BIOCHEMISTRY OF MICROBES

Introduction

Bacteria are small prokaryotic cells with the size range usually varying from 0.5 to 1 µm in diameter and 1 to 6 µm in length. Lately, there are reports related to the existence of even much smaller (about 0.1 µm diameter) bacteria in nature, which are referred as nanobacteria. The smaller size of bacterial cell provides a definite advantage over other organisms with respect to the exchange of nutrients from its environment. Since, a smaller sphere has a higher surface area to volume (S/V) ratio as compared with a larger sphere, the exchange of nutrients will be more efficient in small cells. Thus, per unit of available nutrients, the small cells will typically yield a larger population than larger cells. For instance, spherical bacteria with a diameter of 2 µm, surface area of about 12 µm2 and a volume of 4 µm3, will have the S/V ratio is 3:1. In contrast, a eukaryotic cell with a diameter of 20 µm has a surface area of about 1200 µm2 and volume of 4000 µm3. Their S/V ratio is 0.3:1. The large S/V ratio of bacteria means that internal parts of the cell are very close to surface and, therefore, the nutrients can easily and rapidly reach all parts of the cell. Predominantly, the bacteria are observed in three common shapes: spherical, rods and spiral. These spherical bacteria are called cocci (singular coccus), whereas those shaped like a cylinder are termed as rods or bacilli. Some bacteria are shaped like a spiral and known as helicals. The spiral bacteria also exist in a variety of shapes. A comma-shaped bacterium is called a Vibrio. A rigid, wavy-shaped bacterium is spirillum; and corkscrew-shaped is spirochete. Some other bacteria have atypical morphology, like spindle-shaped, square-shaped (Haloarcula), and star-shaped (Stella). Moreover, those without a well-defined shape are referred as pleomorphic. Morphologically, the bacteria can be further classified based on their colony shape and size variability. They can also be categorized on the basis of their cell wall properties as: Gram-negative; Gram-positive; without a cell wall – the mycoplasmas, and cell wall lacking peptidoglycan – the archaea. The Gram-negative and Gram-positive bacteria are ubiquitous and widely prevalent in soil, water, air and sub-surface environment. However, the Archae bacteria are normally found in harsh environments, like acidic conditions (acidophiles); high salt (halophiles) and dry habitat (xerophiles).

General structure of bacterial cell

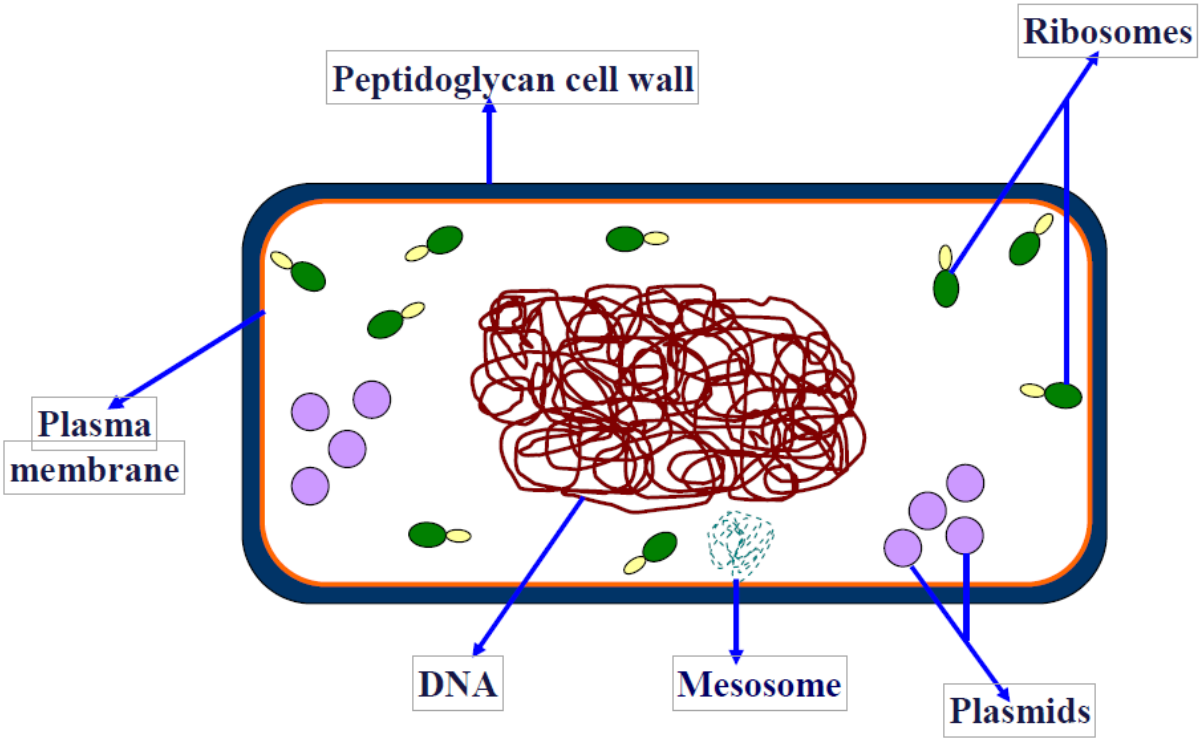
The bacterial cell (Fig below) is comprised of a cell envelope that mainly includes: cell membrane, cytoplasm, ribosomes, and a nucleoid. Most bacteria have a cell wall, although a few do not. Other constituents such as flagella or pili are commonly observed but not essentially present in all bacteria.

