



كلية النور الجامعة
قسم الصيدلة
احياء مجهرية طبية I
2022

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MS.c Medical Microbiology

The Science of Microbiology

- ▣ Microbiology is the study of microorganisms usually less than 1mm in diameter which requires microscope to be seen.
- ▣ It is a large and diverse group of microscopic organisms that exist as single cells or cell clusters; it also includes viruses, which are microscopic but not cellular, bacteria, fungi, algae and protozoan.
- ▣ “**Germ**s” refers to a rapidly growing cell.
- ▣ **The importance of medical microbiology**
- ▣ Medical microbiology basically deals with the study of microorganisms that are both beneficial and harmful to both human and animal majorly. Branches of medical microbiology include Virology, Bacteriology, Parasitology, and Mycology etc.
- ▣ **Following are some of the major important features of medical microbiology:**
 - 1) The major importance of medical microbiology is that it helps in the **identification, isolation, diagnosis and treatment** of pathogenic microorganisms and also produces beneficial organisms such as yeast and some antibiotics.
 - 2) Biologists use microbiology to develop new methods for **preventing illness**.
 - 3) Microbiology gives the information which further use to create the **vaccines** and treatments for different diseases.
 - 4) Companies often employ microbiologists to **develop new products** that kill viruses and bacteria. These scientists help to diagnose diseases such as meningitis and tuberculosis and help to **prevent the spread of diseases** by identifying, containing, and treating disease in human body.
 - 5) Biologists use knowledge obtained from microbiology when **studying the immune system**.

- ▣ 6) Microbiology also used in food production, biodegradation, biotechnology and genetic engineering.
- ▣ 7) Biological weapon use pathogens or organisms that cause disease. Pathogens include bacteria, viruses, fungi, and toxins (poisons produced by animals or plants). Example:
Bacillus anthracis bacteria is one of the most deadly agents to be used as a biological weapon.
- ▣ 8) Chemicals manufacturing, such as production of ethanol, acetone, organic acid, enzymes and perfumes.
- ▣ 9) Clinical microbiology laboratory plays an important role in patient care by providing the cause of infection and antimicrobial susceptibility data to physicians. Rapid diagnosis of pathogens is important for initiating effective antibiotic administration and improving the outcomes of treatment.

Bacillus



Fields of microbes study

- ▣ Microbiologists may be interested in various characteristics or activities of microorganisms:
 - Microbial morphology
 - Microbial cytology
 - Microbial physiology
 - Microbial ecology
 - Microbial genetics and molecular biology
 - Microbial taxonomy

Who discovered microbiology?

- ▣ Antonie van Leeuwenhoek is considered a father of microbiology as he observed and experimented with microscopic organisms in 1676, using simple microscopes of his own design.
- ▣ Louis Pasteur and Robert Koch are the founders of medical microbiology.
- ▣ Louis Pasteur in the 19th century is famous for his experiments when he disproved the theory of spontaneous generation. He offered method for food preservation (pasteurization) and vaccines against anthrax, cholera and rabies.



Antonie van Leeuwenhoek
(1632-1723)



Leeuwenhoek
Microscope
(circa late 1600s)

- ▣ The word prokaryote comes from Greek word meaning “**before nucleus**’. The distinctive feature of prokaryotes is that they can make proteins simultaneously to reading their enteric code because a typical prokaryote does not have a membrane surrounding its genetic material DNA.
- ▣ The main characteristics of Prokaryotes:
 - 1- Their DNA (genetic material) is not enclosed within a membrane and is not circular chromosome.
 - 2- Their DNA is not associated with histones (a special chromosomal protein)
 - 3- They lack membrane-enclosed organelles.
 - 4- Their cell walls almost always contain the complex polysaccharide **peptidoglycan**.
 - 5- They usually divide by **binary fission**. (process which the DNA is copied and the cell splits into two cells).

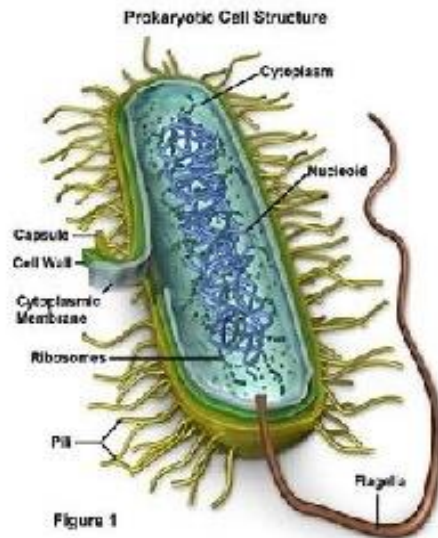
▣ The main characteristics of Eukaryotes:

- 1- Their DNA is found in the cell's nucleus, which is separated from the cytoplasm by nuclear membrane.
- 2- Their DNA is consistently associated with chromosomal proteins called histones.
- 3- They have a number of membrane-enclosed organelles, including mitochondria, endoplasmic reticulum, Golgi complex, lysosome, and sometimes chloroplast.
- 4- Their cell wall, when present, are simple.
- 5- They usually divide by mitosis.

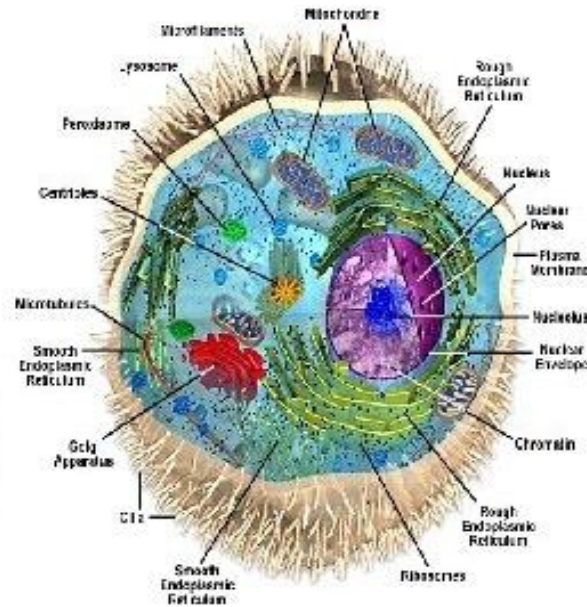
Basis for COMPARISON	PROKARYOTIC CELLS	EUKARYOTIC CELLS
Size	0.5-3um	2-100um
Kind of Cell	Single-cell	Multicellular
Cell Wall	Cell wall present, comprise of peptidoglycan or mucopeptide (polysaccharide).	Usually cell wall absent, if present (plant cells and fungus), comprises of cellulose (polysaccharide).
Presence of Nucleus	Well-defined nucleus is absent, rather 'nucleoid' is present	A well-defined nucleus is present enclosed within nuclear membrane.
Shape of DNA	Circular, double-stranded DNA.	Linear, double-stranded DNA.
Mitochondria	Absent	Present
Ribosome	70S	80S
Golgi Apparatus	Absent	Present
Endoplasmic Reticulum	Absent	Present
Mode of Reproduction	Asexual	Most commonly sexual
Cell Divison	Binary Fission, (conjugation, transformation, transduction)	Mitosis

Eukaryotes VS Prokaryotes

Bacterial cell



Animal cell



Plant cell



Classification.....

- ▣ Binomial system of taxonomic classification
 1. Use only the genus and species
 2. Put line under the genus and species or italicized.
 3. The genus word is always capitilized.
 4. The species is never capitilized

Staphylococcus aureus

Escherichia coli

Types of classification of bacteria...

❑ Bacteria can be classified according to morphology:

1) Cocci... these are spherical cells. On the basis of arrangement they can be described as:

Monococci(Cocci in singles), Diplococci.....(Cocci in pairs), Staphylococci....(Cocci in grape-like clusters), Streptococci...(Cocci in chains), Tetrad.....(Cocci in group of four), Sarcina(Cocci in group of eight)

2) Bacilli these are rod-shaped bacteria. On the basis of arrangement of organisms, they can be described as:

Diplobacilli, Streptobacilli, Chinese-letter form, Coccobacilli, Comma-shaped

3) Spirochaetesthese are relatively longer, slender, non-branched microorganisms of spiral shape having several coils.

4) Mycoplasma.... These bacteria lack a rigid cell wall (cell wall lacking) and are highly pleomorphic (round or oval).

5) Rickettsia and chlamydiae....these are very small parasites, now these are arranged as bacteria

Based on anatomical features:

1. Capsulein to

❑ Capsulate ----- *Streptococcus pneumonia*

Non-capsulate-----*Viridans streptococci*

2. Flagella in to

❑ Flagellate ❑

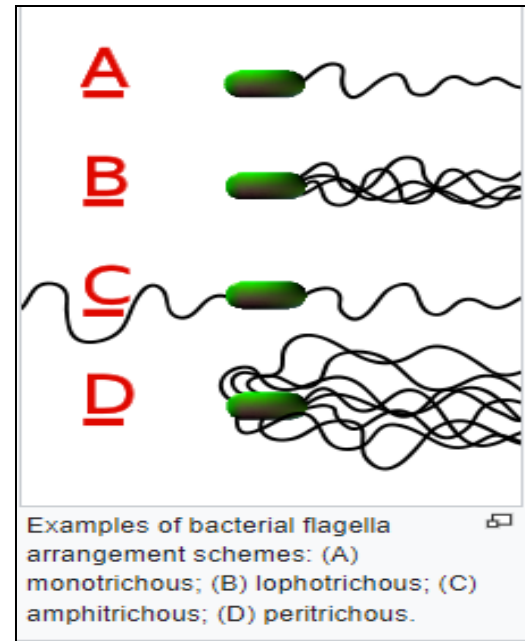
Monotrichous, Lophotrichous, Amphitrichous, Peritrichous

❑ Aflagellate ❑ *Shigella* spp.

3. Spore in to

❑ Spore-forming ❑ *Bacillus* spp.

❑ Non-sporing ❑ *Escherichia coli*



Based on staining reaction:

1. GRAM'S STAIN in to

- **Gram-positive (+) cocci** *Staphylococcus aureus*
- **Gram-negative (-) cocci** *Neisseria gonorrhoeae*
- **Gram-positive rods (+)** *Clostridium* spp.
- **Gram-negative rods (-)** *E. coli*

2. ACID FAST STAIN in to

- **Acid-fast bacilli** *Mycobacterium tuberculosis*
- **Non-acid-fast bacilli** *Staphylococcus aureus*

Based on cultural characteristics

1. Extra growth factor requirements in to

- **Fastidious** – *Hemophilus influenzae*
- **Non-fastidious** – *Escherichia coli*

2. Hemolysis on Sheep Blood Agar in to

Alpha-hemolysis (α) – *Streptococcus pneumoniae*

Beta-hemolysis (β) – *Streptococcus pyogenes*

Gamma-hemolysis (γ) – *Enterococcus faecalis*

3. Utilization of carbohydrates in to

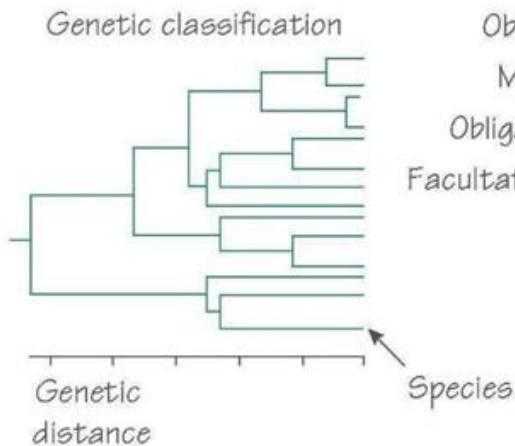
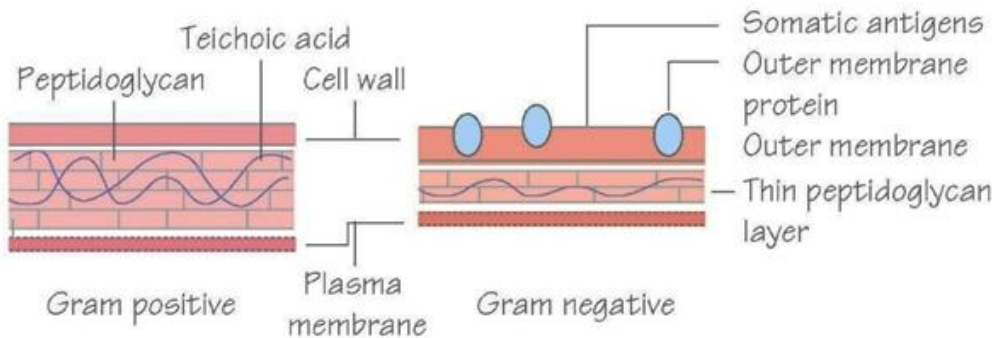
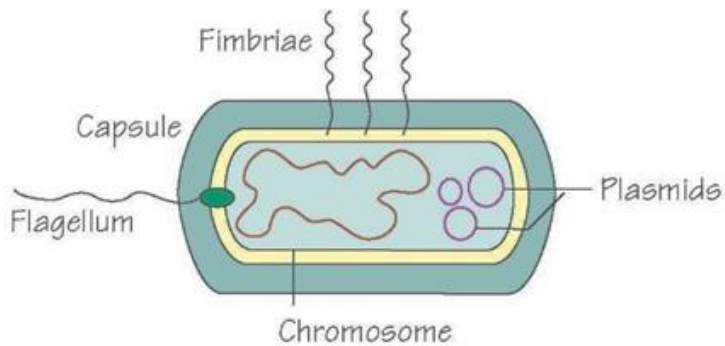
- **Oxidative** – *Micrococcus* (with the presence of O₂)
- **Fermentative** – *Escherichia coli* (with the absence of O₂)

4. Pigment production

- **Pigment producer** – *Staphylococcus aureus*
- **Pigment non-producer** – *Escherichia coli*

Other ways of classification

- **Motile/Non-motile**
- **Pathogenic/Non-pathogenic**
- **Sensitive/Resistant** (to particular antibiotic/chemicals)
- **Lactose fermenter/Lactose non-fermenter**



- Obligate aerobes → Require oxygen
- Microaerophiles → Require reduced oxygen
- Obligate anaerobes → Require no oxygen
- Facultative anaerobes → Anaerobic or aerobic
- Capnophiles → Require increased CO₂

Bacterial classification

Shape

Cocci

Spiral

Bacilli

Cocci are spherical; bacilli are long and thin, with coccobacilli in between; and there are also curved and spiral bacilli with different wavelengths

Gram reaction

Growth

Atmosphere

Spores

Biochemistry

Genetic classification

Serology typing

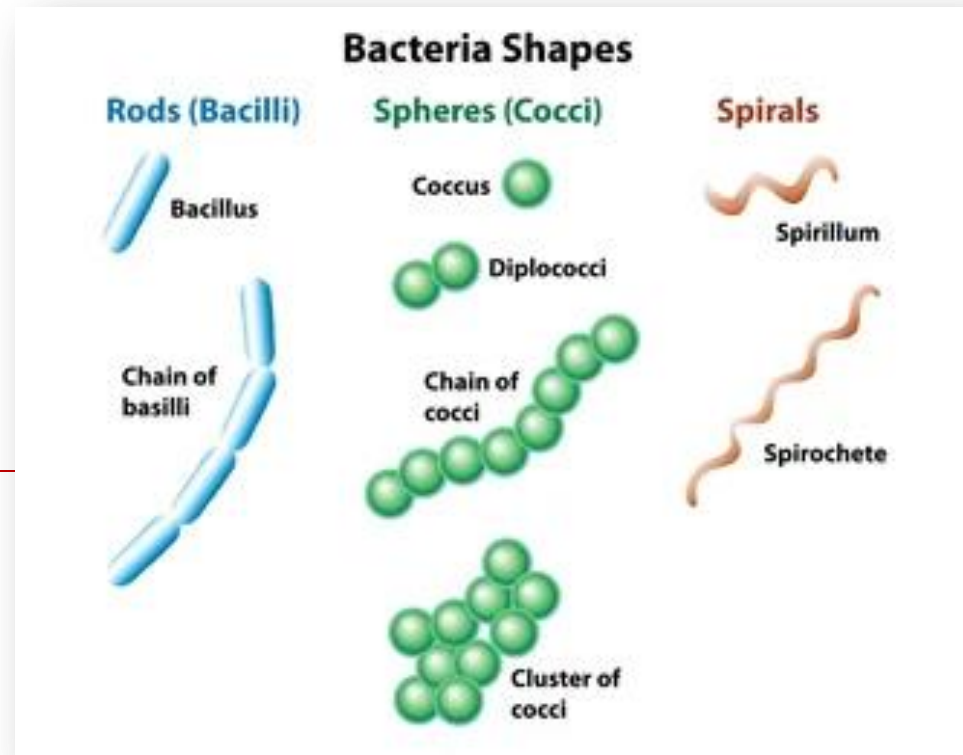
Endospore

Present on *Clostridium* and *Bacillus* spp.


















Batteries of reactions are needed for species identification

Cell shape:

- ❑ Coccus
- ❑ Rod or bacillus
- ❑ Curved or spiral
- ❑ Cell arrangement:
 - ❑ Single- cells found by themselves
 - ❑ Diplo- cells in pairs. Diplococcus, Diplobacillus
 - ❑ Strepto- cells in chains. Streptococcus, Streptobacillus
 - ❑ Staphylo- cells in grape like clusters. Staphylococcus

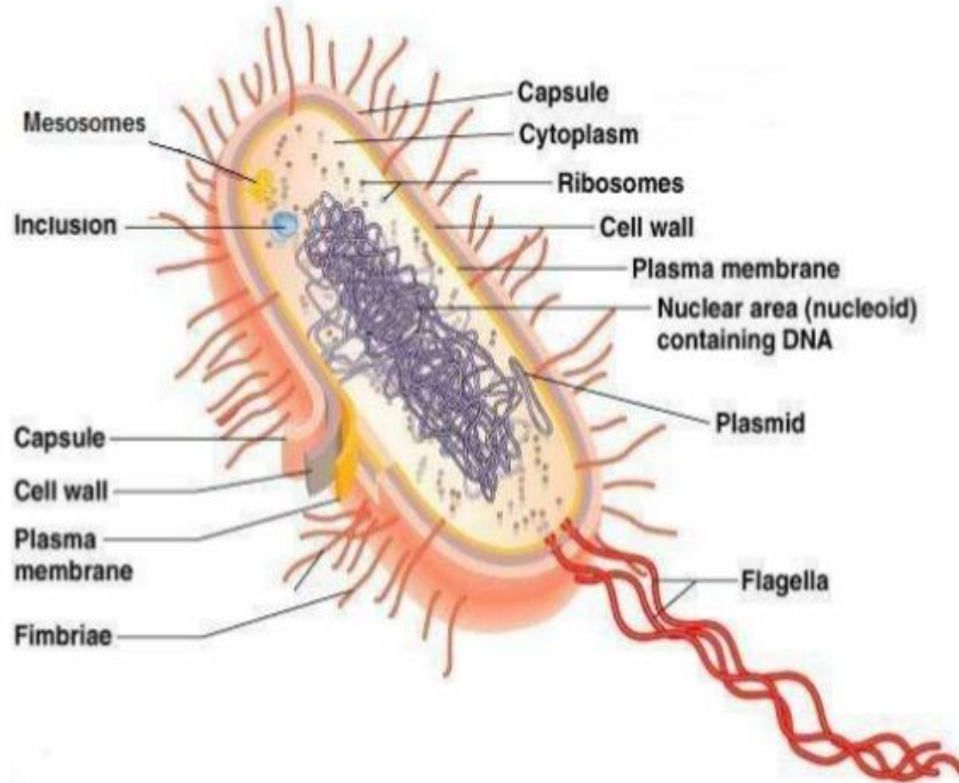


Bacterial shapes and arrangements

 <p>Coccus</p>		 <p>Rod, or Bacillus</p>		 <p>Curved forms: Spirillum/Spirochete</p>
 <p>Diplococci (cocci in pairs)</p>	 <p>Neisseriae (coffee-bean shape in pairs)</p>	 <p>Coccobacilli</p>		 <p>Vibrios (curved rods)</p>
 <p>Tetrads (cocci in packets of 4)</p>	 <p>Sarcinae (cocci in packets of 8, 16, 32 cells)</p>	 <p>Mycobacteria</p>	 <p>Corynebacteria (palisades arrangement)</p>	 <p>Spirilla</p>
 <p>Streptococci (cocci in chains)</p>	 <p>Micrococci and staphylococci (large cocci in irregular clusters)</p>	 <p>Spore-forming rods</p>	 <p>Streptomyces (moldlike, filamentous bacteria)</p>	 <p>Spirochetes</p>

