Bacterial Physiology

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3rd microbiology 2022-2023

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Syllabus:

Bacterial physiology: The science study the fine structures and functions that allow bacteria to survive. This includes everything from the composition of bacterial cell walls to the enzymes they can produce to perform various internal and external functions which include :

1. Studying known organisms as well as learning more about new bacteria.
2. Researchers in bacterial physiology look at bacterial genetics and how bacteria respond to evolutionary pressures.
3. Study the enzymes produced by bacteria. This includes structures necessary for cellular functions like division as well as bacterial enzymes that may be released into the surrounding environment. Some of these are toxic and can play a role in bacterial infections .
4. Some focus specifically on infectious disease and the interactions between bacteria and other organisms like symbiosis and parasites .

5. Physiology describes the role of metabolic reactions in the life processes of a bacterium. The study of bacteria has significance beyond the understanding of bacteria themselves. Since bacteria are abundant, easily grown, and relatively simple in cellular organization, they have been used extensively in biological research (genetic engineering) .
6. Functional analyses of bacterial systems have provided a foundation for much of the current detailed knowledge about molecular biology and genetics.
7. Using of bacterial products in, drug and food industrial like probiotic, antibiotic, bacteriocin, biosurfactant, soil biofertilizer, bioremediation.

Bacteria: are prokaryotes, lacking the complicated cellular organization found in higher organisms; they have no nuclear envelope and no specialized organelles. Yet they employ in all the basic life processes—transport of materials into and out of the cell, catabolism and anabolism of complex organic molecules, and the maintenance of structural integrity. To accomplish this, bacteria must obtain nutrients and convert them into a form of energy that is useful to the cell.

Bacterial species are differentiated by morphology, chemical composition, nutritional requirements, biochemical activities, and source of energy.

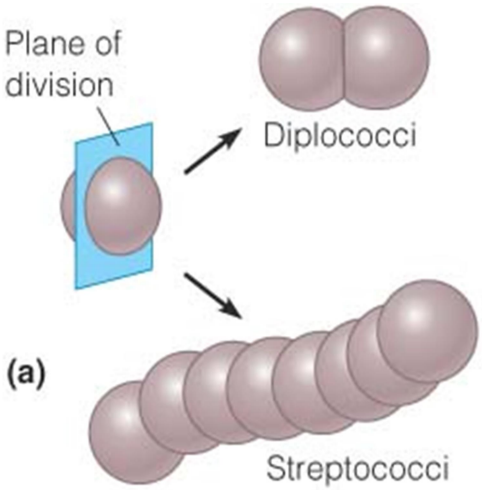
The Size, Shape and Arrangement of Bacterial Cells

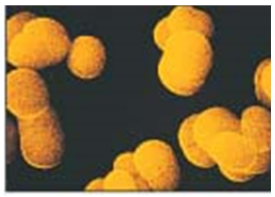
Most bacteria are 0.2 μm in diameter and 2-8 μm in length. The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted), however pleomorphic bacteria can assume several shapes. Nanobacteria and nanoarchaea range from around 0.2 μm to less than 0.05 μm in diameter .The huge bacterium *Epulopiscium fishelsoni* grows as large as 600 by 80 μm. An even larger bacterium, *Thiomargarita namibiensis,* has been discovered in ocean sediment.

Arrangement of cocci

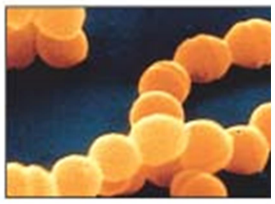
Cocci may be oval, elongated, or flattened on one side. Cocci may remain attached after cell division.

These group characteristics are often used to help identify certain cocci.

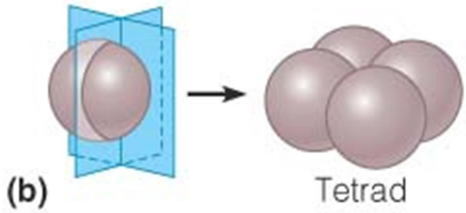




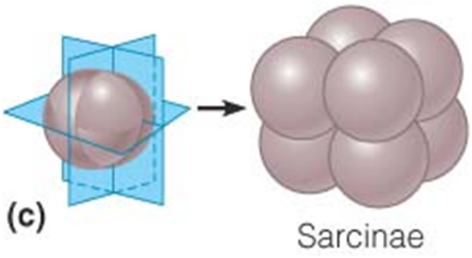
Cocci that remain in pairs after dividing are called diplococci.

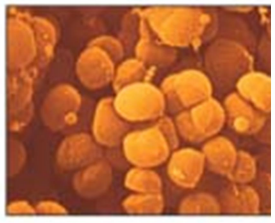


Cocci that remain in chains after dividing are called streptococci.

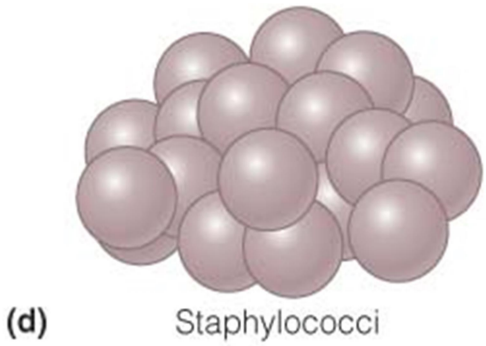


Cocci that divide in two planes and remain in groups of four are called tetrads.





Cocci that divide in three planes and remain in groups cube like groups of eight are called sarcinae.

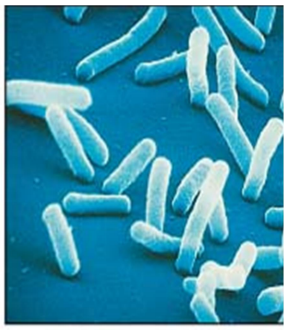


Cocci that divide in multiple planes and form grape like clusters or sheets are called Staphylococci.

Bacilli

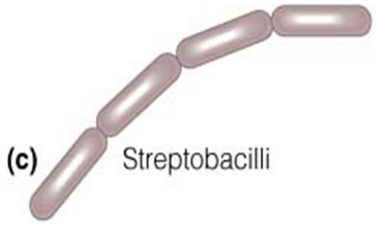
Since bacilli only divide across their short axis there are fewer groupings.

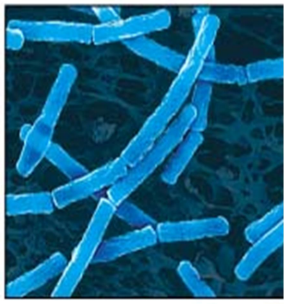
Bacillus is a shape (rod shaped) but there is also a genus of bacteria with the name **Bacillus.**



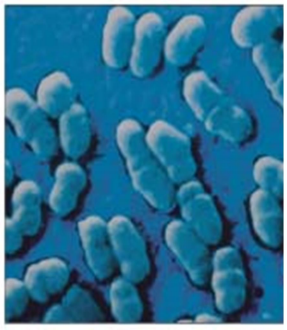
Most bacilli appear as single rods.

Diplobacilli appear in pairs after division.





Streptobacilli appear in chains after division.

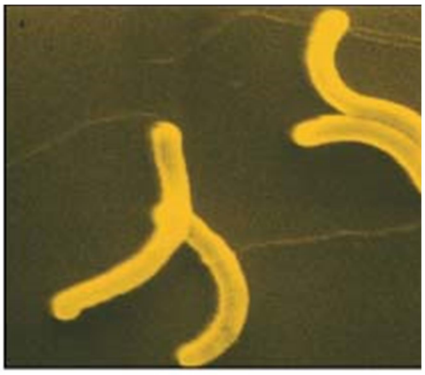


Some bacilli are so short and fat that they look like cocci and are referred to as coccobacilli.

Spiral bacteria

Spiral bacteria have one or more twists.

(a) Vibrio

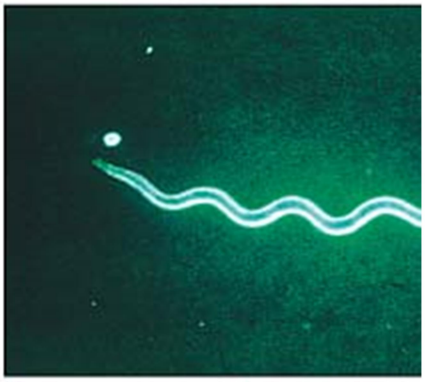


Vibrios look like curved rods.

(b) Spirillum

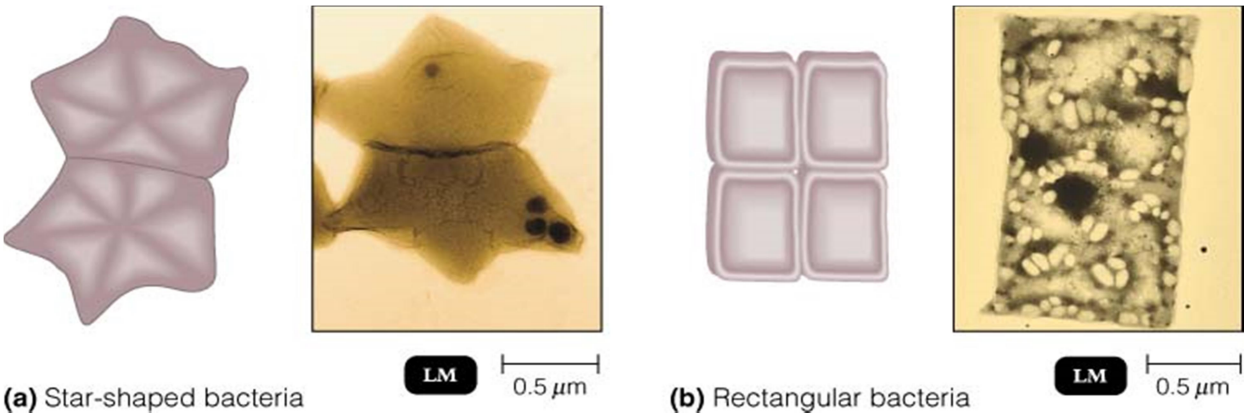
Spirilla have a helical shape and fairly rigid bodies.

(c) Spirochete



Spirochetes have a helical shape and flexible bodies. Spirochetes move by means of axial filaments, which look like flagella contained beneath a flexible external sheath.

Other shapes



(a) Star-shaped bacteria

(b) Rectangular bacteria

Stella are star-shaped.

Haloarcula, a genus of halophilic archaea, are rectangular.

Surface to volume ratio

The size and shape determination are related and have been selected for during the evolutionary history of each bacterial species. The microbes had to be small because being small increases the surface area-to-volume ratio (S/V ratio; figure). As this ratio increases, the uptake of nutrients and the diffusion of these and other molecules within the cell become more efficient, which in turn facilitates a rapid growth rate. Shape affects the S/V ratio. A rod with the same volume as a coccus has a higher S/V ratio than does the coccus. This means that a rod can have greater nutrient flux across its plasma membrane. Also cells that may be are filamentous or have prostheca (e.g., the hypha and bud of a *Hyphomicrobium* sp.) to increase the surface area.

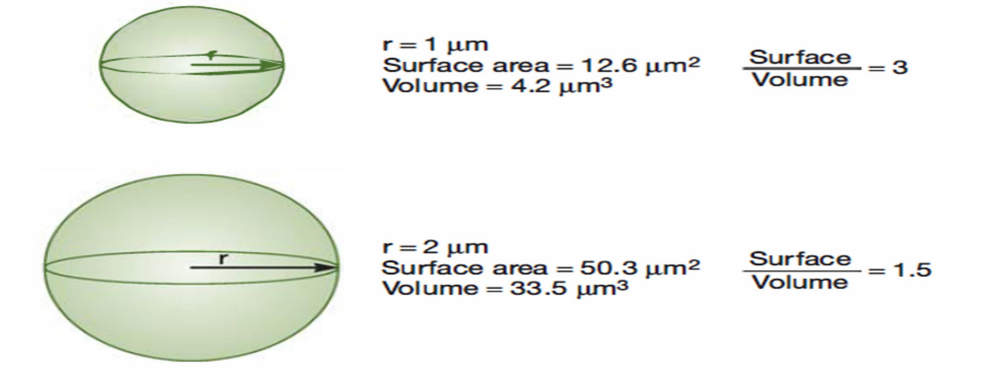
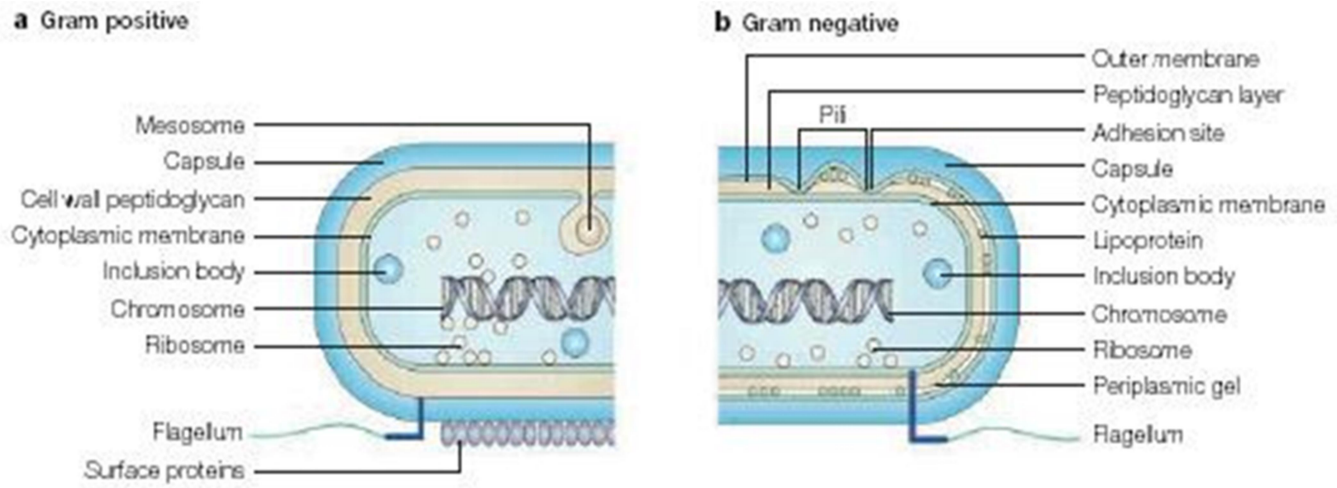


Figure ☐ **The Surface-to-Volume Ratio.** Surface area is calculated by the formula $4\pi r^2$. Volume is calculated by the formula $4/3\pi r^3$. Shape also affects the S/V ratio; rods with the same diameter as a coccus have a greater S/V ratio.

