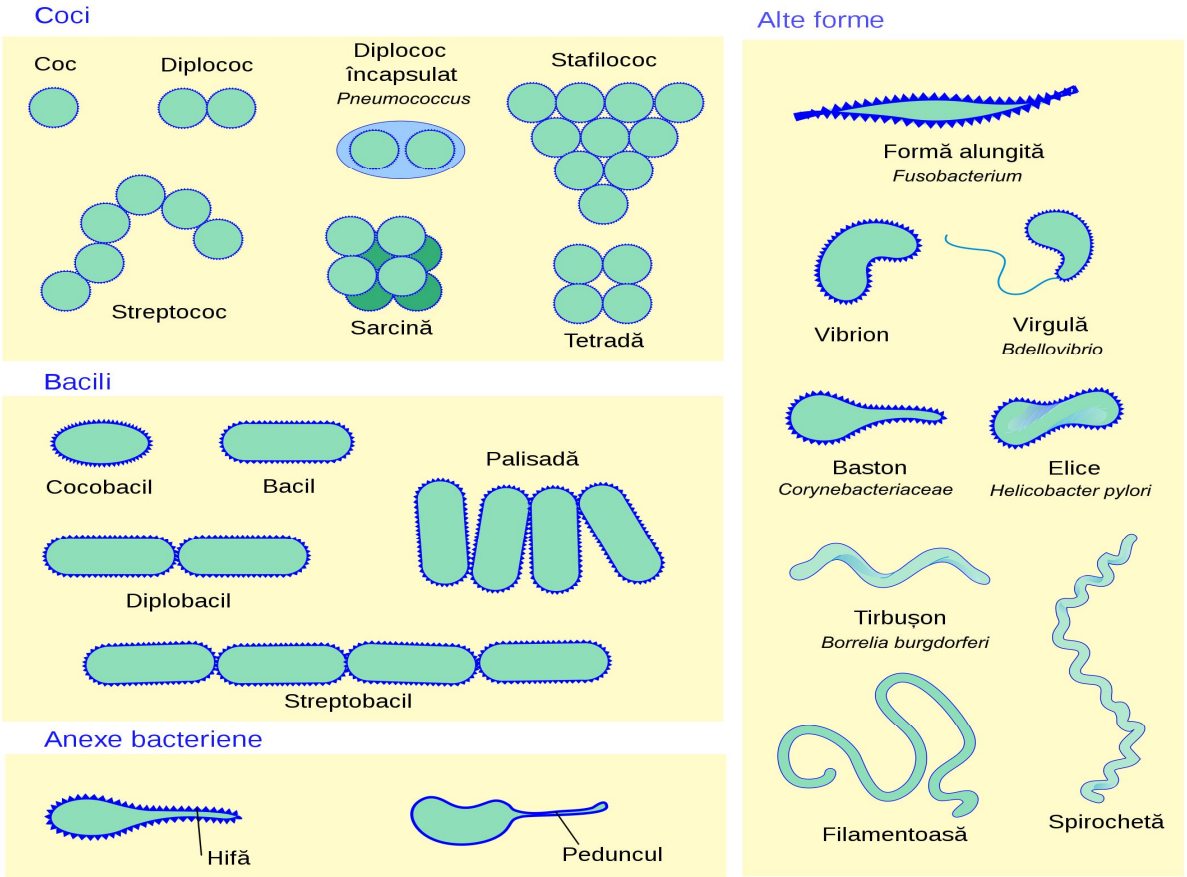
## Main Features of the Bacterial Cell Wall

Bacteria are unicellular organisms, which are spread in a wide variety of environments, from our gut to the deepest layers of the oceanic crust. These organisms can adopt a wide variety of shapes, from cocci (the spherical *Staphylococcus aureus*), to bacilli (the rod-like *Escherichia coli*) or even spirilla (the helically coiled *Helicobacter pylori*). According to the classification by Carl Woese in 1990, bacteria form one of the two domains, which are constituted by prokaryotes (the second one being archaea). As such, bacteria do not have any nucleus and, with the exception of a few species, do not possess any membrane-delimited organelles, unlike eukaryotes. While the latter have a diameter extended usually from 10 to 100 µm, bacteria are in general one order of magnitude smaller, with a size comprised between 1 and 5 µm (although extreme cases exist, like *Thiomargarita namibiensis* which can be detected by naked eyes with a maximum diameter of 750 µm).



