Lesson 1

Definition of "microbiology" and "microorganism"

Microbiology is the study of all living organisms (biological objects) that are too small to be visible with the naked eye. The discipline is used to learn about all aspects of the organisms in order to not only determine how they live in their environment, but also how they impact their respective surroundings and thus other organisms around them (human beings, animals, etc).

Classification of microbiological sciences

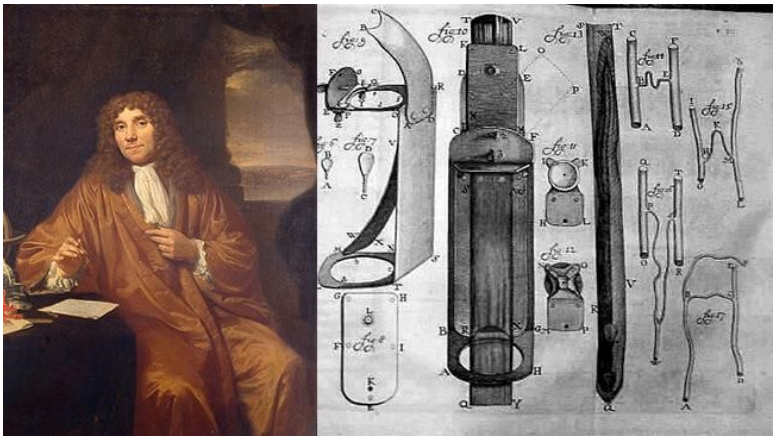
In general the field of microbiology can be divided in the more fundamental branch (pure microbiology, such as General Microbiology, Immunology, Bacteriology, Mycology, Protozoology and Virology) and the applied microbiology (for example, Medical, Sanitary, Veterinary and Industrial Microbiology).

The tasks of Medical Microbiology

This is the branch of microbiology that is concerned with the diagnosis, prevention and treatment of human diseases caused by different types of organisms (infection agents). There are four kinds of microorganisms that cause diseases in human beings: bacteria, fungi, parasites and viruses, and one type of infectious protein called prion.

Methods of microbiological diagnostics

Several diagnostic methods can be used ranging from direct methods, by directly detecting the microorganism causing the infection, such as microscopy, cultures, specific gene detection and antigen detection, to indirect methods, such as serology, in which the levels of specific antibodies against certain microorganism is detected. Animal testing, also known as animal experimentation, animal research and in vivo testing – the use of non-human animals in experiments – can be used at microbiological diagnostics as well.



Anton van Leeuwenhoek and the scheme of his microscope (https://akm-img-a-in.tosshub.com/indiatoday/images/story/201809/hhhh.jpeg?Be0PskP6rQkt1CtpovyrzR07.3Gh7wqs)

History of Microbiology: descriptive period, physiological (Pasteur's) period, immunological period and modern period

In the 1670s and the decades thereafter, a Dutch merchant named Anton van Leeuwenhoek made careful observations of microscopic organisms, which he called animalcules. Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria. From these works the descriptive period was started. Louis Pasteur worked in the middle and late 1800s and put the beginning of the physiological (Pasteur's) period. Many of the etiologic agents of microbial disease were discovered during that period. During the next – immunological – period (from the end of XIX century) the immune response was discovered. The modern period was stared from the middle of XX century with the transition of microbiological investigations on the molecular level.

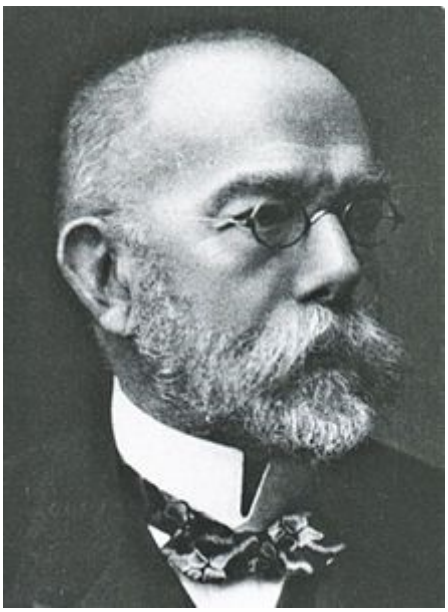
Louis Pasteur, Photograph by Nadar (https://upload.wikimedia.org/wikipedia/commons/thumb/a/a6/Louis_Pasteur%2C_foto_av_Paul_Nadar%2C_Crisco_edit.jpg/220px-Louis_Pasteur%2C_foto_av_Paul_Nadar%2C_Crisco_edit.jpg)

Scientific contribution of Pasteur

He found that each type of fermentation is carried out by a living microorganism. However, before his discovery, people had a misconception about fermentation that it was generated by a series of chemical reactions in which enzymes are produced. Using his work with fermentation, Pasteur was able to devise a process, now known as pasteurization, to kill microbes and preserve certain products. Pasteurization prevents fermenting and spoilage in beer, milk, and other goods. Pasteur successfully identified the organisms that cause diseases in humans: Staphylococcus, Pneumococcus (Streptococcus pneumoniae) and Clostridium. He was the first scientist to create live vaccines for fowl cholera; anthrax, a major livestock disease, and rabies.

Scientific contribution of Koch

A German physician and microbiologist. As one of the main founders of modern bacteriology, he identified the specific causative agents of tuberculosis, cholera, and anthrax and also gave experimental support for the concept of infectious disease, which included experiments on humans and other animals. Koch created and improved laboratory technologies and techniques in the field of microbiology (solid agar media for cultivation of microorganism, aniline dyes for staining microorganisms and equipped the light microscope with immersion objective). His research led to the creation of Koch's postulates, a series of four generalized principles linking specific microorganisms to specific diseases that remain today the "gold standard" in

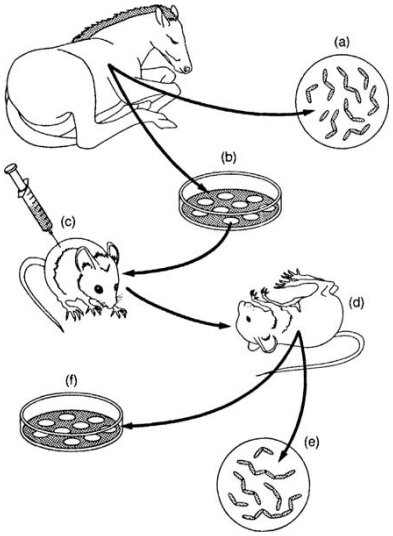


Robert Koch (https://upload.wikimedia.org/wikipedia/commons/thumb/8/8d/RobertKoch_cropped.jpg/220px-RobertKoch_cropped.jpg)

medical microbiology. For his research on tuberculosis, Koch received the Nobel Prize in Physiology or Medicine in 1905.

Classification of microorganisms

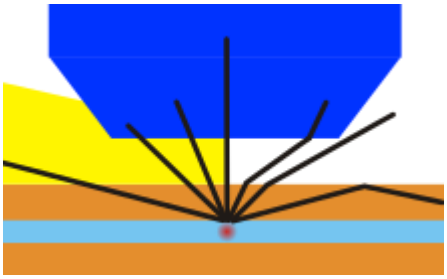
All living organisms are classified into groups based on very basic, shared characteristics. Classification of microorganisms is based on their morphological, biochemical, physiological (cultural), serological and molecular biological features. In science, the practice of classifying organisms is called taxonomy (*taxis* means arrangement and *nomos* means method). The classification of living things includes 7 levels: kingdom, phylum (or division), classes, order, families, genus, and species. Microorganisms belong to kingdoms "Virus" (viruses), "Monera" (bacteria), "Plantae" (fungi) and "Animalia" (protozoans). The basic taxon used for classification of microorganisms is species (the species includes intraspecies subdivisions: variant, strain and clone).



The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab (https://www.cliffsnotes.com/assets/8326.jpg)

Methods of microscopy

There are 3 main microscopic techniques that are used; optical microscopy, electron microscopy and scanning probe microscopy.



Principle of immersion microscopy. Path of rays with immersion medium (yellow) (left half) and without (right half). Rays (black) coming from the object (red) at a certain angle and going through the cover-slip (orange, as is the slide at the bottom) can enter the objective (dark blue) only when immersion is used. Otherwise, the refraction at the cover-slip-air interface causes the ray to miss the objective and its information is lost. (https://upload.wikimedia.org/wikipedia/commons/thumb/4/41/Immersionsvorteil.svg/220px-Immersionsvorteil.svg.png)

Optical microscopy, otherwise known as light microscopy, involves the useage of visible light and one or more lens to produce an enlarged image of an object that is placed in the focal plane of the lens. There are many applications to optical microscopy.

In light microscopy, oil immersion is a technique used to increase the resolving power of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens. Oil immersion microscopy is the main microscopic technique used in bacteriology.

Dark field microscopy is a technique for improving the contrast of unstained, transparent specimens. Dark field illumination uses a carefully aligned light source to minimize the quantity of directly transmitted (unscattered) light entering the image plane, collecting only the light scattered by the sample. As a result, the field around the specimen (i.e., where there is no specimen to scatter the beam) is generally dark. Dark field can dramatically improve image contrast – especially of transparent objects – while requiring little equipment setup or sample preparation.

Phase-contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations.

A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, scattering, reflection, and attenuation or absorption, to study the properties of organic or inorganic substances. "Fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescence image.

Electron microscopy uses electron beams to create an image of the object being used. Electron microscopes have a much higher magnification than light microscopes and so a much higher resolution as a result, this allows us to see smaller specimens in greater detail. The resolution is able to be increased because as the electrons travel faster their wavelength becomes shorter so there is a direct correlation between reducing wavelength and increasing resolution. There are 2 types of electron microscopes used, Transmission and Scanning electron microscopes. TEM involves shooting a high voltage beam through a thin layer of specimen and gathering information about the structure . SEM in contrast produces images by detecting secondary electrons that have been emitted off the surface due to excitation by the primary electron beam.

Scanning probe microscope (SPM) is a branch of microscopy that forms images of surfaces using a physical probe that scans the specimen. Basically it works by being moved around in a rectangular pattern known as raster scanning. A

type of scanning probe microscopy is called STM (scanning tunneling microscopy, an instrument for imaging surfaces at the atomic level) this is when a very sharp conducting tip is brought to the surface and a voltage is applied between them and we are able to find out the tunnel current and if this is maintained we can trace the elevation of the surface and thus produce it on an x-ray.

Methods of staining

Single stain with use of only one dye is applied for simple staining techniques: methylene blue, aqueous fuchsine or other. Several stains with use of several dyes applied in certain order are used for differential staining techniques: i.e. Gram stain, Ziehl-Neelsen stain, Neisser stain and Burry-Hines (using India ink) stain.

Training algorithm of practical skills to be mastered at the lesson

1. Making a smear using bacteria grown on solid agar medium:
   1. degrease the slide with a piece of soap;
   2. put on the slide a drop of water by inoculation loop;
   3. light the alcohol lamp;
   4. take in different hands the culture tube and the inoculation loop;
   5. sterilize the inoculation loop by leaving in the flame of the alcohol lamp until it glows red (sterilization in a fire or "flaming");
   6. open the culture tube near the fire and burn it neck;
   7. touch the inoculation loop to the inside surface of the culture tube wall;
   8. touch the loop to the bacterial culture so as to take a minimal amount of bacterial mass;
   9. remove the inoculation loop from the culture tube, burn it neck and close the culture tube;
   10. put the culture tube in a test tube rack;
   11. touch by the inoculation loop with bacterial culture to the drop of water at the surface of the slide so the drop grown turbid;
   12. burn the rest of the bacterial mass at the loop in the fire of the alcohol lamp;
   13. cool the inoculation loop;
   14. homogenize the drop on the slide;
   15. spread evenly the homogenized drop over the surface of the slide to form a stretched oval;
   16. sterilize the inoculation loop by leaving in the flame of the alcohol lamp until it glows red and then put it in a rack;
   17. dry the smear;
   18. fix the smear.

2. Making a smear using bacteria grown in liquid medium:
   1. degrease the slide with a piece of soap;
   2. light the alcohol lamp;
   3. take in different hands the culture tube and the inoculation loop;
   4. sterilize the inoculation loop by leaving in the flame of the alcohol lamp until it glows red (sterilization in a fire or "flaming");
   5. open the culture tube near the fire and burn it neck;
   6. touch the inoculation loop to the inside surface of the culture tube wall;
   7. take a drop of bacterial culture by the inoculation loop;
   8. remove the inoculation loop from the culture tube, burn it neck and close the culture tube;
   9. put the culture tube in a test tube rack;
   10. touch by the inoculation loop to the slide so as to transfer a drop of bacterial culture on it;
   11. spread evenly the homogenized drop over the surface of the slide to form a stretched oval;
   12. sterilize the inoculation loop by leaving in the flame of the alcohol lamp until it glows red and then put it in a rack;
   13. dry the smear;
   14. fix the smear.

3. Staining the smear with methylene blue:
   1. methylene blue (for a fixed smear), 2-4 minutes;
   2. washing with water;
   3. dry with filter paper.

4. Staining the smear with aqueous fuchsine:
   1. aqueous fuchsine (for a fixed smear), 1-2 minutes;
   2. washing with water;
   3. dry with filter paper.

5. Microscopic investigation of the smears with use of the immersion microscopy:
   1. set the correct lighting, using low magnification;
   2. put a drop of immersion oil on the smear;
   3. put the specimen on the stage of your microscope;
   4. lower immersion objective in a drop of immersion oil prior to contact lens glasses;
   5. looking through the eyepiece, slowly raise the lens with course focus to the discovery of smear;
   6. achieve maximum sharp image using fine focus.

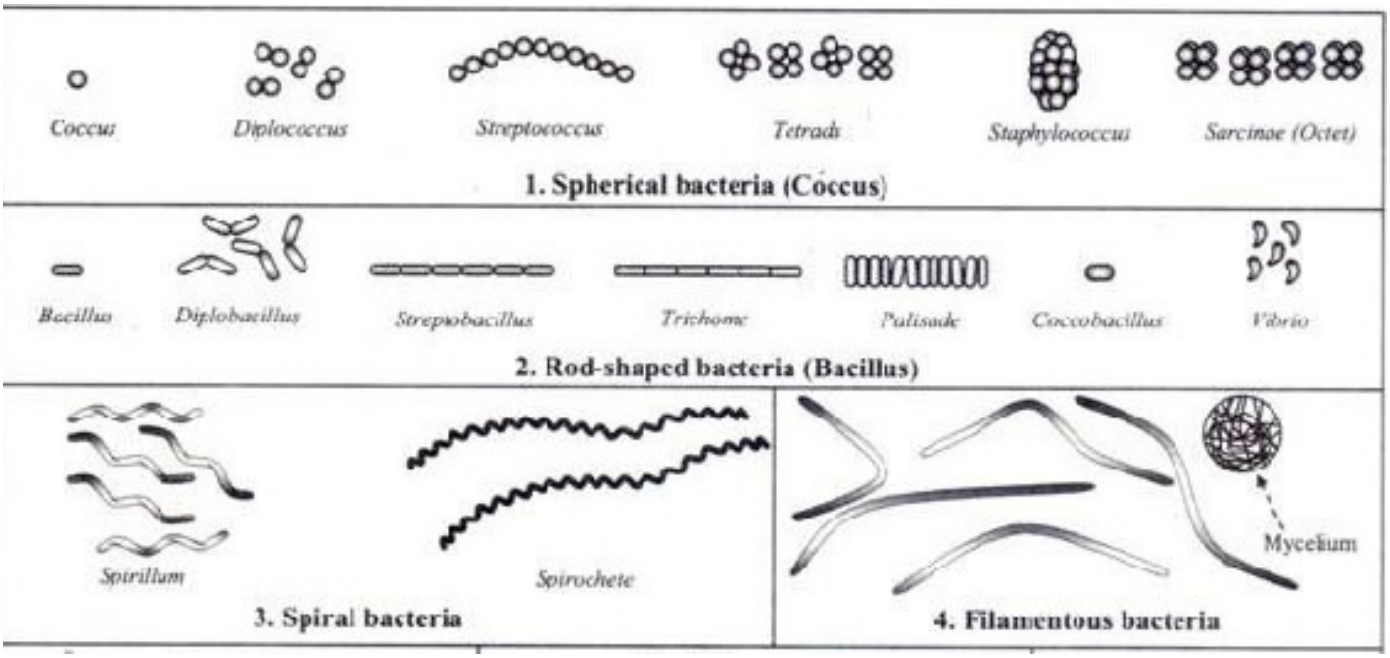
Lesson 2

Morphological features of bacteria

Bacterial morphology deals with Gram stain, shape, size, presence of a spore, presence of a capsule, motility of bacterial cells and arrangement of them in a smear.

Shape of bacteria

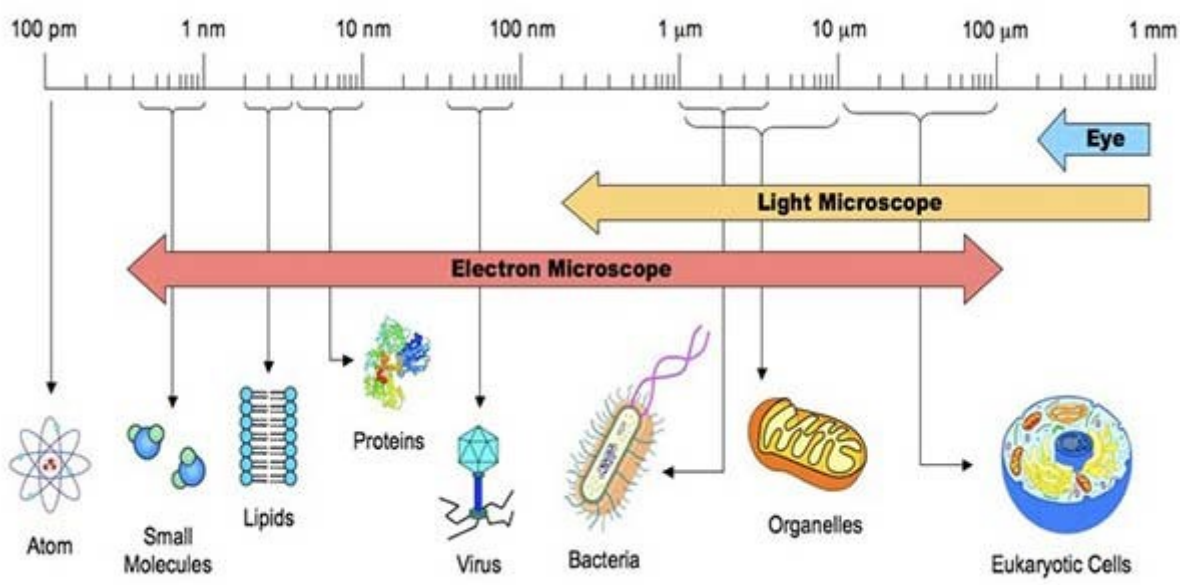
When viewed under light microscope, most bacteria appear in variations of three major shapes: rods/rod-like cells (bacilli), spheres/round cells (cocci), and spirals/spiral-shaped cells (spirochetes).



(https://microbiologyinfo.com/wp-content/uploads/2015/05/Different-Size-Shape-and-Arrangement-of-Bacterial-Cells.jpg)

Size of bacteria

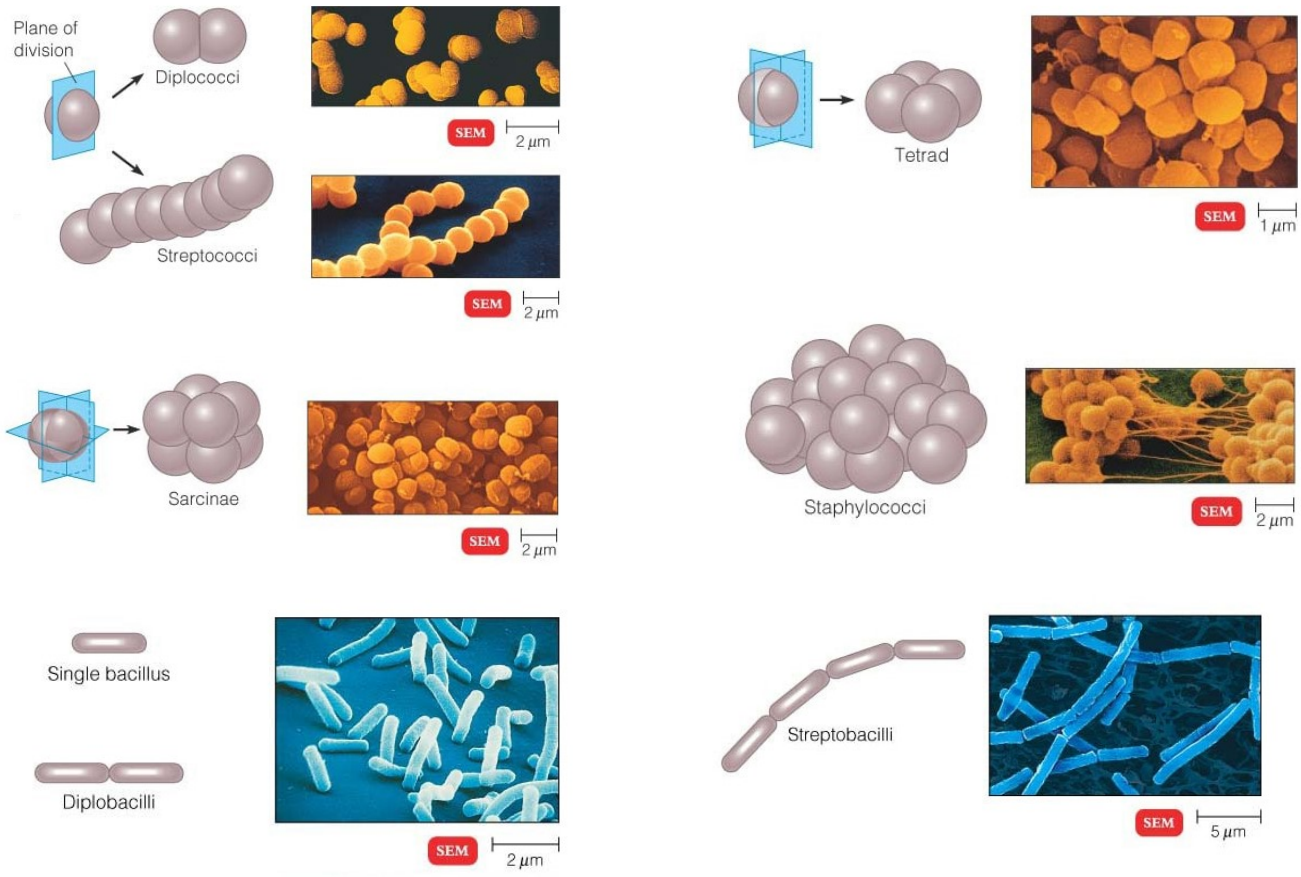
Bacterial cells are about one-tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. Among the smallest bacteria are members of the genus *Mycoplasma*, which measure only 0.3 micrometres, as small as the largest viruses. Given that the limit of resolution for a human eye (naked eye) is between 100 and 200 micrometres (about the diameter of a human hair) then bacteria cannot be seen with the naked eye. The average diameter of spherical bacteria is 0.5-2.0 µm. For rod-shaped bacteria, length is 1-10 µm and diameter is 0.25-1.0 µm. The very small rods, proportionate cocci, are called coccobacilli. Spirochaetes occasionally reach 500 µm in length (but they are very thin).



Size of bacteria (https://microbiologyinfo.com/wp-content/uploads/2015/05/Size-of-Bacterial-Cells.jpg)

Arrangement of bacterial cells in a smear

Cocci can exist singly, in pairs (as diplococci), in groups of four (as tetrads), in chains (as streptococci), in clusters (as staphylococci), or in cubes consisting of eight cells (as sarcinae). Most cylindrical or rod-shaped bacteria which are called 'bacillus' (plural: bacilli) appear as single rods. Diplobacilli appear in pairs after division. Streptobacilli are arranged in chains.

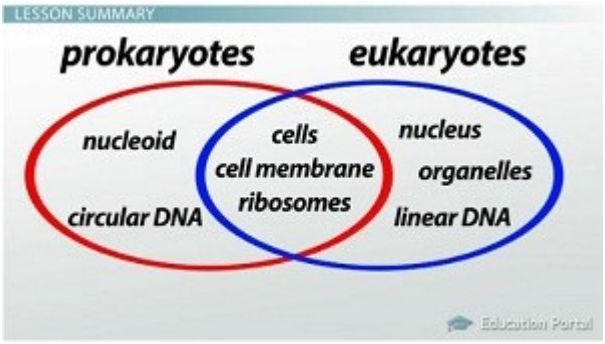


Arrangement of bacterial cells in a smear
(https://microbiologyinfo.com/different-size-shape-and-arrangement-of-bacterial-cells/)

Differences between eukaryotic and prokaryotic cells

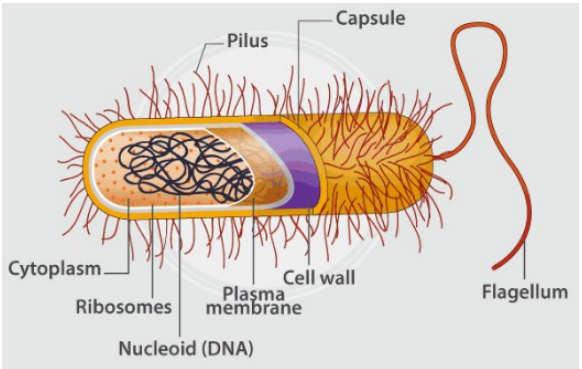
Prokaryotic cells and eukaryotic cells are the two types of cells that exist on Earth. There are several differences between the two, but the biggest distinction between them is that eukaryotic cells have a distinct nucleus (containing the cell's genetic material) and other intracellular membrane-bound organelles, while prokaryotic cells don't have a nucleus and have free-floating genetic material instead. Prokaryotic cells don't have intracellular membrane-bound organelles at all.

Prokaryotes also differ from eukaryotes in that they contain only a single loop of stable chromosomal DNA stored in an area named the nucleoid, while eukaryote DNA is found on tightly bound and organized chromosomes.



(https://study.com/cimages/multimages/16/eukaryotic-and-prokaryotic-cells-similarities-and-differences.jpg)

Most prokaryotic cells have a rigid cell wall that surrounds the plasma membrane and gives shape to the organism. In eukaryotes, vertebrates don't have a cell wall but plants do. The cell walls of prokaryotes differ chemically from the eukaryotic cell walls of plant cells, which are primarily made of cellulose. In bacteria, for example, the cell walls are composed of peptidoglycans (sugars and amino acids).



Organelles of bacterial cell
(https://cdn1.byjus.com/wp-content/uploads/2017/08/Prokaryotic-Cell-And-Eukaryotic-Cell.png)

Organelles of bacterial cell

The bacterial cell is surrounded by a cell membrane, which is made primarily of phospholipids. This membrane encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell.

Additionally, bacteria have a multi-component cytoskeleton to control the localization of proteins and nucleic acids within the cell, and to manage the process of cell division.

Bacteria do not have a membrane-bound nucleus, and their genetic material is typically a single circular bacterial chromosome of DNA located in the cytoplasm in an irregularly shaped body called the nucleoid. The nucleoid contains the chromosome with its associated proteins and RNA. Like all other organisms, bacteria contain ribosomes for the production of proteins, but the structure of the bacterial ribosome is different from that of eukaryotes.

Some bacteria produce intracellular nutrient storage granules, such as glycogen, polyphosphate etc.

Around the outside of the cell membrane is the cell wall. Bacterial cell walls are made of peptidoglycan (also called murein), which is made from polysaccharide chains cross-linked by peptides containing D-amino acids.

Flagella are rigid protein structures, about 20 nanometers in diameter and up to 20 micrometers in length, that are used for motility. Flagella are driven by the energy released by the transfer of ions down an electrochemical gradient across the cell membrane.

Fimbriae (sometimes called "attachment pili") are fine filaments of protein, usually 2–10 nanometers in diameter and up to several micrometers in length. They are distributed over the surface of the cell, and resemble fine hairs when seen under the electron microscope. Fimbriae are believed to be involved in attachment to solid surfaces or to other cells, and are essential for the virulence of some bacterial pathogens. Pili (sing. pilus) are cellular appendages, slightly larger than fimbriae, that can transfer genetic material between bacterial cells in a process called conjugation where they are called conjugation pili or sex pili. They can also generate movement where they are called type IV pili.

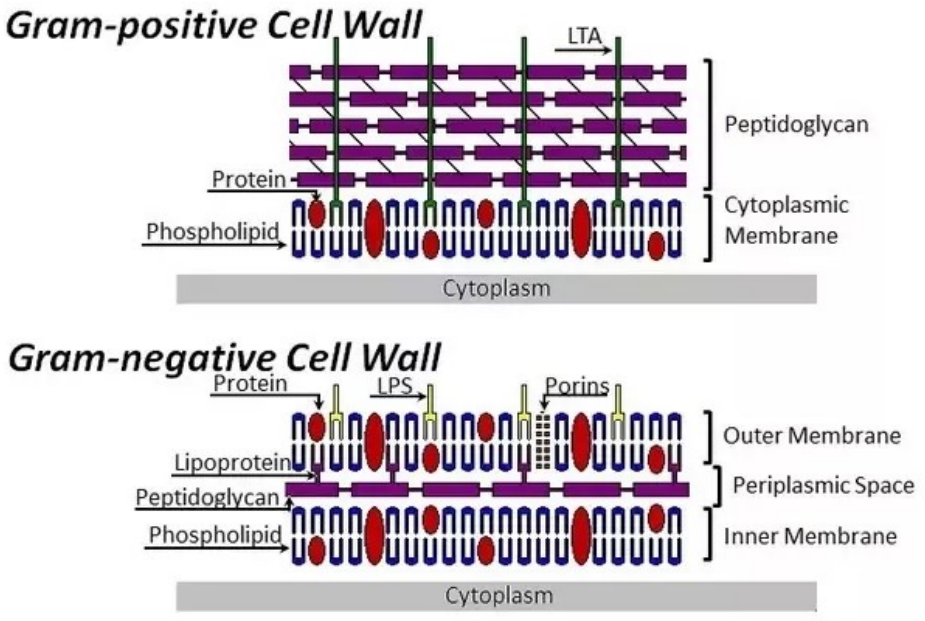
Glycocalyx is produced by many bacteria to surround their cells, and varies in structural complexity: ranging from a disorganized slime layer of extracellular polymeric substances to a highly structured capsule. These structures can protect cells from engulfment by eukaryotic cells such as macrophages (part of the human immune system). They can also act as antigens and be involved in cell recognition, as well as aiding attachment to surfaces and the formation of biofilms.

Two genera of Gram-positive bacteria – *Bacillus* and *Clostridium* – can form highly resistant, dormant structures called endospores. Endospores develop within the cytoplasm of the cell; generally a single endospore develops in each cell. Each endospore contains a core of DNA and ribosomes surrounded by a cortex layer and protected by a multilayer rigid coat composed of peptidoglycan and a variety of proteins. Endospores show no detectable metabolism and can survive extreme physical and chemical stresses. Endospores even allow bacteria to survive exposure to the vacuum and radiation in space.

Composition of bacterial cell wall

The cell envelope is composed of the plasma membrane and cell wall. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycan which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is made up of a polysaccharide backbone consisting of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in equal amounts. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for small substrates. While all bacterial cell walls (with a few exceptions e.g. *Mycoplasma*) contain peptidoglycan, not all cell walls have the same overall structures. There are two main types of bacterial cell walls, those of gram-positive bacteria and those of gram-negative bacteria, which are differentiated by their Gram staining characteristics. For both these types of bacteria, particles of approximately 2 nm can pass through the peptidoglycan.

Gram-positive cell walls are thick and the peptidoglycan layer constitutes almost 95% of the cell wall in some gram-positive bacteria and as little as 5-10% of the cell wall in gram-negative bacteria. The gram-positive bacteria take up the crystal violet dye and are stained purple. The matrix substances in the walls of gram-positive bacteria may be polysaccharides or teichoic acids which have been found only in gram-positive bacteria. Teichoic acids that are anchored to the lipid membrane are referred to as lipoteichoic acids (LTAs), whereas teichoic acids that are covalently bound to peptidoglycan are referred to as wall teichoic acids (WTA). There are two main types of teichoic acid: ribitol teichoic acids and glycerol teichoic acids. The latter one is more widespread. These acids are polymers of ribitol phosphate and glycerol phosphate, respectively, and only located on the surface of many gram-positive bacteria. A major component of the gram-positive cell wall is lipoteichoic acid. One of its purposes is providing an antigenic function. The lipid element is to be found in the membrane where its adhesive properties assist in its anchoring to the membrane.



(https://qph.fs.quoracdn.net/main-qimg-0ca776692098bbb1ac3e8beccca5fee5.webp)

Gram-negative cell walls are thin and unlike the gram-positive cell walls, they contain a thin peptidoglycan layer adjacent to the cytoplasmic membrane. Gram-negative bacteria are stained as pink color. The chemical structure of the outer membrane's lipopolysaccharide is often unique to specific bacterial sub-species and is responsible for many of the antigenic properties of these strains.

