Course BSc (Biotechnology) I Year
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Prokaryotic cells

Prokaryotic cells are found in bacteria, archaea and cynobacteria (blue-green algae) and eukaryotic cells include all other cells, such as protista, fungal, plant and animal cells. The structures of typical prokaryotic cells are described in more detail as :

Common Cell Morphologies and Arrangements: Cell morphologies commonly found in prokaryotic cells are Coccus (pl. cocci) or round, Bacillus (plural: bacilli) or rod, Vibrio (plural vibrios) or curved rod, Coccobacillus (plural coccobacilli) or short rod, Spirillum (plural spirilla) or spiral, Spirochete (plural spirochetes) or long, loose, helical spiral, etc. In addition to cellular shape, prokaryotic cells may group together in certain arrangements like Coccus (pl. cocci) or single coccus, Diplococcus (pl. diplococci) or pair of two cocci, Tetrad (pl. tetrads) or grouping of four cells arranged in a square, Streptococcus (pl. streptococci) or chain of cocci, Staphylococcus (pl. staphylococci) or cluster of cocci, Bacillus (pl. bacilli) or single rod, Streptobacillus (pl. streptobacilli) or a chain of rods.

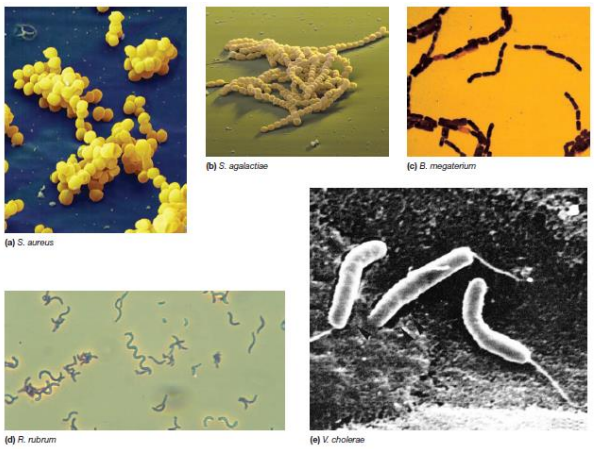


Figure: Common Procaryotic Cell Shapes. (a) *Staphylococcus aureus* cocci arranged in clusters; color-enhanced scanning electron micrograph; average cell diameter is about 1 _m. **(b)** *Streptococcus agalactiae,* the cause of Group B streptococcal infections; cocci arranged in chains; color-enhanced scanning electron micrograph (X 4,800). **(c)** *Bacillus megaterium,* a rod-shaped bacterium arranged in chains, Gram stain (X600). **(d)** *Rhodospirillum rubrum,* phase contrast (X 500). **(e)** *Vibrio cholera,* curved rods with polar flagella; scanning electron micrograph.

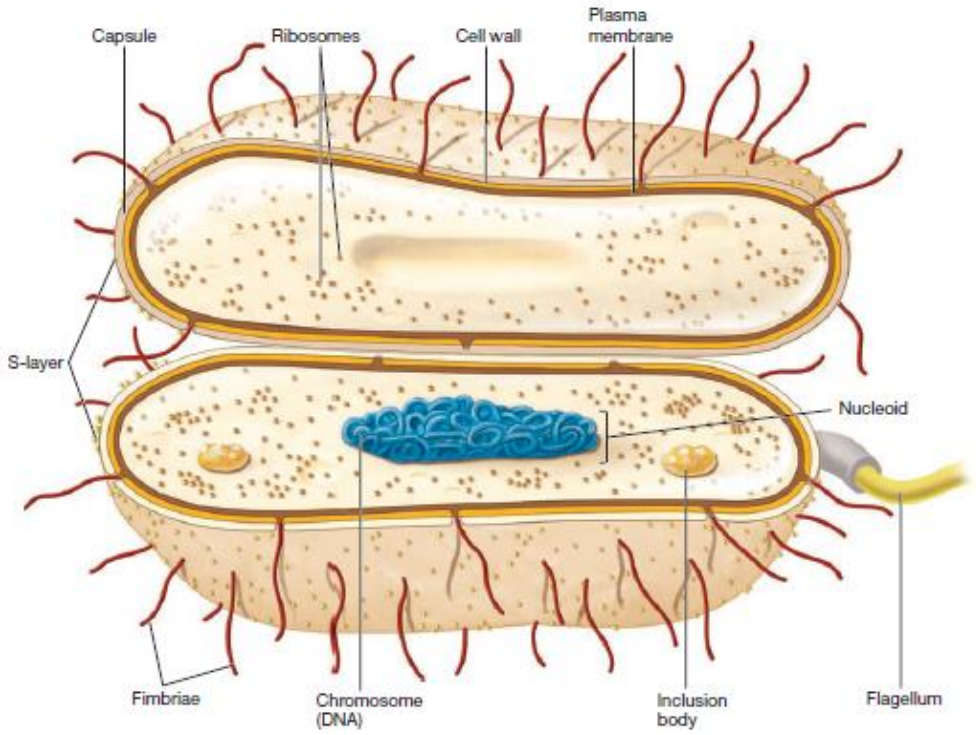
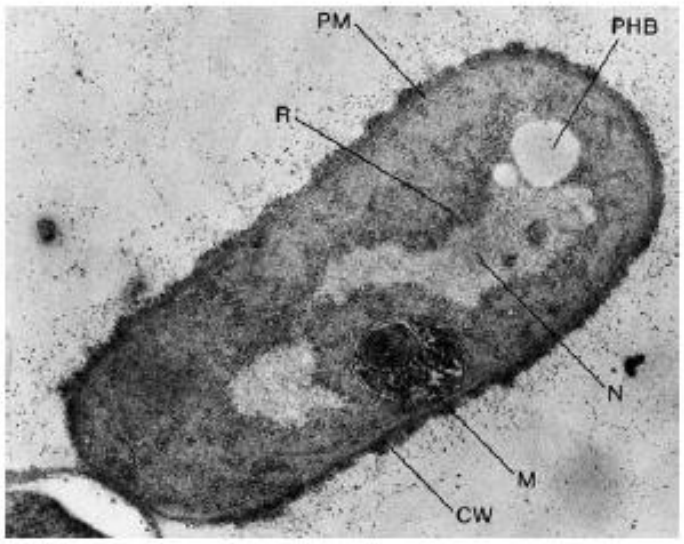


Figure: Morphology of a Procaryotic Cell.