Procaryotic Cell Organization

Procaryotic cells usually are bounded by a chemically complex cell wall, which covers the plasma membrane. The plasma membrane in turn surrounds the cytoplasm and its contents. Because most procaryotic cells do not contain internal, membrane bound organelles, their interior appears morphologically simple. The genetic material is localized in a distinct region, the nucleoid, and usually is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusion bodies are scattered about the cytoplasm. Many procaryotes use flagella for locomotion. In addition, many are surrounded by a capsule or slime layer external to the cell wall.

General bacterial structure as in the figure :



Overview of Bacterial Cell Wall Structure

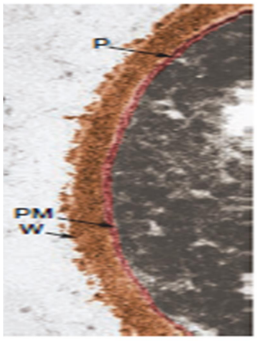
After Christian Gram developed the Gram stain in 1884, it soon became evident that most bacteria could be divided into two major groups based on their response to the Gram-stain. **Gram-positive bacteria stained purple**, whereas **gram-negative bacteria were colored pink or red** by the technique. The true structural difference between these two groups did not become clear until the advent of the transmission electron microscope. **Archaea have different types of cell walls (gram positive or negative).**

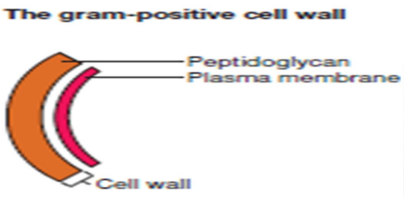
The gram-positive cell wall consists of a single, 20 to 80 nm thick homogeneous layer of peptidoglycan (murein) lying outside the plasma membrane (figure) involve 90% of cell wall .

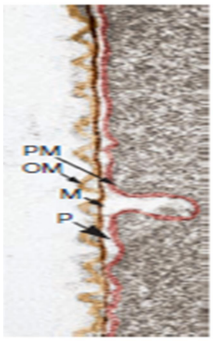
In contrast, the gram-negative cell wall is quite complex. It has a 2 to 7 nm peptidoglycan layer involve 5-20% of cell wall, covered by a 7 to 8 nm thick outer membrane . **Because of the thicker peptidoglycan layer, the walls of gram-positive cells are more resistant to osmotic pressure than those of gram-negative bacteria.** One important feature of the cell envelope is a space that is frequently seen **between the plasma membrane and the outer membrane**. This space is called the periplasmic space from 1 to as great as 70 nm thick covered 20-40 % of all cell volume. The substance that occupies the periplasmic space is the periplasm.

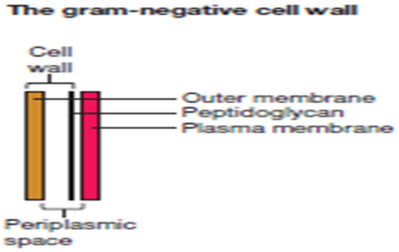
It also is sometimes observed **between the plasma membrane and the wall in gram-positive** bacteria. The nature of the periplasmic space and periplasm differs in gram-positive and gram-negative bacteria. filled with gelatinous substance contain units of peptidoglycan, proteins and enzymes that have an important role in the cell live such as proteolysis enzymes and transport proteins specially in G- but less number of proteins in G +.

in Archaea may have an outer membrane with a very wide periplasmic space especially within the genus Ignicoccus.









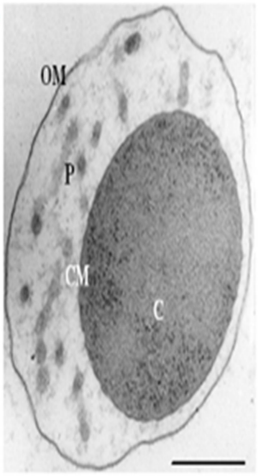


Figure 2: *Ignicoccus hospitalis*, ultrathin section

C = Cytoplasm

CM = cytoplasmic membrane

P = Periplasm

OM = outer membrane

Scale bar: 0.5µm

The cell walls function of Bacteria and Archaea

1- The cell wall surrounds the plasma membrane and protect the cell from osmotic lysis .
2- Give the bacteria shape and rigidity .
3- Have antigenic structure that stimulate the immune system (have a role in pathogenicity).
4- Have phage receptors site .
5- Protects the cell from toxic substances.
6- Has the ability to rebuild the wall again when destroyed or parts of it during elongation.
7- Non selective for permeable.
8- Site of action of several antibiotics.

Peptidoglycan Structure:

Peptidoglycan is an enormous, mesh like polymer composed of many identical subunits. The polymer contains two sugar derivatives, ***N* -acetylglucosamine** and ***N* -acetylmuramic acid**, and several different amino acids. Three of these amino acids are not found in proteins:

D -glutamic acid, D -alanine, and *meso* –diaminopimelic acid. (The presence of D -amino acids protects against degradation by most peptidases, which recognize only the L -isomers of amino acid residues).

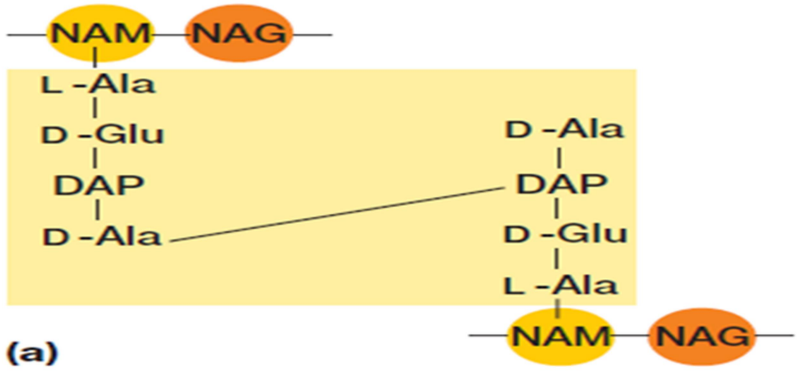
The peptidoglycan subunit present in most gram-negative and many gram-positive bacteria is shown in figure . The backbone of this polymer is composed of alternating *N* -acetylglucosamine and *N* -acetylmuramic acid residues bind in glycoside bound β 1-4 . A peptide chain of four alternating D - and L -amino acids is connected to the carboxyl group of *N* -acetylmuramic acid. Many bacteria replace *meso* -diaminopimelic acid (the precursor of lysine) with another diamino acid, usually L -lysine (figure).

To make a strong, mesh like polymer, peptidoglycan chains must be joined by cross-links between the peptides. Often the carboxyl group of the terminal D -alanine is connected directly to the amino group of diaminopimelic acid, but a peptide inter bridge may be used instead (figure). Most gram-negative cell wall peptidoglycan lacks the peptide inter bridge . With or without a peptide inter bridge, cross-linking results in an enormous peptidoglycan sac that is actually one dense, interconnected network (figure). These sacs

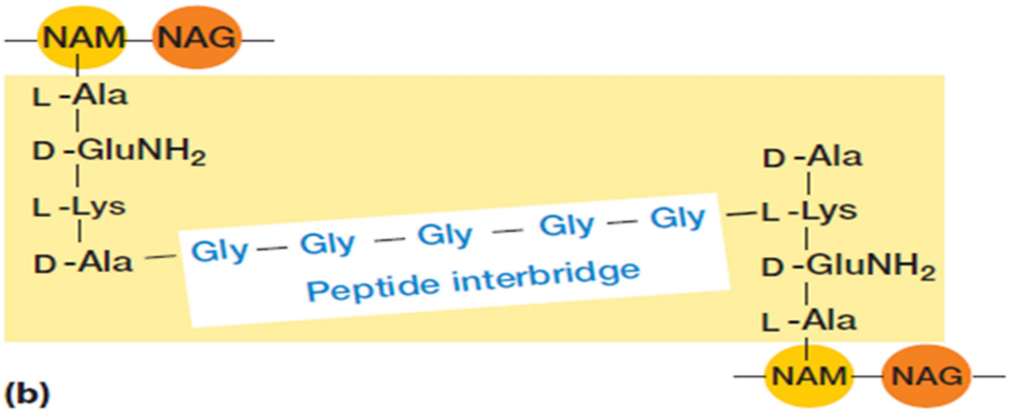
have been isolated from gram-positive bacteria and are strong enough to keep their shape and integrity, yet they are relatively permeable and elastic (figure).

The differences between G+ & G- in possession of the amino acid Lysine in third position instead of DAP and the possession of cross bridges consisting of Penta glycine , which varies from one type to another and may be consist of a single amino acid, which are most common in Gr + than Gr-. Some of the Gr + have D.DAP in a third location bound with D-alanine, as in Corynebacterium, Clostridium, Lactobacillus , Bacillus and some replace D.DAP by L.DAP.

In plant pathogen Corynebacterium the cross bridges site 2 and 4 instead of 3 and 4. (((**Most proteins are composed of amino acids L-type while in the brain and the bacteria are of type D, which prevents them from attacking by Peptidases and also give different and distinctive antigenic structure of the bacteria from eukaryotic))).**



Typical cell wall peptidoglycan in *Escherichia coli*



Typical cell wall peptidoglycan in *Staphylococcus aureus*

Gram-Positive Cell Walls

Gram-positive bacteria normally have cell walls that are thick and composed primarily of peptidoglycan. Peptidoglycan in gram-positive bacteria often contains a peptide inter bridge. In addition, gram-positive cell walls usually contain large amounts **of teichoic acids** , polymers of glycerol or ribitol joined by phosphate groups (figure). Amino acids such as D - alanine or sugars such as glucose are attached to the glycerol and ribitol groups.

The teichoic acids are covalently connected to the peptidoglycan itself are called **Wall Teichoic acid** are polymer of ribitol found in some G+ bacteria or bound to plasma membrane lipids are polymer of glycerol they are called **lipoteichoic acids**(found in all G+).

Teichoic acids function:

1- Because they are negatively charged, they help give the gram-positive cell wall its negative charge.

2- The functions of techoic acids are still unclear, but they may be important in conserving the structure of the wall.

3- Many covalently attached proteins, such as the M protein of pathogenic streptococci, bind with lipoteichoic acid and form Micro fibrils that have roles in virulence, such as assisting in adhesion to host tissues and interfering with host defenses. In staphylococci, these surface proteins are covalently joined to the pentaglycine interbridge of the peptidoglycan .

4- bind and regulate movement of cations into and out of the cell lick ions of magnesium and calcium .

5- prevent extensive wall breakdown and possible cell lysis during cell growth.

6- provide much of the cell wall's antigenicity.

7- Assistance in transformation.

8- Phage receptor site in some bacteria.

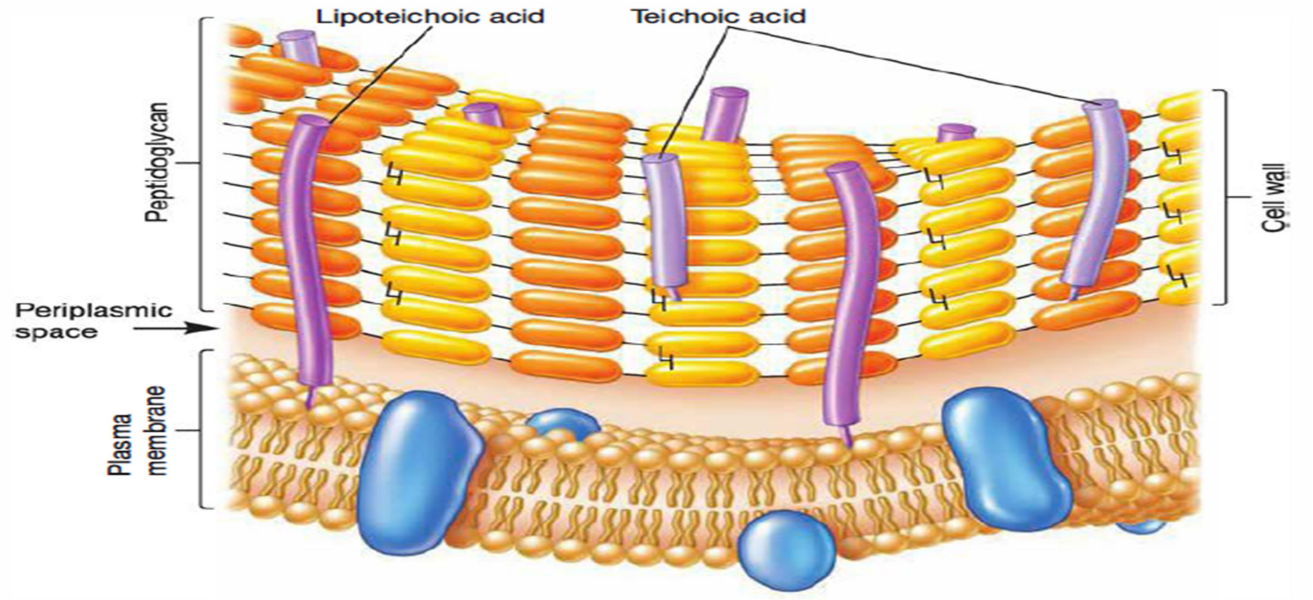


Figure 3.23 Typical Gram-Positive Cell Wall. This component of the cell envelope lies just outside the plasma membrane.

Gram-Negative Cell Walls

The **(figure)** shows that gram-negative cell walls are much more complex than gram-positive walls. The thin peptidoglycan layer next to the plasma membrane and bounded on either side by the periplasmic space usually constitutes only 5 to 10 % of the wall weight. In *E. coli,* it is about 2 nm thick and contains only one or two sheets of peptidoglycan.

The periplasmic space of gram-negative bacteria is also strikingly different from that of gram-positive bacteria. It ranges in width from 1 nm to as great as 71 nm. Some recent studies indicate that it may constitute about 20 to 40% of the total cell volume, and it is usually 30 to 70 nm wide. When cell walls are disrupted carefully or removed without disturbing the underlying plasma membrane, periplasmic enzymes and other proteins are released and may be easily studied. Some periplasmic proteins participate in nutrient acquisition—for example, hydrolytic enzymes and transport proteins. Some periplasmic proteins are involved in energy conservation. For example, the denitrifying bacteria, which convert nitrate to nitrogen gas, and bacteria that use inorganic molecules as energy sources (chemolithotrophs) have electron transport proteins in their periplasmic. Other periplasmic

proteins are involved in peptidoglycan synthesis and the modification of toxic compounds that could harm the cell.

The outer membrane lies outside the thin peptidoglycan layer and is linked to the cell in two ways (**figure**). **The first is by Braun's lipoprotein**, the most abundant protein in the outer membrane. This small lipoprotein is covalently joined to the underlying peptidoglycan and is embedded in the outer membrane by its hydrophobic end.

