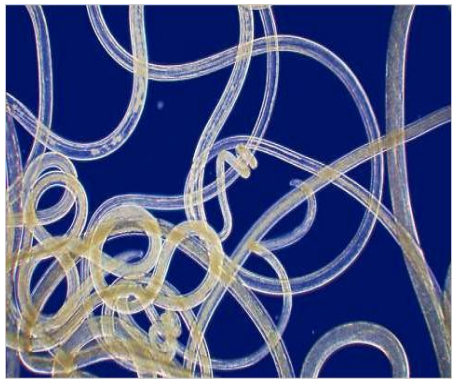
BASIC BACTERIOLOGY

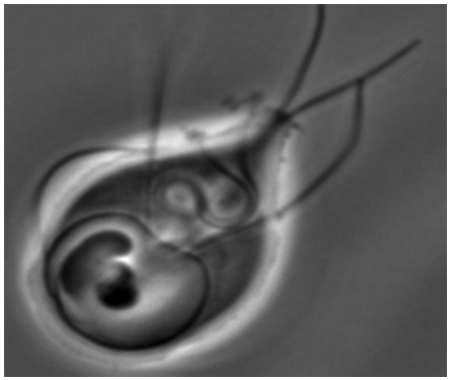
Assoc. Prof. Emrah Şefik Abamor

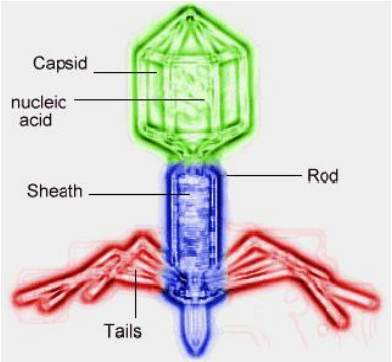
- The word microbiology comes from the combination of the words "micros", "bios" and "logos". In Greek, micros, small; bios, life; logos means science.
- Microbiology is a branch of science that studies small living things called microorganisms, many of which can only be seen under a microscope.

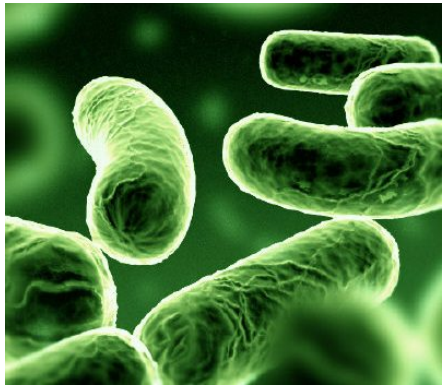
Disease factors, which are called microorganisms and cause infectious diseases in humans, are examined under five groups:

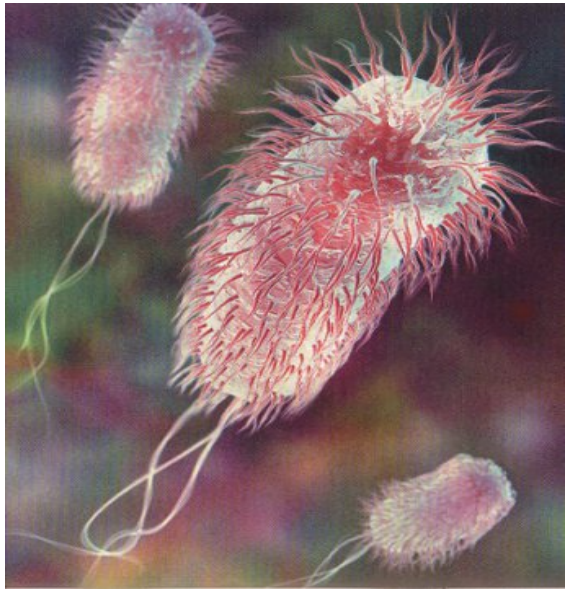
- Bacteria
- Fungi (fungi and yeasts)
- Protozoa
- Helminths
- Viruses

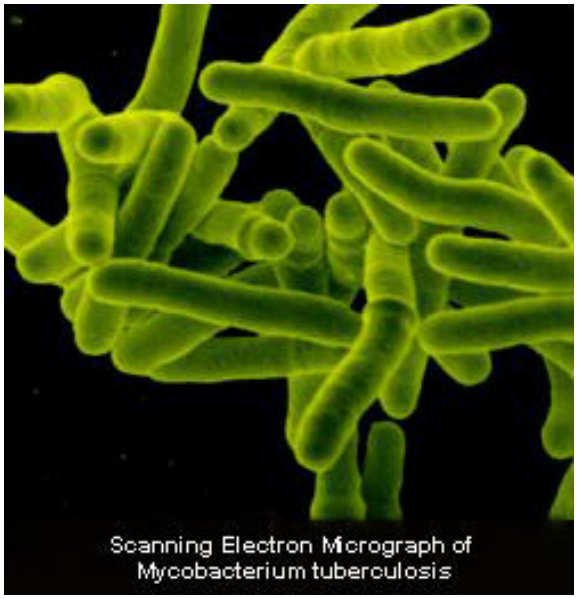




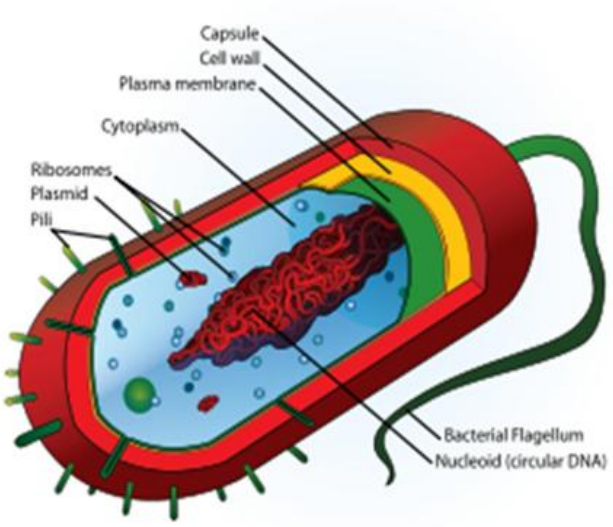







Scanning Electron Micrograph of
Mycobacterium tuberculosis

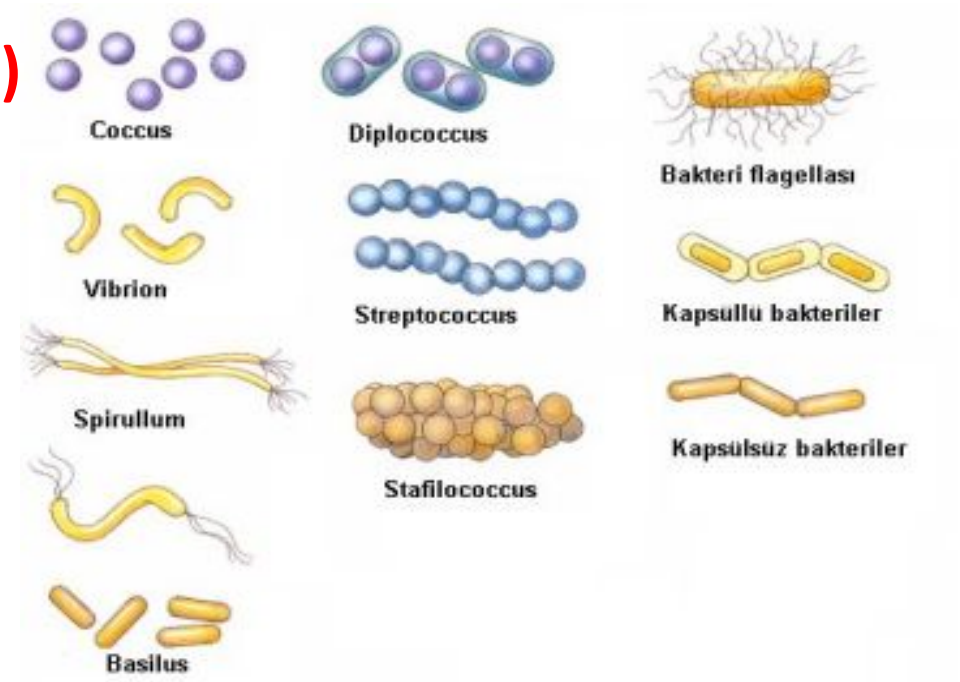
BACTERIA


Capsule
Cell wall
Plasma membrane
Cytoplasm
Ribosomes
Plasmid
Pili
Bacterial Flagellum
Nucleoid (circular DNA)

Morphology of Bacterial Cells

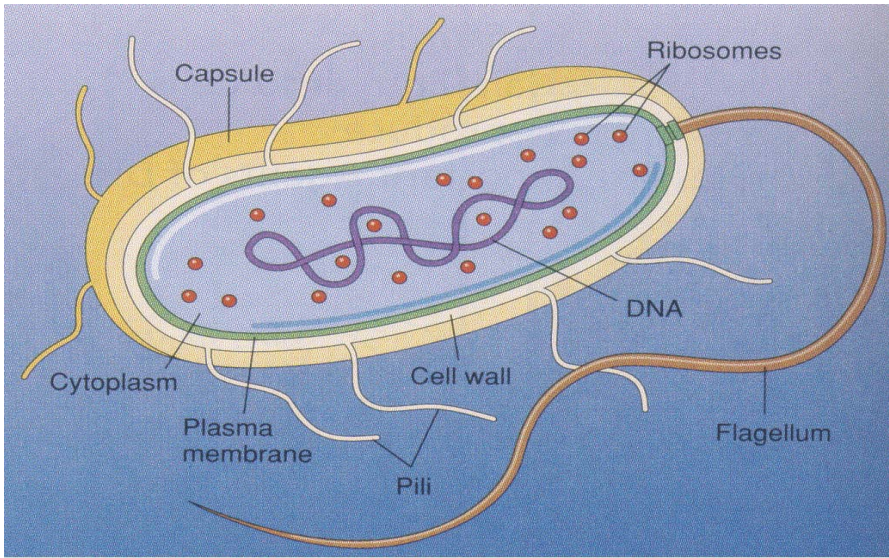
SHAPE AND SIZE

- **Cocci**
  - ❖Diplococcus (double)
  - ❖Streptococcus (chain-shaped)
  - ❖Staphylococcus (grape cluster)
- **Bacille (rod)**
- **Spirochet (Spirillum)**
- **Pleomorphic (polymorphic)**



