MICROBIOLOGY

Subject : Microbiology
(For undergraduate students)

.

Composition, Structure & Bio-synthesis of Cell Wall in Gram(+) & Gram(-) Bacteria

Lecture No. & Title : 1
Structure & Function of Bacterial Cell Wall (Gram + & - Bacteria)

SCRIPT

Bacterial cell wall (Part-1)

Location: The bacterial cell wall is the outermost rigid protective layer of a bacterial cell, it is the outermost layer in case of majority of bacteria, only in case of some bacteria, it is protected by an outer capsule or glycocalyx

layer. The cell wall covers the cell membrane below it. It can constitute up to 50% of the bacterial cell and can actually provide the characteristic shape of the bacterial cell.

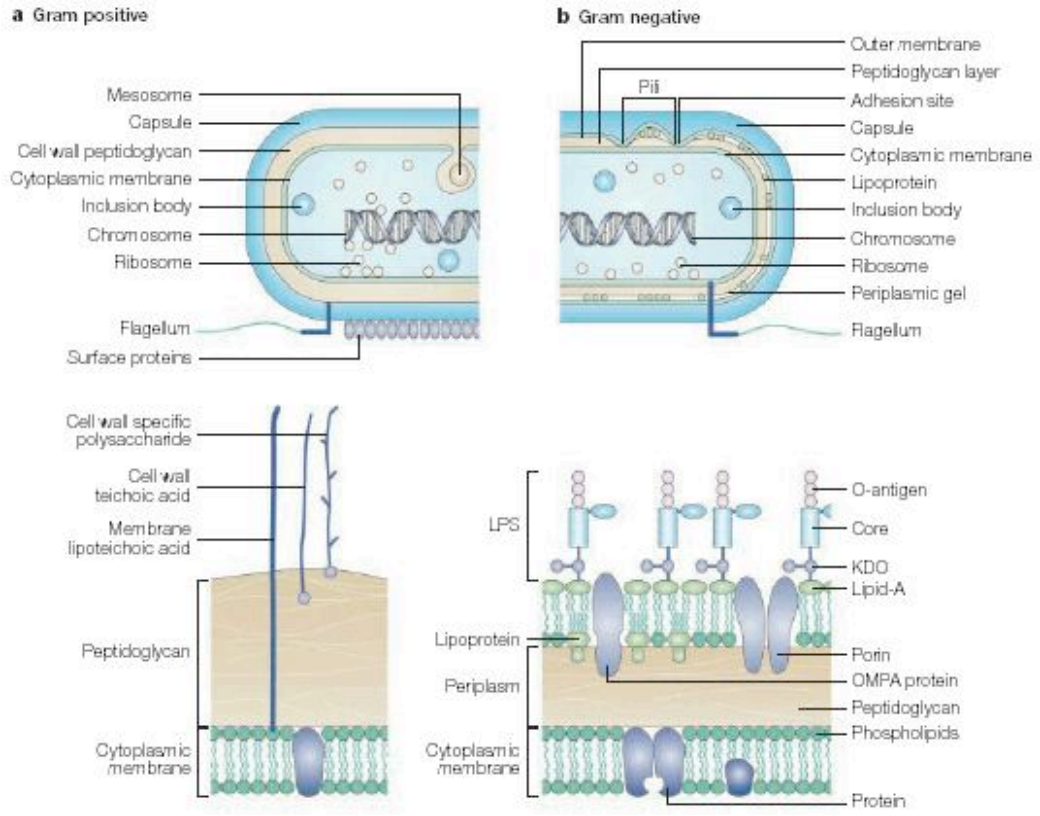

Fig.1. Basic pattern of Gram positive and Gram negative bacterial cell wall.

Types: The structure and the function of the Gram positive and Gram negative bacteria are discussed in the ongoing paragraphs: The cell wall structures of the other prokaryotes including the structure of acid fast bacteria will be discussed in the subsequent lectures.

i) The Gram-positive cell wall

The Gram positive cell wall is thicker and has the following components:

- Major component (50%) is peptidoglycan
- No lipid and often no protein.
- Accessory polymers (Teichoic acid and/or teichuronic acid) remains covalently linked to peptidoglycan.

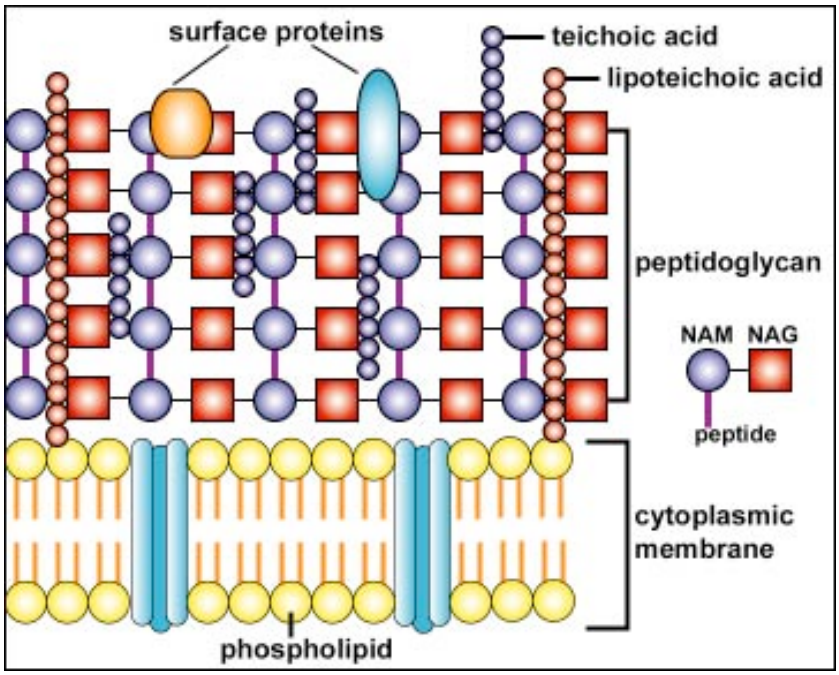

Fig.2 Pattern of Gram positive cell wall

Peptidoglycan (also called murein or mucopeptide).

Present in almost all bacteria (exceptions: wall-less Mycoplasma; archaebacteria). It is unique to bacteria providing the common architecture of the bacterial cell.

It is formed by the repetitive units of a disaccharide formed of amino sugars like N Acetyl Glucosamine and N Acetyl Muramic acid , linked by Beta -1,4 glycoside linkage. These heteropolysaccharides are joined by amino acid cross linkage, the proportion of chains cross-linked varies, in *Staphylococcus aureus*; it may be up to 100% of the total chains. Up to 10 glycan chains may be attached via the cross linkages.

The cross linkage is usually formed of L Lys-D Ala (Glu)-L: Lys-D Ala and is usually termed as the tetrapeptide.These links are either directly connected to the glycan chain or they may be interconnected by the pentaglycine chain connecting the third and the fourth amino acid of the tetra peptide. Bacteria within the Deinococcus-Thermus group may also exhibit Gram positive staining behavior but contain some cell wall structures typical of Gram negative organisms.