The second linking mechanism involves the many **adhesion sites** joining the outer membrane and the plasma membrane. The two membranes appear to be in direct contact at these sites. In *E. coli,* 20 to 100 nm areas of contact between the two membranes can be seen. Adhesion sites may **be regions of direct contact or possibly true membrane fusions**. Possibly the most unusual constituents of the outer membrane are its **lipopolysaccharides (LPSs) .** These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain. The LPS from Salmonella has been studied most, and its general structure is described here (**figure**).

The **lipid A** region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. The fatty acids of lipid A are embedded in the outer membrane, while the remainder of the LPS molecule projects from the surface. The **core polysaccharide** is joined to lipid A. In Salmonella*,* it is constructed of 10 sugars, many of them unusual in structure. The **O side chain** or **O antigen** is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains.

Some bacteria didn't have O-antigen in the layer of LPS which form Lipooligosaccharides (LOS) but it have strong antigenicity .

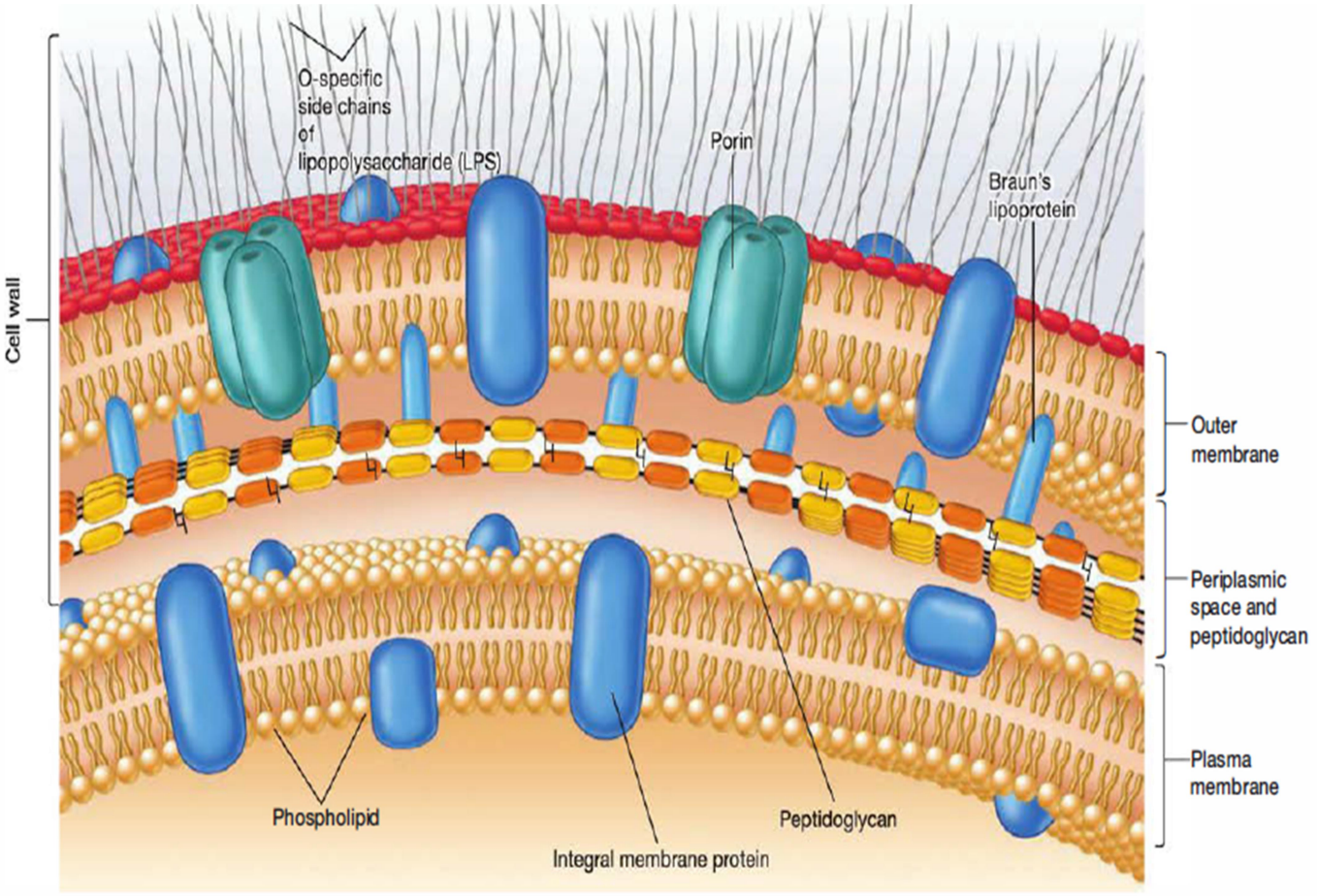


Figure 3.25 Typical Gram-Negative Cell Wall. Notice that these bacteria are bounded by two membranes, the plasma membrane and the outer membrane of the cell wall.

LPS & O.M has many important functions:

1- Because the core polysaccharide usually contains charged sugars and phosphate LPS contributes to the negative charge on the bacterial surface.

2- LPS helps stabilize outer membrane structure because lipid A is a major constituent of the exterior leaflet of the outer membrane.

3- LPS may contribute to bacterial attachment to surfaces and biofilm formation.

4- A major function of LPS is that it helps create a permeability barrier. The geometry of LPS (figure) and interactions between neighboring LPS molecules are thought to restrict the

entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium.

5- LPS also plays a role in protecting pathogenic gram-negative bacteria from host defenses. The O side chain of LPS is also called the **O antigen** because it produces an immune response by an infected host. This response involves the production of antibodies that bind the strain-specific form of LPS that elicited the response. However, many gram-negative bacteria can rapidly change the antigenic nature of their O side chains, thus preventing host defenses.

6- Despite the role of LPS in creating a permeability barrier, the outer membrane is more permeable than the plasma membrane and permits the passage of small molecules such as glucose and other monosaccharides. This is due to the presence of **porin proteins .** Most porin proteins cluster together to form a **trimer** in the outer membrane. Each porin protein spans the outer membrane and is more or less tube-shaped; its narrow channel allows passage of molecules smaller than about 600 to 700 daltons. However, larger molecules such as vitamin B 12 also cross the outer membrane. Such large molecules do not pass through porins; instead, specific carriers transport them across the outer membrane.

((((Porins are proteins that permit small molecules to pass through the outer membrane; specific channel proteins allow other molecules to move through the outer membrane. The permeability varies with the species, for example, ***Pseudomonas aeruginosa*** has an external membrane less than 100 times the permeability of the outer membrane of ***E. coli*** (for this to be more resistant to antibiotics) depending on the type of genes responsible for encoding transported proteins .

7- the lipid A portion of LPS is toxic; as a result, LPS can act as an endotoxin and cause some of the symptoms that arise in gram-negative bacterial infections. If LPS or lipid A

enters the bloodstream, a form of septic shock develops, for which there is no direct treatment.

8- Protecting enzymes in periplasmic space from loss .

9- Have a sex pili as protein receptor for Bacteriophage .

10- The outer membrane protects the cell from phagocytosis and from penicillin, lysozyme, and other chemicals.

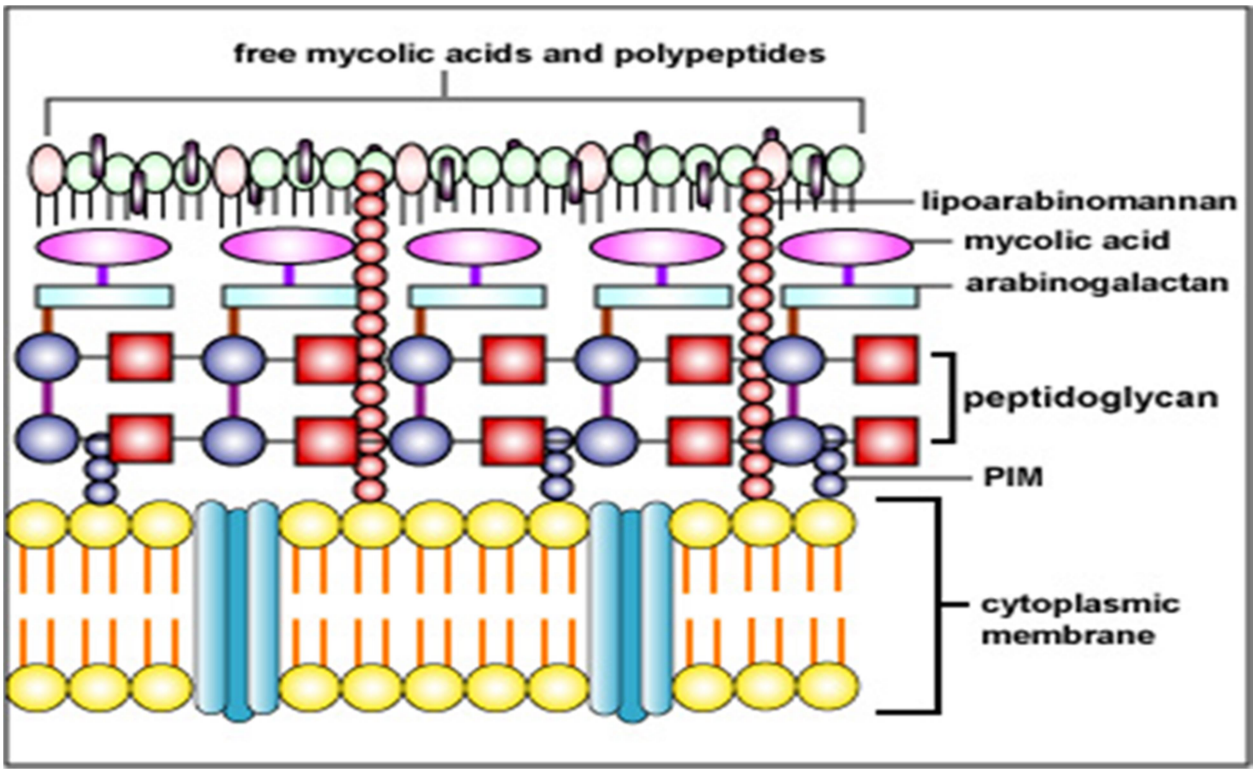
A typical Cell Walls

Mycoplasma is a bacterial genus that naturally lacks cell walls; has a triple-layered "unit membrane" that contains a sterol (mycoplasmas require the addition of serum or cholesterol to the medium to produce sterols for growth);the presence of sterols in the plasma membrane protects from osmotic lysis. mycoplasmas are completely resistant to penicillin because they lack the cell wall structures at which penicillin acts, but they are inhibited by tetracycline or erythromycin

Mycobacterium: is a genus that has mycolic acids in its cell walls, giving it a "waxy" cell wall that is resistant to decolorization with acid-alcohol when stained with carbol fuchsine (and so is designated "acid-fast"). Their cell walls have a very high lipid content and contain waxes with 30 to 90 carbon **mycolic acids .** Mycolic acids are a group of complex branched-chain hydroxylated lipids covalently bound to peptidoglycan in the cell wall; The mycolic acid forms a layer outside of a thin layer of peptidoglycan that are held together by a polysaccharide (arabinogalactans).

In **Mycobacterium:** They have three kinds of it composes 60% of the cell wall outside the peptidoglycan layers is called the **cord factor** and also has a role in:

1. Resistant to digestion by enzymes of Phagocytic cell (prevents fusion of lysosome to Phagocytic gap).
2. Activate the alternative complement pathway.
3. adjuvant
4. Makes the bacteria less permeability and this prevents the entry of medicines, chemicals and antibiotics.
5. Mycolic acid are so stabile that Resistant to desiccation and the different circumstances where used in screening for TB in ancient skeletons .
6. Protects the cell from osmotic lysis.
7. Reduces entry of nutrients leading to slow growth.



Archaeal cell wall

The Archaea were characterized as being either gram positive or gram negative. However, their staining reaction does not correlate as reliably with a particular cell wall structure. Archaeal wall structure and chemistry differ from those of the Bacteria. Have many types of cell walls which are **mostly consist of S-layer(protein or glycoprotein) in about 20-40 nm of thickness addition to other layers which differ according to the species** as in the (figure a) **.**

Types of archaeal cell walls:

1. Glycoprotein (S-layer) with the accumulation of large amounts of amino acids, especially Aspartate as negatively in the wall of Halobacterium that has a role in positive charge equation resulting from the presence of sodium ions, they need 10-20 % of NaCl, some sources indicate it needs from 20-35% salt to form a wall and hardness which comes from the interaction of sodium ions with negative charge amino acids. when the salt NaCl concentration reach to 15 percent the wall lose their solidity and cell transformed into spherical and if the it low for more the cell degrade and die as in (figure : a).
2. In Methanospirillum : Many archaea that stain gram negative have either a layer of **glycoprotein or protein (s-layer) outside their plasma membrane and a sheath** surrounding it. (figure : b).
3. Methanosarcina contain **complex polysaccharides similar to the chondroitin sulfate of animal connective tissue**(figure: c).
4. Methanobacterium and some other methane-generating archaea (methanogens) have walls containing **pseudomurein**, a peptidoglycan-like polymer that has L -amino acids instead of D -amino acids in its cross-links, N -acetyltalosaminuronic acid instead of N -acetylmuramic acid, and β (1→3) glycosidic bonds instead of β(1→4) glycosidic bonds{{ **They lack peptidoglycan , NAM and D-amino acids}}** (figure: d).
5. Many archaea have a wall with a single, thick homogeneous polysaccharides layer resembling that in gram-positive bacteria **without s-layer** (**figure :e**).
6. The most unique walls archaeon is *Ignicoccus hospitalis*. Its envelope consists only of the plasma membrane and an outermost membrane, with an intermembrane compartment between them (figure f and figure 4.8). The outermost membrane contains protein complexes that form pores, much like bacterial porin proteins in the outer membrane of typical Gram-negative bacteria.

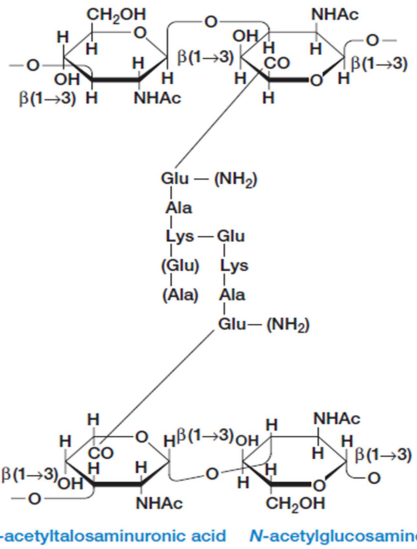


Figure 3.30 The Structure of Pseudomurein. The amino acids and amino groups in parentheses are not always present. Ac represents the acetyl group.

