B.Sc I
Paper II

The cell wall (outer Membrane)

Unit I

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The cell wall of bacteria is a semingid & complex structure present beneath capsule and external to the plasma. It is responsible for characteristic shape of the cell walls. The cell walls protects the plasma membrane and other cytoplasmic inclusions from adverse environment. It also protects the bacterial cell from bursting when the osmotic pressure of cytoplasm is higher than that of outside of cell wall.
It provides support for attachment to the flagella.
It rescues the cell from antibodies and harmful chemicals.

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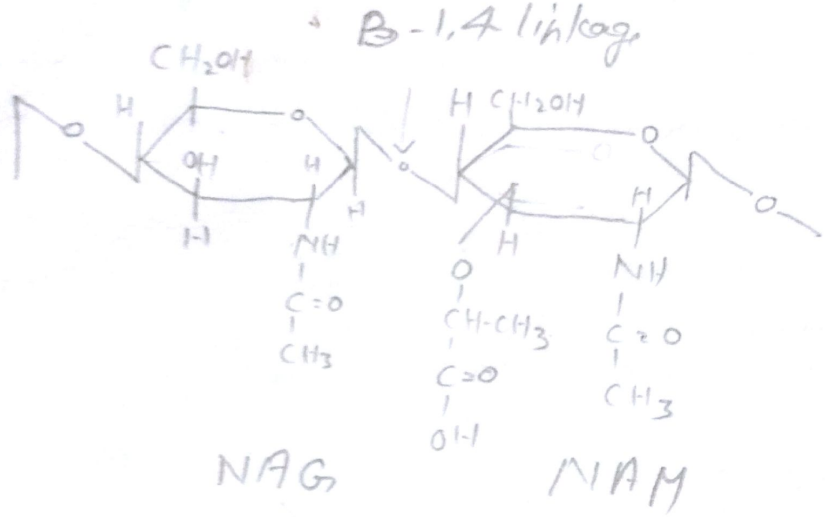
The cell wall of Gram-negative Eubacteria is comparatively thinner than the cell wall of Gram-positive bacteria. Similar is the situation of Gram negative archaebacteria. In addition, chemical composition of cell wall of archebacteria differ from Eubacteria. ~~Also~~ Also cell walls of eukaryotic microorganism (e.g. algae, fungi) differ chemically from those of prokaryotes. The cell envelope of Gram-negative bacteria consists of two unit membrane of 75 A° wide & separated by 100 A° wide periplasmic space. Peptidoglycan is present in the periplasmic space in Gram-negative bacteria.
Difference between cellwalls of Gram-positive & Gram-negative bacteria are as mentions in table.

Character	Gram positive (crystal violet)	Gram-negative
1 Gram's staining	Retain crystal violet and appear dark violet	Pass crystal violet and counter stained by safranin and appear red
2. outer membrane	Absent	Present
3. Peptidoglycan	Several layers thick	Thin (single layer)
4. Lipid and lipoproteins	Low	high
5) Lipopolysaccharides	Absent	high
6) Teichoic acid	mostly present	absent.
7) Periplasmic space	Absent	Present.
8) Flagella.	Contain 2 rings in basal body	Contains 4 rings in basal body
9) Toxin production	Exotoxin	Endotoxin
10) Strength	High	Low
11) Resistance to drying	high	Low
12 Susceptibility to penicillin & Sulfonamide	High	Low
13 susceptibility to streptomycin, Tetracycline & chloramphenicol.	Low	High
14) Inhibition by basic dye.	Marked	very less Marked

Chemical composition and wall characteristics

The cell wall of bacteria is made up of network of peptidoglycan (murein, murus wall). It is present almost in all bacterial cell wall except Halobacterium and Halococcus. Because these bacteria live in marine water which contains high salt concentration. The osmotic pressure of cytoplasm is more or less similar to outside the cell environment.

