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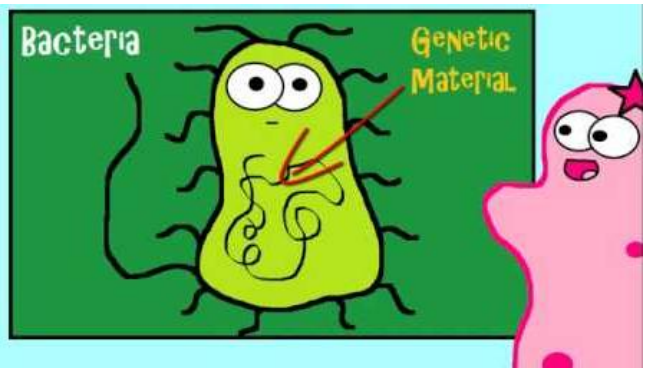

General Bacteriology

By

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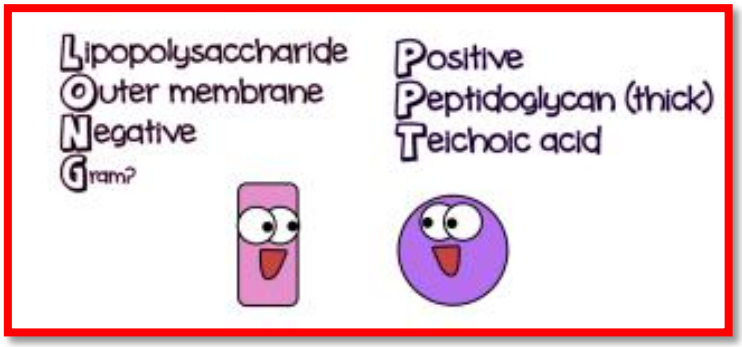
For

The Second Stage



Biology Department
College of Science
University of Baghdad
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General Bacteriology

Introduction

We may define microbiology as the study of organisms too small to be seen clearly as individuals by the unaided human eye. It deals with structure, morphology, and the relationships between microorganisms and other organisms, importance of microorganisms, as well as control methods. Such organisms include: Bacteria, Fungi, Viruses, Protozoa and Algae .

In general, any organism has a diameter of less than 1 mm will be considered as microorganism.

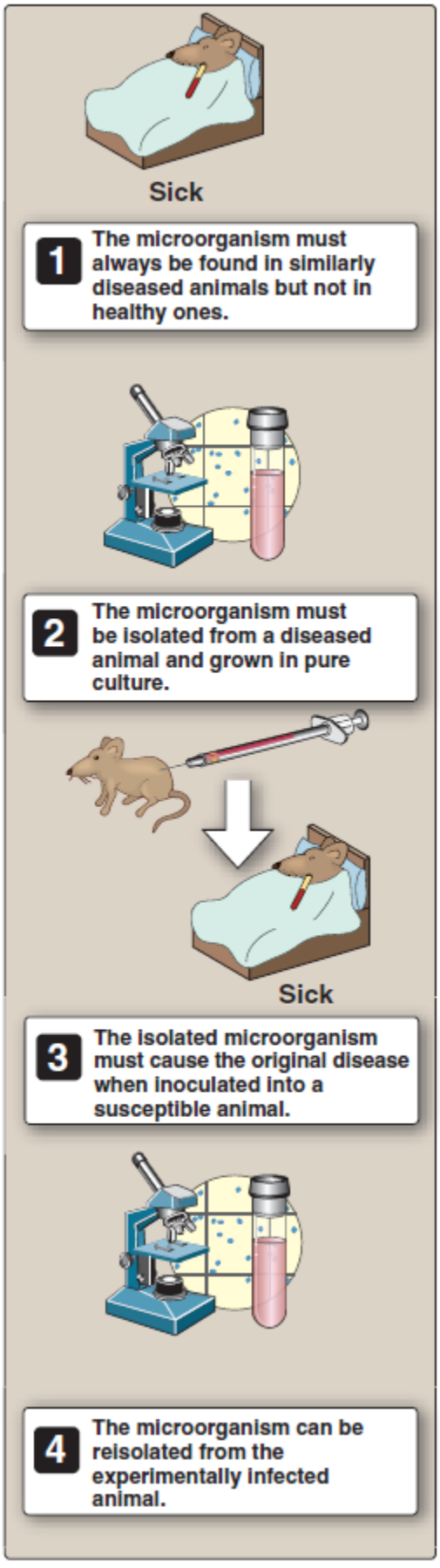
Although microbiology dawn in the end of 19th century, many civilizations like Mesopotamia and Egypt dealt with such organism without seeing them. They used some of them to produce wine without knowing their role in the fermentation process, also they tried to cure disease caused by those microorganisms by medicinal plants. Furthermore, they used to protect their food from spoilage by salting or drying without knowing the causes of this spoilage. Bacteriology: is the branch and specialty of biology that studies the morphology, ecology, genetics and biochemistry of bacteria as well as many other aspects related to them. This subdivision of microbiology involves the identification, classification, and characterization of bacterial species. Because of the similarity of thinking and working with microorganisms other than bacteria, such as protozoa, fungi, and viruses, there has been a tendency for the field of bacteriology to extend as microbiology.

Beginning of microscopy

The very best unaided human eyes fail to see object less than 100 µm in diameter. Nor can the eye clearly perceive as separate objects (i.e. resolve) particles separated by distances less than this. Microorganisms

range downward in diameter from the ca. 50 µm in diameter of animal tissue cells to the diameters of bacteria (1-5 µm) and those of viruses (ca. 0.25 µm). They are beyond the unaided eye ability. Louis Pasteur (1822–1895) was a French biologist, microbiologist and chemist renowned for his discoveries of the principles of vaccination, microbial fermentation and pasteurization. He is remembered for his remarkable breakthroughs in the causes and prevention of diseases, and his discoveries have saved many lives ever since. He reduced mortality from puerperal fever, and created the first vaccines for rabies and anthrax. His medical discoveries provided direct support for **the germ theory of disease** and its application in clinical medicine. He is best known to the general public for his invention of the technique of treating milk and wine to stop bacterial contamination, a process now called **pasteurization**. He is popularly known as the "**father of microbiology"**

In 1884, **Robert Koch** proposed a series of postulates that have been applied broadly to link many specific bacterial species with particular diseases known as **Koch's postulates:**



1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

2. The microorganism must be isolated from a diseased organism and grown in pure culture.

3. The cultured microorganism should cause disease when introduced into a healthy organism.

4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch's postulates played a role into identifying the relationships between bacteria and specific diseases. Since then, bacteriology has had many successful advances like effective vaccines, for example, diphtheria toxoid and tetanus toxoid. Bacteriology has also provided discovery of antibiotics.

Structure of bacteria

Morphology

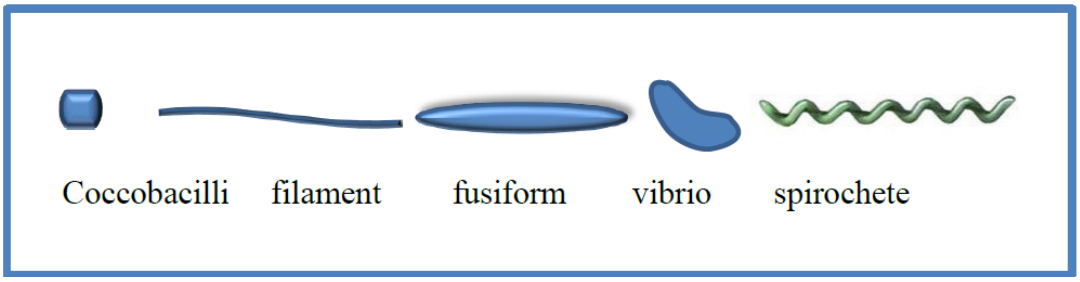
Bacteria are the smallest organisms that all machinery required for growth and self-replication, their diameter is usually about 1 µm.

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 **tetrads** or cubical packets are known as **sarcina**.

	Coccus (single-celled)
	Diplococci (occur in pairs)
	Tetrad (group of 4 cocci)
	Sarcina (cube-like shape)
	Streptococci (chain-like morphology)
	Staphylococci (grape-like cluster)

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** or **spirochete**.



Arrangement of bacilli

In 1981, **square** bacteria had been discovered; they 2-4 µm in

diameter, halophilic (Archaebacteria), 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**.

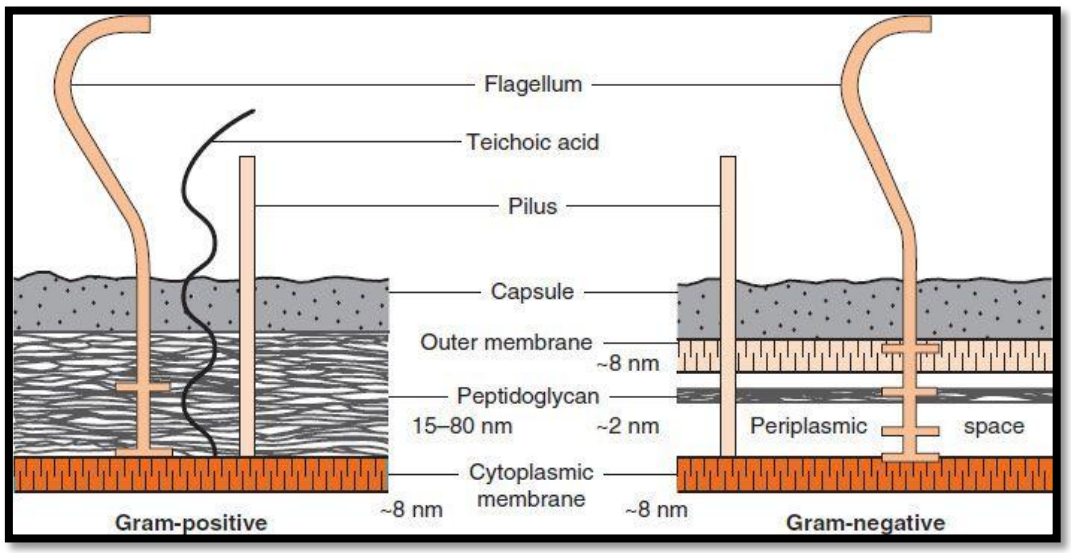
Extracellular polysaccharides

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 polysaccharides which is also called **glycocalyx**. When the glycocalyx forms a condensed well defined layer closely surrounding the cell, it is called **capsule**; when it forms masses of polymers are formed that appear to be totally detached from the cell in which cells may be entrapped, in these instances the extracellular polymers may be referred to simply as a **slime layer**.

The glycocalyx layer contributes to the invasiveness of pathogenic bacteria in protecting them from phagocytosis. Furthermore, it plays a role in the adherence of bacteria to surfaces in their environment, including the cells of plant and animal hosts. A **biofilm** is an aggregate of interactive bacteria attached to a solid surface or to each other. Biofilms are important in human infections that are persistent and difficult to treat.

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 acids, and polysaccharides. While in Gram negative bacteria, the cell wall includes the peptidoglycan, outer membrane, lipopolysaccharide (LPS), and lipoprotein.



Gram positive and Gram negative cell wall

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.

4- Lipopolysaccharide is responsible for the endotoxin activity.

Chemical composition of the cell wall

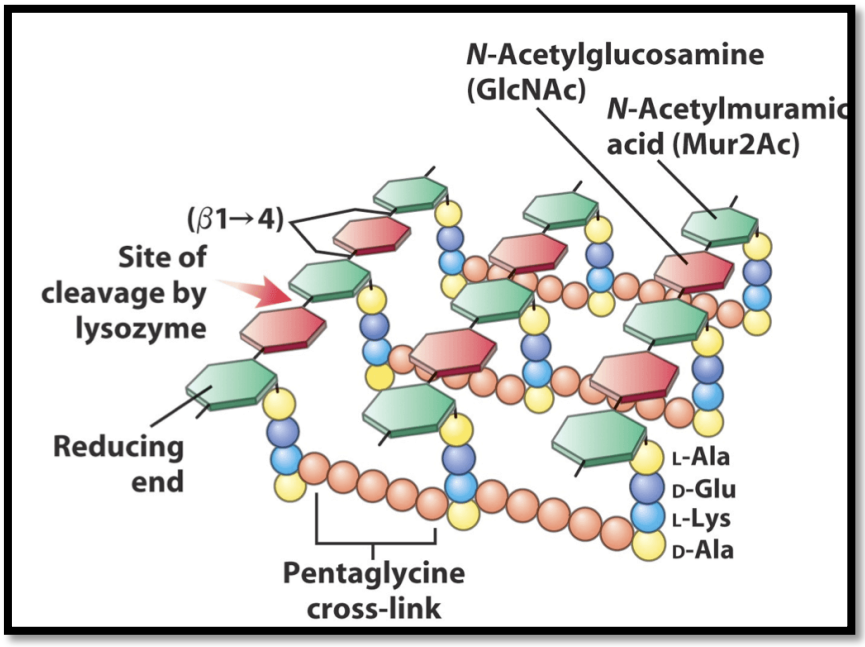
A- The peptidoglycan layer

It is a complex polymer consisting or 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 NH2 of neighboring tetrapeptide).