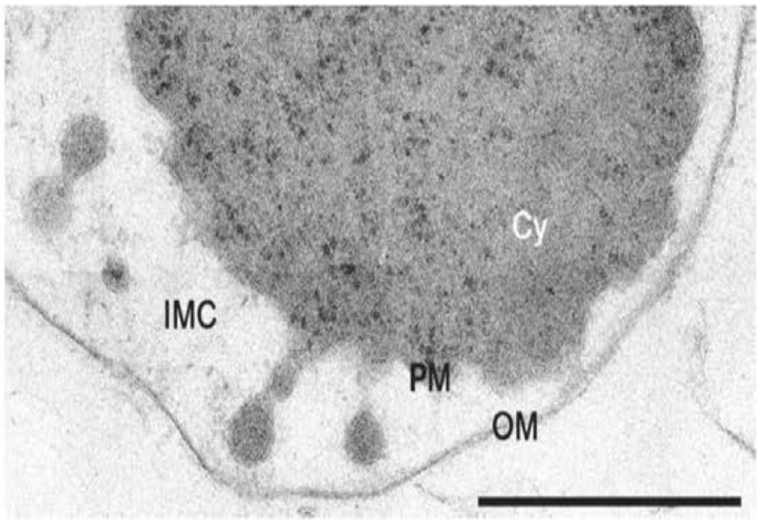


Figure 4.8 Cell Envelope of the Wall-less Archaeon *Ignicoccus hospitalis*. Cy is cytoplasm, PM is plasma membrane, IMC is intermembrane compartment, and OM is outermost membrane. TEM, Bar = 200 nm.

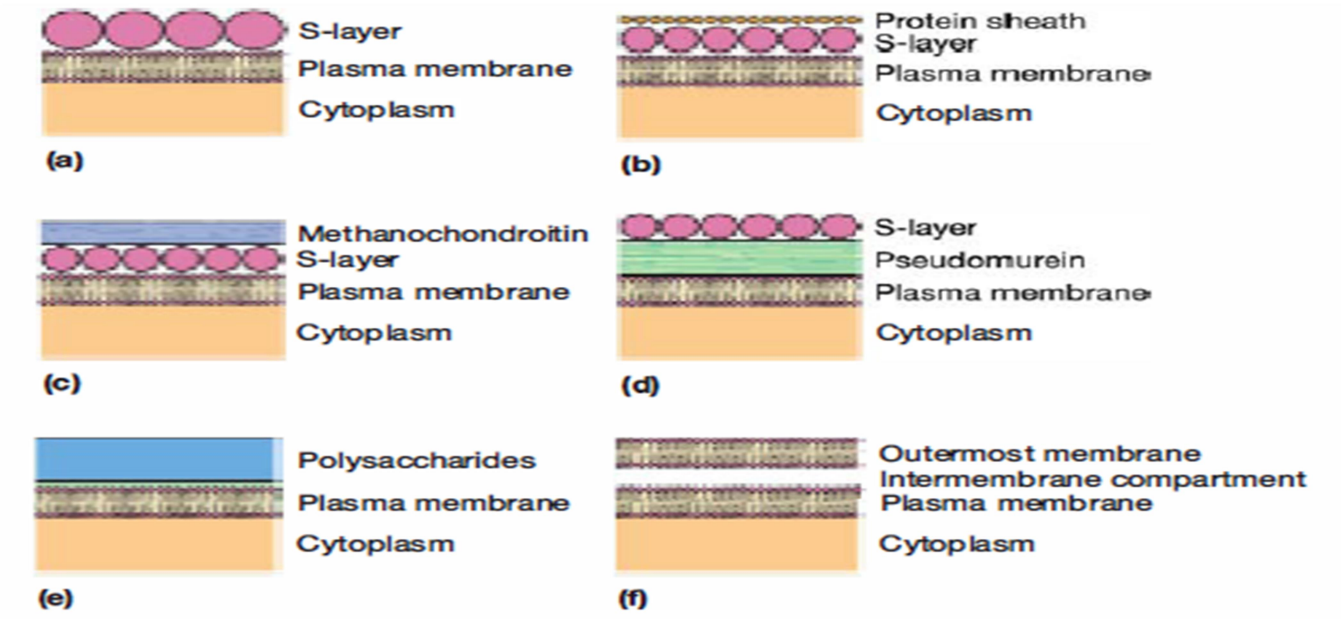


Figure 4.6 Archaeal Cell Envelopes. (a) *Methanococcus, Halobacterium, Pyrodictium, Sulfolobus,* and *Thermoproteus* cell envelopes. (b) *Methanospirillum* cell envelope. (c) *Methanosarcina* cell envelope. (d) *Methanothermus* and *Methanopyrus* cell envelopes. (e) *Methanobacterium, Methanosphaera, Methanobrevibacter, Halococcus,* and *Natronococcus* cell envelopes. For *Methanosphaera,* the polysaccharide layer is composed of pseudomurein. (f) *Ignicoccus* cell envelope. The outermost membrane contains protein complexes that form pores.

Biosynthesis of Peptidoglycan

peptidoglycan is a large, complex molecule consisting of long polysaccharide chains made of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues. **Pentapeptide chains are attached to the NAM groups**. The polysaccharide chains are **connected through their pentapeptides or by interbridges** (figure).

Not surprisingly, such an intricate structure requires an equally intricate biosynthetic process, especially because some **reactions occur in the cytoplasm, others in the membrane,** and others in the **periplasmic space**. Peptidoglycan synthesis involves two carriers (figure).

The first, uridine diphosphate (UDP), functions in the cytoplasmic reactions.

The second bactoprenol phosphate in cytoplasmic membrane

Three stages of cell wall synthesis :

A- Cytoplasmic stage : synthesis of precursors (NAG, NAM)

1- In the first step of peptidoglycan synthesis, UDP derivatives of NAM and NAG are formed.

2- Amino acids are then added sequentially to UDP-NAM to form the pentapeptide chain.

B- Membrane stage : (elongation and transfer)

3- NAM-pentapeptide is then transferred to the second carrier, **bactoprenol** phosphate, which is located at the cytoplasmic side of the plasma membrane. The resulting intermediate is often **called Lipid I.** Bactoprenol is a 55- carbon alcohol and is linked to NAM by a pyrophosphate group .

4- Next, UDP transfers NAG to the bactoprenol- NAM-pentapeptide complex (Lipid I) to generate Lipid II. This creates the peptidoglycan repeat unit. The repeat unit is transferred across the membrane by bactoprenol. If the peptidoglycan unit requires an **inter bridge**, it is added while the repeat unit is within the membrane. Bactoprenol stays within the membrane and does not enter the periplasmic space.

5- After releasing the peptidoglycan repeat unit into the periplasmic space, bactoprenol-pyrophosphate is **dephosphorylated** and returns to the cytoplasmic side of the plasma membrane, where it can function in the next round of synthesis.

C- Extracellular stage: (crosslinking)

6- Meanwhile, the peptidoglycan repeat unit is added to the growing end of a peptidoglycan chain. The final step in peptidoglycan synthesis is transpeptidation , which creates the peptide cross-links between the peptidoglycan chains. The enzyme that catalyzes the reaction removes the terminal D-alanine as the cross-link is formed. To grow and divide efficiently, a bacterial cell must add new peptidoglycan to its cell wall in a precise and well-regulated way while maintaining wall shape and integrity in the presence of high osmotic pressure.

7- Because the cell wall peptidoglycan is essentially a single, enormous network, the growing bacterium must be able to degrade it just enough to provide acceptor ends for the incorporation of new peptidoglycan units. It must also reorganize peptidoglycan structure when necessary. This limited peptidoglycan digestion is accomplished by **enzymes known as autolysins** , **some of which attack the polysaccharide chains, while others hydrolyze the peptide cross-links**.

8- Autolysin inhibitors are produced to keep the activity of these enzymes under tight control. Because of the importance of peptidoglycan to bacterial cell wall structure and function, its synthesis is a particularly effective target for antimicrobial agents. Inhibition of any stage of synthesis weakens the cell wall and can lead to lysis.

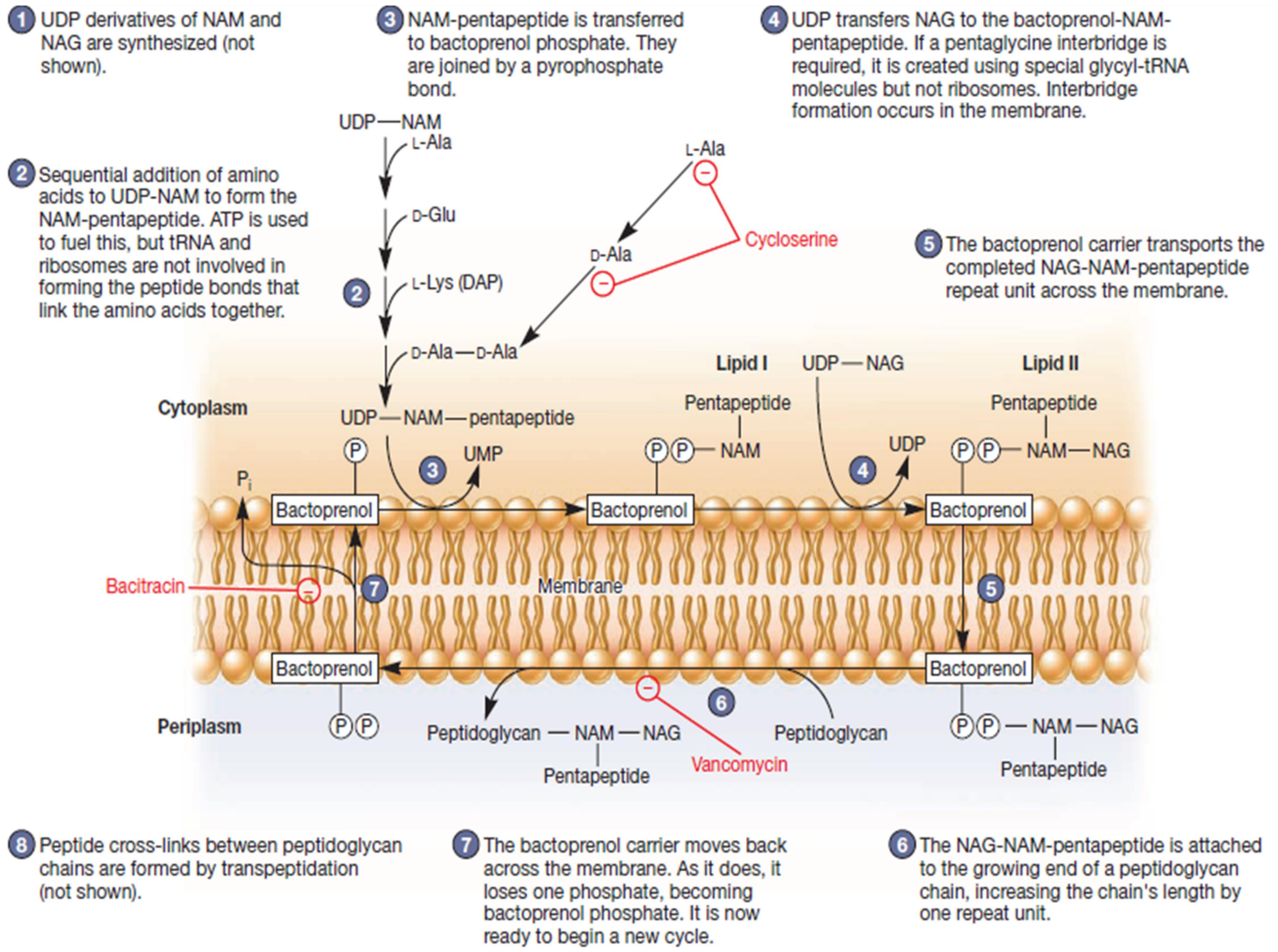


Figure 11.11 Peptidoglycan Synthesis. NAM is *N*-acetylmuramic acid and NAG is *N*-acetylglucosamine. The pentapeptide contains L-lysine in *Staphylococcus aureus* peptidoglycan, and diaminopimelic acid (DAP) in *E. coli*. Inhibition by bacitracin, cycloserine, and vancomycin also is shown. Stage eight is depicted in figure 11.13.

Enzymes and antibiotics that attack bacterial cell wall

Many commonly used antibiotics interfere with peptidoglycan synthesis.

1. In the presence of lysozyme, gram-positive cell walls are destroyed by hydrolyzed glycoside bond B 1-4 the remaining cellular contents are referred to as a protoplast. In the presence of lysozyme (after disruption of the outer membrane), gram-negative cell walls are not completely destroyed, and the remaining cellular contents are referred to as

spheroplasts. Protoplasts and spheroplast are subject to osmotic lysis. Proteus and some other genera can lose their cell walls spontaneously or in response to penicillin and swell into L forms (Lister Institute). L forms can live and divide and/or return to the normal walled state.

2. **Fosfomycin** is bactericidal and inhibits bacterial cell wall biogenesis by inhabit formation glycosidic bound between UDP –NAGA and UDP- NAMA in cytoplasm.
3. The antibiotic **cycloserine** is an analog of D-alanine and interferes with enzymatic conversion of L-alanine to D-alanine in the cytoplasm. Thus, subsequent synthesis of peptidoglycan cannot occur.
4. The peptidoglycan subunit (containing one side-chain and an attached peptide to be used in cross-bridge formation) is passed across the cytoplasmic membrane attached to Bactoprenol diphosphate. After the growing peptidoglycan monomer leaves the carrier on reaching the cell wall, the **Bactoprenol diphosphate is dephosphorylated to its monophosphate form**. **Bacitracin** inhibits the dephosphorylation reaction and in the absence of monophosphorylated carrier peptidoglycan subunit synthesis stops.
5. The final step in peptidoglycan synthesis involves **(**transpeptidases),which include **linking the sugar portion of the peptidoglycan subunit to the glycan backbone of the existing cell wall polymer.** Cross-linking of the peptide portion of the subunit to a peptide in the cell wall then occurs. **During this process D-alanine is enzymatically excised** from the end of a pre-existing peptide side chain allowing it to be cross-linked (by a new peptide bond) to the recently synthesized peptidoglycan subunit. **Vancomycin binds to D-alanine-D-alanine thus inhibits transpeptidation (cross-linking).**
6. **Penicillin's** also bind to several periplasmic proteins (penicillin-binding proteins, or PBPs) PBPs; also known as transpeptidases, which add disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecules and cross-link adjacent peptide strands of immature peptidoglycan units, respectively. The beta lactam antibiotics include penicillin's (e.g. ampicillin), cephalosporin's and monobactams. Thus formation

of a complete cell wall is blocked, leading to osmotic lysis. This mechanism is consistent with the observation that penicillin's act only on growing bacteria that are synthesizing new peptidoglycan. However, the mechanism of penicillin action is actually more complex.

7. penicillins may also destroy bacteria by activating their own **autolytic enzymes**.

