ANATOMY OF BACTERIA CELL

Any bacterial cell whether it is a coccus or a bacillus will have some structures common. These structures are cell wall, cell membrane, cytoplasm, ribosomes and the chromosome. Other intra-cellular structures such as plasmid, inclusion bodies and extra-cellular structures such as capsule, fimbriae and flagella are possessed only by some bacteria.

Glycocalyx/Capsule/Slime:
A gelatinous polysaccharide or polypeptide outer covering of certain bacteria is called glycocalyx. These are the structures that surround the outside of the cell envelope. The glycocalyx is referred to as a capsule if it is firmly attached to the cell wall, or as a slime layer if loosely attached.

The chemical nature of bacterial capsules is diverse but majority of them are polysaccharides. These polymers are composed of repeating oligosaccharide units. However, the capsule of *Bacillus anthracis* is composed of a polypeptide (polyglutamic acid). *Yersinia pestis* produces a capsule of mixed amino acids. Capsules may be weakly antigenic to strongly antigenic, depending on their chemical complexity. Capsules may be covalently linked to the underlying cell wall or just loosely bound to it. They have no net charge and will not bind charged dye particles, hence they can't be stained. Bacteria with capsules form smooth (S) colonies while those without capsules form rough (R) colonies. A given species may undergo a phenomenon called S-R variation whereby the cell loses the ability to form a capsule. Some capsules are very large and absorb water (e.g., *Klebsiella pneumoniae*) forming mucoid (M) colonies. Capsules are often lost during in vitro culture. They are not essential to cell viability and some strains within a species will produce a capsule, while others do not. Capsules are sometimes referred as K antigens (in Enterobacteriaceae) or as Vi antigen (in *Salmonella typhi*). Capsular antigens may either be specific to a species or may be shared by few different bacteria. For example, K2 antigen of Klebsiella cross-reacts with Pneumococcal type2 antigen.

Significance:

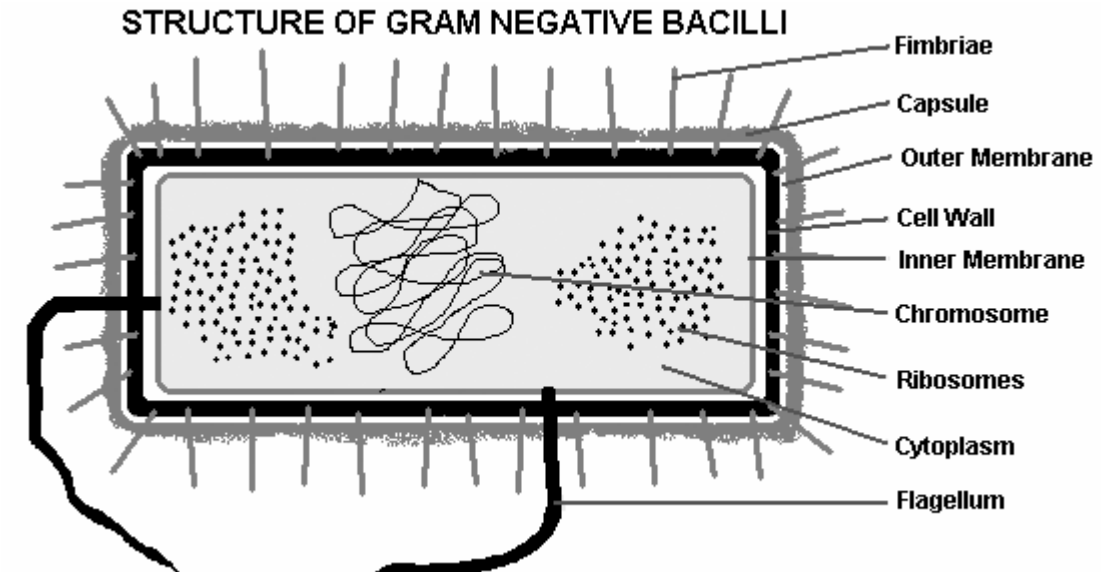
- Virulence factor. Capsules of pathogenic bacteria inhibit ingestion and killing by phagocytes. It can also prevent complement-mediated bacterial cell lysis. Capsules protect the cells from lysozyme. Mutant strains lacking capsule are avirulent.
- Permit bacteria to adhere to cell surfaces and structures such as medical implants and catheters. This is a first step in colonization and sometimes leads to disease.
- Capsules can be a source of nutrients and energy to microbes. *Streptococcus mutans*, which colonizes teeth, ferments the sugar in the capsule and acid byproducts contribute to tooth decay.
- Prevent cell from drying out (desiccation)
- Toxicity to the host cell; capsule of *Bacteroides fragilis* induces abscess formation.
- Capsules may protect cells from bacteriophages.
- Capsules play a role in antigenic mosaic.
- Capsules may trap ions.