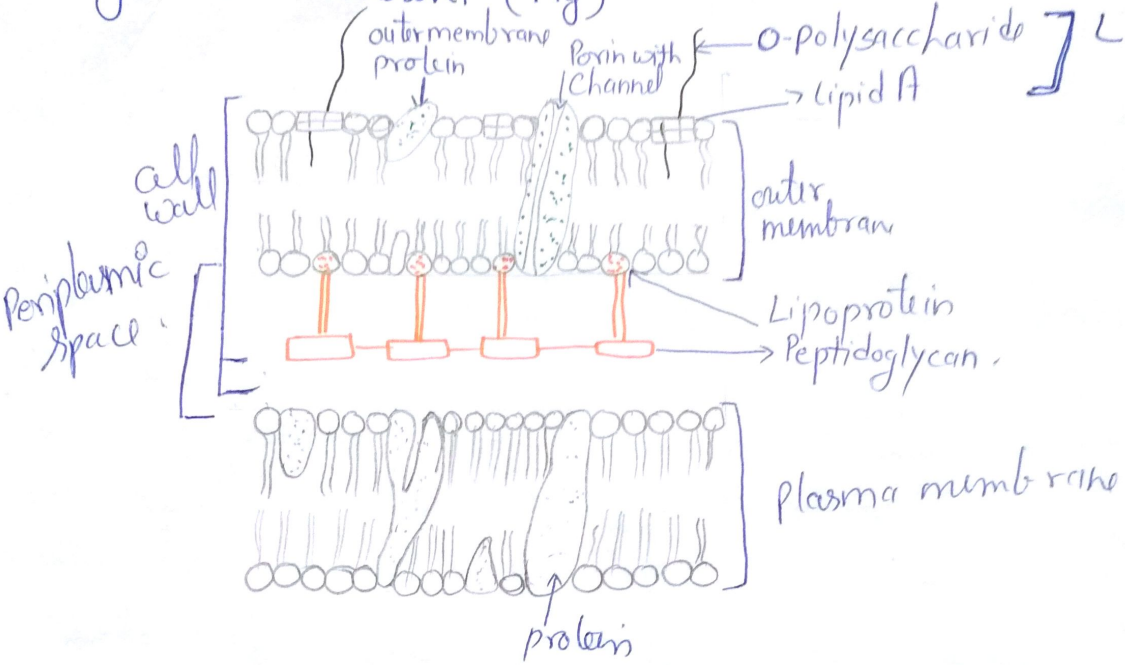
Peptidoglycan determines the shape of a cell. It accounts for 40-80% of total dry weight of cell. Its thickness is about 30-80 nm. It is insoluble and ~~soluble~~ porous polymer that provides rigidity. It is a mucopolysaccharide. However, its chemical composition differ from species to species. It consists of repeating disaccharides attached to chains of four or 5 Amino acids. The monosaccharides, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) are linked by β-1,4-glycosidic bond. There are related to glucose attached with amino groups. The chemical structure is



A tetrapeptide side chain containing four amino acid (L-alanine, D-glutamate, L-Lysine and D-alanine) is attached to each NAM. The third Aminoacid varies with different bacteria and may be lysine, diaminopemutic acid or threonine. For example in E.coli instead of L-lysine (the third amino acid) there is mesodiaminopimetic acid. The D and L forms of amino acid alternate to each other. Except peptidoglycan the amingacid found in protein are L-forms. The parallel tetrapeptide side chains are linked by a pentaglycine peptide cross bridge (PPCB) that contain five amino acid. The PPCB links L-lysine of one tetrapeptide with D-alanine at the terminal end. Due to extensive cross linking the peptidoglycan becomes a rigid macromolecule of the cell wall.

(ii) Gram negative bacteria G-bacteria

The Gram negative bacteria contains peptidoglycan but in very low amount (Fig)



They totally lack teichoic acids. Peptidoglycan is situated in periplasmic space and covalently linked to lipoproteins in the outer membrane. The periplasmic space is a space between the outer membrane and plasma membran which appears like gel and contains a high amount of enzymes and transport proteins. Due to the prusence of low amount of peptidoglycan, the cell wall of Gram-negative bacteria ~~is a bilayered structure consisting of mainly~~ can easily be disintegrated

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The cell envelop of Gram-negative bacteria is a bilayered structure consisting of mainly lipoproteins, lipopolysaccharides (LPS) & phospholipids. The chemical constituents and arrangements are described in detail as below:

(1) **Lipoproteins** - occurs freely and in bound forms as well. In lipoproteins of outer membrane protein binds to lipid non-covalently, whereas in lipoproteins of plasma membrane, protein bind to lipid covalently. Lipoproteins have a molecular weight of about 7000 daltons and consists of about 58 Amino acids. Lipoproteins together with matrix protein form a complex which contains diffusion channels. (Di Rienzo et al 1978). A diffusion channel is enclosed by three molecules of matrix proteins having a diameter of about 1·5-2 nm.