Structure and classification of bacteria:

Anatomy of a Bacterial Cell



Bacterial structure:

- Flagella
- Pili
- Fimbriae
- Capsule or Slime layer
- Plasma membrane
- Cytoplasm
- Cell wall
- Spores
- Nucleoid
- Inclusions

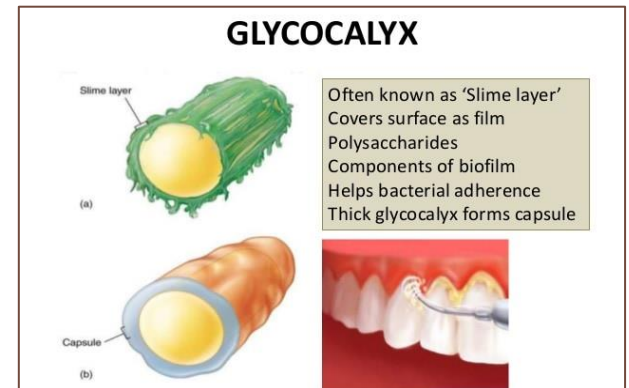
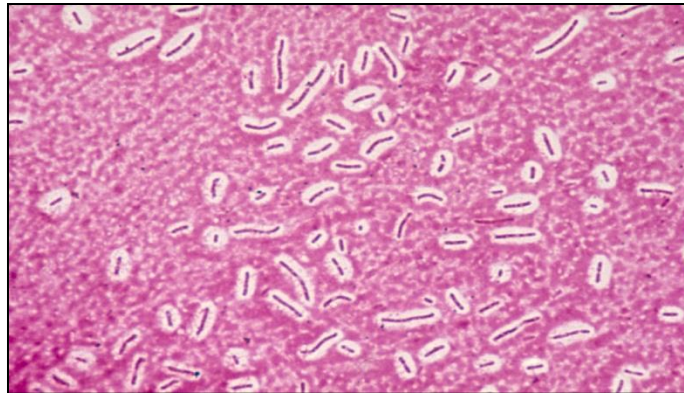
Typical prokaryotic cell (Bacteria)

Bacterial organelles:

- ✦ **Capsule:** Some cells have a gelatinous, sticky substance that surrounds the outside of the cell. This substance is known as a **glycocalyx**, which means (sweet cup). The glycocalyx composed of polysaccharides, polypeptides, or both. These chemicals are produced inside the cell and are release onto the cell's surface.
- ✦ Thus we can say that, there are two types of glycocalyx:
 - (1) Capsule:
 - a- Composed of organized repeating units of organic chemicals.
 - b- Firmly attached to cell surface.
 - c- May prevent bacteria from being recognized by host.
 - (2) Slime layer:
 - a- Loosely attached to cell surface.
 - b- Water soluble.
 - c- Sticky layer allows prokaryotes to attach to surface as biofilm.
- ✦ These chemicals protect cells from desiccation (drying) and can also play a role in ability of pathogens to survive and cause disease. For example, slime layer are often sticky and provide one means for bacteria to attach to surfaces as **biofilm**, which are aggregates of many bacterial living together on surface.
- ✦ Oral bacteria colonize the teeth as a biofilm called **dental plague**. The bacteria in the biofilm produce acid and cause **dental caries** (cavities).

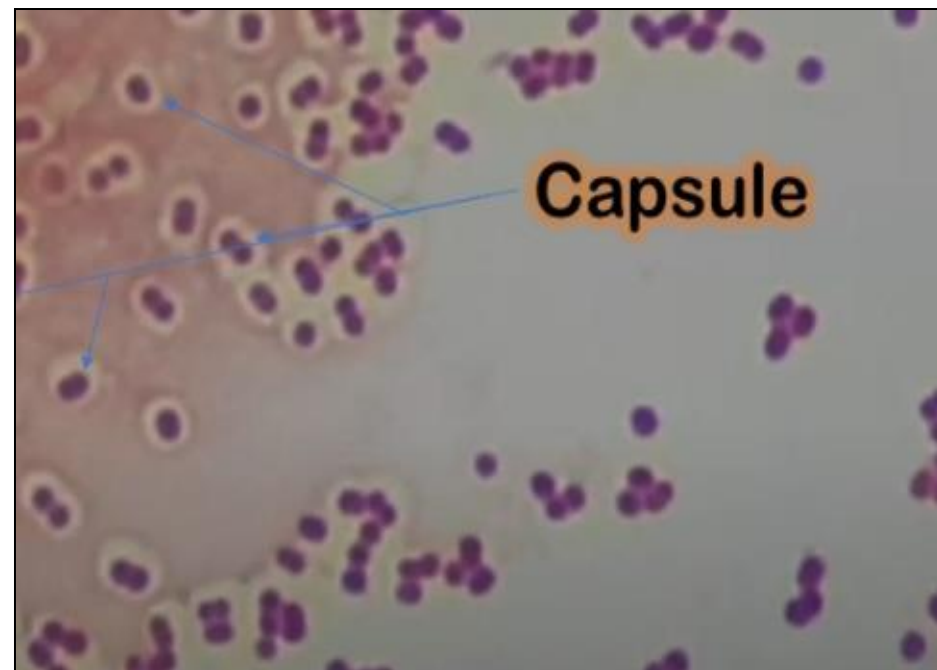
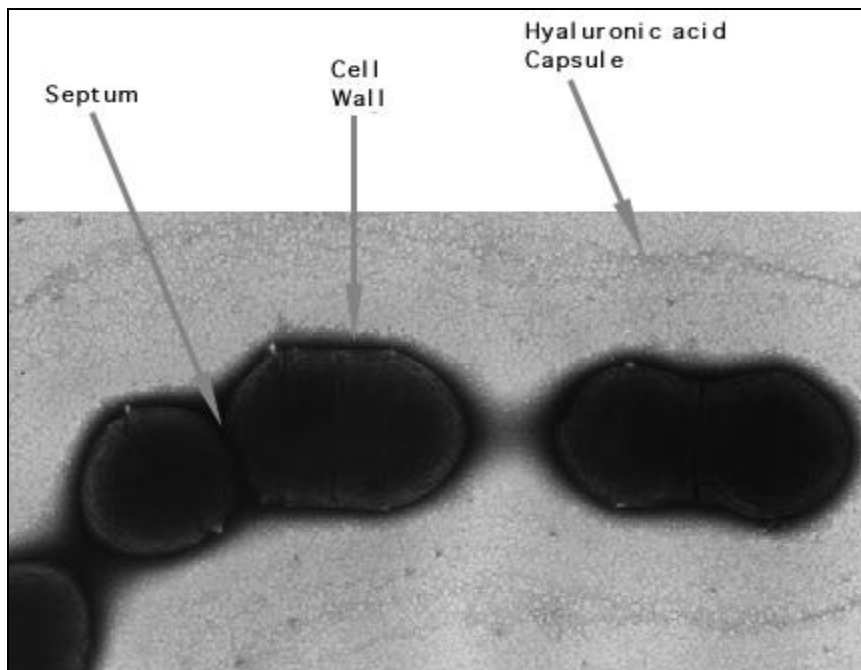
Glycocalyx (meaning sugar coat)

- Glycocalyx is viscous, gelatinous polymer that is external to the cell wall and composed of polysaccharide, polypeptide, or both.
- **If** substances is organized and is firmly attached to the wall, the glycocalyx is described as a **capsule**.
- If the substances is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer**.



For example; the capsules of *streptococcus pneumoniae* and *Klebsiella pneumoniae*, enable these prokaryotes to avoid destruction by defensive cells in respiratory tract and to cause pneumonia.

Un- encapsulated strains of these same bacterial species do not cause disease because the bod's defensive cells destroy them.



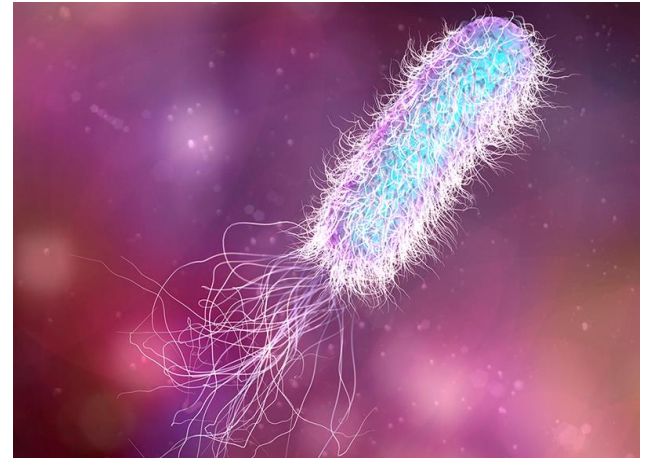
Thank you





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Flagella

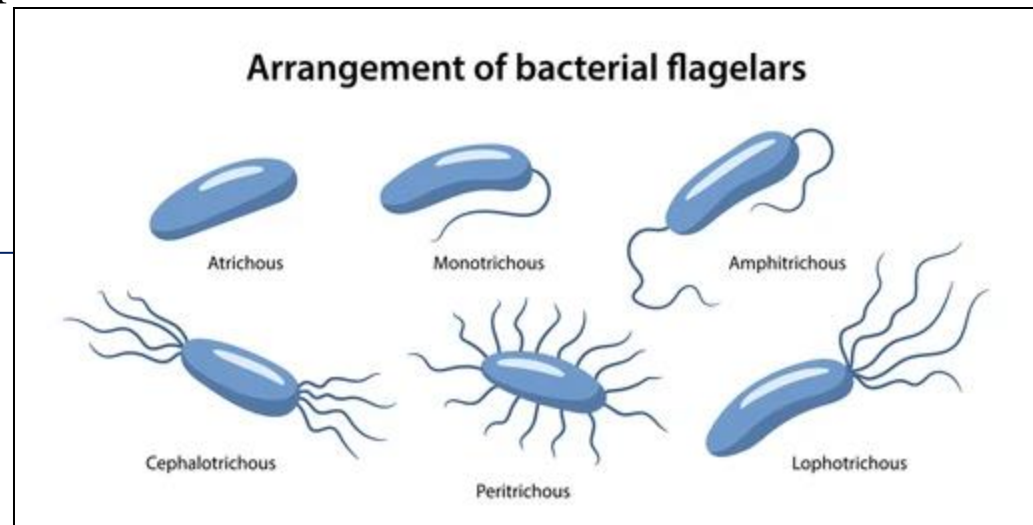
Bacterial flagella are thread-like appendages composed entirely of protein, 12–30 nm in diameter. They are the organs of locomotion for the forms that possess them.

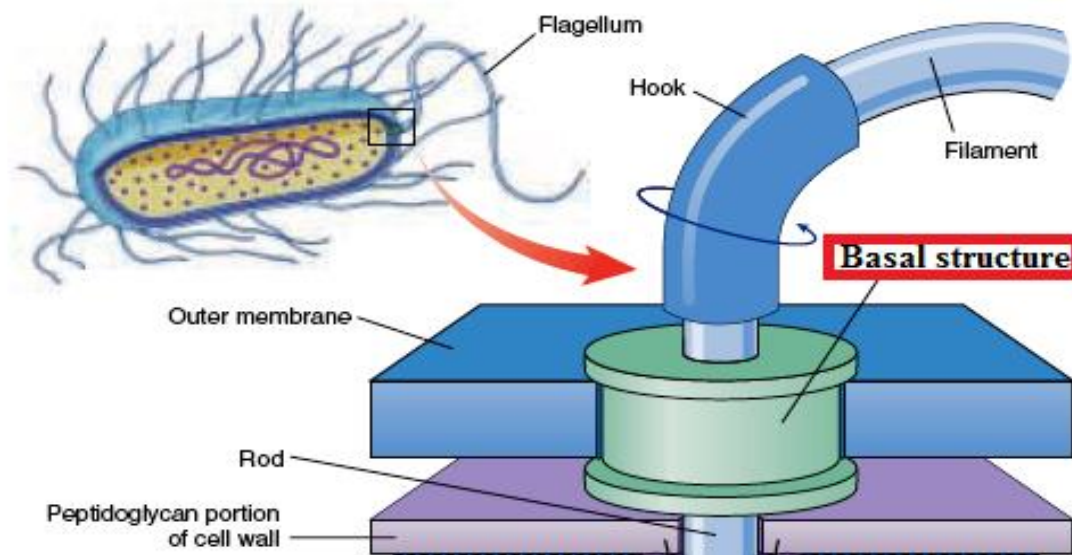
Three types of arrangement are known:

- (1) Monotrichous (single polar flagellum).
- (2) Amphitrichous (flagella @ both ends).
- (3) Lophotrichous (cluster at one end).
- (4) Peritrichous (flagella covering the cell).
- (5) Atrichous (with no flagella).

Bacterial flagella are composed of three parts:

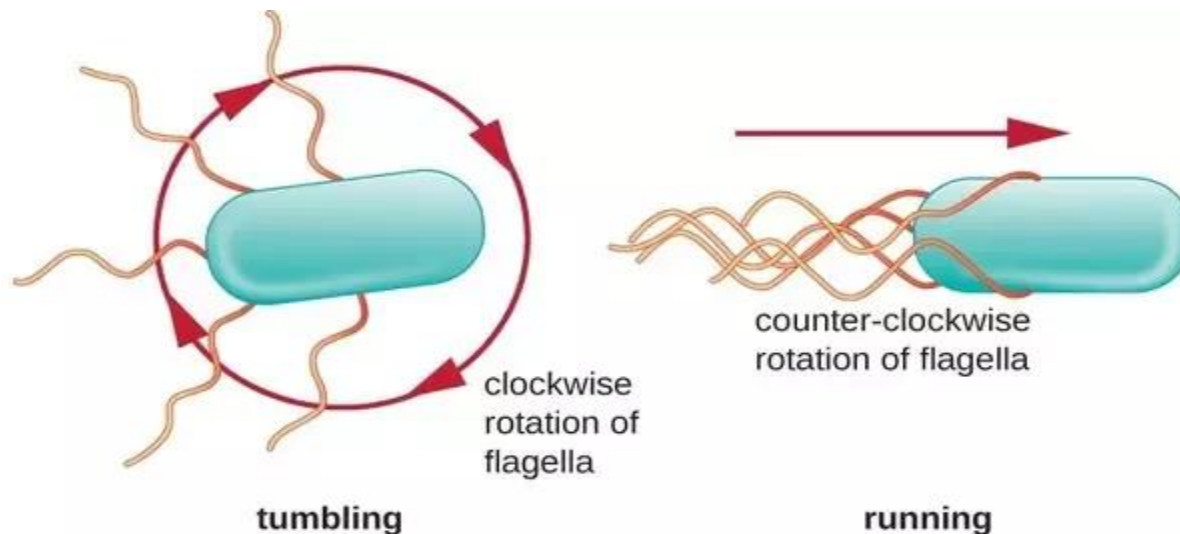
- a- Filament
- b- Hook
- c- Basal body





Flagella function:

- a- Rotation propels bacterium through environment.
- b- The movement of bacterium toward or away from a particular stimulus is called taxis. Such stimulus include chemicals and light.



Fimbriae and pili

Fimbriae: rod like, protein extensions, these projections adhere to one another and to substances in the environment or to their host, colonize the mucous membrane and cause disease. Some fimbriae carry enzymes that render soluble, toxic ions into insoluble, nontoxic form. There may be hundreds of fimbriae per cell, and they are usually shorter than flagella.

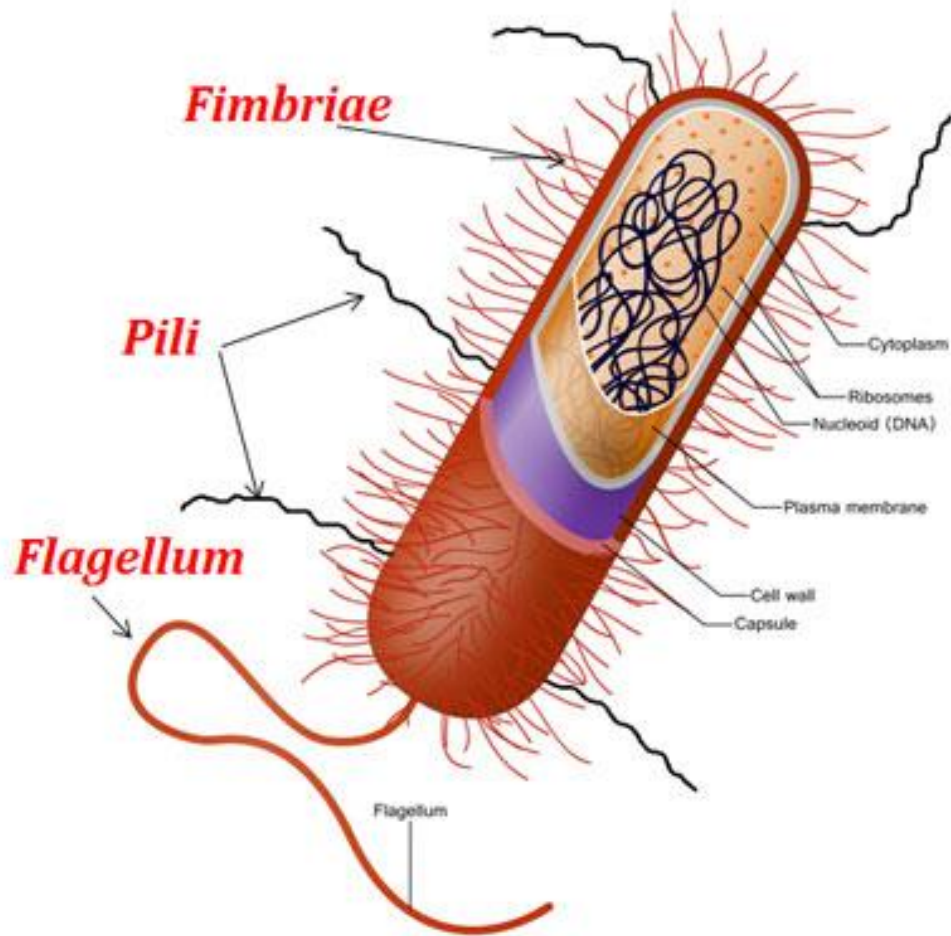
- a- Sticky, bristle-like projections.
- b- Used by bacteria to adhere to one another and to substances in environment.
- c- Used by bacteria to move across a surface.
- d- Serve an important function in biofilm.

Pili: is a type of fimbria, longer than fimbria but shorter than flagella. Typically only one to a few pili are present per cell.

- a- Special type of fimbria.
- b- Also known as conjugation pili.
- c- Longer than fimbria but shorter than flagella.
- d- Bacteria typically have only one or two per cell.
- e- Transfer DNA from one cell to another (conjugation).

Q?? How are pili different from bacterial flagella?

Answer: Bacterial flagella are flexible structures that rotate to propel (push) the cell, pili are hollow tubes used to transfer DNA from one cell to another.



Cell-Surface Appendages of a Bacterial Cell

Bacterial Cell wall

The cells of most prokaryotes are surrounded by a cell wall that provides structure and shape to the cell and protects it from osmotic forces. In addition, a cell wall assists some cells in attaching to other cells or in resisting antimicrobial drugs. Note that animal cells do not have walls, a difference that plays a key role in treatment of many bacterial diseases with certain types of antibiotics. For example, penicillin attacks the cell wall of bacteria but is harmless to human cells, which lack walls.

Function of bacterial cell wall:

- 1- Provide structure and shape and protect cell from osmotic force.
- 2- Assist some cells in attaching to other cells or in resisting antimicrobial drugs.
- 3- Can target cell wall of bacteria with antibiotics.
- 4- Give bacterial cell characteristics shape.
- 5- Composed of peptidoglycan (polysaccharide).

