

STRUCTURE OF BACTERIA

Bacteria are the smallest organisms that all machinery required for growth and self-replication, their diameter is usually about $1\mu\text{m}$.

Bacterial cell have rigid cell wall surrounding the cytoplasmic membrane. The membrane provides the osmotic barrier while the wall protects the cell against osmotic rupture in dilute media and against mechanical damage. The cell wall and membrane are often referred together as the cell envelope, while capsule, flagella, and pilli are considered appendages.

MORPHOLOGY OF BACTERIA

The high microscope reveals two principles forms of Eubacteria, spherical organisms called cocci and cylindrical ones called bacilli.

Cocci appear in number of different patterns depending upon the planes in which they divide. When cocci appear in pairs they are known as diplococci, while if in chain they are called streptococci, and they are called staphylococci if they were in cluster.

Cocci that remain adherent often splitting successively in two or three perpendicular direction yielding square (tetrads) of cubical packets are known as sarcinae (bundles).

Bacillus when unusually short are referred as coccobacilli, when tapered at both ends as fusiform, when growing in long threads as filaments form, when curved as vibrio and when spiral as spirillum.

In 1981, square bacteria had been discovered; they are $2-4\mu\text{m}$ in diameter, halophilic (Archaeobacteria), produce stains similar to bacterial rhodopsin.

PLEOMORPHISM

Bacteria appear in number of different forms. Environmental conditions are affecting the size and shape of bacteria, which is seen obviously in bacilli forms other than cocci forms.

STRUCTURE OF BACTERIAL CELL

The cell envelope:

The layers that surround the prokaryotic cell are called cell envelope. The structure and organization of the cell envelope differ in gram positive and gram negative bacteria.

The gram positive cell envelope

It is relatively simple, consisting of two or three layers: the cytoplasmic membrane, a thick peptidoglycan layer (PG) and in some bacteria an outer layer called capsule.

The gram negative cell envelope

It is a highly complex, multilayered structure. The cytoplasmic membrane (called inner membrane) is surrounded by a single layer of peptidoglycan to which is anchored a complex layer known as outer membrane, and the capsule may also be present. The space between inner membrane and outer membrane referred to as periplasmic space.

Capsule and glycocalyx:

Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environment. With one exception (the poly D-glutamic acid capsule of *Bacillus anthracis*) the extracellular material is polysaccharide. When the polymer forms a condensed well defined

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layer closely surrounding the cell, it is called capsule; when it forms a loose meshwork of fibrils extending outward from the cell, it called the glycocalyx. Negative staining using Indian ink could demonstrate capsules.

In some cases, masses of polymers are formed that appear to be totally detached from the but in which cell may be entrapped, in these instances the extracellular polymers may be referred to simply as a slime layer.

Capsule contributes to the invasiveness of pathogenic bacteria in protecting them from phagocytosis.

The glycocalyx plays a role in the adherence of bacteria to surfaces in their environment, including the cells of plant and animal hosts.

The cell wall

The layers of the cell envelope lying between the cytoplasmic membrane and the capsule are called cell wall.

In gram positive bacteria, the cell wall consists mainly of peptidoglycan, teichoic acid, and polysaccharide. While in gram negative bacteria, the cell wall includes the peptidoglycan, outer membrane, lipopolysaccharide (LPS), and lipoprotein.

The functions of cell wall

- 1- Protects the cell from osmotic pressure.
- 2- Plays an essential role in cell division.
- 3- Various layers of the wall are the sites of major antigenic determination of the cell surface.

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4- Lipopolysaccharide is responsible for the non specific endotoxin activity.

Chemical composition of the cell wall

1- The peptidoglycan layer

It is a complex polymer consisting of three parts:

- 1- A backbone composed of alternating subunit of N-acetyl glucosamine and N-acetylmuramic acid linked together by β 1-4 glycosidic bond.
- 2- A ~~set of identical~~ tetrapeptide side chains attached to N-acetylmuramic acid.
- 3- A set of identical peptide cross-bridge (the terminal COOH to NH_2 of neighboring tetrapeptide).

The backbone is the same in all bacteria species, the tetrapeptide side chain and the peptide cross bridge vary from species to species.

The tetrapeptide side chains of all species however have certain important features in common:

Most of them have L-alanine at position 1 (attached to N-acetylmuramic acid). D-glutamine is at position 2 and D-alanine at position 4. Position 3 is the most variable one, most gram negative bacteria have diaminopemelic acid at this position, and gram positive bacteria may have ~~The amino acid~~ L-lysine, or any of several other L-amino acids at position 3.

All peptidoglycan layers are cross linked, which means that each peptidoglycan layer represents a single giant-molecule.