Bacteria consist of four main layers from the outside to the inside:

1. Special structures outside the cell wall
   a. Capsule (found in some species)
   b. Flagellum,
   c. Pilus
   d. Glycocalyx
2. Cell wall
3. Cytoplasmic cell membrane
4. Cytoplasm



1. Special Structures Outside the Cell Wall

A. CAPSULE:

- **It is a gelatinous layer that covers the entire bacteria.**
- **Capsules of encapsulated bacteria are composed of polysaccharide (except Anthrax bacillus).**
- **The sugar constituents of the polysaccharide vary from one bacterial species to another and often determine the serological type within a species.**

  For example, there are 84 different serological types of Streptococcus pneumoniae, which are distinguished from each other by the antigenic difference of the sugars in the polysaccharide capsule.

Functions of Capsules

1. It is a virulence determinant in many bacteria as it limits the ability of phagocytes to phagocytize (swallow) bacteria.
2. Identification of the species of an organism can be done by using antiserum against capsule polysaccharide.
3. Capsule polysaccharides are used as antigens in some vaccines because they can cause the formation of protective antibodies in humans.
4. Capsule allows infection-causing bacteria to attach to human tissues.

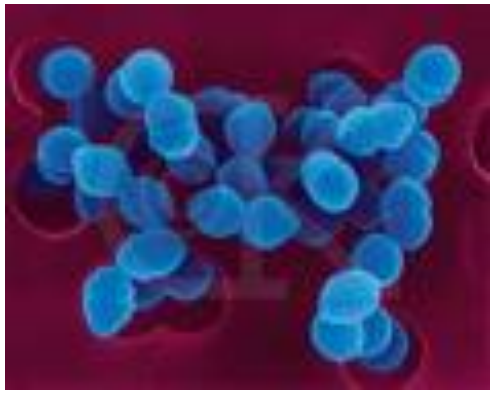
1. Special Structures Outside the Cell Wall

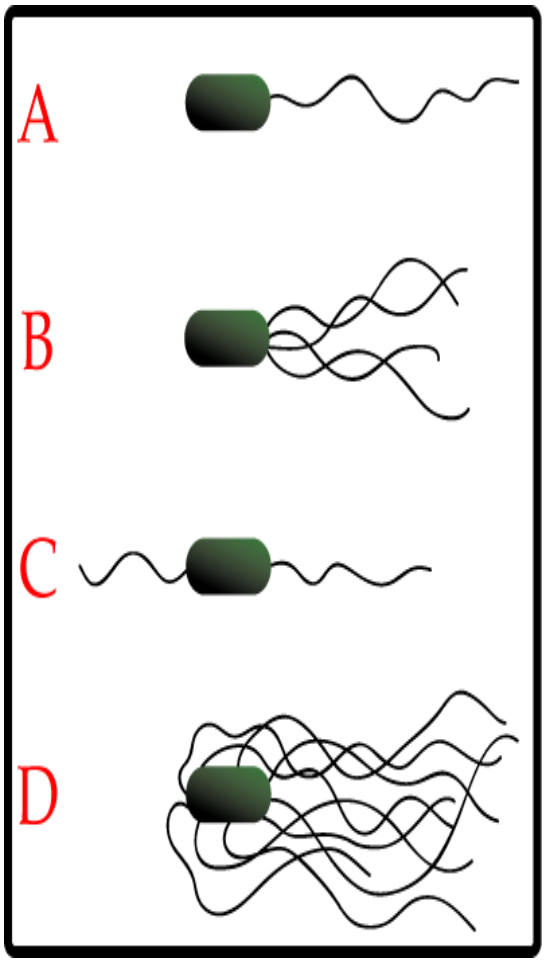
B. WHIP (FLAGELLA):

- **They are long, string-like appendages that move bacteria towards food or other attractants. This phenomenon is called chemotaxis.**
- **The flagellum is made up of many subunits of a single protein called flagellin.**
- **The energy required for movement (proton driving power) is provided by adenosine triphosphate (ATP), which is produced by the passage of ions across the membrane.**

Only some bacteria have flagella; While many bacilli have flagella, most cocci do not, and therefore they are immobile.

- Flagellated bacteria carry flagella in a characteristic (depending on species) position and number of flagella.
- Some bacteria have a single flagella, while others have many.
- In some bacteria, the flagellum is located at one end of the bacterium, while in others it is found along the entire outer surface.





From a medical point of view, flagellas have two important properties:

1. Some strains of motile bacteria (Escherichia coli and Proteus) are common causes of urinary tract infections. In these bacteria, flagella may play a role in the pathogenesis by pushing the bacteria from the urethra to the bladder.
2. Some bacterial species (Salmonella species) are typed in the laboratory using specific antibodies against flagellar proteins.

1. Special Structures Outside the Cell Wall

C. PILUS (FIMBRIAS):

- They are hair-like filaments that emerge from the surface of the cell.
- It is shorter and straighter than a flagellum and is made of subunits of a protein called pilin, arranged in helical strips.
- Pilus have two important roles:

- It mediates the binding of bacteria to specific receptors on the human cell surface, a necessary step for some organisms to initiate infection.
- The sex pilus, a specific type of pilus, provides binding between male (donor) and female (recipient) bacteria during conjugation.

1. Special Structures Outside the Cell Wall

D. GLYCOCALYX:

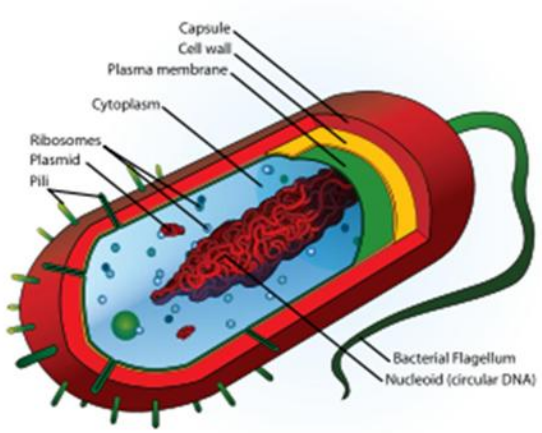
- **It is a polysaccharide cover secreted by many bacteria.**
- **It is present as a thin layer on the surface of the bacteria and allows the bacteria to adhere tightly to various structures such as the skin, heart valves and catheters.**
- **This layer also mediates the adhesion of some bacteria, such as Streptococcus mutans, to the tooth surface. This event plays an important role in the formation of plaque, which is the precursor of dental caries.**

2. Cell Wall

❖It is located in the outermost layer of the bacterial cell.

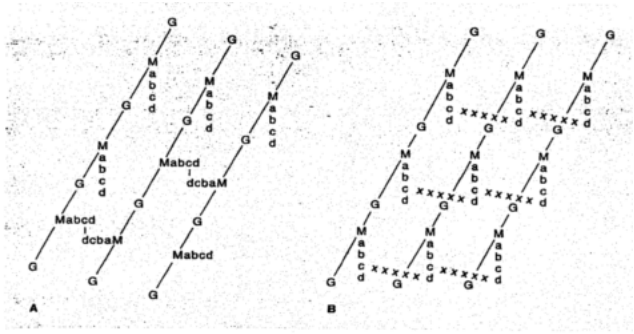
❖It gives mechanical support to the cell and helps it maintain its morphology. It consists of three important structures:

1. Peptidoglycan
2. lipopolysaccharide
3. Teichoic acid



1. Peptidoglycan

- The term 'peptidoglycan' is derived from the peptide and sugars (glycans) that make up the molecule.
- It is in the form of a net that completely surrounds the cell.
- Consists of a single covalently bonded macromolecule
- It provides support to the cell and is important in maintaining the cell's typical shape.
- It allows the cell to resist low osmotic pressure environment such as water.

