مادة الاحياء المجهرية ١

المرحلة الثانية

الكورس الاول

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Lecture one **Microbiology 1** **Dr. Israa Zaidan**

An Introduction to Microbiology

The Scope of Microbiology:

- **Microbiology**: The study of living things too small to be seen without magnification
  - **Microorganisms** or **microbes**- these microscopic organisms
  - Commonly called "germs, viruses, agents…" but not all cause disease and many more are useful or essential for human life

How Can Microbes Be Classified?

Carolus Linnaeus (Swedish) developed taxonomic system for naming plants and animals and grouping similar organisms together

Leeuwenhoek's microorganisms grouped into six categories as follows:

Fungi

Protozoa

Algae

Bacteria

Virus

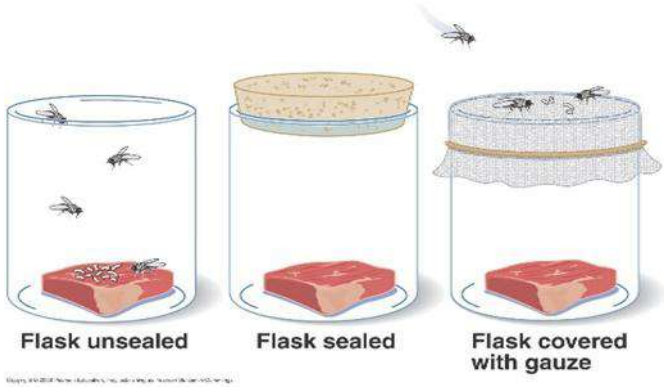
- **Protozoa**
  - Single-celled eukaryotes
  - Similar to animals in nutrient needs and cellular structure
  - Live freely in water; some live in animal hosts
  - Asexual (most) and sexual reproduction
  - Most are capable of locomotion by
    - Pseudopodia – cell extensions that flow in direction of travel
    - Cilia – numerous, short, hairlike protrusions that propel organisms through environment
    - Flagella – extensions of a cell that are fewer, longer, and more whiplike than cilia

- **Algae**
  - Unicellular or multicellular

- Photosynthetic
- Simple reproductive structures
- Categorized on the basis of pigmentation, storage products, and composition of cell wall.

- **Bacteria and Archaea**
  - Unicellular and lack nuclei
  - Much smaller than eukaryotes
  - Found everywhere there is sufficient moisture; some found in extreme environments
  - Reproduce asexually
  - Two kinds
    - Bacteria – cell walls contain peptidoglycan; some lack cell walls; most do not cause disease and some are beneficial
    - Archaea – cell walls composed of polymers other than peptidoglycan

- **Redi's Experiments**
  - When decaying meat was kept isolated from flies, maggots never developed
  - Meat exposed to flies was soon infested
  - As a result, scientists began to doubt Aristotle's theory



- **What Causes Disease?**
  - Pasteur developed germ theory of disease
  - Robert Koch studied causative agents of disease
    - Anthrax
    - Examined colonies of microorganisms
- **What Role Do Microorganisms Play in the Environment?**
  - Bioremediation uses living bacteria, fungi, and algae to detoxify polluted environments
  - Recycling of chemicals such as carbon, nitrogen, and sulfur

- **How Do We Defend Against Disease?** ٣
  - Serology
    - The study of blood serum
    - Von Behring and Kitasato – existence in the blood of chemicals and cells

Cellular Organization

1. Prokaryotic
2. Eukaryotic

Types of Microorganisms Based on Acquiring Nutrition

- Autotrophic
- Heterotrophic
- Saprophytic or Saprobic

Microbial Nutrition & Growth

- Nutrient Requirements
- Nutrient Transport Processes
- Culture Media
- Growth in Batch Culture
- Mean Generation Time and Growth Rate
- Measurement of Microbial Growth
- Continuous Culture
- Factors Influencing Growth

Nutrient Requirements

1-Energy Source

Phototroph: Uses light as an energy source

Chemotroph:Uses energy from the oxidation of reduced chemical compounds

2-Electron (Reduction potential) Source

Organotroph: Uses reduced organic compounds as a source for reduction potential

Lithotroph: Uses reduced inorganic compounds as a source for reduction potential

3 Carbon source

Autotroph Can use CO_2 as a sole carbon source (Carbon fixation)

Heterotroph Requires an organic carbon source; cannot use CO_2 as a carbon source

4- **Nitrogen source**

- Organic nitrogen:Primarily from the catabolism of amino acids
- Oxidized forms of inorganic nitrogen
- Nitrate ($NO3^{-2}$) and nitrite (NO_2-)
- Reduced inorganic nitrogen
- Ammonium (NH_4+)
- Dissolved nitrogen gas (N_2) (Nitrogen fixation)

5-Phosphate source

Organic phosphate

Inorganic phosphate ($H_2PO_4^{-2}$ and HPO_4^{-2})

6-Sulfur source

- Organic sulfur
- Oxidized inorganic sulfur
- Sulfate (SO_4^{-2})
- Reduced inorganic sulfur
- Sulfide (S_2- or H_2S)
- Elemental sulfur (So)

7-Special requirements

- Amino acids
- Nucleotide bases
- Enzymatic cofactors or "vitamins"

- **History of microbiology**

The existence of microorganisms was unknown until the invention of Microscope that is an optical instrument which can magnify small objects which cannot be seen by naked eye. Microscopes were invented in the beginning of 17^{th} century. Early Microscopes were of two types; Simple Microscope, with a single lens of very short focal length and Compound Microscope, with two double convex lens system including ocular and objective lens with higher magnifying power.

Historical Developments in Microbiology

- **Robert Hooke** (1635 - 1703), he made many scientific discoveries in the 17th century, including making one of the first microscopes to see and draw details of the structure of plant cells and some microbes.

- Antony van Leeuwenhoek (1632-1723) : he made the first useful microscopes in the 17th century, he used such a microscope to see the first microscopic cells.

- After the discovery of microorganisms by Leeuwenhoek, scientists began to study the origin of these small microorganisms.

- **Louis Pasteur** (1822-1895) was born in France, he emerged as one of the greatest biologists of the 19th century. His contributions are the most significant in the history of science and industry and his work with germs and microorganisms opened new areas of scientific studies. When he examined the sediments microscopically he observed the presence of small oval shaped bodies.

1857 – Lactic acid fermentation is due to a microorganism

1860 – Yeasts are involved in alcoholic fermentation

1861 – Disproved the theory of spontaneous generation

1861 – Introduction of the terms aerobic and anaerobic for yeasts.

Production of more alcohol in the absence of oxygen during sugar fermentation- The Pasteur Effect

1862 – Proposed germ theory of disease

1867 – Pasteur devised the process of destroying bacteria known

as pasteurization.

1881 – Development of anthrax vaccine.

1885 – Development of a special vaccine for rabies (the Pasteur

treatment)

- In Germany, **Robert Koch (**1843-1910) confirmed Pasteur's germ theory and took it several steps further. He was put of four basic principles or postulates of bacteriology known **as Koch's postulates** and they are:

1. Microorganism must be present in every case of the disease.

2. Microorganism must be isolated from the diseased host and grown in pure culture.

3. The specific disease must be reproduced when a pure culture of microorganism is injected into healthy susceptible host.

4. Microorganism must be recovered once again from experimentally inoculated host.

Branches of Microbiology:

Microbiology is the study of microorganisms, which includes a large and diverse group of microscopic organisms that exist as a single cells or cell clusters and divided into 8 branches :

1. **Virology**: the science that study of viruses and virus-like agents, including their taxonomy, disease-producing properties, cultivation and genetics.
2. **Bacteriology**: is the branch that studies the types ,morphology, ecology, genetics and biochemistry of bacteria as well as many other aspects related to them.
3. **Mycology**: the science that study fungi, there are medical, agricultural, biotechnological specializations.
4. **Parasitology**: the science that study of parasites, their hosts, and the relationship between them, but also used to describe those who study protozoan and bacterial pathogens.
5. **Protozoology**: the science that study small "animal - like" single celled organisms such as amoeba, and various disease causing parasites.
6. **Nematology**: the study of nematodes (roundworms).
7. **Immunology :** medical and biological sciences. The immune system protects us from infection through various lines of defense.
8. **Phycology** : study algae.

- **Main characteristics of microorganisms**

1. Their size is very small.
2. Microorganisms are present everywhere on the bodies of animals and humans, on plant surfaces, in the air, water, dust, soil, and even inside the intestinal canal of all insects, birds, animals and human beings.
3. Each type has a characteristic cellular composition, morphology, mean of locomotion, and reproduction.
4. Some of microorganism are pathogenic and causing disease but almost others are beneficial which play a roles in many ways such as making

life-saving drugs, the manufacture of biofuels, cleaning up pollution, and producing/processing food and drink.

Eukaryotes and prokaryotes:

Cells have evolved into two fundamentally different types: eukaryotes and prokaryotes, which can be distinguished up on the basis structure and the complexity of their organization. Fungi and protozoa are eukaryotic, whereas bacteria and virus are prokaryotic.

1. The eukaryotic cell has a true nucleus with multiple chromosomes surrounded by nuclear membrane and uses a mitotic apparatus to ensure equal allocation of the chromosomes to progeny cells.
2. The nucleoid of a prokaryotic cell consist of a single circular molecule of loosely organized DNA lacking a nuclear membrane and mitotic apparatus.

In addition to the different types of nuclei, the two classes of cells are distinguished by several other characteristics:

1. Eukaryotic cells contain organelles, such as mitochondria and lysosomes, and larger (80S) ribosomes, whereas prokaryotes bacterial cells contain no organelles and smaller (70S) ribosomes.
2. Most prokaryotes bacteria cells have a rigid cell wall that contain peptidoglycan, a polymer of amino acids and sugars, as its unique structural component. Eukaryotes, on the other hand, do not contain peptidoglycan. Either they are bound by a flexible membrane or, in the case of fungi have rigid cell wall with chitin, a homopolymer of N – acetylglucosamine.
3. The eukaryotic cell membrane contains sterols, whereas no of prokaryote, except the wall of mycoplasma, has a sterol in its membrane.

The best example of prokaryotes is the bacteria

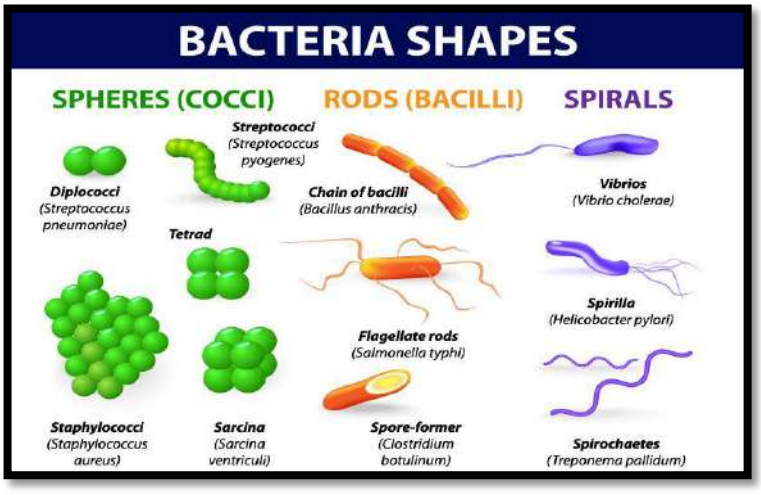
Bacteria: are prokaryotic, unicellular microorganisms, which lack chlorophyll pigments. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles.

Cell morphology include:

- **Shape :**

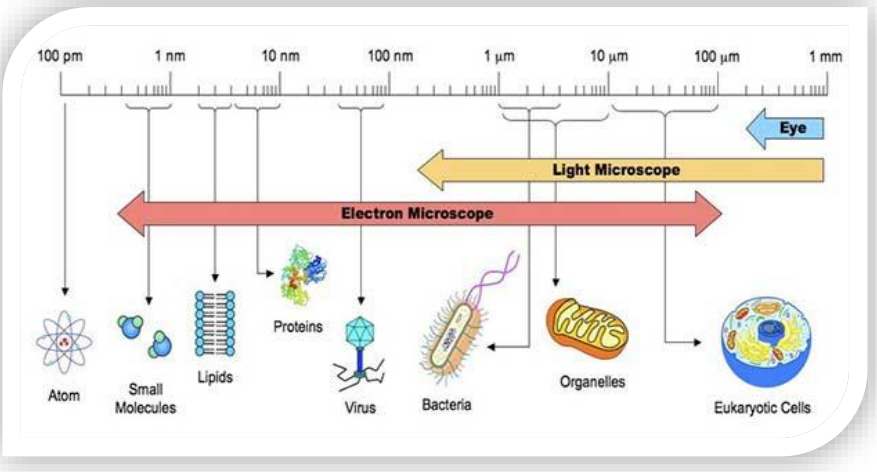
When viewed under light microscope, most bacteria appear in variations of three major shapes based on planes of division:

1. **Cocci** (or coccus for a single cell) are round cells, sometimes slightly flattened when they are adjacent to one another.
2. **Bacilli** (or bacillus for a single cell) are rod-shaped bacteria.
3. **Spirilla** (or spirillum for a single cell) are curved bacteria which can range from a gently curved shape to a corkscrew-like spiral. Many spirilla are rigid and capable of movement. A special group of spirilla known as spirochetes are long, slender, and flexible. The microscopic appearance of a bacterium is one of the most important criteria used in its identification.



- **Size of Bacterial Cell:** Average diameter of spherical bacteria is 0.5-2.0 µm. For rod-shaped or filamentous bacteria, length is 1-10 µm and diameter is 0.25-1 .0 µm.

Bacterial size



- **Arrangement of the bacteria (especially Cocci) :**

Cocci bacteria can exist singly, in pairs (as diplococci), in groups of four (as tetrads), in chains (as streptococci), in clusters (as stapylococci), or in cubes consisting of eight cells (as sarcinae).