A schematic representation of the bacterial cell structure is shown in Figure 1, emphasizing on one side the 20- to 530-nm-thick cell envelope (cell wall and capsule) and on another side the cytoplasm, the two parts being delimited by a 70-Å-thick plasma (or cytoplasmic) membrane which consists of a lipid bilayer containing embedded proteins.

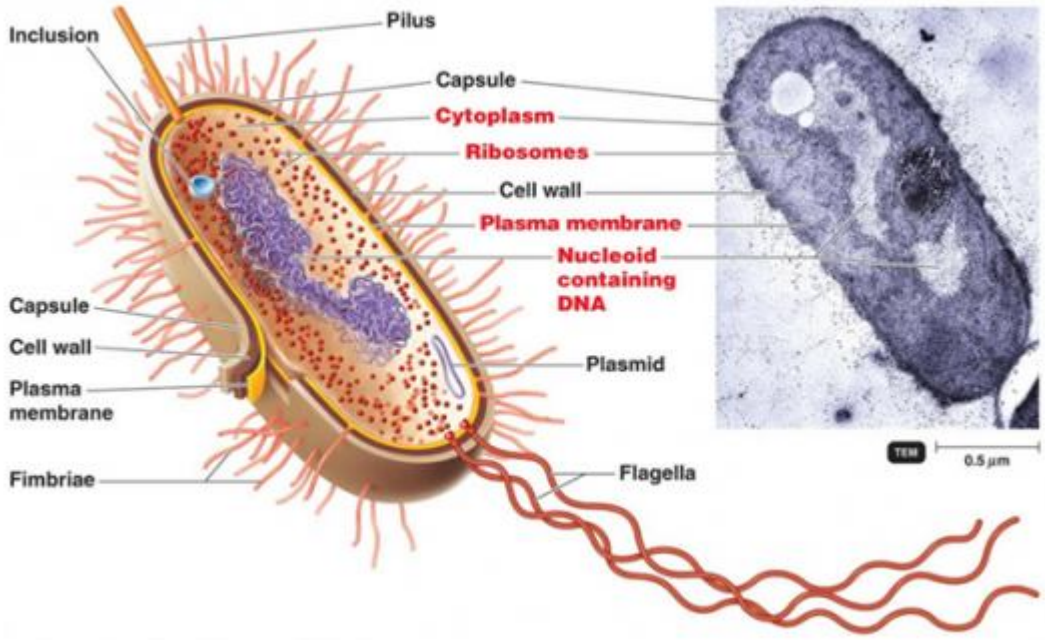





***Figure 1: Schematic representation of the bacterial cell (taken from Tortora et al., 2012)***

As bacteria do not have membrane-bound organelles, most of the metabolic reactions occur in the cytoplasm. It is also in this space that the genetic information, DeoxyriboNucleic Acid (DNA), is located. Bacterial DNA is usually present as a single, double-stranded, circular chromosome. The cell is relatively small comparatively to its genetic material content (about 4.6 million of base pair for 4400 genes for the bacterial model *Escherichia coli)*, it is therefore compacted by and with its interaction partners in a poorly defined region called the nucleoid. As there are no compartments in the bacterial cell, transcription of DNA in RiboNucleic Acid (RNA) can be coupled to translation into proteins. This last step is performed by 70S ribosomes (denominated from the value of their sedimentation rate by ultracentrifugation, in Svedberg), which are made of two subunits, 30S and 50S. Each of these is composed of ribosomal RNA

(rRNA) complexed to numerous proteins. Interestingly, eukaryotic cells have 80S ribosomes with 40S and 60S subunits, which result from different assemblies. These structural divergences enable the specific targeting of the bacterial ribosome with antibiotics without perturbing the patient's cells. The genome can also be supplemented by one or more smaller circular DNA molecules called plasmids. Contrary to the bacterial chromosome, plasmids are not essential for cell survival but bring additional genes which can be an advantage to cope with environmental stress or to gain resistance (to antibiotics, bacteriophages, and/or chemicals...). This genetic material can be shared rapidly among bacteria by horizontal transfer to promote genetic diversity. This process can occur through three different mechanisms. Some bacteria have the faculty to naturally absorb fragments of DNA, which are present in their environment. This competence is called transformation. They can also directly transfer genes from one to another through a sex pilus, an extended surface structure, by conjugation. Finally, genetic material can be acquired by transduction. In this case, DNA can be conveyed by a bacteriophage from a donor bacterium to a recipient cell.