(a)

Figure: Inclusion Bodies in Bacteria. (a) Electron micrograph of *Bacillus megaterium* (X30,500). Poly hydroxybutyrate inclusion body, PHB; cell wall,CW; nucleoid, N; plasma membrane, PM; "mesosome,"M; and ribosomes, R. **(b)** Ultrastructure of the cyanobacterium *Anacystis nidulans*.

Structures external to the cell wall

Glycocalyx (Capsules, Slime layers and S-layers)

It is a viscous (sticky), gelatinous polymer composed of polysaccharide, polypeptide or both. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule** (negative staining). If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer**. Capsules protect pathogenic bacteria from phagocytosis (process by which certain white blood cells engulf and destroy microbes) and contribute to virulence. Unencapsulated *Streptomyces pneumoniae* and *Bacillus anthracis* does not cause disease because the cells are readily phagocytosized. This allows the bacteria to attach to various surfaces, such as rocks in fast-moving streams, plant roots, human tooth and tissues and even other bacteria. Capsules also contain water which prevents them from desiccation. Other examples are *Streptococcus mutans* (dental caries), *Klebsiella pneumoniae* (respiratory tract). These can protect a cell against dehydration. Capsules and slime layers usually are made up of polysaccharides, but they may be constructed of othermaterial, like *Bacillus anthracis* has a capsule of poly D-glutamic acid. Capsules are clearly visible in the light microscope by using stains or special capsule stains.

A regularly structured layer called S-layer is usually seen in many gram positive and gram negative bacteria. It consists of proteins or glycoproteins and resembles a pattern something similar to floor tiles. The S-layer adheres directly to the outer membrane in case of gram negative bacteria and with the peptidoglycan surface in gram positive bacteria. These protect the bacteria against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio* . The S layer also helps maintain the shape and envelope rigidity of at least bacterial cells and also promotes cell adhesion to surfaces. Sometimes, the layer also seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

Glycocalyx: The glycocalyx (capsule, slime layer, or extra cellular polysaccharide) is a gelatinous polysaccharide and/or polypeptide covering lying externally to the cell wall. The exact chemical composition varies depending on the species. Capsules may protect pathogens from phagocytosis and enable adherence to surfaces, prevent desiccation, and may provide nutrients

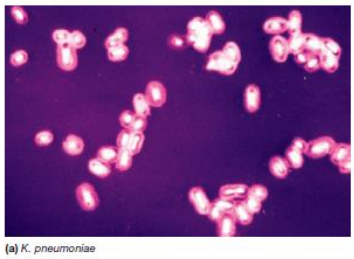


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Figure Bacterial Capsules. (a) *Klebsiella pneumoniae* with its capsule stained for observation in the light microscope (_1,500). **(b)** *Bacteroides* glycocalyx (gly), TEM (X71,250).

Fimbriae and Pili:

Many gram negative bacteria have hairlike appendages that are shorter, straighter and thinner than flagella and are used for attachment rather than for motility. They are usually called fimbriae. These structures contain a protein called pilin.

Fimbriae - occur at the poles of the bacterial cell, or they can be evenly distributed over the entire surface of the cell. Fimbriae of *Neisseria gonorrhoeae* the causative agent of gonorrhea help the microbe to colonize mucous membranes to cause the disease. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

Pilior sex pili or pilus - usually longer than fimbriae and number only one to ten per cell. Pili function to join bacterial cells prior to the transfer to DNA from one cell to another (sometimes called sex pili). They are genetically determined by sex factors or conjugative plasmids and are required for bacterial mating. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

Flagella:

Flagella are long filamentous appendages consisting of a filament, hook, and basal body and help to push the cell by rotating. The filament is composed of the globular protein flagellin, which is arranged in several intertwined chains that form a helix around a hollow core. Motile bacteria move by taxis in two ways: positive taxis or movement towards an attractant and negative taxis or movement Motile bacteria move by use of flagella, threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20 µm long. Bacterial species often differ distinctively in their patterns of flagella distribution .

Monotrichous- single polar flagellum located at one end

Amphitrochous- With two flagella, one at each end

Lophotrichous - With two or more flagella at one or both ends

Peritrichous - flagella all over the surface

Atrichous - Bacteria without flagella (Cocci rarely have flagella)

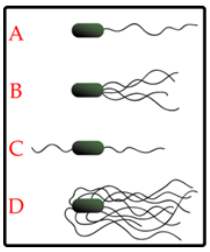

Fig. Flagellar arrangement. A. Monotrichous B. Lophotrichous C. Amphitrichous D. Peritrichous

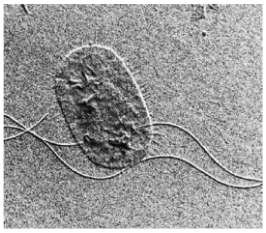

Figure : Flagella and Fimbriae. The long flagella and the numerous shorter fimbriae are very evident in this electron micrograph of the bacterium *Proteus vulgaris* (X39,000).

Structure:

Transmission electron microscopic studies have shown that the bacterial flagellum is composed of three parts. 1) **Filament** – outermost region and contain the globular protein flagellin2) **Hook** – the filament is attached to hook, which consists of a different protein 3) **Basal body** - which anchors the flagellum to the cell wall and plasma membrane. It consists of a small central rod inserted into it are a series of rings (Fig. 16). The filament is a hollow, rigid cylinder constructed of a single protein called **flagellin** (MW from 30,000 to 60,000). Some bacteria have sheaths surrounding their flagella. For example *Bdellovibrio* has a membranous structure surrounding the filament. *Vibrio cholerae* has a lipopolysaccharide sheath.

The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex structure of the flagellum. In *E.coli* and Gram negative bacteria, the body has four rings connected to central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers. The inner M ring connects the plasma membrane.Gram positive have only twp basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan.

The synthesis of flagella is a complex process involving atleast 20 to 30 genes. Flagellin subunits are transported through the filament's hollow internal core. When they reach the tip, the subunits spontaneously aggregate under the direction of a special filament cap so that the filament grows at its tip rather than at the base. Filament synthesis is an excellent example of self-assembly.