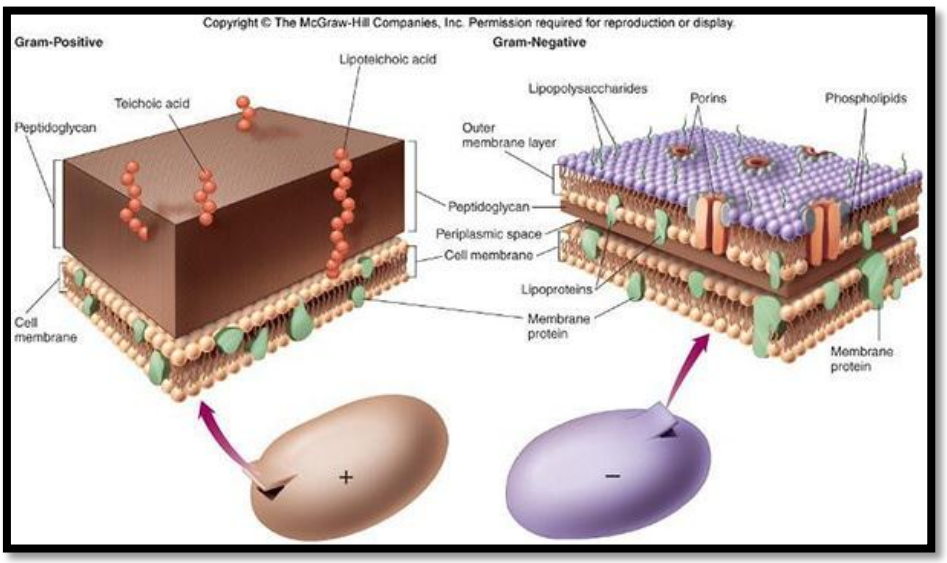


Peptidoglycan structure

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

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

1- 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 **lipoteichoic acid** (membrane teichoic acid), covalently linked to membrane glycolipid and concentrated in mesosome. Some Gram positive species lack wall teichoic acid but all appears to contain lipoteichoic acid. The function of teichoic acids is still a matter of speculation:

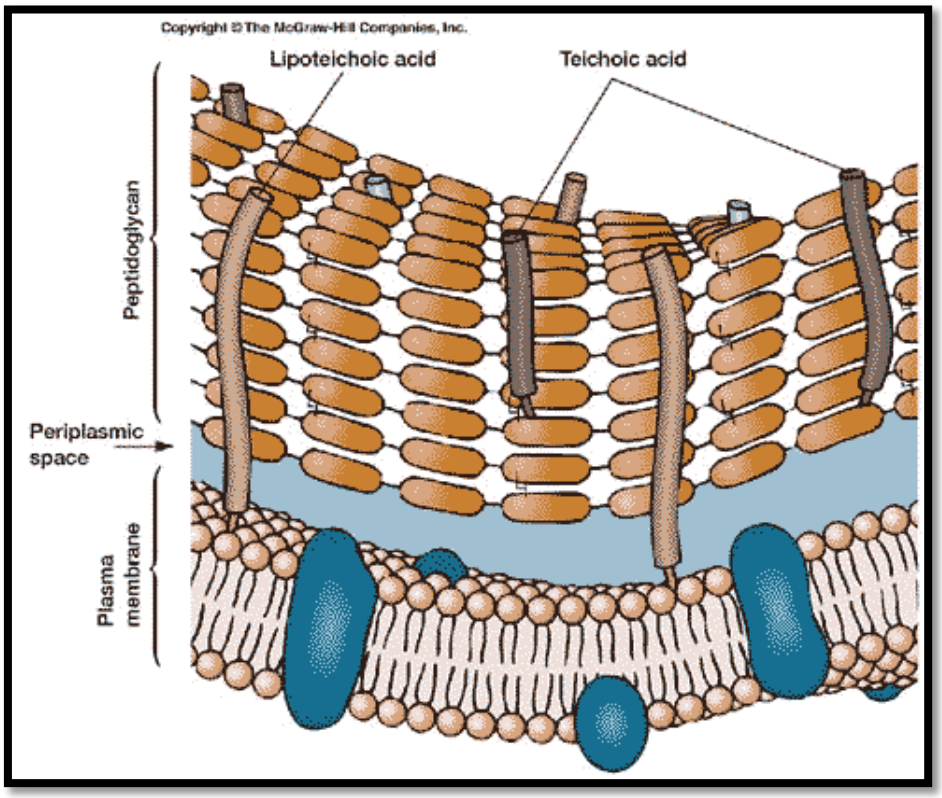
a. The main function of teichoic acids is to provide rigidity to the cell wall by attracting cations such as magnesium and sodium.

b. Teichoic acids provide an external permeability barrier to Gram positive bacteria.

c. Limiting the ability of autolysins to break the β (1-4) bond between the *N*-acetyl glucosamine and the *N*-acetylmuramic acid.

d. They have role in cell elongation and division.

e. Functions in biofilm formation and adhesion.



2- 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.

3- Polysaccharides

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

C- Special components of Gram negative cell wall

1- 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.

2- 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**:

a. Prevents leakage of periplasmic space proteins.

b. Protects the enteric bacteria from bile salts and hydrolytic enzymes.

c. Contains the minor proteins, which are involved in the transport of specific molecules such as vitamin B12 and iron-siderophore complexes.

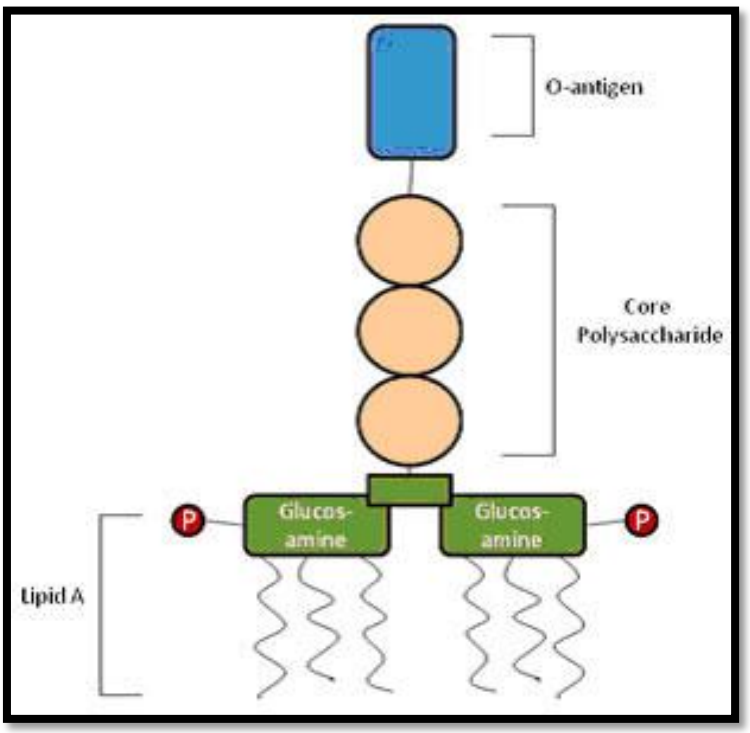
d. Has a special channels, consisting of proteins called **porins** that permit the passive diffusion of low molecular weight hydrophilic compounds like sugars, amino acids, and certain ions.

e. Contains numbers of enzymes like proteases and phospholipases.

3- Lipopolysaccharide

The lipopolysaccharide of Gram negative 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 (O antigen). Lipopolysaccharide is attached to the outer membrane by hydrophobic bound.



Lipopolysaccharides structure

The function of lipopolysaccharide:

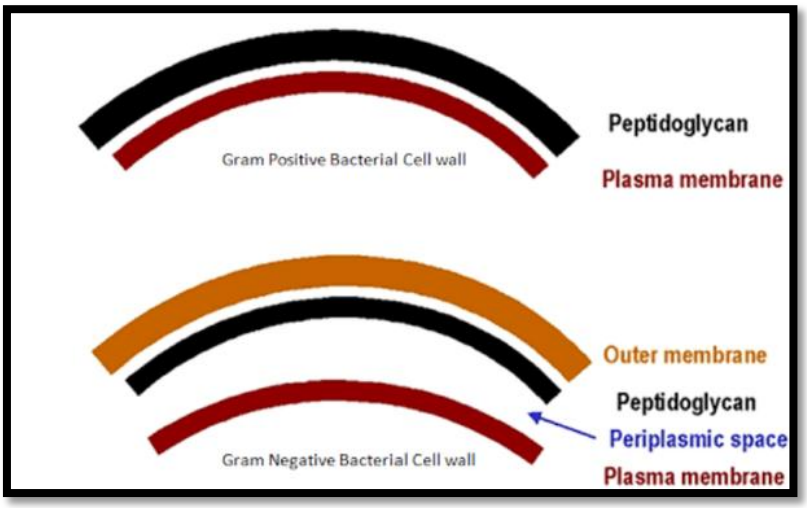
a) Stabilizes the membrane and provides a barrier to hydrophobic molecules.

b) Lipopolysaccharide, which is toxic to animals, has been called the endotoxin of Gram negative bacteria because it is 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.

c) Polysaccharide represents a major surface antigen of the bacterial cell so called O-antigen, and is responsible for the antigenic specificity.

The periplasmic space

The space between the cytoplasmic membrane and outer membrane, called the periplasmic space, contains the peptidoglycan 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.



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 are:

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.

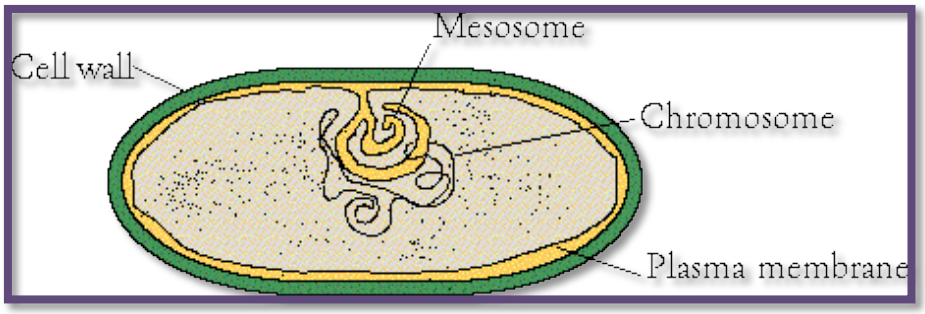
Differences between Gram positive and Gram negative bacteria

Characteristics	Gram Positive	Gram Nagative
Gram Reaction	Retain crystal violet dye and stain blue-purple	Can be decolorized to accept counterstain (Safranin) and stain pink-red
Cell wall thickness	20-30 nm	8-12
Cell wall	Smooth	Wavy
Peptidoglycan Layer	Thick (Multi-layered)	Thin (single layer= unilayer)
Teichoic acid	Present in many	Absent
Periplasmic space	Absent	Present
Outer membrane	Absent	Present
Porins	Absent	Occurs in outer membrane
Lipopolysaccharide (LPS) content	None	High
Lipid and lipoprotein content	Low (Acid fast bacteria have lipids linked to peptidoglycan	High (Presence of outer membrane)
Toxins	Exotoxins	Exotoxins & Endotoxin
Resistance to drying	High	Low