Bacterial shapes

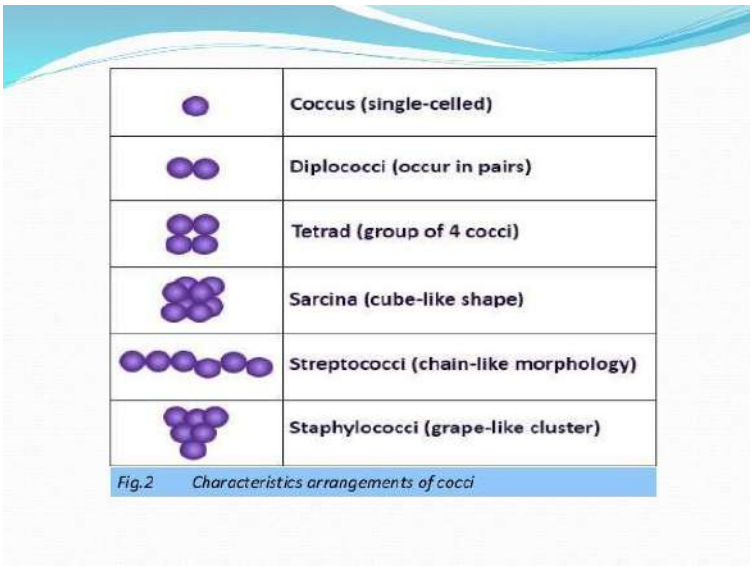
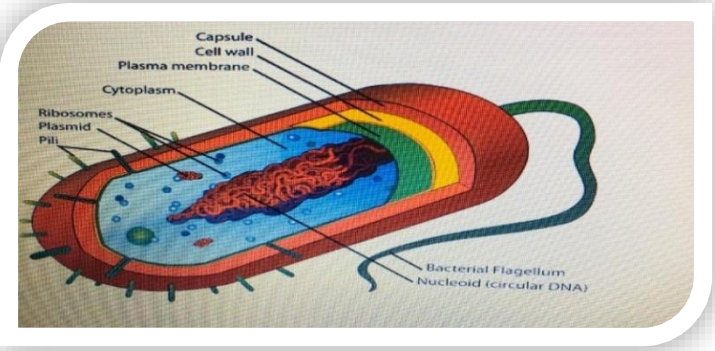


Fig.2 Characteristics arrangements of cocci

Structures of the bacteria :

1. **Cell wall:** the cell wall is the outermost component common to the bacteria except Mycoplasma specie's, which are bounded by a cell membrane, not a cell wall. The major component of the bacterial cell wall is peptidoglycan or murein gives the cell shape and provides structural support and surrounds the cytoplasmic membrane. The cell wall provides important ligands for adherence and receptor sites for viruses or antibiotics. The bacterial cell wall is often a target for antibiotic treatment.



The Anatomy of the bacterial structure

The cell walls of bacteria deserve special attention for several reasons:

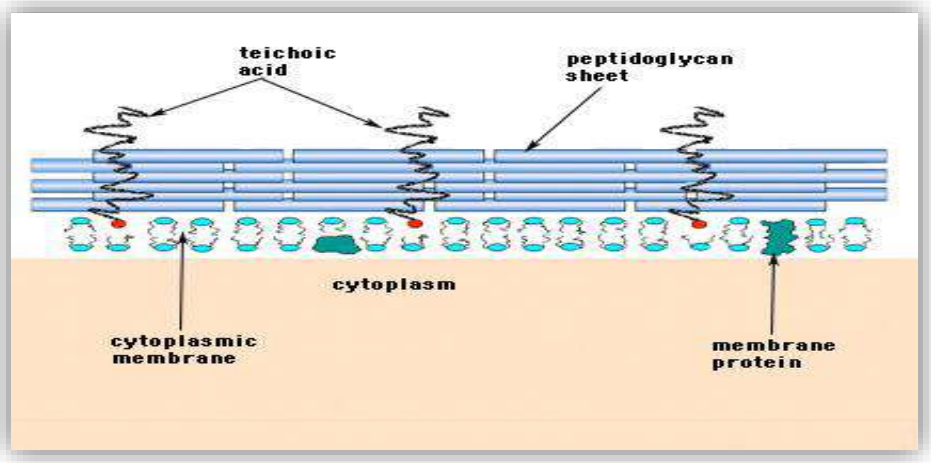
1. They are an essential structure for viability.
2. They are composed of unique components found nowhere else in nature.
3. They are one of the most important sites for attack by antibiotics.
4. They provide ligands for adherence and receptor sites for drugs or viruses.
5. They cause symptoms of disease in human and animals.

6. They provide for immunological distinction and immunological variation among strains of bacteria.

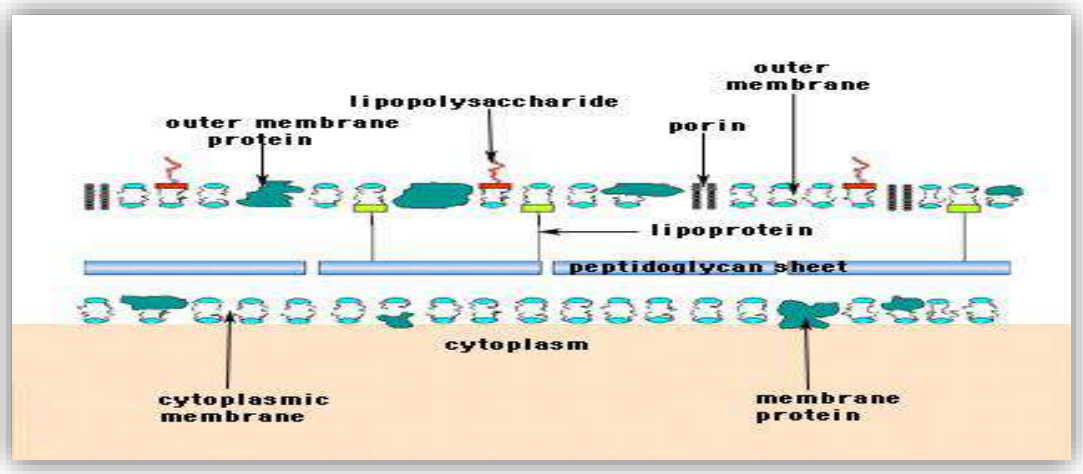
What are the components of the cell wall ???

Peptidoglycan: Many types of peptidoglycan exist. is a huge polymer of disaccharides (glycan) cross-linked by short chains of identical amino acids (peptides) monomers. The backbone of the peptidoglycan molecule is composed of two derivatives of glucose: N-acetylglucosamine (NAG) and N-acetlymuramic acid (NAM) with a pentapeptide coming off NAM and varying slightly among bacteria. The NAG and NAM strands are synthesized in the cytosol of the bacteria. They are connected by inter-peptide bridges. All Bacterial peptidoglycans contain N-acetylmuramic acid, which is the definitive component of murein. The cell walls of Archaea may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never do they contain murein. This feature distinguishes the Bacteria from the Archaea. Going further out, the bacterial world divides into two major classes: Gram positive (Gram +) and Gram negative (Gram -).

a. **Cell walls of Gram** – positive bacteria: The peptidoglycan layer is much thicker in Gram positive than in Gram – negative bacteria. The Gram-positive Bacteria (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure), the cell wall consists of several layers of peptidoglycan. Running perpendicular to the peptidoglycan sheets is a group of molecules called **teichoic acids** which are unique to the Gram-positive cell wall. (see figure below).



b. **In the Gram-negative Bacteria** (which do not retain the crystal violet), the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is toxic to human and animals. (see figure below).



2. **Cytoplasmic membrane**: Just inside peptidoglycan, the **bacterial cytoplasmic membrane** is composed of a phospholipid bilayer and proteins and encloses the contents of the **bacterial** cell. Hydrophobic in nature, it acts as a barrier, preventing the leakage of the hydrophilic **cytoplasmic** constituents and protecting the inside of the cell from environmental in salt.
3. **Mesosome:** this invagination of the cytoplasmic membrane is important during cell division.

4. **Cytoplasm:** the cytoplasm has two distinct areas when seen in the electron microscope:

a. An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmid.

b. An inner, nucleoid region which composed of DNA.

5. **Ribosomes :** bacterial ribosomes are the site of protein synthesis as eukaryotic, but they are differ from eukaryotic ribosomes in size and chemical composition. Bacterial ribosomes are 70S in size, with 30S and 50S subunits. Whereas the eukaryotic ribosomes are 80S in size, with 40S and 60S subunits.

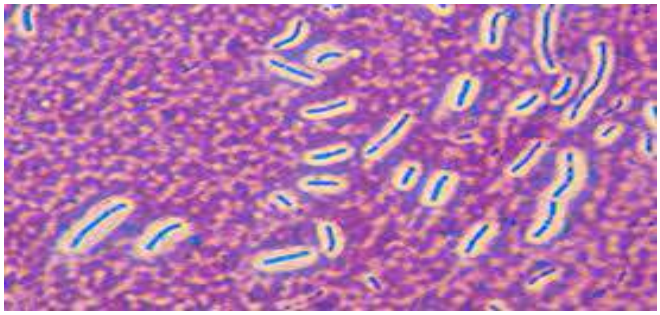
6. **Granules :** the cytoplasm contains several different types of granules that serve as storage areas for nutrient and stain characteristically with certain dyes.

7. **Nucleoid:** the nucleoid is the area of the cytoplasm in which DNA is located. The DNA of prokaryotes is a single , circular molecule.

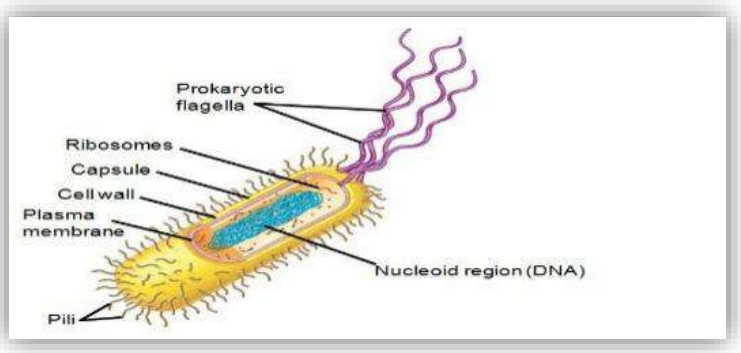
8. **Plasmids:** extrachromosomal, double- stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome.

Specialized structures outside the cell wall:

1. **Capsule:** The **bacterial capsule** is a very large structure of many **bacteria**. It is a polysaccharide layer that lies outside the cell envelope. It considered as antiphagocytic agent and it can be the cause of various diseases.



2. **Flagella:** A **flagellum** (plural: **flagella**) is a long, whip-like structure that helps some single celled organisms move. It is composed of microtubules. They help propel cells and organisms in a whip-like motion. The **flagellum** of eukaryotes usually moves with an "S" motion, and is surrounded by cell membrane.



3. **Pili:** The pilus is a hair-like structure associated with bacterial adhesion and related to bacterial colonization and infection. Pili are primarily composed of oligomeric pilin proteins, which arrange helically to form a cylinder.
4. **Glycocalyx (slime layer):** The **glycocalyx**, is a glycoprotein and glycolipid covering that surrounds the cell membranes of some bacteria to adhere firmly to various structures.
5. **Spores : Spores** are single-celled reproductive units produced by many different organisms, including plants, fungi, and bacteria. **Spores** are primarily used for asexual reproduction, although some bacterial groups use **spores** to survive harsh conditions.

Growth and Nutrition of the Bacteria

- **Growth of the bacteria :**

Bacteria reproduce by binary fission, a process by which one parent cell divides to form two progeny cells. Because one cell gives rise to two progeny cells, bacteria are said to undergo exponential growth (logarithmic growth). The concept of exponential growth can be illustrated by the following relationship:

Number of cells : 1 2 4 8 16

Exponential: 2^0 2^1 2^2 2^3 2^4

Thus, one bacterium will produce 16 bacteria after generations. The doubling (generation) time of bacteria ranges from as little as 20 minutes for ex. *Escherichia coli* (*E coli*) to more than 24 hours for *Mycobacterium tuberculosis*. The exponential growth and the short doubling time of some organisms result in rapid production of very large numbers of bacteria. For example, one E coli organism will produce over 100 progeny in about 3 hours and over 1 million 7 hours. The doubling time varies not only with species but also with amount of nutrients, the temperature, the pH, and other factors.

The growth cycle of bacteria has four major phases. If a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at a frequent intervals, the typical phases of a standard growth curve are:

1. The first phase is lag, during which vigorous metabolic activity occurs but cells do not divide. This can last for a few minutes to many hours.

2. The log phase (logarithmic) is when rapid cell division occurs. Beta lactam drugs, such as penicillin, act during this phase because the drugs are effective when cells are making peptidoglycan i.e, when they are dividing.
3. The stationary phase occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state. cells grown in a special apparatus called chemostat, into which waste products are removed continuously , and can remain in the log phase and do not enter the stationary phase.
4. The final phase is the death phase, which is marked by a decline in the number of viable bacteria.

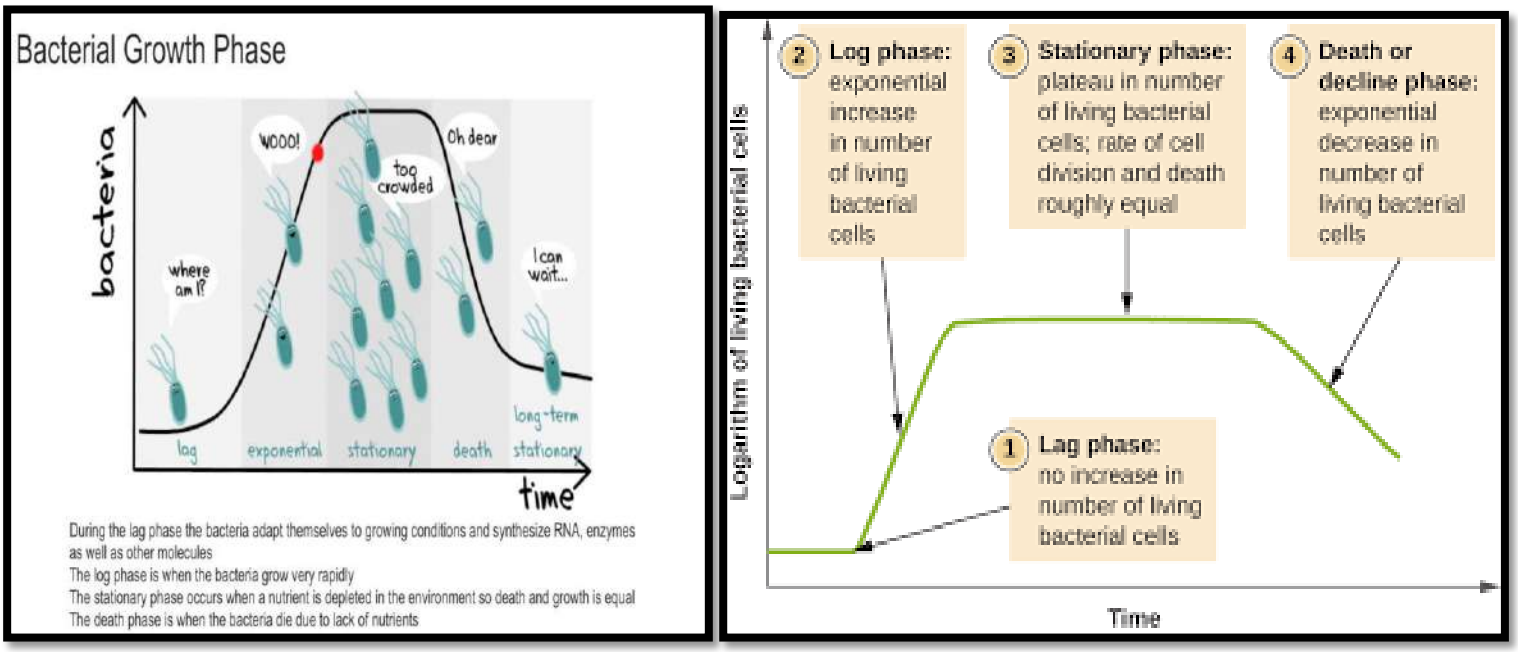


Figure of the bacterial growth phases

Factors affecting bacterial growth:

I. Nutrition.

II. Physical factors.

I. Nutritional requirements of the bacteria :

Generally, every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. Many bacteria can be grown the laboratory in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth.