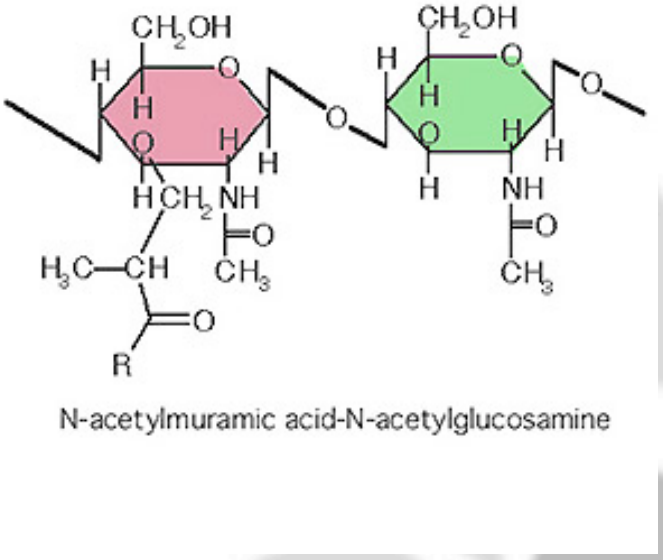

Fig.3. Disaccharide of NAG and NAM

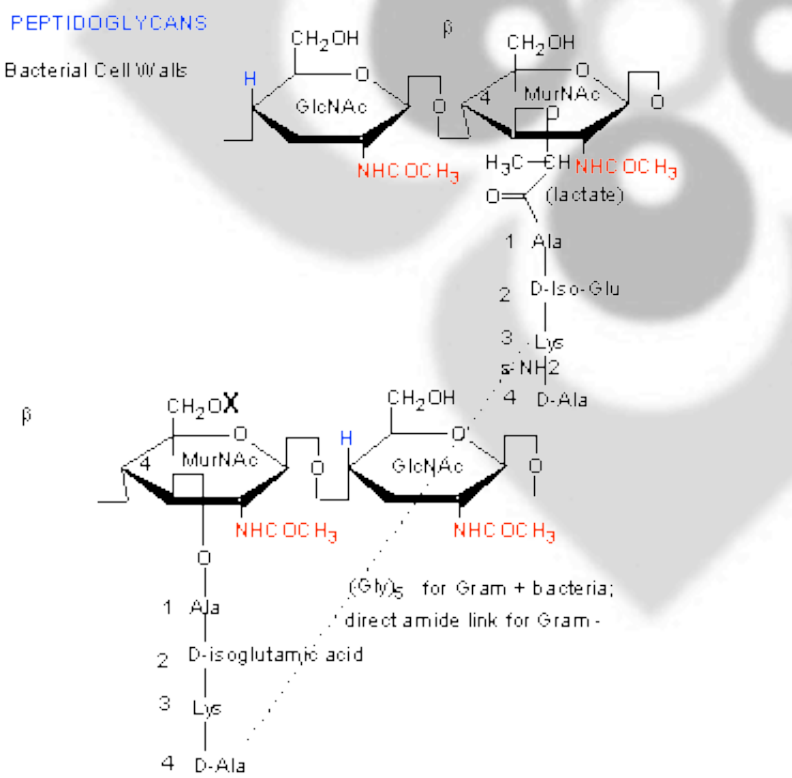

Fig.4: NAG-NAM layer with tetra peptide cross linkage.

Teichoic acids: They are embedded in the Gram positive cell wall and sometimes may be connected to lipid typically have a backbone of (polyol-phosphate)$_n$, usually with sugars and/or the amino acid D-alanine as substituents. The polyol is usually ribitol (C5) or glycerol (C3), but a few examples of mannitol (C6) are also known. They are probably involved in uptake of Mg2+ by the cell and also connect the cell wall with the cytoplasmic membrane. They also give the Gram positive cell wall an overall negative charge due to the presence of phosphodiester bonds between Teichoic acid monomers.

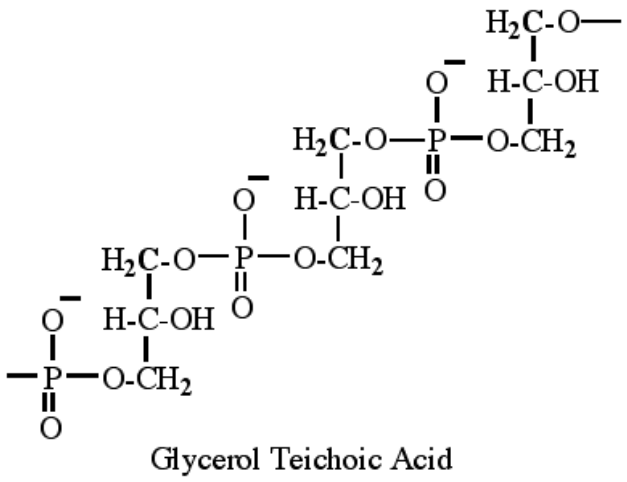

Fig.5: Structure of Glycerol Teichoic acid.

Teichuronic acids: Acidic polysaccharides (contain uronic acids). Production stimulated when cell growth limited by supply of P (the available P is used for DNA, RNA,

phospholipids rather than Teichoic acids. They are functionally interchangeable with Teichoic acid.

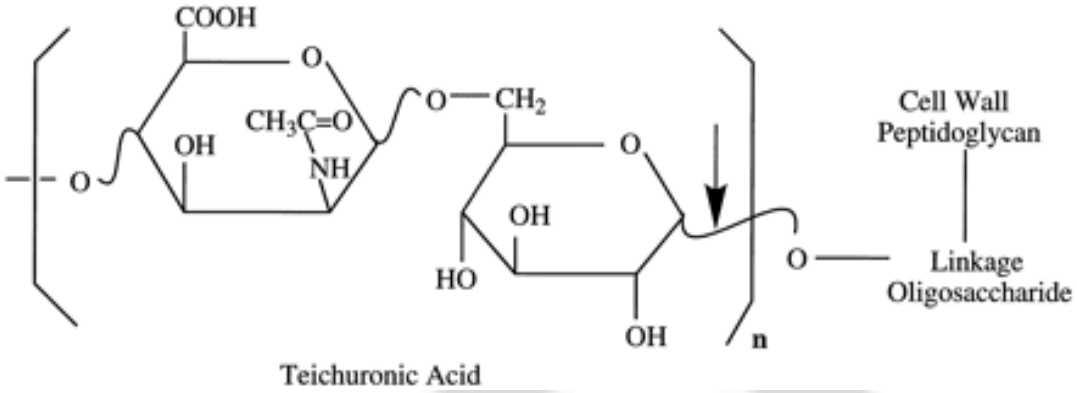

Fig.6: Structure of Teichuronic acid.

Surface Layer: The Surface layer is associated with the peptidoglycan, IS-layers contain surface layer homology (SLH) domains, through which the binding occurs to the peptidoglycan and to a secondary cell wall polymer (e.g., Teichoic acids). In the absence of SLH domains, the binding occurs via electrostatic interactions between the positively charged N-terminus of the S-layer protein and a negatively charged secondary cell wall polymer. It provides a provision for periplasmic compartment in Gram-positive bacteria together with the peptidoglycan and the cytoplasmic membranes.

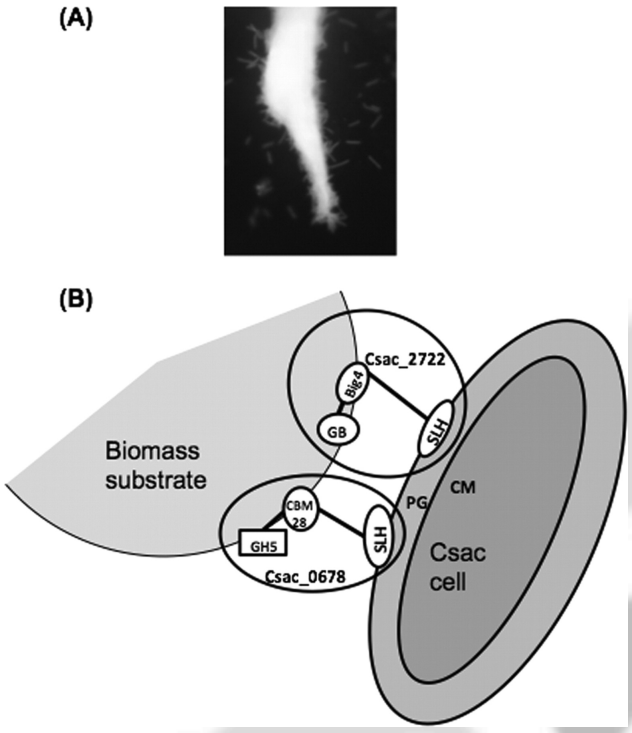

Fig.7: Surface layer homology domain represented by Cesc domain protein

Periplasmic space: There is no **periplasmic space** in Gram-positive bacteria because there is only one biological membrane, the cytoplasmic membrane, but a region termed **inner wall zone (IWZ)** has been observed between the cytoplasmic membrane and the mature cell wall.