Cell wall-deficient (CWD) bacteria

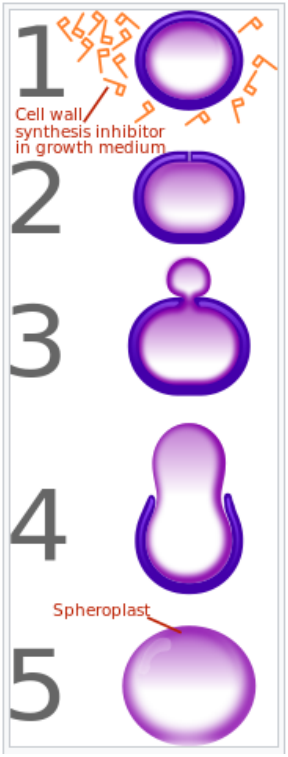
L-form bacteria, also known as wall-deficient (CWD) bacteria, are strains of bacteria that lack cell walls. They were first isolated in 1935 by Emmy Klieneberger-Nobel, who named them "L-forms" after the Lister Institute in London where she was working. The bacterial cell wall can be removed as by the action of penicillin or lysozyme.

Mollicutes is a class of bacteria, such as mycoplasma, distinguished by the absence of a cell wall, but these are not considered L-forms since they are not derived from bacteria that normally have cell walls.

Bacterial morphology is determined by the cell wall. Since the L-form has no cell wall, its morphology is different from that of the strain of bacteria from which it is derived. Typical L -form cells are spheres or spheroids.

Two types of L-forms are distinguished: unstable L-forms, that are capable of dividing, but can revert to the original morphology, and stable L-forms, L-forms that are unable to revert to the original bacteria.

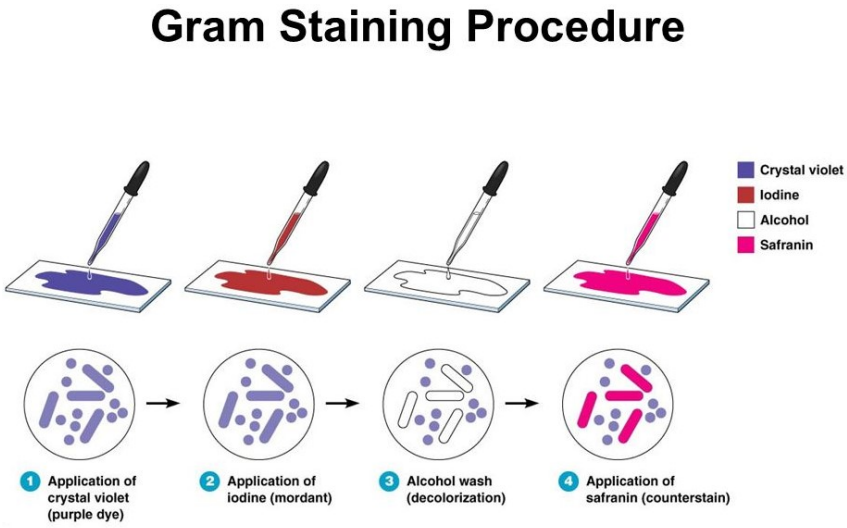
L-forms can develop from Gram-positive (protoplasts) as well as from Gram-negative bacteria (spheroplasts), in a Gram stain test, the L-forms always color Gram-negative, due to the lack of a cell wall. Protoplasts differ from spheroplasts in that their cell wall has been completely removed. Spheroplasts retain part of their cell wall: the peptidoglycan component of the cell wall has been removed but the outer membrane component has not.



Gram-negative bacteria attempting to grow and divide in the presence of peptidoglycan synthesis-inhibiting antibiotics (e.g. penicillin) fail to do so, and instead end up forming spheroplasts. (https://upload.wikimedia.org/wikipedia/commons/thumb/c/c9/Penicillin_spheroplast_generation.svg/170px-Penicillin_spheroplast_generation.svg.png)

Gram-staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.



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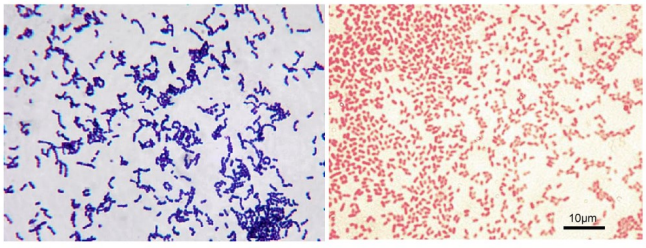
Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolorization, and counterstaining, usually with safanin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. The process involves four steps:

1) Cells are stained with crystal violet dye.

2) Next, a Gram's iodine solution (iodine and potassium iodide) is added to form a complex between the crystal violet and iodine. This complex is a larger molecule than the original crystal violet stain and iodine and is insoluble in water.

3) A decolorizer such as ethyl alcohol or acetone is added to the sample, which dehydrates the peptidoglycan layer, shrinking and tightening it. The large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria. Conversely, the the outer membrane of Gram negative bacteria is degraded and the thinner peptidoglycan layer of Gram negative cells is unable to retain the crystal violet-iodine complex and the color is lost.



Gram Positive Bacteria Gram Negative Bacteria

(https://images.squarespace-cdn.com/content/v1/5ada4d1770e8028585294334/1528063373406-TJYIM7IH818HFVOWZ56W/ke17ZwdGBToddI8pDm48kIjBzQvvRJYNfG-ryWJg05wUqsxRUqqbr1mO-JYKfIPR7LoDQ9mXPOjoJoqy81S2I8N_N4V1vUb5AoIIIbLZhVYxCRW4BPu10St3TBAUQYVKcM3mv19O0r48uCrzfIr8ouBZe1nwjGyEOhPAQ9-RWqLpZrITjROBn8OyBN82yuLR-/image-asset.png)

4) A counterstain, such as the weakly water soluble safranin, is added to the sample, staining it red. Since the safranin is lighter than crystal violet, it does not disrupt the purple coloration in Gram positive cells. However, the decolorized Gram negative cells are stained red.

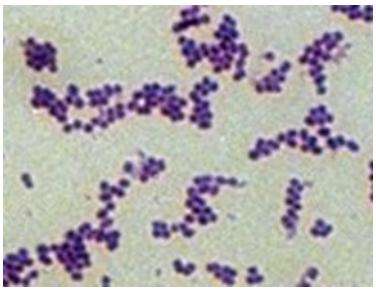
Gram-positive and Gram-negative bacteria

Gram-positive bacteria are: all cocci (except *Neisseria*) as well as some rods (spore-forming, branch-forming, *Listeria*). Gram-negative bacteria are: *Neisseria*, the majority of rods, spirochetes.

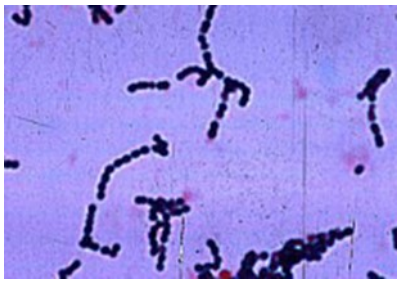
Training algorithm of practical skills to be mastered at the lesson

1. Gram staining method (stages):
   1. gentian violet (for a fixed smear), 1-2 minutes;
   2. Lugol's iodine, 1-2 minutes;
   3. washing with water;
   4. alcohol, 20 seconds;
   5. safranin or water fuchsine, 2 minutes;
   6. washing with water;
   7. dry with filter paper.

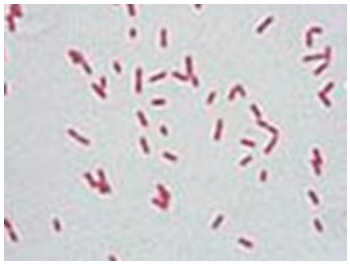
2. Identification of *Staphylococcus* by smear:
   points, located in clusters



3. Identification of *Streptococcus* by smear:
   cocci, located in chains



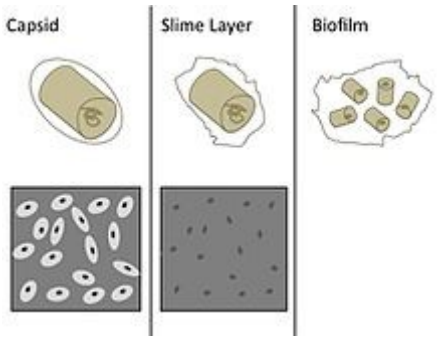
4. Identification of rod-like bacteria by smear: rods without any additional features



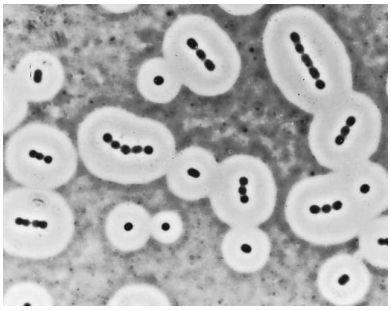
Lesson 3

Bacterial capsule

Many bacterial cells secrete some extracellular material in the form of a capsule or a slime layer. A slime layer is loosely associated with the bacterium and can be easily washed off, whereas a capsule is attached tightly to the bacterium and has definite boundaries. Capsules can be seen under a light microscope by placing the cells in a suspension of India ink. The capsules exclude the ink and appear as clear halos surrounding the bacterial cells. Capsules are usually polymers of simple sugars (polysaccharides), although the capsule of *Bacillus anthracis* is made of polyglutamic acid. Most capsules are hydrophilic ("water-loving") and may help the bacterium avoid desiccation (dehydration) by preventing water loss. Capsules can protect a bacterial cell from ingestion and destruction by white blood cells (phagocytosis) and from antibodies.



A bacterial capsule has a semi-ridged border that follows the contour of the cell. The capsule excludes India Ink when dyed. A slime layer is a non-ridged matrix that is easily deformed and is not able to exclude India Ink. Biofilms are composed of many cells and their outer barriers. The primary functions of both capsules and slime layers are for protection and adhesion. (https://upload.wikimedia.org/wikipedia/commons/thumb/3/3f/Bacteria_Capsules_and_Slime_Layers.jpg/220px-Bacteria_Capsules_and_Slime_Layers.jpg)

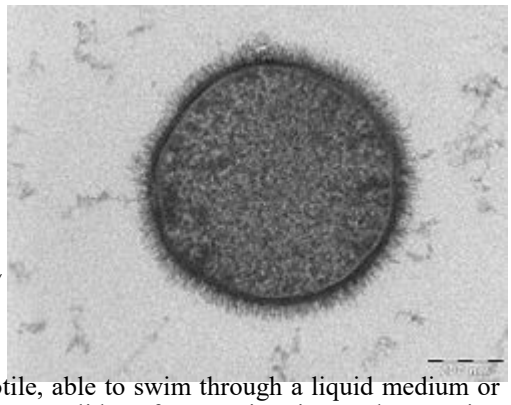


The capsular material surrounding these bacteria (Acinetobacter calcoaceticus) is revealed in a suspension of India ink and viewed through a light microscope (magnified about 2,500×). From W.H. Taylor and E. Juni, "Pathways for Biosynthesis of a Bacterial Capsular Polysaccharide," Journal of Bacteriology (May 1961) (https://cdn.britannica.com/s:700x500/03/58703-050-77C73F3C/material-bacteria-suspension-ink-India-light-microscope.jpg)

When the amorphous viscid secretion (that makes up the capsule) diffuses into the surrounding medium and remains as a loose undemarcated secretion, it is known as a slime layer. Capsule and slime layer are sometimes summarized under the term glycocalyx.

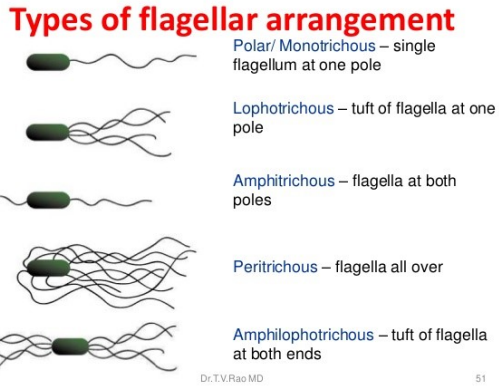
A glycocalyx, literally meaning "sugar coat" (*glykys* = sweet, *kalyx* = husk), is a network of polysaccharides that project from cellular surfaces of bacteria, which classifies it as a universal surface component of a bacterial cell, found just outside the bacterial cell wall. A distinct, gelatinous glycocalyx is called a capsule, whereas an irregular, diffuse layer is called a slime layer.

A capsular layer of extracellular polysaccharide material can enclose many bacteria into a biofilm and serves many functions.



TEM micrograph of a B. subtilis bacterium, with the hair-like glycocalyx visible surrounding the cell membrane (scale bar = 200 nm) (https://upload.wikimedia.org/wikipedia/commons/thumb/9/91/Bacillus_subtilis.jpg/250px-Bacillus_subtilis.jpg)

Bacterial flagella



(https://3.bp.blogspot.com/-80gvkQmDYG4/V9z7Aly7-CI/AAAAAAAAAyU/EcoJ0ACGv7Ey1u5TisaIBjne2SxgQ-5ZwCLcB/s1600/Flagella.jpg)

Many bacteria are motile, able to swim through a liquid medium or glide or swarm across a solid surface. Swimming and swarming bacteria possess flagella, which are the extracellular appendages needed for motility.

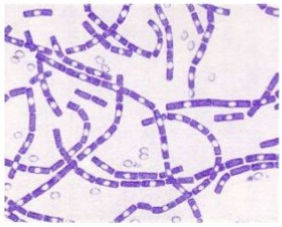
Different species of bacteria have different numbers and arrangements of flagella. Monotrichous bacteria have a single flagellum. Lophotrichous bacteria have multiple flagella located at the same spot on the bacterial surfaces which act in concert to drive the bacteria in a single direction. Amphitrichous bacteria have a single flagellum on each of two opposite ends (only one flagellum operates at a time, allowing the bacterium to reverse course rapidly by switching which flagellum is active). Peritrichous bacteria have flagella projecting in all directions.

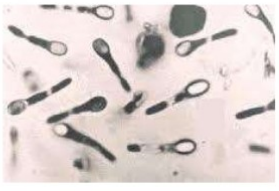
The bacterial flagellum is made up of the protein flagellin.

Bacterial spore

Endospores and exospores are two types of spores produced by bacteria. Endospores (or simply a spores) are dormant, tough structures made by some bacteria. These structures allows the bacteria to survive during unfavorable conditions, such as intense heat, disinfectants and even UV radiation. Endospores are so named because they are formed intracellularly. There are two genera which can produce endospores: *Bacillus* (spore doesn't change the cell diameter) and *Clostridium* (size of the spore is bigger than the cell diameter).

Visualizing endospores under light microscopy can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colorless. To combat this, a special stain technique called a





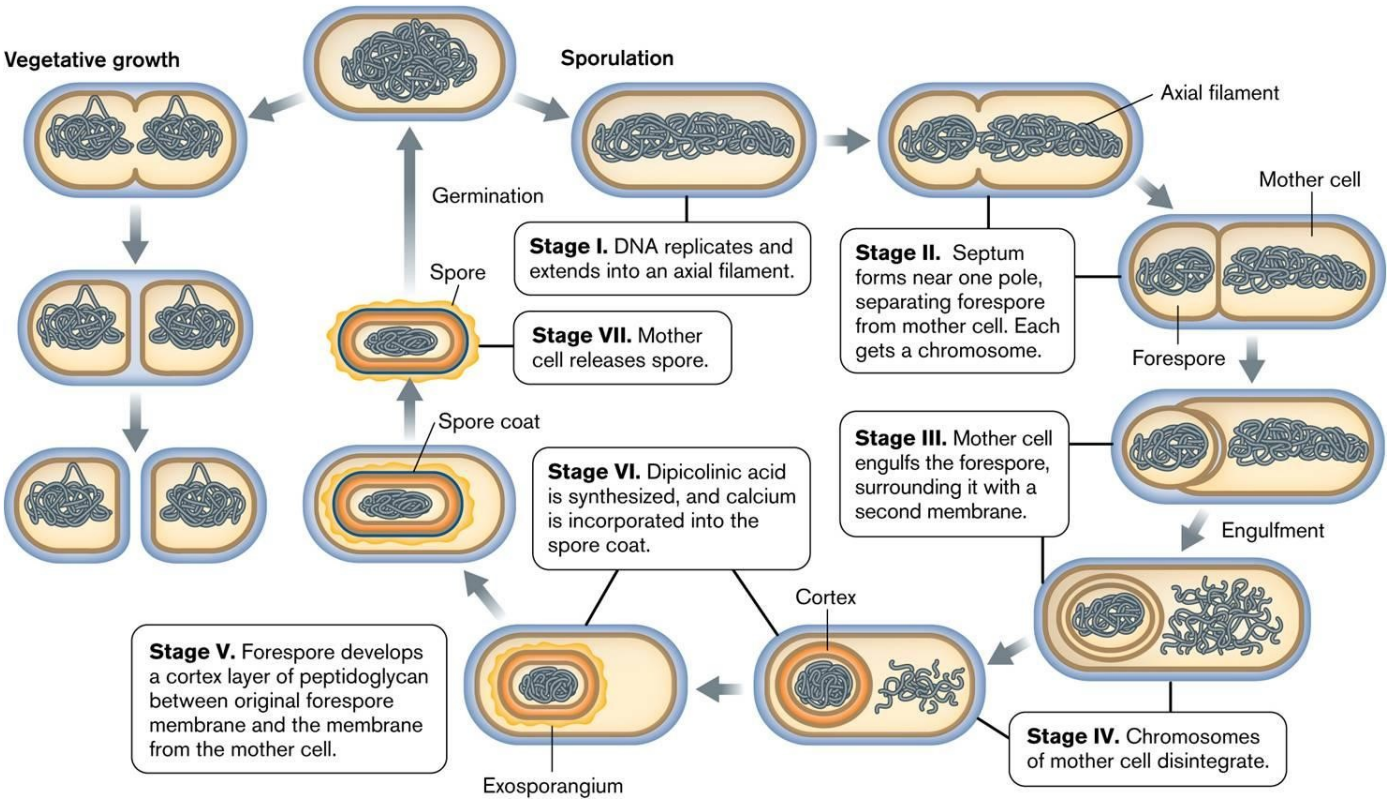
Bacillus *Clostridium*
(https://image.slidesharecdn.com/aula6-bacilosgrampositivos-110310174902-phpapp02/95/aula-6-m-31-728.jpg?cb=1299779375)

Ziehl-Neelsen stain (a type of acid-fast stain) is used. That allows the endospore to show up as red, while the rest of the cell stains blue.

Streptomycetes form reproductive structures which are called exospores and produced outside the cell.

Sporulation in bacteria

Sporulation can be divided into several stages. In *Bacillus subtilis*, entire process of sporulation takes 8 hours to complete all these stages (see the picture below).



Stages of sporulation
(https://www.onlinebiologynotes.com/wp-content/uploads/2017/07/sporulation.jpg)

Stage I – axial filament formation stage. In this stage bacterial chromosome become thread like known as axial filament. Axial filaments attached to cytoplasmic membrane by mesosome. Elongation of cell take places. PHBA (4-hydroxybenzoic acid, also known as p-hydroxybenzoic acid) is the reserved food material in *Bacillus spp* is utilized in sporulation.

Stage II – forespore formation. Asymmetric cell division occurs. Cell membrane forms septum near one end which encloses a small portion of DNA forming forespore.

Stage III – engulfment of forespore. Mother cell membrane grow around the forespore engulfing it. Fore spore now has two membrane layer.

Stage IV – synthesis of exosporium. Chromosome of mother cell disintegrates. Exosporium synthesis occurs (it is the outermost layer made up of protein that encloses spore coat; in some bacterial spore, exosporium is made up of polysaccharide and lipid). Forespore starts forming primodial cortex (made up of loosely arranged peptidoglycan layer) between two membrane. Dehydration of cell.

Stage V – synthesis of dipicolonic acid. Production of SASPs (small acid soluble proteins: SASP is synthesized during sporulation and it binds to DNA in core and protect it from potential damage caused by UV radiation, desiccation and drying) and dipicolinic acid occurs. Incorporation of calcium ions with dipicolonic acid occur forming calcium dipicolonate. Further dehydration of cytoplasm. Formation of coat layer.

Stage VI – maturation. Maturation of endospore.

Stage VII – release of endospore. Cell lysis and release of endospore.

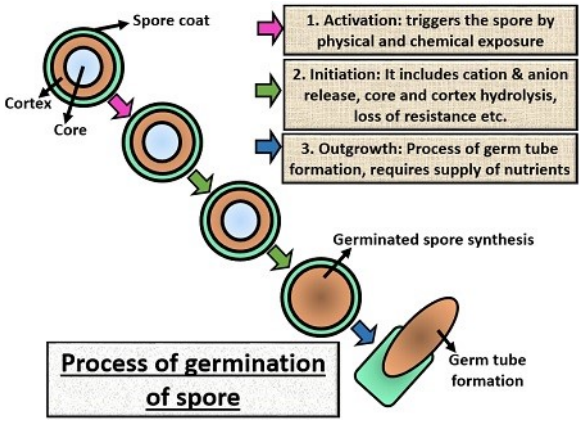
Spore germination

Endospore remains dormant for years. But under favorable conditions each endospore germinates to give rise to a vegetative cell.

Spore germination involves 3 process: activation, germination, outgrowth.

Activation of endospore: the germination of bacterial spore do not occur even when the environment is favorable unless it is first activated; at first the spore coat must be damaged by heating for several minutes.

Germination: the activated spore initiates germination after binding of effector molecules; binding of effectors molecules activates



(https://biologyreader.com/wp-content/uploads/2018/12/germination-of-spore.jpg)

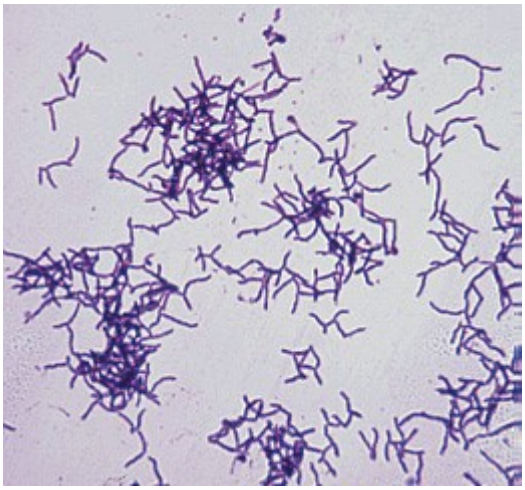
autolysis that destroy peptidoglycan of cortex; after destruction of peptidoglycan, water is taken up and calcium dipicolinic acid is released.

Outgrowth: after uptake of water swelling of spore occurs; along with swelling, synthesis of DNA, RNA and proteins also occurs; a small germ cell emerges out after breaking the spore coat and begins to grow into vegetative cell.

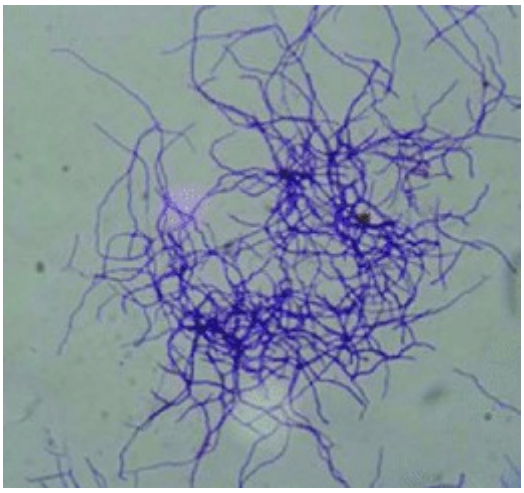
Morphological and ultra-structural peculiarities of actinomycetes

Actinomycetes belong to a heterogeneous group of gram-positive bacteria noted for a filamentous and branching form of their cells. There are two medically important genera in these group of bacteria: *Actinomyces* and *Streptomyces*.

Actinomyces species, while individual bacteria are rod-shaped, their colonies form fungus-like branched networks of hyphae.



Actinomyces, Gram staining (https://2.bp.blogspot.com/_-zO0z54Jsk0/TPuMfbE78DI/AAAAAAAAAE0/_LhshYR_Ol0/s1600/nfA.israelii.jpg)



Microscopic view of Streptomyces sp. isolate (100×) showing long aerial hyphae, after Gram staining (https://www.researchgate.net/profile/Vijay_Kumar473/publication/319905985/figure/fig1/AS:571156046794752@1513185621152/A-Morphological-micrograph-of-isolating-Streptomyces-sp-strain-BHUMBU-80-after-14-days.png)

The genus *Streptomyces* includes filamentous bacteria that produce well-developed vegetative hyphae with branches. Although the mycelia and the aerial hyphae that arise from them are amotile, mobility is achieved by dispersion of spores. In some species, aerial hyphae consist of long, straight filaments, which bear 50 or more spores. Some strains form short chains of spores on substrate hyphae.

Several species of *Actinomyces* cause the disease actinomycosis in humans and cattle. Species of *Streptomyces* are beneficial sources of antibiotics.

Morphological and ultra-structural peculiarities of spirochetes

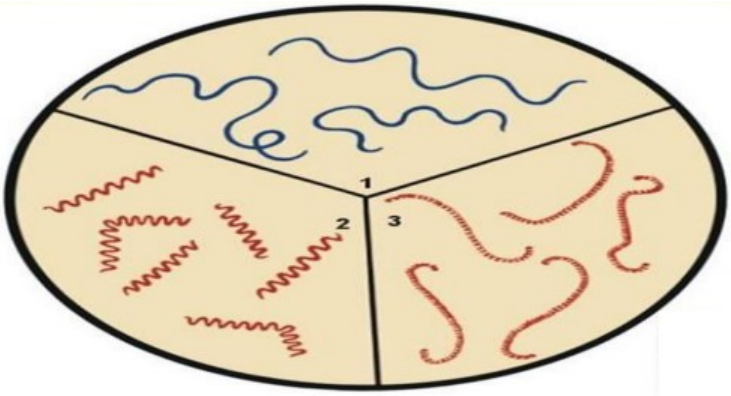
Spirochetes are thin, spiral-shaped or wave-like, highly motile bacteria. They are distinguished from other bacteria by the location of their flagella, sometimes called axial filaments, which run lengthwise between the bacterial inner membrane and outer membrane in periplasmic space. These cause a twisting motion which allows the spirochete to move about.

Diseases in humans are causing by some species from genera *Treponema*, *Borrelia* and *Leptospira*.

Treponema are slender with tight coils; *Borrelia* are somewhat thicker with fewer and looser coils; and *Leptospira* resemble *Borrelia* except for their hooked ends.

Romanowsky–Giemsa staining can be used as the differential one for spirochetes: *Treponema* get pink, *Borrelia* – blue and *Leptospira* – red by this staining technique.

Treponema and *Leptospira* are best visualized by darkfield microscopy.

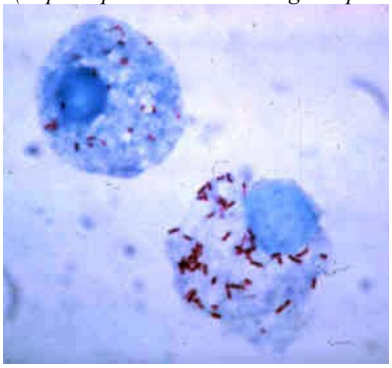


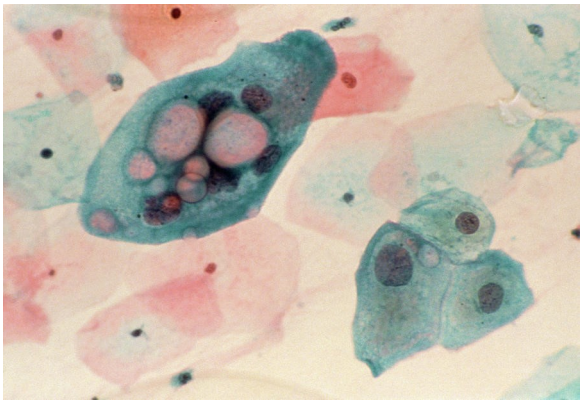
Borrelia (1), Treponema (2) and Leptospira (3) (https://image.slidesharecdn.com/spirillaceae-120919212113-phpapp02/95/spirillaceae-7-728.jpg?cb=1348089790)

Morphological peculiarities of rickettsia and chlamydia

Rickettsia and chlamydia are obligate intracellular parasites (which cannot reproduce outside their host cell, meaning that the parasite's reproduction is entirely reliant on intracellular resources). Chlamydia form cytoplasmic inclusions.

Gimenez stain of cells infected with Rickettsia (https://upload.wikimedia.org/wikipedia/commons/8/86/Rickettsia_rickettsii.jpg)



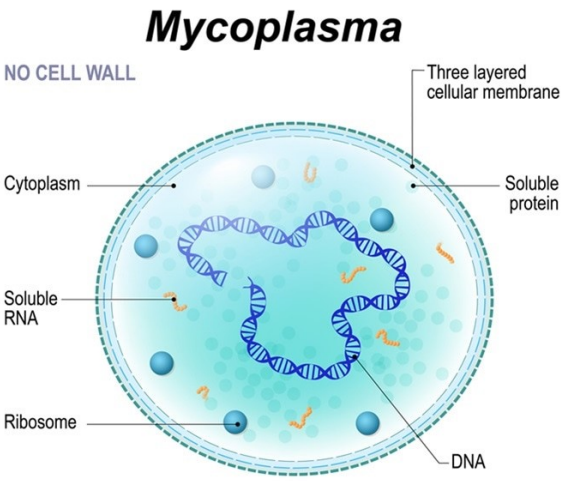


Chlamydia infective (or inclusion) bodies (spherical, large pink and small dark pink) are seen inside the epithelial cells of the vagina (light pink and light blue). Papanicolaou stain. (https://images.fineartamerica.com/images-medium-large-5/lm-of-cervical-smear-chlamydia-infection-science-photo-library.jpg)

Morphological and ultra-structural peculiarities of mycoplasmas

Mycoplasmas are the bacteria distinguished by the absence of a cell wall. Unlike other bacteria the most of mycoplasmas have sterols inside their cell membrane that make the last somewhat more rigid.

Analysis of the genomes of mycoplasmas gives solid support for the hypothesis that mycoplasmas have developed from Gram-positive bacteria by a process of reductive evolution. By adopting a parasitic mode of life with use of nutrients from their hosts, mycoplasmas were able to reduce their genetic material considerably. On the other hand, mycoplasmas lost the genes for many assimilative processes. Thus, mycoplasmas possibly became the smallest self-replicating organisms in nature.



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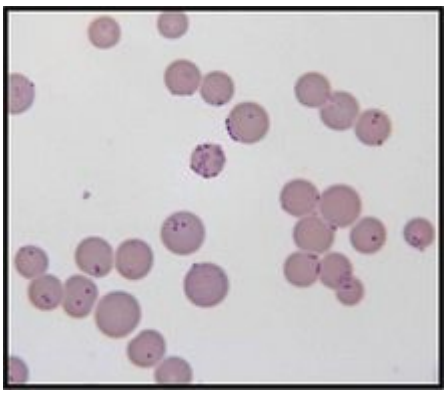


Cells of mycoplasmas (https://upload.wikimedia.org/wikipedia/commons/thumb/f/ff/M._haemofelis_IP2011.jpg/220px-M._haemofelis_IP2011.jpg)

Classification and taxonomy of fungi

A fungus (plural: fungi or funguses) is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds. These organisms are classified as a kingdom, fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls.

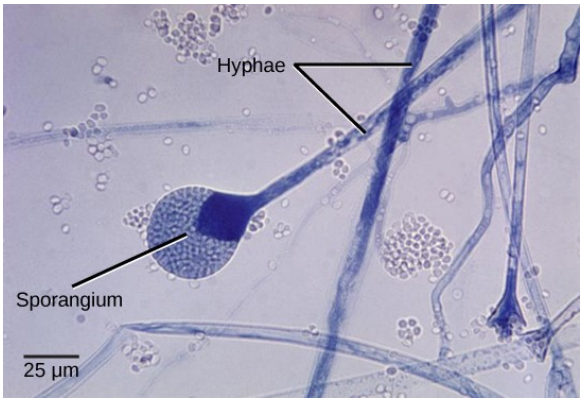
These and other differences place fungi in a single group of related organisms, named the Eumycota (true fungi or Eumycetes). This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology (from the Greek μύκης *mykes*, mushroom).

The fungi imperfecti or imperfect fungi, also known as *Deuteromycota* or deuteromycetes, are fungi which do not fit into the commonly established taxonomic classifications of fungi that are based on biological species concepts or morphological characteristics of sexual structures because their sexual form of reproduction has never been observed. Only their asexual form of reproduction is known, meaning that these fungi produce their spores asexually, in the process called sporogenesis. The most pathogenic fungi belongs to deuteromycetes.

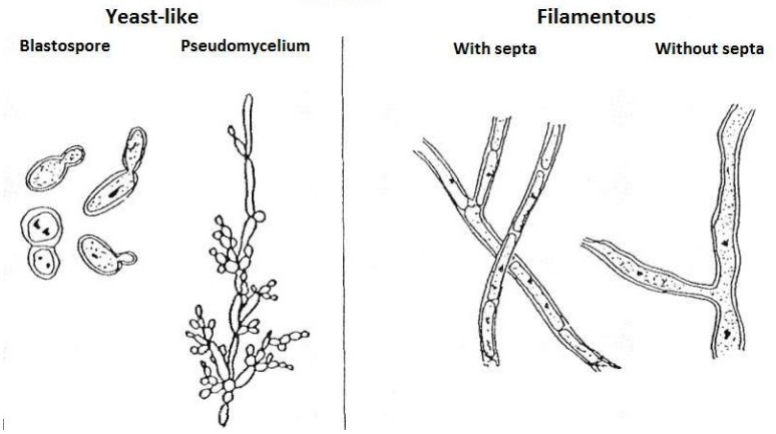
Morphological peculiarities of fungi

A mold is a fungus that grows in the form of multicellular filaments called hyphae. In contrast, fungi that can adopt a single-celled growth habit are called yeasts.