Bacteria, like all living cells, require energy and nutrients to build proteins and structural membranes and drive biochemical processes. Bacteria require sources of carbon, nitrogen, phosphorous, iron and a large number of other molecules. Carbon, nitrogen and water are used in the highest quantities.

All bacteria requires two things of the growth :

A. **Source of energy.**
B. **Source of a matter for building cells ex. C,O,N,S,P,H, in addition to trace minerals, water and growth factors.**

A.Source of energy.

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2.

Based on the carbon source bacteria are classified as:

1. **Autotrophs**: Organisms that use CO_2 as a sole source of carbon needed for growth.
2. **Heterotrophs :** Organisms that use organic carbon as source of carbon needed for growth .

Thus, on the basis of carbon and energy sources for growth four major nutritional types of bacteria are defined in the following table:

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Organisms that use radiant energy (light) and CO2 are called Photoautotrophs. Organisms that use radiant energy (light) and (oxidize) an organic form of carbon are called Photoheterotrophs ,while the organisms that utilize Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S as source of energy and use CO2 as the source of carbon is called chemoautotrophs and the organisms that utilized organic compounds as the source of both energy and carbon source is called Chemoheterotrophs.

What are the organic and inorganic compounds?

In chemistry, **organic compounds** are generally any chemical compounds that contain carbon-hydrogen bonds in which one or more atoms of carbon are covalently linked to atoms of other elements, most commonly hydrogen, oxygen, or nitrogen. Due to carbon's ability to catenate (form chains with other carbon atoms), millions of organic compounds are known such as: carbohydrates, lipids, proteins, and nucleic acids.

Inorganic compounds: are generally any chemical compounds that lack carbon-hydrogen bonds such as water.

B.Source of a matter for building cells ex. C,O,N,S,P,H, in addition to trace minerals, water and growth factors :

- **Carbon (C)**: The carbon is the structural backbone of organic compounds that make up a living cells.

- **Oxygen (O):** bacteria show a great deal of variation in their gaseous requirements. Oxygen is used by aerobic bacteria during cellular respiration as a final electron receptor.
- **Nitrogen (N)** : nitrogen is used for the synthesis of proteins, amino acids , DNA, RNA and ATP.
- **Minerals:** ex. Sulfur : needed to synthesizes sulfur – containing amino acids and certain vitamins.
- **Phosphorus** : is a phosphorus is an essential elements for nucleic acid synthesis and others processes such as construction of the phospholipids ,DNA and RNA.
- **Trace elements** : to support bacterial growth such as potassium magnesium, calcium and iron.
- **Water** : is essential for cell cmponents.
- **Growth factors** : are organic compounds such as:

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

2. Amino acids: required for the synthesis of proteins.

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

II. Physical conditions required for growth :

Certain physical conditions affects the type and amount of growth which includes:

a. Temperature: enzyme activity depends on the temperature of the environment, and microorganisms are classified in three groups according to their temperature performances :

- Psychrophilic organisms : prefer cold temp. about 0 C° to 20 C°.

- Mesophilic organisms : prefer temp. at 20 C°40C°. most pathogenic bacteria grows best at 37 C°.
- Thermophilic organisms : prefer temp. higher than 40 C°.

b. Gaseous requirement such as:

- Oxygen (O) : bacteria may be classified into four groups based on their needed or not for O:
  1. Obligate aerobes : bacteria that grow only in the presence of oxygen, they obtain energy through aerobic respiration.
  2. Microaerophilic bacteria : bacteria that requires a low concentration of the oxygen (2% to 10%) for their growth.
  3. Obligate anaerobes bacteria : bacteria that grow only in the absence of oxygen, they obtain their energy through anaerobic respiration or fermentation.
  4. Facultative anaerobes bacteria: are bacteria that grow with or without the presence of oxygen, but grow best with oxygen.
  - Carbon dioxide (CO2): all bacteria requires CO2 for their growth. But certain bacteria are called capnophilic it means that of does not require more / additional supplementation of CO2 ex, Brucella spp. which needed only (5-10%)of CO2 for their growth.

c. pH: microorganisms can be placed in one of the following groups based on their optimum pH requirements:

1. Neutrophils: grow best at a pH range 5-8. Most pathogenic bacteria are neutrophils.
2. Acidophils : grow best ate a pH below 5.5.
3. Alkaliphils : : grow best ate a pH above 8.5.

d. Osmosis : Osmosis is a physical phenomenon of great importance in biology, and describes the movement of water across a semi-permeable membrane. A semi-permeable membrane is a barrier that allows water molecules to pass through, but not salt ions (sodium and chloride).

Fourth lecture of Microbiology 1

Prepared by Assist. Prof. Dr. Aida Hussain

Physiology and Metabolism of the bacteria:

Not too many of us think about the survival of bacteria or how it takes place. All living organisms need ways to get nutrients and energy, including bacteria. Microbial growth requires the polymerization of biochemical building blocks into proteins, nucleic acids, polysaccharides, and lipids. The building blocks must come preformed in the growth medium or must be synthesized by the growing cells.

Microbial metabolism: Is the means by which a microbe obtains the energy and nutrients (e.g. carbon) it needs to live and reproduce.

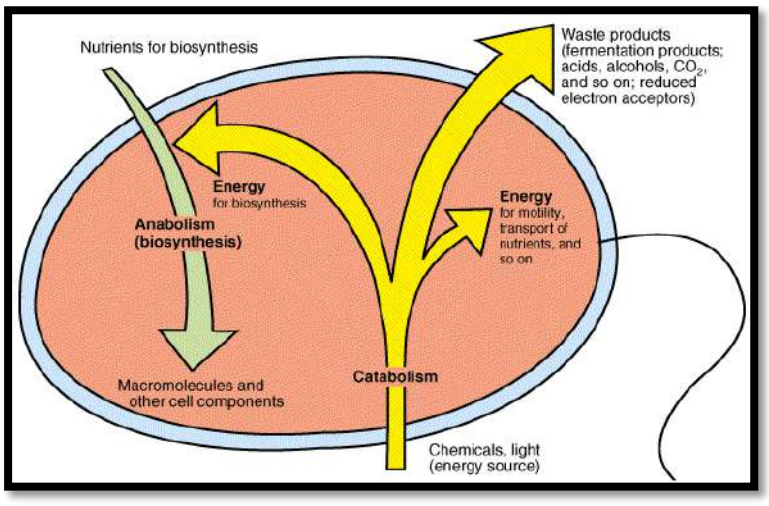
Microbes use many different types of metabolic strategies and species can often be differentiated from each other based on metabolic characteristics.

All living cells depend on biochemical reactions to maintain homeostasis. All of the biochemical reactions in an organism are collectively referred to as metabolism, **which is of 2 basic types:**

Catabolism : Is the set of metabolic pathways that breaks down molecules into smaller units that are either oxidized to release energy or used in other anabolic reactions.

Anabolism: Synthesis of more complex compounds by using of energy.

The relationship between catabolism and anabolism is illustrated in the fowling figure.



Catabolism and anabolism relationship

Energy Generating Patterns: After sugars are made or obtained, they are the energy source of life. Breakdown of sugar (catabolism) occurs in different ways:

1. Cellular respiration:

Cellular respiration is a process that uses oxygen, nitrate, or sulfate to break down nutrients to generate a cell's energy. If oxygen is used, it is called aerobic cellular respiration (that utilized by aerobic bacteria and even facilitative bacteria). If oxygen is not used, it is called anaerobic cellular respiration or fermentation.

In the process of breaking down nutrients such as glucose, carbon dioxide and water are generated. Carbon dioxide and water are waste products of aerobic cellular respiration. When people exhale carbon dioxide (CO2), or sweat, you are seeing by-products of cellular respiration in human cells. Bacteria does the same thing, it just doesn't breathe or sweat, so we don't usually think about it generating energy in the same way we do.

Preparing of Cellular respiration:

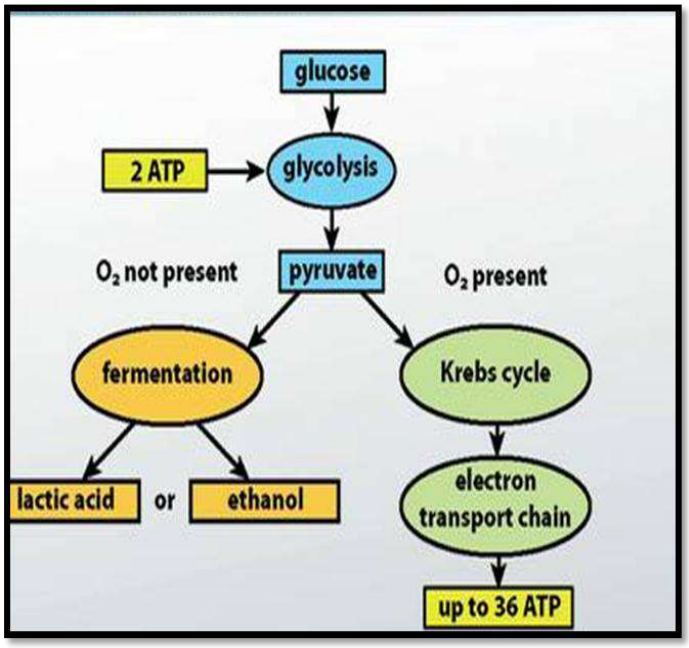
Three steps of cellular respiration make us an understanding of how cells get energy from glucose in short. As you know, plants get their food by a process called photosynthesis. Photosynthesis helps plants to store energy in the form of glucose. Animals also have glucose where energy is stored and used for growing.

Now, you might have a few questions about living things. Living things make use of this energy by a process called **cellular respiration**. Cellular respiration plays an important role in releasing the energy to break down glucose to make ATP (Adenosine Triphosphate).

Adenosine Triphosphate, also knew as, ATP is an organic compound, which provides energy in living cells in the body.

a. Glycolysis : The term of glycolysis means, "splitting glucose" and it is important for cellular respiration. It's the first step in breaking down of glucose to obtain enough energy through cellular respiration in bacteria. This set of reactions occurs **in the cytosol of the cytoplasm** of bacteria. Glycolysis is a catabolic pathway by which sugars such as glucose (& several other "food" sources) are broken down into two 3 - Carbon molecules of pyruvic acid (or pyruvate) releases energy to yield 2 ATP per glucose also transfers high energy electrons (+ H) to NAD (nicotinamide adenine dinucleotide) + to yield 2 NADH.

Process of cellular respiration in the bacteria

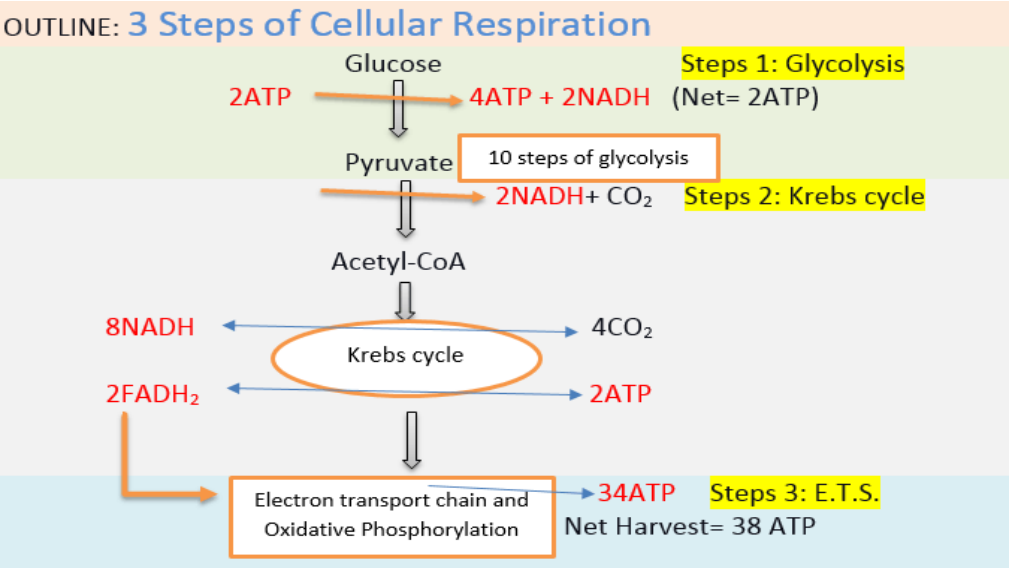


The product of glycolysis, pyruvate, can be broken down further to generate even more energy. Two possible outcomes for pyruvate are the fermentation or Krebs cycle. **Fermentation** : generates some energy for the cell, but it also generates toxic products like alcohol, acetic acid (or vinegar), and lactic acid that have to be cleaned up, or removed from the cell.

b. The Krebs cycle : Is the second preparatory step for cellular respiration in aerobic bacteria. The Krebs cycle also occurs in the cytoplasm of bacteria. it released the stored energy by the method of oxidation of acety l- CoA.

The Krebs cycle starts with acetyl-CoA, which reacts with the four-carbon molecule known as OAA (Oxaloacetate). During the bonding with OAA, it produces citric acid that includes six carbon atoms. Consequently, the Krebs cycle is also known as the Citric Acid cycle. This acetyl-CoA comes from pyruvic acids, the final product of glycolysis. Pyruvic acid don't participate directly in the reactions of Krebs cycle. It first convert to acetyl-CoA. Acetyl-CoA enters the Krebs cycle.

In addition to generating ATP, the Krebs cycle and glycolysis generate hydrogen ions (H+), and electrons. The electrons and H+ are harvested in cellular respiration to generate energy on the electron transport chain.