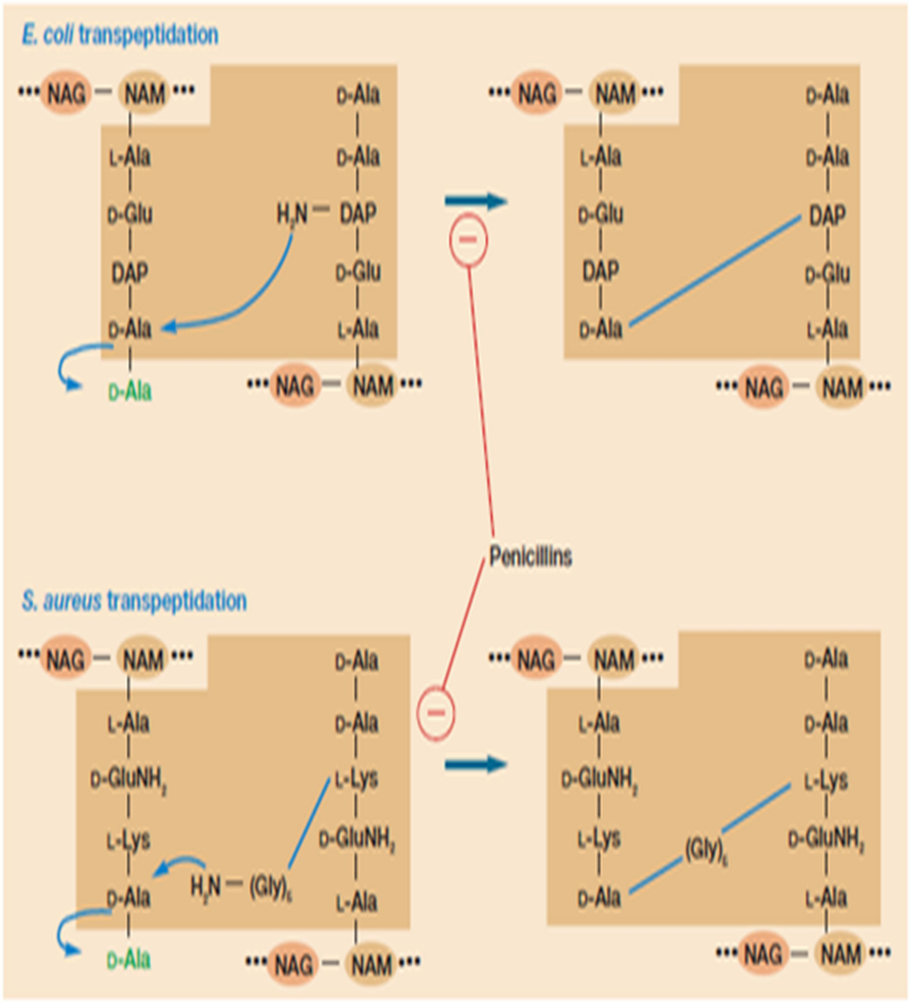


Figure 10.29 Transpeptidation. The transpeptidation reactions in the formation of the peptidoglycans of *Escherichia coli* and *Staphylococcus aureus*.

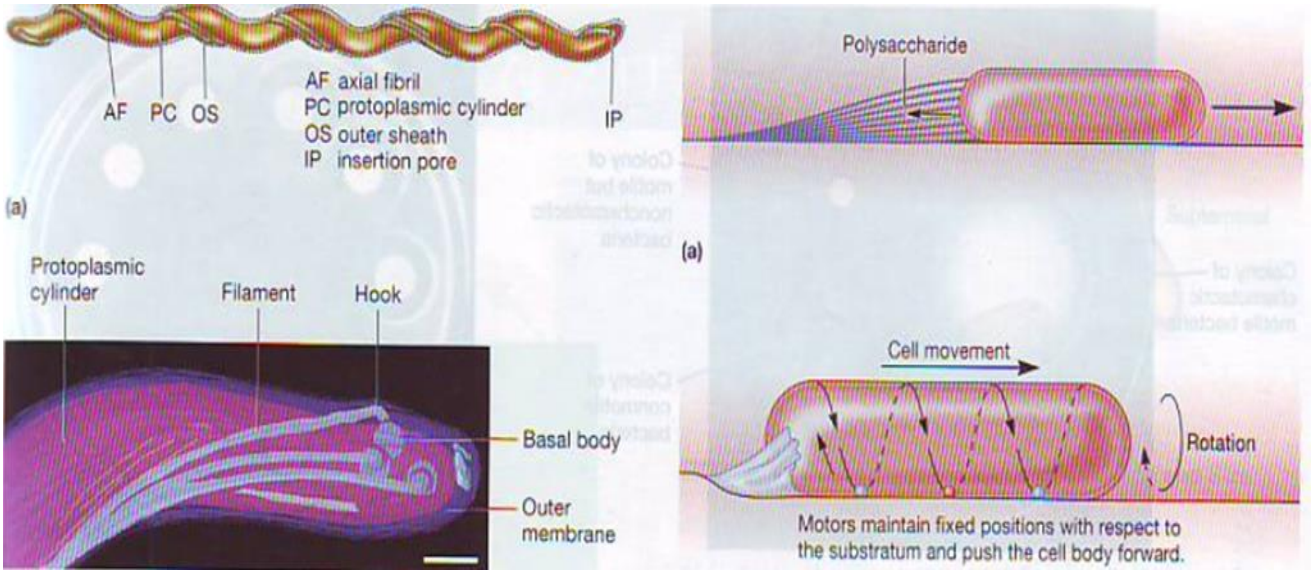
External Structures

Flagella and Motility

Several structures outside the cell wall contribute to the motility of procaryotes. Four major methods of movement have been observed in Bacteria:

a. The swimming movement conferred by flagella .The bacterial flagellar filament is a rigid helix that rotates like a propeller to push the bacterium through water .
b. The corkscrew movement of spirochetes is brought by flagella that are wound around the cell and remain within the periplasmic space. When they rotate, the outer sheath of the spirochete is thought to rotate, thus moving the cell **(figure).**
c. Twitching and
d. gliding motility are similar in that both occur on moist surfaces and can involve type IV pili and the secretion of slime. Twitching motility is a **jerky movement**, whereas gliding motility is **smooth.**

e. Tumbling motility in *Listeria monocytogenes* get by actin filaments like the actin protein of muscle through producing of the ACT A protein which accumulates in the form of tale at the end of the cell that can enter and spread for 11 **micrometer/per minute** in host cell.



Bacterial flagella :

Bacterial flagella (s., **flagellum**), are slender, rigid structures, about 20 nm across and up to 20 μm long. Flagella are so thin they cannot be observed directly with a bright-field

microscope but must be stained with special techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope.

The flagellar protein called H antigen is useful for distinguishing among serovars, or variations within a species, of gram-negative bacteria . For example, there are at least 50 different H antigens for *E. coli*. Those serovars identified as *E. coli* 0157:H7 are associated with foodborne epidemics **.**

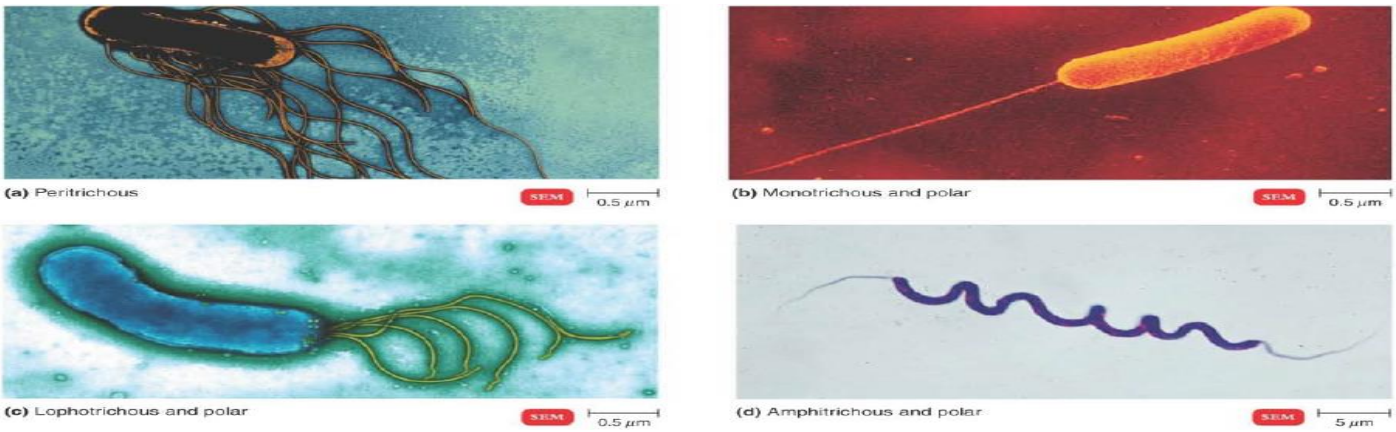
Bacterial species often differ distinctively in their patterns of flagella distribution, and these patterns are useful in identifying bacteria.

Flagella are spread evenly over the whole surface of **peritrichous** (**peri** means around) bacteria (figure a).

Monotrichous bacteria (**trichous** means hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum** (figure b).

lophotrichous bacteria (lopho means tuft**) have a cluster of flagella at one or both ends (** figure c **).**

Amphitrichous bacteria (**amphi** means on both sides) have a single flagellum at each pole **(** figure d **).**



Flagellar ultrastructure transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts.

(1) The longest and most obvious portion is the **flagellar filament ,** which extends from the cell surface to the tip.

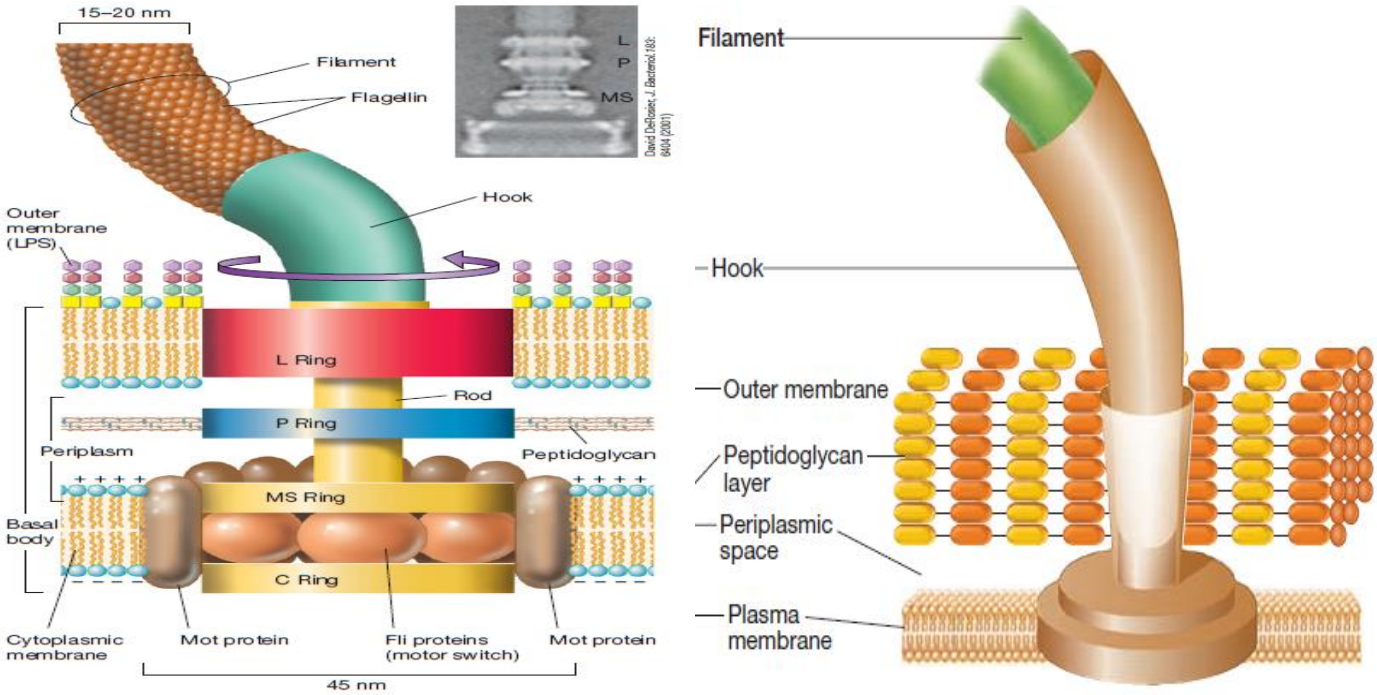
(2) The **basal body** is embedded in the cell; and

(3) a short, curved segment, the **flagellar hook ,** links the filament to its basal body and acts as a flexible coupling.

The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin ,** which ranges in molecular weight from 30,000 to 60,000 daltons, depending on the bacterial species. **The filament ends with a capping protein**. Some bacteria have sheaths surrounding their flagella. For example, *Vibrio cholerae* has a lipopolysaccharide sheath.

The hook and basal body are quite different from the filament (**figure**). Slightly wider than the filament, the hook is made of different protein subunits.

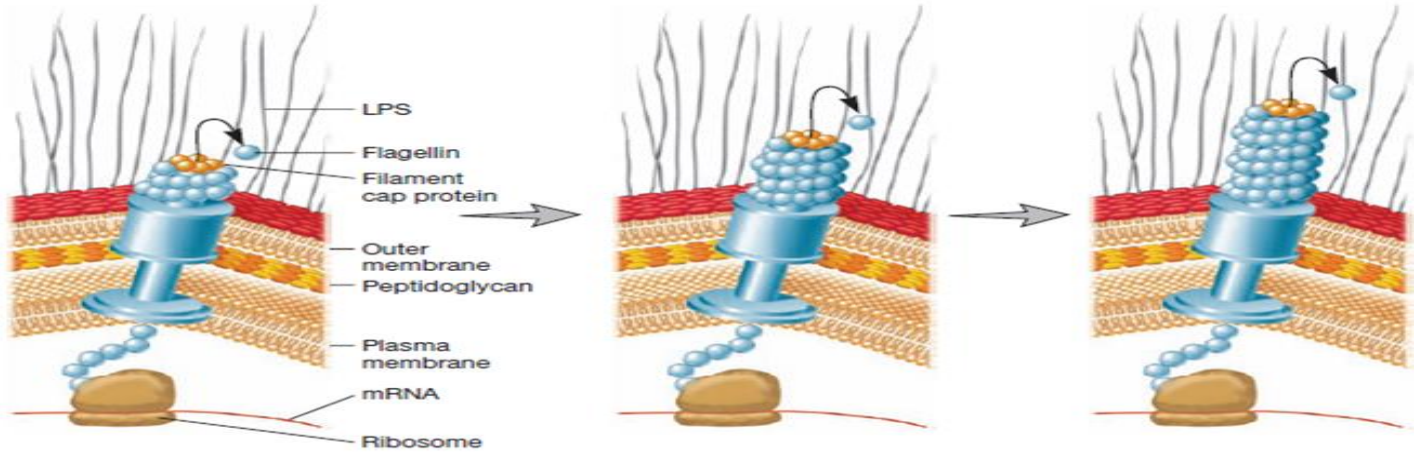
The basal body is the most complex part of a flagellum. In transmission electron micrographs of the basal bodies of *E. coli* and most other gram-negative bacteria, the basal body appears to have five rings **, L ring, P ring, S ring, and M ring, connected to a central rod & C ring** (figure *a*). It is now known that the S ring and M ring are different portions of the same protein, and they are now referred to as the MS ring. On the cytoplasmic side of the MS ring is **the C ring**, which was discovered later. Gram-positive bacteria have only two rings—**an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan** (figure *b*).



