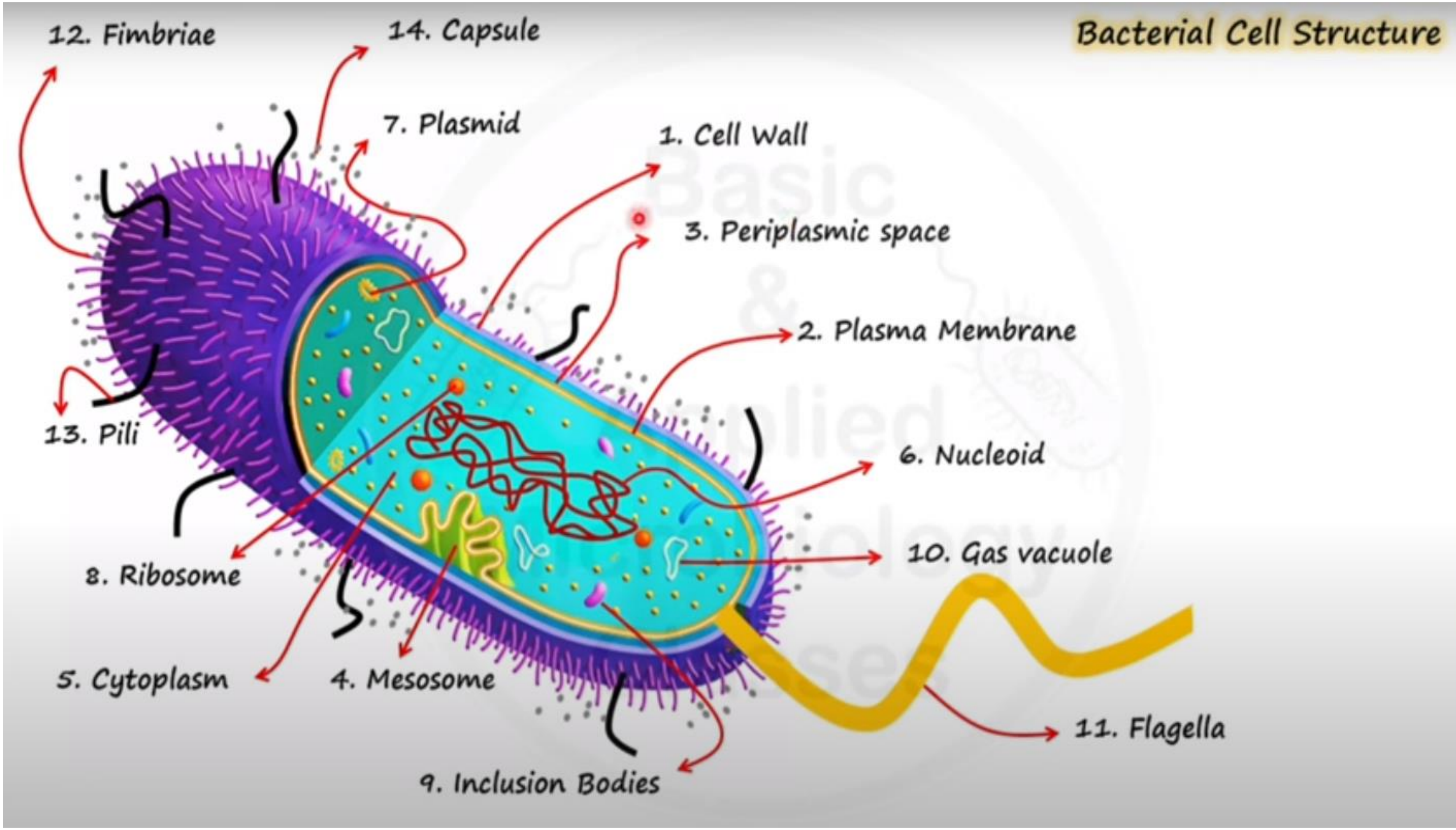


Flagellum
Capsule
Cell Wall
Cell Membrane
Ribosome
Chromosomal DNA
in the Nucleoid
Pili



Typical Eukaryotic Cell. By Mediran (Own work) [CC BY-SA 3.0], *via Wikimedia Commons*

Eukaryotes have numerous additional components called organelles, such as the nucleus, the mitochrondria, the endoplasmic reticulum, the Golgi apparatus, etc. These are all membrane-bound compartments that house different activities for the cell. Because each structure is bounded by its very own plasma membrane, it provides the cell with multiple locations for membranous functions to occur.