Fungi can reproduce asexually by fragmentation, budding, or producing spores, or sexually with homothallic (when male and female reproductive structures are present in the same mycelium) or heterothallic mycelia. Perfect fungi reproduce both sexually and asexually, while imperfect fungi reproduce only asexually (by mitosis). Asexual spores (conidia) are genetically identical to the parent and may be released either outside or within a special reproductive sac called a sporangium.



Release of spores from a sporangium: This bright field light micrograph shows the release of spores from a sporangium at the end of a hypha called a sporangiophore. The organism depicted is a Mucor sp. fungus: a mold often found indoors (https://s3-us-west-2.amazonaws.com/courses-images/wp-content/uploads/sites/1842/2017/05/26231521/figure-24-01-08.jpeg)



Fungi (https://www.researchgate.net/profile/Antoine_Cogulet/publication/323111630/figure/fig2/AS:655640041103361@1533328175101/Yeast-like-and-filamentous-fungi-Kendrick-1985.png)

Most yeasts reproduce asexually by budding: a small bump protrudes from a parent cell, enlarges, matures, and detaches. An asexual fungal spore produced by budding is named a blastospore A few yeasts reproduce by fission, the parent cell dividing into two equal cells.

Ultra-structural peculiarities of fungi

A hypha (plural hyphae, from Greek ὑφή, huphḗ, "web") is a long, branching filamentous structure of a fungus. A hypha consists of one or more cells surrounded by a tubular cell wall. In most fungi, hyphae are divided into cells by internal cross-walls called "septa" (singular septum). Septa are usually perforated by

pores large enough for ribosomes, mitochondria and sometimes nuclei to flow between cells. In most fungi, hyphae are the main mode of vegetative growth, and are collectively called a mycelium. There are three types of hyphae:

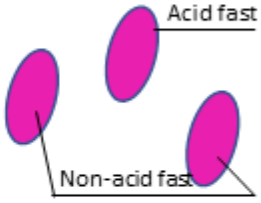
1. septate (with septa);
2. aseptate or coenocytic (without septa – fungi with nonseptate hyphae can named as phycomycetes);
3. "pseudohyphae" are distinguished from true hyphae by their method of growth, relative frailty and lack of cytoplasmic connection between the cells.

Yeast can form pseudohyphae. They are the result of a sort of incomplete budding where the cells elongate but remain attached after division.

Ziehl-Neelsen (acid-fast) staining

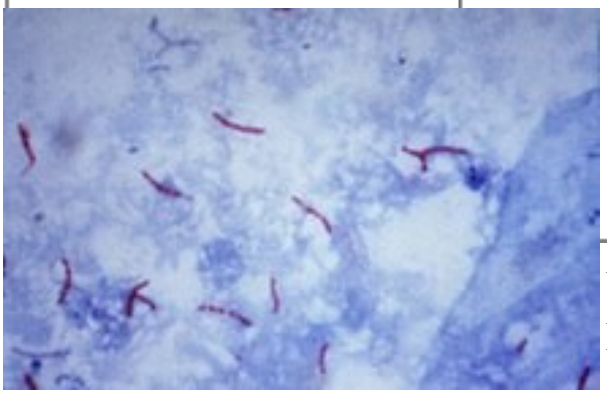
Ziehl-Neelsen staining is a type of acid-fast stain. Ziehl–Neelsen staining is a bacteriological stain used to identify acid-fast organisms, mainly *Mycobacteria*, and bacterial endospores.

To begin the staining process, a bacterial smear must be done. The smear should be evenly spread across the center of the slide. The smear is covered in a piece of bibulous paper and the smear is stained with carbol fuchsin. The slide is heated for five minutes while keeping the bibulous paper moist with the carbol fuchsin. The bibulous paper is removed and the slide is rinsed with distilled water. Acid-alcohol is used to decolorize the slide until the runoff is clear. The decolorizer removes the stain from non-acid-fast cells. The slide is rinsed with distilled water to insure all of the decolorizer is off of the slide. The slide is stained with the counter stain of methylene blue for one minute. Rinse the slide with distilled water. Blot, do not rub, the slide dry in a tablet of bibulous paper. When the slide is dry observe the bacteria under a microscope with oil immersion.

Procedure:	Purpose:	Cell Appearance:
Heat fix dried smear and cover with fuchsin stain. Then heat smear to 60 degrees Celsius and allow it to sit for five minutes after heating.	Heat fixes the sample to the slide, allows carbol fuchsin to enter cell walls.	
Use water to wash the stain and cover smear with 3% by volume acid-alcohol alcohol solution until smear is decolorized to pale pink; about 2-5 minutes.	This clears the dye from the cells so that the non-acid fast cell can be cleared of dye for counterstaining.	
Wash smear with clear water and cover with 0.5% w/v methylene blue for 1-2 minutes.	The methylene blue will only color the non-acid fast stains and make them visible.	
Wash smear with clean water and air dry.	Prepare to analyze slide with oil immersion objective lens.	

Acid fast staining mechanism (https://upload.wikimedia.org/wikipedia/commons/thumb/3/39/HO.png/800px-HO.png)

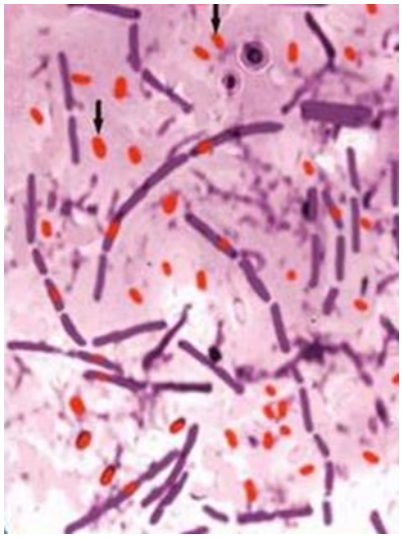
Initially, carbol fuchsin stains every cell. When they are de-stained with acid-alcohol, only non-acid-fast bacteria get de-stained since they do not have a thick, waxy lipid layer like acid-fast bacteria. When counter stain is applied, non-acid-fast bacteria pick it up and become blue (methylene blue) or green (malachite green) when viewed under the microscope. Acid-fast bacteria retain carbol fuchsin so they appear red.



Mycobacterium tuberculosis visualization using the Ziehl–Neelsen stain (https://upload.wikimedia.org/wikipedia/commons/thumb/7/71/Mycobacterium_tuberculosis_Ziehl-Neelsen_stain_02.jpg/220px-Mycobacterium_tuberculosis_Ziehl-Neelsen_stain_02.jpg)

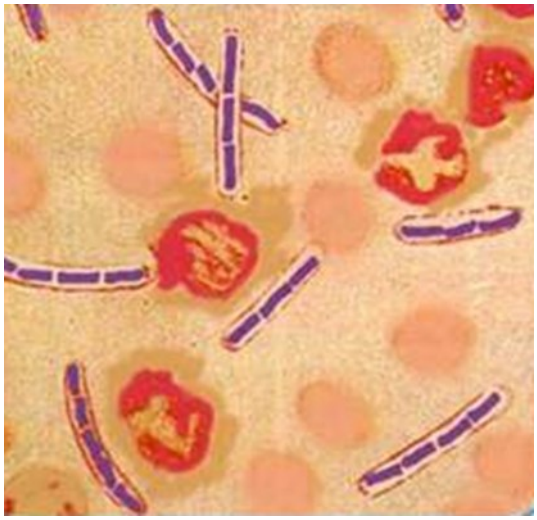
Training algorithm of practical skills to be mastered at the lesson

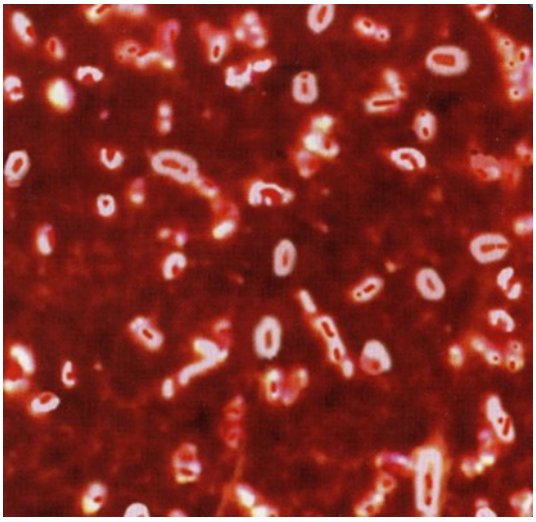
1. Ziehl-Neelsen staining method:
   1. carbol fuchsine (for a fixed smear), hold a flame beneath the slide until steam appears but do not allow it to boil;
   2. cool smear;
   3. washing with water;
   4. acid, 5 seconds;
   5. washing with water;
   6. methylene blue, 4 minutes
   7. washing with water;
   8. dry with filter paper.



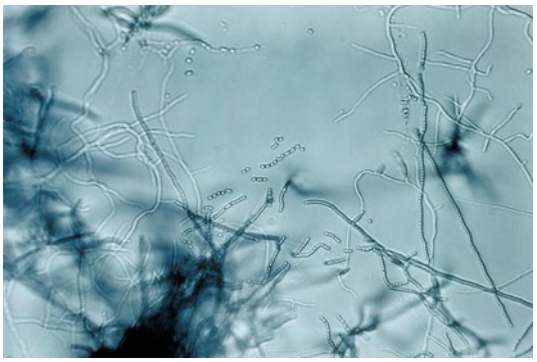
2. Identification of *Bacillus* by smear:
   large rods, forming chains.

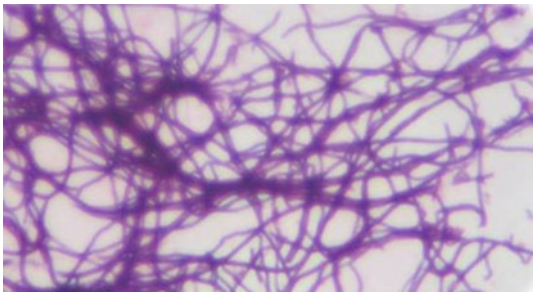
3. Identification of encapsuled bacteria by smear:
   on colored background – unpainted halos around bacterial cells.



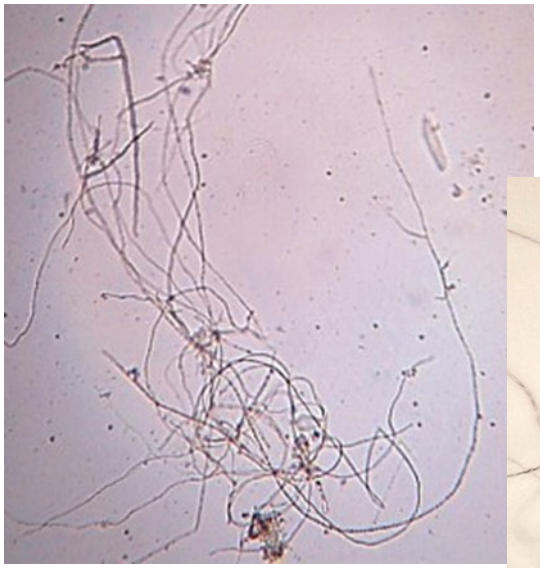


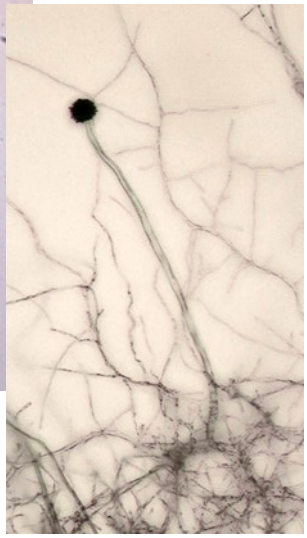
4. Identification of *Streptomyces* by smear:
   strongly branched threads.





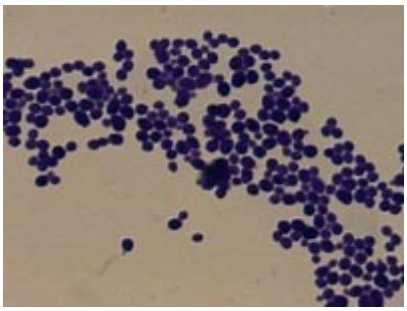
5. Identification of mold fungus by smear:
   hyphae (at low magnification).

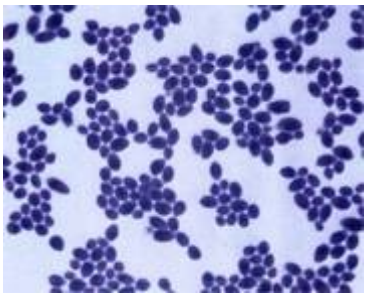




6. Identification of yeast (Candida) by smear:

oval and round large cells of different sizes (with the heterogeneity inside).



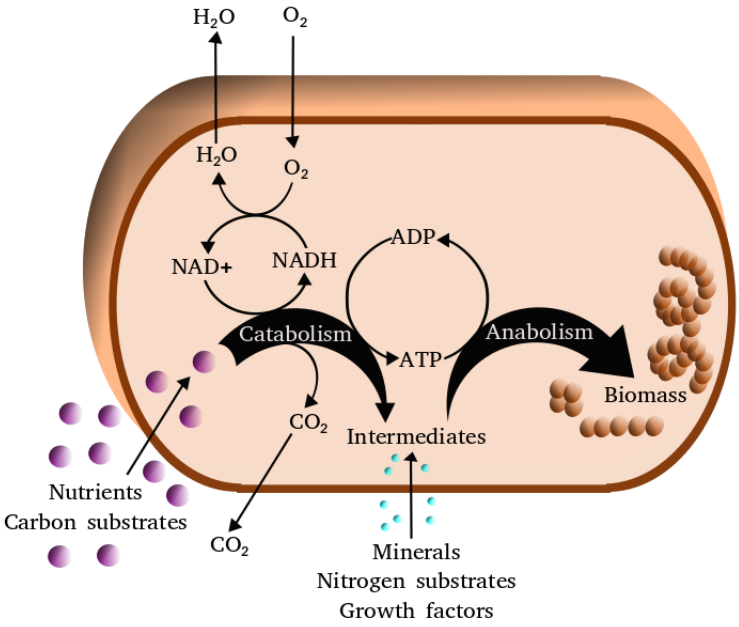


Lesson 4

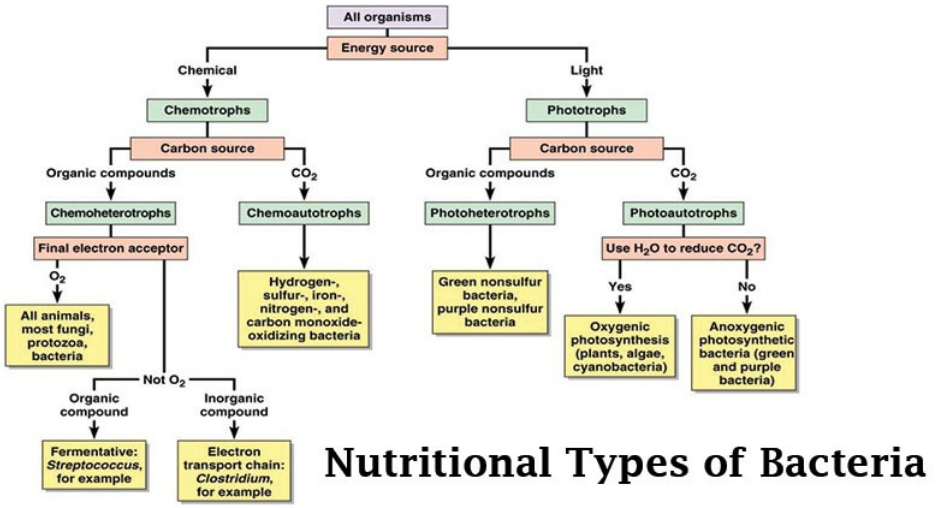
Features of metabolism in microorganisms

Bacteria differ dramatically with respect to the conditions that are necessary for their optimal growth. In terms of nutritional needs, all cells require sources of carbon, nitrogen, sulfur, phosphorus, numerous inorganic salts (e.g., potassium, magnesium, sodium, calcium, and iron), and a large number of other elements called micronutrients (e.g., zinc, copper, manganese, selenium, tungsten, and molybdenum). Carbon is the element required in the greatest amount by bacteria since hydrogen and oxygen can be obtained from water, which is a prerequisite for bacterial growth. Also required is a source of energy to fuel the metabolism of the bacterium. One means of organizing bacteria is based on these fundamental nutritional needs: the carbon source and the energy source.

There is can be formulated that metabolism means the ability to collect and use energy. But the separate type of microorganisms, viruses, are too small and simple to collect or use their own energy – they just steal it from the cells they infect. Viruses only need energy when they make copies of themselves inside a host cell, and they don't need any energy at all when they are outside of a cell.



Simplified view of the cellular metabolism (https://doi.org/10.6084/m9.figshare.7138037.v1)



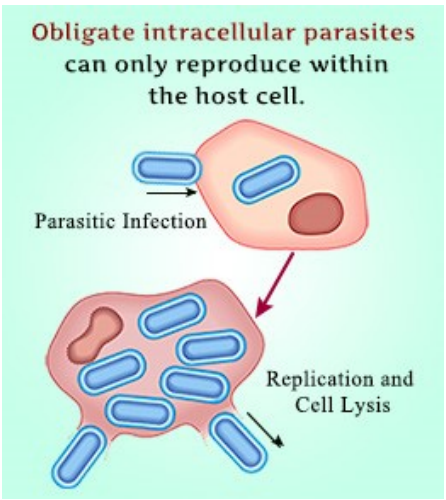
(https://163602-560839-raikfcquaxqncofqfm.stackpathdns.com/wp-content/uploads/2019/02/Classification-of-Bacteria-on-the-basis-of-Nutrition.jpg)

Classification of bacteria by the source of carbon

All organisms require carbon in some form for use in synthesizing cell components. All organisms require at least a small amount of CO_2. However, some can use CO_2 as their major or even sole source of carbon; such organisms are termed as Autotrophs (Autotrophic bacteria). Others require organic compounds as their carbon source and are known as Heterotrophs (Heterotrophic bacteria).

There are three main categories that differ in how chemoheterotrophs obtain their organic nutrients: saprophytic bacteria, parasitic bacteria and symbiotic bacteria.

Saprophytic bacteria obtain their food from the dead and organic decaying matter such as leaves, fruits, vegetables, meat, animal feces, leather, humus etc. These bacteria secrete enzymes to digest the food and absorb it. The enzymes secreted to break down the complex compounds such as carbohydrate and protein, into simpler soluble compounds, which are easily absorbed.



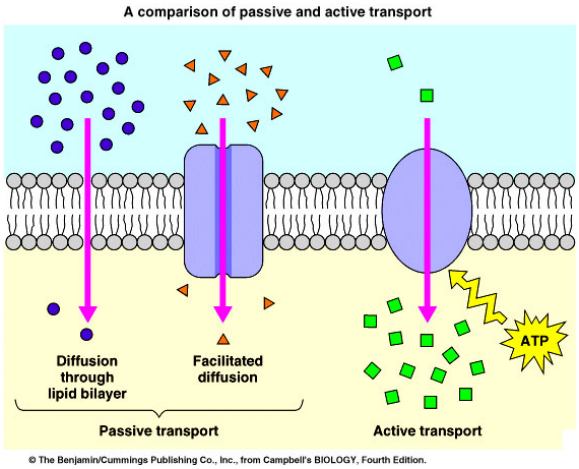
(https://www.buzzle.com/img/articleImages/610122-5969-16.jpg)

Parasitic bacteria obtain their nutrition from the tissues of the hosts on which they grow. They may be harmless or may cause serious diseases. Parasitic bacteria which cause various diseases in plants and animals are known as pathogens. An obligate parasite or holoparasite is a parasitic organism that cannot complete its life-cycle without exploiting a suitable host. If an obligate parasite cannot obtain a host it will fail to reproduce. Whether one regards viruses as living organisms or not, they cannot reproduce except by means of resources within living cells. Accordingly, it is convenient and customary to regard them as obligate intracellular parasites. This is opposed to a facultative parasite, which can act as a parasite but does not rely on its host to continue its life-cycle. More intimately, normally free-living microbes may opportunistically live as facultative parasites in other organisms.

Symbiotic bacteria live in close association with other organisms as symbionts. They are beneficial to the organisms. The common examples are the nitrogen-fixing bacteria. These bacteria live inside the roots of leguminous plants. They fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria.

Main routes of penetration of nutrients into bacterial cell

There are two types of the movement of molecules across a membrane and a bacterial cell wall: passive transport and active transport.



(https://cardenasbio.weebly.com/uploads/2/7/3/6/27363379/7395215_orig.jpg)

Passive transport (diffusion) is a movement of ions and other atomic or molecular substances across cell membranes without need of energy input. The difference of concentration between the two areas is often termed as the concentration gradient, and diffusion will continue until this gradient has been eliminated. Since diffusion moves materials from an area of higher concentration to an area of lower concentration, it is described as moving solutes "down the concentration gradient" (compared with active transport, which often moves material from area of low concentration to area of higher concentration, and therefore referred to as moving the material "against the concentration gradient"). Unlike active transport, it does not require an input of cellular energy because it is instead driven by the tendency of the system to grow in entropy. The rate of passive transport depends on the permeability of the cell membrane, which, in turn, depends on the organization and characteristics of the membrane lipids and proteins. The two main kinds of passive transport are simple diffusion and facilitated diffusion.

Simple diffusion is the passive movement of solute from a high concentration to a lower concentration until the concentration of the solute is uniform throughout and reaches equilibrium. Facilitated diffusion, also called carrier-mediated osmosis, is the movement of molecules across the cell membrane via special transport proteins that are embedded in the plasma membrane by actively taking up or excluding ions. The permeases are membrane transport proteins, a class of multipass transmembrane proteins that allow the diffusion of a specific molecule in or out of the cell in the direction of a concentration gradient, a form of facilitated diffusion.

Active transport is the movement of molecules across a membrane from a region of lower concentration to a region of higher concentration—against the concentration gradient. Active transport requires cellular energy to achieve this movement. There are two types of active transport: primary active transport that uses adenosine triphosphate (ATP), and secondary active transport that uses an electrochemical gradient.

Classification of bacteria by their growth factors needs

Prototrophic bacteria are self sufficient producers of all required metabolites – growth factors (e.g. amino acids, lipids, cofactors), while auxotrophic bacteria require to be on medium with the metabolite that they cannot produce.

Classification of bacteria by the feature of their energy metabolism

On the basis of energy source organisms are designated as: phototrophs (these bacteria can utilize light as an energy source) and chemotrophs (these bacteria cannot carry out photosynthesis – they gain energy from chemical compounds).

Some chemotrophs can use reduced inorganic compounds as electron donors and are termed as lithotrophs. Some chemotrophs can use organic compounds as electron donors and are termed as organotrophs.

There are two types of heterotrophic metabolism: respiration (or oxidation) and fermentation. Respiration takes place when any organic compound (usually carbohydrate) is oxidized completely to CO_2 and H_2O. At this case the terminal electron acceptor is oxygen. In fermentation an organic compound rather than oxygen is the terminal electron (or hydrogen) acceptor. Less energy is generated from this incomplete form of carbohydrate degradation, but the process supports anaerobic growth.

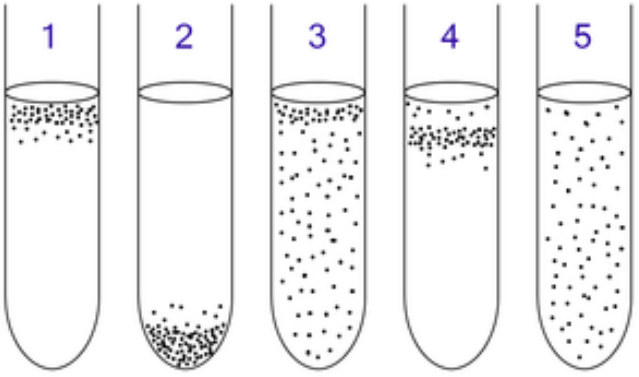
Classification of bacteria by their requirements of the oxygen in the air

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.

For practical purposes, there are two categories of anaerobe: obligate and aerotolerant Obligate anaerobes are harmed by the presence of oxygen. Aerotolerant bacteria cannot use oxygen for growth, but tolerate its presence.

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.

Facultative anaerobes grow in either the presence or absence of oxygen.



Aerobic and anaerobic bacteria can be identified by growing them in test tubes of thioglycollate broth:
1: Obligate aerobes need oxygen because they cannot ferment or respire anaerobically. They gather at the top of the tube where the oxygen concentration is highest.
2: Obligate anaerobes are poisoned by oxygen, so they gather at the bottom of the tube where the oxygen concentration is lowest.
3: Facultative anaerobes can grow with or without oxygen because they can metabolise energy aerobically or anaerobically. They gather mostly at the top because aerobic respiration generates more adenosine triphosphate (ATP) than either fermentation or anaerobic respiration.
4: Microaerophiles need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top.
5: Aerotolerant organisms do not require oxygen as they metabolize energy anaerobically. Unlike obligate anaerobes however, they are not poisoned by oxygen. They can be found evenly spread throughout the test tube.

(https://upload.wikimedia.org/wikipedia/commons/thumb/9/90/Anaerobic.png/300px-Anaerobic.png)

Features of metabolism in rickettsia, chlamydia and mycoplasmas

The rickettsia are bacteria which are obligate intracellular parasites. The basis for the obligate parasitism of these cells is that they require the rich cytoplasm to stabilize an unusually permeable cell membrane. The rickettsia have many of the metabolic capabilities of bacteria, but require an exogenous supply of cofactors to express these capabilities. The response to exogenous cofactors implies an unusually permeable cytoplasmic membrane.

The chlamydia are bacteria which are obligate intracellular parasites of higher animals (mammals and birds). The basis of their obligate intracellular parasitism is due to a lack of ATP-generating ability and the need to obtain ATP from the host cell.

The mycoplasmas are essentially bacteria lacking a rigid cell wall during their entire life cycle, although they are also much smaller than bacteria.

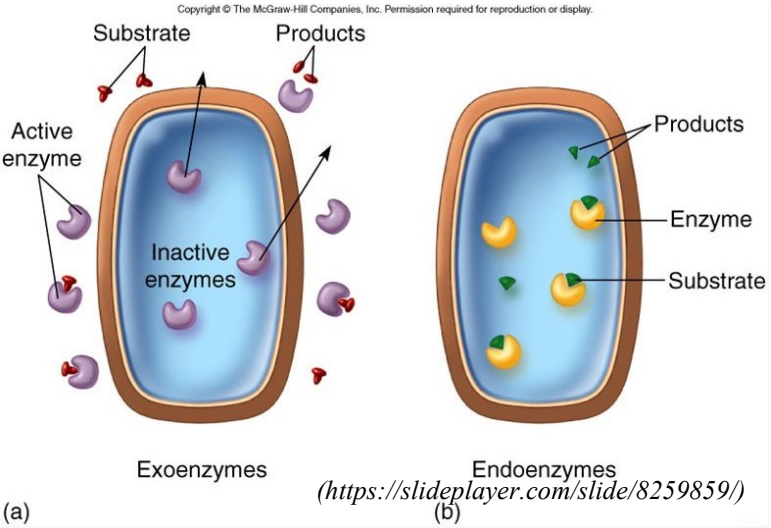
The parasitic mycoplasmas have truncated respiratory systems, lacking quinones and cytochromes and need for sterols (mainly cholesterol) for their cell membrane building.

Classification of bacterial enzymes

According to the International Union of Biochemists (IUB), enzymes are divided into seven functional classes and are classified based on the type of reaction in which they are used to catalyze. The 7 types of enzymes are oxidoreductases, hydrolases, transferases, lyases, isomerases, ligases and translocases.

In microbiology bacterial enzymes are also divided by their location of action to exoenzymes and endoenzymes. Exoenzymes are inactive while inside the cell, but upon release from the cell they become active. In contrast, endoenzymes remain in the cell and are active.

Additionally, bacterial enzymes are divided to constitutive and inducible ones. Constitutive enzymes are present in constant amounts (even in the absence of the substrate), while inducible (regulated) enzymes are either induced or repressed (that is its expression level is dependent of the substrate concentration).



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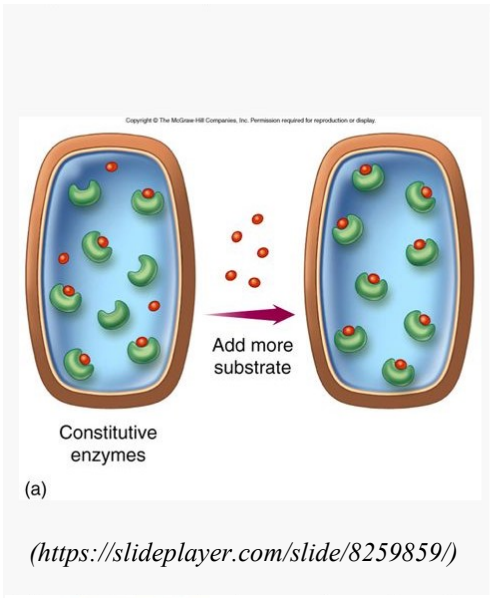

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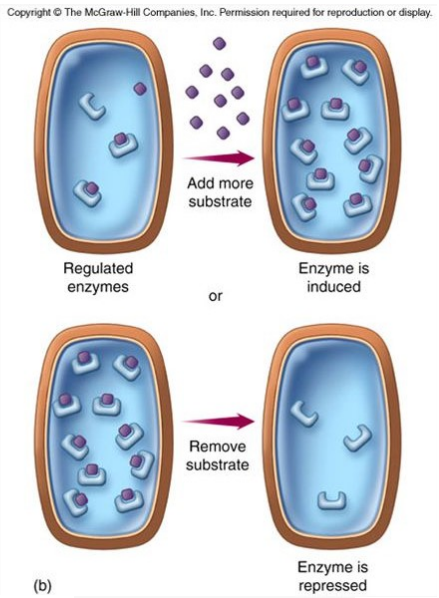


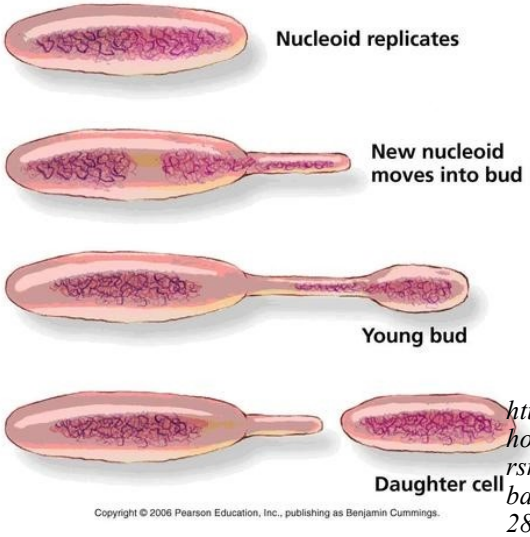
Forms of reproduction of bacteria

Most bacteria rely on binary fission for propagation. Conceptually this is a simple process; a cell just needs to grow to twice its starting size and then split in two. But, to remain viable and competitive, a bacterium must divide at the right time, in the right place, and must provide each offspring with a complete copy of its essential genetic material.

Some bacteria can reproduce by budding. Each bacterium divides following unequal cell growth; the mother cell is retained, and a new daughter cell is formed. In budding, the cell wall grows from one point on the cell (polar growth).

Streptomyces multiply by exospores.

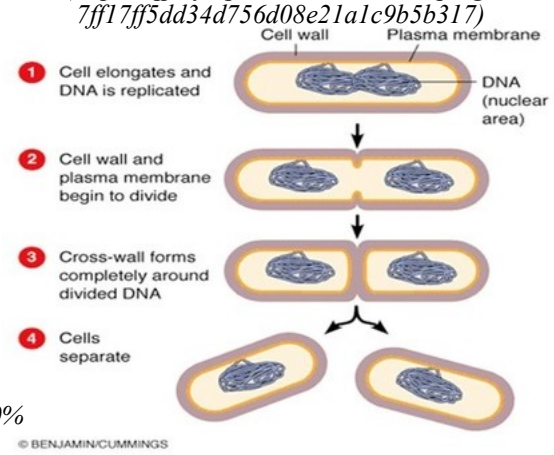
About *Chlamydia* – see below.



https://sites.google.com/site/howdowebeatbacteria/_/rsrc/1414483858117/home/bacterial-growth/budding%20%281%29.JPG

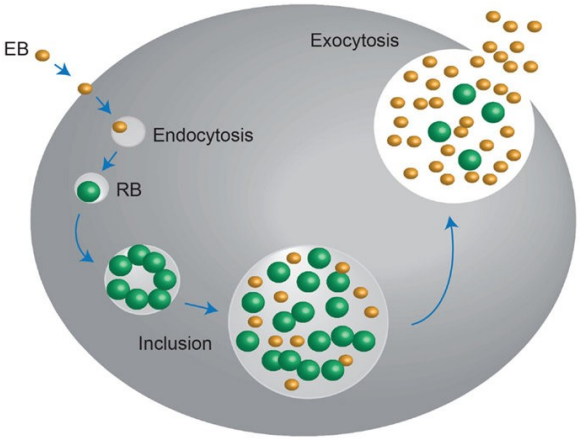
Binary Fission

(https://qph.fs.quoracdn.net/main-qimg-7ff17ff5dd34d756d08e21a1c9b5b317)

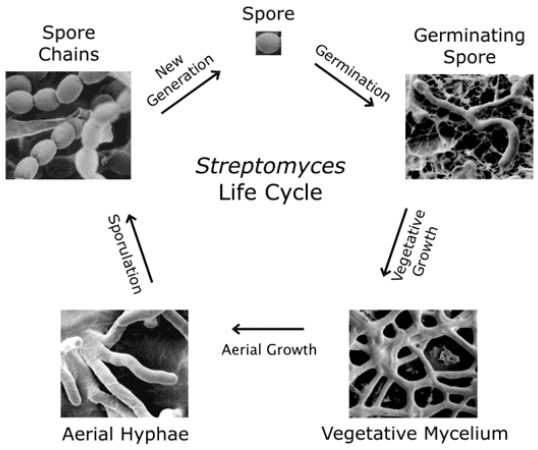


Developmental cycle of Chlamydia

Chlamydia undergo a unique biphasic developmental cycle. The infectious form of Chlamydia, the elementary body (EB) enters into the host cell via endocytosis. Upon entry, the EB convert into the metabolically active, non-infectious reticulate body (RB), which binary replicates within a vacuolar compartment, termed the inclusion. Once the developmental cycle is almost complete, the RBs revert back into EBs, stimulating host cell lysis and release of the infectious EBs into the extracellular space.