Cell envelope

The cell envelope is an illustrative term used to depict the multiple layers of biological material that surrounds or envelope the protoplasm of the cell. In other words, the cell protoplasm (cytoplasm) is protected by the plasma membrane, a cell wall and a capsule. The bacterial cell wall itself is a layered structure. It protects the underlying protoplast from all kinds of damage. Many bacteria have a polysaccharide capsule, or at least a glycocalyx present at the outer surface of the cell wall. The details of the cell envelope are discussed below.

Capsules

Most bacteria form a capsular polysaccharide layer external to the cell. A true capsule as a discrete detectable layer of polysaccharides deposited outside the cell wall is easily detectable upon staining with Indian ink (Fig. 2). In some bacteria, a less discrete structure or matrix is formed, which embed the cells and is a called a slime layer or a biofilm. The thin layer of tangled polysaccharide is also called as glycocalyx. The capsule exhibits several functions, including (i) adherence of cells to surfaces, (ii) protection of bacterial cells from engulfment by predatory protozoa or phagocytes, (iii) protection against the attack by antimicrobial agents of plant or animal origin, (iv) protection of cells from perennial effects of drying or desiccation and (v) acts as a reserve of carbohydrate for metabolism.



Structure of a bacterial cell

Bacteria can attach to surface, produce slime, divide and produce microcolonies within the slime layer, and construct a biofilm, which provides an enriched and protected environment for their growth. A typical example of biofilm construction in nature is the formation of dental plaque mediated by the oral bacterium, Streptococcus mutans. The bacteria adhere specifically to the pellicle of the tooth by means of a protein on the cell surface. The bacteria grow and synthesize a dextran capsule which binds them to the enamel and forms a biofilm, some 300-500 cells in thickness. The bacteria are able to hydrolyze sucrose present in diet into glucose plus fructose. The fructose serves as an energy source for bacterial growth, whereas the glucose is polymerized into an extracellular dextran polymer that cements the bacteria to tooth enamel and becomes the matrix of dental plaque. The dextran slime on de-polymerization to glucose produces lactic acid within the biofilm or plaque that decalcifies the enamel and promotes dental caries.

Another important characteristic of capsule is the ability to block the phagocytic process and thereby prevent bacterial cells from being engulfed or destroyed by phagocytes. For example, the primary determinant of virulence of the pathogen Streptococcus pneumoniae is its

polysaccharide capsule, which prevents ingestion of Pneumococci by alveolar macrophages. Similarly, the Bacillus anthracis survives phagocytic engulfment because the lysosomal enzymes of the phagocyte cannot initiate an attack on the poly-D-glutamate capsule of the bacterium. Bacteria such as Pseudomonas aeruginosa produce extracellular slime upon colonization and form a biofilm refractory to phagocytes.