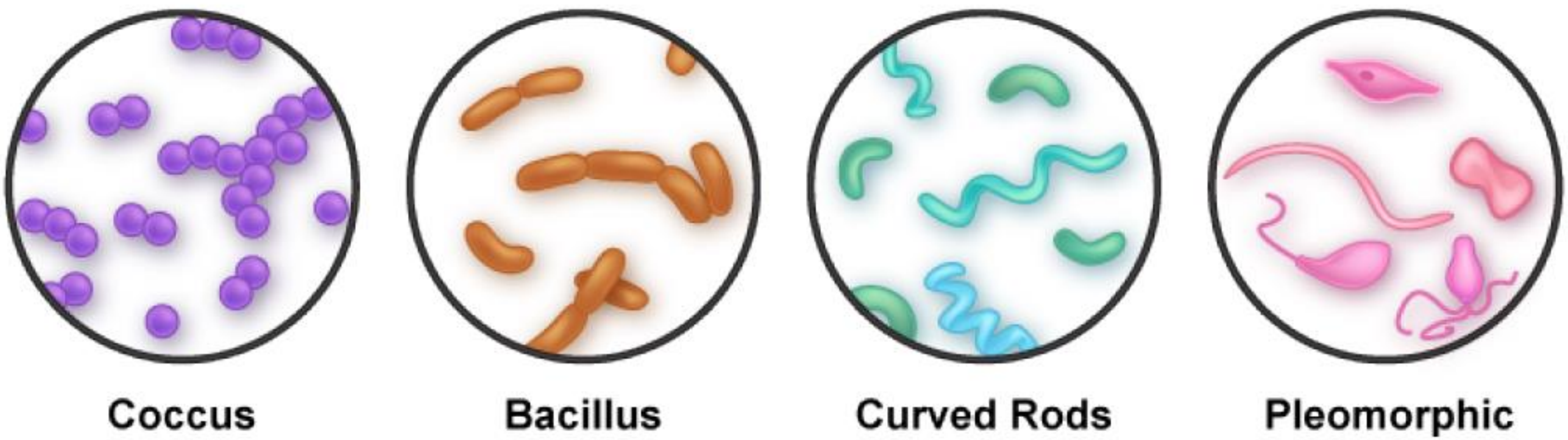

Bacterial Cell Structure
12. Fimbriae
14. Capsule
7. Plasmid
1. Cell Wall
3. Periplasmic space
2. Plasma Membrane
6. Nucleoid
10. Gas vacuole
11. Flagella
13. Pili
8. Ribosome
5. Cytoplasm
4. Mesosome
9. Inclusion Bodies

Bacterial morphological types

Cell Morphology

Cell **morphology** is a reference to the shape of a cell. It might seem like a trivial concept but to a cell it is not. The shape dictates how that cell will grow, reproduce, obtain nutrients, move, and it's important to the cell to maintain that shape to function properly. Cell morphology can be used as a characteristic to assist in identifying particular microbes but it's important to note that cells with the same morphology are not necessarily related.

Bacteria tend to display the most representative cell morphologies, with the most common examples listed here:



Coccus (pl. cocci) – a **coccus** is a spherically shaped cell.

Bacillus (pl. bacilli) – a **bacillus** is a rod-shaped cell.

Curved rods – obviously this is a rod with some type of curvature. There are three sub-categories: the **vibrio**, which are rods with a single curve and the **spirilla/spirochetes**, which are rods that form spiral shapes. Spirilla and spirochetes are differentiated by the type of motility that they exhibit, which means it is hard to separate them unless you are looking at a wet mount.

Pleomorphic – **pleomorphic** organisms exhibit variability in their shape.

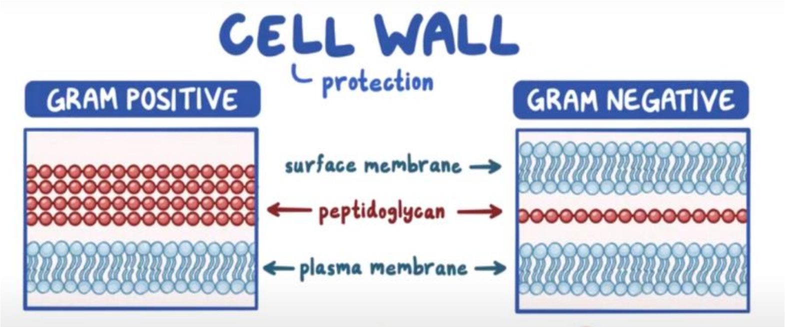
There are additional shapes seen for bacteria, and an even wider array for the archaea, which have even been found as star or square shapes. Eukaryotic microbes also tend to exhibit a wide array of shapes, particularly the ones that lack a cell wall such as the protozoa.

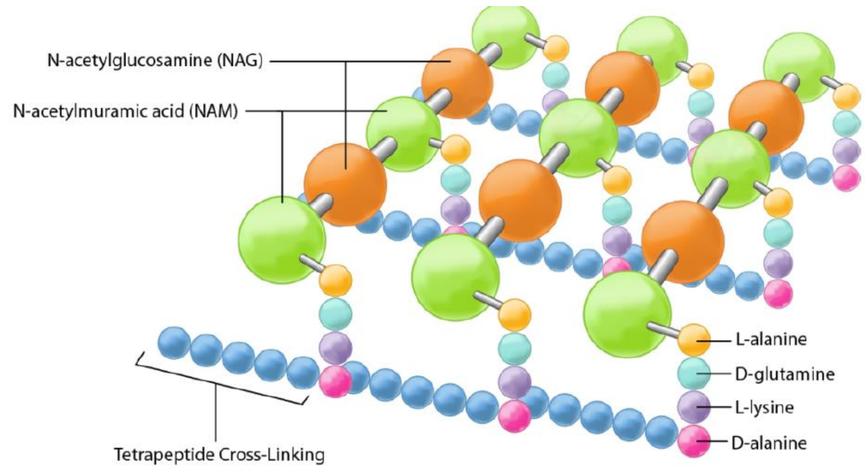
Cell Wall structure of bacteria

A cell wall, not just of bacteria but for all organisms, is found outside of the cell membrane. It's an additional layer that typically provides some strength that the cell membrane lacks, by having a semi-rigid structure.

Both gram positive and gram negative cell walls contain an ingredient known as **peptidoglycan** (also known as **murein**). This particular substance hasn't been found anywhere else on Earth, other than the cell walls of bacteria. But both bacterial cell wall types contain additional ingredients as well, making the bacterial cell wall a complex structure overall, particularly when compared with the cell walls of eukaryotic microbes. The cell walls of eukaryotic microbes are typically composed of a single ingredient, like the cellulose found in algal cell walls or the chitin in fungal cell walls.

The bacterial cell wall performs several functions as well, in addition to providing overall strength to the cell. It also helps maintain the cell shape, which is important for how the cell will grow, reproduce, obtain nutrients, and move. It protects the cell from **osmotic lysis**, as the cell moves from one environment to another or transports in nutrients from its surroundings. Since water can freely move across both the cell membrane and the cell wall, the cell is at risk for an osmotic imbalance, which could put pressure on the relatively weak plasma membrane. Studies have actually shown that the internal pressure of a cell is similar to the pressure found inside a fully inflated car tire. That is a lot of pressure for the plasma membrane to withstand! The cell wall can keep out certain molecules, such as toxins, particularly for gram negative bacteria. And lastly, the bacterial cell wall can contribute to the pathogenicity or disease –causing ability of the cell for certain bacterial pathogens.





