Bacteriology

For third year students, general education

(Biological and Geological Sciences Division)

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رؤية الكلية

تسعى الكلية الى مساعدة الجامعة فى تحقيق اهدافها الاستراتيجية من خلال ان تكون واحدة من الكليات المتميزة والمنافسة داخليا وخارجيا فى التعليم وخدمة المجتمع والبحث العلمى من خلال تحقيق مستوى رفيع من الاداء وتقديم خريج متميز يقابل الاحتياجات المتعددة بسوق العمل الداخلى والاقليمى والخارجى

رسالة الكلية

تهدف كلية التربية بالغردقة الى التميز من خلال:

- **إعداد المربين والمعلمين المتخصصين والقادة إعداداً أكاديمياً ومهناً وثقافياً فى مختلف التخصصات التربوية.**
- **تنمية القدرات المهنية والعلمية للعاملين فى ميدان التربية والتعليم بتعريفهم بالاتجاهات التربوية الحديثة.**
- **إجراء البحوث والدراسات فى التخصصات التربوية والمختلفة بالكلية.**
- **نشر الفكر التربوى الحديث واسهاماتة لحل مشكلات البيئة والمجتمع.**
- **تبادل الخبرات والمعلومات مع الهيئات والمؤسسات التعليمية والثقافية المختلفة.**
- **تنمية جوانب شخصية الطلاب ورعاية الموهوبين والمبعدين.**

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Introduction

Microbes are tiny living things that are found all around us and are too small to be seen by the naked eye. They live in water, soil, and in the air. The human body is home to millions of these microbes too, also called microorganisms. Some microbes make us sick, others are important for our health. The most common types are bacteria, viruses and fungi. There are also microbes called protozoa. These are tiny living things that are responsible for diseases such as toxoplasmosis and malaria.

Microorganism is a term that is difficult to define precisely. Operationally, it refers to any organism that is too small to be seen by the unaided eye. For most people, that would be about 0.1 to 0.2 mm in diameter. Anything less than this cannot be seen without a microscope of some kind and is in a loose sense a microorganism. In practice, however, the term microorganism is often used to include some macroscopic forms that belong to a group that is largely microscopic (e.g., the fungi, most of the algae), and it excludes some microscopic forms (e.g., some microscopic animals that despite their small size are multicellular with differentiated tissues and organ systems).

The vast majority of microbes are unicellular that is, the entire organism consists of a single cell. Also fairly common are filaments of cells attached end to end in a row. In some cases, especially in the fungi and the algae (most of which are considered microbes), there are representatives that are multicellular (often macroscopic as well). Even in these forms, however, the cells that make up the organism are not organized into highly differentiated tissues and organs. The differentiation of diverse and very different types of tissues and the organization of these different tissues into organs seem to have been an evolutionary invention of the plants and animals alone (with some rudimentary tissue differentiation in the algae and fungi).

Cosmologists agree that the earth is about 4.5 billion years old, originating from the coalescence of debris left over after the formation of the sun. It was originally very hot too hot for liquid water from the heat of gravitational collapse and from radioactive decay in its core. It was also continually bombarded with large meteorites whose impact released so much energy that nothing living would have been able to survive. It is generally agreed that the earth cooled to habitable temperatures (less than 100°C) about 4.0 billion years ago and that the meteoritic bombardment abated by about 3.8 billion years ago. Coincidentally, this is about the age of the oldest rocks, and these rocks show chemical evidence of microbial life. Shortly thereafter (in geological time), at about 3.5 billion years ago, fossil evidence of microbes exists. Clearly life originated on earth almost immediately after conditions permitted. The microscope has been as much a tool for geologists as for biologists. Starting in the 19th century, the microscopic organization of mineral grains in rocks was examined by putting very small shards of rock under the microscope and looking through the very thin edges. When the rock is very thin (on the order of 0.1 mm), it is translucent, and light can pass through it, revealing its structure. It was thus natural that at some point geologists would search shards of rock for fossil microorganisms. The earth's mantle consists of layer on layer of rock, with, unless the rock has been rearranged by tectonic movements, the most recent rock on top and the oldest on the bottom. The top rocks, corresponding to the last quarter of the earth's history, contain macroscopic fossils; the bottom rock, three quarters of the history of the earth, is barren of visible fossils. This discontinuity was recognized even in Darwin's time, and Darwin commented on the apparently sudden appearance of life in the geological era called the Cambrian. It was an obvious possibility that Precambrian life was microscopic, and thus, geologists were attentive to the possibility of microscopic fossils. They are quite rare, however, and it was not until the 20th century that the first Precambrian microfossils were discovered. In

1918, E. Moore saw fossil cyanobacteria in Precambrian rocks, and similar observations were made by John Gruner in 1923 and Burton Ashley in 1937 (Figure 1). Interest waned, however; the point had been made, and the attention of geologists moved on. After the Second World War, however, nuclear sciences produced new and precise ways to date rocks (rock dates before this had been little more than educated guesses). This led to the realization that a systematic study of microfossils might reveal when life on earth originated, and there was a resurgence of interest in microbial fossils. These studies have revealed that complex, filamentous organisms similar to modern prokaryotes are present in rocks from nearly 3.6 billion years ago (Figure 2). Older rocks exist up to about 3.8 billion years old. To date, these have not shown unambiguous fossils. Modern micropaleontology is a complex science. Rocks to be examined are cleaned many times and shattered into small pieces with a hammer, and then thin sections (typically about 100 μm thick) are cut with a fine saw for microscopy. The rest of the rock sample is pulverized and analyzed chemically for the amount of carbon present and for the ratio of 13C to 14C (which can indicate whether the carbon is of biological origin). Sometimes the rock is assayed for specific compounds, like derivatives of chlorophylls or other complex molecules, which indicate the presence of life.

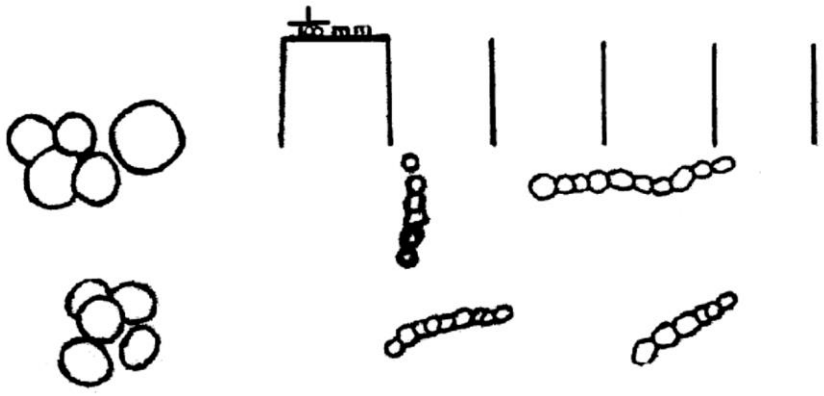


Figure.1: Moore's 1918 drawing of microbial fossils

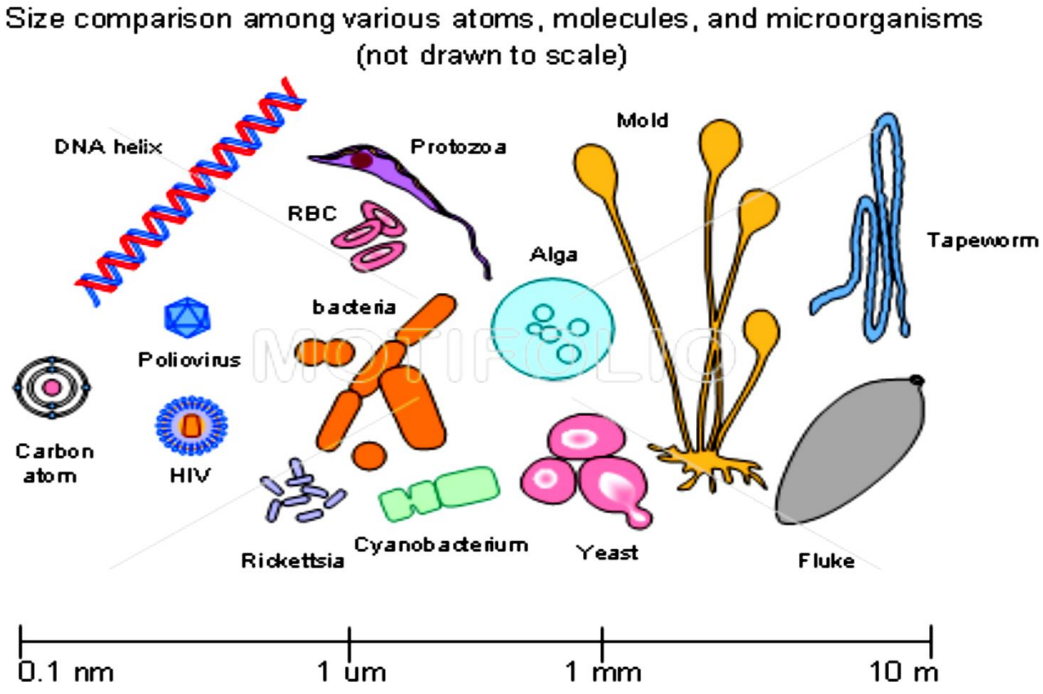
Figure .2: Modern micrograph of fossil microbes 3.5 billion years old.

In fact, historians are not sure of the identity of the person who recorded the first observations of microorganisms, as it is mentioned that between 1590-1608 a Dutch person named **Zacharias Janssen** managed to develop the first compound microscope, but what is certain is that the microscope became available in the middle of the seventeenth century, when the world recorded The Englishman **Robert Hooke** made the first observations of a fungal hyphae between the cells of one of the specimens he was examining.

In 1676, a Dutch merchant named **Anton van Leeuwenhoek** recorded - more accurate observations of microorganisms, which he called (animals) after he developed the microscope used by Robert Hooke, which magnified objects 30-20 times to be able to magnify more than 200 times and continued This man until his death in 1723 explored this new world and is considered to this day the first to provide an accurate description of protozoa, fungi and bacteria.

Microbiology definition

It is the science that is concerned with studying living organisms that are small in size and that cannot be seen with the naked eye. (Bacteria, Viruses, Protozoa, The rickettsia, Algae and Fungi).



The importance of Microorganisms

Microorganisms are one of the indispensable components of the ecosystem. Without them, the cycles of some elements such as carbon, oxygen, nitrogen and phosphorous cannot be completed in the land and water ecosystem. They are also the main source of nutrients for every food chain in the ecosystem. Microbiology is used on a large industrial scale in the production of food, antibiotics, vaccines, vitamins, enzymes and many other products. In fact, the science of biotechnology depends mainly on microbiology.

Branches of Microbiology

Microbiology can be classified into two main branches, namely (pure) microbiology and applied microbiology, and each includes a group of secondary branches, which are as follows:

Purified Microbiology includes:

- Bacteriology
- Mycology
- Animal primatology
- Algae science
- Parasitology
- Immunology
- Virology

Bacteriology

Bacteria (bacteriology) is one of the branches of microbiology, which in turn is considered one of the most important branches of life science (biological).

Biology is the science that studies all living things, including animals, plants and microorganisms.

The word biology is a Greek word derived from two words or two syllables:

Bio=Bios = Life, **logy= Logos = Science**,

This discovery was made by the two scientists: Lamark and Treviranus.

Biology is divided into the following branches with respect to the type of living organisms we study:

A- Zoology: concerned with the study of animals from all angles

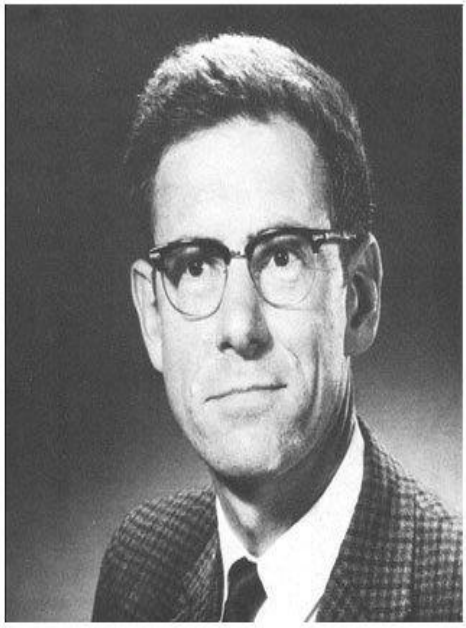
2- Botany: It is concerned with the study of plants from all angles

3- Microbiology: concerned with the study of microorganisms

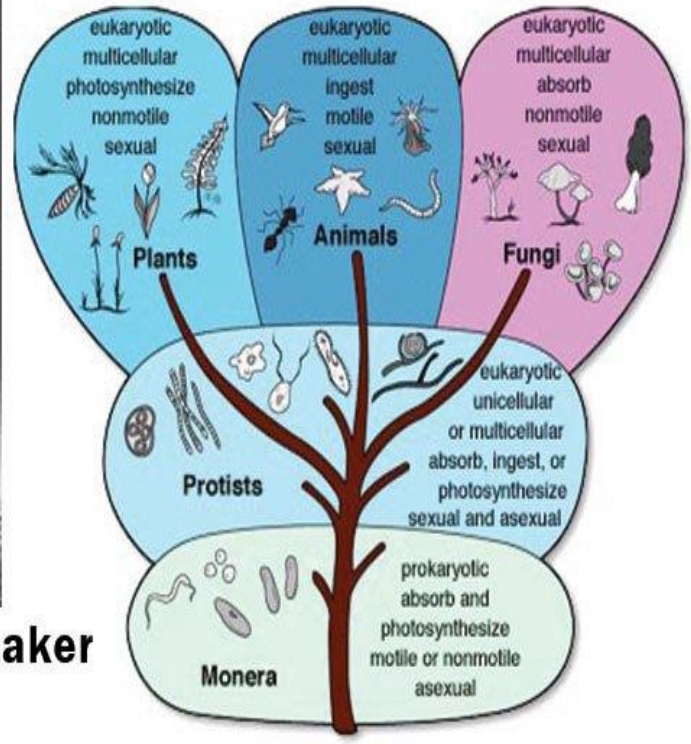
Microbiology is the study of microorganisms that cannot be seen with the naked eye, such as bacteria, algae, and fungi. Some scientists prefer to include this science within botany and do not tend to separate microorganisms from plants.

However, others tend to separate these organisms in a kingdom independent of the plant kingdom and study them under this branch, because some living organisms such as the euglena algae share with the animal the characteristic of movement and share with the plant in the presence of chlorophyll, so they were placed in a separate branch. **Anton van Leeuwenhoek** was the first to discover living things in 1675, with a microscope he designed. In 1866, the German zoologist **Ernst Heinrich** Haeckel divided organisms into three kingdoms, the animal kingdom - the plant kingdom and the Protista kingdom. He was the first to put living things into an independent kingdom. He also talked about the idea of Protista or protozoa for the first time, and included in his group many types of organisms, including fungi and sponges. After the discovery of the electron microscope, it was found that there are prokaryotes, including eukaryotes, which are single-celled organisms that have a specific nucleus and organelles. Organelles are structures that perform certain functions, such as Protista, which include algae. Among them are prokaryotes, which are single-celled organisms that do not have a specific nucleus or organelles in a group called monera, which is bacteria.

After that came the world. The Whittaker system for classifying living things is a classification developed by the American scientist Robert Whittaker) in 1969 AD, which places living organisms within five kingdoms: Unitarians, Protista, fungi, plants and animals. Prokaryotes have lived alone on the planet for more than a billion years. It remained the most numerous and widespread among other living organisms on Earth. The total living mass of prokaryotes is ten times more than that of eukaryotes. Both primitives and eukaryotes today evolved from a common ancestor through a complex process of gene transfer between prokaryotic lineages. Some genes of primitives are similar to those of bacteria, some are similar to those of eukaryotes, and some are limited to archaea.



Robert Harding Whittaker
(1920 – 1980)

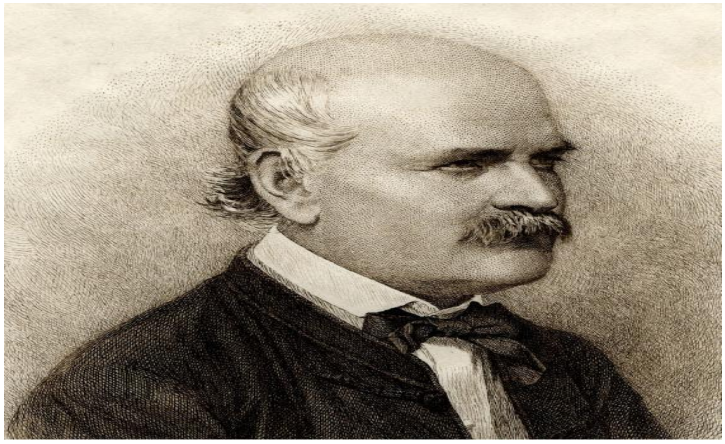


Discovery of bacteria

Anton van Leeuwenhoek, a Dutch cloth merchant, was the first person to see bacteria in 1660, and he used a magnifying glass so that he could examine the fabric of the cloth more easily, achieving magnifications up to 500 times, using his best lens to look at a sample of pond water, and seeing small living things Leeuwenhoek sent a report of his observations of bacteria and algae to the Royal Society of London in the late 1670s. With many detailed drawings, which still exist today, he also saw some single-celled plants and animals, and he also saw some of the largest bacteria, and for nearly two centuries, it was known that the world contained small living organisms, but was discovered Recently, bacteria can be a factor in causing some diseases, and they can transmit diseases from one person to another.

The scientist *Semmelweis* and the germ theory of disease

The germ theory of the disease was not fully developed until 1870, but 30 years before that, a Viennese doctor named Ignaz Semmelweis made a very important but totally unacceptable discovery at the time, as Semmelweis was working as an obstetrician in a Vienna hospital. The maternal and infant mortality rate was very high due to the well-known infection Puerperal Fever, and Semmelweis noted that the room run by midwives (health care providers or midwives) had a much lower death rate than the ward run by doctors, and at that time, doctors were going from sick to another without washing their hands. Thus the bacteria that cause puerperal fever are easily transmitted around the ward, and Semmelweiss suggestion that physicians should sanitize their hands after each patient, wear clean ward coats, and different room clothes, and this was carried out after the death of a large number of patients, led to this leads to a significant reduction in mortality rates.



However, Semmelweis said that the doctors were doing worse than the midwives, and that his colleagues hated him, and he was fired, after which he got another job in another hospital, and made the same notes and improvements, Semmelweis again made heavy criticism and lost his job, and died in In 1865 in a mental institution where they considered him crazy, he did not live to see the theory of small particles

invisible to the naked eye like bacteria that can carry infectious diseases from person to person.

Louis Pasteur, Robert Koch, **and the discovery of bacteria**

Conclusive evidence was obtained by many scientists of the presence of bacteria, but the most important evidence came from the work of Louis Pasteur and Robert Koch in the 1860s and 1870s, Pasteur showed that microorganisms grow in the broth, but no growth or spoilage of the broth occurs if it is Boiling it, because boiling kills bacteria and other microorganisms. The boiling process to keep food fresh is called pasteurization. Robert Koch showed that anthrax is caused by bacteria, setting the basic criteria that proved the germ theory and are now called Koch's concepts and are still used today to prove that Bacteria is an infectious agent that causes diseases.

Definition of bacteria

- Bacteria are living organisms that are not visible to the naked eye, prokaryotic, and in the absence of a nuclear membrane
- In the cell, DNA is found in a double helix strand.