Flagellar Synthesis

The synthesis of bacterial flagella is a complex process involving at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes are concerned with the control of flagellar construction or function. How the cell regulates or determines the exact location of flagella is not known. Because many components of the flagellum lie outside the cell wall, they must be transported

across the plasma membrane and cell wall. Transport of many flagellar components is carried out by a tool in the basal body. It is thought that 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, as does an animal hair. The MS ring is synthesized first and inserted into the cytoplasmic membrane. Then other anchoring proteins are synthesized along with the hook before the filament forms** (**figure**). Thus filament synthesis, like S-layer formation, is an example of **self-assembly. The flagellum grows more or less continuously until it reaches its final length.** Broken flagella still rotate and can be repaired with new flagellin units passed through the filament channel to replace the lost ones.



Flagellar Movement

Procaryotic flagella operate differently from eucaryotic flagella. Eucaryotic flagella flex and bend, resulting in a whiplash that moves the cell. The filament of a procaryotic flagellum is in the shape of a rigid helix, and the cell moves when this helix rotates like a propeller on a boat. The flagellar motor can rotate very rapidly. The *E. coli* motor rotates 270 revolutions per second (rps); *Vibrio alginolyticus* averages 1,100 rps. The direction of flagellar rotation determines the nature of bacterial movement.

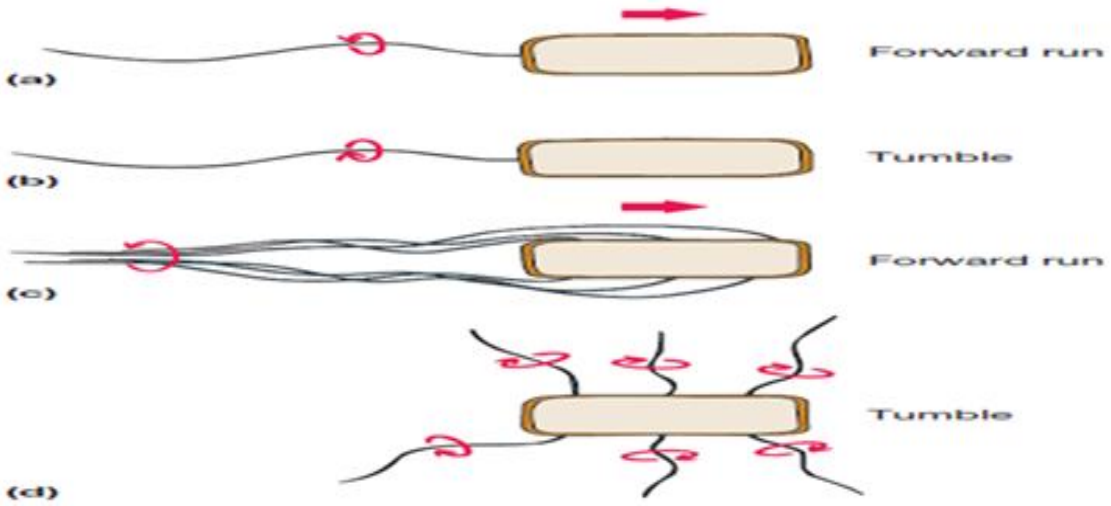
Monotrichous, polar flagella rotate counterclockwise (when viewed from outside the cell) during normal forward movement, whereas the cell itself rotates slowly clockwise. The rotating helical flagellar filament pushes the cell forward with the flagellum trailing behind (**figure**). Monotrichous bacteria **stop and tumble** randomly **by reversing the direction of flagellar rotation.**

Peritrichously flagellated bacteria operate in a somewhat similar way. To move forward, the flagella rotate counterclockwise. As they do so, they bend at their hooks to form a rotating

bundle that propels the cell forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.

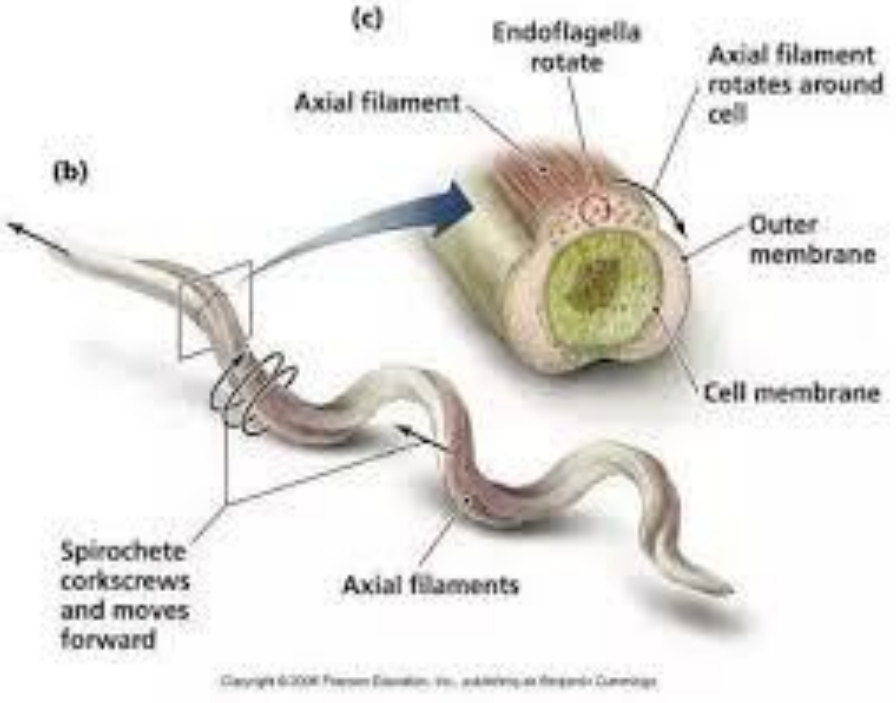
The motor that drives flagellar rotation is located at the base of the flagellum, where it is associated with the basal body. Torque generated by the motor is transmitted by the basal body to the hook and filament. The motor is composed of two components: the **rotor and the stator**. It is thought to function like an electrical motor, where the rotor turns in the center of a ring of electromagnets, the stator. **In gram-negative bacteria, the rotor is composed of the MS ring and the C ring** (**figure**). The power used by most flagellar motors is a difference in charge and pH across the plasma membrane. This difference is called the proton motive force (PMF).

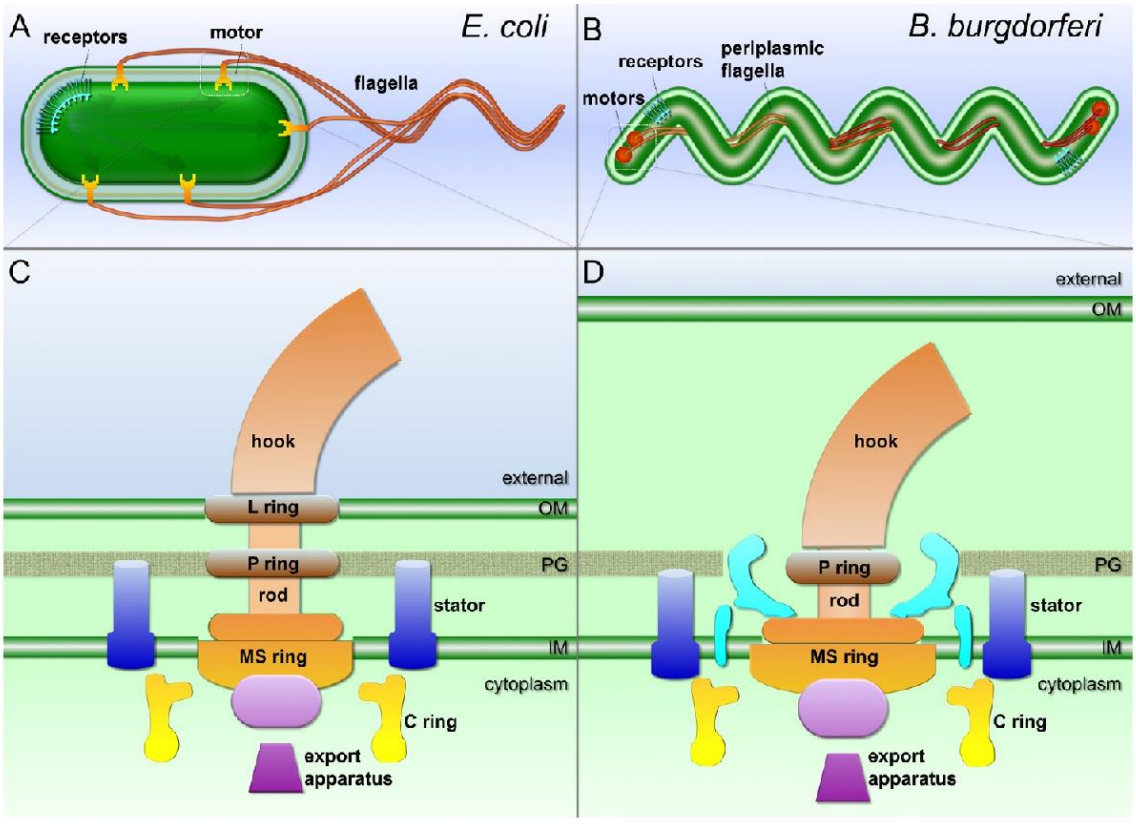
The flagellum is a very effective swimming device. From the bacterium's point of view, swimming is quite a difficult task because the surrounding water seems as viscous as molasses. The cell must bore through the water with its corkscrew-shaped flagella, and if flagellar activity finishes, it stops almost directly.



Spirochete Motility

Although spirochetes have flagella, they work in a different manner. In many spirochetes, multiple flagella arise from each end of the cell and associate to form an axial fibril, which winds around the cell **(figure)**. The flagella do not extend outside the cell wall but rather remain in the periplasmic space and are covered by an outer sheath. The way in which axial fibrils propel the cell has not been fully established. They are thought to rotate like the external flagella of other bacteria, causing the corkscrew-shaped outer sheath to rotate and move the cell through the surrounding liquid, even very viscous liquids. Flagellar rotation may also flex or bend the cell and account for the crawling movement observed when spirochetes are in contact with a solid surface.


(c)
Endoflagella rotate
Axial filament rotates around cell
Axial filament
(b)
Outer membrane
Cell membrane
Spirochete corkscrews and moves forward
Axial filaments


A
receptors
motor
E. coli
flagella
B
B. burgdorferi
receptors
periplasmic flagella
motors
C
hook
external
L ring
OM
P ring
PG
rod
stator
MS ring
IM
cytoplasm
C ring
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D
external
OM
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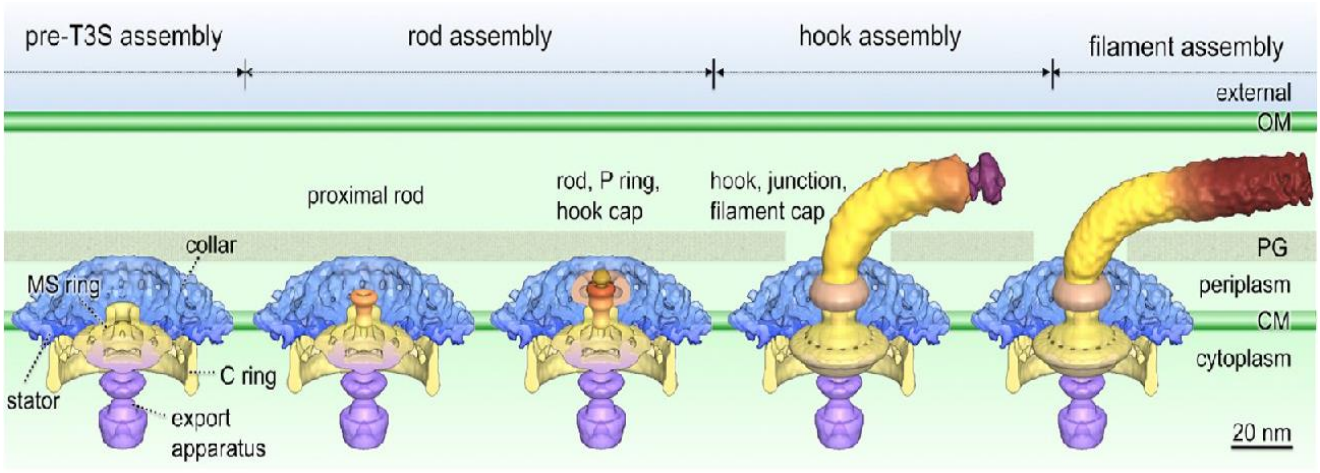
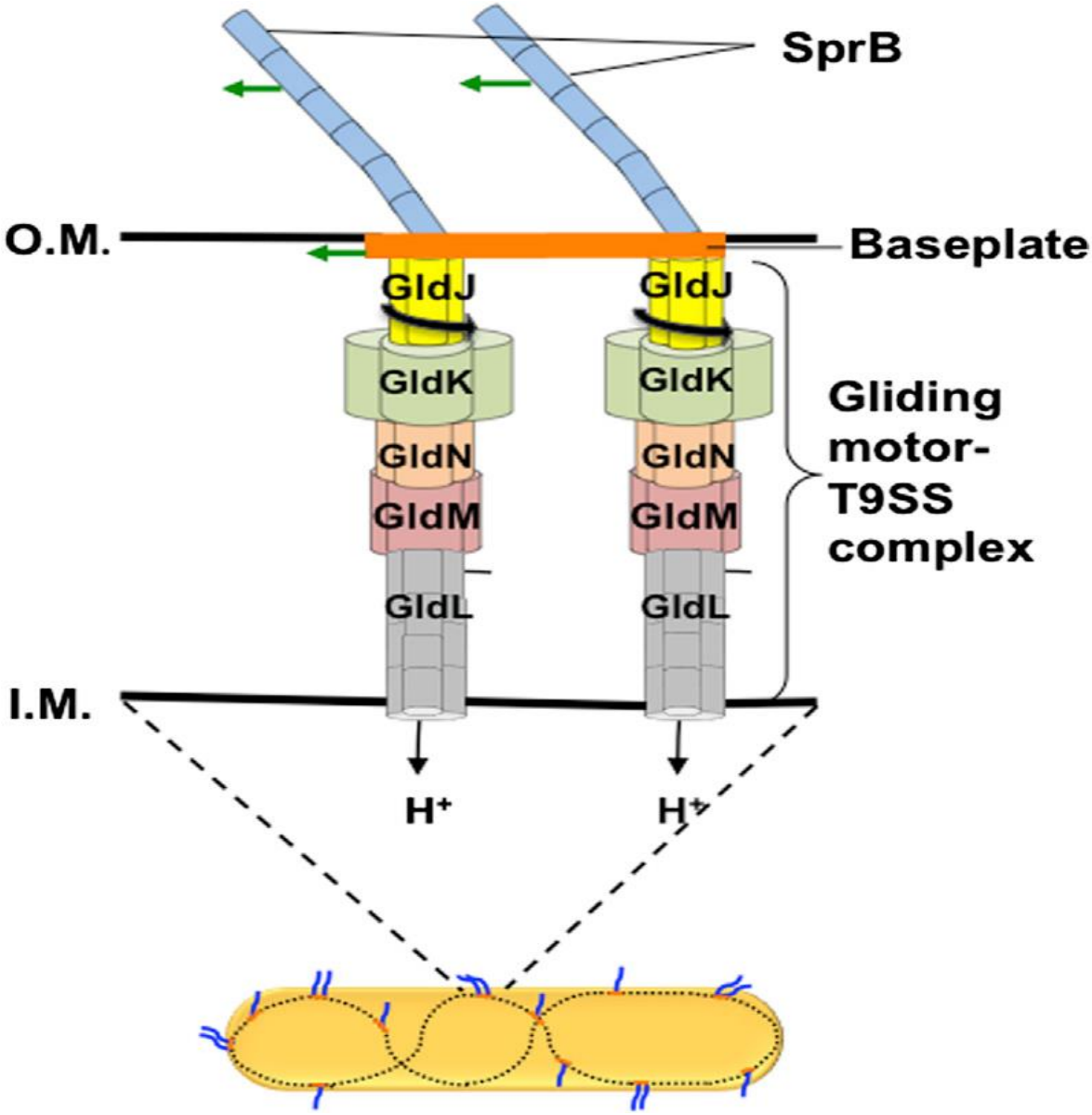