Peptidoglycan Structure.

Structure of Peptidoglycan

Let us start with peptidoglycan, since it is an ingredient that both bacterial cell walls have in common.

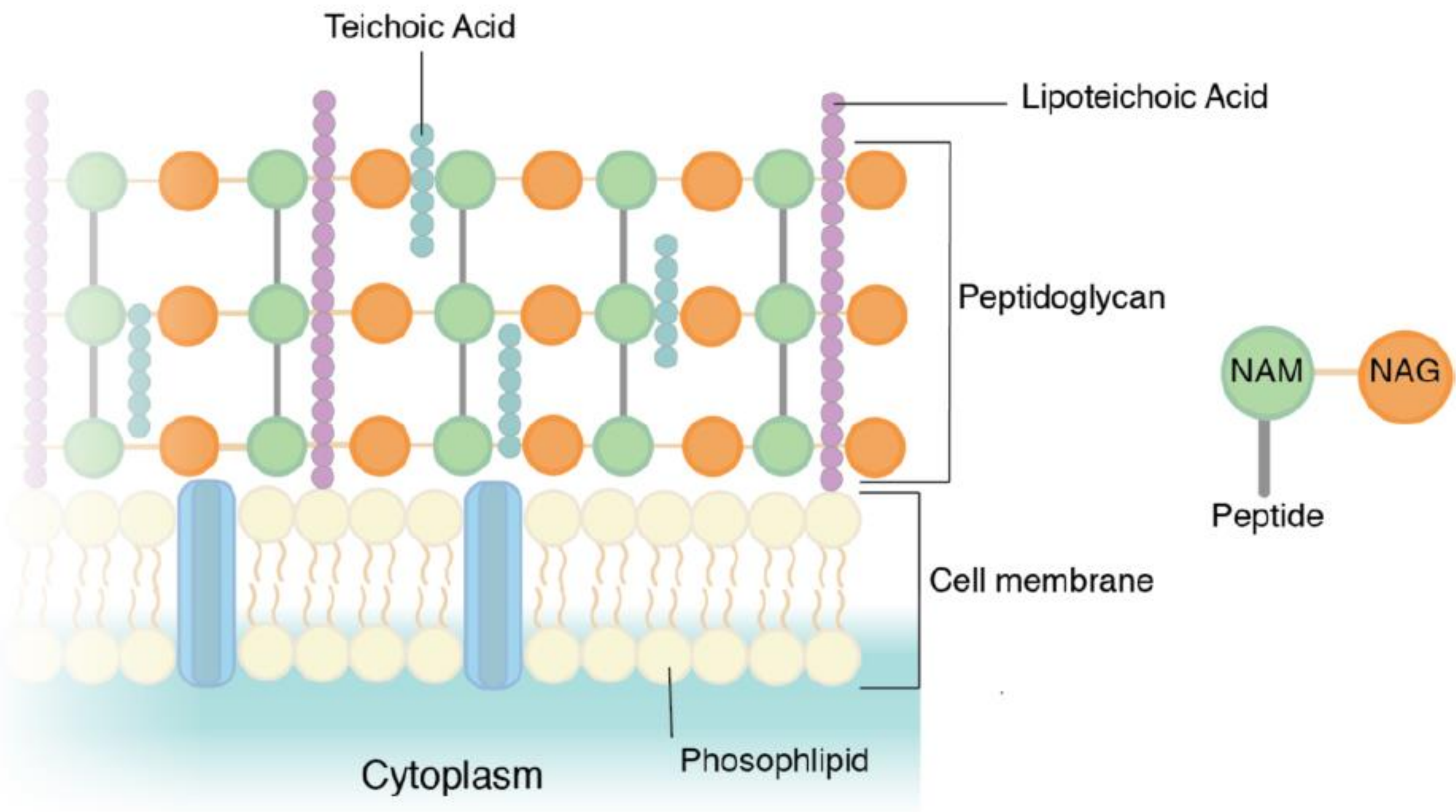
Peptidoglycan is a polysaccharide made of two glucose derivatives, **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**, alternated in long chains. The chains are cross-linked to one another by a **tetrapeptide** that extends off the NAM sugar unit, allowing a lattice-like structure to form. The four amino acids that compose the tetrapeptide are: **L-alanine, D-glutamine, L-lysine or *meso*-diaminopimelic acid (DPA),** and **D-alanine**. Typically only the L-isomeric form of amino acids are utilized by cells but the use of the mirror image D-amino acids provides protection from proteases that might compromise the integrity of the cell wall by attacking the peptidoglycan. The tetrapeptides can be **directly cross-linked** to one another, with the D-alanine on one tetrapeptide binding to the L-lysine/ DPA on another tetrapeptide. In many gram positive bacteria there is a cross-bridge of five amino acids such as glycine (**peptide interbridge**) that serves to connect one tetrapeptide to another. In either case the cross-linking serves to increase the strength of the overall structure, with more strength derived from **complete cross-linking**, where every tetrapeptide is bound in some way to a tetrapeptide on another NAG-NAM chain.

CELL WALL STRUCTURE OF GR +VE & GR –VE EUBACTERIA

Gram Positive Cell walls

The cell walls of gram positive bacteria are composed predominantly of peptidoglycan. In fact, peptidoglycan can represent up to 90% of the cell wall, with layer after layer forming around the cell membrane. The NAM tetrapeptides are typically cross-linked with a peptide interbridge and complete cross-linking is common. All of this combines together to create an incredibly strong cell wall.