- Plasmids of single-celled bacteria with a cellular structure that perform all vital functions and are either singly or in clusters and are heterotrophic, either accumulating, autotrophic, parasitic or symbiotic.
- They multiply by binary fission, meaning that their number doubles every certain period, and the period separating two successive divisions is called generation time.

Prokaryotic Vs. Eukaryotic

No	Eukaryotic cell	Prokaryotic cell
1	The chromosome are enclosed in a double- layered membrane (nucleus)	The chromosome is in the cytoplasm (no membranes)
2	Chromosome structure is relatively complex	More simple structure
3	Cell division involve meiosis and mitosis	Cell division does not involve meiosis or mitosis
4	Two types of ribosomes are present; a larger type in the cytoplasm and a smaller type in the chloroplasts and mitochondria	Only one small type in the cytoplasm
5	Presence of cell organelles for specific functions such as photosynthesis (chloroplasts) and respiration (mitochondria)	No such organelles

The importance of bacteria in nature and in human life

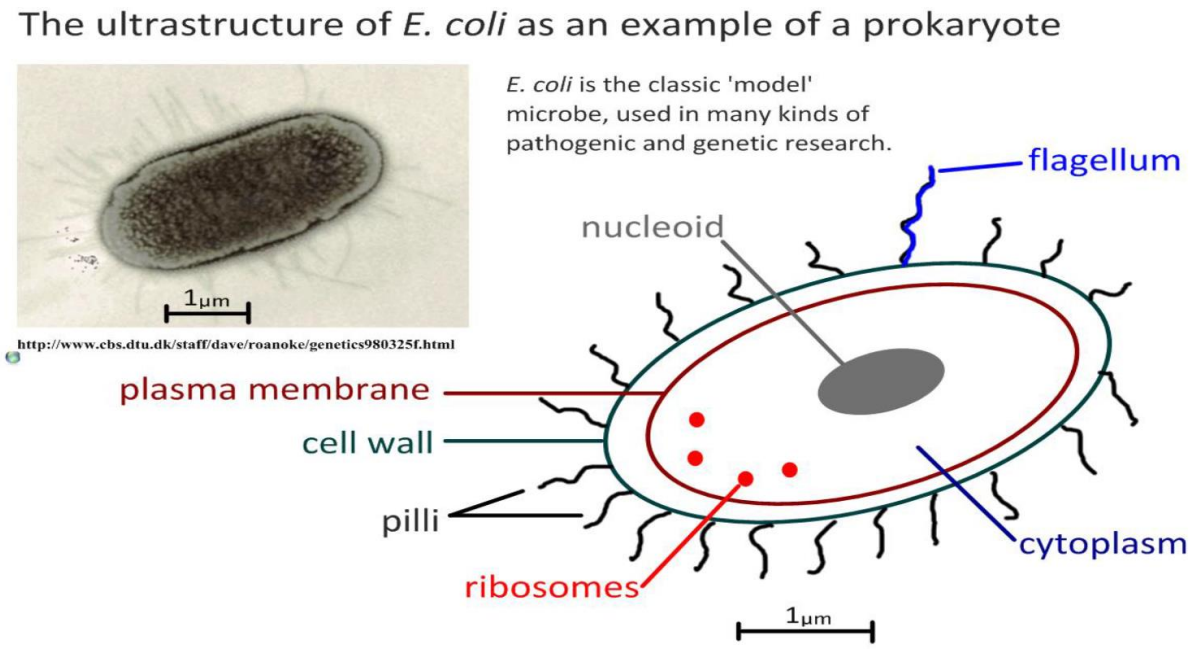
1) It is used in the dairy industry, the production of vinegar and butter, and the fermentation of animal feed
2) Bacteria ferment sugars, proteins and organic compounds and is used in the production of acetone, crackers, imaging films, alcohol, lactic and citric acid.

3) Decompose the remains of plants, animals and humans and return their elements to the soil in a simple form where the plants feed on them by absorbing them in their simple form
4) Nitrogen fixation in plants and municipal fertilizer production
5) Some of them are used in the analysis of complex hydrocarbon compounds, disposal of explosive residues, wastewater treatment and pollutant disposal.
6) Some bacteria live in the roots of plants and fix nitrogen and make it easy to use.
7) Bacteria are used to eliminate pests harmful to agricultural crops (biological resistance), which eliminate worms, larvae and pathogenic fungi in the soil.
8) The genetic content of these organisms can be controlled in what is known as genetic engineering to be able to produce important therapeutic compounds such as insulin hormone and interferon compounds in the elimination of agricultural pests. Harmful resistance to pathogenic fungi, worms and larvae (biological resistance).
9) It lives in the human intestine, reduces the incidence of cancer, helps produce lactic acid, and inhibits the growth of rotting bacteria. It also produces vitamins B1, B2, B6, B12 and folic acid.
10) It is used in the production of important proteolytic enzymes and enzymes that degrade carbohydrates and fats, and in the production of medical drugs such as antibiotics that inhibit types of pathogenic bacteria.

Harmful activities of bacteria

1) It is used in the production of important enzymes such as proteolytics. It causes plant diseases such as pear fire blight, cotton root rot and potato scab
2) Cause food spoilage such as milk, meat and vegetables.

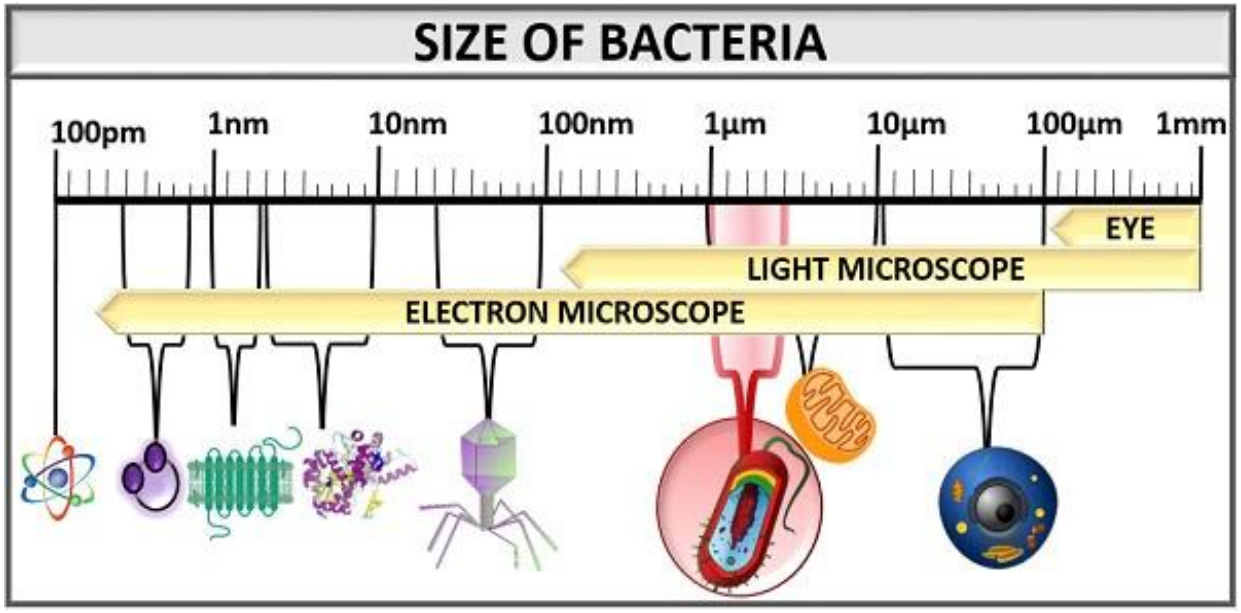
3) *Staphylococcus aureus* produces an exotoxin that is not affected by boiling iodine for food poisoning
4) *Salmonella* produces an endotoxin as a result of eating food with microbes
5) *Clostridium botulinum* bacteria cause botulinum toxin caused by an exogenous toxin that affects the nervous system and leads to death after 36 hours
6) Bacteria cause some serious diseases transmitted by droplets such as tuberculosis and pneumonia, and *Clostridium tetani* bacteria cause tetanus.
7) There is a bacteria responsible for diseases such as gonorrhea, syphilis, anthrax and brucellosis, which are transmitted through milk and meat of infected animals.



Morphology of bacteria

The study of the morphology (sizes - shapes - groups) of bacteria is one of the basic branches of bacteriology and is considered the first openness in classification.

- **Bacterial Size**

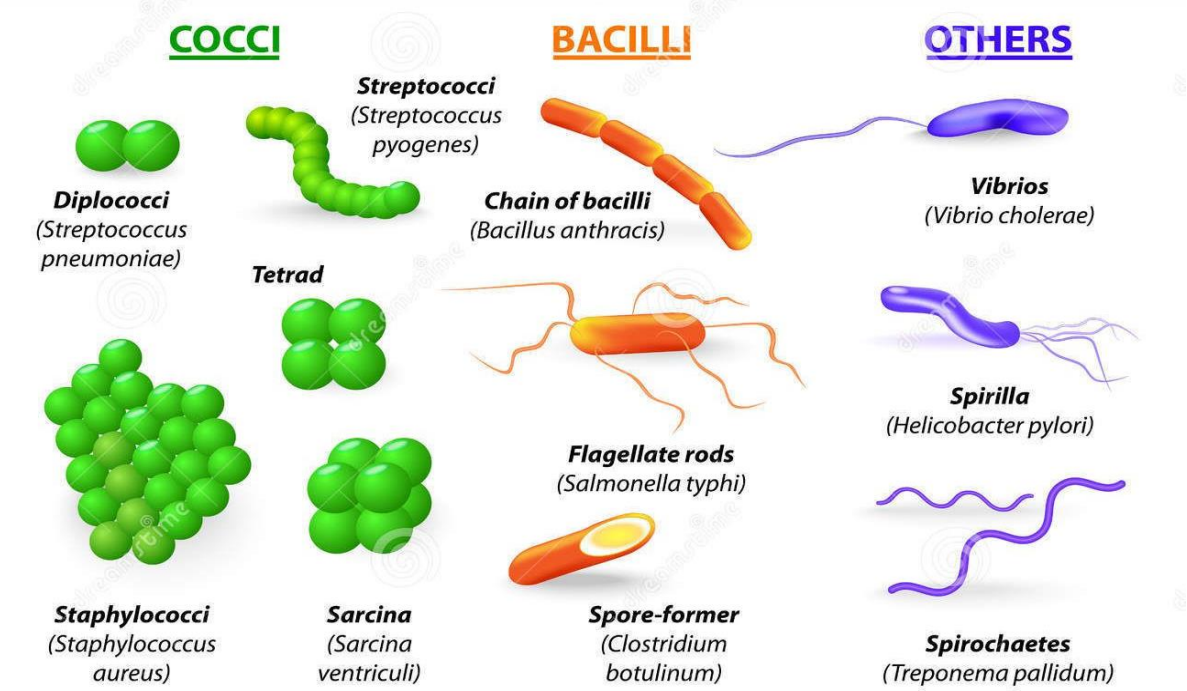


The relative sizes of various microscopic and nonmicroscopic objects. Note that a typical virus measures about 100 nm, 10 times smaller than a typical bacterium (~1 µm), which is at least 10 times smaller than a typical plant or animal cell (~10–100 µm). An object must measure about 100 µm to be visible without a microscope.

- The unit of measurement used in bacteriology is the micron (micrometer) which is one-thousandth of a millimeter.
- Bacteria are, in general one-tenth the size of the eukaryotic cell. On average, the size of bacteria ranges from 0.5 to 5 µm.
- However, they can be as tiny as 0.3 µm and as large as 0.7mm.
- The limit of resolution with the unaided eye is about 200 microns, and as many bacteria are smaller than this size, they are not visible with naked eyes.

- The size of common bacteria like *Escherichia coli* ranges in size from 1.1 to 1.5 µm in diameter.
- It has been observed that the size of bacteria has a significant role in the survival of the organisms.
- Owing to their tiny size, they are capable of surviving and even thriving in various unlikely environments like the vertical sediments in the marine environment.
- Since other organisms are absent in such an environment, bacteria can utilize the available resources.
- Besides, the small size of bacteria favors parasitism and the ability to survive in areas with low nutrition.
- The high surface area-volume ratio also allows the bacteria to take up all the nutrients required for survival while allowing the steady growth and reproduction.

- **Bacterial Shape**



- Most of the bacteria have a rigid cell wall that provides a definite shape to the bacteria while protecting the internal components.
- Even though this characteristic is valid for the majority of bacteria, they vary in shape that allows them to be classified into different groups based on their forms.
- This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton.
- Even though bacteria have a wide variety of shapes, anyone genus typically exhibits a limited subset of morphologies, indicating that, with a universe of shapes to choose from, individual bacteria adopt only those that are adaptive.
- Bacteria with different shapes present different physical features to the outside world, and these features help cells cope with and adapt to external conditions.
- It has been observed that bacterial shape contributes a measure of survival value in the face of nutrient acquisition, cell division, predators, attachment to surfaces, passive dispersal, active motility, and internal or external differentiation.

The common categories of bacteria based on their shapes are:

1. **Cocci**
    - The bacteria that are oval or spherical in shape are included called cocci bacteria.
    - These may either remain single or attached to one another in groups. They appear flattened when placed in groups.
    - It is assumed that coccoid forms were derived from rod-shaped organisms through evolutionary time.
2. **Bacilli (Rod-shaped)**
    - These are rod-shaped cells that also like cocci, remain either single or attached to other cells.

- Bacilli bacteria are among the first bacteria to have arisen, and this shape is said to be not as advantageous as other shapes. This has been assumed upon the observation of the behavior of filamentous *E. coli* cells which, though motile and chemotactic, move slowly and cannot tumble to change direction.

3. Spiral

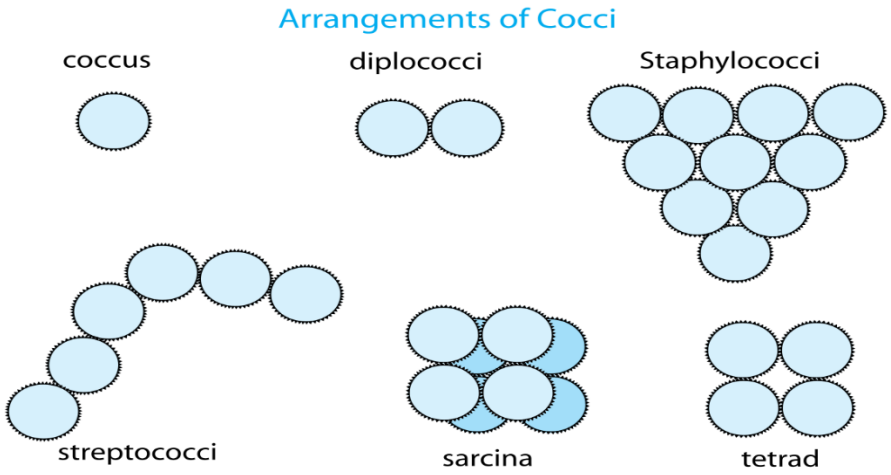
- This group includes bacteria that are either helical-shaped or curved (comma-shaped).
- The bacteria can range from slightly curved to corkscrew-like spiral.

Arrangements of Cocci

- Cocci bacteria can be arranged either singly, in pairs, in groups of four, in chains, in clusters or cubes consisting of eight cells.
- These cells remain attached during cell division.

Coccus

- This group includes bacteria that are present as a single cell.



Diplococci

- This arrangement results when two bacterial cells occur as a pair (joined together).

- Some of the cells in this arrangement might remain spherical while some might appear flattened, elongated, or bean-shaped.
- Examples: *Streptococcus pneumonia, Moraxella catarrhalis, Enterococcus spp, Neisseria gonorrhea.*

Tetrad

- Tetrad bacteria are arranged in a group of four cells that remain attached and grow in the attachment after cell division.
- This arrangement results when the cells divide into two planes.
- Examples: Aerococcus, Pediococcus, and Tetragenococcus.

Sarcina

- In this arrangement, the bacterial cells form a group of eight cells.
- This happens when the cells divide in a perpendicular plane.
- The common characteristic associated with these organisms is being strict anaerobe.
- Examples: *Sarcina aurantiaca, Sarcina lutea*, *Sarcina ventriculi*.

Streptococci

- Here, the bacteria are arranged in long chains.
- These bacteria are present in family Streptococcaceae, which is characterized by a lack of motility and Gram-positive bacteria.
- Examples: *Streptococcus pyogenes, Streptococcus pneumonia,Streptococcus mutans.*

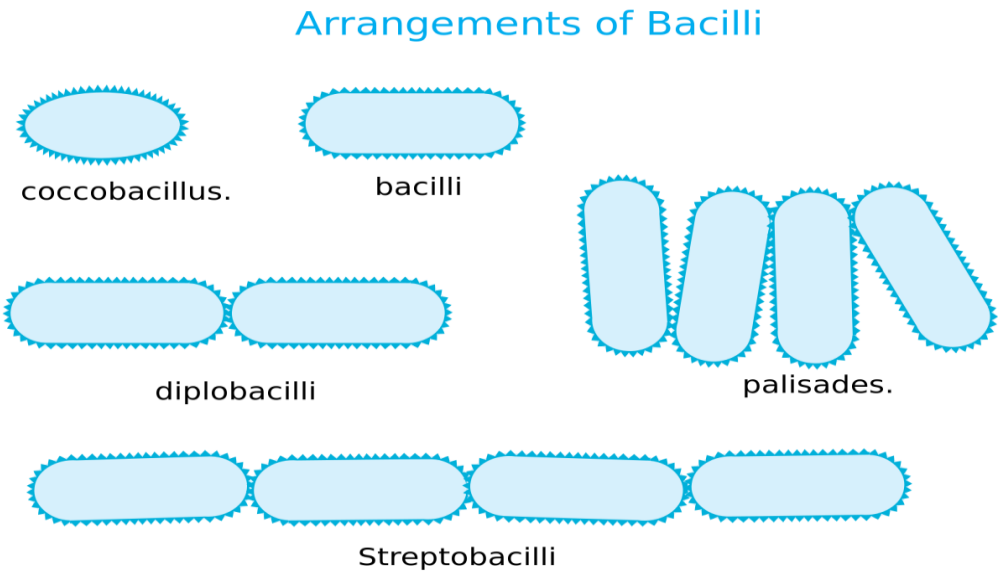
Staphylococci

- This type includes bacteria that are arranged in grape-like clusters.
- This results from cell division in both the planes and are characterized by organisms which are immotile and Gram-positive.
- Examples*: Staphylococcus epidermidis,Staphylococcus haemolyticus, Staphylococcus aureus, Staphylococcus capitis.*

Arrangement of Bacilli

Bacillus

- Bacilli are the bacteria which are rod-shaped and are present as single cells.
- These bacteria can form endospores and are facultative anaerobes.
- Examples: *Salmonella enterica subsp,Bacillus cereus, and Salmonella choleraesuis.*



Diplobacilli

- As in Diplococci, Diplobacilli also exists in pairs.
- After cell division, the two cells do not divide and grow in an attached arrangement.
- Examples: *Coxiella burnetii, Klebsiella rhinoscleromatis, Moraxella bovis.*

Streptobacilli

- In this group, bacteria are arranged in chains.
- This results from cell division in a single chain.
- Examples: *Streptobacillus moniliformis, Streptobacillus Levaditi, Streptobacillus felis, Streptobacillus hongkongensis.*

Coccobacilli

- As the name suggests, coccobacilli resemble both cocci as well as bacilli.
- These are shorter in size and thus, appear stumpy.
- Examples: Chlamydia trachomatis, Haemophilus influenza, Gardnerella vaginalis.