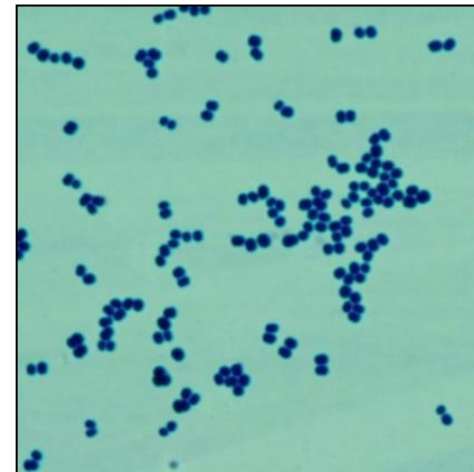
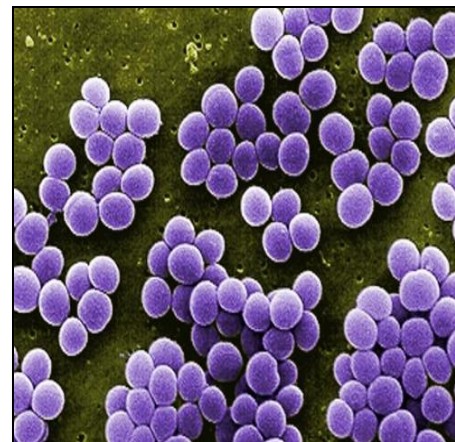
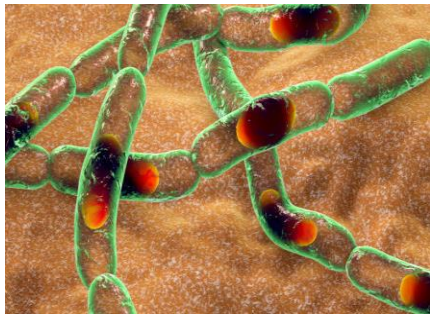
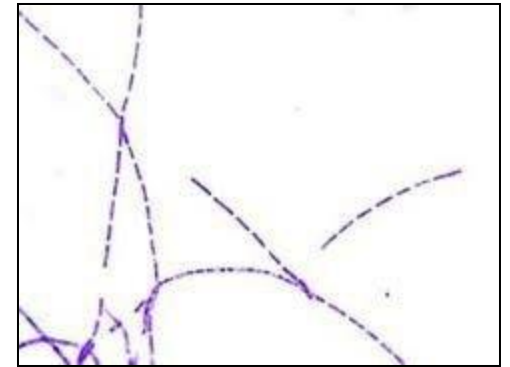
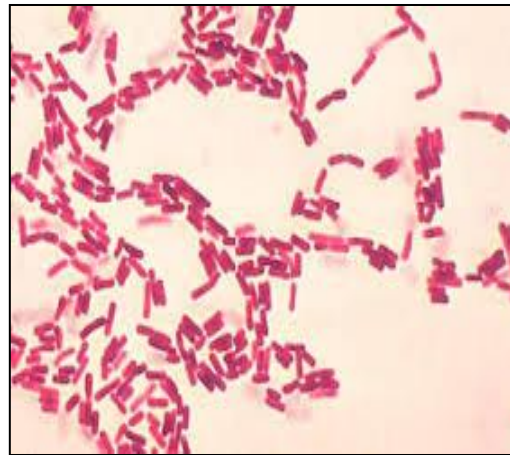
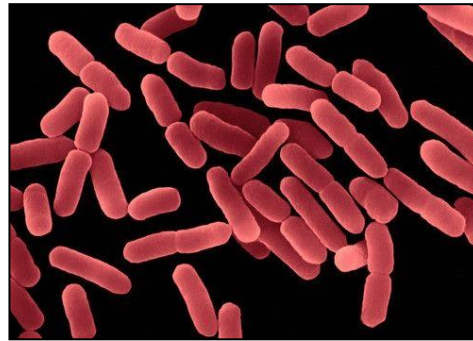
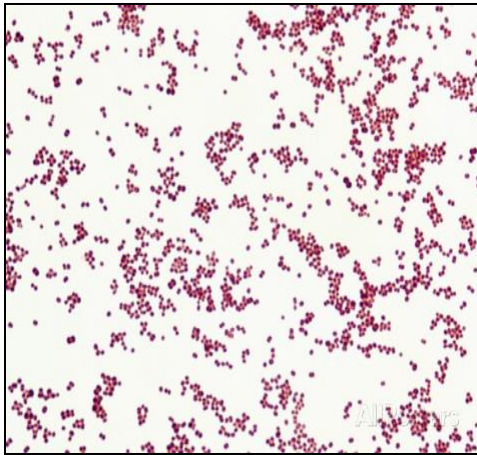
Scientists describe two basic types of bacterial **cell wall**; Gram-positive & Gram-negative :

Gram-positive bacterial cell wall:

- 1- Relatively thick layer of peptidoglycan.
- 2- Contain unique polyalcohols called teichoic acid.
- 3- Appear **purple** following Gram staining procedure.
- 4- Presence of up to 60% mycolic acid in acid-fast bacteria (T.B), helps cells survive desiccation.

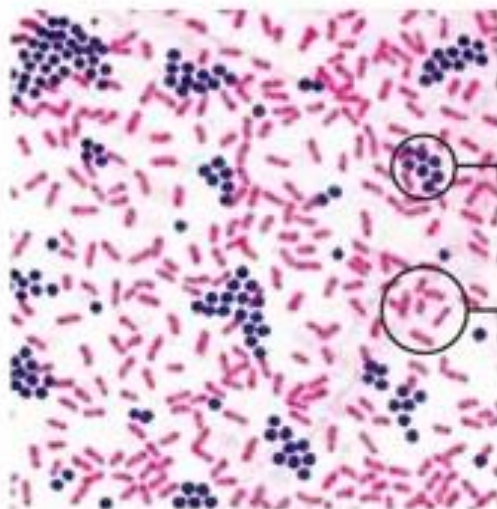
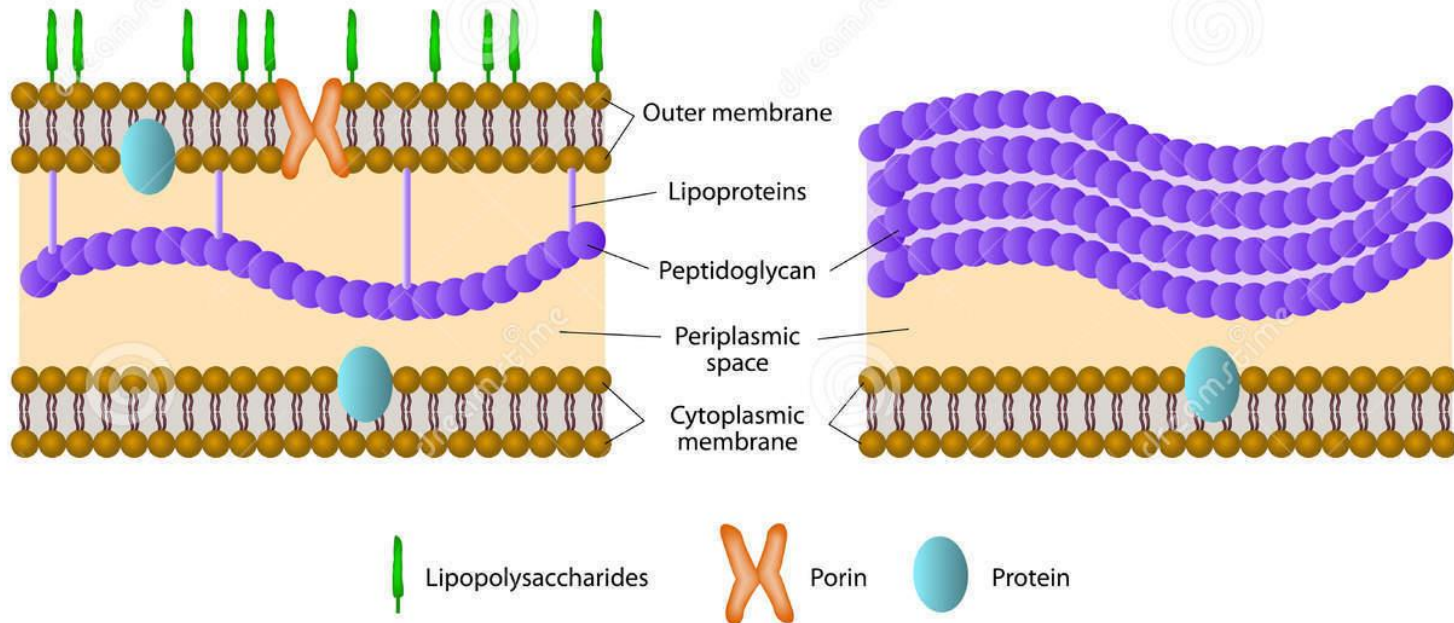
Gram-negative bacterial cell wall:

- 1- Have only a thin layer of peptidoglycan.
- 2- Bilayer membrane outside the peptidoglycan contain phospholipids, proteins, and lipopolysaccharide (LPS). Lipids in LPS can cause fever, vasodilation, inflammation, shock, and blood clotting.
- 3- Appear **pink** following Gram staining procedure.



GRAM-NEGATIVE

GRAM-POSITIVE



Gram-positive
Cocci (spherical)

Gram-negative
Bacilli (rod-shaped)

Comparative characteristic between Gr+ and Gr -

Property	Gram + bacteria	Gram - bacteria
Peptidoglycan layer	Thick (multilayered)	Thin (single layer)
Outer membrane	Absent	Present
Teichoic acid in wall	present	Absent
protein/lipoprotein content	low	high
Sensitivity to penicillin	sensitive	resistant
Sensitivity to lysozyme	sensitive	resistant
Color by Gram stain	Purple	pink

Cell wall function

- 1- Control passage of substances into and out of the cell.
- 2- Energy storage.
- 3- Harvest light energy in photosynthetic bacteria.
- 4- Selectively permeable.
- 5- Naturally impermeable to most substances.
- 6- Proteins allow substances to cross membrane.
- 7- Maintain concentration and electrical gradient.

Bacterial cytoplasmic membranes

Beneath the glycocalyx and the cell wall is a cytoplasmic membrane. It's about 8nm thick and composed of phospholipid bilayer and associated proteins.

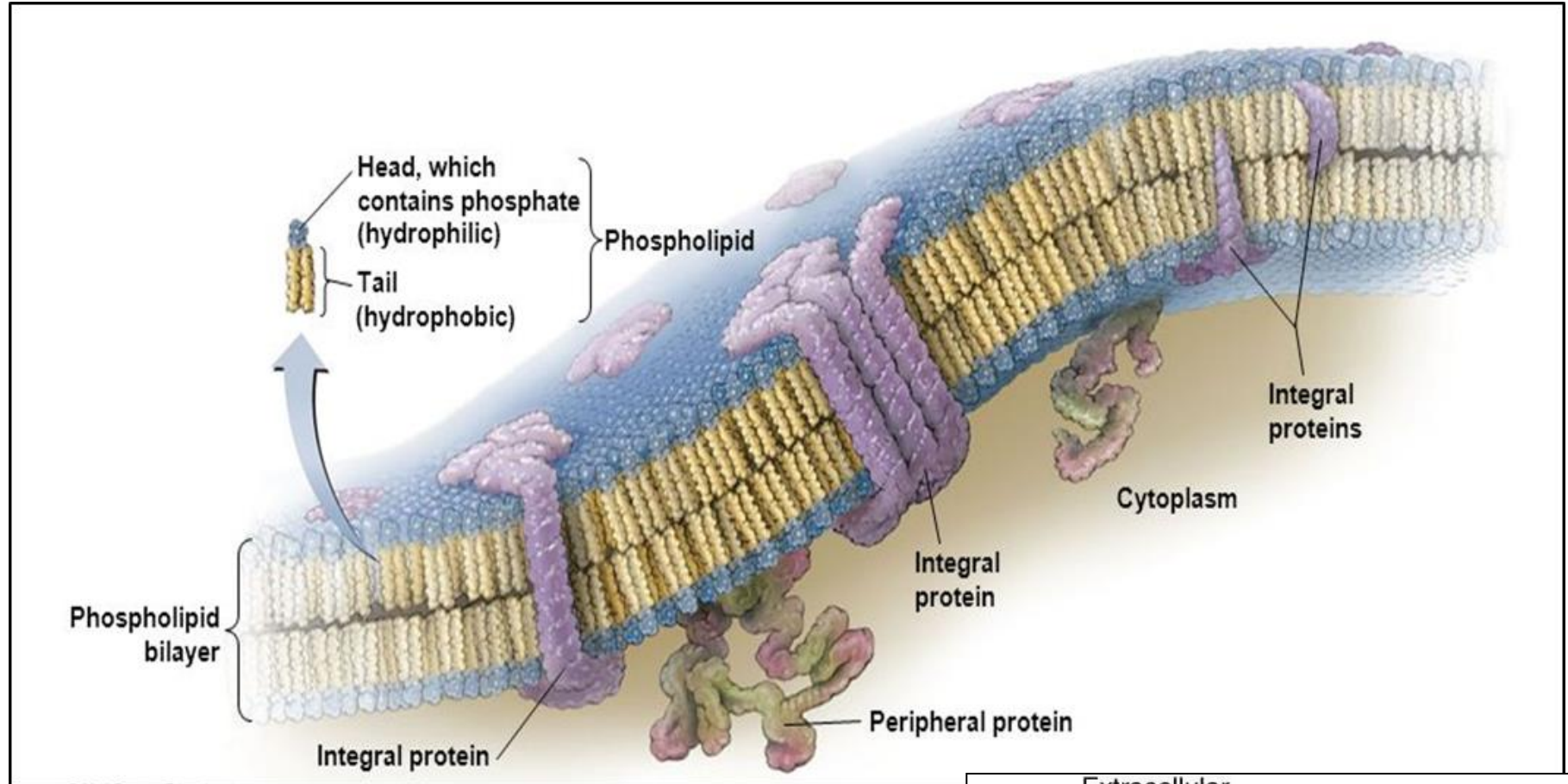
The cytoplasmic membrane of bacterial cells are flexible structure composed of phospholipids and proteins.

Structure:

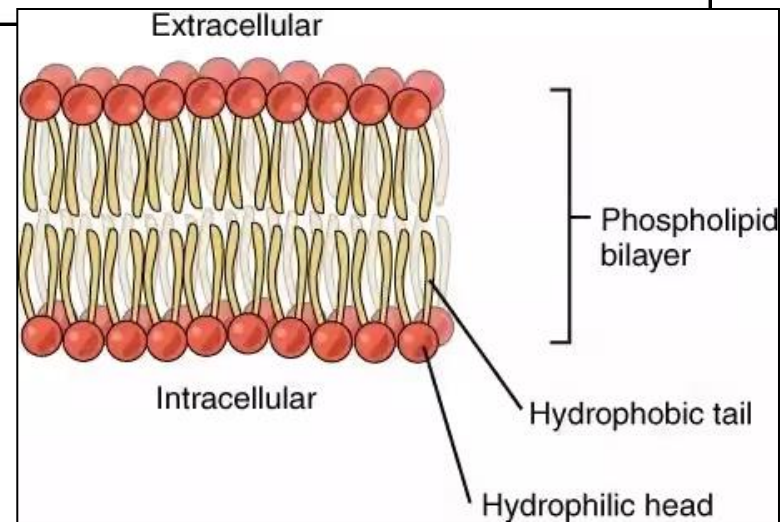
- 1- Referred to as phospholipid bilayer.
- 2- Composed of lipids and associated proteins (integral proteins and peripheral proteins).
- 3- Fluid mosaic model describes current understanding of membrane structure.

Function:

- 1- Control passage of substances into and out of the cell.
- 2- Energy storage.
- 3- Harvest light energy in photosynthetic bacteria.
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The structure of a prokaryotic cytoplasmic membrane: a phospholipid bilayer



Cytoplasm

- Cytoplasm, which is enclosed by the cytoplasmic membrane, is essentially an aqueous fluid containing the nuclear material, ribosomes, nutrients, enzymes and other molecules involved in synthesis, cell maintenance and metabolism.
- The nucleoid region.
 1. Circular loop of naked DNA.
 2. Plasmids.
- Ribosomes: protein factories, different from our own.
- Inclusions- other visible structures: like storage granules and vacuoles.

Nucleoid

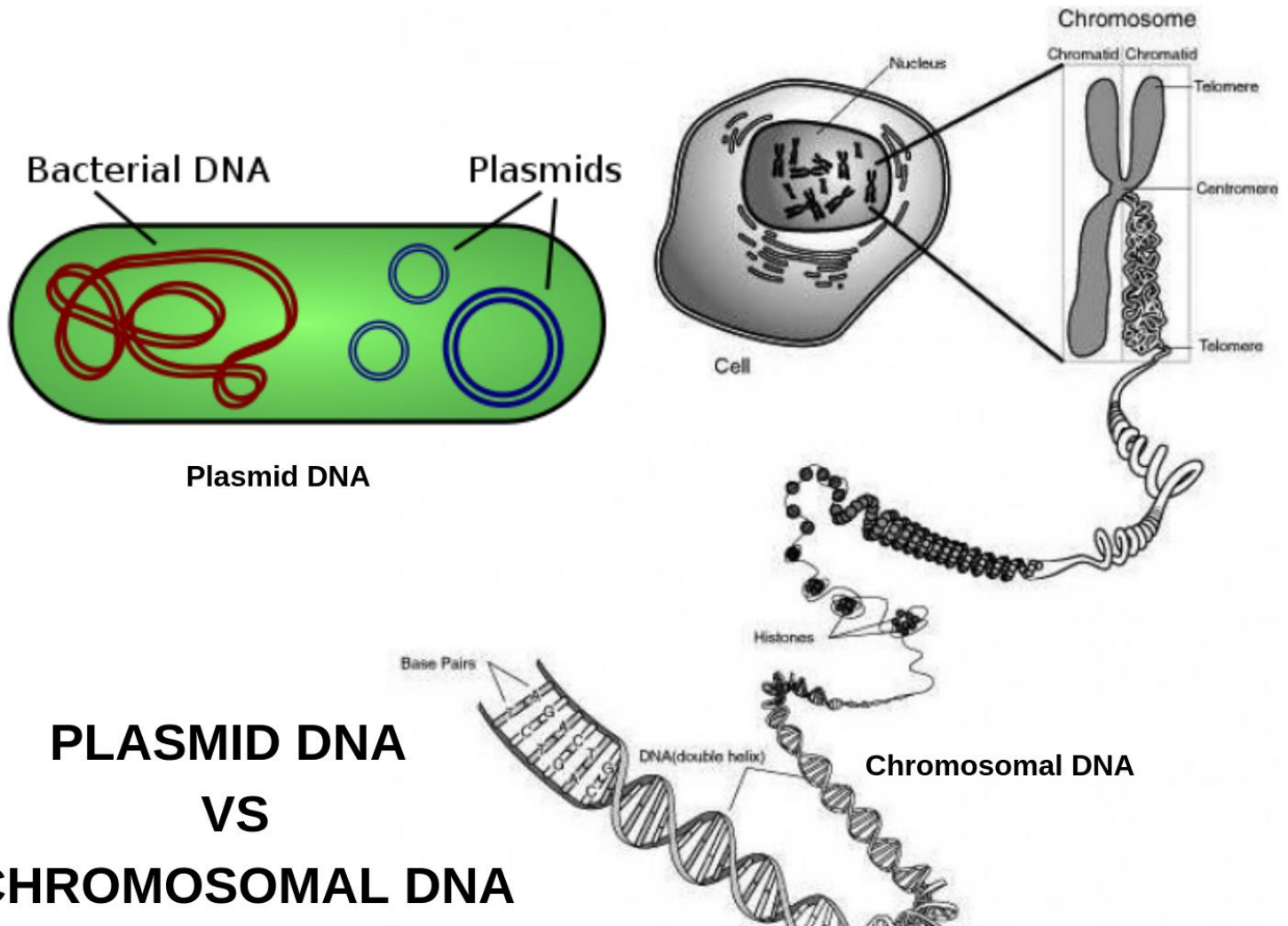
- The bacterial genome is composed of a single circular chromosome containing double-stranded DNA (bacterial chromosome), which carry all the information required for the cell structure and function.
- Bacterial chromosome are not surrounded by nuclear envelope and don't contain histones.
- Because of its length, the bacterial chromosome is extensively folded to form a dense body which can be seen by electron microscope.

Plasmid – Extrachromosomal DNA

Small circular pieces of double-stranded DNA which are separated from the genome.