The additional component in a gram positive cell wall is **teichoic acid**, a glycopolymer, which is embedded within the peptidoglycan layers. Teichoic acid is believed to play several important roles for the cell, such as generation of the net negative charge of the cell, which is essential for development of a proton motive force. Teichoic acid contributes to the overall rigidity of the cell wall, which is important for the maintenance of the cell shape, particularly in rod-shaped organisms. There is also evidence that teichoic acids participate in cell division, by interacting with the peptidoglycan biosynthesis machinery. Lastly, teichoic acids appear to play a role in resistance to adverse conditions such as high temperatures and high salt concentrations, as well as to β-lactam antibiotics. Teichoic acids can either be covalently linked to peptidoglycan (**wall teichoic acids or WTA**) or connected to the cell membrane via a lipid anchor, in which case it is referred to as **lipoteichoic acid**.

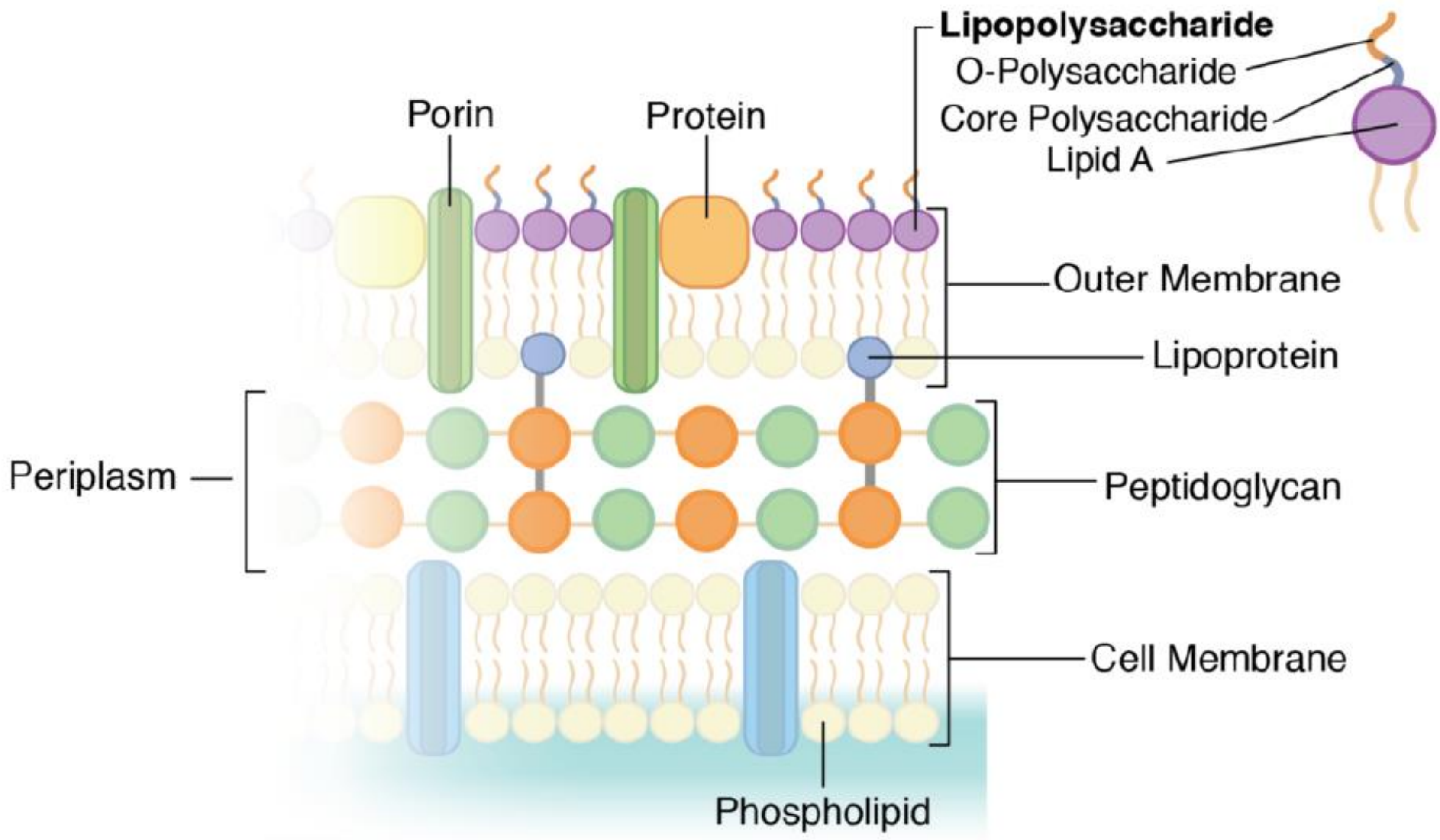


Gram Positive Bacteria Cell Wall

Since peptidoglycan is relatively porous, most substances can pass through the gram positive cell wall with little difficulty. But some nutrients are too large, requiring the cell to rely on the use of **exoenzymes**. These extracellular enzymes are made within the cell's cytoplasm and then secreted past the cell membrane, through the cell wall, where they function outside of the cell to break down large macromolecules into smaller components.