Steps of cellular respiration

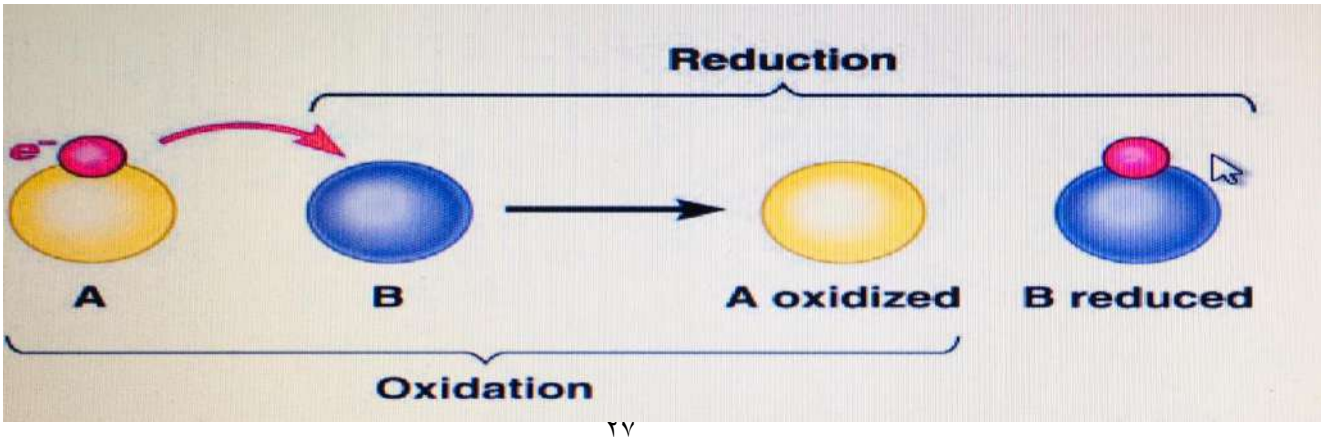
- **NAD**H (Nicotinamide adenine dinucleotide) : High energy electron carrier or (is a crucial coenzyme in making ATP) used to transport electrons generated in Glycolysis and Krebs Cycle to the Electron Transport Chain. It exists in two forms in the cell: NAD+ and NADH. In cellular respiration, NAD+ is one of the oxidizing agents used to remove electrons from organic molecules. When NAD+ gains electrons, it is reduced to NADH .
- **FADH2** : (flavin adenine dinucleotide) : High energy electron carrier (is a crucial coenzyme in making ATP along with NADH molecule) used to transport electrons generated in Glycolysis and Krebs Cycle to the Electron Transport Chain.

c. **Electron transport chain (**ETC): Cellular respiration requires the electron transport chain, or ETC, which is a series of enzymes that pump electrons and hydrogen (H+) generated in glycolysis and the Krebs cycle.

The net harvested of cellular respiration : each molecule of glucose makes 38 molecules of ATP. Here is the equation below:

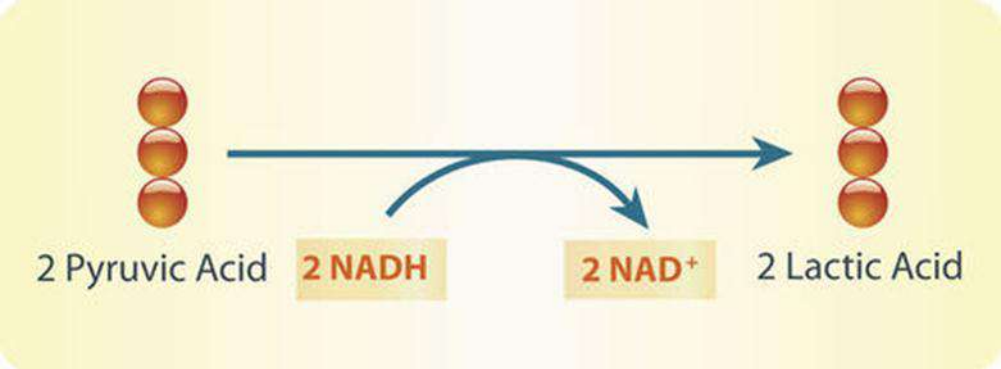
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \approx 38$ ATP

Oxidation/Reduction: glucose is captured as high energy electrons (e -) by electron carriers such as NADH & FADH2 • when a molecule receives or gains electrons it is said to be reduced • while a molecule that gives up electrons (i.e., loses H) is said to be oxidized.



2. Anaerobic respiration:

All anaerobic bacteria make energy without oxygen. They do this in one of two ways, either through lactic acid or alcoholic fermentation. During lactic acid fermentation, cells use a molecule of NADH to take electrons from glucose. The NADH use the energy stored in the electrons to make ATP, and convert glucose to pyruvate. This process is called glycolysis and is the first step in all forms of cellular respiration. In the Lactic acid fermentation converts pyruvate to lactic acid, and regenerates NAD+ from NADH (Lactic acid is formed by the reduction of pyruvate). *Only the Lactobacillus bacteria use this type anaerobic respiration which ultimately produce a yogurt as the waste of the bacteria.*



The other way an aerobes make energy is through alcoholic or ethanol fermentation, like in lactic acid fermentation, NADH takes electrons from glucose and turns it into pyruvate during glycolysis. From here, the pyruvate is converted to ethanol instead of lactic acid. This is the same ethanol that we find in beverages like wine and beer.

Lecture Fifth **Microbiology**

Prepared by : Assist. Prof. Dr. Aida Hussain Ibrahim

Bacterial pathogenesis and virulence factors

Pathogenesis of the bacteria:

Microbial pathogenicity has been defined as either the structural and biochemical mechanisms whereby microorganisms can cause disease or the infectious process and mechanisms that's leading to the development of signs and symptoms of bacterial disease.

Pathogen: A pathogen is usually defined as a tiny living organism, such as a bacterium, virus, fungi, and parasitic that causes or can causes disease.

Another definition of pathogen: A microbe that can cause damage in a host tissue. Some microbes are highly pathogenic, whereas others cause disease rarely (low pathogenic).

Pathogens can be classified as either primary pathogens or opportunistic pathogens.

1. **Primary pathogen**: Primary pathogen can cause disease in a host regardless of the host's resident microbiota or immune system.
2. **Opportunistic pathogen**: Opportunistic pathogen can only cause disease in situations that compromise the host's defenses, such as the body's protective barriers, immune system, or normal microbiota.

Individuals susceptible to opportunistic infections include the very young, the elderly, women who are pregnant, patients undergoing chemotherapy, people with immunodeficiencies (such as acquired immunodeficiency syndrome [AIDS]), patients who are recovering

from surgery, and those who have had a breach of protective barriers (such as a severe wound or burn).

Characters of pathogen:

1. Pathogenic organism should be able to enter the host body.
2. Pathogenic organism should be able to multiply in tissue.
3. Pathogenic organism should be able to damage the tissue.
4. They must be capable to resist the host defense.

Virulence: It is referred to as the degree of the ability of microbe to produce disease. The virulence is determined by virulence factors such as capsule, enzymes and toxins.

Infection, in general, is the presence and multiplication of microbe within host.

Virulence factors help bacteria to:

(1) Invade the host,

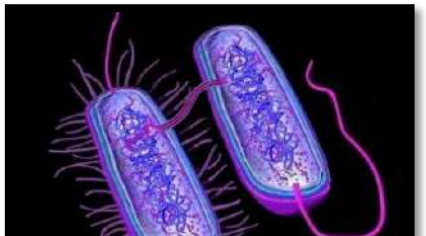
(2) Cause disease.

(3) Evade host defenses mechanisms.

Some important virulence factors that have a role in pathogenesis of bacteria:

a. **Adherence Factors**: Many pathogenic bacteria colonize mucosal sites by using *pili* (fimbriae) to adhere to cells.

A fimbriae is a hair like appendages (short pilus) found on the surface of many bacteria, that is used to attach the bacterium to a surface. See the following figure:



Pili (Fambriae) of bacteria

b. **Invasion Factors**: Microbial invasion can be facilitated by virulence factors such as microbial adherence, resistance to antimicrobials, and defects in host defense mechanisms.

c. **Capsules**: Many bacteria are surrounded by capsules, it is a polysaccharide layer that lies outside the cell wall of bacteria. Capsule mediate adherence of pathogen to specific receptors on host cell surface. so that it prevent the phagocytes from adhering to bacteria, therefore act as antiphagocytic factor.

d. **Enzymes**: Several enzymes secreted by bacteria play a role in the pathogenesis of bacteria which facilitate local tissue damage such as (protease, hyaluronidase, coagulase, elastase, ect.).

e. Toxins: Toxins produced by bacteria are generally classified into two groups:

1. **Endotoxins**: Integral part of the cell wall of gram - negative bacteria. Released on bacterial death and in part during growth such as polysaccharides of gram negative bacteria.

2. **Exotoxins**: Excreted by both gram - negative and gram positive bacteria. There are several types of proteins: toxins and enzymes produced and /or secreted from pathogenic bacteria. Major categories include cytotoxins, neurotoxins, and enterotoxins.

f. **Siderophores**: Siderophores are iron-binding factors that allow some bacteria to compete with the host cells for iron, which is bound to hemoglobin, transferrin, and lactoferrin such as *Pseudomonas aeruginosa*.

Source of Infection: The starting point for the occurrence of a disease or infection is the existence of source of **infection**.

The source of infection is defined as "the person, animal, object or substance from which an infectious agent passes or is disseminated to the host (immediate source)."

There are two types of source of infection:

1. **Endogenous source**: a person can be a source of infection (person serve as the origin from which a host acquires the infection, either for him /herself.
2. **Exogenous source:** in contrast to endogenous source, exogenous source involves a pathogen entering a patient's body from his/her environment. These pathogens can be introduced through another infected persons, contaminated device, healthcare worker, surface, or other vector.

Objects may be sources of infection; food, water, air-conditioning systems, showers, medical instruments, recreational waters, door knobs, cotton handkerchiefs, etc,

Steps of initiation of infection or pathogenesis:

1. Transmission from an external source into the portal of entry.
2. Colonization of the bacteria to the host cells.
3. Adherance of the bacteria to the host cells.
4. Invasiveness of the bacteria to host tissues.

5. Toxigenity of the bacteria.
6. Ability to evade host's immune system.

1. Transmission from an external source into the portal of entry:

An infectious agent may be transmitted from its natural reservoir to a susceptible host in different ways. There are different classifications for modes of transmission. Here is one classification:

a. Direct.
   - Direct contact.
   - Droplet spread.

b. Indirect
   - Airborne.
   - Vehicleborne.
   - Vectorborne (mechanical or biologic).

a. Direct transmission, In direct transmission an infectious agent is transferred from a reservoir to a susceptible host by direct contact or droplet spread. Direct contact occurs through :
   - skin-to-skin contact, kissing, and sexual intercourse.
   - Direct contact also refers to contact with soil or vegetation harboring infectious organisms.
   - Droplet spread refers to spray with relatively large, short-range aerosols produced by sneezing, coughing, or even talking. Droplet spread is classified as direct because transmission is by direct spray over a few feet, before the droplets fall to the ground. Such as Covid 19 can be easily transmitted by areosoles and dropplets.

b. Indirect transmission: refers to the transfer of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects (vehicles), or animate intermediaries (vectors). Airborne

transmission occurs when infectious agents are carried by dust or droplet nuclei suspended in air. Airborne dust includes material that has settled on surfaces and become resuspended by air currents as well as infectious particles blown from the soil by the wind.

2. **Colonization** : The first stage of microbial infection is colonization, the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human and animal hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva.
3. **Bacterial Adherence to Mucosal Surfaces** : Adherence of the bacteria to mucosal surfaces of host helps microorganisms to establish a base from which it penetrate tissues. In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a receptor and an ligand. The receptors so far defined are usually specific carbohydrate or peptide residues on the eucaryotic cell surface. The bacterial ligand, called an adhesin, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion.
4. **Invasiveness of the bacteria** : Is the ability of a pathogen to invade host tissues. Invasiveness encompasses (1) mechanisms for colonization (adherence and initial multiplication), (2) production of extracellular substances ("invasins"), that promote the immediate invasion of tissues and (3) ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process.
5. **Toxigenesis** : Is the ability to produce toxins. Toxic substances produced by bacteria, both soluble and cell-associated, may be

transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth.

6. **Ability to evade host's immune system**: Pathogens have developed mechanisms to evade the phagocytes in the immune system by many ways such as grow in areas where there are no phagocytes, suppress the inflammatory response, inhibit phagocyte mobility, and "trick" the immune system into thinking the bacteria is a "self" cell.

STERILIZATION AND DISINFECTION

INTRODUCTION

Disinfection and sterilization are essential for ensuring that medical and surgical

instruments do not transmit infectious pathogens to patients. Because sterilization of all patient-care items is not necessary, health-care policies must identify, primarily on the basis of the items' intended use, whether cleaning, disinfection, or sterilization is indicated.

OBJECTIVES

After reading this lesson, you will be able to:

- define terms related to Sterilization and Disinfection
- classify items to be sterilised or disinfected
- discuss different Methods of sterilisation
- discuss Methods of disinfection

DEFINITIONS OF TERMS

Sterilization: *Sterilization* describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.

Disinfection: *Disinfection* describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, **on inanimate** objects.

Cleaning*: Cleaning* is removal of visible soil (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.

Decontamination: *Decontamination* removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

Classification of Materials to be Sterilised / Disinfected

Earle H. Spaulding devised a rational approach to disinfection and sterilization of patient-care items and equipment. This has three categories

1- Critical Items

Critical items confer a high risk for infection if they are contaminated with any microorganism. Thus, objects that enter sterile tissue or the vascular system must be sterile because any microbial contamination could transmit disease. This category includes surgical instruments, etc.

2- Semi-critical Items

Semi-critical items contact mucous membranes or non-intact skin. This category includes respiratory therapy and anaesthesia equipment, some endoscopes, laryngoscope blades, esophageal manometry probes, cystoscopes, anorectal manometry catheters, and diaphragm fitting rings etc.

3- Noncritical Items

Noncritical items are those that come in contact with intact skin but not mucous membranes. Intact skin acts as an effective barrier to most microorganisms; therefore, the sterility of items coming in contact with intact skin is "not critical." They can be Non-critical patient care items: bedpans, blood pressure cuffs, crutches and Computers Non-critical environmental surfaces.