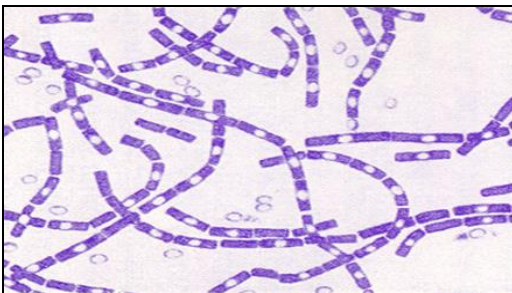
Copies of plasmids can be transferred from cell to cell during binary fission or through conjugation.

Plasmid DNA may code for characteristics such as antibiotic resistance and exotoxin production.



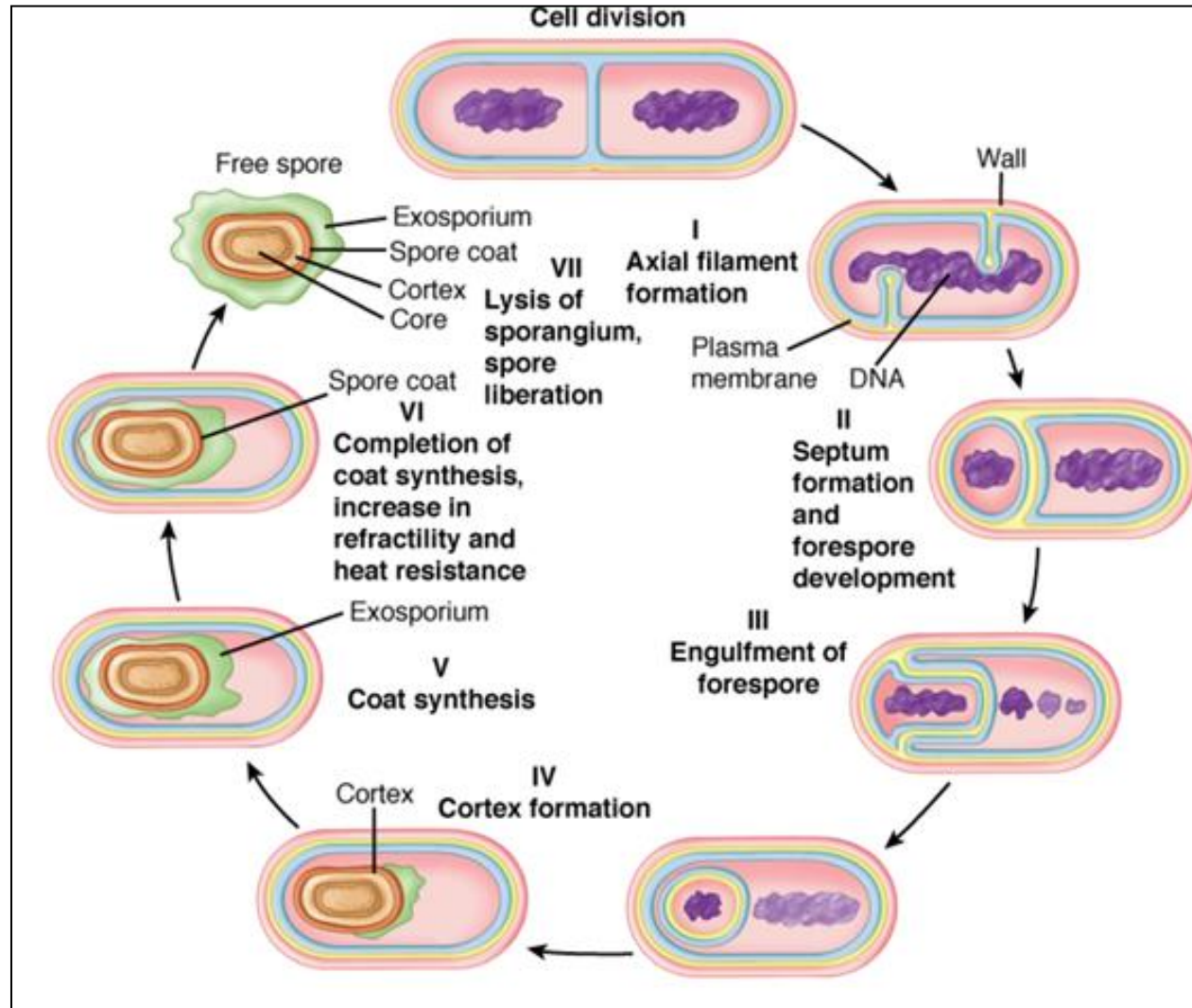
Endospores

- 1- Unique structure produced by some bacteria, contains all the important parts of the cell.
- 2- Defensive strategy against unfavorable condition.
- 3- Vegetative cells transform into endospores when nutrients are limited and the process of endospore formation called *sporulation*.
- 4- Resistant to extreme conditions such as heat, radiation, and chemicals.
- 5- Only made by certain bacteria (*Bacillus* and *Clostridium*) called endospore-forming bacteria that produce deadly toxins that cause such fatal diseases as *anthrax*, *tetanus* and *gangrene*.
- 6- Not reproduction !! No increase in #'s
- 7- The resistant of endospores is attributed to their layered structure, their dehydrated state, negligible metabolic activity and high content of dipicolinic acid (DPA) occurs in the spore wall with high amounts of calcium.
- 8- Because spores are thermostable they can be destroyed only by moist heat at 121°C for 15 minutes.



Bacillus anthracis

Formation of endospore



Q???

What is the process of **sporulation**?

It is the production of spores. The one vegetative cell forms a single spore, which, after germination, develops into a new cell.

What is **germination** in microbiology?

In bacteria, germination is the process in which a spore begins to grow vegetative cells.

Non-membranous organelles

(a) Ribosomes:

- 1- Sites of protein synthesis.
- 2- Composed of polypeptides and ribosomal RNA.
- 3- 70S ribosome composed of smaller 30S and 50S subunits.
- 4- Many antibacterial drugs act on bacterial ribosomes without affecting larger eukaryotic ribosomes.

(b) Cytoskeleton:

- 1- Composed of three or four types of protein fibers.
- 2- Play different roles in the cell:

Cell division

Cell shape

Segregation of DNA molecules

Movement through the environment

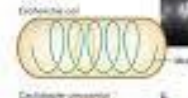
The Bacterial Cytoskeleton

Shape-determining proteins

- FtsZ = Forms a "Z ring" in spherical cells

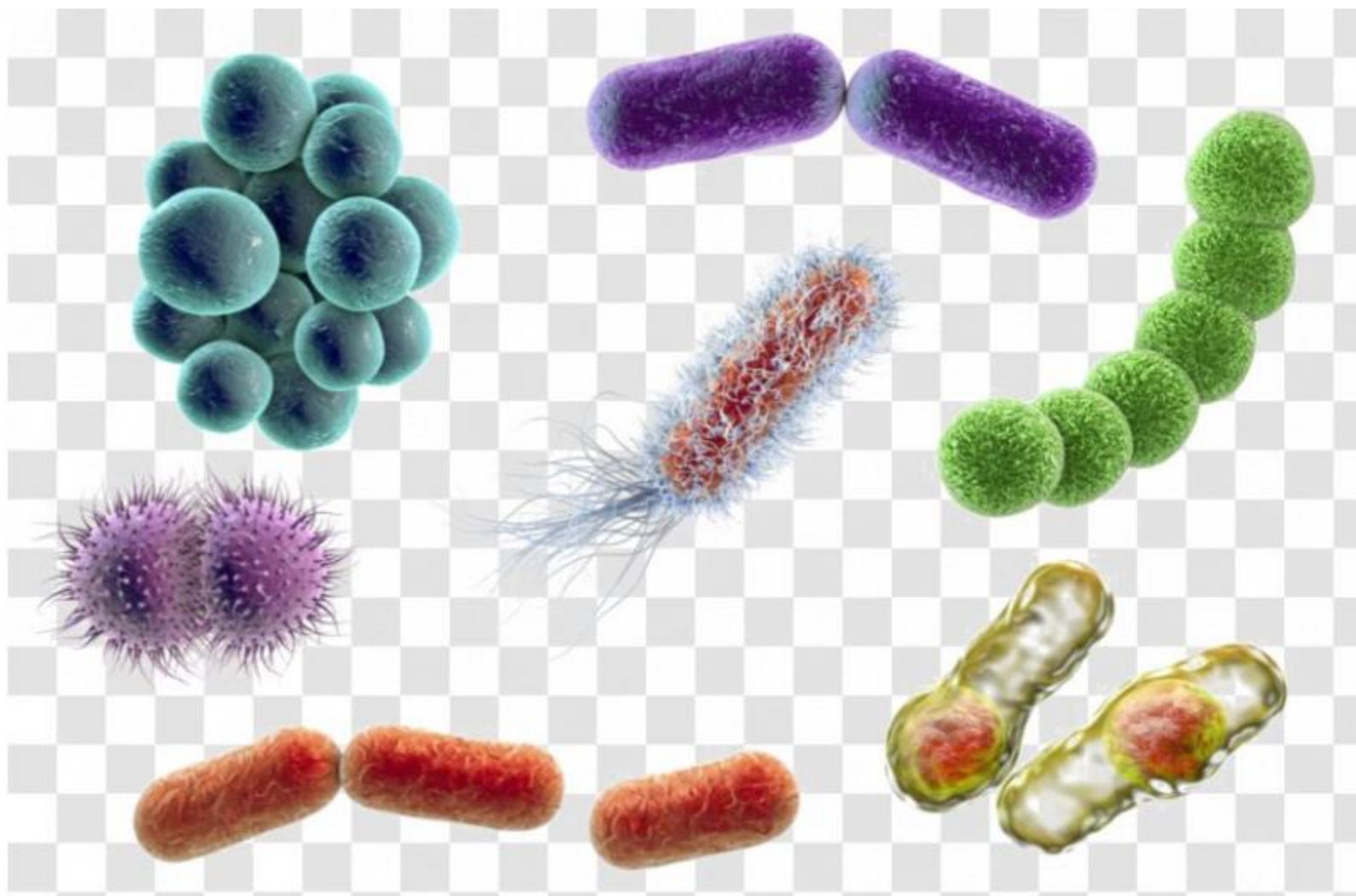


- MreB = Forms a coil inside rod-shaped cells



- CreS "Crescentin" = Forms a polymer along the inner side of crescent-shaped bacteria







كلية النور الجامعة
قسم الصيدلة
احياء مجهرية طبية ١
2022



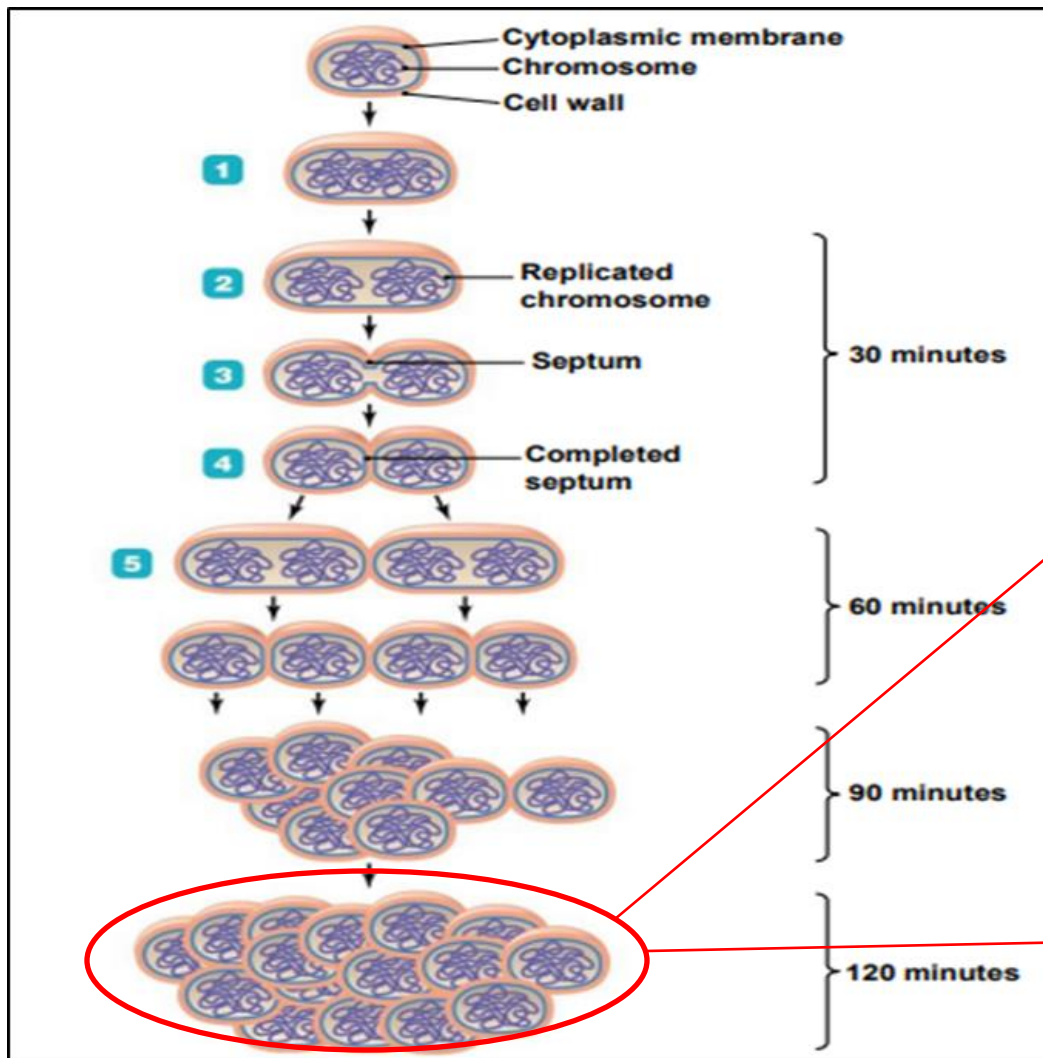
م.م ندى خيرى يونس الاسعدي
MS.c Medical Microbiology

Growth of microbial populations

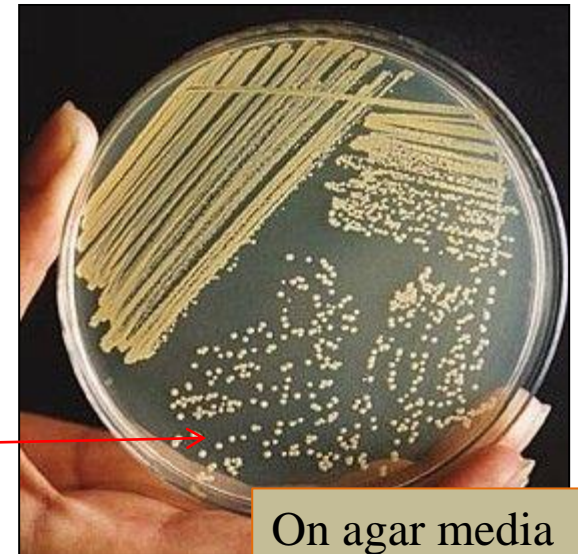
Most unicellular microorganisms reproduce by binary fission, a process in which a cell grows to twice its normal size and divides in half to produce two daughter cells of equal size.

Binary fission generally involves four steps:

- 1- The cell replicates its chromosome (DNA molecule).
- 2- The cell elongates and growth.
- 3- The cell forms a new cytoplasmic membrane and wall (septum) across the midline.
- 4- When the septum is completed, the daughter cells remain attached or they may separate completely.



In human tissues



On agar media

The events of binary fission, all the cell divide in parallel plane and remain attached to form a chain, or they may divide in different planes to form a cluster

Phases of microbial population growth

- When bacteria are inoculated in a liquid medium, there are four distinct phases to population's growth curve:

1- Lag phase

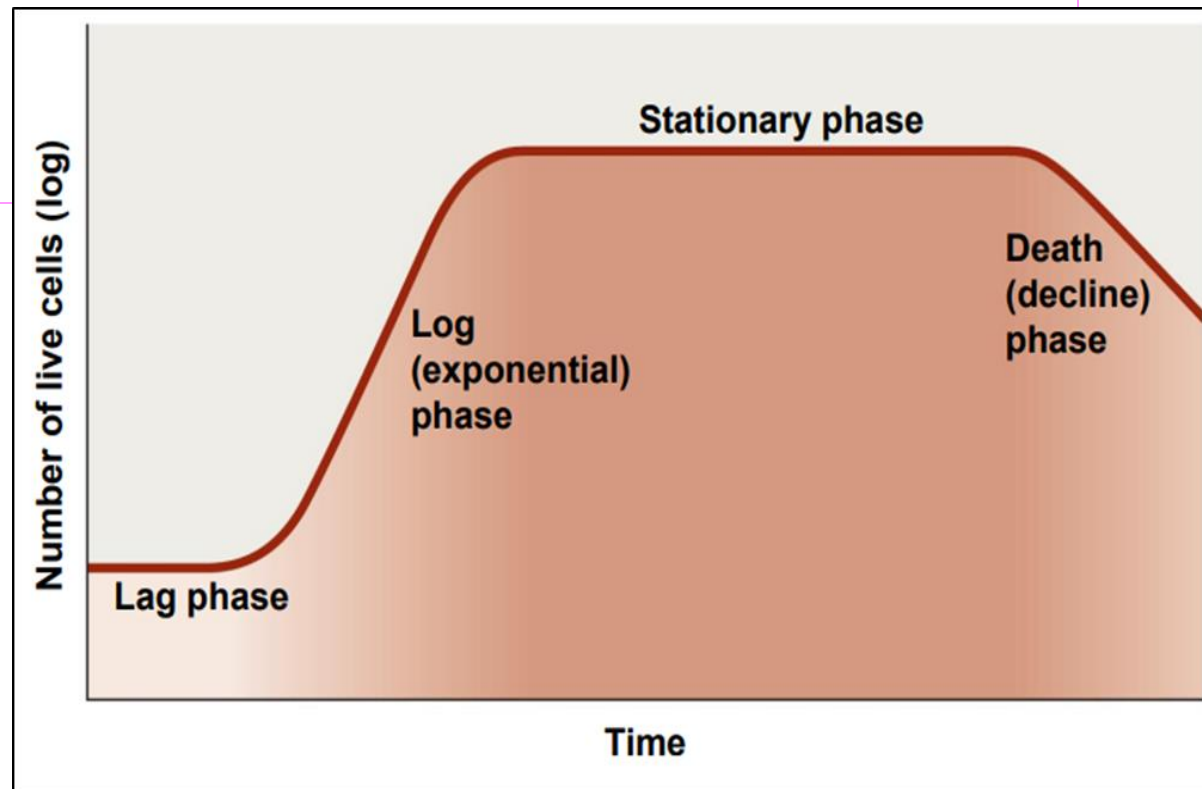
2- Log phase

3- Stationary phase

4- Death phase



A typical microbial growth



- 1) Lag phase: most cells do not reproduce immediately but instead actively synthesize enzymes to utilize novel nutrients in the medium.
- 2) Log phase: the population of bacterial cells increases and reproductive rate reaches a constant as DNA and protein syntheses are maximized.
- 3) Stationary phase: The rate of bacterial cell reproduction decreases, the number of dying cells equals the number of cells being produced, the nutrients are depleted and wastes accumulate, and the metabolic rate of surviving cells declines.
- 4) Death phase: If nutrients are not added and wastes are not removed, population reaches a point at which cells die at a faster rate than they are produced.

Growth requirements

✱ Organisms use variety of nutrients for their energy needs and to build organic molecules and cellular structures. Most common nutrients contain necessary elements such as carbon, oxygen, nitrogen, and hydrogen. Microbes obtain nutrients from variety of sources.