Developmental cycle of Chlamydia (https://www.researchgate.net/profile/Kate_Redgrove/publication/268229035/figure/fig3/AS:272596512014385@1442003481788/Chlamydia-undergo-a-unique-biphasic-developmental-cycle-The-infectious-form-of.png)



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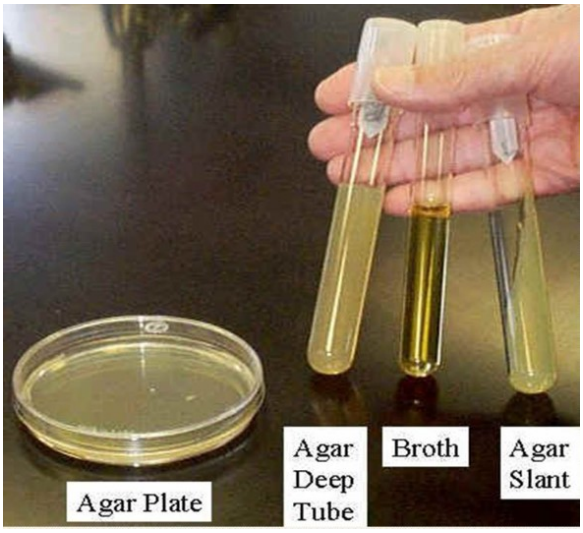
Cultivation of microorganisms

When microorganisms are cultivated in the laboratory, a growth environment called a medium is used. The medium may be purely chemical (a chemically defined medium), or it may contain organic materials, or it may consist of living organisms such as fertilized eggs (cultivation of obligate parasites: *Rickettsia*, *Chlamydia*, viruses; this type of cultivation is named *in vivo* cultivation, opposite to *in vitro* cultivation – which uses nutrient media without any living organisms). Microorganisms growing in or on such a medium form a culture. A culture is considered a pure culture if only one type of organism is present and a mixed culture if populations of different organisms are present. When first used, the culture medium should be sterile, meaning that no form of life is present before inoculation with the microorganism.

Classification of culture media

For the cultivation of bacteria, a commonly used medium is nutrient broth, a liquid containing proteins, salts, and growth enhancers that will support many bacteria. To solidify the medium, an agent such as agar is added. Agar is a polysaccharide that adds no nutrients to a medium, but merely solidifies it. The medium that results is nutrient agar. Agar does not melt until near boiling point; this means that cultures can be incubated at 37°C or above without the medium melting. Moreover, when it cools, agar remains molten until just over 40°C, allowing heat-sensitive media components such as blood to be added. In addition, most bacteria can tolerate a short exposure to temperatures in this range, so they too can be inoculated into molten agar (see pour plate method below). Crucially, agar is more or less inert nutritionally; only a very few organisms are known that are able to use agar as a food source; consequently, it is the near ideal setting agent, resisting both thermal and microbial breakdown.



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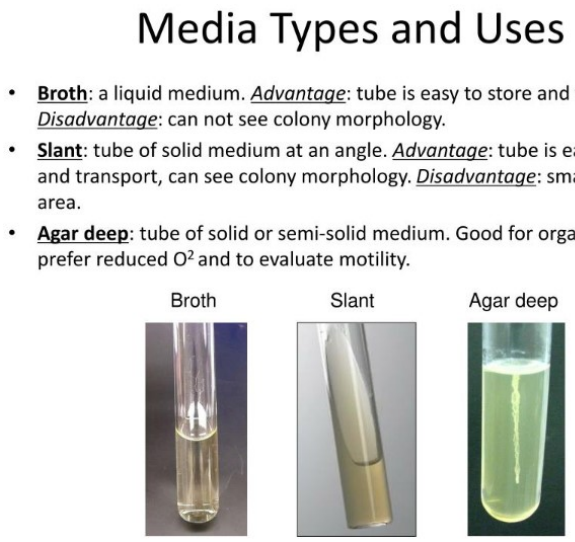


(https://image1.slideserve.com/2742784/media-types-and-uses-l.jpg)

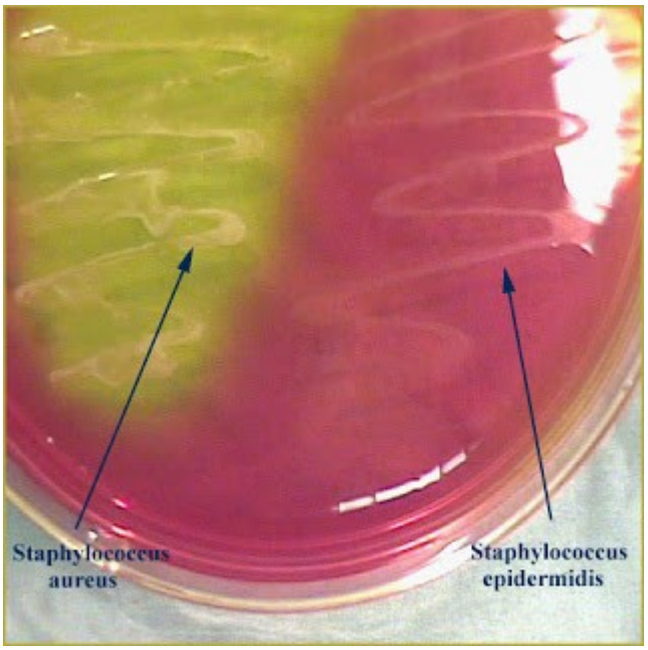
A solid medium contains 2% agar, a semi-solid one contains 0.5% agar, there are no agar inside a liquid medium at all. Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate. Semi-solid media have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility. Broth (liquid) media serve various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. It was the **classification of bacterial culture media on the basis of consistency**.

Classification of culture media on the basis of composition. A synthetic (chemically defined) medium is one prepared from purified ingredients and therefore its exact composition is known. Non-synthetic (or chemically undefined) medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Synthetic medi-

um may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Classification of Bacterial Culture media on the basis of purpose/functional use/application. Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria.

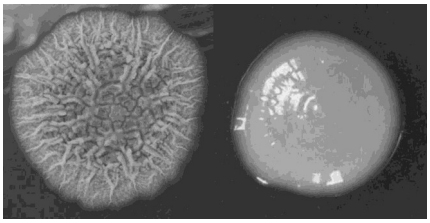
1) Basic (General purpose) media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.
2) Enriched (Added growth factors) medium. Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc. are few of the enriched media.
3) Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from fecal specimens.
4) Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated. Thayer Martin Agar used to recover *Neisseria gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin. Mannitol Salt Agar and Salt Milk Agar used to recover *S. aureus* contains 10% NaCl. Potassium tellurite medium used to recover *C. diphtheriae* contains 0.04% potassium tellurite. MacConkey's Agar used for *Enterobacteriaceae* members contains bile salt that inhibits most gram positive bacteria. Lowenstein Jensen Medium used to recover *M. tuberculosis* is made selective by incorporating malachite green. Wilson and Blair's Agar for recovering *S. typhi* is rendered selective by the addition of dye brilliant green. Selective media such as TCBS Agar used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.
5) Differential (indicator) media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various approaches include incorporation of dyes, metabolic substrates etc., so that those bacteria that utilize them appear as differently colored colonies. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies. Examples of differential media include: Mannitol salts agar (mannitol fermentation = yellow), Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis), MacConkey agar (lactose fermenters, pink colonies whereas non-lactose fermenter produces pale or colorless colonies, TCBS (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose).
6) Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Examples: Cary Blair transport medium and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected cholera patients, Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery, Pike's medium is used to transport streptococci from throat specimens.



Mannitol salts agar (https://i1.wp.com/3.bp.blogspot.com/_cCU1vJAsuTc/TEsQkgljtkI/AAAAAAAAAxY/h1GbpNpylwc/s320/mannitol+salt+staph.jpg?resize=320%2C320)

Bacterial culture requirements

A characteristic of microorganisms is their ability to grow and form a population of organisms. One of the results of microbial metabolism is an increase in the size of the cell. The many requirements for successful growth include the nutritional needs (simple or complex) of the concrete bacteria, the cultivation temperature, the extent of acidity or alkalinity, referred to as the pH of a solution, and the specific type of aeration.

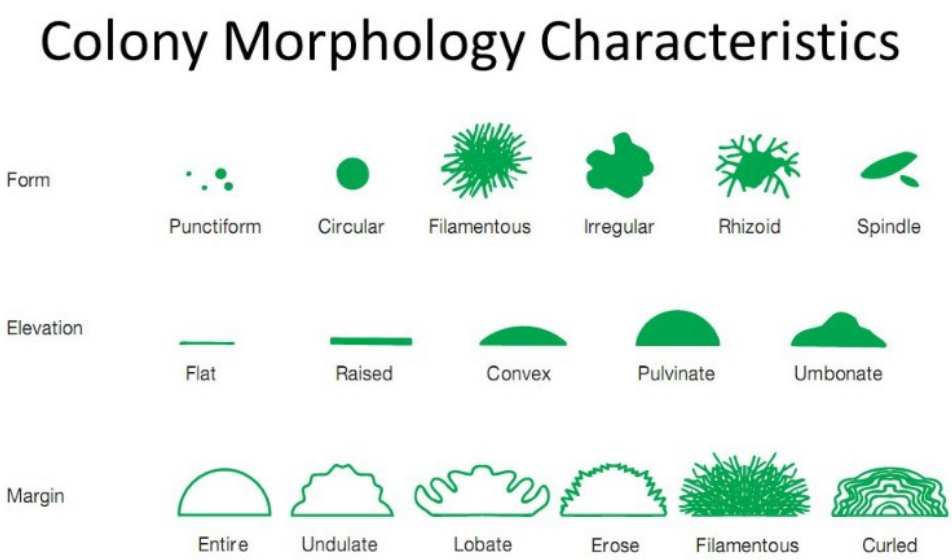


R-(left) and S-form of a colony (https://jcm.asm.org/content/jcm/52/1/244/F1.large.jpg)

The character of bacterial growth in nutrient media

Bacteria grown in liquid cultures often form colloidal suspensions.

Bacteria grow on solid media as lawn (with dense seeding) or as colonies (while rare seeding). A microbial colony is defined as a visible cluster of microorganisms growing on the surface of or within a solid medium, presumably cultured from a single cell. Because the colony is clonal, with all organisms in it descending from a single ancestor (assuming no contamination), they are genetically identical, except for any mutations (which occur at low frequencies). Obtaining such genetically identical organisms (or pure strains) can be useful; this



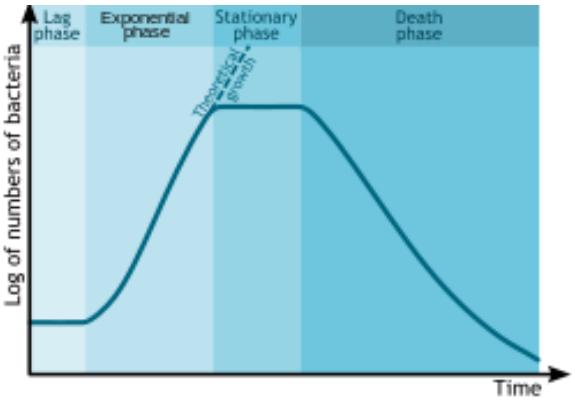
(https://slideplayer.com/6152380/18/images/17/Colony+Morphology+Characteristics.jpg)

is done by spreading organisms on a culture plate and starting a new stock from a single resulting colony.

There are two main types of colonies: S-form colonies ("smooth") and R-form colonies – ("rough").

Phases of bacterial growth in batch culture

The growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: lag phase, log phase or exponential phase, stationary phase, and death phase.



Bacterial growth curve\Kinetic Curve (https://upload.wikimedia.org/wikipedia/commons/thumb/c/c0/Bacterial_growth_en.svg/250px-Bacterial_growth_en.svg.png)

⇒ During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

⇒ The log phase (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

⇒ The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase.

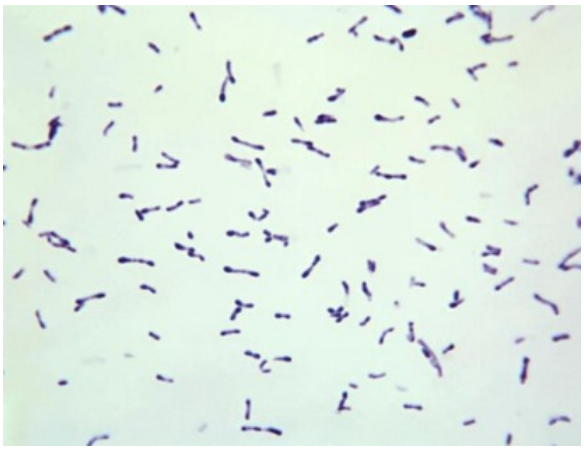
⇒ At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

Training algorithm of practical skills to be mastered at the lesson

1. Neisser staining technique:
   1. Neisser's methylene blue (at a fixed smear), 1-2 minutes;
   2. Lugol's iodine solution, 1-2 minutes;
   3. rinse the slide clean with water;
   4. vesuvin (Bismarck Brown counter stain), 1-2 minutes;
   5. rinse the slide clean with water and shake off the excess water;
   6. dry with filter paper.

2. Identification of Corynebacterium by smear (stained by Neisser or Loeffler's Blue stain):
rods with points.

Neisser Staining



Loeffler's Blue staining

Lesson 5

Definition of the term “bacteriophage (phage)”

A bacteriophage is a type of virus that infects bacteria. In fact, the word "bacteriophage" literally means "bacteria eater," because bacteriophages can destroy their host cells.

Discovery of bacteriophage

n 1896, Ernest Hanbury Hankin reported that something in the waters of the Ganges and Yamuna rivers in India had a marked antibacterial action against cholera and it could pass through a very fine porcelain filter.

In 1915, British bacteriologist Frederick Twort, superintendent of the Brown Institution of London, discovered a small agent that infected and killed bacteria. He believed the agent must be one of the following: a stage in the life cycle of the bacteria, an enzyme produced by the bacteria themselves, or a virus that grew on and destroyed the bacteria. Twort's research was interrupted by the onset of World War I and a shortage of funding.

Independently, French-Canadian microbiologist Félix d'Hérelle, working at the Pasteur Institute in Paris, announced on 3 September 1917, that he had discovered a virus parasitic on bacteria. D'Hérelle called the virus a bacteriophage, from the Greek φαγεῖν (phagein), meaning "to devour" . It was D'Herelle who conducted much research into bacteriophages and introduced the concept of phage therapy.

Félix d'Herelle
(https://upload.wikimedia.org/wikipedia/commons/thumb/b/b5/F%C3%A9lix_d%27H%C3%A9relle.jpg/220px-F%C3%A9lix_d%27H%C3%A9relle.jpg)

Classification of bacteriophage

Bacteriophages occur abundantly in the biosphere, with different genomes, and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid.

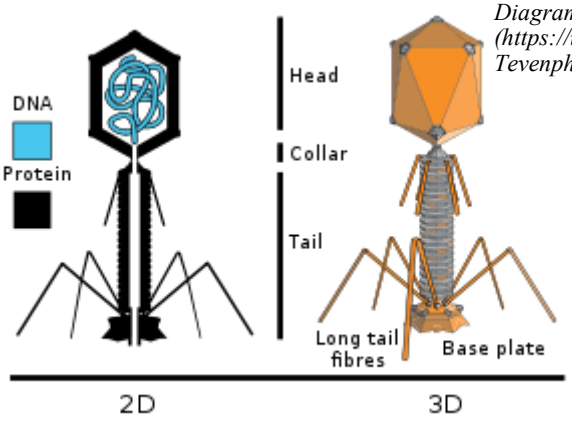
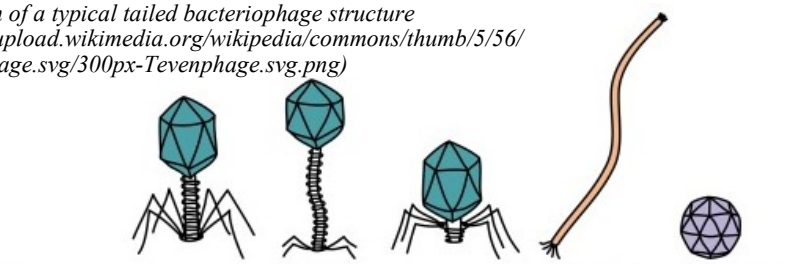


Diagram of a typical tailed bacteriophage structure
(https://upload.wikimedia.org/wikipedia/commons/thumb/5/56/Tevenphage.svg/300px-Tevenphage.svg.png)



Some forms of bacteriophages
(https://ars.els-cdn.com/content/image/1-s2.0-S1359644617302970-gr1.jpg)

Structure of bacteriophage

Like all viruses, phages are simple organisms that consist of a core of genetic material (nucleic acid) surrounded by a protein capsid. The nucleic acid may be either DNA or RNA and may be double-stranded or single-stranded.

Morphological types of bacteriophages

There are three basic structural forms of phage: an icosahedral (20-sided) head with a tail, an icosahedral head without a tail, and a filamentous form.

Host specificity of bacteriophages

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn, determines the phage's host range. Phages are usually species-specific and even strain-specific, however, some polyvalence is observed, predominantly among phages of enterobacteria and staphylococci. These polyvalent phages are able to infect strains from either different genera or species.

Life cycles of bacteriophages

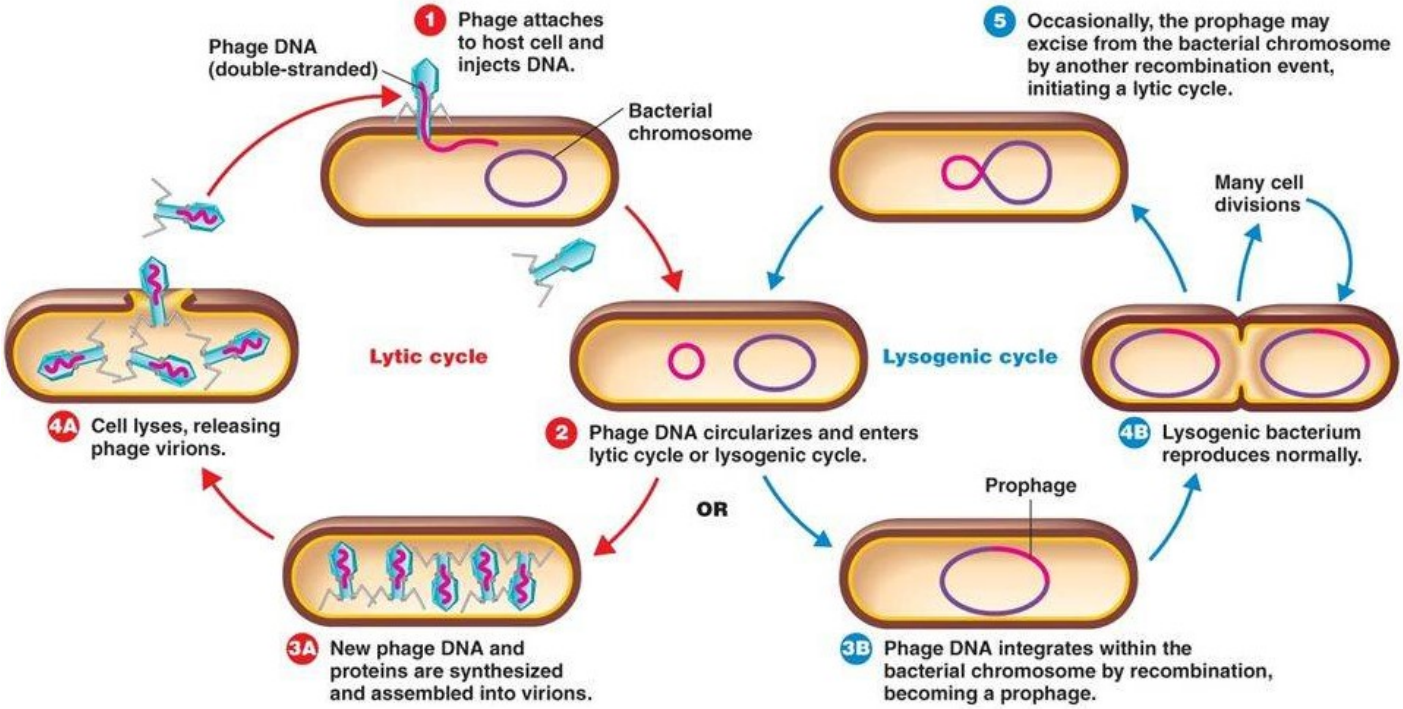
Bacteriophages may have a lytic cycle or a lysogenic cycle. With lytic phages bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy.

In contrast, the lysogenic cycle does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it, relatively harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients, then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is replicated in all offspring of the cell.

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome, in a phenomenon called lysogenic conversion. Examples are the conversion of harmless strains of Corynebacterium

Lesson 5

Lytic and Lysogenic Cycles



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(https://krystalbio11.weebly.com/uploads/2/3/3/9/23399170/6820224_orig.jpg)

diphtheriae or Vibrio cholerae by bacteriophages, to highly virulent ones that cause diphtheria or cholera, respectively.

Other life cycles, including pseudolysogeny and chronic infection, also exist. In pseudolysogeny a bacteriophage enters a cell but neither co-opts cell-replication machinery nor integrates stably into the host genome. Pseudolysogeny occurs when a host cell encounters unfavorable growth conditions and appears to play an important role in phage survival by enabling the preservation of the phage genome until host growth conditions have become advantageous again. In chronic infection new phage particles are produced continuously over long periods of time but without apparent cell killing.

Practical application of phages in medicine

Soon after making their discovery, Twort and d'Hérelle began to use phages in treating human bacterial diseases such as bubonic plague and cholera. Phage therapy was not successful, and after the discovery of antibiotics in the 1940s, it was virtually abandoned. With the rise of antibiotic-resistant bacteria, however, the therapeutic potential of phages has received renewed attention.

Phages are used in diagnostics (to identify the pure culture), and prevention (use per os, such as dysentery, typhoid bacteriophages).

In the 1980s American biochemist George P. Smith developed a technology known as phage display, which allowed for the generation of engineered proteins. Such proteins were produced by fusing foreign or engineered DNA fragments into phage gene III. Gene III encodes a protein expressed on the phage virion surface. Thus, gene III fusion proteins taken up by phages were displayed on the surfaces of virion particles. Researchers could then use antibodies developed to recognize the foreign protein fragment to purify fusion phage cultures, thereby effectively amplifying the foreign gene sequence for further study. British biochemist Gregory P. Winter subsequently refined phage display technology for the development of human antibody proteins. Such proteins could be used to treat diseases in humans with less risk of inducing potentially dangerous immune reactions compared with previous therapeutic antibodies derived from animals. For their discoveries relating to phage display, Smith and Winter were awarded a share of the 2018 Nobel Prize in Chemistry.

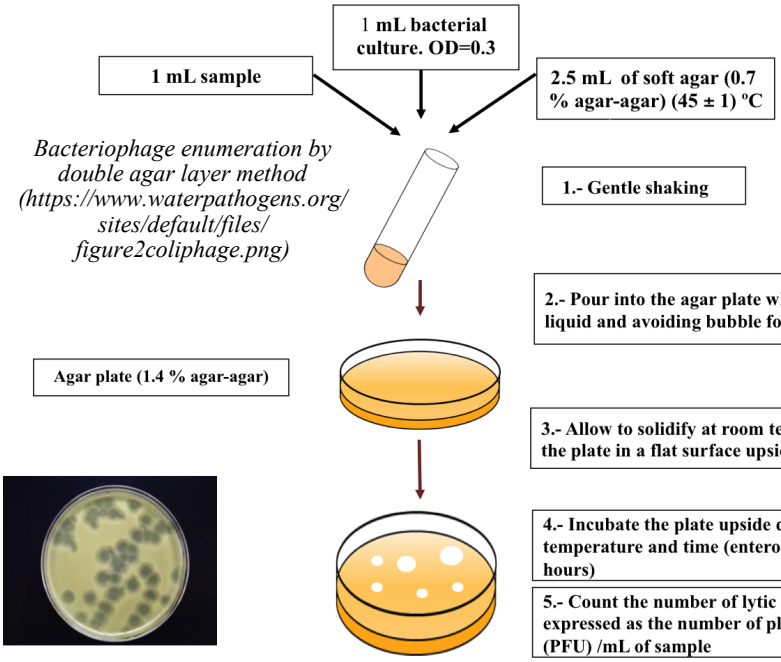
Bacteriophage isolation

Material containing bacteriophage (object of the environment or bacterial culture) is filtered through a bacterial filter. Filtrate is placed in a liquid medium, which is inoculated by susceptible to this phage bacterial culture. Seeded medium is incubated. Growth of bacteria indicates the absence of the bacteriophage in the material. No growth of bacteria indicates that the bacteriophage is present in the material.

For bacteriophage isolation a plating technique can be used. By this technique specific phages from the sample can be detected and counted. The plating technique is referred to as "plaque assay" and involves seeding a "lawn" of host bacteria with a small volume of sample containing phage. When phages seeded on the lawn infect and lyse the host cells, they produce a plaque within the confluent opaque lawn of bacteria. The number of plaques multiplied by the dilution is equal for the amount of phage particles in 1 ml of the source material (i.e. - phage titer).

Phage indication

Species-specific phage is used to identify the species of bacteria: after seeding of the investigated bacterial species over the surface of agar plate to get bacterial lawn a drop of the solution containing species-specific phage are placed to stream down over



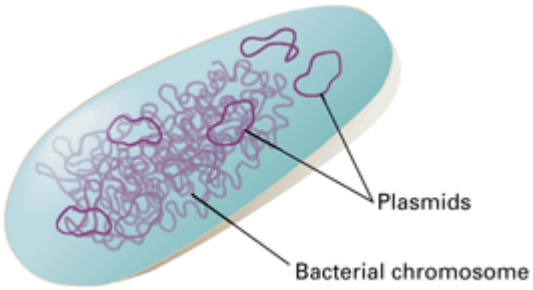
Bacteriophage enumeration by double agar layer method (https://www.waterpathogens.org/sites/default/files/figure2coliphage.png)

(https://slideplayer.com/slide/14061598/86/images/14/Phage+typing+is+most+often+used+for+the+two+common+salmonella+types%2C+S.jpg)

susceptible phage regions will show a circular clearing where the bacteria have been lysed, and this is used in differentiation.

Organization of genetic material in bacteria

All cellular activities are encoded within a cell's DNA.



Bacterial DNA (https://bodell.mtchs.org/OnlineBio/BIOCD/text/chapter13/13images/13-03.gif)

While the basic structure of DNA is the same, the organization of the DNA in bacterial cells is very different than in human or animal cells. Recall that while eukaryotic chromosomes are housed in the membrane-bound nucleus, prokaryotes contain a single chromosome that is found in an area of the cytoplasm called the nucleoid.

Chromosomes in bacteria are circular, and a prokaryotic cell contains only a single chromosome within the nucleoid. Since there is only one copy of the chromosome, bacterial cells are haploid. As in eukaryotic cells, DNA supercoiling is necessary for the genome to fit within the prokaryotic cell. As with eukaryotes, topoisomerases are involved in supercoiling DNA. DNA gyrase is a type of topoisomerase found in bacteria that helps prevent the over winding of DNA. Some antibiotics kill bacteria by targeting DNA gyrase.

Although most DNA is contained within a cell's chromosomes, bacteria have additional molecules of DNA outside the chromosomes, called extrachromosomal DNA (plasmids, transposons, IS-elements, temperate phages) that may contain one or a few genes not essential for normal growth.

Extrachromosomal factors of heredity and their integration into the nucleoid

Extrachromosomal DNA exists in prokaryotes outside the nucleoid region as plasmids, transposons, IS-elements and temperate phages. DNA molecules that replicate as discrete genetic units in bacteria are called replicons (or autonomous factors of heredity). The bacterial chromosome and plasmids are self-replicating genetic elements (replicons), transposons and IS-elements cannot replicate themselves so they are not be named as replicons (they are non-autonomous factors of heredity). In addition, plasmids and temperate phages can be inserted into the nucleoid only in homologous regions, in contrast to transposons and IS-elements that can be integrated into the nucleoid in any parts of latter.

Plasmids

Plasmids may replicate autonomously (i.e. outside the nucleoid, as independent plasmid molecules) or become integrated into the chromosome (the integrative plasmids). Plasmids carry out two possible functions in a bacterial cell: regulatory (compensate infringements of the function of DNA of the bacterial nucleoid) and coding (introduce new information into the genotype of bacteria). Plasmids can be broadly classified into conjugative plasmids and non-conjugative plasmids. Conjugative plasmids contain a set of transfer or tra genes which promote conjugation between different cells. In the complex process of conjugation, plasmids may be transferred from one bacterium to another via sex pili encoded by some of the tra genes (see below "Functions of tra-operon"). Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids.

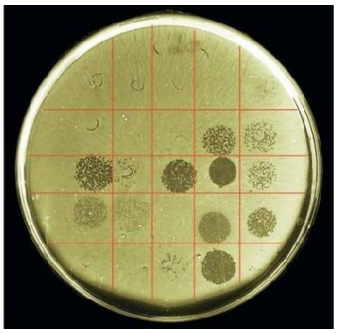
the bacterial lawn. After incubation the bacterial growth over the streaming down area is registered: if it is – the test result is negative, if it is not – a positive.

Phage typing

Phage typing is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. The phages which can only infect a single strain of bacteria are used to identify different strains of bacteria within a single species.

A culture of the strain is grown in the agar and dried. A grid is drawn on the base of the Petri dish to mark out different regions. Inoculation of each square of the grid is done by a different phage. The phage drops are allowed to dry and are incubated: The

Phage typing is most often used for the two common salmonella types, *S. Enteritidis* and *S. Typhimurium*. But phage typing systems also exist for some other *Salmonella* serotypes and a few other bacteria such as *Mycobacteria, Listeria, Staphylococci* and *Pseudomonas*.



Functions of tra-operon

Transfer operon, commonly called tra-operon, or tra genes, are some genes necessary for non-sexual transfer of genetic material in both gram-positive and gram-negative bacteria. The tra locus includes the pilin gene and regulatory genes, which together form pili on the cell surface, polymeric proteins that can attach themselves to the surface of F-bacteria and initiate the conjugation. Tra-operon can determine the process of one-way transfer of plasmids or a site of the nucleoid.

F-plasmids

The fertility factor (also called the sex factor or F-plasmid) allows genes to be transferred from one bacterium carrying the factor to another bacterium lacking the factor by conjugation.

The most common functional segments constituting F factors are:

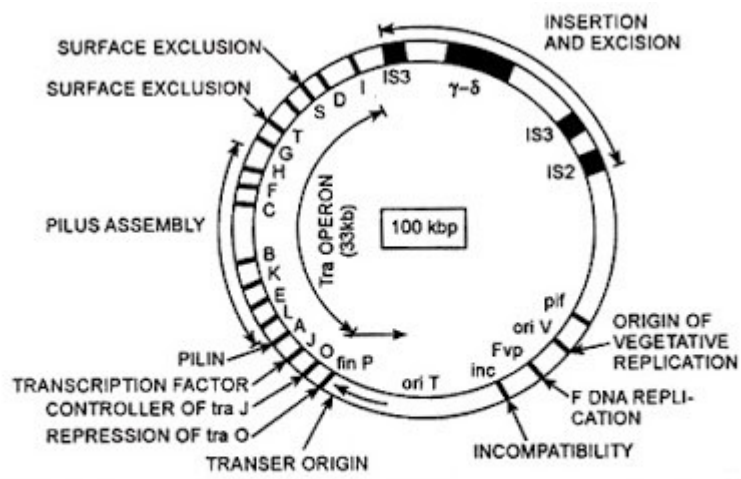
OriT (Origin of Transfer): the sequence which marks the starting point of conjugative transfer;

OriC (Origin of Replication): the sequence starting with which the plasmid-DNA will be replicated in the recipient cell;

tra-region (transfer genes): genes coding the F-Pilus and DNA transfer process;

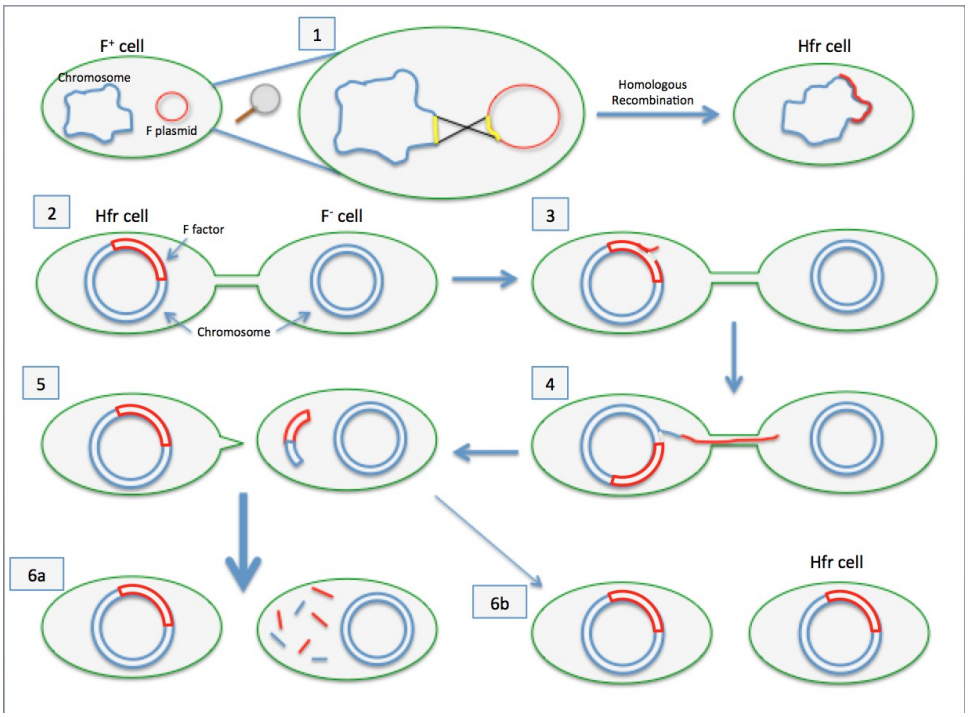
IS (Insertion Elements) composed of one copy of IS2, two copies of IS3, and one copy of IS1000: so-called "selfish genes" (sequence fragments which can integrate copies of themselves at different locations).