Gliding motility :

is smooth and varies greatly in rate (from 2 to over 600 μm per minute) and in the nature of the motion. Although first observed over 100 years ago, the mechanism by which many bacteria glide remains a mystery. Some glide along in a direction parallel to the longitudinal axis of their cells. Others travel with a screw like motion or even move in a direction perpendicular to the long axis of the cells. Still others rotate around their longitudinal axis while gliding. Such diversity in gliding movement correlates with the observation that more than one mechanism for gliding motility exists. Some types involve type IV pili, some involve slime, and some involve mechanisms that have not yet been elucidated. Gliding is powered by a proton motive force.

In Flavobacterium gliding motility form by [1]surface adhesion protein Spr ,[2] **baseplate** , [3] gliding motor complex T9SS consist of (**GldJ** GldK, GldL, GldM, and GldN) are interacts with the Type-IX protein secretion system (T9SS) interacts with the Type-IX protein secretion system (T9SS) and is important for the movement of cell surface adhesions. GldK, GldL, GldM, and GldN are core T9SS proteins, and cells lacking these proteins do not exhibit motility. The macromolecular structure of the gliding motor and its exact interaction with T9SS is unclear. GldL localizes to the cytoplasmic membrane, and it might act as an anchor for the gliding motor. Besides the core T9SS proteins, other Gld and Spr (surface adhesion) proteins might associate with this motor. The gliding motor appears to associate with T9SS in a manner analogous to the association of the bacterial flagellar

motor with the Type-III secretion system (T3SS). In flagellated bacteria, T3SS is required for secretion of axial components of the flagellum. in which rotary motors drive baseplates, to which SprB filaments are attached. In our model, gliding motors formed complexes with T9SS, which spanned the inner and outer membranes, harvesting proton motive force to power SprB rotation. The baseplates moved along the inner surface of the outer membrane (Figure).



Cell Speed and Motion

In Bacteria, flagella do not rotate at a constant speed but instead increase or decrease their rotational speed in relation to the strength of the proton motive force. Flagella can rotate at up

to 300 revolutions per second and propel cells through a liquid at 2 to over 100 cell lengths per second . By contrast, the fastest known animal, the cheetah, moves at a maximum rate of about 25 body lengths/sec. In contrast, an exceptionally fast human might be able to run around 5 to 6 body lengths per second.

The swimming motions of polarly and lophotrichously flagellated organisms differ from those of peritrichously flagellated organisms, and these can be distinguished microscopically (**Figure)**.

Peritrichously flagellated organisms typically move in a straight line in a slow fashion.

Polarly flagellated organisms, on the other hand, move more rapidly, spinning around and seemingly dashing from place to place.

Swimming speed is a genetically governed property because different motile species, even different species that are the same cell size, can swim at different maximum speeds. When assessing the capacity of a laboratory culture of a bacterium for swimming motility and swimming speed, observations should **only be made on young cultures**. In old cultures, otherwise motile cells often stop swimming and the culture may appear to be nonmotile.

Distribution of flagella when cell division:

when cell division each new cell receives the same number of flagella, in polar flagella the new flagella arise from the polar region of the new cell, and in the peripheral flagella the flagella distributed in both new cells and each cell after division begins to filled with anew flagella between the old one.

Archaeal Flagella

Besides Bacteria, flagellar motility is also widespread among species of Archaea; major genera of methanogens, extreme halophiles, thermoacidophiles, and hyperthermophiles are all capable of swimming motility.

1- Archaeal flagella are half the diameter of bacterial flagella, measuring only 10–13 nm in width (**Figure**), but impart movement to the cell by rotating, as do flagella in Bacteria.
2- unlike Bacteria, in which a single type of protein makes up the flagellar filament, several **different flagellin proteins** are known from Archaea, and their amino acid sequences and genes that encode them bear no relationship to those of bacterial flagellin.
3- grow from the base not from the tip.
4- difficulty to distinguishing hook from the filaments it is longer than the hook in bacterial.

5- Unable to distinguish the basal body and some have a knob like structure in the embedded end of the cell.
6- Movement of all flagella as a single unit, while in bacteria flagella works separately.
7- flagella are powered directly by ATP rather than by the proton motive force, the source of energy for the flagella of Bacteria

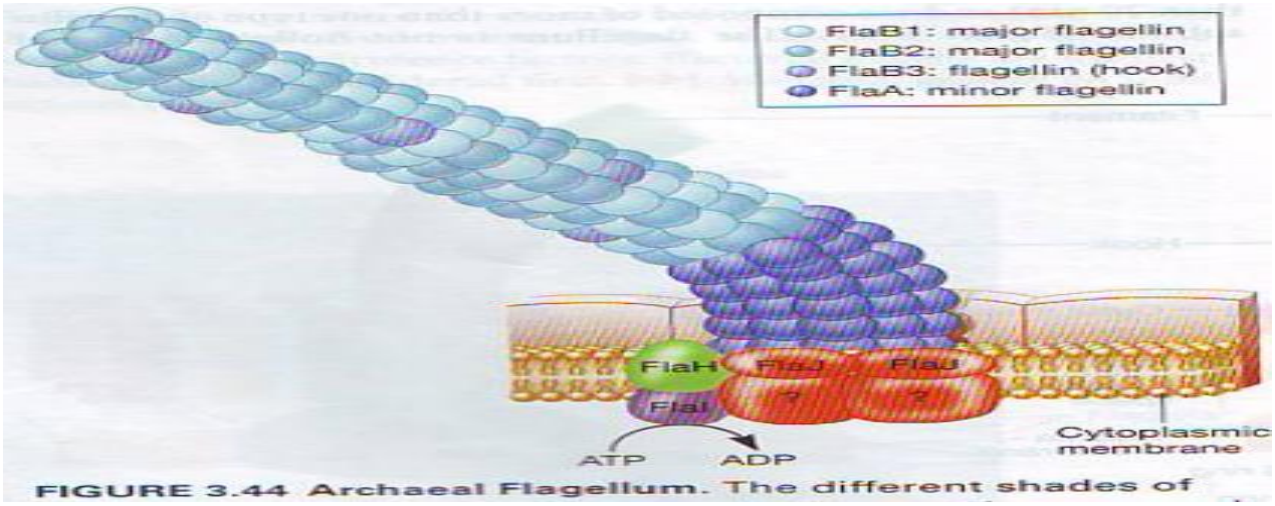


FIGURE 3.44 Archaeal Flagellum. The different shades of

Chemotaxis

The movement of a microorganism toward chemical attractants and away from chemical repellents.

Bacteria have not evolved motility to move aimlessly. Rather, motility is used to move toward nutrients such as sugars and amino acids and away from many harmful substances and bacterial waste products.Bacteria also can respond to environmental signals such as temperature (thermotaxis), light (phototaxis), oxygen (aerotaxis), osmotic pressure (osmotaxis), and gravity (Magnetotaxis).

Movement toward chemical attractants and away from repellents is known as chemotaxis. Attractants and repellents are **detected by chemoreceptors** (**methyl accepting chemotaxis proteins)** , proteins that bind chemicals and transmit signals to other components of the **chemosensing system**. The chemosensing systems are very sensitive and allow the cell to respond to very **low levels** of attractants (about 10 −8 M for some sugars) & for (high level of repellent). In gram-negative bacteria, the chemoreceptor proteins are **located in the periplasmic space or in the plasma membrane**. Some receptors also participate in the initial stages of sugar transport into the cell. The chemotactic behavior of bacteria has been studied using the tracking microscope, a microscope with a moving stage that automatically keeps an individual bacterium in view.

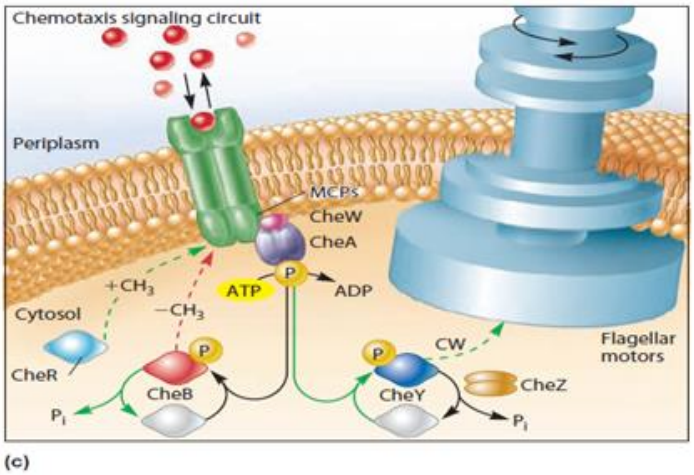


Figure 8.28 Proteins and Signaling Pathways of the Chemotaxis Response in *E. coli*. **(a)** The methyl-accepting chemotaxis proteins (MCPs) form clusters associated with the CheA and CheW proteins. CheA is a sensor kinase that when activated phosphorylates CheB, a methylesterase, or CheY. Phosphorylated CheY interacts with the FliM protein of the flagellar motor, causing rotation of the flagellum to switch from counterclockwise (CCW) to clockwise (CW). This results in a switch from a run (CCW rotation) to a tumble (CW rotation). **(b)** MCPs, CheW, CheA complexes form large clusters of receptors at either end of the cell, as shown in this electron micrograph of *E. coli*. Gold-tagged antibodies were used to label the receptor clusters, which appear as black dots (encircled). **(c)** The chemotactic signaling pathways of *E. coli*. The pathways that increase the probability of CCW rotation are shown in red. CCW rotation is the default rotation. It is periodically interrupted by CW rotation, which causes tumbling. The pathways that lead to CW rotation are shown in green. Molecules shown in gray are unphosphorylated and inactive. Note that MCP, CheA, and CheZ are homodimers. CheW, CheB, CheY, and CheR are monomers.

In the absence of a chemical gradient, bacteria move randomly, switching back and forth between a phase called a run and a phase called a tumble. During a run, the bacterium travels in a straight or slightly curved line. After a few seconds, the flagella "fly apart" and the bacterium will stop and tumble . The tumble randomly reorients the bacterium so that it often is facing in a different direction. Therefore when it begins the next run, it usually goes in a different direction (figure a). In contrast, when the bacterium is exposed to an attractant, it tumbles less frequently (or has longer runs) when traveling toward the attractant. Although the tumbles can still orient the bacterium away from the attractant, over time, the bacterium gets closer and closer to the attractant (figure b). The opposite response occurs with a repellent. Tumbling frequency decreases (the run time lengthens) when the bacterium moves away from the repellent.

The bacterium moves toward the attractant because it senses that the concentration of the attractant is increasing. Likewise, it moves away from a repellent because it senses that the concentration of the repellent is decreasing. The bacterium's chemoreceptors play a critical role in this process. **when there are Attractants and repellent substances at the same time they are moving to the affected concentration** .

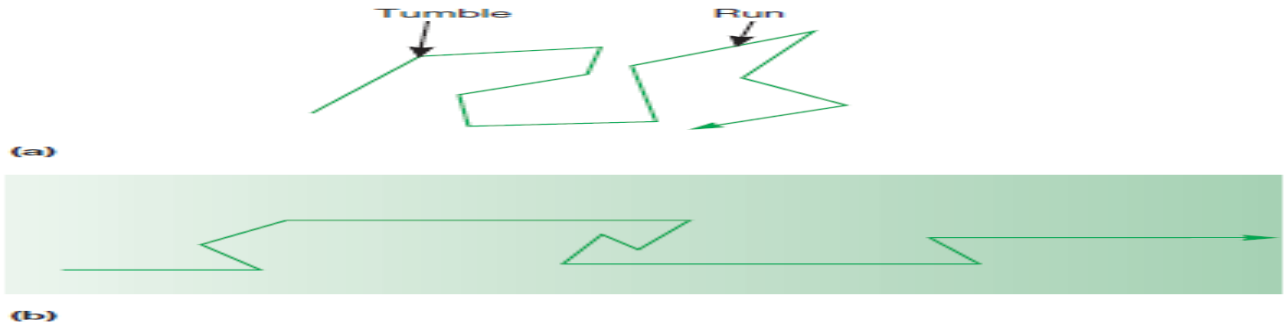


Figure 3.42 Directed Movement in Bacteria. (a) Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. (b) Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium is moving up the gradient. Therefore runs in the direction of increasing attractant are longer.

