- **The periplasmic space:**

1) The space between the inner and outer membranes, called the periplasmic space, contains the peptidoglycan layer and a gel-like solution of proteins.
2) The periplasmic space is approximately 20–40% of the cell volume, which is far from insignificant.
3) The periplasmic proteins include:
   a) Binding proteins for specific substrates (ex, amino acids, sugars, vitamins, and ions).
   b) Hydrolytic enzymes (ex, alkaline phosphatase and 5′-nucleotidase) that break down non-transportable substrates into transportable ones.
   c) Detoxifying enzymes (ex, β-lactamase and aminoglycoside-phosphorylase) that inactivate certain antibiotics.
4) The periplasmic space also contains membrane-derived oligosaccharides appear to play a role in osmo-regulation.

- **The cell wall**

1) The internal osmotic pressure of most bacteria ranges from 5 to 20 atm as a result of solute concentration via active transport.
2) In most environments, this pressure would be sufficient to burst the cell were it not for the presence of a high-tensile-strength cell wall.
3) The bacterial cell wall owes its strength to a layer composed of a substance variously referred to as murein, mucopeptide, or peptidoglycan.
4) Most bacteria are classified as gram positive or gram negative according to their response to the Gram-staining procedure.
5) This procedure was named for the histologist Hans Christian Gram, who developed this differential staining procedure in an attempt to stain bacteria in infected tissues.

6) The Gram stain depends on the ability of certain bacteria (the gram-positive bacteria) to retain a complex of crystal violet (a purple dye) and iodine after a brief wash with alcohol or acetone.
7) Gram-negative bacteria do not retain the dye–iodine complex and become translucent, but they can then be counterstained with safranin (a red dye).
8) Thus, gram-positive bacteria look purple under the microscope, and gram-negative bacteria look red. The distinction between these two groups turns out to reflect fundamental differences in their cell envelopes (Table 1).
9) In addition to giving osmotic protection, the cell wall plays an essential role in cell division as well as serving as a primer for its own biosynthesis.
10) Various layers of the wall are the sites of major antigenic determinants of the cell surface, and one component the lipopolysaccharide of gram-negative cell walls is responsible for the nonspecific endotoxin activity of gram-negative bacteria.
11) The cell wall is, in general, non-selectively permeable; one layer of the gram-negative wall, however the outer membrane hinders the passage of relatively large molecules.

**Table 1: comparison of features of gram negative and gram-positive bacterial cell wall.**

| **Character** | **Gram positive** | **Gram negative** |
|---|---|---|
| Color of Gram Stained Cell | Purple | Reddish-pink |
| Peptidoglycan | Thick layer | Thin layer |
| Teichoic acids | Present | Absent |
| Outer membrane | Absent | Present |

| | | |
|---|---|---|
| Lipopolysaccharide (endotoxin) | Absent | Present |
| Porin proteins | Absent (unnecessary because there is no outer membrane) | Present; allow passage of molecules through outer membrane |
| Periplasm | Absent | Present |
| Sensitivity to Penicillin | Generally more susceptible (with notable exceptions) | Generally less susceptible (with notable exceptions) |
| Sensitivity to Lysozyme | Yes | no |

- **Outer membrane**

1) The outer membrane is chemically distinct from all other biological membranes.
2) It is a bilayered structure; its inner leaflet resembles in composition that of the cell membrane and its outer leaflet contains a distinctive component, a lipopolysaccharide (LPS).
3) As a result, the leaflets of this membrane are asymmetrical, and the properties of this bilayer differ considerably from those of a symmetrical biologic membrane such as the cell membrane.
4) The ability of the outer membrane to exclude hydrophobic molecules is an unusual feature among biologic membranes and serves to protect the cell (in the case of enteric bacteria) from deleterious substances such as bile salts.
5) Because of its lipid nature, the outer membrane would be expected to exclude hydrophilic molecules as well.
6) However, the outer membrane has special channels, consisting of protein molecules called porins that permit the passive diffusion of low-molecular weight hydrophilic compounds such as sugars, amino acids, and certain ions.

- **The exterior components of the cell wall**

- **Extracellular polymeric substance (EPS)**

  ❖ **Capsule and Glycocalyx**

1) Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environments.
2) The terms capsule and slime layer are frequently used to describe polysaccharide layers; the more inclusive term glycocalyx is also used.
3) Glycocalyx is defined as the polysaccharide-containing material lying outside the cell.
4) A condensed, well-defined layer closely surrounding the cell that excludes particles, such as India ink, is referred to as a capsule.
5) If the glycocalyx is loosely associated with the cell and does not exclude particles, it is referred to as a slime layer.
6) Extracellular polymer is synthesized by enzymes located at the surface of the bacterial cell.
7) The capsule contributes to the invasiveness of pathogenic bacteria encapsulated cells are protected from phagocytosis unless they are coated with anticapsular antibody.
8) The glycocalyx plays a role in the adherence of bacteria to surfaces in their environment, including the cells of plant and animal hosts.
9) Because outer polysaccharide layers bind a significant amount of water, the glycocalyx layer may also play a role in resistance to desiccation.

- **Flagella**

1) Bacterial flagella are thread-like appendages composed entirely of protein, 12–30 nm in diameter.
2) They are the organs of locomotion for the forms that possess them.
3) Three types of arrangement are known:

a) Monotrichous (single polar flagellum).
b) Lophotrichous (multiple polar flagella).
c) Peritrichous (flagella distributed over the entire cell).

4) A bacterial flagellum is made up of several thousand molecules of a protein subunit called flagellin.
5) The flagellum is formed by the aggregation of subunits to form a helical structure.
6) If flagella are removed by mechanically agitating a suspension of bacteria, new flagella are rapidly formed by the synthesis, aggregation, and extrusion of flagellin subunits.
7) Motility is restored within 3–6 minutes.
8) The flagellins of different bacterial species presumably differ from one another in primary structure.
9) They are highly antigenic (H antigens), and some of the immune responses to infection are directed against these proteins.

- **Pili and Fimbriae**

1) Pili and fimbriae cell surface appendages present in bacterial cell other than flagella. These structures are not for locomotion.
2) They are shorter and finer than flagella; similar to flagella, they are composed of structural protein subunits termed pilins.
3) The differences between pili and fimbriae are listed in the following table 2:

**Table 2: The differences between pili and fimbriae**

| Pili | Fimbriae |
|---|---|
| Long, thicker, tubular structures made up of protein pilin | Thin and shorter than pili |
| Found only in gram negative bacteria | Found in both gram negative and gram positive bacteria |
| The number of pili are less (3-5) per cell | The number of fimbriae are 300-400 per cell |
| Formation of pili is governed by plasmid genes | Formation of fimbriae is governed by bacterial genes in nucleoid region |
| Pili involved in cell to cell attachment during bacterial conjugation so called sex-pili | Fimbriae involved in the adherence of symbiotic and pathogenic bacteria to host cells |