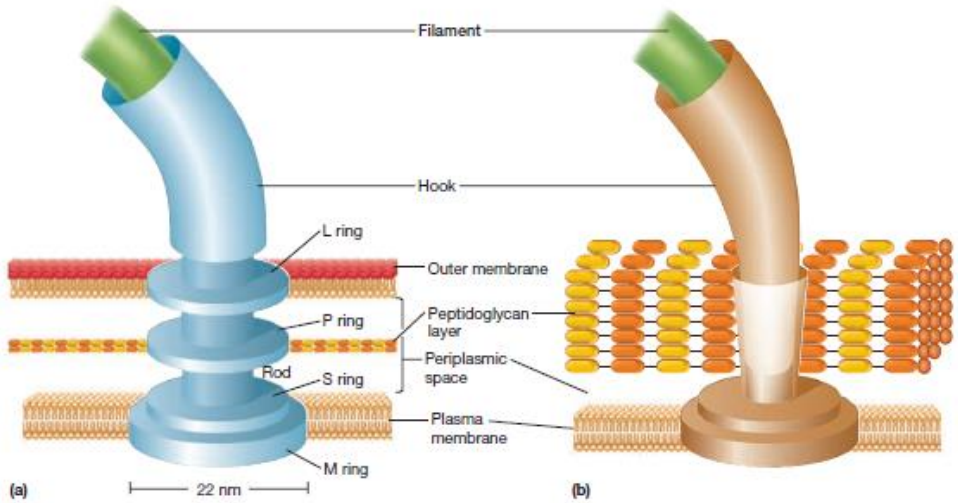

Figure The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in **(a)** gram-negative and **(b)** gram-positive bacteria.

Flagellar movement:

The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial movement. The movement in monotrichous bacteria stop and tumble randomly by reversing the flagellar rotation. The polar flagella, rotate counter clockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. Peritrichous bacteria also operate in a similar way. To move forward, the flagella rotate counter clockwise. As they do so, they bend at their hooks to for a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles

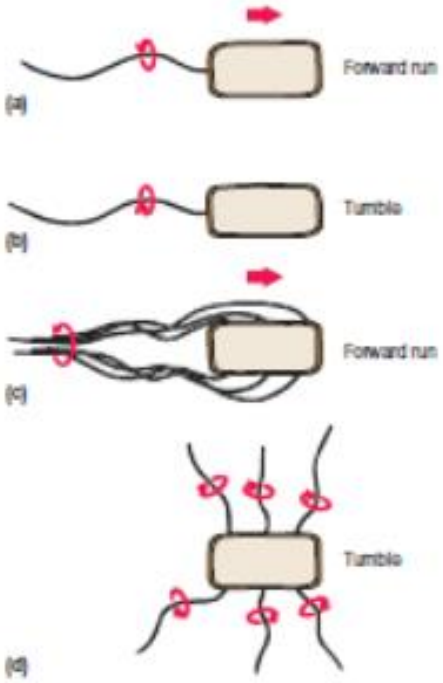

Figure 3.41 Flagellar Motility. The relationship of flagellar rotation to bacterial movement. Parts (a) and (b) describe the motion of monotrichous, polar bacteria. Parts (c) and (d) illustrate the movements of peritrichous organisms.

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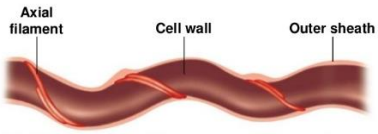
Motility enables the bacterium to move toward a favorable environment or away from a particular stimulus called taxis . Chemotaxis (include chemicals) and phototaxis (include light). Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as **chemotaxis**. The mechanism ofchemotaxis in *E.coli* has been studied most. Forward swimming is due to counterclockwise rotation of the flagellum, whereas tumbling results from clockwise rotation. The bacteria must

be able to avoid toxic substances and collect in nutrient-rich regions and at the proper oxygen levels. *E.coli* has four different chemoreceptors that recognize serine, aspartate and maltose, ribose and galactose and dipeptides respectively. These chemoreceptors often are called methyl-accepting chemotaxis proteins (MCPs)

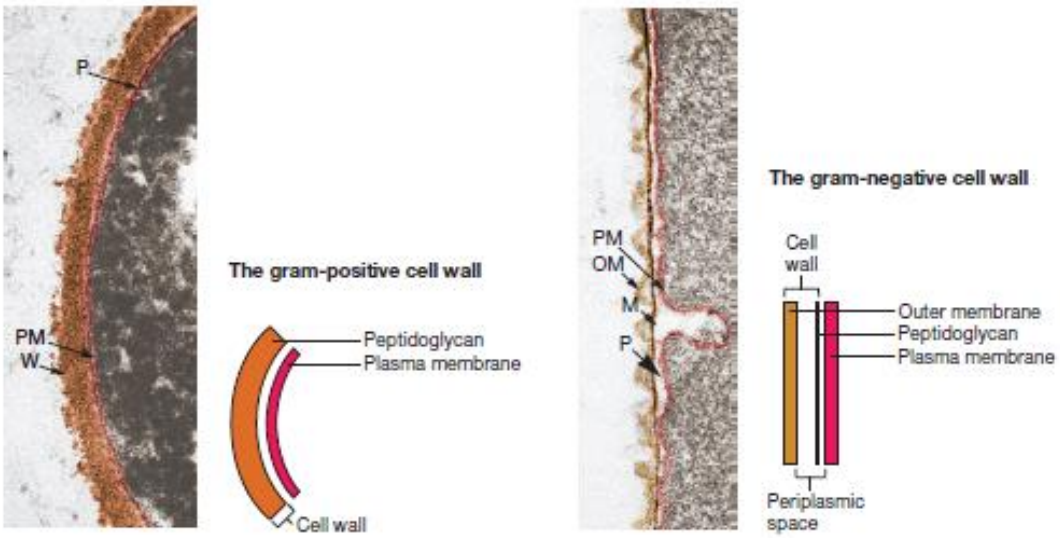
Axial Filaments: Axial filaments are similar to flagella, except that they wrap around the cell and spiral cells that move by means of an axial filament (endoflagellum) are called spirochetes

Some bacteria can move bymechanisms other than flagellar rotation. Spirochetes are a group of bacteria that have unique structure and motility (*Treponemapallidum*, the causative agent of syphilis and *Borrelia burgdorgeri* , the causative agent of Lyme disease).Spirochetes travel through viscous substances such as mucus or mud by flexing and spinning movement caused by special a xial filaments - bundles of fibrils that arise at the ends of the cell beneath the outer sheath and spiral around the cell (fig. 18).The rotation of the filaments produces an opposing movement of the outer sheath that propels the spirochetes by causing them to move like corkscrews.