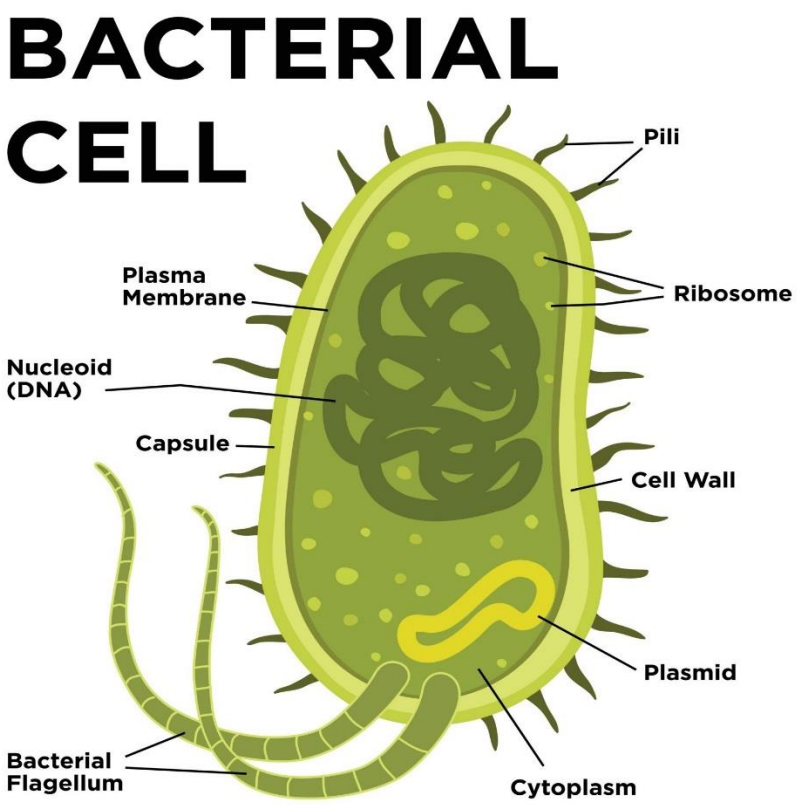
Pallisades

- Pallisades are the type of bacilli bacteria that resemble a picket fence structure as a result of the bent at the point of division during cell division.
- They appear similar to Chinese letters.
- Example: *Corynebacterium diphtheria that causes diphtheria.*

Chapter 2. Bacterial cell structure

- **Bacterial Cell Structure**
- **Flagella**
- **Detecting Bacterial Motility (directly or indirectly)**
- **Pili**
- **Capsular material and the environment**
- **The cell envelopes**
- **Plasma membrane**
- **The cytoplasm**
- **Bacterial endospores**

Bacterial Cell Structure



A bacterial cell shows various parts and these parts have specific structure and functions. Some structures are present in that particular species and hence that structure is characteristic feature of that species. Various parts are as follows:

1. **Capsules**
2. **Cell Wall**
3. **Plasma Membrane**
4. **Cytoplasm & Cytoplasmic Inclusions**
5. **Ribosomes**
6. **Bacterial DNA**
7. **Pili**
8. **Flagella**

1. ***Flagella***

- Flagella are unbranched filaments of uniform thickness (about 20 nm) throughout their length.
- Not essential for viability
- Flagella have three basic parts:

A. Filament

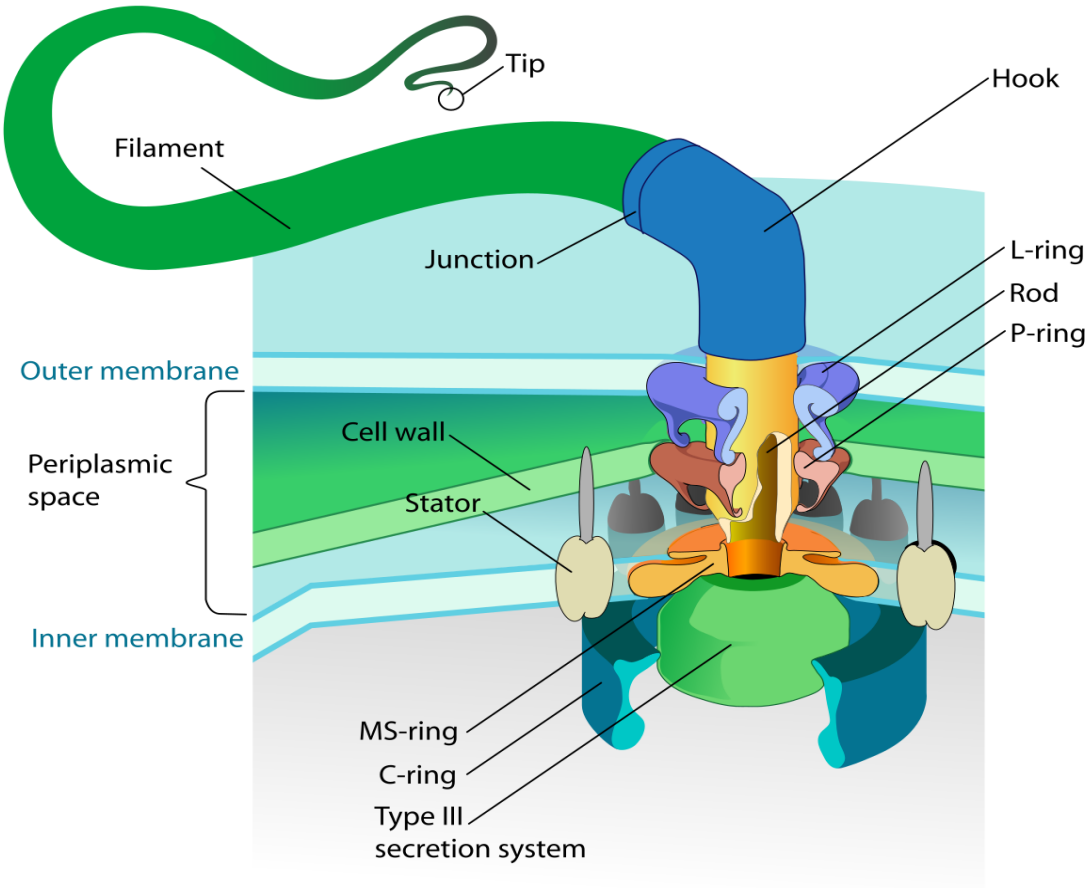
- Extends to exterior
- Made of proteins called flagellin

B. Hook

- Connects filament to cell

C. Basal body (system of rings)

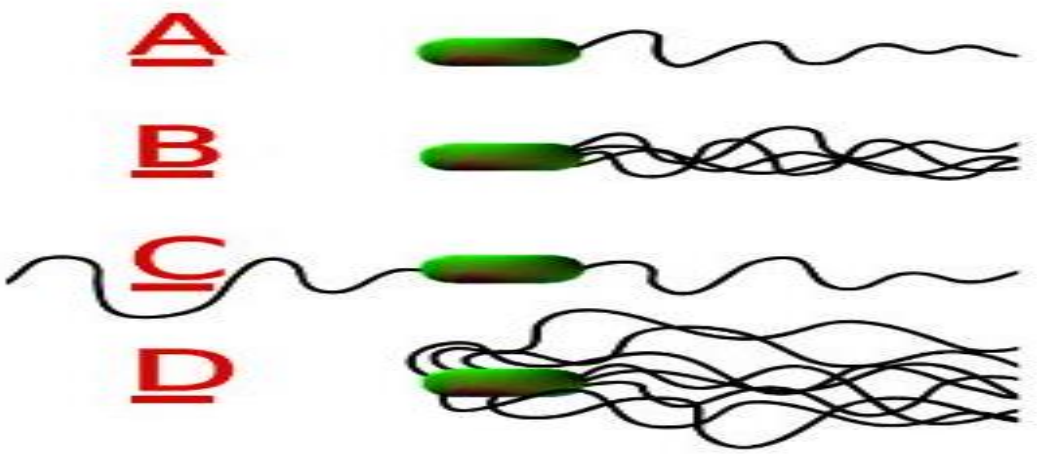
- Anchors flagellum into cell wall



- More than 98% of filament components is protein with high acidic amino acid content with some aromatic amino acids.
- The protein unit of filament (seen as beads under electron microscope) is called flagellin.
- Flagellin is synthesized within the cell and moves out through the hollow central core of the flagellum to its tip to be assembled there.
- The full length can be completed within 10 to 20 minutes.

Bacteria are divided according to the number of flagella and the distribution of flagella on their surface

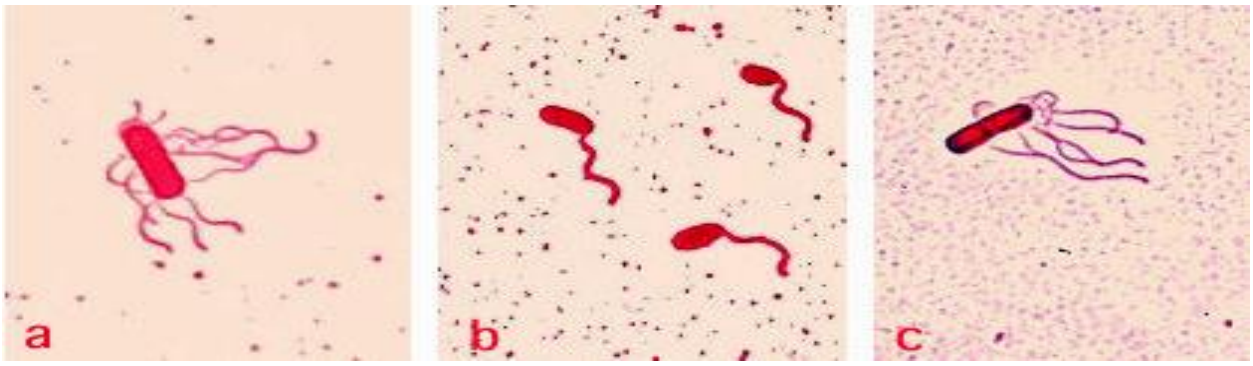
1. **Atrichous**: non- motile bacteria with no flagella.
2. **Monotrichous**: with one polar flagellum at one end.
3. **Lophotrichous**: with one group of polar flagella at one end.
4. **Amphitrichous**: with polar flagellation at both ends.
5. **Peritrichous**: the flagellation is distributed around the cell.



Detecting Bacterial Motility (directly or indirectly)

A. *Flagella staining*

Since the bacterial flagellum is below the resolving power of the light microscope, staining techniques such as Leifson's method utilize dyes and other components that precipitate along the protein filament to increase its effective diameter.

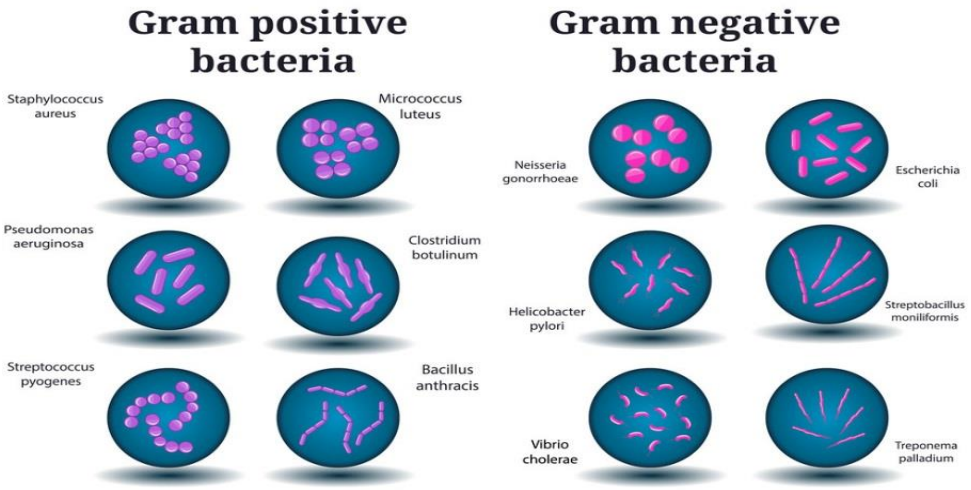


B. Motility test medium

A semisolid medium is inoculated with the bacteria in a straight- line stab with a needle. After incubation, if turbidity (cloudiness) or indicator colour change, due to bacterial growth, can be observed away from the line of the stab it is evidence that the bacteria are motile.

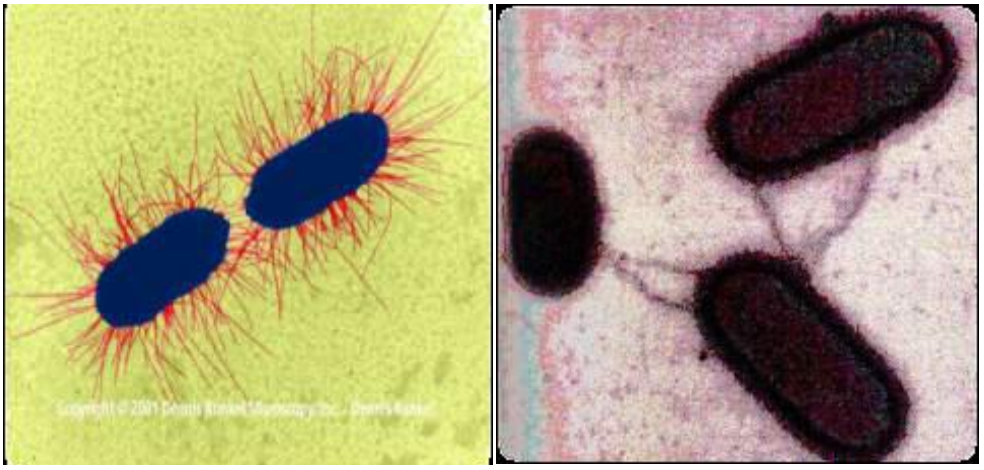
C. Direct microscopic observation

By using a “hanging- drop slide”. Most unicellular bacteria, because of their small size, will shake back and forth in a wet mount observed at 400X or 1000X. This is Brownian movement, due to random collisions between water, molecules and bacterial cells. True motility is confirmed by observing the bacterium swim from one side of the microscope field to the other side.



2. ***Pili***

Very fine and smaller filaments or appendages than flagella and found only in some freshly isolated Gram- negative bacteria (less than 10µm in diameter and one µm long). They have a role in sexual conjugation of bacterial cells (make cells stick together). Their number vary between one to 400 per cell. Common pili (almost always called fimbriae) are usually involved in specific adherence (attachment) of prokaryotes to surfaces in nature. In medical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to (colonize) tissues and/ or to resist attack by phagocytic white blood cells.



Movement in bacteria that not contain flagella

I. Sliding movement

It is defined as the smooth transfer of cells to surfaces through an active process; That is, it requires energy consumption, but it does not require the presence of flagella, a movement that occurs as a result of typical contractions in the protoplasm under the cell wall and occurs when the bacteria are in a solid medium, and to speak in a liquid medium as in gelatinous bacteria.

II. Random Brownian Motion

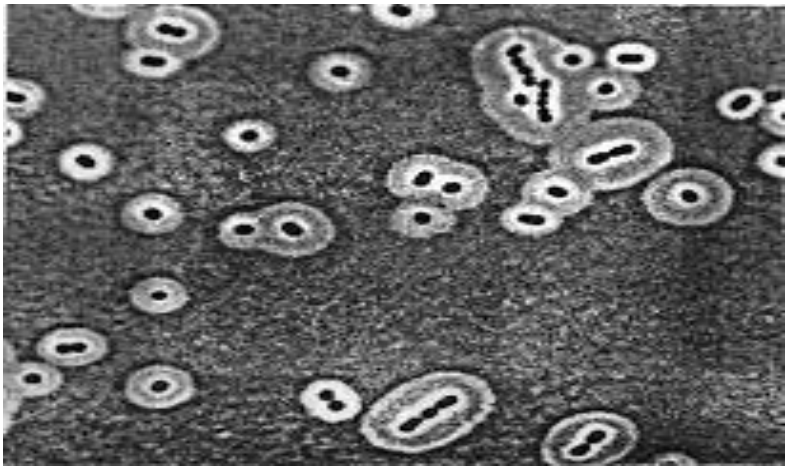
Motion caused by the collision of bacteria with the particles of the medium in which they live

III. Attractiveness

In this case, the bacterial cell is unable to move, but it is attracted to certain stimuli such as chemical attraction, where the bacterial cell begins to sense some chemical compounds in the surrounding environment and is attracted to them if they are beneficial to them and stay away from them if they are harmful. There is also optical attraction, magnetic attraction, and others

3. Capsules and Slime Layer

- General functions
- Protection: Protects bacteria from host defenses
- Attachment: Enables bacteria to adhere to specific surfaces
- Capsule is a distinct gelatinous layer
- Slime layer is an irregular diffuse layer
- (Chemical composition of capsules and slime layers varies depending on the bacterial species. Most are made of polysaccharide)



- **Chemical composition of some bacterial capsules**

Bacterium	**Capsule composition**	**Structural subunits**
Gram-positive Bacteria		
Bacillus anthracis	polypeptide (polyglutamic acid)	D-glutamic acid
Bacillus megaterium	polypeptide and polysaccharide	D-glutamic acid, amino sugars, sugars
Streptococcus mutans	polysaccharide	(dextran) glucose
Streptococcus pneumoniae	polysaccharides	sugars, amino sugars, uronic acids
Streptococcus pyogenes	polysaccharide (hyaluronic acid)	N-acetyl-glucosamine and glucuronic acid
Gram-negative Bacteria		
Acetobacter xylinum	polysaccharide	(cellulose) glucose
Escherichia coli	polysaccharide (colonic acid)	glucose, galactose, fucose glucuronic acid
Pseudomonas aeruginosa	polysaccharide	mannuronic acid
Azotobacter vinelandii	polysaccharide	glucuronic acid
Agrobacterium tumefaciens	polysaccharide	(glucan) glucose

4. ***Capsular material and the environment***

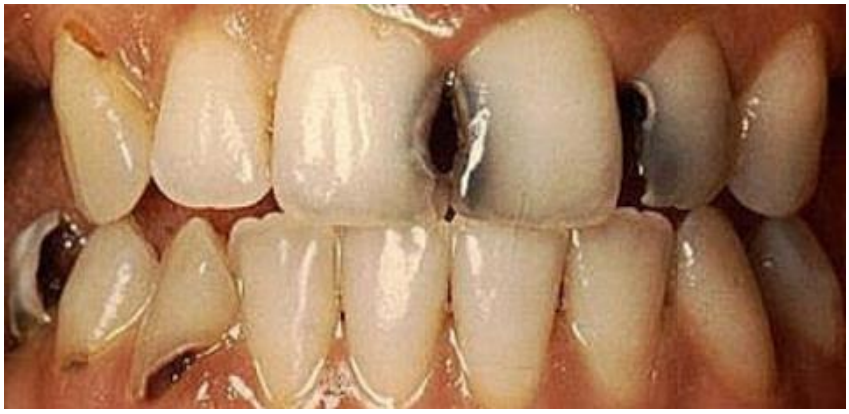
- Mucoid bacterial colonies in the rhizospheric region of desert plants increase the ability of these plants to resist drought and keep the moisture content of the root surface.
- Cements water bacteria in films and facilitates its adhesion to solid surfaces.