Although the cytoplasm is lacking of complex internal structures, like a nucleus or a Golgi apparatus, bacteria can sometimes present other elementary compartments in addition to the nucleoid, for which a short and non-exhaustive selection is given here. Among them, thylakoids can be found in cyanobacteria, where they are formed by an ensemble of membranes. Similarly to those observed in chloroplasts, these are the home of photosynthesis and respiration processes. Another compartment involved in converting light into chemical energy is the chlorosome, which is bound to the neighboring cytoplasmic membrane in green sulfur bacteria. It contains large pigment assemblies which are absorbing photons with a very high efficiency to deal with poorly light exposed waters, where these cells are living. Magnetotactic bacteria are characterized by the presence of magnetosomes, lipid bilayers enclosing each a single magnetic mineral such as magnetite ($Fe_3O_4$) or greigite ($Fe_3S_4$).

The bacterial envelope is made of a cell wall layer and various extracellular structures, which are mainly implied in virulence. Among them, one can count the viscous polysaccharide layer, the capsule that covers the most exposed portion of the bacterial cell. During an infection, this layer enables bacteria to avoid phagocytosis and to resist to antimicrobial peptides and proteins. In addition, the capsule has the faculty to promote adhesion to host cells, other colony individuals, or substrates. This last function can also be fulfilled by excrescences, called pili, and their shorter

counterparts fimbriae, which are both resulting from the polymerization of the pilin protein. Flagella are longer hair-like appendages, which are anchored in the cytoplasm and serve as a mean of locomotion. They are the result of a complex assembly of more than 30 proteins using the electrochemical potential difference in protons from both sides of the cytoplasmic membrane to generate motility. While these structures are not essential for bacterial survival, the wall delimiting the bacterial cell is fundamental and its chemical structure is highly specific, thus being an attractive target for antibiotics.

## The Bacterial Cell Wall

In 1884, the Danish bacteriologist, Hans Christian Gram, while trying to set up a protocol to stain bacteria for observation under the microscope, developed a technique, which became fundamental to discriminate bacteria according to the composition of their cell wall. Heat-fixed bacterial cells are first treated with a purple dye, gentian violet, which penetrates through the cell wall and plasma membrane, thus staining the cytoplasmic compartment. The addition of iodine, which binds to the violet dye through an ion pair, traps it into the cell. When a decolorizer such as ethanol is added to the fixed cells, two behaviors can be observed, either (i) the purple color is retained, or (ii) the purple color is washed out and a secondary staining with safranin or fuchsin is necessary to give decolorized bacteria a pink or red color for visualization. The permeability of the cells to the decolorizer and the washing-out of the primary dye-iodine complex depend on the architecture of the cell wall. The latter contains an essential and ubiquitous peptidoglycan -or murein- layer, which results from the polymerization of β-1,4-linked N-acetyl-glucosamine (GlcNAc) and N-acetyl-muramic acid (MurNAc) disaccharide units, cross-linked by short peptide stems. If differences in the chemical structure of the individual motifs are rather small, the thickness of the peptidoglycan layer nevertheless drastically differs within bacteria. Bacteria with a thick peptidoglycan layer tend to retain the primary dye of the Gram protocol, while a thin peptidoglycan layer favors the washing-out of the dye-iodine complex. Nevertheless, peptidoglycan is not the exclusive component of the cell wall. Bacteria that give a negative staining in the Gram protocol tend to surrender their thin peptidoglycan layer with a lipid-containing outer-membrane, which is destabilized and washed-out by the addition of the ethanol decolorizer. In contrast, bacteria that give a positive staining in the Gram protocol present a surface layer that tends to be dehydrated upon the ethanol treatment. Some bacteria, such as the genera *Actinomyces*, *Corynebacterium* or *Mycobacterium*, yield a Gram-variable pattern with

this protocol. A detailed presentation of the cell wall of typical Gram-positive and Gram-negative bacteria follows, completed by a description of some of these specific Gram-indeterminate bacteria.