(b) A diagram of axial filaments wrapping around part of a spirochete

Cell wall: Prokaryotic cell walls are composed of peptidoglycan in bacteria or pseudopeptidoglycan in archaea. Cell wall in gram-positive bacteria are characterized by a thick peptidoglycan layer, whereas in gram-negative bacterial cells, the cell walls are characterized by a thin peptidoglycan layer surrounded by an outer membrane.



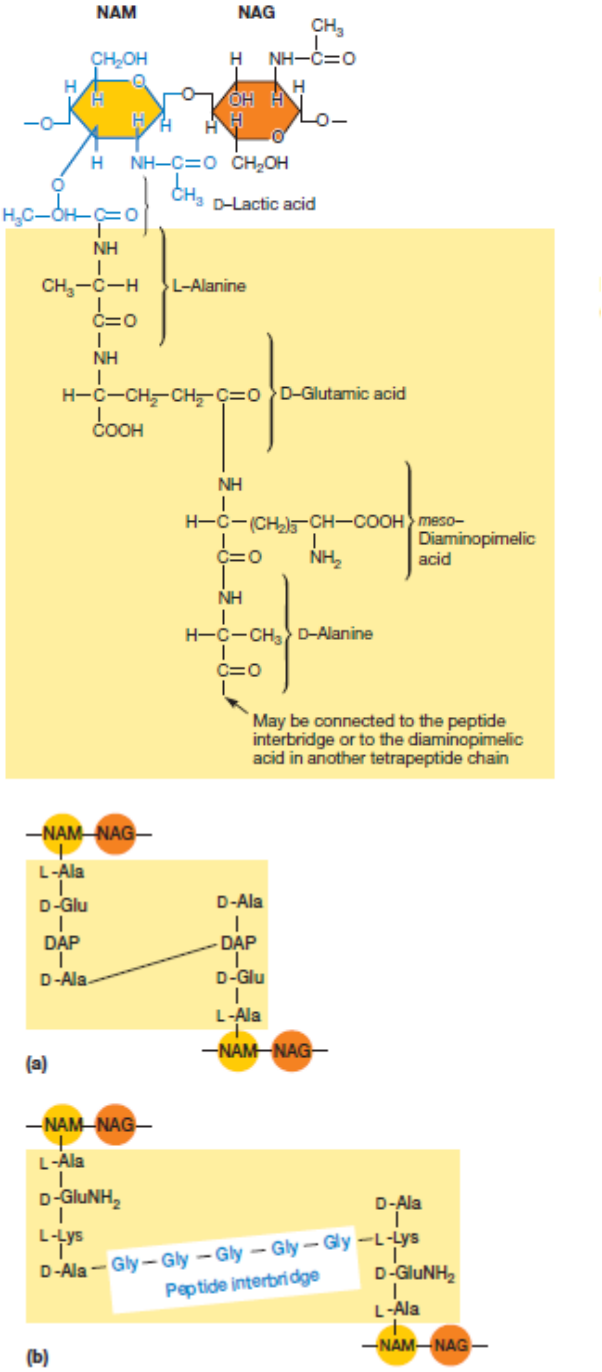


Figure Peptidoglycan Subunit Composition. The peptidoglycan subunit of *E. coli*, most other gram-negative bacteria, and many gram-positive bacteria. NAG is *N* acetylglucosamine. NAM is *N*-acetylmuramic acid. The tetrapeptide side chain is composed of alternating D- and L-amino acids since *meso*-diaminopimelic acid is connected through its L-carbon. NAM and the tetrapeptide chain attached to it are shown in different shades of color for clarity.

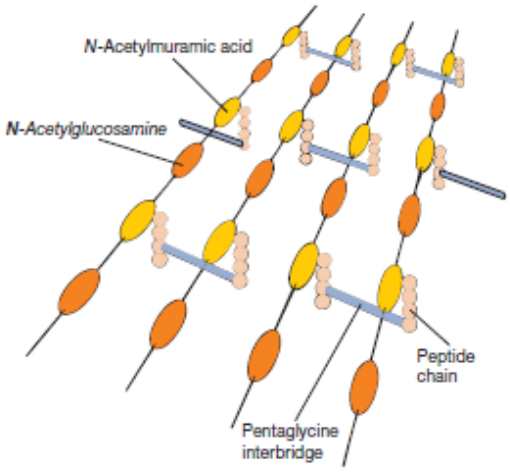


Figure Peptidoglycan Structure. A schematic diagram of one model of peptidoglycan.

Gram positive cell walls

The cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure, which contains a peptide interbridge. Gram positive cell walls also contain **teichoic acids,** polymers of glycerol and ribitol joined by phosphate groups. To the glycerol and ribotol groups, the amino acids such as D-alanine or sugars like glucose are attached. There are two classes to teichoic acids; lipoteichoic acid (if they are attached to the lipid of the plasma membrane) and wall teichoic acid (which extend to the surface of the peptidoglycan and are negatively charged). Teichoic acids are not found in gram-negative bacteria.

Some bacteria like *Staphylococci* and most other gram-positive bacteria have a layer of proteins on the surface of their cell wall peptidoglycan. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids and other receptors and these proteins are involved in the interactions of the cell with its environment. Ex. S-layer proteins. Other surface proteins are covalently attached to the peptidoglycan and in gram positive pathogens these have roles such as aiding in adhesion to host tissues, preventing opsonization and blocking phagocytosis.