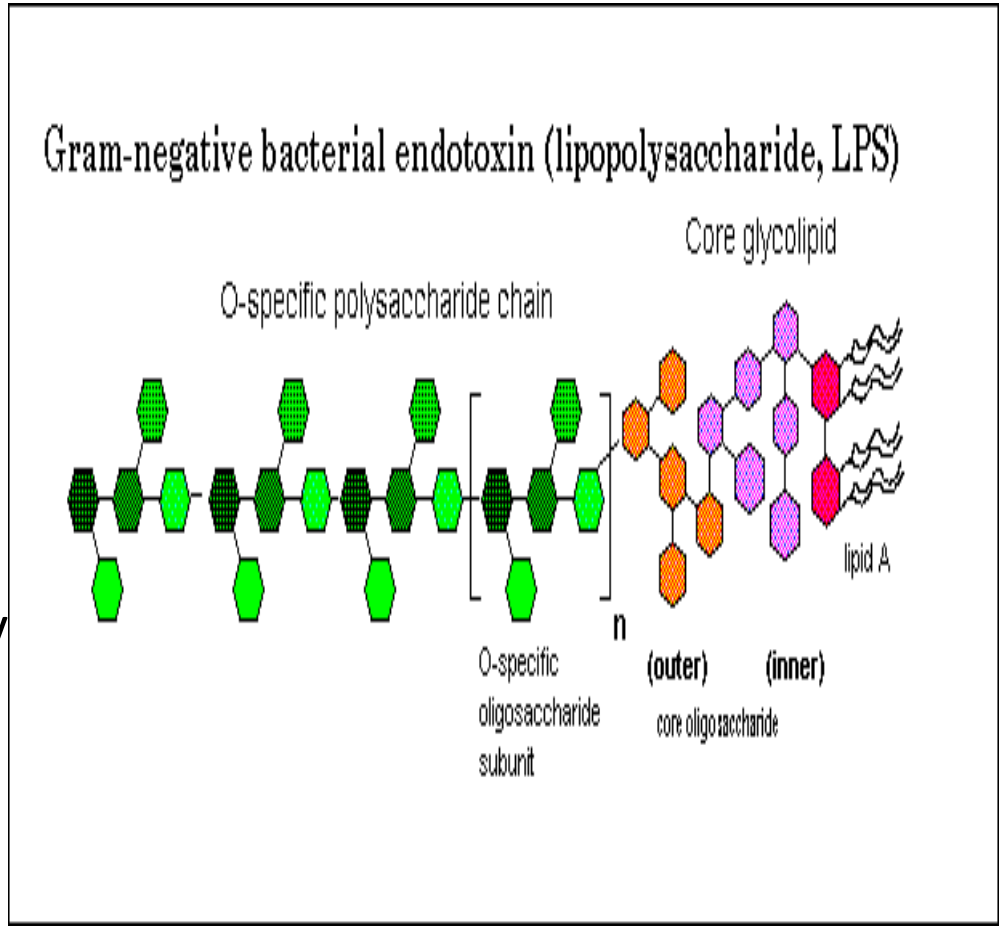


Şekil 2-5. Peptidoglikan çatısı. *Escherichia coli* **(A)** *Staphylococcus aureus*'dan **(B)** farklı bir çapraz bağlanmaya sahiptir. *E.coli*'de, c doğrudan d'ye çapraz bağlanırken *S.aureus*'da c ve d, beş glisinle birbirlerine çapraz bağlanır. Öte yandan, her iki organizmada sonculD-alanin, bağın bir bölümünü yapar. M, muramik asit; G, glukozamin; a, L-alanin; b, D-glutamik asit; c, diaminopimelik asit **(A)** veya L-lizin **(B)**; d, D-alanin; x, pentaglisin köprüsü. (Joklik WK ve ark: *Zinsser Microbiology*, 20. baskı'dan izinle değiştirerek alındı. İlk basım Appleton ve Lange. ? 1992, McGraw-Hill Firması.)

- Peptidoglycan is found in bacteria but not in human cells.
- This makes it a good target for antibacterial drugs. These drugs, such as penicillins, spherosporins, and vancomycins, inhibit peptidoglycan production.
- The enzyme lysozyme found in human tears, mucus and saliva can cleave the peptidoglycan backbone by breaking down glycosyl bonds, thus contributing to the host's natural resistance to microbial infections.

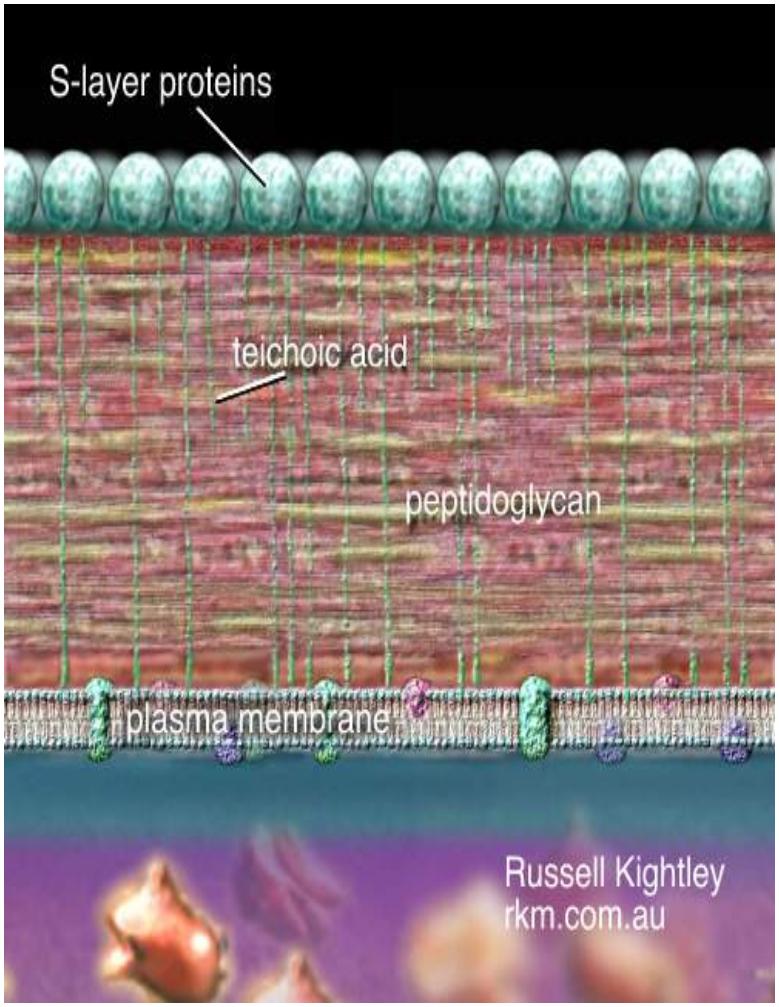
2. LIPOPOLYSACCARIDE (LPS)

- LPS is found in the outer layer of the cell wall of Gram negative bacteria.
- Unlike exotoxins that are freely released by bacteria, LPS, an integral part of the cell wall, is an endotoxin.
- Due to its toxic nature, LPS causes many patient symptoms such as fever, shock and especially hypotension.
- The pathological effects of endotoxin are seen in the same way in all microorganisms.



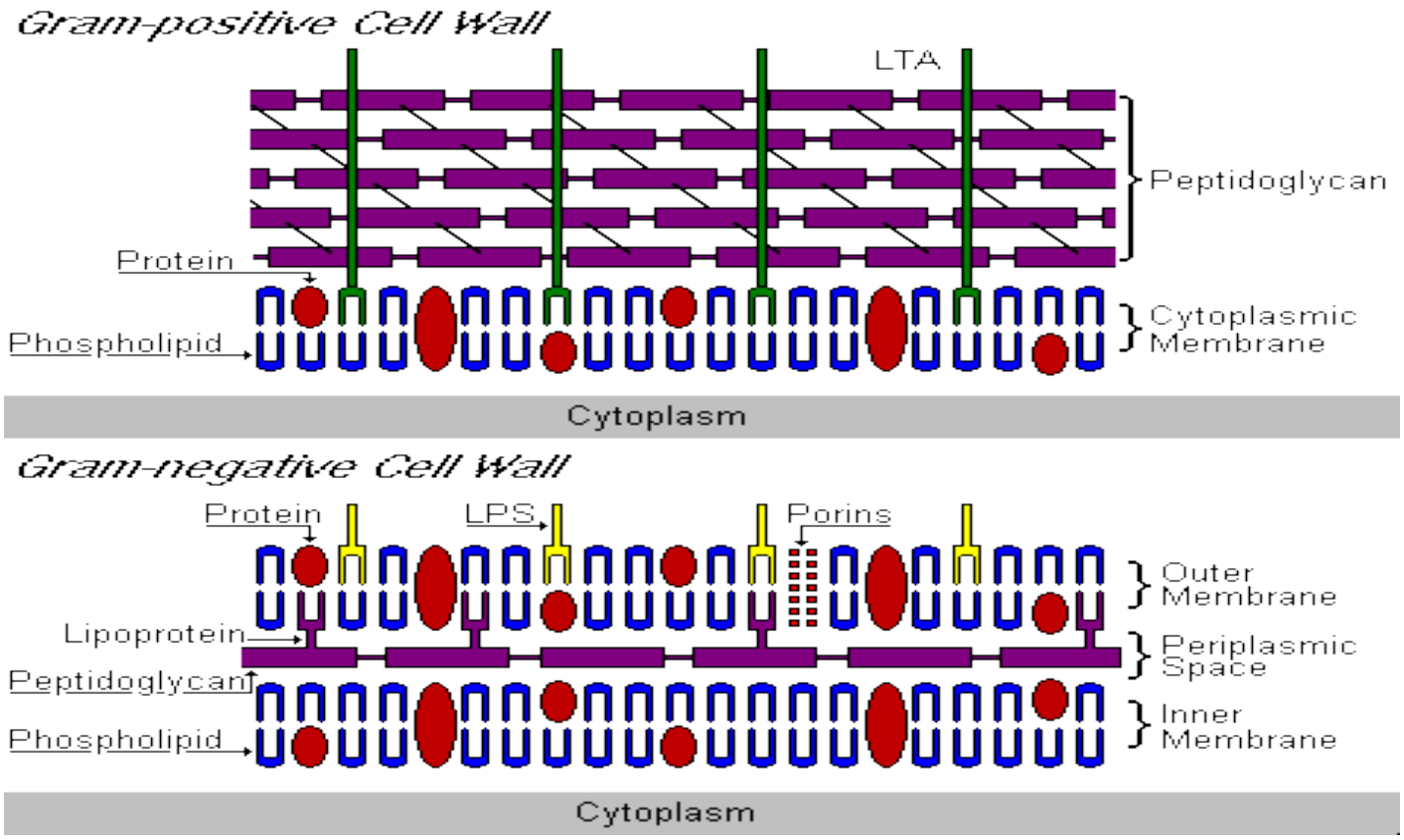
3. TEICHOIC ACID

- The medical significance of teichoic acids is that they cause septic shock to some Gram-positive bacteria.
- Teichoic acids also mediate the adhesion of staphylococci to mucosal cells.