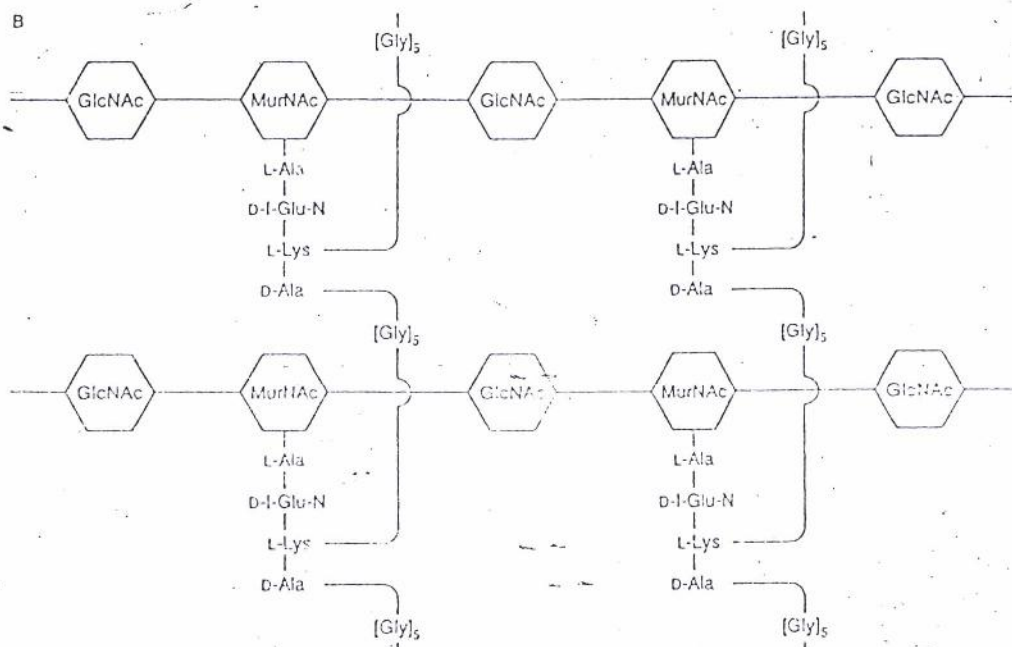
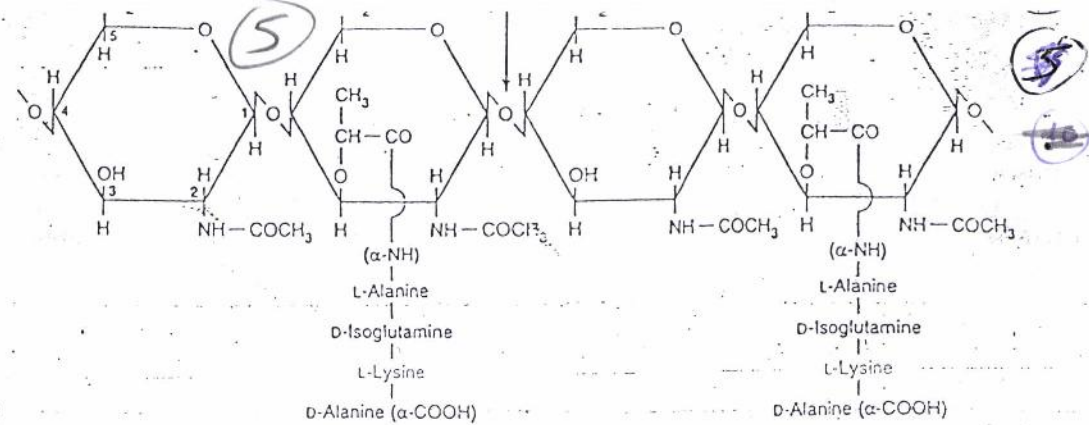


Figure 2-15. A: A segment of the peptidoglycan of *Staphylococcus aureus*. The backbone of the polymer consists of alternating subunits of N-acetylglucosamine and N-acetylmuramic acid connected by β 1 \rightarrow 4 linkages. The muramic acid residues are linked to short peptides, the composition of which varies from one bacterial species to another. In some species, the L-lysine residues are replaced by diaminopimelic acid, an amino acid that is found in nature only in prokaryotic cell walls. Note the D-amino acids, which are also characteristic constituents of prokaryotic cell walls. The peptide chains of the peptidoglycan are cross-linked between parallel polysaccharide backbones, as shown in Figure 2-15B. B: Schematic representation of the peptidoglycan lattice that is formed by cross-linking. Bridges composed of pentaglycine peptide chains connect the α -carboxyl of the terminal D-alanine residue of one chain with the ϵ -amino group of the L-lysine residue of the next chain. The nature of the cross-linking bridge varies among different species.

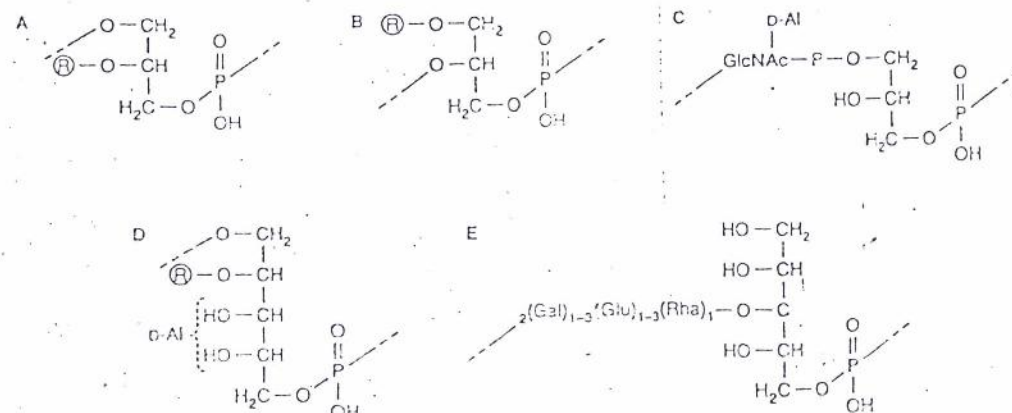


Figure 2-16. Repeat units of some teichoic acids. A: Glycerol teichoic acid of *Lactobacillus casei* 7469 (R, D-alanine). B: Glycerol teichoic acid of *Actinomyces antibioticus* (R, D-alanine). C: Glycerol teichoic acid of *Staphylococcus lactis* 13, D-Alanine occurs on the 6 position of N-acetylglucosamine. D: Ribitol teichoic acids of *Bacillus subtilis* (R, glucose). E: Ribitol teichoic acids of *Bacillus subtilis* (R, glucose).