**Gram-positive bacteria**

Gram-positive bacteria encompass organisms such as the rod-shaped model *Bacillus subtilis*, the spheroid *Staphylococcus aureu*s, or the ovococcus *Streptococcus pneumoniae*. Because of their thick peptidoglycan layer, which prevents washing of the gentian violet dye, Gram-positive bacteria appear in purple with Gram-staining. Their wall is further characterized by a single membrane (plasma or inner-membrane) and the presence of different glycopolymers that are connected either to the peptidoglycan or to lipids of the plasma membrane. These cell wall glycopolymers threads through the peptidoglycan layers towards the bacterial cell surface, where they can shape physicochemical surface properties and biofilm formation, mediate interaction with host receptors or binding to phages, and initiate innate host defenses and inflammation, T-cell or complement activation, or opsonization. In addition, the surface of Gram-positive bacteria can be covered with protective surface structures, such as capsular polysaccharides or surface layer (S-layer) proteins, which are highly variable among bacterial species and modulate all the previously described activities.

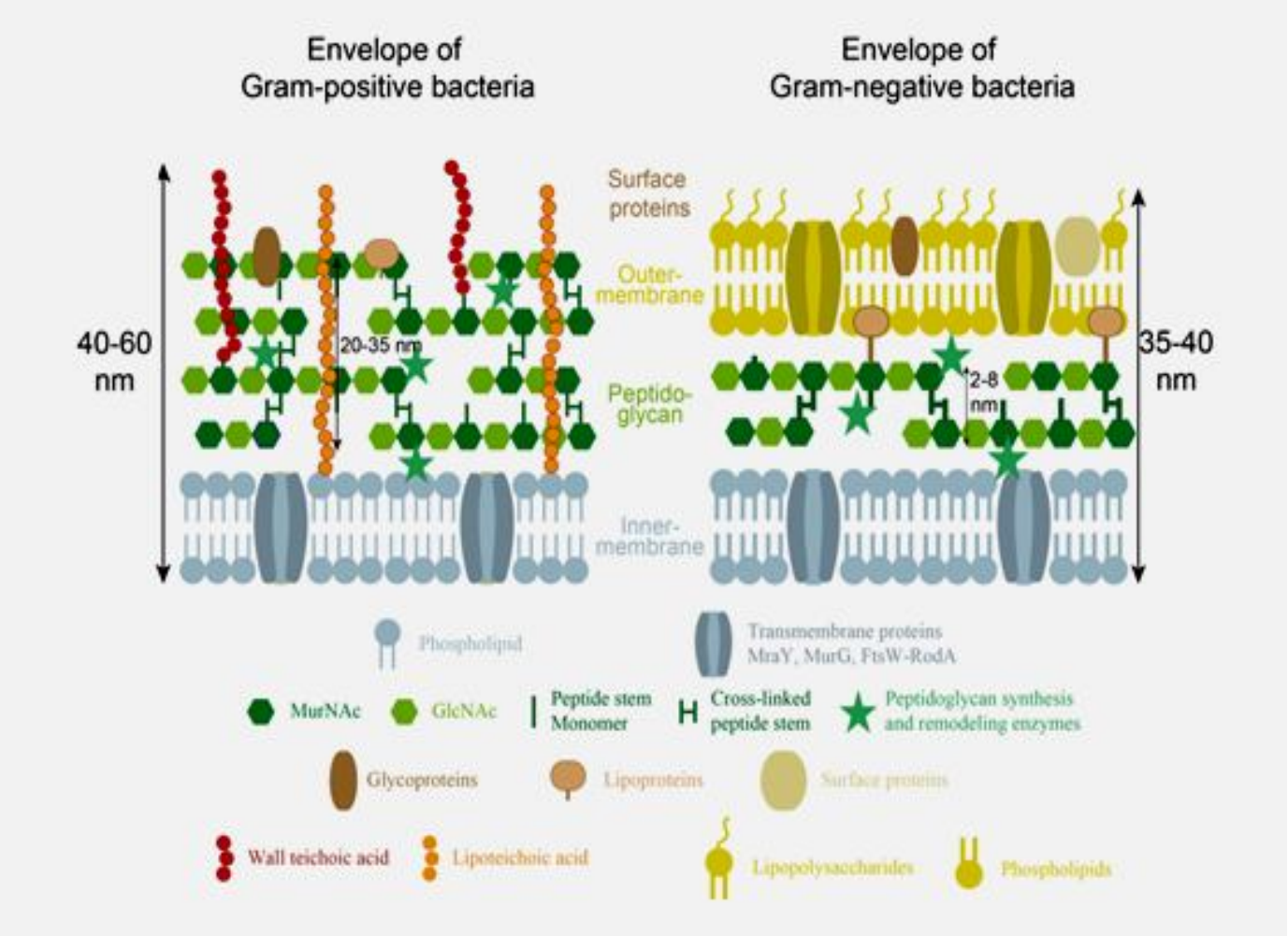



***Figure 2 : Schematic organization and main components of the bacterial cell-envelope of (A) Gram-positive and (B) Gram-negative bacteria. Details on the typical thicknesses of the cell envelope and constituting layers of different bacterial species can be found in Vollmer & Seligman, 2010. Adapted from Silhavy et al., 2010.***

The peptidoglycan-anchored cell wall glycopolymers are usually covalently linked to the peptidoglycan N-acetylmuramic acid through a phosphorylated disaccharide composed of N-acetylglucosamine and another sugar. The glycopolymer itself can be zwitterionic, as in most teichoic acids, anionic as in most teichuronic acids, or neutral when other sugars such as mannose or galactose are involved. Teichoic acids, as in *S. aureus* Xia et al., 2010, are generally formed by repeats of polyglycerol and/or polyribitol phosphate residues bound by phosphodiesters. Their zwitterionic properties come from the negative charge of their phosphate groups in physiological conditions, balanced with the amino extremities of the D-alanine polyol elements. The structure of the membrane-anchored glycopolymers is usually less diverse than

their peptidoglycan-linked analogs. They usually consist of lipoteichoic acids containing glycerol-phosphate repeating units that are connected to lipids through a glycerol-disaccharide anchor. However, more complicated lipoteichoic acid structures have also been described, such as the ribitol tetrasaccharide motif of *Streptococcus pneumoniae*.