Depending on the structure, chemical composition and thickness of the cell wall, bacteria are divided into two:

Gram-positive and Gram-negative bacteria.



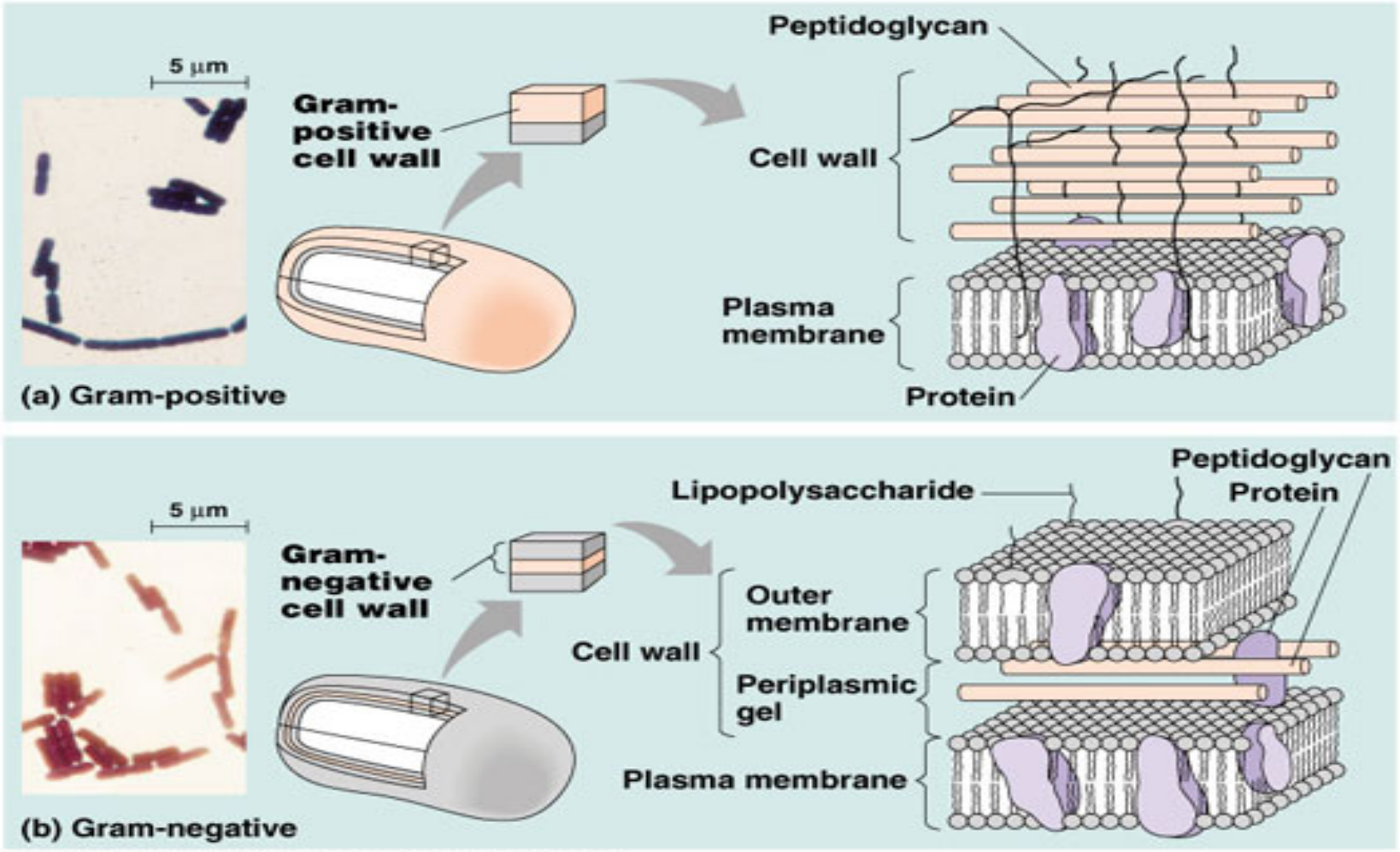
Gram positive

- The peptidoglycan layer is thicker.
- Teichoic acid fibers are also found in some Gram-positive bacteria.
- The outer layers are not as complex as Gram-negative bacteria.
- No LPS layer
- Periplasm is absent.

Gram negative

- The peptidoglycan layer is thinner.
- Teichoic acid is not found.
- It has a complex outer layer of lipopolysaccharide, lipoprotein and phospholipid.
- Between the outer membrane layer and the cytoplasmic membrane is the periplasm.

Differences Between Cell Wall Structures of Gram(+) and Gr(-) Bacteria



3. CYTOPLASMIC MEMBRANE

- It is located just inside the peptidoglycan layer.
- It consists of a bilayer of phospholipid structure.
- The microscope image is similar to the membrane in eukaryotic cells.
- Prokaryotic cells generally do not contain sterols in their cell membranes. The only prokaryotes that contain sterols in their membranes are bacteria of the genus *Mycoplasma.*

The cytoplasmic membrane has four important functions:

1. Efficient transport of molecules into the cell
2. Energy generation by oxidative phosphorylation
3. Synthesis of cell wall precursors
4. Secretion of enzymes and toxins

- The cytoplasm contains the following organelles:
  - a. Ribosomes
  - b. Particles
  - c. Nucleoid
  - d. Plasmids
  - e. Transposons

a. Ribosomes

- It is the organelle where protein synthesis takes place.
- Bacterial ribosomes have 50S and 30S subunits and are 70S in size.

b. Particles

- Originally painted with some dyes and serving as a storage area for nutrients various types of granules contain particles of cytoplasm creates.

c. Nucleoid

- It is the region in the cytoplasm where DNA is located. Prokaryotic DNA is a single circular molecule and contains as many as 2000 genes (human DNA contains 100,000 genes).
- Nucleoid lacks nuclear membrane, mitotic spindle, histone proteins

d. Plasmids

- They are **extrachromosomal, double-stranded, circular DNA molecules that have the ability to replicate independently of the bacterial chromosome.**
- Plasmids occur in both Gram-negative and Gram-positive bacteria, and multiple different plasmids can coexist in a cell.

Plasmids carry genes for the following functions and structures:

1. Antibiotic resistance mediated by various enzymes;
2. Resistance to some heavy metals such as mercury and silver through a reductase enzyme;
3. Resistance to ultraviolet light mediated by DNA repair enzymes;
4. Pilus (fimbriae) mediating the adhesion of bacteria to epithelial cells;
5. It carries genes for the production of exotoxins, including various enterotoxins.

e. Transposons

- DNA fragments that can easily move from one point to another within or between bacteria, plasmid and bacteriophage DNAs.
- Transposons are not capable of independent replication and replicate as part of the recipient DNA.
- They can encode drug resistance enzymes, toxins or various metabolic enzymes and either cause mutations in the gene into which they are inserted or alter the expression of neighboring genes.

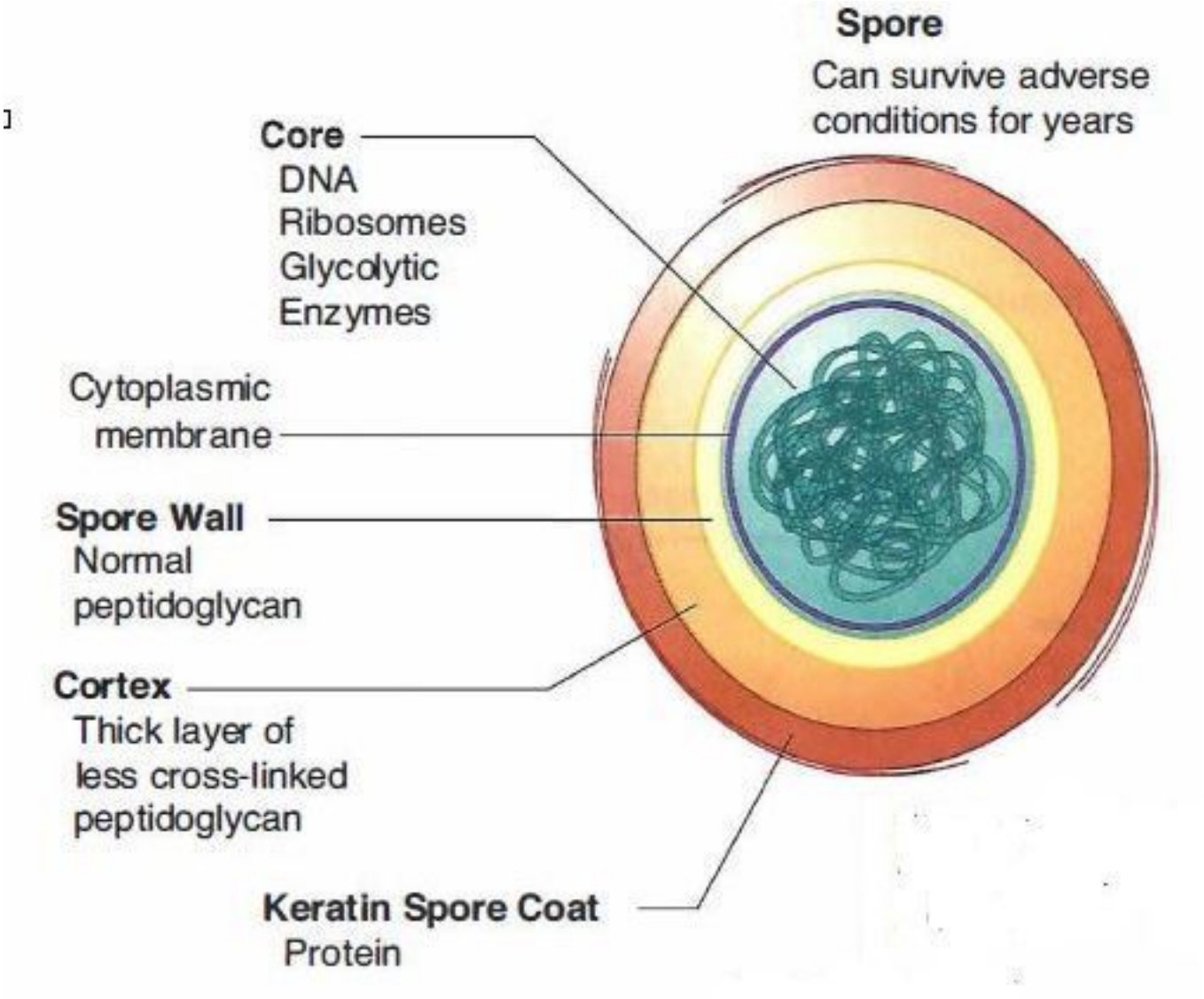
Spores

When nutrients such as carbon and nitrogen sources are depleted, in response to adverse conditions, some bacterial cells go to spore formation (sporulation) to secure their generation.