Examples:
Streptococcus pneumoniae, *Streptococcus mutans, Klebsiella pneumoniae, Bacillus anthracis*, *Neisseria meningitidis*

Demonstration:
Since capsules don't take up any stain, they can be demonstrated by negative staining techniques. In Gram stain, they may appear as a clear halo around the bacteria where they represent capsule. They are best demonstrated by Negative staining using India ink, Nigrosine, Maneval's method (using Congo Red, acetic acid and acid fuchsin) or Welch method (involving crystal violet and copper sulfate solution). They can also be demonstrated immunologically by Quellung reaction.

Application:

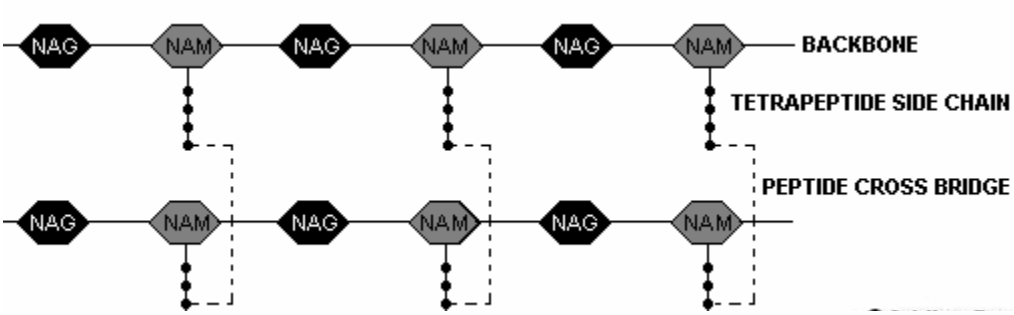
- Since the polysaccharides are good antigens, their presence on bacteria may be used to identify or type them by serological methods. Detection of capsular antigen in clinical specimens such as CSF, blood provides rapid method of diagnosis. Rapid identification of *Streptococcus pneumoniae* in CSF can be made by serological tests such as coagglutination. At least 13 serogroups of *Neisseria meningitidis* are identified on the basis of antigenicity of capsular polysaccharides.
- Since polysaccharides from certain capsules can be good antigen, they have been used in vaccines. Purified polysaccharide vaccines are available against *Neisseria meningitidis* and *Streptococcus pneumoniae.* They can also be conjugated with other vaccine as in *Hemophilus influenzae* vaccine.



CELL WALL:

The layers of cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as cell wall. In gram positive bacteria, the cell wall mainly consists of peptidoglycan and teichoic acid while the cell wall in gram negative bacteria includes peptidoglycan, lipoprotein, outer membrane and lipopolysaccharide layers. Cell wall does not take up any stain and hence are not seen by light microscope.
Most bacteria have a complex cell wall consisting of peptidoglycan (also called murein, mucopeptide). This complex polymer consists of three parts,

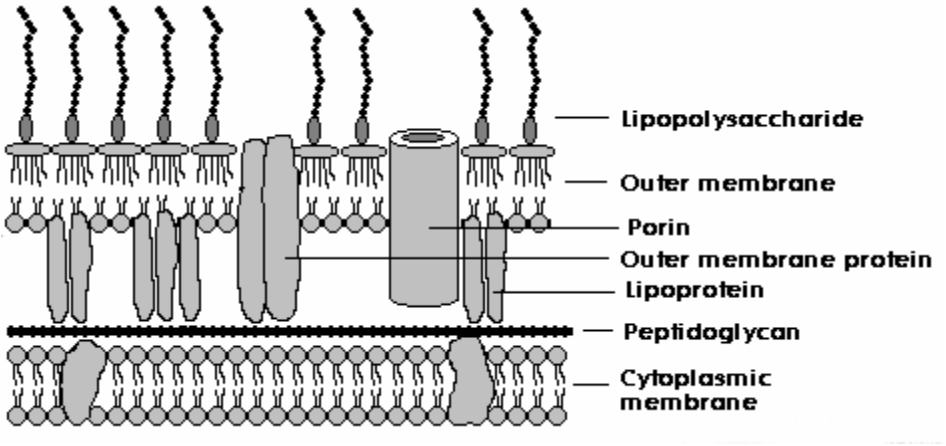
- A backbone consisting of alternating units of NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid).
- Tetrapeptide side chain attached to NAM
- Peptide cross-bridges, which are short chains of amino acids that crosslink the backbone.