METHODS OF STERILIZATION

The various methods of sterilization are:

1. Physical Method

(a) Thermal (Heat) methods

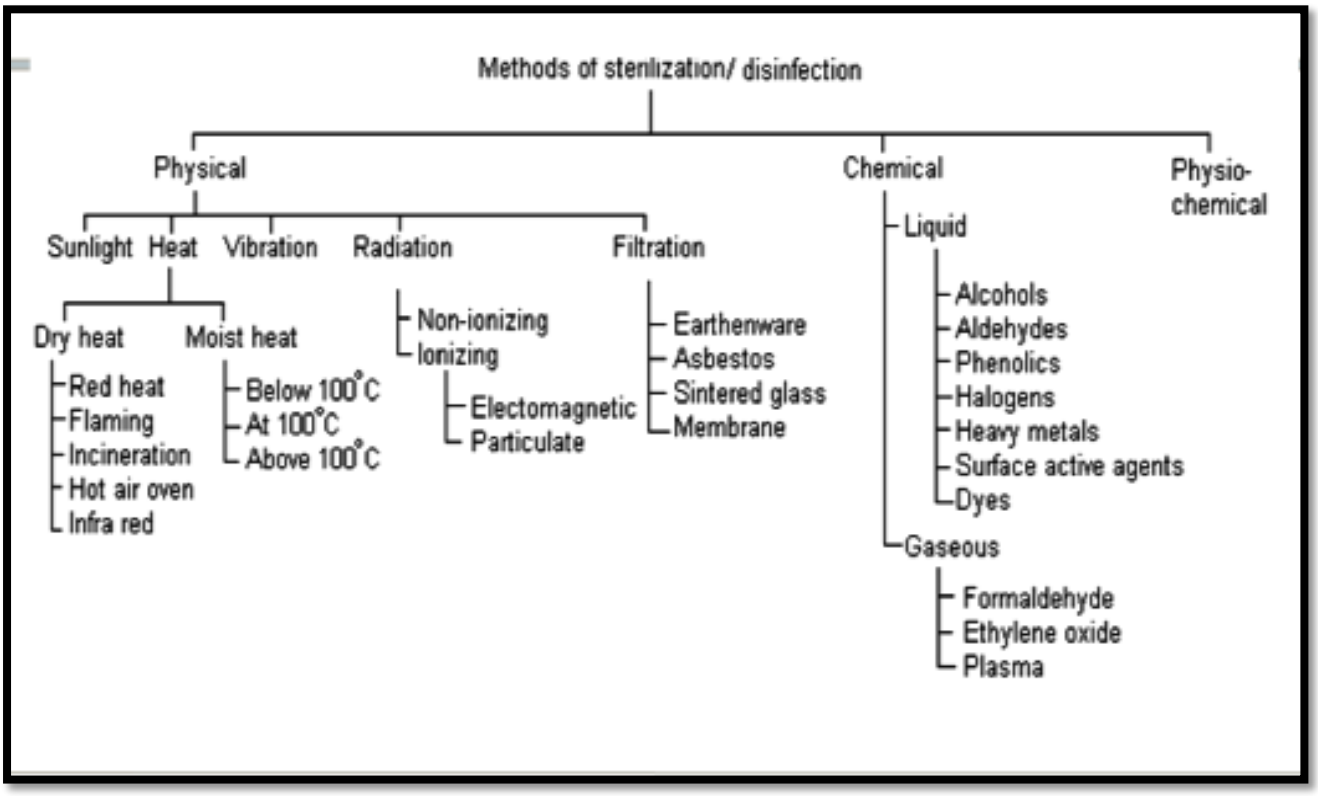
(b) Radiation method

(c) Filtration method

(d) Sunlight

2. Chemical Method

3. Gaseous method.



Heat Sterilization

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This method of sterilization can be applied only to the thermostable products,

but it can be used for moisture-sensitive materials for which dry heat (160- 180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used. The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

Dry Heat Sterilization: Examples of Dry heat sterilization are :

1. Incineration
2. Red heat
3. Flaming
4. Hot air oven

It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments. It is also used for sterilizing non-aqueous thermo-stable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Hot-air oven

Dry heat sterilization is usually carried out in a hot air oven, which consists of An insulated chamber surrounded by an outer case containing electric circle.

Moist Heat Sterilization: Moist heat may be used in three forms to achieve

microbial inactivation

1. Autoclaving
2. Boiling water/ steam at atmospheric pressure
3. Hot water below boiling point

Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

RADIATION STERILIZATION

Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light), particulate radiation (e.g. accelerated electrons).The major target for these radiation is microbial DNA. Gamma rays and electrons cause ionization and free radical production while UV light causes excitation.

Radiation sterilization with high energy gamma rays or accelerated electrons has proven to be a useful method for the industrial sterilization of heat sensitive products. But some undesirable changes occur in irradiated products, an example is aqueous solution where radiolysis of water occurs. Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastic syringes and dry pharmaceutical products. UV light, with its much lower energy, and poor penetrability finds uses in the sterilization of air, for surface sterilization of aseptic work areas, for

treatment of manufacturing grade water, but is not suitable for sterilization of pharmaceutical dosage forms.

Filtration Sterilization

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers.

Membrane filters are used for sterility testing.:

Application of filtration for sterilization of gases: HEPA (High efficiency particulate air) filters can remove up to 99.97% of particles >0.3 micrometer in diameter. Air is first passed through prefilters to remove larger particles and then passed through HEPA filters. The performance of HEPA filter is monitored by pressure differential and airflow rate measurements.

There are two types of filters used in filtration sterilization:

(a) Depth filters: Consist of fibrous or granular materials so packed as to form twisted channels of minute dimensions. They are made of diatomaceous earth, unglazed porcelain filter, sintered glass or asbestos.

(b) Membrane filters: These are porous membrane about 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or some other synthetic material.The membranes are supported on a frame and held in special holders. Fluids are made to

transverse membranes by positive or negative pressure or by centrifugation.

Application of filtration for sterilization of liquids: Membrane filters of 0.22 micrometer nominal pore diameter are generally used, but sintered filters are used for corrosive liquids, viscous fluids and organic solvents. The factors which affects the performance of filter is the titre reduction value, which is the ratio of the number of organism challenging the filter under defined conditions to the number of organism penetrating it. The other factors are the depth of the
membrane, its charge and the tortuosity of the channels.

CHEMICAL METHODS OF DISINFECTION

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used at appropriate concentration for appropriate duration can be used for sterilization and are called sterilant liquids. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

An ideal antiseptic or disinfectant should have following properties:

1. Should have wide spectrum of activity
2. Should be able to destroy microbes within practical period of time
3. Should be active in the presence of organic matter
4. Should make effective contact and be wettable
5. Should be active in any pH
6. Should be stable
7. Should have long shelf life
8. Should be speedy
9. Should have high penetrating power
10. Should be non-toxic, non-allergenic, non-irritative or non-corrosive
11. Should not have bad odour

12. Should not leave non-volatile residue or stain

13. Efficacy should not be lost on reasonable dilution

14. Should not be expensive and must be available easily

Such an ideal disinfectant is not yet available. The level of disinfection achieved depends on contact time, temperature, type and concentration of the active ingredient, the presence of organic matter, the type and quantum of microbial load. The chemical disinfectants at working concentrations rapidly lose their strength on standing.

Classification of disinfectants:

1. Based on consistency

(a) Liquid (E.g., Alcohols, Phenols)

(b) Gaseous (Formaldehyde vapour)

2. Based on spectrum of activity

(a) High level

(b) Intermediate level

(c) Low level

3. Based on mechanism of action

(a) Action on membrane (E.g., Alcohol, detergent)

(b) Denaturation of cellular proteins (E.g., Alcohol, Phenol)

(c) Oxidation of essential sulphydryl groups of enzymes (E.g., H2O2, Halogens)

(d) Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Formaldehyde)

(e) Damage to nucleic acids (Formaldehyde).

Alcohols

Mode of action: Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol

Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores,hence is useful in disinfecting inoculation hoods.

Disadvantages: Skin irritant, volatile (evaporates rapidly), inflammable

Phenol

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol).

Applications: Joseph Lister used it to prevent infection of surgical wounds.Phenols are coal-tar derivatives. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal,mycobactericidal but are inactive against spores and most viruses.

Disadvantages: It is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water.

Hydrogen Peroxide

Mode of action: It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.

Application: It is used at 6% concentration to decontaminate the instruments, equipments such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection and deodorising wounds and ulcers. Strong solutions are sporicidal.

Disadvantages: Decomposes in light, broken down by catalase, proteinaceous organic matter drastically reduces its activity.

Antibiotics and chemotherapeutic agents

An antimicrobial agent: Is an agent that kills microorganisms or inhibits their growth.

Antimicrobial medicines can be grouped according to the microorganisms they act primarily against them. For example, antibacterials (antibiotics) are used against bacteria and antifungals are used against fungi and antiviral drugs against viruses. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus *Penicillium notatum* but its discovery was first reported in 1929, and clinical trials first conducted on humans in 1940.

The main classes of antimicrobial agents are:

1. **Disinfectants** ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness.
2. **Antiseptics** (which are applied to living tissue and help to reducing the infection during surgery) such as ethyl alcohol at 95% or isopropanol.
3. **Antibiotics** (which destroy microorganisms within the body).

The term antibiotic was coined from the word „antibiosis" which literally means „ against life". In the past, antibiotics were considered to be organic

compounds produced by one microorganism which are toxic to other microorganisms. And recently the antibiotic can be defined as:

Antibiotics or anti - bacterial are a type of antimicrobial used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. Several antibiotics are also effective against fungi and protozoans, and some are toxic to humans and animals, even when given in therapeutic dosage.

The ideal antibacterial agent should be nontoxic to the host (selective toxicity), non-allergenic, soluble in body fluids, able to be maintained at therapeutic levels, have a low probability of eliciting resistance, long shelf life, and low cost.

Any chemical agent (natural or synthetic) that is used in medicine. Ideally, it should attack microorganisms selectively and not harm host cells.

Sources of antibiotics:

a. Natural drug- one made by microorganisms.

b. Synthetic drugs that is made in the laboratory.

c. Semisynthetic drug-one synthesized partly in the laboratory and partly by microorganisms.

Classification of the antibiotics:

There are several ways of classifying antibiotics but the most common classification schemes are based on their molecular structures, mode of action and spectrum of activity. Others include route of administration (injectable, oral and topical). Antibiotics within the same structural class will generally show similar pattern of effectiveness, toxicity and allergic potential side effects. Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides,

Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones .

A. Classification based on the spectrum of activity :

Each antibacterial drug has a range of microorganisms that it affects. According for this reason antibiotics can be divided into:

1. Broad spectrum antibiotics: Antibiotics are active against several types of microorganisms, e.g, tetracycline are active against many gram negative rods.

2. Narrow spectrum antibiotics*:* Antibiotics are active against one or very few types of microorganisms, eg, vancomycin is primarily used against certain gram - positive cocci namely *Staphylococcus aureus.*

The antibacterial drugs can be subdivided into :

- **Bactericidal antibiotics** : the drugs that kill bacteria.
- **B**acteriostatic antibiotics: the drugs that inhibit their growth or reproduction of bacteria but does not kill them.

B. **Classification based on the Mechanisms of action of antibiotics:**

Microbes are killed through various means:

1. **Inhibition of the cell wall:**

Specific antibacterials interfere with the synthesis of the cell wall, weakening the peptidoglycan scaffold within the bacterial wall so that the structural integrity eventually fails. For example : penicillin's and cephalosporins are β – lactam drugs act by inhibiting transpeptidases, the enzymes that catalyze the final cross – linking step in the synthesis of peptidoglycan.

2. **Inhibition of protein synthesis:**

(**Major groups:** aminoglycosides, tetracyclines, macrolides).

Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human and animals.

This selectivity is due to the differences between bacterial cell (prokaryotic cell) and eukaryotic cell such as human and animal. The ribosomal proteins, RNAs, and associated enzymes of bacteria have 70S ribosome's with large 50S and small 30S sub units, whereas Eukaryotes have 80S ribosome's, each consisting of a small (40S) and large (60S) subunit.

There are two types of dugs :

a. Drugs that act on the 50S subunit such as : Macrolides (erythromycin, etc.) bind reversibly to the 50S subunit. They can inhibit elongation of the protein by the peptidyltransferase, the enzyme that forms peptide bonds between the amino acids.

b. ***Drugs that act on the 30S subunit such as*** : Aminoglycosides (tetracycline) blocks protein translation by tRNAs through binding reversibly to the 30S subunit.

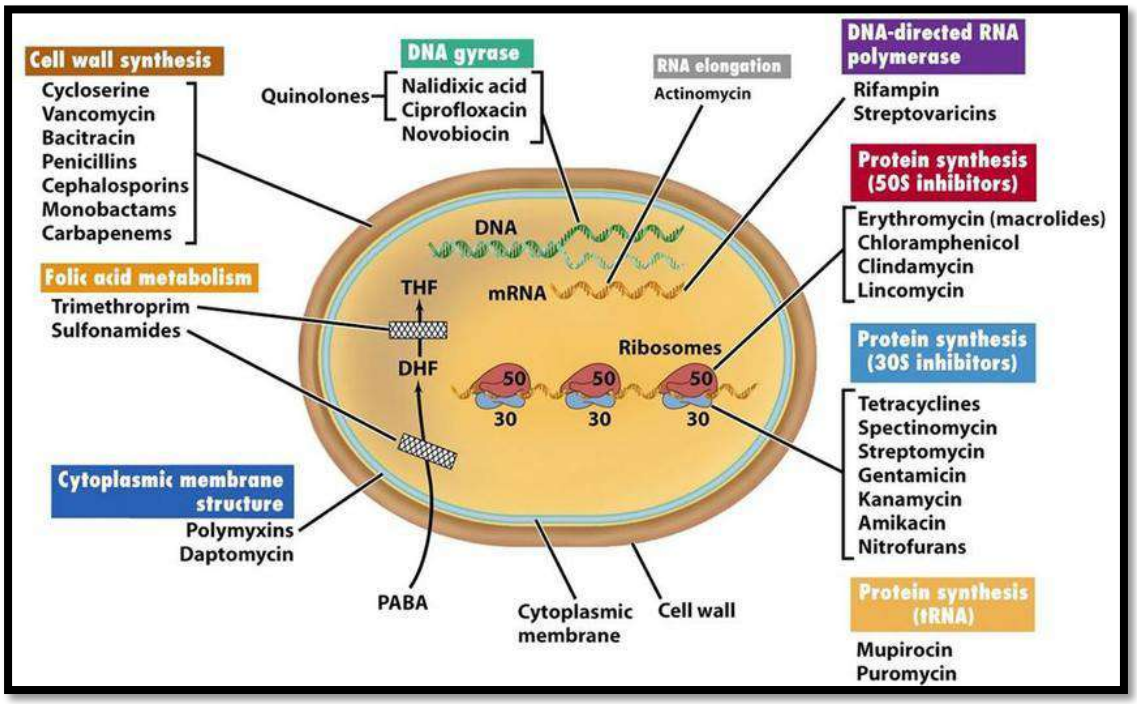
3. Inhibition of nucleic acid synthesis (DNA/RNA):

By three modes:

a. By block the synthesis of tetrahydrofolic acid : which is required as methyl donor in the synthesis of the nucleic acid precursors adenine, guanine, and thymine. For example sulfonamides.
b. Inhibition the DNA synthesis: by inhibiting DNA grase (topoisomerase) such as fluoroquinolones.

c. Inhibition of mRNA synthesis : blocking mRNA synthesis by RNA polymerase such as rifampin drug.