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In gram positive bacteria there are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall materials. In gram negative bacteria, it appears to be only one or two sheets, comprising 5-10% of the wall materials.

B- special components of gram positive cell wall

Teichoic acid

Most gram positive cell walls contain amount of teichoic acid, which may account for up to 50% of the dry weight of the wall and 10% of the dry weight of total cell.

Teichoic acids are water soluble polymers containing ribitol or glycerol residues joined through phosphodiester linkage. There are two types of teichoic acids; wall teichoic acid covalently linked to peptidoglycan; and membrane teichoic acid (lipoteichoic acid), covalently linked to membrane glycolipid and concentrated in mesosome. Some gram positive species lack wall teichoic acid but all appears to contain membrane teichoic acid.

The function of teichoic acids: is still a matter of speculation:

- 1- Teichoic acids bind magnesium ion and may play a role in the supply of this ion to the cell.
- 2- They also play a role in the normal functioning of the cell envelope.
- 3- They enable the cell to resist the autolysis and to lose the ability to take up transforming DNA.

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- 4- Teichoic acid cell wall layer provides an external permeability barrier to gram positive bacteria, functionally equivalent to the outer membrane of gram negative bacteria.
 - 5- Membrane teichoic acids may serve to anchor the wall to the underlying cell membrane.

Teichuronic acid

The teichuronic acids are similar polymers, but the repeat units include sugar acids instead of phosphoric acids. They are synthesized in place of teichoic acids when phosphate is limiting.

Polysaccharides

The hydrolysis of gram positive cell wall has yielded neutral sugars such as mannose, arabinose, galactose, rhamnose, and glucosamine and acidic sugars.

C. special components of gram negative cell wall

Lipoprotein

Molecules of an unusual lipoprotein cross-link the outer membrane and peptidoglycan layers. The lipoprotein contains 57 amino acids. Their function is to stabilize the outer membrane and anchor it to the peptidoglycan layer.

Outer membrane

The outer membrane is a bilayered structure; its inner leaflet resembles in composition that of the cytoplasmic membrane while the

phospholipids of the outer leaflet are replaced by lipopolysaccharide (LPS) molecules.

The functions of outer membrane are:

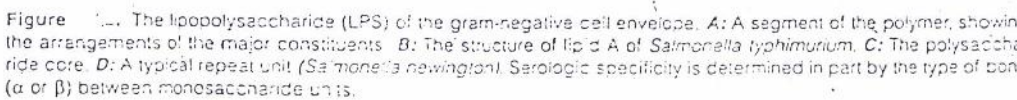
- 1- Prevents leakage of periplasmic space proteins.
- 2- Protects the enteric bacteria from bile salts and hydrolytic enzymes.
- 3- Contains the minor proteins, which are involved in the transport of specific molecules such as vitamin B₁₂ and iron-siderophore complexes.
- 4- Has a special channels, consisting of proteins called porins that permit the passive diffusion of low molecular weight hydrophilic compounds like sugars, amino acids, and certain ions.
- 5- Contains numbers of enzymes like proteases and phospholipases.

Lipopolysaccharide

The lipopolysaccharide of gram -ve cell wall consists of a complex lipid called lipid A, to which is attached a polysaccharide made up of a core and a terminal series of repeat units.

The function of lipopolysaccharide:

- 1- Stabilizes the membrane and provides a barrier to hydrophobic molecules.
- 2- Lipopolysaccharide, which is toxic to animals, has been called the endotoxin of gram negative bacteria because it is