Gram positive bacterial cell wall: In gram positive bacteria, there may be as many as 40 sheets of peptidoglycan, comprising up to 50% of cell wall material. Electron micrographs show the peptidoglycan of Gram positive cells to be 20-80 nm thick. Most gram positive cell walls contain additional substances such as teichoic acid and teichuronic acid. These are water soluble polymers of ribitol or glycerol. There are two types of teichoic acid, wall teichoic acid (linked to peptidoglycan) and lipoteichoic acid (linked to membrane). Some gram positive bacteria may lack wall teichoic acid but all contain lipoteichoic acid. The teichoic acid constitutes major antigens of cells that possess them. Teichoic acid binds to Magnesium ions and plays a role in supply of this ion to the cell. Teichuronic acids are

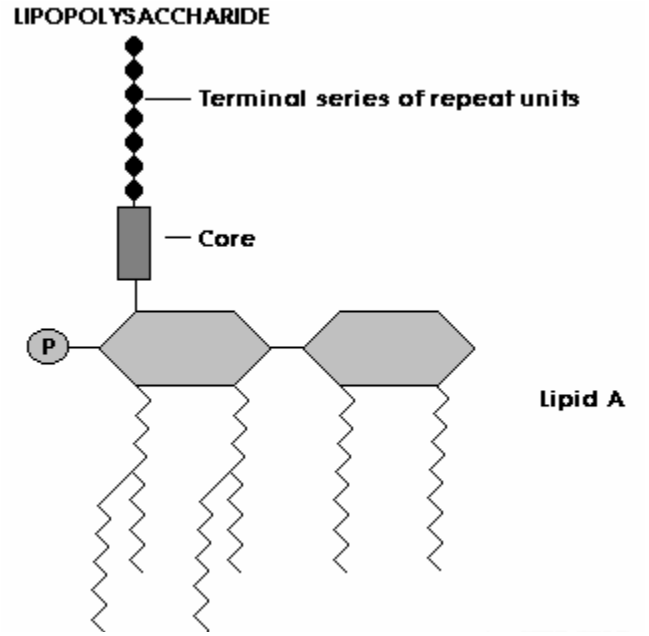
produced in place of teichoic acid when phosphate is limiting. Teichoic acid in *Streptococcus pneumoniae* bears the Forssman antigen. Gram positive cells stain purple due to retention of the crystal violet dye during the Gram stain procedure. If peptidoglycan is digested away from the cell, gram positive cells lose their cell walls and become protoplasts while the gram negative cells become spheroplasts. In some cases the cell wall of Gram-positive bacteria may contain proteins of special significance such as M, T and R proteins of the group A streptococci and Protein A of *Staphylococcus aureus.*

Gram negative bacterial cell wall: Gram negative cells consist of a relatively thin layer of peptidoglycan (approximately 10 nm). There appears to be only one or two sheets of peptidoglycan, comprising 5-10% of cell wall material. Gram negative bacteria do not retain the primary dye in Gram stain and hence appear pink. Gram negative bacteria have an additional outer membrane. The outer membrane is the major permeability barrier in Gram negative bacteria. The space between the inner and outer membranes is known as the periplasmic space, which contains digestive enzymes and other transport proteins.



Gram negative cell walls contain three components that lie outside the peptidoglycan layer: lipoprotein, outer membrane and lipopolysaccharide. Lipoprotein stabilizes the outer membrane by anchoring it to peptidoglycan. Outer membrane is phospholipid bilayer in which the outer phospholipids are replaced by lipopolysaccharides. It is structurally similar to cytoplasmic membrane and serves to prevent leakage of periplasmic proteins and protects the cell from bile salts and protelolytic enzymes. The outer membranes contain several important porins, which specifically allow transport of solutes.

Lipopolysaccharide consists of a polysaccharide core, a complex lipid called Lipid A and a terminal series of repeat units. The polysaccharide core is similar in all gram negative bacteria. Each species contains unique terminal repeat units. Lipopolysaccharide (LPS) is toxic in nature and is called endotoxin because it is firmly bound to the cell wall and released only when cell is lysed. Endotoxin can trigger fever and septic shock in gram negative infections. Endotoxin can be detected in IV fluids by Limulus lysate reaction. The lysate of amebocytes (circulating cells) from the horseshoe crab (*Limulus polyphemus*) are highly sensitive to endotoxin and gels (clots) immediately on exposure to it. LPS also protects the cell from phagocytosis and lysozyme.



LPS confers a negative charge and also repels hydrophobic molecules such as bile in the intestine. If LPS is split into Lipid A and polysaccharide, all the toxicity is associated with Lipid A and polysaccharide represents the major surface antigen of bacterial cell. This antigen is designated as somatic "O" antigen and is used in serological typing of species. It was formerly known as Boivin antigen. Antigenic specificity is conferred by the terminal repeat units

The cell envelopes of Mycobacteria are more complex than other bacteria. Long chained branched fatty acids (Mycolic acid) are covalently bound via a polysaccharide to peptidoglycan. Other mycolic acid containing compounds and complex lipids form a thick waxy membranous layer outside the peptidoglycan layer.