(2) **Lipopolysaccharides (LPS)**

outer membrane of Gram-negative bacteria is covered by LPS or which is made up of Polysaccharides covalently linked to lipid A. (Fig Lipid A. It consists of glucosamine, phosphate and fatty acids. The ~~fatty~~ acid β-1, 6 D glucosamine disaccharide units constitute the carbohydrate components of lipid A.

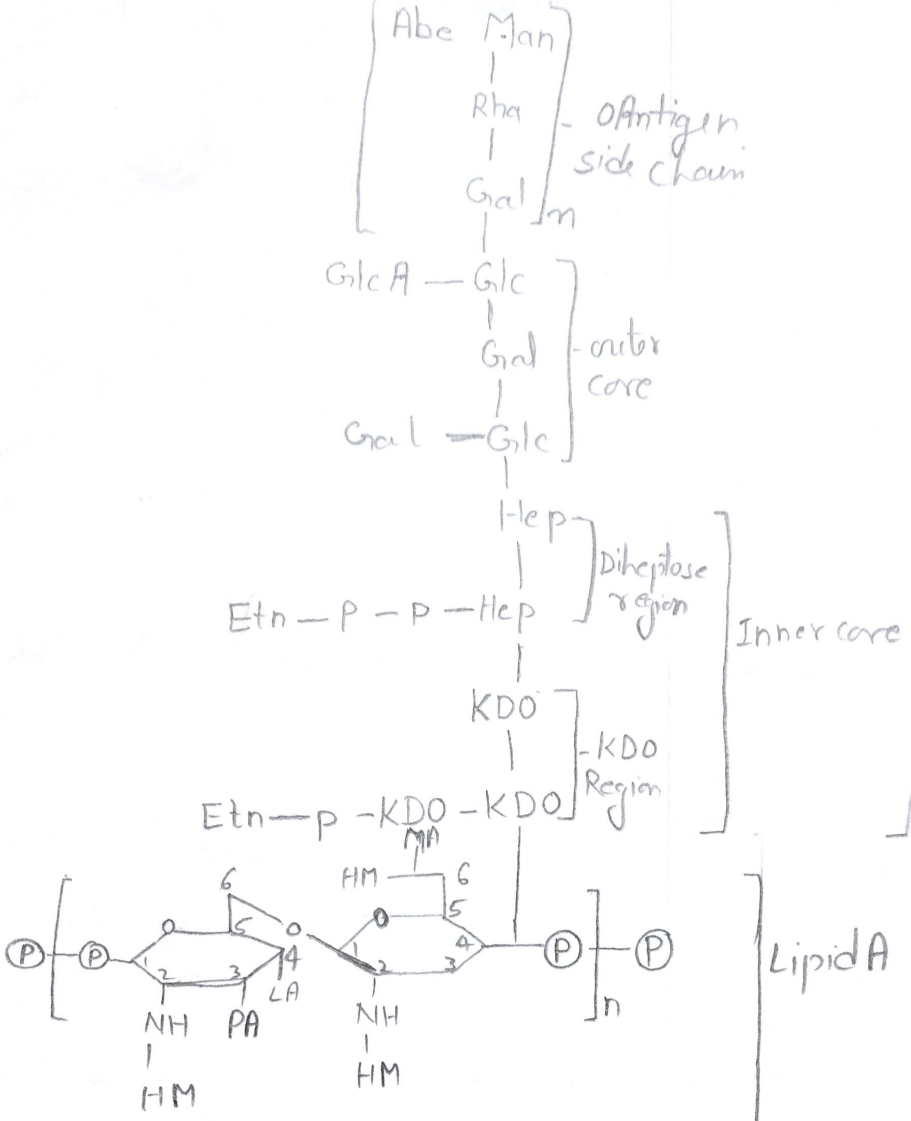

Fig. chemical organisation of a lipopolysaccharide molecule Polysaccharide (Abe - abequose; Etn, ethanolamine; Gal, galactose; GlcA, glucose acetyl; glucose, GlcN, glucosamine; Hep (L-glycero-D-mannoheptose); KDO (2-Keto-3-deoxyoctonate); Fatty acid - HM, 3-hydroxymyristic acid (12C) LA Lauric acid (12C); M, myristic acid (14C) PA palmitic acid.