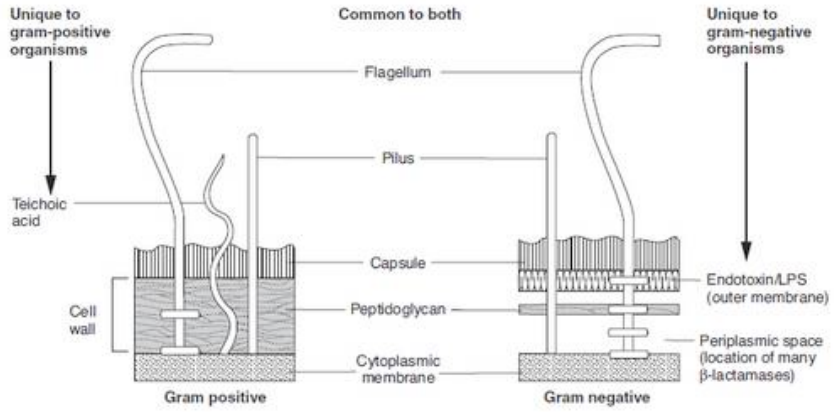

Fig.8: Comparison of Periplasmic space in Gram positive and Gram negative bacteria

Functions:

1. The peptidoglycan serves as the store house of polysaccharides.
2. They also store important amino acids.
3. They impart definite shape to the bacterial cell.
4. They act as the suitable site for the action of different cell wall biosynthesis inhibiting β lactam antibiotics.
5. They make the cell surface negatively charged.
6. They supply phosphates for the different cellular metabolic activities.

ii) The Gram negative cell wall

The cell envelope consists of a pair of membranes (cytoplasmic and the outer membrane) with a thin, intermediate layer of **peptidoglycan.**

The outer membrane contains **lipopolysaccharide (LPS)** as well as lipids and proteins. LPS is located exclusively in the outer leaflet: lipid embedded in the membrane, polysaccharide protruding.

Lipids: They may include the phospholipids, confined to the inner leaflet of the outer membrane

Proteins: Several types asymmetrically placed in the outer membrane. The two major types are the transmembrane proteins or porins - (often trimeric assemblies) form aqueous channels across the outer membrane. The lipoproteins anchor the outer membrane to the peptidoglycan layer (lipid inserted into the inner leaflet, protein partly covalently attached to peptidoglycan, *e.g.* 1 in 10 molecules in *E. coli*).

The detailed structure of the Gram-negative bacterial cell wall includes the following layers:

1. Lipopolysaccharide layer.
2. Surface membrane layer with porin.
3. Outer periplasmic space.
4. Peptidoglycan layer.
5. Inner periplasmic space.
6. Inner membrane.

1. Lipopolysaccharide Layer:

It is a tripartite structure conserved in Gram-negative bacteria consisting of O-antigens, core polysaccharide and lipid A. It is essential for the structural integrity and viability of the bacteria. The chemical structure of the outer membrane lipopolysaccharides is often unique to specific bacterial strains (i.e. sub-species) and is responsible for many of the antigenic properties of these strains.

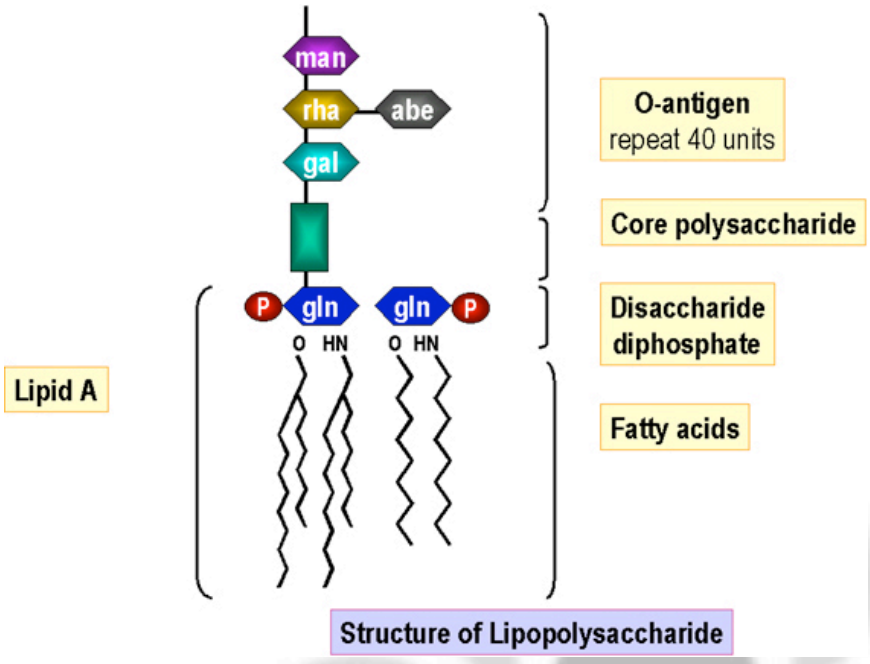

Fig.9: Different parts of LPS.

LPS acts as an endotoxin, and hence majority of Gram negative bacteria induce a strong response from normal animal immune systems. It binds the different receptors like CD14, TLR4, MD2 receptor complex, and promotes the secretion of pro-inflammatory cytokines in many cell types like macrophages and B cells. It has also been implicated in non-pathogenic aspects of bacterial ecology, including surface adhesion, bacteriophage sensitivity, and interactions with predators such as amoebae.

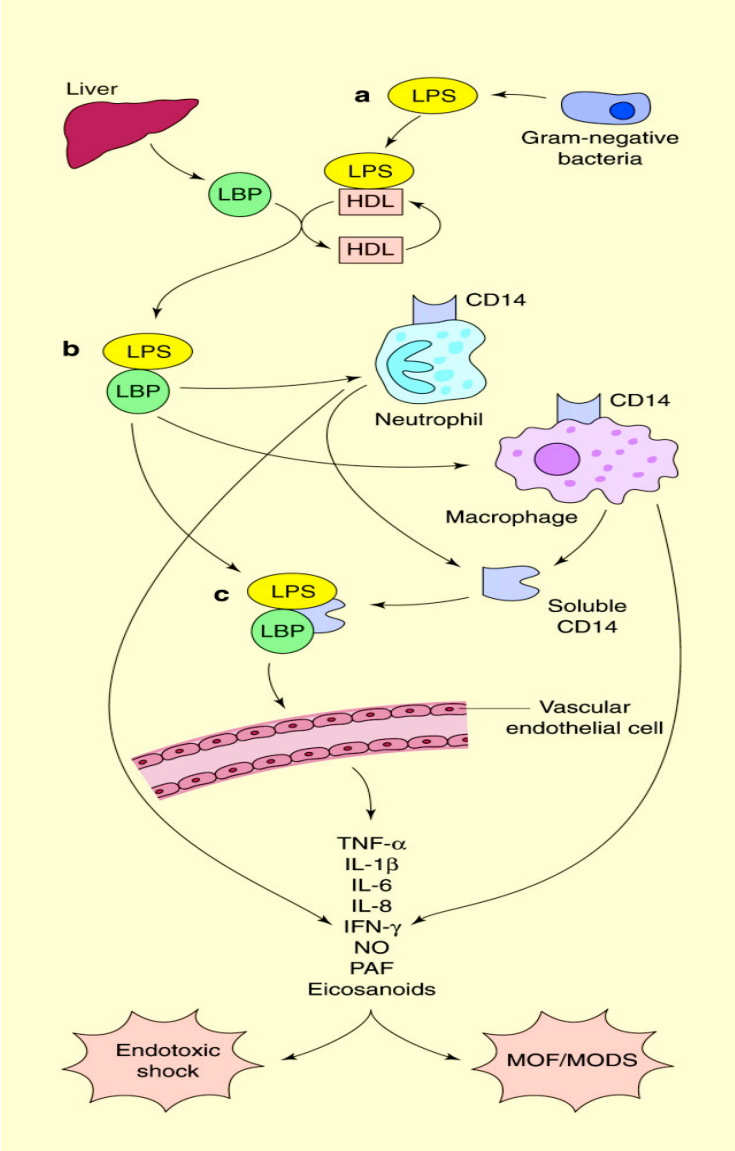

Fig.10: Binding of LPS with cell receptor and activation of immune system, finally causing sepsis.