- The encapsulated bacteria of the oral microflora, in the presence of polysaccharides, stimulate dental caries processes.
- Hinders the attack by bacteriophages specific for O- antigens and lipopolysaccharide regions of the cell wall.
- The capsule may enclose a huge number of cells forming colonies called "zooglea". Zooglea will form in concentrated sugar solutions in the sugar refinery plants. These zooglea cause problems in these factories by stopping the flow of sugar solution in the pipes.
- Capsule formation may be responsible for considerable economic loss in dairy and other food industries. Carbohydrate- containing materials become "ropy" when encapsulated organisms grow on it.
- Some organisms such as Leuconostoc species, are employed commercially in the production of dextran (polymer of glucose). Dextrans are used as plasma extenders in the treatment of chock resulting from blood loss.

Relation of capsule to bacterial pathogenicity

- If the smooth colonies are pathogenic (disease- causing), so the rough mutants are not. This means that the virulence is associated with the occurrence of the capsule.
- *Streptococcus mutans* is the bacteria responsible for caries. Where these bacteria are attached to the thin membrane surrounding the teeth by a group of proteins that are located on the surface of the cell.
- The bacterial cell grows and creates a type of capsule called dextran capsule, which helps the cell bind to the enamel layer and then form a biofilm of 300-500 cells in thickness.

- Bacteria break down the sugar sucrose and then convert it into glucose and fructose, where they use fructose as an energy source in their biological processes.
- Lime layers accumulate on the teeth, causing yellowing.
- Yellowing turns into caries:
- The process of depolymerizing dextron into glucose and then using glucose as a carbon source.
- The depolymerization process results in the formation of lactic acid, which works to remove calcium from the teeth, and then decay occurs.



5. ***The cell envelopes***

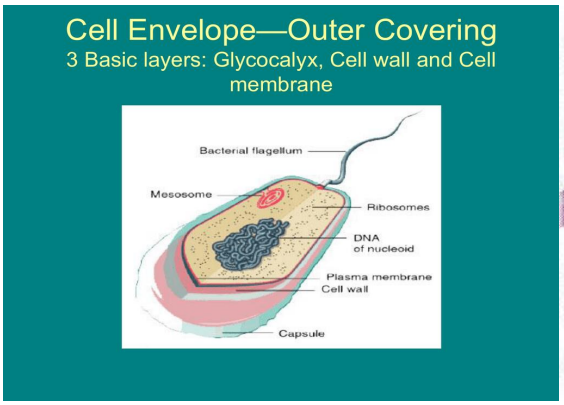
The "cell envelopes" are the several layers of material that envelope or enclose the protoplasm of the cell. The cell protoplasm (cytoplasm + membrane) is surrounded by the plasma membrane, a cell wall and a capsule. Almost all prokaryotes have a cell wall to prevent damage to the underlying protoplast.

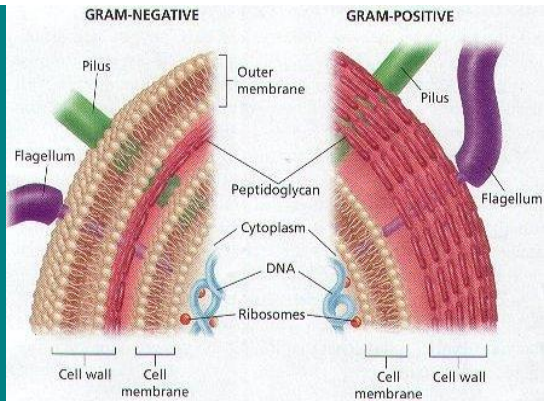
The wall is rigid but ductile.

1- It gives the cell the required mechanical strength

2- Determines the cell shape and encloses the cytoplasm

3- The characteristic antigens of each bacterium are also located on their cell walls.



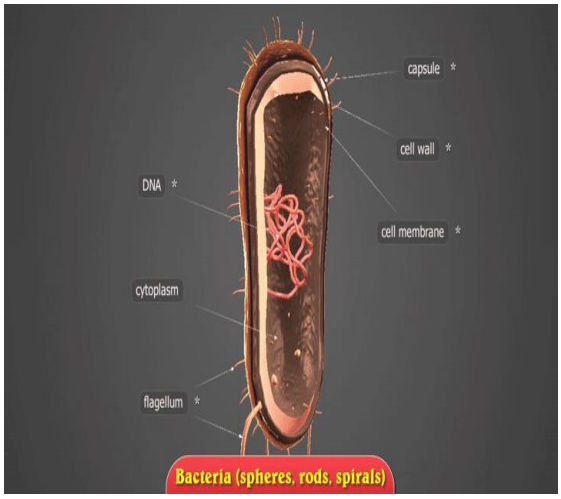


The presence of the cell wall can be evidenced by many ways

- Staining with special stains: mordant a heat- fixed film with tannic acid (5-10%), washing with distilled water (protein is altered and will not take up the stain), the wall can be stained then with 0.2% aqueous crystal violet.
- Plasmolysis of the cell: cell contents will contract in hypertonic solution leaving the wall without contraction and then, can be stained easily.
- Cell destruction by various methods: by ultrasonic waves in the presence of powdered glass, or autolysis and digestion of the protoplasm without affecting the wall. Then wall can be separated from the destroyed cell by centrifugation. Investigation of wall preparations can be carried out by electron microscope.

Cell walls are unique structures

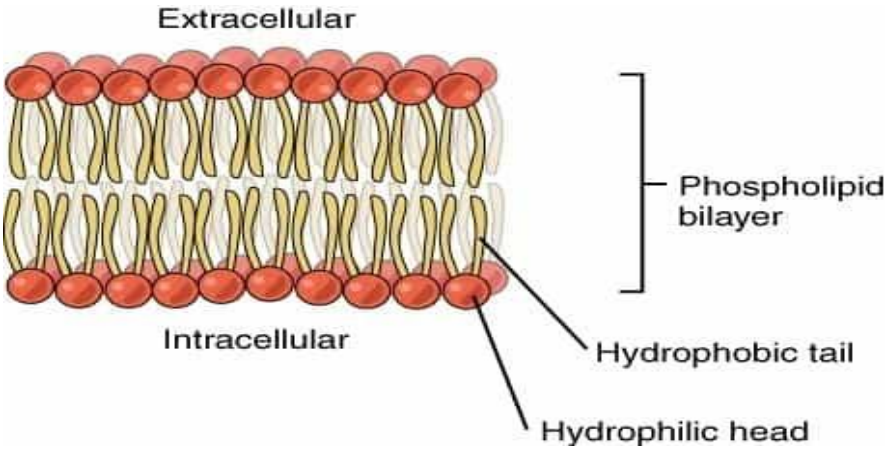
- They are essential structures for viability, as described above.
- They are composed of unique components found nowhere else in nature.
- They are one of the most important sites for attack by antibiotics.
- They provide ligands for adherence and receptor sites for drugs or viruses.
- They cause symptoms of disease in animals.
- They provide the immunological distinction and variation among strains of bacteria.



6. Plasma membrane

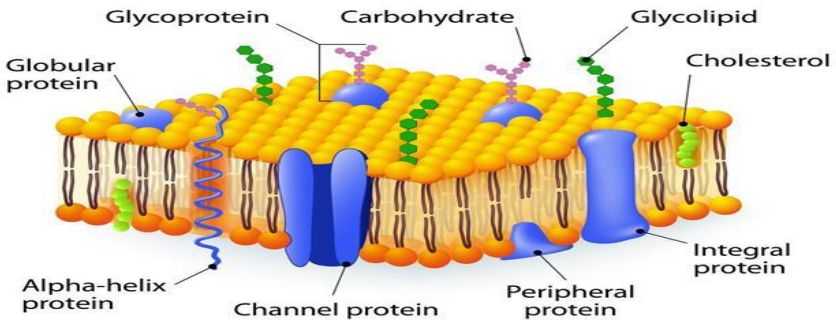
Functions

1. Osmotic or permeability barrier
2. Location of transport systems for specific solutes (nutrients and ions)
3. Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous ATP-synthesizing ATPase
4. Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)
5. Synthesis of murein (cell wall peptidoglycan)
6. Assembly and secretion of extracytoplasmic proteins
7. Coordination of DNA replication and segregation with septum formation and cell division
8. Chemotaxis (both motility and sensing functions)
9. Location of specialized enzyme system



- Cytoplasmic membrane is a bi- layered membrane that is stabilized by the hydrophobic forces between the fatty acid residues and electrostatic forces between the hydrophilic heads.
- Bacterial membranes are composed of 40% phospholipid and 60% protein. The phospholipids are amphoteric molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments.
- Dispersed within the bilayer are various structural and enzymatic proteins which carry out most membrane functions.
- It is considered as soft, elastic fluid structure and the active site of transportation or substrate permeability system. The influx and outflux of substances is mediated by membrane proteins.

CELL MEMBRANE

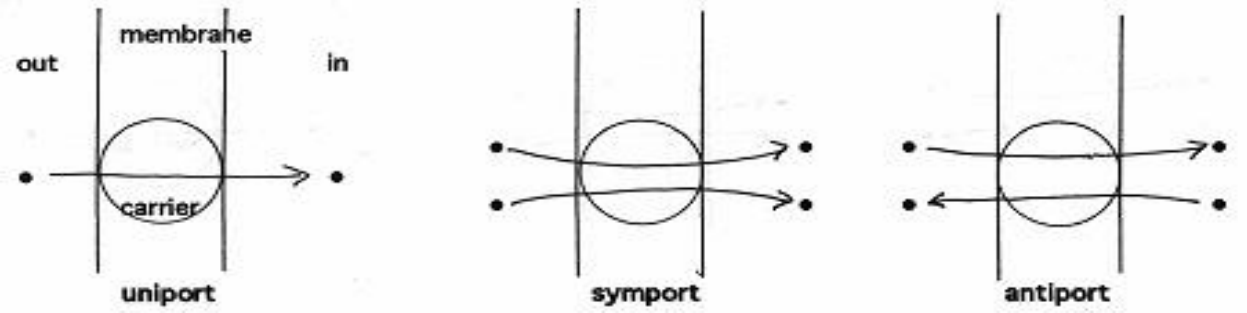


Transport processes

The proteins that mediate the passage of solutes through membranes are referred to as transport systems, carrier proteins, porters, and permeases.

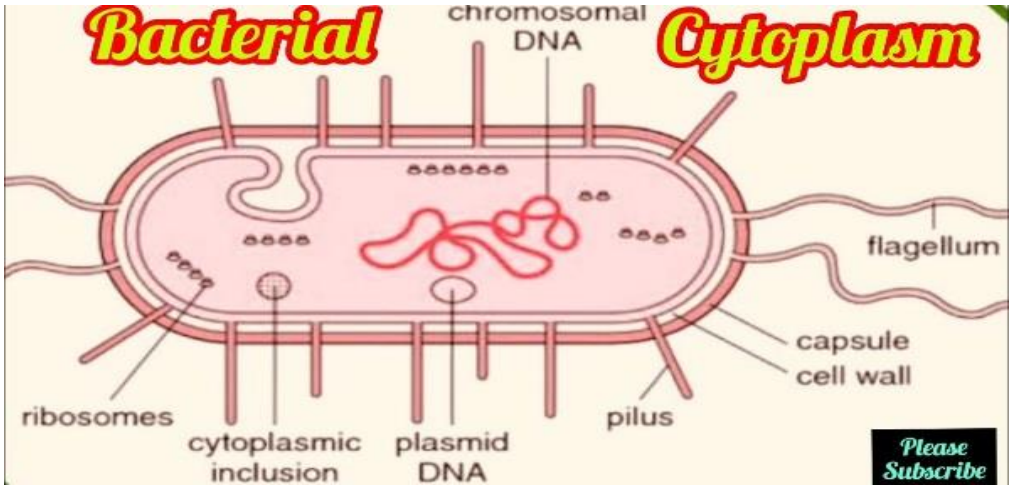
Transport systems operate by one of three transport processes.

1. In a uniporter process, a solute passes through the membrane unidirectionally.
2. In symport processes (also called cotransport) two solutes must be transported in the same direction at the same time
3. In antiport processes (also called exchange diffusion), one solute is transported in one direction simultaneously as a second solute is transported in the opposite direction.



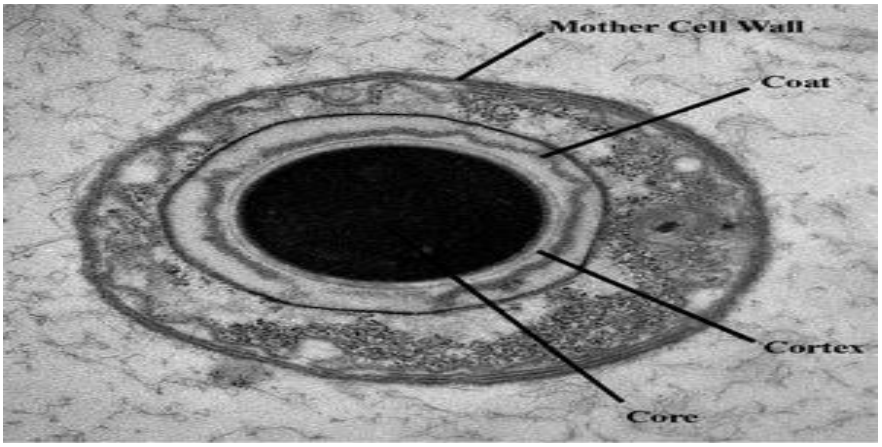
7. The cytoplasm

The cytoplasmic constituents of prokaryotic cells invariably include the prokaryotic chromosome and ribosomes. The chromosome is one large circular molecule of DNA, free in the cytoplasm and sometimes with smaller extrachromosomal pieces of DNA called plasmids (totally, the genome). The distinct granular appearance of prokaryotic cytoplasm is due to the presence and distribution of ribosomes. Prokaryotic ribosomes are 70S (Svedberg unit) in size, being composed of 30S and 50S subunits. The 80S ribosomes of eukaryotes are made up of 40S and 60S subunits.



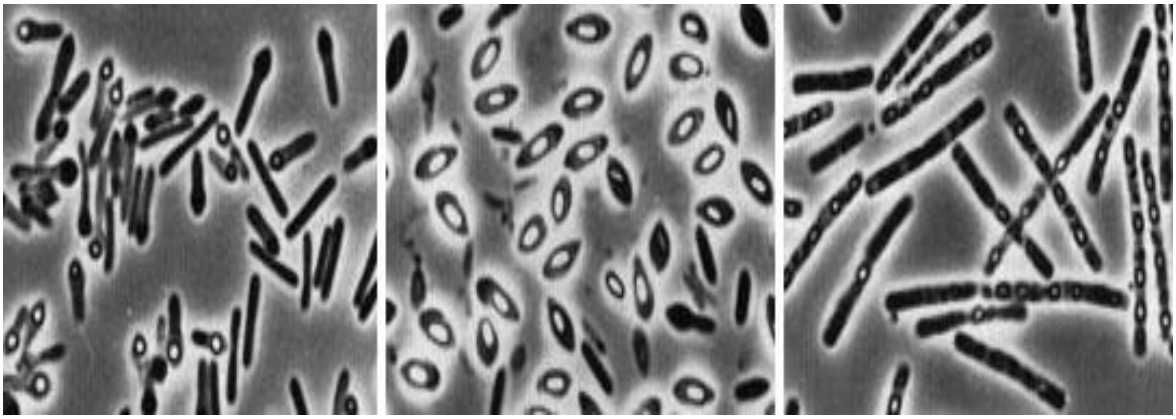
Bacterial endospores

- Endospores are formed by a few groups of Bacteria as intracellular structures, but ultimately they are released as free endospores.
- Endospores exhibit no signs of life, being described as cryptobiotic.
- They are highly resistant to environmental stresses such as high temperature, irradiation, strong acids, disinfectants, etc.
- They germinate and become vegetative cells when the environmental stress is relieved.
- Endospore-formation is a mechanism of survival rather than a mechanism of reproduction.



Endospore formation and position

A vegetative cell is converted to a heat- resistant spore through many stages starting from the exponential phase (the optimum growth phase) where the chromosomal content is doubled. In the following Figure, bacterial endospores (phase- contrast microscopy image) the refractivity as well as characteristic spore shapes and locations within the mother cell.

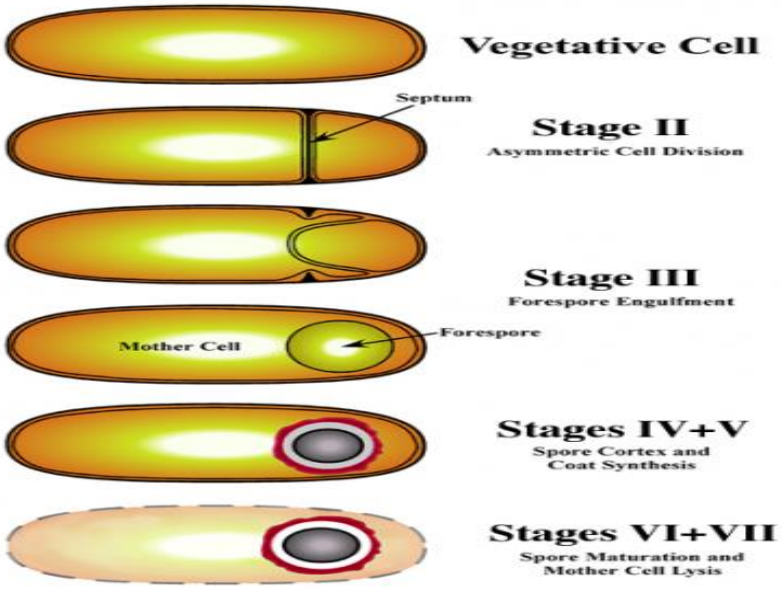


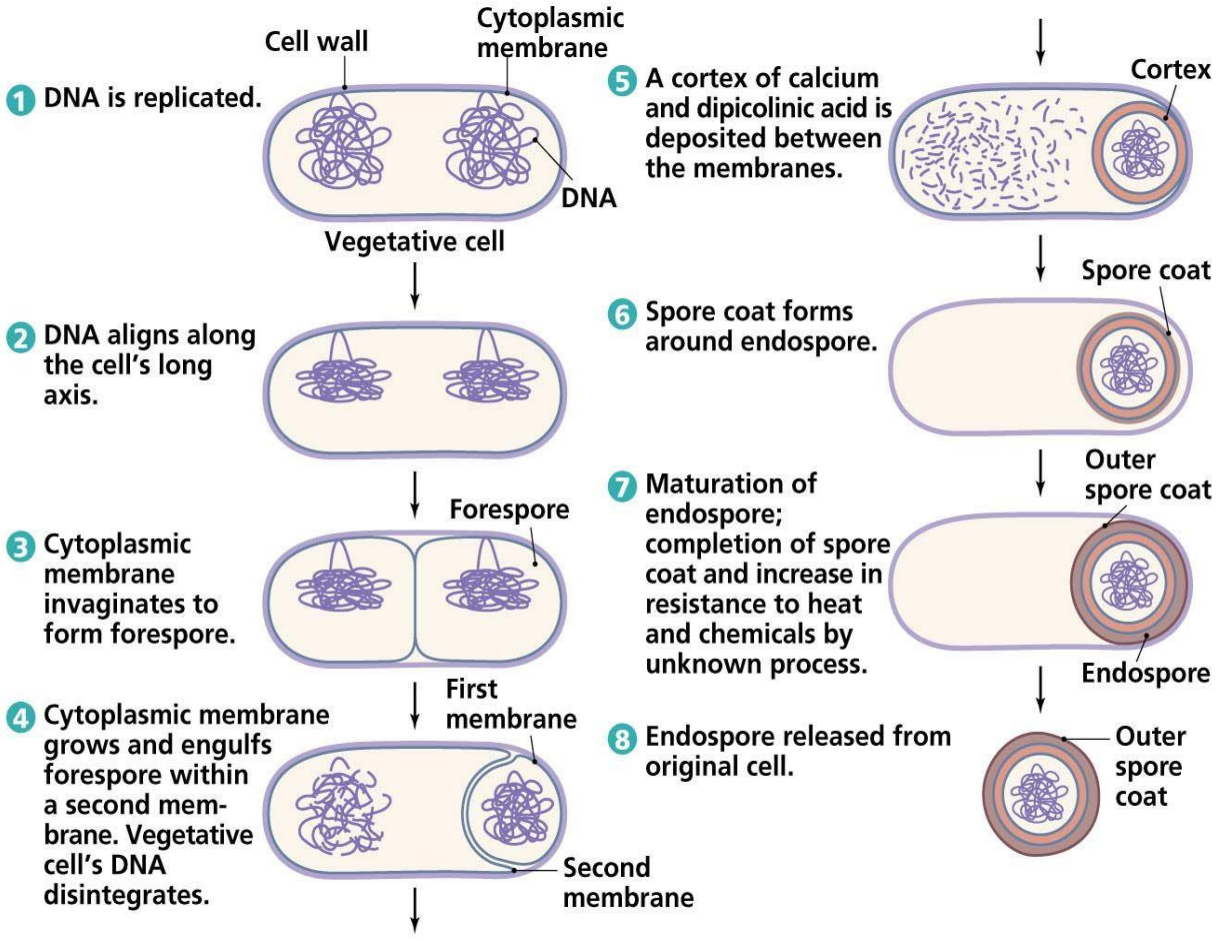
Microorganisms sense and adapt to changes in their environment. When favored nutrients are exhausted, some bacteria may become motile to seek out nutrients, or they may produce enzymes to exploit alternative resources. One example of an extreme survival strategy employed by certain low G+C Gram-positive bacteria is the formation of endospores. This complex developmental process is often initiated in response to nutrient deprivation. It allows the bacterium to produce a dormant and highly resistant cell to preserve the cell's genetic material in times of extreme stress. Endospores can survive environmental assaults that would normally kill the bacterium. These stresses include high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction. The extraordinary resistance properties of endospores make them of particular importance because they are not readily killed by many antimicrobial treatments. A variety of different

microorganisms form "spores" or "cysts", but the endospores of low G+C Gram-positive bacteria are by far the most resistant to harsh conditions.