Cell wall

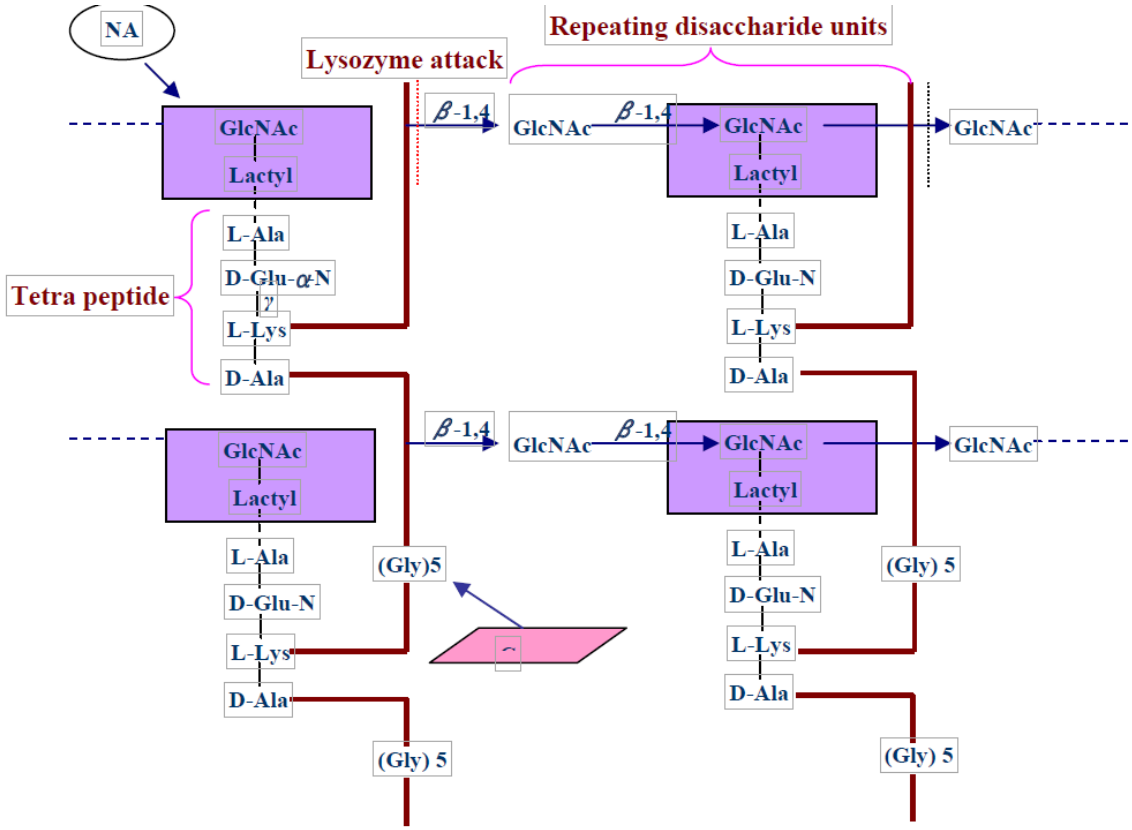
The cell wall is an important structural component that protects the bacterial cell protoplast from mechanical damage and osmotic rupture. It is made up of a porous, rigid material that has high tensile strength, which can withstand the osmotic pressure equivalent to about 10-25 atmosphere against the inside of the plasma membrane. It also plays some role in determining the shape of the bacterial cell. The unique constituents of cell wall are not found elsewhere in nature. There are two types of cell walls found in bacteria. Based on the properties of their cell wall, the bacteria are categorized as Gram-positive and Gram-negative. Nevertheless, in both cases, the rigid protective nature of the wall is due to the macromolecule called as peptidoglycan or murein. This polymer is composed of alternating glycan molecules cross-linked by short peptides. The two glycans are N-acetylmuramic acid and N-acetylglucosamine. The Gram-positive cell wall consists of mostly the peptidoglycan plus acidic polysaccharides including teichoic acids. However, certain prokaryotes like the mycoplasma lack a cell wall, or the archaea with a modified cell wall (pseudomurein), do not contain peptidoglycan. *The cell wall is an important bacterial cell component, which provides (i) an important site for attack by antibiotics, (ii) ligands for adherence and receptor sites for drugs or viruses, (iii) cause symptoms of disease in animals and (iv) immunological distinction and variation among strains of bacteria.*