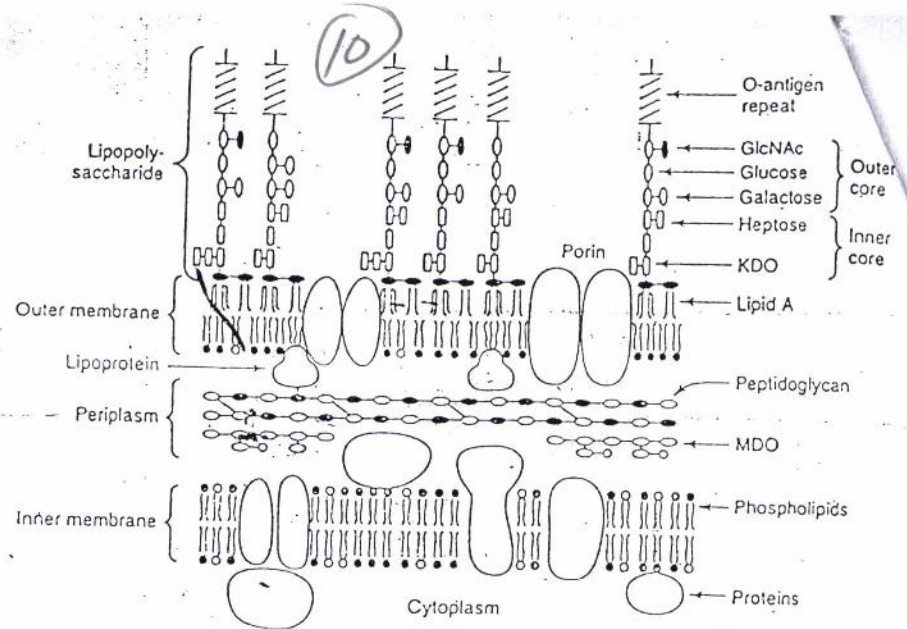


Figure 1. Molecular representation of the envelope of a gram-negative bacterium. Ovals and rectangles represent sugar residues, whereas circles depict the polar head groups of the glycerophospholipids (phosphatidylethanolamine and phosphatidylglycerol). (MDO, membrane-derived oligosaccharides.) The core region shown is that of *E. coli* K-12, a strain that does not normally contain an O-antigen repeat unless transformed with an appropriate plasmid. (Reproduced, with permission, from Raetz CRH: Bacterial endotoxins: Extraordinary lipids that activate eucaryotic signal transduction. *J Bacteriol* 1993;175:5745.)

firmly bound to the cell surface and is released only when the cells are lysed, all of the toxicity is associated with the lipid A.

- 3- Polysaccharide represents a major surface antigen of the bacterial cell so called O-antigen, and is responsible for the antigenic specificity. Lipopolysaccharide is attached to the outer membrane by hydrophobic bound.

The periplasmic space

The space between the cytoplasmic membrane and outer membrane, called the periplasmic space, contains the murein layer and a gel-like solution of proteins. The periplasmic space is approximately 20-40% of the cell volume. Its proteins include binding proteins for specific substrates (e.g. amino acids, sugars, vitamins, and ions) and the hydrolytic enzymes.

Enzymes that attack cell wall

The β 1-4 linkage of the peptidoglycan backbone is hydrolyzed by the enzyme lysozyme, which is found in the animal secretions (tears, saliva, and nasal secretions).

Bacteria themselves possess a number of autolysins, hydrolytic enzymes that attack the peptidoglycan, including glycosidases, amylases, and peptidases.

These enzymes play an essential role in cell growth and division. However, their activity is apparent during the dissolution of cell death (autolysis).

Protoplast, Spheroplast, and L-Forms

Removal of bacterial wall is accomplished either by hydrolysis with lysozyme or treating with penicillin, in supporting media of high solute concentration, such treatments will liberate protoplast from gram positive bacteria and spheroplast (which retains portions of the outer membrane and entrapped peptidoglycan) from gram negative bacteria.

Protoplast preparation could be accomplished as in the following manners:

- 1- Removal of the cell wall by treating the cell with an enzyme (lysozyme), which selectively dissolve the cell wall material.
- 2- Cultivate the organism in the presence of penicillin, which prevents the synthesis of cell wall material and doesn't interfere with growth and reproduction.

If such cells are able to grow and divide, they are called L-Forms. L-Forms are difficult to cultivate and usually require a medium that is solidified with agar as well as giving the right osmotic strength. L-Forms are produced more readily with penicillin than with lysozyme, suggesting the need for residual peptidoglycan.

Some L-Forms can revert to the normal bacillary form upon removal of inducing the stimulus. Thus they are able to resume normal cell synthesis. Others, however, are stable and never revert. Some bacterial species produce L-Form spontaneously. The spontaneous or antibiotic induced formation of L-Forms in the host may produce chronic infections, since their resistance to the antibiotics presents special problems in chemotherapy.

Cytoplasmic membrane:

It is also called cell membrane, composed of proteins and phospholipids. The membrane of prokaryotic cell is differing from those of eukaryotic cells by the absence of sterols except Mycoplasma.

Function of cytoplasmic membrane:

- 1- Selective permeability and transport of solutes.
- 2- Electron transport and oxidative phosphorylation, in aerobic species.
- 3- Excretion of hydrolytic exoenzymes.
- 4- Bearing the enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers, and membrane lipids.
- 5- Bearing the receptors and other proteins of the chemotactic and other sensory transduction systems.

At least 50% of the cytoplasmic membrane must be in the semifluid state in order for cell growth to occur.

Cytoplasmic ultrastructures

MESOSOMES:

One or more large irregular invaginations of the plasma membrane often are seen in the thin section of bacteria, called mesosomes. There are two types of them, *septal mesosome* which function in the formation of a transverse cell membrane during cell division. Septal mesosomes always seen attached to DNA, and *lateral mesosome*.

function of
mesosome types

Ribosomes:

Roughly, spherical densely stained objects about 18 nm in diameter. They are mostly grouping in chain called polysomes.

The ribosomes are designating according to their sedimentation coefficient as 70s ($s = 10^{13}$ cm/sec.) in prokaryotes and 80s in eukaryotes.

Ribosomes are responsible for the synthesis of proteins.

GRANULAR INCLUSION:

Many species of prokaryotes and eukaryotes store up reserve food substance in intracellular granules such as:

i- LIPID: in bacteria many of the inclusions formerly regarded as fat are actually a highly polymerized form of fatty acid and β -hydroxybutric acid.