Endospore Structure

The resilience of an endospore can be explained in part by its unique cellular structure. The outer proteinaceous coat surrounding the spore provides much of the chemical and enzymatic resistance. Beneath the coat resides a very thick layer of specialized peptidoglycan called the cortex. Proper cortex formation is needed for dehydration of the spore core, which aids in resistance to high temperature. A germ cell wall resides under the cortex. This layer of peptidoglycan will become the cell wall of the bacterium after the endospore germinates. The inner membrane, under the germ cell wall, is a major permeability barrier against several potentially damaging chemicals. The center of the endospore, the core, exists in a very dehydrated state and houses the cell's DNA, ribosomes and large amounts of dipicolinic acid. This endospore-specific chemical can comprise up to 10% of the spore's dry weight and appears to play a role in maintaining spore dormancy. Small acid-soluble proteins (SASPs) are also only found in endospores. These proteins tightly bind and condense the DNA, and are in part responsible for resistance to UV light and DNA-damaging chemicals. Other species-specific structures and chemicals associated with endospores include stalks, toxin crystals, or an additional outer glycoprotein layer called the exosporium.



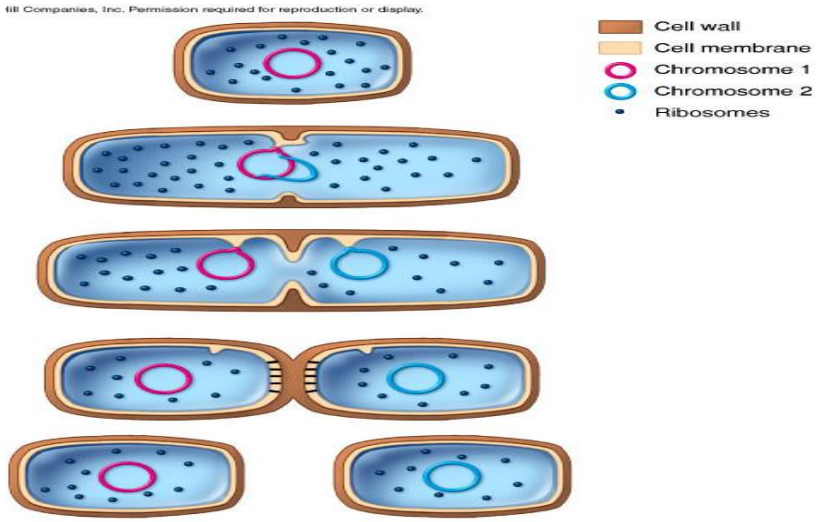


Chapter 3. Bacterial growth & reproduction

- **Growth**
- **Growth requirements**
- **Culturing Microorganisms**
- **Types of Culture Media**
- **Physical and environmental requirements for growth**
- **Measuring bacterial growth**
- **Bacterial growth curve**
- **Modes of cell division**
- **Bacterial metabolism**

Growth

Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth. The time required for the formation of a generation.



Growth requirements

Growth essential requirements are: nutrients, energy, water, optimum temperature, pH, oxygen level and, sometimes, specific vitamins and growth factors. Interaction may occur between different factors.

- ***(1) Nutrients***

  Nutrients are essential for cell growth, maintenance and division. Bacterial requirements for different nutrients are variable according to the available enzymes. Macro elements are required for all the bacteria (such as nitrogen, carbon, sulphur and phosphorus) in greater amounts than microelements (cobalt, zinc, nickel, etc.).

Chemical	Function
Carbon, oxygen, and hydrogen	Component of cellular constituents including amino acids, lipids, nucleic acids, and sugars.
Nitrogen	Component of amino acids and nucleic acids.
Sulfur	Component of some amino acids.
Phosphorus	Component of nucleic acids, membrane lipids, and ATP.
Potassium, magnesium, and calcium	Required for the functioning of certain enzymes; additional functions as well.
Iron	Part of certain enzymes.

How Microbes Obtain Nutrients

- Heterotroph: uses organic carbon source
- Autotroph: uses inorganic carbon dioxide
- Phototroph: uses light as energy source
- Chemotroph: uses chemical compounds (ie. glucose)
- Parasite

- **(2) Energy**

Energy is required for carrying out all the metabolic reactions, motility and nutrient uptake. Bacterial cells derive their energy from the surrounding environmental sources. This energy may be stored in the cell in the form of high- energy compounds such as ATP.

- **(3) Growth Factors**

- An organism (autotroph or a heterotroph) may require small amounts of certain organic compounds for growth because the organism is unable to

synthesize from available nutrients. Such compounds are called growth factors. Growth factors are required in small amounts by cells. Some bacteria (e.g *E. coli*) do not require any growth factors

- Growth factors are organized into three categories.

1. **Purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)
2. **Amino acids**: required for the synthesis of proteins
3. **Vitamins:** needed as coenzymes and functional groups of certain enzymes

Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan is a tryptophan auxotroph and would be designated *E. coli* trp.

Common vitamins required in the nutrition of certain bacteria are

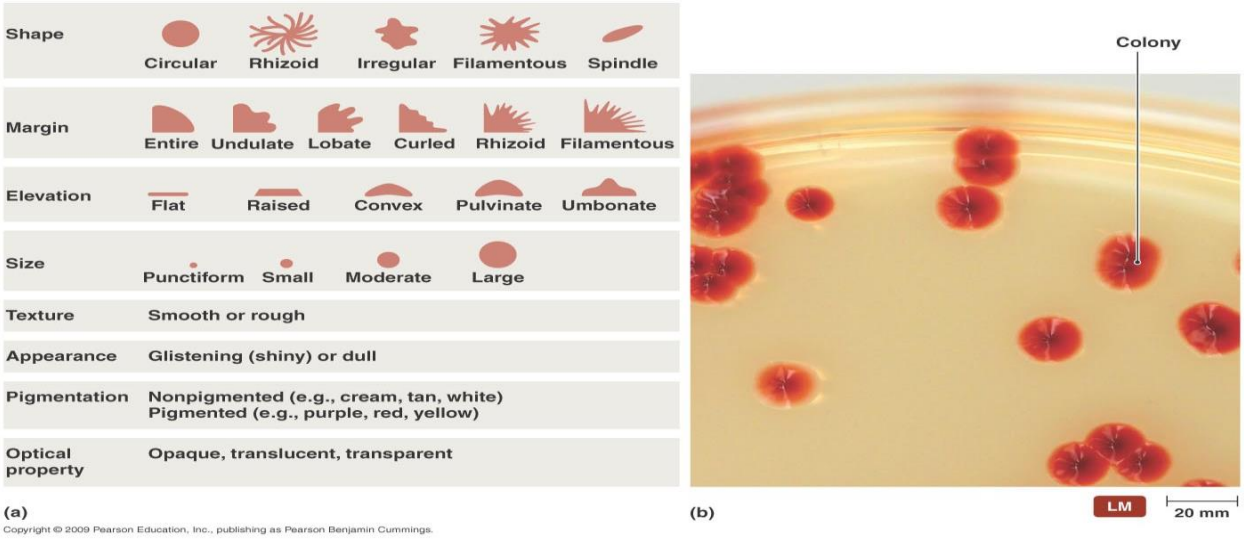
- p-Aminobenzoic acid (PABA)
- Folic acid
- Biotin
- Lipoic acid
- Mercaptoethane-sulfonic acid
- Nicotinic acid
- Pantothenic acid
- Pyridoxine (B6), Riboflavin (B2), Thiamine (B1), Vitamin B12 and Vitamin K

Culturing Microorganisms

- ✓ **Inoculum introduced into medium (broth or solid)**
- Environmental specimens
- Clinical specimens

- Stored specimens

Culture: refers to act of cultivating microorganisms or the microorganisms that are cultivated



Culture media for bacterial growth

Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties. The usual gelling agent for solid or semisolid medium is agar, a hydrocolloid derived from red algae. Agar is used because it melts at 100 degrees and remains liquid until 40 degrees and because it cannot be metabolized by most bacteria.

Types of Culture Media

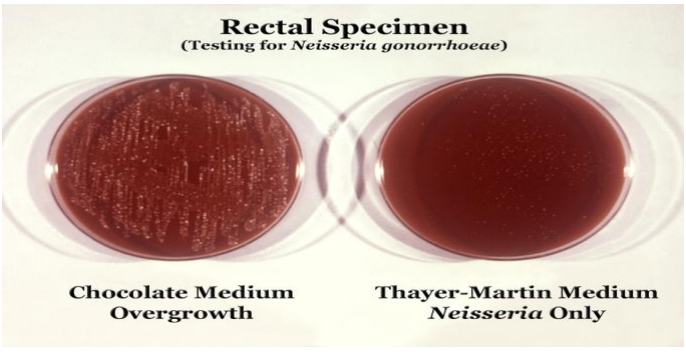
- Culture media may be classified into several categories depending on their composition or use.
- A chemically- defined (synthetic) medium is one in which the exact chemical composition is known.
- A complex (undefined) medium is one in which the exact chemical constitution of the medium is not known.

1- Enriched Media

Added nutrient encourages the growth of microorganisms.

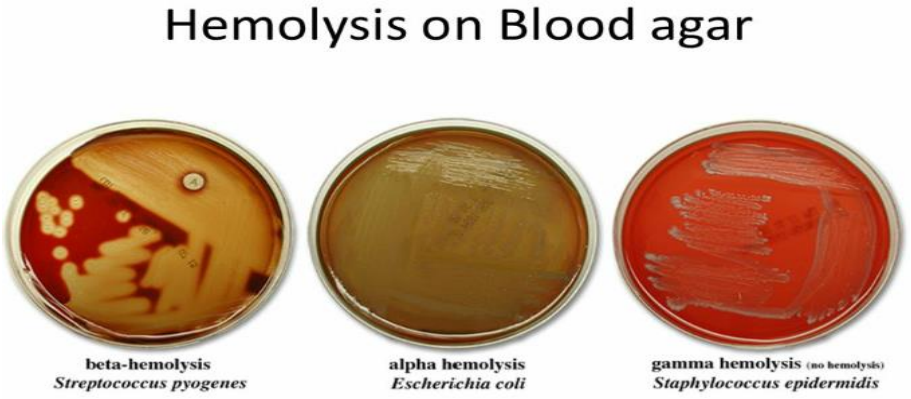
2- Selective Media

Selects a microorganism while inhibiting most others. Examples: Phenol Ethanol Agar, Deoxycholate Agar



3- Differential Media

Allow for the differentiation of microorganisms based on action that occurs on the media or a color change within the media based on a pH change. Examples Mannitol Salt Agar, MacConkey Agar.



Physical and environmental requirements for growth

1- Growth temperature

Bacteria have adapted to a wide range of temperatures. Bacteria that grow at temperatures of less than about 15 °C (59 °F) are psychrophiles. The ability of bacteria to grow at low temperatures is not unexpected, since the average subsurface temperature of soil in the temperate zone is about 12 °C (54 °F) and 90 percent of the oceans measure 5 °C (41 °F) or colder. Obligate psychrophiles, which have been isolated from Arctic and Antarctic Ocean waters and sediments, have optimum growth temperatures of about 10 °C (50 °F) and do not survive if exposed to 20 °C (68 °F). The majority of psychrophilic bacteria are in the gram-negative genera *Pseudomonas*, *Flavobacterium* and *Achromobacter*. Mesophilic bacteria are those in which optimum growth occurs between 20 and 45 °C (68 and 113 °F), although they usually can survive and grow in temperatures between 10 and 50 °C (50 and 122 °F). Animal pathogens are mesophiles. Thermophilic prokaryotes can grow at temperatures higher than 60 °C (140 °F).These temperatures are encountered in rotting compost piles, hot springs, and oceanic geothermal vents. In the runoff of a hot spring, thermophiles such as the bacterium Thermus aquaticus (optimum temperature for growth, 70 °C [158 °F]; maximum temperature, 79 °C [174 °F]) are found near the source where the temperature has fallen to about 70 °C. The archaeon *Sulfolobus acidocaldarius* has a high tolerance for acidic conditions,

which allows growth in a pH range of about 1.0 to 6.0 and a temperature optimum of 80 °C (176 °F). Numerous bacteria and archaea are adapted to the temperature range of 50 to 70 °C (122 to 158 °F), including some members of the genera *Bacillus*, *Thermoactinomyces* , *Methanobacterium*, *Methylococcus*, and *Sulfolobus* Most striking was the discovery in the mid-1980s of bacteria and archaea in nutrient-rich. The archaea in the genus *Pyrodictium* thrive in the temperature range of 80 to 110 °C (176 to 230 °F), temperatures at which the water remains liquid only because of the extremely high pressures.

- **Psychrophiles** (cold loving): 5-20 ºC

Psychrotrophs: 20-30ºC. Contribute to food spoilage in refrigerator

- **Mesophiles** (mod.-temp): 25-40 ºC

Most common. Often in animals

- **Thermophiles** (hot): 45-60 degrees ºC

Obligate thermophiles: only above 50ºC. Extreme thermophiles: above 80 ºC Archaea

Descriptive term	Property	Example
Psychrophilic	Low temp $<10^0$C	*Flavobacterium* spp
Thermophilic	High temp $>60^0$C	*B. stearothermophilus*
Mesophilic	$20\text{-}40^0$C	Most bacterial pathogens

2- pH

Most bacteria grow in the range of neutral pH values (between 5 and 8), although some species have adapted to life at more acidic or alkaline extremes. An example of an acidophilic bacterium is *A. ferrooxidans*. When coal seams are exposed to air through mining operations, the pyritic ferrous sulfide deposits are attacked by *A. ferrooxidans* to generate sulfuric acid, which lowers the pH to 2.0 or even 0.7.

However, acid tolerance of *A. ferrooxidans* applies only to sulfuric acid, since these bacteria die when exposed to equivalent concentrations of other acids such as hydrochloric acid. Many bacteria cannot tolerate acidic environments, especially under anaerobic conditions, and, as a result, plant polymers degrade slowly in acidic (pH between 3.7 and 5.5) bogs, pine forests, and lakes. In contrast to acidophilic bacteria, alkalophilic bacteria are able to grow in alkaline concentrations as great as pH 10 to 11. Alkalophiles have been isolated from soils, and most are species of the gram-positive genus *Bacillus*.

3- <u>Water activity (osmotic pressure)</u>

- The water activity (Aw) of pure H2O is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, dissolved in the water.
- Microorganisms live over a range of Aw from 1.0 to 0.7. The only common solute in nature that occurs over a wide concentration range is salt (NaCl), and some microorganisms are named based on their growth response to salt.
- Microorganisms that require some NaCl for growth are halophiles. Mild halophiles require 1-6% salt, moderate halophiles require 6-15% salt. Extreme halophiles that require 15-30% NaCl for growth are found among the Archaea.
- Bacteria that are able to grow at moderate salt concentrations, but they grow best in the absence of NaCl, are called halotolerant. Although halophiles are "osmophiles" (and halotolerant organisms are "osmotolerant"), the term osmophiles is usually reserved for organisms that are able to live in environments high in sugar. Organisms which live in dry environments (i. e. lack of water) are called xerophiles.

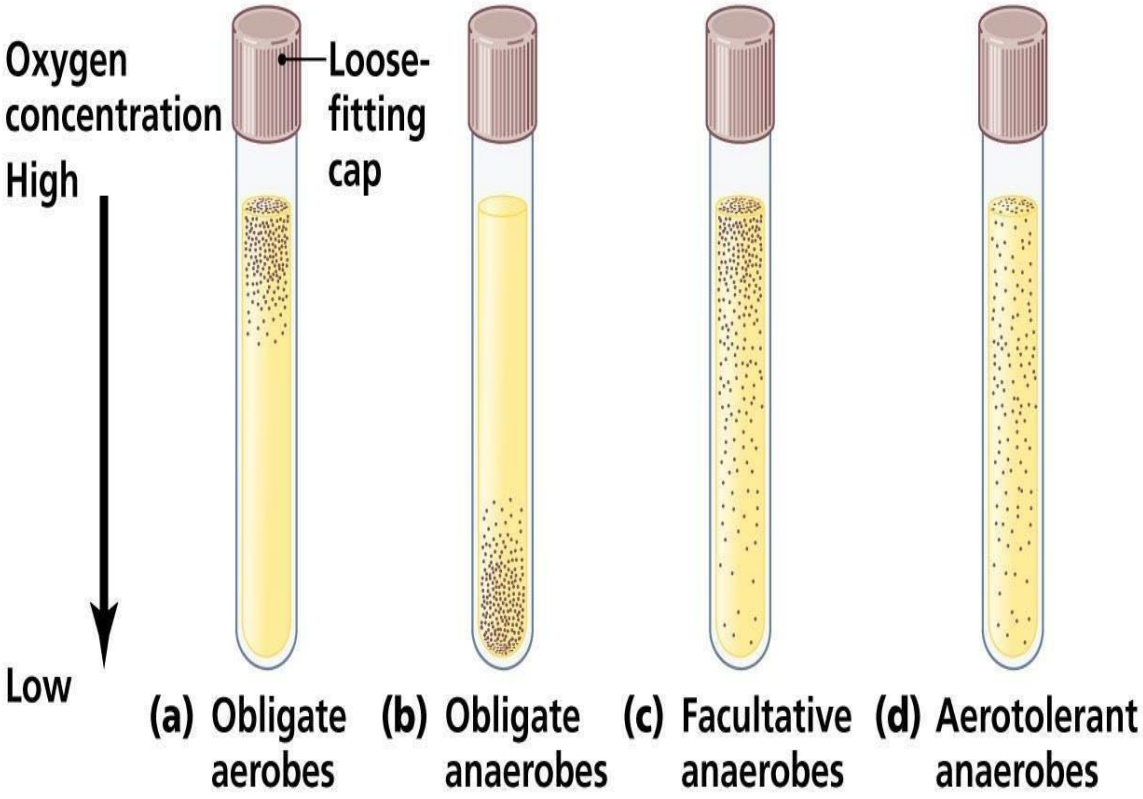
4- Oxygen

Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor. For aerobic organisms, oxygen is an absolute requirement for their energy-yielding properties. Certain microorganisms grow in oxygen-free environments and are described as anaerobic.