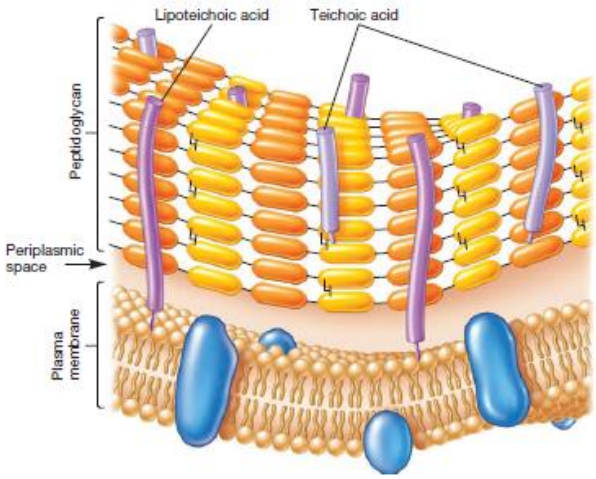


Figure: The Gram-Positive Envelope.

Gram negative cell walls

The cell wall contains only a thin layer of peptidoglycan. In *E. coli,* it is about 2-3nmthick and contains only one or two layers of sheets of peptidoglycan. The peptidoglycan is bounded to lipoproteins in the outer membrane and is embedded in a soft material, the **periplasmic gel.** Gram negative cell walls do not contain teichoic acids. They are more susceptible to mechanical breakage because of small amount of peptidoglycan. **Outer membrane -** found primarily in gram-negative cell consists of lipoproteins, liposaccharides and phospholipids and lies outside the thin peptidoglycan layer. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane e and plasma membrane appear to be in direct contact at many locations in the cell wall. The adhesion sites may be regions of direct contact or possibly true membrane fusions. It is proposed that substances can move into the cell through these adhesions sites rather than travelling through the periplasm.

The most unusual constituents of the outermembrane are its lipopolysaccharides. (LPSs). These contain both lipid and carbohydrate and consist of three parts: 1. Lipid A, 2. the core polysaccharide, and 3. the O side chain. The lipid A region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. This region is buried in the outer membrane and the remaining portion projects from the surface. The second region or the core polysaccharide is joined to lipid A and the third region or the O side chain which is a polysaccharide chain extends outward from the core. The O side chain is readily recognized by host antibodies, gram negative bacteria may thwart host defenses by readily changing the nature of their O side chains to avoid detection. The core polysaccharide contains charged sugars and phosphate, LPS contributes to the negative charge on the bacterial surface (Fig. 8). Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize the membrane structure. Lipid A is often toxic and as a result LPS can act as an endotoxin and cause some symptoms that arise in gram-negative bacterial infections. The function of the outer membrane is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. Provides a barrier to certain antibiotics (like penicillin), lysozyme detergents, heavy metals, bile salts and digestive. **Porins** are proteins that permit small molecules to pass through the outer membrane (about 800 MW). S **pecific channel proteins –** allow specific substances like vitamin B12, iron, nucleotides and maltose.

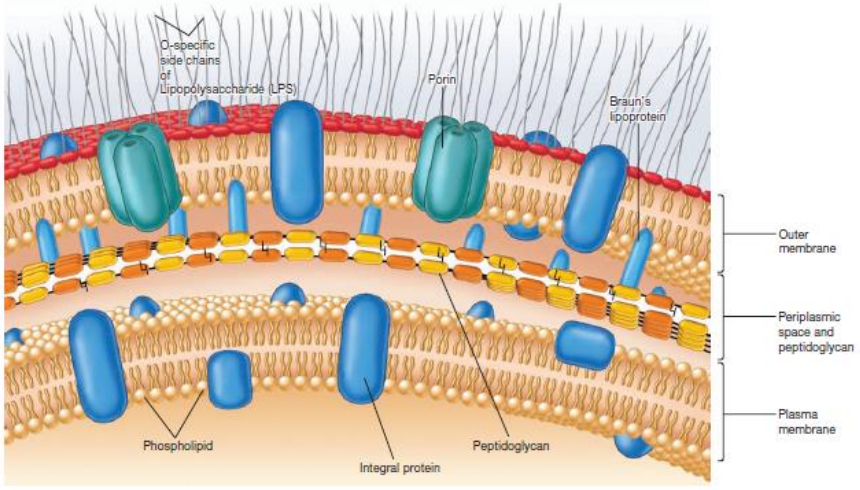


Figure: The Gram-Negative Envelope

Atypical cell walls:

- *Mycoplasma* – is a bacterial genus that naturally lacks cell walls. Their plasma membranes have lipids called sterols, which protect them from osmotic lysis.

- Archaeabacteria – have pseudomurein (N-acetylalosaminuronic acid) but no peptidoglycan.

- L forms - are mutant bacteria with defective cell walls .

Damage to the cell wall

The pres ence of cell wall is essential to protect bacteria against destruction by osmotic pressure. The bacterial cytoplasm is much more concentrated with solutes than in most microbial habitats which are hypotonic. During osmosis, water moves across selectively permeable membranes such as the plasma membrane from dilute solutions (higher water concentration) to more concentrated solutions (lower water concentration). Usually water generally enters the bacterial cells and the osmotic pressure may reach 20 atmospheres. Plasma membrane cannot resist such high pressures and the cell will swell and be physically disrupted and destroyed, a process called lysis. In hypertonic habitats, the water flows outward, and the cytoplasm shrivels up and pulls away from the cell wall. This phenomenon is called plamolysis and is useful in food preservation because many microorganisms cannot grow in dried foods and jellies as they cannot avoid plasmolysis. The importance of the cell wall in protecting bacteria can be demonstrated by treatment with lysozyme (naturally occurs in eukaryotic cells and is a constituent of tears, mucus and saliva), which attacks the peptidoglycan by hydrolyzing the bond that connects NAM and NAG units. Penicillin inhibits peptidoglycan synthesis. Gram positive cell walls are destroyed and the remaining cellular contents are referred to as **protoplast** .Gram negative cells are not completely destroyed and the remaining cellular contents are referred to as **spheroplast**. Protoplast and spheroplast are subject to osmotic lysis.