Sardin III
Stain -
Vibrio cholerae
inclusions

ii- VOLUTIN: is an especially rich in organic phosphate, it consists largely of polymerized metaphosphates associated with nucleic acid and lipid in various linkage. Volutin appeared in large granule called metachromatic granules and usually abundant under condition of good nutrition and slower metabolism.

Albert stain
Corynebacterium
diphtheriae

iii- POLYSACCHARIDE: many species synthesize and store up excess soluble carbohydrate food substance; these are polymers of glucose.

names of
Dyes
Iodine
Enterobacteriaceae

NUCLEAR BODY (NUCLEOID):

The nucleoid in prokaryotes does not enclosed by a membrane. On contrary, in eukaryotes it is surrounded by the nuclear membrane.

membranous structures

It is so known that the prokaryotes, in general, didn't contain membrane-enclosed organelles. However, a few specialized bacterial groups contain extensive internal membranes. Such groups of bacteria include nitrifying bacteria and photosynthetic bacteria, in the later these membranes represent the sites of oxidative phosphorylation. These membranes may appear in spherical vesicles or known as chromatophores, which contains a mixture of lipids, proteins, and photosynthesis dyes. Cyanobacteria contain multilayered membrane structures, known as thylakoids. Some bacteria formed gas vacuoles are surrounded by a protein rather than real membranes.

PLASMIDS

In addition to the bacterial chromosome, bacteria may contain one or more small, circular macromolecules of DNA known as plasmids. Plasmids contain specific genetic information such as mating capabilities, resistance to antibiotics, production of toxins, and tolerance to toxic metals.

Typical plasmid
Factor F
R- resistance
AD high frequency
transmission

Sheaths: it is a filamentous or tubule structure enables bacteria to attach to solid surfaces. These sheaths afford protection against predators and parasites. In some cases, they may be covered with metal oxides such as iron or manganese oxides.

Stalks: some of bacterial species have appendages with an adhesive material at the far end of the cell by which the organisms can attach to a substrate. In some cases stalks may permit cells to adhere to each other, forming rosettes.

Flagella: the bacterial flagellum is a thread-like appendage (a long filamentous appendage) extending outward from the cytoplasmic membrane that propels bacteria; hence their main function is motility. It's usually several times longer than the cell, is generally only 12-25 nm in diameter. Thus flagella are too thin to be seen by ordinary microscope unless heavily coated by a special stain.

Bacterial flagellum composed of many subunits of the protein flagellin with a molecular weight ranging from 20,000 to 40,000, which confers a specific antigenicity.

Arrangement of flagella

Flagella may be arranged in various ways on bacterial cells. The flagellation is said to be **monotrichous** if only one flagellum protrudes from one end, or pole, of the cell; **lophotrichous** if several or numerous flagella protrude from one pole; **amphitrichous** if at least one flagellum is at each end; and **peritrichous** if the flagella protrude from all portions of the bacterial surface.

The number and distribution of the flagella is a stable genetic character so it is used in classification of bacteria. Flagella may be modified into what is called the **axial filament**, which does not extrude outward (e.g. *Spirochete*) or could be sheathed within a sheath (e.g. *Vibrio*).

Structure of flagella

Prokaryotic flagella unlike eucaryotic flagella, has no definite membrane and consists of a single very small filament made up of three

or more parallel or intertwined longitudinal fibers of protein flagellin. Each fibril is a polypeptide chain in α -helix form. At least some, and perhaps all, prokaryotic flagella appear to be attached to a structure called the hook, which in turn is attached to the basal body, which has a set of rings that attach to the cytoplasmic membrane and a rod that passes through the rings to anchor the flagellum to the cell. In gram-negative bacteria the basal body has a second set of rings that attaches to the outer membrane of the cell envelope. In Gram-positive bacteria there is only one set of rings that attaches to the cytoplasmic membrane.

MOTILITY

Bacterial flagella are semirigid helical rotors to which the cell imparts a spinning (rotatory) movement (quick motion) occur in bacteria with polar flagella (monotrichous and lophotrichous). When the bacteria are peritrichous flagella, drive the cell forward in a straight-line counterclockwise rotation (slow motion). At intervals, the flagella reverse their direction of rotation and momentarily dissociate, causing the cell to tumble. Twitching motion, it is seen in *Pseudomonas*, when *Streptococcus* ^{attach} to a solid surface by aid of pili. There is another kind of motions, Gliding motion; this kind of motility is found in numerous species of Cyanobacteria and Myxobacteria. It occurs when the bacteria are contact with a solid surface.