Gram Negative Cell Walls

The cell walls of gram negative bacteria are more complex than that of gram positive bacteria, with more ingredients overall. They do contain peptidoglycan as well, although only a couple of layers, representing 5-10% of the total cell wall. What is most notable about the gram negative cell wall is the presence of a plasma membrane located outside of the peptidoglycan layers, known as the **outer membrane**. This makes up the bulk of the gram negative cell wall. The outer membrane is composed of a lipid bilayer, very similar in composition to the cell membrane with polar heads, fatty acid tails, and integral proteins. It differs from the cell membrane by the presence of large molecules known as **lipopolysaccharide (LPS)**, which are anchored into the outer membrane and project from the cell into the environment. LPS is made up of three different components: 1) the **O-antigen or O-polysaccharide**, which represents the outermost part of the structure , 2) the **core polysaccharide**, and 3) **lipid A**, which anchors the LPS into the outer membrane. LPS is known to serve many different functions for the cell, such as contributing to the net negative charge for the cell, helping to stabilize the outer membrane, and providing protection from certain chemical substances by physically blocking access to other parts of the cell wall. In addition, LPS plays a role in the host response to pathogenic gram negative bacteria. The O-antigen triggers an immune response in an infected host, causing the generation of antibodies specific to that part of LPS (think of E. *coli* **O**157). Lipid A acts as a toxin, specifically an **endotoxin**, causing general symptoms of illness such as fever and diarrhea. A large amount of lipid A released into the bloodstream can trigger endotoxic shock, a body-wide inflammatory response which can be life-threatening.



Gram Negative Bacteria Cell Wall

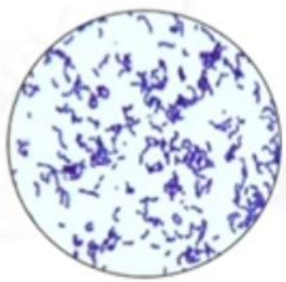
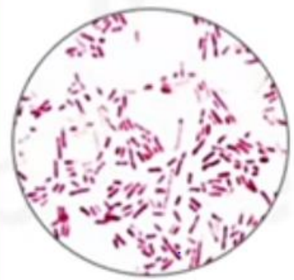
The outer membrane does present an obstacle for the cell. While there are certain molecules it would like to keep out, such as antibiotics and toxic chemicals, there are nutrients that it would like to let in and the additional lipid bilayer presents a formidable barrier. Large molecules are broken down by enzymes, in order to allow them to get past the LPS. Instead of exoenzymes (like the gram positive bacteria), the gram negative bacteria utilize **periplasmic enzymes** that are stored in the **periplasm**. Where is the periplasm, you ask? It is the space located between the outer surface of the cell membrane and the inner surface of the outer membrane, and it contains the gram negative peptidoglycan. Once the periplasmic enzymes have broken nutrients down to smaller molecules that can get past the LPS, they still need to be transported across the outer membrane, specifically the lipid bilayer. Gram negative cells utilize **porins**, which are transmembrane proteins composed of a trimer of three subunits, which form a pore across the membrane.

Some porins are non-specific and transport any molecule that fits, while some porins are specific and only transport substances that they recognize by use of a binding site. Once across the outer membrane and in the periplasm, molecules work their way through the porous peptidoglycan layers before being transported by integral proteins across the cell membrane.

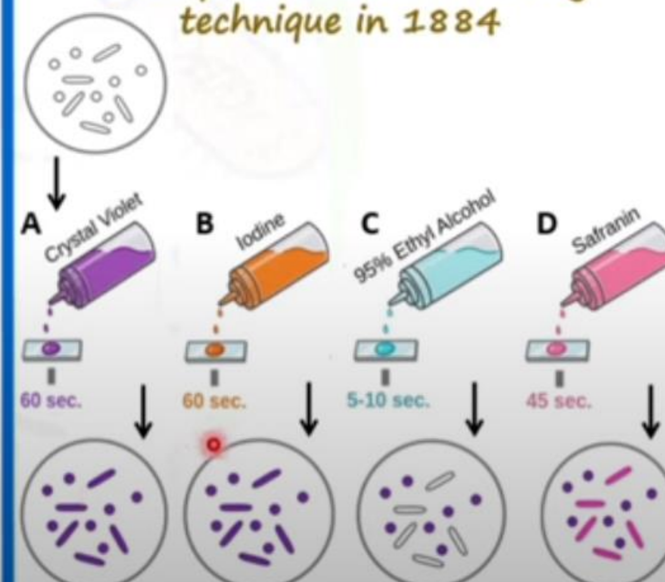
The peptidoglycan layers are linked to the outer membrane by the use of a lipoprotein known as **Braun's lipoprotein** (good ol' Dr. Braun). At one end the lipoprotein is covalently bound to the peptidoglycan while the other end is embedded into the outer membrane via its polar head. This linkage between the two layers provides additional structural integrity and strength.

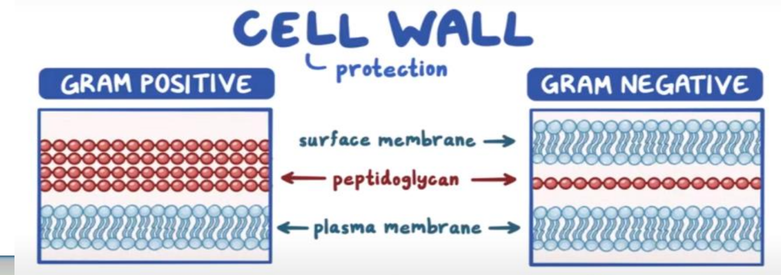
Gram + Bacteria

Gram - Bacteria

Terms of Comparison	Gram positive bacteria	Gram negative bacteria
1. Colour retention (After Gram staining)	Purple 	Pink 
2. Gram reaction	Positive (G+ve) (Retain crystal violet)	Negative (G-ve) (Don't retain crystal violet)

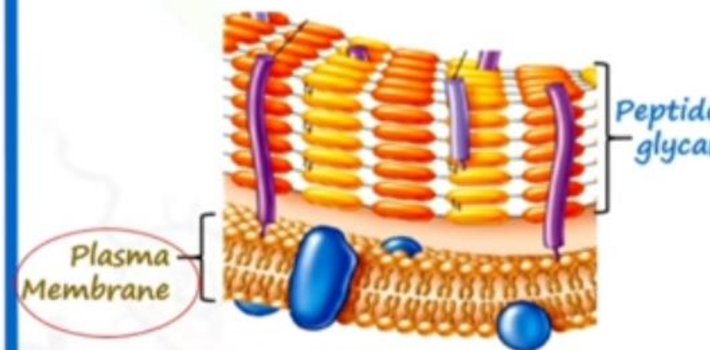
Dr. Hans Christian Gram
Developed the Gram staining technique in 1884