Organisms such as these produce odoriferous gases in their metabolism, including hydrogen sulfide gas and methane. Certain pathogenic species, such as *Clostridium* species, are anaerobic.

Certain species of microorganisms are said to be facultative. These species grow in either the presence or absence of oxygen. Some bacteria species are microaerophilic, meaning that they grow in low concentrations of oxygen. In some cases, these organisms must have an environment rich in carbon dioxide. Organisms such as these are said to be capnophilic.

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


Oxygen concentration
High
Low
Loose-fitting cap
(a) Obligate aerobes
(b) Obligate anaerobes
(c) Facultative anaerobes
(d) Aerotolerant anaerobes


KIMAX
KIMAX

Measuring bacterial growth

Growth can be measured in terms of two different parameters: changes in cell mass and changes in cell numbers

Methods for measuring cell mass

1. Direct physical measurement of dry weight, wet weight, or volume of cells after centrifugation (packed cell volume or PCV).
2. Direct chemical measurement of some chemical component of the cells such as total N, total protein, or total DNA content.
3. Indirect measurement of chemical activity such as rate of O2 production or consumption, CO2 production/ consumption, etc.
4. Turbidity measurements employ a variety of instruments to determine the amount of light scattered by a suspension of cells.

Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria.

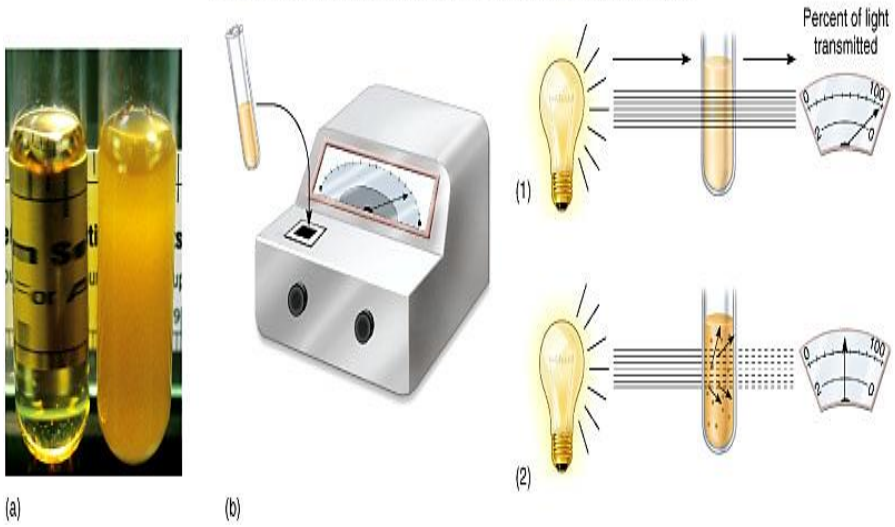
- ***Turbidity***
  - As bacteria multiply in media, it becomes turbid.
  - Use a spectrophotometer to determine % transmission or absorbance.
  - Multiply by a factor to determine concentration.

Advantages

- No incubation time required.

Disadvantages

- Cannot distinguish between live and dead bacteria.
- Requires a high concentration of bacteria (10 to 100 million cells/ml).



- ***Direct microscopic counts***

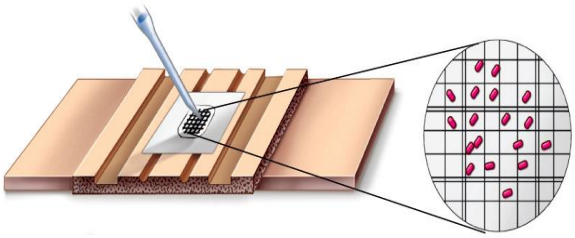
Using special slides known as counting chambers. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted (>10^7 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity. A variation of the direct microscopic count has been used to observe and measure growth of bacteria in natural environments.

Advantages

- No incubation time required

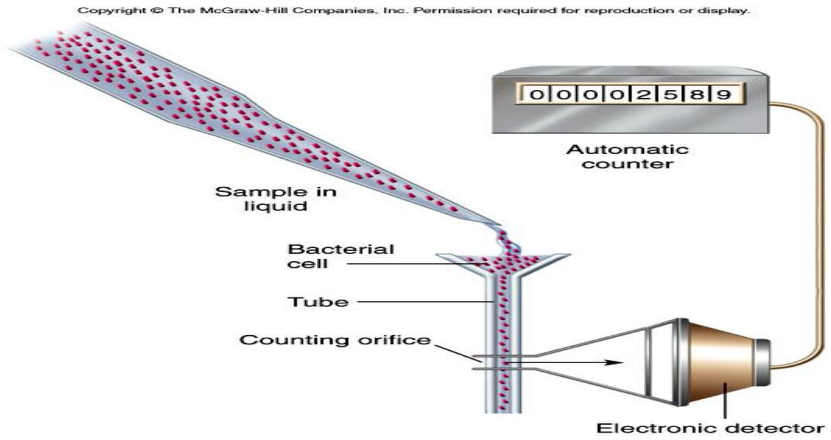
Disadvantages

- Cannot always distinguish between live and dead bacteria.
- Motile bacteria are difficult to count.
- Requires a high concentration of bacteria (10 million/ml).



○ ***Electronic counting chambers***

Count numbers and measure size distribution of cells. The suspending medium must be very clean. Such electronic devices are more often used to count eukaryotic cells such as blood cells.



○ ***Indirect viable cell counts***

Also called plate counts, involve plating out (spreading) a known volume of sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each viable unit grows and forms a colony. Each colony that can be counted is called a colony forming unit (CFU) and the number of cfu's is related to the viable number of bacteria in the sample.

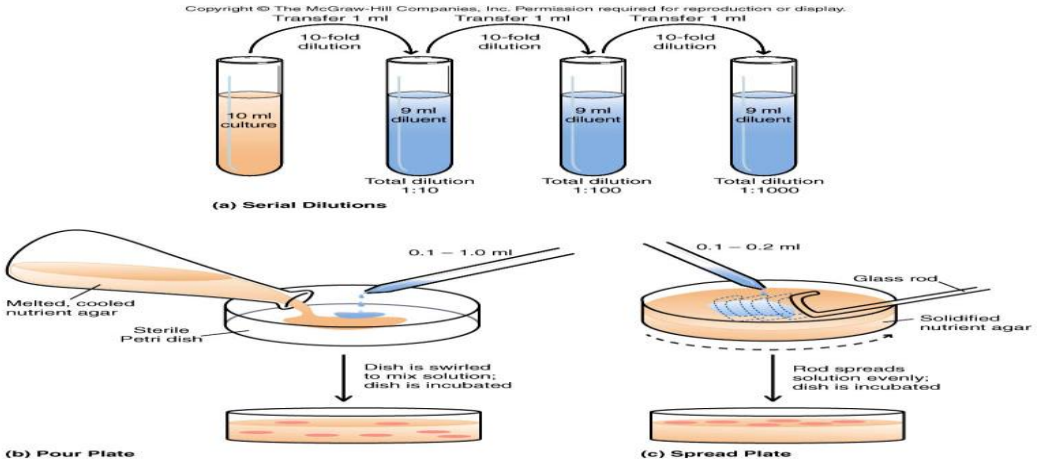
Advantages

- Sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted.

Disadvantages

- Only living cells develop colonies that are counted
- Clumps or chains of cells develop into a single colony

- Colonies develop only from those organisms for which the cultural conditions are suitable for growth.
- time- consuming



Bacterial growth curve

Bacterial population growth studies require cultivation of viable cells in a fresh sterile broth medium and incubation in a closed culture vessel with a single batch of medium under optimum temperature, pH, and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation and can be used to delineate stages of the growth cycle. Growth involves an increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division.

Generation time

Generation time is the time required for bacteria to grow and divide i.e. one complete cell division. Some microbes are able to divide as rapidly as once every 12 to 15 minutes, others require up to several hours, and a few very slow growing

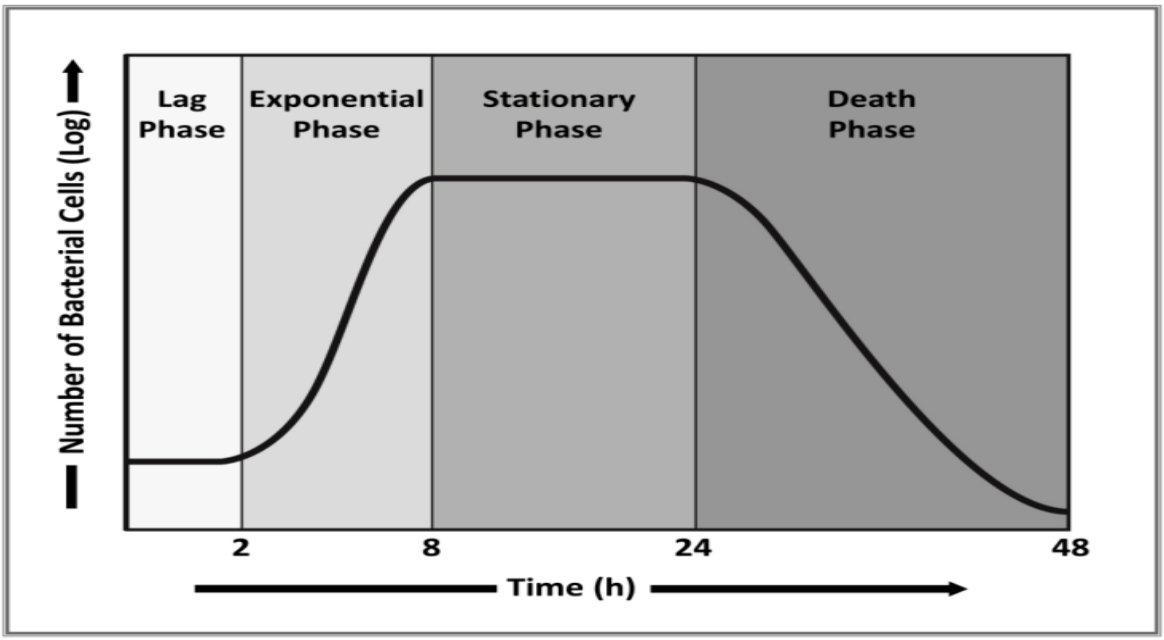
bacteria may require more than 24 hours per cell division. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases. The growth of microorganism can be measured by:

1. Increase in size but this a poor criterion of growth.
2. Increase in the number of bacterial cell by either counting the number of living cells (viable count) or all cells (total count).
3. Measurement of some component of cell structures such as protein or DNA as an indication of microbial increase (growth) or decrease (death).

Bacterial Growth Curve

When microorganisms are grown in a suitable liquid medium (batch culture or closed system) and incubated its growth follows a definite process. If bacterial counts are carried out at intervals after inoculation and plotted in relation to time, a growth curve is obtained. The typical growth curve is divided into the following phase:

- **Lag phase**
- **Log phase or Exponential phase**
- **Stationary Phase**
- **Death or decline phase**



1. Lag phase

When a bacterial population is inoculated into new fresh media the cells do not reproduce immediately in a new medium. During the lag phase, bacteria take some time adapt themselves to the new growth conditions. The lag phase is characterized by:

• No cell division

• No increase in the number of cells.

• Increase in size of bacteria

• Synthesis of RNA, enzymes, and co-enzymes for physiological activities.

• Duration of the lag phase varies according to conditions and species of bacteria. For example, if the culture microorganism is taken from old culture, the duration will be longer but if the culture is fresh, duration is short. Likewise, if the culture media is different from the previous culture then duration is long because bacteria takes some more time to adapt to the fresh media.

2. Log or exponential phase

During exponential phase,

• Microorganisms start dividing at a constant rate

• Bacterial cell numbers double with time

• Rate of growth remains constant

• Bacteria have smallest size

• Generation time is shortest during this phase

• The rate of exponential growth varies between bacterial genera and is also influenced by cultural conditions.

3. Stationary phase

A stationary phase is attained at a bacterial population level of around 10^9 cells per ml. During stationary phase, there is no net increase in the number of bacterial cells

• Cell division stops due to nutrient exhaustion and accumulation of toxic products.

• The viable count remains stationary as equilibrium exists between the dying cells and the newly formed cells.

• Production of antibiotics such as Penicillin, streptomycin etc and enzymes by certain bacteria occur during this phase

• In endospore forming bacteria, sporulation occur as the bacteria enter stationary phase.

4. Phase of decline

This is the phase when the population decreased due to cell death. Since it is a closed system, there is no way to add nutrients or remove the waste products. Eventually, this leads to unfavorable conditions and a decrease in the number of living cells in the population.

Modes of cell division

(1) Division by forming a septum (fission):

- **Binary fission** resulting in two identical cells.
- **Asymmetrical** binary fission (non- identical).
- **Multiple fission** (repeated binary fission resulting in bag- shaped colonies) as in cyanobacteria.
- **Ternary fission** resulting in three cells.

(***2) Budding:***

- **Outgrowth of daughter cell from mother cell like buds in higher plants.**

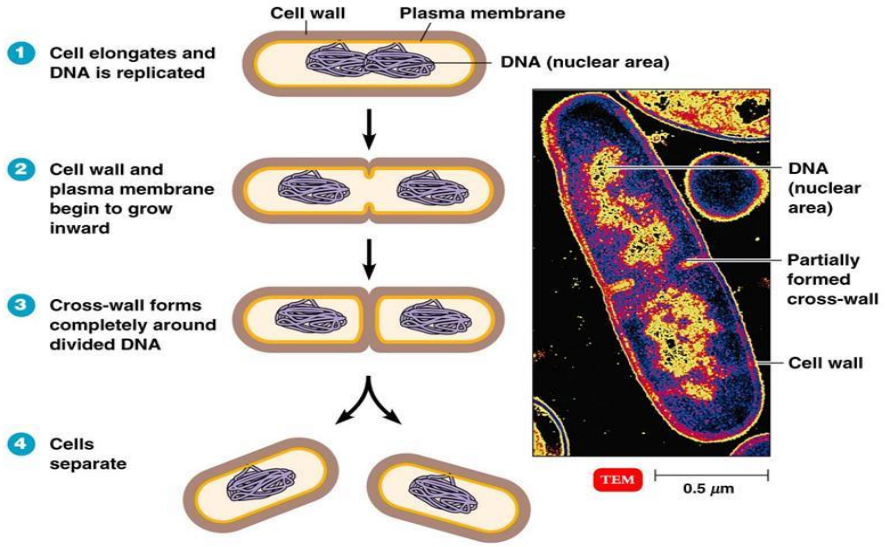
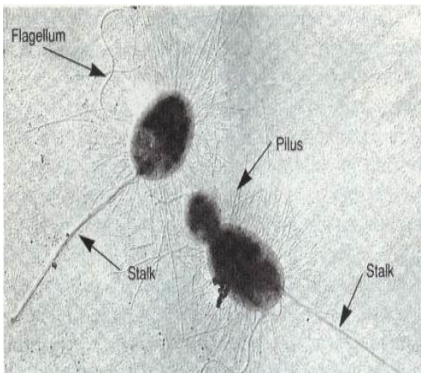
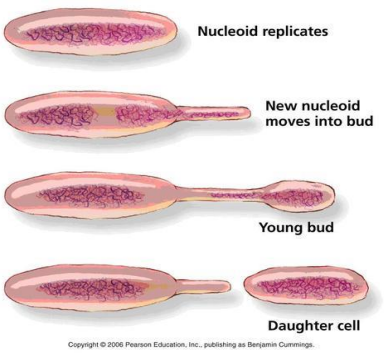
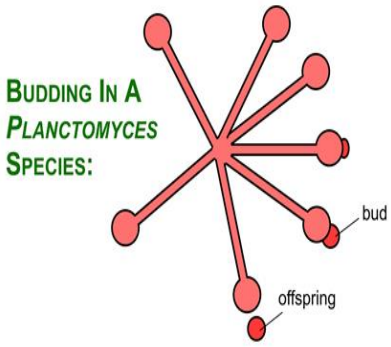


Figure 6.11 - Overview







Doubling time (generation time) "The time required for one complete cell cycle"

Optimum growth conditions will result in minimum doubling time. Generation times for bacteria vary from about 12 minutes to 24 hours or more.

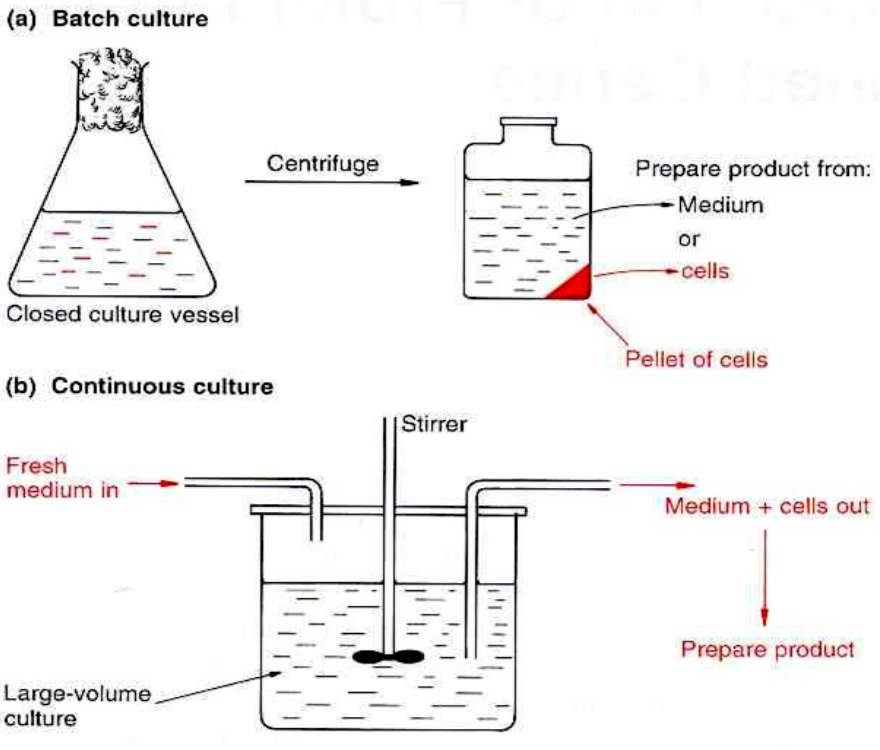
Bacterium	Medium	Generation Time (minutes)
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

Batch culture

When growth from lag phase to death phase occurs in the same batch of medium it is called a "batch culture". This includes growth under normal incubating conditions and is known as balanced growth giving rise to a normal growth curve.

Continuous culture

It is called also "continuous- flow culture" or "open culture". This is because the bacteria is grown in an apparatus called the chemostat that is producing a continuous flow of fresh, sterile medium with simultaneous outflow of the old medium. Therefore, cells are kept in balanced growth conditions or optimum growth for an extended period of time.