Cytoplasmic Ultra-structures

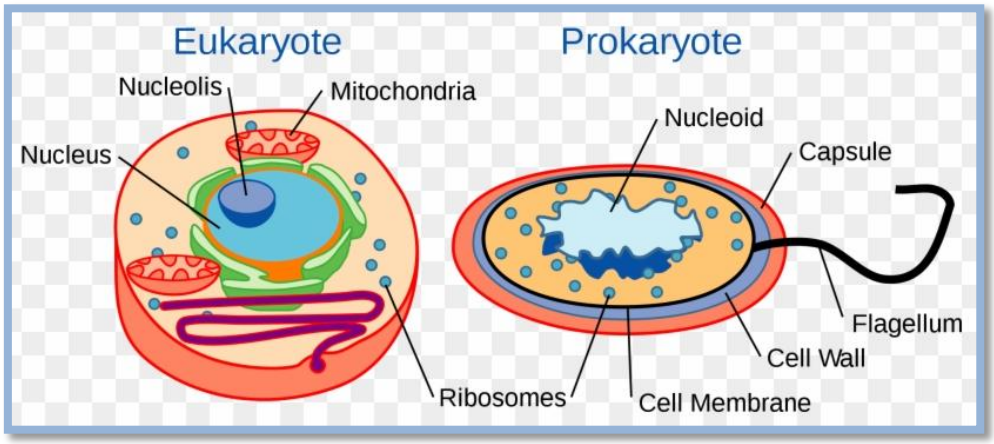
1- Mesosomes

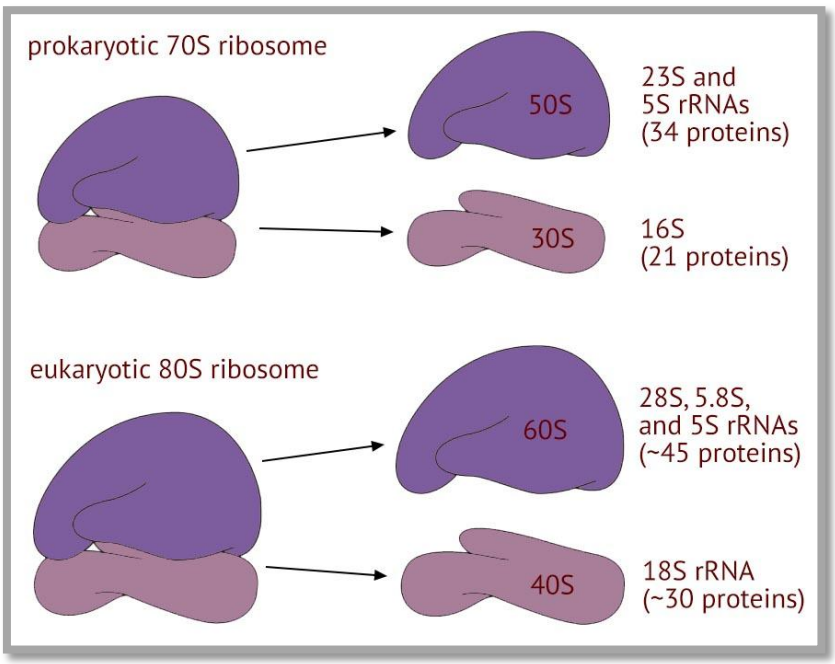
One or more large irregular invaginations of the plasma membrane often are seen in the thin section of bacteria, called mesosomes. There are two types of them, septal mesosome and lateral mesosome. Septal mesosomes function in the formation of a transverse cell membrane during cell division and always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



2- Ribosomes

Spherical densely stained objects mostly grouping in chain called polysomes. The ribosomes are designating according to their sedimentation coefficient as 70S (Svedberg unit = 10-13 cm/sec) in prokaryotes and 80S in eukaryotes. Ribosomes are responsible for the synthesis of proteins.





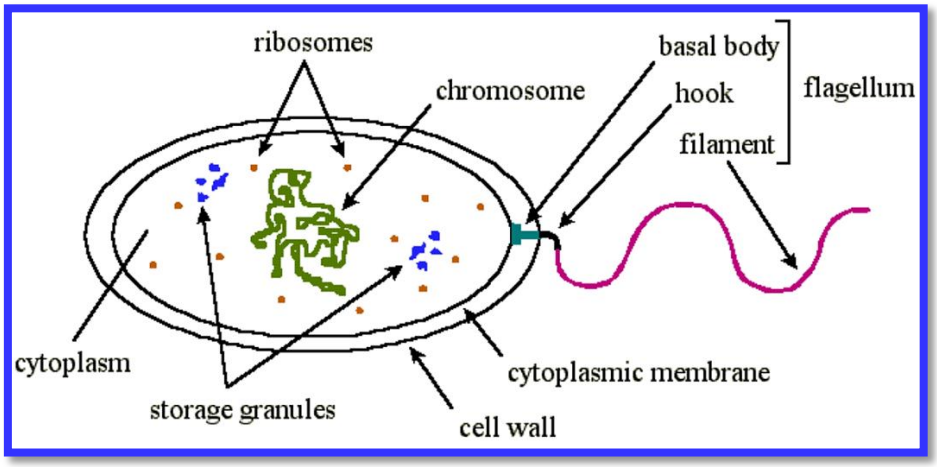
3- Granular inclusion

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.

ii- VOLUTIN: is metachromatic granules especially rich in organic phosphate.

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



4- Nuclear body (Nucleoid)

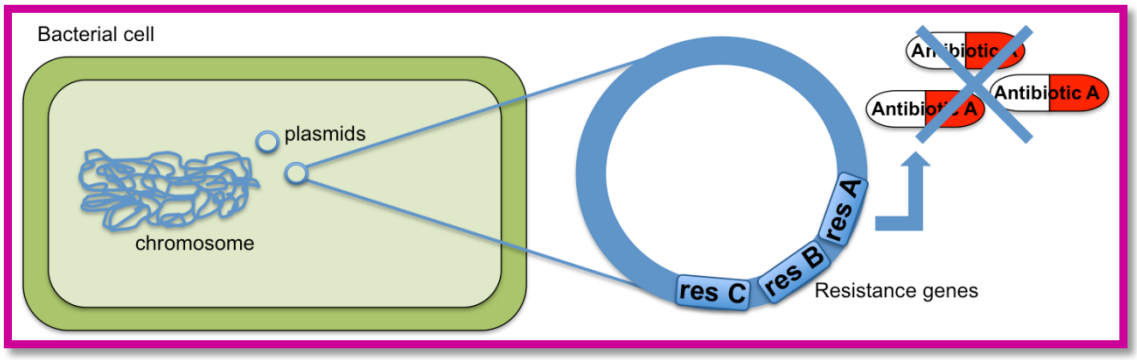
The nucleoid in prokaryotes does not enclosed by a membrane. On contrary, in eukaryotes it is surrounded by the nuclear membrane.

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.

Plasmids

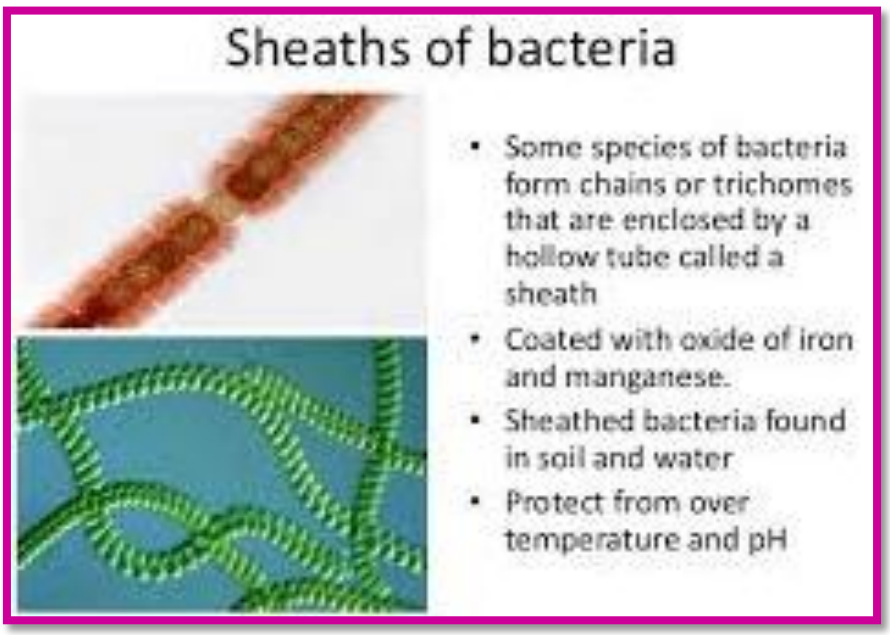
In addition to the bacterial chromosome, bacteria may contain one or more small, circular macromolecules of DNA known as plasmids. Plasmids contain specific genetic information such as mating capabilities, resistance to antibiotics, production of toxins, and tolerance to toxic metals.



Sheaths

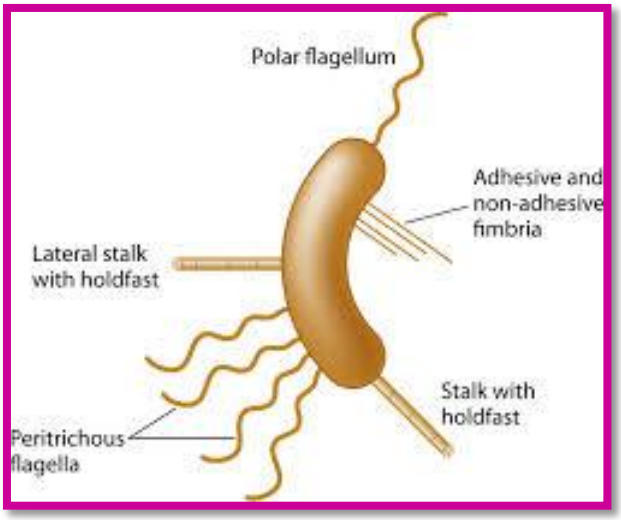
It is a filamentous or tubule structure enables bacteria to attach to solid surfaces. These sheaths afford protection

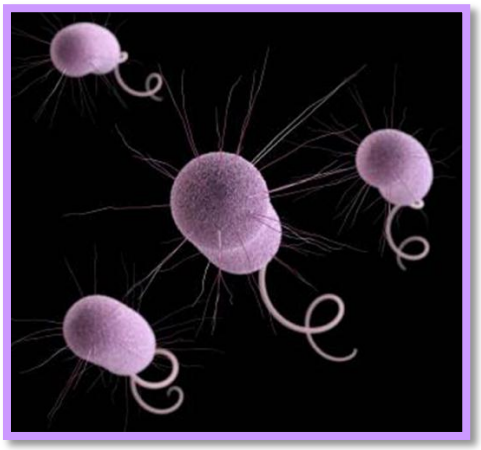
against predators and parasites. In some cases, they may be covered with metal oxides such as iron or manganese oxides.



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'susually 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, which confers a specific antigenicity.

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 used in classification of bacteria.

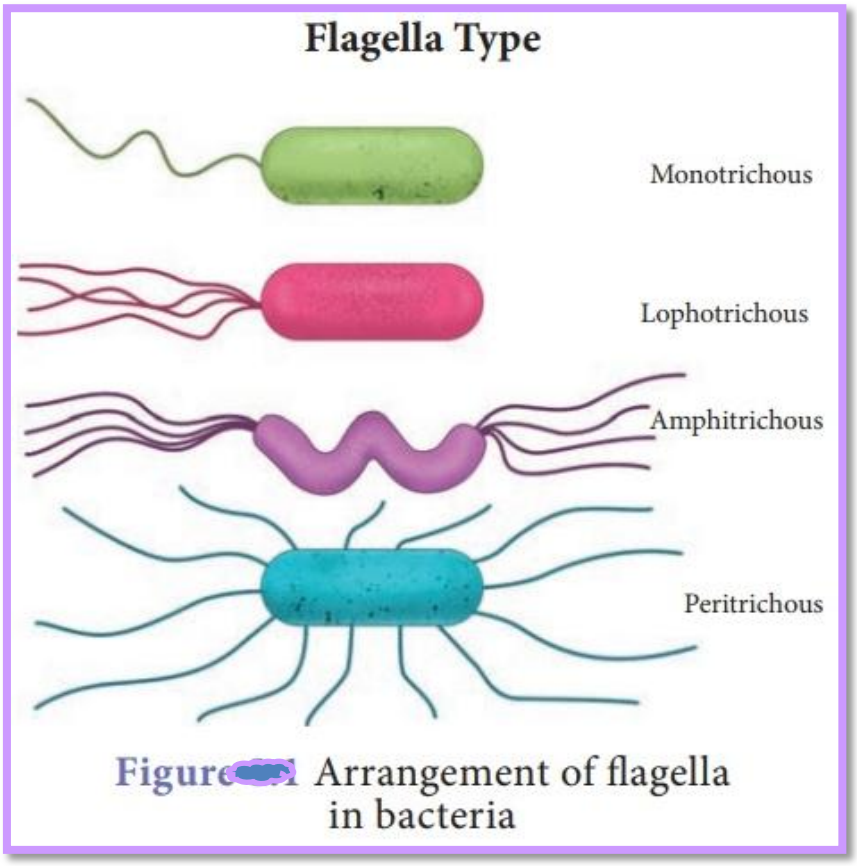
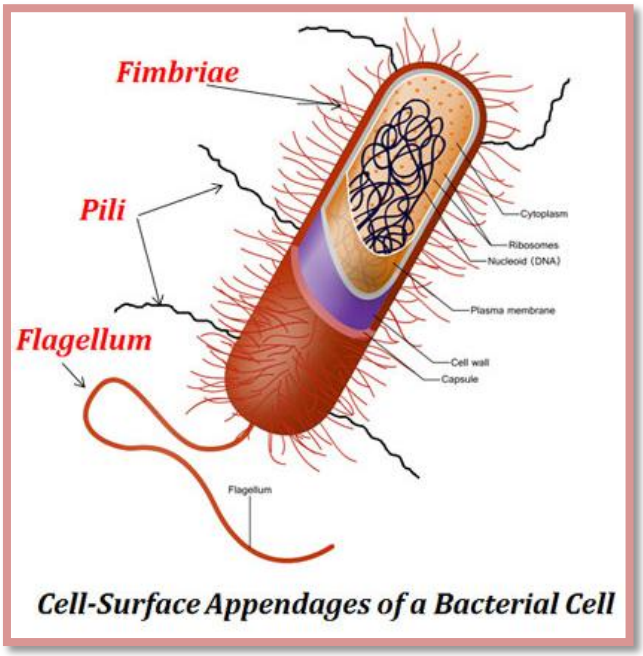


Figure 3: Arrangement of flagella in bacteria

Pili

Pili are short, thin, straight, hair like projections that emanate from the surface of some bacteria and are involved in attachment processes. Pili shorter and finer than the flagella. Pili are composed of structural protein pilins. Two classes of pili can be distinguished: ordinary pili, which play a role in the adherence of 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.