✱ Nutrients: can be classified into

1- Chemical requirements (Carbon, Oxygen, Nitrogen, and others)

2- Physical requirements (Temperature, pH, Osmotic pressure)

Chemical requirements

Carbon

they use in cell synthesis and maintenance

Energy source	Carbon source	
	Carbon dioxide	Organic substances
Light	<i>Photoautotrophs</i> Higher plants, algae, photosynthetic bacteria	<i>Photoheterotrophs</i> Purple and green bacteria
Chemical	<i>Chemoautotrophs</i> Energy from reduced organic compounds such as NH_3 , NO_2^- , H_2 , reduced forms of sulphur (H_2S , S , $\text{S}_2\text{O}_3^{2-}$) or ferrous iron	<i>Chemoheterotrophs</i> Carbon and energy can usually be derived from the metabolism of a single organic carbon

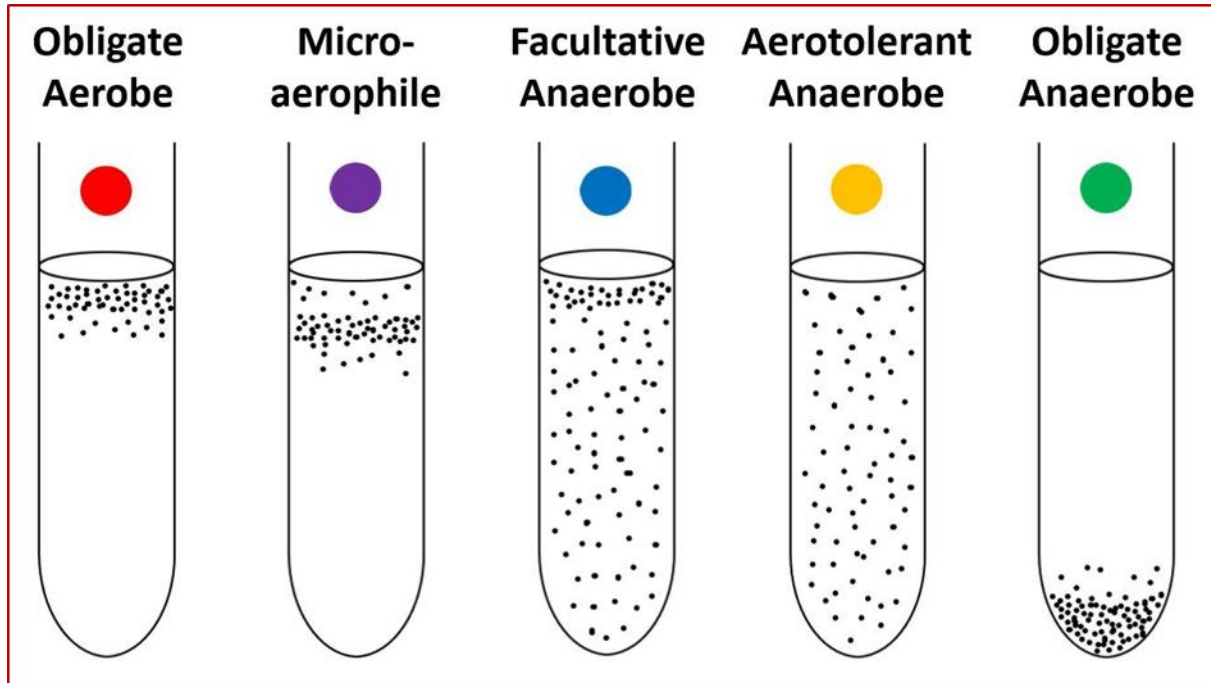
Classification of microorganisms based on the type of energy and carbon sources

Oxygen

Oxygen is essential for **obligate aerobes** bacterial cells because it serves as the final electron acceptor of electron transport chain, which produce most of the ATP in these organism. By contrast, Oxygen is a deadly poison for **obligate anaerobes**. Bacteria can produce enzymes like superoxide dismutase, catalase, peroxidase, and other antioxidants such as vitamin C & E to protect themselves against toxic oxygen products.

Bacteria can be identify into:

- 1- **Obligate aerobes**: Oxygen is essential for growing. e.g. *Mycobacterium tuberculosis* (TB)
- 2- **Obligate anaerobes**: Oxygen is deadly poison for bacterial cells. e.g *Clostridium spp*
- 3- **Facultative anaerobes**: Some aerobic bacteria can maintain life via fermentation or anaerobic respiration, through their metabolic efficiency is often reduced in the absence of oxygen. e.g. *Escherichia coli*.
- 4- **Aerotolerant anaerobes**: Bacteria do not use aerobic metabolism, but they tolerant oxygen by having some of enzymes that detoxify oxygen's poisonous form. e.g *Lactobacillus spp*.
- 5- **Microaerophiles**: such as the ulcer- causing pathogen *Helicobacter pylori*, require oxygen level of 2% to 10%. This concentration of oxygen is found in the stomach. Microaerophiles are damaged by the 21% concentration of oxygen in the atmosphere, because they have limited ability to detoxify hydrogen peroxide H_2O_2 .



Identification of bacteria according to oxygen requirements

Nitrogen

- Another essential element is nitrogen, which is contained in many organic compounds, including the amino group of amino acids and as part of nucleotide bases. Nitrogen makes up about 14% of the dry weight of microbial cells.
- Nitrogen is often a growth-limiting nutrient for many organisms; that is, their anabolism ceases because they do not have sufficient nitrogen to build proteins and nucleotides.
- *Other chemical requirements*
- Together, carbon, oxygen, and nitrogen make up more than 95% of the dry weight of the cells; phosphorous (P), sulfur (S), calcium (Ca), manganese (Mn), magnesium (Mg), copper (Cu), iron (Fe), and a few other elements constitute the rest.

Physical requirements

In addition to chemical nutrients, organisms have physical requirements for growth, including specific conditions of temperature, pH, and osmolarity.

Temperature

Temperature plays an important role in microbial life through its effects on the configuration of microbial molecules (proteins).

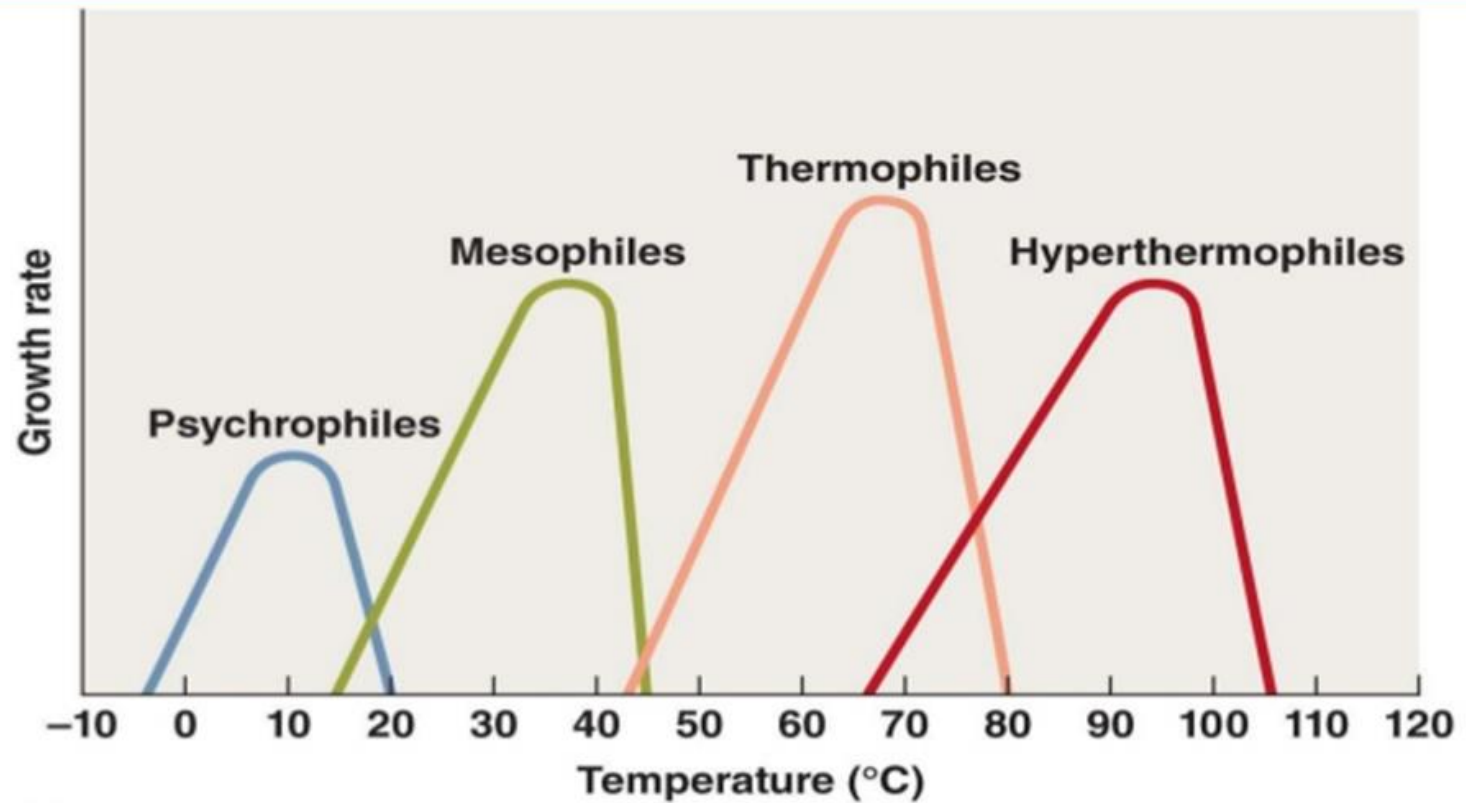
Based on their preferred temperature ranges, microbes can be categorized into four groups:

1- **Psychrophiles**: cells grow best at temperature below about 15°C and can even continue to grow at temperature below 0°C. They die at temperatures much above 20°C. There are some types of bacteria can grow in snowfields, ice, and cold water. They do not cause diseases in humans because they cannot thrive at body temperature; some do cause food spoilage in refrigerators.

2- **Mesophiles**: cells grow best at temperatures range from 20°C to 40°C. Because normal body temperature is 37°C, human pathogens are mesophiles.

3- **Thermophiles**: cells grow at temperature above 45°C.

4- **Hyperthermophiles**: some organisms can grow at temperature 80°C and 100°C. They called Heat-loving organisms, do not cause disease because they “freeze” at body temperature.



Four categories of microbes based on temperature ranges for growth

pH

- pH: is a measure of the concentration of hydrogen ions in a solution; that is, it is a measure of the acidity or alkalinity of substances
- Organisms are sensitive to changes in acidity because hydrogen ions and hydroxyl ions interfere with hydrogen bonding within proteins and nucleic acid; as a result, organisms have ranges of acidity that they prefer and can tolerate.
- Most bacteria and protozoa, including most pathogens, grow best in a narrow range around a neutral pH _that is, between pH 6.5 and 7.5, which is also the pH range of most tissues and organs in human body.

Organisms can be identified into:

- 1- **Neutrophiles**: bacteria like to grow between pH 6.5 – 7.5.
- 2- **Acidophiles**: bacteria can grow below pH 7.2.
- 3- **Alkaliphile**: bacteria grow in media up to pH 11.5

Osmotic pressure

Osmosis: is the diffuse of water across a membrane and is driven by unequal solute concentrations on the two sides of such a membrane.

According to this osmotic pressure, bacteria classified into:

1- **Obligate halophiles**: bacteria are adapted to growth under high osmotic pressure such as exists in the Great Salt Lake. They may grow in up to 30% salt and will burst if placed in freshwater.

2- **Facultative halophiles**: this bacteria although they do not require high salt concentrations, they can tolerate them .e.g *Staphylococcus aureus* can tolerate up to 20% salt, which allow it to colonize the surface of the skin_ an environment that too salty for most microbes.

Microbial genetics

- **Genetics:** is the study of inheritance and inheritable traits.
- **Genes:** are composed of specific sequences of nucleotides that code for polypeptides or RNA molecules.
- **Genome:** is the sum of all the genes and linking nucleotide sequences in a cell.
- A typical prokaryotic chromosome consists of circular molecule of DNA localized in region of the cytoplasm called **nucleoid**. With the absence of membrane surrounds a nucleoid. Chromosomal DNA is folded into loops that are 50,000 to 100,000 bp long held in place by molecules of protein and RNA.
- **Plasmids:** are a small molecule of chromosome, circular and 1% to 5% of the size of a prokaryotic chromosome.

Researchers have identified many types of plasmids (sometimes called factors):

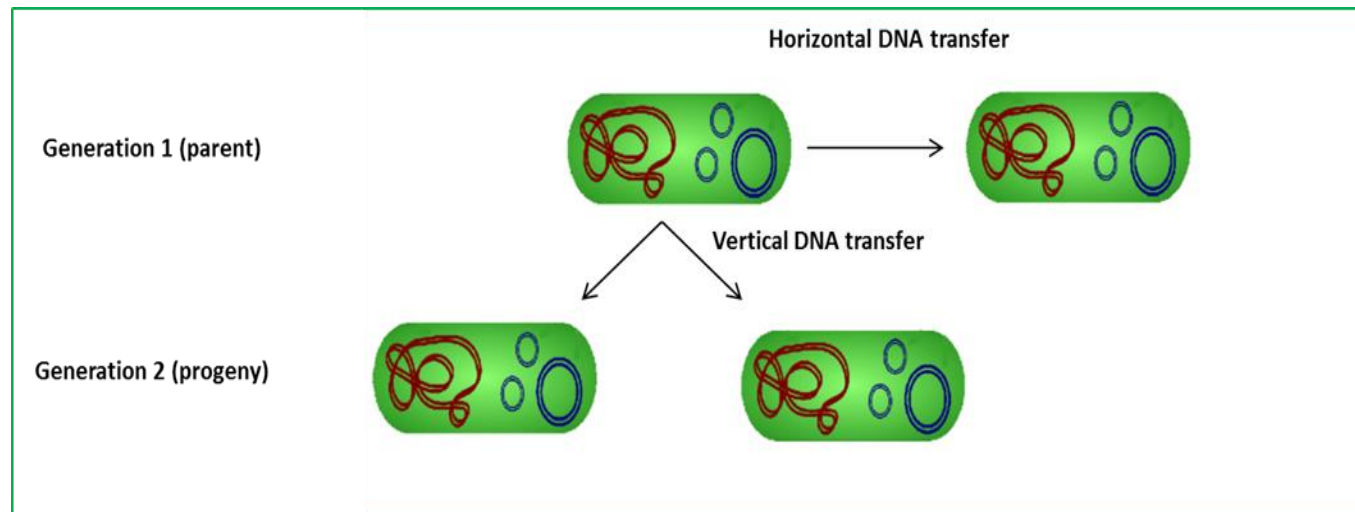
- 1- *Fertility (F) plasmid*: carry instruction for conjugation.
- 2- *Resistance(R) plasmid*: carry genes for resistance to or more antimicrobial drugs.
- 3- *Bacteriocin plasmid*: carry genes for proteinaceous toxins.
- 4- *Virulence plasmid*: carry instructions for structures, enzymes, or toxins that enable a bacterium to become pathogenic.

Gene transfer among bacterial cell

- Normally, genes and the characteristics they code for are passed down from parent to progeny.

This is called

1) vertical gene transfer (the passing of genes to the next generation) and is why you have half of the characteristics of your mother, and half of your father. Bacteria and some lower eukaryotes are unique in that they can pass DNA from one cell of the same generation to another. We refer to this as Horizontal Gene Transfer.



2) Horizontal gene transfer

- Many prokaryotes acquire genes from other microbes of the same generation_ a process termed horizontal gene transfer, a **donor cell** contributes part of its genome to a **recipient cell**, which may be a different species from the donor.
- Typically, the recipient cell inserts part of the donor's DNA into its own chromosome, becoming a **recombinant cell**. Cellular enzymes then usually degrade unincorporated DNA.

There are three types of horizontal gene transfer:

- (a) Transformation (naked DNA uptake by bacteria)
- (b) Transduction (bacterial DNA transferred by viruses -phage-)
- (c) Conjugation (DNA transfer between bacterial cells)

(a) **Transformation:** Bacteria are capable of taking up DNA directly from their environment and incorporating it into their genomes. This process is known as natural transformation.

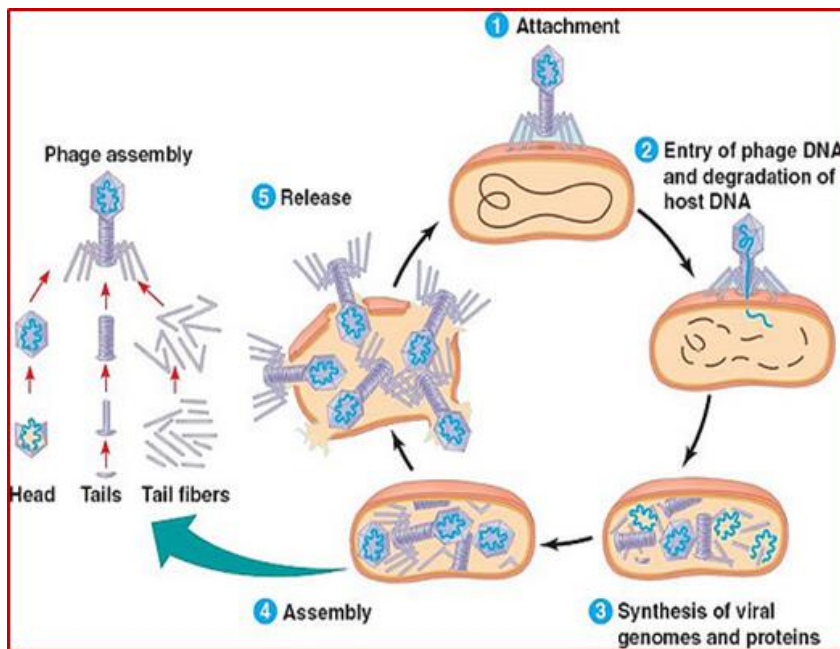
This DNA usually comes from dead bacteria lysing (splitting open) and releasing their genetic contents into the surrounding area.

(b) **Transduction:** is the transfer of DNA from one cell to another by a virus. These viruses are known as bacteriophage and they specifically infect bacteria. Bacteriophage don't have the machinery to replicate their own genomes or express their own genes, so instead, they hijack the bacterial machinery to do so.

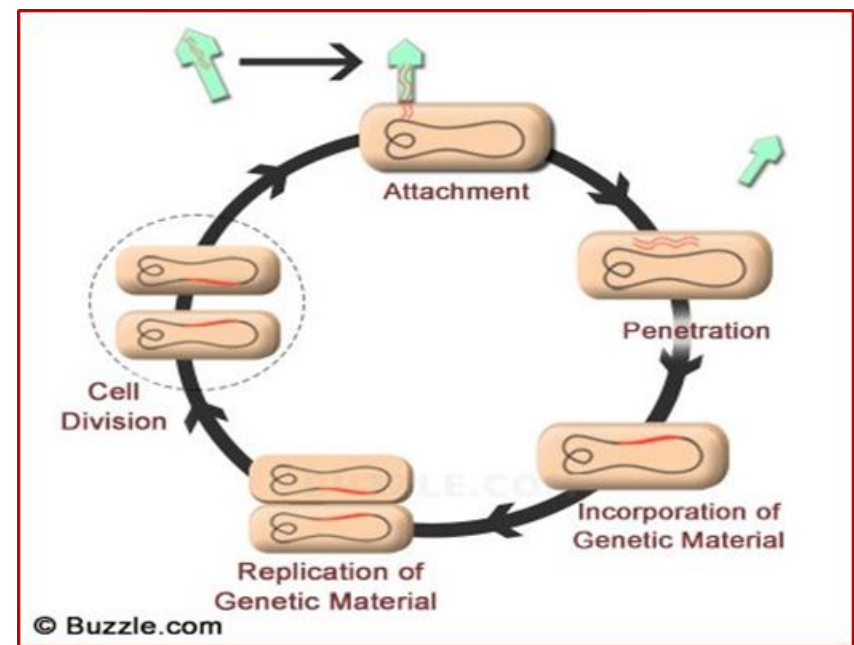
Host cells will continue to express phage proteins and replicate the phage genome forming new virus particles. This process continues until the cell is so full of phage particles that it splits open (**lyses**), releasing phage into the surrounding area. This is known as the **lytic cycle**.

Some phage can switch between this life cycle and a state of **lysogeny**, where they combine their genome with the bacterial chromosome, and remain silent for many generations. When lysogenic phage remove (excise) their genomes from the host chromosome, they occasionally take small sequences of bacterial DNA with them.

Phage genome containing bacterial DNA is then packaged into phage coat proteins to form a complete, recombinant virus particle. When these phage lyse the bacterial cell and re-infect a new host, they take bacterial DNA with them.