Chemotaxis: a cell that is moving toward or away from the source of chemical stimuli, behavior known as Chemotaxis. Bacteria move away from certain chemicals, (chemorepellents) like phenol and acids is called negative chemotaxis, and when the cell is moving toward other

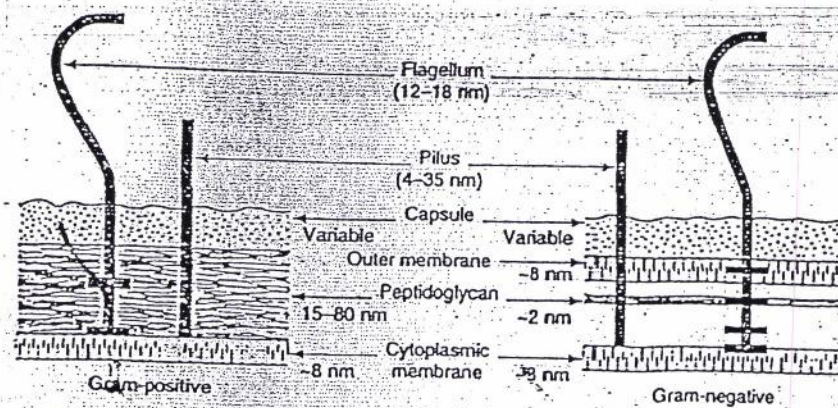


Figure Comparison of the structures of gram-positive and gram-negative cell envelopes. The region between the cytoplasmic membrane and the outer membrane of the gram-negative envelope is called the periplasmic space. (Reproduced, with permission, from Ingraham JL, Maaløe O, Neidhardt FC: *Growth of the Bacterial Cell*. Sinauer Associates, 1983.)

enolpyruvate: the phosphorylated carrier protein then

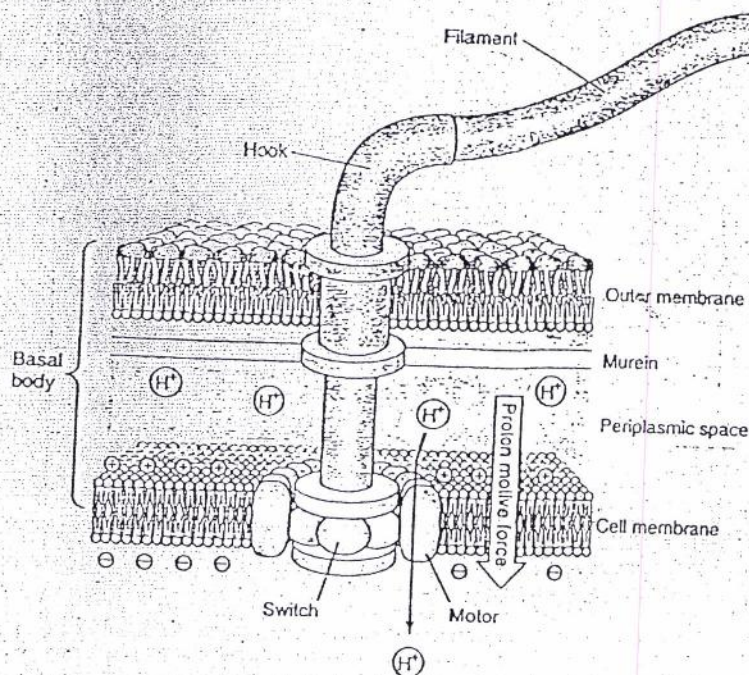


Figure Structural components within the basal body of the flagellum allow the inner portion of this structure, the rods of the basal body, and the attached hook-filament complex to rotate. The outer rings remain statically in contact with the inner and outer cell membranes and cell wall (murein), anchoring the flagellum complex to the bacterial cell envelope. Rotation is driven by the flow of protons through the motor from the periplasmic space, outside the cell membrane, into the cytoplasm in response to the electric field and proton gradient across the membrane which together comprise the proton motive force. A switch determines the direction of rotation, which in turn determines whether the bacteria swim forward (due to counterclockwise rotation of the flagellum) or tumble (due to clockwise rotation of the flagellum). (Reproduced, with permission, from Saier MH Jr: Peter Mitchell and his chemiosmotic theories. *ASM News* 1997;63:13.)

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chemicals (*chemoattractants*) like sugars and amino acids, it known as positive chemotaxis.

Aerotaxis: movement toward the optimum oxygen concentration.

Phototaxis: movement of photosynthetic bacteria toward the light.

Magnetotaxis: some motile bacteria contain inclusions of crystalline magnetic iron oxide (Fe_3O_4) called magnetosomes.

Magnetosomes permit these bacteria to orient their movement in response to magnetic fields, a phenomenon known as magnetotaxis.

Pili:

Pili are short, thin, straight, hair-like projections that emanate from the surface of some bacteria and are involved in attachment processes.

They shorter and finer than the flagella.

Like flagella, they are composed of structural protein subunits termed pilins. Two classes of pili can be distinguished: ordinary pili, which play a role in the adherence of symbiotic and pathogenic bacteria to host cells; and sex pili (F or fertility pilus), which are responsible for the attachment of donor and recipient cells in bacterial conjugation. Pili also act as receptor sites for some bacteriophages.

Sometimes a distinction is made between types of attachment processes, with the term *pilus* referring only to attachment between mating bacterial cells and the term *fimbriae* referring to all other attachment.

Spores:

Under conditions of limitation in supply of carbon, nitrogen, or phosphorus, (in a process known as sporulation) certain gram positive

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aerobic *Bacillus* and anaerobic *Closteridium* and others form highly resistant dehydration, heating, and chemical agent called endospores.

All bacterial spores contain large amount of dipicolinic acid and calcium, whereas these substances are undetectable in vegetative cell, the spore germinate to produce a single vegetative cell.

Stages on endospores formation:

1- DNA of mother cell condenses.

2- Transverse wall begins to form.

3- Spore material separated; formation of forespore at one end of a cell.

4- Vegetative cell grows around spore.

5- Spore forms multilayered coating.