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.



Cell-Surface Appendages of a Bacterial Cell

Spores

Under conditions of limitation in supply of carbon, nitrogen, or phosphorus, (in a process known as sporulation) certain Gram positive aerobic *Bacillus* and anaerobic *Clostridium* 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. Spore integument consists of the following layers:

i- Inner membrane surrounds the core.

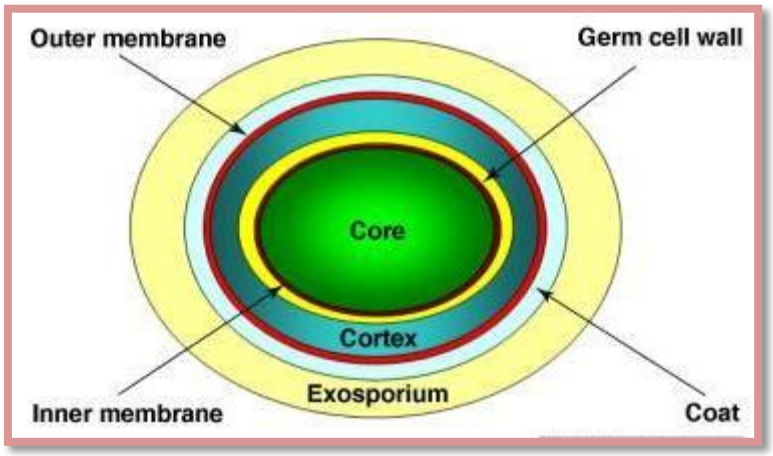
ii- Spore wall (germ cell wall) is the innermost layer surrounding the inner spore membrane.

iii- Cortex is the thickest layer of the spore envelope.

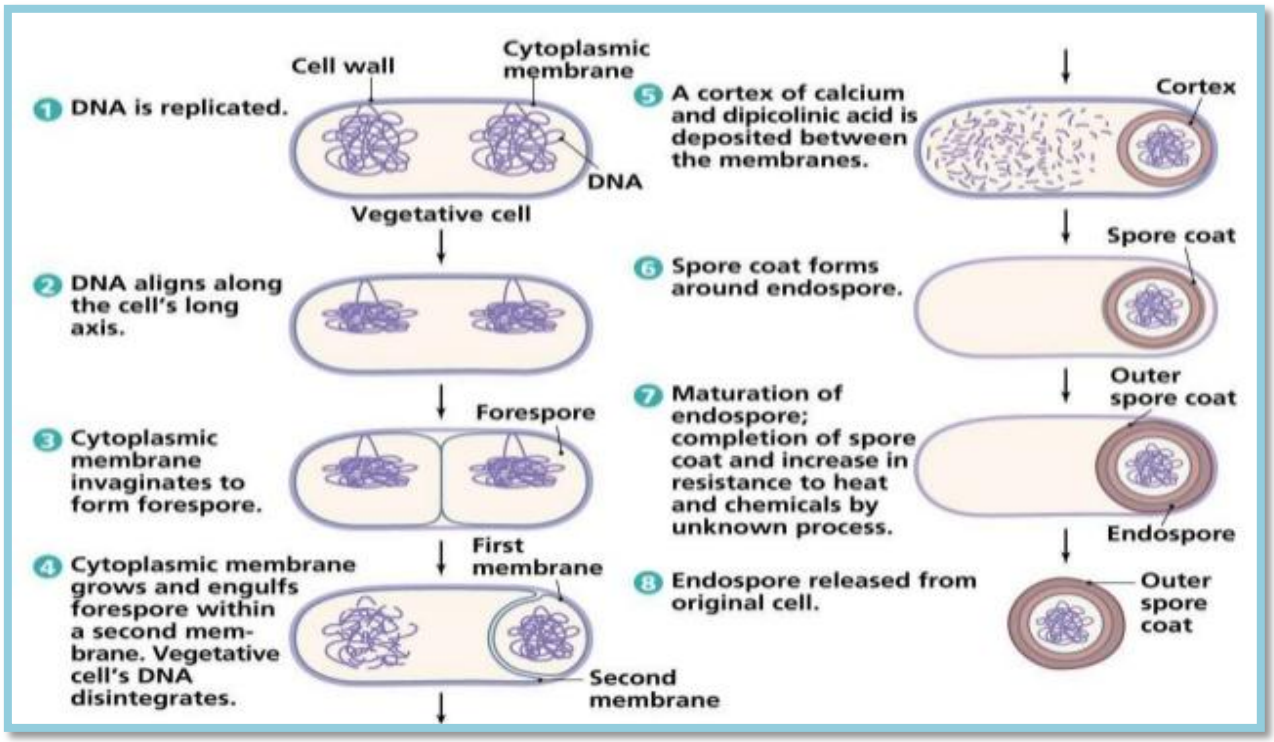
iv- Outer membrane layer is densely stained called coat.

v- Between the inner and outer layers there is a thick shell or cortex.

vi- Outermost layer found in some species called exosporium.



Stages of endospores formation, mature spore is completed in 6-8 h.



Microbial genetics

Nucleic acids types

The genetic information of prokaryotic and eukaryotic microorganisms encoded within the DNA (deoxyribonucleic acid) molecule and sometimes (as in viruses) in the RNA (ribonucleic acid) molecule. These molecules are known as macromolecules and they are responsible for the transition of hereditary information from one generation to the other. Another macromolecule found in the cell is the protein, which is the result of the genetic code into its structural or functional form.

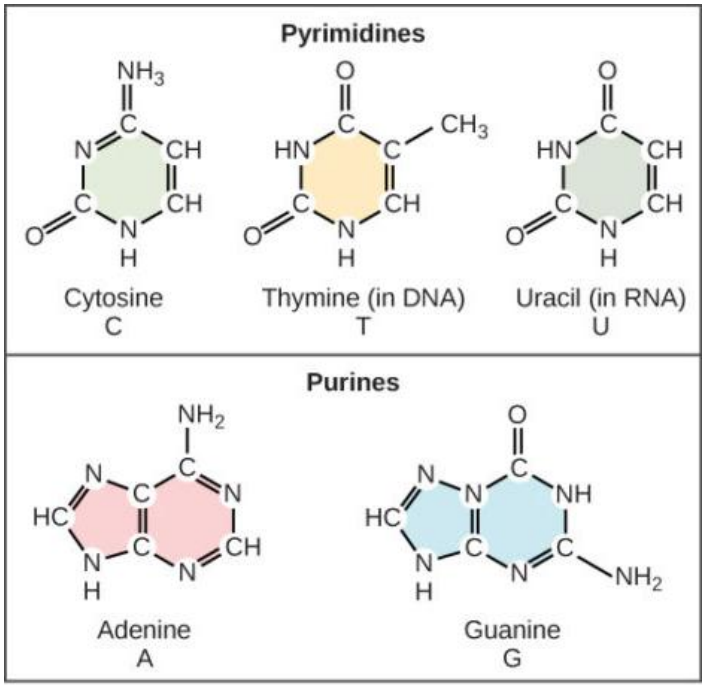
The structure of nucleic acids and their replication

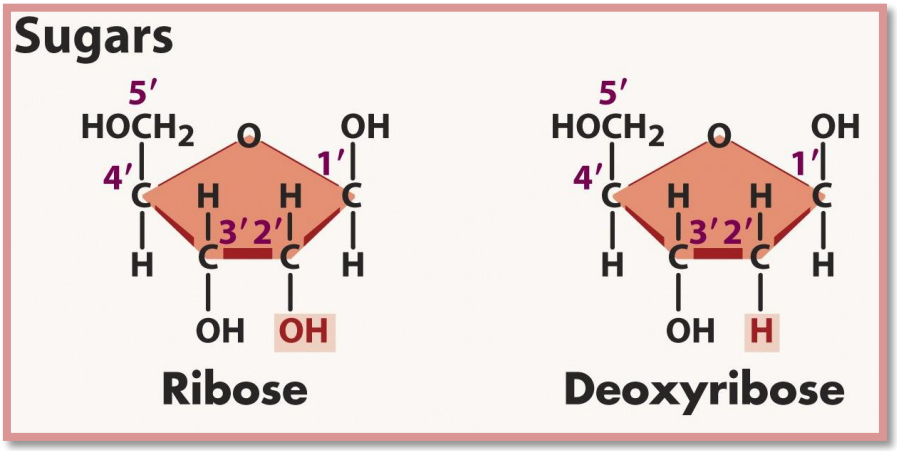
The genetic information of a cell forms a GENOME. The genome of a microorganism is divided into segments consisting of DNA nucleotides sequences known as a GENE. These genes may have structural or functional, metabolic functions .

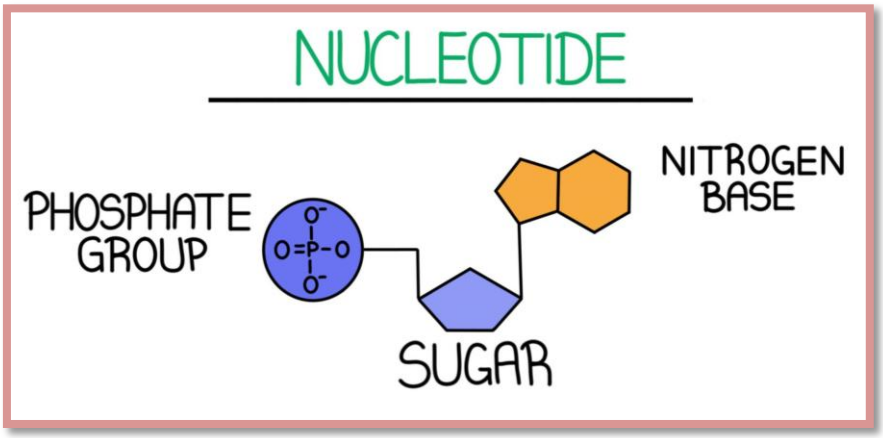
DNA structure

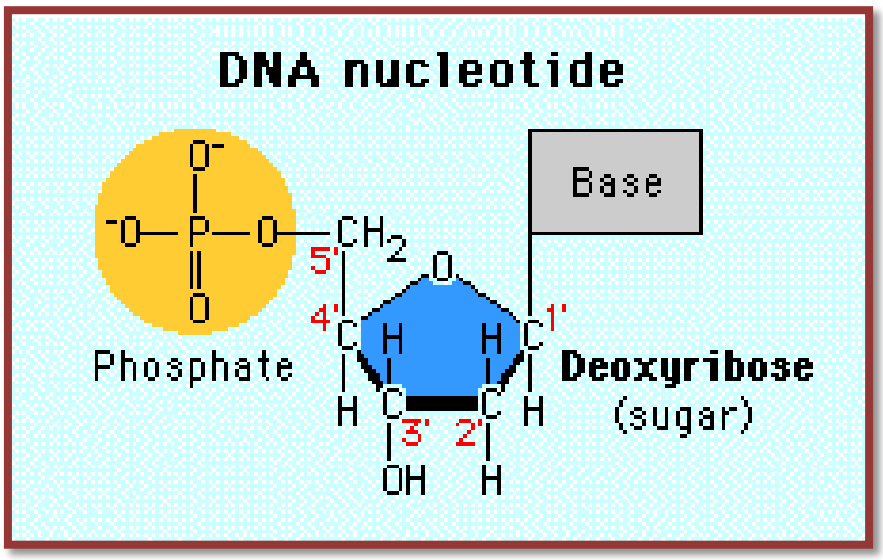
The DNA is a double helix where each strand is composed of a sequence of nucleotides; phosphodiester bonds link these nucleotides to each other. Each nucleotide is formed of **a deoxyribose sugar, a nitrogen base** and **a phosphate group**.