The structure of the cell walls of bacteria is shown below. In the Gram-positive bacteria the cell wall is thick (15 - 80 nm) and consists of several layers of peptidoglycan. It makes up about 40 to 80 % of dry weight of the wall, depending upon the species. It retains the purple crystal violet dye when subjected to the Gram-staining. On the contrary, the Gram-negative bacteria have a relatively thinner wall (10 nm), and contain a mono layer of murein, which does not allow the retention of crystal violet. They do not retain the primary dye, and appear pink-red when counter-stained with safranin dye (Fig. 4). In Gram-negative bacteria, the single layer of peptidoglycan is surrounded by a membranous structure called the outer membrane. The

outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which exhibits toxic effects. Chemically, the peptidoglycan in Gram-negative bacteria is made up of alternating molecules of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) connected with a beta 1,4-glycosidic bond. The 3-carbon of NAM is substituted with a lactyl ether group derived from pyruvate. The lactyl ether connects the glycan backbone to a peptide side chain that contains L-alanine, (L-ala), D-glutamate (D-glu), and D-alanine (D-ala). Strands of murein are assembled in the periplasm from about 10 muramic acid subunits. The strands are then connected to form a continuous glycan molecule that encompasses the cell. The tetrapeptide chains that project from the glycan backbone can be cross-linked by an interpeptide bond between L-Lys and D-ala aminoacids.

The Gram-negative bacteria are less vulnerable to attack by lysozyme because their peptidoglycan is shielded by the outer membrane. The exact site of lysozyme attack is the beta 1,4 bond between (NAM) and (NAG) on bacterial peptidoglycan. The peptidoglycans in Gram-positive bacteria exhibit several different peptide arrangements. Considerable variation exists in the amino acids that form the cross-linking peptides of peptidoglycan. Certain amino acids are never found in peptide bridge including sulfur-containing amino acids, aromatic amino acids and branched-chain amino acids, as well as arginine, proline and histidine. Since the peptidoglycan is not protected by an outer membrane, the Gram-positive bacteria are more sensitive to penicillin than Gram-negative bacteria. Some Gram-positive bacteria are also very sensitive to lysozyme. The glycan backbone of the peptidoglycan molecule is susceptible to cleavage by this enzyme present in animal serum, tissues and secretions, and in the phagocytic lysosome. The function of lysozyme is to lyse bacterial cells as a constitutive defense against bacterial pathogens. Closely associated with the layers of peptidoglycan in Gram-positive bacteria are a group of molecules called teichoic acids. Teichoic acids are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars. The teichoic acid polymers are often anchored to the plasma membrane called lipoteichoic acids apparently directed outward at right angles to the layers of peptidoglycan. The functions of teichoic acid are not very well understood. However, they are considered essential to viability of Gram-positive bacteria and provide a channel of

regularly-oriented negative charges for threading positively charged substances through the complicated peptidoglycan network. It is also speculated that teichoic acids are in some way involved in the regulation and assembly of muramic acid subunits on the outside of the plasma membrane. Furthermore, in some specific cases, like in case of Streptococci, the teichoic acids are implicated in the adherence of the bacteria to tissue surfaces.