6- Cell lysis free spore.

Mature spore is completed in 6-8 h.

Spore integument consists of the following layers:

i- Innermost layer in germ cell membrane.

ii- Outer layer is densely stained called coat.

iii- Between the inner and outer layers there is laid down a thick shell or cortex.

iv- Delicate layer found in some species called exosporium.

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Layers of spore integument:

Core : protoplast, DNA, Enzymes

The resistant to dehydration is belonged to the presence of calcium dipicolinate (5-15%).

Thus the spore resist heat.

- The spore wall (the inner most layer) consists of peptidoglycan.
- cortex : unusual type of peptidoglycan less cross linked, sensitive to lysozyme
- coat : Keratin like protein with many S-S bonds, resist to antibiotics
- exosporium : lipoprotein membrane

Origin

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Growth and multiplication

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— Growth means an increase in size, numbers, weight, and mass.

— Reproduction means an increase in individual number. Cell growth is a group of reactions and events led to an increase the macromolecules number and then cell division and reproduction.

Cell cycle

A group of steadily successive events are interrupted with periods which depending on environmental conditions. The required time from the beginning to the end of division known as generation time and the resulting growth called growth rate.

Eukaryotic cell cycle

It includes several stages:

i- First stage:

A-It is the period that is preceded the multiplication of DNA. It is called first gap (G₁).

B- It constitutes 50% of generation time.

C-In it the cell is preparing for DNA multiplication.

D-It depends on environmental conditions, since it short in optimal conditions that result in shortening the generation time.

ii- Second stage:

A-It is the period in which the DNA is synthesized. It is abbreviated as S.

B-It constitutes 20-25% of generation time.

C-It does not depend too much on environmental conditions.

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iii- Third stage:

A-It the period in which the precursors of mitosis spindle and cytoplasmic division is synthesized, it referred to as G_2

B-It constitutes about 25% of generation time.

C-The environmental conditions do not greatly affect it.

iv- Fourth stage:

A-Mitosis takes place, referred to as M .

B-It constitutes about 5% of generation time.

C-Separation of the two daughter cells.

Prokaryotic cell cycle

Most of studies on prokaryotic cell cycle were done on *E. coli* because of it is easy to handle. Prokaryotic cell cycle includes:

i- First stage

This period is still under speculating. Mostly, under the optimal conditions it disappears due to the shortage of generation time.

Also the environmental conditions greatly affect the cell.

ii- Second stage

A-A stage of DNA synthesis abbreviated as C instead of S , it means chromosome replication.

B-It required most of cycle time.

C-It controls the continuity of the cycle, since when the DNA synthesis is interrupted the cell will not divide. Sometimes

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it is possible to prevent cell division (by adding penicillin to the medium) without inhibiting the DNA synthesis.

D-It is affected, a little bit, by the environmental conditions.

iii Third and fourth stages

A-After the DNA synthesis stage, there is a gap before the cell is dividing into two daughter cells.

B-It represents both third and fourth stages, G_2 and M .

C-It referred as to D .

D-It is affected, a little bit, by the environmental conditions.

N.B. it possible to control the bacterial cell division period or its generation time by alteration the environmental conditions such as: nutrients concentration, pH, O_2 , temperature, and others.

Methods of cell cycle study

- 1- Cultures are used to study the cell cycle.
- 2- In these cultures, there are cells at different cell cycle stages in the same culture.
- 3- For a study, it is preferable that all culture cells being at one stage.
- 4- A synchronous culture should be chosen for a study, since all culture cells are at the same growth phase. (multiplying at one time)

Methods of synchronous growth obtaining

To obtain a synchronous growth all its cells are multiplying at one time, it should be chose one of the following:

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1- Induction methods

It depends on the chemical or physical treatment of the cells' environment.

2- choosing methods

they depend on physical dimensions of the cell, since the daughter cell has the smallest size. It can be obtain the cells by:

A- Using microbial filter that pass the medium and hold the cells.

B- Using density gradient method. A 30% density gradient sucrose solution is used, thereafter the cells are centrifuged, and so the small size cell will be separated from the big ones in the sugar solution according to their sizes.

Batch culture

When liquid media is inoculated with bacteria (the nutrients are expended and metabolic products accumulate in the closed environment) so that the normal bacterial growth curve is a characteristic of the batch culture.

Growth curve of bacteria

When a bacteria are inoculated into a new culture media it shows a characteristic growth curve which has four phases:

1- Lag phase

During this phase bacteria exhibit growth in size but no increase in cell number and the bacteria are preparing for synthesis of DNA, various enzymes, and other components, which are needed for cell division. The lag phase varies in length with the condition of the

microorganism and the nature of the media this mean that the phase may be long if the inoculum is from an old culture or if the culture is refrigerated.

2- Logarithmic (exponential) phase

During this period the cells divide steadily at a constant rate. The log of the number of cells is plotted against time results in a straight line. Under appropriate conditions the growth rate is maximal during this phase, and the population is most nearly uniform in terms of chemical composition of cells, metabolic activity and other physiological characteristics.

3- Stationary phase

During this phase the growth rate is equal to the death rate. Food begins to run out; poisonous waste products accumulate, pH changes, hydrogen acceptors are used up, energy transfers are diminished. The rate of fission begins to decline, and the organisms die in increasing numbers.