LPS is required for the proper conformation of Omptin (barrel like protein representing bacterial aspartate protease) activity; however, smooth LPS will sterically hinder omptins. It also acts as an exogenous pyrogen

(external fever-inducing substance). It can be used as a potential target to design novel anti-microbial substances.

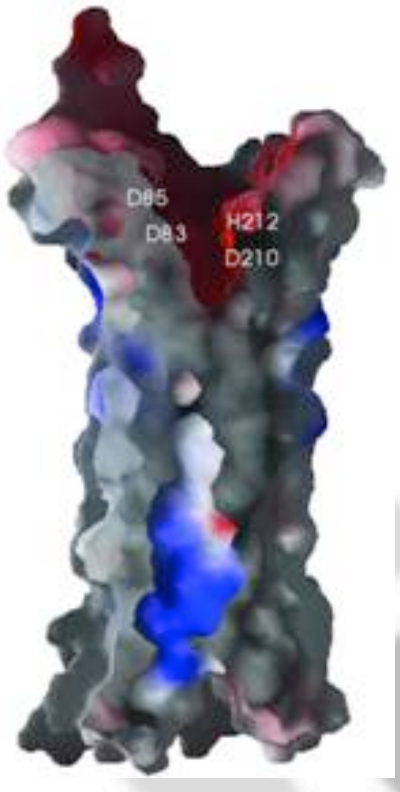

Fig.11: Structure of Omptin.

O-antigen

A repetitive glycan polymer contained within an LPS is referred to as the O antigen or O polysaccharide, or O side-chain of the bacteria. It is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different *E. coli* strains The

presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more hydrophobic. As because it is representing the outermost molecule of the Gram-negative bacterial cell, they are easily targeted by the antibodies. O-antigens (the outer carbohydrates) are the most variable portion of the LPS molecule, imparting the antigenic specificity.

Core oligosaccharide

The Core domain always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO *i.e.* keto-deoxyoctulosonate). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substituents.

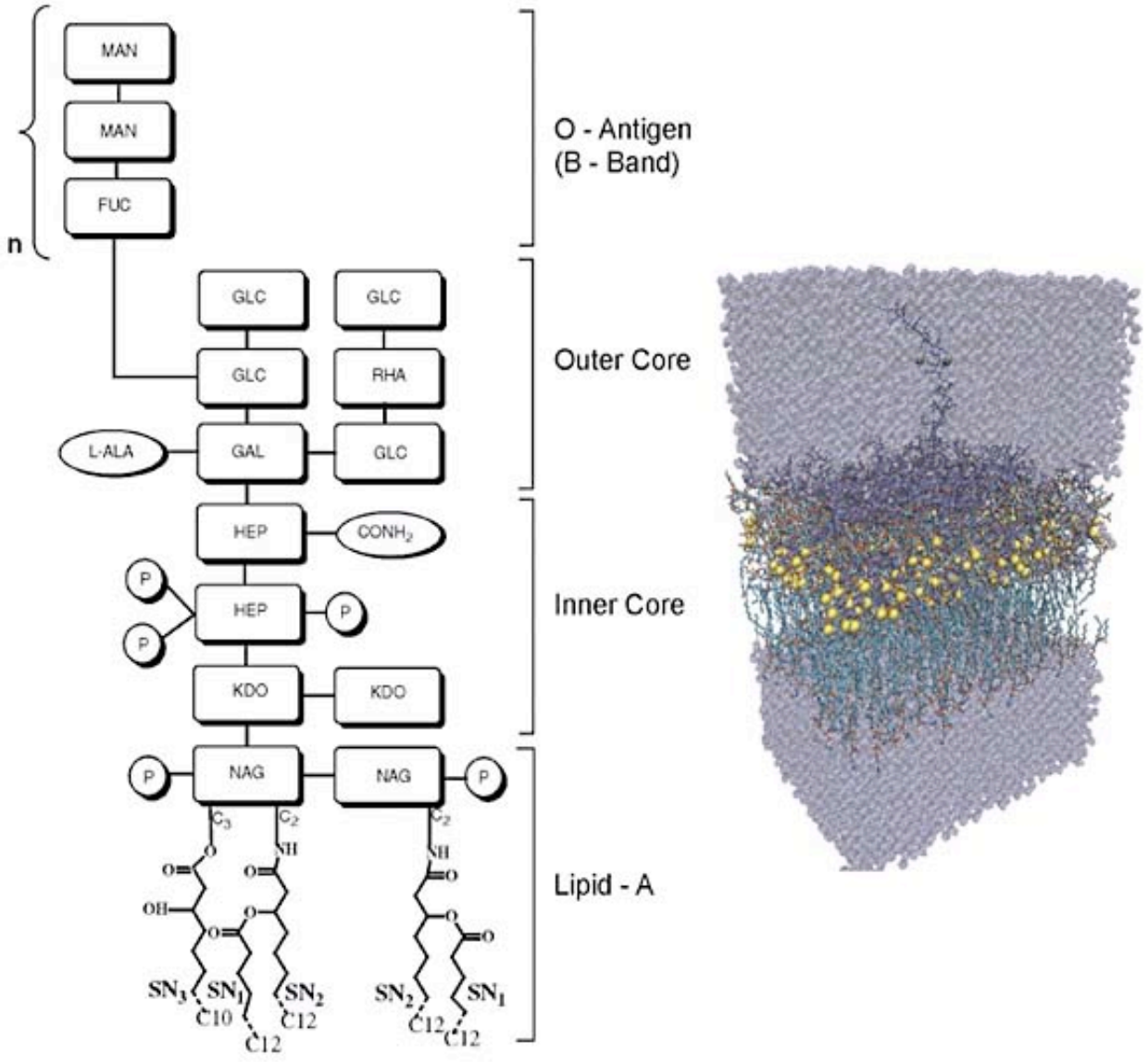

Figure 1. Schematic representation of a LPS unit (NAG: N-acetyl-D-glucosamine; P: phosphatidyl group; KDO: 3-Deoxy-D-manno-octulosonic Acid; HEP: heptose; GAL: D-galactose; GLC: D-glucose; L-ALA: L-alanine; RHA: D-rhamnose; FUC: D-fucose; MAN: D-manose). Acyl lipid chains SN_1,SN_2 and SN_3 are labeled (right). Atomistic model of the A-B+ LPS membrane of *Pseudomonas aeruginosa* (left). Membrane atoms are represented in "sticks", Ca^{++} ions in filled yellow CPK and water in transparent blue CPK model.