They are highly resistant structures.

- Bacillus containing the anthrax agent
- Clostridium containing agents of tetanus and botulism breeds form spores.

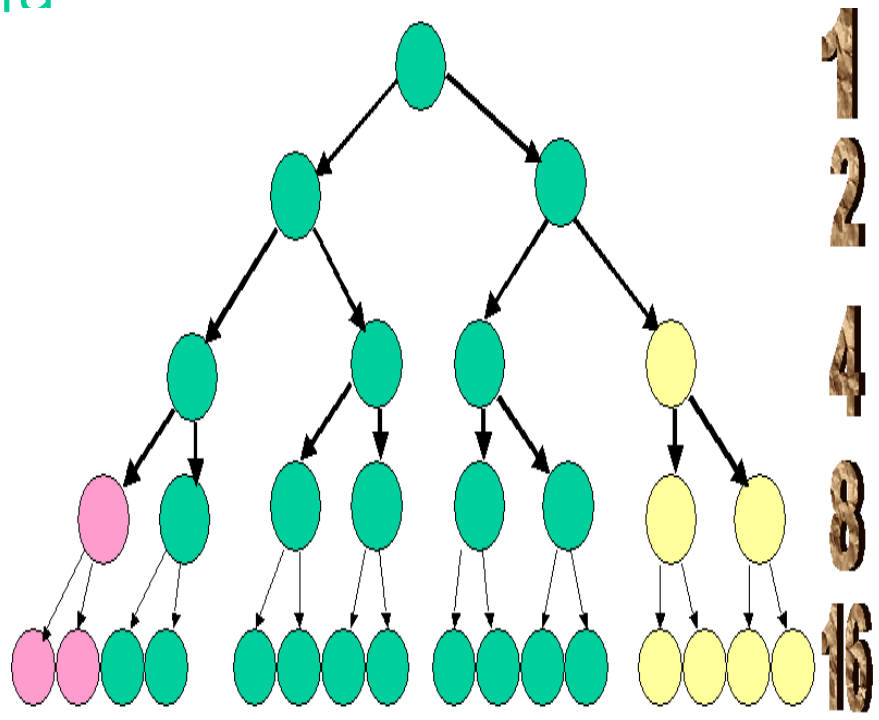
Spores


Spore
Can survive adverse conditions for years
Core
DNA
Ribosomes
Glycolytic Enzymes
Cytoplasmic membrane
Spore Wall
Normal peptidoglycan
Cortex
Thick layer of less cross-linked peptidoglycan
Keratin Spore Coat
Protein

Reproduction in Bacteria

REPRODUCTION CYCLE

- ✓**Bacteria reproduce by binary fission, in which a parent cell divides to produce two daughter cells.**
- ✓**Thus, a bacterium will produce 16 bacteria after 4 generations.**

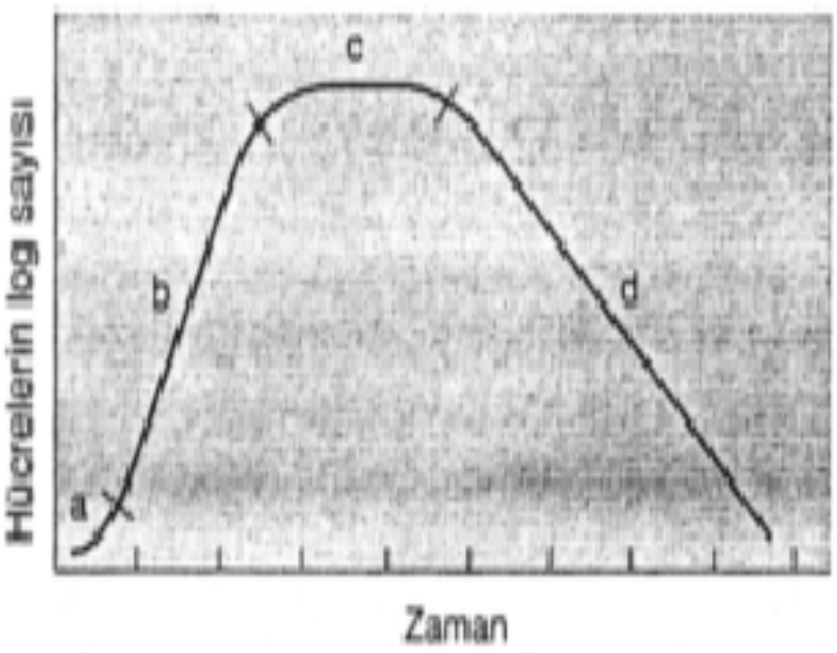


Cell Replication

Cell Replication

- The reproductive cycle of bacteria includes 4 major stages:

1. lag phase
2. log phase
3. stationary phase
4. death phase



Şekil 3-1. Bakterinin üreme eğrisi. a, lag faz; b, log faz; c, stasyoner faz; d, ölüm fazı. (Joklik WK ve ark: Zinsser Microbiology, 20. baskı'dan izinle alınmıştır. İlk baskı Appleton ve Lange. © 1992, McGraw-Hill firması.)

Bacteria reproductive cycles

1. The first phase is the lag phase, and although a very intense metabolic activity is observed in this phase, the cells do not divide. This phase can last from a few minutes to several hours.

2. The log (logarithmic) phase is the phase in which rapid cell division is seen. Beta-lactam drugs such as penicillin act at this stage.

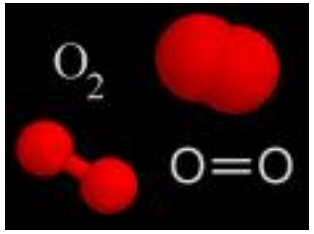
3. The stationary phase occurs when the nutrients in the environment are depleted or when toxic products slow growth. In this phase, newly formed cells and dying cells balance each other.

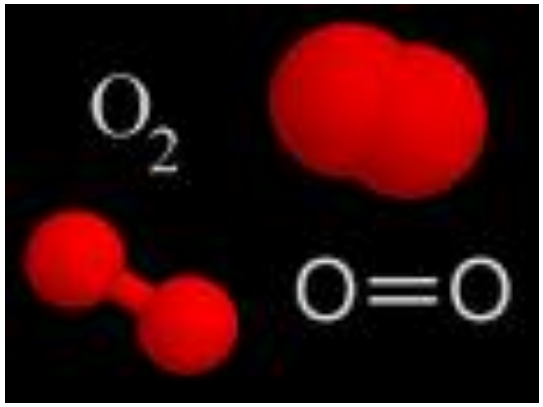
4. The final stage is the death phase, manifested by a decrease in the number of viable bacteria.

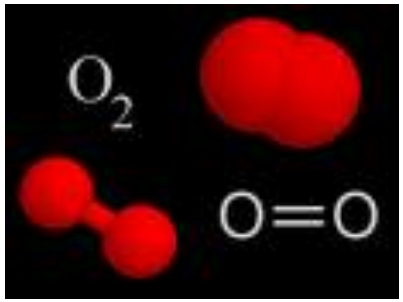
- **The availability of sufficient oxygen for most organisms accelerates metabolism and reproduction.**

- Bacteria are divided into 3 classes according to their oxygen requirement:

1. Obligate Aerobic bacteria
2. Facultative bacteria
3. Obligate Anaerobic bacteria

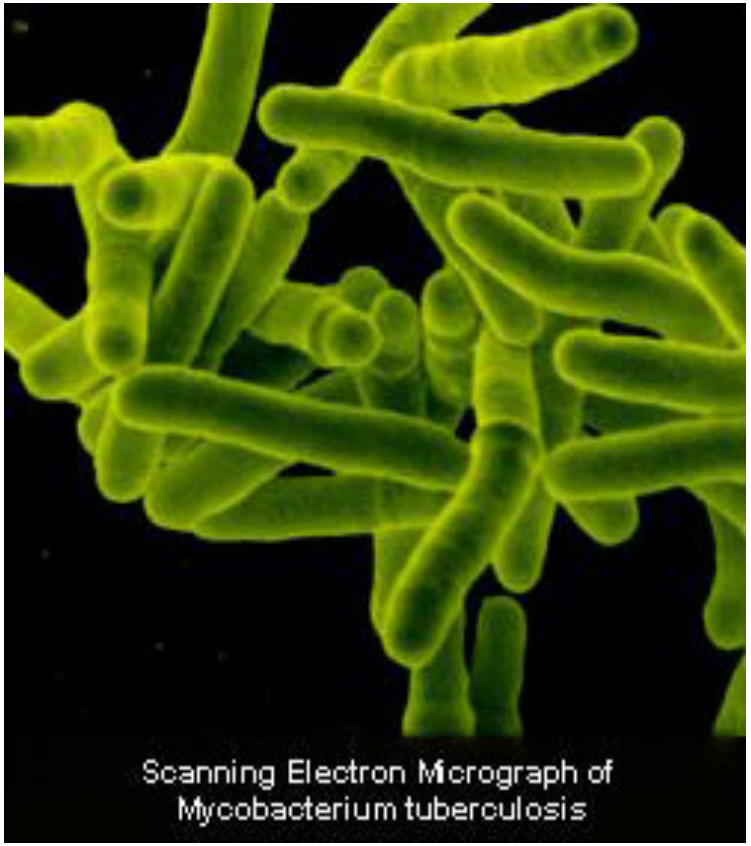






1. Obligate Aerobic bacteria

Some bacteria, such as M. tuberculosis, are obligate aerobes, meaning oxygen is required for reproduction as their ATP generating system is dependent on oxygen as a hydrogen acceptor.