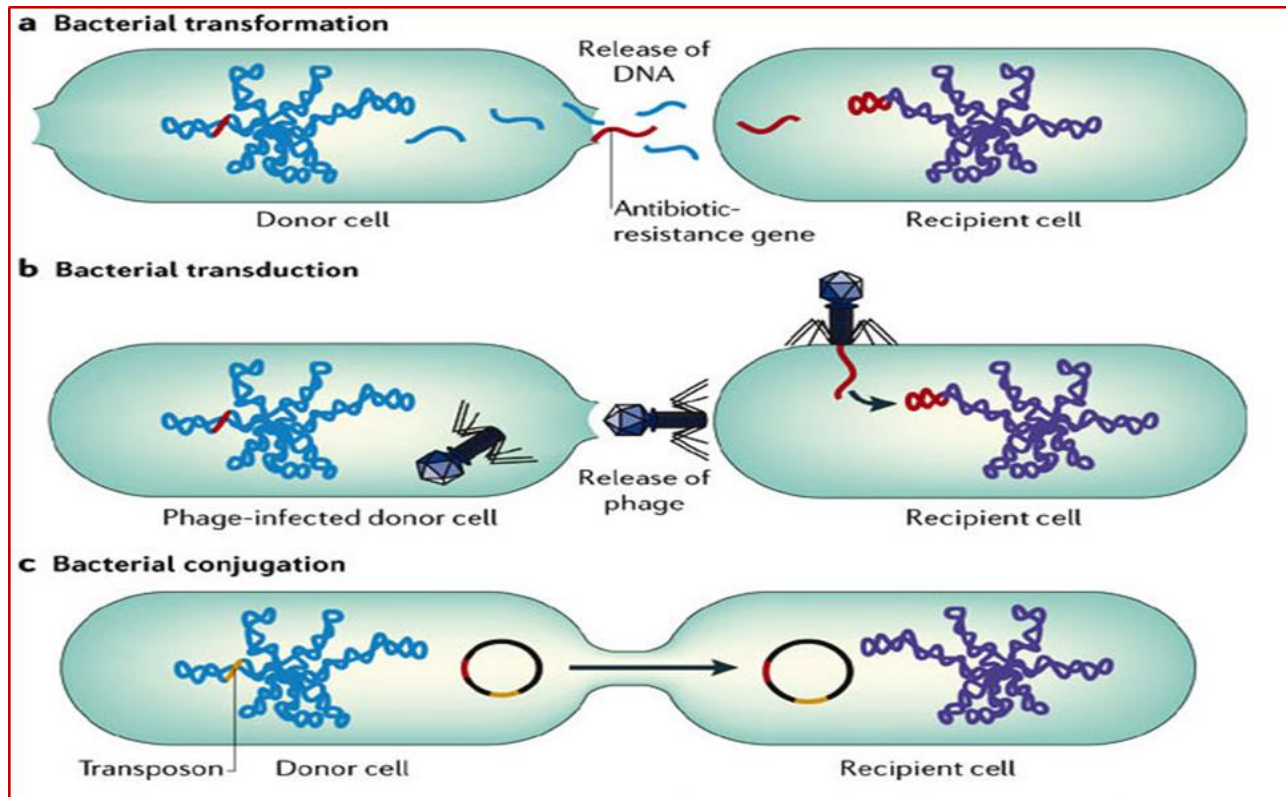


Lytic cycle



Lysogenic cycle

(c) **Conjugation** is the transfer of DNA directly from one cell to another through cell-cell contact. The DNA transferred by conjugation often involve plasmids. Plasmids are circular pieces of DNA that can replicate in the bacterial cell, independently of the chromosome. The conjugative transfer of plasmids is carried out by cell surface structures that act like syringes, injecting the plasmid into neighboring cells.



The three ways bacteria can acquire DNA horizontally. Bacteria are acquiring genes which make them resistant to antibiotics.

THANK YOU

The text 'THANK YOU' is rendered in a bold, purple, serif typeface. It is centered horizontally and features a subtle, semi-transparent reflection directly beneath it, creating a slight 3D effect. The background is a solid, light cream or off-white color.



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2022-2023

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MS.c Medical Microbiology

Mutation

Mutation is a change in a DNA sequence.

Mutation can result from DNA copying mistakes made during cell division, exposure to ionizing radiation, exposure to chemicals called **mutagens**, or infection by viruses.

Features of mutation

- 1- Mutations may or may not produce visible changes in the observable characteristics (phenotype) of an organism.
- 2- Mutations play a part in both normal and abnormal biological process including: evolution, cancer, and the development of immune system.
- 3- Mutation in genes can have no effect, alter the product of the gene, or prevent the gene from functioning properly or completely.
- 4- Some mutations may improve an organism's survival (beneficial) example: Antibiotic resistant.

Types of mutation:

Small changes results from mutations:

1) Point mutation

Point mutation: in which just a single nucleotide base pair is affected.

Point mutation types: two types:

1- Substitutions: when one or more bases in the sequence is replaced by the same number of bases.

2- Frameshift mutations:

a) Inversion: when a segment of a chromosome is reversed end to end.

b) Insertions: when a bases is added to the sequence.

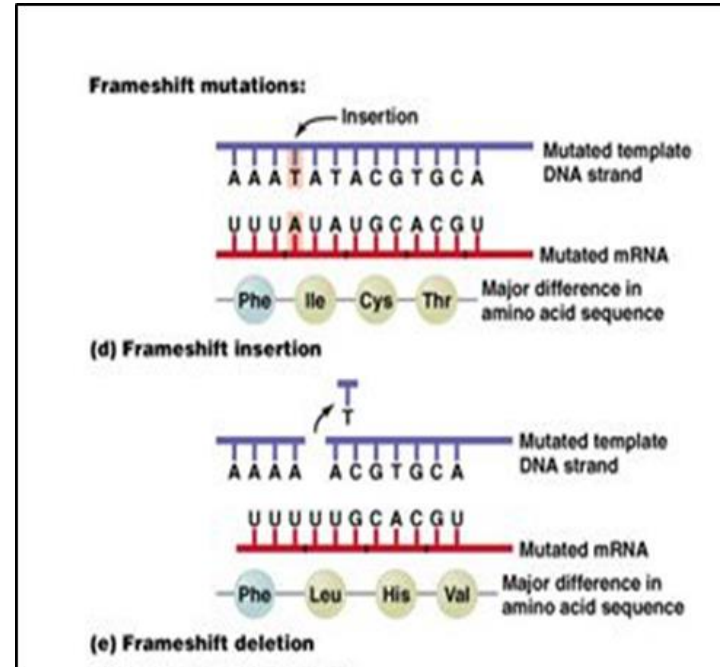
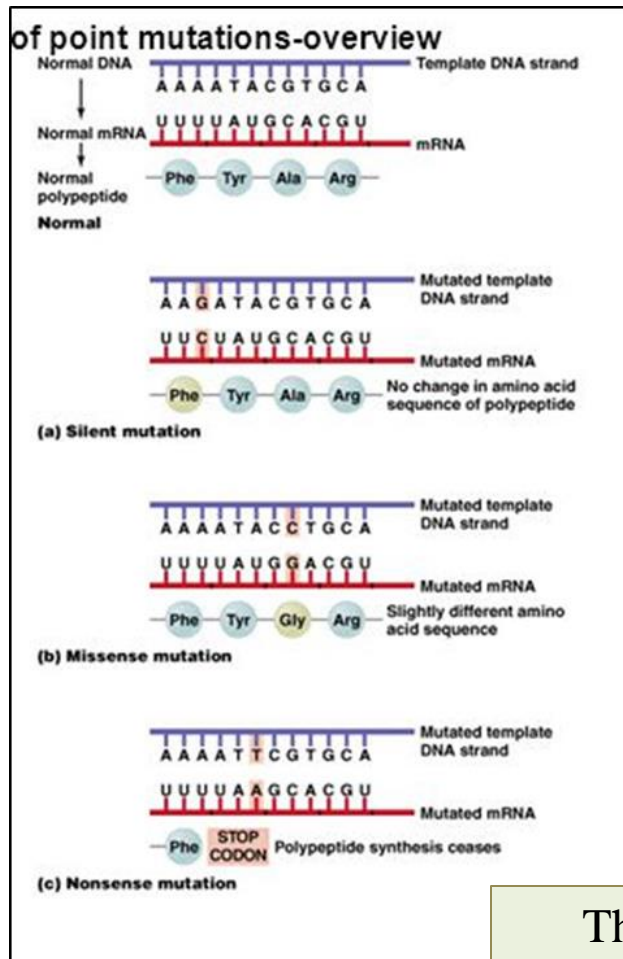
c) Deletions: when a base is deleted from the sequence.

Point mutation can be categorized by their effects into:

1) Silent mutations: when there's a change in the sequence of nucleotide bases that make up DNA, but that doesn't change the amino acid or the function of the protein as a whole. There is no change in the phenotype- the mutation is silent because it affect the genotype only.

2) Missense mutations: some changes cause a change in our amino sequence. These could bring about slight changes, or major changes. However, they definitely cause a change in our amino acid sequence.

3) Nonsense mutations: Some mutations lead to the coding of a STOP codon, instead of a normal amino acid. This leads to formation of shorter protein sequences which maybe dysfunctional, or not functional at all.



The effects of the various types of point mutation

- Normal gene.
- Base pair substitutions can result in silent mutation.
- Missense mutation.
- Nonsense mutation.
- Frameshift insertions.
- Frameshift deletions.

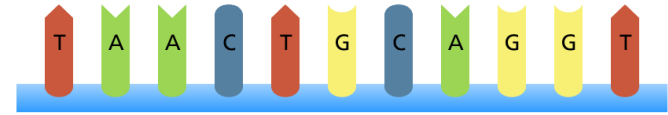
Large changes occurs in cell by mutations

Cross mutations

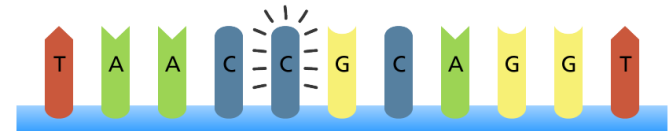
Cross mutations: include major changes to the DNA sequence, such as:

- a) **Deletions**: in which a part of a chromosome or a sequence of DNA is lost during DNA replication.
- b) **Insertions**: the addition of one or more nucleotide base pairs into a DNA sequence.

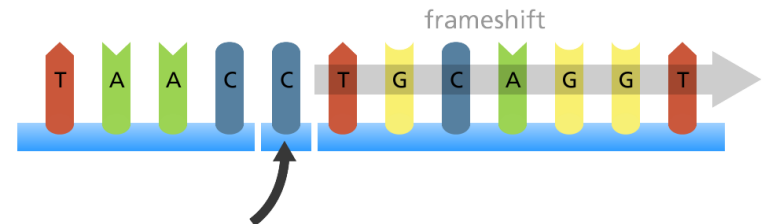
Original sequence



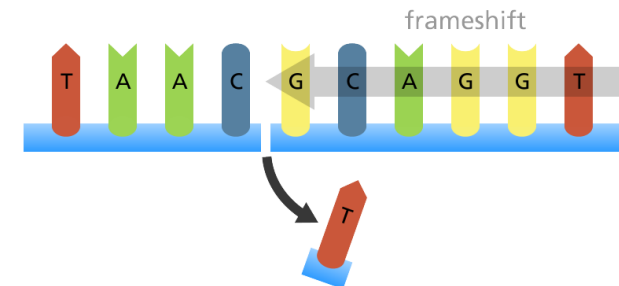
Base substitution



Base addition



Base deletion





Original chromosome



Deletion



Duplication



Translocation



Inversion

Conclusion

- Changes in the DNA can change the proteins made by the cell.
- A random change in the sequence of nucleotides in DNA is called a mutating.
- Some mutations have little or no effect on the organism, others are harmful and very few are beneficial.
- Still other proteins are used as activators or repressors, turning genes on or off.
- Therefore, a change in a cell's proteins could have dramatic effects on the cell's structure or function.
- Gene mutations are errors that occur within individual genes in a chromosome.
- Gene mutations can involve a single nucleotide or they can affect sections of DNA that include many nucleotides.
- The deletion or addition of nucleotides that disrupts codons is called a frameshift mutation.
- Because mRNA is read in codons (three-base sections) during translation, an addition or deletion of nucleotides can alter the sequence of bases, or reading frame, of the genetic message.

Recombinant DNA and biotechnology

“Genetic engineering”

Biotechnology: is the use of microorganisms to make useful products such as bread, wine, and cheese.

During the 20th century, scientists industrialized the natural metabolic reactions of bacteria to make large quantities of acetone, butanol, and antibiotics.

Recombinant DNA technology: is a new type of biotechnology in which scientists change the genotypes and phenotypes of organisms to benefit humans. Today, scientists isolate specific genes from almost any so-called donor organism, such as bacterium or plants and insert it into the genome of almost any kind of recipient organism.

The role of recombinant DNA technology in biotechnology

Scientists who manipulate genomes have three main goals:

- ✳ To eliminate undesirable phenotypic traits in humans, animals, plants and microbes. For example, scientists have inserted genes from microbes into plants to make them resistant to pests or freezing, and since 1999 they have cured children born with a fatal and previously untreatable genetic disorder called severe combined immunodeficiency disease (SCID).
- ✳ To combine beneficial traits of two or more organisms to create valuable new organisms, such as laboratory animals that mimic human susceptibility to HIV.
- ✳ To create organisms that synthesize products that humans need, such as vaccines, antibiotics, hormones, and enzymes. For instance, gene therapists have successfully inserted the human gene for insulin into bacteria so that the bacteria synthesize human insulin.

Overview of recombinant DNA technology

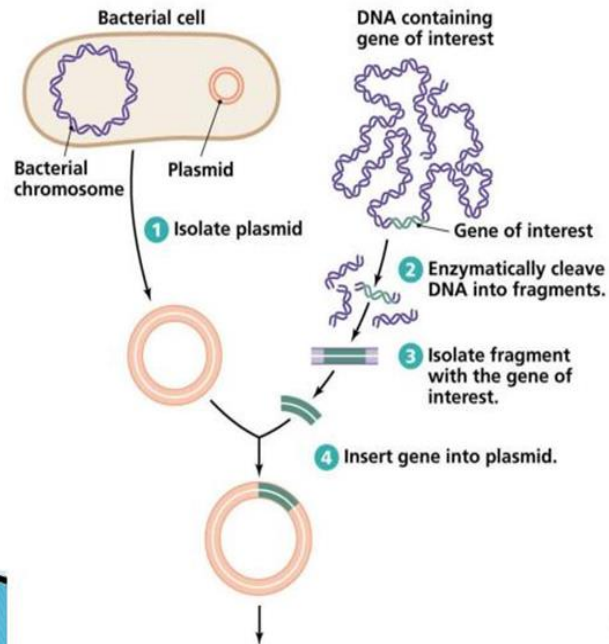


FIGURE 8.1.1

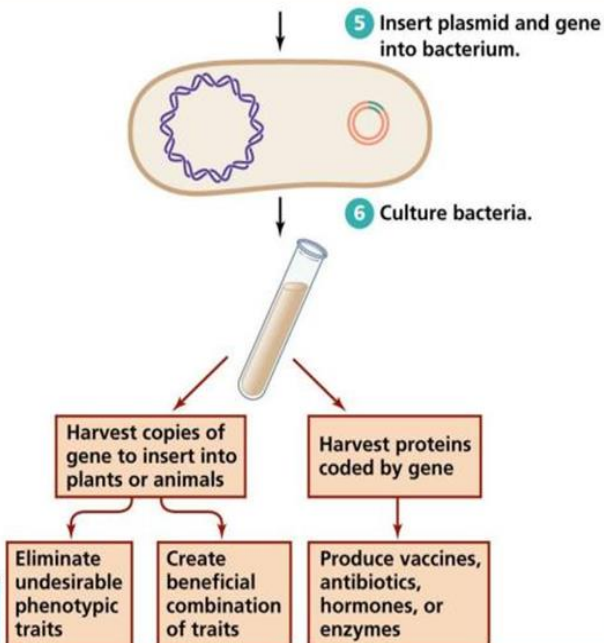


FIGURE 8.1.2

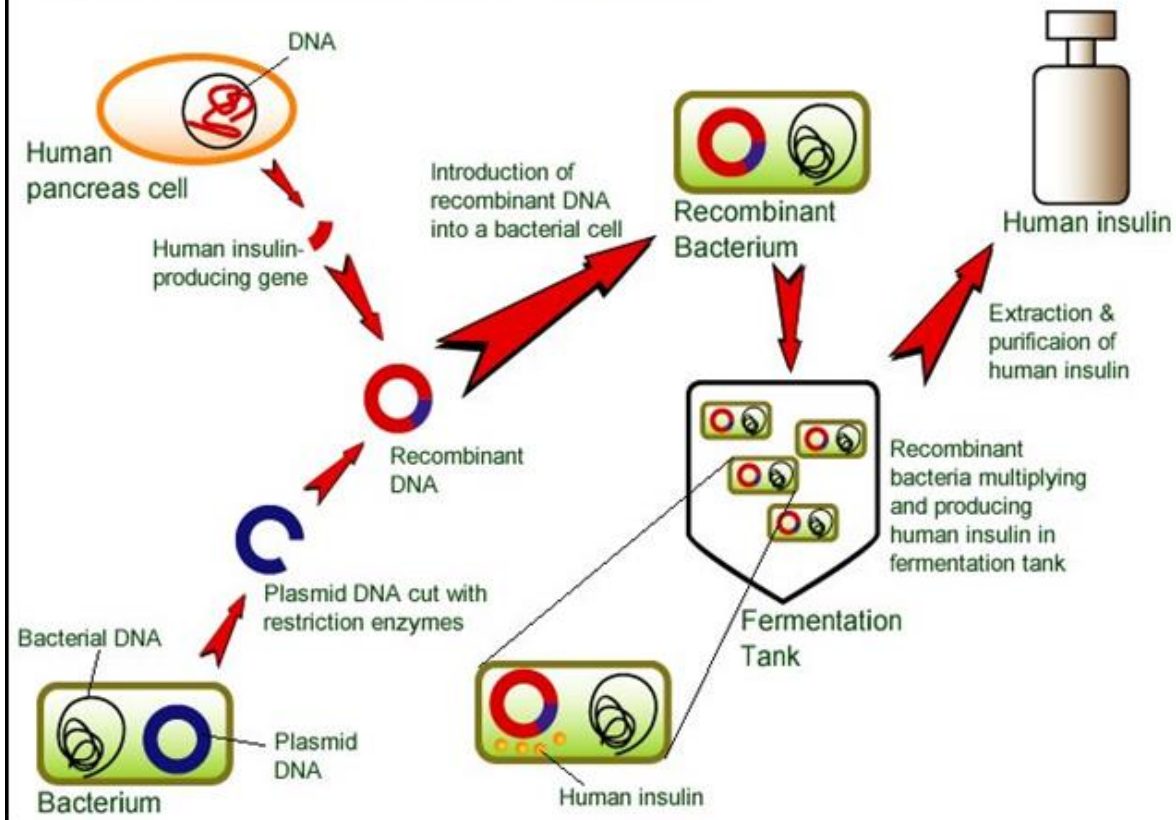
Human Insulin Production

The hormone insulin is essential for the control of blood sugar levels. Diabetes mellitus is a disease in which some people cannot make insulin themselves. This disease kills many people in the world every year. Insulin has been used in the treatment of diabetes mellitus since 1922 when Leonard Thompson became the first human to receive an injection of man-made insulin.

Production of Genetic Engineered Insulin:

1. Human insulin is extracted from pancreas cells and an insulin-producing gene is isolated.
2. A plasmid DNA is extracted from a bacterium and cut with restriction enzyme, forming plasmid vector.
3. Insert human insulin-producing gene into the bacterial plasmid vector to form the recombinant DNA of human insulin-producing gene.
4. Introduce this recombinant DNA into a bacterial cell to form the recombinant bacterium.
5. The recombinant bacteria multiply in a fermentation tank and produce human insulin.
6. Insulin is extracted, purified and bottled. It is then ready to be injected into diabetic patients.