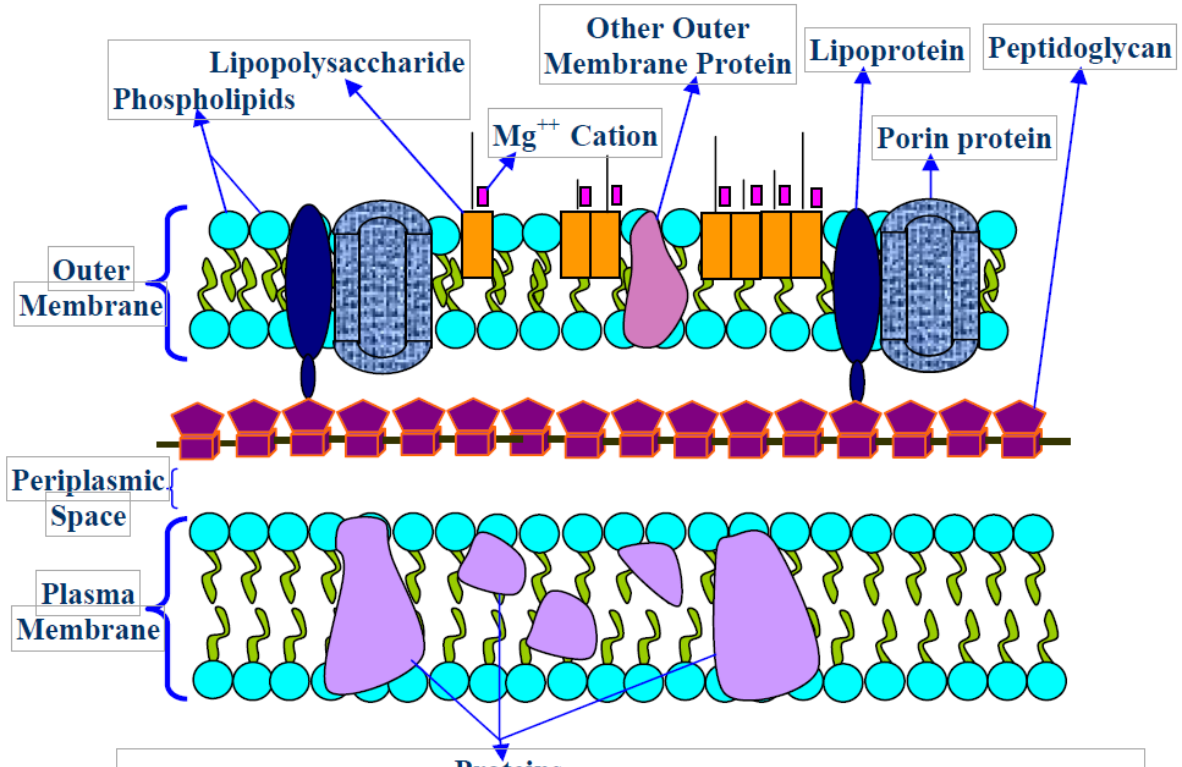
A typical cell wall composition of Gram + ve bacteria

Cell wall-less forms

A group of bacteria called Mycoplasma exist without a cell wall. They have sterol-like molecules incorporated into their membranes and are usually inhabitants of osmotically-protected environments. Without a cell wall, they are polymorphic and mostly acquire the slender or branched filamentous growth. Some bacteria under pressure of antibiotic therapy lose their ability to form cell walls. These wall-deficient strains are called as L-forms, after the Lister Institute, where they were discovered. Moreover, some members of group Archaea may also lack cell wall.

Outer membrane of gram-negative bacteria

The outer membrane of Gram-negative cell wall is a discrete bilayered structure external to the peptidoglycan layer (Fig. 6). Indeed, it is the first and foremost permeability barrier. Owing to its lipopolysaccharide content, it possesses many interesting and important characteristics of Gram-negative bacteria. The outer membrane is a lipid bilayer intercalated with proteins, apparently similar to the plasma membrane. The internal side of the outer membrane is composed of phospholipids similar to the phosphoglycerides that compose the plasma membrane. The external face of the outer membrane may contain some phospholipids, but mainly it is formed by a different type of amphiphilic molecule, which is composed of lipopolysaccharide (LPS).