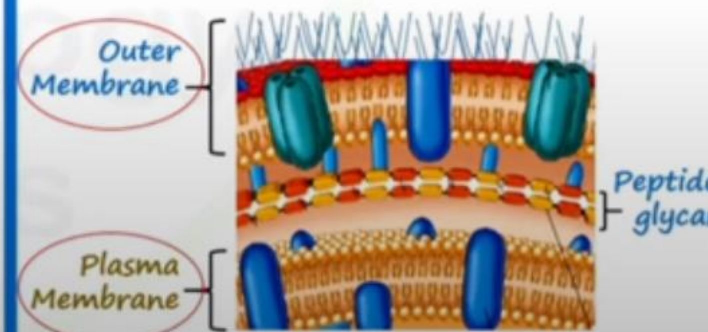


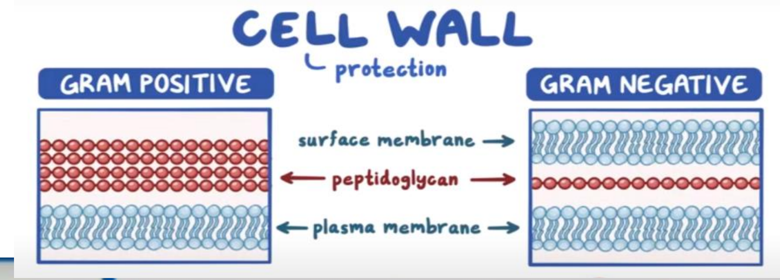
Terms of Comparison	Gram positive bacteria	Gram negative bacteria
3. Cell wall	Single layered	Two layered
4. Peptidoglycan	Thick layered (20 to 80 nm), extensively cross-linked	Thin layered (2 to 7 nm) with few cross-links
5. Outer membrane	Absent	Present, 7 to 8 nm
6. Lipid content	Low	High
7. Lipopolysaccharide (LPS)	Absent	Present

Gram +ve

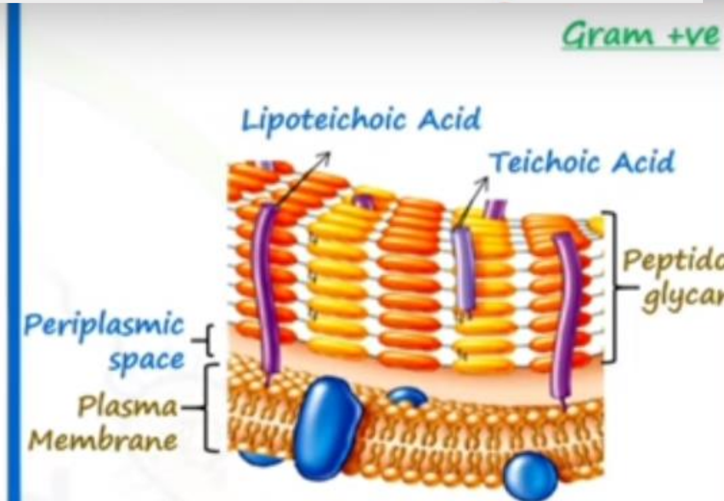


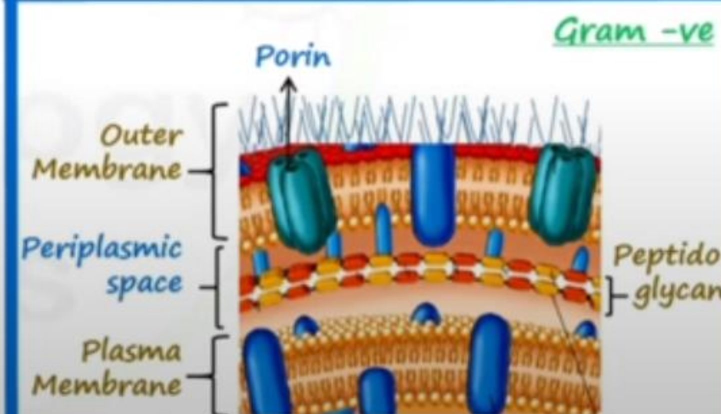
Gram -ve



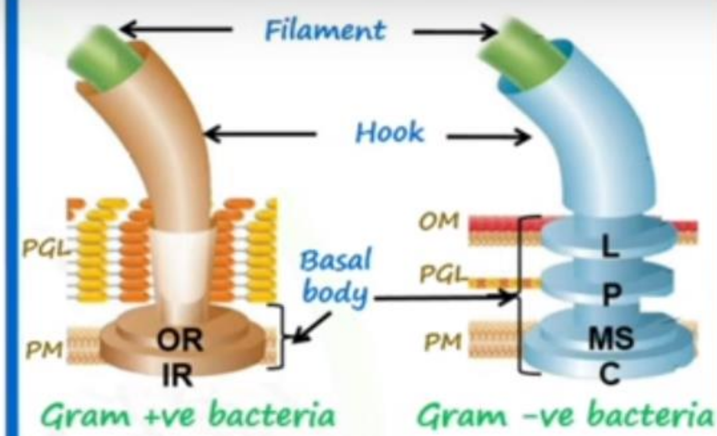


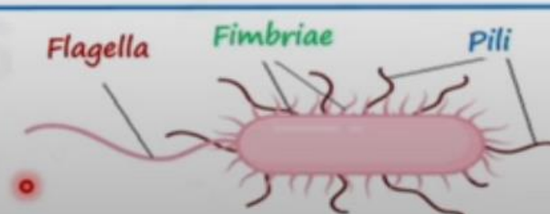
Terms of Comparison	Gram positive bacteria	Gram negative bacteria
8. Periplasmic space	Narrow (present in some bacteria)	Wide (Present in all bacteria)
9. Teichoic acid	Present	Absent
10. Porin proteins	Absent	Present
11. Toxin production	Exotoxin	Endotoxin (LPS-Lipid A)