Fig.12: Different parts of LPS molecule.(NAG-N-acetyl-D-glucosamine-Phosphate group, KDO-3-deoxy-D-mannooctulosonic Acid, HEP-heptose, GAL-D Galactose, GLC-D-glucose, L-ALA-L-alanine, RHA-D-Rhamnose,FUC-D-Fucose, MAN-D-manose)

Lipid A

Lipid A is, in normal circumstances, a phosphorylated glucosamine disaccharide attached to multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock). It is the innermost lipid component of the LPS, hydrophobic in nature and acts as an anchor of the LPS to the outer membrane. lipid A is the most conserved part. However, lipid A composition also may vary (e.g., in number and nature of acyl chains even within or between genera). Some of these variations may impart antagonistic properties to these LPS. For example *Rhodobacter sphaeroides* diphosphoryl lipid A (RsDPLA) is a potent antagonist of LPS in human cells, but is an agonist in hamster and equine cells.

It has been speculated that conical Lipid A (e.g., from *E. coli*) are more agonistic, less conical lipid A like those of *Porphyromonas gingivalis* may activate a different signal (TLR2 instead of TLR4), and completely cylindrical lipid A like that of *Rhodobacter sphaeroides* is antagonistic to TLRs.

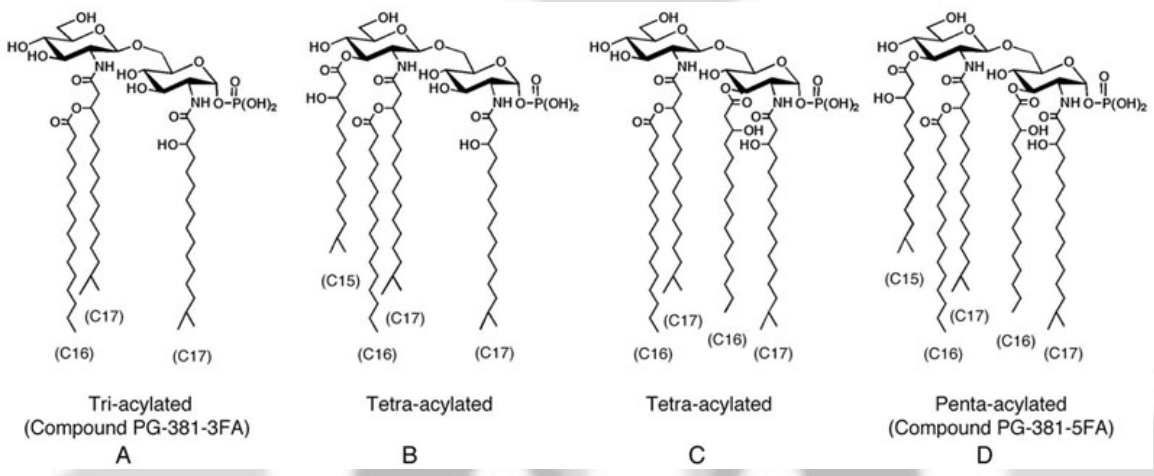

Fig.13: Different types of Lipid A with glucosamine disaccharide along with acetyl chain

Assembly of LPS:

LPS Final Assembly: O-antigen subunits are translocated across the inner membrane (by Wzx) where they are polymerized (by Wzy, chain length determined by Wzz) and ligated (by WaaL) on to complete Core-Lipid A molecules (which were translocated by MsbA).

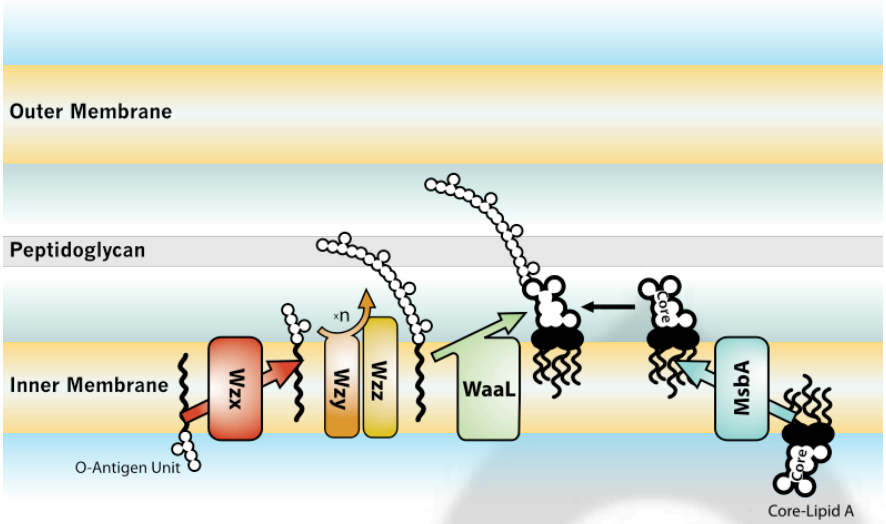

Fig.14: Assembly of different components of LPS

Completed LPS molecules are transported across the periplasm and outer membrane by the proteins Lpt A, B, C, D, E, F, and G.

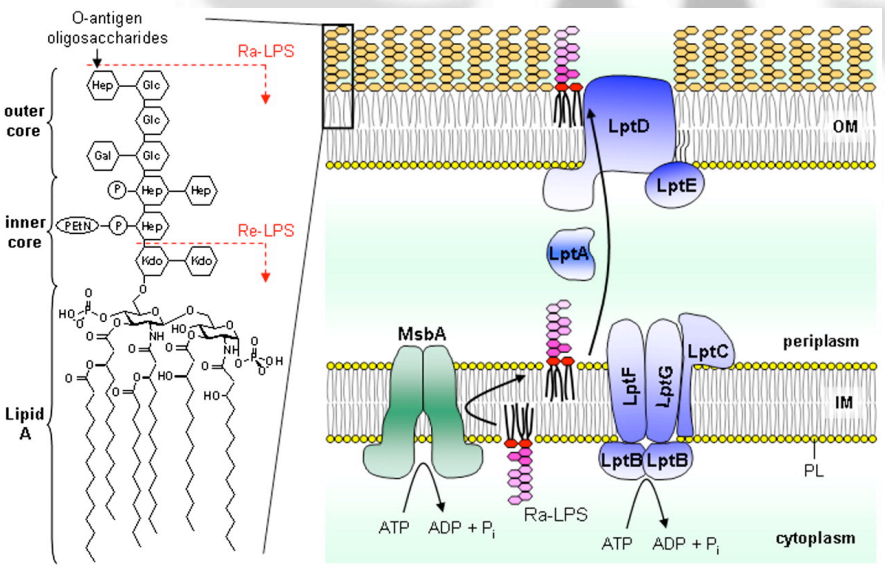

Fig.15: Transport of LPS from the inner to the outer membrane.

LPS modifications

The making of LPS can be modified in order to present a specific sugar structure. Those can be recognized by either other LPS (which enables to inhibit LPS toxins) or glycosyltransferases that use that sugar structure to add more specific sugars. It has recently been shown that a specific enzyme in the intestine (alkaline phosphatase) can detoxify LPS by removing the two phosphate groups found on LPS carbohydrates. This may function as an adaptive mechanism to help the host manage potentially toxic effects of Gram-negative bacteria normally found in the small intestine.