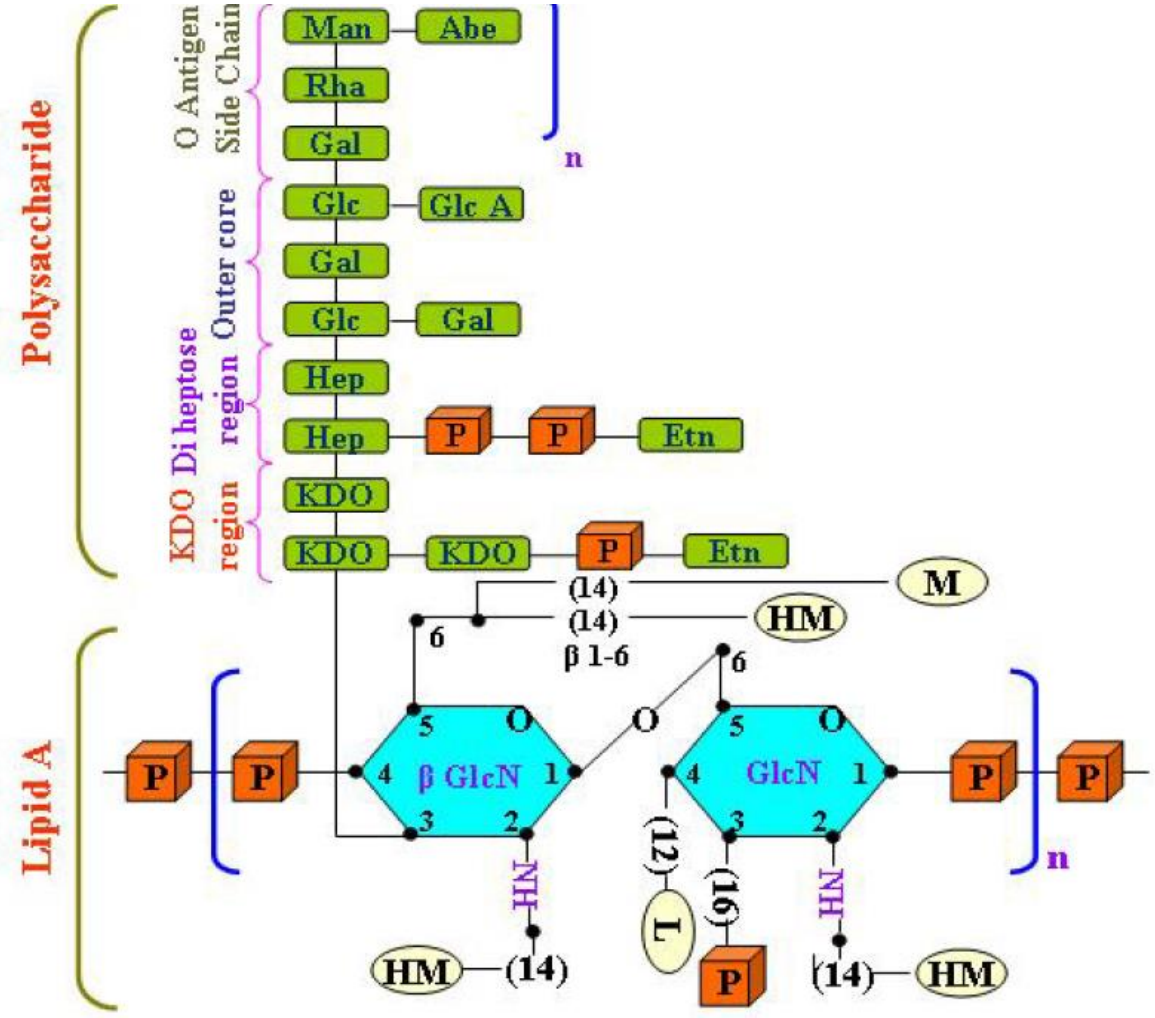


Structure of Gram negative bacterial membrane and cell wall

The LPS molecule is composed of a hydrophobic region, called Lipid A that is attached to a hydrophilic linear polysaccharide region, consisting of the core polysaccharide and the O-

specific polysaccharide. The Lipid A molecule inserts into the interior of the membrane, and the polysaccharide part of the molecule is exposed to the aqueous environment. At the junction of lipid A and polysaccharide there is an accumulation of negative charge that provides the lateral stability to the outer membrane. The bacterial LPS are toxic to animals, and activate the (i) macrophages to produce pyrogens, (ii) complement cascade causing inflammation, and (iii) blood factors resulting in intravascular coagulation and hemorrhage, even when injected in small amounts. The toxic component of endotoxin (LPS) is Lipid A. The O-specific polysaccharide may provide ligands for bacterial attachment and confer some resistance to phagocytosis. Variation in the exact sugar content of the O-polysaccharide also referred to as the somatic or O-antigen, accounts for multiple antigenic types (serotypes) among Gram-negative bacterial pathogens.