2. Facultative bacteria

Other bacteria, such as E. coli, are facultative anaerobes; If there is oxygen, they use it to produce energy by respiration, but if there is not enough oxygen in the environment, they can also use the fermentation way to produce ATP.

3. Obligate Anaerobic bacteria

A third group of bacteria, such as Clostridium tetani, are obligate anaerobes, meaning they lack either or both of the enzymes superoxide dismutase or catalase and cannot grow in the presence of oxygen. Obligate anaerobes differ in their response to the presence of oxygen; some can survive but fail to reproduce while others die quickly.

Bacteria Genetics

- Bacteria are haploid, that is, they have a single chromosome.
- They contain a circular hereditary material.
- There is only one copy of each gene.
- In haploid cells, any gene being mutated and thus not expressed causes that cell to lose its quality.

TRANSFER OF DNA BETWEEN BACTERIAL CELLS

- The transfer of genetic information from one cell to another is accomplished in three ways:

1. conjugation,
2. transduction and
3. transformation

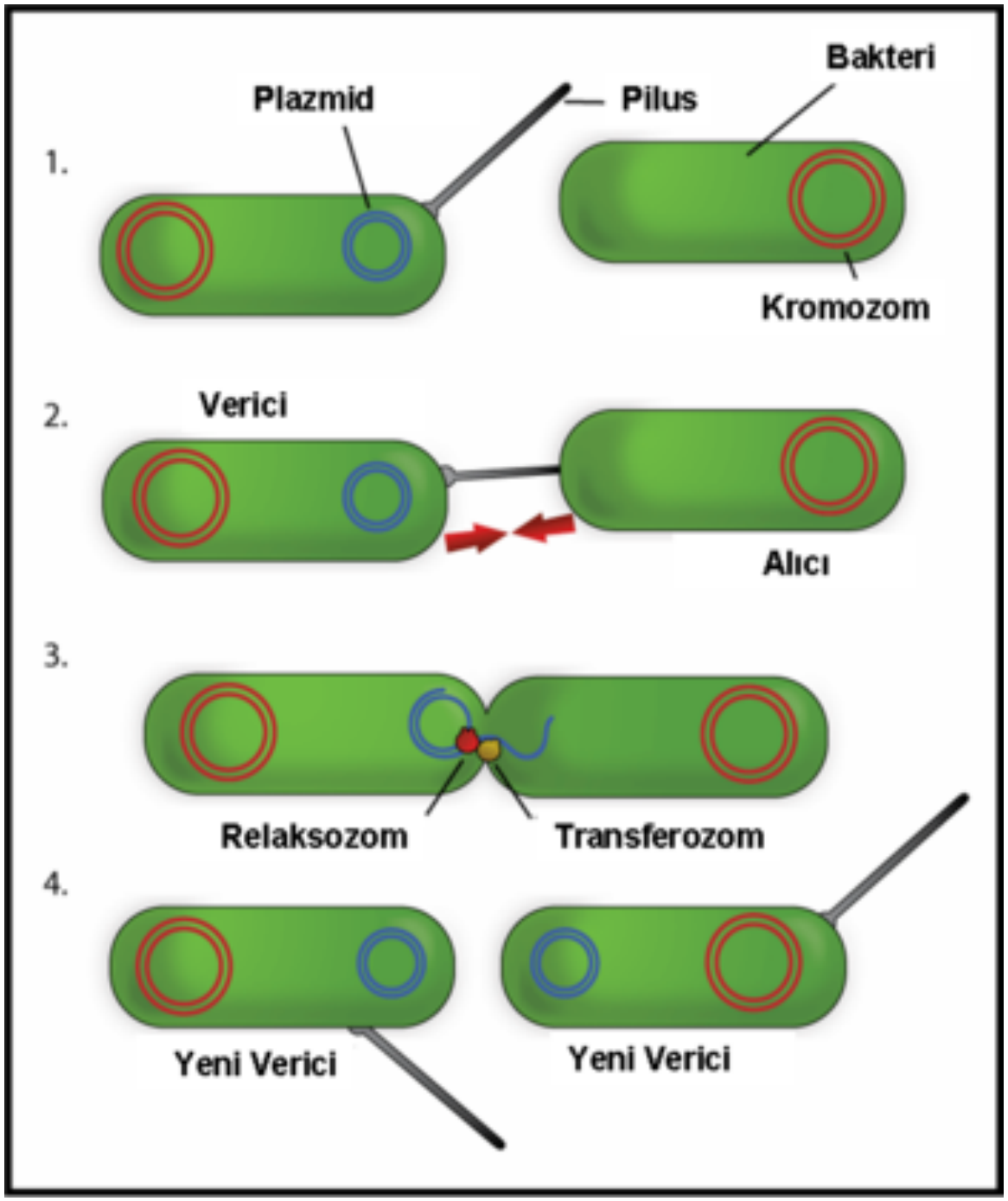
- The most important consequence of DNA transfer from a medical point of view is the spread of antibiotic resistance genes from one bacterium to another in this way.

Conjugation

It is the mating of two bacterial cells. Meanwhile, DNA is transferred from the donor cell to the recipient cell.

Conjugation is controlled by an F (fertility) plasmid (F factor) that carries genes for proteins required for mating action.

One of the most important proteins for conjugation is pilin. It forms the sex pilus (conjugation tube) of the pilin.



Conjugation

Mating begins when the pilus of the donor (F+) bacterium carrying the F factor attaches to a receptor (F") on the surface of the bacterium that does not carry the F factor.

Here, only the F factor is transferred and the bacterial chromosome is not transferred.

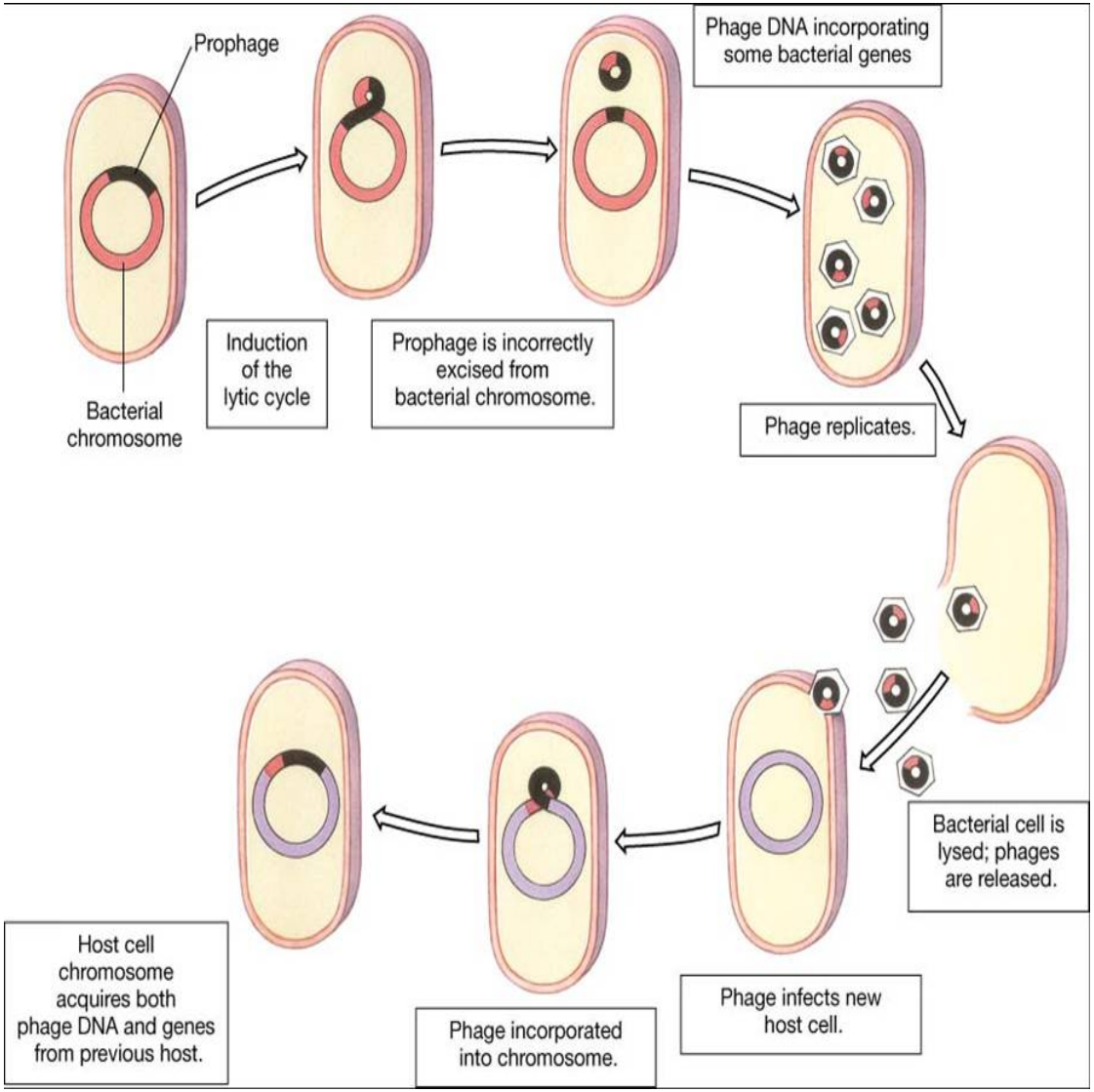
Transduction

- It is the transfer of cell DNA into another bacteria by means of a bacterial virus (bacteriophage, phage).