When an F+ cell conjugates/mates with an F− cell, the result is two F+ cells, both capable of transmitting the plasmid to other F− cells by conjugation. The tra operon includes genes required for conjugation and plasmid transfer. This means that an F+ bacteria can always act as a donor cell.



A map of *F* plasmid. The transfer operon (*tra* operon) has genes *A* to *T*. Insertion sequences *IS2*, *IS3*, *γ - δ* provide the sites of integration into the chromosome of various *Hfr* strains. The entry during conjugation with an *F'* cell is in the order of *ori T – inc - frp - ori V* etc., the *tra* operon entering at the end.

(https://lh3.googleusercontent.com/proxy/Zf1NeyyEqruu8TUKx8qZ3PuB28u-bOTb1me7-RhmNegqkaWkX_IuW2LLTJUtYky0W_zGbPUVysnipMZp5L1VxEUCKCYIeEnz6x2IrPJjFt4sh9OM7Gt4R271RDJFleHXptHMNlU9EQdpiO_nn0Opc)



1.The insertion sequences (yellow) on both the F factor plasmid and the chromosome have similar sequences, allowing the F factor to insert itself into the genome of the cell. This is called homologous recombination and creates an Hfr (high frequency of recombination) cell.
2.The Hfr cell forms sex pilli a pilus and attaches to a recipient F- cell.
3.A nick in one strand of the Hfr cell's chromosome is created.
4.DNA begins to be transferred from the Hfr cell to the recipient cell while the second strand of its chromosome is being replicated.
5.The pilus detaches from the recipient cell and retracts. The Hfr cell ideally wants to transfer its entire genome to the recipient cell. However, due to its large size and inability to keep in contact with the recipient cell, it is not able to do so.
6.The F- cell remains F- because the entire F factor sequence was not received. Since no homologous recombination occurred, the DNA that was transferred is degraded by enzymes. In very rare cases, the F factor will be completely transferred and the F- cell will become an Hfr cell.
(https://upload.wikimedia.org/wikipedia/commons/thumb/f/ff/Hfr_Recombination.png/220px-Hfr_Recombination.png)

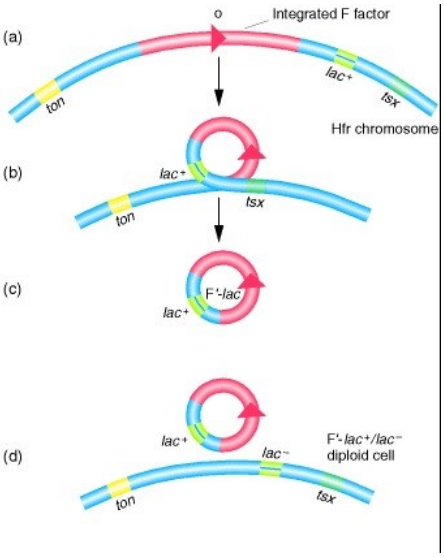
F+ cells are usually inhibited from making contact with other F+ cells; therefore the F plasmid is not transferred from F+- to F+-cell.

In the case of Hfr transfer, the resulting transconjugates are rarely Hfr. The result of Hfr/F− conjugation is a F− strain with a new genotype. When F-prime plasmids are transferred to a recipient bacterial cell, they carry pieces of the donor's DNA that can become important in recombination. Bioengineers have created F plasmids that can contain inserted foreign DNA; this is called "a bacterial artificial chromosome".

Variants of the location of F-plasmids in the bacterial cell

The episome that harbors the F factor can exist as an independent plasmid or integrate into the bacterial cell's genome. There are several names for the possible states:

Hfr bacteria possess the entire F epi-



Origin and reintegration of the F′ factor – in this case, F′ lac.
(a) F is inserted in an Hfr strain between the ton and lac+ alleles.
(b,c) Abnormal "outlooping" and separation of F occurs to include the lac locus, producing the F′-lac+ particle.
(d) An F lac+ / lac− partial diploid is produced by the transfer of the F′-lac particle to an F− lac− recipient.
From G. S. Stent and R. Calendar, Molecular Genetics, 2d ed. Copyright © 1978 by W. H. Freeman and Company, New York. (https://www.ncbi.nlm.nih.gov/books/NBK21351/bin/ch9f11.jpg)

some integrated into the bacterial genome;

F+ bacteria possess F factor as a plasmid independent of the bacterial genome – the F plasmid contains only F factor DNA and no DNA from the bacterial genome;

F' (F-prime) bacteria are formed by incorrect excision from the chromosome, resulting in F plasmid carrying bacterial sequences that are next to where the F episome has been inserted;

F− bacteria do not contain F factor and act as the recipients.

A high-frequency recombination cell (Hfr cell) (also called an Hfr strain) is a bacterium with a conjugative plasmid (for example, the F-factor) integrated into its chromosomal DNA. The integration of the plasmid into the cell's chromosome is through homologous recombination. A conjugative plasmid capable of chromosome integration is also called an episome (a segment of DNA that can exist as a plasmid or become integrated into the chromosome). When conjugation occurs, Hfr cells are very efficient in delivering chromosomal genes of the cell into recipient F− cells, which lack the episome.

Occasionally, the integrated F factor of an Hfr strain exits from the bacterial chromosome. Usually this event is a clean excision regenerating an intact F plasmid. However, in some cases, the excision event is not a precise reversal of the original insertion, and a part of the bacterial chromosome is incorporated into the liberated plasmid. Such plasmids carrying bacterial genes are called F′. If an F′ plasmid is transferred upon conjugation with an F− strain, the recipients generated are stable merozygotes, carrying a complete bacterial genome plus a donor fragment on the autonomously replicating plasmid. The process of creating a merozygote by an F′ element is called sexduction or F′-duction.

R-plasmids

Resistance (R) plasmids contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors. Often, R-factors code for more than one antibiotic resistance factor: genes that encode resistance to unrelated antibiotics may be carried on a single R-factor, sometimes up to 8 different resistances. It consists of two components: the resistance transfer factor (RTF) required for transfer of the plasmid between bacteria, and the r-determinants (genes conferring antibiotic resistance).

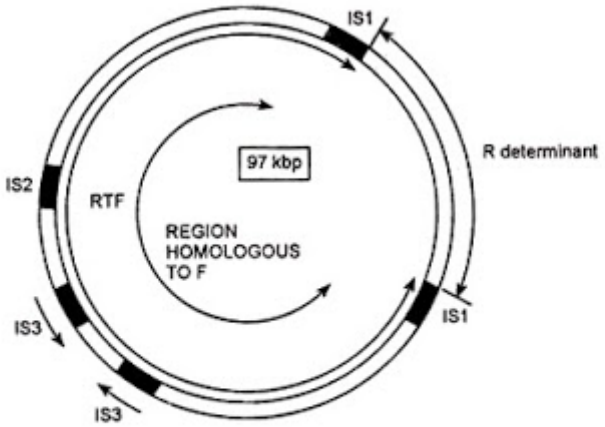


Diagram showing the map of an *R* factor (*R6*) that has 97 kbp. The *RTF* (resistance transfer factor) constitutes the major portion of the *R-RTF* molecule. The *RTF* region is largely homologous to *F* plasmid but it contains a *fin O* gene not found in *F* elements. The *RTF* has two *IS3* elements which are in reverse orientations. The *R* determinant is separated from *RTF* by *IS1* elements. *(https://lh3.googleusercontent.com/proxy/4GERC6GqsSkqjrq5ZxUGhgBFqLbzPOP4TFM_8GjNPuXOUem4UxKQvB9JvwhI3lN0hoUtt0I7fI_20a55uVNlz0p0JHLYyV3bpxfUDSc7TisXG0prDOT9SfXRw7WiCYX8fodiVMu99KshM6LmB5pv)*

The composition of r-operon

This operon contains genes that determine resistance to antibiotics, as well as its composition can include a transposon or its part – IS-elements.

Mechanisms of bacterial resistance to antibiotics due to the presence of R-plasmids

R-plasmid can determine: the ability of bacterial cells to inactivate the antibiotic, the ability of bacterial cells to modify the antibiotic with loss of the last of its antibacterial activity, the ability of bacterial cells to reduce the permeability of the cell wall to the antibiotic.

Plasmids of bacteriocynogenity (example with Col-plasmids of E.coli)

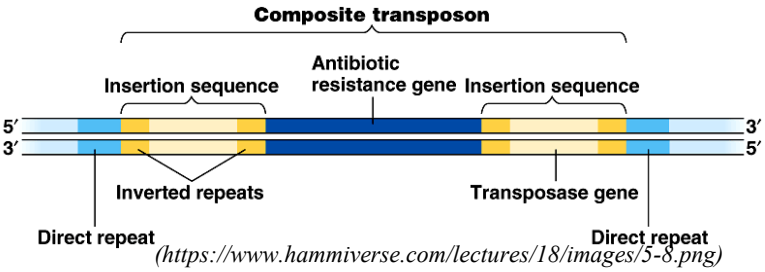
Plasmids of bacteriocynogenity contain genes that code for bacteriocins, proteins that can kill other bacteria. They are found in many types of bacteria. Bacteriocins are proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). The bacteriocins from *E. coli* are called colicins (formerly called "colicines", meaning "coli killers") and the plasmids which contain genes that code for colicins are named Col-plasmids.

Characteristics of colicins

Colicins are released into the environment to reduce competition from other bacterial strains. Colicins bind to outer membrane receptors, using them to translocate to the cytoplasm or cytoplasmic membrane, where they exert their cytotoxic effect, including depolarization of the cytoplasmic membrane, DNase activity, RNase activity, or inhibition of murein synthesis. Colicins kill bacterial cells, but do not lyse them.

Transposons

A transposon (transposable element, TE or "jumping gene") is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations. DNA transposons include IS-elements encoding the protein transposase, which they require for insertion and excision, genes that determine a trait (e.g., antibiotic resistance gene or genes) and the special terminal structures by which transposons can be identified (thanks to the "sticky ends" transposons can be closed into a ring). So, IS-elements determine the ability of transposons to change the place of localization in the DNA molecule, as well as "jump" from one DNA molecule to another one.



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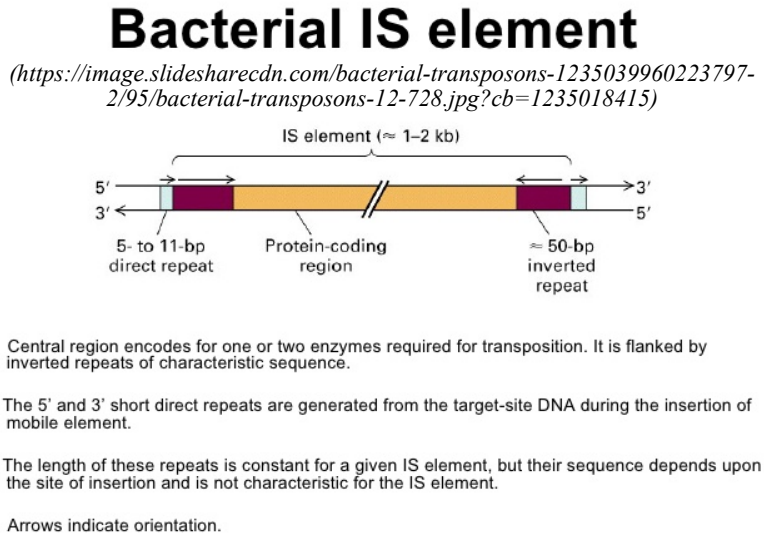
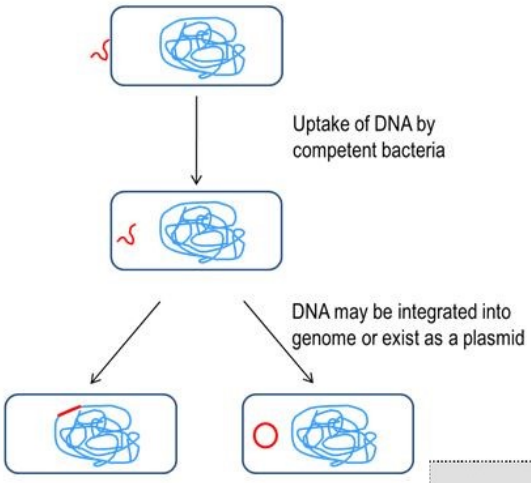


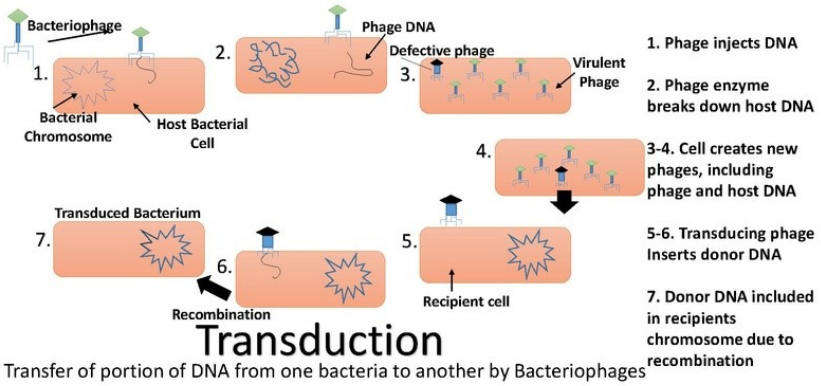
(https://image.slidesharecdn.com/bacterial-transposons-1235039960223797-2/95/bacterial-transposons-12-728.jpg?cb=1235018415)

IS-elements

Insertion element (also known as an IS, an insertion sequence element, or an IS element) is a short DNA sequence that acts as a simple transposable element. Insertion sequences have two major characteristics: they are small relative to other transposable elements (generally around 700 to 2500 bp in length) and only code for proteins implicated in the transposition activity (they are thus different from other transposons, which also carry accessory genes such as antibiotic resistance genes). These proteins are usually the transposase which catalyzes the enzymatic reaction allowing the IS to move, and also one regulatory protein which either stimulates or inhibits the transposition activity. The coding region in an insertion sequence is usually flanked by inverted repeats.

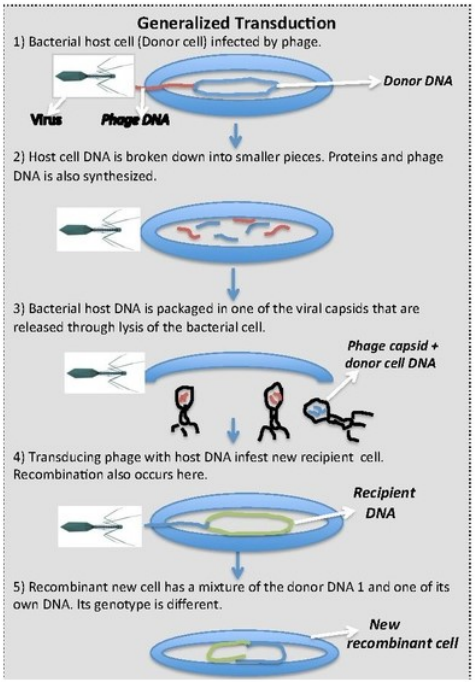


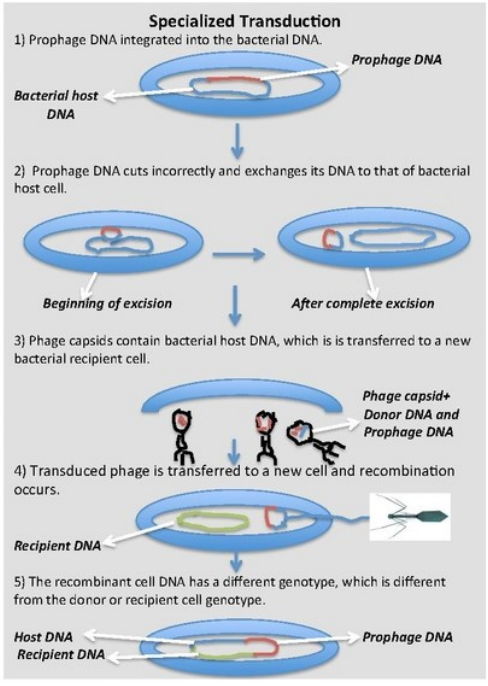
Transformation (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/articles/protocols/biology/transformation-of-bacteria.jpg)



(https://upload.wikimedia.org/wikipedia/commons/thumb/7/76/Transduction_image.pdf/page1-800px-Transduction_image.pdf.jpg)

This is an illustration of the difference between generalized transduction, which is the process of transferring any bacterial gene to a second bacterium through a bacteriophage and specialized transduction, which is the process of moving restricted bacterial genes to a recipient bacterium. While generalized transduction can occur randomly and more easily, specialized transduction depends on the location of the genes on the chromosome and the incorrect excision of the a prophage. (https://upload.wikimedia.org/wikipedia/commons/thumb/4/47/Transduction_illustration.pdf/page1-436px-Transduction_illustration.pdf.jpg)





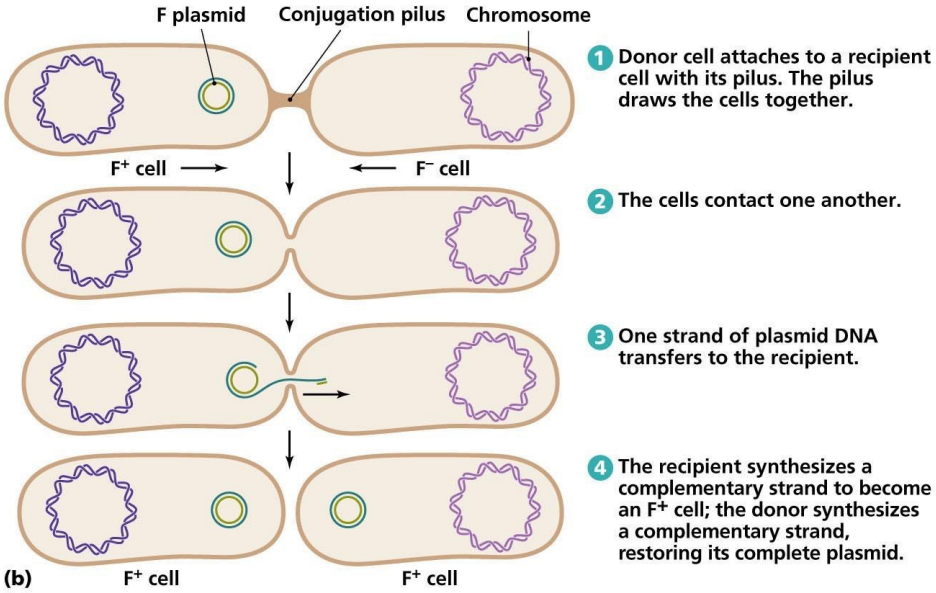
Modifications in bacteria

Phenotypic variations in bacteria are called modifications.

Mutations in bacteria

A mutation is a change in the nucleotide sequence and can create new cellular functionalities or lead to the dysfunction of others. Mutations can occur spontaneously or be caused by exposure to mutation-inducing agents (mutagens). Spontaneous mutations occur without mutation induction by mutagens. Most of them are the result of errors during DNA replication (errors of DNA polymerase). Some of them – insertion mutations (the addition of one or more nucleotide base pairs into a DNA sequence) – can be caused by extra-chromosomal hereditary factors during their integration in the nucleoid.

Induced mutations are alterations in the gene after it has come in contact with mutagens and environmental causes (e.g., during an experiment).



Conjugation
(https://i.pinimg.com/originals/e0/63/4e/e0634e13c9e6c4cf150ae16be66b5802.jpg)

SR-dissociations

SR-dissociation is a phenomenon when R-shaped colonies appear in pure culture forming S-shaped colonies. It is an insertion mutation resulted in the loss of the genes controlling synthesis of carbohydrate chains of LPS of the outer membrane of the cell wall.

Mutagens

Mutagen are physical or chemical agents that change the genetic material, usually DNA, of an organism and thus increase the frequency of mutations above the natural background level. Mutagens cause pre-mutative changes in DNA structure. These pre-mutative changes turn into mutation by repaired enzymes errors or by infringements in the proceeding of the reparation processes.

Reparations in bacteria

This term refers to the recovery of damaged DNA by enzyme repair systems of the bacterial cell.

Bacterial recombination

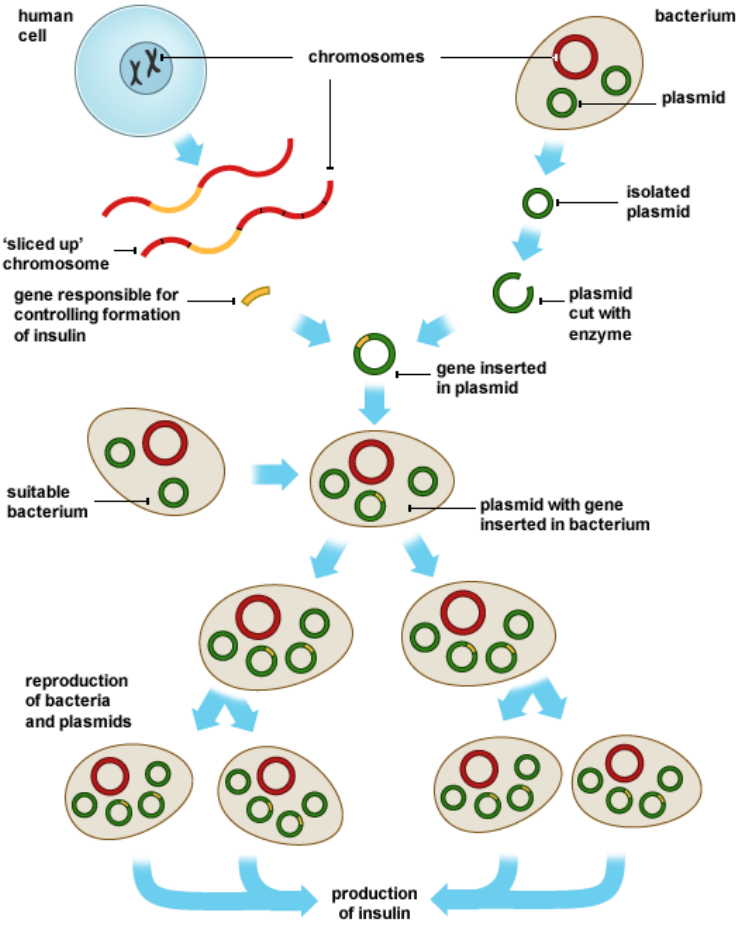
Bacterial recombination is a type of genetic recombination in bacteria characterized by DNA transfer from one organism called donor to another organism as recipient. The final result of bacterial recombination is the production of genetic recombinants, individuals that carry not only the genes they inherited from their parent cells but also the genes introduced to their genomes by the process of bacterial recombination.

This process occurs in five main ways:

- ⇒ transformation (direct transfer of genetic material from the donor to the recipient cell),
- ⇒ transduction (transfer of genetic material from the donor to the recipient cell with bacteriophages),
- ⇒ conjugation (transfer of genetic material from the donor to the recipient cell through conjugative pili),
- ⇒ lysogenization (if temperate phage genome inserts into the genome of the recipient cell),
- ⇒ phage conversion (different from lysogenization only that the recipient cell phenotype changes).

Genetic engineering in medical microbiology

Genetic engineering means the manipulation of organisms to make useful products and it has broad applications. In medicine, genetic engineering has been used to



Genetic engineering
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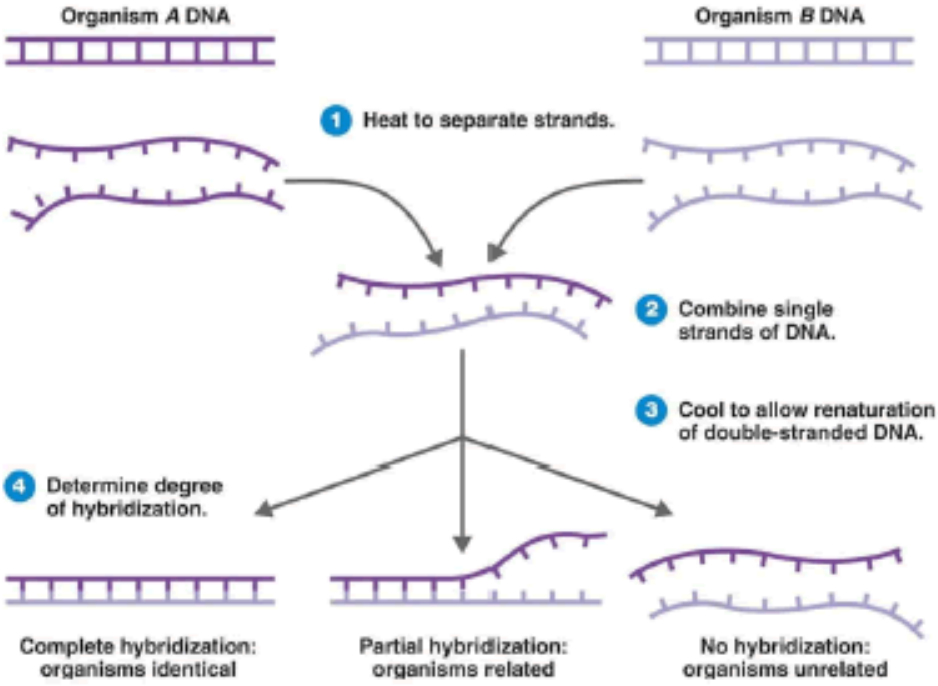


Figure 1: Digrammatic representation of hybridization technique for strain relatedness

(https://www.omicsonline.org/articles-images/molecular-genetic-medicine-hybridization-technique-strain-relatedness-08-142-g001.png)

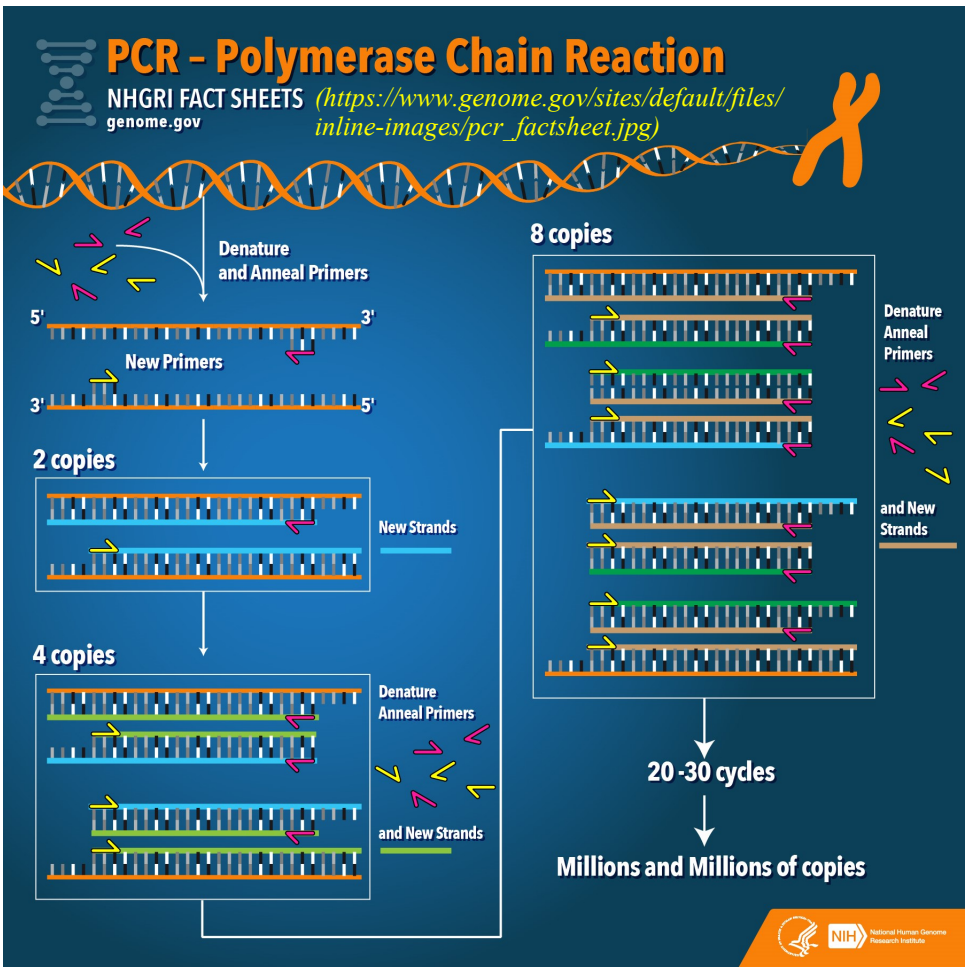
mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, anti-hemophilic factors, vaccines, and many other drugs. Industrial applications include transforming microorganisms such as bacteria or yeast with a gene coding for a useful protein. Mass quantities of the protein can be produced by growing the transformed organism in bioreactors using fermentation, then purifying the protein.

Methods of genetics applied in microbiological diagnostics

In modern medicine are becoming more common genetic methods for microbiological diagnosis: percentage of guanine and cytosine in the bacterial genome determination, method of molecular hybridization and especially – polymerase chain reaction (PCR).

Method of molecular hybridization

In molecular biology, hybridization (or hybridisation) is a phenomenon in which single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to complementary DNA or RNA. Though a double-stranded DNA sequence is generally stable under physiological conditions, changing these conditions in the laboratory (generally by raising the surrounding temperature) will cause the molecules to separate into single strands. These strands are complementary to each other but may also be complementary to other sequences present in their surroundings. Lowering the surrounding temperature allows the single-stranded molecules to



anneal or “hybridize” to each other.

DNA–DNA hybridization generally refers to a molecular biology technique that measures the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two organisms. This has been used extensively in phylogeny and taxonomy.

DNA–DNA hybridization was once used as a primary method to distinguish bacterial species; but it it's not always that easy to perform in routine laboratories.

Polymerase chain reaction

Polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" - copy - small segments of DNA. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification.

Often heralded as one of the most important scientific advances in molecular biology, PCR revolutionized the study of DNA to such an extent that its creator, Kary B. Mullis, was awarded the Nobel Prize for Chemistry in 1993.

Once amplified, the DNA produced by PCR can be used in many different laboratory procedures including detection of bacteria or viruses.

To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes – builds – two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment.

The entire cycling process of PCR is automated and can be completed in just a few hours. It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.

So, the principle of PCR is to increase the amount of the target gene (its amplification) at the positive reaction (if this gene was in the test material) that is detected by electrophoresis.

Training algorithm of practical skills to be mastered at the lesson

There are no new practical skills to be mastered at this lesson.

Lesson 6

Definition of the term "ecology of microorganisms" (microbial ecology, environmental microbiology)

- Environmental Microbiology
  - Study of microbes in their natural habitats
  - Microbial Diversity – study of the different types of microbes in an environment
- Microbial Ecology
  - Studies the interactions between microbes & their environments
  - Involving biotic & abiotic components
  - Distribution
  - Abundance – numbers of bacteria

(https://image.slideserve.com/1174938/slide2-l.jpg)

Ecology (from Greek: οἶκος, "house", or "environment"; -λογία, "study of") is a branch of biology concerning interactions among organisms and their biophysical environment, which includes both biotic and abiotic components. Topics of interest include the biodiversity, distribution, biomass, and populations of organisms, as well as cooperation and competition within and between species.

Microbial ecology (or environmental microbiology) is the ecology of microorganisms: their relationship with one another and with their environment (including the built environment and human organism). In other words environmental microbiology is the study of microbial processes in the environment, microbial communities and microbial interactions. This includes: structure and activities of microbial communities, microbial interactions and interactions with macroorganisms, population biology of microorganisms, microbes and surfaces (adhesion and biofilm formation), microbial community genetics and evolutionary processes, (global) element cycles and biogeochemical processes, microbial life in extreme and unusual little-explored environments.

Basic concepts of microbial ecology (environmental microbiology)

A **population** is all the microorganisms of the same species, which live in a particular biotope.

A **biotope** is an area of uniform environmental conditions providing a living place for a specific assemblage of organisms. Biotope is almost synonymous with the term **habitat**, which is more commonly used in English-speaking countries. However, in some countries these two terms are distinguished: the subject of a habitat is a population, the subject of a biotope is a biocoenosis or biological community.