1. Structures Internal To The Cell Wall

Plasma (cytoplasmic) membrane

Membranes are absolute requirement of all living organisms. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. Plasma membrane – encloses the cytoplasm and consists of phospholipids and proteins (**fluid mosaic model**).Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends. The polar ends interact with water and are hydrophilic and the nonpolar hydrophobic ends are insoluble in water. The lipid composition of bacterial membranes varies with environmental temperature in such a way that the membrane remains fluid during growth. Bacterial membranes usually differ from eukaryotic membranes in lacking sterols such as cholesterol and they contain pentacyclic sterol-like molecules called hopanoids and these are said to stabilize the bacterial membranes. Cell membranes are very thin structures about 5 to 10 nm thick and can be seen only with electron microscope. Plasma membranes have a complex internal structure; the small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer (Fig. 9).

The most widely accepted current model for membrane structure is the fluid mosaic model of S. Jonathan Singer and Garth Nicholson. Two types of membrane proteins are seen, **Peripheral proteins** - which are loosely connected to the membrane and can be easily removed and are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These cannot be easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings. Plasma membranes serve as a selectively permeable barrier; it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The plasma membrane also is the location of a variety of crucial metabolic processes; respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation.The bacterial plasma membrane can be destroyed by alcohols and polymixins which cause leakage of intracellular contents and subsequent cell death of the organism.

Internal membrane systems: Prokaryotes do not contain complex membrane systems as present in eukaryotes like chloroplast and mitochondria. They contain membranous structures like the one observed most is **mesosome**. Mesosomes – irregular infoldings or invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae. They can be seen in both gram positive and gram-negative bacteria. These are often found next to the septa or cross-walls in dividing bacteria and sometimes seems attached to the bacterial chromosome. Thus they seem to be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells.

Some bacteria have internal membrane systems quite different from the mesosomes. The infoldings of the plasma membrane can become extensive and complex in

photosynthetic bacteria such as the cyanobacteria and purple bacteria or in bacteria with very high respiratory activity like the nitrifying bacteria. They may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes. Their function may be to provide a larger membrane surface for greater metabolic activity.

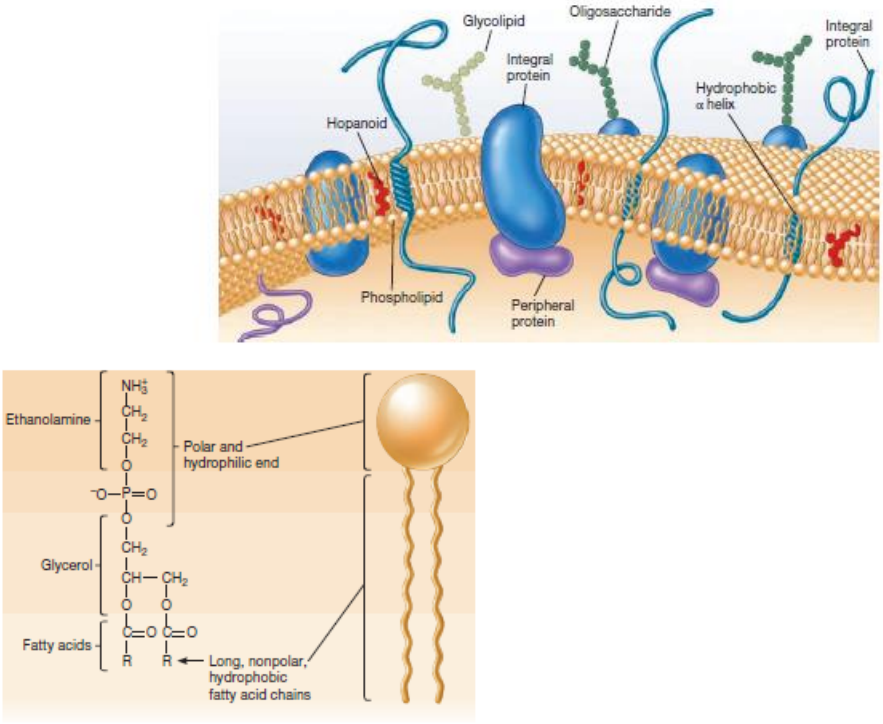


Figure: Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains.Other membrane lipids such as hopanoids (red) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

Function of Bacterial Plasma Membrane Uptake of the required nutrients by the microbial cell is important. Since microorganisms live in nutrient poor habitats, they must be able to transport nutrients from dilute solutions into the cell against concentration gradient. Finally, they must pass through a selectively permeable plasma membrane. Microorganisms use different transport mechanisms like facilitated diffusion, active transport and group translocation. Eukaryotic microorganisms do not employ group translocation but take up nutrients by endocytosis.

Movement of materials across the plasmamembrane is mostly done by two processes:

Passive processes : Substances cross the area from an area of high concentration to an area of low concentration without any expenditure of energy (ATP). Example, simple diffusion, osmosis and facilitated diffusion.

Active process: The cell must use energy (ATP) to move substances from areas of low concentration to areas of high concentration. Example, Group translocation.

Passive processes:

Passive or simple diffusion :Often called diffusion, is the process in which molecules move from a region of higher concentration to one of lower concentration. The rate is dependent on the size of the concentration gradient between a cell's exterior and its interior. Very small molecules such as water and oxygen and carbon dioxide move across membranes by simple or passive diffusion. Larger molecules, ions, and polar substances do not cross membranes by this method.

Osmosis: Is the net movement of solvent molecules across a selectively permeable membrane from an area in which the solvent molecules are highly concentrated to an area of low concentration until equilibrium is reached. In living systems the chief solvent is water. The three types of solutions which are normally found are isotonic, hypotonic and hypertonic.

Facilitated diffusion: The rate of diffusion across selectively permeable membrane is greatly increased by using carrier proteins, sometimes called **permeases** which are embedded in the plasma membrane. Because a carrier aids the diffusion process, it is called as **facilitated diffusion.** Carrier proteins also resemble enzymes in their specificity for the substances to be transported; each carrier is selective and will transport only closely related solutes. Because there is no energy input, molecules will continue to enter only as long as their concentration is greater on the outside. Two widespread major intrinsic protein channels in bacteria are aquaporins that transport water and glycerol facilitators which aid glycerol diffusion. The carrier protein complex spans the membrane (Figure 1). After the solute molecule binds to the outside, the carrier may change conformation and release the molecule on the cell interior. The carrier would subsequently change back to its original shape and be ready to pick up another molecule. The mechanism is driven by concentration gradients and therefore is reversible.