During transduction;

1. **The virus enters the bacterial cell.**
2. **The virus genome integrates into part of the bacterial DNA.**
3. **During infection, a part of the virus and bacterial DNA is transferred to the recipient cell.**

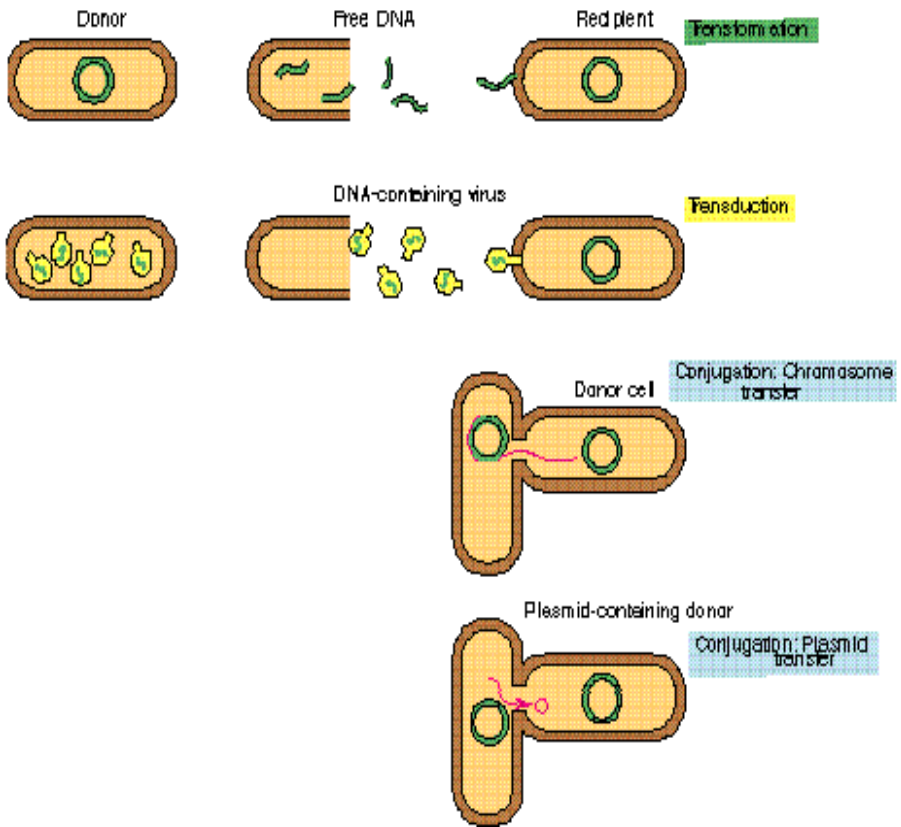
Transduction



In the recipient cell, the phage DNA can integrate with the cell's DNA and the cell can acquire a new character; This phenomenon is called isogenic transformation.

Transformation

It is the complete transfer of bacterial DNA from one cell to another, unlike conjugation and transduction.



Transformation occurs in two ways:

1. **In nature, as a result of the dying bacteria giving their DNA to the external environment, DNA can be captured by the recipient cells.**
2. **In the laboratory, DNA from one type of bacteria can be extracted and this DNA can be inherited into another bacterium. These applications are often used in genetic engineering. One of these applications, transfection, is the introduction of purified DNA into the nucleus of a eukaryotic cell.**

Normal Flora

- Various bacteria and fungi that **continuously** live in some parts of the human body (such as skin, esophagus, colon and vagina) form the flora of the region where they are located.
- Viruses and parasites, the other two major groups of microorganisms, are not considered members of the normal flora.

Tablo 6-1. Normal flora üyeleri ve bunların anatomik yerleşimleri (Alfabetik sıra ile).

Normal Flora Üyeleri	Anatomik Yerleşim
Bacteroides türleri	Kolon, boğaz, vagina
Candida albicans	Ağız, kolon, vagina
Clostridium türleri	Kolon
Corynebacterium türleri (difteroidler)	Nazofarenks, deri, vagina
Enterococcus faecalis	Kolon
Escherichia coli ve diğer koliformlar	Kolon, vagina, dış üretra
Gardnerella vaginalis	Vagina
Haemophilus türleri	Nazofarenks, konjonktiva
Lactobacillus türleri	Ağız, kolon, vagina
Neisseria türleri	Ağız, nazofarenks
Propionibacterium acnes	Deri
Pseudomonas aeruginosa	Kolon, deri
Staphylococcus aureus	Burun, deri
Staphylococcus epidermidis	Deri, burun, ağız, vagina, üretra
Viridans streptokoklar	Ağız, nazofarenks

Roles of normal flora members on human health:

1. Normal flora members, which do not cause any disease in healthy people, can cause disease, especially in people with weak immune systems and bedridden people.

   In addition, although these organisms are not pathogenic in their usual anatomical locations, they can cause disease in other parts of the body.

2. **Normal flora members also form a protective host defense mechanism.** Bacteria in the normal flora can prevent disease-causing bacteria from colonizing by occupying the attachment points on the skin and mucous membranes.

   The ability of normal flora members to limit the growth of pathogens is called resistance to colonization.

- If the normal flora is suppressed, pathogens can reproduce and cause disease.
- For example, antibiotics predispose to **pseudomembranous colitis** caused by Clostridium difficile by reducing the normal colony flora.

Clostridium difficile

3. Normal flora members can also function as nutrients for their host.

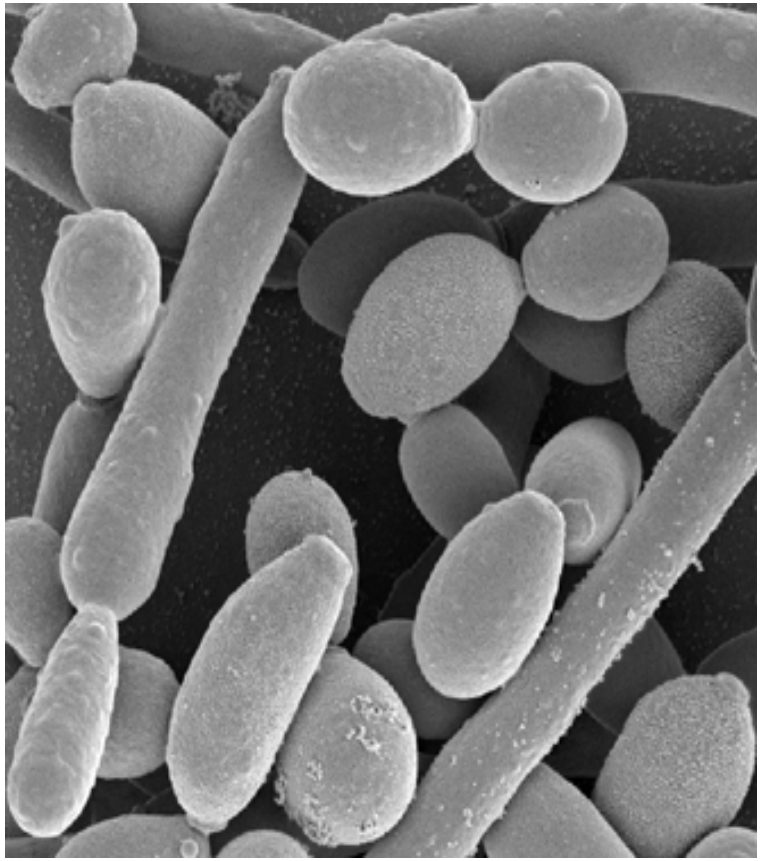
   Gut bacteria produce many B vitamins and vitamin K.

   Vitamin deficiencies may develop due to decreased normal flora in malnourished patients treated with oral antibiotics.

NORMAL FLORA OF SKIN

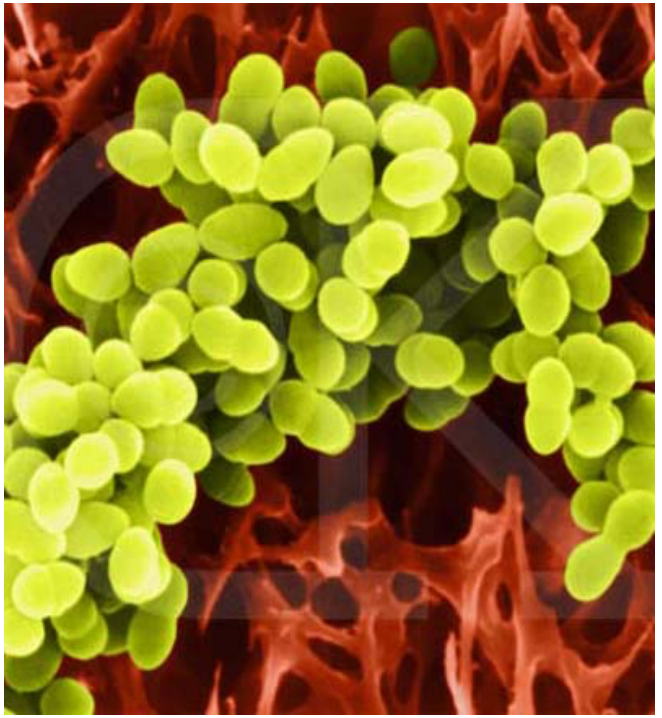
- The dominant organism is ***Staphylococcus aureus.***
- It is not pathogenic on the skin but can cause disease when it reaches certain areas.
- In the skin, it is located superficially, but some of it is located in the hair follicles.
- Anaerobic organisms such as *Peptococcus* and *Propionibacterium* are found in the deeper follicles of the dermis, where the oxygen pressure is lower.