Significance of cell wall:

- Maintains cell shape, any cell that loses its cell wall, loses its shape as well.
- Protects bacteria from osmotic lysis
- Acts as a barrier, protects cell contents from external environment
- Determines reactivity to Gram stain, cells become gram negative if they lose cell wall
- Attachment site for flagella
- Site of action of certain antimicrobial agents (E.g. Penicillins, Cephalosporins)
- Bacteria may attach to surface, produce slime, divide and produce microcolonies within the slime layer and construct a biofilm. E.g. formation of dental plaque mediated by the bacterium *Streptococcus mutans*.
- Confer specific antigenicity to a strain/species that can be exploited to detect and identify an isolate.

Substances acting against cell wall:

- ✓ Lysozyme, an enzyme found in tears and saliva breaks down a component of cell walls
- ✓ Antibiotics that inhibits cell wall synthesis such as Penicillins and cephalosporins
- ✓ Autolytic enzymes produced by some bacteria such as *Streptococcus pneumoniae*

Demonstration of cell wall:

Since cell wall does not take up stain, they can't be demonstrated by light microscopy. Their presence can be demonstrated by placing a cell in hypertonic solution, where they undergo plasmolysis. The cytoplasm shrinks as the water is lost by osmosis but the cell wall retains its original shape (due to its rigidity). This is described as "bacterial ghost". The cell wall may also be demonstrated by micro-dissection, electron microscopy and immunological reactivity.

CELL MEMBRANE

Cell membrane or cytoplasmic membrane is a typical unit membrane composed of phospholipids (40%) and proteins (60%). It measures approximately 5-10 nm in thickness. It lies below the peptidoglycan layer of the cell wall and encloses the cytoplasm. The arrangement of proteins and lipids to form a membrane is called the fluid mosaic model. The membranes of bacteria (except Mycoplasma) do not contain sterols. It is a phospholipid bilayer with polar heads on either side of the membrane. Hydrophobic tails are oriented to the interior of the membrane. Specialized structures called mesosomes or chondroids are formed from the convoluted invaginations of cytoplasmic membrane. There are two types of mesosomes, septal mesosome and lateral mesosome. The bacterial chromosome is attached to the septal mesosome. During cell division, the septal mesosome participates in the formation of cross-walls. Mesosomes are more prominant in gram positive bacteria. They are believed to be analogous to eukaryotic mitochondria since they are rich in respiratory enzymes.

Functions of cell membrane:

- A selectively permeable barrier: substances that pass through the membrane are limited by pore sizes and the hydrophobic nature of the membrane
- Integral (transmembrane) proteins form channels and act as carriers
- Peripheral proteins can act as receptors and as enzymes for metabolic reactions
- Electron transport and oxidative phosphorylation: cytochromes and dehydrogenases of respiratory chain are located in the cell membrane
- Excretion of hydrolytic enzymes
- Site of initiation of cell wall synthesis
- Site of attachment of the chromosome
- Site of synthesis of phospholipids
- Bear receptors and proteins of sensory transduction system

Substances acting on cell membrane:

- ❑ Detergents that contain lipophilic and hydrophilic groups disrupt cytoplasmic membranes
- ❑ Antibiotics such as Polymyxin B and Gramicidin selectively damages membrane
- ❑ Ionophores (E.g. Valinomycin) are compounds that permit rapid diffusion of cations through the membrane.
- ❑ Chemical agents such as alcohols and quaternary ammonium compounds

CYTOPLASM

The cytoplasm or protoplasm is the portion of the cell that lies within the cytoplasmic membrane. It is gel-like in consistency and includes the procaryotic chromosome and ribosomes. Constituents of cytoplasm include proteins (including enzymes), vitamins, ions, nucleic acids and their precursors, amino acids and their precursors, carbohydrates and their derivatives, fatty acids and their derivatives. The cytoplasm does not exhibit any internal

mobility (cytoplasmic streaming). The cytoplasm also lacks organelles such as mitochondria, golgi apparatus or endoplasmic reticulum. Cytoplasm stains uniformly in young cultures. Recent studies suggest that some bacteria (*Bacillus subtilis*) possess cytoskeleton.