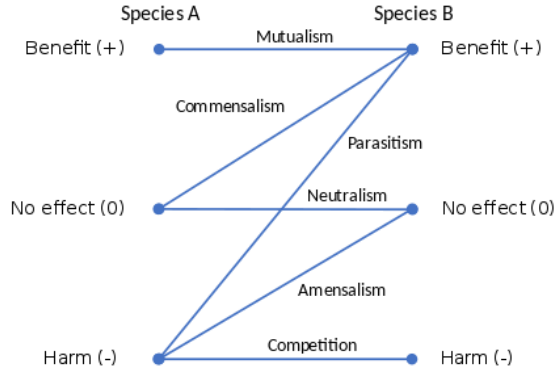
A biocenosis (UK English, biocoenosis, also biocenose, biocoenose, biotic community, biological community, ecological community, life assemblage,) describes the interacting organisms living together in a habitat (biotope). Based on the concept of biocenosis, ecological communities can take in various forms: for the microbial community the term "**microbiocenosis**" is used. This term describes the interacting microorganisms living together in a habitat (biotope).

In ecological microbiology an **ecosystem** is a community of microorganisms in conjunction with the living and nonliving components of their environment (a biotope), interacting as a system.

Ecological interactions in microbiocenoses

In ecology, a biological interaction is the effect that a pair of organisms living together in a community have on each other. They can be either of the same species (intraspecific interactions), or of different species (interspecific interactions). Species of microorganisms or separate cells of microorganisms inside a species interact in various ways. These effects may be short-term, like predation (when one organism, the predator, kills and eats another organism, its prey), or long-term. A long-term interaction is called a symbiosis.

The six possible types of symbiosis are mutualism, commensalism, parasitism, neutralism, amensalism, and competition. These are distinguished by the degree of benefit or harm they cause to each partner.



The six possible types of symbiotic relationship, from mutual benefit to mutual harm (https://upload.wikimedia.org/wikipedia/commons/thumb/6/64/Symbiotic_relationships_diagram.svg/440px-Symbiotic_relationships_diagram.svg.png)

Mutualism is an interaction between two or more species, where species derive a mutual benefit. Similar interactions within a species are known as co-operation. Mutualism may be classified in terms of the closeness of association, the closest being symbiosis, which is often confused with mutualism. One or both species involved in the interaction may be obligate, meaning they cannot survive in the short or long term without the other species.

Commensalism benefits one organism and the other organism is neither benefited nor harmed. Numerous genera of bacteria and fungi live on and in the human body as part of its natural flora. The fungal genus *Aspergillus* is capable of living under considerable environmental stress, and thus is capable of colonizing the upper gastrointestinal tract where relatively few examples of the body's gut flora can survive due to highly acidic or alkaline conditions produced by gastric acid and digestive juices. While *Aspergillus* normally produces no symptoms, in individuals who are immunocompromised or suffering from existing conditions such as tuberculosis, a condition called aspergillosis can occur, in which populations of *Aspergillus* grow out of control. *Staphylococcus aureus*, a common bacterial species, is known best for its numerous pathogenic strains that can cause numerous illnesses and conditions. However, many strains of *S. aureus* are metabiotic commensals, and are present on roughly 20 to 30% of the human population as part of the skin flora. *S. aureus* also benefits from the variable ambient conditions created by the body's mucous membranes, and as such can be found in the oral and nasal cavities, as well as inside the ear canal. Other *Staphylococcus* species including *S. epidermidis*, will also engage in commensalism for similar purposes.

Parasitism is a relationship between species, where one organism, the parasite, lives on or in another organism, the host, causing it some harm, and is adapted structurally to this way of life. The parasite either feeds on the host, or, in the case of intestinal parasites, consumes some of its food. Many bacteria are parasitic, though they are more generally

thought of as pathogens causing disease. Parasitic bacteria are extremely diverse, and infect their hosts by a variety of routes. Viruses are obligate intracellular parasites, characterized by extremely limited biological function, to the point where, while they are evidently able to infect all other organisms from bacteria and archaea to animals, plants and fungi, it is unclear whether they can themselves be described as living. They lack all the usual machinery of the cell such as enzymes, relying entirely on the host cell's ability to replicate DNA and synthesize proteins.

Neutralism describes the relationship between two species that interact but do not affect each other. Examples of true neutralism are virtually impossible to prove; the term is in practice used to describe situations where interactions are negligible or insignificant.

Amensalism is an interaction where an organism inflicts harm to another organism without any costs or benefits received by itself. A clear case of amensalism is where sheep or cattle trample grass. Whilst the presence of the grass causes negligible detrimental effects to the animal's hoof, the grass suffers from being crushed. Amensalism is often used to describe strongly asymmetrical competitive interactions.

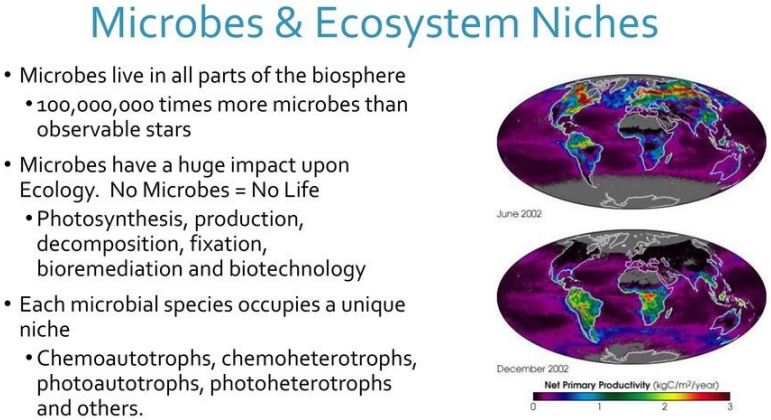
Competition can be defined as an interaction between organisms or species, in which the fitness of one is lowered by the presence of another. Competition is often for a resource such as food and water. Competition among members of the same species is known as intraspecific competition (when members of the same species compete for the same resources in an ecosystem), while competition between individuals of different species is known as interspecific competition. According to the competitive exclusion principle, species less suited to compete for resources should either adapt or die out. According to evolutionary theory, this competition within and between species for resources plays a critical role in natural selection.

Ecological niches of microorganisms

Every ecosystem on Earth contains microorganisms that occupy unique niches based on their specific metabolic properties.

Microbial life is amazingly diverse and microorganisms quite literally cover the planet. In fact, it has been estimated that there are 100,000,000 times more microbial cells on the planet than there are stars in the observable universe! Microbes live in all parts of the biosphere where there is liquid water, including soil, hot springs, the ocean floor, acid lakes, deserts, geysers, rocks, and even the mammalian gut.

Each species in an ecosystem is thought to occupy a separate, unique niche. The ecological niche of a microorganism describes how it responds to the distribution of resources and competing species, as well as the ways in which it alters those same factors in turn. In essence, the niche is a complex description of the ways in which a microbial species uses its environment.



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Microorganisms are found on practically every habitable square inch of the planet. They live and thrive in all parts of the biosphere where there is liquid water, including hostile environments such as the poles, deserts, geysers, rocks, and the deep sea. Additionally, while microbes are often free-living, many have intimate symbiotic relationships with other larger organisms. Clearly, microbes have adapted to extreme and intolerant conditions, and it is this adaptation that has yielded tremendous biological diversity among microorganisms.

Bacteria and fungi play key roles in maintaining a healthy **soil**. They act as decomposers that break down organic materials to produce detritus and other breakdown products. Soil detritivores, like earthworms, ingest detritus and decompose it. Saprotrophs, well represented by fungi and bacteria, extract soluble nutrients from detritus. Many more microorganisms exist in topsoil, where food sources are plentiful, than in subsoil. They are especially abundant in the area immediately next to plant roots (called the rhizosphere), where sloughed-off cells and chemicals released by roots provide ready food sources. These organisms are primary decomposers of organic matter, but they do other things, such as provide nitrogen through fixation to help growing plants, detoxify harmful chemicals (toxins), suppress disease organisms, and produce products that might stimulate plant growth. Soil microorganisms have had another direct importance for humans—they are the source of most of the antibiotic medicines we use to fight diseases.

There are many types of **watery environments** ranging from freshwater ponds, streams, puddles, lakes, rivers, and swamps to salty seas with three times the salt concentration of the ocean. Microbes live in overgrown slime, on pipes and in open oceans with few nutrients to support microbial life. Microbes thrive in streams with lots of oxygen to murky bogs that have no oxygen. In ponds there is a rich thriving ecosystem of microbial life including green and purple bacteria and algae, sulfate reducers, methane producers, and others. Many microbes live in the bottom of lakes and rivers in sediments. Many microbes cannot survive except in the presence of high concentrations of salt. The largest watery place on earth is the ocean. Oceans cover 71% of the Earth's surface and are responsible for producing about half of the world's organisms, which includes the plants, animals, fungi, and microbes. Most life in the oceans lives at the sunlit ocean surface. Below 25 meters there is little light in the ocean, and life productivity decreases. As well as little light, deeper waters are cooler, which supports less life. Below 50 meters, the temperature is less than 10 degrees Celsius.

Bacteria have no active mechanisms for becoming airborne. They are dispersed on dust particles disturbed by physical agencies, in minute droplets of water generated by any process which leads to the formation of an aerosol. **Air** is mainly transport medium for microorganisms. They occur in small numbers in air when compared with soil or water.

The total bacteria count is one of the key indicators in the field of hygiene management. It indicates how many microorganisms are present in a sample. Monitoring the total bacteria count is necessary, because the number of microorgan-

isms shouldn't exceed certain guide values. These guide values are expressed in CFU (colony-forming units) per gram or milliliter. A colony-forming unit (CFU, cfu, Cfu) is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Viable is defined as the ability to multiply via binary fission under the controlled conditions. Counting with colony-forming units requires culturing the microbes and counts only viable cells, in contrast with microscopic examination which counts all cells, living or dead. The visual appearance of a colony in a cell culture requires significant growth, and when counting colonies it is uncertain if the colony arose from one cell or a group of cells. Expressing results as colony-forming units reflects this uncertainty.

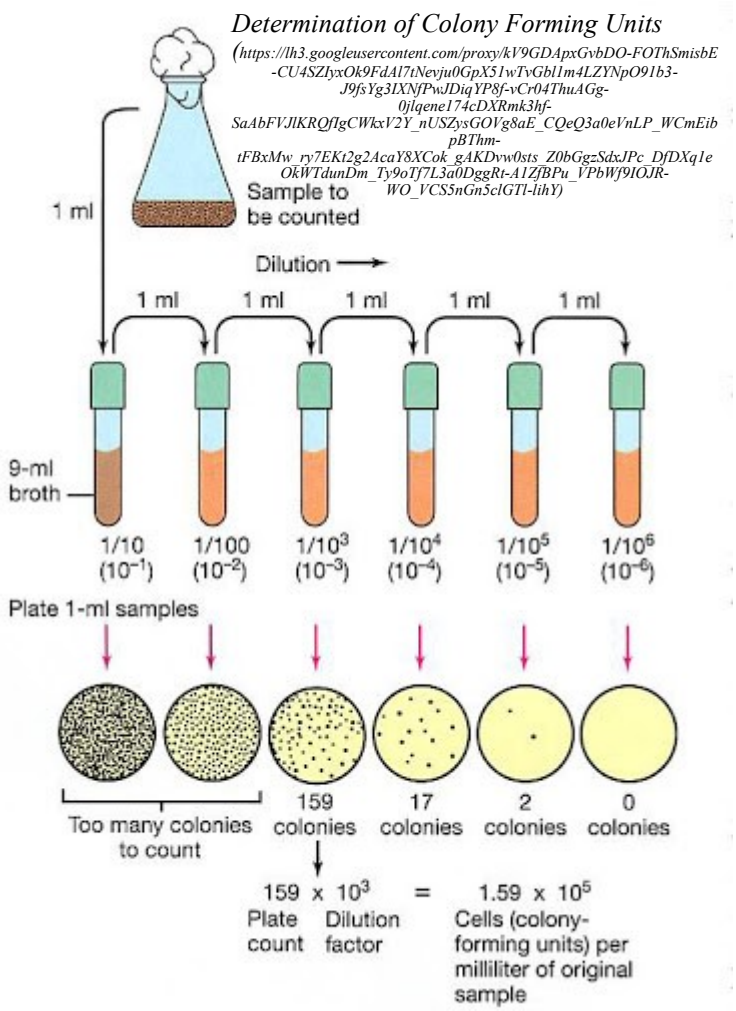
Indicator bacteria are types of bacteria used to detect and estimate the level of fecal contamination of soil, food and water. They are not dangerous to human health but are used to indicate the presence of a health risk: their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites.

Commonly used indicator bacteria include total coliforms, or a subset of this group, fecal coliforms, which are found in the intestinal tracts of warm blooded animals. The **coliform index** is a rating of the purity of water, soil or food based on a count of fecal bacteria.

Escherichia coli (*E. coli*) and enterococci are also used as indicators. *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination. **Coli-titer** is the smallest amount of investigated sample where present 1 CFU of *E. coli*. **Coli-index** is the amount of *E. coli* in 1 volume unit of investigated sample.

Determination of Colony Forming Units

(https://lh3.googleusercontent.com/proxy/kV9GDApxGvbDO-FOThSmisbE-CU4SZIyxOk9FdAl7tNevju0GpX51wTvGbl1m4LZYNpO91b3-J9fsYg3lXNfPwJDiqYP8f-vCr04ThuAGg-0jlqene174cDXRmk3hf-SaAbFVJlKRQfIgCWkxV2Y_nUSZysGOVg8aE_CQeQ3a0eVnLP_WCmEibpBThm-tFBxMw_ry7EKt2g2AcaY8XCok_gAKDvw0sts_Z0bGgzSdxJPc_DfDXq1eOkWTdunDm_Ty9oTf7L3a0DggRt-A1ZfBPu_VPbWf9IOJR-WO_VCS5nGn5clGTl-lihY)



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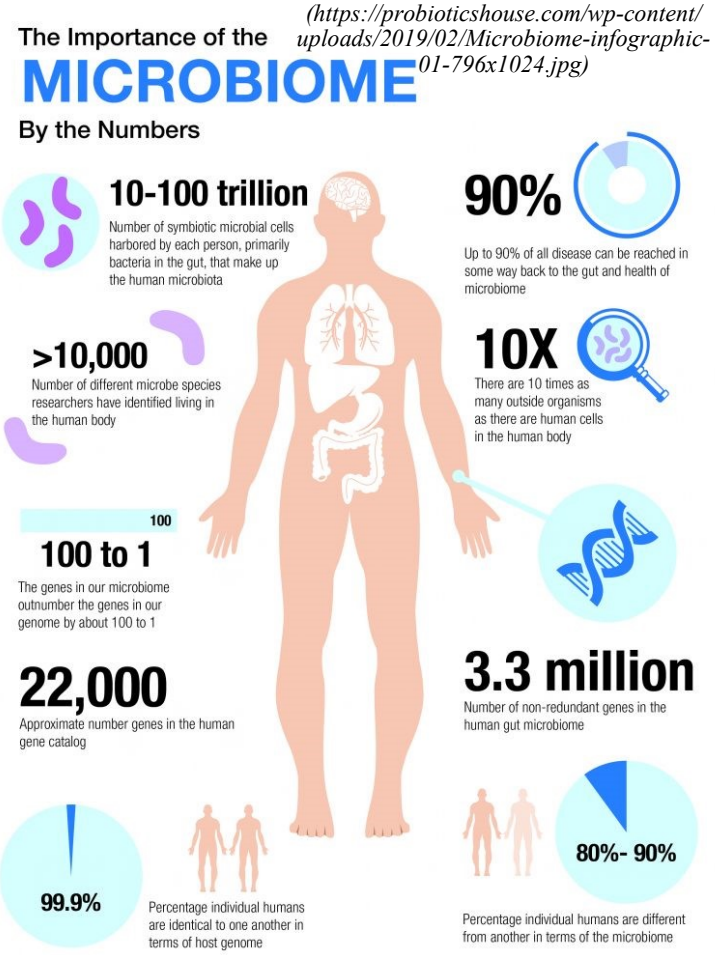


Microbiological aspects of environmental protection

Environmental protection has, among others, and microbiological aspects of protecting some microbes, fight with others, as well as protecting the biosphere from getting into it certain microorganisms.

General characteristics of the microflora of human body

In microbiology, collective bacteria and other microorganisms in a host are known as flora. Although microflora is commonly used, the term microbiota is becoming more common as microflora is a misnomer (flora pertains to the kingdom *Plantae*).

The human microbiome is the aggregate of all microbiota that reside on or within human tissues and biofluids along with the corresponding anatomical sites in which they reside. Types of human microbiota include bacteria, archaea, fungi, protists and viruses. Though microanimals can also live on the human body, they are typically excluded from this definition. In the context of genomics, the term human microbiome is sometimes used to refer to the collective genomes of resident microorganisms; however, the term human metagenome has the same meaning.

Microflora are grouped into two categories based on the origin of the microorganism: au-

tochthonous flora (bacteria and microorganisms native to the host environment) and allochthonous flora (temporary microorganisms non-native to the host environment).

Microflora are a community of bacteria that exist on or inside the body, and possess a unique ecological relationship with the host. This relationship encompasses a wide variety of microorganisms and the interactions between microbes. These interactions are often a mutualistic relationships between the host and autochthonous flora. Microflora responsible for harmful diseases are often allochthonous flora.

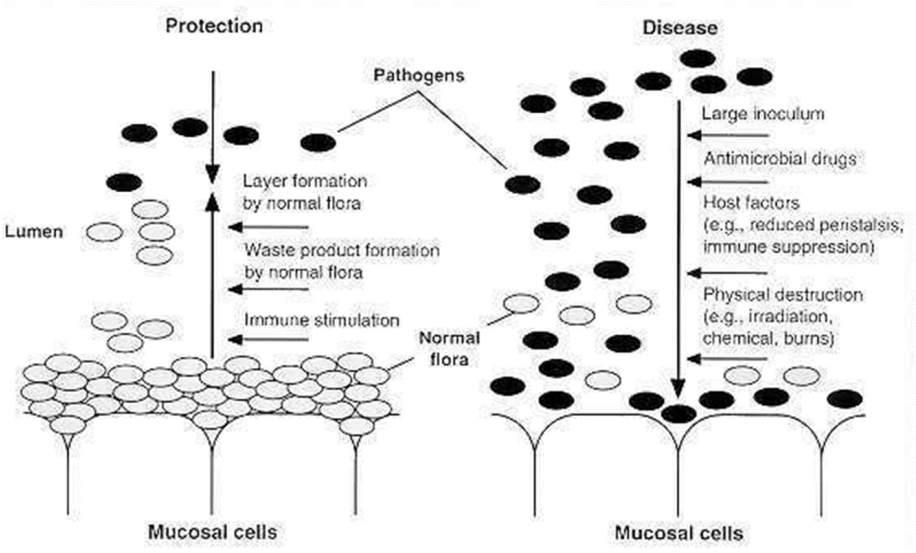
The role of normal microflora in human organism

The adult human body contains 10^{14} cells, of which only 10% compose the body proper and 90% are accounted for by members of the microflora.

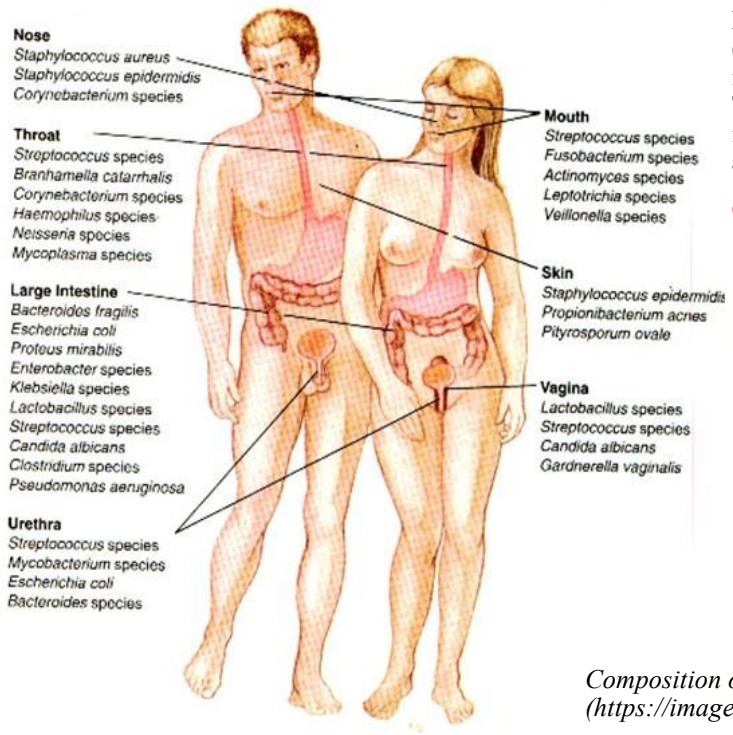
The functions of the normal flora include digestion of substrates, production of vitamins, stimulation of cell maturation, stimulation of the immune system, aid in intestinal transit and colonization resistance.

The fact that the normal flora substantially influences the well-being of the host was not well understood until germ-free animals became available. Germ-free animals were obtained by cesarean section and maintained in special isolators; this allowed the investigator to raise them in an environment free from detectable viruses, bacteria, and other organisms. Two interesting observations were made about animals raised under germ-free conditions. First, the germ-free animals lived almost twice as long as their conventionally maintained counterparts, and second, the major causes of death were different in the two groups. Infection often caused death in conventional animals, but intestinal atonia frequently killed germ-free animals. Other investigations showed that germ-free animals have anatomic, physiologic, and immunologic features not shared with conventional animals. For example, in germ-free animals, the alimentary lamina propria is underdeveloped, little or no immunoglobulin is present in sera or secretions, intestinal motility is reduced, and the intestinal epithelial cell renewal rate is approximately one-half that of normal animals (4 rather than 2 days).

Although the foregoing indicates that bacterial flora may be undesirable, studies with antibiotic treated animals suggest that the flora protects individuals from pathogens. Investigators have used streptomycin to reduce the normal flora and have then infected animals with streptomycin-resistant *Salmonella.* Normally, about 10^6 organisms are needed to establish a gastrointestinal infection, but in streptomycin-treated animals whose flora is altered, fewer than 10 organisms were needed to cause infectious disease.



Mechanisms by which the normal flora competes with invading pathogens
(https://www.ncbi.nlm.nih.gov/books/NBK7617/bin/ch6f2.jpg)



Composition of the microflora of human body
(https://images.slideplayer.com/24/7042854/slides/slide_38.jpg)

Further studies suggested that fermentation products (acetic and butyric acids) produced by the normal flora inhibited *Salmonella* growth in the gastrointestinal tract. The figure overhead shows some of the factors that are important in the competition between the normal flora and bacterial pathogens.

Composition of the micro-flora of human body

In number organs the presence of microorganisms is a norm: skin (is populated in the greatest quantity by staphylococci and *Candida*), oral cavity (one of the "most dirty" places in the human body, there are more than 100 different species of microbes), stomach (micro-flora presents, but is relatively poor – because of the acid in gastric contents), intestines from the point of view, both the number of its microorganisms and their role in the life of the human being, is the leading body biotope (bifidobacteria, lactobacilli and bacteroides predominate; *E. coli* and enterococci are founded in high quantities; in minor quantities in the gut

contains other representatives of enterobacteria, clostridia, staphylococci, fungi *Candida*), airways (are mainly inhabited by streptococci, staphylococci, and *Corynebacterium*), conjunctiva has very few microorganisms because tear fluid contains lysozyme which has antibacterial activity, urethra contains bacteria mainly in its lower third, vaginal flora is dominated by lactic acid bacilli (lactobacilli). Other organs of the human body (middle and inner ear, bladder, ureters, kidneys, uterus, etc.) are aseptic.

Disturbances in composition of normal micro-flora and approaches to its normalization

Dysbiosis (also called dysbacteriosis) is a term for a microbial imbalance or maladaptation on or inside the body, such as an impaired microbiota. For example, a part of the human microbiota, such as the skin flora, gut flora, or vaginal flora, can become deranged, with normally dominating species underrepresented and normally outcompeted or contained species increasing to fill the void. Dysbiosis is most commonly reported as a condition in the gastrointestinal tract, particularly during small intestinal bacterial overgrowth (SIBO) or small intestinal fungal overgrowth (SIFO).

Dysbiosis may be caused by such diverse things as antibiotic exposure, alcohol misuse, or inappropriate diet. Disruptions in the microbiome can allow outside factors or even pathogenic members of the microbiome to take hold in the gut environment. Dysbiosis can be associated with illnesses, such as periodontal disease, inflammatory bowel disease, chronic fatigue syndrome, obesity, cancer, bacterial vaginosis, and colitis.

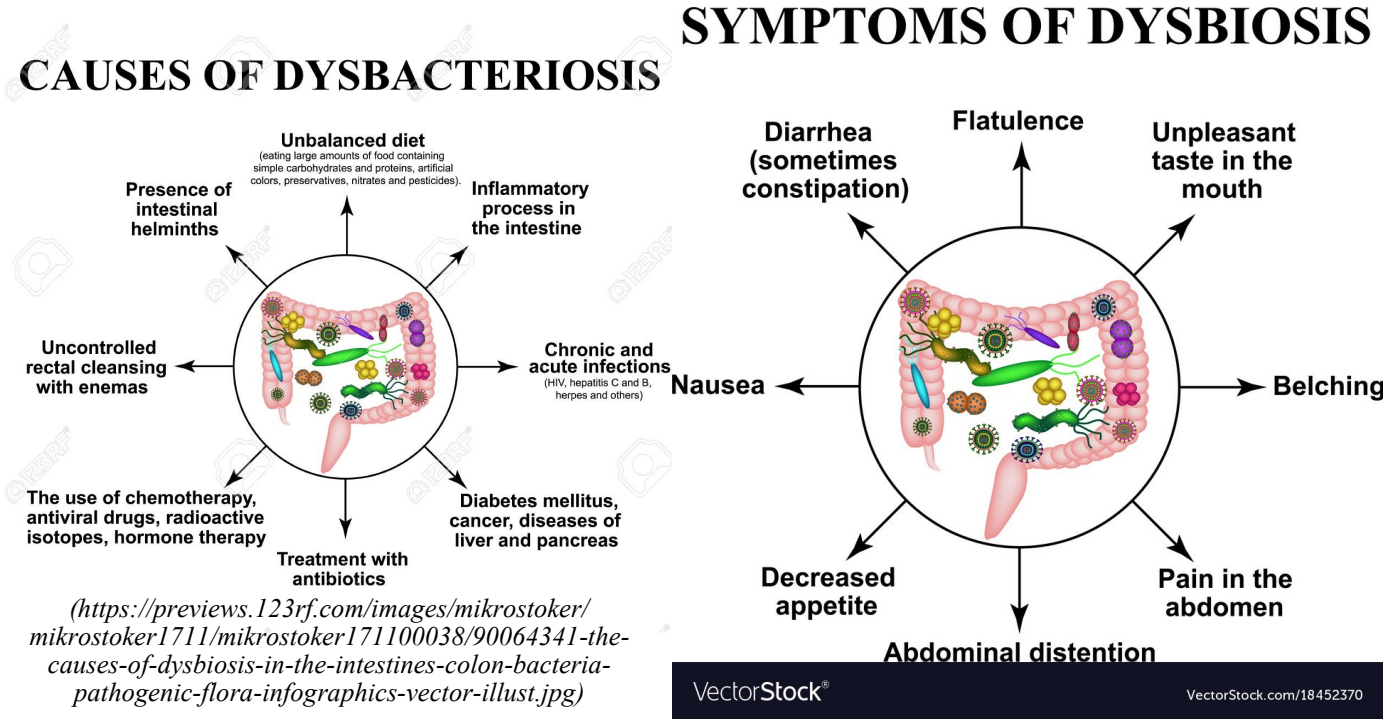
For dysbiosis treatments prebiotics and probiotics are usually used.

Prebiotics are compounds in food that induce the growth or activity of beneficial microorganisms such as bacteria and fungi. The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

The World Health Organization defines probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". The benefit of using probiotics to treat dysbiosis related diseases lies in its ability to treat the underlying cause of said diseases. Some benefits include their ability to suppress inflammation in the microbiome and disrupt colonization by pathogens.

Using prebiotics and probiotics in combination may be described as synbiotic, but the United Nations Food & Agriculture Organization recommends that the term "synbiotic" be used only if the net health benefit is synergistic.

Fecal microbiota transplants (FMT) use the same line of reasoning as probiotics; to recreate a healthy balance of microbiota in the microbiome by inserting beneficial microbes into the environment. FMT accomplishes this by taking a donation of fecal matter from a healthy individual, diluted, strained and introduced to a diseased patient. FMTs are currently used to treat patients with Clostridium difficile infections, who have proved resistant to other therapies. Because the process is not sterile and contaminations can pass from donor to patient, there is a push to isolate key microbiota and culture them independently.



(https://previews.123rf.com/images/mikrostoker/mikrostoker1711/mikrostoker171100038/90064341-the-causes-of-dysbiosis-in-the-intestines-colon-bacteria-pathogenic-flora-infographics-vector-illust.jpg)

Influence of physical environmental factors on microorganisms

Temperature is the most important factor that determines the rate of growth, multiplication, survival, and death of all living organisms. High temperatures damage microbes by denaturing enzymes, transport carriers, and other proteins. Microbial membrane are disrupted by temperature extremes. At very low temperatures membranes also solidify and enzymes also do not function properly. Microbes live in all sorts of environments around the world and have adapted to survival in harsh environments. A mesophile is an organism that grows at temperatures between 20°C and 45°C. A psychrophile is an organism that grows best at temperatures below 20°C. An organism that lives in extreme heat is a thermophile, which grows best between 50°C and 60°C, while hyperthermophiles grow at hot temperatures of up to 120°C.

Microbial growth is strongly affected by the pH of the medium. Drastic variations in cytoplasmic pH disrupt the plasma membrane or inhibit the activity of enzymes and membrane transport proteins. An organism that grows in acidic (low pH of usually 2 or below) conditions is called an acidophile. An organism that grows in basic (high pH of usually 8.5–11) conditions is called an alkaliphile. An organism that grows in neutral pH (between 6.5 and 7.5) conditions is called

a neutrophile. Most microbes grow in neutral pH conditions of around pH 7.0, but a number of bacteria can grow in acidic or basic conditions.

Drying (desiccation) causes dehydration (loss of the water) of the cytoplasm with violation of enzyme activity as a consequence, damages the cytoplasm membrane and the ribosomes.

Ultraviolet causes mutations in the genome due to the formation of thymine dimmers.

Ultrasound causes mechanical damage, both of the microbial cell and its intracellular structures.

Influence of chemical environmental factors on microorganisms

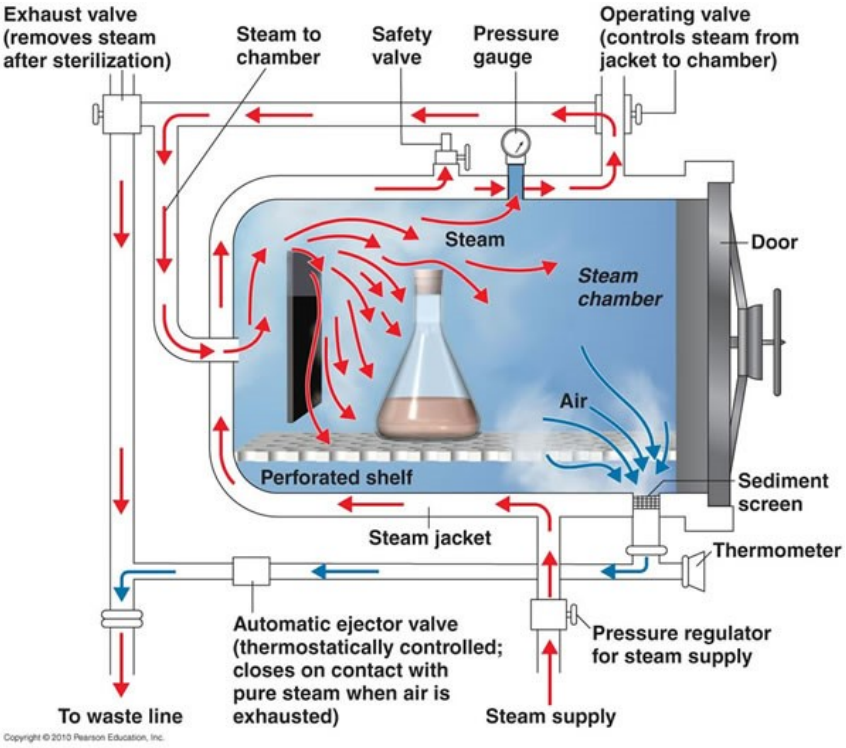
The antimicrobial action of chemical substances is reduced to three main effects: denaturation of the protein and solubilization of the lipid components of cytoplasm membrane (e.g., alcohol), damage of structure and disturbance of the function of the cytoplasm membrane (detergents, which include fatty acids, soaps, organic polymers with a length of the carbohydrate chain of 8 to 12 atoms), protein denaturation (practically all other antiseptics and disinfectants).

Definition and types of microbial decontamination

Microbial decontamination is a process of complete or partial removal of microorganisms from unanimated objects of surroundings (sterilization, disinfection) or from the human organism (antisepsis, chemotherapy) with use of the factors causing direct damage of microorganisms.

Sterilization

Sterilization refers to any process that eliminates, removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents like prions present in a specific surface, object or fluid, for example food or biological culture media. Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic.