Examples. Glycerol is transported by facilitated diffusion in *E.coli, Salmonella typhimurium, Pseudomonas, Bacillus* and many other bacteria. This is prominent in eukaryotes where it is used to transport a variety of sugars and amino acids.

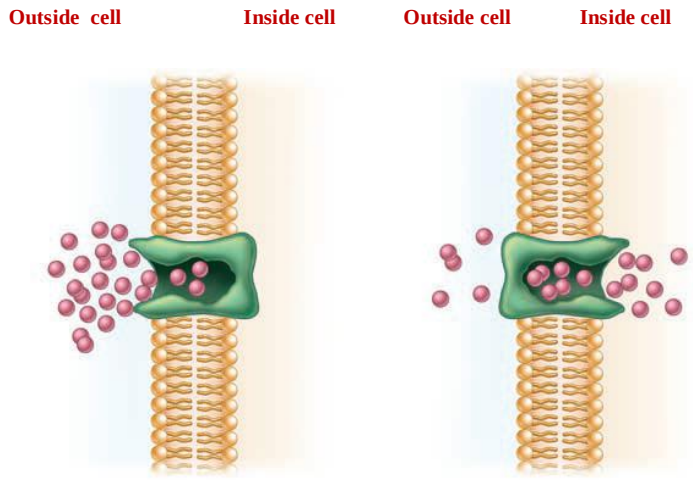


Fig. Facilitated diffusion. The carrier proteins aid in the release of solute molecules from extracellular space to the intracellular space.

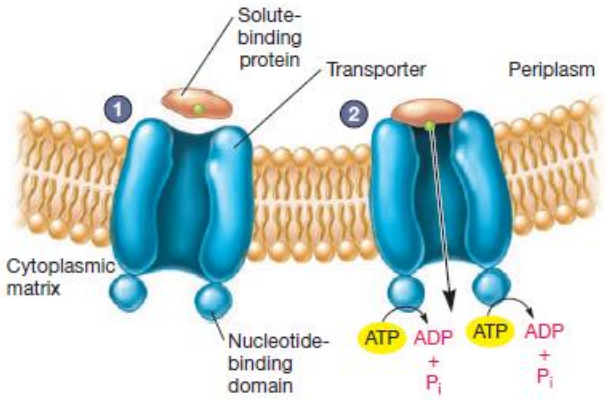


Figure ABC Transporter Function. (*1*) The solute binding protein binds the substrate to be transported and approaches the ABC transporter complex. (*2*) The solute binding protein attaches to the transporter and releases the substrate, which is moved across the membrane with the aid of ATP hydrolysis.

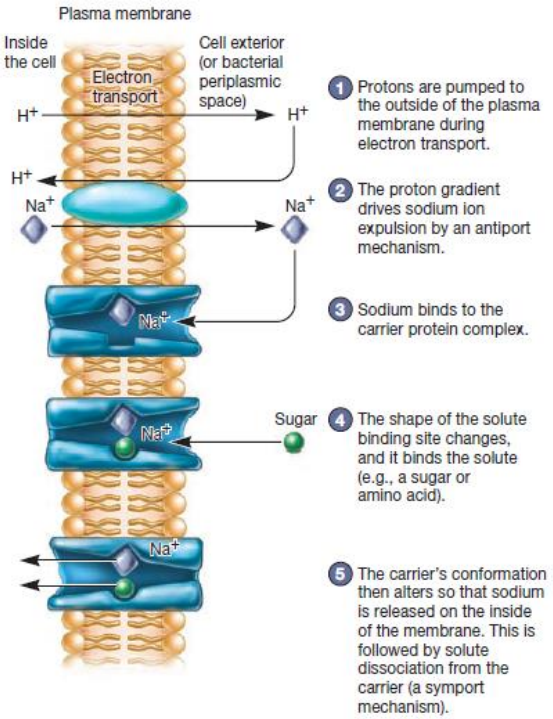


Figure Active Transport Using Proton and Sodium Gradients.

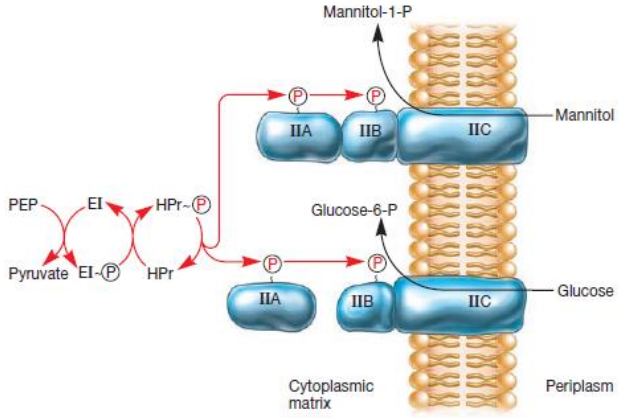


Figure. Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated.The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heatstable protein (HPr), and enzyme II (EII).The highenergy phosphate is transferred from HPr to the soluble EIIA. EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system. In either case the phosphate moves from EIIA to EIIB, and then is transferred to the sugar during transport through the membrane.Other relationships between the EII components are possible. For example, IIA and IIB may form a soluble protein separate from the membrane complex; the phosphate still moves from IIA to IIB and then to the membrane domain(s).

Cytoplasm: Cytoplasm is the fluid component of the cell surrounded by the plasma membrane. The cytoplasm is composed mostly of water along with inorganic and organic molecules, DNA, ribosomes, and inclusions.

The Nucleoid: Prokaryotic DNA and DNA-associated proteins are not bound by a complex nuclear membrane and occur as the **nucleoid** region in the cytoplasm of the cell. Prokaryotic chromosomes are typically circular and haploid (unpaired). In bacteria, Nucleoid-associated proteins (NAPs) interacts with prokaryotic DNA and function like histones of eukaryotic cells assisting in the organization and packaging of the chromosome. However, in archaea, either NAPs or histone-like DNA organizing proteins associate with the chromosomal DNA to organize the nucleoid.