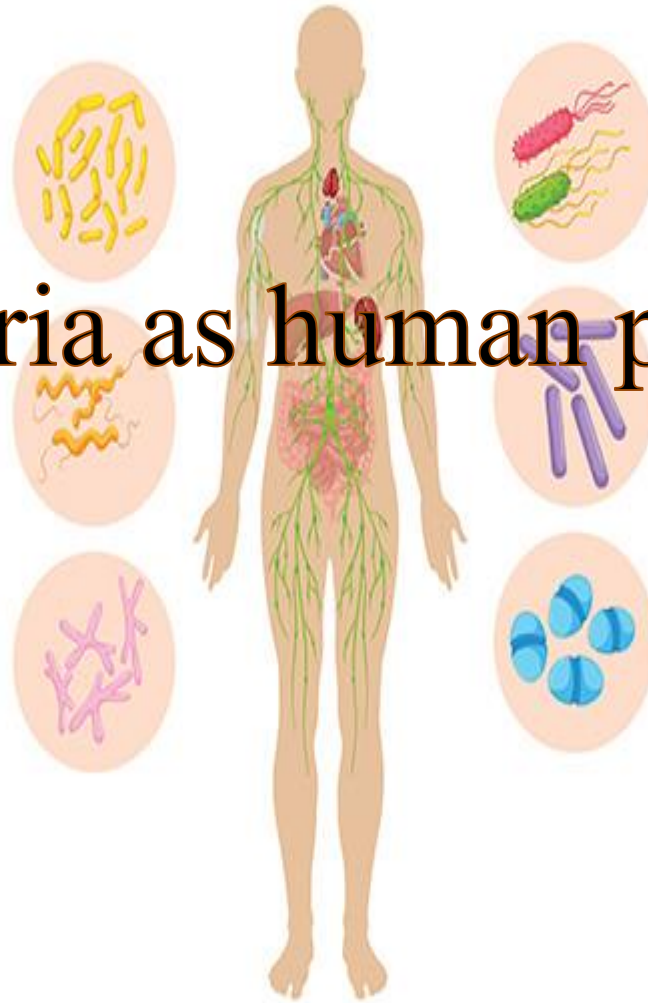
Human Insulin Production



Pharmaceutical and therapeutic applications

- Scientists now use recombinant DNA technology to synthesize selected proteins, produce vaccines, screen for genetic diseases, match DNA specimen to the organisms from which they came, treat genetic illnesses, and aid in organ transplantation.
- **Gene therapy**
- An exciting use of recombinant DNA technology is gene therapy, in which missing or defective genes are replaced with normal copies. Theoretically, physician could remove a few genetically defective cells – for example, cells that produce a defective protein- from a patient, insert normal genes, and replace the cells into the patient-curing the disease. Alternatively, plasmid or viral vectors could deliver genes directly to target cells within a patient.
- Unfortunately, gene therapy has proven difficult in practice because of unexpected results. Specially, some patient's immune systems react out of control to the presence of vectors, resulting in the death of the patient.
- Nevertheless, doctors have successfully treated patients for severe immunodeficiency disease. Other diseases that respond well to gene therapy are cystic fibrosis, sickle cell anemia, and some types of hemophilia and diabetes.

Bacteria as human pathogens

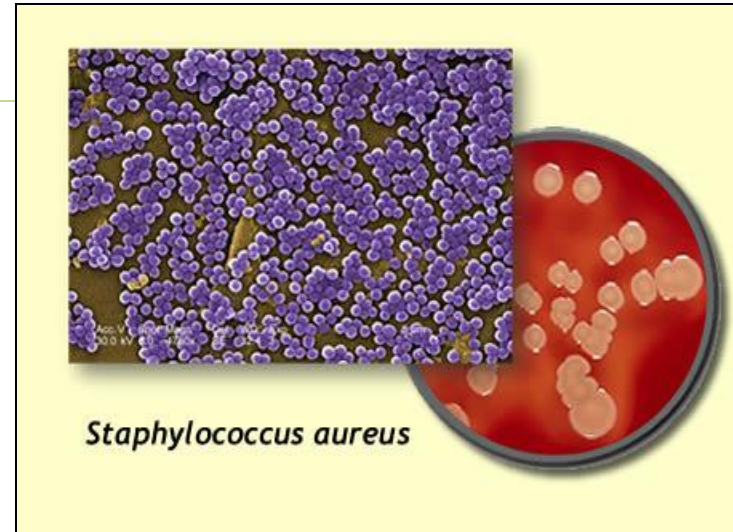


STAPHYLOCOCCUS



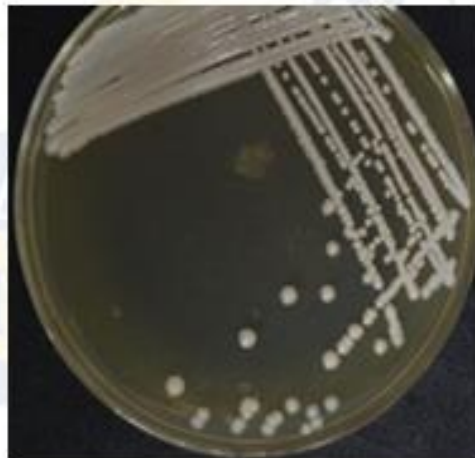
Staphylococcus : General characteristics

- Normal flora of every human
- Gr + cocci in cluster
- Catalase +
- Facultative anaerobe
- Tolerant of salt and desiccation.
- Additionally, Staphylococci cells are tolerant of drying out, solar radiation, and heat (up to 60°C for 30 min), allowing them to survive on environmental surfaces in addition to skin.
- Two species are commonly associated with staphylococcal diseases in humans:
 - *Staphylococcus aureus* ----- more virulent , some people are "carriers" in nose and on skin, MRSA is a resist form of this bacteria
 - *Staphylococcus epidermidis*----- normal microbiota of human skin,



Staphylococcus bacteria show pigments colony on agar surface:

- 1- Golden yellow colonies .e.g. *Staphylococcus aureus*
- 2- White colonies .e.g. *Staphylococcus epidermidis* & *S. albus*
- 3- Lemon yellow colonies *Staphylococcus citreus*



The most important species from the viewpoint of human medicine is *S. aureus*.

S. aureus

S. epidermidis

S. citreus

Pathogenicity

- A number of extracellular enzymes and exotoxins such as **coagulase, alphatoxin, leukocidin, exfoliatins, enterotoxins, and toxic shock toxin** are responsible for the clinical symptoms of infections by this pathogen, which are observed in the three types:
 - (1) invasive infections.
 - (2) pure toxicoses.
 - (3) mixed forms.
- The antibiotics of choice for therapy of these infections are penicillinase-resistant penicillins. Laboratory diagnosis involves identification of the pathogen by means of microscopy and culturing.
- *S. aureus* is a frequent pathogen in nosocomial infections and limited outbreaks in hospitals. Hand washing by medical staff is the most important prophylactic measure in hospitals.
- Coagulase-negative staphylococci are classic opportunists. *S. epidermidis* and other species are frequent agents in foreign body infections due to their ability to form biofilms on the surfaces of inert objects. *S. saprophyticus* is responsible for between 10 and 20% of acute urinary tract infection in young women.

Species	Parameter
<i>S. aureus</i>	Coagulase-positive; colonies golden yellow. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, postinfluenza pneumonia, sepsis. Toxin-caused illnesses: food poisoning, dermatitis exfoliativa, toxic shock syndrome
<i>S. epidermidis</i>	Coagulase-negative; sensitive to novobiocin; most frequent CNS* pathogen; opportunist; infection requires host predisposition; foreign body infections with discrete clinical symptoms
<i>S. saprophyticus</i>	Coagulase-negative; resistant to novobiocin. Urinary tract infections in young women (10–20%); occasional nonspecific urethritis in men
* CNS: coagulase-negative staphylococci	

Overview of Staphylococcus species that affect humans most frequently

Extracellular toxins and enzymes.

Staphylococcus aureus secretes numerous enzymes and toxins that determine, together with the fine structures described above, the pathogenesis of the attendant infections.

The most important are:

Plasma coagulase is an enzyme that functions like thrombin to convert fibrinogen into fibrin. Tissue microcolonies surrounded by fibrin walls are difficult to phagocytose.

α -toxin: can have lethal CNS effects, damages membranes (resulting in, among other things, hemolysis), and is responsible for a form of dermonecrosis.

Leukocidin: damages microphages and macrophages by degranulation.

Exfoliatins are responsible for a form of epidermolysis.

Food poisoning symptoms can be caused by eight serologically differentiated enterotoxins (A-E, H, G, and I). These proteins (MW: 35 kDa) are not inactivated by heating to 100 °C for 15–30 minutes. *Staphylococcus* enterotoxins are superantigens.

Toxic shock syndrome toxin-1 (TSST-1): is produced by about 1% of *Staphylococcus* strains. TSST-1 is a super antigen that induces clonal expansion of many T lymphocyte types (about 10%), leading to massive production of cytokines, which then give rise to the clinical symptoms of toxic shock.



Pathogenesis and clinical pictures.

The pathogenesis and symptoms of *S. aureus* infections take one of three distinct courses:

Invasive infections: In this type of infection, the pathogens tend to remain on site after penetrating through the derma or mucosa and to cause local infections characterized by purulence. Examples include furuncles, carbuncles, wound infections, sinusitis, otitis media, and mastitis puerperalis.

Other kinds of invasive infection include postoperative or post-traumatic osteitis, osteomyelitis, endocarditis following heart surgery (especially valve replacement), postinfluenza pneumonia, and sepsis in immunocompromised patients.

Toxicoses: Food poisoning results from ingestion of food contaminated with enterotoxins. The onset a few hours after ingestion takes the form of nausea, vomiting, and massive diarrhea.

Mixed forms: Dermatitis exfoliativa (staphylococcal scalded skin syndrome(SSSS), Ritter disease), and bullous impetigo are caused by exfoliatin-producing strains that infect the skin surface.

Toxic Shock Syndrome (TSS) is caused by strains that produce TSST-1. These strains

can cause invasive infections, but may also only colonize mucosa.

The main symptoms are hypotension, fever, and rash.

Diagnosis:

This requires microscopic and culture-based pathogen identification.

Differentiating *S. aureus* from the coagulase-negative species is achieved by detection of the plasma coagulase and/or the clumping factor. The enterotoxins and TSST-1 can be detected by means of immunological and molecular biological methods (special laboratories).

Epidemiology and prevention.

Staphylococcus aureus is a frequent colonizer of skin and mucosa. High carrier rates (up to 80%) are the rule among hospital patients and staff. The principle localization of colonization in these persons is the anterior nasal mucosa area, from where the bacteria can spread to hands or with dust into the air and be transmitted to susceptible persons.

S. aureus is frequently the causal pathogen in nosocomial infections. Certain strains are known to cause hospital epidemics.

The most important preventive measure in hospitals is washing the hands thoroughly before medical and nursing procedures.

On Blood Agar.....

Staphylococcus aureus on Columbia agar with 5% sheep blood. Individual colonies on agar are round, convex, and 1-4 mm in diameter with a sharp border. On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of clear beta-hemolysis. The golden appearance of colonies of some strains is related to the name; aureus meaning "golden" in Latin.

Methicillin-resistant strains of *Staphylococcus aureus* (i.e. MRSA) often have only weak or no beta-hemolysis and special cultivation media with oxacillin, mannitol and NaCl for their isolation are used. MRSA is able to grow on this media and produce colonies of certain color, depending on used pH indicator.

β -hemolysis of *Staphylococcus aureus*

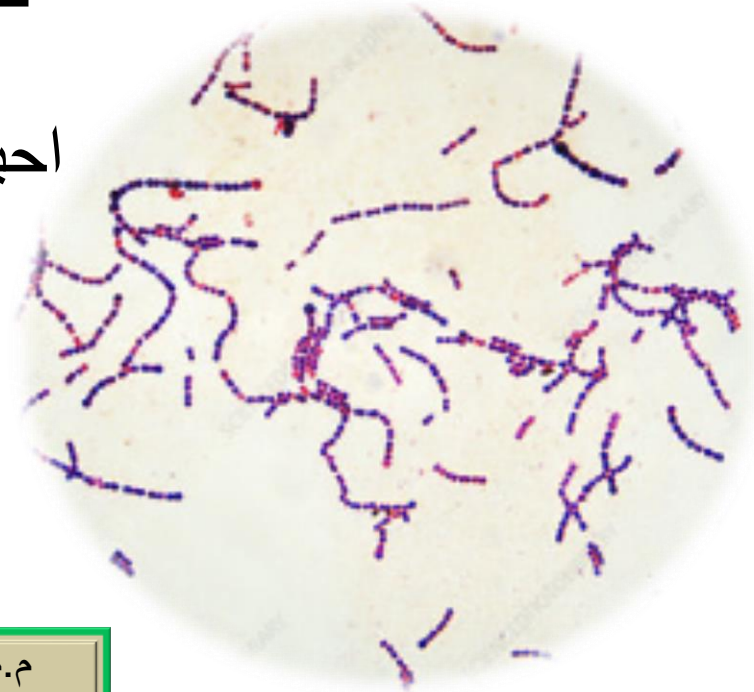




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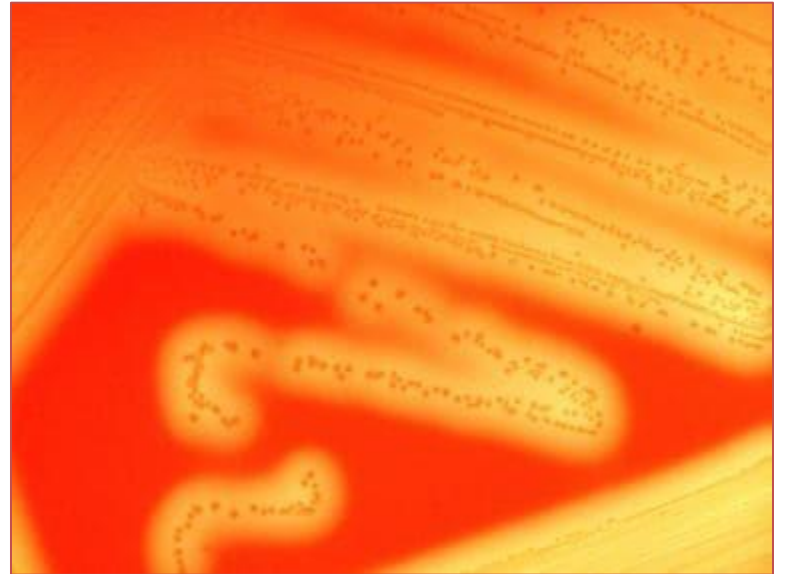
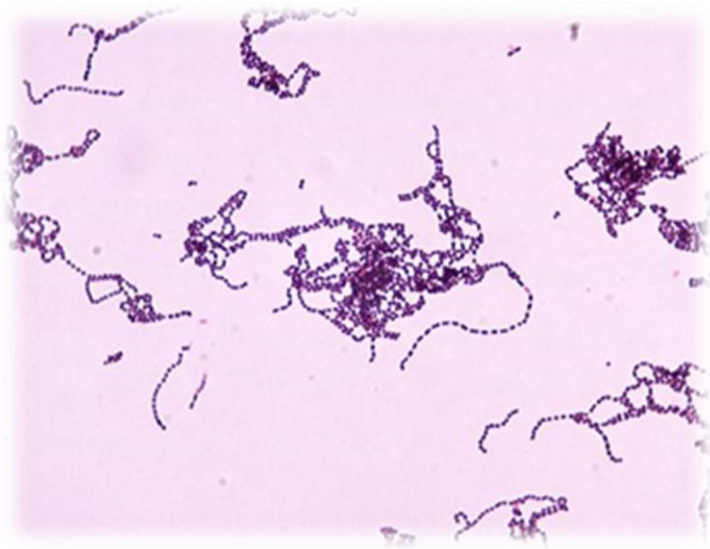


كلية النور الجامعة
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Streptococcus and Enterococcus



- **Streptococci** are Gram-positive, non-motile, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs.
- Researchers differentiate species of streptococcus based on the reactions of antibodies to specific bacterial antigens, type of hemolysis (α - β - γ -hemolysis). This serological classification scheme, developed in 1938 by Rebecca Lancefield (Lancefield antigen).

Streptococcus Classification

On the basis of hemolysis, streptococci can be divided into 3 groups:-

- ✱ • α Hemolytic: (Partial or green hemolysis), e.g. *Streptococcus viridans*, *Streptococcus pneumoniae*.
- ✱ • β Hemolytic: (Complete or yellowish hemolysis), e.g. *Streptococcus agalactiae* and *Streptococcus pyogenes*.
- ✱ • γ Hemolytic: (No hemolysis is seen), e.g. *Enterococci*

In the medical setting, the most important groups are the alpha-hemolytic streptococci *S. pneumoniae* and *Streptococcus viridans* group, and the beta-hemolytic streptococci of Lancefield groups A and B (also known as “group A strep” and “group B strep”).

Types of streptococcus on blood agar

Hemolysis Sheep Blood Agar



(1) Group A streptococci (GAS)(*Streptococcus pyogenes*) (β - haemolytic)

This coccus forms 1-2 mm white colonies surrounded by large zone of β - hemolysis after 24 hr on blood agar plate.

Pathogenic strains of this species often form a capsules.

Pathogenicity:

Strains of *streptococcus pyogenes* have a number of structures, enzymes, and toxins that enable them to survive as pathogens in the human body.

Two main structural features enable cells of *S. pyogens* evade phagocytosis:

- 1- Protein M Virulent group A streptococcus.
- 2- Hyaluronic acid capsule.

There are many enzymes had been identified by researchers:

- 1) Streptokinasethat break down blood clot, enabling streptococci cells to spread through infected & damaged tissue.
- 2) Deoxyribonucleases depolymerize DNA.....that released from dead cells that reducing the firmness of the pus surrounding the bacteria.
- 3) C5a peptidasebreaks down the complement C5a.
- 4) Hyaluronidasefacilitates the spread of streptococcal cells through tissue by breaking down hyaluronic acid.

So, *S. pyogenes* decreases the movement of white blood cells into a site of infection.

Group A *S. pyogenes* secrete three toxins which cause blood capillaries near the surface to dilate, producing a red rash and shock.

1- Erythrogenic toxins

2- Fever-stimulating pyrogenic-toxin.

3- Pus-producing pyrogenic-toxin.

Group A-Streptococcal diseases:

Cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue (pyoderma & erysipelas....pus producing lesion)

Pharyngitis

Scarlet fever

Streptococcus Toxic Shock Syndrome(STSS).

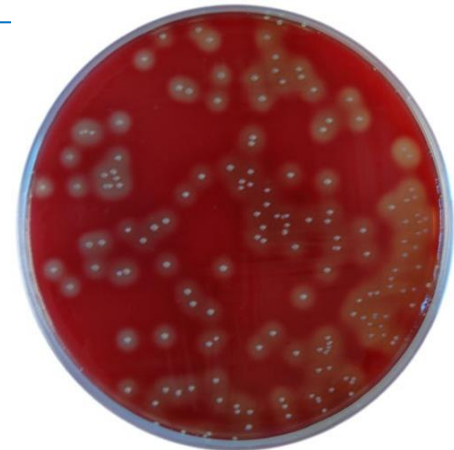
Necrotizing fasciitis “**flesh eating strep**”

Rheumatic fever

Glomerulonephritis

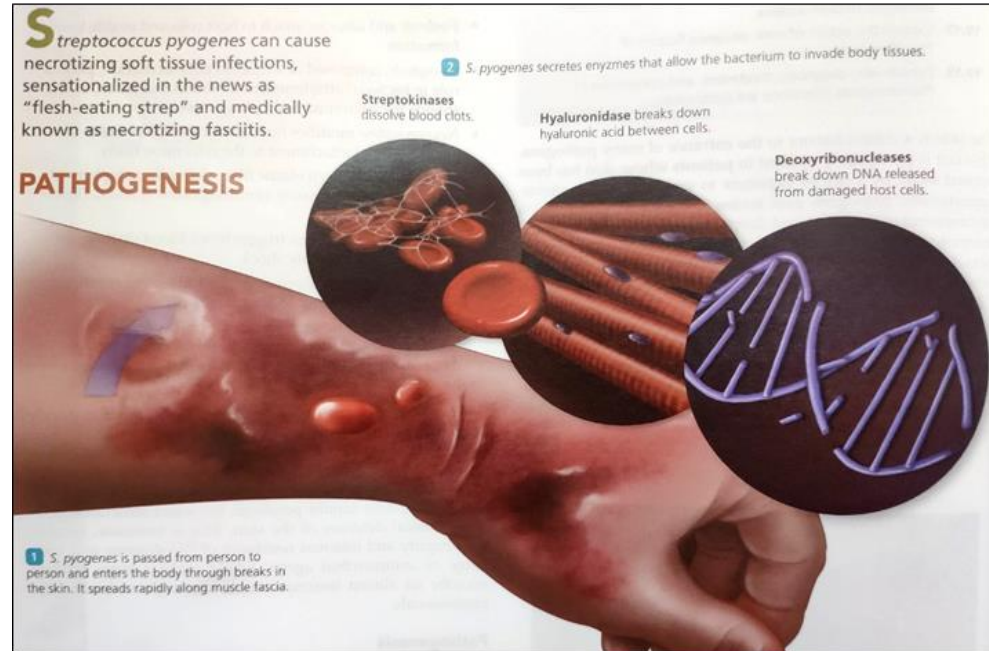


To identify *S. pyogenes* in clinical samples, blood agar plates are screened for the presence of β -hemolytic colonies. The typical appearance of *S. pyogenes* colonies after 24 hours of incubation at 35-37°C is dome-shaped with a smooth or moist surface and clear margins.