4- Death (decline) phase

Eventually the number of viable bacterial cells begins to decline, signaling the onset of the Death phase. The kinetics of bacterial death, like those of growth, are exponential. The cells reproduce more slowly and death overtakes them in ever-increasing numbers. Environmental conditions interfere within this phase.

Continuous culture:

Bacteria may also be grown in continuous culture where nutrients are supplied and end products and end products removed continuously so that the logarithmic growth phase is maintained and the bacteria never reach

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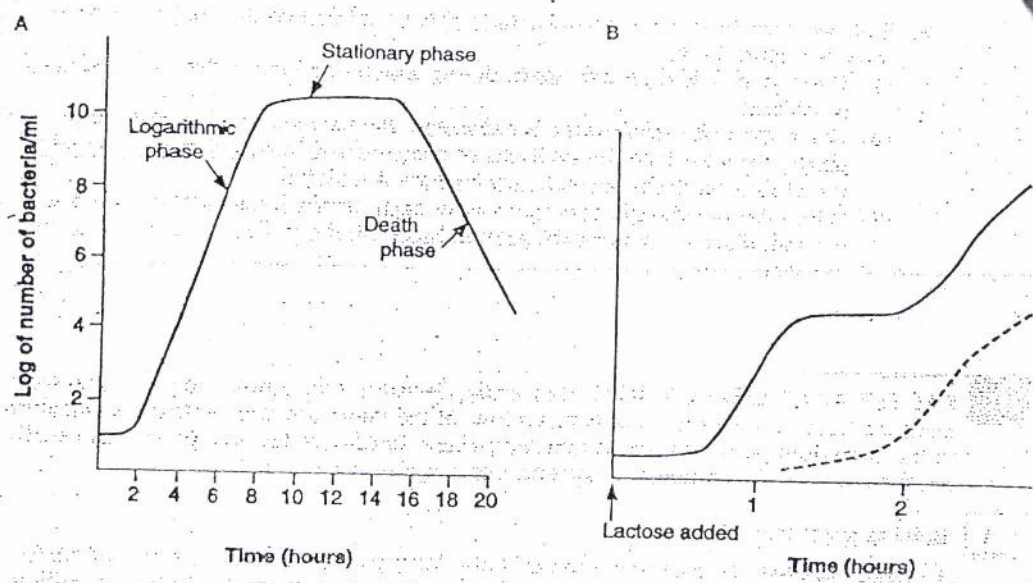


FIGURE (A) Bacterial growth curve. (B) Biphasic growth curve of an organism that may use both glucose and lactose as energy sources. The **bold line** represents the optical density of the suspension, which is indicative of the number of bacteria present in the culture. The **dashed line** represents the breakdown of substrate, which is indicative of the relative activity of β -galactosidase.

stationary phase because liquid medium is continuously fed into the bacterial culture and this can be done by using:

1- Chemostat:

Apparatus is arranged so that there is slow, but adjustable, admixture of new and nutrient fluid (containing a limiting conditions) of some growth controlling micronutrients. In the presence of the limiting micronutrients, exponential growth proceeds, as concentration of the limiting micronutrients approaches exhaustion, growth slows. By a concomitant and equal removal of old medium with its accumulation of toxic metabolic products and older and dead cells, a constant volume of culture, a constant volume of culture, concentration of micronutrients and number of exponential growing cell is maintained in the main culture.

2- Turbidostat:

(growth & removal of cells in main culture)
(Constant electronic monitoring of changes in turbidity) due to growth or removal of cells in the main culture vessel maintains the steady state of the culture. Changes in turbidity retard or increase passage of light through the culture. These changes activate mechanisms that control the flow of nutrients into it, and flow of waste out of the main culture vessel.

Growth rate and generation time:

Generation time (doubling time): the time for a single cell to undergo fission.

It takes short time in prokaryotes (e.g. 20-25 min. in *E. coli*). While in eukaryotes it takes long time.

Generation time varies with:

- 1- Species of microorganism.
- 2- Nutrients.
- 3- Environmental conditions: pH, and temperature.
- 4- Growth phase.

Number of microorganisms increased exponentially, i.e. one cell will become two cells after one (gt), and after two gt it will become four cells:

$$2^0 - 2^1 - 2^2 - 2^3 - 2^4 \text{ ----- } 2^f$$

$$f = \frac{\log_{10} C^2 - \log_{10} C^1}{\log_{10} 2}$$

f = generations number

C¹ = cells number in the first time

C² = cells number in the second time

For example:

$$\begin{aligned} f &= \log_{10} (800) - \log_{10} (100) / 0.3 \\ &= 2.4 - 2 / 0.3 \\ &= 3 \end{aligned}$$

$$\text{Growth rate (gr)} = f / t$$

Enumeration of bacteria

In order to observe microbial reproduction rates it is necessary to determine numbers of microorganisms. There are different methods that can be employed which include:

1- Viable plate count

In this method ~~serial dilution~~ of a serial dilution of a suspension of bacteria are prepared. The suspension is spread over the surface of the agar, or mixed with the agar before solidify and pouring into the plate. Multiplication of bacteria on solid medium results in the formation of microscopic colony visible to eye.

2- Direct microscopic count

Cells can be counted in a stained smear as in the Breed method, or they counted by the Petroff-Haüsser counting chamber (haemocytometer).

In these two methods it is so impossible to differentiate living from non-living bacteria.