Synchronous growth

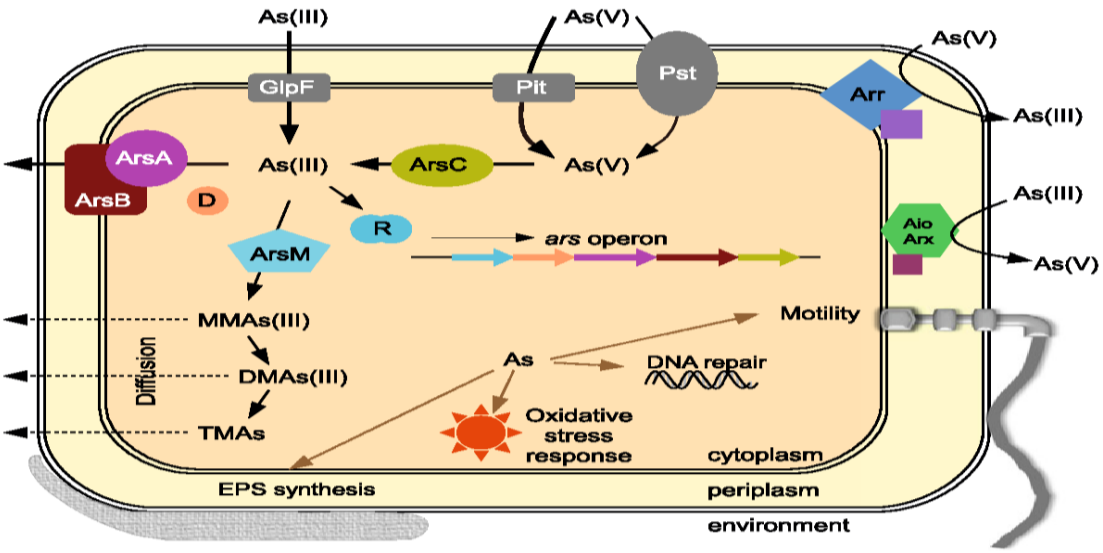
Synchronized growth is obtaining synchronized cell division (division at the same time) approximately by mechanical, physical or chemical ways. For example, mechanically by filtration through membrane filter that retain all the cells bigger than its pore size. The passing cells will be of the same size and, therefore, will grow and divide synchronously.

Diauxic growth

In a mixture of two different carbon sources, cells of some species prefer to start with one of them and then, growth slows down or stops then, starts again on the second source. This advantage can be useful for culture maintaining purposes (i. e. for longer time).

Bacterial metabolism

Metabolism is the sum total of all the chemical transformations that occur in the cell. Metabolism includes anabolism (building up macromolecules or biosynthesis) and catabolism (breaking down molecules). Many of the cell reactions require the activation of their reactants to an increased energy status. The common chemical form of energy used is adinosine triphosphate (ATP). Other energy- rich compounds are NAD (nicotinamide adenine dinucleotide), NADP, FAD (flavine adenine dinucleotide). Metabolic activity or reactions also involves specific proteins called enzymes.



Bacterial cells are made up basically of macromolecules (proteins, nucleic acids, lipids, polysaccharides, etc.). These components are always accompanied by a proportion of low molecular weight compounds that will become one of the following:

1- Part of the new generation macromolecules.

2- To catalyze macromolecular synthesis

3- Take part of the energy metabolism of the cell.

Eukaryotes produce energy (ATP) through alcohol fermentation (e.g. yeast), lactic acid fermentation (e.g. muscle cells, neutrophils), aerobic respiration (e.g. molds, protozoa, animals) or oxygenic photosynthesis (e.g. algae, plants). These modes of energy-generating metabolism exist among prokaryotes, in addition to all the following types of energy production:

1- Unique fermentations proceeding through the Embden- Meyerhof pathway.

2- Other fermentation pathways such as the phosphoketolase (heterolactic) and Entner-Doudoroff pathways.

3- Anaerobic respiration: respiration that uses substances other than 0_2 as a final electron acceptor.

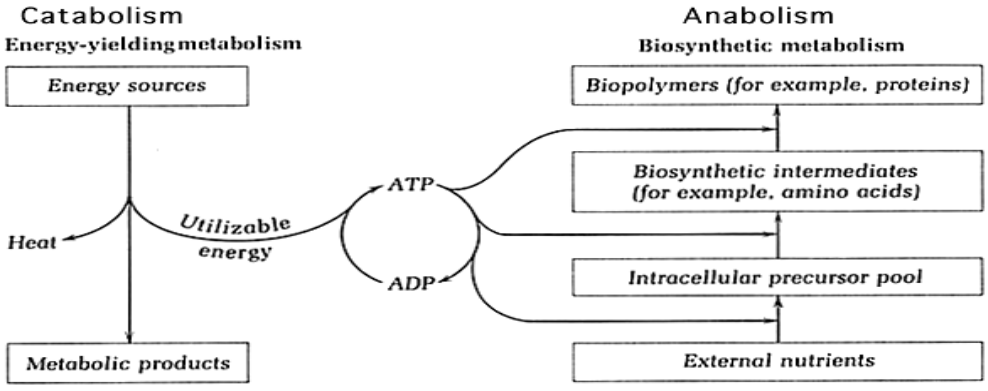
4- Lithotrophy: use of inorganic substances as sources of energy.

5- Photoheterotrophy: use of organic compounds as a carbon source during bacterial photosynthesis.

6- Anoxygenic photosynthesis: photophosphorylation in the absence of O^2.

7- Methanogenesis: an ancient type of aracheon metabolism that uses H_2 as an energy source and produces methane.

8- Light-driven nonphotosynthetic photophosphorylation: unique aracheon metabolism that converts light energy into chemical energy.



ATP Synthesis in prokaryotes

The objective of a catabolic pathway is to make ATP: to transform either chemical energy or electromagnetic (light) energy into the chemical energy contained within the high- energy bonds of ATP. Cells can produce ATP in two ways: substrate level phosphorylation (SLP) and electron transport phosphorylation (ETP).

1- SLP

- ATP is made during the conversion of an organic molecule from one form to another.
- SLP occurs during fermentations and respiration (the TCA cycle), and during some lithotrophic transformations of inorganic substrates

2- ETP

- More complicated
- Takes place during respiration, photosynthesis, lithotrophy and possibly other types of bacterial metabolism

ETP requires that electrons removed from substrates be dumped into an electron transport system (ETS) contained within a membrane. The electrons are transferred through the ETS to some final electron acceptor in the membrane (like O2 in aerobic respiration), while their traverse through the ETS results in the extrusion of protons and the establishment of a proton motive force (pmf) across the membrane. An essential component of the membrane for synthesis of ATP is a membrane-bound ATPase (ATP synthetase) enzyme. The ATPase enzyme transports protons, by utilizing the pmf during the synthesis of ATP. The idea in electron transport

phosphorylation is to drive electrons through an ETS in the membrane, establish a pmf, and use the pmf to synthesize ATP.

Heterotrophic types of metabolism

Heterotrophic bacteria are the masters of decomposition and biodegradation in the environment. Heterotrophic metabolism is driven mainly by two metabolic processes: fermentations and respirations.

1- Fermentation

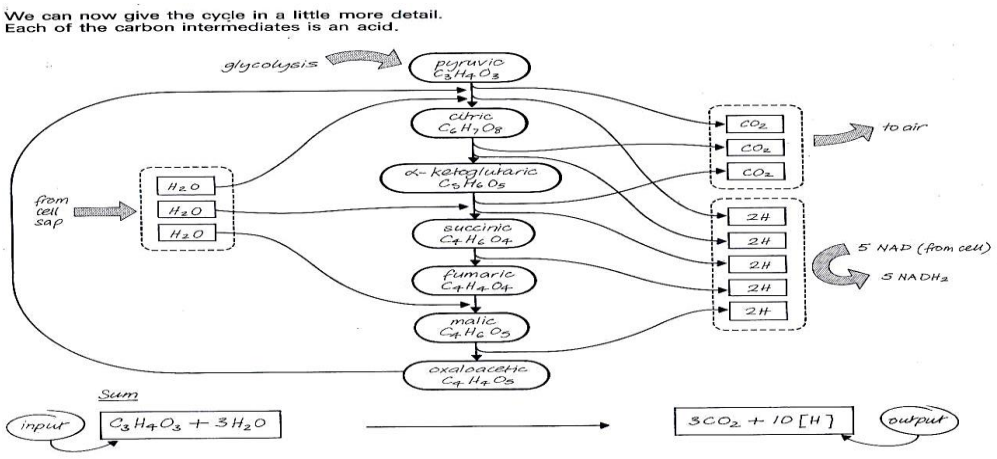
Fermentation is metabolism in which energy is derived from the partial oxidation of an organic compound using organic intermediates as electron donors and electron acceptors. No exogenous electron acceptors are involved; no membrane or electron transport system is required; all ATP is produced by substrate level phosphorylation (SLP). It may be simple or complex. Fermentation pathways start with glucose because it is the simplest molecule, requiring the fewest catalytic steps, to enter into a pathway of glycolysis and central metabolism. In prokaryotes there are three major pathways of glycolysis (the dissimilation of sugars): the classic Embden- Meyerhof pathway (EMP), which is also used by most eukaryotes, including yeast (Saccharomyces), the phosphoketolase or heterolactic pathway and the Entner-Doudoroff pathway.

2- Respiration

Respirations result in the complete oxidation of the substrate by an outside (exogenous) electron acceptor. In addition to a pathway of glycolysis, four essential structural or metabolic components are needed:

1. ***The tricarboxylic acid (TCA) cycle***

(also known as the citric acid cycle or the Kreb's cycle). When an organic compound is utilized as a substrate, the TCA cycle is used for the complete oxidation of the substrate. The end product that always results from the complete oxidation of an organic compound is CO2.



2. ***A membrane and an associated electron transport system (ETS).***

The ETS is a sequence of electron carriers in the plasma membrane that transports electrons taken from the substrate through the chain of carriers to a final electron acceptor.

3. ***An exogenous electron acceptor***

For aerobic respiration the final electron acceptor is O2. Molecular oxygen is reduced to H2O in the last step of the electron transport system. In the anaerobic respiration, the final electron acceptors may be SO4 or S or NO3 or NO2 or certain other inorganic compounds, or even an organic compound, such as fumarate.

4. ***A transmembranous ATPase enzyme (ATP synthetase).***

This enzyme utilizes the proton motive force established on the membrane (by the operation of the ETS) to synthesize ATP in the process of electron transport phosphorylation.

Lithotrophic types of metabolism

Lithotrophy is the use of an inorganic compound as a source of energy. (Some lithotrophs are facultative lithotrophs, meaning they are able to use organic compounds, as well, as sources of energy). CO_2 is the sole source of carbon for the methanogens and the nitrifying bacteria and a few other species in other groups. These lithoautotrophs are sometimes referred to as "chemoautotrophs“. The nitrifying bacteria are represented by two genera, *Nitrosomonas* and *Nitrobacter*. Together these bacteria can accomplish the oxidation of NH_3 to NO_3, known as the process of nitrification. Lithotrophic sulfur oxidizers include both Bacteria (e.g. *Thiobacillus*) and Archaea (e.g. *Sulfolobus*). Sulfur oxidizers oxidize H_2S (sulfide) or S (elemental sulfur) as a source of energy. Similarly, the purple and green sulfur bacteria oxidize H_2S or S as an electron donor for photosynthesis. Iron bacteria oxidize Fe++ (ferric iron) to Fe+++ (ferrous iron).

Phototrophic metabolism

The cyanobacteria conduct plant photosynthesis, called oxygenic photosynthesis; the purple and green bacteria conduct bacterial photosynthesis or anoxygenic photosynthesis; the extreme halophilic archaea use a type of nonphotosynthetic photophosphorylation mediated by bacteriorhodopsin to transform light energy into ATP. Photosynthesis is a type of metabolism involving both catabolic and anabolic component. The catabolic component is the light reaction, where light energy is transformed into electrical energy, then chemical energy. The anabolic component involves the fixation of CO2 and its use as a

carbon source for growth, usually called the dark reaction. In photosynthetic procaryotes, there are two types of photosynthesis and two types of CO2 fixation.

The main differences between plant and bacterial photosynthesis

Process	**plant photosynthesis**	**bacterial photosynthesis**
organisms	plants, algae, cyanobacteria	purple and green bacteria
type of chlorophyll	chlorophyll a absorbs 650-750nm	Bacteriochlorophyll absorbs 800-1000nm
Produces O_2	yes	no
Photosynthetic electron donor	H_2O	H_2S, other sulfur compounds or certain organic compounds

Autotrophic Co_2 fixation

The use of RUBP (ribulose biphosphate) carboxylase and the Calvin cycle is the most common mechanism for CO_2 fixation among autotrophs. RUBP carboxylase is the most abundant enzyme on the planet (nitrogenase, which fixes N_2 is second most abundant). This is the only mechanism of autotrophic CO_2 fixation among eukaryotes, and it is used by all cyanobacteria, purple bacteria and lithoautotrophic bacteria. Green bacteria and the methanogens, as well as a few isolated groups of procaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase. The methanogens, on the other hand, fix CO_2 by means of the enzyme CODH (carbon monoxide dehydrogenase) and the Acetyl CoA pathway.

Biosynthesis of cell molecules

Steps for biosynthesis of cell molecules:

1- Biosynthesis of low molecular weight organic molecules.

2- Biosynthesis of the macromolecules.

3- Supplying the appropriate energy form to achieve biosynthesis.

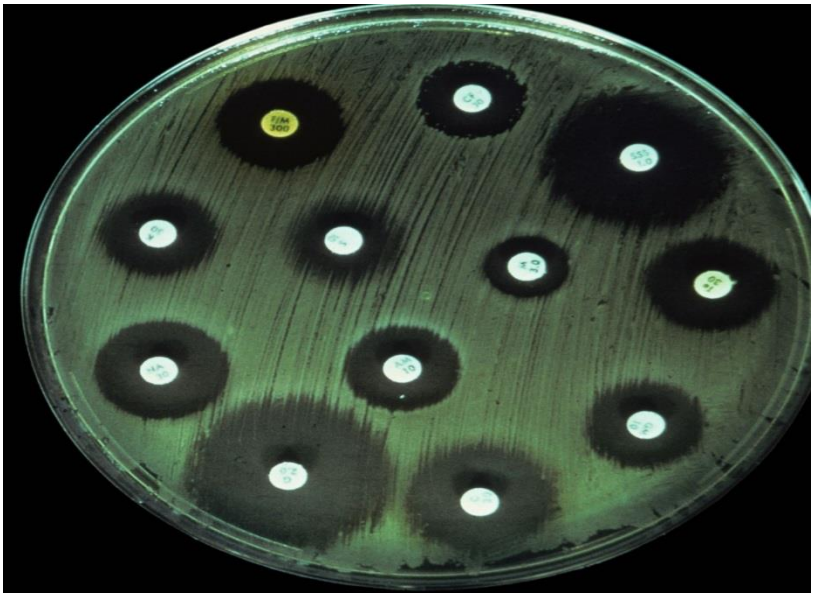
In these processes one of the reactants must provide the necessary energy for the polymerization step. The low molecular weight component of the reaction is the molecule activated to provide the energy. The complexity of the synthesis of macromolecules is related to the complexity of the macromolecule itself.

Chapter 4. Bacterial growth inhibition

- **Bacterial growth inhibition**
- **Factors that influence effectiveness**
- **Methods of Control**
- **Types of antimicrobial agents**
- **Examples of bacterial growth inhibitors**
- **Mode of antibiotic action**
- **Bacterial resistance to antibiotics**
- **Antibiotic sensitivity tests**

Bacterial growth inhibition

Chemical substances that stop or slow down the process of bacterial growth are called "bacteriostatic", while if its effect is unrecoverable are called "bactericidal".



The mechanism is by one of the following:

1- Interference with the energy source: e.g. poisons for oxidation reactions. Because oxidation liberates energy and the cell cannot utilize it.

2- Interference with precursors or intermediates: such as vitamins (cannot be replaced by another substance).

3- Interference with biosynthesis itself: such as interference with protein and nucleic acid synthesis.

4- Damaging the cell wall or cell membrane: all the intake, uptake and growth in cell size and mass processes will be damaged and stopped.

Terms

- **Biocide**: kills biologicals

- **Bactericide or Germicide**: Kills bacteria
- **Bacteriostatic**: stops growth
- **Sterilization**: destruction of all life forms
- **Disinfecting**: destruction of vegetative pathogens
- **Antisepsis**: disinfection of living tissue
- **Sepsis:** refers to microbial contamination.
- **Asepsis**: absence of growth

Factors that influence effectiveness

- Number of microbes
- Environmental conditions
- Time of exposure
- Microbial characteristics

Methods of Control

- Physical
- Chemical
- Biological

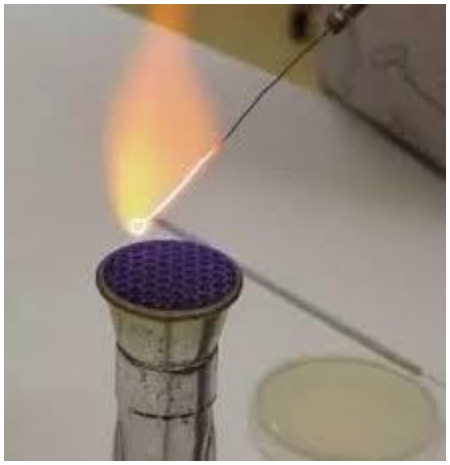
A. Physical methods

- Not for use on living organisms
- Somehow, alter membrane permeability and / or structure of proteins and nucleic acids

I. Sterilization by heat.

- Different species of bacteria differ in their susceptibility to heat. Spores of spore- forming bacteria also differ in their resistance to heat.
- Therefore, the process depends on both the temperature and time.

- Sterilization by dry heat involve the use of hot- air ovens (e.g.: for soil and glassware) and incineration or alcoholic flaming of different tools such as the triangular spreader and inoculation loops.

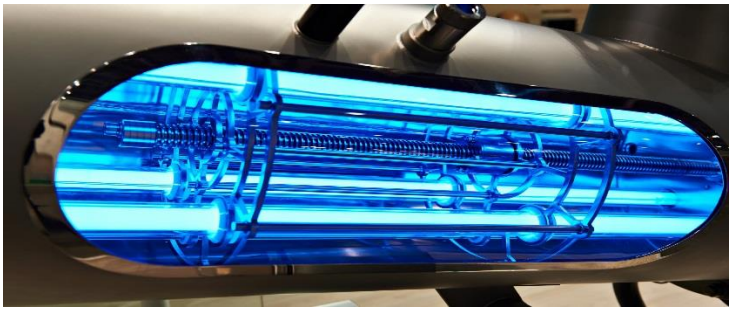


II. Moist heat and pressure (the autoclave)

15psi, 121ºC, 15 minutes (or more). Thermal death point (TDP): Lowest temperature at which all cells in a culture are killed in 10 min. Thermal death time (TDT): Time to kill all cells in a culture.

III. Irradiation

Including X- ray, UV, and gamma rays. UV is used for surface sterilization purposes only because it cannot penetrate inside surfaces. The bacterial cells that have been exposed to UV or X- rays can be reactivated. In case of UV, the reactivation is carried out by visible light and the process is called "photoreactivation". The X- ray effect can be reversed by exposing the bacteria to a sub- optimum temperature.Gamma rays are involved in some sterilization process such as disposable plastic dishes with around one year of expiry.