Four nitrogen bases are found in DNA: adenine (A), guanine (G), cytosine (C), and thymine (T). A and G are purines, while C, T and U are pyrimidines.


Pyrimidines
Cytosine
C
Thymine (in DNA)
T
Uracil (in RNA)
U
Purines
Adenine
A
Guanine
G


Sugars
Ribose
Deoxyribose


NUCLEOTIDE
PHOSPHATE GROUP
NITROGEN BASE
SUGAR



The primary structure of DNA

It is resembled by the sequence of nucleotides in a single strand. In this structure when the nitrogen base is bound to the sugar it is known as a **nucleoside**, when a phosphate group is linked to the nucleoside it is known as **nucleotide**.

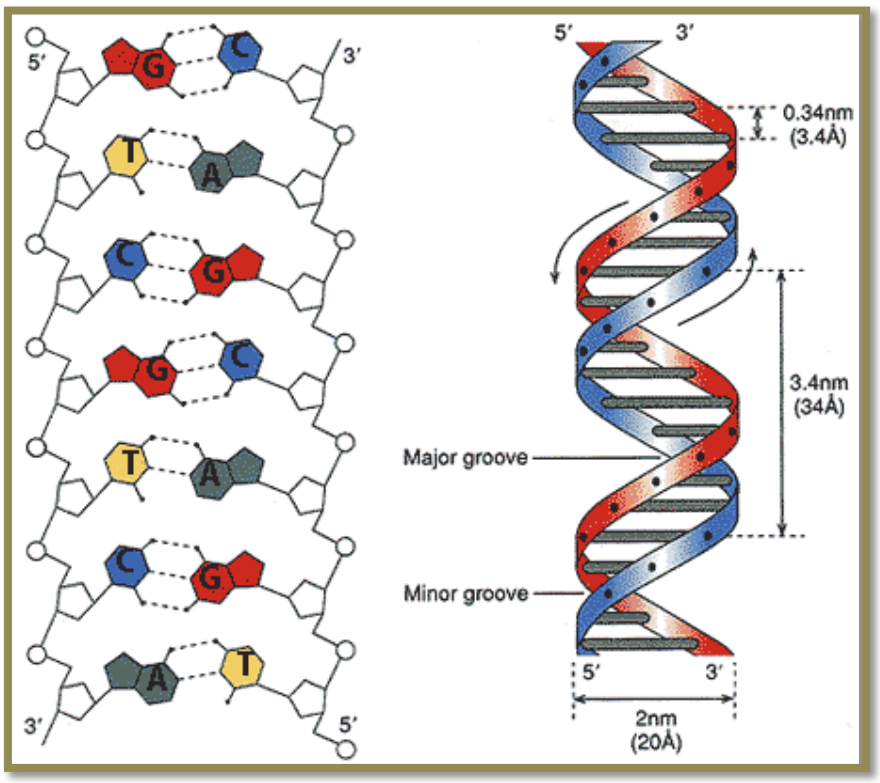
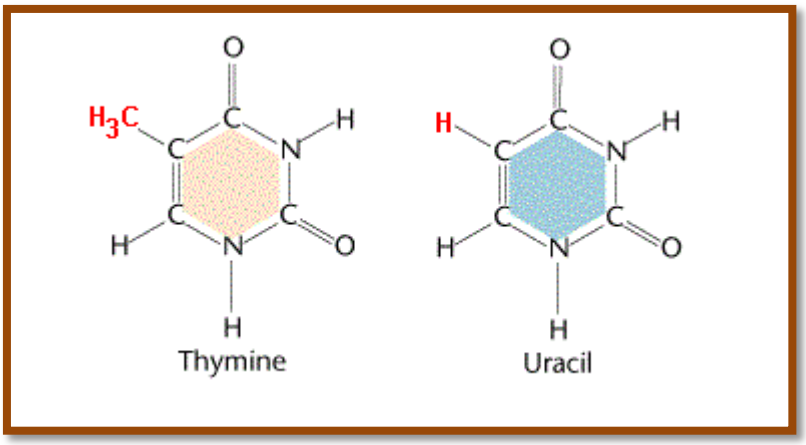


Figure: Secondary structure of DNA

A DNA molecule always carries a negative charge due to the PO4 groups. These charges are neutralized by alkaline proteins known as histones in eukaryotes, histone-like proteins in prokaryotes.

Structure of RNA molecule

An RNA molecule is usually single stranded; it has a sequence of ribonucleotides each is formed of a ribose sugar, a nitrogen base (A, G, C, and **Uracil (U) instead of thymine**), and a phosphate group. A ribonicleoside is formed of a **ribose** sugar and a nitrogen base.



DNA replication

DNA replicates when the cell is ready for division. Meselsen and Stahl first studied this process that proved experimentally using N15-labeled DNA that the DNA replication is semi-conservative.

The process of replication begins when the cell membrane is formed for the new cell; the chromosome is attached to the cell membrane before replication (in prokaryotes the chromosome is mostly circular except in some viruses it is linear) in eukaryotes DNA is linear.

The initiation stage in DNA replication is the unwinding of the double helix when each strand serves as a template. An enzyme known as primase is needed to form a primer (a small segment of

RNA) which serves as a leader to which the DNA nucleotides link and bind together in sequence.

The point where DNA starts unwinding is known as the **replicon** (or origin of replication) when the opened double helix with strands apart forms the replication fork. The enzyme that participate in unwinding the double helix at the replication fork are; DNA **gyrase** and helix destabilizing protein **helicase**.

Another protein takes part in holding the two strands apart it is the replication protein (single strand binding protein). The new strand will be complementary to the template strand and antiparallel.

The following step will be the elongation stage. In this stage the leading strand will be in the 5' 3' direction synthesis of DNA in this direction is continuous but for the lagging strand, which will be in the 3' 5' direction DNA synthesis is not possible so along this strand small DNA fragments of separate primers are synthesized in the 5' 3' direction, these are known as **Okazaki fragments**.

DNA synthesis in both strands requires **DNA polymerase III** to join the nucleotides 3'-OH-5'-PO4= and **DNA polymerase I** is required to remove the primers. The gaps are then filled with the proper nucleotides and joined by **ligase**.

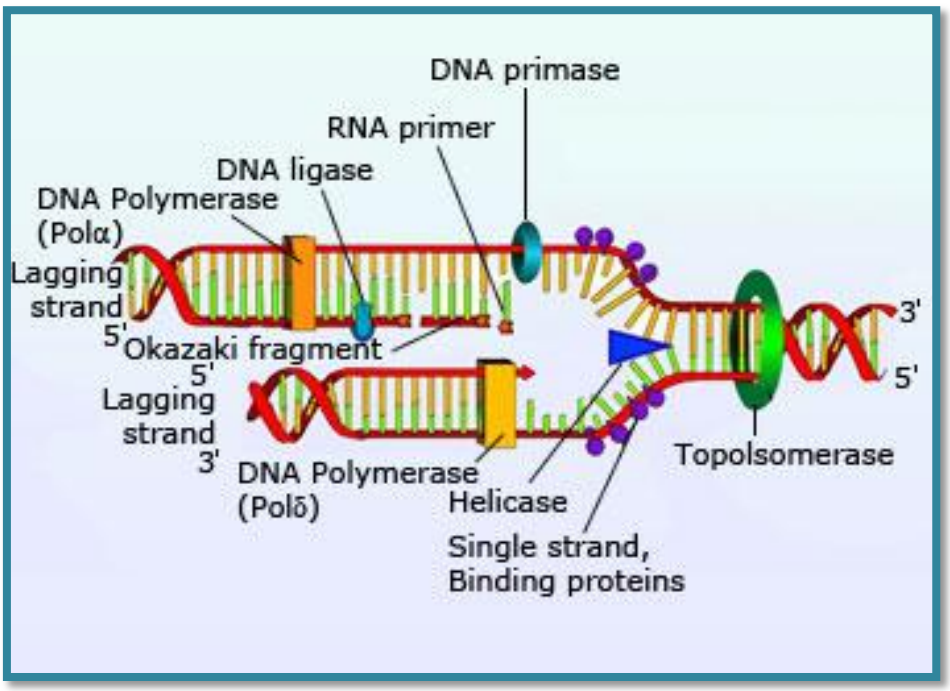


Figure: Semi-conservative DNA replication

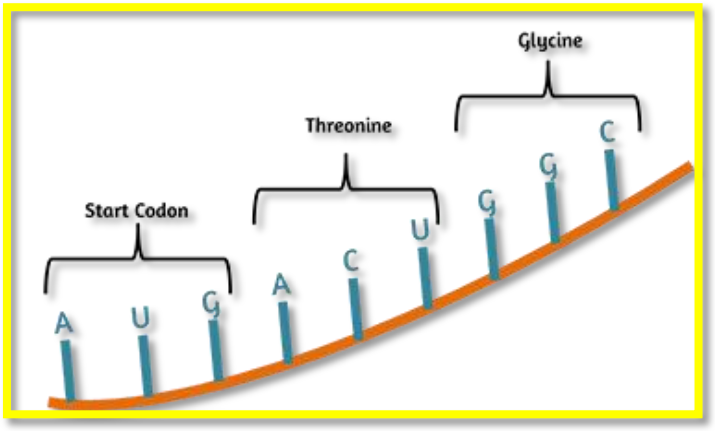
Types of RNA and steps in proteins synthesis

There are three types of RNA (mRNA, tRNA, and rRNA). Their roles will be described within the process of protein synthesis.

1-Messenger RNA (mRNA):

It is formed in the nucleus of eukaryotes and nuclear region of prokaryotes. It carries the information transcribed from the DNA to the ribosomes (in the cytoplasm) where protein is synthesized. It is transcribed from a single strand of DNA and is complementary to that strand.

mRNA is a single strand with a sequence of ribonucleotides to be translated by the ribosomes to the required protein.

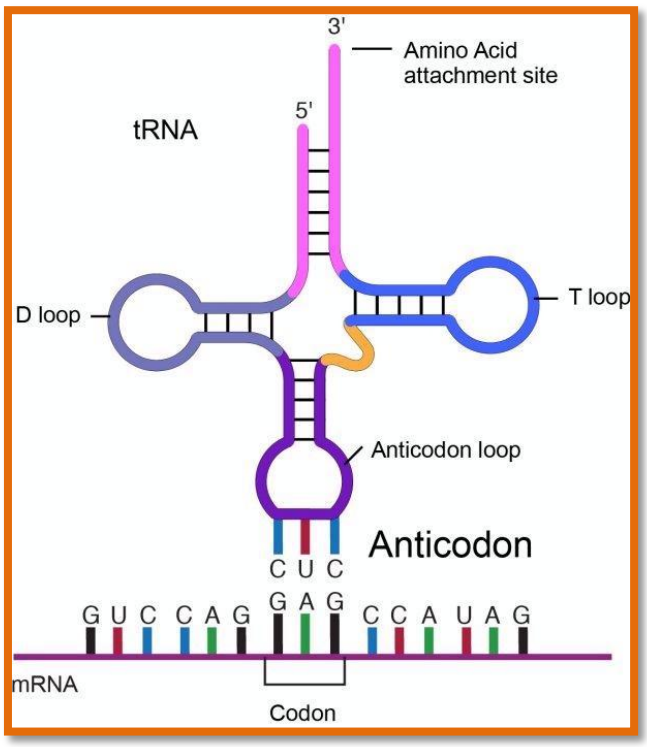


Transcription is a non-symmetric process since only one strand of DNA is transcribed (except in some viruses where DNA is single stranded by nature). mRNA is synthesized by a DNA-dependent RNA polymerase known also as **transcriptase**. Transcription is the first step in protein synthesis.

The second step in protein synthesis is translation; it requires the presence of the other two types of RNA; transfer RNA tRNA and ribosomal RNA (rRNA).