Chromosome:
The chromosome in bacteria is typically a single, closed circle DNA that is concentrated in a nucleoid region. It is not membrane bound as in eukaryotes. Some bacteria possess smaller extrachromosomal pieces of DNA called plasmids. Plasmids replicate independently of the chromosome and carry genes that are not essential for cell survival but may give some advantage to an organism. The chromosome is attached to an invagination of the cytoplasmic membrane called mesosome. Mitotic apparatus and nuclear membrane are completely lacking. The length of *E.coli* chromosome is approximately 1.4 mm but is condensed inside the cell by supercoiling. DNA is mainly negatively charged hence bind readily to basic dyes. It can be demonstrated by Feulgen stain or by electron microscopy.

Ribosomes:
Bacterial cells can contain thousands of ribosomes, which are the sites of protein synthesis. The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of ribosomes. Often they aggregate to form structures known as polysomes. Bacterial ribosomes are termed 70 S (Svedberg units) and eukaryotic ribosomes are termed 80S. The difference between bacterial and eukaryotic ribosomes is often exploited during antibiotic therapy.

Inclusion bodies:
Intracytoplasmic inclusions can be vacuoles, crystals or storage bodies. Bacteria often store reserve material in the form of insoluble cytoplasmic granules. Inclusions accumulate when a cell is grown in the presence of excess nutrients and they are often observed under laboratory conditions. Various examples of these bodies are:

- Starch/Glycogen granules - blue-greens and enteric bacteria
- Poly-ß-hydroxybutyrate granules - Azotobacter and Rhizobium
- Nitrogen-reserve granules - blue-greens
- Sulphur inclusions – Thiotrix
- Lipid inclusions
- Volutin granules – *Corynebacterium diphtheriae*

The inclusion bodies can be appreciated using phase contrast microscope or using special stains such as Albert's stain (volutin granules) or Sudan black (lipid inclusion).

FLAGELLA
Some bacteria are motile and some are not. Almost all motile bacteria possess flagella as the organ of locomotion. Such bacteria tend to move towards or away from the source of stimulus. These stimuli can be chemicals (chemotaxis), light (phototaxis), air (aerotaxis) or magnetism (magnetotaxis).

Structure:
Procaryotic flagella are much thinner than eukaryotic flagella and they lack the typical 9 + 2 arrangement of microtubules. Over 40 genes are involved in its assembly and function. They are approximately 3-20µm long and end in a square tip. Since flagella are very thin (20-30 nm in diameter), they are below the resolution limits of a normal light microscope and cannot be seen. The bacterial flagellum is a non contractile, composed of single kind of protein subunit called flagellin. It is anchored to the bacterial cytoplasmic membrane and cell well by means of disk-like structures. A flagellum comprises of three parts, filament, hook and basal body. The flagellum is attached to the cell body by hook and basal body. While the hook and basal body are embedded in the cell envelope, the filament is free. If a flagellum is cut off it will regenerate until reaches a maximum length. This is so because the growth is not from base, but from tip. The basal body bears a set of rings, one pair in gram positive bacteria and two pairs in gram negative bacteria. While the rings named S and M are common to both, the rings names P and L are found only in gram negative bacteria. Rings in the basal body rotate relative to each other causing the flagella to turn like a propeller. The energy to drive the basal body is obtained from the proton motive force. Bacteria move at average speed of 50µm/sec, the fastest being *Vibrio cholerae* that moves 200µm/sec.

The numbers of flagella, as well as their location on the cell surface are characteristic of a species.

Flagella arrangements are:
1. Monotrichous - a single flagellum at one pole (also called polar flagellum) E.g. *Vibrio cholerae*
2. Amphitrichous - single flagellum at both poles. Eg. Spirilla
3. Lophotrichous - two or more flagella at one or both poles of the cell E.g. *Spirillum undula*
4. Peritrichous - completely surrounded by flagella E.g. *E.coli*

Other mechanisms of bacterial locomotion include gliding and motion by axial filament contraction. Gliding is movement of bacteria along solid surfaces by an unknown mechanism. Spirochetes have internally-located axial filaments or endoflagella. Axial filaments wrap around the spirochete towards the middle from both ends. They are located above the peptidoglycan cell wall but below the outer membrane.