The hydroxyl and amino groups of this disaccharide are substituted by the constituents such as polysaccharide chain, phosphate or pyrophosphate and fatty acids. The fatty acids provide hydrophilic property to Lipid A. There are six fatty acids such as lauric acid (12C) mysristic acid (14C) and palmitic acid (16C) (present in the ratio of 1:1:1), 3 three molecules 7

of 3-D-Hydromyristic acid (C_{14}) two of which contain amide ($C-NH_2$) linkages with each amino group of two glucosamine. The third is esterified through its hydroxyl ($-OH$) group to myristic acid (De Rienzo et al. 1978).

The esterified group of lipid A is referred to as endotoxin which is toxic when present in blood. Lipid A of LPS is responsible to induce fever & shocks

③ Polysaccharide

The polysaccharide portion of LPS of Salmonella cell wall is composed of three important components, the inner core, the outer core and the O-antigen side chains in rough strains of Gram-negative bacteria are absent, whereas the smooth strains of Salmonella have O-antigen side chains which may extend out the cell wall surface and attain about 30 nm length. These chains have antigenic property and, therefore, can be distinguished serologically (eg species of Salmonella). Its role is comparable to that of teichoic acids in gram positive bacterial cell walls.

The outer membrane consists of two molecules of glucose sandwitching a molecule of galactose. one glucose subunit is linked to glucose acetyl and the others to galactose. The inner core consists of two regions, the Ketodeoxyoctonate (KDO) region and diheptose region. The KDO region comprises of three units of KDO and eight carbon α-keto sugar. The diheptose region consists of two units of L-glycero-D-mannoheptose, the seven carbon heptose sugar. Fig

(4) Matrix proteins

The outer membrane of cell envelop does not warrant the entry of all substances since the nutrients are to pass across the membrane. It is impermeable only to macromolecules such as proteins, lipids, etc. The permeability of outer membrane is due to the presence of proteins called porins that forms channels. The porins are not specific and allow the small molecules. Certain porins are specific and permit only the specific substances such as Vitamin B12, nucleotides, etc. Porins also act as receptor sites for bacteriophage and bacteriocins (The proteins produced by certain bacteria that inhibit or kill the related species.)

ii) Function of cell wall

- Following are the functions of the cell wall (the outer membrane)

(a) Peptidoglycan provides structural integrity to cell by forming a rigid layer in outer membrane. The matrix proteins to some extent also contribute to structure with peptidoglycan.

(b) The cell envelop acts as barrier for diffusion to certain molecules across the envelope

(c) The matrix proteins act as receptor sites for bacteriophages and bacteriocins.

(d) The O-antigen side chain of polysaccharide of LPS determines the antigen specificity of Gram negative bacteria

a Archaebacterial cell wall

All archaebacterial lack murein (Konig 1988). Most of them possess cell wall that lack peptidoglycan. Cell wall structure and chemical composition of archaebacteria differ from that of Eubacteria.

Moreover, the archaebacteria possess no common cell wall polymer. Usually cell wall is composed of proteins, glycoproteins or polysaccharides. Due to unusual chemical composition the cell envelopes show a high degree of resistance against cell wall antibiotics & lytic agents.

The cell walls of the Gram-positive archaebacteria consists of Pseudomurein, methanochondroitin, or heteropolysaccharide. All Gram negative archaebacteria have cell envelops which are composed of single layered or more complex crystallin protein or glycoprotein subunits. In most organism of methanogenic branch and extreme thermophilic glycoprotein metabolizers. Single S-layer is found in cell wall envelop which provides remarkable resistance. The organism tolerate the extremes of environmental conditions such as high salt, low pH & high temperature (Konig 1988 b).

Peptidoglycan of staphylococcus aureus consists of linear carbohydrate ~~breakdown~~ backbone the glycan chain. The glycan chains contains alternate residues of NAG and NAM linked by β-1,4-linkage. The amino acid residues are connected by tetrapeptide and pentaglycine bridge (Fig). The wall of most Eubacteria contains very low amount of lipid except mycobacterium and corynebacterium. Mycobacterium exhibits acid fast staining ie the stain from cell wall is not easily decolourized with dilute acid. This is due to the presence of mycolic acid in cell wall. A mycolic acid derivative ie. trehalose dimycolate plays a role in disease caused by M. tuberculosis & C. ~~diphth~~ C. diphtheriae.