Components External to the Cell Wall

Glycocalyx: A network of polysaccharides extending from the surface of procaryotes This layer has different names depending on its characteristics:

Capsule :

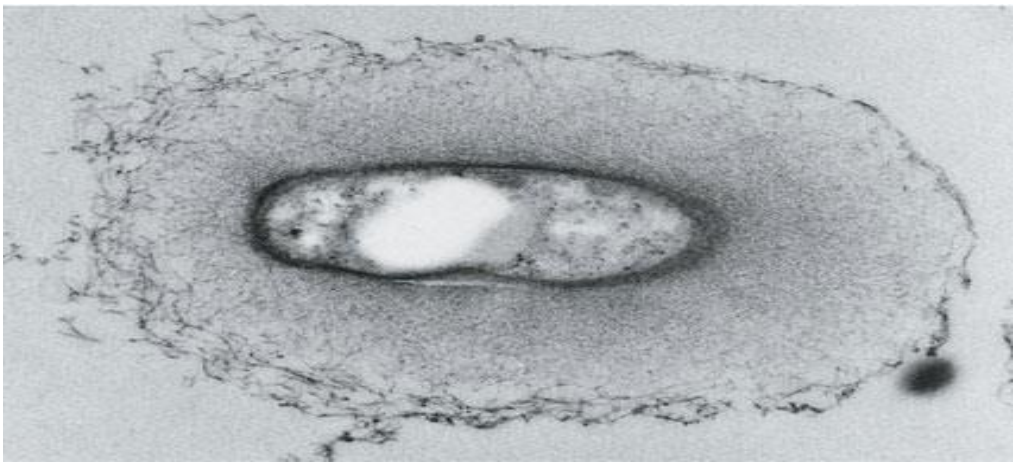
The layer is well organized and not easily washed off (figure) Some types of bacteria have an external structure surrounding the cell wall compose of mono saccharide such as Dextrans and Glucans or hetero saccharides like five or sex carbon sugars and Ribitol (5C), Glycerol (3C) or other sugar alcohols additional to phosphate or other materials like protein, For example, *Bacillus anthracis* has a capsule composed of poly- D -glutamic acid. Usually built in the cytoplasm and pass out through the carrier isoprenoid lipid to aggregate outside the cell wall. Capsules are clearly visible in the light microscope when negative stains or special capsule stains are employed., they also can be studied with the electron microscope. Although capsules are not required for growth and reproduction in laboratory cultures, they confer several advantages when procaryotes grow in their normal habitats *Streptococcus pneumoniae* provides a dramatic example. When it lacks a capsule, it is destroyed easily and does not cause disease.

Slime layer : it is a zone of diffuse, unorganized material that is removed easily. Consist of polysaccharides ,lipopolysaccharides , glycoproteins.

The function of Glycocalyx are :

1- They can protect procaryotes from certain environmental conditions.
2- allow procaryotes to attach to surfaces. Through attachment, bacteria can grow on diverse surfaces such as rocks in fast-moving streams, plant roots, human teeth, medical implants, water pipes, and even other bacteria. *Vibrio cholera* produces a glycocalyx that helps it attach to the cells of the small intestine.
3- may provide nutrients (utilizing the sugars when energy stores are low).
4- viscosity may inhibit the movement of nutrients out of the cell.
5- very important component of biofilms.
6- Capsules contain a great deal of water and can protect against desiccation (dehydration).

7- They exclude viruses and most hydrophobic toxic materials such as detergents.
8- Participate in Gliding motility of some bacteria (slime layer).
9- Protect pathogenic bacteria from phagocytosis.
10- Antigenic structure: detect by **Quellung reaction** (The increase in visibility or the swelling of the capsule of a microorganism in the presence of antibodies against capsular antigens).



Biofilm

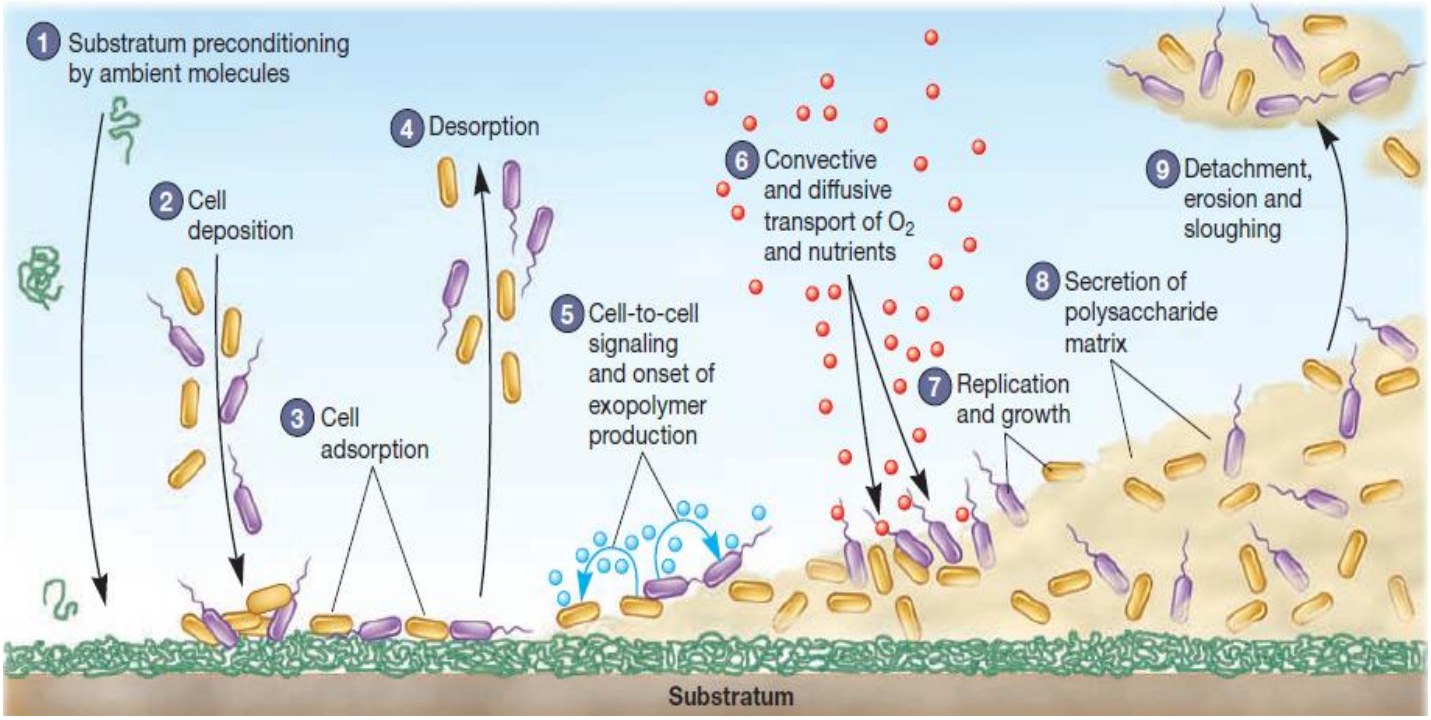
Biofilms are universal in nature, where they are most often seen as layers of slime on rocks or other objects in water (**figure**). When they form on the hulls of boats and ships, they cause decomposition, which limits the life of the ships and results in economic losses. Of major concern is the formation of biofilms on medical devices such as hip and knee implants. These biofilms often cause serious illness and failure of the medical device. Biofilm formation is apparently an ancient ability among the microbes, as evidence for biofilms can be found in the fossil record from about 3.4 billion years ago.

Initially microbes attach to the conditioned surface but can readily separate. Eventually they begin releasing polysaccharides, proteins, and DNA and these polymers allow the microbes to stick more stably to the surface. As the biofilm thickens and matures, the microbes reproduce and secrete additional polymers. The result is a complex, dynamic (active) community of microorganisms.

The microbes interact in a variety of ways. For example, the waste products of one microbe may be the energy source for another microbe. The cells also communicate with each other, as described next. Finally, DNA present in the extracellular slime can be taken up by members of the biofilm community. **Thus genes can be transferred**

from one cell (or species) to another. While in the biofilm, microbes are protected from numerous harmful agents such as UV light, antibiotics, and other antimicrobial agents. This is due in part to the extracellular matrix in which they are embedded, but it also is due to physiological changes.

Indeed, numerous proteins synthesized or activated in biofilm cells are not observed in planktonic cells and vice versa. The resistance of biofilm cells to antimicrobial agents has serious values. When biofilms form on a medical device such as a hip implant , they are difficult to kill and can cause serious illness. Often the only way to treat patients in this situation is by removing the implant. Another problem with biofilms is that cells are regularly sloughed off (figure). This can have many values. For instance, biofilms in a city's water distribution pipes can serve as a source of contamination after the water leaves a water treatment facility.



Cell-Cell Communication

Bacteria often communicate with one another in a density dependent way and carry out a particular activity only when a certain population density is reached. This phenomenon is called quorum sensing

Within Microbial Populations For decades, microbiologists tended to think of bacterial populations as collections of individual cells growing and behaving independently. But about 30 years ago, it was discovered that the marine luminescent bacterium *Vibrio fischeri* controls its ability to glow by producing a small, diffusible substance called autoinducer. The autoinducer molecule was later identified as an *N*-acylhomoserine lactone (AHL). It is now

known that many gram-negative bacteria make AHL molecular signals that vary in length and substitution at the third position of the acyl side chain . In many of these species, AHL is freely diffusible across the plasma membrane. Thus at a low cell density, it diffuses out of the cell. However, when the cell population increases and AHL accumulates outside the cell, the diffusion gradient is reversed so that the AHL enters the cell. Because the influx of AHL is cell density– dependent, it enables individual cells to assess population density. This is referred to as quorum sensing —a quorum usually **refers to the minimum number of members in an organization.** When AHL reaches a threshold level inside the cell, it induces the expression of target genes that regulate a number of functions, depending on the microbe. These functions are most effective only if a large number of microbes are present. For instance, the light produced by one cell is not visible, but cell densities within the light organ of marine fish and squid reach 10 10 cells per milliliter. This provides the animal with a flashlight effect while the microbes have a safe and nutrient-enriched habitat . In fact, many of the processes regulated by quorum sensing involve host-microbe interactions such as symbioses and pathogenicity.

For instance, while only gram-negative bacteria are known to make AHLs, both gram-negative and gram-positive bacteria make autoinducer-2 (AI-2). Gram-positive bacteria also exchange short peptides called oligopeptides instead of autoinducer-like molecules. The soil microbe *Streptomyces griseus* produces a γ-butyrolactone known as A-factor. This small molecule regulates both morphological differentiation and the production of the antibiotic streptomycin. Other examples of such complex behavior are pattern formation in colonies and fruiting body formation in the myxobacteria.

S-layer (Surface layer)

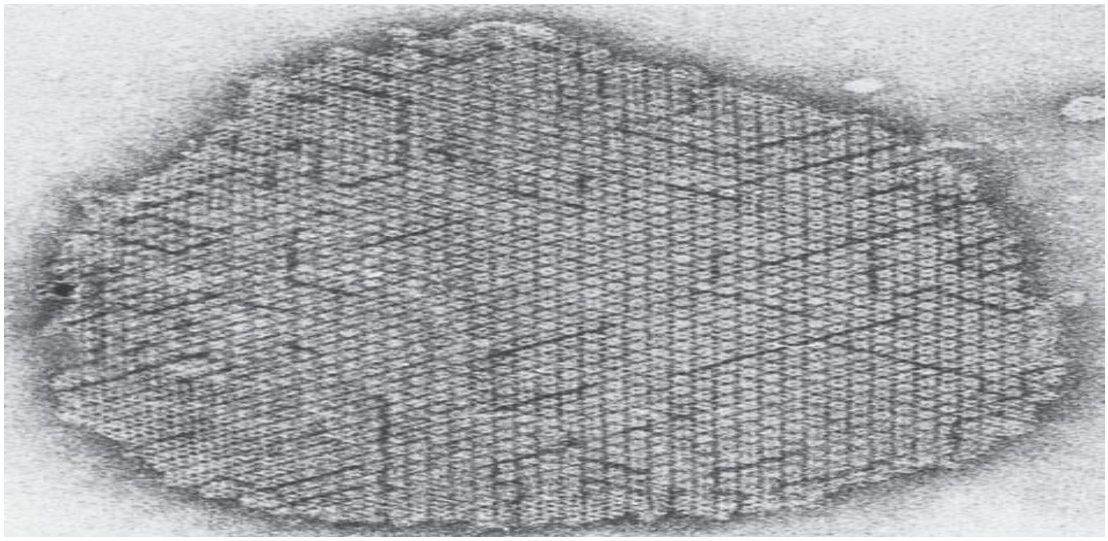
S-layers are observed in some bacteria and many archaea. They are composed of proteins or glycoprotein and have a characteristic geometric shape(has a pattern something like floor tiles). In many archaea, the S-layer serves as the cell wall (figure). Have the ability to aggregate its proteins by self-assemble.

In gram-negative bacteria, the S-layer adheres directly to the outer membrane; it is associated with the peptidoglycan surface in gram-positive bacteria.

Their biological roles include:

1- protecting the cell against ion and pH fluctuations, osmotic stress, Enzymes, predacious bacteria.
2- helps maintain the shape and envelope rigidity of some cells.
3- Adhesion to surfaces.

4- Protect some bacterial pathogens against host defenses, thus contributing to their virulence.
5- May play a role as permeability a barrier that allows the passage of small molecules.
6- Antigenic structure.
7- The potential use of S-layers in nanotechnology is due to the ability of S-layer proteins to self-assemble. That is, the S-layer proteins contain the information required to associate and form the S-layer without the aid of any special enzymes or other factors. Thus S-layer proteins could be used as building blocks for the creation of technologies such as drug-delivery systems and novel detection systems for toxic chemicals or bio terrorism agents.



Pili and Fimbriae

structure out of the cell wall are fine, hair like protein appendages on some procaryotes some help attach cells to surfaces and others are involved in a type of twitching motility. (figure).

Fimbriae (singular, Fimbria, **short attachment pili**, or fimbriae), often uses the term Pili and Fimbriae **in the same time** , are composed of protein the secondary units arranged in a hollow spiral shape. Have phenotypic heterogeneity : many species have the ability to change the types of antigenic protein that responsible for building the **Pili and Fimbriae** this process is called **phase variation** are controlled genetically. The cell may cover by more than **1,000 Fimbriae** but sees only by electron microscope because of their small size and short length with diameter 3-10 nm and in several micrometer in length:

Pili and Fimbriae function are :

1- Adhesion to **diverse surfaces**.

2- Have a role in Twitching motility by type IV Pili in some kinds of bacteria, including *Escherichia coli*, Myxobacteria, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* when the bacteria presence on the surfaces and lead to the movement for several micrometer (slow motion).

3- Transformation.

F pili (also called sex pili s:Pilus) long conjugation pili, A pilus is Thin protein appendages composed of subunits of the protein pilin differ from **fimbriae** in :

1- fewer and longer than **fimbriae** more than 10 pili for each cell.

2- Have large diameter than **fimbriae** (9-10 nm).

3- required for bacterial conjugation.

4- Considered as receptors for certain viruses.