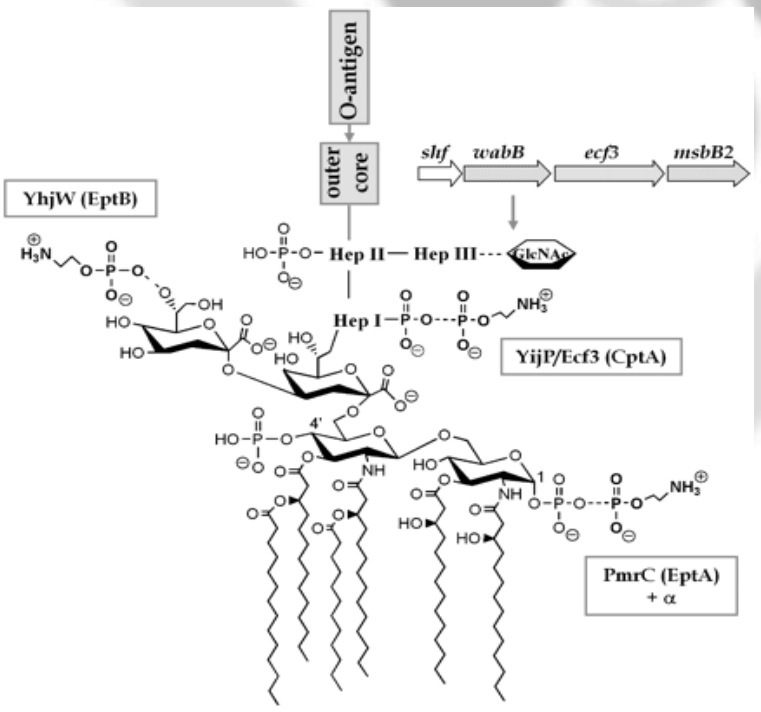

Fig.16: Probable sites of LPS modification

Functions of LPS:

1. It is acting as a hydrophobic receptor.
2. It activates the immune system.
3. It act as an endotoxin.
4. It is a target for antibodies.
5. It helps in antimicrobial drug design.
6. It brings about the septic shock.

2. Outer Membrane:

It is a phospholipid bilayer; the lipid portion of the outer membrane is largely impermeable to all charged molecules. However, channels called porins are present in the outer membrane that allow for passive transport of many ions, sugars and amino acids across the outer membrane. In Gram negative bacteria, it is a very important constituent of the outer membraneIt consists of a monomolecular layer composed of identical proteins or glycoproteins. This two-dimensional structure is formed by self-assembly and encloses the whole cell surface. Thus, the S-layer protein can represent up to 10–15% of the whole protein content of a cell. S-layer proteins are poorly conserved and can differ markedly even between related species. Depending on

species, the S-layers have a thickness between 5 and 25 nm and possess identical pores with 2–8 nm in diameter. S-layers exhibit an oblique (p1, p2), square (p4) or hexagonal (p3, p6) lattice symmetry. Depending on the lattice symmetry, the S-layer is composed of one (P1), two (P2), three (P3), four (P4), or six (P6) identical protein subunits, respectively. The space between these subunits ranges between 2.5 and 35 nm.

Function of S layer:

1. It provides protection against bacteriophage, and phagocytes
2. It gives resistance against low pH
3. It provides a barrier against high-molecular-weight substances (e.g., lytic enzymes)
4. It is a medium of adhesion (for glycosylated S-layers)
5. It stabilizes the membrane
6. It provides a provision of adhesion sites for exoproteins.

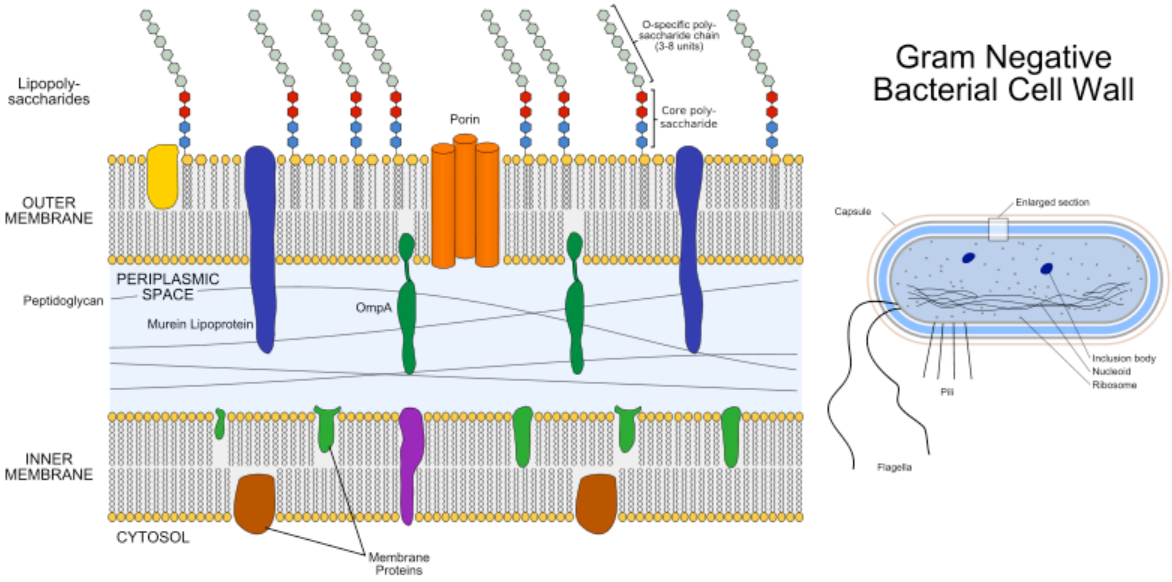

Fig.17: Different layers of Gram negative bacteria showing the surface layer

Porins: They are cylindrical proteins that cross a cellular membrane and act as a pore through which molecules can move in or move out. Unlike other membrane transport proteins, porins are large enough to allow passive diffusion, i.e., they act as channels that are specific to different types of molecules. They are present in the outer membrane of Gram-negative bacteria.

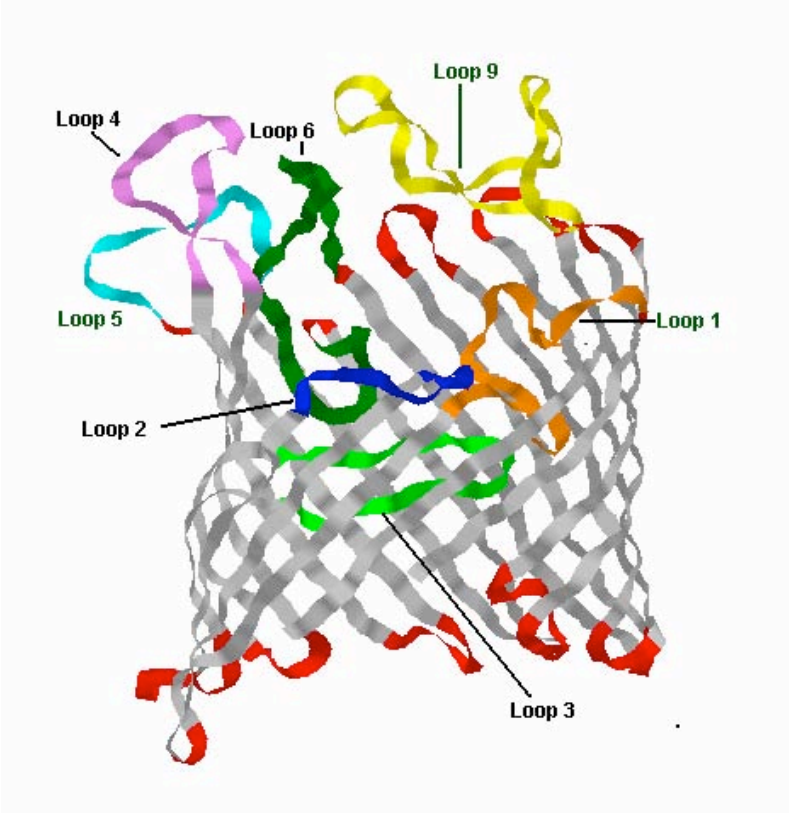

Fig.18: Different loops of Porin

Porins are composed of β strand protein which is, in general, linked together by beta turns on the cytoplasmic side and long loops of amino acids on the other. The β strands lie in an antiparallel fashion and form a cylindrical tube, called a β barrel. The amino acid composition of the porin β strands is unique in that polar and nonpolar residues alternate between themselves. This means that the non-polar residues face outward so as to interact with the non-polar lipid membrane, whereas the polar residues face inwards

into the center of the beta barrel to interact with the aqueous channel. The phospholipids that compose the outer membrane give it the same semi-permeable characteristics as the cytoplasmic membrane.