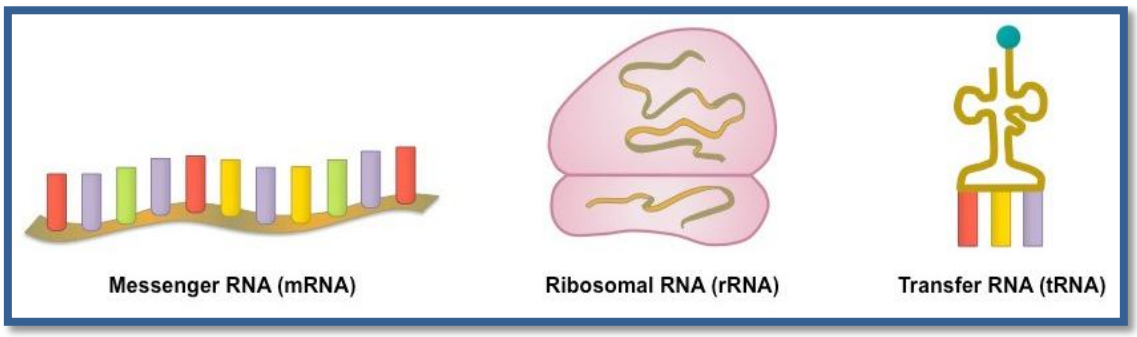
2-Transfer RNA (tRNA)

It is also known as soluble RNA has a distinguished clover leaf structure and two recognition sites; one binds to an activated amino acid, the second is known as the anticodon that recognizes the codon on the mRNA.



3- Ribosomal RNA (rRNA)

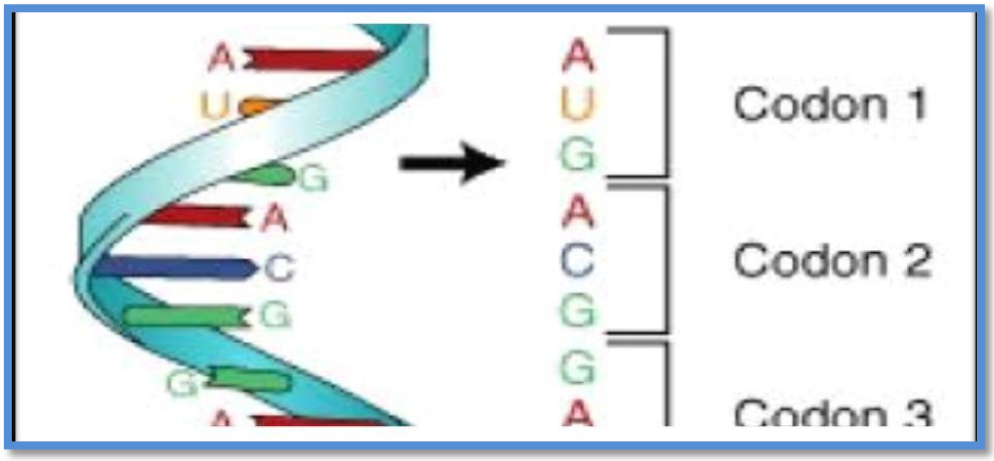
rRNA is a type of non-coding RNA that is a primary and permanent component of ribosomes. As non-coding RNA, rRNA itself is not translated into a protein, but it does provide a mechanism for decoding mRNA into amino acids and interacting with the tRNAs during translation by providing peptidyl transferase activity.



The genetic code

Every **codon** is made of three nucleotides coding for one amino acid, and since there are four nitrogen bases the probabilities of

the number of genetic codes are 43 = 64. There are 20 amino acids therefore there could be more than one code for most amino acids. For each amino acid there is one or more tRNA that carries the specific activated amino acid to the ribosome.



Protein synthesis

There are three stages in proteins synthesis:

1- **Activation**: when each amino acid is activated by a specific amino acyl synthetase. This is also known as the **initiation stage**. The starting signal is f-met (formyl-methionine). This stage requires If1, If2, and If3 (If= initiation factor, which is a protein).

2- **Elongation stage**: it is achieved by the following steps:

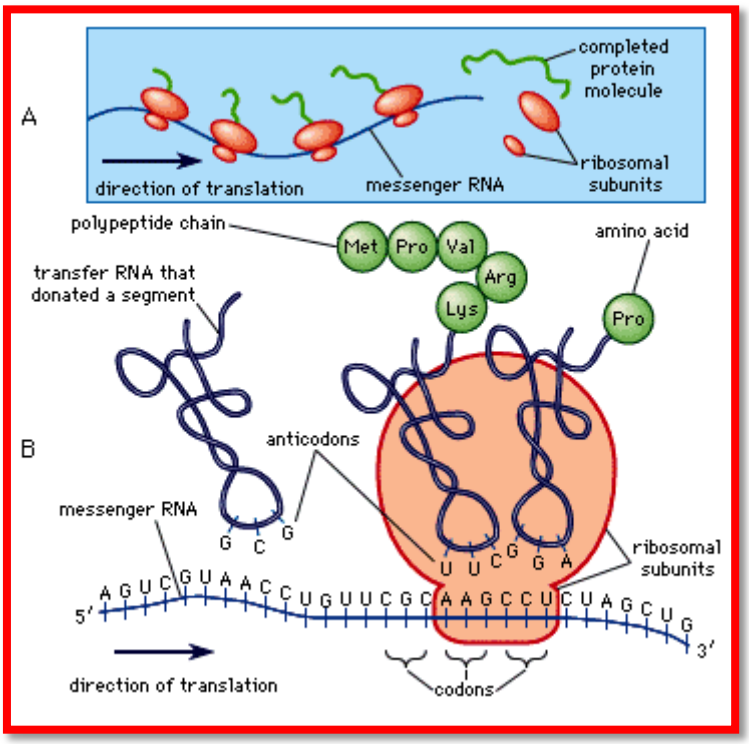
i) Attachment of the activated amino acid to a tRNA molecule. The specificity of the tRNA is determined by the anticodon sequence on the anticodon arm.

ii) The mRNA is attached to the ribosome at a specific site.

iii) The amino acid is transferred and carried by the tRNA to the ribosome at the site bound to the mRNA codon complementary to the anticodon of the tRNA where each C is bound to G and each A to U.

iv) Translocation on the ribosome takes place and other amino acids are placed in position, these amino acids are then connected together by a peptide bond formed by the **peptidyl transferase** enzyme.

3- **Termination stage**: termination of translation occurs when a stop codon enters the ribosome and three release factors (Rf1, Rf2, and Rf3) help in releasing the tRNA, mRNA, and proteins from the ribosomes.



Plasmids

They are DNA molecules other than the chromosomal DNA and are found in prokaryotic microorganisms and some eukaryotes such as yeasts. A plasmid is a double stranded DNA segment found in the cytoplasm unrelated to the chromosome and can replicate independently. Plasmids can be divided into two types according to size:

1- Large size plasmids with high molecular weight 60-120 (kb) known as R-plasmids (resistance) carrying genes responsible for antibiotic resistance or F-plasmids (fertility) which have the ability to transfer some chromosomal genes within the cells. These plasmids are also known as **Conjugative** plasmids.

2- Small size plasmids are 2.5-15 kb they carry bacteriocinogenic elements and resistance to some antibiotics or chemicals, they are also known as **non-conjugative** plasmids.

Transfer of genetic materials bacteria

There are three different mechanisms for genetic exchange in bacteria:

1-Transformation A free DNA molecule is transferred to a recipient cell of bacteria. The double stranded DNA could enter as it is like in Gram negative *Haemophilus* cells or it could enter single stranded as mostly happens in Gram positive cells. After entering the cell the single strand DNA recombines with the DNA of the recipient cell and the new cell is known as the **transformed cell**. A special treatment is needed to facilitate the entrance of the DNA to the recipient cell; this could be accomplished by treatment of recipient cells with CaCl2, then it is known as a competent cell (ready to receive the new DNA).

2-Transduction

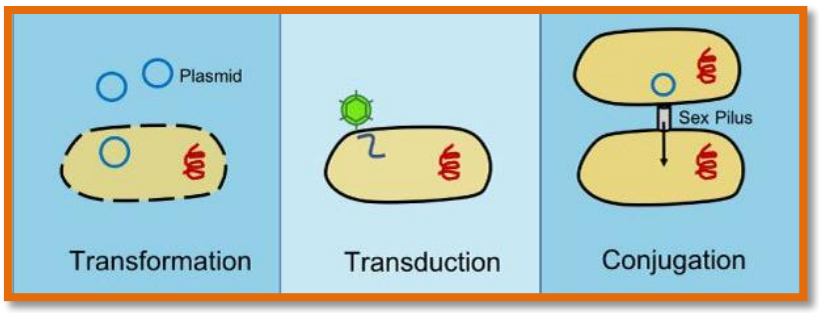
It occurs when the DNA of a donor is transferred to the recipient bacteria by a bacteriophage. Transfer of the genetic material by this method is more frequent than by transformation. There are two mechanisms for transduction:

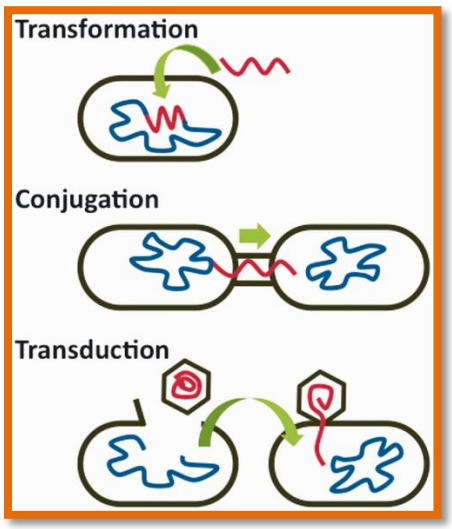
i. **General transduction**: when a piece of DNA at random is carried by a phage and is transferred to the recipient cell later forming transduced cells.

ii. **Specialized transduction**: it occurs when the bacterial DNA has a specific site for the phage DNA to incorporate; therefore, when the phage DNA is released it carries the bacterial DNA genes that are closely attached to the phage DNA and transfers it to the recipient cell (an example is the lambda (λ) phage that infects *Escherichia coli*).

3-Conjugation

Another method for genetic transfer, this method requires the presence of the F (fertility or sex) factor present on the F-plasmid, which is responsible for the formation of sex pili or Conjugation Bridge from donor (r) cells to the recipient (F) cells. Some donor (r) cells are known as high frequency recombinants (HFr) they are (1000) times more competent than the recipient cells in transferring the genetic material.





Mutation in bacteria

The term mutation applies to all heritable changes in nucleotide sequence arising within an organism, they may be: 1- Spontaneous (naturally occurring) 2- Induced by some mutagenic agents, this could be chemical or physical. When the mutant is not altered phenotypically but only genotypically it is known as **Silent mutation**. A mutation could occur by deletion, insertion of a nucleotide, transition, (purine into pyrimidine or pyrimidine into purine) or transversion (purine into purine or pyrimidine into pyrimidine). ***A* point mutation** occurs when one nucleotide is inserted or deleted.

Bacterial mutants

Several types of mutants are known:

1- Antibiotic or drug resistant mutants.

2- Mutants that differ in their fermentation products.

3- Auxotrophs: are mutants that lack the ability to synthesize organic compounds necessary for their growth therefore they are supplemented with vitamins or amino acids and need to grow on rich media.

4- Phenotypic mutants: they are altered phenotypically by a change in morphology or color of the colonies.

5- Mutation in cell surface and antigenic structure.

6- Phage resistant mutants.

7- Mutations with altered structures (i.e. loss of flagella, capsule, or spore formation).

Microbial metabolism

Metabolism is the chemical activity by which an organism synthesizes its constituents and converts energy from outer sources to energy rich chemical bonds. It is the sum of chemical and physical processes that occur within a living cell that cause production and consumption of energy that helps in building cell components or fulfills functional requirements such as motility or transport of molecule across the cytoplasmic membrane or other cell functions such as growth, repair and reproduction. Metabolism represents two processes:

1- Anabolism or Assimilation:

The process of manufacturing protoplasmic constituents. It is the constructive activity by which food forms. This process consumes energy.

2- Catabolism or Dissimilation:

It is intracellular break down of food materials; that is degradation of complex organic components to simpler molecules, which could be organic or inorganic. The process is usually accompanied by release of energy in the form of ATP (adenosine triphosphate), the cell store this energy until in need. This is an energy producing reaction.

The enzymes

Enzymes are proteins of very important functions; they are responsible for **catalyzing** biochemical reactions. Every microbial cell must possess many enzymes. Enzymes control and accelerate the rate of biochemical (metabolic) reactions in the cell, and in order to do so they must function under specific physiological conditions, which should be optimum such as temperature, pH, and ionic strength. Any change in these physiological conditions may lead to loss of enzyme activity.

Every enzyme catalyzes a specific biochemical reaction and some enzymes are highly specific towards their **substrates** (the substance acted upon by an enzyme) to produce an end product.

Enzyme classification

Enzymes are classified according to their mode of biochemical reaction; they are divided into six main categories:

1- Oxidizing and reducing enzymes (**Oxidoreductases**).

2- Transferring enzymes (**Transferases**).

3- Hydrolyzing enzymes (**Hydrolases**).