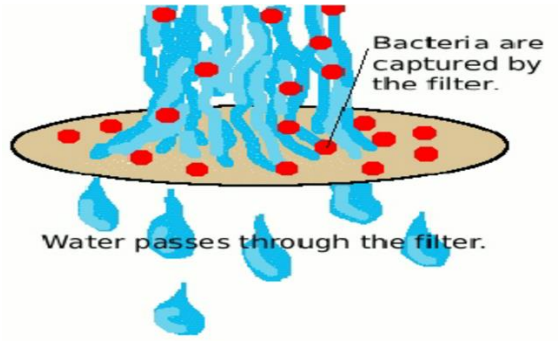


B. Mechanical methods

Heat- sensitive solutions and chemicals such as vitamins and amino acids can be sterilized by filtration.

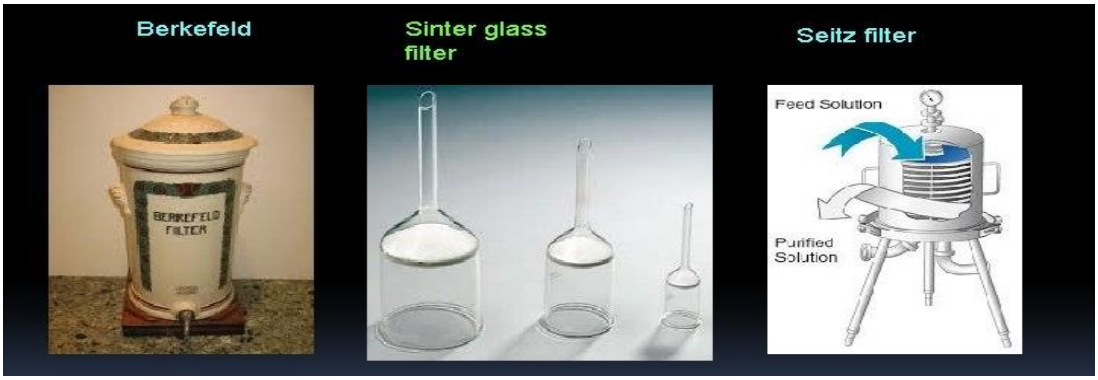
I. Membrane filtration

Different pore sizes. The most commonly used pore sizes are 0.2 and 0.45 µm. Most of the membrane filter materials are biologically inert such as cellulose acetate or cellulose nitrate.



II. Seitz filters

0.45 µm pore- sized, asbestos filters with a stainless steel holder (asbestos is forbidden nowadays).



III. Sintered- glass filters

A disk of sintered glass in a funnel of different pore sizes (grades). Normally grade 5 or G5 size is used for bacteriology because it has a pore size of 0.45 µm.

C. Chemical methods

Antimicrobial agents are chemicals that kill microorganisms or inhibit its growth. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

- May be used on living organisms
- Factors that may affect disinfection:
- Concentration of the disinfectant
- What is to be disinfected
- pH
- Organic materials
- Contact time with the microbe

Types of antimicrobial agents

A. Antiseptics: microbiocidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internally. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

B. Disinfectants: Agents that kill microorganisms, but not necessarily their spores, not safe for application to living tissues; they are used to sterilize objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, copper sulfate, quaternary ammonium compounds.

C. Note that disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. For example, sodium

hypochlorite (chlorine), as added to water is safe for drinking, but "chlorox" (5% hypochlorite), is not.

D. **Preservatives:** static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic (conc.?). Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate and sulfur dioxide.

E. **Chemotherapeutic agents:** antimicrobial agents of natural or synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonamides and antibiotics.

Examples of bacterial growth inhibitors

1- <u>Surface active substance</u>

- Usually these substances are bacteriostatic and rarely, bactericidal.
- They include phenols, soap and detergents, salts of some organic acids and some amines.
- Their action is expressed as "phenol coefficient" in comparison to the same effect of phenol.
- These substances reduce the surface tension of bacterial cells. Therefore, the surface will be wet and the substance will foam with some other effects in few cases.
- Their action may be due to adsorption to cell surface and then make cells leak their solutes into the medium (open pores in cell membranes).

2- <u>Dyes</u>

- Dyes inhibit growth of bacteria but Gram positive are more resistant to their effect than Gram negative.

- Their action may be due to combination with cell proteins or nucleic acids.
- The effect is then related to the concentration of dye, bacterial species and type of the used dye.

3- <u>Sulfonamides</u>

- A dye was discovered to inhibit growth of both Gm -ve & Gm +ve bacteria (prontosil or sulfanilamide), converts into animal body to p- amino-benzene sulfonamide.
- The most useful sulfonamides are sulfadiazine, sulfapyridine and sulfathiazole.
- An important component of the vitamin folic acid is p- aminobenzoic acid. The sulfonamide action is to replace p- aminobenzoic acid and, hence, the folic acid molecule is not formed.

4- Antibiotics

- Antimicrobial agents produced by a living microorganism that inhibit growth of another organism.
- Low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in soil.
- According to their action against bacterial populations they are divided into two groups:

1- <u>Bactericidal antibiotics</u>: Having a lethal action such as penicillin, streptomycin, cephalosporin, neomycin, and polymyxin. Erythromycin is also lethal in high concentrations.

2- Bacteriostatic antibiotics: These inhibit growth and their action is depending on the organism and the drug concentration. Examples include tetracycline and chloramphenicol.

Mode of antibiotic action

(1) Cell wall inhibitors:

Inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan including:

1- Beta lactam antibiotics

Products of two groups of fungi, *Penicillium* and *Cephalosporium*, and are represented by the penicillins and cephalosporins. The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between peptide side chains.

2- Natural penicillins

Such as Penicillin G or Penicillin V, are produced by fermentation of *Penicillium chrysogenum*. They are effective against *Streptococcus*, *Gonococcus* and *Staphylococcus*, except where resistance has developed. They are considered narrow spectrum since they are not effective against Gram -ve rods.

3- Semi-synthetic penicillins

A mold produces the main part of the molecule (6-aminopenicillanic acid) then modified chemically by the addition of side chains for increased spectrum activity. Amoxycillin and ampicillin have broadened spectra against Gram-negatives and are effective orally; methicillin is penicillinase- resistant. Clavulanic acid is a chemical

sometimes added to a semi-synthetic penicillin preparation. Thus, amoxycillin plus clavulanate is clavamox or augmentin.

4- Cephalosporins

Beta lactam antibiotics with a similar mode of action to penicillins produced by species of Cephalosporium. They have a lower toxicity and a broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram-negative bacteria, and in surgical prophylaxis.

5- Bacitracin

Is a polypeptide antibiotic produced by Bacillus species. It prevents cell wall growth by inhibiting the release of the mucopeptide subunits of peptidoglycan from the lipid carrier molecule that carries the subunit to the outside of the membrane. Teichoic acid synthesis, which requires the same carrier, is also inhibited.

(2) Cell membrane inhibitors:

Disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells. However, due to the similarities in phospholipids in eubacterial and eukaryotic membranes, this action is rarely specific enough to permit these compounds to be used systemically. Polymyxin, produced by Bacillus polymyxis is effective mainly against Gram-negative bacteria. Polymyxins bind to membrane phospholipids and thereby interfere with membrane function. The balance between effectiveness and damage to the kidney and other organs is dangerously close, and the drug should only be given under close supervision in the hospital.

(3)- Protein synthesis inhibitors:

Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. Most have an affinity or specificity for 70S (as opposed to 80S) ribosomes. The most important ones are the tetracyclines, chloramphenicol, the macrolides (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

The aminoglycosides are products of Streptomyces species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Tetracycline, chlortetracycline and doxycycline are the best known. The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both Gram +ve and Gram –ve bacteria.

Chloramphenicol has a broad spectrum of activity but it exerts a bacteriostatic effect. It is effective against intracellular parasites such as the rickettsiae. Macrolides are represented by erythromycin and oleandomycin Erythromycin is active against most Gram +ve bacteria, Neisseria, Legionella and Haemophilus, but not against the Enterobacteriaceae. Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. Macrolides are bacteriostatic for most bacteria but are cidal for a few Gram +ve bacteria.

(4) Inhibitors of nucleic acids

Some affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. Either case, can block the growth of cells. The majorities of these drugs is unselective and, therefore, unapplicable. Two nucleic acid synthesis inhibitors which have selective activity against prokaryotes and some

medical utility are nalidixic acid and rifamycins. Nalidixic acid is a synthetic chemotherapeutic agent which has activity mainly against Gram -ve bacteria.The rifamycins are also the products of Streptomyces.

Rifampicin is a semisynthetic derivative of rifamycin that is active against Gram +ve bacteria (including Mycobacterium tuberculosis) and some Gram -ve bacteria. Rifampicin acts quite specifically on eubacterial RNA polymerase and is inactive towards RNA polymerase from animal cells or towards DNA polymerase. The antibiotic binds to the beta subunit of the polymerase and blocks the entry of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis. It has been found to have greater bactericidal effect against M. tuberculosis than other anti-tuberculosis drugs.

(5) Competitive Inhibitors

The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are "growth factor analogs" which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some are bacteriostatic and some are bactericidal (sulfonamides were discussed above).

Bacterial resistance to antibiotics

Resistance to penicillin today occurs in as many as 80% of all strains of all strains of Staphylococcus aureus. Gram –ve bacteria are inherently resistant because their vulnerable cell wall is protected by an outer membrane that prevents permeation of the penicillin molecule the problems of multiple-drug resistant pathogens still emerge. The most important pathogens to emerge in multiple drug resistant forms so far have been Mycobacterium tuberculosis and Staphylococcus aureus.

The basis of resistance

1. Bacterial cells lack the target on which the antibiotic acts.
2. The production of specific enzymes by the bacterial species that inactivate these antibiotics.
3. Acquired resistance through bacterial mutation.

There is a continuous need to produce new antibiotics and a need to prevent or delay bacterial resistance by avoiding inappropriate use of antibiotics.

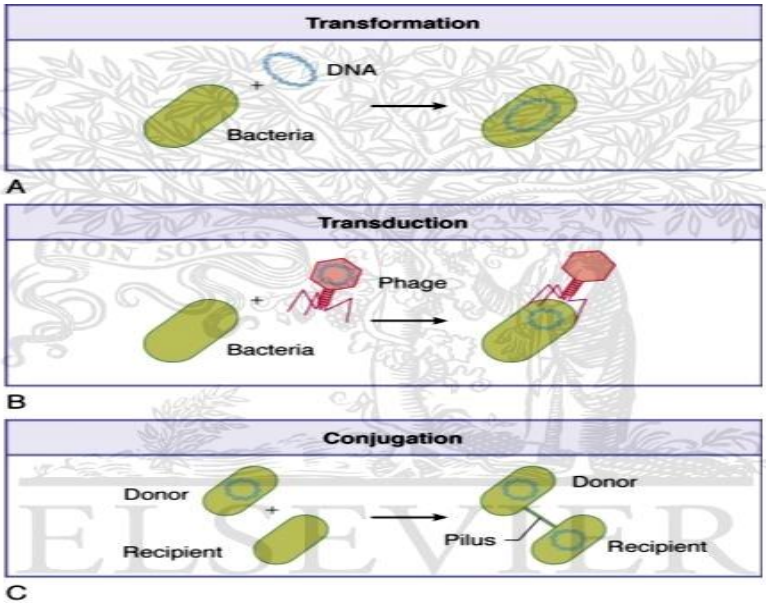
These kinds of resistance are:

Inherent (Natural) Resistance: Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete has a gene that is responsible for resistance to its own antibiotic; or a Gram - ve bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic.

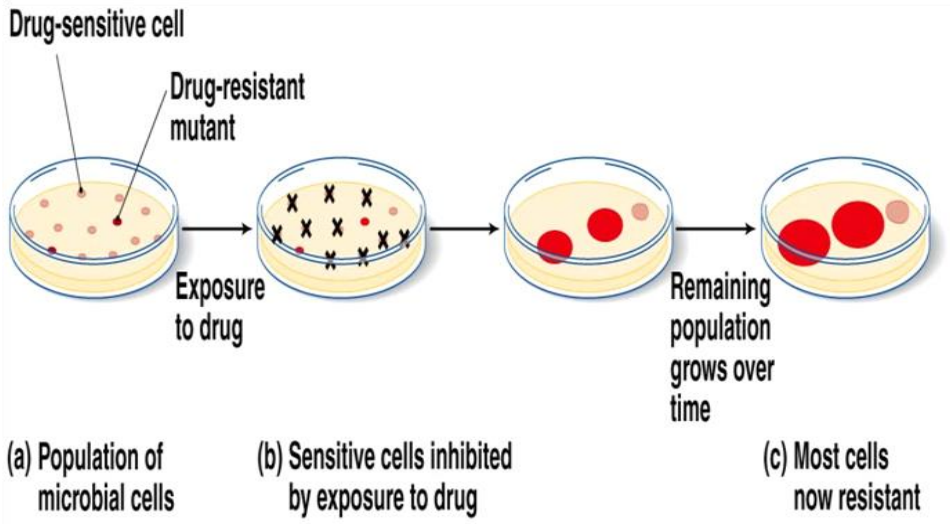
Acquired Resistance: Bacteria can develop resistance to antibiotics, e.g. bacterial populations, previously sensitive to antibiotics, become resistant. Acquired resistance is driven by two genetic processes in bacteria: (1) mutation and selection (sometimes referred to as vertical evolution); (2) exchange of genes between strains and species (sometimes called horizontal evolution).

- Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation.
- Conjugation involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient.

- During transduction, a virus transfers the genes between mating bacteria.
- In transformation, DNA is acquired directly from the environment, having been released from another cell.
- Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant).

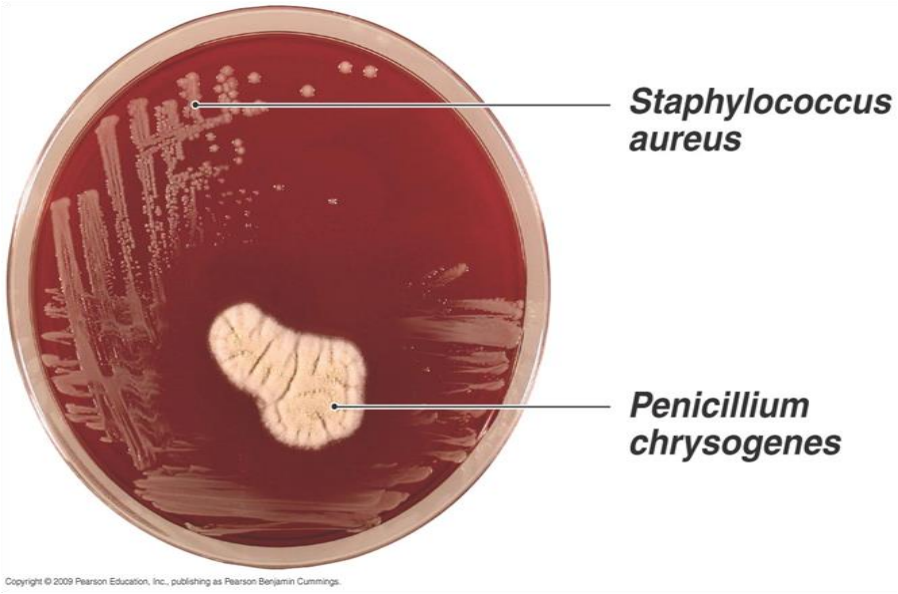


The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly.



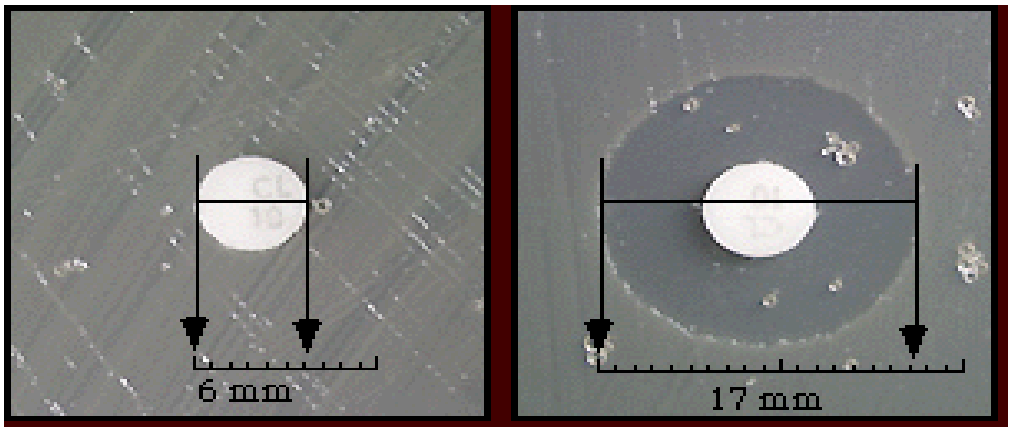
Synergism and antagonism between antibiotics

Synergism is the inhibiting and inactivating action of two different antibiotics on an organism simultaneously. Antagonism is the action of an antibiotic against the action of another antibiotic. Some antibiotics act synergistically and the others antagonistically.

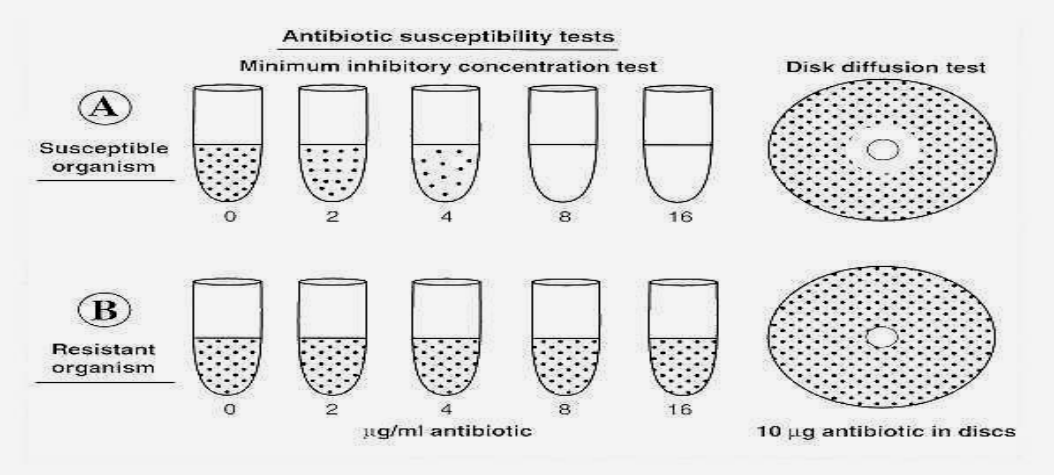


Antibiotic sensitivity tests

These tests are useful in selecting the appropriate antibiotic for specific disease (chemotherapy). The most common test is the “disc diffusion test” that is only suitable for fast– growing organisms. A plate of the appropriate agar medium is inoculated with a suspension of a pure culture, of the tested bacteria, and the inoculum is spread all over the plate. Before incubating the plates a number of small absorbent paper discs, dipped in different antibiotics, are placed apart from each other in the plate. The antibiotics start to diffuse from each disc giving a growth-inhibition zone if that bacterium is sensitive to the antibiotic. These inhibition zones are measured and compared but the conditions should be standardized for the inoculum quantity and medium type, etc. The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration (MIC). The MIC and the inhibition zone diameter are inversely correlated.



The more susceptible the microorganism, to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. On the contrary, the more resistant microorganism require higher MIC and have smaller inhibition zone.


Antibiotic susceptibility tests
Minimum inhibitory concentration test
Disk diffusion test
A
Susceptible organism
0
2
4
8
16
B
Resistant organism
0
2
4
8
16
μg/ml antibiotic
10 μg antibiotic in discs

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