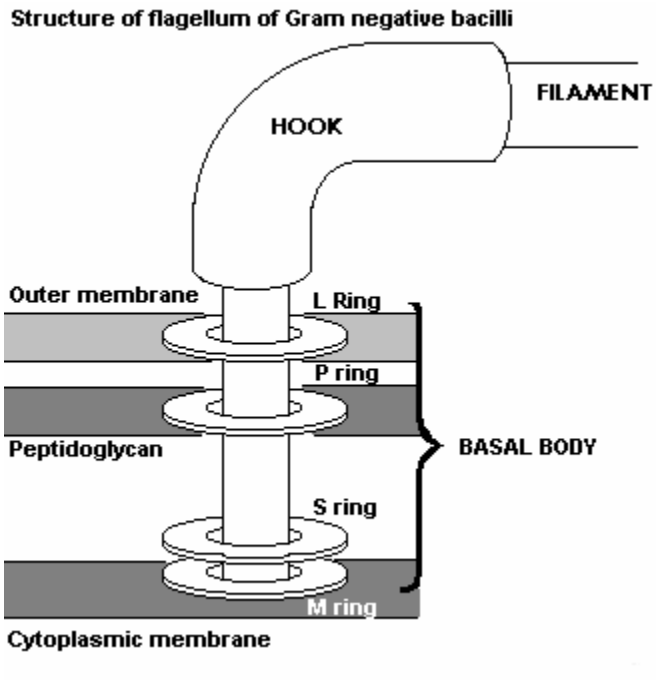
Detection bacterial motility:

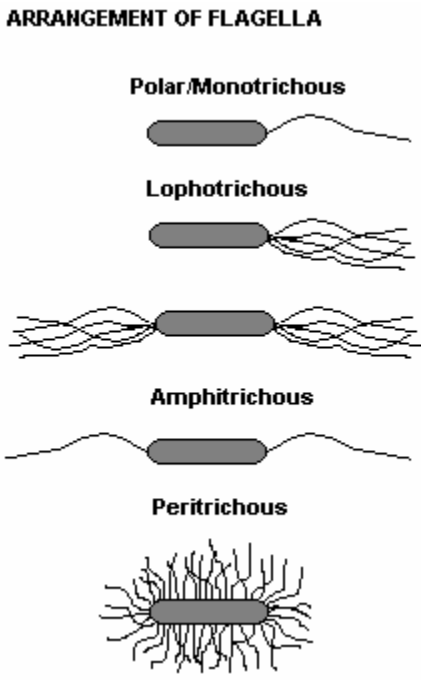
1) Direct observation by means of hanging drop preparation
2) Special-purpose microscopes (phase-contrast and dark-field)
3) Motility media (semi solid agar)
4) Indirectly, by demonstration of flagella
    - Flagella staining (Silver impregnation, Leifson's method)
    - Electron microscopy
    - Immunological detection of flagellar "H" antigen

Types of bacterial motility:

- Stately motility: *Bacillus sps*
- Active motility: *Pseudomonas sps*
- Darting motility: *Vibrio cholerae*
- Tumbling motility: *Listeria monocytogens*
- Corkscrew, extension-flexion motility: Spirochetes

Examples of non-motile bacteria: Most cocci, Shigella, Klebsiella





Significance of flagella:

- Primarily function is motility (chemotaxis, aerotaxis, phototaxis etc). Positive taxis is movement toward a favorable environment whereas negative taxis is movement away from a repellent.
- Flagella can help in identifying certain types of bacteria. For example, Proteus species show 'swarming' type of growth on solid media.
- Flagellar antigens are used to distinguish different species and strains of bacteria (serovars). Variations in the flagellar H antigen are used in serotyping.

FIMBRIAE AND PILI

Fimbriae are short, hair-like structures made up of protein pilin and are present in many gram negative bacteria. Even though pili arise from plasma membrane they are not considered part of the plasma membrane. They are anchored in the membrane and protrude through the cell wall to the outside of the cell. Fimbriae are shorter and straighter than flagella and are more numerous. They are 0.5µm long and 10 nm thick. Since they are made up of protein, they are antigenic. Bacteria from different genera may possess common fimbrial antigens. Fimbriae are

usually seen in young cultures and lost on subcultures on solid media. While some authors use the two terms (fimbriae and pili) interchangeably, some restrict the term pili to denote sex pili. Sex pili acts to join bacterial cells for transfer of DNA from one cell to another by a process called conjugation.

Significance:

- They act as adhesins and allow bacteria to colonize cells. For example*, Neisseria gonorrhoea* uses its fimbriae to attach to the lining of the genital tract and initiate an infection.
- Fimbriae can also detect chemical signals and are important in bacterial cell communication and biofilm formation.
- Fimbriae also act as receptors for bacteriophages.
- Fimbriae of *Streptococcus pyogenes* are coated with M protein, which acts as an important virulence factor by adhering to host cells and resisting phagocytosis.
- Fimbriated bacteria form surface pellicle of liquid media.
- Some fimbriae can agglutinate RBC of guinea pigs, horses, pigs and fowls. This haemagglutination may or may not be inhibited by mannose.