Moist heat sterilization
(https://ouo1927.files.wordpress.com/2015/04/figure_07_02_labeled.jpg)

Moist heat sterilization describes sterilization techniques that uses hot air that is heavily laden with water vapor and where this moisture plays the most important role in the sterilization. The various procedures used to perform moist heat sterilization process cause destruction of micro-organisms by denaturation of macromolecules. A widely used method for heat sterilization is the autoclave, sometimes called a converter or steam sterilizer. Autoclaves use steam heated to 121–134 °C under pressure. To achieve sterility, the article is placed in a chamber and heated by injected steam until the article reaches a temperature and time setpoint. Almost all the air is removed from the chamber, because air is undesired in the moist heat sterilization process (this is one trait that differs from a typical pressure cooker used for food cooking). The article is held at the temperature setpoint for a period of time which varies depending on what bioburden is present on the article being sterilized and its resistance to steam sterilization. Following sterilization, liquids in a pressurized autoclave must be cooled slowly to avoid boiling over when the pressure is released. This may be achieved by gradually depressurizing the sterilization chamber and allowing liquids to evaporate under a negative pressure, while cooling the contents.

Dry heat sterilizer
(https://image.made-in-china.com/2f0j00YRlGTPHtBsqU/Biobase-China-70L-Heating-up-Fast-Hot-Air-Dry-Heat-Sterilizer-for-Clinics.jpg)

Dry heat was the first method of sterilization and is a longer process than moist heat sterilization. At higher temperatures, shorter exposure times are required to kill organisms. The standard setting for a hot air oven is at least two hours at 160 °C. Dry

heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (e.g. it does not cause rusting of steel objects).

Flaming is done to inoculation loops and straight-wires in microbiology labs for streaking. Leaving the loop in the flame of a Bunsen burner or alcohol burner until it glows red ensures that any infectious agent is inactivated. This is commonly used for small metal or glass objects, but not for large objects.

Incineration is a waste treatment process that involves the combustion of organic substances contained in waste materials. This method also burns any organism to ash. It is used to sterilize medical and other biohazardous waste before it is discarded with non-hazardous waste. Bacteria incinerators are mini furnaces that incinerate and kill off any microorganisms that may be on an inoculating loop or wire.

Chemicals are also used for sterilization. Heating provides a reliable way to rid objects of all transmissible agents, but it is not always appropriate if it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. In these situations chemicals, either in a gaseous or liquid form, can be used as sterilants. While the use of gas and liquid chemical sterilants avoids the problem of heat damage, users must ensure that the article to be sterilized is chemically compatible with the sterilant being used.

Ethylene oxide (EO, EtO) gas treatment is one of the common methods used to sterilize, pasteurize, or disinfect items because of its wide range of material compatibility. It is also used to process items that are sensitive to processing with other methods, such as radiation (gamma, electron beam, X-ray), heat (moist or dry), or other chemicals. Ethylene oxide treatment is the most common chemical sterilization method, used for approximately 70% of total sterilizations, and for over 50% of all disposable medical devices.

Chemical sterilization can be also implemented by using nitrogen dioxide (NO_2), ozone, glutaraldehyde, formaldehyde, hydrogen peroxide and peracetic acid.

Sterilization can be achieved using electromagnetic radiation, such as electron beams, X-rays, gamma rays, or irradiation by subatomic particles. Electromagnetic or particulate radiation can be energetic enough to ionize atoms or molecules (ionizing radiation), or less energetic (non-ionizing radiation).

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful for sterilization of surfaces and some transparent objects. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas.

Fluids that would be damaged by heat, irradiation or chemical sterilization, such as drug solution, can be sterilized by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and protein solutions in medicinal drug processing.

Disinfection

Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. Disinfectants (chemical antimicrobial agents designed to inactivate or destroy microorganisms on inert surfaces) are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissue. Disinfectants work by destroying the cell wall of microbes or interfering with their metabolism.

Antisepsis

Antisepsis (from the Greek roots *anti-* against + *sepsis* putrefaction = literally, against putrefaction) means prevention of infection by inhibiting or arresting the growth and multiplication of germs (infectious agents). Antisepsis implies scrupulously clean and free of all living microorganisms. Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter's ability to safely destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects. Some antiseptics are true germicides, capable of destroying bacteria (bactericidal), while others are bacteriostatic and only prevent or inhibit their growth.

Asepsis

Asepsis is the state of being free from disease-causing microorganisms (such as pathogenic bacteria, viruses, pathogenic fungi, and parasites). Asepsis refers to any procedure that is performed under sterile conditions. This includes medical and laboratory techniques (such as with bacterial cultures). This can incorporate techniques such as flame sterilization, and methods to protect wounds and other susceptible sites from organisms that could cause infection. This ensures that only sterile equipment and fluids are used during invasive medical and nursing procedures.

For this process a set of direct (sterilization, disinfection and antisepsis) and indirect (for example, separative events in medical and diagnostic centers – restricted areas, etc.) methods are applied.

Training algorithm of practical skills to be mastered at the lesson

There are no new practical skills to be mastered at this lesson.

Preparation of a smear and stain it by a simple method (repetition of the skill guide to fix it).

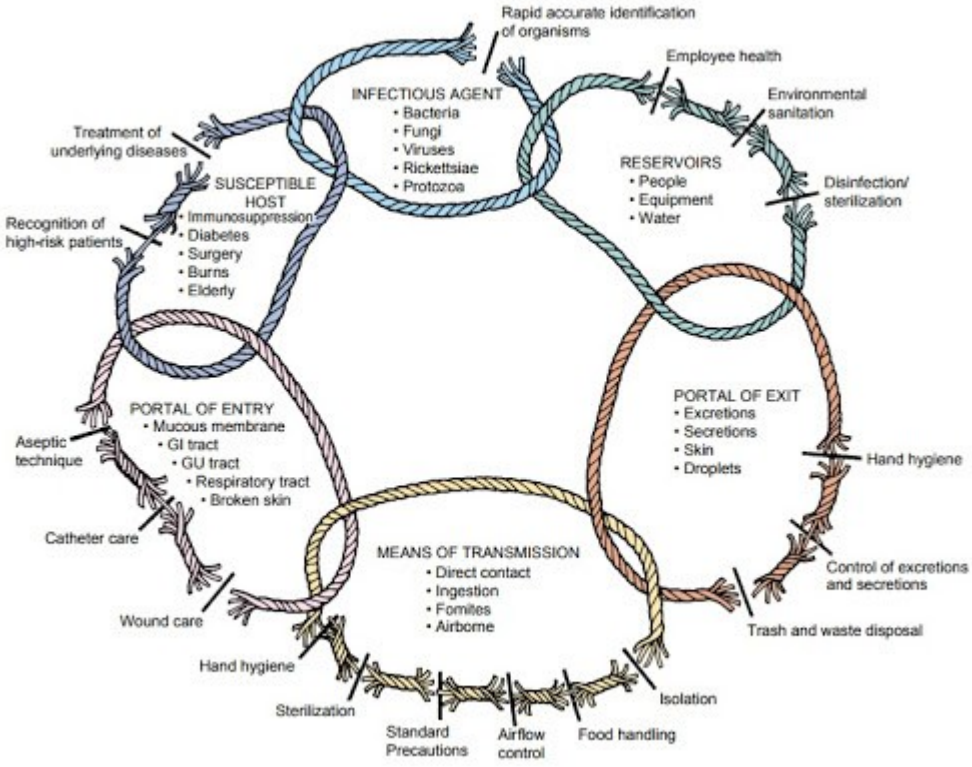
Lesson 7

Infectious process (infection, infectious disease process)

The infection process is the interaction of a microorganism with a particular macro-organism: the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to the infectious agents and the toxins they produce. Infectious disease, also known as transmissible disease or communicable disease, is illness resulting from an infection.

Epidemiological process

This term means a complex specific process of disseminating the causative agents of infectious diseases in human society (human population) that is manifested externally as a continuous chain of sequential interrelated infectious processes on a particular territory. This is the main form of existence of pathogenic microorganisms and their survival as a biological species.



Epidemiological process: its stages (chains) and methods of prevention (https://lh3.googleusercontent.com/proxy/vK64IJZOt4Y4dndS2cmxEIRYmO7i_IBZJ_o1TMSe7SGvL_ejkiYj-bM9Q-V-4f18VBNzkq8YGjJR2PmsqzM2kTgbBkMMeO0vArC5T-G3Q2u6WvpEg9EIb9vR5WUrwiQJMdX2)

Mechanism of transmission of infection

Mechanism of transmitting the infection – that is a process though which the elimination of the pathogenic microorganisms from the recipient organism or other source of infection, their passing through the environment and penetration into another susceptible organism is done.

The stages (phases) of the mechanism of transmission of the infection

These are specific stages of the mechanism of transmission of infection: elimination of the causative agent from the infected organism; passing of the causative agent through the environment; penetration of the causative agent into another susceptible organism.

Factors of transmission, ways of transmission, portals (place of entry) of the infection

Transmission is a process in which several events happen one after the other in the form of a chain. Hence, this process is known as a chain of transmission. Six major factors can be identified: the infectious agent, the reservoir, the route of exit, the mode of transmission, the route of entry and the susceptible host.

Many infectious agents can survive in different organisms, or on non-living objects, or in the environment. Some can only persist and multiply inside human beings, whereas others can survive in other animals, or for example in soil or water. The place where the infectious agent is normally present before infecting a new human is called a reservoir. Without reservoirs, infectious agents could not survive and hence could not be transmitted to other people. Humans and animals which serve as reservoirs for infectious agents are known as infected hosts. Non-living things like water, food and soil can also be reservoirs for infectious agents, but they are called vehicles (not infected hosts) because they are not alive.

Before an infectious agent can be transmitted to other people, it must first get out of the infected host. The site on the infected host through which the infectious agent gets out is called the route (portal) of exit.

Once an infectious agent leaves a reservoir, it must get transmitted to a new host if it is to multiply and cause disease. The route by which an infectious agent is transmitted from a reservoir to another host is called the mode (mean, way) of transmission.

Direct transmission refers to the transfer of an infectious agent from an infected host to a new host, without the need for intermediates such as air, food, water or other animals. Direct modes of transmission can occur in two main ways: 1) person to person (the infectious agent is spread by direct contact between people through touching, biting, kissing, sexual intercourse or direct projection of respiratory droplets into another person's nose or mouth during coughing, sneezing or talking); 2) transplacental transmission (this refers to the transmission of an infectious agent from a pregnant woman to her fetus through the placenta).

Indirect transmission is when infectious agents are transmitted to new hosts through intermediates such as air, food, water, objects or substances in the environment, or other animals. Indirect transmission has three subtypes: 1) airborne transmission (the infectious agent may be transmitted in dried secretions from the respiratory tract, which can remain suspended in the air for some time); 2) vehicle-borne transmission (a vehicle is any non-living substance or object that can be contaminated by an infectious agent, which then transmits it to a new host – contamination refers to the presence of an infectious agent in or on the vehicle); 3) vector-borne transmission (a vector is an organism, usually an arthropod – houseflies, mosquitoes, lice and ticks, – which transmits an infectious agent to a new host).

Successful transmission of the infectious agent requires it to enter the host through a specific part of the body before it can cause disease. The site through which an infectious agent enters the host is called the route (portal) of entry.

After an infectious agent gets inside the body it has to multiply in order to cause the disease. In some hosts, infection leads to the

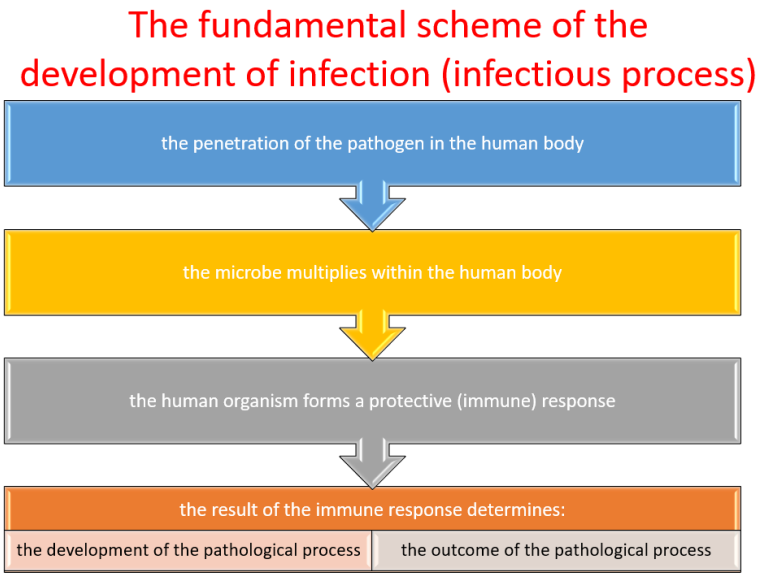
disease developing, but in others it does not. Individuals who are likely to develop a communicable disease after exposure to the infectious agents are called susceptible hosts. Different individuals are not equally susceptible to infection, for a variety of reasons. Factors that increase the susceptibility of a host to the development of a communicable disease are called risk factors. Some risk factors arise from outside the individual – for example, poor personal hygiene, or poor control of reservoirs of infection in the environment. Factors such as these increase the exposure of susceptible hosts to infectious agents, which makes the disease more likely to develop. Additionally, some people in a community are more likely to develop the disease than others, even though they all have the same exposure to infectious agents. This is due to a low level of immunity within the more susceptible individuals.

Structure of epidemiological process

So, an epidemiological process consists of three links: source of infection – mechanism of transmission – susceptible organism.

The fundamental scheme of the development of infection (infectious process)

Infection begins with the penetration of the pathogen in the human body, then the microbe multiplies within it, in response to this the human organism forms a protective (immune) response. The result of the immune response determines the development of the pathological process and its outcome.



Ecological and epidemiological classification of infectious processes

Reservoir (ecological basis) and source (epidemiological basis) of infection – an object, a living organism serving as a place of natural and sustainable existence, development and reproduction of pathogenic microorganisms from which these could separate and cause a new disease.

Anthroponoses – diseases where the only source of infection is infected man (diseased or carrier of infection).

Zoonoses – diseases which are common for man and animals, with a common source of infection. In zoonoses, the main form of existence of pathogenic microorganisms is the epizootic process (their dissemination among the animal population). The epidemic process in these diseases depends on the epizootic process – people are infected by animals which have diseased or are carriers of the infection.

Sapronoses are infectious diseases caused by pathogenic microorganisms that inhabit aquatic ecosystems and/or soil rather than a living host.

Classification of infections according to the mechanism of transmission, ways of transmission and portals of entry of the infection

Agents that cause infectious diseases can be transmitted in many ways. Infections are divided into five groups according to their mechanism of transmission, each group has the corresponding ways of transmission and the portal of infection.

Fecal-oral mechanism of transmission can be implemented by alimentary (with food), by water and by contact; the portal of infection – intestines.

Air born mechanism of transmission can be implemented by droplet and by dust; the portal of infection – respiratory tract.

Blood born mechanism of transmission can be implemented by transmissive way, by parenteral way, by sexual way; the portal of infection – blood.

Contact mechanism of transmission can be implemented by wound, by contact, by sexual ways; the portal of infection – skin and mucous membranes.

Vertical mechanism of transmission can be implemented by transplacental way; the portal of infection – fetal tissues.

Forms of infection

In addition to the above two classifications of diseases they are classified according to other features.

According to the nature of infectious agent: bacterial, viral, fungal or mycosis, protozoan or invasion.

According to their origin and ways of spreading. Exogenous infection is caused by a disease-producing organism from outside the human organism. Endogenous infection is a disease arising from an infectious agent already present in the body but previously asymptomatic.

According to the localization of the pathogen in the human body. Local – confined to a specific area of the body. Systemic – a generalized illness that affects most of the body with pathogens distributed widely in tissues.

According to the number of species of the pathogen. Monoinfection – infection with a single species of microorganism. Mixed infection is where a single infection is caused by a variety of bacterial species which are simultaneous causing the same infection.

According to the duration. Acute disease, pathologic changes occur over a relatively short time (e.g., hours, days, or a few weeks) and involve a rapid onset of disease conditions. Chronic – develops more slowly and is usually less severe, but may persist for a long, indefinite period of time.

According to the character of the spreading of the infection and covered territory. An infection is said to be endemic (from Greek ἐν en "in, within" and δῆμος demos "people") in a population when that infection is constantly maintained at a baseline level in a

geographic area without external inputs. Sporadic infection – when the most infectious disease episodes occur not as part of any apparent outbreaks. An epidemic (from Greek ἐπί epi "upon or above" and δῆμος demos "people") is the rapid spread of disease to a large number of people in a given population within a short period of time. A pandemic (from Greek πᾶν pan "all" and δῆμος demos "people") is a disease epidemic that has spread across a large region, for instance multiple continents, or worldwide.

The last pandemic has been caused by the new strain of coronavirus (SARS-CoV-2) – Coronavirus disease 2019, or COVID-19. Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). COVID-19 was declared a pandemic by the WHO on 11 March 2020. Some coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans, and MERS-CoV from dromedary camels to humans. Several known coronaviruses are circulating in animals that have not yet infected humans. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath, and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Standard recommendations to prevent the spread of infection include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs and avoiding close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing.

And there are some other forms of infections which are classified according to other features.

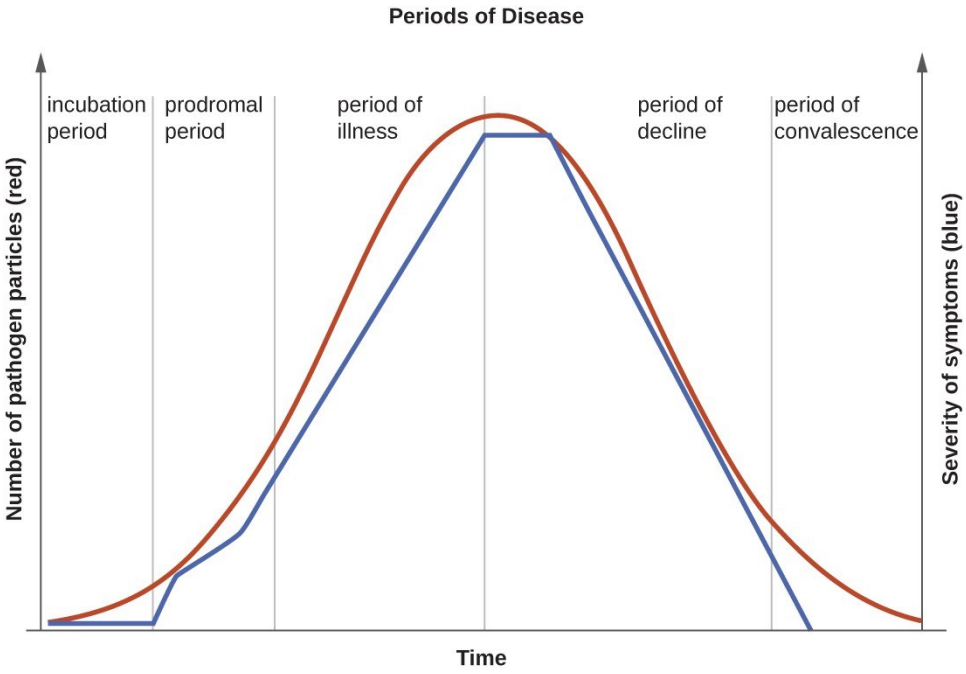
Characteristic properties of the infectious diseases

Infectious diseases differ from other microbial diseases by following features: specificity, contagiousness, cyclic recurrence, and the formation of specific immunity.

Periods of infectious diseases and their characteristics

The five periods of disease (sometimes referred to as stages or phases) include the incubation, prodromal, illness, decline, and convalescence periods.

The incubation period occurs in an acute disease after the initial entry of the pathogen into the host (patient). It is during this time the pathogen begins multiplying in the host. However, there are insufficient numbers of pathogen particles (cells or viruses) present to cause signs and symptoms of disease. Incubation periods can vary from a day or two in acute disease to months or years in chronic disease, depending upon the pathogen. Factors involved in determining the length of the incubation period are diverse, and can include strength of the pathogen, strength of the host immune defenses, site of infection, type of infection, and the size infectious dose received. During this incubation period, the patient is unaware that a disease is beginning to develop.



The progression of an infectious disease can be divided into five periods, which are related to the number of pathogen particles (red) and the severity of signs and symptoms (blue)
(https://s3-us-west-2.amazonaws.com/courses-images/wp-content/uploads/sites/1094/2016/11/03165321/OSC_Microbio_15_01_Stages.jpg)

The prodromal period occurs after the incubation period. During this phase, the pathogen continues to multiply and the host begins to experience general signs and symptoms of illness, which typically result from activation of the immune system, such as fever, pain, soreness, swelling, or inflammation. Usually, such signs and symptoms are too general to indicate a particular disease.

Following the prodromal period is the period of illness, during which the signs and symptoms of disease are most obvious and severe.

The period of illness is followed by the period of decline, during which the number of pathogen particles begins to decrease, and the signs and symptoms of illness begin to decline. However, during the decline period, patients may become susceptible to developing secondary infections because their immune systems have been weakened by the primary infection.

The final period is known as the period of convalescence. During this stage, the patient generally returns to normal functions, although some diseases may inflict permanent damage that the body cannot fully repair.

Infectious diseases can be contagious during all five of the periods of disease. Which periods of disease are more likely to associated with transmissibility of an infection depends upon the disease, the pathogen, and the mechanisms by which the disease develops and progresses. Depending upon the pathogen, the disease, and the individual infected, transmission can still occur during the periods of decline, convalescence, and even long after signs and symptoms of the disease disappear.

The concept of pathogenicity and virulence

Pathogenicity refers to the ability of an organism to cause disease (i.e., harm the host). This ability represents a genetic component of the pathogen.

Virulence, a term often used interchangeably with pathogenicity, refers to the degree of pathology caused by the organism. The extent of the virulence is usually correlated with the ability of the pathogen to multiply within the host and may be affected by other factors (ie, conditional). In summary, a microorganism (species or strain) is defined as being pathogenic (or not), and depending upon conditions, may exhibit different levels of virulence.

Evaluation of the virulence

Direct evaluation of virulence is done by biological test, indirectly – by detecting the presence of specific enzymes of virulence in the culture of microorganisms.

Measurement of the virulence

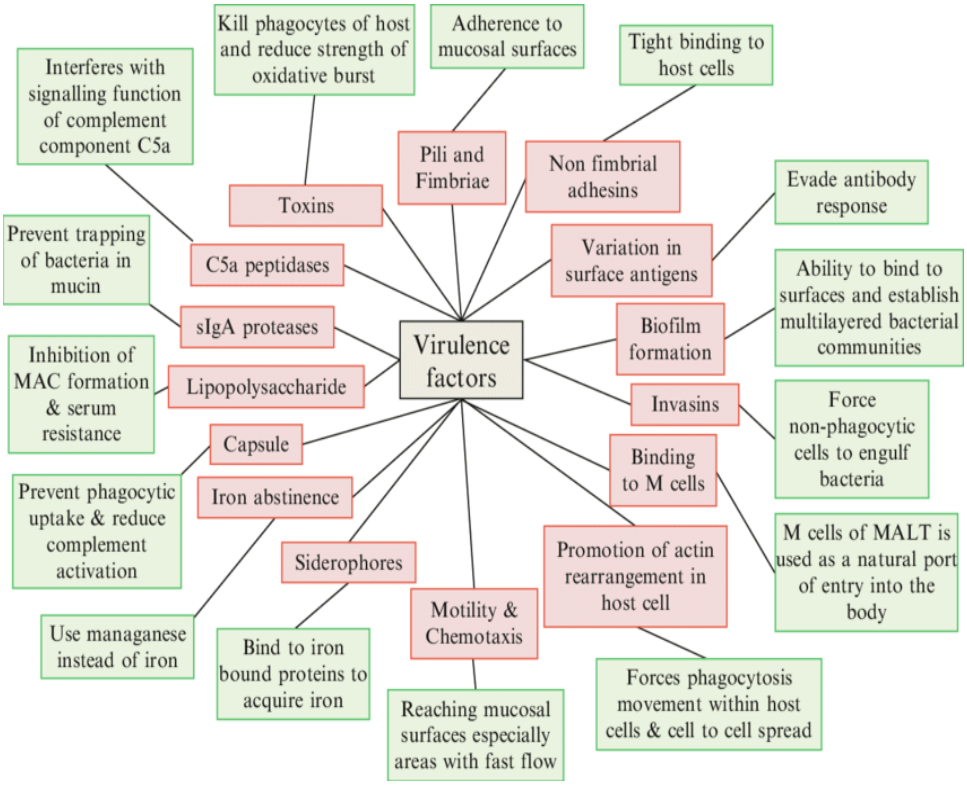
Virulence is measured by determining a 50% lethal dose (LD50), DLM and DCL.

Units of measurement of virulence

The most commonly used measurement of virulence is the lethal dose (usually per body weight) required to kill 50% of the test population, referred to as the LD50 or median lethal dose. Other units: DLM (or LDmin, minimum lethal dose), DCL (absolutely lethal dose). During the calculation the following condition are taken into account: the way of contamination of laboratory animal, laboratory animal species, its weight, and time of its death after contamination.

Factors of virulence

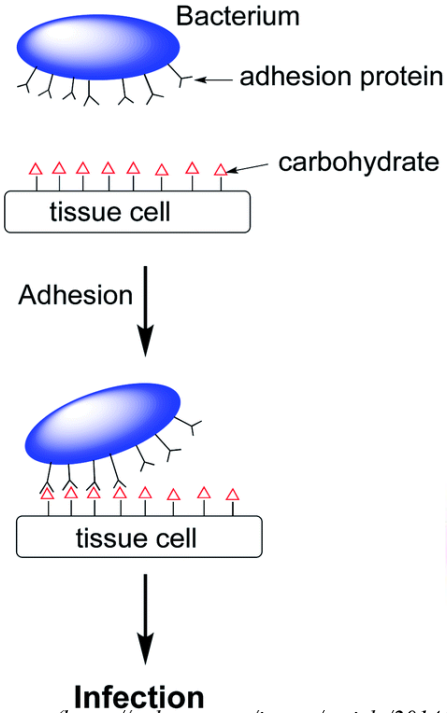
Virulence factors are molecules produced by bacteria, viruses, fungi, and protozoa that implement the interaction latter with a macro-organism, which lead to the development of the pathological process. The main steps of this interaction are: adhesion, colonization, penetration, invasion, aggression.



(https://www.researchgate.net/profile/Prasanth_Rathinam/publication/283672435/figure/fig1/AS:614009250267137@1523402621208/arious-virulence-factors-utilized-by-pathogens-during-infection.png)

Characteristics of adhesion factors

Adhesins are proteins or glycoproteins found on the surface of a pathogen that attach to receptors on the host cell. So,



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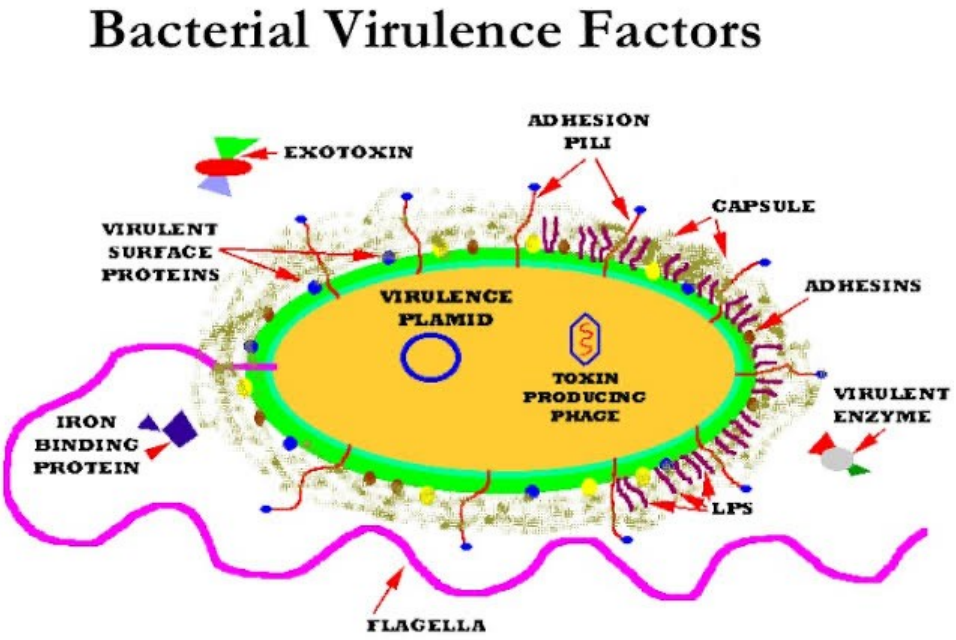

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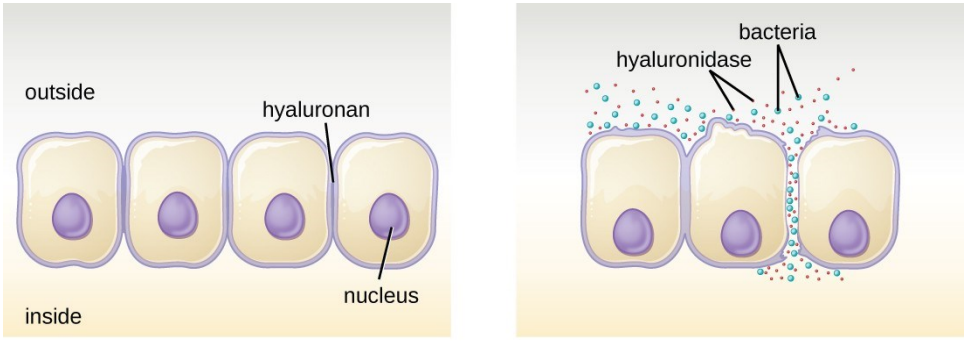
adhesion is the specific interaction of two molecules: one of which (adhesin) is located on the surface of the bacterial cells, and the other (the adhesion receptor) – on the surface of the host's cells.

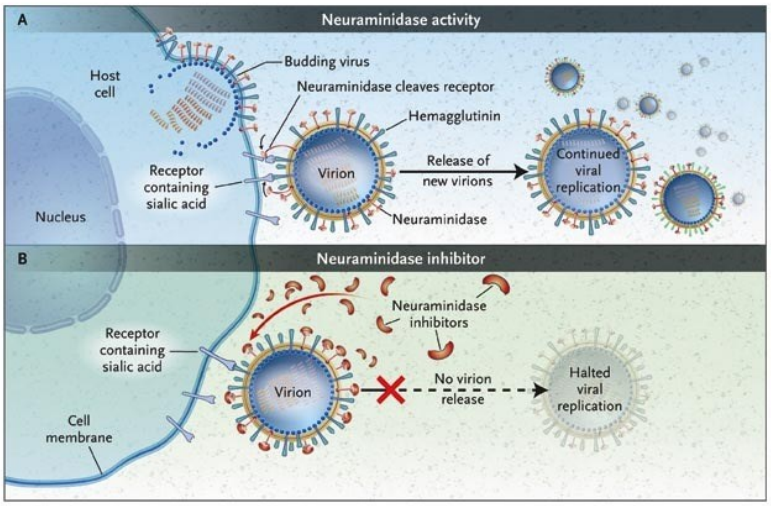
Characteristics of aggression factors

Microorganism can resist a protective response of macro-organism thanks to the two groups of factors: substances that make up its cellular structures, and enzymes of aggression, the effect of which prevents the host's defense response.

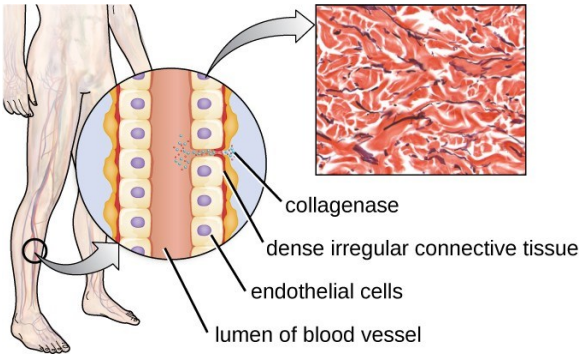


(a) Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (b) Hyaluronidase produced by bacteria degrades this adhesive polymer in the extracellular matrix, allowing passage between cells that would otherwise be blocked (https://s3-us-west-2.amazonaws.com/courses-images/wp-content/uploads/sites/1094/2016/11/03165349/OSC_Microbio_15_03_hyaluronan.jpg)

Characteristics of the main enzymes of invasion and aggression



Mechanism of action of neuraminidase inhibitors (https://www.nejm.org/na101/home/literatum/publisher/mms/journals/content/nejm/2005/nejm_2005.353.issue-13/nejmra050740/production/images/img_medium/nejmra050740_f1.jpeg)



The illustration depicts a blood vessel with a single layer of endothelial cells surrounding the lumen and dense connective tissue (shown in red) surrounding the endothelial cell layer. Collagenase produced by C. perfringens degrades the collagen between the endothelial cells, allowing the bacteria to enter the bloodstream (https://s3-us-west-2.amazonaws.com/courses-images/wp-content/uploads/sites/1094/2016/11/03165352/OSC_Microbio_15_03_CollClost.jpg nejmra050740/production/images/img_medium/nejmra050740_f1.jpeg)

They include hyaluronidase (destroys the intercellular substance of connective tissue), neuraminidase (facilitates the interaction of cell surface receptors of the mucous membranes and microbes), fibrinolysin and collagenase (create conditions for the generalization of infection), coagulase (protects bacterial cells from antibodies and phagocytes), protease (destroys antibodies).

General characteristics of bacterial toxins

Bacterial toxins are toxins produced by bacteria. These toxins promote infection and disease by directly damaging host tissues and by disabling the immune system.