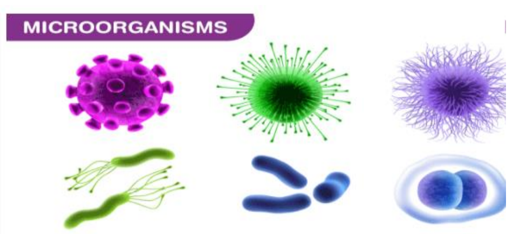


Terms of Comparison	Gram positive bacteria	Gram negative bacteria
12. No. of rings in basal body of flagella	2 rings Outer and Inner	4 rings L, P, MS and C
13. Mesosome	More prominent	Less prominent
14. Pili	Absent	Present





Terms of Comparison	Gram positive bacteria	Gram negative bacteria
15. Resistance to osmotic pressure	More	Less
16. Susceptibiity to anionic detergents and antibiotics	More	Less
17. Examples	✓ Bacillus ✓ Clostridium ✓ Lactobacillus ✓ Listeria ✓ Staphyloccus ✓ Streptococcus etc.	✓ E.coli ✓ Enterobacter ✓ Klebsiella ✓ Pseudomonas ✓ Salmonella ✓ Shigella etc.



Cell Wall structure of Archae

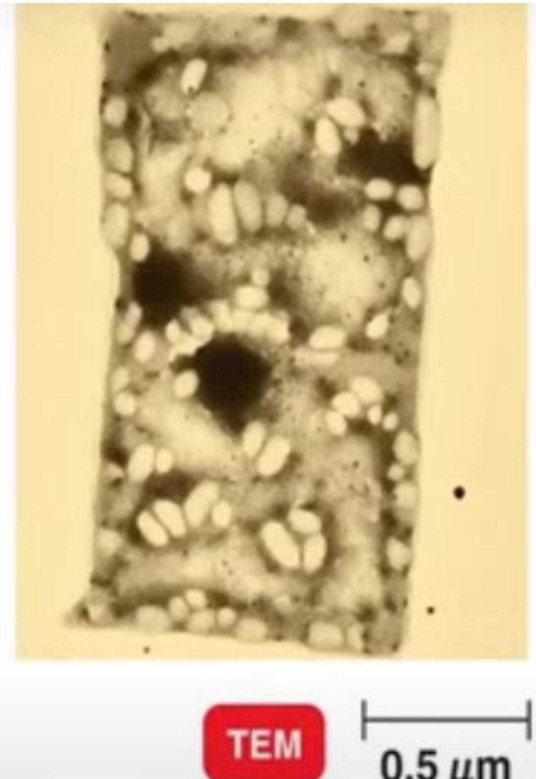
Archebacteria

Archaea

Archaea is a relatively new domain, since these organisms used to be grouped with the bacteria. There are some obvious similarities, since they are mostly unicellular, cells lack a nucleus or any other organelle, they have 70S ribosomes, and all *Archaea* are microbes. But they have completely different cell walls that can vary markedly in composition (but notably lack peptidoglycan and might have pseudomurien instead). In addition, their rRNA sequences have shown that they are not closely related to *Bacteria* at all.

ARCHEA

- Prokaryotic
- Cell wall lacks **peptidoglycan** - pseudomurein instead
- Unicellular
- Reproduces by binary fission (asexual) circular DNA
- Extremophiles:
  - Thermophiles - heat
  - Halophiles salt
  - Methanogens (produce methane as a waste product of respiration)



Cell Wall of Archaea

- Present in most archaea except *Thermoplasma* sp. and *Ferroplasma* sp.
- Surrounding the cell outside the cytoplasmic membrane and is mediating the interaction with the environment.
- Involved in Cell shape maintenance, Protection against Virus, Heat, Acidity or Alkalinity.
- Do not have Peptidoglycan but have Pseudomurein and different in its composition to Murein.

Cell Wall of Archaea

- Do not have Peptidoglycan but have Pseudopeptidoglycan and it is similar to bacterial peptidoglycan (morphologically, functionally) but is chemically distinct.
- Instead of NAM, it contains N-Acetyltalosamine Uronic Acid (NAT) linked to NAG, with peptide interbridges.
- In some Archaea, the Cell wall is composed of Glycan polymers like Glutaminylglycan, Heterosaccharide, Methanochondroitin or Pseudomurein, which can be further supported by a S-layer.