Demonstration of fimbriae:

- Electron microscopy
- Haemagglutination
- Immunological detection of fimbrial antigen

SPORE:

In poor growth conditions some bacteria such as Bacillus and Clostridium produce resistant survival forms termed endospores. This process is known as sporulation. Bacterial spores are endospores in contrast to fungal spores, which are usually exospores. Unlike the spores of fungi, bacterial spores do not serve reproductive function. They are resistant to extreme environmental conditions such as high temperatures, dryness, toxic chemicals (disinfectants, antibiotics), and UV radiation. Once the endospore is formed, the vegetative portion of the bacterium is degraded and the dormant endospore is released. The endospore is able to survive for long periods of time until environmental conditions again become favorable for growth. The endospore then germinates, producing a single vegetative bacterium. Spores can be killed by sterilization methods such as autoclave and hot air oven. Some chemical disinfectants such as formaldehyde and ethylene oxide can also kill spores.

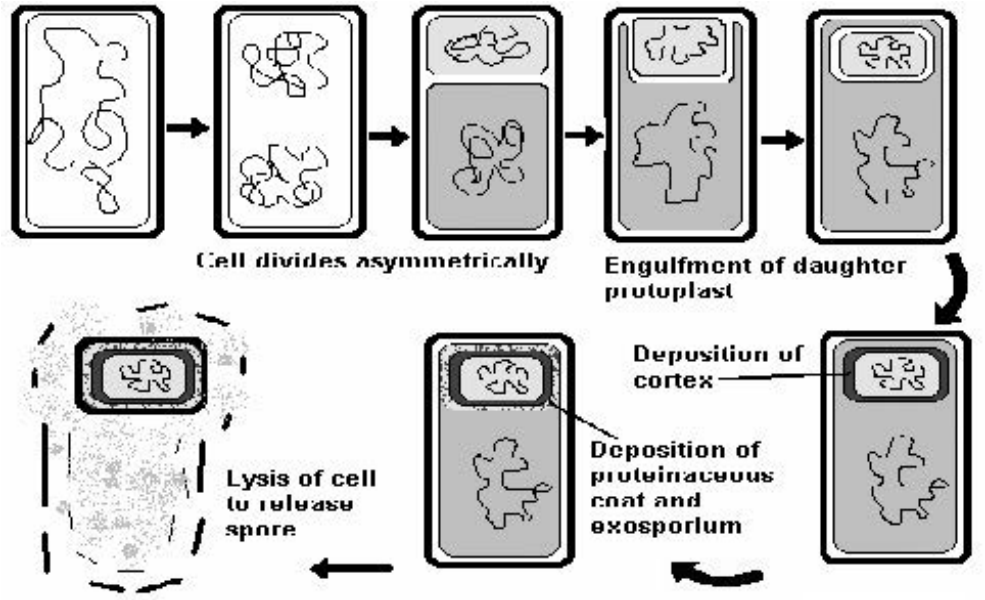
Mechanism of sporulation:

First the DNA replicates and the cell divides asymmetrically. A cytoplasmic membrane septum forms at one end of the cell. A second layer of cytoplasmic membrane then forms around one of the DNA molecules (the one that will become part of the endospore) to form a forespore. Both of these membrane layers then synthesize peptidoglycan in the space between them to form the cortex. Calcium dipocolinate is also incorporated into the forming endospore. A spore coat composed of a keratin-like protein then forms around the cortex. Sometimes an outer membrane composed of lipid and protein and called an exosporium is also formed. Finally, the remainder of the bacterium is degraded and the endospore is released. There is no metabolic activity until the spore is ready to germinate. Single vegetative cell gives rise to a single spore. Sporulation generally takes around 15 hours.

Germination:

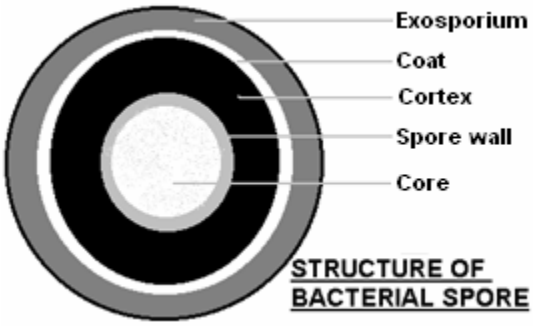
Favorable growth conditions signal the process of endospore germination. Germination of a spore results in a break in the spore wall and the outgrowing of a new vegetative cell. The newly formed vegetative cell is capable of growth and reproduction. A single spore upon germination forms a single vegetative cell. Germination occurs in following steps:

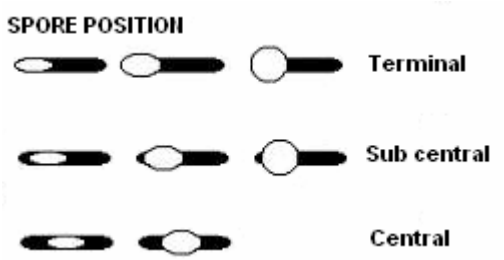
1. Activation: Even in the presence of favorable conditions, the spore will not germinate until its protective spore coat is not damaged. Conditions such as heat, acidity, abrasion or compounds containing free sulphydryl groups activate the spore to germinate.
2. Initiation: once activated, the spore will germinate provided the environment is suitable. Different signaling effectors exist for different species. Binding of effector stimulates autolytic enzymes that degrade the peptidoglycan of cortex. Water is absorbed and calcium dipicolinate is released.
3. Outgrowth: once the cortex and outer layers is degraded, a new vegetative cell consisting of spore protoplast and its wall emerges. This is followed by active biosynthetic activity and process terminates with cell division.



The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals. The resistance of endospores is due to a variety of factors:

- Calcium-dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Specialized DNA-binding proteins saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation.
- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation.
- DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.





The size of the endospore and its position within the vegetative cell is characteristic for a given species. The position of spore in a bacterium can be central, sub terminal or terminal. The shape of the spore can be spherical or oval. Sometimes the width of the spore is slightly more than the width of the bacillus such that spore appears to be bulging from the cell, as seen in Clostridium. In *Bacillus sps*, the spore does not cause the bacillus to bulge.
Bacteria with central or sub terminal spores: *Clostricium welchii, Clostridium sporogenes*
Bacteria with oval and terminal spores: *Clostridium tertium*
Bacteria with spherical and terminal spores: *Clostridium tetani*

Demonstration of spores:
The dense endospore is impenetrable by basic dyes. Strong dyes and vigorous staining conditions such as heat are needed. Once stained, however, endospores are equally hard to decolorize.

- In the Gram stain the spore is not stained and may appear as a clear space
- Appear as refractile bodies when seen through phase contrast microscope
- Spores can be stained by Malachite Green
- Modified acid fast stain

Significance of spores:

- Since they are resistant forms of bacteria, they can survive unfavourable conditions for long period.
- Since spores occur in soil, wounds contaminated by soil can lead to infections like gangrene or tetanus.

- Since spores survive ordinary disinfection, they may contaminate surgical wounds.
- Since spores are everywhere, they may contaminate bacterial culture media.
- Since they are highly heat resistant, they can be used to monitor the efficacy of sterilization process in autoclave (*Bacillus stearothermophilus*) and hot air oven (*Clostridium tetani* var *niger*).
- They have also been used in biological warfare.

L-FORMS, PROTOPLAST AND SPHEROPLASTS:

When bacteria are treated with enzymes that hydrolyze the cell wall (e.g. lysozyme) or antibiotics that interfere with biosynthesis of peptidoglycan (penicillin), wall-less bacteria are often produced. Such a treatment of bacteria in osmotically protective medium liberates protoplasts from gram positive bacteria and spheroplasts from gram negative bacteria. Spheroplasts retain the outer membrane. Usually these treatments generate wall-less non-viable organisms that do not multiply. However, if such cells can grow and divide, they are called L forms. L forms were first reported by Klieneberger Nobel in cultures of *Streptobacillus monoliformis*. They are named L forms after Lister Institute, where they were discovered. They are produced more readily with penicillin than with lysozyme, suggesting that need for residual peptidoglycan. Some L forms are stable and some are unstable. Unstable forms are those which revert back to cell wall containing state when inducing stimulus (penicillin) is removed. Such forms usually have small amounts of residual peptidoglycan that serves as primer for building cell wall. Stable forms do not revert back to normal form since they completely lack peptidoglycan. While some L forms form spontaneously (e.g *Streptobacillus monoliformis*) others are inducible. Since they lack cell wall, they don't have a definite shape. L forms are difficult to cultivate and require medium that has right osmotic strength and low concentration of agar, inactivated serum and sucrose. L forms resemble mycoplasma in morphology, type of growth on agar "fried-egg colony". While mycoplasma lack cell wall and have sterols in their membrane, the L forms may have reminiscent of cell wall but do not have sterols in their membrane.

Significance of L forms: L forms may produce chronic infections in the host. They may persist in protective regions of the body. Since L forms are relatively resistant to antibiotics, they are difficult to treat. Their reversion to normal form can result in relapse of infection.

INVOLUTION FORMS AND PLEOMORPHISM:

Certain species of bacteria are known to exhibit variation in shape and size of individual cells. This variation is known as pleomorphism. Swollen and aberrant forms may be seen in ageing cultures of *Nesseria gonorrhoeae* and *Yersinia pestis* or in the presence of high salt concentration. Such forms are known as involution forms. Pleomorhism as well as involution forms are believed to be the result of defective cell wall synthesis or due to the action of autolytic enzymes that digest their own cell wall.