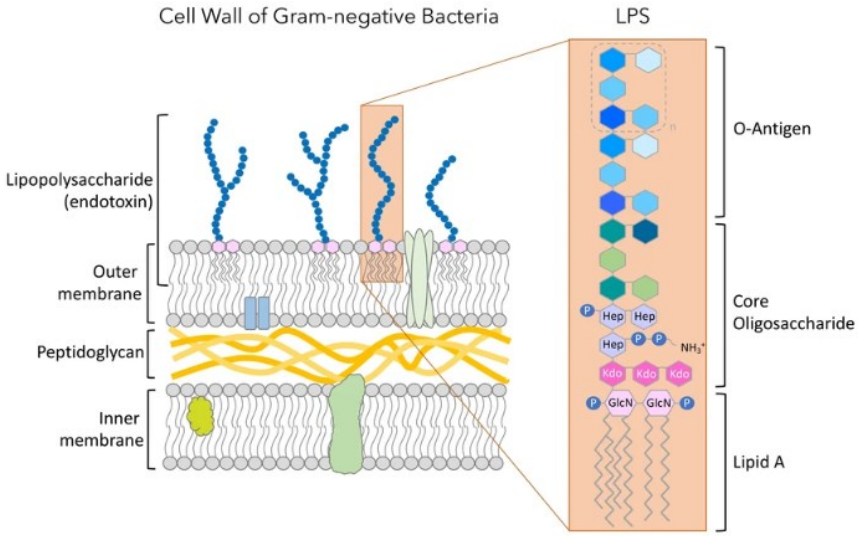
There are two groups of bacterial toxins: protein toxins (metabolites of the bacterial cell) and endotoxin (lipopolysaccharide, which is part of the outer membrane of the cell wall of gram-negative bacteria).

Properties of protein toxins

Protein toxins are characterized by specificity of their effect, high toxicity, high immunogenicity, the ability to convert into toxoid.

Classification of protein toxins

The simplest classification divides protein toxins on neurotoxins (affect on the nervous system cells), enterotoxins (affect on intestinal cells), cytotoxins (block protein synthesis at the subcellular level).



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General characteristics of endotoxin

Endotoxins are heat stable lipopolysaccharide-protein complexes which form structural components of cell wall (they are found in the outer membrane) of Gram-negative bacteria and liberated only on cell lysis or death of bacteria. These are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond. Endotoxin toxicity is associated primarily with the fragment of its molecule containing lipid.

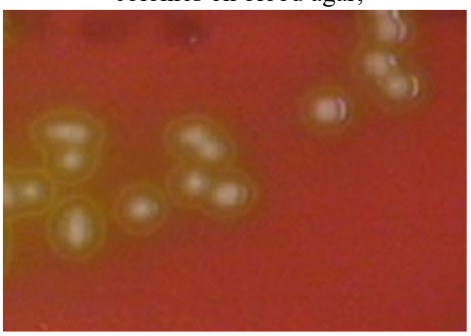
Differences between endotoxins and protein toxins

In contrast to protein toxins endotoxins are more heat stable (on boiling they cannot be denatured), less toxic, weak immunogens, have less specificity of action, and are not converted into toxoids.

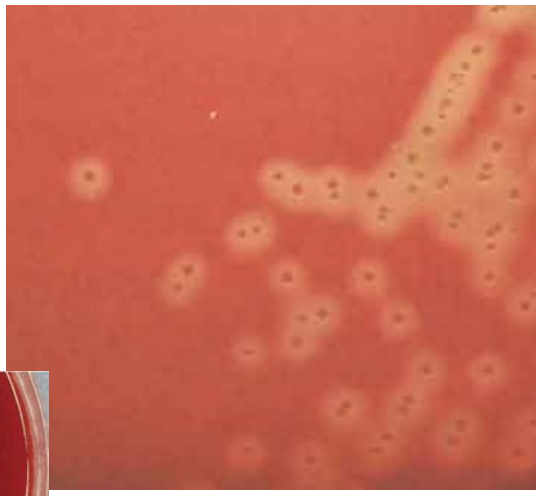
Training algorithm of practical skills to be mastered at the lesson

1. Evaluation of the virulence of the bacterial culture by indirect signs (availability of hemolytic activity, availability of lecithinase activity, availability of plasmocoagulase activity):

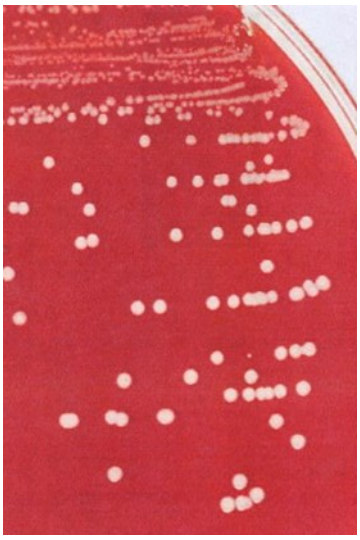
1. hemolytic activity – hemolytic bacteria can cause α-hemolysis, when a zone of greenish coloration forms around colonies growing on blood agar, or β-hemolysis, when a zone of clear hemolysis with no greenish color forms around colonies on blood agar;



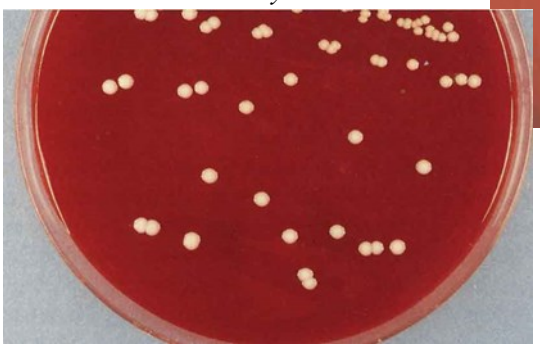
α-hemolysis



β-hemolysis

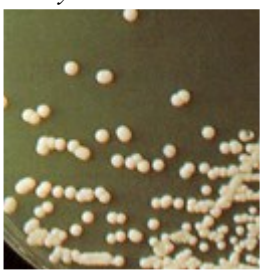


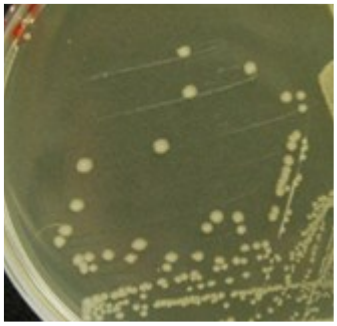
the absence of hemolysis



the absence of hemolysis

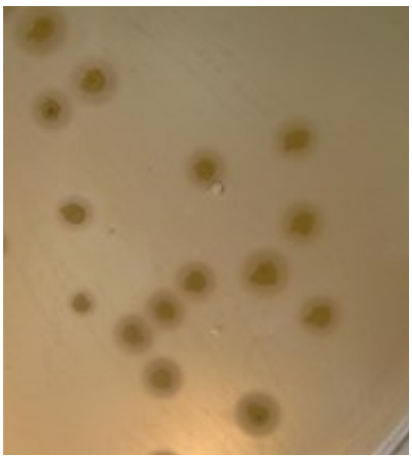
2. lecithinase activity – the opaque opalescent zones appear around the colonies on the yolk-salt agar;

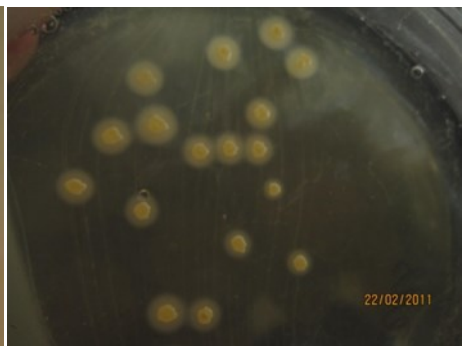




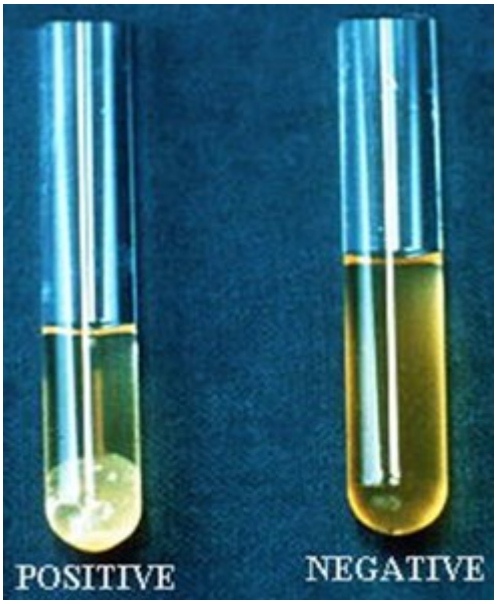
lecithinase activity is absent

3. plasma coagulase activity – clotting of blood plasma.





positive lecithinase activity



coagulase activity

Lesson 9

Antimicrobial chemotherapy and antimicrobial agents: definition of the terms

Antimicrobial chemotherapy is the clinical application of antimicrobial agents to treat infectious disease. There are four types of antimicrobial chemotherapy:

⇒ antibacterial chemotherapy, the use of antibacterial drugs to treat bacterial infections,

⇒ antifungal chemotherapy, the use of antifungal drugs to treat fungal infections,

⇒ antiprotozoal chemotherapy, the use of antiprotozoal drugs to treat protozoan infections

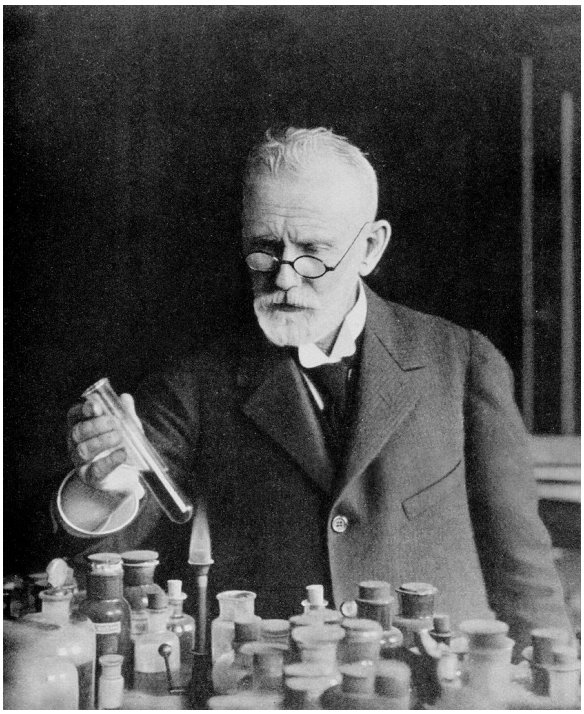
⇒ antiviral chemotherapy, the use of antiviral drugs to treat viral infections.

Antimicrobial agents are agents (drugs) that selectively destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action. "Selectively" means: a chemotherapeutic drug inhibits a vital function of microorganisms that differs qualitatively or quantitatively from functions of host cells.

These agents include natural compounds, called antibiotics, as well as synthetic compounds. An antibiotic (as formerly was, when the term was arrived) is a substance produced by one microbe that can inhibit the growth or viability of another microbe. The earliest use of antibiotics was probably in the treatment of skin infections with moldy bean curd by the ancient Chinese. The development of modern antibiotics can be traced to the work of Louis Pasteur, who observed that the in vitro growth of one microbe was inhibited when another microbe was added to the culture. Pasteur called this phenomenon antibiosis and predicted that substances derived from microbes would someday be used to treat infectious diseases.

Sir Alexander Fleming (1881-1955), the Scottish scientist famous for the discovery of penicillin. Photo taken between 1939 and 1945 (https://upload.wikimedia.org/wikipedia/commons/thumb/b/bf/Synthetic_Production_of_Penicillin_TR1468.jpg/220px-Synthetic_Production_of_Penicillin_TR1468.jpg)

Several decades later, Alexander Fleming observed that the growth of his staphylococcal cultures was inhibited by a Penicillium contaminant. Fleming postulated that the fungus produced a substance, which he called penicillin, and that this substance inhibited the growth of staphylococci. His observations eventually led to the isolation and use of penicillin for treating bacterial infections. The discovery of penicillin stimulated the discovery and development of many other antibiotics and revolutionized the treatment of infectious diseases.



Paul Ehrlich (1854-1915) (https://upload.wikimedia.org/wikipedia/commons/8/83/Paul_Ehrlich%2C_c._1910.jpg)

Synthetic drugs have also provided major advances in the treatment of infectious diseases. During the Renaissance, Paracelsus used mercury compounds for the treatment of syphilis. In the late 19th and early 20th centuries, Paul Ehrlich pioneered the search for selectively toxic compounds. After many failed attempts, he discovered arsphenamine (salvarsan), an arsenical compound for the treatment of syphilis (the first successful application of metalloid complexes in chemotherapy). Ehrlich, who became known as the father of chemotherapy, also studied bacterial stains as potential antimicrobial agents. He reasoned that a stain's selective affinity for bacteria could be coupled with an inhibitory action to halt microbial metabolism and destroy invading organisms. This concept led to the discovery of sulfonamides, drugs that were originally derived from a bacterial stain called prontosil. The sulfonamides were the first effective drugs for the treatment of systemic bacterial infections, and their development accelerated the search for other antimicrobial agents.

The basic characteristics of antimicrobial agents

Antimicrobial agents have no appreciable toxic effect on human organism, they have different antimicrobial spectrum, and there is a constant formation of drug-resistant to any species of microorganisms.

The most important groups of chemotherapeutic agents

All chemotherapeutic agents used in modern medicine can be classified into seven major groups: antibiotics, sulfanilamide preparations (antimetabolites of folic acid in microbial cell), metal-based antimicrobial agents (inactivate enzymes of microorganisms), nitrofurans (infringe bioenergetic processes in bacterial cell), antifungal, antiparasitic and antiviral drugs.

Antibiotics: definition of the term

An antibiotic is a type of antimicrobial substance active against bacteria and is the most important type of antibacterial agent for fighting bacterial infections. Antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiproto-

zoal activity. Antibiotics are not effective against viruses; drugs which inhibit viruses are termed antiviral drugs or antivirals rather than antibiotics.

Sometimes, the term antibiotic which means "opposing life", based on Greek roots, (ἀντι-) anti: "against" and (βίος-) biotic: "life", is broadly used to refer to any substance used against microbes, but in the usual medical usage, antibiotics (such as penicillin) are those produced naturally (by one microorganism fighting another), whereas nonantibiotic antibacterials (such as sulfonamides and antiseptics) are fully synthetic. However, both classes have the same goal of killing or preventing the growth of microorganisms, and both are included in antimicrobial chemotherapy. "Antibacterials" include antiseptic drugs, antibacterial soaps, and chemical disinfectants, whereas antibiotics are an important class of antibacterials used more specifically in medicine and sometimes in livestock feed.

Classification of antibiotics according to the source of their isolation

According to the source of isolation antibiotics are classified on: produced by fungi, produced by actinomycetes (the biggest group of antibiotics), produced by bacteria, produced by animals, produced by plants, synthetic antibiotics.

Classification of antibiotics according to the method of their production

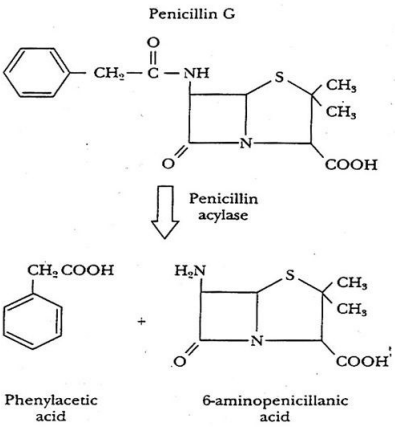
Industrial microbiology can be used to produce antibiotics via the process of fermentation (biological synthesis), where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. Antibiotics produced by biological synthesis are named natural ones.

A photo of researcher in a fermentation lab
(https://acs-h.assetsadobe.com/is/image//content/dam/cen/97/38/WEB/09738-feature2-ajinomoto.jpg/?$responsive$&wid=700&qlt=90,0&resMode=sharp2)

A common form of antibiotic production in modern times is semi-synthetic. Semi-synthetic production of antibiotics is a combination of natural fermentation and laboratory work (antibiotics produced by this combined method are named semisynthetic ones). An example of semi-synthetic production involves the drug ampicillin. A beta lactam antibiotic just like penicillin, ampicillin was developed by adding an addition amino group (NH2) to the R group of penicillin. This additional amino group gives ampicillin a broader spectrum of use than penicillin. Methicillin is another derivative of penicillin, the key difference between penicillin and methicillin being the addition of two methoxy groups to the phenyl group. These methoxy groups allow methicillin to be used against penicillinase producing bacteria that would otherwise be resistant to penicillin.

Not all antibiotics are produced by live organisms; some are made completely synthetically in the lab (synthetic antibiotics). These include the quinolone class, of which nalidixic acid is often credited as the first to be discovered.

SEMISYNTHETIC PENICILLINS



Cleavage of penicillin G generated by fermentation to phenylacetic acid and 6-aminopenicillanic acid. The latter can be chemically modified to produce effective antibacterials.

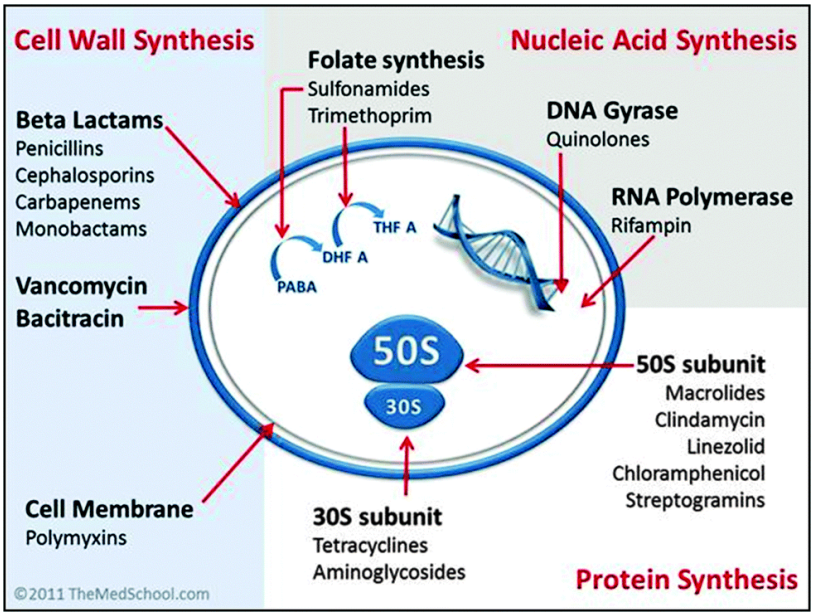
(https://slideplayer.com/slide/4732093/15/images/27/SEMISYNTHETIC+PENICILLINS.jpg)

Classification of antibiotics according to the mechanism of their action

Existing antibacterials are classified into four major groups based upon their intracellular target and their mechanism of action.

(1) Cell wall synthesis inhibition; e.g. penicillin and derivatives, cephalosporins, carbapenems and glycopeptides.These compounds are more effective against infection by Gram positive bacteria.

(2) Cell membrane disruption; e.g. the family of polycationic peptide antibiotics called polymyxins. Polymyxins are used in the treatment of infection by Gram negative bacteria, and are considered a last-line therapy against Gram negative ‘superbugs’.



Targets of the main antibacterial drugs at the bacteria cell [Image by Kendrick Johnson: Creative Commons Attribution-ShareAlike 3.0 Unported license] (https://pubs.rsc.org/image/article/2015/CS/c4cs00343h/c4cs00343h-f2_hi-res.gif)

(3) Nucleic acid synthesis inhibition; e.g. quinolones, rifampicin and sulphonamides. The fluoroquinolones are one of a few examples of a broad-spectrum synthetic antimicrobial in clinical use.

(4) Protein synthesis inhibition; e.g. tetracycline, aminoglycosides, chloramphenicol and macrolides. A large proportion of clinically-used antibacterials inhibit protein synthesis by targeting the ribosomal-RNA rich surfaces of ribosomes.

Classification of antibiotics according to the spectrum of their antimicrobial activity

The spectrum of antimicrobial activity of a drug is the primary determinant of its clinical use. Antimicrobial agents that are active against a single species or a limited group of pathogens are called narrow-spectrum drugs, whereas agents that are active against a wide range of pathogens are called broad-spectrum drugs. Agents that have an intermediate range of activity are sometimes called extended-spectrum drugs. Narrow-spectrum drugs are sometimes preferred because they target a specific pathogen without disturbing the normal flora of the gut or respiratory tract. Broad-spectrum drugs are sometimes preferred for the initial treatment of an infection when the causative pathogen is not yet identified.

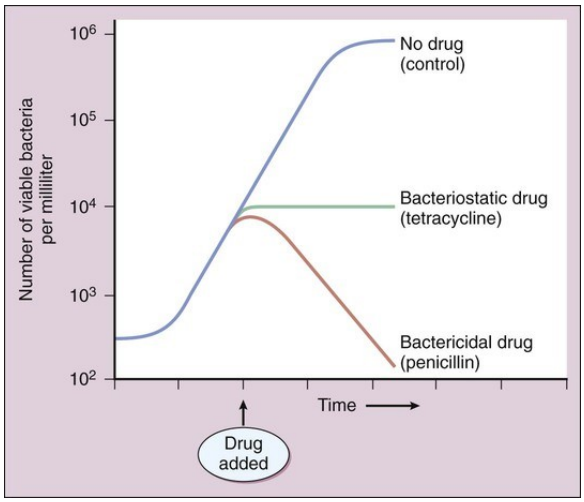
Classification of antibiotics according to the result of their influence on microorganism

A bactericidal drug kills sensitive organisms so that the number of viable organisms falls rapidly after exposure to the drug. In contrast, a bacteriostatic drug inhibits the growth of bacteria but does not kill them. For this reason, the number of bacteria remains relatively constant in the presence of a bacteriostatic drug, and immunologic mechanisms are required to eliminate organisms during treatment of an infection with this type of drug. (The same principle applies to a drug that kills or inhibits the growth of fungi and is referred to as a fungicidal drug or a fungistatic drug, respectively).

A bactericidal drug is usually preferable to a bacteriostatic drug for the treatment of most bacterial infections. This is because bactericidal drugs typically produce a more rapid microbiologic response and more clinical improvement and are less likely to elicit microbial resistance. Bactericidal drugs have actions that induce lethal changes in microbial metabolism or block activities that are essential for microbial viability. For example, drugs that inhibit the synthesis of the bacterial cell wall (e.g., penicillins) prevent the formation of a structure that is required for the survival of bacteria. In contrast, bacteriostatic drugs usually inhibit a metabolic reaction that is needed for bacterial growth but is not necessary for survival. For example, sulfonamides block the synthesis of folic acid, which is a cofactor for enzymes that synthesize DNA components and amino acids.

Drugs that reversibly inhibit bacterial protein synthesis (e.g., tetracyclines) are also bacteriostatic, whereas drugs that irreversibly inhibit protein synthesis (e.g., streptomycin) are usually bactericidal.

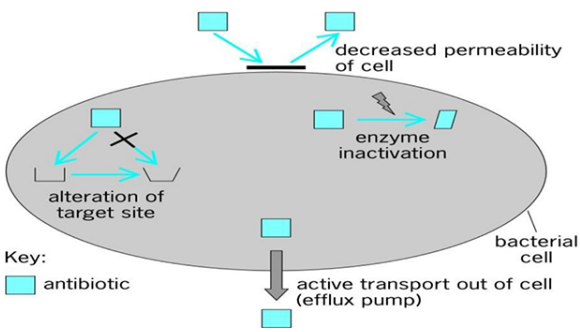
Some drugs can be either bactericidal or bacteriostatic, depending on their concentration and the bacterial species against which they are used.



In vitro effects of bactericidal and bacteriostatic drugs. In the absence of an antimicrobial drug, bacteria exhibit logarithmic growth in a broth culture. The addition of a bacteriostatic drug (tetracycline) inhibits further growth but does not reduce the number of bacteria. The addition of a bactericidal drug (penicillin) reduces the number of viable bacteria (https://basicmedicalkey.com/wp-content/uploads/2016/07/B9781455702824000037X_f037-002-

Complications of antibiotic therapy

Complications of antibiotic therapy include toxic reactions, development of dysbiosis, immunopathological reactions, adverse effects on the fetus, the appearance of atypical forms of bacteria, and the formation antibiotic resistance in microbes.



Four common mechanisms of antibiotics resistance. McGraw-Hill Concise Encyclopedia of Bioscience. 2002 by the McGraw-Hill Companies, Inc. (https://www.intechopen.com/media/chapter/50951/media/fig2.png)

Mechanisms of bacterial resistance to antimicrobial agents

Primary (naturally, specific) resistance of bacteria to antimicrobial agents is due to the absence of the target of the latest, secondary (acquired) resistance may be due to mutation or recombination (associated with R-plasmid, transposon).

Determination of the susceptibility of bacteria to antimicrobial agents

Because susceptibility can vary even within a species (with some strains being more resistant than others), antibiotic susceptibility testing is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo.

Tests for antibiotic sensitivity include:

⇒ Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Müeller-Hinton agar is frequently used in this antibiotic susceptibility test.

⇒ E-test (also based on antibiotic diffusion)

⇒ Agar and Broth dilution methods for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination.

The results of the test are reported on the antibiogram. Once a culture is established, there are two possible ways to get an antibiogram:

⇒ a semi-quantitative way based on diffusion (**Kirby-Bauer method**); small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible. Since the concentration of the antibiotic was the highest at the centre, and the lowest at the edge of this zone, the diameter is suggestive for the Minimum Inhibitory Concentration, or MIC, (conversion of the diameter in millimeter to the MIC, in µg/ml, is based on known linear regression curves).

⇒ a quantitative way based on **dilution**: a dilution series of antibiotics is established (this is a series of reaction vials with progressively lower concentrations of antibiotic substance). The last vial in which no bacteria grow contains the antibiotic at the Minimal Inhibiting Concentration.

E-test, (previously known as Epsilometer test) is a manual in vitro diagnostic device used by laboratories to determine the MIC (Minimum Inhibitory Concentration) and whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antimicrobial. This type of test is most commonly used in healthcare settings to help guiding physicians in treatment of patients by indicating what concentration of antimicrobial would successfully treat an infection.

E-test is a quantitative technique that is based on combination of concept of both dilution and diffusion principle for susceptibility testing. E test strip is placed on to an inoculated agar plate; there is an immediate release of antibiotics from the plastic carrier surface into the agar surface. After incubation, bacterial growth becomes visible, symmetrical inhibition ellipse along the strip is seen. The MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip.

E-test Caspofungin against Candida albicans
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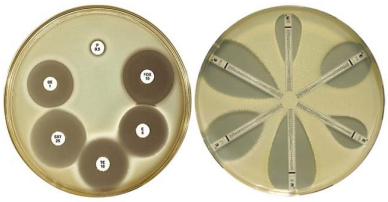

Antimicrobial Susceptibility Testing (AST)
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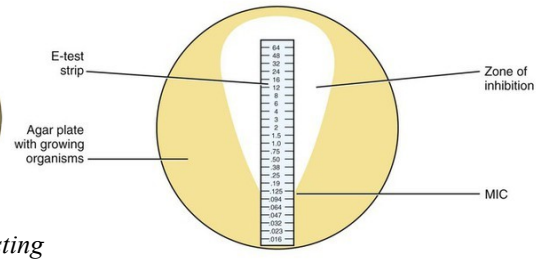

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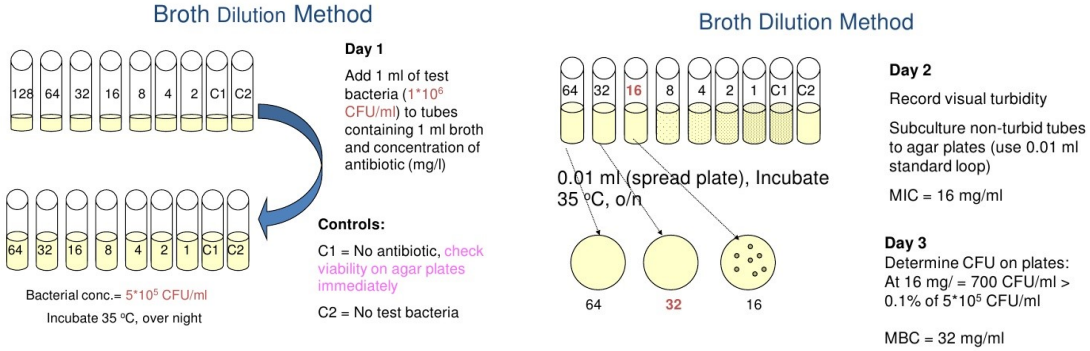

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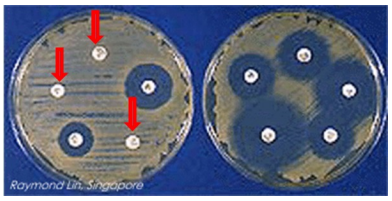
Lesson 8

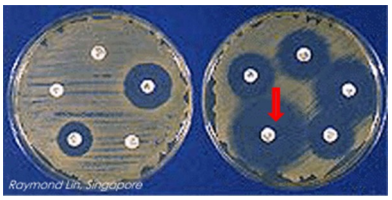
Training algorithm of practical skills to be mastered at the lesson

1. The Kirby-Bauer disk-diffusion method for determination of the susceptibility of bacteria to antimicrobial agents (techniques and experience records)
   1. seeding the test strain on agar plate for making a lawn growth;
   2. placement paper disks with antibiotics on the surface of the seeded agar plate;
   3. thermostating;
   4. experience records: the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the disk, the more effective the compound.

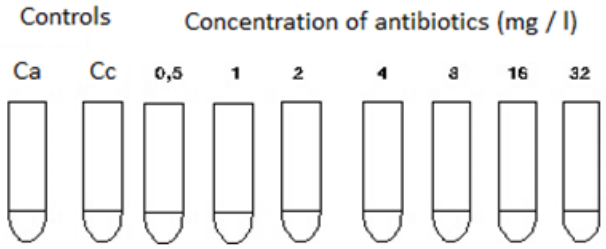


For these antibiotics (marked with red arrows) this strain is total stability

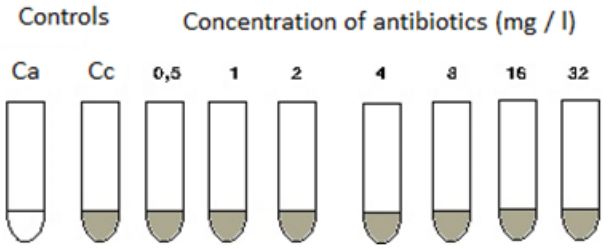
2. Broth dilution method for determination of the susceptibility of bacteria to antimicrobial agent (algorithm of techniques, calculation of MIC and MBC)
   1. successive dilution of antibiotic in liquid medium (twofold for example);
   2. seeding obtained solutions by test strain;
   3. thermostating;
   4. calculation of MIC – the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism;
   5. seeding material from the solutions in which there is no growth on the agar plate or into broth without antibiotic;
   6. thermostating;
   7. calculation of MBC – the lowest concentration of the antibiotic required to kill a particular bacterium.



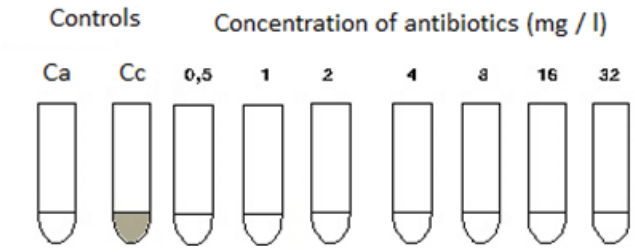
This strain is most sensitive to the antibiotic marked with a red arrow



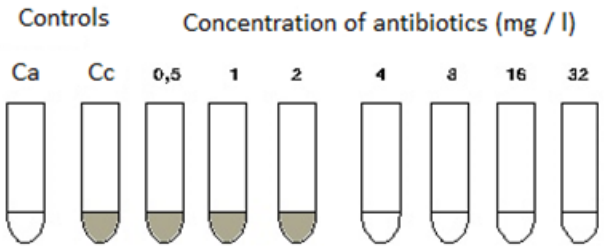
Results cannot be taken into account – the tested bacterial strain has lost viability (in the control of the bacterial culture and, respectively, in all dilutions of the antibiotic bacterial growth is not observed); a different strain of the test species should be taken and the experience should be repeated



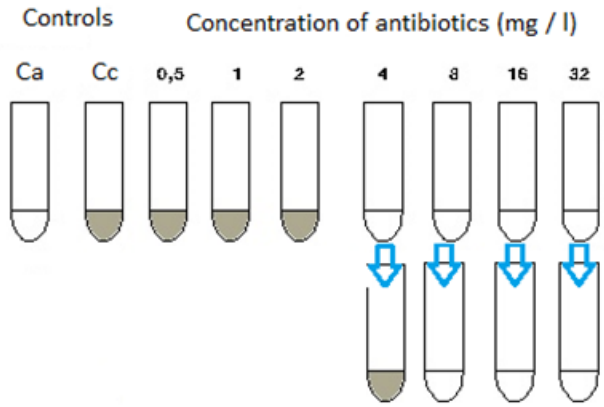
MIC of the antibiotic relative to the test strain is higher than 32 mg/l; a new series of antibiotic with concentration greater than 32 mg/l should be prepared, and the experience should be repeated



MIC of the antibiotic relative to the test strain is less than 0,5 mg/l; a new series of antibiotic with concentration less than 0.5 mg/l should be prepared and the experience should be repeated



MIC of the antibiotic relative to the test strain is = 4 mg/l (the lowest concentration of the antibiotic at which no growth)



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MBC of this antibiotics for this strain – 8 mg/l (after seeding material from the solution of concentration of the antibiotic 4 mg/l bacterial growth is observed; and from solution of concentration of the antibiotic 8, 16 and 32 mg/l – no growth)