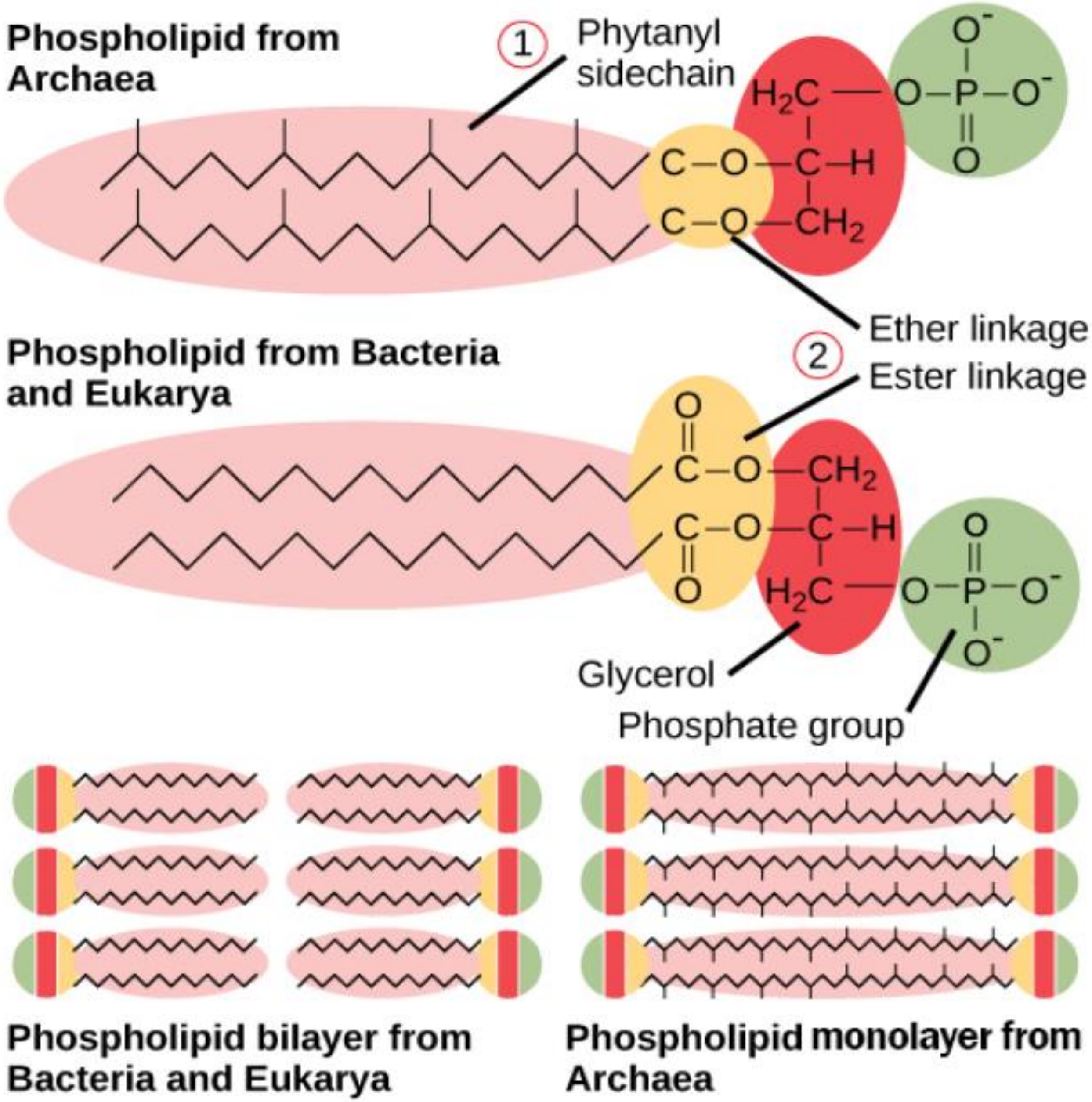
Cell Wall of Archaea

- **S-Layer** - Archaea possess a Protein or Glycoprotein Surface layer (S-Layer) as their sole cell wall structure.
- Consists of hexagonal patterns of protein or glycoprotein that self-assemble into a crystalline lattice (5 to 25 nm thick).
- **Role of the S-layer** - Physical and chemical protection.

- **Methanochondroitin** - Cell wall polymer found in some archaeal cells, similar in composition to the connective tissue component chondroitin, found in vertebrates.

Plasma Membrane

There are several characteristics of the plasma membrane that are unique to *Archaea*, setting them apart from other domains. One such characteristic is chirality of the glycerol linkage between the phopholipid head and the side chain. In archaea it is in the **L-isomeric form**, while bacteria and eukaryotes have the **D-isomeric form**. A second difference is the presence of an **ether-linkage** between the glycerol and the side chain, as opposed to the **ester-linked lipids** found in bacteria and eukaryotes. The ether-linkage provides more chemical stability to the membrane. A third and fourth difference are associated with the side chains themselves, unbranched fatty acids in bacteria and eukaryotes, while **isoprenoid chains** are found in archaea. These isoprenoid chains can have **branching side chains**.



Comparison of Plasma Membrane Lipid Between Bacteria and Archaea. OpenStax, Structure of Prokaryotes. OpenStax CNX. Mar 28, 2014 http://cnx.org/contents/9e7c7540-5794-4c31-917d-fce7e50ea6dd@11.

Difference between Gram positive cell wall, Gram negative cell wall & Archaeal cell wall

Features	Gram Positive Cell wall	Gram Negative Cell wall	Archaea cell wall
Gram Staining	Violet colour	Pink colour	Not applicable.
Thickness	15 – 18 nm	7 – 8 nm	20 – 40 nm
Peptidoglycan	Thick and Multilayered	Thin and Single layered	Absent
Pseudomurein	Absent	Absent	Present
Teichoic acids	Present	Absent	Absent
Periplasmic space	Absent	Present	Absent

Difference between Gram positive cell wall, Gram negative cell wall & Archaeal cell wall

Features	Gram Positive Cell wall	Gram Negative Cell wall	Archaea cell wall
Outer membrane	Absent	Present	Absent
LPS	Low	High	Absent
Lipids and Lipoproteins	Low	High	Low
Porins	Absent	Present	Absent
Cell wall disruption by Lysosomes	High	Low	Resistant to Lysosomes

Difference between Gram positive cell wall, Gram negative cell wall & Archaeal cell wall

Features	Gram Positive Cell wall	Gram Negative Cell wall	Archaea cell wall
Glycosidic linkage	β-1,4 glycosidic linkage	β-1,4 glycosidic linkage	β-1,3 glycosidic linkage
S - Layer	Absent	Absent	Present
Methanochondrotin	Absent	Absent	Present
Protein sheaths	Absent	Absent	Present
Sensitivity to Penicillin	Sensitive	Resistant	Resistant

References:

General Microbiology , Linda Bruslind , ebook supported from Oregon state University Campus.

https://www.youtube.com/watch?v=b15Hy3jCPDs

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Thank You