- *Candida albicans,* a yeast, is also a member of the normal skin flora. It enters the patient's bloodstream when the needle is inserted into the skin.
- It is an important cause of systemic infections in people with reduced cellular immunity.



NORMAL FLORA OF RESPIRATORY TRACT

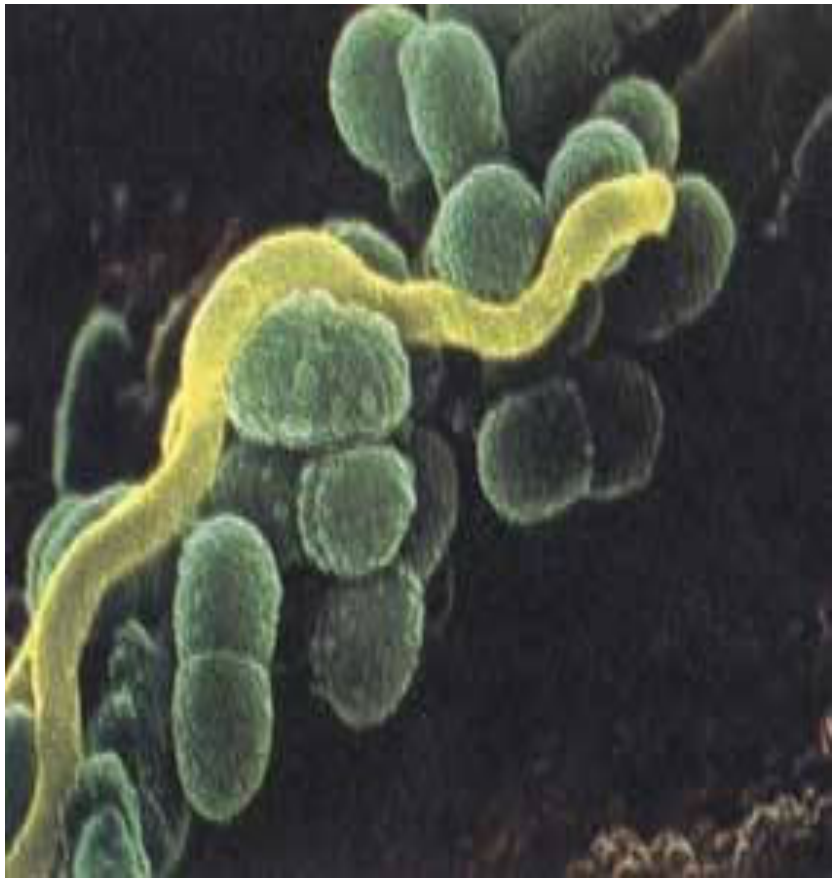
- Although a wide array of organisms colonize the nose, throat, and mouth, the lower bronchi and alveoli typically contain few or even no organisms.



- **The nose** is colonized by various streptococcal and staphylococcal species, the most prominent pathogen being S. aureus.

- **Throat flora includes a mixture of streptococci, *Neisseria* species and *S. epidermidis.***
- These non-pathogenic organisms occupy the attachment points in the pharyngeal (throat) mucosa and suppress the growth of pathogens *Streptococcus pyogenes, Neisseria meningitidis* and *S. aureus* in this area.

- Streptococci make up about half of the **oral flora.**
- *Streptococcus mutans,* a member of the Streptococcus group, is found in large numbers in dental plaque, the precursor of dental caries.

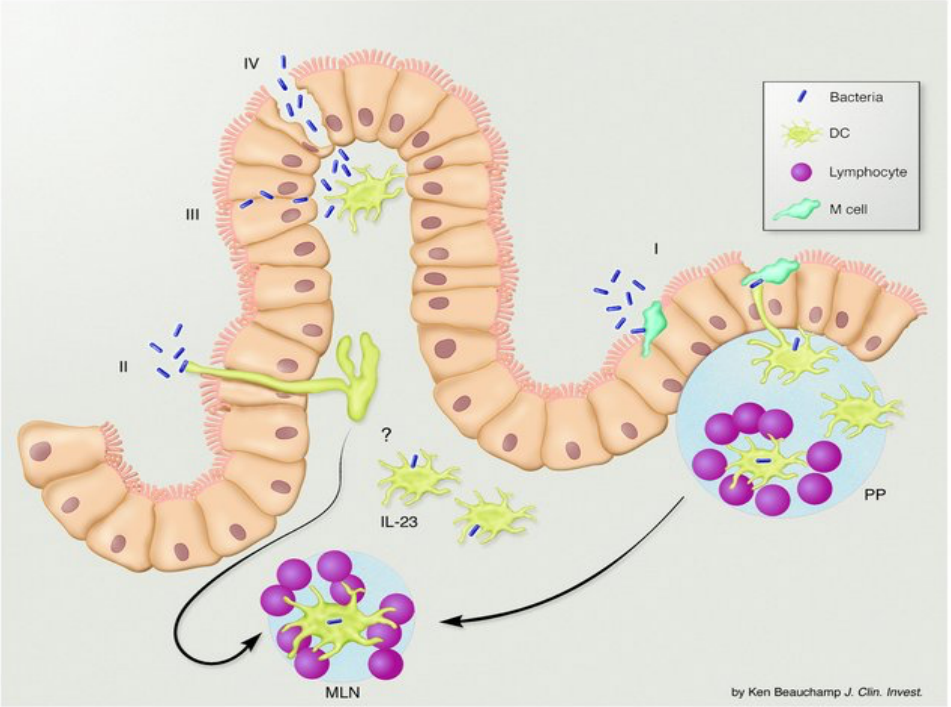


- Anaerobic bacteria such as *Bacteroides, Fusobacterium, Clostridium and Peptostreptococcus* species are found in gingival fissures where oxygen concentration is very low..

- If these organisms are removed, they can cause lung abscesses, especially in bedridden patients with poor dental care.

NORMAL FLORA OF THE BOTTOM CHANNEL

- Healthy people have very few organisms in the stomach due to the low pH and the presence of enzymes. In the foregut there are generally few streptococci, lactobacilli and yeasts, especially C.albicans.

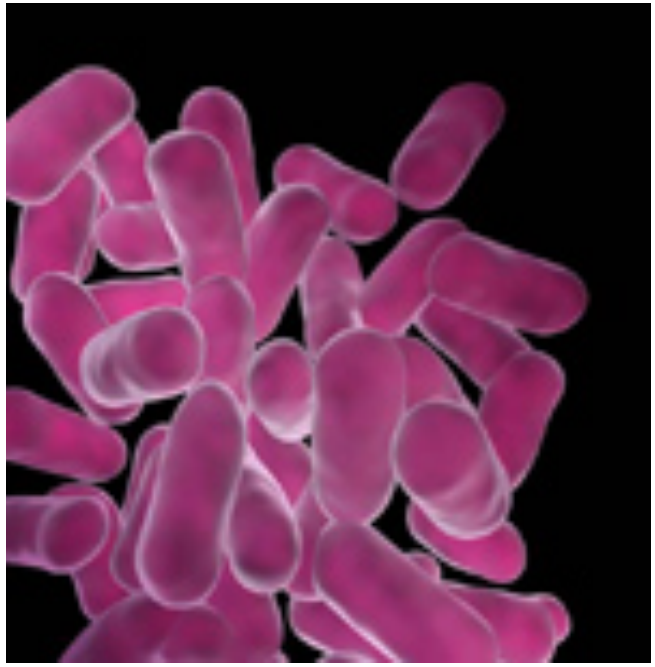


- The large intestine is the main site of bacteria in the body. Roughly 20% of stool is made up of bacteria.
- The normal flora of the intestinal tract plays an important role in extraintestinal diseases.
- For example, Escherichia coli is the leading cause of urinary tract infections. *Bacteroides fragilis* trauma is an important cause of appendicitis, diverticulitis and peritonitis.

- Other organisms include *Enterococcus faecalis,* which causes urinary tract infections and endocarditis, and *Pseudomonas aeruginosa,* which can cause various infections, especially in hospitalized patients with reduced host defenses. P.aeruginosa is found in 10% of normal stools as well as soil and water.

NORMAL FLORA OF THE GENITOURINAR CHANNEL

- The vaginal flora of an adult woman consists mainly of *Lactobacillus* species:
- Lactobacilli are responsible for the production of acid that balances the vaginal pH in the adult woman. Before puberty and after menopause, when estrogen levels are low, lactobacilli are rare and vaginal pH is high.



- Since their suppression with antibiotics can lead to overgrowth of *C. albicans*, lactobacilli prevent the growth of potential pathogens. Overgrowth of this yeast can cause ***inflammation*** in the area.
- The vagina is located close to the anus and can be colonized by members of the fecal flora.
- E.g; Organisms such as *E. coli* and *Enterobacter* are resident in the vagina of women prone to recurrent urinary tract infections.