Structure of Lipopolysaccharide (LPS)



The space between the cell wall and cell membrane is called the periplasm. The periplasmic space is important to the physiology of the cell wall. It contains several enzymes and also has a role in the synthesis of cell wall. Most proteins usually traverse the membrane and anchor the outer membrane to the peptidoglycan layer. The Braun lipoprotein are covalently attached to the peptidoglycan sheet at one end and inserted into the hydrophobic interior of the membrane at the opposite end. A group of trimeric proteins called porins form pores of a fixed diameter through the lipid bilayer of the membrane. Porins in Gram-negative bacteria are responsible for the passage of nutrients through the barrier of the outer membrane, and to exclude the entry of deleterious substances from the environment. The ubiquitous omp A protein in the outer membrane of E. coli has a porin like structure, which may function in uptake of specific ions, and as a receptor to the F pilus for attachment of bacterial viruses. Furthermore, the omp C and omp F porins of E. coli facilitate the entry of hydrophilic molecules of MW ~750 Daltons. Some proteins are responsible for the entry of specific compounds into the cell, such as vitamin B12, iron chelates, disacchararides or phosphorylated compounds.

Plasma membrane

The plasma membrane is also called as the cytoplasmic membrane. It is the most dynamic component of a prokaryotic cell, consisting of 40 percent phospholipids and 60 percent proteins. The phospholipids are amphoteric molecules with a polar hydrophilic glycerol "head" attached to two non-polar hydrophobic fatty acid tails via an ester bond. The arrangement of proteins and lipids to form a bilayer in aqueous environments is represented in the fluid mosaic model. In bacteria, the plasma membrane invaginates into the cytoplasm and forms stack or vesicles attached to the inner membrane surface. These structures are called as mesosomes. Such internal membrane systems increase the surface area of membranes, like in cristae of mitochondria or the thylakoids of chloroplasts, to which enzymes are bound for specific functions. Mesosomes also represent the specialized membrane regions involved in DNA replication and segregation, cell wall synthesis, or increased enzymatic activity. Since bacteria do not have any intracellular organelles for respiration, photosynthesis or secretion, the plasma membrane is playing a role in accompanying these tasks for the cell. It performs a variety of functions related to energy

generation, and biosynthesis. The electron transport system that couples aerobic respiration and ATP synthesis occurs in bacterial membrane. The light energy harvesting photosynthetic chromophores responsible for conversion of light into chemical energy are also localized in the membrane. Thus, the plasma membrane is the site of oxidative phosphorylation and photophosphorylation in bacteria, analogous to the functions of mitochondria and chloroplasts in eukaryotic cells. The bacterial membrane also contains the sensing proteins that measure concentrations of molecules in the environment and binding proteins that translocate signals to genetic and metabolic machinery in the cytoplasm. Membranes also contain enzymes involved in many metabolic processes such as cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO2 fixation and ammonia oxidation. An important function of plasma membrane is to provide a selective permeability barrier and to regulate the passage of substances into and out of the cell. The bacterial membrane freely allows the flow of water and uncharged molecules with MW of about 100 Daltons, and restricts the entry of larger molecules or any charged substances. Such restricted molecules are transferred only through the specialized membrane transport processes.

The specialized membrane-bound proteins that mediate the passage of solutes through cellular membranes are referred as carrier proteins, porters, and permeases. The transport systems operate by one of three transport processes: uniport, symport and antiport (Fig. 8). In a uniport process, a solute passes through the membrane unidirectionally. In symport or cotransport processes, the two solutes must be transported in the same direction at the same time; in antiport processes or exchange diffusion, one solute is transported in one direction and a second solute is transported in the opposite direction, simultaneously.