Streptococcus pyogenes can cause necrotizing soft tissue infections, sensationalized in the News as “flesh-eating strep” and medically known as necrotizing fasciitis

PATHOGENESIS



1- *S. pyogenes* is passed from person to person and enters the body through Breaks in the skin. It spreads rapidly along muscle fascia.

2- *S. pyogenes* secretes enzymes that allow bacterium to invade body tissues.

Streptokinases { dissolve blood clot }

Hyaluronidase { breaks down hyaluronic acid between cells }

Deoxyribonucleases { breaks down DNA released from damaged host cells }

(2) Group B Streptococcus, *Streptococcus agalactiae* (β -hemolytic)

This coccus is a Gram positive, 0.6-1.2 μm in diameter that divides to form chains. Like group A streptococcus, this coccus is also beta-hemolysis, but it can be distinguished from the *S.pyogenes* by three qualities:

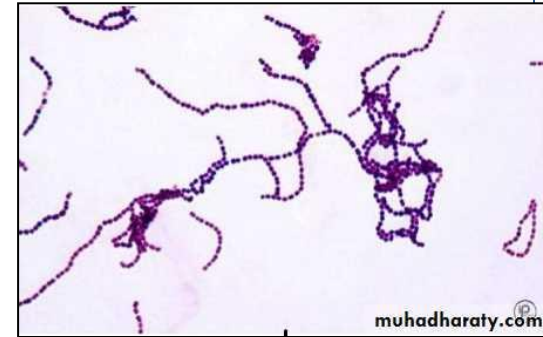
- 1- It has group specific, polysaccharide cell wall antigens.
- 2- It forms buttery colonies that are 2-3 mm in diameter and have a small zone of β -hemolysis after 24 hr of growth on blood agar.
- 3- It is bacitracin resistant.

This bacteria produce enzymes:

1. Proteases(that catabolize proteins).
2. Hemolysins.....(that lysis red blood cells).
3. Deoxyribonuclease
4. Hyaluronidase

Group B-streptococcus bacteria can colonize:

1. The lower gastrointestinal (GI), genital, and urinary tracts.
- 2- In adults, primarily follow wound infections.
3. In newborns, cause early-onset disease& late onset disease.



(3) α -hemolytic Streptococci, *Streptococcus viridans*

- This bacteria lack group-specific carbohydrates, and thus they are not part of any Lancefield group.
- This viridans cocci so called because of producing a green pigment when grow on blood media.
- This bacteria inhabit the mouth and cause dental caries, pharynx, GI tract, genital tract and urinary tract of humans.
- (Streptococcus viridans or viridans streptococci) display alpha-hemolysis. Alpha-hemolysis is also termed incomplete hemolysis or partial hemolysis because the cell membranes of the red blood cells are left intact.



(4) α -hemolytic pneumococci (*Streptococcus pneumoniae*) (Diplococcus)

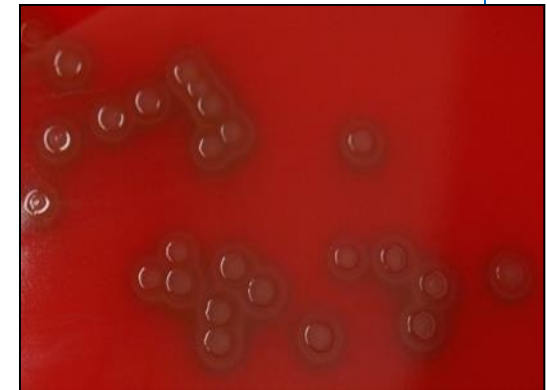
1. Lack Lancefield antigens
 2. Cause pneumoniainfections of the respiratory tract.
 3. It is Gram-positive, short chains commonly pairs.
 4. Colonies of *S. pneumoniae* grown for 24 hr are 1-3 mm in diameter, round, mucoid, un-pigmented, and dimpled in the middle because of the death of older cells.
- This colonies are α -hemolytic on blood agar surface when grown aerobically, and β -hemolytic when grown an-aerobically.

Pathogenicity:

1. This diplococcus has polysaccharide capsule, which protects the bacterial cells from digestion after phagocytosis. Pathogenic pneumococci secrete protein adhesion that mediate binding bacteria to epithelial cells of the pharynx.
2. It secrete IgA proteasewhich destroy IgA antibodies.
3. It secrete pneumolysin.....which lysis the epithelial cells.

So *Streptococcus pneumoniae* cause:

1. Pneumococcal pneumonia.
2. Sinusitis and otitis media.
3. Bacteremia & endocarditis.
4. Pneumococcal meningitis.



Streptococcus pneumoniae Gr + (diplococcus), colonies on blood agar

(5) Group C streptococci (GCS)

- Not important in human pathogenicity.
- Livestock pathogens and they often cause zoonotic diseases in humans. They are Gram-positive, in mostly β -hemolytic and facultative anaerobes.

(6) Group D streptococci (Enterococcus) γ -hemolytic cocci (non-hemolysis)

- 1- Enterococcus, so named because all enterococci are spherical & live in the intestinal tract of animals.
- 2- It is Gram +, un-capsulated bacterial cells, grow at temperatures up to 45°C, at pH 9.6, and in 6.5% NaCl.
- 3- There are two types of enterococcus: *E.faecalis* & *E.faecium*.
- 4- Enterococcus faecalis ubiquitous in the human colon.
- 5- This bacteria cause nosocomial infections (in hospitals), bacteremia, endocarditis, and wound infections.

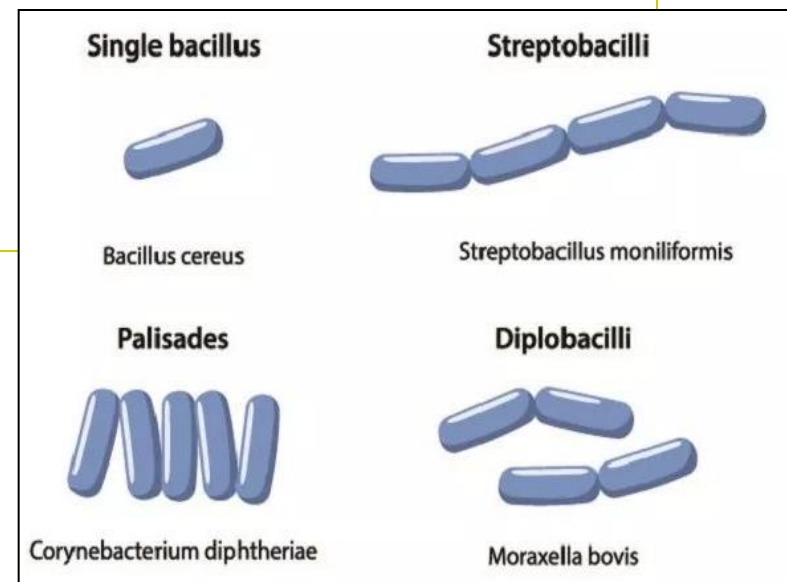
ble 19.2 Characteristics of Pathogenic Streptococci

Lancefield Group	Scientific Name	Hemolytic Pattern	Significant Characteristics	Characteristic Diseases
A	<i>S. pyogenes</i>	Large zone of beta-hemolysis	1- to 2-mm white colonies on blood agar; bacitracin sensitive	Pharyngitis, scarlet fever, pyoderma, erysipelas, streptococcal toxic shock syndrome, necrotizing fasciitis, rheumatic fever, glomerulonephritis
B	<i>S. agalactiae</i>	Small zone of beta-hemolysis	2- to 3-mm buttery colonies on blood agar; bacitracin resistant	Puerperal fever, neonatal bacteremia, meningitis, pneumonia
C	<i>S. equisimilis</i>	Large zone of beta-hemolysis	1- to 2-mm white colonies on blood agar	Pharyngitis, glomerulonephritis
C, F, or G	<i>S. anginosus</i>	Small zone of beta-hemolysis	1- to 2-mm white colonies on blood agar	Purulent abscess
-	<i>S. mutans</i>	Alpha-hemolysis	Viridans group (produce green pigment when grown on blood agar)	Dental caries; rarely bacteremia, meningitis, endocarditis
-	<i>S. pneumoniae</i>	Alpha-hemolysis (aerobic); beta-hemolysis (anaerobic)	Diplococci; capsule required for pathogenicity; bile sensitive	Pneumonia, sinusitis, otitis media, bacteremia, endocarditis, meningitis
D	<i>Enterococcus faecalis</i> , <i>E. faecium</i>	None (gamma-hemolysis)	Diplococci; no capsule; bile insensitive	Urinary tract infections, bacteremia, endocarditis, wound infections

Bacillus



- Bacillus, (genus Bacillus), any of a **genus of rod-shaped, gram-positive, aerobic** or (under some conditions) anaerobic bacteria widely found in soil and water.
- The term bacillus has been applied in a general sense to all cylindrical or rod-like bacteria. ... Bacillus frequently occur in chains.
- Scientists divide Gram-positive bacilli into:
 - 1- Endospore-forming bacteriasuch as *Bacillus* & *Clostridium*
 - 2- Non endospore-forming bacteria.... Such as *Corynebacterium* & *Actinomyces*
- Bacillus classified into two major species:
 1. *Bacillus anthracis*-----cause Anthrax
 2. *Bacillus cereus*----- cause food poisoning



Bacillus anthracis

It is Gram + bacilli, in chain, have characteristics squared ends, endospores are central do not cause bulging.

Bacteria in clinical specimens lack endospores and possess capsules.

The capsule prevents effective phagocytosis by white blood cells.

Pathogenic strains of *B. anthracis* secrete (anthrax toxin).

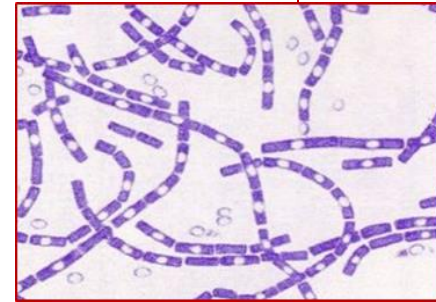
Anthrax can be deadly even after treatment, because antimicrobial drugs do not inactivate accumulated anthrax toxin.

Anthrax can be invade human body via one of three routes:

- 1- Inhalation of spores through respiratory system.
- 2- Inoculation of spores into the body through a break in the skin.
- 3- Ingestion of spore through digestive system.

On agar media *B. anthracis* is an aerobe, and facultative anaerobe with temperature 12-45°C, it is irregularly round colonies, about 2-3 mm in diameter, greyish white, with a frosted glass appearance.

Under the low power microscope, the edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of matted hair. This is called the Medusa head appearance.



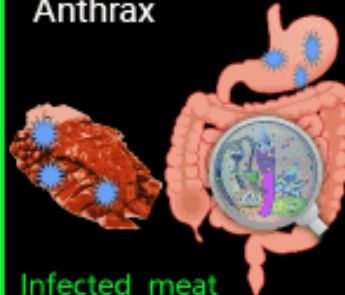
Pathology of Anthrax Infection

Inhalation Anthrax



Anthrax Spores
in surrounding air

Gastrointestinal Anthrax



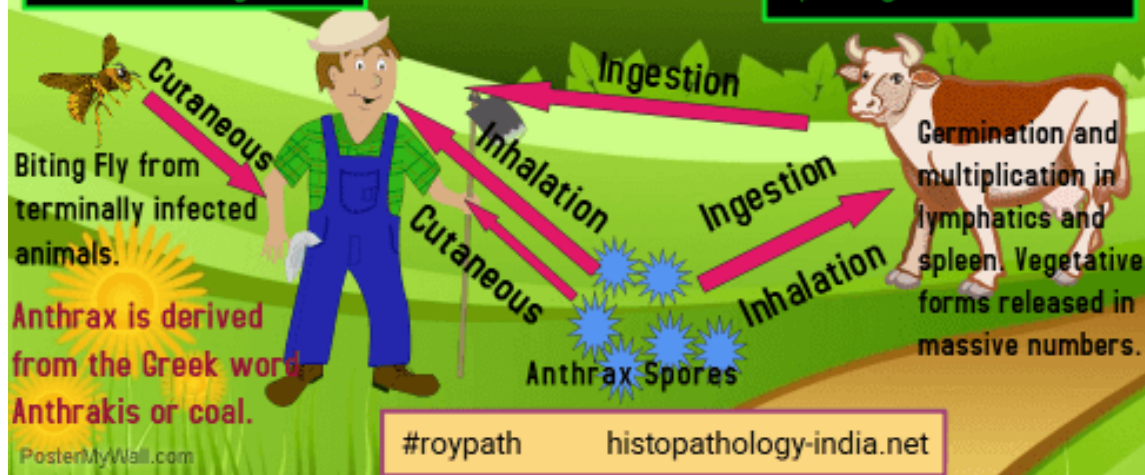
Infected meat

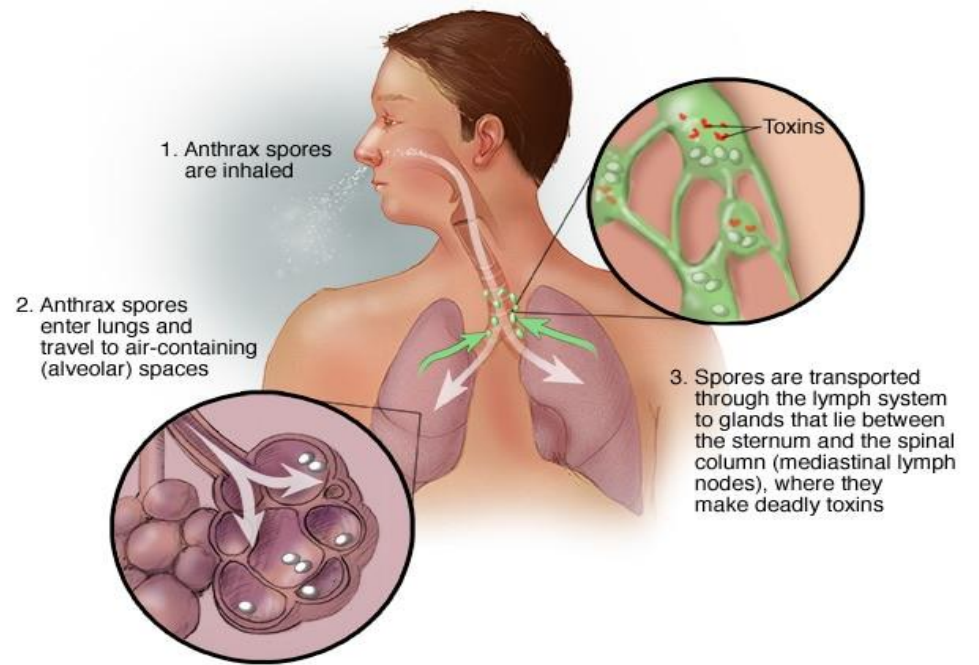
Cutaneous Anthrax



Anthrax
Spores

Infection can also spread
by biting insects





Bacillus cereus

Bacillus cereus is a toxin-producing facultatively anaerobic gram-positive bacteria.

The bacterium is commonly found in the environment, is often found in soil and vegetation, and can be present in foods. It can quickly multiply at room temperature.

There are two main types of an intestinal illness caused by *B. cereus*. One is diarrheal, and one leads more to nausea/vomiting.

B. cereus has also been implicated in infections of the eye, respiratory tract, and in wounds. The pathogenicity of *B. cereus*, whether intestinal or non-intestinal, is intimately associated with the production of tissue-destructive exo-enzymes such as hemolysins, phospholipases, and proteases.

B. cereus illness is related to many foods - beef, turkey, rice, beans, and vegetables. Specifically, the diarrheal illness is often related to meats, milk, vegetables, and fish. There are two types of food-borne *B. cereus* illness.

In the first, contaminated food (many types of food, often left at room temperature) makes its way to the small intestine where the toxin, in this case, a large-molecular-weight protein, is released. This can lead to diarrhea, cramps, and sometimes nausea. Usually, vomiting is not present in this form of illness.

The second type, affected food, most often starchy food, and classically, rice, contains a different type of toxin.

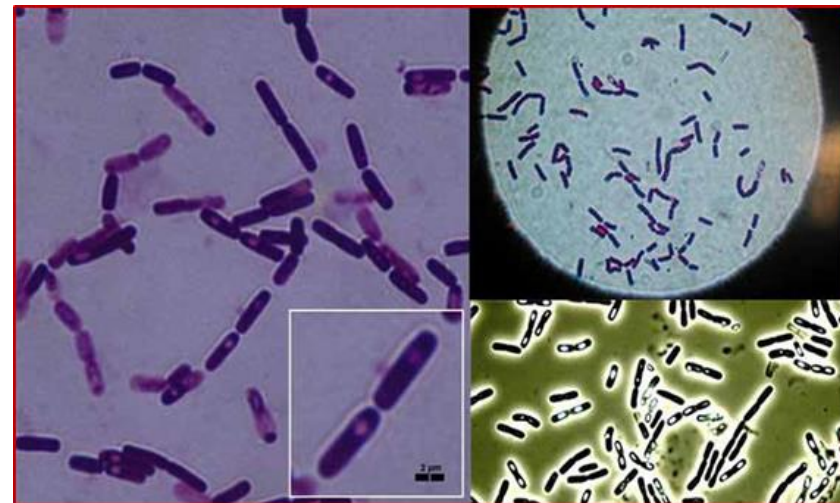
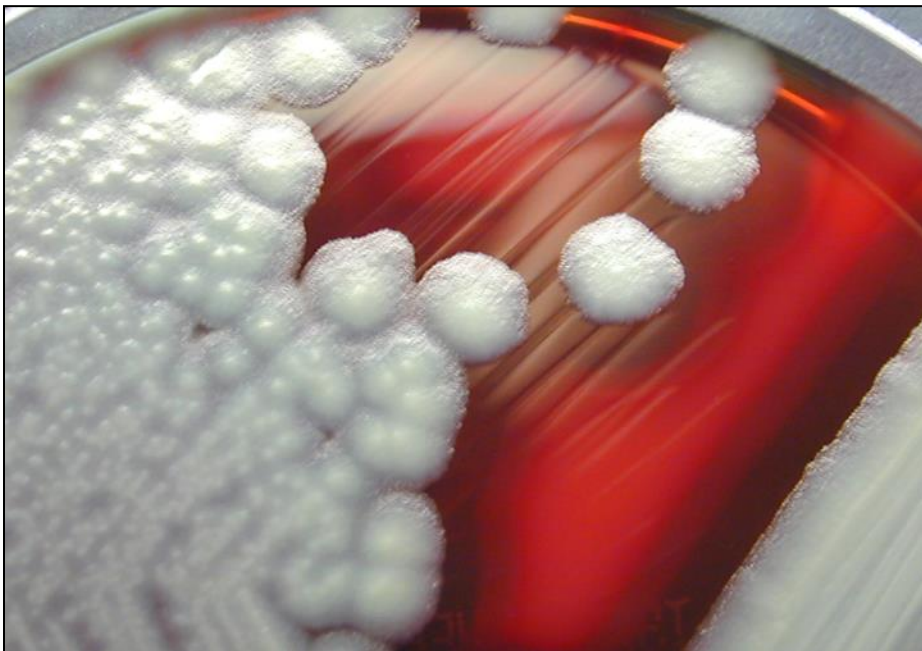
In other infections like lung abscess and endophthalmitis (of eye), evaluation is made with whatever fluid is available.



Morphology of *Bacillus cereus*



Endophthalmitis (of eye)





Thank you