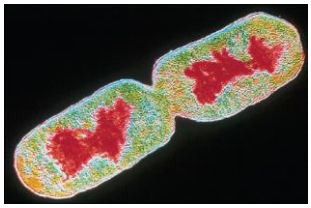
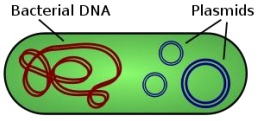


Figure Procaryotic Nucleoids and Chromosomes

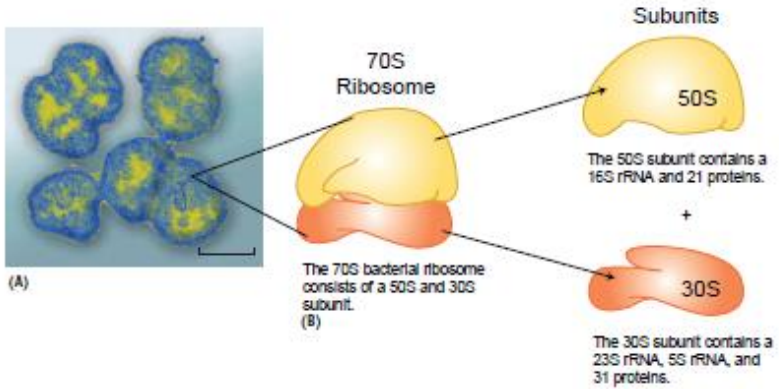
Plasmids: Prokaryotic cells may also contain small, circular, double-stranded extrachromosomal DNA molecules called **plasmids**. Plasmids are generally many in number per cell and often carry genes that confer advantageous traits such as antibiotic resistance.



There are five main classes:

- Fertility F-plasmids, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili.
- Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen. e.g. Ti plasmid in *Agrobacterium tumefaciens*

Ribosomes: The cytoplasm of a prokaryotic cells contains numerous ribosomes of the type 70S. Ribosomes are made up of rRNA and proteins and serve as the site for protein synthesis. Ribosomes are composed of two subunits, each subunit being composed of protein and a type of RNA called ribosomal RNA (rRNA).They are comm. Only 70S: 30S subunit (1 molecule of rRNA) and 50S subunit (2 molecules of rRNA) and have dimensions of about 14 to 15 nm, a molecular weight of approximately 2.7 million.The S in 70S stands for Svedberg value or sedimentation coefficient. It is the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater is its Svedberg value. The Sedimentation coefficient is a function of a particle's molecular weight, volume and shape. Several antibiotics, such as streptomycin, neomycin and tetracyclines, exert their antimicrobial effects by inhibiting protein synthesis on ribosomes.



Inclusions: Inclusions are reserve deposits found in the cells and some examples of prokaryotic cell inclusions found in bacteria are metachromatic granules (inorganic phosphate), polysaccharide granules (usually glycogen or starch), lipid inclusions, sulfur granules, carboxysomes (ribulose 1,5-diphosphate carboxylase), magnetosomes (Fe_3O_4 or magnetite), and gas vacuoles.

Organic inclusion bodies:

Glycogen: Polymer of glucose units composed of long chains formed by alpha (1-4) glycosidic bonds and branching chains connected to themby alpha (1-6)glycosidic bonds. Ex. glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells (glycogen granules appear reddish brown and starch granules appear blue).

Poly B- hydroxybutyrate: Contains beta-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Appear in various species of *Mycobacterium, Bacillus, Azotobacter* , *Spirillum* and other genera. Lipid inclusions are revealed by use of fat-soluble dyes, such as Sudan dyes.

Glycogen and PHB are carbon storage reservoirs providing material for energy and biosynthesis.

Inorganic inclusion bodies:

Polyphosphate granules orMetachromatic granules:

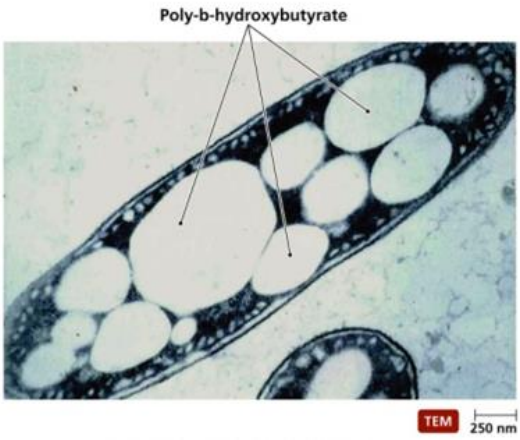
Linear polymer of organo phosphates joined by ester bonds. Reservoirs for phosphate, an important component of cell nucleic acids and also energy reserves. Represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. Stain red with certain blue dyes, such as methylene blue, and are collectively known as volutin. Found in algae, fungi and protozoans, as well as bacteria. These granules are quite large and are characteristic of *Corynebacterium diphtheriae*, the causative agent of diphtheria, thus they have diagnostic significance.

Sulphur granules:

Sulphur bacteria, which belong to the genus *Thiobacillus*, derive energy by oxidizing sulfur and sulfur containing compounds. These bacteria may deposit sulfur granules in the cell, where they serve as an energy reserve. Purple photosynthetic bacteria use H_2S as electron donor and accumulate resulting sulfurin either the periplasmic space or in special cytoplasmic globules.

Magnetosomes:

Not for storage, but these are used by some bacteria to orient in the earth's magnetic field. These inclusion bodies contain iron in the form of magnetite (greigite or pyrite) (Fig. 13). Ex. *Aquaspirillum magnetotacticum.* Also present in heads of birds, dolphins, and turtles etc which aid in navigation.



Cyanophycin granules:

Cyanobacteria are composed of large amino acids containing approximately equal amounts of amino acids arginine and aspartic acid. These are used to store extra nitrogen for the bacteria.

Carboxysomes:

These are polyhedral and hexagonal inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. ·Bacteria that use carbon dioxide as their sole source of carbon require this enzyme for carbon dioxide fixation during photosynthesis (Ex.nitrifying bacteria, cyanobacteria, and *Thiobacilli*).

Gas vacuoles: These are hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria. Each vacuole consists of rows of several individual gas vesicles, which are hollow cylinders covered by protein. Their function is to maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light and nutrients. They are impermeable to water and permeable to atmospheric gases.

Reference Books:

1. Lansing M. Prescott, John P. Harley and Donald A. Klein. Microbiology. Mc Graw Hill companies.