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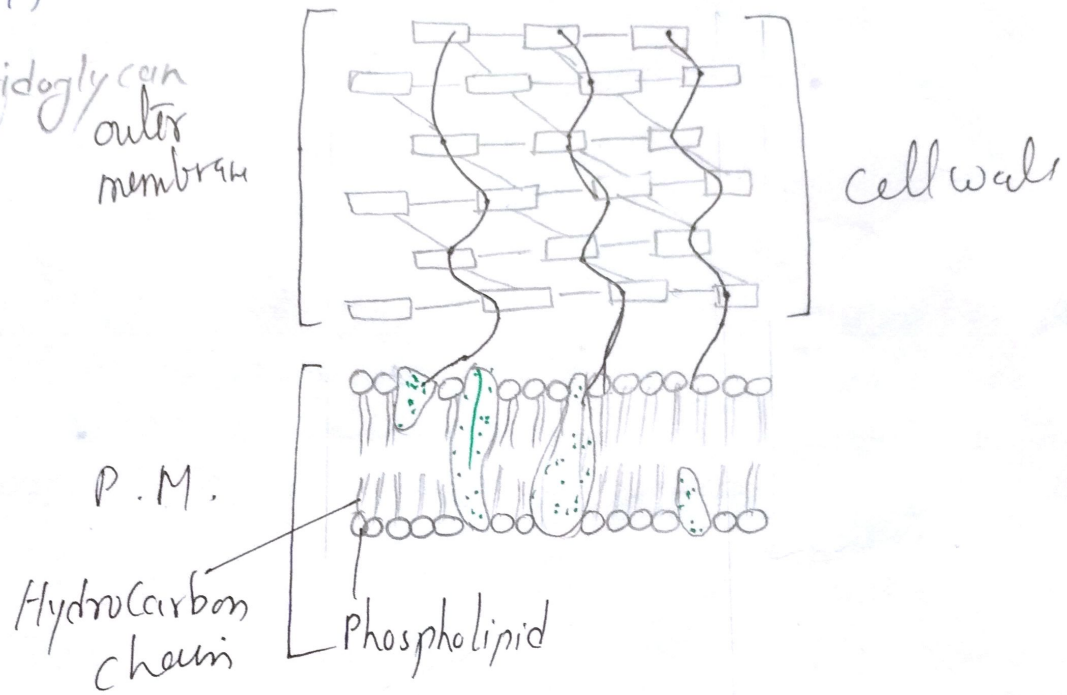
(iv) Gram-positive bacteria

In most of Gram-positive bacteria, the cell wall contains several layers of peptidoglycan which is inter-connected by side chains and cross bridges. (Fig)



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Peptidoglycan accounts for 40-90% of total dry weight of cell wall^{G+} However, the thickness may vary with types of species from 30 nm to 8 nm. The thickness of peptidoglycan provides rigidity to cell wall. The layers of peptidoglycan are thicker in Gram-positive bacteria than that in Gram negative bacteria.

In most of Gram-positive bacteria peptidoglycan is associated with acidic polymers containing phosphorus called teichoic acid or acid polysaccharides such as teichuronic acids. Teichoic acids are hydrophilic, flexible and linear molecules. The presence of teichoic acid makes easy to dignose the bacteria serologically.

Theichoic acids consists of an alcohol (eg. glycerol or ribitol) and phosphate. Therefore, it is the polymer of glycerol phosphate or ribitol phosphate. Thichoic acids are mainly of three types (i) ribitol teichoic acids (found in S. aureus & B. subtilis ii) glycerol teichoic acid. (eg. B. subtilis. iii) glucosylglycerol phosphate teichoic acid B. licheniformis). out of these three only one type is found in a particular bacterium. The acids are linked to layers of peptidoglycan of plasma membrane. The phosphate groups provide negative charge which in turn controls the movement of cations i.e. positive ions across the cells. Teichoic acids possibly play a role in growth of bacterial cell by regulating the activity of an enzyme autolysin. The acids prevent the extensive breakdown and possibly the lysis of cell wall. They also store phosphorus. (Rogers et al. 1978.)