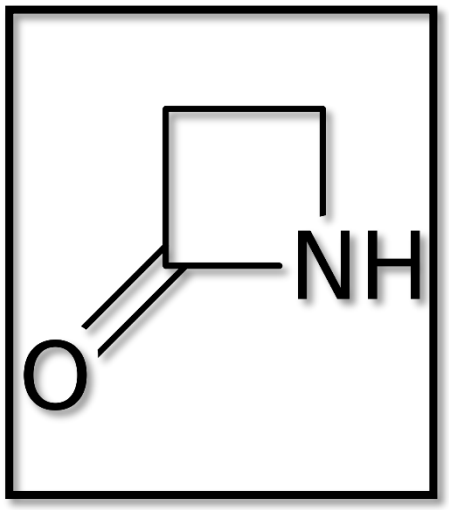
4. **Damaging the plasma membrane** (cell membrane) : Such as polymyxin and Polyenes drugs incorporate into cell membranes causing membrane integrity damage (Pores formation) resulting in cellular lysis.



Antibiotic target sites

C. Classification based on the molecular structure :

1. **Beta-lactams class**: Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive. They interfere with proteins essential for synthesis of bacterial cell wall, and in the process either kills or inhibits their growth. More succinctly, certain bacterial enzymes termed penicillin-binding protein (PBP) are responsible for cross linking peptide units during synthesis of peptidoglycan. Members of beta-lactam antibiotics are able to bind themselves to these PBP enzymes, and in the process, they interfere with the synthesis of peptidoglycan resulting to lysis and cell death.



B - Lactams ring

The most prominent representatives of the beta-lactam class include:

- **Penicillins**: penicillin is the oldest and one of the most commonly used groups of antibiotics at present. They are derived from the mold/fungi Penicillium. Penicillin can be divided into two groups, namely natural and semisynthetic penicillins. Natural penicillins are produced from the fermentation of the fungus *Penicillium chrysogenum*. The semisynthetic penicillins, on the other hand, are prepared from (+)-6-aminopenicillanic acid.
- **Cephalosporins:** Cephalosporin Members of this group of antibiotics are similar to penicillin in their structure and mode of action. They form part of the most commonly prescribed and administered antibiotics; more succinctly, they account for one-third of all antibiotics prescribed and administered.
- **Monobactams:** The discovery of this class of antibiotics was first reported by Skyes and co-workers. The antibiotic was obtained from the bacterium *Chromobacterium violaceum*. They are part of beta-lactam compounds but unlike most other beta-lactams, the beta-lactam ring of monobactams stand alone and is not fused to another ring Aztreonam is the only commercially available monobactam

antibiotic, with a narrow spectrum of activity. Aztreonam is active only against aerobic Gram-negative bacteria such as Neisseria and Pseudomonas.

- **Carbapenems** : This class of antibioticswas discovered out of necessity in 1976. Carbapenems occupy a very important place in our fight against bacterial infections. This is because they are able to resist the hydrolytic action of beta-lactamase enzyme.

2. **Macrolides** : Although, Macrolides are generally broad spectrum, some bacterial species such as *Streptococcus pneumoniae*, have resistance against the antibiotics. Example of members includes Erythromycin, Azithromycin and Clarithromycin.
3. **Tetracyclines** : Tetracycline was discovered in 1945 from a soil bacterium of the genus Streptomyces by Benjamin Duggar .The first member of this class was chlorotetracycline (Aureomycin). Historically, members of this class of antibiotics are grouped into different generations based on the method of synthesis. Those obtained by biosynthesis are said to be First generation such as Tetracycline and Chlortetecycline, ect. Minocycline, and Rolitetracycline are considered Second generation because they are derivatives of semi-synthesis. Those obtained from total synthesis such as Tigecycline are considered to be Third generation. Their target of antimicrobial activity in bacteria is the ribosome. They disrupt the addition of amino acids to polypeptide chains during protein synthesis in this bacterial organelle.
4. **Quinolones**: This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs. They are able to interfere with DNA replication and transcription in bacteria such as norfloxacin, ofloxacin, and ciproxacin.

5. **Sulphonamides** : are reportedly, the first group of antibiotics used in therapeutic medicine, and they still play very important role in medicine and veterinary practice Sulphonamides inhibit both Gram-positive and Gram-negative bacteria such as Nocardia, *E. coli*, Klebsiella, Salmonella, Shigella and Enterobacter, Chlamydia trachomatis and some Protozoa, and are widely used in the treatment of various infections including tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery and some urinary tract infections. Their mode of action is based on the Inhibition of metabolic processes. Sulfonamides interfere with folic acid synthesis by preventing addition of para-aminobenzoic acid (PABA) into the folic acid molecule through competing for the enzyme dihydropteroate synthetase.
6. **Aminoglycosides** : The first drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943 . Streptomycin has been greatly used against *Mycobacterium tuberculosis,* the causal agent of tuberculosis among humans. Aminoglycoside have a broad spectrum of antibacterial activity. They are able to inhibit the protein synthesis in bacteria by binding to one of the ribosomal subunits and are effective against aerobic Gram-negative rods and certain Gram-positive bacteria such as Gentamicin, Neomycin, Tobramycin and Amikacin.

MICROBIAL GENETICS

• **Genetics**: is the science concerned with the cell characteristics, and how they are passed from one generation to the next.

Gene: it is the unit of heredity. It is a segment of DNA that carries, in its nucleotide sequence, information for specific biochemical or physiologic property.

Phenotype: All the heritable physical characters of the organism (Eye colour in humans, resistance to antibiotic in bacteria ….. etc.)

Genotype: It means the information in the DNA that control the phenotype

Compartments of microbial genetics curriculum

• Basic Genetics

• Genetic variation and gene rearrangements.

• Advanced genetics

Molecules of Genetics

• The main molecules of genetics are called nucleic acids.

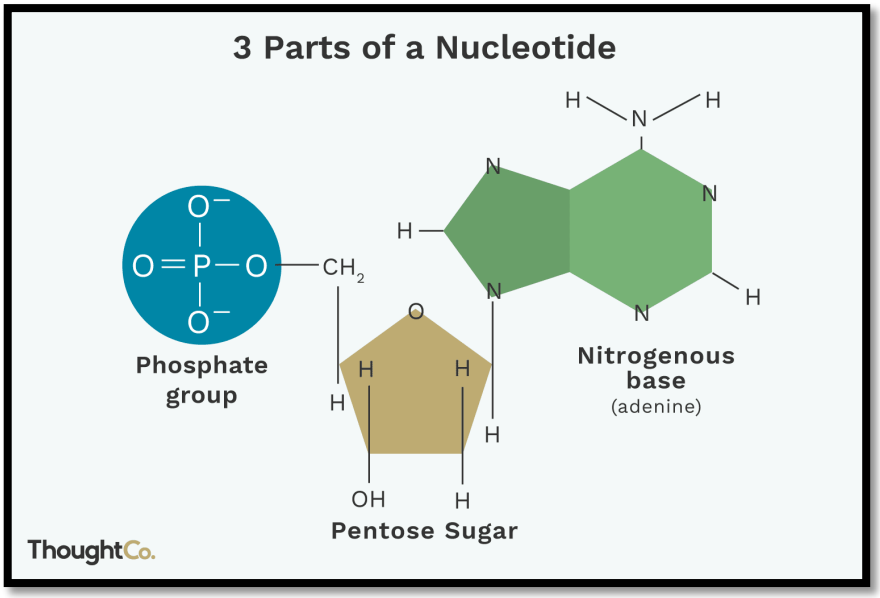
• All the genetic information are stored as a sequence of bases through nucleic acids mainly in DNA and in RNA in some RNA viruses.

- DNA (Deoxyribonucleic acid serves as organism's genetic material.

- It is divided into functional units (genes).

- Most of DNA is double stranded. The two strands held together by hydrogen bonds between A and T or G and C

- It consists of non- identical, complementary base sequences .



The basic structure of DNA molecules is the Nucleotide

1- Sugar: It is a cyclic form of 2-deoxyribose sugar that forms the backbone of the DNA.

2- Phosphate

3- Nitrogenous Bases

- cyclic structure of purine and pyrimidine rings. There are two major purines, adenine (A) andguanine (G),

•Three major pyrimidines, cytosine (C), uracil (U), and thymine (T).

Sugar back bones are linked to each other by phosphodiester bonds, i.e., a single phosphateconnected by ester linkage to two sugars; DNA consists of alternating units of phosphate and 2 deoxy ribose phosphate connects two sugars: bonding the 3' carbon of one sugar and the 5' carbon of the next sugar. DNA is only polymerized 5' to 3' and as antiparallel i.e. one strand in the direction 5' to 3' and the other strand polymerized in the opposite direction.

Prokaryotic DNA molecules: are double helix and circular. This circular double stranded DNA molecules are twisted and compacted through a

process called super coiling "super helicity this is done naturally by topoisomerase enzymes

RNA (Ribonucleic acid):

- Structurally similar to DNA except.

- Most of RNAs are single stranded.

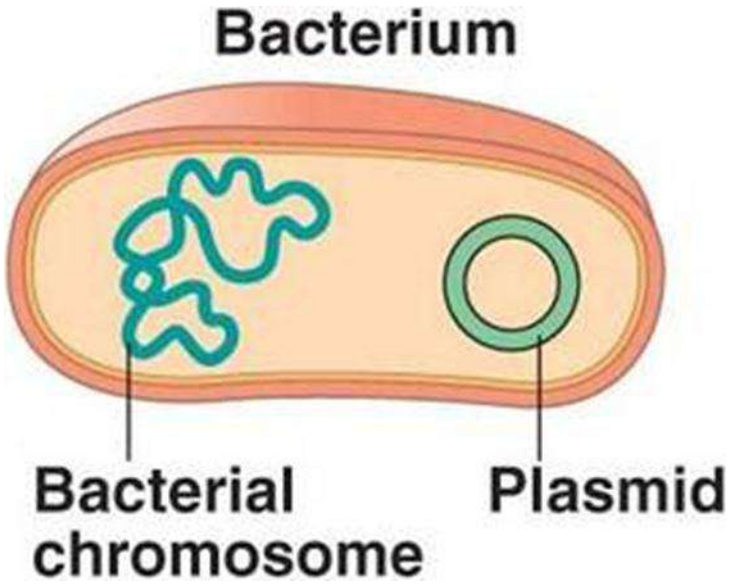
- Sugar is ribose instead of Deoxyribose Uracil base instead of thymine Base.

GENOME

It is the total genetic information in an organism.

Prokaryotic genome (Bacterial): consist of a single copy (Haploid) ciruclar DNA molecule. Range from 580-4600 Kbp

Many bacteria contain extra chromosomal DNA materials as apart of genome called plasmid and transposons.



Eukaryotic genome (e.g Some fungi):

- carried on two or more linear chromosomes separated from the cytoplasm by nuclear membrane.

- Diploid eukaryotic cells contain two homologues copies of each chromosome.

- Eukaryotic cells contain mitochondria and chloroplast .

- Some eukaryotes like yeasts contains plasmids.

- Eukaryotic genes unlike prokaryotes are interrupted by introns.

Viral genome :-

- unlike others, may be of DNA or RNA.

- Capable of survival but no growth in absence of a host cell

- Replication of viral genomes depends on the metabolic energy and the synthetic machinery of the host cell.

- Most viral genomes range in size from 40- 300 Kbp

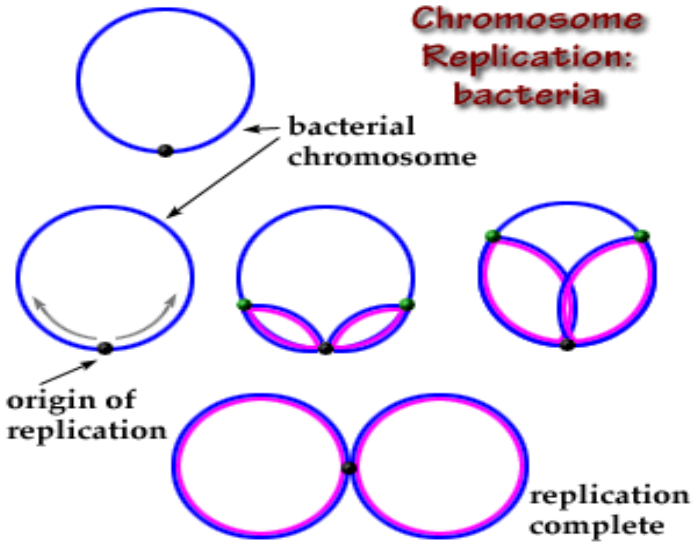
DNA Functions in microbiology

I- Replication
II- Gene expression (protein synthesis)
A. Transcription
B. Translation
C. post-translational processes.

DNA Replication

Semi conservative which means that one DNA molecule gives two DNA molecules each one consists of one strand from the original DNA and the other strand is newly formed one.

Replication starts at a fixed point called origin of replication (ori C) and terminated at fixed point called (Ter) point.



Steps of replication

1. The two old strands of DNA are separated by helicase enzyme to form the replication fork.

2. Replication of one strand template in 5' to 3' direction can proceed in a continuous fashion starting by addition of short segment of small RNA at the starting point called primer.On this primer, DNA polymerase enzyme start to synthesize the new strand in a continuous manner from 5' to 3'; this is called the leading strand.

3.Replication of the other template strand can only proceed discontinuously. This strand is called the lagging strand, many primers (RNA primers) are used to synthesize the lagging new strand as fragments called Okazaki fragments

4. Then all the RNA Primers are removed by DNA polymerase 1 and the gaps are sealed by DNA ligase to make a continuous DNA strand.

5. After formation of the two strands of DNA the old one and thenew one, DNA gyrase Enzyme (Topoisomerase II) make twist and super helicity of each DNA molecules.

Enzymes of replication:

- Helicase enzyme
- RNA polymerase
- DNA polymerase
- DNA ligase
- DNA gyrase (topoisomerase II).

Mutation:

is defined as any change in base sequence of DNA. it occur in two form: Transition (purine replaced by purine or pyrimidine replaced by pyrimidine) or Transversion (purine replaced by pyrimidine or vice versa) Mutations result from damage to DNA which is not repaired, errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile

genetic elements. Mutations play a part in both normal and abnormal biological processes including: evolution, cancer.

Exchange of Genetic Information:

1- Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell.

2- Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage.

3- Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient.

Mycology : Mycology: is the science that study of fungi, which are eukaryotic organisms that evolved in tandem with the animal kingdom. However, unlike animals, most fungi are non - motile and possess a rigid cell wall. Unlike plants, fungi are non - photosynthetic.

- **Introduction to Fungi:**

The word fungus comes from the Latin word Mykes (Greek word) : and used for Mushroom Indeed, the familiar mushroom is a reproductive structure used by many types of fungi. However, there are also many fungi species that don't produce mushrooms at all. Being eukaryotes, a typical fungal cell contains a true nucleus and many membrane-bound organelles. The kingdom Fungi includes an enormous variety of living organisms collectively referred to as Ascomycota, or true Fungi. Approximately 100,000 species of fungi have been described, but fewer than 400 are medically important, and less than 50 species cause more than 90% of the fungal infections of humans and other animals. Edible mushrooms, yeasts, black mold, and the producer of the antibiotic penicillin, *Penicillium notatum,* are all members of the kingdom Fungi.