4- Enzymes that catalyze addition or deletion of groups from double bonds (**Lyases**).

5- Enzymes that catalyze isomerization (**Isomerases**).

6- Enzymes that bind or remove free groups (**Ligases**).

Structure and activity of the enzyme

Sometimes the enzyme activity depends on the protein part only known as the **apoenzyme**, while in other enzymes; to be active they must consist of another part known as a **cofactor**; a small

non-protein inorganic agent that activate the enzyme. The cofactor could be an inorganic substance such as metallic ions: Fe, Mg, Cu, Mo, Co, Zn (known as **prosthetic Group**) or hydrogen ions or it could be an organic substance known as a **coenzyme** such as a vitamin. The apoenzyme with the cofactor is known as the **holoenzyme**. Cofactors bind to a specific site on the three dimensional structure of the enzyme known as the active site this is the site where the substrate binds to start a reaction.

Many enzymes occur in various forms these are called **isoenzymes** or **isozymes** they carry out the same function but have different structural features.

Allosteric enzymes have a regulatory role in unidirectional metabolic pathways. It has an active site where the substrate binds and another site known as the regulatory site to which the regulatory molecule (effectors) binds to regulate the biochemical reaction either by allowing it to proceed or by preventing the attachment of the substrate molecule according to the need of the cell to that reaction.

Factors affecting enzyme activity

These include five factors:

1- Temperature

2- pH

3- Enzyme concentration

4- Substrate concentration

5- Presence of inhibitors

Enzyme inhibitors are substances that cause inhibition of enzyme activity because of their ability to bind to some enzymes

preventing them from binding to their substrate and thus stopping the biochemical reaction. Such inhibitors could be antibiotics, drugs, or toxins, which may affect the apoenzyme or its cofactor.

The energy

In a microbial cell energy can exist in a many forms. Of these forms are the energy rich chemical bonds found in ATP, which when hydrolysed produces high energy consumed by the cell for growth, reproduction, and other biological function.

Energy: is the ability to accomplish work, it exists in many forms in nature it could be heat, radiation, electric or light' energy. Living organisms can utilize two forms of energy and according to the source of energy they can be divided into:

1- Phototrophic microorganisms: which utilize light as a source of energy and convert it to chemical energy by photosynthesis.

2- Chemotrophic microorganisms: Those obtain their energy by biological oxidation through chemical reactions where energy is transferred from organic or inorganic compounds to specific acceptor molecules.

Anabolic reactions and energy consumption

Energy formed by catabolism is used for many purposes:

1- Motility of cilia and flagella in motile microorganisms.

2- Active transport across the membrane.

3- Construction of macromolecules such as proteins and

peptidoglycan.

Energy production in microorganisms

Microorganisms can be divided into two groups according to their nutritional requirements of carbon sources; autotrophs and heterotrophs.

I- Energy Production by Heterotrophs

In these cells energy production depends on oxidation-reduction reactions where some molecules donate electrons (oxidized) and other molecule accept those electrons (reduced). Along this process energy is released and electrons are transferred from the more negatively charged compounds to the more positively charged (i.e. the negatively charged is oxidized and the positively charged is reduced). Most heterotrophic microorganisms can generate ATP through many metabolic pathways depending on the electron acceptors. Energy producing pathways include **Fermentation, Respiration,** and **Photosynthesis.**

A. Fermentation

In fermentation organic substance serves both as electron donor and acceptor; however the yield of energy is lower than in respiration. It is also an anaerobic process where oxygen is not included. Fermentation is considered as an incomplete oxidation process of which its end products contain (low) considerable amounts of energy, the end products could be organic acids such as lactic acid, acetic acid, propionic acid or alcohols and all are released to the surrounding environment. An important intermediate that serves as a terminal electron acceptor and which is of importance in energy production is pyruvate.

There are three main pathways that produce pyruvate or pyruvic acid through fermentation:

1. Glycolysis:

The best-known process by which energy is obtained from glucose anaerobically also known as Embden-Meyerhof pathway. In this pathway one molecule of glucose is converted to two molecules of pyruvate and two NADH and a net of two ATP molecules. Pyruvate is an intermediate compound that participates in many fermentation processes that produce energy.

The overall equation for glycolysis by Embden-Meyerhof

pathway could be written as:

Glucose + 2 ATP + 2 NAD ⟶ 2 Pyruvate + 2 NADH + 2 ATP

2. Phosphogluconate pathway (pentose phosphate pathway):

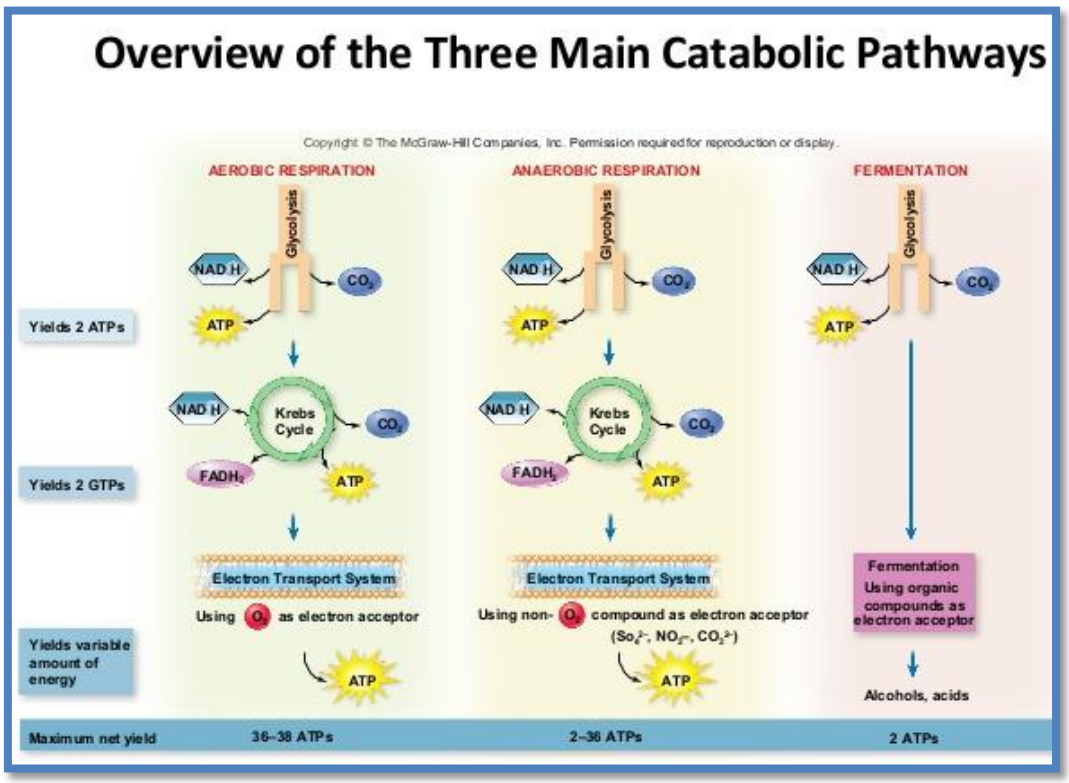
Glucose metabolism proceeds by decarboxylation when glucose-6-phosphate is converted to ethanol, lactic acid, and CO2. Only one pyruvate and one ATP molecule are generated. Through this process ribose-5-phosphate is generated with NADPH, which is required for reductive steps in cell biosynthesis, while ribose-5-phosphate is of importance in nucleic acids biosynthesis. The overall reaction of this process is:

$$G\text{-}6\text{-}P + 2NADP^{+} + H2O \rightarrow \text{ribulose 5-phosphate} + 2NADPH + 2H^{+} + CO2$$

3. Entner-Doudoroff pathway:

Glycolysis by this pathway results in net production of one ATP molecule per molecule of glucose metabolized. The equation could be written as:

G-6-p + 2NAD + ADP + Pi ⟶ 2Pyruvate + 2NADH + ATP + Ethanol



Fates of pyruvate

Microorganisms have evolved a variety of pathways through which pyruvate has a key role in principle fermentations; depending on different nutritional conditions and available enzymes the pathways are:

1. Lactic acid fermentation: this pathway is characteristics in *Lactobacillus* and *Streptococcus* bacteria.
2. Alcoholic fermentation: This fermentation is characteristics of yeasts and uncommon in bacteria.
3. Mixed acid fermentation: is a characteristic of most Enterobacteriaceae.
4. Other types of fermentation are methane fermentation as by Methanobacterium, and acetoin fermentation by *Bacillus* spp., and *Enterobacter*.

Pyruvate could be completely oxidized through the Krebs cycle or (Tricarboxylic acid (TCA) cycle), which is considered to be of the most important pathways for producing ATP in aerobic bacteria.

B. Respiration

In fermentation the electron acceptor is an organic compound while in respiration the electron acceptor is usually O2 (aerobic respiration). By respiration, electrons are donated by one organic or inorganic source leading to oxidation of these compounds, electron acceptors are inorganic compounds that will be reduced.

Types of respiration

1- Aerobic respiration:

The electron donor could be an organic or inorganic compound and it could be similar to that in fermentation (pyruvate) and the electron acceptor is oxygen, this is known as complete oxidation and the energy yield is obtained by complete conversion of the organic substance (the electron donor) to CO2 and H2O.

Substance (oxidized pyruvate) $\longrightarrow$ $2H^+ + 2e^- + CO2 + H2O$

Pyruvate (generated from the glycolytic pathway) is oxidized through the Kreb's or TCA cycle to acetyl CoA and CO2.

2- Anaerobic respiration:

The terminal electron acceptor is an inorganic substrate other than O2 such as nitrate (NO3), nitrite (NO2), sulphate (SO4), or carbonate (CO3).

Some common enzymes that participate in reducing these molecules are: nitrate reductase that reduces nitrate to nitrite

and nitrite reductase that reduces nitrite to molecular nitrogen (N2), while sulphate reductase reduces sulphate to H2S.

According to types of respiration bacteria fall into several groups:

a) Obligate (strict) aerobes: grow only in the presence of O2 such as *Mycobacterium tuberculosis* and some spore forming bacteria.

b) Facultative anaerobes: they can survive anaerobically but in the presence of air shift from fermentation to aerobic oxidation as in enterobacteria and yeasts.

c) Obligate (strict) anaerobes: grow only in the absence of O2 the terminal electron acceptors are sulphate and carbonate as in *Clostridium*.

d) Microaerophiles: bacteria grow in the presence of minute quantities of free O2 as well as CO2.

C. Photosynthesis:

Energy from light is used to provide cellular energy such as ATP molecules. The light is absorbed by special pigments (chlorophyll) in which electrons are transferred through a chain of electron carriers similar to that in phosphorylation.

Photosynthesis occurs in eukaryotes (plants) and prokaryotes (cyanobacteria, purple and green bacteria). In eukaryotes O2 is released by photosynthesis while in prokaryotes no O2 is released.

II- Energy Production by Autotrophs

This type of microorganisms could be divided into two groups:

A. Chemoautotrophs (chemolithotrophs): the sources of energy of such microorganisms are chemical inorganic substrates such as H2, ferrous, ammonia, nitrate, and sulphate.

B. Photoautotrophs: are photosynthetic bacteria that convert light energy into chemical energy which, in turn, converts CO2 into organic compounds in order to synthesize cellular constituents via photosynthesis.

Growth and multiplication

Growth means an increase in size, number, weight, and mass. It 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.

Growth rate and generation time

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

It takes short time in prokaryote (ex: 20-25 min in *E coli*). While in eukaryotes it takes two hours to several days.

Generation time varies with:

1- Species of M.O.

2- Nutrients.

3- Environmental conditions: PH, and temperature.

4- Growth phase.

Eukaryotic cell cycle:

It includes several stages:

1-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.

2-Second stage:

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

B- It constitutes20-25% of generation time.

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

3- Third stage:

A- It is period in which the precursors of mitosis spindle and cytoplasmic division is synthesized, it referred to as G2.

B- It constitutes about 25% of generation time.

C- The environmental condition does not greatly affect it.

4-Fourth stage:

A-Mitosis take place, referred to as M.

B-It constitutes about 5% of generation time.

C- Separation of the tow 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:

a. First stage :

This period is still under speculating. Mostly, under the optimal condition it disappears due to the shortage of generation time, also the environmental conditions greatly affect the cell.

b. 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.

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

2- Third and fourth stages:

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

B- It represents both third and fourth stages, G2 and M.

C- It referred as to D.