**Gram-negative bacteria**

Gram-negative bacteria include the well-known *Escherichia coli* or the crescent-shaped model *Caulobacter crescentus*. The lighter color of these bacteria by Gram coloration is due to the inability of these organisms to retain the purple gentian violet dye during the washing step. Indeed, they possess a much thinner peptidoglycan layer than Gram-positive bacteria, but this is enclosed in the periplasmic space between an inner (cytoplasmic) and an outer membrane. The outer-membrane is an asymmetrical membrane composed of phospholipids and glycolipids (or LipoPolySaccharides ; LPS), at the inner and outer leaflets, respectively. The latter molecules consist of a lipid A anchor linked to a core oligosaccharide, that can be extended with an O-antigen polysaccharide of variable length which protects the cell against macrophages and complement system from the innate immune response . Lipopolysaccharides form an almost impermeable layer at the surface of Gram-negative bacteria that acts as a protective barrier against antibiotics and other antimicrobial molecules, and more generally hydrophobic molecules.

Embedded in the outer-membrane, proteins can also be found. With a few exceptions, they can be divided in two classes, lipoproteins and β-barrel proteins. The former contain a lipid moiety generally covalently linked to a cysteine residue and are mainly localized in the inner leaflet of the membrane. One of them, the highly abundant Braun's lipoprotein (also known as Lpp), is actually essential in *E. coli* for cell wall integrity as it is covalently linked to peptidoglycan to tether the outer membrane to the murein layer. Alternatively, β-barrel proteins are transmembrane proteins that allow the passive diffusion of small molecules, such as sugars and amino acids, through the outer membrane (porins), or that function as gated channels for the transport of high affinity ligands such as Fe-chelates or vitamins. Some additional proteins, usually specific to the bacterial species can also be found in the outer membrane.

The periplasmic space delimited by the inner- and outer-membranes not only contains the peptidoglycan polymer, but also a high density of various proteins and chaperones, which are involved in the biosynthesis or maturation of the peptidoglycan and cell envelope polymers, but

also in sugar and amino acid transport and chemotaxis. Secretion systems for example recruit a number of proteins from the cytoplasm to the outer-membrane, that associate to form a dynamical trans-envelope assembly which can release proteins, DNA or toxins in the medium or to prokaryotic or eukaryotic cell.