In humans, fungal infections are generally considered challenging to treat. Unlike bacteria, fungi do not respond to traditional antibiotic therapy because they are eukaryotes. Fungal infections may prove deadly for individuals with compromised immune systems.

Fungi have many commercial applications. The food industry uses yeasts in baking, brewing, and cheese and wine making. Many industrial compounds are byproducts of fungal fermentation. Fungi are the source of many commercial enzymes and antibiotics.

A fungus : plural: **fungi** or **funguses**is includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, which is separate from the other eukaryotic life kingdoms of plants and animals.

PROPERTIES OF FUNGI:

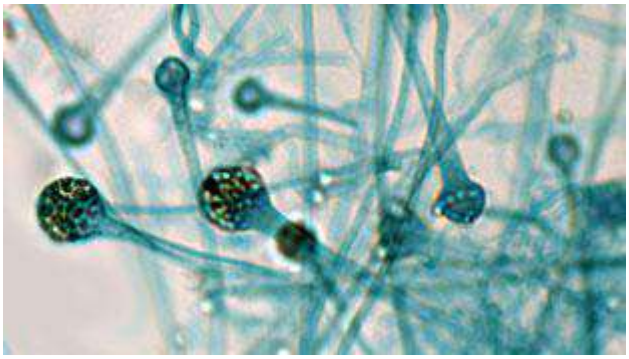
Following are the important characteristics of fungi:

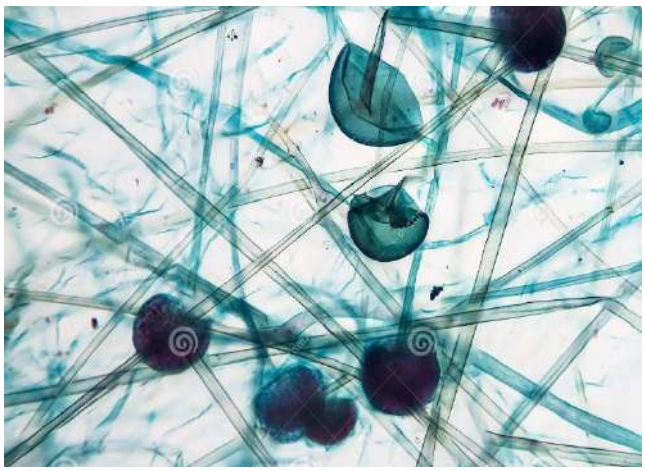
1. Fungi are eukaryotic, non-vascular, non-motile and heterotrophic organisms.
2. They may be unicellular or multicellular (filamentous).
3. They reproduce by means of spores.
4. Fungi exhibit the phenomenon of alternation of generation.
5. Fungi lack chlorophyll and hence cannot perform photosynthesis.
6. Fungi store their food in the form of starch.
7. Biosynthesis of chitin occurs in fungi.
8. The nuclei of the fungi are very small.
9. The fungi have no embryonic stage. They develop from the spores.
10. The mode of reproduction is sexual or asexual.
11. Some fungi are parasitic and can infect the host.
12. Examples include mushrooms, moulds, yeast.

Fungi grow in two basic forms: as

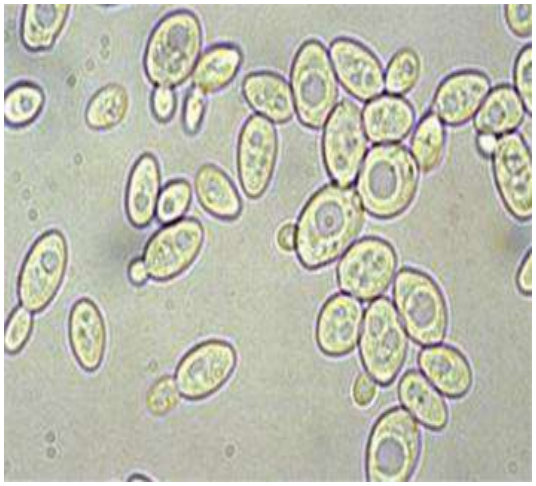
1. **Molds** (or **moulds**). Growth in the mold form occurs by the production of multicellular filamentous colonies. These colonies consist of branching cylindric tubules called **hyphae**, varying in diameter from 2 to 10 μm. The mass of intertwined hyphae that accumulates during active growth is a **mycelium**. Some hyphae are divided into cells by cross-walls or **septa**, which typically form at regular intervals during hyphal growth. Vegetative or substrate hyphae penetrate the

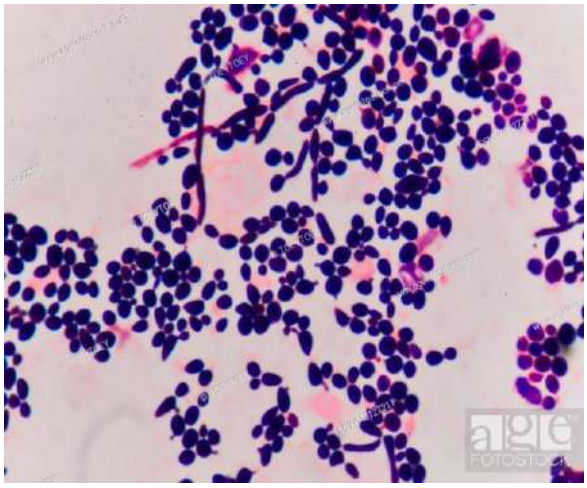
supporting medium, anchor the colony, and absorb nutrients. In contrast, aerial hyphae project above the surface of the mycelium and usually bear the reproductive structures of the mold. When a mold is isolated from a clinical specimen, its growth rate, macroscopic appearance, and microscopic morphology are usually sufficient to determine its genus and species (see below).





2. Yeasts are single cells, usually spherical to ellipsoid in shape and varying in diameter from 3 to 15 μm. Most yeasts reproduce by budding. Some species produce buds that characteristically fail to detach and become elongated; continuation of the budding process then produces a chain of elongated yeast cells called **pseudohyphae**. Yeast colonies are usually soft, opaque, 1–3 mm in size, and cream-colored. Because the colonies and microscopic morphology of many yeasts are quite similar, yeast species are identified on the basis of physiologic tests and a few key morphologic differences. Some species of **fungi are dimorphic** and capable of growth as a yeast or mold depending on environmental conditions especially temperature.





Yeast cells

Definition of important terms:

- **mycelium**: the vegetative part of any fungus, consisting of a mass of branching, threadlike hyphae, often underground
- **hypha**: a long, branching, filamentous structure of a fungus that is the main mode of vegetative growth
- **septum**: cell wall division between hyphae of a fungus
- **saprophyte**: any organism that lives on dead organic matter, as certain fungi and bacteria
- **chitin**: a complex polysaccharide, a polymer of N-acetylglucosamine, found in the exoskeletons of arthropods and in the cell walls of fungi; thought to be responsible for some forms of asthma in humans.

Classification of Fungi

Kingdom Fungi are classified based on different modes. The different classification of fungi is as follows:

- **Based on Mode of nutrition**

On the basis of nutrition, kingdom fungi can be classified into 3 groups.

1. **Saprophytic** – The fungi obtain their nutrition by feeding on dead organic substances. Examples: Rhizopus, Penicillium and Aspergillus.
2. **Parasitic** – The fungi obtain their nutrition by living on other living organisms (plants or animals) and absorb nutrients from their host. Examples: Taphrina and Puccinia.
3. **Symbioti:** These fungi live by having an interdependent relationship association with other species in which both are mutually benefited. Examples: Lichens and mycorrhiza. Lichens are the symbiotic association between algae and fungi. Here both algae and fungi are mutually benefited as fungi provide shelter for algae and in reverse algae synthesis carbohydrates for fungi.

- **Based on Spore Formation**

Kingdom Fungi are classified into the following based on the formation of spores:

1. **Zygomycetes** – These are formed by the fusion of two different cells. The sexual spores are known as zygospores while the asexual spores are known as sporangiospores. The hyphae are without the septa.
2. **Ascomycetes** – They are also called as sac fungi. They can be coprophilous, decomposers, parasitic or saprophytic. The sexual spores are called ascospores. Asexual reproduction occurs by conidiospores. Example – Saccharomyces spp.
3. **Basidiomycetes** – Mushrooms are the most commonly found basidiomycetes and mostly live as parasites. Sexual reproduction occurs by basidiospores. Asexual reproduction occurs by conidia, budding or fragmentation. Example- Agaricus

4. **Deuteromycetes** – They are otherwise called imperfect fungi as they do not follow the regular reproduction cycle as the other fungi. They do not reproduce sexually. Asexual reproduction occurs by conidia. Example – Trichoderma.

Uses of Fungi

Fungi are one of the most important groups of organisms on the planet as it plays a vital role in the biosphere and has great economic importance on account of their both benefits and harmful effects.

Following are some of the important uses of fungi:

1. **Recycling** – They play a major role in recycling the dead and decayed matter.
2. **Food** – Mushrooms species are edible which are cultured and are used as food by humans.
3. **Medicines** – There are many fungi which are used to produce antibiotics, to control diseases in humans and animals. Penicillin antibiotic is derived from a common fungi Penicillium.
4. **Biocontrol Agents** – Fungi are involved in exploiting insects, other small worms and help in controlling pests. Spores of fungi are used as spray-on crops.
5. **Food spoilage** – Fungi play a major role in recycling organic material and are also responsible for major spoilage and economic losses of stored food

Mycosis: Mycosis is a fungal infection of humans and domestic animals. Caused by any fungus that invades the tissues, causing superficial, subcutaneous, or systemic disease. **Mycoses** are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases**.**

The predisposing factors for fungal infections:

Immunosuppression and breakdown of anatomical barriers such as the skin are the major risk factors for fungal infections. Health care workers encounter at-risk patients in various settings, including AIDS clinics and intensive care, transplantation and oncology units. Patients with prolonged and deep neutropenia (haematological malignancy patients) are most at risk and are therefore most likely to receive prophylactic therapy.

Classification of Mycoses

The clinical nomenclatures used for the mycoses are based on the

(1) site of the infection, (2) route of acquisition of the pathogen, and (3) type of virulence exhibited by the fungus.

1. Classification Based on Site :

Mycoses are classified as superficial, cutaneous, subcutaneous, or systemic (deep) infections depending on the type and degree of tissue involvement and the host response to the pathogen.

2. Classification Based on Route of Acquisition :

Infecting fungi may be either exogenous or endogenous. Routes of entry for exogenous fungi include airborne, cutaneous or percutaneous. Endogenous infection involves colonization by a member of the normal flora or reactivation of a previous infection.

3. Classification Based on Virulence :

Primary pathogens can establish infections in normal hosts. Opportunistic pathogens cause disease in individuals with compromised host defense mechanisms.

Epidemiology:

The primary pathogens have relatively well-defined geographic ranges; the opportunistic fungi are ubiquitous. Fungal infections are neglected by social and political communities. However, they affect more than a billion people, resulting in approximately 11·5 million life-threatening infections and more than 1·5 million deaths annually. There have been enormous advances in fungal diagnostics and antifungal drug development over the past 20 years, but most of the world's population has not yet benefited from these advances.

Structure and reproduction of fungi

Structure and growth:

Because fungi (yeasts and molds) are eukaryotic organisms whereas bacteria are prokaryotic, they differ in several fundamental respects (see below table :

Some differences between fungi & bacteria

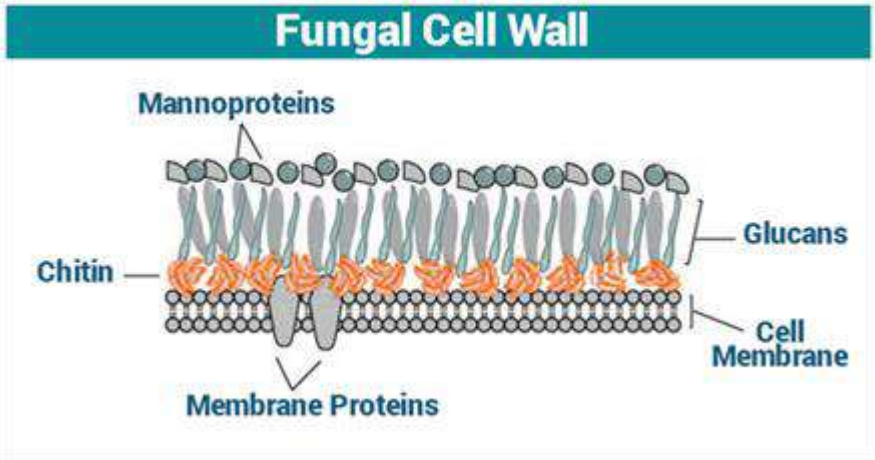
Properties	Fungi	Bacteria
Nucleus	Eukaryotic; nuclear membrane; more than one chromosome; mitosis	Prokaryotic; no membrane; nucleoid; only one "chromosome"
Cytoplasm	Mitochondria; endoplasmic reticulum; 80S ribosomes	No mitochondria; no endoplasmic reticulum; 70S ribosomes
Cytoplasmic membrane	Sterols (ergosterol)	No sterols
Cell wall	Glucans, mannans, chitin, chitosan	Murein, teichoic acids (Gram-positive), proteins
Metabolism	Heterotrophic; mostly aerobes; no photosynthesis	Heterotrophic; obligate aerobes and anaerobes, facultative anaerobes
Size, mean diameter	Yeast cells: 3–5–10 µm. Molds: indefinable	1–5 µm
Dimorphism	In some species	None

Two fungal cell structure are important medically:

1. Fungi have an essential rigid **cell wall** (unlike the cell wall of the bacteria which composed from the peptidoglycan) that determines their shape and protects them from osmotic and environmental stress. Cell walls are composed largely of carbohydrate layers—long chains of polysaccharides - as well as glycoproteins and lipids. Some sugar polymers are found in the cell walls of many fungi, such as chitin (an

unbranched polymer of β-1,4-linked *N*-acetylglucosamine); glucans, which are glucose polymers (eg, β-1,3-glucan and β-1,6-glucan); and mannans, polymers of mannose (eg, α-1,6-mannose). In addition, other polysaccharides may be unique to specific fungal species.

2. The fungal cell membrane contains ergosterol and zymosterol in contrast to human cell membranes, which contain cholesterol.



Cell Structure and Function

Fungi are eukaryotes and have a complex cellular organization. As eukaryotes, fungal cells contain a membrane-bound nucleus where the DNA is wrapped around histone proteins. A few types of fungi have structures comparable to bacterial plasmids (loops of DNA). Fungal cells also contain mitochondria and a complex system of internal membranes, including the endoplasmic reticulum and Golgi apparatus. Unlike plant cells, fungal cells do not have chloroplasts or chlorophyll. Many fungi display bright colors arising from other cellular pigments, ranging from red to green to black. The poisonous *Amanita muscaria* (fly agaric) is recognizable by its bright red cap with white patches. Pigments in fungi are associated with the cell wall. They play a protective role against ultraviolet radiation and can be toxic.