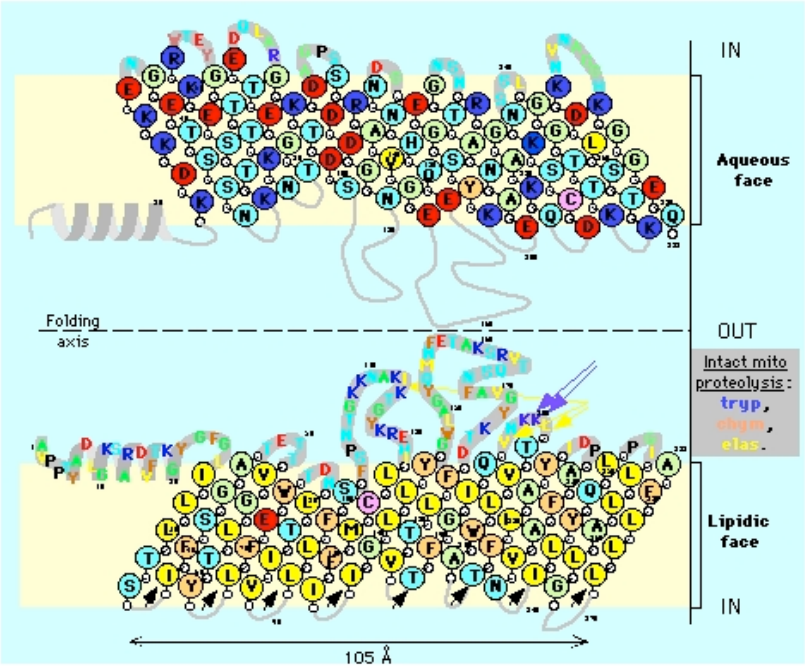

Fig.19: Inner lipophilic and outer hydrophilic surface of the porin channel.

The porin channel is partially blocked by a loop, called the eyelet, which projects into the cavity. In general, it is found between strands 5 and 6 of each barrel, and it defines the size of solute that can traverse the channel. It is lined almost exclusively with charged amino acids arranged on opposite sides of the channel, creating a transversal electric

field across the pore. The eyelet has a local surplus of negative charges from four glutamic acid and seven aspartic acid residues (in contrast to one histidine, two lysine and three arginine residues) is partially compensated for by two bound calcium atoms, and this asymmetric arrangement of molecules is thought to have an influence in the selection of molecules that can pass through the channel.

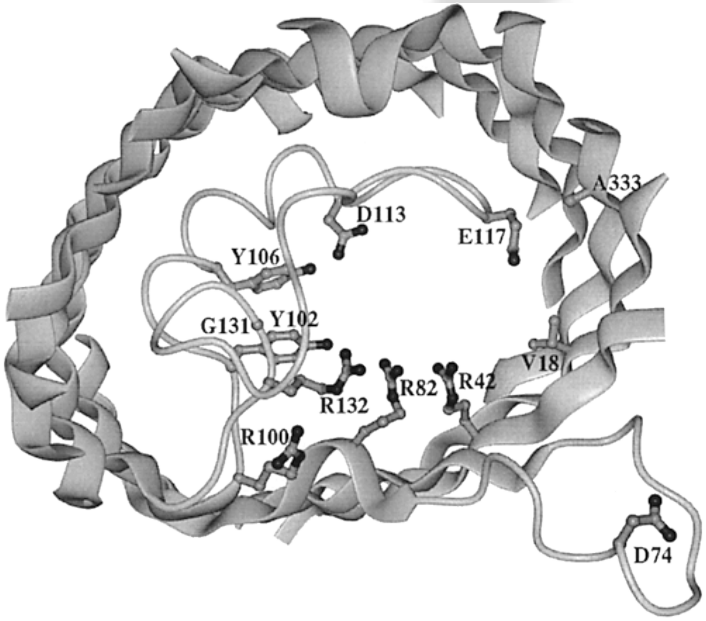

Fig.20: Surface loop of porin channel

Functions:

1. They act channel proteins helping in inter cellular transport.
2. Porins typically control the diffusion of small metabolites like sugars, ions, and amino acids.

3. In gram-negative bacteria, the inner membrane is the major permeability barrier, whereas the outer membrane contains porins, which render it largely permeable to molecules less than about 1.5 kDa.
4. Beta-lactam and fluoroquinolone antibiotics usually pass through porins to reach their targets in gram negative bacteria[. Bacteria can develop resistance to these antibiotics by mutating the gene that encodes the porin – the antibiotics are then excluded from passing through the outer membrane and thus become ineffective to kill the bacterium.
5. Balances the hydrophobicity and the hydrophilicity of the multi-layered cell wall and thus provide structural stabilization.

3. Periplasmic Space:

The periplasmic space is actually the intermediate space between the outer and inner membrane of the didermic bacteria or the Gram negative bacteria having two layered protective layers. This layer represents a homogeneous matrix having the peptidoglycan layer and many proteins responsible for substrate binding or hydrolysis and reception

of extra cellular signals. The periplasm is thought to exist as a gel-like state rather than a liquid due to the high concentration of proteins and peptidoglycan found within it. Because of the presence of the peptidoglycan within, the periplasm may be classified as external and internal periplasmic space.

In addition to the peptidoglycan, the periplasm includes solutes such as ions and proteins, which are involved in wide variety of functions ranging from nutrient binding, transport, folding, degradation, substrate hydrolysis, to peptidoglycan synthesis, electron transport. The periplasm is devoid of ATP.

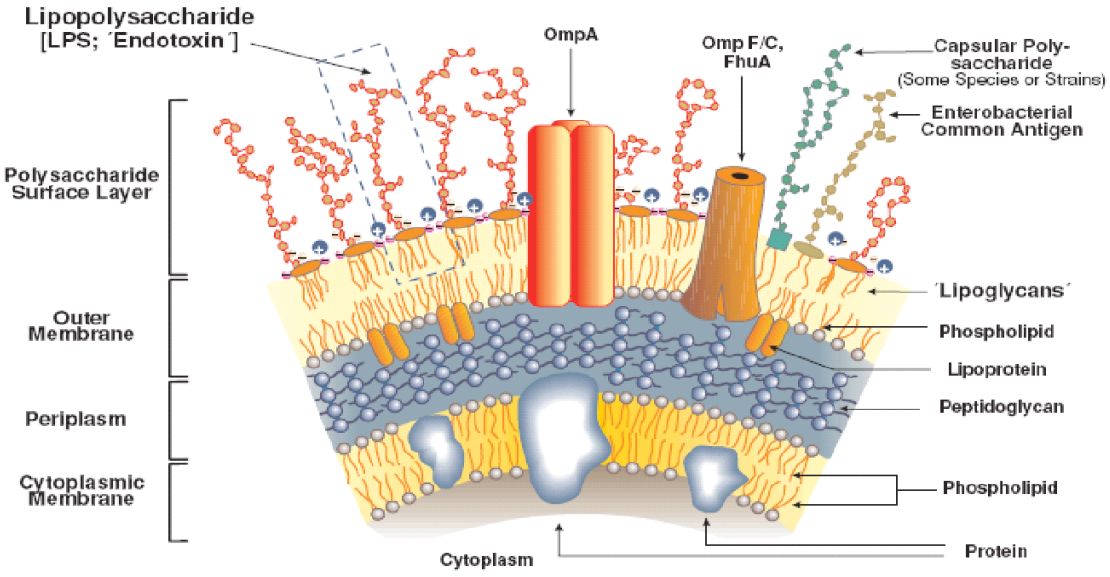

Fig.21: Periplasmic space having peptidoglycan within.