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

Growth curve of bacteria:

When 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 for cell division .The lag phase varies in length with the conditions of the M.O 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:

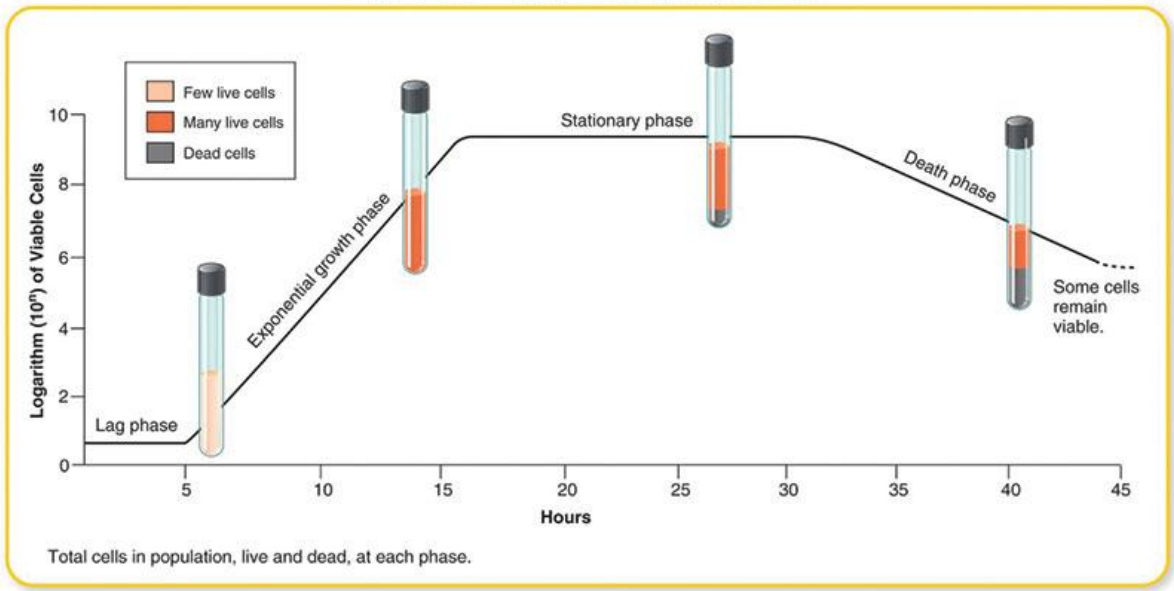
During this period the cells divide steadily at a constant rate. The log of the number of cells is plotted against time results in a straight line. Under appropriate conditions, the growth rate is maximal during this phase, and the population is most nearly uniform in terms of chemical compositions of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



Total cells in population, live and dead, at each phase.

When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new

medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase).

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.

Continuous culture:

Bacteria may also be grown in continuous culture where nutrients are supplied and end products removed continuously, so that the logarithmic growth phase is maintained and the bacteria never reach stationary phase because liquid medium is continuously fed into the bacterial culture and this can be done by using:

1- Chemostat 2- Turbidostat 3- Dialysis technique.

Differences between batch culture and continuous culture

Characteristics	**Batch culture**	**Continuous culture**
Cultivation system	Closed type	Open type
Addition of fresh nutrition	No	Yes
Volume of culture	Constant	Constant
Removal of wastes	No	Yes
Chance of contamination	minimum	Maximum
Growth phase	Lag, log, stationary and decline phase	Lag and log phase
Log phase	Shorter	Longest and Continuous
Density of bacteria	Change with time	Remain same
Product yield	Low	High

Methods of cell cycle study:

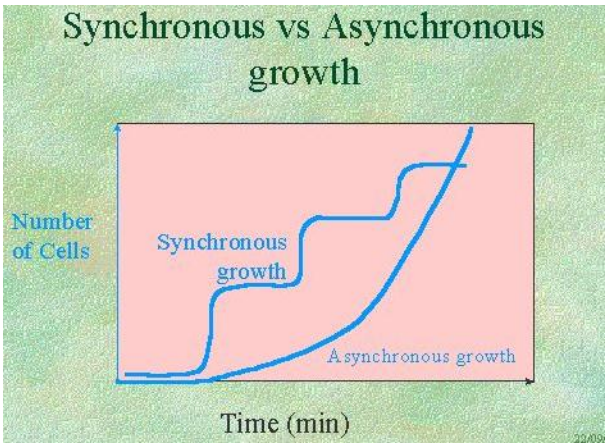
For a study, it is preferable that all culture cells being at one stage, a synchronous culture should be chosen, and this would be bone by:

1- Induction methods

2- Choosing methods.

Synchronous culture:

A synchronous or synchronized culture is a microbiological culture or a cell culture that contains cells that are all in the same growth stage. Normal, non-synchronous cultures have cells in all stages of the cell cycle. Obtaining a culture with a unified cell cycle stage is very useful for biological research. A synchronous culture can be treated as a single cell; the number of cell in the culture can be easily estimated.



Factors affecting growth

1-Temperature

Temperature affects the growth of bacteria by various ways.

- The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.

- There is no growth below minimum and above maximum temperature.
- Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.
- Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.

Microorganisms have been divided into three groups based on their optimum temperature:

A-Psychrophiles:

psychrophilic (cold loving) microorganisms, have a preferential temperature for growth at less than 15 °C exhibit an optimum range of growth between 0°C and 20; over this temperature the ribosome will be unstable. Psychrophiles found in Antarctic, cold soil, deep sea, stream, rivers and lake mud they are growing in a household refrigerator where they are important agents of food spoilage. These microorganisms are unable to live in high temperature because of the inhibition of enzymes. The enzymes of the bacteria are structurally unstable and fail to operate even at room temperature. The enzymes of these organisms allow functions such as the cleaning of clothes in cold water. Psychrophiles are adapted to their cold environment because the cytoplasmic membrane of psychrophile contains much more of a certain kind of unsaturated fatty acid which generally does not occur in prokaryotes.

B-Mesophiles

They have an optimal temperature range between 20-50°C. Most of them grown in the microbiological laboratories, some of

them involved in biodegradation (digestion and decomposition of organic matter),they take part in the web of micro-organic activity that form the humus layer forests and other fertile soils, by decomposing both vegetable and animal matter.

Many mesophile have an optimal temperature of about 37°C.Many of normal resident M.O of the human body such as *E.coli* is mesophilic. Mesophile bacteria are also involved in food contamination and degradation such as bread,and meat, in addition to bacterial infection in humans. The ability of mesophiles to survive at cold as well as hot temperature appears to be related to the composition of the fatty acids in the cell membrane.

C-Thermophiles:

Thermophiles are classified into obligate and facultative **thermophiles**: obligate thermophiles (also called extreme thermophiles) require such high temperatures for growth (above 40C), and the temperature span for optimuim growth of different thermophiles is 40-80C, such as *Thermus aquaticus*, whereas facultative thermophiles (also called **moderate thermophiles**) can thrive at high temperature, but also at lower temperatures. **Hyperthermophiles** are particularly extreme thermophiles for which the optimal temperatures is 90-100°C. Their membranes and proteins are unusually stable at these extremely high temperatures. Resistance to heat is a property associated with the endospores of such bacteria as the *Clostridium.*

2-Osmotic pressure:

Changing the solute concentration not only alters the availability of water but also alters the osmotic pressure. The cell wall structure of bacteria makes them resistant to changes in osmotic pressure but extremity in osmotic pressure can result in the death of M.O. In hypertonic solutions M.O may shrink and in hypotonic solution the cell may burst. M.O that can grow in solution with high solute concentration is called **Osmotolerant**. Some M.O are osmophilic requiring a high solute concentration to grow like some fungi. Some microorganisms known as **Halophiles** require NaCl for growth. Extreme halophiles show maximum growth rate in saturated solutions.

3-Hydrostatic pressure:

It refers to the pressure exerted by a water column as a result of the weight of the water column. Most M.O are relatively tolerant to the hydrostatic pressure, but cannot tolerate the extremely high hydrostatic pressure of 200 atm since this will inactivates the enzymes and disrupt membrane transport system. Some microorganisms are referred to as **barotolerant** (they are classified into obligate and facultative barotolerant) can grow at high hydrostatic pressure. The inhibition of microbial cell is attributed to the accumulation of acids inside the cell.

4-pH: M.O may divide into three categories:

1-Alkalophiles: Grow in a pH range of 7-11 with an optimum of 10.

2-Neutrophiles: Grow in a pH grow of 4-9 with an optimum near neutrality.

3-Acidophiles: Live at low pH values and can be divided into facultative such as fungi, obligate such as *sulfolobus.*

5-Radiation:

The electromagnetic spectrum divided into certain categories of radiation including gamma rays, X-ray, UV light, high energy, and short-wave length radiation disrupt DNA molecules and exposure to short wavelength radiation may cause mutation many of which are lethal.

The visible light is a source of energy for photosynthetic M.O such as cyanbacteria and purple and green bacteria where the light energy is converted into chemical energy in the cell.

6-Nutrient concentration:

If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.

Nutrition of microorganisms

Ways of food entrance to the cells:

Phagotrophic cells

Animal cells are typically without cell walls, have the ability to ingest solid particles of food by drawing them into the cell through the cell membrane by the process called **phagocytosis**. Phagocytic cells are said to have a phagotrophic type of nutrition.

Pinocytosis:

Many kinds of animas cell, though lacking cell walls, are not phagocyte. However, they can engulf fluids, and possibly pass minute particles inward through the cell membrane by a process called **pinocytosis**.

A similar process called endocytosis, large, complex molecules such as proteins, nucleic acids, some phages, and possibly colloids like sulfur, taken into the mammalian cell via minute invaginations of the cytoplasm membrane.

Carbon and energy sources for bacterial growth:

All living M.O require a source of energy. M. O that use energy (light) is called **phototrophs**. Microorganisms that uses an organic form of carbon are called **chemotroph**. Microorganisms that oxidize inorganic compounds are called **lithotrophs**. Microorganisms that use organic carbon are heterotrophs and Microorganisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**. Thus, on the basis of carbon and energy sources for growth five major nutritional types of prokaryotes may be defined in this table:

Nutrional types of prokaryotes

Nutritional type	Energy source	Carbon source	Examples
Photoautotroph photolithotroph	light	Co_2	*Cyanobacteria , Clorobium, Chromatium*
photoheterotoph photoorganotroph	light	Organic compounds	*Rhodospirillum*
Chemoautotrophs or lithotrophs (lithoautotrophs)	Inorganic compounds,e.g.H_2, NH_3,NO_2,H_2S.	CO_2	Archaea
Chemoheterotrophs or heterotrophs, chemoorganotroph	Organic compounds	Organic compounds	Pathogenic bacteria, some archaea.
Mixotroph	Inorganic	Organic	-----

Growth factors:

An autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**. This is required in small amounts by cells because they play a specific role in biosynthesis. Growth factors are organized into three categories:

1-Purines and pyrimidines: Required for synthesis of nucleic acids (DNA and RNA).

2-Amino acid: Required for the synthesis of proteins.

3-Vitamins: Needed as coenzymes and functional groups of certain enzymes.

Auxotrophs:

Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotroph**. Thus, a strain of *Ecoli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph.

Mineral salts:

Microorganisms need mineral salts in a small quantity of inorganic ions (cat ions and anions) such as:

1-Macronutrients elements: They including Mg^{+2}, Ca^{+2}, Na^{+}, and Cl^{-}. Macronutrients are required in con. 0.1-1 mMol.

2-Micronutrients elements: They are required in con. About10^{-3}-10^{-5}mM, such as Co^{+2}.

There is a group of M.O, which needed Na^{+} and Cl^{+} in high concentration Called **Halophiles that can be classified into:**

1-**Slightly halophiles:** Those who needed a small quantity of NaCl (2-5%), including most of marine bacteria.

2-Moderately halophiles: NaCl is needed in a range about (5-10%) such as ***Pseudomonas***, ***Lactobacillus.***

3-Extremely halophiles: NaCl is needed in a range about (20-30%) such as***Halobacterium*** and ***Micrococcus.*** NaCl is necessary to stabilize the binding of cell wall proteins to each other.

The effect of oxygen :

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. Procaryotes display a wide range of responses to molecular O_2. as in the table:

Group	Aerobic	Anaerobic	O_2 effect
Obligate aerobe	Growth	No growth	Required (utilized for aerobic respiration)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate anaerobe	No growth	Growth	
Facultative anaerobe (Facultative aerobe)	Growth	Growth	Not required for growth but utilized when available
Aerotolerant anaerobe	Growth	Growth	Not required and not utilized

Uptake of nutrients by the cell:

Microorganisms used several different transport mechanisms, the most important of them are:

1-Facilitated diffusion: A few substances can cross the cytoplasm membrane by passive diffusion. In this process molecules move from a region of higher concentration to one of lower con.

2-Active transport:

It is transport of solute to higher concentration or against the concentration gradient with the use of metabolic energy in input.

3-Group translocation:

A substrate is becoming phosphorylated during the transport process.