The structure of fungi can be explained in the following points:

1. Almost all the fungi have a filamentous structure except the yeast cells.
2. They can be either single-celled or multicellular organism.
3. Fungi consist of long thread-like structures known as hyphae. These hyphae together form a mesh-like structure called mycelium.
4. Fungi possess a **cell wall** which is made up of chitin and polysaccharides.
5. The cell wall comprises protoplast which is differentiated into other cell parts such as cell membrane, cytoplasm, cell organelles and nuclei.
6. The nucleus is dense, clear, with chromatin threads. The nucleus is surrounded by a nuclear membrane

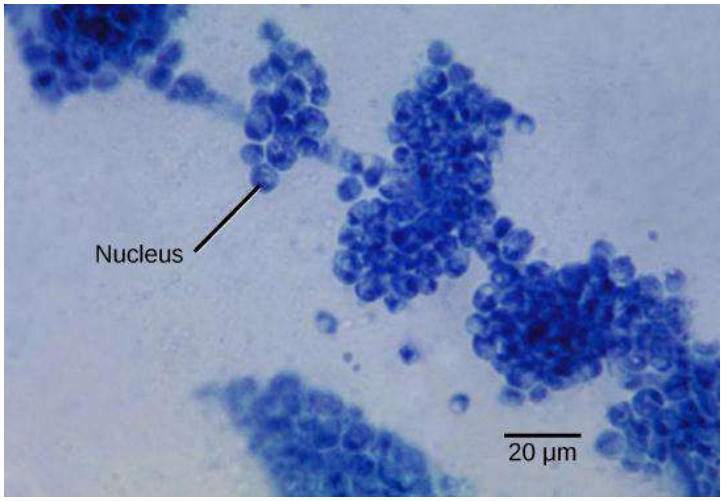
The poisonous *Amanita muscaria*: The poisonous *Amanita muscaria* is native to temperate and boreal regions of North America.

The rigid layers of fungal cell walls contain complex polysaccharides called chitin and glucans. Chitin, also found in the exoskeleton of insects,

gives structural strength to the cell walls of fungi. The wall protects the cell from desiccation and predators. Fungi have plasma membranes similar to other eukaryotes, except that the structure is stabilized by ergosterol: a steroid molecule that replaces the cholesterol found in animal cell membranes. Most members of the kingdom Fungi are non- motile.

Growth:

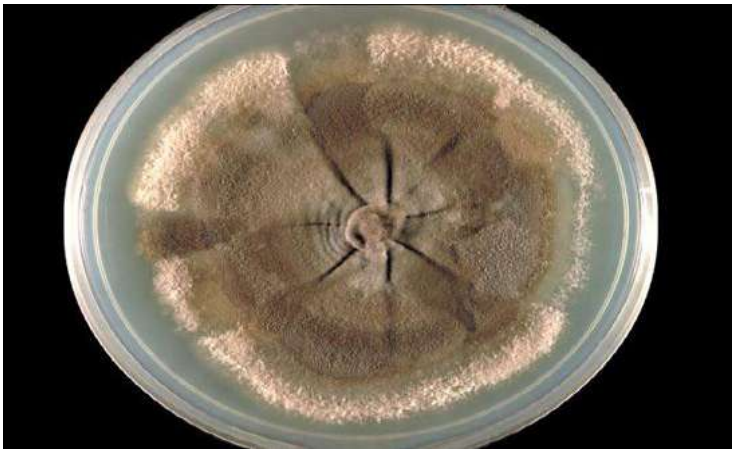
The vegetative body of a fungus is a unicellular or multicellular thallus. Dimorphic fungi can change from the unicellular to multicellular state depending on environmental conditions. Unicellular fungi are generally referred to as yeasts. *Saccharomyces cerevisiae* (baker's yeast) and *Candida* species (the agents of thrush, a common fungal infection) are examples of unicellular fungi.



Example of a unicellular fungus: *Candida albicans* is a yeast cell and the agent of candidiasis and thrush. This organism has a similar morphology to coccus bacteria; however, yeast is a eukaryotic organism (note the nucleus).

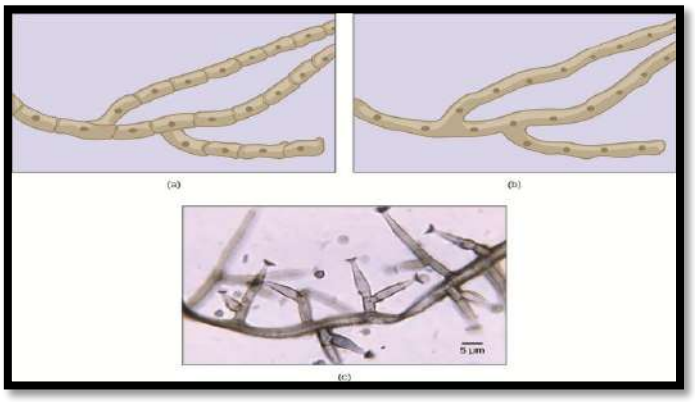
Most fungi are multicellular organisms. They display two distinct morphological stages: the vegetative and reproductive. The vegetative stage consists of a tangle of slender thread-like structures called hyphae (singular,hypha), whereas the reproductive stage can be more conspicuous. The mass of hyphae is a mycelium. It can grow on a surface, in soil or

decaying material, in a liquid, or even on living tissue. Although individual hyphae must be observed under a microscope, the mycelium of a fungus can be very large, with some species truly being “the fungus humongous.” The giant *Armillaria solidipes* (honey mushroom) is considered the largest organism on Earth, spreading across more than 2,000 acres of underground soil in eastern Oregon; it is estimated to be at least 2,400 years old.



Example of a mycelium of a fungus: The mycelium of the fungus *Neotestudina rosati* can be pathogenic to humans. The fungus enters through a cut or scrape and develops a mycetoma, a chronic subcutaneous infection.

Most fungal hyphae are divided into separate cells by endwalls called septa (singular, septum) (a, c). In most phyla of fungi, tiny holes in the septa allow for the rapid flow of nutrients and small molecules from cell to cell along the hypha. They are described as perforated septa. The hyphae in bread molds (which belong to the Phylum Zygomycota) are not separated by septa. Instead, they are formed by large cells containing many nuclei, an arrangement described as coenocytic hyphae (b). Fungi thrive in environments that are moist and slightly acidic; they can grow with or without light.



Division of hyphae into separate cells: Fungal hyphae may be (a) septated or (b) coenocytic (coeno- = “common”; -cytic = “cell”) with many nuclei present in a single hypha. A bright field light micrograph of (c) *Phialophora richardsiae* shows septa that divide the hyphae.

Nutrition

Like animals, fungi are heterotrophs: they use complex organic compounds as a source of carbon, rather than fix carbon dioxide from the atmosphere as do some bacteria and most plants. In addition, fungi do not fix nitrogen from the atmosphere. Like animals, they must obtain it from their diet. However, unlike most animals, which ingest food and then digest it internally in specialized organs, fungi perform these steps in the reverse order: digestion precedes ingestion. First, exoenzymes are transported out of the hyphae, where they process nutrients in the environment. Then, the smaller molecules produced by this external digestion are absorbed through the large surface area of the mycelium. As with animal cells, the polysaccharide of storage is glycogen rather than the starch found in plants.

Fungi are mostly saprobes (saprophyte is an equivalent term): organisms that derive nutrients from decaying organic matter. They obtain their nutrients from dead or decomposing organic matter, mainly plant material. Fungal exoenzymes are able to break down insoluble polysaccharides, such as the cellulose and lignin of dead wood, into readily-absorbable glucose molecules. The carbon, nitrogen, and other elements are thus released into the environment. Because of their varied metabolic pathways, fungi fulfill

an important ecological role and are being investigated as potential tools in bioremediation.

Some fungi are parasitic, infecting either plants or animals. Smut and Dutch elm disease affect plants, whereas athlete's foot and candidiasis (thrush) are medically important fungal infections in humans.

Fungi Reproduction

Fungi can reproduce asexually by fragmentation, budding, or producing spores, or sexually with homothallic or heterothallic mycelia. Perfect fungi reproduce both sexually and asexually, while imperfect fungi reproduce only asexually (by mitosis).

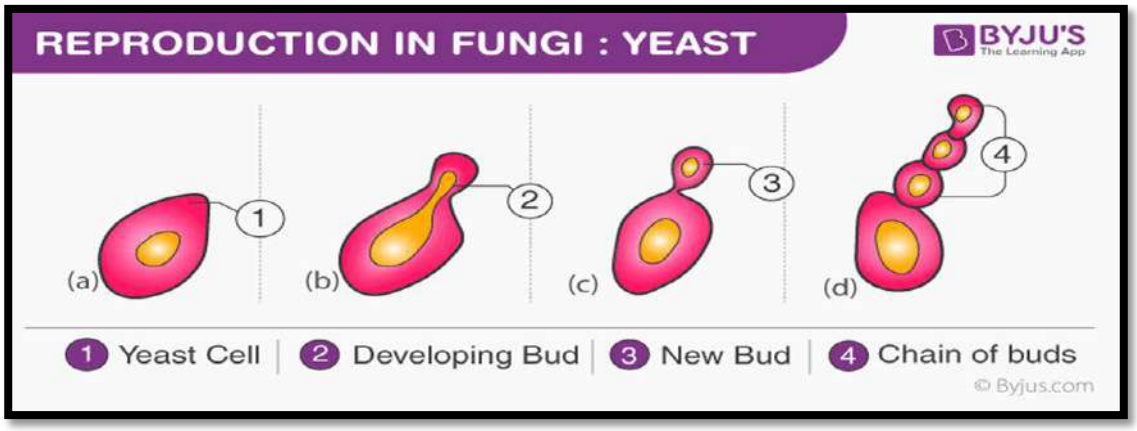
In both sexual and asexual reproduction, fungi produce spores that disperse from the parent organism by either floating on the wind or hitching a ride on an animal. Fungal spores are smaller and lighter than plant seeds.

The sexual mode of reproduction is referred to as teleomorph and the asexual mode of reproduction is referred to as anamorph.

Vegetative reproduction – By budding, fission and fragmentation.

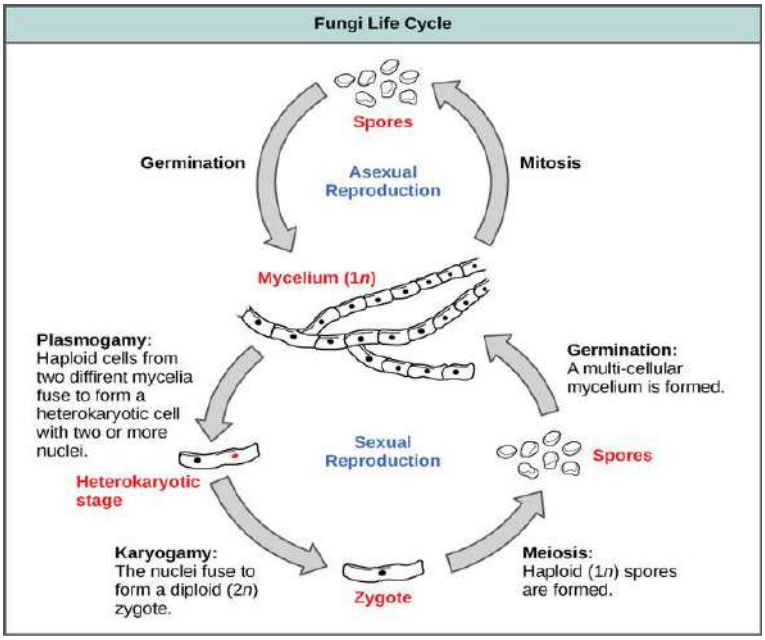
Asexual reproduction : The most common mode of asexual reproduction is through the formation of asexual spores, which are produced by one parent only (through mitosis) and are genetically identical to that parent. Spores allow fungi to expand their distribution and colonize new environments. They may be released from the parent thallus, either outside or within a special reproductive sac called a sporangium.

This takes place with the help of spores called conidia or zoospores or sporangiospores, Fungi reproduce asexually by fragmentation, budding, or producing spores. Fragments of hyphae can grow new colonies. Mycelial fragmentation occurs when a fungal mycelium separates into pieces with each component growing into a separate mycelium. Somatic cells in yeast form buds. During budding (a type of cytokinesis), a bulge forms on the side of the cell, the nucleus divides mitotically, and the bud ultimately detaches itself from the mother cell.

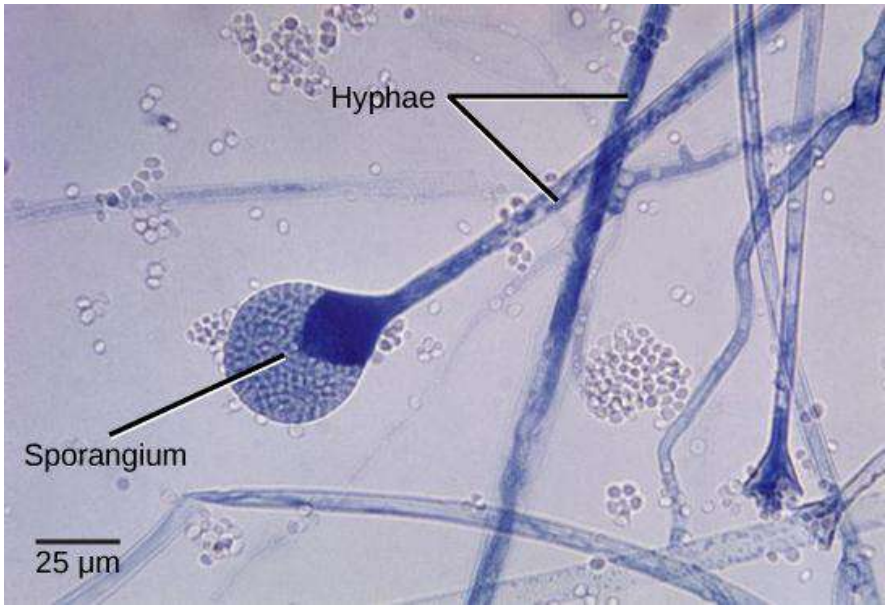


Sexual reproduction – ascospores, basidiospores, and oospores

The conventional mode of **sexual reproduction** is not always observed in the kingdom Fungi. In some fungi, the fusion of two haploid hyphae does not result in the formation of a diploid cell. In such cases, there appears an intermediate stage called the dikaryophase. This stage is followed by the formation of diploid cells.



Types of fungal reproduction: Fungi may utilize both asexual and sexual stages of reproduction; sexual reproduction often occurs in response to adverse environmental conditions.



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.