Functions:

1. Binding, transport and degradation of nutrients.
2. Reception and transport of signals across the membrane.
3. Sometimes it may help in the degradation of xenobiotic molecules (molecules harmful for the cell).

4. Peptidoglycan layer

It constitutes only 15% of the entire cell wall of the Gram negative cell wall. It is formed of hetero-polysaccharides of NAG and NAM joined by β 1,4 glycoside linkage, the number of layers remain within five and the tetrapeptide cross linkage is formed of L Ala-D Glu-meso DAP (Di amino pimelic acid)-D Ala. The horizontal pentaglycine linkage is absent.

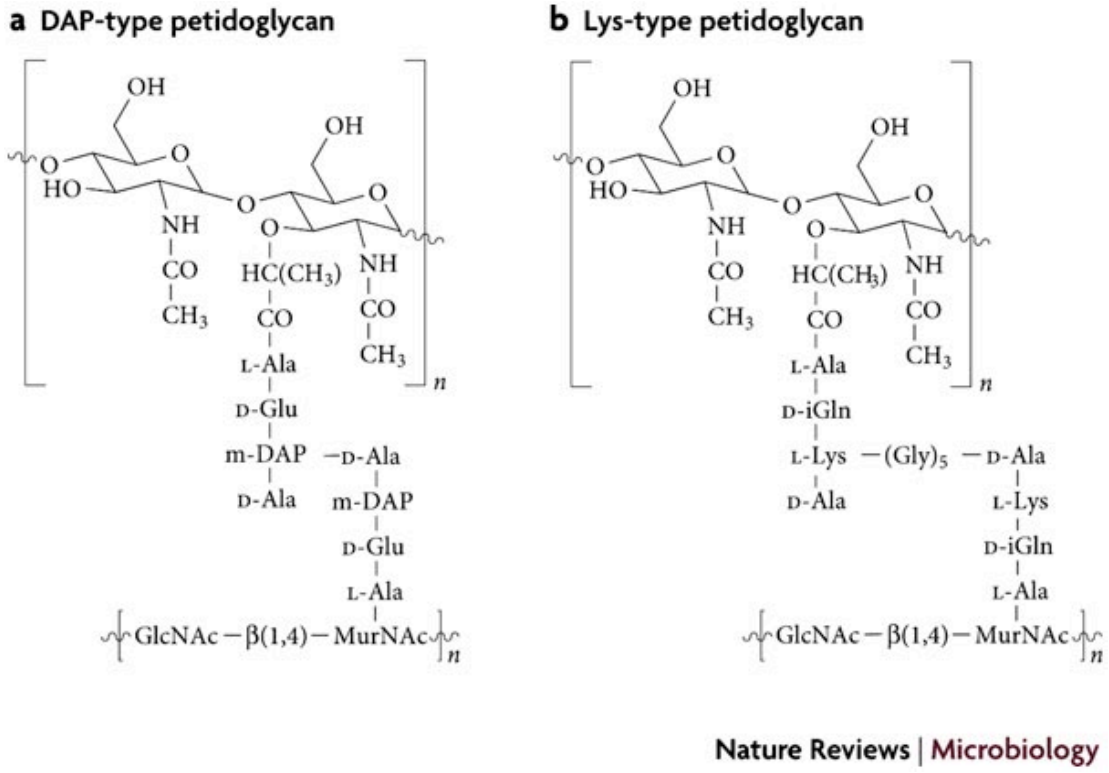

Fig.22: Cross linkage in Gram negative and Gram positive bacteria.

Braun's lipoprotein (BLP or Murein Lipoprotein), found in some Gram-negative cell walls, is one of the most abundant membrane proteins; its molecular weight is about 7.2 kDa. It is bound at its C-terminal end (a lysine) by a covalent bond to the peptidoglycan layer (specifically to diaminopimelic acid molecules and is embedded in the outer membrane by its hydrophobic head (a cysteine with lipids attached). BLP tightly links the two layers and provides structural integrity to the outer membrane.

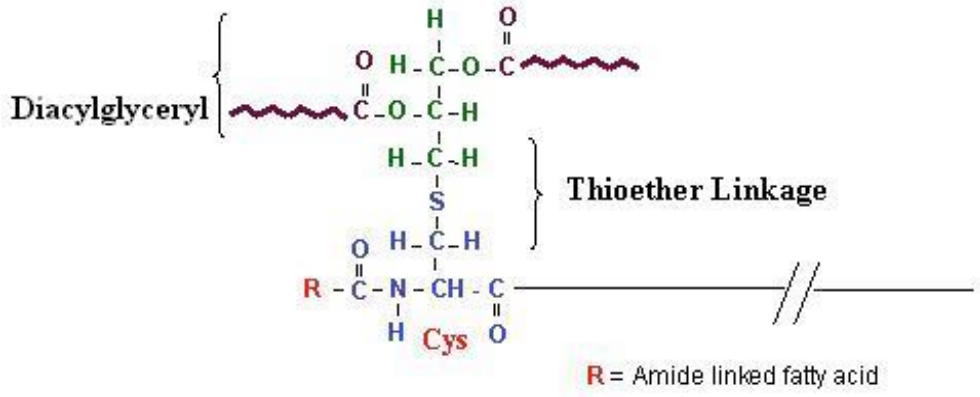

Fig.23: Structure of Apo lipoprotein

5. Inner Membrane

The inner lipo-protein membrane is the innermost layer of the cell wall consisting of the phospholipid bilayer along with surface protein and integral protein molecule. It also has the steroid molecules in between.

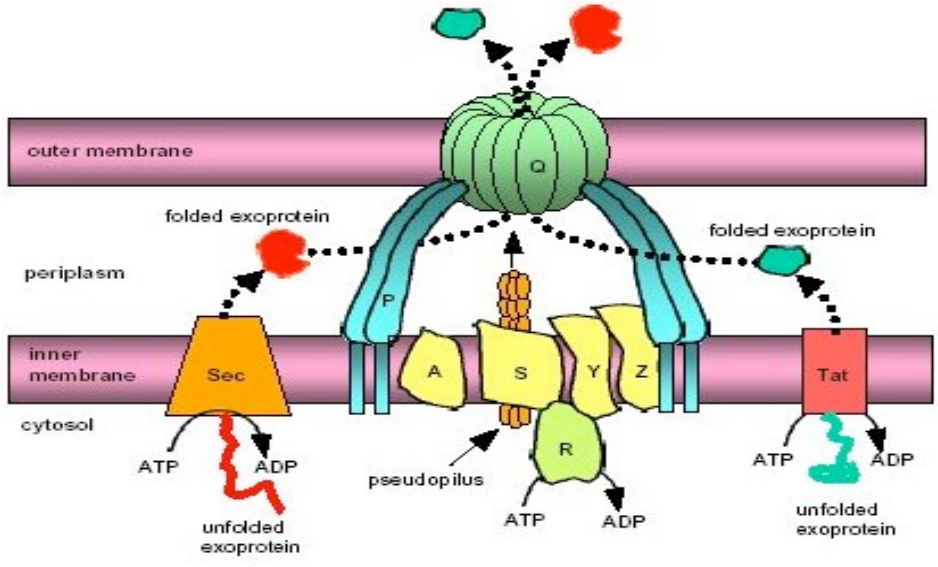

Fig.24: Transport of Sec(Secretion) protein and Tat (Trans activating protein) from the inner to the outer membrane.

Function:

1. It is the selectively permeable membrane.
2. It has the mesosome for the purpose of respiration.
3. It produces the sub-cellular membranous organelles.
4. It attaches the nuclear material.
5. It also helps in protein synthesis.
6. It has 5 different secretion systems to deliver proteins to the external environment.

Gram positive bacteria may be transformed artificially with the help of plasmids from Gram negative bacteria, this might change their nutrient utilization ability but not the Gram characteristics of the cell wall.